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Preparation of novel antibacterial agents. Replacement of the central aromatic ring with heterocycles

Jianke Li, Brian D. Wakefield, J. Craig Ruble, Cory M. Stiff, Donna L. Romero, Keith R. Marotti, Michael T. Sweeney, Gary E. Zurenko, Douglas C. Rohrer and Atli Thorarensen*

Medicinal Chemistry and Infectious Diseases Biology, Pharmacia Corporation, 301 Henrietta Street, Kalamazoo, MI 49001, USA

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Abstract—Discovery of novel antibacterial agents is a significant challenge. We have recently reported on our discovery of novel antibacterial agents in which we have rapidly optimized potency utilizing a parallel chemistry approach. These advanced leads suffer from high affinity for human serum albumin (HSA). In an effort to decrease the affinity for HSA we have prepared a series of heterocyclic analogs, which retained antibacterial activity and demonstrated reduced affinity for HSA.

Bacterial resistance is a significant problem in the treatment of bacterial infections.[1–3] This emerging resistance has fueled a continuous search for new antibiotics resulting in numerous commercially available products. The pharmaceutical industry rapidly took advantage of the wealth of novel targets available as a result of the genomic revolution. Despite the expectation that these new targets would decrease the hurdle in identifying novel classes of antibacterial agents, discovery of compounds that act via novel mechanisms remains a significant challenge. The major obstacle appears to be the identification of novel drugable chemical matter.[4] In the past several years, we have had an extensive research program utilizing a range of approaches in search of novel antibacterials. This includes our work directed toward specific targets such as efflux pumps,[5] PDF,[6] as well as the identification of whole cell active compounds coupled with a reverse pharmacology approach to try to identify the targets against which such compounds are acting.[7]

An attractive target for antibacterial drug discovery is bacterial protein synthesis. The Pharmacia compound collection was screened utilizing a transcription translational assay[8] which resulted in the identification of a hit that was a complex mixture exhibiting both whole cell antibacterial activity and inhibition in the transcription translation screening assay. After extensive fractionation, the structure of the active component was elucidated and determined to be **1** (Fig. 1). Our medicinal chemistry group rapidly transformed this complex unattractive compound into the lead anthranilic amide molecule **2**, which is very attractive for lead optimization utilizing array chemistry. We have recently reported[9] on the optimization of **2** resulting in the identification of the advanced compound **3** which displayed potent broad-spectrum antibacterial activity, but not in vivo

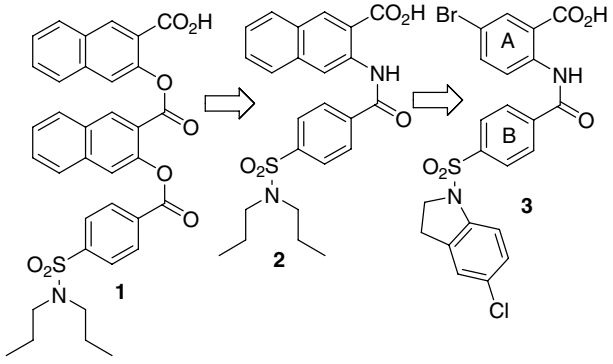

Figure 1. Progression in lead optimization.

Keywords: Anthranilic acid; Antibacterial; Protein binding; Human serum albumin.
* Corresponding author at present address: Pfizer, 700 Chesterfield Parkway West AA2G, Chesterfield, MO 63017, USA. Tel.: +1 636 247 3962; fax: +1 636 247 0250; e-mail: atli.thorarensen@pfizer.com

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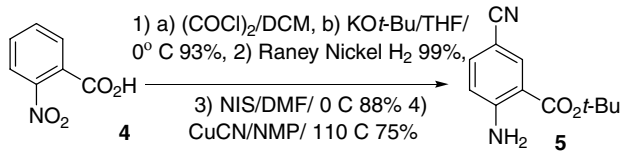
Table 1. Antibacterial activity of initial lead matter

Compound	MIC (μg/mL)[a]					
	SAUR[b]	SAUR[c]	EFAE[d]	SEPI[e]	SPNE[f]	HINF[g]
1	16	ND	32	16	8	>128
2	64	>128	128	16	64	>128
3	0.5	>128	32	4	4	128
Vancomycin	0.5	2.0	1.0	1.0	0.25	0.015

[a] Minimal inhibitory concentration.
[b] *S. aureus* UC 9218.
[c] *S. aureus* UC 9218 + 5% 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 then filtered using a 0.2 micron filtration system.
[d] *Enterococcus faecalis* UC 9217.
[e] *S. epidermidis* UC 12084.
[f] *S. pneumoniae* UC 9912.
[g] *Haemophilus influenzae* 30063.



Scheme 1.

activity in a standard *Staphylococcus aureus* mouse model (Table 1). The lack of in vivo activity was attributed to the high human serum albumin (HSA) binding affinity of compound **3**. In this letter, we will describe our approach for replacing the central ring with a variety of heterocycles.

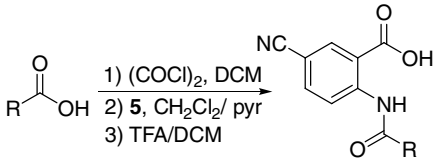
Previous optimization of the A-ring led to the identification of several 5-substituents with good activity.[9] For the purposes of this work, we elected to utilize a 5-cyano substituent.[10] In order to rapidly evaluate the replacement of the B-ring with various heterocycles, we required a suitable A-ring building block where the acid is protected as an acid-labile ester to simplify the final deprotection. Nitrile **5** was synthesized via a simple 4-step procedure (Scheme 1). Conversion of 2-nitrobenzoic acid to the corresponding acid chloride utilizing oxalyl chloride was uneventful; subsequent treatment with potassium *tert*-butoxide in THF cleanly afforded the desired *tert*-butyl ester. This acylation has been scaled uneventfully to >60 g. Reduction followed by iodination provided an intermediate suitable for the installment of the cyanide. The iodide was converted to the nitrile utilizing CuCN in variable yield. We utilized this route to generate over 25 g of the desired nitrile. Subsequently this route was improved by Pharmacia's process research group to provide kg quantity in a reliable manner.[11]

Criteria for the selection of building blocks included (a) commercial availability, (b) existence in our compound collection, or (c) easy preparation from known procedures. Furthermore, previous SAR studies had illustrated that the sulfonamide attached to the B-ring could be replaced with a variety of groups such as a phenyl ring. Therefore, we elected to use a phenyl substituent in place of the sulfonamide due to availability of the appropriate building blocks.

The analogs were prepared as outlined in Scheme 2, acylation followed by acidic deprotection provided the final analogs in high yields and purities, Table 2.

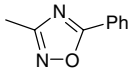
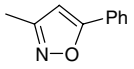
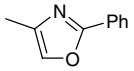
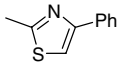
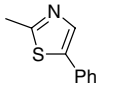
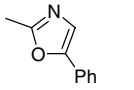
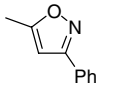
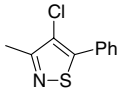
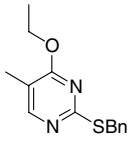
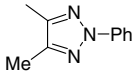
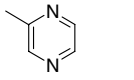
In general most of the building blocks contained a comparable substitution pattern. Table 2 contains selected examples and activities of the various 5- and 6-membered heterocyclic analogs that we prepared, and Table 3 contains selected examples and activities of fused bicyclic analogs. In monitoring progress, we decided early on that improving potency in the whole cell assay versus *S. aureus* would be the driving factor instead of potency in the transcription translation assay. Furthermore, after evaluating many approaches to measuring albumin affinity, we decided the best measure of progress in reducing protein binding was to run the *S. aureus* assay in the presence of 10% serum. Utilizing this gauge it is clear that several heterocyclic replacements were extremely successful replacements for the B-phenyl ring. For example in the monocyclic series (Table 2), isoxazole **7**, thiazole **10**, and oxazole **11** provided potent antibacterial agents with decreased affinity for HSA. In the bicyclic series (Table 3), the indole **17**, benzisoxazole **18**, and benzthiazole derivative **20** demonstrated very promising antibacterial activity.

The identification of several potent heterocyclic replacements for the B-ring phenyl of **3** validated our approach which was designed to improve the potency of new analogs, while reducing lipophilicity and maintaining or reducing the molecular weight. In order to further validate this approach, we elected to prepare more specific analogs containing one of the promising new



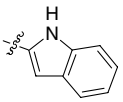
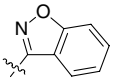
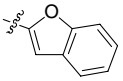
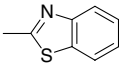
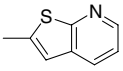
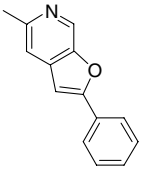
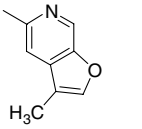
Scheme 2.

Table 2. Antibacterial activity of selected 5- and 6-membered heterocyclic derivatives

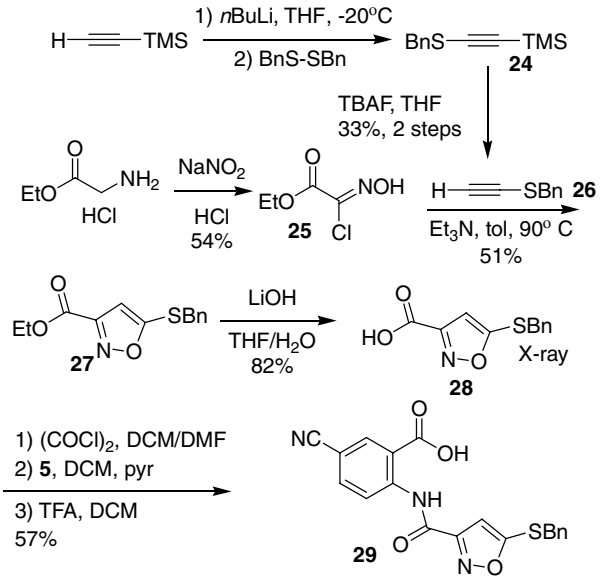
R	Compound	Yield %	MIC (μg/mL)[a]	
			SAUR[b]	10%[c] Serum
	**6**	37	4	32
	**7**	76	0.25	8
	**8**	70	64	>128
	**9**	45	8	32
	**10**	41	1	16
	**11**	42	0.5	8
	**12**	17	0.5	8
	**13**	37	0.5	32
	**14**	12	8	>128
	**15**	60	4	>128
	**16**	17	128	>128

[a] Minimal inhibitory concentration.
[b] *S. aureus* UC 9218.
[c] *S. aureus* UC 9218 + 10% serum.

Table 3. Antibacterial activity of selected heterobicyclic derivatives

R	Compound	Yield %	MIC (μg/mL)[a]	
			SAUR[b]	10%[c] Serum
	**17**	36	2	32
	**18**	ND	0.5	16
	**19**	27	2	32
	**20**	30	0.25	8
	**21**	48	4	32
	**22**	10	>128	>128
	**23**	17	16	64

[a] Minimal inhibitory concentration.
[b] *S. aureus* UC 9218.
[c] *S. aureus* UC 9218 + 5% serum.



Scheme 3.

heterocycles, but with a more optimal substitution pattern. We had previously identified benzyl thioether as a favorable substituent which resulted in very potent analogs. Therefore, we elected to prepare the benzylthioether analog of isoxazole **7**. The synthesis relies on a cycloaddition to form the isoxazole core (Scheme 3). The requisite benzyl ethynyl sulfide **25** was prepared by the addition of lithium acetylide to a disulfide forming **24**, which was followed by deprotection of the acetylene. The isoxazole **27** was then prepared by a [3+2] cycloaddition.[12] The cycloaddition reaction was extensively optimized to minimize the formation of isomeric and dimeric products.

The optimal reaction conditions afforded a single regioisomeric product **27** when Et_3N was added dropwise to a heated toluene solution containing both reaction partners.[13] In order to secure the assignment of the regiochemistry, an X-ray structure was obtained of the

Table 4. Antibacterial properties of **29**

Compound	MIC (μg/mL)[a]				
	SAUR[b]	10%[c] Serum	SPNE[d]	EFAE[e]	SEPI[f]
29	0.125	4	>128	>128	0.125

[a] Minimal inhibitory concentration.
[b] *S. aureus* UC 9218.
[c] *S. aureus* UC 9218 + 10% 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 then filtered using a 0.2 micron filtration system
[d] *Streptococcus pneumoniae* UC 9912.
[e] *Enterococcus faecalis* UC 9217.
[f] *S. epidermidis* UC 12084.

corresponding acid **28**. The subsequent conversion was performed as described for **7** affording **29** in modest yield.

Compound **29** demonstrated potent antibacterial activity against *S. aureus* both in presence and absence of serum. Despite this potent activity, this compound was not broad-spectrum and there were several strains of important pathogens where **29** demonstrated sub-optimal activity (Table 4). The improvement of spectrum of activity against medically important pathogens required further optimization in this series of novel antibacterial agents.

In summary, we have prepared a diverse set of heterocyclic B-ring replacements. Several of these replacements have potent antibacterial activity with significantly decreased affinity for HSA as measured by adding 10% serum to the assay. This has been accomplished by reducing the molecular weight by 40% from MW 625 for **1** to MW 380 for **29**. Simultaneously, we were successful in reducing the lipophilicity of the compounds. Thus, we were successful in transforming a non-drug-like screening hit, **1**, into a lead series with promising drug-like properties.[14] Through the synthesis of **29** containing a more optimal benzyl ether substituent, we have demonstrated that heterocyclic B-ring replacements are a viable option for further exploration. Several of these heterocyclic B-ring replacements were subsequently selected for further optimization, by variation of the B-ring substitution and the anthranilic acid substitution in an effort to improve the spectrum of antibacterial activity with reduced affinity for HSA. These efforts will be disclosed in due course.

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