Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1265–1268

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Conformational Restriction of Methionyl tRNA Synthetase Inhibitors Leading to Analogues with Potent Inhibition and Excellent Gram-Positive Antibacterial Activity

Richard L. Jarvest,[a,*] John M. Berge,[a] Pamela Brown,[a] Catherine S. V. Houge-Frydrych,[a] Peter J. O'Hanlon,[a] David J. McNair,[a] Andrew J. Pope[a] and Stephen Rittenhouse[b]

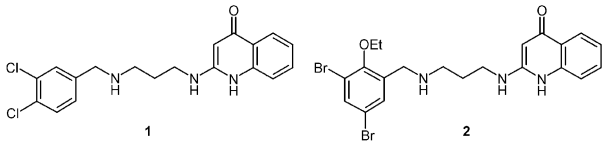
[a]*GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK*
[b]*GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA*

Received 12 December 2002; revised 23 January 2003; accepted 23 January 2003

Abstract—Conformationally restricted analogues of the central linker unit of bacterial methionyl tRNA synthetase (MRS) inhibitors have been prepared. The (1*S*,2*R*)-cyclopentylmethyl moiety was identified as the preferred cyclic linker, with significant diastereo- and enantioselectivity of activity. Combination of this linker with an optimal substituted aryl right-hand side has resulted in a compound with exceptionally good antibacterial activity against staphylococci and enterococci, including antibiotic resistant strains.

New classes of antibiotics acting at novel molecular targets are sought after to counteract the growing threat of bacterial resistance. In the hospital context, resistant pathogens of particular concern are methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci (VRE). At some locations MRSA is now responsible for 20–40% of all *S. aureus* infections,[1] whilst many strains of VRE have developed resistance to all established classes of antibiotics.[2] An underexploited class of antibacterial targets are the aminoacyl tRNA synthetases, essential enzymes in protein biosynthesis. The topical antibiotic mupirocin (marketed as Bactroban®) acts by inhibition of bacterial isoleucyl tRNA synthetase and selective inhibitors of other aminoacyl tRNA synthetases are of interest for their potential as novel-acting antibacterials.

We have reported inhibitors of bacterial methionyl tRNA synthetase (MRS) as a potential new class of antibiotic agents with good Gram-positive antibacterial activity.[3,4] The quinolone **1** was an early chemistry lead for MRS inhibition with improved inhibition, target-related Gram-positive antibacterial activity, and increased chemical manipulability.[3] Optimisation of the left hand side aryl substitution pattern resulted in 2,3,5-trisubstituted analogues such as **2** which had potent antibacterial activity.[4] Here we describe the identification of a constrained central linker with diastereo- and enantioselective activity which can confer exceptionally potent Gram-positive antibacterial activity.



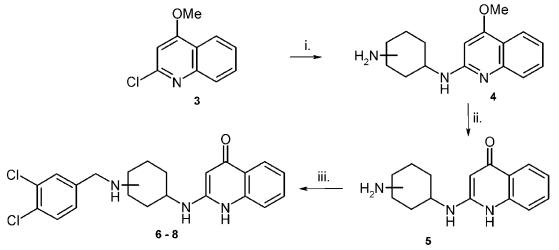
The first constrained analogues to be prepared were the three cyclohexanes **6**–**8**. Reaction of the methoxyquinoline **3** with an excess of the appropriate diaminocyclohexane followed by acid hydrolysis of the methoxyquinoline to the quinolone and subsequent reductive alkylation afforded the desired cyclohexane analogues (Scheme 1).

The cyclohexanes were tested as inhibitors in an MRS assay[3] and tested for antibacterial activity against *S. aureus* and *Enterococcus faecalis* in a standard assay for minimum inhibitory concentration (MIC), Table 1.

The *cis*-1,3-diamino **6** and *trans*-1,4-diamino **8** cyclohexane analogues, both corresponding to extended linker conformations, were poor MRS inhibitors (Table 1).

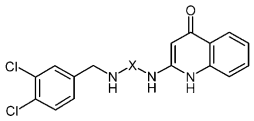
*Corresponding author. Tel.: +44-1279-627629; fax: +44-1279-622260; e-mail: richard_l_jarvest@gsk.com

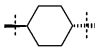
0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00093-3



Scheme 1. Reagents and conditions: (i) 1,3-diaminocyclohexane or *t*-1,3-diaminocyclohexane/Et_2iPrN or *t*-1,4-diaminocyclohexane 70 °C; (ii) c.HCl reflux, 20 h; (iii) 3,4-diCl-benzaldehyde/$NaCNBH_3$/NaOAc/AcOH/ MeOH, 20 h.

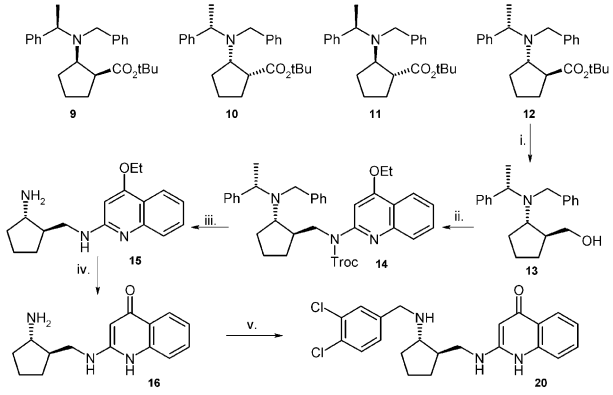
Table 1. MRS inhibition and antibacterial activity of cyclohexane linker analogues



No.	Stereo	X	IC_{50} (nM)	MIC (μg/mL)	
			S. aureus MRS	*S. aureus* Oxford	*E. faecalis* 1
6	rac		150	64	32
7	rac		43	>64	>64
8	—		2300	>64	>64

The *trans*-1,3-diamino **7** was a moderately potent inhibitor, with an IC_{50} value of 43 nM, just 3-fold down on the propyl linker. This compound corresponds to a more-folded linker conformation.

The molecular modelling program Catalyst[5] was used to analyse conformational data from a number of linker analogues, including the cyclohexyl compounds **6**–**8**, to generate a hypothesis of the biologically active linker conformation.[6] This hypothesis was used to prioritise further conformationally restricted targets. The 1,2-*trans* cyclopentylmethyl linkers were predicted to have the correct geometry and scored highly on the list.

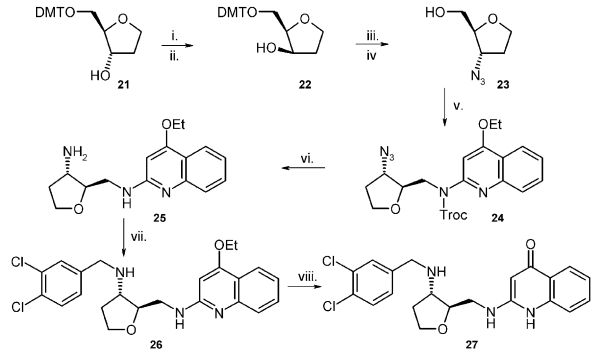


Scheme 2. Reagents and conditions: (i) $LiAlH_4/Et_2O$; (ii) TEAQ/ADDP/Bu_3P/PhH; (iii) NH_4HCO_2/Pd-C/MeOH/reflux; (iv) c.HCl/reflux; (v) 3,4-diCl-benzaldehyde/$NaCNBH_3$/NaOAc/AcOH/MeOH.

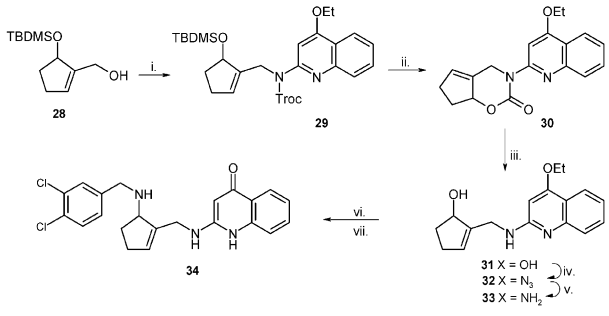
The separate stereoisomers of the cyclopentylmethyl linker were prepared as shown in Scheme 2. All four diastereoisomers of the aminocyclopentane ester **9**–**12** were prepared by the route of Davies et al.[7] Each isomer was separately taken through the reaction sequence as illustrated for **12**, starting with lithal reduction to the alcohol **13**. Mitsunobu coupling of alcohols to amino-heterocycle carbamates[8,9] was then exploited, using the preferred conditions of tributylphosphine and 1,1-azodicarbonylpiperidine (ADDP).[9] The trichloroethoxycarbonyl derivative of 2-amino-4-ethoxy quinoline (TAEQ)[10] was used as the coupling partner to afford the intermediate **14**. Removal of the Troc and benzylic groups by catalytic hydrogen transfer followed by the standard hydrolysis and reductive alkylation steps afforded the four stereoisomers **17**, **18**, **19**, **20**.

A tetrahydrofuran analogue of the preferred cyclopentyl stereochemistry was prepared from the deoxyribose derivative **21**[11] (Scheme 3). The left hand side amine was introduced as azide which was reduced down with concomitant cleavage of the Troc group. The tributylphosphine/ADDP method was again used for coupling the linker to the quinoline moiety.

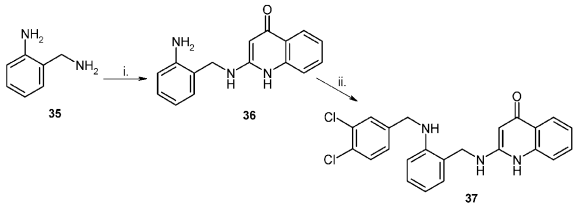
An unsaturated analogue **34** was prepared from the alcohol **28**[12] (Scheme 4). Removal of the protecting



Scheme 3. Reagents and conditions: (i) $PhCO_2H$/DEAD/PPh_3/THF; (ii) NaOH/MeOH; (iii) Ph_2PON_3/DEAD/PPh_3/THF; (iv) TFA/CH_2Cl_2; (v) ADDP/Bu_3P/PhH; (vi) NH_4HCO_2/Pd-C/MeOH/reflux; (vii) 3,4-diCl-benzaldehyde/$NaCNBH_3$/AcOH/MeOH; (viii) c.HCl/reflux.



Scheme 4. Reagents and conditions: (i) ADDP/Bu_3P/benzene; (ii) Bu_4NF/THF; (iii) 2M NaOH/dioxane/45 °C; (iv) $(PhO)_2P(O)N_3/Ph_3P$/DEAD/THF; (v) $NaBH_4$/EtOH; (vi) c.HCl/reflux; (vii) 3,4-diCl-benzaldehyde/$NaCNBH_3$/AcOH/MeOH.

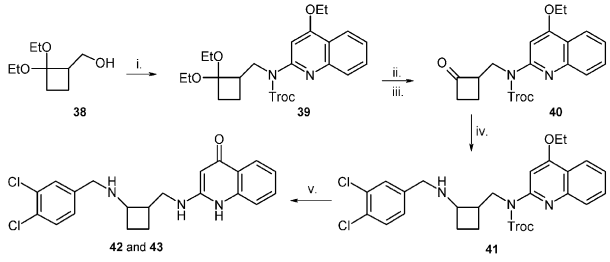


Scheme 5. Reagents and conditions: (i) 2-Cl-4-quinolone/Et_2iPrN/dioxane/75 °C; (ii) 3,4-diCl-benzaldehyde/$NaCNBH_3$/NaOAc/AcOH/MeOH.

groups from **29** resulted in unexpected formation of the cyclic carbamate **30**, which nonetheless could be ring opened and taken through to the target **34**.

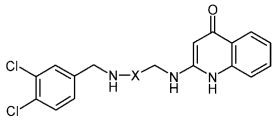
Due to the constraint of the acid lability of a benzylic bond to the quinoline 2-amine, the aromatic analogue **37** was prepared via amine displacement on 2-chloro-4-quinolone followed by reductive alkylation (Scheme 5).

Cyclobutane analogues were prepared from the acetal **38**.[13] Mitsunobu coupling to the Troc-quinoline intermediate (in this instance DEAD/PPh_3 was the preferred reagent combination) gave **39** which was deprotected to the afford the ketone **40**. The reductive amination was carried out with inverted functionality to the usual method and afforded a *cis*/*trans* mixture **41**, which was separated into the two diastereomers by HPLC. The *cis* and *trans* compounds were separately hydrolysed by the standard procedure to give the racemates **42** and **43** (Scheme 6).[14]



Scheme 6. Reagents and conditions: (i) TEAQ/DEAD/PPh_3/THF; (ii) NH_4HCO_2/Pd-C/MeOH/reflux; (iii) 3M H_2SO_4; (iv) 3,4-diCl-benzylamine/$NaCNBH_3$/NaOAc/AcOH/MeOH; (v) c.HCl reflux.

Table 2. MRS inhibition and antibacterial activity of cyclic-methyl linker analogues

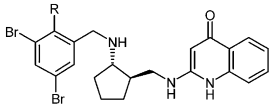


No.	Stereo	X	IC_{50} (nM)	MIC (μg/mL)	
			S. aureus MRS	*S. aureus* Oxford	*E. faecalis* 1
17	1*R*,2*R*		18	16	8
18	1*S*,2*S*		100	8	1
19	1*R*,2*S*		700	>64	32
20	1*S*,2*R*		9	1	0.125
27	1*S*,2*R*		78	64	4
34	rac		10	>64	>64
37	—		56	>64	>64
42	rac		12	2	0.25
43	rac		110	>64	16

The cyclic-methyl compounds were tested in the same way as the cyclohexanes (Table 2). Of the four cyclopentanes, the (1*S*,2*R*) isomer **20** was preferred, whilst its antipode **19** was worst. There is a moderately high degree of stereoselectivity with a range of about two orders of magnitude of IC_{50} and MIC values across the isomers. The antibacterial activity of **20** is clearly best and is significantly improved over that of the straight propyl linker. This suggests that, as predicted, the *trans*-cyclopentylmethyl linker is locking the preferred conformation of the linker moiety.

The tetrahydrofuran analogue of the preferred stereoisomer, **27**, had significantly reduced MRS inhibition and little antibacterial activity. Introduction of planarity—either unsaturation **34** or aromaticity **37**—resulted in loss of antibacterial activity. The cyclobutane analogues were tested in racemic form, **42** and **43**. Taking into account the fact that **42** is racemic, it appears to have very similar enzyme inhibition and antibacterial activity to the cyclopentane **20**. The cyclobutane did not have any benefits in other properties over the cyclopentane and in view of the increased synthetic difficulties, it was not pursued further.

Table 3. MRS inhibition and antibacterial activity of cyclopentylmethyl left-hand side analogues



No.	R	IC_{50} (nM)	MIC (μg/mL)	
		S. aureus MRS	*S. aureus* Oxford	*E. faecalis* 1
44	H	10	≤0.06	≤0.06
45	EtO	7.6	≤0.06	≤0.06

The (1*S*,2*R*)-cyclopentylamine intermediate **16** was coupled by the standard procedure to 3,5-dibromo and 2-ethoxy-3,5-dibromo benzaldehydes.[3] The products **44** and **45** were highly potent antibacterial agents (Table 3). The antibacterial activity of **44** was about 4-fold better than that of the corresponding propyl linker analogue.

The linker analogues retained selectivity for the bacterial enzyme, none of the compounds giving significant inhibition of mammalian (rat liver) MRS up to the highest concentration tested (either 1 or 10 μM).

Compound **45** was tested against panels of clinical isolates of *S. aureus*, *Staphylococcus epidermidis*, *E. faecalis* and *Enterococcus faecium* that include a range of resistant organisms.[3] The MIC90 values (the concentration required to inhibit 90% of the organisms) of the compound were determined. Excellent activity was seen against all the organisms, with MIC90 values at or below 0.125 μg/mL: MIC90's *S. aureus*, 0.06 μg/mL; *S. epidermidis*, 0.125 μg/mL; *E. faecalis*, 0.03 μg/mL; and *E. faecium* $\leq$0.016 μg/mL. The activity against the panel of staphylococci was the best seen for this series, again confirming the beneficial effect of the constrained linker.

In conclusion, we have identified the (1*S*,2*R*)-cyclopentylmethyl moiety as the preferred conformationally restricted linker for the MRS inhibitor series. Incorporation of this linker has resulted in a compound with exceptionally good antibacterial activity against staphylococci and enterococci, including antibiotic resistant strains.

References and Notes

1. Public Health Laboratory Service *Commun. Dis. Rep.* **2001**, *11*, 7.
2. Low, D. E.; Keller, N.; Barth, A.; Jones, R. N. *Clin. Infect. Dis.* **2001**, *32* (Suppl. 2), S133.
3. Jarvest, R. L.; Berge, J. M.; Berry, V.; Boyd, H. F.; Brown, M. J.; Elder, J. S.; Forrest, A. K.; Fosberry, A. P.; Gentry, D. R.; Hibbs, M. J.; Jaworski, D. D.; O'Hanlon, P. J.; Pope, A. J.; Rittenhouse, S.; Sheppard, R. J.; Slater-Radosti, C.; Worby, A. *J. Med. Chem.* **2002**, *45*, 1959.
4. Jarvest, R. L.; Berge, J. M.; Brown M. J.; Brown, P.; Elder, J. S.; Forrest, A. K.; Houge-Frydrych, C. S. V.; O'Hanlon, P. J.; McNair, D. J.; Rittenhouse, S.; Sheppard, R. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 665.
5. Catalyst® 3.1 Accelrys Inc. www.accelrys.com.
6. Conformations of 52 representative molecules including the cyclic linkers (**6**, **7** and **8**) were generated within SYBYL 6.4,[15] and used in hypothesis generation in Catalyst 3.1.[5] Test molecules under consideration were then subject to conformer generation within SYBYL and scored against the hypothesis.
7. Davies, S. G.; Ichihara, O.; Lenoir, I.; Walters, A. S. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1411.
8. Arnould, J. C.; Landier, F.; Pasquet, M. J. *Tetrahedron Lett.* **1992**, *33*, 7133.
9. Abarghaz, M.; Kerbal, A.; Buorguignon, J.-J. *Tetrahedron Lett.* **1995**, *36*, 6463.
10. TEAQ was prepared by reaction of 2-amino-4-ethoxyquinoline with Troc chloride.
11. Takeshita, M.; Chang, C. N.; Johnson, F.; Will, S.; Grollman, A. P. *J. Biol. Chem.* **1987**, *262*, 10171.
12. Tao, T.; Parry, R. J. *Organic Lett.* **2001**, *3*, 3045.
13. Amice, P.; Conia, J. M. *Tetrahedron Lett.* **1974**, *6*, 479.
14. The stereochemistry of the isomers **42** and **43** could not be unambiguously assigned by NMR. The assignment shown is based on their biological activity.
15. *SYBYL® 6.4*. Tripos Inc, St Louis, MO 63144, USA.