current ratio (ip2/ip1, refer to Table 4) increases with increasing scan rate because the chemical dimerization of radical (reaction (7)) is fast enough. Furthermore, when the potential sweeping rate is very slow the coverage of monomer is much lower than that of dimer on the electrode surface, the current ratio (ip2/ip1) is thus probably close to zero. On the other hand, when the rate is fast enough, the coverage of monomer is much higher and the current ratio approaches to unity.

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Synthetic β-Lactam Antibiotics II. Synthesis and Antibacterial Activity of 7β-[2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[1-(halosubstitutedphenyl)-1H-tetrazol-5-yl]thiomethyl-cephalosporins

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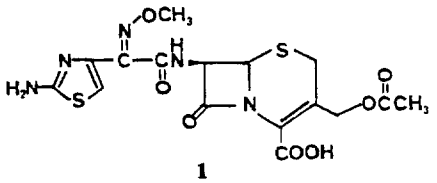
Syntheses of cefotaxime analogs with halosubstituted phenyltetrazolthiomethyl at the C_3-position are described. Their *in vitro* potency was established. The compounds exhibited a broad antibacterial spectrum. Some of these compounds showed activity against Gram-positive bacteria superior to the parent cefotaxime. Against Gram-negative bacteria, these compounds are less effective than cefotaxime.

Recently, a number of β-lactamase-stable, extended-spectrum cephalosporins referred to as third generation cephalosporins have been developed for clinical use. Among them, cephalosporins bearing (Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl group at the C_7 position of a cephem nucleus, such as cefotaxime(1),[1,2] ceftizoxime,[3,4] cefmenoxime[5] and ceftriaxone[6,7] are characterized by their excellent activity and marked resistance to β-lactamases. They are, however, relatively weak in Gram-positive activity, especially in anti-Staphylococcal activity as compared to older cephalosporins such as cephalothin.[8,9] Also only a few exhibit substantial activity against Pseudomonas species.[10]



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For the continuous work of extensive study on the development of broad spectrum cephalosporins, the primary aim in the present work was to prepare cefotaxime analogs by varing the C_3-substituent with enhanced potency against Gram-positive bacteria and Pseudomonas species, and high

β-lactamase stability with retaining its broad antibacterial spectrum. In general, changes at the C_3-position of cephem nucleus have more influence on the level of antibacterial activity and on metabolic and pharmacokinetic properties with limited extension of the spectrum of activity.[11]

Modification in the C_3-position, we became interested in the effect of the mercaptotetrazole having the fluoroaromatic group since we observed these substituents showed a favorable effect on the *in vitro* antibacterial activity from the previous work.[12] Thus a series of cefotaxime derivatives having halosubstituted phenyltetrazolthiomethyl groups at the C_3-position were prepared.

This paper describes the synthesis and the structure activity relationships of cefotaxime analogs and their *in vitro* antibacterial activities against 20 selected strains of Gram-positive and Gram-negative bacteria.

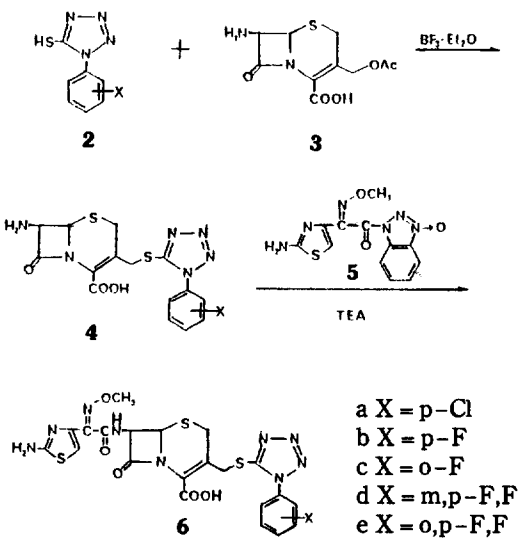
Synthesis

The synthesis of cefotaxime analogs(6a-6e) is shown in Scheme 1. The mercaptotetrazoles(2a-2e) and the tetrazolthiomethylcephalosporanic acids(4a-4e) were prepared by the previously reported method.[12]

For acylation of the cephem nucleus(4a-4e) with 2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetic acid, the acid-HOBT (1-hydroxybenzotriazole) adduct(5)[13] was used. The C_3-substituted cephalosporanic acids(4a-4e) were reacted with 5 in 50% aqueous acetone in the presence of triethylamine to yield the corresponding cephalosporins(6a-6e) in satisfactory yields.[14] Using other activated acids such as anhydrides, acid chlorides, and mixed anhydrides, the coupling reactions gave poor yields or did not work at all.

Antibacterial Activity and Discussion

Minimum inhibitory concentrations(MICs) of the cephalosporins were determined by the standard two fold agar dilution method. Mueller Hinton Agar was generally used for bacteria. The size of inoculum used for MIC determination was adjusted to Macfarland No. 0.5. The lowest concentration inhibiting the visible growth after 18hr incubation at 37°C was expressed as the MIC.



Scheme 1

The MIC values of this series of new cephalosporins against a variety of Gram-positive and Gram-negative bacteria in comparison with those of piperacillin(PIP), cefoperazone (CEF), and cefotaxime(CTX) are shown in Table 1.

As can be seen from Table 1, this series of compounds possess a broad antibacterial spectrum including many species of important Gram-positive and Gram-negative organisms. This data allow examination of the effects of C_3-substituent variation as well as the effects of fluorine atom at the

Table 1. Antibacterial Activity of five Cephalosporins in Comparison with Piperacillin (PIP), Cefoperazone (CEF), and Cefotaxime (CTX)

0.	strains	ATCC No.	6a	6b	6c	6d	6e	PIP	CEF	CTX
1	*Bacillus cereus*	27348	256	128	64	128	128	32	8	256
2	*Bacillus subtilis*	6633	32	16	1	2	4	2	1	1
3	*Micrococcus luteus*	9341	0.5	0.03125	0.03125	0.03125	0.0625	0.0625	0.25	0.0625
4	*Micrococcus luteus*	10240	0.5	0.25	0.25	0.25	0.5	2	2	0.5
5	*Staphylococcus aureus*	6538P	2	2	2	2	1	0.5	1	2
6	*Staphylococcus epidermidis*	12228	2	2	1	1	1	16	2	1
7	*Acinetobacter calcoaceticus*	15473	128	64	32	32	64	16	128	16
8	*Bordetella bronchiseptica*	4617	256	128	256	256	256	1	4	128
9	*Enterobacter aerogenes*	29751	16	64	16	16	16	16	8	4
10	*Escherichia coli*	10536	1	0.5	0.5	0.5	0.5	0.5	0.0625	0.015625
11	*Escherichia coli*	25922	8	8	1	1	2	2	0.125	0.03125
12	*Escherichia coli*	31030	16	32	2	2	4	4	0.25	0.125
13	*Klebsiella pneumoniae*	10031	0.0625	0.03125	0.0625	0.0625	0.03125	8	0.125	0.015625
14	*Proteus mirabilis*	25933	2	2	0.5	0.5	1	0.5	0.5	0.015625
15	*Providencia rettgeri*	9919	0.5	0.25	0.25	0.25	0.5	0.5	0.25	0.015625
16	*Pseudomonas aeruginosa*	10145	256	128	64	128	128	8	8	16
17	*Pseudomonas aeruginosa*	25619	16	32	8	8	16	0.5	0.5	0.5
18	*Pseudomonas aeruginosa*	27853	256	128	64	128	128	4	8	16
19	*Salmonella typhimurium*	14023	8	4	4	4	8	2	0.5	0.125
20	*Serratia marcescens*	27117	4	4	2	2	2	1	0.5	0.0625

phenyl group. Of the cefotaxime analogs(6a-6e), the two compounds(6c and 6d) had about the same range of antibacterial activity and showed activity against Gram-positive bacteria superior to the parent cefotaxime. The four compounds (6b-6e) containing one or two fluorine atoms at the phenyl ring gave better activity than the compound(6a) containing a chlorine atom.

It is worth noting that the substituent of the mercaptotetrazoles containing electron-withdrawing and hydrophobic fluorine atoms results in enhancement of antibacterial activities against Gram-positive bacteria such as *Bacillus cereus* ATCC No. 27348, *Micrococcus luteus* ATCC No. 9341, *Micrococcus luteus* ATCC No. 10240, and *Staphylococcus aureus* 6538P (penicillin G sensitive) compared with cefotaxime while retaining the same activity against other Gram-positive bacteria such as *Bacillus subtilis* ATCC No. 6633 and *Staphylococcus epidermidis* ATCC No. 12228. It can be seen that replacement of the acetoxy group of cefotaxime with mercaptotetrazoles resulted in a decrease of the activities against Gram-negative bacteria. Against *Pseudomonas aeruginosa,* 6c was about four to sixteen times less active than cefotaxime.

In spite of this, these new cephalosporins are generally more effective than piperacillin against most of Gram-positive and Gram-negative organisms except *Pseudomonas aeruginosa.* 6c and 6d are superlor to cefoperazone against Gram-positive bacteria. Although the activities of 6c and 6d are comparable to those of cefoperazone against *Proteus mirabilis, Providencia rettgeri* and superior against *Klebsiella pneumoniae,* in general the new cephalosporins are less effective than cefoperazone against Gram-negative bacteria.

When this series of cephalosporins is classified by the arylsubstituted mercaptotetrazole ring containing fluorine atoms, the trend of enhancement in activities against Gram-positive bacteria was observed similary with cephalothin analogs described in the previous paper.[12] It could be that attempts at chemical modification to increase the intrinsic Gram-positive activity has often resulted in a decrease in anti-Gram-negative activity.

Our further studies on development of the cephalosporins having new substituents at the C_3-position with enhanced activity will be presented in near future.

Experimental

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR-spectra were recordered at 200MHz on Bruker AM 200 NMR using TMS as an internal standard. IR spectra were taken on Analect Instrument fx-6160 FT-IR. Mass spectra were obtained by use of Hewlett-Packard 5985 GC-Mass spectroscopy. No effort was made to improve the yields.

1-(3′,4′-Difluorophenyl)-1H-tetrazole-5-thiol(2d): To a solution of 3,4-difluoroaniline(6.45g, 50mmol) in 50m*l* of water were added potassium hydroxide(5.5g, 100mmol) and carbon disulfide(3.8g, 50mmol) at room temperature. After being stirred for 18hr, sodium azide(3.9g, 60mmol) in 20m*l* of water was added to the above solution. The reaction mixture was refluxed for 12hr. The aqueous solution was washed with ethyl acetate and acidified to pH 2 with 1N hydrochloric acid. The precepitate was filtered and washed with water and acetone to give 5.0g(47%) of 2d, a white solid. MS 214(M^+).

1-(2′,4′-Difluorophenyl)-1H-tetrazol-5-thiol(2e): 2e was obtained (56%) from 2,4-difluoroaniline by a similar procedure as described for the preparation of 2d. MS 214(M^+).

7-Amino-3-[1-(3′,4′-difluorophenyl)-1H-tetrazol-5-yl] thiomethyl-3-cephem-4-carboxylic acid(4d): To a stirred solution of borontrifluoride etherate(2.11g, 15mmol) in 15m*l* of anhydrous acetonitrile under nitrogen were added successively 1.15g(5mmol) of 2d and 1.36g(5mmol) of the 7-ACA (3) at room temperature. The resulting solution was allowed to react at 50°C for 4hr. After cooling it, the residue was filtered off and washed with 5m*l* of acetonitrile. The filtrate was diluted with 10m*l* of water and adjusted to pH 4.0 by addition of 28% ammonium hydroxide solution. The precipitate was filtered and washed with water and acetone. The solid was dried *in vacuo* to give 1.68g(79%) of 4d, mp 185-188°C (decom). IR(KBr) 1801(β-lactam), 1619(carboxylic acid) cm^{-1}; NMR(TFA-d_1) δ (ppm) 7.46-7.83(3H, m, phenyl-H), 5.44(2H, br s, C_6-H, C_7-H), 4.76-4.83(1H, d, J=13.6H, C_3-CH_2), 4.58-4.65(1H, d, J=13.6H, C_3-CH_2), 3.91 (2H, br s, C_2-H).

7-Amino-3-[1-(2′,4′-difluorophenyl)-1H-tetrazol-5-yl] thiomethyl-3-cephem-4-carboxylic acid(4e): This compound was prepared(81%) from 2e by the same procedure used for 4d, mp 180-185°C(decom). IR(KBr) 1801(β-lactam), 1617 (carboxylic acid); NMR(TFA-d_1) (ppm) 7.16-8.03(3H, m, phenyl-H), 5.43-5.50(2H, m, C_6-H, C_7-H), 4.74-4.81(1H, d, J=14.0H, C_3-CH_2), 4.54-4.61(1H, d, J=14.0H, C_3-CH_2), 3.88 (2H, br s, C_2-H).

7β-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino) acetamido]-3-[1-(4′-chlorophenyl)-1H-tetrazol-5-yl] thiomethyl-3-cephem-4-carboxylic acid(6a): 4a(0.85g, 2mmol) was suspended in 20m*l* of 50% aqueous acetone and triethylamine (0.18g, 1.8mmol) was added dropwise to the stirred solution. After the mixture became a clear solution, 0.66g (2.1 mmol) of 5 was added portionwise at 5°C while the pH of the solution was adjusted at pH 7.5 with 45% potassium phosphate solution. After 3hr, the mixture was allowed to warm to room temperature and stirred for 6hr. The acetone was removed and the resulting aqueous solution was acidified to pH 2.5 with 1N hydrochloric acid. The precipitate solid was collected by filtration, washed with water and acetone and dried *in vacuo* over phosphorus pentoxide to provide 0.77g (64%) of a pale yellow solid, mp 155-158°C (decom). IR (KBr) 1778 cm^{-1} (β-lactam); NMR (DMSO-d_6) δ(ppm) 9.73-9.77(1H, d, J=7.9H, amide-H), 7.71-8.01 (6H, m, phenyl-H, thiazole-NH_2), 6.86(1H, s, thiazole-H), 5.61-5.82(1H, dd, J=4.8H, 7.9H, C_7-H), 5.11-5.14(1H, d, J=4.8H, C_6-H), 4.57-4.64(1H, d, J=13.7H, C_3-CH_2), 4.23-4.30(1H, d, J=13.7H, C_3-CH_2), 3.92 (3H, s, N-OCH_3), 3.67-3.73 (2H, d, J=10.9H, C_2-CH_2).

7β-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[1-(4′-fluorophenyl)-1H-tetrazol-5-yl] thiomethyl-3-cephem-4-carboxylic acid (6b): 6b was obtained (59%) from 4b by a similar procedure as described for the preparation of 6a, mp 162-165°C(decom). IR(KBr) 1776cm^{-1}(β-lactam); NMR (DMSO-d_6) δ (ppm) 9.60-9.64(1H, d, J=8.0H, amide-H), 7.33-8.05 (6H, m, phenyl-H, thiazole-NH_2), 6.76(1H, s, thiazole-H), 5.75-5.83(1H, dd, J=4.8H, 8.0H, C_7-H), 5.10-5.13 (1H, d, J=4.8H, C_6-H), 4.50-4.57 (1H, d, J=13.4H, C_3-CH_2), 4.22-4.29(1H, d, J=13.4H, C_3-CH_2), 3.85(3H, s, N-OCH_3), 3.67-3.72(2H, d, J=10.7H, C_2-CH_2).

7β-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[1-(2′-fluorophenyl)-1H-tetrazol-5-yl] thiomethyl-3-

cephem-4-carboxylic acid(6c): 6c was obtained(61%) from 4c by a similar procedure as described for the preparation of 6a, mp 165-168°C(decom). IR(KBr) 1777cm^{-1}(β-lactam); NMR(DMSO-d_6) δ(ppm) 9.59-9.63(1H, d, J=8.0H, amide-H), 7.40-7.79(4H, m, phenyl-H), 7.24(2H, br s, thiazole-NH_2), 6.74(1H, s, thiazole-H), 5.76-5.83(1H, dd, J=4.8H, 8.0H, C_7-H), 5.05-5.11(1H, d, J=4.8H, C_6-H), 4.53-4.60(1H, d, J=13.5H, C_3-CH_2), 4.24-4.31(1H, d, J=13.5H, C_3-CH_2), 3.84 (3H, s, N-OCH_3), 3.64-3.71(2H, d, J=13.5H, C_2-CH_2).

7β-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[1-(3′,4′-difluorophenyl)-1H-tetrazol-5-yl] thiomethyl-3-cephem-4-carboxylic acid(6d): 6d was obtained (65%) from 4d by a similar procedure as described for the preparation of 6a, mp 190-195°C(decom). IR(KBr) 1775 cm^{-1} (β-lactam); NMR(DMSO-d_6) δ (ppm) 9.58-9.62(1H, d, J=8.0H, amide-H), 7.59-7.96(4H, m, phenyl-H), 7.25(2H, br s, thiazole-NH_2), 6.78(1H, s, thiazole-H), 5.76-5.83(1H, dd, J=4.8H, 8.0H, C_7-H), 5.01-5.12(1H, d, J=4.8H, C_6-H), 4.49-4.56 (1H, d, J=13.4H, C_3-CH_2), 4.22-4.29 (1H, d, J=13.4H, C_3-CH_2), 3.84(3H, s, N-OCH_3), 3.66-3.72(2H, d, J=12.3H, C_2-CH_2).

7β-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[1-(2′,4′-difluorophenyl)-1H-tetrazol-5-yl] thiomethyl-3-cephem-4-carboxylic acid(6e): 6e was obtained (60%) by a similar procedure as described for the preparation of 6a, mp 194-197°C. IR(KBr) 1775cm^{-1}(β-lactam); NMR (DMSO-d_6) δ (ppm) 9.59-9.63(1H, d, J=7.9H, amide-H), 7.30-7.96(3H, m, phenyl-H), 7.23(2H, br s, thiazole-NH_2), 6.78(1H, s, thiazole-H), 5.74-5.83(1H, dd, J=4.8H, 7.9H, C_7-H), 5.07-5.18(1H, d, J=4.8H, C_6-H), 4.51-4.58(1H, d, J=13.4H, C_3-CH_2), 4.24-4.31(1H, d, C_3-CH_2), 3.84(3H, s, N-OCH_3), 3.64-3.71(2H, d, J=14.5H, C_2-CH_2).

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Enantioselective Reduction of Racemic Three-Membered Heterocyclic Compounds. 3. Reaction of Epoxides with B-Isopinocampheyl-9-borabicyclo[3.3.1]nonane–Potassium Hydride and Potassium B-Isopinocampheyl-9-boratabicyclo[3.3.1]nonane Systems[1]

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The chiral B-isopinocampheyl-9-borabicyclo[3.3.1]nonane—potassium hydride (IPC-9-BBN—KH) and potassium B-isopinocampheyl-9-boratabicyclo [3.3.1] nonane (K IPC-9-BBNH) systems were applied to the enantioselective reduction of representative racemic epoxides, namely 1,2-epoxybutane, 1,2-epoxyoctane, 3,3-dimethyl-1,2-epoxybutane and styrene oxide. In the case of IPC-9-BBN—KH system, the optical yields are in the range of 8.3-37.4% ee. However, the system of K IPC-9-BBNH provides significantly lower optical yields, showing 7-22.5% ee. These results strongly suggest that the enantioselective coordination of chiral organoborane to the epoxy oxygen of racemic epoxides plays an important role in this resolution.

Introduction

In the previous note[1], we have reported that the optically-active (-)-diisopinocampheylborane-lithium chloride (1:0.1) system reduced enantioselectively various racemic epoxides in the range of 5.5-30.5% ee and racemic episulfide in the