Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 4040–4043
Bioorganic & Medicinal Chemistry Letters

Structure–activity relationships of bioisosteres of a carboxylic acid in a novel class of bacterial translation inhibitors

J. Craig Ruble,[a] Brian D. Wakefield,[a] Gregg M. Kamilar,[a] Keith R. Marotti,[b] Earline Melchior,[b] Michael T. Sweeney,[b] Gary E. Zurenko[b] and Donna L. Romero[a,*]

[a]*Medicinal Chemistry, Pharmacia Corporation, 301 Henrietta Street, Kalamazoo, MI 49001, USA*
[b]*Infectious Diseases Biology, Pharmacia Corporation, 301 Henrietta Street, Kalamazoo, MI 49001, USA*

Received 3 April 2007; accepted 24 April 2007
Available online 27 April 2007

Abstract—The discovery and initial optimization of a novel anthranilic acid derived class of antibacterial agents which suffered from extensive protein binding has been previously reported. The structure–activity relationships around the carboxylic acid substituent are described herein. This acid was replaced by several alternative functional groups in attempts to retain bioactivity while reducing protein binding. Only groups with an acidic proton retained activity, and analogs containing those groups maintained the protein binding inherent to this class of antibacterial agents.

In efforts to overcome the rising problem of bacterial resistance,[1–3] our antibacterial program has focused on identifying lead compounds with novel mechanisms of action. We successfully identified a novel class of translation inhibitors through the use of high throughput screening and medicinal chemistry optimization (Fig. 1).[4] Advanced compounds in this series failed to show activity in a standard mouse bacteremia model of infection, likely due to their high protein binding. Subsequent optimization efforts focused on further improving the potency and reducing the affinity of these leads for serum proteins and led to the synthesis of analog **3**.[5,6] Since these compounds contain a carboxylic acid and since human serum albumin is known to bind aromatic carboxylic acids such as salicylates and ibuprofen,[7] we began systematically replacing the carboxylic acid in our series with alternative functional groups in an effort to reduce the extent of protein binding. This paper describes the results of these efforts.

Initial attempts to replace the 2-carboxylic acid focused on replacing the acid with a variety of other functional groups to ascertain the impact on bioactivity. Synthesis of the requisite analogs was accomplished through the use of common intermediate **4** which can be made in large quantities and easily stored (Scheme 1). Conversion of acid intermediate **4** to the acid chloride with oxalyl chloride in methylene chloride with catalytic DMF afforded **5**. Reaction of **5** with commercially available methyl 2-amino-5-bromobenzoate followed by hydrolysis yielded carboxylic acid **6** which we used as our standard for activity comparisons. Reaction of **5** with other appropriately substituted anilines afforded the desired analogs of interest, **7–19**.

In general, the requisite bromoanilines were easily prepared via bromination of the 2-substituted anilines with NBS in DMF at room temperature. The non-commercially available trifluoromethyl-sulfonamido(aniline) was synthetically accessible via monosulfonylation of

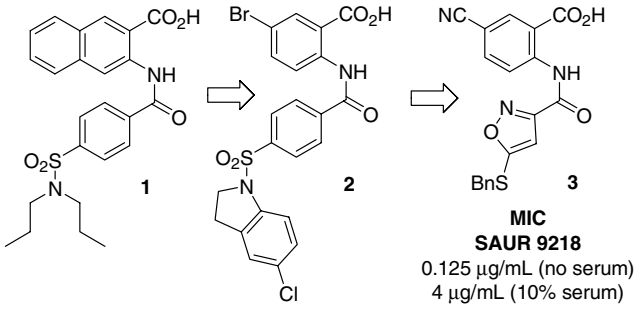
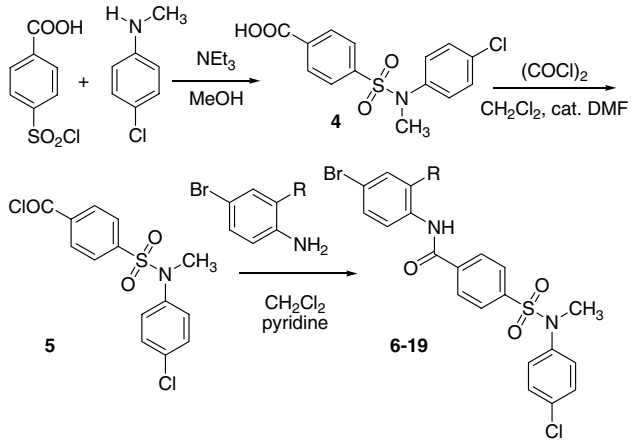


Figure 1. Evolution of translation inhibitor lead series.

Keywords: Bioisostere; Antibacterial; Translation inhibitor; Protein binding; Carboxylic acid.
* Corresponding author. E-mail: donna.romero@sbcglobal.net

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.04.074

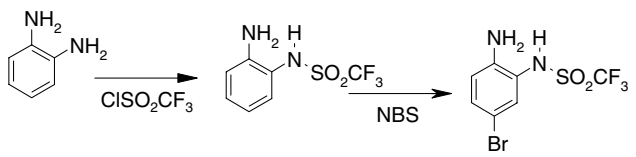


Scheme 1. Preferred synthesis of 5-bromo aryl analogs containing carboxyl replacements.

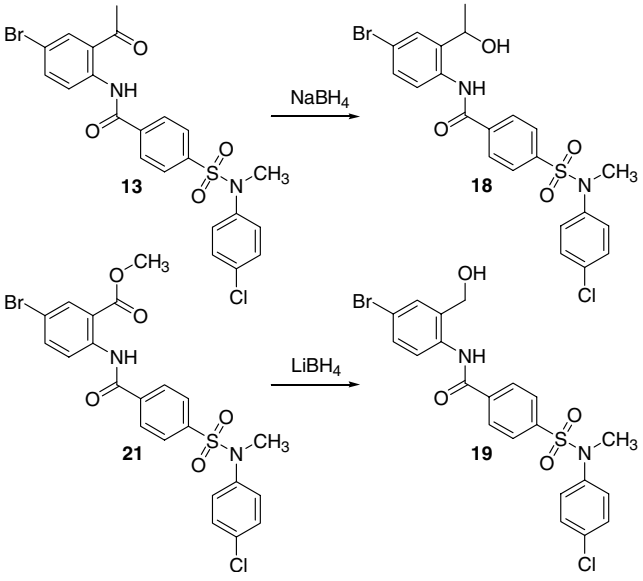
1,2-phenylenediamine (Scheme 2). Some of the desired analogs were not synthesized utilizing the route shown in Scheme 1 either because the functional groups were not compatible with the amide bond-forming conditions, or because they were more readily synthesized via an alternate route. For example, reduction of the methylketone **13** or the methyl ester **21** led to the secondary or primary alcohols **18** and **19**, respectively (Scheme 3).

Analogs synthesized by these routes and the corresponding bioassay data in the transcription/translation (T/T) inhibition assay[8] and the antibacterial assay versus *Staphylococcus aureus* are shown in Table 1. Substitution of the carboxylic acid moiety by most functional groups resulted in analogs devoid of activity (**7**–**16**; **18**–**19**). The only analog showing significant activity in both the T/T inhibition assay and the antibacterial assay was the 2-(trifluoromethyl)sulfonamido analog **17**, however it was less potent in the antibacterial assay. These initial results indicated that the acidic moiety is critical for activity (Table 1). The pK_a's of the only two active compounds are calculated to be 2.96 for the acid compound **6** and 4.28 for the (trifluoromethyl)sulfonamido analog **17**.[9]

In addition to their high overall lipophilicity and molecular weight, we continued to believe that analogs in this series suffered from extensive protein binding in part due to the presence of the carboxylic acid. After confirming that many of the active analogs were bound tightly to human serum albumin (HSA), which accounts for 60% of the total mass of plasma proteins, we felt that there was still a compelling reason to investigate acid replace-



Scheme 2. Synthesis of a non-commercially available aniline.



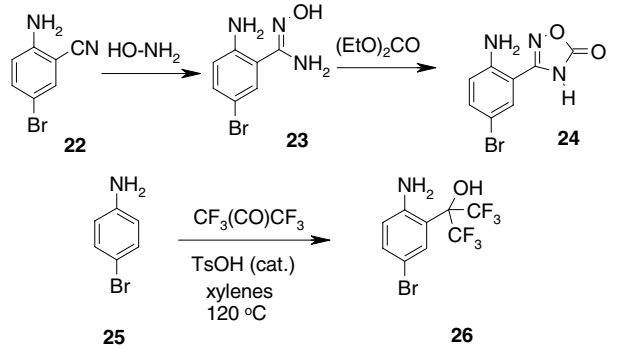
Scheme 3. Alternate synthesis of analogs via direct elaboration of 2-substituent.

Table 1. Activity of compounds synthesized according to Schemes 1–3

Compound	R	% T/T inhibition[a] at 100 μM	SAUR MIC[b] (μg/mL)
6	–COOH	79–82	8–16
7	–H	11	>128
8	$-CH_3$	12	>128
9	–Br	6	>128
10	–CN	0	>128
11	$-NO_2$	0	>128
12	$-SO_2NH_2$	0	>128
13	$-COCH_3$	0	>128
14	$-CONH_2$	13	>128
15	$-CONHOCH_3$	7	>128
16	$-NHSO_2CH_3$	19	>128
17	$-NHSO_2CF_3$	72	32
18	$-CH(OH)CH_3$	7	>128
19	$-CH_2OH$	4	>128

[a] *Staphylococcus aureus* coupled transcription–translation assay (Ref. 8).

[b] Minimum inhibitory concentration versus *Staphylococcus aureus* UC9218.



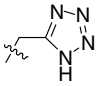
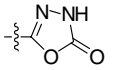
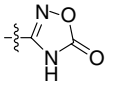
Scheme 4. Synthesis of specialized anilines bearing bioisosteres.

ments. Therefore, we next turned our attention to the synthesis of a variety of known carboxylic acid bioisosteric replacements[10] to explore their activity and protein binding properties.

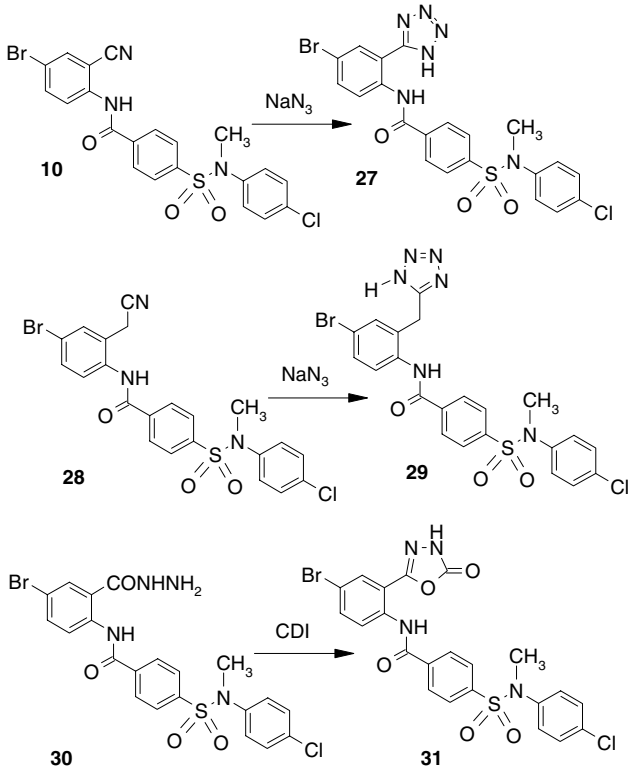
Some of the requisite analogs (**32**, **33**) were prepared via Scheme 1 using the appropriately substituted anilines synthesized as depicted in Scheme 4. For example, nitrile **22** was treated with hydroxylamine to afford amidoxime **23**, which when treated with diethyl carbonate afforded the elaborated aniline **24**. Similarly, treatment of 4-bromoaniline **25** with hexafluoroacetone and catalytic toluenesulfonic acid provided elaborated aniline **26**. Tetrazoles **27** and **29** were prepared via elaboration of the corresponding nitriles **10** and **28** (Scheme 5). Oxadiazolone **31** was prepared from the corresponding hydrazide **30** with CDI.

Table 2 contains the results of the T/T inhibition assay and the antibacterial activity of these bioisostere analogs. The extended tetrazole analog **29** exhibited the poorest activity from this group. Tetrazole **27** and oxadiazolone **32** exhibited good T/T inhibition and antibacterial activity comparable to the carboxylic acid (MICs ~16 μg/mL). Oxadiazolone **31** and the hexafluoro-isopropanol **33** exhibited more modest T/T inhibition but good antibacterial activities (MICs of 2 and 0.5 μg/mL, respectively).[11] The calculated pK_a's for the bioisosteres are also shown in Table 2. Even though the bioisosteres' calculated pK_a's are not as low as that of the carboxylic acid, several appear to be suitable replacements in terms of potency and seemed to offer promise in terms of reducing the protein binding.

Table 2. Activity of compounds synthesized according to Scheme 1

Compound	R	% T/T Inhib. at 100 μM	SAUR 9218 MIC[a] (μg/mL)	pK_a[b]
6	–COOH	79–82	8–16	2.96
27		96	16	3.75
29		46	>128	4.85
31		60	2	6.37
32		98	8–16	6.55
33	–C(OH)(CF_3)$_2$	41	0.5	8.50

[a] Minimum inhibitory concentration. *Staphylococcus aureus* UC9218.
[b] pK_a calculated using PALLAS.



Scheme 5. Synthesis of bioisosteres via direct elaboration of 2-substituent.

To determine whether the bioisosteres' performance in the presence of serum was superior to that of the parent acid, they were tested in the antibacterial assay in the absence and presence of both 5% and 10% serum (Table 3). By evaluating the ratio of MICs in the presence and absence of 5% serum (column entitled 'Ratio') it is apparent that relative to the acid, the bioisosteres have a similar (compound **32**) or even greater degree (compound **33**) of protein binding. The increased antibacterial potency[11] of compound **33** is helpful in that some antibacterial activity in the presence of 5% HSA is retained. Vancomycin and novobiocin were used as standards to gauge what level of antibacterial activity in the presence of HSA was needed to observe an in vivo effect. Clearly, MICs in the presence of 10% HSA for both these standards were significantly lower

Table 3. Activity of selected bioisosteres in the presence of serum

Compound	MIC		Ratio[c]	MIC
	SAUR[a]	5% HSA[b]		10% HSA[d]
6	8–16	128	8–16	>128
31	2	—	—	>128
32	8–16	>128	>8–16	>128
33	0.5	64	128	>128
Vancomycin[e]	1	1	1	1
Novobiocin	>0.125	0.5	—	1

[a] Minimum inhibitory concentration (μg/mL). *Staphylococcus aureus* UC9218.
[b] *Staphylococcus aureus* UC9218 + 5% pooled human serum. Human serum (male, from Sigma) was thawed at room temperature, then placed in a 56 °C water bath for 30 min. The serum was filtered using a 0.2 μm filtration system.
[c] Ratio = MIC (5% HSA)/MIC (0% HSA).
[d] *Staphylococcus aureus* UC9218 + 10% pooled human serum.
[e] Positive control vancomycin.

than the acid or its bioisosteres. For the translation inhibitor compounds, even the addition of just 10% HSA caused all antibacterial activity to be lost.

Conclusion. In an effort targeted at reducing the protein binding of this series of translation inhibitors, the SAR surrounding the acid substituent was explored to probe whether it could be replaced with functional groups less likely to bind to HSA. A trifluoromethylsulfonamide replacement (**17**) was the only functional group that came close to the activity of the carboxylic acid. Our results indicate that a functional group containing an acidic proton is required for good potency. In addition, some bioisosteres, such as a oxadiazolone (**32**), could replace the acid and offered comparable bioactivity. The bioisosteres did not offer any advantages over the acid in terms of reducing protein binding. These results led to a future chemistry strategy which focused on the use of the carboxylic acid moiety in the design of new analogs.

References and notes

1. Cohen, M. L. *Science* **1992**, *257*, 1050.
2. (a) Allen, N. E. In *Progress in Medicinal Chemistry*; Ellis, G. P., Luscombe, D. K., Eds.; Elsevier Science: New York, 1995; Vol. 32, p 157, Chapter 4; (b) Hayes, J. D.; Wolf, C. R. *Biochem. J.* **1990**, *272*, 281; (c) Spratt, B. G. *Science* **1994**, *264*, 388; (d) Nikaido, H. *Science* **1994**, *264*, 382.
3. (a) Russell, A. D. In *Progress in Medicinal Chemistry*; Ellis, G. P., Luscombe, D. K., Oxford, A. W., Eds.; Elsevier Science: New York, 1998; Vol. 35, Chapter 4 (b) Fuchs, T. M. *Naturwissenschaften* **1998**, *85*, 99; (c) Cunha, B. A. *Drugs Today* **1998**, *34*, 691.
4. Larsen, S. D.; Hester, M. R.; Ruble, J. C.; Kamilar, G. M.; Romero, D. L.; Wakefield, B.; Melchior, E. P.; Sweeney, M. T.; Marotti, K. R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6173.
5. Li, J.; Wakefield, B. D.; Ruble, J. C.; Stiff, C. M.; Romero, D. L.; Marotti, K. R.; Sweeney, M. T.; Zurenko, G. E.; Rohrer, D. C.; Thorarensen, A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2347.
6. Thorarensen, A.; Wakefield, B. D.; Romero, D. L.; Marotti, K. R.; Sweeney, M. T.; Zurenko, G. E.; Rohrer, D. C.; Han, F.; Bryant, G. L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2823.
7. Peters, T., Jr. *All About Albumin: Biochemistry, Genetics, and Medical Applications*; Academic Press: New York, 1996.
8. Murray, R. W.; Melchior, E. P.; Hagadorn, J. C.; Marotti, K. R. *Antimicrob. Agents Chemother.* **2001**, *45*, 1900.
9. ACD Labs' pK_a predictor was used for these calculations: http://www.acdlabs.com/.
10. (a) Chen, Xiaoqi; Wang, Weibo The Use of Bioisosteric Groups in Lead Optimization. In *Annual Reports in Medicinal Chemistry*; Doherty, A., Ed.; Elsevier, 2003; Vol. 38, pp 333–346; (b) Wermuth, Camille G. Molecular Variations Based on Isosteric Replacements. In *Practice of Medicinal Chemistry*; Wermerth, C., Ed.; Academic Press, 2003; pp 189–214.
11. The lack of correlation of T/T activity with antibacterial activity as well as previous reports of 1,1,1,3,3,3-hexafluoro-2-phenyl-2-propanol serving as an antibacterial agent suggest that compound **33** may act via a different mechanism, see: Sidorenko, S. S.; Aizenberg, S. Z.; Blakitnyi, A. N.; Fialkov, Y. A.; Yagupol'skii, L. M. *Fiziologicheski Aktivnye Veshchestva* **1972**, *4*, 41.