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Synthesis and Antimicrobial Activity of Cephalosporin Derivatives

K.R. DESAI*, BHAVNA MISTRY and B.R. MODI,
Department of Chemistry,
South Gujarat University, Surat-395 007, India

Attempts have been made to prepare cephalosporin derivatives of type 1 and of type 2 by substitution at 7-amino group of 7-amino-3′-deacetoxycephalosporanic acid (7-ADCA) and at 7-(2-amino-2-phenylacetamido) side chain of cephalexin by substituted heterocyclic nucleus-s-triazine. These compounds were evaluated for their antibacterial activity against *S.aureus*, *S. pyogenes* (gram positive bacteria) and *E. coli* and *K. pneumoniae* (gram negative bacteria). The structures of these compounds have been confirmed by elemental analysis and IR, NMR spectral studies.

INTRODUCTION

The discovery and development of the cephalosporins began in 1948 with the finding by Brotzy that a cephalosporium species produced antibiotic materiai that was active against certain gram negative as well as gram positive bacteria[1]. 7-Aminocephalosporanic acid (6-APA) has given rise to thousands of new semisynthetic cephalosporins. Only a few cephalosporins are known to be efficiently absorbed when administered orally. Cephalexin, a clinical agent with the best absorption characteristic, is a derivative of 7-amino-3′-deacetoxy-cephalosporanic acid (7-ADCA), which has a methyl group at the 3′-position. It has broad spectrum activity against a large number of gram positive and gram negative organisms including penicillinase producing *staphylococci* and is indicated for the treatment of mild to moderate infections, including infections of the urinary tract[2, 3]. Keeping in view the wide spectrum activities of this system, it was considered worthwhile to synthesise cephalosporin derivatives of type (1) and of type (2) as possible antimicrobial agents.

EXPERIMENTAL

All the melting points were determined by open capillary method and are not corrected. IR-spectra were recorded in KBr pellets on Perkin-Elmer spectrophotometer. PMR spectra (DMSO) were run on Varian 300 spectrometer using TMS as internal standard. The purity of the compounds in addition to elemental analysis was checked by TLC.

Preparation of 7-[4′, 6′-dichloro-s-triazine-2′-ylamino]-3′-deacetoxycephalosporanic acid (C)

To a stirred solution of cyanuric chloride (A) (1.845 g, 0.01 mole) in acetone at 0–5°C, the solution of 7-amino-3′-deacetoxycephalosporanic acid (B) (2.142 g, 0.01 mole) in acetone was added slowly and neutral pH was maintained. After

complete addition, the stirring was continued at the same temperature for 2 h. Then the stirring was stopped and the solution was treated with crushed ice. The solid product thus obtained was filtered, dried and recrystallized from ethanol (1.74 g, 48%), m.p. 235°C.

Preparation of 7-[4′-(4″-metoxyphenylureido)-6′-chloro-s-triazine-2′-ylamino]-3′-dacetoxycephalosporanic acid (E)

To a stirred solution of 7-[4′,6′-dichloro-s-triazine-2′-ylamino]-3′-deacetoxycephalosporanic acid (C) (3.623 g, 0.01 mole) in acetone at 35°C the solution of *p*-methoxyphenylurea (D) (1.661 g, 0.01 mole) dissolved in acetone was added slowly for 1/2 h. Neutral pH was maintained. The temperature was gradually raised to 45°C during the stirring for 2 h. Then the solution was poured in ice-cold water. The solid product thus obtained was filtered, dried and recrystallized from ethanol (2.657 g, 54%), m.p. 223°C.

Preparation of 7-[2-(4′, 6′-dichloro-s-triazine-2′-ylamino)-2-phenylacetamido]-3′-deacetoxycephalosporanic acid (H)

To a stirred solution of cyanuric chloride (A), (1.845 g, 0.01 mole) in acetone at 0–5°C the solution of cephalexin (G) (3.474 g, 0.01 mole) in acetone was added slowly and neutral pH was maintained. After complete addition, the stirring was continued at the same temperature for 2 h. Then the stirring was stopped and the solution was treated with crushed ice. The solid product thus obtained was filtered, dried and recrystallized from ethanol (2.428 g, 49%), m.p. 243°C.

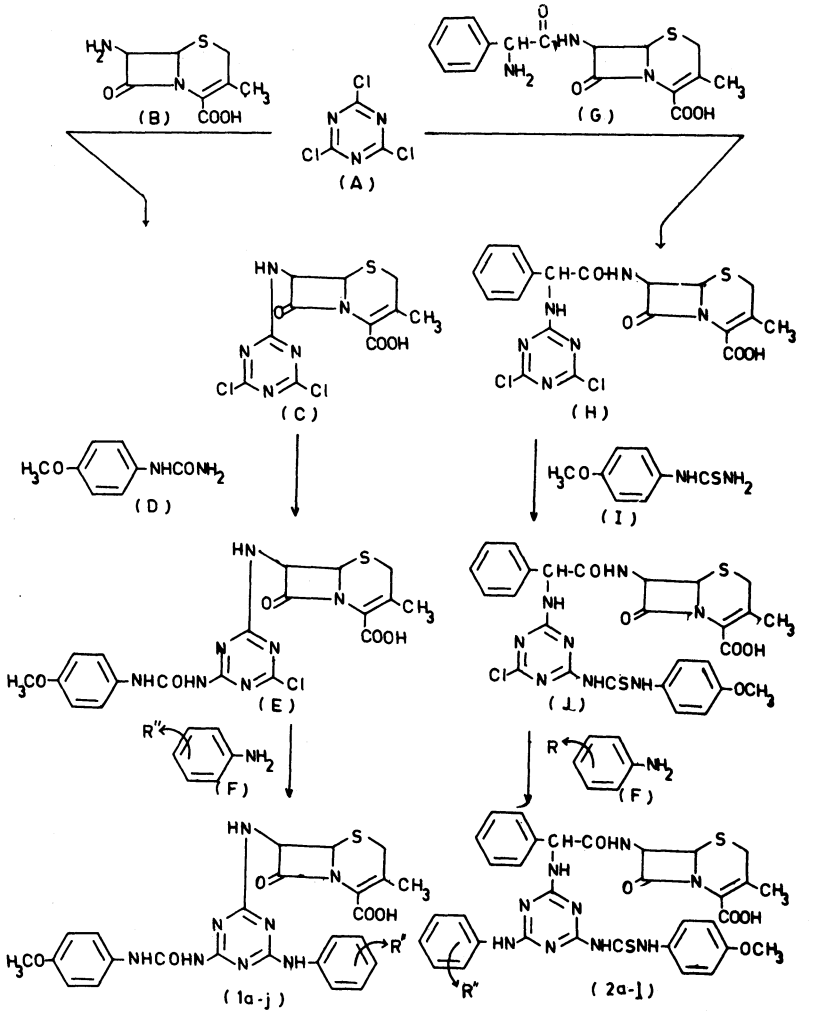
Preparation of 7-[2-{4′(4″-methoxyphenylthioureido)-6′-chloro-s-triazine-2′-ylamino}-2-phenylacetamido]-3′-deacetoxycephalosporanic acid (J)

To a stirred solution of 7-[2-{4′, 6′-dichloro-s-triazine-2′-ylamino}-2 phenylacetamido]-3′-deacetoxycephalosporanic acid (H) (4.954 g, 0.01 mole) in acetone at 35°C the solution of *p*-methoxyphenylthiourea (I) (1.882 g, 0.01 mole) dissolved in acetone was added slowly for $\frac{1}{2}$ h. Neutral pH was maintained. The temperature was gradually raised to 45°C during the stirring for 2 h. The solution was poured in ice-cold water. The solid product thus obtained was filtered, dried and recrystallized from ethanol (5.643 g, 88%), m.p. 196°C.

General procedure for preparation of 7-[4′-(4″-Methoxyphenyl-ureido)-6′-(arylamino)-s-triazine-2′-ylamino]-3′-deacetoxycephalosporanic acid (1a–j)/ 7-[2-{4′-(4″-methoxyphenylthioureido)-6′-(arylamino)-s-triazine-2′-ylamino}-2-phenyl acetamido]-3′-deacetoxycephalosporanic acid (2a–l)

A mixture of 7-[4′-(4″-methoxyphenylureido)-6′-chloro-s-triazine-2′-ylamino]-3′-deacetoxycephalosporanic acid (E) (0.492 g, 0.001 mole)/7-[2-{4′-(4″-methoxypenylthioureido)-6′-chloro-s-triazine-2′-ylamino}-2-phenylacetamido]-3′-deacetoxycephalosporanic acid (J) (0.0641 g, 0.001 mole) and arylamine (F) (0.01 mole) in dioxane was refluxed for 3 h, cooled and poured into ice-cold water. The separated solid was filtered and recrystallized from ethanol to furnish 1a-j/2a-1.

For compounds 1a–j, IR (KBr): ν_{max} (cm^{-1}) 795–800 (C_3N_3); 1770–1880 (β-lactam C=O); 1605 (urea C=O); 1490–1530 (*sec.* amine NH). NMR),



(DMSO): (ppm), 3.83 (—OCH_3), 7.66 (—NH—C(=O)—NH—), 7.3 (—NH, aromatic 7.84 (—NH, heterocyclic ring), 2.72 (—CH_3, aromatic ring).

For compounds 2a–l; IR (KBr): ν_{max} (cm^{-1}) 790–795 (C_3N_3); 1740–1760 (β-lactam C=O); 1155–1165 (thiourea C=S); 3370 (*sec.* amine NH) and 1610 (*sec.* amine NH). NMR (DMSO): δ (ppm), 3.83 (—OCH_3), 4.0 (NH—CS—NH), 5.4 (—NH, aromatic ring), 2.72 (—CH_3, aromatic ring), 8.4 (NH—CO—).

RESULTS AND DISCUSSION

The antimicrobial activity of 1a–j and 2a-l was determined against *S. aureus* and *S. pyogenes i.e.* gram-positive bacterial strain and *E. coli* and *K. pneumoniae*

i.e. gram-negative bacterial strain by agar diffusion (paper disc) method[4] at a concentration of 15 mcg/d. Solution for testing was prepared by using dimethyl sulfoxide as solvent. The results are given in Table 3. The activity of four standard drugs measured by the same procedure as for the above compounds is also given in Table 3.

Evaluation of bacterial activity reveals that the compound 1a having phenylamino group in 6-position of s-triazine ring showed activity upto 4.0 mm. It is nearly 50–67% as active as ampicillin and gentamycin and 30% as active as cephalexin. However, the compounds 1b–j having substituted phenylamino groups in 4-position of s-triazine ring showed slight decrease in activity and maximum inhibitory zone is upto 2.0 mm. The results also indicate that compounds 2a–l are more active against gram-negative bacilli than the gram-positive *cocci*.

The physical and analytical data of compounds 1a–j and 2a–l are presented in Table 1 and Table 2 respectively.

TABLE 1
PHYSICAL AND ANALYTICAL DATA OF COMPOUNDS 1a–j

Compd. No.	R″	Molecular formula	m.p. (°C)	Yield (%)	% of nitrogen Found (Calcd.)
1 a	H	$C_{25}H_{24}O_5N_8S$	234	28	20.24 (20.42)
1 b	2″—CH_3	$C_{26}H_{26}O_5O_8S$	225	75	19.88 (19.91)
1 c	3″—CH_3	$C_{26}H_{26}O_5N_8S$	231	71	19.82 (19.91)
1 d	4″—CH_3	$C_{26}H_{26}O_5N_8S$	242	76	19.70 (19.91)
1 e	2″—NO_2	$C_{25}H_{23}O_7N_9S$	227	61	21.04 (21.23)
1 f	3″—NO_2	$C_{25}H_{23}O_7N_9S$	238	59	20.91 (21.23)
1 g	4″—NO_2	$C_{25}H_{23}O_7N_9S$	221	54	21.07 (21.23)
1 h	2″—Cl	$C_{25}H_{23}O_5N_8SCl$	229	65	19.08 (19.22)
1 i	3″—Cl	$C_{25}H_{23}O_5N_8SCl$	245	49	19.44 (19.22)
1 j	4″—Cl	$C_{25}H_{23}O_5N_8SCl$	233	65	19.38 (19.22)

TABLE 2
PHYSICAL AND ANALYTICAL DATA OF COMPOUNDS 2a–l

Compd. No.	R″	Molecular formula	m.p. (°C)	Yield (%)	% of Nitrogen Found (calcd.)
2 a	H	$C_{33}H_{31}O_5N_9S_2$	207	74	18.38 (18.06)
2 b	2″—CH_3	$C_{34}H_{33}O_5N_9S_2$	212	44	17.87 (17.71)
2 c	3″—CH_3	$C_{34}H_{33}O_5N_9S_2$	256	21	17.49 (17.71)
2 d	4″—CH_3	$C_{34}H_{33}O_5N_9S_2$	208	41	17.53 (17.71)
2 e	2″—OCH_3	$C_{34}H_{33}O_6N_9S_2$	238	26	17.21 (17.32)
2 f	4″—OCH_3	$C_{34}H_{33}O_6N_9S_2$	182	54	17.13 (17.32)
2 g	2″—NO_2	$C_{33}H_{30}O_7N_{10}S_2$	204	47	18.60 (18.85)
2 h	3″—NO_2	$C_{33}H_{30}O_7N_{10}S_2$	188	59	18.80 (18.85)
2 i	4″—NO_2	$C_{33}H_{30}O_7N_{10}S_2$	195	56	18.64 (18.85)
2 j	2″—Cl	$C_{33}H_{30}O_5N_9S_2Cl$	197	48	17.15 (17.21)
2 k	3″—Cl	$C_{33}H_{30}O_5N_9S_2Cl$	236	33	16.80 (17.21)
2 l	4″—Cl	$C_{33}H_{30}O_5N_9S_2Cl$	241	48	17.50 (17.21)

TABLE 3
ANTIMICROBIAL ACTIVITY OF COMPOUNDS 1 AND 2

Compd. No.	*S. aureus*	Zone of inhibition (mm)		*K. pneumoniae*
		S. pyogenes	*E. Coli*	
1 a	2.5	2.0	4.0	0.5
1 b	2.0	1.0	2.0	0.5
1 c	1.0	0.5	1.0	0.0
1 d	0.5	0.0	0.0	1.0
1 e	1.5	0.5	1.0	2.5
1 f	2.0	1.0	2.0	0.0
1 g	2.0	1.5	2.5	0.0
1 h	0.0	0.5	0.5	0.5
1 i	1.0	1.0	2.0	1.5
1 j	1.0	0.0	1.5	1.0

Compd. No.	*S. aureus*	Zone of inhibition (mm)		*K. pneumoniae*
		S. pyogenes	*E. Coli*	
2 a	2.0	1.5	3.0	1.0
2 b	1.0	1.0	1.5	0.5
2 c	2.0	1.0	2.0	3.0
2 d	1.0	1.0	2.5	2.0
2 e	1.0	1.5	4.0	1.5
2 f	0.0	1.0	2.0	2.0
2 g	2.0	0.0	0.0	2.5
2 h	2.0	2.0	3.5	3.0
2 i	2.0	1.5	3.0	2.5
2 j	2.0	1.5	2.0	1.0
2 k	1.0	2.0	4.0	1.5
2 l	5.0	1.0	2.5	0.0
Ampicillin	5.0	—	—	—
Cephalexin	—	6.0	—	—
Gentamycin	—	—	6.0	—
Chloramphenicol	—	—	—	6.0

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