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Synthesis, antibacterial and antifungal activities of some newer cephlosporin derivatives

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ABSTRACT

7-(D-5-Amino-5-carboxy-valeramido)-3-(acetylhydrazido)-8-oxo-5-thio-1-azabicyclo [4,2,0]-oct-2-ene-2-carboxylic acid (1). 7-(D-5-Amino-5-carboxy-valeramido)-3-(substitutedarylacetylhydrazido)-8-oxo-5-thio-1-azabicyclo[4,2,0]-oct-2-ene-2-carboxylic acid.(2-5). 7-(D-5- Amino-5- carboxy-valeramido)- 3- (2'- substituted aryl-3'-chloro-4'-oxo-azetidin-1'-yl)-acetylamino]-8-oxo-5-thio-1-azabicyclo[4,2,0]-oct-2-ene-2-carboxylic acid (6-9). 7-(D-5- Amino-5- carboxy- valeramido)- 3-[(2'-substitutedaryl-4'-oxo-thiazolidin-3'-yl)-acetyl amino]- 8-oxo- 5-thio- 1-azabicyclo [4, 2, 0]- oct-2-ene-2- carboxylic acid.(10-13) have been synthesized in present study. All the developed congeners of cephalosporin acid evaluated for their antibacterial and antifungal activity. Compound 11 was found to possess potent bactericidal activity in comparison to clinically used chemotherapeutic agents viz. ampicillin; norfloxacin and fluconazole.

Key words: Cephlosporin derivatives, Antibacterial and Antimicrobial activities.

INTRODUCTION

Cephlosporin derivatives[1-4] have been reported to exhibit potent antibacterial and antimicrobial activities. However azetidinone[5-8] and thiazolidinone[9-10] congeners of different heterocyclic nuclei possessed the potent bactericidal as well as bacteriostatic activities. Furthermore, substitution of heterocyclic moiety at 3rd position markedly influenced the antibacterial and antimicrobial activities. In view of above observation we have synthesized a new series of cephalosporin derivatives by incorporating different heterocyclic moieties at cephalosporin derivatives with the hope to develop better antibacterial and antimicrobial agents with lesser side effects. The structure of all newly cephlosporin derivatives were confirmed on the basis of spectral and analytical data. All the compounds were tested pharmacologically for their antibacterial and antimicrobial activities.

Chemistry

The reaction sequence leading to the formation of different title compounds is outlined in scheme.The starting compound 1, ie 7-(D-5-Amino-5-carboxy-valeramido)-3-(acetylhydrazido)-8-oxo-5-thio-1-azabicyclo [4,2,0]-oct-2-carboxylic acid was prepared by hydrazine hydrate. Compound 1 with various substituted aromatic aldehydes in presence of glacial acetic acid afforded the corresponding 7-(D-5-Amino-5-carboxy-valeramido)-3-(substituted aryl acetyl hydrazido)-8-oxo-5-thio-1-azabicyclo [4,2,0]-oct-2-ene-2-carboxylic acid 2-5. On the other hand, reaction between compound 2-5 and chloroacetyl chloride in the presence of 2-3 drops of triethylamine to yielded a cyclized product, ie

azetidinone 7-(D-5- Amino-5- carboxy- valeramido)-3- [(2'- substituted aryl-3'- chloro-4'-oxo- azetidin-1'-yl)- acetylamino]- 8-oxo-5-thio-1-aza-bicyclo [4,2,0]-oct-2-ene-2-carboxylic acid 6-9. Cyclocondensation of compound 2-5 with thioglycolic acid in the presence of anhydrous zinc chloride, gave the compounds 7-(D-5- Amino-5-carboxy- valeramido)- 3-[(2'-substituted aryl-4'-oxo-thiazolidin-3'-yl)-acetyl amino]- 8-oxo- 5-thio- 1-azabicyclo [4, 2, 0]- oct-2-ene- 2- carboxylic acid 10-13.

EXPERIMENTAL

General

The melting points of the compounds were determined in open glass capillaries with the help of thermonic melting points apparatus (Campbell Electronics, Mumbai, India) and are uncorrected. The Purity of all the newly synthesized compounds was routinely checked by TLC on silica gel G plates and spots were located by using iodine chamber. IR spectra were recorded on Perkin Elmer 881 spectrophotometer in KBr. ^{1}H-NMR spectra were recorded on Bruker DPX-300MHz spectrometer and mass spectra were determined on JEOL-JMS-D-300 spectrometer. 3

Step-I: Synthesis of 7-(D-5-Amino-5-carboxy-valeramido)-3-(acetylhydrazido)-8-oxo-5-thio-1-azabicyclo [4,2,0]-oct-2-carboxylic acid (1)

A methanolic solution of 7-(D- 5- Amino-5- carboxy- valeramido)- 3-(acetylhydrazido)- 8- oxo-5- thio- 1- azabicyclo [4, 2, 0]- oct- 2- ene- 2-carboxylic acid (.001 mole) was refluxed with hydrazine hydrate (.001 mole) for 2 hrs. The completion of the reaction was checked by TLC. Excess of methanol was distilled off and reaction mixture was cooled, poured into ice water, filtered, washed with water, dried and recrystallized from methanol to afford.

Compound 1

m.p. 156^{o}C, Yield: 74%, (r.s): methanol, (m.f.): $C_{15}H_{21}N_5O_7S$. Elemental analysis: Calcd. C 43.37, H 5.06, N 16.86%, Found: C 43.68, H 5.20, N 16.90%. IR (KBr) (cm^{-1}): 680 (C-S-C), 1425 (C-N), 1600 (C=C), 1700 (COOH), 1720 (side chain CONH), 1750 (CO of ^{2}-lactam), 3350 (NH_2).^{1}H-NMR ($CDCl_3$ + DMSO-d_6) ´ (ppm): 1.218-1.264 (m, 4H$_{c,d,}$ 2x CH_2), 1.280-1.368 (m, 1H$_e$, CH), 3.540 (s, 2H-4, CH_2-S), 3.600-3.620 (t, 2H$_b$, CH_2), 3.880-3.910 (t, 2H$_a$, CH_2-C), 5.01 (bs, 2H$_f$, NH_2), 5.39 (bs, 2H$_c$, NH_2-NH), 5.40-5.43 (d, 1H, H-6 of lactam), 5.82-5.89 (q, 1H, H-7 of lactam) 6.56-6.65 (d, 1H$_a$, CONH), 8.46 (s, 1H$_b$, CONH), 9.56 (s, 1H$_g$, COOH), 11.94 (s, 1H, COOH-C_2), MS: $[M]^+$ at m/z 415.

Step-II: Synthesis of 7-(D-5-Amino-5-carboxy-valeramido)-3-(substituted aryl acetyl hydrazido)-8-oxo-5-thio-1-azabicyclo [4,2,0]-oct-2-ene-2-carboxylic acid (2-5)

Equimolar DMF solution of of compound 1 (.001 mole), substituted aromatic aldehydes (.001 mole) in presence of few drops of glacial acetic acid was allowed to reflux for 6-8 hrs. The completion of the reaction was checked by TLC. Excess of DMF was distilled under vaccuo. The reaction mixture was diluted with ice water, filtered, washed with water, dried and recrystallized from appropriate solvents.

Compound 2

m.p. 160^{o}C, Yield: 80%, (r.s): ethanol, (m.f): $C_{22}H_{25}N_5O_7S$. Elemental analysis: Calcd. C 52.48, H 4.97, N 13.91%, Found: C 52.10, H 5.16, N 13.70%. IR (KBr) (cm^{-1}): 681 (C-S-C), 1424 (C-N), 1698 (COOH), 1718 (COOH), 3352 (NH_2).^{1}H-NMR ($CDCl_3$ + DMSO-d_6) ´ (ppm): 1.218 (m, 4H$_{c,d,}$ 2x CH_2), 1.28 (m, 1H$_e$, CH), 3.6 (t, 2H$_b$, CH_2), 3.5 (s, 2H-4, CH_2-S), 3.8 (t, 2H$_a$, CH_2-C), 5.01 (bs, 2H$_f$, NH_2),), 5.40 (d, 1H, H-6 of lactam ring), 5.8 (q, 1H, H-7 of lactam ring), 6.58 (bs, 1H$_a$, CONH), 6.8 (m, 5H, ArH), 8.2 (1H, N=CH-Ar), 8.4 (s, 1H$_b$, CONH), 9.5 (s, 1H$_g$, COOH), 11.9 (s, 1H, COOH-C_2),MS: $[M]^+$ at m/z 503

Compound 3

7-(D-5-Amino-5-carboxy-valeramido)-3-[(o-hydroxy) phenyl]-acetyl hydrazido- 8- oxo- 5- thio-1- azabicyclo [4, 2, 0]- oct- 2- ene- carboxylic acid. m.p.146^{o}C, Yield: 68%, (r.s): Methanol, (m.f): $C_{22}H_{25}N_5O_8S$. Elemental analysis: Calcd. C 50.86, H 4.81, N 13.48%, Found: C 43.68, H 5.20, N 16.90%. IR (KBr) (cm^{-1}): 683 (C-S-C), 1426 (C-N), 1600 (C=C), 1722 (side chain CONH), 1750 (C=O of ^{2}-lactam ring), 3290 (OH), 3349 (NH_2). ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) ´ (ppm): 1.218 (m, 4H$_{c,d,}$ 2x CH_2), 1.28 (m, 1H$_e$, CH), 3.5 (s, 2H-4, CH_2-S), 3.6 (t, 2H$_b$, CH_2), 3.8 (t, 2H$_a$, CH_2-C), 5.01 (bs, 2H$_f$, NH_2), 5.40 (d, 1H, H-6 of lactam ring), 5.8 (q, 1H, H-7 of lactam ring), 6.52 (d, 1H$_a$, CONH), 6.58 (bs, 1H$_a$, CONH),

), 6.8 (m, 4H, ArH), 8.2 (s, 1H, N=CH-Ar), 8.4 (s, $1H_b$, CONH), 9.5 (s, $1H_g$, COOH), 11.9 (s, 1H, COOH-C_2), 12.5 (ss, 1H, OH-Ar), MS: $[M]^+$ at m/z 519.

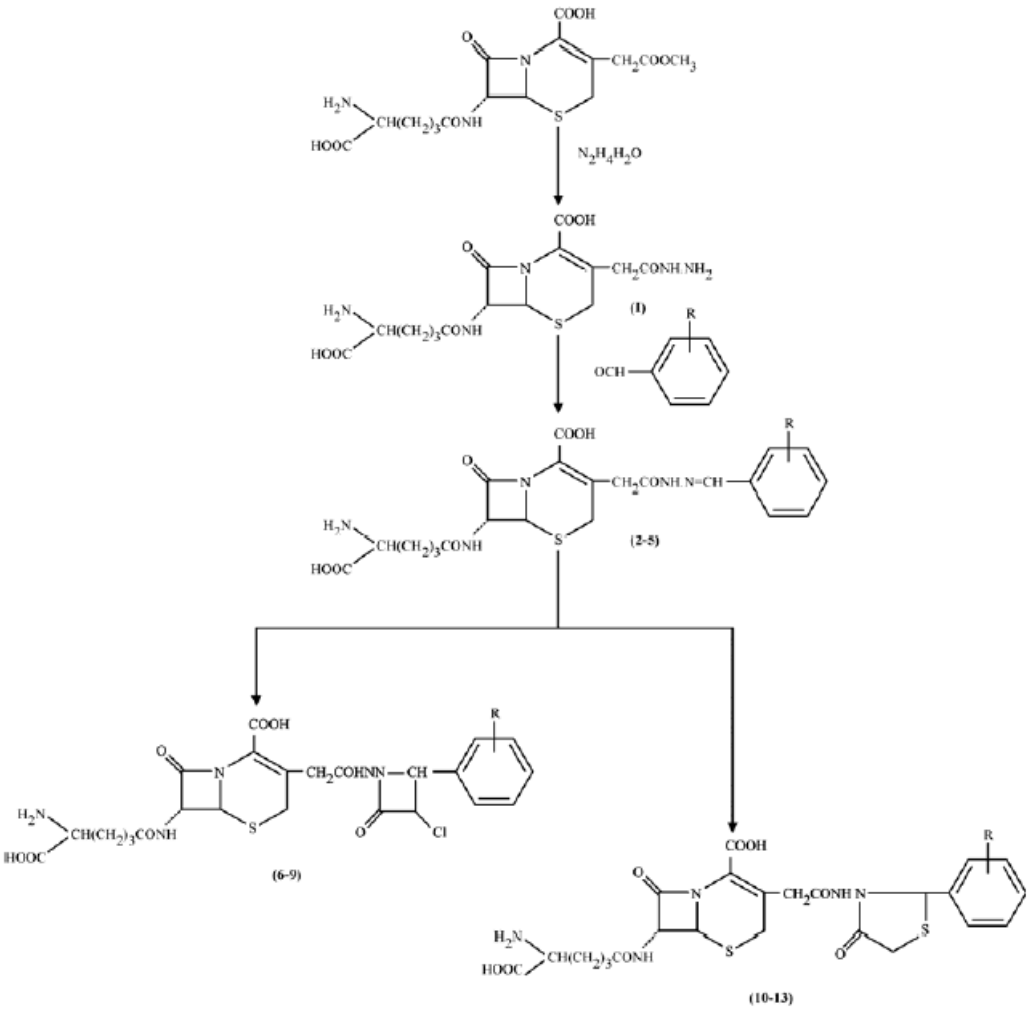
Compound-4

7-(D-5-Amino-5-carboxy- valeramido)-3-[(p-methoxy) phenyl]-acetyl hydrazido- 8- oxo- 5-thio- 1- azabicyclo [4, 2, 0]- oct- 2- ene- carboxylic acid.

m.p.162°C, Yield: 72%, (r.s): ethanol, (m.f): $C_{23}H_{27}N_5O_8S$. Elemental analysis: Calcd. C 51.78, H 5.06, N 13.13%, Found: C 51.98, H 5.13, N 12.24%. IR (KBr) (cm^{-1}): 684 (C-S-C), 1186 (C-O-C), 1422 (C-N), 1700 (C=C), 1719 (side chain CONH), 1747 (C=O of ²-lactam ring), 3290 (OH), 3350 (NH_2).¹H-NMR ($CDCl_3$ + DMSO-d_6) ´ (ppm): 1.218 (m, $4H_{c,d,}$ 2x CH_2), 1.28 (m, $1H_e$, CH), 3.4 (s, 3H, Ar-OCH_3), 3.5 (s, 2H-4, CH_2-S), 3.6 (t, $2H_b$, CH_2), 3.8 (t, $2H_a$, CH_2-C=O), 5.01 (bs, $2H_f$, NH_2), 5.40 (d, 1H, H-6 of lactam ring), 5.8 (q, 1H, H-7 of lactam ring), 6.52 (d, $1H_a$, CONH), 6.8 (m, 4H, ArH), 8.2 (s, 1H, N=CH-Ar), 8.4 (s, $1H_b$, CONH), 9.5 (s, $1H_g$, COOH), 11.9 (s, 1H, COOH-C_2], MS: $[M]^+$ at m/z 533.

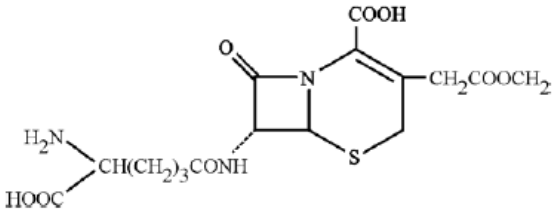
Compound-5

7-(D-5-Amino-5-carboxy- valeramido)-3- (p-hydroxy, m-methoxy) acetyl hydrazido- 8- oxo- 5- thio-1- azabicyclo [4, 2, 0]- oct- 2- ene- carboxylic acid.



Scheme 1.

Table 1(a): *Antibacterial* and *antifungal* activity of the compounds: 7-(D-5-Amino-5-carboxy-valeramido)-3-acetyl hydrazide-8-oxo-5-thio-1-azabicyclo [4,2,0]-oct-2-ene-2-carboxylic acid (1), 7-(D-5-Amino-5-carboxy-valeramido)-3-(substituted aryl acetyl hydrazide)-8-oxo-5-thio-1-azabicyclo [4,2,0]- oct-2-ene-2-carboxylic acid (2-5)



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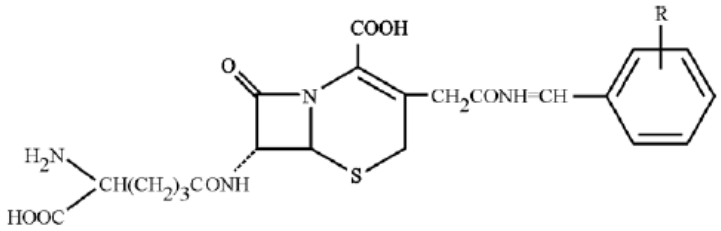


(2-5)

Comp. No.	R	Bacterial growth inhibition (diameter)				Fungal growth inhibition (diameter)			
		S.aureus	*E. coli*	P. vulgaris	*K. pneumoniae*	A. fumigatus	C.albicans	C. albicans *ATCC*	C. Krusei *G03*
1	-	6 mm	*6 mm*	-	5 mm	5 mm	6 mm	-	-
2	H	-	-	-	-	8 mm	9 mm	7 mm	-
3	o-OH	6 mm	*9 mm*	-	-	9 mm	6 mm	9 mm	6 mm
4	p-OCH_3	9 mm	*8 mm*	-	-	7 mm	5 mm	8 mm	7 mm
5	m- OCH_3 & p-OH	5 mm	*6 mm*	7 mm	-	-	6 mm	6 mm	5 mm

m.p.170°C, Yield:70%, (r.s): ethanol, (m.f): $C_{23}H_{27}N_5O_9S$. Elemental analysis: Calcd. C 50.27, H 4.91, N 12.75%, Found: C 50.51, H 5.00, N 12.64%. IR (KBr) (cm^{-1}): 686 (C-S-C), 1186 (C-O-C), 3348 (NH_2), 1425 (C-N), 1600 (C=C), 1697 (COOH), 1722 (side chain CONH), 1750 (C=O of ²-lactam ring), 3290 (OH), 3350 (NH_2). ^{1}H-NMR ($CDCl_3$ + 5

DMSO-d_6) ´ (ppm): 1.218 (m, 4$H_{c,d,}$ 2x CH_2), 1.28 (m, 1H_e, CH), 3.4 (s, 3H, Ar-OCH_3), 3.5 (s, 2H-4, CH_2-S), 3.6 (t, 2H_b, CH_2), 3.8 (t, 2H_a, CH_2-COO), 5.01 (bs, 2H_f, NH_2), 5.40 (d, 1H, H-6 of lactam ring), 5.8 (q, 1H, H-7 of lactam ring), 6.52 (d, 1H_a, CONH), 6.8 (m, 3H, ArH), 8.2 (s, 1H, N=CH-Ar), 8.4 (s, 1H_b, CONH), 9.5 (s, 1H_g, COOH), 11.9 (s, 1H, COOH-C_2], 12.4 (ss, 1H, HO-Ar), MS: $[M]^+$ at m/z 549.

Step-III: 7-(D-5- Amino-5- carboxy- valeramido)- 3- [(2'- substituted aryl-3'- chloro-4'-oxo- azetidin-1'-yl)- acetylamino]- 8-oxo-5-thio-1-aza-bicyclo [4,2,0]-oct-2-ene-2-carboxylic acid (6-9)

A solution of compounds 2-5 (.001 mole) in methanol (50 ml) was refluxed with chloro acetyl chloride (.002 mole) in presence of triethyl amine for 8-12 hrs. The completion of the reaction was checked by TLC. The excess of methanol was distilled off, poured into ice water, filtered, washed with water, dried and recrystallized with appropirate solvents to obtain compounds 6-9.

Compound-6: 7-(D-5- Amino-5- carboxy- valeramido)- 3- [(2'- substituted aryl-3'- chloro-4'-oxo- azetidin-1'-yl)- acetylamino]- 8-oxo-5-thio-1-aza-bicyclo [4,2,0]-oct-2-ene-2-carboxylic acid

m.p. 174°C, Yield: 67%, (r.s): DMF-water, (m.f): $C_{24}H_{26}N_5O_8SC1$. Elemental analysis: Calcd. C 49.74, H 4.49, N 12.08%, Found: C 49.48, H 4.60, N 12.20%. IR (KBr) (cm^{-1}): 686 (C-S-C), 690 (C-C1), 1191 (C-O-C), 1425 (C-N), 1600 (C=C), 1698 (COOH), 1718 (side chain CONH), 1750 (C=O of ²-lactam ring), 3350 (NH_2). ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) ´ (ppm): 1.205-1.260 (m, 4$H_{c,d,}$ 2x CH_2), 1.280—1.368 (m, 1H_e, C), 3.504 (s, 2H-4, CH_2-S), 3.600-3.631 (t, 2H_b, CH_2), 3.869-3.900 (d, 2H_a, CH_2), 4.2 (d, 1H, CH-C1), 5.01 (bs, 2H_f, NH_2), 5.40 (d, 1H, H-6 of lactam ring), 5.8 (q, 1H, H-7 of lactam ring), 6.2 (d, 1H, N-CH-Ar), 6.52 (d, 1H_a, CONH), 6.8 (m, 5H, ArH), 8.4 (s, 1H_b, CONH), 9.5 (s, 1H_g, COOH), 11.9 (s, 1H, COOH-C_2), MS: $[M]^+$ at m/z 579.

Compound-7

7-(D-5- Amino-5- carboxy- valeramido)- 3-[{(2'- o-hydroxy) phenyl-3'-chloro-4'-oxo- azetidin-1'-yl}- acetylamino]- 8-oxo-5-thio-1-aza-bicyclo [4,2,0]-oct-2-ene-2-carboxylic acid.

m.p.162°C, Yield: 70%, (r.s): ethanol, (m.f): $C_{24}H_{26}N_5O_8SC1$. Elemental analysis: Calcd. C 48.40, H 4.36, N 11.76%, Found: C 48.65, H 4.40, N 11.61%. IR (KBr) (cm^{-1}): 625 (C-C1), 686 (C-S-C), 690 (C-S-C), 1425 (C-N), 1600 (C=C), 1698 (COOH), 1718 (side chain amide), 1750 (C=O of ²-lactam ring), 3350 (NH_2). ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) ´ (ppm): 1.205 (m, 4$H_{c,d,}$ 2x CH_2), 1.28 (m, 1H_e, CH), 3.5 (s, 2H-4, CH_2-S), 3.6 (t, 2H_b, CH_2), 3.8 (d, 2H_a, CH_2), 4.2 (d, 1H, CH-C1), 5.01 (bs, 2H_f, NH_2), 5.40 (d, 1H, H-6 of ²-lactam ring), 5.8 (q, 1H, H-7 of ²-lactam ring), 6.2 (d, 1H, N-CH-Ar), 6.52 (d, 1H_a, CONH), 6.8 (m, 4H, ArH), 8.4 (s, 1H_b, CONH), 9.5 (s, 1H_g, COOH), 11.9 (s, 1H, COOH-C_2], 12.4 (ss, 1H, ArOH). MS: $[M]^+$ at m/z 595.

Compound-8

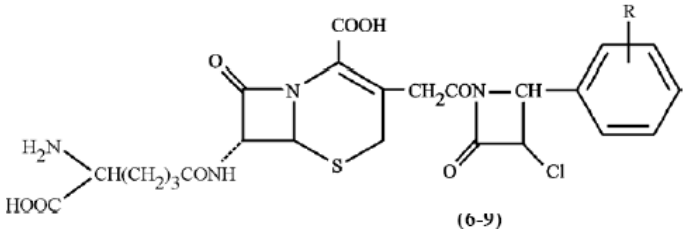
7-(D-5- Amino-5- carboxy- valeramido)- 3-[{(2'- p-methoxy) phenyl-3'-chloro-4'-oxo- azetidin-1'-yl}- acetylamino]- 8-oxo-5-thio-1-azabicyclo [4,2,0]-oct-2-ene-carboxylic acid.

m.p.179°C, Yield: 64%, (r.s): methanol, (m.f): $C_{25}H_{28}N_5O_9SC1$. Elemental analysis: Calcd. C 49.26, H 4.59, N 11.49%, Found: C 49.59, H 4.68, N 11.29%. IR (KBr) (cm^{-1}): 624 (C-C1), 686 (C-S-C), 1190 (C-O-C), 1423 (C-N), 1597 (C=C), 1700 (COOH), 1720 (side chain CONH), 1749 (C=O of ²-lactam ring), 3348 (NH_2). ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) ´ (ppm): 1.210-1.244 (m, 4$H_{c,d,}$ 2x CH_2), 1.287-1.369 (m, 1H_e, CH), 3.410 (s, 3H, Ar-OCH_3), 3.470 (s, 2H-4, CH_2-S), 3.600-3.642 (t, 2H_b, CH_2), 3.865-3.900 (d, 2H_a, CH_2), 4.241-4.253 (d, 1H, CH-C1), 4.986 (bs, 2H_f, NH_2), 5.412-5.440 (d, 1H, H-6 of ²-lactam ring), 5.889-5.856 (q, 1H, H-7 of ²-lactam ring), 6.230-6.253 (d, 1H, N-CH-Ar), 6.540 (bs, 1H_a, CONH), 6.800-7.110 (m, 3H, ArH), 8.450 (s, 1H_b, CONH), 9.581 (s, 1H_g, COOH), 11.978 (s, 1H, COOH-C_2). MS: $[M]^+$ at m/z 609.

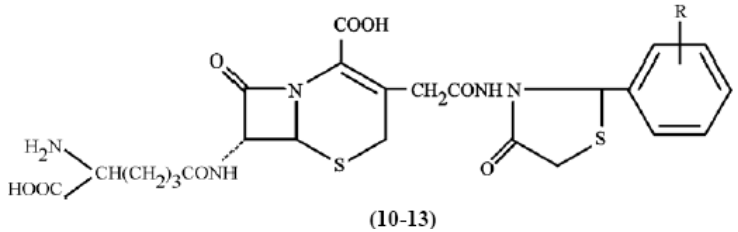
Compound-9

7-(D-5- Amino-5- carboxy- valeramido)- 3-[{(2'- p-hydroxy, m-methoxy) phenyl-3'-chloro-4'-oxo- azetidin-1'-yl}- acetylamino]- 8-oxo-5-thio-1-

Table 1(b): *Antibacterial* and *antifungal* activity of the compounds: 7-(D-5-Amino-5-carboxy-valeramido)-3-[(2'-substituted aryl-3'-chloro-4'-oxo-azetidin-1'-yl)-acety1 amino]-8-oxo-5-thio-1-azabicyclo [4,2,0]-oct-2-ene-2-carboxylic acid (6-9), 7-(D-5-Amino-5-carboxy-valeramido)-3-[(2'-substituted aryl-4'-oxo-thiazolidin-3'-yl)-amino acetyl]-8-oxo-5-thio-1-azabicyclo [4,2,0]-oct-2-ene-2-carboxylic acid (10-13)



(6-9)



(10-13)

Comp. No.	R	Bacterial growth inhibition (diameter)				Fungal growth inhibition (diameter)			
		S.aureus	*E. coli*	*P. vulgaris*	*K. pneumoniae*	*A. fumigatus*	*C.albicans*	*C. albicans ATCC*	*Krusei G03*
6	H	-	*8 mm*	-	9 mm	8 mm	-	6 mm	-
7	o-OH	12 mm	*18 mm*	-	-	-	-	-	-
8	p-OCH_3	14 mm	*22 mm*	10 mm	14 mm	10 mm	10 mm	10 mm	-
9	m- OCH_3 & p-OH	10 mm	-	-	10 mm	-	10 mm	9 mm	-
10	H	-	*8 mm*	-	-	-	-	-	-
11	o-OH	16 mm	*26 mm*	7 mm	-	10 mm	12 mm	10 mm	8 mm
12	p-OCH_3	17 mm	*26 mm*	-	-	-	10 mm	9 mm	12 mm
13	m- OCH_3 & p-OH	16 mm	-	12 mm	16 mm	-	-	-	-
Ampicillin	20 mm	*18 mm*	18 mm	14 mm	-	-	-	-	
Norfloxacin	16 mm	*17 mm*	13 mm	16 mm	-	-	-	-	
Fluconazole	-	-	-	-	-	29 mm	25 mm	19 mm	

* 250 ¼g/ml. – Drug concentration

azabicyclo [4,2,0]-oct-2-ene-carboxylic acid.

m.p.190°C, Yield: 65%, (r.s): methanol, (m.f): $C_{25}H_{28}N_5O_{10}SC1$. Elemental analysis: Calcd. C 48.00, H 4.48, N 11.20%, Found: C 48.29, H 4.40, N 11.59%. IR (KBr) (cm^{-1}): 622 (C-C1), 688 (C-S-C), 1187 (C-O-C), 1421 (C-N), 1599 (C=C), 1698 (C=OH), 1718 (side chain CONH), 1747 (C=O of ²-lactam ring), 3287 (OH), 3347 (NH_2). ¹H-NMR ($CDCl_3$ + DMSO-d_6) ´ (ppm): 1.210-1.242 (m, $4H_{c,d,}$ 2x CH_2), 1.289-1.365 (m, $1H_e$, CH), 3.400 (s, 3H, Ar-OCH_3), 3.462 (s, 2H-4, CH_2-S), 3.602-3.634 (t, $2H_b$, CH_2), 3.815-3.867 (d, $2H_a$, CH_2-CO), 4.210-4.247 (d, 1H, CH-C1), 5.000 (bs, $2H_f$, NH_2), 5.410-5.442 (d, 1H, H-6 of ²-lactam ring), 5.810-5.855 (q, 1H, H-7 of ²-lactam ring), 6.224-6.258 (d, 1H, N-CH-Ar), 6.514 (bs, $1H_a$,CONH), 6.792-7.088 (m, 3H, ArH), 8.434 (s, $1H_b$, CONH), 9.573 (s, $1H_g$, COOH), 11.970 (s, 1H, COOH-C_2), 12.500 (ss, 1H, Ar-OH). MS: $[M]^+$ at m/z 625.

Step-IV: 7-(D-5- Amino-5- carboxy- valeramido)-3-[(2'-substituted aryl-4'-oxo-thiazolidin-3'-yl)-acetyl amino]- 8-oxo- 5-thio- 1-azabicyclo [4, 2, 0]- oct-2-ene- 2- carboxylic acid(10-13)

In a ethanolic mixture of compounds 6-9 (.001 mole) and a pinch of anhydrous zinc chloride, thioglycolic acid (.002 mole) were added dropwise. This reaction mixture was refluxed for 8-12 hrs and completion of the reaction was checked by TLC. The excess of ethanol was distilled off. The remaining reaction residue was poured into ice water, filtered, washed with water and recrystallized from appropriate solvents to yield compounds 10-13.

Compound-10

7-(D-5- Amino-5- carboxy- valeramido)- 3-[(2'- phenyl-4'-oxo-thiazolidin-3'-yl)-acetylamino]-8-oxo-5-thio-1-azabicyclo [4,2,0]-oct-2-ene-carboxylic acid.

m.p.169°C, Yield: 67%, (r.s): methanol, (m.f): $C_{24}H_{27}N_5O_8S_2$. Elemental analysis: Calcd. C 49.91, H 4.67, N 12.13%, Found: C 50.20, H 4.54, N 12.22%. IR (KBr) (cm^{-1}): 622 (C-C1), 690 (C-S-C), 1425 (C-N), 1599 (C=C), 1697 (COOH), 1721 (side chain CONH), 1749 (C=O of ²-lactam ring), 3346 (NH_2). ¹H-NMR ($CDCl_3$ + DMSO-d_6) ´ (ppm): 1.200-1.245 (m, $4H_{c,d,}$ 2x CH_2), 1.269-1.370 (m, $1H_e$, CH), 3.504 (s, 2H-4, CH_2-S), 3.600-3.639 (t, $2H_b$, CH_2), 3.658 (s, 2H, CH_2 of ²-lactam ring), 3.826-3.880 (d, $2H_a$, CH_2-CO), 4.983 (bs, $2H_f$, NH_2), 5.400-5.432 (d, 1H, H-6 of ²-lactam ring), 5.802-5.845 (q, 1H, H-7), 6.225 (s, 1H, N-CH-Ar) 6.540 (bs, $1H_a$,CONH), 6.822-7.230 (m, 5H, ArH), 8.450 (s, $1H_b$, CONH), 9.608 (s, $1H_g$, COOH), 11.960 (s, 1H, COOH-C_2). MS: $[M]^+$ at m/z 577.

Compound-11

7-(D-5- Amino-5- carboxy- valeramido)- 3-[(2'-(o-hydroxy)phenyl-4'-oxo-thiazolidin-3'-yl)-acetylamino]-8-oxo-5-thio-1-azabicyclo [4,2,0]-oct-2-ene-carboxylic acid

m.p.172°C, Yield: 69%, (r.s): methanol, (m.f): $C_{24}H_{27}N_5O_9S_2$. Elemental analysis: Calcd. C 49.26, H 4.59, N 11.49%, Found: C 49.59, H 4.68, N 11.29 IR (KBr) (cm^{-1}): 687 (C-S-C), 1422 (C-N), 1598 (C=C), 1699 (COOH), 1720 (side chain CONH), 1730 (C=O of ²-lactam ring), 1746 (C=O of ²-lactam ring), 3285 (OH), 3350.2 (NH_2).

¹H-NMR ($CDCl_3$ + DMSO-d_6) ´ (ppm): 1.200-1.245 (m, $4H_{c,d,}$ 2x CH_2), 1.268-1.364 (m, $1H_e$, CH), 3.472 (s, 2H-4, CH_2-S), 3.610-3.650 (t, $2H_b$, CH_2), 3.628 (s, 2H, CH_2 of ²-thialactam ring), 3.816-3.850 (d, $2H_a$, CH_2-CO), 4.962 (bs, $2H_f$, NH_2), 5.391-5.420 (d, 1H, H-6 of ²-lactam ring), 5.800-5.846 (q, 1H, H-7), 6.247 (s, 1H, N-CH-Ar) 6.524 (bs, $1H_a$,CONH), 6.928-6.220 (m, 4H, ArH), 8.422 (s, $1H_b$, CONH), 9.600 (s, $1H_g$, COOH), 11.990 (s, 1H, COOH), 12.465 (ss, 1H, Ar-OH). MS: $[M]^+$ at m/z 593.

Compound-12

7-(D-5- Amino-5- carboxy- valeramido)- 3-[(2'-(p-methoxy)phenyl-4'-oxo-thiazolidin-3'-yl)-acetylamino]-8-oxo-5-thio-1-azabicyclo [4,2,0]-oct-2-ene-carboxylic acid

m.p. 169°C, Yield: 67%, (r.s): ethanol, (m.f): $C_{25}H_{29}N_5O_9S_2$. Elemental analysis: Calcd. C 49.42, H 4.77, N 11.53%, Found: C 49.21, H 4.72, N 11.20%. IR (KBr) (cm^{-1}): 686 (C-S-C), 1188 (C-O-C), 1424 (C-N), 1600 (C=C), 1700 (COOH), 1720 (side chain CONH), 1725 (C=O of ²-lactam ring), 1748 (C=O of ²-lactam ring), 3350 (NH_2). ¹H-NMR ($CDCl_3$ + DMSO-d_6) ´ (ppm): 1.130-1.264 (m, $4H_{c,d,}$ 2x CH_2), 1.230-1.345 (m, $1H_e$, CH), 3.400 (s, 3H, Ar-OCH_3), 3.458 (s, 2H-4, CH_2-S), 3.607 (s, 2H,

CH_2 of ²-thialactam ring), 3.600-3.648 (t, $2H_b$, CH_2), 3.838-3.876 (d, $2H_a$, CH_2-CO), 4.994 (bs, $2H_f$, NH_2), 5.387-5.404 (d, 1H, H-6 of ²-lactam ring), 5.840-5.842 (q, 1H, H-7), 6.225 (s, 1H, N-CH-Ar) 6.508 (bs, $1H_a$,CONH), 6.772-7.000 (m, 3H, ArH), 8.458 (s, $1H_b$, CONH), 9.585 (s, $1H_g$, COOH), 12.000 (ss, 1H, COOH-C_2). MS: $[M]^+$ at m/z 607.

Compound-13

7-(D-5- Amino-5- carboxy- valeramido)- 3-[(2'-(p-hydroxy, m-methoxy) phenyl- 4'-oxo-thiazolidin- 3'-yl)-acetylamino]-8-oxo-5-thio-1-azabicyclo [4,2,0]-oct-2-ene-carboxylic acid.

m.p. 160°C, Yield: 80%, (r.s): ethanol, (m.f): $C_{25}H_{29}N_5O_{10}S_2$. Elemental analysis: Calcd. C 48.15, H 4.65, N 11.23%, Found: C 48.40, H 4.52, N 11.44 IR (KBr) (cm^{-1}): 688 (C-S-C), 1190 (C-O-C), 1597 (C=C), 1698 (COOH), 1718 (side chain CONH), 1725 (C-N), 1727 (C=O of ²-lactam ring), 1748 (C=O of ²-lactam ring), 3288 (OH), 3350.2 (NH_2). 9

^{1}H-NMR ($CDCl_3$ + DMSO-d_6) ´ (ppm): 1.122-1.260 (m, $4H_{c,d,}$ 2x CH_2), 1.254-1.362 (m, $1H_e$, CH), 3.407 (s, 3H, Ar-OCH_3), 3.475 (s, 2H-4, CH_2S), 3.620 (s, 2H, CH_2 of ²-thialactam ring), 3.620-3.664 (t, $2H_b$, CH_2), 3.856-3.895 (d, $2H_a$, CH_2-CO), 4.991 (bs, $2H_f$, NH_2), 5.400-5.438 (d, 1H, H-6 of ²-lactam ring), 5.786-5.828 (q, 1H, H-7), 6.245 (s, 1H, N-CH-Ar) 6.505 (bs, $1H_a$,CONH), 6.892-7.100 (m, 4H, ArH), 8.427 (s, $1H_b$, CONH), 9.600 (s, $1H_g$, COOH), 12.450 (ss, 1H, Ar-OH). MS: $[M]^+$ at m/z 623.

Methods for biological activity

Filter paper disc method (Gould and Bowie, 1950)

Antibacterial activity

Antibacterial activity of methanolic solution of compounds and standard drug was performed by preparing standard size of blank Whatmann filter paper-1 discs (6.5 mm). Paper discs sterilized by dry heat at 140°C for 1h, saturated with the test solution and the known standard reference antibiotic solution separately. These discs were air-dried at room temp. to remove any residual solvent which might interfere with the determination. The discs were then placed on the surface of a sterilized agar nutrient medium that had been inoculated with the test organism (by using a sterile swab) and air-dried to remove the surface moisture. Thickness of the agar medium was kept equal in all Petri dishes and standard discs, Ampicillin norfloxacin and Fluconazole were used in each plate as a control. Before incubation, Petri dishes were placed for 1h in a cold room (5°C) to allow diffusion of the compounds from the disc into the agar plate. These discs were now incubated at 37°C for 20-24h after which the zone of inhibition or depressed growth was measured.

Antifungal activity

For antifungal screening, spore suspension (5mL) of each test organisms (72 h culture) was added to sterilised Sabouraud dextrose agar (Himedia Lab. Ltd., Mumbai) medium at 35-40°C by thorough shaking. The peteri dishes were seeded with the mixture and the paper discs of the methanolic solution of compound and the reference antibiotic (Fluconazole) as the control was placed in the same manner as in antibacterial activity determination. These Petri dishes were incubated at 30°C for 48h. The zone of inhibition was considered as an indicator the antifungal activity[10].

Biological Evaluation

Various compounds synthesized in scheme were evaluated for biological activities. Results are mentioned in tables Ia-Ib. Compound 1 on screening against various bacterial and antifungal strains like S. aureus, E., coli, K. pneumoniae and A. fumigatus, C. albicans exhibited very less antibacterial as well as antifungal activities respectively. Compound 2 gave mild antifungal activity against used fungal species and devoid of antibacterial activity. Compounds 3 and 4 possessed equipotency. Compound 5 possessed a lesser biological activity than compound 3 and 4.

In corporation of ²-lactam moieties to compounds 2-5 resulted into formation of compounds 6-9. Among all the for synthesized compounds, Compound 8 showed higher antibacterial as well as antifungal activities. Compound 6, 7 and 9 exhibited lesser bioactivity than compound 8.

Parent compounds 2-5 resulted in the formation of thiazolidinone congeners 10-13. Among the compounds 10-13, compound 10 was active against A. fumigatus (i.z. 8mm) only and lack off

antibacterial activity. Compound 11 and 12 showed highest antibacterial activity comparatively to remaining compounds of the scheme. Compound 11 exhibited more and wide spectrum of action in compare to compound 12. Compound 13 showed lesser activity than compounds 11 and 12.

DISCUSSION

Structural analysis relationship resulted that-

- Hydroxy substitution is beneficial for antibacterial and antifungal activity.
- Incorporation of β-lactam (azetidinone) moieties are beneficial to antibacterial as well as β-thialactams (thiazolidinones) moieties for antifungal activities.
- Screening data elicited that synthesized congeners (β-lactams) are more potent against gram negative bacteria (*E.coli*) as compared to Gram positive bacteria (*S. aureus*).
- Compound 11 and 12 were found more potent antibacterial than all above mentioned clinically used chemotherapoeutic agents viz ampicillin and norfloxacin.

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