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Discovery and initial development of a novel class of antibacterials: Inhibitors of *Staphylococcus aureus* transcription/translation

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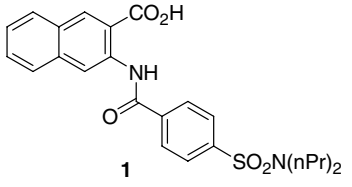
Abstract—The novel bacterial transcription/translation (TT) inhibitor **1** was identified through a combination of high throughput screening and exploratory medicinal chemistry. Initial optimization of the anthranilic acid moiety and sulfonamide amine diversity was accomplished via 1- and two-dimensional solution phase libraries, resulting in an improvement in the MIC of the lead from 64 to 8 μg/mL (compound **4l**). Subsequent modification of the central aromatic ring and further refinement of the sulfonamide amines required the development of a solid phase route on Wang resin. The resulting libraries generated a number of potent antibacterials with MICs of $\leqslant$1 μg/mL (e.g., **10b**, **12**, and **13**). During the course of this work, it became apparent that the antibacterial activity of the series is not fully correlated with TT inhibition, suggesting that at least one additional mechanism of action is operative.

Bacterial protein synthesis has proven to be a fruitful target for antibiotic discovery.[1] A number of marketed antibiotics inhibit bacterial growth through inhibition of prokaryotic RNA transcription and protein translation. Rifampin is a potent inhibitor of bacterial RNA polymerase, and the macrolides, lincosamides, aminoglycosides, tetracyclines, and oxazolidinones all have protein translation as their site of action. Unfortunately, the increase in the antibiotic resistance of Gram-positive bacteria threatens to reduce the effectiveness of these and other antibiotics. These concerns act as an incentive to discover new and more effective transcription and protein translation inhibitors.

Through a combination of high throughput screening and exploratory medicinal chemistry,[2] **1** was identified as a novel inhibitor of bacterial transcription/translation[3] (TT) with modest antibacterial activity against *Staphylococcus aureus*.[4] The presence of easily modified carboxamide and sulfonamide bonds encouraged us to rapidly expand the structure–activity relationships (SAR) of this lead through parallel medicinal chemistry. A straightforward 2-step solution phase route was developed that was suitable for preparing 1- and two-dimensional libraries of 50–150 compounds from



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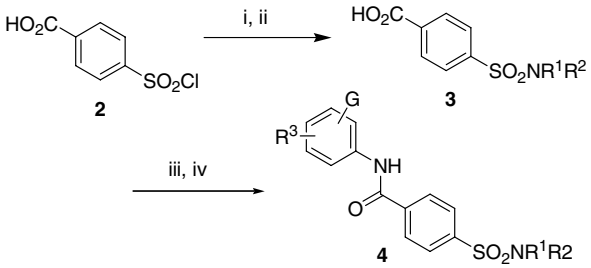

2 **3** **4**

Scheme 1. Reagents and conditions: (i) $R^1R^2NH_2$, MeOH, 0 °C, rt, 3 h; (ii) aq HCl; (iii) $SOCl_2$, DMF, 60 °C, 3 h or $(CO)_2Cl_2$, cat. DMF, CH_2Cl_2, rt, 2 h; (iv) $(R^3)(G)PhNH_2$, rt, 3 h.

Keywords: *Staphylococcus aureus*; Athranilic acid.
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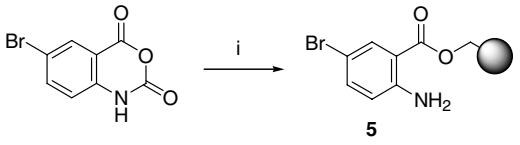
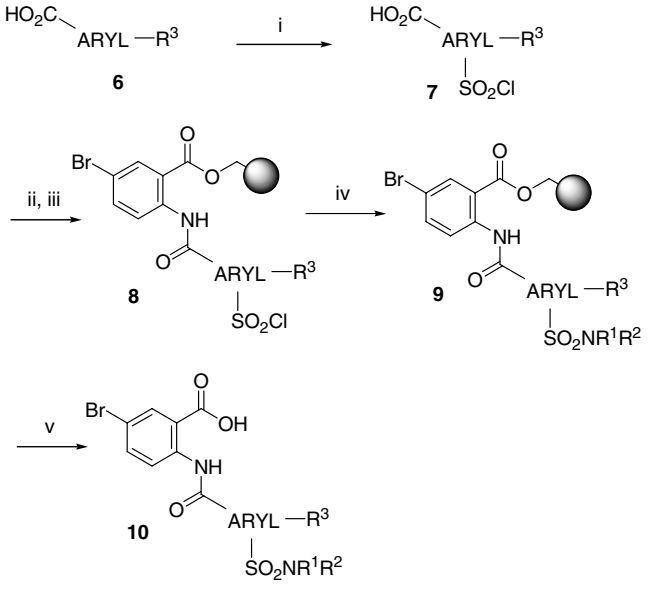
Table 1. Biological activity of selected library analogs **4**

Compound	G	R^3	R^1	R^2	% TT inh[a]	SAUR MIC[b] (μg/mL)
1	2-COOH	4,5-fused Ph	*n*-Pr	*n*-Pr	30	64
4a	H	4,5-fused Ph	*n*-Pr	*n*-Pr	10	>128
4b	2-OH	4,5-fused Ph	*n*-Pr	*n*-Pr	10	>128
4c	2-COOH	4-Br	*n*-Pr	*n*-Pr	61	8
4d	2-COOH	4-Br	$(CH_2)_5$		35	16
4e	2-COOH	4,6-diOMe	*n*-Pr	*n*-Pr	0	>128
4f	2-COOH	4-Br	Me	4-(MeO)Ph	50	32
4g	2-COOH	4-Br	H	3-(CF_3)Ph	65	8
4h	2-COOH	4-OMe	H	3-(CF_3)Ph	35	>128
4i	2-COOH	4,5-diF	H	3-(CF_3)Ph	55	>128
4j	2-COOH	4-Br	H	3-(CN)Ph	30	64
4k	2-COOH	4-Br	MeO–$(CH_2)_2$	MeO–$(CH_2)_2$	10	32
4l	2-COOH	4-Br	Me	4-(Cl)Ph	75 80[c]	16 8[c]
4m	2-Me	4-Br	Me	4-(Cl)Ph	12	>128
4n	2-COOH	4-Br	H	4-(Cl)Ph	45	16
4o	2-COOH	4-Cl	*n*-Pr	Ph	50	32
4p	2-COOH	4-Br	*n*-Pr	Ph	80	16
4q	2-COOH	4-I	*n*-Pr	Ph	90	16
4r	2-COOH	4-Me	*n*-Pr	Ph	34	128
4s	2-COOH	4-Cl	Me	4-(Cl)Ph	85	16
4t	2-COOH	5-Cl	Me	4-(Cl)Ph	47	64
4u	3-COOH	4-Cl	Me	4-(Cl)Ph	10	>128
4v	2-COOH	4-F	Me	4-(Cl)Ph	30	>128
4w	2-COOH	4-Br	H	3-(BnO)Ph	75	8
4x	2-COOH	4-Br	H	3-(MeO)Ph	20	16
4y	2-COOMe	4-Br	H	3-(BnO)Ph	4	>128

[a] Percent inhibition of *S. aureus* coupled transcription/translation at 100 μM (Ref. 3).
[b] Minimum Inhibitory Concentration against *S. aureus* (UC 9218, ATCC 29213) (Ref. 4).
[c] Values are for resynthesized singleton.



Scheme 2. Reagents and conditions: (i) Wang resin, DMF, DMAP, 60 °C, 24 h.



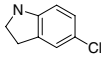
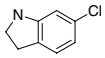
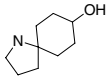
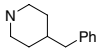
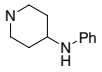
Scheme 3. Reagents and conditions: (i) $ClSO_3H$, 0–80 °C, 2 h; (ii) $(ClCO)_2$, cat. DMF, CH_2Cl_2, rt, 18 h; (iii) resin **5**, CH_2Cl_2, pyr, rt, 4 h; (iv) $R^1R^2NH_2$, Et_3N, CH_2Cl_2, rt, 15 h; (v) TFA, CH_2Cl_2, rt, 1 h.

commercially available 4-(chlorosulfonyl)benzoic acid **2** and diverse amines and anilines (Scheme 1). The method was sufficiently robust that 282 compounds out of 362 possible targets were pure enough for biological assay (av purity 80%) without HPLC purification (av yield 72% overall).

Most of the aniline diversity was selected to include one carboxyl or hydrogen-bonding group (G = CO_2H, CO_2Me, OH, OMe, CN, and PhCO) and at least one other substituent (R^3 = halogen, alkyl, Ph, OMe, OH, fused phenyl). Amine diversity was designed to span a range of hydrophilicities and MWs (R^1, R^2 = H, alkyl, alkyl-OMe, morpholine, piperidine, NHAr, NMeAr, NCH_2Ar) with varying substitution on the Ar groups. Representative SAR is presented in Table 1.

The carboxylic acid, positioned ortho to the NH, proved to be essential for activity (compare **1**, **4a**, **4b**, **4s**, **4u**, **4w**, and **4y**). The naphthyl group of the lead could be successfully replaced by 4-haloanthranilic acid (**4c**), and the halide was optimally placed at the 4-position (**4s**, **4t**). The lipophilic halides Br and I were superior to the more hydrophilic Cl, F, Me or OMe (**4g**, **4h**, **4o**–**4r**). With regard to the sulfonamide substituents, secondary amines were slightly better than primary (**4l**, **4n**) and aromatics could replace aliphatic. Lipophilic amines were necessary for good TT inhibition and antibacterial activity (e.g., **4g**, **4j**). One of the best compounds from the library was resynthesized and found to inhibit TT by 80% at 100 μM with an MIC of 8 μg/mL (**4l**).

Table 2. Biological activity of selected validation library analogs **10** (ARYL = 1,4-disubstitued Ph; R^3 = H)

Compound	NR^1R^2	% TT inh at 100 μM (IC_{50})[a]	SAUR MIC (μg/mL)
10a		71 (80)	1
10b		96 (40)	1
10c		87 (55)	2
10d		90 (60)	4
10e		88 (60)	4
10f		77	4
10g		22	8
10h		96 (55)	8
10i		84 (60)	8
10j		57	8
10k		34	16
10l		98 (28)	16
10m		96 (30)	16
10n		62	32
10o		19	>128

[a] IC_{50} values are in μM.

To facilitate an evaluation of the central aromatic ring, a solid phase route was developed that would also permit additional modification of the sulfonamide amine, potentially allowing the evaluation of a full two-dimensional array. Because of the clear superiority of the 5-bromoanthranilic acid in the solution phase libraries, this diversity element was fixed in the solid phase libraries. Commercially available 5-bromoisatoic anhydride was loaded onto Wang resin with DMAP catalysis, affording resin-bound bromoanthranilic acid **5** (Scheme 2).[5] Commercially available aryl carboxylic acids **6** were chlorosulfonylated with chlorosulfonic acid, and the resulting sulfonyl chlorides **7** were loaded onto resin **5** via the corresponding acid dichlorides (Scheme 3). Consistent with literature precedent, the resin-bound aniline reacted preferentially with the carbonyl chloride vs the sulfonyl chloride.[6] The resulting resin-bound sulfonyl chlorides **8** were reacted with diverse amines to afford sulfonamides **9**. Cleavage from the resin was then effected with TFA, giving the desired analogs **10**.

A one-dimensional validation library was first prepared as in Scheme 3 by combining the aryl sulfonyl chloride

derived from **2**, resin **5**, and 46 diverse amines that were selected based on the SAR of the solution phase libraries. Average overall yield of the resulting analogs **10** was 78% with 25 final compounds being pure enough (>70%) for initial biological assay. Representative SAR is presented in Table 2.

It is clear from these initial library results that reducing the lipophilicity of the sulfonamide amine, either through addition of polar functionality or simply lowering the molecular weight, attenuates antibacterial activity. Interestingly, fused aromatic bicyclic systems (e.g., indoline and isoindoline) were superior to aromatic substituted cycloalkylamines. It is noteworthy that this library revealed that some analogs in this series possess antibacterial activity without significant TT inhibition (e.g., **10g**), suggesting that an additional mechanism for antibacterial activity is likely operative. Nevertheless, a significant advancement in both TT and antibacterial activity was realized in this validation library relative to the best compound from the solution phase libraries (**10b** vs **4l**).

Encouraged by the results of the validation library, a larger two-dimensional array was designed and put into production. A total of 12 central aromatic rings and 45 amines were selected as diversity elements. The central rings are presented in Figure 1. Amines were selected to maintain some of the successful elements of the previous libraries (bicyclics, aromatics) along with additional attempts to reduce lipophilicity and incorporate some new diversity. It was assumed that production on solid phase would facilitate the isolation of more analogs with more divergent physicochemical properties.

The success rate of the larger library was more modest than those of the solution phase or solid phase validation libraries, presumably due to the production format (96-well plates) and the wider diversity of the amines, which spanned a broad range of reactivities. Although all 12 acid chloride diversity elements got successfully incorporated into products, only 23 amines yielded products (Fig. 2). A total of 156 compounds were obtained in purities sufficient for biological assay (>70%).

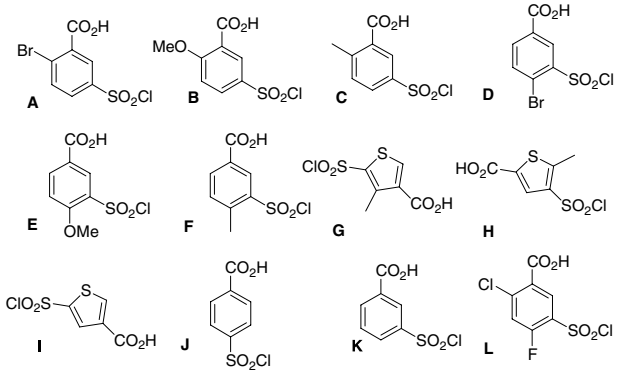


Figure 1. Central aromatic ring diversity.

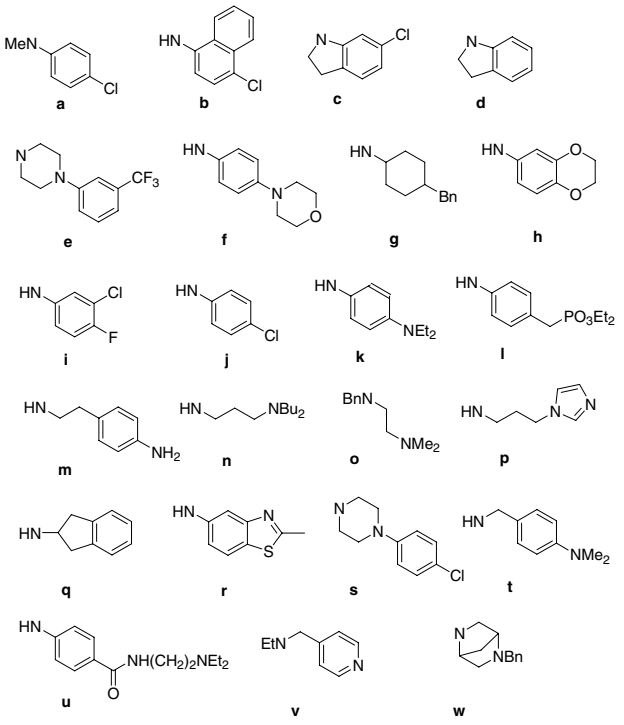


Figure 2. Successful amine diversity elements in two-dimensional library.

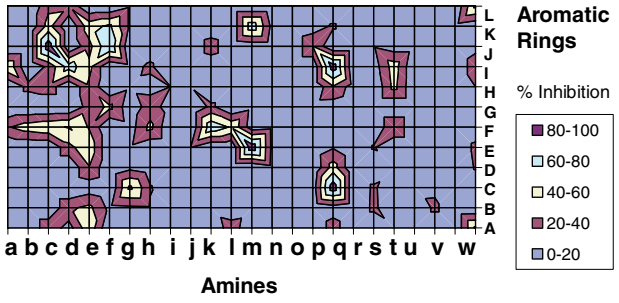


Figure 3. Contour plot of % TT inhibition (at 30 μM) vs amine and aromatic ring diversity elements for two-dimensional library.

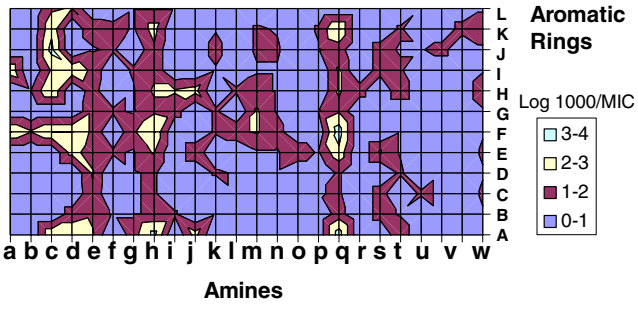


Figure 4. Contour plot of log1000/MIC versus amine and aromatic ring diversity elements for two-dimensional library.

Key SAR from this library is summarized in the Excel® contour plots depicted in Figures 3 and 4. These plots use color to depict levels of activity, as indicated by the legends in the figures. It is important to note that there are only 156 data points contained within the

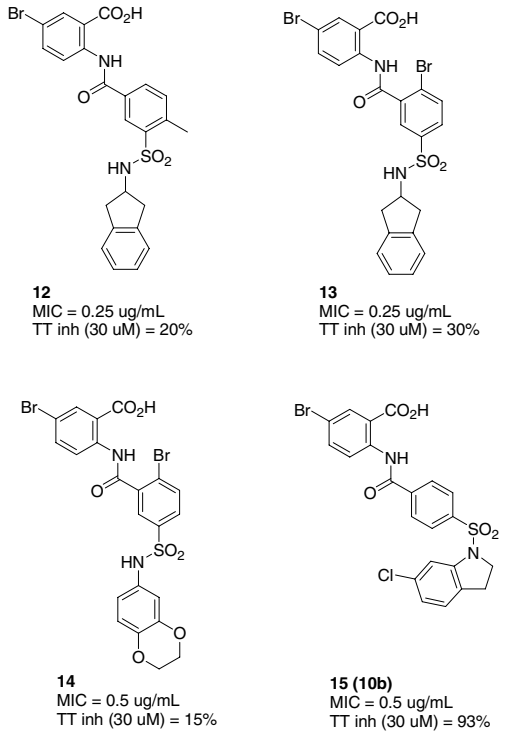


Figure 5. Most potent antibacterial compounds from the two-dimensional library.

$12 \times 23 = 276$ possible grid intersections depicted in these plots. This is because not all possible combinations were tested due to insufficient purity or simple absence of product. *Thus these plots are only useful for identifying pockets of significant activity corresponding to particular diversity elements*. Regions where no activity is apparent can either be due to genuine lack of activity or to simple lack of testing data. As can be seen in Figure 3, the amines associated with good inhibition of TT are c, e, m, and q (identified in Fig. 2). The aromatic rings most associated with good TT inhibition are F, I, and J. In Figure 4 it can be seen that the amines associated with the best antibacterial activity were c, e, h, and q, while the best central aromatic rings were A and F. Although the contour plots bear some resemblance, indicating a correlation of TT inhibition with antibacterial activity, there are significant areas of diversion where antibacterial activity exists without significant TT inhibition (e.g., for amine h). It is apparent from the two contour plots that the amines associated with the best combination of TT and antibacterial activity are 6-chloroindoline (c) and 2-aminoindane (q), while the best central aromatic ring is F.

Despite the apparent disconnect between TT and antibacterial activity, this library was successful in identifying four compounds with MICs less than 1 μg/mL (Fig. 5). One of these compounds (**15**) proved to be identical to one of the best compounds from the validation library (**10b**) that was included as a control.

In summary, through a series of both solution and solid phase libraries, we were successful in rapidly converting a modest bacterial TT inhibitor with weak antibacterial activity (MIC = 64 μg/mL) into a number of highly potent antibacterials (MICs = 0.25–1 μg/mL). In the process we discovered that the antibacterial activity within the series is not fully coupled with TT inhibition. This apparent disconnect is suggestive of additional mechanisms of antibacterial activity. Studies are underway to better understand this.

Acknowledgments

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