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Synthesis and Antimicrobial Activity of 7-Amino cephalosporanic Acid Derivatives of Amino Acids and Peptides

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A novel series of 7-amino cephalosporanic acid derivatives of amino acid and peptides were synthesized by solution phase technique. The synthesized compounds were tested for their biological activities against bacterial and fungal organisms. All the compounds showed potent antifungal activity and most of the compounds have shown moderate antibacterial activities. The structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR and mass spectral analysis.

Key Words: 7-Aminocephalosporanic acid, Amino acids, Dipeptides, Antibacterial and Antifungal activities.

INTRODUCTION

Cephalosporins are known class of antibiotics against bacterial organisms. Of many structural variations that have been performed, the most important in terms of numbers and useful biological properties have been those involving the 7-acylamino side chains of cephalosporins[1,2]. 7-Amino cephalosporanic acid is the key intermediate for the semisynthetic production of many cephalosporins. Peptide antibiotics are used as novel therapeutic agents to combat drug resistant microbial infections[3]. Most of these peptides exhibit their biological activities through binding to the corresponding acceptor molecules (receptors or enzymes) and hence allow bioactive peptides to act as therapeutic agents. An attempt has been made to synthesize 7-aminocephalosporanic of amino acids and peptide derivatives.

EXPERIMENTAL

All the reactions requiring anhydrous conditions were conducted in flame dried apparatus. Solvents and reagents were purified by standard methods. Organic extracts were dried over anhydrous sodium sulphate. Melting points were determined by an open capillary method and are uncorrected. The completion of the reaction and purity of the compounds were checked by thin layer chromatography. IR spectra were recorded on Nicolet impact 400 FT/IR spectrometer using KBr pressed pellet technique. ^{1}H NMR spectra were recorded on JEOL-JMS D-300 (MHz) NMR spectro-

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meter. MASS spectra were recorded on Shimadzu GC-MS (at 70 eV) mass spectrometer using xenon as the carrier gas.

Synthesis of *p*-nitrophenyl esters of Boc-amino acids and peptides: The Boc-peptide-OMe was hydrolyzed with LiOH to get Boc-peptide. Boc-amino acids/Boc-peptides were converted into the corresponding *p*-nitrophenyl (PnP-) derivatives by treating with *p*-nitrophenol in presence of DIPC and N-methylmorpholine (NMM) as a base. This additional step is carried out to retain the stability of 7-amino cephalosporanic acid since it is unstable in the acidic condition when the Boc-amino acids/Boc-peptides were added directly. The Boc-amino acid (1.5 mmol) was dissolved in $CHCl_3$ (15 mL) at 0 ºC. Then *p*-nitrophenol was added (0.27 g, 2 mmol) and stirred for 12 h at room temperature. The reaction mixture was filtered and the filtrate was washed with $NaHCO_3$ solution (10 %) until excess of *p*-nitrophenol was removed and finally washed with 5 % HCl (5 mL) to get Boc-amino acid-pnp-ester (**Scheme-I**).

Synthesis of 7-amino cephalosporanic acid derivatives of amino acids/peptides[4]: To the Boc-amino acid/peptide-PnP-ester (10 mmol) in $CHCl_3$ (25 mL), 7-amino cephalosporanic acid (ACA) (10 mmol) Et_3N (2.69 mL, 20 mmol) was added and stirred at room temperature for 12 h. The reaction mixture was washed with 10 % $NaHCO_3$ until the byproduct *p*-nitrophenol was removed completely and finally washed with 5 % HCl (5 mL). The organic layer was dried over anhydrous Na_2SO_4. The resulting solution is evaporated to dryness. The crude cyclized compound was then recrystallized by using $CHCl_3$ at 0 ºC.

7-Seryl-amino cephalosporanic acid: IR (ν_{max}, cm^{-1}): 3274 (-OH stretch), 2934 (-CH, aliphatic), 2855(-NH; asymmetric) 1750 (C=O in COOH), 1697 (C=O in ester), 1616 (C=O of amide), 1515 (-NH bend), 1451 (-CH bend). ^1H NMR ($CDCl_3$): δ 11.0 (1H, s, COOH), 8.0 (1H, s, -NH), 5.45 (1H, d, CH of lactam ring), 5.1 (1H, d, N-CH-S), 4.73 (2H, s, $-CH_2O$), 4.16-3.65(4H, m, aliphatic CH_2), 3.0 (2H, s, $S-CH_2$), 2.0 (6H, m, -OH, $-NH_2$ and $-CH_3$). Mass (EI): m/z 359 (M^+ peak).

7-Trptophanyl-amino cephalosporanic acid: IR (ν_{max}, cm^{-1}): 2932 (-CH, aliphatic), 2855 (-NH; asymmetric) 1757 (C=O in COOH), 1698 (C=O in ester), 1617 (C=O of amide), 1640, 1505, 1481 (Ar -CH str.), 1515 (-NH bend.), 1451 (-CH bend.). ^1H NMR ($CDCl_3$): δ 11.0 (1H, s, COOH), 10.3 (1H, s, NH of indole), 8.0 (1H, s, -NH), 7.18 (4H, m, Ar-H), 6.6 (1H, s, CH in indole), 5.45 (1H, d, CH of lactam ring), 5.1 (1H, d, $S-CH_2$), 4.72 (2H, s, $-CH_2O$), 3.98 (4H, m, $-CH_2$ in tryptophan, $-S-CH_2$), 2.0 (5H, m, $-NH_2$ and $-CH_3$).

7-Tyrosinyl-amino cephalosporanic acid: IR (ν_{max}, cm^{-1}): 3442 (-OH stretch), 2932 (-CH, aliphatic), 1759 (C=O in COOH), 1698 (C=O in ester), 1617 (C=O of amide), 1515 (-NH bend.), 1451 (-CH bend). ^1H NMR ($CDCl_3$): δ 11.0 (1H, s, COOH), 8.0 (1H, s, NH), 7.0 -6.7 (4H, m, Ar-H), 5.5 (1 H, s, CH in lactam ring), 3.0 (4H, d, $S-CH_2$), 2.0-1.9 (5H, m, NH_2 and CH_3). (m/z): 436 $(M + 1)^+$.

7-(N, O-CH_3) Tyrosinyl-amino cephalosporanic acid: IR (ν_{max}, cm^{-1}): 2932 (-CH, aliphatic), 2855(-NH; asymmetric) 1759 (C=O in COOH), 1698 (C=O in ester), 1617 (C=O of amide), 1515 (-NH bend), 1451 (-CH bend). ^1H NMR ($CDCl_3$):

δ 11.0 (1H, s, COOH), 8.0 (1H, s, NH), 7.2 (2H, d, Ar-H), 6.7 (2H, d, Ar-H adjacent to C-O), 5.45 (1 H, s, CH in lactam ring), 5.1 (1H, d, N-CH-S), 4.75 (2H, s, CH_2 attached to ester), 2.47 (3H, d, N-CH_3) 2.0-1.9 (5H, m, NH_2 and CH_3).

7-(Valyl-prolyl)-amino cephalosporanic acid: IR (ν_{max}, cm^{-1}): 3145; 3017 (asymmetric and symmetric -NH), 2974 (aliphatic -CH stretch), 2933 (symmetric -NH), 1766(CO in COOH), 1633.41 (C=O of amide), 1555 (-NH bending), 1453 (-CH bending), 1045 (-CH bend). ^{1}H NMR ($CDCl_3$): δ 11.0 (1H, s, COOH), 8.0 (1H, s, NH), 5.45 (1H, s, CH of lactam ring), 5.2 (1H, d, N-CH-S) δ 4.7 (2H, s, O-CH_2), 3.8-3.4 (5H, m, CH_2-N-CH_2 in proline, CH in valine), 3.0 (2H, s, S-CH_2), 2.2-1.9 (5H, m, CH_2-CH_2 of proline and CH of valine), 1.0-0.9 (6H, s, CH_3 group of valine). (m/z): 469 $(M + 1)^+$.

7-(D-Alanyl-D-alanyl)-amino cephalosporanic acid: IR (ν_{max}, cm^{-1}): 3141, 3017 (asymmetric and symmetric -NH), 2975 (aliphatic -CH stretch), 1758 (CO in COOH), 1633 (C=O of amide), 1555 (-NH bend), 1453 (-CH bend). ^{1}H NMR ($CDCl_3$): δ 11.0 (1H, s, COOH), 8.0 (1H, s, CONH), 5.45 (1H, s, CH of lactam ring), 5.1 (1H, d, N-CH-S), 4.7 (3H, m, CH and CH_2), 3.74 (1H, s, CH in alanine), 3.0 (2H, s, S-CH_2), 2.2-1.9 (5H, m, NH_2 and CH_3), 1.5 (3H, m, CH_3 group of alanine).

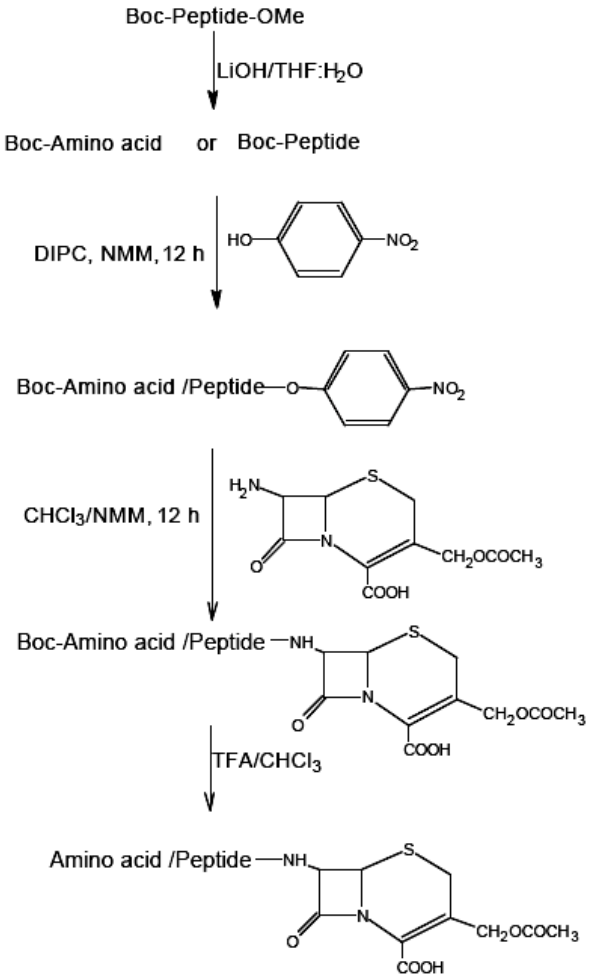
7-(Leucyl-D-alanyl)-amino cephalosporanic acid: IR (ν_{max}, cm^{-1}): 3145, 3014 (asymmetric and symmetric -NH), 2975 (aliphatic -CH), 1758 (CO in COOH), 1697 (C=O in ester), 1633 (C=O of amide), 1559 (-NH bend.), 1453 (-CH bend.). ^{1}H NMR ($CDCl_3$): δ 11.0 (1H, s, COOH), 8.0 (1H, s, CONH), 5.45 (1H, s, CH of lactam ring), 5.1 (1H, d, N-CH-S), 4.72 (3H, m, CH of alanine and CH_2 adjacent to ester), 3.74 (1H, m, CH in leucine), 3.0 (2H, s, S-CH_2), 2.1-1.9 (5H, m, NH_2 and CH_3), 1.48 (3H, m, CH_3 group of alanine), 1.0 (6H, d, CH_3 in leucine) (m/z): 456 (M^+ peak).

7-(Tyrosyl-glycyl)-amino cephalosporanic acid: IR (ν_{max}, cm^{-1}): 3329 (aliphatic C-H), 2933 (asymmetric -NH), 1759 (C=O stretch), 1687 (CO in ester), 1556 (C=O of amide), 1456 (-NH bend), 1089 (-CH bend). ^{1}H NMR ($CDCl_3$): δ 11.0 (1H, s, COOH), 8.0 (1H, s, NH), 5.0 (2H, s, Ar-OH and aliphatic CH of tyrosine), 3.64 (2H, s, CH in glycine), 3.0 (4H, d, -CH_2 in tyrosine and S-CH_2-C), 2.0 (5H, m, NH_2 and CH_3).

Evaluation of antimicrobial activity: Agar disc diffusion method was used for the evaluation of antimicrobial activity of the synthesized compounds[5]. The strains used for carrying out the antimicrobial activity are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* for antibacterial and *Candida albicans* for antifungal activity using the standards of cefotaxime and grieseofulvin for antibacterial and antifungal activity, respectively. All the test compounds were tested at 50 μg level.

RESULTS AND DISCUSSION

A series of 7-amino cephalosporanic acid containing amino acids and peptides were synthesized and their structures were characterized by elemental analysis ^{1}H NMR, IR and mass spectral data. The synthesized compounds were subjected to antimicrobial evaluation by disc diffusion method. Physical data of all the 7-amino cephalosporanic acid containing amino acids and peptides are given in Table-1.



Scheme-I

All the compounds have shown very potent antifungal activity which can be comparable to the standard drug. In fact all the bacterial strains used for the study were susceptible to the synthesized compounds but the *E. coli* is much more sensitive and *P. aeruginosa* is less sensitive to the compounds. In general the dipeptide substituted 7-amino cephalosporanic acid derivatives resulted in slight increase in antimicrobial activity compared to the amino acid substituted 7-amino cephalosporanic acid (Table-2).

Among the four dipeptide substituted compounds synthesized, compound **VII** and **VIII** were more potent. However the compound **IV** having (N, O-CH_3) tyr unit as a substituent showed better activity equally to the dipeptide substituted ones. In addition to that, compound **IV** may likely be less susceptible to acid hydrolysis since it has N-methylation adjacent to the peptide bond.

TABLE-1
PHYSICAL DATA OF SYNTHESIZED AMINO ACID/DIPEPTIDE
7-AMINO CEPHALOSPORANIC ACID

Comp. No.	Amino acid/Dipeptide-7-ACA	m.f.	m.w.	m.p. (ºC)	Yield (%)
I	7-Ser-ACA	$C_{13}H_{17}N_3SO_7$	359	193	77.5
II	7-Try-ACA	$C_{21}H_{24}N_4SO_6$	460	110	76.9
III	7-Tyr-ACA	$C_{19}H_{22}N_3SO_7$	436	95	83.6
IV	7-(N,O-CH_3) Tyr-ACA	$C_{20}H_{23}N_3SO_7$	449	93	56.2
V	7-(Val-Pro) ACA	$C_{20}H_{29}N_4SO_7$	469	155	61.3
VI	7-(D-Ala-D-Ala) ACA	$C_{16}H_{20}N_4SO_7$	412	132	65.5
VII	7-(Leu-D-Ala) ACA	$C_{19}H_{28}N_4SO_7$	456	–	60.8
VIII	7-(Tyr-Gly) ACA	$C_{21}H_{24}N_4SO_8$	492	112	79.2

TABLE-2
RESULTS OF ANTIMICROBIAL ACTIVITY

Compound	Zone of inhibition in diameter (mm)				
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
I	13	13	17	22	13
II	14	13	19	21	13
III	13	12	15	17	14
IV	14	13	17	22	13
V	12	12	16	15	15
VI	13	14	17	16	13
VII	16	15	16	20	15
VIII	16	12	18	17	16
Cefotaxime	14	18	16	32	–
Griseofulvin	–	–	–	–	12

'–' indicates no activity.

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