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Optimisation of Aryl Substitution Leading to Potent Methionyl tRNA Synthetase Inhibitors with Excellent Gram-Positive Antibacterial Activity

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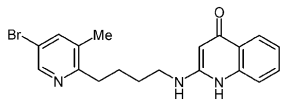
Abstract—Optimisation of the left-hand-side aryl moiety of a file compound screening hit against *Staphylococcus aureus* methionyl tRNA synthetase led to the identification of a series of potent nanomolar inhibitors. The best compounds showed excellent antibacterial activity against staphylococcal and enterococcal pathogens, including strains resistant to clinical antibiotics.

Bacterial resistance to established antibiotics continues to pose an increasing problem in clinical practice. This is particularly evident in the hospital context, with the rise of methicillin resistant *Staphylococcus aureus* (MRSA)[1] and of vancomycin resistant enterococci (VRE),[2] many strains of which have become resistant to all antibiotics. The resulting medical need has stimulated the search for new classes of antibacterial agents that act at novel molecular targets.

We recently reported the discovery of a new class of inhibitors of bacterial methionyl tRNA synthetase (MRS) that had useful activity against staphylococci and enterococci, including in vivo efficacy.[3] Compound **1** was identified as a high throughput screening hit with an IC_{50} value of 350 nM against *S. aureus* MRS but no Gram-positive antibacterial activity.



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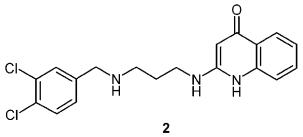

2

Compound **2** was derived as an early chemistry lead with improved MRS inhibition and target-related antibacterial activity. Here we report the structure–activity relationships of the left-hand side moiety that greatly enhanced MRS inhibition and resulted in potent Gram-positive antibacterial activity.

Alternative modes of attachment of the aryl moiety were investigated in order to identify the preferred linkage and chemistry for array amplification of the lead **2**. Direct acylation of the key amine intermediate **3** afforded the amide **4** (Scheme 1).

The ether and thioether linked analogues **11** and **12** were synthesised by first preparing the appropriate aryl linker units **7** and **8** (Scheme 2). These were then reacted with the methoxybenzyloxy quinoline **10**,[4] utilised to allow unmasking of the quinolone functionality under mild conditions (TFA at room temperature).

The compounds **4**, **11** and **12** were tested for inhibition of MRS in an aminoacylation assay.[3] They were also tested for antibacterial activity against *S. aureus* and *Enterococcus faecalis* in a standard MIC assay (Table 1). The amide and ether analogues were clearly less potent than the amine and were without significant antibacterial activity.

Array amplification was thus carried out around the secondary amine scaffold of **2**. The reductive amination

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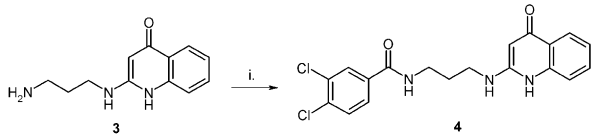
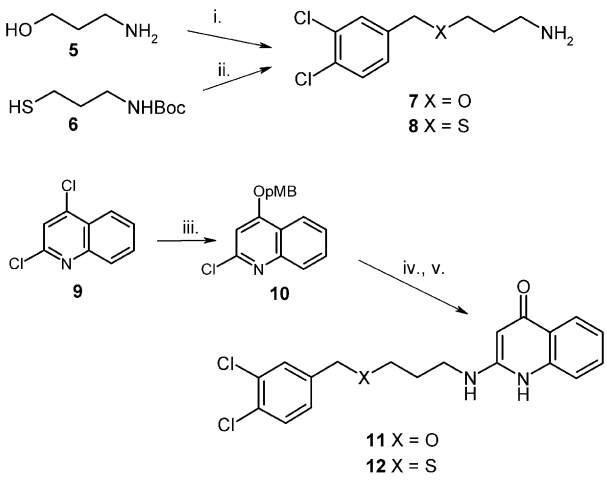


Scheme 1. Reagents: (i) 3,4-diClPhCO$_2$H/DIC/*N*-HO-succinimide/DMF.



Scheme 2. Reagents: (i) 3,4-diClbenzyl-Cl/NaH/THF/Δ; (ii) 3,4-diClbenzyl-Cl/NaH/THF then TFA; (iii) 4-methoxybenzyl-OH/NaH/15-crown-5/THF; (iv) **7** or **8** toluene/Δ; (v) TFA.

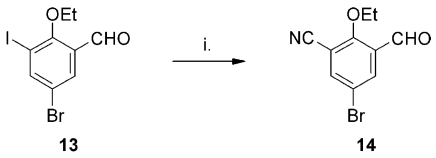
Table 1. MRS IC_{50} values and in vitro minimum inhibitory concentration (MIC) of compounds against strains of *S. aureus* and *E. faecalis*

Compd	IC_{50} (nM)	MIC (μg/mL)	
	S. aureus MRS	*S. aureus* Oxford	*E. faecalis* 1
2	16	4	2
4	140	>64	>64
11	50	>64	>64
12	170	>64	>64

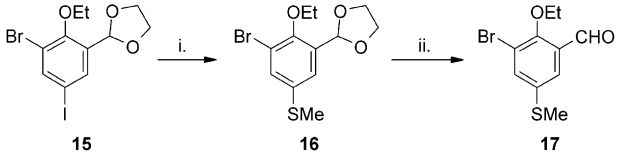
of **3** was optimised for array synthesis using resin-bound cyanoborohydride as the reducing agent.[5] Purification was performed by parallel chromatography[6] and the products were converted to their hydrochloride salts by addition of 1 M HCl in methanol to a methanolic solution of the amine.

A range of commercially available aldehydes was utilised in the initial arrays. As the preferred substitution pattern became evident, targetted 2,3,5-trisubstituted aldehydes were synthesised. 2-Alkoxy-3,5-substituted benzaldehydes were prepared by three routes: (i) 2-*O*-alkylation of 3,5-substituted salicaldehydes (or salicyclic esters followed by conversion of the ester to aldehyde); (ii) electrophilic substitution of 3-substituted salicaldehydes; (iii) copper-catalysed aryl substitution reactions.

An example of the copper-catalysed chemistry is the formation of the 3-cyano analogue **14** (Scheme 3). In some cases protection of the aldehyde functionality was necessary, as in the synthesis of the 5-methylthio analogue **17** (Scheme 4). Oxidation of the intermediate **16**



Scheme 3. Reagents: (i) CuCN/DMF/80 °C.



Scheme 4. Reagents: (i) NaSMe/Cu_2O/DMF/80 °C; (ii) pyridinium tosylate/H_2O/acetone.

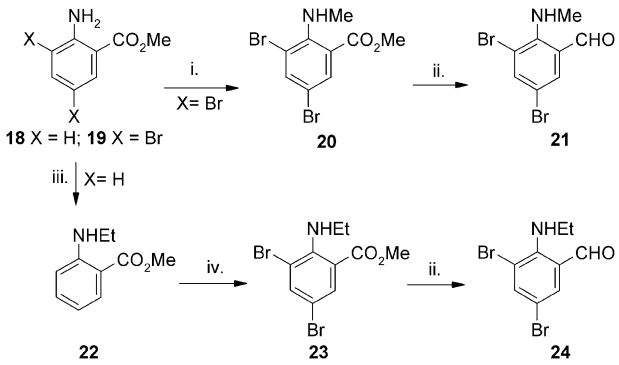
with MCPBA followed by deprotection gave the 5-sulfoxide and 5-sulfone aldehydes.

2-Amino 3,5-disubstituted aldehyde intermediates were prepared by the specific chemistries shown in Scheme 5, due to the aniline nitrogen of the 3,5-dibromo anthranilate **19** being resistant to standard alkylation methods.

The substituted phenyl analogues were tested in the standard way (Table 2). Many of the compounds were very potent MRS inhibitors. However, due to the enzyme concentration in the assay (3 nM), many of the IC_{50} values are approaching the observable tight binding limit and there is thus little differentiation for the most potent compounds.

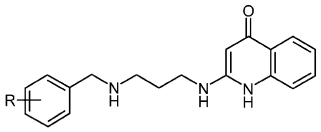
The unsubstituted phenyl derivative **25** was a poor MRS inhibitor and had no antibacterial activity (Table 2). Of a set of dichlorophenyl isomers tested **26**–**30**, the 3,5-isomer gave the most potent MRS inhibition and the best antibacterial activity. More lipophilic 3,5-dihalo analogues **31**–**33** gave potent inhibition and increased antibacterial activity.

By elaboration of the 3,5-dichloro and 3,5-dibromo analogues it was found that 2,3,5-trisubstitution was optimal, for example **34**. At the 2-position, small alkoxy groups such as ethoxy (**35** and **38**) were preferred over branched alkoxy or amino substituents. Variation at the



Scheme 5. Reagents: (i) $(CO_2Me)_2$/KOBu^t/DMA/reflux; (ii) NaAl$(NEt_2)_3$H/THF/−78 °C; (iii) MeCHO/NaBH$(OAc)_3$/CH_2Cl_2; (iv) Br_2/MeOH.

Table 2. MRS IC_{50} values and in vitro minimum inhibitory concentration (MIC) of compounds against strains of *S. aureus* and *E. faecalis*



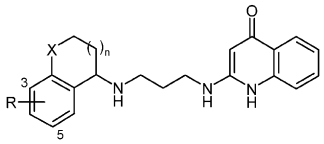
	R	IC_{50} (nM)	MIC (μg/mL)	
		S. aureus MRs	*S. aureus* Oxford	*E. faecalis* 1,7
25	—	300	>64	>64
26	2,3-diCl	15	8	2[a]
27	2,4-diCl	15	4	4[a]
28	2,5-diCl	9	4	16
29	3,4-diCl	16	4	2
30	3,5-diCl	3	1	0.13
31	3,5-diBr	<3	0.25	≤0.06
32	3-Br, 5-I	3.2	<0.06	≤0.06
33	3,5-diI	6.4	<0.06	≤0.06
34	2,3,5-triCl	<3	0.25	≤0.06[a]
35	2-EtO,3,5-diCl	14	0.13	≤0.06
36	2-AllylO,3,5-diCl	7.2	0.5	≤0.06
37	2-iPrO,3,5-diCl	29	2	0.5[a]
38	2-EtO,3,5-diBr	4.4	≤0.06	≤0.06
39	2-CF_3CH_2O,3,5-diBr	11	0.25	≤0.06
40	2-NH_2,3,5-diBr	9.1	0.13	≤0.06
41	2-NHMe,3,5-diBr	11	0.5	0.13
42	2-NHEt,3,5-diBr	18	1	≤0.06
43	2-EtO,3-Cl,5-MeO	4.3	0.25	≤0.06
44	2-EtO,3-Br,5-I	8.8	≤0.06	≤0.06
45	2-EtO,3-Br,5-Me	4.9	2	0.25
46	2-EtO,3-Br,5-CN	4.6	4	1
47	2-EtO,3-Br,5-MeO	3.1	0.13	≤0.06
48	2-EtO,3-Br,5-EtO	61	8	4
49	2-EtO,3-Br,5-MeS	4.7	≤0.06	≤0.06
50	2-EtO,3-Br,5-MeSO	23	4	2
51	2-EtO,3-Br,5-$MeSO_2$	72	>64	8
52	2-EtO,3-I,5-Br	5.9	≤0.06	≤0.06
53	2-EtO,3,5-diI	<3	≤0.06	≤0.06
54	2-EtO,3-I,5-Me	7.2	2	0.5
55	2-EtO,3-I,5-CH_2OH	14	>64	>64
56	2-EtO,3-I,5-CH_2OMe	17	8	2
57	2-EtO,3-I,5-CO_2Et	170	32	8
58	2-EtO,3-I,5-MeO	3.3	0.5	0.13
59	2-EtO,3-SMe,5-Br	21	0.5	1
60	2-EtO,3-Me,5-Cl	6.8	0.25	≤0.06
61	2-EtO,3-Me,5-Br	5.7	0.13	≤0.06
62	2-EtO,3-Me,5-I	3.2	≤0.06	≤0.06
63	2-EtO,3-Me,5-MeS	5.1	≤0.06	≤0.06
64	2-EtO,3-Me,5-CN	3.9	8	4
65	2-EtO,3-Me,5-CF_3	6.8	0.5	0.25
66	2-EtO,3-CH_2OH,5-I	4.1	32	32
67	2-EtO,3-CH_2OMe,5-I	9.4	16	8
68	2-EtO,3-allyl,5-I	<3	0.13	1
69	2-EtO,3-tBu,5-I	120	32	8
70	2-EtO,3-CN,5-Br	4	0.5	0.25
71	2-EtO,3-CO_2Et,5-I	21	32	8
72	2-EtO,3-MeO,5-Br	13	4	2
73	2-EtO,3-MeO,5-I	12	2	≤0.06

[a]MIC against *E. faecalis* 1 not usable; the MIC given is for the strain *E. faecalis* 7.

3- and 5-positions showed halogen and methylthio to give the best antibacterial activity.

More polar substituents were investigated to reduce lipophilicity. A methoxy group was tolerated in the 5-position, giving good antibacterial activity, **47**. Other small polar groups of slightly higher polarity, such as cyano and hydroxymethyl were reasonably well tolerated by the enzyme but generally resulted in significant reductions in antibacterial activity. Larger substituents resulted in loss of MRS inhibition, especially large polar groups such as ester, sulfoxide and sulfone. Overall it seems that the binding pockets for the 3- and 5-positions are quite constrained, preferring relatively compact, non-polar substituents.

Table 3. MRS IC_{50} values and in vitro minimum inhibitory concentration (MIC) of compounds against strains of *S. aureus* and *E. faecalis*



	R	X	*n*	IC_{50} (nM)	MIC (μg/mL)	
				S. aureus MRS	*S. aureus* Oxford	*E. faecalis* 1
74	3,5-diCl	CH_2	0	5.1	0.25	≤0.06
75	3,5-diCl	O	0	6.0	2	0.125
76	3,5-diCl	CH_2	1	3.8	0.25	≤0.06
77	3,5-diCl	O	1	<3	0.13	≤0.06
78	3,5-diCl	NH	1	23	0.13	≤0.06
79	3,5-diBr	O	1	7.8	≤0.06	≤0.06
80	3-Cl,5-I	O	1	12	0.13	≤0.06
81	3-I,5-Cl	O	1	9.4	≤0.06	≤0.06
82	3,5-diBr	NH	1	8.0	≤0.06	≤0.06
83	3-Cl,5-Br	NH	1	<3	≤0.06	≤0.06
84	3-Br,5-Cl	NH	1	<3	≤0.06	≤0.06
85	3-Br,5-Me	NH	1	8.0	1	0.25
86	3-I,5-Et	NH	1	12	0.25	≤0.06
87	3-Me,5-Br	NH	1	9.4	0.5	≤0.06
88	3-Et,5-Br	NH	1	4.3	0.25	≤0.06

Conceptual cyclisation of the 2-substituent onto the benzylic α-position results in compounds of the general structure shown in Table 3. Analogues of this type were synthesised by reductive amination from cyclic ketones under more forcing conditions than used for the aldehydes, typically in refluxing methanol.[3] The cyclic ketones were generally known compounds or prepared by the route described for substituted tetrahydroquinolines.[3] The tetralone starting material for **76** was isolated in low yield from the reaction of butyrolactone and *m*-dichlorobenzene[7] while the iodine in the precursor to **80** was introduced into the known chlorochromanone using iodine tris-trifluoroacetate.[8]

From the series of 3,5-dichloro analogues **74**–**78**, it was found that the six-membered ring compounds were more active than the five-membered (Table 3). The tetrahydro-naphthalene, chroman and tetrahydroquinoline scaffolds all afforded compounds exhibiting potent MRS inhibition and good antibacterial activity.

A smaller range of substituents were investigated at the 3- and 5-positions on the chroman and tetrahydroquinoline scaffolds, based on those that were preferred in the phenyl series. The preference for halogen was more pronounced than in the phenyl series, with only an ethyl substituent approaching the level of antibacterial activity of the

Table 4. Antibacterial activity of selected compounds against panels of staphylococci and enterococci, where MIC90 is the concentration required to inhibit 90% of the organisms[9]

	MIC90 (μg/mL)			
	S. aureus	*S. epidermidis*	*E. faecalis*	*E. faecium*
Amoxicillin	>16	8–16	>16	>16
35	2	2	0.06	≤0.016
47	2	2	0.06	0.03
49	0.25	0.25	0.5	0.5
81	0.25	0.25	nd	nd
82	0.5	0.5	0.03	≤0.016
86	1	1	0.25	0.06
84	1	1	0.06	≤0.016

nd, not done.

dihalo analogues. Mono-chloro substituted analogues appeared optimal for MRS IC_{50} and antibacterial activity.

The excellent Gram-positive antibacterial activity of some of these compounds was confirmed by testing against panels of clinical isolates to determine their MIC90 values (the concentration required to inhibit 90% of the organisms; Table 4). These panels of *S. aureus*, *Staphylococcus epidermidis*, *E. faecalis* and *Enterococcus faecium* include a range of resistant organisms.[9] In all cases, the MRS inhibitors maintain good activity against these pathogens. Excellent activity was seen against the enterococci. Particularly good antibacterial activity against staphylococci was seen with the 5-methylthiophenyl analogue **49** and the chroman **81**.

All the MRS inhibitors described were highly selective for the bacterial enzyme, with no significant inhibition of mammalian (rat liver) MRS up to the highest concentration tested (either 1 or 10 μM).

In summary, the topology of a 2,3,5-trisubstituted benzylamino group has been identified as an optimal left-hand side moiety, affording potent nanomolar MRS inhibition and Gram-positive antibacterial activity. Cyclisation to the benzylic α-position is tolerated, affording very active chroman and tetrahydroquinoline analogues. The 3- and 5-positions are relatively constrained, and display a preference for non-polar substituents. Analogues from both the phenyl series and the cyclised chroman and tetrahydroquinoline series afford very good antibacterial activity against panels of staphylococcal and enterocccal clinical isolates. These results provide encouragement for the potential of MRS as a target for a novel class of Gram-positive antibacterial agents.

Acknowledgements

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References and Notes

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4. In the preparation of **10**, addition of the crown ether was designed to increase the amount of 4-substitution by reducing the postulated complexation of sodium to the quinoline nitrogen[10] which favours 2-substitution. Under these conditions, the crown ether increased the ratio of 4-substitution to 2-substitution from 1:7 to 1:1.
5. The preferred array procedure utilised the aldehyde (0.1 mmol), amine **3** (0.12–0.15 mmol), and resin-bound cyanoborohydride in methanol containing 1% acetic acid.
6. The crude reaction mixture was adsorbed onto Biotage Quad 3 samplets followed by drying in a dessicator overnight. Subsequent parallel chromatography was carried out on silica gel cartridges eluting with increasing concentrations of methanolic ammonia in dichloromethane (0–10%).
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9. The number of isolates in each profile were *S. aureus*, $n=31$, *S. epidermidis*, $n=10$, *E. faecalis*, $n=10$, *E. faecium*, $n=10$. The resistance profile has been reported.[3] Testing was carried out using the NCCLS protocol.
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