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SAR Studies of Anti-MRSA Non-zwitterionic 3-Heteroarylthiocephems

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SAR studies in a series of 3-heteroarylthio substituted cephalosporins established that high activity against methicillin-resistant *Staphylococcus aureus* (MRSA) can be achieved with various heteroaryl substituents. Early results showed that highly lipophilic 3-heteroarylthio substituents, which were necessary for anti-MRSA activity, caused high affinity of such cephems toward serum proteins. Our earlier published efforts described discovery of zwitterionic cephems MC-02,331 and RWJ-54428 (MC-02,479), where serum binding was reduced by employing basic, positively charged functionalities attached to the 3-heteroarylthio substituent. In order to avoid low solubility problems associated with most such zwitterionic cephalosporins a wide variety of non-basic heteroaryl substituents was tested (non-zwitterionic cephems are more easily formulated as water soluble sodium salts for intravenous administration). Considerable reduction in serum binding was obtained in some analogs while maintaining high anti-MRSA potency.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common cause of serious nosocomial infections[1]. Restrictions in the use of vancomycin, a drug of choice against MRSA infections, are presently recommended as a measure to decrease the emergence of vancomycin resistant Gram-positive organisms[2]. Reports of infections caused by *S. aureus*, with varying degrees of glycopeptide resistance (GISA), point to the urgent need for the development of novel, anti-MRSA agents[3]. Efforts directed toward discovery and development of such drugs within the cephalosporin class are exemplified by publications from Bristol-Myers Squibb[4] (**1**), Meiji Seika[5] (**2**) and Microcide[6] (**3**). (Fig. 1).

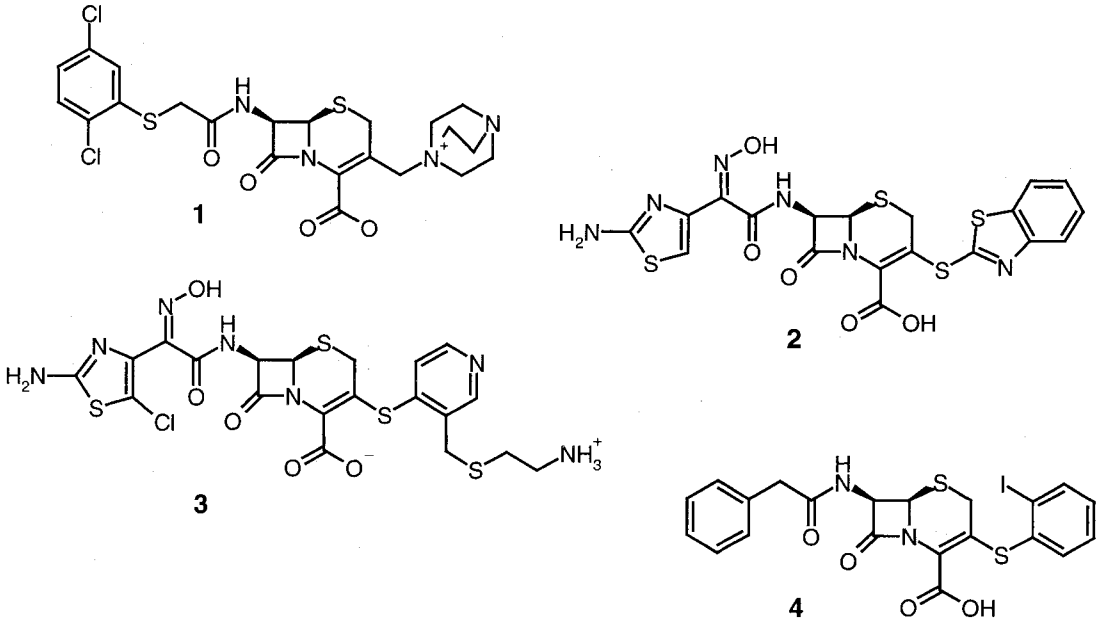
The structural modifications introduced to these cephalosporins aim to increase the affinity of the drug molecule toward penicillin binding protein 2a (PBP 2a), which is the main mediator of resistance to methicillin. Recently described kinetic studies of β-lactam binding to PBP 2a have provided important insight into the resistance mechanism at the molecular level[7]. In our early SAR studies of anti-MRSA cephalosporins, it became apparent that relatively high lipophilicity was required for high anti-MRSA potency but was also one of the factors responsible for high serum binding of most potent compounds. The 3-(2-iodophenyl)thio cephalosporin derivative **4**[8] exemplifies an anti-MRSA cephalosporin agent of high *in vitro* potency with extremely high serum protein binding, which precluded its antibacterial application *in vivo*. An avenue to reducing serum binding of such cephalosporins, by introducing positively charged functionalities, was successfully employed by us in the discovery of RWJ 54428 (**3**). This approach resulted in a zwitterionic molecule, which requires low pH to achieve solubility necessary for parenteral administration. An alternative strategy seeks a non-zwitterionic analog which can be formulated as a highly soluble sodium salt at neutral pH. The present publication describes such an alternative strategy in which we tested the limits of reduction of the overall lipophilicity of the molecule without sacrificing anti-MRSA potency.

Preparation of key intermediates and general synthetic schemes of 3-heteroaryl-thiocephems are outlined in Scheme 1.

Syntheses of aminothiazoleacetic acid and aminochlorothiazoleacetic acid intermediates **I** and **II**, used

Dedicated to the memory of Sir EDWARD ABRAHAM.

Fig. 1. Cephalosporins with high anti-MRSA activity.



for the introduction of C-7 acyl functionalities, have been previously described[6]. Synthesis of the corresponding (5-amino-[1,2,4]thiadiazol-3-yl)-*Z*-trityloxyimino-acetic acid intermediate **III** was accomplished using a modified Katayama route[9].

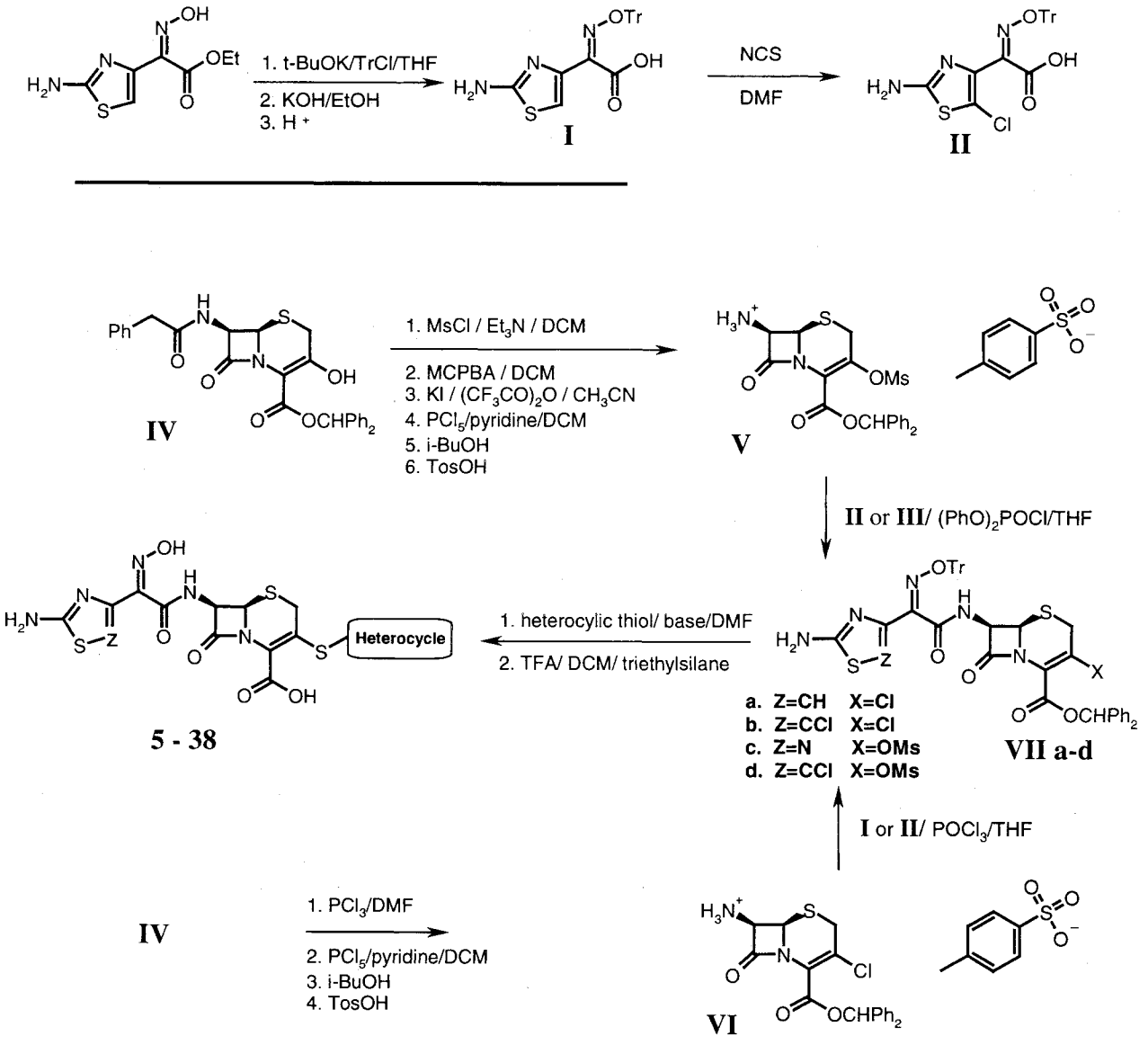
Acids **I**~**III** were coupled to the key benzhydryl protected 7-amino-3-methanesulfonyloxy- (**V**) or 7-amino-3-chloro- (**VI**) cephem intermediates to produce the corresponding amides **VII a**~**d**. In the ensuing reactions with thiols, depending on the character of the heterocyclic thiol, base induced isomerization leading to the undesired Δ^2 cephem isomer (up to 80%) often could not be suppressed, and the desired Δ^3 isomers of protected 3-heteroarylthiocephems had to be isolated by chromatography. The final 3-heteroarylthio cephalosporins **5**~**38** were obtained by deprotection with trifluoroacetic acid and, when necessary, were converted into the corresponding sodium salts.

In our early SAR studies of 3-arylthio and 3-heteroarylthio substituted cephalosporins[8] we determined that the *ortho* substitution present in the aromatic substituent generally improved anti-MRSA potency relative to other attachment positions. Therefore we have explored a class of 3-(*ortho*-substituted-phenylthio)cephems (Table 1) with variations in phenyl ring and oxime substitution. High anti-MRSA activity was associated with either unsubstituted oxime (compound **7**) or large lipophilic substituents attached to the oxime functionality (compounds **9**, **11**). However, analogs from this group were highly lipophilic, which adversely affected serum protein binding (*e.g.* human serum binding for the 2-cyanophenyl analog **12** is 99%).

Potent anti-MRSA compounds were also found among a series of 3-(*N*-phenylpyrazol-5-yl)-thiocephems (Table 2). Particularly potent were analogs **13** and **18** bearing lipophilic alkyl groups at the oxime functionality as well as the 2-fluoroethyl oxime derivative **16**, where additional improvement in potency is due to the chlorine atom present in the aminothiazole moiety. None of the *N*-phenylpyrazolyl compounds (**13**, **17**, **19**) tested in animal model of infection was efficacious *in vivo*. Compared to 3-phenylthio analogs, there was no substantial reduction in lipophilicity and this group of analogs suffered from essentially the same problem of high affinity for serum proteins (by correlating the decrease in growth inhibition zone on agar culture plate to the magnitude of serum binding, the serum binding of compounds **13**, **14** was estimated to be >95% and of compounds **15**, **16**, **17** was estimated to be >99%).

Greater reductions in serum binding were achieved in the class of 3-(pyrid-4-yl) thiocephems (Table 3). The analogs of this class can be compared to the very potent, zwitterionic cephalosporin RWJ-54428. Truncation of the

Scheme 1. Synthesis of 3-heteroarylthiocephems.

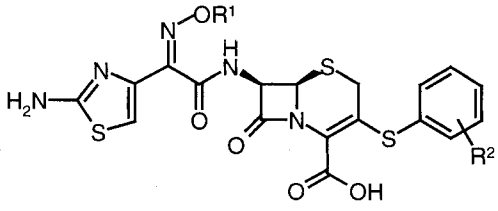


2-(2-aminoethylthiomethyl) substituent present in RWJ-54428 (*e.g.* **21**) results in a small drop in anti-MRSA activity and a dramatic increase in human serum binding (truncation of the side-chain had much smaller effect on mouse serum protein binding, which was determined to be 54% for **21** *vs.* 59% for RWJ-54428). The formyl derivative of RWJ-54428 (**24**) showed noticeable reduction in serum binding relative to **21** but did not possess the desired *in vivo* efficacy. An even larger reduction in serum binding was achieved in the des-chloro analog **23**, but was accompanied by a considerable loss of anti-MRSA activity. A similar decrease in potency paralleling reduction in serum binding was observed in other 3-(pyrid-4-yl)thio analogs in Table 3.

In an attempt to further reduce serum binding without the loss of anti-MRSA activity, a series of polyaza-6π-thiocephalosporins was studied (Table 4). Interestingly, introduction of electron rich imidazole substituents (*e.g.* **31** and **32**) resulted in a dramatic decrease of anti-MRSA activity while the thiadiazole **34** and aminothiazole **36** derivatives showed rather high activity. The [1,2,4]triazolo[4,3-a]pyridine **35** and 1H-[1,2,4]triazole **38** analogs showed greater reduction in serum protein binding but also displayed somewhat lower anti-MRSA activity.

In summary, we have identified among a series of non-zwitterionic, potent anti-MRSA cephalosporins, analogs with lipophilicity and binding to human serum proteins

Table 1. Substituted 3-phenylthiocephems.



Compound No.	R^1	R^2	MIC (μg/ml)						ED_{50}, mg/kg (95% C.I)
			S. a. Smith	*S. a.* 29213	*S. a.* COL	*S. a.* 76	*E. f.* 29212	*E. f.* 35667	
imipenem	-	-	≤0.25	<0.02	16	32	1	4	0.15 (0.06-0.25)
vancomycin	-	-	0.5	0.5	1	0.5	1	0.25	2.1 (1.3-2.9)
5	methyl	2-bromo	-	0.5	16	16	>32	32	-
6	methyl	4-bromo	-	<0.015	-	32	16	16	-
7	H	2-iodo	-	<0.25	2	4	-	2	-
8	methyl	2-iodo	-	1	32	32	-	32	-
9	cyclopentyl	2-iodo	-	<0.25	1	2	2	2	-
10	H	2-phenyl	-	0.5	4	8	2	2	-
11	3,3-dichloro-propen-2-yl	2-iodo	-	-	2	2	4	8	-
12	cyclopentyl	2-cyano	-	1	4	8	8	8	-

Abbreviations: S.a. Smith, *Staphylococcus aureus* Smith (MSSA); *S. a.* 29213, *Staphylococcus aureus* ATCC 29213 (MSSA); *S. a.* COL, *Staphylococcus aureus* COL (MRSA, non-β-lactamase producing); *S. a.* 76, *Staphylococcus aureus* 76 (MRSA, β-lactamase producing); *E. f.* 29212, *Enterococcus faecalis* ATCC 29212; *E. f.* 35667, *Enterococcus faecium* ATCC 35667; ED_{50}, 50% efficacious dose, *S. aureus* Smith, mg/kg; 95% C.I – 95% confidence interval.

considerably reduced relative to initially synthesized compounds. None of these non-zwitterionic cephalosporins possessed a combination of anti-MRSA potency and serum binding comparing favorably with the properties of RWJ-54428.

Materials and Methods

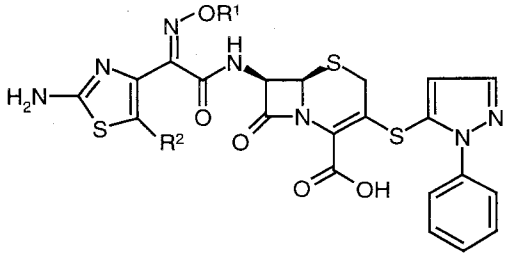
Susceptibility Testing

Compounds were evaluated for antimicrobial activity against a panel of bacterial strains using a broth microdilution assay performed as recommended by the NCCLS[10]. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of a compound that prevents the growth of the bacteria.

Mouse Model of Sepsis

Inoculum Preparation: *Staphylococcus aureus* strain Smith (ATCC 13709, penicillin-susceptible) or strain 76 (methicillin-resistant) was grown overnight at 37°C in brain-heart infusion broth (BHIB). The following morning, it was subcultured to fresh BHIB and incubated for 4~5 hours at 37°C. The cells were harvested by centrifugation, washed twice with PBS, and adjusted to the desired inoculum. The cell suspension was mixed with an equal volume of sterile 14% hog-gastric mucin[11]. The inoculum was kept in an ice bath until used (<1 hour).

Table 2. Substituted 3-(*N*-phenylpyrazol-5-yl)thiocephems.



Compound No.	R^1	R^2	MIC (μg/ml)						ED_{50}, mg/kg
			S. a. Smith	*S. a.* 29213	*S. a.* COL	*S. a.* 76	*E. f.* 29212	*E. f.* 35667	
13	cyclopentyl	H	0.5	0.5	2	4	2	4	>10
14	2-fluoroethyl	H	-	1	8	16	32	16	-
15	H	H	-	1	16	32	2	8	-
16	2-fluoroethyl	Cl	0.5	0.5	2	4	1	4	-
17	1-ethylpropyl	H	-	0.5	2	4	2	4	>10
18	1,1-dimethylethyl	H	1	1	8	16	2	16	
19	cyclopropylmethyl	H	0.5	0.5	4	8	16	16	>10
20	2-propenyl	H	0.5	1	8	16	32	32	-

Abbreviations: S.a. Smith, *Staphylococcus aureus* Smith (MSSA); *S. a.* 29213, *Staphylococcus aureus* ATCC 29213 (MSSA); *S. a.* COL, *Staphylococcus aureus* COL (MRSA, non-β-lactamase producing); *S. a.* 76, *Staphylococcus aureus* 76 (MRSA, β-lactamase producing); *E. f.* 29212, *Enterococcus faecalis* ATCC 29212; *E. f.* 35667, *Enterococcus faecium* ATCC 35667; ED_{50}, 50% efficacious dose, *S. aureus* Smith, mg/kg.

Experimental Infection

Male Swiss-Webster mice were challenged intraperitoneally with 0.5 ml of bacterial suspension of *S. aureus* strain Smith (LD_{50}). Test compounds were administered subcutaneously in 0.1 ml volumes immediately after inoculation and 2 hours later. Animals were then observed for 72 hours. The total dose associated with 50% survival (ED_{50}) was determined using the probit method[12].

Human Serum Binding

For selected compounds, the binding in pooled human serum was determined using ultrafiltration. Compounds were incubated in serum for 10 minutes at 37°C in a shaking water bath. Serum ultrafiltrate was obtained by centrifugation of ultrafiltration units (Amicon Centrifree) for 20 minutes at 25°C. Drug content in ultrafiltrate was quantified by HPLC using standards prepared in blank ultrafiltrate undergoing similar processing.

For some compounds estimated human serum binding (HSB*) was calculated using the MIC values against *S. aureus* ATCC 29213 determined in growth medium (GM) and in a 1 : 1 mixture of growth medium and human serum (GM+HS).

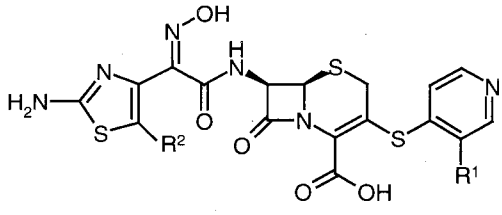
$$HSB^* = (MIC_{GM+HS} - MIC_{GM}) / MIC_{GM} \cdot 100\%$$

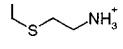
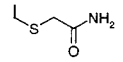
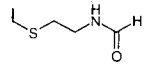
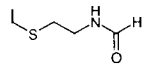
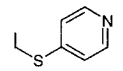
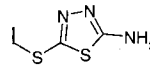
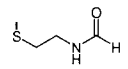
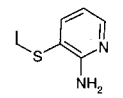
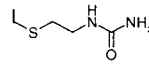
The synthetic methods utilized to prepare the compounds described herein are exemplified with the synthesis of compound **34**.

(7*R*)-7-[2-(2-Amino-5-chloro-thiazol-4-yl)-2-trityloxyimino-acetylamino]-3-(4-methyl-[1,2,3]thiadiazol-5-ylsulfanyl)-3-cephem-4-carboxylic Acid Trityl Ester

To a stirred solution of sodium 4-methyl-[1,2,3]thiadiazole-5-thiolate (330 mg, 2.17 mmol) in DMF (5 ml) was added at room temperature 3-metanesulfonyloxy cephem **VII d** (1.98 g, 2.17 mmol). After 1 hour the

Table 3. Substituted 3-(pyrid-4-yl)thiocephems.



Compound No.	R^1	R^2	MIC (μg/ml)						HSB (HSB*)	ED_{50}, mg/kg (95% C.I)
			S. a. Smith	*S. a.* 29213	*S. a.* COL	*S. a.* 76	*E. f.* 29212	*E. f.* 35667		
RWJ 54428		Cl	0.125	0.25	0.5	1	≤0.06	0.25	84% (67%)	1.0 (0.7-1.6)
21	H	Cl	0.25	0.25	2	2	0.5	2	98%	3.6 (1.1-6.7)
22		Cl	0.25	0.5	4	4	0.5	1	94% (88%)	1.15 (0.7-1.6)
23		H	0.5	0.5	8	8	0.5	4	86%	-
24		Cl	0.25	0.5	2	2	0.125	0.5	95% (88%)	>5
25		Cl	0.25	0.25	1	2	0.125	0.25	>99%	>5
26		Cl	0.06	0.25	1	2	0.25	0.5	>99%	0.38 (01-0.7)
27		Cl	0.25	0.25	2	4	0.25	1	94%	2.27 (1.0-5.5)
28	hydroxymethyl	Cl	0.125	0.5	2	2	0.25	1	>99%	-
29		Cl	0.25	0.125	1	2	0.125	0.5	-	>5
30		Cl	0.5	0.5	4	4	0.125	-	96%	-

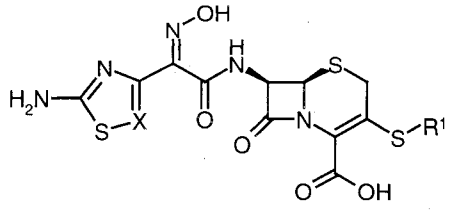
Abbreviations: S.a. Smith, *Staphylococcus aureus* Smith (MSSA); *S. a.* 29213, *Staphylococcus aureus* ATCC 29213 (MSSA); *S. a.* COL, *Staphylococcus aureus* COL (MRSA, non-β-lactamase producing); *S. a.* 76, *Staphylococcus aureus* 76 (MRSA, β-lactamase producing); *E. f.* 29212, *Enterococcus faecalis* ATCC 29212; *E. f.* 35667, *Enterococcus faecium* ATCC 35667; HSB, human serum binding; HSB*, human serum binding calculated using serum effect on MIC; ED_{50}, 50% efficacious dose, *S. aureus* Smith, mg/kg; 95% C.I – 95% confidence interval.

reaction mixture was partitioned between ethyl acetate and water, and the organic layer was dried over anhydrous sodium sulfate. After evaporating the solvent under reduced pressure the residue was chromatographed on silica gel column (ethyl acetate/hexane 1/1) to produce the title product (990 mg, 48%). ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H); 2.98 (d, 1H, J=16 Hz); 3.18 (d, 1H, J=16 Hz); 5.02 (d, 1H, J=6 Hz); 5.98 (d, 1H, J=6 Hz); 6.92 (s, 1H); 7.10~7.40 (m, 25H).

(7*R*)-7-[2-(2-Amino-5-chloro-thiazol-4-yl)-2-hydroxyimino-acetylamino]-3-(4-methyl-[1,2,3]thiadiazol-5-ylsulfanyl)- 3-cephem-4-carboxylic Acid (**34**).

To a solution of (7*R*)-7-[2-(2-amino-5-chloro-thiazol-4-yl)-2-trityloxyimino-acetylamino]-3-(4-methyl-[1,2,3]thiadiazol-5-ylsulfanyl)-3-cephem-4-carboxylic acid

Table 4. Substituted 3-heteroarylthiocephems.



Compound No.	R^1	X	MIC (μg/ml)						HSB (HSB*)
			S. a. Smith	*S. a.* 29213	*S. a.*. COL	*S. a.* 76	*E. f.* 29212	*E. f.* 35667	
31		CCl	0.125	0.5	16	32	1	16	-
32		CCl	0.125	0.25	32	>32	2	16	-
33		CCl	0.5	1	4	8	1	4	-
34		CCl	0.125	0.125	2	2	0.25	2	- (98%)
35		CCl	0.25	0.5	4	4	0.25	4	- (88%)
36		CCl	0.125	0.125	2	8	1	4	- (97%)
37		N	0.125	0.125	8	8	1	2	- (94%)
38		CCl	0.5	0.25	8	8	1	4	92% (88%)

Abbreviations: S.a. Smith, *Staphylococcus aureus* Smith (MSSA); *S. a.* 29213, *Staphylococcus aureus* ATCC 29213 (MSSA); *S. a.* COL, *Staphylococcus aureus* COL (MRSA, non-β-lactamase producing); *S. a.* 76, *Staphylococcus aureus* 76 (MRSA, β-lactamase producing); *E. f.* 29212, *Enterococcus faecalis* ATCC 29212; *E. f.* 35667, *Enterococcus faecium* ATCC 35667; HSB, human serum binding; HSB*, human serum binding calculated using serum effect on MIC.

trityl ester (990 mg, 1.05 mmol) in dichloromethane (21 ml) was added triethylsilane (11 ml) followed by addition of trifluoroacetic acid (21 ml). After 1 hour reaction at room temperature the reaction mixture was concentrated in vacuum and diisopropyl ether was added to the oily residue. The precipitated title product was filtered, thoroughly washed with additional diisopropyl ether and dried in vacuum (528 mg, 95%). ^{1}H NMR (CD_3OD) δ 2.65 (s, 3H); 3.30 (d, 1H, J=16 Hz); 3.60 (d, 1H, J=16 Hz); 5.22 (d, 1H, J=6 Hz); 5.98 (d, 1H, J=6 Hz).

Acknowledgements

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