

A Bump and Hole Approach to the Development of Selective Degrons



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Abstract

The aim of this study was to develop novel immunomodulatory imide drug (IMiD) analogues which would not induce degradation of endogenous proteins but would induce degradation of a suitably modified zinc finger (ZF) degron motif, therefore providing an IMiD/ZF degron system that could be reliably used for target validation. To achieve this, we employed ‘bump and hole’ methodology to identify a ligand that selectively induces degradation of a mutant degron but not the wild type (WT) ZF. Traditional IMiDs, such as thalidomide, lenalidomide and pomalidomide, are unsuitable for target validation due to off-target degradation of transcription factors IKZF1 and IKZF3, as well as other endogenous proteins.

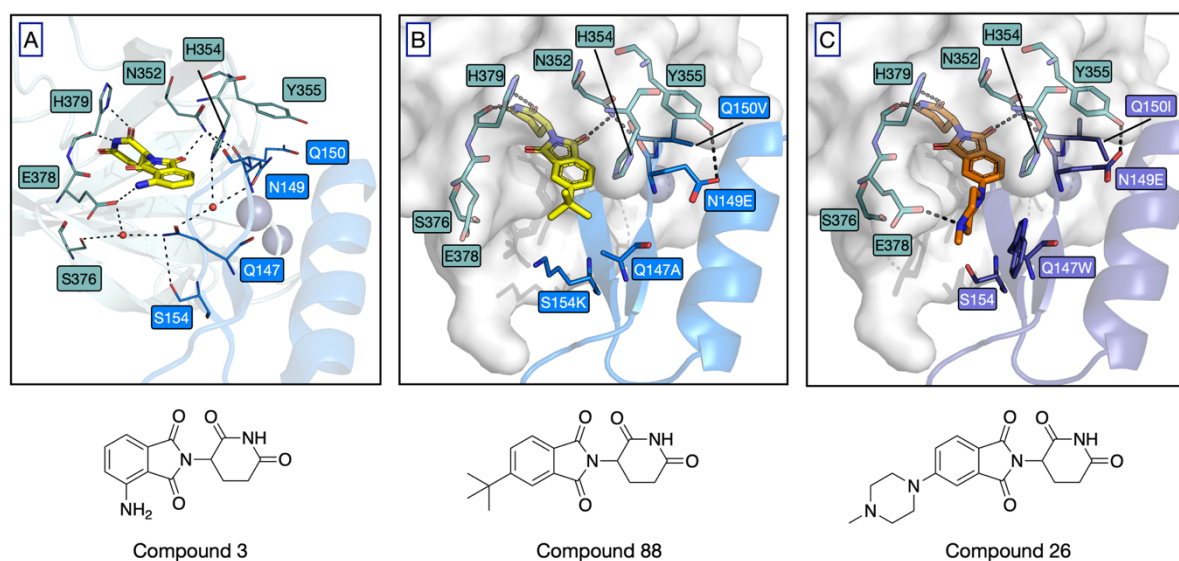


Figure i. **A)** Pomalidomide (yellow sticks) binds to cereblon (teal) facilitating a protein-protein interaction with a ZF degron (blue) derived from IKZF1/IKZF3. Hydrogen bonds (dashed lines) between the two proteins are enabled *via* water molecules (red spheres). **B)** Compound **88** features a tertiary butyl ‘bump’ group, which is accommodated by Q147A ‘hole’ mutation in a mutant ZF degron. **C)** Compound **26** features a methyl piperazine group, in which the tertiary amine is protonated. This forms a cation- π interaction with a Q147W mutation in a mutant ZF degron. The cereblon/ZF interface is also stabilised by ZF mutations at positions 149 and 150 in both **B)** and **C)**.

Abstract

A library of 'bumped' IMiD analogues were screened by molecular docking, after which a refined library of 'bumped' IMiD analogues was chemically synthesised and tested for degradation ability against the WT ZF degron sequence, and a 'hole'-containing glutamine-to-alanine (Q147A) mutant, using a flow cytometry-based fluorescence assay. The four most promising candidate molecules were then screened against a library of 8380 ZF mutant sequences using a fluorescence activated cell sorting (FACS) degradation assay, yielding IMiD/degron pairs with high selectivity.

Further flow cytometry and western blot assays confirmed the high selectivity of these IMiD/degron pairs. No degradation of endogenous IKZF1 or IKZF3 was observed in Jurkat cells incubated with either compound **88** or **26** for 18 h at 10 μ M concentration. The most selective IMiD/degron pair – compound **26** and a Q147W/N149E/Q150I mutant ZF – was then used to degrade TRIM28, a disease-relevant protein with no known small molecule ligands, with $D_{MAX} > 80\%$.

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Abbreviations

Å	Angström
Ac	Acetyl
ATP	Adenosine triphosphate
β-ME	β-Mercaptoethanol
BET	Bromo- and extra-terminal
Bn	Benzyl
BP	Beta propeller
BSA	Bovine serum albumin
BSL2	Biosafety level 2
CAR	Chimeric antigen receptor
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
CRBN	Cereblon
Cryo-EM	Cryogenic electron microscopy
CTG	CellTiter-Glo
DAPI	4',6-Diamidino-2-phenylindole
DC ₅₀	Half-maximal degradation concentration
DDB1	Damaged DNA-binding protein
DIPEA	<i>N,N</i> -Diisopropylethylamine
D _{MAX}	Maximum percentage of degradation
DMEM	Dulbecco's modified eagle medium
DMF	Dimethylformamide

Abbreviations

DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded deoxyribonucleic acid
DTT	Dithiothreitol
E1	Ubiquitin-activating enzyme
E2	Ubiquitin-conjugating enzyme
E3	Ubiquitin-protein ligase
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
eGFP	Enhanced green fluorescent protein
eq.	Equivalents
ESI	Electrospray ionisation
FACS	Fluorescence activated cell sorting
FBS	Fetal bovine serum
FKBP	FK506 binding protein
g	Grams
GSK	GlaxoSmithKline
$\Delta\Delta G$	Change in Gibbs free energy
h	Hours
HB	Helical bundle
HECT	Homologous to E6AP C-terminus
HEK	Human embryonic kidney
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectrometry

Abbreviations

HRP	Horseradish peroxidase
IC ₅₀	Half maximal inhibitory concentration
IKZF1	Ikaros family zinc finger 1
IKZF2	Ikaros family zinc finger 2
IKZF3	Ikaros family zinc finger 3
IMiD	Immunomodulatory imide drug
IPA	Isopropyl alcohol
IPiD	IMiD proximity-induced degradation
IRES	Internal ribosomal entry site
<i>J</i>	Coupling constant
L	Litres
lit.	Literature value
Log2FC	Fold change at log base 2
-log10p	p-Value at log base 10
Lon	Lon protease-like domain
LRMS	Low resolution mass spectrometry
M	Molar
MFI	Median fluorescence intensity
MLKL	Mixed lineage kinase domain-like pseudokinase
m.p.	Melting point
Me	Methyl
MEM	Modified eagle medium
mg	Milligrams
min	Minutes
mL	Millilitres

Abbreviations

mol	Moles
mRNA	Messenger ribonucleic acid
MW	Molecular weight
NGS	Next generation sequencing
NMP	<i>N</i> -methyl-2-pyrrolidone
NMR	Nuclear magnetic resonance
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
Ph	Phenyl
PHD	Plant homeodomain
POI	Protein of interest
PPI	Protein-protein interaction
Pr	Propyl
PROTAC	Proteolysis targeting chimera
PTM	Post-translational modification
PVDF	Polyvinylidene fluoride
RBR	Ring between ring
R _f	Retention factor
RING	Really interesting new gene
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute 1640 Growth Medium
rt	Room temperature
SAR	Structure activity relationship
s.e.m.	Standard error of mean
sgRNA	Single guide ribonucleic acid

Abbreviations

SMILES	Simplified molecular input line entry system
S _N Ar	Nucleophilic aromatic substitution
SPRI	Solid phase reversible immobilisation
<i>t</i> -Bu	Tertiary butyl
TAE	Tris-acetate-EDTA
TBD	Thalidomide-binding domain
TBS	Tris-buffered saline
TEV	Tobacco Etch Virus
TGX	Tris-glycine-eXtended
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMT	Tandem mass tag
TRIM28	Tripartite motif-containing 28
Tris	Tris(hydroxymethyl)aminomethane
Ub	Ubiquitin
WT	Wild type
ZF	Zinc finger

Abbreviations

Chapter 1: Introduction

1.1 Overview

The ability to rapidly and reversibly degrade proteins of interest (POIs) is a useful tool in chemical biology.¹ Protein degradation can be achieved using small molecule degradation inducers that recruit a protein motif called a degron. Immunomodulatory imide drugs (IMiDs), such as thalidomide and its analogues lenalidomide and pomalidomide, can be used to induce degradation of a POI that has been tagged with a genetically encoded degron.^{2–5}

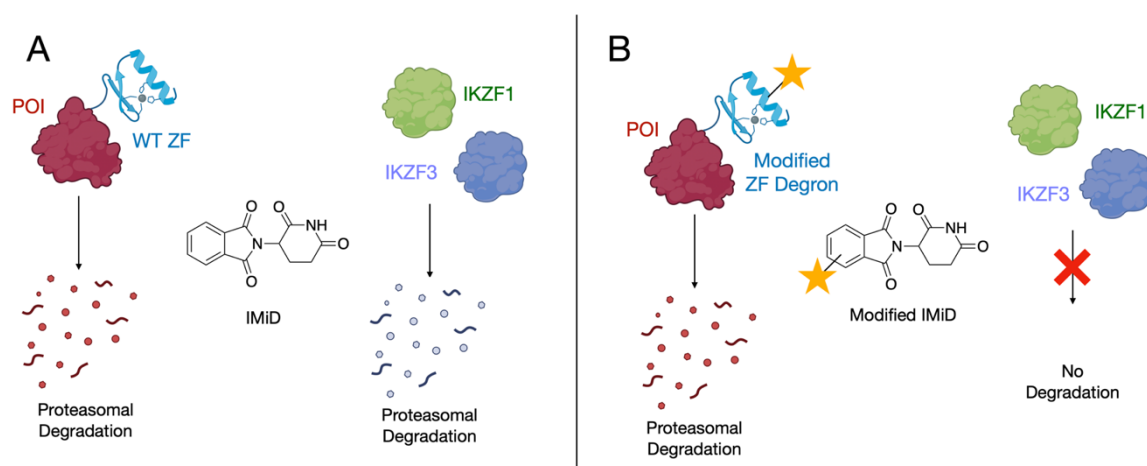


Figure 1.1. A) IMiDs can be used to induce degradation of a POI (red) fused to a ZF degron (light blue), but these molecules also induce degradation of endogenous transcription factors such as IKZF1 (dark blue) and IKZF3 (green). **B)** An IMiD analogue could be modified in such a way that it does not induce degradation of endogenous proteins but will induce degradation of a suitably modified ZF degron.

However, traditional IMiDs possess off-target effects that make their use in targeted protein degradation less than ideal.^{6–9} The work presented in this dissertation documents the development of new IMiD molecules that efficiently degrade POIs

fused to a suitably modified degron motif, but do not exhibit the off-target effects possessed by other IMiDs

1.2 Protein Degradation and the Role of Cereblon

1.2.1 Polyubiquitination and the Proteasome

Polyubiquitination is a method by which eukaryotic cells label proteins for degradation by the 26S proteasome.^{10–12} In this process, multiple copies of a small protein, ubiquitin, are covalently linked to a substrate protein that has been identified as suitable for degradation. This process is controlled by an enzyme cascade (figure 2).¹³ In this cascade, ATP hydrolysis drives the loading of a ubiquitin E1 ligase with a single ubiquitin protein. This ubiquitin is transferred from the E1 ligase to an E2 ligase; the ubiquitin is then transferred from the E2 ligase to the substrate *via* an E3 ligase, which recognises the specific protein to be degraded. In some cases the E3 ligase plays an enzymatic role in the transferring of the ubiquitin from the E2 ligase to the substrate; in other cases the E2 ligase transfers the ubiquitin directly onto the substrate and the E3 ligase acts solely as an adapter between these two proteins.

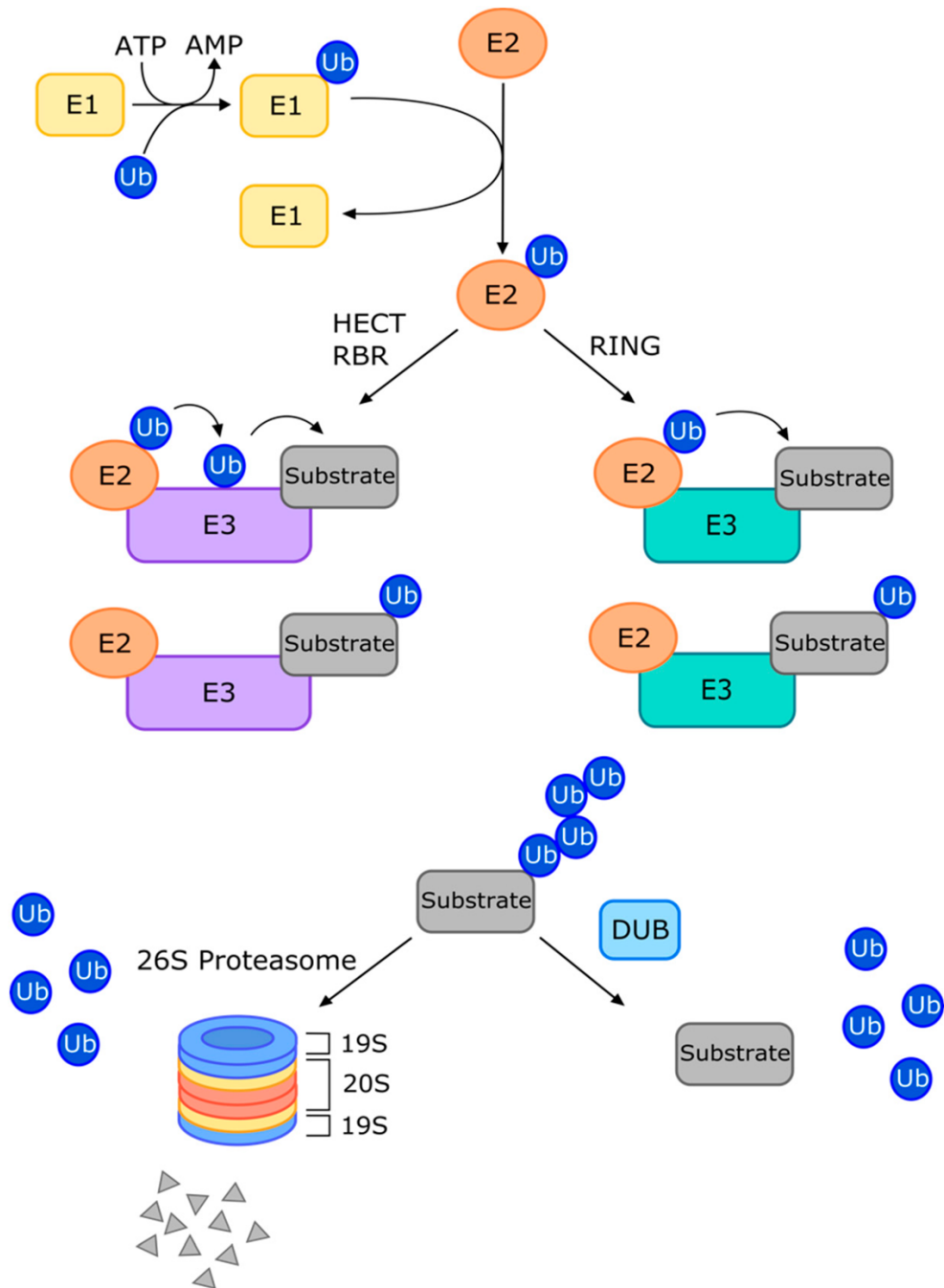


Figure 1.2. Enzyme cascade leading to polyubiquitination of a protein, labelling it for degradation by the 26S proteasome. An E1 ubiquitin ligase (yellow) catalyses the attachment of a ubiquitin protein (Ub – dark blue) onto a ubiquitin E2 ligase (orange), driven by the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP). HECT and RBR-type ubiquitin E3 ligases (violet) partake in the catalysis of the transfer of a ubiquitin protein from a ubiquitin E2 ligase onto a substrate protein (grey); RING-type ubiquitin E3s bridge a ubiquitin E2 ligase to a substrate, facilitating a ubiquitin transfer, but are not involved catalytically. After multiple ubiquitination events (polyubiquitination), a substrate is either degraded by the 26S proteasome or deubiquitinated by a deubiquitinating enzyme (DUB – light blue). Reprinted with permission by Laplante *et al.*¹³

Once the substrate has been polyubiquitinated it is shuttled to the 26S proteasome,¹⁴ a large assembly which cleaves the substrate into its constituent amino acids. These free amino acids are then used for other cellular processes.

1.2.2 Cereblon

Cereblon (CRBN) is a protein found in eukaryotic cells. CRBN acts as an E3, but possesses no ligase activity. It binds to damaged DNA binding protein, (DDB1), which in turn binds to three other proteins to form a complex, including an E2 ligase. This complex can then polyubiquitinate the substrate recruited by CRBN.

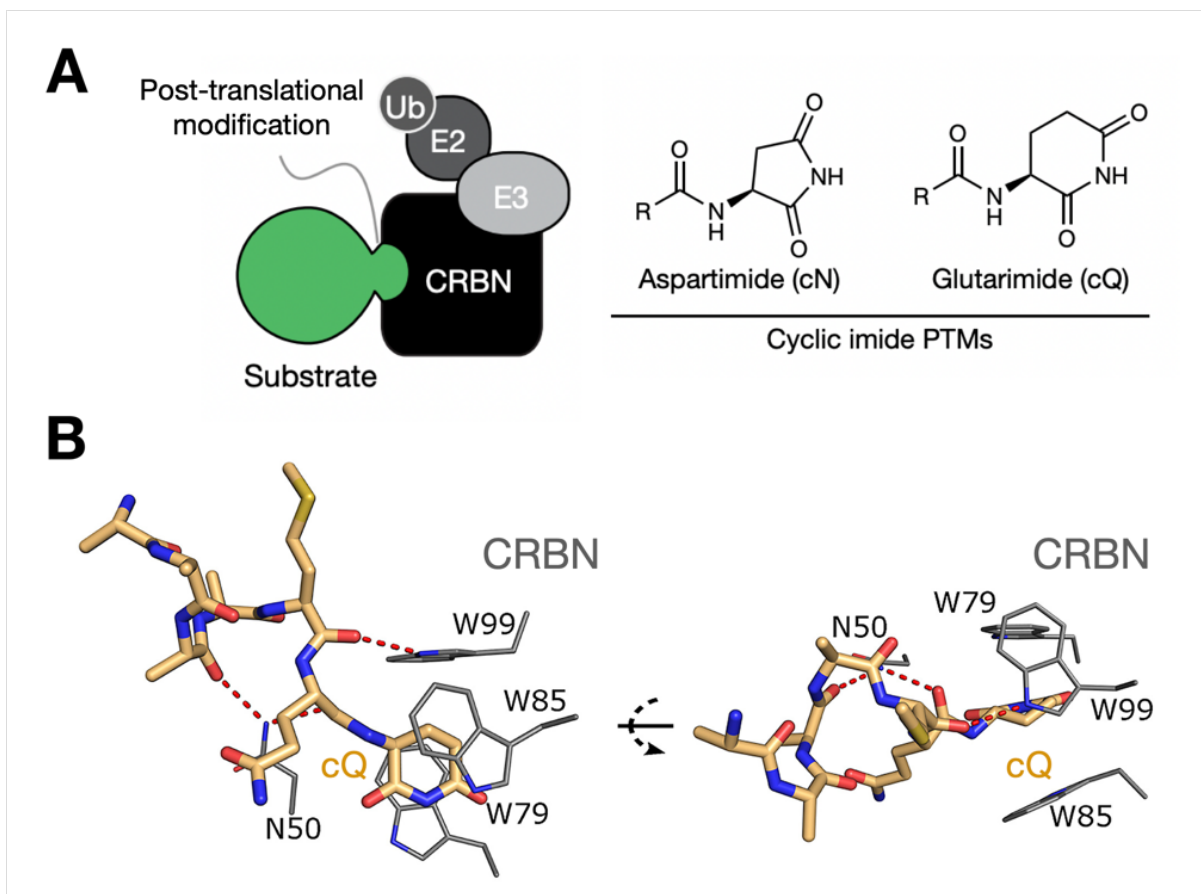


Figure 1.3. A) Cyclisation of a C-terminal asparagine or glutamine residue in a substrate protein (green), results in a cyclic imide group (cN or cQ) PTM, which is recognised by the ubiquitin E3 ligase cereblon (CRBN - black) and facilitates attachment of ubiquitin (Ub) to the substrate protein by a ubiquitin E2 ligase (E2). **B)** Crystal structure of peptide (beige) with a C-terminal cyclised glutamine bound to the conserved pocket of CRBN (grey) (PDB ID: 8BC7). Adapted from Ichikawa *et al.*¹⁵ and Fischer *et al.*¹⁶ with permission.

The endogenous target of CRBN has been the focus of much speculation, with a number of hypotheses being put forward, including the small molecule uridine.¹⁷ However, recent work has proposed that the endogenous substrate is neither a small molecule nor a specific protein, but instead a post-translational modification (PTM) that arises in many proteins (figure 1.3A).^{15,18} To form this PTM, a C-terminal asparagine or glutamine residue cyclises to form a cyclic imide: a five-membered ring for asparagine, or a six-membered ring for glutamine. This cyclic imide motif is recognised by a highly conserved region in CRBN, in which three tryptophan side chains create a hydrophobic pocket (figure 1.3B).¹⁶ This pocket also recognises an analogous ring moiety found in IMiDs.

1.3 Immunomodulatory Imide Drugs (IMiDs)

1.3.1 Thalidomide and its Analogues

IMiDs are a class of CRBN-binding molecules that include thalidomide (**1**) and its analogues lenalidomide (**2**) and pomalidomide (**3**). More recently developed molecules, with enhanced or modulated therapeutic effects, include avadomide (**4**), CC-220 (also known as iberdomide) (**5**),^{19,20} and CC-885 (**6**)²¹ (figure 1.4).

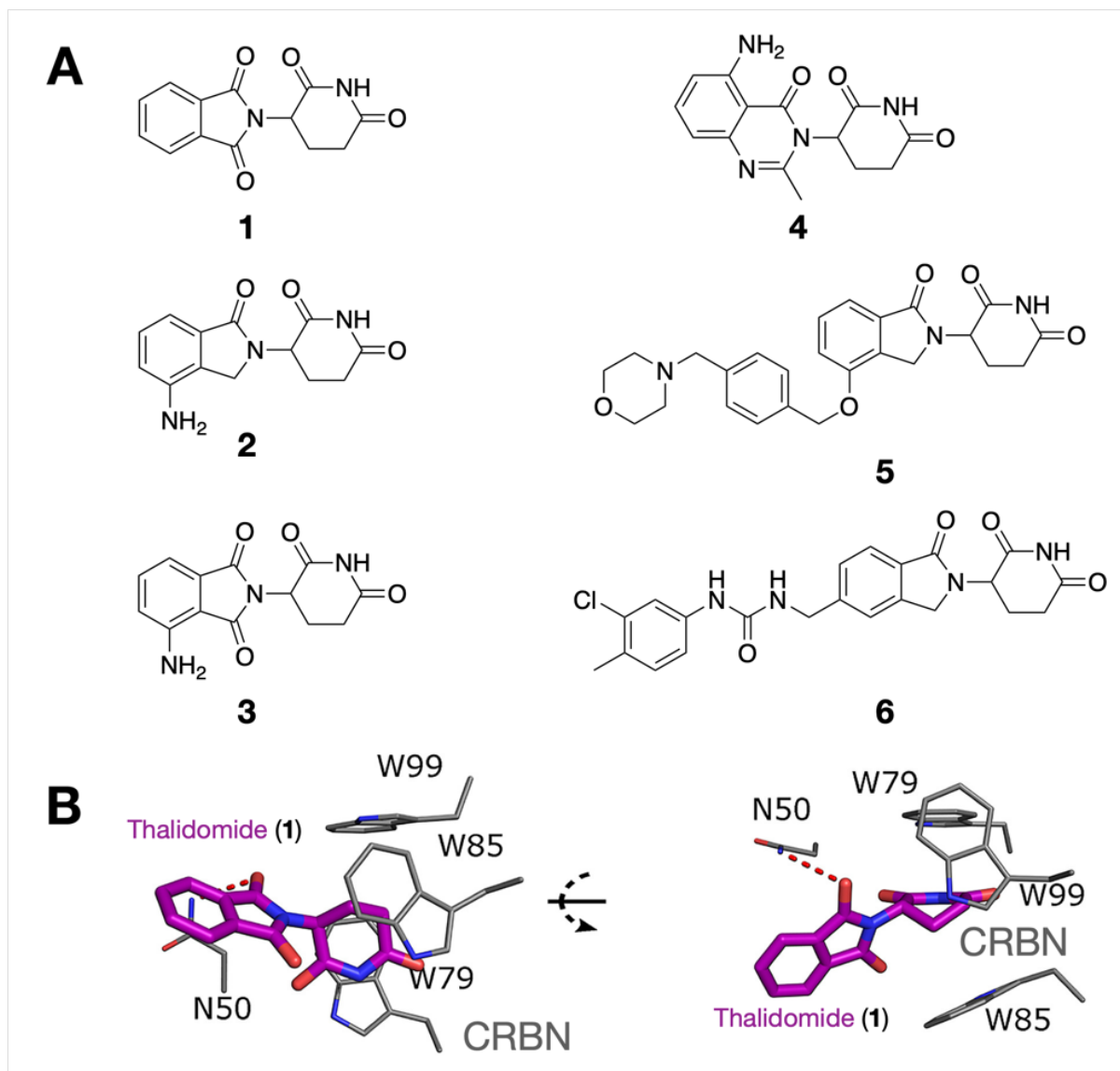


Figure 1.4. A) Examples of IMiDs: thalidomide (**1**), lenalidomide (**2**), pomalidomide (**3**), avadomide (**4**), CC-220 (also known as iberdomide) (**5**), and CC-885 (**6**). **1-4** are currently approved for use in humans. **B)** Crystal structure of thalidomide (purple) bound to the conserved pocket of CRBN (grey); the recognition of the aminoglutarimide ring in thalidomide by CRBN is analogous to the recognition of the cyclic glutamine moiety of a substrate peptide by CRBN in Figure 1.3B. Adapted from Fischer *et al.* with permission.¹⁶

All IMiD molecules possess the six-membered cyclic imide ring, which mimics the C-terminal cyclic glutamine PTM found in the endogenous targets of CRBN. This is also referred to as a glutarimide ring. The IMiD glutarimide ring binds in the same hydrophobic pocket in CRBN as the cyclic glutamine PTM.²²

Binding of an IMiD to CRBN can affect the conformation of CRBN. Work by Watson *et al.* has shown that, in the absence of a small molecule binder, CRBN adopts an open conformation. Binding of an IMiD induces a closed conformation (figure 1.5).²³

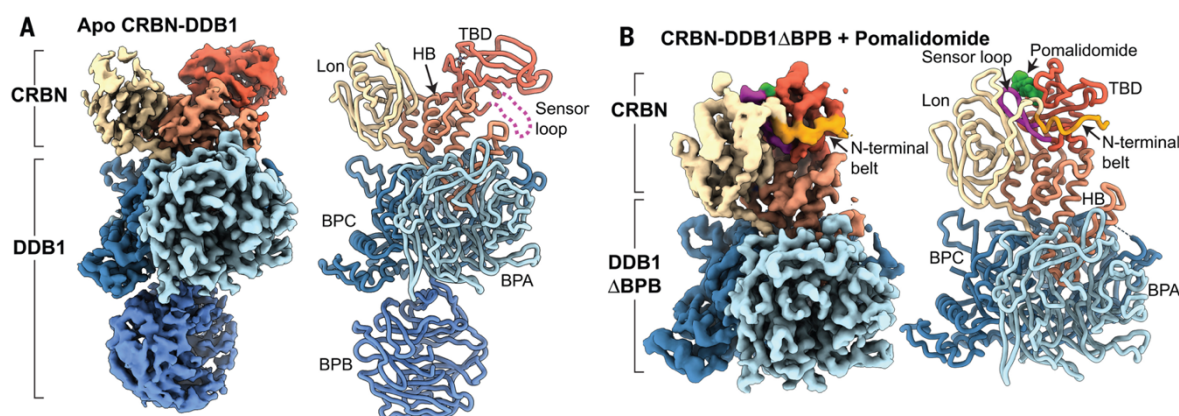


Figure 1.5. A) Cryo-EM structure of CRBN (Lon domain, tan; TBD domain, red; HB domain, orange) bound to DDB1 (BPA domain, light blue; BPB domain, medium blue; BPC domain, steel blue) with CRBN in an open (apo) conformation (*PDB ID: 8CVP*). **B)** Cryo-EM structure of CRBN bound to DDB1 and pomalidomide (**3**); pomalidomide induces CRBN to adopt a closed conformation, in which it can bind neosubstrates (*PDB ID: 8D81*). Adapted from Watson *et al.* with permission.²³

1.3.2 IMiD Recruitment of Neosubstrates

Once the IMiD has bound to CRBN, the CRBN/IMiD duplex possesses an altered affinity profile for other proteins relative to unoccupied CRBN; it has been shown that at least eleven proteins that do not usually interact significantly with CRBN will bind more strongly to the CRBN/IMiD duplex, forming a ternary complex.^{2,6,24–26} This number is likely far larger. These proteins are known collectively as neosubstrates. CRBN only possesses affinity for these neosubstrates in the IMiD-induced closed conformation.²³

Upon formation of this ternary complex, the neosubstrate is then in a suitable position for monoubiquitination and subsequent polyubiquitination by the E2 ligase associated

with CRBN. Therefore, the net effect of IMiD treatment is the depletion of intracellular levels of any susceptible neosubstrates *via* proteasomal degradation.

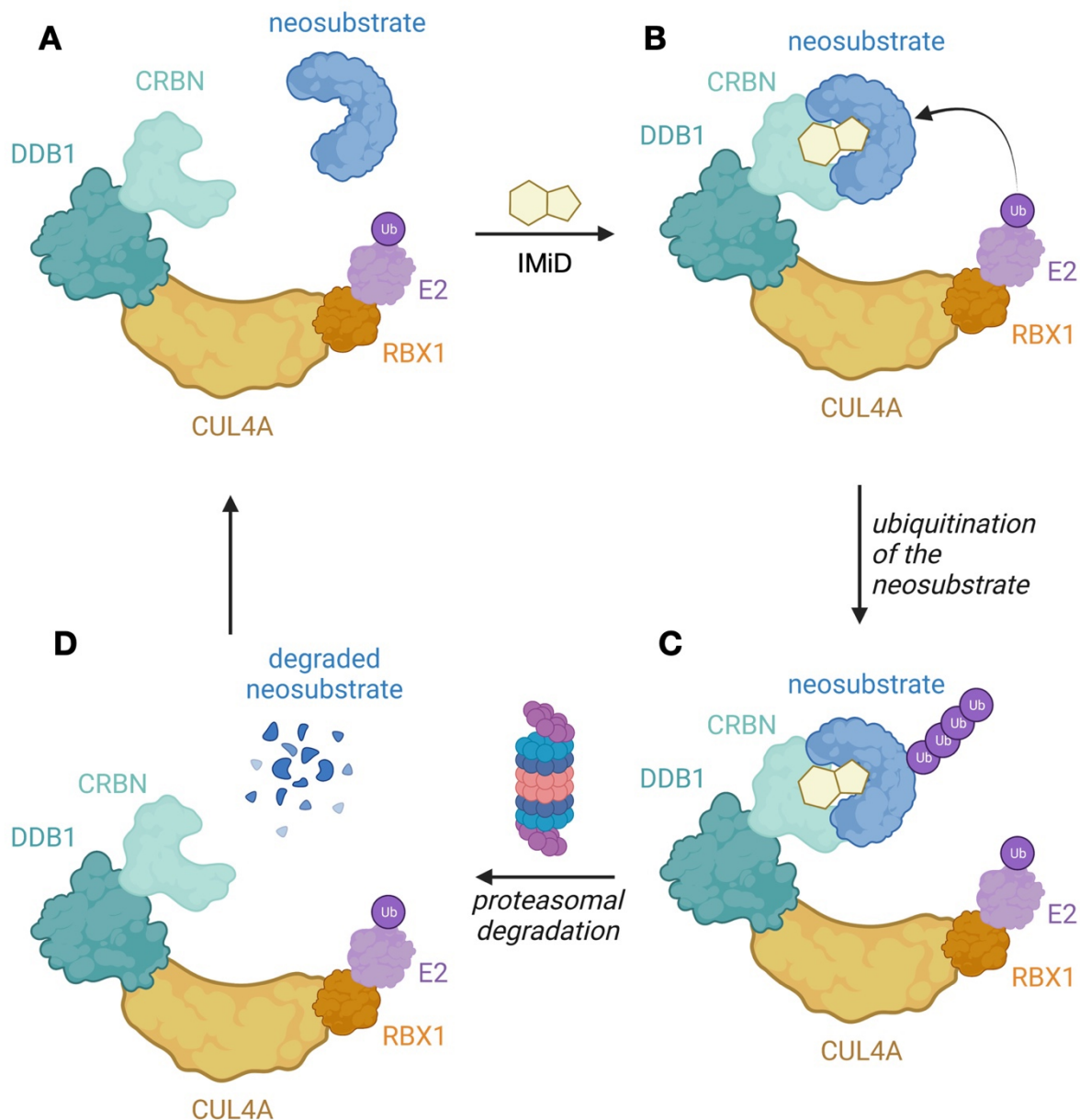


Figure 1.6. **A)** IMiDs (beige) act as molecular glues, effecting the formation of a ternary complex between cereblon (CRBN – light green) and a neosubstrate (blue); **B)** the neosubstrate then becomes monoubiquitinated by a ubiquitin E2 ligase (violet), which is linked to CRBN via DDB1 (dark green), CUL4A (yellow) and RBX1 (orange). Ubiquitin is represented by Ub (purple); **C)** after monoubiquitination, if the neosubstrate is not deubiquitinated, it will become polyubiquitinated; **D)** polyubiquitination marks the neosubstrate for degradation by the 26S proteasome (purple/blue/pink).

This method of action has been repurposed by proteolysis targeting chimera (PROTACs).²⁷ PROTACs are heterobifunctional molecules, where a ligand that binds

to a POI is attached to an E3 ligase ligand by a linker region. A PROTAC tethers the POI to an E3 ligase, whereupon the POI is polyubiquitinated and degraded. Many examples of PROTACs use IMiD-derived moieties as their E3 ligase ligands.²⁸

While IMiDs as standalone molecules effect the same outcome, they act in a different way – they are an example of a molecular glue,^{29,30} where a small molecule stabilises a protein-protein interaction (figure 1.7). Unlike PROTACs, IMiDs do not bind to neosubstrates on their own; the neosubstrate will bind to the CRBN/IMiD duplex, but not to either one by themselves. The IMiD tips the balance, facilitating a protein-protein interaction between CRBN and the neosubstrate.

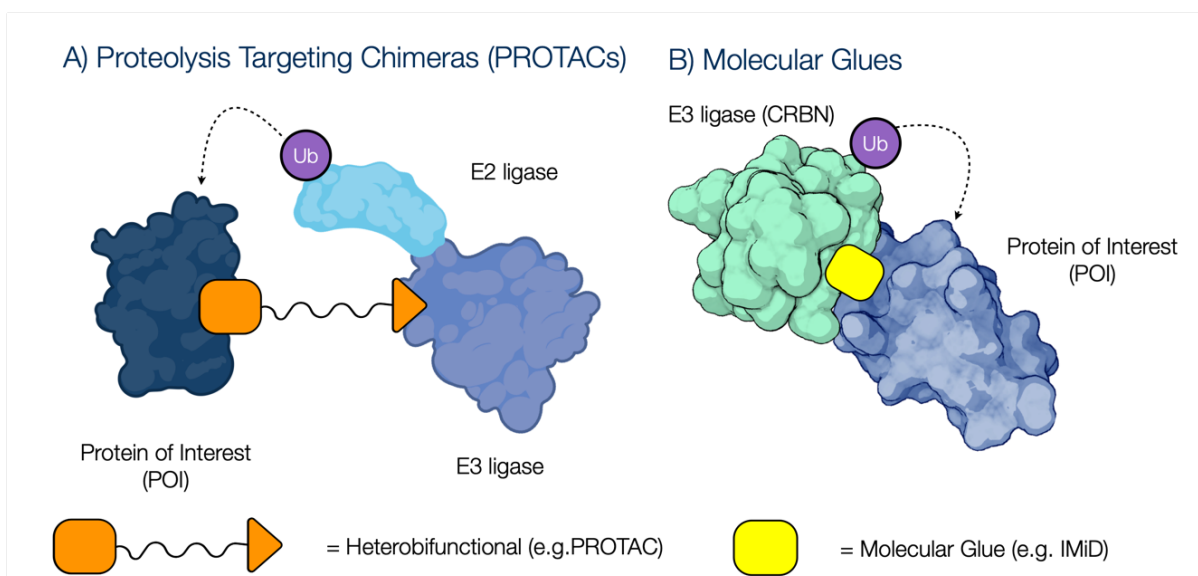


Figure 1.7. **A)** Proteolysis targeting chimera (PROTACs – orange) are heterobifunctional small molecules that comprise two binding regions bridged by a linker region, one for a protein of interest (POI, dark blue), and one for a ubiquitin E3 ligase (mid blue). **B)** IMiDs (yellow) act as molecular glues, stabilising a protein-protein interaction between a ubiquitin E3 ligase (CRBN, green) and a POI.²⁷

1.3.3 The CRBN/IMiD/ZF interface

The nature of the neosubstrates recruited to cereblon by IMiDs differs significantly, and depends partly on the structure of the IMiD used.^{2,31} However, despite overall structural diversity, many of these neosubstrates share a common zinc finger (ZF)

motif through which they interact with the CRBN/IMiD duplex. This region is known as a degron, as it is required for ternary complex formation and subsequent degradation of the neosubstrate. Neosubstrates are known which bind through other kinds of structural motifs, GSPT1 and CK1 α being examples,^{32–34} but it is the ZF degron which will be discussed further.

Figure 1.8 shows the interface between CRBN, pomalidomide and the ZF degron motif that is common to two transcription factors: IKZF1 (also known as ikaros) and IKZF3 (also known as aiolos).² Residues are numbered according to the IKZF3 convention.

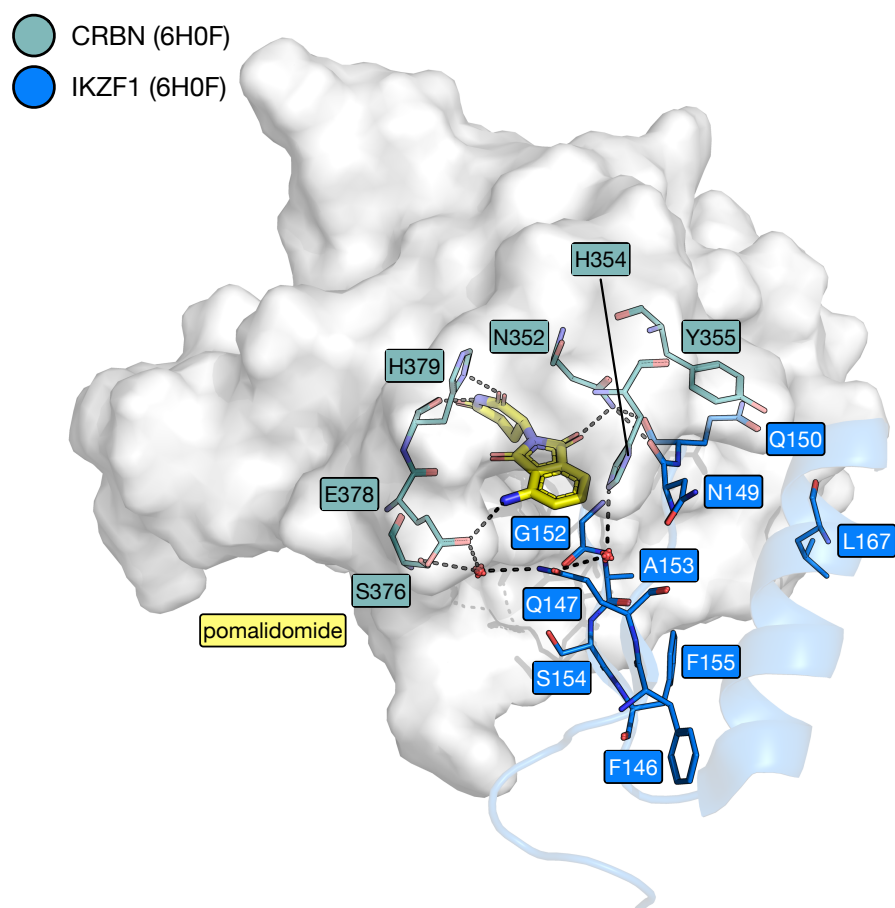


Figure 1.8. The interface between CRBN (teal) and the IKZF1/3 ZF degron (blue), bridged by pomalidomide (yellow) with key residues in stick form. Polar interactions are shown as dotted lines and two key water molecules are shown as red spheres (*PDB ID: 6H0F*).

A number of key interactions can be seen at this interface, both between the ZF and pomalidomide, and between the ZF and CRBN. The glycine at position 152 is close to the phthalimide region of pomalidomide that protrudes from the CRBN binding pocket. As can be seen in Table 1.1, this glycine is highly conserved across ZF degnon motifs from a variety of neosubstrates.² It has also been shown that mutation from glycine to alanine at this position drastically reduces the ability of IMiDs to effect degradation of a ZF degnon.³⁵ Key hydrogen bonds can be seen between pomalidomide and CRBN residues in the IMiD binding pocket region: H379, N352 and E378. Hydrogen bonds between N352 in CRBN and the backbones of N149/Q150 in the ZF can also be seen, through which these two proteins interact directly.

Table 1.1. Residue identities from position 146 to 168 of the IKZF3 ZF degnon and in the equivalent positions of 13 homologous ZF structures found in other endogenous neosubstrates.²

	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168
IKZF1	F	Q	C	N	Q	C	G	A	S	F	T	Q	K	G	N	L	L	R	H	I	K	L	H
IKZF2	F	H	C	N	Q	C	G	A	S	F	T	Q	K	G	N	L	L	R	H	I	K	L	H
IKZF3	F	Q	C	N	Q	C	G	A	S	F	T	Q	K	G	N	L	L	R	H	I	K	L	H
SALL4	F	V	C	S	V	C	G	H	R	F	T	T	K	G	N	L	K	V	H	F	H	R	H
ZNF654	F	A	C	V	I	C	G	R	K	F	R	N	R	G	L	M	Q	K	H	L	K	N	H
ZNF787	F	V	C	P	R	C	G	R	G	F	S	Q	P	K	S	L	A	R	H	L	R	L	H
ZNF653	L	Q	C	E	I	C	G	Y	Q	C	R	Q	R	A	S	L	N	W	H	M	K	K	H
ZNF276	L	Q	C	E	V	C	G	F	Q	C	R	Q	R	A	S	L	K	Y	H	M	T	K	H
ZNF692	L	Q	C	E	I	C	G	F	T	C	R	Q	K	A	S	L	N	W	H	Q	R	K	H
ZFP91	L	Q	C	E	I	C	G	F	T	C	R	Q	K	A	S	L	N	W	H	M	K	K	H
ZNF582	Y	Q	C	K	V	C	G	R	A	F	K	R	V	S	H	L	T	V	H	Y	R	I	H
ZNF517	Y	R	C	R	A	C	G	R	A	C	S	R	L	S	T	L	I	Q	H	Q	K	V	H
E4F1	H	E	C	K	L	C	G	A	S	F	R	T	K	G	S	L	I	R	H	H	R	R	H
ZNF827	F	Q	C	P	I	C	G	L	V	I	K	R	K	S	Y	W	K	R	H	M	I	I	H

Other key interactions are also notable from Figure 1.8. The side chain of ZF residue 147 (a glutamine in IKZF1/3) is close to the phthalimide region of pomalidomide and forms a hydrogen bonding network with nearby water molecules, residues H354 and E378 in CRBN, and the aniline NH₂ group in pomalidomide. The side chains of three residues in the ZF also form interactions with CRBN: The side chain of ZF residue 149 (an asparagine in IKZF1/3) interacts with CRBN residue H354; the side chain of ZF

residue 150 (a glutamine in IKZF1/3) interacts with CRBN residue W355; the side chain of ZF residue 153 (an alanine in IKZF1/3) interacts closely with the CRBN surface.

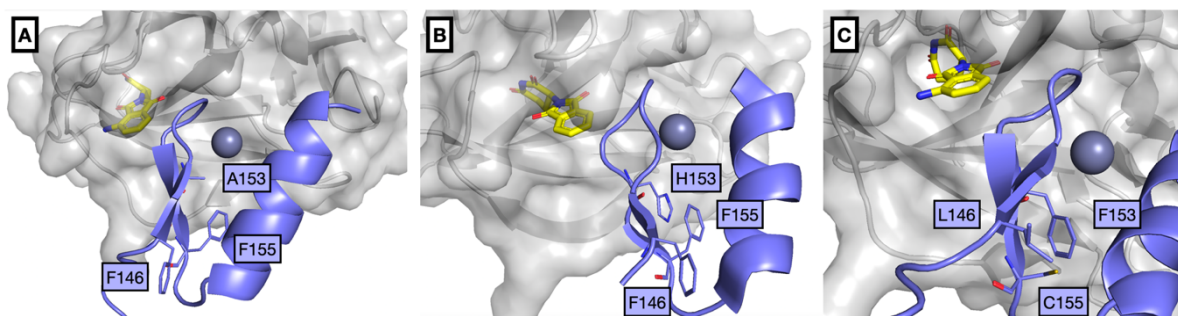


Figure 1.9. Tripartite motif comprising the internal core region of the ZF degron regions of **A)** IKZF1, **B)** SALL4, and **C)** ZNF692. All residues are numbered according to IKZF3 convention. CRBN (grey), ZF degrades (lilac), IMiD molecules (yellow), zinc (grey). (PDB IDs: 6H0F, 7BQU and 6H0G).

ZF residues 146, 153 and 155 (phenylalanine, alanine and phenylalanine, respectively, in IKZF1/3) form a tripartite motif constituting a hydrophobic core of the ZF. The residues making up this motif differ between different ZF degrades. Three examples are shown in Figure 1.9: IKZF1, SALL4 and ZNF692. For simplicity, all residues are labelled according to IKZF3 convention.

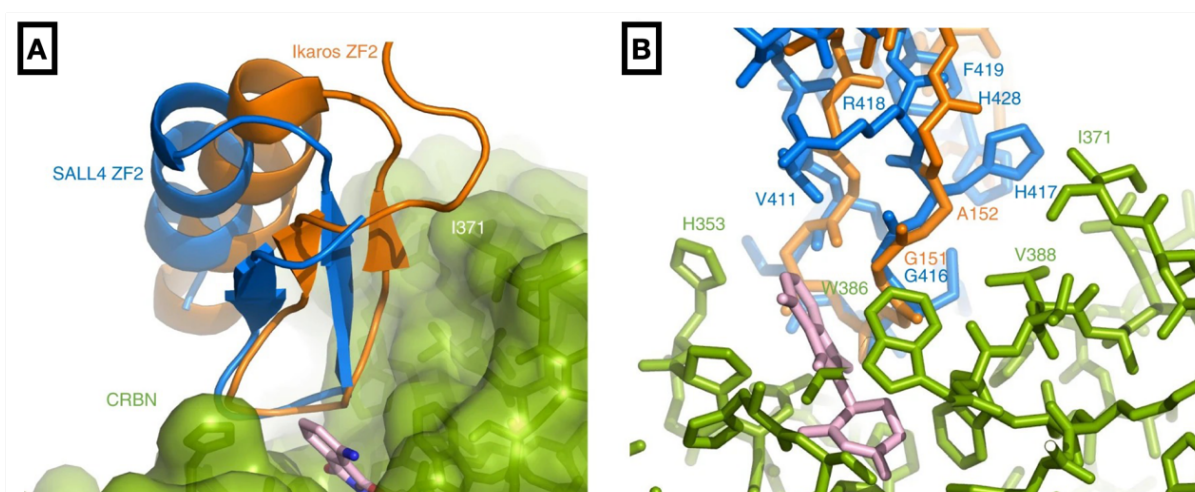


Figure 1.10. **A)** Superimposition of ZF degrades from IKZF1 (orange) and SALL4 (blue) shows a deviation in the binding by an angle of 15°. **B)** Adoption of this angle by SALL4 is due to clash between CRBN (green) and H417 in SALL4 (equivalent of position A152 in IKZF1 and A153 in IKZF3). Pomalidomide is shown as pink sticks. (PDB IDs: 6H0F and 6UML). Adapted from Matyskiela *et al.* with permission.³⁵

In SALL4 – the neosubstrate whose degradation is proposed to be responsible for the teratogenic effects of thalidomide^{6,7} – there is a histidine residue at position 153, instead of the alanine present at this position in IKZF1/3. The larger side chain of this histidine pushes against the CRBN surface, forcing the entire ZF into a different orientation by 15° compared to IKZF1/3 (figure 1.10).³⁵

In ZNF692 there is a phenylalanine at the equivalent of position 153, but instead of pushing against the CRBN surface like the H417 of SALL4 does, its side chain occupies the internal space of the ZF. In IKZF1/3 this space is occupied by the side chain of F155, but in ZNF692 it is free for occupation as residue 155 is a cysteine, which possesses a smaller side chain and so occupies less space.

Table 1.1 shows that many neosubstrates that exhibit either the F146/A153/F155 or L146/F153/C155 tripartite motif, with several others possessing a F146/R153/F155 motif. Attention has been given to this particular motif in this chapter as these three positions were considered during design of the ZF degron library (chapter 4).

1.4 Degrons for Targeted Protein Degradation

1.4.1 Established Degron Technologies

POIs can be specifically degraded *via* the use of genetically encodable degron tags. This allows for rapid and reversible knocking down of POIs in applications such as target validation. Many small molecule/degron technologies are already well established.^{36,37} The most common strategy involves employing heterobifunctional small molecules that are analogous to PROTACs in their mechanism of action (Figure

1.11). Table 1.2 shows four examples of this heterobifunctional approach. HaloTag/HaloPROTAC³⁸ (7) and dTag³⁹ (8) systems have already seen use in phenotypic knockdown studies,^{40,41} while BromoTag⁴² (9) and NanoTAC⁴³ (10) are more recent developments. It is notable that HaloTag/HaloPROTAC differs slightly from the other methods mentioned, as a covalent bond is formed between the chloroalkane moiety of the HaloPROTAC molecule and the HaloTag degen.

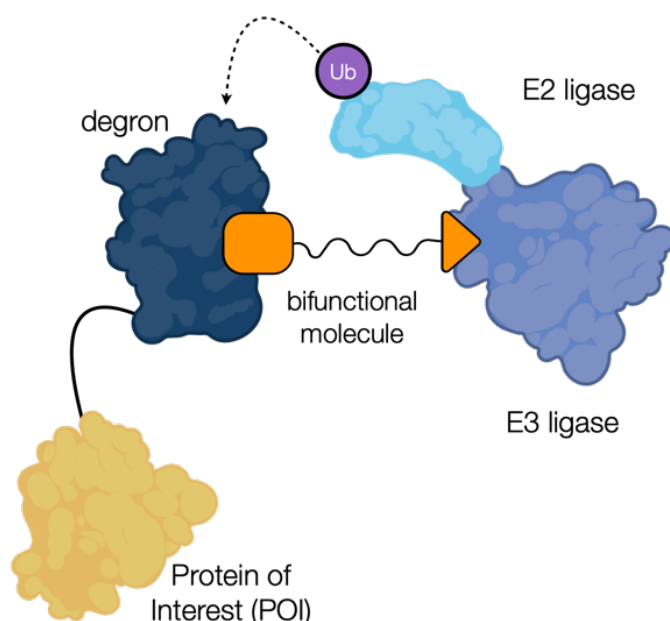
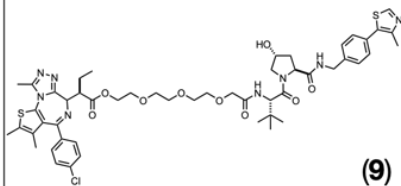
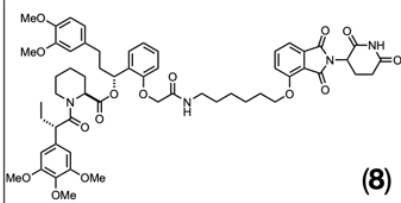
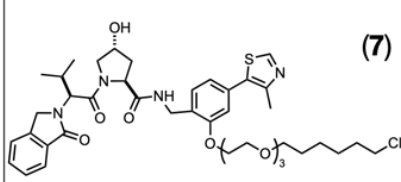
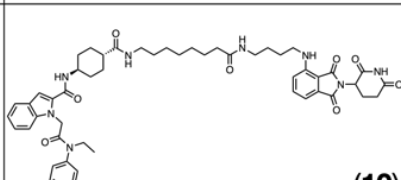


Figure 1.11. A POI (yellow) is fused to a degron motif (dark blue), allowing a heterobifunctional degrader molecule (orange) to recruit a ubiquitin E3 ligase (mid blue), facilitating addition of ubiquitin (purple) to the fusion protein *via* a ubiquitin E2 ligase (light blue).

Table 1.2. Four examples of degron systems using heterobifunctional small molecule degraders.

System	Degrone	Example Ligand
BromoTag	BRD4(2) ^{L387A}	 (9)
dTag	FKBP12 ^{F36V}	 (8)
HaloTag/HaloPROTAC	<i>Rhodococcus</i> haloalkane dehalogenase	 (7)
NanoTAC	NanoLuc	 (10)

Each of these methods possesses the same two limitations: the small molecules employed are large in size and do not possess ideal pharmacokinetic properties; and the degrons employed are large and can potentially interfere in the endogenous functionality of the protein they are fused to.

It has also been shown in work by Grohmann *et al.* that fusion of a degron region to a POI can negatively affect the level of protein expression (figure 1.12).⁴²

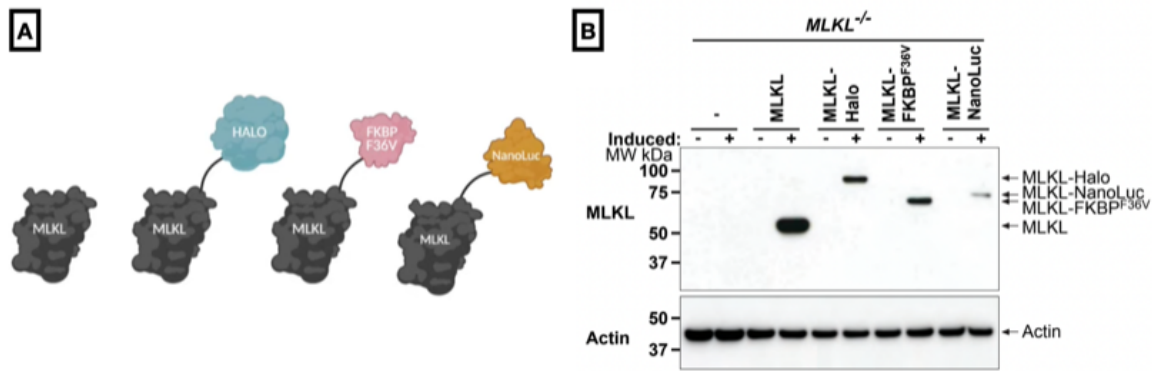


Figure 1.12. A) MLKL (a pro-necroptotic pseudokinase – black) is tagged with different established degron domains: HaloTag (blue), F36V mutant FKBP (pink), and NanoLuciferase (yellow). **B)** Western blot data for MLKL^{-/-} HT29 cells stably expressing doxycycline-inducible MLKL (either untagged or C-terminal degron fusion proteins) induced with 40 ng/mL doxycycline: MLKL-degron fusion protein expression is diminished compared to the untagged MLKL. Adapted from Grohmann *et al.* with permission.⁴²

1.4.2 IMiD Proximity Induced Degradation (IPID)

In addition to the ability to degrade endogenous neosubstrates possessing a ZF degron motif, IMiDs can also induce degradation of other proteins onto which a ZF degron has been genetically fused (Figure 1.13).^{2,5} This approach has been named IMiD proximity induced degradation (IPID).⁴⁴

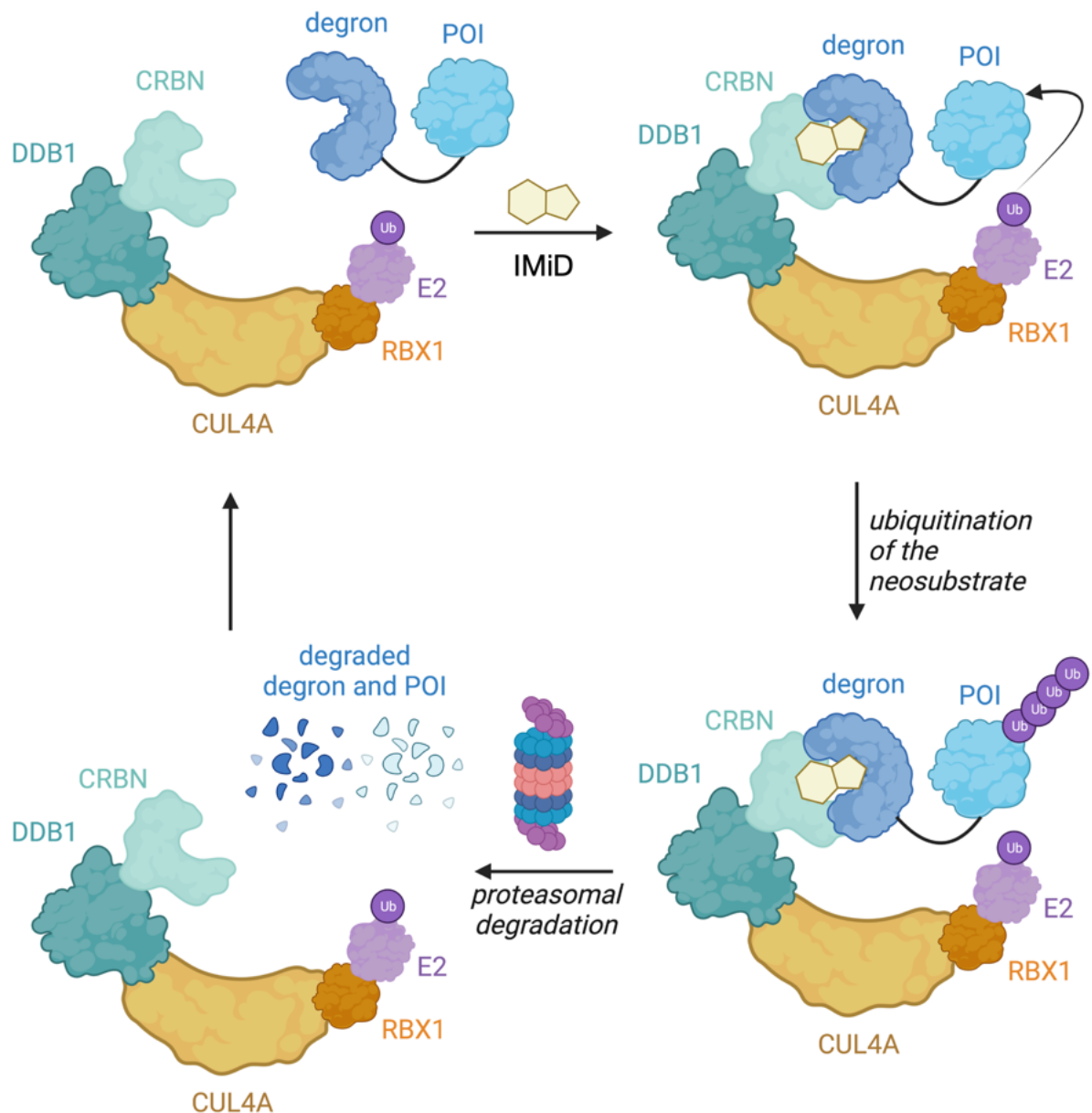


Figure 1.13. The ZF degron region from IKZF1/3 (dark blue) can be fused to a POI (light blue), whereupon addition of an IMiD (beige) can be used to effect degradation of the ZF-POI fusion protein.

The ZF motif common to both IKZF1 and IKZF3 which acts as a degron is called C2H2 ZF2. In both IKZF1 and IKZF3 this degron motif is flanked on either side by other ZF motifs, which do not act as degrons by themselves. The 23-residue region from 146-168 in IKZF3 (145-167 in IKZF1), that comprises ZF2, is therefore the minimal region required to act as a genetically encodable degron. However, inclusion of some of the

flanking region can increase the level of degradation seen in assays. Figure 1.14 shows three lengths of degron used by Sievers *et al.* for different degradation assays.² The longest degron sequence – used by Sievers *et al.* for a saturation mutagenesis assay – includes segments of both flanking non-degron ZFs, and provides more efficient degradation compared to the other two, smaller degron lengths.

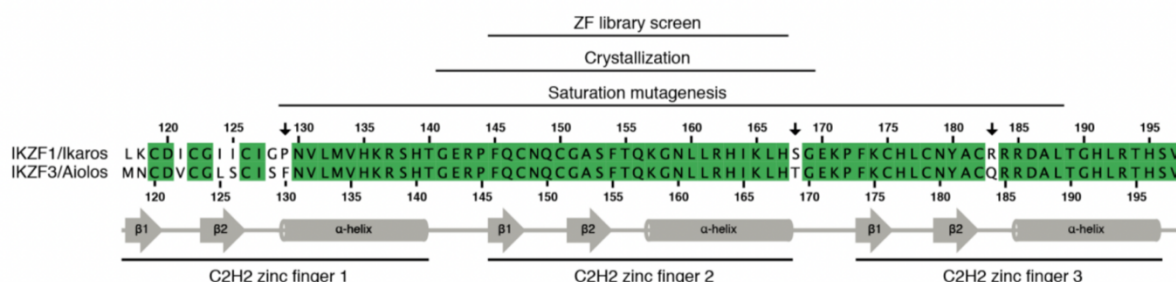


Figure 1.14. Residues 118-198 of IKZF3 and corresponding residues (117-197) of homologous IKZF1. Conserved residues are highlighted in green. Three ZF structures adopted by this region are shown in grey below. C2H2 ZF 2 (residues 146-168 in IKZF3) is the minimal degron, but inclusion of flanking regions can improve degron efficiency. Lengths used for three assays by Sievers *et al.* are shown above; the region used for saturation mutagenesis provided the best degradation. Adapted from Sievers *et al.* with permission.²

1.4.3 Application of IPID

The use of IPID bypasses both of the disadvantages associated with other degron systems, as IMiD molecules are small and drug-like, and the ZF degron is small and therefore less likely to perturb endogenous functionality of the POI.⁴⁵ IPID has been applied to both the regulation of CRISPR/Cas9,³ and to the regulation of T-cell activity in chimeric antigen receptor (CAR) T cell therapy (Figure 1.15).^{4,46}

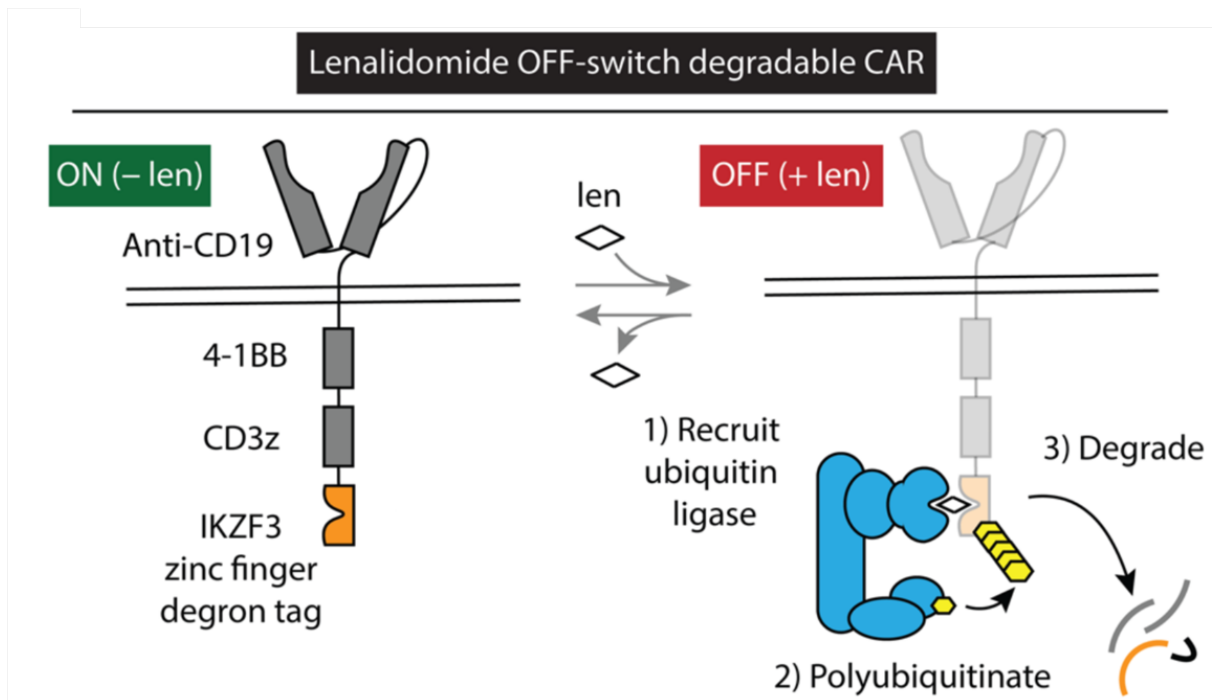


Figure 1.15. A transmembrane chimeric antigen receptor (CAR) comprising Anti-CD19, 4-1BB and CD3z regions (grey) is fused to a ZF degron region from IKZF3 (orange). Addition of lenalidomide induces degradation of the CAR via recruitment of CRBN and associated proteins (blue), and subsequent polyubiquitination. Adapted from Jan *et al.* with permission.⁴

In CAR-T cell therapy a population of T cells is removed from a cancer patient, transduced with genes required to express CARs which recognise the patient's cancer, then expanded and transfused back into the patient. This recognition ability endows the modified T-cells with the ability to selectively kill the cancer cells. The ability to degrade CARs is a potentially useful way of down-regulating T-cell activity during therapy, as too much T cell activity can result in toxic side effects in patients.⁴⁷ The ability to reversibly 'switch off' the T cells without killing them is therefore highly desirable.

The small, drug-like nature of thalidomide, lenalidomide, and pomalidomide makes IPID an attractive candidate technology for use in CAR-T cell therapy. Use of a

clinically approved drug molecule – which these IMiDs all are – is also essential for any technology that will be used in patients.

However, these established IMiD molecules do not differentiate between the engineered IKZF1/3-derived ZF degron used in the CAR construct, and the ZF degron regions found in endogenous IKZF1/3, along with other neosubstrates. Therefore, degradation of the desired POI-degron fusion is inevitably accompanied by unwanted degradation of endogenous proteins. This is a particular issue in CAR-T cell therapy, as degradation of IKZF1 and IKZF3 leads to T cell stimulation,^{8,9} negating the effects of the CAR degradation.

1.4.4 Benefits of Orthogonality

Development of an orthogonal IPID system which does not induce degradation of endogenous IKZF1 and IKZF3 is therefore highly desirable for use in CAR-T cell therapy. In this case the beneficial effects of CAR degradation would not be offset by T cell stimulation caused by off-target effects.

Such a system would also be useful in wider applications, such as target validation during drug discovery.⁴⁸ In target validation, a potential druggable target protein is selectively knocked down, or knocked out, to examine the resulting phenotype. Only if desired effects are observed in the absence of a protein is it a suitable target for inhibition or degradation by a drug. IPID using established IMiDs is not ideal for use in this scenario, as it would not be clear whether observed phenotypes were due to degradation of the degron-tagged POI, or due to degradation of IKZF1 or IKZF3 and other neosubstrates.

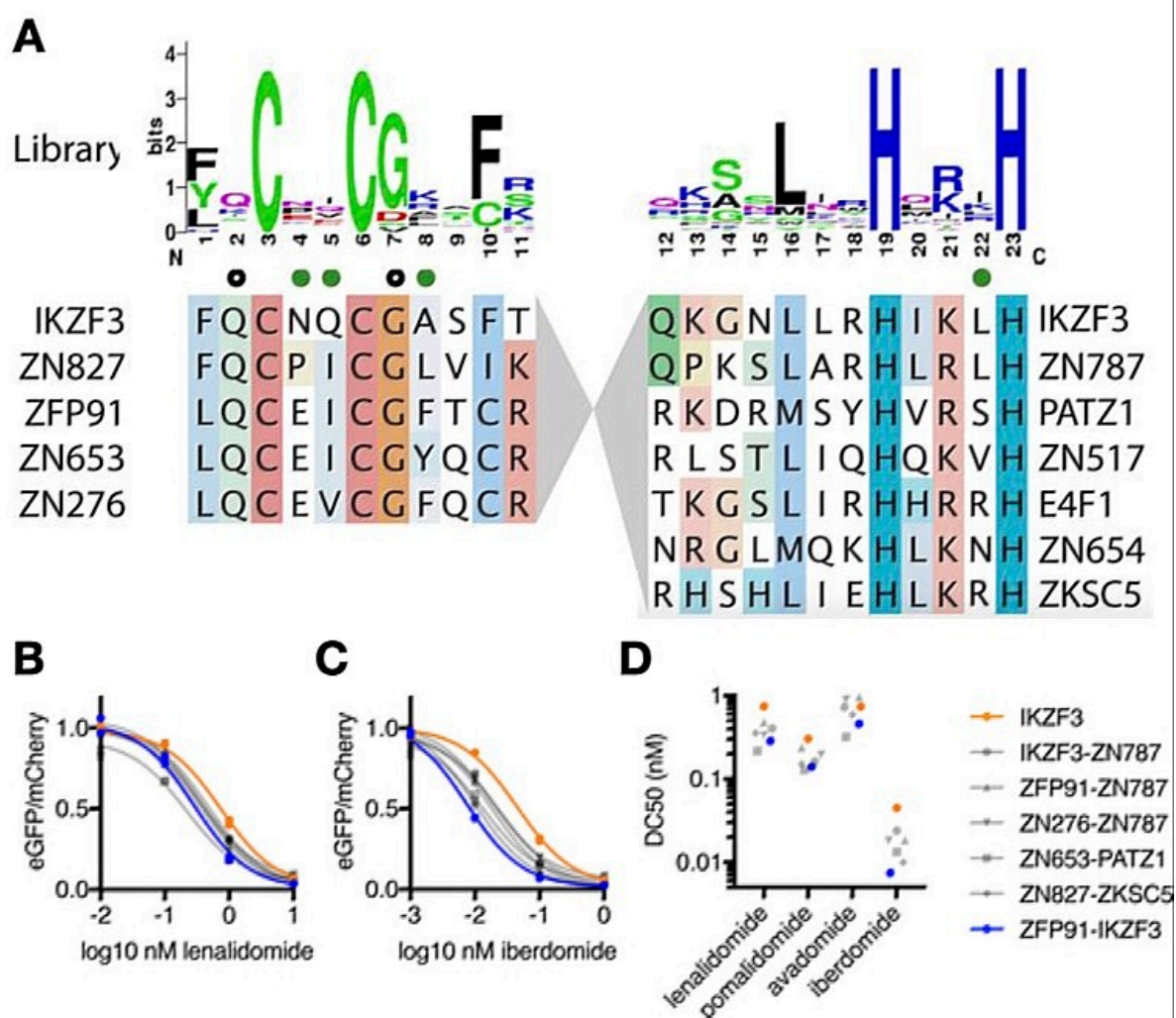


Figure 1.16. A) ZF degrons composed of N- and C- terminal regions derived from different endogenous neosubstrates. Green circles indicate residues that interact directly with CRBN during ternary complex formation; black circles indicate residues that interact directly with the IMiD during ternary complex formation. **B)** Degradation profiles for hybrid degrons treated with lenalidomide (2). **C)** Degradation profiles for hybrid degrons treated with CC-220, also known as iberdomide (5). **D)** DC₅₀ values for pairs of hybrid ZF degrons and four IMiDs: lenalidomide (2), pomalidomide (3), avadomide (4) and iberdomide (5). Adapted from Jan *et al.* with permission.⁴

Some degree of selectivity has already been achieved in an IPiD system, as seen in work carried out by Jan *et al.*: in this study, hybrid ZF degrons derived from differing N and C terminal regions from endogenous neosubstrates were screened for enhanced degradation by various IMiDs, including lenalidomide, pomalidomide, avadomide, and CC-220/iberdomide (Figure 1.16), resulting in the development of ‘super-degrons’.⁴ These ‘super-degrons’ are degraded more efficiently by established

IMiDs than the WT sequence from IKZF1/3, meaning a lower concentration of IMiD can be used to induce the same level of POI-degron degradation, hence resulting in less degradation of endogenous neosubstrates.

However, a greater degree of selectivity would be needed for an IPID system to be orthogonal, meaning that a degron-tagged POI would be completely degraded while endogenous neosubstrates are completely unaffected. To further enhance selectivity of a degron system, methodology that alters both the degron and the small molecule degrader is required.

1.5 Bump and Hole for Orthogonality

1.5.1 General Principle for Bump and Hole

‘Bump and Hole’ is a well-established method of achieving orthogonality in chemical biology (Figure 1.17).^{49,50} In a scenario where a ligand/protein interaction is desirable to study, but the ligand in question possesses undesirable off-target effects, a chemical ‘bump’ modification can be made to the ligand. This modification – usually the addition of a sterically bulky group – renders it unable to bind any of the proteins to which the original ligand could bind.

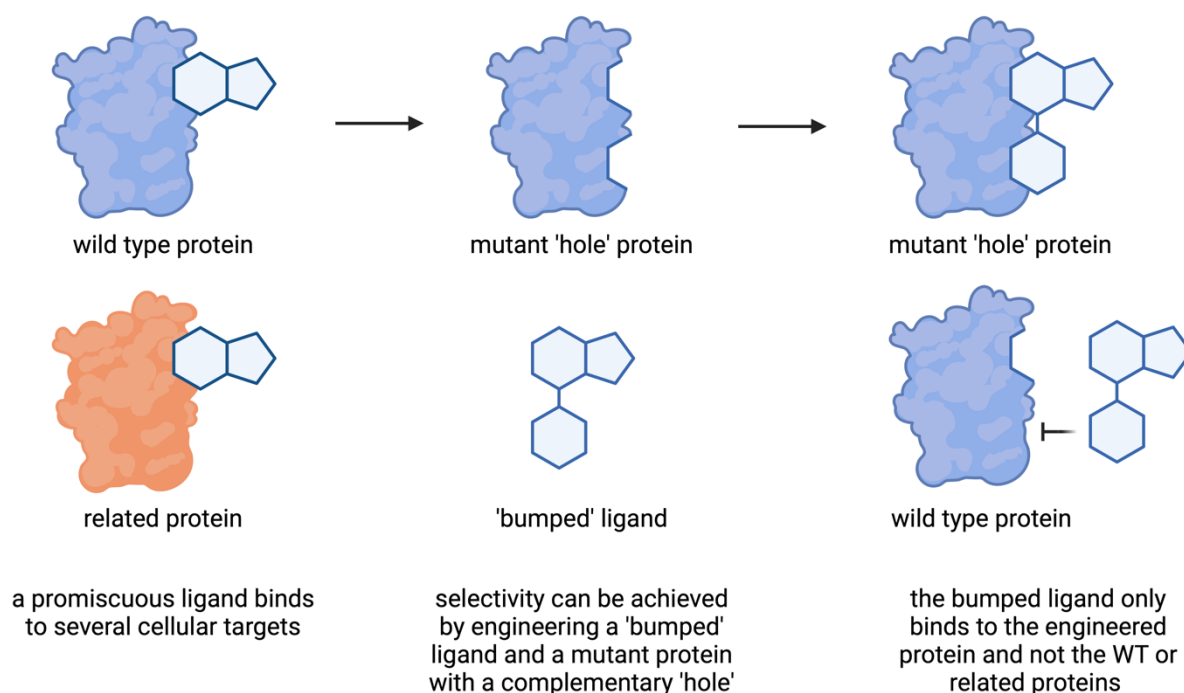


Figure 1.17. Addition of a new chemical moiety – a ‘bump’ – onto a ligand prevents it from binding to its original protein partner(s). Mutation from a large residue to a small residue at an appropriate position – a ‘hole’ – can accommodate this ‘bump’ group.^{49,50}

To accommodate the ‘bump’ group, a complementary ‘hole’ mutation is made in the desired protein. This is usually a residue with a large side chain being mutated to a residue with a small side chain, such as alanine or glycine. Ideally, the ‘bumped’ ligand will no longer have any off-target effects, but will still interact with its intended target protein due to the accommodating ‘hole’. This approach is only applicable in settings where genetic modification of proteins is possible.

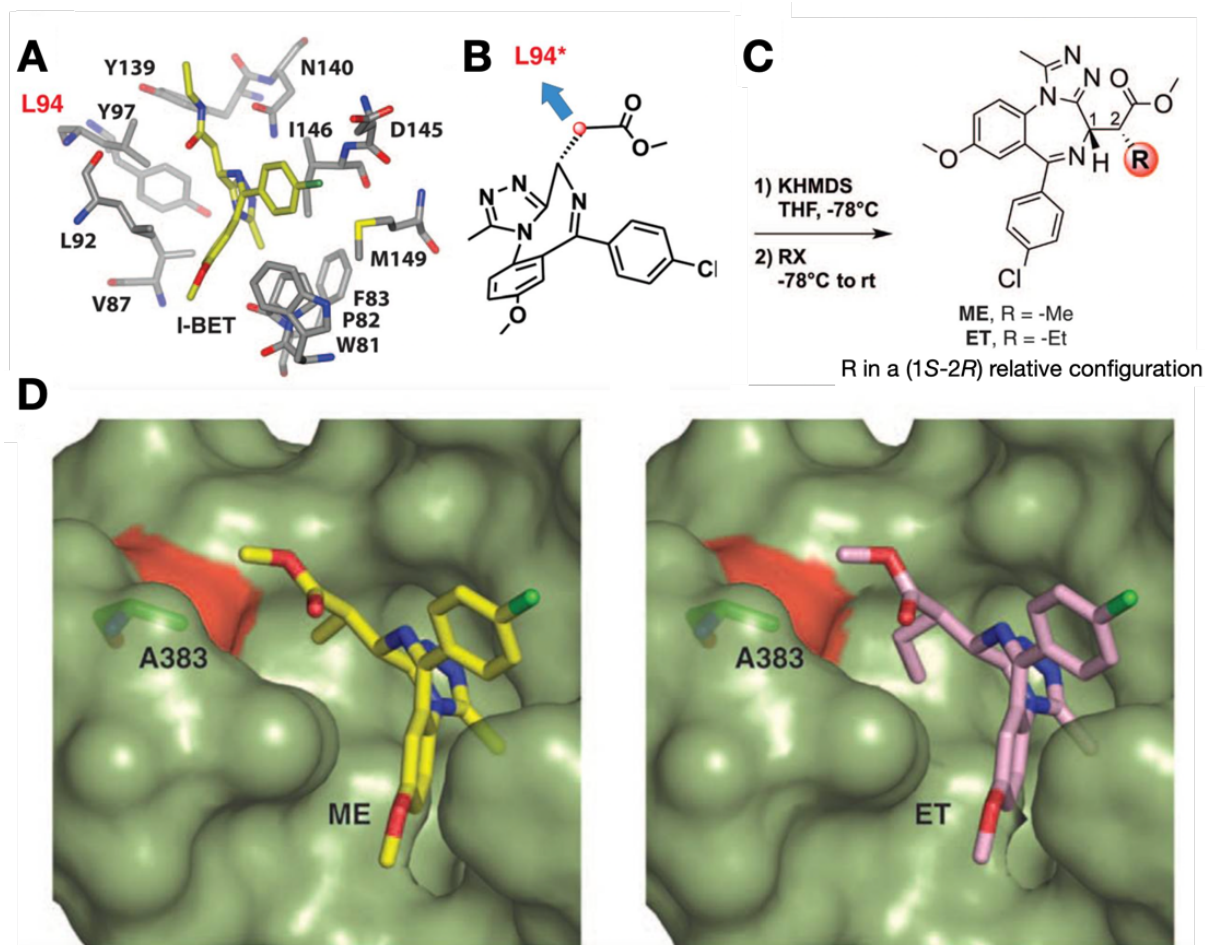


Figure 1.18. **A)** Compound **11** (yellow sticks) occupies a pocket in the BRD4 bromodomain (grey sticks). **B)** Compound **12**, an analogue of **11**; the highlighted region is proximal to the bromodomain L94 residue. **C)** Addition of a methyl or ethyl moiety at position 2 yields two ‘bumped’ analogues, **13** and **14**. **D)** Compounds **13** and **14** occupy the pocket in the ‘hole’-containing mutant bromodomain, where L383 has been mutated to A383. Adapted from Matthias *et al.* with permission.⁵¹

BromoTag (Section 1.4.1) employs ‘bump and hole’ to achieve selectivity over endogenous bromodomain-containing proteins. The BRD4-binding moiety (based on (+)-JQ1) in the heterobifunctional inducer molecule contains an ethyl group which clashes with a conserved leucine found in endogenous bromodomain motifs.^{43,51} However, the bromodomain employed as a degron contains a leucine-to-alanine ‘hole’ mutation at this position, which accommodates the ethyl ‘bump’ on the ligand (Figure 1.18). This system is therefore orthogonal, as the degradation of tagged POIs can be achieved without modulation of endogenous bromodomain-containing proteins.⁵¹

1.5.2 Application of Bump and Hole to IPID

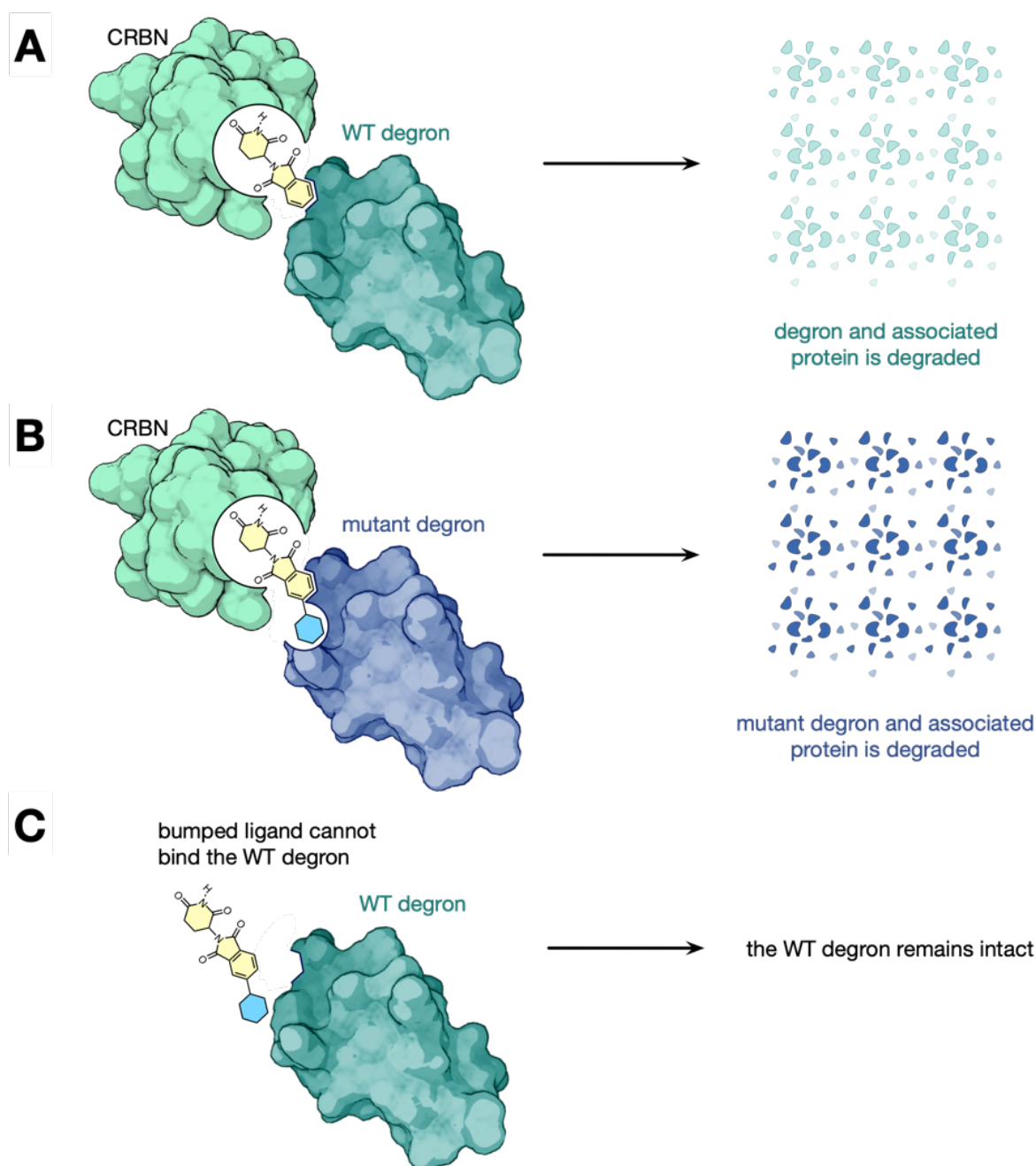


Figure 1.19. The concept of a 'bump and hole' approach applied to the CRBN/ZF degron system. **A)** An IMiD (thalidomide pictured) recruits IKZF1/3 ZF degron to CRBN, effecting degradation of the degron. **B)** A 'Bumped' IMiD analogue recruits a 'hole'-containing mutant ZF degron to CRBN, effecting degradation of the degron. **C)** A 'Bumped' IMiD cannot bind the WT IKZF1/3 degron as the 'bump' group cannot be accommodated.

As ‘bump and hole’ has previously been applied to numerous ligand/protein systems,⁴⁹ it was decided that applying this approach to the IMiD/ZF interface would be a suitable way to achieve orthogonality in IPID (Figure 1.19). A suitable IMiD analogue would not induce degradation of any endogenous neosubstrates, but would still induce degradation of a POI fused to a suitably modified ZF degron.

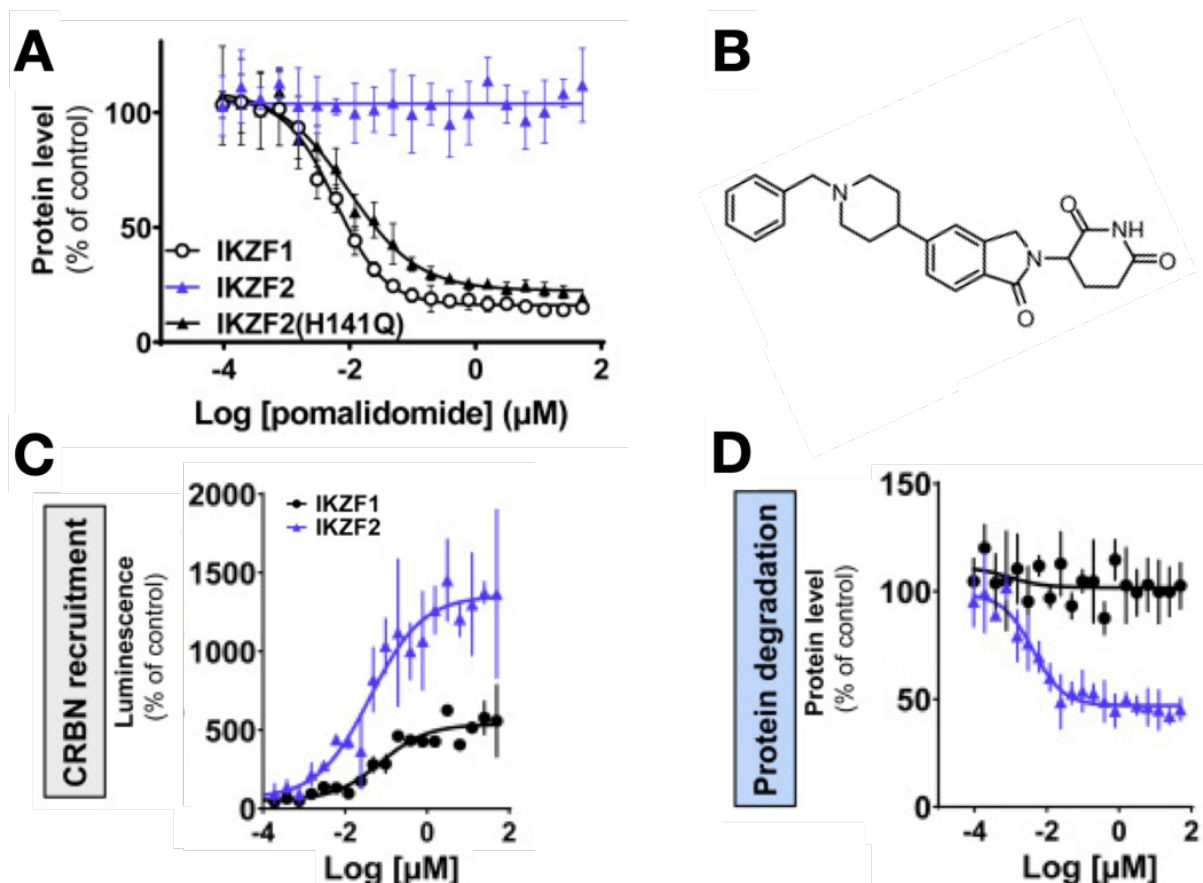


Figure 1.20. **A**) Pomalidomide effectively degrades IKZF1 but does not degrade IKZF2, which possesses a single Q to H mutation; mutation from H to Q at this position in IKZF2 renders the protein susceptible to degradation by pomalidomide. Data obtained through addition of pomalidomide to engineered 293T cells expressing ProLabel-tagged IKZF1, IKZF2, or IKZF2 (H141Q) **B**) Structure of IMiD analogue DKY709 (15). **C**) CRBN recruitment to IKZF1 or IKZF2 upon addition of DKY709 (15). **D**) Degradation of ProLabel-tagged IKZF1 and IKZF2 in engineered 293T cells; IKZF1 levels are unaffected whilst >50% degradation of IKZF2 is observed. Adapted from Bonazzi *et al.* with permission.³¹

A ‘bump’ approach has been applied to IMiDs to achieve selectivity over traditional neosubstrates in favour of new endogenous neosubstrates.^{31,52} In work carried out by Bonazzi *et al.*, a novel IMiD degrader, DKY709 (15), was developed to selectivity

degrade IKZF2 over IKZF1 and IKZF3. The degron region of IKZF2 shares a near identical sequence with the degron regions in IKZF1/3, but with a key Q147H mutation (IKZF3 numbering). The *N*-benzylpiperidine chemical group featured on the 5-position of DKY709 is an example of a ‘bump’ group that clashes with glutamine 147 in IKZF1/3, but is accommodated by the analogous histidine in IKZF2. As a result, DKY709 does not significantly degrade endogenous IKZF1, but degrades endogenous IKZF2 by over 50% (Figure 1.20).

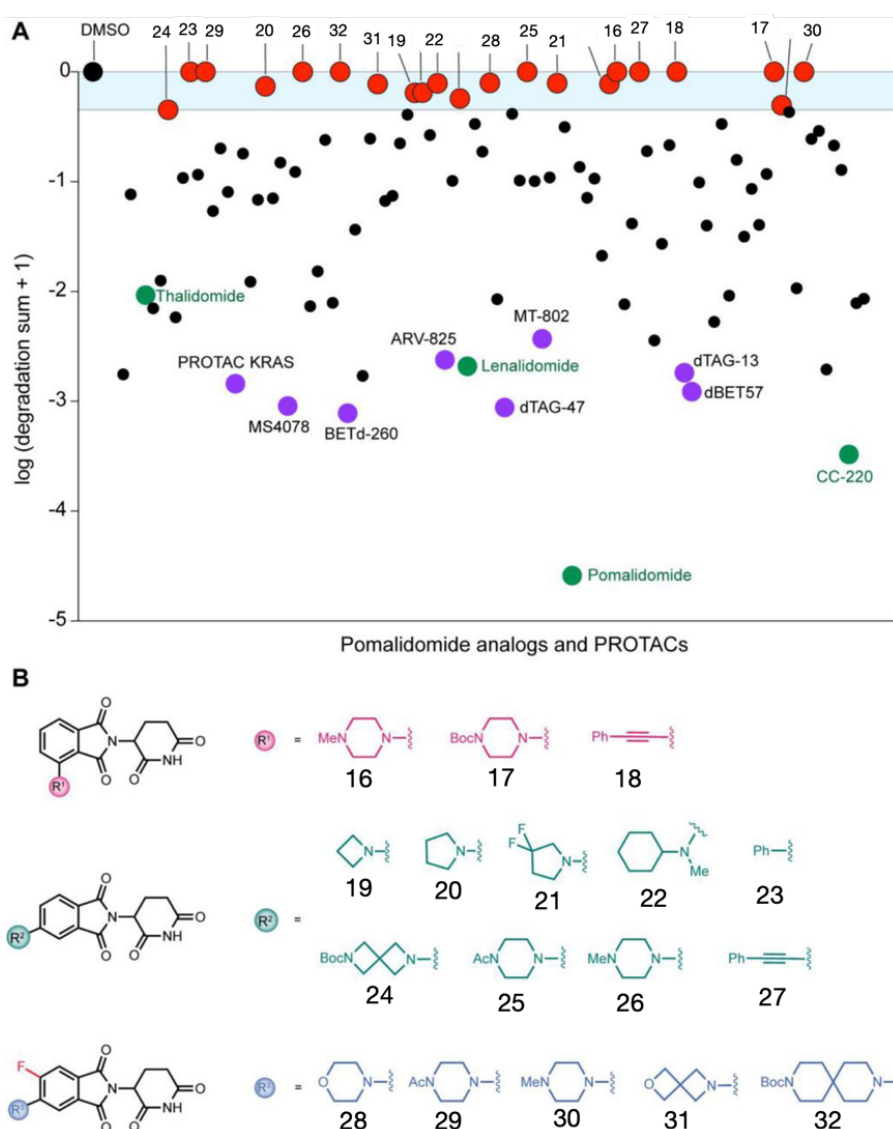


Figure 1.21. A) IMiD analogues with ‘bump’ groups at either the 4- or 5- position were screened for degradation against 14 ZF motifs known to be sensitive to pomalidomide; Red circles represent IMiD analogues with close to zero off-target ZF degradation. **B)** Chemical structures of IMiD analogues with low level off-target ZF degradation. Adapted from Nguyen *et al.* with permission.⁵³

A 'bump approach has also been applied to IMiDs in the context of PROTACs.^{53,54} it has been observed that PROTACs based off traditional IMiD designs display undesired degradation of endogenous neosubstrates as well as their desired target proteins. In work carried out by Nguyen *et al.*, IMiDs with 'bump' groups at either the 4 or 5 position were screened for degradation against 14 ZF degrons known to be sensitive to pomalidomide. The screen yielded 17 IMiD analogues with low degradation across all 14 degrons (Figure 1.21). These IMiD analogues were then explored for use in PROTACs with reduced off-target effects.

1.6 Project Aims

The aim of this study is to develop a novel IMiD/degron pair that can be used to degrade degron-tagged POIs without inducing off-target degradation of endogenous proteins such as IKZF1 and IKZF3. To achieve this we have completed the following aims:

- Screening a virtual library of IMiD analogues, aiding in the design of a refined IMiD analogue library for chemical synthesis.
- Synthesising this library of IMiD analogues and determining their ability to degrade a 'hole'-containing Q147A degron compared to a degron based on the wild type (WT) IKZF1/3 degron using a fluorescence-based flow cytometry assay.
- Designing a large scale mutant ZF library and using a fluorescence activated cell sorting (FACS) assay to screen for promising compound/mutant combinations.

Chapter 1: Introduction

- Using flow cytometry and western blotting-based degradation assays to validate the selectivity of the IMiD/degron pairs identified as most selective by the library screen.
- Applying the best performing IMiD/degron pair to the degradation of an unliganded protein target.

Chapter 2: Design and Synthesis of IMiD

Analogues

2.1 Introduction and Aims

2.1.1 Introduction

The previous chapter provided an overview of IMiDs and their molecular glue mechanism in action. In this chapter, work undertaken to determine suitable IMiD modification and degron mutation for 'bump and hole' methodology will be explored, culminating in the design of a library of IMiD analogues for chemical synthesis and use in biological testing.

This chapter will first discuss the initial computational work carried out to identify potential suitable pairs of 'bumped' IMiD analogues and mutant, 'hole'-containing degrons. Only mutations at position 147 of the ZF degron are considered at this initial stage.

2.1.2 Overall Aims

The 'bump and hole' approach traditionally relies on identifying a suitable residue for mutation in the POI, usually one with a large side chain such as leucine or isoleucine. Mutation at this position to a residue with a smaller side chain, such as alanine, provides space to accommodate a suitably placed 'bump' group on the modified ligand. This bump group should be similar in dimension to the side chain that

previously occupied this space: for example, in the case of a leucine-to-alanine mutation, an ethyl ‘bump’ group could be a suitable choice. Structural data can be used to identify a suitable location on the small molecule onto which this ‘bump’ group can be added, such that it is adjacent to the residue being mutated.

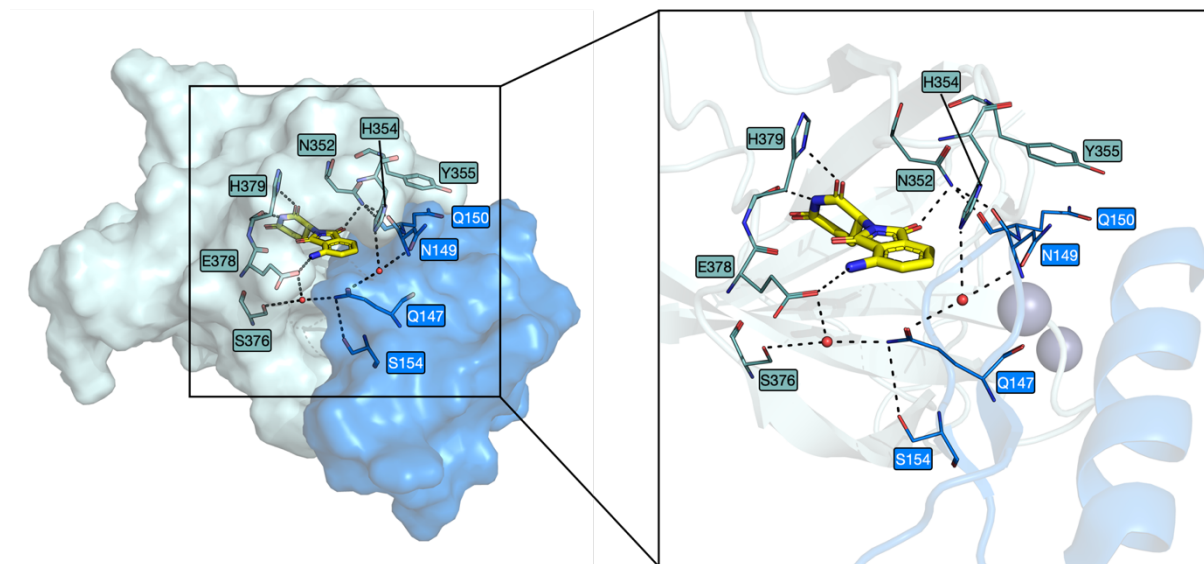


Figure 2.1. Residues on the IKZF1/3 ZF degron proximal to pomalidomide (*PDB ID: 6H0F*). CRBN, teal; IKZF1/3 ZF degron, blue; pomalidomide, yellow; hydrogen bonds, dashed lines; water molecules, red spheres.

Inspection of an X-ray crystal structure of CRBN and an IKZF1-derived ZF degron bridged by pomalidomide² (Figure 2.1) provides insight into which degron residues are closest to the IMiD, and hence which are most suitable for mutation to accommodate a ‘bumped’ IMiD derivative. After inspection, the conclusion was reached that a glutamine residue, Q147, was the most suitable residue for mutation, as it comes directly into contact with the phthalimide region of the IMiD molecule.

Inspection of the structural data also suggested that a ‘traditional’ approach to ‘bump and hole’ may not be sufficient for this particular system. This was for several reasons:

- Q147 is not hydrophobic, so mutation to alanine may remove beneficial interactions such as hydrogen bonds.
- Q147 is not a buried residue, so providing a 'bump' group to clash with it will not guarantee a steric clash that reduces binding potential, as the residue could potentially adopt a new rotamer to inhabit a different region of space.
- The region being targeted is solvent exposed, so if a 'bump' group on the ligand is flexible, it could adopt a conformation where it points away from the degon.
- The system is a ternary complex with two proteins, instead of a ligand/protein duplex, adding to the complexity of the problem.

It was therefore decided that a computational approach should initially be taken in order to screen a wide variety of both potential bump groups and potential degron mutations at position 147. The decision was taken to screen a library of 'bumped' IMiD analogues against a library of 20 degrons with all 20 residue types at position 147 (i.e. 19 mutants and the WT) in a manner that would highlight any particularly well matched IMiD/degron pairings.

2.1.3 ZF Degron Numbering Convention

It should be noted that numbering conventions differ between homologous ZF degron regions derived from different endogenous neosubstrates. For example, the equivalent residue to Q147 in IKZF3 is at position 146 in IKZF1, position 141 in IKZF2, and position 411 in SALL4. Throughout this study, the IKZF3 numbering system will be adopted to refer to all degron mutants.

Table 2.1. Four examples of ZF degron homologues with differing numbering conventions. Residues conserved across all known ZF degrons (beyond those in this table) are highlighted in red; residue 147 in IKZF3 and residues at the equivalent position in IKZF1, IKZF2 and SALL4 are highlighted in blue.

Degron	Sequence
IKZF1 145-167	–F Q CN Q CGASFTQKGNLLR H IKL H –
IKZF3 146-168	–F Q CN Q CGASFTQKGNLLR H IKL H –
IKZF2 140-162	–F H CN Q CGASFTQKGNLLR H IKL H –
SALL4 410-432	–F V C S VCGHRFTTKGNLKV H FHR H –

2.2 Computational Docking Screen Setup

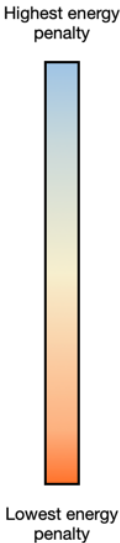
2.2.1 Receptor Setup

It was decided that a molecular docking screen would be the best way to assess a large number of bumped IMiD analogues against all 20 of the position 147 mutant degron variants. To conduct this study, a region of crystal structure 6H0F was used, comprising the region of CRBN containing the IMiD binding pocket (residues 319-426), and the IKZF1 ZF degron (residues 138-169). It should be noted that the relative global position of the two proteins was not altered during the workflow, and they were treated as one static, multi-chain protein. While this is not representative of the binding dynamics observed in nature, where the degron only binds CRBN once the IMiD has already bound, it was decided that this approach was sufficient for this initial screen.

Prior to docking, the residue at position 147 was mutated using Foldx software.⁵⁵ The software's 'position scan' functionality was used to replace the native glutamine with all 20 residue types (for consistency, glutamine was also mutated to glutamine again), providing both the 3D coordinates of the new mutant, and a predicted energy penalty for the mutation compared to the unmutated protein. The results (Table 2.2) indicate that all mutations at this position are energetically favourable, as all energy changes (favourable or unfavourable) are small in magnitude.

Table 2.2. Foldx was used to perform all possible mutations at position 147 and evaluate the resultant energy penalty. Negative values represent energetically favourable mutations. All energy penalties shown here are small in magnitude, indicating all mutations at position 147 will be well tolerated.

Mutation	Energy Penalty (kcal/mol)
Q147A	0.45
Q147C	0.24
Q147D	1.06
Q147E	0.54
Q147F	0.72
Q147G	0.79
Q147H	0.63
Q147I	0.21
Q147K	-0.21
Q147L	-0.32
Q147M	0.18
Q147N	0.43
Q147P	1.10
WT	0.00
Q147R	-0.19
Q147S	0.53
Q147T	0.66
Q147V	0.45
Q147W	0.49
Q147Y	0.72



During 3D coordinate generation, the mutated residue was placed in the most energetically favourable rotamer (according to Foldx). Spatially adjacent residues were also assessed, and their side chains were repositioned if deemed energetically favourable by Foldx. As both the region of CRBN and the ZF were treated as one

protein, the residues on CRBN proximal to position 147 on the ZF (e.g. E378 in CRBN) were also assessed by Foldx during this process. In some cases these residues were assigned new rotamers that differed from the original pose in the crystal structure, so as to accommodate the new residue at position 147. In all cases, mutant protein 3D coordinate generation and energetic evaluation were carried out after removal of the original pomalidomide ligand from the receptor pocket.

2.2.2 Docking Setup

An assumption was made during this study that the glutarimide ring region of any screened IMiD analogues would adopt the same conformation and occupy the same region of the CRBN binding pocket as the native pomalidomide ligand. Therefore, it was decided that a constrained docking approach would be suitable for this screen.

GOLD (Genetically Optimised Ligand Docking) software from CCDC (Cambridge Crystallographic Data Centre)⁵⁶ was chosen to perform the constrained docking, using crystal structure 6H0F as a starting point. The scaffold constraint option of GOLD was used to hold the conserved glutarimide region of the IMiD analogues in the same position as the identical glutarimide region from the original pomalidomide molecule from the crystal structure. For each IMiD analogue, the phthalimide region was allowed to rotate around the C-N bond connecting the two halves of the core thalidomide structure, and any bump groups branching off the phthalimide region were also allowed complete rotational freedom (Figure 2.2).

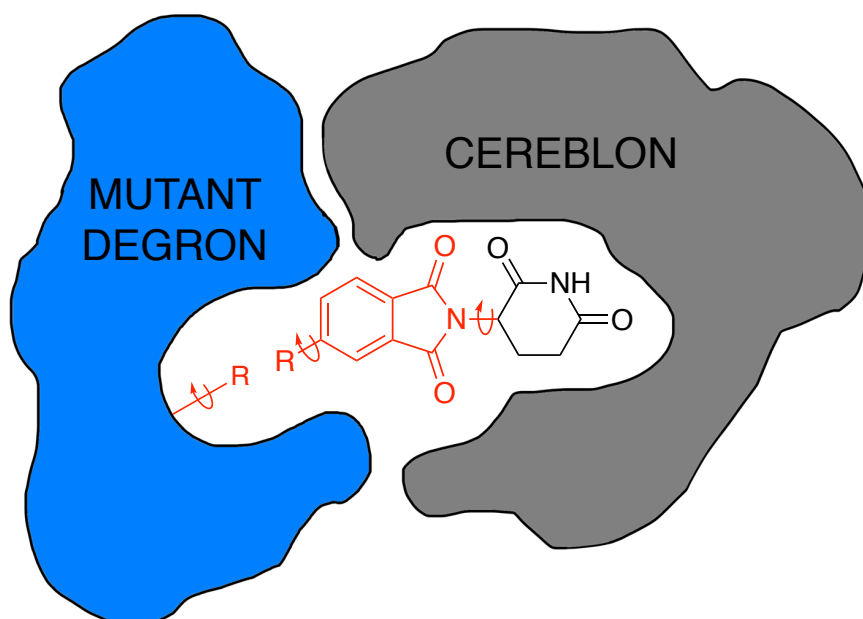


Figure 2.2. Constrained docking holds the black portion of the IMiD molecule in place while the red section of the molecule can move. The residue side chain at position 147 in the degron (also in red) can also move.

It should be noted that constrained docking does not ‘fix’ the scaffolded region of the ligand in place during pose generation: instead, poses are generated randomly, as in non-constrained docking, but an additional scaffold constraint parameter is used during scoring to assess how well this pose adheres to the desired scaffold. The ‘weight’ at which this parameter is enforced, determining how likely GOLD is to reject poses in which the ligand does not align with the original molecule, is set by the user. As we only wished to consider aligning poses, a relatively high scaffold constraint weight of 10 (compared to the default of 5) was set.

2.2.3 Starting Pose Generation

In a non-constrained docking scenario, it is preferable to generate a random pose for a ligand as a starting point for the docking algorithm to improve upon. This pose can be any conformation, position and orientation. Software such as OpenBabel⁵⁷ and

RDKit⁵⁸ can be used to generate such ligand poses from 2D structural information alone - e.g. a simplified molecular input line entry system (SMILES) string. However, in this scenario neither of these pieces of software were able to recreate the glutarimide ring conformation observed in pomalidomide in the original crystal structure. Therefore, an alternative approach was taken: to generate starting poses for the IMiD analogues, the 'builder' function from PyMOL⁵⁹ was used to 'draw' the structure of the IMiD analogue in 3D, using the original pomalidomide atom coordinates as a scaffold. No attempt was made to position these new atoms in a manner that seemed energetically favourable.

2.2.4 Residue Flexibility

For this screen, two alternative strategies regarding side chain flexibility were adopted. In one setup, all residue side chains were kept rigid throughout docking; in the other setup, the side chain of residue 147 was allowed to change rotamer (with all others remaining fixed), then scored by GOLD to assess rotamer favourability. Advantages and disadvantages of these two alternative approaches will be discussed in section 2.3.4.

2.2.5 Workflow Summary

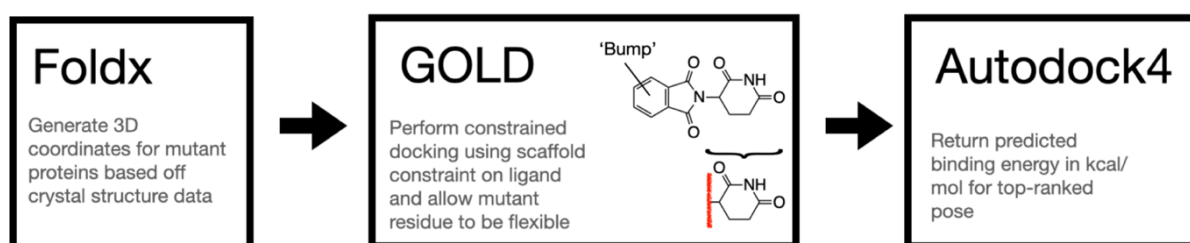


Figure 2.3. The three main steps of the docking screen workflow.

The full mutant docking screen workflow is summarised in Figure 2.3. To ensure comparability across multiple docking runs, it was decided that the scoring function from AutoDock4⁶⁰ should be used to rank poses generated by GOLD, as it returns a predicted binding energy value in kcal/mol, rather than arbitrary units generated by the internal scoring function in GOLD.

2.3 Bump and Hole Docking Test

2.3.1 Motivation for Testing Docking Workflow

Before carrying out the docking screen on a library of IMiD analogues and ZF mutant deignons, we sought to test the workflow on an example ‘bump and hole’ system that had already been implemented in a biological situation. If the docking workflow could emulate the energy differences observed, this would provide confidence in the methodology.

2.3.2 Literature Example of Bump and Hole

The example chosen was from a study by Matthias *et al.*⁵¹ In this study a bromodomain ligand (**11**) was modified with a ‘bump’ group that clashed with a leucine residue in the WT bromodomain, but could not be accommodated by the L94A bromodomain mutant (Figure 2.4). Both methyl (**13**) and ethyl (**14**) groups were employed as bump groups in this study. It should be noted that the ‘bumped’ ligands differ slightly from the original ligand, as they contain a methyl ester group in place of an ethyl amide group in an adjacent region molecule. However, this modification does not affect the ‘bump and hole’ effect.

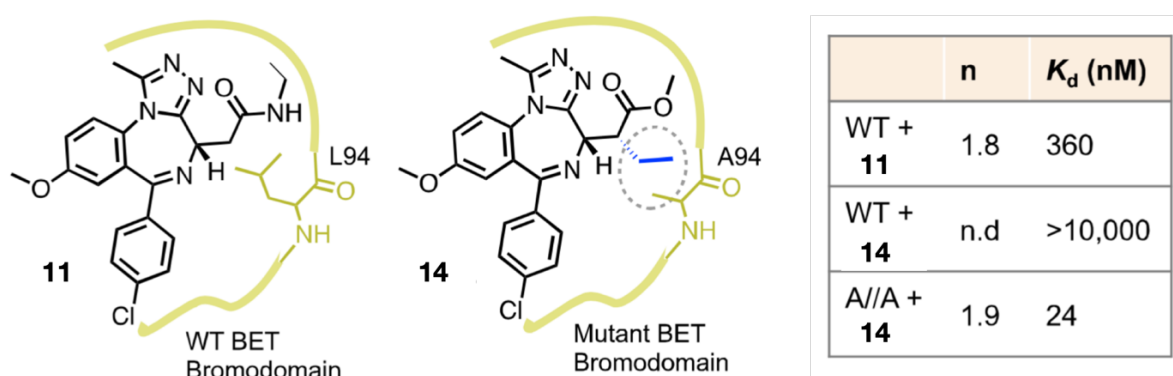


Figure 2.4. Compound **11** binds to the WT BET Bromodomain, but an ethyl 'bumped' version (compound **14**) does not, due to a steric clash. Compound **14** is able to inhibit the L94A mutant of the bromodomain due to the 'bump and hole' effect. Adapted from 'The Bump-and-Hole' Tactic: Expanding the Scope of Chemical Genetics' by K. Islam with permission.⁴⁹

Crystal structures exist for both the original inhibitor (**11**) in the WT bromodomain and the ethyl-'bumped' ligand (**14**) in the L94A mutant bromodomain – aligning these structures shows the 'bump and hole' effect (Figure 2.5).

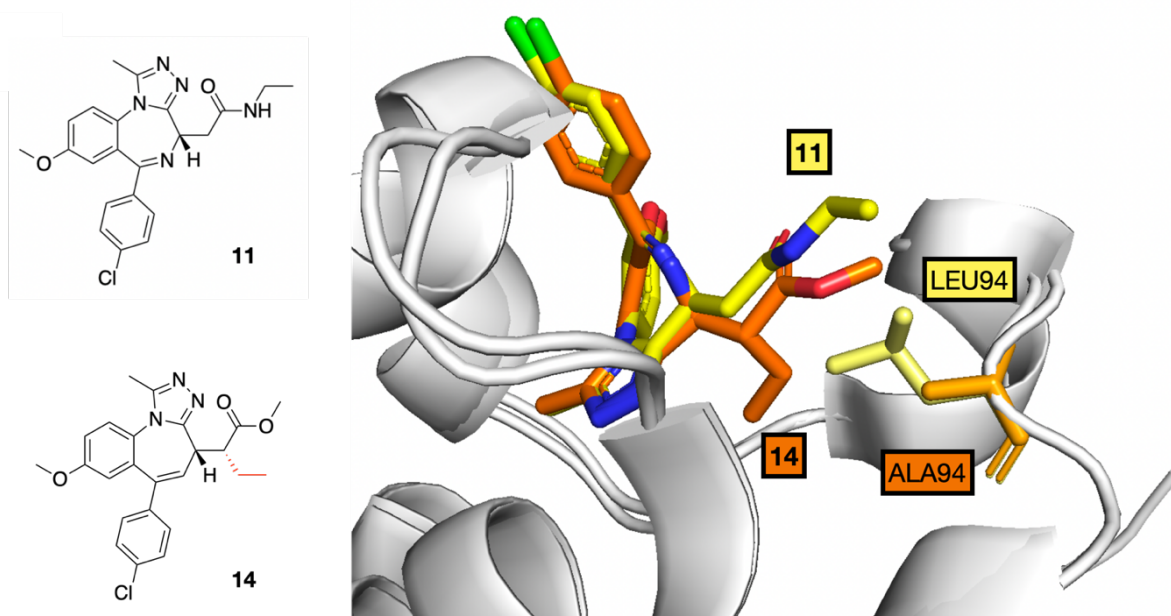


Figure 2.5. Crystal structures of compound **11** bound to a WT bromodomain (yellow) and compound **14** bound to a mutant L94A bromodomain (orange) are overlaid to show orthogonal binding poses. (PDB IDs: 3P5O and 4QEW).

2.3.3 Docking Test Setup

Our workflow (Figure 2.3) was used to dock the original, methyl-‘bumped’ and ethyl-‘bumped’ ligands into both the WT bromodomain and the L94A mutant. Despite crystal structures existing for both bromodomains, just the WT protein and its Foldx-derived L94A mutant were used for this docking test, to prevent any small variations in atom coordinates between the crystal structure proteins affecting the predicted binding energy values returned.

In a similar manner, instead of comparing scores for the original ligand (**11**) against the methyl (**13**) and ethyl (**14**) ‘bumped’ ligands used in the Matthias *et al.* study, which differed by featuring methyl ester groups in place of ethyl amide groups, the original ligand was modified using the builder functionality of PyMOL to generate methyl (**33**) and ethyl (**34**) ‘bumped’ versions of the original, ethyl amide-containing ligand (Figure 2.6). This ensured that differences in scores were purely due to ‘bump and hole’ interactions, and not due to differences in other regions of the ligand molecules.

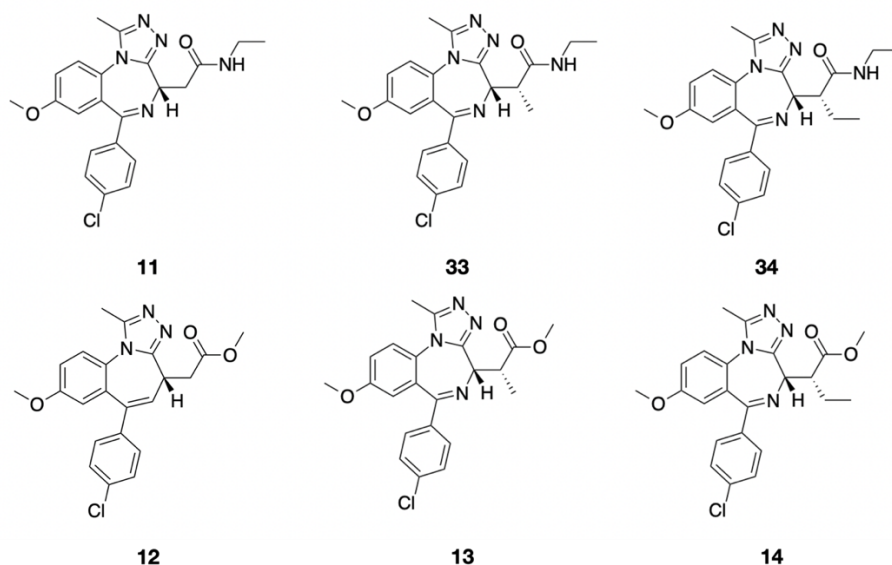


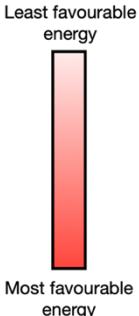
Figure 2.6. Original bromodomain inhibitor (**11**); methyl (**33**) and ethyl (**34**) bumped inhibitors used in docking; analogue of the original ligand (**12**); methyl (**13**) and ethyl (**14**) inhibitors seen in literature.

2.3.4 Docking Test Results

This docking test was carried out twice: once ‘rigid’, where the side chain of residue 94 was maintained in a fixed position, and once ‘flexible’, where residue 94 was allowed to switch rotamer (see 2.2.4).

Table 2.3. Constrained docking was performed using GOLD with residue 94 allowed to be either rigid or flexible. A colour gradient shows favourability of energy for the predicted ligand poses, with red representing more favourable binding energies.

Compound	Predicted Binding Energy (kcal/mol)			
	Residue 94 Rigid		Residue 94 Flexible	
	WT	L94A	WT	L94A
11	-9.18	-8.42	-9.13	-8.42
33	-7.92	-8.82	-9.95	-8.87
34	-5.48	-9.20	-9.74	-9.20



The results (Table 2.3) suggest that the ‘rigid’ test was able to recreate the results as seen in the original study: the bumped ligands (**33** and **34**) paired with the WT bromodomain exhibit unfavourable predicted binding energies compared to both the other ligand/protein combinations.

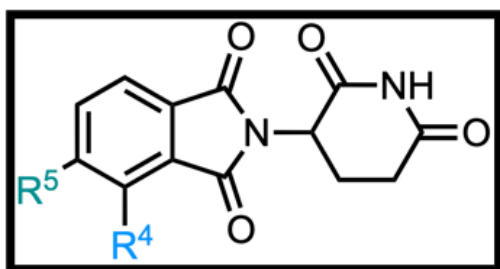
The ‘flexible’ test did not predict the binding energy trends seen in literature, as the bumped ligands (**33** and **34**) showed even more favourable scores for the WT protein than the original ligand (**11**). This highlights a shortcoming of using this workflow for assessment of ‘bump and hole’ options: if the side chain of a residue is flexible, it will adopt the rotamer that minimises the energy during docking, and the score produced will reflect that particular ligand/side chain pose. However, this does not mean that this particular rotamer is the most energetically favourable overall – other rotamers that would clash with the ‘bumped’ ligand may be more prevalent in nature.

This test has shown that use of the 'rigid' workflow is preferable for recreation of existing 'bump and hole' data, as it can highlight steric clashes. However, this 'rigid' approach lacks the ability to flag potential favourable ligand/residue interactions that would only become apparent if the residue side chain can adopt new rotamers. The 'flexible' option, whilst less likely to highlight clashes between 'bump' groups and a bulky WT side chain, could prove more suitable for discovering beneficial mutations that might interact more favourably with a ligand 'bump' group than the original residue. Therefore a decision was made to carry out all screens twice: once using the 'rigid' workflow and once using the 'flexible' workflow.

2.4 Large Scale IMiD/Degron Constrained Docking Screen

2.4.1 Bumped IMiD Analogue Library Design

A library of 41 IMiD analogues was designed to assess all 20 residue options at position 147 of the degron. A variety of bump groups were chosen (Figure 2.7) to place at both the 4 and 5 position of the core thalidomide structure, ranging from very small (e.g. fluoro, methyl) to larger (e.g. benzylamino). Synthetic feasibility, reactivity and potential off-target effects were not considered at this stage.



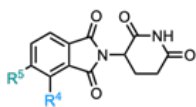
Compound	R ⁴	R ⁵	Compound	R ⁴	R ⁵	Compound	R ⁴	R ⁵
1	H	H	47	H	CH ₂ OH	61	H	C≡N
3	NH ₂	H	48	CH ₂ CH ₂ CH ₃	H	62	NHCH ₂ CH ₃	H
35	H	NH ₂	49	H	CH ₂ CH ₂ CH ₃	63	H	NHCH ₂ CH ₃
36	OH	H	50	CH ₂ CH ₂ CH ₃	H	64	NHCH ₂ CH ₂ CH ₃	H
37	H	OH	51	H	CH ₂ CH ₂ CH ₃	65	H	NHCH ₂ CH ₂ CH ₃
38	F	H	52	CHO	H	66	NHCH ₂ CH ₂ CH ₃	H
39	H	F	53	H	CHO	67	H	NHCH ₂ CH ₂ CH ₃
40	Me	H	54	CH ₃ CO	H	68	NHCH ₂ CH ₂ Ph	H
41	H	Me	55	H	CH ₃ CO	69	H	NHCH ₂ CH ₂ Ph
42	Et	H	56	CH ₃ COOCH ₃	H	70	NHCH ₂ CH ₂ CH ₂ CH ₃	H
43	H	Et	57	H	CH ₃ COOCH ₃	71	H	NHCH ₂ CH ₂ CH ₂ CH ₃
44	CH ₂ NH ₂	H	58	CH ₃ COOCH ₃	H	72	NHCH ₂ CH ₂ C≡N	H
45	H	CH ₂ NH ₂	59	H	CH ₃ COOCH ₃	73	H	NHCH ₂ CH ₂ C≡N
46	CH ₂ OH	H	60	C≡N	H			

Figure 2.7. A library of the 41 IMiD analogues used in the first docking screen.

2.4.2 IMiD/Degron Docking Screen Results

Table 2.4 ('rigid' results) and Table 2.5 ('flexible' results) show the absolute predicted binding energies for every compound/mutant combination. Combinations where the docking failed to run contain 'N/A' instead of a value.

Table 2.4. Predicted energies for the 41 thalidomide analogues shown binding 19 mutants and the WT at position 147 of the IKZF1/3 ZF degnon; the residue at this position was kept rigid during docking. The predicted binding energies were generated using AutoDock4. 'N/A' is shown when the docking failed to run.



**Predicted Binding
Energies (kcal/mol)
Residue 147 Rigid**

lower predicted
affinity

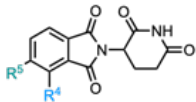


higher predicted
affinity

R ⁴	R ⁵	147	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
H	H	1	-11	-11.12	-10.99	-11.08	-11.24	-10.94	-11.03	-11.15	-11.07	-10.17	-11.4	-11.02	-11	-11.65	-11.28	-10.99	-11.02	-11.14	-11.07	-11.11
NH ₂	H	3	-10.84	-11.00	-10.86	-10.92	-11.07	-10.80	-10.87	-11.01	-10.92	-11.06	-11.22	-10.87	-10.88	-11.46	-10.47	-10.84	-10.09	-11.00	-10.92	-10.97
H	NH ₂	35	-10.72	-10.71	-10.76	-10.86	-10.84	-10.63	-10.74	-11.13	-10.58	-9.41	-10.98	-10.72	-10.73	-11.45	-11.35	-10.73	-10.77	-10.95	-10.80	-10.90
OH	H	36	-11.08	-11.28	-11.19	-11.01	-11.17	-11.01	-11.01	-11.27	-10.72	-9.75	-11.04	-11.09	-11.10	-11.55	-11.29	-11.02	-11.12	-11.21	-11.06	-11.19
H	OH	37	-10.82	-11.11	-10.80	-10.97	-11.31	-10.85	-10.89	-11.15	-11.10	-11.12	-11.09	-10.87	-10.84	-11.52	-11.29	-10.85	-10.95	-11.15	-10.90	-11.11
F	H	38	-10.94	-11.11	-10.94	-11.00	-11.17	-10.89	-10.95	-11.10	-10.92	-10.37	-11.09	-10.95	-10.94	-11.40	-11.10	-10.94	-10.96	-11.06	-10.99	-11.05
H	F	39	-10.98	-11.09	-11.02	-10.81	-11.23	-11.04	-10.95	-11.20	-11.04	-10.24	-11.21	-10.98	-11.08	-11.48	-11.37	-10.99	-11.03	-11.10	-11.05	-11.11
Me	H	40	-11.42	-11.60	-11.41	-11.52	-11.68	-11.38	-11.48	-11.62	-11.45	-10.94	-11.85	-11.46	-11.44	-12.03	N/A	-11.45	-11.48	-11.60	-11.54	-11.57
H	Me	41	-11.36	-11.49	-11.40	-11.32	-11.64	-11.27	-11.39	-11.57	-11.25	-10.55	-10.93	-11.33	-11.35	-11.52	-11.82	-11.30	-11.43	-11.51	-11.50	-11.55
Et	H	42	-11.54	-11.76	-11.62	-11.72	-11.85	-11.50	-11.63	-11.79	-11.69	-11.29	-11.80	-11.64	-11.62	-12.12	-10.65	-11.58	-11.69	-11.83	-11.74	-11.75
H	Et	43	-11.32	-11.56	-11.40	-11.72	-11.78	-11.54	-11.70	-11.60	-11.43	-10.77	-10.92	-11.68	-11.74	-11.60	-12.17	-11.36	-11.41	-11.55	-11.97	-11.70
CH ₂ NH ₂	H	44	-11.35	-11.60	-11.89	-11.74	-11.70	-11.36	-11.47	-11.70	-11.38	-11.22	-11.55	-11.51	-11.87	-11.73	N/A	-11.56	-11.81	-11.55	-11.43	-11.31
H	CH ₂ NH ₂	45	-10.39	-10.42	-11.21	-11.88	-11.06	-10.62	-10.22	-11.05	-10.48	-9.90	-10.12	-10.11	-10.74	-11.47	-11.68	-10.37	-11.25	-10.51	-10.90	-10.75
CH ₂ OH	H	46	-11.67	-11.77	-11.74	-11.74	-11.90	-11.57	-11.67	-11.80	-11.80	-11.04	-11.87	-11.77	-11.63	-11.90	N/A	-11.67	-11.74	-11.57	-11.70	-11.75
H	CH ₂ OH	47	-10.94	-11.00	-10.90	-11.19	-11.45	-10.83	-11.07	-11.22	-11.20	-10.36	-10.42	-10.91	-10.83	-11.14	-11.99	-10.89	-10.98	-11.22	-11.20	-11.16
CH ₂ CCH	H	48	-11.99	-12.06	-11.96	-12.15	-12.29	-11.95	-12.06	-12.22	-11.96	-11.71	-12.11	-12.05	-11.97	-12.62	-9.68	-11.99	-12.01	-12.21	-12.20	-12.13
H	CH ₂ CCH	49	-11.80	-11.92	-11.70	-11.99	-12.15	-12.05	-11.93	-11.96	-11.87	-11.18	-11.16	-11.70	-11.70	-11.82	-12.48	-11.63	-11.87	-11.93	-12.04	-12.21
CH ₂ CN	H	50	-11.78	-11.98	-11.82	-11.98	-12.03	-11.71	-11.83	-12.01	-11.65	-11.45	-11.90	-11.81	-11.79	-12.30	-9.50	-11.77	-11.83	-12.01	-11.97	-11.95
H	CH ₂ CN	51	-11.58	-11.78	-11.68	-11.96	-12.07	-11.49	-11.71	-11.85	-11.64	-10.99	-10.77	-11.66	-11.79	-11.78	-12.25	-11.65	-11.62	-11.81	-11.95	-12.12
CHO	H	52	-11.14	-11.31	-11.15	-11.26	-11.40	-11.04	-11.21	-11.30	-11.24	-10.82	-11.42	-11.18	-11.15	-11.76	-10.91	-11.15	-11.16	-11.28	-11.24	-11.30
H	CHO	53	-11.23	-11.36	-11.28	-11.42	-11.47	-10.91	-11.10	-11.42	-11.05	-10.56	-11.17	-11.36	-11.35	-11.63	-11.87	-11.06	-11.28	-11.37	-11.57	-11.43
COMe	H	54	-11.75	-11.93	-11.73	-11.98	-12.02	-11.68	-11.81	-11.98	-11.75	-12.10	-11.92	-11.78	-11.73	-12.43	N/A	-11.74	-11.77	-11.90	-11.89	-11.86
H	COMe	55	-11.69	-11.65	-11.65	-12.22	-11.85	-11.68	-11.79	-11.73	-12.12	-10.38	-10.97	-11.73	-11.78	-11.52	-12.39	-11.72	-11.79	-11.66	-12.08	-11.80
COOMe	H	56	-11.56	-11.76	-11.56	-11.71	-11.78	-11.55	-11.63	-11.76	-11.72	-12.25	-12.18	-11.61	-11.59	-12.24	N/A	-11.60	-11.64	-11.71	-11.72	-11.73
H	COOMe	57	-11.38	-11.31	-11.43	-11.56	-11.67	-11.26	-11.43	-11.47	-11.35	-10.11	-10.74	-11.32	-11.47	-11.22	-12.08	-11.40	-11.49	-11.42	-11.76	-11.47
OCOMe	H	58	-11.60	-11.83	-11.55	-11.74	-11.83	-11.49	-11.63	-11.81	-11.62	-11.89	-11.56	-11.69	-11.56	-11.98	N/A	-11.58	-11.61	-11.78	-11.78	-11.70
H	OCOMe	59	-11.17	-11.09	-11.24	-11.14	-11.74	-11.91	-11.47	-11.37	-11.27	-9.81	-10.73	-11.19	-11.39	-11.23	-12.30	-11.24	-11.39	-11.26	-11.41	-11.61
CN	H	60	-11.76	-11.85	-11.71	-11.82	-11.96	-11.72	-11.74	-11.88	-11.77	-11.87	-12.26	-11.72	-11.78	-12.34	N/A	-11.70	-11.79	-11.89	-11.84	-11.83
H	CN	61	-11.32	-11.52	-11.35	-11.68	-11.70	-11.33	-11.55	-11.69	-11.39	-10.99	-11.09	-11.50	-11.36	-11.36	-12.01	-11.52	-11.48	-11.55	-11.79	-11.66
NHMe	H	62	-11.18	-11.34	-11.18	-11.35	-11.43	-11.13	-11.21	-11.34	-11.33	-11.46	-11.39	-11.20	-11.19	-11.82	N/A	-11.19	-11.21	-11.32	-11.31	-11.31
H	NHMe	63	-11.18	-11.17	-11.17	-11.54	-12.01	-11.02	-11.24	-11.34	-11.19	-9.94	-11.45	-11.22	-11.23	-11.56	-11.67	-11.18	-11.24	-11.30	-11.41	-11.92
NHEt	H	64	-11.34	-11.49	-11.38	-11.43	-11.60	-11.32	-11.35	-11.51	-11.47	-11.04	-11.73	-11.40	-11.38	-12.00	N/A	-11.36	-11.40	-11.49	-11.46	-11.44
H	NHEt	65	-11.30	-11.30	-11.88	-11.59	-12.28	-11.16	-11.22	-11.24	-11.27	-10.48	-11.57	-11.41	-11.12	-11.86	-11.75	-11.06	-11.90	-11.16	-11.67	-12.14
NH ⁱ Pr	H	66	-11.64	-11.77	-11.66	-11.77	-11.86	-11.57	-11.65	-11.75	-11.67	-11.27	-11.88	-11.64	-11.65	-12.30	N/A	-11.64	-11.67	-11.77	-11.90	-11.75
H	NH ⁱ Pr	67	-11.39	-11.15	-12.10	-11.66	-12.34	-11.32	-11.66	-11.56	-11.23	-9.80	-11.43	-11.40	-12.13	-11.35	-11.95	-11.44	-12.06	-11.42	-12.37	-12.41
NHBn	H	68	-12.53	-12.74	-12.64	-12.69	-12.81	-12.49	-13.35	-13.47	-12.64	-12.99	-12.92	-12.58	-12.54	-13.21	N/A	-12.47	-12.68	-13.37	-13.70	-12.67
H	NHBn	69	-12.26	-12.50	-13.06	-12.70	-13.65	-12.15	-12.96	-12.69	-12.35	-12.90	-12.77	-13.06	-12.29	-11.79	-12.71	-12.85	-12.31	-12.72	-13.52	-12.96
NHCN ₂ CCH	H	70	-11.81	-11.96	-11.81	-11.87	-12.02	-11.76	-11.78	-11.89	-11.83	-11.46	-12.15	-11.78	-11.78	-12.41	N/A	-11.77	-11.81	-11.93	-12.22	-11.95
H	NHCN ₂ CCH	71	-11.24	-11.54	-12.29	-11.87	-12.75	-11.68	-12.37	-11.91	-11.54	-10.36	-11.87	-11.15	-11.46	-11.82	-11.98	-11.45	-12.23	-11.78	-12.64	-12.59
NHCH ₂ CN	H	72	-11.68	-11.85	-11.71	-11.77	-11.93	-11.66	-11.70	-11.82	-11.66	-11.35	-11.91	-11.67	-11.69	-12.28	N/A	-11.67	-11.68	-11.80	-11.77	-11.81
H	NHCH ₂ CN	73	-11.20	-11.43	-12.15	-11.80	-12.55	-11.44	-11.14	-11.31	-11.25	-10.68	-11.70	-11.19	-11.35	-12.00	-11.92	-11.19	-12.18	-11.18	-12.50	-12.46

Chapter 2: Design and Synthesis of IMiD Analogues

Table 2.5. Predicted energies for the 41 thalidomide analogues shown binding 19 mutants and the WT at position 147 of the IKZF1/3 ZF degron; the residue at this position was allowed to be flexible during docking. The predicted binding energies were generated using AutoDock4. 'N/A' is shown when the docking failed to run.



**Predicted Binding
Energies (kcal/mol)
Residue 147 Flexible**

lower predicted
affinity

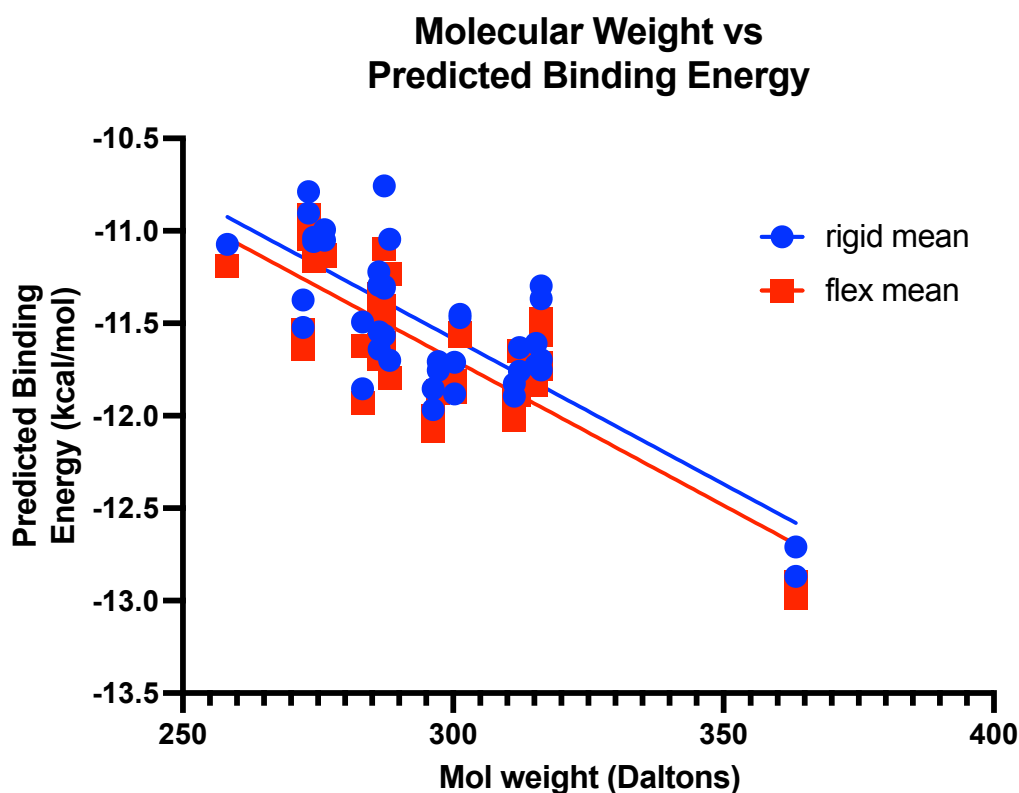


higher predicted
affinity

R ⁴	R ⁵	147	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
H	H	1	-10.96	-11.15	-11.04	-11.34	-11.26	-10.93	-11.17	-11.32	-11.18	-11.15	-11.36	-11.08	-11.02	-11.44	-11.55	-11.07	-11.08	-11.18	-11.22	-11.27
NH ₂	H	3	-10.85	-11	-10.91	-11.2	-11.11	-10.79	-11	-11.17	-11.22	-10.98	-11.27	-10.92	-10.87	-11.32	-11.12	-10.91	-10.96	-11.03	-11.07	-11.13
H	NH ₂	35	-10.72	-10.93	-11.23	-11.18	-10.86	-10.64	-11.11	-11.16	-10.16	-10.91	-11.01	-10.77	-10.73	-10.84	-11.61	-10.78	-10.89	-11.01	-10.85	-10.84
OH	H	36	-11.09	-11.07	-10.98	-11.4	-11.24	-11.01	-11.16	-11.39	-11.1	-11.27	-11.32	-11.05	-11.04	-10.92	-11.34	-11.15	-11.23	-11.23	-11.04	-11.24
H	OH	37	-10.84	-11.14	-10.85	-11.42	-11.25	-10.77	-11.15	-11.32	-11.25	-11.05	-11.13	-10.95	-10.86	-11.22	-11.6	-10.9	-11.02	-11.16	-11.18	-11.26
F	H	38	-10.93	-11.11	-10.99	-11.27	-11.21	-10.87	-11.09	-11.27	-11.09	-11.1	-11.36	-10.99	-10.96	-11.37	-11.41	-11	-11.05	-11.11	-11.17	-11.21
H	F	39	-10.93	-11.19	-11.04	-10.98	-11.22	-11.02	-11.16	-11.28	-11.07	-11.14	-11.19	-11.14	-11.01	-11.14	-11.55	-10.99	-11.06	-11.16	-11.2	-11.23
Me	H	40	-11.45	-11.58	-11.48	-11.81	-11.74	-11.36	-11.65	-11.78	-11.61	-11.61	-11.98	-11.57	-11.47	-11.93	N/A	-11.5	-11.54	-11.63	-11.69	-11.72
H	Me	41	-11.35	-11.55	-11.41	-11.43	-11.67	-11.33	-11.54	-11.71	-11.5	-11.51	-11.63	-11.38	-11.33	-11.49	-12.01	-11.46	-11.53	-11.54	-11.66	-11.66
Et	H	42	-11.59	-11.78	-11.58	-11.95	-11.88	-11.5	-11.81	-12	-11.78	-11.8	-11.98	-11.7	-11.57	-12.03	-8.55	-11.59	-11.72	-11.78	-11.84	-11.9
H	Et	43	-11.45	-11.54	-11.39	-11.64	-11.77	-11.53	-11.68	-11.79	-11.67	-11.79	-11.86	-11.65	-11.73	-11.7	-12.28	-11.64	-11.52	-11.61	-11.77	-11.82
CH ₂ NH ₂	H	44	-11.74	-11.94	-11.63	-12.76	-12.06	-11.7	-11.31	-12.04	-11.71	-11.63	-11.54	-11.82	-11.42	-11.7	-8.44	-11.25	-11.38	-12	-12	-12.07
H	CH ₂ NH ₂	45	-10.84	-10.64	-12.29	-12.83	-10.78	-10.21	-11.04	-10.78	-10.34	-10.64	-10.91	-10.82	-10.76	-11.57	-11.83	-11.63	-11.29	-10.81	-11.06	-10.85
CH ₂ OH	H	46	-11.61	-11.83	-11.67	-11.81	-11.98	-11.34	-11.88	-12.03	-11.76	-11.62	-12.13	-11.74	-11.72	-12.1	N/A	-11.78	-11.74	-11.77	-11.83	-11.8
H	CH ₂ OH	47	-11.01	-11.22	-11.16	-11.28	-11.48	-10.88	-11.29	-11.43	-11.03	-11.25	-11.33	-11.05	-11.04	-11.44	-11.93	-10.95	-10.98	-11.04	-11.43	-11.45
CH ₂ CCH	H	48	-11.96	-12.19	-12.04	-12.26	-12.28	-11.88	-12.23	-12.41	-12.21	-12.23	-12.44	-12.17	-11.99	-12.36	-10	-12.05	-12.13	-12.25	-12.3	-12.29
H	CH ₂ CCH	49	-11.69	-11.86	-11.8	-11.98	-12.16	-12.04	-12	-12.09	-12.09	-12.09	-12.22	-11.71	-11.69	-12	-12.6	-11.72	-11.96	-11.91	-12.22	-12.19
CH ₂ CN	H	50	-11.77	-11.94	-11.84	-12.18	-12.12	-11.7	-12.01	-12.2	-11.83	-12.04	-12.15	-11.9	-11.82	-12.31	-9.78	-11.87	-11.91	-12.02	-12.05	-12.1
H	CH ₂ CN	51	-11.58	-11.8	-11.67	-11.87	-12.05	-11.51	-11.94	-11.98	-11.74	-11.89	-11.9	-11.74	-11.62	-11.87	-12.45	-11.83	-11.89	-11.89	-12.08	-12.07
CHO	H	52	-11.14	-11.3	-11.19	-11.56	-11.39	-11.06	-11.35	-11.52	-11.43	-11.32	-11.65	-11.21	-11.17	-11.74	-11.1	-11.24	-11.25	-11.34	-11.38	-11.47
H	CHO	53	-11.22	-11.26	-11.37	-11.3	-11.43	-11.18	-11.32	-11.56	-11.37	-11.27	-11.6	-11.4	-11.42	-11.52	-12.1	-11.17	-11.27	-11.45	-11.44	-11.42
COMe	H	54	-11.76	-11.89	-11.81	-12.28	-12.03	-11.71	-11.98	-12.15	-12.01	-11.94	-12.24	-11.81	-11.74	-12.1	-9.01	-11.81	-11.91	-11.93	-12.04	-12.04
H	COMe	55	-11.68	-11.65	-11.72	-11.87	-11.86	-11.67	-11.82	-11.93	-11.92	-11.94	-12.03	-11.73	-11.84	-12.09	-12.62	-11.65	-11.63	-11.67	-12.2	-11.95
COOMe	H	56	-11.59	-11.78	-11.62	-12.02	-11.8	-11.54	-11.78	-12.02	-12.03	-11.84	-12.16	-11.67	-11.59	-12.28	-10.32	-11.6	-11.69	-11.76	-11.84	-12.02
H	COOMe	57	-11.38	-11.39	-11.42	-11.61	-11.7	-11.28	-11.43	-11.56	-11.58	-11.69	-11.82	-11.31	-11.48	-11.84	-12.18	-11.49	-11.37	-11.42	-11.68	-11.68
OCOMe	H	58	-11.6	-11.77	-11.59	-11.97	-11.9	-11.5	-11.79	-11.91	-11.88	-11.79	-12.04	-11.7	-11.58	-12.02	-10.29	-11.67	-11.72	-11.81	-11.86	-11.9
H	OCOMe	59	-11.21	-11.33	-11.21	-11.49	-11.76	-11.91	-11.57	-11.53	-11.25	-11.29	-11.22	-11.42	-11.32	-11.4	-12.18	-11.19	-11.29	-11.42	-11.81	-11.74
CN	H	60	-11.69	-11.86	-11.75	-12.16	-11.97	-11.67	-11.95	-12.05	-12.07	-11.88	-12.28	-11.84	-11.77	-12.28	N/A	-11.82	-11.83	-11.89	-11.94	-11.97
H	CN	61	-11.41	-11.49	-11.5	-11.57	-11.77	-11.26	-11.61	-11.78	-11.56	-11.72	-11.69	-11.54	-11.51	-11.68	-12.24	-11.4	-11.52	-11.61	-11.79	-11.78
NHMe	H	62	-11.17	-11.35	-11.23	-11.62	-11.47	-11.14	-11.38	-11.64	-11.55	-11.47	-11.72	-11.2	-11.19	-11.78	N/A	-11.24	-11.29	-11.39	-11.44	-11.48
H	NHMe	63	-11.15	-11.25	-11.72	-11.55	-11.99	-11.08	-11.61	-11.45	-11.24	-11.41	-11.54	-11.23	-11.24	-11.63	-11.86	-11.18	-11.38	-11.28	-11.61	-12.02
NHEt	H	64	-11.32	-11.5	-11.4	-11.71	-11.65	-11.27	-11.55	-11.7	-11.66	-11.55	-11.95	-11.56	-11.34	-12.01	N/A	-11.42	-11.44	-11.54	-11.61	-11.62
H	NHEt	65	-11.05	-11.29	-11.82	-11.22	-12.25	-11.21	-11.59	-11.5	-11.24	-11.3	-11.49	-11.39	-11.14	-11.62	-12.31	-11.1	-11.25	-11.97	-12.05	-12.25
NH ⁱ Pr	H	66	-11.63	-11.78	-11.68	-12.06	-11.93	-11.59	-11.85	-12.04	-11.87	-11.8	-12.09	-11.81	-11.59	-12.22	N/A	-11.66	-11.71	-11.75	-11.85	-11.9
H	NH ⁱ Pr	67	-11.41	N/A	-11.8	-11.37	-12.44	-11.31	-11.79	-11.85	-11.36	-11.56	-11.57	-11.22	-11.31	-11.99	-12.21	-11.49	-12.09	-12.05	-12.16	-12.46
NHBn	H	68	-12.55	-12.67	-12.52	-13.09	-12.86	-12.62	-12.76	-13.43	-13.24	-13	-13.44	-12.74	-12.57	-13.23	N/A	-12.66	-12.72	-13.35	-14.29	-12.95
H	NHBn	69	-12.26	-12.45	-13.19	-12.57	-13.3	-12.54	-13.49	-13.21	-12.76	-13.12	-12.92	-12.42	-12.8	-12.62	-13.01	-12.37	-13.04	-13.08	-13.21	-13.58
NHCN ₂ CCH	H	70	-11.86	-11.97	-11.84	-12.3	-12.09	-11.79	-11.98	-12.13	-12.21	-12.01	-12.4	-11.92	-11.81	-12.3	N/A	-11.86	-11.9	-11.99	-12.03	-12.08
H	NHCN ₂ CCH	71	-11.31	-11.64	-12.02	-11.91	-12.74	-11.65	-12.64	-12.43	-11.47	-11.46	-11.51	-11.15	-11.51	-11.97	-12.1	-11.4	-11.71	-12.35	-12.36	-12.73
NHCH ₂ CN	H	72	-11.69	-11.84	-11.76	-12.12	-11.98	-11.65	-11.85	-12.05	-11.84	-11.86	-12.2	-11.82	-11.73	-12.22	N/A	-11.73	-11.77	-11.87	-11.91	-11.92
H	NHCH ₂ CN	73	-11.1	-11.43	-11.97	-11.75	-12.62	-11.45	-11.68	-11.47	-11.27	-11.33	-11.32	-11.23	-11.37	-11.83	-12.26	-11.16	-11.39	-11.64	-12.23	-12.55

In both tables there appears to be a correlation between size of the ligand and the predicted binding energy. Plotting molecular weight vs binding energy (Figure 2.8)

confirms this to be the case. This is to be expected, as larger ligands have more surface area for favourable interactions to take place across.



Figure

2.8. Molecular weight of the 41 compounds used in the docking screen, plotted against average predicted binding energy across all 19 mutants and WT for that compound. Positive correlation observed between molecular weight and favourability of binding for both 'rigid' and 'flexible' docking screens. Correlation significant ($p < 0.0001$) for both lines of best fit; R^2 values of 0.6197 (rigid) and 0.6541 (flex).

The average predicted binding energies for 4-‘bumped’ vs 5-‘bumped’ compounds for the WT degron (Table 2.6) shows that, for both the ‘rigid’ and ‘flexible’ screens, the 4-‘bumped’ compounds have, on average, more favourable predicted binding energies than 5-‘bumped’ compounds. This result suggests that steric clashes with the Q147 side chain are forcing the 5-‘bumped’ compounds to adopt slightly less favourable poses compared to their 4-‘bumped’ regioisomers. These data indicate that 5-‘bumped’ IMiD analogues may be a more reliable option when designing ‘bumped’ IMiD analogues for chemical synthesis and subsequent biological testing.

Table 2.6. Mean predicted binding energy scores for 4- and 5- ‘bumped’ compounds for WT (Q147) only.

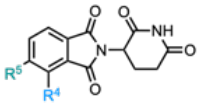
	Estimated Binding Energy (kcal/mol)			
	Rigid		Flexible	
	Mean	SD	Mean	SD
4 bumped	-12.09	0.43	-12.01	0.48
5 bumped	-11.56	0.23	-11.67	0.39

2.4.3 Highlighting Ligand/Mutant Combinations

Data from these screens can be interpreted in two ways: as absolute predicted binding energies, or as differences in predicted binding energies between mutants and the WT. The measurement of the difference in predicted binding energies, regardless of the overall magnitude of those energies, is important for finding selective ligands: i.e. those that will possess higher binding affinity to a mutant degron over the WT. The differences in predicted binding energies for the ‘rigid’ screen are shown in Table 2.7, and the differences in predicted binding energies for the ‘flexible’ screen are shown in Table 2.8.

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Table 2.7. Difference in predicted binding energies between mutant and WT (Q147) for the docking screen where residue 147 has been kept rigid. A favourable difference in energy indicates a more favourable energy for that compound/mutant combination the compound/WT combination.



**Difference in mutant and WT
Predicted Binding Energies
(kcal/mol)
Residue 147 Rigid**

Unfavourable difference
in energies

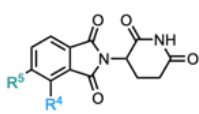


Favourable difference
in energies

R ⁴	R ⁵	147	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
H	H	1	0.65	0.53	0.66	0.57	0.41	0.71	0.62	0.5	0.58	1.48	0.25	0.63	0.65	N/A	0.37	0.66	0.63	0.51	0.58	0.54
NH ₂	H	3	0.62	0.46	0.60	0.54	0.39	0.66	0.59	0.45	0.54	0.40	0.24	0.59	0.58	N/A	0.99	0.62	1.37	0.46	0.54	0.49
H	NH ₂	35	0.73	0.74	0.69	0.59	0.61	0.82	0.71	0.32	0.87	2.04	0.47	0.73	0.72	N/A	0.10	0.72	0.68	0.50	0.65	0.55
OH	H	36	0.47	0.27	0.36	0.54	0.38	0.54	0.54	0.28	0.83	1.80	0.51	0.46	0.45	N/A	0.26	0.53	0.43	0.34	0.49	0.36
H	OH	37	0.70	0.41	0.72	0.55	0.21	0.67	0.63	0.37	0.42	0.40	0.43	0.65	0.68	N/A	0.23	0.67	0.57	0.37	0.62	0.41
F	H	38	0.46	0.29	0.46	0.40	0.23	0.51	0.45	0.30	0.48	1.03	0.31	0.45	0.46	N/A	0.30	0.46	0.44	0.34	0.41	0.35
H	F	39	0.50	0.39	0.46	0.67	0.25	0.44	0.53	0.28	0.44	1.24	0.27	0.50	0.40	N/A	0.11	0.49	0.45	0.38	0.43	0.37
Me	H	40	0.61	0.43	0.62	0.51	0.35	0.65	0.55	0.41	0.58	1.09	0.18	0.57	0.59	N/A	N/A	0.58	0.55	0.43	0.49	0.46
H	Me	41	0.16	0.03	0.12	0.20	-0.12	0.25	0.13	-0.05	0.27	0.97	0.59	0.19	0.17	N/A	-0.30	0.22	0.09	0.01	0.02	-0.03
Et	H	42	0.58	0.36	0.50	0.40	0.27	0.62	0.49	0.33	0.43	0.83	0.32	0.48	0.50	N/A	1.47	0.54	0.43	0.29	0.38	0.37
H	Et	43	0.28	0.04	0.20	-0.12	-0.18	0.06	-0.10	0.00	0.17	0.83	0.68	-0.08	-0.14	N/A	-0.57	0.24	0.19	0.05	-0.37	-0.10
CH ₂ NH ₂	H	44	0.38	0.13	-0.16	-0.01	0.03	0.37	0.26	0.03	0.35	0.51	0.18	0.22	-0.14	N/A	N/A	0.17	-0.08	0.18	0.30	0.42
H	CH ₂ NH ₂	45	1.08	1.05	0.26	-0.41	0.41	0.85	1.25	0.42	0.99	1.57	1.35	1.36	0.73	N/A	-0.21	1.10	0.22	0.96	0.57	0.72
CH ₂ OH	H	46	0.23	0.13	0.16	0.16	0.00	0.33	0.23	0.10	0.10	0.86	0.03	0.13	0.27	N/A	N/A	0.23	0.16	0.33	0.20	0.15
H	CH ₂ OH	47	0.20	0.14	0.24	-0.05	-0.31	0.31	0.07	-0.08	-0.06	0.78	0.72	0.23	0.31	N/A	-0.85	0.25	0.16	-0.08	-0.08	-0.02
CH ₂ CCH	H	48	0.63	0.56	0.66	0.47	0.33	0.67	0.56	0.40	0.66	0.91	0.51	0.57	0.65	N/A	2.94	0.63	0.61	0.41	0.42	0.49
H	CH ₂ CCH	49	0.02	-0.10	0.12	-0.17	-0.33	-0.23	-0.11	-0.14	-0.05	0.64	0.66	0.12	0.12	N/A	-0.66	0.19	-0.05	-0.11	-0.22	-0.39
CH ₂ CN	H	50	0.52	0.32	0.48	0.32	0.27	0.59	0.47	0.29	0.65	0.85	0.40	0.49	0.51	N/A	2.80	0.53	0.47	0.29	0.33	0.35
H	CH ₂ CN	51	0.20	0.00	0.10	-0.18	-0.29	0.29	0.07	-0.07	0.14	0.79	1.01	0.12	-0.01	N/A	-0.47	0.13	0.16	-0.03	-0.17	-0.34
CHO	H	52	0.62	0.45	0.61	0.50	0.36	0.72	0.55	0.46	0.52	0.94	0.34	0.58	0.61	N/A	0.85	0.61	0.60	0.48	0.52	0.46
H	CHO	53	0.40	0.27	0.35	0.21	0.16	0.72	0.53	0.21	0.58	1.07	0.46	0.27	0.28	N/A	-0.24	0.57	0.35	0.26	0.06	0.20
COMe	H	54	0.68	0.50	0.70	0.45	0.41	0.75	0.62	0.45	0.68	0.33	0.51	0.65	0.70	N/A	N/A	0.69	0.66	0.53	0.54	0.57
H	COMe	55	-0.17	-0.13	-0.13	-0.70	-0.33	-0.16	-0.27	-0.21	-0.60	1.14	0.55	-0.21	-0.26	N/A	-0.87	-0.20	-0.27	-0.14	-0.56	-0.28
COOMe	H	56	0.68	0.48	0.68	0.53	0.46	0.69	0.61	0.48	0.52	-0.01	0.06	0.63	0.65	N/A	N/A	0.64	0.60	0.53	0.52	0.51
H	COOMe	57	-0.16	-0.09	-0.21	-0.34	-0.45	-0.04	-0.21	-0.25	-0.13	1.11	0.48	-0.10	-0.25	N/A	-0.86	-0.18	-0.27	-0.20	-0.54	-0.25
OCOMe	H	58	0.38	0.15	0.43	0.24	0.15	0.49	0.35	0.17	0.36	0.09	0.42	0.29	0.42	N/A	N/A	0.40	0.37	0.20	0.20	0.28
H	OCOMe	59	0.06	0.14	-0.01	0.09	-0.51	-0.68	-0.24	-0.14	-0.04	1.42	0.50	0.04	-0.16	N/A	-1.07	-0.01	-0.16	-0.03	-0.18	-0.38
CN	H	60	0.58	0.49	0.63	0.52	0.38	0.62	0.60	0.46	0.57	0.47	0.08	0.62	0.56	N/A	N/A	0.64	0.55	0.45	0.50	0.51
H	CN	61	0.04	-0.16	0.01	-0.32	-0.34	0.03	-0.19	-0.33	-0.03	0.37	0.27	-0.14	0.00	N/A	-0.65	-0.16	-0.12	-0.19	-0.43	-0.30
NHMe	H	62	0.64	0.48	0.64	0.47	0.39	0.69	0.61	0.48	0.49	0.36	0.43	0.62	0.63	N/A	N/A	0.63	0.61	0.50	0.51	0.51
H	NHMe	63	0.38	0.39	0.39	0.02	-0.45	0.54	0.32	0.22	0.37	1.62	0.11	0.34	0.33	N/A	-0.11	0.38	0.32	0.26	0.15	-0.36
NHEt	H	64	0.66	0.51	0.62	0.57	0.40	0.68	0.65	0.49	0.53	0.96	0.27	0.60	0.62	N/A	N/A	0.64	0.60	0.51	0.54	0.56
H	NHEt	65	0.56	0.56	-0.02	0.27	-0.42	0.70	0.64	0.62	0.59	1.38	0.29	0.45	0.74	N/A	0.11	0.80	-0.04	0.70	0.19	-0.28
NH ⁱ Pr	H	66	0.66	0.53	0.64	0.53	0.44	0.73	0.65	0.55	0.63	1.03	0.42	0.66	0.65	N/A	N/A	0.66	0.63	0.53	0.40	0.55
H	NH ⁱ Pr	67	-0.04	0.20	-0.75	-0.31	-0.99	0.03	-0.31	-0.21	0.12	1.55	-0.08	-0.05	-0.78	N/A	-0.60	-0.09	-0.71	-0.07	-1.02	-1.06
NHBn	H	68	0.68	0.47	0.57	0.52	0.40	0.72	-0.14	-0.26	0.57	0.22	0.29	0.63	0.67	N/A	N/A	0.74	0.53	-0.16	-0.49	0.54
H	NHBn	69	-0.47	-0.71	-1.27	-0.91	-1.86	-0.36	-1.17	-0.90	-0.56	-1.11	-0.98	-1.27	-0.50	N/A	-0.92	-1.06	-0.52	-0.93	-1.73	-1.17
NHCN ₂ CCH	H	70	0.60	0.45	0.60	0.54	0.39	0.65	0.63	0.52	0.58	0.95	0.26	0.63	0.63	N/A	N/A	0.64	0.60	0.48	0.19	0.46
H	NHCN ₂ CCH	71	0.58	0.28	-0.47	-0.05	-0.93	0.14	-0.55	-0.09	0.28	1.46	-0.05	0.67	0.36	N/A	-0.16	0.37	-0.41	0.04	-0.82	-0.77
NHCH ₂ CN	H	72	0.60	0.43	0.57	0.51	0.35	0.62	0.58	0.46	0.62	0.93	0.37	0.61	0.59	N/A	N/A	0.61	0.60	0.48	0.51	0.47
H	NHCH ₂ CN	73	0.80	0.57	-0.15	0.20	-0.55	0.56	0.86	0.69	0.75	1.32	0.30	0.81	0.65	N/A	0.08	0.81	-0.18	0.82	-0.50	-0.46

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Table 2.8. Difference in predicted binding energies between mutant and WT (Q147) for the docking screen where residue 147 has been allowed to be flexible. A favourable difference in energy indicates a more favourable energy for that compound/mutant combination the compound/WT combination.



Difference in mutant and WT
Predicted Binding Energies (kcal/
mol)
Residue 147 Flexible

Unfavourable difference
in energies



Favourable difference in
energies

R ⁴	R ⁵	147	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
H	H	1	0.48	0.29	0.4	0.1	0.18	0.51	0.27	0.12	0.26	0.29	0.08	0.36	0.42	N/A	-0.11	0.37	0.36	0.26	0.22	0.17
NH ₂	H	3	0.47	0.32	0.41	0.12	0.21	0.53	0.32	0.15	0.1	0.34	0.05	0.4	0.45	N/A	0.2	0.41	0.36	0.29	0.25	0.19
H	NH ₂	35	0.12	-0.09	-0.39	-0.34	-0.02	0.2	-0.27	-0.32	0.68	-0.07	-0.17	0.07	0.11	N/A	-0.77	0.06	-0.05	-0.17	-0.01	0
OH	H	36	-0.17	-0.15	-0.06	-0.48	-0.32	-0.09	-0.24	-0.47	-0.18	-0.35	-0.4	-0.13	-0.12	N/A	-0.42	-0.23	-0.31	-0.31	-0.12	-0.32
H	OH	37	0.38	0.08	0.37	-0.2	-0.03	0.45	0.07	-0.1	-0.03	0.17	0.09	0.27	0.36	N/A	-0.38	0.32	0.2	0.06	0.04	-0.04
F	H	38	0.44	0.26	0.38	0.1	0.16	0.5	0.28	0.1	0.28	0.27	0.01	0.38	0.41	N/A	-0.04	0.37	0.32	0.26	0.2	0.16
H	F	39	0.21	-0.05	0.1	0.16	-0.08	0.12	-0.02	-0.14	0.07	0	-0.05	0	0.13	N/A	-0.41	0.15	0.08	-0.02	-0.06	-0.09
Me	H	40	0.48	0.35	0.45	0.12	0.19	0.57	0.28	0.15	0.32	0.32	-0.05	0.36	0.46	N/A	N/A	0.43	0.39	0.3	0.24	0.21
H	Me	41	0.14	-0.06	0.08	0.06	-0.18	0.16	-0.05	-0.22	-0.01	-0.02	-0.14	0.11	0.16	N/A	-0.52	0.03	-0.04	-0.05	-0.17	-0.17
Et	H	42	0.44	0.25	0.45	0.08	0.15	0.53	0.22	0.03	0.25	0.23	0.05	0.33	0.46	N/A	3.48	0.44	0.31	0.25	0.19	0.13
H	Et	43	0.25	0.16	0.31	0.06	-0.07	0.17	0.02	-0.09	0.03	-0.09	-0.16	0.05	-0.03	N/A	-0.58	0.06	0.18	0.09	-0.07	-0.12
CH ₂ NH ₂	H	44	-0.04	-0.24	0.07	-1.06	-0.36	0	0.39	-0.34	-0.01	0.07	0.16	-0.12	0.28	N/A	3.26	0.45	0.32	-0.3	-0.3	-0.37
H	CH ₂ NH ₂	45	0.73	0.93	-0.72	-1.26	0.79	1.36	0.53	0.79	1.23	0.93	0.66	0.75	0.81	N/A	-0.26	-0.06	0.28	0.76	0.51	0.72
CH ₂ OH	H	46	0.49	0.27	0.43	0.29	0.12	0.76	0.22	0.07	0.34	0.48	-0.03	0.36	0.38	N/A	N/A	0.32	0.36	0.33	0.27	0.3
H	CH ₂ OH	47	0.43	0.22	0.28	0.16	-0.04	0.56	0.15	0.01	0.41	0.19	0.11	0.39	0.4	N/A	-0.49	0.49	0.46	0.4	0.01	-0.01
CH ₂ CCH	H	48	0.4	0.17	0.32	0.1	0.08	0.48	0.13	-0.05	0.15	0.13	-0.08	0.19	0.37	N/A	2.36	0.31	0.23	0.11	0.06	0.07
H	CH ₂ CCH	49	0.31	0.14	0.2	0.02	-0.16	-0.04	0	-0.09	-0.09	-0.09	-0.22	0.29	0.31	N/A	-0.6	0.28	0.04	0.09	-0.22	-0.19
CH ₂ CN	H	50	0.54	0.37	0.47	0.13	0.19	0.61	0.3	0.11	0.48	0.27	0.16	0.41	0.49	N/A	2.53	0.44	0.4	0.29	0.26	0.21
H	CH ₂ CN	51	0.29	0.07	0.2	0	-0.18	0.36	-0.07	-0.11	0.13	-0.02	-0.03	0.13	0.25	N/A	-0.58	0.04	-0.02	-0.02	-0.21	-0.2
CHO	H	52	0.6	0.44	0.55	0.18	0.35	0.68	0.39	0.22	0.31	0.42	0.09	0.53	0.57	N/A	0.64	0.5	0.49	0.4	0.36	0.27
H	CHO	53	0.3	0.26	0.15	0.22	0.09	0.34	0.2	-0.04	0.15	0.25	-0.08	0.12	0.1	N/A	-0.58	0.35	0.25	0.07	0.08	0.1
COMe	H	54	0.34	0.21	0.29	-0.18	0.07	0.39	0.12	-0.05	0.09	0.16	-0.14	0.29	0.36	N/A	3.09	0.29	0.19	0.17	0.06	0.06
H	COMe	55	0.41	0.44	0.37	0.22	0.23	0.42	0.27	0.16	0.17	0.15	0.06	0.36	0.25	N/A	-0.53	0.44	0.46	0.42	-0.11	0.14
COOMe	H	56	0.69	0.5	0.66	0.26	0.48	0.74	0.5	0.26	0.25	0.44	0.12	0.61	0.69	N/A	1.96	0.68	0.59	0.52	0.44	0.26
H	COOMe	57	0.46	0.45	0.42	0.23	0.14	0.56	0.41	0.28	0.26	0.15	0.02	0.53	0.36	N/A	-0.34	0.35	0.47	0.42	0.16	0.16
OCOMe	H	58	0.42	0.25	0.43	0.05	0.12	0.52	0.23	0.11	0.14	0.23	-0.02	0.32	0.44	N/A	1.73	0.35	0.3	0.21	0.16	0.12
H	OCOMe	59	0.19	0.07	0.19	-0.09	-0.36	-0.51	-0.17	-0.13	0.15	0.11	0.18	-0.02	0.08	N/A	-0.78	0.21	0.11	-0.02	-0.41	-0.34
CN	H	60	0.59	0.42	0.53	0.12	0.31	0.61	0.33	0.23	0.21	0.4	0	0.44	0.51	N/A	N/A	0.46	0.45	0.39	0.34	0.31
H	CN	61	0.27	0.19	0.18	0.11	-0.09	0.42	0.07	-0.1	0.12	-0.04	-0.01	0.14	0.17	N/A	-0.56	0.28	0.16	0.07	-0.11	-0.1
NHMe	H	62	0.61	0.43	0.55	0.16	0.31	0.64	0.4	0.14	0.23	0.31	0.06	0.58	0.59	N/A	N/A	0.54	0.49	0.39	0.34	0.3
H	NHMe	63	0.48	0.38	-0.09	0.08	-0.36	0.55	0.02	0.18	0.39	0.22	0.09	0.4	0.39	N/A	-0.23	0.45	0.25	0.35	0.02	-0.39
NHEt	H	64	0.69	0.51	0.61	0.3	0.36	0.74	0.46	0.31	0.35	0.46	0.06	0.45	0.67	N/A	N/A	0.59	0.57	0.47	0.4	0.39
H	NHEt	65	0.57	0.33	-0.2	0.4	-0.63	0.41	0.03	0.12	0.38	0.32	0.13	0.23	0.48	N/A	-0.69	0.52	0.37	-0.35	-0.43	-0.63
NH ⁱ Pr	H	66	0.59	0.44	0.54	0.16	0.29	0.63	0.37	0.18	0.35	0.42	0.13	0.41	0.63	N/A	N/A	0.56	0.51	0.47	0.37	0.32
H	NH ⁱ Pr	67	0.58	N/A	0.19	0.62	-0.45	0.68	0.2	0.14	0.63	0.43	0.42	0.77	0.68	N/A	-0.22	0.5	-0.1	-0.06	-0.17	-0.47
NHBn	H	68	0.68	0.56	0.71	0.14	0.37	0.61	0.47	-0.2	-0.01	0.23	-0.21	0.49	0.66	N/A	N/A	0.57	0.51	-0.12	-1.06	0.28
H	NHBn	69	0.36	0.17	-0.57	0.05	-0.68	0.08	-0.87	-0.59	-0.14	-0.5	-0.3	0.2	-0.18	N/A	-0.39	0.25	-0.42	-0.46	-0.59	-0.96
NHCN ₂ CCH	H	70	0.44	0.33	0.46	0	0.21	0.51	0.32	0.17	0.09	0.29	-0.1	0.38	0.49	N/A	N/A	0.44	0.4	0.31	0.27	0.22
H	NHCN ₂ CCH	71	0.66	0.33	-0.05	0.06	-0.77	0.32	-0.67	-0.46	0.5	0.51	0.46	0.82	0.46	N/A	-0.13	0.57	0.26	-0.38	-0.39	-0.76
NHCH ₂ CN	H	72	0.53	0.38	0.46	0.1	0.24	0.57	0.37	0.17	0.38	0.36	0.02	0.4	0.49	N/A	N/A	0.49	0.45	0.35	0.31	0.3
H	NHCH ₂ CN	73	0.73	0.4	-0.14	0.08	-0.79	0.38	0.15	0.36	0.56	0.5	0.51	0.6	0.46	N/A	-0.43	0.67	0.44	0.19	-0.4	-0.72

Compound **69** stands out in the 'rigid' difference table (Table 2.7) as interacting especially well with F147 and W147. The docking poses for these interactions are shown in Figures 2.9E and 2.9F respectively. In both cases, the benzyl group of the ligand is predicted to stack against the aromatic residue at position 147, resulting in a

more favourable interaction relative to the binding pose formed between compound **69** and Q147 in the WT degnon.

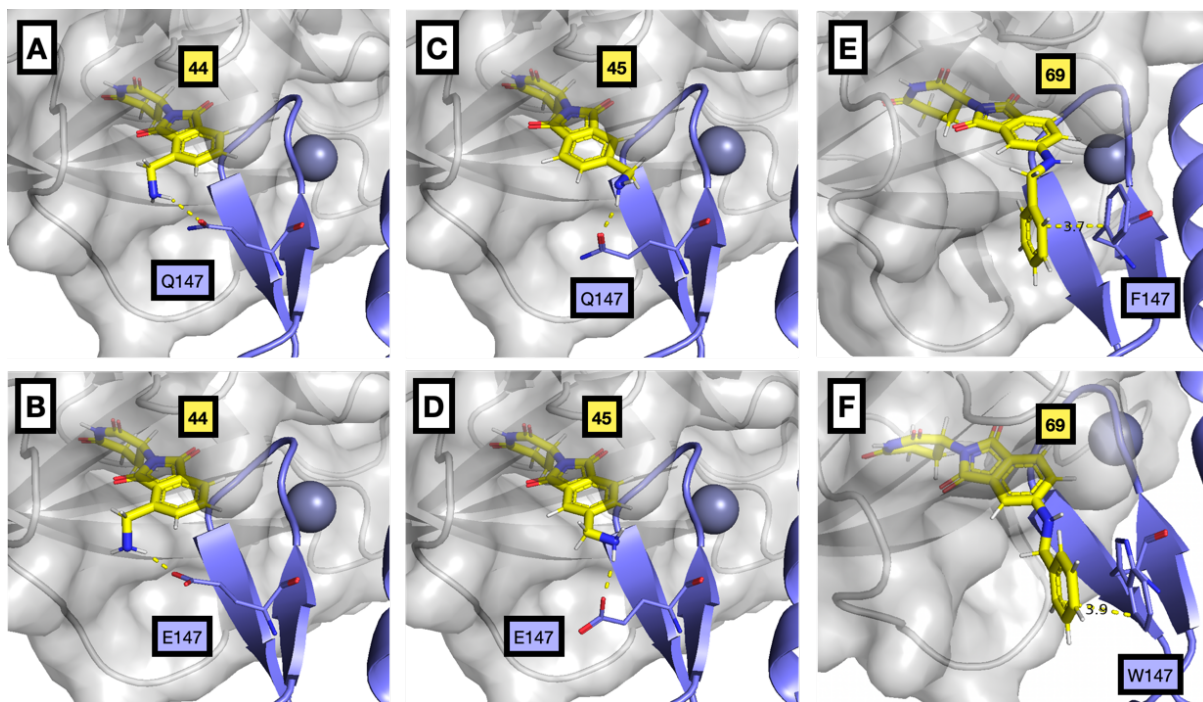


Figure 2.9. Ternary complexes generated by constrained docking in GOLD. Compounds as yellow sticks, ZF mutants in lilac with residue 147 in sticks, CRBN as grey surface. **A)** **44** with Q147, **B)** **44** with E147, **C)** **45** with Q147, **D)** **45** with E147, **E)** **69** with F147, and **F)** **69** with W147. Hydrogen bonds and ionic interactions shown as dashed lines; π -stacking interactions shown as dashed lines with distances (in Å).

The ‘flexible’ screen data in the ‘flexible’ difference table (Table 2.8) highlight three further IMiD/degnon combinations that are not as pronounced in the ‘rigid’ data table (Table 2.7). Compounds **44** and **45**, which are regioisomers, both have enhanced predicted binding energy values for E147 relative to the WT. Poses generated for these combinations can be seen in Figures 2.9A-D. In all four cases hydrogen bonding is predicted between the ligand’s ‘bump’ group amine and the side chain of the residue at position 147. It is likely that this interaction is strong between the anionic glutamate and a primary amine, which will be protonated at physiological pH, and could therefore be classed as a salt bridge rather than a hydrogen bond for E147.

In both of these cases it is possible that the ‘flexible’ docking screen has identified a ligand/side chain interaction which was only possible to detect by allowing the side chain of the residue at position 147 to change rotamers.

Another interaction which was less noticeable in the ‘rigid’ difference data (Table 2.7), but which stands out more in both the absolute predicted binding energy data (Table 2.5) and the predicted binding energy difference data (Table 2.8) for the ‘flexible’ screen is the pairing of compound **68** and W147. The pose generated can be seen in Figure 2.10. Here a predicted π -stacking interaction between the phenyl ring of the ligand and the aromatic side chain of W147 can be seen.

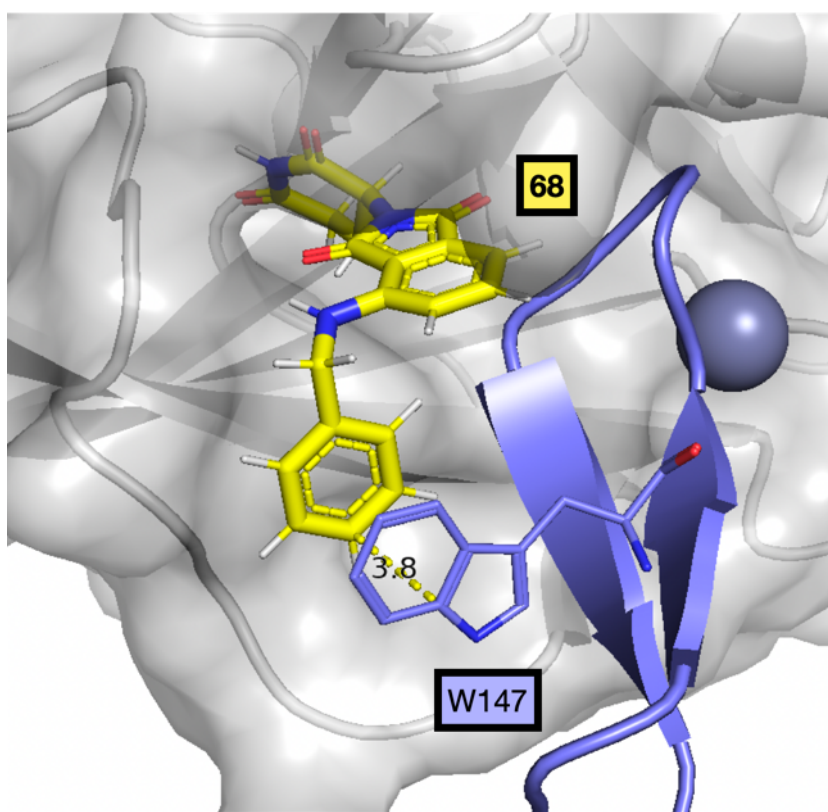


Figure 2.10. Ternary complex pose generated by constrained docking using GOLD for compound 68 (yellow) against the Q147W mutant IKZF1/3 ZF degron (lilac). Residue 147 shown as lilac sticks; π -stacking interaction shown as dashed line with distance (in Å); CRBN shown as grey surface.

The pairing of compound **68** and W147 is especially noticeable in the ‘flexible’ absolute predicted binding energy data table (Table 2.5), as it exhibits the largest predicted binding energy value of any IMiD/degron pair in either of Tables 2.4 or 2.5. Due to the high absolute value of the predicted binding energy for this pair, it was decided that this IMiD/degron pair should be explored further.

2.5 Refinement of Bumped IMiD Design

2.5.1 Compound **68** Analogue Docking Screen

It was hypothesised that a suitably modified analogue of compound **68** could interact more favourably with W147 than compound **68** itself. In order to test this hypothesis, a library of analogues of compound **68** was designed (Table 2.9)

Table 2.9. A library of 12 variations on compound **68** and three controls (**1**, **3**, **68**) which were screened against the WT and Q147W ZF degrons using constrained docking in GOLD.

A						B			
Compound	R ⁴	Compound	R ⁴	Compound	R ⁴		Q147	Q147W	Difference
1		76		81		1	-11.03	-11	0.03
3		77		82		3	-11.31	-11.05	0.26
68		78		83		68	-11.54	-13.98	-2.44
74		79		84		74	-11.84	-14.74	-2.9
75		80		85		75	-13.31	-14.29	-0.98
						76	-10.75	-11.73	-0.98
						77	-11.9	-11.21	0.69
						78	-13.24	-13.53	-0.29
						79	-12.37	-12.34	0.03
						80	-11.59	-11.21	0.38
						81	-12.91	-13.76	-0.85
						82	-12.29	N/A	N/A
						83	-11.31	-12.97	-1.66
						84	-10.81	-13.95	-3.14
						85	-12.73	-12.79	-0.06

The results shown in Table 2.9 indicate that either of two modifications to compound **68** – an *ortho*-methyl group (**74**) or a *meta*-pyridine nitrogen (**84**) – give greater favourable differences in predicted binding energy than compound **68** for W147 compared to the WT. The poses generated can be seen in Figure 2.11.

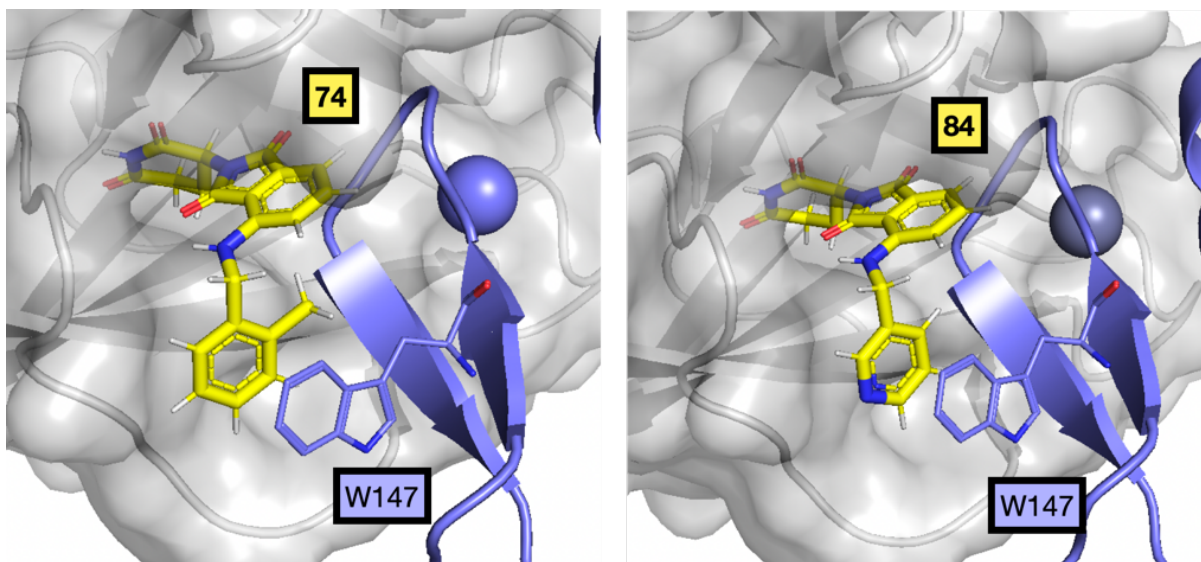


Figure 2.11. Ternary complex pose generated by constrained docking using GOLD for compounds **74** (A) and **84** (B) against the Q147W mutant IKZF1/34 ZF degron (lilac). Residue 147 shown as lilac sticks; CRBN shown as grey surface.

2.5.2 Design of Compounds **86** and **87**

Due to the flexible nature of the benzylamino group used in compound **68** and its analogues, it was unclear whether this group would adopt the conformation required for the predicted π -stacking interaction with W147. To explore this further, another round of constrained docking was performed on compound **68**, using just the CRBN region as the protein receptor.

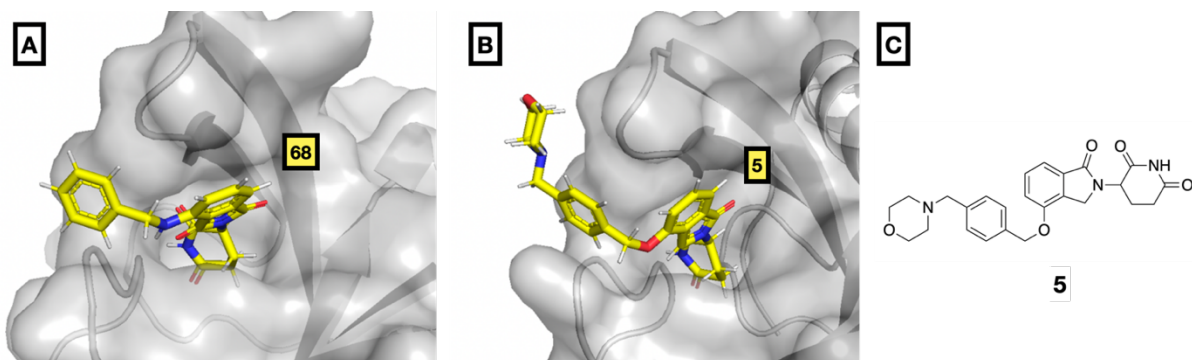


Figure 2.12. **A)** The docking pose generated for compound **68** (yellow sticks) in CRBN (grey surface) only; **B)** Crystal structure of compound **5** (yellow sticks) bound to CRBN (grey surface) (*PDB ID: 5V3O*). **C)** Compound **5** (CC-220, also known as iberdomide), a highly potent IMiD.

The result of this docking (Figure 2.12A) shows that the aminobenzyl group can also occupy a cleft in the CRBN surface, in a manner analogous to that of compound **5** – also known as iberdomide or CC-220²⁰ – bound to CRBN (*PDB ID: 5V3O*). This result suggests that the aminobenzyl group in compound **68** might be unavailable to interact with W147 in the mutant degron, and therefore also unable to provide a steric clash with Q147 in the WT degron.

To address these concerns, two new IMiD analogue designs were proposed (**88** and **87**), each containing a benzhydryl ‘bump’ group. This group was predicted to occupy both the CRBN surface cleft and simultaneously interact with W147. Constrained docking showed that such a pose could be achieved (Figure 2.13).

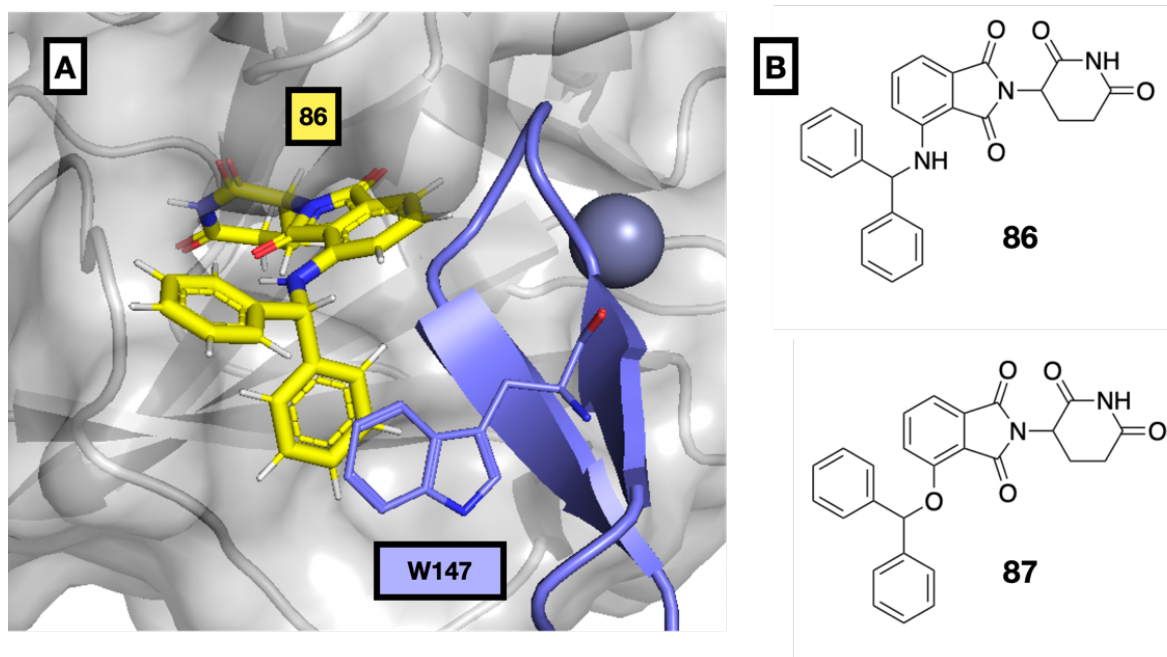


Figure 2.13. A) Ternary complex pose generated by constrained docking using GOLD for compound **86** (yellow sticks) against the Q147W mutant IKZF1/3 ZF degron (lilac). This pose suggests that the π -stacking interaction is maintained while also filling out the external CRBN cleft. Residue 147 shown as lilac sticks; CRBN shown as grey surface. **B)** Chemical structures of compounds **86** and **87**.

2.6 IMiD Library for Chemical Synthesis

Of the IMiD design strategies highlighted by the docking screen, two strategies were chosen to explore further.

2.6.1 Refined IMiD Library Design Strategy 1

As trends observed in Table 2.6 suggested that addition of a 'bump' group at the 5 position of the IMiD may be more effective at providing a steric clash with Q147 in the WT ZF than the equivalent 'bump' groups placed at the 4 position, the decision was made to synthesise a set of IMiD analogues with a variety of 'bump' groups at the 5 position. Superposition of an example 5-'bumped' IMiD (**88**), docked into CRBN, over the crystal structure conformation of the WT ZF shows this clash clearly (Figure 2.14).

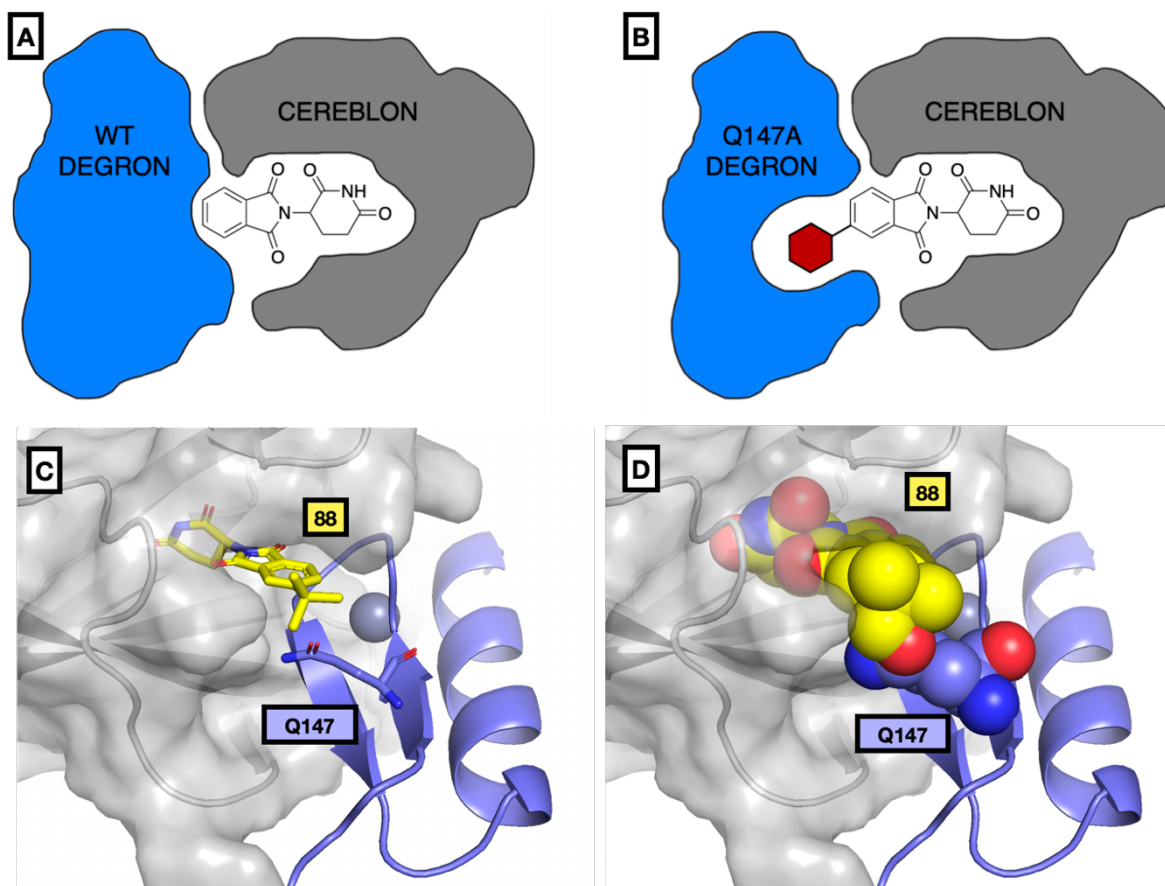


Figure 2.14. **A)** Cartoon representation of the ternary complex for CRBN (grey), thalidomide and the WT ZF degron (blue). **B)** Cartoon representation of the ternary complex for CRBN (grey), a 5-'bumped' IMiD analogue, and the Q147A degron (blue). **C)** Addition of a tertiary butyl 'bump' group to the 5-position of a thalidomide scaffold (compound **88**, yellow sticks) using the 'builder' functionality in PyMOL shows such a group would clash significantly with Q147 (lilac sticks) in the WT ZF degron (lilac cartoon). CRBN in grey. **D)** As previous but with compound **88** and Q147 represented as spheres.

2.6.2 Refined IMiD Library Design Strategy 2

As IMiD designs derived from compound **68** were predicted to interact favourably with A Q147W mutant ZF degron, the decision was made to synthesise a variety of analogues of compound **68**.

2.6.3 Final IMiD Analog Library Design

A library of 36 compounds was chosen for chemical synthesis (Figure 2.15).

Chapter 2: Design and Synthesis of IMiD Analogues

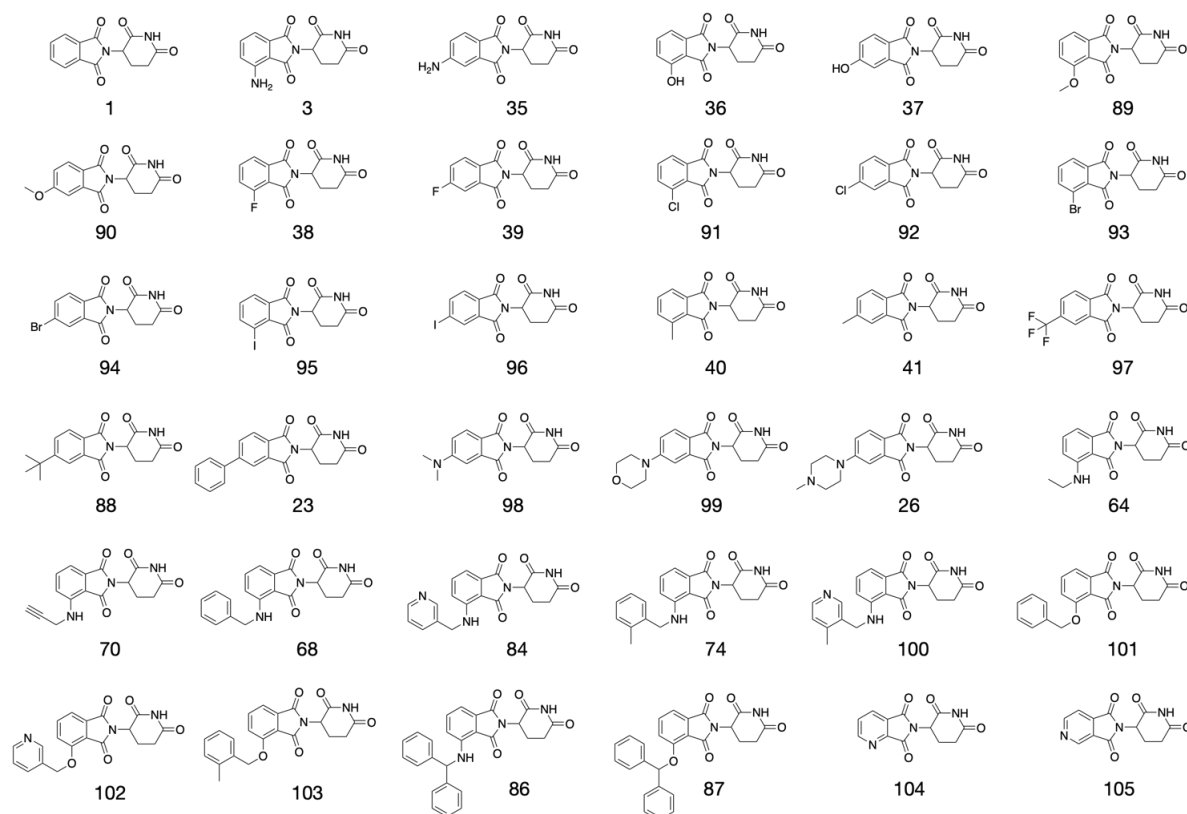


Figure 2.15. Full compound library of 36 IMiD analogues for biological testing.

Compounds **23**, **26**, **35**, **37**, **39**, **41**, **88**, **90**, **92**, **94**, **96**, **97**, **98** and **99** arose from design strategy 1.

Compounds **68**, **74**, **84**, **86**, **87**, **100**, **101**, **102** and **103** arose from design strategy 2.

As well as compound designs derived from the docking results, a variety of control compounds were included.

Compounds **64** and **70** were included because they are structural analogues of compound **68**, but with neither containing a phenyl ring as part of their 'bump' group.

Compounds **3** (pomalidomide), **36**, **38**, **40**, **89**, **91**, **93** and **95** were included because they are regioisomers of compounds **35**, **37**, **39**, **41**, **90**, **92**, **94** and **96** respectively, but with the 'bump' groups on the 4 position instead of the 5 position.

Thalidomide (**1**) and compounds **104** and **105** were also included as control compounds with no 'bump' groups.

2.7 Conclusions

The IMiD compound library design arrived upon through the work described in this chapter covers two distinct strategies for identification of 'bumped' IMiD analogues, as well as a variety of control compounds. Through testing of all 36 compounds in this library, not only can promising 'bump and hole' candidate molecules be identified, but some overall trends in the structure activity relationship (SAR) of IMiD analogues can also be explored. Chapter 3 will discuss the chemical synthesis of these IMiD analogues and the subsequent testing carried out to assess their biological activity.

Chapter 3: IMiD Synthesis and Degradation Screen

3.1 Introduction and Aims

Chapter 2 summarised the design of 36 IMiD analogues for use in biological testing (Figure 3.1). This chapter will describe the synthesis of the IMiD analogues, the methodology used to assess the ability of compounds to induce ZF degradation, and the trends seen in the results. Comparison of the ability of these compounds to degrade both the WT ZF and a 'hole'-containing Q147A mutant ZF was assessed using a flow cytometry assay to identify 'bump and hole' activity within the compound library. The data gathered from these initial tests highlighted several candidate molecules, including two clear 'hit' compounds, four of which were taken forward for further testing in Chapter 4.

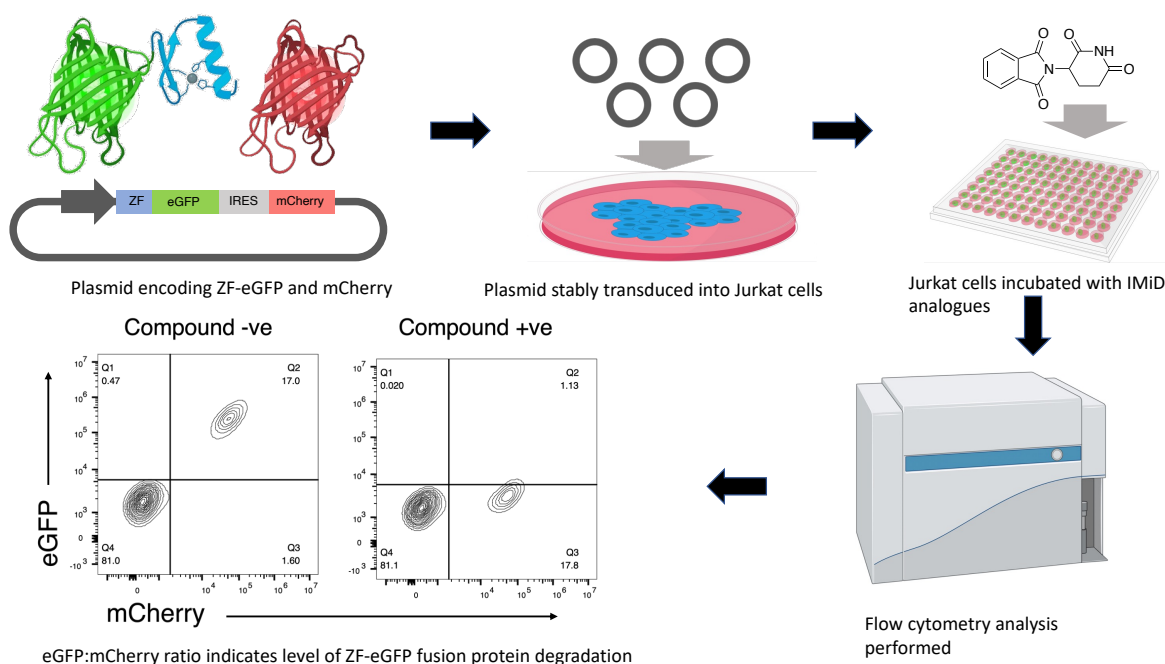
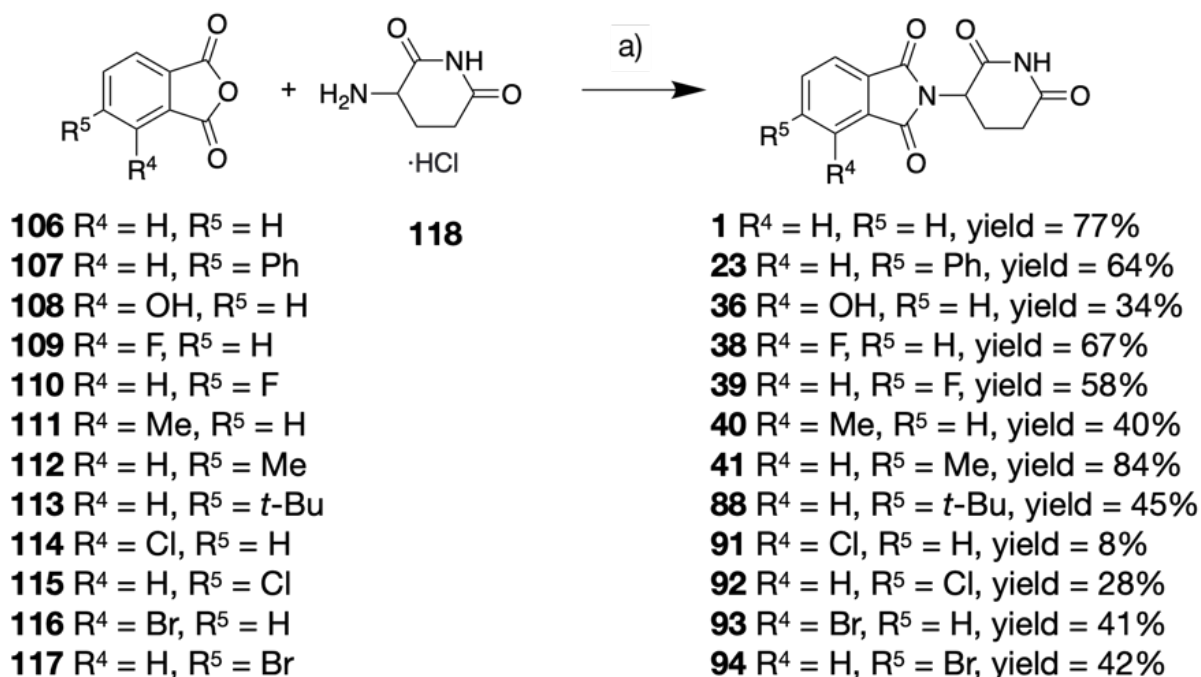


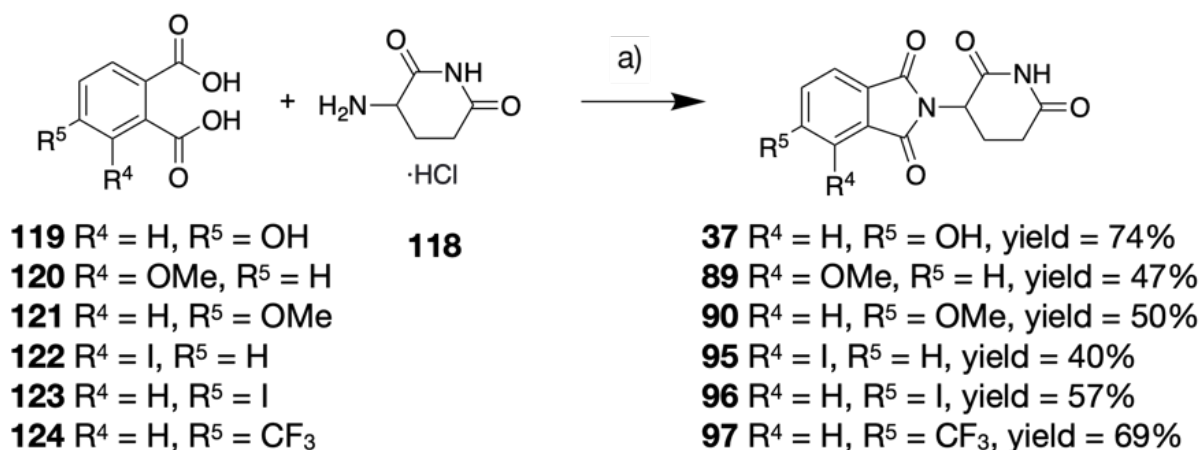
Figure 3.1 Full workflow for compound screening: a plasmid coding for a ZF-eGFP fusion protein is stably transduced into Jurkat cells; the cells are incubated for 18 h with an IMiD concentration curve; the cells are analysed by flow cytometry; the eGFP/mCherry median fluorescence intensity (MFI) ratio is used to determine the level of ZF degradation.

3.2 Chemical Synthesis of IMiD Analogues

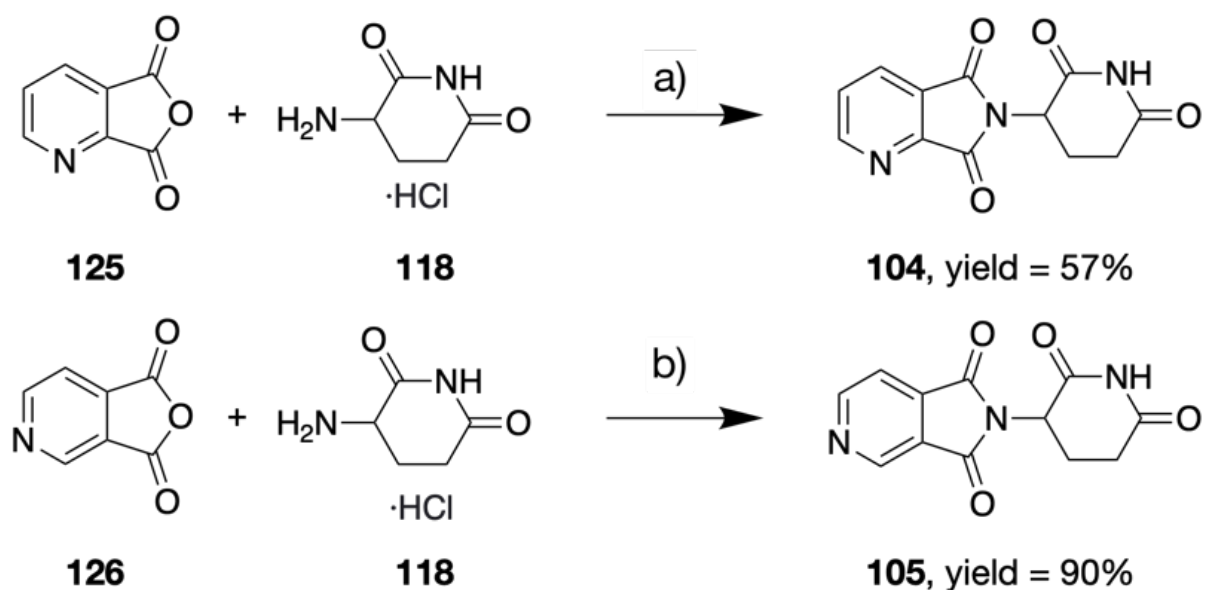
The synthesis of 20 of the 36 IMiD analogues (compounds **1**, **23**, **36**, **37**, **38**, **39**, **40**, **41**, **88**, **89**, **90**, **91**, **92**, **93**, **94**, **95**, **96**, **97**, **104** and **105**) was completed using a one-step condensation reaction between 3-aminopiperidine-2,6-dione hydrochloride (**118**) and a suitably substituted phthalic anhydride or phthalic acid (Schemes 3.1, 3.2 and 3.3). these reactions, completed using acetic acid and sodium acetate, resulted in yields ranging from 28%-90%. In all cases purity was sought over quantity.



Scheme 3.1. Synthesis of IMiD analogues **1**,⁶¹ **23**,⁶² **36**,⁶³ **38**,⁶⁴ **39**,²⁸ **40**,⁶⁵ **41**,⁶⁵ **88**,⁶⁵ **91**,⁶⁶ **92**,⁶⁷ **93**⁶⁸ and **94**.⁶⁵ *Reagents and conditions:* a) NaOAc, AcOH, 120 °C, 17 h.

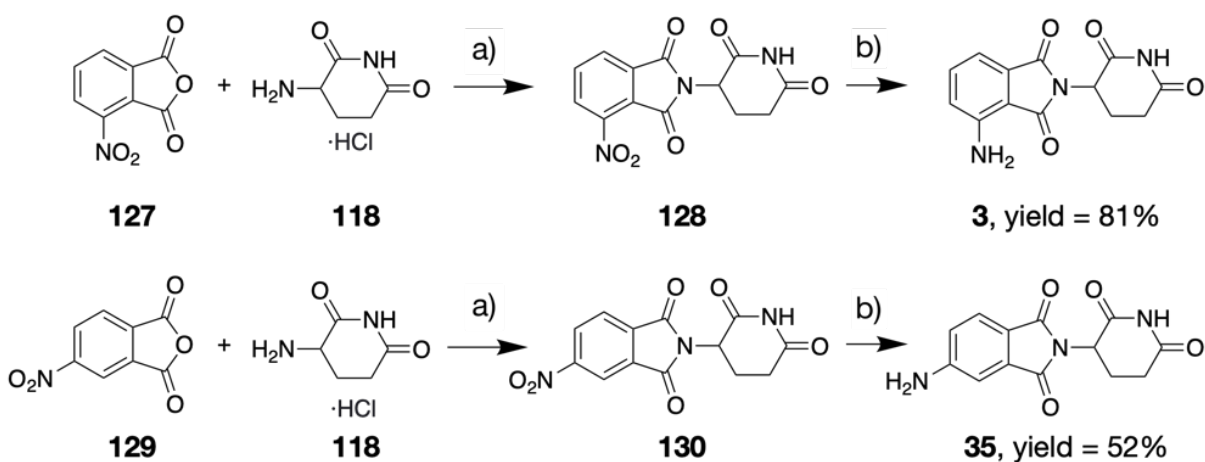


Scheme 3.2. Synthesis of IMiD analogues **37**,⁶⁵ **89**,⁶⁶ **90**,⁶⁶ **95**,⁶⁹ **96** and **97**. *Reagents and conditions:* a) NaOAc, AcOH, 120 °C, 17 h.



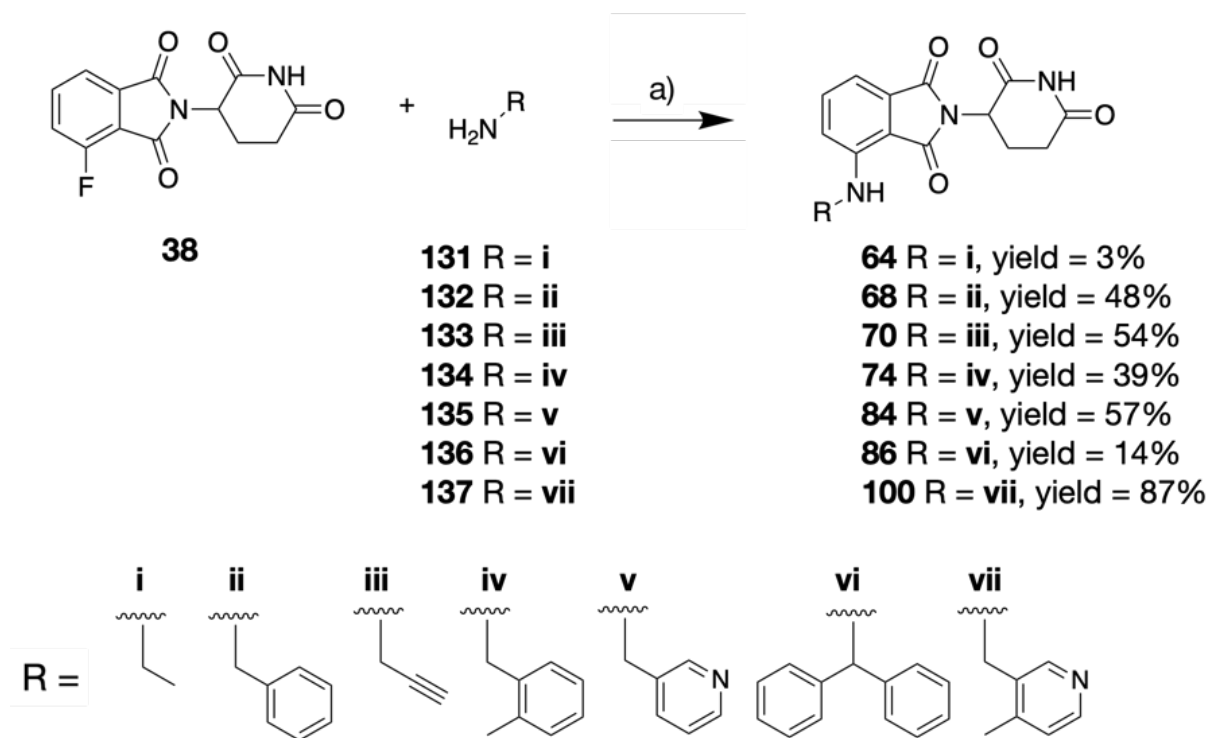
Scheme 3.3. Synthesis of IMiD analogues **104**⁶⁵ and **105**.⁶⁵ *Reagents and conditions:* a) NaOAc, AcOH, 120 °C, 17 h.

For the synthesis of the remaining IMiD analogues in the library, further steps were required. Pomalidomide (**3**) and its regioisomer compound **35** were synthesised using palladium-catalysed hydrogenation of the corresponding nitrothalidomide (Scheme 3.4), giving yields of 81% and 52%, respectively.

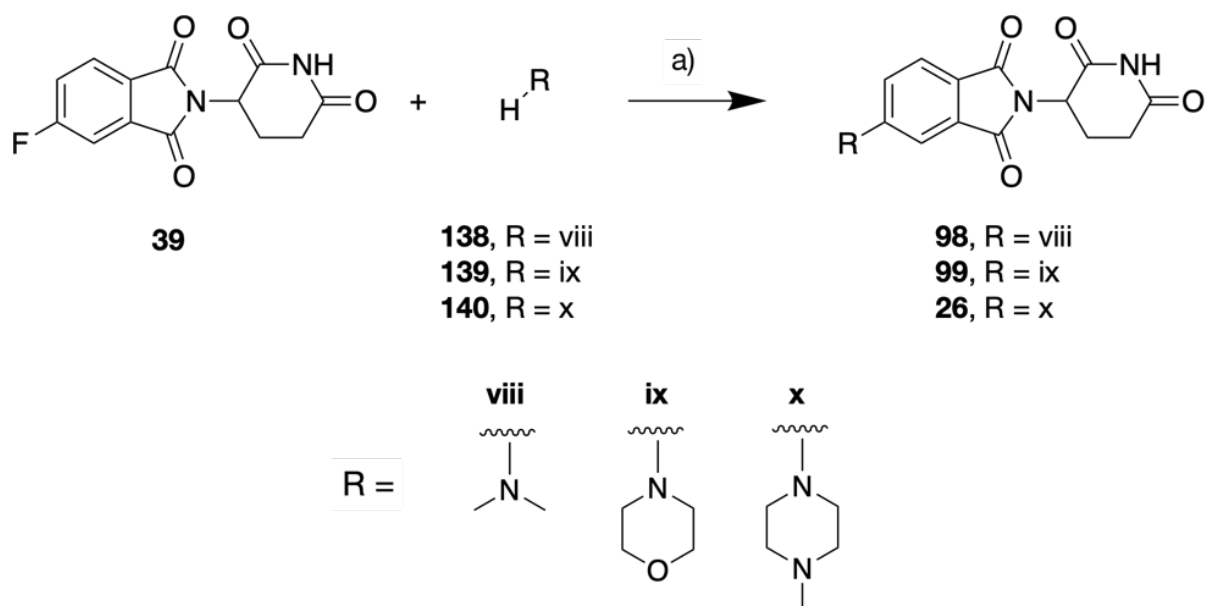


Scheme 3.4. Synthesis of pomalidomide (**3**)⁷⁰ and its regioisomer (**35**)⁷¹. *Reagents and conditions:* a) NaOAc, AcOH, 120 °C, 17 h; b) Pd/C (10%), DMF, H₂ (1 atm), rt, 16 h.

For IMiD analogues possessing an aniline substituent at either the 4 or 5 position (compounds **26**, **64**, **68**, **70**, **74**, **84**, **86**, **98**, **99** and **100**), an aromatic nucleophilic substitution (S_NAr) reaction was carried out on the appropriate regioisomer of fluorothalidomide using the appropriate amine (Schemes 3.5 and 3.6). N-methyl-2-pyrrolidone (NMP) was used instead of dimethylformamide (DMF), as DMF can degrade to form dimethylamine at the elevated temperature used. This can react with the fluorothalidomide in place of the desired amine. Yields ranged from 3%-88%, with yield mainly being determined by how easily separable the product was from any remaining fluorothalidomide starting material.



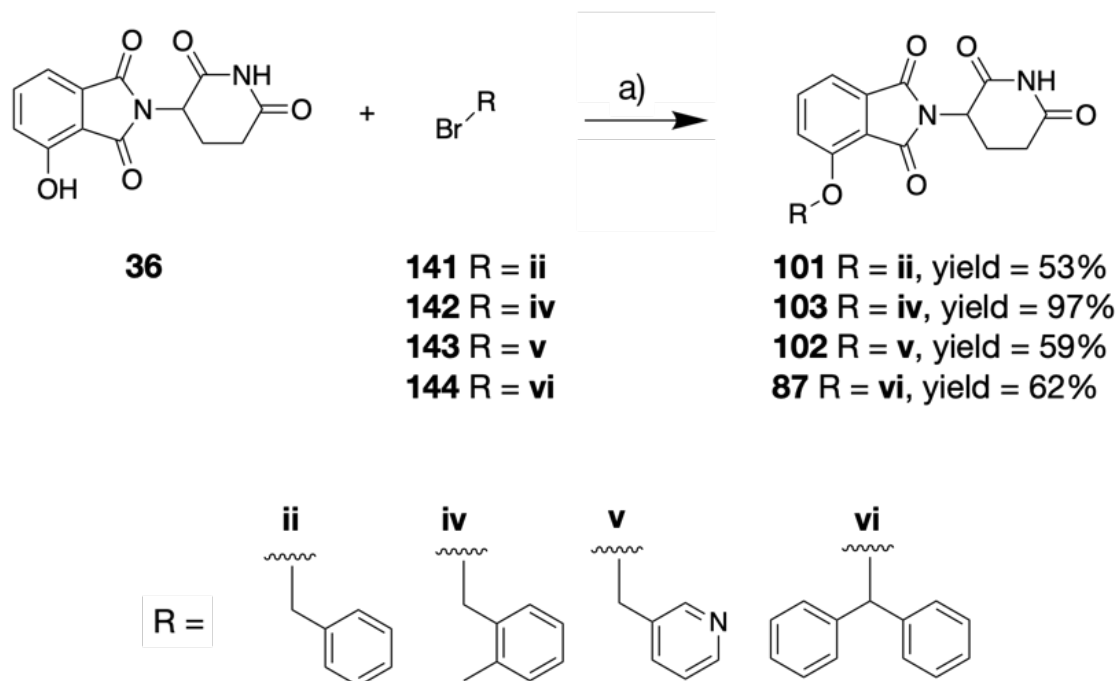
Scheme 3.5. Synthesis of IMiD analogues **64**,⁷² **68**,⁷³ **70**,⁷⁴ **74**, **84**, **86** and **100**. *Reagents and conditions:* a) DIPEA, 110 °C, 2 h.



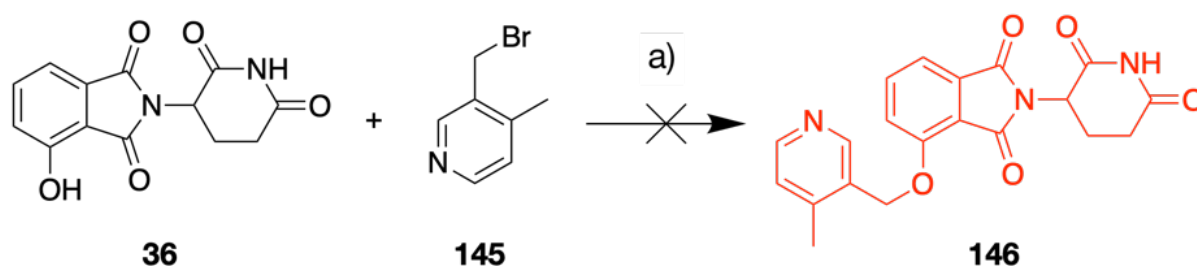
Scheme 3.6. Synthesis of IMiD analogues **98**,⁷² **99** and **26**. *Reagents and conditions:* a) DIPEA, 110 °C, 2 h.

Four IMiD analogues with phenolic ether substituents (compounds **86**, **101**, **102** and **103**) were obtained using an alkylation involving 4-hydroxythalidomide and an appropriate benzyl bromide (Scheme 3.7). This approach was only attempted using benzyl bromides and not with alkyl bromides, due to the enhanced reactivity of the former. Synthesis of compound **146** was attempted but the reaction did not occur (Scheme 3.8). The reason why is unclear, as its analogues (**101**, **102** and **103**) were synthesised.

NMR Assignments for all compounds (see chapter 7) for which definitive assignments were not possible from spectral inspection alone were obtained via analogy to literature assignment.



Scheme 3.7. Synthesis of IMiD analogues **101**,⁷⁵ **103**, **102** and **87**. *Reagents and conditions:* a) K_2CO_3 , DMF, rt, 1.5 h.



Scheme 3.8. Attempted synthesis of compound **146**. *Reagents and conditions:* a) K_2CO_3 , DMF, rt, 1.5 h.

3.3 Plasmid Design

3.3.1 Protocol Overview

Having synthesised the IMiD analogues described above, we required an efficient method to assess their ability to induce degradation of the WT degron and a 'hole'-containing Q147A mutant. Previous studies probing the degradation capabilities of

IMiDs on ZF degrons have utilised a ratiometric fluorescence-based assay,² where a ZF degron is fused to enhanced green fluorescent protein (eGFP) and expressed alongside mCherry. These two fluorescent proteins possess excitation and emission spectra that do not significantly overlap, allowing fluorescence levels to be assessed independently.

In this method, the ZF-eGFP fusion protein gene is encoded on the same gene as mCherry, with an internal ribosomal entry site (IRES) inserted between the two sequences. This means that the two fluorescent proteins can be translated from the same piece of messenger RNA (mRNA), but the translation products exist as separate proteins in the cell. Consequently there is a consistent ratio of eGFP:mCherry across the cell population. Expressing these two proteins using different genes would not guarantee this consistent ratio, as a change in environmental conditions could cause the expression levels of the two genes to differ. A consistent protein ratio is advantageous for studying degradation dynamics as the data gathered display lower levels of background noise, meaning small differences in pDC_{50} can be reliably observed.

DC_{50} is defined as the concentration of an IMiD required to induce degradation of 50% of the ZF-eGFP fusion protein, and pDC_{50} is defined in Equation 3.1. It must also be noted that 50% degradation refers to halfway between 0% (no degradation) and D_{MAX} – i.e. the minimum level of protein achievable by that compound. Therefore pDC_{50} value depends on the percentage degradation at which the curve plateaus. Not all IMiD analogues degrade the ZF-eGFP fusion protein 100%.

Equation 3.1: $pDC_{50} = -\log_{10}(DC_{50})$

Upon addition of an IMiD to cells expressing this plasmid, the ZF-eGFP fusion protein is polyubiquitinated and subsequently degraded *via* the proteasome (Figure 3.2). After an 18 hour incubation period, cells are analysed using flow cytometry to obtain an eGFP:mCherry ratio. This ratio is then compared against the eGFP:mCherry ratio obtained from untreated cells (DMSO control). This normalisation ensures that readings for different compounds and different mutant degrons are directly comparable.

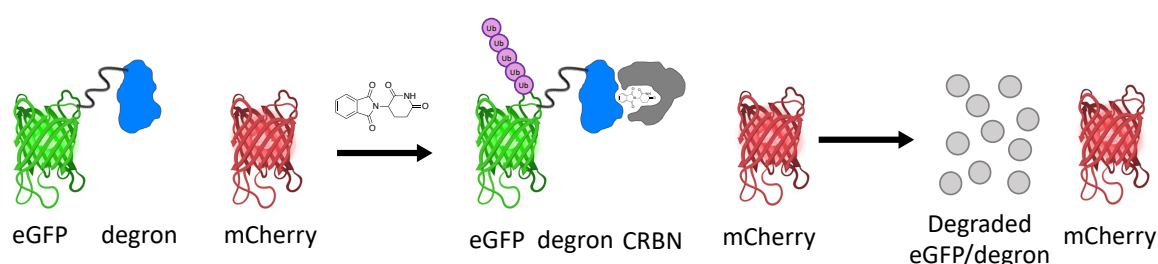


Figure 3.2. ZF-eGFP fusion protein and mCherry are expressed at a consistent ratio; addition of IMiD leads to ZF-eGFP polyubiquitination and subsequent degradation. The eGFP/mCherry ratio is used to determine the degrader efficacy.

This system can be introduced into cells *via* a plasmid construct, allowing for the degradation activity of IMiDs to be assessed in living cells.

3.3.2 Artichoke and Sprout Plasmids

The system described above can be implemented using a commercially available plasmid called Artichoke (addgene #73320)². This plasmid is an empty backbone that can be amplified in bacteria and transduced into human cells lentivirally (see section 3.3.1). There is a restriction site upstream of the eGFP gene that is recognised by the endonuclease enzyme BsmBI. Golden Gate assembly⁷⁶ can be used to clone the sequence for any desired degron into this site by insertion of a gBlock (a double stranded DNA fragment 125-3000 base pairs in length) (Figure 3.3). A version of

Artichoke into which the WT IKZF1/3 ZF degnon is already inserted is also commercially available (Addgene #74451)².

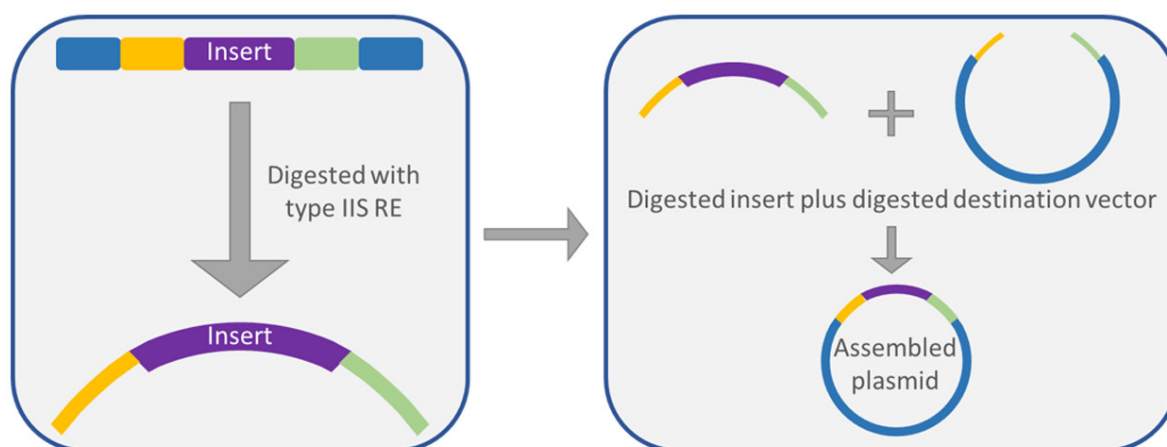


Figure 3.3. Golden Gate assembly is a scarless insertion technique suitable for addition of a DNA fragment mid-gene. Yellow and green regions in the destination vector (blue) are cut sites for BsmBI endonuclease, allowing integration of an insert (purple) with complementary cut-site regions. Adapted from Bird *et al.* with permission.⁷⁷

For this study, a similar plasmid called Sprout was developed (Figure 3.4). Sprout functions identically to Artichoke, except that, for the empty backbone, the region immediately upstream of eGFP is not empty; instead it encodes residues 130-143 of the WT IKZF1/3 degnon, followed by a BsmBI restriction site, followed by residues 171-189 of the WT IKZF1/3 degnon. This means that a shorter DNA gBlock – covering residues 144-170 of the IKZF1/3 degnon – can be used to insert any desired mutations within this residue 144-170 region into the ZF degnon. The use of a smaller gBlock allows costs to be significantly reduced when screening a large number of ZF mutants – this measure is necessary for the protocol discussed in chapter 5.

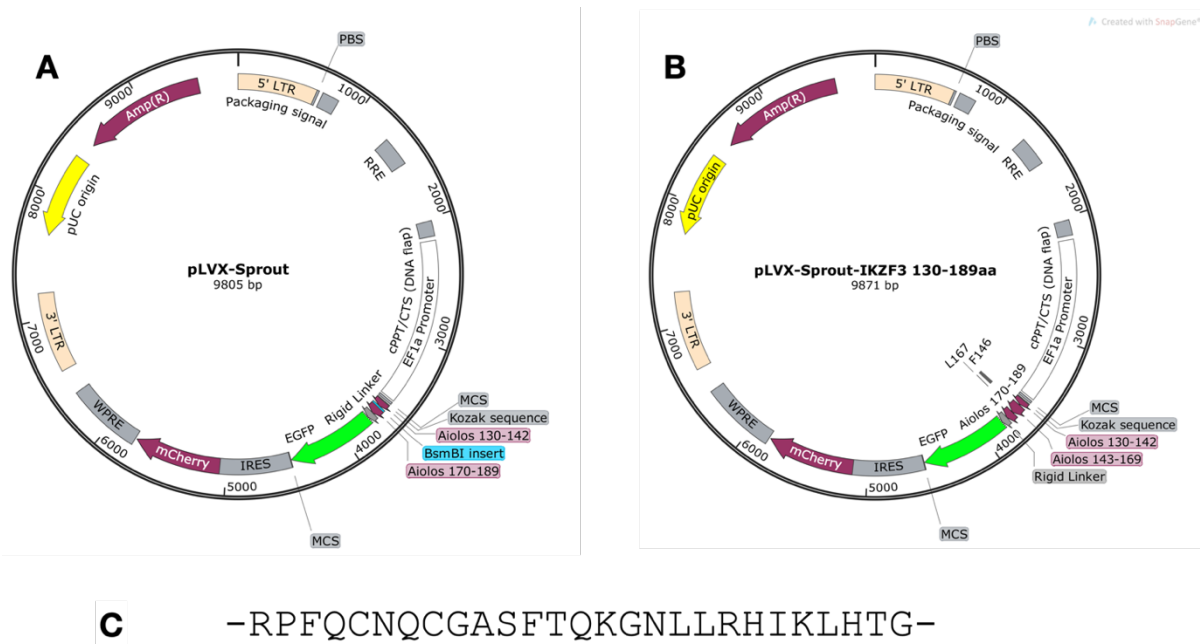


Figure 3.4. **A)** Empty Sprout plasmid with BsmBI restriction site. **B)** Sprout plasmid after insertion of gBlock DNA. **C)** WT gBlock sequence (residues 144-170).

3.4 Transduction

Two common methods are often used when introducing new DNA into a human cell line: transient transfection (e.g. *via* electroporation or using a chemical reagent such as lipofectamine), and stable transduction (e.g. by using a lentiviral construct).⁷⁸

Transient transfection is quicker and easier to carry out, but does not lead to the DNA being incorporated into the genome of the cell – therefore any expression of the gene products is temporary, and the DNA is lost after a few passages of the cell culture. Stable transduction is a longer process, which must be carried out in a biosafety level 2 (BSL2) laboratory, but as the DNA is incorporated into the genome of the cell, this results in a cell line that stably expresses the gene for as many generations as the cells are viable (Figure 3.5).

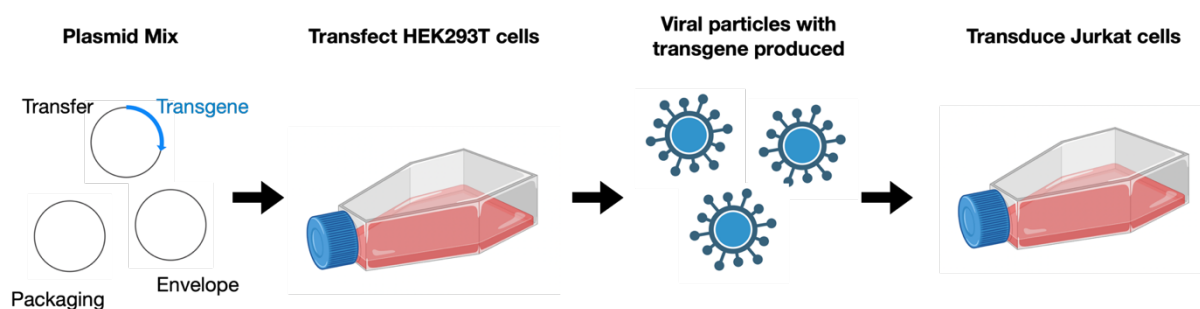


Figure 3.5 Jurkat cell transduction *via* lentiviral construct to create stably transduced cell lines.

The decision was made to use lentiviral transduction for all assays involving the Sprout plasmid, owing to the longevity of the cell lines generated. Having identified a suitable method for introduction of the plasmid into our chosen cell line (Jurkat cells)⁷⁹, assessment of the degradation abilities of the compounds in the IMiD analogue library could be undertaken.

3.5 Compound Incubation and Flow Cytometry

3.5.1 Assay Overview

To determine which compounds out of the library of 36 would be most suitable for 'bump and hole' development, all 36 were screened against both the WT degran and a Q147A mutant. The Q147A mutation was chosen as it was perceived to be the simplest option for pursuing the 'traditional bump and hole' approach. It was hypothesised that any IMiD analogues with a sterically bulky 'bump' group that degraded the Q147A mutant more effectively than the WT degran would be showing the 'bump and hole' effect, and would therefore be suitable for further testing.

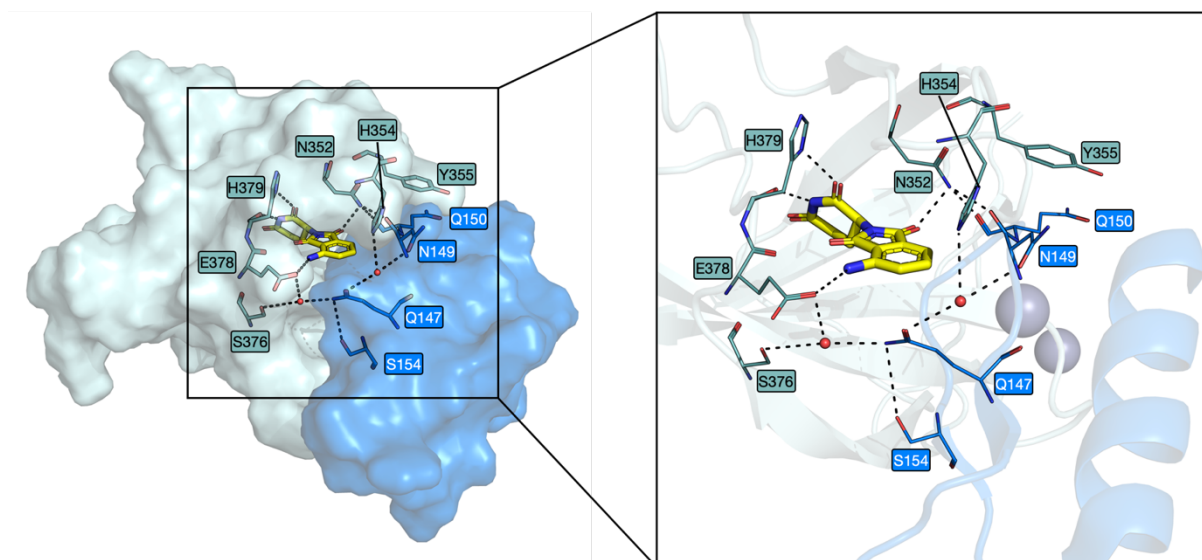


Figure 3.6. Residues on the IKZF1/3 ZF degnon proximal to pomalidomide (*PDB ID: 6H0F*).

3.5.2 Compound Incubation and Flow Cytometry

To obtain a DC_{50} value for both the WT and Q147A mutant degnons for all 36 compounds, concentration curves were acquired *via* the following method: Jurkat cells that were stably transduced with either the WT or Q147A Sprout plasmid were seeded into 96 well plates, along with one of the following concentrations of an IMiD analogue: 1 nM, 10 nM, 100 nM, 1 μ M, or 10 μ M. Six wells of untreated cells were also incubated to provide values for normalisation. All wells contained 0.1% dimethyl sulfoxide (DMSO). All concentration curves were acquired in triplicate.

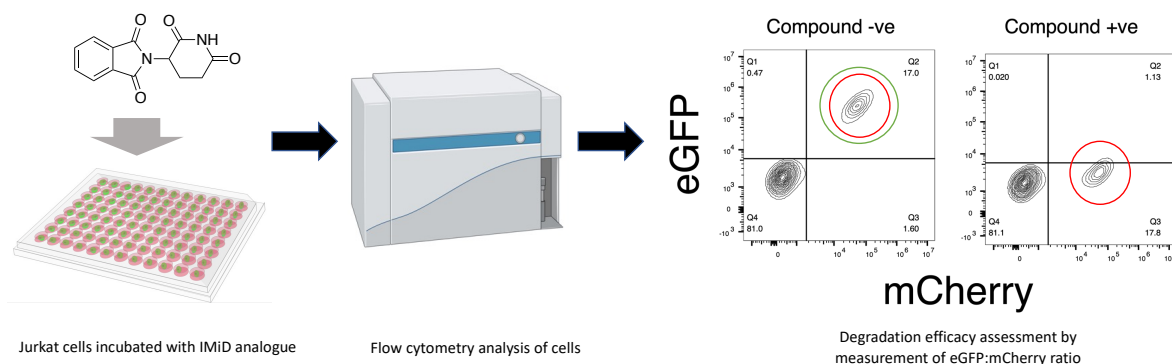


Figure 3.7. Jurkat cell incubation with concentration curve of IMiD analogue for 18 h. Flow cytometry analysis yields an eGFP:mCherry ratio, which determines degradation efficacy. Low eGFP fluorescence while mCherry fluorescence remains high is indicative of effective ZF degradation.

After 18 hours of incubation each well of cells was resuspended in a buffer comprising 10% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), treated with 4',6-diamidino-2-phenylindole (DAPI) – a dye used to detect dead cells – and analysed using flow cytometry.

3.5.3 FlowJo Analysis

Once the raw data for each well was gathered, it could be processed to provide a ratio of eGFP:mCherry. This was achieved using the flow cytometry data analysis software FlowJo.⁸⁰

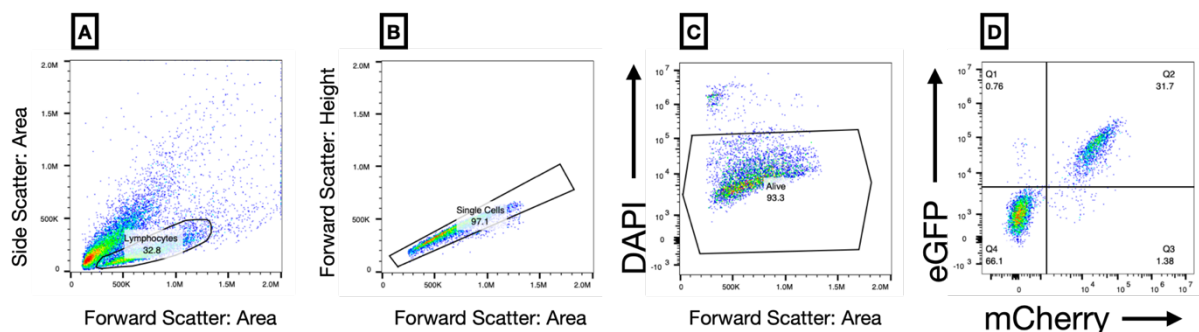


Figure 3.8. Processing flow cytometry data by successive gating. **A)** cells gated from all events. **B)** Single cells gated from all cells. **C)** Alive cells gated from all single cells. **D)** mCherry positive cells gated from all alive, single cells.

Figure 3.8 shows the workflow for how gates are sequentially applied to the raw data from each well to obtain an eGFP:mCherry fluorescence ratio. The gating protocol is summarised below:

1. Cells are gated out from all events. The circled population (labelled 'lymphocytes') are cells, while the population to the left are smaller entities, such as dust, debris and cell fragments. The identity of each event is determined by measuring its dimensions *via* forward and side-scattered light.
2. Single cells are gated out from the cells by measuring the dimensions of the events. The gated population (labelled 'single cells') are individual cells, and any events to the bottom right are cell clumps. Signals obtained from cell clumps are not representative of the data we wish to acquire, as the signal intensities are larger than those of single cells by a factor of how many cells are in the analysed clump.
3. Alive cells are gated out from the single cells. The circled population (labelled 'Alive') are living cells, whereas the events above this population are cells that have taken up DAPI, indicating that they are dead. Signals obtained from dead cells are not useful for this assay.
4. Separation of cells based on eGFP and mCherry fluorescence. The bottom left population of cells, in this case making up roughly two thirds of the 'alive, single cells' population that has been gated, represents all untransduced cells, which exhibit neither eGFP nor mCherry fluorescence. The population to the top right, which makes up most of the remaining third of the 'alive, single cells' population, represents all cells that have been transduced with the Sprout plasmid.

The level of eGFP degradation can be qualitatively gauged by observing how far the top right population has moved down the y-axis compared to the DMSO control. The plot in Figure 3.8 is taken from a DMSO control well, which explains why the population is towards the top of the y-axis.

A more accurate way of determining the eGFP:mCherry ratio is to gate all mCherry positive cells (i.e. all events to the right of the vertical line in Figure 3.8D) and calculate the median fluorescence intensity (MFI) for both eGFP and mCherry. The final value is obtained using the following formula:

$$\text{Equation 3.1: } \textit{Degradation Value} = \frac{(eGFP \text{ MFI}/mCherry \text{ MFI})_{\text{compound}}}{(eGFP \text{ MFI}/mCherry \text{ MFI})_{DMSO}}$$

Where a value of 1 indicates no degradation and a value of 0 indicates full degradation. Once values are obtained for all concentrations of a compound, a curve can be fitted and DC₅₀ and D_{MAX} values can be determined.

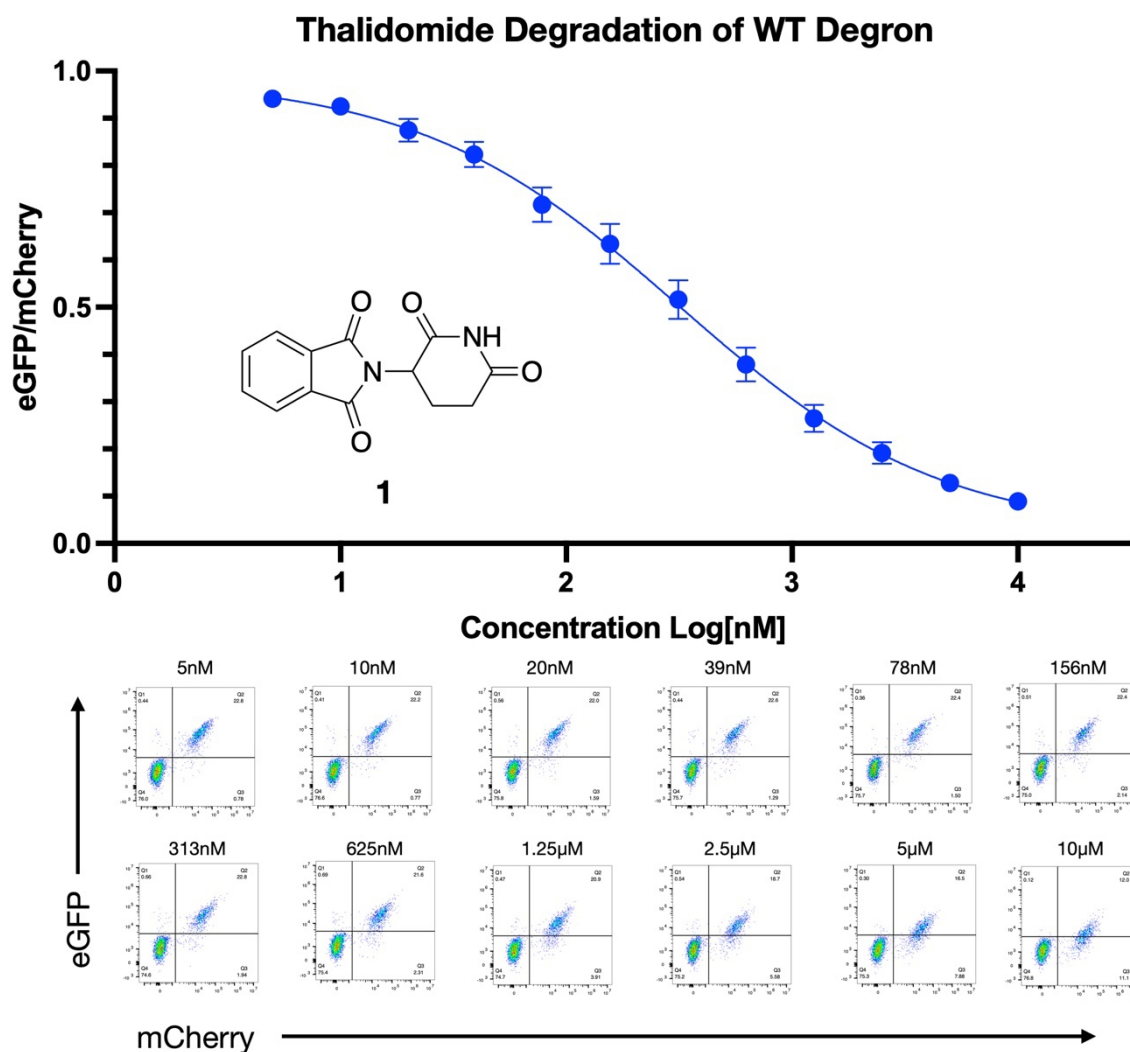


Figure 3.9. A) Degradation curve for thalidomide (**1**) against WT ZF degron; eGFP:mCherry ratio normalised against untreated DMSO control to gain value between 0 and 1 (y-axis). **B)** Flow cytometry plots for each concentration of thalidomide used showing eGFP fluorescence on y-axis and mCherry fluorescence on x-axis. Population is observed to move down the y-axis as thalidomide concentration is increased.

Figure 3.9 shows the generation of a degradation curve for thalidomide from the raw eGFP/mCherry flow plots. This curve was obtained from a larger number of compound concentrations (5 nM, 10 nM, 20 nM, 39 nM, 78 nM, 156 nM, 312 nM, 625 nM, 1.25 µM, 2.5 µM, 5 µM and 10 µM), like those obtained in Chapter 5 – it is included in this chapter for illustrative purposes.

3.5.4 Selectivity Value

Once a pDC_{50} value has been obtained for both the WT and Q147A degrons for a certain IMiD analogue, a value comparing a compound's performance for both degrons can be acquired. This value – known as a selectivity value – indicates how much more effective an IMiD analogue is at degrading the 'hole'-containing Q147A mutant vs the WT degron. A positive value indicates that a compound degrades Q147A more effectively than the WT. The selectivity value is obtained *via* the following formula:

$$\text{Equation 3.3: } \textit{Selectivity Value} = 100 * \frac{pDC_{50Q147A}}{pDC_{50WT}}$$

The compounds with the highest selectivity values were interpreted as being the most promising for 'bump and hole' interaction. This will be discussed further in section 3.6.2.

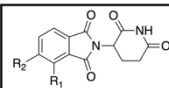
3.6 Results of Degradation Screens

3.6.1 WT and Q147A Degron Screens

3.6.1.1 Overview of Results

Table 3.1 shows the 'bump' groups present on either the 4 or 5 position of each IMiD analogue used in the compound library. The compound library contains thalidomide (**1**) and pomalidomide (**3**), against which the degradation capabilities of the other IMiDs are compared.

Table 3.1. Summary of all IMiD analogues synthesised for this project.

											
Compound	R ₁	R ₂	Compound	R ₁	R ₂	Compound	R ₁	R ₂	Compound	R ₁	R ₂
1			92			98			102		
3			93			99			103		
35			94			26			86		
36			95			64			87		
37			96			70			104		
89			40			68			105		
90			41			84					
38			97			74					
39			88			100					
91			23			101					

pDC₅₀ values for all 36 library compounds are shown for the WT screen and Q147A in Figure 3.10 and Table 3.2.

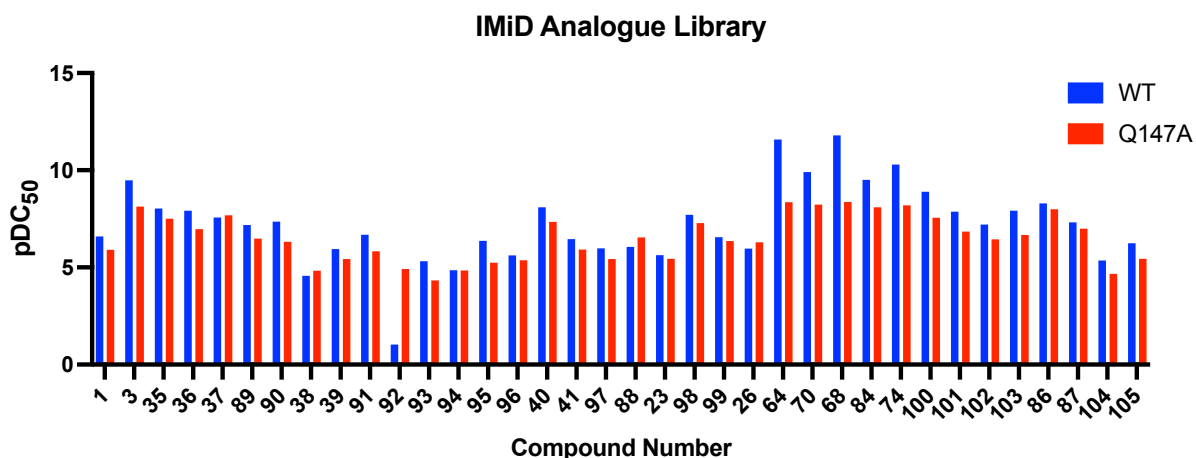
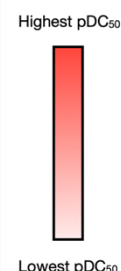


Figure 3.10. pDC₅₀ values for all compounds from IMiD analogue library against WT (blue) and Q147A (red) mutant ZF degrons.

Table 3.2. pDC₅₀ values for all IMiD analogues against WT and Q147A degrons. Colour gradient from red (highest pDC₅₀) to white (lowest pDC₅₀).

	pDC ₅₀ WT	pDC ₅₀ Q147A		pDC ₅₀ WT	pDC ₅₀ Q147A		pDC ₅₀ WT	pDC ₅₀ Q147A	
1	6.60	5.91	94	4.86	4.84	70	9.91	8.23	
3	9.48	8.13	95	6.37	5.24	68	11.79	8.37	
35	8.03	7.50	96	5.62	5.37	84	9.51	8.10	
36	7.92	6.97	40	8.09	7.34	74	10.29	8.20	
37	7.57	7.68	41	6.45	5.92	100	8.89	7.56	
89	7.18	6.48	97	5.99	5.43	101	7.87	6.84	
90	7.35	6.32	88	6.05	6.54	102	7.21	6.45	
38	4.57	4.83	23	5.63	5.44	103	7.92	6.67	
39	5.94	5.43	98	7.71	7.28	86	8.29	7.99	
91	6.68	5.83	99	6.56	6.35	87	7.32	6.99	
92	1.03	4.92	26	5.96	6.30	104	5.36	4.67	
93	5.32	4.33	64	11.58	8.36	105	6.25	5.44	



The first general trend observed is that almost all compounds in the library induce degradation of the WT degron more efficiently than the Q147A mutant. The mean pDC₅₀ value for the WT screen is 7.26, vs a mean pDC₅₀ value of 6.55 for Q147A. This is unsurprising as the absence of the glutamine side chain at position 147 likely decreases the affinity of the ZF degron for the CRBN/IMiD duplex due to loss of favourable interactions such as hydrogen bonds. This decreases prevalence of the tertiary complex and hence the level of polyubiquitination. There are some key exceptions to this trend which will be discussed in section 3.6.2.

The following sections will examine trends observed in smaller subsets of the compound library, which have been grouped by similarities in 'bump' group characteristics. It should be noted that the sigmoidal four parameter logistic (4PL) curves generated for the following compound combinations most likely do not accurately reflect the actual degradation dynamics observed: Figure 3.11: WT/**94**, Q147A/**38**, Q147A/**93** and Q147A/**94**; Figure 3.12: Q147A/**104**. In all cases this is due to a full sigmoidal curve with a high minimum plateau (i.e. low D_{MAX}) being assigned to data for which an incomplete sigmoidal curve should be assigned. As these compounds show weak degradation, it is plausible that data for further, higher

concentrations could have yielded data suggestive of a sigmoidal curve with a lower minimum plateau and a D_{MAX} closer in value to the other ZF/compound combinations tested.

3.6.1.2 Halogenated IMiD Analogues

Figure 3.11 shows degradation curves for halogenated IMiD analogues (**38**, **39**, **91**, **92**, **93**, **94**, **95** and **96**) compared to thalidomide (**1**) and pomalidomide (**3**). For both the WT and Q147A screens, it is evident that halogenated IMiDs are generally less efficient at inducing degradation than either thalidomide or pomalidomide.

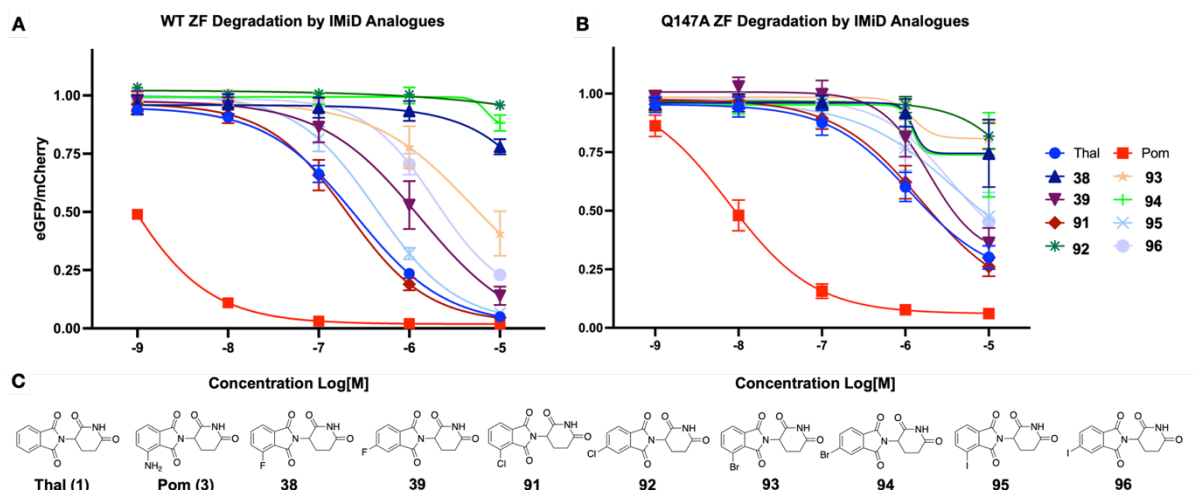


Figure 3.11. **A)** Degradation curves for halogenated IMiD analogues against WT ZF degen. **B)** Degradation curves for halogenated IMiD analogues against Q147A ZF degen.

3.6.1.3 IMiD Analogues with Small Bump Groups

Figure 3.12 shows degradation curves for IMiD analogues with small ‘bump’ groups at either the 4- or 5- position (**35**, **36**, **37**, **40**, **41**, **64**, **70**, **89**, **90**, **104** and **105**) compared to thalidomide (**1**) and pomalidomide (**3**). All of these IMiD analogues induced degradation of the WT more efficiently than the Q147A mutant. Compounds **64** and **70** are particularly potent degraders, each more potent than pomalidomide, suggesting

that substitution of a small aliphatic group to the aniline nitrogen on pomalidomide does not disrupt its activity and can sometimes enhance it.

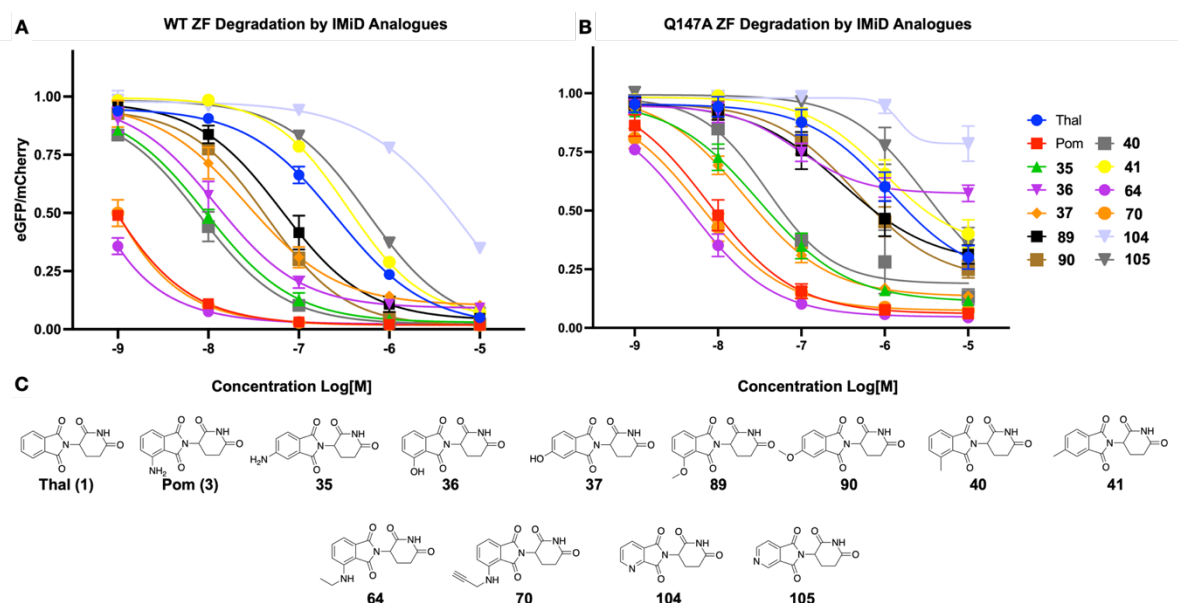


Figure 3.12. **A)** Degradation curves for IMiD analogues with small 'bump' groups against WT ZF degran. **B)** Degradation curves for IMiD analogues with small 'bump' groups against Q147A ZF degran.

The two regioisomers **104** and **105** both show weak degradation, suggesting that replacement of a carbon with a nitrogen in the phthalimide structure is detrimental to degradation ability regardless of the position of placement. It is noteworthy that both of these compounds exhibit poor CRBN pIC₅₀ (Figure S.1), suggesting that ability to bind CRBN well is required for a compound to exhibit high degradation ability.

Compound **35** causes lower degradation than its structural analogue pomalidomide (**3**), suggesting that the placement of the aniline nitrogen at the 4 position is important for ensuring efficient degradation. This can be observed in both the WT and Q147A screens. The phenolic analogue of pomalidomide (**36**) also shows stronger degradation of the WT compared to Q147A. However, the regioisomer (**37**), against expectations, degrades both WT and Q147A with similar potencies. It is unclear why

this is the case. One possibility is that the steric clash caused by a 5-OH group is counteracted by a favourably polar interaction with Q147 in the WT, and that the loss of both of these interactions in the Q147A mutant roughly cancels out in terms of energetics.

Regioisomers **89** and **90**, with methoxy groups at positions 4- or 5-, each exhibit similar degradation profiles in both the WT and Q147A screens. It is possible that the methoxy group is not large or rigid enough to be acting as an effective 'bump' group, and favourable interactions with Q147, such as hydrogen bonding, are compensating for any steric clash.

Regioisomers **40** and **41**, which have methyl groups at positions 4- or 5-, show different degradation profiles from one another. For both the WT and Q147A screens, the 4-methylated analogue possesses a far higher pDC₅₀ value than the 5-methylated analogue. This could be evidence of the 'bump and hole' effect, where the 5-methyl group does not provide the same benefit as a 4-methyl group as it is offset by steric clash. However, if this was the case one might expect the gap between pDC₅₀ values to be closed further in the Q147A screen. In both screens **41** performs similarly to thalidomide, whereas **40** is far more potent than either. Exploring the reasons for this could be an interesting area for future study.

3.6.1.4 IMiD Analogues with Larger Bump Groups at Position 5

Figure 3.13 shows degradation curves for IMiD analogues with larger 'bump' groups exclusively at the 5 position (**23**, **26**, **88**, **97**, **98** and **99**) compared to thalidomide (**1**) and pomalidomide (**3**). In most cases, the decrease in degradation ability for the Q147A mutant compared to the WT is small, suggesting that alleviation in steric clash

between the bulky ‘bump’ group and the Q147 side chain is contributing towards the relatively high pDC₅₀ values seen in the Q147A screen for these compounds.

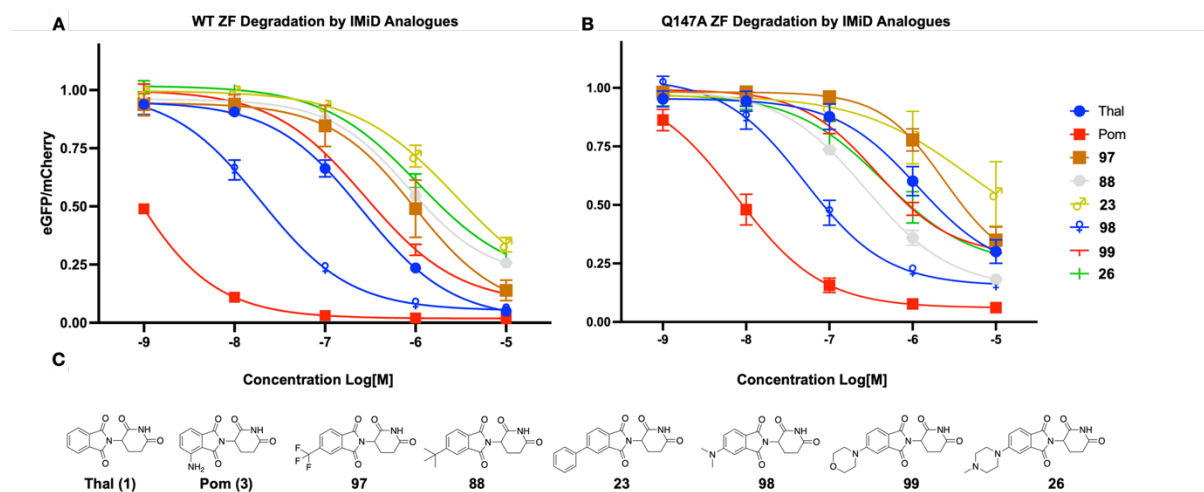


Figure 3.13. **A)** Degradation curves for IMiD analogues with large ‘bump’ groups at the 5-position against WT ZF degen. **B)** Degradation curves for IMiD analogues with large ‘bump’ groups at the 5-position against Q147A ZF degen.

For most of these compounds the lack of steric clash when interacting with Q147A is not enough to compensate for the loss of favourable interactions provided by Q147 in the WT. However, compounds **26** and **88** – which feature a methylpiperazine group and a tertiary-butyl group at position 5, respectively – are notable because they both induce degradation of the Q147A mutant more efficiently than the WT. Their degradation curves are both seen to ‘swap positions’ relative to the curve for thalidomide between graphs 3.13A and 3.13B (this ‘swap’ is also illustrated in figure 3.16). The degradation profiles shown by these compounds will be explored further in section 3.6.2.

3.6.1.5 IMiD Analogues Derived from Compound **68**

Figure 3.14 shows degradation curves for analogues of compound **68**, the hit compound identified in the docking results (Chapter 2) (**74**, **84**, **86**, **87**, **100**, **101**, **102**

and **103**) compared to thalidomide (**1**) and pomalidomide (**3**). In general, all of these compounds induce degradation of the WT degron efficiently, with compound **68** exhibiting the highest pDC₅₀ value of all 36 library compounds. All compounds with an aniline nitrogen at the 4- position were more efficient degraders than their corresponding 4-phenolic oxygen-containing analogues. All compounds in this set degrade the WT more efficiently than the Q147A mutant.

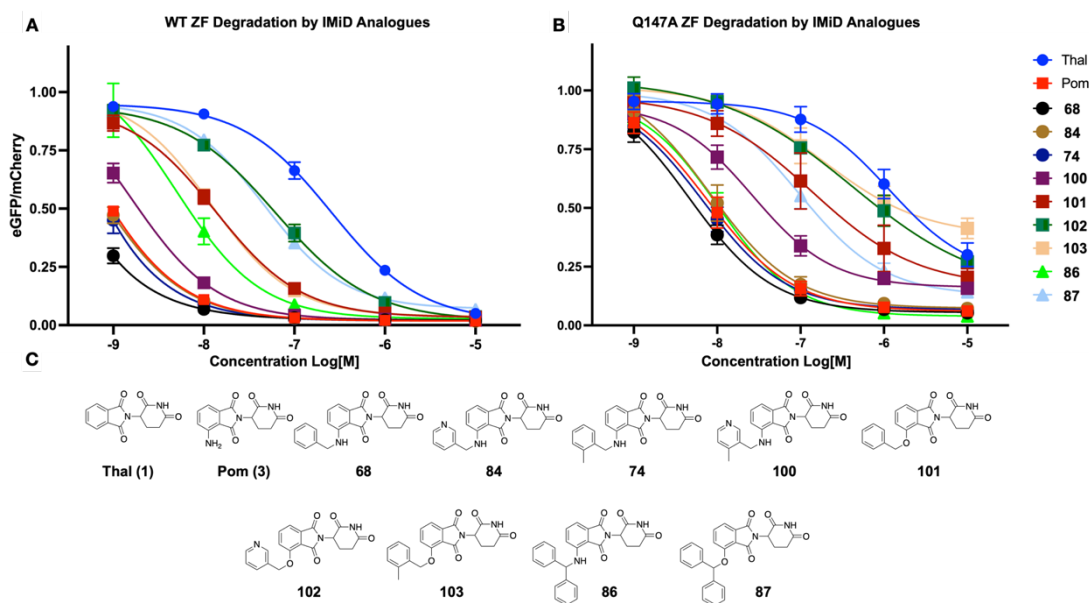


Figure 3.14. A) Degradation curves for analogues of compound **68** against WT ZF degron. B) Degradation curves for analogues of compound **68** against Q147A ZF degron.

Any substitution to the benzyl group in this compound set is detrimental to degradation. Compound **74**, which incorporates a 2-methyl substituent, and compound **84**, which incorporates a 3-pyridyl nitrogen, are both less efficient degraders of both the WT and Q147A mutant degrons than their unmodified counterpart (**68**). It is also notable that compound **100**, which incorporates both of these modifications simultaneously, is less efficient seemingly by an amount that is equivalent to the losses of compounds **74** and **84** combined. A similar trend is observed across compounds **101**, **103** and **102**, which contain the same modifications on the phenyl ring as **68**, **74** and **84**, respectively, but

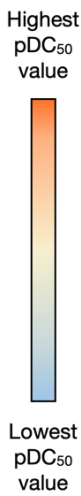
contain a phenolic oxygen atom in place of an aniline nitrogen. Compounds **86** and **87** exhibit the same hierarchy (aniline nitrogen-containing **86** is more potent than phenolic oxygen-containing **87**); both are less efficient degraders than the other compounds in this group, and the gap in pDC₅₀ values between WT and Q147A for both compounds are considerably smaller than for other compounds in this group, suggesting that the benzhydryl ‘bump’ groups are providing more steric clash than the benzyl-derived ‘bump’ groups, corroborating docking results discussed in section 2.5.2.

3.6.1.6 4- and 5- Bump Group Direct Comparison

Table 3.3 shows pDC₅₀ values for the structural isomers within the library (i.e. compounds with the same bump group on either the 4- or 5- position). For both WT and Q147A, the mean pDC₅₀ value for the 4- ‘bumped’ compounds is higher than for 5-bumped compounds, suggesting that substitution at the 5- position is generally detrimental towards degradation capability.

Table 3.3. pDC₅₀ values for structural isomers within the IMiD analogue library.

	pDC ₅₀			
	WT		Q147A	
	4	5	4	5
NH ₂	9.48	8.03	8.13	7.50
OH	7.92	7.57	6.97	7.68
Me	8.09	6.45	8.09	5.92
OMe	7.18	7.35	6.48	6.32
F	4.57	5.94	4.83	5.43
Cl	6.68	1.03	5.83	4.92
Br	5.32	4.86	4.33	4.84
I	6.37	5.62	5.24	5.37
mean	6.95	5.86	6.24	6.00

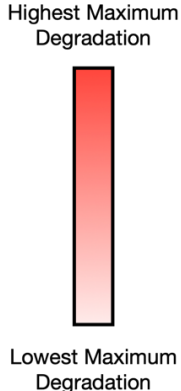


3.6.2 IMiD Selectivity Assessment

To select suitable candidate compounds for 'bump and hole' development, their selectivity for degradation of the Q147A mutant over the WT degnon was assessed. Comparing the degradation of the two degnons for each IMiD analogue returned a 'selectivity value' (Section 3.5.4). Only compounds which exhibited a D_{MAX} value for the Q147A mutant greater than 60% degradation were assessed in this way. D_{MAX} values for Q147A for all compounds are shown in Table 3.4

Table 3.4. D_{MAX} values for all compounds from the IMiD analogue library against the Q147A mutant ZF degnon.

	D_{MAX} Q147A		D_{MAX} Q147A		D_{MAX} Q147A
1	82%	94	56%	70	93%
3	94%	95	86%	68	95%
35	90%	96	77%	84	93%
36	46%	40	87%	74	94%
37	88%	41	72%	100	86%
89	75%	97	91%	101	85%
90	82%	88	87%	102	82%
38	57%	23	66%	103	65%
39	89%	98	86%	86	96%
91	88%	99	76%	87	90%
92	37%	26	79%	104	60%
93	66%	64	96%	105	91%



The resulting selectivity values are shown in Figure 3.15. As discussed in section 3.6.1.4, two IMiD analogues outperform the rest of the compound library in terms of selectivity for the Q147A degnon: **88** (a tertiary-butyl 'bump' on the 5-position) and **26** (a methylpiperazine 'bump' on the 5-position). It should be noted that due to inherent variability and error in this screening measurement, definitive selectivity of these two compounds over other similarly performing compounds (e.g. compounds **23** and **99**) should not be inferred from these relatively modest selectivity values.

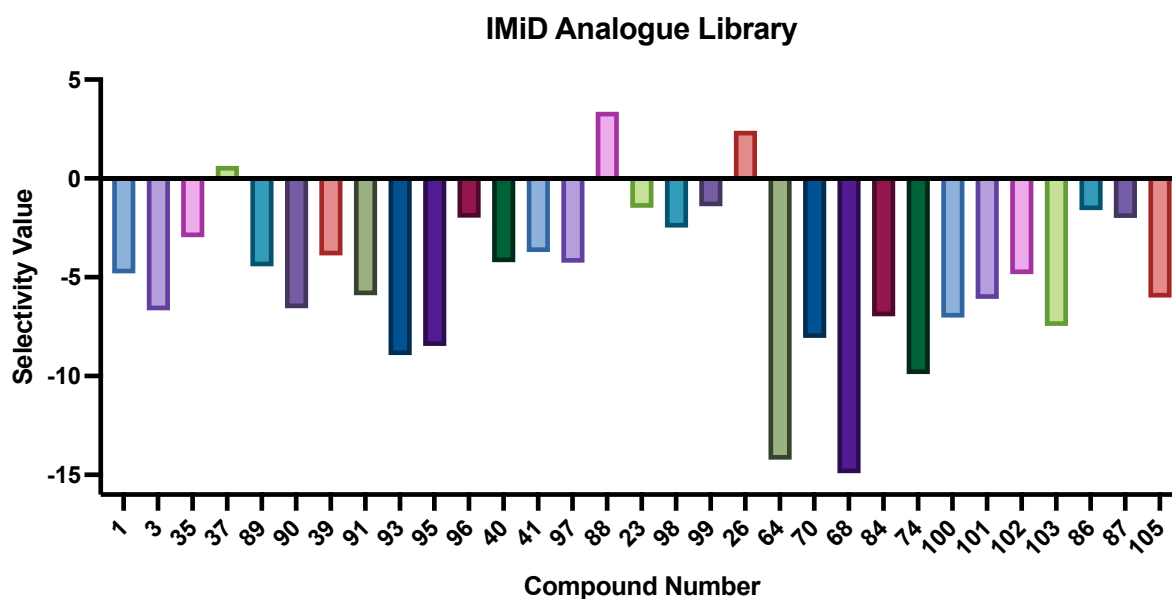


Figure 3.15. Selectivity values for all compounds from IMiD analogue library with D_{MAX} values for Q147A >60%.

Figure 3.16 shows the WT and Q147A degradation curves for compounds **1** (thalidomide), **88** and **26**. Thalidomide does not induce degradation of Q147A as efficiently as the WT; in this sense it is representative of most of the IMiD analogues in the compound library. However, the WT and Q147A degradation curves for compounds **88** and **26** are ‘swapped’ in position compared to the curves for thalidomide, indicating that the alleviation of the steric clash by mutation of Q147 to alanine is sufficiently beneficial to outweigh the loss of favourable Q147 interactions.

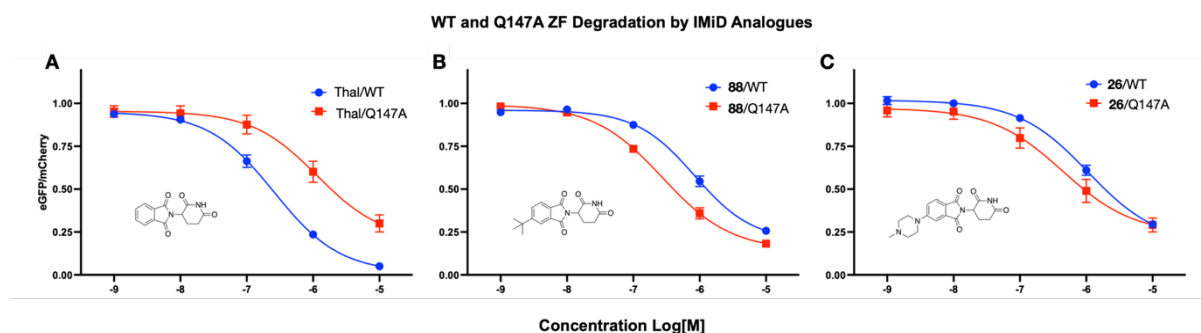


Figure 3.16. Comparison of degradation curves for both WT and Q147A mutant ZF degron for A) thalidomide (**1**), B) compound **88**, and C) compound **26**.

The relative selectivity of compound **88** compared to other library compounds can be explained by considering the amount of space taken up by a *t*-Bu group compared to other groups; due to the symmetry exhibited by the *t*-Bu group, a rotation of the group by 120° will not reduce the amount of space it takes up in the area that Q147 occupies in the original crystal structure (see Figure 2.14). This hypothesis suggests that it can act as a more potent 'bump' group than a 'bump' that can change conformation to take up less space in the clashing region.

The reason for the relative selectivity of compound **26**, compared to other library compounds with large 5-position 'bump' groups, is less clear. It is notable that the structural analogue **99** (a morpholine group at the 5-position) does not display a preference for Q147A. As these two compounds differ only in the replacement of an ether oxygen for a methyl tertiary amine, it is reasonable to conclude that this tertiary amine is working to increase selectivity towards Q147A.

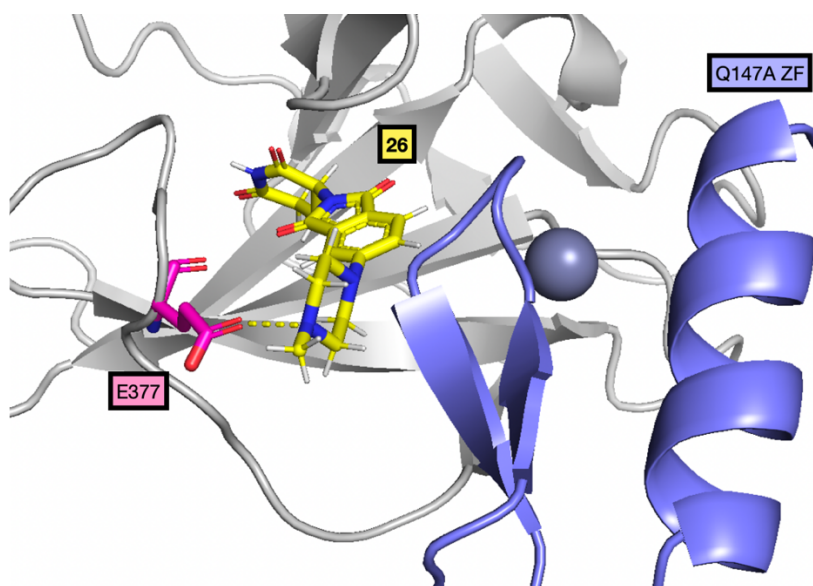


Figure 3.17. Constrained docking result shows predicted salt bridge between tertiary amine in compound **26** (yellow sticks) and E377 (pink sticks) from CRBN (grey cartoon). Ionic interaction represented as dashed line; ZF degnon as lilac cartoon.

At physiological pH, this tertiary amine will be protonated. One possible effect of this protonation is the likely formation of a salt bridge between this amine and residue E377 in CRBN (Figure 3.17). This salt bridge might encourage the IMiD analogue to adopt a pose which is either unsuitable for favourable interaction with Q147 in the WT degron, or particularly suitable for accommodation by the alanine 'hole' in the Q147A mutant.

3.7 Endogenous IKZF1 Degradation Test

3.7.1 Motivation and Assay Description

Having screened all 36 compounds in the IMiD analogue library for 'bump and hole' activity, an assay was performed to aid in the process of narrowing down the candidate compounds for further study. Ten compounds from the IMiD analogue library (**23**, **26**, **41**, **68**, **86**, **87**, **88**, **98**, **99** and **101**) were selected for an assay to assess their ability to degrade endogenous IKZF1 in Jurkat cells, alongside thalidomide (**1**), lenalidomide (**2**) and pomalidomide (**3**) controls. This set of compounds was selected to include the two best performing IMiD analogues by selectivity value (**26** and **88**), four other 5- 'bump' compounds (**23**, **41**, **98** and **99**), and four analogues of compound **68** (**68**, **86**, **87** and **101**)

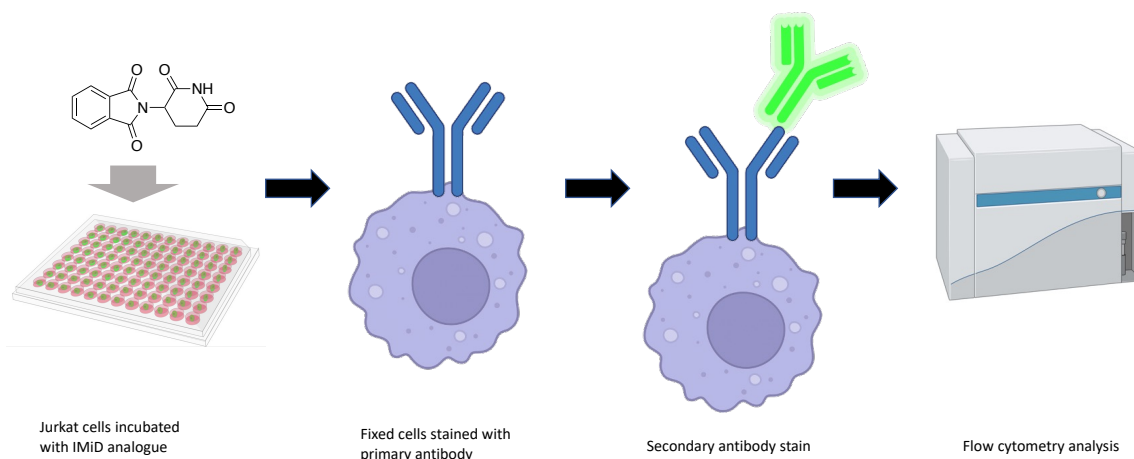


Figure 3.18. Jurkat cells incubated with concentration curves of IMiDs and subsequent cell fixing, antibody staining, secondary antibody staining and flow cytometry analysis.

Flow cytometry was used to assess the ability of these IMiD analogues to induce degradation of endogenous IKZF1 in Jurkat cells. These data were obtained *via* antibody staining of the endogenous IKZF1 protein, followed by further staining by a fluorescent secondary antibody, all carried out in cells which has been fixed post-compound incubation (Figure 3.18). Secondary antibody fluorescence was then measured using flow cytometry and normalised against an untreated control to return a concentration curve (1 nM, 10 nM, 100 nM, 1 μ M and 10 μ M).

3.7.2 Results of Endogenous IKZF1 Degradation Test

The data obtained from this screen showed that the most selective compounds in the WT/Q147A screens (**26** and **88**) also did not degrade endogenous IKZF1 significantly for any concentration tested. This result suggests that use of either of these two compounds at 10 μ M would be suitable for a target validation assay, as levels of endogenous IKZF1 would not be affected.

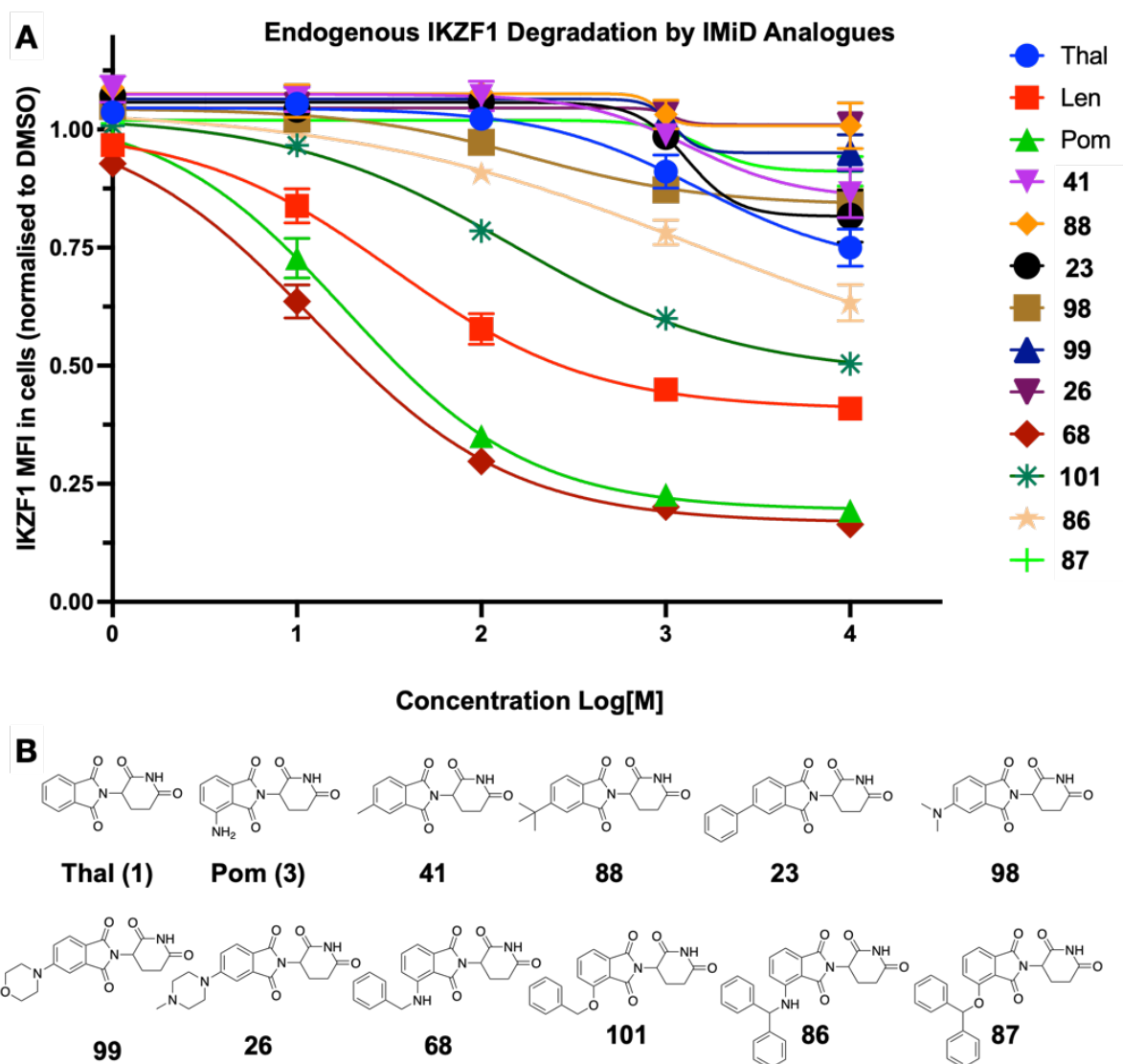


Figure 3.19. A) Degradation curves for a subset of IMiD analogue library compounds (**1**, **3**, **41**, **88**, **23**, **98**, **99**, **26**, **68**, **101**, **86**, **87** and commercially acquired lenalidomide) against endogenous IKZF1. **B)** Chemical structures for compounds **1**, **3**, **41**, **88**, **23**, **98**, **99**, **26**, **68**, **101**, **86** and **87**.

The other four 5-‘bump’-containing compounds tested in this assay (**23**, **41**, **98** and **99**) also exhibited low degradation of endogenous IKZF1, but all were more potent IKZF1 degraders than compounds **26** and **88**. This result was also observed for compound **87**.

Compounds **68**, **86** and **101** all showed greater degradation ability against endogenous IKZF1. Compound **68** was seen to be more potent than pomalidomide, corroborating results from the WT/Q147A screen.

It is notable that all compounds were far less potent degraders of endogenous IKZF1 in this assay than the ZF-eGFP fusion protein in the WT/Q147A screen. This observation suggests that, as a general rule, IMiDs are more potent degraders of this design of ZF-eGFP fusion protein than of the ZF degron in the context of the endogenous protein from which it originated. However more data would be required to determine whether this rule extends to other fusion protein constructs (e.g. with different linker regions) or the ZF degron fused to other POIs.

3.8 Summary and Conclusions

The data gathered by these initial compound degradation screens facilitated identification of IMiD analogues that exhibited the ‘bump and hole’ effect to at least a small degree. As well as the two most selective IMiD analogues from these screens (**26** and **88**), several other promising compounds were identified that exhibited a relatively small preference for the WT over Q147A. In particular, compounds **86** and **99** were decided upon as the best compounds to explore further alongside **26** and **88**: **99** was chosen due to its overall similarity to **26** in both structure and selectivity value; **86** was chosen as it was the best performing analogue of **68** in terms of selectivity value.

The next chapter will discuss the high throughput methodology used to screen a large number of mutant ZF degrons against these candidate IMiD analogues (**26**, **86**, **88** and **99**), as well as thalidomide (**1**) and lenalidomide (**2**) controls (lenalidomide was chosen over pomalidomide to maximise structural dissimilarity to thalidomide).

Chapter 4: Mutant Degron Library Screen

4.1 Introduction and Aims

Chapter 3 summarised the degradation studies that were conducted on a library of 36 IMiD analogues. This work allowed four molecules (**26**, **86**, **88** and **99**) to be identified as promising candidates for further development. Of these, two compounds (**26** and **88**) were clear 'hits', as both showed modest selective degradation of the Q147A mutant over the WT protein. However, for an IMiD/degron pair to be useful in a biological setting, a greater degree of selectivity is required in order to ensure minimal degradation of endogenous neosubstrates. It was therefore decided that further combinations of degron mutations should be explored to identify ligand/degron pairs with greater selectivity over the WT protein.

This chapter will describe the design and implementation of a mutant degron library screen. The purpose of this screen was to measure the degradation capabilities of IMiD analogues **26**, **86**, **88** and **99** – as well as thalidomide (**1**) and lenalidomide (**2**) controls – against a large pool of ZF mutants, to identify mutant degrons which were efficiently degraded in the presence of these compounds.

4.2 Library Design

4.2.1 Candidate Positions

To design a library of mutant degrons we needed to consider the number of positions we wished to mutate on the ZF degron, which particular combinations of mutations we

wished to prioritise, and how many combinations of mutations could be tested within the size allowed by the library methodology. Figure 4.1 highlights nine residues of the ZF degron that are close to either the IMiD molecule or the CRBN interface. Mutations at these positions were all considered potentially appropriate for inclusion in the library screen. However, all possible combinations of all possible mutations at all nine positions would result in 512,000,000,000 sequences. A library size of <10,000 was desired, which constrained how inclusive our library could be for different combinations of mutations at these nine positions.

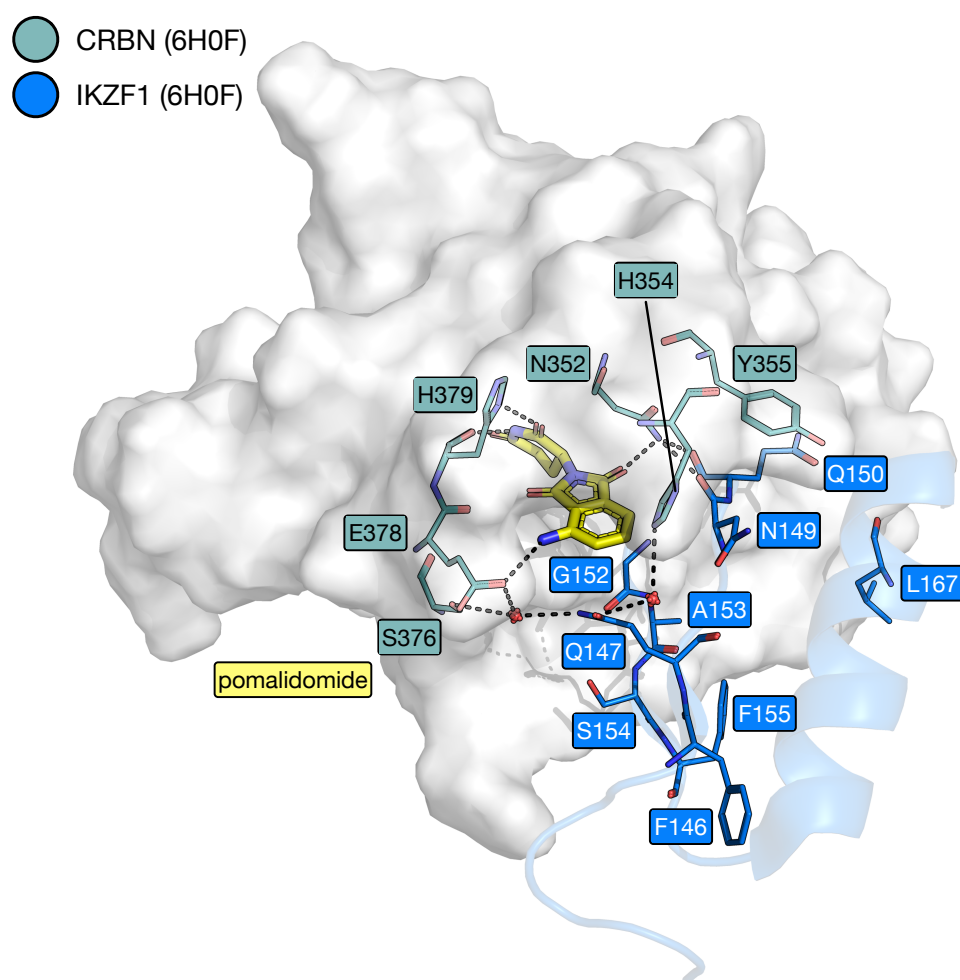


Figure 4.1. Crystal structure of CRBN (grey surface, teal sticks), the IKZF1/3 ZF degron (blue cartoon and sticks) and pomalidomide (yellow sticks). Nine key residues from the IKZF1/3 were deemed suitable for mutation in a mutant ZF degron library: G152 and Q147 were chosen due to their proximity to the IMiD; S154 was chosen due to its proximity with Q147; N149, Q150, A153 and L167 were chosen due to their interaction with CRBN; A153 was also chosen, along with F146 and F155, as the side chains of these residues comprise a hydrophobic core of the ZF. Polar interactions shown as dashed lines; waters represented as red spheres. (PDB ID: 6H0F).

Figures 4.2 and 4.3 show the IKZF1/3 ZF degron sequence alongside sequences observed in equivalent positions of other endogenous neosubstrates.^{2,4} Inspiration was drawn from the prevalence of residues types at key positions in these endogenous neosubstrates during the library design process.

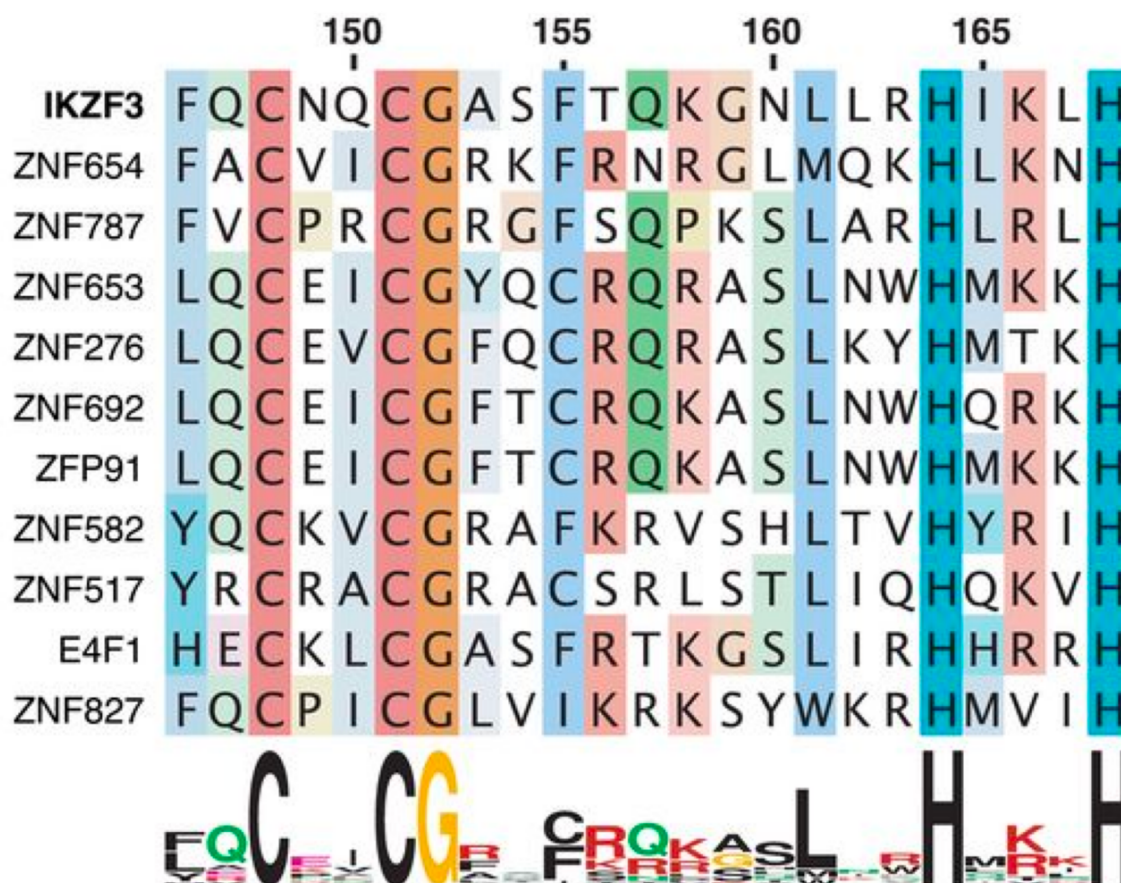


Figure 4.2 Residue identities from position 146 to 168 of the IKZF3 ZF and in the equivalent positions of 10 homologous ZF structures found in other endogenous neosubstrates. Colours are given to residues which occur frequently in ZF degron sequences. The C148, C151, H164 and H168 residues that comprise the C2H2 zinc-binding motif are highly conserved, as is G152. Adapted from Sievers *et al.* with permission.²

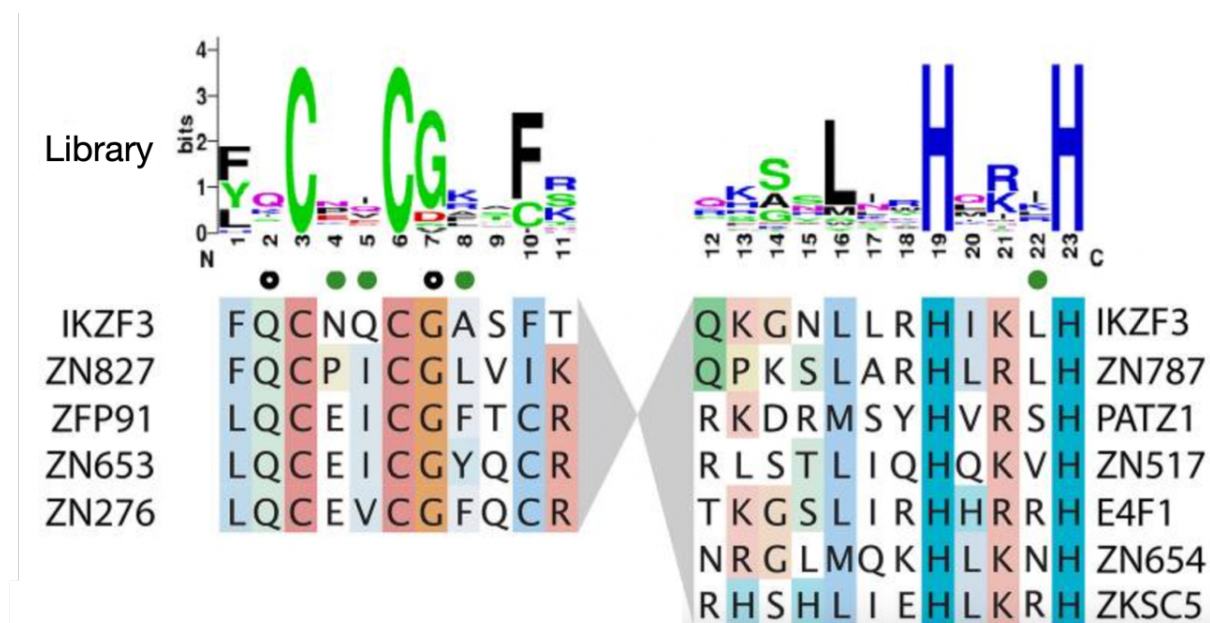


Figure 4.3 Residue identities from positions 146 to 156 and 157 to 168 exhibited by IKZF3 and in analogous positions in other neosubstrates. Colours are given to residues which occur frequently in ZF degon sequences. The C148, C151, H164 and H168 residues that comprise the C2H2 zinc-binding motif are highly conserved, as is G152. Adapted from Jan *et al.* with permission.⁴

Given Q147 is located at the protein-ligand-protein interface, it is considered a key residue. This is demonstrated by the observation that a Q147A mutation is sufficient to allow a ‘bump and hole’ effect to be observed when using an appropriately ‘bumped’ IMiD analogue. It was therefore decided that the mutation combinations comprising our library should contain significant focus on position 147, including all possible mutations at this position. However, proline was excluded from consideration at this position, as the Φ and Ψ angles exhibited by the native glutamine in the WT structure (-111.3° and 148.4° , respectively) did not fall into the range of angles preferred by proline (Φ angle of around -60° ; Ψ angle of around either -45° or 135°).

Residue 154 was also considered important for the library. As the side chain of residue 154 is proximal to the side chain of residue 147, it was concluded that an appropriate mutation at 154 could be beneficial for optimal positioning of the side chain of residue 147. Therefore all possible mutations at this position were explored in the library.

Residues 149, 150, 153 and 167 have all previously been identified as possessing side chains that interact with the surface of CRBN during ternary complex formation.⁴ Exploring full combinatorial space for these regions would have returned too many sequence for the desired capacity of our library, so studies were performed to narrow down mutations to those considered to possess the most potential for enabling favourable ZF/CRBN interaction. This is explored in section 4.2.2.

Residues 146, 153 and 155 possess side chains that occupy internal regions of the IKZF1/3 ZF, as shown in figure 4.1. The arrangement of the side chains of these three residues (F146/A153/F155 in IKZF1/3) comprises a hydrophobic core of the ZF. Inspection of equivalent positions in the ZF degrons of other neosubstrates shows variations on this motif that occur frequently: in particular an F146/H153/F155 motif seen in SALL4 (Figure 4.4A) and an L146/F153/C155 motif seen in ZNF692 (Figure 4.4B). Inspection of sequences in Figures 4.2 and 4.3 also highlights not only several instances of this L146/F153/C155 motif, but also a frequently observed F146/R153/F155 motif. Exploration of these hydrophobic core variants was incorporated into the design of the library, as it was hypothesised that deviation from the F146/A153/F155 motif seen in the IKZF1/3 ZF degron could potentially alter the overall structure of the ZF in such a way that aided in selectivity towards a 'bumped' IMiD analogue.

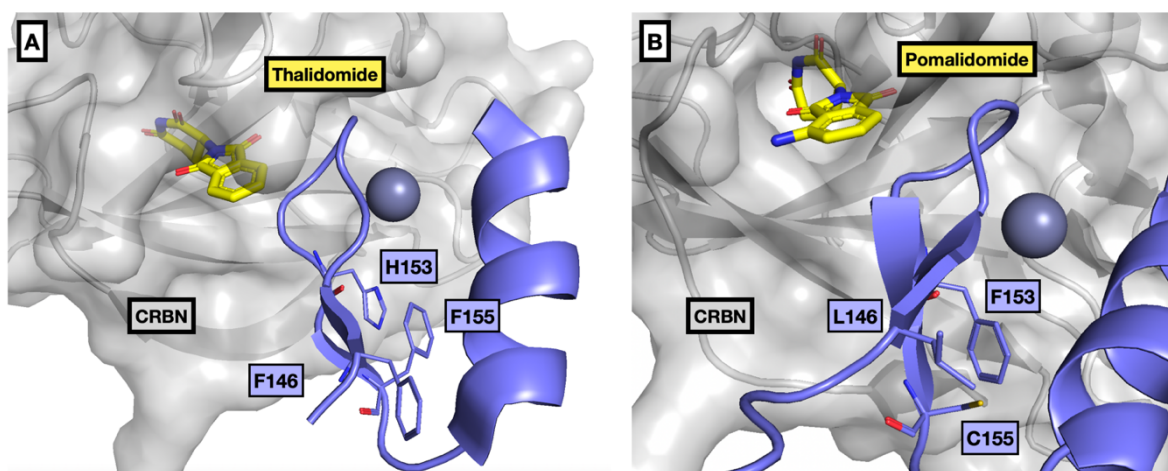


Figure 4.4. Tripartite motif comprising the internal core region of the ZF degrons of **A)** SALL4 and **B)** ZNF692. Ligand (thalidomide in **A**, pomalidomide in **B**) shown as yellow sticks; CRBN shown as grey surface and cartoon; ZF shown as lilac cartoon and sticks; zinc atom shown as grey sphere. (*PDB IDs: 7BQU and 6H0G*).

As discussed in Chapter 1, a mutation at position 152 to anything with a larger side chain than glycine significantly reduces capacity for ZF degradation by CRBN. We included a sequence in our library that comprised the G152W mutation at this position (with all other residues remaining unmutated) as a negative control that we predicted would be inactive.

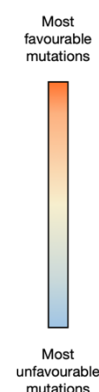
4.2.2 Computational Studies

To refine the combinations of residues explored at positions 149, 150, 153 and 167, the energetic penalties of all 20 point mutations at these four positions in the 6H0F crystal structure were calculated the Position Mutation functionality in Rosetta^{81,82} and the Position Scan functionality in Foldx.⁵⁵ The results of these energetic assessments are shown in Table 4.1. No significantly favourable mutations (with $\Delta\Delta G$ values <-1) were observed for either position 153 or position 167. For position 149, Foldx returned two mutations with $\Delta\Delta G$ values <-1 (N149E and N149P) and Rosetta returned two mutations with $\Delta\Delta G$ values <-1 (N149D and N149P). For position 150, Foldx returned

three mutations with $\Delta\Delta G$ values <-1 (Q150F, Q150L and Q150M), and Rosetta returned five mutations with $\Delta\Delta G$ values <-1 (Q150A, Q150E, Q150I, Q150T and Q150V). Attention was only given to those mutations for which there was a convergence between the two methods. Furthermore, attention was paid to residue abundance in ZF degron sequences observed in endogenous neosubstrates (Figures 4.2 and 4.3) to aid in determination of whether these mutations should be included in our library design. Consideration of both endogenous protein sequence data and computational results directed us towards the following mutations: N149D (mean $\Delta\Delta G$ = -1.25), N149E (mean $\Delta\Delta G$ = -0.99) and N149P (mean $\Delta\Delta G$ = -1.84) for position 149; Q150I (mean $\Delta\Delta G$ = -1.65) and Q150V (mean $\Delta\Delta G$ = -1.00) for position 150.

Table 4.1. Change in free energies in complex with CRBN for single mutations at positions 149, 150, 153 and 167 of the IKZF1/3 ZF degron, as predicted by Foldx and Rosetta. Colour scale highlights favourable mutations in orange and unfavourable mutations in blue.

$\Delta\Delta G$				$\Delta\Delta G$				$\Delta\Delta G$				$\Delta\Delta G$			
Mutation	Foldx	Rosetta	Mean	Mutation	Foldx	Rosetta	Mean	Mutation	Foldx	Rosetta	Mean	Mutation	Foldx	Rosetta	Mean
N149A	-0.26	-0.23	-0.25	Q150A	-0.22	-1.51	-0.87	A153A	0.01	N/A	0.01	L167A	0.41	1.61	1.01
N149C	0.02	1.76	0.89	Q150C	0.20	1.46	0.83	A153C	2.32	N/A	2.32	L167C	0.46	3.64	2.05
N149D	-0.91	-1.59	-1.25	Q150D	0.53	1.30	0.92	A153D	3.76	N/A	3.76	L167D	1.34	2.91	2.13
N149E	-1.28	-0.71	-0.99	Q150E	-0.34	-1.27	-0.81	A153E	5.33	N/A	5.33	L167E	0.81	2.67	1.74
N149F	-0.58	1.98	0.70	Q150F	-1.11	4.65	1.77	A153F	23.77	N/A	23.77	L167F	0.29	3.04	1.67
N149G	0.45	2.50	1.48	Q150G	0.46	2.66	1.56	A153G	1.42	5.32	3.37	L167G	0.71	3.49	2.10
N149H	0.74	0.98	0.86	Q150H	-0.59	1.44	0.43	A153H	49.24	N/A	49.24	L167H	0.85	2.26	1.55
N149I	-0.23	1.89	0.83	Q150I	-0.99	-2.31	-1.65	A153I	8.08	N/A	8.08	L167I	0.38	1.42	0.90
N149K	-0.91	-0.59	-0.75	Q150K	-0.92	1.36	0.22	A153K	8.20	N/A	8.20	L167K	0.53	0.96	0.75
N149L	-0.58	3.55	1.48	Q150L	-1.32	2.36	0.52	A153L	5.25	N/A	5.25	L167L	0.00	N/A	0.00
N149M	-0.29	1.65	0.68	Q150M	-1.30	1.63	0.16	A153M	4.46	N/A	4.46	L167M	-0.05	1.32	0.63
N149N	0.00	N/A	0.00	Q150N	0.47	1.47	0.97	A153N	5.33	N/A	5.33	L167N	0.83	1.17	1.00
N149P	-1.49	-2.19	-1.84	Q150P	0.60	N/A	0.60	A153P	5.46	N/A	5.46	L167P	0.50	N/A	0.50
N149Q	-0.56	-0.19	-0.38	Q150Q	0.00	N/A	0.00	A153Q	7.81	N/A	7.81	L167Q	0.11	1.79	0.95
N149R	-0.71	-0.04	-0.38	Q150R	-0.27	1.71	0.72	A153R	15.58	N/A	15.58	L167R	0.23	0.89	0.56
N149S	0.41	-0.25	0.08	Q150S	0.15	-0.09	0.03	A153S	1.99	4.85	3.42	L167S	0.26	1.18	0.72
N149T	0.72	0.04	0.38	Q150T	0.02	-1.06	-0.52	A153T	4.97	N/A	4.97	L167T	0.04	2.25	1.14
N149V	0.12	0.51	0.32	Q150V	-0.64	-1.37	-1.00	A153V	4.64	N/A	4.64	L167V	0.83	2.33	1.58
N149W	0.11	2.13	1.12	Q150W	-0.94	3.42	1.24	A153W	22.72	N/A	22.72	L167W	0.56	0.93	0.74
N149Y	0.19	1.22	0.71	Q150Y	0.28	3.88	2.08	A153Y	25.84	N/A	25.84	L167Y	0.08	1.57	0.82



4.2.3 Final Library Design

Figure 4.5 shows the full design of the mutant degron library, totalling 8380 mutant sequences.

The first, and largest section of the library, A, consists of a fully combinatorial exploration of certain mutations at residues 147, 149, 150 and 154 only, with every other residue kept the same as in IKZF1/3. All 20 residues are included at position 154, and all 20 except proline for position 147. For positions 149 and 150, only the residues identified in section 4.2.2 are included. This results in a library section of $19 \times 4 \times 3 \times 20$ mutants: 4560 in total.

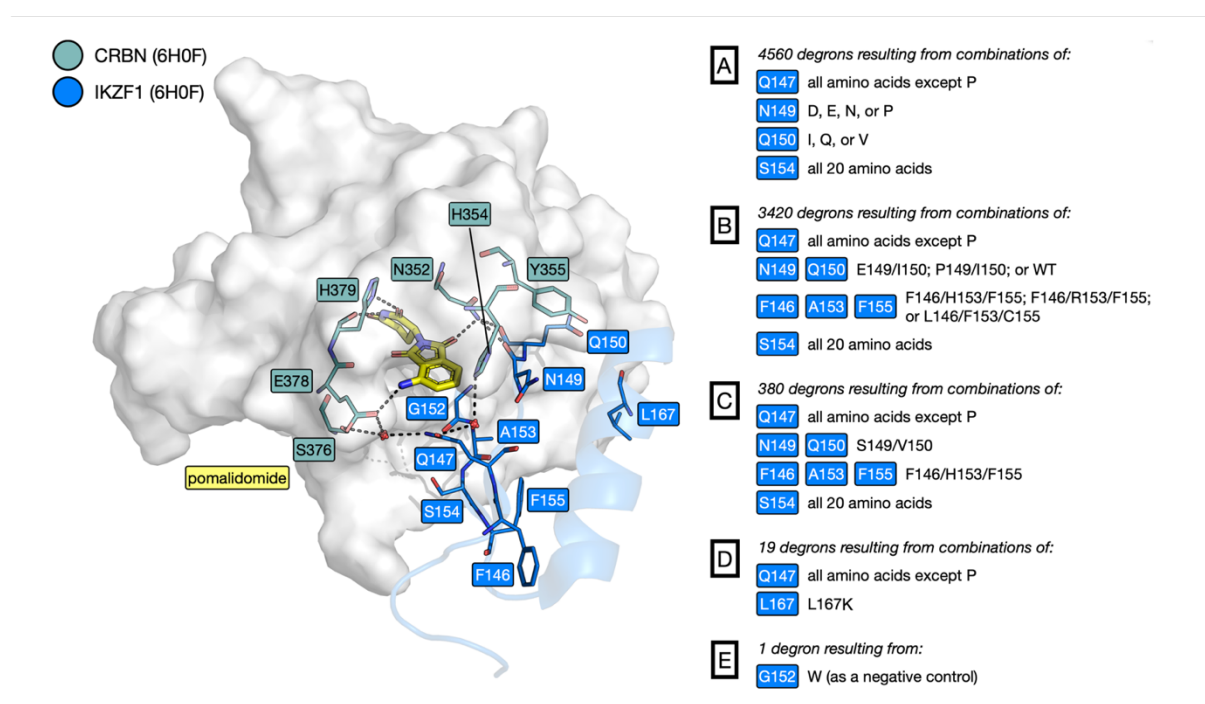


Figure 4.5. Full mutant ZF library design totalling 8380 sequences. **A)** 4560 sequences comprising combinations of mutations made at positions 147, 149, 150 and 154. **B)** 3420 sequences comprising combinations of mutations made at positions 146, 147, 149, 150, 153, 154 and 155. **C)** 380 sequences comprising combinations of mutations made at positions 146, 147, 149, 150, 153, 154 and 155. **D)** 19 sequences comprising combinations of mutations made at positions 147 and 167. **E)** 1 sequence comprising a single G152W mutation. Pomalidomide shown as yellow sticks, CRBN shown as grey surface and teal sticks, IKZF1/3 ZF shown as blue cartoon and sticks, polar interactions shown as dashed lines, water molecules shown as red spheres.

The second library component, B, takes a subsection of A and explores different motifs at positions 146, 153 and 155. Instead of the WT F146/A153/F155 (FAF) motif, FHF,

FRF and LFC motifs are included. As before, all 20 residues are explored at position 154, and all 20 except proline at position 147. In order to reduce the number of sequences tested in this section, only certain pairs of mutations are included at positions 149 and 150: E149/I150, P149/I150, or WT. These two non-WT 149/150 pairs were picked as it was suspected that these mutation pairs may be the most beneficial of the full set of 149/150 mutation combinations from library subsection A. The total number of mutant sequences in subsection B is $19 \times 3 \times 3 \times 20$: 3420 in total.

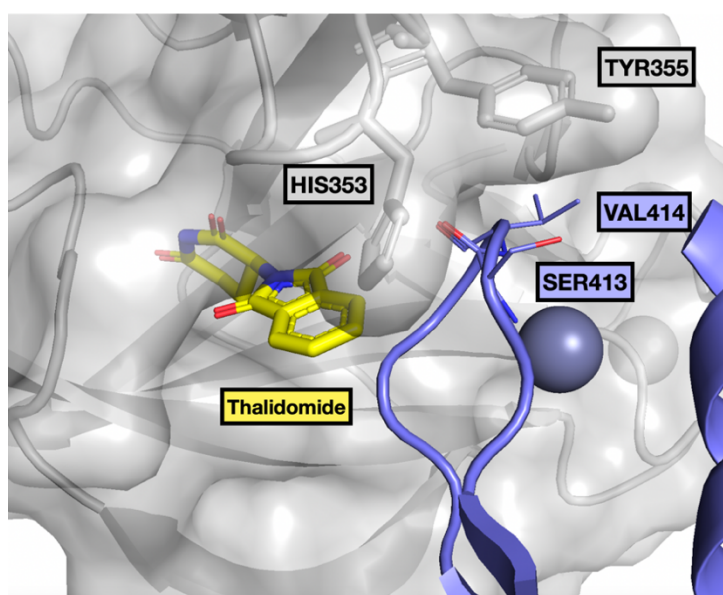


Figure 4.6. SALL4 exhibits residues S and V at positions 413 and 141: equivalent positions to 149 and 150 in IKZF3. Thalidomide shown as yellow sticks; CRBN shown as grey surface and cartoon, residues 353 and 355 shown as grey sticks; IKZF1/3 ZF shown as lilac cartoon, residues 413 and 414 shown as lilac sticks; zinc atom shown as grey sphere. (PDB ID: 7BQU).

Library section C was designed to emulate the residues observed in the SALL4 ZF at positions 146, 149, 150, 153 and 154, while exploring full combinations at 147 and 154, and keeping other residues the same as the WT. This strategy was chosen because SALL4 is also a neosubstrate of traditional IMiDs such as thalidomide (**1**) or pomalidomide (**3**). SALL4 has an FHF motif at positions 146/153/155, but possesses residues with smaller side chains at positions 149 and 150 compared to IKZF1/3:

serine and valine, respectively. (Figure 4.6). The total number of mutant sequences in subsection C is 19×20 mutants: 380 in total.

Library section D keeps every position the same as IKZF1/3 except 167, which is mutated from leucine to lysine. This was included due to observation in Figure 4.2 that K167 occurs frequently in endogenous neosubstrate ZF degrons. This results in a library subsection of 19 mutants in total.

Finally, one further G152W mutant sequence is included as a negative control (see section 4.2.1).

4.3 Library Screen

4.3.1 Library Screen Workflow

Library screening was carried out at GlaxoSmithKline (GSK) in Stevenage in partnership with Dr Lewis Brayshaw. Figure 4.7 shows the full procedure that was employed for the mutant library screen. After synthesis of the Sprout plasmid (see Chapter 3) encoding each library mutant, the full plasmid library was transduced into a population of Jurkat cells. Care was taken during transduction to ensure that the plasmid copy number per cell was no higher than 1, as the presence of multiple plasmids encoding different mutants in the same cell would compromise the screening methodology. This was achieved by using an appropriate concentration of viral vector and an appropriate incubation time of the Jurkat cells with the viral particle solution (full details are included in the experimental section).

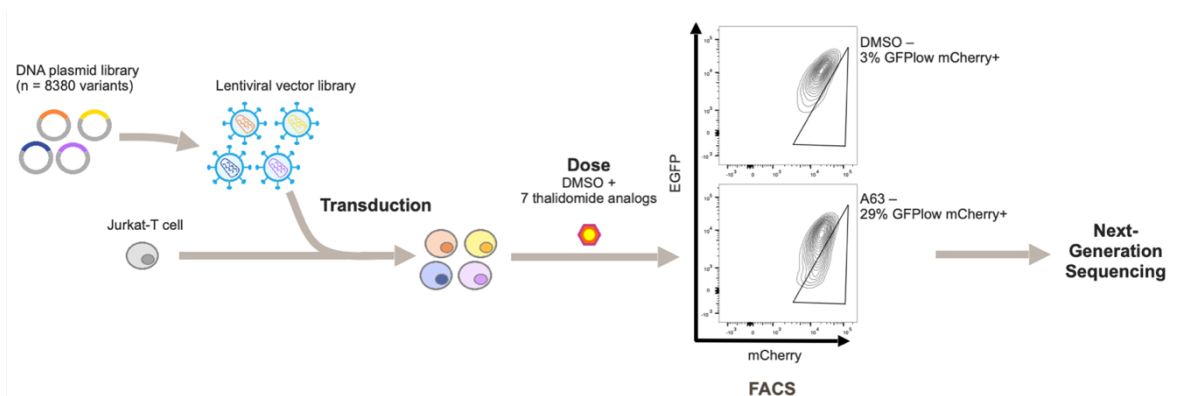


Figure 4.7. Full library screen workflow. Jurkat cells are transduced with plasmid library (8380 sequences) and cultured as one population; cells cultured and incubated with IMiD analogue for 18 h; fluorescence activated cell sorting (FACS) used to separate cells in which ZF-eGFP fusion protein levels are low ('GFPlow' cells) from remaining population. Figure made by Dr Lewis Brayshaw (GSK), reprinted with permission.

After transduction, an initial round of fluorescence activated cell sorting (FACS) was conducted to remove any untransduced cells from the library population by gating so as to only collect mCherry positive cells. The resultant population was cultured until enough cells were present for >500× library coverage (i.e. each library sequence was transduced into at least 500 individual cells in the population). Compound incubations were then performed, in which cells were resuspended in media containing an appropriate concentration of an IMiD analogue for 18 hours (details on IMiD analogue concentrations used discussed in section 4.3.2).

After incubation, FACS was performed to separate cells in which significant degradation of the ZF-eGFP fusion protein had occurred from cells in which minimal degradation of the ZF-eGFP fusion protein had occurred. This was achieved by separating cells showing a low eGFP:mCherry ratio from those exhibiting a similar eGFP:mCherry ratio to untreated cells. As seen in Figure 4.8, the population of cells that exhibit low eGFP:mCherry ratios (in which degradation of the ZF degron has occurred) appears as a 'shoulder' on the bottom of the main population. The gate used

to separate the cells also determines what percentage of the entire population this 'shoulder' comprises.

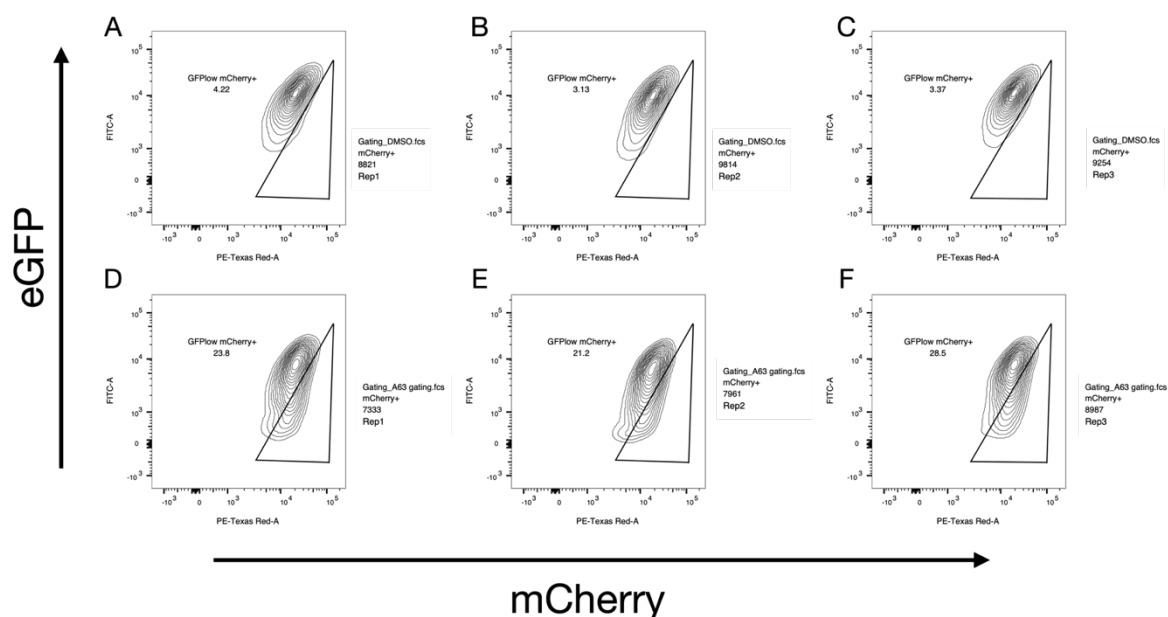


Figure 4.8. Triplicate data showing eGFP and mCherry fluorescence for stably transduced mutant library ZF-eGFP-expressing Jurkat cells either untreated (**A**, **B** and **C**) or treated with 313 nM compound **88** (**D**, **E** and **F**). FACS was performed using a gate that did not significantly infringe on the untreated population but collected a 'shoulder' of cells with low eGFP fluorescence after compound treatment.

Each compound screen was compared to a control screen in which no compound had been added (DMSO control). For each incubation, enough cells were used such that each mutant would be encountered a minimum of 500 times during screening. After separation, these populations were then quantitatively sequenced by Next Generation Sequencing (NGS) to obtain the relative abundance of mutants in each population. NGS was carried out by staff at GSK in Stevenage. Each screen was performed in triplicate.

4.3.2 Compound Concentration

Six compounds were selected for library screening: thalidomide (**1**), lenalidomide (**2**), **26**, **86**, **88** and **99** (Figure 4.9).

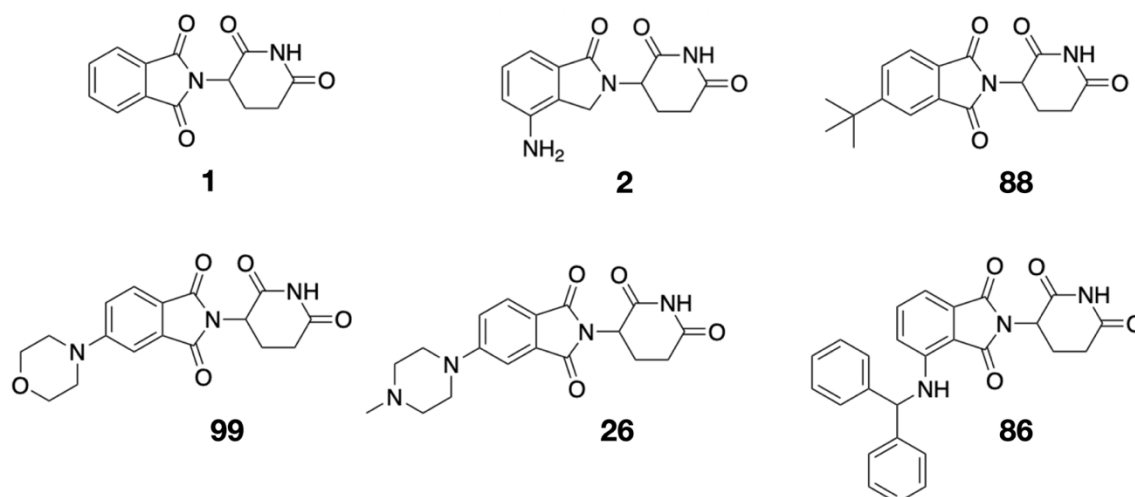


Figure 4.9. The chemical structures of the six compounds screened against the mutant degron library.

Using an appropriate concentration of each compound was an important consideration during protocol design: using too low a concentration would fail to yield many hit sequences, whilst too high a concentration would fail to distinguish exceptional degrons from merely good degrons.

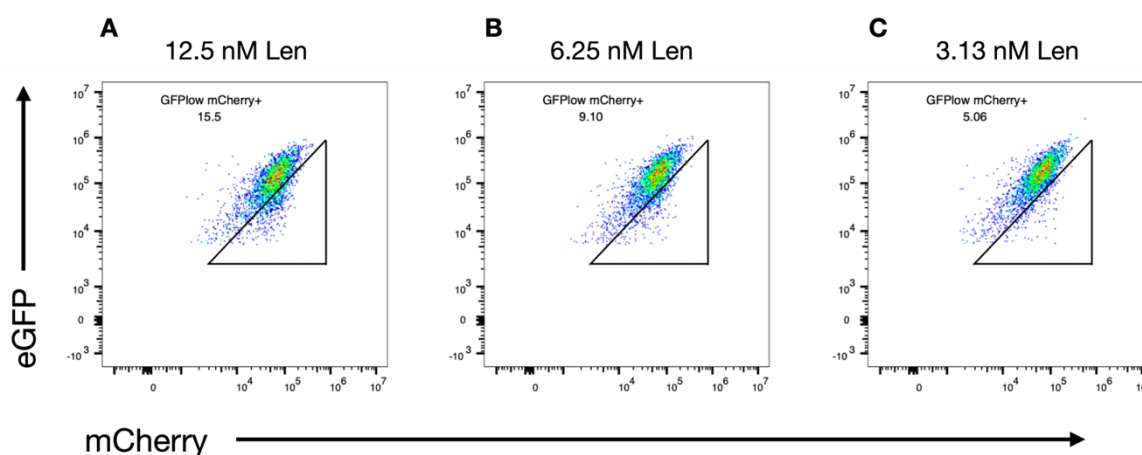


Figure 4.10. eGFP and mCherry fluorescence for stably transduced mutant library ZF-eGFP-expressing Jurkat cells treated with either **A)** 12.5 nM, **B)** 6.25 nM or **C)** 3.13 nM lenalidomide. The same gate was applied to each population of mCherry positive cells after treatment with a concentration curve of an IMiD analogue (in this case lenalidomide). The concentration which resulted in the closest to 10% of the Jurkat population falling within the gate was chosen for use in the library screen.

It was decided that a concentration of compound which induces a 'shoulder' of around 10% during FACS would in all cases be a suitable concentration to yield a suitable number of 'hit' sequences. Figure 4.10 shows flow cytometry plots that dictate 6.25 nM is a suitable concentration to use for lenalidomide (**2**), as this yields the 'shoulder' percentage closest to 10%.

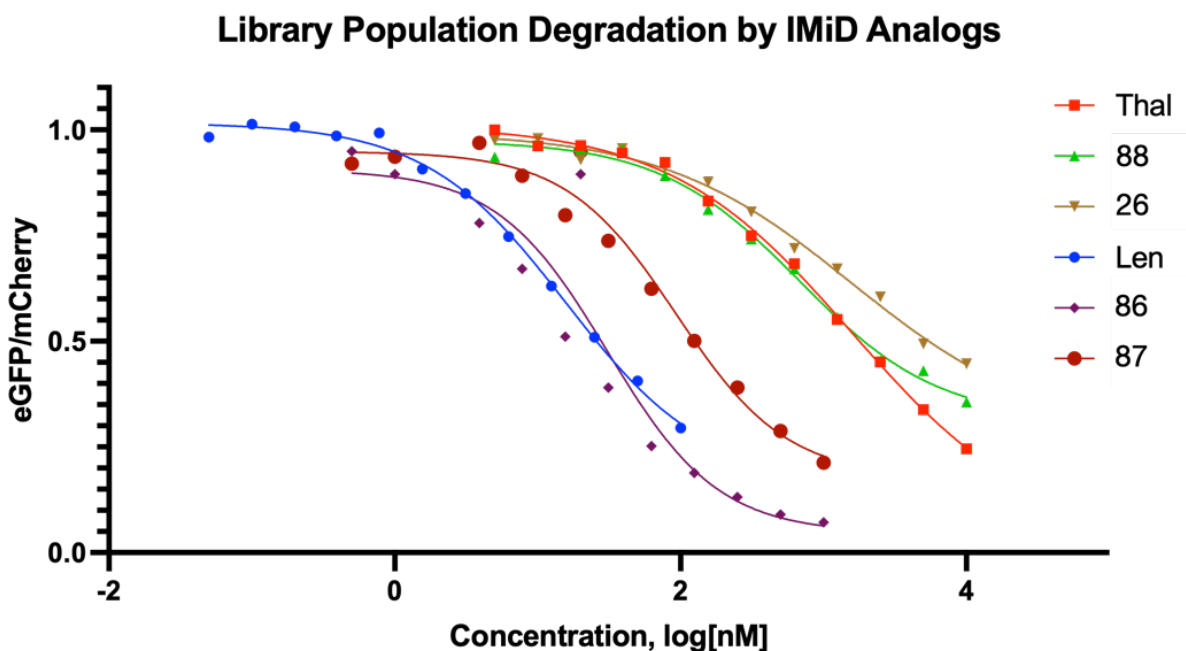


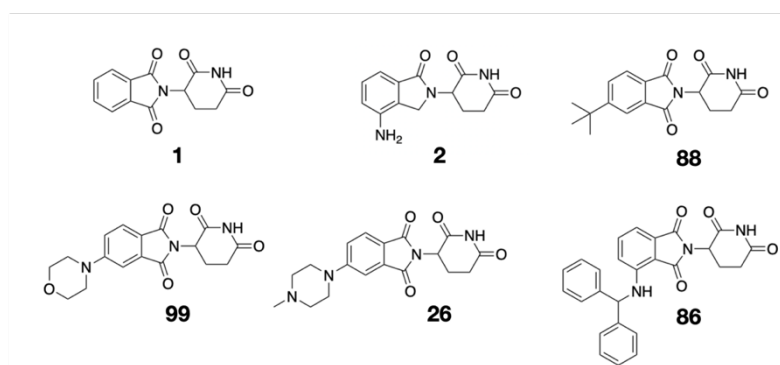
Figure 4.11. Populations of stably transduced mutant library ZF-eGFP-expressing Jurkat cells were incubated with a concentration curve of one of six candidate IMiD analogues (thalidomide, **88**, **26**, lenalidomide, **86** and **87**) to obtain single replicate concentration curves. Curve shapes were used to aid the decision making process for which concentration should be used for each compound in the ZF mutant library screen.

A single set of incubations was performed to obtain degradation curves (Figure 4.11) for compounds **1**, **2**, **26**, **86**, **87** and **88**. The concentration ranges chosen for each compound were determined using data obtained from the initial screen discussed in Chapter 2. At the time this assay was carried out it had not yet been decided whether to include compound **86** or **87**, so curves for both were obtained. Similarly, compound **99** had not yet been chosen, so a curve was not obtained for it. Eventually compound **86** was chosen over compound **87** to represent the analogues of compound **68**, and

the concentration of compound **99** to use was chosen by using the same concentration chosen for compound **26**. Inspection of the curves yielded the concentrations seen in Table 4.2.

Table 4.2. Concentrations used for compounds **1**, **2**, **26**, **86**, **88** and **99**, which were employed in the mutant library screen.

Compound	Concentration
Thalidomide (1)	313 nM
Lenalidomide (2)	6.25 nM
88	313 nM
99	313 nM
26	625 nM
86	7.81 nM



4.4 Library Screen Results

4.4.1 Quality Control

After next generation sequencing was performed on all replicates of the sorted populations, raw sequencing data were processed by Dr Becky Saunders at GSK.

Figure 4.12 shows similarity of sequences between all library replicates for populations with low eGFP:mCherry ratios (i.e. populations in which high ZF degradation had occurred). A higher value in this figure represents a higher similarity of component sequences, and a value of 1 would indicate that two replicates are identical in their sequence content. This plot shows not only that replicates of the same compound screen have high similarity, but that replicates of screens of related compounds also possess a high degree of similarity, which correlates with how structurally similar they are. For instance, it can be seen that the thalidomide and lenalidomide screens have

high similarity, indicating that these two compounds preferentially degrade many of the same mutant degrons in the library. The same can also be seen for the three 5-‘bumped’ compounds (**26**, **88** and **99**).

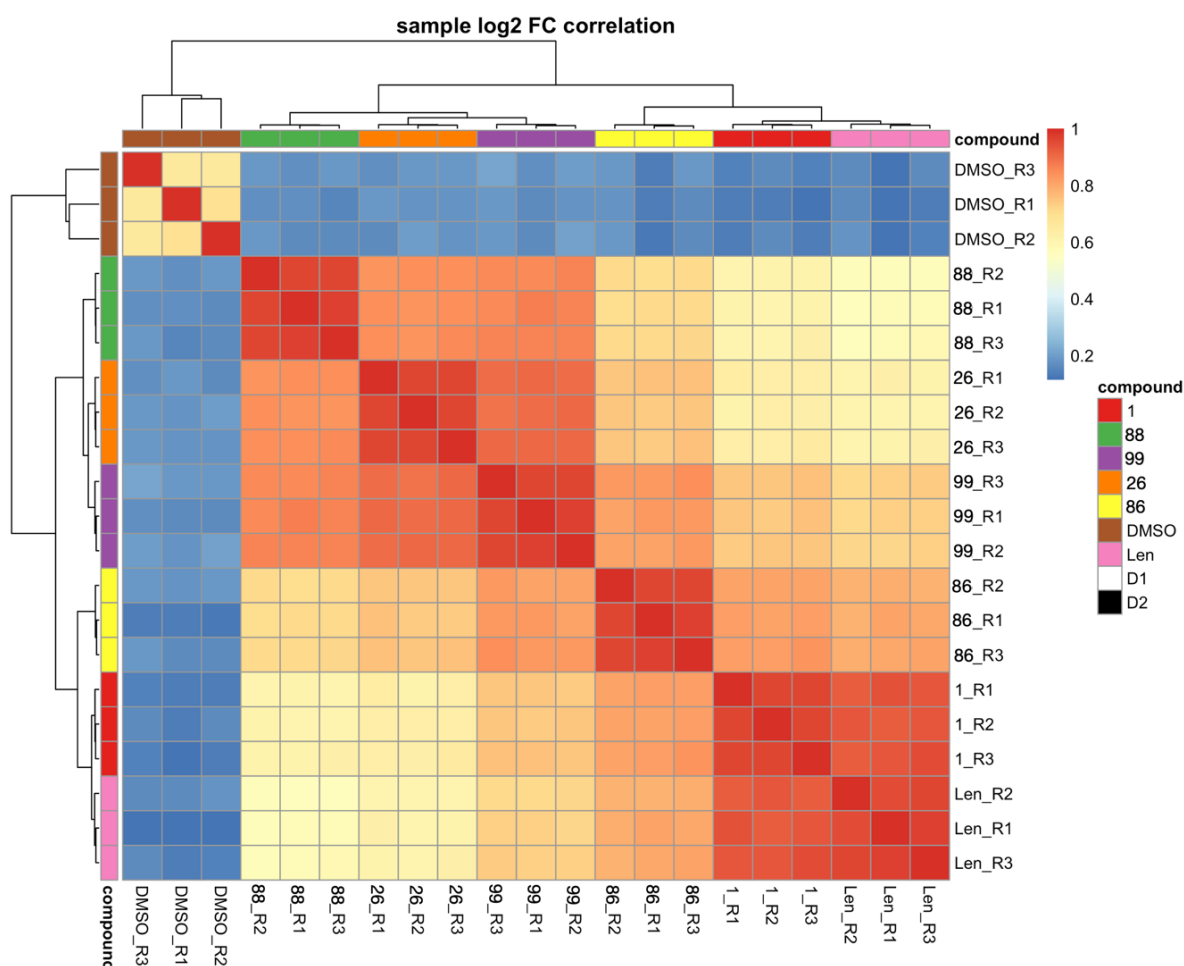


Figure 4.12. Similarity of enriched sequences observed in populations of library ZF-eGFP-expressing Jurkat cells with low eGFP fluorescence. Data shown for three replicates of each compound screen and three DMSO control replicates. A value of 1 represents identical sequences, with 0 meaning no sequence similarity. High sequence similarity is observed between replicates of each compound screen but not between DMSO controls. High sequence similarity is also observed for similarly ‘bumped’ compounds (e.g. **88**, **99** and **26**) and the two control IMiDs (thalidomide (**1**) and lenalidomide).

4.4.2 Volcano Plots

Figure 4.13 shows volcano plots for all compound screens and the DMSO control. All values shown are the mean across all three replicates. The ‘log2FC’ (fold-change at log base 2) value represents how over-represented a sequence is in the population of

cells exhibiting low eGFP:mCherry ratios (the separated ‘shoulder’ population) compared to the remaining cells, in which degradation of the ZF-eGFP fusion protein was low. A log₂FC value of 0 indicates equal representation of a sequence in both populations; a value of +4 would indicate a 16-fold enrichment of a sequence in the ‘shoulder’ population. The ‘-log₁₀p’ (p value at log base 10) value represents the confidence in the observed log₂FC value. In this case, the vast majority of p values are >1, so log₂FC values can be used confidently to infer sequence performance. The dark brown data points represent those for which some degradation is observed when there is no compound treatment (log₂FC > 1.5 for the DMSO control). The single green data point seen in each plot highlights the position of the WT (IKZF1/3 ZF degron) sequence.

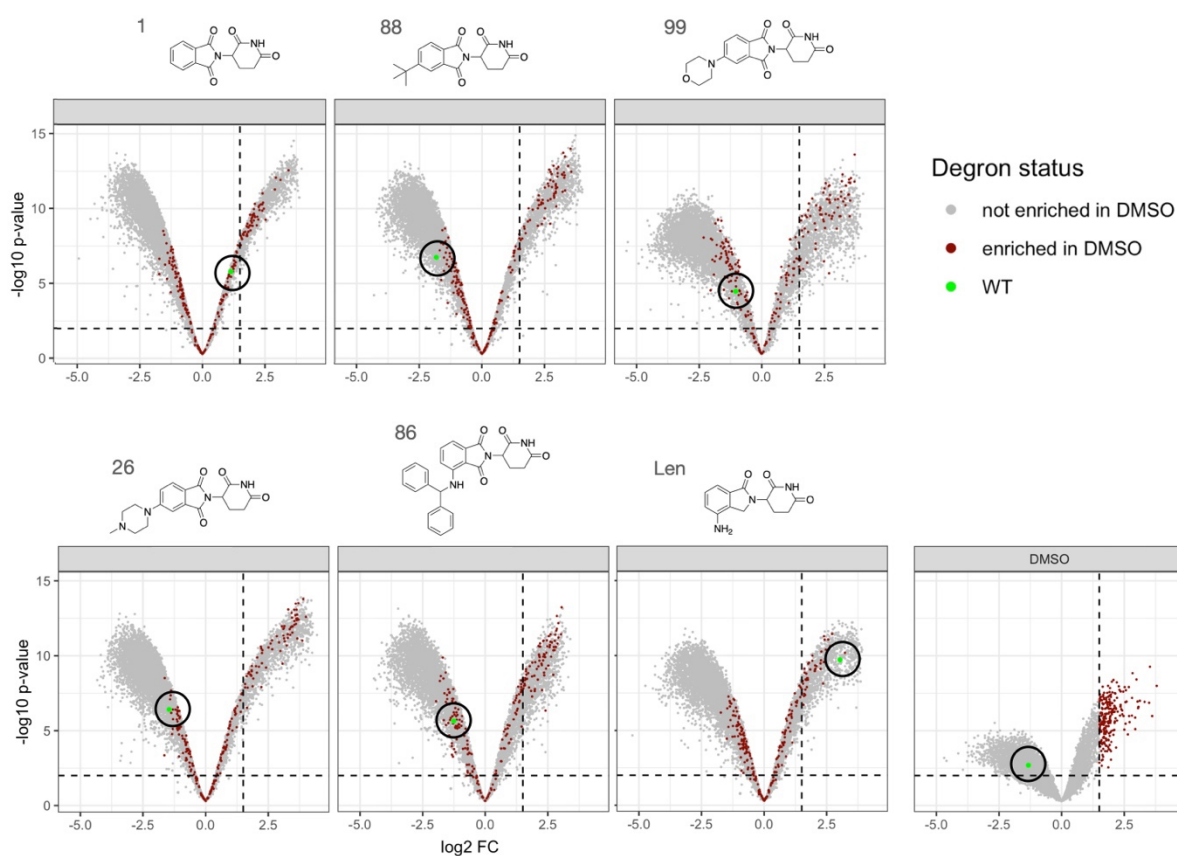


Figure 4.13. Volcano plots for the six compounds (thalidomide (**1**), lenalidomide (**2**), **26**, **86**, **88** and **99**) tested against the mutant library, and a DMSO control. Brown data points represent sequences that were enriched in the DMSO control; the green data point in each plot represents the WT sequence (i.e. no change in sequence from the original IKZF1/3 ZF degron).

In the case of lenalidomide, the WT sequence has a high log₂FC value, indicating that lenalidomide degrades the WT sequences more efficiently than most of the mutant sequences present in the library. There are also some sequences with higher log₂FC values, that are degraded more efficiently than the WT by lenalidomide. This proportion of mutants with log₂FC scores higher than the WT sequences increases for thalidomide, indicating that even without a 'bump' group present on the thalidomide structure, selectivity can be achieved through degron mutation alone. However, the WT sequence possesses a positive log₂FC value, so is still degraded more efficiently by thalidomide compared to the majority of library sequences. The separation in log₂FC values between the WT and top performing mutants is far larger for the four bumped compounds (**26**, **86**, **88** and **99**). In all four cases, the WT sequence possesses a negative log₂FC value, indicating that no significant degradation has been observed at the compound concentrations used. Furthermore, these data indicate that, for all four bumped compounds, a large proportion of the library comprises sequences that are efficiently degraded in their presence.

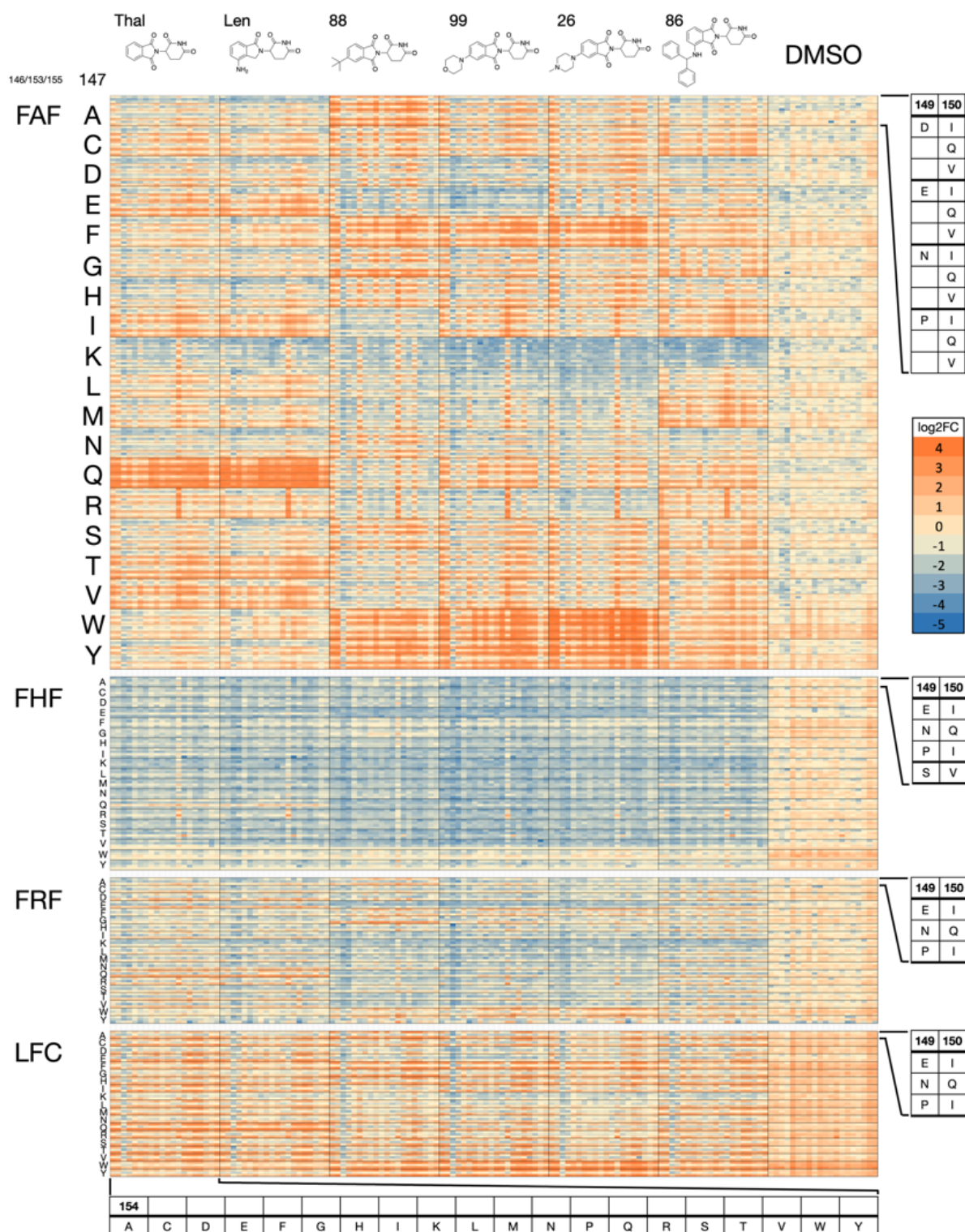
4.3.3 Complete Library Screen Data

Table 4.3 shows the full library data obtained from all the compound screens and the DMSO control. In all cases the log₂FC values shown are derived from the mean of three replicates. Sections D and E of the library (see section 4.2.3) are not included in this figure as the six tested compounds induced no significant degradation for any of these 20 mutants.

Table 4.3 is intended as a map, which can be used to navigate the full log₂FC data for all sequences (Appendix S.3) and to reveal overall patterns in the library screen data.

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Table 4.3. Log2FC data for six compounds and a DMSO control against all library mutants except sections D and E – 8360 mutants in total. Log2FC values are arranged according to compound and residue 154 on the x-axis, and values are arranged according to residues 146/153/155, 147, 149 and 150 on the y-axis. Colour scale shows high log2FC as orange, representing high sequence enrichment, and low log2FC as blue, representing low sequence occurrence. A log2FC score of zero, coloured as tan, represents equal representation of a sequence in the ‘degraded’ cell population and the remaining cell population after FACS.



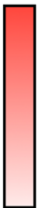
The four main sections of this figure group the sequences according to the residues present at positions 146, 153 and 155 – the ‘core’ region of the ZF. Sequences with the ‘FHF’ motif at these positions are generally not degraded efficiently by any of the six tested compounds. Sequences with the ‘LFC’ motif are, by comparison, more efficiently degraded in the presence of all six compounds; however, many of the sequences with the ‘LFC’ motif also exhibit high log₂FC values in the DMSO control screen, indicating either that they are degraded at least moderately even in the absence of compound, or that they are not expressed well by the cell in the first place.

Table 4.4 shows the difference in mean log₂FC values for all sequences with a given motif at the 146/153/155 positions compared between treatment with all IMiD analogues (all values combined) and the DMSO control. A more positive mean log₂FC value represents higher mean degradation of the sequences by either IMiDs, or in the absence of compound (DMSO). The greater the difference value (mean ‘IMiD’ log₂FC minus mean ‘DMSO’ log₂FC), the greater the separation between the mean ‘IMiD’ degradation level and the mean ‘DMSO’ degradation; meaning that a set of sequences is degraded relatively efficiently in the presence IMiDs, but degradation in the absence of IMiDs is comparatively low.

Table 4.4. Mean log₂FC values for all mutants in the library with specified motifs at positions 146, 153 and 155. Mean taken across all six compound screens (IMiD column) and for untreated control screen (DMSO column). Difference column shows IMiD value minus DMSO value. Of particular note are the FAF and LFC rows: despite LFC having a higher mean log₂FC value in the IMiD column than the FAF row, LFC possesses a smaller ‘difference’ value than FAF.

Residues as 146/153/155	Mean log ₂ FC		Difference
	IMiD	DMSO	
FAF	-0.287	-0.828	0.541
FHF	-2.305	-0.357	-1.949
FRF	-1.392	-0.494	-0.898
LFC	0.016	0.652	-0.636

Highest Mean Degradation Relative to DMSO Control



Lowest Mean Degradation Relative to DMSO Control

These values indicate that the mutants with 'FAF' at these positions (i.e. same as WT) show the maximum overall degradation upon compound treatment, while not also being degraded (or not expressing well) when no compound is added. Consequently, attention was focused on this group for subsequent work.

Table 4.5 shows degradation trends for the 'FAF' library subset (Section A from figure 4.5). For each compound, mean log2FC values are shown for all mutations except for the position specified on the Y axis (residue 147). For instance, the top left cell of Table 4.5A shows the mean log2FC value for all the library sequences in the 'FAF' subset, with alanine at position 147 for the thalidomide screen.

Table 4.5. Mean log2FC values for the 'FAF' section of the mutant library. **A)** Mean log2FC values for all mutants with specified residue at position 147. **B)** Mean log2FC values for all mutants with specified residue at position 149. **C)** Mean log2FC values for all mutants with specified residue at position 150. **D)** Mean log2FC values for all mutants with specified residue at position 154. Colour scale shows high mean log2FC as orange, representing high mean sequence enrichment (and hence a high level of degradation), and low mean log2FC as blue, representing low mean sequence occurrence.

A	147	Thal	Len	88	99	26	86	DMSO
	A	-1.06	-1.22	1.15	-0.26	-0.47	-0.75	-0.93
	C	-0.10	-0.30	0.13	-0.11	-0.04	0.43	-0.88
	D	-0.85	-0.95	-0.76	-1.69	0.04	-1.10	-0.93
	E	0.22	0.44	-1.53	-2.22	-0.83	-0.31	-1.51
	F	-0.59	-0.39	1.31	1.35	1.67	0.31	-0.29
	G	-0.76	-0.82	0.44	-0.40	-0.46	0.22	-0.62
	H	-1.33	-1.17	0.00	-0.37	0.12	-0.44	-0.92
	I	0.46	0.28	-1.57	0.14	-0.56	0.60	-0.87
	K	-1.85	-2.14	-1.85	-2.75	-2.75	-2.83	-1.19
	L	-0.37	-0.38	-1.20	-1.70	-2.12	-0.31	-0.85
	M	-0.50	-0.63	-1.39	-1.09	-1.35	0.88	-0.77
	N	-1.30	-1.12	-0.16	-1.20	-1.08	-1.19	-0.89
	Q	2.35	2.93	-0.94	0.42	-0.17	0.42	-1.07
	R	-0.04	-0.09	-1.23	-1.18	-1.61	0.22	-0.77
	S	-0.56	-0.83	0.30	-0.26	-0.11	0.27	-0.96
	T	0.63	0.66	-0.82	0.07	-0.18	0.50	-1.01
	V	0.66	0.55	-1.16	0.10	-0.40	0.38	-1.06
	W	-0.50	-0.36	1.39	1.96	3.01	0.65	0.18
	Y	-0.67	-0.62	1.53	1.66	2.04	0.87	-0.43
B	149	Thal	Len	88	99	26	86	DMSO
	D	-1.33	-1.75	-0.82	-1.03	-0.67	-0.95	-1.27
	E	0.21	0.14	0.25	0.01	0.14	0.32	-1.38
	N	-0.71	-0.41	-1.09	-0.89	-0.66	-0.47	-0.43
	P	0.53	0.72	0.32	0.33	0.08	0.85	-0.24
C	150	Thal	Len	88	99	26	86	DMSO
	I	0.55	0.36	0.67	0.60	0.63	0.69	-0.66
	Q	-1.57	-1.26	-1.70	-1.83	-1.59	-1.20	-1.01
	V	0.04	-0.06	0.03	0.04	0.13	0.32	-0.81
D	154	Thal	Len	88	99	26	86	DMSO
	A	0.39	0.42	0.88	0.72	1.00	0.79	-1.03
	C	-0.03	-0.04	0.12	0.09	0.25	0.67	-0.81
	D	-1.47	-1.37	-1.92	-2.00	-1.65	-1.19	-1.46
	E	-0.71	-0.76	-0.87	-1.05	-0.71	-0.65	-1.72
	F	-1.06	-1.11	-1.02	-1.24	-1.22	-0.40	-0.15
	G	-0.39	-0.51	0.65	-0.23	0.12	0.15	-0.97
	H	-0.63	-0.36	-0.49	-0.65	-0.68	-0.26	-0.87
	I	-0.42	-0.56	-0.85	-0.66	-0.53	-0.02	-0.25
	K	0.01	-0.08	0.09	-0.18	-0.42	-1.25	-1.18
	L	-0.82	-0.81	-1.15	-1.13	-0.99	-0.13	-0.48
	M	-0.61	-0.52	-0.52	-0.63	-0.49	-0.08	-0.69
	N	-0.18	0.03	-0.46	-0.16	-0.06	0.21	-1.14
	P	0.71	0.59	1.17	0.99	1.07	0.49	-0.50
	Q	0.15	0.47	0.06	0.30	0.33	0.57	-1.17
	R	0.36	0.32	0.33	0.29	-0.12	-0.44	-0.85
	S	0.16	0.20	0.66	0.46	0.72	0.56	-1.28
	T	-0.24	-0.31	-0.57	-0.22	0.02	0.16	-1.23
	V	-0.20	-0.34	-0.63	-0.29	-0.16	0.47	-0.68
	W	-1.20	-1.26	-1.45	-1.63	-1.47	-0.94	0.06
	Y	-0.36	-0.48	-0.61	-0.72	-0.53	0.03	-0.17

LogFC
3
2
1
0
-1
-2
-3

Table 4.5A shows clear differences in preference for residue at position 147 between different compounds. Glutamine at 147 is preferred for thalidomide and lenalidomide, while compound **88** (5-*tert*-butyl ‘bump’) exhibits a preference for alanine and the three aromatic residues (phenylalanine, tryptophan and tyrosine). Compounds **26** (5-methylpiperazine ‘bump’) and **99** (5-morpholine ‘bump’) show similar preference for aromatic residues at 147, with tryptophan being particularly favoured by compound **26**. However, compound **86** does not possess a clear preference for any particular residue or subset of residues at position 147. A lysine residue at position 147 is generally disfavoured across all compounds. It can also be seen that, in the absence of compound, mutants with phenylalanine, tryptophan or tyrosine at position 147 are generally slightly overrepresented in the low eGFP:mCherry population compared to other mutants.

Table 4.5B indicates a universal preference for glutamic acid or proline at position 149 over aspartic acid or the WT asparagine. As the residue at this position does not come into direct contact with the IMiD, it is unsurprising that this preference is common to both ‘bumped’ and non-‘bumped’ IMiDs. A similar uniformity in preference is seen in Table 4.5C, where all compounds show clear preference for either isoleucine or valine at position 150, over the WT glutamine. It is also notable that, without compound treatment, sequences with asparagine or proline at position 149 are slightly overrepresented in the ‘shoulder’ population, compared to aspartic acid or glutamic acid.

Figure 4.5D shows an apparent modest universal preference for alanine, proline, or serine at position 154. As residue 154 is proximal to residue 147, residue preference at this position is dependent on the residue present at position 147, so mean log2FC

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values that do not consider 147 mutations individually do not provide insight for studying the effects of choice of residue at position 154.

Table 4.6. Mean log2FC values from 'FAF' mutant library section for all mutants with specified residue positions 147 and 154. Compound screen indicated in top left of each table (screens for thalidomide, lenalidomide, **88**, **99**, **26** and **86** shown, as well as a DMSO control). Colour scale shows high mean log2FC as orange, representing high mean sequence enrichment, and low mean log2FC as blue, representing low mean sequence occurrence.

Thal	154																			
	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A	-0.42	-1.12	-2.16	-1.66	-1.86	-1.19	-1.21	-1.41	-0.87	-1.90	-1.34	-1.16	-0.24	-0.65	-0.72	-0.78	-1.32	-1.11	-2.00	-1.30
C	0.53	0.19	-1.45	-0.90	-1.43	-0.59	-0.63	-0.38	0.42	-0.81	-0.39	0.07	0.11	0.43	0.84	0.35	0.18	-0.05	-1.54	-0.89
D	-0.33	-0.82	-2.04	-1.68	-1.40	-1.51	-0.79	-1.32	-0.56	-1.45	-0.90	-0.78	-1.00	0.05	0.61	-0.33	-1.08	-1.00	-1.63	-0.97
E	1.37	0.92	-0.68	-0.47	-1.14	0.03	0.14	0.52	0.97	-0.27	0.06	1.33	0.76	1.28	1.91	1.20	0.63	0.83	-1.00	-0.49
F	0.06	0.04	-1.89	-0.78	-1.09	-0.96	0.24	-0.55	-0.20	-0.69	-0.78	0.38	-0.37	0.66	0.37	0.10	0.06	-0.26	-1.33	-0.78
G	-0.21	-0.54	-1.75	-1.17	-1.08	-1.41	-1.01	-0.61	-0.33	-1.15	-0.93	-0.50	-1.42	-0.38	-0.25	-0.47	-0.67	-0.40	-1.57	-0.57
H	-0.54	-0.79	-2.69	-1.71	-1.76	-1.54	-1.06	-1.40	-0.80	-1.45	-1.09	-1.45	0.29	0.04	-0.45	-0.99	-1.45	-1.24	-2.03	-1.21
I	1.10	0.53	-0.98	0.24	-0.80	0.64	0.32	-0.65	0.57	-0.32	-0.03	1.01	1.80	1.48	0.91	1.04	0.01	-0.26	-0.17	0.01
K	-1.16	-2.26	-2.28	-1.97	-2.61	-1.48	-2.35	-2.47	-2.49	-3.21	-2.70	-1.96	0.15	-1.61	-2.47	-1.78	-2.55	-2.64	-2.47	-2.41
L	-0.21	-0.07	-1.89	-0.66	-1.34	-0.73	-0.74	-0.32	-0.03	-0.25	-0.50	-0.11	1.35	0.09	0.59	-0.19	-0.16	-0.44	-1.37	-0.71
M	0.59	-0.51	-1.38	-0.86	-2.04	-0.11	-0.97	-0.93	-0.76	-1.25	-1.00	-0.11	1.30	0.17	-0.43	0.29	-0.56	-0.69	-1.81	-1.33
N	-0.58	-0.96	-2.24	-1.45	-1.38	-1.50	-1.14	-1.49	-1.14	-1.33	-1.10	-1.24	0.17	-0.44	-0.61	-0.91	-1.51	-1.30	-1.53	-0.78
Q	3.45	3.43	2.27	3.08	1.80	2.32	2.55	3.45	3.26	2.39	3.25	2.98	2.82	3.35	3.33	3.29	3.46	3.47	1.67	2.42
R	1.17	-0.21	-0.19	-0.58	-0.52	0.51	-0.70	-0.44	-0.34	-0.27	-0.62	-0.43	2.99	0.10	-0.83	0.39	-0.55	-0.24	-0.88	-0.25
S	0.19	-0.43	-1.82	-1.49	-1.74	-1.22	-1.06	-1.08	-0.51	-1.48	-0.97	-0.59	0.03	-0.18	-0.01	-0.23	-0.71	-0.71	-1.69	-0.89
T	1.33	1.09	-0.42	0.10	-0.21	0.42	0.52	0.07	1.01	-0.41	0.29	0.91	1.34	1.25	1.83	1.51	1.08	0.78	-1.18	0.93
V	1.62	0.74	-0.57	0.34	-0.64	1.17	0.52	-0.39	0.86	-0.31	0.31	1.20	2.05	1.61	1.22	1.31	0.29	0.08	-0.93	0.58
W	0.18	-0.04	-1.98	-1.46	-0.77	-1.22	0.53	-0.36	-0.06	-0.80	-0.67	0.87	0.43	1.08	0.38	0.28	-0.55	-0.53	-1.01	-0.69
Y	-0.20	-0.01	-1.94	-1.40	-1.14	-1.25	-0.03	-0.97	-0.49	-0.86	-0.75	0.20	-0.19	0.52	-0.13	-0.23	-0.49	-0.66	-1.48	-0.88
88	154																			
	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A	2.37	1.87	-0.26	0.12	0.23	1.65	1.00	0.94	2.30	0.06	1.31	0.70	1.60	1.63	2.57	2.13	1.52	1.24	-0.62	0.75
C	1.74	0.73	-1.32	-0.56	-1.11	1.40	-0.34	-0.52	1.20	-1.58	0.00	0.22	1.53	0.76	1.14	1.42	-0.08	-0.32	-1.22	-0.57
D	-0.02	-0.59	-2.33	-1.93	-1.66	-0.39	0.78	-1.55	-0.15	-1.71	-1.32	-0.19	-0.40	-0.37	1.21	0.51	-0.69	-1.27	-1.94	-1.14
E	0.43	1.30	-2.19	-2.19	-2.45	-0.27	-2.15	-2.11	-1.28	-2.79	-1.18	-1.73	0.06	-1.78	0.71	0.02	-1.63	-1.64	-2.68	-1.48
F	1.81	2.08	-1.19	1.33	0.75	1.82	1.05	0.91	1.98	0.93	1.28	1.19	2.63	2.00	2.39	1.82	1.19	1.29	0.19	1.09
G	0.80	1.54	-1.64	-0.72	0.50	0.20	-0.32	0.94	1.67	0.50	1.22	-0.33	0.04	1.27	1.57	0.85	0.69	-1.00	0.08	0.88
H	1.25	0.72	0.32	-0.34	0.66	1.31	-0.07	-0.87	0.99	0.51	0.08	-0.70	2.11	0.27	0.78	0.42	0.32	1.65	-0.30	1.17
I	-1.02	-1.58	-2.75	-1.88	-1.87	-1.30	-1.74	-1.81	-1.52	-1.88	-1.57	-0.31	-1.31	-1.13	-1.19	-1.38	-1.75	-1.97	-1.67	-1.67
K	0.09	-1.54	-2.46	-2.33	-2.69	0.24	-2.14	-2.58	-2.35	-2.89	-2.43	-2.04	0.59	-1.44	-2.49	-0.42	-2.39	-2.70	-2.39	-2.54
L	-0.23	-0.87	-2.56	-1.14	-1.63	0.16	-1.26	-1.57	-1.20	-1.82	-1.64	-1.16	0.40	-0.95	-1.04	-0.32	-2.33	-1.86	-1.72	-2.19
M	0.22	1.38	-2.80	-1.84	-2.42	0.58	-1.71	-1.85	-1.27	-2.30	-1.78	-1.53	1.29	-1.28	-1.09	-0.47	-2.09	-1.95	-2.11	-1.94
N	1.00	0.36	-2.24	-0.66	-0.80	1.19	0.35	-1.33	0.14	-1.20	-0.48	0.13	1.32	0.37	0.57	0.98	-0.56	-0.76	-1.65	-0.02
Q	0.84	0.62	-1.69	-0.92	-2.30	0.49	-1.59	-1.43	-0.85	-1.95	-1.37	-1.00	1.33	-0.80	-1.21	0.50	-1.33	-1.15	-1.93	-0.96
R	0.85	1.36	-2.34	-1.90	-1.54	0.02	-1.53	-1.22	-1.59	-2.40	-1.64	-1.54	2.13	1.27	0.82	0.29	-1.89	-2.00	-1.62	-1.71
S	1.51	0.95	-1.41	-0.60	-0.71	1.07	0.21	-0.15	0.86	1.00	0.15	0.15	1.18	0.61	1.50	1.52	0.81	0.23	-1.19	0.30
T	1.06	0.32	-1.96	-1.49	-1.42	0.21	-1.11	-1.57	-0.86	-1.75	-1.23	-1.22	0.71	-1.15	0.09	0.41	-1.13	-1.17	-1.64	-0.86
V	-0.24	-1.23	-2.38	-1.68	-1.57	0.45	-1.38	-1.64	-0.89	-1.80	-1.40	-1.30	0.71	-1.09	-0.34	-0.68	-1.47	-1.72	-1.13	-1.13
W	2.05	2.46	-1.20	0.83	1.17	1.39	1.17	2.41	0.89	1.75	1.72	1.75	1.90	2.10	2.29	0.97	1.36	0.11	1.37	1.37
Y	2.20	2.43	-0.90	1.34	1.14	2.13	1.39	2.01	2.06	1.36	1.59	1.52	2.87	1.96	2.18	2.14	1.33	1.55	0.01	1.39
26	154																			
	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A	1.17	0.24	-1.52	-0.68	-1.49	-0.36	-1.04	-0.45	-0.76	-1.43	-0.74	-0.61	0.05	-0.51	0.62	0.37	0.22	-1.81	-0.76	-0.76
C	1.42	0.67	-1.39	-0.32	-1.44	-0.97	-0.25	-0.16	-0.24	-0.27	0.21	0.62	0.07	0.64	1.16	0.72	0.22	-1.30	-0.59	-0.59
D	-1.45	-0.56	-1.48	-0.39	-0.93	0.34	0.30	-0.54	-0.41	-0.57	-0.18	0.43	0.25	0.71	1.07	1.36	0.14	-0.13	-1.18	-0.17
E	1.84	0.33	-2.45	-1.79	-2.18	-0.69	-1.85	-1.38	-1.11	-2.46	-1.10	-0.81	0.92	-0.51	-1.09	-1.16	-0.98	-0.75	-2.17	-0.15
F	2.52	2.57	-0.07	1.93	0.39	1.30	1.68	1.48	1.70	1.20	1.31	2.57	1.83	2.70	1.86	2.74	2.65	0.33	1.13	1.13
G	0.84	0.49	-1.59	-0.50	-1.13	-0.67	-0.82	-0.77	-1.30	-0.80	-0.47	-0.63	-0.65	-0.07	-0.93	0.47	0.27	0.30	-1.61	-0.40
H	1.74	1.28	-2.29	-1.05	-1.16	-0.45	-1.00	-0.28	-0.33	-0.25	0.20	1.59	1.40	0.83	1.67	0.51	0.41	-1.67	-0.44	-0.44
I	-1.25	-0.41	-1.47	-1.31	-1.22	-0.67	-0.88	-1.00	-0.15	-1.24	-0.83	-0.70	0.21	-0.16	-0.18	-0.18	-0.18	-0.18	-0.18	-0.18
K	-2.40	-2.32	-1.26	-3.02	-2.41	-2.36	-2.81	-2.92	-3.10	-2.77	-2.99	-2.83	-1.99	-2.80	-2.78	-2.62	-3.00	-3.10	-2.43	-2.78
L	-1.81	-2.05	-3.01	-2.75	-2.26	-1.86	-2.44	-2.50	-2.13	-2.15	-2.24	-0.17	-2.18	-2.37	-1.98	-2.52	-2.32	-2.13	-1.92	-1.92
M	0.17	-1.42	-2.35	-1.97	-2.02	-0.93	-1.98	-1.65	-1.94	-1.52	-1.77	-1.07	1.35	-0.94	-1.57	-0.41	-1.50	-1.65	-2.08	-1.84
N	0.02	-0.65	-2.44	-1.67	-1.64	-0.50	-1.65	-1.59	-1.28	-1.77	-1.40	-0.90	0.25	-0.32	-0.87	-0.19	-0.99	-1.12	-1.97	-0.96
Q	1.30	0.75	-1.75	-1.24	-1.81	-0.10	-0.13	-0.93	-0.52	-0.57	0.01	0.74	1.70	0.18	0.00	0.69	0.53	0.81	-2.00	-0.49
R	-0.54	-1.81	-2.54	-2.84	-1.79	-0.36	-1.99	-1.90	-1.87	-1.91	-1.96	-1.89	2.39	-1.78	-2.14	-1.20	-1.75	-1.83	-1.78	-1.78
S	1.51	0.95	-1.41	-0.60	-0.71	1.07	0.21	-0.15	0.86	1.00	0.15	0.15	1.18	0.61	1.50	1.52	0.81	0.23	-1.19	0.30
T	1.06	0.32	-1.96	-1.49	-1.42	0.21	-1.11	-1.57	-0.86	-1.75	-1.23	-1.22	0.71	-1.15	0.09	0.41	-1.13	-1.17	-1.64	-0.86
V	-0.24	-1.23	-2.38	-1.68	-1.57	0.45	-1.38	-1.64	-0.89	-1.80	-1.40	-1.30	0.71	-1.09	-0.34	-0.68	-1.47	-1.72	-1.13	-1.13
W	2.05	2.46	-1.20	0.83	1.17	1.39	1.17	2.41	0.89	1.75	1.72	1.75	1.90	2.10	2.29	0.97	1.36	0.11	1.37	1.37
Y	2.20	2.43	-0.90	1.34	1.14	2.13	1.39	2.01	2.06	1.36	1.59	1.52	2.87	1.96	2.18	2.14	1.33	1.55	0.01	1.39
DMSO	154																			
	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A	-0.07	-0.38	-1.58	-1.64	-0.25	-0.96	-0.88	-0.52	-1.19	-0.67	-0.70	-1.80	-0.38	-1.42	-0.77	-1.50	-1.15	-0.80	-0.11	-0.27
C	-1.07	-0.74	-1.38	-1.76	-0.24	-0.77	-1.05	-1.22	-1.34	-0.87	-0.71	-1.33	-0.58	-1.24	-0.85	-1.55	-12			

and 150 are shown. For instance, the top left cell of Table 4.6A shows the mean log₂FC value for all 12 sequences that have alanine at position 147 and alanine at position 154, within the 'FAF' library subsection. This provides more insight into the cooperativity between residues 147 and 154. Certain pairings of residues at 147 and 154 are revealed to be particularly beneficial for facilitating degradation, such as W147/S154 for compound **26**. A suitable 154 residue can sometimes 'rescue' (i.e. enable degradation) a mutant with an otherwise unsuitable residue at position 147, such as K147/P154 for compound **99**.

Table 4.7 shows the residues present at positions 146, 147, 149, 150, 152, 153, 154, 155 and 167 for the top 20 ranked sequence according to log₂FC score for each compound screen. The WT sequence is included in each list for comparison, including the log₂FC score for the WT for that particular compound screen – this is included for comparison against the log₂FC scores of the top ranked sequences in that screen. Trends that can be seen in Tables 4.5 and 4.6 are also seen represented in the sequences in Table 4.7, such as the high prevalence of Q147 for the thalidomide and lenalidomide, the high prevalence of W147 for compound 26, or the high prevalence of both E149 and P149 across all compounds.

Chapter 4: Mutant Degron Library Screen

Table 4.7. Residues present at positions 146, 147, 149, 150, 152, 153, 154, 155 and 167 for the top 20 sequences, ranked by log2FC score, for all six compounds screened against the mutant ZF library (thalidomide, lenalidomide, **88**, **99**, **26** and **86**), as well as the DMSO control. Each amino acid type is coloured according to a key. The WT sequence and log2FC score for each compound screen is included at the bottom of the screen table for reference.

Compound		Thalidomide										Log2FC	Lenalidomide										Log2FC	Compound 88										Log2FC
Rank		146	147	149	150	152	153	154	155	167	146		147	149	150	152	153	154	155	167	146	147		149	150	152	153	154	155	167				
A		1	F	Q	P	V	G	A	K	F	L	3.9618	F	Q	N	I	G	A	E	F	L	3.9142	F	H	E	I	G	A	P	F	L	4.0013		
C		2	F	Q	P	V	G	A	P	F	L	3.8717	F	Q	P	V	G	A	D	F	L	3.8922	F	F	E	V	G	A	P	F	L	3.9979		
D		3	F	Q	N	I	G	A	T	F	L	3.8667	F	Q	N	V	G	A	V	F	L	3.8861	F	W	E	I	G	A	K	F	L	3.9155		
E		4	F	Q	N	I	G	A	I	F	L	3.8114	F	Q	P	V	G	A	T	F	L	3.8852	F	F	D	I	G	A	P	F	L	3.9195		
F		5	F	Q	P	I	G	A	D	F	L	3.8059	F	Q	N	I	G	A	Q	F	L	3.8639	F	A	E	V	G	A	K	F	L	3.9176		
G		6	F	Q	P	V	G	A	T	F	L	3.7941	F	Q	P	V	G	A	N	F	L	3.8156	F	F	P	I	G	A	P	F	L	3.9164		
H		7	F	Q	P	I	G	A	I	F	L	3.7924	F	Q	P	I	G	A	L	F	L	3.8345	F	A	E	V	G	A	R	F	L	3.9084		
I		8	F	Q	P	I	G	A	S	F	L	3.7900	F	Q	N	I	G	A	T	F	L	3.8290	F	Y	E	I	G	A	K	F	L	3.9035		
K		9	F	Q	E	V	G	A	Q	F	L	3.7719	F	Q	N	I	G	A	A	F	L	3.8170	F	G	P	I	G	A	C	F	L	3.9002		
L		10	F	Q	P	I	G	A	C	F	L	3.7711	F	Q	E	V	G	A	A	F	L	3.7915	F	A	E	I	G	A	A	F	L	3.8965		
M		11	F	Q	P	V	G	A	E	F	L	3.7612	F	Q	N	I	G	A	I	F	L	3.7833	F	G	P	V	G	A	C	F	L	3.8841		
N		12	L	Q	P	I	G	F	V	C	L	3.7517	F	Q	E	I	G	A	R	F	L	3.7803	F	Y	E	V	G	A	A	F	L	3.8726		
P		13	F	Q	E	I	G	A	S	F	L	3.7484	F	Q	E	Q	G	A	C	F	L	3.7802	F	A	E	V	G	A	S	F	L	3.8698		
Q		14	F	Q	P	V	G	A	V	F	L	3.7460	F	Q	P	I	G	A	S	F	L	3.7741	F	Y	N	I	G	A	P	F	L	3.8168		
R		15	F	Q	E	V	G	A	T	F	L	3.7446	F	Q	P	V	G	A	V	F	L	3.7703	F	Y	P	I	G	A	P	F	L	3.8626		
S		16	F	Q	E	V	G	A	P	F	L	3.7224	F	Q	E	V	G	A	I	F	L	3.7619	F	Y	E	I	G	A	P	F	L	3.8608		
T		17	F	Q	E	V	G	A	V	F	L	3.7106	F	Q	P	Q	G	A	V	F	L	3.7592	F	G	E	V	G	A	R	F	L	3.8587		
V		18	F	Q	N	I	G	A	P	F	L	3.7088	F	Q	P	Q	G	A	C	F	L	3.7152	F	G	P	V	G	A	M	F	L	3.8585		
W		19	F	Q	E	V	G	A	R	F	L	3.7061	F	Q	E	Q	G	A	V	F	L	3.7519	F	W	E	I	G	A	C	F	L	3.8152		
Y		20	F	Q	E	I	G	A	R	F	L	3.7041	F	Q	P	Q	G	A	A	F	L	3.7513	F	A	E	I	G	A	R	F	L	3.8372		

WT	F	Q	N	Q	G	A	S	F	L	1.1337	F	Q	N	Q	G	A	S	F	L	3.0311	F	Q	N	Q	G	A	S	F	L	-1.8291
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Compound		Compound 99										Log2FC	Compound 26										Log2FC	Compound 86										Log2FC
Rank		146	147	149	150	152	153	154	155	167	146		147	149	150	152	153	154	155	167	146	147		149	150	152	153	154	155	167				
1		F	Y	E	V	G	A	T	F	L	4.1392	F	W	E	I	G	A	S	F	L	4.4149	F	R	E	V	G	A	P	F	L	3.3928			
2		F	Y	E	I	G	A	T	F	L	4.0154	F	W	D	I	G	A	N	F	L	4.2762	F	R	D	I	G	A	P	F	L	3.3226			
3		F	R	E	I	G	A	P	F	L	3.9835	F	W	N	I	G	A	Q	F	L	4.2715	F	R	E	I	G	A	P	F	L	3.3123			
4		F	I	P	V	G	A	P	F	L	3.9370	F	W	D	I	G	A	A	F	L	4.2601	F	R	P	V	G	A	P	F	L	3.2684			
5		F	W	E	I	G	A	Q	F	L	3.9298	F	W	E	V	G	A	S	F	L	4.2567	F	M	N	I	G	A	P	F	L	3.2358			
6		F	W	P	V	G	A	Q	F	L	3.9190	F	W	D	V	G	A	Q	F	L	4.2369	F	R	D	V	G	A	P	F	L	3.2095			
7		F	W	E	V	G	A	H	F	L	3.9186	F	Y	E	V	G	A	S	F	L	4.2356	F	M	E	I	G	A	A	F	L	3.1949			
8		F	W	E	V	G	A	A	F	L	3.9041	F	W	D	I	G	A	S	F	L	4.2199	L	Y	E	I	G	F	T	C	L	3.1905			
9		L	Y	E	I	G	F	R	C	L	3.9018	F	W	D	V	G	A	S	F	L	4.2017	L	C	P	I	G	F	Y	C	L	3.1590			
10		F	W	D	V	G	A	N	F	L	3.8903	F	W	P	V	G	A	Q	F	L	4.1974	L	C	P	I	G	F	T	C	L	3.1410			
11		F	Y	E	V	G	A	N	F	L	3.8762	F	W	E	Q	G	A	S	F	L	4.1915	F	Y	E	I	G	A	Q	F	L	3.1346			
12		F	Y	E	V	G	A	S	F	L	3.8615	F	W	E	I	G	A	H	F	L	4.1861	F	M	E	I	G	A	P	F	L	3.1328			
13		F	W	E	V	G	A	K	F	L	3.8571	F	W	E	I	G	A	C	F	L	4.1830	F	M	E	V	G	A	P	F	L	3.1157			
14		F	W	E	V	G	A	N	F	L	3.8564	F	Y	E	I	G	A	T	F	L	4.1715	F	G	P	V	G	A	F	F	L	3.1139			
15		F	W	P	V	G	A	N	F	L	3.8155	F	W	D	V	G	A	A	F	L	4.1742	F	Y	E	V	G	A	C	F	L	3.1049			
16		F	W	D	V	G	A	Q	F	L	3.8513	F	W	E	I	G	A	K	F	L	4.1692	L	Y	E	I	G	F	S	C	L	3.0997			
17		F	Y	E	V	G	A	C	F	L	3.8457	F	Y	E	V	G	A	E	F	L	4.1417	L	Y	P	I	G	F	S	C	L	3.0902			
18		F	Y	E	I	G	A	K	F	L	3.8391	F	Y	E	I	G	A	S	F	L	4.1371	F	M	P	I	G	A	P	F	L	3.0896			
19		F	F	E	I	G	A	C	F	L	3.8315	F	W	N	V	G	A	S	F	L	4.1357	F	M	P	V	G	A	P	F	L	3.0869			
20		F	W	E	I	G	A	A	F	L	3.8306	F	W	E	V	G	A	Q	F	L	4.1344	F	M	P	V	G	A	A	F	L	3.0783			

WT	F	Q	N	Q	G	A	S	F	L	-1.0370	F	Q	N	Q	G	A	S	F	L	-1.4561	F	Q	N	Q	G	A	S	F	L	-1.2556
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Compound		DMSO Control										Log2FC
Rank		146	147	149	150	152	153	154	155	167		
1		L	W	P	I	G	F	F	C	L	3.7923	
2		L	W	P	I	G	F	L	C	L	3.6101	
3		L	W	P	I	G	F	P	C	L	3.5229	
4		L	W	P	I	G	F	Y	C	L	3.4893	
5		L	W	P	I	G	F	I	C	L	3.3061	
6		L	W	P	I	G	F	D	C	L	3.1185	
7		L	W	P	I	G	F	M	C	L	3.0653	
8		L	W	P	I	G	F	W	C	L	3.0491	
9		L	W	E	I	G	F	F	C	L	3.0278	
10		L	W	P	I	G	F	C	C	L	2.9859	
11		L	W	N	Q	G	F	I	C	L	2.9385	
12		L	W	N	Q	G	F	F	C	L	2.9343	
13		L	W	E	I	G	F	I	C	L	2.8293	
14		L	W	P	I	G	F	P	C	L	2.8090	
15		L	W	N	Q	G	F	W	C	L	2.8031	
16		F	T	P	I	G	H	Q	F	L	2.7099	
17		F	W	P	I	G	H	G	F	L	2.6906	
18		L	W	N	Q	G	F	L	C	L	2.6802	
19		F	W	P	I	G	A	F	F	L	2.6417	
20		L	W	P	I	G	F	V	C	L	2.5893	

WT	F	Q	N	Q	G	A	S	F	L	-1.325
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Thal

O=C1C(=O)N2C(=O)C(=O)N2C1=O

Len

Nc1ccc2c(c1)nc(=O)[C@@H]3CC[C@H](C2=O)N3=O

88

CC(C)(C)c1ccc2c(c1)nc(=O)[C@@H]3CC[C@H](C2=O)N3=O

99

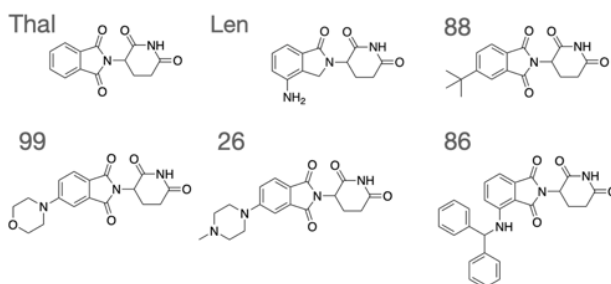
C1CCNCC1c2ccc3c(c2)nc(=O)[C@@H]4CC[C@H](C3=O)N4=O

26

C1CCNCC1c2ccc3c(c2)nc(=O)[C@@H]4CC[C@H](C3=O)N4=O

86

c1ccc(cc1)Nc2ccc3c(c2)nc(=O)[C@@H]4CC[C@H](C3=O)N4=O



4.4 Summary and Conclusions

Having identified suitable IMiD analogue candidates that exhibited 'bump and hole' behaviour paired with a Q147A mutant, a large scale library screen was performed to identify optimised degron sequences for enhanced selectivity. The library screen provided high quality results that reveal a variety of interesting trends regarding IMiD degradation of this ZF degron motif.

The purpose of this study was to identify the most selective IMiD/degron pairs. The data provided by this library have highlighted which sequences are most efficiently degraded by each of the tested compounds. For the purposes of identifying the most selective IMiD/degron pair, a choice now needed to be made of which IMiD/degron pairs identified by the library screen as selective should be carried forward for further testing. As compounds **26** and **88** performed best in the initial WT/Q147A screen, and both induced high levels of degradation for distinct sequences in the library screen, the decision was made to explore a set of the top sequences for each of these two compounds. Compounds **86** and **99** will not be pursued further: **99** because it appeared to be very similar in sequence preference to **26** but not quite as potent; and **86** because it did not display a clear preference for a specific subsection of library sequences.

The next chapter will discuss assays carried out to validate the selectivity of these chosen IMiD/degron pairs.

Chapter 5: IMiD/Degron Pair Validation

5.1 Introduction and Aims

Chapter 4 provided an overview of the mutant degron library screen which was conducted for four promising IMiD analogues (**26**, **86**, **88** and **99**). The screen identified sequences of mutant degrons that were degraded efficiently by the ‘bumped’ analogues; the top performing mutant degrons for each of the four analogues were identified, and a decision was made to further pursue the degrons for compounds **26** and **88** – the two compounds with the highest ‘selectivity values’ from tests discussed in Chapter 3. This chapter will discuss the assays carried out to validate the degradation efficiencies of these top-performing mutants.

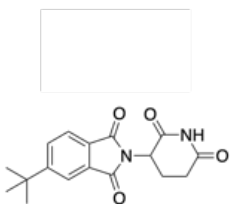
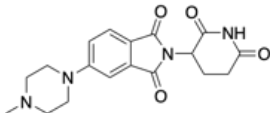
5.2 Further Flow Cytometry Studies

5.2.1 Selection of Top Library Mutants

The library mutants with the top five log₂FC scores for their respective compound screens are shown in Table 5.1. Using methods described previously in Chapter 3, Sprout plasmids coding for eight of these ten mutants were assembled and transduced into Jurkat cells, so that the same ratiometric eGFP:mCherry fluorescence assays could be carried out to obtain pDC₅₀ values for each IMiD/degron pair. The data gathered from these assays were required to validate the potency of these IMiD/degron pairs; the log₂FC values from the library screen were used to identify

which sequences were most efficiently degraded, but could not be used to obtain pDC₅₀ values for the IMiD/degron pairs.

Table 5.1. Five highest ranked sequences by log₂FC value for compounds 88 and 26. Sequences are named according to residues present at positions 147, 149, 150 and 154 (all other positions are the same as WT). Sequences highlighted in red were not successfully cloned.

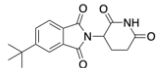
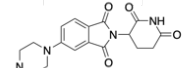
Compound	Rank	Mutant (147/149/150/154)	log ₂ FC
 88	1	HEIP	4.00
	2	FEVP	4.00
	3	WEIK	3.96
	4	FDIP	3.92
	5	AEVK	3.92
 26	1	WEIS	4.45
	2	WDIN	4.28
	3	WNIQ	4.27
	4	WDIA	4.26
	5	WEVS	4.26

Jurkat cell lines for all five of the top sequences for compound **88** were created, as were Jurkat cell lines for the first, third and fourth best performing mutants for compound **26**. However, the mutants with the second and fifth highest log₂FC scores for compound 26 were not successfully cloned, and so transduction could not be attempted. It is not believed that these sequences are inherently inexpressible, only that their plasmids were not synthesised on this particular occasion, and that a second

cloning attempt would have been required to obtain them. However, in the interests of time, it was decided that the first, third and fourth most efficiently degraded sequences for compound **26** would be sufficient for the purposes of this study.

In addition to obtaining Jurkat lines for these top performing library mutants, five control degron sequences were also selected for testing. The full list of degrons for which Jurkat cell lines were made is shown in Table 5.2.

Table 5.2. Sequences selected for further testing with their corresponding IMiD analogues. Values for log₂FC, DC₅₀, pDC₅₀, and selectivity are coloured to highlight sequences that are optimally degraded by their respective compound, with red representing optimal sequences and blue representing sequences that are not well degraded.

Compound	Mutant (147/149/150/154)	Log ₂ FC	DC ₅₀ (nM)	pDC ₅₀	Selectivity (WT/mutant)
Thal	WT	1.13	316	6.50	-
	QPIS	3.79	66.1	7.18	4.8
 88	WT	-1.83	759	6.12	-
	ANQS	-0.98	537	6.27	1.4
	APIS	3.73	74.1	7.13	10.2
	HEIP	4.00	30.2	7.52	25.1
	FEVP	4.00	50.1	7.30	15.1
	WEIK	3.96	42.0	7.38	18.1
	FDIP	3.92	63.1	7.20	12.0
	AEVK	3.92	27.5	7.56	28
 26	WT	-1.46	631	6.20	-
	WNQS	3.90	126	6.90	5.0
	WEIS	4.45	18.2	7.74	35
	WNIQ	4.27	53.7	7.27	11.8
	WDIA	4.26	28.8	7.54	22.0

From this point onwards, all mutant degrons discussed contain a maximum of four mutations, localised entirely to positions 147, 149, 150 and 154; all other positions are the same as the WT IKZF1/3 degron. Therefore a new degron naming system will be

used going forward, in which the residues at these four positions are stated as single letters to make one four-letter name. For example, the single residue mutant Q147A is called 'ANQS', referring to the alanine, asparagine, glutamine and serine residues present at positions 147, 149, 150 and 154, respectively. While the WT degron would be called 'QNQS' under this naming system, it will still be referred to as 'WT' going forward.

Two degrons featuring single mutations at position 147 – ANQS and WNQS – were selected for use with compounds **88** and **26**, respectively. We decided to test these degrons to determine how much extra selectivity resulted from the additional mutations in the top library sequences compared to the mutants with single mutations at position 147.

To highlight the importance of beneficial mutations at positions 149 and 150, a double mutant QPIS was chosen for testing with thalidomide, and for comparison to the degradation of the WT degron by thalidomide.

To show the combined efficacy of the 'bump and hole' effect between ANQS and compound **88**, and the beneficial mutations at positions 149 and 150, a triple mutant APIS was chosen for testing with compound **88** and for comparison with both **88**/ANQS and thalidomide/WT degradation efficiencies.

The top library-identified mutants chosen for testing were HEIP, FEVP, WEIK, FDIP and AEVK for compound **88**; and WEIS, WNIQ and WDIA for compound **26**.

5.2.2 Degradation Curves

Having obtained Jurkat lines stably transduced with these sequences, degradation curves were obtained using the same flow cytometry assay as detailed in Chapter 3.

Instead of the previously used concentration curve rising by intervals of log₁₀ from 1 nM to 10 μ M, a smaller range with more concentrations was employed, so that the most relevant region of the degradation curve could be observed at higher resolution. This concentration gradient starts at 5nM, and increases by log₂ (doubling) up to 10 μ M, yielding 12 data points. All data shown are a mean of three biological repeats, $\pm$ standard error of mean (s.e.m.)

The degradation curves obtained are shown in Figure 5.1. Figure 5.1B shows that thalidomide degrades the QPIS mutant degron more efficiently than the WT degron, highlighting the beneficial nature of the N149P and Q150I mutations.

Figure 5.1C highlights two important results. Firstly, as previously shown in Chapter 3, there is a very weak bump and hole effect observed for the degradation of the WT and ANQS degrons by compound **88**. Secondly, the benefits of the N149P and Q150I mutation are very significant in this context, as compound **88** degrades the APIS triple mutant degron with a 10.2-fold increase in efficiency compared to the WT. It should be noted that, in the full library results (Appendix), the AEIS mutant degron possesses a very similar log₂FC value to APIS (3.67 and 3.73, respectively), indicating that either N149P or N149E mutations provide similar magnitudes of degradation enhancement.

Figure 5.1D highlights the major contribution made by the single Q147W mutation for degradation induced by compound **26**, with the WNQS mutant degron being degraded with a 5.0-fold increase in efficiency compared to the WT.

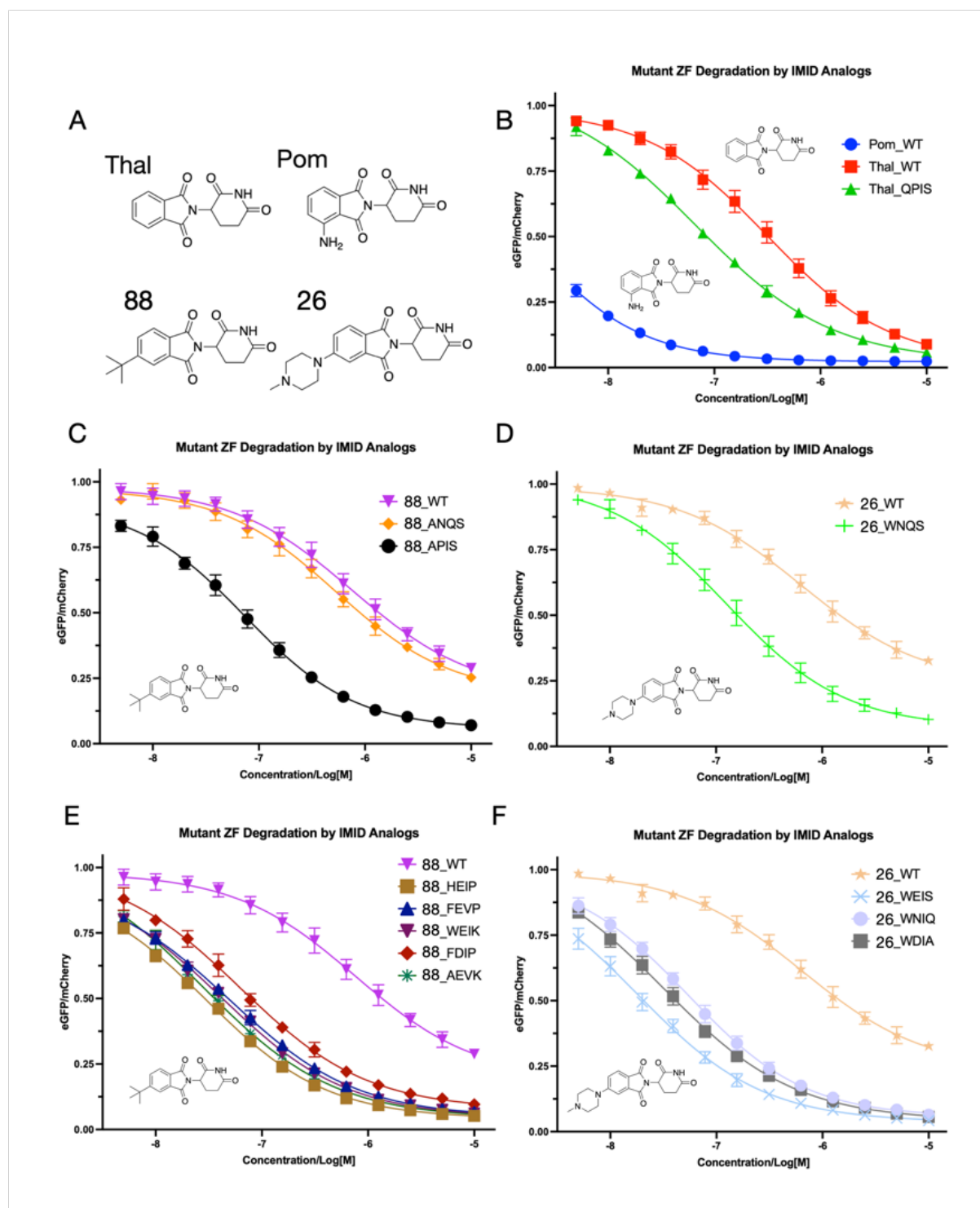


Figure 5.1. **A)** Chemical structure of four IMiD analogues. Curves for: **B)** pomalidomide and thalidomide degradation of the WT degon; thalidomide degradation of the QPIS degon. **C)** Compound **88** degradation of the WT, ANQS and APIS degrons. **D)** Compound **26** degradation of the WT and WNQS degrons. **E)** Compound **88** degradation of the WT degon and five top ranking mutant degrons from the compound **88** mutant library screen. **F)** Compound **26** degradation of the WT degon and three (of five) top ranking mutant degrons from the compound **26** mutant library screen.

Figure 5.1E shows the efficient induction of degradation by compound **88** of all of the top five library mutant degrons from the compound **88** library screen. The HEIP and AEVK degrons possess the greatest selectivity of the five; a small gap between curves for the HEIP and AEVK degrons in favour of HEIP is observed, but the pDC₅₀ values obtained (7.52 for HEIP, 7.56 for AEVK) suggest that AEVK is degraded slightly more efficiently. As the difference in selectivity compared to the WT is so small (25.1-fold for HEIP, 28-fold for AEVK), both of these sequences will be discussed later in this chapter.

Figure 5.1F shows the efficient induction of degradation by compound **26** of the first, third and fourth highest ranked mutants from the compound **26** library screen. The top ranked library mutant, WEIS, is the most efficiently degraded mutant degron. Table 5.2 and Figure 5.2 both show that both the pDC₅₀ value and the selectivity over WT for WEIS are the highest of all degrons tested.

It should be noted that, in the full library results (Appendix), in the screen of compound **26**, the WPIS mutant possesses a similarly high log₂FC value to WEIS (4.09 and 4.45, respectively), again indicating that both N149P and N149E mutations contribute substantially to the increase in degradation efficiency.

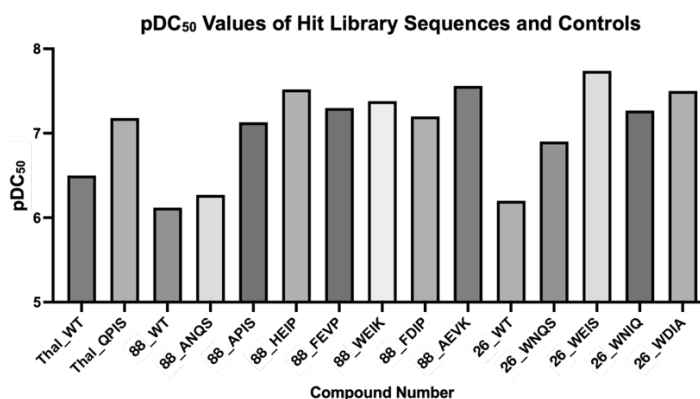


Figure 5.2. pDC₅₀ values for IMiD analogue/degron pairs. For all compounds shown, all mutant sequences possess greater pDC₅₀ values than the WT.

5.2.3 Molecular Docking Results

Having identified which mutant degrons are most effectively degraded in the presence of compounds **88** and **26**, constrained docking using GOLD was performed to predict the arrangement of the CRBN/IMiD/degron interface for three IMiD analogue/degron pairs: **88**/AEVK (Figure 5.3A), **88**/HEIP (Figure 5.3B) and **26**/WEIS (Figure 5.3C). The docking protocol used was the same as that described in Chapter 2, employing Foldx to perform the mutations in the ZF degron and position the side chains optimally, followed by constrained docking of the ‘bumped’ IMiD using GOLD.

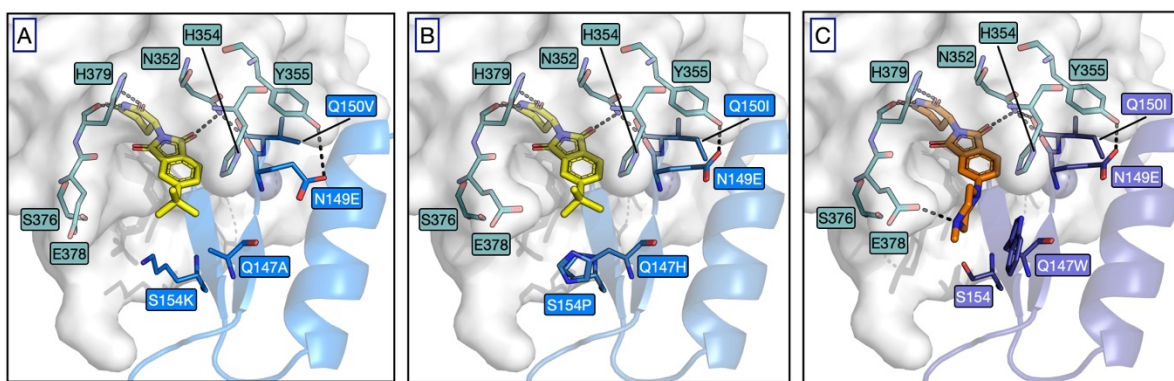


Figure 5.3. Constrained docking performed in GOLD and mutations performed in Foldx. **A)** Compound **88** (yellow) bridges CRBN (teal) and the AEVK degron (blue). **B)** Compound **88** (yellow) bridges CRBN (teal) and the HEIP degron (blue). **C)** Compound **26** (orange) bridges CRBN (teal) and the WEIS degron (violet). Notable proposed interactions are highlighted by black dashed lines. (Derived from PDB ID: 6H0F).

In all three cases, an N149E mutation at position 149 introduces a new polar interaction between the side chain of E149 and the side chain of Y355 in CRBN. The mutations at position 150 – either Q150I or Q150V – introduce a hydrophobic interaction between the side chain of residue 150 and Y355 in CRBN. It is predicted that these interactions aid the stabilisation of the CRBN/ZF interface in the ternary complex.

In 5.3A, the tertiary butyl ‘bump’ of **88** can be seen occupying the space left by the absence of the side chain of Q147, with the replacement alanine side chain providing no clash. Similarly, in 5.3B, the side chain of H147 can be seen occupying space away from the tertiary butyl group of **88**. This space is perhaps available due to the proximal S154P mutation, which provides extra space for the H147 side chain to inhabit.

In 5.3C, the tertiary amine nitrogen of the methyl piperazine of **26** is predicted to form a polar interaction with E378 from CRBN. As this tertiary amine group will be protonated at physiological pH, it is likely that the interaction it forms with the glutamate side chain will be ionic, and therefore strong. The aromatic side chain of W147 of the degron can be seen lining up parallel with the methyl piperazine of **26**, suggesting a cation- π interaction is taking place. As the indole side chain group is more electron-rich than other aromatic side chains (phenylalanine, tyrosine and histidine), the tryptophan cation- π will be the most energetically favourable. This could explain the observation that the most highly ranked sequences for the compound **26** library screen – 40 from the top 50 – contain tryptophan at position 147.

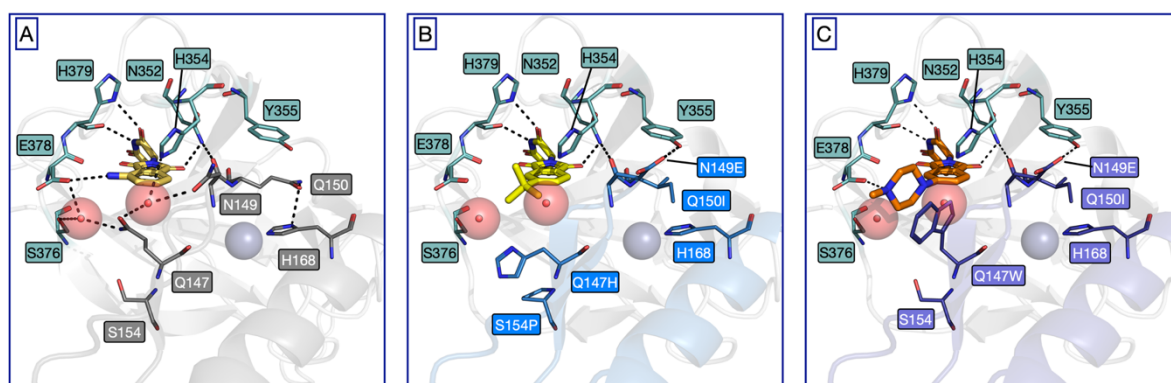


Figure 5.4. A) X-ray crystal structure (PDB ID: 6H0F) shows two water molecules (red spheres) are present at the interface between IKZF1/3 (grey) and CRBN (teal) in the presence of a bridging pomalidomide (gold). Superimposition of these two waters over docking results for **A)** compound **88** (yellow) and the HEIP degron (blue), and **B)** compound **26** (orange) and the WEIS degron (violet), shows that these water molecules clash with the ‘bump’ groups of the IMiD analogues, and would therefore not be accommodated in these systems.

It is also notable that the two water molecules seen in the crystal structure from the CRBN-pomalidomide-IKZF1/3 interface cannot be accommodated at the proposed interface of these novel IMiD/degron pairs (Figure 5.4). It can therefore be hypothesised that the 'hole' which is being occupied by the 'bump' groups of these IMiD analogues, is partly a consequence of the absence of water molecules that would occupy this region in the presence of Q147 in the degron.

5.3 Western Blots

Having demonstrated by flow cytometry analysis that the top performing mutants from the library screens are degraded efficiently by their corresponding IMiD analogues, attention was then turned towards the endogenous target IKZF3, also known as aiolos.² As the aim of this project was to identify IMiD analogues which will not degrade endogenous IKZF3 while still degrading mutant degrons, western blotting was identified as the best method for showing these two events occurring simultaneously in the same cell line. Compound incubation and cell pellet preparation were performed by Patrick Brennan, and subsequent immunoblotting were carried out by Dr Marta Serafini, Oxford University.

Firstly, four Jurkat cell lines each stably transduced with the Sprout plasmid constructs for one of three mutant degrons (AEVK, HEIP and WEIS) and the WT degron, were sorted using FACS (detecting mCherry) to enrich the population for transduced cells. This yielded four cell lines with close to 100% transduction efficiency. Next, cultures of these Jurkat lines were incubated for 18 hours with the relevant IMiD analogues using compound concentration gradients of 1 nM to 10 μ M for compounds **88** and **26**, and 1 nM to 100 nM for pomalidomide, with log₁₀ intervals (Figure 5.5).

The results show that, in all cases, the ZF-eGFP fusion protein is degraded more efficiently than endogenous IKZF3 (Figure 5.5). However, the difference in degradation efficiencies between endogenous IKZF3 and the eGFP-degion fusion protein is far greater for the ‘bumped’ IMiD/mutant degion pairings compared to the pomalidomide/WT degion pairing, i.e. the window of selectivity is far greater.

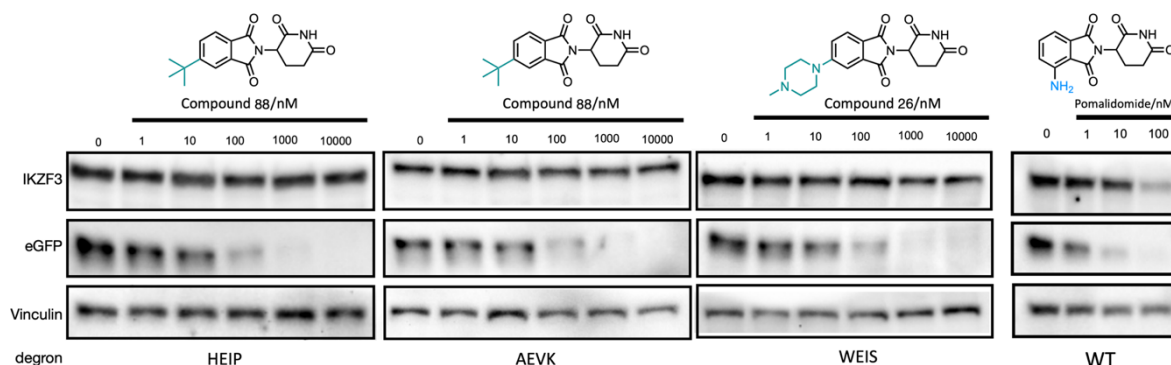


Figure 5.5. Western blots show degradation of endogenous IKZF3 and ZF-eGFP fusion protein induced by three IMiD analogues, in Jurkat cells transduced with the corresponding degion construct; vinculin is included as the loading control.

In Figure 5.5 it can be seen that none of the concentrations of pomalidomide used induce full degradation of the WT-eGFP fusion protein while also leaving endogenous IKZF3 completely unaffected. A 10 nM concentration of pomalidomide does not significantly reduce IKZF3 levels, but WT-eGFP is still present; meanwhile, a concentration of 100nM of pomalidomide has completely degraded WT-eGFP, but IKZF3 is also significantly degraded. It could be inferred that a concentration between 10 and 100 nM might be more selective, but this is not an ideal solution and cannot be said to be an ‘orthogonal’ system. However, for all three pairings of ‘bumped’ IMiD analogues and mutant degions, at concentrations of 1 μ M and 10 μ M, the ZF-eGFP fusion protein has been completely degraded, while endogenous IKZF3 levels are not

affected. This demonstrates a larger window of selectivity, and suggests that these systems are orthogonal in the context of IKZF3 degradation.

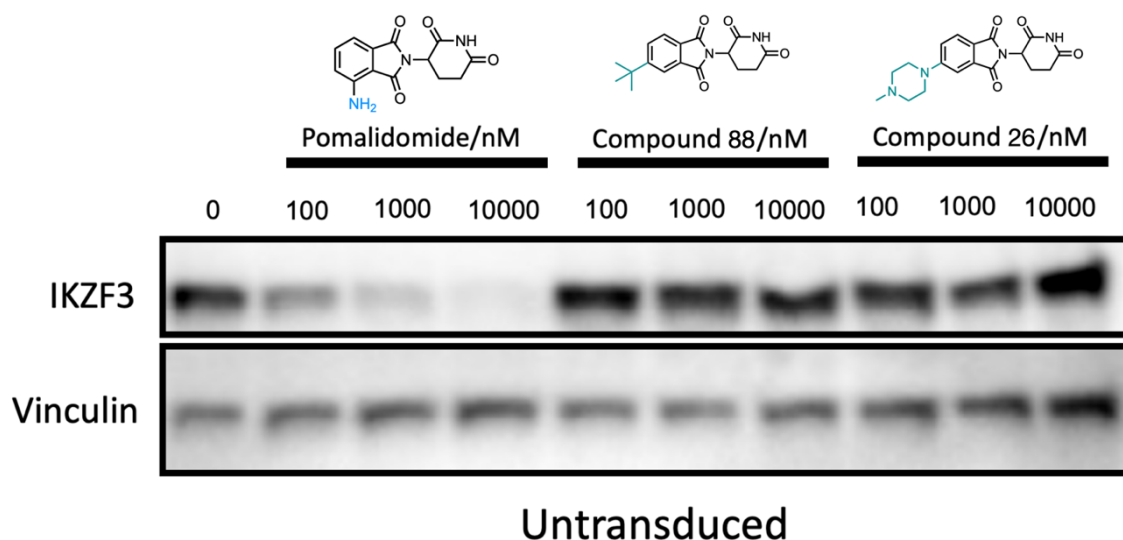


Figure 5.6. Western blot shows degradation of endogenous IKZF3 by the three IMiD analogues shown (pomalidomide, **88** and **26**) in untransduced Jurkat cells; vinculin included as a loading control.

Figure 5.6 shows further confirmation of the inability of these IMiD analogues to induce degradation of endogenous IKZF3, this time in untransduced Jurkat cells. Pomalidomide, also included in this blot, induces efficient IKZF3 degradation at the same concentrations. This extra assay in untransduced Jurkat cells was undertaken to show conclusively that neither **88** nor **26** affected levels of endogenous IKZF3 in cell lines that had not had any extra genetic material introduced into them.

5.4 Application of IMiD/Degron Pair to Protein of Interest

At this point it had been demonstrated that top performing mutants from the library screens are efficiently degraded in the presence of their corresponding IMiD analogues in the context of a ZF-eGFP fusion protein. It has also been shown that these IMiD analogues do not induce degradation of endogenous IKZF3 in Jurkat cells.

However, demonstration that the high degree of selectivity of this IMiD/degron system is also observed for a protein other than eGFP is desirable, particularly as we wish to demonstrate the potential of this system for applications such as target validation. Therefore, the best performing IMiD/degron pair (26/WEIS) was chosen to test for two proteins of interest. These tests were carried out by Dr Lewis Brayshaw and staff at GSK.

The first protein chosen was β -catenin,⁸³ which has previously been used to test other degron systems (known via personal communication from Dr Lewis Brayshaw). The second protein chosen was TRIM28,⁸⁴ a plant homeodomain (PHD) and bromodomain-containing protein found in human cells which currently possesses no known small molecule binders, and cannot therefore be inhibited or degraded using small molecule degraders. Three constructs were made for both proteins: one with only the POI, one with the degron at the N terminus, and one with the degron at the C terminus. A small 11 amino acid peptide, HiBit, was included in the fusion protein to provide a simple way of monitoring degradation. HiBit will bind strongly to a larger protein called LgBit, and when together this complex possesses luciferase activity. Addition of LgBit along with a luciferin small molecule can then be used to assess abundance of HiBit in a population of cells.⁸⁵

To test both β -catenin and TRIM28, plasmids containing genes coding for POI-WEIS-HiBit fusion proteins were assembled and transduced into Jurkat cells. A HiBit luminescence assay was employed to monitor degradation of the fusion protein across a concentration gradient of compound **26** (Figure 5.7 A and B). Cell survival rates, determined using CellTiter-Glo,⁸⁶ are also shown, indicating that results observed are

due to selective degradation of the desired target, and not due to artefacts caused by cell death (Figure 5.7 C and D).

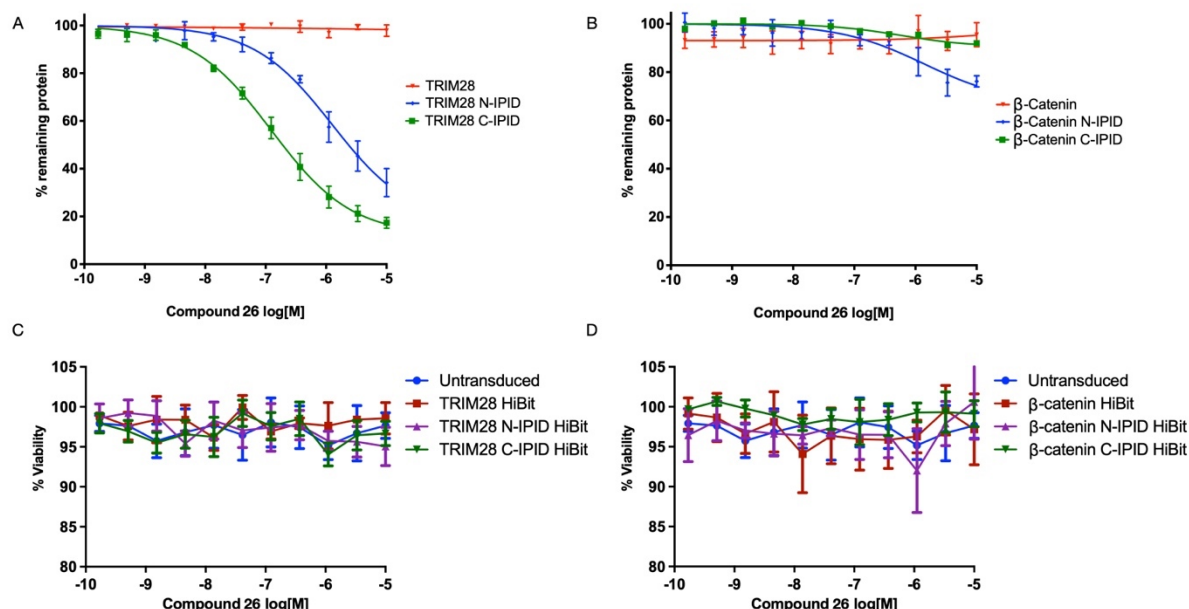


Figure 5.7. HiBit luminescence data for Jurkat cells treated with compound **26**. **A)** Jurkat cells transduced with a construct for TRIM28 tagged with the WEIS degon at either the N or C terminus and an untagged control. **B)** Jurkat cells transduced with a construct for β-catenin tagged with the WEIS degon at either the N or C terminus and an untagged control. CellTiter-Glo cell viability assay results for Jurkat cells transduced with **C)** TRIM28 constructs and **D)** β-catenin constructs, and untransduced controls.

Results indicate that degradation of TRIM28 occurred, with a 10 μM concentration of **26** conferring 83% degradation of the TRIM28 fusion protein with the degon at the C terminus, and 66% degradation for the TRIM28 fusion protein with the degon at the N terminus. No degradation was observed for the untagged TRIM28.

Degradation of β-catenin was very modest with the degon attached at either terminus. It must be noted that no optimisation was carried out prior to these two tests; the degon motif used was exactly the same as that used for the eGFP protein. The linker region bridging the POI and the degon was also kept exactly the same. Therefore, it is perhaps unsurprising that one of the POIs tested was not degraded well. This result

does not rule out the possibility that β -catenin could be degraded using an optimised degron design – potentially a design with changes made to the length or flexibility of the linker region.

5.5 Summary and Conclusions

The data acquired during the work described in this chapter have enabled us to identify nine viable pairings of IMiD analogues and degrons that show high selectivity (>10-fold increase in degradation) over the WT degron sequence derived from IKZF1/3. Three of these pairings (**88**/AEVK, **88**/HEIP, and **26**/WEIS) have been shown to achieve complete selectivity over endogenous IKZF3 in Jurkat cells expressing degron-eGFP fusion proteins. Endogenous IKZF3 remains completely undegraded even at a 10 μ M concentration of either compound **88** or compound **26** – a stark contrast to the efficient degradation of endogenous IKZF3 by pomalidomide. The most selective of these systems, **26**/WEIS, induces efficient degradation of a WEIS degron-tagged POI for which no small molecule inhibitors are known, proving that this system could be applied to a target validation scenario. We predict that optimisation of the linker region of this WEIS degron construct could facilitate improved degradation for POIs in which the initial design does not effect potent degradation.

Chapter 6: Conclusions and Future Work

6.1 Conclusions

The aim of this study was to develop an orthogonal IMiD/degron pair which would promote targeted degradation of a degron-tagged POI without unwanted off-target degradation of endogenous proteins. Computational techniques were initially used to screen potential suitable IMiD analogues, after which flow cytometry assays were used to refine this selection further. Four of the most promising IMiD analogues were then screened against 8380 mutant ZF degron sequences, yielding IMiD/degron pairs with high selectivity over the WT ZF. Further flow cytometry assays, as well as western blotting and HiBit assays, then confirmed the selectivity of the most potent IMiD/degron pairs over endogenous IKZF1 and IKZF3 in Jurkat cells.

The three most selective IMiD/degron pairs are shown in Figure 6.1. Compound **88** comprises a tertiary butyl ‘bump’ group at the 5-position of a thalidomide scaffold; it induces degradation of two mutant ZF degrons, AEVK or HEIP, with similar selectivity (28-fold and 25.1-fold selectivity, respectively, compared to the WT ZF degron). Each of these mutant ZF degrons differs from the original WT IKZF1/3 sequences by four residues. Compound **26** comprises a methylpiperazine group at the 5-position of a thalidomide scaffold; it induces degradation of the WEIS degron with a higher selectivity (35-fold compared to the WT ZF degron) than either of the other two IMiD/degron pairs.

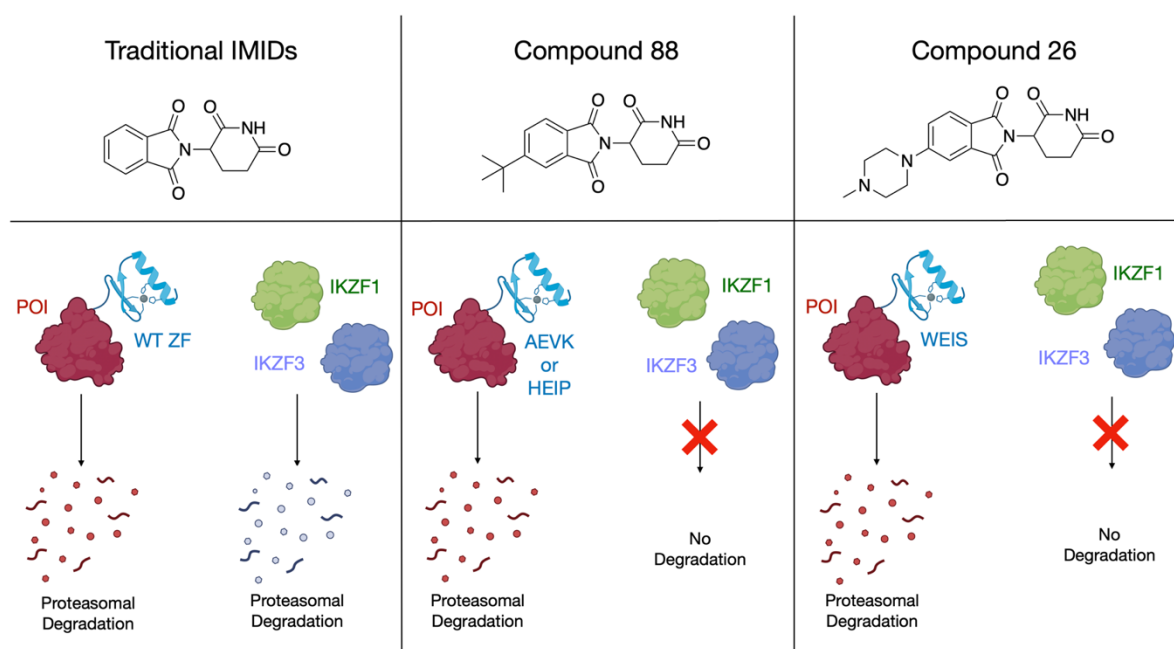


Figure 6.1. IMiDs such as thalidomide can be used to facilitate degradation of a POI (maroon) tagged with a ZF degnon (light blue) derived from IKZF1/3, but will also cause degradation of endogenous IKZF1 and IKZF3 (green and lilac, respectively). Compound **88** will facilitate degradation of a POI tagged with either the AEVK or HEIP ZF degnon mutant, but will not cause endogenous IKZF1 or IKZF3 to be degraded. Compound **26** will facilitate degradation of a POI tagged with the WEIS ZF degnon mutant, but will not cause endogenous IKZF1 or IKZF3 to be degraded.

Compound **26** paired with the WEIS ZF degnon mutant was the most selective IMiD/degnon pair, therefore this combination was used for the HiBit degradation assay discussed in section 5.4. The results from this assay showed that the **26**/WEIS combination could facilitate over 80% degradation of TRIM28 even when no optimisation of the degnon had been carried out. It is expected that higher levels of degradation could be achieved by altering the rigid linker region that bridges the POI to the ZF degnon, which would allow for greater flexibility of the ZF-POI fusion protein, and hence greater accessibility of surface lysine residues for polyubiquitination, leading to increased likelihood of proteasomal degradation.

It is anticipated that these selective IMiD/degron pairs could be used in target validation assays, where rapid and reversible compound-induced degradation of a POI is invaluable for scrutinising the roles of proteins in the cell.

6.2 Future Work

6.2.1 Proteomics

The work described in this dissertation has shown that compounds **88** and **26** do not significantly alter the levels of endogenous IKZF1 and IKZF3 in Jurkat cells, after 18 h incubation at 10 μ M. However, further work will be required to explore whether these compounds cause degradation of other endogenous proteins in human cell lines. Tandem mass tag (TMT) mass spectrometry-based proteomics is an ideal assay for assessment of the cellular interactome of a compound, as the full proteome of a cell can be assessed simultaneously. Previous work by Donovan *et al.* employed this method to assess protein abundance relative to a DMSO control for 10 μ M thalidomide, 5 μ M lenalidomide and 1 μ M pomalidomide.⁶ To minimise secondary effects that may arise from extended drug exposure, an incubation of 5 h was employed for all conditions. Use of this protocol for assessment of compounds **88** and **26** is currently being investigated.

6.2.2 Analogues of Compound **26**

As seen in Figure 5.3C, the exterior tertiary amine nitrogen in compound **26** will be protonated at physiological pH, allowing both an ionic interaction to occur between it at E378 in CRBN, and a cation- π interaction to occur between it and W147 in the WEIS degron. It would therefore be interesting to explore the degradation profiles of

analogues of compound **26** that include alkyl substituents with different lengths and degrees of branching (Table 6.1), as the nature of this group will influence the pK_a of the tertiary amine. We predict that use of a suitable alkyl group to optimise the pK_a of this amine would allow for optimal interaction between the IMiD analogue, E378 and W147, thus enhancing the efficiency of WEIS degron degradation observed. Therefore a suitable analogue of compound **26** could provide greater selectivity (>35-fold) when paired with WEIS, compared to the original **26**/WEIS pairing.

Table 6.1. Potential analogues of compound 26 proposed for further degradation studies.

Compound	R	Compound	R
26		150	
147		151	
148		152	
149		153	

6.2.3 Incorporation into CAR-T Cell Therapy

The use of IMiD Proximity-Induced Degradation (IPID) in CAR-T cell therapy has already been explored in literature (see section 1.4.3).^{4,46} The main drawbacks of using the WT degron paired with either pomalidomide or lenalidomide as the small molecule degrader are the undesirable side effects resulting from the degradation of

endogenous neosubstrates that occurs in the presence of these compounds. In particular, degradation of endogenous IKZF1 and IKZF3 can have the net effect of activating T cells, which negates the desired deactivation of CAR-T cells *via* IPID-CAR degradation.^{8,9} Therefore, incorporation of this new system would provide a potential solution to this, whereby an orthogonal IMiD molecule such as compound **26** could be used to degrade IPID-CARs incorporating the WEIS degnon, without affecting levels of endogenous IKZF1 and IKZF3 in the patient. The main disadvantage of this approach is that compound **26** is not yet approved for use in humans. However, should initial *in vivo* testing in suitable animal studies show the desired effect, it is possible that both compound **26** and an IPID-CAR incorporating the WEIS degnon could be explored for use in human therapies.

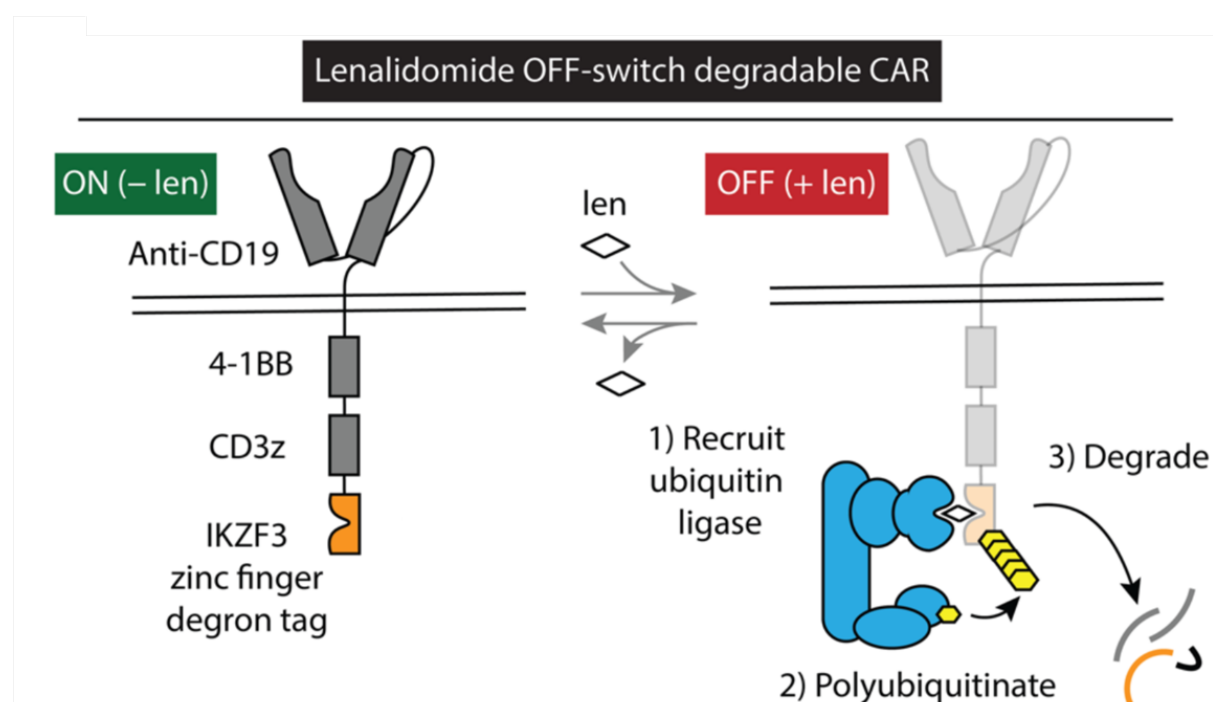


Figure 6.2. Incorporation of a ZF degnon (orange) into a chimeric antigen receptor (CAR, grey). CAR degradation can be induced *via* addition of an IMiD, whereupon ubiquitin (yellow) is added to the CAR via CRBN and other affiliate proteins (blue). Adapted from Jan *et al.* with permission.⁴

Chapter 7: Experimental

7.1 General Chemistry Experimental

Reagents and solvents used were of commercially available reagent grade quality from Alfa Aesar, Fluorochem, Merck (formally known as Sigma-Aldrich) and Tokyo Chemical Industry, and were used without further purification, unless stated. Anhydrous solvents were obtained from an MBRAUN Solvent Purification Systems 5 and stored under an argon atmosphere over 3 Å molecular sieves. Concentration *in vacuo* was performed at 40 °C for organic solvents using a Buchi™ rotary evaporator. Brine refers to a saturated aqueous solution of sodium chloride. Petroleum ether refers to the fraction of light petroleum ether boiling in the range 40–60 °C. Celite® refers to Celite® 545 filter aid, treated with sodium carbonate, flux-calcined (Merck).

¹H NMR spectra were measured on a Bruker AV400 (400 MHz), a Bruker AVII 500 (500 MHz) or a Bruker AV600 (600 MHz) spectrometer in the stated solvents as a reference for the internal deuterium lock. The chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to tetramethylsilane (TMS) where $\delta(\text{TMS}) = 0.00$. The spectra are calibrated using the solvent peak with the data provided by Fulmer *et al.*⁸⁷ The multiplicity of each signal is indicated by: s (singlet); bs (broad singlet); d (doublet); t (triplet); q (quartet); p (pentet); sept (sept); m (multiplet) or combinations thereof. The number of protons (n) for a given resonance signal is indicated by nH. Where appropriate, coupling constants (*J*) are quoted in Hz, recorded to the nearest 0.1 Hz, and were determined by analysis using Bruker TopSpin v3.2 software or MestreNova software. The mean value of identical coupling

constants is reported. Spectra were assigned using COSY, HSQC and HMBC experiments as necessary. Spectra acquired at high temperature (353 K or 363 K) are indicated.

^{13}C NMR spectra were measured on a Bruker AV400 (101 MHz), a Bruker AVII 500 (126 MHz) or a Bruker AV600 (151 MHz) spectrometer in the stated solvents as a reference for the internal deuterium lock using the standard ^{13}C experiment. The chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to tetramethylsilane (TMS) where $\delta(\text{TMS}) = 0.00$. The spectra are calibrated using the solvent peak with the data provided by Fulmer *et al.*⁸⁷ Signals are quoted to one decimal place unless peaks are indistinguishable, in which case two decimal places are used. Where appropriate, coupling constants (J) are quoted in Hz, recorded to the nearest 0.1 Hz, and were determined by analysis using Bruker TopSpin v3.2 software or MestreNova software. Spectra were assigned using HSQC and HMBC experiments as necessary. Spectra acquired at high temperature (353 K or 363 K) are indicated.

^{19}F NMR spectra were measured on a Bruker AVIII HD 400 (376 MHz) spectrometer in the solvent stated, and are ^{13}C decoupled. Chemical shifts are given as δ in units of parts per million (ppm), to the nearest 0.1 ppm. The multiplicity of each signal is a singlet unless stated otherwise.

Mass spectra were acquired on either an Agilent 6120 (low resolution) or Bruker microToF spectrometer (high resolution) using electrospray ionisation (ESI) from solutions of either methanol or water. m/z values are reported in Daltons and are followed by their percentage abundance in parentheses.

Melting points were determined using a Griffin capillary tube melting point apparatus (Registered Design No. 889339) and are uncorrected. The solvent(s) from which the sample was crystallised is given in parentheses. Dec. indicates that the sample decomposed at the stated temperature.

Infrared spectra were obtained from thin films, using a diamond ATR module on a Bruker Tensor 27 spectrometer. Absorption maxima are reported in wavenumbers (cm^{-1}) and reported as s (strong), m (medium), w (weak) or br (broad).

Analytical high performance liquid chromatography (HPLC) method 1 was performed on a PerkinElmer Flexar system with a Binary LC Pump and UV/vis LC Detector set at 254 nm. To determine compound purity, a Dionex Acclaim[®] 120 C18 [5 μm , 12 Å, 150 mm $\times$ 4.6 mm] reverse phase column was used with a constant flow rate of 1.5 mL min^{-1} and gradient method of 10 min from 95:5 H_2O :acetonitrile (0.1% TFA) to 5:95 H_2O :acetonitrile (0.1% TFA) with a 5 min hold. Samples injected were prepared by dissolving in methanol, water or acetonitrile, and filtered. All samples are run with 0.1% TFA added unless indicated.

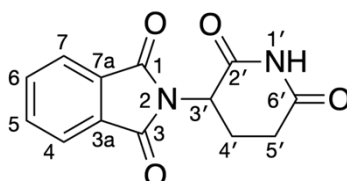
All biologically assessed compounds had a purity of $\geq 95\%$.

Normal Phase silica gel analytical Thin Layer Chromatography (NP TLC) to monitor reaction progress was carried out on normal phase Merck silica gel 60 F254 aluminium-supported thin layer chromatography sheets. Visualisation was carried out using absorption of UV light (λ_{max} = 254 nm and 365 nm) or thermal development after staining in an ethanolic solution of ninhydrin or an aqueous solution of potassium permanganate.

Normal phase silica gel flash column chromatography was carried out manually on Merck Geduran® silica gel 60 (40–63 μm), eluting with solvents as supplied, under a positive pressure of compressed, gaseous nitrogen.

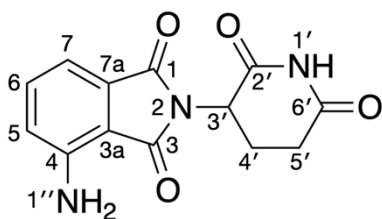
7.2 Compound Synthesis Procedures and Data

2-(2,6-Dioxopiperidin-3-yl)isoindoline-1,3-dione (1)



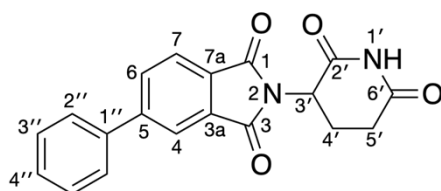
Phthalic anhydride (200 mg, 1.35 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (222 mg, 1.35 mmol, 1.0 eq) and NaOAc (166 mg, 2.03 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and stirred at 120 °C for 16.5 h. The reaction was cooled to rt and the solvent removed *in vacuo* (azeotrope with cyclohexane). The product was purified using flash column chromatography (2–10% MeOH:CH₂Cl₂) to afford the title compound (269.8 mg, 77%) as a colourless solid: *R_f* 0.60 (MeOH:CH₂Cl₂ 1:9); m.p. 263–266 °C (from CH₂Cl₂) [lit.⁶¹ 269–271 °C, lit.⁸⁸ 258–260 °C, lit.⁸⁹ 270–272 °C]; ¹H NMR (400 MHz, D₆-DMSO) δ 11.12 (1H, s, H-1'), 7.98 – 7.85 (4H, m, H-4, H-5, H-6 and H-7), 5.16 (1H, dd, *J* 12.9, 5.4 Hz, H-3'), 2.90 (1H, ddd, *J* 17.5, 14.1, 5.4 Hz, H-5'^a), 2.66 – 2.52 (2H, m, H-4'^a and H-5'^b), 2.13 – 1.97 (1H, m, H-4'^b); LRMS *m/z* (ESI[−]) 257 ([M-H][−], 100%); HPLC Retention time 220: 7.0 min, 98.7%; 254: 7.0 min, 100.0%. These data are in good agreement with the literature values.⁶¹

4-Amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (3)



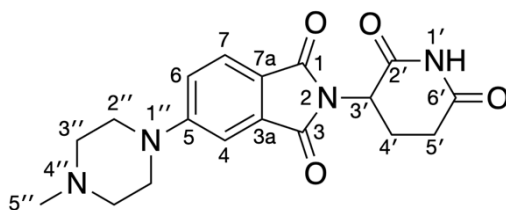
2-(2,6-Dioxopiperidin-3-yl)-4-nitroisoindoline-1,3-dione (497 mg, 1.64 mmol, 3.0) and palladium on carbon (10% w/w; 50 mg, 0.47 mmol, 1.0 eq) were dissolved in DMF (20 mL) and heated to 35 °C under an atmosphere of H₂ atmosphere for 16 h. The mixture was cooled to rt and filtered through Celite®. The DMF was removed from the filtrate *in vacuo* (azeotrope with toluene). The residual light green powder was dissolved in 20 mL of EtOAc and heated under reflux at 77 °C for 30 min. After this time, the solution was cooled, filtered, and the solvent was removed *in vacuo* to afford the title compound (363 mg, 81%) as a yellow, fluorescent powder: *R_f* 0.22 (EtOAc:petroleum ether 1:1); m.p. 290 °C – dec. (from CH₂Cl₂) [lit.⁹⁰ 319-322 °C, lit.⁹¹ 252 °C]; ¹H NMR (400 MHz, D₆-DMSO) δ 11.07 (1H, s, H-1'), 7.47 (1H, dd, *J* 8.5, 7.0 Hz, H-6), 7.05 – 6.97 (2H, m, H-5 and H-7), 6.51 (2H, s, H-1''), 5.04 (1H, dd, *J* 12.9, 5.4 Hz, H-3'), 2.88 (1H, ddd, *J* 17.4, 14.4, 5.4 Hz, H-5'^a), 2.64 – 2.51 (2H, m, H-4'^a and H-5'^b), 2.07 – 1.96 (1H, m, H-4'^b); LRMS *m/z* (ESI⁻) 272 ([M-H]⁻, 100%); HPLC Retention time 220: 6.4 min, 99.9%; 254: 6.4 min, 100.0%. These data are in good agreement with the literature values.⁷⁰

2-(2,6-Dioxopiperidin-3-yl)-5-phenylisoindoline-1,3-dione (23)



5-Phenylisobenzofuran-1,3-dione (125 mg, 0.56 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (92 mg, 0.43 mmol, 1.0 eq) and NaOAc (69 mg, 0.84 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and heated at 120 °C under reflux for 2 h. The AcOH was removed *in vacuo* and the residue purified using flash column chromatography (2% MeOH:CH₂Cl₂) to afford a colourless solid (119 mg, 64%): *R_f* 0.65 (MeOH:CH₂Cl₂ 1:19); m.p. 216–220 °C (from CH₂Cl₂) [lit.⁶² 230 °C]; ¹H NMR (400 MHz, D₆-DMSO) δ 11.15 (1H, s, H-1'), 8.23 – 8.15 (2H, m, Ar-CH), 8.05 – 7.96 (1H, m, Ar-CH), 7.88 – 7.81 (2H, m, Ar-CH), 7.59 – 7.44 (3H, m, Ar-CH), 5.19 (1H, dd, *J* 12.9, 5.5 Hz, H-3'), 2.91 (1H, ddd, *J* 17.3, 14.0, 5.5 Hz, H-5'^a), 2.66 – 2.52 (2H, m, H-4'^a and H-5'^b), 2.14 – 2.03 (1H, m, H-4'^b); LRMS *m/z* (ESI[−]) 333 ([M-H][−], 100%); HPLC Retention time 220: 9.5 min, 98.2%; 254: 9.5 min, 99.7%. These data are in good agreement with the literature values.⁶²

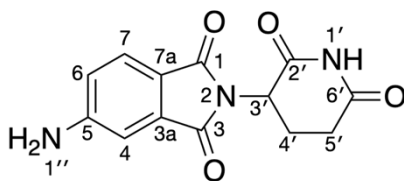
2-(2,6-Dioxopiperidin-3-yl)-5-(4-methylpiperazin-1-yl)isoindoline-1,3-dione (26)



2-(2,6-Dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione (50mg, 0.18 mmol, 1.0 eq), 1-methylpiperazine (40 µL, 0.36 mmol, 2.0 eq) and DIPEA (126 µL, 0.72 mmol, 4.0 eq) were dissolved in NMP (2 mL) and heated at 110 °C for 2 h in a microwave. The NMP was removed by vigorously blowing nitrogen over the reaction vessel whilst heating to 60 °C. The residual dark brown oil was purified using flash chromatography (2-10% MeOH:CH₂Cl₂) to afford a bright yellow solid (40 mg, 62%): *R_f* 0.44 (MeOH:CH₂Cl₂ 1:9); m.p. 192–194 °C (from EtOAc); *ν*_{max} (thin film)/cm^{−1} 1709 (C=O, s); ¹H NMR (400 MHz, CDCl₃) δ 8.40 (1H, s, H-1'), 7.69 (1H,

d, J 8.6 Hz, H-7), 7.28 (1H, d, J 2.4 Hz, H-4), 7.06 (1H, dd, J 8.6, 2.4 Hz, H-6), 4.98 – 4.88 (1H, m, H-3'), 3.47 – 3.40 (4H, m, H-2''), 2.93 – 2.64 (3H, m, H-4'^a and H-5'), 2.62 – 2.53 (4H, m, H-3''), 2.35 (3H, s, H-5''), 2.19 – 2.02 (1H, m, H-4'^b); ^{13}C NMR (151 MHz, CDCl_3) δ 171.0 (s, C-6'), 168.3 (s, C-2'), 168.0 (s, C-3), 167.3 (s, C-1), 155.6 (s, C-5), 134.4 (s, C-3a), 125.5 (s, C-7), 119.7 (s, C-7a), 118.2 (s, C-6), 108.9 (s, C-4), 54.6 (s, C-3''), 49.3 (s, C-3'), 47.6 (s, C-2''), 46.1 (s, C-5''), 31.6 (s, C-5'), 22.9 (s, C-4'); HRMS m/z (EI^-) [Found: 355.1417, $\text{C}_{18}\text{H}_{19}\text{N}_4\text{O}_4$ requires M^- 355.1412]; LRMS m/z (ESI^-) 355 ($[\text{M}-\text{H}]^-$, 100%); HPLC Retention time 220: 5.1 min, 100.0%; 254: 5.1 min, 100.0%.

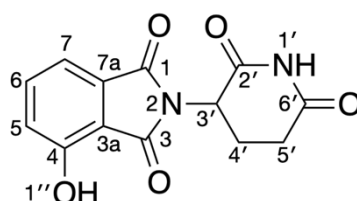
5-Amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (35)



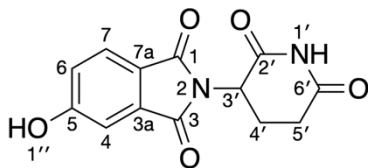
2-(2,6-Dioxopiperidin-3-yl)-5-nitroisoindoline-1,3-dione (691 mg, 2.28 mmol, 3.0 eq) and palladium on carbon (10% w/w; 70 mg, 0.74 mmol, 1.0 eq) were dissolved in DMF and heated to 35 °C under an atmosphere of argon. H_2 (g) was then bubbled through the solution for 10 min and the reaction was stirred at r.t. for 16 h under an atmosphere of H_2 (g). The mixture was then filtered through Celite® and the residue washed with toluene. The DMF was removed from the filtrate *in vacuo* (azeotrope with toluene). The residual dark green powder was dissolved in 25 mL of EtOAc and heated under reflux at 77 °C for 30 min. The solution was cooled, filtered, and the solvent was removed *in vacuo* to afford a yellow, fluorescent powder (369 mg, 52%): R_f 0.40 (MeOH: CH_2Cl_2 1:9); m.p. >300 °C – dec. (from CH_2Cl_2) [lit.⁹² 318-320 °C, lit.⁶⁷ 320-322 °C]; ^1H NMR (400 MHz, D_6 -DMSO) δ 11.06 (1H, s, H-1'), 7.52 (1H, d, J 8.3 Hz, H-

7), 6.94 (1H, d, J 2.1 Hz, H-4), 6.83 (1H, dd, J 8.3, 2.1 Hz, H-6), 6.55 (2H, s, H-1''), 5.01 (1H, dd, J 12.8, 5.5 Hz, H-3'), 2.94 – 2.80 (1H, m, H-5'^a), 2.62 – 2.52 (2H, m, H-4'^a and H-5'^b), 2.04 – 1.93 (1H, m, H-4'^a); LRMS m/z (ESI⁻) 272 ([M-H]⁻, 100%); HPLC Retention time 220: 6.2 min, 98.7%; 254: 6.2 min, 100.0%. These data are in good agreement with the literature values.⁷¹

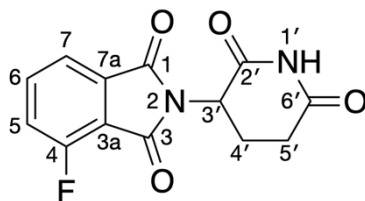
2-(2,6-Dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (36)



3-Hydroxyphthalic anhydride (2 g, 12.19 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (2.01 g, 12.19 mmol, 1.0 eq) and NaOAc (1.50 g, 18.29 mmol, 1.5 eq) were dissolved in AcOH (50 mL) and heated at 140 °C under reflux for 17.5 h. The AcOH was removed *in vacuo* (azeotrope with cyclohexane). The residue was dissolved in water and extracted with EtOAc, washed with water and brine, then dried with sodium sulfate and filtered. The EtOAc was removed *in vacuo* to afford a salmon-pink solid (1.14 g, 34%). No further purification was required: R_f 0.31 (MeOH:CH₂Cl₂ 1:19); m.p. 257–259 °C (from CH₂Cl₂) [lit.⁹³ 243–244 °C, lit.⁶⁷ 275–276 °C, lit.⁷⁵ 281–282 °C]; ¹H NMR (400 MHz, D₆-DMSO) δ 11.19 (1H, s, H-1''), 11.08 (1H, s, H-1'), 7.65 (1H, dd, J 8.4, 7.2 Hz, H-6), 7.32 (1H, dd, J 7.2, 0.8 Hz, H-5), 7.25 (1H, dd, J 8.4, 0.8 Hz, H-7), 5.07 (1H, dd, J 12.8, 5.4 Hz, H-3'), 2.95 – 2.81 (1H, m, H-5'^a), 2.64 – 2.50 (2H, m, H-4'^a and H-5'^b), 2.08 – 1.96 (1H, m, H-4'^b); LRMS m/z (ESI⁻) 273 ([M-H]⁻, 100%); HPLC Retention time 220: 6.2 min, 97.0%; 254: 6.2 min, 100.0%. These data are in good agreement with the literature values.⁶³

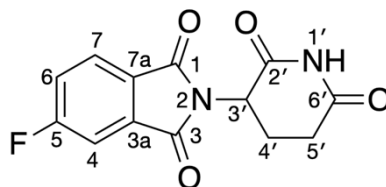
2-(2,6-Dioxopiperidin-3-yl)-5-hydroxyisoindoline-1,3-dione (37)

4-Hydroxyphthalic acid (246 mg, 1.35 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (222 mg, 1.35 mmol, 1.0eq) and NaOAc (166 mg, 2.03 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and heated at 120 °C under reflux for 3 h. The AcOH was removed *in vacuo* and the residue purified using flash column chromatography (5% MeOH/CH₂Cl₂) to afford a colourless solid (274 mg, 74%) which fluoresces yellow when dissolved: *R_f* 0.43 (MeOH:CH₂Cl₂ 1:9); m.p. >300 °C (from H₂O/MeCN) [lit.⁹⁴ 317-318 °C]; ¹H NMR (400 MHz, D₆-DMSO) δ 11.14 (1H, bs, H-1''), 11.11 (1H, s, H-1'), 7.74 (1H, dt, *J* 7.9, 1.0 Hz, H-6), 7.16 (2H, d, *J* 7.9 Hz, H-4 and H-7), 5.08 (1H, dd, *J* 12.9, 5.3 Hz, H-3'), 2.88 (1H, ddd, *J* 17.1, 13.7, 5.3 Hz, H-5'^a), 2.64 – 2.51 (2H, m, H-4'^a and H-5'^b), 2.11 – 1.97 (1H, m, H-4'^b). LRMS *m/z* (ESI⁻) 273 ([M-H]⁻, 100%); HPLC Retention time 220: 6.3 min, 95.3%; 254: 6.4 min, 99.7%. These data are in good agreement with the literature values.⁶⁵

2-(2,6-Dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (38)

3-Fluorophthalic anhydride (2.00 g, 12.04 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (1.98 g, 12.04 mmol, 1.0 eq) and NaOAc (1.27 g, 18.06 mmol, 1.5 eq) were dissolved in AcOH (50 mL) and heated at 140 °C under reflux for 17 h. The mixture was cooled to rt and the AcOH removed *in vacuo* (azeotrope with cyclohexane). The residue was extracted from 0.5 M LiCl solution with EtOAc. The EtOAc was removed *in vacuo* and the residue purified using flash column chromatography (60% EtOAc in petroleum ether) to afford the title compound (3.181 g, 67%) as a colourless solid: R_f 0.50 (MeOH:CH₂Cl₂ 1:9); m.p. 243–247 °C (from CH₂Cl₂) [lit.⁹⁵ 255–257 °C, lit.⁶⁹ 289 °C]; ¹H NMR (400 MHz, D₆-DMSO) δ 11.16 (1H, s, H-1'), 7.95 (1H, td, J 8.2, 5.9 Hz, H-6), 7.81 – 7.70 (2H, m, H-5 and H-7), 5.16 (1H, dd, J 12.7, 5.5 Hz, H-3'), 2.89 (1H, ddd, J 17.4, 13.8, 5.5 Hz, H-5'^a), 2.70 – 2.50 (2H, m, H-4'^a and H-5'^b), 2.10 – 2.02 (1H, m, H-4'^b); ¹⁹F NMR (376 MHz, D₆-DMSO) δ -114.69; LRMS m/z (ESI⁻) 275 ([M-H]⁻, 100%); HPLC Retention time 220: 6.8 min, 97.5%; 254: 6.8 min, 98.5%. These data are in good agreement with the literature values.⁶⁴

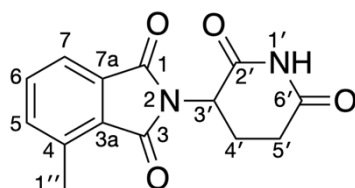
2-(2,6-Dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione (39)



4-Fluorophthalic anhydride (2.00 g, 12.04 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (1.98 g, 12.04 mmol, 1.0 eq) and NaOAc (1.27 g, 18.06 mmol, 1.5 eq) were dissolved in AcOH (50 mL) and heated at 120 °C under reflux for 1.5 h. The

AcOH was removed *in vacuo* and the residue purified using flash column chromatography (5% MeOH:CH₂Cl₂) to afford a light lilac solid (1.922 g, 58%): *R*_f 0.50 (MeOH:CH₂Cl₂ 1:19); m.p. 245–250 °C (from CH₂Cl₂); ¹H NMR (400 MHz, D₆-DMSO) δ 11.15 (1H, s, H-1'), 8.01 (1H, dd, *J* 8.2, 4.5 Hz, H-4), 7.86 (1H, dd, *J* 7.5, 2.4 Hz, H-7), 7.73 (1H, ddd, *J* 9.4, 8.2, 2.4 Hz, H-6), 5.17 (1H, dd, *J* 12.7, 5.4 Hz, H-3'), 2.89 (1H, ddd, *J* 17.1, 13.6, 5.4 Hz, H-5'^a), 2.65 – 2.51 (2H, m, H-4'^a and H-5'^b), 2.07 (1H, ddt, *J* 11.5, 6.4, 4.0 Hz, H-4'^b); ¹⁹F NMR (376 MHz, D₆-DMSO) δ -102.35; LRMS *m/z* (ESI⁻) 275 ([M-H]⁻, 100%); HPLC Retention time 220: 7.1 min, 99.5%; 254: 7.1 min, 100.0%. These data are in good agreement with the literature values.²⁸

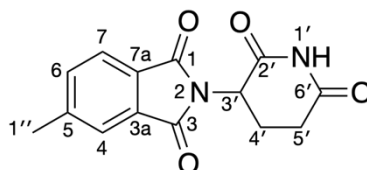
2-(2,6-Dioxopiperidin-3-yl)-4-methylisoindoline-1,3-dione (40)



3-Methylphthalic anhydride (200 mg, 1.204 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (203 mg, 1.204 mmol, 1.0eq) and NaOAc (152 mg, 1.806 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and heated at 140 °C under reflux for 24 h. The residue was extracted from 0.5M LiCl solution with EtOAc. The EtOAc was removed *in vacuo* and the residue purified using flash column chromatography (2% MeOH:CH₂Cl₂) to afford an off-white solid (135 mg, 40%): *R*_f 0.64 (MeOH:CH₂Cl₂ 1:9); m.p. 284–288 °C (from CH₂Cl₂) [lit.⁶⁶ 290-292 °C]; ¹H NMR (400 MHz, D₆-DMSO) δ 11.11 (1H, s, H-1'), 7.72 – 7.64 (3H, m, H-5, H-6 and H-7), 5.13 (1H, dd, *J* 12.9, 5.4 Hz, H-3'), 2.89 (1H, ddd, *J* 17.4, 14.1, 5.4 Hz, H-5'^a), 2.63 (3H, s, H-1''), 2.61 – 2.50

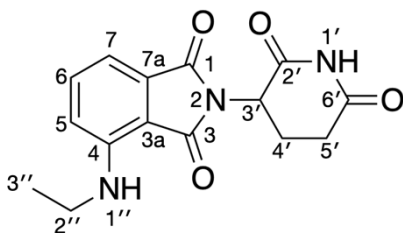
(2H, m, H-4'^a and H-5'^b), 2.11 – 1.99 (1H, m, H-4'^b); LRMS m/z (ESI⁻) 271 ([M-H]⁻, 100%); HPLC Retention time 220: 7.9 min, 96.7%; 254: 7.9 min, 100.0%. These data are in good agreement with the literature values.⁶⁵

2-(2,6-Dioxopiperidin-3-yl)-5-methylisoindoline-1,3-dione (41)



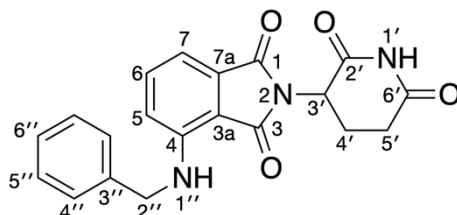
4-Methylphthalic anhydride (200 mg, 1.204 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (203 mg, 1.204 mmol, 1.0eq) and NaOAc (152mg, 1.81 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and heated at 120 °C under reflux for 2 h. The AcOH was removed *in vacuo* and the residue purified using flash column chromatography (5% MeOH:CH₂Cl₂) to afford a colourless solid (277 mg, 84%): R_f 0.60 (MeOH:CH₂Cl₂ 1:19); m.p. 260–266 °C (from CH₂Cl₂) [lit.⁹⁶ 265-267 °C]; ¹H NMR (400 MHz, D₆-DMSO) δ 11.12 (1H, s, H-1'), 7.84 – 7.78 (1H, m, H-7), 7.76 (1H, dt, J 1.5, 0.8 Hz, H-4), 7.69 (1H, ddd, J 7.6, 1.5, 0.8 Hz, H-6), 5.18 – 5.09 (1H, m, H-3'), 2.96 – 2.82 (1H, m, H-5'^a), 2.65 – 2.52 (2H, m, H-4'^a and H-5'^b), 2.11 – 2.00 (1H, m, H-4'^b); LRMS m/z (ESI⁻) 271 ([M-H]⁻, 100%); HPLC Retention time 220: 7.8 min, 98.7%; 254: 7.8 min, 98.4%. These data are in good agreement with the literature values.⁶⁵

2-(2,6-Dioxopiperidin-3-yl)-4-(ethylamino)isoindoline-1,3-dione (64)



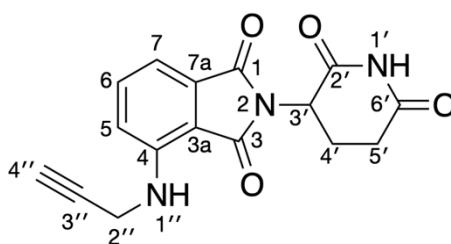
2-(2,6-Dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (200 mg, 0.72 mmol, 1.0 eq), ethylamine (2.0 M in THF; 1.44 mL, 2.90 mmol, 4.0 eq) and DIPEA (504 μ L, 2.90 mmol, 4.0 eq) were dissolved in NMP (4 mL) and heated at 110 $^{\circ}$ C for 2 h in a microwave. The NMP was removed by vigorously blowing nitrogen over the reaction vessel whilst heating to 60 $^{\circ}$ C. The residual oil was purified using flash column chromatography (40% EtOAc in petroleum ether) to afford a yellow powder (6 mg, 3%): R_f 0.30 (EtOAc:petroleum ether 3:7); m.p. 209–211 $^{\circ}$ C (from EtOAc) [lit.⁷² 221–224 $^{\circ}$ C]; ^1H NMR (400 MHz, CDCl_3) δ 7.96 (1H, s, H-1'), 7.50 (1H, ddd, J 8.5, 7.1, 0.6 Hz, H-6), 7.10 (1H, dd, J 7.1, 0.6 Hz, H-5), 6.89 (1H, d, J 8.5 Hz, H-7), 6.17 (1H, s, H-1''), 4.96 – 4.87 (1H, m, H-3'), 3.32 (2H, qd, J 7.2, 5.4 Hz, H-2''), 2.98 – 2.64 (3H, m, H-4'^a and H-5'), 2.19 – 2.08 (1H, m, H-4'^b), 1.31 (3H, t, J 7.2 Hz, H-3''); LRMS m/z (ESI^-) 300 ($[\text{M}-\text{H}]^-$, 100%); HPLC Retention time 220: 8.5 min, 97.9%; 254: 8.5 min, 98.6%. These data are in good agreement with the literature values.⁷²

4-(Benzylamino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (68)



2-(2,6-Dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (200 mg, 0.72 mmol, 1.0 eq), benzylamine (158 μ L, 1.45 mmol, 2.0 eq) and DIPEA (504 μ L, 2.90 mmol, 4.0 eq) were dissolved in NMP (4 mL) and heated at 110 °C for 2 h in a microwave. The NMP was removed by blowing nitrogen over it overnight whilst heating the vessel to 60 °C. The resultant oil was purified using column chromatography (50% EtOAc:petroleum ether) to afford a yellow solid (125 mg, 48%): R_f 0.40 (EtOAc:petroleum ether 1:1); m.p. 199–201 °C (from EtOAc) [lit.⁹⁷ 209-211 °C]; ^1H NMR (400 MHz, CDCl_3) δ 8.00 (1H, s, H-1'), 7.45 (1H, dd, J 8.5, 7.1 Hz, H-6), 7.40 – 7.27 (5H, m, H-4'', H-5'' and H-6''), 7.12 (1H, d, J 7.1 Hz, H-5), 6.84 (1H, d, J 8.5 Hz, H-7), 6.70 (1H, t, J 6.0 Hz, H-1''), 4.97 – 4.88 (1H, m, H-3'), 4.52 (2H, d, J 6.0 Hz, H-2''), 2.94 – 2.68 (3H, m, H-4'^a and H-5'), 2.20 – 2.10 (1H, m, H-4'^b); LRMS m/z (ESI^-) 362 ($[\text{M}-\text{H}]^-$, 100%); HPLC Retention time 220: 9.8 min, 99.5%; 254: 9.8 min, 97.7%. These data are in good agreement with the literature values.⁷³

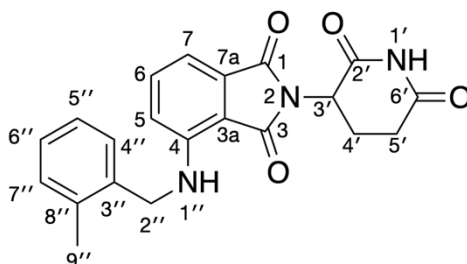
2-(2,6-Dioxopiperidin-3-yl)-4-(prop-2-yn-1-ylamino)isoindoline-1,3-dione (70)



2-(2,6-Dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (200 mg, 0.72 mmol, 1.0 eq), propargylamine (186 μ L, 2.90 mmol, 4.0 eq) and DIPEA (504.4 μ L, 2.896 mmol, 4.0 eq) were dissolved in NMP (4 mL) and heated at 110 °C for 2 h in a microwave. The NMP was removed by vigorously blowing nitrogen over the reaction vessel while heating to 60 °C. The residual oil was purified using flash column chromatography (30-

50% EtOAc:petroleum ether) to afford a bright yellow solid (123 mg, 54%): R_f 0.55 (EtOAc:petroleum ether 3:2); m.p. 174–176 °C (from EtOAc); ^1H NMR (400 MHz, CDCl_3) δ 8.03 (1H, s, H-1'), 7.57 (1H, dd, J 8.5, 7.2 Hz, H-6), 7.20 (1H, d, J 7.2 Hz, H-5), 7.03 (1H, d, J 8.5 Hz, H-7), 6.45 (1H, t, J 6.2 Hz, H-1''), 4.92 (1H, dd, J 12.2, 5.3 Hz, H-3'), 4.09 (2H, dd, J 6.2, 2.5 Hz, H-2''), 2.94 – 2.67 (3H, m, H-4'^a and H-5'), 2.27 (1H, t, J 2.5 Hz, H-4''), 2.18 – 2.08 (1H, m, H-4'^b); LRMS m/z (ESI⁻) 310 ([M-H]⁻, 100%); HPLC Retention time 220: 8.0 min, 96.7%; 254: 8.1 min, 97.7%. These data are in good agreement with the literature values.⁷⁴

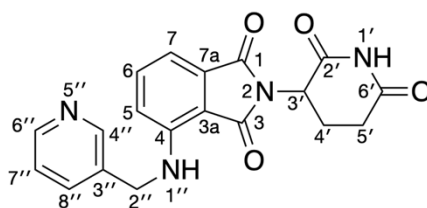
2-(2,6-Dioxopiperidin-3-yl)-4-((2-methylbenzyl)amino)isoindoline-1,3-dione (74)



2-(2,6-Dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (200 mg, 0.724 mmol, 1.0 eq), 2-methylbenzylamine (180 μL , 1.45 mmol, 2.0 eq) and DIPEA (504.4 μL , 2.90 mmol, 4.0 eq) were dissolved in NMP (4 mL) and heated at 110 °C for 2 h in a microwave. The NMP was removed by vigorously blowing nitrogen over the reaction vessel whilst heating to 60 °C. The residual orange oil was purified using flash column chromatography (40-60% EtOAc:petroleum ether) to afford a bright, light yellow solid (107 mg, 39%): R_f 0.50 (EtOAc:petroleum ether 1:1); m.p. 218–221 °C (from EtOAc); ν_{max} (thin film)/ cm^{-1} 1690 (C=O, s); ^1H NMR (400 MHz, CDCl_3) δ 7.97 (1H, s, H-1'), 7.48 (1H dd, J 8.5, 7.1 Hz, H-6), 7.24 – 7.16 (4H, m, H-4'', H-5'', H-6'', and H-7''), 7.12 (1H, d, J 7.1 Hz, H-5), 6.85 (1H, d, J 8.5 Hz, H-7), 6.52 (1H, t, J 5.6 Hz, H-1''), 4.96 – 4.86 (1H, m, H-3'), 4.45 (2H, d, J 5.6 Hz, H-2''), 2.95 –

2.66 (3H, m, H-4^{ra} and H-5'), 2.38 (3H, s, H-9''), 2.20 – 2.09 (1H, m, H-4^{rb}); ¹³C NMR (151 MHz, CDCl₃) δ 171.0 (s, C-6'), 169.6 (s, C-2'), 168.3 (s, C-1), 167.7 (s, C-3), 146.88 (s, C-4), 136.4 (s, C-8''), 136.1 (s, C-3''), 135.3 (s, C-6), 132.6 (s, C-7a), 130.8 (s, C-7''), 127.9 (s, C-4''), 127.6 (s, C-6''), 126.5 (s, C-5''), 117.1 (s, C-5), 112.1 (s, C-7), 110.6 (s, C-3a), 49.1 (s, C-3'), 45.1 (s, C-2''), 31.6 (s, C-5'), 23.0 (s, C-4'), 19.2 (s, C-9''); HRMS *m/z* (EI⁺) [Found: 376.1306, C₂₁H₁₈N₃O₄ requires M⁺ 376.1303]; LRMS *m/z* (ESI⁺) 376 ([M-H]⁺, 100%); HPLC Retention time 220: 10.3 min, 95.4%; 254: 10.3 min, 98.8%.

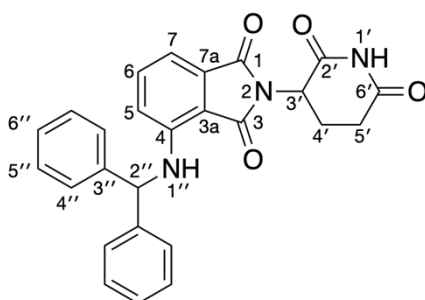
2-(2,6-Dioxopiperidin-3-yl)-4-((pyridin-3-ylmethyl)amino)isoindoline-1,3-dione (84)



2-(2,6-Dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (200 mg, 0.72 mmol, 1.0 eq), 3-picolylamine (147.4 μ L, 1.45 mmol, 2.0 eq) and DIPEA (504 μ L, 2.90 mmol, 4.0 eq) were dissolved in NMP (4 mL) and heated at 110 °C for 2 h in a microwave. The NMP was removed by vigorously blowing nitrogen over the reaction vessel whilst heating to 60 °C. The residual orange oil was purified using flash column chromatography (100% EtOAc:petroleum ether) to afford a bright, light yellow solid (149.2 mg, 57%): *R_f* 0.31 (EtOAc); m.p. 217–220 °C (from EtOAc); ν_{max} (thin film)/cm⁻¹ 1696 (C=O, s); ¹H NMR (400 MHz, CDCl₃) δ 8.63 (1H, d, *J* 2.2 Hz, H-4''), 8.56 (1H, dd, *J* 4.8, 1.6 Hz, H-6''), 8.05 (1H, s, H-1'), 7.68 (1H, dt, *J* 7.9, 2.2 Hz, H-8''), 7.47 (1H, dd, *J* 8.5, 7.2 Hz, H-6), 7.30 (1H, dd, *J* 7.9, 4.8 Hz, H-7''), 7.16 (1H, d, *J* 7.2 Hz, H-5), 6.82 (1H, d, *J* 8.5 Hz, H-7), 6.71 (1H, t, *J* 6.0 Hz, H-1''), 4.93 (1H, dd, *J* 12.2, 5.3 Hz, H-3'), 4.55 (2H, d, *J* 6.0 Hz, H-2''), 2.95 – 2.68 (3H, m, H-4^{ra} and H-5'), 2.20 – 2.12 (1H, m, H-4^{rb}); ¹³C NMR (151 MHz, CDCl₃) δ 170.9 (s, C-6'), 169.6 (s, C-2'), 168.3 (s, C-1), 167.5 (s, C-3), 149.4 (s, C-4''), 149.0 (s, C-6''), 146.4 (s, C-4), 136.5 (s, C-6), 134.8 (s, C-8''), 133.4 (s, C-3''),

132.7 (s, C-7a), 123.9 (s, C-7''), 117.0 (s, C-5), 112.7 (s, C-7), 111.1 (s, C-3a), 49.1 (s, C-3'), 44.6 (s, C-2''), 31.6 (s, C-5'), 22.9 (s, C-4'); HRMS m/z (EI⁺) [Found: 363.1100, C₁₉H₁₅N₄O₄ requires M⁺ 363.1099]; LRMS m/z (ESI⁺) 363 ([M-H]⁺, 100%); HPLC Retention time 220: 5.9 min, 99.5%; 254: 5.9 min, 100.0%.

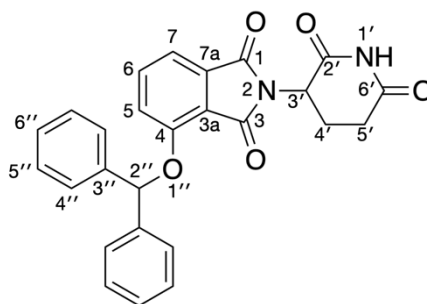
4-(Benzhydrylamino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (86)



2-(2,6-Dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (100 mg, 0.36 mmol, 1.0 eq), benzhydrylamine (250 μ L, 1.45 mmol, 4.0 eq) and DIPEA (252. μ L, 1.45 mmol, 4.0 eq) were dissolved in NMP (4 mL) and heated at 110 °C for 20 h. The NMP was removed by vigorously blowing nitrogen over the reaction vessel whilst heating to 60 °C. The residual dark brown oil was purified using flash column chromatography (50% EtOAc:petroleum ether) from which an impure product was gained; recolumn (30-40% EtOAc:petroleum ether) yields a bright yellow powder (22 mg, 14%): R_f 0.36 (EtOAc:petroleum ether 1:1); m.p. 86–88 °C (from EtOAc); $\nu_{\max}$ (thin film)/cm⁻¹ 1701 (C=O, s); ¹H NMR (600 MHz, CDCl₃) δ 7.92 (1H, s, H-1'), 7.41 – 7.27 (11H, m, H-6, H-4'', H-5'' and H-6''), 7.12 (1H, d, J 8.5 Hz, H-7), 6.86 (1H, d, J 5.5 Hz, H-1''), 6.71 (1H, d, J 8.5 Hz, H-5), 5.69 (1H, d, J 5.5 Hz, H-2''), 4.92 (1H, dd, J 12.5, 5.4 Hz, H-3'), 2.92 – 2.68 (3H, m, H-4'^a and H-5'), 2.13 (1H, dd, J 12.4, 6.4 Hz, H-4'^b); ¹³C NMR (151 MHz, CDCl₃) δ 170.8 (s, C-6'), 169.6 (s, C-2'), 168.3 (s, C-3), 167.6 (s, C-1), 145.9 (s, C-4), 141.44 (s, C-3''), 141.41 (s, C-3''), 136.2 (s, C-6), 132.5 (s, C-7a), 129.22 (s, C-5''), 129.21 (s, C-5''), 128.06 (s, C-6''), 128.03 (s, C-6''), 127.35 (s, C-4''), 127.31 (s, C-4''), 118.3 (s, C-5), 112.4 (s,

C-7), 111.0 (s, C-3a), 61.9 (s, C-2''), 49.1 (s, C-3'), 31.6 (s, C-5'), 23.0 (s, C-4'); HRMS m/z (EI^-) [Found: 438.1460, $\text{C}_{26}\text{H}_{20}\text{N}_3\text{O}_4$ requires M^- 438.1459]; LRMS m/z (ESI^-) 438 ($[\text{M}-\text{H}]^-$, 100%); HPLC Retention time 220: 11.1 min, 96.0%; 254: 11.1 min, 99.1%.

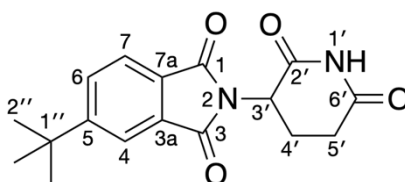
4-(Benzhydryloxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (87)



2-(2,6-Dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (100 mg, 0.36 mmol, 1.0 eq) was dissolved in dry DMF (5 mL) under an argon atmosphere. Benzhydryl bromide (89 mg, 0.36 mmol, 1.0 eq) and potassium carbonate (76mg, 0.550 mmol, 1.5 eq) were added and the mixture stirred at room temperature for 1.5 h. The DMF was then removed by vigorously blowing nitrogen over the reaction vessel. The crude product was purified using flash chromatography (2% MeOH: CH_2Cl_2) to afford a colourless solid (100 mg, 62%): R_f 0.53 (MeOH: CH_2Cl_2 1:9); m.p. 201–204 °C (from CH_2Cl_2); ν_{max} (thin film)/ cm^{-1} 1713 (C=O, s); ^1H NMR (400 MHz, D_6 -DMSO) δ 11.15 (1H, s, H-1'), 7.72 (1H, dd, J 8.6, 7.3 Hz, H-6), 7.63 – 7.56 (4H, m, H-4''), 7.50 (1H, d, J 8.6 Hz, H-7), 7.42 (1H, d, J 7.3 Hz, H-5), 7.37 (4H, td, J 7.7, 1.5 Hz, H-5''), 7.27 (2H, td, J 7.3, 1.4 Hz, H-6''), 6.90 (1H, s, H-2''), 5.14 (1H, dd, J 12.9, 5.4 Hz, H-3'), 2.90 (1H, ddd, J 17.1, 13.9, 5.4 Hz, H-5'^a), 2.67 – 2.51 (2H, m, H-4'^a and H-5'^b), 2.07 (1H, ddt, J 13.4, 6.1, 3.6 Hz, H-4'^b); ^{13}C NMR (151 MHz, D_6 -DMSO) δ 173.3 (s, C-6'), 170.4 (s, C-2'), 167.2 (s, C-3), 165.9 (s, C-1), 154.8 (s, C-4), 141.37 (s, C-3''), 141.35 (s, C-3''), 137.2 (s, C-6), 133.8 (s, C-7a), 129.20 (s, C-5''), 129.18 (s, C-5''), 128.30 (s, C-6''), 128.28 (s, C-6''), 126.67 (s, C-4''), 126.64 (s, C-4''), 121.7 (s, C-7), 117.9 (s, C-3a), 116.2 (s, C-5),

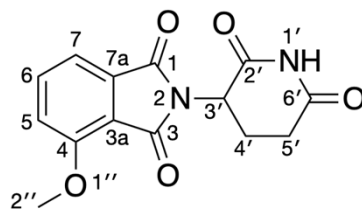
80.8 (s, C-2''), 49.3 (s, C-3'), 31.5 (s, C-5'), 22.5 (s, C-4'); HRMS m/z (EI^-) [Found: 439.1300, $\text{C}_{26}\text{H}_{19}\text{N}_2\text{O}_5$ requires M^- 439.1299]; LRMS m/z (ESI^-) 439 ($[\text{M}-\text{H}]^-$, 100%); HPLC Retention time 220: 10.5 min, 95.9%; 254: 10.5 min, 96.3%.

5-(*tert*-Butyl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (88)



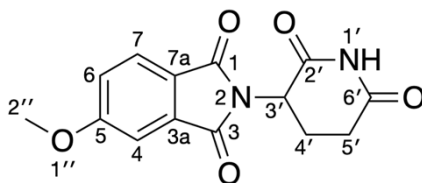
4-*tert*-Butylphthalic anhydride (200 mg, 0.98 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (161mg, 0.98 mmol, 1.0 eq) and NaOAc (121mg, 1.47 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and heated at 120 °C under reflux for 21 h. The AcOH was removed *in vacuo* and the residue purified using flash column chromatography (10% MeOH: CH_2Cl_2) to afford a colourless solid (139 mg, 45%): R_f 0.69 (MeOH: CH_2Cl_2 1:9); m.p. 145–155 °C (from CHCl_3); ^1H NMR (400 MHz, D_6 -DMSO) δ 11.12 (1H, s, H-1'), 7.96 – 7.82 (3H, m, H-4, H-6 and H-7), 5.14 (1H, dd, J 12.9, 5.4 Hz, H-3'), 2.96 – 2.82 (1H, m, H-5'^a), 2.65 – 2.52 (2H, m, H-4'^a and H-5'^b), 2.11 – 1.99 (1H, m, H-4'^b), 1.36 (9H, s, H-1''); LRMS m/z (ESI^-) 313 ($[\text{M}-\text{H}]^-$, 100%); HPLC Retention time 220: 9.7 min, 99.8%; 254: 9.7 min, 100.0%. These data are in good agreement with the literature values.⁶⁵

2-(2,6-Dioxopiperidin-3-yl)-4-methoxyisoindoline-1,3-dione (89)



3-Methoxyphthalic acid (125 mg, 0.70 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (115 mg, 0.70 mmol, 1.0 eq) and NaOAc (86 mg, 1.05 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and heated at 120 °C under reflux for 16.5 h. The AcOH was removed *in vacuo* and the residue purified using flash column chromatography (2% MeOH/CH₂Cl₂) to afford a colourless solid (96 mg, 47%): *R_f* 0.50 (MeOH:CH₂Cl₂ 1:49); m.p. 276–278 °C (from CH₂Cl₂) [lit.⁶⁶ 281–282 °C]; ¹H NMR (400 MHz, D₆-DMSO) δ 11.10 (1H, s, H-1'), 7.84 (1H, dd, *J* 8.6, 7.3 Hz, H-6), 7.53 (1H, dd, *J* 8.6, 0.7 Hz, H-5), 7.46 (1H, dd, *J* 7.3, 0.7 Hz, H-7), 5.09 (1H, dd, *J* 12.7, 5.4 Hz, H-3'), 3.97 (3H, s, H-2''), 2.89 (1H, ddd, *J* 16.8, 13.7, 5.4 Hz, H-5'^a), 2.63 – 2.51 (2H, m, H-4'^a and H-5'^b), 2.11 – 1.97 (1H, m, H-4'^b); LRMS *m/z* (ESI[−]) 287 ([M-H][−], 100%); HPLC Retention time 220: 6.9 min, 96.7%; 254: 6.9 min, 100.0%. These data are in good agreement with the literature value.⁶⁶

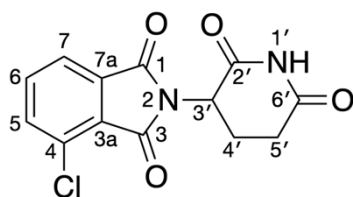
2-(2,6-Dioxopiperidin-3-yl)-5-methoxyisoindoline-1,3-dione (90)



4-Methoxyphthalic acid (167 mg, 0.85 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (140 mg, 0.85 mmol, 1.0 eq) and NaOAc (105 mg, 1.28 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and heated at 120 °C under reflux for 20 h. The AcOH

was removed *in vacuo* and the residue purified using flash column chromatography (2% MeOH:CH₂Cl₂) to afford a colourless solid (122 mg, 50%): *R_f* 0.61 (MeOH:CH₂Cl₂ 1:49); m.p. 218–219 °C (from CH₂Cl₂); $\nu_{\max}$ (thin film)/cm⁻¹ 1717 (C=O, s); ¹H NMR (400 MHz, D₆-DMSO) δ 11.11 (1H, s, H-1'), 7.85 (1H, d, *J* 8.3 Hz, H-7), 7.44 (1H, d, *J* 2.3 Hz, H-4), 7.36 (1H, dd, *J* 8.3, 2.3 Hz, H-6), 5.12 (1H, dd, *J* 12.9, 5.5 Hz, H-3'), 3.94 (3H, s, H-2''), 2.89 (1H, ddd, *J* 17.5, 14.1, 5.5 Hz, H-5'^a), 2.65 – 2.51 (2H, m, H-4'^a and H-5'^b), 2.04 (1H, dtd, *J* 13.5, 6.3, 3.7 Hz, H-4'^b); ¹³C NMR (151 MHz, D₆-DMSO) δ 172.73 (s, C-6'), 169.89 (s, C-2'), 166.86 (s, C-3), 166.79 (s, C-1), 164.67 (s, C-5), 133.92 (s, C-3a), 125.29 (s, C-7), 123.02 (s, C-7a), 120.26 (s, C-6), 108.53 (s, C-4), 56.38 (s, C-2''), 48.95 (s, C-3'), 30.93 (s, C-5'), 22.04 (s, C-4'); HRMS *m/z* (EI⁻) [Found: 287.0676, C₁₄H₁₁N₂O₅ requires M⁻ 287.0673]; LRMS *m/z* (ESI⁻) 287 ([M-H]⁻, 100%); HPLC Retention time 220: 7.6 min, 95.5%; 254: 7.6 min, 100.0%.

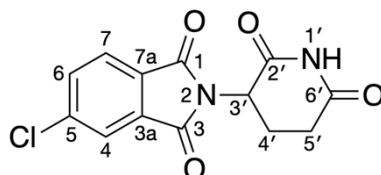
4-Chloro-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (91)



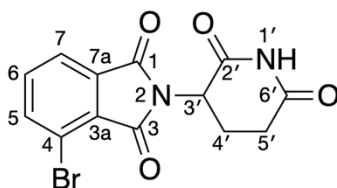
3-Chlorophthalic anhydride (155 mg, 0.85 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (140 mg, 0.85 mmol, 1.0 eq) and NaOAc (105 mg, 1.28 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and heated at 120 °C under reflux for 20 h. The AcOH was removed *in vacuo* and the residue purified using flash column chromatography (2% MeOH:CH₂Cl₂) to afford a colourless solid (19 mg, 8%): *R_f* 0.64 (MeOH:CH₂Cl₂ 1:19); m.p. 273–277 °C (from CH₂Cl₂) [lit.⁶⁶ 290-291 °C]; ¹H NMR (400 MHz, D₆-

DMSO) δ 11.15 (1H, s, H-1'), 7.95 – 7.84 (3H, m, H-5, H-6 and H-7), 5.17 (1H, dd, J 12.8, 5.5 Hz, H-3'), 2.89 (1H, ddd, J 17.0, 13.9, 5.5 Hz, H-5'^a), 2.66 – 2.50 (2H, m, H-4'^a and H-5'^b), 2.07 (1H, dtd, J 13.0, 5.3, 2.2 Hz, H-4'^b); LRMS m/z (ESI⁻) 291 ([M-H]⁻, 100%), 293 ([M-H]⁻, 33%); HPLC Retention time 220: 7.7 min, 100.0%; 254: 7.7 min, 96.4%. These data are in good agreement with the literature values.⁶⁶

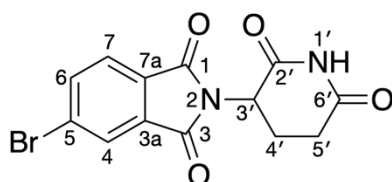
5-Chloro-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (92)



4-Chlorophthalic anhydride (155 mg, 0.85 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (140 mg, 0.85 mmol, 1.0 eq) and NaOAc (105 mg, 1.28 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and heated at 120 °C under reflux for 20 h. The AcOH was removed *in vacuo* and the residue purified using flash column chromatography (2% MeOH:CH₂Cl₂) to afford a pale pink solid (68 mg, 28%): R_f 0.50 (MeOH:CH₂Cl₂ 1:49); m.p. >300 °C (from CH₂Cl₂) [lit.⁶⁷ 312-313 °C]; ¹H NMR (400 MHz, D₆-DMSO) δ 11.15 (1H, s, H-1'), 8.04 (1H, t, J 1.2 Hz, H-4), 7.95 (2H, d, J 1.3 Hz, H-6 and H-7), 5.17 (1H, dd, J 12.8, 5.5 Hz, H-3'), 2.89 (1H, ddd, J 17.0, 13.9, 5.5 Hz, H-5'^a), 2.65 – 2.52 (2H, m, H-4'^a and H-5'^b), 2.06 (1H, dtd, J 12.9, 5.2, 2.2 Hz, H-4'^b); LRMS m/z (ESI⁻) 291 ([M-H]⁻, 100%), 293 ([M-H]⁻, 33%); HPLC Retention time 220: 7.9 min, 95.2%; 254: 7.9 min, 100.0%. These data are in good agreement with the literature values.⁶⁷

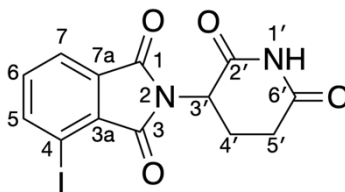
4-Bromo-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (93)

3-Bromophthalic anhydride (200 mg, 0.84 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (137 mg, 0.84 mmol, 1.0 eq) and NaOAc (103 mg, 1.25 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and heated at 120 °C under reflux for 15 h. The AcOH was removed *in vacuo* and the residue purified using flash column chromatography (2% MeOH:CH₂Cl₂) to afford a colourless solid (115 mg, 41%): *R_f* 0.68 (MeOH:CH₂Cl₂ 1:19); m.p. 283–286 °C (from CH₂Cl₂); ¹H NMR (400 MHz, D₆-DMSO) δ 11.15 (1H, s, H-1'), 8.07 (1H, dd, *J* 8.1, 0.9 Hz, H-5), 7.94 (1H, dd, *J* 7.4, 0.9 Hz, H-7), 7.78 (1H, dd, *J* 8.1, 7.4 Hz, H-6), 5.17 (1H, dd, *J* 12.8, 5.4 Hz, H-3'), 2.89 (1H, ddd, *J* 17.0, 13.9, 5.4 Hz, H-5'^a), 2.66 – 2.50 (2H, m, H-4'^a and H-5'^b), 2.12 – 2.01 (1H, m, H-4'^b); LRMS *m/z* (ESI[−]) 335 ([M-H][−], 97%), 337 ([M-H][−], 100%); HPLC Retention time 220: 7.9 min, 100.0%; 254: 7.9 min, 98.8%. These data are in good agreement with the literature values.⁶⁸

5-Bromo-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (94)

4-Bromophthalic anhydride (1g, 4.18 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (688 mg, 4.18 mmol, 1.0 eq) and NaOAc (514 mg, 6.27 mmol, 1.5 eq) were dissolved in AcOH (30 mL) and heated at 120 °C under reflux for 1 h. The AcOH was removed *in vacuo* and the residue purified using flash column chromatography (5% MeOH:CH₂Cl₂) to afford a colourless solid (585 mg, 42%): *R_f* 0.53 (MeOH:CH₂Cl₂ 1:19); m.p. 292–297 °C – dec. (from CH₂Cl₂) [lit.⁶² 230 °C, lit.⁹³ 240–241 °C, lit.⁹⁸ 303 °C]; ¹H NMR (400 MHz, D₆-DMSO) δ 11.15 (1H, s, H-1'), 8.15 (1H, dd, *J* 1.7, 0.6 Hz, H-4), 8.10 (1H, dd, *J* 7.9, 1.7 Hz, H-6), 7.87 (1H, dd, *J* 7.9, 0.6 Hz, H-7), 5.17 (1H, dd, *J* 12.8, 5.4 Hz, H-3'), 2.89 (1H, ddd, *J* 17.0, 13.8, 5.4 Hz, H-5'^a), 2.65 – 2.52 (2H, m, H-4'^a and H-5'^b), 2.06 (1H, dtd, *J* 13.0, 5.3, 2.2 Hz, H-4'^b); LRMS *m/z* (ESI[−]) 335 ([M-H][−], 97%), 337 ([M-H][−], 100%); HPLC Retention time 220: 8.3 min, 96.2%; 254: 8.3 min, 97.6%. These data are in good agreement with the literature values.⁶⁵

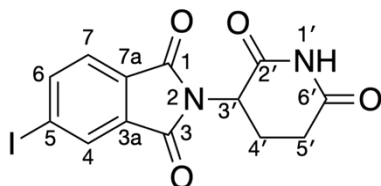
2-(2,6-Dioxopiperidin-3-yl)-4-iodoisoindoline-1,3-dione (95)



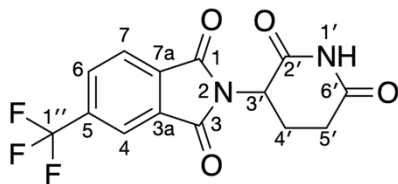
3-Iodophthalic acid (300 mg, 1.03 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (169 mg, 1.03 mmol, 1.0 eq) and NaOAc (126 mg, 1.54 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and heated at 120 °C under reflux for 1 h. The AcOH was removed *in vacuo* and the residue purified using flash column chromatography (2% MeOH:CH₂Cl₂) to afford a colourless solid (156 mg, 40%): *R_f* 0.60 (MeOH:CH₂Cl₂ 1:19); m.p. 286–290 °C (from CH₂Cl₂) [lit.⁶² 304–305 °C]; ¹H NMR (400 MHz, D₆-

DMSO) δ 11.15 (1H, s, H-1'), 8.28 (1H, dd, J 7.9, 0.9 Hz, H-5), 7.93 (1H, dd, J 7.4, 0.9 Hz, H-7), 7.58 (1H, dd, J 7.9, 7.4 Hz, H-6), 5.16 (1H, dd, J 12.8, 5.4 Hz, H-3'), 2.89 (1H, ddd, J 17.0, 13.8, 5.4 Hz, H-5'^a), 2.65 – 2.52 (2H, m, H-4'^a and H-5'^b), 2.06 (1H, dtd, J 12.9, 5.1, 2.1 Hz, H-4'^b); LRMS m/z (ESI⁻) 382.9 ([M-H]⁻, 100%); HPLC Retention time 220: 8.2 min, 95.9%; 254: 8.2 min, 100.0%. These data are in good agreement with the literature values.⁶⁹

2-(2,6-Dioxopiperidin-3-yl)-5-iodoisoindoline-1,3-dione (96)

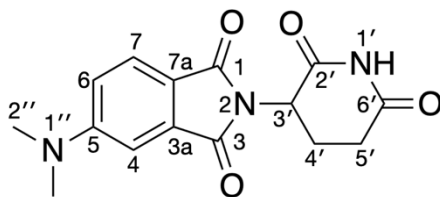


4-Iodophthalic acid (124 mg, 0.43 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (70 mg, 0.43 mmol, 1.0 eq) and NaOAc (53 mg, 0.64 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and heated at 120 °C under reflux for 2 h. The AcOH was removed *in vacuo* and the residue purified using flash column chromatography (2% MeOH:CH₂Cl₂) to afford a colourless solid (93 mg, 57%); R_f 0.48 (MeOH:CH₂Cl₂ 1:49); m.p. 243–245 °C (from CH₂Cl₂); $\nu_{\max}$ (thin film)/cm⁻¹ 1726 (C=O, s); ¹H NMR (400 MHz, D₆-DMSO) δ 11.14 (1H, s, H-1'), 8.31 – 8.24 (2H, m, H-4 and H-6), 7.69 (1H, d, J 8.1 Hz, H-7), 5.15 (1H, dd, J 12.9, 5.4 Hz, H-3'), 2.88 (1H, ddd, J 17.0, 13.9, 5.4 Hz, H-5'^a), 2.65 – 2.51 (2H, m, H-4'^a and H-5'^b), 2.05 (1H, dtd, J 12.9, 5.2, 2.1 Hz, H-4'^b); ¹³C NMR (151 MHz, D₆-DMSO) δ 172.69 (s, C-6'), 169.67 (s, C-2'), 166.76 (s, C-3), 165.92 (s, C-1), 143.47 (s, C-6), 132.65 (s, C-3a), 131.83 (s, C-4), 130.43 (s, C-7a), 124.92 (s, C-7), 102.76 (s, C-5), 49.07 (s, C-3'), 30.88 (s, C-5'), 21.89 (s, C-4'); HRMS m/z (EI⁻) [Found: 382.9538, C₁₃H₈IN₂O₄ requires M⁻ 382.9534]; LRMS m/z (ESI⁻) 382.9 ([M-H]⁻, 100%); HPLC Retention time 220: 8.6 min, 96.6%; 254: 8.6 min, 98.7%.

2-(2,6-Dioxopiperidin-3-yl)-5-(trifluoromethyl)isoindoline-1,3-dione (97)

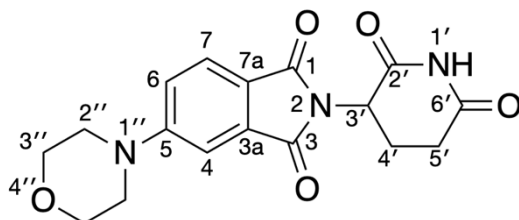
4-(Trifluoromethyl)phthalic acid (200 mg, 0.850 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (140 mg, 0.85 mmol, 1.0 eq) and NaOAc (105 mg, 1.28 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and heated at 120 °C under reflux for 20 h. The AcOH was removed *in vacuo* and the residue purified using flash column chromatography (5% MeOH:CH₂Cl₂) to afford a pale pink solid (192 mg, 69%): *R_f* 0.75 (MeOH:CH₂Cl₂ 1:9); m.p. 190–192 °C (from CH₂Cl₂); $\nu_{\max}$ (thin film)/cm⁻¹ 1730 (C=O, s); ¹H NMR (400 MHz, D₆-DMSO) δ 11.18 (1H, s, H-1'), 8.29 (2H, d, *J* 8.1 Hz, H-4 and H-6), 8.16 (1H, d, *J* 7.7 Hz, H-7), 5.22 (1H, dd, *J* 12.8, 5.4 Hz, H-3'), 2.90 (1H, ddd, *J* 17.0, 13.8, 5.4 Hz, H-5'^{ra}), 2.67 – 2.52 (2H, m, H-4'^{ra} and H-5'^{rb}), 2.08 (1H, dtd, *J* 13.0, 5.3, 2.1 Hz, H-4'^{rb}); ¹³C NMR (151 MHz, CDCl₃) δ 172.7 (s, C-6'), 169.6 (s, C-2'), 166.0 (s, C-1), 165.8 (s, C-3), 134.7 (s, C-3a), 134.5 (q, *J*_{C-F} = 32.804 Hz, C-5), 132.2 (s, C-7a), 132.0 (d, *J*_{C-F} = 21.204 Hz, C-6), 124.5 (d, *J*_{C-F} = 22.369 Hz, C-7), 123.2 (q, *J*_{C-F} = 273.104 Hz, C-1''), 120.4 (d, *J*_{C-F} = 21.203 Hz, C-4), 49.3 (s, C-3'), 30.87 (s, C-5'), 21.8 (s, C-4'); ¹⁹F NMR (565 MHz, CDCl₃) δ -56.61; HRMS *m/z* (EI⁻) [Found: 325.0439, C₁₄H₈F₃N₂O₄ requires M⁻ 325.0442]; LRMS *m/z* (ESI⁻) 325 ([M-H]⁻, 100%); HPLC Retention time 220: 8.5 min, 100.0%; 254: 9.5 min, 100.0%.

5-(Dimethylamino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (98)



2-(2,6-Dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione (50mg, 0.181 mmol, 1.0 eq), dimethylamine (2.0 M in THF, 2.90 mmol, 16.0 eq) and DIPEA (504 μ L, 2.90 mmol, 16.0 eq) were dissolved in DMF (4 mL) and heated at 110 $^{\circ}$ C for 21 h. The DMF was removed by vigorously blowing nitrogen over the reaction vessel. The residual dark brown oil was purified using flash chromatography (45-50% EtOAc:petroleum ether) to afford a bright yellow powder (48.1 mg, 88%): R_f 0.07 (EtOAc:petroleum ether 9:11); m.p. 241–243 $^{\circ}$ C (from EtOAc) [lit.⁷² 240-242 $^{\circ}$ C]; 1 H NMR (400 MHz, CDCl_3) δ 7.99 (1H, s, H-1'), 7.67 (1H, d, J 8.6 Hz, H-7), 7.09 (1H, d, J 2.4 Hz, H-4), 6.82 (1H, dd, J 8.6, 2.4 Hz, H-6), 4.98 – 4.89 (1H, m, H-3'), 3.13 (6H, s, H-2''), 2.94 – 2.64 (3H, m, H-4'^a and H-5'), 2.21 – 2.08 (1H, m, H-4'^b); LRMS m/z (ESI⁻) 300 ($[\text{M}-\text{H}]^-$, 100%); HPLC Retention time 220: 7.5 min, 98.9%; 254: 7.5 min, 98.6%. These data are in good agreement with the literature values.⁷²

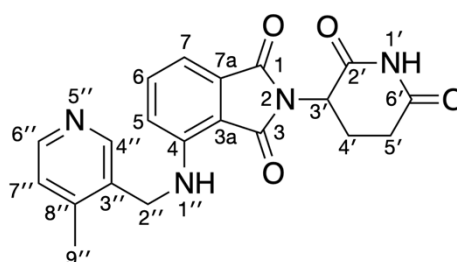
2-(2,6-Dioxopiperidin-3-yl)-5-morpholinoisoindoline-1,3-dione (99)



2-(2,6-Dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione (50mg, 0.18 mmol, 1.0 eq), morpholine (32 μ L, 0.36 mmol, 2.0 eq) and DIPEA (126 μ L, 0.72 mmol, 4.0 eq) were dissolved

in NMP (2 mL) and heated at 110 °C for 2 h in a microwave. The NMP was removed by vigorously blowing nitrogen over the reaction vessel while heating to 60 °C. The residual dark brown oil was purified using flash chromatography (40% EtOAc:petroleum ether) to afford a bright yellow solid (23 mg, 37%); R_f 0.09 (EtOAc:petroleum ether 1:1); m.p. 207–208 °C (from EtOAc); $\nu_{\max}$ (thin film)/ cm^{-1} 1707 (C=O, s); ^1H NMR (400 MHz, CDCl_3) δ 7.95 (1H, s, H-1'), 7.73 (1H, d, J 8.5 Hz, H-7), 7.30 (1H, d, J 2.4 Hz, H-4), 7.08 (1H, dd, J 8.5, 2.4 Hz, H-6), 4.95 (1H, dd, J 12.3, 5.3 Hz, H-3'), 3.88 (4H, dd, J 6.0, 3.8 Hz, H-3''), 3.41 – 3.34 (4H, m, H-2''), 2.95 – 2.68 (3H, m, H-4'^a and H-5'), 2.19 – 2.09 (1H, m, H-4'^b); ^{13}C NMR (151 MHz, CDCl_3) δ 170.9 (s, C-6'), 168.2 (s, C-2'), 167.9 (s, C-1), 167.3 (s, C-3), 155.8 (s, C-5), 134.4 (s, C-3a), 125.5 (s, C-7), 120.5 (s, C-7a), 118.1 (s, C-6), 108.8 (s, C-4), 66.5 (s, C-3''), 49.4 (s, C-3'), 47.8 (s, C-2''), 31.6 (s, C-5'), 22.9 (s, C-4'); HRMS m/z (EI^-) [Found: 342.1098, $\text{C}_{17}\text{H}_{16}\text{N}_3\text{O}_5$ requires M^- 342.1095]; LRMS m/z (ESI^-) 342 ($[\text{M}-\text{H}]^-$, 100%); HPLC Retention time 220: 7.4 min, 96.5%; 254: 7.4 min, 97.5%.

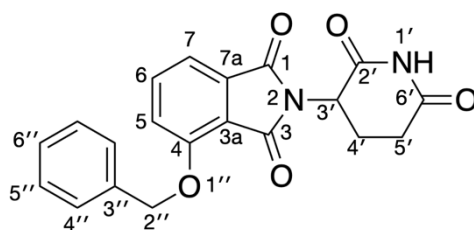
2-(2,6-Dioxopiperidin-3-yl)-4-(((4-methylpyridin-3-yl)methyl)amino)isoindoline-1,3-dione (100)



2-(2,6-Dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (282 mg, 1.02 mmol, 1.0 eq), (4-methylpyridin-3-yl)methylamine (250 mg, 2.05 mmol, 2.0 eq) and DIPEA (713 μL , 4.09 mmol, 4.0 eq) were dissolved in NMP (4 mL) and heated at 110 °C for 2 h in a microwave. The NMP was removed by vigorously blowing nitrogen over the reaction vessel whilst heating to 60 °C. The residual orange oil was purified using flash column chromatography (100%

EtOAc:petroleum ether) to afford a bright yellow oil. TLC showed this was impure; recolumn (2% MeOH:CH₂Cl₂) yielded a bright yellow/green glass-like oil, which was placed under vacuum for 72 h. Redissolving this in CHCl₃ and removal of solvent *via* nitrogen stream yielded a bright yellow powder (338mg, 87%): *R_f* 0.21 (MeOH:CH₂Cl₂ 1:49); m.p. 199–202 °C (from EtOAc); ν_{max} (thin film)/cm⁻¹ 1694 (C=O, s); ¹H NMR (400 MHz, CDCl₃) δ 8.51 (1H, s, H-1'), 8.50 (1H, s, H-4''), 8.45 (1H, d, *J* 4.9 Hz, H-6''), 7.50 (1H, dd, *J* 8.5, 7.1 Hz, H-6), 7.19 – 7.10 (2H, m, H-5 and H-7''), 6.89 (1H, d, *J* 8.4 Hz, H-7), 6.45 (1H, t, *J* 5.6 Hz, H-1''), 4.90 (1H, dd, *J* 12.1, 5.3 Hz, H-3'), 4.47 (2H, d, *J* 5.6 Hz, H-2''), 2.92 – 2.66 (3H, m, H-4'^a and H-5'), 2.39 (3H, s, H-9''), 2.18 – 2.08 (1H, m, H-4'^b); ¹³C NMR (151 MHz, CDCl₃) δ 170.9 (s, C-6'), 169.6 (s, C-2'), 168.3 (s, C-1), 167.5 (s, C-3), 149.3 (s, C-4''), 148.9 (s, C-6''), 146.4 (s, C-4), 146.3 (s, C-8''), 136.5 (s, C-6''), 132.7 (s, C-3''), 131.4 (s, C-7a), 125.8 (s, C-7''), 116.8 (s, C-5), 112.6 (s, C-7), 111.1 (s, C-3a), 49.1 (s, C-3'), 43.1 (s, C-2''), 31.6 (s, C-5'), 22.9 (s, C-4'), 18.8 (s, C-9''); HRMS *m/z* (EI⁻) [Found: 377.1253, C₂₀H₁₇N₄O₄ requires M⁻ 377.1255]; LRMS *m/z* (ESI⁻) 377 ([M-H]⁻, 100%); HPLC Retention time 220: 5.9 min, 99.5%; 254: 5.9 min, 100.0%.

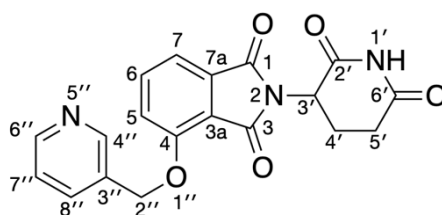
4-(Benzyloxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (101)



2-(2,6-Dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (100 mg, 0.36 mmol, 1.0 eq) was dissolved in dry DMF (5 mL) under an argon atmosphere. Benzyl bromide (43 μ L, 0.36 mmol, 1.0 eq) and potassium carbonate (76 mg, 0.55 mmol, 1.5 eq) were added and the mixture stirred at room temperature for 1.5 h. The reaction mixture was dissolved in aqueous 0.5 M LiCl and extracted with EtOAc. The organic layer was washed with water, then brine, then

dried with sodium sulfate, filtered and the solvent removed *in vacuo*. The residue was purified using flash chromatography (5% MeOH:CH₂Cl₂). Further purification was achieved using a second round of flash chromatography (3% MeOH:CH₂Cl₂), as residual benzyl bromide was still present after first column. This afforded a colourless solid (71 mg, 53%): *R_f* 0.65 (MeOH:CH₂Cl₂ 1:9); m.p. 223–226 °C (from CH₂Cl₂); ¹H NMR (400 MHz, D₆-DMSO) δ 11.10 (1H, s, H-1'), 7.83 (1H, dd, *J* 8.5, 7.3 Hz, H-6), 7.60 (1H, d, *J* 8.5 Hz, H-7), 7.55 – 7.30 (6H, m, H-5, H-4'', H-5'' and H-6''), 5.38 (2H, s, H-2''), 5.09 (1H, dd, *J* 12.8, 5.4 Hz, H-3'), 2.88 (1H, ddd, *J* 17.2, 14.0, 5.4 Hz, H-5'^a), 2.64 – 2.51 (1H, m, H-4'^a and H-5'^b), 2.04 (1H, dp, *J* 11.4, 3.5 Hz, H-4'^b); LRMS *m/z* (ESI[−]) 363 ([M-H][−], 100%); HPLC Retention time 220: 9.2 min, 96.7%; 254: 9.2 min, 96.8%. These data are in good agreement with the literature values.⁷⁵

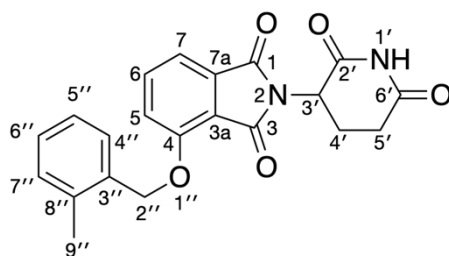
2-(2,6-Dioxopiperidin-3-yl)-4-(pyridin-3-ylmethoxy)isoindoline-1,3-dione (102)



2-(2,6-Dioxopiperidin-3-yl)-4-hydroxy-1*H*-isoindole-1,3(2*H*)-dione (100 mg, 0.36 mmol, 1.0 eq) was dissolved in DMF (5 mL). Potassium carbonate (76 mg, 0.55 mmol, 1.5 eq) was then added and mixed. 3-(Bromomethyl)pyridine hydrobromide (82 mg, 0.32 mmol, 0.9 eq) was added and the mixture stirred at room temperature for 1.5 h. The DMF was removed by vigorously blowing nitrogen over the reaction vessel, and the residue was purified using flash chromatography (2% MeOH:CH₂Cl₂) to afford a colourless solid (68.8mg, 59%): *R_f* 0.38 (MeOH:CH₂Cl₂ 1:9); m.p. 217–220 °C (from CH₂Cl₂); *ν*_{max} (thin film)/cm^{−1} 1709 (C=O, s); ¹H NMR (400 MHz, D₆-DMSO) δ 11.12 (1H, s, H-1'), 8.73 (1H, s, H-4''), 8.57 (1H, d, *J* 4.8 Hz, H-6''), 7.93 (1H, d, *J* 6.8 Hz, H-8''), 7.86 (1H, t, *J* 7.7 Hz, H-6), 7.64 (1H, d, *J* 8.6 Hz, H-7), 7.50 (1H, d, *J* 8.6 Hz H-5), 7.46 (1H, dd, *J* 6.8, 4.8 Hz, H-7''), 5.42 (1H, s, H-2''), 5.09 (1H, dd, *J*

12.9, 5.2 Hz, H-3'), 2.88 (1H, t, J 15.6 Hz, H-5'^a), 2.65 – 2.52 (2H, m, H-4'^a and H-5'^b), 2.07 – 1.99 (1H, m, H-4'^b); ¹³C NMR (151 MHz, D₆-DMSO) δ 172.7 (s, C-6'), 169.9 (s, C-2'), 166.7 (s, C-3), 165.3 (s, C-1), 155.3 (s, C-4), 149.3 (s, C-4''), 148.7 (s, C-6''), 137.1 (s, C-8''), 135.3 (s, C-6), 133.3 (s, C-7a), 131.8 (s, C-3''), 123.6 (s, C-7''), 120.2 (s, C-7), 116.8 (s, C-5), 115.8 (s, C-3a), 67.9 (s, C-2''), 48.8 (s, C-3'), 30.9 (s, C-5'), 22.0 (s, C-4'); HRMS m/z (EI⁺) [Found: 364.0940, C₁₉H₁₄N₃O₅ requires M⁺ 364.0939]; LRMS m/z (ESI⁺) 364 ([M-H]⁺, 100%); HPLC Retention time 220: 5.7 min, 98.3%; 254: 5.7 min, 100.0%.

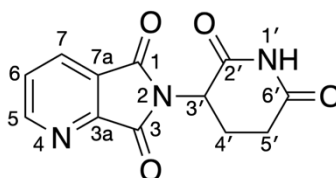
2-(2,6-Dioxopiperidin-3-yl)-4-((2-methylbenzyl)oxy)isoindoline-1,3-dione (103)



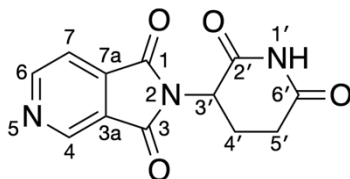
2-(2,6-Dioxopiperidin-3-yl)-4-hydroxy-1*H*-isoindole-1,3(2*H*)-dione (100 mg, 0.36 mmol, 1.0 eq) was dissolved in DMF (5 mL). Potassium carbonate (76 mg, 0.55 mmol, 1.5 eq) was then added and mixed. 2-Methylbenzyl bromide (44 μ L, 0.32 mmol, 0.9 eq) was then added and the mixture stirred at room temperature for 1.5 h. The DMF was removed by vigorously blowing nitrogen over the reaction vessel, and the residue was purified using flash chromatography (2-5% MeOH:CH₂Cl₂) to afford a colourless solid (117 mg, 97%): R_f 0.56 (MeOH:CH₂Cl₂ 1:9); m.p. 213–215 °C (from CH₂Cl₂); $\nu_{\max}$ (thin film)/cm⁻¹ 1704 (C=O, s); ¹H NMR (400 MHz, D₆-DMSO) δ 11.12 (1H, s, H-1'), 7.85 (1H, dd, J 8.6, 7.2 Hz, H-6), 7.68 (1H, d, J 8.6 Hz, H-7), 7.55 (1H, d, J 7.0 Hz, H-7''), 7.48 (1H, d, J 7.2 Hz, H-5), 7.31 – 7.18 (3H, m, H-4'', H-5'' and H-6''), 5.35 (2H, s, H-2''), 5.09 (1H, dd, J 12.8, 5.4 Hz, H-3'), 2.88 (1H, ddd, J 17.0, 13.7, 5.4 Hz, H-5'^a), 2.63 – 2.51 (2H, m, H-4'^a and H-5'^b), 2.36 (3H, s, H-9''), 2.08 – 1.99 (1H, m, H-4'^b); ¹³C NMR (151 MHz, D₆-DMSO) δ 172.7 (s, C-6'), 169.9 (s, C-2'), 166.8 (s, C-

3), 165.3 (s, C-1), 155.5 (s, C-4), 137.0 (s, C-6"), 136.5 (s, C-8"), 134.1 (s, C-3"), 133.3 (s, C-7a), 130.1 (s, C-7"), 128.2 (s, C-6"), 128.0 (s, C-4"), 125.8 (s, C-5"), 120.2 (s, C-7), 116.6 (s, C-3a), 115.5 (s, C-5), 68.8 (s, C-2"), 48.8 (s, C-3'), 30.9 (s, C-5'), 22.0 (s, C-4'), 18.5 (s, C-9"); HRMS m/z (EI^-) [Found: 377.1144, $\text{C}_{21}\text{H}_{17}\text{N}_2\text{O}_5$ requires M^- 377.1143]; LRMS m/z (ESI^-) 377 ($[\text{M}-\text{H}]^-$, 100%); HPLC Retention time 220: 9.7 min, 96.2%; 254: 9.7 min, 97.4%.

6-(2,6-Dioxopiperidin-3-yl)-5H-pyrrolo[3,4-*b*]pyridine-5,7(6H)-dione (104)



2,3-Pyridinedicarboxylic anhydride (201 mg, 1.35 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (222 mg, 1.35 mmol, 1.0eq) and NaOAc (166 mg, 2.03 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and heated at 120 °C under reflux for 19 h. The AcOH was removed *in vacuo* and the residue purified using flash column chromatography (5% MeOH: CH_2Cl_2) to afford a colourless solid (200 mg, 57%): R_f 0.50 (MeOH: CH_2Cl_2 1:9); m.p. 245–247 °C (from $\text{H}_2\text{O}/\text{MeCN}$) [lit.⁹⁹ 258–259 °C, lit.¹⁰⁰ 266–267 °C]; ^1H NMR (400 MHz, D_6 -DMSO) δ 11.17 (1H, s, H-1'), 9.04 (1H, dt, J 5.0, 1.2 Hz, H-5), 8.42 – 8.34 (1H, m, H-7), 7.85 (1H, ddd, J 7.5, 4.8, 0.7 Hz, H-6), 5.24 (1H, dd, J 12.9, 5.4 Hz, H-3'), 2.90 (1H, ddd, J 17.2, 13.8, 5.4 Hz, H-5'^a), 2.67 – 2.52 (2H, m, H-4'^a and H-5'^b), 2.08 (1H, dtd, J 13.1, 5.2, 2.2 Hz, H-4'^b); LRMS m/z (ESI^-) 258 ($[\text{M}-\text{H}]^-$, 100%); HPLC Retention time 220: 5.1 min, 99.7%; 254: 5.2 min, 100.0%. These data are in good agreement with the literature values.⁶⁵

2-(2,6-Dioxopiperidin-3-yl)-1*H*-pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-dione (105)

3,4-Pyridinedicarboxylic anhydride (201 mg, 1.35 mmol, 1.0 eq), 3-aminopiperidine-2,6-dione hydrochloride (222 mg, 1.35 mmol, 1.0eq) and NaOAc (166 mg, 2.03 mmol, 1.5 eq) were dissolved in AcOH (5 mL) and heated at 120 °C under reflux for 19.5 h. The AcOH was removed *in vacuo* and the residue purified using flash column chromatography (5% MeOH:CH₂Cl₂) to afford a colourless solid (314.3 mg, 90%): *R_f* 0.61 (MeOH:CH₂Cl₂ 1:9); m.p. 219–221 °C (from H₂O/MeCN) [lit.¹⁰¹ 233–235 °C, lit.¹⁰⁰ 234–235 °C]; ¹H NMR (400 MHz, D₆-DMSO) δ 11.18 (1H, s, H-1'), 9.22 – 9.11 (2H, m, H-4 and H-7), 7.97 (1H, dt, *J* 4.8, 1.1 Hz, H-6), 5.22 (1H, dd, *J* 12.9, 5.4 Hz, H-3'), 2.89 (1H, ddd, *J* 17.3, 13.9, 5.4 Hz, H-5'^a), 2.67 – 2.50 (2H, m, H-4'^a and H-5'^b), 2.07 (1H, ddq, *J* 11.1, 6.0, 2.8 Hz, H-4'^b); LRMS *m/z* (ESI[−]) 258 ([M-H][−], 100%); HPLC Retention time 220: 5.2 min, 99.4%; 254: 5.2 min, 100.0%. These data are in good agreement with the literature values.⁶⁵

7.3 Biological Methods

Cell culture

Jurkat cells were cultured in a media of RPMI (Gibco), 10% heat-inactivated FBS (Gibco), 1% Glutamax (Gibco), 1% MEM non-essential amino acids (Gibco), 1% sodium pyruvate (Gibco) and 1% PenStrep (Sigma). Lenti-X 293-T cells (Takara) were cultured in a media of DMEM high glucose (Gibco), 10% Tet-free FBS (Takara), 1% sodium pyruvate and 1% PenStrep. HEK293T cells were cultured in a media of DMEM, 10% heat-inactivated FBS, 1% MEM non-essential amino acids and 1% PenStrep. Cells were incubated at 37 °C and 5% CO₂.

Cloning

The degradation reporter plasmid, pLVX-Sprout, was generated by inserting a gene fragment encoding for IKZF3 130aa-142aa 170-189aa fused to EGFP *via* a linker into pLVX-EF1a-IRES-mCherry (Takara) using XbaI/BamHI restriction sites (*carried out by Dr Lewis Brayshaw at GlaxoSmithKline, Stevenage, UK*). Gene fragments encoding for IKZF3 143aa-169aa sequences were cloned into pLVX-Sprout using the Golden Gate Assembly Kit BsmBI-v2 (New England Biolabs). Gene fragments encoding for either HiBit-TRIM28, HiBit-TRIM28 N-terminal IPID degron or HiBit-TRIM28 C-terminal IPID degron were inserted into pLVX-EF1a-IRES-mCherry (Takara) using XbaI/BamHI restriction sites (*carried out by Dr Lewis Brayshaw at GlaxoSmithKline, Stevenage, UK*).

Lentivirus vector production and transduction

The below method (*carried out by Dr Lewis Brayshaw at GlaxoSmithKline, Stevenage, UK*) was used to generate lentiviral vectors for plasmids used in initial compound screening (WT and Q147A) and TRIM28 degradation (TRIM28, TRIM28 N-IPID and TRIM28 C-IPID). The following method was also used to generate the library lentiviral vector pool. Lenti-X 293-T

cells were seeded into 100 mm TC-treated cell culture dishes (Falcon). When cells were 90% confluent, 7 µg transgene plasmid was mixed with Lenti-X™ Packaging Single Shot (VSV-G) (Takara) and incubated for 10 minutes at room temperature before being added to cells. 6 hours later, Viralboost (Alstem) was added at a 500X dilution. After 3 days, the supernatant was harvested and centrifuged at 1000 x g for 10 minutes, then supernatant was passed through a 0.22 µm PES filter. Lenti-X concentrator (Takara) was added at a 4X dilution and the supernatant was incubated at 4 °C for 6 hours. Supernatant was centrifuged at 4 °C 1500 x g for 45 minutes, supernatant was removed and virus pellet was resuspended at a 50X concentration in cell culture media used for Jurkat cells. Lentivirus vector aliquots were frozen at -80 °C. For transductions, lentivirus vectors were added to Jurkat cells in 6 well TC-treated cell culture dishes (Falcon) with 3×10^6 cells per well and incubated for 2 days at 37 °C and 5% CO₂. After 2 days, the supernatant was removed and replaced with fresh media.

The following method (*carried out by Dr Ryan Beveridge at the Weatherall Institute of Molecular Medicine, Oxford, UK*) was used to generate lentiviral vectors for plasmids tested in library hit confirmation experiments: WT, QPIS, ANQS, APIS, HEIP, FEVP, WEIK, FDIP, AEVK, WNQS, WEIS, WNIQ, and WDIA. HEK293T cells were seeded into 6 well TC-treated cell culture dishes (Falcon) at 7×10^5 cells per well and incubated overnight at 37 °C and 5% CO₂. 888 ng of transgene plasmid, 444 ng of psPAX2 (Addgene Cat# 12260), and 222 ng of pMD2.G (Addgene Cat#12259) were combined in 100 µL of OptiMEM (Thermo). In a separate tube 2 µL of PEIPro (Polyplus) was added to 100 µL of OptiMEM. The solution containing PEIPro and DNA was mixed and incubated at room temperature for 15 minutes to create the transfection mixture. The transfection mixture was added to cells and incubated for 5-6 hours at 37 °C and 5% CO₂. After 2 days, the supernatant was harvested and fresh media was added to cells. 24 hours later, the supernatant was harvested and combined with the first supernatant harvested. Supernatant was centrifuged at 2000 x g room temperature for 5 minutes, then the supernatant was filtered through a 0.45 µm cellulose acetate syringe filter and frozen at -80 °C. For transductions, lentivirus vectors were added to Jurkat cells in 6 well TC-treated

cell culture dishes (Falcon) with 2.5×10^6 cells per well in media containing 4 $\mu\text{g/mL}$ polybrene (Sigma). The cells were centrifuged at $500 \times g$ for 60 minutes at 30°C . Cells were incubated for 3 days at 37°C and 5% CO_2 prior to replacing supernatant with fresh media. (*psPAX2* and *pMD2.G* were a gift from Didier Trono's lab).

GFP degradation flow cytometry assay

Test compounds (10 mM stock in DMSO) were either serially diluted 10-fold to create a 6 point dilution spanning 1 nM-100 μM (final assay concentration) or serially diluted 2-fold to create a 12 point dilution spanning 5 nM-10 μM . Jurkat cells were added to 96 well U-bottom assay plates (Falcon) at 1×10^5 cells per well and treated with compounds for 18 hours at 37°C and 5% CO_2 . Cells were stained with 1 $\mu\text{g/mL}$ DAPI (ThermoFisher) and acquired on a Cytoflex S (Beckman Coulter). Data were analysed using FlowJo 10.8.1 (BD).

Compound solubility (*carried out by Laurie Gordon at GlaxoSmithKline, Stevenage, UK*)

A DMSO stock solution of IMiD analogue (5 μL , 10 mM) was diluted to a volume of 100 μL with pH 7.4 PBS, equilibrated for 1 h at room temperature and filtered through Millipore Multiscreen HTS-PCF filter plates (MSSL BPC). The filtrate was quantified by suitably calibrated Charged Aerosol Detector (**ref**).

Protein expression and purification (*carried out at GlaxoSmithKline, Stevenage, UK*)

Wild-type human 6xHis-TEV-Cereblon and 6xHis-TEV-DDB1 Δ BPB were cloned in pFastbac-HTb (Genscript), and recombinant proteins were expressed in Super 9 SFX insect cells (SH3A3187.02, Cytiva) using the BacMam baculovirus expression system (ThermoFisher). For protein purification cells were resuspended in buffer containing 500 mM NaCl (Sigma), 10% glycerol (Sigma), 50 mM Tris pH 7.7 (Sigma), 15 mM imidazole (Sigma), 90.5 mM TCEP (Sigma), 5 $\mu\text{L/mL}$ protease inhibitor cocktail (Sigma) and 0.66 $\mu\text{L/mL}$ benzonase (Novagen). Cells were lysed by sonication and pelleted by ultra-centrifugation. The supernatant was passed over Ni-NTA affinity resin (Qiagen) and eluted in buffer containing 500 mM NaCl, 10%

glycerol, 50 mM Tris pH 7.7 and 90.5 mM TCEP supplemented with 350 mM imidazole. Proteins were further purified *via* size exclusion chromatography in 50 mM Tris pH 7.5, 5% glycerol, 2 mM DTT (Sigma), and 150 mM NaCl. Protein fractions were concentrated by ultrafiltration using an Ultra 10 K MWCO device (Amicon), then flash frozen in liquid nitrogen and stored at -80°C .

CRBN binding (*carried out by Laurie Gordon at GlaxoSmithKline, Stevenage, UK*)

Test compounds (10 mM stock in DMSO) were serially diluted 4-fold to create an 11 point dilution spanning 0.1 nM-100 μM . 100 nL of compounds were added to black 384 well low volume assay plates (Greiner) using an Echo 555 acoustic dispenser (Labcyte). 5 μL 6xHis-TEV-Cereblon/6xHis-TEV-DDB1 Δ BPB (5 nM final assay concentration) containing Alexa647 labeled lenalidomide (50 nM final assay concentration) were added to the assay plates using a Multidrop Combi dispenser (ThermoFisher) in assay buffer (50 mM HEPES (Sigma), 150 mM NaCl (Sigma), 5% Glycerol (Sigma), 1 mM CHAPS (Sigma) and 1 mM DTT (Sigma) pH 7.4). Assay plates were centrifuged at 1000 rpm for 1 minute and then incubated for 15 minutes at room temperature. 5 μL Eu-W1024-labeled Anti-6xHis antibody (1 nM final assay concentration) (Perkin Elmer) in assay buffer was added to all wells, plates were centrifuged at 1000 rpm for 1 minute and then incubated for 30 min at room temperature. After excitation of europium fluorescence at 337 nm, emission at 615 nm (donor, europium) and 665 nm (acceptor, Alexa647) were recorded with a 20 μs delay to reduce background fluorescence on an Envision 2104 microplate reader (Perkin Elmer). The data was reported as a 665/615 ratio and normalised between high (no test compound) and low (no protein) controls.

Endogenous IKZF1 immunostaining

Test compounds (10 mM stock in DMSO) were serially diluted 10-fold to create a 6 point dilution spanning 1 nM-100 μM (final assay concentration). Jurkat cells were added to 96 well U-bottom assay plates (Falcon) at 1×10^5 cells per well and treated with compounds for 18 hours at 37°C and 5% CO_2 . Following incubation, cells were stained with LIVE/DEAD™

Fixable Aqua Dead Cell Stain (Thermo) diluted 1000X in PBS for 30 minutes at room temperature and then incubated with Human TruStain FcX block (Biolegend) diluted 40X in PBS + 1% FBS (Gibco) for 10 minutes at room temperature. Intranuclear staining of IKZF1 was carried out using the Transcription Factor Buffer Set (BD Bioscience) according to the manufacturer's protocol and staining with IKZF1 AF488 antibody (clone R32-1149, BD Bioscience) diluted 40X for 30 minutes at room temperature. Cells were acquired on a Cytotflex S (Beckman Coulter) and data analysed using FlowJo 10.8.1 9 (BD).

Pooled library construction (*performed by Twist Bioscience*)

A library of 8,380 IKZF3 degrons was cloned into pLVX-Sprout to create the eGFP/mCherry protein degradation reporter plasmid pool. A lentivirus pool was then generated using the Lenti-X lentiviral vector production method.

Pooled library screen

Jurkat cells were transduced at a multiplicity of infection of < 0.3 with the lentivirus vector library in order to maximise the number of cells with a single integration (*carried out by Dr Lewis Brayshaw at GlaxoSmithKline, Stevenage, UK*). Transductions were carried out in 6 well TC-treated cell culture dishes (Falcon) with 3×10^6 cells per well. Cells were incubated for 2 days at 37 °C and 5% CO₂ before the supernatant was removed and replaced with fresh media. 6 days post-transduction, mCherry+ cells were isolated *via* FACS using the FACS Aria Fusion (BD) in a buffer containing PBS (Gibco), 2 mM EDTA (Gibco), 25 mM HEPES (Sigma) and 1% BSA (Sigma). 8 days later, cells were treated with DMSO or a thalidomide analog for 18 hours at 37 °C and 5% CO₂. mCherry+GFPlow and NOT(mCherry+GFPlow) cells were sorted by FACS using the FACS Aria Fusion (BD). DMSO treated cells were used to set the mCherry+GFPlow gate and dead cells were excluded from sorting by staining cells with 1 µg/mL DAPI (ThermoFisher). gDNA was isolated using the Maxwell RSC 48 (Promega) (*carried out by Dr Lewis Brayshaw at GlaxoSmithKline, Stevenage, UK*). gDNA from cells that were unsorted and untreated were also isolated to determine the library representation on the

day of compound treatment. Throughout the screen >600X coverage of the library was maintained. 3 biological replicates were carried out with a new transduction taking place each time.

Next-generation sequencing (NGS) *(carried out by Dr Lewis Brayshaw at GlaxoSmithKline, Stevenage, UK)*

A two-step PCR was used where the first PCR (PCR1) amplified the integrated degron sequence from gDNA and the second PCR (PCR2) added Illumina adaptors and barcodes to permit multiplexing. See Supp F for PCR1 and PCR2 primers. Q5 Hot Start High-Fidelity 2X Master Mix (New England Biolabs) was used for both PCRs. Sufficient gDNA was amplified in PCR1 in order to maintain >600X coverage and amplicons were purified using solid phase reversible immobilization (SPRI) beads (Beckman Coulter). Purified PCR1 products were analysed on the Tapestation 4200 (Agilent) to confirm purity prior to proceeding to PCR2. Sufficient PCR1 product was amplified in PCR2 in order to maintain >600X coverage and amplicons were run on a 2% agarose TAE gel. Target bands were excised and purified using the NucleoSpin Gel and PCR Clean-up Kit (Machery Nagel). Amplicons were analysed on the Tapestation 4200 and quantified using Qubit dsDNA HS kit (ThermoFisher). PCR products were pooled and sequenced as paired-reads on the NovaSeq (Illumina) using a SP2 200 cycles kit (Illumina).

NGS data analysis *(carried out by Dr Becky Saunders, Dr Mary Spanou and Dr Guillaume Heger at GlaxoSmithKline, Stevenage, UK)*

Sample fastq files were processed to align reads to reference degron sequences using the alignment tool Vsearch (<https://pubmed.ncbi.nlm.nih.gov/27781170/>) in order to count the number of times each degron was present within each sample. Count distributions, percentage of read mapping, library coverage and replicate correlation per sample were assessed for QC purposes. Counts for each degron were then subject to Limma Voom linear regression analysis (<https://genomebiology.biomedcentral.com/articles/10.1186/gb-2014-15->

2-r29) to generate an estimated log fold change between replicate samples in the positively (mCherry+GFPlow) sorted fraction vs replicate samples in the negatively (NOT(mCherry+GFPlow) sorted fraction for each degron sequence.

Immunoblotting

Test compounds (10 mM stock in DMSO) were serially diluted 10-fold to create a 3 or 6 point dilution spanning 100 nM-10 μ M or 1 nM-10 μ M respectively (final assay concentration). Jurkat cells were added to T25 flasks (Falcon) at 2×10^6 cells per flask and were incubated with compounds for 18 hours at 37 °C and 5% CO₂. Cells were lysed in buffer containing 9 M urea (Sigma), 75 mM Tris-HCl (Sigma), 0.15 M β -mercaptoethanol (Merck) and 10X diluted protease inhibitor cocktail (Merck). *(Procedure after this point carried out by Dr Marta Serafini, Oxford University, UK)* Cells lysates were sonicated for 10 seconds 3 times, centrifuged at 13000 rpm for 10 minutes at 4 °C and the supernatants were transferred into fresh tubes. Protein concentrations were measured using a Pierce BCA Protein Assay Kit (Thermo). Proteins were boiled in Laemmli sample buffer (Bio-Rad) for 5 minutes at 100 °C and separated by SDS-PAGE (7.5% Mini-PROTEAN TGX gels, Bio-Rad) and compared to the PageRuler Prestained Protein Ladder (Thermo). Semi-dry blotting was done on a Trans-Blot Turbo Transfer System (Bio-Rad) with the Trans-Blot Turbo RTA Mini 0.2 μ m PVDF Transfer Kit (Bio-Rad) using the default mixed molecular weight mode. Membranes were blocked in 5% non-fat dry milk (Bio Rad) in TBS-T. Primary IKZF3 (D1C1E, Cell Signaling Technology Europe), eGFP (F56-6A1.2.3, Thermo) and vinculin (E1E9V, Cell Signaling Technology Europe and sc-25336, Santa Cruz Biotechnologies) antibodies were diluted 1000X, 500X and 50X respectively in 5% non-fat dry milk in TBS-T. Membranes were incubated with primary antibodies overnight at 4 °C, washed with TBS-T 3 times, incubated with HRP conjugated secondary antibodies (Promega) for 1 hour at room temperature, washed with TBS-T 3 times and imaged with Clarity Western ECL substrate (Bio-Rad). Membranes were scanned with a Bio-Rad ChemiDoc XRS+ Imaging System (Bio-Rad) and signal intensities were quantified

using Image Lab software (Bio Rad). Band densitometry was assessed, normalized to vinculin bands, and reported as percentage of the DMSO control lane.

CRISPR editing (*carried out by Dr Lewis Brayshaw and Dr Agnieszka Konopacka at GlaxoSmithKline, Stevenage, UK*)

Jurkat cells were edited 8 days after lentiviral transduction. For each gene, 3 sgRNAs targeting a single exon were designed using the Synthego CRISPR Design tool. See Supp F for sgRNA sequences. sgRNA were resuspended to 100 μ M in IDT duplex buffer and then combined to create pools of sgRNA. Cas9 RNPs were prepared by combining 2.5 μ L 36.6 μ M Alt-R[®] S.p. Cas9 Nuclease V3 (IDT) and 2 μ L 100 μ M sgRNA pool in IDT duplex buffer and incubating for 10 minutes at room temperature. 1.5×10^6 cells in 20 μ L SE buffer (Lonza) were mixed with the Cas9 RNPs and then electroporated using a 4D-Nucleofector (Lonza) on pulse code CL-120. Estimation of knockout efficiency was performed by PCR amplification of the sgRNA target site using primers described in Supp F. PCR products were Sanger sequenced and editing efficiency was assessed using the online ICE webtool (<https://ice.synthego.com>).

HiBit and Cell Titre Glo (CTG) (*carried out by Dr Lewis Brayshaw and Dr Agnieszka Konopacka at GlaxoSmithKline, Stevenage, UK*)

Compound (10 mM stock in DMSO) was serially diluted 3-fold to create a 11 point dilution spanning 169 pM-10 μ M (final assay concentration). Jurkat cells transduced with Hibit-TRIM28 lentiviral vectors were added to white opaque-bottom 384-well plates (ThermoFisher) at 1×10^4 cells per well in 25 μ L volume, and treated with compound for 24 hours at 37 °C and 5% CO₂. The Hibit signal was measured by adding 25 μ L of the lytic detection reagent from the Nano-Glo[®] HiBiT Extracellular Detection System (Promega), prepared as per manufacturer protocol. The CTG signal was developed using 25 μ L of CTG reagent (Promega). Both assays were read using PHERAstar microplate luminescence reader (BMG Labtech). The data have been expressed as % of DMSO control and represent three independent biological replicates.

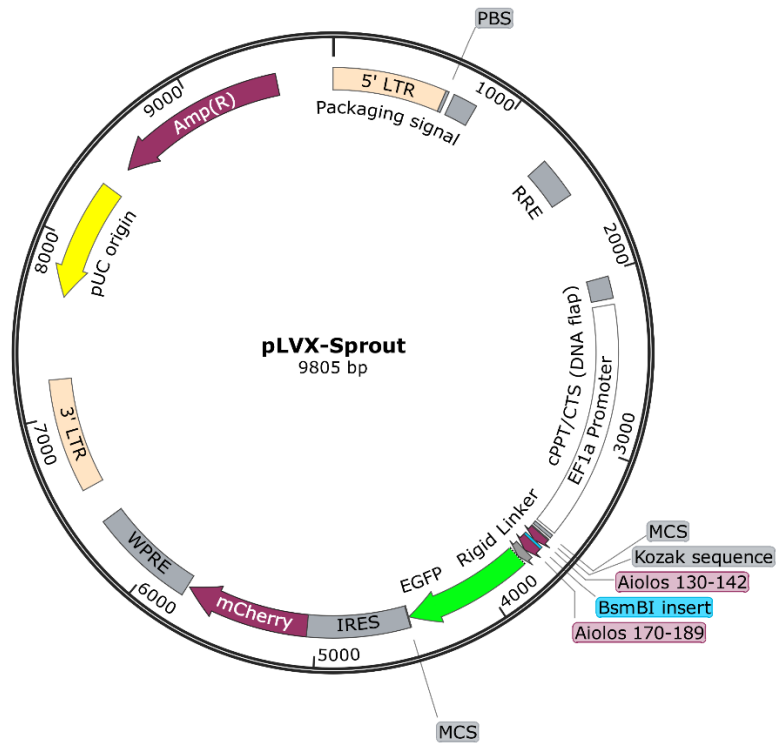
Plasmid information

Plasmid: pLVX-Sprout

Backbone: pLVX-EF1a-IRES-mCherry (Takara)

Insert sequence cloned into MCS:

cagtgtctagaggaatagccaccatgtccgattcaatgtcttaatggttcataagcgaagccatactggtgaagagacggacg
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actgtataaataaggatccccacat



Chapter 7: Experimental

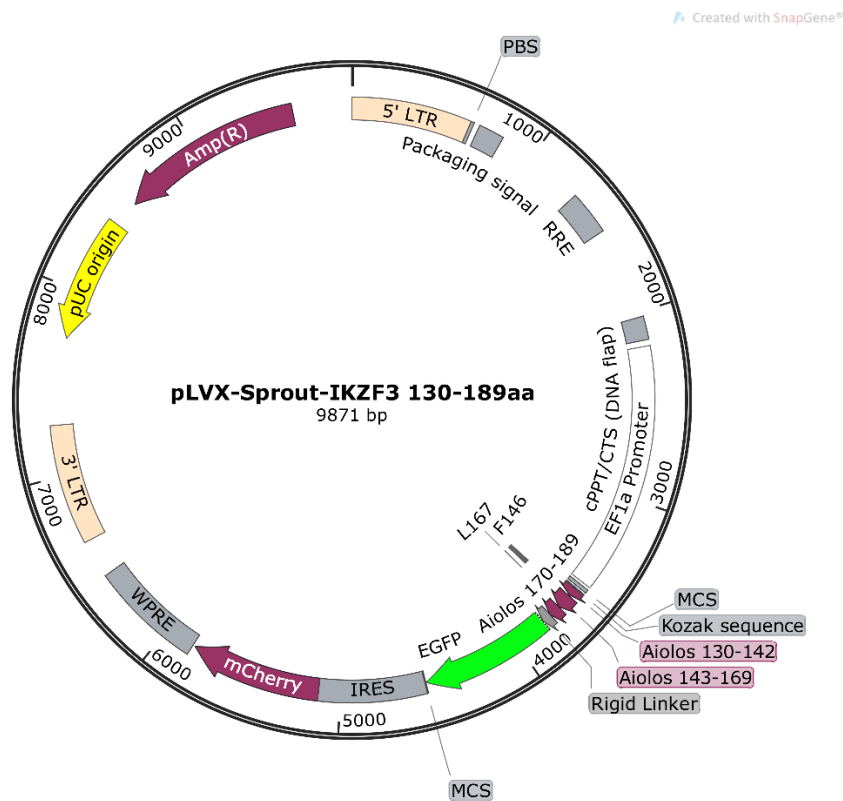
Plasmid: pLVX-Sprout-IKZF3 130-189aa (WT)

Backbone: pLVX-Sprout

Insert sequence cloned into pLVX-Sprout:

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Plasmid map:



Plasmid: pLVX-Sprout-IKZF3 130-189aa Q147A (ANQS)

Backbone: pLVX-Sprout

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Backbone: pLVX-Sprout

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Plasmid: pLVX-Sprout-IKZF3 130-189aa Q147W (WNQS)

Backbone: pLVX-Sprout

Insert sequence cloned into pLVX-Sprout:

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Backbone: pLVX-Sprout

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Plasmid: pLVX-Sprout-IKZF3 130-189aa Q147F N149E Q150V S154P (FEVP)

Backbone: pLVX-Sprout

Insert sequence cloned into pLVX-Sprout:

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Plasmid: pLVX-Sprout-IKZF3 130-189aa Q147W N149E Q150I S154K (WEIK)

Backbone: pLVX-Sprout

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Backbone: pLVX-Sprout

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Backbone: pLVX-EF1a-IRES-mCherry (Takara)

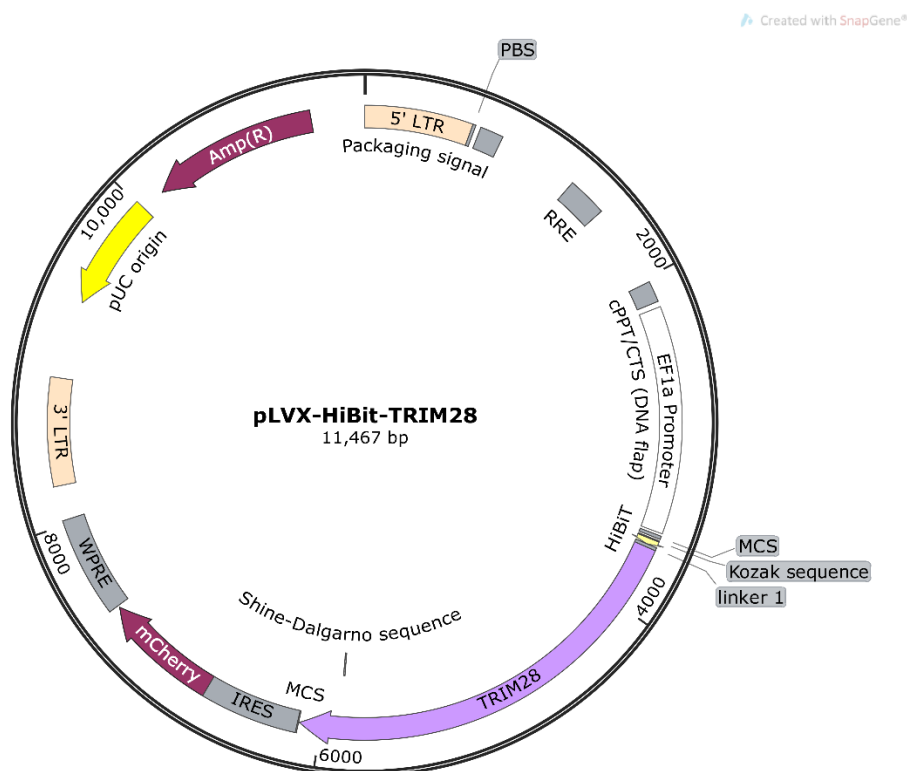
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Plasmid map:



Plasmid: pLVX-HiBit-TRIM28 N-IPID

Backbone: pLVX-EF1a-IRES-mCherry (Takara)

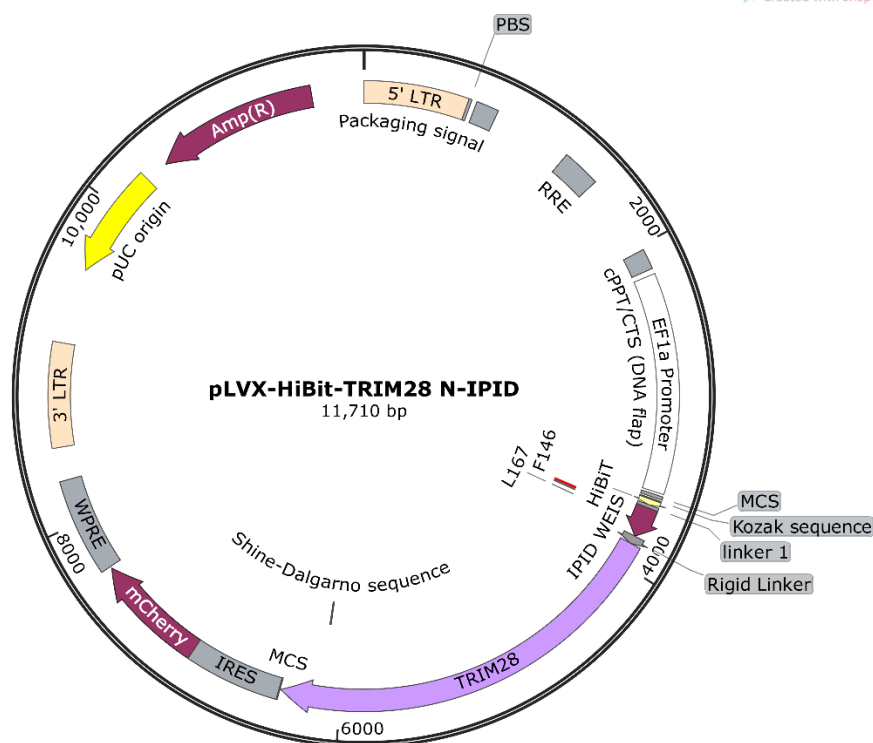
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Plasmid map:



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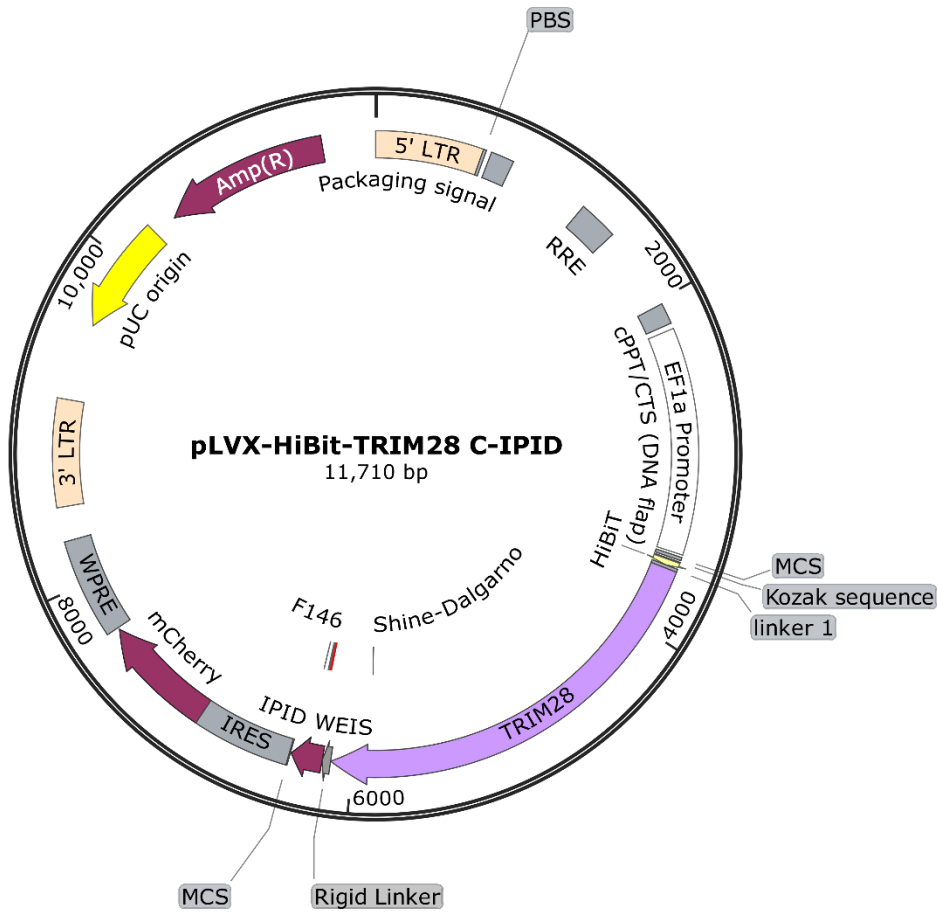
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Chapter 7: Experimental

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Plasmid map:



7.4 Computational Experimental

Docking was carried out using the crystal structure *DDB1-CRBN-pomalidomide complex bound to IKZF1(ZF2)* from the Protein Data Bank (PDB ID: 6h0f) as a template.

Protein energetics and mutation evaluation were carried out using the PositionScan function in FoldX 5. The 3D coordinate files generated for the mutant proteins were then used for subsequent docking.

Docking was carried out using GOLD version 2020.1 from the Cambridge Crystallographic Data Centre. The position of the original pomalidomide ligand in the template crystal structure was used to define the binding pocket for new ligands using GOLD's 'cavity' functionality. The imide ring region of the original pomalidomide ligand was used to specify the position onto which the imide region of screened ligands should be superimposed using GOLD's 'scaffold' functionality. All parameters were kept as default during docking screens, except constraint weight, which was increased from the default value of 5 to 10. For every ligand/mutant combination in the large scale high throughput screens, 10 poses were generated, from which the highest ranked was taken forward for further analysis. 10 poses were also used in the validation screen study. In the smaller scale screen of benzyl pomalidomide variants, a constraint weight of 20 was used, and 100 poses were generated, from which the highest ranked was taken forward for further analysis.

Energetic evaluation of docked poses produced by GOLD was carried out using AutoDock4.

Mutational analysis for use in the lentiviral library screen was performed using the PositionScan functionality in foldx and the PositionMutation functionality in Rosetta. The same crystal structure (PDB ID: 6Hh0F) was used with the pomalidomide ligand removed.

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Appendices

S.1 Cereblon Binding and Solubility Data

Table S.1 (CRBN binding and solubility data) shows CRBN binding affinity values and solubility values for all library compounds, as well as commercially-acquired lenalidomide. These tests were carried out by Laurie Gordon at GSK, Stevenage, UK.

Table S.1. CRBN pIC₅₀ and solubility values for all compounds from IMiD analogue library

Compound	Cereblon pIC ₅₀	Solubility/ μ M	Compound	Cereblon pIC ₅₀	Solubility/ μ M	Compound	Cereblon pIC ₅₀	Solubility/ μ M	Compound	Cereblon pIC ₅₀	Solubility/ μ M
1	6.9	321	92	6.47	53	98	7	254	102	7.08	22
3	7.19	341	93	7.10	23	99	6.96	254	103	7.65	17
35	7.32	301	94	6.58	9	26	7.00	287	86	7.61	1
36	7.57	342	95	7.23	18	64	7.47	307	87	7.48	2
37	7.39	348	96	6.79	153	70	7.78	389	104	6	2
89	7.02	387	40	7.14	48	68	7.79	54	105	6.05	1
90	7.08	287	41	7.12	279	84	7.61	377			
38	6.62	162	97	6.32	68.5	74	7.85	20			
39	6.73	190.5	88	7.07	265	100	7.64	257			
91	6.86	111	23	7.06	34	101	7.57	7			

There is a strong correlation between compound molecular weight and CRBN pIC₅₀ (P value <0.0001) within the compounds tested (Figure S.1).

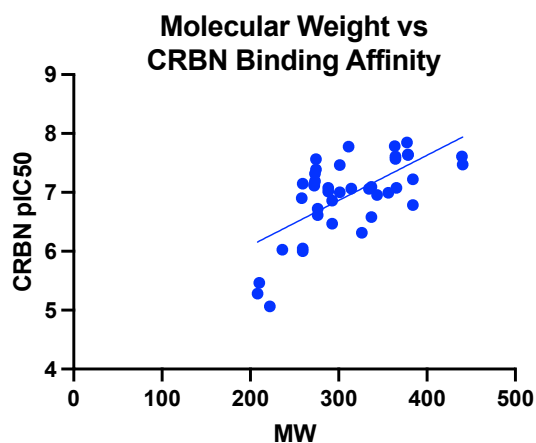


Figure S.1. Relationship between molecular weight and CRBN binding affinity of IMID analogues

Figure S.2 shows a strong general correlation between CRBN binding affinity and degradation ability (P value <0.0001) for both WT and Q147A.

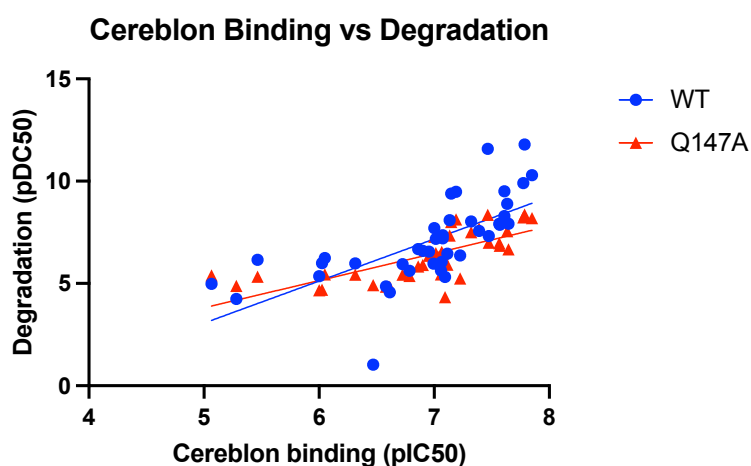


Figure S.2. Relationship between CRBN binding affinity and degradation capability of IMID analogues

There is no significant correlation between compound solubility and pDC₅₀ values for either WT or Q147A (P value = 0.15 and 0.06 respectively)

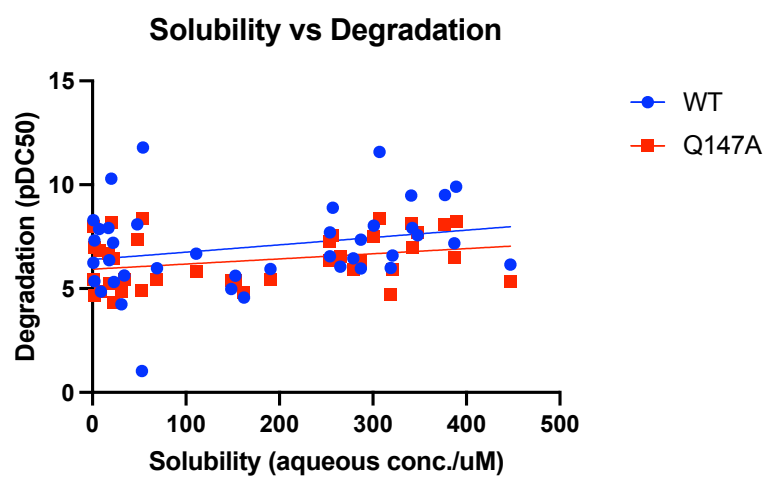


Figure S.3. Solubility vs degradation capability of IMID analogues

S.2 Western Blot repeats

Full biological repeat data for western blots discussed in Chapter 5.

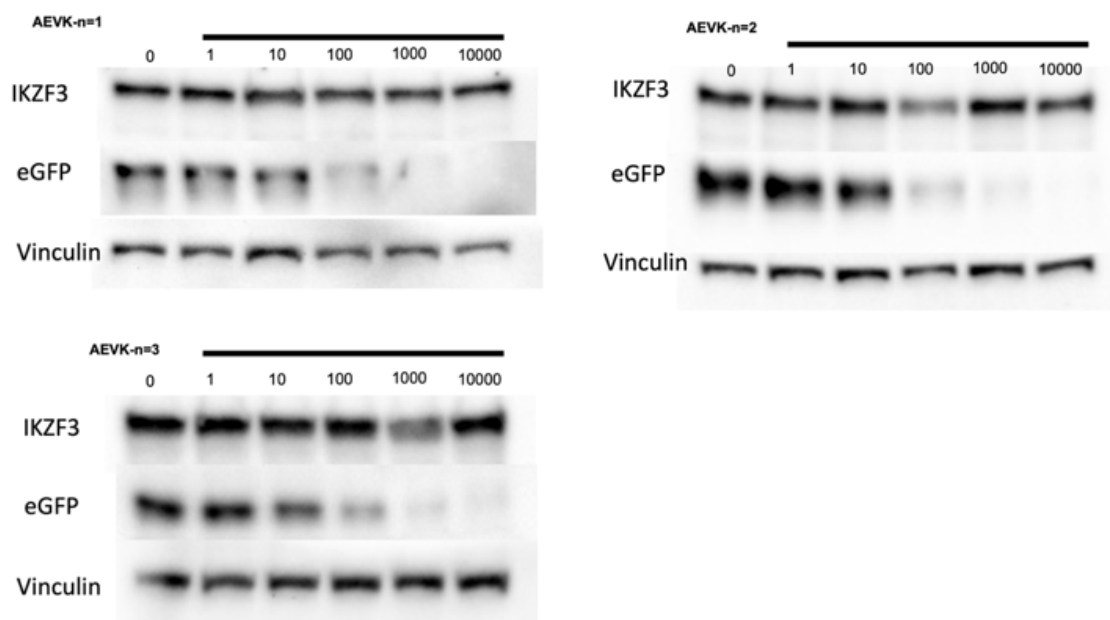


Figure S.4. Transduced Jurkat cells expressing a ZF-eGFP fusion protein with the AEVK Degrone.

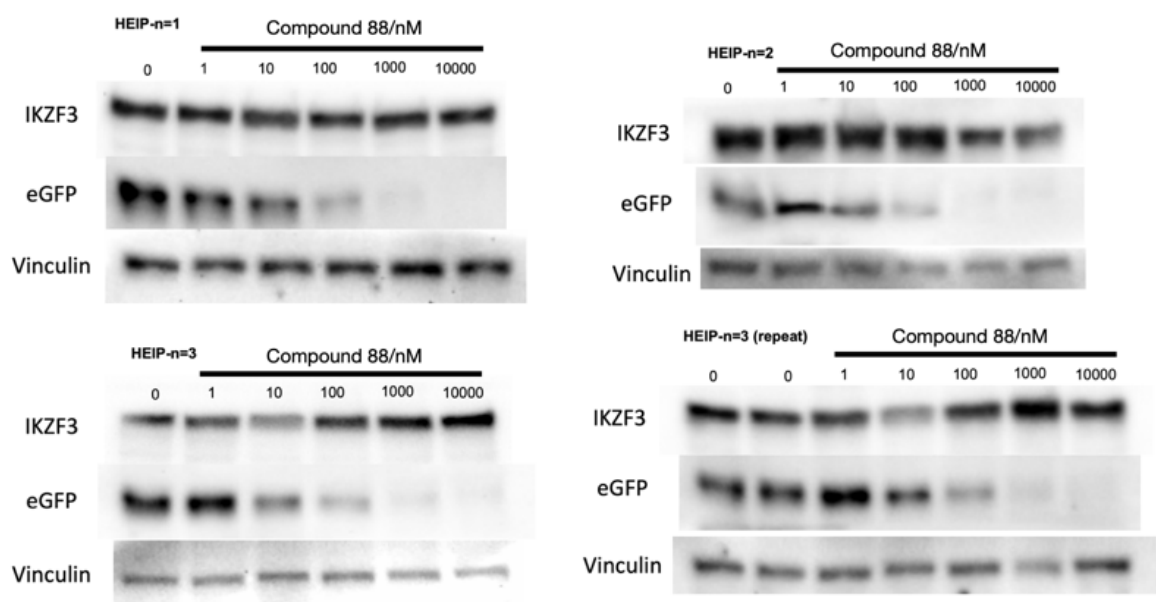


Figure S.5. Transduced Jurkat cells expressing a ZF-eGFP fusion protein with the HEIP Degrone.

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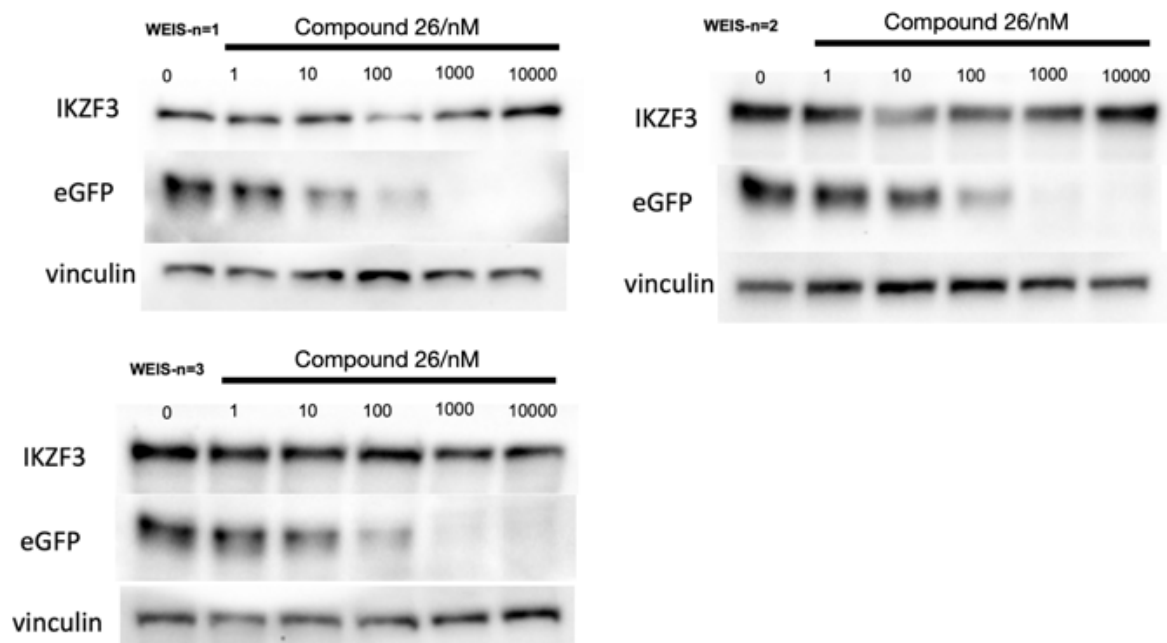


Figure S.6. Transduced Jurkat cells expressing a ZF-eGFP fusion protein with the WEIS Degron.

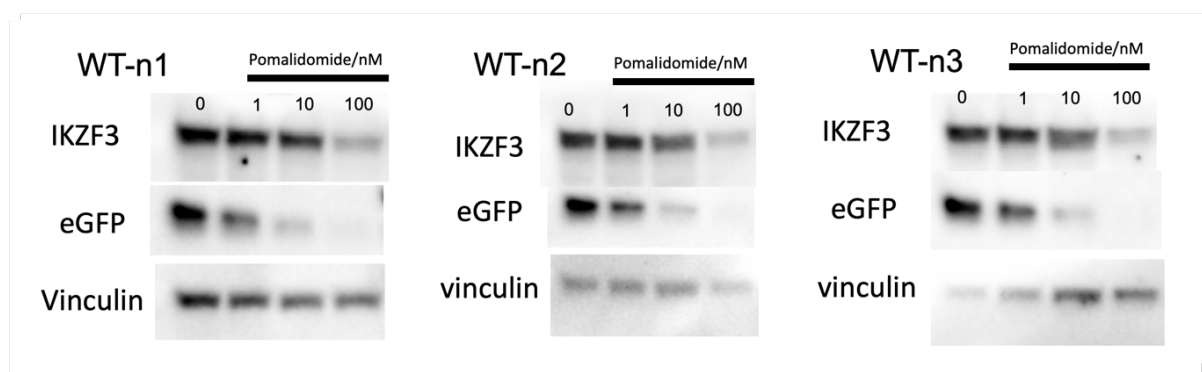


Figure S.7. Transduced Jurkat cells expressing a ZF-eGFP fusion protein with the WT Degron.

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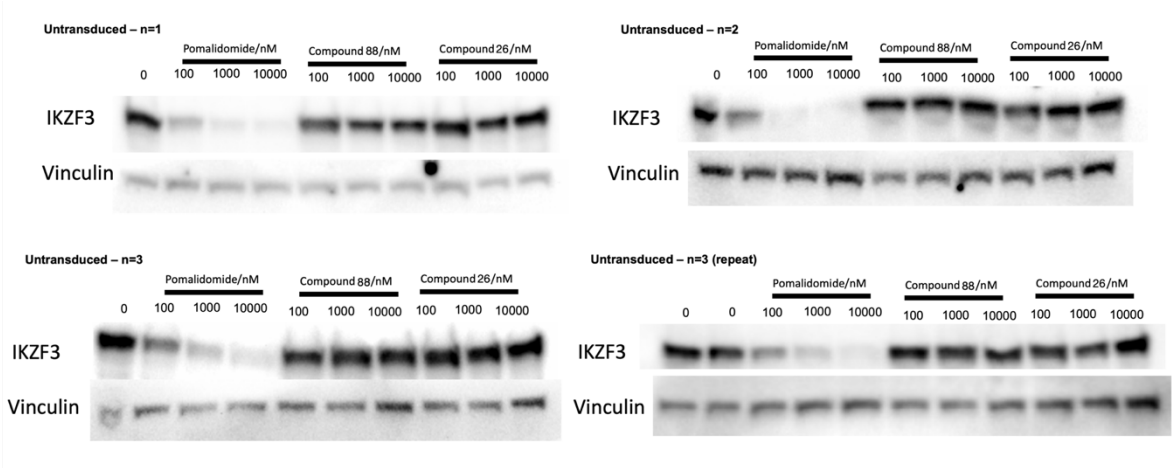


Figure S.8. Untransduced Jurkat cells.

S.3 Raw Mutant Library Data

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[illegible]

Appendices

	V	0.48	-0.75	-1.91	-1.02	-2.24	0.35	-1.68	-1.70	-1.57	-2.14	-1.96	-1.31	1.95	-0.41	-1.87	0.15	-1.11	-1.47	-1.61	-2.34
	N	-0.57	-1.43	-1.94	-1.35	-2.64	-1.05	-1.98	-1.78	-2.17	-2.16	-2.29	-1.51	1.84	-1.13	-1.40	-1.12	-1.21	-1.81	-1.78	-1.82
	Q	-2.55	-2.61	-3.30	-2.30	-2.98	-2.65	-2.83	-2.22	-2.90	-2.68	-2.72	-2.82	-1.06	-2.91	-2.80	-2.95	-3.20	-2.52	-2.22	-2.05
	V	-1.49	-2.32	-3.01	-2.10	-2.60	-1.59	-2.54	-2.70	-2.44	-2.68	-2.63	-2.19	0.54	-1.60	-2.64	-1.88	-2.42	-2.00	-2.28	-2.03
	P	1.01	-0.71	-0.38	0.04	-1.32	0.60	-0.55	-1.63	-1.08	-2.18	-1.28	-0.51	2.87	0.09	-1.57	0.42	-1.17	-1.10	-1.08	-1.20
	Q	-1.05	-2.34	-3.39	-2.49	-2.44	-1.65	-2.62	-2.44	-2.69	-2.93	-2.79	-2.24	-0.18	-2.24	-2.54	-1.89	-1.86	-2.84	-2.41	-2.51
	V	0.80	-0.83	-0.88	-1.00	-1.50	0.07	-1.22	-1.54	-1.18	-1.61	-1.83	-0.84	2.38	0.02	-1.48	0.15	-0.96	-1.20	-1.52	-1.29
	L	-0.37	-0.57	-2.22	-0.62	-1.62	-1.03	-1.20	-0.51	0.02	-0.61	-1.09	-0.53	1.98	0.06	0.43	-0.52	-0.56	-0.41	-1.65	-0.78
	D	-1.65	-2.30	-3.52	-2.92	-2.87	-2.54	-2.80	-2.14	-2.32	-2.11	-2.70	-2.43	-1.23	-2.05	-1.12	-3.07	-2.84	-2.35	-2.59	-2.41
	I	-0.80	-0.88	-2.99	-1.84	-2.38	-1.42	-1.54	-1.17	-0.93	-1.23	-1.19	-1.15	1.16	-1.04	-0.24	-0.97	-1.14	-1.41	-1.72	-1.03
	E	1.59	1.57	-0.41	1.49	-0.46	0.80	0.79	1.60	2.07	1.46	0.89	1.75	3.17	2.07	2.33	1.42	1.80	1.55	0.01	1.03
	Q	-0.76	-0.71	-2.62	-1.63	-1.87	-1.55	-1.96	-0.72	-0.71	-1.01	-1.31	-1.40	-0.07	-0.78	-0.01	-0.94	-1.01	-0.91	-2.01	-1.87
	V	1.55	1.59	-0.83	1.01	-0.69	1.08	0.29	0.91	1.49	0.98	0.15	1.25	2.97	1.57	2.09	1.45	1.34	1.47	-1.08	0.30
	N	0.23	0.39	-1.15	0.04	-0.97	-0.19	-0.38	0.30	0.06	-0.03	-0.36	0.26	2.49	0.67	0.84	0.40	0.10	0.14	-1.20	-0.25
	Q	-1.96	-1.96	-2.29	-2.47	-1.88	-1.89	-2.04	-1.65	-1.61	-1.48	-2.21	-2.43	-0.93	-1.98	-1.15	-2.25	-2.19	-2.00	-1.93	-2.10
	V	-0.52	-0.53	-1.57	-1.09	-1.59	-0.98	-1.46	-1.17	-1.03	-0.85	-1.04	-0.72	1.32	-0.81	-0.21	-0.83	-0.64	-1.40	-2.19	-1.04
	P	1.59	1.62	0.37	1.67	0.78	1.01	1.07	1.29	1.25	1.19	1.08	1.77	3.18	1.91	1.92	1.57	1.68	1.17	-0.03	1.31
	Q	-1.00	-0.92	-2.72	-1.72	-1.24	-0.75	-1.21	-0.88	-0.83	-1.12	-1.59	0.91	0.72	-0.58	-0.01	-0.90	-0.95	-1.18	-1.33	-0.69
	V	1.42	1.62	-0.17	-1.24	0.41	0.95	0.94	0.80	0.80	0.94	0.69	1.54	3.41	1.61	1.68	1.37	1.09	0.78	0.01	0.79
	M	0.34	-0.55	-1.47	-1.29	-2.19	-0.29	-1.87	-0.62	0.43	-1.42	-1.45	0.50	1.89	-0.15	0.24	0.04	-0.34	-0.45	-1.82	-1.53
	D	-2.32	-2.62	-3.24	-2.63	-3.05	-2.29	-2.88	-2.62	-2.27	-2.11	-2.89	-2.26	-1.30	-1.90	-2.40	-2.12	-2.03	-2.33	-2.53	-3.07
	I	-0.15	-1.10	-1.93	-2.10	-2.66	-1.24	-1.96	-1.70	-1.55	-1.90	-2.22	-0.87	0.72	-0.93	-0.77	-0.53	-1.29	-1.28	-2.52	-1.54
	E	2.46	1.09	-0.15	1.29	-1.43	1.31	0.55	0.95	1.27	0.05	0.91	1.56	2.84	1.97	1.50	2.00	1.15	1.57	-1.25	-0.03
	Q	-0.27	-1.20	-2.74	-1.90	-2.63	-1.42	-2.00	-1.44	-1.40	-2.34	-1.53	-0.76	-0.40	-1.16	-1.11	-0.53	-1.30	-1.09	-2.16	-2.25
	V	2.28	1.45	-0.74	0.36	-1.37	1.41	-0.09	0.29	0.60	-0.22	0.08	1.11	2.60	1.55	1.48	2.05	0.94	0.96	-1.27	-0.10
	N	1.40	0.24	-0.65	-0.04	-1.54	0.38	-0.76	0.26	-0.37	-0.65	-0.65	0.38	2.42	0.85	0.08	0.90	0.22	0.37	-1.08	-0.64
	Q	-1.34	-1.90	-2.44	-2.11	-2.64	-1.80	-2.26	-1.47	-2.11	-1.89	-2.50	-1.91	-0.82	-2.27	-0.98	-1.50	-2.08	-2.10	-1.91	-2.11
	V	0.27	-0.80	-1.76	-1.06	-1.83	-0.51	-1.28	-1.17	-0.93	-1.20	-1.26	-0.79	1.08	-0.48	-0.85	-0.35	-0.85	-0.81	-1.77	-1.53
	P	2.32	1.41	1.23	1.67	-0.38	1.64	0.95	0.98	0.89	0.05	0.91	1.98	2.88	1.97	1.11	2.41	1.34	1.36	-0.22	0.38
	Q	-0.12	-0.99	-2.44	-1.49	-1.67	-0.76	-1.32	-1.46	-1.18	-1.45	-1.40	-0.65	0.07	-0.52	-0.60	-0.24	-0.87	-0.96	-1.55	-1.59
	V	2.11	0.96	0.87	1.14	-0.48	1.42	0.77	0.43	0.80	-0.38	0.37	1.69	2.96	1.78	0.72	2.02	1.06	1.09	-0.22	0.38
	N	-1.32	-1.23	-2.94	-2.45	-2.25	-2.07	-1.76	-2.65	-2.02	-2.24	-1.79	-1.21	-0.15	-1.53	-1.10	-1.35	-1.95	-2.03	-2.05	-1.53
	D	-2.92	-2.67	-3.18	-3.44	-2.53	-2.46	-3.32	-3.71	-2.90	-2.49	-3.13	-2.90	-2.55	-2.54	-2.09	-2.74	-3.68	-2.62	-2.68	-2.46
	I	-1.66	-2.07	-3.05	-2.79	-2.42	-2.61	-2.36	-2.46	-1.81	-2.04	-2.74	-2.09	-1.34	-1.97	-1.71	-2.18	-2.19	-2.68	-2.92	-1.93
	E	0.72	0.10	-1.40	-0.17	-1.26	-0.25	-0.42	-0.48	0.45	-1.13	-0.55	0.46	1.58	0.56	0.65	0.46	-0.32	-0.34	-1.90	0.73
	Q	-1.83	-2.41	-3.51	-2.21	-2.61	-2.80	-2.76	-3.04	-2.19	-3.30	-2.21	-2.41	-2.17	-2.22	-1.68	-2.28	-2.48	-2.04	-2.13	-2.53
	V	-0.88	-0.55	-1.86	-0.89	-1.17	-0.27	-0.58	-0.95	0.14	-1.46	-0.74	-0.32	0.85	0.33	0.25	0.44	-0.31	-0.66	-1.59	-0.21
	N	-0.13	-0.35	-0.99	-0.84	-1.15	-1.03	-0.81	-1.09	0.48	-1.17	-1.15	0.94	0.97	-0.25	-0.27	-0.42	-0.58	-0.58	-1.31	0.05
	Q	-2.35	-2.07	-3.10	-3.03	-2.35	-2.42	-2.17	-1.94	-1.99	-2.05	-2.45	-2.23	-1.92	-2.60	-1.76	-2.71	-2.70	-1.97	-2.70	-1.97
	V	-1.97	-1.53	-2.49	-1.82	-1.71	-1.40	-1.77	-1.40	-1.50	-1.89	-1.56	-1.83	-0.29	-1.35	-1.07	-1.60	-1.72	-1.24	-1.84	-1.02
	P	1.13	0.84	-0.06	0.90	-0.12	0.35	0.60	0.08	0.72	0.07	0.48	0.65	2.43	1.10	0.97	0.88	0.65	0.43	-0.58	0.82
	Q	-0.60	-1.84	-3.69	-1.94	-2.24	-2.07	-2.04	-2.32	-1.98	-2.23	-2.25	-2.31	-1.81	-1.62	-1.52	-1.68	-2.26	-2.18	-2.27	-1.43
	V	2.42	1.61	-0.85	0.15	-0.31	0.05	0.14	-0.32	0.35	-0.32	0.11	0.31	2.25	0.95	0.61	0.43	0.07	0.20	-0.83	0.57
	Q	3.01	3.30	1.54	2.44	0.87	1.69	1.82	3.47	3.28	2.36	2.91	2.72	3.35	2.90	3.03	2.99	3.25	3.17	1.02	1.82
	D	1.16	0.90	-1.34	-0.01	-1.43	-0.55	-0.93	1.41	1.09	-0.22	0.68	-0.26	-0.15	0.41	1.33	0.79	1.31	1.30	-1.39	-0.61
	I	2.84	3.01	0.54	1.77	0.03	1.06	1.00	2.87	2.87	1.92	2.37	1.97	2.48	2.41	2.81	2.89	3.16	3.01	0.03	0.88
	E	3.64	3.48	3.00	3.53	2.71	3.09	3.17	3.68	3.46	3.53	3.57	3.59	3.72	3.56	3.70	3.75	3.26	3.22	2.26	3.33
	Q	3.01	3.02	-0.31	1.66	0.01	0.87	0.80	2.72	2.70	1.20	2.31	1.59	1.31	2.30	3.03	2.84	3.10	2.92	-0.18	0.93
	V	3.65	3.69	3.09	3.70	2.25	3.21	3.05	3.70	3.40	3.21	3.52	3.56	3.53	3.77	3.71	3.53	3.74	3.71	2.06	2.96
	N	3.39	3.37	2.72	3.24	1.91	2.44	2.32	3.81	3.26	2.82	2.91	3.08	3.71	3.28	3.26	3.34	3.87	3.47	1.81	3.08
	Q	1.66	1.54	-1.05	-0.07	-1.28	-0.19	-0.57	1.49	1.15	0.23	0.78	-0.01	0.42	1.10	1.43	1.13	1.47	1.68	-1.06	-0.23
	V	3.09	3.21	1.68	2.44	0.39	1.66	1.49	3.37	2.80	1.70	2.69	2.55	2.69	2.83	2.71	2.88	2.74	3.41	0.79	1.41
	P	3.53	3.77	3.81	3.61	2.67	3.69	3.32	3.79	3.44	3.37	3.62	3.58	3.65	3.65	3.55	3.79	3.56	3.67	2.87	3.36
	Q	3.27	3.26	0.71	2.07	0.22	1.20	1.43	2.95	2.85	1.61	2.53	2.12	1.78	2.67	2.99	2.81	3.17	3.42	0.36	1.25
	V	3.69	3.46	3.40	3.76	2.40	3.38	3.27	3.53	3.96	3.50	3.58	3.48	3.87	3.59	3.63	3.60	3.79	3.75	2.70	2.88
	R	1.21	0.12	-0.63	-0.77	0.16	0.15	-1.18	-0.03	0.17	-0.56	-0.69	-0.33	3.52	0.12	-0.57	0.39	-0.33	0.42	0.02	0.62
	D	-1.29	-2.52	-3.13	-2.48	-1.67	-2.24	-2.48	-2.16	-2.09	-2.63	-2.18	-2.62	1.03	-2.38	-2.18	-2.20	-2.43	-2.50	-1.94	-1.48
	I	0.64	-0.34	-1.43	-1.26	-1.02	-0.61	-1.13	-0.77	-0.73	-1.08	-1.12	-0.94	2.88	-0.85	-1.08	0.06	-0.92	-0.63	-0.89	-0.67
	E	2.49	1.53	0.59	1.31	1.37	1.70	0.73	1.56	1.68	0.68	0.83	1.36	3.58	1.94	0.85	2.12	1.42	1.71	1.33	1.98
	Q	0.19	-1.19	-2.71	-1.35	-1.33	-0.65	-1.40	-1.17	-1.27	-1.64	-1.56	-1.63	1.81	-1.30	-1.25	-0.52	-1.31	-1.05	-1.44	-1.22
	V	2.62	1.50	0.53	0.82	1.09	1.57	0.20	1.07	1.09	0.45	0.61	0.85	3.82	1.56	0.52	2.07	1.06	1.60	0.75	1.47
	N	1.32	0.41	-0.23	0.29	0.79	0.55	-0.39	0.73	-0.12	-0.02	-0.17	0.22	3.38	0.11	-0.77	0.84	0.03	0.63	0.41	1.07
	Q	-0.64	-1.60	-2.65	-2.36	-1.26	-1.53	-1.87	-1.39	-1.53	-2.02	-1.70	-1.49	1.54	-1.54	-1.16	-1.91	-1.75	-1.36	-1.18	-1.18
	V	0.62	-0.05	-1.03	-1.40	-0.30	-0.74	-1.07													

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E	Q	-1.67	-0.83	-3.25	-2.61	-1.95	-2.75	-1.66	-1.02	-1.82	-1.76	-1.12	-2.00	-1.92	-1.65	-1.65	-1.91	-1.99	-1.97	-1.58	-1.91							
	V	-0.88	-0.93	-2.20	-2.31	-1.89	-2.13	-1.02	-0.75	-0.49	-1.68	-1.36	-0.76	-1.33	0.03	-0.75	-1.19	-1.99	-1.97	-1.61	-1.55							
	I	1.57	1.16	-1.50	-0.52	-0.45	-0.29	1.08	0.52	1.31	-0.08	0.82	1.57	1.23	0.24	1.46	1.42	0.79	0.92	0.93	0.14							
	Q	0.61	-1.00	-2.54	-2.56	-1.69	-2.14	1.14	-1.07	-0.55	-1.51	-0.72	-1.39	-0.63	-0.59	-1.07	-1.68	-1.35	-1.97	-1.23	-1.33							
	V	1.54	1.25	-1.26	-0.65	-0.48	-0.33	0.94	1.18	1.54	-0.07	0.32	1.61	1.01	2.03	1.59	1.37	0.33	0.59	-1.03	-0.03							
	N	0.35	-0.44	-1.29	-0.35	-0.28	-0.74	0.32	0.25	0.12	-0.36	-0.46	0.44	0.25	0.83	0.52	0.48	0.27	-0.28	-0.86	-0.08							
	Q	-1.49	-1.26	-2.57	-2.85	-1.18	-2.37	-1.19	-0.90	-1.53	-1.26	-1.76	-1.00	-1.13	-1.17	-0.93	-1.90	-2.00	-1.28	-1.19	-1.05							
	V	-0.34	-0.34	-1.99	-1.67	-0.83	-1.23	-0.91	-0.57	-0.49	-0.77	-0.83	-0.10	-0.59	-0.07	-0.29	-0.30	-1.73	-0.94	-0.75	-0.90							
	P	I	1.20	1.46	-0.82	0.11	0.77	0.52	0.88	0.25	0.83	0.28	0.33	1.51	1.37	1.76	1.15	1.35	0.52	0.66	0.17	0.97						
	Q	-0.50	-0.24	-2.59	-1.75	-0.75	-1.78	0.80	-0.20	-0.26	-0.97	-0.75	-0.36	-1.33	0.16	0.06	-0.64	-1.24	-0.60	-1.10	-0.57							
Y	V	1.11	1.36	-0.86	0.60	0.61	0.05	0.84	0.41	0.81	0.11	0.32	1.69	1.06	2.09	1.26	1.35	0.82	0.57	-0.68	0.63							
	I	-0.55	-0.70	-0.24	-1.71	-1.43	-1.59	-1.03	-1.75	-0.63	-1.19	-1.55	-0.68	0.27	-0.01	-0.31	-0.76	-1.35	-1.18	-1.79	-1.54							
	Q	-2.28	-2.45	-3.05	-3.08	-2.48	-2.43	-2.33	-2.56	-2.66	-2.49	-2.69	-2.04	-2.24	-2.11	-2.00	-2.43	-2.35	-1.83	-1.99	-2.23							
	V	-1.42	-1.11	-3.95	-2.51	-1.66	-1.54	-1.53	-1.86	-1.15	-1.55	-1.95	-0.41	-0.59	-0.78	-0.57	-0.01	-0.49	-1.56	-1.73	-2.00							
	I	1.64	1.43	-1.13	0.38	-0.23	0.71	1.24	0.40	1.59	0.36	0.91	1.54	2.44	2.12	1.80	1.64	1.19	1.07	-0.93	0.31							
	Q	-0.92	-0.91	-3.08	-2.41	-1.67	-1.66	-1.60	-1.73	-0.89	-1.75	-1.70	-1.29	-1.07	-0.76	-0.65	-1.03	-1.40	-1.53	-0.92	-1.44							
	V	1.80	1.37	-1.36	-0.08	-0.43	-0.44	0.32	0.38	1.54	0.43	0.38	1.50	1.63	1.94	1.95	1.59	1.12	0.69	-1.17	-0.04							
	N	0.26	0.28	-2.33	-1.03	-0.84	-0.73	0.21	0.75	0.68	-0.61	-0.58	0.01	0.14	0.34	0.64	-0.16	-0.96	-0.49	-1.14	-0.47							
	Q	-1.86	-1.79	-2.94	-2.78	-1.15	-1.97	-1.98	-1.70	-1.76	-2.43	-1.66	-1.68	-1.40	-1.56	-1.56	-1.91	-2.43	-1.97	-1.79	-2.22							
	V	-0.95	-0.45	-2.65	-1.92	-1.28	-1.32	-1.14	-1.43	-1.57	-1.14	-1.62	-0.66	-1.11	-0.76	-0.42	-0.61	-1.27	-1.56	-1.66	-1.16							
P	I	0.58	1.33	-0.52	0.78	0.46	-0.12	0.70	0.38	0.50	0.51	0.04	1.32	2.20	1.70	0.07	1.24	1.16	0.65	-0.42	1.01							
	Q	-0.43	-0.45	-2.64	-1.93	-1.07	-1.27	-0.93	-0.84	-0.55	-0.84	-1.06	-1.13	-0.41	-0.26	-0.60	-0.38	-0.73	-0.95	-1.33	-1.07							
	V	0.97	1.29	-0.49	0.48	0.23	-0.08	0.93	0.25	0.35	0.35	0.24	1.30	1.62	1.69	0.14	1.12	1.02	0.56	-0.62	0.81							
	A	C	D	E	F	G	H	I	J	K	L	M	N	P	Q	R	S	T	V	W	Y							
	F	H	I	F	A	N	E	Q	-3.30	-2.97	-3.42	-2.91	-1.82	-2.72	-3.14	-2.47	-2.65	-2.64	-2.85	-3.10	-1.07	-2.93	-2.51	-3.06	-3.65	-2.72	-2.27	-2.50
	N	I	Q	-0.87	-1.14	-2.80	-3.42	-1.85	-2.26	-2.01	-2.56	-1.75	-2.83	-2.67	-2.20	-2.14	-2.92	-2.28	-2.81	-2.76	-2.55	-1.86	-2.25	-1.86	-2.25	-2.50		
	P	I	V	-1.40	-2.17	-2.74	-1.81	-1.93	-1.68	-2.04	-1.55	-1.52	-2.19	-1.90	-1.88	0.35	-3.02	-1.38	-1.60	-2.56	-1.67	-1.70	-1.51	-1.51	-1.51	-1.51		
	S	V	-2.82	-2.40	-3.45	-3.30	-1.93	-2.93	-2.75	-2.45	-2.39	-2.52	-2.41	-2.74	-1.96	-3.42	-2.18	-2.33	-2.98	-2.58	-2.30	-2.69	-2.69	-2.69	-2.69	-2.69		
	C	E	I	-2.79	-2.48	-2.41	-2.97	-1.89	-2.24	-3.19	-1.61	-1.75	-2.10	-2.35	-2.76	-1.04	-2.06	-1.19	-2.96	-2.70	-2.16	-2.44	-2.58	-2.58	-2.58	-2.58		
	N	Q	V	-2.51	-2.05	-2.80	-3.65	-2.00	-2.05	-2.83	-1.98	-2.06	-1.91	-2.67	-2.37	-2.29	-2.63	-2.17	-2.75	-2.53	-2.84	-1.76	-1.93	-1.93	-1.93	-1.93		
P	I	V	-1.62	-1.16	-2.01	-3.61	-1.61	-1.48	-1.75	-2.19	-1.01	-1.84	-1.56	-1.21	-1.28	-0.04	-1.09	-0.60	-1.45	-1.62	-1.03	-1.19	-1.40	-1.40	-1.40			
S	V	-1.28	-2.69	-3.33	-2.69	-1.85	-2.44	-2.60	-2.74	-2.04	-2.25	-2.55	-2.21	-2.46	-2.21	-2.54	-2.92	-2.21	-2.54	-2.92	-2.21	-2.54	-2.92	-2.21	-2.54			
D	E	I	-2.62	-2.51	-3.06	-2.81	-1.38	-2.57	-2.44	-2.16	-2.97	-1.62	-2.14	-3.13	-1.66	-3.14	-1.87	-3.03	-3.23	-2.20	-1.24	-1.82	-1.82	-1.82	-1.82			
N	Q	V	-2.94	-2.37	-2.78	-2.82	-1.29	-2.42	-1.85	-1.77	-2.27	-1.64	-1.59	-2.04	-2.21	-2.65	-2.44	-2.62	-2.82	-2.21	-0.87	-1.18	-1.18	-1.18	-1.18			
P	I	V	-1.61	-1.77	-2.25	-2.07	-1.75	-1.53	-2.10	-1.37	-0.79	-1.33	-1.66	-2.02	-0.23	-1.60	-0.58	-2.01	-1.80	-1.08	-1.39	-1.66	-1.66	-1.66	-1.66			
S	V	-2.40	-2.14	-2.43	-2.75	-1.18	-2.61	-2.41	-2.28	-2.73	-1.15	-2.42	-2.60	-1.90	-2.30	-2.00	-2.47	-2.75	-2.80	-2.16	-0.65	-1.49	-1.49	-1.49	-1.49			
E	E	I	-2.69	-2.74	-2.92	-3.52	-3.00	-2.45	-3.14	-2.43	-1.99	-2.55	-2.93	-2.88	-1.68	-2.61	-1.77	-3.14	-2.57	-2.63	-2.30	-2.87	-2.87	-2.87	-2.87			
N	Q	V	-3.31	-2.57	-2.97	-2.77	-2.28	-3.03	-2.48	-2.36	-2.71	-3.18	-3.32	-3.46	-2.50	-3.35	-2.72	-3.43	-3.70	-3.32	-2.36	-2.64	-2.64	-2.64	-2.64			
P	I	V	-1.53	-1.63	-1.70	-1.82	-1.85	-1.84	-1.57	-1.03	-1.19	-1.54	-1.54	-0.88	-1.17	-1.16	-0.82	-1.65	-1.60	-0.90	-1.92	-1.20	-1.20	-1.20	-1.20			
S	V	-2.93	-3.04	-3.32	-2.89	-1.29	-2.83	-3.11	-2.68	-2.84	-3.04	-3.03	-2.95	-2.73	-2.46	-2.87	-3.33	-2.62	-2.87	-3.31	-2.50	-2.50	-2.50	-2.50	-2.50			
F	E	I	-2.43	-2.57	-3.06	-2.81	-1.72	-2.71	-2.80	-1.04	-1.69	-2.15	-2.01	-2.84	-1.23	-3.11	-1.72	-2.65	-2.09	-1.87	-2.21	-2.06	-2.06	-2.06	-2.06			
N	Q	V	-2.06	-2.10	-2.80	-3.56	-1.60	-2.52	-1.93	-1.51	-2.42	-1.30	-2.13	-2.15	-1.36	-2.95	-1.98	-2.00	-2.55	-2.00	-1.49	-1.83	-1.83	-1.83	-1.83			
P	I	V	-1.29	-1.30	-2.25	-1.41	-1.10	-1.48	-1.37	-1.05	-1.05	-1.02	-1.00	-1.74	-0.26	-1.18	-1.16	-1.42	-1.65	-1.12	-1.17	-1.02	-1.02	-1.02	-1.02			
G	E	I	-2.36	-1.99	-2.40	-2.02	-2.35	-2.42	-2.73	-1.91	-2.27	-2.11	-2.44	-2.45	-2.28	-2.99	-2.78	-2.50	-2.14	-2.49	-1.88	-2.56	-2.56	-2.56	-2.56			
N	Q	V	-1.93	-1.34	-2.37	-2.58	-0.89	-1.95	-1.83	-1.44	-1.84	-1.45	-1.65	-1.85	-1.37	-1.77	-1.33	-1.93	-2.43	-1.81	-0.96	-1.39	-1.39	-1.39	-1.39			
P	I	V	-1.74	-2.06	-2.21	-2.26	-1.93	-2.10	-1.98	-1.42	-1.75	-1.39	-1.82	-1.12	-0.76	-2.38	-2.11	-2.13	-2.08	-2.24	-1.77	-1.69	-1.69	-1.69	-1.69			
S	V	-2.25	-1.82	-2.07	-2.86	-1.72	-2.46	-1.84	-2.00	-2.63	-1.91	-2.14	-2.33	-2.70	-2.20	-2.19	-2.97	-2.55	-2.29	-1.52	-1.63	-1.63	-1.63	-1.63	-1.63			
H	E	I	-2.30	-2.30	-2.72	-3.17	-2.41	-2.65	-2.85	-1.96	-1.84	-2.20	-2.36	-3.29	-0.93	-2.45	-1.97	-3.22	-2.52	-2.36	-2.30	-3.01	-3.01	-3.01	-3.01			
N	Q	V	-2.40	-2.25	-3.18	-3.10	-1.66	-2.39	-1.81	-2.33	-2.45	-2.05	-2.20	-2.52	-2.46	-2.57	-1.87	-2.96	-2.11	-2.56	-1.77	-1.62	-1.62	-1.62	-1.62			
P	I	V	-1.70	-1.51	-2.35	-1.75	-1.35	-1.62	-1.88	-1.19	-1.71	-2.07	-1.49	-1.83	-0.08	-3.13	-1.75	-3.32	-1.65	-1.39	-1.62	-1.30	-1.30	-1.30	-1.30			
S	V	-2.18	-2.04	-3.24	-2.97	-2.05	-2.59	-2.56	-2.14	-2.67	-2.41	-2.47	-2.67	-2.71	-2.64	-2.14	-2.50	-3.02	-2.18	-1.87	-2.39	-2.39	-2.39	-2.39	-2.39			
I	E	I	-1.67	-2.24	-3.18	-2.11	-1.79	-2.34	-2.65	-1.68	-1.04	-1.74	-1.96	-2.36	-0.75	-1.49	-1.19	-2.17	-2.12	-2.07	-2.80	-1.94	-1.94	-1.94	-1.94			
N	Q	V	-2.70	-2.63	-3.26	-3.58	-2.52	-2.66	-2.92	-1.95	-3.11	-2.95	-2.33	-2.30	-2.27	-3.11	-2.26	-2.90	-2.79	-2.61	-2.19	-1.96	-1.96	-1.96	-1.96			
P	I	V	-1.22	-1.46	-1.87	-1.49	-1.31	-1.16	-1.25	-1.30	-1.00	-1.29	-1.01	-1.42	0.03	-1.12	-0.80	-1.28	-1.76	-0.93	-1.72	-1.18	-1.18	-1.18	-1.18			
S	V	-2.33	-2.40	-3.12	-3.41	-2.04	-2.62	-2.54	-1.71	-2.45	-2.59	-2.47	-2.94	-1.50	-4.93	-2.54	-3.51	-2.67	-2.39	-2.26	-2.22	-2.22	-2.22	-2.22	-2.22			
K	E	I	-2.86	-2.98	-2.38	-3.19	-2.85	-2.89	-3.72	-2.62	-3.65	-2.70	-3.48	-2.89	-0.95	-2.88	-3.20	-3.17	-3.68	-2.85	-2.64	-2.64	-2.64	-2.64	-2.64			
N	Q	V	-2.58	-2.42	-3.02	-3.32	-2.37	-2.46	-2.57	-2.52	-2.66	-2.48	-2.66	-3.01	-2.30	-3.25	-2.52	-3.37	-2.71	-2.60	-2.12	-2.31	-2.31	-2.31	-2.31			
P	I	V	-2.00	-2.11	-2.05	-2.42	-3.34	-1.69	-2.16	-2.86	-3.42	-2.43	-2.38	-2.54	0.03	-2.01	-2.52	-2.61	-2.90	-2.44	-1.86	-2.54	-2.54	-2.54	-2.54			
S	V	-2.82	-2.60	-3.22	-3.06	-2.66	-2.47	-2.65	-2.42	-2.56	-2.99	-2.70	-2.25	-1.70	-2.96	-2.70	-3.05	-2.										

Appendices

						A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
F	R	F	A	E	I	-1.66	-1.89	-3.19	-2.35	-1.48	-2.16	-1.88	-1.01	-0.46	-1.37	-1.56	-1.66	-0.34	-1.01	-0.32	-2.38	-1.75	-1.32	-1.43	-1.22
				N	Q	-2.83	-2.45	-2.52	-3.47	-1.95	-2.40	-2.93	-2.28	-2.59	-2.56	-2.80	-2.38	-2.02	-2.27	-2.11	-3.30	-2.38	-2.75	-1.57	-1.61
				P	I	-0.04	-0.80	-1.10	-0.49	-1.00	-0.16	-0.46	0.07	0.14	-0.47	0.12	0.72	1.31	0.31	0.53	0.13	-0.33	0.41	-0.64	0.04
			C	E	I	-1.41	-1.20	-2.42	-1.56	-1.24	-1.10	-0.87	-0.35	0.21	-0.88	-0.48	-0.62	-0.60	-0.42	0.84	-1.15	-0.97	-0.33	-1.33	-0.80
				N	Q	-2.65	-1.91	-2.44	-3.50	-2.02	-2.18	-2.29	-1.70	-1.99	-2.09	-2.11	-2.08	-2.08	-2.31	-1.82	-2.76	-2.49	-2.12	-1.88	-1.83
				P	I	0.12	0.19	-0.53	0.16	-0.28	0.01	-0.05	0.66	0.71	0.55	0.27	1.02	0.69	1.25	1.26	0.42	0.43	0.83	0.19	0.38
			D	E	I	-1.27	-1.16	-2.46	-2.31	-0.88	-1.68	-0.86	-0.95	0.57	-0.81	-0.67	-1.62	-1.33	-1.14	0.99	-2.06	-1.64	-0.75	-1.11	0.79
				N	Q	-2.41	-1.93	-1.99	-3.24	-1.20	-2.15	-2.64	-1.72	-2.14	-1.69	-1.74	-2.11	-2.01	-2.39	-1.55	-2.93	-3.13	-1.91	-0.87	-1.23
				P	I	0.84	1.27	-0.85	0.27	1.28	0.10	0.41	2.04	2.68	1.77	1.70	1.07	1.03	1.91	2.66	0.58	1.51	1.97	0.73	1.36
			E	E	I	-1.41	-1.15	-2.82	-1.84	-1.93	-1.58	-0.86	-0.40	0.61	-0.37	-1.05	-0.73	-0.97	-0.37	0.94	-1.32	-0.71	-0.61	-1.84	-1.21
				N	Q	-3.19	-2.77	-3.35	-3.19	-2.37	-2.96	-2.98	-2.23	-2.57	-3.08	-3.18	-2.69	-2.98	-2.78	-2.00	-2.98	-3.65	-3.07	-2.66	-2.33
				P	I	0.80	1.11	-0.18	0.22	0.63	0.56	0.82	1.64	2.28	1.09	0.74	1.85	0.96	1.62	2.58	1.14	1.43	1.53	0.25	1.06
			F	E	I	-1.11	-0.77	-2.69	-1.42	-1.25	-1.63	-1.28	-0.32	0.40	-0.67	-0.75	-1.08	-0.13	-0.27	0.23	-0.89	-0.81	-0.35	-1.23	-0.89
				N	Q	-2.49	-1.85	-2.85	-2.95	-1.72	-1.89	-1.74	-1.01	-2.57	-1.60	-1.89	-2.16	-1.86	-2.67	-1.68	-2.53	-2.59	-1.66	-1.01	-1.78
				P	I	-1.31	-0.29	-1.56	0.06	-0.82	-1.95	-0.50	-0.06	-1.31	0.05	-0.94	-0.21	0.42	0.57	-0.64	-0.15	0.22	0.10	-0.69	-0.47
			G	E	I	-3.57	-2.03	-2.87	-2.84	-2.04	-2.19	-2.18	-1.00	-0.88	-1.50	-1.71	-1.87	-1.77	-1.42	-0.88	-1.60	-1.72	-1.41	-1.30	-1.31
				N	Q	-1.91	-1.70	-2.21	-2.80	-1.53	-1.70	-1.65	-1.45	-1.90	-1.56	-1.96	-1.49	-1.74	-1.99	-1.95	-2.39	-2.51	-2.12	-1.15	-1.64
				P	I	-0.49	-0.56	-1.05	-0.76	-0.17	-0.87	-0.86	-0.16	-0.61	-0.92	-0.43	0.15	0.28	-0.40	-0.42	-0.54	-0.83	-0.12	-0.43	0.38
			H	E	I	-1.02	-1.24	-2.42	-2.15	-2.02	-2.03	-1.95	-0.54	-0.66	-0.92	-1.36	-1.48	-0.47	-0.73	-0.14	-2.11	-1.48	-0.53	-2.30	-1.74
				N	Q	-2.28	-2.28	-3.17	-2.84	-1.71	-2.30	-2.81	-1.85	-2.51	-1.72	-2.00	-3.17	-2.05	-2.65	-1.69	-2.29	-2.30	-1.87	-2.29	-1.99
				P	I	-1.26	-0.86	-1.35	-0.18	-1.66	-1.74	-0.83	0.25	-1.13	-0.16	-1.01	-0.53	0.84	0.29	-0.38	-1.06	-0.46	0.32	-0.56	-0.82
			I	E	I	-0.31	-0.60	-2.55	-1.69	-0.59	-1.12	-0.53	-0.16	1.11	-0.61	-0.74	-0.37	-0.41	0.20	0.95	-0.77	-0.22	-0.22	-1.36	-0.41
				N	Q	-2.57	-1.95	-3.73	-3.43	-2.00	-2.96	-2.39	-1.54	-2.15	-1.85	-1.95	-2.60	-2.21	-2.34	-1.82	-3.61	-2.66	-2.12	-1.84	-1.67
				P	I	0.30	0.42	-0.58	0.38	0.24	0.06	0.57	0.93	0.84	0.49	0.49	0.70	0.32	1.10	1.50	0.51	0.54	0.64	-0.22	0.73
			K	E	I	-2.08	-2.73	-2.51	-3.29	-2.89	-2.21	-2.46	-2.23	-1.91	-2.70	-2.46	-2.37	-0.43	-2.04	-1.89	-1.99	-2.33	-2.41	-2.58	-2.58
				N	Q	-2.98	-2.76	-3.28	-3.35	-2.33	-2.64	-2.39	-2.44	-2.81	-2.48	-2.60	-2.74	-2.21	-2.78	-2.87	-2.82	-2.85	-2.81	-2.38	-1.96
				P	I	-0.31	-1.46	-1.19	-1.48	-1.32	-0.84	-1.10	-1.89	-0.85	-1.51	-1.39	-0.46	0.83	-0.82	-1.51	-0.71	-1.25	-1.26	-1.43	-1.59
			L	E	I	-1.22	-0.92	-2.58	-1.65	-0.77	-1.55	-0.55	-0.06	0.31	-1.09	-1.18	-0.41	0.10	-0.28	-0.07	-1.22	-0.78	-0.55	-0.64	-0.65
				N	Q	-2.34	-2.11	-2.68	-3.24	-1.50	-2.59	-2.00	-1.34	-1.94	-1.99	-1.95	-2.53	-2.28	-2.91	-2.19	-3.06	-2.18	-1.87	-1.62	-2.08
				P	I	0.28	-0.03	-1.16	0.04	0.16	-0.72	0.40	0.28	0.50	0.26	0.31	0.20	1.16	0.57	0.84	0.43	-0.07	0.31	0.09	0.64
			M	E	I	-1.10	-1.79	-2.87	-1.87	-1.98	-1.76	-1.64	-0.64	-0.53	-0.92	-1.41	-1.02	-0.45	-1.10	-0.70	-1.40	-1.71	-1.37	-1.62	-1.36
				N	Q	-2.21	-2.40	-3.37	-3.01	-1.84	-2.71	-2.39	-1.69	-2.66	-1.96	-2.41	-2.40	-2.17	-2.97	-1.93	-2.42	-3.00	-2.24	-1.47	-2.17
				P	I	0.63	-0.16	-1.05	-0.30	-0.56	0.04	-0.02	-0.13	0.38	-0.50	-0.21	0.51	0.86	0.44	0.28	0.56	-0.11	-0.07	-0.77	0.04
			N	E	I	-1.83	-1.85	-2.53	-2.64	-1.71	-2.43	-1.60	-1.74	0.77	-1.46	-1.51	-1.82	-0.83	-1.49	-0.31	-1.77	-2.00	-1.48	-1.50	-1.45
				N	Q	-2.82	-2.35	-2.92	-3.88	-1.60	-2.88	-1.93	-3.26	-2.18	-2.12	-2.52	-2.61	-2.05	-2.72	-1.87	-2.64	-2.71	-1.71	-1.80	-1.57
				P	I	-0.22	-0.22	-1.09	0.17	-0.08	-1.03	-0.42	0.38	0.25	0.27	-0.18	0.09	1.02	0.30	0.91	-0.32	0.12	-0.59	-0.25	1.06
			Q	E	I	0.26	1.08	-0.91	0.35	1.17	-0.08	0.77	2.12	1.95	1.39	1.19	0.92	1.31	0.98	1.79	0.57	0.95	1.86	0.44	1.25
				N	Q	-2.58	-2.14	-2.69	-2.95	-2.20	-2.17	-2.33	-1.60	-1.32	-2.06	-1.75	-2.19	-2.06	-2.34	-1.77	-3.12	-1.98	-1.52	-1.97	-1.86
				P	I	2.15	2.29	1.58	2.20	2.22	1.80	2.15	3.18	2.52	2.29	2.62	2.58	2.92	2.33	2.36	2.46	2.43	2.88	1.84	2.33
			R	E	I	0.08	-1.04	-1.86	-1.79	0.69	-0.56	-1.05	-0.94	0.11	-0.56	-0.61	-0.80	2.06	-0.45	-0.63	-0.50	-1.35	-0.70	0.78	1.27
				N	Q	-1.91	-2.22	-3.06	-3.65	-1.44	-2.56	-2.07	-2.11	-2.65	-1.80	-2.15	-2.56	-0.87	-2.31	-1.96	-2.70	-2.44	-1.61	-1.22	-1.25
				P	I	1.60	-0.08	-0.29	-0.19	1.43	0.75	0.16	0.33	0.80	0.69	0.32	0.70	2.97	0.52	-0.18	0.50	-0.50	0.56	1.56	1.49
			S	E	I	-1.91	-1.80	-2.15	-2.01	-2.03	-1.92	-1.61	-1.37	-0.38	-1.00	-1.31	-1.79	-0.70	-0.91	0.12	-1.71	-2.06	-1.39	-2.04	-1.44
				N	Q	-2.41	-2.73	-2.97	-2.97	-1.77	-2.70	-2.33	-2.52	-2.05	-2.23	-2.79	-2.60	-2.12	-2.96	-1.75	-2.96	-2.34	-2.20	-1.82	-1.61
				P	I	0.06	-0.39	-0.87	-0.21	-1.17	-0.37	-0.39	0.17	-0.02	-0.36	-0.15	0.24	0.79	0.34	1.01	-0.16	-0.05	0.56	-0.45	0.05
			T	E	I	-0.78	-0.89	-1.81	-1.02	-1.11	-1.03	-0.40	-0.04	0.75	-0.34	-0.40	-0.01	-0.46	-0.29	1.75	-0.87	-0.81	-1.14	-1.02	-0.34
				N	Q	-2.39	-2.43	-3.15	-3.22	-2.36	-2.93	-2.70	-2.61	-2.34	-2.31	-2.15	-2.64	-2.55	-2.53	-1.42	-2.90	-2.66	-2.08	-1.92	-2.32
				P	I	0.59	0.70	0.06	0.58	0.22	0.36	0.57	1.17	0.96	0.55	0.63	1.38	1.08	0.81	2.08	0.73	1.13	1.23	0.31	0.78
			V	E	I	-0.47	-0.61	-2.11	-1.28	-0.70	-0.55	-0.21	0.44	1.30	0.21	-0.30	-0.06	-0.04	0.35	1.44	-0.56	-0.44	-0.06	-1.03	-0.14
				N	Q	-1.51	-2.43	-3.28	-3.45	-2.10	-3.12	-2.01	-2.16	-2.24	-1.68	-2.39	-2.75	-2.42	-2.55	-2.05	-3.35	-2.60	-2.90	-1.90	-1.80
				P	I	0.70	0.74	-0.15	0.53	0.04	0.33	0.84	1.32	1.15	1.08	0.61	1.47	0.62	1.51	2.08	0.84	0.83	1.32	0.21	0.92
			W	E	I	-1.28	-0.55	-2.92	-2.02	-0.94	-1.81	-0.90	-0.03	0.52	-0.42	-0.58	-1.11	-0.91	-0.43	0.08	-1.30	-0.83	-0.43	-0.98	-0.84
				N	Q	-2.10	-1.50	-2.79	-2.22	-0.81	-1.68	-1.55	-0.55	-2.07	-1.41	-1.69	-2.01	-1.50	-1.87	-1.25	-2.07	-1.45	-1.22	-1.07	-1.18
				P	I	-0.77	-0.11	-1.35	-0.49	-0.99	-1.30	-0.79	-0.24	-0.62	-0.20	-0.50	-0.51	-0.19	0.90	-0.36	0.01	-0.44	-0.12	-0.15	-0.69
			Y	E	I	-1.58	-0.96	-2.67	-1.93	-1.29	-1.95	-1.43	-0.29	-0.73	-0.61	-0.73	-1.19	-0.02	-0.54	-1.31	-1.26	-1.02	-0.56	-1.72	-1.20
				N	Q	-2.29	-2.28	-3.30	-2.81	-1.47	-2.63	-2.05	-1.32	-2.35	-1.70	-2.19	-2.63	-1.68	-2.01	-2.29	-2.21	-2.08	-1.41	-1.65	-1.87
				P	I	-3.40	-1.68	-1.28	-0.48	-0.27	-0.33	-2.17	-0.34	-2.39	-0.62	-1.57	-1.49	-0.40	-0.14	-4.19	-2.40	-1.01	-1.30	-1.43	-2.21
						A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
146	153	155	147	149	150	154																			
L	F	C	A	E	I	0.92	-0.11</																		

Appendices

	N	Q	-1.44	-1.95	-2.84	-2.57	-1.05	-2.13	-1.74	-1.61	-1.58	-1.36	-1.60	-2.20	-1.81	-1.82	-1.02	-1.37	-1.48	-2.24	-1.24	-0.76
	P	I	2.54	2.02	-0.62	1.71	2.39	1.55	2.28	0.85	1.77	0.54	1.42	1.41	1.62	1.93	2.27	2.80	2.59	1.29	2.08	2.93
T	E	I	2.46	2.05	-2.46	0.76	0.98	1.18	1.95	1.10	2.39	-0.22	1.60	1.01	-0.76	2.60	2.73	3.20	3.14	1.46	0.54	1.86
	N	Q	-1.30	-1.48	-3.19	-2.34	-1.27	-1.62	-1.37	-1.61	-1.79	-1.91	-1.23	-1.62	-1.71	-1.47	-0.56	-1.02	-0.79	-1.74	-1.12	-1.14
	P	I	3.02	3.00	-0.29	2.40	2.70	2.89	2.78	1.78	2.65	1.12	2.30	2.86	1.64	2.88	2.92	3.49	3.44	2.24	3.20	3.24
V	E	I	3.09	2.44	-1.81	1.89	1.42	2.41	2.91	1.53	2.95	0.64	2.41	0.96	-0.42	3.13	3.28	3.33	3.20	1.62	0.56	2.73
	N	Q	-0.60	-1.34	-2.71	-2.25	-1.12	-1.29	-1.65	-1.21	-0.95	-2.03	-1.69	-1.89	-1.93	-1.00	-0.48	-0.09	-1.21	-1.58	-1.28	-0.83
	P	I	3.56	2.97	0.00	2.99	2.52	3.18	2.57	1.79	2.78	1.48	2.63	2.74	1.90	3.09	3.20	3.59	3.21	1.94	1.77	2.84
W	E	I	1.06	0.75	-1.38	-0.53	0.91	-0.01	1.12	1.03	1.24	0.29	0.84	0.11	-0.29	1.13	1.80	1.89	1.83	0.61	0.41	0.93
	N	Q	-0.89	-0.77	-1.82	-1.76	-0.11	-0.91	-0.47	-0.03	-0.86	-0.11	-0.62	-0.80	-1.27	-1.22	-0.06	-0.53	-0.81	-0.29	0.04	-0.16
	P	I	1.84	2.17	0.79	0.38	2.05	1.05	1.65	1.24	1.58	1.41	1.56	1.53	1.22	1.92	2.05	2.33	2.09	1.14	1.06	2.00
Y	E	I	1.71	1.36	-1.72	-0.22	0.85	0.29	1.66	0.75	1.42	-0.30	1.05	0.87	-0.66	1.80	1.90	2.45	2.25	0.95	0.14	1.64
	N	Q	-1.50	-1.48	-2.15	-2.14	-0.79	-1.27	-1.42	-0.88	-1.40	-1.29	-1.29	-1.26	-1.60	-2.14	-1.14	-1.38	-1.63	-1.76	-0.88	-0.77
	P	I	1.77	1.99	-0.67	0.74	1.26	1.46	1.83	0.94	1.01	0.32	1.14	1.56	1.36	1.87	1.15	2.70	2.63	1.20	0.75	1.82
	A	C	D	E	F	G	H	I	J	K	L	M	N	P	Q	R	S	T	U	V	W	Y

[illegible]

Appendices

I	D	I	0.19	-0.80	-2.34	-1.12	-1.76	-0.54	-0.78	-1.41	0.01	-1.28	-1.16	0.07	1.54	0.42	0.09	-0.23	-0.96	-1.45	-2.38	-1.22
		Q	-1.43	-1.92	-2.99	-2.16	-2.69	-1.75	-2.35	-2.03	-1.83	-1.97	-2.20	-1.46	-0.76	-1.06	-1.04	-1.32	-2.10	-2.57	-2.52	-2.40
		V	-0.56	-1.47	-2.80	-2.17	-1.78	-1.01	-1.17	-2.61	-1.09	-1.33	-1.63	-0.35	0.62	0.10	-0.34	-0.48	-2.10	-1.74	-2.37	-1.41
E	I	I	2.50	2.13	0.11	2.02	0.21	2.03	1.88	0.81	2.26	0.96	1.69	2.45	3.26	2.70	2.28	2.53	1.60	1.22	-0.65	1.43
		Q	1.32	0.63	-1.57	-0.07	-1.23	0.73	0.46	-1.23	0.71	-0.80	-0.53	0.78	1.31	1.54	1.12	0.98	0.00	-0.60	-1.33	-0.07
		V	2.58	2.24	0.11	1.77	0.36	2.18	1.99	0.60	2.22	0.86	1.74	2.41	3.06	2.78	2.40	2.61	1.48	1.02	-0.07	1.60
N	I	I	1.85	1.51	-0.02	1.38	-0.14	1.44	1.23	0.50	0.91	0.42	0.99	1.76	2.90	2.25	1.39	1.81	0.99	0.67	-0.47	0.93
		Q	-0.22	-0.58	-2.67	-1.09	-1.50	-0.57	-1.03	-1.65	-0.54	-1.41	-1.20	-0.08	0.79	0.46	-0.33	0.02	-1.25	-1.15	-1.46	-1.10
		V	0.92	0.30	-1.39	0.00	-1.64	0.44	-0.20	-1.20	0.04	-0.71	-0.69	0.77	1.73	1.27	0.18	0.54	-0.21	-0.67	-1.69	-0.19
P	I	I	2.43	1.94	1.68	2.39	0.72	2.13	2.07	0.83	1.98	1.20	1.54	2.38	2.85	2.73	2.12	2.51	1.62	1.44	0.59	1.86
		Q	1.24	0.32	-1.06	-0.10	-0.72	0.65	-0.08	-0.72	0.55	-0.69	-0.17	1.11	1.54	1.66	1.04	1.11	-0.30	-0.24	-0.64	0.21
		V	2.39	2.01	1.18	1.98	0.51	1.91	1.86	0.28	1.65	0.85	1.25	2.32	2.74	2.93	1.96	2.34	1.40	0.91	0.16	1.82
K	D	I	-2.24	-2.91	-3.21	-3.22	-2.78	-2.34	-2.70	-2.67	-2.86	-3.82	-2.41	-2.74	-0.37	-2.58	-2.58	-2.48	-3.90	-3.00	-2.93	-3.32
		Q	-3.33	-2.94	-4.02	-3.21	-2.75	-2.84	-3.46	-3.27	-2.95	-3.05	-4.35	-3.91	-2.47	-3.37	-2.83	-4.44	-3.68	-3.55	-3.12	-3.97
		V	-2.34	-3.20	-3.23	-3.30	-3.32	-2.51	-3.32	-3.07	-2.91	-3.94	-3.76	-3.02	-1.48	-2.45	-3.39	-3.49	-2.82	-3.33	-3.32	-3.04
E	I	I	0.05	-1.47	-1.40	-1.31	-2.47	-0.40	-1.62	-1.69	-1.83	-3.39	-2.95	-1.04	1.78	-0.69	-1.68	-0.79	-1.73	-2.47	-2.37	-1.50
		Q	-1.68	-2.68	-3.29	-2.56	-3.13	-2.24	-3.32	-3.25	-2.72	-3.34	-2.99	-2.26	-1.25	-2.93	-2.69	-2.45	-3.43	-3.68	-3.47	-3.33
		V	0.21	-1.64	-1.39	-1.19	-2.73	-0.20	-1.73	-2.49	-2.39	-2.75	-2.04	-0.73	1.25	-0.57	-2.29	-0.45	-1.95	-2.05	-2.17	-2.58
N	I	I	-0.68	-1.93	-1.34	-1.11	-2.60	-1.46	-2.32	-1.67	-2.24	-3.20	-2.58	-1.57	1.33	-2.86	-1.06	-1.78	-1.83	-2.25	-2.24	
		Q	-2.92	-2.82	-2.84	-2.37	-3.03	-2.62	-2.88	-2.33	-2.64	-3.08	-2.74	-2.95	-1.26	-2.26	-2.62	-2.06	-3.44	-3.08	-2.31	-2.35
		V	-1.42	-2.07	-2.88	-2.57	-2.37	-1.69	-2.32	-3.03	-3.54	-3.41	-2.78	-2.19	0.23	-1.98	-2.65	-2.70	-2.45	-2.98	-2.24	-1.12
P	I	I	0.71	-1.35	-0.10	0.09	-1.73	0.20	-0.79	-1.59	-1.57	-2.82	-1.49	-0.19	2.33	0.26	-1.55	0.14	-1.45	-1.44	-1.42	-1.29
		Q	-1.15	-2.89	-2.70	-2.21	-2.78	-1.55	-2.48	-2.35	-2.41	-3.22	-2.58	-2.28	-0.30	-1.66	-2.74	-1.42	-2.83	-2.91	-2.26	-2.68
		V	0.45	-1.18	-1.00	-0.64	-1.57	-0.15	-1.28	-1.60	-1.86	-2.53	-1.69	-0.84	1.94	0.23	-1.71	-0.21	-1.18	-1.33	-1.82	-1.46
L	D	I	-1.54	-0.92	-2.98	-1.86	-2.16	-1.40	-1.61	-0.84	0.77	-1.00	-0.85	0.96	0.99	-0.74	0.33	-1.31	-0.79	-1.44	-1.52	-1.66
		Q	-2.50	-2.42	-3.60	-4.17	-2.66	-2.98	-2.45	-1.98	-1.52	-2.12	-2.22	-2.39	-1.07	-2.85	-1.17	-2.86	-2.12	-2.50	-2.49	-2.59
		V	-1.50	-1.16	-3.87	-2.37	-2.55	-2.69	-2.32	-1.60	-0.97	-1.48	-1.94	-1.65	-0.10	-1.22	-0.51	-1.25	-2.07	-1.67	-2.17	-2.35
E	I	I	1.07	1.26	-0.97	0.91	-0.58	0.19	0.64	1.33	1.70	0.87	0.37	1.63	2.63	1.83	1.97	1.15	1.38	1.08	-0.83	0.83
		Q	-0.46	-0.19	-3.04	-0.98	-2.18	-0.89	-1.38	-0.45	-0.07	-0.59	-0.53	-0.91	0.29	-0.01	0.75	-0.26	-0.21	-0.91	-1.97	-1.06
		V	1.15	1.29	-1.04	0.80	-1.06	0.67	0.28	0.71	1.41	0.78	0.36	1.37	2.83	1.67	1.84	1.55	1.37	1.02	-1.48	-0.19
N	I	I	0.39	0.83	-0.70	0.45	-0.58	-0.18	-0.43	0.26	0.33	0.45	0.07	0.70	2.60	0.95	1.21	0.47	0.48	0.54	-0.32	0.08
		Q	-0.97	-1.58	-2.36	-2.02	-2.10	-1.51	-2.21	-1.42	-1.59	-0.98	-1.51	-1.49	-0.14	-1.53	-0.97	-1.55	-1.83	-1.85	-2.12	-1.65
		V	-0.38	-0.71	-2.72	-0.55	-1.48	-0.99	-1.07	-1.01	-0.78	-0.78	-0.68	-0.64	1.32	-0.39	0.04	-0.72	-0.48	-0.94	-1.99	-1.23
P	I	I	1.50	1.68	0.68	1.60	0.48	1.01	1.37	1.06	1.18	1.14	1.12	1.31	2.59	1.83	2.08	1.74	1.78	1.07	0.06	1.28
		Q	-0.57	-0.26	-2.30	-0.99	-1.12	-0.61	-0.66	-0.72	-0.22	-0.47	-0.96	-0.65	1.00	1.04	0.26	-0.65	-0.58	-0.67	-1.20	-0.62
		V	1.34	1.36	0.25	1.22	0.19	0.65	1.02	0.75	0.98	1.16	0.76	1.76	2.87	1.46	1.94	1.44	1.19	0.94	0.20	0.90
M	D	I	-0.79	-1.93	-2.62	-2.23	-2.73	-1.17	-2.31	-1.54	-1.47	-2.08	-2.34	-1.30	0.61	-0.95	-1.34	-1.21	-1.75	-1.51	-2.57	-2.11
		Q	-2.11	-2.69	-3.15	-2.96	-3.12	-2.72	-2.83	-2.50	-2.45	-2.36	-2.78	-2.49	-1.81	-1.98	-2.39	-2.32	-2.21	-2.32	-2.97	-2.61
		V	-1.10	-2.39	-2.61	-2.64	-2.63	-1.58	-2.61	-2.15	-2.21	-2.39	-2.79	-1.33	-0.37	-1.90	-2.21	-1.45	-2.34	-2.20	-2.43	-2.10
E	I	I	1.90	0.73	-0.47	0.27	-1.67	0.96	0.10	-0.19	0.44	-0.69	-0.46	1.02	2.44	1.43	0.95	1.40	0.66	0.55	-1.74	-0.75
		Q	0.19	-0.90	-3.13	-1.85	-3.08	-0.95	-2.06	-1.71	-2.31	-1.18	-0.36	-0.07	-0.31	-0.58	0.02	-1.47	-1.09	-2.03	-2.15	
		V	2.20	0.89	-0.73	0.11	-1.42	1.13	0.12	-0.19	0.31	-0.73	-0.25	0.99	2.32	1.27	1.00	1.71	0.57	0.04	-1.50	-0.75
N	I	I	1.73	0.51	-0.36	0.57	-1.43	0.97	-0.17	0.03	0.60	-0.52	-0.24	0.77	2.66	1.01	0.08	1.23	0.58	0.59	-1.21	-0.99
		Q	-0.87	-1.37	-2.20	-2.01	-2.42	-1.11	-1.96	-1.45	-1.77	-1.77	-1.96	-1.59	-0.19	-1.00	-1.67	-0.99	-1.71	-1.67	-2.34	-0.03
		V	0.40	-0.93	-1.41	-1.12	-2.49	-0.27	-1.19	-1.36	-1.11	-1.26	-1.21	0.60	1.42	0.06	-0.64	0.08	-0.73	-1.06	-2.27	-1.69
P	I	I	2.37	1.17	1.10	1.43	-0.49	1.61	1.25	0.59	1.00	0.22	0.77	1.87	3.03	2.06	0.76	2.42	1.27	0.86	-0.44	0.09
		Q	0.77	-0.39	-1.79	-1.01	-2.21	0.10	-0.83	-0.89	-0.77	-0.95	-0.98	-0.18	0.41	0.35	-0.12	0.36	-0.67	-1.01	-1.65	-1.32
		V	2.43	1.13	0.75	1.15	-0.78	1.68	0.84	0.23	0.67	-0.17	0.48	1.61	2.76	1.99	0.99	2.25	1.08	0.57	-0.56	0.43
N	D	I	-1.93	-1.49	-2.69	-3.48	-2.26	-2.22	-2.25	-2.17	-2.76	-2.37	-2.00	-2.54	-0.38	-1.78	-2.04	-2.11	-2.79	-2.34	-2.31	-1.54
		Q	-2.89	-2.94	-3.25	-3.90	-2.52	-3.13	-3.41	-3.34	-3.29	-2.62	-3.30	-3.10	-2.87	-2.75	-2.19	-3.02	-3.49	-2.96	-2.29	-2.78
		V	-1.13	-2.25	-3.56	-2.84	-2.24	-3.36	-2.50	-2.63	-2.75	-2.27	-2.73	-2.65	-1.38	-2.53	-1.93	-2.45	-2.80	-2.83	-2.95	-2.05
E	I	I	0.34	-0.18	-1.66	-0.43	-1.22	-0.89	-0.60	-0.52	-0.05	-1.07	-0.40	-0.38	1.65	0.65	0.37	0.03	-0.73	-0.73	-1.66	-0.05
		Q	-1.53	-2.43	-3.35	-3.09	-2.93	-2.18	-1.90	-2.74	-2.12	-2.92	-1.71	-2.23	-2.11	-1.49	-1.77	-1.69	-2.17	-2.69	-1.85	-2.20
		V	0.78	-0.03	-2.07	-0.78	-0.97	-0.55	-0.44	-1.09	0.07	-0.85	-0.42	-0.50	1.22	0.94	0.39	0.08	-0.93	-0.70	-1.40	-0.12
N	I	I	0.23	-0.14	-1.34	0.01	-0.83	-0.82	-0.41	-0.52	-0.29	-0.53	-0.40	0.17	1.89	0.51	0.17	0.06	-0.50	0.13	-1.19	-0.21
		Q	-1.67	-1.99	-2.75	-2.54	-2.20	-2.07	-1.91	-2.31	-2.04	-2.04	-1.88	-2.10	-1.47	-1.99	-1.69	-2.22	-2.81	-1.97	-2.48	-1.85
		V	-0.70	-1.33	-2.33	-1.80	-1.40	-1.70	-1.36	-1.34	-1.48	-1.63	-1.50	-1.29	0.10	-0.83	0.81	-1.06	-1.68	-1.77	-1.66	-0.65
P	I	I	1.76	1.36	0.00	1.60	0.87	0.40	1.29	0.58	0.99	1.06	1.40	1.17	3.20	2.28	1.45	1.31	0.99	1.22	0.32	1.79
		Q	-0.94	-1.24	-3.33	-1.35	-1.52	-1.68	-1.06	-1.72	-0.77	-1.50	-1.25	-0.98	-0.49	-0.27	-1.74	-1.08	-1.37	-		

Appendices

	V	1.55	1.15	-0.46	0.14	-0.34	0.03	0.59	0.02	0.47	-0.74	0.26	0.79	1.63	1.15	1.72	1.51	0.83	0.74	-0.34	1.33				
P	I	2.92	3.04	2.61	2.55	2.29	2.45	2.49	2.46	2.41	1.61	2.12	2.78	3.03	3.00	3.01	3.11	2.81	2.87	2.25	3.24				
	Q	1.35	1.42	-1.09	-0.01	-0.41	0.27	0.77	-0.33	0.84	-0.89	0.23	1.05	0.43	1.84	1.91	1.85	1.36	0.75	0.07	1.13				
	V	3.06	2.68	2.24	2.28	1.89	2.28	2.65	2.17	2.33	1.49	2.02	2.81	3.03	2.86	3.12	2.83	2.88	2.93	2.34	2.79				
V	D	0.60	-0.30	-2.14	-0.99	-1.64	0.15	-0.55	-1.61	-0.09	-1.23	-0.75	0.07	1.96	0.71	0.30	0.29	-0.73	-0.73	-1.64	-0.67				
	I	-1.07	-1.95	-2.32	-2.88	-2.51	-1.12	-2.12	-2.16	-1.37	-2.53	-2.26	-0.94	-0.20	-0.94	-1.41	-0.92	-0.46	-2.45	-2.26	-2.07				
	V	0.03	-0.93	-2.24	-1.77	-1.96	-0.52	-1.04	-2.00	-0.86	-2.05	-1.25	-0.74	0.80	0.01	0.06	-0.58	-1.73	-1.72	-2.68	-0.50				
E	I	2.54	2.26	0.63	1.86	0.34	2.69	1.96	0.93	2.65	1.12	1.64	2.53	3.28	2.90	2.71	2.41	1.84	1.68	-0.19	2.03				
	Q	1.73	0.61	-1.32	0.05	-1.30	1.25	0.27	-1.03	1.07	-0.60	0.22	1.07	1.63	1.78	1.56	1.64	0.35	-0.45	-1.43	0.38				
	V	3.19	2.56	0.65	2.02	0.65	2.39	1.83	1.03	2.55	1.00	1.94	2.82	3.06	2.79	2.90	1.88	1.56	-0.56	-0.36	2.03				
N	I	2.48	1.51	0.16	1.77	0.00	2.07	1.42	0.60	1.50	0.64	1.12	1.88	3.18	2.42	1.82	2.13	1.19	1.04	-0.31	1.41				
	Q	0.44	-0.64	-2.40	-1.17	-1.74	-0.29	-0.69	-1.62	-0.42	-1.38	-1.00	-0.18	1.09	0.27	-0.27	0.02	-0.97	-1.01	-1.72	-1.06				
	V	1.66	0.41	-0.91	0.18	-0.94	0.84	0.03	0.70	0.42	-0.67	0.18	0.91	2.01	1.23	0.57	1.09	-0.17	-0.35	-1.42	0.20				
P	I	2.89	2.11	2.02	2.59	1.27	2.59	2.46	1.30	2.09	1.52	1.86	2.77	3.09	2.98	2.43	2.68	1.97	1.81	0.64	2.37				
	Q	2.03	0.91	-0.63	0.21	-0.84	1.50	0.58	-0.51	0.87	-0.44	0.22	1.49	1.63	1.98	1.41	2.09	0.51	-0.13	-0.67	0.60				
	V	2.97	2.31	1.62	2.26	0.93	2.49	2.06	1.06	1.90	0.95	1.79	2.67	3.02	2.91	2.23	2.66	1.72	1.70	0.63	2.24				
W	D	-1.06	-1.14	-2.57	-2.30	-1.22	-2.04	-0.38	-0.82	-0.84	-1.33	-1.24	-0.17	-1.03	-0.13	-0.04	-0.42	-1.56	-1.18	-1.38	-1.56				
	I	-1.97	-1.90	-3.01	-3.29	-1.98	-2.55	-1.65	-1.24	-2.05	-2.17	-2.39	-1.74	-1.68	-1.40	-1.62	-2.38	-2.23	-1.90	-1.66	-2.06				
	V	-0.99	-1.39	-2.73	-2.68	-2.14	-2.53	-0.65	-1.15	-1.11	-1.49	-2.07	-0.60	-1.47	-0.29	-0.70	-1.22	-1.83	-1.38	-1.67	-1.33				
E	I	1.53	0.84	-1.25	-0.71	-0.38	-0.27	1.79	0.48	1.22	-0.55	0.10	1.98	1.00	2.54	1.68	1.82	0.72	0.50	-1.14	-0.18				
	Q	0.04	0.52	-3.35	-3.31	-1.44	-1.61	0.28	0.73	0.01	-1.27	-1.08	0.28	-1.75	0.45	0.24	-0.13	-0.97	-1.01	-1.63	-1.57				
	V	1.73	1.19	-1.00	-0.76	-0.58	-0.52	1.86	0.20	1.44	-0.46	-0.03	2.25	0.56	2.75	1.82	1.75	0.69	0.67	-1.02	-0.61				
N	I	1.05	0.74	-1.37	-0.49	0.21	-0.93	1.03	0.35	0.27	-0.29	0.15	1.80	0.99	1.74	0.82	1.15	0.14	-0.01	-0.56	-0.05				
	Q	-0.77	-0.93	-2.30	-2.62	-1.01	-2.15	-0.21	-1.28	-1.07	-1.09	-1.15	0.30	-1.53	0.01	-0.75	-0.68	-1.57	-1.52	-0.64	-1.08				
	V	0.00	0.00	-2.53	-1.35	-0.82	-1.15	0.67	-0.62	-0.36	-1.01	-0.49	1.22	-0.79	1.00	-0.21	-0.16	-0.34	-0.88	-0.77	-0.78				
P	I	1.25	1.25	-0.57	0.08	0.46	0.42	1.52	0.15	0.84	0.33	0.43	2.11	1.32	2.54	1.39	1.79	0.71	0.45	-0.21	0.67				
	Q	-0.06	0.03	-2.22	-1.15	-0.49	-1.60	0.46	-0.17	0.10	-0.55	-0.62	0.79	-0.97	1.24	0.54	0.09	-1.03	-0.48	-0.85	-0.66				
	V	1.45	1.35	-0.79	-0.02	0.58	0.23	1.60	0.52	0.89	0.26	0.69	2.11	0.83	2.66	1.37	1.43	0.46	0.40	-0.64	-0.77				
Y	D	-0.14	-1.45	-2.62	-2.98	-2.03	-1.95	-0.98	-2.19	-1.29	-1.73	-1.81	-0.75	-1.09	-0.30	-0.76	-1.17	-1.76	-2.09	-1.69	-1.69				
	I	-2.80	-1.93	-2.74	-3.31	-2.68	-2.69	-2.32	-2.24	-1.93	-2.41	-2.50	-1.82	-2.40	-1.93	-1.78	-2.27	-3.12	-2.19	-2.33	-2.23				
	V	-1.55	-1.05	-2.83	-3.03	-1.92	-2.30	-1.66	-2.49	-2.01	-1.91	-2.18	-1.01	-1.62	-0.96	-0.92	-1.82	-1.89	-1.68	-2.43	-2.18				
E	I	1.29	1.29	-1.05	0.05	0.80	0.08	1.42	0.11	1.26	0.13	0.22	1.18	1.43	2.23	1.80	1.49	1.14	0.58	-1.25	-0.43				
	Q	-0.36	-0.23	-2.77	-2.22	-2.21	-1.71	-0.74	-1.45	-0.51	-1.49	-1.24	-0.56	-0.87	0.11	0.16	-0.86	-1.10	-2.15	-1.61	-1.86				
	V	1.60	1.37	-1.36	-0.22	-0.58	-0.12	1.54	0.10	1.33	-0.14	0.66	1.83	1.09	2.34	2.01	1.59	1.05	0.70	-1.48	-0.17				
N	I	0.09	0.44	-1.78	-0.83	0.85	-1.05	0.52	-1.00	-0.72	-0.37	-0.32	0.70	0.75	-0.62	-1.00	-0.72	-0.36	-0.81	-0.31	-0.31				
	Q	-1.46	-1.56	-2.39	-2.55	-1.18	-1.87	-0.88	-1.37	-1.86	-1.75	-1.62	-1.10	-1.17	-1.33	-1.08	-1.74	-1.54	-1.85	-1.64	-1.71				
	V	-0.89	-0.56	-2.44	-2.02	-1.44	-1.67	-0.21	-1.64	-1.24	-1.21	-1.49	0.09	-0.63	-0.08	-1.11	-0.57	-1.29	-1.19	-1.33	-1.03				
P	I	0.94	1.72	-0.33	1.14	0.55	-0.29	1.54	0.77	0.70	0.67	0.90	1.88	1.79	2.25	0.10	1.37	1.45	0.92	-0.49	0.90				
	Q	-0.08	0.41	-2.67	-1.45	-0.74	-1.11	-0.03	-0.51	-0.01	-0.69	-0.51	0.05	-0.78	0.94	0.39	0.15	0.01	-0.81	-1.14	-0.78				
	V	0.97	1.39	-0.28	0.64	0.25	-0.31	1.38	0.53	0.42	0.61	0.92	1.89	1.21	2.24	0.20	1.19	1.46	0.79	-0.64	0.68				
	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y					
146	153	155	147	149	150	154																			
F	H	F	A	E	I	-4.35	-2.66	-3.14	-3.27	-3.23	-3.52	-2.94	-3.46	-3.14	-2.89	-3.10	-3.15	-1.96	-3.88	-2.82	-3.64	-3.44	-3.53	-2.45	-3.27
	N	Q	-1.15	-2.65	-2.37	-3.73	-2.17	-2.03	-2.80	-2.30	-1.76	-2.43	-3.04	-2.49	-2.36	-3.81	-2.40	-2.52	-3.37	-2.54	-2.04	-1.94	-2.04	-1.94	
	P	I	-1.97	-2.52	-2.42	-2.44	-1.67	-2.13	-2.10	-1.78	-2.20	-1.98	-1.84	-2.06	-0.10	-3.17	-1.81	-1.91	-3.10	-2.49	-1.52	-2.00	-1.52	-2.00	
	S	V	-3.73	-2.55	-3.41	-2.92	-2.24	-2.25	-2.76	-2.42	-2.88	-2.59	-2.62	-2.66	-2.10	-3.03	-2.25	-2.64	-3.08	-2.93	-2.14	-2.07	-2.07	-2.07	
C	E	I	-3.05	-2.63	-3.35	-3.65	-2.16	-2.33	-3.04	-2.50	-3.03	-2.44	-2.64	-3.07	-1.73	-2.70	-2.63	-3.36	-3.16	-2.85	-2.72	-2.15	-2.15	-2.15	
	N	Q	-1.93	-1.82	-3.14	-3.53	-2.19	-2.06	-2.43	-2.45	-2.63	-2.18	-2.73	-2.48	-2.67	-2.50	-2.18	-2.37	-3.01	-2.65	-1.73	-2.01	-1.73	-2.01	
	P	I	-1.71	-1.70	-2.53	-2.15	-1.66	-1.81	-2.08	-1.33	-2.10	-2.09	-1.37	-2.21	-0.51	-1.55	-1.34	-2.01	-1.86	-1.50	-1.26	-1.73	-1.26	-1.73	
	S	V	-2.83	-2.30	-3.18	-3.11	-1.91	-2.70	-2.30	-2.14	-2.14	-2.58	-2.42	-2.75	-2.56	-2.88	-2.10	-3.23	-2.66	-2.38	-2.09	-1.68	-1.68	-1.68	
D	E	I	-2.71	-3.10	-2.40	-2.76	-1.57	-2.49	-3.01	-1.43	-3.15	-2.04	-3.02	-3.36	-1.42	-3.42	-2.82	-3.31	-3.03	-2.76	-1.31	-1.86	-1.31	-1.86	
	N	Q	-2.04	-2.42	-2.84	-2.73	-0.98	-2.05	-2.20	-1.71	-1.84	-1.70	-1.80	-2.31	-2.46	-2.49	-2.48	-2.74	-2.46	-1.96	-0.86	-1.34	-0.86	-1.34	
	P	I	-2.44	-1.70	-2.67	-2.60	-1.88	-2.41	-2.49	-1.80	-1.56	-2.58	-2.38	-2.38	-1.22	-1.88	-0.94	-2.26	-2.11	-2.04	-1.91	-2.27	-1.91	-2.27	
	S	V	-2.08	-2.10	-2.65	-2.78	-1.19	-2.62	-2.62	-1.30	-2.72	-1.45	-2.03	-2.34	-2.43	-3.46	-2.71	-2.79	-2.68	-2.47	-0.83	-2.71	-0.83	-2.71	
E	E	I	-3.27	-2.94	-3.61	-3.83	-2.65	-3.14	-2.98	-3.43	-2.94	-3.58	-3.52	-3.66	-2.05	-3.46	-2.62	-3.07	-3.11	-2.93	-2.37	-2.77	-2.37	-2.77	
	N	Q	-3.01	-3.04	-3.14	-3.42	-2.51	-2.73	-3.53	-2.73	-2.72	-2.96	-2.90	-2.78	-2.55	-3.43	-3.53	-3.21	-3.35	-3.51	-2.09	-2.26	-2.09	-2.26	
	P	I	-1.20	-2.13	-1.84	-1.72	-1.83	-1.99	-1.41	-1.73	-1.43	-2.02	-1.60	-1.17	-0.15	-1.20	-0.75	-1.72	-1.81	-1.52	-1.97	-1.29	-1.29	-1.97	
	S	V	-3.22	-2.97	-3.78	-3.16	-2.57	-3.00	-3.79	-3.47	-2.87	-2.64	-3.21	-2.96	-2.75	-3.80	-3.53	-3.20	-3.10	-2.77	-2.04	-2.62	-2.04	-2.62	
F	E	I	-2.83	-2.67	-3.05	-2.96	-2.14	-3.22	-2.57	-0.85	-1.80	-1.76	-2.37	-2.58	-2.08	-2.84	-1.94	-2.98	-2.57	-1.99	-1.62	-2.53	-1.62	-2.53	
	N	Q	-3.06	-2.30	-3.39	-3.48	-1.34	-2.01	-2.38	-1.52	-2.18	-1.97	-1.70	-2.38	-1.50	-2.60	-2.08	-2.54	-2.14	-2.32	-1.64	-1.57	-1.64	-1.57	
	P	I	-1.59	-1.45	-2.60	-1.47	-1.20	-1.43	-1.80	-1.21	-1.44	-1.39	-1.08	-1.55	-0.54	-0.96	-1.19	-1.73	-0.91	-1.38	-1.22	-1.22	-1.22	-1.22	
	S	V	-2.15	-2.20	-3.26	-3.03	-1.49	-2.14	-1.88	-1.78	-2.01	-1.57													

Appendices

	N	Q	-2.77	-2.58	-3.49	-2.85	-2.24	-2.81	-2.46	-2.77	-2.53	-2.51	-2.78	-3.06	-2.32	-3.64	-2.26	-2.64	-3.16	-2.71	-2.61	-2.45					
	P	I	-1.60	-1.65	-2.36	-2.19	-1.34	-2.00	-1.81	-1.57	-1.54	-1.88	-1.64	-1.74	-0.44	1.60	-0.56	-1.58	-1.76	-1.27	-2.55	-1.42					
	S	V	-3.35	-2.60	-3.46	-3.37	-2.09	-3.16	-2.97	-3.64	-3.04	-2.49	-2.63	-3.76	-2.45	-3.17	-2.10	-3.39	-3.34	-2.67	-2.57	-2.33					
V	E	I	-2.78	-2.55	-2.80	-3.14	-2.94	-3.05	-3.30	-2.50	-2.22	-3.69	-2.67	-3.45	-1.46	-2.76	-2.14	-2.96	-3.43	-3.01	-3.52	-2.66					
	N	Q	-3.74	-2.60	-3.49	-3.31	-2.73	-2.59	-2.50	-2.12	-3.18	-2.36	-3.08	-3.20	-2.75	-3.13	-2.42	-2.73	-2.94	-2.69	-3.08	-2.02					
	P	I	-1.61	-1.83	-2.09	-1.73	-1.50	-1.69	-2.03	-1.41	-1.30	-1.82	-1.88	-1.73	-0.39	-1.36	-1.38	-1.50	-1.77	-1.09	-2.49	-1.70					
	S	V	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96					
W	E	I	-2.43	-2.32	-2.72	-3.72	-1.89	-2.71	-2.15	-1.15	-2.54	-1.39	-2.26	-2.23	-1.24	-2.28	-2.28	-3.24	-2.65	-2.04	-1.66	-1.65					
	N	Q	-2.01	-1.86	-1.87	-2.24	-1.14	-1.93	-1.42	-0.82	-2.04	-1.35	-1.37	-1.69	-0.81	-1.86	-1.25	-2.00	-1.60	-1.96	-0.94	-1.73					
	P	I	-1.04	-0.44	-1.64	-2.05	-0.10	-0.17	-1.17	-0.71	-0.95	-1.04	-0.34	-1.31	-0.79	-0.96	-0.77	-1.65	-1.95	-1.51	-0.45	-1.07					
	S	V	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96					
Y	E	I	-2.57	-2.49	-3.06	-3.36	-1.16	-3.19	-2.29	-1.92	-2.26	-2.04	-2.29	-2.58	-1.46	-2.41	-2.82	-3.71	-2.70	-2.00	-1.97	-2.15					
	N	Q	-2.59	-2.25	-2.54	-3.03	-1.61	-2.35	-1.98	-2.05	-2.11	-1.90	-2.45	-1.98	-2.03	-2.86	-2.10	-2.66	-2.62	-2.34	-1.82	-1.96					
	P	I	-2.79	-1.28	-2.12	-1.50	-1.03	-2.34	-1.67	-0.99	-2.02	-1.37	-1.30	-2.01	-0.86	-1.05	-3.72	-2.03	-1.53	-1.88	-1.26	-1.94					
	S	V	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96	-0.96					
	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	X	Y						
146	153	155	147	149	150	154	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	X	Y
	F	R	F	A	E	I	-2.59	-2.60	-3.12	-3.12	-3.08	-2.50	-2.32	-1.97	-1.22	-1.92	-2.05	-1.81	-0.98	-2.94	-0.96	-2.76	-2.56	-2.51	-2.17	-1.68	
				N	Q		-3.16	-2.48	-2.61	-3.36	-2.09	-2.24	-3.10	-2.37	-2.26	-2.76	-2.29	-2.49	-2.34	-1.86	-3.22	-2.80	-2.81	-1.57	-1.88		
				P	I		-0.29	-0.86	-1.02	-0.81	-1.12	-0.35	-0.45	0.06	-0.37	-0.41	-0.15	0.49	0.99	0.12	0.48	-0.17	-0.51	0.10	-0.81	-0.41	
	C	E	I	-2.14	-2.33	-3.79	-2.53	-1.82	-1.91	-2.31	-1.48	-0.54	-1.59	-1.43	-2.02	-1.66	-1.71	-0.30	-2.89	-1.52	-1.42	-1.93	-1.81				
				N	Q		-2.89	-2.31	-2.54	-2.89	-1.57	-2.41	-2.45	-2.00	-2.30	-2.14	-1.99	-2.42	-2.41	-2.18	-2.11	-2.69	-2.35	-2.36	-2.11	-1.92	
				P	I		0.31	-0.20	-0.78	-0.19	-0.55	-0.47	-0.50	0.37	0.40	0.19	-0.01	0.76	0.32	0.85	0.85	0.07	0.26	0.65	-0.27	-0.25	
	D	E	I	-1.82	-1.74	-2.87	-2.65	-1.36	-2.20	-1.51	-1.88	-0.31	-1.56	-1.53	-2.37	-2.03	-1.67	0.03	-2.27	-2.81	-1.76	-1.47	-1.09				
				N	Q		-2.59	-2.13	-2.22	-3.06	-1.07	-2.14	-2.33	-1.84	-2.14	-1.62	-2.20	-2.34	-1.96	-2.23	-1.99	-2.94	-2.31	-1.90	-1.13	-1.18	
				P	I		-0.80	1.07	-0.87	-0.08	0.94	-0.33	0.41	1.54	2.80	1.44	1.55	1.01	0.58	2.13	2.64	0.70	1.67	1.60	0.19	1.33	
	E	E	I	-2.15	-2.04	-3.31	-2.84	-2.10	-2.23	-1.74	-0.89	0.24	-1.23	-1.79	-1.37	-1.61	-1.12	0.68	-1.50	-1.83	-1.80	-1.99	-1.60				
				N	Q		-3.05	-2.56	-2.84	-2.91	-2.69	-2.63	-3.12	-2.70	-2.73	-2.99	-3.21	-2.71	-2.50	-3.00	-2.13	-2.91	-3.75	-2.98	-3.01	-2.39	
				P	I		1.24	1.03	0.04	0.28	0.44	0.57	1.22	1.25	2.52	1.22	0.93	1.84	1.21	1.84	2.71	1.33	1.40	1.76	0.05	1.27	
	F	E	I	-2.58	-1.57	-2.35	-2.06	-1.42	-2.76	-1.76	-0.67	-0.42	-0.74	-1.46	-1.28	-1.18	-0.74	-0.36	-0.87	-1.17	-1.20	-1.26	-1.65				
				N	Q		-1.54	-1.84	-2.89	-2.52	-1.49	-2.72	-1.87	-1.08	-1.83	-1.72	-2.30	-2.51	-2.00	-2.52	-1.76	-2.81	-2.05	-1.69	-1.34	-2.17	
				P	I		-1.18	-0.40	-1.38	-0.05	-1.29	-2.63	-0.64	-0.06	-1.40	-0.16	-0.78	0.14	-0.05	0.85	-0.87	-0.32	0.29	0.13	-0.60	-0.65	
	G	E	I	-2.32	-2.12	-2.61	-3.63	-2.26	-3.09	-3.12	-1.44	-1.91	-2.07	-2.85	-2.27	-2.55	-2.62	-1.74	-2.66	-2.00	-2.08	-2.04	-2.07				
				N	Q		-1.98	-1.51	-2.03	-3.03	-1.49	-2.29	-2.16	-2.06	-2.11	-1.69	-1.97	-2.76	-2.19	-2.37	-1.76	-2.62	-2.76	-1.97	-1.51	-1.25	
				P	I		-0.50	-0.91	-1.13	-0.63	-0.80	-1.10	-1.03	-0.51	-1.05	-0.76	-0.53	-0.31	-0.09	-0.48	-0.78	-0.94	-0.97	-0.39	-1.17	-0.48	
	H	E	I	-1.84	-1.81	-2.83	-2.93	-2.23	-2.46	-2.48	-1.04	-0.98	-1.60	-1.60	-2.09	-1.02	-1.24	-0.50	-2.38	-2.23	-1.33	-2.33	-1.91				
				N	Q		-2.40	-2.54	-3.65	-3.21	-1.76	-2.55	-2.33	-1.69	-2.77	-1.67	-2.51	-2.67	-2.01	-2.90	-2.10	-2.77	-2.35	-1.74	-2.23	-2.03	
				P	I		-1.30	-0.44	-1.32	-0.06	-2.07	-1.70	-0.80	0.33	-0.83	0.19	-0.74	-0.43	0.70	0.39	-0.40	-1.20	-0.65	0.56	-1.13	-1.15	
	I	E	I	-1.50	-1.53	-2.82	-2.89	-1.90	-2.48	-1.29	-1.02	0.14	-1.72	-1.40	-1.66	-1.49	-0.83	-0.12	-1.82	-0.89	-1.23	-2.28	-1.24				
				N	Q		-2.66	-2.35	-3.14	-3.73	-1.97	-3.34	-2.09	-1.81	-2.44	-1.66	-2.50	-2.30	-2.54	-3.10	-1.96	-2.77	-2.88	-2.00	-1.85	-1.72	
				P	I		-0.31	-0.22	-1.05	-0.20	-0.69	-0.57	0.44	0.46	0.58	0.22	-0.01	0.21	-0.36	0.73	0.95	0.09	0.06	0.43	-0.72	0.06	
	K	E	I	-3.11	-3.83	-2.99	-3.11	-3.10	-2.91	-3.41	-3.30	-2.59	-2.83	-2.85	-1.58	-3.08	-3.33	-2.10	-2.90	-3.42	-2.48	-3.03					
				N	Q		-2.40	-2.84	-3.18	-3.11	-2.59	-2.89	-2.53	-2.34	-3.86	-2.51	-2.84	-2.85	-2.22	-2.20	-3.31	-2.81	-2.86	-2.53	-2.33	-2.43	
				P	I		-0.94	-2.77	-1.46	-1.34	-1.54	-0.29	-1.54	-2.78	-1.82	-1.87	-2.17	-0.51	-0.03	-1.23	-1.21	-1.08	-2.00	-2.00	-2.54	-1.94	
	L	E	I	-2.17	-1.85	-2.91	-2.38	-1.30	-2.74	-1.68	-0.49	-0.28	-1.41	-2.16	-1.58	-0.89	-1.41	-0.78	-2.16	-1.58	-1.28	-1.55	-1.16				
				N	Q		-2.81	-2.22	-2.85	-2.78	-1.36	-2.39	-2.23	-1.36	-2.80	-2.06	-2.07	-3.02	-2.05	-2.70	-2.32	-3.22	-3.05	-1.98	-1.55	-2.45	
				P	I		0.15	-0.17	-1.46	-0.15	0.10	-0.56	0.28	0.18	0.38	0.29	0.17	0.49	0.68	0.55	0.55	0.34	-0.15	-0.01	-0.22	0.49	
	M	E	I	-2.22	-2.45	-4.07	-3.00	-2.39	-3.21	-2.21	-1.36	-1.33	-2.06	-1.94	-1.98	-1.37	-1.99	-1.97	-2.18	-2.24	-1.84	-2.55	-2.49				
				N	Q		-2.29	-2.92	-2.91	-3.81	-1.74	-2.42	-2.74	-1.96	-2.74	-2.34	-2.20	-2.46	-2.93	-2.87	-2.28	-2.32	-3.02	-2.80	-2.11	-2.21	
				P	I		0.84	-0.37	-1.34	-0.30	-0.80	-0.26	0.12	-0.13	0.28	-0.56	-0.16	0.26	0.42	0.41	0.23	0.79	-0.38	-0.13	-1.18	-0.42	
	N	E	I	-2.48	-2.14	-3.51	-3.07	-1.82	-2.86	-2.49	-1.44	-1.02	-2.01	-2.26	-2.98	-1.17	-2.57	-0.84	-2.83	-2.63	-1.91	-2.13	-1.92				
				N	Q		-2.71	-3.02	-2.66	-2.54	-1.70	-2.56	-2.08	-2.55	-2.48	-2.09	-2.26	-2.81	-2.15	-2.90	-2.26	-2.19	-2.50	-1.68	-1.89	-1.60	
				P	I		0.52	0.22	-1.20	0.91	0.48	-0.96	0.22	0.94	0.57	0.82	0.54	-0.01	1.61	0.91	1.39	0.17	0.46	1.21	0.54	1.45	
	Q	E	I	-0.03	1.06	-1.57	0.11	0.72	-1.83	3.00	2.02	1.69	1.57	1.27	0.82	0.90	0.65	1.68	0.24	0.71	1.71	0.17	1.11				
				N	Q		-2.28	-1.76	-2.81	-3.02	-1.65	-2.10	-2.19	-0.53	-1.02	-1.22	-1.37	-2.40	-1.70	-1.88	-1.34	-2.29	-1.99	-1.11	-2.28	-1.58	
				P	I		2.56	2.73	1.95	2.88	2.62	2.01	2.26	3.23	2.68	2.96	3.12	3.03	2.88	2.95	2.60	2.81	2.88	3.42	2.15	2.74	
	R	E	I	-0.48	-1.88	-2.28	-2.61	-0.83	-0.74	-1.69	-1.42	-0.48	-0.76	-0.98	-1.92	1.74	-1.17	-0.88	-1.33	-2.09	-1.36	-0.89	-0.81				
				N	Q		-2.24	-2.35	-2.67	-3.29	-1.69	-2.19	-2.36	-2.28	-2.41	-1.79	-2.38	-2.12	-0.83	-2.32	-3.01	-2.47	-2.13	-2.24	-1.41	-2.15	
				P	I		2.53	0.17	-0.23	0.15	0.80	1.51	-0.12	0.52	1.28	1.24	0.53	0.89	2.96	0.98	0.37	0.76	-0.69	0.65	0.19	0.72	
	S	E	I	-2.93	-2.75	-3.03	-3.00	-2.55	-2.88	-2.45	-																

Appendices

		P	I	A	D	E	F	G	H	I	J	K	L	M	N	P	Q	R	S	T	V	W	X
L	E	0.73	0.33	-1.01	-0.02	0.34	1.02	-0.02	-1.77	-1.11	-1.15	-0.99	-0.19	0.82	0.00	-1.23	1.07	0.06	1.37	-0.27	0.21		
	N	0.85	-0.56	-2.41	-0.33	0.12	-0.06	0.18	0.40	1.21	-0.19	0.67	-0.67	-0.95	1.40	1.75	1.82	1.24	0.22	-0.06	0.74		
	P	-1.78	2.56	-2.22	-0.68	1.22	-1.73	-1.14	1.80	1.09	-1.64	2.01	-1.54	-1.63	0.02	-1.53	-1.48	1.12	-1.02	-0.89			
M	E	1.57	1.54	-0.77	0.88	1.46	1.85	1.14	0.81	1.11	0.53	1.13	0.77	1.24	1.83	1.95	2.06	2.75	1.06	0.86	1.51		
	N	1.17	0.47	-2.33	-0.95	0.00	0.12	0.09	-0.25	0.40	-0.68	0.14	-0.55	-0.89	1.28	1.43	1.54	1.94	0.13	-0.23	0.30		
	P	-1.58	2.38	2.70	-2.96	-0.71	-2.20	1.52	-1.17	-1.84	2.34	-1.06	-1.82	-1.78	1.77	1.23	-1.25	-1.47	1.80	-0.82	-1.24		
N	E	2.77	1.54	-0.84	0.93	1.46	2.00	1.71	0.58	0.97	0.29	1.21	1.55	1.44	1.92	1.41	2.59	2.13	0.78	1.01	1.74		
	N	-0.38	-0.92	-0.71	-1.27	-0.14	-1.17	-0.58	-0.89	-0.89	-0.67	-1.91	-1.52	-0.57	-0.17	0.74	-0.33	-1.20	-0.47	-0.08			
	P	-2.07	-1.87	-0.72	-2.36	-0.97	-1.43	-1.16	-1.50	-1.85	-1.12	-1.58	-2.46	-1.92	-2.04	-1.41	-1.87	-2.09	-2.01	-0.57	-1.24		
Q	E	1.93	1.19	-0.77	1.10	2.09	0.75	1.51	0.24	0.38	0.66	1.55	0.76	1.04	1.15	1.27	1.88	1.93	0.19	1.64	2.09		
	N	3.24	3.12	-1.10	2.79	2.15	1.70	2.26	3.14	3.33	2.56	3.24	1.38	-0.37	3.56	3.53	3.31	3.50	3.13	1.72	2.53		
	P	0.28	-0.22	-2.53	0.30	-0.66	-1.47	-0.80	0.19	0.36	-0.15	0.47	-1.49	-1.67	0.44	0.90	0.90	1.29	0.42	-0.59	-0.17		
R	E	3.40	3.54	2.26	3.52	3.40	3.36	3.36	3.48	3.59	3.34	3.61	3.01	2.71	3.50	3.51	3.70	3.63	3.52	2.94	3.36		
	N	1.88	0.83	-1.60	0.21	0.60	1.12	0.40	-0.15	0.62	0.31	0.49	-0.30	1.75	0.99	-0.07	2.20	1.71	0.34	0.33	1.15		
	P	-1.04	-2.11	-2.54	-2.40	-1.00	-1.05	-1.35	-1.65	-1.23	-1.48	-1.43	-1.64	0.51	-1.87	-1.35	-1.19	-1.62	-1.63	-0.42	-1.01		
S	E	2.81	1.89	1.89	1.46	2.56	2.85	2.87	0.74	1.80	2.25	1.74	1.57	3.23	1.72	3.28	2.78	2.30	0.86	2.02	2.64		
	N	0.01	0.26	-2.72	-1.02	-0.02	-0.74	-0.11	-1.02	-0.04	-1.06	-0.25	-1.11	-1.54	0.10	0.59	0.84	0.66	-0.98	-0.33	0.44		
	P	-1.56	-2.05	-2.87	-2.73	-0.90	-2.00	-1.64	-1.54	-1.76	-1.65	-1.58	-1.89	-1.92	-2.04	-1.32	-1.86	-2.02	-2.46	0.02	-0.87		
T	E	2.02	1.44	-0.91	0.50	1.82	1.04	1.78	-0.03	0.91	0.09	0.84	1.17	0.53	1.28	1.56	2.32	1.78	0.57	1.59	2.39		
	N	1.89	1.29	-3.94	-0.13	0.55	0.62	1.25	-0.01	1.64	-0.71	0.07	-1.24	-2.11	1.94	2.75	2.52	3.31	0.08	1.0			
	P	-1.58	-1.88	-2.68	-2.34	-1.09	-2.20	-1.81	-1.82	-1.76	-2.03	-1.49	-1.52	-2.21	-1.48	-0.82	-1.01	-0.94	-2.06	-1.49	-1.05		
V	E	2.73	2.71	-0.39	1.95	2.12	2.32	2.73	0.92	2.12	0.72	2.18	2.37	1.01	2.60	2.56	3.08	2.98	1.88	2.73	2.85		
	N	2.48	1.74	-2.54	1.03	0.90	1.76	1.38	0.49	2.10	-0.03	1.61	0.41	-0.87	2.50	2.42	2.97	2.53	0.61	-1.12	1.83		
	P	-0.80	-1.52	-3.53	-2.19	-1.09	-1.71	-1.76	-1.57	-1.35	-1.77	-2.19	-2.39	-1.11	-0.67	-0.57	-1.26	-1.54	-1.41	-1.23			
W	E	3.25	2.47	-0.39	2.40	2.75	2.95	2.24	1.12	2.28	1.12	2.24	2.28	1.09	2.70	2.75	3.18	2.68	1.50	1.61	2.54		
	N	0.70	0.37	-1.63	-0.89	0.69	-0.04	1.22	0.75	0.81	0.04	0.63	0.17	-0.71	0.95	1.57	1.53	1.51	0.49	0.37	0.61		
	P	-1.09	-0.92	-2.19	-1.66	-0.22	-1.02	-0.44	0.02	-0.69	-0.10	-0.81	-0.76	-0.93	-1.18	-0.17	-0.47	-0.73	-0.42	-0.21	-0.25		
Y	E	1.68	1.84	0.81	0.07	1.66	0.88	2.05	1.11	1.28	1.03	1.59	1.55	0.72	2.03	1.92	2.29	1.93	1.10	0.86	1.76		
	N	1.29	0.78	-1.83	-0.72	0.37	-0.12	1.58	0.19	0.83	-0.51	0.52	0.30	-1.28	1.28	1.62	2.07	1.78	0.21	-1.18	0.81		
	P	-1.81	-1.55	-2.23	-2.16	-0.68	-1.64	-1.35	-0.98	-1.39	-1.21	-1.38	-1.46	-1.69	-1.91	-0.97	-1.58	-1.72	-1.80	-1.36	-1.04		
	I	1.33	1.58	-0.96	0.48	1.26	0.96	2.02	0.42	0.60	0.08	0.64	1.49	0.46	1.70	0.84	2.31	2.26	0.76	0.71	1.57		

[illegible]

Appendices

	Q	-2.39	-2.07	-1.94	-2.63	-1.54	-2.16	-2.35	-1.83	-1.14	-2.03	-1.91	-2.40	-2.13	-1.83	-1.25	-1.56	-2.05	-2.09	-1.70	-1.47
	V	-0.60	0.22	-2.57	-2.62	-0.89	-1.21	-1.48	-0.36	0.26	-0.65	-0.10	-2.04	-1.00	-0.08	0.13	-0.70	-0.34	-0.80	-1.56	-1.29
	I	3.57	3.90	1.78	2.60	3.17	3.28	2.89	3.81	3.59	3.01	3.33	2.79	3.74	3.58	3.39	3.27	3.46	3.56	1.54	2.93
	P	0.83	2.80	-2.08	-0.40	-0.13	-0.18	-0.26	1.75	2.19	0.74	1.86	-0.53	-1.03	1.70	2.43	1.02	1.41	1.25	-1.21	-0.19
	V	3.32	3.98	1.15	3.04	2.95	3.26	2.85	3.73	3.82	3.14	3.96	2.84	3.34	3.80	3.60	3.39	3.66	3.56	1.42	3.00
	H	2.17	1.53	-2.89	-0.17	-0.57	2.14	0.11	-0.26	2.00	0.00	0.81	-0.87	3.18	1.04	1.82	1.72	0.24	0.43	-1.73	-0.05
	D	-1.25	-1.69	-2.89	-2.40	-2.92	-1.29	-2.52	-2.66	-1.02	-2.63	-1.90	-3.03	-0.67	-2.00	-1.25	-1.97	-2.73	-2.18	-2.39	-2.57
	I	1.10	0.38	-2.70	-0.98	-1.48	1.22	-1.28	-1.54	0.91	-0.71	-0.45	-1.73	2.36	-0.28	0.45	0.29	-0.97	-1.05	-2.81	-1.08
	E	3.24	2.97	-1.38	1.80	0.88	3.51	1.68	1.06	2.99	1.63	2.37	1.01	4.00	2.66	2.72	3.13	1.72	1.69	-0.98	1.35
	V	-0.29	-0.90	-3.84	-1.77	-1.90	-0.06	-1.68	-2.31	-0.62	-2.17	-1.32	-2.06	-0.02	-1.26	-0.96	-0.84	-2.20	-1.95	-2.55	-2.45
	Q	3.08	2.22	-1.53	0.99	0.22	3.18	1.19	0.55	2.92	0.66	1.44	0.85	3.54	1.71	2.39	2.93	1.12	0.99	-1.47	0.73
	N	1.66	1.16	-2.71	-0.36	-0.42	1.76	0.23	-0.57	1.29	0.12	0.39	-0.55	3.64	0.66	1.44	1.10	-0.28	0.20	-1.62	-0.21
	I	-1.24	-1.49	-2.74	-2.84	-1.88	-1.21	-2.14	-2.35	-1.43	-1.80	-1.87	-2.38	-0.27	-1.73	-1.49	-1.60	-2.08	-1.89	-2.06	-1.91
	V	0.61	0.07	-3.07	-1.24	-1.27	0.42	-1.43	-1.31	0.09	-1.06	-0.73	-1.40	2.22	-0.61	0.14	0.08	-1.31	-1.26	-1.45	-1.11
	P	3.05	2.71	-0.22	2.40	1.88	3.12	2.16	1.02	2.60	1.19	1.97	1.83	3.84	2.48	2.37	2.77	1.64	1.77	-0.22	2.30
	Q	-0.05	-0.73	-3.16	-1.21	-1.40	0.07	-0.79	-2.23	-0.02	-1.74	-1.14	-1.74	0.46	-1.17	-0.20	-0.38	-1.34	-1.55	-1.82	-0.88
	V	2.96	2.41	-0.68	-1.73	0.94	2.85	1.69	0.16	2.35	0.44	1.40	1.66	3.20	1.78	1.99	2.48	-1.14	0.97	-0.74	2.21
	I	-0.22	-1.39	-2.77	-1.59	-1.81	0.60	-1.27	-1.76	-1.20	-1.72	-1.52	-1.53	1.28	-0.90	-0.56	-0.49	-1.98	-1.48	-2.21	-1.28
	D	-1.23	-2.37	-3.13	-2.15	-2.06	-1.95	-2.22	-2.15	-1.81	-1.82	-2.35	-1.59	-0.15	-1.72	-1.35	-1.49	-2.47	-2.39	-2.55	-2.56
	V	0.76	-0.12	-2.33	-0.18	-1.14	0.51	-0.48	-0.71	0.42	-0.35	-0.36	0.19	2.83	0.51	0.08	0.54	-0.67	-1.79	-0.27	0.73
	E	-1.94	-2.15	-3.09	-2.28	-2.76	-2.55	-2.68	-2.03	-2.04	-2.44	-2.67	-2.09	-1.33	-2.20	-2.08	-2.77	-2.65	-2.86	-2.84	-2.47
	I	0.08	-0.74	-2.24	-1.38	-1.40	0.14	-1.03	-1.14	-0.59	-1.87	-1.34	-0.80	2.03	-0.28	0.11	0.03	-1.28	-1.66	-1.88	-1.09
	N	-1.14	-1.09	-2.47	-1.06	-1.81	0.59	-1.84	-1.36	-1.48	-2.10	-1.72	-1.16	1.31	-1.02	0.96	0.38	-1.75	-1.19	-1.55	-1.50
	V	-2.19	-2.25	-3.68	-3.37	-2.38	-2.57	-2.73	-2.25	-2.61	-2.09	-2.72	-2.92	-1.84	-2.68	-2.42	-2.65	-3.41	-2.25	-1.85	-2.37
	I	-2.11	-2.35	-3.44	-2.81	-2.24	-2.05	-2.23	-2.95	-2.37	-2.12	-2.55	-2.12	-0.34	-1.95	-2.17	-1.60	-2.59	-2.28	-2.01	-2.22
	P	0.27	-0.83	-0.65	-0.63	-0.83	0.22	-0.48	-1.03	0.38	-1.19	-0.71	0.55	1.84	-0.15	0.08	-0.08	-1.06	-1.26	-1.88	-0.48
	V	-2.06	-2.23	-3.68	-2.78	-1.61	-2.14	-2.78	-1.72	-2.27	-2.62	-1.79	-1.72	-1.17	-2.37	-1.71	-1.75	-2.40	-1.84	-1.65	-2.23
	Q	-0.39	-0.98	-1.77	-1.00	-1.35	-0.52	-1.07	-1.69	-1.08	-1.65	-1.13	-1.07	1.18	-0.74	-0.54	-0.33	-1.38	-1.47	-1.35	-0.57
	K	0.75	-1.67	-2.39	-3.02	-3.10	0.73	-2.12	-2.93	-2.45	-3.07	-2.25	-2.13	1.21	-1.27	-2.49	-0.29	-3.33	-2.36	-2.52	-2.64
	D	-2.36	-2.75	-3.72	-2.81	-2.98	-1.83	-2.36	-3.05	-3.26	-3.32	-3.31	-3.71	-1.92	-2.90	-3.04	-2.18	-2.94	-3.18	-2.73	-3.53
	I	-0.22	-1.81	-2.97	-3.02	-3.00	0.06	-2.22	-2.64	-2.52	-3.12	-2.82	-2.43	-0.15	-1.69	-2.89	-1.03	-2.47	-3.26	-3.06	-2.99
	V	-1.51	-0.17	-1.37	-0.28	-2.34	2.69	-1.24	-2.19	1.08	2.68	-1.66	1.06	2.93	0.11	-1.50	1.57	-1.88	-2.51	-1.79	-0.27
	E	-1.51	-2.66	-3.04	-3.70	-3.39	-1.08	-2.97	-3.40	-3.50	-2.84	-2.50	-2.53	-1.28	-2.76	-2.91	-1.73	-3.48	-3.73	-3.54	-3.21
	I	2.13	-0.02	-1.76	-1.42	-2.61	2.32	-2.01	-2.44	-1.41	-2.68	-1.98	-1.14	2.02	-0.06	-1.76	1.43	-1.63	-2.30	-2.24	-2.58
	N	0.12	-1.31	-2.22	-2.30	-2.69	0.32	-1.74	-2.52	-2.40	-2.66	-1.96	-1.76	1.74	-0.96	-2.72	-0.44	-1.19	-2.43	-1.98	-2.05
	V	-2.19	-2.45	-3.37	-3.11	-2.73	-2.02	-2.55	-2.30	-2.91	-2.78	-2.81	-2.63	-1.33	-2.57	-3.42	-2.53	-3.16	-2.58	-2.46	-2.29
	I	-1.02	-2.21	-3.13	-3.18	-2.65	-0.75	-2.90	-2.36	-2.45	-3.36	-3.34	-2.45	0.20	-2.17	-2.81	-1.29	-3.12	-2.44	-2.21	-2.39
	P	2.04	-0.51	-0.97	-0.97	-1.46	2.08	-1.18	-2.31	-1.55	-2.68	-1.51	0.89	2.58	-0.04	-2.04	1.49	-1.47	-2.44	-1.51	-1.78
	V	-1.50	-2.36	-3.48	-2.81	-3.05	-1.32	-2.93	-2.78	-3.03	-2.69	-2.84	-2.49	-0.97	-2.31	-2.49	-1.16	-2.05	-3.66	-2.05	-2.56
	Q	0.79	-0.72	-1.15	-1.33	-2.30	1.69	-1.43	-2.04	-1.68	-2.49	-2.20	-1.23	2.06	-0.60	-1.78	1.07	-1.61	-2.10	-1.95	-0.98
	L	0.69	-0.48	-2.36	-0.63	-1.99	1.14	-1.16	-1.45	-0.85	-1.79	-1.33	-1.24	1.62	-0.36	-0.59	0.33	-1.67	-1.45	-1.79	-1.16
	D	-1.92	-2.50	-3.34	-3.23	-2.98	-1.58	-2.62	-2.31	-2.43	-3.11	-3.11	-2.40	-2.15	-2.75	-2.47	-2.00	-3.51	-3.31	-2.89	-3.04
	I	-0.47	-1.03	-3.19	-1.56	-2.47	-0.04	-1.61	-2.12	-1.99	-2.27	-2.20	-1.84	-0.40	-1.20	-1.59	-0.56	-3.21	-2.36	-2.12	-2.05
	V	2.02	1.19	-1.50	1.48	-0.64	2.55	0.73	-0.59	0.83	-0.92	-0.29	0.58	2.96	1.29	0.57	1.80	-1.30	-0.39	-0.91	0.40
	E	-1.39	-1.81	-3.15	-2.34	-2.15	-1.36	-2.14	-2.09	-1.93	-2.56	-2.20	-2.52	-0.93	-1.61	-1.68	-1.51	-2.96	-2.54	-2.19	-2.05
	I	1.91	0.44	-2.24	0.49	-1.26	2.28	0.04	-1.59	0.27	-1.39	-0.92	0.08	2.39	0.56	0.11	1.64	-1.71	-1.19	-1.90	-2.23
	N	-0.36	-1.09	-2.94	-1.21	-1.50	-0.12	-1.65	-1.50	-1.28	-1.79	-1.89	-1.17	1.46	-1.38	-1.10	-0.32	-2.93	-1.53	-1.66	-1.33
	V	-2.31	-2.22	-2.76	-3.48	-1.95	-1.95	-2.48	-2.15	-2.20	-2.15	-2.83	-2.11	-1.62	-2.48	-2.45	-2.17	-2.95	-2.62	-1.99	-2.36
	I	-1.60	-2.06	-3.11	-1.30	-2.17	-1.11	-2.15	-1.70	-2.00	-2.40	-1.67	-1.61	-0.29	-2.31	-1.90	-1.47	-2.27	-2.82	-2.39	-2.16
	P	1.31	0.66	-1.15	0.78	-0.21	1.86	0.26	-0.61	0.11	-0.61	0.19	0.74	2.02	0.75	0.41	1.51	-1.02	-0.87	-0.73	0.26
	Q	-1.51	-1.76	-3.37	-2.54	-1.44	-1.11	-2.01	-1.63	-2.44	-1.81	-2.53	-2.20	-1.51	-2.20	-1.40	-1.88	-3.01	-2.28	-1.30	-1.29
	V	0.86	0.24	-1.67	-0.14	-0.81	1.38	-0.30	-1.08	-0.54	-1.10	-0.85	-0.13	1.29	0.29	0.18	0.82	-1.39	-1.27	-0.83	-0.49
	M	0.96	-1.13	-2.82	-1.70	-2.96	1.34	-1.62	-1.97	-0.74	-2.28	-1.51	-1.46	2.71	-1.10	-0.89	0.12	-2.06	-2.40	-2.40	-1.89
	D	-1.96	-2.85	-3.54	-3.30	-2.76	-1.59	-3.46	-2.71	-2.59	-2.86	-2.85	-3.17	-1.44	-2.53	-2.31	-2.32	-2.77	-3.41	-3.01	-3.22
	I	-0.05	-1.81	-3.31	-2.92	-3.17	-0.01	-2.54	-2.28	-1.64	-3.12	-2.49	-1.96	0.67	-1.67	-1.48	-1.00	-2.12	-2.09	-2.11	-2.59
	V	2.64	0.32	-1.98	-0.09	-1.97	2.81	-0.20	-1.18	0.75	-1.96	-0.96	0.12	3.59	0.49	0.02	1.61	-1.21	-1.10	-1.97	-0.88
	E	-1.40	-2.46	-4.22	-2.79	-3.29	-1.00	-2.55	-2.60	-2.32	-3.57	-2.18	-2.25	-0.39	-2.88	-1.50	-1.60	-2.84	-2.46	-2.55	-2.39
	I	-2.23	-0.99	-2.30	-0.89	-2.24	2.54	-0.75	-1.69	0.07	-2.07	-1.51	0.73	2.75	0.02	0.17	1.99	-1.56	-1.47	-1.57	-1.36
	N	0.32	-1.57	-2.58	-2.08	-2.52	1.72	-1.61	-1.68	-1.58	-1.87	-1.68	-1.52	2.52	-1.38	-1.02	-0.62	-1.98	-1.55	-1.88	-1.68
	V	-2.24	-2.29	-3.57	-2.80	-2.34	1.58	-2.62	-1.99	-2.87	-2.38	-2.53	-2.58	-1.21	-2.72	-1.93	-2.55	-3.02	-2.03	-2.34	-2.49
	I	-1.07	-1.95	-2.78	-2.26	-2.51	0.52	-2.03	-1.77	-2.05	-1.95	-2.09	-2.22	0.93	-1.95	-2.93	-1.44	-2.40	-2.82	-2.55	-2.38
	P	-2.51	-0.17	-1.76	0.10	-1.36	2.70	-0.43	-1.72	0.06	-1.79	-0.41	-0.79	3.35	0.24	-0.05	1.53	-1.31	-1.13	-1.26	-0.52
	V	-0.87	-2.10	-3.62	-2.82	-2.44	0.51	-2.09	-1.92	-2.16	-2.34	-1.65	-0.40	-1.64	-1.45	-1.81	-2.74	-1.11	-2.11	-1.99	-1.99
	Q	1.72	-0.82	-1.61	-0.48	-1.48	2.20	-0.69	-1.27												

Appendices

E	I	3.56	3.31	0.28	1.69	1.33	3.39	2.47	2.19	3.23	0.85	2.32	2.11	3.54	2.86	3.25	3.56	2.83	2.73	0.26	2.46
	Q	0.45	-0.53	-2.96	-2.08	-2.11	-0.48	-1.34	-1.25	-0.34	-2.21	-1.40	-1.57	-0.96	-0.81	0.71	0.07	-0.35	-1.49	-2.54	-1.69
	V	3.60	3.26	0.18	1.22	0.64	3.22	2.19	1.27	3.08	0.30	2.43	2.26	2.89	2.77	3.53	3.58	2.73	2.19	-0.34	2.03
	I	1.82	0.63	-1.35	-0.85	-0.86	1.15	0.20	-0.13	0.85	-0.86	0.17	0.16	2.52	0.81	1.42	1.66	0.86	0.30	-1.43	0.33
N	I	-1.54	-1.76	-3.08	-2.92	-1.84	-1.47	-2.02	-2.31	-1.55	-2.07	-2.06	-2.24	-1.16	-1.99	-1.02	-1.46	-2.29	-1.79	-2.31	-1.46
	Q	0.52	-0.23	-2.57	-1.82	-1.72	-0.26	-1.25	-1.25	-0.21	-1.83	-0.81	-0.71	0.82	-0.65	0.49	0.53	-0.29	-0.87	-1.74	-0.84
	V	3.12	2.84	1.30	1.92	1.19	2.83	2.35	1.58	2.33	0.49	1.66	2.46	3.29	2.37	2.63	3.35	2.78	2.16	0.60	2.23
	I	0.14	-0.44	-3.32	-2.11	-1.57	0.18	-1.06	-1.87	-0.40	-2.25	-1.41	-1.19	-1.05	-0.92	0.26	0.55	-0.53	-1.14	-1.65	-1.08
P	I	3.23	2.64	0.69	1.12	0.90	2.73	2.42	1.34	2.14	-0.37	1.68	1.92	3.04	2.24	2.78	3.33	2.72	1.86	0.28	2.16
	Q	2.39	1.04	-1.32	-0.35	-1.28	1.51	-0.52	-1.07	-0.13	-1.08	-0.37	-0.85	2.10	-0.50	1.44	1.38	-0.35	-0.38	-1.99	-0.02
	V	-1.14	-2.29	-3.70	-3.45	-2.38	-1.44	-2.22	-3.12	-2.20	-3.11	-2.67	-2.94	-1.81	-2.58	-1.73	-1.41	-3.23	-2.97	-2.44	-3.07
	I	1.62	-0.47	-2.29	-1.47	-2.23	0.14	-1.41	-2.20	-0.59	-1.70	-1.40	-1.32	0.63	-1.35	0.31	0.90	-1.33	-1.44	-2.44	-1.17
T	I	3.16	1.77	-0.35	0.50	-0.33	2.35	0.47	-0.13	0.91	-0.16	0.14	0.23	2.99	0.56	1.84	2.27	0.58	0.42	-0.79	0.60
	Q	-0.29	-1.75	-3.52	-3.11	-2.36	-1.02	-1.82	-2.67	-1.64	-3.13	-1.95	-2.22	-1.07	-2.06	-1.20	0.93	-2.43	-2.33	-2.45	-2.34
	V	2.80	1.37	-1.27	-0.30	-0.93	2.39	-0.09	-0.79	0.58	-0.89	-0.15	-0.30	2.16	-0.19	1.77	2.27	-0.04	-0.21	-1.42	0.56
	I	1.19	-0.12	-1.50	-1.16	-1.39	0.38	-1.12	-1.00	-0.99	-1.46	-1.09	-0.96	2.03	-1.07	0.31	0.34	-0.51	-0.76	-1.19	-0.97
N	I	-1.54	-2.29	-2.81	-2.71	-2.21	-2.45	-3.07	-2.47	-2.12	-2.64	-2.50	-2.31	-1.47	-2.96	-1.90	-1.67	-2.78	-2.42	-2.66	-2.18
	Q	0.18	-1.69	-2.59	-2.70	-1.76	-0.93	-1.83	-1.68	-1.67	-2.25	-2.08	-2.15	0.15	-1.87	-0.78	-0.69	-1.41	-1.76	-1.98	-1.10
	V	2.47	1.29	-0.66	0.39	0.33	1.55	0.38	-0.57	0.63	-0.70	-0.13	0.01	2.68	0.22	1.24	2.17	0.23	0.10	-0.07	1.01
	I	-0.48	-1.63	-3.31	-3.27	-2.12	-1.44	-2.22	-2.17	-2.22	-2.72	-2.29	-1.90	-1.71	-2.03	-1.15	-1.20	-2.00	-1.91	-1.97	-1.91
V	I	2.30	0.95	-0.77	-0.29	-0.46	1.42	0.17	-1.19	-0.32	-1.18	-0.32	0.10	1.88	0.03	0.93	1.45	-0.27	-0.44	-0.29	0.30
	Q	0.45	-0.63	-1.87	-1.18	-1.33	0.23	-0.94	-1.26	-0.40	-1.73	-0.96	-0.56	1.63	-0.37	0.23	-0.16	-0.56	-0.79	-2.06	-0.85
	V	-1.73	-2.75	-3.59	-3.02	-2.60	-2.79	-2.88	-2.98	-1.95	-3.05	-2.96	-3.28	-1.33	-2.83	-1.80	-2.83	-0.91	-2.73	-2.14	-3.03
	I	-0.53	-1.45	-2.64	-2.72	-2.55	-0.79	-1.76	-2.46	-1.57	-2.09	-2.08	-1.78	-0.06	-1.63	-0.86	-1.45	-2.43	-2.20	-2.63	-1.38
E	I	1.93	0.54	-1.49	0.26	0.34	1.53	0.30	0.22	1.42	-0.13	0.20	0.58	3.26	0.91	1.67	1.36	0.83	0.42	-1.20	0.48
	Q	-1.76	-2.59	-3.51	-2.80	-2.01	-1.40	-2.89	-2.11	-1.39	-2.46	-2.29	-2.52	-0.78	-1.93	-1.60	-1.43	-2.60	-2.55	-1.99	-2.26
	V	1.64	-0.06	-1.81	-0.61	-0.98	0.88	-0.24	-1.03	0.61	-1.10	-0.34	-0.01	2.23	0.15	1.35	2.21	-0.25	-0.57	-1.70	-0.07
	I	-0.11	-1.18	-2.49	-1.13	1.08	-0.02	-0.92	-1.34	-0.61	-1.32	-1.19	-0.84	1.87	-0.60	0.07	-0.47	-0.58	-1.12	-1.18	-0.92
N	I	-2.28	-2.19	-3.46	-3.53	-2.55	-2.26	-2.66	-2.30	-2.09	-2.42	-1.67	-2.76	-1.48	-2.77	-2.15	-2.59	-2.74	-2.58	-1.99	-1.98
	Q	-1.10	-2.03	-2.85	-2.44	-1.85	-1.19	-1.72	-1.27	-1.71	-2.20	-1.91	-1.83	-0.11	-2.00	-0.69	-1.27	-1.92	-2.21	-2.28	-1.79
	V	1.31	0.01	-1.19	0.16	0.54	1.03	0.22	0.60	-0.28	-0.95	-0.50	0.33	2.40	0.27	0.92	0.68	-0.25	-0.35	-1.28	0.32
	I	-1.44	-1.94	-2.71	-2.41	-1.99	-1.30	-2.08	-2.01	-2.19	-2.37	-2.14	-2.47	-0.94	-1.74	-1.22	-1.71	-2.02	-2.28	-1.70	-2.26
W	I	0.71	-0.46	-0.97	-0.77	-0.96	0.55	-0.62	-1.21	-0.59	-1.75	-0.83	-0.41	1.79	-0.53	0.05	0.22	-1.12	-0.83	-1.13	0.15
	Q	3.12	3.31	-0.38	1.93	2.42	2.54	2.34	2.50	3.22	2.43	2.78	2.81	2.91	2.88	3.15	3.28	2.74	2.43	0.62	2.24
	V	0.43	0.91	-2.31	-1.11	-0.51	-0.45	-0.54	-0.32	1.22	-0.43	0.22	-0.25	-0.67	0.25	1.15	0.76	0.11	-0.02	-1.24	-0.65
	I	2.61	2.78	-1.56	0.78	0.95	1.66	1.38	1.53	2.92	1.18	2.00	1.91	1.39	2.68	2.89	2.69	1.94	1.86	-0.51	1.39
E	I	3.77	3.85	0.35	2.97	3.03	3.64	3.45	3.17	3.58	2.93	3.33	3.41	3.82	3.54	3.43	3.85	3.05	3.33	1.28	3.24
	Q	0.84	1.69	-3.00	-0.54	-0.59	0.17	-0.07	-0.20	2.40	-0.32	0.66	0.10	0.10	0.92	1.69	1.47	-0.33	0.22	-1.06	0.08
	V	3.39	3.64	-0.30	2.37	2.44	3.22	3.10	2.66	3.74	2.17	3.14	3.33	3.06	3.44	3.36	3.77	2.75	2.83	0.36	2.85
	I	2.29	2.95	-1.37	1.39	1.60	1.80	1.65	1.21	2.61	0.91	2.03	2.27	2.88	2.02	2.19	2.60	-0.07	1.68	0.02	1.83
N	I	-0.40	0.04	-2.44	-1.48	-0.53	-0.95	-0.77	-0.19	0.09	-0.86	-0.30	-0.46	-0.53	-0.49	-0.18	-0.37	-1.71	-0.73	-0.95	-0.59
	Q	1.25	1.84	-2.14	-0.50	0.25	0.60	0.55	0.14	1.78	0.09	1.35	1.20	1.34	0.71	1.19	1.46	-0.87	-0.40	-0.61	0.74
	V	3.12	3.58	0.49	2.47	2.39	3.30	2.66	1.76	2.94	1.82	2.54	2.99	3.28	3.01	2.78	3.29	2.22	2.24	1.04	2.72
	I	1.01	1.49	-2.04	-0.51	0.33	0.35	0.30	0.08	1.29	-0.57	0.49	0.25	0.15	0.80	0.93	1.40	-0.37	-0.18	-0.78	0.12
P	I	3.13	3.40	0.33	2.23	2.23	2.91	2.57	1.69	2.74	1.30	2.70	3.05	3.23	2.98	2.67	3.31	2.12	2.22	0.48	2.82
	Q	3.37	3.33	0.05	2.65	2.23	3.19	2.51	1.94	3.20	2.47	2.46	2.78	3.86	3.18	3.31	3.19	2.69	2.92	0.37	2.30
	V	0.50	0.67	-2.33	-1.15	-0.97	0.07	-0.71	-1.03	0.82	-0.74	-0.69	-0.59	1.02	0.27	1.34	0.38	-0.51	-0.55	-1.26	-0.74
	I	2.59	2.49	-1.39	1.18	0.78	2.56	1.20	0.75	2.68	1.60	1.53	1.74	3.09	2.41	3.21	2.66	2.06	1.75	-0.45	1.43
Y	I	3.59	3.73	1.04	3.55	3.38	3.64	3.61	3.43	3.90	3.29	3.62	3.74	3.86	3.60	3.70	3.72	3.83	3.66	1.78	3.25
	Q	1.67	2.11	-2.62	-0.25	-0.40	1.38	-0.13	0.08	1.64	-0.08	0.64	-0.50	1.97	1.49	2.19	0.98	0.37	0.67	-1.14	0.02
	V	3.67	3.74	0.69	3.40	2.76	3.31	3.12	2.89	3.48	3.18	3.32	3.26	3.69	3.53	3.84	3.70	3.10	3.58	-1.44	2.99
	I	2.66	2.86	-1.40	1.90	1.22	2.74	1.66	0.72	1.97	1.66	2.14	1.69	3.87	2.24	1.83	2.45	0.43	1.85	-0.52	1.72
D	I	-0.29	-0.04	-2.54	-1.24	-1.34	-0.15	-1.28	-1.24	-0.55	-0.98	-0.54	-0.98	1.31	-0.97	-0.37	-0.55	-1.80	-1.00	-1.08	-1.02
	Q	1.57	1.86	-2.47	0.14	0.02	1.70	0.21	0.30	0.94	0.46	0.83	0.97	2.95	0.95	0.82	1.35	-0.44	-0.04	-1.12	0.29
	V	2.86	3.50	1.73	3.16	3.08	2.99	3.12	2.75	2.61	2.83	2.65	3.19	3.63	3.20	2.38	3.25	3.20	2.88	1.88	3.26
	I	1.03	1.60	-2.67	-0.12	0.24	1.08	0.45	-0.06	1.46	0.06	0.52	0.14	1.74	0.80	1.42	1.44	0.45	0.23	-0.59	0.28
V	I	2.93	3.26	1.09	2.83	2.69	3.05	2.89	2.17	2.58	2.53	2.59	2.82	3.47	2.88	2.50	3.09	2.82	2.66	1.36	2.90
	Q																				

Appendices

N	E	I	-2.46	-2.62	-3.14	-3.22	-1.97	-2.44	-2.57	-2.47	-3.12	-2.18	-2.81	-3.17	-1.17	-3.10	-2.42	-2.29	-3.13	-2.32	-2.26	-1.99			
N	Q	I	-2.21	-2.42	-2.39	-3.10	-1.73	-2.86	-2.29	-2.01	-2.94	-1.94	-1.83	-2.55	-2.08	-2.57	-1.52	-2.75	-2.83	-2.33	-1.29	-1.52			
P	I	I	-1.92	-2.10	-2.71	-2.74	-1.39	-2.56	-1.59	-2.06	-1.66	-2.07	-1.93	-2.43	-0.64	-1.69	-2.17	-2.27	-2.31	-2.17	-2.29	-1.60			
S	V	I	-2.45	-2.36	-3.56	-3.09	-1.50	-1.70	-2.29	-1.80	-1.76	-1.83	-1.26	-2.71	-1.62	-3.10	-1.91	-2.80	-2.71	-2.33	-1.39	-1.44			
Q	E	I	-3.35	-3.29	-3.94	-4.10	-2.69	-3.85	-3.40	-3.04	-3.61	-2.78	-3.13	-2.88	-2.42	-3.41	-2.86	-3.69	-3.91	-2.89	-3.10	-3.12			
N	Q	I	-3.21	-2.87	-3.61	-3.09	-2.91	-2.24	-2.21	-2.78	-3.13	-3.12	-2.67	-2.53	-2.31	-2.70	-3.06	-2.93	-3.03	-3.32	-2.60	-3.07			
P	I	I	-2.15	-2.31	-3.28	-2.46	-2.15	-2.52	-2.25	-2.01	-1.95	-2.12	-2.27	-2.90	-1.59	-2.57	-2.33	-2.37	-2.53	-2.35	-2.18	-2.22			
S	V	I	-2.64	-2.48	-3.43	-3.89	-2.44	-2.48	-3.05	-3.13	-2.78	-2.53	-2.61	-2.96	-2.28	-3.15	-2.28	-2.71	-3.12	-2.55	-2.58	-1.99			
R	E	I	-3.15	-3.15	-3.77	-3.30	-2.88	-2.68	-2.83	-3.04	-3.27	-3.29	-2.48	-3.92	-1.32	-3.28	-2.94	-2.67	-3.41	-2.92	-2.50	-2.71			
N	Q	I	-2.37	-2.48	-2.95	-3.26	-2.11	-2.72	-2.62	-2.18	-2.59	-2.18	-2.38	-2.02	-1.26	-2.41	-2.19	-2.89	-2.99	-2.27	-1.98	-2.24			
P	I	I	-2.07	-2.21	-2.96	-2.68	-2.06	-2.03	-2.39	-2.22	-2.46	-2.22	-2.14	-2.21	-0.86	-2.97	-2.11	-2.63	-2.68	-2.24	-1.75	-1.99			
S	V	I	-2.73	-2.45	-3.21	-3.05	-2.15	-1.95	-2.17	-2.21	-2.52	-2.53	-2.17	-2.20	-1.61	-3.07	-2.02	-2.28	-2.44	-2.45	-2.31	-1.93			
S	E	I	-2.67	-3.69	-3.65	-3.48	-3.03	-2.20	-2.45	-2.49	-2.20	-3.13	-3.23	-3.26	-1.27	-2.64	-2.02	-2.72	-4.11	-2.99	-2.92	-2.13			
N	Q	I	-2.17	-2.50	-2.70	-3.07	-2.27	-2.24	-1.95	-2.10	-2.24	-2.19	-2.76	-2.51	-2.29	-2.67	-2.24	-2.96	-2.44	-2.59	-1.84	-1.92			
P	I	I	-2.14	-2.27	-2.51	-2.39	-1.77	-1.76	-1.98	-2.28	-2.77	-2.04	-2.18	-2.44	-0.75	-2.30	-1.69	-2.10	-2.42	-2.55	-2.00	-1.64			
S	V	I	-2.27	-2.17	-2.64	-3.14	-1.86	-2.42	-2.25	-2.27	-2.19	-2.24	-2.17	-2.98	-1.67	-2.44	-1.73	-3.32	-2.58	-2.47	-1.76	-1.56			
T	E	I	-2.41	-3.58	-3.18	-3.57	-2.91	-3.85	-3.98	-2.87	-2.74	-2.72	-3.47	-3.67	-2.05	-3.80	-2.44	-3.77	-3.84	-3.37	-2.99	-3.06			
N	Q	I	-3.03	-2.80	-3.22	-3.27	-1.82	-3.03	-3.13	-3.22	-3.06	-2.62	-2.77	-2.88	-2.54	-3.06	-2.17	-3.06	-3.38	-3.23	-2.86	-2.34			
P	I	I	-2.55	-2.62	-2.69	-2.10	-2.09	-2.24	-2.50	-2.54	-2.75	-2.23	-1.93	-2.35	-1.82	0.28	-2.24	-2.59	-2.39	-2.49	-2.98	-2.35			
S	V	I	-2.60	-3.07	-3.78	-2.91	-1.80	-2.66	-2.43	-3.36	-2.52	-2.96	-2.72	-3.69	-1.21	-3.34	-2.34	-2.98	-3.68	-3.01	-2.41	-2.22			
V	E	I	-3.06	-3.82	-3.18	-2.97	-2.82	-2.86	-3.28	-3.09	-3.19	-3.22	-3.22	-3.71	-1.95	-3.52	-2.85	-3.82	-3.57	-2.36	-4.05	-2.70			
N	Q	I	-3.02	-2.82	-3.29	-2.81	-2.34	-2.56	-2.92	-1.87	-3.22	-2.72	-3.19	-3.00	-2.55	-3.07	-2.83	-3.37	-3.49	-2.66	-3.35	-3.08			
P	I	I	-2.48	-2.65	-2.84	-2.48	-2.20	-2.33	-2.42	-1.87	-2.74	-2.18	-2.12	-2.72	-1.64	-3.06	-2.70	-2.11	-2.57	-1.93	-2.88	-2.70			
S	V	I	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21			
W	E	I	-1.50	-1.40	-2.45	-2.75	-1.12	-2.19	-1.69	-0.45	-0.24	-1.08	-1.52	-2.28	0.30	-2.17	-1.13	-2.07	-2.23	-1.24	-1.25	-1.28			
N	Q	I	-2.13	-1.93	-1.97	-1.85	-1.40	-2.07	-1.52	-0.49	-1.61	-1.29	-1.27	-2.06	-1.41	-2.03	-1.40	-2.32	-1.74	-1.79	-1.14	-1.39			
P	I	I	-0.32	-0.13	-1.77	-1.83	-0.04	0.35	-0.88	-0.50	-0.25	-1.02	-0.11	-1.41	0.61	-1.19	-0.61	-1.12	-1.68	-1.19	-0.78	-0.54			
S	V	I	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21			
Y	E	I	-1.66	-1.99	-3.09	-2.18	-1.04	-1.36	-1.89	-0.68	-0.72	-1.15	-1.34	-2.98	1.14	-1.84	-0.91	-2.01	-2.51	-1.05	-1.78	-1.15			
N	Q	I	-2.34	-2.30	-3.18	-3.06	-1.68	-2.38	-2.11	-1.89	-2.07	-2.04	-2.21	-2.47	-2.19	-2.41	-2.02	-2.65	-2.32	-2.11	-1.74	-2.37			
P	I	I	-1.60	-1.24	-2.30	-1.12	-0.33	-1.37	-1.55	-0.40	-1.19	-0.91	-1.06	-1.61	0.56	-1.22	-2.44	-1.69	-1.83	-0.84	-0.98	-0.99			
S	V	I	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21	-1.21			
			A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y			
146	153	155	147	149	150	154																			
F	R	F	A	E	I	-0.35	-0.73	-1.66	-1.72	0.64	0.22	-0.24	0.37	1.52	0.56	0.05	-0.44	0.96	0.45	1.50	-0.24	-0.42	-0.41	0.34	1.02
			N	Q	I	-2.74	-2.32	-2.11	-2.72	-1.74	-2.53	-2.50	-2.12	-2.58	-2.35	-2.90	-2.38	-2.82	-1.70	-3.17	-2.65	-2.28	-1.45	-1.22	
			P	I	I	1.06	0.21	-0.40	0.10	0.82	1.07	0.98	1.61	1.32	0.77	1.20	1.07	2.40	1.29	1.44	1.05	0.56	1.47	0.83	1.45
C	E	I	-1.63	-2.07	-3.21	-2.44	-1.80	-1.17	-1.46	-1.00	0.27	-1.83	-0.98	-2.24	-0.25	-1.29	-0.08	-1.64	-2.06	-1.06	-1.41	-1.20			
N	Q	I	-2.96	-2.79	-3.33	-3.21	-1.56	-2.64	-2.18	-2.01	-2.35	-2.31	-2.20	-2.49	-2.43	-2.52	-2.50	-2.98	-2.96	-2.29	-2.25	-2.09			
P	I	I	-0.06	-0.61	-1.56	-0.71	-0.55	-0.17	-0.43	-0.10	0.54	-0.53	-0.21	-0.18	0.88	0.40	0.31	-0.28	-0.75	-0.11	-0.19	-0.26			
D	E	I	-1.75	-1.92	-3.08	-2.21	-1.62	-1.69	0.38	-1.81	-0.56	-2.24	-1.73	-2.44	-0.90	-1.57	0.19	-1.61	-2.33	-2.46	-1.38	-1.48			
N	Q	I	-2.69	-2.40	-1.98	-2.85	-1.09	-2.46	-2.61	-2.14	-2.64	-1.54	-1.70	-2.33	-2.11	-2.61	-2.72	-3.26	-3.26	-2.33	-0.92	-1.41			
P	I	I	-0.38	-0.81	-2.41	-1.43	-0.87	-0.37	1.31	-0.77	1.56	-0.77	-0.39	0.07	1.53	-0.25	2.15	-0.11	-0.33	-0.73	-1.10	-0.44			
E	E	I	-2.85	-3.10	-3.10	-3.38	-3.25	-2.59	-3.43	-2.38	-1.98	-1.72	-3.05	-2.61	-2.42	-2.82	-1.67	-2.51	-3.39	-2.69	-2.99	-2.46			
N	Q	I	-3.75	-3.36	-3.19	-2.90	-2.65	-2.76	-2.89	-2.75	-3.76	-3.48	-3.18	-3.17	-3.42	-3.35	-3.16	-2.91	-3.88	-3.11	-2.89	-2.75			
P	I	I	-1.39	-1.78	-2.35	-3.21	-1.85	-1.82	-1.72	-1.37	-0.94	-2.03	-1.94	-2.02	-1.09	-2.29	-0.66	-1.57	-1.77	-1.50	-1.63	-1.38			
F	E	I	0.60	0.79	-2.41	-0.55	0.87	0.41	0.42	1.37	2.27	1.57	1.25	-0.14	2.53	1.49	2.12	0.60	0.49	0.81	0.16	0.94			
N	Q	I	-2.55	-1.86	-2.83	-2.82	-1.63	-2.39	-1.95	-0.88	-2.10	-1.33	-1.61	-2.26	-1.64	-1.73	-1.82	-2.31	-2.31	-1.73	-1.43	-2.53			
P	I	I	-0.76	0.16	-1.18	0.71	0.74	-1.13	0.21	0.98	-0.34	1.16	0.21	0.36	2.46	1.17	-0.16	0.41	1.10	0.72	1.04	1.17			
G	E	I	-1.09	-0.63	-2.74	-2.14	-0.34	-1.02	-1.44	0.87	1.54	1.41	0.87	-1.20	-0.61	-0.21	0.98	-0.94	-0.17	0.00	-0.59	-0.50			
N	Q	I	-2.18	-1.79	-2.27	-3.02	-1.53	-1.91	-2.15	-1.52	-1.95	-1.56	-1.92	-1.49	-1.83	-1.78	-2.04	-2.77	-2.44	-2.37	-1.26	-2.02			
P	I	I	2.41	2.65	0.46	1.83	2.53	2.07	1.70	3.47	3.06	3.15	3.20	1.95	3.01	2.87	2.49	2.28	2.83	3.06	1.92	2.49			
H	E	I	0.00	-0.75	-3.86	-1.70	-0.89	-0.11	-0.86	-0.54	0.66	-0.64	-0.73	-1.97	1.69	-0.92	0.08	-1.49	-1.40	-0.36	-1.63	-0.78			
N	Q	I	-2.57	-2.84	-3.11	-2.44	-1.69	-2.24	-2.75	-2.31	-2.37	-1.98	-2.18	-3.15	-2.32	-2.64	-1.91	-2.65	-1.82	-1.73	-1.43	-2.20			
P	I	I	-0.54	-0.63	-1.55	0.04	-0.74	-0.60	-0.05	0.04	-0.37	-0.29	-0.50	-0.82	2.08	0.09	-0.38	-0.75	-0.78	0.00	-0.43	-0.07			
I	E	I	-2.83	-2.73	-3.12	-3.40	-1.91	-3.28	-1.85	-1.03	-1.59	-2.48	-2.48	-3.42	-2.25	-2.14	-1.79	-2.87	-3.05	-2.22	-0.54	-2.04			
N	Q	I	-2.99	-2.12	-3.13	-2.93	-2.03	-3.02	-2.53	-1.95	-2.91	-1.92	-2.79	-2.87	-2.09	-2.55	-2.09	-3.32	-2.82	-2.25	-2.14	-2.16			
P	I	I	-1.86	-1.98	-2.20	-2.20	-1.43	-2.06	-1.55	-1.16	-1.78	-1.28	-1.51	-1.98	-2.22	-1.97	-1.38	-2.15	-1.62	-1.47	-1.61	-2.29			
K	E	I	-2.33	-2.88	-3.16	-3.99	-2.83	-2.10	-2.88	-2.74	-2.33	-2.52	-2.79	-3.35	-1.01	-2.81	-2.87	-2.35	-2.58	-3.54	-2.80	-2.70			
N	Q	I	-2.77	-2.99	-3.15	-3.47	-2.43	-2.31	-2.42	-2.52	-3.44	-2.81	-3.05	-3.10	-2.13	-3.44	-2.98	-2.86	-2.99	-2.69	-2.23	-2.18			
P	I	I	-2.26	-2.39	-2.59	-3.22	-2.81	-1.46	-1.93	-2.27	-2.33	-2.19	-2.45	-1.69	-0.83	-2.24									

Appendices

E	E	I	0.67	-0.82	-3.74	-2.40	-0.91	-0.96	-1.23	-1.50	-0.07	-1.74	-1.18	-1.89	-2.20	-0.90	0.73	0.46	0.26	-0.78	-1.41	-0.86
	N	Q	-2.55	-2.87	-2.97	-2.41	-1.90	-2.37	-2.29	-2.78	-1.92	-2.37	-2.33	-2.21	-2.72	-2.94	-2.32	-2.68	-2.40	-2.92	-1.99	-2.20
	P	I	1.75	0.46	-2.52	-1.64	0.02	0.70	-0.15	-0.83	0.76	-1.58	-0.46	-0.88	-1.29	-0.15	1.29	1.90	1.10	-0.51	-0.58	0.41
F	E	I	3.18	3.37	-1.80	1.88	2.27	2.19	2.74	2.79	3.22	1.31	3.20	1.16	0.56	3.43	3.71	3.66	3.64	3.27	1.42	2.44
	N	Q	-0.74	-0.47	-2.56	-2.00	-0.48	-1.23	-1.31	-0.43	-1.31	-0.61	-1.18	-1.81	-1.53	-1.10	-0.36	-0.32	-0.96	-1.04	-0.82	-0.73
	P	I	3.05	3.44	-0.61	2.80	2.50	3.06	2.94	2.63	2.81	1.52	2.71	2.31	2.02	3.36	3.24	3.55	3.39	3.02	1.68	2.88
G	E	I	0.46	-0.15	-2.62	-0.94	1.15	-1.54	-0.35	0.76	1.82	0.08	1.40	-0.55	-1.16	2.06	2.14	1.24	2.28	1.01	0.67	1.27
	N	Q	-1.76	-1.74	-2.48	-2.05	-0.32	-1.78	-1.46	-1.47	-1.95	-1.24	-1.57	-1.91	-1.90	-1.96	-1.05	-1.87	-1.90	-1.38	-0.42	-0.77
	P	I	3.10	3.17	0.58	1.95	3.27	1.51	2.72	3.01	3.67	2.27	3.42	2.96	1.94	3.74	3.17	3.62	3.65	3.04	3.20	3.53
H	E	I	2.53	1.84	-2.60	0.77	0.99	1.59	1.32	1.05	2.11	-0.06	1.75	-0.34	-0.29	2.39	2.71	3.19	2.89	1.71	-0.09	1.51
	N	Q	-1.77	-2.38	-3.26	-2.67	-0.98	-1.46	-1.77	-1.91	-1.87	-1.63	-1.84	-2.25	-1.95	-2.02	-1.15	-1.59	-2.07	-1.75	-0.83	-1.13
	P	I	2.75	2.70	-1.62	2.03	2.35	2.82	2.33	1.31	2.19	0.71	2.17	1.26	1.86	2.57	2.28	3.33	2.75	1.99	1.34	2.56
I	E	I	0.69	0.03	-2.70	-1.11	-0.40	0.32	-0.29	-0.61	0.24	-1.09	0.01	-2.16	-1.41	0.76	1.24	1.33	0.79	-0.51	-0.75	0.04
	N	Q	-2.05	-2.32	-3.41	-2.91	-0.99	-1.68	-1.95	-1.85	-2.18	-1.63	-2.11	-2.70	-2.14	-2.29	-1.54	-2.17	-2.33	-1.86	-1.16	-1.62
	P	I	0.75	0.31	-1.67	-0.54	0.46	0.71	-0.26	-0.46	0.11	-0.19	0.25	-0.79	0.11	0.32	0.75	1.00	0.37	-0.65	0.09	0.50
K	E	I	0.64	-0.78	-2.45	-1.28	-1.13	0.61	-1.27	-1.88	-1.07	-1.61	-1.23	-2.22	-1.15	-2.27	-1.07	-1.70	-1.00	-1.72	-1.29	-1.68
	N	Q	-1.74	-2.31	-3.17	-2.84	-1.29	-1.97	-1.62	-2.44	-1.60	-1.80	-2.21	-2.35	-1.80	-2.34	-1.69	-1.83	-2.28	-2.13	-1.30	-1.44
	P	I	1.20	-0.23	-1.99	-0.74	-0.52	-1.97	-1.01	-1.88	-1.33	-1.62	-1.07	-1.09	0.34	-0.04	-1.28	-1.33	-0.86	-1.29	-1.05	-0.43
L	E	I	0.64	0.42	-2.90	-1.20	-0.36	0.63	-0.44	-0.41	0.10	-0.60	-0.45	-1.18	-1.57	0.49	0.78	1.68	0.01	-0.93	-0.70	-0.29
	N	Q	-2.26	-1.90	-2.04	-2.60	-1.11	-1.65	-2.22	-1.52	1.88	-1.61	-1.85	-2.44	-1.89	-2.02	-1.19	-1.99	-1.91	-1.67	-1.00	-1.05
	P	I	0.70	0.77	-1.82	-0.54	0.50	1.45	-0.42	0.37	-0.12	-0.47	0.04	-1.15	-0.67	0.59	0.66	1.38	0.15	-0.36	-0.15	0.31
M	E	I	0.78	-0.10	-2.43	-1.49	-0.46	0.47	-0.67	-1.00	0.01	-1.21	-0.35	-1.15	-1.20	0.29	0.99	1.25	0.14	-0.57	-0.75	-0.81
	N	Q	-1.80	-1.66	-2.84	-3.10	-1.23	-1.94	-1.82	-1.27	-1.71	-1.69	-1.58	-2.77	-2.18	-2.32	-1.72	-2.00	-2.17	-1.36	-1.04	-1.54
	P	I	1.55	0.47	-1.51	-0.92	0.39	1.88	0.02	-0.33	0.15	-0.66	-0.08	-0.47	0.39	0.28	0.59	1.63	-0.03	-0.23	-0.11	0.60
N	E	I	1.39	0.29	-2.51	-0.43	0.47	0.43	0.65	-0.17	0.55	-0.66	0.34	-0.86	-1.20	1.46	1.31	1.58	1.73	0.43	-0.21	0.60
	N	Q	-1.63	-1.92	-2.69	-2.42	-0.99	-1.75	-1.47	-1.64	-1.80	-1.27	-1.90	-2.47	-2.09	-1.80	-1.19	-2.04	-1.96	-2.10	-0.70	-1.01
	P	I	2.30	1.60	-1.95	1.07	1.27	2.19	1.34	0.12	1.00	-0.11	1.27	0.03	0.78	1.88	1.52	2.90	2.74	1.01	0.55	1.76
Q	E	I	2.27	0.66	-2.82	0.72	-0.64	0.93	-0.24	-0.41	0.76	-1.11	0.13	-1.53	-0.91	0.99	1.29	2.41	1.59	-0.08	-0.87	-1.41
	N	Q	-1.96	-2.28	-2.77	-3.17	-1.85	-2.51	-2.12	-2.79	-2.06	-2.33	-1.97	-2.28	-2.34	-1.61	-1.41	-1.84	-2.28	-2.85	-1.83	-1.62
	P	I	2.66	1.14	-1.36	1.35	0.40	2.46	0.55	-0.02	0.79	-0.59	0.65	-0.45	1.27	1.01	0.32	2.67	1.75	0.32	0.03	1.00
R	E	I	2.22	0.25	-2.41	-0.68	-0.29	2.02	-0.54	-0.55	-0.15	-0.90	0.07	-1.42	1.28	0.69	-0.03	2.23	0.25	-0.72	-0.54	1.12
	N	Q	-1.46	-1.69	-2.40	-2.51	-1.45	-1.13	-1.37	-1.68	-2.03	-1.18	-1.62	-1.83	-1.25	-2.19	-1.44	-1.43	-0.73	-1.27	-1.55	-1.19
	P	I	2.16	0.81	-1.49	-0.28	1.26	2.74	-0.01	-0.41	-0.02	-0.23	0.28	-0.49	2.94	0.72	0.00	2.43	-0.33	-0.31	0.71	1.07
S	E	I	2.20	1.68	-2.04	0.01	0.58	0.56	0.93	0.62	2.27	-0.12	1.72	-0.33	-0.98	2.40	2.82	2.75	3.05	1.20	-0.05	1.18
	N	Q	-1.99	-2.19	-2.58	-2.77	-1.14	-1.74	-2.08	-2.08	-1.85	-1.62	-1.50	-2.44	-2.47	-1.88	-1.15	-1.50	-1.70	-2.24	-1.48	-1.09
	P	I	2.81	2.56	-1.41	1.08	2.05	2.40	2.37	1.06	2.15	0.32	1.79	1.11	1.11	2.31	2.79	3.04	3.13	1.49	1.49	2.68
T	E	I	2.12	0.85	-3.24	-0.33	0.08	0.78	0.57	-0.46	0.90	-0.54	0.68	-0.89	-1.52	0.84	1.66	2.45	1.29	-0.01	-0.65	0.66
	N	Q	-1.90	-2.24	-2.95	-2.52	-1.72	-2.15	-1.81	-1.80	-2.23	-2.17	-2.05	-2.11	-2.43	-2.38	-1.82	-2.06	-2.55	-2.10	-1.35	-1.50
	P	I	2.49	1.64	-1.73	0.60	0.94	1.84	1.10	-0.03	0.78	-0.18	0.99	-0.01	0.15	0.77	1.45	2.51	1.57	0.40	0.36	1.69
V	E	I	1.99	1.06	-3.07	-0.01	0.18	1.26	0.19	0.12	1.43	-0.70	1.06	-0.87	-1.74	1.83	2.50	2.39	1.83	0.31	-1.05	0.94
	N	Q	-2.13	-1.80	-2.99	-2.78	-1.59	-2.19	-2.54	-1.64	-2.22	-2.24	-2.20	-2.81	-2.29	-1.85	-1.53	-1.87	-2.33	-2.51	-1.33	-1.48
	P	I	1.96	1.02	-1.45	0.13	0.41	1.94	0.16	0.19	0.66	-0.51	0.69	-0.38	0.14	1.16	1.86	2.25	1.27	0.18	1.00	1.01
W	E	I	2.64	2.90	-1.69	1.65	2.37	1.70	2.73	2.49	3.29	1.13	3.05	1.50	-0.01	3.20	3.39	3.66	3.55	2.88	1.30	2.23
	N	Q	-0.71	-0.55	-2.07	-1.40	-0.03	-0.87	-0.32	0.24	-0.59	-0.02	-0.44	-0.74	-0.74	-0.62	0.19	0.06	-0.44	-0.23	0.12	0.13
	P	I	2.95	3.20	0.35	2.12	2.66	2.74	2.74	2.27	2.63	1.91	2.86	2.43	1.54	3.21	3.02	3.31	3.24	2.87	1.49	2.78
Y	E	I	3.17	3.35	-2.07	2.22	3.00	2.73	3.13	2.99	3.60	1.45	3.38	2.00	0.75	3.53	3.55	3.63	3.57	3.30	1.69	1.90
	N	Q	-1.21	-0.81	-2.39	-1.92	-0.21	-1.33	-1.03	-1.17	-0.86	-1.29	-0.79	-1.61	-1.31	-1.36	-0.46	-0.07	-0.85	-0.98	-0.92	-0.86
	P	I	2.98	3.56	-0.77	2.78	2.85	3.31	3.13	2.94	2.56	1.74	3.14	2.19	2.42	3.45	3.08	3.73	3.60	3.29	2.73	3.72
			A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	X

			99																											
			154																											
146	153	155	147	149	150	A	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
F	A	F	A	D	I	2.04	-0.60	-1.82	-0.45	-1.28	0.46	-0.62	0.25	0.52	-1.37	0.29	0.33	1.66	0.70	1.91	1.47	0.86	0.78	-1.95	-0.27					
					Q	-2.01	-2.78	-3.11	-3.28	-3.11	-2.73	-2.95	-2.79	-1.91	-3.23	-2.51	-3.45	-2.93	-2.65	-1.90	-1.83	-2.51	-2.15	-3.06	-2.94					
					V	1.22	-0.41	-2.46	-0.63	-1.78	-0.94	-1.30	-1.16	-0.48	-2.42	-0.87	-0.97	-0.42	-0.29	1.19	0.33	-0.38	-1.31	-2.28	-1.97					
	E				I	3.03	2.60	-0.33	1.11	0.56	1.86	1.02	1.96	1.86	0.32	1.74	1.89	2.51	2.36	2.89	2.80	2.04	2.39	0.11	1.38					
					Q	-0.45	-1.68	-1.32	-2.25	-2.96	-1.93	-2.68	-1.96	-1.51	-2.69	-1.51	-2.55	-2.54	-1.68	-0.48	-1.23	-2.05	-1.83	-2.78	-2.44					
					V	2.99	2.16	-1.42	0.88	-0.15	1.13	0.66	1.02	2.02	-0.61	1.34	1.91	1.62	2.06	3.03	2.73	2.03	2.04	-1.03	0.72					
	N				I	2.64	1.95	-0.08	0.42	0.26	0.96	1.18	1.29	0.97	-0.46	1.00	1.17	2.25	1.62	2.42	2.03	1.89	2.05	-0.57	1.23					
					Q	-1.24	-1.88	-2.65	-2.95	-2.14	-2.05	-3.11	-1.40	-1.87	-2.72	-2.43	-2.37	-2.79	-1.92	-1.46	-1.89	-2.40	-1.95	-2.50	-1.89					
					V	1.44	0.26	-2.01	-1.39	-1.66	-0.83	-0.83	-0.34	-0.46	-1.63	-0.72	-0.57	0.23	-1.24	1.08	0.64	-0.05	0.04	-1.67	-0.46					
	P				I	2.93	2.08	0.74	1.22	0.01	1.67	1.00	1.21	1.44	-0.53	1.12	1.85	3.03	2.33	2.53	2.24	1.82	1.96	-0.43	1.05					
					Q	-0.79	-1.27	-2.90	-2.64	-2.49	-1.31	2.00	-1.49	-1.34	-3.35	-2.45	-2.58	-1.93	-1.50	-2.21	-1.24	-1.32	-1.36	-2.49	-2.61					
					V	2.87	1.64	0.69	0.42	-0.04	1.04	0.05	0.68	1.28	-1.02	0.81	1.30	2.58	1.78	2.28	2.15	1.68	1.74	-0.41	0.46					
C	D				I	2.17	1.36	-1.30	-0.29	-1.43	0.67	-0.46	1.06	1.65	-1.33	0.00	0.59	2.21	1.58	2.23	1.84	1.05	1.00	-1.29	-0.08					
					Q	-1.49	-1.99	-4.01	-3.43	-3.16	-2.25	-3.70	-2.73	-1.73	-3.03	-2.51	-2.54	-2.25	-1.68	-1.12	-1.94	-2.23	-1.90	-2.95	-2.29					
					V	1.25	0.30	-2.37	-1.91	-1.88	-0.23	-1.02	-1.13	0.58	-1.74	-0.62	0.09	0.35	0.28	1.50	0.77	0.05	-0.40	-2.33	-2.28					
	E				I	3.10	2.06	-0.40	1.26	-0.07	2.09	0.68	1.57	2.52	0.25	1.35	1.66	2.99	2.58	3.01	2.70	2.25	1.91	-0.74	0.31					
					Q	-0.69	-0.99	-3.53	-2.58	-2.89	-1.77	-2.93	-1.49	-0.42	-2.46	-2.03	-1.89	-2.38	-1.61	-0.40	-1.00	-0.97	-1.52	-2.23	-0.89					
					V	2.96	2.27	-0.87	0.83	-0.77	1.74	1.06	0.82	2.43	-0.44	1.34	1.89	2.09	2.23	3.02	2.85	1.22	1.80	-0.58	0.53					
	N				I	2.36	1.61	-0.69	0.39	-0.62	1.06	-0.20	0.86	0.95	-0.52	0.58	1.33	2.73	1.91	1.76	1.91	1.41	1.48	-0.27	-0.18					
					Q	-1.13	-1.48	-3.19	-2.52	-2.23	-1.89	-2.87	-1.73	-2.09	-2.67	-1.64	-2.15	-1.33	-1.49	-1.34	-1.92	-1.39	-1.96	-1.99	-2.33					
					V	0.95	0.41	-1.62	-0.97	-1.66	-0.32	-1.19	-0.61	0.06	-1.49	-0.90	-0.41	0.69	0.11	0.60	0.45	-0.03	-0.18	-1.43	-1.21					
	P				I	2.84	2.23	1.10	1.36	-0.01	1.92	0.95	1.25	2.05	0.29	1.26	2.16	3.16	2.66	2.75	2.71	1.82	1.91	0.20	1.19					
					Q	-0.94	-0.95	-3.11	-1.78	-2.39	-1.07	-2.19	-1.84	-1.03	-1.96	-1.69	-1.28	-1.58	-0.99	0.22	-0.30	-0.97	-1.37	-0.20	-1.85					
					V	2.58	1.98	0.51	0.92	-0.49	1.65	0.75	0.73	1.99	0.09	0.96	1.64	2.60	2.25	2.41	2.24	1.86	1.66	-0.16	-0.63					
D	D				I	-1.32	-1.40	-3.65	-3.09	-2.45	-2.24	-0.86	-1.63	-1.13	-2.05	-2.58	-2.14	-1.45	-1.35	0.02	-1.22	-1.86	-2.03	-2.31	-1.69					
					Q	-3.36	-3.26	-3.14	-3.70	-2.99	-2.71	-2.82	-2.71	-2.91	-2.67	-2.77	-3.38	-3.30	-2.58	-2.34	-3.90	-2.67	-3.52	-2.92	-2.78					
					V	-2.09	-2.28	-3.05	-3.37	-2.49	-2.79	-1.72	-2.75	-2.35	-2.95	-2.98	-2.46	-2.67	-2.45	-0.48	-1.80	-2.15	-2.99	-2.15	-2.69					
	E				I	0.42	-0.31	-2.74	-1.94	-1.12	-1.13	0.82	-0.89	0.28	-1.67	-1.04	-0.71	0.89	0.95	1.74	0.78	0.14	-0.16	-1.97	-1.26					
					Q	-3.12	-2.86	-2.62	-3.18	-2.78	-3.98	-3.07	-3.53	-2.70	-2.62	-2.93	-2.81	-0.44	-2.14	-1.25	-2.32	-3.18	-3.40	-2.72	-3.67					
					V	0.14	-1.47	-3.36	-1.82	-2.24	-1.45	0.71	-1.32	0.74	-1.69	-0.96	-1.08	-1.29	-0.57	0.52	0.54	-0.57	-0.95	-2.82	-1.50					
	N				I	-0.35	-0.90	-1.61	-1.82	-1.63	-1.59	-0.01	-1.22	0.73	-1.36	-1.10	0.83	0.55	-0.13	0.63	0.02	-0.69	-0.67	-1.42	-1.15					
					Q	-2.91	-2.17	-3.28	-2.55	-1.99	-2.63	-2.75	-3.15	-3.14	-2.25	-2.83	-2.24	-2.66	-2.57	-2.19	-2.73	-3.15	-3.49	-1.76	-2.18					
					V	1.67	0.40	-2.25	-2.16	-2.29	-1.08	-1.28	-1.44	0.88	-1.83	-0.29	0.73	1.50	1.89	1.08	1.02	-0.12	-0.10	-0.45	-0.32					
	P				I	3.65	0.40	-1.56	-3.55	-1.15	-0.15	1.28	-0.44	0.88	-0.33	0.29	0.73	1.50	1.89	1.08	1.02	-0.12	-0.10	-0.45	-0.32					
					Q	-3.34	-2.80	-3.50	-3.27	-3.38	-3.43	-1.94	-2.67	-2.67	-3.44	-2.86	-3.70	-2.95	-2.24	-1.58	-2.72	-2.62	-2.65	-2.57	-3.35					
					V	0.40	-0.36	-2.42	-1.74	-2.30	-0.89	1.04	-0.86	0.13	-1.26	-0.87	-0.42	0.49	0.83	1.66	0.65	-0.46	-0.35	-1.00	-1.02					
E	D				I	-2.01	-2.76	-3.31	-2.72	-2.98	-2.55	-3.04	-1.75	-2.12	-2.90	-2.46	-2.43	-1.62	-2.42	-1.60	-2.24	-1.79	-2.16	-3.03	2.06					
					Q	-3.38	-3.55	-3.62	-2.63	-3.16	-2.30	-3.67	-1.15	-3.57	-3.86	-3.45	-4.58	-3.31	-3.15	-2.83	-3.77	-3.44	-3.51	-3.60	-3.99					
					V	-2.63	-2.72	-3.19	-2.56	-2.69	-3.71	-4.09	-2.90	-2.27	-2.78	-3.65	-2.52	-2.97	-2.69	-2.97	-2.72	-3.12	-2.48	-3.71	-2.51					
	E				I	-0.83	-1.14	-2.74	-3.25	-1.77	-1.47	-2.34	-1.08	-1.41	-1.71	-1.94	-1.05	-0.51	-0.87	-0.53	-0.32	-0.76	-2.35	-1.28						
					Q	-2.29	-2.73	-4.35	-4.70	-4.02	-2.94	-3.00	-2.97	-2.48	-2.69	-3.32	-2.52	-3.27	-2.33	-1.75	-2.62	-2.86	-3.16	-2.79	-2.37					
					V	-0.65	-1.58	-3.12	-2.20	-2.52	-1.52	-1.81	-1.32	-1.55	-1.94	-1.93	-1.03	-1.41	-1.16	-0.69	-1.03	-1.74	-0.89	-2.52	-1.30					
	N				I	-0.89	-1.44	-2.95	-2.28	-2.76	-1.92	-2.29	-1.38	-1.33	-2.22	-2.95	-1.27	-1.22	-1.38	-1.38	-1.42	-1.58	-1.85	-2.48	-1.39					
					Q	-3.53	-3.12	-3.90	-4.25	-3.66	-2.79	-3.01	-3.36	-3.49	-3.54	-2.39	-3.60	-2.90	-2.17	-2.62	-2.70	-3.02	-2.49	-2.99	-2.84					
					V	-2.13	-2.30	-4.02	-2.74	-2.84	-2.48	-2.72	-1.70	-2.39	-3.14	-2.50	-1.81	-2.20	-2.04	-2.81	-1.70	-0.80	-2.46	-3.02	-2.33					
	P				I	-0.05	-0.33	-1.03	-0.74	-1.51	-0.95	-1.36	-0.19	-0.65	-0.86	-1.23	0.03	0.30	-0.47	0.11	-0.12	-0.54	-0.04	-1.76	-1.09					
					Q	-1.98	-2.56	-3.58	-3.03	-3.69	-3.17	-2.81	-1.51	-2.70	-3.24	-2.69	-2.39	-2.37	-2.58	-1.44	-2.51	-2.65	-2.41	-2.99	-1.80					
					V	-0.45	-0.61	-1.56	-1.45	-1.48	-1.41	-2.45	-0.81	-1.21	-1.78	-1.18	-0.52	-0.29	-0.59	-0.35	-0.73	-0.00	-2.24	-0.92	-1.36					
F	D				I	2.55	3.14	-0.94	1.70	0.75	1.78	2.59	1.35	3.15	1.43	1.39	3.23	3.16	3.28	3.35	2.99	2.77	2.66	-0.22	1.36					
					Q	-0.34	0.20	-2.38	1.50	-1.44	-1.95	-0.63	-1.28	-0.45	-2.27	-1.83	-0.25	-1.00	0.27	1.12	-0.37	0.32	-0.09	-1.72	-1.41					

Appendices

	V	2.58	2.54	-1.60	0.75	0.27	0.81	1.68	1.20	2.44	0.69	0.95	2.70	1.95	3.16	3.15	2.73	2.26	1.98	-1.03	0.53
E	I	3.64	3.84	1.48	3.11	2.78	2.90	3.33	3.61	3.65	2.91	3.12	3.63	3.34	3.67	3.66	3.72	3.80	3.76	1.57	2.84
	Q	1.01	1.16	-2.47	-0.13	-0.01	-0.70	0.42	0.68	1.21	-0.22	-0.05	1.06	-0.21	1.93	2.47	1.58	1.81	1.10	-1.29	-0.88
	V	3.39	3.51	0.23	3.05	2.33	2.72	3.12	3.02	3.42	2.63	3.15	3.45	3.18	3.60	3.71	3.66	3.74	3.44	0.94	2.41
N	I	2.68	2.69	-0.63	1.94	1.34	1.73	1.69	1.65	1.78	1.18	1.50	2.61	3.29	2.81	2.56	2.55	2.32	2.75	0.13	1.63
	Q	-0.70	-0.67	-3.39	-2.07	-1.53	-1.14	-1.59	-1.12	-0.93	-1.39	-1.65	-0.97	-0.73	-0.60	0.01	-1.04	-0.85	-1.49	-1.88	-1.96
	V	1.43	1.66	-1.71	0.24	-0.18	-0.11	0.57	0.49	0.68	-0.08	0.27	1.79	1.44	1.75	1.30	1.80	0.88	0.86	-0.91	0.23
P	I	2.91	3.34	0.80	2.67	2.22	2.03	2.84	2.07	2.64	1.64	2.30	3.51	2.84	3.47	3.29	3.18	3.54	2.72	1.23	2.34
	Q	0.84	1.25	-2.64	-1.02	-0.40	-0.70	0.45	-0.07	0.81	-0.59	-0.38	1.36	0.11	1.68	1.64	1.41	1.55	0.90	-0.64	-0.25
G	D	0.58	1.27	-3.02	-1.57	-0.92	-1.28	-1.51	0.58	0.13	-0.45	0.27	-0.47	-0.82	0.79	0.82	-0.10	0.30	0.83	-2.23	-0.72
	Q	-2.48	-2.47	-3.47	-3.93	-2.79	-3.16	-3.31	-2.22	-3.28	-2.59	-2.82	-3.25	-2.83	-2.03	-2.53	-2.90	-2.27	-2.37	-2.82	-2.79
	V	-0.23	0.53	-3.50	-1.82	-1.35	-1.88	-2.08	-0.17	-0.81	-1.26	-0.56	-1.53	-2.27	-0.34	-0.38	-0.86	0.00	-0.27	-2.80	-1.71
E	I	2.59	2.93	-1.87	0.34	1.05	0.31	0.31	2.72	1.63	1.24	2.34	0.76	0.80	2.64	2.26	2.12	2.59	2.60	-1.17	0.23
	Q	-1.78	-1.37	-3.20	-3.52	-2.12	-2.72	-2.45	-1.94	-2.26	-2.28	-1.47	-2.44	-4.08	-0.99	-0.70	-1.91	-1.22	-1.60	-3.87	-2.39
	V	2.24	2.80	-2.17	-0.05	0.51	-0.10	-0.11	1.65	1.30	0.89	2.09	0.77	-0.56	2.36	2.74	2.27	2.54	2.44	-1.51	-0.35
N	I	1.29	2.09	-1.50	-0.26	0.41	0.16	-0.34	1.61	0.31	0.46	1.31	0.72	0.35	1.78	1.42	1.06	1.69	1.81	-1.28	0.06
	Q	-2.05	-1.69	-2.33	-3.16	-1.79	-2.80	-2.62	-1.94	-2.11	-2.16	-2.61	-3.27	-2.28	-1.43	-2.31	-1.69	-1.58	-1.47	-1.70	-2.21
	V	0.38	0.43	-1.90	-1.85	-1.00	-1.59	-1.49	0.14	-0.60	-0.59	0.15	-1.08	-1.44	0.26	0.13	-0.94	0.16	0.43	-2.00	-1.17
P	I	2.61	2.82	0.58	1.34	1.17	1.32	1.31	2.51	1.76	1.25	2.19	1.65	2.36	2.85	2.58	2.28	2.40	2.54	-0.46	-0.55
	Q	-1.09	-0.67	-1.86	-2.37	-1.28	-3.06	-1.44	-1.35	-1.29	-1.93	-1.68	-2.35	-2.61	-1.36	-1.07	-1.21	-1.19	-1.03	-2.68	-2.29
	V	2.34	3.16	0.19	0.70	0.78	1.13	0.90	2.12	1.67	0.88	2.08	1.40	1.90	2.65	2.10	2.36	2.41	2.40	-0.91	0.80
H	D	1.89	1.18	-3.51	-1.53	-1.25	-0.22	-0.68	-0.50	0.89	-0.92	-0.11	-0.62	2.85	1.14	1.31	1.17	0.17	0.36	-1.85	-0.84
	Q	-1.63	-1.96	-3.03	-3.36	-2.95	-2.79	-2.91	-2.52	-2.59	-2.99	-2.65	-3.67	-1.82	-1.91	-1.69	-2.65	-2.67	-2.23	-3.42	-3.37
	V	0.91	0.30	-3.55	-2.59	-1.49	-1.08	-1.65	-1.91	-0.11	-2.08	-0.63	-1.72	1.64	0.38	0.59	0.17	-1.29	-1.15	-2.54	-1.42
E	I	3.00	2.54	-3.23	0.18	-0.28	1.13	0.61	0.75	2.33	0.60	1.87	0.94	3.46	2.64	2.42	2.67	1.62	1.65	-1.57	0.68
	Q	-0.69	-1.58	-4.23	-2.18	-2.01	-2.81	-2.44	-2.15	-1.98	-2.60	-1.96	-2.01	-1.21	-0.80	-1.06	-1.52	-1.62	-1.83	-2.60	-2.71
	V	2.89	2.25	-2.72	-0.27	-0.60	1.07	0.35	0.39	1.93	-0.30	1.27	0.81	3.17	2.60	2.38	2.64	1.35	1.24	-1.81	0.49
N	I	2.30	1.38	-2.07	-0.67	-0.82	0.10	-0.23	0.35	0.21	-0.52	0.47	0.40	3.01	1.29	1.15	2.01	0.54	0.91	-1.30	0.14
	Q	-1.82	-1.87	-3.60	-3.41	-1.98	-2.43	-2.57	-2.26	-1.66	-2.97	-2.21	-2.72	-1.39	-1.99	-1.78	-1.95	-2.33	-2.19	-2.36	-2.73
	V	0.77	0.12	-2.95	-1.64	-1.44	-1.01	-1.71	-1.16	-0.61	-1.21	-0.89	-0.98	1.30	0.00	-0.20	0.47	-1.38	-0.73	-2.37	-1.39
P	I	2.78	2.64	0.86	1.62	1.24	1.19	1.72	1.34	2.09	1.18	1.90	2.48	3.39	2.80	2.50	2.54	1.89	1.77	-0.45	1.16
	Q	-0.04	-0.79	-3.04	-2.01	-1.74	-1.59	-1.45	-2.30	-0.42	-2.24	-1.05	-1.55	-0.11	-0.86	-0.24	-0.39	-1.81	-1.66	-2.04	-1.97
	V	2.40	2.44	-1.25	0.90	0.60	1.03	1.29	0.68	1.96	0.47	1.46	1.96	2.86	2.55	2.23	2.53	1.21	1.67	-0.50	1.68
I	D	2.29	0.87	-1.44	0.54	-0.83	2.03	0.58	-0.99	1.29	-0.25	0.33	1.74	3.46	2.19	1.75	1.93	0.33	0.39	-1.05	0.62
	Q	-0.75	-1.59	-3.84	-1.96	-2.50	-0.95	-2.55	-2.17	-1.86	-2.22	-2.92	-1.39	1.00	-1.05	-0.91	-1.07	-2.61	-2.49	-3.40	-1.97
	V	-1.78	-0.12	-2.03	-0.77	-0.85	0.91	-0.36	-1.61	0.28	-0.92	-0.47	1.29	3.08	1.38	0.93	1.22	-0.75	-0.89	-1.74	-0.68
E	I	2.95	2.02	-0.89	1.83	0.18	2.54	1.87	0.43	2.07	0.95	1.35	2.75	3.53	2.86	2.66	2.73	1.48	1.25	-0.92	1.41
	Q	0.32	-0.60	-3.46	-1.92	-2.13	-0.85	-1.10	-1.40	-0.95	-1.74	-1.70	-0.32	1.67	-0.10	-0.43	-0.50	-1.11	-1.39	-2.62	-1.05
	V	3.00	2.10	-0.91	1.58	0.07	2.66	1.63	0.04	2.35	0.58	1.23	2.54	3.48	2.80	2.04	2.88	1.21	0.66	-0.31	1.56
N	I	1.56	0.24	-1.85	0.04	-1.09	1.17	-0.37	0.75	0.17	-0.71	0.09	1.28	3.43	1.66	0.71	1.14	0.01	-0.24	-1.35	0.10
	Q	-0.99	-1.71	-3.02	-3.09	-1.91	-1.54	-2.22	-2.01	-1.93	-2.70	-2.75	-1.57	1.07	-1.36	-1.56	-1.62	-2.23	-2.42	-2.45	-1.81
	V	0.44	-0.44	-2.88	-1.73	-1.56	0.09	-0.91	-2.03	-0.73	-1.66	-1.10	0.49	2.49	0.39	-0.67	0.11	-1.10	-1.69	-2.10	-0.88
P	I	3.15	2.02	0.97	2.17	0.86	2.67	2.15	0.61	1.85	0.93	1.48	2.74	3.65	2.86	2.32	2.87	1.53	0.84	0.38	2.03
	Q	0.53	-0.60	-2.36	-1.72	-1.44	0.11	-0.54	-1.21	-0.61	-1.29	-0.61	0.75	2.36	0.27	0.22	0.28	-1.33	-1.53	-0.85	-0.52
	V	2.86	2.06	0.38	1.73	0.70	2.57	1.75	-0.21	1.60	0.59	1.11	2.63	3.94	2.74	2.09	2.74	1.38	0.68	-0.18	1.65
K	D	-2.12	-2.92	-3.71	-3.75	-3.35	-1.83	-3.02	-3.42	-3.31	-4.20	-3.55	-3.10	0.29	-2.18	-3.93	-2.66	-3.49	-3.08	-2.19	-3.12
	Q	-3.50	-3.31	-3.52	-1.60	-3.22	-3.93	-3.44	-4.06	-2.95	-3.60	-3.98	-3.78	-3.07	-3.70	-3.42	-4.11	-2.22	-4.05	-2.77	-3.89
	V	-1.97	-3.03	-3.35	-3.06	-3.68	-2.06	-2.77	-4.92	-2.95	-3.95	-3.68	-3.51	-0.61	-3.09	-3.15	-3.01	-3.71	-3.42	-3.40	-3.36
E	I	-0.81	-3.02	-3.36	-2.59	-2.94	-0.47	-2.78	-2.74	-3.10	-3.79	-3.24	-1.95	1.73	-2.17	-3.60	-0.81	-3.54	-2.83	-3.51	-3.47
	Q	-3.07	-3.69	-4.54	-3.71	-4.48	-3.08	-3.13	-3.41	-3.88	-3.19	-4.00	-3.34	-2.18	-3.28	-3.72	-3.45	-4.30	-2.97	-3.74	-3.90
	V	-0.69	-2.37	-2.84	-2.94	-3.02	-0.51	-3.17	-3.13	-3.28	-3.49	-3.85	-2.91	1.13	-2.24	-3.02	-1.05	-3.47	-3.46	-2.89	-3.08
N	I	-0.82	-2.26	-2.39	-2.39	-2.43	-1.32	-2.18	-2.81	-3.54	-2.69	-2.51	-2.20	0.55	-2.11	-3.31	-1.93	-2.52	-2.68	-2.92	-3.61
	Q	-3.18	-3.39	-3.61	-3.42	-3.90	-3.28	-3.85	-2.75	-3.53	-3.28	-2.92	-3.20	-2.45	-3.29	-3.81	-2.72	-3.10	-2.71	-2.76	-2.79
	V	-0.82	-3.17	-3.28	-3.10	-3.10	-1.86	-3.59	-3.33	-2.64	-2.98	-3.02	-2.95	-1.20	-2.82	-2.69	-2.06	-3.25	-2.91	-3.34	-2.84
P	I	-0.32	-2.33	-1.78	-2.53	-1.87	-0.36	-2.04	-3.23	-2.53	-3.21	-2.40	-1.21	2.24	-1.47	-2.60	-0.57	-2.70	-2.91	-1.95	-1.80
	Q	-1.99	-3.00	-3.95	-3.22	-3.52	-3.07	-2.17	-2.63	-3.29	-3.44	-3.17	-3.66	-1.90	-2.77	-3.80	-2.37	-2.58	-3.33	-2.65	-3.28
	V	-0.20	-1.85	-1.67	-1.70	-1.97	-0.89	-1.80	-2.78	-2.82	-3.35	-2.82	-1.70	1.94	-2.43	-2.92	-1.40	-2.57	-2.69	-2.04	2.57
L	D	-1.84	-1.68	-3.49	-2.41	-3.49	-1.03	-2.10	-1.86	-1.86	-1.85	-2.15	-1.68	2.15	-1.53	-2.06	-1.41	-1.74	-2.17	-2.51	-2.51
	Q	-3.45	-3.14	-2.87	-3.72	-3.56	-3.24	-3.88	-3.15	-3.31	-2.96	-2.68	-3.23	-1.74	-3.24	-3.33	-2.92	-3.76	-3.19	-3.41	-2.52
	V	-1.90	-2.95	-1.10	-2.62	-2.50	-1.55	-2.36	-2.19	-3.59	-2.29	-2.29	-1.75	0.77	-1.87	-2.29	-1.99	-2.37	-2.95	-2.93	-2.41
E	I	0.30	-0.21	-2.89	-1.55	-1.48	-0.34	-1.65	-0.86	-0.87	-1.27	-1.63	-0.67	2.82	-0.49	-1.16	-0.02	-1.41	-0.90	-2.85	-0.70
	Q	-1.88	-1.94	-2.62	-3.66	-2.60	-2.53	-3.15	-2.68	-3.38	-2.90	-2.48	-2.67	-0.90	-2.15	-3.07	-2.26	-2.87	-2.50	-3.33	-3.29
	V	0.17	-1.49	-2.88	-1.20	-2.25	-0.04	-1.45	-1.27	-1.55	-1.22	-1.47	0.42	2.45	-1.35	-1.55	-1.32	-1.32	-2.32	-1.51	-1.51
N	I	-1.33	-1.17	-2.80	-1.61	-															

Appendices

[illegible]

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Appendices

			N	Q	-2.38	-2.02	-2.28	-2.76	-2.04	-2.26	-1.46	-0.88	-1.59	-0.64	-1.66	-2.01	-2.17	-1.30	-0.85	-2.11	-1.36	-1.19	-1.83	-1.54				
			P	I	0.50	0.91	-1.05	-0.13	0.05	-0.80	0.12	0.77	1.10	0.47	0.69	1.39	0.78	2.96	1.78	1.23	0.98	1.14	0.60	0.65				
		Y	E	I	-0.05	0.53	-2.76	-1.07	1.15	-0.95	0.17	1.56	1.60	1.51	1.03	-0.03	1.62	3.37	-0.53	-0.32	1.09	0.43	-0.15	0.39				
			N	Q	-2.27	-2.08	-3.14	-2.90	-1.83	-3.07	-2.71	-1.62	-2.69	-2.28	-2.34	-2.50	-2.21	-2.32	-2.55	-2.47	-1.65	-1.77	-1.96	-2.00				
			P	I	-2.69	-1.16	-1.65	-0.50	-0.85	-0.13	-2.44	0.80	2.12	-0.03	-1.23	-0.72	1.65	1.50	-2.59	-2.36	1.43	-0.15	-0.07	-1.30				
			A	C	D	E	F	G	H	I	J	K	L	M	N	P	Q	R	S	T	V	W	X	Y				
146	153	155	147	149	150	154	A	C	D	E	F	G	H	I	J	K	L	M	N	P	Q	R	S	T	V	W	X	Y
L	F	C	A	E	I	2.30	0.96	-3.36	-0.74	0.59	-0.23	0.42	0.46	1.50	-0.69	1.33	-0.82	-1.46	2.06	2.79	2.96	3.30	1.25	0.98	0.90			
			N	Q	-2.01	-2.42	-2.79	-2.34	-1.22	-2.50	-2.54	-2.17	-1.99	-2.34	-2.53	-2.62	-2.48	-2.03	-1.50	-2.10	-1.63	-2.36	-1.31	-1.56				
			P	I	2.95	2.22	-0.87	1.33	2.49	1.64	1.99	0.84	1.75	-0.08	1.98	1.61	1.16	2.59	2.70	3.18	3.23	1.68	2.34	2.79				
	C	E	I	1.67	0.65	-3.26	-2.07	-2.09	-0.07	0.57	1.83	-0.68	1.14	-1.02	-1.65	1.72	2.33	3.27	2.78	2.96	-0.08	0.73						
			N	Q	-2.13	-2.67	-2.72	-2.78	-1.17	-1.81	-2.61	-1.97	-1.54	-1.89	-2.27	-2.61	-2.43	-2.50	-1.65	-1.64	-1.90	-2.58	-1.34	-1.44				
			P	I	2.68	2.10	-0.93	1.38	1.75	1.89	1.94	1.17	1.91	1.00	2.02	1.48	1.08	2.42	2.67	3.15	2.71	1.57	1.38	2.29				
	D	E	I	-1.63	-2.74	-3.15	-2.68	-0.88	-2.36	-1.23	-1.92	-1.70	-1.38	-2.42	-2.54	-2.44	-1.68	-0.50	-1.04	-0.83	-1.72	-1.05	-1.61					
			N	Q	-2.60	-3.41	-3.83	-3.38	-1.49	-2.38	-2.42	-0.88	-2.99	-1.76	-1.90	-2.64	-2.62	-2.61	-2.29	-2.40	-2.79	-2.20	-1.04	-2.49				
			P	I	-0.37	-0.39	-2.53	-2.15	0.07	-1.42	-0.38	-0.63	0.04	-0.87	-0.53	-1.04	-1.79	0.24	0.93	0.45	0.30	-0.60	-0.62	-0.36				
	E	E	I	-1.22	-1.56	-3.36	-2.34	-1.20	-2.31	-1.96	-1.58	-1.67	-1.91	-2.25	-2.68	-2.28	-1.36	-0.83	-1.92	-0.87	-1.63	-1.41	-1.43					
			N	Q	-2.90	-2.97	-4.03	-3.15	-2.47	-3.22	-3.12	-3.01	-2.89	-2.91	-3.17	-3.16	-2.49	-2.99	-2.57	-3.24	-2.82	-3.56	-1.69	-2.10				
			P	I	-0.13	-0.50	-0.45	-1.83	-0.13	-0.66	-0.66	-0.81	-0.76	-1.49	-0.68	-0.84	-1.77	-0.25	0.49	0.45	0.01	-0.45	-0.58	-0.10				
	F	E	I	3.02	3.39	-2.40	2.18	2.12	1.74	1.60	1.32	0.34	3.15	1.54	3.12	2.21	-0.39	3.41	3.64	3.51	3.67	3.45	1.31	2.72				
			N	Q	-1.36	-0.61	-1.32	-2.64	-0.70	-1.12	-1.26	-0.96	-1.29	-0.92	-1.13	-1.88	-1.97	-0.87	-0.31	0.15	-0.78	-0.88	-1.20	-0.67				
			P	I	3.52	3.46	-0.23	2.89	2.69	2.74	3.37	2.72	2.98	1.54	2.77	3.04	1.55	3.29	3.20	3.56	3.54	2.99	1.85	2.73				
	G	E	I	-0.19	-0.36	-2.57	-1.33	-0.46	-2.01	-0.59	-0.04	0.34	-0.55	0.35	-1.21	-1.81	1.04	1.52	0.53	2.37	0.54	0.12	0.52					
			N	Q	-1.76	-2.02	-3.34	-2.40	-0.83	-1.84	-1.67	-1.73	-1.82	-1.61	-1.85	-2.41	-1.90	-1.83	-1.22	-2.27	-2.17	-2.06	-0.98	-1.31				
			P	I	2.10	2.03	-1.42	0.37	2.15	-0.23	1.37	1.37	1.98	0.78	1.83	1.49	0.42	2.31	2.04	2.62	2.87	1.82	2.02	2.70				
	H	E	I	2.12	1.64	-2.87	-0.11	0.67	0.10	1.17	0.82	1.46	-0.46	1.42	-0.33	-0.69	2.27	2.40	3.16	2.93	1.51	-1.00	1.06					
			N	Q	-1.97	-2.20	-3.70	-2.69	-1.41	-2.11	-2.61	-2.10	-2.17	-1.34	-2.14	-2.24	-2.25	-2.49	-1.70	-1.48	-2.32	-2.16	-1.30	-1.26				
			P	I	2.63	2.21	-1.37	1.28	2.51	2.64	2.45	1.76	2.18	0.78	2.35	2.04	1.88	2.67	2.40	3.49	2.97	1.96	1.54	2.84				
	I	E	I	2.24	1.26	-2.58	0.13	0.03	1.70	0.62	-0.02	1.62	-0.50	1.04	-0.80	-0.58	2.30	2.26	3.02	2.24	0.25	-0.43	0.74					
			N	Q	-1.97	-2.20	-3.70	-2.69	-1.41	-2.11	-2.61	-2.10	-2.17	-1.34	-2.14	-2.24	-2.25	-2.49	-1.70	-1.48	-2.32	-2.16	-1.30	-1.26				
			P	I	2.69	1.98	-1.42	1.53	1.48	2.89	1.42	0.79	1.80	0.43	1.92	0.85	1.65	2.57	2.24	3.08	2.36	1.80	2.07	1.72				
	K	E	I	-1.28	-2.23	-3.72	-2.55	-1.41	-1.44	-1.76	-2.73	-2.80	-1.82	-2.41	-3.15	-1.42	-2.01	-2.46	-1.26	-2.29	-2.04	-2.16	-1.79					
			N	Q	-2.24	-2.82	-3.28	-3.18	-1.65	-2.62	-2.50	-2.47	-2.48	-1.92	-2.46	-2.48	-2.22	-2.76	-2.11	-2.35	-2.78	-2.86	-1.39	-2.20				
			P	I	-0.78	-1.30	-1.82	-1.19	-0.41	0.52	-1.50	-2.17	-2.29	-1.82	-2.03	-1.05	-0.96	-1.47	-2.03	-1.19	-1.65	-1.93	-0.78	-0.60				
	L	E	I	-0.73	-0.89	-3.73	-2.46	-0.96	-0.79	-1.44	-0.96	-1.51	-0.60	-1.13	-1.96	-1.25	-0.72	-1.31	-0.03	-2.21	-1.38	-1.05	-1.11					
			N	Q	-2.60	-1.97	-2.44	-2.84	-1.20	-2.13	-2.49	-1.71	-2.26	-1.76	-1.98	-2.88	-1.87	-1.96	-1.70	-1.12	-2.50	-2.23	-1.03	-1.35				
			P	I	0.25	0.49	-1.43	-1.15	0.65	1.37	0.09	-0.54	-0.65	-0.43	-0.30	-0.40	-1.10	-0.16	-0.35	0.88	0.29	-0.43	-0.18	0.57				
	M	E	I	1.33	0.28	-2.63	-1.33	-0.23	0.58	-0.47	0.55	-0.21	-0.08	-0.14	-1.04	-0.39	0.91	0.81	2.14	1.50	-0.06	-0.53	0.39					
			N	Q	-1.87	-1.93	-3.38	-3.14	-1.61	-1.93	-2.01	-1.80	-1.93	-2.32	-1.89	-2.99	-2.49	-2.34	-1.64	-1.63	-2.12	-2.13	-1.23	-1.76				
			P	I	2.48	1.34	-1.38	0.39	1.07	2.56	1.21	0.29	0.87	-0.37	0.58	0.99	2.34	1.65	1.13	2.78	1.97	0.39	0.42	1.77				
	N	E	I	0.11	-0.86	-2.84	-1.95	-0.23	-1.12	-0.94	-0.69	-1.09	-1.00	-0.90	-2.12	-1.86	-0.09	0.06	-0.02	0.62	-0.92	-0.65	-0.10					
			N	Q	-1.99	-2.31	-2.62	-2.82	-1.31	-2.73	-2.01	-2.56	-2.46	-1.64	-2.39	-3.15	-2.28	-2.33	-1.61	-2.96	-3.01	-2.25	-1.04	-1.79				
			P	I	1.32	0.64	-1.67	-0.03	1.65	0.53	0.56	0.09	0.22	0.15	0.81	-0.53	0.72	1.12	0.80	2.01	1.48	0.17	1.01	1.72				
	Q	E	I	1.95	1.19	-2.45	-0.03	-0.23	-0.10	-0.46	1.21	1.87	0.38	1.31	-0.85	-1.04	0.22	1.55	1.96	2.54	1.31	-1.01	0.04					
			N	Q	-2.01	-2.29	-3.11	-3.18	-2.00	-2.68	-2.37	-2.40	-1.91	-2.34	-1.82	-2.12	-3.00	-1.80	-1.72	-1.70	-1.53	-2.00	-1.73	-1.69				
			P	I	2.96	2.25	-0.48	1.29	1.20	2.07	1.17	2.22	2.43	1.20	2.15	0.21	1.94	1.71	1.54	2.76	2.59	2.16	0.59	1.74				
	R	E	I	1.45	-0.37	-2.89	-1.31	-0.45	1.04	-1.18	-1.40	-0.97	-1.11	-0.57	-1.56	-2.40	-0.33	-1.11	1.56	-0.03	-1.12	-0.93	-0.04					
			N	Q	-1.70	-1.92	-3.02	-2.83	-1.65	-1.80	-2.21	-1.86	-1.51	-1.92	-1.55	-2.23	-1.22	-2.05	-1.89	-1.82	-1.68	-2.74	-1.40	-1.63				
			P	I	2.14	0.60	-1.07	-0.36	1.30	2.14	0.25	-0.89	-0.15	0.16	0.36	-0.46	3.54	0.19	-0.25	1.90	0.59	-0.15	0.75	1.60				
	S	E	I	2.05	1.58	-3.05	-0.18	0.32	-0.23	0.36	0.75	1.70	-0.55	1.32	-0.50	-1.51	1.95	2.76	2.53	2.87	1.38	-0.36	0.71					
			N	Q	-2.25	-2.22	-2.82	-2.96	-1.37	-2.41	-1.93	-1.97	-2.17	-1.88	-1.92	-2.66	-2.69	-2.40	-1.47	-1.48	-1.94	-2.61	-1.61	-1.62				
			P	I	2.91	2.36	-1.48	0.73	1.71	1.34	2.02	1.05	1.87	0.07	1.66	1.29	0.56	2.18	2.57	3.05	2.83	1.63	1.52	2.68				
	T	E	I	2.47	1.53	-2.70	0.10	1.03	1.21	-0.66	1.56	-1.50	0.97	0.00	-1.31	1.86	2.10	3.22	2.89	0.61	-0.50	-1.20						
			N	Q	-2.08	-2.28	-2.97	-2.70	-1.87	-2.23	-2.39	-1.97	-1.85	-2.63	-1.99	-2.08	-2.96	-2.53	-1.33	-1.95	-1.46	-2.22	-1.63	-1.33				
			P	I	3.12	2.62	-1.63	1.64	1.52	2.44	2.57	0.72	1.78	-0.01	1.84	2.03	1.30	2.50	2.61	3.37	3.19	1.59	1.28	2.58				
	V	E	I	2.70	1.69	-2.83	0.68	0.39	1.94	0.79	-0.08	1.70	-0.44	1.36	-0.48	-0.97	2.58	2.55	3.11	2.47	0.56	-0.38	1.35					
			N	Q	-2.02	-2.08	-3.00	-2.78	-1.85	-2.39	-1.60	-2.27	-2.28	-2.32	-2.85	-2.29	-1.50	-1.43	-1.31	-2.26	-2.29	-1.83	-1.37					
			P	I	3.22	2.52	-0.87	2.20	1.81	3.18	2.06	1.67	2.13	0.49	1.61	0.72	3.01	3.63	3.61	3.11	2.21	2.30						
	W	E	I	2.04	3.31	-1.94	1.52	2.35	1.25	3.48	2.61	3.25	1.39	3.20	2.46	-0.32	3.49	3.50	3.79	3.79	3.17	1.35	2.48					
			N	Q	-0.55	-0.13	-2.42	-2.22	-0.07	-1.47	0.19	0.01	-0.29	-0.58	-0.43	-0.37	-1.09	0.02	1.12	0.99	0.37	-0.08	-0.31	-0.09				
			P	I	3.32	3.49	0.76	2.64	3.04	2.56	3.54	2.70	3.18	2.18	3.37	3.50	1.47	3.52	3.70	3.52	3.71	3.15						

[illegible]

Appendices

P	I	2.54	2.03	-0.11	0.25	-0.40	1.33	1.07	-0.07	1.08	-0.14	0.40	1.50	1.77	2.16	2.15	2.37	1.10	0.97	-0.75	0.33
	Q	-1.36	-1.69	-2.91	-2.84	-2.68	-2.41	-1.72	-2.18	-2.94	-2.42	-2.17	-1.73	-2.64	-1.72	-1.70	-1.49	-2.20	-2.30	-1.99	-1.81
	V	2.38	1.09	-0.92	-0.14	-1.19	0.97	0.80	-0.49	0.30	-0.57	0.15	1.19	1.20	1.52	1.74	2.17	0.57	0.44	-1.35	0.22
E	D	3.04	0.68	-1.99	-0.88	-1.47	1.89	-1.43	-0.80	-0.46	-2.38	-0.43	-0.05	2.55	0.57	-0.49	2.48	-0.26	0.22	-1.65	0.84
	I	-0.42	-2.20	-3.63	-2.87	-2.47	-1.39	-3.61	-2.86	-2.79	-3.24	-2.44	-2.46	-1.89	-2.51	-3.07	-1.06	-2.55	-2.78	-3.01	-1.99
	V	2.33	-0.24	-2.77	-2.63	-2.37	0.85	-2.39	-1.78	-1.74	-2.42	-1.36	-0.75	1.25	-0.43	-1.30	1.65	-1.34	-1.17	-2.70	0.17
E	I	3.57	2.10	-1.38	0.34	-0.82	3.00	-0.39	0.89	0.96	-1.03	1.00	1.24	3.44	1.83	0.84	3.45	1.13	1.54	-1.21	2.31
	Q	0.92	-1.67	-3.37	-3.58	-3.82	-0.59	-2.98	-2.09	-1.80	-2.95	-2.22	-2.28	-0.98	-1.95	-2.23	0.21	-2.36	-2.22	-2.90	-1.26
	V	3.78	1.56	-2.13	-0.31	-1.33	2.85	-0.68	-0.43	0.62	-1.97	0.41	0.99	2.56	1.52	0.34	3.15	0.70	1.08	-1.45	1.69
N	D	3.02	0.76	-2.25	-0.87	-1.99	2.19	-1.19	-0.44	-0.76	-1.96	-0.20	-0.16	2.75	0.42	-0.86	2.33	0.13	0.56	-2.04	0.42
	I	-0.33	-2.37	-2.59	-2.83	-3.24	-1.11	-2.22	-3.15	-2.52	-3.32	-1.72	-1.99	-1.63	-2.54	-2.65	-1.26	-2.41	-2.32	-2.71	-1.86
	V	2.04	-0.58	-3.39	-2.08	-2.75	0.77	-2.11	-1.35	-1.51	-2.94	-1.53	-1.44	0.79	-1.40	-1.45	1.06	-1.76	-1.13	-2.21	-0.54
P	I	2.34	0.19	-1.08	-0.89	-1.43	1.21	-0.96	-0.92	-0.49	-1.90	-0.75	0.04	2.44	0.37	-0.44	1.90	-0.10	0.21	-1.21	0.23
	Q	-0.45	-2.12	-2.97	-2.98	-2.66	-2.22	-2.91	-2.64	-2.17	-3.21	-2.87	-2.59	-2.05	-2.29	-1.14	-1.38	-2.53	-2.70	-2.95	-1.90
	V	2.24	-0.07	-1.84	-1.94	-1.79	0.61	-1.30	-1.01	-0.68	-2.20	-1.08	-0.31	1.82	0.25	-0.67	1.44	-0.41	-0.28	-2.06	0.05
F	D	3.41	3.68	0.83	3.18	0.70	2.27	2.85	1.68	2.97	1.93	2.15	3.37	3.25	3.60	3.06	3.44	3.55	3.08	-0.22	1.70
	I	1.20	0.96	-1.79	-0.04	-1.81	-0.67	-0.53	-0.53	0.03	-0.72	-0.88	1.10	-0.45	1.14	0.27	0.99	1.46	0.17	-1.76	-0.74
	V	3.21	2.98	0.28	2.38	0.01	1.57	2.21	1.47	2.50	1.27	1.45	3.41	2.33	3.63	2.76	3.55	3.22	2.35	-0.90	1.00
E	D	3.73	4.00	3.23	3.66	2.33	3.16	3.62	3.48	3.70	3.53	3.58	4.09	3.21	3.53	3.13	3.71	3.67	3.74	0.98	3.91
	I	1.95	2.28	-1.83	1.24	-0.82	-0.11	0.90	0.83	0.95	0.43	0.54	1.62	-0.07	2.09	1.04	2.28	2.63	1.61	-1.64	-0.02
	V	3.92	3.87	2.30	3.81	1.84	3.05	3.18	3.32	3.27	3.04	3.07	3.58	3.55	3.73	3.26	4.02	3.70	3.60	0.69	2.74
N	D	2.85	3.05	-0.30	2.59	0.87	2.22	2.22	1.75	1.81	1.45	1.72	2.90	3.33	2.96	2.02	2.88	2.70	2.40	-1.41	1.47
	I	-0.01	0.07	-2.80	-1.05	-1.23	-0.91	-0.97	-0.30	-0.90	-0.95	-0.90	-0.14	-0.54	0.21	-0.29	-0.29	-0.29	-0.78	-1.39	-1.19
	V	2.13	1.90	-1.08	0.82	-0.54	0.72	1.03	0.41	1.08	0.00	0.29	2.08	1.56	2.13	0.75	2.47	1.42	0.94	-1.06	0.27
P	I	3.23	3.22	2.16	3.27	2.05	2.33	2.79	2.70	2.55	2.65	2.49	3.53	3.31	3.64	2.72	3.64	3.58	3.29	1.20	2.82
	Q	0.99	1.55	-0.01	0.06	-0.21	-0.28	0.17	0.58	0.11	-0.27	0.00	1.74	-0.32	1.84	0.67	1.96	2.40	1.12	-0.47	-0.22
	V	3.48	3.34	1.88	3.26	1.53	2.19	2.75	2.37	2.28	2.02	2.44	3.57	2.80	3.66	2.95	3.41	3.63	3.04	0.74	2.41
G	D	1.53	0.97	-1.37	-0.04	-1.36	-0.33	-1.03	0.46	-1.31	-0.49	0.02	-0.05	-0.18	0.61	-0.66	0.90	0.99	0.92	-1.57	-0.82
	I	-1.81	-1.89	-3.82	-2.93	-2.55	-2.50	-2.66	-2.14	-2.69	-2.03	-2.81	-2.81	-2.53	-2.08	-2.22	-2.29	-2.16	-2.00	-2.00	-2.00
	V	0.92	0.25	-1.61	-0.83	-1.15	-1.16	-1.69	-0.35	-1.77	-1.40	-0.71	-0.79	-1.70	-0.41	-1.57	0.23	0.00	-1.00	-2.62	-1.50
E	D	2.62	2.41	-0.45	1.33	-0.48	0.66	0.85	1.61	-0.28	0.75	1.11	1.10	0.57	1.68	0.43	2.38	2.44	2.37	-1.36	1.11
	I	-0.87	-1.89	-3.22	-2.30	-2.31	-2.32	-1.89	-1.96	-3.06	-2.35	-2.24	-2.62	-2.17	-1.61	-2.21	-2.28	-1.51	-1.81	-2.15	-1.63
	V	2.61	2.06	-0.89	0.96	-0.47	0.22	0.31	1.04	0.02	0.13	0.87	0.85	-0.38	-1.43	0.41	2.38	1.98	1.76	-1.50	0.37
N	D	1.98	1.83	0.65	0.67	-0.67	0.26	0.13	0.81	0.36	0.05	0.42	0.08	0.78	0.87	0.14	1.42	1.40	1.82	-1.24	0.31
	I	-1.71	-1.46	-2.10	-2.61	-1.59	-2.40	-2.35	-1.54	-2.06	-1.30	-1.96	-2.32	-2.41	-2.20	-1.91	-0.95	-1.26	-1.41	-1.66	-1.22
	V	0.89	0.32	-1.64	-0.88	-1.13	-0.82	-1.12	-0.21	-1.86	-0.57	-0.80	-0.84	-0.79	-0.24	-1.25	0.31	0.02	0.13	-1.60	-0.33
P	I	2.62	2.08	0.41	1.30	-0.02	1.19	0.75	1.61	0.22	0.41	1.21	1.23	2.08	1.46	0.48	2.24	1.82	1.84	0.68	1.26
	Q	-1.10	-0.79	-3.10	-1.56	-1.67	-1.65	-1.57	-1.31	-1.82	-2.85	-1.70	-2.39	-2.21	-1.58	-2.48	-0.94	-1.33	-1.15	-1.79	-1.68
	V	2.43	2.19	-0.59	0.86	-0.11	0.74	0.43	1.18	-0.12	-0.02	0.97	1.03	1.17	1.25	0.51	2.11	1.66	1.73	-0.96	1.14
H	D	0.71	2.38	-3.25	-0.01	-0.94	0.85	-0.10	0.43	1.07	-0.45	1.09	0.31	3.14	2.14	1.24	2.56	1.42	1.35	-1.56	-0.49
	I	-2.62	-1.27	-2.41	-2.26	-3.12	-1.67	-2.47	-1.84	-1.39	-2.34	-1.82	-1.79	-1.34	-1.19	-1.28	-1.05	-2.10	-1.48	-2.56	-2.61
	V	2.22	1.19	-2.54	-1.08	-1.66	0.04	-0.87	-0.93	0.33	-0.92	-0.05	0.02	2.05	1.70	0.28	1.86	0.27	-0.15	-2.40	-0.85
E	D	3.37	3.06	-1.69	1.49	-0.03	2.16	1.07	1.52	1.94	1.07	2.39	1.71	3.42	3.44	2.08	3.63	2.83	2.31	-1.10	0.91
	I	0.13	-0.58	-3.07	-2.55	-2.24	-1.80	-2.13	-1.58	-1.47	-2.68	-1.46	-1.47	-0.85	-0.33	-1.47	0.04	-1.37	-1.49	-2.83	-2.48
	V	3.48	2.90	-1.73	0.90	-0.11	1.87	0.99	0.87	2.06	0.32	1.73	1.66	2.90	3.02	2.30	3.52	2.33	1.87	-1.28	0.99
N	D	2.43	2.22	-2.53	0.34	-0.48	1.19	0.19	0.95	0.52	-0.17	0.88	0.54	2.78	2.26	1.03	2.75	1.76	1.54	-1.37	0.17
	I	-0.32	-1.07	-3.09	-2.92	-1.89	-1.54	-1.82	-2.17	-1.55	-2.09	-1.76	-1.80	-0.92	-1.30	-1.40	-0.76	-1.69	-1.27	-2.27	-2.05
	V	1.59	1.23	-2.61	-1.17	-1.26	-0.30	-1.07	-0.55	-0.01	-1.14	-0.14	0.30	1.44	1.22	0.05	1.99	0.45	-0.05	-2.02	-0.86
P	I	3.00	2.58	-0.57	1.25	0.56	1.49	1.11	1.26	1.61	0.62	1.90	2.35	3.49	3.10	2.13	2.77	2.16	2.05	-0.20	0.73
	Q	0.17	-0.39	-2.74	-1.88	-1.78	-1.48	-1.25	-2.23	-1.08	-2.42	-1.31	-0.98	-0.28	-0.13	-0.52	0.18	-1.24	-1.22	-1.77	-1.20
	V	2.75	2.82	-1.26	0.82	0.32	1.16	0.95	0.66	1.32	0.21	1.50	2.16	2.95	2.85	1.90	2.52	1.55	1.50	-0.67	1.41
I	D	1.35	-0.21	-2.15	-0.55	-1.39	1.54	-0.46	-1.30	-0.26	-1.01	-0.73	0.67	3.20	1.04	0.47	0.83	-0.90	-0.76	-1.60	-0.14
	I	-2.32	-2.10	-4.09	-2.02	-2.52	-1.61	-2.22	-2.35	-2.45	-2.33	-2.39	-1.79	0.16	-1.56	-2.42	-1.82	-2.47	-2.69	-3.07	-2.50
	V	0.54	-0.60	-2.08	-1.26	-1.65	0.50	-1.21	-2.23	-1.27	-1.65	-0.98	0.23	2.45	0.02	0.31	0.11	-1.22	-2.17	-2.08	-1.48
E	D	2.07	1.03	-1.21	0.53	-0.82	2.01	0.91	-0.78	1.16	0.10	0.33	1.29	3.79	2.42	1.45	2.00	0.55	0.03	-1.33	0.62
	I	-0.92	-1.70	-3.51	-2.07	-2.79	-1.32	-2.20	-2.30	-1.75	-2.38	-2.35	-1.39	0.71	-1.48	-1.53	-1.17	-2.07	-2.55	-2.32	-1.71
	V	2.35	1.06	-1.36	0.19	-0.57	2.25	0.66	-0.77	1.19	-0.53	0.41	1.65	3.45	1.98	1.45	1.96	0.16	-0.38	-0.83	0.60
N	D	0.65	-0.15	-2.17	-0.68	-1.56	0.51	-0.74	-0.95	-0.70	-1.09										

Appendices

		V	I	N	P	Q	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
		2.04	0.51	-1.80	-0.90	-1.23	0.84	-0.82	-1.34	-0.12	-1.20	-0.61	0.19	1.78	1.17	0.10	1.59	0.34	-0.54	-1.81	-0.01			
	N	0.73	0.15	-1.89	-1.07	-1.30	0.21	-0.92	-0.68	-0.56	-1.42	-0.87	-0.34	1.43	0.81	-0.28	0.82	0.09	0.59	-1.30	-0.14			
	Q	-2.26	-1.60	-2.65	-3.17	-2.09	-1.83	-2.21	-2.11	-2.28	-2.10	-2.21	-2.20	-2.29	-2.06	-2.06	-2.35	-2.73	-1.98	-1.99	-2.02			
	V	-0.17	-0.80	-2.81	-1.89	-1.95	-0.55	-1.65	-1.41	-1.56	-1.97	-1.59	-0.75	-0.31	-0.85	-0.78	-0.39	-1.21	-1.07	-2.11	-1.17			
	P	1.36	0.72	-1.20	0.22	-0.23	0.99	-0.33	-0.38	0.28	-0.41	-0.59	0.40	0.82	0.13	0.26	1.13	0.55	0.20	1.04	-0.47			
	Q	-1.76	-1.92	-3.80	-2.89	-2.53	-0.20	-2.50	-2.46	-2.26	-2.54	-2.57	-2.51	-1.60	-1.69	-1.95	-1.74	-2.56	-2.20	-2.45	-2.12			
	V	-1.14	0.09	-1.51	-0.50	-0.86	0.26	-0.87	-0.74	-0.27	-0.89	-0.62	0.07	0.33	0.80	0.09	0.97	-0.01	-0.44	-1.02	-1.09			
	Q	2.28	1.77	-1.59	-0.44	-1.92	0.64	-0.65	1.77	1.33	0.03	0.58	-0.23	2.92	0.90	0.74	1.43	1.28	1.81	-0.88	-0.81			
	D	-0.92	-1.78	-3.28	-2.99	-2.56	-2.03	-2.60	-1.35	-1.65	-2.36	-1.82	-2.63	-1.02	-2.62	-1.95	-1.63	-1.69	-1.31	-2.60	-2.41			
	V	1.78	0.75	-2.31	-1.35	-2.21	-0.26	1.74	0.85	0.42	-0.55	-0.05	-0.51	2.00	0.08	0.19	1.06	1.00	0.78	-1.17	-1.69			
	E	2.77	2.51	-0.74	0.40	-0.30	1.64	0.03	2.58	2.16	1.13	1.78	0.66	3.03	1.78	2.66	2.24	2.68	-1.93	-2.45	-1.88			
	Q	0.12	-0.57	-3.06	-2.62	-2.80	-1.71	-2.80	-1.71	-2.80	-1.71	-2.80	-1.71	-2.80	-1.71	-2.80	-1.71	-2.80	-1.71	-2.80	-1.71			
	V	3.00	2.33	-3.51	0.09	-1.32	1.57	0.27	2.70	1.67	0.81	1.53	0.45	2.32	1.13	1.93	2.11	2.23	1.90	2.91	-1.26			
	N	1.59	-1.26	-1.71	0.44	-0.86	0.36	-0.51	1.67	0.81	0.23	0.26	-0.49	2.66	0.70	0.15	1.18	1.42	1.58	1.38	-1.26			
	Q	-1.05	-1.10	-3.17	-2.82	-2.01	-1.96	-2.37	-0.84	-1.61	-2.19	-1.32	-2.13	-0.88	-2.04	-1.42	1.46	-1.42	-1.01	-2.42	-2.71			
	V	1.17	0.39	-1.66	-1.99	-2.16	-1.56	-1.58	0.33	0.11	-1.06	-0.63	-0.94	-1.41	-0.32	-0.80	0.34	1.14	0.23	-0.02	-1.70			
	P	2.43	2.25	0.15	0.25	-0.63	1.29	0.44	2.93	2.00	0.82	1.50	0.47	3.40	1.68	1.24	2.12	1.87	2.41	-0.86	-0.42			
	Q	-0.38	-0.62	-2.68	-2.73	-2.28	-1.38	-1.47	-0.79	-0.55	-1.73	-1.34	-1.06	-2.11	-0.83	-0.97	-0.99	-0.69	-2.20	-2.51				
	V	2.71	1.95	-0.46	-0.28	-0.58	1.28	0.29	2.35	1.82	0.58	1.20	0.50	3.18	1.80	0.99	1.88	1.96	2.27	-1.20	-2.30			
	R	-0.30	-1.81	-2.96	-2.70	-2.22	-0.93	-2.49	-1.58	-1.90	-2.01	-2.00	-2.08	3.22	-1.78	-2.48	-1.45	-2.07	-1.52	-2.23	-2.30			
	Q	-3.10	-3.08	-3.25	-3.43	-2.69	-2.77	-2.58	-2.61	-2.83	-2.63	-2.21	-3.21	-0.03	-2.92	-2.84	-2.60	-2.82	-3.40	-2.35	-2.84			
	V	-0.77	-2.51	-2.28	-2.81	-2.73	-1.45	-2.49	-2.70	-2.17	-2.51	-2.56	-2.60	2.82	-2.20	-2.76	-1.88	-2.40	-2.31	-1.86	-1.89			
	E	0.49	-1.04	-2.19	-2.04	-2.51	-0.04	-1.26	-1.31	-1.04	-1.32	-1.57	-1.45	-0.83	-0.91	-1.90	-0.38	-1.58	0.57	-1.94	-1.78			
	Q	-1.85	-2.89	-3.86	-3.12	-1.16	-1.86	-2.86	-2.85	-2.86	-2.99	-2.83	-2.72	0.36	-0.01	-2.13	-2.86	-2.96	-2.42	-2.94	-2.80			
	V	0.79	-0.93	-2.95	-1.96	-1.38	-0.26	-1.97	-1.48	-1.32	-1.26	-1.75	-1.99	3.73	-1.41	-2.46	-0.58	-1.46	-1.13	-1.72	-1.72			
	N	0.38	-1.68	-2.25	-2.54	-1.45	-1.08	-2.21	-1.41	-1.86	-1.50	-1.81	-1.51	2.69	-1.47	-1.87	-1.35	-1.43	-1.80	-1.55	-1.80			
	Q	-1.54	-2.63	-3.22	-3.16	-1.92	-2.73	-2.74	-2.58	-1.94	-2.60	-2.24	-2.26	0.17	-2.84	-2.22	-0.02	-2.72	-2.25	-1.93	-2.23			
	V	-0.85	-1.44	-2.94	-2.95	-1.61	-1.58	-1.75	-2.09	-2.31	-2.42	-2.46	-1.87	2.17	-2.17	-2.55	-2.05	-2.36	-1.84	-1.57	-1.70			
	P	1.21	-0.42	-0.89	0.70	-0.45	0.83	0.75	-0.82	-0.87	-0.89	-0.72	-1.42	3.89	-0.41	-1.28	0.14	-1.25	-0.81	-0.76	-0.27			
	Q	-1.22	-2.21	-2.78	-2.77	-1.80	-0.93	-1.77	-2.14	-2.33	-1.93	-2.26	-1.91	0.05	-1.80	-2.41	-1.86	-1.95	-2.14	-2.02	-1.72			
	V	1.01	-1.10	-0.88	-1.74	-0.62	0.75	-0.99	-1.23	-1.06	-0.92	-1.12	-0.92	3.64	-0.48	-1.41	0.20	-0.87	-1.04	-0.88	-0.32			
	S	2.82	2.40	-0.47	0.79	-1.39	1.02	-0.51	1.22	0.46	-0.06	0.42	1.13	1.72	1.86	1.11	2.44	2.51	1.88	-1.56	-2.08			
	Q	-0.74	-1.59	-3.22	-2.68	-2.78	-1.88	-2.87	-1.92	-2.12	-2.45	-1.95	-1.52	-2.07	-1.48	-1.73	-1.38	-0.90	-1.74	-3.17	-1.87			
	V	2.18	1.07	-1.54	-0.17	-1.67	0.22	-1.71	-0.20	-0.51	-0.10	-0.29	0.46	0.04	0.75	0.26	1.74	1.33	0.71	-2.20	-1.97			
	E	3.42	2.83	0.25	1.83	-0.73	1.94	0.58	1.85	1.56	0.53	1.57	1.89	2.61	2.75	2.05	2.84	3.00	2.64	1.24	1.02			
	Q	0.15	-0.01	-3.08	-1.87	-2.54	-1.82	-2.37	-1.74	-2.11	-2.31	-1.58	-1.10	-1.88	-1.35	-1.28	-0.57	-0.83	-1.50	-2.61	-1.97			
	V	3.37	2.59	0.11	1.44	-1.23	1.72	0.42	1.16	1.28	0.21	1.48	1.68	1.63	2.22	1.92	3.25	2.71	2.10	-1.55	0.72			
	N	2.62	1.89	-0.63	0.67	-1.25	1.05	-0.49	1.05	-0.11	-0.08	0.42	0.76	1.90	1.41	0.74	2.00	2.12	1.67	-1.66	0.45			
	Q	-0.94	-1.27	-2.78	-2.41	-1.95	-1.69	-2.13	-1.49	-1.95	-1.67	-1.95	-1.95	-1.53	-1.80	-1.48	-1.14	-1.36	-1.26	-2.42	-1.59			
	V	1.39	0.36	-1.73	-0.80	-1.91	-0.73	-1.94	-0.61	-0.84	-1.10	-0.64	-0.28	0.15	-1.00	-0.37	1.12	0.69	-0.05	-1.84	-1.11			
	P	2.55	1.77	-0.05	0.90	-0.85	1.09	0.14	0.78	0.97	0.02	0.58	1.17	2.47	2.07	1.31	2.43	2.10	1.46	-0.92	-0.49			
	Q	-0.66	-1.29	-2.91	-2.57	-2.57	-1.36	-2.44	-1.78	-1.36	-1.75	-2.27	-2.02	-1.99	-1.10	-1.07	-0.50	-1.30	-1.17	-2.04	-2.22			
	V	2.43	1.76	-0.80	0.27	-1.02	0.72	-0.05	0.50	0.58	-0.63	0.34	0.63	1.78	1.66	1.10	2.12	1.80	-0.98	-0.98	0.15			
	T	2.75	1.48	-0.26	0.79	-1.61	1.87	0.75	-0.42	0.43	-1.01	0.01	1.03	0.04	1.04	1.80	2.22	3.05	0.68	-1.74	0.31			
	Q	-0.75	-1.42	-3.27	-2.86	-2.44	-1.34	-2.76	-2.40	-2.08	-2.74	-2.23	-2.02	-1.15	-1.75	-1.14	-0.99	-2.02	-2.01	-2.48	-2.56			
	V	2.20	0.64	-1.60	-0.29	-1.94	0.98	-1.44	-1.54	-0.17	-1.96	-0.64	0.30	1.96	0.34	0.95	1.81	0.37	0.41	-1.92	-0.70			
	E	3.22	2.07	0.51	1.44	-0.93	2.37	0.24	0.53	1.53	-0.33	0.62	1.52	3.28	2.05	2.42	2.95	2.13	1.50	-0.90	-0.85			
	Q	-0.18	-1.80	-2.91	-2.51	-2.38	-1.11	-2.39	-2.34	-1.69	-2.92	-2.25	-1.56	-0.81	-2.07	-0.87	-0.55	-1.24	-2.06	-2.40	-1.80			
	V	3.04	2.14	-0.17	1.04	-0.82	2.51	0.15	-0.08	1.52	-0.63	0.63	1.65	1.38	1.86	2.54	2.98	1.36	-1.35	-1.28	0.11			
	N	2.01	0.92	-0.92	-0.17	-1.41	1.01	-0.93	-0.52	-0.22	-1.28	-0.29	0.20	2.86	0.54	0.84	1.66	0.88	0.29	-1.56	-0.20			
	Q	-0.65	-1.44	-2.77	-2.53	-2.20	-1.44	-2.23	-2.39	-1.93	-2.23	-2.01	-1.79	-1.04	-1.90	-1.33	-1.21	-1.88	-2.21	-2.71	-2.24			
	V	2.53	2.02	-1.50	-1.70	-1.01	1.91	-1.50	-1.52	-1.13	-1.81	-1.52	-1.80	3.30	-0.90	-0.22	0.12	0.26	0.12	-0.99	-0.99			
	P	2.38	2.02	0.30	1.29	0.05	1.80	0.38	0.58	0.96	-0.29	-0.74	1.87	2.84	2.95	2.21	1.76	1.51	1.51	1.51	1.51			
	Q	-0.29	-0.83	-3.02	-1.64	-2.21	-0.86	1.63	2.23	-1.39	-1.67	-1.71	-1.26	-1.06	-0.70	-0.28	-0.06	-0.65	-1.26	-1.83	-1.63			
	V	2.60	1.57	0.10	0.67	-0.30	1.86	0.15	0.24	0.94	-1.15	0.44	1.42	2.76	1.75	0.29	2.40	1.92	1.03	0.41	0.94			
	W	2.19	0.36	-1.34	0.10	-1.70	1.79	0.02	-1.16	0.03	-1.19	-0.25	0.84	3.39	1.43	0.70	1.47	0.14	-0.26	-0.20	-0.16			
	D	-0.94	-1.85	-3.14	-2.87	-2.28	-0.92	-2.47	-3.00	-2.06	-2.88	-2.34	-2.44	0.15	-2.11	-2.00	-1.29	-2.27	-2.54	-2.22	-2.56			
	Q	1.27	-0.66	-2.65	-1.28	-1.87	1.03	-0.88	-2.06	-1.10	-1.92	-1.39	0.42	2.76	0.41	-0.39	0.87	-1.36	-1.63	-2.74	-0.64			
	V	2.67	1.19	-1.27	0.65	-0.63	2.80	0.78	-0.31	1.27	-0.37	0.43	1.65	3.50	2.01	1.58	2.30	0.94	0.58	-1.13	-1.54			
	E	-0.38	-1.64	-3.26	-2.58	-2.02	-0.44	-2.52	-1.99	-1.23	-2.59	-1.44	-1.43	-0.47	-0.89	-1.54	-0.93	-2.10	-2.26	-1.93	-2.20			
	Q	3.00	1.20	-1.00	0.87	-0.70	2.25	0.69	-0.71	1.22	-0.44	0.75	1.92	3.28	2.02	1.84	2.81	0.63	-0.04	-1.27</				

Appendices

		P	I	-2.80	-2.10	-2.24	-3.25	-2.15	-2.62	-2.89	-2.42	-2.32	-2.36	-3.00	-2.83	-1.71	-2.52	-2.09	-3.16	-2.65	-2.23	-1.90	-2.53
		S	V	-2.87	-1.86	-2.39	-2.95	-1.11	-2.48	-2.50	-1.57	-2.81	-1.74	-1.95	-2.27	-2.40	-2.94	-2.64	-2.66	-2.85	-2.14	-0.79	-1.59
E	E	I	-3.14	-3.58	-2.81	-3.97	-3.15	-2.78	-3.11	-3.22	-3.22	-4.33	-3.31	-3.45	-2.81	-3.75	-2.93	-3.29	-2.92	-3.10	-2.52	-3.13	
	N	Q	-3.31	-2.95	-3.11	-2.66	-2.42	-2.96	-2.72	-2.76	-3.16	-2.94	-3.42	-2.89	-2.51	-3.03	-2.93	-3.31	-3.57	-3.65	-2.16	-2.35	
	P	I	-2.85	-2.68	-3.04	-2.88	-2.59	-2.91	-2.71	-2.87	-2.64	-2.84	-2.92	-2.74	-2.58	-2.81	-2.83	-2.89	-2.62	-2.94	-2.50	-2.30	
	S	V	-2.87	-2.74	-3.68	-3.79	-3.26	-3.10	-3.58	-3.56	-3.57	-3.61	-2.82	-2.84	-2.45	-3.11	-3.05	-3.70	-2.96	-2.98	-2.54	-2.84	
F	E	I	-1.74	-2.04	-2.40	-1.57	-1.52	-2.37	-1.80	-0.77	-0.95	-1.44	-1.27	-1.97	-0.56	-1.57	-1.12	-1.73	-1.46	-1.28	-2.22	-1.80	
	N	Q	-2.88	-1.66	-3.20	-3.54	-1.60	-2.32	-2.15	-2.00	-2.27	-1.60	-1.62	-2.48	-1.74	-2.25	-1.63	-2.29	-2.44	-2.16	-1.77	-1.86	
	P	I	-1.05	-1.06	-2.01	-1.66	-1.01	-1.43	-1.53	-0.70	-1.12	-0.98	-1.23	-1.25	-0.39	-0.77	-1.38	-1.02	-1.54	-0.82	-0.83	-1.01	
	S	V	-1.90	-1.42	-2.69	-2.66	-1.71	-1.88	-1.76	-1.06	-2.16	-1.53	-1.36	-2.08	-1.40	-2.10	-2.07	-2.27	-2.10	-2.54	-1.31	-1.65	
G	E	I	-2.47	-2.74	-2.80	-1.79	-2.12	-2.35	-2.42	-2.06	-2.60	-2.07	-2.58	-2.26	-1.39	-2.58	-2.55	-2.76	-3.54	-2.29	-1.98	-2.14	
	N	Q	-1.98	-1.57	-2.48	-2.93	-0.83	-1.68	-1.85	-1.23	-1.52	-1.76	-1.72	-1.77	-1.81	-2.08	-1.67	-2.20	-2.58	-2.01	-0.78	-0.91	
	P	I	-1.77	-2.12	-2.38	-2.58	-1.98	-2.07	-1.95	-1.46	-1.94	-2.32	-1.83	-2.55	-0.55	-2.41	-2.58	-2.21	-2.12	-2.14	-1.69	-1.60	
	S	V	-2.75	-2.36	-2.45	-3.03	-1.80	-2.39	-1.74	-2.07	-2.35	-1.88	-1.94	-2.44	-2.25	-2.61	-1.87	-2.61	-2.98	-2.26	-1.38	-1.62	
H	E	I	-2.53	-2.64	-3.15	-3.05	-2.31	-3.19	-3.10	-1.91	-2.38	-2.17	-2.11	-3.64	-0.73	-2.92	-2.00	-2.50	-2.91	-2.10	-2.47	-2.74	
	N	Q	-2.23	-2.25	-2.99	-2.83	-1.94	-2.04	-1.93	-1.90	-2.55	-1.96	-2.12	-2.53	-2.03	-1.94	-1.88	-2.82	-2.72	-3.01	-1.67	-2.01	
	P	I	-1.83	-1.89	-2.43	-2.74	-1.43	-1.59	-1.81	-1.47	-2.12	-1.97	-1.96	-2.39	-0.58	-1.88	-1.65	-2.00	-2.11	-1.76	-1.78	-1.43	
	S	V	-2.20	-2.14	-3.02	-2.83	-1.98	-2.66	-2.79	-2.57	-2.42	-2.22	-2.30	-3.06	-2.18	-1.49	-2.12	-2.30	-2.84	-2.24	-1.87	-2.15	
I	E	I	-2.97	-3.19	-3.55	-2.53	-2.95	-2.76	-2.98	-2.44	-2.41	-2.04	-3.08	-3.18	-1.94	-3.29	-2.75	-3.24	-3.12	-2.55	-3.03	-2.98	
	N	Q	-3.12	-2.48	-3.38	-3.69	-2.43	-2.43	-2.74	-2.42	-2.75	-2.87	-2.72	-2.90	-2.46	-3.35	-2.09	-2.94	-2.81	-2.96	-2.25	-2.46	
	P	I	-1.74	-2.28	-2.64	-3.01	-1.94	-2.23	-2.37	-1.75	-2.43	-1.74	-2.06	-2.23	-1.30	-2.02	-1.99	-2.50	-2.69	-1.78	-2.46	-1.97	
	S	V	-2.90	-2.19	-3.37	-3.21	-1.87	-2.59	-2.18	-2.33	-2.98	-2.46	-2.85	-3.09	-1.55	-3.40	-2.96	-3.33	-2.85	-2.41	-1.88	-2.53	
K	E	I	-3.33	-3.23	-3.59	-2.87	-2.69	-3.78	-3.41	-3.00	-3.89	-2.78	-3.46	-3.13	-2.82	-3.06	-3.16	-3.53	-3.35	-3.48	-2.61	-2.93	
	N	Q	-2.54	-2.69	-3.34	-3.44	-2.12	-2.58	-2.38	-2.69	-3.02	-2.12	-2.81	-2.67	-2.41	-2.93	-2.27	-3.07	-3.13	-3.18	-2.09	-2.53	
	P	I	-2.70	-2.79	-2.83	-3.02	-1.95	-2.49	-2.38	-2.79	-2.72	-2.32	-2.84	-2.63	-1.53	-2.89	-2.89	-2.82	-2.98	-3.16	-2.00	-2.48	
	S	V	-2.64	-2.41	-3.02	-2.75	-2.06	-2.31	-2.88	-2.56	-2.83	-2.34	-2.63	-2.67	-2.07	-3.01	-2.79	-2.98	-2.92	-2.76	-2.12	-2.25	
L	E	I	-3.24	-3.29	-2.94	-4.06	-2.71	-2.92	-2.81	-2.57	-2.81	-3.02	-2.76	-3.16	-2.04	-3.31	-3.34	-3.91	-3.86	-2.37	-2.40	-3.08	
	N	Q	-3.16	-2.54	-3.27	-2.23	-1.40	-2.26	-2.34	-2.28	-2.54	-2.43	-2.47	-2.45	-2.04	-2.36	-2.35	-2.94	-2.60	-2.38	-1.78	-2.15	
	P	I	-2.89	-2.10	-2.39	-2.57	-1.54	-3.15	-2.39	-1.86	-2.45	-2.23	-2.57	-2.68	-1.56	-2.89	-2.23	-2.52	-2.48	-2.07	-1.83	-2.28	
	S	V	-2.94	-2.62	-3.53	-2.95	-1.63	-3.06	-2.60	-2.03	-3.32	-2.30	-2.36	-2.67	-2.22	-2.92	-2.42	-2.80	-2.32	-2.02	-1.92	-2.09	
M	E	I	-3.30	-3.72	-3.44	-3.90	-2.17	-2.92	-2.99	-2.36	-2.26	-2.60	-2.93	-2.34	-1.44	-3.51	-3.08	-3.13	-3.59	-2.84	-2.72	-2.69	
	N	Q	-2.54	-3.01	-2.59	-3.33	-2.06	-3.15	-2.36	-1.84	-2.81	-1.95	-2.65	-2.05	-1.91	-3.06	-2.38	-2.99	-2.96	-2.84	-2.15	-2.45	
	P	I	-2.46	-2.27	-2.09	-1.98	-1.68	-2.17	-2.19	-1.77	-2.02	-2.12	-1.61	-2.77	-0.57	-2.09	-2.68	-2.03	-2.69	-2.40	-1.67	-2.05	
	S	V	-2.48	-2.60	-2.98	-3.64	-1.57	-2.93	-2.22	-1.69	-2.52	-2.55	-2.35	-2.57	-2.00	-2.98	-2.42	-3.22	-2.94	-2.40	-1.88	-2.24	
N	E	I	-2.86	-2.94	-3.39	-3.43	-2.18	-3.26	-2.92	-1.80	-3.25	-2.62	-2.73	-3.29	-2.07	-2.45	-2.55	-2.97	-3.20	-2.74	-1.87	-2.24	
	N	Q	-1.98	-2.27	-2.60	-2.97	-1.35	-2.12	-1.92	-2.07	-2.51	-1.94	-1.83	-2.21	-2.23	-2.59	-1.91	-2.62	-2.84	-2.38	-1.35	-1.56	
	P	I	-2.35	-2.09	-2.81	-2.59	-1.72	-2.65	-2.52	-1.85	-2.18	-2.38	-2.22	-2.59	-1.27	-1.89	-2.03	-2.60	-2.68	-2.05	-1.60	-1.80	
	S	V	-2.87	-2.19	-2.96	-3.47	-2.07	-2.37	-2.34	-1.88	-3.32	-2.65	-2.47	-2.79	-1.99	-3.07	-2.19	-2.97	-2.71	-2.71	-1.54	-1.93	
Q	E	I	-3.18	-3.28	-3.62	-3.38	-2.94	-3.82	-3.24	-2.80	-3.16	-2.58	-2.85	-3.42	-2.11	-3.51	-2.55	-2.97	-3.70	-2.88	-1.48	-2.49	
	N	Q	-2.49	-2.55	-3.09	-3.43	-2.63	-2.62	-2.45	-2.61	-2.65	-2.33	-2.95	-2.48	-2.62	-1.12	-2.67	-2.56	-3.01	-2.24	-2.81	-2.75	
	P	I	-2.17	-2.07	-2.89	-2.44	-1.75	-2.48	-2.38	-1.57	-1.80	-2.09	-1.57	-2.48	-1.33	-2.46	-2.37	-2.13	-2.46	-1.94	-2.09	-1.99	
	S	V	-2.69	-2.43	-2.95	-3.74	-2.44	-2.80	-3.13	-3.11	-2.43	-2.33	-2.75	-2.89	-2.24	-2.99	-2.62	-2.94	-3.47	-2.83	-2.06	-2.05	
R	E	I	-3.03	-3.14	-3.44	-3.60	-2.73	-2.99	-2.87	-3.29	-2.81	-2.77	-2.50	-3.05	-0.38	-2.68	-3.82	-2.29	-3.56	-2.63	-2.39	-2.72	
	N	Q	-2.08	-2.31	-2.75	-2.86	-2.07	-2.45	-2.16	-2.06	-2.12	-2.37	-2.21	-2.13	-1.71	-2.88	-2.34	-2.25	-2.61	-2.67	-1.68	-2.05	
	P	I	-2.31	-2.00	-2.97	-2.69	-1.61	-2.40	-2.10	-2.61	-2.33	-1.91	-1.52	-2.46	1.25	-2.62	-2.14	-2.84	-2.65	-2.63	-1.55	-1.95	
	S	V	-2.73	-2.10	-2.53	-2.98	-2.12	-2.05	-1.87	-2.37	-2.99	-2.40	-2.51	-2.37	-0.81	-2.78	-2.36	-2.40	-2.59	-1.86	-2.19	-3.06	
S	E	I	-2.50	-3.15	-3.22	-3.34	-2.78	-2.51	-2.98	-2.45	-2.70	-2.48	-3.01	-3.26	-2.23	-3.43	-2.32	-2.56	-2.94	-2.87	-2.84	-2.46	
	N	Q	-2.47	-2.61	-3.00	-3.36	-1.75	-2.55	-1.46	-2.24	-2.47	-2.57	-2.93	-2.87	-2.37	-2.21	-2.20	-2.55	-2.84	-3.09	-1.62	-1.90	
	P	I	-1.96	-2.17	-3.07	-3.24	-2.01	-2.32	-2.63	-2.14	-2.29	-1.93	-1.96	-2.66	-1.53	-2.18	-1.70	-2.25	-2.22	-2.66	-1.90	-2.08	
	S	V	-2.57	-2.33	-2.26	-3.80	-1.66	-2.49	-2.60	-2.30	-2.92	-2.71	-2.22	-2.60	-2.39	-2.88	-2.11	-2.83	-2.67	-2.44	-1.76	-2.21	
T	E	I	-2.55	-3.66	-2.64	-2.74	-2.87	-3.29	-3.42	-2.71	-2.87	-2.87	-3.42	-3.42	-1.50	-3.08	-2.38	-3.35	-3.31	-3.45	-2.87	-2.65	
	N	Q	-2.36	-2.89	-3.73	-3.18	-2.15	-2.57	-2.65	-2.78	-3.28	-2.50	-2.67	-2.69	-2.80	-3.72	-1.75	-3.20	-2.82	-3.35	-2.63	-2.22	
	P	I	-2.62	-2.14	-2.53	-1.98	-2.17	-2.26	-2.89	-2.32	-2.49	-1.92	-1.91	-2.59	-1.26	0.47	-1.60	-2.41	-2.70	-2.17	-2.44	-1.85	
	S	V	-2.77	-2.57	-3.46	-3.54	-2.27	-2.76	-2.31	-2.93	-2.50	-2.66	-2.48	-3.21	-1.96	-2.90	-2.58	-3.18	-3.45	-2.74	-3.00	-2.19	
V	E	I	-2.54	-2.63	-3.24	-3.63	-3.25	-2.48	-3.09	-2.87	-2.84	-2.88	-3.49	-3.30	-1.74	-3.72	-3.10	-2.81	-2.85	-3.17	-3.66	-3.41	
	N	Q	-2.91	-2.56	-3.76	-2.83	-2.56	-2.98	-2.87	-1.83	-3.06	-3.											

Appendices

Q	P	I	-1.27	-1.75	-2.24	-2.15	-1.44	-1.29	-1.65	-0.99	-0.95	-0.70	-1.00	-1.40	0.15	-1.19	-0.48	-1.58	-1.47	-1.06	-1.24	-0.44						
	E	I	-2.38	-2.13	-2.60	-2.51	-1.31	-2.63	-2.04	-1.86	-1.45	-1.82	-2.03	-2.49	-0.92	-2.36	-1.29	-2.49	-2.54	-1.87	-2.53	-1.94						
	N	Q	-2.78	-2.87	-2.97	-3.84	-2.55	-2.50	-2.23	-2.14	-2.62	-2.88	-2.70	-3.06	-2.59	-3.00	-2.29	-3.03	-2.95	-2.64	-2.71	-2.41						
	P	I	-1.16	-1.36	-2.22	-1.52	-1.21	-1.32	-1.47	-0.57	-0.46	-0.83	-0.82	-1.29	0.14	-1.12	-1.12	-1.12	-0.94	-0.92	-1.58	-1.18						
R	E	I	-2.12	-2.79	-2.33	-3.18	-2.69	-2.29	-2.51	-2.24	-2.30	-2.06	-2.14	-2.40	0.72	-2.67	-2.55	-2.69	-2.79	-2.29	-2.61	-1.90						
	N	Q	-2.91	-2.52	-2.93	-3.40	-2.04	-2.47	-2.52	-2.46	-2.93	-1.59	-1.95	-2.52	-1.77	-2.77	-2.83	-2.80	-2.38	-2.23	-1.79	-1.40						
	P	I	-0.33	-1.97	-2.22	-2.04	-1.04	-0.96	-1.69	-1.64	-1.18	-1.25	-1.37	-1.21	2.25	-1.69	-1.44	-1.51	-2.10	-1.43	-1.68	-1.24						
	E	I	-3.14	-1.79	-2.49	-2.22	-1.71	-2.11	-1.69	-1.25	-1.43	-1.25	-1.15	-2.03	-1.26	-1.39	-0.90	-1.79	-1.50	-1.53	-1.79	-1.20						
S	N	Q	-2.76	-2.51	-2.79	-3.20	-1.93	-2.49	-2.48	-2.29	-2.70	-2.49	-2.57	-2.89	-2.42	-2.52	-2.48	-2.18	-2.70	-2.31	-1.53	-1.71						
	P	I	-1.15	-1.80	-1.86	-1.73	-1.87	-1.14	-1.39	-0.84	-0.99	-1.44	-1.15	-1.22	-0.35	-0.61	-0.02	-1.08	-1.18	-0.91	-1.19	-1.02						
	E	I	-1.96	-2.09	-2.44	-2.54	-2.76	-2.11	-2.05	-1.49	-1.26	-1.79	-1.67	-1.71	-0.96	-1.67	0.28	-1.63	-2.29	-1.51	-2.51	-1.56						
	N	Q	-2.81	-2.35	-3.40	-3.44	-2.63	-3.04	-2.39	-3.05	-3.13	-2.29	-2.22	-2.48	-1.82	-2.65	-1.99	-3.05	-2.60	-2.58	-2.43	-2.28						
T	P	I	-0.91	-1.25	-1.81	-1.49	-1.97	-0.77	-1.64	-0.91	-0.93	-1.37	-1.02	-0.79	-0.31	-1.04	0.53	-1.06	-1.32	-0.94	-1.17	-1.15						
	E	I	-2.74	-2.24	-3.19	-2.66	-1.54	-1.95	-2.37	-1.27	-0.82	-1.64	-2.03	-1.94	-1.33	-1.91	-1.37	-2.27	-2.79	-1.99	-1.80	-1.74						
	N	Q	-2.20	-2.52	-3.21	-3.77	-2.31	-3.18	-2.02	-2.16	-3.10	-1.99	-2.69	-3.12	-2.10	-2.51	-2.81	-3.75	-2.82	-2.40	-2.38	-1.88						
	P	I	-1.14	-1.37	-2.60	-1.46	-1.69	-1.12	-1.30	-1.07	-0.85	-1.03	-1.40	-1.16	-0.78	-0.71	-0.05	-1.14	-1.58	-0.98	-1.20	-0.88						
W	E	I	2.12	2.26	-0.96	1.10	1.23	0.51	1.52	2.14	3.01	2.35	2.28	2.13	1.93	3.21	2.77	2.27	2.11	2.21	0.59	2.36						
	N	Q	-1.24	-0.86	-2.58	-1.98	-0.78	-1.68	-1.05	-0.27	-0.34	-1.06	-0.90	-1.03	-1.03	-0.60	-0.16	-1.00	-1.18	-0.83	-1.03	-0.59						
	P	I	0.91	1.21	-0.39	0.78	0.18	-0.54	0.26	0.93	1.09	0.40	0.88	1.38	1.37	3.18	1.65	1.67	1.34	1.12	0.85	0.97						
	E	I	0.68	1.05	-1.21	0.60	0.30	0.03	0.97	2.03	1.55	1.36	1.38	0.76	1.85	2.10	-0.07	0.46	1.53	1.59	-0.38	1.15						
Y	N	Q	-2.33	-1.84	-3.04	-2.76	-1.41	-2.24	-2.32	-1.20	-1.75	-1.48	-1.80	-2.40	-1.77	-1.98	-3.29	-1.46	-2.01	-1.41	-1.66	-1.66						
	P	I	-2.63	-1.51	-0.61	-0.43	-1.53	-3.74	-2.12	0.38	-2.23	-0.69	-1.15	-0.85	1.39	1.17	-4.23	-2.72	1.01	-0.43	-0.84	-1.10						
	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y								
146	153	155	147	149	150	154																						
L	F	A	C	A	E	I	1.48	0.81	-3.17	-0.05	-0.22	-0.57	0.04	0.24	0.73	-0.94	0.79	-1.06	-1.40	1.57	1.38	2.18	2.78	1.01	0.40	0.12		
	N	Q	-1.52	-1.75	-2.66	-2.32	-0.96	-1.88	-2.31	-1.92	-1.73	-1.51	-1.78	-1.93	-1.97	-1.86	-1.19	-1.57	-1.32	-1.96	-0.59	-1.56						
	P	I	2.18	1.32	-1.47	0.26	1.24	0.66	0.88	0.57	0.80	-0.17	1.22	0.48	0.52	1.61	1.43	2.58	2.50	0.82	1.26	2.04						
	E	I	1.24	0.60	-3.23	-0.08	-0.17	-0.36	-0.24	0.61	0.93	-0.38	0.78	-1.05	-1.46	1.44	1.57	2.13	2.64	0.95	-0.32	0.16						
C	N	Q	-1.63	-1.96	-2.83	-2.75	-1.02	-1.94	-1.82	-1.49	-1.96	-1.33	-1.94	-1.91	-1.98	-1.86	-1.21	-1.44	-1.56	-1.63	-1.12	-1.00						
	P	I	2.05	1.60	-1.68	0.63	1.20	1.31	0.94	0.76	1.02	0.09	1.35	0.59	0.62	1.64	1.66	2.45	2.25	1.11	0.84	1.61						
	E	I	0.85	-0.78	-3.23	-1.11	0.23	-1.31	0.05	0.26	0.10	-0.22	-0.22	-1.60	-1.54	0.68	1.13	1.56	1.44	0.57	-0.10	-0.08						
	N	Q	-1.83	-2.76	-2.90	-2.86	-0.78	-1.91	-2.72	-1.31	-2.11	-1.34	-2.04	-2.37	-2.15	-2.24	-1.68	-1.85	-2.11	-1.95	-1.15	-1.56						
D	P	I	1.11	0.20	-2.21	-1.26	0.50	-0.47	0.32	-0.16	-0.02	-0.46	-0.02	-0.91	-1.44	0.98	1.16	1.52	1.08	0.16	-0.26	0.60						
	E	I	2.05	0.27	-2.63	-1.73	-0.24	-0.15	-0.80	0.14	0.18	-0.92	0.46	-1.41	-1.66	0.76	0.38	2.30	1.43	0.38	-0.55	0.22						
	N	Q	-1.49	-3.37	-2.83	-2.85	-1.59	-2.38	-2.40	-2.46	-2.52	-2.08	-1.94	-2.35	-2.73	-2.43	-2.15	-1.97	-2.12	-2.50	-1.71	-1.71						
	P	I	1.58	0.31	-2.25	-1.50	-0.43	0.48	-0.62	-0.54	-0.19	-1.26	-0.18	-0.84	-1.22	0.04	0.29	1.80	0.38	-0.40	-0.11	0.41						
E	E	I	3.48	3.38	-1.50	3.00	2.20	1.93	3.03	3.12	3.05	1.90	3.29	2.29	-0.20	3.64	3.06	3.91	3.83	3.68	1.32	2.46						
	N	Q	-0.39	-0.20	-2.26	-1.15	-0.06	-1.29	-0.59	-0.39	-1.22	-0.58	-0.82	-1.35	-1.75	-0.47	-0.19	0.75	0.02	0.45	-0.65	-0.36						
	P	I	3.30	3.55	-0.31	3.20	2.22	2.68	3.11	2.99	2.59	1.82	2.80	3.10	1.38	3.44	2.96	3.75	3.68	3.33	1.70	2.66						
	E	I	0.39	0.07	-1.82	-0.86	0.40	-1.36	0.16	-0.22	-0.37	-0.12	-0.05	-0.95	-1.13	0.54	0.40	0.79	2.01	0.55	0.19	0.36						
G	N	Q	-1.62	-1.97	-2.42	-2.07	0.10	-2.02	-1.42	-1.16	-1.42	-1.20	-1.24	-1.73	-1.98	-2.06	-0.91	-1.76	-1.63	-1.38	-0.18	-0.71						
	P	I	1.82	1.36	-1.35	0.63	1.57	-0.01	1.03	0.91	0.96	0.52	1.09	0.59	0.89	1.75	1.17	2.34	2.51	1.48	1.30	1.84						
	E	I	2.08	1.67	-2.50	0.49	0.55	0.21	1.06	1.14	1.21	-0.03	1.26	-0.15	-0.95	2.07	1.80	3.05	2.75	1.70	0.05	1.21						
	N	Q	-1.34	-2.21	-2.73	-2.24	-1.05	-1.85	-1.50	-1.33	-1.66	-1.37	-1.56	-2.03	-2.02	-1.71	-2.04	-0.79	-2.30	-1.77	-0.62	-1.09						
H	P	I	2.43	2.64	-1.45	1.02	2.13	1.68	2.10	1.08	1.54	0.70	2.15	1.67	1.63	2.38	1.55	3.23	2.60	1.73	1.16	2.27						
	E	I	1.33	0.50	-1.90	-1.05	-0.09	-0.86	-0.04	-0.19	0.28	-1.30	0.34	-1.37	-0.60	1.59	1.07	2.47	0.94	-0.39	-0.52	0.74						
	N	Q	-1.73	-1.90	-2.97	-2.68	-1.04	-1.66	-1.85	-1.43	-1.82	-1.57	-1.80	-2.43	-2.17	-1.92	-1.06	-1.93	-1.94	-1.91	-1.15	-1.30						
	P	I	1.74	1.36	-1.60	0.48	0.86	1.90	0.51	0.22	0.88	0.16	1.08	0.04	1.18	1.71	1.34	1.22	1.27	0.23	0.34	0.96						
I	E	I	-1.76	-1.68	-2.80	-2.43	-1.15	-1.67	-1.94	-2.00	-2.32	-1.80	-2.33	-3.11	-1.47	-2.07	-1.89	-2.02	-2.95	-2.57	-1.90	-1.61						
	N	Q	-2.01	-1.81	-3.11	-2.27	-1.19	-1.81	-1.74	-1.70	-2.06	-1.84	-1.97	-2.06	-1.91	-2.39	-1.59	-1.83	-2.54	-2.11	-1.13	-1.39						
	P	I	-1.13	-1.40	-1.99	-1.55	-0.94	-0.32	-1.27	-2.01	-2.45	-1.57	-1.98	-1.18	0.34	-1.44	-2.20	-0.97	-1.52	-1.68	-0.96	-0.88						
	E	I	-1.39	-1.32	-2.94	-2.41	-0.76	-0.81	-2.13	-1.24	-1.92	-1.22	-1.27	-1.72	-1.36	-1.88	-1.07	-0.99	-1.06	-1.86	-0.73	-1.24						
J	N	Q	-1.95	-1.55	-2.37	-2.40	-0.73	-1.51	-2.20	-1.09	-1.95	-1.35	-1.70	-1.81	-1.86	-2.24	-1.08	-2.02	-2.23	-1.91	-1.34	-1.12						
	P	I	-0.51	-0.03	-1.11	-1.27	0.25	0.11	-0.54	-0.53	-1.26	-0.34	-0.72	-1.05	0.47	-0.85	-0.80	-0.36	-0.86	-0.06	0.18							
	E	I	0.56	-0.16	-2.16	-1.17	-0.16	-0.03	-1.08	-0.73	-0.84	-1.08	-0.72	-1.21	-0.63	0.32	0.18											

Appendices

	Q	0.24	1.14	-2.48	-2.07	-1.29	-0.84	-1.16	0.27	-2.04	-0.59	-0.28	-0.60	-1.75	0.54	-0.53	0.23	0.52	0.42	-1.80	-1.55
	V	-2.10	2.88	0.06	0.33	0.98	-1.47	1.05	2.36	0.57	1.58	1.70	1.89	0.73	2.45	-1.45	2.25	2.55	2.68	0.16	-1.71
N	I	1.77	2.45	0.10	0.31	0.45	0.81	0.27	1.91	-1.32	1.23	1.49	1.70	1.32	1.87	0.55	1.76	2.06	2.14	-0.44	0.57
	Q	-0.13	0.21	-2.37	-2.22	-1.48	-1.74	-1.71	-0.79	-2.33	-1.00	-0.97	-0.88	-1.75	-0.38	-1.64	-0.45	-0.28	-0.11	-1.49	-1.31
	V	0.92	1.96	-1.07	-0.60	-0.35	0.28	-0.31	1.29	1.33	0.38	0.56	0.80	-0.01	1.35	-0.06	0.99	1.43	1.49	-1.27	-0.26
P	I	2.48	2.82	1.77	1.47	1.54	1.85	1.85	2.67	0.12	2.05	2.22	2.33	2.16	2.87	1.80	2.44	2.36	2.68	1.02	1.52
	Q	1.25	2.02	-1.61	-1.26	-0.48	-0.04	-0.41	0.77	-1.87	0.25	0.34	0.13	-1.91	1.23	0.22	1.42	1.49	1.29	-1.19	-0.59
	V	2.48	2.99	1.45	1.40	1.39	1.80	1.65	2.52	-0.23	2.17	2.19	2.32	1.70	2.84	1.79	2.49	2.81	3.00	1.05	1.38
D	D	I	-1.46	-0.97	-2.06	-2.31	-1.91	-2.07	-1.83	-1.66	-2.97	-1.09	-2.07	-1.31	-1.51	-1.71	-1.13	-1.21	-1.66	-1.35	-1.78
	Q	-2.90	-2.91	-2.80	-3.04	-2.54	-3.13	-3.28	-2.62	-3.37	-2.57	-2.92	-3.24	-3.04	-3.81	-2.48	-4.03	-3.15	-2.94	-2.27	-2.57
	V	-1.66	-1.77	-2.88	-2.58	-2.12	-2.37	-2.68	-1.77	-3.42	-1.74	-2.10	-1.63	-2.24	-2.03	-1.77	-2.37	-2.38	-1.73	-2.44	-1.67
E	I	0.79	0.63	-1.41	-0.86	-0.32	-0.60	-0.33	-0.13	-2.01	0.19	-0.41	0.52	-0.24	0.00	0.65	0.44	0.19	0.79	-1.70	-0.23
	Q	-2.18	-2.08	-3.41	-2.99	-2.09	-2.62	-2.51	-2.43	-3.03	-2.76	-2.47	-2.31	-3.14	-2.10	-1.59	-1.95	-2.62	-1.74	-2.23	-2.21
	V	0.43	0.55	-1.51	-0.95	-0.99	-1.35	-0.16	-0.55	-2.09	0.23	0.08	0.17	-1.15	-0.02	0.57	0.13	-0.02	0.43	-1.47	-1.02
N	I	0.44	0.31	-0.94	-0.38	-0.68	-0.73	-0.03	-0.17	-2.13	0.33	0.18	0.54	-0.25	-0.24	-0.12	-0.13	0.02	0.61	-1.38	0.09
	Q	-1.62	-2.28	-2.30	-2.22	-2.03	-2.29	-2.20	-2.35	-3.16	-1.91	-1.92	-2.07	-2.37	-3.20	-2.21	-2.35	-3.02	-2.47	-1.72	-1.71
	V	-0.53	-0.76	-1.80	-1.57	-1.25	-1.65	-1.44	-1.20	-2.78	-0.75	-1.24	-0.44	-1.22	-0.82	-0.99	-1.21	-1.24	-0.34	-1.75	-0.37
P	I	1.79	2.22	1.67	1.14	0.82	0.56	0.89	1.09	-0.26	1.62	1.07	2.30	1.54	1.26	1.60	1.22	1.11	1.99	0.04	1.41
	Q	-1.39	-1.20	-2.59	-2.74	-2.27	-2.45	-1.72	-2.22	-2.90	-1.36	-1.76	-1.24	-2.39	-1.62	-0.90	-1.25	-2.20	-1.53	-2.08	-1.73
	V	1.30	1.46	0.77	0.60	0.46	0.31	0.82	0.67	-0.73	1.33	0.84	2.04	0.89	1.04	1.40	1.35	0.76	1.32	0.78	1.28
E	D	I	-0.16	0.06	-1.31	-1.85	-1.75	-0.91	-0.90	-0.96	-2.83	-1.35	-1.41	0.54	-0.03	-0.79	-0.32	-0.44	-0.72	0.04	-2.03
	Q	-1.76	-1.71	-3.56	-3.06	-3.01	-2.05	-2.61	-2.18	-3.46	-3.38	-2.73	-1.92	-2.56	-2.35	-2.38	-2.52	-2.48	-2.39	-3.42	-1.13
	V	-0.30	-0.51	-1.99	-2.69	-2.16	-1.25	-1.55	-1.62	-2.62	-1.92	-2.05	0.03	-1.24	-1.62	-0.88	-0.52	-1.23	-0.86	-2.20	-0.62
E	I	2.05	1.90	0.27	0.12	0.00	0.90	1.10	1.05	0.53	0.76	0.39	2.35	1.41	1.45	1.59	1.74	1.16	1.88	-0.10	1.06
	Q	-0.15	0.04	-2.06	-2.03	-2.17	-1.37	-1.40	-1.26	-2.32	-1.47	-1.89	-0.04	-1.90	-0.93	-0.08	-0.18	-0.89	-0.74	-2.58	-0.54
	V	1.75	1.91	-0.24	-0.38	-0.36	0.85	1.16	0.92	-0.97	0.00	0.33	2.04	0.68	1.28	1.27	1.48	0.89	1.58	-0.67	1.50
N	I	1.05	1.07	0.24	-0.23	-0.75	0.39	0.16	0.52	-1.82	-0.63	0.05	1.75	0.75	0.89	0.50	0.85	0.43	1.43	-1.38	1.20
	Q	-1.37	-1.67	-2.82	-2.49	-2.52	-2.43	-2.49	-2.49	-2.92	-2.65	-1.83	-1.14	-2.41	-2.23	-1.99	-1.96	-2.92	-1.72	-2.70	-1.48
	V	0.42	0.44	-1.06	-0.92	-1.69	-0.52	-0.57	-0.35	-1.99	-0.82	-0.99	0.84	-0.22	-0.18	-0.48	0.10	-0.56	0.39	-1.55	0.07
P	I	2.39	2.66	2.32	1.72	0.91	1.72	1.84	2.17	0.02	1.70	1.39	2.52	2.23	2.11	1.75	2.38	1.91	2.77	0.84	2.00
	Q	0.35	0.73	-1.11	-0.99	-1.62	-1.41	-0.74	-0.34	-2.13	-0.95	-1.35	1.17	-1.67	-0.60	0.14	0.07	-0.46	0.28	-1.45	-0.42
	V	2.32	2.52	2.05	1.33	0.64	1.30	1.44	1.94	-0.31	1.27	0.98	2.70	1.64	2.09	1.42	2.20	1.74	2.46	0.71	1.90
F	D	I	0.97	1.33	-2.01	-0.34	-0.49	0.35	0.75	-0.51	0.23	0.36	0.15	0.55	1.06	1.67	0.99	0.92	0.34	0.50	-1.19
	Q	-0.62	-0.92	-3.25	-2.48	-2.52	-1.34	-1.81	-2.05	-1.94	-1.40	-2.28	-1.24	-2.00	-0.88	-1.43	-0.98	-1.40	-1.19	-1.95	-1.56
	V	0.95	0.79	-2.49	-1.21	-0.71	-0.26	0.19	-1.10	-0.13	-0.29	-0.29	0.24	0.31	1.31	0.55	0.58	-0.03	-0.22	-1.62	-1.13
E	I	2.55	2.33	-0.41	0.95	1.27	1.61	2.07	1.62	1.37	1.74	1.91	1.75	2.28	2.56	1.66	2.31	1.79	2.20	-0.32	1.44
	Q	0.32	0.33	-3.31	-1.31	-1.17	-0.86	-0.10	-1.15	-0.98	-0.74	-0.71	-0.24	-0.64	-0.59	-0.39	0.26	-0.23	-0.40	-1.63	-1.13
	V	2.35	2.56	-0.85	0.82	1.27	1.51	2.12	1.40	1.26	1.79	2.00	1.84	1.98	2.42	1.69	2.48	1.90	1.92	-0.54	1.43
N	I	1.25	1.37	-1.58	0.37	0.28	0.82	0.97	0.47	0.03	0.55	0.77	0.74	1.81	1.63	0.47	1.20	0.71	1.05	-1.00	0.52
	Q	-0.81	-0.93	-2.98	-1.77	-1.39	-1.18	-1.34	-1.15	-1.59	-1.01	-1.31	-1.42	-1.04	-0.60	-1.12	-0.93	-1.48	-0.93	-1.85	-1.45
	V	0.70	0.64	-2.22	-0.64	-0.44	-0.30	0.37	-0.42	-0.61	0.13	0.03	0.12	0.47	1.06	0.18	0.54	-0.50	0.17	-1.23	0.14
P	I	2.18	2.28	0.30	1.77	1.71	1.63	1.81	1.16	1.20	1.65	1.76	1.86	2.55	2.49	1.51	2.18	1.65	1.64	0.71	2.21
	Q	1.41	1.10	-1.87	-0.94	0.15	0.46	0.50	-0.41	-0.26	-0.25	0.06	0.42	-0.41	1.12	0.27	1.39	0.72	0.25	-0.53	0.39
	V	2.41	2.16	0.21	1.45	1.68	1.80	2.10	0.85	0.95	1.45	1.70	1.89	2.23	2.43	1.72	2.27	1.59	1.64	0.40	2.15
G	D	I	0.96	0.88	-1.78	-0.78	1.74	-0.09	-0.84	0.85	-1.87	0.63	0.14	0.03	-1.38	0.04	-1.24	0.02	-0.07	1.33	-0.60
	Q	-0.73	-0.56	-3.23	-2.84	0.03	-1.47	-2.28	-0.68	-2.73	-0.91	-1.84	-2.38	-2.36	-1.85	-2.52	-1.39	-1.30	-0.59	-1.38	-0.42
	V	1.05	0.85	-2.13	-1.14	1.65	0.38	-0.97	0.81	-2.43	0.28	-0.23	-0.59	-2.07	-0.46	-1.65	0.28	-0.05	0.86	-0.74	1.14
E	I	2.15	2.06	-0.75	0.27	2.58	1.02	0.73	1.73	-1.11	1.79	1.39	1.12	-0.56	1.18	-0.58	1.77	1.76	2.14	0.51	2.58
	Q	-0.25	-0.64	-3.30	-2.25	0.61	-1.44	-1.74	-0.89	-2.15	-0.48	-1.19	-1.66	-2.88	-1.35	-1.92	-1.11	-0.78	-0.17	-1.74	-0.04
	V	1.96	1.86	-0.91	0.05	2.77	0.54	0.43	1.16	-1.12	1.40	1.16	0.42	-1.51	1.12	-0.60	1.58	0.98	2.02	0.58	2.29
N	I	1.79	1.61	-0.41	0.43	2.36	0.90	0.39	1.84	-1.73	1.60	1.13	0.84	0.00	1.10	-0.61	1.16	1.52	2.10	-0.14	2.13
	Q	-0.52	-0.28	-2.27	-2.55	0.24	-1.17	-1.79	-0.62	-2.91	-0.26	-1.43	-2.15	-2.09	-1.73	-2.64	-0.90	-1.24	-0.14	-1.19	-0.12
	V	1.27	1.03	-1.13	-0.61	1.65	0.03	-0.58	0.81	1.45	0.98	0.11	-0.23	-1.20	0.14	-1.27	0.52	0.37	1.18	-0.75	1.31
P	I	2.88	2.74	1.70	1.63	2.88	2.32	1.51	2.55	-0.62	2.19	1.89	1.94	1.84	1.84	0.02	2.49	2.17	2.82	1.47	2.83
	Q	1.77	2.27	-1.63	-0.22	2.09	0.75	-0.37	0.89	-1.74	1.28	0.26	-0.32	-1.98	-0.01	-1.72	1.52	0.87	1.58	-0.01	1.87
	V	2.97	2.81	1.08	1.54	3.11	2.32	1.38	2.50	-0.46	2.02	2.22	2.03	1.27	1.73	-0.12	2.57	2.06	2.72	1.67	2.84
H	D	I	0.13	-0.20	-3.09	-1.51	-1.54	0.58	-1.24	-1.35	-1.85	-1.00	-0.90	-1.82	1.67	-0.02	-0.87	-0.37	-0.89	-0.60	-2.37
	Q	-1.85	-2.17	-2.78	-3.44	-3.14	-1.71	-2.65	-2.79	-2.91	-2.83	-2.77	-3.01	-1.80	-2.11	-2.76	-2.20	-3.05	-2.13	-2.72	-2.54
	V	-0.38	-1.01	-2.23	-2.02	-1.88	0.21	-1.59	-1.78	-2.32	-1.76	-1.84	-2.67	0.64	-0.66	-1.42	-0.85	-1.57	-1.15	-2.69	-1.86
E	I	1.87	1.42	-1.06	1.00	0.20	1.93	0.60	0.49	-0.36	0.46	1.06	0.13	2.38	2.00	0.44	1.62	1.12	1.31	-0.78	0.58
	Q	-0.66	-0.97	-3.09	-2.03	-1.93	-0.53	-1.36	-2.01	-2.50	-1.81	-1.82	-2.04	-1.00	-0.47	-2.08	-1.22	-0.92	-1.38	-0.01	-2.17
	V	1.84	1.89	-1.28	-0.56	0.67	2.01	0.44	0.82	-0.60	0.23	0.51	-0.03	2.63	1.97	-0.47	1.25	1.15	1.22	-0.82	0.93
N	I	1.64	0.66	-1.64	-0.06	-0.52	1.20	-0.01	-0.53	-0.14	-0.10	0.51	1.94	1.05	-0.45	0.55	0.45	0.74	-1.40	-0.29	0.33
	Q	-1.53	-1.86	-2.65	-3.03	-2.18	-1.35	-													

Appendices

M	D	I	2.60	1.57	1.30	1.23	-0.93	1.79	0.77	1.06	-0.57	0.17	0.65	1.92	3.03	2.21	0.61	2.10	1.13	1.30	-0.36	0.88
		Q	0.44	-0.83	-1.83	-1.60	-2.62	-0.32	-1.73	-1.10	-2.03	-1.58	-1.88	-0.59	0.62	-0.23	-1.84	-0.05	-0.83	-1.03	-2.47	-1.09
		V	2.19	0.98	0.12	0.59	-1.08	1.48	0.18	0.17	-0.91	-0.58	0.01	1.30	2.45	1.50	0.01	2.02	0.56	0.71	-0.84	0.35
E	I	I	3.19	2.53	1.98	2.02	0.58	2.73	1.94	2.14	0.58	1.34	1.90	2.76	3.13	2.70	1.34	2.90	2.20	2.50	0.69	1.93
		Q	1.87	0.75	-1.11	-0.36	-1.84	1.13	-0.41	-0.32	-1.03	-1.16	-0.14	0.59	1.41	1.15	-0.26	1.54	0.10	0.30	-1.34	-0.30
		V	2.75	2.45	1.99	1.91	0.50	2.74	1.79	1.89	0.45	1.07	1.84	2.72	3.12	2.77	1.48	2.94	2.09	2.27	0.63	1.90
N	I	I	2.54	1.63	1.62	1.56	-0.41	2.00	0.83	1.36	-0.57	0.33	1.03	1.99	3.24	2.02	0.40	2.22	1.25	1.53	-0.03	1.14
		Q	0.57	-0.81	-1.37	-1.04	-1.97	-0.31	-1.57	-1.03	-2.42	-1.47	-1.08	-0.43	1.13	-0.20	-1.11	0.17	-1.33	-0.84	-1.87	-0.97
		V	2.00	0.81	1.31	1.09	-1.30	1.44	0.35	0.58	-1.39	-0.33	0.12	1.64	2.52	1.65	-0.40	1.89	0.54	0.81	-0.87	0.52
P	I	I	2.94	2.49	2.99	2.81	1.42	2.76	2.01	1.79	0.82	1.27	1.72	2.87	3.09	2.62	1.05	2.91	2.08	2.20	1.30	1.63
		Q	2.36	1.14	0.34	0.71	-0.90	1.52	0.21	0.28	-0.87	-0.25	-0.10	1.47	1.49	1.60	0.32	1.75	0.72	0.65	-0.51	0.29
		V	3.08	2.31	2.93	2.65	1.23	2.81	2.13	2.07	0.66	1.20	2.00	2.92	3.09	2.90	1.25	2.75	1.90	1.97	1.30	2.30
N	D	I	-1.11	-0.82	-2.68	-2.21	-1.91	-1.73	-1.51	-2.08	-2.65	-1.81	-1.85	-1.02	-0.68	-1.45	-2.00	-0.97	-1.19	-1.29	-2.22	-1.74
		Q	-2.80	-2.72	-3.53	-3.78	-2.39	-2.54	-3.03	-2.82	-3.82	-2.94	-3.20	-2.39	-2.82	-2.69	-2.62	-2.24	-2.11	-2.47	-3.36	-3.11
		V	-1.38	-1.60	-3.10	-2.46	-1.91	-1.77	-1.95	-1.96	-3.27	-1.80	-2.37	-1.39	-1.71	-1.80	-2.21	-1.21	-1.41	-1.73	-2.81	-2.07
E	I	I	0.07	0.51	-1.14	-0.59	-0.73	-0.04	-0.03	-0.47	-1.68	-0.74	-0.47	-0.16	0.57	0.53	-0.69	0.78	0.33	0.00	-1.73	0.00
		Q	-2.12	-2.07	-3.67	-3.48	-2.41	-2.33	-2.26	-2.37	-3.11	-3.19	-2.24	-2.50	-2.69	-2.13	-2.86	-1.31	-1.80	-2.04	-2.98	-2.90
		V	0.63	0.10	-1.81	-1.23	-0.61	-0.01	-0.56	-0.94	-2.12	-0.80	-0.54	-0.25	0.03	0.41	-0.81	0.49	0.17	-0.09	-1.59	-0.50
N	I	I	0.00	0.55	-1.09	-0.69	-0.90	-0.54	-0.55	-0.57	-1.47	-0.20	-0.54	-0.39	0.57	0.28	-1.05	0.37	0.29	0.36	-1.29	-0.38
		Q	-2.35	-1.54	-3.16	-2.52	-2.01	-2.16	-2.37	-2.00	-2.17	-1.80	-2.58	-2.19	-2.06	-1.84	-2.45	-1.90	-2.26	-1.72	-2.23	-1.18
		V	-0.92	-0.35	-2.04	-1.77	-1.50	-1.68	-1.05	-1.22	-2.41	-1.26	-1.53	-0.86	-0.91	-1.14	-1.84	-0.67	-0.82	-0.32	-1.10	-1.59
P	I	I	0.95	1.37	0.30	0.48	0.47	0.58	0.59	0.50	-1.08	0.19	0.72	0.98	1.82	1.27	-0.35	1.33	1.69	1.11	-0.01	0.72
		Q	-1.22	-0.54	-2.75	-2.32	-2.27	-1.35	-1.43	-1.74	-2.53	-1.68	-1.97	-1.52	-1.80	-0.98	-1.63	-0.25	-0.69	-1.46	-2.32	-1.42
		V	0.64	1.00	-0.44	0.00	0.11	0.63	0.25	0.01	-1.26	-0.11	0.17	0.77	1.33	0.87	-0.30	1.06	1.21	0.73	-0.18	0.47
Q	D	I	0.98	1.17	-0.18	0.24	0.64	-0.59	-0.60	1.70	-0.88	0.05	1.25	0.36	0.97	1.09	0.07	1.15	0.81	1.28	-0.56	-0.46
		Q	-1.26	-1.59	-2.33	-2.46	-1.37	-2.39	-2.88	-0.95	-3.11	-1.80	-0.88	-2.34	-2.06	-1.35	-1.94	-1.12	-1.23	-1.08	-1.93	-1.91
		V	0.61	0.49	-0.90	-0.81	-0.31	-0.82	-1.09	1.26	-1.46	-0.54	0.63	-0.25	-0.07	0.64	-0.25	0.41	0.03	0.42	-1.54	-0.00
E	I	I	2.34	2.71	1.28	1.73	2.27	1.48	0.88	2.84	0.28	1.74	2.45	1.94	2.63	2.41	1.34	2.23	2.20	2.34	0.52	1.76
		Q	0.31	0.54	-1.83	-0.87	-0.37	-0.96	-1.66	1.08	-1.62	-0.68	0.57	-0.56	-0.67	0.60	-0.70	0.41	0.18	0.40	-1.86	-1.46
		V	2.22	2.23	1.04	1.61	1.67	1.38	0.73	2.65	0.14	1.44	2.44	1.34	1.41	2.21	1.42	2.13	2.01	2.39	0.18	0.85
N	I	I	1.53	1.65	0.97	1.25	1.11	0.36	0.21	2.21	-0.24	0.85	1.28	1.05	1.71	1.62	0.40	1.22	1.32	1.99	-0.44	0.34
		Q	-0.75	-0.97	-2.08	-2.00	-1.62	-1.60	-2.17	-0.52	-2.57	-1.93	-0.73	-1.76	-1.66	-1.09	-1.28	-1.26	-0.95	-0.58	-2.08	-1.84
		V	0.97	0.87	0.02	-0.04	-0.06	-0.58	-0.64	1.44	-1.28	0.01	0.85	0.30	0.74	0.93	-0.40	0.88	0.61	1.22	-1.33	-0.85
P	I	I	2.56	2.53	2.48	2.51	2.30	1.77	1.32	2.84	0.56	1.86	2.51	2.19	2.53	2.41	1.18	2.76	2.33	2.70	1.05	1.61
		Q	0.68	0.81	-0.88	-0.44	-0.12	-0.67	-0.86	1.25	-1.37	-0.57	0.83	-0.12	-0.76	0.91	-0.14	0.66	0.85	0.72	-1.30	-0.99
		V	2.52	2.39	2.37	2.30	2.08	1.81	1.32	2.64	0.36	1.66	2.34	1.90	2.34	2.51	1.08	2.38	2.57	2.75	0.87	1.24
R	D	I	2.34	1.03	0.39	0.37	-0.39	1.79	0.16	-0.06	-0.22	0.93	-0.25	0.42	3.32	0.20	-1.21	0.43	0.50	1.36	-1.13	-0.08
		Q	0.69	-1.12	-2.20	-2.48	-2.20	-0.21	-0.01	-1.76	-2.31	-1.26	-1.88	-2.59	2.12	-1.77	-2.58	-1.10	-1.51	-1.13	-2.44	-1.89
		V	2.13	0.64	-0.45	0.12	-0.68	1.34	-0.68	-0.58	-0.97	0.23	-0.76	-0.16	3.21	-0.26	-1.47	0.87	-0.06	0.47	-1.47	-0.35
E	I	I	2.62	1.83	1.47	1.71	0.58	2.46	0.98	1.17	0.56	1.47	0.88	1.48	3.31	1.38	-0.31	2.01	1.54	1.95	-0.34	0.75
		Q	1.84	-0.23	-1.58	-0.52	-1.04	0.99	-1.14	-1.23	-1.27	-0.18	-1.59	-0.91	2.25	-0.67	-1.47	0.51	-0.60	-0.24	-2.05	-1.00
		V	2.86	1.72	1.33	1.22	0.78	2.33	0.67	0.81	0.47	1.76	0.65	1.07	3.39	1.48	-0.46	2.15	1.42	1.68	-0.45	0.72
N	I	I	1.96	0.53	0.32	0.44	0.33	1.45	-0.45	-0.12	-0.75	0.55	-0.54	0.23	3.05	-0.16	-1.43	0.89	1.21	0.97	-1.05	0.77
		Q	0.51	-1.29	-2.06	-1.76	-1.57	-0.33	-2.14	-1.79	-1.93	-1.25	-1.89	-1.05	2.33	-1.72	-2.34	-0.70	-1.57	-1.19	-2.05	-1.10
		V	1.73	0.29	-0.43	0.02	-0.92	0.86	-0.65	-0.49	-1.40	-0.11	-1.04	0.06	2.94	-0.66	-2.19	0.37	-0.55	0.12	-1.31	-0.44
P	I	I	2.48	1.61	1.92	1.59	0.85	2.28	1.06	0.89	0.42	1.47	0.73	1.12	3.04	1.17	-0.33	1.79	1.07	1.51	0.08	0.99
		Q	1.78	0.36	-0.62	-0.40	-0.78	1.32	-0.52	-1.06	-0.89	-0.10	-1.04	-0.53	2.70	-0.17	-1.13	0.77	-1.05	0.16	-1.49	-0.27
		V	2.44	1.52	1.65	1.50	1.19	2.25	0.97	0.61	0.33	1.32	0.68	1.60	3.27	0.97	-0.25	1.91	0.98	1.54	0.21	1.02
S	D	I	1.40	1.40	-1.11	-1.01	-0.88	-0.14	-0.76	0.38	-1.63	0.77	-0.22	0.27	0.27	0.20	-0.82	0.83	0.78	1.20	-1.60	0.68
		Q	0.12	-0.12	-2.10	-1.87	-1.77	-1.35	-2.04	-1.04	-3.11	-0.18	-1.87	-1.51	-2.54	-2.51	-2.05	-1.09	-0.53	-0.24	-2.65	-0.93
		V	1.14	1.22	-1.55	-1.13	-0.94	-0.44	-0.91	-0.10	-2.39	0.46	-0.74	0.16	-0.90	-0.54	-1.04	0.32	1.04	-1.72	0.22	0.22
E	I	I	2.24	2.56	-0.22	0.22	0.96	1.23	0.78	1.65	-0.76	2.41	1.02	1.31	1.29	1.48	0.49	2.11	1.79	2.49	-0.05	1.69
		Q	0.86	0.66	-2.27	-1.74	-1.30	-0.70	-1.26	-0.50	-2.19	0.79	-0.95	-0.84	-2.17	-0.83	-1.36	0.24	0.45	0.76	-1.88	-0.21
		V	2.57	2.54	-0.32	0.44	0.70	1.24	0.51	1.41	-0.98	2.23	1.15	1.38	0.36	1.32	0.37	2.27	1.63	2.31	-0.32	1.61
N	I	I	1.82	2.11	-0.40	0.44	0.11	0.92	0.19	1.48	-1.29	1.75	0.77	0.98	1.18	1.09	-0.15	1.15	1.81	2.04	-0.46	1.43
		Q	-0.14	0.12	-2.46	-2.44	-1.69	-1.23	-1.52	-0.82	-2.51	-0.16	-1.32	-1.46	-1.62	-1.42	-1.76	-0.71	-0.35	-0.01	-1.91	-0.84
		V	1.12	1.41	-1.22	-0.49	-0.48	-0.48	-0.33	0.36	-1.60	0.63	-0.13	0.12	-0.10	0.19	-1.03	0.71	0.88	1.27	-0.99	0.63
P	I	I	2.70	2.64	1.52	1.41	1.42	1.77	1.50	2.26	-0.46	2.59	1.50	1.97	2.02	1.87	0.56	2.17	2.34	2.72	0.79	2.17
		Q	1.89	2.14	-1.45	-0.61	0.01	0.71	-0.65	0.73	-1.52	1.52	1.00	0.20	-1.73	0.10	0.49					

Appendices

	V	1.33	1.12	-1.78	-0.20	0.11	1.11	0.62	-0.13	-0.20	0.40	0.31	0.92	1.69	1.29	-0.29	1.25	0.05	0.45	-1.14	0.41					
	I	2.38	2.59	1.25	2.43	2.25	2.10	2.10	1.68	1.08	2.19	2.02	2.38	2.84	2.65	0.41	2.54	2.42	2.42	1.00	2.39					
	Q	1.86	2.01	-1.32	-0.02	0.84	1.48	1.13	0.69	0.14	0.19	0.60	0.93	1.30	1.75	0.59	2.04	1.43	1.32	-0.41	0.88					
	V	2.36	2.69	1.16	2.28	2.24	2.22	2.31	1.68	1.14	2.22	2.08	2.32	2.82	2.38	0.83	2.34	2.28	1.18	0.95	2.48					
146	153	155	147	149	150	154	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
F	H	F	A	E	I		A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
	N	Q					-3.76	-3.11	-2.92	-3.77	-2.54	-3.25	-3.07	-2.90	-3.61	-2.84	-2.87	-3.22	-1.94	-2.43	-3.53	-3.17	-3.45	-3.16	-2.63	-2.76
	P	I					-0.60	-2.67	-2.89	-3.11	-2.29	-2.44	-2.62	-2.62	-2.05	-2.36	-2.77	-2.75	-2.51	-3.27	-3.15	-3.08	-2.99	-2.62	-2.43	-2.14
	S	V					-2.13	-2.74	-2.74	-2.80	-2.17	-2.01	-2.07	-1.74	-2.53	-2.36	-1.91	-2.03	-0.84	-1.30	-2.52	-1.87	-2.44	-2.02	-2.03	-2.11
C	E	I					-2.99	-3.20	-3.08	-3.21	-2.40	-3.24	-2.87	-3.00	-2.78	-3.06	-2.86	-3.07	-2.78	-2.96	-2.70	-2.75	-3.27	-2.59	-2.19	-2.97
	N	Q					-2.87	-2.29	-3.46	-2.64	-2.15	-2.18	-3.06	-1.42	-3.24	-2.10	-2.43	-3.13	-1.49	-1.88	-2.10	-2.91	-2.66	-2.09	-2.56	-2.88
	P	I					-2.30	-2.19	-3.04	-3.52	-2.08	-2.34	-2.72	-2.25	-2.48	-2.16	-2.78	-2.94	-2.69	-2.96	-2.25	-3.52	-3.73	-2.85	-2.07	-2.06
	S	V					-1.78	-1.00	-1.92	-1.56	-1.42	-1.73	-1.87	-0.63	-2.00	-1.28	-0.61	-1.70	-0.37	-0.85	-1.58	-1.62	-1.63	-0.73	-1.86	-1.83
D	E	I					-2.95	-2.45	-3.07	-3.52	-2.25	-2.54	-2.93	-1.90	-2.77	-2.22	-2.30	-2.54	-2.65	-2.25	-1.80	-2.74	-2.57	-2.47	-2.49	-2.20
	N	Q					-2.99	-2.42	-3.20	-3.40	-1.61	-3.00	-2.86	-1.78	-3.30	-1.90	-1.84	-3.64	-2.31	-2.98	-2.39	-3.42	-3.30	-2.48	-1.78	-2.48
	P	I					-2.47	-2.79	-2.73	-2.70	-1.82	-2.45	-2.13	-2.16	-2.81	-1.84	-2.43	-2.32	-2.42	-2.64	-2.29	-2.34	-2.39	-2.40	-1.31	-1.71
	S	V					-2.06	-1.60	-2.13	-2.14	-1.79	-2.23	-2.07	-1.80	-2.16	-1.27	-1.76	-1.99	-1.00	-2.48	-1.27	-2.53	-1.81	-1.29	-1.72	-1.76
E	E	I					-2.56	-2.56	-3.06	-2.28	-1.70	-2.85	-2.61	-1.76	-2.61	-2.23	-2.76	-2.48	-2.33	-3.54	-2.32	-2.58	-3.24	-2.20	-1.28	-1.73
	N	Q					-2.68	-2.70	-3.39	-3.46	-2.73	-2.59	-3.12	-3.34	-3.43	-3.14	-3.14	-2.55	-1.95	-2.05	-3.00	-3.41	-2.68	-2.71	-2.71	-2.77
	P	I					-3.36	-2.90	-3.52	-3.40	-2.86	-3.01	-2.58	-3.01	-3.47	-3.01	-3.50	-3.26	-2.73	-3.68	-3.15	-3.65	-3.73	-3.26	-2.44	-2.73
	S	V					-1.62	-1.71	-1.37	-1.58	-2.03	-2.00	-1.68	-1.53	-2.67	-1.79	-1.51	-0.72	-0.99	-1.56	-1.63	-1.75	-1.88	-1.19	-2.35	-1.81
F	E	I					-2.15	-2.83	-3.25	-3.28	-2.98	-3.39	-3.71	-3.43	-3.53	-3.61	-3.20	-3.00	-2.52	-3.55	-3.48	-3.40	-3.06	-2.87	-2.66	-3.03
	N	Q					-1.84	-2.30	-3.18	-2.52	-1.77	-2.60	-2.59	-0.96	-1.71	-1.90	-1.72	-2.38	-0.86	-2.03	-1.47	-2.73	-2.37	-2.03	-2.35	-1.97
	P	I					-2.42	-2.23	-2.94	-3.16	-1.61	-2.79	-2.32	-2.13	-2.31	-1.87	-2.26	-2.23	-1.76	-3.09	-1.97	-2.57	-2.74	-2.26	-2.00	-2.00
	S	V					-0.60	-0.65	-2.39	-1.31	-0.59	-0.97	-1.25	-0.85	-1.02	-0.83	-0.62	-1.60	0.37	-0.75	-1.11	-0.77	-1.34	-1.19	-1.06	-0.89
G	E	I					-2.35	-2.07	-3.04	-3.20	-1.59	-2.33	-2.17	-1.54	-2.50	-1.59	-1.99	-2.79	-1.56	-2.31	-1.98	-2.36	-2.30	-2.02	-1.38	-1.75
	N	Q					-2.98	-2.89	-2.93	-2.17	-2.60	-2.89	-3.09	-2.02	-2.58	-2.33	-2.75	-2.85	-2.17	-2.44	-2.92	-2.77	-3.48	-2.51	-2.02	-2.15
	P	I					-2.08	-1.94	-2.56	-2.82	-1.82	-2.05	-1.76	-2.40	-2.12	-1.75	-2.40	-2.04	-1.71	-2.33	-1.85	-2.19	-2.55	-2.18	-1.20	-1.63
	S	V					-1.54	-1.49	-2.39	-2.07	-1.86	-2.18	-1.81	-1.74	-2.41	-2.15	-2.08	-2.18	-0.89	-2.17	-2.58	-1.64	-2.01	-1.47	-1.60	-1.37
H	E	I					-3.20	-2.59	-2.69	-2.93	-1.84	-2.64	-2.57	-2.06	-2.53	-2.26	-2.04	-2.46	-2.46	-2.80	-2.58	-3.21	-2.58	-1.56	-1.44	-2.06
	N	Q					-2.20	-2.05	-3.35	-2.28	-2.01	-2.50	-2.93	-1.50	-2.58	-2.33	-1.92	-2.88	-0.12	-1.98	-2.04	-2.79	-2.54	-2.00	-2.26	-2.75
	P	I					-2.64	-2.75	-3.64	-3.35	-2.38	-2.40	-2.18	-2.31	-2.84	-2.31	-2.58	-2.46	-2.83	-2.47	-2.36	-3.30	-2.61	-2.76	-1.96	-1.16
	S	V					-0.96	-0.59	-1.84	-0.76	-0.92	-0.95	-1.22	-0.61	-1.66	-1.34	-1.14	-1.73	1.11	-0.88	-1.30	-1.36	-1.31	-0.50	-1.12	-0.87
I	E	I					-2.55	-2.41	-3.45	-2.75	-2.10	-2.71	-2.40	-2.10	-2.46	-2.35	-2.48	-2.72	-1.53	-2.33	-2.45	-2.74	-2.86	-2.34	-2.33	-2.32
	N	Q					-2.31	-2.44	-3.20	-3.24	-1.89	-2.93	-2.69	-1.68	-1.58	-2.23	-2.26	-3.16	-1.09	-1.59	-2.00	-2.20	-2.68	-2.23	-2.79	-2.32
	P	I					-3.27	-2.79	-3.72	-3.89	-2.06	-2.88	-3.18	-2.64	-2.67	-3.16	-2.83	-2.97	-2.72	-2.90	-2.49	-3.07	-3.19	-3.00	-2.90	-2.40
	S	V					-1.40	-1.74	-2.45	-2.05	-1.13	-1.71	-2.04	-1.50	-1.88	-1.67	-0.78	-2.16	-0.56	-1.50	-1.41	-2.06	-1.90	-1.68	-2.26	-1.58
K	E	I					-2.12	-2.80	-3.70	-3.21	-2.16	-3.14	-2.79	-2.43	-2.97	-2.53	-2.99	-3.34	-1.96	-3.44	-3.06	-3.57	-3.14	-2.62	-2.65	-2.68
	N	Q					-3.91	-3.48	-3.60	-3.95	-2.97	-2.92	-3.56	-3.20	-3.52	-2.86	-4.13	-3.42	-2.61	-3.74	-2.93	-3.82	-3.83	-3.36	-2.70	-2.92
	P	I					-3.03	-2.86	-3.88	-3.66	-2.50	-2.70	-3.05	-2.88	-3.28	-2.73	-3.30	-2.88	-2.72	-3.34	-2.56	-3.31	-3.05	-2.72	-2.36	-2.64
	S	V					-3.02	-3.16	-2.67	-2.68	-2.76	-2.14	-3.03	-2.92	-3.14	-3.86	-3.12	-2.71	-1.86	-2.61	-3.36	-3.04	-3.41	-3.57	-2.24	-2.57
L	E	I					-3.05	-3.08	-3.17	-3.88	-2.70	-2.88	-2.65	-2.55	-2.93	-3.30	-3.02	-2.51	-2.23	-3.44	-2.34	-3.65	-3.34	-3.11	-2.25	-2.76
	N	Q					-3.31	-3.16	-3.14	-3.93	-2.17	-2.64	-2.89	-2.93	-3.30	-2.84	-2.56	-2.68	-0.99	-3.53	-2.95	-3.69	-3.33	-2.44	-2.77	-2.56
	P	I					-2.25	-2.66	-3.48	-2.42	-2.13	-2.66	-2.41	-2.50	-2.59	-2.16	-3.40	-2.95	-1.90	-3.09	-2.42	-3.12	-3.07	-2.90	-2.06	-2.86
	S	V					-1.66	-1.87	-2.33	-2.04	-1.01	-2.29	-1.82	-1.52	-2.17	-1.86	-1.54	-2.02	0.03	-2.11	-2.02	-2.03	-2.61	-1.85	-1.64	-1.41
M	E	I					-3.29	-2.65	-3.56	-3.26	-1.85	-2.94	-2.88	-2.76	-3.23	-2.10	-2.72	-2.97	-1.81	-2.54	-2.69	-2.89	-3.22	-2.80	-2.30	-2.46
	N	Q					-1.64	-2.75	-2.40	-2.51	-2.32	-2.19	-2.21	-1.97	-2.42	-2.20	-2.15	-1.82	0.43	-1.95	-2.28	-2.18	-2.78	-2.18	-2.34	-3.02
	P	I					-2.92	-2.91	-2.87	-2.68	-2.04	-3.11	-3.29	-2.10	-3.43	-2.34	-2.56	-2.73	-2.01	-2.92	-2.22	-3.19	-3.09	-3.09	-2.26	-2.70
	S	V					-0.06	-1.24	-0.07	-0.65	-1.41	-0.86	-1.14	-0.86	-1.70	-1.34	-1.01	-0.72	1.61	-0.62	-1.10	-0.60	-1.67	-0.98	-1.25	-1.11
N	E	I					-2.54	-2.83	-3.17	-3.88	-2.70	-2.88	-2.65	-2.55	-2.93	-3.30	-3.02	-2.51	-2.23	-3.44	-2.34	-3.65	-3.34	-3.11	-2.25	-2.76
	N	Q					-3.16	-3.25	-3.79	-3.22	-2.29	-3.60	-3.20	-2.24	-3.55	-2.77	-2.79	-2.95	-1.57	-3.65	-3.13	-3.61	-3.43	-2.71	-2.96	-2.57
	P	I					-2.49	-2.22	-2.94	-2.87	-1.92	-2.34	-2.47	-2.58	-2.62	-2.20	-2.55	-2.63	-2.75	-2.47	-2.24	-2.76	-2.79	-2.77	-1.73	-2.02
	S	V					-3.41	-1.84	-2.81	-2.19	-1.43	-2.25	-2.24	-2.15	-2.18	-1.94	-1.92	-1.94	-0.69	-1.59	-2.04	-2.63	-2.08	-1.58	-1.67	-1.47
Q	E	I					-2.35	-2.67	-3.74	-2.97	-2.34	-2.88	-2.40	-2.03	-2.42	-2.36	-1.98	-3.10	-3.09	-3.32	-2.50	-3.33	-2.80	-2.91	-2.07	-1.90
	N	Q					-2.87	-2.57	-2.98	-2.70	-1.83	-2.95	-2.51	-2.03	-2.99	-2.36	-2.07	-2.83	-2.16	-2.65	-2.93	-2.91	-3.44	-1.74	-1.85	-2.61
	P	I					-3.54	-2.74	-3.82	-3.48	-3.20	-2.57	-2.63	-2.91	-2.89	-3.01	-3.76	-2.93	-3.05	-2.56	-2.83	-3.25	-3.92	-3.40	-2.89	-2.90
	S	V					-1.64	-1.29	-1.51	-1.44	-1.09	-1.99	-1.75	-0.70	-1.82	-1.72	-1.03	-1.21	-0.47	-1.14	-1.62	-1.37	-1.64	-1.03	-1.59	-1.61
R	E	I					-2.89	-2.63	-3.18	-3.86	-2.17	-3.17	-2.78	-2.98	-3.18	-2.94	-2.74	-2.68	-2.97	-3.03	-2.78	-2.97	-3.4			

Appendices

H	E	I	0.16	-0.37	-2.11	-0.90	-0.51	-0.11	-1.35	0.42	-1.13	-0.37	-0.43	-1.48	0.31	0.52	-0.39	-0.91	-0.41	-0.03	-1.67	-1.27
	N	Q	-2.55	-2.09	-3.52	-3.54	-2.02	-2.17	-2.84	-1.74	-2.92	-2.01	-2.08	-3.06	-2.18	-2.30	-2.27	-3.22	-2.37	-1.72	-2.12	-2.29
	P	I	0.40	0.25	-0.27	1.46	-0.74	0.30	0.00	0.96	-1.19	0.53	-0.30	-0.21	1.88	1.45	-0.52	0.37	0.59	1.13	-0.13	-0.33
I	E	I	-1.38	-0.86	-2.89	-2.61	-0.25	-1.89	-1.24	0.14	-0.72	-0.53	-0.24	-1.61	-0.94	0.43	-0.61	-1.31	-0.79	-0.19	-1.72	-1.06
	N	Q	-3.02	-2.29	-3.58	-3.08	-2.07	-3.62	-2.85	-1.83	-3.34	-2.23	-2.57	-2.66	-2.96	-2.67	-2.08	-3.35	-2.95	-2.46	-2.32	-2.06
	P	I	-0.29	-0.16	-1.54	-0.77	0.39	-0.78	-0.55	1.08	-0.89	0.26	0.72	-0.25	-0.14	1.04	0.04	-0.08	0.37	-0.53	-0.17	-0.42
K	E	I	-3.25	-3.46	-3.24	-4.15	-3.42	-2.84	-2.62	-3.06	-3.65	-3.30	-3.65	-3.38	-2.58	-2.92	-3.00	-2.47	-3.14	-3.66	-3.01	-3.12
	N	Q	-3.78	-2.50	-3.53	-3.30	-2.70	-2.99	-2.92	-2.71	-3.72	-2.92	-3.43	-3.00	-2.67	-3.25	-3.61	-3.70	-3.23	-2.84	-2.97	-2.73
	P	I	-2.65	-2.84	-1.62	-2.81	-2.21	-1.81	-2.06	-2.53	-2.60	-2.70	-2.78	-1.48	-1.58	-2.12	-2.36	-2.13	-2.69	-3.13	-2.79	-2.42
L	E	I	-1.36	-1.92	-2.97	-2.18	-1.04	-1.71	-1.80	-0.45	-1.61	-1.54	-1.69	-1.84	-0.31	-1.27	-1.89	-1.64	-2.48	-1.30	-1.93	-1.31
	N	Q	-2.91	-3.08	-2.91	-3.41	-1.91	-3.43	-2.64	-1.87	-2.46	-2.15	-2.18	-3.00	-2.46	-2.91	-2.40	-2.96	-3.37	-2.51	-2.03	-2.59
	P	I	0.01	-0.43	-1.36	-0.69	0.14	-0.79	-0.54	0.00	-0.45	0.08	0.36	-0.59	0.58	0.19	-0.21	-0.08	-0.53	-0.10	-0.50	-0.70
M	E	I	0.00	-1.29	-2.10	-1.22	-1.00	-0.77	-1.36	-0.31	-0.89	-1.18	-0.70	-0.58	0.57	-0.41	-0.69	-0.38	-1.25	-0.82	-1.68	-1.59
	N	Q	-2.33	-2.97	-3.54	-2.97	-2.20	-2.41	-2.60	-1.79	-2.88	-2.11	-2.87	-2.77	-2.36	-3.00	-2.20	-2.57	-3.02	-2.22	-2.14	-2.05
	P	I	1.16	0.24	0.88	0.39	-0.10	0.92	0.30	0.62	0.08	-0.10	0.45	0.83	1.39	1.17	0.22	1.18	0.06	0.52	0.04	0.15
N	E	I	-2.13	-1.89	-2.39	-2.09	-0.83	-2.30	-2.30	-1.52	-1.89	-1.45	-1.79	-2.33	-1.06	-1.83	-1.19	-1.77	-1.78	-1.26	-1.77	-1.77
	N	Q	-2.84	-2.79	-3.42	-3.61	-1.90	-2.72	-2.35	-2.54	-2.87	-2.72	-2.65	-2.71	-2.66	-3.25	-2.17	-3.04	-2.78	-2.18	-1.98	-1.71
	P	I	-0.54	0.06	-1.07	-0.57	0.51	-0.69	-0.91	0.35	-1.09	0.17	-0.16	-0.82	0.17	0.03	-0.44	-0.53	0.20	0.38	0.23	0.53
Q	E	I	-1.28	-1.07	-2.16	-1.55	0.61	-1.44	-1.51	0.09	-1.04	-0.46	-0.50	-0.77	-1.07	-0.48	-1.08	-1.05	-1.13	-0.28	-0.97	-0.66
	N	Q	-3.01	-2.71	-2.81	-3.68	-2.29	-2.70	-3.07	-2.61	-3.68	-2.81	-2.43	-3.08	-3.05	-3.19	-2.65	-2.54	-3.05	-2.52	-3.02	-2.89
	P	I	0.23	0.44	0.20	0.04	1.79	-0.11	-0.24	1.09	-0.70	0.78	1.11	0.72	0.38	0.85	-0.60	0.50	0.57	1.01	0.31	0.60
R	E	I	0.84	-0.65	-1.50	-1.03	-1.20	-0.08	-1.24	0.59	-0.88	-0.18	-1.11	-1.31	2.22	-0.71	-1.21	-0.23	-1.07	-0.25	-1.89	-1.07
	N	Q	-2.15	-2.71	-2.99	-3.56	-1.70	-2.27	-2.74	-2.46	-2.57	-1.86	-2.43	-2.38	-0.70	-2.68	-3.34	-2.47	-2.81	-2.54	-2.22	-1.98
	P	I	1.65	0.26	-0.46	0.04	0.04	1.14	-0.19	0.18	0.23	0.76	-0.20	0.44	2.29	0.29	-0.70	0.56	-0.41	0.64	-1.05	-0.23
S	E	I	-1.24	-0.64	-2.10	-1.90	-0.91	-1.51	-2.12	0.18	-1.69	0.78	-0.87	-1.69	-1.18	-0.43	-1.43	-1.36	-0.78	-0.26	-1.47	-1.28
	N	Q	-2.43	-2.83	-2.59	-3.45	-1.91	-3.18	-2.84	-2.25	-2.73	-1.96	-2.32	-2.61	-2.51	-2.81	-2.43	-3.16	-2.31	-2.12	-2.07	-1.74
	P	I	0.65	0.84	-0.86	-0.13	-0.48	0.02	-0.64	1.06	-1.71	1.44	0.31	-0.11	0.45	1.00	0.10	0.57	0.78	1.53	0.10	0.15
T	E	I	-2.00	-1.10	-2.87	-2.69	-1.26	-1.41	-1.63	-0.73	-1.76	-0.94	-1.03	-1.43	-1.05	-0.80	-0.05	-1.23	-1.44	-0.70	-1.55	-0.90
	N	Q	-2.79	-2.98	-3.44	-3.95	-2.74	-3.26	-2.80	-2.61	-3.06	-3.15	-3.36	-3.01	-2.61	-3.56	-1.95	-2.89	-3.10	-2.33	-2.16	-2.33
	P	I	-0.09	0.55	-0.84	-0.52	0.08	0.00	-0.30	0.96	-1.19	0.36	0.49	0.00	0.54	0.50	0.37	0.39	0.51	1.15	0.29	0.49
V	E	I	-1.15	-0.67	-2.80	-2.09	-0.70	-1.74	-2.13	0.06	-1.16	-0.14	-0.32	-1.81	-1.28	0.04	-0.92	-1.63	-0.88	0.14	-1.48	-1.15
	N	Q	-2.32	-2.59	-3.62	-3.61	-2.14	-3.93	-2.69	-1.92	-2.88	-1.99	-2.78	-3.35	-2.78	-2.85	-2.66	-3.63	-2.85	-2.56	-2.42	-2.41
	P	I	-0.68	0.31	-1.26	-0.62	-0.21	-0.77	-0.76	1.04	-1.05	0.85	0.34	-0.31	-0.52	0.94	0.03	-0.28	0.10	0.92	-0.28	-0.34
W	E	I	0.58	1.05	-1.96	-0.60	0.27	-0.97	0.03	1.26	1.02	1.18	1.04	0.29	0.52	1.15	0.93	0.63	0.42	1.14	-0.36	0.17
	N	Q	-1.76	-1.83	-2.72	-2.74	-1.33	-2.14	-1.56	-0.51	-1.77	-1.29	-1.51	-1.80	-1.71	-1.46	-1.16	-0.99	-1.37	-1.22	-1.73	-1.58
	P	I	1.02	0.97	-0.24	1.23	-0.02	-0.29	-0.04	0.83	-0.41	0.53	0.90	0.72	1.24	1.53	-0.52	1.28	0.43	0.92	0.43	0.12
Y	E	I	0.76	1.13	-1.95	-0.33	0.31	0.31	0.02	1.32	0.55	0.97	1.08	-0.59	1.39	1.50	-0.63	0.32	1.39	-0.88	-0.52	-0.52
	N	Q	-2.36	-1.97	-3.52	-3.01	-1.74	-2.27	-2.01	-1.27	-2.00	-2.01	-1.71	-2.48	-1.82	-1.81	-2.60	-1.47	-1.89	-1.97	-1.93	-1.84
	P	I	-2.69	-0.72	-0.04	0.93	-1.00	-2.65	-1.75	0.89	-2.49	-0.09	-0.68	-0.61	2.14	0.98	-3.95	-1.67	1.00	0.58	-0.27	-0.99
A			C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	
146			153	155	147	149	150	154														
L	E	I	0.35	-0.03	-2.97	-1.36	0.24	-1.38	0.86	-0.53	-1.07	-1.12	0.10	-0.69	-1.59	0.63	0.16	0.73	1.55	-0.22	1.23	0.54
	N	Q	-1.89	-2.59	-3.34	-2.97	-1.37	-2.30	-2.09	-1.95	-2.37	-2.08	-1.95	-2.38	-2.39	-2.28	-1.86	-2.02	-1.63	-2.32	-1.31	-1.58
	P	I	2.05	1.83	-0.77	0.18	2.27	0.53	2.59	0.97	-0.21	1.18	1.43	1.93	0.58	1.86	1.18	2.48	2.51	1.48	2.58	2.52
C	E	I	1.00	1.13	-2.73	-0.57	1.11	-0.75	1.51	1.54	-0.18	0.33	1.45	0.05	-1.16	1.60	1.37	1.93	2.65	1.53	0.93	1.68
	N	Q	-1.86	-1.90	-2.80	-2.70	-1.27	-1.87	-1.70	-1.03	-2.07	-1.48	-1.57	-2.13	-2.47	-1.92	-1.27	-1.69	-1.36	-1.96	-1.18	-0.85
	P	I	2.80	2.79	0.29	1.36	2.77	1.99	3.06	2.57	0.93	2.15	2.86	2.73	1.35	2.76	2.28	3.00	3.14	2.59	2.47	3.16
D	E	I	-1.61	-1.75	-2.79	-2.74	-0.54	-2.60	-1.48	-0.72	-1.79	-0.99	-1.03	-2.01	-2.10	-1.53	-0.62	-1.04	-0.73	-0.91	-0.51	-1.14
	N	Q	-2.75	-2.85	-3.00	-3.00	-1.66	-2.25	-2.72	-1.44	-2.57	-1.72	-2.02	-2.58	-2.88	-2.96	-1.72	-2.47	-2.41	-2.20	-1.26	-1.83
	P	I	0.65	0.64	-2.01	-0.75	1.13	-0.83	0.52	0.86	-0.53	0.24	0.59	-0.14	-1.15	0.58	1.11	1.19	1.70	0.97	0.68	1.46
E	E	I	0.46	0.01	-2.57	-2.20	-0.02	-1.15	0.08	-0.14	-0.53	-0.61	-0.24	-0.79	-1.97	0.77	2.02	1.25	1.66	0.29	-0.31	0.55
	N	Q	-2.33	-2.52	-3.26	-3.12	-2.21	-2.59	-2.86	-2.50	-3.09	-2.47	-2.34	-1.66	-2.97	-2.38	-1.63	-2.05	-1.52	-2.68	-1.79	-1.83
	P	I	2.17	1.98	-1.18	0.35	1.74	1.10	1.91	1.37	0.96	0.68	1.22	1.24	-0.15	2.16	2.73	2.42	2.64	1.81	1.46	2.47
F	E	I	1.63	2.01	-2.23	-0.49	1.44	0.28	2.05	1.03	0.66	0.43	1.56	0.77	-0.60	1.96	1.36	2.53	2.59	1.38	0.56	1.41
	N	Q	-1.06	-1.04	-2.24	-2.42	-0.74	-1.61	-1.24	-0.78	-1.74	-0.70	-1.29	-1.92	-1.52	-1.43	-0.65	-0.75	-1.34	-1.39	-1.19	-0.84
	P	I	2.44	2.75	0.11	1.47	2.44	2.03	2.60	1.55	1.20	1.44	2.05	2.11	1.52	2.33	1.81	2.79	2.49	1.66	1.78	2.34
G	E	I	-0.78	-0.97	-2.67	-1.59	0.63	-1.97	-0.67	-0.44	-1.60	-0.64	-0.86	-1.47	-2.14	-0.46	-0.38	-0.28	0.52	-0.45	0.39	0.48
	N	Q	-1.84	-1.87	-2.38	-2.15	-0.76	-2.06	-1.43	-1.48	-1.65	-1.32	-1.68									

Appendices

		A	C	D	F	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
F	A	-1.63	-1.29	-1.96	-1.41	-0.37	-1.41	-1.88	-0.19	-1.46	-0.69	-0.10	-1.89	0.08	-1.37	-0.63	-0.93	-2.10	-1.82	-0.54	-0.55
	Q	-1.70	-1.62	-0.90	-1.83	-0.98	-0.82	-1.88	-1.59	-1.67	-1.15	-1.19	-2.09	-1.41	-2.00	-1.18	-2.27	-1.33	-0.59	-0.09	-0.69
	D	-1.84	-1.16	-1.38	-2.01	-1.78	-1.62	-1.09	-0.07	-0.72	-1.40	-1.49	-1.67	-0.75	-1.50	-1.06	-1.35	-1.18	-1.53	-0.42	-1.00
E	I	-1.49	-1.34	-1.95	-2.36	-0.56	-1.04	-2.44	-1.38	-2.26	-1.08	-1.37	-1.65	-0.55	-2.83	-0.78	-1.57	-3.61	-1.09	-0.35	-0.29
	Q	-1.70	-1.73	-0.90	-2.25	-0.26	-3.27	-2.44	-1.25	-2.25	-1.95	-1.97	-1.94	-2.61	-1.66	-1.84	-2.63	-2.15	-1.09	-0.82	-1.24
	V	-1.67	-1.75	-1.13	-2.71	-1.11	-1.53	-1.72	-1.09	-2.17	-1.64	-0.27	-1.68	-0.51	-2.28	-1.18	-1.42	-0.54	-1.54	-0.09	-0.20
N	I	-1.20	-0.21	-2.57	-0.91	0.46	-0.59	-0.11	-0.16	0.55	0.01	-0.70	-0.93	0.64	-0.83	0.51	-0.94	-0.83	-0.72	0.62	0.46
	Q	-1.06	-0.89	-1.40	-1.03	0.02	0.42	-0.66	-0.56	-0.71	0.09	-0.36	-1.03	-0.24	-1.10	-1.62	-1.35	-0.96	-0.23	0.53	0.28
	V	-0.58	-0.63	-0.89	-1.17	-0.02	0.92	-0.49	0.20	1.09	-0.40	-0.54	-1.04	0.68	-1.52	-0.92	-0.98	0.06	-0.74	0.72	-0.06
P	I	-0.37	-0.10	-0.43	-0.36	0.58	-0.05	-0.06	0.44	-0.85	0.42	0.17	0.02	0.73	-0.14	-0.51	-0.28	-0.49	0.13	0.91	0.97
	Q	-0.79	-0.90	-1.96	-2.46	0.25	-0.57	-0.17	-0.54	-0.58	-0.46	-0.56	-4.60	-1.26	-1.13	-0.41	-3.94	-0.76	-0.18	0.14	-0.61
	V	-0.36	0.00	-0.50	-1.12	0.78	-0.09	0.03	0.01	0.07	0.23	0.02	-0.70	0.52	-0.70	-0.01	-0.33	0.04	-0.17	0.68	-0.30
C	D	-1.75	-1.58	-0.87	-2.49	-0.95	-0.77	-1.73	-0.63	-0.45	-0.81	-0.72	-0.93	-1.41	-1.67	-0.29	-1.77	-1.95	-0.70	-0.37	-0.25
	Q	-1.61	-2.58	-2.20	-2.94	-0.58	-0.62	-2.36	-1.32	-1.93	-1.68	-1.13	-1.99	-1.88	-1.82	-0.78	-3.38	-1.67	-1.07	-0.79	-0.20
	V	-2.64	-0.92	-2.00	-1.74	-0.23	-2.28	-1.94	-0.48	-2.40	-1.29	-2.13	-1.74	-0.92	-1.38	-1.26	-1.92	-2.02	-1.34	-0.42	-0.26
E	I	-0.98	-0.88	-2.47	-1.56	-0.52	-1.28	-1.20	-0.62	-1.93	-1.30	-0.66	-1.48	-0.86	-1.29	-1.55	-1.45	-1.42	-1.19	-0.69	-1.40
	Q	-1.22	-1.69	-2.46	-2.32	-1.34	-1.11	-2.29	-0.63	-1.17	-2.18	-1.98	-3.27	-1.73	-1.75	-1.76	-2.81	-2.06	-1.52	0.23	-1.70
	V	-2.41	-0.38	-1.36	-1.87	-0.72	-1.03	-1.52	-0.72	-2.04	-0.84	-1.15	-2.14	-0.85	-1.93	-1.59	-2.84	-0.98	-1.06	-1.27	-0.53
N	I	-0.64	0.03	-0.52	-1.33	0.26	-0.47	-0.50	0.52	-1.73	-0.33	-0.50	-2.09	0.37	-0.34	-1.29	-0.65	-0.62	0.43	0.33	0.75
	Q	0.01	-0.51	-1.19	-1.97	-0.25	0.43	-0.45	0.03	-1.45	-0.83	-0.44	0.62	0.27	-1.59	0.72	-0.20	-0.88	0.05	0.72	0.16
	V	-1.39	0.08	-1.03	-1.57	-0.02	-0.22	-0.09	-0.05	-0.88	-0.67	-0.19	-0.72	0.07	-1.26	-0.34	-1.23	-1.46	-0.35	0.45	0.29
P	I	0.26	0.18	-0.07	-0.71	0.65	-0.54	-0.31	0.58	0.58	0.15	0.11	0.07	1.12	0.44	0.05	-0.25	-0.03	0.50	1.11	1.04
	Q	-0.46	-0.52	-1.19	-1.87	0.01	0.82	-0.13	-0.09	-1.57	-0.27	0.23	-0.18	-1.28	-1.72	-0.51	-2.03	-1.26	-0.33	0.55	0.04
	V	-0.02	-0.13	-1.26	-0.77	0.84	0.31	-0.10	0.74	0.00	-0.04	0.02	-0.68	0.71	-0.11	-0.02	-0.15	-0.26	-0.16	0.51	0.49
D	D	-1.46	-1.03	-0.58	-1.76	0.72	-0.87	-1.40	0.22	-1.62	0.17	-1.89	0.81	-0.65	-2.05	-2.67	-2.18	-1.38	-0.98	0.48	0.67
	Q	-1.58	-0.79	-0.67	-1.39	0.16	-1.30	-2.12	-0.66	-3.30	-0.44	-0.08	-1.29	-0.96	-2.78	-1.35	-3.58	-2.04	-1.39	0.11	-0.09
	V	-0.80	-1.43	-0.89	-1.90	0.22	-1.29	-1.48	0.23	-2.32	0.02	-0.77	-1.44	-1.63	-1.29	-1.04	-2.42	-1.89	-2.20	0.38	0.50
E	I	-1.04	-1.82	-0.99	-1.08	-0.24	-1.48	-2.14	-0.48	-1.23	-0.70	-0.79	-1.89	-1.19	-1.86	-1.44	-2.09	-0.95	-1.17	0.35	-0.58
	Q	-1.57	-1.78	-1.02	-2.75	-0.14	-2.00	-1.10	-0.72	-1.26	-0.48	-1.54	-2.41	-2.95	-1.66	-1.61	-1.58	-2.93	-1.32	-0.24	-0.28
	V	-2.21	-1.37	-1.18	-2.41	-0.10	-0.91	-1.66	-0.72	-1.52	-0.07	-0.78	-0.93	-1.29	-1.95	-1.47	-2.46	-1.79	-1.19	0.15	-0.27
N	I	-0.75	-0.72	-0.50	-1.45	0.79	-0.60	-0.60	-0.25	-1.39	0.16	0.21	0.48	-0.23	-0.85	0.66	-1.52	-1.96	-0.41	0.83	1.00
	Q	-0.41	0.15	0.29	-1.18	1.07	0.46	-0.70	-0.12	-1.06	0.24	-0.46	0.15	0.14	-1.07	-0.39	-0.79	-1.54	-0.59	1.37	1.13
	V	-1.67	-0.57	-0.18	-1.06	0.44	-0.95	-1.92	0.18	-1.21	0.16	-0.56	-1.44	-0.13	-0.93	-0.86	-2.33	-1.74	-0.75	0.47	0.72
P	I	-1.23	-0.47	-1.14	-0.78	0.03	-0.89	-1.25	-0.28	-0.59	-0.44	-0.40	0.87	-0.06	-1.01	0.89	-0.85	-0.99	0.01	-0.04	-0.29
	Q	-1.43	-1.52	-2.05	-1.91	0.22	-1.38	-0.86	-0.60	-2.69	-0.13	-1.60	-2.64	-1.88	-0.89	-0.90	-2.20	-1.87	-1.51	-0.20	-0.32
	V	-1.02	-0.43	-1.02	-2.01	-0.95	-1.24	-1.03	0.28	-1.22	-0.54	-0.53	-1.19	-0.59	-1.01	-0.57	-0.68	-1.14	-0.20	-0.19	-0.38
E	D	-2.01	-2.56	-1.91	-1.97	-1.62	-2.46	-2.20	-1.96	-0.88	-1.71	-1.68	-2.45	-1.21	-2.09	-1.78	-2.36	-1.66	-2.02	-1.69	-1.44
	Q	-2.64	-2.57	-2.59	-0.98	-0.91	-0.52	-2.37	-1.76	-3.01	-2.93	-2.48	-3.12	-2.27	-2.62	-2.62	-1.85	-2.61	-2.91	-2.16	-1.05
	V	-2.17	-0.67	-3.06	-3.72	-1.99	-1.75	-3.17	-0.83	-2.03	-1.88	-1.95	-1.19	-0.94	-3.48	-1.99	-2.12	-1.11	-2.20	-1.92	-1.31
E	I	-1.23	-1.87	-2.22	-2.60	-0.88	-1.83	-2.35	-1.21	-1.35	-0.96	-1.62	-0.61	-1.47	-1.35	-1.24	-1.25	-2.43	-1.56	-1.25	-0.80
	Q	-1.73	-1.87	-3.18	-3.56	-1.58	-0.88	-2.01	-1.68	-1.54	-1.96	-2.62	-2.08	-1.95	-1.93	-1.90	-3.21	-2.16	-3.07	-0.98	-0.61
	V	-1.59	-1.98	-2.63	-3.08	-1.55	-2.20	-1.10	-1.83	-1.73	-2.78	-2.10	-1.90	-1.42	-1.47	-0.86	-1.99	-1.85	-1.01	-1.06	-1.01
N	I	-0.58	-0.95	-1.03	-2.41	-2.02	-0.04	-1.66	-1.66	-1.88	-0.37	-1.71	-1.32	-0.68	-0.45	-1.10	-1.38	-1.18	-1.13	-0.34	-0.57
	Q	-1.27	-1.70	-1.87	-1.45	-0.88	-1.63	-0.86	-1.28	-1.18	-1.13	-0.06	-2.42	-1.35	-2.49	-2.15	-1.59	-2.17	-1.57	-0.49	-0.52
	V	-2.02	-1.97	-1.20	-1.94	-1.25	-2.33	-2.44	-0.71	-1.07	-1.84	-1.20	-1.79	-0.92	-2.63	-0.86	-1.82	-1.26	-2.59	-0.81	-0.38
P	I	-0.59	-0.88	-0.82	-0.65	-0.31	-0.84	-0.78	-0.37	-1.11	-0.08	-0.62	-0.55	-0.17	-0.71	0.50	-1.38	-0.11	-0.12	-0.16	-1.21
	Q	-1.84	-1.42	-1.83	-2.49	-0.79	-1.82	-0.96	0.05	-1.83	-1.11	-1.39	-1.33	-2.19	-2.03	-0.09	-1.49	-1.00	-1.54	-1.55	-0.95
	V	-1.24	-0.74	-0.93	-1.74	-1.15	-0.77	-0.04	-0.19	-0.40	-1.12	-1.08	-0.77	-0.30	-0.95	-0.52	-0.69	-0.49	-0.07	-0.05	-1.12
F	D	-0.58	-0.64	-2.46	-2.32	-0.17	-1.16	-0.52	-0.29	-0.51	-0.04	-0.57	-0.88	-0.22	0.17	-0.42	-1.55	-0.42	0.00	0.67	-0.40
	Q	-1.75	0.01	-1.65	-1.56	-0.29	-1.08	-0.79	-0.12	-1.77	-0.20	-1.12	-2.70	-0.74	-1.38	-1.45	-1.95	-1.02	-0.39	0.46	-0.30
	V	-0.25	-0.38	-1.89	-1.94	0.38	0.24	-0.73	0.01	1.42	-0.19	-1.63	-0.69	-0.43	-1.51	-1.31	-1.50	-2.46	0.41	0.62	-0.49
E	I	-1.69	-0.50	-1.88	-1.50	-0.34	-0.72	-0.39	0.01	-0.80	0.35	0.00	-0.83	-0.25	-0.76	-0.89	-0.93	-1.95	-0.21	0.41	-0.15
	Q	-1.00	-1.28	-1.26	-2.51	0.44	-1.18	-1.33	-1.25	-1.44	-0.03	-0.62	-1.98	-1.06	-1.53	-1.19	-0.97	-1.01	-0.64	-0.26	-0.51
	V	-0.91	-1.24	-2.37	-0.98	-0.24	-1.13	-1.08	0.04	1.86	-0.16	-0.51	-0.84	-0.73	-1.68	-1.71	-1.31	-0.49	-0.51	0.01	0.02
N	I	0.10	0.31	-1.09	-0.17	1.19	0.71	0.45	1.31	-0.67	0.82	0.33	0.00	0.48	-0.05	0.02	0.33	-0.04	0.68	0.52	0.80
	Q	0.09	0.35	-0.09	-0.21	1.35	0.17	0.18	1.23	-0.17	0.99	0.36	0.15	0.89	-0.16	0.61	-1.21	-0.03	0.18	1.23	0.65
	V	-0.38	0.48	0.14	-0.93	0.96	0														

Appendices

	Q	-1.46	-0.80	-1.26	-1.64	0.11	-0.47	-0.62	-0.47	-1.17	-1.34	-0.43	-1.49	-1.00	-0.56	-0.73	-0.75	-0.68	-0.76	-0.40	-0.76
	V	-0.87	-0.43	-2.37	-1.93	0.06	-0.73	-0.52	0.27	-0.61	-0.35	-1.08	-1.34	0.12	-0.99	-1.27	-1.47	-1.21	-1.28	-0.21	-0.67
	I	-0.43	-0.44	-0.31	-0.44	-0.09	-0.05	-0.51	-0.58	-1.66	-0.52	-0.57	-0.11	0.23	0.02	-1.07	-0.77	-1.17	-0.71	0.47	0.19
P	Q	-0.98	-1.04	-2.12	-1.66	-0.58	-0.83	-1.31	-1.37	-1.23	-0.49	-0.59	-1.54	-1.86	-1.55	-0.36	-0.38	-1.08	-1.14	0.12	0.35
	V	0.07	-0.59	-0.27	-0.64	0.54	-0.55	-0.82	-0.20	-0.90	-0.44	-0.23	-0.25	0.22	-0.68	-0.84	-0.51	-0.89	-0.03	0.35	0.36
L	D	I	-1.64	-1.43	-2.62	-2.83	-0.57	-1.39	-1.47	-0.01	-2.55	-0.04	-1.19	-0.80	-0.59	-1.09	-1.63	-2.52	-1.66	-0.59	-0.01
	V	-1.11	-1.17	-0.97	-3.39	-1.31	-1.76	-0.87	-0.69	-1.47	-0.36	-1.80	-2.79	-1.51	-2.23	-1.40	-2.22	-1.89	-1.32	-0.06	-0.41
	Q	-0.30	-0.83	-1.16	-2.36	0.77	-2.55	-0.97	-0.37	-1.68	-0.87	-0.66	-1.64	-0.93	-1.75	-1.55	-1.55	-2.36	-1.95	-0.52	-0.89
E	I	-1.60	-1.96	-2.68	-2.74	-0.79	-1.53	-1.77	-0.12	-1.53	-0.53	-1.12	-1.09	-0.30	-2.24	-0.87	-1.60	-2.09	-1.09	-0.56	-0.21
	V	-1.24	-1.68	-1.23	-2.90	-0.15	-1.83	-1.54	-1.29	-1.47	-1.21	-1.30	-1.58	-1.01	-2.41	-1.24	-1.66	-1.51	-2.38	-0.22	-0.84
	Q	-1.88	-1.48	-2.54	-1.08	0.94	-1.12	-1.73	-0.71	-1.57	-1.24	-1.20	-1.28	-1.38	-1.99	-1.53	-1.81	-2.29	-0.90	-0.62	-1.44
N	I	-0.59	-1.07	-1.70	-1.65	0.49	-0.57	-0.07	0.29	-1.07	0.48	0.02	-0.43	-0.18	-0.51	-0.81	-0.63	-1.28	-0.15	0.50	0.92
	V	-1.62	-0.48	-0.96	-1.76	0.87	-0.31	-0.58	0.55	-0.65	0.32	-0.05	-0.60	-0.22	-0.99	-0.66	-1.09	-0.66	0.17	0.55	0.08
	Q	-1.34	0.41	-1.80	0.51	0.41	-1.11	-0.56	0.50	-0.51	-0.15	-0.30	-0.72	0.22	-0.73	0.91	-0.22	-0.37	0.02	0.66	0.28
P	I	0.08	0.25	-0.10	-1.12	0.87	0.28	0.00	0.69	-0.39	0.71	0.24	-0.26	0.40	-0.58	-0.38	-0.02	-0.20	-0.24	1.00	1.01
	V	-0.61	-0.50	-1.82	-2.31	0.67	-0.84	-0.63	0.77	-1.37	0.20	0.04	-1.69	-0.47	-1.01	-0.60	-1.04	-2.16	0.05	1.07	-0.18
	Q	-0.26	-0.12	-1.05	-0.76	0.53	-0.29	-0.04	0.64	-0.05	0.44	-0.08	-0.30	0.80	-0.88	0.29	-0.28	-0.32	0.37	1.68	0.72
M	D	I	-0.98	-1.24	-1.23	-2.43	-1.13	-1.24	-1.19	0.86	-0.57	-0.88	-0.82	-1.55	-1.06	-1.91	-0.69	-0.93	-1.90	-0.82	-0.38
	V	-3.10	-0.72	-1.57	-1.35	-1.33	-2.02	-1.49	-0.62	-1.32	-1.12	-0.65	-2.63	-1.14	-0.71	-1.81	-1.46	-1.47	-1.11	-0.66	-0.69
	Q	-1.11	-1.96	-1.10	-2.19	-0.55	-1.56	-2.21	-0.45	-1.13	-0.60	-1.29	-0.59	-0.81	-1.17	-1.23	-2.17	-1.67	-0.86	-0.69	-2.01
E	I	-1.79	-1.59	-1.65	-1.79	0.88	-1.04	-1.13	-0.58	-1.08	-0.71	-1.90	-1.88	-0.38	-1.95	-1.26	-0.93	-1.51	-0.83	-0.61	-0.54
	V	-2.56	-1.30	-2.69	-2.99	-1.16	-2.58	-1.21	-0.36	-2.21	-0.99	-1.20	-1.50	-0.74	-1.39	-0.94	-1.03	-1.71	-1.05	-0.27	-0.89
	Q	-1.33	-1.57	-1.53	-1.72	-0.93	-1.00	-2.17	0.04	-1.73	-0.05	-1.14	-1.97	-0.84	-1.74	-1.65	-2.36	-1.76	-1.06	-0.23	-1.09
N	I	-0.56	-0.18	-1.05	-1.28	0.16	-0.44	-0.73	1.41	-0.98	0.97	-0.26	-0.10	0.49	-0.52	-0.24	-0.91	-0.38	0.20	0.44	0.17
	V	-0.97	-0.58	-1.30	-0.59	0.26	-0.97	-0.69	0.66	-0.32	0.23	-0.18	-0.47	-0.38	-1.56	0.57	-0.15	-0.53	0.20	0.43	0.50
	Q	-1.21	-0.80	-1.36	-1.28	0.16	-0.30	-0.64	0.24	-0.42	0.55	0.43	-1.49	-0.32	-0.98	-0.49	-1.06	-1.35	-0.89	0.27	0.10
P	I	-0.11	-0.39	-0.75	-0.36	0.44	0.00	-0.50	0.65	-0.28	0.43	0.25	-0.02	0.60	-0.24	-0.46	-0.56	-0.12	-0.17	1.41	0.58
	V	-0.75	0.16	-2.06	-1.65	0.78	-0.43	-0.21	0.75	-0.67	0.26	-0.08	-0.96	-1.03	-0.57	-0.65	-1.16	-0.69	-0.29	0.43	0.07
	Q	-0.25	-0.17	-1.32	-1.05	0.43	-0.02	-0.18	0.59	-1.02	0.76	0.99	-0.20	0.49	-0.92	-0.51	-0.28	-0.23	0.13	0.66	0.59
N	D	I	-0.67	-0.67	-1.60	-3.31	0.05	-1.19	-1.04	0.54	-1.52	-0.70	-0.62	-1.85	0.10	-0.99	-0.83	-1.02	-1.33	-1.65	-0.17
	V	-1.78	-1.46	-1.44	-3.34	-0.39	-0.62	-0.90	-1.19	-1.83	-0.13	-1.72	-2.74	-1.73	-1.55	-1.25	-1.74	-1.56	-0.19	-0.73	-0.16
	Q	-1.37	-0.84	-1.49	-2.05	-0.29	-1.60	-0.89	-0.79	-1.47	-1.27	-1.05	-2.42	-0.76	-1.94	-0.10	-1.90	-2.06	-1.19	-0.82	-0.75
E	I	-2.00	-0.83	-1.57	-2.48	0.84	-2.33	-1.70	0.40	-1.48	-1.29	-1.33	-0.62	-0.25	-1.66	-0.98	-1.27	-1.63	-1.41	-1.62	-0.59
	V	-0.80	-2.27	-2.38	-2.96	-1.20	-1.60	-2.72	-1.60	-2.80	-2.47	-1.40	-2.37	-1.65	-1.70	-0.98	-1.92	-1.37	-1.40	-1.14	-0.52
	Q	-2.30	-2.42	-2.16	-2.83	-0.87	-1.99	-1.13	-0.93	-1.13	-1.19	-1.51	-2.07	0.20	-2.05	0.04	-2.12	-2.01	-1.75	-0.92	-0.41
N	I	-0.98	-0.86	-1.53	-0.71	0.16	-0.74	-0.56	0.15	-0.60	0.34	-0.34	-0.75	0.24	-1.14	-0.39	-0.59	-0.71	0.15	0.67	0.86
	V	-1.09	-0.75	-0.62	-1.65	0.70	-0.53	-0.23	-0.22	-0.41	0.25	-0.06	-0.71	-0.16	-1.11	0.01	-1.78	-0.70	-0.44	0.25	-0.13
	Q	-0.23	-0.10	-1.56	-1.99	-0.02	-0.92	-0.59	0.02	-0.61	-0.10	-0.61	-1.42	0.03	-0.79	-0.57	-0.88	-1.40	-0.75	0.57	0.05
P	I	-0.62	-0.04	-0.38	-0.87	0.58	-0.38	-0.06	0.03	-0.30	-0.07	-0.05	-0.96	0.49	-0.52	0.12	-0.08	-0.29	0.26	0.27	0.82
	V	-1.08	-0.82	-2.08	-1.05	-0.10	-1.71	-0.44	-0.33	-1.36	-0.51	-0.79	-1.30	-0.64	-0.71	-0.05	-1.49	-1.17	-0.63	-0.11	0.10
	Q	-0.73	-0.04	-0.31	-0.74	0.45	-0.40	-0.27	0.14	-0.08	-0.04	-0.32	0.39	0.09	-0.32	-0.30	-0.52	-0.69	-0.16	0.34	0.82
Q	D	I	-1.02	-0.71	-1.30	-0.84	-1.16	-2.20	-1.43	-0.38	-1.35	-1.72	-1.16	-1.06	-0.80	-1.47	-2.23	-1.92	-1.67	-1.94	-0.12
	V	-1.74	-2.34	-2.71	-2.51	-1.02	-2.32	-1.78	-1.81	-1.34	-1.66	-0.90	-2.28	-2.13	-1.78	-1.89	-2.69	-1.90	-1.21	-0.48	-0.92
	Q	-1.04	-1.39	-1.89	-2.72	-1.39	-1.29	-1.45	-1.40	-1.67	-0.88	-0.78	-1.66	-1.30	-1.92	-1.89	-2.15	-1.94	-1.47	-0.70	-1.94
E	I	-1.90	-1.98	-1.98	-1.32	-1.60	-1.87	-1.41	-0.87	-1.32	-1.52	-1.98	-1.13	-1.03	-1.33	-0.80	-1.56	-1.40	-1.92	-1.17	-1.09
	V	-1.90	-1.60	-2.22	-1.92	-1.32	-2.84	-2.56	-1.88	-2.83	-2.13	-2.04	-1.88	-1.24	-1.68	-1.93	-1.35	-3.06	-2.33	-1.13	-1.77
	Q	-1.70	-1.68	-2.33	-2.26	-0.53	-1.97	-1.67	-1.75	-1.53	-1.54	-1.84	-2.08	-0.70	-1.37	-0.73	-1.50	-1.59	-1.19	-0.97	-0.53
N	I	-0.84	-0.98	-0.71	-1.23	-0.40	-0.61	-1.30	-0.01	-1.47	-0.83	-0.45	-0.55	0.12	-0.46	0.00	-0.29	-0.06	-0.60	0.06	-0.57
	V	-1.70	-0.04	-1.18	-2.42	-1.07	-1.09	-0.61	-0.35	-1.09	-0.18	-0.85	-0.75	-1.21	-1.48	-0.15	-1.32	-1.70	-1.10	-0.93	-1.18
	Q	-0.87	-0.80	-0.76	-1.84	-0.14	-1.35	-0.34	-0.02	-1.93	0.21	-1.27	-1.29	-0.27	-1.15	-0.44	-0.66	-0.68	-1.29	-1.18	-0.19
P	I	-0.14	0.29	-0.88	-0.60	0.01	0.01	0.06	0.18	-0.16	0.53	0.01	0.34	0.32	-0.41	-0.52	-0.30	-0.33	-0.19	0.25	0.36
	V	-0.83	-0.56	-1.38	-1.66	-0.58	-0.43	-0.61	-0.66	-0.94	-0.54	-1.16	-0.85	-1.09	-0.78	-1.00	-0.85	-1.27	-0.61	-0.12	-0.86
	Q	-0.02	-0.17	-1.35	-1.23	-0.10	-0.12	-0.09	-0.39	-0.63	-0.18	0.12	-0.50	0.53	-0.18	-0.56	-0.04	-0.38	0.13	0.46	0.11
R	D	I	-1.19	-1.33	-1.53	-2.11	-1.07	-1.34	-0.93	-0.61	-1.06	-0.62	-1.15	-0.96	-0.29	-0.73	-1.36	-1.56	-2.13	-0.12	-0.94
	V	-1.64	-1.14	-1.89	-1.70	-0.47	-1.37	-0.38	-1.11	-1.35	-2.04	-0.89	-1.40	-1.13	-1.05	-1.29	-2.21	-1.26	-2.25	-0.16	-0.84
	Q	-0.98	-1.58	-2.19	-2.61	0.83	-1.72	-1.71	-0.73	-1.58	-0.97	-1.50	-1.51	-0.45	-2.33	-1.65	-2.21	-1.68	-1.26	-0.33	-0.42
E	I	-2.27	-1.69	-1.73	-2.75	-0.79	-1.15	-0.32	0.15	-0.79	-1.21	-0.79	-1.17	-0.70	-1.17	-0.85	-1.12	-1.36	-0.11	0.10	-0.40
	V	-2.38	-1.46	-3.12	-1.70	-0.76	-1.63	-2.25	-1.39	-1.43	-1.72	-1.64	-0.89	-1.27	-1.63	-0.95	-2.22	-1.60	-1.05	-0.43	-1.28
	Q	-1.40	-1.59	-1.35	-2.22	0.19	-1.83	-0.87	-0.20	-1.46	-0.81	-1.29	-1.68	-1.35	-1.59	-1.43	-1.53	-2.82	-0.12	-0.97	-0.71
N	I	-0.03	0.05	-0.99	-2.64	0.55	-0.54	-0.62	-0.03	-1.02	-0.29	-0.64	0.13	-0.11	-0.57	-0.73	-0.36	-0.59	-0.40	0.20	0.71
	V	-0.42	-0.61	-1.30	-1.28	0.34	-1.17	-0.24	0.43	-0.12	-0.92	-0.38	-0.53	-0.15	-1.01	-0.01	-0.42	-0.91	0.08	0.69	0.17
	Q	-0.21	0.01	-0.32	-1.03	0.52	-0.73	-0.80	0.28	-0.73	-0.70	0.32	-1.05	0.27	-0.73	-0.54	-1.05	-0.60	-0.24	0.31	0.22
P	I	-0.41	-0.28	0.31	-0.57	1.16	0.38	0.38	0.08	0.22	0.54	-0.26	0.58	-0.50	0.07	-0.70	-0.36	-0.13	-0.54	1.58	1.58
	V	-0.28	-0.16	-1.71	-1.28	-0.32	-0.58	-													

Appendices

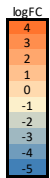
E	I	-1.05	-0.38	-1.16	-1.99	0.96	-1.02	-0.42	0.87	-0.85	0.61	-0.18	-1.27	0.02	-0.98	-0.92	-0.68	-1.06	-0.21	0.66	0.54				
	Q	-0.77	-0.59	-1.05	-2.29	0.60	-0.43	-0.89	0.48	-0.54	0.43	-0.18	-0.93	0.29	-1.52	-1.21	-0.86	-1.51	0.11	1.05	1.01				
	V	-1.21	-0.93	-1.20	-1.61	0.96	-0.75	-0.47	0.79	-1.26	0.18	-0.69	-0.96	-0.12	-0.63	-0.49	-1.85	-0.89	-0.43	0.85	0.26				
	I	0.26	0.78	0.48	0.31	1.86	0.81	1.01	1.74	-0.30	1.35	0.79	-0.01	0.95	-0.16	0.95	0.54	-0.12	1.07	1.48	1.61				
N	I	0.55	0.56	0.17	-0.11	1.67	0.13	0.89	1.24	0.46	1.24	0.39	0.39	1.08	-0.03	0.30	-0.11	0.37	1.14	1.75	1.03				
	Q	0.07	0.37	-0.09	-0.83	1.44	0.69	0.56	1.45	-0.50	1.03	0.59	0.49	1.06	0.35	0.43	0.50	-0.76	0.99	1.69	1.37				
	V	0.71	1.43	0.31	0.89	2.64	1.59	0.85	1.41	0.36	1.13	1.11	0.38	1.54	-0.04	0.13	0.58	0.14	1.34	1.88	1.97				
	I	0.77	0.98	-0.31	-0.38	1.73	0.55	0.40	2.21	-0.65	1.13	0.83	0.30	0.61	0.15	0.49	0.17	0.38	1.02	1.68	0.83				
P	I	0.93	1.21	0.67	-0.48	2.00	1.01	1.18	1.39	-0.32	1.33	1.05	0.41	1.13	0.31	0.51	0.63	0.87	1.03	1.80	1.84				
	Q	-0.90	-0.31	-1.78	-2.15	-0.27	-0.73	-0.47	-0.55	-0.51	0.03	-0.34	-1.28	-0.73	-1.35	-0.69	-1.10	-1.03	-0.49	0.33	0.18				
	V	-2.35	-0.53	-1.55	-1.43	-0.02	-0.76	-1.28	-0.26	-1.03	-1.20	-1.46	-2.04	-0.83	-1.13	-0.93	-1.94	-2.22	-0.15	0.19	0.17				
	I	-1.07	-0.78	-2.14	-1.91	0.06	-0.69	-0.83	-0.80	-1.04	-0.21	-0.81	-1.15	-0.26	-1.30	-0.95	-1.01	-1.02	-0.41	0.62	0.04				
E	I	-1.12	-1.05	-1.55	-2.36	0.56	-1.25	-1.13	-0.14	-1.83	-0.42	-0.52	-0.66	-0.69	-1.63	-1.16	-0.91	-1.10	-0.33	-0.08	0.18				
	Q	-1.22	-0.79	-1.72	-2.85	-0.14	-0.67	-0.52	0.29	-1.72	-0.89	-1.25	-1.81	-0.62	-0.37	-0.65	-2.13	-1.75	-1.36	0.26	-0.63				
	V	-0.88	-0.63	-1.67	-1.84	0.03	-1.66	-1.04	-0.10	-2.29	-0.53	-0.41	-0.95	-0.81	-0.69	-1.53	-1.08	-0.52	-0.30	-0.09	-1.25				
	I	-0.98	0.01	-0.26	-0.94	1.13	-0.14	0.25	0.76	-1.47	0.59	0.44	0.01	0.34	-0.42	-0.82	-0.59	-0.16	0.24	1.15	0.51				
N	I	-0.51	-0.36	-0.47	-1.31	0.70	-0.26	-0.45	1.02	-0.18	-0.31	0.57	0.10	0.20	-0.32	-0.05	-0.05	-0.09	0.29	1.45	0.75				
	Q	-0.75	-0.09	-0.57	-1.10	0.89	-0.74	-0.21	0.66	-1.09	0.49	-0.26	-0.30	0.22	-0.29	-0.71	-0.45	-1.05	-0.54	1.11	0.77				
	V	0.01	0.67	-0.04	-0.04	1.15	-0.21	-0.06	0.84	-1.06	1.07	0.19	-0.13	0.86	0.08	-1.24	-0.26	-0.38	0.48	0.98	0.88				
	I	-0.21	0.00	-1.35	-0.82	1.21	-0.46	0.27	0.45	-0.30	0.78	0.16	-0.11	-0.43	-0.09	-0.33	-0.23	0.07	0.64	0.96	0.58				
P	I	-0.50	0.39	-0.15	0.04	1.47	-0.76	0.56	0.19	-0.69	0.80	-0.10	-0.22	0.30	0.00	-1.02	-0.23	-0.24	0.33	0.77	1.02				
	Q																								
	V																								
	I																								
146 153 155 147 149 150 154																									
A C D E F G H I K L M N P Q R S T V W Y																									
F	H	F	A	E	I	-2.48	-0.86	-1.24	-1.60	0.21	-1.18	-0.91	-0.64	-1.11	-0.95	-1.34	-0.78	-0.60	-1.76	-1.85	-2.35	-2.32	-0.65	-0.22	-1.33
	Q	-0.21	-0.07	0.05	-1.15	0.87	0.17	-0.45	-0.12	0.86	0.05	0.30	0.09	0.26	-0.29	0.29	-0.87	-1.41	-0.22	1.16	0.59				
	I	-0.55	-0.32	-0.74	-0.57	0.36	-0.30	-0.34	0.49	-0.70	-0.21	0.11	-0.50	0.46	-0.12	-0.49	-0.73	-0.36	-0.44	0.60	0.28				
	V	-0.74	-0.88	-0.94	-1.30	0.41	0.15	-0.57	0.89	-1.12	0.41	-0.09	-0.77	0.45	-1.79	-0.72	-0.26	-0.57	-0.48	0.80	-0.38				
C	E	I	-2.46	-1.40	-1.21	-2.82	0.08	-0.99	-2.05	-0.47	-2.36	-1.41	-0.85	-1.62	-0.44	-0.68	-1.93	-1.50	-2.08	-0.97	0.03	-1.19			
	Q	-0.02	0.02	-0.69	-1.21	1.01	0.02	0.16	0.60	0.11	0.49	-0.48	0.09	0.05	0.30	0.41	-0.64	-0.53	-0.52	0.82	0.90				
	V	-0.02	0.14	-0.44	-1.09	0.84	-0.39	0.25	-0.12	-0.95	-0.14	0.51	0.02	0.78	0.04	0.16	-0.38	-1.02	-0.44	0.59	0.43				
	I	-0.70	0.54	-0.49	-1.20	0.38	-0.22	-0.22	0.58	-0.25	-0.07	0.12	-0.54	-0.02	-1.19	0.33	-0.58	-0.74	0.31	0.67	0.43				
D	E	I	-0.28	-0.53	-0.84	-1.19	1.27	-0.62	-0.70	0.70	-0.96	0.86	0.31	-1.08	-0.40	-1.16	-0.96	-1.40	-1.23	-0.33	1.52	0.93			
	Q	0.28	-0.26	-0.21	0.14	1.37	0.46	0.90	0.91	0.29	1.19	0.66	0.63	0.39	-0.57	0.38	-0.21	-0.10	0.71	1.98	1.22				
	V	1.27	-0.56	-0.64	-1.31	0.17	-0.67	-1.26	-0.21	-1.63	-0.53	-1.04	-0.60	-1.66	-1.44	-0.16	-1.34	-0.80	-0.65	0.24	-0.44				
	I	-0.22	0.21	0.43	-0.93	1.66	0.30	-0.27	1.21	-0.11	1.09	0.85	-0.05	0.32	-2.08	-0.62	-0.95	-0.92	0.38	1.59	1.12				
E	I	-1.43	-1.45	-1.02	-2.03	-1.63	-1.92	-1.88	-1.82	-3.31	-1.65	-2.03	-1.18	-2.07	-2.81	-2.52	-1.97	-1.83	-2.24	-1.04	-1.09				
	Q	-1.71	-0.20	-1.09	-0.77	-0.14	-0.79	-0.62	-0.56	-3.29	-1.06	-0.69	-0.76	-0.26	-1.55	-0.95	-1.46	-1.41	-1.20	-0.04	0.16				
	V	-1.16	-0.92	-0.80	-0.94	-0.52	-0.79	-0.87	-0.77	-0.87	-0.78	-0.60	-0.87	-0.45	-1.93	-0.73	-0.67	-0.94	-1.03	-0.52	-0.79				
	I	-1.39	-1.17	-0.97	-1.36	-0.72	-1.84	-0.88	-0.95	-0.91	-1.00	-0.83	-1.33	-1.06	-1.64	-2.48	-1.61	-1.75	-1.32	0.35	-0.66				
F	E	I	-1.09	-0.44	-0.98	-2.00	0.75	-0.91	-1.73	1.03	-0.87	0.50	0.18	-1.21	0.25	-1.08	-1.02	-0.83	-0.92	-0.66	0.78	0.11			
	Q	0.54	0.38	-0.87	-0.61	1.20	0.76	0.64	0.98	0.19	1.01	0.75	0.45	0.98	-0.14	0.42	0.34	0.30	0.51	1.02	1.09				
	V	0.47	0.19	-0.45	-0.71	1.17	0.61	0.34	0.50	-0.26	0.70	0.69	0.55	0.84	-0.13	0.13	0.28	-0.21	0.28	1.55	0.71				
	I	0.20	0.86	-0.26	-0.49	1.48	-0.12	0.75	1.11	-0.16	1.04	1.17	0.13	0.83	0.37	0.00	0.40	-0.10	0.31	1.79	0.82				
G	E	I	-0.47	-0.16	-0.76	0.27	0.30	-0.45	-0.59	0.45	-0.47	0.04	-0.16	-0.55	-0.24	-0.24	-1.41	-1.17	-1.14	-0.45	0.92	-0.10			
	Q	0.90	0.50	0.68	-0.36	1.89	1.32	1.27	1.38	0.97	1.27	0.70	0.78	0.68	0.71	0.95	0.29	-0.08	0.98	1.91	1.96				
	V	-0.59	-0.13	-0.26	-0.61	0.91	-0.07	-0.09	0.75	0.24	0.11	-0.38	-0.17	0.64	-0.48	-0.03	-0.38	-0.44	0.02	0.69	0.65				
	I	0.62	0.16	0.15	-1.13	1.09	0.66	0.92	1.02	0.20	0.41	0.36	0.24	0.65	-0.07	0.29	-0.46	-0.66	0.94	1.68	1.13				
H	E	I	-1.46	-1.16	-1.13	-1.54	0.22	-1.45	-0.77	0.24	-2.03	-0.40	-0.84	-1.67	0.00	-1.79	-0.75	-1.35	-1.38	-1.00	-0.37	-0.27	-1.09		
	Q	-0.25	-0.37	-0.28	-1.25	0.93	0.32	0.93	0.42	0.16	0.30	0.47	0.00	0.18	0.08	0.52	-0.65	0.14	-0.41	1.14	0.12				
	V	-0.62	-0.05	-0.99	-0.08	0.08	0.01	0.04	0.53	-0.46	-0.42	0.05	-0.28	-0.15	-0.47	-0.31	-1.50	-0.15	-0.42	0.53	0.46				
	I	-0.55	-0.18	-0.76	-0.97	0.88	-0.40	0.02	0.31	-1.01	0.44	0.36	-0.42	0.34	-0.16	0.06	-0.01	-0.39	-0.26	0.69	0.57				
I	E	I	-2.28	-1.40	-2.06	-2.52	-0.78	-1.87	-1.57	-0.31	-1.27	-0.72	-1.87	-2.29	-1.22	-1.83	-0.81	-1.23	-2.15	-1.98	-1.53	-1.27			
	Q	-1.82	-0.24	-1.87	-2.15	0.25	-1.20	-0.81	0.50	-0.70	0.04	-0.22	-0.23	-0.13	-2.14	-0.16	-0.59	-0.70	-0.58	0.26	0.58				
	V	0.12	-0.36	-1.01	-0.40	0.57	0.23	-0.78	0.78	-1.40	0.13	0.26	-0.33	-0.68	-0.47	-0.41	-0.33	-1.12	-0.12	-0.09	-0.01				
	I	-0.53	-0.28	-2.59	-1.61	0.80	-0.73	-0.14	0.17	-1.23	-0.08	-0.30	-0.96	-0.03	-1.64	-0.70	-1.15	-1.36	-0.73	0.13	0.26				
K	E	I	-1.88	-2.06	-2.22	-2.05	-0.48	-2.38	-1.70	-1.37	-1.91	-1.56	-2.31	-1.42	-0.32	-2.25	-1.47	-2.43	-2.10	-2.24	-0.09	-0.14	-0.13		
	Q	-0.59	-0.13	-1.68	-1.39	0.62	0.07	0.20	0.11	-1.00															

Appendices

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Appendices

T	E	I	0.11	-0.51	-0.59	-1.14	1.37	-0.11	-0.56	0.41	-1.19	0.58	-0.31	-0.22	-0.25	-0.85	-0.24	-0.80	-0.86	-0.50	0.57	0.87
	N	Q	0.32	0.61	-0.79	-0.52	1.30	0.39	0.26	0.72	-0.28	0.55	0.44	0.35	0.09	-0.18	0.44	-0.99	-0.20	0.33	1.30	1.44
	P	I	0.41	0.83	0.22	-0.04	1.34	0.63	0.46	0.99	-0.04	1.21	1.01	0.66	1.70	0.00	0.66	0.35	0.07	0.69	1.32	1.25
V	E	I	-0.44	-0.06	-0.39	-1.24	1.25	0.05	-0.45	0.60	-1.25	0.04	0.10	-0.29	0.31	-0.19	-0.39	-1.10	-0.76	-0.35	0.34	0.57
	N	Q	-0.36	0.16	-0.96	-0.81	1.92	0.31	-0.23	0.92	0.01	0.94	-0.06	0.18	0.37	-0.24	0.53	0.08	-1.14	-0.61	1.14	1.02
	P	I	0.75	1.03	0.76	0.11	1.92	0.59	0.77	1.71	0.15	1.35	0.50	0.88	1.80	0.36	0.71	0.67	0.05	1.31	1.64	1.46
W	E	I	1.29	1.47	0.90	1.35	3.03	1.67	1.77	2.83	1.10	2.34	1.90	1.63	1.52	0.80	1.34	1.15	1.02	1.80	2.55	2.35
	N	Q	1.97	2.02	1.06	0.92	2.93	1.67	2.20	2.94	1.79	2.68	2.07	2.01	1.74	1.10	2.28	1.66	1.69	2.00	2.80	2.55
	P	I	2.42	2.99	3.12	1.45	3.79	2.56	2.31	3.31	1.76	3.61	3.07	2.46	2.81	1.95	2.40	1.93	1.97	2.59	3.05	3.49
Y	E	I	-0.02	0.80	0.29	0.04	1.63	0.51	0.63	1.62	-0.28	1.51	0.84	0.82	0.86	0.07	0.54	0.20	0.05	0.55	0.80	1.42
	N	Q	0.71	1.17	-0.28	-0.27	2.11	1.18	1.08	1.83	1.00	1.53	1.14	1.28	0.87	0.38	0.84	0.41	0.98	1.16	1.63	1.93
	P	I	0.32	1.38	1.10	0.59	1.92	1.34	0.75	1.81	0.19	1.53	0.84	1.08	1.76	0.84	0.61	1.09	0.83	1.30	1.99	1.69
			A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y



S.4 NMR Spectra

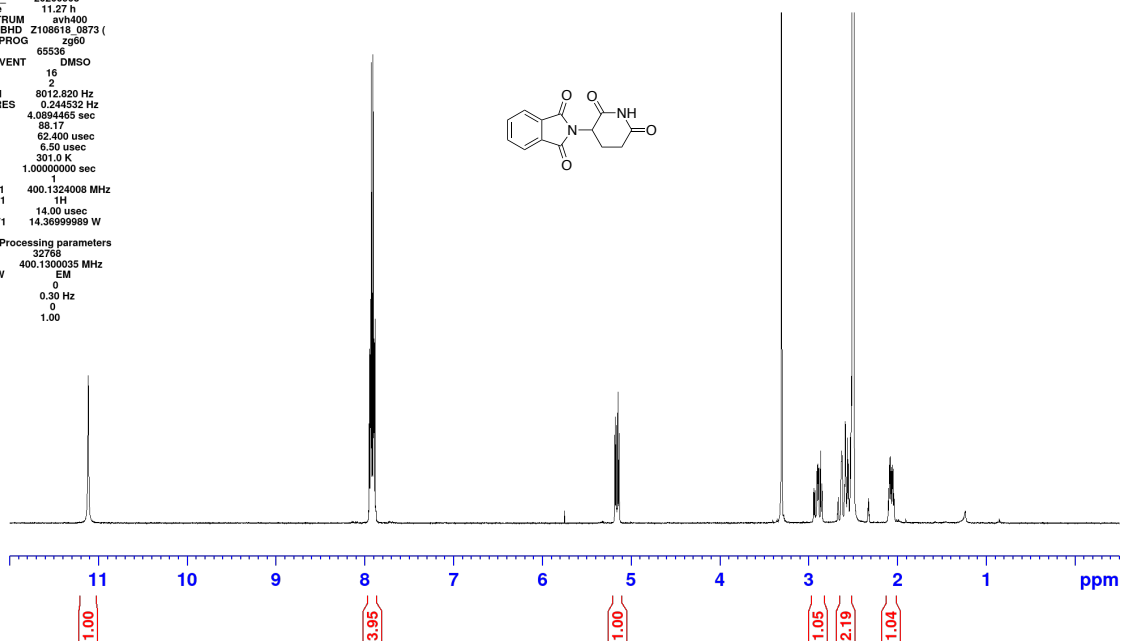
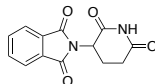
Appendices

Current Data Parameters
NAME Sep03-2020-1-PJBA32
EXPNO 1
PROCNO 1

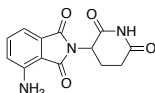
F2 - Acquisition Parameters
Date_ 20200903
Time 11.27 h
INSTRUM avh400
PROBHD Z10618_0873 (Zg60)
PULPROG zg60
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 85.17
DW 62.400 usec
DE 6.50 usec
TE 300.2 K
D1 1.00000000 sec
TD0
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W

F2 - Processing parameters
SI 32768
SF 400.1300035 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)isoindoline-1,3-dione (1)



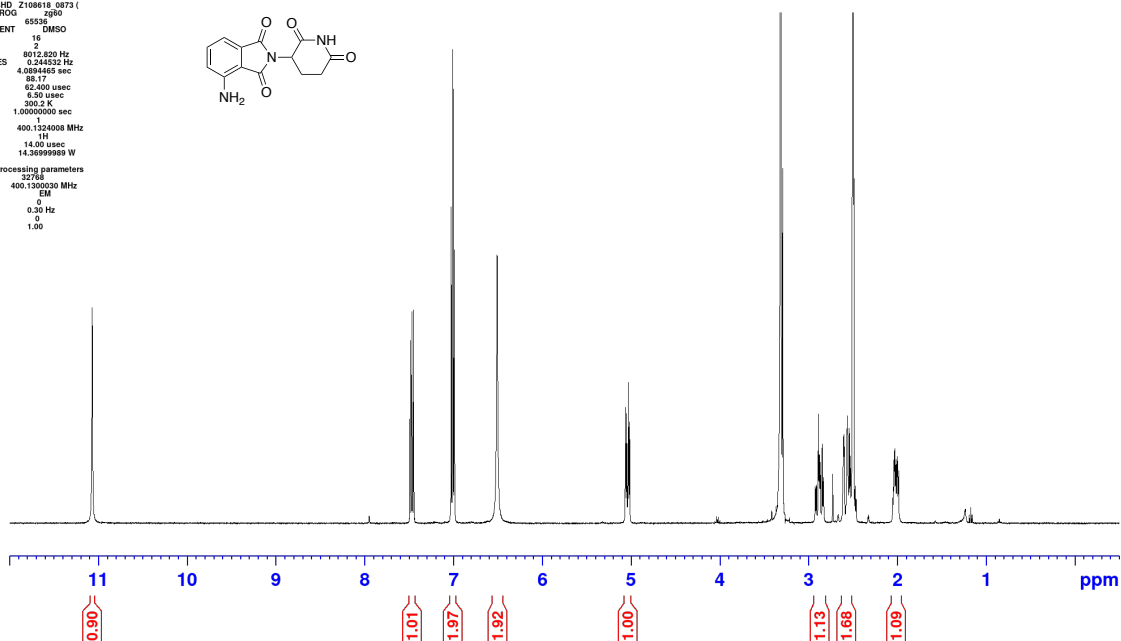
4-Amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (3)



Current Data Parameters
NAME Nov26-2019-1-PJBA03-crude-prod
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20191126
Time 18.56 h
INSTRUM avh400
PROBHD Z10618_0873 (Zg60)
PULPROG zg60
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 85.17
DW 62.400 usec
DE 6.50 usec
TE 300.2 K
D1 1.00000000 sec
TD0
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W

F2 - Processing parameters
SI 32768
SF 400.1300030 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



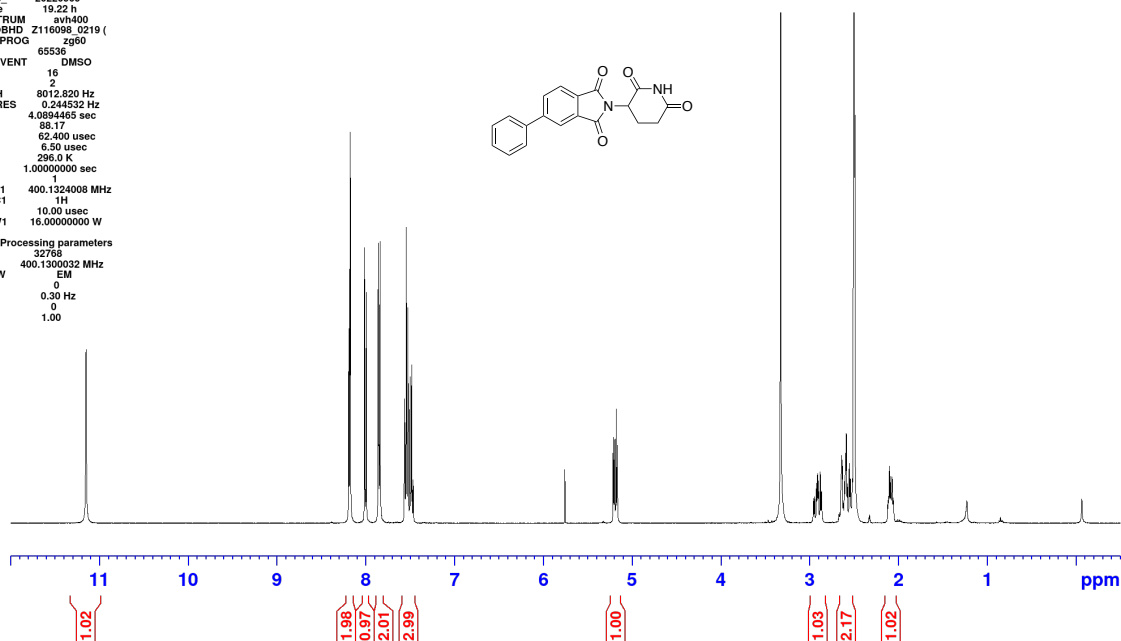
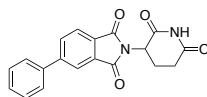
Appendices

Current Data Parameters
NAME Sep09-2022-9-PJB02
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220909
Time 19.22 h
INSTRUM avh400
PROBHD Z116098_0219 (PULPROG zg50)
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 88.17
DW 62.400 usec
DE 6.50 usec
TE 296.0 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 10.00 usec
PLW1 16.00000000 W

F2 - Processing parameters
SI 32768
SF 400.1300032 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-5-phenylisoindoline-1,3-dione (**23**)



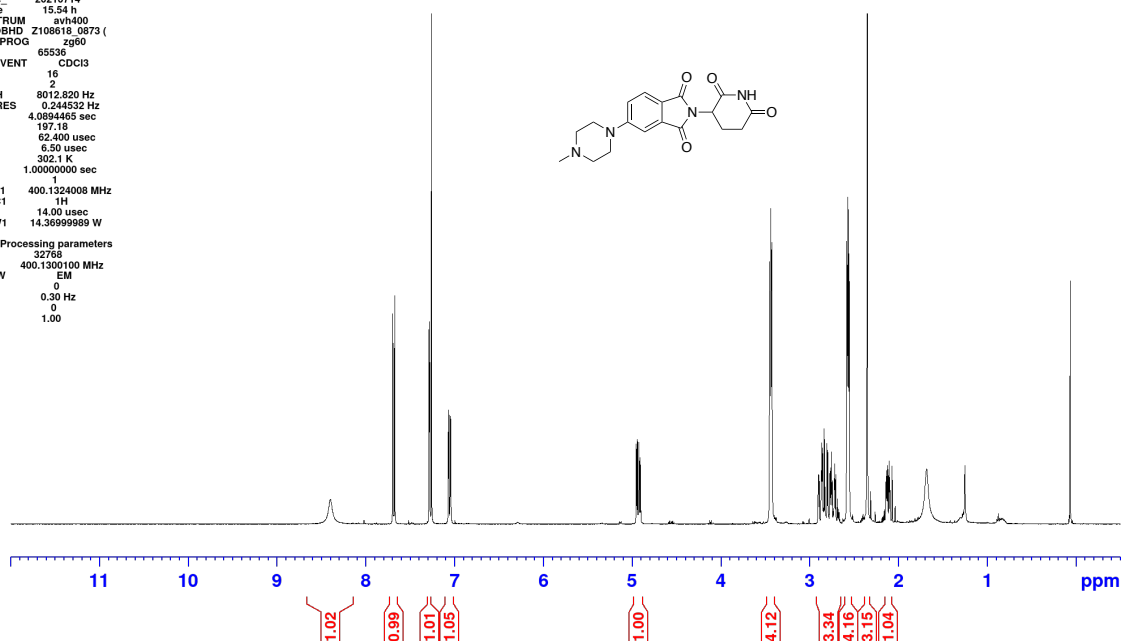
Appendices

Current Data Parameters
NAME Jul14-2021-1-PJBB08
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date 20210714
Time 15.54 h
INSTRUM svt400
PROBHD Z108618_0873 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 197.18
DW 62.400 usec
DE 6.50 usec
TE 302.1 K
D1 1.00000000 sec
TD0
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.3699989 W

F2 - Processing parameters
SI 32768
SF 400.1300100 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-5-(4-methylpiperazin-1-yl)isoindoline-1,3-dione (26)

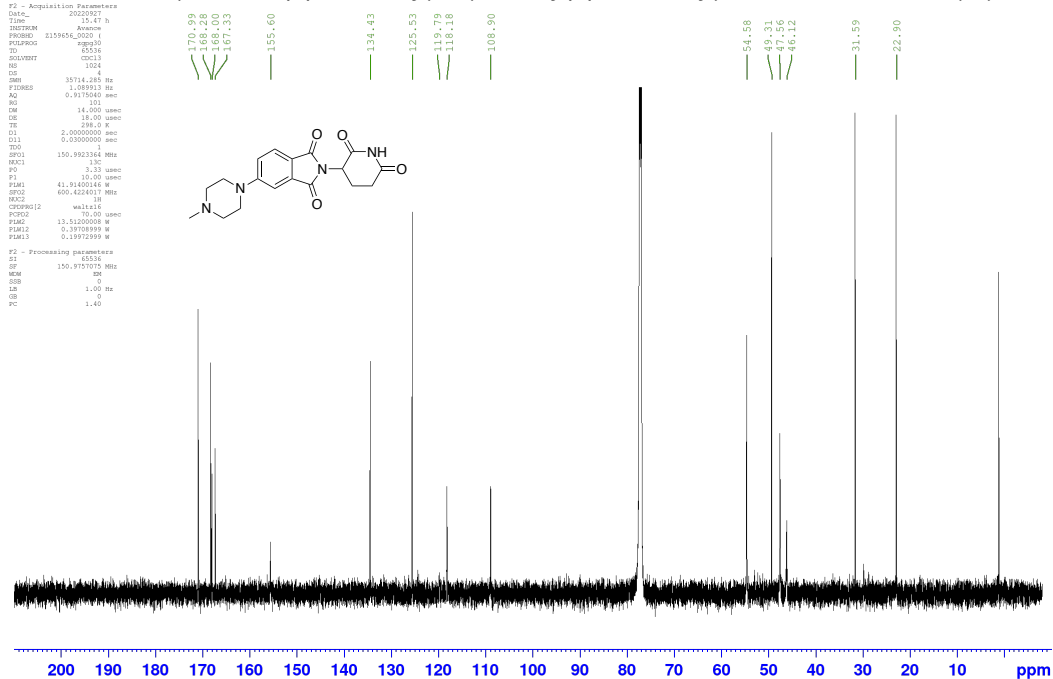


Current Data Parameters
NAME Jul14-2021-1-PJBB08
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date 20210714
Time 15.54 h
INSTRUM svt400
PROBHD Z108618_0873 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 197.18
DW 62.400 usec
DE 6.50 usec
TE 302.1 K
D1 1.00000000 sec
TD0
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.3699989 W

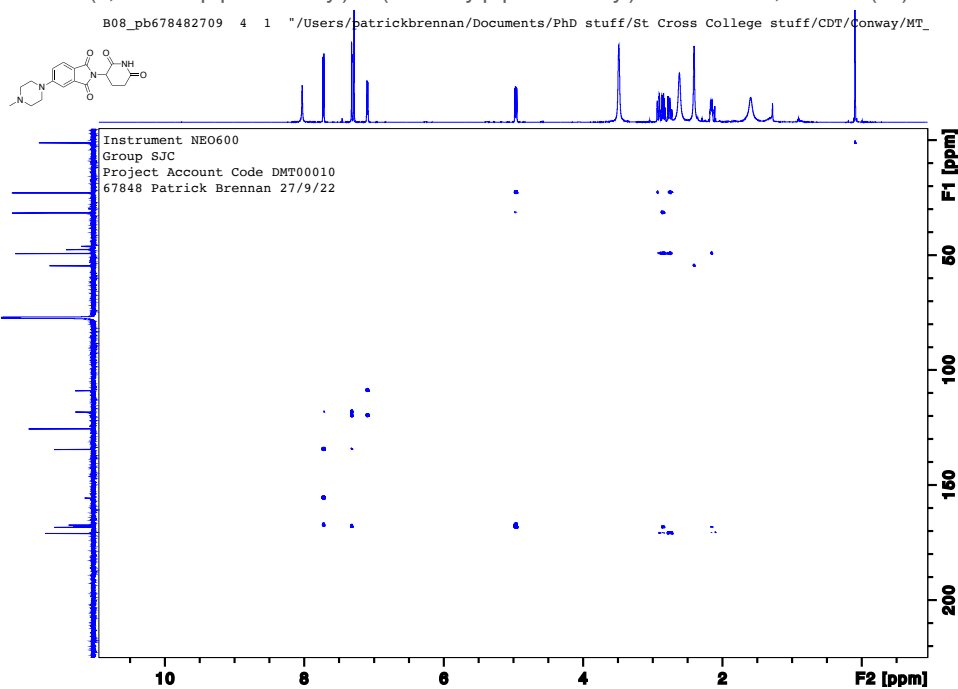
F2 - Processing parameters
SI 32768
SF 400.1300100 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-5-(4-methylpiperazin-1-yl)isoindoline-1,3-dione (26)

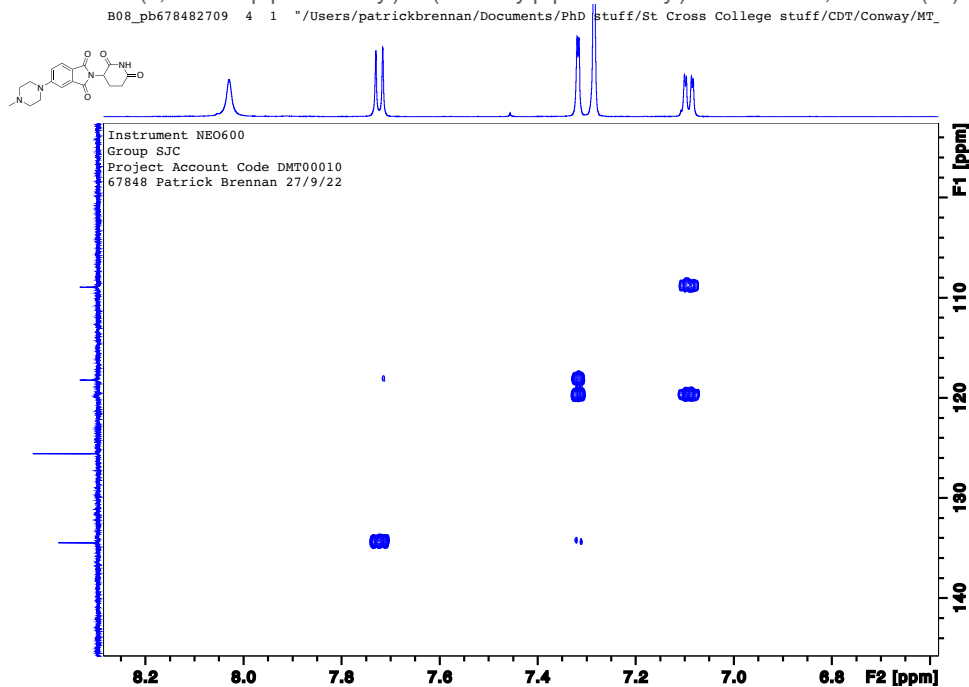


Appendices

2-(2,6-Dioxopiperidin-3-yl)-5-(4-methylpiperazin-1-yl)isoindoline-1,3-dione (26)



2-(2,6-Dioxopiperidin-3-yl)-5-(4-methylpiperazin-1-yl)isoindoline-1,3-dione (26)



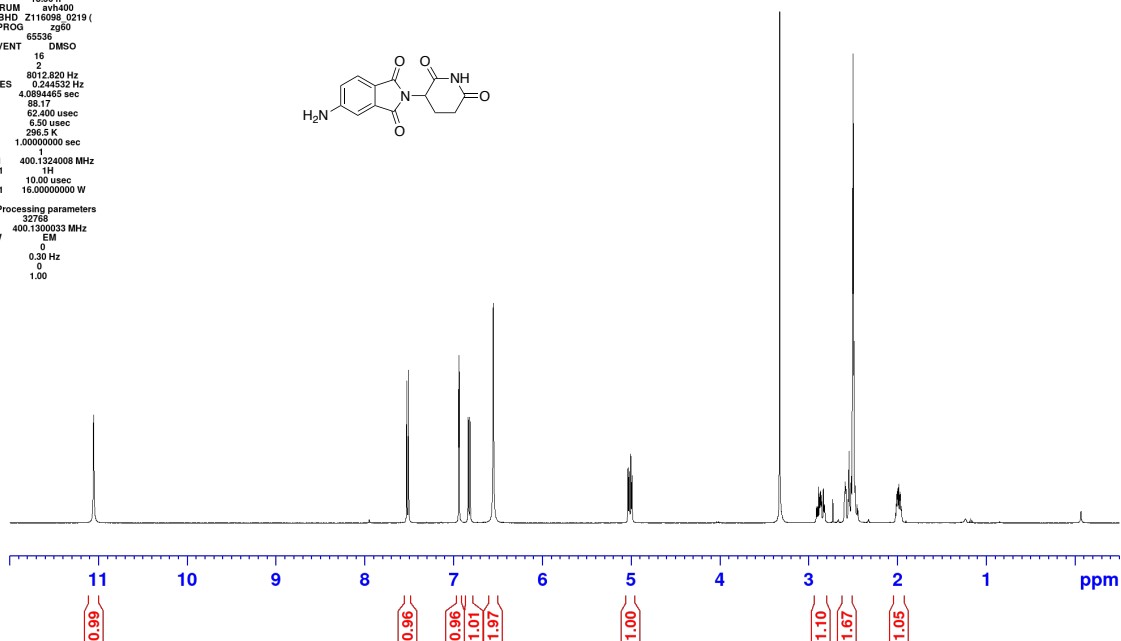
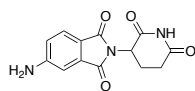
Appendices

Current Data Parameters
NAME Sep09-2022-3-PJBA48_clean
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220909
Time 18.56 h
INSTRUM avh400
PROBHD Z116098 0219 (Z116098)
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244552 Hz
AQ 4.0894465 sec
RG 88.17
DW 62.400 usec
DE 6.50 usec
TE 296.5 K
D1 1.00000000 sec
TDO 1
SFO1 400.1324008 MHz
NUC1 1H
P1 10.00 usec
PLW1 16.00000000 W

F2 - Processing parameters
SI 32768
SF 400.1300033 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

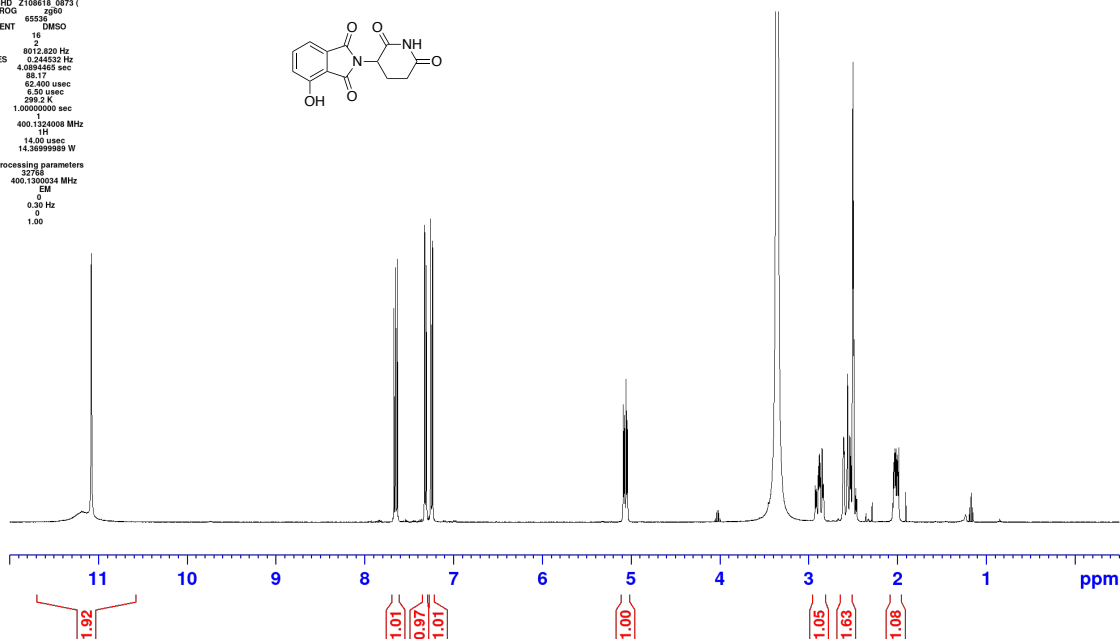
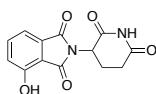
5-Amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (35)



Appendices

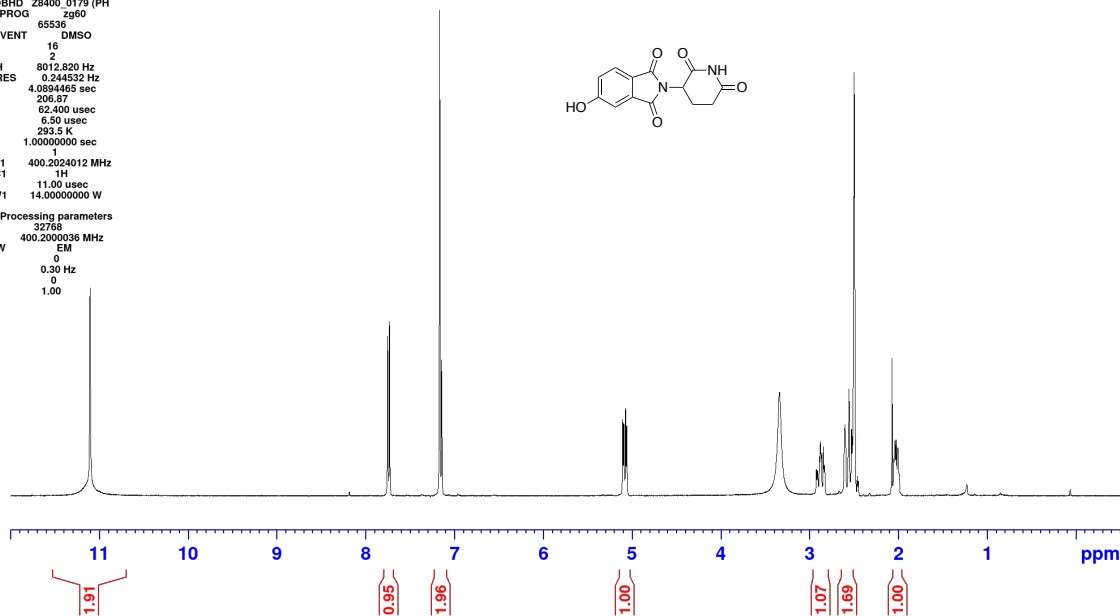
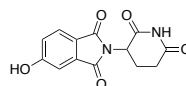
Current Data Parameters
NAME Feb18-2020-6-PJBA17-postextract1
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200219
Time 19.42 h
INSTRUM avq400
PROBHD Z106618_0873 (P)
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 36.17
DW 62.400 usec
DE 6.50 usec
TE 299.2 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324098 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.30959889 W
F2 - Processing parameters
SI 32768
SF 400.1300234 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (36)



Current Data Parameters
NAME Sep20-2022-1-PJBA75
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20220920
Time 11.57 h
INSTRUM avq400
PROBHD Z8400_0179 (PH)
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 36.17
DW 62.400 usec
DE 6.50 usec
TE 293.5 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 11.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000036 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-5-hydroxyisoindoline-1,3-dione (37)



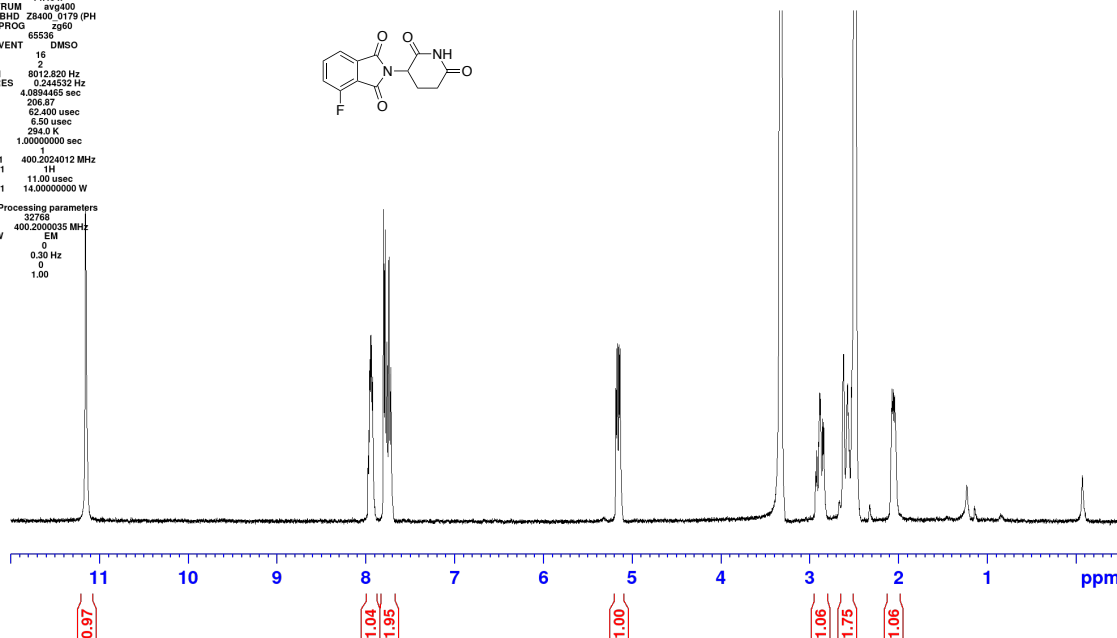
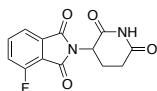
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Current Data Parameters
NAME Sep07-2022-1-PJBA08_clean
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date 20220907
Time 14:46 h
INSTRUM avq400
PROBHD Z8400_0179 (PH)
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 206.87
DW 62.400 usec
DE 6.50 usec
TE 294.0 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 11.00 usec
PLW1 14.00000000 W

F2 - Processing parameters
SI 32768
SF 400.2000035 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-4-fluoroisindoline-1,3-dione (**38**)

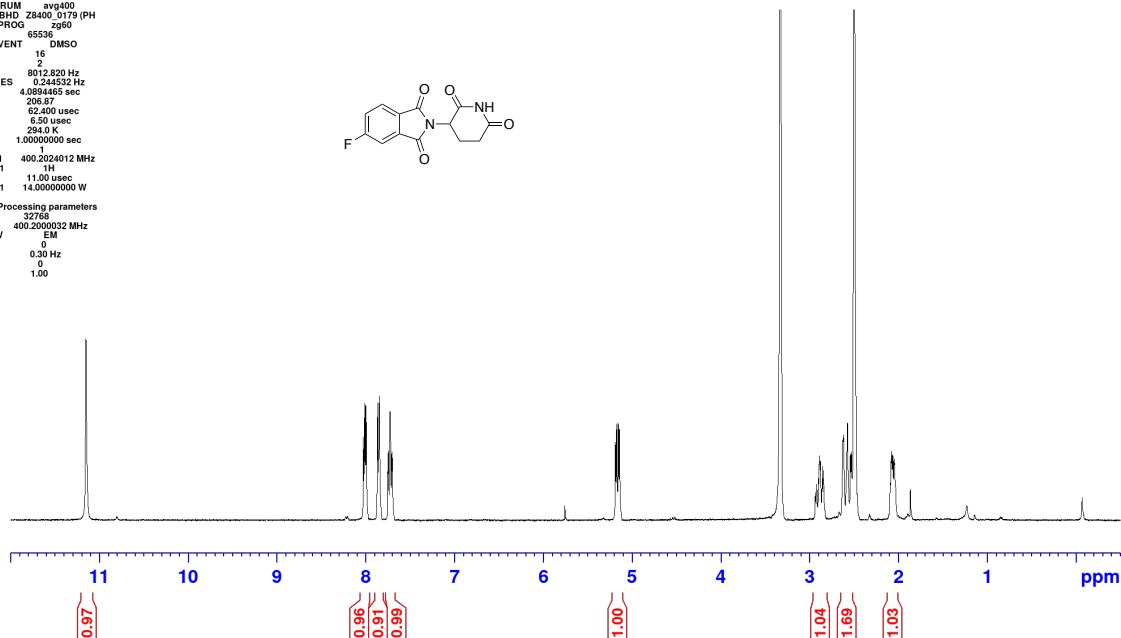
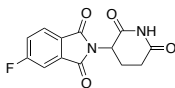


Current Data Parameters
NAME Sep07-2022-1-PJBA46_clean
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date 20220907
Time 14:52 h
INSTRUM avq400
PROBHD Z8400_0179 (PH)
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 206.87
DW 62.400 usec
DE 6.50 usec
TE 294.0 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 11.00 usec
PLW1 14.00000000 W

F2 - Processing parameters
SI 32768
SF 400.2000032 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

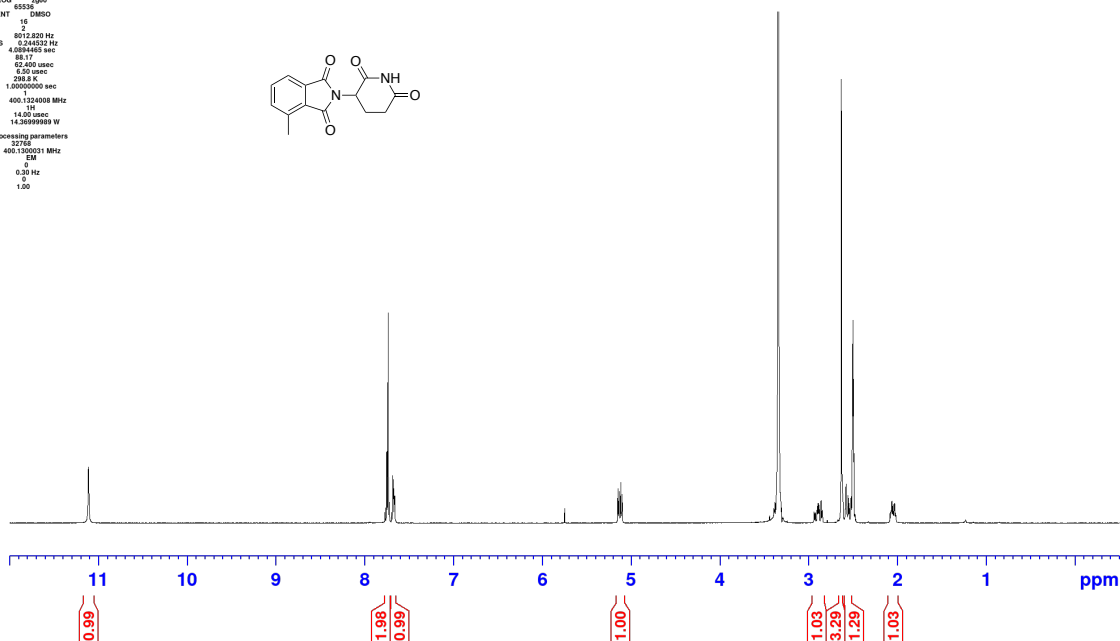
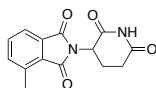
2-(2,6-Dioxopiperidin-3-yl)-5-fluoroisindoline-1,3-dione (**39**)



Appendices

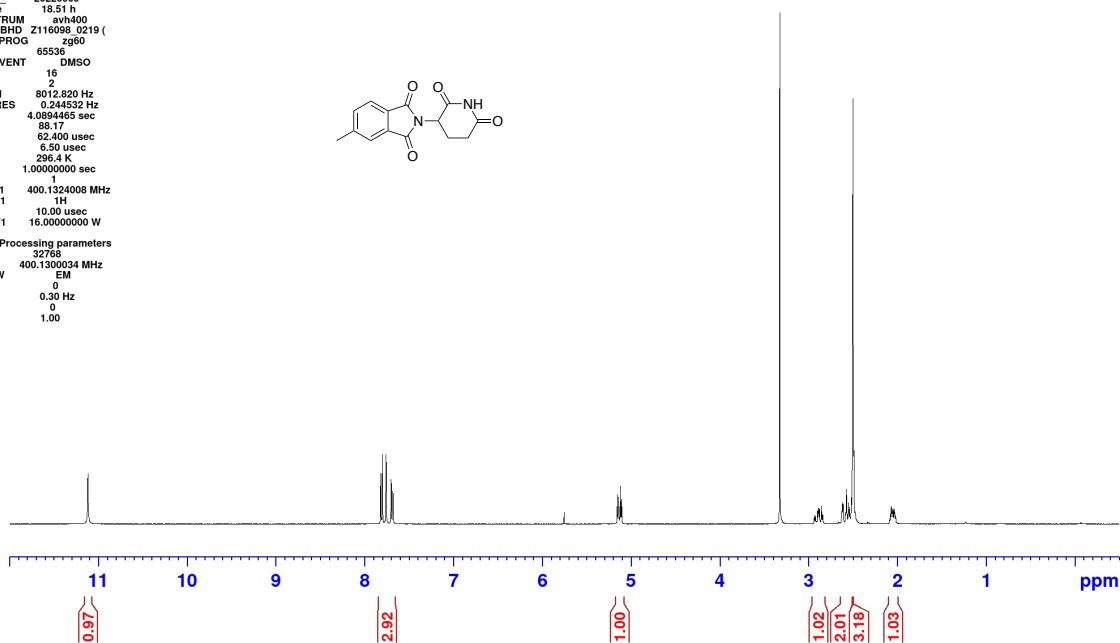
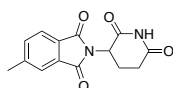
Current Data Parameters
NAME Feb04-2020-1-PJB-postc01-DMSO_A13
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200504
Time 12.15 h
INSTRUM avh400
PROBHD Z116098_0273 (PULPROG zgpg)
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 88.17
DWF 62.400 usec
DE 6.50 usec
TE 296.6 K
D1 1.0000000 sec
TDO 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999998 W
F2 - Processing parameters
SI 32768
SF 400.130034 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-4-methylisoindoline-1,3-dione (40)



Current Data Parameters
NAME Sep05-2022-2-PJBA45
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20220909
Time 18.51 h
INSTRUM avh400
PROBHD Z116098_0219 (PULPROG zgpg)
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 88.17
DWF 62.400 usec
DE 6.50 usec
TE 296.4 K
D1 1.0000000 sec
TDO 400.1324008 MHz
NUC1 1H
P1 10.00 usec
PLW1 16.00000000 W
F2 - Processing parameters
SI 32768
SF 400.1300034 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

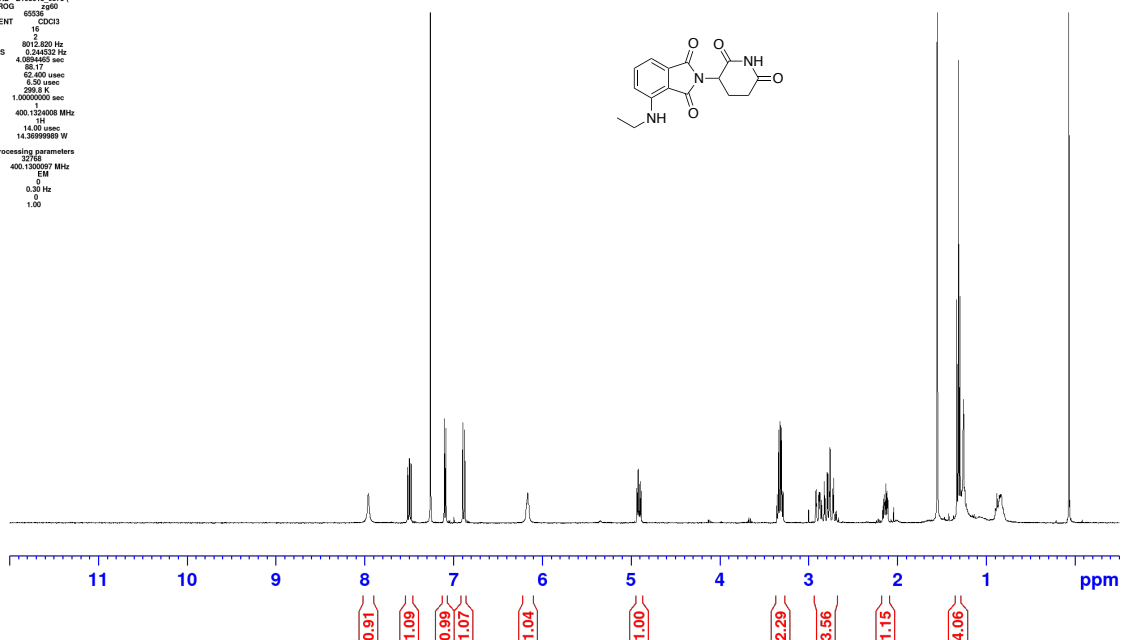
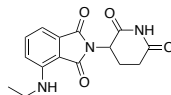
2-(2,6-Dioxopiperidin-3-yl)-5-methylisoindoline-1,3-dione (41)



Appendices

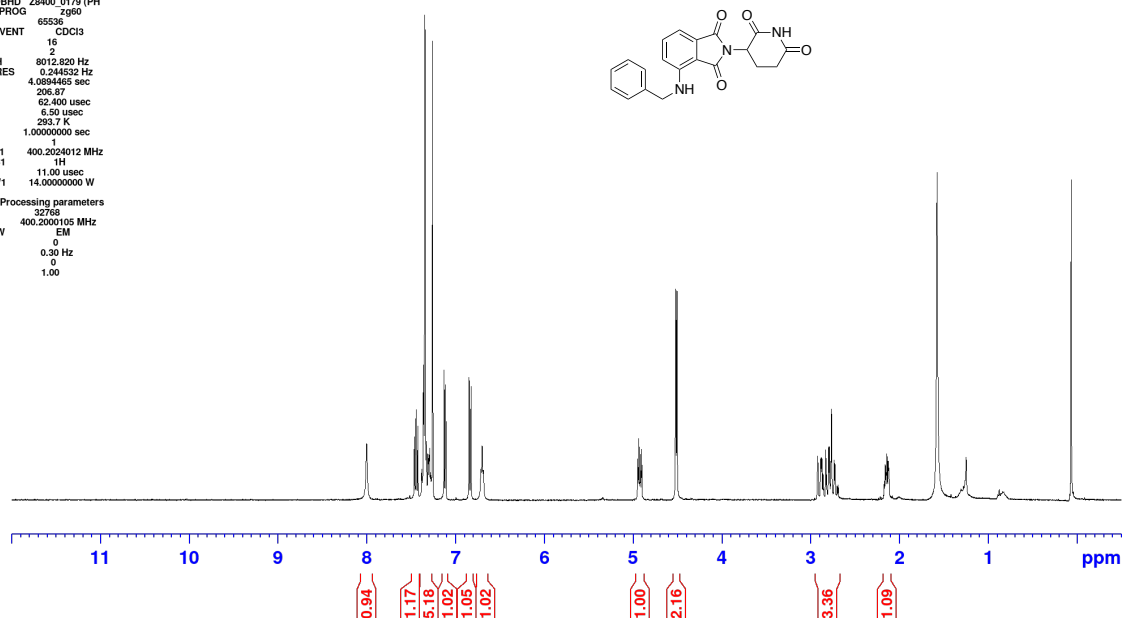
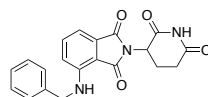
Current Data Parameters
NAME Mar10-2020-1-PJBA27-postcol-29to42
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200310
Time 1:28:11
INSTRUM avn400
PROBHD Z106113 9073 (1
PULPROG zgpg
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 88.17
DW 62.400 usec
DE 6.30 usec
TE 299.3 K
D1 1.0000000 sec
TDO 0
SFO1 400.1324009 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.39999999 W
F2 - Processing parameters
SI 32768
SF 400.1300097 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-4-(ethylamino)isoindoline-1,3-dione (**64**)



Current Data Parameters
NAME Sep13-2022-1-PJBA22_1_4
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20220913
Time 14:24 h
INSTRUM avn400
PROBHD Z8400_0179 (PH
PULPROG zgpg
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 206.67
DW 62.400 usec
DE 6.50 usec
TE 299.7 K
D1 1.0000000 sec
TDO 0
SFO1 400.2034012 MHz
NUC1 1H
P1 11.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000105 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

4-(Benzylamino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**68**)

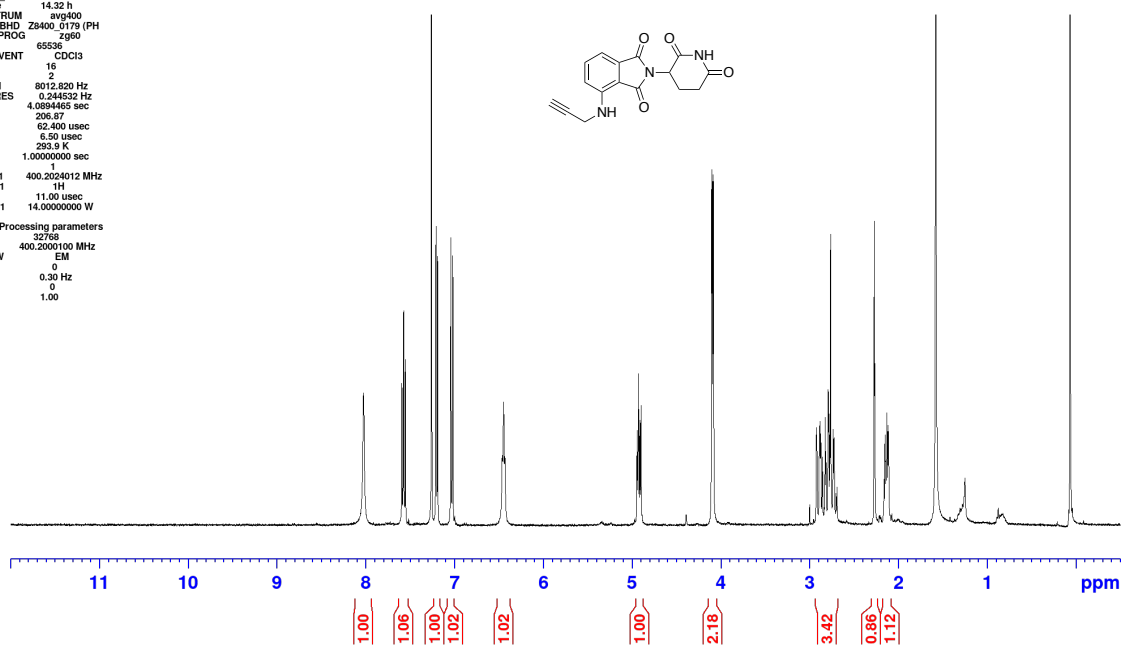


Appendices

Current Data Parameters
NAME Sep13-2022-1-PJBA28_1_4
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220913
Time 14.32 h
INSTRUM avq400
PROBHD ZS400_0179 (PH
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 16
DS 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 296.87
DW 62.400 usec
DE 6.50 usec
TE 293.2 K
D1 1.00000000 sec
TDO 1
SFO1 400.2024012 MHz
NUC1 1H
P1 11.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000100 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-4-(prop-2-yn-1-ylamino)isoindoline-1,3-dione (**70**)



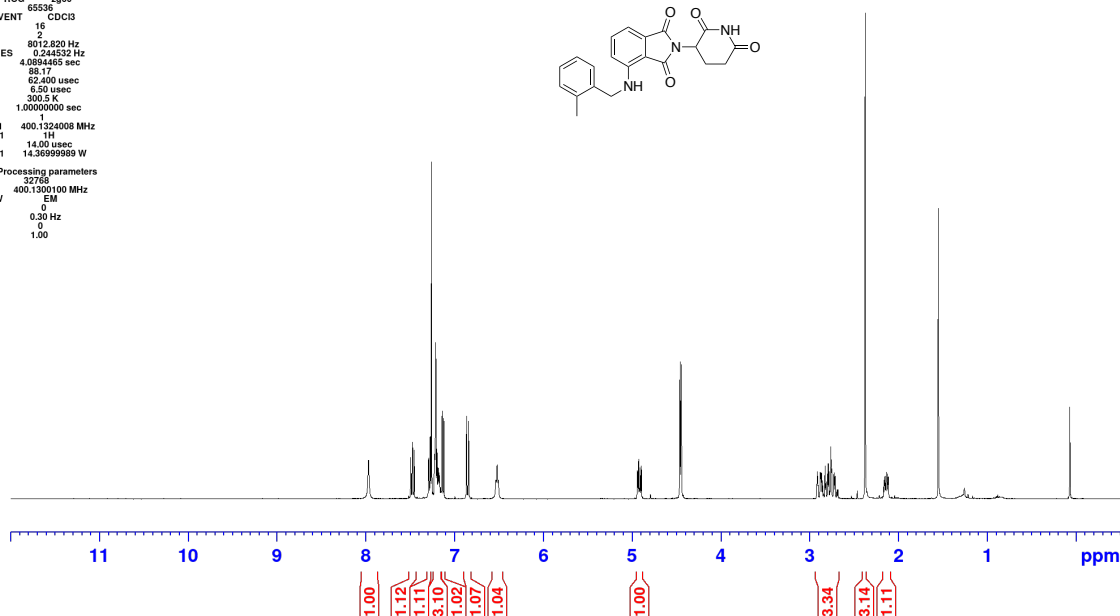
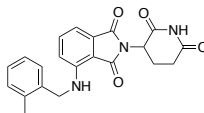
Appendices

Current Data Parameters
NAME Sep01-2020-2-PJBA34-second
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date 20200901
Time 15.00 h
INSTRUM spect
PROBHD Z10818 0873 (1H)
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244552 Hz
AQ 4.089465 sec
RG 88.17
DW 62.400 usec
DE 6.50 usec
TE 300.5 K
D1 1.0000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.3699989 W

F2 - Processing parameters
SI 32768
SF 400.1300100 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-4-((2-methylbenzyl)amino)isoindoline-1,3-dione (74)

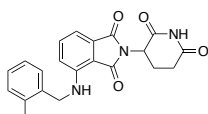
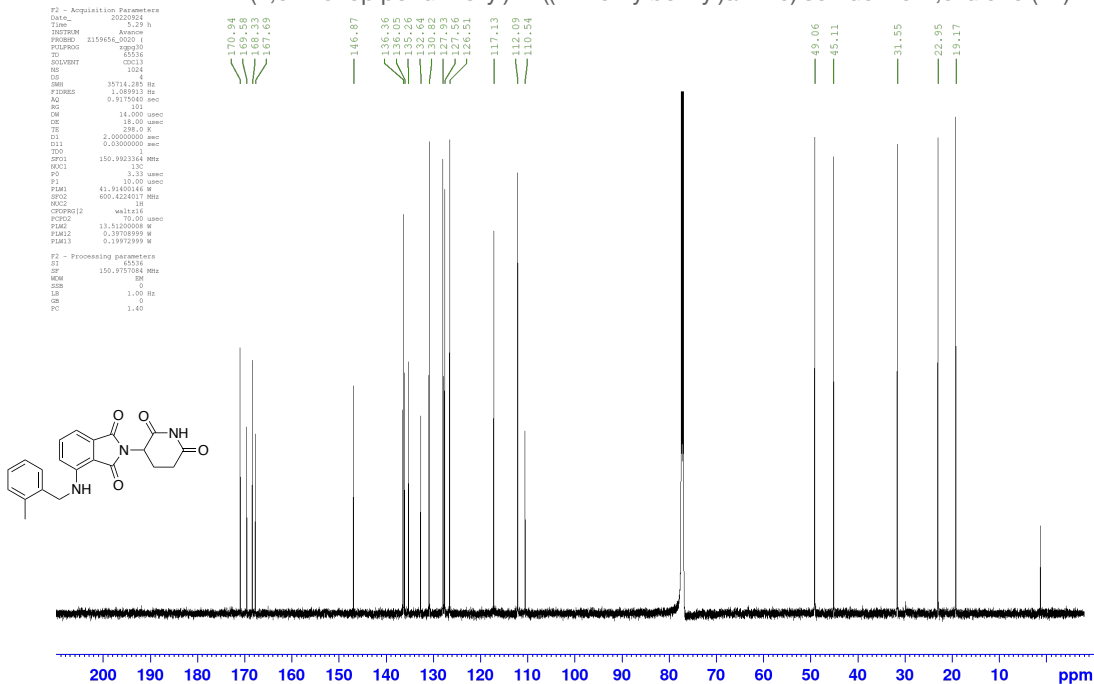


Current Data Parameters
NAME A34_g8478282359
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date 20200901
Time 5.29 h
INSTRUM spect
PROBHD Z10818 0873 (1H)
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244552 Hz
AQ 4.089465 sec
RG 88.17
DW 62.400 usec
DE 6.50 usec
TE 300.5 K
D1 1.0000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.3699989 W

F2 - Processing parameters
SI 32768
SF 400.1300100 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-4-((2-methylbenzyl)amino)isoindoline-1,3-dione (74)



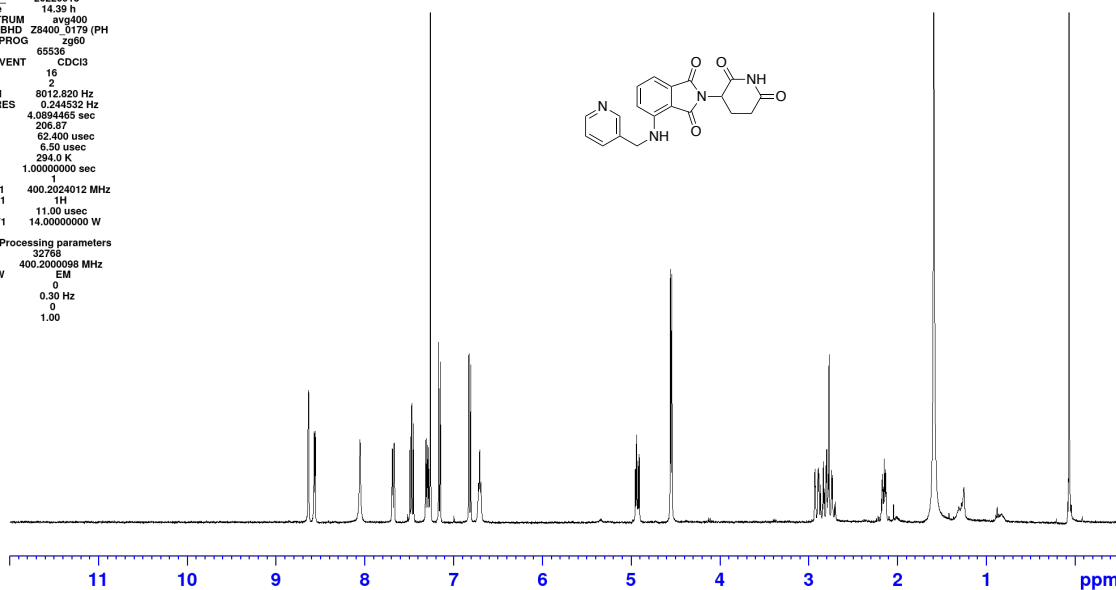
Appendices

Current Data Parameters
NAME Sep13-2022-1-PJBA31
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220913
Time 14.39 h
INSTRUM avq400
PROBHD Z8400_0179 (PH)
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 206.87
DW 62.400 usec
DE 6.50 usec
TE 294.0 K
D1 1.00000000 sec
TD0
SFO1 400.2024012 MHz
NUC1 1H
P1 11.00 usec
PLW1 14.00000000 W

F2 - Processing parameters
SI 32768
SF 400.2000098 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-4-((pyridin-3-ylmethyl)amino)isoindoline-1,3-dione (**84**)

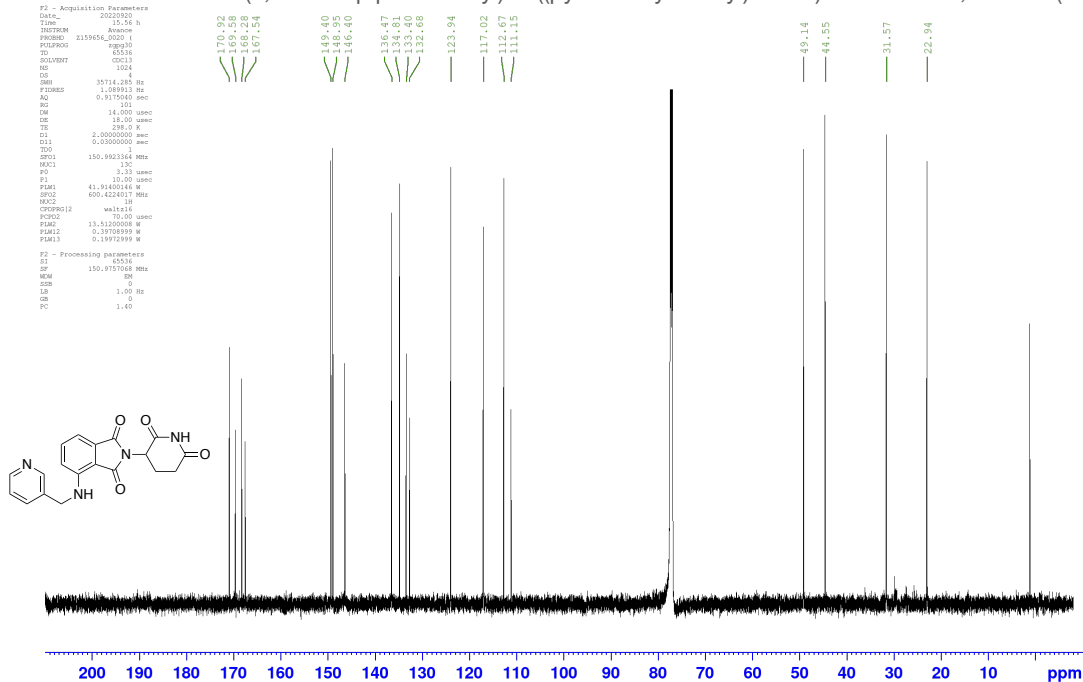


Current Data Parameters
NAME A31_gm77512059
EXPNO 3
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220920
Time 15.56 h
INSTRUM Avance
PROBHD BBO500-0020 (I)
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 35714.820 Hz
FIDRES 1.089913 Hz
AQ 0.9175940 sec
RG 101
SM 14.000 usec
DE 18.00 usec
TE 296.0 K
D1 2.00000000 sec
D11 0.00000000 sec
TD0
SFO1 500.9923364 MHz
NUC1 13C
P1 10.00 usec
PL1 41.81400146 W
PCPGM12 600.4224017 MHz
PCPGM13 10.00 usec
PCPGM14 13.42000008 W
PCPGM15 0.10708999 W
PCPGM16 0.10708999 W

F2 - Processing parameters
SI 65536
SF 500.9757068 MHz
WDW BM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

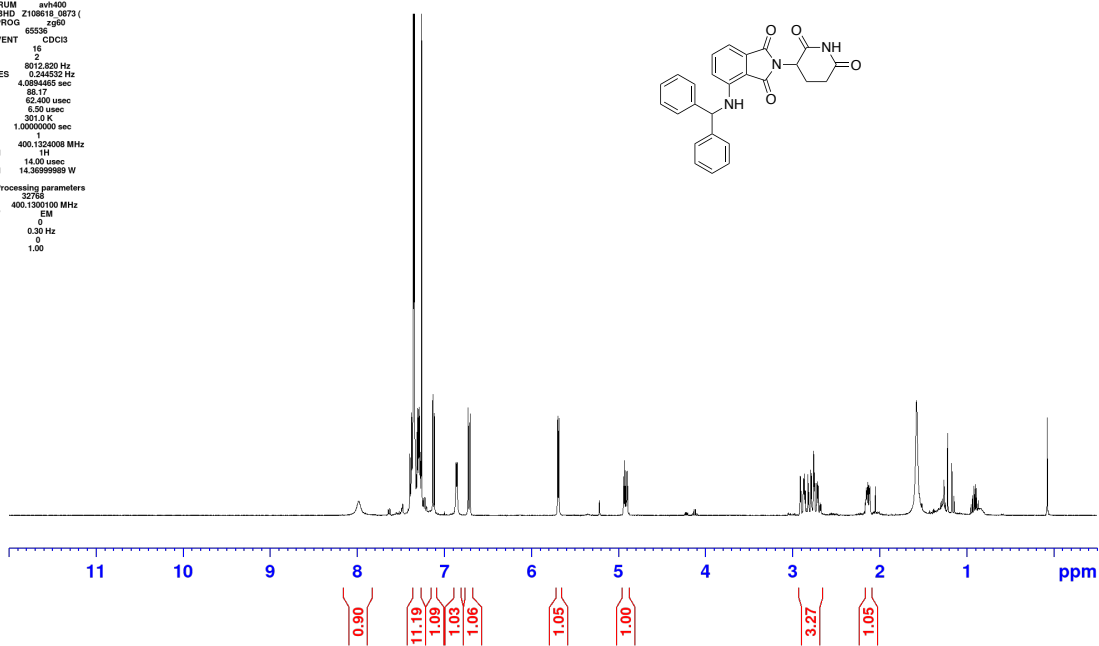
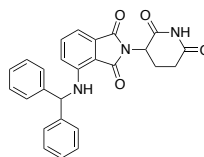
2-(2,6-Dioxopiperidin-3-yl)-4-((pyridin-3-ylmethyl)amino)isoindoline-1,3-dione (**84**)



Appendices

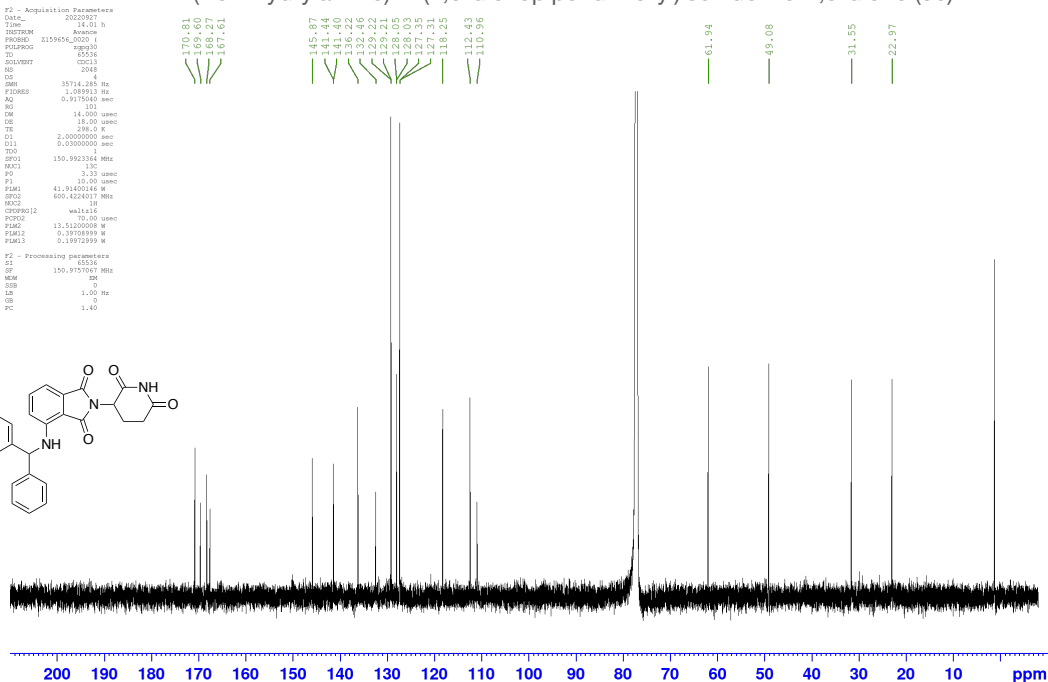
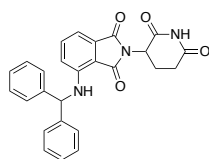
Current Data Parameters
NAME: Jen15-2021-1-PJBA65-postcol2
EXPNO: 1
PROCNO: 1
F2 - Acquisition Parameters
Date_: 20210115
Time: 16:57:5
INSTRUM: avn400
PROBHD: Z10618 (873)
PULPROG: zg60
TD: 65536
SOLVENT: CDCl3
NS: 16
DS: 2
SWH: 8072.820 Hz
FIDRES: 0.244532 Hz
AQ: 4.0894465 sec
RG: 88.17
DW: 62.400 usec
DE: 6.50 usec
TE: 301.0 K
D1: 1.0000000 sec
TD0: 1
SFO1: 400.1324008 MHz
NUC1: 1H
P1: 14.30 usec
PLW1: 14.36999889 W
F2 - Processing parameters
SI: 32768
SF: 400.1300100 MHz
WDW: EM
SSB: 0
LB: 0.30 Hz
GB: 0
PC: 1.00

4-(Benzhydrylamino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (86)



Current Data Parameters
NAME: ASD_20679472709
EXPNO: 5
PROCNO: 5
F2 - Acquisition Parameters
Date_: 20220927
Time: 14:03:14
INSTRUM: Avance
PROBHD: E155956_202001
PULPROG: zgpg30
TD: 65536
SOLVENT: CDCl3
NS: 2048
DS: 4
SWH: 35714.285 Hz
FIDRES: 0.088913 Hz
AQ: 0.9175040 sec
RG: 101
DW: 14.000 usec
DE: 14.00 usec
TE: 298.0 K
D1: 2.00000000 sec
D11: 0.03000000 sec
SFO1: 150.992334 MHz
NUC1: 13C
P1: 1.30 usec
PLW1: 41.81420148 W
SFO2: 800.4224017 MHz
NUC2: 1H
PCPDW12: waltz16
PCPDW2: 30.00 usec
PLW2: 13.5120008 W
PLW12: 0.39708989 W
PLW13: 0.19972999 W
F2 - Processing parameters
SI: 32768
SF: 150.9757077 MHz
WDW: EM
SSB: 0
LB: 1.00 Hz
GB: 0
PC: 1.40

4-(Benzhydrylamino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (86)



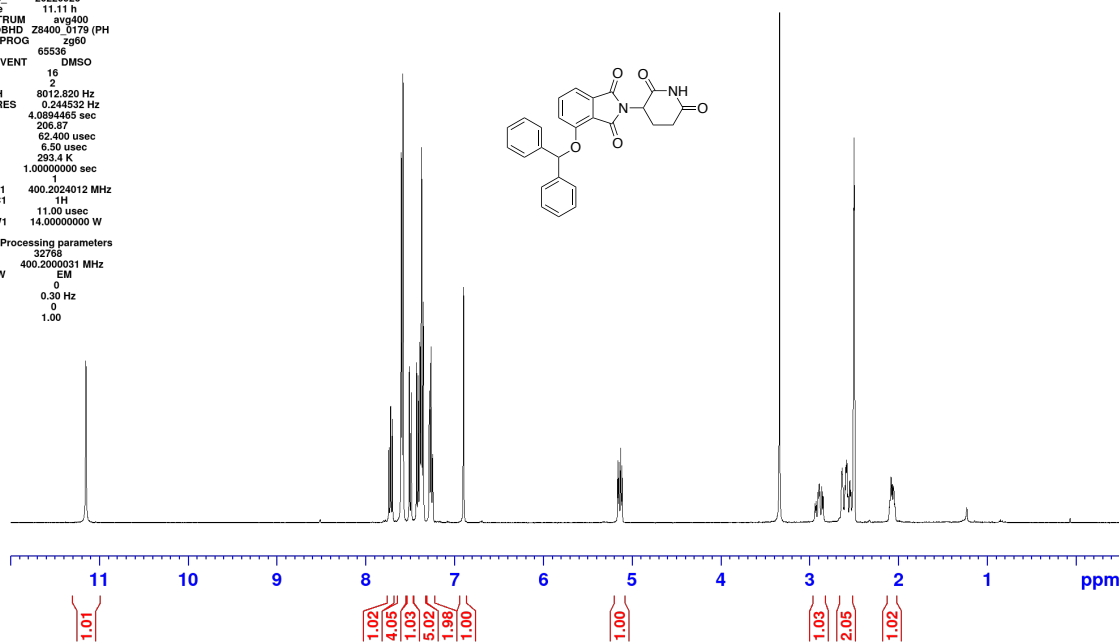
Appendices

Current Data Parameters
NAME Sep26-2022-1-PJBA83
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220926
Time 11.11 h
INSTRUM svp400
PROBHD Z8400_0179 (PH)
PULPROG zgpg
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.284532 Hz
AQ 4.0894465 sec
RG 206.87
DW 62.400 usec
DE 6.50 usec
TE 293.4 K
D1 1.00000000 sec
D11 400.2024012 MHz
NUC1 1H
P1 11.00 usec
PLW1 14.00000000 W

F2 - Processing parameters
SI 32768
SF 400.2000031 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

4-(Benzhydryloxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (87)

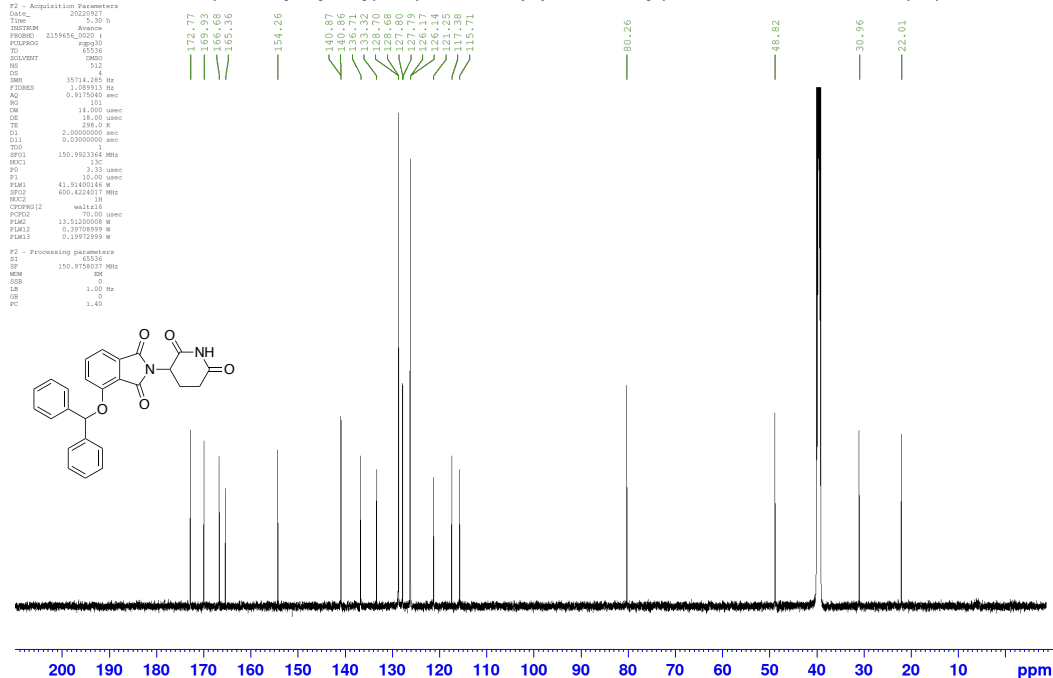


Current Data Parameters
NAME A83_g6784269
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220927
Time 5.30 h
INSTRUM Avance
PROBHD BBO500
PULPROG zgpg
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 35714.180 Hz
FIDRES 1.085913 Hz
AQ 0.1975040 sec
RG 101
DW 14.000 usec
DE 18.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
D12 1
D13 150.9923364 MHz
NUC1 13C
P1 3.33 usec
PL1 41.91450146 W
PL12 600.424017 MHz
PL13 10
PL14 10.00 usec
PL15 13.51200008 W
PL16 0.19708999 W
PL17 0.19708999 W

F2 - Processing parameters
SI 65536
SF 150.9758037 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

4-(Benzhydryloxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (87)



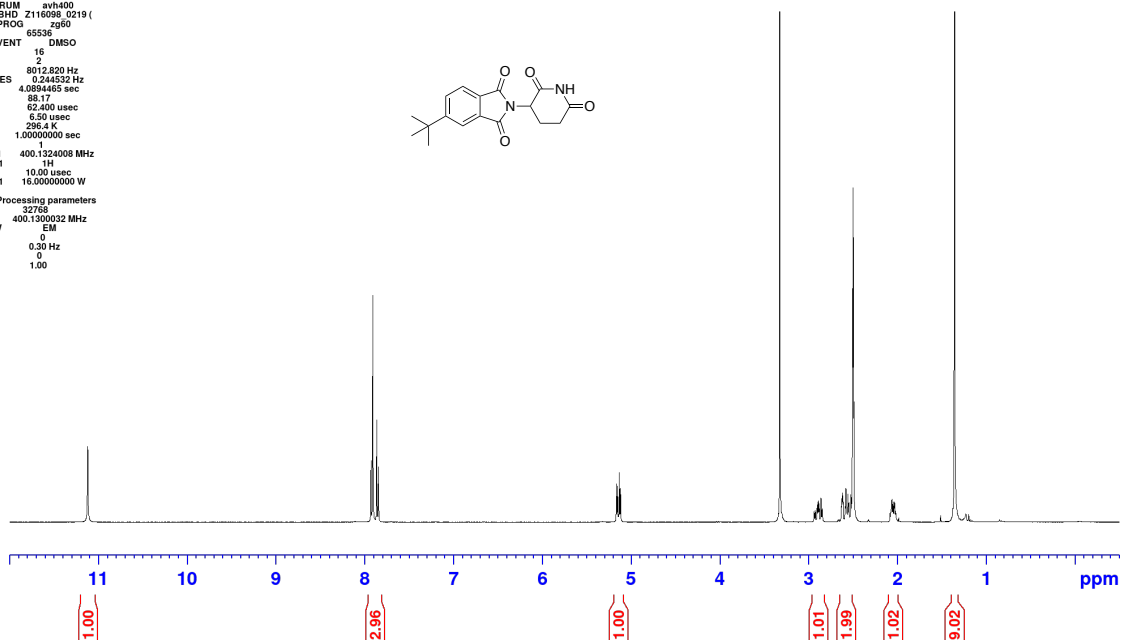
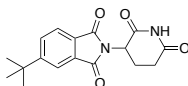
Appendices

Current Data Parameters
NAME Sep09-2022-11-PJBA63_clean
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220909
Time 19.21 h
INSTRUM avh400
PROBHD Z116098_0219 (PULPROG zg60
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 88.17
DW 62.400 usec
DE 6.50 usec
TE 296.4 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 10.00 usec
PLW1 16.00000000 W

F2 - Processing parameters
SI 32768
SF 400.1300032 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

5-(Tert-butyl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**88**)

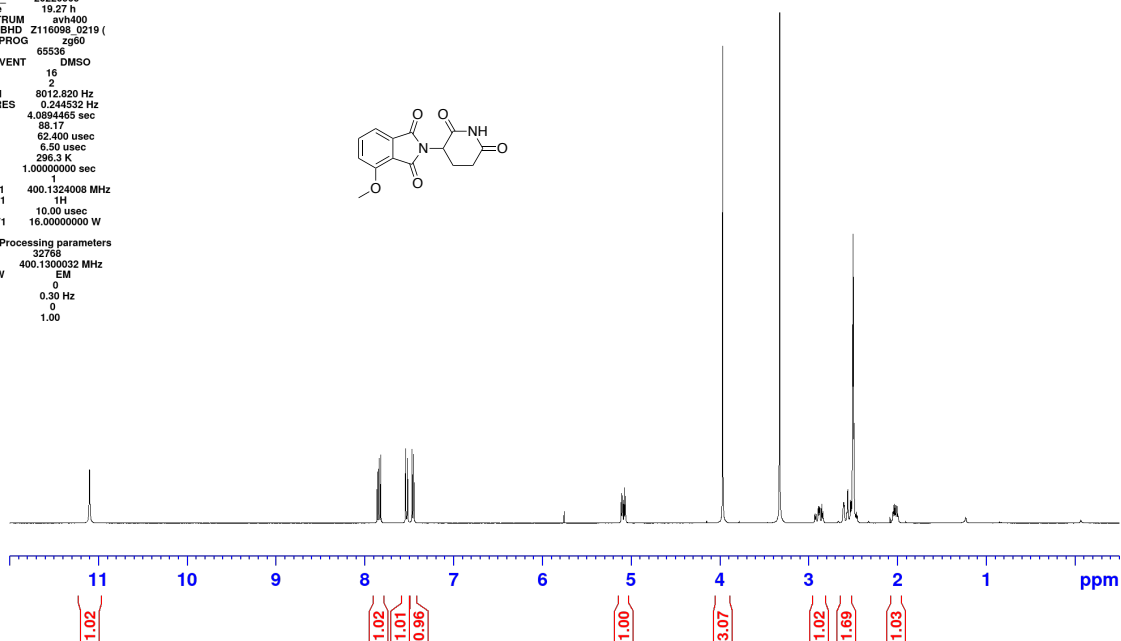
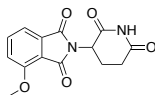


Current Data Parameters
NAME Sep09-2022-10-PJBB05
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220909
Time 19.27 h
INSTRUM avh400
PROBHD Z116098_0219 (PULPROG zg60
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 88.17
DW 62.400 usec
DE 6.50 usec
TE 296.3 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 10.00 usec
PLW1 16.00000000 W

F2 - Processing parameters
SI 32768
SF 400.1300032 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-4-methoxyisoindoline-1,3-dione (**89**)



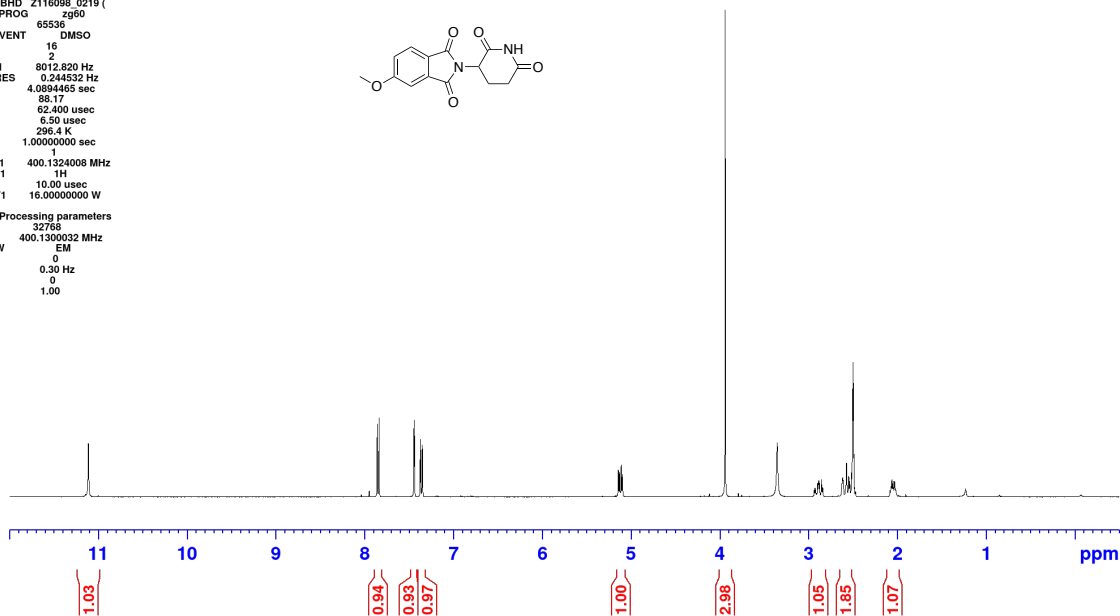
Appendices

Current Data Parameters
NAME Sep16-2022-21-PJBA87
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220916
Time 12.28 h
INSTRUM avh400
PROBHD Z116098_0219 (Z116098)
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 88.17
DW 62.400 usec
DE 6.50 usec
TE 296.4 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 10.00 usec
PLW1 16.00000000 W

F2 - Processing parameters
SI 32768
SF 400.1300032 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-5-methoxyisoindoline-1,3-dione (90)

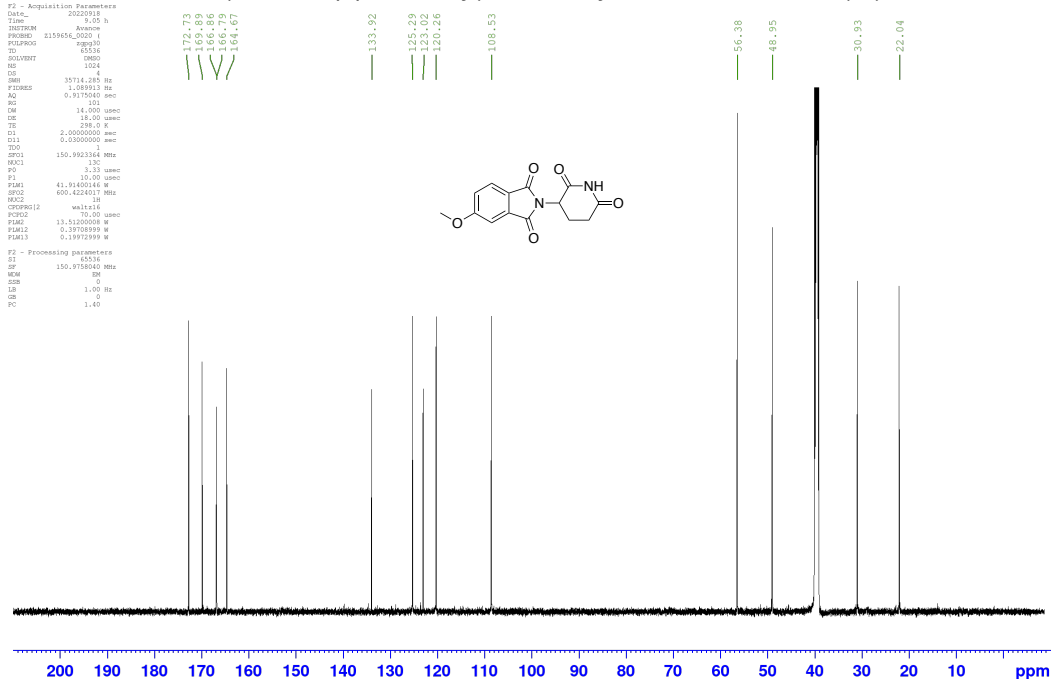


Current Data Parameters
NAME sep16-2022-21-PJBA87
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220916
Time 12.28 h
INSTRUM avh400
PROBHD Z116098_0219 (Z116098)
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 88.17
DW 62.400 usec
DE 6.50 usec
TE 296.4 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 10.00 usec
PLW1 16.00000000 W

F2 - Processing parameters
SI 32768
SF 400.1300032 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

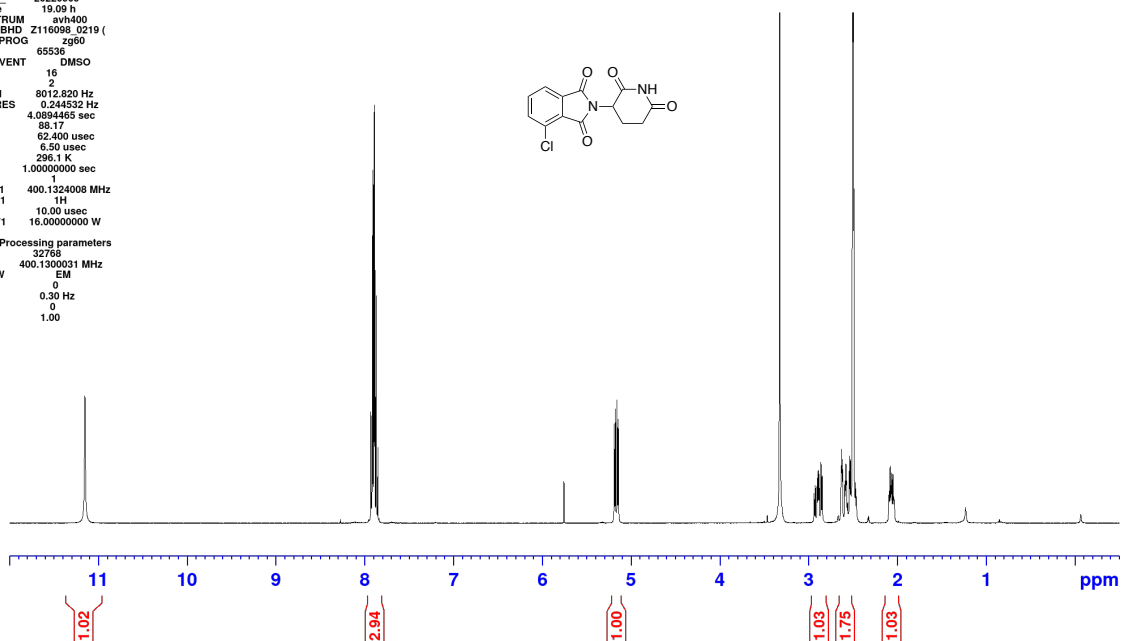
2-(2,6-Dioxopiperidin-3-yl)-5-methoxyisoindoline-1,3-dione (90)



Appendices

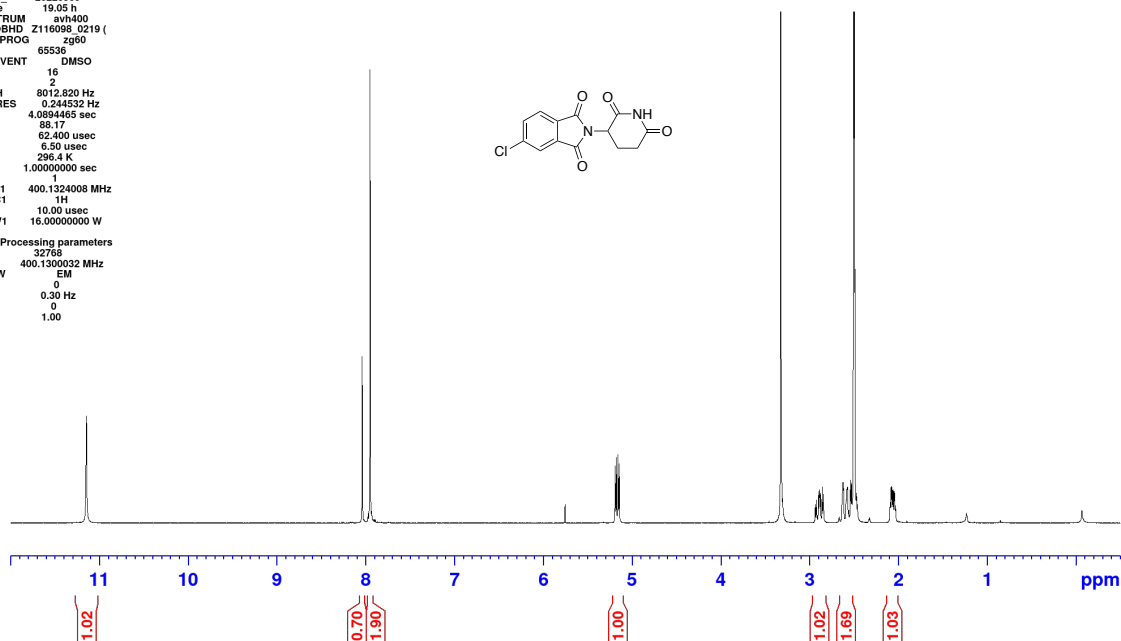
Current Data Parameters
 NAME Sep09-2022-6-PJBA89
 EXPNO 1
 PROCNO 1
 F2 - Acquisition Parameters
 Date_ 20220909
 Time 19.09 h
 INSTRUM avh400
 PROBHD Z116098_0219 ()
 PULPROG zg60
 TD 65536
 SOLVENT DMSO
 NS 16
 DS 2
 SWH 8012.820 Hz
 FIDRES 0.244532 Hz
 AQ 4.0894465 sec
 RG 88.17
 DW 62.400 usec
 DE 6.50 usec
 TE 296.1 K
 D1 1.00000000 sec
 TD0 1
 SFO1 400.1324008 MHz
 NUC1 1H
 P1 10.00 usec
 PLW1 16.00000000 W
 F2 - Processing parameters
 SI 32768
 SF 400.1300031 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

4-Chloro-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**91**)



Current Data Parameters
 NAME Sep09-2022-5-PJBA86
 EXPNO 1
 PROCNO 1
 F2 - Acquisition Parameters
 Date_ 20220909
 Time 19.05 h
 INSTRUM avh400
 PROBHD Z116098_0219 ()
 PULPROG zg60
 TD 65536
 SOLVENT DMSO
 NS 16
 DS 2
 SWH 8012.820 Hz
 FIDRES 0.244532 Hz
 AQ 4.0894465 sec
 RG 88.17
 DW 62.400 usec
 DE 6.50 usec
 TE 296.4 K
 D1 1.00000000 sec
 TD0 1
 SFO1 400.1324008 MHz
 NUC1 1H
 P1 10.00 usec
 PLW1 16.00000000 W
 F2 - Processing parameters
 SI 32768
 SF 400.1300032 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

5-Chloro-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**92**)



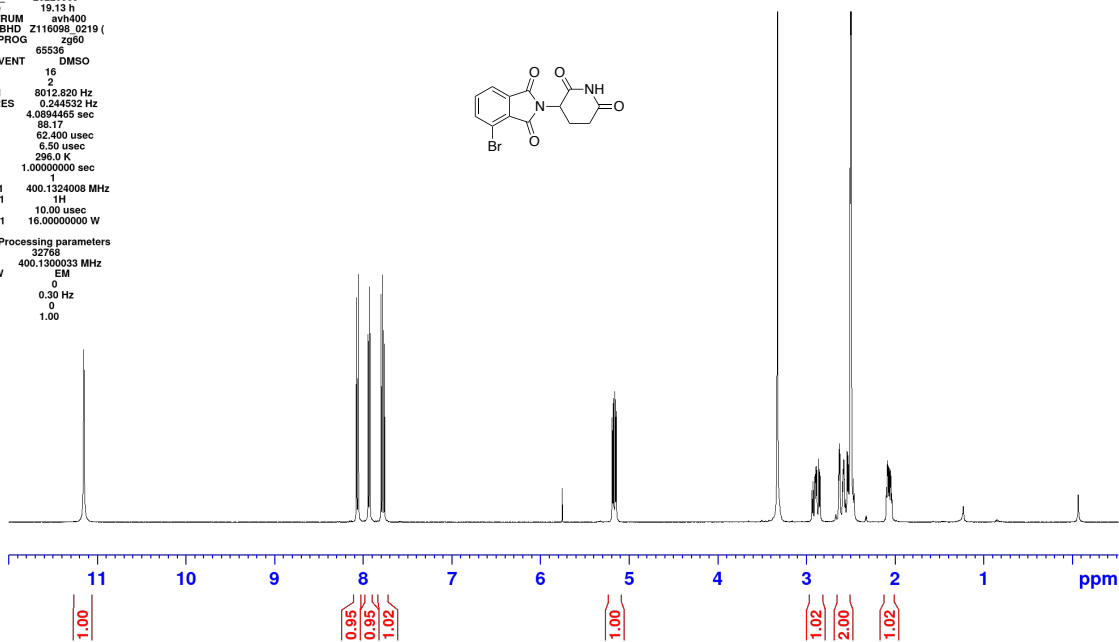
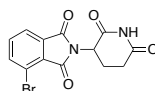
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Current Data Parameters
NAME Sep09-2022-7-PJBA93
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220909
Time 19.13 h
INSTRUM avh400
PROBHD Z116098.0219 (PULPROG zg60)
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0694465 sec
RG 68.17
DW 62.400 usec
DE 6.50 usec
TE 296.0 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 10.00 usec
PLW1 16.00000000 W

F2 - Processing parameters
SI 32768
SF 400.1300033 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

4-Bromo-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**93**)

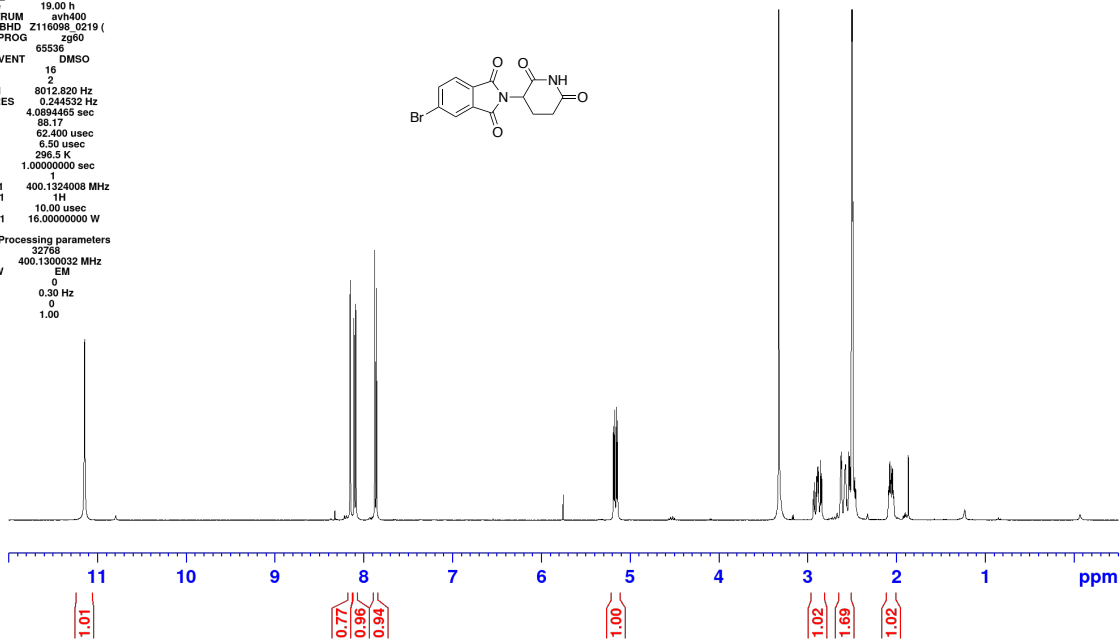
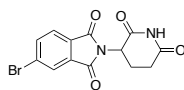


Current Data Parameters
NAME Sep09-2022-4-PJBA52
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220909
Time 19.00 h
INSTRUM avh400
PROBHD Z116098.0219 (PULPROG zg60)
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0694465 sec
RG 68.17
DW 62.400 usec
DE 6.50 usec
TE 296.5 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 10.00 usec
PLW1 16.00000000 W

F2 - Processing parameters
SI 32768
SF 400.1300032 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

5-Bromo-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**94**)



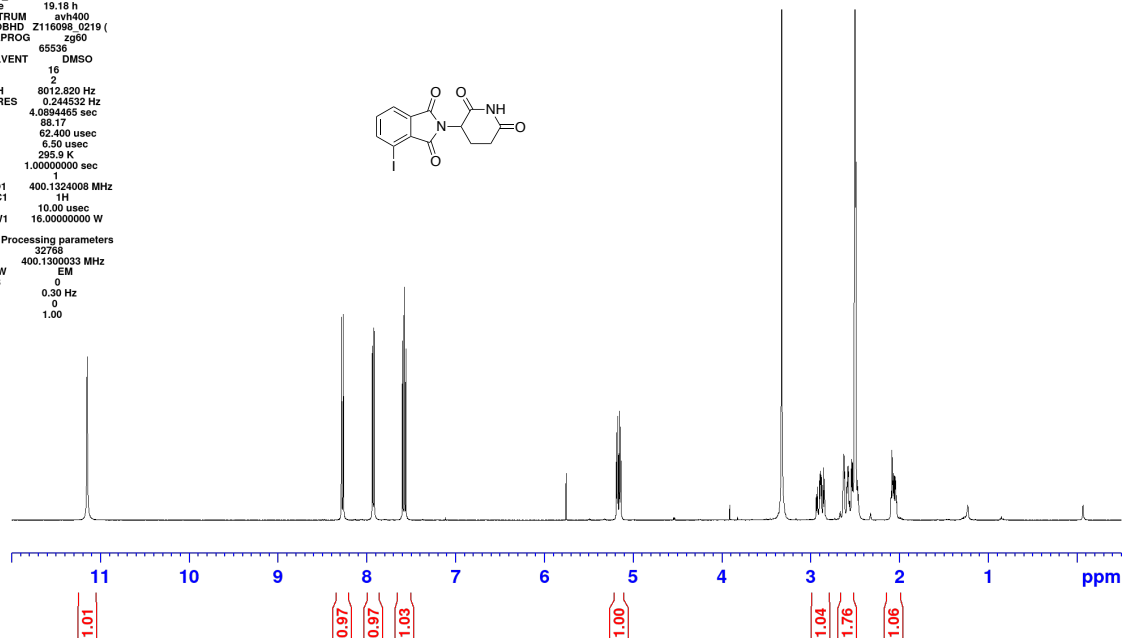
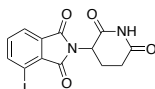
Appendices

Current Data Parameters
NAME Sep09-2022-8-PJBB01
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220909
Time 19.18 h
INSTRUM avh400
PROBHD Z116098_0219 (PULPROG zgpg)
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 88.17
DW 62.400 usec
DE 6.50 usec
TE 295.9 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 10.00 usec
PLW1 16.00000000 W

F2 - Processing parameters
SI 32768
SF 400.1300033 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-4-iodoisoindoline-1,3-dione (**95**)



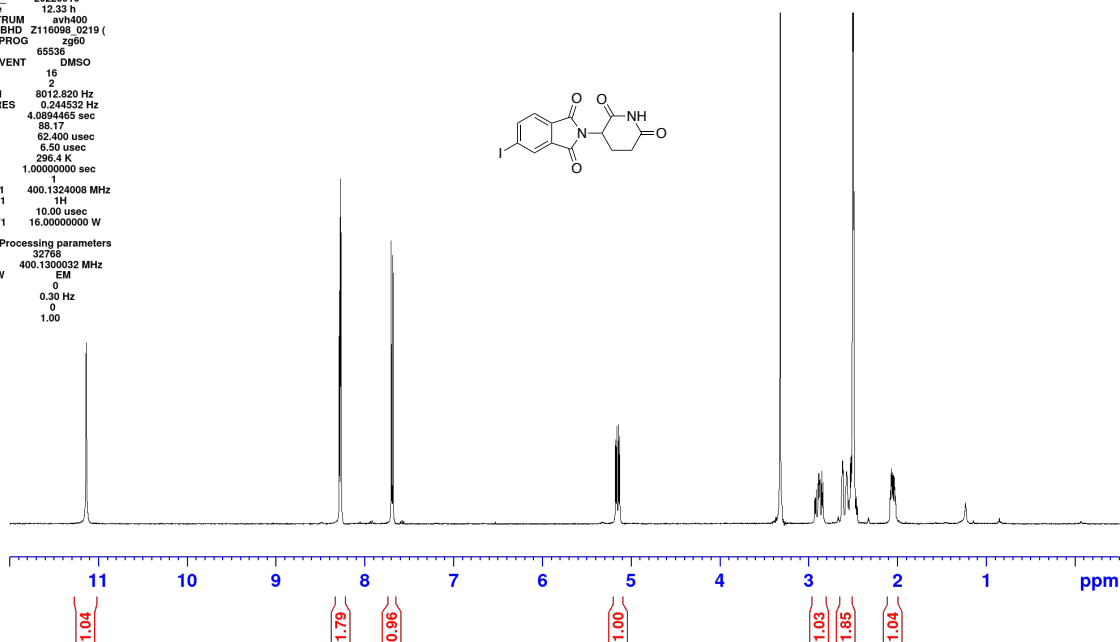
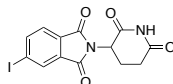
Appendices

Current Data Parameters
NAME Sep16-2022-22-PJBA88
EXPNO 1
PROCNO 1

2-(2,6-Dioxopiperidin-3-yl)-5-iodoisoindoline-1,3-dione (96)

F2 - Acquisition Parameters
Date_ 20220916
Time 12.33 h
INSTRUM avh400
PROBHD Z116098_0219 (PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 88.17
DW 62.400 usec
DE 6.50 usec
TE 296.4 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 10.00 usec
PLW1 16.00000000 W

F2 - Processing parameters
SI 32768
SF 400.1300032 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



Current Data Parameters
NAME ASR_2677811659
EXPNO 5
PROCNO 1

2-(2,6-Dioxopiperidin-3-yl)-5-iodoisoindoline-1,3-dione (96)

F2 - Acquisition Parameters
Date_ 20220918
Time 10.44 h
INSTRUM Avance
PROBHD BBO500-0020 (PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 35714.283 Hz
FIDRES 1.089913 Hz
AQ 0.9175040 sec
RG 101
DW 14.000 usec
DE 18.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 150.9923364 MHz
NUC1 13C
P1 3.33 usec
PLW1 41.91400144 W
SFO2 600.4224017 MHz
NUC2 1H
CPCPGP12 waltz16
PCPD1 70.00 usec
PLM2 13.51200008 W
PLM12 0.3870889 W
PLM13 0.19729299 W

F2 - Processing parameters
SI 65536
SF 150.9754547 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

172.68
166.67
166.76
165.91

143.47

132.65
131.82
130.43

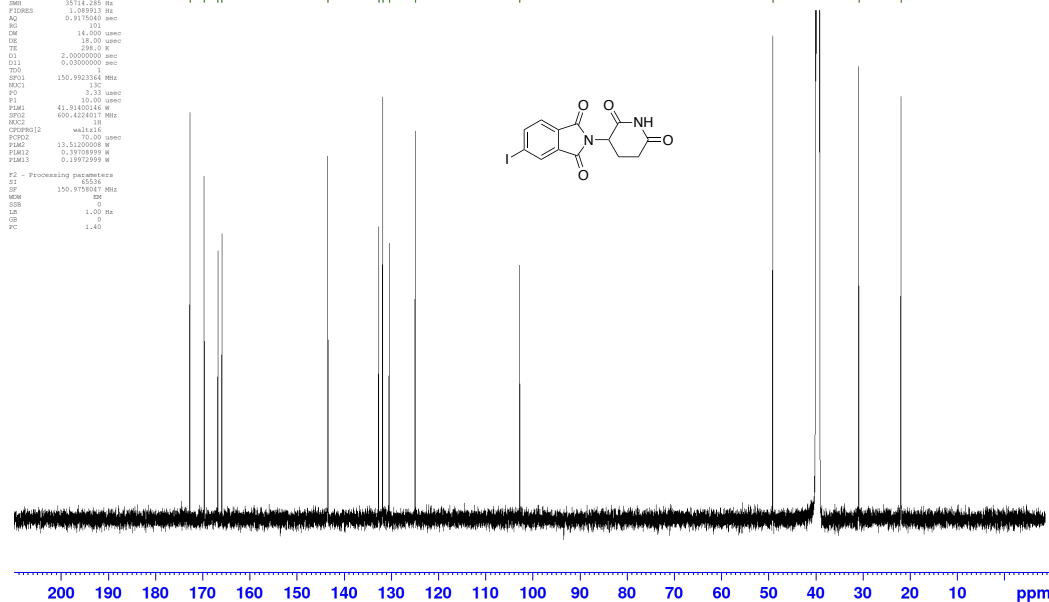
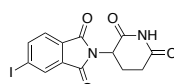
124.91

102.76

49.06

30.87

21.88



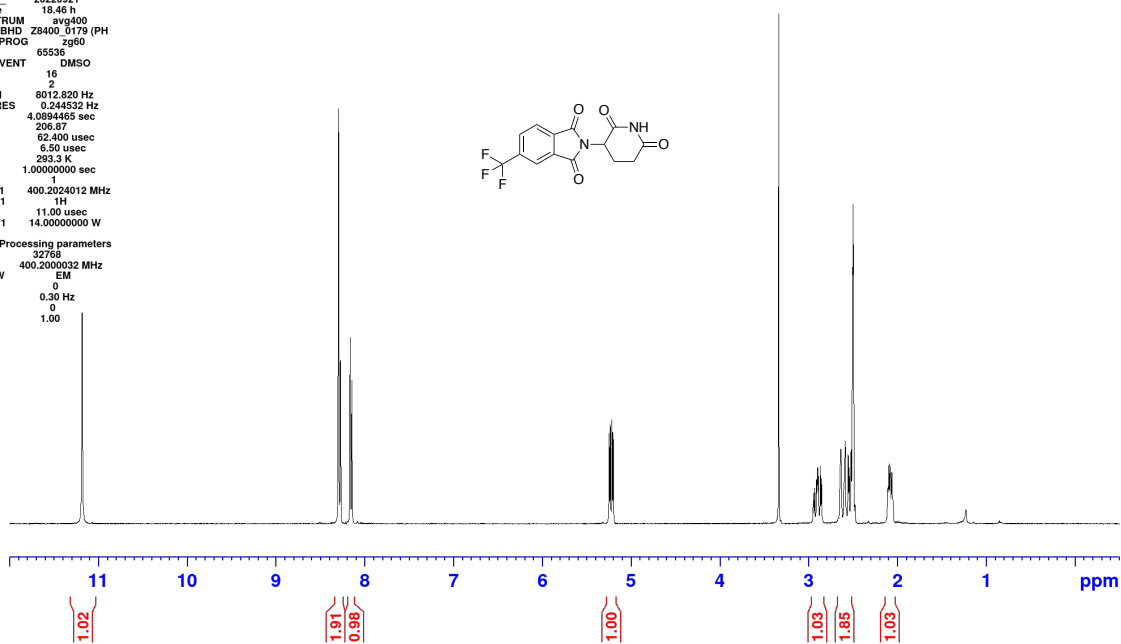
Appendices

Current Data Parameters
NAME Sep21-2022-1-PJBA84
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220921
Time 18.46 h
INSTRUM avq500
PROBHD Z8400_0179 (PH
PULPROG zg90
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 206.67
DW 62.400 usec
DE 6.50 usec
TE 293.3 K
D1 1.0000000 sec
D11 1
SFO1 400.2024012 MHz
NUC1 1H
P1 11.00 usec
PLW1 14.0000000 W

F2 - Processing parameters
SI 32768
SF 400.2000032 MHz
WDW EM
SSB 0
LB 0.90 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-5-(trifluoromethyl)isoindoline-1,3-dione (97)

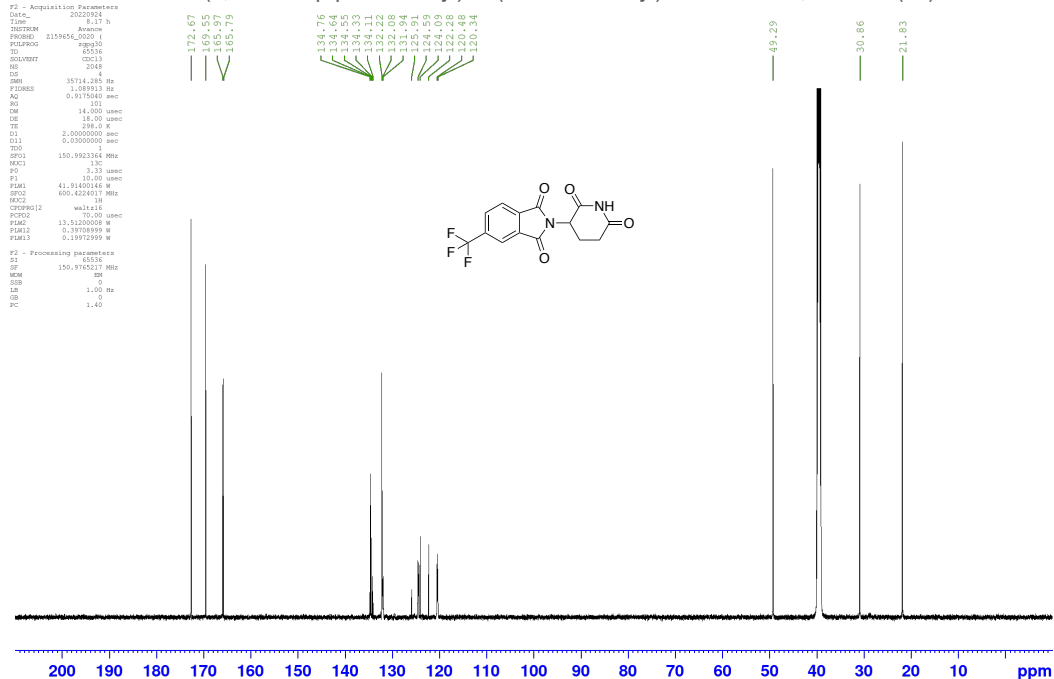


Current Data Parameters
NAME A84_gab78302309
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220924
Time 8.21 h
INSTRUM Avance
PROBHD z13995_0020 f
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 35714.285 Hz
FIDRES 1.089913 Hz
AQ 0.917000 sec
RG 101
DW 14.000 usec
DE 18.00 usec
TE 298.2 K
D1 2.0000000 sec
D11 0.0300000 sec
TD0 3
SFO1 150.9923344 MHz
NUC1 13C
P1 3.33 usec
PL1 0.10 usec
FIR1 41.91400146 W
SFO2 400.6264017 MHz
NUC2 1H
P2 10.00 usec
PL2 13.51200008 W
FIR2 0.19788999 W
PLR13 0.1972999 W

F2 - Processing parameters
SI 65536
SF 150.9765217 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

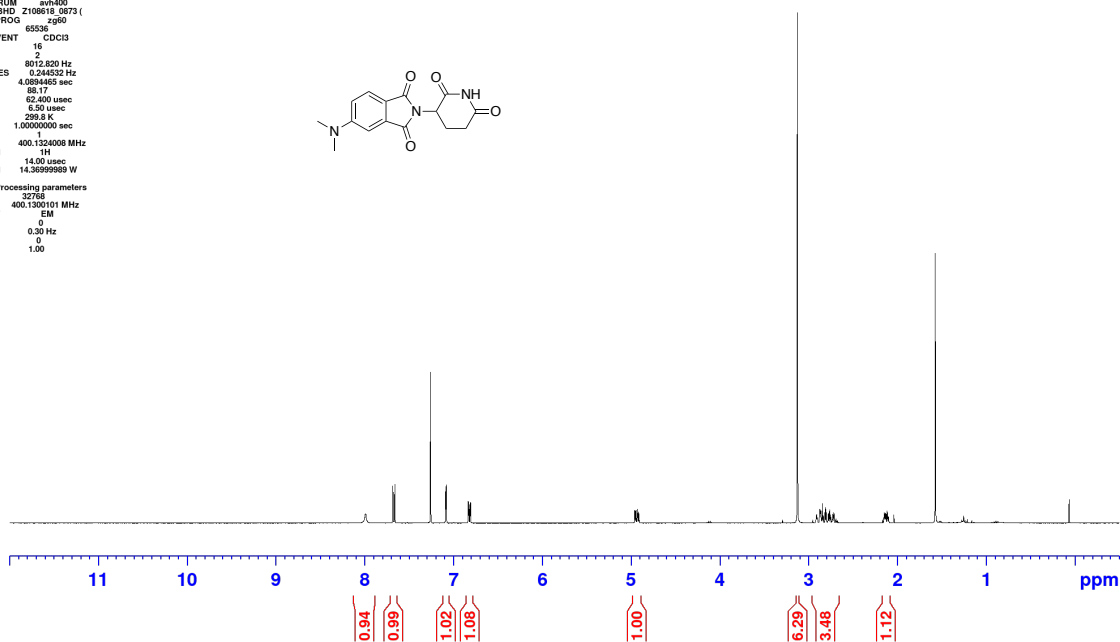
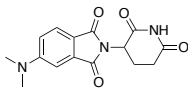
2-(2,6-Dioxopiperidin-3-yl)-5-(trifluoromethyl)isoindoline-1,3-dione (97)



Appendices

Current Data Parameters
 NAME Dec17-2020-3-PJBA62-postcol1
 EXPNO 1
 PROCNO 1
 F2 - Acquisition Parameters
 Date_ 20201217
 Time 18.34 h
 INSTRUM avo400
 PROBRND Z108615 0873 ()
 PULPROG zg60
 TD 65536
 SOLVENT CDCl3
 NS 16
 DS 2
 SWH 8012.820 Hz
 FIDRES 0.244532 Hz
 AQ 4.088465 sec
 RG 88.17
 DW 62.400 usec
 DE 6.50 usec
 TE 299.8 K
 D1 1.0000000 sec
 TDO 400.1324008 MHz
 SFO1 400.1324008 MHz
 NUC1 1H
 P1 14.00 usec
 PLW1 14.36999889 W
 F2 - Processing parameters
 SI 32768
 SF 400.1300101 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

5-(Dimethylamino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**98**)



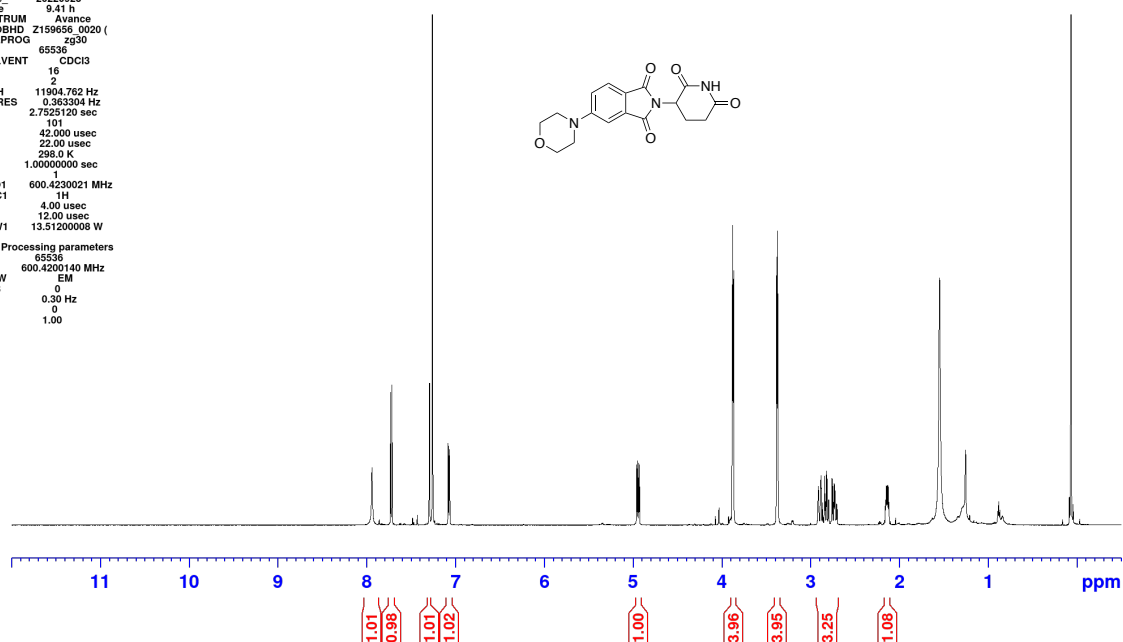
Appendices

Current Data Parameters
NAME B03_pb78232209
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220923
Time 9.41 h
INSTRUM Avance
PROBHD Z159656_0020 (z330)
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 11904.762 Hz
FIDRES 0.363304 Hz
AQ 2.7525120 sec
RG 101
DW 42.000 usec
DE 22.00 usec
TE 298.0 K
D1 1.0000000 sec
D11
SFO1 600.4230021 MHz
NUC1 1H
P0 4.00 usec
P1 12.00 usec
PLW1 13.51200008 W

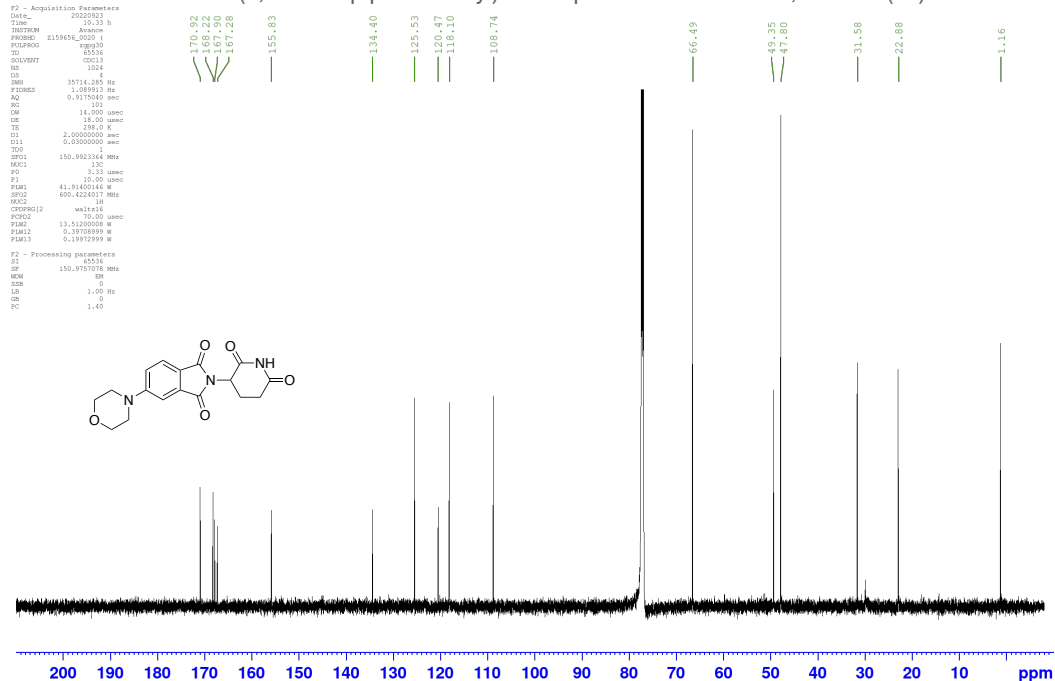
F2 - Processing parameters
SI 65536
SF 600.4200140 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-5-morpholinoisoindoline-1,3-dione (99)



Current Data Parameters
NAME B03_pb78232209
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20220923
Time 10.33 h
INSTRUM Avance
PROBHD Z159656_0020 (z330)
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 11904.762 Hz
FIDRES 0.363304 Hz
AQ 2.7525120 sec
RG 101
DW 42.000 usec
DE 22.00 usec
TE 298.0 K
D1 1.0000000 sec
D11
SFO1 600.4230021 MHz
NUC1 1H
P0 4.00 usec
P1 12.00 usec
PLW1 13.51200008 W

2-(2,6-Dioxopiperidin-3-yl)-5-morpholinoisoindoline-1,3-dione (99)



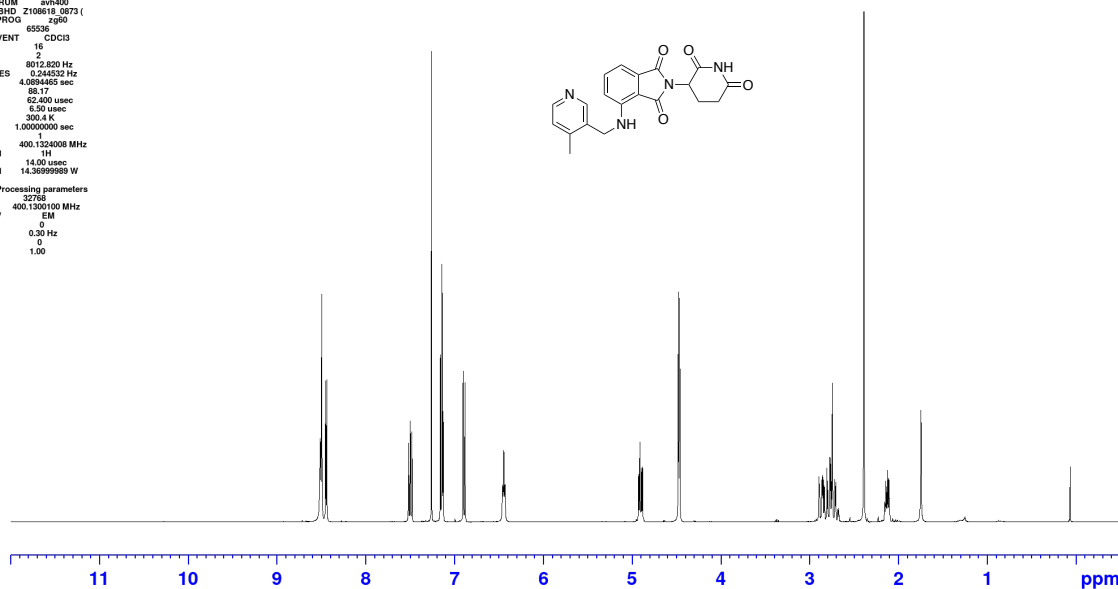
Appendices

Current Data Parameters
NAME Oct12-2020-2-PJBA53_postcol2
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20201012
Time 15:15 h
INSTRUM mv400
PROBHD Z10618-0073 (PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894485 sec
RG 68.17
DW 62.400 usec
DE 6.50 usec
TE 300.4 K
D1 1.00000000 sec
TD0
SFO1 400.132408 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.3699988 W

F2 - Processing parameters
SI 32768
SF 400.1306100 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-4-(((4-methylpyridin-3-yl)methyl)amino)isoindoline-1,3-dione (**100**)

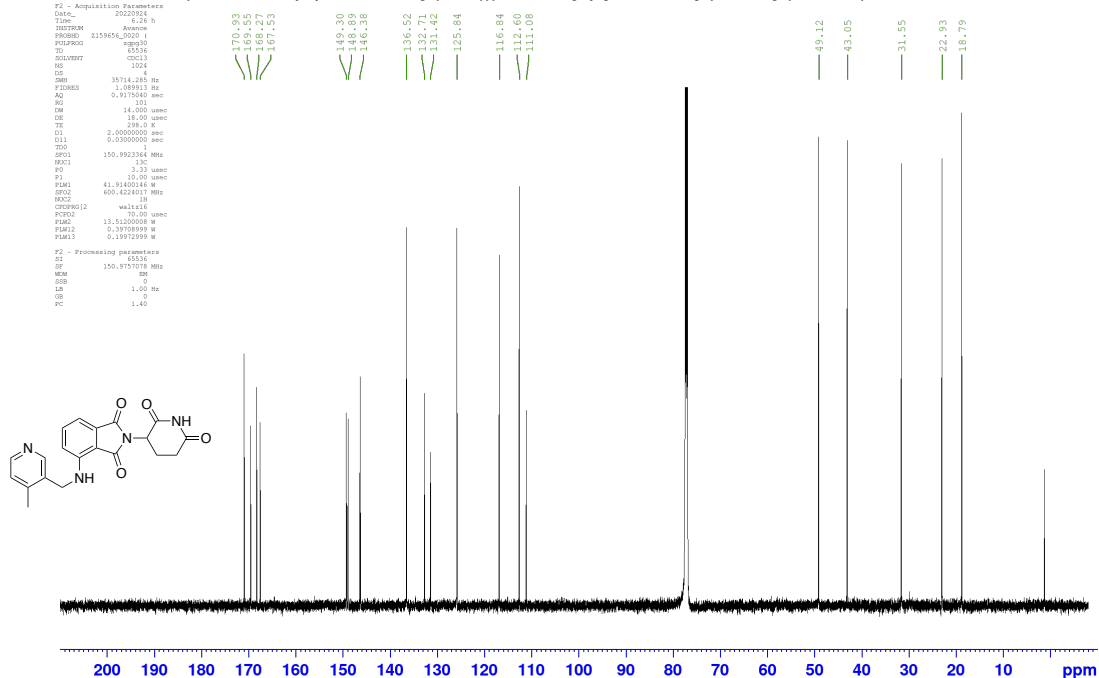


Current Data Parameters
NAME A53_gd78292309
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200504
Time 6:26 h
INSTRUM Avance
PROBHD B1H50056_0020 (PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1604
DS 4
SWH 35714.380 Hz
FIDRES 1.083913 Hz
AQ 0.9176500 sec
RG 101
DW 14.000 usec
DE 18.00 usec
TE 298.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0
SFO1 500.136099 MHz
NUC1 13C
P1 3.33 usec
PLW1 10.30 usec
PLM1 41.91400144 W
PLM2 400.4244017 MHz
PLM3 1H
CPCPRG12 waltz16
PCPRG2
P1M2 33.5120000 W
P1M3 0.3970899 W
P1M4 0.1972839 W

F2 - Processing parameters
SI 65536
SF 500.136099 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

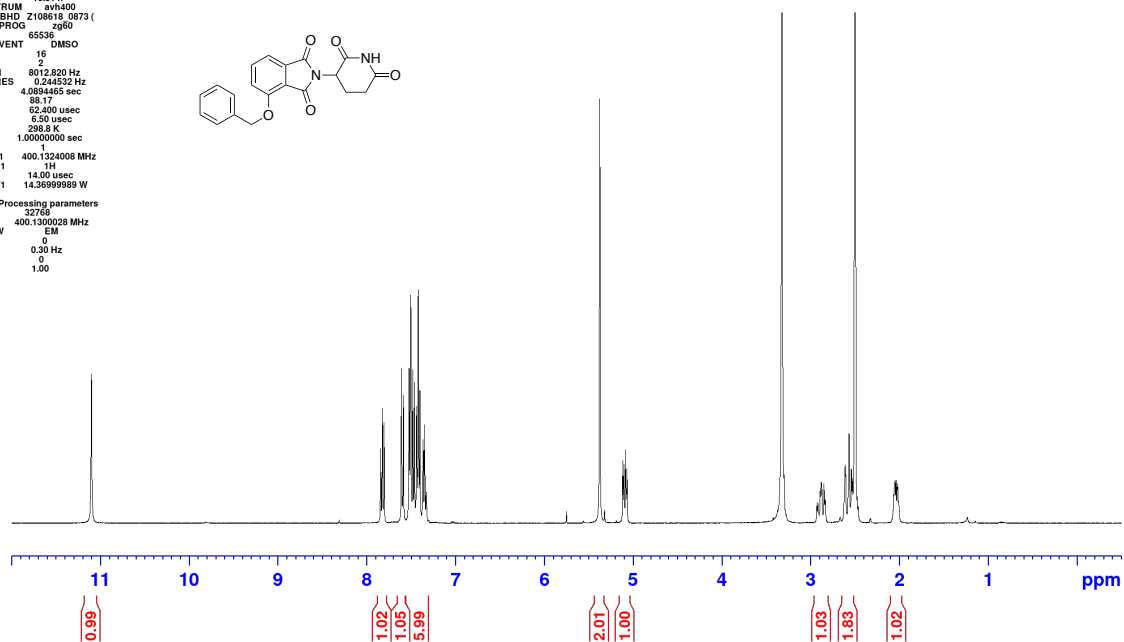
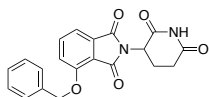
2-(2,6-Dioxopiperidin-3-yl)-4-(((4-methylpyridin-3-yl)methyl)amino)isoindoline-1,3-dione (**100**)



Appendices

Current Data Parameters
 NAME Feb21-2020-6-PJBA20-DMSO
 EXPNO 1
 PROCNO 1
 F2 - Acquisition Parameters
 Date_ 20200221
 Time 18.21 h
 INSTRUM avh400
 PROBHD Z108618_0873 ()
 PULPROG zgpg
 TD 65536
 SOLVENT DMSO
 NS 16
 DS 2
 SWH 8012.820 Hz
 FIDRES 0.244532 Hz
 AQ 4.0894465 sec
 RG 88.17
 DW 62.400 usec
 DE 6.50 usec
 TE 298.2 K
 D1 1.00000000 sec
 TD0
 SFO1 400.1324008 MHz
 NUC1 1H
 P1 14.00 usec
 PLW1 14.36999989 W
 F2 - Processing parameters
 SI 32768
 SF 400.1300028 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

4-(Benzyloxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**101**)



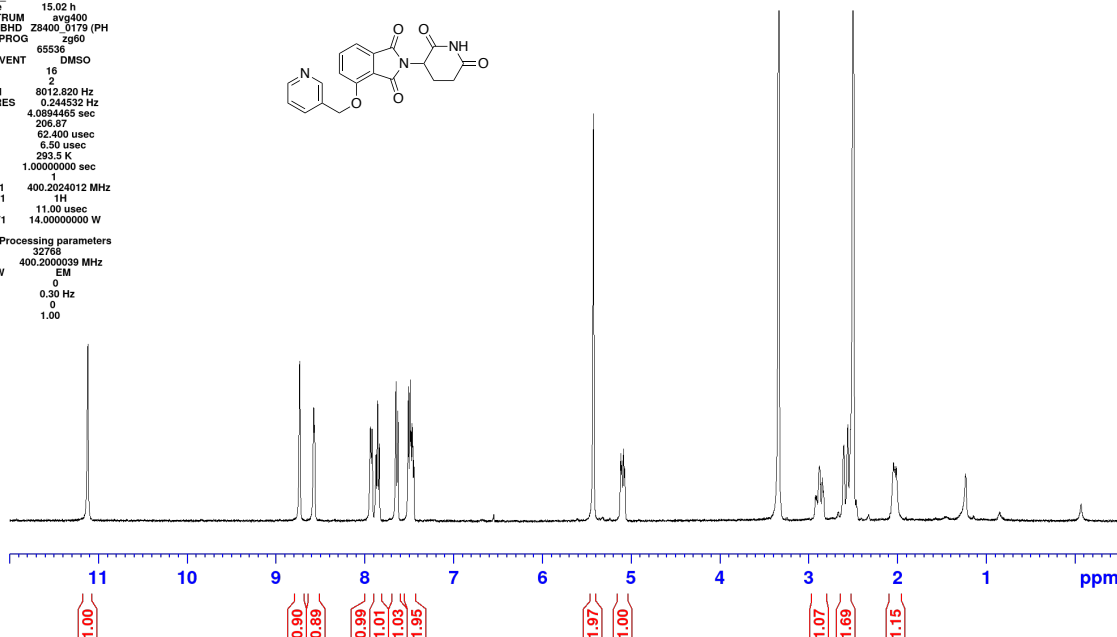
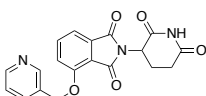
Appendices

Current Data Parameters
NAME Sep21-2022-1-PJBA43
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220921
Time 15.02 h
INSTRUM avq400
PROBHD Z8400_0179 (PH
PULPROG zgpg
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 206.67
DW 62.400 usec
DE 6.50 usec
TE 293.5 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 11.00 usec
PLW1 14.00000000 W

F2 - Processing parameters
SI 32768
SF 400.2000039 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-4-(pyridin-3-ylmethoxy)isoindoline-1,3-dione (**102**)

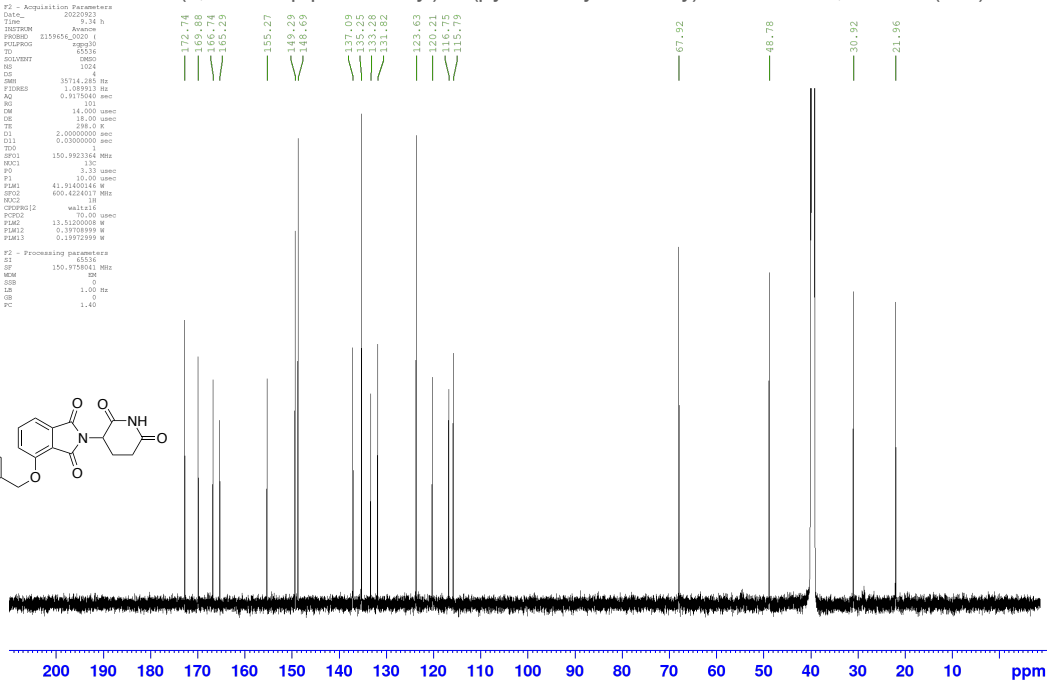
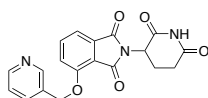


Current Data Parameters
NAME A13_gbf76222209
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220921
Time 9.34 h
INSTRUM Avance
PROBHD BBO500_0020 (H
PULPROG zgpg
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 38714.380 Hz
FIDRES 1.089013 Hz
AQ 0.9179540 sec
RG 101
DW 14.000 usec
DE 18.00 usec
TE 296.0 K
D1 2.00000000 sec
D11 0.05000000 sec
TD0 1
SFO1 500.1362600 MHz
NUC1 13C
P1 3.33 usec
PL1 10.00 usec
PLM1 41.91400146 W
PT02 600.4240177 MHz
NUC2 1H
PCPRG12 waltz16
PCPRG2 70.00 usec
PLM2 13.51200008 W
PLM12 0.30708999 W
PLM13 0.18972999 W

F2 - Processing parameters
SI 65536
SF 500.1362600 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

2-(2,6-Dioxopiperidin-3-yl)-4-(pyridin-3-ylmethoxy)isoindoline-1,3-dione (**102**)



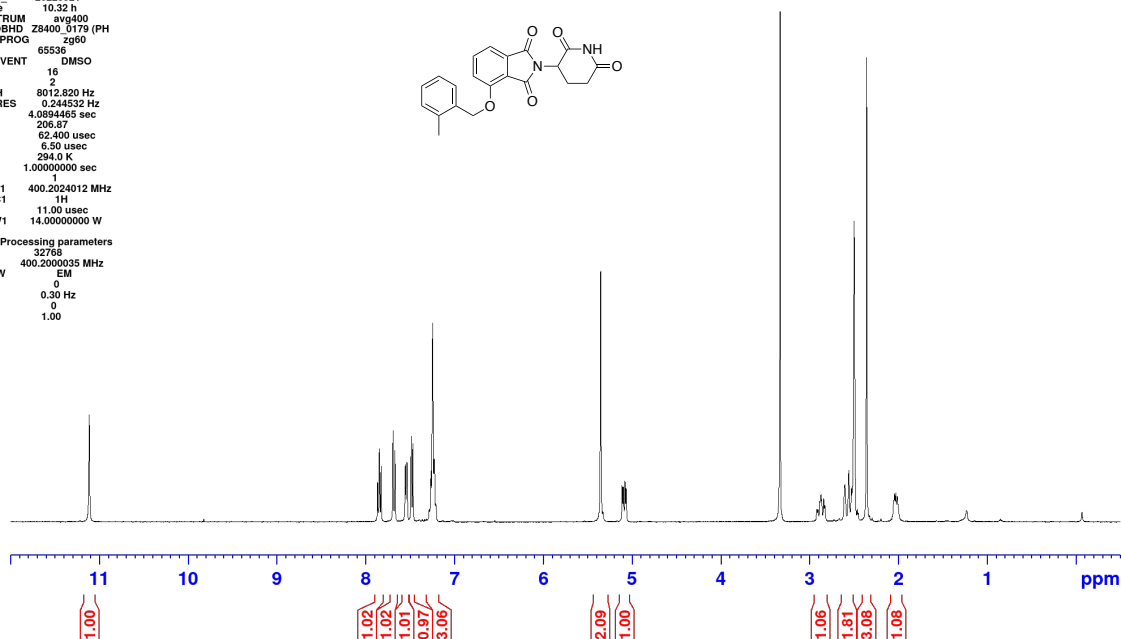
Appendices

Current Data Parameters
NAME Sep21-2022-1-PJBA42
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220921
Time 10.32 h
INSTRUM avg400
PROBHD Z8400 0179 (PH
PULPROG zg60
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 206.67
DW 62.400 usec
DE 6.50 usec
TE 294.0 K
D1 1.00000000 sec
TD0
SFO1 400.2024012 MHz
NUC1 1H
P1 11.00 usec
PLW1 14.00000000 W

F2 - Processing parameters
SI 32768
SF 400.2000035 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

2-(2,6-Dioxopiperidin-3-yl)-4-((2-methylbenzyl)oxy)isoindoline-1,3-dione (103)



Current Data Parameters
NAME A42_gd78172209
EXPNO 5
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220923
Time 4.45 h
INSTRUM Avance
PROBHD 115955_5020 (1
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 16
DS 4
SWH 39714.280 Hz
FIDRES 1.089933 Hz
AQ 6.9170400 sec
RG 101
DW 14.000 usec
DE 18.00 usec
TE 298.2 K
D1 2.00000000 sec
D11 0.05000000 sec
TD0
SFO1 500.1362644 MHz
NUC1 13C
P1 3.33 usec
P2 10.00 usec
PLA1 41.91400144 W
PLA2 400.6224021 MHz
PLA3 0.39708999 W
PLA4 0.19972839 W
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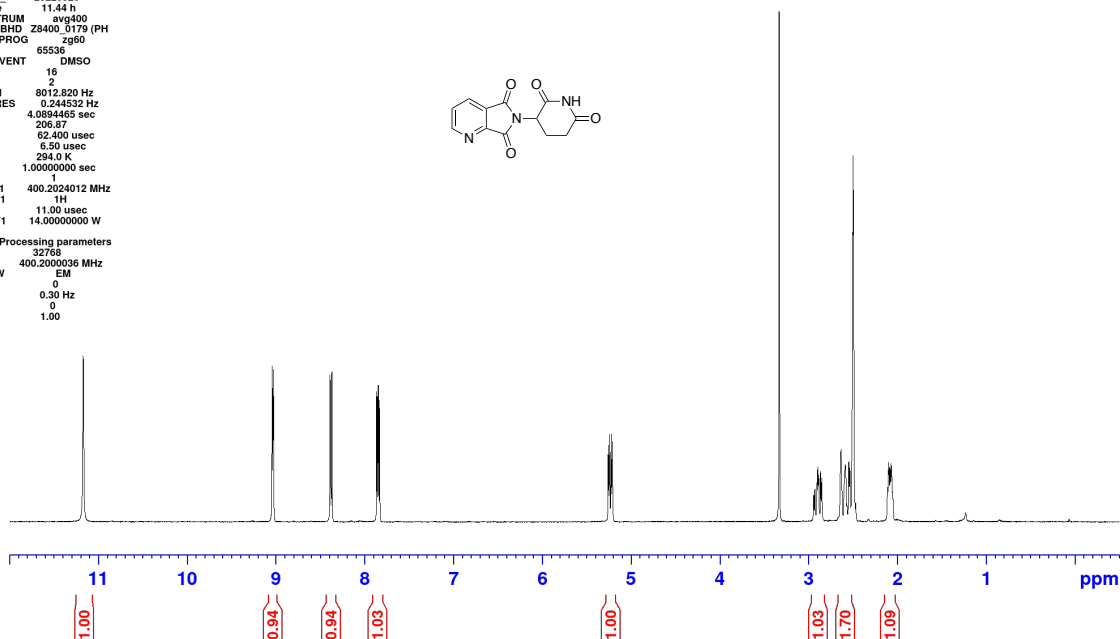
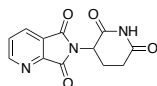
Appendices

Current Data Parameters
NAME Sep20-2022-1-PJBA66
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220920
Time 11.44 h
INSTRUM avg400
PROBHD Z8400_0179 (PH)
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 206.87
DW 62.400 usec
DE 6.50 usec
TE 294.0 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 11.00 usec
PLW1 14.00000000 W

F2 - Processing parameters
SI 32768
SF 400.2000036 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

6-(2,6-Dioxopiperidin-3-yl)-5H-pyrrolo[3,4-b]pyridine-5,7(6H)-dione (**104**)

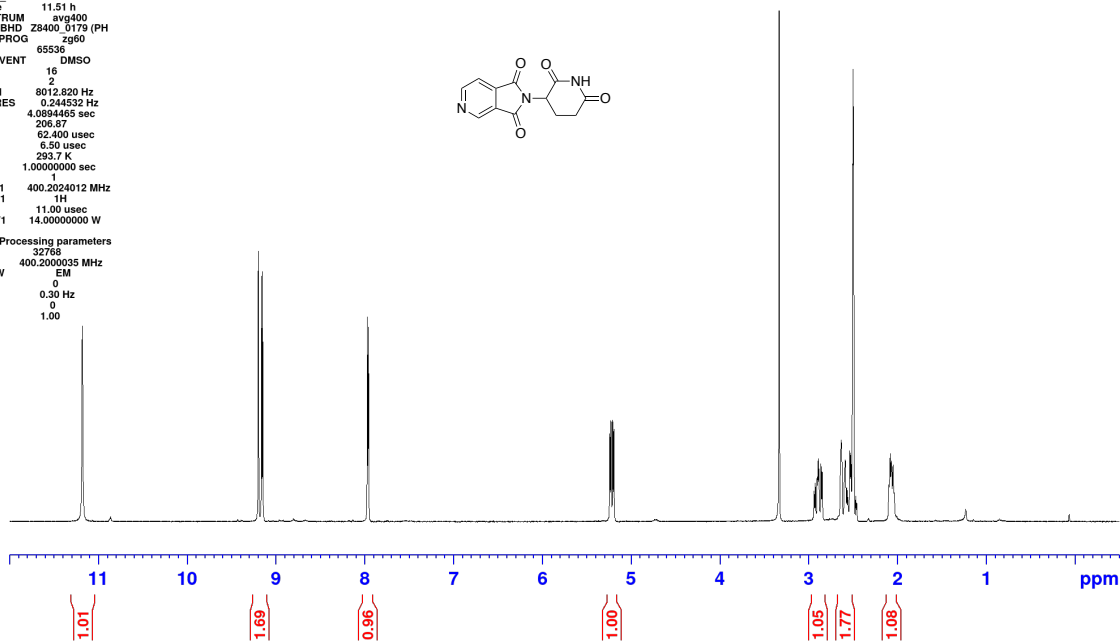
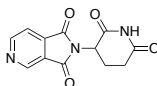


Current Data Parameters
NAME Sep20-2022-1-PJBA74
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220920
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INSTRUM avg400
PROBHD Z8400_0179 (PH)
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 206.87
DW 62.400 usec
DE 6.50 usec
TE 293.7 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 11.00 usec
PLW1 14.00000000 W

F2 - Processing parameters
SI 32768
SF 400.2000035 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

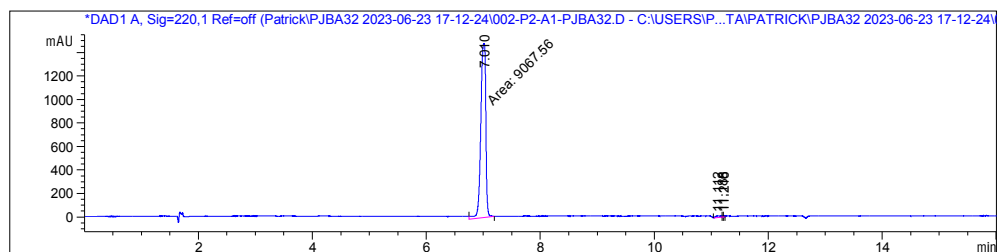
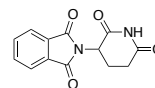
2-(2,6-Dioxopiperidin-3-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**105**)



S.5 HPLC Data

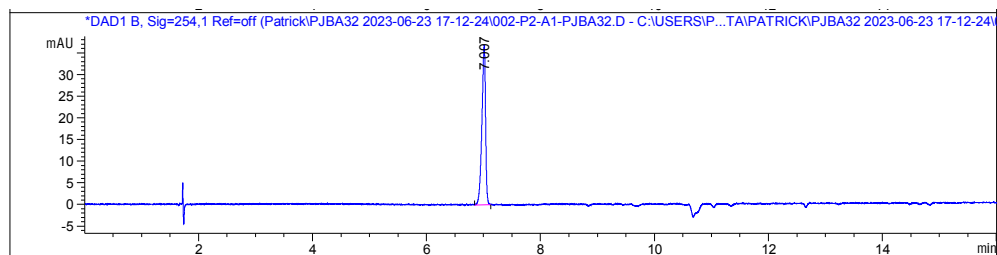
Appendices

2-(2,6-Dioxopiperidin-3-yl)isoindoline-1,3-dione (1)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.010	MM	0.1021	9067.55762	1480.10632	98.7473
2	11.112	BV	0.0775	89.24358	13.60625	0.9719
3	11.188	VV	0.0158	11.46414	9.67118	0.1248
4	11.216	VV	0.0207	14.32457	8.69674	0.1560

Totals : 9182.58990 1512.08049

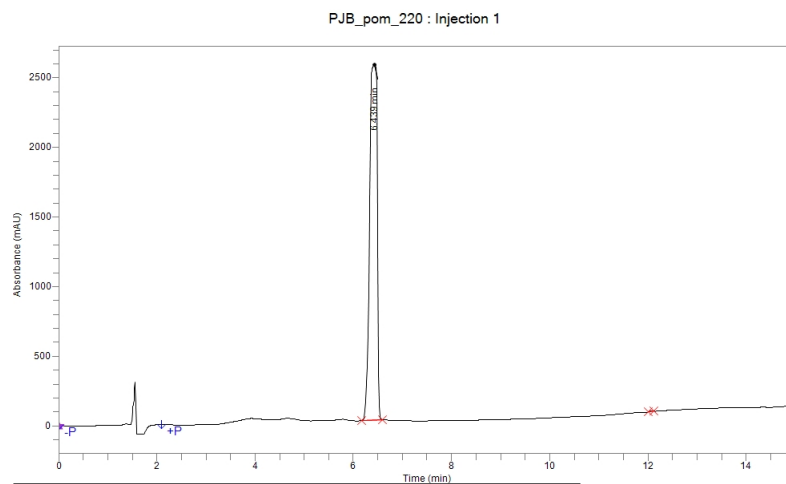
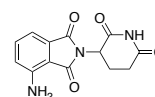


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.007	BB	0.0659	165.96967	36.92858	100.0000

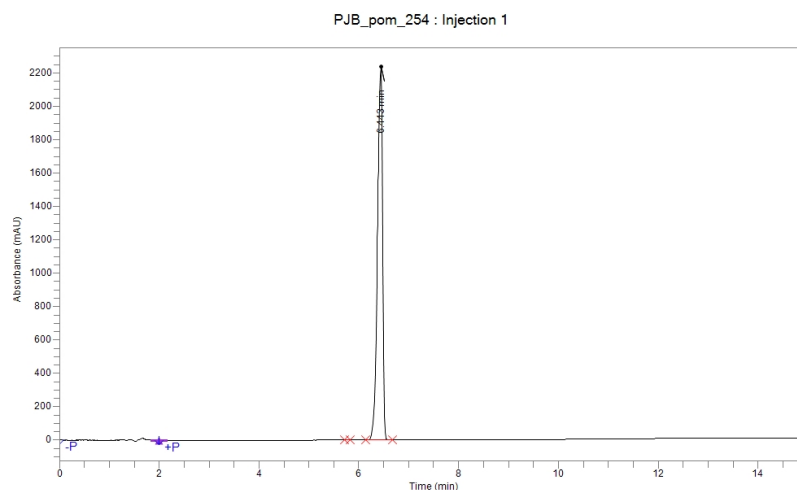
Totals : 165.96967 36.92858

Appendices

4-Amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**3**)



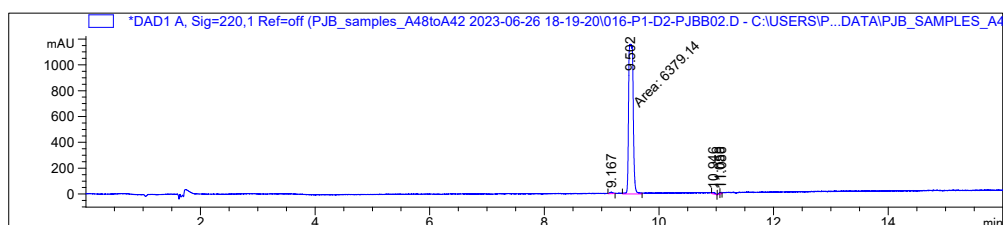
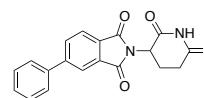
Time	Height	Area	Area %
6.439	2,553,608.8	27,408,154.1	99.93
12.077	4,916.0	19,776.4	0.07
Total		27,427,930.4	100.00



Time	Height	Area	Area %
5.789	340.4	1,487.8	0.01
6.443	2,239,247.0	15,788,772.2	99.99
Total		15,790,260.0	100.00

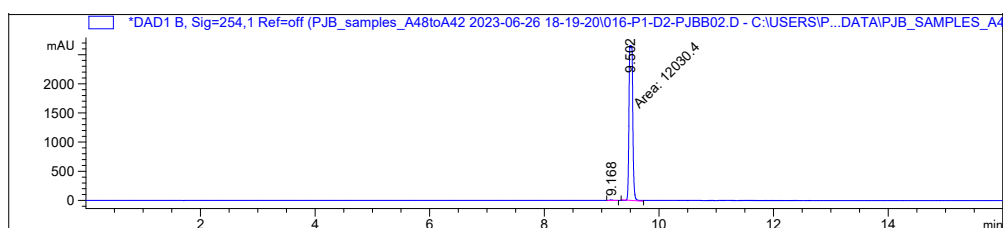
Appendices

2-(2,6-Dioxopiperidin-3-yl)-5-phenylisoindoline-1,3-dione (**23**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.167	VV	0.0397	38.47590	12.08027	0.5924
2	9.502	MM	0.0916	6379.13721	1160.77014	98.2165
3	10.946	VB	0.0532	41.28960	10.78413	0.6357
4	11.059	BV	0.0189	16.44551	11.05400	0.2532
5	11.086	VV	0.0242	19.62358	10.30767	0.3021

Totals : 6494.97180 1204.99620

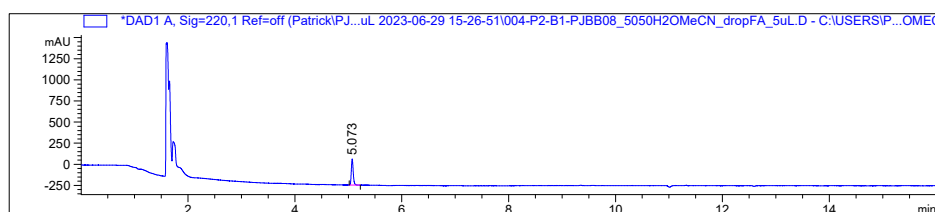
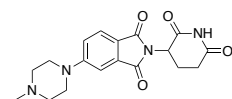


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.168	BB	0.0474	32.15612	10.45554	0.2666
2	9.502	MM	0.0756	1.20304e4	2653.79126	99.7334

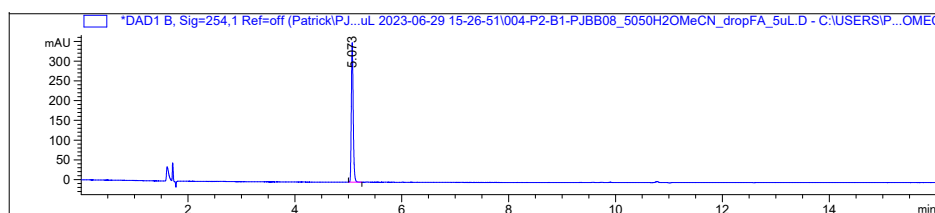
Totals : 1.20626e4 2664.24680

Appendices

2-(2,6-Dioxopiperidin-3-yl)-5-(4-methylpiperazin-1-yl)isoindoline-1,3-dione (**26**)



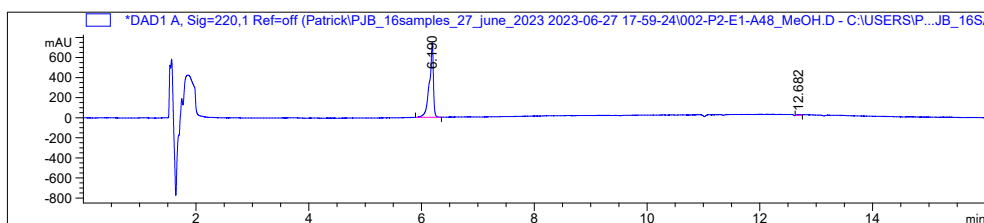
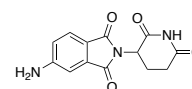
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.073	BB	0.0382	760.88721	309.12369	100.0000
Totals :				760.88721	309.12369	



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.073	BB	0.0380	864.64014	353.40967	100.0000
Totals :				864.64014	353.40967	

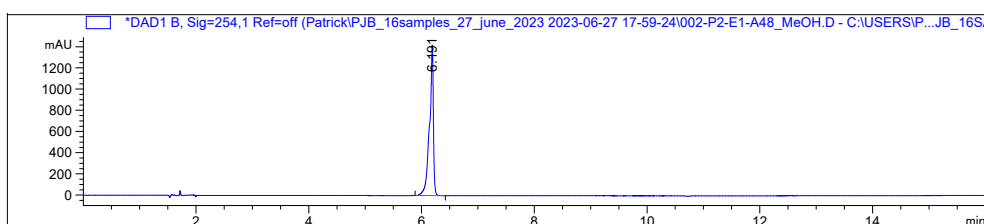
Appendices

5-Amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**35**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.190	BB	0.0657	3589.78223	744.89728	98.7363
2	12.682	BV	0.0706	45.94411	7.98587	1.2637

Totals : 3635.72633 752.88315

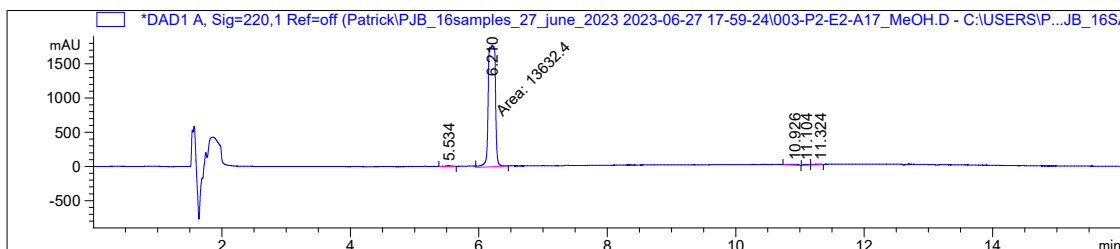
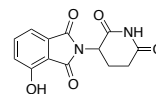


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.191	BB	0.0653	6732.34521	1419.69299	100.0000

Totals : 6732.34521 1419.69299

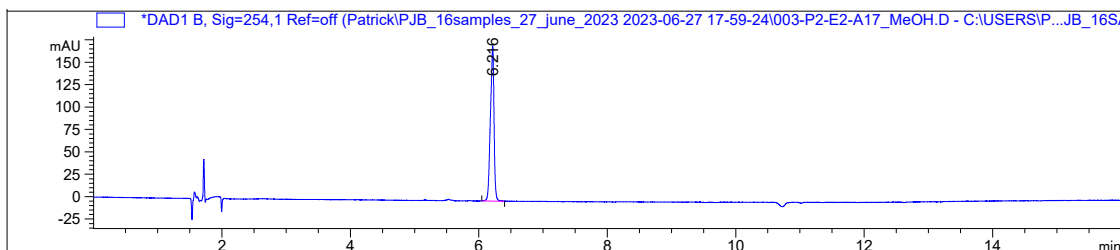
Appendices

2-(2,6-Dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (**36**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.534	BB	0.0646	65.99696	12.70768	0.4697
2	6.210	MM	0.1278	1.36324e4	1777.73010	97.0149
3	10.926	BB	0.1220	141.60933	13.88067	1.0078
4	11.104	BV	0.0815	105.82807	15.42453	0.7531
5	11.324	VV	0.1264	106.02243	9.86394	0.7545

Totals : 1.40519e4 1829.60693

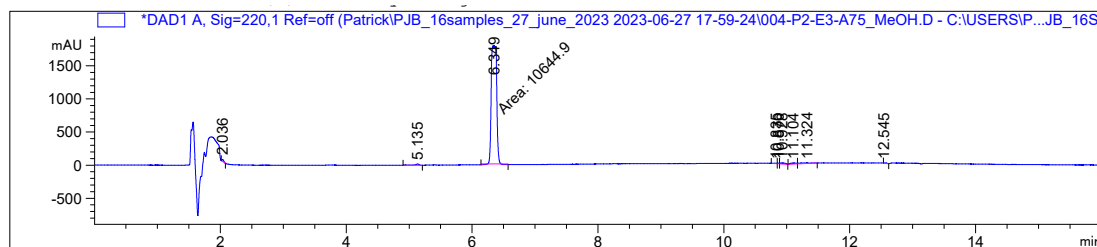
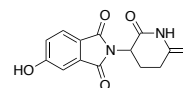


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.216	BB	0.0554	675.15735	173.32211	100.0000

Totals : 675.15735 173.32211

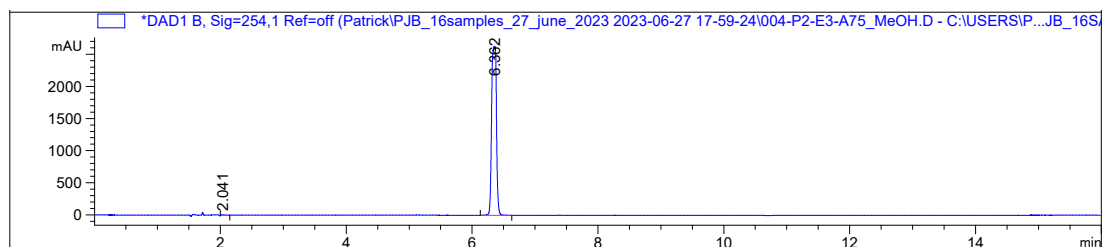
Appendices

2-(2,6-Dioxopiperidin-3-yl)-5-hydroxyisoindoline-1,3-dione (**37**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.036	BB	0.0318	63.73929	32.55661	0.5708
2	5.135	BB	0.0519	80.56284	20.23950	0.7214
3	6.349	MM	0.0991	1.06449e4	1791.00110	95.3208
4	10.835	BV	0.0522	27.88848	7.70735	0.2497
5	10.870	VV	0.0245	18.61944	9.87828	0.1667
6	10.928	VB	0.0819	87.11492	13.66248	0.7801
7	11.104	BV	0.0830	105.74609	15.13966	0.9469
8	11.324	VV R	0.1459	110.80653	9.01607	0.9922
9	12.545	VB	0.0465	28.06925	7.27520	0.2513

Totals : 1.11674e4 1906.47626

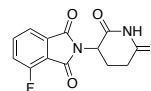


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.041	BB	0.0587	47.90619	10.50316	0.3428
2	6.362	BB	0.0638	1.39278e4	2630.26196	99.6572

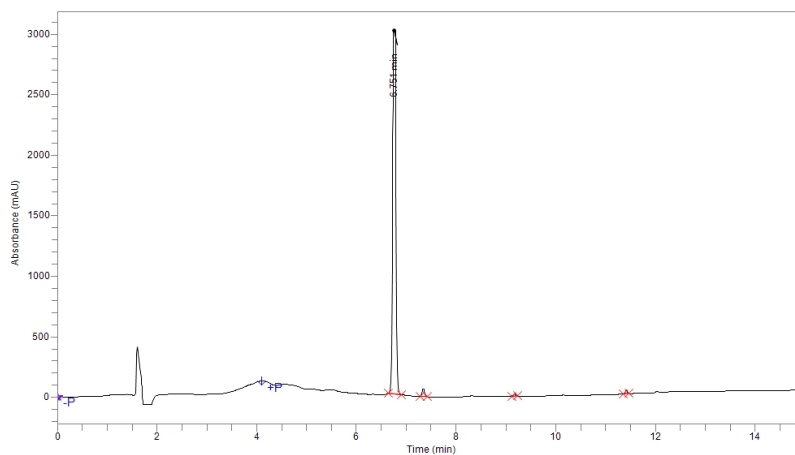
Totals : 1.39757e4 2640.76512

Appendices

2-(2,6-Dioxopiperidin-3-yl)-4-fluoroisindoline-1,3-dione (**38**)

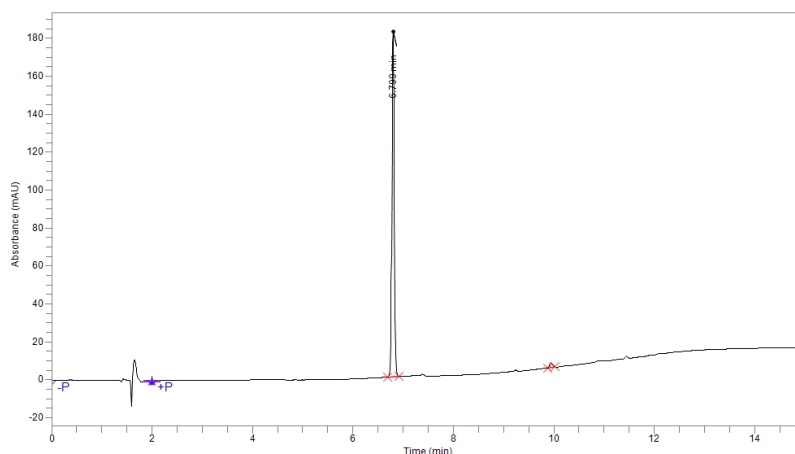


PJBA16_220 : Injection 1



Time	Height	Area	Area %
6.751	3,028,627.9	14,117,851.2	97.52
7.346	63,271.4	219,745.1	1.52
9.173	16,466.0	52,777.1	0.36
11.416	28,380.4	86,527.3	0.60
Total		14,476,900.7	100.00

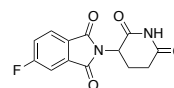
PJBA16_254 : Injection 1



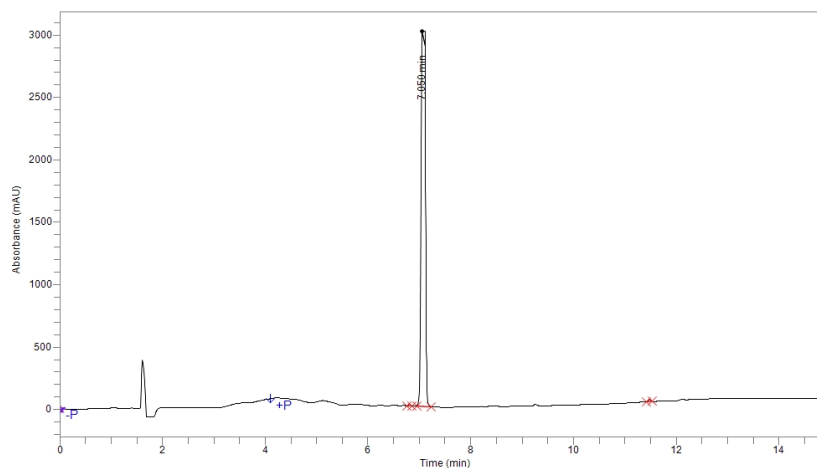
Time	Height	Area	Area %
6.799	182,217.2	630,377.0	98.51
9.945	2,444.7	9,551.0	1.49
Total		639,928.0	100.00

Appendices

2-(2,6-Dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione (39)

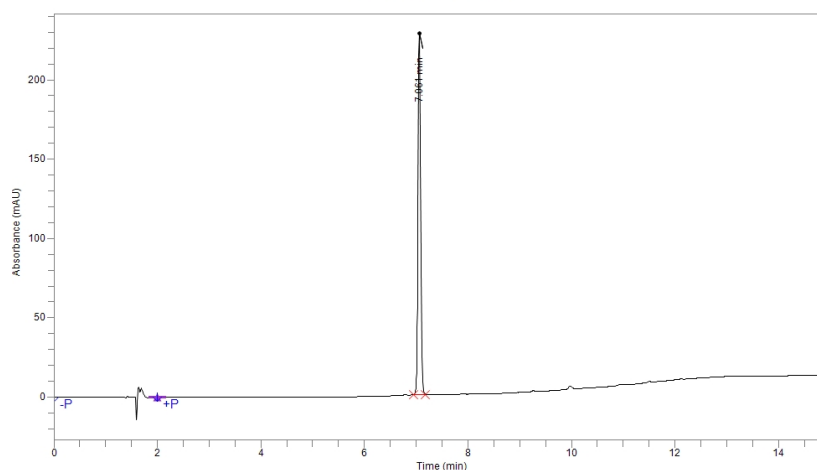


PJBA46_220 : Injection 1



Time	Height	Area	Area %
6.809	13,303.9	39,412.8	0.22
7.050	3,039,466.0	18,002,760.7	99.45
11.482	20,006.3	60,644.8	0.34
Total		18,102,818.3	100.00

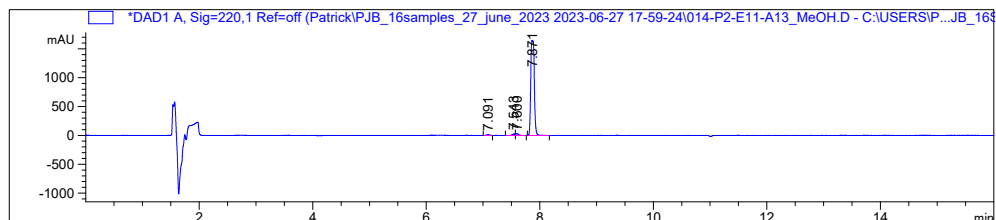
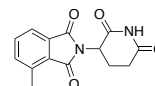
PJBA46_254 : Injection 1



Time	Height	Area	Area %
7.061	227,924.9	796,290.9	100.00
Total		796,290.9	100.00

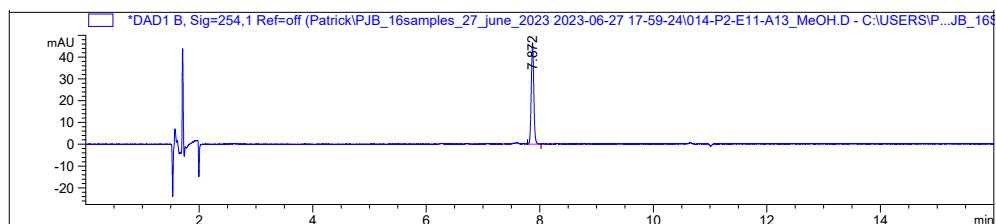
Appendices

2-(2,6-Dioxopiperidin-3-yl)-4-methylisoindoline-1,3-dione (**40**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.091	BB	0.0601	48.40011	12.24416	0.6610
2	7.543	BV	0.0466	84.10289	26.80878	1.1486
3	7.600	VB	0.0487	106.54169	32.09800	1.4550
4	7.871	BB	0.0705	7083.42627	1636.31177	96.7355

Totals : 7322.47095 1707.46271

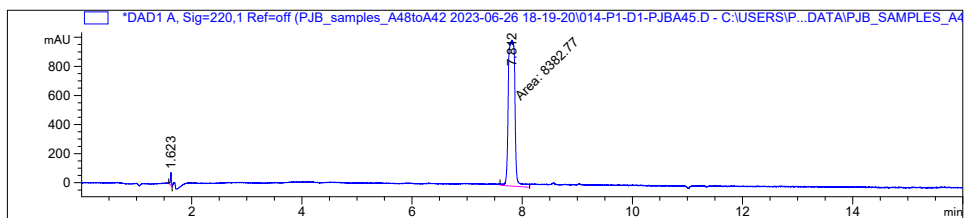
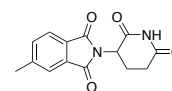


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.872	BB	0.0472	144.07738	46.43835	100.0000

Totals : 144.07738 46.43835

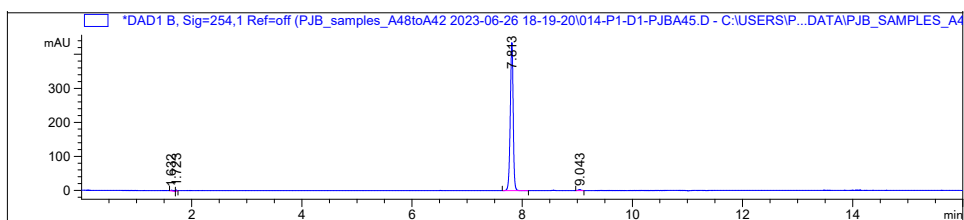
Appendices

2-(2,6-Dioxopiperidin-3-yl)-5-methylisoindoline-1,3-dione (**41**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.623	BB	0.0208	108.92145	85.71591	1.2827
2	7.812	MM	0.1389	8382.77344	1005.74152	98.7173

Totals : 8491.69489 1091.45743

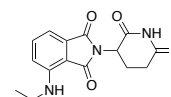


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.632	BB	0.0811	11.08154	1.72119	0.6859
2	1.723	BB	0.0229	5.64052	3.67605	0.3491
3	7.813	BB	0.0567	1589.70142	434.66150	98.3937
4	9.043	BB	0.0501	9.23091	2.82392	0.5713

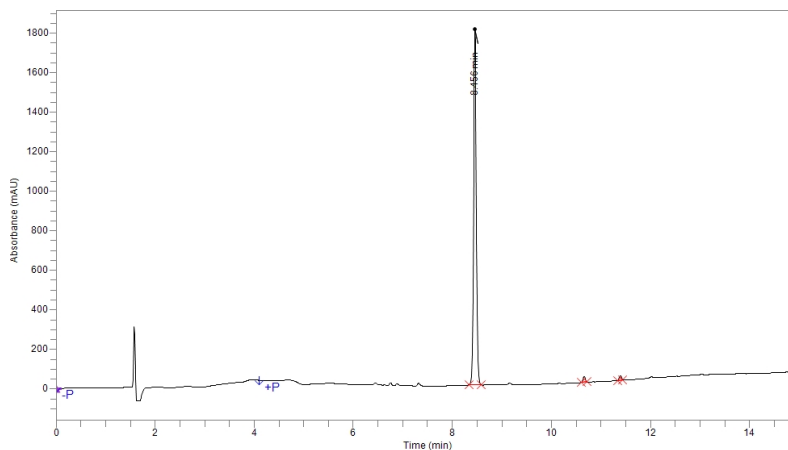
Totals : 1615.65439 442.88267

Appendices

2-(2,6-Dioxopiperidin-3-yl)-4-(ethylamino)isoindoline-1,3-dione (**64**)

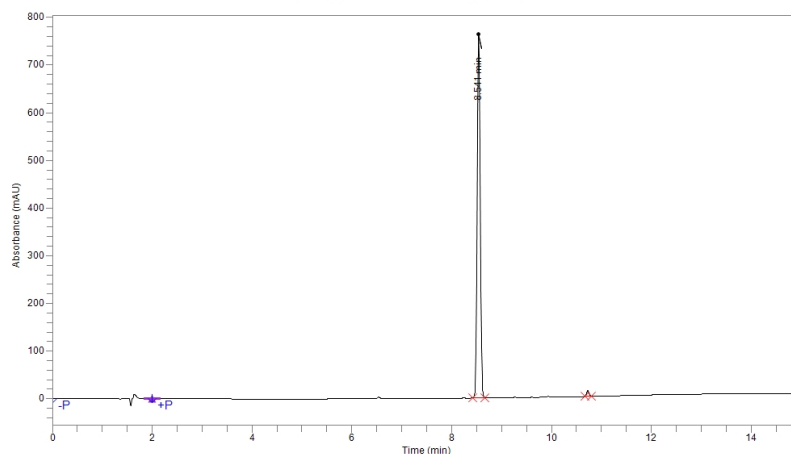


A27_220_13_7_2021 : Injection 1



Time	Height	Area	Area %
8.456	1,800,428.2	7,219,094.3	97.92
10.660	27,073.5	88,235.7	1.20
11.390	22,352.5	65,484.8	0.89
Total		7,372,814.8	100.00

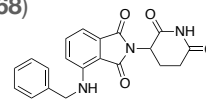
A27_254_13_7_2021 : Injection 1



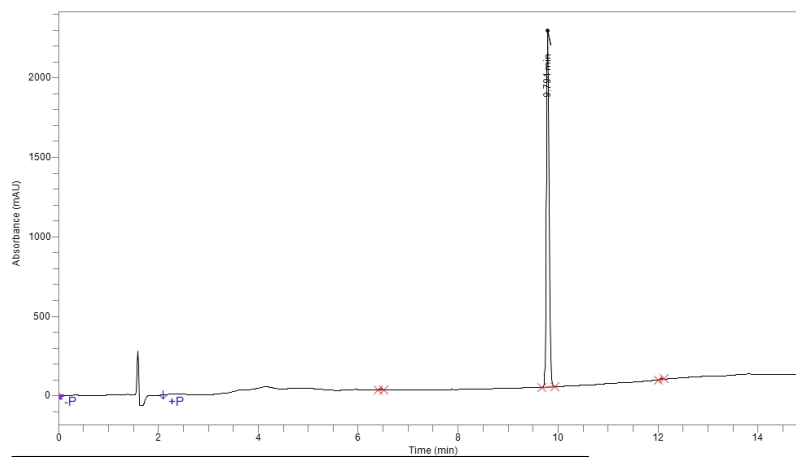
Time	Height	Area	Area %
8.541	763,584.8	3,012,532.3	98.61
10.731	12,178.1	42,392.6	1.39
Total		3,054,924.9	100.00

Appendices

4-(Benzylamino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**68**)

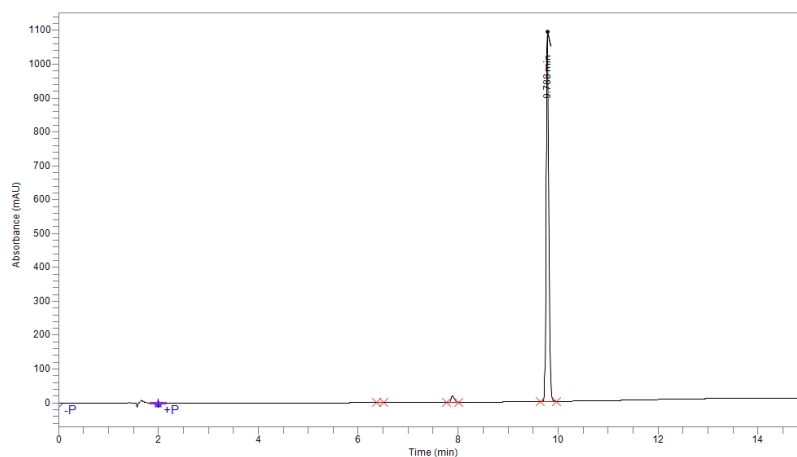


PJBA22_220 : Injection 1



Time	Height	Area	Area %
6.453	5,600.9	19,763.4	0.22
9.794	2,243,243.4	8,880,223.9	99.53
12.077	5,477.1	22,020.7	0.25
Total		8,922,008.0	100.00

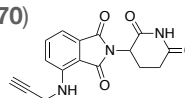
PJBA22_254 : Injection 1



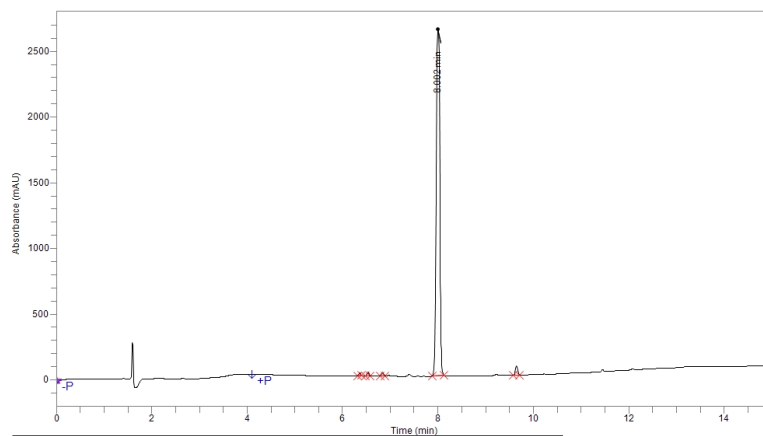
Time	Height	Area	Area %
6.444	1,859.3	7,084.6	0.16
7.894	19,164.5	93,740.9	2.17
9.788	1,093,396.3	4,221,720.4	97.67
Total		4,322,546.0	100.00

Appendices

2-(2,6-Dioxopiperidin-3-yl)-4-(prop-2-yn-1-ylamino)isoindoline-1,3-dione (**70**)

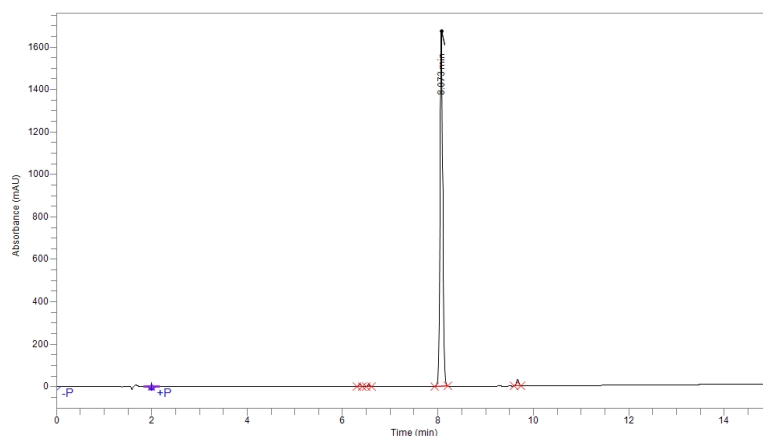


A28_220_13_7_2021 : Injection 1



Time	Height	Area	Area %
6.357	21,251.7	61,668.2	0.45
6.531	27,158.9	83,361.4	0.61
6.838	28,107.5	86,355.7	0.63
8.002	2,641,521.8	13,311,843.4	96.69
9.649	69,316.8	224,418.0	1.63
Total		13,767,646.8	100.00

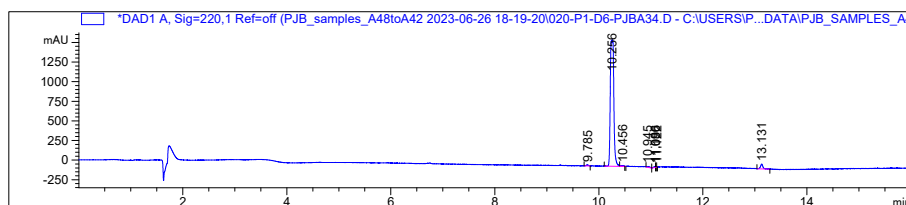
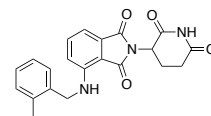
A28_254_13_7_2021 : Injection 1



Time	Height	Area	Area %
6.365	10,841.5	35,164.2	0.49
6.547	8,636.8	27,331.6	0.38
8.073	1,674,556.2	7,007,741.9	97.68
9.670	31,892.5	103,799.2	1.45
Total		7,174,036.9	100.00

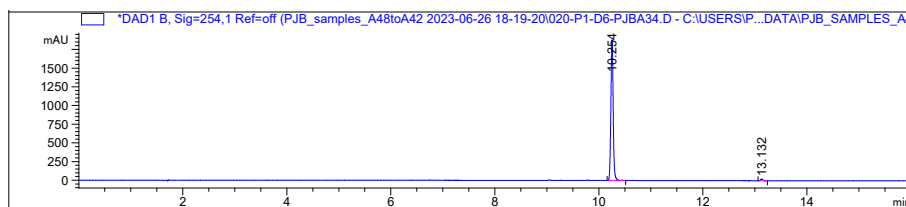
Appendices

2-(2,6-Dioxopiperidin-3-yl)-4-((2-methylbenzyl)amino)isoindoline-1,3-dione (**74**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.785	VV	0.0444	45.78736	12.75951	0.5763
2	10.256	BV R	0.0579	7577.73291	1609.09106	95.3733
3	10.456	VV E	0.0356	30.46284	10.73134	0.3834
4	10.945	VB	0.0530	46.46904	10.63425	0.5849
5	11.086	BV	0.0376	31.33801	10.12394	0.3944
6	11.096	VV	0.0175	9.68551	9.24094	0.1219
7	11.122	VV	0.0131	6.09629	7.05964	0.0767
8	13.131	BV R	0.0402	197.76942	61.97969	2.4891

Totals : 7945.34139 1731.62037

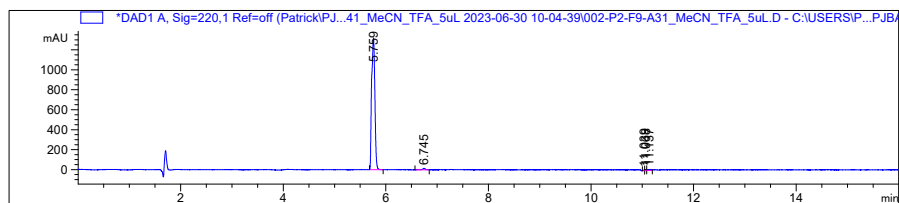
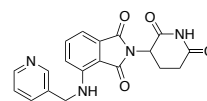


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.254	BV R	0.0481	5814.16699	1877.96265	98.7680
2	13.132	BB	0.0450	72.52560	24.90003	1.2320

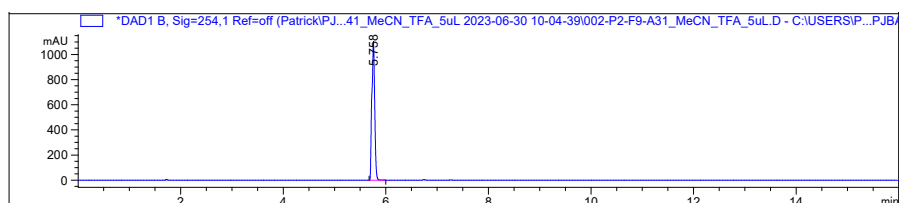
Totals : 5886.69259 1902.86268

Appendices

2-(2,6-Dioxopiperidin-3-yl)-4-((pyridin-3-ylmethyl)amino)isoindoline-1,3-dione (**84**)



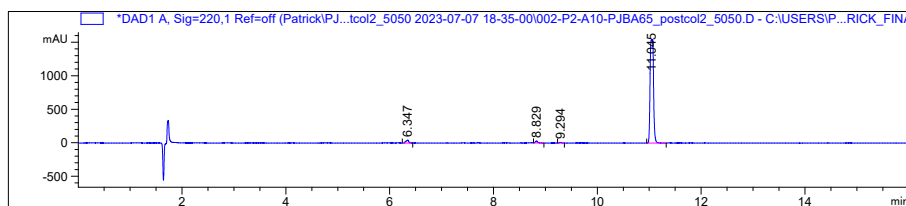
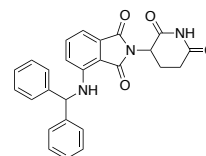
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.759	BB	0.0630	5757.53711	1316.86975	97.6576
2	6.745	VV R	0.0474	57.94011	14.87543	0.9828
3	11.039	BV	0.0169	10.67051	8.06211	0.1810
4	11.068	VV	0.0292	21.24766	9.12582	0.3604
5	11.137	VV	0.0710	48.23938	8.21837	0.8182
Totals :				5895.63477	1357.15148	



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.758	BB	0.0542	4278.00879	1104.08264	100.0000
Totals :				4278.00879	1104.08264	

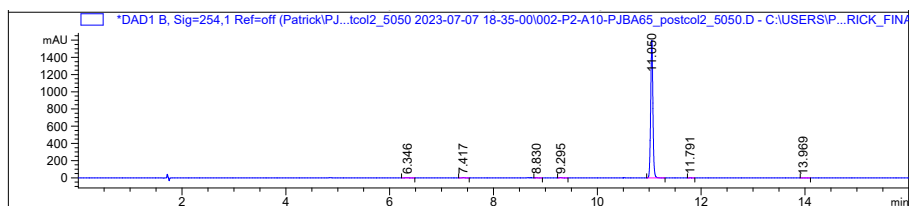
Appendices

4-(Benzhydrylamino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**86**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.347	BB	0.0499	170.95700	46.51646	2.3214
2	8.829	BB	0.0469	96.15903	30.37664	1.3057
3	9.294	BB	0.0451	28.13963	9.34437	0.3821
4	11.045	BB	0.0635	7069.25488	1541.79138	95.9908

Totals : 7364.51055 1628.02885

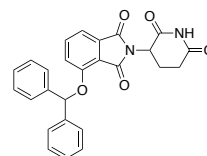


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.346	BB	0.0537	8.49456	2.12141	0.1766
2	7.417	BB	0.0469	9.34295	3.12326	0.1943
3	8.830	BB	0.0450	3.33332	1.05111	0.0693
4	9.295	BB	0.0453	11.41339	3.77203	0.2373
5	11.050	BB	0.0458	4765.96533	1597.53052	99.1084
6	11.791	BB	0.0543	3.98341	9.63068e-1	0.0828
7	13.969	BB	0.0718	6.30632	1.08459	0.1311

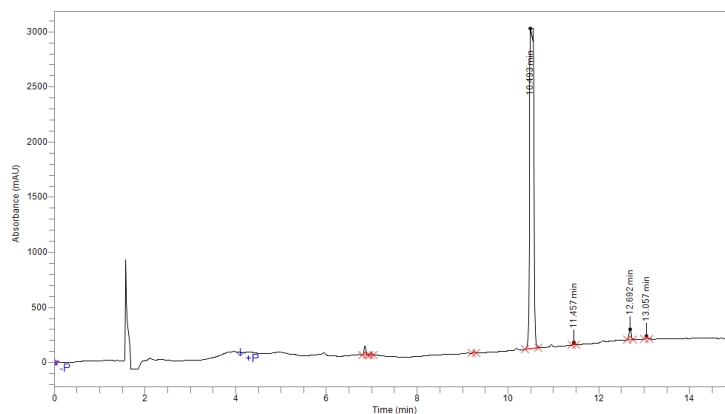
Totals : 4808.83929 1609.64599

Appendices

4-(Benzhydryloxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (87)

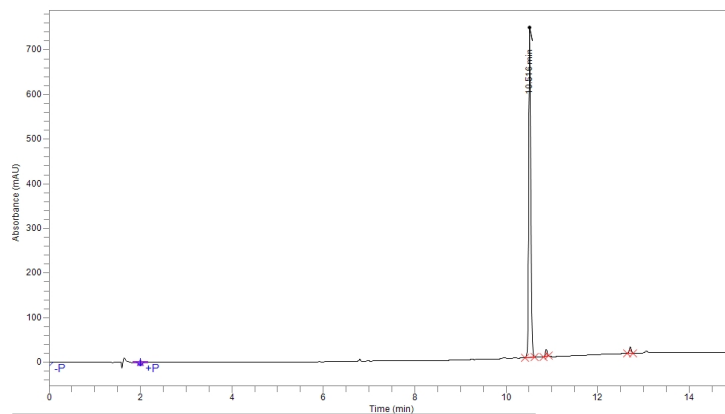


A83_220_14_7_2021 : Injection 1



Time	Height	Area	Area %
6.850	84,966.7	237,594.8	1.19
6.994	19,324.9	50,593.3	0.25
9.255	18,338.3	54,587.1	0.27
10.493	2,942,967.8	19,196,493.1	95.94
11.457	20,516.2	58,680.1	0.29
12.692	95,320.7	316,917.6	1.58
13.057	27,577.0	93,787.6	0.47
Total		20,008,653.7	100.00

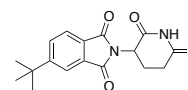
A83_254_14_7_2021 : Injection 1



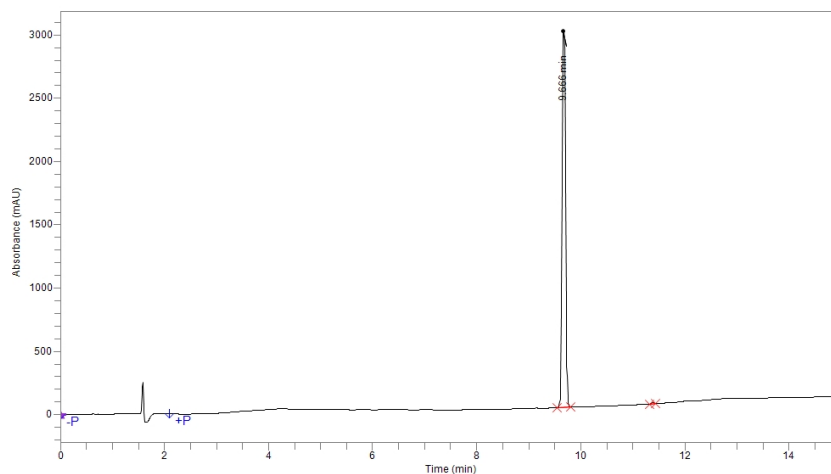
Time	Height	Area	Area %
10.516	739,977.7	2,443,080.6	96.27
10.876	15,065.8	48,078.3	1.89
12.714	14,528.5	46,657.1	1.84
Total		2,537,816.0	100.00

Appendices

5-(Tert-butyl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**88**)

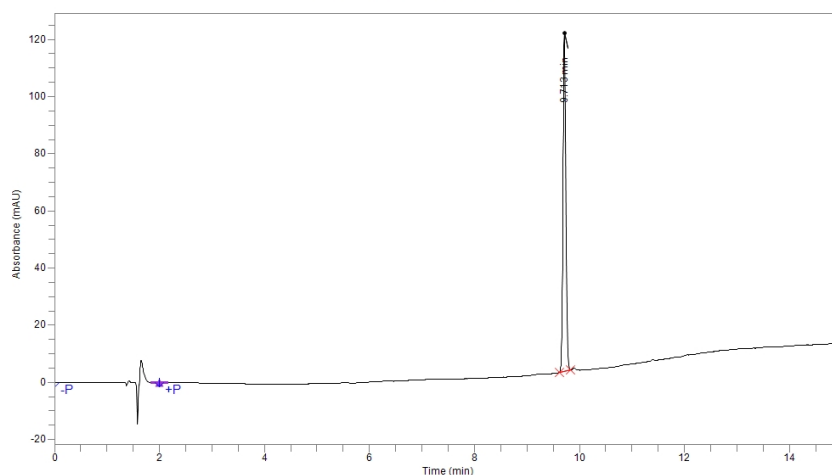


PJB5tBu_220_3_2_2021 : Injection 1



Time	Height	Area	Area %
9.666	3,015,607.0	13,255,833.1	99.79
11.388	8,856.5	28,068.3	0.21
Total		13,283,901.5	100.00

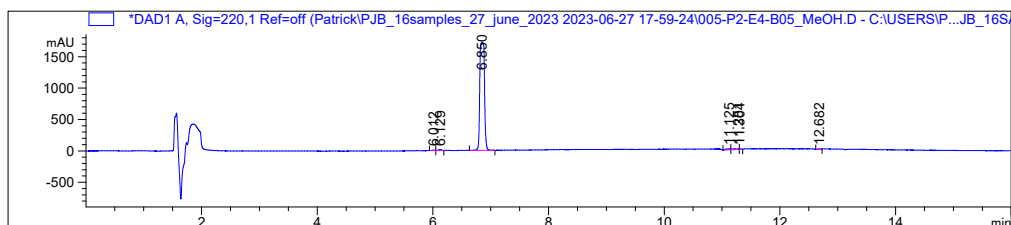
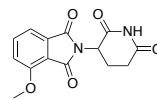
PJB5tBu_254_3_2_2021 : Injection 1



Time	Height	Area	Area %
9.713	118,463.5	453,471.6	100.00
Total		453,471.6	100.00

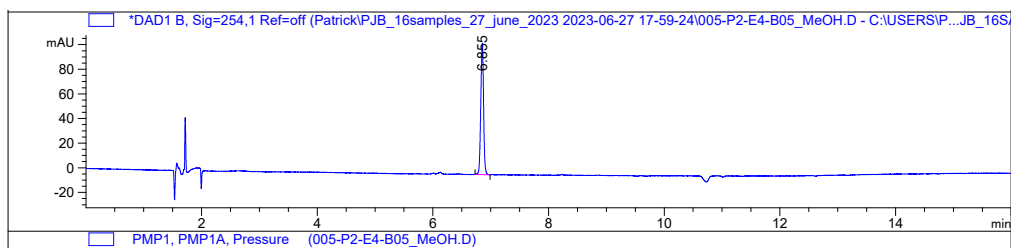
Appendices

2-(2,6-Dioxopiperidin-3-yl)-4-methoxyisoindoline-1,3-dione (**89**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.012	BV	0.0440	25.54466	7.36038	0.2574
2	6.129	VB	0.0474	57.92704	15.93125	0.5838
3	6.850	BB	0.0661	9592.31934	1717.97754	96.6734
4	11.125	BV	0.0756	93.57810	14.94160	0.9431
5	11.281	VV	0.1129	89.21191	9.51512	0.8991
6	11.304	VB	0.0268	18.89025	8.87971	0.1904
7	12.682	BV	0.0587	44.93058	9.40365	0.4528

Totals : 9922.40189 1784.00926



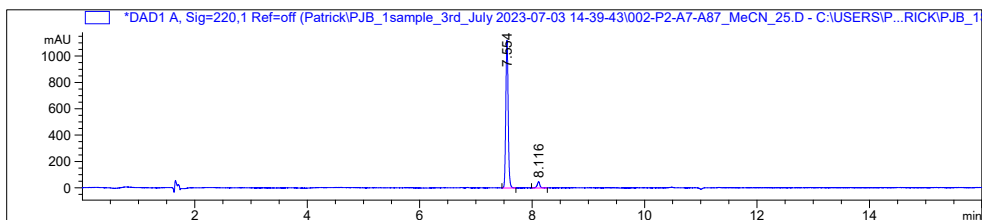
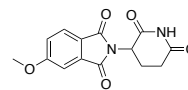
Signal 2: DAD1 B, Sig=254,1 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.855	BB	0.0527	358.38605	106.73189	100.0000

Totals : 358.38605 106.73189

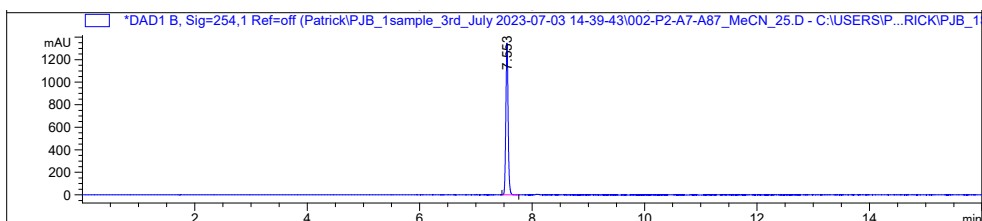
Appendices

2-(2,6-Dioxopiperidin-3-yl)-5-methoxyisoindoline-1,3-dione (**90**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.554	VB R	0.0463	3345.32910	1122.03931	95.5459
2	8.116	BB	0.0463	155.95166	48.19432	4.4541

Totals : 3501.28076 1170.23363

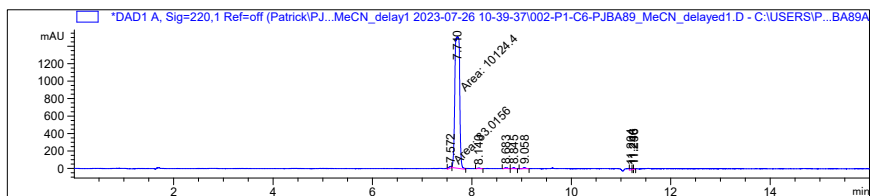
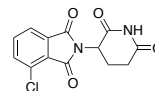


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.553	BB	0.0436	3819.21582	1347.94446	100.0000

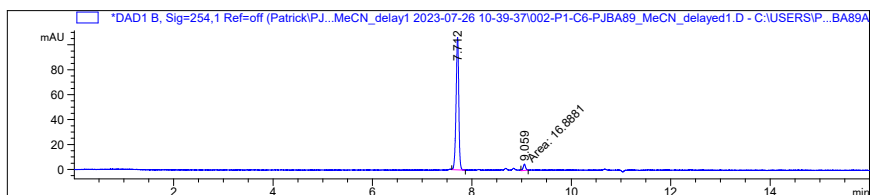
Totals : 3819.21582 1347.94446

Appendices

4-Chloro-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**91**)



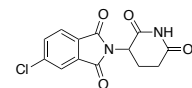
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.572	MM	0.0716	83.01558	19.31462	0.7948
2	7.710	MM	0.1120	1.01244e4	1506.12683	96.9367
3	8.140	BB	0.0456	28.66743	7.59032	0.2745
4	8.683	BV	0.0456	39.57912	10.96288	0.3790
5	8.845	VB	0.0483	35.57435	10.30676	0.3406
6	9.058	BV R	0.0457	46.73374	12.61280	0.4475
7	11.204	VV	0.0401	47.28849	14.10421	0.4528
8	11.246	VV	0.0244	19.43041	9.87937	0.1860
9	11.256	VV	0.0257	19.65645	9.27698	0.1882
Totals :				1.04443e4	1600.17477	



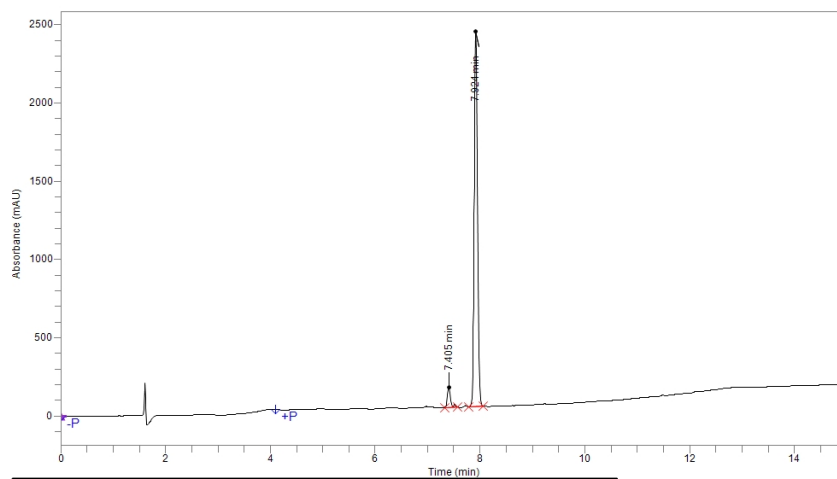
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.712	BB	0.0580	400.58704	106.22004	95.9547
2	9.059	MM	0.0571	16.88810	4.93309	4.0453
Totals :				417.47514	111.15313	

Appendices

5-Chloro-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**92**)

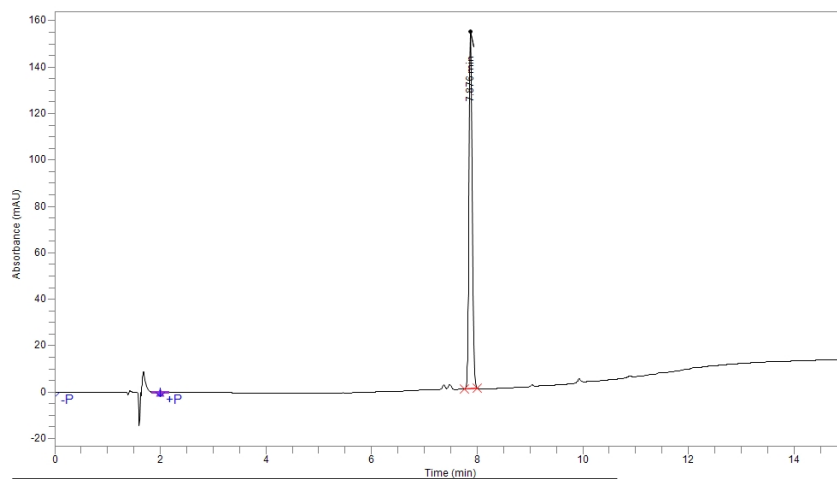


PJBA86_220_actual : Injection 1



Time	Height	Area	Area %
7.405	129,530.5	482,204.2	4.35
7.518	16,496.3	52,657.9	0.48
7.924	2,400,093.6	10,540,490.2	95.17
Total		11,075,352.3	100.00

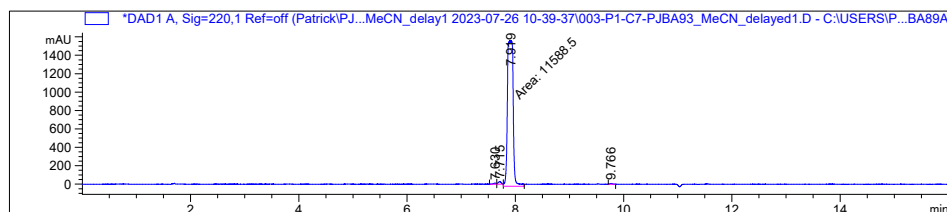
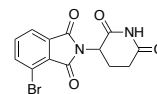
PJBA86_254 : Injection 1



Time	Height	Area	Area %
7.876	153,998.7	642,358.4	100.00
Total		642,358.4	100.00

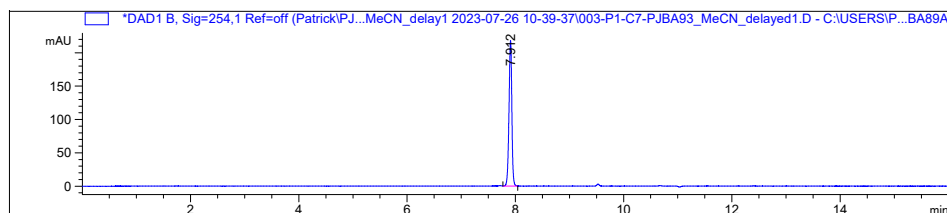
Appendices

4-Bromo-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**93**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.630	BV	0.0488	47.52709	11.97518	0.4039
2	7.715	VB	0.0466	107.22497	28.36489	0.9112
3	7.919	MM	0.1219	1.15885e4	1584.42163	98.4798
4	9.766	BB	0.0395	24.13687	7.50915	0.2051

Totals : 1.17674e4 1632.27086

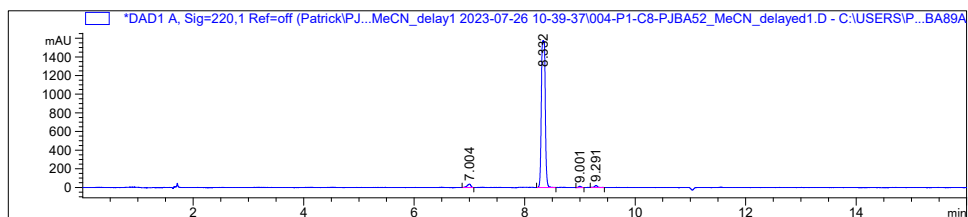
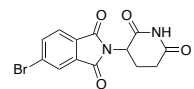


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.912	BB	0.0561	796.89526	218.08075	100.0000

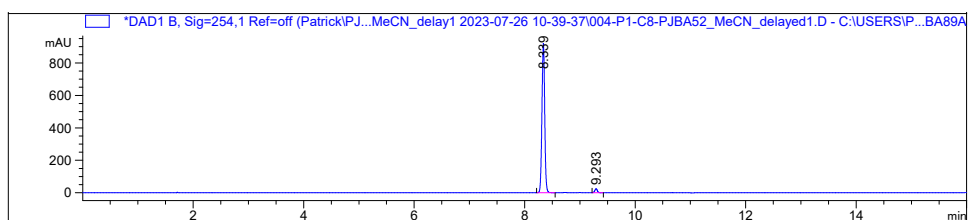
Totals : 796.89526 218.08075

Appendices

5-Bromo-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**94**)



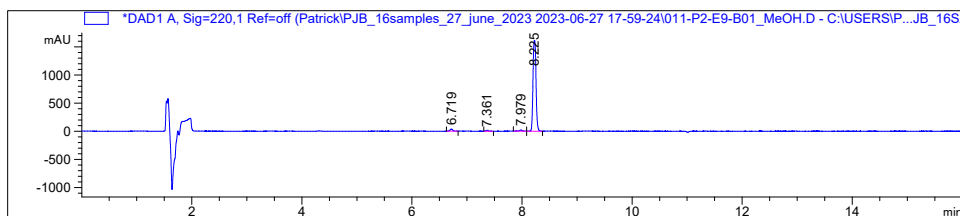
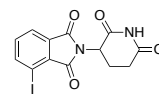
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.004	VB R	0.0601	169.77148	37.28113	2.1269
2	8.332	BB	0.0589	7684.37988	1575.89148	96.2706
3	9.001	BB	0.0400	47.47767	14.40140	0.5948
4	9.291	BV R	0.0449	80.43294	22.11587	1.0077
Totals :				7982.06198	1649.68988	



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.339	BB	0.0550	3232.09131	920.24182	97.5726
2	9.293	BB	0.0479	80.40845	25.42469	2.4274
Totals :				3312.49976	945.66651	

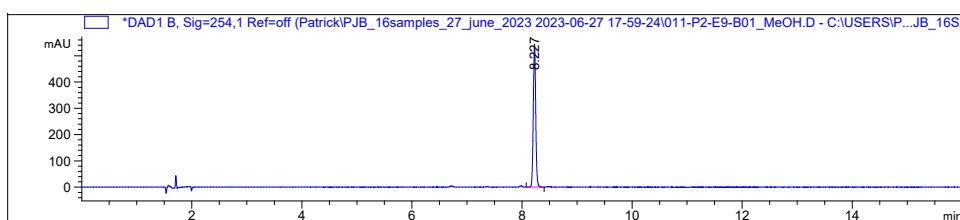
Appendices

2-(2,6-Dioxopiperidin-3-yl)-4-iodoisindoline-1,3-dione (**95**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.719	BB	0.0445	133.82944	37.15325	2.0950
2	7.361	BB	0.0403	49.76325	15.35464	0.7790
3	7.979	BB	0.0524	77.25655	20.54155	1.2094
4	8.225	BB	0.0468	6127.28809	1613.76160	95.9167

Totals : 6388.13733 1686.81104

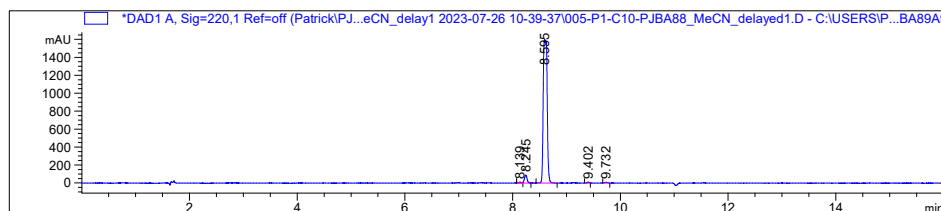
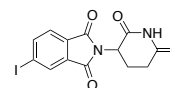


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.227	BB	0.0480	1676.24133	543.46442	100.0000

Totals : 1676.24133 543.46442

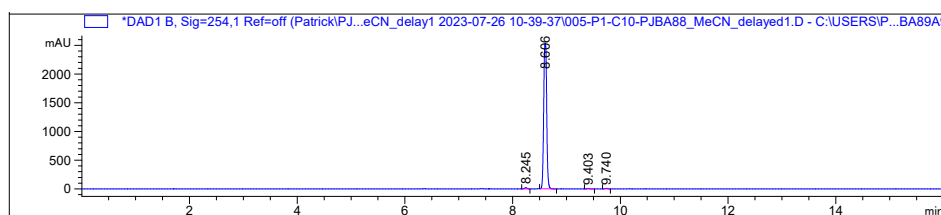
Appendices

2-(2,6-Dioxopiperidin-3-yl)-5-iodoindoline-1,3-dione (**96**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.139	BV E	0.0379	31.36179	10.05772	0.3685
2	8.245	VV R	0.0507	303.46432	89.25816	3.5662
3	8.595	VB R	0.0608	8107.98242	1594.07239	95.2812
4	9.402	BV	0.0366	35.78897	11.90493	0.4206
5	9.732	VB	0.0454	30.93200	8.13432	0.3635

Totals : 8509.52951 1713.42752

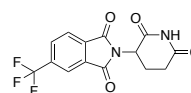


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.245	BB	0.0493	65.21466	19.61108	0.6621
2	8.606	BB	0.0543	9735.32910	2534.51929	98.8364
3	9.403	BB	0.0462	24.64587	7.71999	0.2502
4	9.740	BB	0.0482	24.74964	7.86426	0.2513

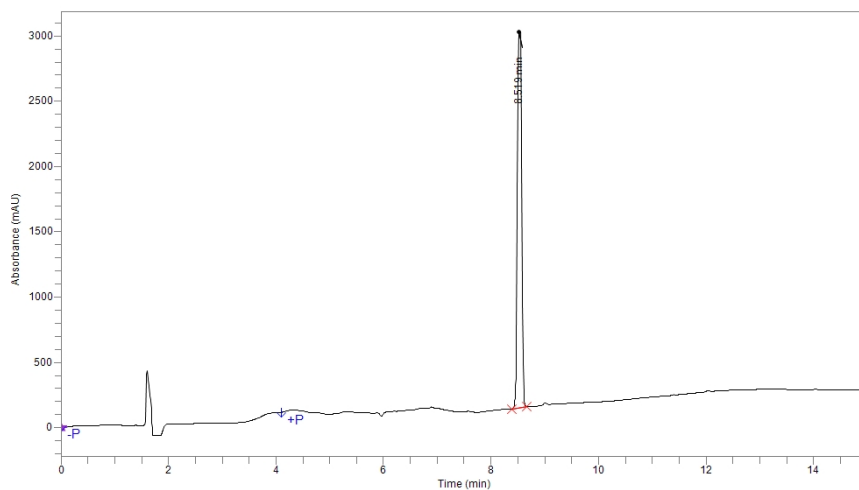
Totals : 9849.93928 2569.71462

Appendices

2-(2,6-Dioxopiperidin-3-yl)-5-(trifluoromethyl)isoindoline-1,3-dione (**97**)

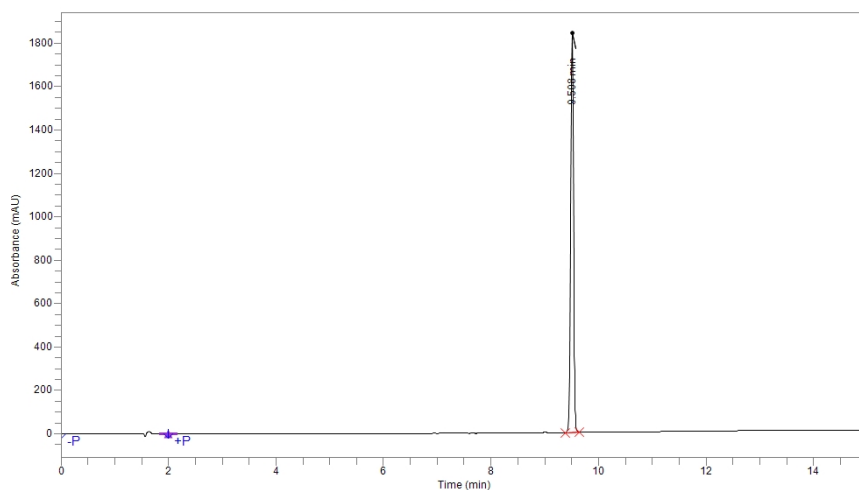


A84_220_12_7_2021 : Injection 1



Time	Height	Area	Area %
8.519	2,921,498.9	15,168,796.2	100.00
Total		15,168,796.2	100.00

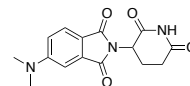
A84_254_12_7_2021 : Injection 1



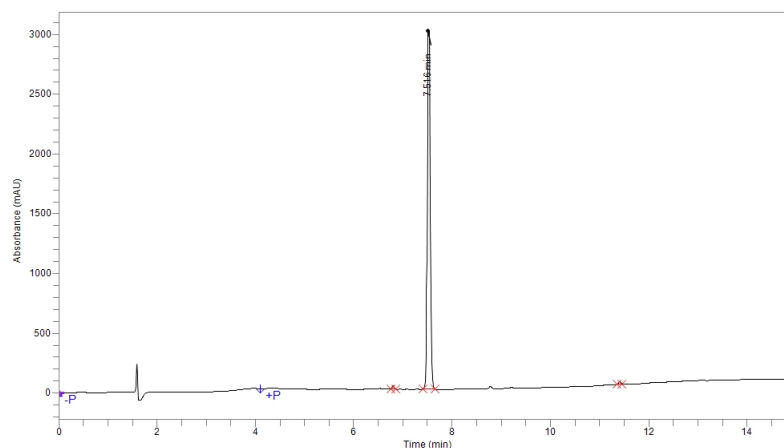
Time	Height	Area	Area %
9.508	1,844,947.1	6,668,456.4	100.00
Total		6,668,456.4	100.00

Appendices

5-(Dimethylamino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**98**)

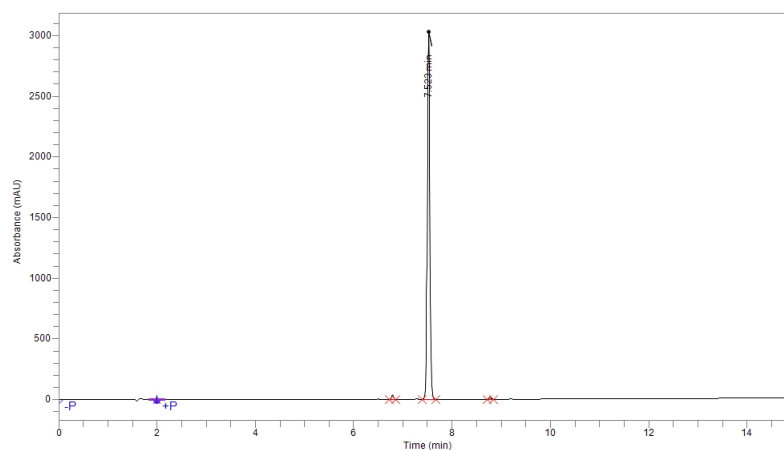


A62_220_13_7_2021 : Injection 1



Time	Height	Area	Area %
6.801	31,486.5	93,221.0	0.68
7.516	3,044,345.1	13,613,467.1	98.89
11.409	20,678.6	59,497.1	0.43
Total		13,766,185.2	100.00

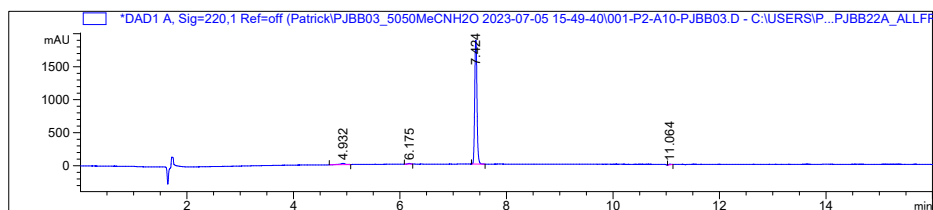
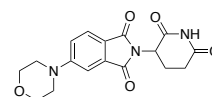
A62_254_13_7_2021 : Injection 1



Time	Height	Area	Area %
6.790	37,642.5	119,915.4	1.03
7.523	3,057,655.3	11,472,276.8	98.60
8.777	11,796.8	42,782.4	0.37
Total		11,634,974.6	100.00

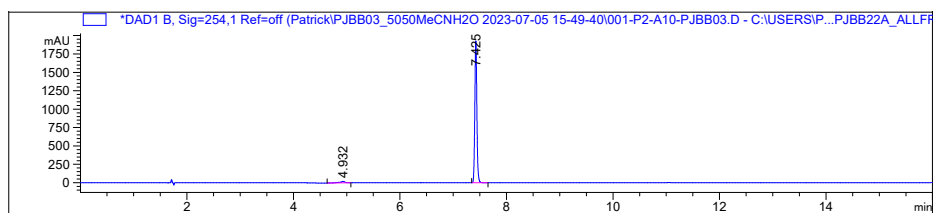
Appendices

2-(2,6-Dioxopiperidin-3-yl)-5-morpholinoisindoline-1,3-dione (**99**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.932	BB	0.0854	133.68457	19.52182	2.2990
2	6.175	BB	0.0472	36.72113	10.41101	0.6315
3	7.424	BB	0.0469	5612.18750	1878.12195	96.5140
4	11.064	BB	0.0438	32.30088	10.53894	0.5555

Totals : 5814.89408 1918.59372

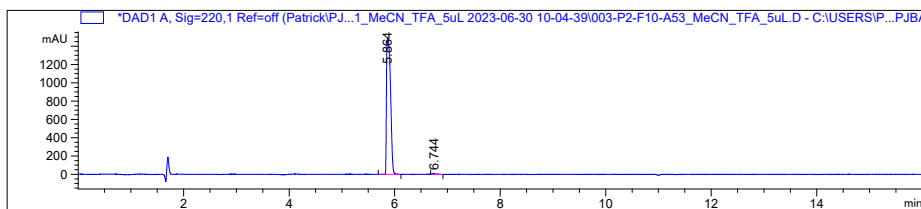
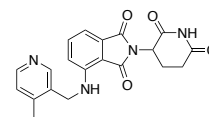


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.932	BB	0.0914	132.48828	19.23407	2.5003
2	7.425	BB	0.0413	5166.39648	1927.11658	97.4997

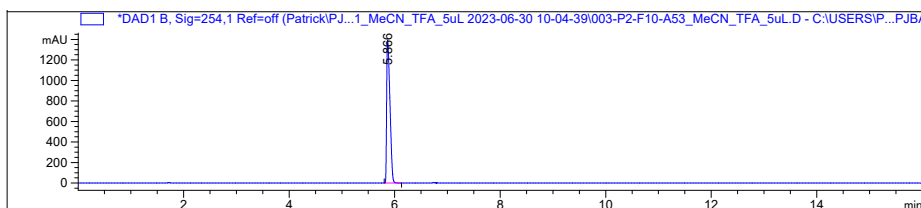
Totals : 5298.88477 1946.35064

Appendices

2-(2,6-Dioxopiperidin-3-yl)-4-(((4-methylpyridin-3-yl)methyl)amino)isoindoline-1,3-dione (**100**)



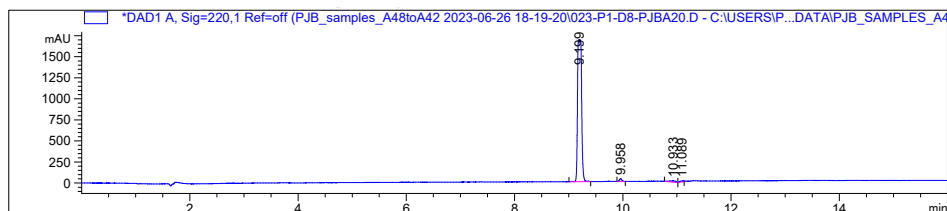
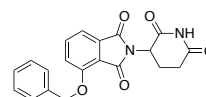
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.864	VB R	0.0630	7864.15283	1479.57996	99.4521
2	6.744	BB	0.0488	43.32656	10.80010	0.5479
Totals :				7907.47939	1490.38006	



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.866	BB	0.0655	6021.60986	1392.17969	100.0000
Totals :				6021.60986	1392.17969	

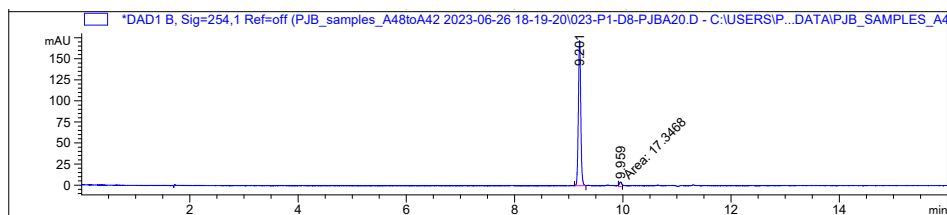
Appendices

4-(Benzyloxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**101**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.199	VV R	0.0616	8759.21680	1687.26782	96.7374
2	9.958	BB	0.0428	104.18281	33.90955	1.1506
3	10.933	BB	0.0947	129.00211	16.15368	1.4247
4	11.089	BV	0.0602	62.22880	12.37132	0.6873

Totals : 9054.63051 1749.70237

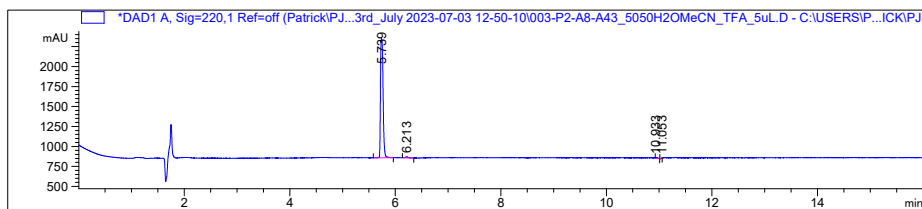
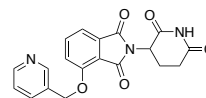


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.201	BB	0.0474	529.15289	171.89610	96.8258
2	9.959	MM	0.0531	17.34685	5.44532	3.1742

Totals : 546.49974 177.34143

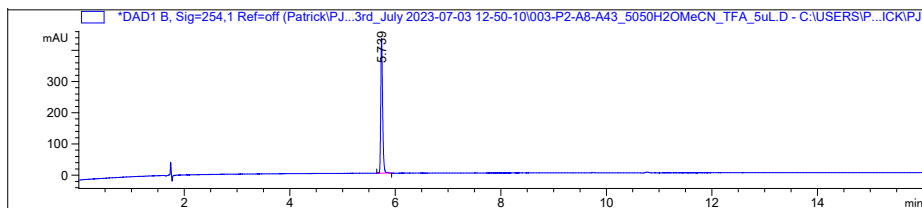
Appendices

2-(2,6-Dioxopiperidin-3-yl)-4-(pyridin-3-ylmethoxy)isoindoline-1,3-dione (**102**)



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.739	BB	0.0422	5070.66211	1490.58447	98.3312
2	6.213	VV R	0.0412	47.04042	14.54279	0.9122
3	10.933	VB	0.0443	27.20971	7.49671	0.5277
4	11.053	BV	0.0186	11.80451	8.07088	0.2289

Totals : 5156.71675 1520.69486

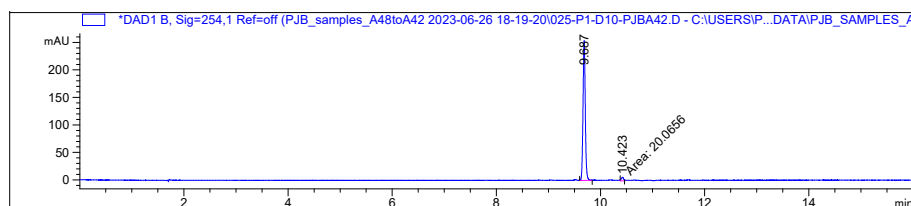
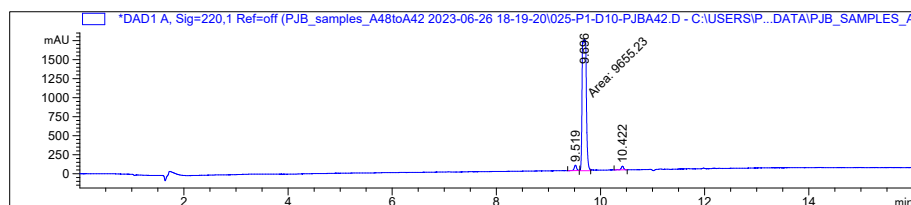
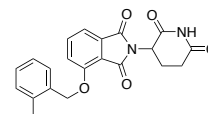


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.739	BV R	0.0364	1051.10193	431.90494	100.0000

Totals : 1051.10193 431.90494

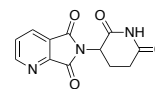
Appendices

2-(2,6-Dioxopiperidin-3-yl)-4-((2-methylbenzyl)oxy)isoindoline-1,3-dione (**103**)

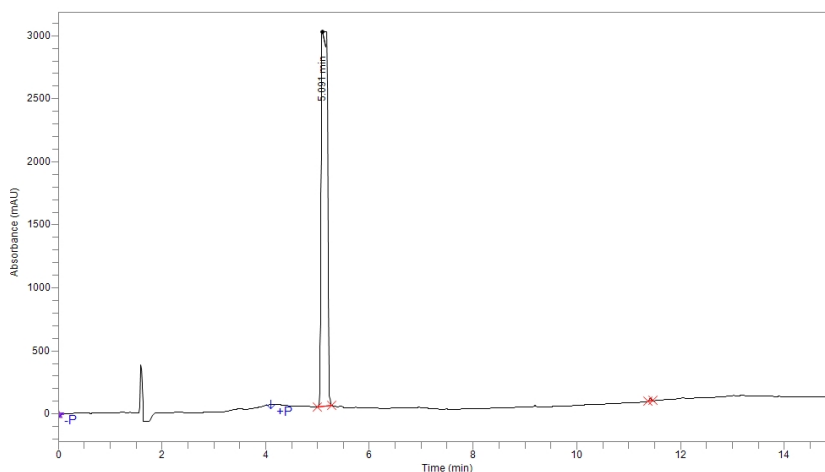


Appendices

6-(2,6-Dioxopiperidin-3-yl)-5H-pyrrolo[3,4-b]pyridine-5,7(6H)-dione (**104**)

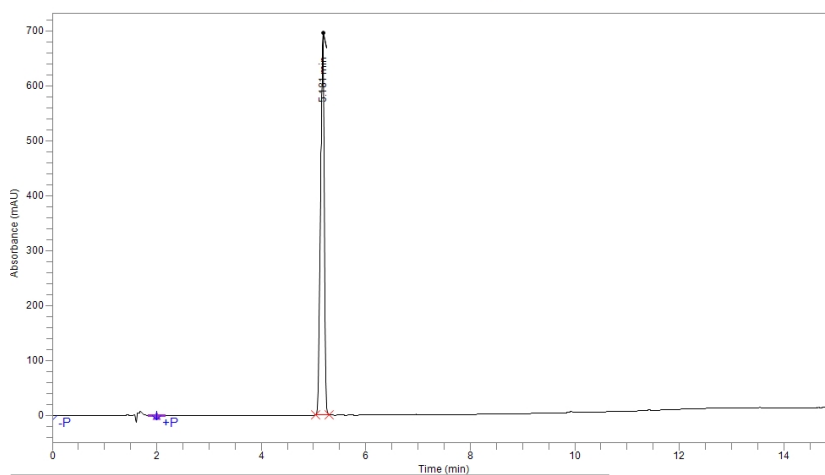


A66_220_13_7_2021 : Injection 1



Time	Height	Area	Area %
5.091	3,000,256.3	24,431,285.3	99.74
11.418	22,738.6	64,527.0	0.26
Total		24,495,812.2	100.00

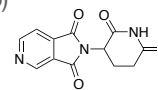
A66_254_13_7_2021 : Injection 1



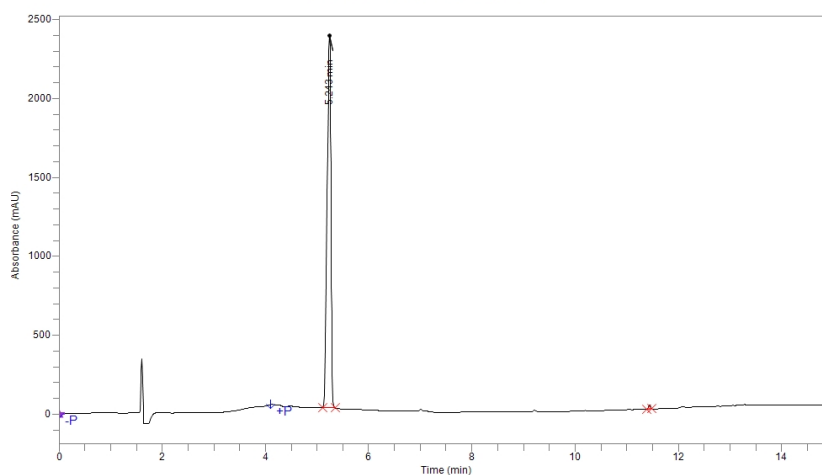
Time	Height	Area	Area %
5.181	697,023.0	3,671,621.6	100.00
Total		3,671,621.6	100.00

Appendices

2-(2,6-Dioxopiperidin-3-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**105**)

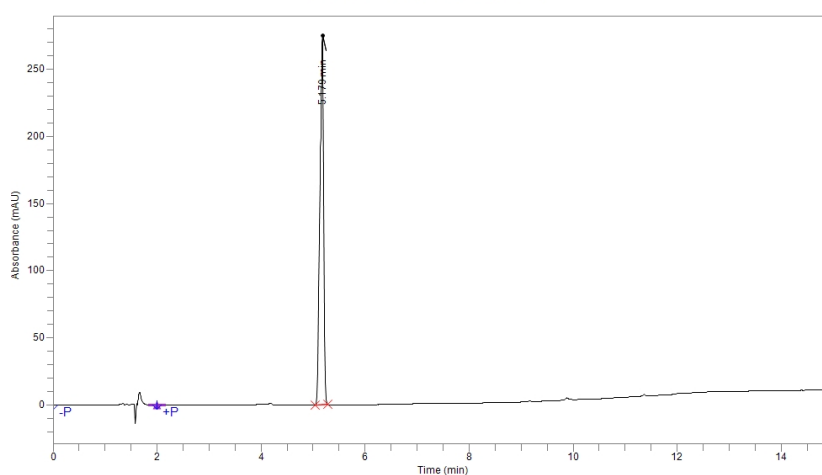


A74_220_13_7_2021 : Injection 1



Time	Height	Area	Area %
5.243	2,361,847.5	12,178,523.5	99.41
11.440	23,934.0	72,061.0	0.59
Total		12,250,584.5	100.00

A74_254_13_7_2021 : Injection 1



Time	Height	Area	Area %
5.179	275,514.1	1,450,941.8	100.00
Total		1,450,941.8	100.00

S.6 NGS Sequences

Appendices

Sequences for NGS

NGS PCR1 primers

Name	Sequence
PCR1 F	CAGGTGTCGTGAGGATCTATTTCCG
PCR1 R	GTGCAGATGAATTCAGGGTCAGG

NGS PCR2 P5 primers

Sample	Name	Sequence
Replicate 1	N502 index P5 0 nt stagger	AATGATACGGCGACCACCGAGATCTACACCTCTCTATACACTCTTTCCCT ACACGACGCTCTTCCGATCTAGCGAAGCCATACTGGTGAA* ^C
Replicate 1	N502 index P5 1 nt stagger	AATGATACGGCGACCACCGAGATCTACACCTCTCTATACACTCTTTCCCT ACACGACGCTCTTCCGATCTAGCGAAGCCATACTGGTGAA* ^C
Replicate 1	N502 index P5 2 nt stagger	AATGATACGGCGACCACCGAGATCTACACCTCTCTATACACTCTTTCCCT ACACGACGCTCTTCCGATCTGACGGAAGCCATACTGGTGAA* ^C
Replicate 1	N502 index P5 3 nt stagger	AATGATACGGCGACCACCGAGATCTACACCTCTCTATACACTCTTTCCCT ACACGACGCTCTTCCGATCTAGCAGCGAAGCCATACTGGTGAA* ^C
Replicate 1	N502 index P5 4 nt stagger	AATGATACGGCGACCACCGAGATCTACACCTCTCTATACACTCTTTCCCT ACACGACGCTCTTCCGATCTCAACAGCGAAGCCATACTGGTGAA* ^C
Replicate 1	N502 index P5 6 nt stagger	AATGATACGGCGACCACCGAGATCTACACCTCTCTATACACTCTTTCCCT ACACGACGCTCTTCCGATCTTGACCCAGCGAAGCCATACTGGTGAA* ^C
Replicate 1	N502 index P5 7 nt stagger	AATGATACGGCGACCACCGAGATCTACACCTCTCTATACACTCTTTCCCT ACACGACGCTCTTCCGATCTACGCAACAGCGAAGCCATACTGGTGAA* ^C
Replicate 1	N502 index P5 8 nt stagger	AATGATACGGCGACCACCGAGATCTACACCTCTCTATACACTCTTTCCCT ACACGACGCTCTTCCGATCTGAAGACCCAGCGAAGCCATACTGGTGAA* ^C
Replicate 2	N505 index P5 0 nt stagger	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGACACTCTTTCCCT ACACGACGCTCTTCCGATCTAGCGAAGCCATACTGGTGAA* ^C
Replicate 2	N505 index P5 1 nt stagger	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGACACTCTTTCCCT ACACGACGCTCTTCCGATCTAGCGAAGCCATACTGGTGAA* ^C
Replicate 2	N505 index P5 2 nt stagger	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGACACTCTTTCCCT ACACGACGCTCTTCCGATCTGACGGAAGCCATACTGGTGAA* ^C
Replicate 2	N505 index P5 3 nt stagger	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGACACTCTTTCCCT ACACGACGCTCTTCCGATCTAGCAGCGAAGCCATACTGGTGAA* ^C
Replicate 2	N505 index P5 4 nt stagger	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGACACTCTTTCCCT ACACGACGCTCTTCCGATCTCAACAGCGAAGCCATACTGGTGAA* ^C
Replicate 2	N505 index P5 6 nt stagger	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGACACTCTTTCCCT ACACGACGCTCTTCCGATCTTGACCCAGCGAAGCCATACTGGTGAA* ^C
Replicate 2	N505 index P5 7 nt stagger	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGACACTCTTTCCCT ACACGACGCTCTTCCGATCTACGCAACAGCGAAGCCATACTGGTGAA* ^C
Replicate 2	N505 index P5 8 nt stagger	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGACACTCTTTCCCT ACACGACGCTCTTCCGATCTGAAGACCCAGCGAAGCCATACTGGTGAA* ^C
Replicate 3	N506 index P5 0 nt stagger	AATGATACGGCGACCACCGAGATCTACACACTGCATAACACTCTTTCCCT ACACGACGCTCTTCCGATCTAGCGAAGCCATACTGGTGAA* ^C
Replicate 3	N506 index P5 1 nt stagger	AATGATACGGCGACCACCGAGATCTACACACTGCATAACACTCTTTCCCT ACACGACGCTCTTCCGATCTAGCGAAGCCATACTGGTGAA* ^C
Replicate 3	N506 index P5 2 nt stagger	AATGATACGGCGACCACCGAGATCTACACACTGCATAACACTCTTTCCCT ACACGACGCTCTTCCGATCTGACGGAAGCCATACTGGTGAA* ^C
Replicate 3	N506 index P5 3 nt stagger	AATGATACGGCGACCACCGAGATCTACACACTGCATAACACTCTTTCCCT ACACGACGCTCTTCCGATCTAGCAGCGAAGCCATACTGGTGAA* ^C
Replicate 3	N506 index P5 4 nt stagger	AATGATACGGCGACCACCGAGATCTACACACTGCATAACACTCTTTCCCT ACACGACGCTCTTCCGATCTCAACAGCGAAGCCATACTGGTGAA* ^C
Replicate 3	N506 index P5 6 nt stagger	AATGATACGGCGACCACCGAGATCTACACACTGCATAACACTCTTTCCCT ACACGACGCTCTTCCGATCTTGACCCAGCGAAGCCATACTGGTGAA* ^C
Replicate 3	N506 index P5 7 nt stagger	AATGATACGGCGACCACCGAGATCTACACACTGCATAACACTCTTTCCCT ACACGACGCTCTTCCGATCTACGCAACAGCGAAGCCATACTGGTGAA* ^C
Replicate 3	N506 index P5 8 nt stagger	AATGATACGGCGACCACCGAGATCTACACACTGCATAACACTCTTTCCCT ACACGACGCTCTTCCGATCTGAAGACCCAGCGAAGCCATACTGGTGAA* ^C
		P5 flowcell attachment sequence
		Illumina sequencing primer
		Vector primer binding sequence
		Barcode region
		Stagger region
		* = Phosphorothioate bond

Appendices

NGS PCR2 P7 primers

Compound	Name	Sequence
DMSO -ve	N703 index P7.3	CAAGCAGAAGACGGCATACGAGATTCTGCCTGTGACTGGAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
A32 -ve	N704 index P7.3	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTGAAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
A63 -ve	N705 index P7.3	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTGACTGGAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
B08 -ve	N706 index P7.3	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTGAAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
Len -ve	N707 index P7.3	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTGACTGGAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
A65 -ve	N710 index P7.3	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTGACTGGAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
B03 -ve	N711 index P7.3	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTGACTGGAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
A33 -ve	N712 index P7.3	CAAGCAGAAGACGGCATACGAGATCCTCTACGTGACTGGAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
DMSO +ve	N714 index P7.3	CAAGCAGAAGACGGCATACGAGATTCATGAGCGTGAAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
A32 +ve	N715 index P7.3	CAAGCAGAAGACGGCATACGAGATCCTGAGATGTGACTGGAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
A63 +ve	N716 index P7.3	CAAGCAGAAGACGGCATACGAGATTAGCGAGTGTGACTGGAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
B08 +ve	N718 index P7.3	CAAGCAGAAGACGGCATACGAGATGTAGCTCCGTGACTGGAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
Len +ve	N719 index P7.3	CAAGCAGAAGACGGCATACGAGATTACTACGCGTGAAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
A65 +ve	N720 index P7.3	CAAGCAGAAGACGGCATACGAGATAGGCTCCGTGACTGGAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
B03 +ve	N721 index P7.3	CAAGCAGAAGACGGCATACGAGATGCAGCGTAGTGAAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
A33 +ve	N722 index P7.3	CAAGCAGAAGACGGCATACGAGATCTGCGCATGTGACTGGAGTTCAGACGT GTGCTCTCCGATCTGTGACACTTAAAGGTTTTCCCT*G
		P7 flowcell attachment sequence
		Illumina sequencing primer
		Vector primer binding sequence
		Barcode region
		* = Phosphorothioate bond

sgRNA

sgRNA	Sequence
CTNNB1 sgRNA 1	UAUAGCUGAUUUGAUGGAGU
CTNNB1 sgRNA 2	CUGGCAGCAACAGUCUUACC
CTNNB1 sgRNA 3	UUGCCUUUACCACUCAGAGA
TRIM28 sgRNA 1	AUCCCUUCUUCUGAAGUGG
TRIM28 sgRNA 2	GUGCUUCUCCAAAGACAUCG
TRIM28 sgRNA 3	CGACGCCCAGGAUGCGAACC

PCR primers for amplifying sgRNA target site

Primer	Sequence
CTNNB1 F	GGAATCTAGTCTGGATGACTGC
CTNNB1 R	CACTATCCACAGTTCAGCATTTACC
TRIM28 F	CGAAGTGATCGGTGCCAC
TRIM28 R	GCATTATCCTCACAGCTAGTGC

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Figure 1 from Watson et al., *SCIENCE*, 3 Nov 2022
Vol 378, Issue 6619, pp. 549-553, DOI: 10.1126/science.add7574

Figure 2 & 3 from Baud et al., *SCIENCE*, 31 Oct 2014
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