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Aryl urea analogs with broad-spectrum antibacterial activity

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Abstract—The preparation and evaluation of novel aryl urea analogs as broad-spectrum antibacterial agents is described. Numerous compounds showed low micromolar minimum inhibitory concentrations (MIC) against both Gram-positive and Gram-negative bacteria. Selected analogs also exhibited in vivo efficacy in a lethal murine model of bacterial septicemia.

The emergence of drug resistant pathogenic bacteria continues to be a serious health problem worldwide.[1] As a result, it has become critical to identify new structural classes of antibacterial agents to combat the growing threat of bacterial resistance.[2] During screening of new compound classes for antibacterial activity, thiourea **1** (Fig. 1) was identified as having good activity[3] (MIC 3–6 μM) against *S. aureus* in our assay.[4] However, thiourea **1** did not show any antibacterial activity in the in vitro assay (MIC > 100 μM) when tested in the presence of 4% bovine serum albumin (BSA). Since the loss of activity in the presence of serum could be attributed to the high lipophilicity of thiourea **1**, we initiated a study to identify alternate structures with reduced lipophilicity and equivalent or improved antibacterial activity. Here we report the initial SAR study around thiourea **1** resulting in analogs with good broad-spectrum antibacterial activities as well as in vivo efficacy in a lethal murine model of bacterial septicemia.

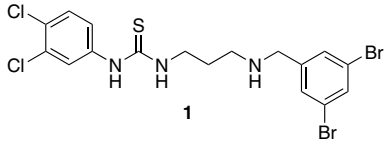
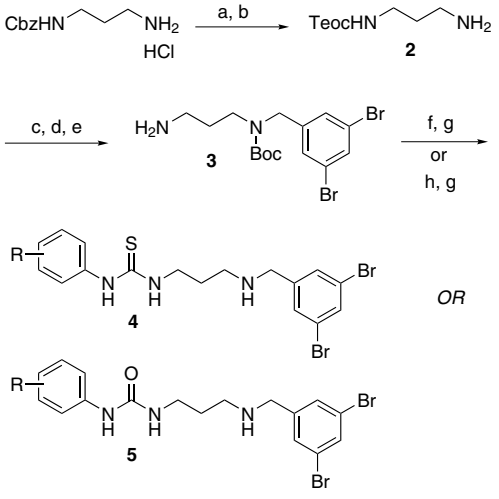


Figure 1.

We first chose to modify the aryl ring of the thiourea moiety without altering the 3,5-dibromo substitution pattern on the *N*-benzyl substituent. Commercially available mono-Cbz protected 1,3-diaminopropane was reacted with Teoc-OSu, followed by removal of the Cbz group by catalytic hydrogenation to provide mono-Teoc protected 1,3-diaminopropane **2** (Scheme 1). Reductive amination of **2** with 3,5-dibromobenzaldehyde,



Scheme 1. Reagents and conditions: (a) Teoc-OSu, Et_3N, CH_2Cl_2; (b) 10% Pd/C, H_2; (c) 3,5-dibromobenzaldehyde, $NaBH_3CN$, MeOH (68%, over three steps); (d) Boc_2O, CH_2Cl_2 (95%); (e) 1 M TBAF, KF, CH_3CN, 40 °C, 10 h (75%); (f) ArNCS, CH_2Cl_2; (g) 30% TFA/CH_2Cl_2; (h) ArNCO, CH_2Cl_2.

Keywords: Aryl urea; Antibacterial; Broad spectrum.
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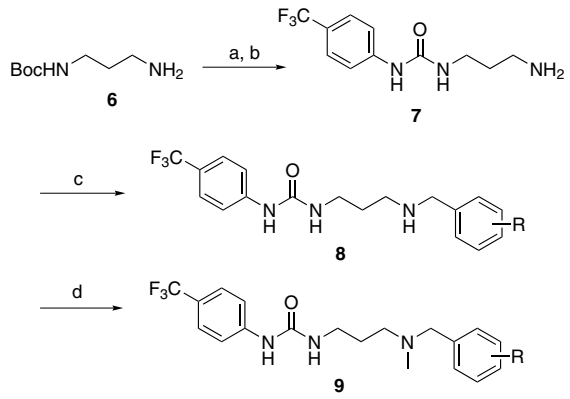
protection of the secondary amine with Boc_2O and deprotection of the Teoc group using TBAF/KF provided amine **3**. Subsequent reaction of **3** with commercially available arylisothiocyanates or arylisocyanates followed by deprotection of the Boc group provided the corresponding thiourea **4** or urea **5** analogs, respectively, in good overall yield (40–55% after reversed phase HPLC purification). All final thiourea **4** and urea **5** analogs were tested for antibacterial activity as their acetate salts.

In the first SAR set, ~30 thiourea/urea analogs with electron donating and electron withdrawing groups at the 2-, 3-, and 4-positions of the aryl ring were prepared and evaluated. The objective was to identify substitutions that would reduce hydrophobicity while maintaining activity. Biological screening revealed that substitution at the 2-position was not tolerated and led to a substantial decrease in antibacterial activity (Table 1). Substitution at the 3- and 4-positions was tolerated and optimal activity was seen with small non-polar groups such as chloro and trifluoromethyl. From this series urea **5d** with a 4-CF_3 group exhibited activity similar to the lead thiourea **1**. Urea analog **5d** also had a lower Clog *P* value[5] (4.89) as compared to thiourea **1** (6.23). As a result we chose urea **5d** as the new lead structure for further optimization.

In the second analog set, we explored the SAR of the benzylamino substituent. Commercially available *N*-Boc-1,3-diaminopropane was reacted with 4-(trifluoromethyl)phenyl isocyanate followed by deprotection of the Boc group to provide urea **7** (Scheme 2). Reductive aminations with ~40 aromatic aldehydes provided ureas **8** (40–55% after reversed phase HPLC purification). All the final compounds were tested for antibacterial activity as their acetate salts.



Scheme 2. Reagents: (a) 4-CF_3-phenyl isocyanate, CH_2Cl_2; (b) 30% TFA, CH_2Cl_2 (90% over two steps); (c) ArCHO, TMOF, $NaBH_3CN$, cat. AcOH, MeOH; (d) 40% HCHO, MeOH, cat. AcOH, $NaBH_3CN$.

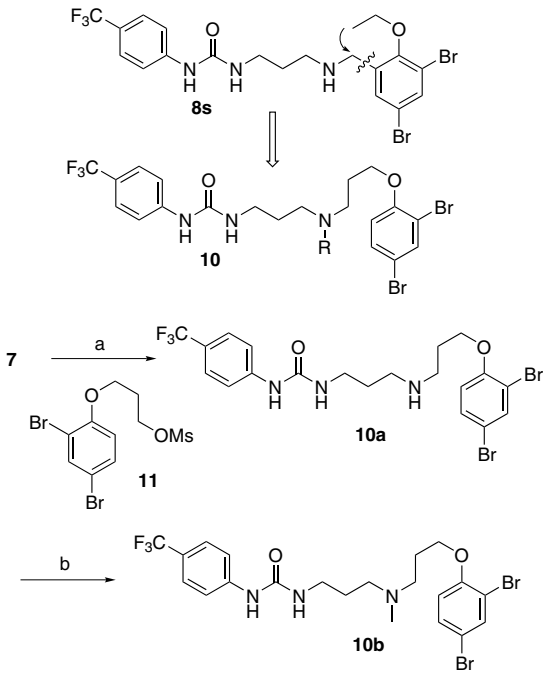
Substitution at the 4-position of the benzylamino group with a variety of hydrophobic substituents such as *t*-butyl, phenyl, phenoxy, and OCF_3 provided analogs that showed good activity against *S. aureus* (Table 2, entries 4–9). We were also surprised that a 4-dimethylamino group also displayed some antibacterial activity suggesting that it may be possible to substitute the 4-position with other hydrophilic groups (entry 10). The best substitution pattern for the halogenated benzylamino analogs was either 3,5-dibromo (**8l**) or 3,4-dichloro (**8q**). 3,5-Dichloro-substitution resulted in a slight loss of activity while 3,5-difluoro, 3,5-ditrifluoromethyl, and 3,5-dimethoxy analogs showed reduced

Table 1. In vitro antibacterial activity of thiourea/urea analogs

Entry	Compd	R	MIC (μM)[4] *S. aureus*
1	**1**	3,4-DiCl	3–6
2	**4a**	2-F	25–50
3	**4b**	2-Cl	12–25
4	**4c**	2-OMe	>100
5	**4d**	3-F	12–25
6	**4e**	3-Cl	6–12
7	**4f**	3-Br	6–12
8	**4g**	3-CF_3	6–12
9	**4h**	3-OMe	25–50
10	**4i**	4-F	12–25
11	**4j**	4-Cl	6–12
12	**4k**	4-Br	12–25
13	**4l**	4-I	12–25
14	**4m**	4-CF_3	6–12
15	**4n**	4-NO_2	12–25
16	**4o**	4-CH_3	12–25
17	**4p**	4-CN	25–50
18	**4q**	4-OMe	25–50
19	**5a**	3-CF_3	6–12
20	**5b**	3,4-DiCl	3–6
21	**5c**	4-Cl	6–12
22	**5d**	4-CF_3	3–6
23	**5e**	4-OCF_3	6–12
24	Linezolid	—	3–6

Table 2. In vitro antibacterial activity of benzylamino group modified analogs

Entry	Compd	R	MIC (μM)[4] *S. aureus*
1	**8a**	H	50–100
2	**8b**	2-OCF_3	12–25
3	**8c**	3-Ph	6–12
4	**8d**	4-OCF_3	3–6
5	**8e**	4-Ph	3–6
6	**8f**	4-(4′-OMe)-Ph	3–6
7	**8g**	4-(2′-OMe)-Ph	6–12
8	**8h**	4-OPh	3–6
9	**8i**	4-*t*-Bu	3–6
10	**8j**	4-NMe_2	12–25
11	**8k**	4-NHCOMe	>100
12	**5d**	3,5-DiBr	3–6
13	**8m**	3,5-DiCl	6–12
14	**8n**	3,5-DiF	50–100
15	**8o**	3,5-DiCF_3	50–100
16	**8p**	3,5-DiOMe	50–100
17	**8q**	3,4-DiCl	3–6
18	**8r**	2-OH-3,5-DiBr	>100
19	**8s**	2-OEt-3,5-DiBr	3–6
20	**8t**	2-OEt-5-Br	6–12
21	**9**	3,5-DiBr	>100
22	**10a**	—	3–6
23	**10b**	—	6–12
24	Linezolid	—	3–6



Scheme 3. Reagents and conditions: (a) **11**, $CaCO_3$, DMF, 40 °C, 48 h; (b) 40% HCHO, MeOH cat. AcOH, $NaBH_3CN$.

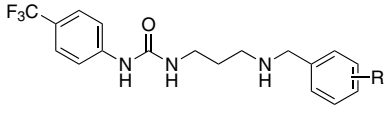
antibacterial activity (entries 12–17). Replacement of the aryl ring with halogenated heteroaromatic rings was also not well tolerated (data not shown). A few urea analogs with substituted and unsubstituted phenolic groups were also prepared and evaluated (entries 18–20). Our intention was to incorporate groups that could potentially provide us with a handle to introduce alkyl chains with polar substituents. In this series, urea **8s** with a 2-ethoxy substituent showed activity comparable to the lead urea **5d**. Interestingly, analogs **10a**–**b** with extended tethers (Scheme 3) were prepared and found to be as active as the 2-ethoxy substituted analog **8s** (entries 22–23). An interesting observation is that replacement of the secondary amino group in the tether with a tertiary amine was permitted in case of urea **10b** but not for urea **9** (entry 21). It would appear that the methyl group in **9** might alter the conformation such that the molecule is not able to bind its target. In contrast, analog **10b** may be flexible enough to adopt the bioactive conformation despite the methyl group on the tether amine.

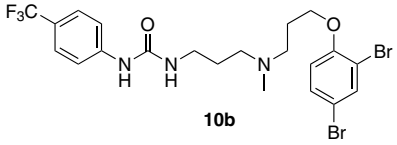
A number of analogs were then evaluated against a broader panel of Gram-positive and Gram-negative bacteria (Table 3). For the most part, urea analogs showed low micromolar activity against *S. aureus*, *S. pyogenes*, and *E. faecalis*. However, only ureas **8i** and **8s** showed good activity against *E. faecium*. Most of the urea analogs showed slightly reduced activities against *E. coli* but good activity against *K. pneumoniae*. All compounds showed weak activity against *P. vulgaris* and no activity against *P. aeruginosa*.

Selected urea analogs were also evaluated for activity in the presence of 4% BSA. Most of the analogs tested showed reduced activity in the presence of serum (Table 3). This was not completely surprising considering the high Clog *P* values for these compounds (Table 3). The greatest reduction in antibacterial activity in the presence of serum was observed for the halogenated analogs **5d**, **8s** and **10b**. Ureas **8e** and **8h** gave the best activity in the presence of serum (4-fold reduction).

Analysis of the in vitro data suggests that the urea analogs are binding in a very lipophilic binding pocket as increasing hydrophobicity improves the activity. The antibacterial activity of the urea analogs is also reduced substantially in the presence of 4% bovine serum albumin. It is conceivable that the reduced activity is likely the result of high serum protein binding of this compound class. These observations are consistent with the previous SAR studies carried out on other urea

Table 3. Broad-spectrum antibacterial activity of urea analogs





Bacteria	MIC (μM)						
	5d 3,5DiBr	**8s** 2-OEt-3,5-DiBr	**8e** 4-Ph	**8h** 4-OPh	**8i** 4-*t*-Bu	**10b**	Linezolid
S. aureus ATCC 13709	3–6	3–6	3–6	3–6	3–6	3–6	3–6
S. pyogenes ATCC 49399	6–12	3–6	3–6	3–6	3–6	3–6	1.5–3
E. faecalis ATCC 29212	6–12	6–12	>100	3–6	6–12	3–6	3–6
E. faecium ATCC 6569	>100	6–12	>100	>100	3–6	>100	>100
E. coli ATCC 25922	12–25	12–25	6–12	6–12	6–12	12–25	>100
K. pneumoniae ATCC 13383	6–12	3–6	6–12	6–12	3–6	3–6	>100
P. vulgaris ATCC 8427	12–25	12–25	12–25	12–25	12–25	12–25	12–25
P. aeruginosa ATCC 25416	>100	>100	>100	>100	>100	>100	>100
S. aureus ATCC 13709 (+4% BSA)	25–50	25–50	12–25	12–25	50–100	25–50	3–6
MTT HUH-7—CC_{50} 48 h (μM)	NT	50–100	25–50	12.5–25	NT	>100	NT
Clog *P*	4.89	5.1	4.91	4.77	4.94	5.3	0.58

Table 4. In vivo antibacterial activity of urea analogs[13]

Entry	Compd	Dose (mg/kg)	Mice alive/total
1	No drug	—	0/10
2	**10b**	75 × 2	4/10
3	**10b**	37.5 × 2	7/10
4	**10b**	18.8 × 2	6/10
5	**10b**	9.4 × 2	6/10
6	**10b**	4.7 × 2	4/10
7	**10b**	2.3 × 2	3/10
8	**8e**	37.5 × 2	3/10
9	**8e**	18.8 × 2	3/10
10	**8e**	9.4 × 2	4/10
11	**8e**	4.7 × 2	2/10
12	**8e**	2.3 × 2	0/10
13	Vancomycin	1 × 2	10/10

based antibacterial compounds.[6–9] While increased serum binding is not necessarily an undesirable property for antibiotic compounds, it indicates that higher doses of compound may be required to elicit a therapeutic response in vivo[10,11] This may narrow the therapeutic window and also raise the aqueous solubility threshold for poorly soluble drugs. During the course of this study we were able to prepare analogs with reduced lipophilicity and improved aqueous solubility relative to thiourea **1**.[12] However, there is still scope for reducing the lipophilicity and serum protein binding as well as increasing the potency of this compound class.

Lastly, to assess the in vivo efficacy of this class, analogs **8e** and **10b** were advanced for testing in a lethal murine model of bacterial infection (Table 4).[13] During the in vivo evaluation, urea **10b** showed efficacy in the murine model with 6/10 mice surviving in the 9.4 mg/kg (dosed 1 and 3 h postinfection) dose group and 7/10 mice surviving in the 37.5 mg/kg (dosed 1 and 3 h postinfection) dose group. Urea **10b**, however, was unable to rescue all mice from infection at the higher doses that were evaluated. Presumably the toxicity of the drug becomes an issue at higher drug concentrations.[13] Both urea analogs **8e** and **10b** were not as potent as the vancomycin positive control, which rescued all the animals at a dose of 1 mg/kg (dosed 1 and 3 h postinfection).

In conclusion, we have described the preparation and evaluation of novel aryl urea analogs as broad-spectrum antibacterial agents. Numerous compounds showed low micromolar activity against both Gram-positive and Gram-negative bacteria. Selected analogs also exhibited in vivo efficacy in a lethal murine model (*S. aureus*) of bacterial infection.

Acknowledgements

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References and notes

1. Chopra, I.; Hodgson, J.; Metcalf, B.; Poste, G. *Antimicrob. Agents Chemother.* **1997**, *41*, 497.
2. Wijkmans, J. C. H. M.; Beckett, R. P. *DDT* **2002**, *7*, 126.
3. Thiourea **1** showed activity comparable to Linezolid (3–6 μM), the positive control for our in vitro antibacterial assays.
4. The MIC assays were carried out in a 150 μL volume in duplicate in 96-well clear flat-bottom plates. The bacterial suspension from an overnight culture growth in the appropriate medium was added to a solution of test compound in 0.5% DMSO in water. Final bacterial inoculum was approximate 10^3–10^4 CFU/well. The percentage growth of the bacteria in the test wells relative to that observed for a control well containing no compound was determined by measuring absorbance at 595 nm (A_{595}) after 20–24 h at 37 °C. The MIC was determined as a range of concentrations where complete inhibition of growth was observed at the higher concentration and the bacterial cells were viable at the lower concentration. The bacterial strains used for the assays include *S. aureus* ATCC 13709, *S. pyogenes* ATCC 49399, *E. faecalis* ATCC 29212, *E. faecium* ATCC 6569, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13383, *P. vulgaris* ATCC 8427, *P. aeruginosa* ATCC 25416.
5. Clog *P* values were calculated using ChemDraw Ultra 7.0 software.
6. Francisco, G. D.; Li, Z.; Albright, D.; Eudy, N. H.; Katz, A. H.; Petersen, P. J.; Labthavikul, P.; Singh, G.; Yang, Y.; Rasmussen, B. A.; Lin, Y.-I.; Mansour, T. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 235.
7. Kane, J. L., Jr.; Hirth, B. H.; Liang, B.; Gourlie, B. B.; Nahill, S.; Barsomian, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4463.
8. Wilson, L. J.; Morris, T. W.; Wu, Q.; Renick, P. J.; Parker, C. N.; Davis, M. C.; McKeever, H. D.; Hershberger, P. M.; Switzer, A. G.; Shrum, G.; Sunder, S.; Jones, D. R.; Soper, S. S.; Dobson, R. L. M.; Burt, T.; Morand, K. L.; Stella, M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1149.
9. Proctor, R. A.; Dalal, S. C.; Kahl, B.; Brar, D.; Peters, G.; Nichols, W. W. *Antimicrob. Agents Chemother.* **2002**, *46*, 2333.
10. Bergogne-Berezin, E. *Clin. Pharmacokinet.* **2002**, *41*, 741.
11. Actor, P.; Uri, J. V.; Zajac, I.; Guarini, J. R.; Phillips, L.; Pitkin, D. H.; Berges, D. A.; Dunn, G. L.; Hoover, J. R. E.; Weisbach, J. A. *Antimicrob. Agents Chemother.* **1978**, *13*, 784.
12. Solubility of **8e**·lactate salt ~8 mg/mL, solubility of **10b**·HCl salt >10 mg/mL.
13. Mouse protection assay: Ten mice/dose group (ICR-CD-1 female mice 18–20 g, Charles River) were infected with a lethal dose (10^6 CFU/mouse) of *S. aureus* (ATCC 13709) suspended in 7.5% hog Gastric Mucin (IP). The infected animals were treated at 1 and 3 h postinfection with either compound **8e**·lactate salt, from 37.5 mg/kg down to 2.3 mg/kg or compound **10b**·hydrochloride salt, from 75 mg/kg down to 2.3 mg/kg (0.1 mL/mouse, IV). The positive control drug was vancomycin 1 mg/kg dosed twice at 1 and 3 h postinfection. The animals were observed for one week and mortality was calculated. Ureas **8e** and **10b** were toxic when dosed at concentrations above 37.5 and 150 mg/kg, respectively.