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Design and synthesis of 2-aminothiazole based antimicrobials targeting MRSA

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ABSTRACT

Privileged structure-based libraries have been shown to provide high affinity lead compounds for a variety of important biological targets. The present study describes the synthesis and screening of a 2-aminothiazole based compound library to determine their utility as antimicrobials, focusing on MRSA. Several of the compounds in this series demonstrated improved antimicrobial activity as compared to ceftriaxone (CTX), a β-lactam antibiotic. The most potent compound (**21**) had MICs in the range of 2–4 μg/ml across a panel of *Staphylococcus aureus* strains. In addition, trifluoromethoxy substituted aminothiazoles and aminobenzothiazoles were found to be potent antimicrobials with MICs of 2–16 μg/ml.

More deaths are caused by methicillin resistant *Staphylococcus aureus* (MRSA) strains in the US each year than by HIV/AIDS.[1] The emergence of antibacterial resistance due to metallo-β-lactamases neutralizing carbapenems is an example of the serious challenges that exist for antibacterial drug discovery. This challenge is further complicated by the exit of major pharmaceutical companies from the antibacterial arena leading to a paucity of new antibiotics to combat resistant bacteria.[2] Hence, there is a need for increased drug discovery efforts in this important area. One commonly used approach for lead generation is the screening of commercial libraries and natural product-based libraries. Commercial libraries commonly result in low hit rates due to false positives, lack of structural diversity and poor physicochemical properties.[3] Natural product-based libraries are often comprised of derivatives of parent compounds and seldom lead to activity that is truly distinct from the parent.[3] Privileged structure-based libraries however, have been shown to be capable of providing high affinity ligands for diverse biological targets. This approach has been used successfully for a variety of biological targets, especially G-protein coupled receptors (GPCRs), which has seen a number of successful developments over the last decade.[4,5]

Our efforts to develop a novel series of antibacterial agents led to the preparation and study of privileged structure-based libraries. We hypothesized that the 2-aminothiazole scaffold (fused and non-fused) would serve as a privileged structure due to their prevalence in antibacterial agents and other biologically active molecules.[6–9] The Kyoto Encyclopedia of Genes and Genomes (KEGG) database reveals the extensive use of the 2-aminothiazole structural motif in numerous drugs, as well as, preclinical and clinical candidates (Fig. 1). In addition there is literature evidence showing that 2-aminothiazole-based compounds serve as muscarinic and serotonergic ligands.[10–12] Herein we report the synthesis and antimicrobial screening of a 2-aminothiazole-based library of 35 compounds.

Our structurally diverse privileged structure-based library was synthesized using both commercially available bromo-2-aminobenzothiazoles (5-bromo and 6-bromosubstituted) and in-house synthesized bromo substituted 2-aminothiazole scaffolds. Both microwave[13] and thermal[14] synthetic approaches were employed to generate the 2-aminothiazole scaffolds (Scheme 1). The microwave assisted synthesis of the scaffold involving α-bromoketones and thiourea or substituted thiourea proceeded in moderate to high yields. Changing the solvent from ethanol to methanol for piperidine, morpholine and pyrrolidine containing α-bromoketones led to a higher conversion to the target aminothiazoles. A fused aminothiazole scaffold (**26a**) was generated from 2-bromocyclohexanone under microwave conditions (refer Supplementary data). In the absence of a commercially available α-bromoketone, the corresponding substituted acetophenone was subjected to thermal conditions with iodine and thiourea to generate the target 2-aminothiazole scaffold. Aminothiazoles containing methyl ester groups (**30**) and (**33**) were synthesized by methylating the corresponding acetyl benzoic acid starting materials with trimethylsilyl diazomethane (refer Supplementary

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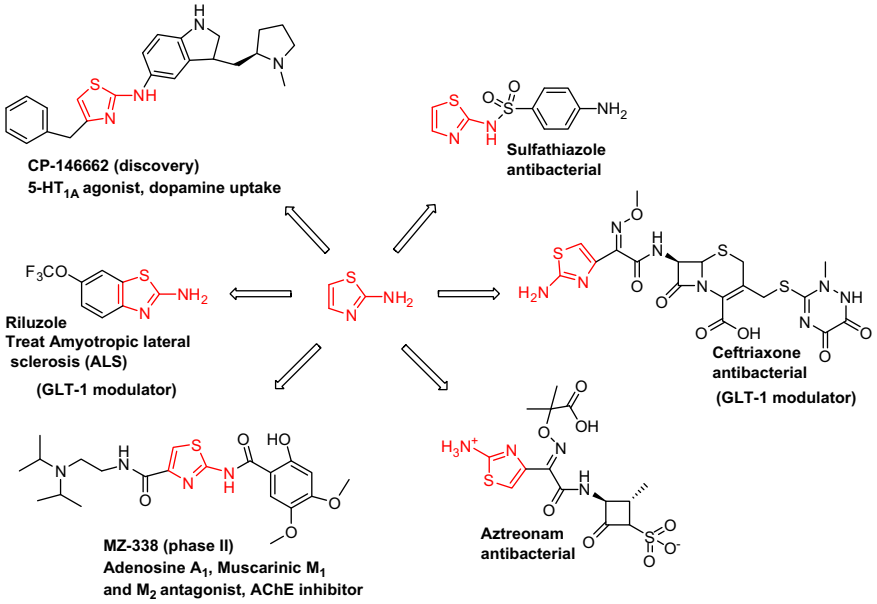
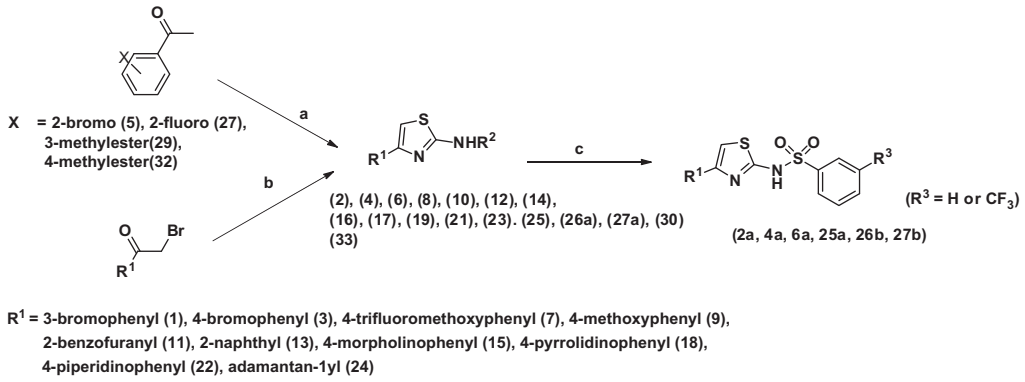


Figure 1. Use of 2-aminothiazole (non-fused and fused) scaffold in ligands/drugs pertaining to the biological targets of interest (adapted and modified from KEGG database).



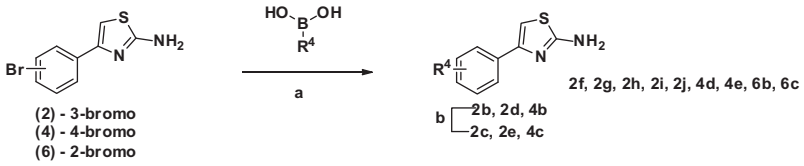
Scheme 1. Synthesis of 2-aminothiazole scaffolds and functionalization. Reagents and conditions: (a) iodine (1 equiv) thiourea (2 equiv) heat at 100 °C or reflux in THF (12–52%); (b) thiourea or 3-fluorophenyl thiourea, ethanol or methanol, MW 50 °C, 10 min (closed) or 30 min (open vessel) (20–96%); (c) benzene sulfonyl chloride or substituted benzene sulfonyl chloride, pyridine, 0 °C to rt (12–64%).

data) and then heated with iodine and thiourea to provide the desired aminothiazole scaffold.

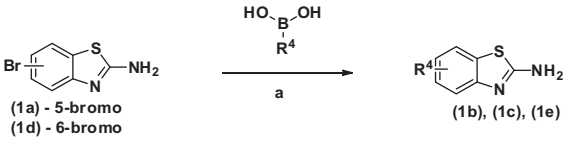
The aminothiazole scaffolds (**2**), (**4**), (**6**), (**25**), (**26**), (**27**), (**30**) and (**33**) were chosen for further functionalization. An aryl sulfonamide fragment was incorporated at the amino end of the scaffolds (**2**), (**4**), (**6**), (**25**), (**26a**) and (**27a**) by condensation with either benzene sulfonyl chloride or substituted benzene sulfonyl chloride in the presence of base (Scheme 1). The yields of the sulfonamidation reactions were found to be low to moderate. LC–MS analysis of the reaction mixture revealed the simultaneous formation of the bis-sulfonamide product in some cases. Literature reports suggest the simultaneous formation of bis-sulfonamide during sulfonamidation reactions may be responsible for the low yields of monosulfonamide product recovered in our hands.[15–17] Alkali mediated recovery of the monosulfonamides has been reported to improve the final yield of the monosulfonamide product but was not attempted here.

Microwave promoted Suzuki coupling reactions were employed in order to incorporate carboxyphenyl and trifluoromethoxyphenyl fragments on the aminothiazole scaffold. The bromoaminothiazoles and bromoamino benzothiazoles were coupled with the corresponding substituted boronic acids in the presence of palladium catalysts (Schemes 2 and 3). *trans*-Dichlorobis(triphenyl phosphine) palladium(II) provided better reaction yields when compared to Tetrakis (triphenylphosphine)palladium(0) in the present series.

There are several methods reported in the literature for coupling the amino group of the β-lactams with carboxyl derivatives. A commonly used method is the activation of the acid by formation of the Vilsmeier reagent using phosphorus oxychloride ($POCl_3$), *N*,*N*-dimethylformamide and tetrahydrofuran.[18] Other methods include the use of coupling reagents like diethyl chlorophosphate and 2-chloro-4,6-dimethoxy-1,3,5-triazine.[19,20] After unsuccessful attempts with the Vilsmeier activation, 2-chloro-4,6-dimethoxy-1,3,5-triazine was used for activation of the carboxyl containing aminothiazoles. An advantage of using 2-chloro-4,6-dimethoxy-1,3,5-triazine is the ability to monitor the formation of the activated ester using LC-MS. Using this approach, β-lactam-based aminothiazoles were synthesized in four steps (Schemes 4 and 5). In the first step the amine functionality was protected with a



Scheme 2. Synthesis of 2-aminothiazole derivatives. Reagents and conditions: (a) substituted boronic acids or boronic pinacol ester, $(Ph_3P)_4Pd$ or $PdCl_2(PPh_3)_2$, anhydrous potassium carbonate or sodium carbonate (2 M), THF:water (1:1), MW 140 °C, 30 min (10–49%); (b) 2 M NaOH (40–76%).

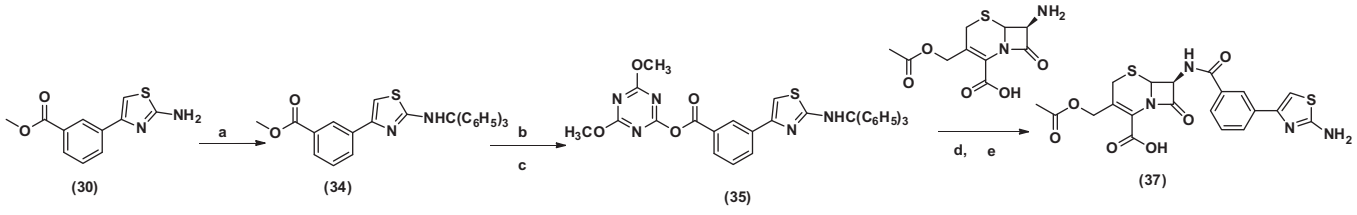


Scheme 3. Synthesis of 2-aminobenzothiazole derivatives. Reagents and conditions: (a) substituted boronic acids or boronic pinacol esters, $(Ph_3P)_4Pd$ or $PdCl_2(PPh_3)_2$, sodium carbonate (2 M), THF:water (1:1), MW 140 °C,30 min (32–49%)

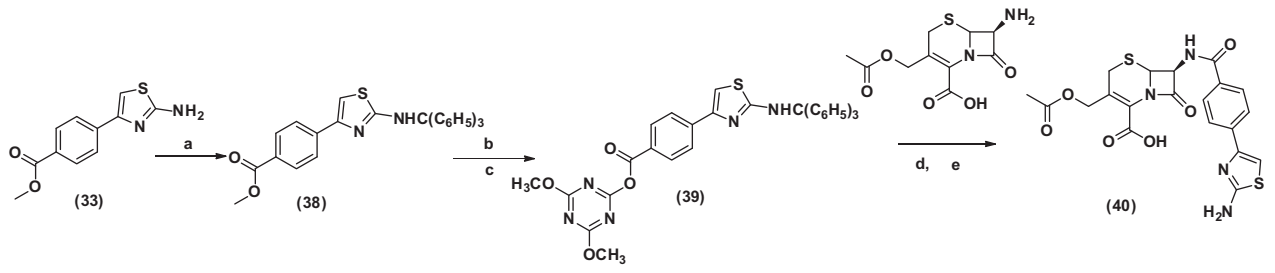
trityl group followed by alkali hydrolysis of the ester to the corresponding acid. The acid was then activated using 2-chloro-4,6-dimethoxy-1,3,5-triazine and the activated ester was coupled to the silylated β-lactam fragment (7-aminocephalosporanic acid) followed by deprotection of the trityl group. The compounds synthesized as described above were evaluated in antimicrobial assays and MIC values determined.

Thirty five compounds were screened for antimicrobial activity against a panel of methicillin resistant or susceptible microorganisms including *S. aureus* (USA300, UAMS-1 & RN8175), *Escherichia coli* C600 N/puc19 and *Acinetobacter baumanii* UNMC 8872. MIC values were determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines using cation adjusted Mueller–Hinton broth. Microtiter plates containing serial dilutions of compounds (0, 2, 4, 8, 16, 32, 64, 128, or 256 μg/ml) were inoculated with 10^5 colony forming units (CFU/ml) and incubated for 24 h at 37 °C. MIC values were determined to be the lowest compound concentration that inhibits bacterial growth (turbidity). MIC data for the test compounds is shown in Tables 1–3. Aminothiazole derivatives possess comparable or superior antimicrobial activity against *S. aureus* strains when compared to the standard CTX [8 μg/ml(UAMS-1);128 μg/ml(USA300);16 μg/ml (RN8175)].

Compound (**21**) was found to be the most potent in the series with MIC values in the range of 2–4 μg/ml across all *S.aureus* strains. Trifluoromethoxyphenyl substituted 2-aminothiazoles (**2j**), (**4d**), (**6c**) and trifluoromethoxy substituted aminobenzothiazoles (**1c**) and (**1e**) showed MIC values in the range of 2–16 μg/ml against *S. aureus* strains. Compound (**8**) containing a 4-trifluoromethoxy phenyl group showed a moderate MIC value of 64 μg/ml against *S. aureus* (USA 300 and RN8175) compared to CTX. Replacement of the 4-trifluoromethoxy phenyl group with a 4-methoxy phenyl group resulted in loss of antimicrobial activity (>256 μg/ml). Additional studies will be required to determine the role of the trifluoromethoxy phenyl fragment in improving antimicrobial potency of 2-aminothiazoles. In addition to the promising effects observed with the trifluoromethoxy phenyl fragment, there were other fragments which resulted in MIC values comparable to CTX. Compound (**20**), a pyrrolidine based compound, demonstrated an MIC value of 2 μg/ml against *S. aureus* (UAMS-1). The MIC for the β-lactam based compound (**37**) was determined to be 16 μg/ml against *S. aureus* (RN8175). Sulfonamides (**4a**) and (**6a**) exhibited MIC values comparable to CTX against *S. aureus* strains. The MIC value for ethylbenzoate (**6b**) was two fold lower than CTX against *S. aureus* (RN8175). The

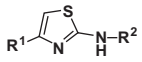


Scheme 4. Synthesis of compound (**37**). Reagents and conditions: (a) Trityl chloride, pyridine, 40 °C; (b) 2 M NaOH, 50 °C; (c) 2-chloro-4, 6-dimethoxy,-1,3,5-triazine, *N, N*-dimethylformamide, 4-methylmorpholine, rt 30 min; (d) N,O-bis(trimethyl silyl) acetamide (2 equiv), tetrahydrofuran, 0 °C, 30 min (silylation of 7-ACA) followed by coupling at 0– −10 °C overnight; (e) Trifluoroacetic acid, anisole (21%).



Scheme 5. Synthesis of compound (**40**). Reagents and conditions: (a) Trityl chloride, pyridine, 40 °C; (b) 2 M NaOH, 50 °C; (c) 2-chloro-4, 6-dimethoxy,-1,3,5-triazine, *N,N*-dimethylformamide, 4-methylmorpholine, rt 30 min; (d) N, O-bis(trimethyl silyl) acetamide (2 equiv), tetrahydrofuran, 0 °C, 30 min (silylation of 7-ACA) followed by coupling at 0–−10 °C overnight; (e) trifluoroacetic acid, anisole (48%).

Table 1
Minimum inhibitory concentration (MIC) data of 2-aminothiazole derivatives



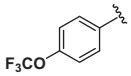
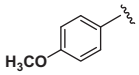
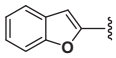
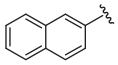
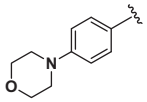
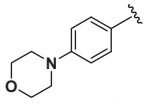
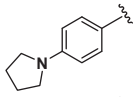
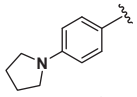
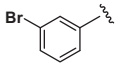
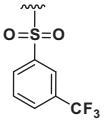
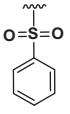
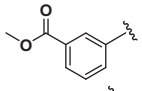
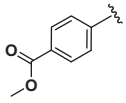
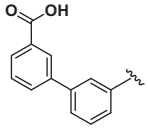
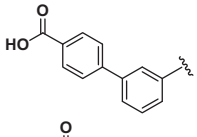
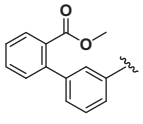
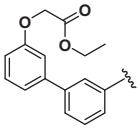
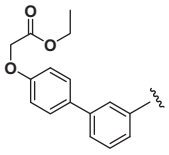
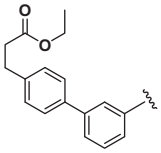
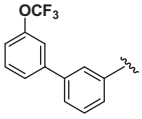
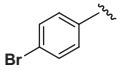
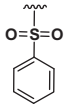
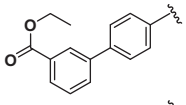
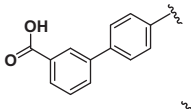
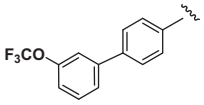
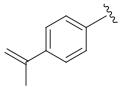
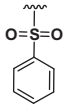
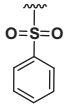
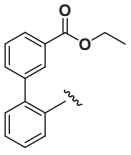
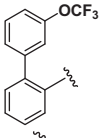
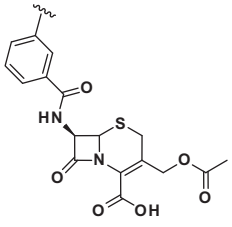
Compd. No.	R^1	R^2	*S.aureus*			*E.Coli*	*A.baumanii*
			UAMS-1 μg/ml	USA-300 μg/ml	RN8175 μg/ml	C600 N/puc19 μg/ml	UNMC 8872 μg/ml
8		H	N.T.	64	64	>256	N.T.
10		H	N.T.	>256	>256	> 256	N.T.
12		H	256	>256	>256	>256	N.T.
14		H	256	>256	>256	>256	N.T.
16		H	128	>256	256	>256	N.T.
17			256	>256	>256	>256	N.T.
19		H	256	>256	>256	>256	N.T
20			2	>256	>256	>256	N.T.
21			2	4	4	>256	N.T.
25a			128	>256	>256	>256	N.T.
27b			N.T.	128	32	> 256	N.T.
30		H	N.T.	>256	>256	>256	N.T.
33		H	N.T.	>256	128	>256	N.T.
2c		H	N.T.	>256	>256	>256	>256
2e		H	N.T.	>256	>256	>256	>256
2f		H	>256	>256	>256	>256	N.T.

Table 1 (*continued*)

Compd. No.	R^1	R^2	*S.aureus*			*E.Coli*	*A.baumanii*
			UAMS-1 μg/ml	USA-300 μg/ml	RN8175 μg/ml	C600 N/puc19 μg/ml	UNMC 8872 μg/ml
2g		H	N.T.	>256	>256	>256	N.T.
2h		H	N.T.	>256	256	>256	N.T.
2i		H	N.T.	>256	256	>256	N.T.
2j		H	N.T.	8	4	>256	N.T.
4a			N.T.	64	16	>256	>256
4b		H	N.T.	>256	>256	>256	>256
4c		H	N.T.	N.T.	N.T.	N.T.	N.T.
4d		H	N.T	>256	2	>256	N.T.
4f			N.T.	N.T.	N.T.	N.T.	N.T.
6a			N.T.	128	32	>256	N.T.
6b		H	N.T.	256	32	>256	>256
6c		H	N.T.	16	16	>256	N.T.
37		H	256	256	16	>256	N.T.

(*continued on next page*)

Table 1 (*continued*)

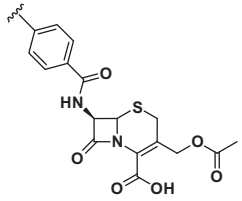
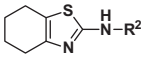
Compd. No.	R^1	R^2	*S.aureus*			*E.Coli*	*A.baumanii*
			UAMS-1 μg/ml	USA-300 μg/ml	RN8175 μg/ml	C600 N/puc19 μg/ml	UNMC 8872 μg/ml
40		H	N.T.	N.T.	N.T.	N.T.	N.T.

Table 2
Minimum inhibitory concentration (MIC) data of fused 2-aminothiazole



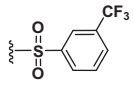
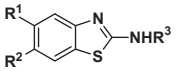
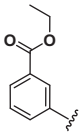
Compd. No.	R^2	*S. aureus*			*E. coli*	*A. baumanii*
		UAMS-1	USA-300	RN8175	C600 N/puc19	UNMC 8872
		μg/ml	μg/ml	μg/ml	μg/ml	μg/ml
26b		>256	>256	>256	>256	N.T

Table 3
Minimum inhibitory concentration of 2-aminobenzothiaozles



Compd. No.	R^1	R^2	R^3	*S. aureus*			*E. coli*	*A. baumanii*
				UAMS-1	USA-300	RN8175	C600 N/puc19	UNMC 8872
				μg/ml	μg/ml	μg/ml	μg/ml	μg/ml
1b		H	H	>256	>256	>256	>256	N.T.
1c		H	H	N.T.	8	8	>256	N.T.
1e	H		H	N.T.	16	8	>256	N.T.
CTX				8	128	16	2	8

CTX-ceftriaxone.
N.T.—not tested.

preliminary antimicrobial data presented herein supports the privileged nature of 2-aminothiazole and 2-aminobenzothiazole scaffolds. Incorporation of suitable peripheral fragments could be a useful approach to generate promising antimicrobial leads especially against methicillin resistant *S. aureus* strains.

In conclusion, the present work describes the synthesis and antimicrobial screening of a privileged structure-based library focused on the aminothiazole (fused and non-fused) ring system. The work provides useful synthetic methods for the preparation of aminothiazole-based ligands that can be used to prepare novel compounds for screening in antibacterial assays as well as for other important biological targets. Biological evaluation of the present library has identified promising antimicrobial leads against methicillin resistant *S. aureus* strains. Efforts are currently underway to

expand the library and screen the compounds for antibacterial activity and to assess their utility in additional biologically relevant targets.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09.095.

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