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Preparation of novel anthranilic acids as antibacterial agents. Extensive evaluation of alternative amide bioisosteres connecting the A- and the B-rings

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Abstract—In the past few years, a significant effort has been devoted by Pharmacia toward the discovery of novel antibiotics. We have recently described the identification of an anthranilic acid lead **1** and the optimization resulting in the advanced lead **2**. In this report, we describe the preparation of several selected amide bioisosteres connecting the A- and the B-rings. The *E*-alkene provided a rigid analog with equal potency to the corresponding amide. This indicates that the amide is not a recognition element rather acts as an appropriate spatial linker of the two important aryl A and B rings. The work here clearly demonstrates that the amide linker can be replaced with several functionalities without significant deterioration in the MIC activity.

Emergence of bacterial resistance is a significant problem in the treatment of bacterial infections,[1–3] and this has fueled a continuous search for novel antibiotics resulting in numerous commercially available products. Pharmacia had significant effort devoted to the discovery of novel antibacterial agents over a period of many years. We recently reported[4] on the optimization of acid **1** resulting in the discovery of the lead compound **2** which displayed potent broad-spectrum antibacterial activity. Subsequent optimization of **2** targeted reducing its affinity for human serum albumin (HSA) resulting in advanced compound **3**.[5]

In the absence of structural information, the relative importance of each functional group embedded in compounds **2** and **3** was unclear, although prior work showed that the carboxylic acid was required for activity. This paper will disclose our work in exploring the role of the linker connecting the A- and the B-rings. The design of amide isosteres was guided by two separate hypotheses; (i) that the amide linker had a key interaction with the target and disruption would result in decreased activity or (ii) that the purpose of the amide linker was to act as a conformational control element connecting key pharmacophores (Fig. 1).

There are numerous examples where replacing functional groups with alternative bioisosteres was successful.[6,7] We selected several functional groups that had been successfully utilized as amide replacements by maintaining the key recognition elements of an amide

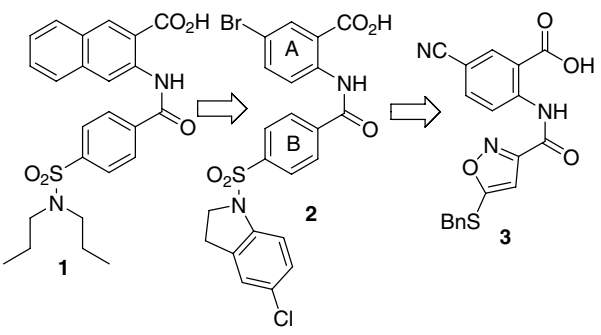

Figure 1. Progression in lead optimization.

Keywords: Antibacterial; Bioisoster; Amide; Cyclopropane.
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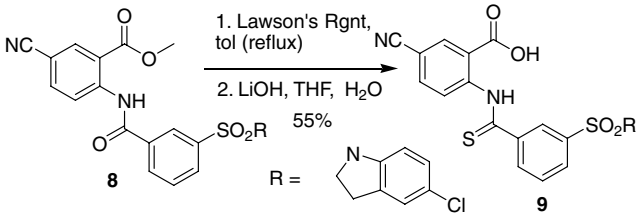
either by incorporating hydrogen accepting or donating properties. The design of novel congeners was aided by extensive modeling overlaying the potential linking moieties. The modeled subunits were overlaid with amide **4** and a similarity score was calculated. The modeling indicated that the linkers that were planned had a conformation available very similar to the ground state conformation of **4**, Table 1. A priori the importance of the differences in the similarity scores was hard to judge. Therefore, a wide range of new linkers were selected and prepared.

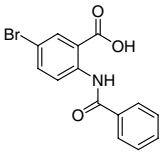
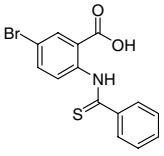
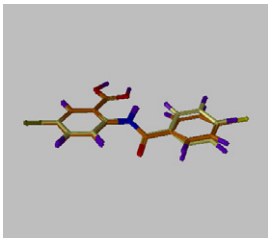
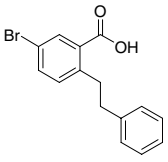
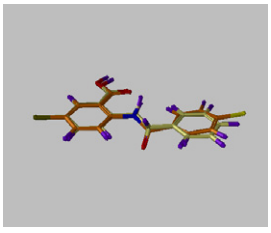
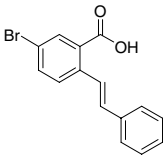
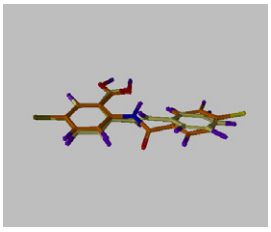
Our goal from the outset was the systematic preparation of analogs containing a linking unit using comparable fragments on the A- and the B-rings. It should be noted that prior work showed that electron withdrawing groups at the 5-position on the A ring such as –Cl, –Br, $–NO_2$, and –CN were interchangeable and had similar antibacterial activity.[4,11] Likewise, prior work had also indicated that 3- versus 4-substituted B-rings provide analogs with similar activity, with the 3-substitution providing slightly improved potency. In addition the selection of a B-ring substituent was guided by the fact that the corresponding amide analog had illustrated reasonable antibacterial activity. For the purposes of this work, the substitution patterns were selected depending on the ease of synthesis, since the initial objective was to illustrate the ability of the linker to replace the amide. It should be recognized that the selected substitution would not illustrate the full potential of the corresponding linker.

The thioamide was readily available from the methyl ester **8** and Lawsson's reagent in refluxing toluene, Scheme 1.[12] The majority of the Lawsson's reagent could be removed by filtration; however, several silica gel flash chromatographies were required to completely purify the product. The ester was hydrolyzed under standard conditions to afford the orange thioamide **9**.



Scheme 1.

Table 1. Selected examples of the overlays utilized in design and selection of linkers

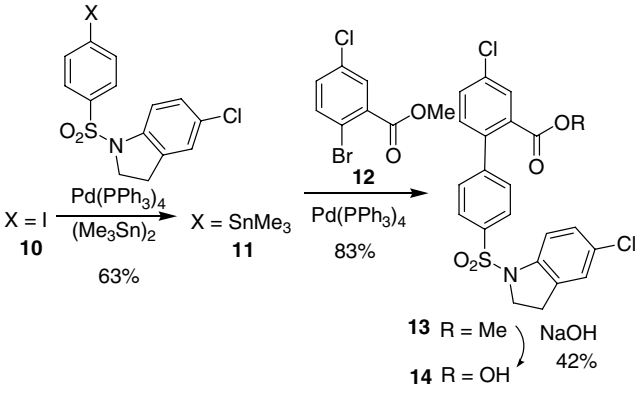
Compound	Structure	#Conf similarity	Best similarity
4		Model	
5		12 0.69	
6		11 0.66	
7		7 0.63	

The minimum energy conformation closest to that of the model compound **2** was obtained through conformational searching retaining those within 3 kcal/mol of the lowest energy[8] followed by ab initio QM geometry optimization at the HF/3-61G level.[9] The resulting analog structures were overlaid upon the structure of compound **2** and the pair-wise similarities were evaluated using a field-based similarity technique.[10]

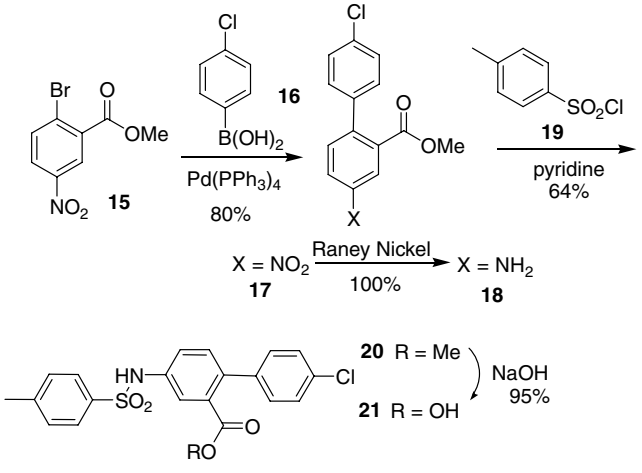
Removing the linking group between the A- and the B-ring results in contracted analogs where the carboxylate could be positioned on either ring. The analog containing the A-ring carboxylate (**14**) was prepared in excellent overall yield via a Stille coupling followed by hydrolysis (Scheme 2). Incorporation of the carboxylate on the B-ring was performed in a similar manner, Scheme 3. The installment of the carboxylate on the B-ring and removing the amide allows the location of the acidic proton in a very similar space while retaining other features. In this instance, the organometallic partner is boronic acid **16** which was coupled in a Suzuki reaction affording the core subunit **17** in 80% yield. Hydrogenation of the nitro group afforded amine **18**, treatment with tolylsulfonylchloride provided **20** which was deprotected to afford the final analog **21** in excellent overall yield.

There are a number of amide bioisosteres described in the literature and among them *E*-alkenes have been successfully utilized to replace amides.[13] Our approach accessing a C–C linked A- and B-ring was based on sequential Sonogashira couplings, Scheme 4. This approach has the potential of providing a series of analogs with differing degrees of saturation via sequential selective hydrogenations. The fully assembled alkynyl analog **24** was easily prepared in several steps, using sequential Sonogashira couplings. Unfortunately hydrolysis and selective partial hydrogenation were unsuccessful, however elevated pressure (50 psi) and prolonged reaction time (24 h) afforded the fully saturated ethylene linked analog **25**.

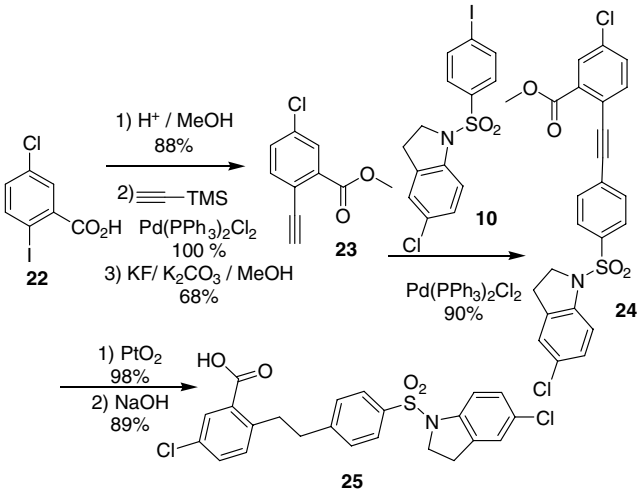
Due to the difficulty with the selective hydrogenation, an alternative route was designed to provide the *E*-alkene linker, Scheme 5. This approach used phosphonylide chemistry which we had previously enjoyed success with in efforts to prepare efflux pump inhibitors.[14] First the requisite nitrile was prepared by hydrogenation of the corresponding nitro compound **26**, followed by



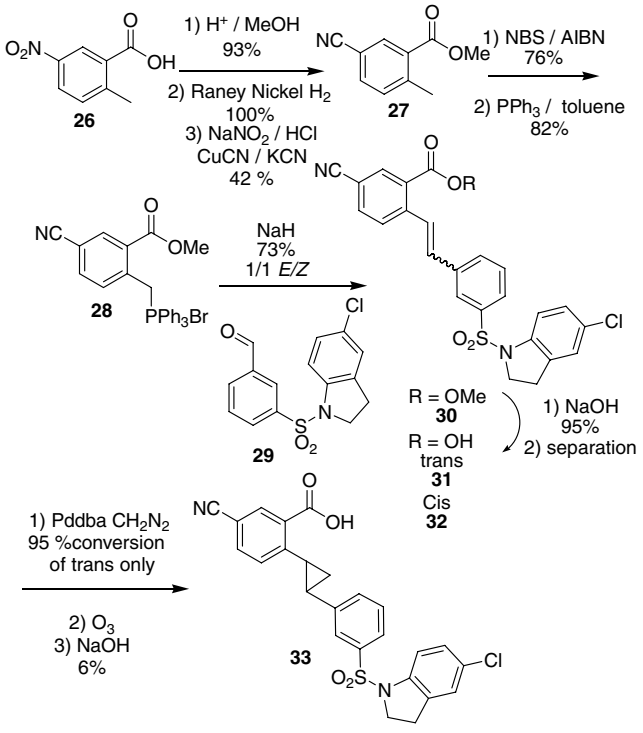
Scheme 2.



Scheme 3.



Scheme 4.



Scheme 5.

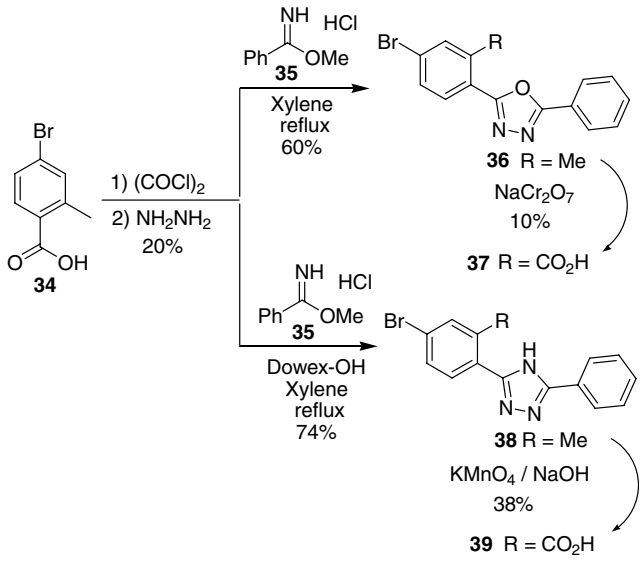
diazotization and cyanation. Bromination and reaction with triphenyl phosphine provided phosphine **28**. Deprotonation with NaH and condensation with the fully functionalized aldehyde **29** afforded the desired alkene **30** as a 1:1 mixture of E/Z isomers. The alkene **30** was hydrolyzed and separated to provide the pure E (**31**) and Z (**32**) isomers.

Since a *trans* substituted cyclopropane yields a very similar spatial arrangement to the amide and the alkene, alkene **30** was converted to a cyclopropane. It was discovered after extensive attempts that alkene **30** was very unreactive. Screening of over 30 methylene transfer conditions found that only Pddba with excess diazomethane was able to achieve cyclopropanation with complete conversion of the desired *E*-alkene.[15] The *Z*-alkene was found to be inert to cyclopropanation under any conditions. Attempts to separate the remaining *Z*-alkene failed, thus ozonization was utilized to convert the remaining alkene to readily separated materials. The desired *trans* cyclopropane **33** was obtained in poor yields due to decomposition during the ozonolysis but with high purity.

Another linker that has been reported in the literature as a viable bioisostere for amides is a triazole.[16] Modeling indicates that the triazole can occupy similar conformations as the amide, although the B-ring would be slightly shifted. In addition the triazole retains the hydrogen bonding pattern of the amide. In order to prepare this linker, we initiated a model study for its assembly, Scheme 6. Fortunately, classical literature observations were reproduced and depending on whether the hydrochloride salt of benzimidate was used or not we obtained either the triazole or oxadiazole as single isomers out of each reaction in modest yields.[17] Conversion of the methyl groups to the acids was troublesome and was achieved with chromium oxidation for the oxadiazole **36**, and under basic conditions with $KMnO_4$ for triazole **38**.[18] The fully functionalized triazole analog was prepared in a similar but lower yielding sequence (not shown but in vitro activity was similar to that of the truncated analog **39**, validating our approach that only linkers that illustrated the potential to replace the amide justified the preparation of the more complex analog for direct comparison across multiple linkers). Unfortunately, the fully functionalized oxadiazole analog resisted any oxidation.

The ability of the new linker as amide replacement was monitored by its activity against *Staphylococcus aureus*. Not surprisingly we found that the thioamide **9** is equipotent with the corresponding amide, Table 2. It is intriguing that the spectrum of activity for the thioamide is improved though the reason for that activity improvement is not clear. The conversion of an amide to a thioamide represents the smallest possible change in linker construction. The removal of the linker as in **14** and **21** results in analogs devoid of activity. Likewise, when the linker is replaced with five-membered ring heterocycles such as **37** and **39** all activity is lost. A flexible linker such as alkane **25** exhibits faint antibacterial activity. The alkenes provide a rigid scaffold and the activity of the *E*-alkene **31** is equal to the potency of the corresponding amide. This indicates that the amide is not essential for activity and serves to hold the pharmacophores at the appropriate distances. Therefore, it is not surprising that the corresponding cyclopropyl linker found in **33** is nearly as active as the corresponding *E*-alkene **31**. The *Z*-alkene **32** possessed poor activity indicating that the A- and the B-rings were inappropriately positioned.

The work described herein clearly demonstrates that the purpose of the amide linker is to appropriately position the aryl rings and it does not have a role as a recognition element. The amide can be replaced with several functionalities such as the *E*-alkene and the *trans* cyclopropyl without significant deterioration in the MIC activity. These findings have enabled us to propose a



Scheme 6.

Table 2. Antibacterial activity of new linkers in this report

Compound	MIC[a] (μg/mL)				
	SAUR[b]	EFAE[c]	SEPId[d]	SPNE[e]	HINF[f]
2	0.5	32	4	4	128
9	0.125	32	0.125	16	64
14	128	>128	128	>128	>128
21	>128	>128	64	>128	>128
25	32	>128	32	32	>128
31	0.25	128	0.5	>128	>128
32[g]	16	>128	32	64	>128
33	4	>128	8	>128	16
37	>128	>128	>128	>128	>128
39	>128	>128	>128	>128	>128

[a] Minimal inhibitory concentration.
[b] *Staphylococcus aureus* UC 9218.
[c] *Enterococcus faecalis* UC 9217.
[d] *Staphylococcus epidermidis* UC 12084.
[e] *Streptococcus pneumoniae* UC 9912.
[f] *Haemophilus influenzae* 30063.
[g] This is a 97.9/2.1 *cis*/*trans* mixture and the activity can be mainly attributed to the *trans* impurity.

pharmacophore model for this series of compounds, details of which will be the topic of future publication.

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