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Abstract: The synthesis and evaluation of 3-enaminetetramic acids as antibacterial agents is reported; contrary to the analogous 3-acyltetramic acids, the enaminetetramic acid class of compound exhibits modest antibacterial activity against a limited spectrum of organisms, and even that activity is strongly dependent on the identity of the tetramate ring substituents. Moreover, these compounds appear to have a different mode of action to the analogous 3-acyltetramic acids, and appear to offer more limited opportunity for further elaboration in drug discovery.

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From: Dr Mark G. Moloney

Professor of Chemistry and EP Abraham Fellow in Chemistry, St Peter's College

March 5, 2014

Professor Stephen Neidle,
Cancer Research UK Professorial Fellow and Professor of Chemical Biology,
The School of Pharmacy,
University of London, UK

Dear Professor Neidle,

I would like to re-submit the attached manuscript for publication in *BiorgMedChemLett*; in the attached letter I answer all the referees criticisms, and hope that these responses are to their satisfaction.

I would like to thank you and the referees for their care in this work; I believe that the revised manuscript is significantly enhanced as a result.

Yours Sincerely,

Mark Moloney

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March 5, 2014

Professor Stephen Neidle,
Cancer Research UK Professorial Fellow and Professor of Chemical Biology,
The School of Pharmacy,
University of London, UK

Dear Professor Neidle,

I would like to submit the revised manuscript for publication in *BiorgMedChemLett*; I hope that I have addressed all of the referee's concerns, and indicate below what I have done to make the necessary corrections

Editor's Comments:

Please ensure that your revised manuscript follows the journal-specific guidelines listed below:

1. Address: If there is more than one affiliation, designate authors to institutions by using superscript 'a', 'b', 'c', etc. NOT RELEVANT
2. Headings: Please do not use common headings, such as 'Introduction', 'Chemistry', or 'Conclusion', other than 'Acknowledgments' and 'References and Notes'. CORRECTED
3. R1, R2: Use a superscript numeral for R1, R2, etc. (not a subscript R1, R2) to designate substituents in graphic structures, tables, and text. CORRECTED
4. Reference Style: For proper reference style, please consult the References and Notes section of the Journal's Guide for Authors found at www.ees.elsevier.com/bmcl. Note that the Journal uses a start page number only for journal articles. For journal title abbreviations, please consult the list provided: Click on Guide for Authors; then click on Journal Title Abbreviation List. CORRECTED
5. X-ray coordinates: X-ray coordinates must be deposited with the RCSB Protein Data Bank (PDB) database (or Cambridge Crystallographic Data Centre for small molecules) and the PDB (or CCDC) deposition number must be placed in the manuscript. NOT RELEVANT
6. Experimental Information: All experimental information should be placed in the References and notes section as an endnote or placed in Supporting Information. CORRECTED

Reviewers' comments:

Reviewer #2: 1. In general, it was very hard indeed to map chemical structures onto the text of the discussion either relating to synthesis or biological activity. The organizational principle by which the analogues are numbered and lettered is not at all apparent, but the reader naturally assumes there must be one, and confusion results. The authors are urged to come up with a strategy for making the paper more reader-friendly. I HAVE SPLIT THE COMPOUNDS INTO STRUCTURALLY BETTER DEFINED GROUPS, AND INCLUDED A DISCUSSION IN THE TEXT OF HOW THIS BREAKDOWN WAS MADE. I HOPE THAT THIS IS NOW MUCH CLEARER.

2. The text is turgid in many places, with overly-long sentences. A prime example is the first paragraph of the Results section. Here, not only is the sentence construction clumsy, but the meaning could be made more immediately transparent with a little forethought. For example, rather than having to cite 3i and 3k as exceptions to the generalization 'all 3ETs', it would be simple to say that the 3ET core was prepared via..., and that 3i and 3g resulted from subsequent acylation of the pendant amine on the side chain. I HAVE GONE THROUGH THE ENTIRE MANUSCRIPT AND SIMPLIFIED ALL TEXT; I HOPE THAT THE REFEREE AGREES THAT IT IS NOW MUCH IMPROVED
Related to this, the manuscript would benefit from more careful proof-reading: for example, the abbreviation 3-ET vs 3ET should be made consistent throughout. CORRECTED

3. In the introduction, refrain from citing the institution at which work was performed (for ref 18). CORRECTED

4. It would be helpful to point out in the text that the results for *P. aeruginosa* and *E. coli* are not shown in Table 1. CORRECTED

It would also be helpful for the reader if the column heading in Table 1 contained the abbreviated bacterial strains, rather than forcing him/her to dig into the footnotes to assimilate such essential information. THIS WOULD BE DIFFICULT TO CHANGE WITHOUT MAKING THE TABLE MUCH BIGGER; I HAVE MODIFIED THE TABLE SO IT ALL APPEARS ON ONE PAGE, WHICH I HOPE ADDRESSES THE REFEREE'S CONCERN

5. The discussion around the SAR (1st paragraph of p5 of the pdf) is very hard to follow - and anyway, the very limited variance in the levels of potency in Table 1 makes any pretence at real SAR interpretation of dubious validity. The most important point would be to convey the magnitude of the loss in potency compared to the specific acyltetramic acid comparators, but this is not addressed in the current manuscript except in very vague terms. I HAVE RESTRUCTURED THIS PARAGRAPH ENTIRELY AND HOPE THAT IT IS CLEARER. I HAVE ILLUSTRATED THE LOSS OF ACTIVITY USING TWO SPECIFIC EXAMPLES.

6. The discussion around the results in the presence of horse blood is deficient in several respects. Firstly, the most active compound is only 2-fold less active under these conditions, so the generalization that '3ETs are highly bound by serum albumin' is unfounded. More importantly, it is naïve to suggest that high serum binding is necessarily an impediment to in vivo efficacy: see D. A. Smith, Nat Rev Drug Discovery, 2010, 9, 929. AGREED, AND I HAVE REMOVED THIS.

7. The very crude physical properties-activity relationship analysis is also problematic.

a. What is meant by disubstitution on C(3)? Is it intended to imply that these disubstituted compounds (whatever they are) are a subset of the 3ETs? If so, what is the subset and where do they fall on the plots? AGREED, AND I HAVE REMOVED THIS.

b. The pKa of the acyltetramic acids is much greater than that of the 3ETs, and as a result the analysis by logD should look very different from that by logP. Some comment should be made in the text about this, and the use of clogP in the main text (relegating the logD to the supplemental) defended. THE REFEREE IS QUITE RIGHT HERE; I HAVE PLACED THE CLOGD DATA IN THE MAIN PAPER AND RELEGATED THE CLOGP TO THE SUPPLEMENTARY DATA.

c. Why is Fig 6B cited in the same sentence (p6, 2nd sentence) as exemplifying both unclear SAR due to low activity, and clearer SAR? CORRECTED

d. The assertion that 'the active 3-ETs... tend to be more lipophilic... and bigger... than their related active 3-acyls... and 3-carboxamides' is uninterpretable, 1) because the direct matched pairs are not exemplified, 2) because the active 3-ETs span a wide range of both clogP and MSA, and 3) because there aren't enough of them to draw any statistically-defensible conclusions anyway. I HAVE MODIFIED THIS SECTION AND FOCUS ON THE COMPARISON WITH REF 28 (SEE F BELOW)

e. The authors are cautioned about drawing conclusions based on their categorization of the data (active/mildly active/inactive) for two reasons: 1) the categorizations span <1 log and are therefore really quite marginal, and 2) arbitrary differentiation of this sort has been recognized to risk inflating correlations - see P.W. Kenny and C.A. Montanari, J Comp. Aided Mol. Des., 2013, 27, 1) I UNDERSTAND THE REFEREE'S POINT, BUT EQUALLY BELIEVE THAT THERE IS ROOM FOR SOME SEEKING OF TRENDS. THE ACTIVE/MILD/INACTIVE CHARACTERISATION IS ARBITRARY, BUT BASED UPON WHAT WOULD BE CONSIDERED A LEVEL OF ACTIVITY SUITABLE FOR ADVANCING A COMPOUND FOR MORE DETAILED INVESTIGATION. MY READING OF THE KENNY PAPER IS THAT IT IS AVERAGES WHICH MUST BE VERY CAREFULLY DEALT WITH.

f. Rather than try to draw conclusions about physical properties SAR within this data set, it would perhaps be more instructive to compare these compound series to the more general analysis in reference 28. I HAVE SUMMARISED WHAT APPEAR TO BE THE "DESIRABLE" PHYSICOCHEMICAL PARAMETERS IN THE TA SYSTEMS, AND COMPARED THESE TO REE 28. I HOPE THAT THIS IS ACCEPTABLE.

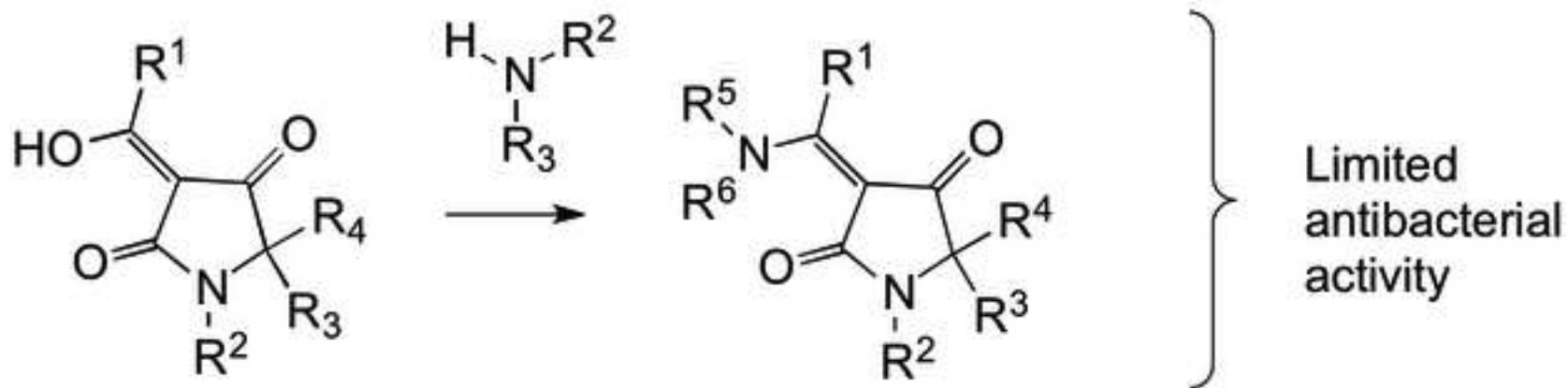
I hope that you will agree that these corrections/modifications address the concerns raised above.

Yours Sincerely,

Mark Moloney

Synthesis, antibiotic activity and structure-activity relationship study of some 3-enaminetetramic acids

Yong-Chul Jeong, Muhammad Anwar and Mark G. Moloney



Synthesis, antibiotic activity and structure-activity relationship study of some 3-enaminetetramic acids

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Abstract

The synthesis and evaluation of 3-enaminetetramic acids as antibacterial agents is reported; contrary to the analogous 3-acyltetramic acids, the enaminetetramic acid class of compound exhibits modest antibacterial activity against a limited spectrum of organisms, and even that activity is strongly dependent on the identity of the tetramate ring substituents. Moreover, these compounds appear to have a different mode of action to the analogous 3-acyltetramic acids, and appear to offer more limited opportunity for further elaboration in drug discovery.

Biologically active natural products are a source of lead compounds for structural optimization in drug discovery,¹⁻⁴ and those containing the 3-acyltetramic acid subunit are of considerable interest since they possess diverse biological activities.⁵⁻¹⁴ Those 3-acyltetramates with antibiotic activity are known to possess a range of modes of action, and include streptolydigin (bacterial RNA polymerase inhibitory activity),¹² kibdelomycin (bacterial type II topoisomerase inhibitory activity)¹³ and signermycin B (histidine kinase WalK inhibitory activity),¹⁴ but all have sufficiently complex structures that their synthesis is challenging (Figure 1). However, appropriate modification of the core tetramate skeleton of these natural products can provide rapid access to antibacterially active small molecule libraries, and this includes derivatives of the 3-acyltetramic acid **1a**, 3-carboxamidotetramic acid **1b** and the piperidine-2,4-dione one **1c** skeletons (Figure 2).¹⁵⁻¹⁸ In order to extend the scope of this work, exploration of the synthesis, bioactivity and structure-activity relationships (SARs) of 3-enaminetetramic acids **1d**, derived by exchanging 3-acyl for 3-enamine functionality, is reported here. Interestingly, unlike 3-acyltetramic acids, 3-ETs are very rarely found in nature;⁸ fischerellin A (Figure 1) is one example and has antifungal and herbicidal activity.¹⁹ Moreover, the biological activity of unnatural 3-ETs has been rarely reported (examples include herbicidal and antifungal activities²⁰ and binding to tubulin²¹) while their antibiotic activity has not been reported, aside from some examples in our earlier work.¹⁵

All of the 3-ETs **2-6** (Figures 3-5) examined in this study were prepared via direct nucleophilic attack of the required primary or secondary amines onto the corresponding 3-acyltetramic acids **1d** (Scheme 1) whose synthesis has been reported previously.^{15,16,20,22} Two types of analogues were prepared in order to be able to directly compare bioactivity of enamines **1d** (Figure 1) with the parent 3-acyl **1a** and 3-carboxamido **1b** systems,

and to provide a range of hydrophobic, hydrophilic and functionalised side chains. In the first set, the moiety R^1 was fixed as a methyl group, while the amine substituent was held as a primary amine ($R^6 = H$) giving 3-ETs **2a-u** (Figure 3) with the R^5 group being varied across alkyl **2a-e**, substituted alkyl **2f-2q**, aryl **2r,s** and substituted aryl **2t,u**. In the second set, the alkyl moiety R^1 of **1d** was varied, giving monocyclic 3-ETs **3a-l**, **4a-k** (Figure 4) and bicyclic 3-ETs **6a-m** (Figure 5). R^1 was varied across a spectrum of aryl (**3a-c**, **6a**), alkyl (**3d-l**, **4a-c**, **4f-j**, **6b-i**), substituted alkyl (**4d-e,k**, **6j-m**) groups, while the amine substituent was again held as a primary amine ($R^6 = H$) but with the R^5 group also being varied across substituted alkyl, aryl and substituted aryl groups (Figures 4 and 5). In two cases, secondary amines were included (**3g**, **6d**). In the case of formation of 3-ET **5b**, by-product 3-ET **5** was also obtained, indicating that the *N*-acyl moiety can also be attacked by nucleophiles. 3-ETs **3i** and **3k** were synthesized from 3-ETs **3h** and **3j**, respectively, using diglycolic anhydride.

3-ETs **1g**, like the analogous 3-acyltetramic acid **1d** and 3-carboxamidotetramic acid **1f** systems, may exist as tautomeric forms A-D (Figure 6).^{15-17,22} In their 1H - and ^{13}C -NMR spectra, two clear sets of signals generally appeared as a 1:1 ratio, although for 3-ETs **3h**, **4e,i** and **6c,e,f,g,i** and *tert*-amine derivatives **2i**, **3g** and **6d**, the signals were broad (see Experimental Section in Supporting Information for details). In order to determine the relevant tautomeric form, HMBC NMR spectra of representative analogues were also determined and as an example, HMBC correlation of 3-ET **3d** is shown in Figure 7 (see S-Figure 1 in Supporting Information for other analogues). Similarly to previous literature reports,^{20,23} 3-ETs were found to exist as *exo*-enol tautomers B and D rather than *endo*-enol tautomers A and C. In the assignment of NMR spectra, chemical shifts of carbonyl carbons on C(2) and C(4) were key, with the expected chemical shifts of keto-carbonyls for tautomers B and D rather than enol-carbonyls for C(4) in tautomer A and C(2) in tautomer C. In 3-ET **3d**, the chemical shift of the carbon on C(4) (197.8 ppm) and the carbon on C(2) (172.9 ppm) were assigned to tautomer B (as compared to tautomer D, 193.3 and 176.7 ppm, respectively). In cases when there was only a single set of signals (e.g. *tert*-amines **2i**, **3g** and **6d**), the favoured *exo*-enol form B or D could not be assigned.

The antibacterial activity of 58 of 3-ETs **2-6** shown in Figures 3-5 was assessed (Table 1). The existence of activity against the Gram-negative *Haemophilus influenzae* (H3) and efflux-negative *Haemophilus influenzae* (H4) and Gram-positive *Staphylococcus aureus* (S1, S26, S4 and S2), *Enterococcus faecalis* (E1), *E. faecium* (E2) and *S. pneumonia* (P1 and P9) strains critically depended on the identity of the ring substituents R^1 , R^2 , R^5 and R^6 . Of interest is that 3-ETs **2-6** tended to be significantly weaker in activity than their corresponding analogues **1a-c**.¹⁵⁻¹⁷ By way of illustration, the magnitude of this activity loss can be seen by comparing compounds **2d** and **6g** with their analogues **7a,b** (Figure 8); both **2d** and **6g** are almost devoid of all activity, while **7a** has MIC values against all organisms in the assay panel not worse than 2 $\mu g/ml$ and **7b** is similar but has some MIC values as low as 0.5 $\mu g/ml$.¹⁵ The activities of enamines **2-6** indicates that the identity of the functionality at C(3) of **1d** is similarly crucial to the observance of antibiotic activity, and appears to be more important than N(1) and C(5)). Interestingly, the activity profile among Gram-positive strains decreases in the order *S. pneumonia* > *E. faecalis* and *E. faecium* >> *S. aureus*; in particular, 3-ETs **3e,g,h**, **4j** and **6e-g** are the

most active. 3-ET (\pm)-**2m** exhibited a broad spectrum of activity, especially against resistant and susceptible strains such as vancomycin susceptible (VSSA) and methicillin resistant (MRSA) *S. aureus*, vancomycin susceptible *E. faecalis* (VSE), vancomycin resistant *E. faecium* (VRE) and multi-drug resistant *S. pneumonia* (MDRSP). Moreover, none of 3-ETs **2-6** proved to be active against Gram-negative *Pseudomonas aeruginosa* and both efflux-positive and -negative *Escherichia coli* (data not shown in Table 1). It has been found that tetramic acids either without any pendant functional groups²⁴ or with mono-, di-alkyl^{25,26} or cyano²⁷ groups on C(3), have no or only weak antibiotic activity, while 3-acyl **1a** and 3-carboxamide tetramic acids **1b** generally exhibit excellent antibiotic activity.^{13-18,27} Unfortunately, the antibacterial activity of the few actives decreased in the presence of 2.5 % horse blood (P9B); a similar effect has been found for 3-acyl and 3-carboxamidetetramic acids.¹⁵⁻¹⁷ Additionally, 3-ETs **2a-c,e,h-j,r**, **3h** and **6a,b,d,e,i,l** were inactive against RNA polymerase ($IC_{50} > 100 \mu M$, although 3ETs **6j,h** exhibited mild RNA polymerase activity ($IC_{50} = 26.0 \mu M$) and 3-ETs **2a,c-e,j,r** were inactive against undecaprenyl pyrophosphate synthase (UPPS, $IC_{50} > 10 \mu M$), suggesting that the mode of action of active 3-ETs is different to 3-acyltetramic acids and 3-carboxamidetetramic acids, systems known to exhibit inhibition of RNA polymerase and UPPS.^{15,16}

Physicochemical properties of 3-enamines **2-6** were compared with 3-acyls **1a,c** and 3-carboxamides **1b**,¹⁵⁻¹⁷ for which the plots of ClogD_{7.4}, ClogP, polar surface area (PSA) and relative-PSA (rel-PSA = PSA/MSA X100) against molecular surface area (MSA) are shown in Figure 9 and S-Figures 2-4 in the Supporting Information, respectively (see also S-Table 1 in Supporting Information). Noteworthy is that almost all active 3-ETs are relatively lipophilic (ClogP > 3, ClogD_{7.4} > 2, rel-PSA < 10, MSA > 600), and since they have a high molecular weight (MW > 400 Da) and a larger number of rotatable bonds (RB > 10), they are already placed at a disadvantage for further optimization. By contrast, active 3-acyls **1a,c** are clustered at more polar values of ClogD_{7.4} and MSA (2-4 and 550-700 respectively); active 3-carboxamides **1b** are even more tightly clustered (-2-0 and 550-650 respectively) (Figure 9 and S-Figure 2). It is worth noting that most known antibacterial compounds are relatively polar, and this might account for the general lack of activity in the relatively lipophilic enamine compound class.²⁸

Overall, 3-ETs **2-6** were easily prepared from 3-acyltetramic acids **1a** and found to exist predominantly in the exo-enol form in solution. However, unlike the parent 3-acyl and 3-carboxamide TAs which exhibited excellent antibacterial activity with novel mode of actions as inhibitors of RNA polymerase and UPPS,¹⁵⁻¹⁷ the antibacterial activity of 3-ETs tended to be poorer and with unclear modes of action. This result suggests that the carbonyl oxygen in the 3-acyl **1a** and 3-carboxamide **1c** TA systems (Figure 2), giving systems with ClogP ≈ 4.5 , ClogD_{7.4} ≈ 3 , MSA ≈ 600 , rel-PSA > 10, and ClogP ≈ 1.5 , ClogD_{7.4} ≈ -1.0 , MSA ≈ 600 , rel-PSA > 10 respectively, are critical for sizeable antibacterial biological activity.¹⁵ Moreover, the parameters for the 3-carboxamide **1c** compound class more nearly overlap with those seen for other antibacterial agents, being relatively more polar, and may therefore be the most suitable for further optimisation.²⁸

Acknowledgements

We are particularly grateful for valuable input by Drs Phil Dudfield and John Lowther, and for funding by Galapagos SASU (France).

Supplementary Information

Supporting Information Available: Experimental detail, spectroscopic data, NMR spectra and Tables of Data.

References

1. Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2012**, *75*, 311.
2. Wetzel, S.; Bon, R. S.; Kumar, K.; Waldmann, H. *Angew. Chem. Int. Ed.* **2011**, *50*, 10800.
3. Danishefsky, S. *Nat. Prod. Rep.* **2010**, *27*, 1114.
4. Nicolaou, K. C.; Chen, J. S.; Edmonds, D. J.; Estrada, A. A. *Angew. Chem. Int. Ed.* **2009**, *48*, 660.
5. Zhao, H.; Cui, Z.; Gu, Y.; Liu, Y.; Wang, Q. *Pest Manag. Sci.* **2011**, *67*, 1059.
6. Schobert, R.; Schlenk, A. *Bioorg. Med. Chem.* **2008**, *16*, 4203.
7. Gänzle, M. G. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 326.
8. Royles, B. J. *Chem. Rev.* **1995**, *95*, 1981.
9. Kumar, R.; Subramani, R. Feussner, K.-D.; Aalbersberg, W. Aurantoside K, *Mar. Drugs* **2012**, *10*, 200.
10. Barnickel, B.; Bayliffe, F.; Diestel, R.; Kempf, K.; Laschat, S.; Pachali, S.; Sasse, F.; Schlenk, A.; Schobert, R. *Chem. Biodivers.* **2010**, *10*, 2830.
11. Yang, S.-W.; Mierzwa, R.; Terracciano, J.; Patel, M.; Gullo, V.; Wagner, N.; Baroudy, B.; Puar, M.; Chan, T.-M.; Chu, M. *J. Antibiot.* **2007**, *60*, 524.
12. Tuske, S.; Sarafianos, S. G.; Wang, X.; Hudson, B.; Sineva, E.; Mukhopadhyay, J.; Birktoft, J. J.; Leroy, O.; Ismail, S.; Clark, A. D.; Dharia, C.; Napoli, A.; Laptenko, O.; Lee, J.; Borukhov, S.; Ebright, R. H.; Arnold, E. *Cell* **2005**, *122*, 541.
13. Phillips, J.W.; Goetz, M. A.; Smith, S. K.; Zink, D. L.; Polishook, J.; Onishi, R.; Salowe, S.; Wiltsie, J.; Allocco, J.; Sigmund, J.; Dorso, K.; Lee, S.; Skwish, S.; de la Cruz, M.; Martin, J.; Vicente, F.; Genilloud, O.; Lu, J.; Painter, R. E.; Young, K.; Overbye, K.; Donald, R. G. K.; Singh, S. B. *Chem. Biol.* **2011**, *18*, 955.
14. Watanabe, T.; Igarashi, M.; Okajima, T.; Ishii, E.; Kino, H.; Hatano, M.; Sawa, R.; Umekita, M.; Kimura, T.; Okamoto, S.; Eguchi, Y.; Akamatsu, Y.; Utsumi, R. *Antimicrob. Agents Chemother.* **2012**, *56*, 3657.
15. Jeong, Y.-C.; Anwar, M.; Bikadi, Z.; Hazai, E.; Moloney, M. G. *Chem. Sci.* **2013**, *4*, 1008.
16. Jeong, Y.-C.; Moloney, M. G. "Antimicrobial compounds", UK Patent Application No 1211203.3; Isis Innovation Limited, PCT application (2012).
17. Jeong, Y.-C.; Moloney, M. G., *Beilstein J. Org. Chem.* 2013, *9*, 1899.
18. Peukert, S.; Sun, Y.; Zhang, R.; Hurley, B.; Sabio, M.; Shen, X.; Gray, C.; Dzink-Fox, J.; Tao, J.; Cebula, R.; Wattanasin, S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1840.
19. Hagmann, L.; Jüttner, F. Fischerellin, A. *Tetrahedron Lett.* **1996**, *37*, 6539.
20. Wang, X.-F.; Si, T.-F.; Li, Q.-B.; Zhu, Z.-Y.; Zhu, X.-J.; Qiang, S.; Yang, C.-L. *ARKIVOC* **2010**, *2*, 31.
21. Dorléans, A.; Gigant, B.; Ravelli, R. B. G.; Mailliet, P.; Mikol, V.; Knossow, M. *Proc. Nat. Acad. Sci. U.S.A.* **2009**, *106*, 13775.

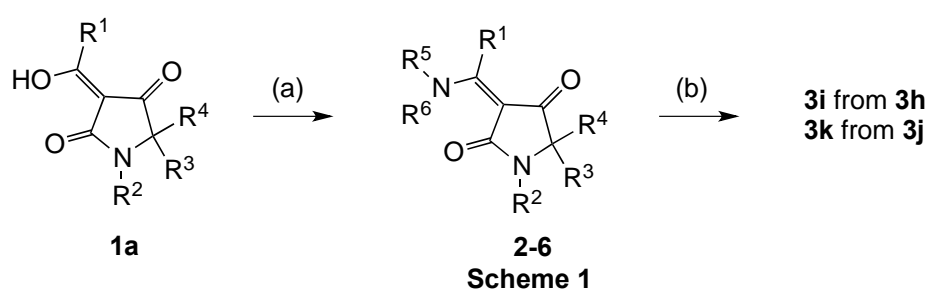
22. Jeong, Y.-C.; Moloney, M. G. *J. Org. Chem.* **2011**, 76, 1342.
23. Tietze, O.; Schiefner, B.; Ziemer, B.; Zschunke, A. *Fresenius J. Anal. Chem.* **1997**, 357, 477.
24. Jeong, Y.-C.; Moloney, M. G. *Synlett* **2009**, 2487.
25. Holloway, C. A.; Matthews, C. J.; Jeong, Y.-C.; Moloney, M. G.; Roberts, C. F.; Yaqoob, M. *Chem. Biol. Drug Des.* **2011**, 78, 229.
26. Moloney, M. G.; Yaqoob, M. *Synlett* **2008**, 2107.
27. Yendapally, R.; Hurdle, J. G.; Carson, E. I.; Lee, R. B.; Lee, R. E. *J. Med. Chem.* **2008**, 51, 1487.
28. O'Shea, R.; Moser, H. E. *J. Med. Chem.* **2008**, 51, 2871.
29. Fernandes, J.; Gattass, R. *J. Med. Chem.* **2009**, 52, 1214.

Table 1. *In vitro* antibiotic activity (MIC, µg/mL) of 3-enaminetetramic acids **2-6**.^{a-e}

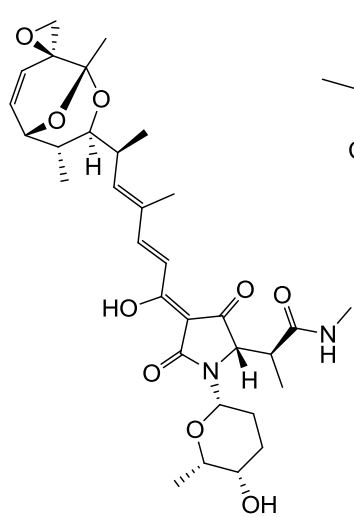
	S1	S26	S4	S2	E1	E2	P1	P9	P9B	H3	H4
2c^e	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	16
2d	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	16
2j	64	64	>64	64	64	8	2	1	2	>64	32
2m	8	4	8	8	4	1	1	1	2	4	<0.06
2p	- ^c	>64	>64	>64	>64	>64	>64	>64	>64	>64	32
2s	64	64	64	64	64	32	8	8	8	>64	8
3e	16	>64	16	64	16	8	4	4	32	>64	2
3g	16	16	16	16	16	8	>64	16	16	64	>64
3h	2	4	4	4	2	1	2	8	32	64	32
3i	>64	>64	>64	>64	16	16	8	16	64	>64	>64
3k	>64	>64	>64	>64	>64	>64	2	2	>64	>64	>64
3l	>64	>64	>64	>64	>64	>64	2	2	64	>64	>64
4a	>64	>64	>64	>64	>64	>64	64	64	64	>64	16
4b	>64	>64	>64	>64	>64	>64	16	16	32	>64	8
4c	64	64	64	64	64	64	32	32	32	>64	32
4h	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	4
4j	16	16	8	16	16	8	8	4	8	>64	8
5	>64	>64	>64	>64	>64	>64	>64	16	>64	>64	8
6b	>64	>64	>64	>64	>64	>64	>64	16	>64	>64	4
6e	16	16	16	16	16	8	4	4	16	>64	>64
6f	32	64	32	64	32	32	32	16	32	>64	16
6i	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	8
6k	32	16	16	32	32	16	16	8	16	>64	8
line^e	2	2	2	2	2	2	1	0.5	0.5	16	4
cipro^e	0.12	0.5	0.12	16	1	32	1	1	1	0.5	≤0.06

a; Abbreviation; **S1**; *S. aureus* 1, ATCC13709 *in vivo* (methicillin sensitive), **S26**; *S. aureus* 26, ATCC25923 (vancomycin susceptible), **S4**; *S. aureus* 4, Oxford, **S2**; *S. aureus* 2, MRSA *in vivo* (methicillin resistant), **E1**; *E. faecalis* 1, ATCC29212 VanS (vancomycin susceptible), **E2**; *E. faecium* 1, VanA (vancomycin resistant), **P1**; *S. pneumonia* 1, ATCC49619 (erythromycin susceptible), **P9**; *S. pneumonia* 9, PenR (penicillin and erythromycin resistant), **P9B**; *S. pneumonia* 9 in presence of 2.5 % horse blood, **H3**; *H. influenzae* 3, ATCC31517 MMSA, **H4**; *H. influenzae* 4, LS2 Efflux knock-out, **line**; linezolid, **cipro**; ciprofloxacin, b; All analogues are inactive against *E. coli* 1, ATCC25922 (non Pathogenic strain), *E. coli* 50, Ec49 No Efflux and *P. aeruginosa* 1, ATCC27853 (MIC > 32 µg/ml), c; Not determined. d; Analogues **2a,b,e-i,k,l,n,o,q,r,t,u**, **3a-d,f,j**, **4d-g,i,k**, and **6a,c,d,g,h,j,l** were inactive against all strains (MIC > 32

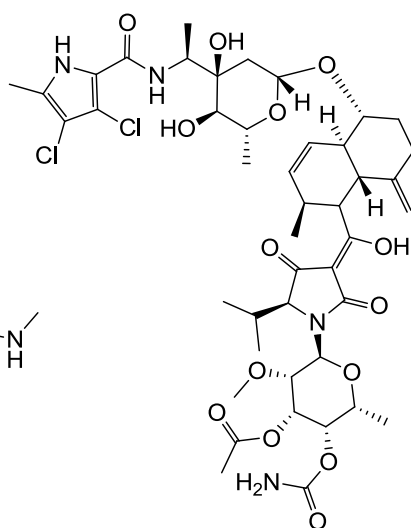
$\mu\text{g/ml}$), e; the activity of analogues **2a,c,d,r** was reported in our previous paper and is included here for comparison.¹⁵



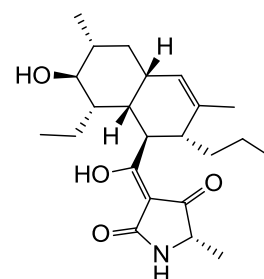
Scheme 1. Synthesis of 3-enaminetetramic acids; reaction conditions (a) NHR^5R^6 (1.1 eq), toluene, reflux; (b) diglycolic anhydride (1.1 eq), toluene, reflux.



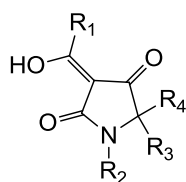
streptolydigin (1a)



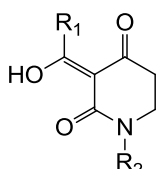
kibdelomycin (1b)



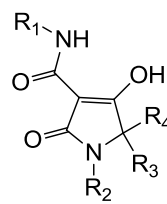
signermycin B (1c)



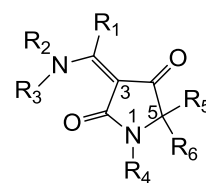
1d



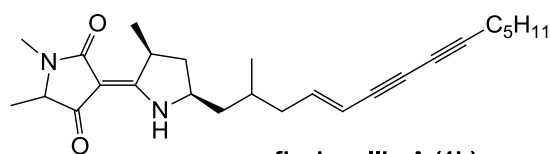
1e



1f

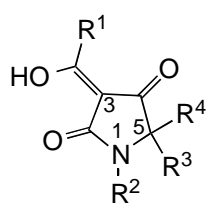


1g

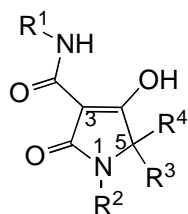


fischerellin A (1h)

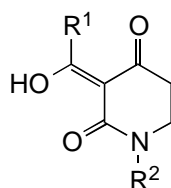
Figure 1.



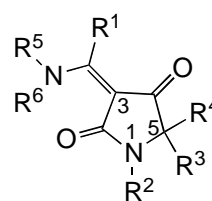
1a



1b



1c



1d

Figure 2

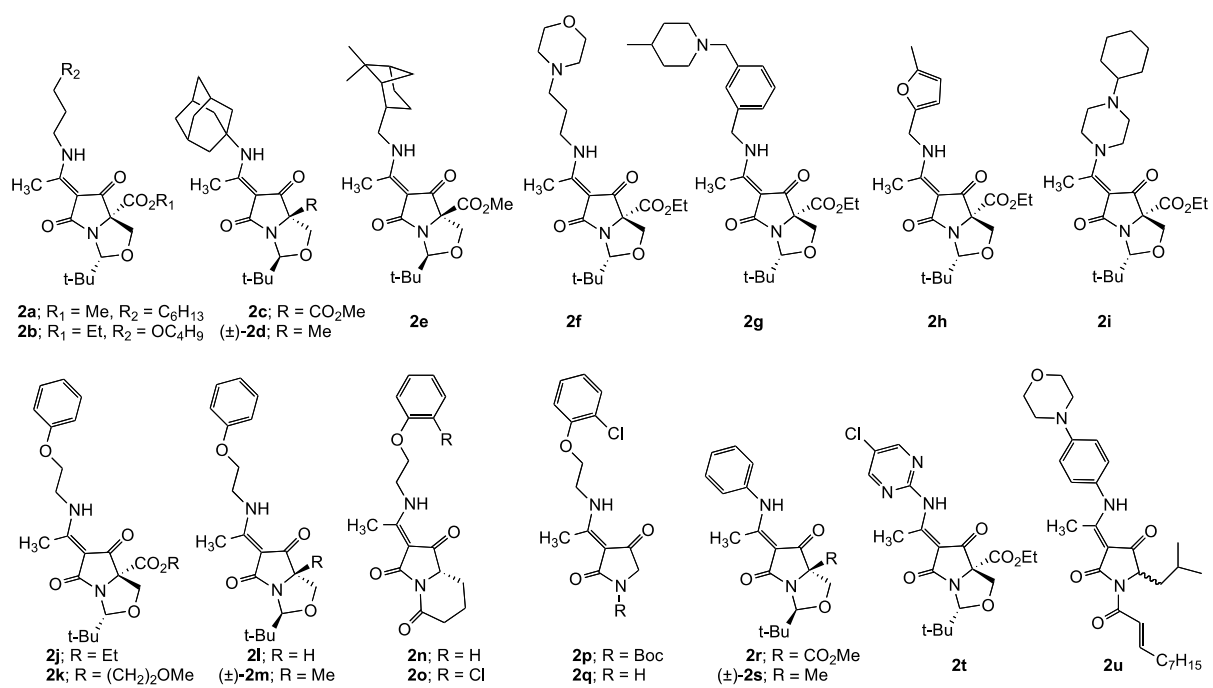


Figure 3. 3-Enaminetetramic acids (21 analogues)

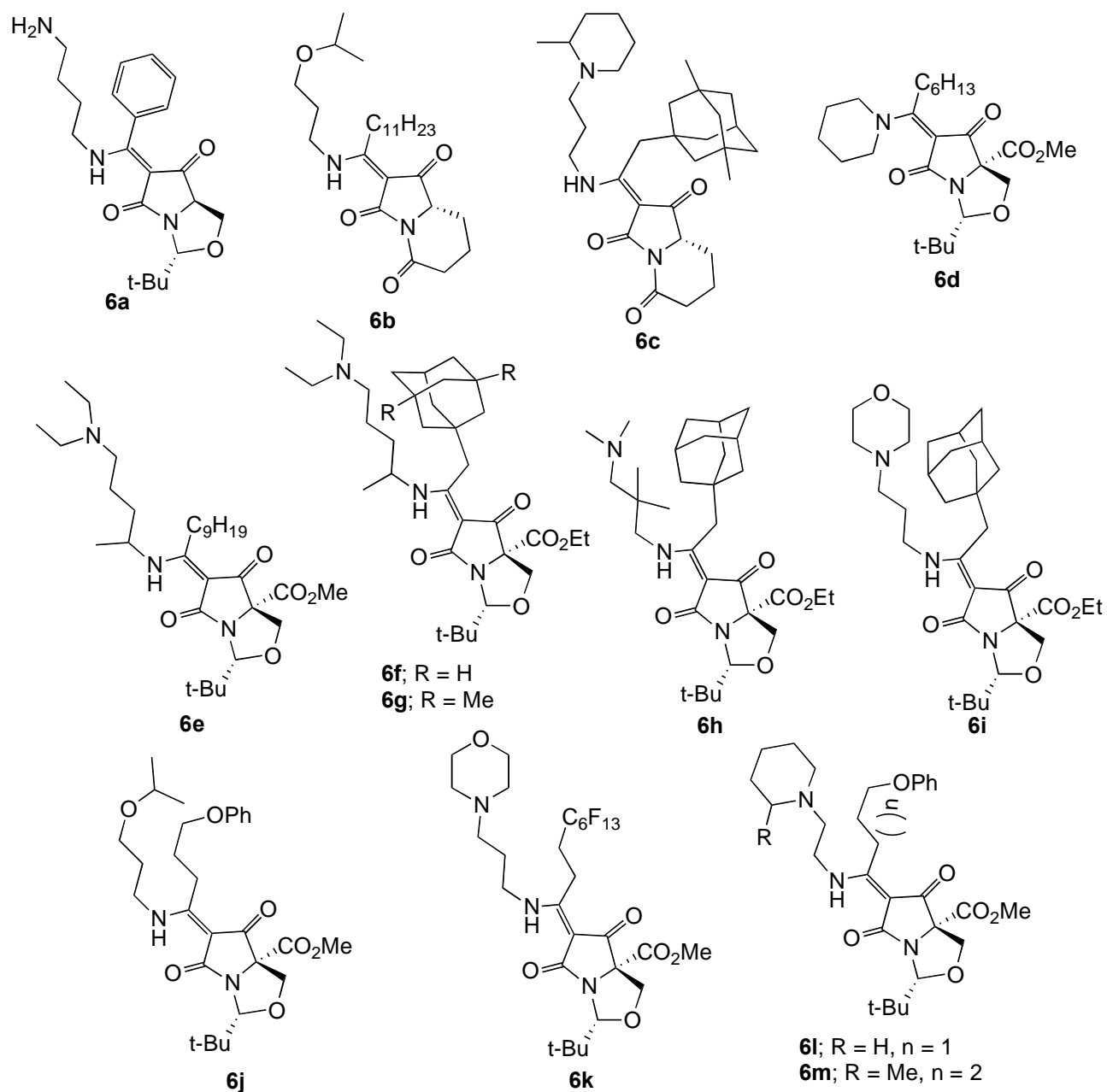


Figure 5

Figure 5. 3-Enaminetetramic acids (13 analogues)

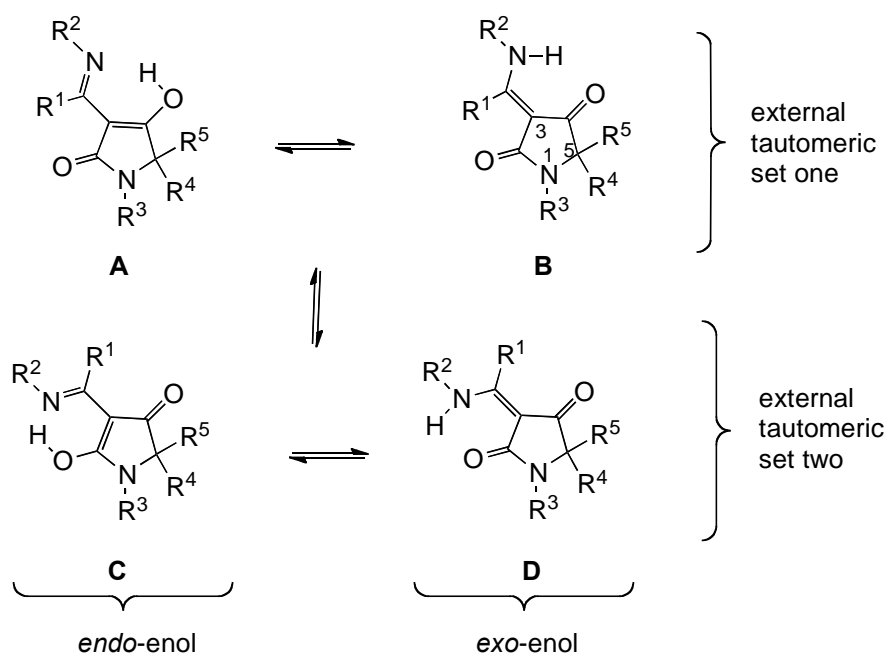


Figure 6. Tautomerism of 3-enaminetetramic acids

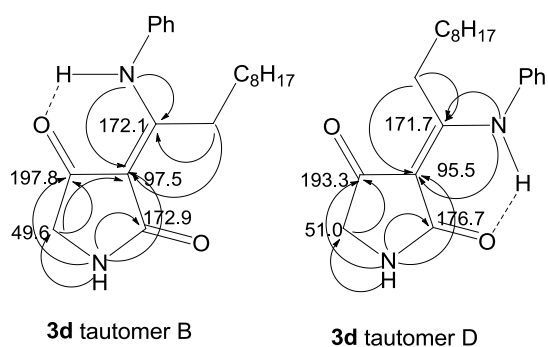


Figure 7. HMBC correlation of 3ET **3d** in CDCl_3

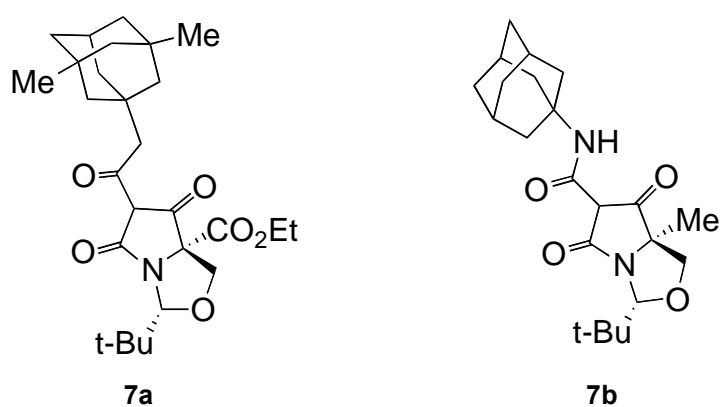


Figure 8

Figure 8. Examples of active acyl and amide analogues

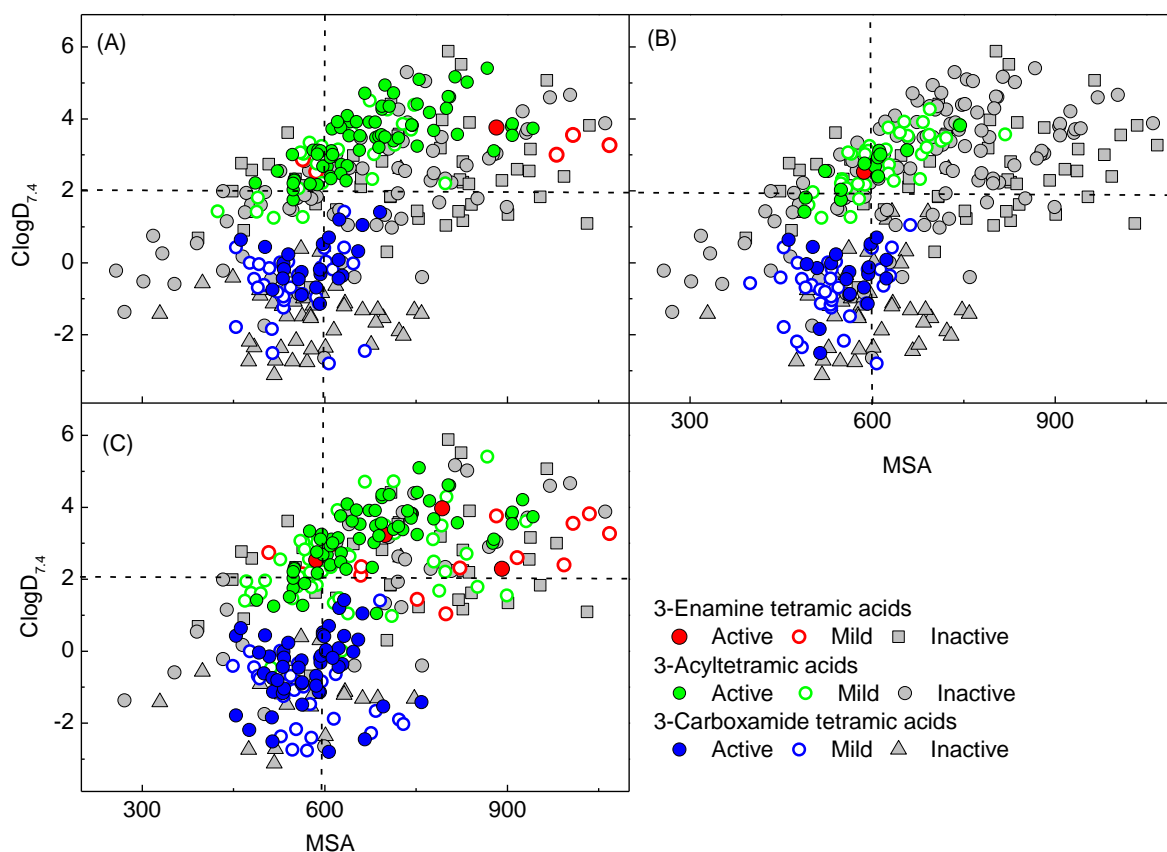


Figure 9. Plot of $\text{ClogD}_{7.4}$ against MSA of 3-enamines **2-6** along with the 3-acyls **1a,c** and 3-carboxamides **1b** reported in our previous papers against (A) MRSA, (B) *H. influenzae* 3 and (C) efflux-negative *H. influenzae* 4. Active, mild and inactive mean that the values are $\text{MIC} \leq 4 \mu\text{g/mL}$, $4 \mu\text{g/mL} < \text{MIC} \leq 32 \mu\text{g/mL}$ and $\text{MIC} > 32 \mu\text{g/mL}$, respectively.

Figure(s)

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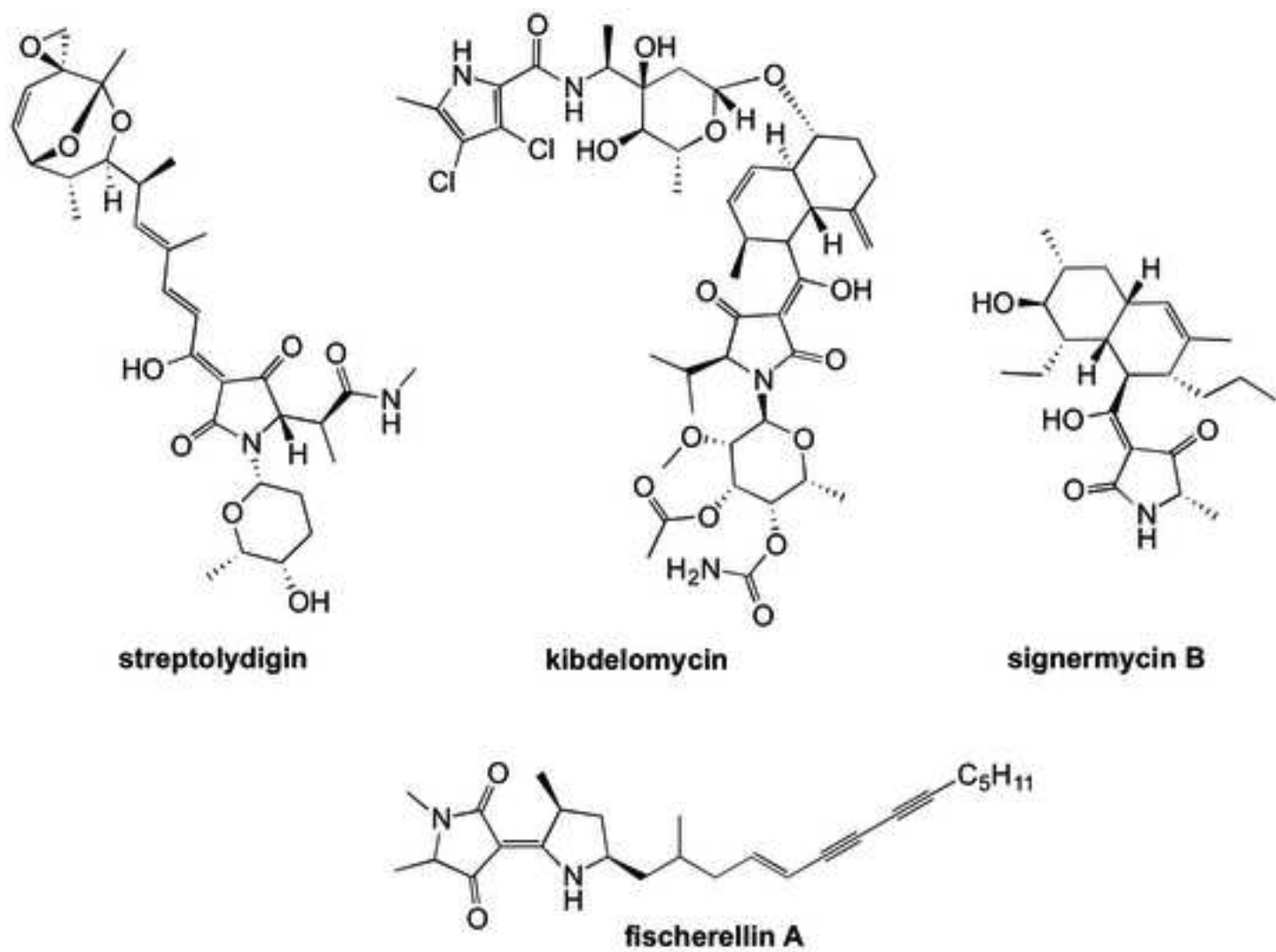


Figure 1

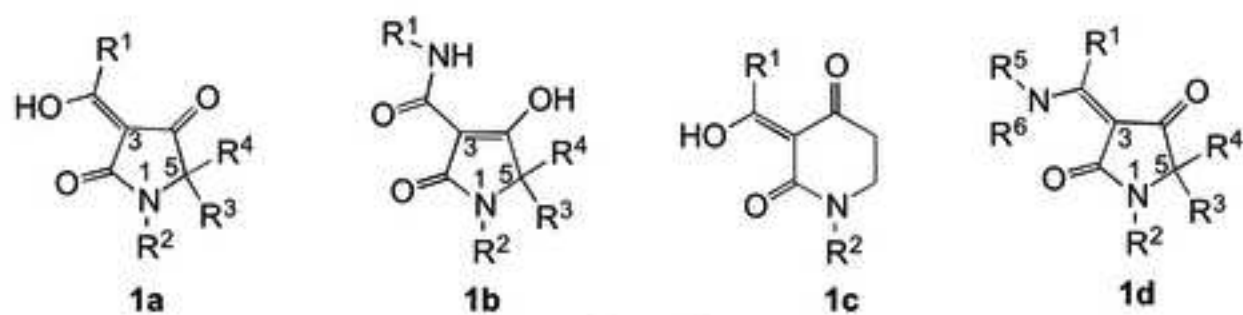


Figure 2

Figure(s)

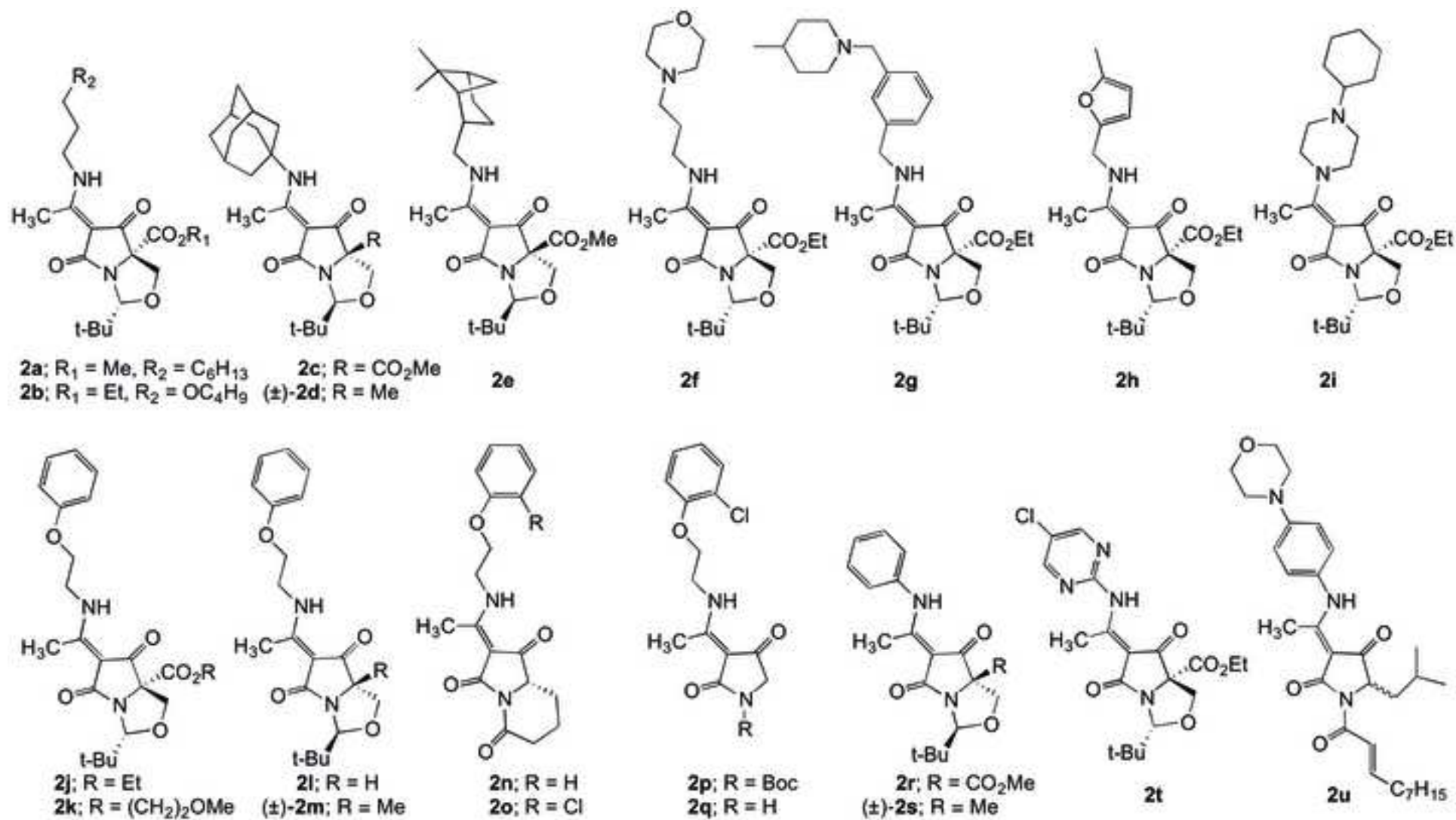
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Figure 3

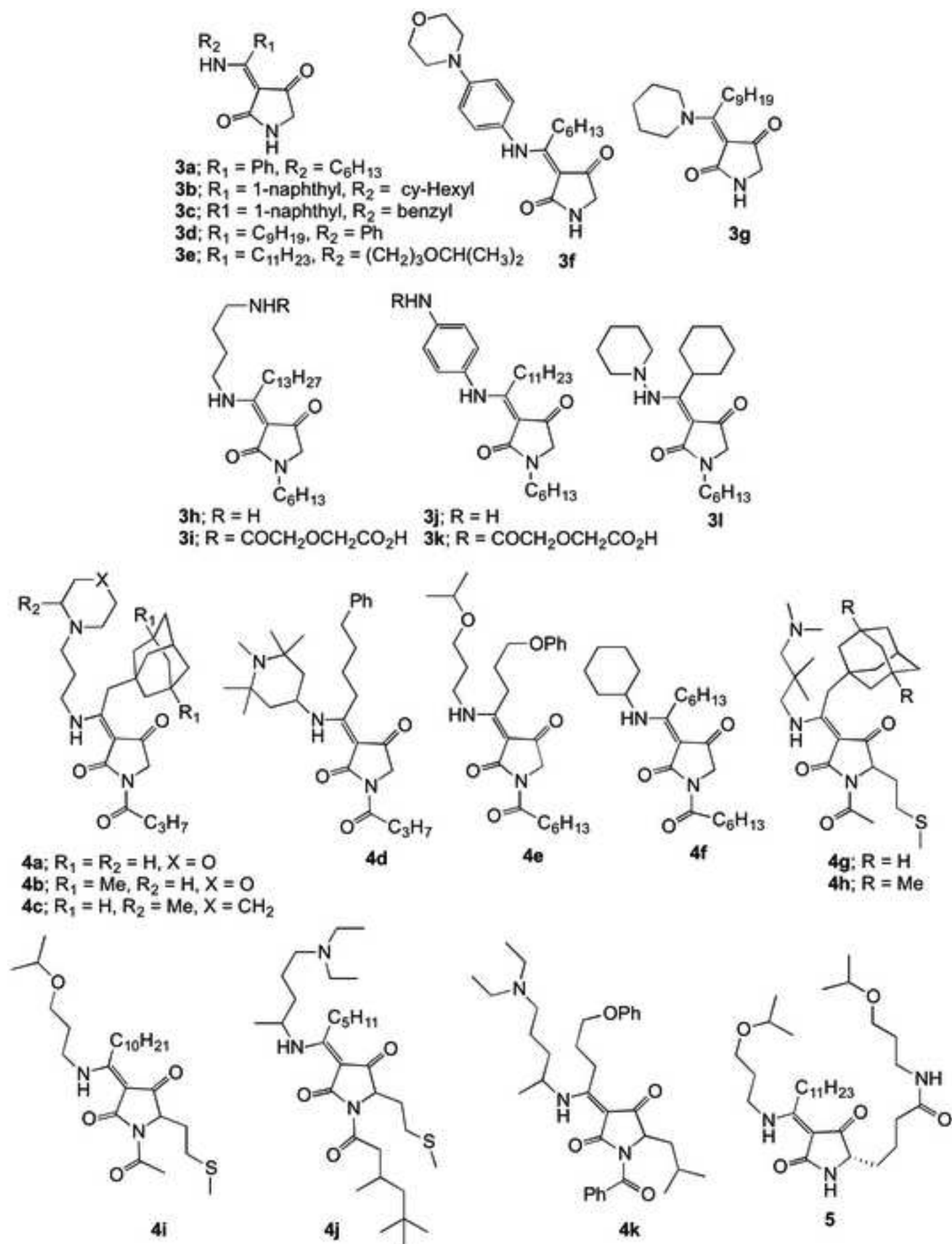


Figure 4

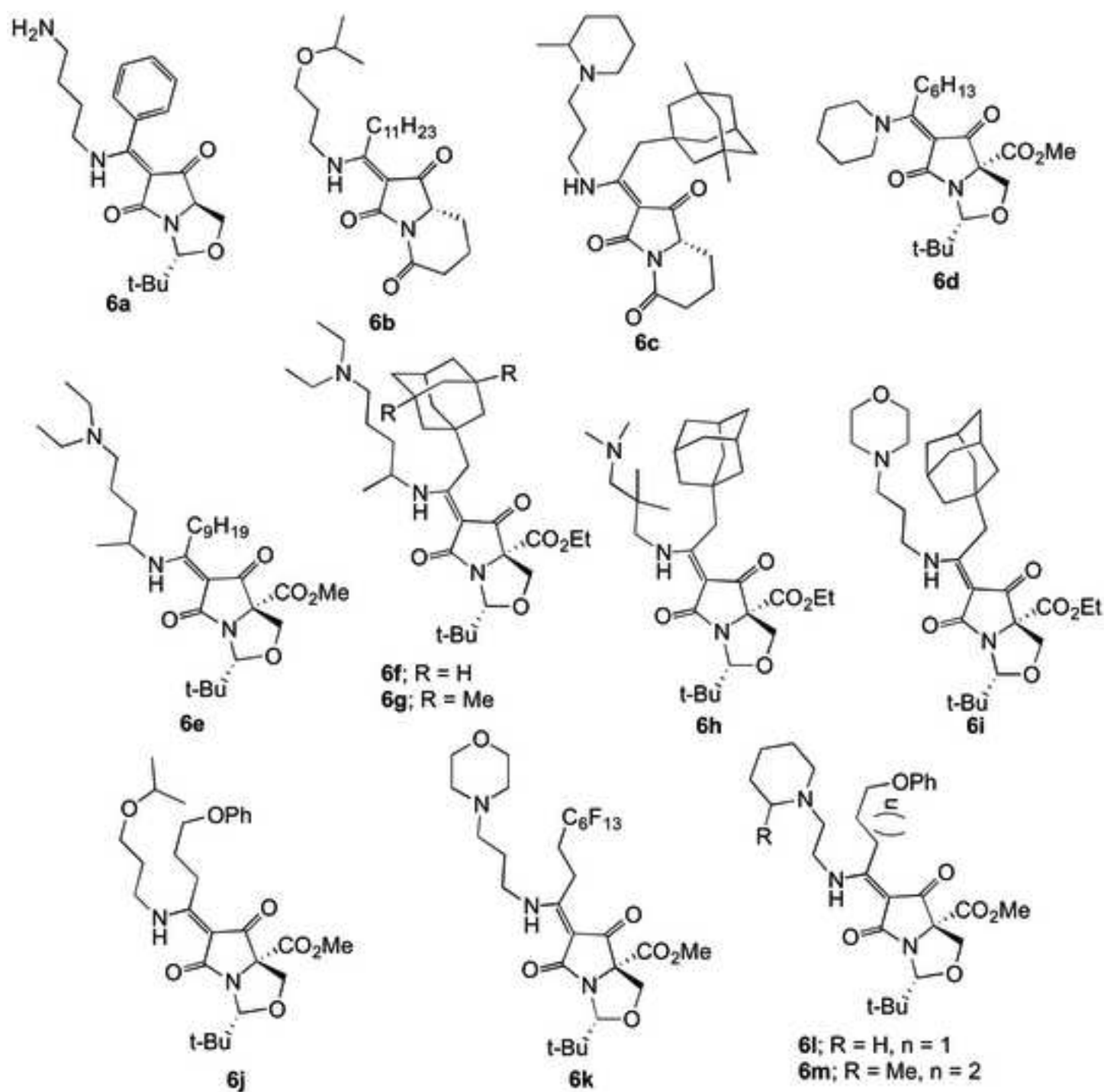


Figure 5

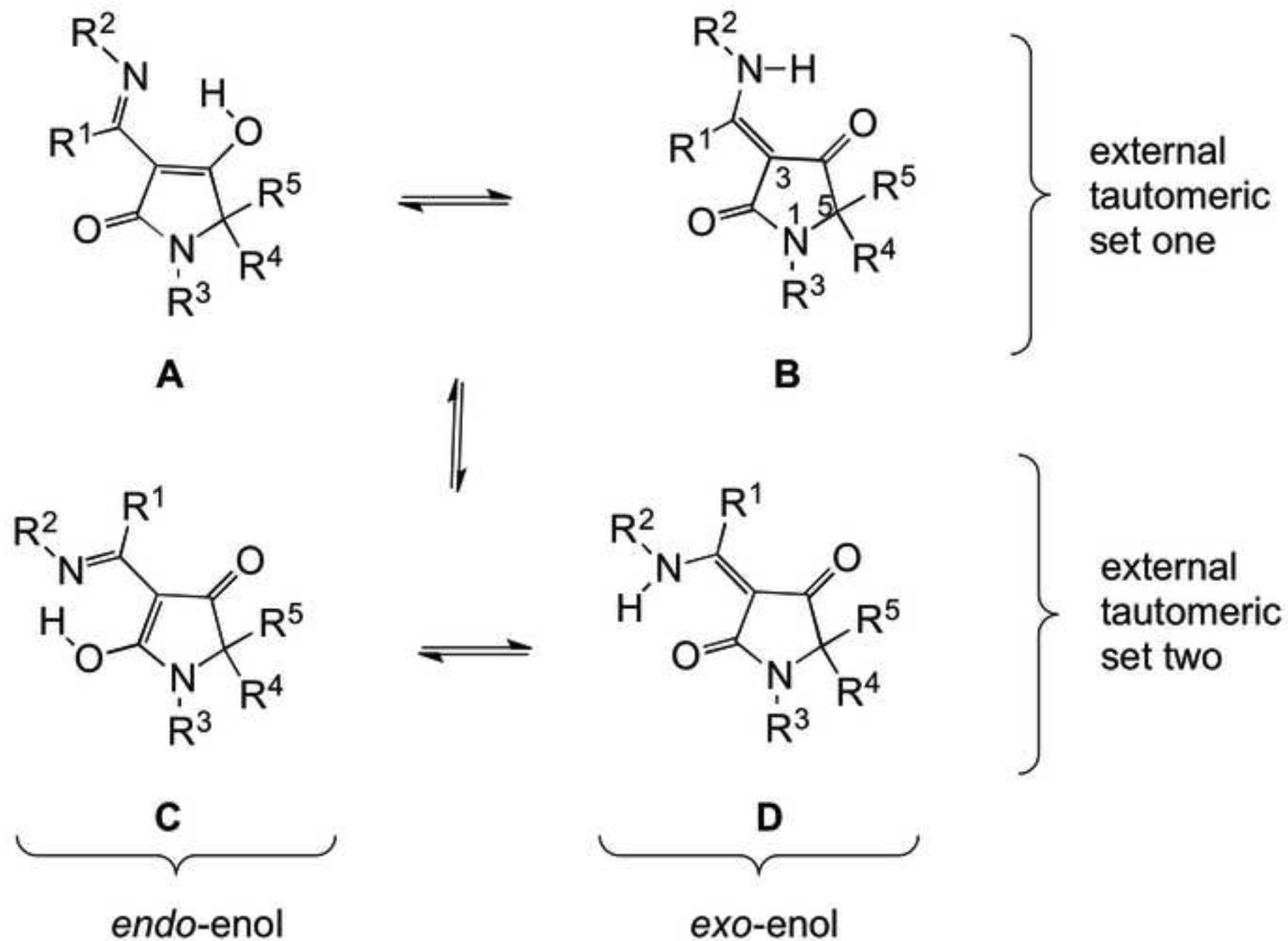


Figure 6

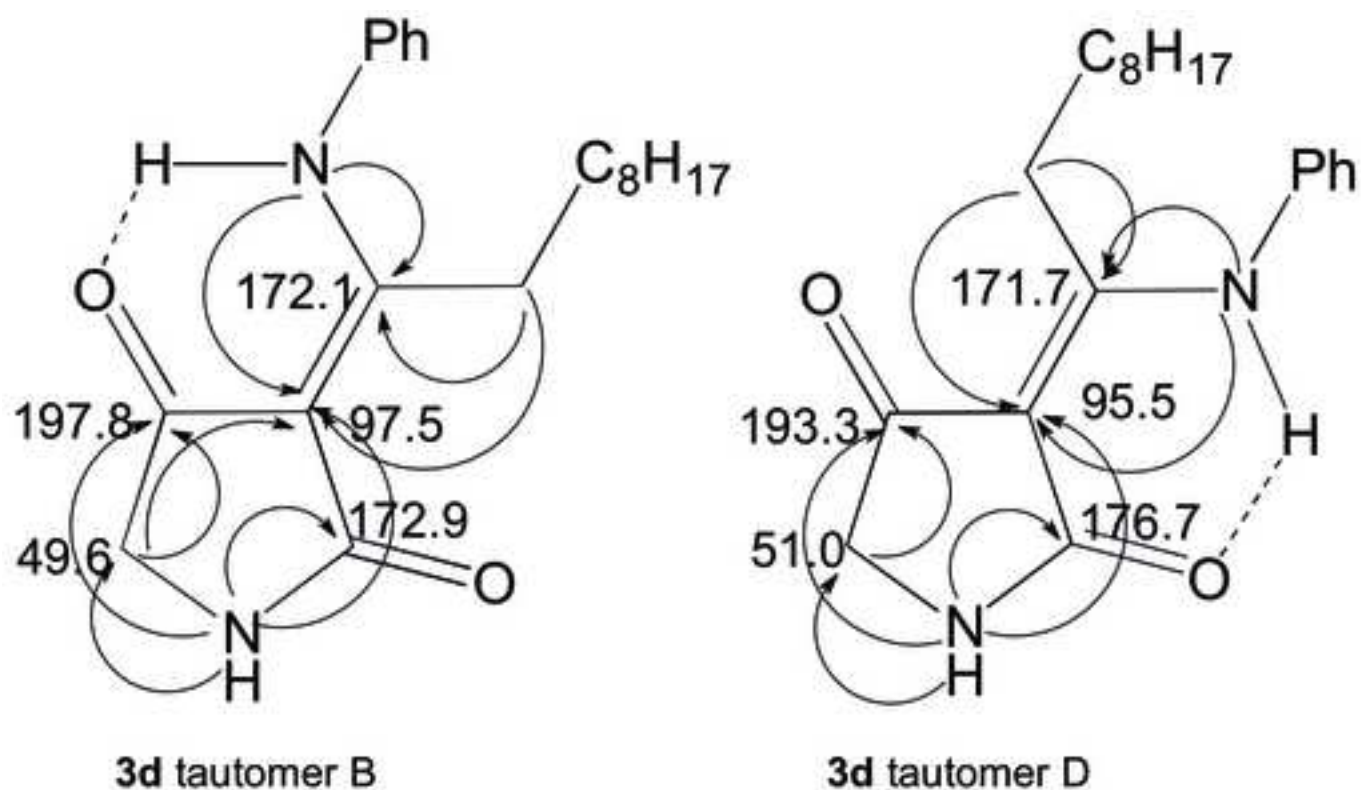


Figure 7

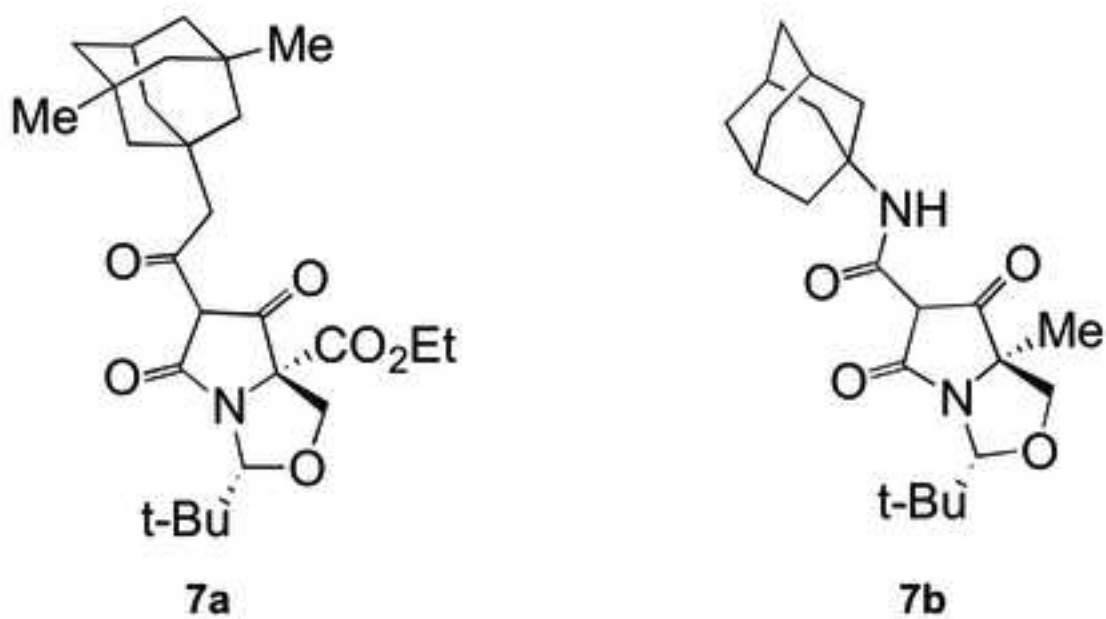
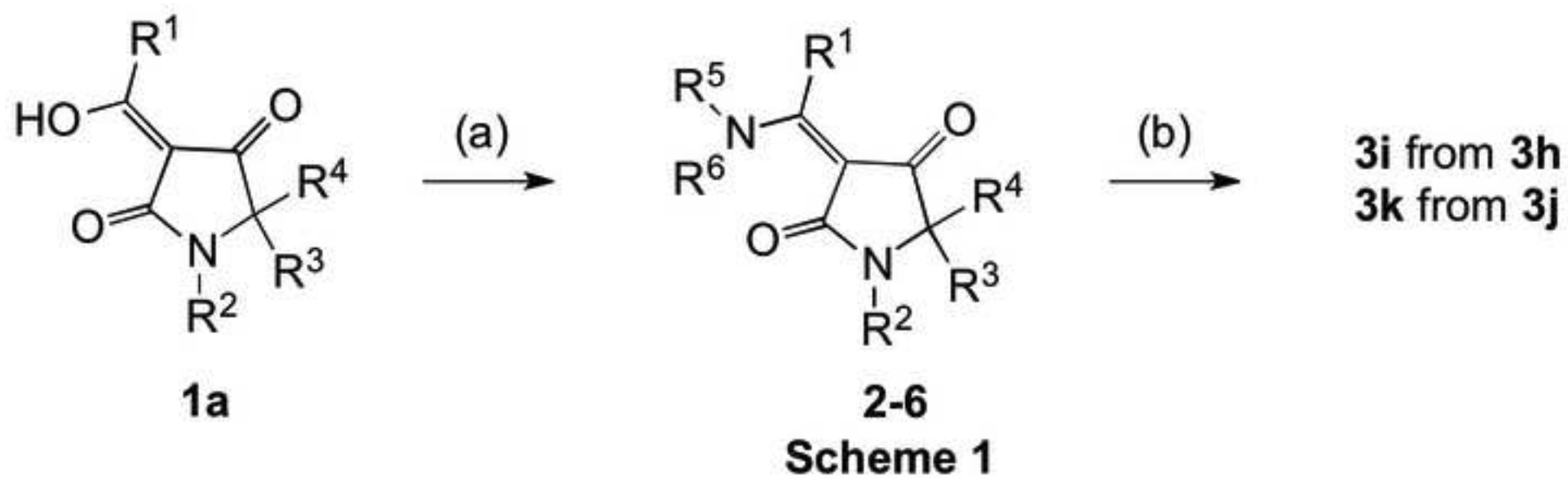


Figure 8



Supplementary Material
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Supplementary Material

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