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Benzothiazolylthio Carbapenems: Potent Anti-MRSA Agents

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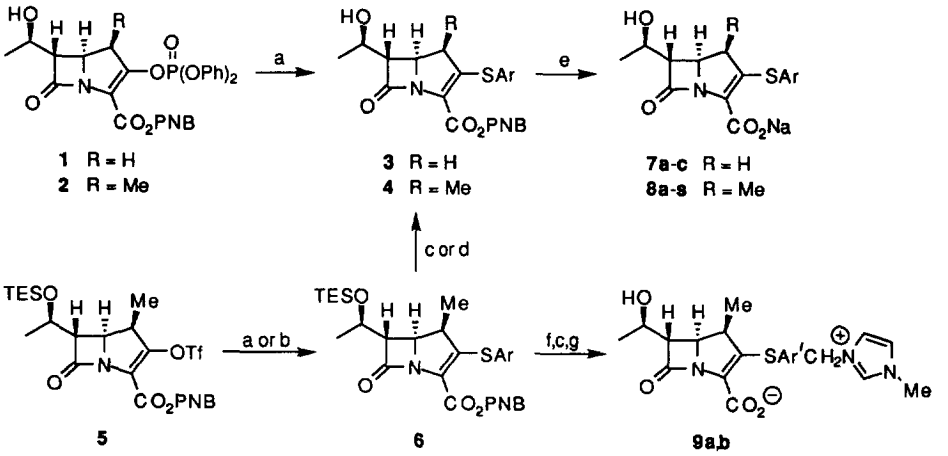
Abstract: A series of sulfur-linked benzothiazolyl carbapenems has been prepared and evaluated against a battery of microorganisms. Many of the compounds displayed good activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Data is presented which delimits the pharmacophore and provides a preliminary SAR.

Imipenem, the prototypical carbapenem antibiotic, is effective against an extremely broad spectrum of bacterial pathogens. An important deficiency, however, is its reduced potency versus methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase negative staphylococci (MRCNS), pathogens of growing clinical importance.[1] A leading hypothesis to account for this lack of potency is that normal carbapenems, as well as most penicillins and cephalosporins, do not bind well to PBP2a, a penicillin binding protein unique to MRS.[2] Introduction of a suitable "binding element" would be expected to overcome this deficiency and confer activity against MRS. A benzothiazole moiety linked by sulfur to the 3-position of a cephalosporin[3] or carbacephalosporin[4] has been observed to confer potency against MRSA, leading us to the hypothesis that this moiety is such a "binding element" and prompting us to investigate the attachment of S-linked benzothiazoles to the 2-position of the carbapenem nucleus. A recent disclosure of a similar approach by another group[5] has prompted us to communicate our efforts in this area.

Synthesis

As shown in the scheme, the general synthesis involved the addition/elimination displacement of a leaving group (diphenylphosphate or triflate) by a heteroaryl thiolate at the 2-position of a suitably protected 1-β-H or 1-β-Me carbapenem, followed by deprotection.[6] The carbapenem-2-diphenylphosphates **1**[7] and **2**[8] had the advantage of requiring no protection of the side-chain hydroxyl, but were insufficiently reactive in the 1-β-methyl series (**2**). Here, much better yields were obtained using the more reactive *O*-TES 1-β-methylcarbapenem-2-triflate **5**.[9] The precursors to the thiolate nucleophiles exist predominantly or exclusively as the thioxo tautomers, and generally required a stronger base than a tertiary amine for thiolate formation. Initially, sodium thiolates were prepared using sodium hydride in tetrahydrofuran, but it was soon found that lithium thiolates gave faster additions and better yields. Lithium hexamethyldisilazide was used successfully, but lithium hydroxide monohydrate in tetrahydrofuran eventually emerged as the reagent of choice for activation of the nucleophile. Following the optimized procedure, isolated yields for the addition reaction of lithium thiolates to the 1-β-methylcarbapenem-2-triflate **5** exceeded 80%. Zwitterionic analogs **9** were prepared from adducts **6** bearing a distal arylic alcohol by one pot activation/displacement using triflic anhydride and an excess of N-methylimidazole. Deprotection of the triethylsilyloxy group was accomplished by treatment with pH 2.2 aq. THF, and the p-nitrobenzyl ester was removed by hydrogenation. Optimized deprotection yields generally exceeded 80%.



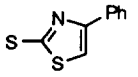
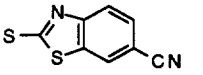
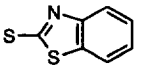
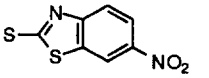
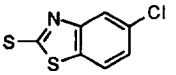
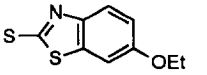
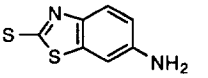
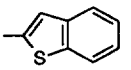
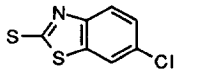
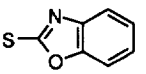
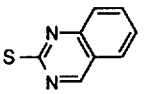
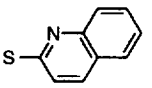
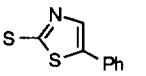
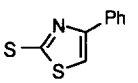
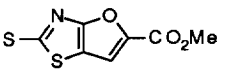
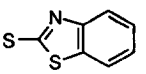
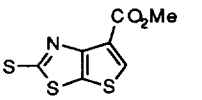
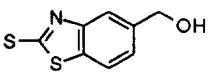
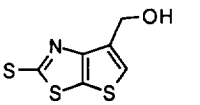
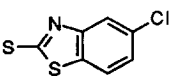
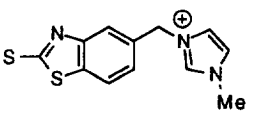
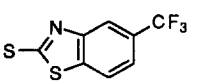
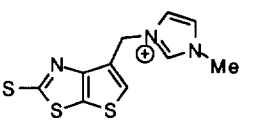
Reagents: (a) NaSAr, THF; (b) LiSAr, THF; (c) pH 2.2 (HCl), THF-H_2O (2:1); (d) TBAF, HOAc, THF; (e) H_2 (1 atm), 10% Pd/C, $NaHCO_3$, THF-EtOH-H_2O; (f) Tf_2O, 1-Me-imidazole, CH_2Cl_2, -78°C to rt; (g) H_2 (1 atm), 10% Pd/C, THF-BuOH-EtOH-H_2O-buffer (pH 7).

Biological Evaluation

The results of the antimicrobial assays, as well as data on PBP2a binding and DHP-I susceptibility,[10] are presented in tables 1 and 2. It should be noted that midway through our program a switch was made from an agar disk diffusion assay[11,12] to a broth microtube dilution assay. The data from the disk diffusion assay is presented in table 1 in the form of computed minimum inhibitory concentrations (MICs, see the footnotes to the table for details), while the data from the microtube dilution assay is presented in table 2 as actual MICs. In order to facilitate comparison of data between the tables, each table includes the same strains of microorganisms. The comparison is inexact but reasonably close, as can be seen by comparing the data for compounds **8f** and **9a**.

From an examination of the biological data presented in tables 1 and 2, several points emerge. First, it can be seen by comparing the pairs **7a/8e**, **7b/8f** and **7c/8h** that the expected increase in DHP stability upon introduction of the β-methyl group at position 1 is realized:[8] the 1-β-methyl compounds are at least six-fold less susceptible than their 1-β-hydrogen analogs. A parallel increase in chemical stability is also observed, e. g. the 1-β-methyl compounds react more slowly with aq. NH_2OH than their 1-β-hydrogen counterparts. Further, the introduction of the 1-β-methyl group appears to enhance potency against MRSA (**7a/8e**, **7c/8h**) and MRCNS (**7a/8e**, **7b/8f**, **7c/8h**).

Entries **8a-f,o-s** serve to delimit the pharmacophore. Of this series, compounds **8e,f,r,s** display acceptable potency against MRSA/MRCNS. This data supports the following conclusions: first, an S-linked thiazole by itself (**8a**) does not confer activity: an aromatic ring must be associated either by fusion (**8f,r,s**) or by direct attachment to position 4 of the thiazole (**8e**), but not to position 5 (**8p**). Second, this potentiating aromatic ring may be derived from benzene (**8e-f**), thiophene (**8r,s**) or less successfully from furan (**8q**). Finally, a series of more or less "homologous" replacements destroy or attenuate activity; thus, replacement of N by CH (**8b**), S by O (**8c**), S by a C-C double bond (**8d**), or S by a C-N double bond (**8o**) all result in compounds with reduced activity compared to the paradigm **8f**.

Compd	SAr	Synthesis	Compd	SAr	Synthesis
7a		a,e	**8j**		b,c,e
7b		a,e	**8k**		footnote[b]
7c		a,e	**8l**		a,c,e
8a		a,e	**8m**		a,e
8b		footnote[a]	**8n**		a,c,e
8c		a,c,e	**8o**		b,d,e
8d		a,c,e	**8p**		b,d,e
8e		a,e	**8q**		b,c,e
8f		a,e	**8r**		b,c,e
8g		a,e	**8s**		b,c,e
8h		a,e	**9a**		b,f,c,g
8i		a,c,e	**9b**		b,f,c,g

[a] Compound **8b** was prepared from carbapenem phosphate **2**, 2-mercaptobenzothiophene and iPr_2NEt in MeCN followed by ester deblocking according to step e.

[b] Compound **8k** was prepared from the allyl ester corresponding to **5**, 2-thioxo-6-nitro-2,3-dihydro-benzothiazole and NaH in THF followed by acid mediated desilylation (step c) and palladium catalyzed deallylation.

Table 1. Computed MIC[a], PBP2a Binding[b], and DHP-I Susceptibility[c] of Carbapenems **7a-c**, **8a-n** and **9a**.

Compd	Computed Minimum Inhibitory Concentration (μg/mL)					PBP2a IC_{50} (μg/mL)	DHP-I (xIPM)
	MSSA (2)	MRSA (1)	MRCNS (1)	Enterococcus (1)	Gram Neg[d] (11)		
7a	0.03	11.3	19.7	2.1	14.0	NT	3.6
7b	0.07	1.9	3.5	1.7	3.7	3.1	1.7
7c	0.02	1.8	3.7	0.7	3.8	1.6	1.2
8a	0.17	18.2	41.6	7.1	2.5	11.7	0.4
8b	0.04	3.9	12.1	5.7	9.8	16.5	0.4
8c	0.23	12.1	34.9	8.9	27.8	43.0	NT
8d	0.05	6.3	14.6	4.9	10.1	28.0	NT
8e	0.05	1.9	3.0	2.6	6.6	10.1	0.4
8f	0.06	1.7	1.6	2.1	5.6	2.0	0.2
8g	0.07	1.0	2.0	3.8	8.0	1.2	0.3
8h	0.02	0.8	1.7	2.7	4.8	2.7	0.2
8i	0.08	1.2	2.8	2.9	9.9	0.9	NT
8j	0.04	0.9	1.9	4.5	8.8	1.6	0.1
8k	0.05	1.1	1.9	4.9	22.4	0.6	0.2
8l	0.10	4.0	7.1	6.0	31.6	4.7	0.3
8m	0.09	9.0	25.2	5.9	18.3	16.8	NT
8n	0.10	2.3	3.5	4.0	17.6	0.9	NT
9a	0.03	1.0	0.8	1.2	3.4	0.4	0.2
Imipenem	0.02	35.9	63.7	1.1	0.6	128	1.0

[a] Agar disk diffusion method, see ref 11. MIC values were computed according to the method of ref 12 using concentrations determined by UV spectroscopy. Where more than one strain was tested, the activity is reported as the geometric mean of the computed MICs for the number of strains indicated in parentheses.

[b] Binding to PBP2a was evaluated by competition analysis with [^{3}H]-benzylpenicillin using cell membrane fractions from the homogeneous MRSA COL strain.

[c] DHP-I (swine kidney enzyme) results are presented as the hydrolysis rate for a given compound divided by the hydrolysis rate with imipenem as substrate, see ref 10.

[d] The gram-negative activity is derived from eleven strains selected from seven enteric genera.

The benzothiazole pharmacophore was chosen as the focus of a more detailed SAR study, and the remaining entries explore the effect of substitution on this basic platform. Grossly, it can be seen that electron withdrawing substituents (**8h-k**) improve anti-MRSA potency, while electron donating substituents (**8l,m**) have a deleterious effect. In the one case where direct comparison is possible (**8h/8n**; substituent is chloro), substitution at the 5' position is preferred over substitution at 6'. All compounds bearing a 5' substituent (**8g-i**) show improved anti-MRSA potency relative to the unsubstituted paradigm (**8f**), even when the substituent should have no pronounced electronic effect (**8g**).

Table 2. MIC[a], PBP2a Binding, and DHP-I Susceptibility of Carbapenems **8f**, **8o-s** and **9a-b**.

Compd	Minimum Inhibitory Concentration (μg/mL) MSSA (2)	MRSA (1)	MRCNS (1)	Enterococcus (1)	Gram Neg (11)	PBP2a IC_{50} (μg/mL)	DHP-I (xIPM)
8f	0.02	1	4	4	9.7	2.0	0.2
8o	0.02	4	16	8	15.0	11.9	0.3
8p	0.03	32	64	8	16.0	100	0.5
8q	0.02	4	16	4	12.4	1.5	NT
8r	0.04	2	4	4	21.9	1.2	0.3
8s	0.02	2	4	4	9.7	1.2	0.5
9a	0.02	2	4	1	5.2	0.4	0.2
9b	0.02	1	2	2	5.5	0.3	0.2
Imipenem[b]	0.02	64	>64	1.3	0.4	128	1.0

[a] Broth microtube dilution method. MRS strains were tested in Mueller-Hinton broth + 2% NaCl at ~10^5 CFU/mL; Enterococcus was tested in brain heart infusion broth at ~10^6 CFU/mL; the remaining strains were tested in Mueller-Hinton broth at ~10^5 CFU/mL. Incubation was at 35°C for 20-22 hours. Where more than one strain was tested, the activity is reported as the geometric mean of the MICs for the number of strains indicated in parentheses.

[b] Imipenem activities are expressed as geometric means of MICs obtained from a series of tests.

Consideration of the PBP2a IC_{50}s reveals a limited relationship between binding constants and potency. With one exception (compound **8e**), compounds with MICs < 2 μg/mL vs. MRSA have PBP2a IC_{50}s less than 4 μg/mL, but within this group there is little further relationship between MIC and PBP2a IC_{50}. Conversely, compounds with PBP2a IC_{50}s greater than 10 μg/mL (again with the exception of compound **8e**) all have MICs > 3 μg/mL, but as before, within this group there is little further correlation. This suggests that while binding constants to PBP2a allow for gross predictions of potency, other factors are also of importance: it seems likely that achieving the greatest potency depends on binding well to an ensemble of PBPs.

Finally, data for compounds **9a** and **9b** show that potency against gram-positive organisms can be maintained despite the incorporation of a positively charged sidechain.[13] The benefits of this transition from anionic to zwitterionic species include an improved gram-negative spectrum, substantially improved binding to PBP2a, and a dramatic reduction in plasma protein binding. These issues will be explored in greater detail in a subsequent report.

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6. The following procedure, for the preparation of **8s**, is typical: To 39.0 mg (0.929 mmol) of finely powdered $LiOH{\cdot}H_2O$ in 13.5 mL of anhydrous tetrahydrofuran at room temperature was added 189 mg (0.931 mmol) of 6-hydroxymethyl-1*H*-thieno[3,2-*d*]thiazole-2-thione as a solid in one portion. The resulting mixture was sonicated and stirred for 5 minutes, after which time it remained a hazy suspension. To this mixture was added 474 mg (0.778 mmol) of triflate **5** as a solid in a single portion. After stirring 25 minutes at room temperature the solvent was removed under reduced pressure and the crude residue was chromatographed on EM silica gel 60 (230-400 mesh), eluting with 8 : 1 CH_2Cl_2-EtOAc. In this manner 478 mg (93%) of **6s** was obtained as a white foam. To 24 mg (0.036 mmol) of **6s** in 5 mL of tetrahydrofuran and 2.5 mL of water was added 2 N aq. HCl until the pH (monitored by a pH meter) reached 2.2. After 5.5 hours the desilylation was judged to be complete by thin layer chromatography (on silica plates, eluting with 6 : 1 hexane-EtOAc). The reaction was added to 100 mL of methylene chloride and extracted twice with saturated aq. sodium bicarbonate and then with brine, and the organic layer was dried over sodium sulfate, filtered, and the solvent was removed under reduced pressure to afford a crude colorless oil. To this material was added 1.5 mL of tetrahydrofuran, 1.5 mL of ethanol, a solution of 3.0 mg (0.036 mmol) of sodium bicarbonate in 1 mL of water, and 20 mg of 10% Pd/C. This mixture was stirred briskly under a H_2 atmosphere for 1 hour at room temperature, then filtered through a Celite pad and the catalyst was washed with water (2 x 5 mL). The filtrate was washed with methylene chloride (2 x 5 mL) and ether (5 mL), filtered through a 0.45 micron CR acrodisc, concentrated under vacuum to about 5 mL volume, and lyophilized to give **8s** (12.0 mg, **74%**) as an amorphous white solid.
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