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The discovery of biaryl acids and amides exhibiting antibacterial activity against Gram-positive bacteria

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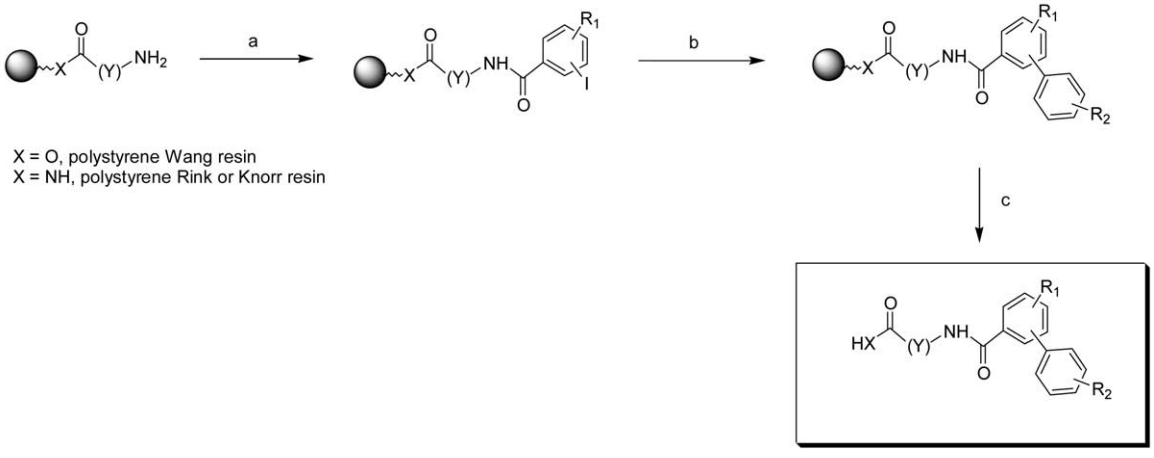
Abstract—Solid-phase synthetic methods for biaryl-based compounds were developed resulting in the construction of two 1000-member libraries. Numerous compounds were identified by high-throughput screening using whole cell screens to exhibit antimicrobial activity against Gram-positive bacteria. A series of biaryl compounds containing natural and unnatural amino acids were made to explore the SAR of the amino acid functionality.

A growing concern about the continuing problem of antibiotic-resistant pathogens is compelling the pharmaceutical industry to search for novel antimicrobial agents.[1] With the availability of complete genome sequences and whole genome comparisons, the number of potential drug targets has greatly increased, and modern antibiotic drug discovery is now heavily focusing on rationally chosen targets. However, broad cell-based screens have been utilized for the discovery of new antimicrobial agents particularly for natural



Scheme 1. General scheme for the preparation of biaryl libraries: (a) iodo-aryl acid, HATU, HOAT, DIEA, NMP; (b) arylboronic acid, $Pd_2(dba)_3$, K_2CO_3, H_2O, DME; (c) 90% TFA, CH_2Cl_2, H_2O.

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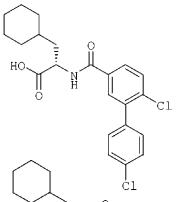
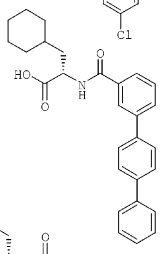
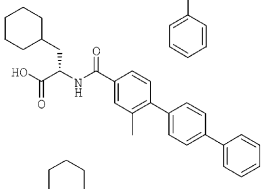
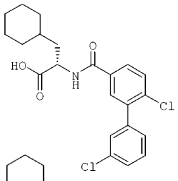
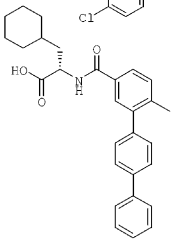
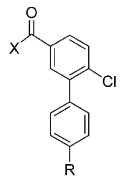
Table 1. Antibacterial activity of selected members of the biaryl acid library

Compd	Structure	IC_{90}[a] vs *S. aureus* 8325 (μg/mL)
PDL117230		10
PDL117279		4
PDL117219		2
PDL117227		8
PDL117199		3

[a] Concentration at which there was 90% inhibition of bacterial growth.

product screening. Libraries of biaryls have been prepared previously by other groups.[2–4] Pertaining to antibacterials, previous work by others have produced inhibitors of purified *E. coli* peptide deformylase which contain a biaryl moiety.[5] Herein, we report on a series of biaryl acids and amides, which were discovered in our high-throughput screening program that exhibit low μg/mL MICs against Gram-positive bacteria.[6] We also discuss SAR data involving the variation of the amino acid moiety of these compounds.



PDL141052, X= NH_2, R=Cl: >50 μg/mL

PDL141021, X=OH, R=Cl: >50 μg/mL

PDL141053, X=NH2, R=Ph: >50 μg/mL

Figure 1. *Des*-amino acid biaryl compounds.

The combinatorial amide and acid libraries were prepared using amino acid-functionalized resin according to the general procedure outlined in Scheme 1. Ten amino acids, 10 iodo-aryl acids, and 10 aryl boronic acids were combined in the split/pool method to form 1000-member libraries. The use of polystyrene resin in polypropylene micromesh reactors, which have been uniquely identified by radio-frequency tags, allows one to perform syntheses of discrete compounds utilizing the advantages of the split/pool technique for library synthesis.[7] The scale of synthesis afforded 10–25 mg of each compound that can be purified as part of a hit follow-up protocol. For the Suzuki coupling step, the phosphine-free conditions of Wallow and Novak were used.[8] Use of the aforementioned conditions afforded solvent compatibility at slightly elevated temperatures with the polypropylene reactors. Approximately, 10% of the members of each of the libraries were evaluated by proton NMR, HPLC, and mass spectrometry. The average yield for the libraries was determined by weighing material from 5% of the members. The anticipated product was found in approximately 90% of the sampled wells and an average yield of synthesis was 70%. The quality of the libraries was sufficient for high-throughput screening purposes with the only two components being the product and the iodo-starting material.[9]

The unpurified biaryl libraries were screened in 384-well format versus *E. coli* and *S. aureus*.[10] The arbitrary threshold of activity for compounds to pursue was 50%

Table 2. Minimal inhibitory concentrations of selected biaryl acids for bacteria

Strain	Description	MIC[a] (μg/mL)		
		PDL117230	PDL117279	PDL117227
S. aureus 8325	Control	20	20	15
S. aureus F2490	Blood culture	20	20	15
S. aureus W30078c	MRSA	20	20	20
E. faecium F24910	VRE (UTI)	>20	20	20
E. spp. T66429	UTI	20	20	20
Grp A Strep T32098	Throat	20	20	20
Grp A Strep S54408	Throat	10	10	10
E. coli H28233	UTI	Not active	Not active	Not active
P. aeruginosa W30298	Cellulitis	Not active	Not active	Not active
P. aeruginosa W30279	UTI	Not active	Not active	Not active

[a] The minimal concentration needed to kill most (99.9%) of the viable organisms after a 24 h incubation.

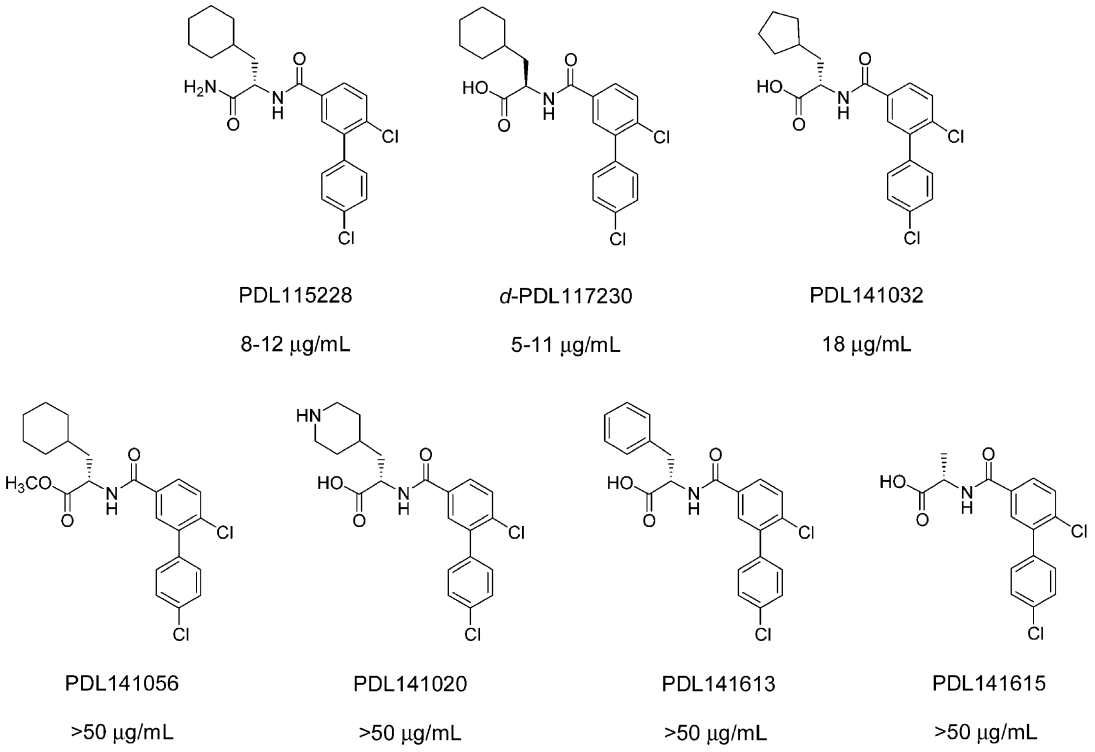


Figure 2. Amino acid variants of PDL 117230 and their IC_{90}'s.

bacterial growth inhibition at approximately 50 μM (limit of solubility) final concentration. No bacterial growth inhibition was seen for either library when screened against *E. coli*. Approximately 30 active wells were identified from each library in the screen using *S. aureus*, and in general, the biaryl amides displayed slightly less activity than their corresponding biaryl acids. However, due to their decreased solubility they were not actively pursued. Six biaryl acids from the original library material were identified based on the recurrence of the cyclohexylalanine moiety as the amino acid for follow-up studies. These compounds were purified by HPLC or, if necessary, resynthesized and purified.[11] Data on the activity of five compounds is presented in Table 1. In comparison, the marketed oxazolidinone antibiotic, Linezolid shows MIC's of 2–4 μg/mL versus *S. aureus* and 1–2 μg/mL versus *S. pneumoniae,* whereas Vancomycin shows activities of 0.5–4 μg/mL and 0.25 μg/mL versus the respective organisms.[12] It was later determined that the compounds are bactericidal in mechanism rather than bacteristatic by removal of the compound with dilution and plating on agar for interpretation of killing endpoints.

A series of amino acid variants based upon the structures of PDL117230 and PDL117279 were prepared and tested against *S. aureus* (Figs. 1–3). The *d*-amino acids gave similar growth inhibition values to the *l*-amino acid containing biaryls. The *des*-amino acid biaryls were prepared (Fig. 1) and tested versus *S. aureus*. None of the compounds exhibited inhibitory activity. Thus, in the case of compound PDL117230, it appears that the antibacterial activity is dependent on the nature of the amino acid side chain.

Variation of PDL117279 (Fig. 3) afforded good potency versus *S. aureus*. Our preliminary studies indicate that the triaryl system tolerates variations in the amino acid side chain more so than in the PDL117230 series.

Three of the original biaryl acid hits were examined for their antibacterial activity against a panel of organisms including clinical isolates.[13] The results are presented in Table 2. None of the biaryl compounds demonstrated activity versus Gram-negative bacteria.

In conclusion, two 1000-member libraries of biaryl acids and amides were screened and active members displayed anti-microbial activity in the low micromolar range toward Gram-positive organisms. Our studies concerning further SAR of this class of compounds as well as

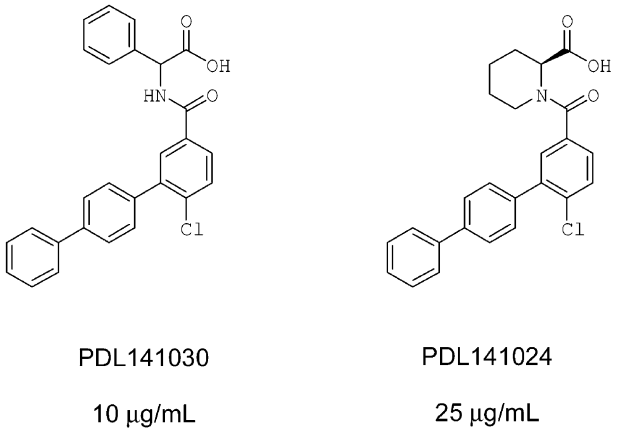


Figure 3. Variants of PDL117279 and IC_{90}'s.

the mode of action of these agents against bacteria will be reported forthcoming.

Acknowledgements

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