A Detailed Study of Antibacterial 3-Acyltetramic Acids and 3-Acylpiperidine-2,4-diones

ARTICLE *in* CHEMMEDCHEM · MAY 2014

Impact Factor: 2.97 · DOI: 10.1002/cmdc.201402093 · Source: PubMed

4 AUTHORS, INCLUDING:

Mark G Moloney
University of Oxford
191 PUBLICATIONS **2,250** CITATIONS

Bioorganic & Medicinal Chemistry Letters 24 (2014) 1901–1906

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

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

Chemistry Research Laboratory, University of Oxford, Mansfield Rd, Oxford OX1 3TA, UK

ARTICLE INFO

Article history:
Received 20 January 2014
Revised 5 March 2014
Accepted 6 March 2014
Available online 14 March 2014

Keywords:
Tetramate
Antibacterial
Enamine

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.

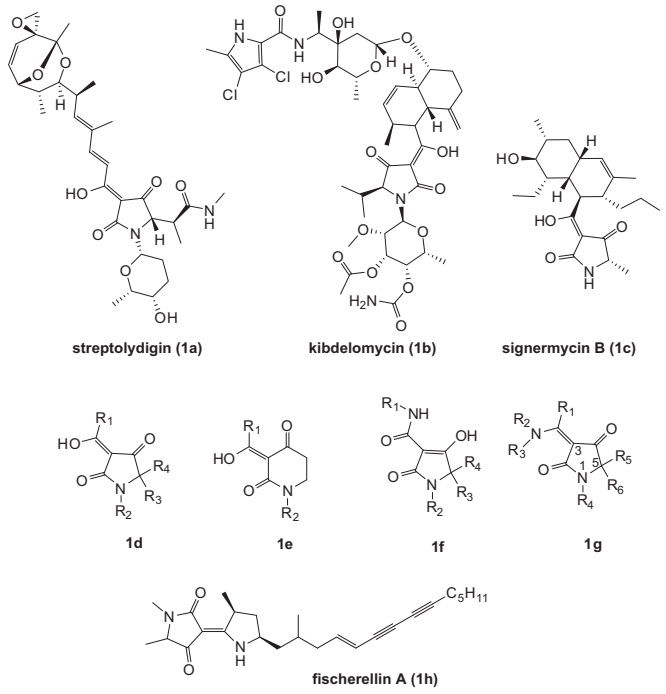


Figure 1. Tetramate-containing natural products.

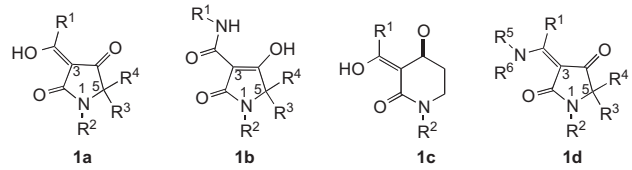


Figure 2. 3-Acyltetramic acid 1a, 3-carboxamidotetramic acid 1b, piperidine-2,4-dione one 1c and 3-enaminetetramic acid 1d skeletons.

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 (Fig. 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 (Fig. 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 (Fig. 1) is one

* Corresponding author. Tel.: +44 1865 275656.
E-mail address: mark.moloney@chem.ox.ac.uk (M.G. Moloney).

http://dx.doi.org/10.1016/j.bmcl.2014.03.013
0960-894X/

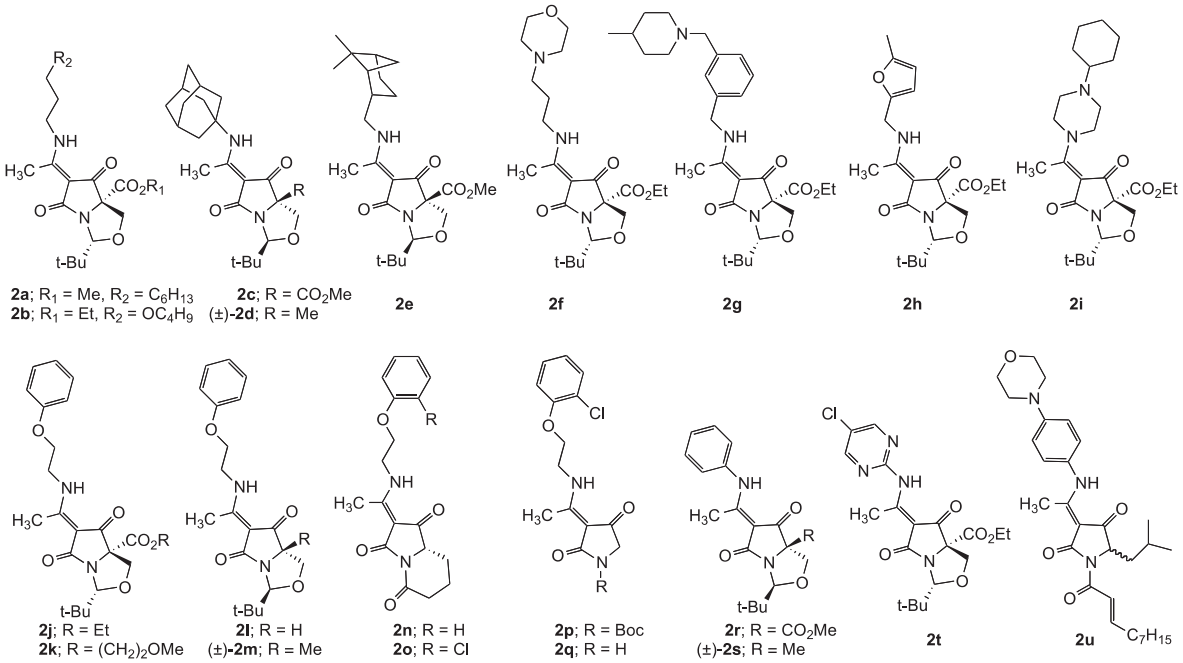


Figure 3. 3-Enaminetetramic acids (21 analogues).

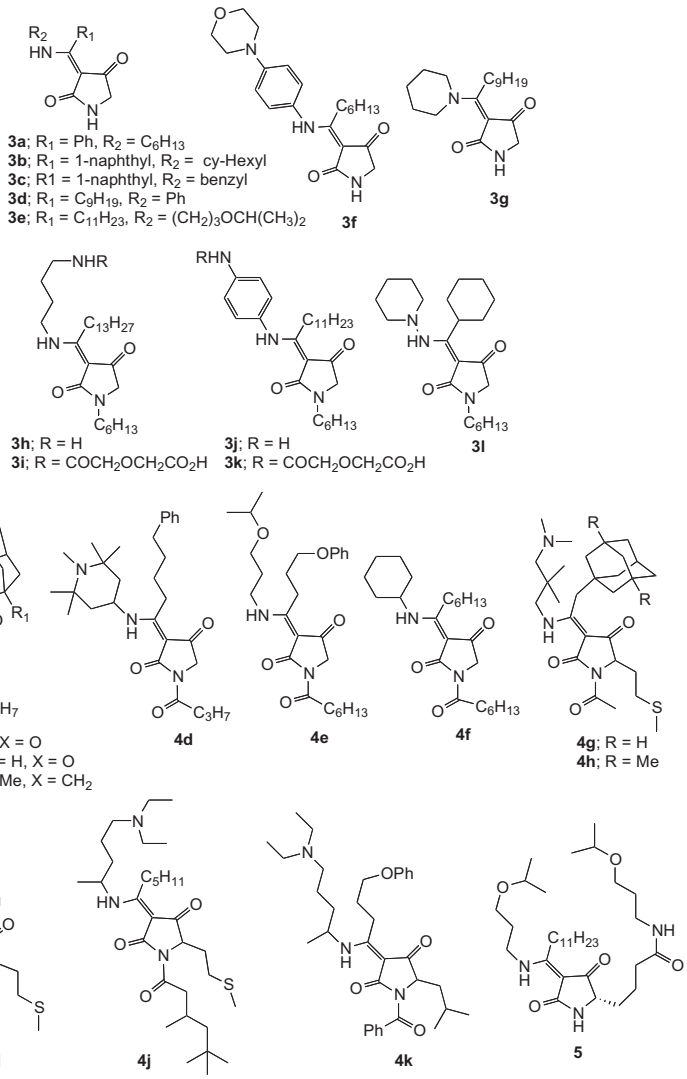


Figure 4. 3-Enaminetetramic acids (24 analogues).

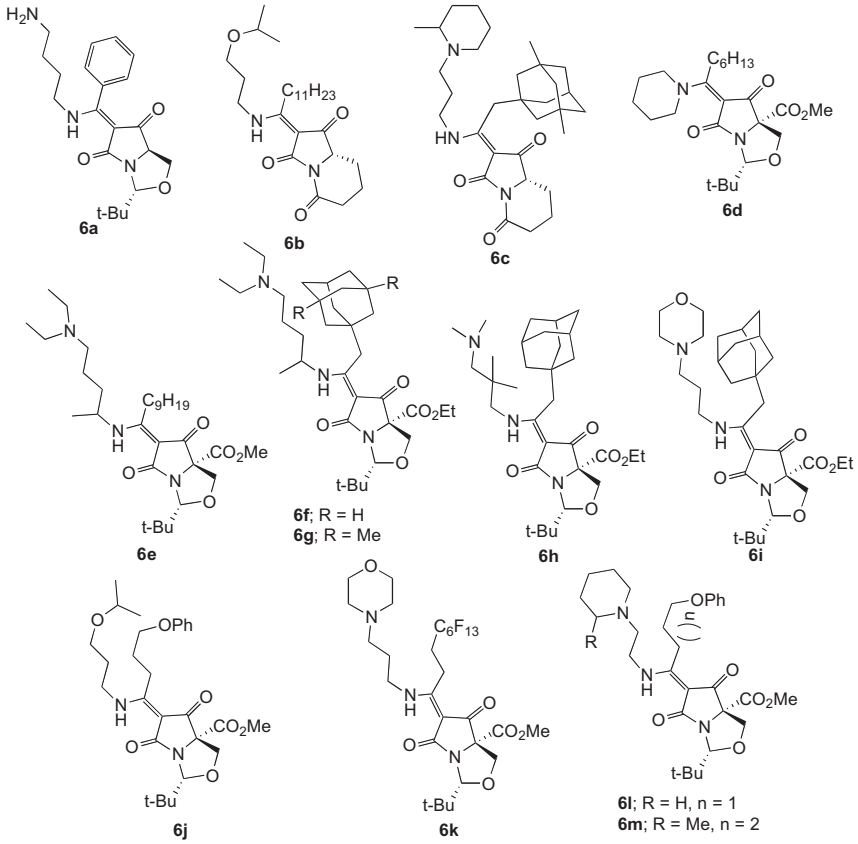
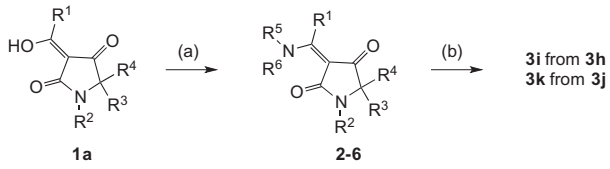


Figure 5. 3-Enaminetetramic acids (13 analogues).



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

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 activities[20] and binding to tubulin[21]) while their antibiotic activity has not been reported, aside from some examples in our earlier work.[15]

All of the 3-ETs **2**–**6** (Figs. 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** (Fig. 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** (Fig. 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** (Fig. 4) and bicyclic 3-ETs **6a**–**m** (Fig. 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

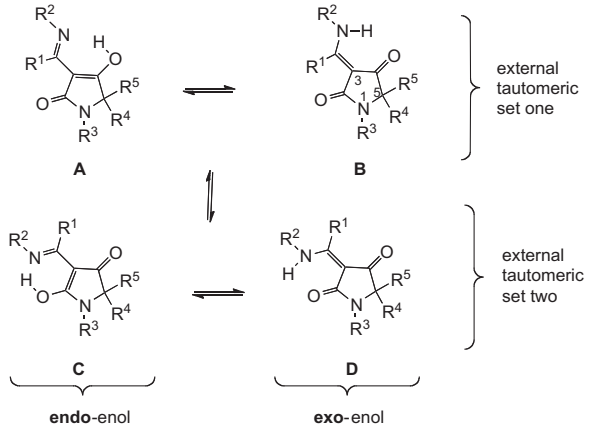


Figure 6. Tautomerism of 3-enaminetetramic acids.

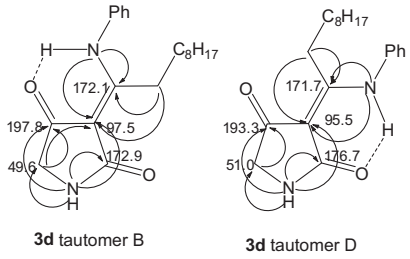


Figure 7. HMBC correlation of 3ET **3d** in $CDCl_3$.

(R^6 = H) but with the R^5 group also being varied across substituted alkyl, aryl and substituted aryl groups (Figs. 4 and 5). In two cases,

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 μg/ml).
[e] The activity of analogues **2a**,**c**,**d**,**r** was reported in our previous paper and is included here for comparison.[15]

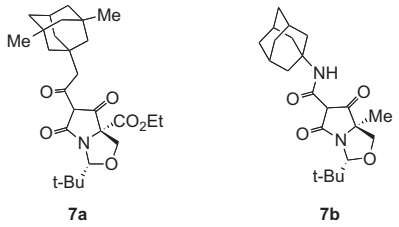


Figure 8. Examples of active acyl and amide analogues.

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 (Fig. 6).[15–17,22] In their ^{1}H 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 Fig. 7 (see S-Fig. 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** (Fig. 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 μg/ml and **7b** is similar but has some MIC values as low as 0.5 μg/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 (±)-**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

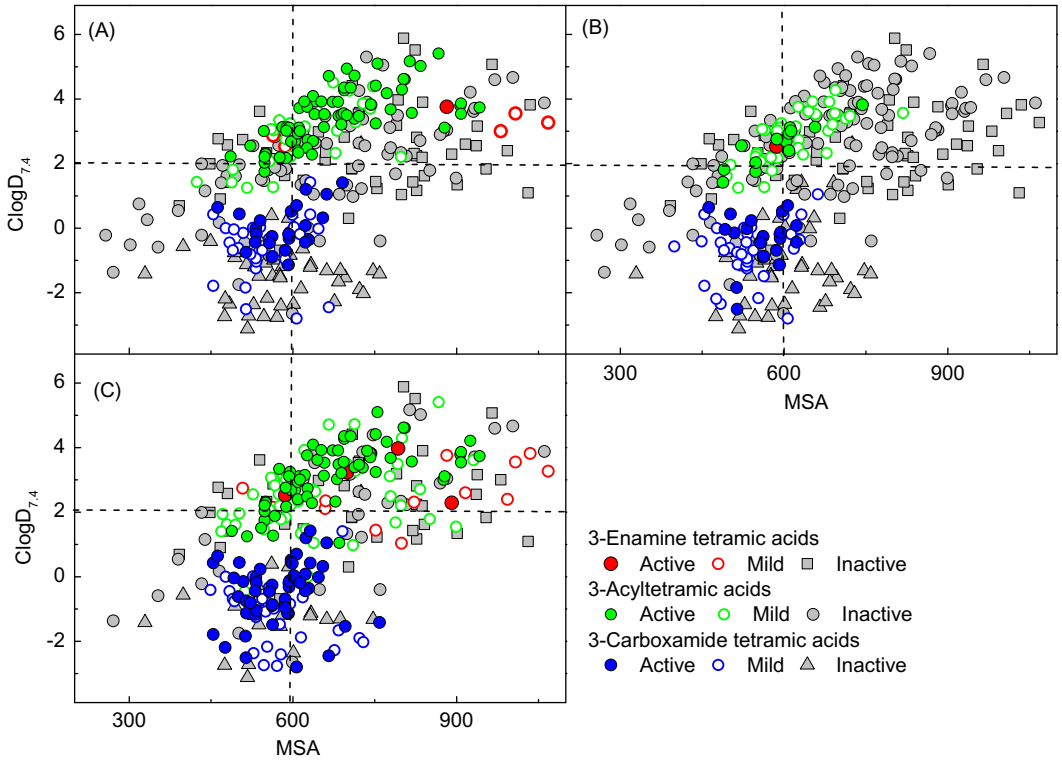


Figure 9. Plot of $C\log D_{7.4}$ against MSA of 3-enamines **2**–**6** along with the 3-acyls **1a**,**c** and 3-carboxamides **1b** reported in our previous papers against (A) MRSA, (B) *H. influenzae 3* and (C) efflux-negative *H. influenzae 4*. Active, mild and inactive mean that the values are MIC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

been found that tetramic acids either without any pendant functional groups[24] or with mono-, di-alkyl[25,26] or cyano[27] 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 μM, although 3ETs **6j**,**h** exhibited mild RNA polymerase activity (IC_{50} = 26.0 μM) and 3-ETs **2a**,**c**–**e**,**j**,**r** were inactive against undecaprenyl pyrophosphate synthase (UPPS, IC_{50} > 10 μM), suggesting that the mode of action of active 3-ETs is different to 3-acyltetramic acids and 3-carboxamidotetramic 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 $C\log D_{7.4}$, 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 ($C\log P$ > 3, $C\log D_{7.4}$>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 $C\log D_{7.4}$ and MSA (2–4 and 550–700, respectively); active 3-carboxamides **1b** are even more tightly clustered (−2–0 and 550–650, respectively) (Fig. 9 and S-Fig. 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 (Fig. 2), giving systems with $C\log P \approx 4.5$, $C\log D_{7.4} \approx 3$, MSA ≈ 600, rel-PSA > 10, and $C\log P \approx 1.5$, $C\log D_{7.4} \approx -1.0$, 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]

Acknowledgments

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

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.03.013.

References and notes

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.; 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.