

EQUISETIN, REUTERICYCLIN AND STREPTOLODYGIN AS NATURAL PRODUCT LEAD STRUCTURES FOR NOVEL ANTIBIOTIC LIBRARIES

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Abstract

The emergence of antimicrobial resistance has created a need for the development of novel antibacterial therapies to treat infection. Natural products which exhibit antibacterial activity offer validated starting points for library generation, and we report here that small molecule mimics of tetramate-containing natural products may show antibacterial activity and offer the potential for further optimisation.

Introduction

There has been a realisation that the antibacterial drug pipeline has begun to run dry [1-3] and that commercially-driven models for drug development within the pharmaceutical industry which have been hitherto highly successful are unlikely to serve so well for new antibacterial drug development in the twenty-first century.[4-8] This, coupled with the emergence of antibacterial resistance,[9-11] has begun to compromise modern medical practice, and a number of government reports have highlighted the urgency, but also the scientific, technological, commercial and societal challenges, of responding to this threat.[4, 12-14] Faced with this need for action, there has been discussion of the most appropriate methodological approach to be deployed[15-21] especially in a resource-limited environment. It has also been recognised that, unlike many other therapeutic areas, there are challenges peculiar to antibacterial drug discovery[22-27] which mitigate against success. Historically, natural products have provided a very important start point in antibacterial therapies and drug discovery[15, 28-34] and of interest is that antibacterially active natural products appear to occupy an unusual chemical property space,[22, 35, 36] offering excellent opportunity for exploitation for drug development.[37] We have become interested in tetramate-containing natural products,[38, 39] many of which have been known for some time, including for example equisetin[40], reutericyclin[41] and streptolodygin[42], but which still continue to be discovered, e.g. kibdelomycin (Figure 1).[43] These exhibit a range of biological activities, importantly including antibacterial properties, for which some, e.g. reutericyclin, vermispurin, equisetin, BU4514N, delaminomycin C, PF1052, oxasetin, and vancoresmycin (Figure 2), are quite active. Inspired by this precedent, small molecule mimics **1-3** of such systems have been reported, and of interest is that these too show antibacterial activity (Figure 3).[44-47] In a statistical analysis, we have shown that, in general, (a) Gram-negative antibacterial agents tend to be more charged than the Gram-positive only agents, presumably to facilitate penetration of the outer membrane via porins as well as prevent efflux, (b) most antibiotics, with the exception of topical agents, tend to

have a higher limit for lipophilicity (ClogD7.4 <2.0 and ClogP <3.0) and a lower limit for polar surface area (PSA >60 Å² and rel-PSA >13%); ~~and (c) Gram-negative antibacterial agents tend to be more charged than the Gram-positive only agents, presumably to penetrate the outer membrane via porins as well as prevent efflux.~~[48]. Against this backdrop, it was of interest to examine small highly acidic tetramate systems, with the aim to introduce several points of diversity into the synthesis (Figure 4) and we report details of this work here.

Discussion

Our first requirement was to establish direct but versatile protocols for tetramate synthesis, and this was done by optimisation of literature approaches;[49] elaboration of a suitably protected glycine with either mono-ethylmalonate or Meldrum's acid gave the corresponding tetramates **4-6** in a short high-yielding sequence. Significantly, however, in order to achieve closer mimics to the natural products, we needed to be able to C-acylate the tetramate core to give derivatives **7,8**, and this was most effectively achieved using either acetone cyanohydrin or an O-acylation-rearrangement sequence which we recently reported (Scheme Figure 54).[50, 51] The latter approach proved to be very general, enabling the ready introduction of a wide variety of aromatic, heteroaromatic, and alkyl carbonyl side chains, and provided access to a library of monocyclic tetramate derivatives (the TA, HTA, CTA, PTA, RTA and BTA series), with variation of substitution at 2-3 points around the heterocyclic ring (Figure 65).[52-55] These libraries were readily structurally characterised using NMR and MS techniques. Of interest is that complex keto-enol behaviour was frequently observed in these compounds, in which different species preponderated, principally depending on the nature of the side chain and the identity of the *N*-substituent. All Thus, *N*-H and *N*-alkyl forms favour exo-enol form CD, but *N*-acyl 3-acyltetramates favour forms AB and CD approximately equally, although for those with β-heteroatom functionality on the 3-acyl unit, pair AB becomes even more preferred, and is sometimes exclusively formed (Figure 76). In order to extend the series, the 3-carboxethoxy and 3-carboxamido series (ETA and ATA series) were also prepared.[52,55] To our surprise, initial antibacterial assay by hole-plate analysis against *S. aureus* and *E. coli* showed activity for a substantial subset of the library, although of interest was that O-acyl derivatives were universally inactive (Table 1). This activity was dose dependent, and in one case superior to the Cephalosporin C control (Figure 87). This outcome compared favourably with structurally similar compounds reported previously.[45, 46]

Table 1: Antibacterial activity of selected compounds from Figure 65.

Entry	Compounds	Minimum inhibition concentration (µg/ml) ^a		
		<i>Staphylococcus aureus</i> D267	<i>Escherichia coli</i> X580	
			Neutral pH	pH 4.5 buffer
1	TA-PH	>4000	4000	800

2	TA-NA	2000	2000	1000
3	TA-CY	800	800	400
4	TA-C6	400	200	20
5	TA-OPH	>4000	800	-
6	TA-ONA	>4000	400	-
7	TA-OCY	>4000	800	-
8	TA-OC6	>4000	800	-
9	Cephalosporin C ^c	200	2	-

a: Determined by disc diffusion method [57]; b: Cephalosporin C was used as the standard

Follow up analysis by [Minimum Inhibitory Concentration \(MIC\)](#) evaluation against a panel of Gram positive and negative bacteria (Gram-positive bacteria including *Staphylococcus aureus* (methicillin sensitive, vancomycin susceptible, non-resistant and methicillin-resistant *in vivo*, MRSA), *Enterococcus faecalis* (vancomycin susceptible, VSE), *E. faecium* (vancomycin resistant, VRE) and *S. pneumonia* (erythromycin susceptible and multi drug resistant, MDRSP) as well as Gram-negative bacteria including *Pseudomonas aeruginosa*, *Escherichia coli* (efflux-positive Ec50 and -negative Ec49) and *Haemophilus influenzae* (efflux-positive and -negative) [using broth assay](#) verified these initial assessments, and showed strong activity against Gram positive but lower efficacy against Gram negative bacteria.[48, 52-55] Cheminformatic analysis [with-comparing](#) a range of parameters [and comparison](#)—to bioactivity was instructive; of interest was to investigate the role of hydrophobicity, steric and electronic effects using the descriptors MSA, PSA, %PSA and clogP. Thus, it was evident that the most active Gram positives clustered around ClogP and MSA values of 4 ± 0.5 and 670 ± 30 , [respectively](#), while the most active Gram negatives had narrower ClogP and MSA values around 2.5 ± 0.5 and 530 ± 50 , [respectively](#) (Figure [98A,B,D,E](#)). This difference in behaviour was reflected in rel PSA (PSA/MSA x 100) values for each of the most active Gram positive and Gram negative systems, with values of $12 \pm 2\%$ and $14 \pm 1\%$, respectively (Figure [98C,F](#)). When grouped by chemical structure, the most active against *S. aureus* ATCC 25923 were the RTA and CTA systems, interestingly with similar preference for ClogP (≈ 3.5), MSA (650), PSA (78) and rel-PSA (12%) (Figure [109](#)). The most active compounds (RTA-C3, RTA-C5, CTA-NR, TA-C11, CTA-C6 and CTA-C9) occupied a narrow region of property space defined by ClogP, MSA and rel-PSA (Figure [110](#) and Table 2).

Table 2: Active compounds and their cheminformatic descriptors.

Compounds	MIC (µg/ml, <i>S. aureus</i>)	ClogP	MSA	rel PSA
Most active: CTA-C9, RTA-C3 & RTA-C5	0.125-0.5	3.6-4.47	631-693	11.2-12.3
Active: TA-C11, CTA-NR & CTA-C6	0.5-2	2.19-2.98	483-545	14.2-14.3

Selectivity between organisms was examined for each of the main compound classes (Figure 124). Noteworthy is that for the Gram positive organisms, SA25923 and SA13709, the identity of the *N*-substituent was very important, with *N*-acyl being much more active than *N*-H, which in turn was more active than *N*-alkyl and shorter chain *N*-acyl (Figure 124A-C). However, this pattern altered for *E. faecalis*, *E. faecium*, and *S. pneumonia* (Figure 124D-F), although the *N*-H system was universally at the lower end of activity. This offers the possibility of the design of species-specific antibacterial tetramate systems. SAR may be summarised as in Figure 4213, in which the C-acyl substituent has a positive effect on antibacterial activity for longer chain length, and enhanced activity is seen in cases with an *N*-acyl substituent over *N*-alkyl or *N*-H, or a C-2 alkyl substituent. α,β -Unsaturated *N*-acyl groups are preferred, while shorter chain lengths at this position are less effective (Figure 132). Of interest is that the headline MIC values for representative systems compare favourably in activity with other lead structures of interest in antibacterial drug discovery (Figure 143).

Extension of this approach from five membered to six membered rings as also examined, since similar behaviour might have been expected (Scheme 2Figure 15). These systems 9-11 were readily accessed by analogous chemistry, and of interest is that many showed high levels of activity.[48]

It might be argued that this approach has merely identified systems by chance, but related small polar molecules, not possessing the highly enolised tetramate core, do not show activity. Thus, 1,5,5-trimethylimidazolidine-2,4-dione systems were examined with a range of *N*-substitution (Figure 164); these systems are arguably structurally similar to the tetramates, but do not possess the highly enolisable tetramate core, and were found to possess only very low activity. This outcome is consistent with the fact that the enolisable tetramate system is necessary but not sufficient for bioactivity. On the other hand, not all systems modelled on natural products show antibacterial activity: pyroglutaminols 11 and 12 modelled on pramanicin 13 were devoid of activity, with the exception of the phenylacetylenyl epoxidesystem, which was weakly active against *S. aureus* (Figure 17).[56]

This phenotypic approach using natural product lead structures to inform library design contrasts with a target-based approach, but nonetheless has led to the identification of novel templates suitable for elaboration to antibacterially active compound libraries. A key benefit of this approach is that bacterial cell wall permeability is automatically built in to active library

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members, and does not need retro-fitting as would be required in a target driven approach. Further investigation has shown that tetramates may exhibit RNA polymerise, undecaprenyl pyrophosphate synthase, topoisomerase or gyrase inhibitory activity, and that some exhibit dual modes of action, although for others no mode of action has yet been identified. It is hoped that future work will permit optimisation against these targets, will enhance biological activity, and provide the identification of suitable lead candidates.

Conclusion

We have shown that antibacterially active natural products provide a useful start point for the identification of novel new chemical entities, suitable for investigation as antibacterial agents, and that modified tetramates in particular offer unusual systems with potential for further exploitation in antibacterial drug development.

Acknowledgements

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

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Key terms:

antibacterial resistance: the loss of susceptibility of bacteria to the lethal effects of antibacterial agents

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Cheminformatic analysis: the use of computational analysis to characterise and uncover trends in the structures of molecules and their bioactivity

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phenotypic approach: drug design based upon the development of molecules with whole cell or whole organism effects

target-based approach: drug design based upon the development of molecules which act against a known molecular (often an enzyme) target

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lipophilicity: the solubility of a compound in non-polar solvents such as hexane or toluene, fats, oils and lipids.

Future perspective: The development of effective antibacterial therapies over the last century must surely rank as one of the major achievements of humankind, and modern medical practice assumes their ready availability. Over the last decade in particular, however, the emergence of resistant and virulent bacterial strains has come to threaten the viability of such treatment, and therefore even of modern society itself. There is now an urgent need to identify and develop new strategies for finding new therapies, along with new therapies themselves, but society would now seem to be badly underprepared for this challenge. We have attempted to show in our work that a return to natural product chemistry – which has been a crucial element in antibacterial development over the last century, but more recently been forgotten - offers interesting and worthwhile opportunities. Tetramate systems in particular seem worthy of more detailed examination, although whether they prove to be a new “magic bullet” will remain to be seen.

Executive Summary:

- The emergence of antimicrobial resistance has created a need for the development of novel antibacterial therapies to treat infection.
- Natural products which exhibit antibacterial activity offer validated starting points for library generation.
- Small molecule mimics of tetramate-containing natural products may be readily prepared with a wide range of chemical diversity.
- Tetramate libraries show high levels of antibacterial activity against a panel of Gram positive and Gram-negative bacteria.
- This strategy is sufficiently general that there is significant potential for further optimisation.

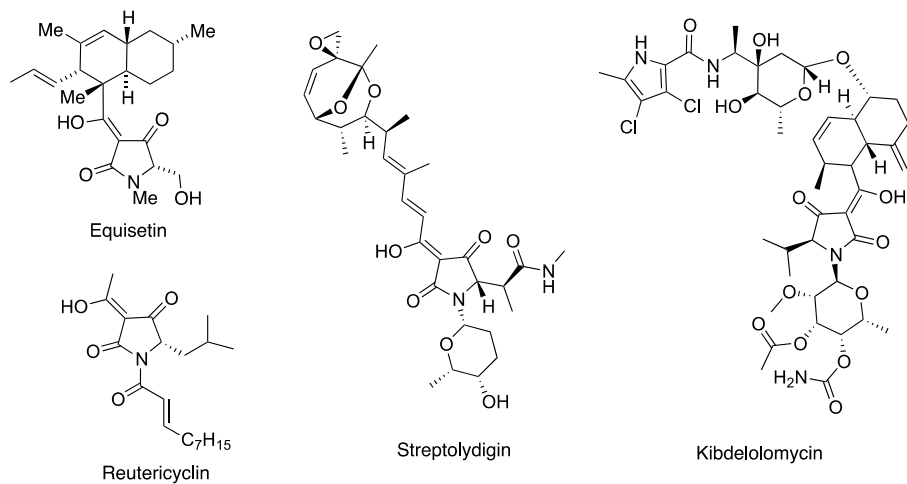


Figure 1

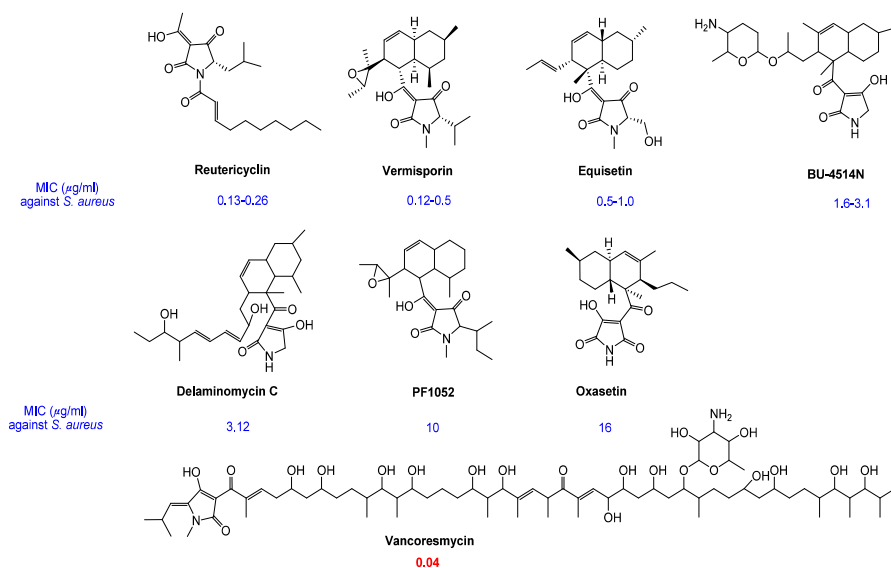


Figure 2

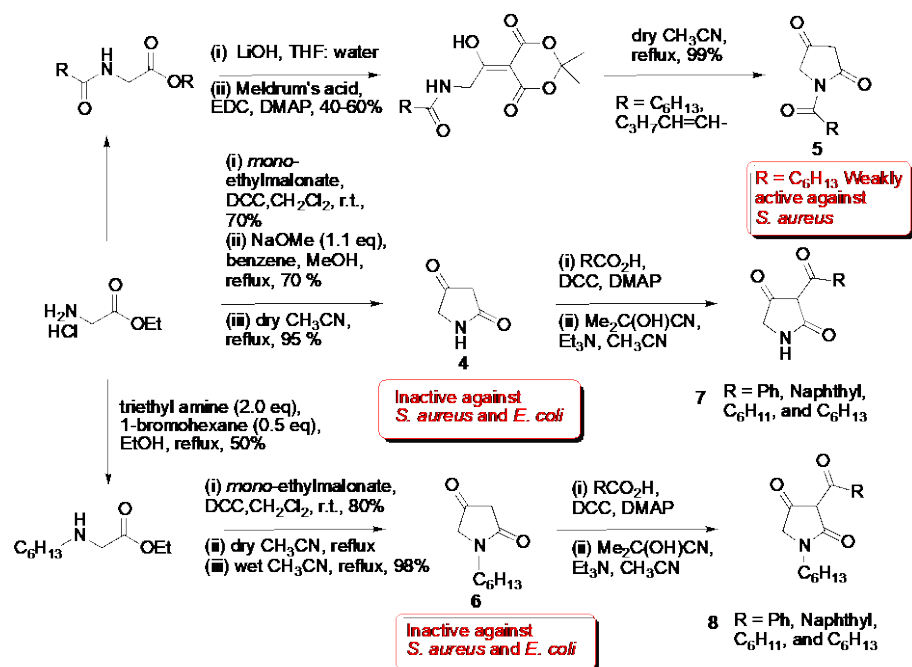
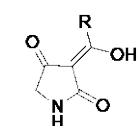
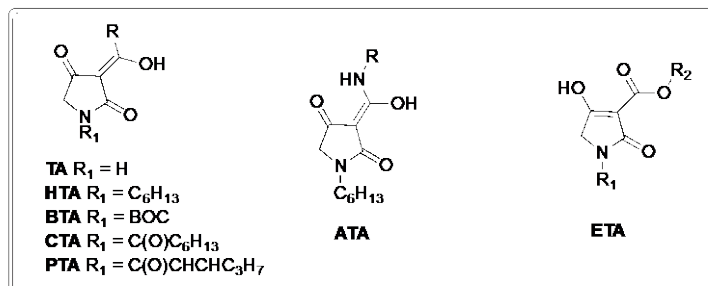
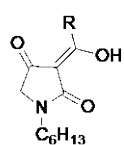


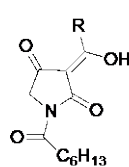
Figure 5



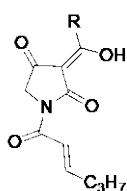
TA-PH; $R = Ph$
TA-NA; $R = 1\text{-Naphthyl}$
TA-CY; $R = \text{cy-Hexyl}$
TA-C6; $R = C_6H_{13}$
TA-C9; $R = C_9H_{19}$
TA-C11; $R = C_{11}H_{23}$
TA-C13; $R = C_{13}H_{27}$



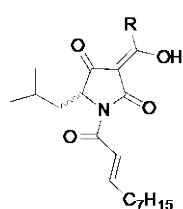
HTA-CY; $R = \text{cy-Hexyl}$
HTA-C6; $R = C_6H_{13}$
HTA-C9; $R = C_9H_{19}$
HTA-C11; $R = C_{11}H_{23}$
HTA-C13; $R = C_{13}H_{27}$



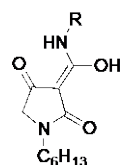
CTA-C6; $R = C_6H_{13}$
CTA-C9; $R = C_9H_{19}$
CTA-C11; $R = C_{11}H_{23}$
CTA-C13; $R = C_{13}H_{27}$
CTA-OE; $R = CH_2(O(CH_2)_2)_2OCH_3$
CTA-NR; $R =$



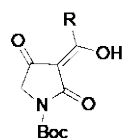
PTA-C6; $R = C_6H_{13}$
PTA-C9; $R = C_9H_{19}$
PTA-C11; $R = C_{11}H_{23}$
PTA-C13; $R = C_{13}H_{27}$



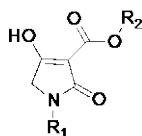
RTA-C1; $R = CH_3$
 (racemic Reutericyclin)
RTA-C3; $R = C_3H_7$
RTA-C5; $R = C_5H_{11}$



ATA-PH; $R = Ph$
ATA-BN; $R = \text{Benzyl}$
ATA-CY; $R = \text{cy-Hexyl}$
ATA-C6; $R = C_6H_{13}$
ATA-C9; $R = C_9H_{19}$
ATA-C12; $R = C_{12}H_{25}$



BTA-C9; $R = C_9H_{19}$
BTA-C11; $R = C_{11}H_{23}$
BTA-C13; $R = C_{13}H_{27}$



ETA-C1; $R_1 = H, R_2 = CH_3$
ETA-C2; $R_1 = C_6H_{13}, R_2 = C_2H_5$
ETA-CY; $R_1 = C_6H_{13}, R_2 = \text{cy-hexyl}$

Figure 6

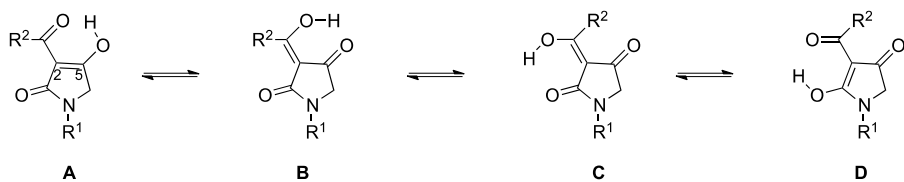


Figure 7

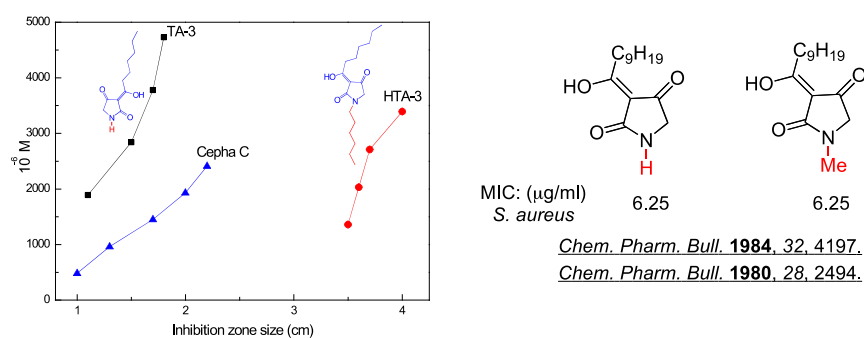


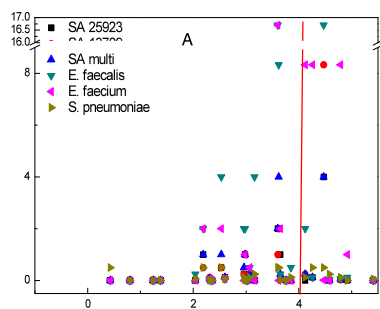
Figure 8

Table 1: Antibacterial activity of selected compounds from Figure 5.

Entry	Compounds	Minimum inhibition concentration (μg/ml) ^a		
		<i>Staphylococcus aureus</i> D267	<i>Escherichia coli</i> X580	
			Neutral pH	pH 4.5 buffer
<u>1</u>	<u>TA-PH</u>	<u>>4000</u>	<u>4000</u>	<u>800</u>
<u>2</u>	<u>TA-NA</u>	<u>2000</u>	<u>2000</u>	<u>1000</u>
<u>3</u>	<u>TA-CY</u>	<u>800</u>	<u>800</u>	<u>400</u>
<u>4</u>	<u>TA-C6</u>	<u>400</u>	<u>200</u>	<u>20</u>
<u>5</u>	<u>TA-OPH</u>	<u>>4000</u>	<u>800</u>	<u>-</u>

<u>6</u>	<u>TA-ONA</u>	<u>>4000</u>	<u>400</u>	<u>:</u>
<u>7</u>	<u>TA-OCY</u>	<u>>4000</u>	<u>800</u>	<u>:</u>
<u>8</u>	<u>TA-OC6</u>	<u>>4000</u>	<u>800</u>	<u>:</u>
<u>9</u>	<u>Cephalosporin C^c</u>	<u>200</u>	<u>2</u>	<u>:</u>

a: Determined by disc diffusion method[]; b: Cephalosporin C was used as the standard



D

B

E

C

-rel PSA (%)

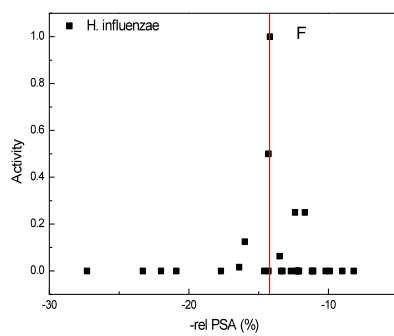
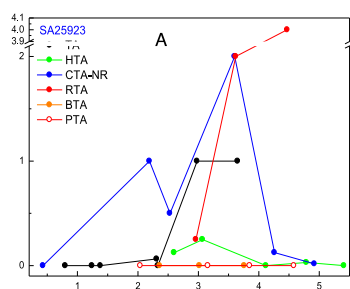


Figure 9

Table 2: Active compounds and their cheminformatic descriptors.

Compounds	MIC ($\mu\text{g/ml}$, <i>S. aureus</i>)	ClogP	MSA	rel PSA
Most active: CTA-C9, RTA-C3 & RTA-C5	0.125-0.5	3.6-4.47	631-693	11.2-12.3
Active: TA-C11, CTA-NR & CTA-C6	0.5-2	2.19-2.98	483-545	14.2-14.3



B

C

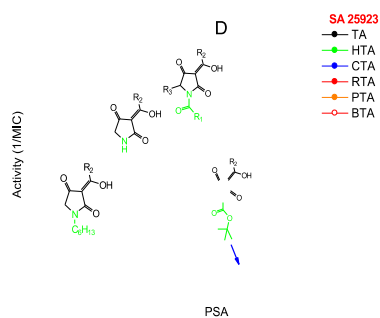


Figure 10

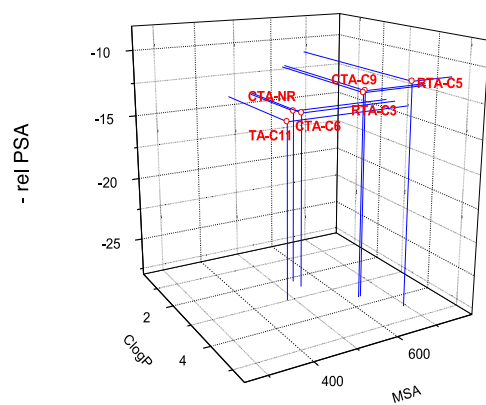
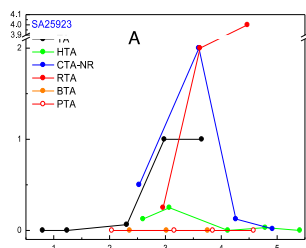


Figure 11

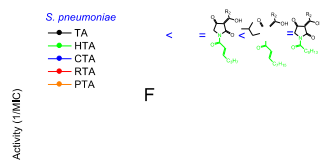


B

D

E

C



ClogP

Figure 12

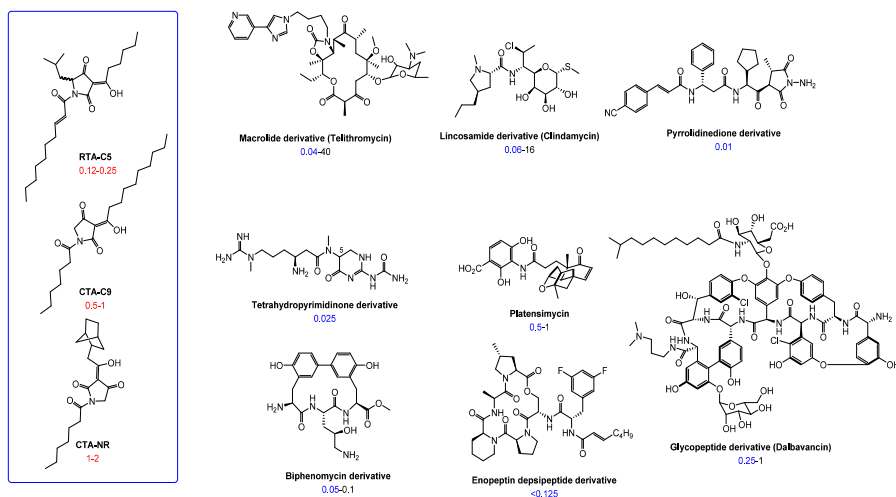


Figure 14

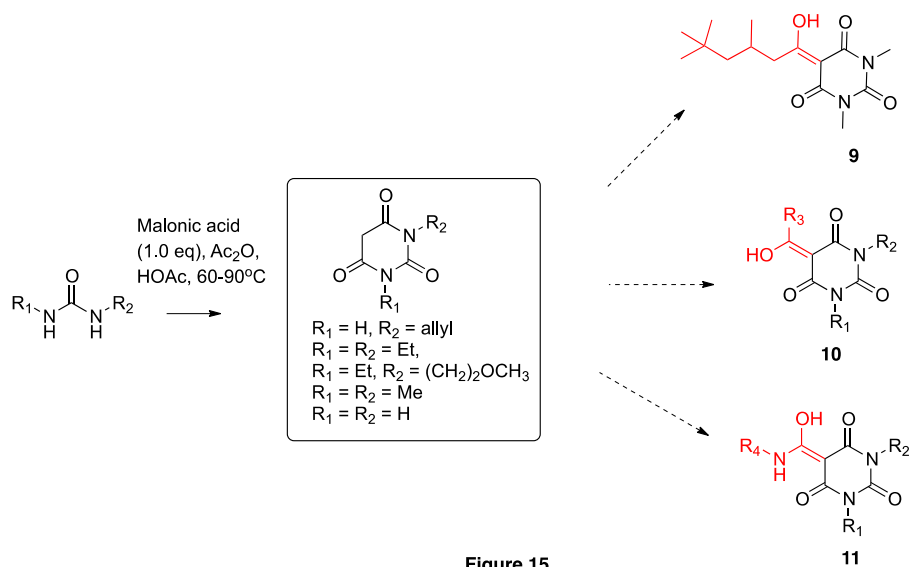


Figure 15

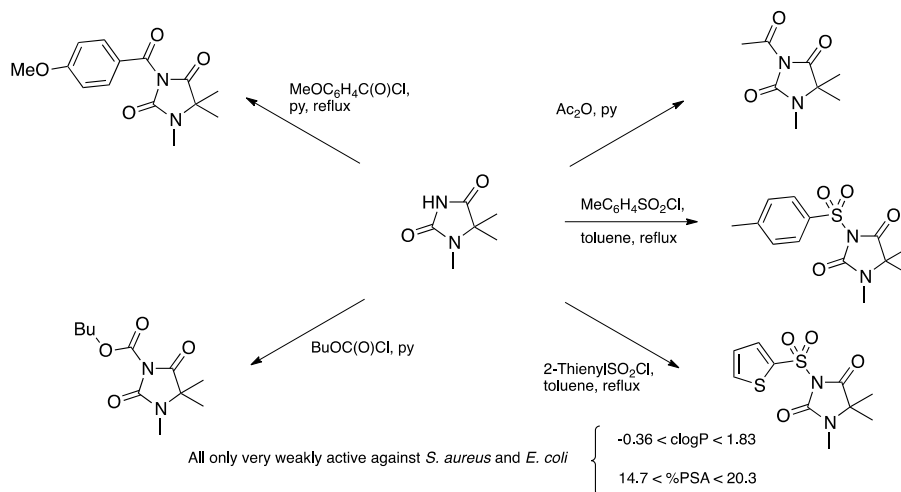


Figure 16

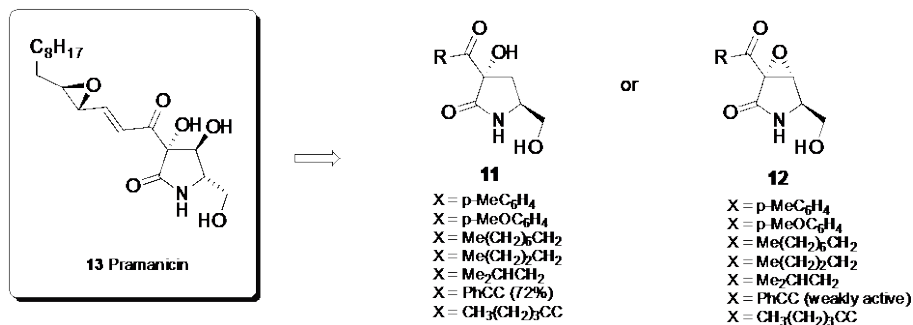


Figure 17