*Eur J Med Chem* (1995) 30, 157–164




# Synthesis and *in vitro* antimicrobial activity of 3-heteroarylsulphonylmethyl cephems: a new class of cephalosporins

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(Received 25 May 1994; final version received and accepted 24 October 1994)

**Summary** — A series of 3-heteroarylsulphonylmethyl and 3-heteroarylsulphenylmethyl cephems were prepared and tested for antimicrobial activities. In contrast to the adverse effect of oxidized ring sulphur of penams and cephems on antimicrobial activities, the oxidized side-chain sulphur of 3-mercaptoheteroaryl cephems retained Gram-negative and slightly decreased Gram-positive activity. The chemical nature of the moieties substituted at C-7 and C-3 positions also influenced the antibacterial activity and spectrum. Compounds with thienyl substitution at C-7 and sulphonylmethylthiazoles or sulphonylmethylthiadiazoles at C-3 exhibited good differentials in antibacterial activity *versus* their unoxidized counterparts.

**β-lactam / 3-heteroarylsulphonylcephem / oxidized cephem / antibacterial agent**

## Introduction

Many of the newer cephalosporins in clinical use and under development have a mercaptoheteroaryl moiety at the C-3 position (fig 1) [1-3], and some of them are found to give rise to problems of disulfiram-like reactions and coagulopathy in humans due to the presence of a mercaptoheteroaryl moiety [4–6]. The substitution of the thioether function at C-3 by a sulphoxide or sulphone group would be expected to change the antimicrobial spectrum as well as the pharmacokinetic properties of the cephalosporins. There are a few reports on *S*- and *R*-sulphoxides of cephalosporin derivatives being biologically active against selected microorganisms [7–8]. Our studies with oxidized penams and cephems have also indicated loss of activity against both Gram-positive and Gram-negative bacteria [9–11]. Several other cephalosporin derivatives with the ring sulphur oxidized to sulphoxide and sulphone have been reported in the literature and no improvements in antimicrobial activity have been claimed [12–19].

Although a small number of cephalosporin derivatives with oxidized ring sulphur and oxidized sulphur at the C-3 side chain are known, only a few compounds with the C-3 side chain sulphur oxidized to sulphone and ring sulphur as sulphide have been reported in the literature [12–16]. Mainly, two types of C-3 sulphone-substituted cephem derivatives are made: one with sulphur directly attached to the C-3 and the other with a methylene bridge between the C-3 and the sulphur of the alkyl or heteroaryl moieties. The first is generally obtained in two steps by the reaction of 3-hydroxy-3-cephems with *p*-toluenesulphonyl chloride and further displacement of the tosylate group with a suitable aliphatic, aromatic or heteroaromatic thiol [14]. The second type of derivative is obtained by the nucleophilic substitution of the acetoxy group of the 3-acetoxymethyl ceph-3-em-4-carboxylic acid by a suitable mercaptan [15–23].

Our extensive studies [11, 24–27] on the oxidation pattern of the 3-heteroarylthiomethyl ceph-3-em-4-carboxylate have resulted in the synthesis of a new

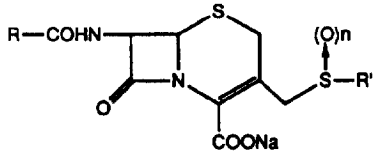


**Fig 1.** General structure of 3-mercaptoheteroaryl cephems (R = 2-thienyl methyl, 2-amino-4-thiazolyl (methoxyimino) methyl, aryl methyl; R' = heteroaryl; and $n = 0$).

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class of cephalosporins which exhibited very good antibacterial activity against Gram-negative organisms. Some of this information was presented at the 28th ICAAC [28–29]. The present paper deals with the synthesis and antimicrobial activity of the new compounds synthesized and tested in our laboratory.

## Chemistry

The 3-heteroarylthiomethyl-7β-(2-thienyl acetamido)-ceph-3-em-4-carboxylate **2** required as starting material was synthesized by nucleophilic substitution of 7-amino ceph-3-em-4-carboxylic acid, followed by acylation and esterification with diphenyl diazomethane. The oxidations of compound **2** with three equivalents of *m*-chloroperbenzoic acid (*m*-CPBA) gave mixture of products which were separated by silica-gel column chromatography using ethyl acetate and methylene chloride as eluent. Compound **3** was deoxygenated using phosphorus pentasulphide to yield the desired compound **4** which was then deacylated using phosphorus pentachloride, *N*-*N*-dimethylaniline and methanol to obtain the important intermediate, 7-amino-3-heteroarylsulphonylmethyl ceph-3-em-4-carboxylate **5**. A number of acylated products **6** were synthesized by using the intermediate **5** according to the standard procedures described previously [9–11]. The unoxidized samples of the acylated cephems **8** were prepared for comparative biological activities. Finally, the hydrolysis of the diphenylmethyl ester of cephems **2**, **4**, **6** and **8** were carried out in anisole with trifluoroacetic acid at 0°C to obtain the corresponding acids which were then converted into their sodium salts **1** by the addition of sodium 2-ethylhexanoate (scheme 1).

## Results and discussion

The 3-heteroarylsulphonylmethyl cephems representing a new class of cephems are listed in the table I. The unoxidized counterparts of each cephem were also synthesized for comparative biological activities. Our earlier reports have suggested substantial loss of antibacterial activity due to oxidation of ring and side-chain sulphur in cephems [11].

Synthesis of **1b** ($n$ = 2) enabled us to compare its activity with other previously reported oxidized cephems (fig 2). Since the antibacterial activity of **1b** against Gram-negative bacteria seemed encouraging, we synthesized and tested several compounds with different substitution patterns at C-7 and C-3 positions. The minimal inhibitory concentrations (MICs) of synthesized compounds **1a–j** against wild-type and β-lactam-resistant bacterial strains are given in tables II and III, respectively. The cephems with 2-thienylacetamido substitution at C-7 and heteroarylsulphonylmethyl substitution at C-3 position (**1b**, **d**, and **f**) were generally 1–3-fold more active against most Gram-negative bacteria and 1–3-fold less active against Gram-positive bacteria than their unoxidized (sulphenyl) counterparts. However, the activity against *Escherichia coli* (TEM2 producer), *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Acinetobacter calcoaceticus*, *Hafnia alvei* and *Flavobacterium meningosepticum* remained unchanged, possibly due to exclusion of the test compounds by the outer membrane permeability barrier of these bacteria (tables I and II).

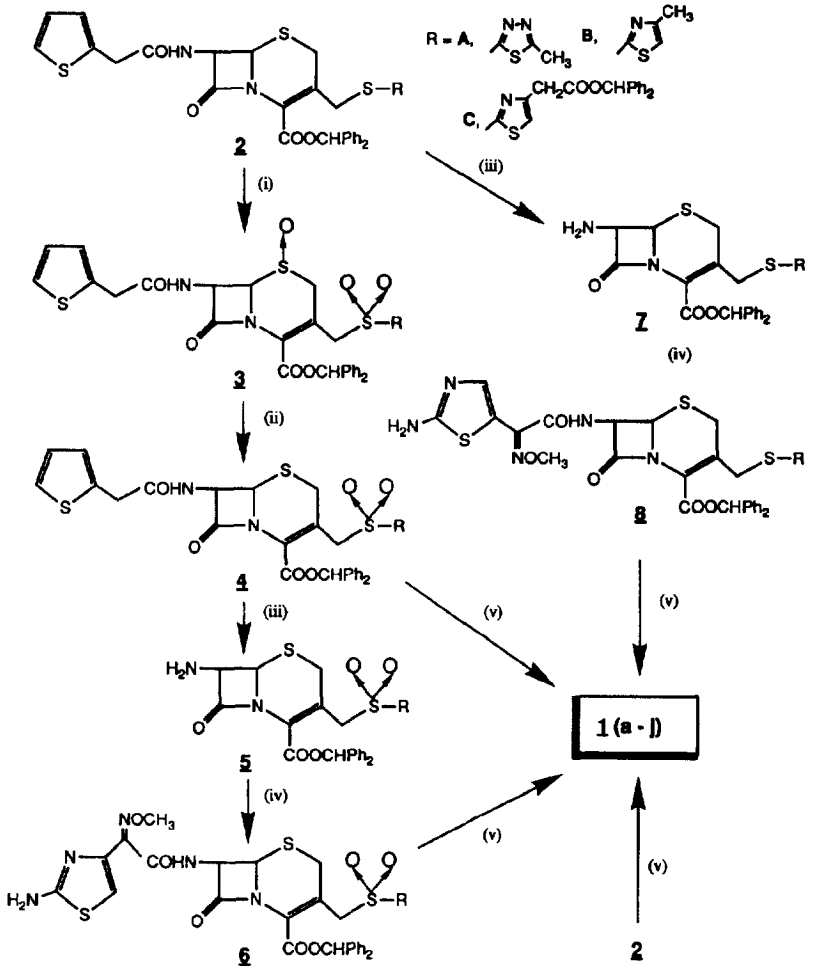
Our earlier observation of *E coli* DC2 (permeability mutant of *E coli* DC0) being more susceptible than *E coli* DC0 to this class of cephems further supports the fact that entry of these compounds is hindered by normal outer membrane [30]. Although most sulphonyl cephems exhibited reduced Gram-positive activity compared to their sulphenyl counterparts, **1b** and **1f** exhibited slightly higher activity against a few selected strains of methicillin-resistant *Staphylococcus aureus* (table III), which is also consistent with our earlier observation with a methicillin-resistant *S aureus* DU4916-K7 [30]. However, the MICs of sulphonylcephems were not low enough to be of clinical use.

In contrast to the 7β-(2-thienyl)acetamido cephems, the 7β-[α-syn-methoxyimino-α-(2-amino-4-thiazolyl)]-acetamido cephems (**1h** and **j**) were found to be 1–3-fold less active than their unoxidized counterparts (**1g** and **i**) against both Gram-positive and Gram-negative bacteria. Although the overall antibacterial activity of methoxyimino(2-amino-4-thiazolyl)acetamido cephems (**1g–i**) were 1–3-fold better than the substituted cephems with 2-thienylacetamido C-7 side chain (**1a–f**), the presence of a sulphonylheteroaryl side chain at C-3 position (**1h** and **j**) was unfavourable to the desirable physicochemical properties for antibacterial activity. The polarity of such compounds is probably determined by the nature of substituents at both C-7 and C-3 positions, which in turn influences their permeability through the outer membrane and their sensitivity to various β-lactamases. Syntheses of several other sulphonylcephems and their evaluation in animal model would reveal their pharmacokinetic–pharmacodynamic profile.

## Experimental protocols

*General methods*

The $^1$H-NMR spectra (δ ppm) were obtained in $CDCl_3$ (for esters) or $D_2O$ (for salts) with tetramethylsilane as an internal standard on a Bruker AM-300 spectrometer.




**Scheme 1.** Synthetic route to a new class of 3-heteroarylcephems. (i) *m*-CPBA, (ii) $P_2S_5$, (iii) $PCl_5$, (iv) $PCl_5/Et_3N$, (v) TFA/anisole and Na-2-ethyl hexanoate.

Analytical results for compounds (unpurified sodium salts **1**) followed by elemental symbols were within the expected range of calculated values unless stated otherwise and were determined on a Perkin-Elmer 240 elemental analyzer.

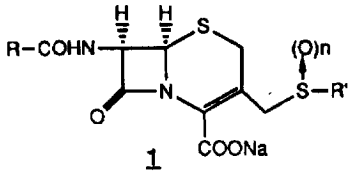
The *in vitro* antibacterial activities of the final compounds were determined by the standard agar dilution method as recommended by the National Committee for Clinical Laboratory Standards [31] using unsupplemented Mueller–Hinton agar (BBL) medium. All reference antibiotics were purchased from Sigma Chemical Company, USA. The Cathra multipoint inoculation system (MCT Medical Inc, Saint Paul, USA) was used to dispense about $10^4$ cfu/spot of the test organism onto the agar plate. The MIC was recorded as the lowest concentration of the antibiotic that prevented visible growth after 18 h of incubation at 35°C disregarding the appearance of a single colony or very hazy growth.

*Chemistry*

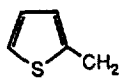
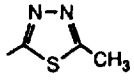
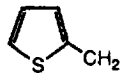
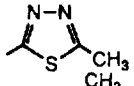
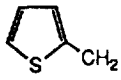
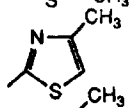
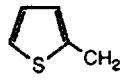
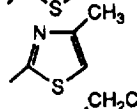
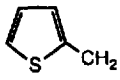
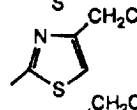
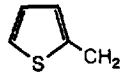
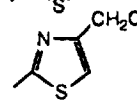
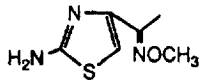
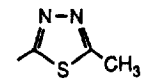
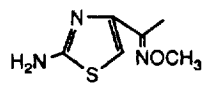
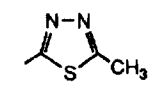
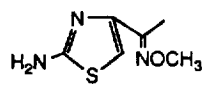
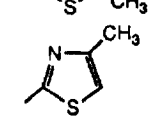
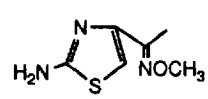
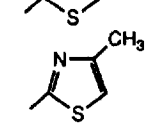
*Diphenyl methyl 7β-(2-thienyl)acetamido-3-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl] ceph-3-em-4-carboxylate* **2A**

A mixture of 7β-(2-thienyl)acetamido-3-acetoxymethyl ceph-3-em-4-carboxylic acid (40 g, 0.1 mol), sodium bicarbonate (16.798 g, 0.2 mol) and 5-methyl-2-mercapto-1,3,4-thiadiazole (12.98 g, 0.1 mol) in phosphate buffer (pH 6.4, 700 ml) was heated at 55–60°C for 6 h, cooled to room temperature, acidified with 3 N HCl to pH 3.0, and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $Na_2SO_4$ and then concentrated *in vacuo*. The residue, the crude 7β-(2-thienyl)acetamido-3-[5-methyl-2-mercapto-1,3,4-thiadiazole] ceph-3-em-4-carboxylic acid, was redissolved in a mixture of methylene chloride (500 ml) and methanol (15 ml). To this solution, a diphenyldiazomethane (17 g, 0.088 mol)

**Table I.** Chemical structures of new 3-heteroarylcephems.




1

| *Compound* | *R* | *R'* | *n* |
|---|---|---|---|
| **1a** |  |  | 0 |
| **1b** |  |  | 2 |
| **1c** |  |  | 0 |
| **1d** |  |  | 2 |
| **1e** |  |  | 0 |
| **1f** |  |  | 2 |
| **1g** |  |  | 0 |
| **1h** |  |  | 2 |
| **1i** |  |  | 0 |
| **1j** |  |  | 2 |

solution in methylene chloride (150 ml) was slowly added while stirring at room temperature. The resulting solution was further stirred for 2 h at the same temperature and then washed with $NaHCO_3$, water and brine. The organic layer was dried over anhydrous $Na_2SO_4$ and was then concentrated *in vacuo*. The residue was chromatographed over a silica-gel column using ethyl acetate/methylene chloride as eluent, yield 26.25 g, 56%. $^1$H-NMR ($CDCl_3$): 2.7 (3H, s, $CH_3$), 3.68 and 3.75 (2H, ABq, $CH_2$), 3.86 (2H, s, $CH_2$), 4.19 and 4.5 (2H, ABq, $CH_2$), 4.96 (1H, d, C6-H), 5.88 (1H, q, C7-H), 6.96 (1H, s, $CH_2$), 6.62 (1H, d, NH), 6.98–7.48 (13H, m, Ar).

*Diphenyl methyl 7β-(2-thienyl)acetamido-3-[(4-methyl-thiazol-2-yl)thiomethyl] ceph-3-em-4-carboxylate* ***2B***

To a mixture of 7β-(2-thienyl)acetamido-3-acetoxymethyl ceph-3-em-4-carboxylic acid (5.92 g, 0.0149 mol) and sodium

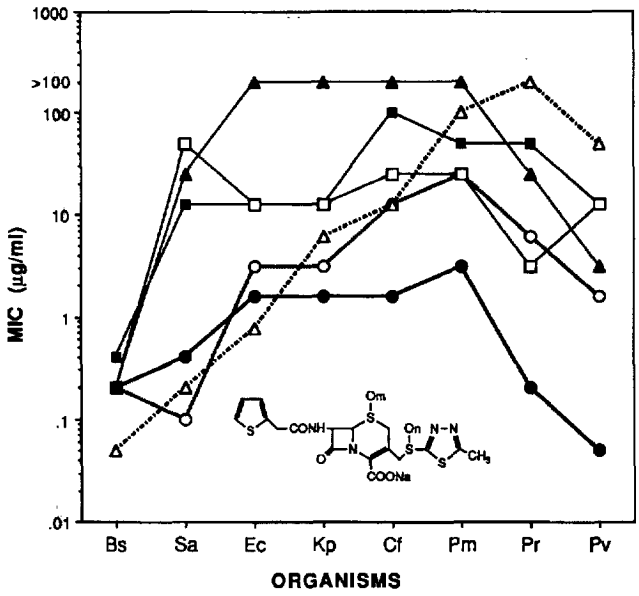



**Fig 2.** Effect of S-oxidation on the antibacterial activity of 3-substituted cephem. Bs, *Bacillus subtilis*; Sa, *S aureus*; Ec, *E coli*; Kp, *Klebsiella pneumoniae*; Cf, *Citrobacter freundii*; Pm, *Proteus mirabilis*; Pr, *P rettgeri*; Pv, *P vulgaris*. Cephalothin (△) $m = n = 0$ (○); $m = n = 1$ (□); $m = n = 2$ (▲); $m = 1$, $n = 0$ (■); $m = 0$, $n = 2$ (●).

bicarbonate (2.51 g, 0.03 mol) in phosphate buffer of pH 6.4 (150 ml), 4-methyl-2-mercaptothiazole (2.089 g, 0.016 mol) was added. The mixture was heated at 55–60°C for 18 h. The reaction mixture was processed (extracted, esterified, and purified) as for **2A**. Yield 5.0 g, 71.63%. $^1$H-NMR ($CDCl_3$): 2.34 (3H, s, $CH_3$), 3.48 and 3.64 (2H, ABq, $CH_2$), 3.84 (2H, s, $CH_2$), 4.08 and 4.44 (2H, ABq, $CH_2$), 4.93 (1H, d, C6-H), 5.84 (1H, q, C7-H), 6.69 (1H, s, thiazole), 6.78 (1H, d, NH), 6.95 (1H, s, $CH_2$), 6.96–7.5 (13H, m, Ar).

*Diphenyl methyl 7β-(2-thienyl)acetamido-3-[(4-diphenyl methyloxy carbonyl methyl-thiazol-2-yl)thiomethyl] ceph-3-em-4-carboxylate* ***2C***

A mixture of 7β-(2-thienyl)acetamido-3-acetoxymethyl ceph-3-em-4-carboxylic acid (5.10 g, 0.01288 mol), sodium bicarbonate (3.24 g, 0.0386 mol), 2-mercapto thiazole-4-acetic acid (2.63 g, 0.015 mol) in phosphate buffer at pH 6.4 (108 ml) was heated at 55–60°C for 20 h. The reaction mixture was processed (extracted, esterified, and purified) as for **2A**. Yield 8.26 g, 97.98%. $^1$H-NMR ($CDCl_3$): 3.42 (2H, s, $CH_2$), 3.8 (4H, bs, 2 x $CH_2$), 4.15 (2H, ABq, $CH_2$), 4.84 (1H, d, C6-H), 5.8 (1H, q, C7-H), 6.93 (3H, bs, thiazole + $CHPh_2$), 7.1–7.6 (23H, m, Ar), 7.8 (1H, bs, NH).

*Diphenyl methyl 7β-(2-thienyl)acetamido-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulphonylmethyl] ceph-3-em-1β-oxide-4-carboxylate* ***3A***

To a stirred solution of compound **2A** (10.0 g, 0.0158 mol) in methylene chloride (250 ml), *m*-CPBA (85%, 10.56 g, 0.048 mol) was added portionwise and the mixture was stirred at room temperature for 7 h. The separated solid was

**Table II.** Antibacterial spectrum (MIC, μg/ml) of reference and new cephems.

| Microorganisms | CET | CXM | 1a | 1b | 1c | 1d | 1e | 1f | 1g | 1h | 1i | 1j |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *S aureus* 209P | 0.10 | 1.56 | 0.05 | 0.10 | ≤0.05 | 0.20 | 0.39 | 3.13 | 0.78 | 3.13 | 1.56 | 3.13 |
| *S epidermidis* IFO 3762 | 0.78 | 6.25 | 0.20 | 1.56 | 0.39 | 1.56 | 6.25 | 25 | 3.13 | 12.50 | 6.25 | 6.25 |
| *B subtilis* ATCC 6633 | ≤0.05 | 12.50 | ≤0.05 | 0.78 | 0.10 | 0.78 | 0.10 | 1.56 | 1.56 | 3.13 | 0.78 | 3.13 |
| *B cereus* IFO 3001 | 25 | 100 | 12.50 | 100 | 25 | 50 | 100 | >100 | 25 | 50 | 25 | 100 |
| *E faecalis* IFO 12968 | 25 | >100 | 12.50 | 50 | 25 | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| *M flavus* ATCC10240 | 0.39 | 0.20 | ≤0.05 | 0.78 | 0.20 | 0.78 | 0.39 | 1.56 | 0.20 | 0.78 | 0.39 | 0.78 |
| *E coli* NIHJ (Taiho CC) | 3.13 | 0.20 | 0.20 | 0.78 | 0.10 | 0.10 | 6.25 | 0.20 | ≤0.05 | ≤0.05 | ≤0.05 | 0.39 |
| *S typhimurium* G 46 | 1.56 | 3.13 | 1.56 | 1.56 | 25 | 1.56 | 1.56 | 0.20 | 0.20 | 0.78 | 0.78 | 1.56 |
| *P mirabilis* IFO 3849 | 12.50 | 3.13 | 12.50 | 6.25 | 50 | 3.13 | 3.13 | 0.20 | 0.20 | 0.39 | 0.20 | 0.78 |
| *P morganii* IID kono | >100 | 25 | 100 | 1.56 | >100 | 3.13 | >100 | 6.25 | ≤0.05 | 0.10 | 0.39 | 0.20 |
| *P vulgaris* IID OX-19 | 12.50 | 12.50 | 0.39 | 0.20 | 12.50 | 1.56 | 12.50 | 6.25 | ≤0.05 | ≤0.05 | ≤0.05 | ≤0.05 |
| *P rettgeri* NIH 96 | 0.39 | ≤0.05 | ≤0.05 | ≤0.05 | ≤0.05 | ≤0.05 | 0.10 | ≤0.05 | ≤0.05 | 0.20 | ≤0.05 | ≤0.05 |
| *P inconstans* IFO 12930 | 6.25 | 0.10 | 0.78 | 0.20 | 3.13 | 0.39 | 6.25 | ≤0.05 | ≤0.05 | 0.10 | ≤0.05 | 0.10 |
| *C freundii* IFO 12681 | >100 | 6.25 | 3.13 | 25 | 100 | 12.50 | >100 | 50 | 0.78 | 1.56 | 1.56 | 0.20 |
| *S marcescens* IFO 12648 | >100 | 25 | 50 | 6.25 | >100 | 50 | >100 | 100 | 0.20 | 0.39 | 1.56 | 3.13 |
| *E aerogenes* IFO 13534 | >100 | 6.25 | 50 | 6.25 | 100 | 6.25 | >100 | 12.50 | 0.39 | 1.56 | 1.56 | 1.56 |
| *E cloacae* IFO 13535 | >100 | >100 | >100 | >100 | >100 | 100 | >100 | >100 | 1.56 | 1.56 | 6.25 | 0.10 |
| *P aeruginosa* IFO 12020 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | 25 | 50 | 50 | 50 |
| *A calcoaceticus* IFO 12552 | >100 | 50 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| *H alvei* IFO 3731 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | 25 | 12.50 | 25 | 12.50 |
| *F meningosepticum* IFO 12535 | >100 | >100 | 50 | >100 | >100 | >100 | >100 | >100 | 25 | 25 | 25 | 50 |

CET, cephalothin; CXM, cefuroxime; **1a–j**, cephems as listed in table I.

filtered off and usual work-up of the filtrate followed by solvent removal gave 8.0 g of the crude product which was purified by silica-gel column chromatography using methylene chloride/ethyl acetate (gradient) as eluent. The first fraction was the disulphone and the middle fraction was the desired product **3A**. Yield 3.3 g, 30.68%. $^1$H-NMR ($CDCl_3$): 2.79 (3H, s, $CH_3$), 3.64 and 3.88 (2H, ABq, $CH_2$), 3.86 (2H, s, $CH_2$), 4.22 and 5.58 (2H, ABq, $CH_2$), 5.6 (1H, d, C6-H), 6.12 (1H, q, C7-H), 6.84 (1H, s, $CH_2$), 7.0–7.5 (14H, m, Ar + NH).

*Diphenyl methyl 7β-(2-thienyl)acetamido-3-[(4-methylthiazol-2-yl)sulphonylmethyl] ceph-3-em-1β-oxide-4-carboxylate* ***3B***
To an ice-cold solution of diphenyl methyl 7β-(2-thienyl)-acetamido-3-[(4-methylthiazol-2-yl)thiomethyl] ceph-3-em-4-carboxylate **2B** (15.6 g, 0.0246 mol) in methylene chloride (300 ml), *m*-CPBA (85%, 16.09 g, 0.075 mol) was added portionwise. The reaction mixture was stirred at room temperature for 8 h and was then processed as for **3A**. Yield 10.0 g, 59.63%. $^1$H-NMR ($CDCl_3$), 2.72 (3H, s, $CH_3$), 3.78–4.1 (4H, m, 2 x $CH_2$), 4.58 and 5.28 (2H, ABq, $CH_2$), 5.05 (1H, q, C7-H), 6.78 (1H, s, thiazole), 6.98 (1H, s, $CH_2$), 7.24–7.6 (13H, m, Ar), 8.54 (1H, d, NH).

*Diphenyl methyl 7β-(2-thienyl)acetamido-3-[(4-diphenylmethyl-oxycarbonylmethylthiazol-2-yl)sulphonylmethyl] ceph-3-em-1β-oxide-4-carboxylate* ***3C***
To an ice-cold solution of compound **2C** (5.15 g, 0.006 mol) in methylene chloride (115 ml), *m*-CPBA (84.8%, 4.88 g, 0.024 mol) was added. The mixture was stirred at room temperature for 28 h and was then processed as for **3A**. Yield 1.1 g, 20.22%. $^1$H-NMR ($CDCl_3$): 3.52 (4H bs, 2 x $CH_2$), 3.82 (4H, bs, 2 x $CH_2$), 5.58 (1H, d, C6-H), 6.0 (1H, q, C7-H), 6.78 (1H, s, thiazole), 6.88 (2H, s, $CHPh_2$), 6.95–7.4 (23H, m, Ar), 7.7 (1H, bs, NH).

*Diphenyl methyl 7β-(2-thienyl)acetamido-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulphonylmethyl] ceph-3-em-4-carboxylate* ***4A***
To a solution of diphenyl methyl 7β-(2-thienyl)acetamido-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulphonylmethyl] ceph-3-em-1β-oxide-4-carboxylate **3A** (1.0 g, 0.0015 mol) in dry methylene chloride (40 ml), pyridine (0.47 ml) and phosphorus pentasulphide (0.33 g, 0.0015 mol) were added and the mixture was stirred at room temperature for 18 h. The reaction mixture was washed with water, dilute hydrochloric acid and brine, dried over anhydrous $Na_2SO_4$ and then concentrated *in vacuo*. The residue (1.1 g) was chromatographed over a silica-gel column using methylene chloride/ethyl acetate (gradient) as eluent, yield 0.7 g, 71.7%. $^1$H-NMR ($CDCl_3$): 2.72 (3H, s, $CH_3$), 3.45 and 3.78 (2H, ABq, $CH_2$), 3.86 (2H, s, $CH_2$), 4.49 and 5.22 (2H, ABq, $CH_2$), 5.01 (1H, d, C6-H), 5.9 (1H, q, C7-H), 6.79 (1H, s, $CH_2$), 6.93 (1H, d, NH), 7.0–7.48 (13H, m, Ar).

*Diphenyl methyl 7β-(2-thienyl)acetamido-3-[(4-methylthiazol-2-yl)sulphonylmethyl] ceph-3-em-4-carboxylate* ***4B***
To a solution of diphenyl methyl 7β-(2-thienyl)acetamido-3-[(4-methylthiazol-2-yl)sulphonylmethyl] ceph-3-em-1β-oxide-4-carboxylate **3B** (6.0 g, 0.0088 mol) in methylene chloride

**Table III.** Antibacterial activity (MIC, μg/ml) of reference drugs and new cephems against β-lactam-resistant bacteria.

| Microorganisms | CET | PIPC | 1a | 1b | 1c | 1d | 1e | 1f | 1g | 1h | 1i | 1j |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E coli* SHV1 | 6.25 | 12.50 | 6.25 | 3.13 | 25 | 25 | 25 | 12.50 | 0.10 | 0.39 | 0.20 | 0.39 |
| *E coli* TEM1 | 3.13 | 25 | 6.25 | 12.50 | 25 | 25 | 25 | 12.50 | 0.10 | 0.90 | 0.39 | 0.39 |
| *E coli* TEM2 | 25 | >100 | 100 | >100 | >100 | >100 | >100 | >100 | 0.10 | 0.39 | 0.39 | 0.78 |
| *E coli* OXA1 | 3.13 | 12.50 | 6.25 | 3.13 | 25 | 6.25 | 12.50 | 12.50 | 0.20 | 3.13 | 0.39 | 3.13 |
| *E coli* 1573E | 3.13 | 3.13 | 3.13 | 1.56 | 25 | 6.25 | 12.50 | 6.25 | 0.10 | 0.39 | 0.20 | 0.39 |
| *E coli* OXA3 | 1.56 | 3.13 | 3.13 | 1.56 | 25 | 3.13 | 12.50 | 6.25 | 0.10 | 0.39 | 0.20 | 0.39 |
| *K pneumoniae* SHV1 | 12.50 | 100 | 25 | 12.50 | 50 | 12.50 | 25 | 12.50 | 0.39 | 1.56 | 1.56 | 3.13 |
| *K pneumoniae* TEM1 | 12.50 | >100 | 25 | 12.50 | 50 | 25 | 50 | 25 | 0.10 | 0.39 | 0.39 | 0.78 |
| *P mirabilis* 60 | 0.78 | 1.56 | 6.25 | 0.78 | 50 | 1.56 | 1.56 | 0.20 | 0.05 | 0.39 | 0.78 | 0.78 |
| *S marcescens* TEM+C | >100 | 25 | >100 | 25 | >100 | >100 | >100 | >100 | 0.05 | 0.20 | 0.20 | 0.39 |
| *Citrobacter* sp C | 100 | 12.50 | 100 | 12.50 | >100 | >100 | >100 | >100 | 0.20 | 1.56 | 1.56 | 0.78 |
| *Citrobacter* sp TEM2 | 0.78 | 100 | 6.25 | 1.56 | 25 | 3.13 | 6.25 | 0.78 | 0.20 | 0.39 | 0.78 | 0.78 |
| *E cloacae* P99 C | >100 | 100 | >100 | >100 | >100 | >100 | >100 | >100 | 50 | 100 | 50 | 100 |
| *S aureus* 54 | 0.39 | 12.50 | 0.39 | 0.78 | 0.39 | 1.56 | 1.56 | 12.50 | 3.13 | 12.50 | 3.13 | 6.25 |
| *S aureus* 123K | 0.20 | 0.78 | 0.20 | 0.78 | 0.78 | 3.13 | 1.56 | 12.50 | 3.13 | 6.25 | 1.56 | 6.25 |
| *S aureus* 66K | 3.13 | 6.25 | 0.78 | 3.13 | 6.25 | 12.50 | 50 | >100 | 12.50 | 50 | 12.50 | 50 |
| *S aureus* 86K$^{MR}$ | 1.56 | >100 | 25 | 1.56 | 25 | 12.50 | >100 | 25 | 3.13 | 25 | 6.25 | 12.50 |
| *S aureus* 130K$^{MR}$ | 6.25 | >100 | 0.78 | 12.50 | 100 | 25 | >100 | 100 | 50 | 100 | 50 | 50 |
| *S aureus* JHHDO78$^{MR}$ | 1.56 | >100 | 25 | 3.13 | 3.13 | 6.25 | >100 | >100 | 3.13 | 50 | 6.25 | 25 |
| *S aureus* JHH M241$^{MR}$ | 1.56 | >100 | 0.78 | 3.13 | 12.50 | 25 | 12.50 | 50 | 3.13 | 50 | 6.25 | 50 |
| *S aureus* 157-399$^{MR}$ | 50 | >100 | 100 | 50 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| *S aureus* AMP 81-3$^{MR}$ | 25 | >100 | 50 | 25 | 12.50 | 25 | >100 | >100 | 50 | >100 | >100 | >100 |

CET, cephalothin; PIPC, piperacillin; **1a–j**, cephems as listed in table I; MR = methicillin resistant.

(200 ml), pyridine (3.16 g) and phosphorus pentasulphide (2.22 g, 0.01 mol) were added and the mixture was stirred at room temperature for 18 h. The reaction mixture was processed and purified as for **4A**. Yield 5.0 g, 90.9%. $^1$H-NMR ($CDCl_3$): 2.37 (3H, s, $CH_3$), 3.44 and 3.77 (2H, ABq, $CH_2$), 3.82 (2H, s, $CH_2$), 4.37 and 5.02 (2H, ABq, $CH_2$), 4.97 (1H, d, C6-H), 5.88 (1H, q, C7-H), 6.65 (1H, d, NH), 6.7 (1H, s, thiazole), 6.94–7.0 (3H, m, Ar), 7.09 (1H, s, $CH_2$), 7.22–7.42 (10H, m, Ar).

*Diphenyl methyl 7β-(2-thienyl)acetamido-3-[(4-diphenylmethyl-oxycarbonylmethylthiazol-2-yl)sulphonylmethyl] ceph-3-em-4-carboxylate **4C***

To a solution of diphenyl methyl 7β-(2-thienyl)acetamido-3-[(4-diphenylmethyloxycarbonylmethylthiazol-2-yl)sulphonylmethyl] ceph-3-em-1β-oxide-4-carboxylate **3C** (0.5 g, 0.00056 mol) in methylene chloride (15 ml), pyridine (0.18 ml) and phosphorus pentasulphide (0.124 g, 0.00056 mol) were added and the mixture was stirred at room temperature for 20 h. The reaction mixture was processed and purified as for **4A**. Yield 0.463 g, 93.68%. $^1$H-NMR ($CDCl_3$): 3.5 (2H, d, $CH_2$), 3.78 (2H, s, $CH_2$), 3.83 (2H, s, $CH_2$), 4.25 (2H, ABq, $CH_2$), 4.88 (1H, d, C6-H), 5.84 (1H, q, C7-H), 6.74 (1H, s, thiazole), 6.92 (2H, s, $CHPh_2$), 7.1–7.68 (24H, m, Ar + NH).

*Diphenyl methyl 7β-amino-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulphonylmethyl] ceph-3-em-4-carboxylate **5A***

To a solution of compound **4A** (1.12 g, 0.00168 mol) in methylene chloride (30 ml) and *N,N*-dimethyl aniline (0.612 ml) at –20°C, $PCl_5$ (0.403 g, 0.00193 mol) was added and the mixture was stirred at –20°C for 10 min, then at –10°C for 40 min. After this, methanol (8 ml) and additional portion of dimethylaniline (0.6 ml) were added and stirred at room temperature for 12 h. The hydrolysis was carried out by the addition of 50 ml cold water and stirring at room temperature for 2 h. The pH of the reaction mixture was adjusted to 7.0 and it was extracted with ethyl acetate. The organic phase was separated, washed with brine, dried over anhydrous $Na_2SO_4$ and then concentrated *in vacuo*. The crude product (1.2 g) was purified over a silica-gel column using methylene chloride/ethyl acetate as eluent, yield 0.44 g, 49%. $^1$H-NMR ($CDCl_3$): 1.84 (2H, bs, $NH_2$), 2.78 (3H, s, $CH_3$), 3.53 and 3.86 (2H, ABq, $CH_2$), 4.44 and 5.28 (2H, ABq, $CH_2$), 4.82 (1H, d, C6-H), 5.05 (1H, d, C7-H), 6.81 (1H, s, $CH_2$), 7.3–7.48 (10H, m, Ar).

*Diphenyl methyl 7β-amino-3-[(4-methylthiazol-2-yl)sulphonyl-methyl] ceph-3-em-4-carboxylate **5B***

To a solution of compound **4B** (5.0 g, 0.0075 mol) in methylene chloride (150 ml) and dimethylaniline (2.73 g) at –20°C,

$PCl_5$ (1.80 g, 0.0086 mol) was added and the mixture was stirred at the same temperature for 10 min, and then at −10°C for 1 h. After this, methanol (30 ml) and additional portion of dimethyl aniline (2.73 g) was added and stirred at room temperature for 12 h. The reaction mixture was processed as for **5A**. Yield 2.0 g, 49.16%. $^1$H-NMR ($CDCl_3$): 1.8 (2H, bs, $NH_2$), 2.4 (3H, s, $CH_3$), 3.48 and 3.88 (2H, ABq, $CH_2$), 4.35 and 5.05 (2H, ABq, $CH_2$), 4.78 (1H, d, C6-H), 5.0 (1H, d, C7-H), 6.82 (1H, s, $CH_2$), 7.18 (1H, s, thiazole), 7.3–7.6 (10H, m, Ar).

*Diphenyl methyl 7β-[α-syn-methoxyimino-α-(2-aminothiazol-4-yl)]acetamido-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulphonylmethyl] ceph-3-em-4-carboxylate* ***6A***

A mixture of diphenyl methyl 7β-amino-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulphonylmethyl] ceph-3-em-4-carboxylate **5A** (0.26 g, 0.0005 mol) and 1-benzotriazolyl (*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetate (0.185 g, 0.00033 mol) in tetrahydrofuran (10 ml) was stirred at room temperature for 2 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The extract was washed with water, dried over anhydrous $Na_2SO_4$ and then concentrated *in vacuo*. The residue was chromatographed over a silica-gel column using ethyl acetate as eluent. Yield 0.3 g, 85.8%. $^1$H-NMR ($CDCl_3$): 2.77 (3H, s, $CH_3$), 3.58 and 3.88 (2H, ABq, $CH_2$), 4.05 (3H, s, $OCH_3$), 4.53 and 5.25 (2H, ABq, $CH_2$), 5.18 (1H, d, C6-H), 6.1 (1H, q, C7-H), 5.83 (2H, bs, $NH_2$), 6.83 and 6.84 (2H, ss, $CH_2$ + thiazole), 7.28–7.5 (10H, m, Ar), 8.14 (1H, d, NH).

*Diphenyl methyl 7β-[α-syn-methoxyimino-α-(2-amino-thiazol-4-yl)]acetamido-3-[(4-methylthiazol-2-yl)sulphonylmethyl] ceph-3-em-4-carboxylate* ***6B***

A mixture of diphenyl methyl 7β-amino-3-[(4-methylthiazol-2-yl)sulphonylmethyl] ceph-3-em-4-carboxylate **5B** (1.08 g, 0.00212 mol) and 1-benzotriazolyl (*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetate (0.646 g, 0.0012 mol) in tetrahydrofuran (10 ml) was stirred at room temperature for 2 h. The reaction mixture was processed as for **6A**. Yield 0.9 g, 62.5%. $^1$H-NMR ($CDCl_3$): 2.36 (3H, s, $CH_3$), 3.54 and 3.85 (2H, ABq, $CH_2$), 4.02 (3H, s, $OCH_3$), 4.4 and 5.09 (2H, ABq, $CH_2$), 5.15 (1H, d, C6-H), 5.85 (2H, bs, $NH_2$), 6.08 (1H, q, C7-H), 6.73 (1H, s, thiazole), 6.76 (1H, s, thiazole), 7.12 (1H, s, $CH_2$), 7.27–7.5 (10H, m, Ar), 8.1 (1H, d, NH).

*General process for deblocking and preparation of sodium salts*

To a solution of diphenyl methyl ester **2**, **4**, **6** or **8** (1.0 g, 0.0015 mol) in anisole (2 ml), trifluoroacetic acid (8 ml) was added at 0–4°C. The resulting solution was stirred further for 15–20 min, then diluted with a mixture of solvents (ether/hexane, 1:2, 100 ml). The precipitated solid was filtered off and washed with ether and dried *in vacuo*. The solid was redissolved in ethyl acetate (when necessary, a minimum amount of methanol was added to obtain clear solution) and pH was adjusted to 7.0 by adding a minimal amount of sodium ethylhexanoate. The solution was diluted with ether and the solid that separated out was filtered, washed with ether, and dried *in vacuo*. Sodium salts were obtained in 73–81% yields and their structures were confirmed by $^1$H-NMR spectroscopy and elemental analyses. The compounds prepared are listed below.

*Sodium 7β-(2-thienyl)acetamido-3-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl] ceph-3-em-4-carboxylate* ***1a***. $^1$H-NMR ($D_2O$): 2.74 (3H, s, $CH_3$), 3.38 and 3.75 (2H, ABq, $CH_2$), 3.97 and 4.5 (2H, ABq, $CH_2$), 3.9 and 3.93 (2H, ss, $CH_2$), 5.06 (1H, d, C6-H), 5.62 (1H, d, C7-H), 7.05 (2H, d, thiophene), 7.37 (1H, t, thiophene). Anal $C_{17}H_{15}N_4O_4S_4Na$ (C, H, N).

*Sodium 7β-(2-thienyl)acetamido-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulphonylmethyl] ceph-3-em-4-carboxylate* ***1b***. $^1$H-NMR ($D_2O$): 2.73 (3H, s, $CH_3$), 3.3 and 3.7 (2H, ABq, $CH_2$), 3.74 (2H, bs, $CH_2$), 4.4 and 5.15 (2H, ABq, $CH_2$), 5.0 (1H, d, C6-H), 5.49 (1H, d, C7-H), 6.88–6.92 (2H, m, ar), 7.22–7.28 (1H, m, Ar). Anal $C_{17}H_{15}N_4O_6S_4Na$ (C, H, N).

*Sodium-7β-(2-thienyl)acetamido-3-[(4-methylthiazol-2-yl)thiomethyl] ceph-3-em-4-carboxylate* ***1c***. $^1$H-NMR ($D_2O$): 2.3 (3H, s, $CH_3$), 3.13 and 3.53 (2H, ABq, $CH_2$), 3.78 and 4.37 (2H, ABq, $CH_2$), 3.85 (2H, bs, $CH_2$), 4.93 (1H, d, C6-H), 5.58 (1H, d, C7-H), 6.85–7.0 (3H, multiplate overlapped with a broad singlet, thiazole + thiophene protons), 7.14–7.2 (1H, m, thiophene). Anal $C_{18}H_{16}N_3O_4S_4Na$ (C, H, N).

*Sodium 7β-(2-thienyl)acetamido-3-[(4-methylthiazol-2-yl)sulphonylmethyl] ceph-3-em-4-carboxylate* ***1d***. $^1$H-NMR ($D_2O$): 2.5 (3H, s, $CH_3$), 3.39 and 3.63 (2H, ABq, $CH_2$), 3.76 and 3.86 (2H, ABq, $CH_2$), 4.09 (2H, s, $CH_2$), 4.98 (1H, d, C6-H), 5.57 (1H, d, C7-H), 6.94–7.05 (2H, m, thiophene), 7.34–7.43 (1H, m, thiophene), 7.8 (1H, s, thiazole). Anal $C_{18}H_{16}N_3O_6S_4Na$ (C, H, N).

*Disodium 7β-(2-thienyl)acetamido-3-[(4-carboxymethylthiazol-2-yl)thiomethyl] ceph-3-em-4-carboxylate* ***1e***. $^1$H-NMR ($D_2O$): 3.18 and 3.59 (2H, dd, $CH_2$), 3.47 (2H, s, $CH_2$), 3.67 and 4.3 (2H, ABq, $CH_2$), 3.73 and 3.78 (2H, ABq, $CH_2$), 4.88 (1H, d, C6-H), 5.43 (1H, d, C7-H), 7.1 (1H, s, thiazole), 6.9 (2H, m, thiophene), 7.25 (1H, m, thiophene).

*Disodium 7β-(2-thienyl)acetamido-3-[(4-carboxymethylthiazol-2-yl)sulphonylmethyl] ceph-3-em-4-carboxylate* ***1f***. $^1$H-NMR ($D_2O$): 3.18 and 3.52 (2H, ABq, $CH_2$), 3.65 (2H, s, $CH_2$), 3.7 (2H, d, $CH_2$), 4.23 and 5.14 (2H, ABq, $CH_2$), 4.96 (1H, d, C6-H), 5.45 (1H, d, C7-H), 6.86–6.9 (2H, m, thiophene), 7.18–7.24 (1H, m, thiophene), 7.7 (1H, s, thiazole).

*Sodium 7β-[α-syn-methoxyimino-α-(2-aminothiazol-4-yl)]acetamido-3-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl] ceph-3-em-4-carboxylate* ***1g***. $^1$H-NMR ($D_2O$): 2.58 (3H, s, $CH_3$), 3.28 and 3.68 (2H, ABq, $CH_2$), 3.8 and 4.34 (2H, ABq, $CH_2$), 3.85 (3H, s, $OCH_3$), 5.02 (1H, d, C6-H), 5.64 (1H, d, C7-H), 6.88 (1H, s, thiazole). Anal $C_{17}H_{16}N_7O_5S_4Na$ (C, H, N).

*Sodium 7β-[α-syn-methoxyimino-α-(2-aminothiazol-4-yl)]acetamido-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulphonylmethyl] ceph-3-em-4-carboxylate* ***1h***. $^1$H-NMR ($D_2O$): 2.93 (3H, s, $CH_3$), 3.56 and 3.98 (2H, ABq, $CH_2$), 4.03 (3H, s, $OCH_3$), 4.62 and 5.35 (2H, ABq, $CH_2$), 5.3 (1H, d, C6-H), 5.84 (1H, d, C7-H), 7.06 (1H, s, thiazole). Anal $C_{17}H_{16}N_7O_7S_4Na$ (C, H, N).

*Sodium 7β-[α-syn-methoxyimino-α-(2-aminothiazol-4-yl)]acetamido-3-[(4-methylthiazol-2-yl)thiomethyl] ceph-3-em-4-carboxylate* ***1i***. $^1$H-NMR ($D_2O$): 2.4 (3H, s, $CH_3$), 3.4 and 3.87 (2H, ABq, $CH_2$), 3.83 and 4.57 (2H, ABq, $CH_2$), 4.05 (3H, s, $OCH_3$), 5.18 (1H, d, C6-H), 5.78 (1H, d, C7-H), 7.05 (1H, s, thiazole), 7.18 (1H, s, thiazole). Anal $C_{18}H_{17}N_6O_5S_4Na$ (C, H, N).

*Sodium 7β-[α-syn-methoxyimino-α-(2-aminothiazol-4-yl)]acetamido-3-[(4-methylthiazol-2-yl)sulphonylmethyl] ceph-3-em-4-carboxylate* ***1j***. $^1$H-NMR ($D_2O$): 2.5 (3H, s, $CH_3$), 3.42 and 3.84 (2H, ABq, $CH_2$), 4.0 (3H, s, $OCH_3$), 4.35 and 5.38 (2H, ABq, $CH_2$), 5.2 (1H, d, C6-H), 5.79 (1H, d, C7-H), 6.97 (1H, s, thiazole), 7.7 (1H, s, thiazole). Anal $C_{18}H_{17}N_6O_7S_4Na$ (C, H, N).

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