Supporting Information

Structural Basis for the Inhibition of Factor-Inhibiting Hypoxia-Inducible Factor by Cyclic 2-Oxoglutarate Analogues

Crystallographic Analyses

Crystals were grown by vapour diffusion in sitting drops from mother liquor containing 1.6M ammonium sulfate, 0.1M HEPES at pH 7.5, 5% PEG400, 1mM $FeSO_4$, with 2mM of the appropriate inhibitor. The data for the three structures presented here were collected at the Synchrotron Radiation Source (SRS), Daresbury, UK. All crystals were cryo-cooled to 100K. Images were integrated with MOSFLM[1] and reduced with programs from the CCP4 suite[2]. Initial models were obtained by molecular replacement with a previously determined FIH structure (PDB-ID 1H2N). Models were rebuilt using Coot[3], and refined using the programs CNS[4], REFMAC[5], and PHENIX[6]. TLS groups were assigned with the TLSMD[7] server.[8]

For the structure of **3** with FIH, besides the 'primary' binding site involving iron coordination, extra electron density near the iron opposite His 279 was apparent. The extra density possibly reflects more than one mode of binding, a second molecule of **3** at 66% occupancy was built and appears to account reasonably well for some of the extra density. In support of this binding mode is the fact that Asp 201 seems to be (partially) detached and turned away from the iron as a result of the substrate binding at this position. The crystal structure for **2** (Fig 2b) also reveals evidence for binding of a second inhibitor molecule, but the electron density was again fragmented.

Table S1. X-Ray data collection and crystallographic statistics

Ligand	**4**		**3**		**2**	
X-ray source	SRS Daresbury 14.1		SRS Daresbury 10.1		SRS Daresbury 10.1	
Wavelength	1.4880		1.4880		1.4880	
PDB ID Code	2w0x		2wa4		2wa3	
Space group	$P4_12_12$		$P4_12_12$		$P4_12_12$	
Unit Cell Dimensions (*a* = *b* Å, *c* Å)	86.47, 147.24		87.12, 150.91		86.94, 148.42	
Resolution shell (Å)	61–2.2	2.32–2.2	38–2.6	2.74–2.6	37–2.52	2.66–2.52
Total Number of Reflections	249631	26224	171072	24334	183482	25936
Number of Unique Reflections	29151	4156	18595	2639	19959	2841
Average $I/\sigma(I)$	19.4	3.7	12.6	2.8	9.4	2.7
Completeness (%)	100	99.0	100	100	100.0	100.0
R_{merge} (%)[§]	6.0	41.2	9.5	64.1	13.7	67.8
R_{meas} (%)[9]	6.3	45.0	10.0	67.9	14.5	71.8
R_{pim} (%)[9]	2.1	17.7	3.3	22.2	4.7	23.6
R_{cryst} (%)[*]	18.3		19.5		19.3	
R_{free} (%)(based on 5% of reflections)	22.4		26.1		23.6	
RMS deviation[¶]	0.007(1°)		0.008(1.1°)		0.008(1.1°)	
B factors (Å^2)[‖]	52.2, 57.9, 36.5, 57.7		76.0, 80.9, 64.8, 67.0		70.5, 74.8, 64.2, 61.3	
Number of Water Molecules	200		59		84	
Ramachandran favoured/outliers[10] %	96.8/0.3		93.2/0.0		95.8/0.6	

[§] $R_{\text{merge}} = \sum_j\sum_h| I_{h,j}-\langle I_h\rangle| /\sum_j\sum_h\langle I_h\rangle \times 100$

[*] $R_{\text{cryst}} = \sum||F_{\text{obs}}| - |F_{\text{calc}}||/\sum|F_{\text{obs}}| \times 100$

[¶] RMS deviation from ideality for bonds (followed by the value for angles).

[‖] Average *B* factors in order: main chain, side chain, iron and substrate, solvent

Figure S1. LIGPLOT[11] diagrams of the mode of binding of compounds (**a**) **4** (**b**) **2** (**c**) **3** to FIH.

Figure S1a

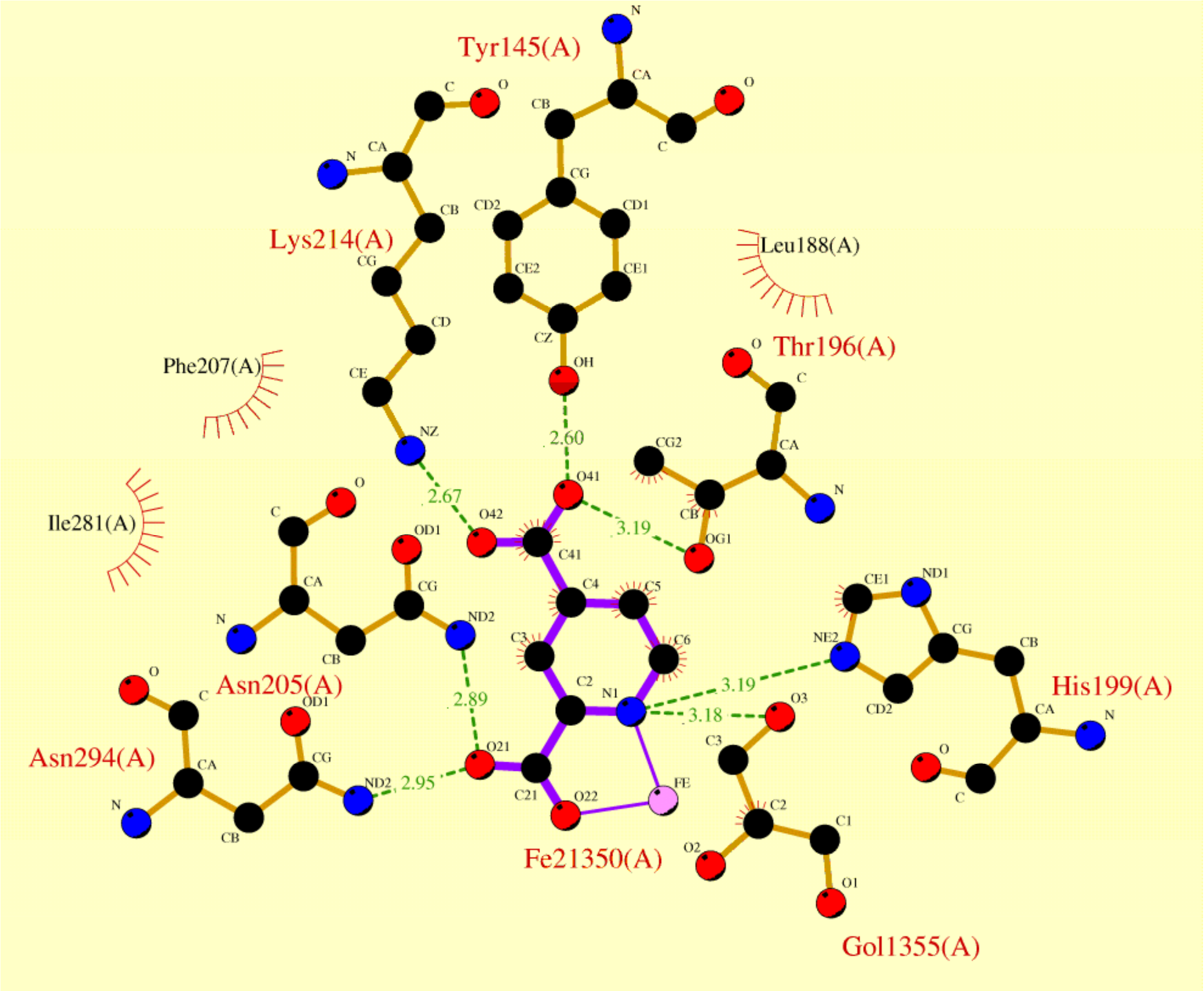


Figure S1b

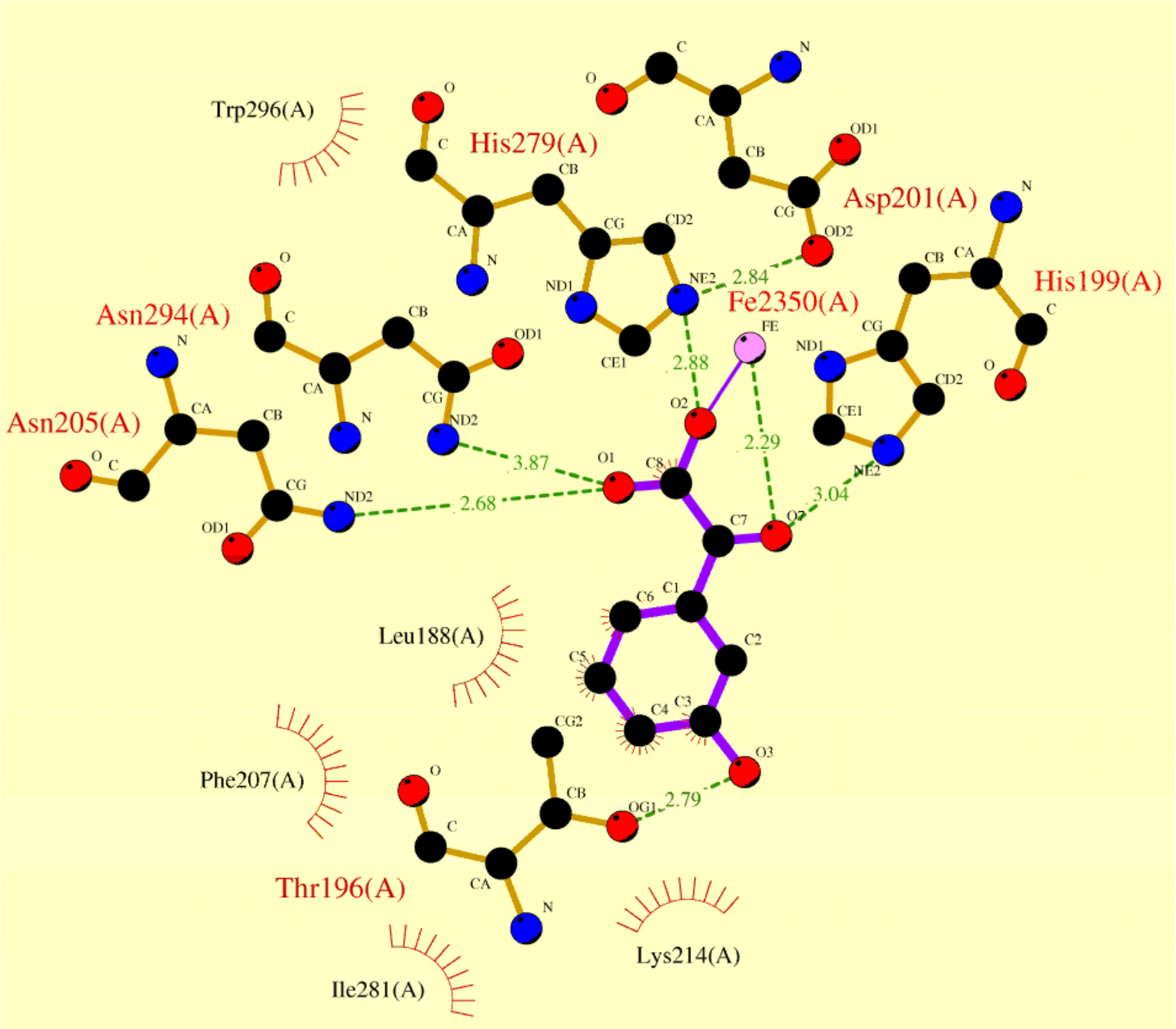
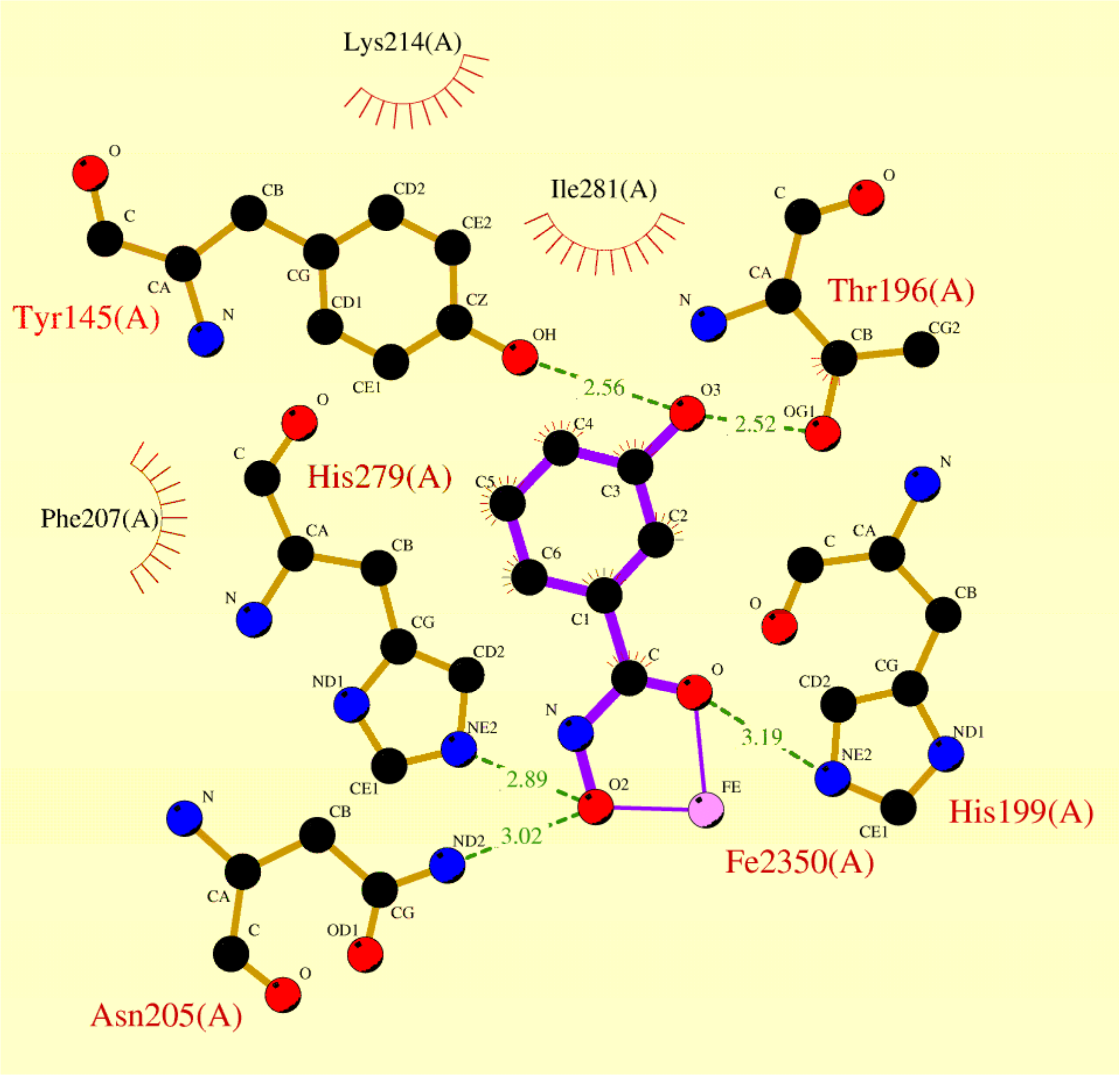
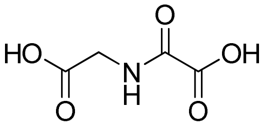
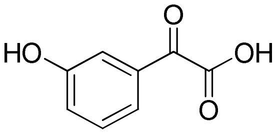
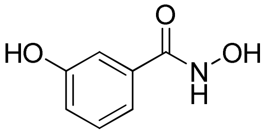
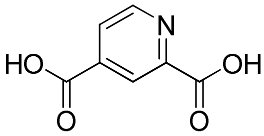
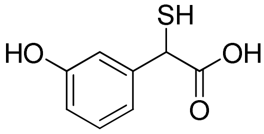
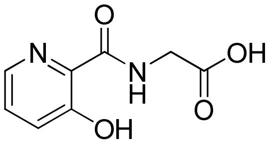
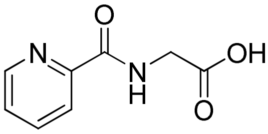
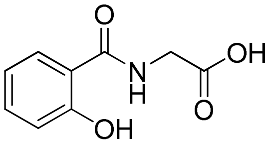

Trp296(A)
His279(A)
Asp201(A)
His199(A)
Fe2350(A)
Asn294(A)
Asn205(A)
Leu188(A)
Phe207(A)
Thr196(A)
Ile281(A)
Lys214(A)
2.84
2.88
2.29
3.87
2.68
3.04
2.79
FE
O1
O2
O3
C1
C2
C3
C4
C5
C6
C7
C8
O7
N
CA
CB
CG
CD2
CE1
ND1
NE2
OD1
OD2
ND2
OG1
CG2
C
O

Figure S1c


Lys214(A)
O
CB
CD2
Ile281(A)
C
O
C
CE2
CA
CG
Thr196(A)
CA
CD1
CZ
N
Tyr145(A)
N
CB
CG2
OH
CE1
2.56
O3
O
2.52
OG1
C4
His279(A)
N
C5
C3
C2
Phe207(A)
CA
C
CA
CB
C6
CB
N
C1
O
CG
CD2
C
O
CD2
CG
ND1
N
NE2
3.19
ND1
NE2
2.89
O2
FE
CE1
N
CB
His199(A)
CE1
ND2
3.02
Fe2350(A)
CA
CG
C
OD1
O
Asn205(A)

Biochemical assays

Table S2. IC_{50}s for compounds vs FIH as determined by MALDI MS

		IC_{50} (μM)
1		0.842
2		>1000
3		18.37
4		624.8
5		~500
6		91.09
6a		>1000
6b		>1000

Synthesis of Inhibitors

General procedures. All solvents were either dried solvents purchased from Aldrich or dried by passing under nitrogen pressure over aluminate column. All reagents were used as obtained from commercial sources unless otherwise stated. Measurement of pH was carried out using Prolabo Rota pH 1-10 paper. Flash chromatography was performed using silica gel (0.125-0.25 mm, 60-120 mesh) as the stationery phase. Thin layer chromatography (TLC) was performed on aluminium plates pre-coated with silica gel (Merck silica gel 60 F_{254} 1.05554), which were visualised by the quenching of UV fluorescence (using an irradiation wavelength λ_{max} = 254 nm), and/or by staining with iodine or 10% ammonium molybdate in 2 M sulphuric acid, followed by heating. Melting points (mp) were obtained using a Büchi

510 Cambridge Instruments Gallen III hot stage melting point apparatus. Infrared (IR) spectra were recorded as thin films between NaCl plates or KBr disc using a Tensor 27 FT-IR Brüker spectrometer and only selected peaks are reported. Proton magnetic resonance spectra (^{1}H NMR) were recorded on Brüker DPX 250 (250 MHz), Brüker DPX 400 (400 MHz), Brüker DQX 400 (400 MHz), Brüker DRX 500 (500 MHz), and Brüker AMX500 (500 MHz) spectrometers at ambient and variable temperature. The central point of multiplets is reported. ^{1}H NMR spectral assignments are supported by ^{1}H-^{1}H COSY where necessary. Carbon magnetic resonance spectra (^{13}C NMR) were recorded on Brüker DPX 250 (62.9 MHz), Brüker DPX 400 (100.6 MHz), Brüker DQX 400 (100.6 MHz), Brüker DRX 500 (125.8 MHz), and Brüker AMX500 (125.8 MHz) spectrometers at ambient temperature. Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to the residual solvent peak. High-resolution mass spectra (HRMS) were recorded on a VG Autospec spectrometer by chemical ionisation (CI) or on a Micromass LCT electrospray ionisation (ES) mass spectrometer operating at a resolution of 5000 full width half height. Intermediates **8**[12, 13] and **12**[14] were synthesized as reported.

Cyano (3-*tert*-butyldimethylsilyloxyphenyl) methyl ethyl carbonate (9). To a solution of compound **8** (1.00 g, 4.203 mmol), ethyl chloroformate (0.68 g, 6.340 mmol) and benzyltrimethyl ammonium chloride (52.0 mg, 0.280 mmol) in THF (8 mL) cooled in an ice-bath was slowly added a solution of sodium cyanide (414.6 mg, 8.459 mmol) in water (8 mL). The mixture was stirred overnight at room temperature. Sodium bicarbonate was added to the mixture and the THF removed by evaporation. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers washed with saturated solution of $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated to afford **9** as an oil (1.2 g, 87%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.22 (s, 9H), 1.01 (s, 9H), 1.36 (t, J = 7 Hz, 3H), 4.31 (dq, J = 7 Hz, 2H), 6.21 (s, 1H), 6.93 (dd, J = 7.5, 2 Hz, 1H), 7.01 (t, J = 2 Hz, 1H), 7.12 (d, J = 7.5 Hz, 1H), 7.31 (t, J = 7.5 Hz, 1H); ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ -4.5 (x2), 14.1, 18.1, 25.6 (x3), 65.6, 66.1, 115.7, 119.5, 120.6, 122.2, 130.3, 132.5, 153.4, 156.3; HRCIMS (*m/z*): $[M + NH_4]^+$ calculated for $C_{17}H_{29}N_2O_4Si$, 353.1897; found, 353.1890; IR (neat): 1702 cm^{-1}.

Ethyl (3-*tert*-butyldimethylsilyloxyphenyl)(oxo)acetate (10). A solution of lithium diisopropylamide (2.978 mmol) was prepared at 0° C from diisopropyl amine (0.42 mL, 2.978 mmol) in dry THF (7 mL) and 1.6 M *n*-butyllithium (1.80 mL, 2.978 mmol). After 40 minutes the solution was cooled to -78° C and a solution of **9** (0.91 g, 2.708 mmol) in dry THF (5 mL) was added dropwise. The solution was stirred at -78° C for an hour then at room temperature for two hours, then quenched with saturated aqueous ammonium chloride. The THF was evaporated and the residue extracted with dichloromethane. The combined organic layers were washed with water and brine, dried (Na_2SO_4) and evaporated. The crude product was purified by flash column chromatography on silica gel using hexane-ethyl acetate (1:9) as eluent to afford **10** as an oil (450 mg, 55%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.22 (s, 9H), 1.00 (s, 9H), 1.43 (t, J = 7 Hz, 3H), 4.45 (dq, J = 7 Hz, 2H), 7.14 (m, 1H), 7.37 (t, J = 8 Hz, 1H), 7.47 (m, 1H), 7.58 (dd, J = 1.5, 8 Hz, 1H); ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ -4.5 (x2), 14.1, 18.1, 25.6 (x3), 62.3, 120.6, 123.4, 127.0, 130.0, 133.7, 156.1 163.8, 186.2; HRCIMS (*m/z*): $[M + NH_4]^+$ calculated for $C_{16}H_{28}N_1O_4Si$, 326.1788; found, 26.1790; IR (neat): 1740 cm^{-1}.

(3-Hydroxyphenyl)(oxo)acetic acid (2). NaOH (1M, 1 mL) was added to a solution of **10** (0.11g, 0.354 mmol) in MeOH (1 mL) and stirred at room temperature for 3 hours. The MeOH was evaporated and water was added to the residue before washed with ethyl acetate. The aqueous layer was then acidified with 1M HCl and extracted with ethyl acetate dried ($NaSO_4$) and the solvent evaporated to afford **2** as a pale yellow solid (53.1 mg, 90%). ^{1}H-

NMR (400 MHz, *d*$_6$-acetone): δ 7.23 (d, *J* = 7.5 Hz, 1H), 7.45 (t, *J* = 7.5 Hz, 1H), 7.52 (m, 2H), 8.52 (bs, 2H, OH); ^{13}C-NMR (100.6 MHz, *d*$_6$-acetone): δ 115.8, 121.7, 122.6, 130.7, 134.1, 158.3, 165.3, 187.9; HRESMS (*m/z*): [M − H]$^-$ calculated. for $C_8H_5O_4$, 165.0188; found, 165.0184; IR (acetone): 1715 cm^{-1}.

3-(2-Methoxy-2-oxoethyl)phenyl benzoate (13). To a solution of **12** (5.50 g, 0.033 mol) in dry CH_2Cl_2 (120 mL) were added benzoyl chloride (5.10 g, 0.036 mol) and dimethylaminopyridine (2.00 g, 0.016 mol), triethylamine (8.30 g, 0.082 mol) was added dropwise and the mixture was stirred under nitrogen overnight. A saturated solution of $NaHSO_4$ was then added and the organic layer was separated. The aqueous layer was extracted back with ethyl acetate. The combined organic layers were dried (Na_2SO_4), filtered and evaporated. The crude product was purified by flash column chromatography on silica gel using as eluent hexane-ethyl acetate (6:1, 5:1 and 3:2) to afford **13** as colourless oil (8.30 g, 92%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.68 (s, 1H), 3.72 (s, 3H), 7.19 (m, 3H), 7.40 (t, *J* = 7.5 Hz, 1H), 7.53 (t, 2H, *J* = 7.5 Hz, 2H), 7.65 (t, *J* = 7.5 Hz, 1H), 8.22 (d, *J* = 7.5 Hz, 2H); ^{13}C NMR (100.6 MHz, Cl_3CD): δ 40.9, 52.1, 120.5, 122.6, 126.8, 128.6 (x2), 129.5, 129.5 (x2), 130.1, 133.6, 135.5, 151.0, 165.1, 171.5; HRCIMS (*m/z*): [M + NH$_4$]$^+$ calculated for $C_{16}H_{18}N_1O_4$, 288.1235; found, 288.1240; IR (neat) 1732 cm^{-1}.

3-(1-Bromo-2-methoxy-2-oxoethyl)phenyl benzoate (14). A mixture of **13** (8.10 g, 0.030 mol), dibenzoyl peroxide (30.0 mg, 0.124 mmol) and *N*-bromosuccinimide (5.60 g, 0.030 mol) in CCl_4 (250 mL) was refluxed with UV radiation overnight. The reaction mixture was cooled to room temperature and the succinimide removed by filtration. The solvent was removed *in vacuo* and the remaining oil was purified by flash column chromatography on silica gel using as eluent hexane-ethyl acetate (10:1 and 10:2) to afford **14** as a slightly yellow oil (5.90 g, 57%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.72 (s, 3H), 5.38 (s, 1H), 7.25 (m, 1H), 7.45 (m, 3H), 7.53 (t, 2H, *J* = 8 Hz, 2H), 7.66 (t, *J* = 7.5 Hz, 1H), 8.22 (d, *J* = 8 Hz, 2H); ^{13}C-NMR (100.6 MHz, Cl_3CD): δ 45.5, 53.8, 122.1, 122.8, 126.1, 128.7 (x2), 129.2, 129.8 (x2), 130.2, 133.7, 137.2, 151.1, 164.8, 168.5; HRCIMS (*m/z*): [M + NH$_4$]$^+$ calculated for $C_{16}H_{17}N_1O_4Br$, 366.0341; found, 366.0350; IR (neat): 1739 cm^{-1}.

3-{1-[(Ethoxycarbonothioyl)sufanyl]-2-methoxy-2-oxoethyl}phenyl benzoate (15). Potassium *o*-ethyldithiocarbonate (2.30 g, 0.014 mol) was dissolved in warm methanol (15 mL). **14** (4.00 g, 0.011 mol) in dioxane (10 mL) was then added to the cooled solution. The suspension was stirred under nitrogen atmosphere for 18 hours. The solvent was removed; CH_2Cl_2 was added and washed with water and brine. The aqueous layer was then extracted back with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and evaporated. The crude product was purified by flash column chromatography on silica gel using as eluent hexane-CH_2Cl_2 (3:1, 2:1 and 3:3) to afford **15** as slightly yellow oil (2.50 g, 57%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.39 (t, *J* = 8 Hz, 3H), 3.78 (s, 3H), 4.63 (q, *J* = 8 Hz, 2H), 5.53 (s, 1H), 7.25 (m, 1H), 7.33 (m, 2H), 7.43 (t, *J* = 7.5 Hz, 1H), 7.52 (t, *J* = 8 Hz, 2H), 7.65 (m, 1H), 8.22 (d, *J* = 8 Hz, 2H); ^{13}C-NMR (100.6 MHz, Cl_3CD): δ 13.6, 53.2, 56.4, 70.4, 95.7, 122.0, 122.3, 126.1, 128.6, 129.2 (x2), 130.0, 130.2 (x2), 133.7, 135.0, 151.2, 164.8, 169.5; HRCIMS (*m/z*): [M + NH$_4$]$^+$ calculated for $C_{19}H_{22}N_1O_5S_2$, 408.0939; found, 408.0938; IR (neat): 1740 cm^{-1}.

Methyl (3-hydroxyphenyl)[(2-methoxy-2-oxo-1-phenylethyl)disulfanyl] acetate (16). To a solution of **15** (550.0 mg, 1.408 mmol) in ethanol (10 mL) was added concentrated NH_4OH (1.2 mL), the mixture was then stirred at room temperature for two days under nitrogen. After the solvent was removed, dichloromethane was added and washed with saturated $NaHSO_4$. The aqueous layer was then extracted back with dichloromethane and the combined organic layers washed with water and brine dried ($NaSO_4$) and evaporated. The crude product was fraction by flash column chromatography on silica gel using CH_2Cl_2: MeOH (10:0.1 and 10:0.2) as eluent. Further purification by column chromatography using CH_2Cl_2: Ethyl

Acetate (6:4 and 5:5) as eluent afford **16** as a colourless oil (165.0 mg, 30%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.72 (s, 3H), 3.74 (s, 3H), 4.41 (s, 1H), 4.47 (s, 1H), 6.52 (bs, 2H), 6.87 (m, 6H), 7.20 (t, *J* = 8 Hz, 2H); ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 53.1 (x2), 57.9, 58.0, 115.8, 115.9, 116.1, 116.2, 121.0, 121.1, 130.2 (x2), 135.9, 136.0, 155.9 (x2), 170.9, 171.0; HRESMS (*m/z*): $[M - H]^-$ calculated for $C_{18}H_{17}O_6S_2$, 393.0467; found, 393.0468; IR (neat): 1719 cm^{-1}. The analysis of the ^{1}H-NMR revealed the presence of about 25% of diester **17.** Separation of **17** from **16** by chromatography proved imposible; therefore the mixture was treated with NaOH resulting in complete conversion to **18**.

{[Carboxy(3-hydroxyphenyl)methyl]disulfanyl}(phenyl)acetic acid (18). To a solution of **16 + 17** (135.0 mg, 0.342 mmol) in MeOH (2 mL) NaOH was added (1M, 2 mL) and stirred at room temperature for 3 hours then heated at 60 ºC for another 2 hours. The MeOH was then removed *in vacuo* and water was added to the residue before washed with ethyl acetate. The aqueous layer was then acidified using acidic Dowex 50 resin and extracted with ethyl acetate, dried ($NaSO_4$) and evaporated to afford **18** as a brown oil (120.0 mg, 96%). ^{1}H-NMR (400 MHz, CD_3OD): δ 4.45 (s, 1H), 4.58 (s, 1H), 6.77 (m, 2H), 6.87 (m, 4H), 7.17 (m, 2H); ^{13}C-NMR (100.6 MHz, CD_3OD): δ 58.5, 58.6, 115.5 (x2), 115.6 (x2), 120.1, 120.2, 129.7 (x2), 137.1, 137.3, 157.8 (x2), 172.3, 172.4; HRESMS (*m/z*): $[M - H]^-$ calculated. for $C_{16}H_{13}O_6S_2$, 365.0154; found, 365.0139.

The diastereoisomers were separated by semipreparative reverse-phase HPLC (C-18 column). First peak: ^{1}H-NMR (500 MHz, CD_3OD): δ 4.41(s, 1H), 6.68 (dd, *J* = 8, 1.5 Hz, 1H), 6.91 (m, 2H), 7.11 (t, *J* = 8, 1H); ^{13}C-NMR (128.5 MHz, CD_3OD): δ 62.5, 114.5, 115.8, 120.4, 129.1, 140.6, 157.2.

Second peak: ^{1}H-NMR (500 MHz, CD_3OD): δ 4.30 (s, 1H), 6.67 (dd, *J* = 8, 2.5 Hz, 1H), 6.85 (d, *J* = 8 Hz, 1H), 6.92 (s, 1H), 7.10 (t, *J* = 8 Hz, 1H); ^{13}C-NMR (128.5 MHz, CD_3OD): δ 62.6, 114.4, 115.7, 120.7, 129.0, 141.4, 157.2.

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