Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 2305–2309
Bioorganic & Medicinal Chemistry Letters

Discovery and optimisation of potent, selective, ethanolamine inhibitors of bacterial phenylalanyl tRNA synthetase

Richard L. Jarvest,[a,*] Symon G. Erskine,[b] Andrew K. Forrest,[a] Andrew P. Fosberry,[a] Martin J. Hibbs,[a] Joanna J. Jones,[a] Peter J. O'Hanlon,[a] Robert J. Sheppard[a] and Angela Worby[a]

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

Received 13 December 2004; revised 25 February 2005; accepted 2 March 2005
Available online 30 March 2005

Abstract—High throughput screening of *Staphylococcus aureus* phenylalanyl tRNA synthetase (FRS) identified ethanolamine **1** as a sub-micromolar hit. Optimisation studies led to the enantiospecific lead **64**, a single-figure nanomolar inhibitor. The inhibitor series shows selectivity with respect to the mammalian enzyme and the potential for broad spectrum bacterial FRS inhibition.

The continued spread of bacterial antibiotic resistance and its attendant threat to antimicrobial and general medicine, has been well-documented in medical and scientific publications. The rise of methicillin resistant *S. aureus* (MRSA) has received particular attention but antibiotic resistance in the major pathogens responsible for respiratory tract infections is also of concern. In the search for new molecular targets for novel antibacterial agents that would not be compromised by established resistance mechanisms, we have focused on bacterial aminoacyl tRNA synthetases. These enzymes couple proteinogenic amino acids to their cognate tRNA in an essential step in protein biosynthesis. The enzyme family is validated clinically by the topical antibiotic mupirocin (marketed as Bactroban®) which acts by inhibition of isoleucyl tRNA synthetase.

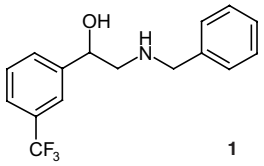
We targeted high throughput screening of the aminoacyl tRNA synthetases from *S. aureus*. For example, we have described the identification of a file compound hit against *S. aureus* methionyl tRNA synthetase and optimisation of the series to give highly potent inhibitors with excellent antibacterial activity.[1–3] Here we describe the discovery and optimisation of a hit against *S. aureus* FRS. Others have also very recently reported the discovery of new inhibitors of bacterial FRS by high throughput screening.[4,5]

In a pioneering series of studies, Santi et al. characterised FRS from *Escherichia coli*[6] and went on to identify phenethylamine derived inhibitors with potency down to 140 nM against the *E. coli* enzyme.[7] Structurally, FRS is a large complex heterodimeric $\alpha_2\beta_2$ enzyme[8] whose subunits are encoded by a linked transcriptional operon in *S. aureus*.[9] Because of the complexity of the enzyme, the high throughput screening of the Smith-Kline Beecham compound bank was carried out with a crude preparation of FRS isolated from wild-type *S. aureus* Oxford. Subsequently, the enzyme from *S. aureus* WCUH29 was cloned, overexpressed in *E. coli*, purified and characterised.[9] This recombinant enzyme was used for testing compounds in IC_{50} assays using the full aminoacylation reaction with an SPA readout.[10,11]

The ethanolamine **1** was identified as potent hit in the high throughput screen. When tested in the standard aminoacylation assay, **1** was found to inhibit *S. aureus* FRS with an IC_{50} value of 160 nM. Further kinetic characterisation of the enzyme inhibition by **1** showed that its mechanism was best described as competitive with respect to phenylalanine but non-competitive with respect to ATP. Compound **1** thus formed an excellent starting point for a hit-to-lead optimisation programme.

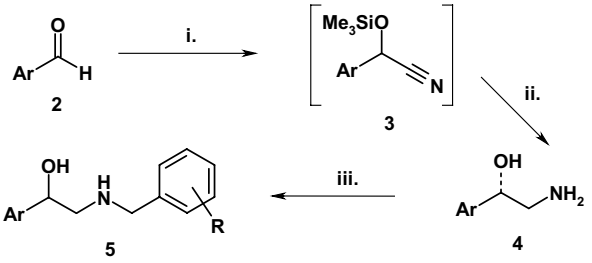
* Corresponding author. Tel.: +44 01438 762074; fax: +44 01438 763620; e-mail: richard.l.jarvest@gsk.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.03.003



1

The most efficient preparation of arrays of analogues of **1** was by reductive alkylation of ethanolamines with aryl aldehydes, as shown in step (iii) of Scheme 1. The limiting factor for this chemistry was the availability of the ethanolamine starting materials **4**. An array methodology for the synthesis of these compounds was developed as shown in steps (i) and (ii) of Scheme 1. In a one-pot process, in situ formation of the silylated cyanohydrins **3** was followed by LAH reduction and parallel normal phase chromatography to afford the ethanolamines **4** in moderate yields and good purity.

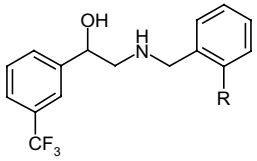


2 3 4 5

Scheme 1. Reagents and conditions: (i) TMSCN (1.1 equiv)/ZnI_2 (0.1 equiv)/Et_2O; (ii) $LiAlH_4/Et_2O/\Delta$; (iii) R-PhCHO/$NaCNBH_3$ or resin $CNBH_3$/AcOH/MeOH.

Initial studies of the SAR of the right hand side phenyl ring showed that substitution was only tolerated at the *ortho* position. The enzyme assay results for *ortho* substituted analogues are reported in Table 1. The carbon-linked and nitrogen-linked substituents of compounds **6–8** resulted in a significant reduction in inhibition. However, several oxygen-linker substituents, such as those of compounds **12** and **13**, afforded a significant enhancement in potency.

Table 1. FRS inhibition of 3-(trifluoromethyl)phenyl ethanolamines



No.	R	IC_{50} (nM) *S. aureus* FRS
6	Me	790
7	CO_2H	2500
8	Morpholino	~10,000
9	OH	100
10	OCF_2H	260
11	OMe	160
12	Oallyl	58
13	OCH_2Ph	50

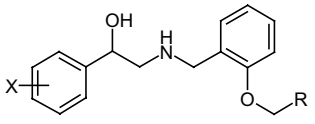
The role of the *meta*-trifluoromethyl group in the left hand side ring was investigated with a series of analogues containing an *ortho* alkoxy substituent in the right hand side ring, as shown in Table 2. It is clear from the positional isomers and di-substitution, that only *meta* substitution is favoured and that further substitution is highly detrimental.

Further analogues to identify the preferred *meta* substituent were then prepared (Table 3). Compact non-polar substituents, such as halogen, methyl and trifluoromethyl were preferred at this position. Larger (**21**) or polar (**25**) substituents resulted in a significant reduction in potency.

Amongst the compounds synthesised in the *meta*-substituted array was the bromothiophene analogue **34**. This had an FRS IC_{50} value of 50 nM, a 3-fold improvement over the corresponding (trifluoromethyl)phenyl analogue. The consistent improvement in properties of the bromothiophene resulted in this becoming the preferred left hand side moiety.

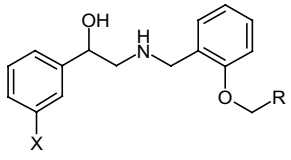
Thus to enhance potency further, the SAR of the 2-alkoxy position was developed in the bromothiophene series. Several groups of analogues were prepared via array chemistry at the benzaldehyde level. Direct alkylations of salicylaldehyde (**26**) resulted in final compounds such

Table 2. FRS inhibition of (trifluoromethyl)phenyl ethanolamines

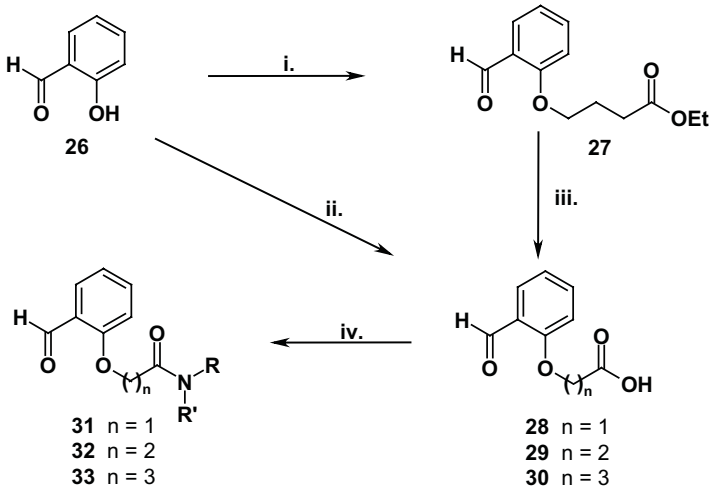


No.	X	R	IC_{50} (nM) *S. aureus* FRS
14	2-CF_3	H	>10,000
11	3-CF_3	H	150
15	4-CF_3	H	>10,000
16	2,5-Di-CF_3	H	>10,000
17	3,5-Di-CF_3	Ph	>10,000

Table 3. FRS inhibition of 3-(substituted)phenyl ethanolamines



No.	X	R	IC_{50} (nM) *S. aureus* FRS
11	CF_3	H	150
18	Br	H	110
19	Me	H	110
20	OCF_3	H	190
21	OCH_2Ph	H	>10,000
13	CF_3	Ph	50
22	Cl	Ph	31
23	I	Ph	32
24	OMe	Ph	83
25	CH_2OH	Ph	570



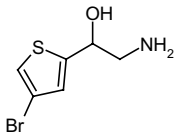
Scheme 2. Reagents and conditions: (i) $Br(CH_2)_3CO_2Et/K_2CO_3/DMF/\Delta$ (96%); (ii) propiolactone/NaH/DMF/Δ (35%); (iii) KOH/DMF (94%); (iv) RR′NH/EDC/HOAt/THF/DMF (50–70%).

as **44**–**48**. More remote diversity was introduced by amide arrays as shown in Scheme 2. The acid intermediates were prepared by bromoester alkylation and base hydrolysis (**30**), or by propiolactone alkylation (**29**), whilst **28** was commercially available. The acids were converted to amides by standard water-soluble carbodiimide coupling methodology.

In contrast to the relatively tight SAR constraints elsewhere in the molecule, a wide variety of *ortho*-alkoxy substituents was tolerated as evidenced by many analogues with IC_{50} values <50 nM in Table 4. A diverse range of functional groups is accommodated, including hydrogen bond donors and acceptors and heterocycles, as well as ionisable groups such as amine and carboxylic acid. Reduced activity was seen only for the phenoxy compound **35** and several of the analogues with a nitrogen atom separated by two carbons from the *ortho* oxygen (**39**, **40**, **51**, **52**). The diverse functionality possible at this position allows tuning of the overall physicochemical properties of the inhibitors. The tolerance for such a range of polar functional groups suggests that in the enzyme–inhibitor complex, the alkoxy group is likely to be at least partially solvent-exposed on the protein surface.

Representative compounds from the series were tested for selectivity against mammalian FRS, isolated from rat liver. Very little inhibition of the mammalian enzyme was observed. For example, compound **47** had an IC_{50} of 90,000 nM, resulting in a selectivity ratio of roughly 5000-fold in favour of the bacterial enzyme.

The racemic amine **61** was resolved by chiral HPLC on a Chiralpak AD column, affording the enantiomers **62** (faster eluting) and **63** (slower eluting) with 99.8% ee and 98.0% ee, respectively. The absolute configuration of the enantiomers was assigned by ab initio optical rotation.[12] The enantiomeric amines were taken through in the usual way to the (*R*)- and (*S*)-enantiomers of **38**, compounds **64** and **65**, respectively. When tested against *S. aureus* FRS, **64** had an IC_{50} value of 3.7 nM whereas for **65** the IC_{50} was >1000 nM. Thus the inhibition is highly enantiospecific with a ratio of over 250-fold between the two isomers.

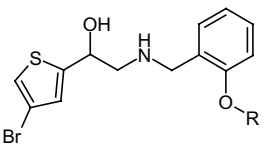


61 racemic
62 (*R*)-enantiomer
63 (*S*)-enantiomer

In view of the high affinity of the compounds for *S. aureus* FRS, we wished to test the spectrum of inhibition against FRS enzymes from organisms that are important pathogens in respiratory tract infections. The FRS enzymes from *Streptococcus pneumoniae* and *Haemophilus influenzae*, the key Gram-positive and Gram-negative RTI pathogens, were overexpressed and purified essentially as described for the *S. aureus* enzyme.[9] Selected analogues were tested in assays against these enzymes and the results are shown in Table 5.[13] The initial hit **1** did not inhibit the *H. influenzae* enzyme at the highest concentration tested. In contrast, the optimised compounds **48** and **64** showed a relatively balanced spectrum of inhibition across the three species. All three enzymes had a strong preference for the (*R*) stereochemistry. These results are encouraging for the potential of an RTI spectrum of FRS inhibition.

Despite potent inhibition of *S. aureus* FRS, none of these compounds showed whole cell antibacterial activity against the *S. aureus* organism, when tested at concentrations up to 64 μg/mL. Although it is possible that the target potency needs improving still further, the extremely poor antibacterial activity seems likely to be due to issues of bacterial penetration or efflux. In artificial membrane assays, representative analogues from the series showed high permeability (data not shown). Thus bacterial efflux may be the major factor contributing to the large discrepancy between enzyme potency and antibacterial activity for the ethanolamine inhibitors.

Table 4. FRS inhibition of 4-bromo-2-thienyl ethanolamines



No.	R	IC_{50} (nM) *S. aureus* FRS
34	Me	50
35	Ph	120
36	CH_2OMe	35
37	CH_2CN	49
38	$(CH_2)_2OH$	8
39	$(CH_2)_2NH_2$	220
40	$(CH_2)_2$phthalimido	250
41	$(CH_2)_2$morpholino	18
42	Allyl	18
43	CH_2Ph	10
44	$CH_2(4\text{-}MeSO_2Ph)$	26
45	CH_2(2-pyridyl)	43
46	CH_2(3-pyridyl)	18
47	CH_2(4-pyridyl)	16
48	CH_2(2-benzimidazolyl)	26
49	CH_2CONH_2	42
50	$CH_2CONHEt$	32
51	$CH_2CONHCH_2CONHMe$	120
52	$CH_2CONMe(CH_2)_2OH$	110
53	$(CH_2)_2CO_2H$	58
54	$(CH_2)_2CONHCH_2CONHMe$	36
55	$(CH_2)_2CONMe(CH_2)_2OH$	25
56	$(CH_2)_3CO_2H$	43
57	$(CH_2)_3CONHCH_2CONHMe$	30
58	$(CH_2)_3CONMe(CH_2)_2OH$	26
59	$(CH_2)_4NH_2$	22
60	$(CH_2)_4$phthalimido	69

Table 5. Inhibition of various bacterial FRS enzymes by selected analogues

	Stereochem.	FRS IC_{50} (nM)		
		Streptococcus aureus	*Streptococcus pneumoniae*	*Haemophilus influenzae*
1	*RS*	160	ND	NI @ 300
43	*RS*	10	280	1500
48	*RS*	26	250	150
64	*R*	3.7	190	56
65	*S*	>1000	>1000	>1000

ND—not done; NI—no inhibition.

In summary, a sub-micromolar HTS hit against *S. aureus* FRS was identified and optimised to give a highly potent sub-10 nM enantiospecific inhibitor. Whilst the series was selective with respect to mammalian FRS and showed significant broad spectrum inhibition, useful antibacterial activity against *S. aureus* was not attained.

Acknowledgments

We thank Margarita Rodriguez-Lens and David McNair for assistance with preparation of compounds **39/40** and **64/65**, respectively, Greg Jonas for the chiral preparative chromatography to resolve **62/63**; Doug Minick for assignment of absolute configuration; Stephen Rittenhouse and his team for antibacterial testing; and Nicola Wallis and Christine Richardson for useful discussions.

References and notes

1. 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.
2. 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.
3. Jarvest, R. L.; Berge, J. M.; Brown, P.; Houge-Frydrych, C. S. V.; O'Hanlon, P. J.; McNair, D. J.; Pope, A. J.; Rittenhouse, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1265.
4. (a) Yu, X. Y.; Finn, J.; Hill, J. M.; Wang, Z. G.; Keith, D.; Silverman, J.; Oliver, N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1339; (b) Yu, X. Y.; Finn, J.; Hill, J. M.; Wang, Z. G.; Keith, D.; Silverman, J.; Oliver, N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1343.
5. Beyer, D.; Kroll, H.-P.; Endermann, R.; Schiffer, G.; Siegel, S.; Bauser, M.; Pohlmann, J.; Brands, M.; Ziegelbauer, K.; Haebich, D.; Eymann, C.; Brötz-Oesterhelt, H. *Antimicrob. Agents Chemother.* **2004**, *48*, 525.
6. (a) Santi, D. V.; Danenberg, P. V.; Satterly, P. *Biochemistry* **1971**, *10*, 4804; (b) Santi, D. V.; Danenberg, P. V. *Biochemistry* **1971**, *10*, 4813; (c) Santi, D. V.; Danenberg, P. V.; Montgomery, K. A. *Biochemistry* **1971**, *10*, 4821.
7. (a) Anderson, R. T., Jr.; Santi, D. V. *J. Med. Chem.* **1976**, *19*, 1270; (b) Santi, D. V.; Cunnion, S. O.; Anderson, R. T., Jr.; Webster, R. W., Jr. *J. Med. Chem.* **1979**, *22*, 1260.
8. Mosyak, L.; Reshetnikova, L.; Goldgur, Y.; Delarue, M.; Safro, M. G. *Nat. Struct. Biol.* **1995**, *2*, 537.
9. Savopoulos, J. W.; Hibbs, M.; Jones, E. J.; Mensah, L.; Richardson, C.; Fosberry, A.; Downes, R.; Fox, S. G.; Brown, J. R.; Jenkins, O. *Protein Expr. Purif.* **2001**, *21*, 470.
10. Macarrón, R.; Mensah, L.; Cid, C.; Carranza, C.; Benson, N.; Pope, A.; Diez, E. *Anal. Biochem.* **2000**, *284*, 183.
11. Assay conditions used $1 \times K_m$[Phe], $10 \times K_m$[ATP] and a large excess of tRNA.
12. Specific rotations were calculated for models of 2-amino-1-(4-chlorothien-2-yl)ethanol at the sodium D line using HF/SCF wavefunctions with the 6-31G* basis set (Cl was used in place of Br as fourth row elements are not supported in the current version of the Dalton program).[14] Predicted specific rotations were averaged using Boltzmann statistics. Experimental values were measured in methanol (c = 0.004 mg/mL, 20 °C) at the sodium D line and are compared to the predicted values:

	$[\alpha]_D$ (calcd)		$[\alpha]_D$ (exp)	$[\alpha]_D$ (calcd − exp)	
	R	*S*		*R*	*S*
62	+9	−9	+13	−4	−22
63	+9	−9	−14	+22	+5

Both the sign and magnitude of rotation are in good agreement for **62** as (*R*) and **63** as (*S*). The sign of the rotation predicted in this way is correctly predicted in about 95% of cases for small molecules, although the exact magnitude may be more variable.[15,16] It is considered that the current configurational assignment is highly reliable. Moreover, this assignment is consistent with the sign of rotation of all literature aryl and heteroaryl ethanolamine enantiomers.

13. For each bacterial enzyme assay, the concentration of phenylalanine was equal to K_m for that enzyme, to allow comparison across the species.
14. 'Dalton', a molecular electronic structure program, Release 1.2, **2001**, written by T. Helgaker et al.
15. Polavarapu, P. L. *Mol. Phys.* **1997**, *91*, 551.
16. Stephens, P. J.; Devlin, F. J.; Cheeseman, J. R.; Frisch, M. J. *J. Phys. Chem. A* **2001**, *105*, 5356.