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SYNTHESIS AND ANTIBACTERIAL PROPERTIES OF β-DIKETONE ACRYLATE BIOISOSTERES OF PSEUDOMONIC ACID A

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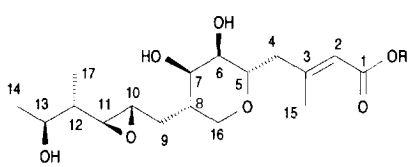
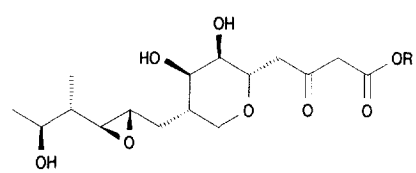
Abstract: A series of β-diketone acrylate bioisosteres **4** of pseudomonic acid A **1** have been synthesized and evaluated for their ability to inhibit bacterial isoleucyl-tRNA synthetase and act as antibacterial agents. A number of analogues have excellent antibacterial activity. Selected examples were shown to afford good blood levels and to be effective in a murine infection model.

Pseudomonic acid A **1** is a naturally occurring antibiotic[1] that exerts its mode of action through selective inhibition of bacterial isoleucyl-tRNA synthetase (IRS).[2] *In vivo* it has been shown that rapid breakdown to the parent monic acid **2** occurs[3] and as a result a number of C1-ester isosteres have been synthesised.[4] Having defined that the *E*-stereochemistry is an essential requirement for activity the isostere programme was extended to include the C1 to C3 α,β-unsaturated system.[5] β-Keto esters **3** are not inhibitors of IRS and are essentially not enolised. However, it was postulated that β-diketones **4** would exist mainly in the enolic form and that the form **4"** could be considered as a 3-hydroxy C1-ketone variant locked in the preferred double bond geometry through hydrogen bonding. We have synthesized a series of β-diketones and investigated their antibacterial properties in an attempt to discover a systemic agent.



1 R = $(CH_2)_8CO_2H$ **2** R = H



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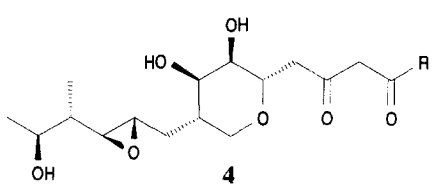

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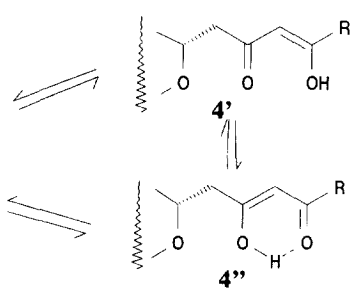

4'

4"

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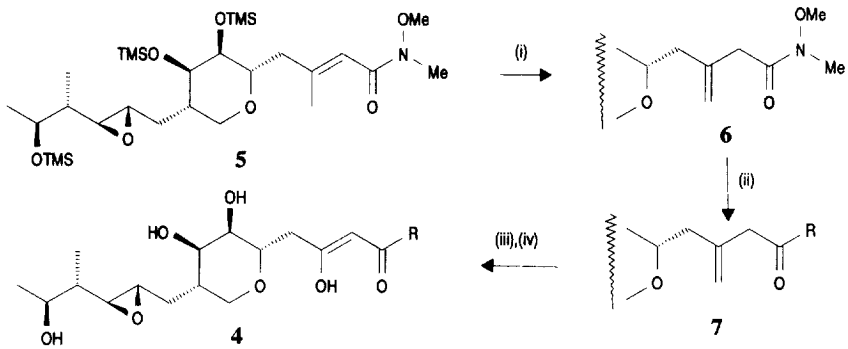
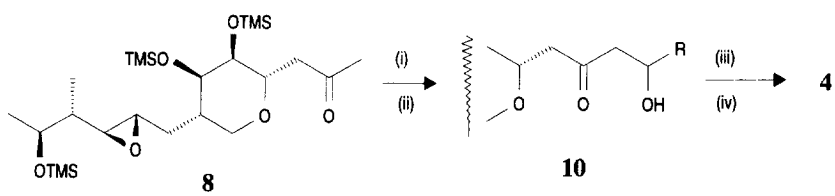


Reagents:- (i) tBuLi, $^{i}Pr_2NH$(cat), THF, -70 °C; (ii) RLi, THF, -70 °C; (iii) O_3, CH_2Cl_2, -70 °C, then Ph_3P; (iv) H^+

Scheme 1

Chemistry

The first synthesis of the diketone system **4** used a base catalysed deconjugation process (Scheme 1). Thus treatment of the Weinreb derivative **5**[7] with LDA followed by quenching at low temperature gave a 4:1 mixture of **6** to **5**. Conversion of this amide to a ketone was easily achieved by treatment with an organolithium species, and then ozonolysis revealed the diketone system. Brief acid treatment provided the target system **4**. ^1H NMR ($CDCl_3$) of these compounds confirmed that the systems were totally in the enolic form e.g. **4b** δ 6.20 (1H, s, 2-H). The process was successful for aryl and heteroaryl derivatives. However, as some systems, e.g. 3-furyl, were unstable to the ozonolysis step, an alternative sequence was devised.

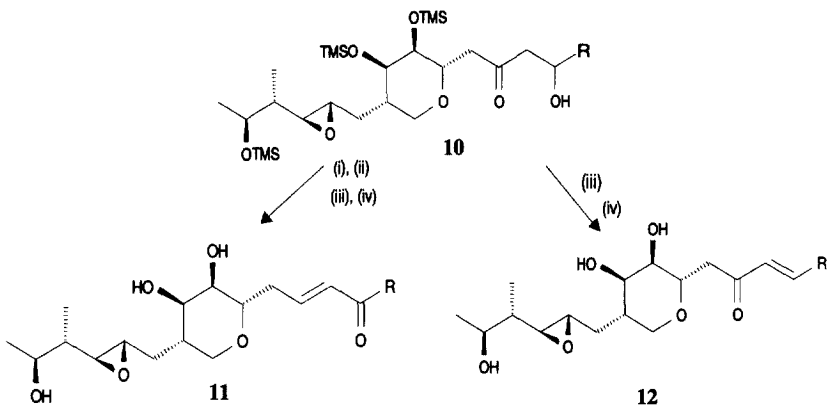


Reagents:- (i) LDA, THF, -70 °C; (ii) RCHO 9 ; (iii) MnO_2, C_6H_6, reflux; (iv) H^+

Scheme 2

Reaction of the enolate derived from the readily available ketone **8**[6] with an aldehyde **9** afforded the β-hydroxyketone **10**. Oxidation of the benzylic alcohol followed by removal of the silyl protecting groups furnished the diketone **4** in good overall yield (Scheme 2). With the electron rich 4-dimethylaminophenyl group the manganese dioxide procedure failed but oxidation using DDQ in dioxane was found to be the method of choice. This approach is complementary to that shown in Scheme 1 since the nucleophiles and electrophiles have been transposed and the ready availability of the aldehydes **9** makes Scheme 2 the preferred sequence.

The intermediate **10** also afforded access to analogues lacking an oxygen substituent at either the 3-position **11** or the 1-position **12** (Scheme 3).



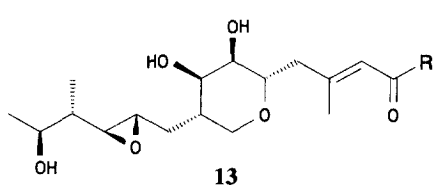
Reagents:- (i) $NaBH_4$; (ii) MnO_2, C_6H_6, reflux; (iii) MsCl, NEt_3; DBU; (iv) H^+

Scheme 3

Biological Activity

All the β-diketones **4** were potent inhibitors of IRS from *Staphylococcus aureus* Oxford[5] (IC_{50}: 5-15nM) and this has translated into excellent antibacterial activity (Table 1). In comparison with pseudomonic acid A **1** and the previously reported C1-ketones[7] **13**, the diketones were generally less potent *in vitro* against the majority of organisms, with the exception of *Enterococcus faecalis*. The desmethyl ketone **11b** was also less effective than the 3-Me counterpart **13b**, especially against the gram negative organisms *Haemophilus influenzae* and *Moraxella catarrhalis*. The reversed analogue **12b** was both a poor inhibitor and antibacterial agent. This reflects the importance of the interactions between the target enzyme and the α,β-unsaturated carbonyl system.[5]

In contrast to the structure activity relationships observed for C1-ketones **13**, the aryl diketones **4a-f** were generally more active than the heteroaryl derivatives **4g-l**, possibly as a result of the greater polarity of the diketones. Optimal activity was observed with small para phenyl substituents (**4b**, **4f**). Also we have shown that the ring does not have to be aromatic (**4n**), but that an alkyl chain was poor (**4m**).



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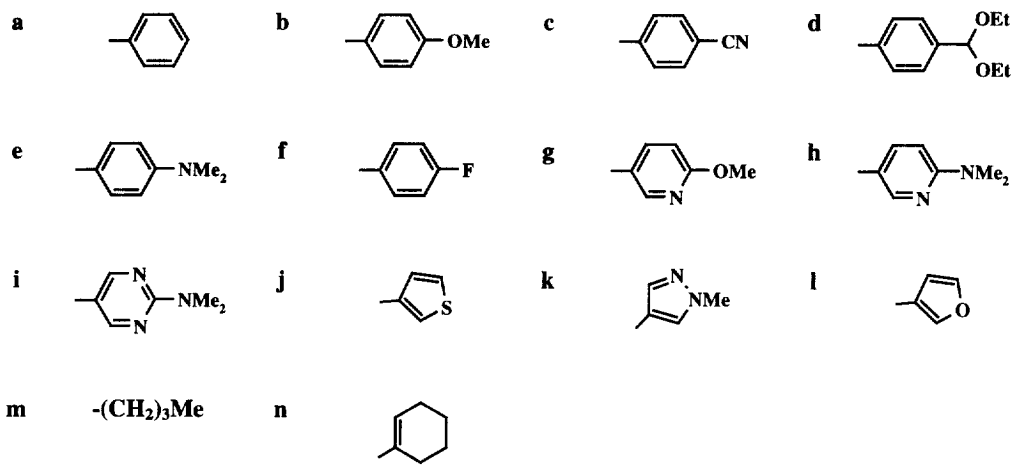

Table 1: Antibacterial activity (Minimum Inhibitory Concentration (MIC) μgml^{-1})

Compound	Preparative Scheme	*Staphylococcus aureus* Oxford	*Streptococcus pyogenes* CN10	*Streptococcus pneumoniae* PU7	*Haemophilus influenzae* Q1	*Moraxella catarrhalis* 1502
1	-	0.13	0.13	0.13	0.06	0.13
4a	1	1	0.5	0.5	0.25	1
4b	1/2	0.5	0.25	0.25	0.06	0.25
4c	2	2	0.13	0.25	0.06	0.5
4d	2	1	0.5	1	1	0.5
4e	2	2	1	0.5	0.06	0.5
4f	2	0.5	0.25	0.13	0.06	0.5
4g	1	1	0.13	0.13	0.13	0.25
4h	2	1	1	0.5	0.06	1
4i	2	2	1	1	0.25	2
4j	2	1	1	0.5	0.13	1
4k	2	4	1	1	0.13	0.5
4l	2	1	1	2	0.13	2
4m	1	8	2	8	0.5	2
4n	2	2	0.5	0.5	0.13	2
11b	3	1	0.5	0.25	0.13	2
12b	3	128	8	8	1	16
13b	-	0.5	0.25	0.25	0.03	0.25

Several compounds were examined *in vivo* in mouse pharmacokinetic and infection models. As can be seen from Table 2, in the 4-methoxyphenyl series the diketone **4b** has superior mouse blood levels and more favourable serum binding than the C1-ketone analogue **13b**. This has translated into **4b** being more effective in the mouse at eradicating a *S. aureus* infection dosing subcutaneously.

Table 2

Compound	Mouse Blood Level[7] 50 mg kg^{-1} dose AUC (μg ml^{-1} minute)		Mouse *S. aureus* i.p. infection [a] CD_{50} (mg kg^{-1})		Serum Binding [b] % bound	
	oral	s.c.	oral	s.c.	mouse	human
4b	355	894	28	3.8	51	75
4f	296	408	19	12	NT	80
4h	431	798	23	12	66	84
13b	106	336	18	12	79	96

[a] Non fasted, male, Charles Rivers CD1 mice were infected intraperitoneally with 2~9 x 10^6 cfu of *S. aureus* Smith contained in 0.5ml of brain heart infusion broth. Compounds were administered as solutions or suspensions in 10% ethanol in hydroxypropylmethyl cellulose (p.o.) or pH 7.3 phosphate buffered saline (s.c.) at 1 and 5 hours post infection. The CD_{50} was calculated on the second day post infection as the total dose required to protect 50% of the mice from death. [b] By ultrafiltration (Amicon microfree partition apparatus) using sterile pooled serum; initial compound concentration 40 μg ml^{-1}. **NT** – not tested

Conclusion

We have synthesized a range of aryl- and heteroaryl- β-diketone acrylate bioisosteres of pseudomonic acid A, and discovered structural factors which influence *in vitro* antibacterial activity and murine pharmacokinetics. As a result of this we have identified an example of a β-diketone **4b** that shows advantages over the previously described C1-ketone **13b**. A further comparison of the data suggests that **4b** would be better than **13b** in a human infection, due to its lower human serum binding, and thus it may be a useful systemic antibacterial agent in man.

References and Notes

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