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Research Article

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF 6-AMINOPENICILLANIC ACID DERIVATIVES OF N-METHYLATED AMINO ACIDS AND DIPEPTIDES

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ABSTRACT

A series of 6-aminopenicillanic acid derivatives of N-methylated amino acid and D-amino acid incorporated dipeptides were synthesized by solution phase technique. The structure of the newly synthesized compounds was confirmed by IR, ^{1}H NMR, and Mass spectral analysis. The synthesized compounds were tested for their biological activities against bacterial and fungal organisms. The compounds showed potent antibacterial activity against *E.Coli*.

Keywords: 6- Aminopenicillanic acid, Amino acids, peptides, Antibacterial and Antifungal activity.

INTRODUCTION

Penicillin antibiotics are historically significant because they are the first drugs that were effective against many diseases such as syphilis and Staphylococcus infections. Penicillin's are β-lactam antibiotics, used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms. 6-aminopenicillanic acid (6-APA), is a key intermediate in the synthesis of clinically useful semi-synthetic penicillins such as ampicillin or amoxicillin[1] and 6-APA plays a viral role in the antimicrobial activities. In the penicillin structure, various structural variations had been performed, to increase the biological properties of the penicillins[2-6]. Peptide antibiotics are used as novel therapeutic agents to combat drug resistant microbial infections[7]. Hence an attempt has been made to synthesize the 6-aminopenicillanic acid derivatives of amino acids (commonly found in natural peptide antibiotics) and peptides.

MATERIALS AND METHODS

All the reactions requiring anhydrous conditions were conducted in flame dried apparatus.The amino acid used are L-amino acid , except D-alanine, purchased from Spectrochem Private Limited, Mumbai, India. Solvents and reagents were purified by standard methods. Boc-amino acids, amino acid methyl ester hydrochlorides and nitro-arginine were prepared by standard procedures[8]. N-methylated amino acids were prepared using NaH/CH_3I by Benoiton method[9]. 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 GEOL-JMS D–300 (MHz) NMR spectrometer. MASS spectra were recorded on Shimandzu GC-MS (at 70 eV) Mass Spectrometer using xenon as the carrier gas.

Preparation of the Dipeptides:

Amino acid methyl ester hydrochloride (10mmol) was dissolved in chloroform (20ml)**.** To this, N-methylmorpholine (1.3ml) was added at 0^0C and the reaction mixture was stirred for 15 minutes**.** Boc-amino acid (10mmol) in $CHCl_3$ (20ml) and DIPC (Diisopropylcarbodiimide) (10mmol) were added with stirring**.** After 24 hours, the reaction mixture was filtered. The filtrate was washed with 5% $NaHCO_3$ (20ml), 5% HCl (20ml) and distilled H_2O (20ml)**.** The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum**.** The residue was purified by recrystallization from $CHCl_3$ and petroleum ether.

Synthesis of p-nitrophenyl(pnp) esters of Boc-Amino acids and peptides:

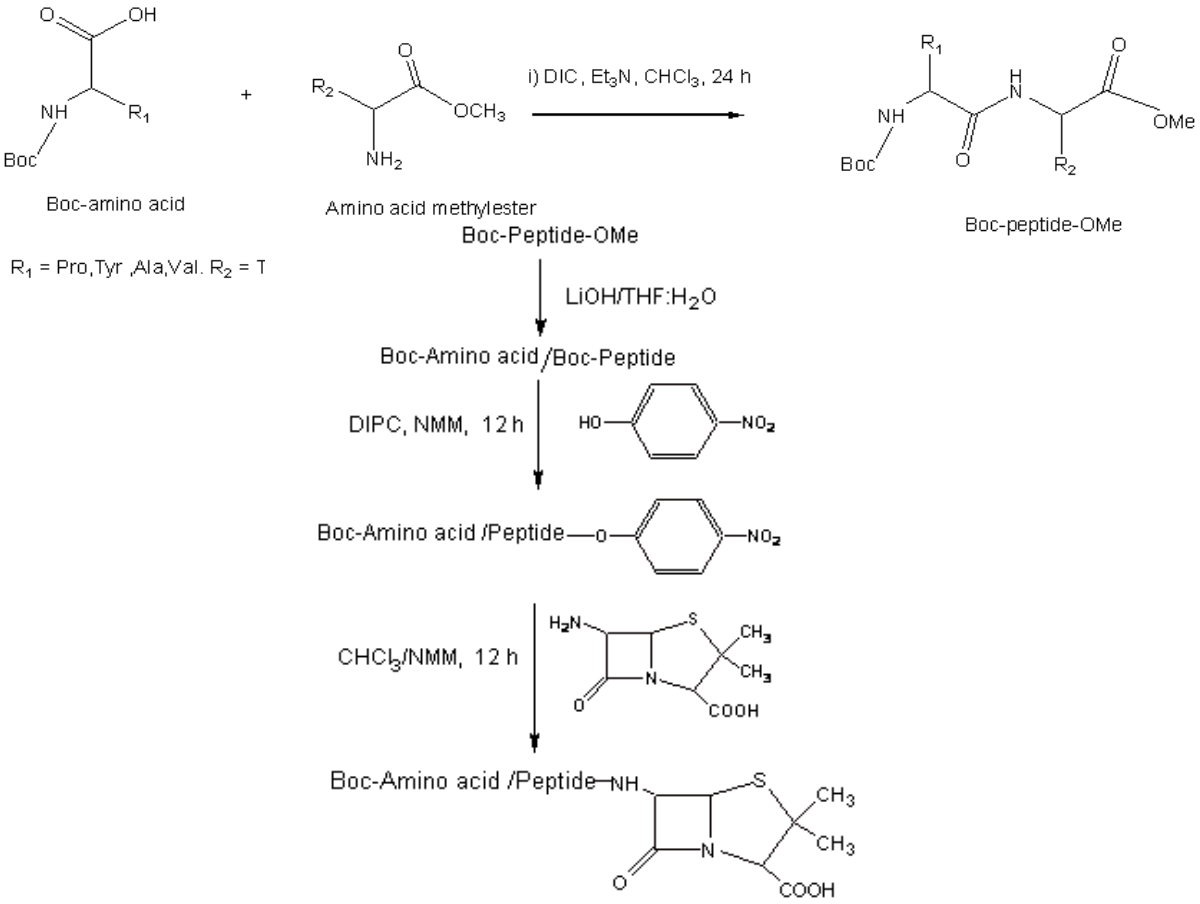
The Boc-amino acid/peptide (1.5mmol) was dissolved in $CHCl_3$ (15ml) at 0^0C**.** Then p-nitrophenol was added (0.27g,

2mmol) and stirred for 12 hours 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 (5ml) to get Boc-amino acid/Peptide-pnp-ester[10].

Synthesis of 6-APA derivatives of Boc-Amino acids/peptides:

To the Boc-amino acid/peptide-pnp-ester (10mmol) in $CHCl_3$ (25ml), 6-APA (10mmol) Et_3N (2.69 ml, 20mmol) was added and kept at 0^0C for 12 hours. The reaction mixture was washed with 10% $NaHCO_3$ until the byproduct p-nitrophenol was removed completely and finally washed with 5% HCl (5ml). The organic layer was dried over anhydrous Na_2SO_4. Chloroform and Et_3N were distilled off to get the crude product of the cyclised compound, which was then recrystallised from $CHCl_3$. To the above (1.2mmol) in $CHCl_3$ (15ml), CF_3COOH (0.274 g, 2.4mmol) was added, stirred for 1 hour at room temperature and washed with 10% $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4.

Scheme I



Evaluation of Antimicrobial Activity:

Agar disk diffusion method was used for the evaluation of antimicrobial activity of the synthesized compounds. 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

Physical Data and Spectral Analysis:

Compound-1: 6-[Boc-(N-Me)Val]aminopenicillanic acid: **IR (KBr Pallets):** 3288.7(s, N-H str), 2932.6(s, C-H str), 2857.9(s, C-H str), 1776.9(m,C=O of COOH), 1705.6(br, s, C=O of Boc), 1657(s, C=O of amide), 1528(N-H bend), 1454(C-H bend) cm^{-1}.

^{1}H NMR (300MHz, $CDCl_3$): δ 7.6(1H, br, s, N-H), 5.0(1H, d, J = 1.1Hz, α-H), 4.8(1H, s, α-CH of 6-APA), 4.4(1H, d, N-CH-CO of β-lactam ring), 4.1(1H, d, -CH-S- of β-lactam), 2.2(3H, s, N-CH_3), 1.5(6H, s, -C-$(CH_3)_2$), 1.45(9H, s, $(CH_3)_3$C-), 1.4-1.2(1H, m, β-CH of Val), 0.95(6H, d, -$C(CH_3)_2$ of Val). **FABMass:** m/z = 429.

Compound-2: 6-(Boc-Ala-Tyr)aminopenicillanic acid: **IR (KBr Pallets):** 3520.2(s, O-H str), 3329.3(s, N-H str), 2932.6(s, C-H str), 2855.9(s, C-H str), 1772.3(m, C=O of COOH), 1719.0(br, s, C=O of Boc), 1658(s, C=O of amide), 1630(s, Ar-C-C str), 1532(N-H bend), 1450(C-H bend) cm^{-1}.

^{1}H NMR (300MHz, $CDCl_3$): δ 7.3(1H, br, s, N-H), 7.1-6.8

(4H, m, Ar-H), 5.2(1H, q, -CH- of Ala), 4.9(1H, s, α-H of 6-APA), 4.7(1H, s, α-H), 4.5(1H, d, N-CH-CO of β-lactam ring), 4.4(1H, d, -CH-S- of β-lactam), 4.3(1H, t, α-H), 4.2(2H, br, s, NH), 3.2(2H,d,β-CH_2 of Tyr), 1.45(9H, s, $(CH_3)_3C$-). **FABMass:** m/z = 550.

Compound-3 : 6-(Boc-Pro-Tyr)aminopenicillanic acid : **IR (KBr Pallets):** 3540.3(s, O-H str), 3327.6(s, N-H str), 2934.5(s, C-H str), 2859.4(s, C-H str), 1776.1(m, C=O of COOH), 1717.0(br, s, C=O of Boc), 1655(s, C=O of amide), 1635(s, Ar-C-C str), 1538(N-H bend), 1455(C-H bend) cm^{-1}.

^{1}H NMR (300MHz, $CDCl_3$): δ 7.4(1H, br, s, N-H), 7.0-6.8 (4H, m, Ar-H), 4.8(1H, s, α-H of 6-APA), 4.6(1H, s, α-H), 4.6(1H, d, N-CH-CO of β-lactam ring), 4.5(1H, d, -CH-S- of β-lactam), 4.3(2H, m, -CH_2- of pro), 4.2(2H, m, -CH_2), 4.1(1H, t, α-H), 4.2(2H, br, s, NH), 3.2(2H,d,β-CH_2 of Tyr). **FABMass:** m/z = 576.

Compound-4: 6-[Boc-(N-Me,O-Me)Tyr]aminopenicillanic acid: **IR (KBr Pallets):** 3545.7(s, O-H str), 3326.8(s, N-H str), 2939.9(s, C-H str), 2855.1(s, C-H str), 1774.3(m, C=O of COOH), 1720.2(br, s, C=O of Boc), 1659(s, C=O of amide), 1638(s, Ar-C-C str), 1540 (N-H bend), 1459(C-H bend) cm^{-1}.

^{1}H NMR (300MHz, $CDCl_3$): δ 7.4(1H, br, s, N-H), 7.2-6.9 (4H, m, Ar-H), 4.8(1H, s, α-H of 6-APA), 4.9(1H, s, α-H), 4.7(1H, d, N-CH-CO of β-lactam ring), 4.5(1H, d, -CH-S- of β-lactam), 4.4(1H, t, α-H), 4.3(2H, br, s, NH), 3.5(2H,d,β-CH_2 of Tyr), 2.5(3H, s, (CH_3).**FABMass: m/z** 507.

Compound-5: 6-(Boc-Pro-(NO_2)Arg)aminopenicillanic acid: **IR (KBr Pallets):** 3325.8(s, N-H str), 3256.8(s, NH_2 str), 2940.9(s, C-H str), 2859.1(s, C-H str), 1778.3(m, C=O of COOH), 1721.2(br, s, C=O of Boc), 1659(s, C=O of amide), 1542 (N-H bend), 1457(C-H bend) cm^{-1}.

^{1}H NMR (300MHz, $CDCl_3$): δ 7.3(1H, br, s, N-H), 4.9(1H, s, α-H of 6-APA), 4.7(1H, s, α-H), 4.5(1H, d, N-CH-CO of β-lactam ring), 4.6(1H, d, -CH-S- of β-lactam), 4.3(2H, m, -CH_2- of Arg), 4.2(2H, m, -CH_2 of Arg), 4.2(2H, m, -CH_2 of pro), 4.2(2H, m, -CH_2 of pro), 4.1(1H, t, α-H), 4.2(2H, br, s, NH),3.2 (2H,s,NH_2) . **FABMass:** m/z = 614.

Table 1: Physical data of synthesized amino acid/dipeptide 6-amino penicillinic acid

Sl. No	Dipeptides	Mol For.	Mol. Wt	Physical state	M.P (^{0}C)	% yield
1.	6-[Boc-(N-Me,O-Me)Tyr] APA	$C_{23}H_{33}N_3O_7S$	507	Yellow semisolid	-	87.2
2.	6-[Boc-(N-Me)Val]APA	$C_{19}H_{31}N_3O_6S$	429	Orange crystals	134	82.4
3.	6-[Boc-Pro-(NO_2)Arg]APA	$C_{24}H_{38}N_8O_9S$	614	Brown semisolid	-	78.9
4.	6-(Boc-Pro-Tyr)APA	$C_{27}H_{36}N_4O_8S$	576	Yellow crystals	92	84.6
5.	6-(Boc-D-Ala-Tyr)APA	$C_{25}H_{34}N_4O_8S$	550	Pale brown crystals	127	81.2

Table 2: Antimicrobial Activity of 6–APA Derivatives

Sl. No	Compound no	Diameter of zone of Inhibition (mm)				
		B. sub	*S. aur.*	*E. coli*	*P.aer*	*C. alb.*
1	6-[Boc-(N-Me,O-Me)Tyr] APA	09	09	12	10	10
2	6-[Boc-(N-Me)Val]APA	09	09	14	09	07
3	6-[Boc-Pro-(NO_2)Arg]APA	10	10	13	10	08
4	6-(Boc-Pro-Tyr)APA	10	09	15	09	9
5	6-(Boc-D-Ala-Tyr)APA	09	8	13	09	08
6	Ampicillin	15	15	14	17	–
7	Griseofulvin	–	–	–	–	12

(–) indicates no inhibition zone (no activity)

CONCLUSION

Structural modification of 6-amino pencillanic acid was carried out by coupling N-methylated amino acids and dipeptides with the amino group of 6-APA and the synthesized compounds were characterized by IR, ^{1}H NMR and Mass spectral analysis. The compounds were subjected to antimicrobial evaluation by Disk Diffusion method[11]. All the compounds had shown potent antibacterial activity against *E.Coli* which can be comparable to the standard drug (Ampicillin). In fact all the bacterial strains used for the study were susceptible to the synthesized compounds but *E.coli* was more sensitive than other strains to the synthesized compounds. However the compound I and II having (N, O-CH_3) Tyr unit as a substituent showed better activity equally to the dipeptide substituted ones. Final conclusion was made, based on the antimicrobial activities of the newly synthesized 6-amino penicillanic acid derivatives, the methylated and D-amino acid substituted compounds had shown potent antibacterial and antifungal activities.

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