β-Lactams in the New Millennium. Part-II: Cephems, Oxacephems, Penams and Sulbactam

G.S. Singh*

Chemistry Department, University of Botswana, Private Bag 0022, Gaborone, Botswana

Abstract: β-lactam ring-containing compounds such as penicillins, ampicillin, amoxicillin, cephalosporins and carbapenems are among the most famous antibiotics. This article reviews the recent developments in study of cephems, oxacephems, penams and sulbactam. Many of the compounds reviewed have potential antibacterial activity, even against resistant strains such as MRSA, and enzyme inhibitory activity.

Keywords: β-Lactam, cephem , penams, sulbactam, antibacterial, enzyme inhibitors, anticancer.

1. INTRODUCTION

In the preceding part of this review, synthesis and biological activity of monobactams and carbapenems are described. Cephalosporins (cephems), the well-known antibiotics with broad spectrum, have maintained their glamour among medicinal chemists in the previous more than forty years [1]. As a result of extensive research three generations of cephalosporins are already in clinical use and fourth generation (cefepime) has already been proposed. The main approaches in design of the new cephem derivatives involve structural modifications at positions C-3 and C-7, and the development of cephem prodrugs. The compounds with a methoxy [2], carbamoyloxy [3] or heteroaryl ring such as tetrazole [4] or thiazole [5] in the C-3 side chain are known to have potent antibacterial activity.

This article aims to review the current status of research in synthesis and biological activity of cephems (including oxacephems) and other β-lactams not described in Part-I of this review.

2. SYNTHESIS AND BIOLOGICAL ACTIVITY OF CEPHALOSPORINS

2.1 Synthesis and Antibacterial Activity

Yamamoto and coworkers have exploited the reports cited in the introductory paragraph and published a series of synthetic and biological studies on orally active cephalosporins [6-10]. In order to improve the low efficacy of cefdinir (CFDN) discovered by their group earlier [11], a series of 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-hydoxyiminoacetamido]-3-[(*E*) and (*Z*)-2-substituted vinyl] cephalosporin derivatives **1,2** have been synthesized using palladium-catalyzed coupling reaction of a 3-methanesulfonoxy-3-cephem and an *E* substituted vinyl stannane (**Scheme 1**) or Wittig reaction of a 3-triphenylphosphoniummethyl cephem and an aldehyde (**Scheme 2**) as key steps [7].

These compounds were evaluated for *in vitro* antibacterial activity and oral absorption in rats using CFDN as reference. All of the synthesized compounds exhibited potent antibacterial activity against both Gram-(+) and Gram-(-) bacteria. The compound having a (*Z*)-2-(3-pyridyl)vinyl moiety at C-3 position, FR86524 (**2j**) exhibited the most balanced activity (MIC for *S.a.* = 0.29*, E. f.* = 7.3, *M. c.* = 0.195, *H. i.* = 0.047, *K. p.* = 0.041 and *E. c.* = 0.167 μg/mL) The effect of C-3 double bond stereochemistry on the antibacterial activity was significant; the activity of *Z* isomers against Gram-(-) bacteria, including *H. influenzae* was far superior to that of the corresponding *E* isomers, whereas in terms of activity against *E. faecalis*, the *E* isomers were more potent. The study of urinary and biliary recovery of these compounds after oral administration in rats showed that compounds with aromatic ring moieties and functional groups in the C-3 side chain had relatively low absorption, except for carbamoyloxy cephem, which exhibited better oral absorption in comparison to CFDN.

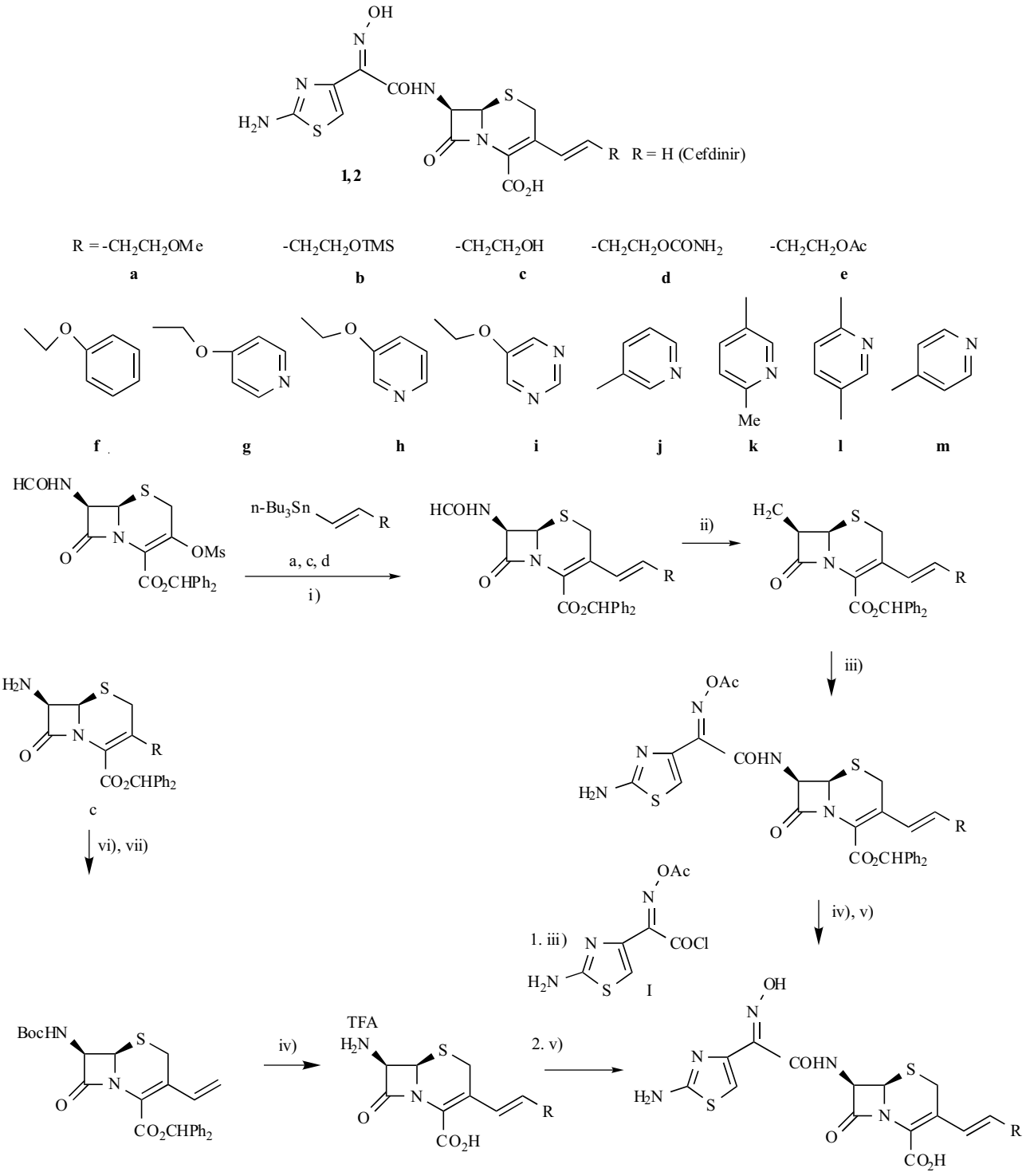
Looking at the important role of a pyridine moiety at C-3 and C-3 double bond stereochemistry in antibacterial activity and oral absorption of cephems, Yamamoto and coworkers extended their work to the study of a series of 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]cephalosporins **3-18,** having a pyridine moiety attached through various spacers to the C-3 position. The compounds **3-18** were synthesized (**Scheme 3, 4**) using more or less similar protocols employed earlier (**Scheme 1,2**) [8].

All of the synthesized compounds exhibited potent antibacterial activity against both Gram-(+) and Gram-(-) bacteria (MIC = 0.052-46.0 μg/mL). In particular, they all exhibited almost equal or improved antibacterial activity against *S. aureus* (MIC = 0.12-0.39 μg/mL) compared with CFDN (MIC = 0.33 μg/mL) and FR86524 (MIC = 0.29 μg/mL) irrespective of both the spacer moiety between pyridine and the cephem nucleus and the position of the pyridine nitrogen. However, the effect of the spacer moiety on the antibacterial activity against Gram-(-) bacteria was distinct from Gram-(+) bacteria. Compounds with a carbon atom directly attached to the C-3 position (**3-6**) showed decreased antibacterial activity against *M. catarrhalis*. Although **3** and **4** showed improved antibacterial activity against *H. influenzae* (MIC = 0.21, 0.11 μg/mL) compared with CFDN (MIC = 0.41μg/mL) the 4-pyridyl compound **4** was slightly superior to the 3-pyridyl compound **3** against

*Address correspondence to this author at the Chemistry Department, University of Botswana, Private Bag 0022, Gaborone, Botswana; Tel: +267-3552501; Fax: +267-3552836; E-mail: singhgs@mopipi.ub.bw

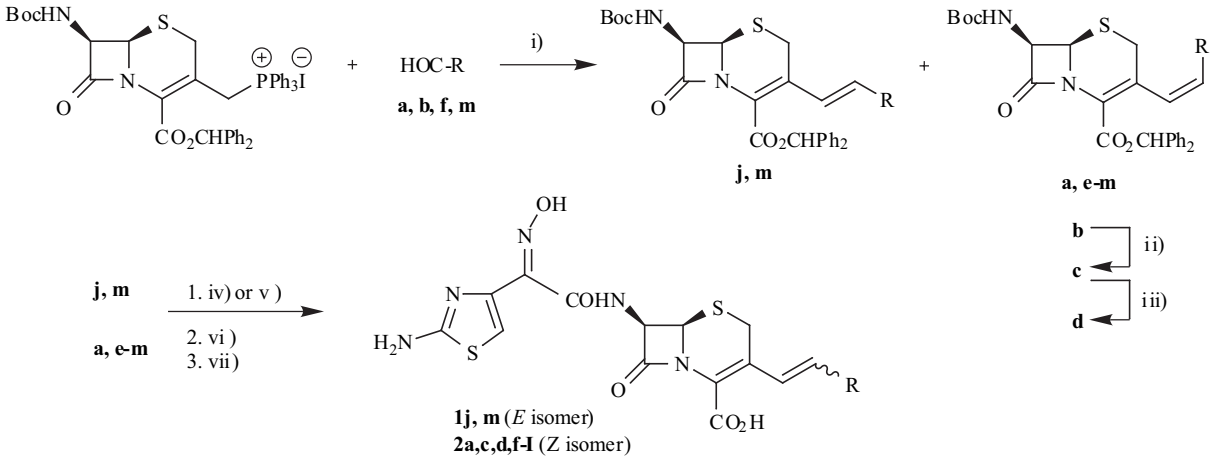
1389-5575/04 $45.00+.00

H. influenzae, *E. coli* and *K. pneumoniae*. Compounds **11-18** with the sulfur atom directly attached to the C-3 position also showed improved antibacterial activity against *H. influenzae*. Among all the compounds synthesized, FR86830 (**14**) exhibited the most well balanced activity against both Gram-(+) and Gram-(-) bacteria (MIC = 0.041, 5.8 μg/mL, respectively). It was 7-fold more active against *H. influenzae* (MIC = 0.067 μg/mL) than CFDN (MIC = 0.41 μg/mL). Although it was slightly less active against *H. influenzae* compared to FR86524 (MIC= 0.047 μg/mL) it exhibited equal or improved antibacterial activity against the other strains tested. Its oral absorption was also higher than FR86524.



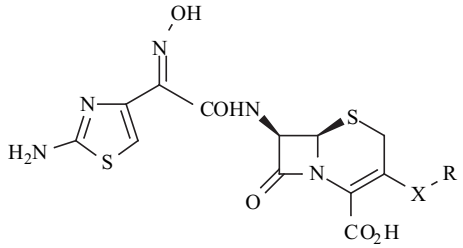
Reagnts: i) $Pd(CH_3CN)_2Cl_2$, LiBr, DMF ii) cHCL, MeOH iii) **I**, BSA, CH_2Cl_2 iv) TFA, anisole, CH_2Cl_2 v) $NaHCO_3$, NH_4Cl, MeOH-H_2O, vi) Boc_2O, MSA, THF vii) CH_3COCl, ET_3N, CH_2Cl_2.

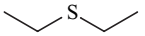
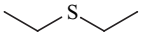
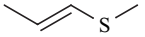
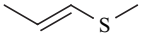
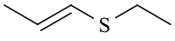
Scheme 1.

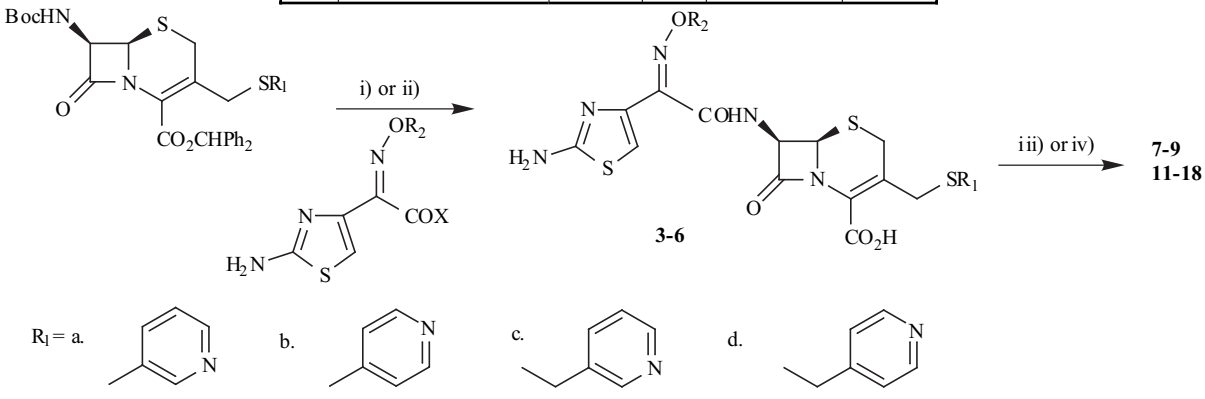


[i]Reagents: i) a) 1N NaOH, aq. NaCl(sat.), CH_2Cl_2, b) separation ii) cHCl, MeOH iii) Cl_3CONCO, CH_2Cl_2, b) SiO_2, $CHCl_3$, MeOH iv) TFA, anisole, CH_2Cl_2 v) HCO_2H,cHCl, vi) BSA or MSA, (Z)-2-(2-aminothiazol-4-yl)-2-acetoxyi-minoacetyl chloride hydrochloride, CH_2Cl_2 vii) $NaHCO_3$, NH_4Cl, MeOH, H_2O.*i*

Scheme 2.

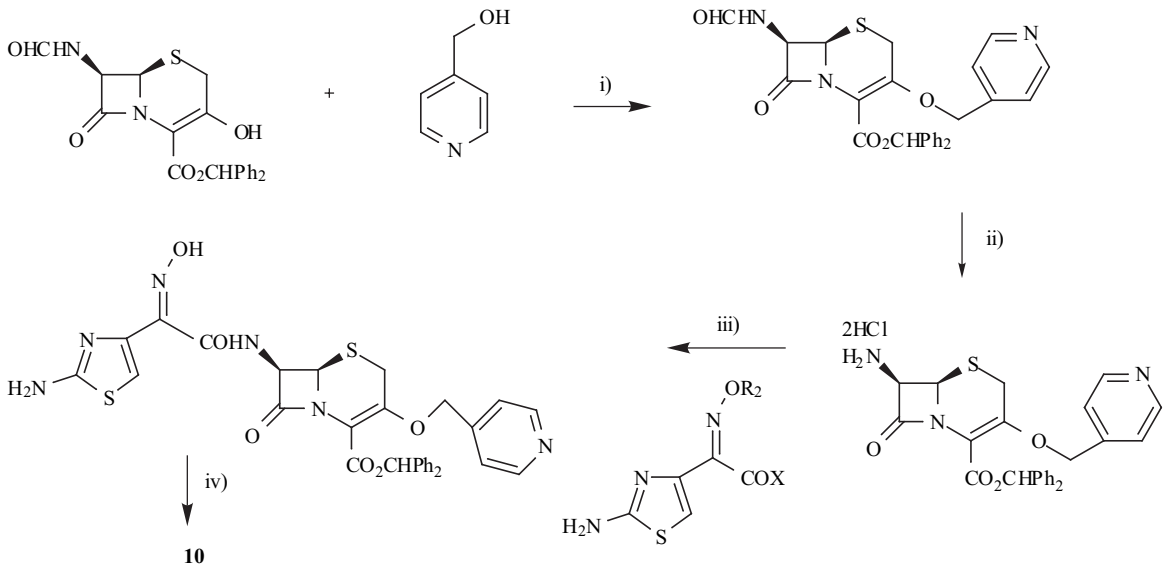


	X	R		X	R
3	$-CH_2S-$	3-pyridyl	11	-S-	3-pyridyl
4	-do-	4-pyridyl	12	-do-	4-pyridyl
5		5-pyridyl	13	$-SCH_2$	3-pyridyl
6		6-pyridyl	14	-do-	4-pyridyl
7		7-pyridyl	15	$-SCH_2CH_2-$	-do
8		8-pyridyl	16	$-SCH{=}CHCH_2$	3-pyridyl
9		9-pyridyl	17	$-SCH_2S-$	-do-
10	$-OCH_2-$	10-pyridyl	18	-do-	4-pyridyl



Reagents: i) TMSCl, Et_3N, THF , DMF ii) BSA, CH_2Cl_2 iii) $NaHCO_3$, NH_4Cl, H_2O iv) 90% HCO_2H

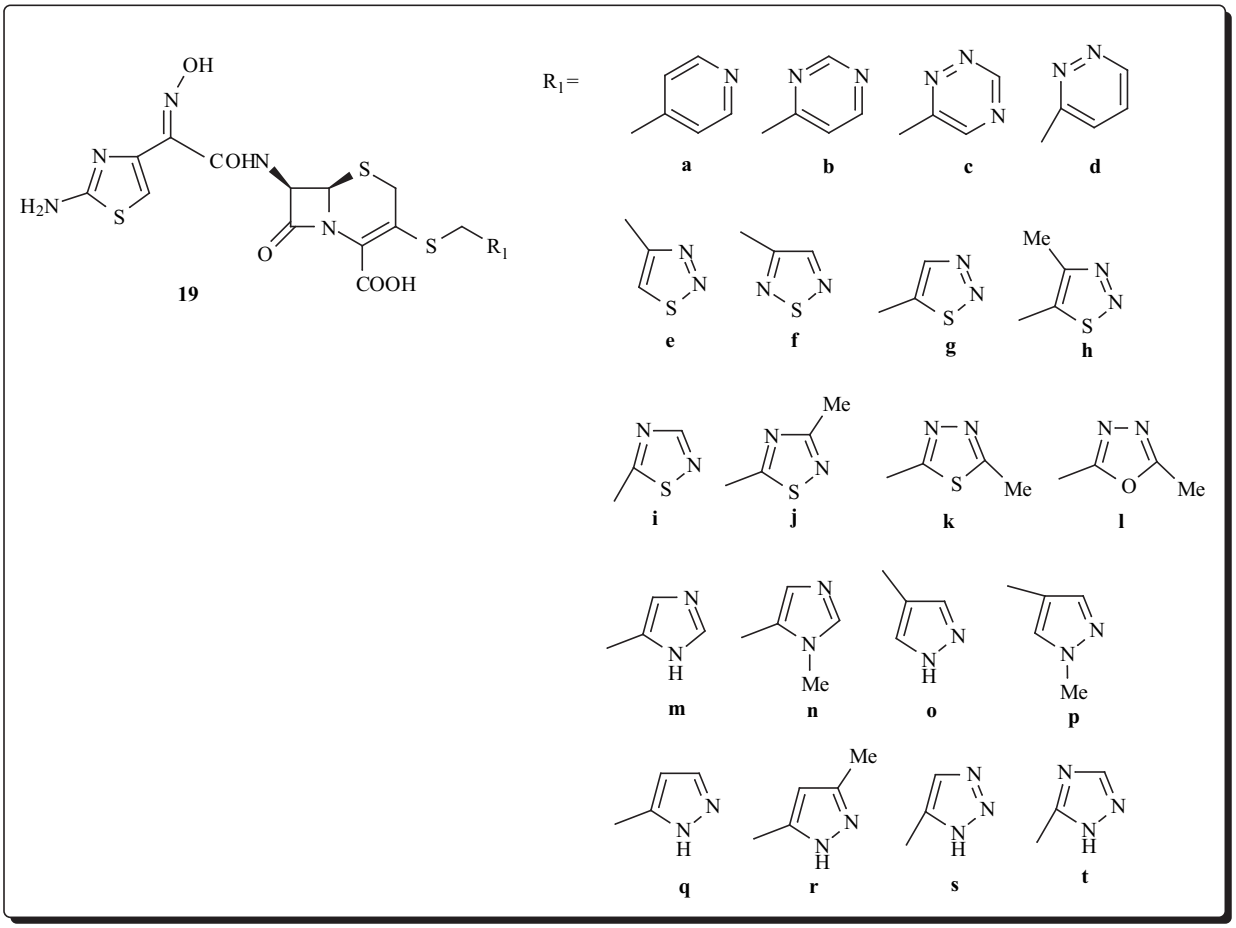
Scheme 3.

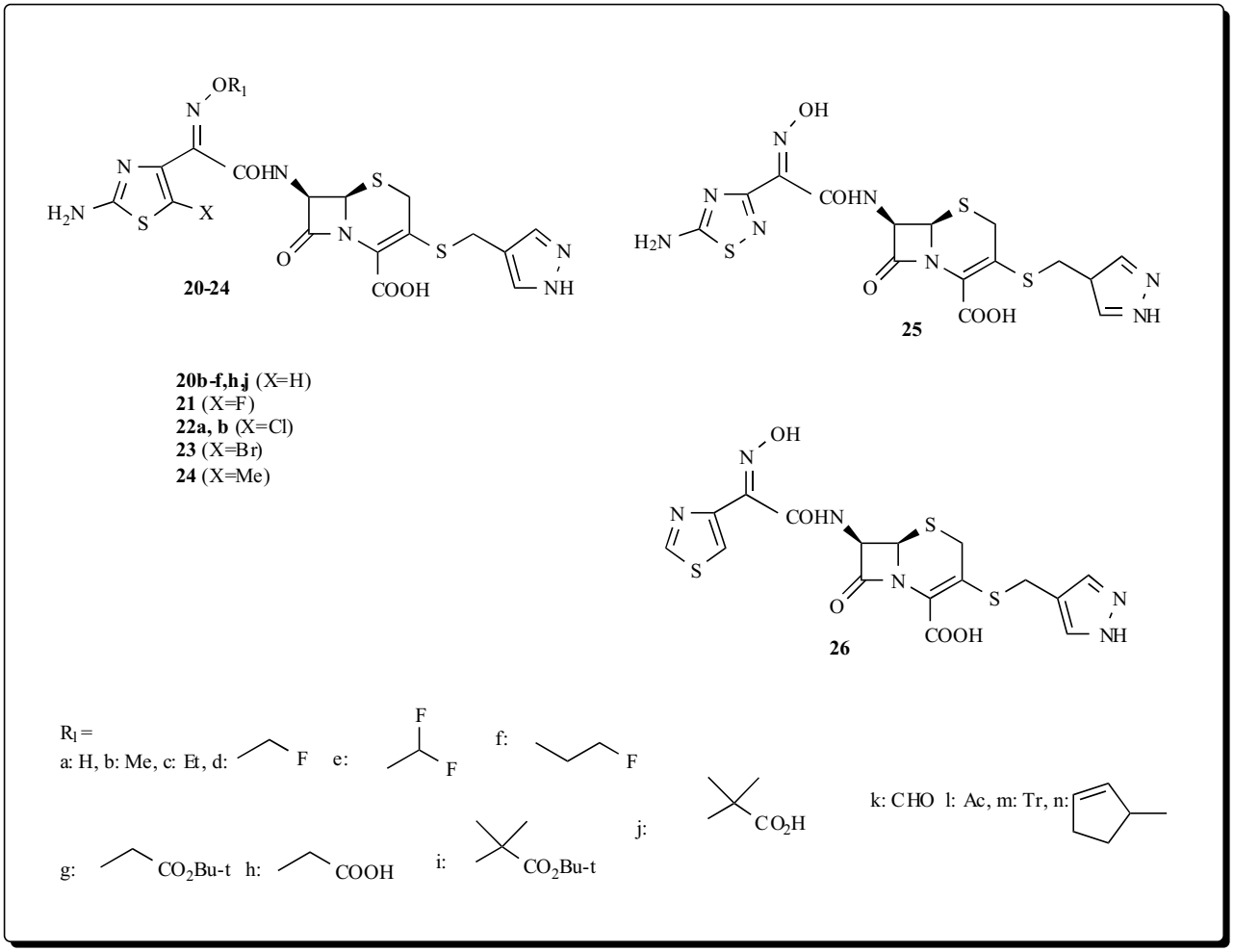


Reagents: i) PPh_3, DEAD, THF ii) cHCl, MeOH iii) a. BSA, CH_2Cl_2 b. c. HCl. MeOH iv) TFA

Scheme 4.

In order to maximize the effectiveness of the methylthio linkage present in FR86830 (**14**), a number of similar cephalosporins **19** were synthesized and screened against Gram-(+) and Gram-(-) bacteria [9]. Among these compounds, **19e**, **19g,h** and **19o** showed potent antibacterial activity against both Gram-(+) and Gram-(-) bacteria. Although **19h** exhibited equal antibacterial activity against tested strains compared with FR 86830, it was 7-fold more active (MIC = 0.069 μg/mL), against *H. influenzae* than CFDN (MIC = 0.41 μg/mL). However, only **19o** (FK 041) exhibited higher oral absorption. At this stage they directed their study towards variation on C-7 side chain and reported

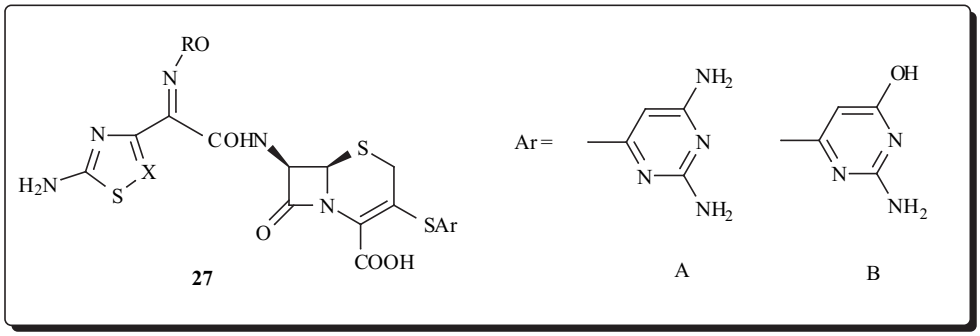




the synthesis, SAR and oral absorption studies of 3-(4-pyrazolylmethylthio)cephalosporins (**20-26**) with various C-7 side chains [10]. Again, all of the synthesized compounds exhibited potent antibacterial activity against both Gram-(+) and Gram-(-) bacteria. Compounds **20b-f,j** and **22b** with an alkoxyimino moiety had dramatically improved activity against *H. influenzae* (MIC = 0.025 μg/mL), and similar potent activity against the other Gram-(-) bacteria compared to FK041 (**190**, MIC = 0.41 μg/mL). In contrast, these compounds had decreased antibacterial activity against *S. aureus* (MIC = 0.78-12.5 μg/mL) and *E. faecalis* (MIC = 19.1 - >100 μg/mL). The study also showed that introduction of an electron-withdrawing halogen substituent to the aminothiazole moiety increased the antibacterial activity while an electron-donating methyl substituent decreased the antibacterial activity. Only compound **22a** had higher oral absorption than both CFDN and FK041 (**190**).

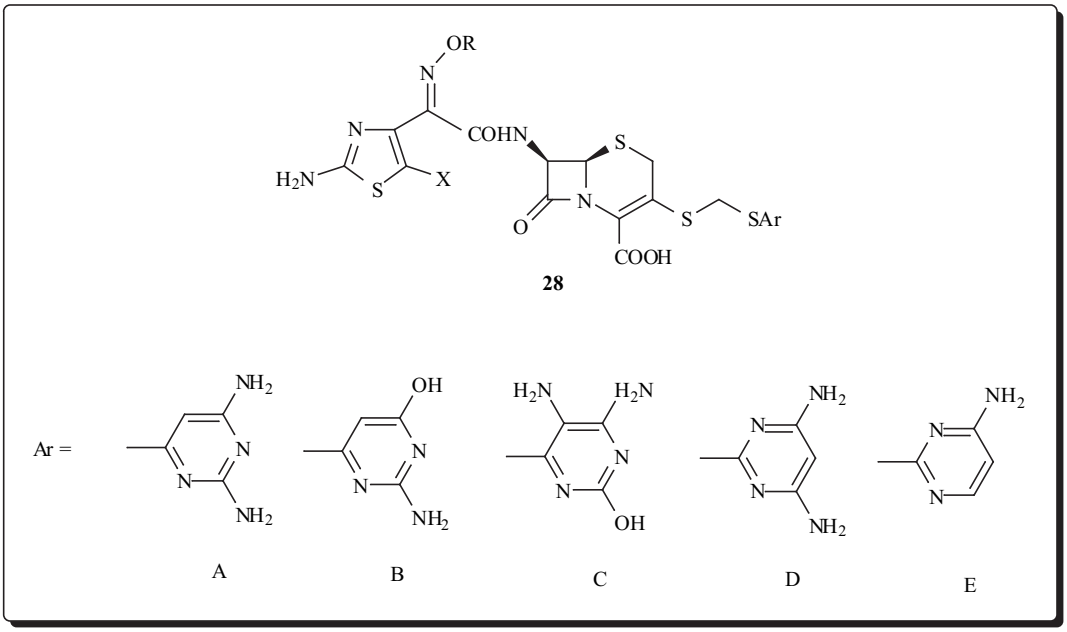
A similar type of cephalosporins with C(3)-aminopyrimidinyl substituent has been synthesized and evaluated for their antibacterial activity, including activity against respiratory tract pathogens, using CFDN as standard drug, by Lee and coworkers at Biotech Research Institute, Taejon, South Korea [12]. These compounds exhibited MIC comparable to CFDN against the Gram-(+) bacteria such as MRSA and Gram-(-) organisms including *E. coli*. Furthermore, the compounds **27** showed much better activities for the respiratory tract pathogens, penicillin-resistant *S. pneumoniae, M. catarrhalis,* and *H. influenzae* (MIC = <0.006-1.0 μg/mL) than CFDN (MIC = 0.5-4.0 μg/mL). Among the compounds of series **28**, compounds **28a-d** (4-pyrimidinethiol substituents) showed better activity than **28e-g**. However, **28c-e,g,h** exhibited good and balanced activity against respiratory tract pathogens, penicillin-resistant *S. pneumoniae*, *M. catarrhalis* and *H. influenzae* (MIC = 0.016-1.0 μg/mL). Some of the compounds showed good pharmacokinetic value and bioavailability. Especially, the compound **28c** showed an excellent oral absorption (C_{max} ~13.8 mg/mL) and half-life ($t_{1/2}$ = 139 min) in rats (20 mg/kg administration), and was selected for further evaluation.

Andrea and coworkers have exploited the research findings at Lilly in the nineteen seventies that 2, 5-dichlorophenylthioacetamido at C-7 as a lipophilic side chain conferred excellent Gram-(+) activity to the cephem class [13]. Through a series of optimization at C-3 and C-7, they have reported four cephalosporins **39, 40, 43** and **44** possessing a 2, 5-dichlorophenylthioacetamido group at C-7 and a polar thiopyridinium group at C-3 with potent *in vitro* and *in vivo* anti-MRSA activity [14]. The C-3 thiopyridinium ring was substituted with amino acid and pyruvic acid groups that were designed to provide aqueous solubility as required for IV formulation. The syntheses of compounds **107** and **40** are shown in **Scheme 5** while that of compounds **43** and **44** are shown in **Scheme 6**. These



27

Compds	R	X	Ar	Compds.	R	X	Ar
27a	Me	CH	A	27e	Et	N	A
27b	H	CH	A	27f	H	CHCl	A
27c	Propargyl	Ch	A	27g	H	CHCl	B
27d	Et	CH	A	27h	H	CHCl	B

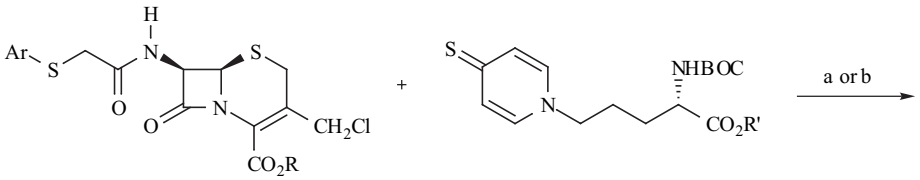


28

Compds	R	X	Ar	Compds.	R	X	Ar
28a	H	H	A	28e	H	Cl	C
28b	Me	H	A	28f	H	H	D
28c	H	Cl	A	28g	H	Cl	E
28d	H	Cl	B	28h	H	Cl	D

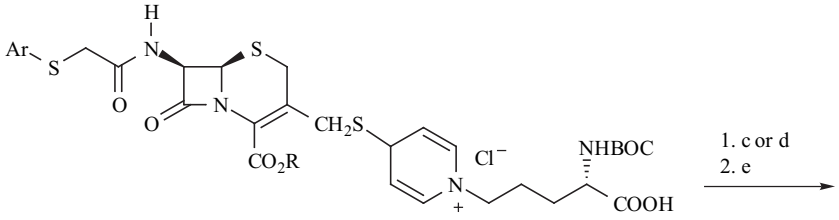
compounds have excellent *in vitro* activity against a variety of Gram-(+) bacteria including resistant strains such as penicillin-resistant *S. pneumoniae*, methicillin-resistant *S. epidermitis* and *S. haemolyticus* (**Table 1**). Furthermore, all of them were efficacious in a systemic murine model of infection with PD_{50}s ranging from 4.8-9.6 mg/kg. The aqueous solubility of **43** and **44** was much more (23 and 40 mg/mL, respectively at pH 7) in comparison to **39** (2-3 mg/mL at pH 7 and at room temperature).

Koh and coworkers have been interested in the design of 3-isoxazolylvinylcephalosporins as potent antibacterial agents [15,16]. Since Microcide Pharmaceutical Inc. reported that the chlorine-substituted aminothiazole moiety of the cephalosporin MC-02479 was responsible for its excellent activity against *S. aureus* [17], Koh and coworkers synthesized and studied the antibacterial activity of 3-isoxazolyl-, 3-isothiazolyl- and 3-thiadazolylvinylcephalo-

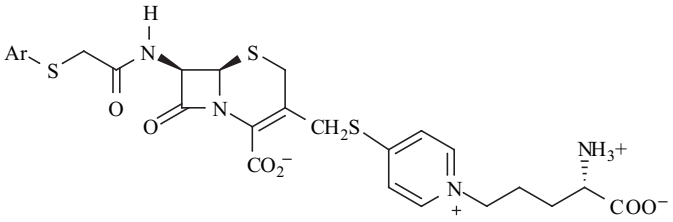


Ar = diClPh **29**: R = DPM, **30**: R = H
Ar = diClpyr **31**: R = DPM, **32**: R = H

33: R1 = Na
34: R1 = H



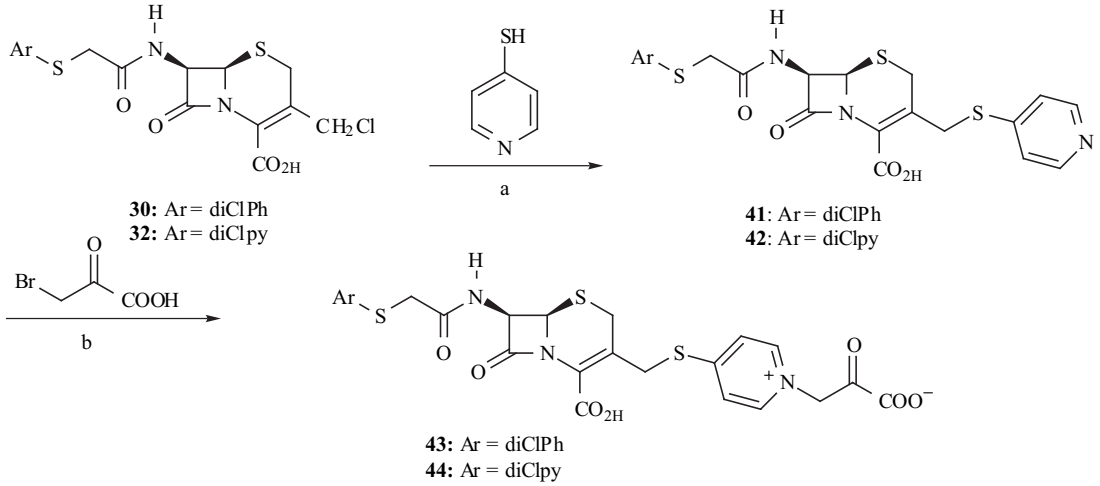
Ar = diClPh **35**: R = DPM, **36**: R = H
Ar = diClpyr **37**: R = DPM, **38**: R = H



39: Ar = diClPh: (double zwitterion)
40: Ar = diClpyr

Reagents and conditions: a. **32**+**33** ⟶ **38**: DMF, 5h, rt; b. **30**+**34** ⟶ **36**: THF, 3h, rt; c. TFA, anisole, CH_2Cl_2 d. HCOOH, HCl; e. NaOAc, MeOH

Scheme 5.



30: Ar = diClPh
32: Ar = diClpy

41: Ar = diClPh
42: Ar = diClpy

43: Ar = diClPh
44: Ar = diClpy

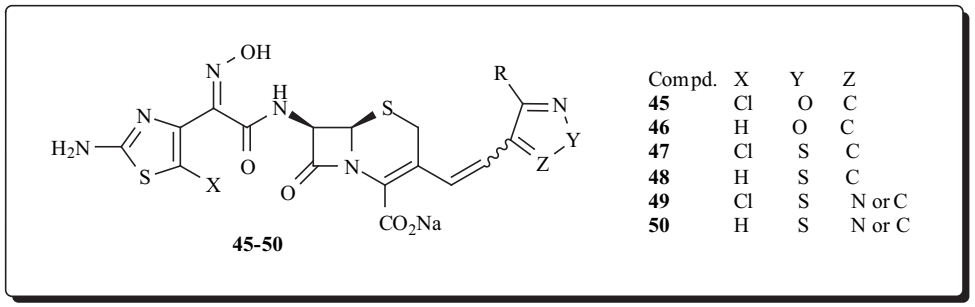
Reagents and conditions: a. 2,6-lutidine, NaI, DMF, 25°C, 40min; b. BSA, THF, 25°C, 2h.

Scheme 6.

sporins **45-50** with 2-amino- and 2-amino-5-chlorothiazole at C-7 [18]. Most of the compounds showed good *in vitro* antibacterial activity against Gram-(+) bacterial strains including MRSA and CRSA. The MIC was in the range of 0.12 to 4.0 μg/mL with a few exceptions. The introduction of a chlorine atom on the 2-aminothiazole ring of the C-7 enhanced the activity slightly against MRSA and considerably against CRSA. The compound **45** with R = 2-thiophenyl was superior to vancomycin in activity against all tested strains. The replacement of the C-3 isoxazole by an isothiazole or thiadiazole resulted in slightly reduced *in vitro* activities.

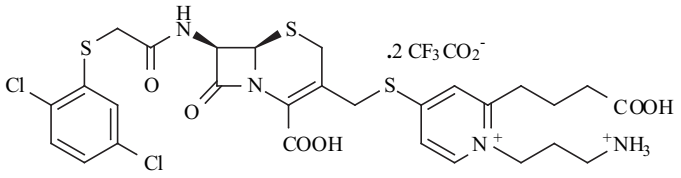
Table 1. MIC in μg/mL, MR = Methicillin-Resistant, P- = Penicillin Negative, M = Methicillin, IM = Imipenem, NT = Not Tested

Organism	A No.	39	40	43	44	M	IM
S. aureus/Hetero MR	A27218	0.5	1	0.5	1	32	1
S. aureus/+50% calf serum	A27218	0.5	1	1	1	8	NT
S. aureus/Hetero MR	A27217	0.5	0.5	0.5	1	64	1
S. aureus/Hetero MR	A25795	1	2	1	1	128	8
S. aureus/Homo MR	A27223	2	2	2	2	128	32
S. aureus/Hetero MR	A27223	4	4	16	2	64	NT
S. aureus/Homo MR	A27621	1	2	2	2	64	16
S. aureus/Homo MR	A27295	2	4	4	2	128	64
S. aureus/Homo MR	A27226	1	2	1	2	64	4
S. aureus/ MR, P-	A27225	1	2	2	2	128	NT
PBP2a IC_{50} (μg/mL)		28	NT	10	4.5	100	NT



Compd.	X	Y	Z
45	Cl	O	C
46	H	O	C
47	Cl	S	C
48	H	S	C
49	Cl	S	N or C
50	H	S	N or C

45-50

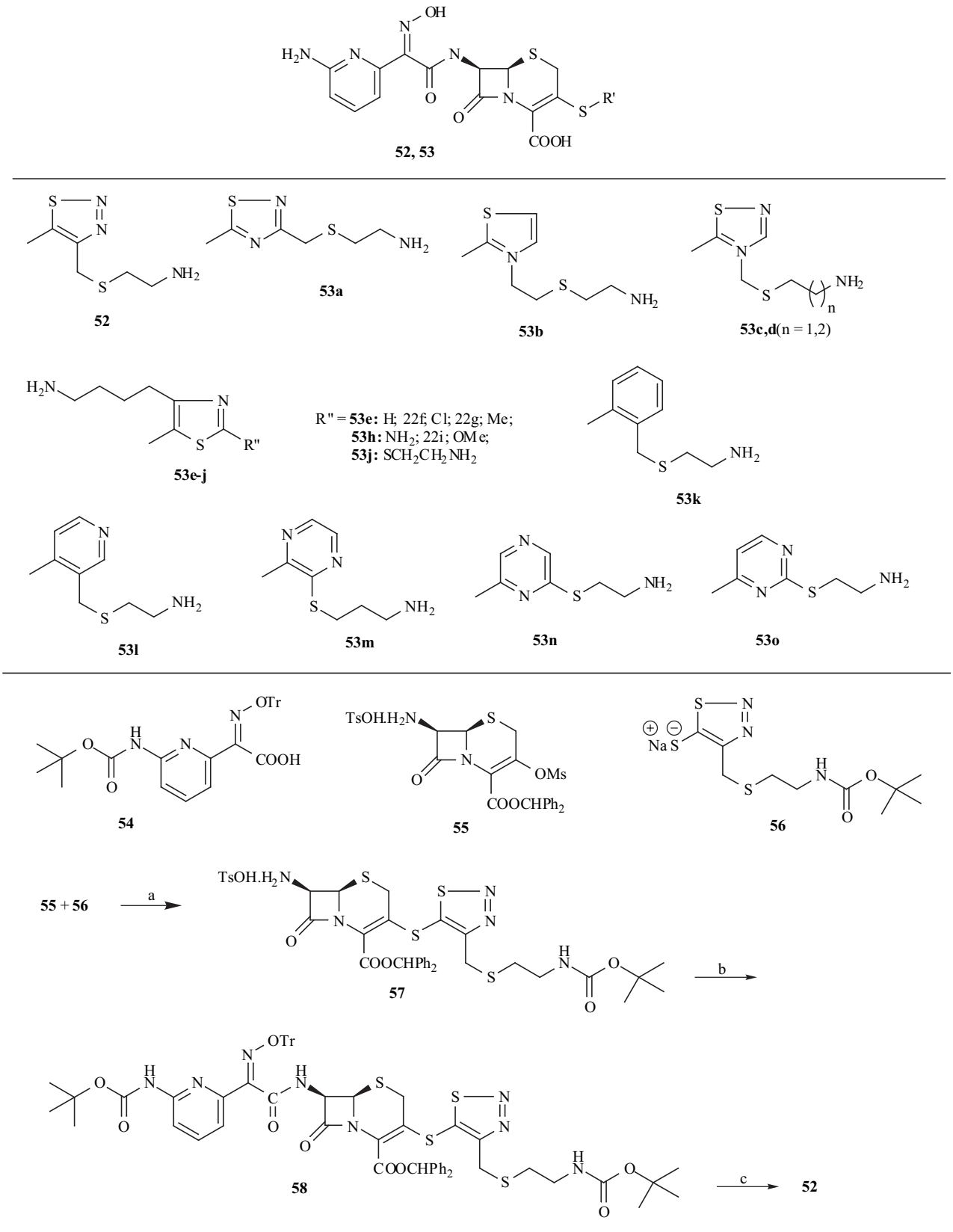


51

Some other variations at C-3 include introduction of thiopyridinium, 3-pyrazolylpyridinium, and nitrogen-linked quarternary ammonium salt [19-21]. In the thiopyridinium series, the most active compound **51** displayed an *in vivo* MIC of 0.5 μg/mL against MRSA (A27223).

Goto and coworkers had reported an aminopyridine ring as viable isosteres of the aminothiazole at the C-7 position of cephalosporins [22]. Based on this report, Cho and coworkers expected that placing aminopyridine at C-7 of cephalosporin would give more solubility and required lipophilicity for anti-MRSA activity to the molecule. Accordingly, they synthesized several cephalosporins with aminopyridine at C-7 **52**,**53** (**Scheme 7**) and studied their anti-MRSA activity using vancomycin and imipenem as reference drugs [23]. Most of the compounds tested displayed good to excellent anti-MRSA activity (MIC = 1-2 μg/mL). The compound **52** displayed an excellent anti-MRSA activity (MIC = 1 μg/mL) as well as dramatically increased solubility (> 20 mg/mL at pH 4.5). Compounds **53h**,**53i**,**53n** and **53o** had MICs in the range of 4-16 μg/mL. The MICs of imipenem and vancomycin were 32 μg/mL and 0.5-1.0 μg/mL, respectively, against these strains. The trend of activity in **53k** (1.0 μg/mL)>**53l** (1.0-2.0 μg/mL)>**53m** (2.0 μg/mL) has been explained on the basis of lipophilicity. The electronic factor has been proposed responsible for a bit more activity of the compound **52** (MIC = 1 μg/mL) with thiadiazole moiety at C-3 in comparison to compound **53a** (MIC = 2-4 μg/mL) with thiazole moiety at C-3.

Kobayashi and coworkers have studied the *in vitro* and *in vivo* activity of 7α-methoxy-cephems and 7α-methoxy-oxacephems **59-62** and their demethoxy congeners **59a**-**62a** *on H. felis* and *H. pylori*, human pathogens associated with type B gastritis, peptic ulcer disease and gastric cancer and showed the significance of 7α-methoxy substituents in dealing with these bacteria [24]. The *in vivo* antibacterial activity was studied on a mouse helibacter infection model after oral administration, in which mice were infected with *H. felis*. All of the compounds except **62** and **62a** exhibited very similar MICs for both *H. felis* (0.25-0.5 mg/L) and *H. pylori* (0.5-1.0 mg/L). Compounds **62** and **62a** had lower MIC for *H. felis* (0.13 mg/L) than for *H. pylori* (1.0-2.0 mg/L). Even though the MICs of all four pairs of compounds were within 1to 2-fold dilution for *H.felis* and *H. pylori*, the 7α-methoxy compounds were at least 4-fold more active at bacterial eradication than their demethoxy counterparts (**Table 2**). Intravenous administration of flomoxef resulted in extremely low eradication activity compared with oral administration. These results, together with the fact that flomoxef is not absorbed orally, indicated

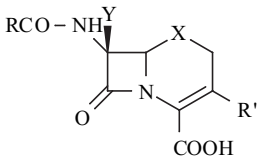


Reagents and conditions: a. EtOAc-aq. $NaHCO_3$; b. **54**, $POCl_3$, Hunig's base. THF; c. TFA, Et_3SiH, CH_2Cl_2.

Scheme 7.

that the compound had direct access to the bacteria in the stomach after oral administration.

Swenson and Tenover have investigated the activity of RWJ-54428, a new parenteral cephalosporin originally



59-62

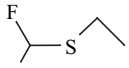
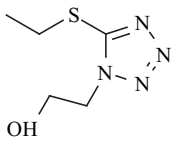
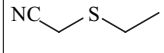
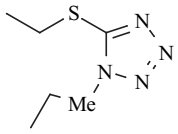
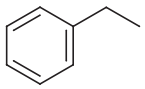
Compounds	X	Y	R	R'
59 Flomoxef	O	OMe		
59a Demethoxy-flomoxef	O	H		
60 1-Thia-flomoxef	O	OMe		
60a Demethoxy-1-thia-flomoxef	S	H		
61 Cefmetazole	S	OMe		
61a Demethoxy-cefmetazole	S	H		
62 M-1	O	OMe		
62a H-1	O	H		

Table 2.

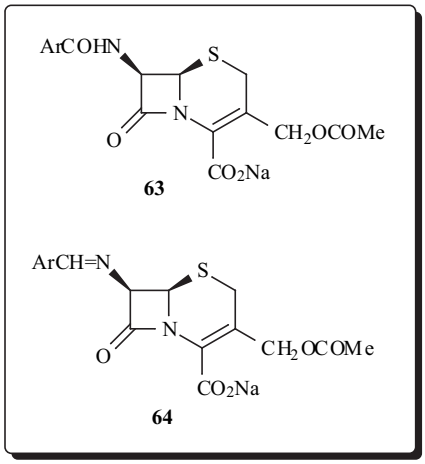
Compounds	50% Clearance dose (mg/kg/dose)[a]	50% Eradication dose (mg/kg/dose)[b]
59 Flomoxef	1.00	3.67
59a Demethoxyflomoxef	4.00	17.4
60 1-thia-flomoxef	0.97	3.38
60a Demethoxy-1-thiaflomoxef	3.84	>60
61 Cefmetazole	1.00	3.67
61a	5.79	58.8
Demethoxycefmetazole	0.47	Not tested
62 M-1	3.59	Not tested
62a H-1	1.92	9.12[c]
Amoxicillin	>15.0	58.8
59 Flomoxef (iv)[c]		

[a]Compounds were administered orally twice a day for 1 day and mice were killed on the following day.

[b]Compounds were administered orally twice a day for 5 days and mice were killed after 14 days.

developed by the R. W. Johnson Pharmaceutical Research Institute, NJ, USA, against recent isolates of Gram-(+) bacteria, including staphylococci with decreased susceptibility to vancomycin [25]. This compound has been shown to be active against a wide range of multiply resistant Gram-(+) pathogens, including oxacillin-resistant *S. aureus* (MRSA), *E. faecalis* (MIC_{90} = 0.5 mg/L), vancomycin-resistant *E. faecalis* (MIC_{90} = 0.25 mg/L), and penicillin-resistant pneumococci and streptococci (MIC_{90} = 1 mg/L). The only group of organisms for which the MIC_{90} was > 2 mg/L was ampicillin-resistant *E. faecium*. Reinert and coworkers have evaluated cefditoren against penicillin-susceptible strains of *S. pneumoniae* and penicillin-intermediate strains of *S. pneumoniae* isolated from patients with respiratory tract infections and suggested it as a promising agent for the treatment of infections caused by pneumococci with reduced penicillin susceptibility [26]. Gerber and coworkers have reported cefepime, considered as fourth generation cephalosporin, having an excellent CSF penetration with level ranging between 10 and 16 mg/L after two intravenous injections (100 mg/kg). The bactericidal activity of cefepime was superior to ceftriaxone and vancomycin in the treatment of rabbits with meningitis caused by an isolate highly resistant to penicillin [27]. Schito and coworkers have studied the activity of many cephalosporins against some common respiratory tract pathogens such as *S. pneumoniae*, *H. influenzae* and *M. catarrhalis,* etc., isolated from the patients in Italy, Spain, and Austria [28]. Cefpodoxime has been reported as a suitable choice for use.

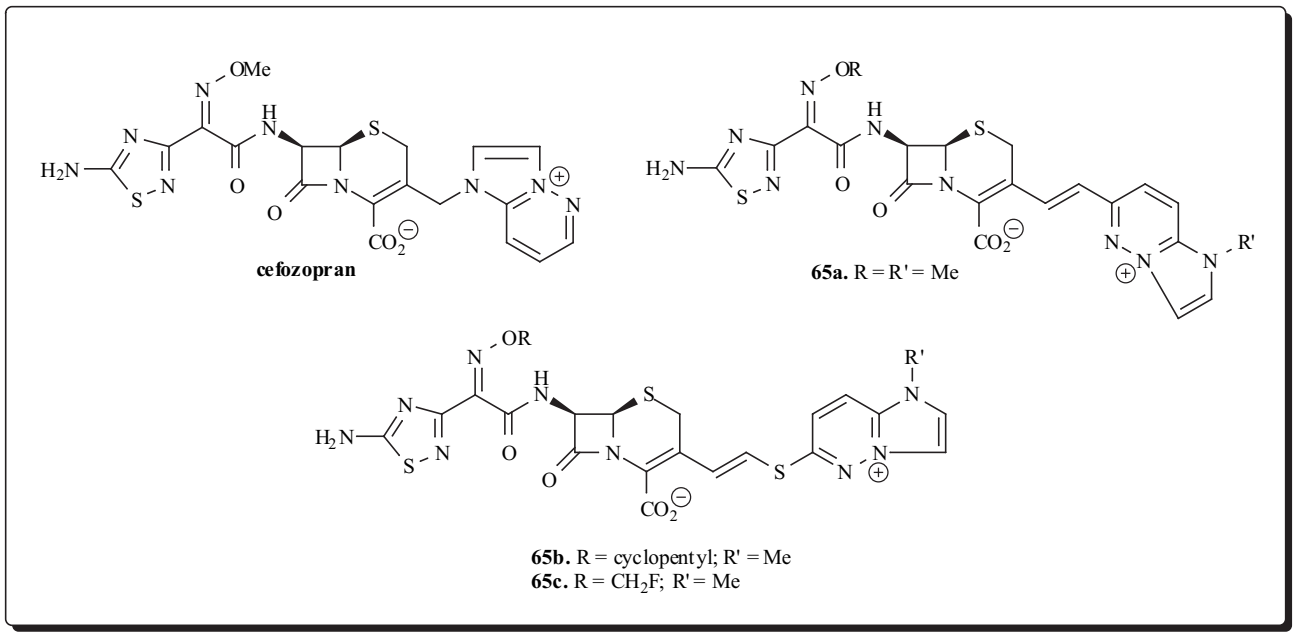
Stoyanova and coworkers have synthesized some amides **63** and imines **64** containing 5-nitrofuryl and 3-methoxy-2-nitrophenyl groups from 7β-aminocephalosporanic acid and 7β-aminodesacetoxycephalosporanic acid and evaluated them for antibacterial activity [29]. Many compounds, especially with 5-nitrofuryl moiety, exhibited an activity equal to or better than those of ampicillin or cephalexin against the majority of Gram-(+) organisms tested. None of the compounds showed appreciable activity against *E. coli*.



63

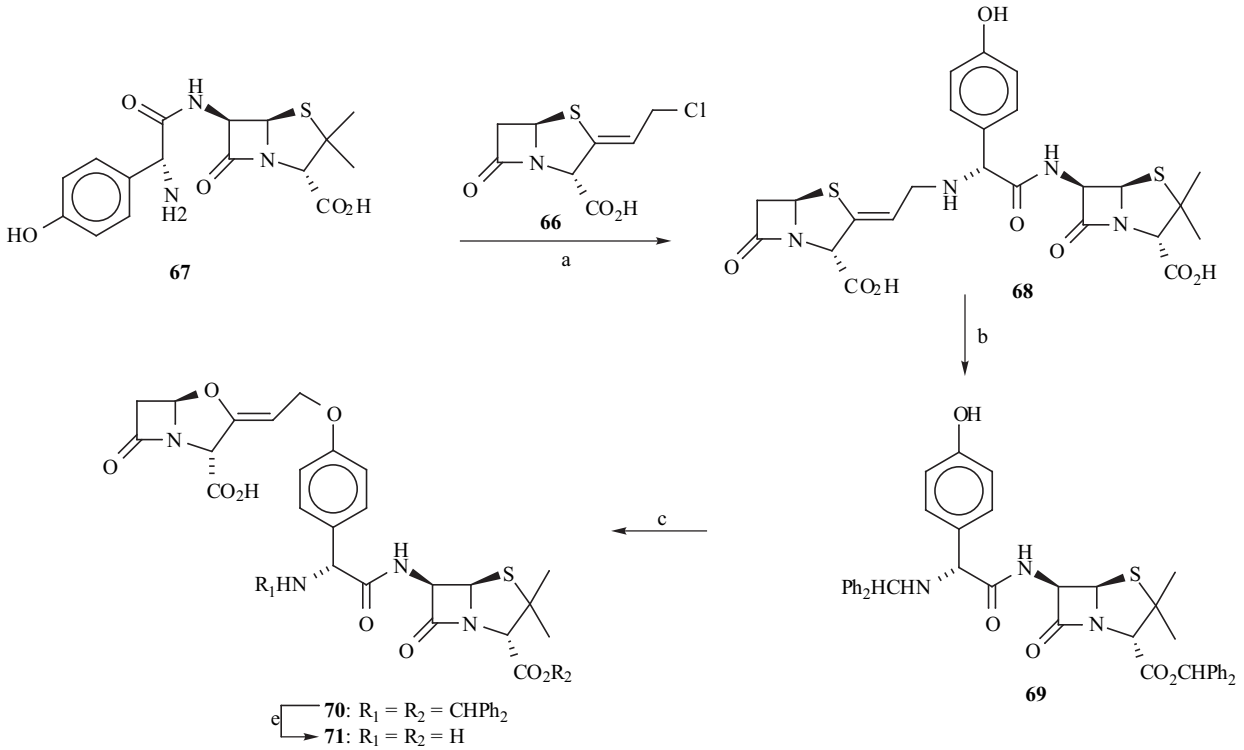
64

Ishikawa and coworkers are involved in the development of new cefozopran (CZOP) derivatives for use against MRSA [30,31]. They observed that the CZOP with lipophilic alkoxyimino groups in the C-7 acyl moiety showed potent antiMRSA activity. Cyclopentyloxyimino derivatives with amino-based substituent(s) in the C-3' azole moiety had anti-MRSA activity comparable to vancomycin. In order to further increase the activity they have modified the C-3 linked spacers of cephem derivatives bearing a 1-methylimidazo[1,2-*b*]pyridazinium-6-yl group at the C-3' position and a 2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(Z)-cyclopentyloxyiminoacetyl group at the C-7 position [32]. They have found that the optimal spacers are (*E*)-2-vinyl and (*E*)-2-thiovinyl groups. The anti-MRSA activity of the compounds **65a,b** bearing these spacers were 16-32 times higher than CZOP. Taking these two spacers they have modified the alkoxyimino group in the C-7 acyl moiety and the 1-alkylimidazo[1,2-*b*]pyridazinium moiety at C-3' and discovered compound **65c** with anti-MRSA activity comparable to vancomycin both *in vitro* and *in vivo*, high affinity (IC_{50} = 2.7 mg/mL) for PBP2' of MRSA and potent activity against Gram- (-) bacteria as well.



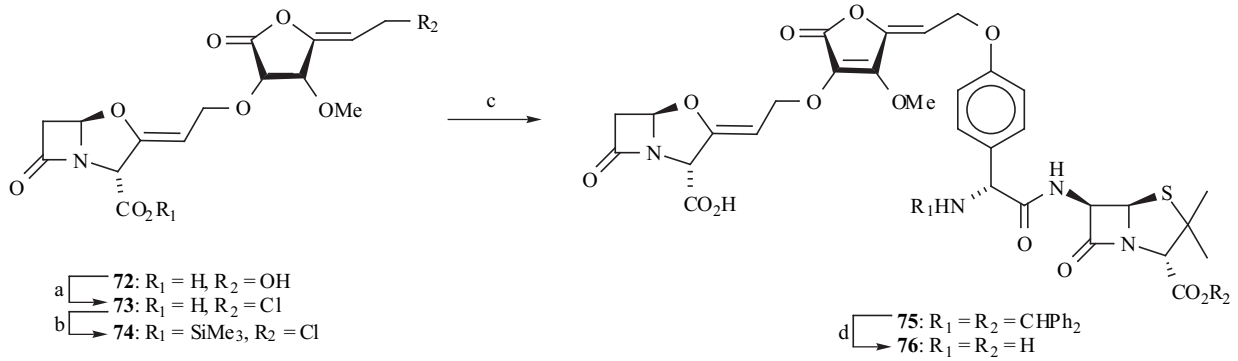
2.2 Cephalosporins prodrugs (Antibacterial and Anticancer Activity)

Hakimelahi and coworkers are working on development of antibacterial and anticancer prodrugs. They have reported the synthesis, antibacterial and β-lactamase inhibitory activity of clavulanate derivatives of amoxicillin **68**, **71** and **76**, as well as cephalosporin-containing amoxicillin **80** (**Scheme 8-10**) [33]. All of these compounds have shown better antibacterial activity (MIC = 0.03-8.53 μg/mL) than amoxicillin and clavulinic acid combination, augmentin (MIC = 0.58-8.74μg/mL) against *S. aureus* A9606, *S. aureus* A15091, *S. aureus* A20309, *S. aureus* 95, *E. coli* A9675, *E. coli* A21223, *E. coli* 27C7, *P. aeruginosa* 18S-H and *K. pneumoniae* A20634 TEM. Furthermore, their lipophilicity and water solubility are much greater in comparison to clavulanic acid or amoxicillin. These newly synthesized compounds also possess β-lactamase inhibitory



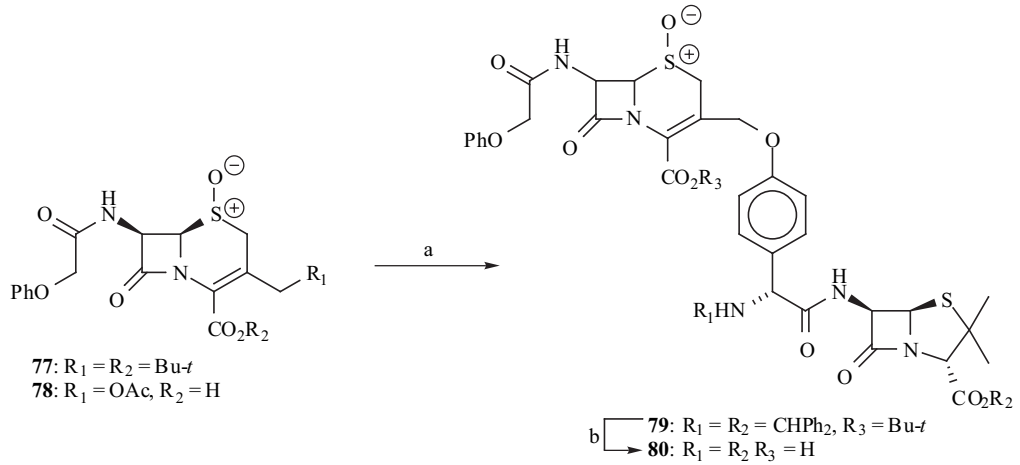
Reagents and conditions: a. (i) Me_3SiCl, Et_3N, 25°C, 1h; ii) trimethylsilyl ester of **66**, Et_3N, 25°C, 6h; b. Ph_2CHCl, Et_3N, MeCN, 25°C, 3h; c. K_2CO_3, trimethylsilyl ester of **66**, MeCN, 25°C, 13h; d. CF_3CO_2H-anisole, CH_2Cl_2, 25°C, 30 min

Scheme 8.



Reagents and conditions: a. $MeSO_2Cl$, pyridine, MeCN, 25°C, 20h; b. Me_3SiCl, Et_3N, MeCN, 25°C, 1h; c. K_2CO_3, **69**, MeCN, 25°C, 15h; d. CF_3CO_2H-amisole, CH_2Cl_2, 25°C, 20 mn.

Scheme 9.

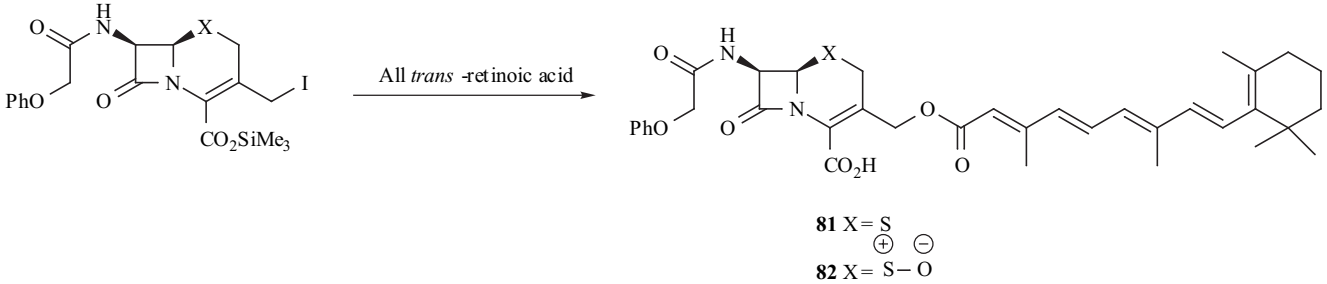


Reagens and conditions: . K_2CO_3, **69**, MeCN, 25°C, 13h, b. CF_3CO_2H-anisole. CH_2Cl_2, 25°C, 1.5h.

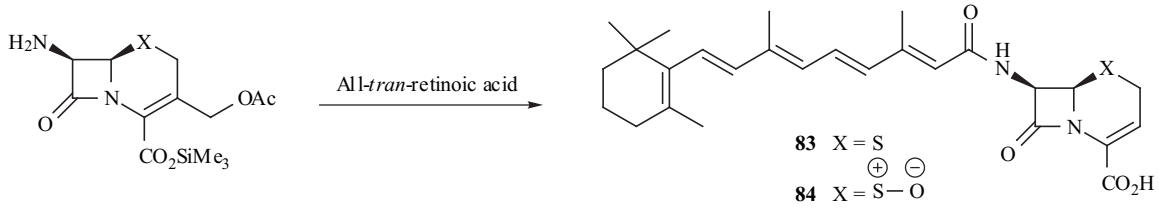
Scheme 10.

activity comparable to clavulanic acid and cephalosporin-1-oxide.

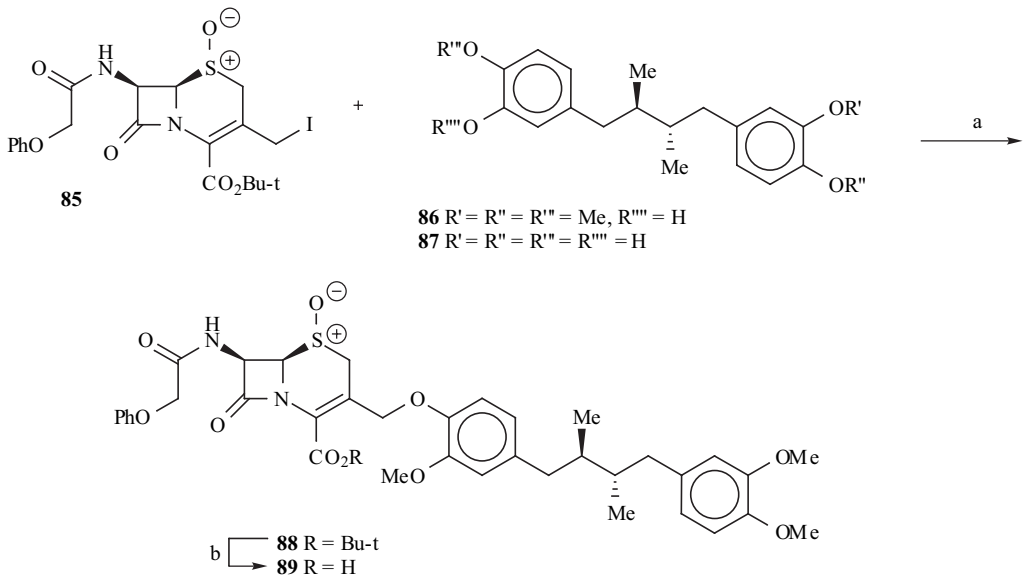
In order to develop new anticancer cephalosporin prodrugs, Hakemelahi and coworkers have synthesized and studied the anticancer activity of four cephalosporin derivatives of retinoic acid, cephalosporin 3'-retinoic esters **81** and **82** (**Scheme 11**), and 7-(retinamido) cephalosporins **83** and **84** (**Scheme 12**) [34]. One cephalosporin derivative of nordihydroguaearetic acid (NDGA) **89** (**Scheme 13**) has



Scheme 11.



Scheme 12.



Reagents and conditions: a. K_2CO_3, DMF, 25°C, 6h; b. CF_3CO_2H-anisole, CH_2Cl_2, 25°C, 2h

Scheme 13.

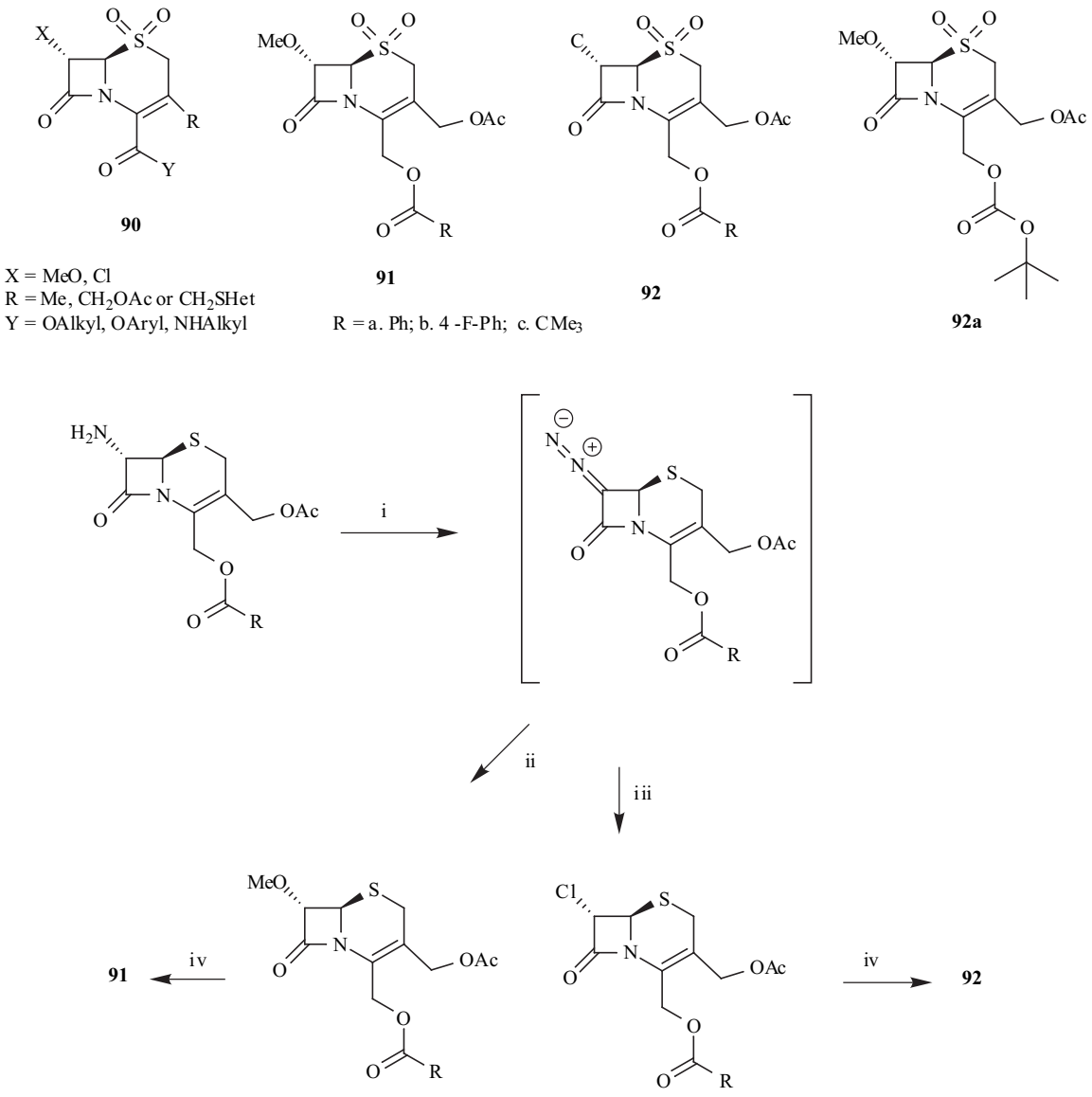
also been studied [35]. Retinoic acid and NDGA were known to possess anticancer activity [36, 37]. Although these new prodrugs have been found to exhibit interesting activity against murine leukemias (L1210 and P388), sarcoma 180, breast carcinoma MCF7 and human T-lymphocytes (Molt4/C8 and CEM/0) they exhibit less inhibition on the examined tumor cell lines in comparison to reference compounds ara-c and all *trans*-β-retinoic acid. However, they are less toxic against human lung cells. Among these compounds, oxacephem-retinoic acid conjugate **82** and NDGA derivative **89** are found to be activated (IC_{50} = 0.01-1.35 μM) by a β-lactamase or the targeting fusion protein, dsFv3-blactamase. Moreover, cephalosporin 3'-retinoic esters **82** exhibit enhanced activity against keratinization with $ED_{50} = 3.91X10^{-11}$M in the presence of a β-lactamase from *S. aureus* 95.

2.3 Enzyme Inhibitor Cephems and Oxacephems

Cephalosporins and synthetic β-lactams are being investigated thoroughly for their enzyme inhibition activity. They are among the most promising β-lactam derivatives studied as human leukocyte elastase (HLE) inhibitors in the past decade [38-40]. Buynak and coworkers are involved in the development of 2- and 3-substituted-7-(alkylidene) cephalosporin sulfones as β-lactamase inhibitors [41,42].

Cephalosporins **90** were the first β-lactam derivatives studied for HLE inhibitory activity. Their molecular modeling studies indicated the importance of the C-2 substituent, which appeared to be involved in the binding process with the S1'-S2' sites of HLE [43]. Balsamo and coworkers have reported the synthesis and *in vitro* HLE and porcine pancreatic elastase (PPE) inhibitory activity of analogues of **90**, **91** and **92** in which the esterified C-2 carboxylic group of **90** is replaced by a hydroxymethyl group esterified by aromatic or aliphatic acyls (**Scheme 14**) [44]. These substituents were observed by them to increase the nucleophilicity of the natural cephalosporin ring which appeared important for the HLE inhibition activity. All the tested cephem esters **91a-c** and **92a,b** were found to possess moderate inhibitory activity against HLE, with k_{inact}/K_I values ranging from 1540 $M^{-1}S^{-1}$ for **90a** to 7600 $M^{-1}S^{-1}$ for **92b** which had the highest activity in the series. The compounds showed a poor activity against PPE, with k_{inact}/K_I values ranging from 240 $M^{-1}S^{-1}$ for **91a** to 1800 $M^{-1}S^{-1}$ for **91b**. The reference drug **92a** had a k_{inact}/K_I value of 25000 $M^{-1}S^{-1}$ against HLE and 131000 $M^{-1}S^{-1}$ against PPE. Thus, even though the substitution of a stronger electron-withdrawing group with a weaker electron-withdrawing group did not hinder the inhibition of HLE considerably, it did so in the case of PPE.

The first β-lactam compound known to be exhibiting Human Chimase Inhibitor (HCI) activity is 1-oxacephem derivative **93** (IC_{50} = 0.25 μM). It was synthesized at Shionogi Research Laboratory, Osaka, Japan [45]. Now Ayoma and coworkers from the same laboratory have reported the structure-activity relationship by structural modifications at the 3'-, 4- and 7β-positions of the 1-oxacepham nucleus of **93** [46]. The structural changes at 7β-position did not lead to any improvement in activity. Next, they made changes at 4-position in order to optimize a substituent for 7β-*p*-hydroxyphenylacetamide. It led to a lowest IC_{50} value of 0.05 μM in case of **94a** and **94b** with $OCH_2C_6H_4$-3-Me and $OCH_2C_6H_4$-3-CF_3 moieties, respectively. Finally, in order to enhance the potency of **94**, they made modifications at C-3'. Two compounds, **95** and **96,** showed roughly the same potency (IC_{50} = 0.07 and 0.08 μM, respectively). Considering the match-mismatch between 3', 4 and 7β-sustituents, they prepared a hybrid compound **97**, which was 40-fold more active (IC_{50} = 0.006 μM) than the lead compound **93**. It was an extremely selective inhibitor, causing weak or no absorption of several other serine proteases. However, its *in vivo* evaluation was limited by its lability in human plasma ($t_{1/2}$ < 10 min). They



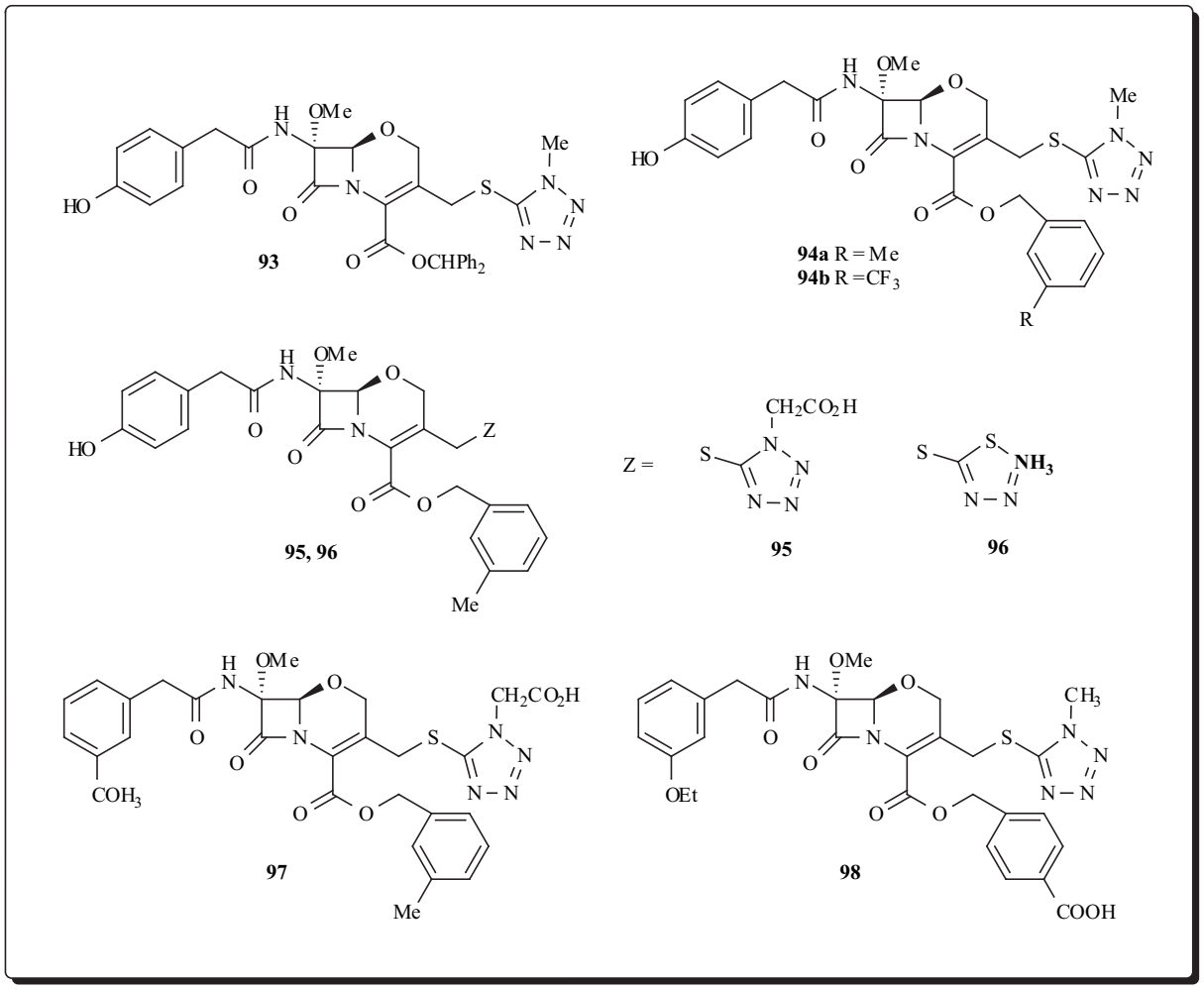
Scheme 14.

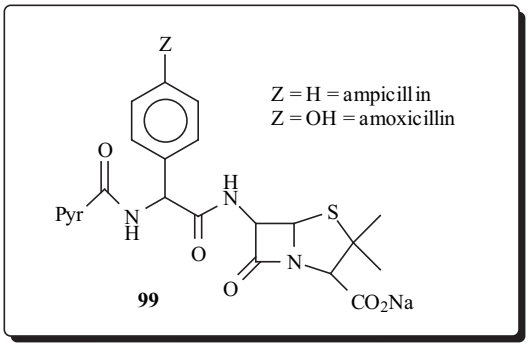
addressed this problem by making several other structural modifications and finally discovered the compound **98** [47]. Even though the compound **98** has 4-fold less inhibition of chymase (IC_{50} = 0.027 μM) than compound **97**, it has very high selectivity for HCE and high stability in human plasma (stability % = 84; $t_{1/2}$ = 1.5 h). As a result of a series of kinetic studies they have proposed the mechanism of action, which shows that the active site serine residue (serine 195) in the enzyme approaches the β-lactam ring of 1-oxacephem, followed by generation of an acyl-enzyme.

3. AMINOPENICILLINS AND OTHER β-LACTAM ANTIBIOTICS

Aminopenicillins are β-lactamase sensitive drugs. Because of their β-lactamase sensitivity, some commercial preparations contain β-lactamase-inhibitors. For example, augmentin, a combination of amoxicillin and clavulanic acid, has been mentioned earlier. Ampicillin is used both orally and parenterally whereas amoxicillin is used mainly orally. These drugs are not very active against *B. fragilis* but effective against other anaerobes. There are only a few recent reports on structural modification of such compounds. Some clinical studies on their activity against various strains are also reported.

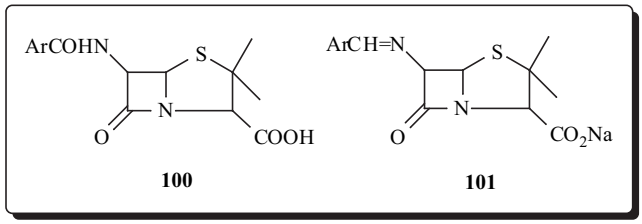
Bijev and Hung have reported the synthesis of twelve new pyrrole carboxylic acid derivatives **99** of ampicillin and amoxicillin [48]. The parent compounds have been *N*-acylated using activated pyrrole carboxylic acid. *In vitro* antibacterial test against standard and clinical Gram-(+) strains showed their MIC in the range of 0.62-16.0 mg/mL which was much higher in comparison of the parent compounds. Preliminary investigations have shown low toxicity as well.





Stoyanova and coworkers have synthesized some amides **100** and imines **101** containing 5-nitrofuryl and 3-methoxy-2-nitrophenyl groups from 6β-aminopenicillanic acid and evaluated them for antibacterial activity [29]. The compounds with 5-nitrofuryl moiety exhibited an activity equal to cephalexin against *B. subtilis* HB_2. It also showed ten times better activity (MIC = 1000.00 μg/mL) against *S. aureus* Cow, *S. aureus* 209 and *S. aureus* ATCC25923 in comparison to ampicillin (MIC = 10000.00 μg/mL). However, none of the compounds showed appreciable activity against *E. coli*.

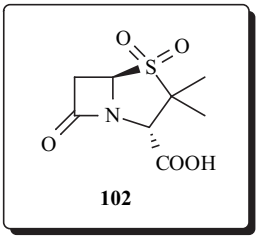
In a clinical study, Brook and Gober have compared the effects of amoxicillin and co-amoxiclav (amoxicillin + clavulanic acid) on the nasopharyngeal flora of children with acute otitis media (AOM) [49]. Co-amoxiclav was able to cure 92% of the patients whereas amoxicillin cured only 64% of the patients.



A new co-amoxiclav single-dose sachet formulation containing 1.0 g of amoxicillin and 0.125 g of clavulanic acid has been evaluated *in vitro*/*ex vivo* against a β-lactamase-producing strain of *H. influenzae* by Bronner and coworkers [50]. The evaluation covered a 12 h period after the drug administration. For all the serum samples, bactericidal activity was fast (3-6 h), marked (3-6 $\log_{10}$ reduction in the initial inoculum) and sustained over 12 h.

Hu and coworkers have reported the synergy of epigallocatechin gallate (EGCg) with a combination of ampicillin and sulbactam (**102**) [51]. The latter is a β-lactamase inhibitor which acts primarily by irreversible inactivation of β-lactamases and thus increases the spectrum of ampicillin. EGCg is the main constituent of tea catechins. The combination of ampicillin and sulbactam in 2:1 ratio showed MICs in the range of 16-32 mg/L against 28 clinical isolates of MRSA. After the addition of EGCg to this

combination, the MIC_{90} was reduced to 4 mg/L. The fractional inhibitory indices were between 0.19 and 0.56 in combination with 6.25 and 25.00 mg/L of EGCg, respectively indicating the effectiveness of ampicillin + sulbactam +EGCg combination in treating MRSA infections.



102

Hernandez and coworkers have compared sulbactam's efficacy with imipenem in an experimental pneumonia model in immunocompetent mice, using a susceptible strain of *Acinetobacter baumanii*, and in an experimental endocarditis model in rabbits, using an intermediary susceptible strain [52]. In the former, sulbactam was almost as efficacious as imipenem in terms of survival, sterility of lungs and in bacterial clearance from the lungs and blood if the t>MIC for sulbactam (1.84 h) was similar to that of imipenem (2.01 h). However, in the endocarditis model it was less efficacious (t>MIC, 1.17 h) than imipenem (t>MIC, 2.12h) in bacterial clearance from vegetations.

4. CONCLUSION

The principal strategy in designing the new cephem drugs is the structural modification at C-3 and C-7 in order to impart appropriate lipophilicity and basicity. In some cases, the stereochemistry of the substituents has been observed to play a role in determining the activity. The structural modifications in cephem have led to the synthesis of some promising cephems. Among them, **70j**, **82**, **87o**, **107-112, 120** and **132c** can be counted as the promising antibacterial drugs for further studies. There is significant enzyme inhibitory activity in some of the compounds, such as **57** and **58**.

I would like to apologize to those scientists whose work may not have appeared either due to limited scope of the review or oversight.

5. ACKNOWLEDGEMENT

I am grateful to Professor Berhanu M. Abegaz, Head, Chemistry Department, University of Botswana, for providing the facilities and to Professor Jamil Ahmad for helpful suggestions. My sincere thanks are due to those scientists who assisted me by sending the reprints of their papers. I shall be indebted to Professor C. A. L. Becker, Chemistry Department, University of Botswana, for reading the manuscript and suggesting changes in its language. I am thankful to my wife, Mrs. Geeta K. Singh, and daughter, Surabhi, for their moral support. The financial assistance from the Faculty of Science Research and Publications Committee, University of Botswana, is gratefully acknowledged.

6. REFERENCES

[1] Bryskier, A. *J. Antibiotics,* **2000**, *53*, 1028.
[2] Fujimoto, K.; Ishihara, S.; Yanagisawa, H.; Ide, J.; Nakayama, E.; Nakao, H.; Sugawara, S. *J. Antibiotics,* **1987**, *40*, 70.
[3] Negi, S.; Yamanaka, M.; Sugiyama, I.; Komatsu, Y.; Sasho, M.; Tsuruoka, A.; Kamada, A.; Tsukada, I.; Himura, R.; Katsu, K.; Machida, Y. *J. Antibiotics,* **1994**, *47*, 1507.
[4] Sadaki, H.; Imazumi, T.; Inaba, T.; Hirakawa, T.; Murotani, Y.; Watanabe, Y.; Minami, S.; Saikawa, I. *Yakugaku Zasshi,* **1986**, *106*, 129.
[5] Sakagami, K.; Atsumi, K.; Tamura, A.; Yoshida, T.; Nishihata, K.; Fukayasu, S. *J. Antibiotics,* **1990**, *43*, 1047.
[6] Yamamoto, H.; Kawabata, K.; Tawara, S.; Takasugi, H.; Tanaka, H. *J. Antibiotics,* **2000**, *53*, 1223.
[7] Yamamoto, H.; Terasawa, T.; Ohki, O.; Shirai, F.; Kawabata, K.; Sakane, K.; Matsumoto, S.; Matsumoto, Y.; Tawara, S. *Bioorg. Med. Chem.,* **2000**, *8*, 43.
[8] Yamamoto, H.; Terasawa, T.; Nakamura, A.; Kawabata, K.; Sakane, K.; Matsumoto, S.; Matsumoto, Y.; Tawara, S. *Bioorg. Med. Chem.,* **2000**, *8*, 1159.
[9] Yamamoto, H.; Terasawa, T.; Nakamura, A.; Kawabata, K.;Takasugi, H.; Tanaka, H.; Matsumoto, S.; Matsumoto, Y.; Tawara, S. *Bioorg. Med. Chem.,* **2001**, *9*, 465.
[10] Yamamoto, H.; Eikyu, Y.; Okuda, S.; Kawabata, K.; Takasugi, H.; Tanaka, H.; Matsumoto, S.; Matsumoto, Y.; Tawara, S. *Bioorg. Med. Chem.,* **2002**, *10*, 1535.
[11] Inamoto, Y.; Chiba, T.; Kamimura, T.; Takaya, T. *J. Antibiotics,* **1988**, *41*, 828.
[12] Lee, C-S.; Ryu, E-J.; Oh, H.; Paek, K-S.; Kim, M. Y.; Youn, H. *Bioorg. Med. Chem. Lett.,* **2000**, *10*, 2123.
[13] Huffman, G. *Patent US 3907784*, Sept. 23, **1975**.
[14] D'Andrea, S-V. *Tetrahedron,* **2000**, *56*, 5687.
[15] Choi, K. I.; Cha, J. H.; Pae, A. N.; Cho, Y. S.; Kim, Y. S.; Chang, M. H.; Koh, H. Y. *J. Antibiotics,* **1998**, *51*, 1117.
[16] Cho, K. I. *Drug Future,* **1999**, *24*, 287.
[17] Glinka, T.; Cho, I.; Zhang, Z.; Price, M.; Case, L.; Crase, J.; Frith, R.; Liu, N.; Ludwikow, M.; Rea, D.; Chamberland, S.; Lee, V.; Hecker, S. Abstract No. F176, 37th *Intersci. Antimicrob. Agents Chemother.,* Toronto, Ontario, Sept. 28, **1997**.
[18] Pae, A. N.; Lee, J. E.; Kim, B. H.; Cha, J. H.; Kim, H. Y.; Cho, Y. S.; Choi, K. I.; Koh, H. Y.; Lee, E.; Kim, J. H. *Tetrahedron,* **2000**, *56*, 5667.
[19] Springer, D. M.; Luh, B-Y.; Bronson, J. J. *Bioorg. Med. Chem. Lett.,* **2001**, *11*, 797.
[20] Chang, K. Y.; Kim, S. H.; Nam, G.; Seo, J. H.; Kim, J. H.; Ha, D-C. *Bioorg. Med. Chem. Lett.,* **2000**, *10*, 1211.
[21] Kim, S. H.; Son, H.; Nam, G.; Chi, D. Y.; Kim, J. H. *Bioorg. Med. Chem. Lett.,* **2000**, *10*, 1143.
[22] Goto, J.; Sakane, K.; Nakai, Y.; Teraji, T.; Kamiya, T. *J. Antibiotics,* **1984**, *37*, 532.
[23] Cho, A.; Glinka, T.; Ludwikow, M.; Fan, A. T.; Wang, M.; Hecker, S. *Bioorg. Med. Chem. Lett.,* **2001**, *11,* 137.
[24] Kobayashi, Y.; Doi, M.; Nagata, H.; Kubota, T.; Kume, M.; Murakami, K. *J. Antimicrob. Chemother.,* **2000**, *45*, 807.
[25] Swenson, J. M.; Tenover, F. C. *J. Antimicrob. Chemother.,* **2002**, *49*, 845.
[26] Reinert, R. R.; Al-Lahham, A.; Lutticken, R. *J. Antimicrob. Chemother.,* **2001**, *48*, 279.
[27] Gerber, C. M.; Cottagnoud, M.; Neftel, K.; Tauber, M. G.; Cottagnoud, P. *J. Antimicrob. Chemother.,* **2000**, *45*, 63.
[28] Schito, G. C.; Georgopoulos, A.; Prieto, J. *J. Antimicrob. Chemother.,* **2002**, *50*, 7.
[29] Stoyanova, R.; Kaloyanov, N.; Traldi, P.; Bliznakov, M. *Arzneimittel-Forschung-Drug Res.,* **2001**, *51*, 991.
[30] Ishikawa, T.; Iizawa, Y.; Okonogi, K.; Miyake, A. *J. Antibiotics,* **2000**, *53*, 1053.
[31] Ishikawa, T.; Kamiyama, T.; Matsumoto, T.; Matsunaga, N.; Tawada, H.; Kawano, Y.; Iizawa, Y.; Okonogi, K.; Miyake, A. *J. Antibiotics,* **2000**, *53*, 1071.
[32] Ishikawa, T.; Kamiyama, T.; Nakayama, Y.; Iizawa, Y.; Okonogi, K.; Miyake, A. *J. Antibiotics,* **2001**, *54*, 257.
[33] Hakimelahi, G. H.; Shia, K. S.; Xue, C.; Hakimelahi, S.; Moosavi-Movahedi, A. A.; Saboury, A.A; Khalafi-Nehzad, A.; Soltani-Rad, M.; Osyetrov, V.; Wang, K-P.; Liao, J-H.; Luo, F-T. *Bioorg. Med. Chem.*, **2002**, *10*, 3489.

[34] Hakimelahi, G. H.; Ly, T.; Yu, S-F.; Zakerinia, M.; Khalafi-Nezhad, A.; Soltani, M. N.; Gorgani, M. N.; Chadegani, A. R.; Moosavi-Movahedi, A. A. *Bioorg. Med. Chem.,* **2001**, *9*, 2139.

[35] Hakimelahi, G. H.; Shia, K. S.; Pasdar, M.; Hakimelahi, S.; Khalafi-Nehzad, A.; Soltani, M. N.; Mei, N-W.; Mei, H-C.; Saboury, A. A.; Rezaei-Tavirani, M.; Moosavi-Movahedi, A. A. *Bioorg. Med. Chem.,* **2002**, *10*, 2927.

[36] Moon, R. C.; Grubbs, C. J.; Sporn, M. B.; Goodman, D. G. *Nature,* **1977**, *267*, 620.

[37] Rillema, J. A. *Leukotr. Med.,* **1984**, *16*, 89.

[38] Finche, P. E.; Shah, S. K.; Ashe, B. M.; Ban, R. G.; Blacklock, T. J.; Bonney, R. J.; Brause, K. A.; Chandler, G. O.; Cotton, M.; Davies, P.; Dellea, P. S.; Dorn, C. P.; Fletcher, D. S.; O'Grady, L. A.; Munford, R. A.; Osinga, D. G.; Sohar, P.; Thompson, K. R.; Weston, H.; Doherty, J. B. *J. Med. Chem.,* **1992**, *35*, 3731.

[39] Tompson, K. R.; Finche, P. E.; Shah, S. K.; Ashe, B. M.; Dahlgren, M. E.; Maycock, A. L.; Doherty, J. B. *Bioorg. Med. Chem. Lett.,* **1993**, *3*, 2283.

[40] Macchia, B.; Gentili, D.; Macchia, M.; Mamone, F.; Martinelli, A.; Orlandini, E.; Rossello, A.; Cercignani, G.; Pierotti, R.; Allegretti, M.; Asti, C.; Caselli, G. *Euro. J. Med. Chem.,* **2000**, *35*, 53.

[41] Buynak, J. D.; Doppalapudi, V. R.; Rao, A. S.; Nidamarthy, S. D.; Adam, G. *Bioorg. Med. Chem. Lett.,* **2000**, *10*, 847.

[42] Buynak, J. D.; Doppalapudi, V. R.; Adam, G. *Bioorg. Med. Chem. Lett.,* **2000**, *10*, 853.

[43] Finche, P. E.; Ashe, B. M.; Knight, W. B.; Maycock, A. L.; Navia, M. A.; Shah, S. K.; Tompson, K. R.; Underwood, D.; Weston, H.; Zimmerman, M.; Doherty, J. B. *J. Med. Chem.,* **1990**, *33*, 2522.

[44] Balsamo, A.; Cercignani, G.; Gentili, D.; Lapucci, A.; Macchia, M.; Orlandini, E.; Rapposelli, S.; Rossello, A. *Euro. J. Med. Chem.,* **2001**, *36*, 185.

[45] Narisada, M.; Yoshida, T.; Onoue, H.; Ohtani, M.; Okada, T.; Tsuji, T.; Kikkawa, I.; Haga, N.; Satoh, H.; Itani, H.; Nagata, W. *J. Med. Chem.,* **1979**, *22*, 757.

[46] Aoyama, Y.; Uenaka, M.; Konoike, T.; Iso, Y.; Nishitani, Y.; Kanda, A.; Naya, N.; Nakajima, M. *Bioorg. Med. Chem. Lett.,* **2000**, *10*, 2397.

[47] Aoyama, Y.; Uenaka, M.; Konoike, T.; Iso, Y.; Nishitani, Y.; Kanda, A.; Naya, N.; Nakajima, M. *Bioorg. Med. Chem. Lett.,* **2000**, *10*, 2403.

[48] Bijev, A. T.; Hung, V. *Arzeim. Forsch Drug Res.,* **2001**, *51*, 667.

[49] Brook, I.; Gober, A. E. *J. Antimicrob. Chemother.,* **2002**, *49*, 689.

[50] Bronner, S.; Pompei, D.; Elkhaili, H.; Dhoyen, N.; Monteil, H.; Jehl, F. *J. Antimicrob. Chemother.,* **2001**, *48*, 501.

[51] Hu. Z.-Q.; Zhao, W.-H.; Hara, Y.; Shimamura, T. *J. Antimicrob. Chemother.,* **2001**, *48*, 361.

[52] Rodriguez-Hernandez, M.-J.; Cuberos, L.; Pichardo, C.; Caballero, F. J.; Moreno, I.; Jimenez-Mejias, M. E.; Garcia-Curiel, A.; Pachon, J. *J. Antimicrob. Chemother.,* **2001**, *47*, 379.