

Full Length Research Paper

**DESIGN AND SYNTHESIS OF 4-[2'-(5'- NITRO)] IMIDAZOLYL
BENZOYL (N-METHYL) AMINO ACIDS AND PEPTIDES**

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ABSTRACT

In the past two decades, a wide variety of bioactive peptides have been discovered. Condensation of heterocyclic moieties viz nicotinic acid, thiazole coumarin, quinolin, furan, imidazole etc. with amino acids and peptides resulted in compounds with potent biological activities. Many of the heterocyclic found to exhibit antifungal, antibacterial, cytotoxic, antineoplastic, insectisidal, antiinflammatory, anthelmintic, tyrosinase inhibitory and melanin production inhibitory

activities. Metronidazole, serconidazole, flucanazole are well known marketed drugs. Introduction of D-amino acids and N-methylation of amino acids like tyrosine, valine, alanine etc enhanced antimicrobial activity. Hence an attempt is made towards the synthesis of 5-nitroimidazolyl-benzoic acid derivative of N-methylamino acids and peptide using solution phase technique of peptide synthesis. The method includes the introduction of tert-butyloxy carboxyl group (Boc) to amino acids to protect the amino group forming Boc-amino acids. The protection of carboxyl group was done by converting the amino acids into corresponding methyl ester. The protected amino acids were coupled using diisopropylcarbodiimide and triethylamine to get protected dipeptides. N-methylation was done by treating with methyl iodide and sodium hydride. The ester group was then removed by lithium hydroxide. The Boc(N-methyl)dipeptide were coupled to amino acids or Boc(N-methyl)dipeptide were coupled to 4-[2-(5-nitro)imidazolyl]benzoic acids.

Keywords: *Imidazole, N-Methylation, Antibacterial, Antifungal, Anthelmintic*

INTRODUCTION

4-[2'-(5'-NITRO)IMIDAZOLYL] BENZOYL(N-METHYL) AMINO ACIDS AND PEPTIDES were prepared by coupling 4-[2'-(5'-nitro)imidazolyl] benzoic acids with corresponding N-methylated amino acids methyl ester hydrochloride or dipeptide methyl ester. Representative compounds have been characterized for antifungal and anthelmintic activities.

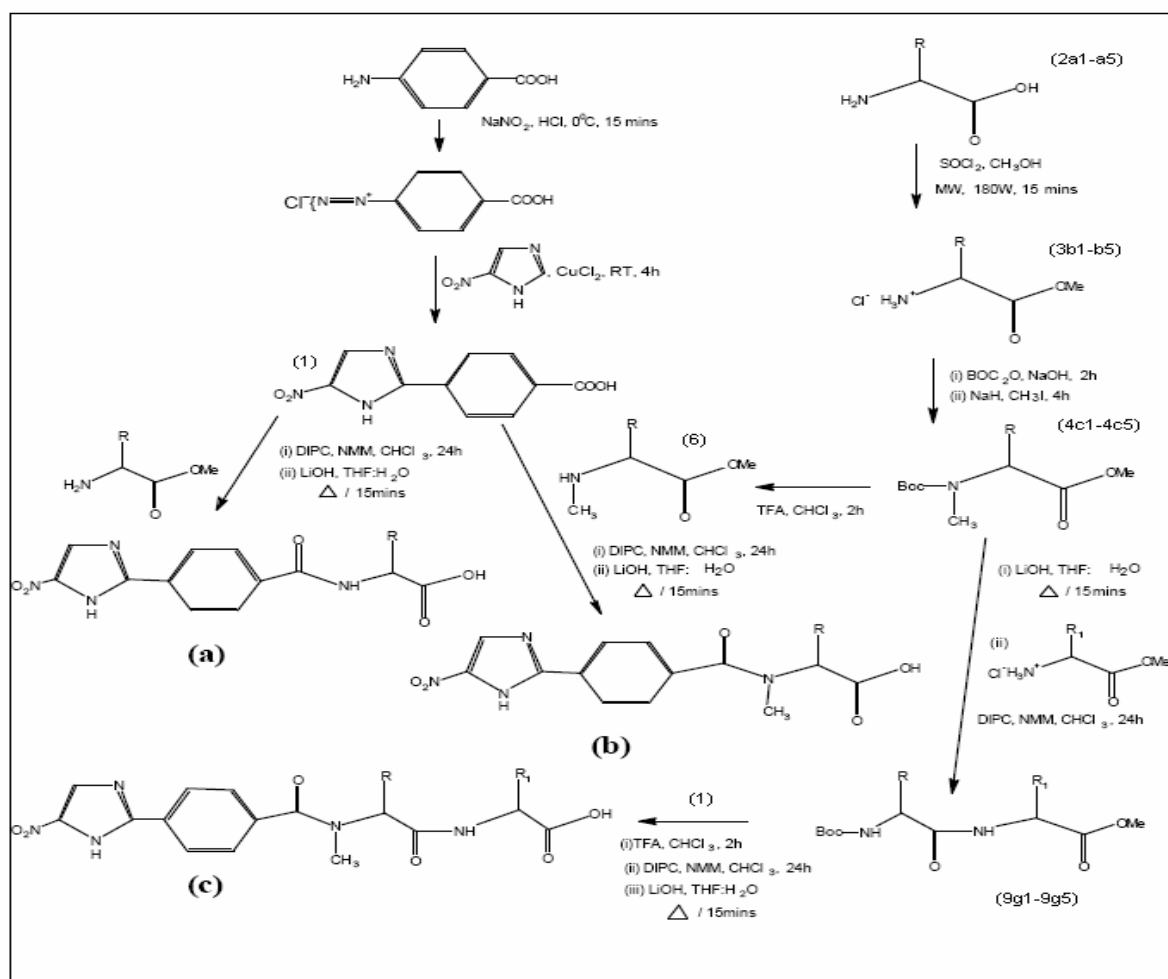
Imidazole or imidazoline, earlier called glyoxalin was prepared from glyoxal and ammonia. Imidazole nucleus, found in essential amino acids, exhibit potent antiprotozoal, antifungal and antibacterial^[1] activities besides acting as adrenergic and anthelmintic agents. In continuation of earlier work^[2] on the synthesis of a new series of 5-nitromidazole of amino acids and peptides, the synthesis of 4-[2'-(5'-NITRO)IMIDAZOLYL] BENZOYL(N-METHYL) AMINO ACIDS AND PEPTIDES along with their anti microbial and anthelmintic activities.

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Imidazole was nitrated with nitrating mixture [Conc.H₂SO₄ and HNO₃ (1:1)] to get 5-nitroimidazole. P-amino benzoic acid was diazotized with sodium nitrite and dilutes hydrochloric acid at 0° C. The resulting diazonium salt was stirred with 5-nitroimidazole in the presence of an aqueous solution of cupric chloride for 4hrs at room temperature to get 4-[2'-(5'-NITRO)IMIDAZOLYL]BENZOIC ACID (2). Amino acids (2a₁-a₅) were converted into the corresponding methyl ester hydrochloride (3b₁-b₅) using thionyl chloride and methanol. N-methylation was done by treating with methyl iodide and sodium hydride by following methods of Benoit^[3] and

Julie^[4] to get Boc-(N-Me) amino acids methyl ester. The Boc group of N-methylated amino acids methyl ester (4c₁-c₅) was removed by trifluoroacetic acid. The required dipeptides(10h₁-h₅) were prepared by coupling Boc N-methylated-amino acid with the respective amino acid ester hydrochloride using di- isopropylcarbodiimide and N-methyl morpholine as per Bodanszky's^[5] procedure. The N-methylated amino acid methyl ester were coupled with 4-[2'-(5'-NITRO)IMIDAZOLYL]BENZOIC ACID using isopropyl-di carbodiimide, N-methyl morpholine and triethylamine to obtain the title compounds which were characterized on the basis of spectral data.



Scheme 1

R = side chain at (a) Val(7e₁), Phe(7e₂), Tyr(7e₃), Thr(7e₄), Leu(7e₅)

R = side chain at (b) Val(8f₁), Phe(7e₄), Tyr(8f₃), Thr(8f₄), Leu(8f₅)

R, R₁ = side chain at (c) Phe-Pro (10h₁), Thr-Phe(10h₂), Leu-Tyr(10h₃)

MATERIAL AND METHOD

Melting points were recorded in open capillary tubes. Purity of compound was confirmed by TLC on silica gel-G using chloroform: glacial acetic acid: water (3:2:5) as solvent and iodine vapor as visualizing agent. The IR spectra were recorded on JASCO FTIR 300 SPECTROMETER and ¹HNMR spectra were recorded on BRUCKER AC NMR spectrometer (300MHz) using CDCL₃ as internal standard. FAB MASS spectra were recorded on a Joel Sx 102/DA 6000 mass spectrometer using xenon as the carrier gas.

GENERAL PROCEDURE

Preparation of Nitroimidazole:

Concentrated sulphuric acid (53ml) and fuming nitric acid (38ml) were taken in a 250 ml round bottom flask and imidazole (75g, 1.1 moles) was added slowly in small portions with regular shaking. The mixture was refluxed shaking for 30 mins, allowed to cool and cautiously poured into 500ml of water. The precipitated nitroimidazole was filtered and washed with water.

Preparation of 4-[2'-(5'-nitro)imidazoly]benzoic acid (2):

A mixture of p-amino benzoic acid (34.25 gm, 250 m mol), dilute hydrochloric acid (120ml) and water (150 ml) was heated to get a clear solution. The solution was cooled to 0°-5°C and diazotized by the addition of 30% of sodium nitrite solution (48ml). To the above diazonium salt solutions, dilute hydrochloric acid (100ml), nitroimidazole (250mmol) and aqueous cupric chloride solution (25% ml) were added with stirring. The mixture was shakes for 6 hrs and kept overnight in the refrigerator. The separated solid was filtered, washed with water and recrystallized with acetone.

Preparation of Amino acid methyl ester hydrochlorides (3b₁-b₅):

The methyl ester hydrochlorides of all five amino (valine, threonine, leucine, tyrosine, phenylalanine) were prepared separately by slowly adding 20 mmol of corresponding amino acid to a mixture was irradiated in microwave (Model M 1739 N) at 180W for 15 minutes to give a pasty mass of methyl ester hydrochloride which was triturated with ether at 0° C to remove excess of dimethyl sulphite. The resulting solid was recrystallised with a mixture of methanol and diethyl ether (1:1)

Preparation of the Boc-Amino acid methyl ester:

To a solution of amino methyl ester hydrochloride (2.2 mmol) in chloroform (20ml) was added triethylamine (4 mmol) followed by (Boc₂)O (4.5 ml, 1.81 m mol) and ether (15 ml). The mixture was stirred for 2 hours at room temperature and washed with 10% NaHCO₃ (2 x 10ml). The organic layer was separated. Dried and concentrated to get Boc-amino acid methyl ester which was recrystallized, with n-hexane at- 15° C. Using their procedure, following Boc-amino acid methyl ester were prepared:

Preparation of Boc-(N-Me) Amino acid methyl ester (4c₁-c₅):

Corresponding Boc-amino acid methyl ester (5.5 mmol) was dissolved in dimethyl formamide (30ml), and sodium hydride (0.75 gm, 16.5 mmol) was added at room temperature followed by methyl iodide (6.3 gm, 44 mmol). To the above mixture 15 ml of ether was added and shaken well for 4 hrs. The solution was washed with saturated NH₄Cl (20 ml) followed by 20% Na₂S₂O₃ (20 ml) and saturated NaCl (20 ml). The ether layer was separated, dried with anhydrous Na₂SO₄ and concentrated under reduced pressure to give the title compound as viscous oil.

Preparation of the Dipeptides (9g₁-g₅):

Amino acid methyl ester hydrochloride (10mmol) was dissolved in chloroform (20ml) and N-methylmorpholine (1.3ml) was added and the

mixture was stirred for 15 minutes. Boc (N-Me) amino acid (10 mmol) in chloroform (20 ml) and di-isopropyl carbamide (10 mmol) was added to the above mixture with stirring. After 24 hours, the reaction mixture was shaken with chloroform (30ml). The chloroform layer was separated and washed with 5% NaHCO₃ (20ml) followed by saturated NaCl (20 ml) solution and dried over anhydrous Na₂SO₄, filtered and evaporated in vacuum to get dipeptide.

Preparation of 4[2'-(5'-nitro) imidazolyl]benzoyl (N-Me) amino acid and peptide methyl ester (7e₁-e₅, 8f₁-f₅, 10h₁-h₃):

A mixture of amino acid methyl ester (7.0 mmol) tetrahydrofuran (20 ml), 4-[2'-(5'-nitro)imidazolyl]benzoic acid (1.631 gm, 7.0 mmol), di-isopropyl carbodiimide and triethylamine (208ml) was stirred at room temperature for 24 hours. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in chloroform, washed with 10% NaHCO₃ (10ml) followed by 5% hydrochloric acid (10ml), dried anhydrous Na₂SO₄ and evaporated under vacuum to get the title compound. The crude product was recrystallized from CHCl₃ and n-hexane. The data are given in the following table:-

Table 1. Physical and analytical data of compounds prepared:

Sl. No	Compound No.	Physical State	Molecular Formula	Molecular Weight	M.P.°C	% Yield
1.	7e ₁	Reddish Brown Solid	C ₁₅ O ₅ N ₄ H ₁₆	332	68-70 C	74.53
2.	7e ₂	Reddish Brown Solid	C ₁₉ O ₅ N ₄ H ₁₆	380	93-95 C	95.5
3.	7e ₃	Yellow Crystals	C ₁₉ O ₆ N ₄ H ₁₆	396	92-95 C	27.41
4.	7e ₄	Brown Crystals	C ₁₄ O ₆ N ₄ H ₁₄	334	80-82 C	37.94
5.	7e ₅	Yellow Solid	C ₁₆ O ₅ N ₄ H ₁₈	346	61-64 C	23.17
6.	8f ₁	Yellow Brown Solid	C ₁₆ O ₅ N ₄ H ₁₈	346	118-120 C	51.23
7.	7e ₄	Brown Semi-solid	C ₂₀ O ₅ N ₄ H ₁₈	394	—	50.73
8.	8f ₃	Brown Semi-solid	C ₂₀ O ₆ N ₄ H ₁₈	410	—	37.94
9.	8f ₄	Brown Semi-solid	C ₁₅ O ₆ N ₄ H ₁₆	348	—	33.33
10.	8f ₅	Brown Semi-solid	C ₁₇ O ₅ N ₄ H ₂₀	360	—	43.95
11.	10h ₁	Yellow viscous liquid	C ₂₅ O ₆ N ₅ H ₂₅	491	—	27.35
12.	10h ₂	Brown Semi-solid	C ₂₄ O ₇ N ₅ H ₂₅	585	—	18.86
13.	10h ₃	Reddish Brown semisolid	C ₂₆ O ₇ N ₅ H ₂₉	523	—	43.95

SPECTRAL RESULTS

1)7e₅: ¹HNMR (300 MHz CDCl₃) δ in PPM: 7.9 (1H,m,- NH), 7.7 (1H, m, Aromatic-H), 7.4 (2H, m, Aromatic-H), 6.8 (2H, m, Aromatic-H), 4.9 (1H, m, ar-H), 3.8 (1H, s, COCH₃), 1.4 (2H, d, β-CH₂ of Leucine), 1.2 (1H, m, β -H), 0.95 (6H, CH₃ group of Leucine)

IR (CHCl₃) in cm⁻¹: 3305.5 (Aromatic C-H stretching), 2930.9 (Aliphatic C-H stretching), 1707.3 (C=O (ester) stretching), 1608 (C=O (amide) stretching), 1545.7 (N-H bending), 1509.2 (C-H bending)

2)7e₁: 7.7 (2H, d, Aromatic-H), 7.6 (2H, d, aromatic-H), 7.14 (1H, s, Aromatic-H), 6.3 (1H, br.s, NH₂), 4.6 (1H, m α-H), 3.8 (3H, s,-COCH₃), 1.2 (1H, m, β-h), 0.85 (6h, CH₃ group of Valine)

IR (CHCl₃) in cm⁻¹: 3292.1 (Aromatic C-H Stretching), 2931.1 (aliphatic C-H stretching), 1701.5 (C=O (ester) stretching), 1628.9 (C=O (amide) stretching), 1543.6 (N-H bending), 1487.3 (C-H bending)

3) 8f₅: ¹H NMR (300 MHz CDCl₃) δ in PPM: 7.9 (1H,m,- NH), 7.7 (1H, m, Aromatic-H), 7.4 (2H, m, Aromatic-H), 6.9 (2H m, Aromatic-H), 4.8 (1H, m,

α -H), 3.8 (1H, s, COCH₃), 2.9 (1H, s, -N-CH₃), 1.4 (2H, β -CH₂ of Leucine), 1.2 (1H, m, γ -H), 0.95 (6H,d, CH₃ group of Leucine)

IR (CHCl₃) in cm⁻¹ : 3305.5 (Aromatic C-H stretching), 2930.9 (Aliphatic C-H stretching), 2870.1 (Aliphatic C-H Stretching), 1707.3 (C=O (ester) stretching), 1609 (C=O (amide) stretching), 1544.7 (N-H bending), 1508.2 (C-H bending)

Mass in m/z: 375, 352, 313, 285, 253, 220, 171, 146, 121, 102 and 100.

4) 8f₁ : (300 MHz CDCl₃) δ in PPM: 7.7 (2H, d, Aromatic-H), 7.6 (2H, d, aromatic-H), 7.15 (1H, s, Aromatic-H), 6.2 (1H, br.s, NH₂), 4.6 (1H, m α -H), 3.08 (3H, s, -COCH₃) 2.9 (3H,s, -NCH₃), 1.2 (1h, m, β -H), 0.85 (6H, CH₃ group of Valine)

IR (CHCl₃) in cm⁻¹ : 3292.1 (Aromatic C-H Stretching), 2931.1 (aliphatic C-H stretching), 2855.4 (aliphatic C-H stretching), 1701.5 (C=O (ester) stretching), 1628.9 (C=O (amide) stretching), 1543.6 (N-H bending), 1487.3 (C-H bending)

Mass in m/z: 361,336,320,306,271,250,238,225,207,190,158,125, 98and 96.

ANTIMICROBIAL ACTIVITY

The synthesized compounds were screened for antibacterial and antifungal activities. The antibacterial and antifungal activity were studied in a concentration of 50 μ g/ml against four bacterial (*S.aureus*, *B.subtilis*, *P.aeruginosa* and *E-coli*) and one fungus (*C.albicans*) by disc diffusion method. Solution of benzil penicillin and fluconazole were used as standard antibacterial and antifungal drugs respectively solvents used for both standards.

The culture media used were nutrient agar and Sabouraud's^[6] medium for bacteria and fungus respectively. All compounds have shown promising antibacterial and antifungal activity. However, the activities 8f₁,8f₂,8f₅ and 10h₁,10h₂,10h₃ of compounds 10h₁,10h₂,10h₃ were more pronounced in comparison to compounds 8f₁,8f₂,8f₅ and found to be equivalent to 90% of standards drugs benzyl penicillin and fluconazole.

Table 2: Results of Antimicrobial Activity

SL.No	Diameter of Zone of inhibition (in mm)					
	Compound No	B.Sub	S.aur	E.coli	P.aer	C.alb
1	31	10	10	11	9	12
2	32	9	-	-	9	18
3	33	10	9	9	10	9
4	34	10	10	11	9	10
5	35	10	-	-	9	8
6	36	16	-	12	-	21
7	37	15	18	11	10	20
8	38	10	21	13	-	18
9	39	11	16	16	15	19
10	40	12	24	13	-	20
11	41	18	20	15	16	16
12	42	19	21	16	16	17
13	43	18	15	14	13	16
14	Benzil penicillin	16	25	16	16	-
15	Fuconzole	-	-	-	-	20

Table 3: Minimum Inhibitory Concentration for Antifungal Activity

Sl. No	Compound No	Presence/Absence of growth						
		Candida albicans						
Dilutions		I	II	III	IV	V	VI	VII
1	31	-	+	+	+	+	+	+
2	32	-	-	+	+	+	+	+
3	33	-	-	-	+	+	+	+
4	34	-	+	+	+	+	+	+
5	35	-	-	+	+	+	+	+
6	36	-	-	+	+	+	+	+
7	37	-	+	+	+	+	+	+
8	38	-	+	+	+	+	+	+
9	39	-	+	+	+	+	+	+
10	40	-	+	+	+	+	+	+
11	41	-	+	+	+	+	+	+
12	42	-	-	+	+	+	+	+
13	43	-	+	+	+	+	+	+
14	Fluconazole	-	-	-	-	-	-	-

'+' indicates presence of growth, '-' indicates absence of growth

ANTHELMINTIC ACTIVITY

All compounds were tested for anthelmintic activity by Garg²⁵[7] method using Mebendazole as standard drug. Anthelmintic activity studies were carried out against *Eudrilus eugeniae*. The study revealed that the compounds 8f₁, 8f₂, 8f₅ have moderate to

significant activities and their efficacy is enough to develop as clinically useful agents. The dipeptide compound 10h₁, 10h₂, 10h₃ showed 95% activity when compared with standard drug and hence requires special attention for developing as therapeutic agents.

Table 4: Results of Anthelmintic Activity:

Sl.No	Compound No	Conc. of the compound (mg)	Mean paralyzing time (min) ± S.E		Mean death time (min) ±S.E	
1	Control	-	N.E			
2	Mebendazole	100	6.00	± 0.34	7.40	± 0.24
3	36	100	6.90	± 0.31	7.35	± 0.47
4	37	100	6.12	± 0.35	7.34	± 0.43
5	38	100	6.10	± 0.51	6.90	± 0.33
6	39	100	7.04	± 0.32	8.29	± 0.24
7	40	100	6.10	± 0.31	7.40	± 0.37
8	41	100	7.05	± 0.29	7.90	± 0.37
9	42	100	6.00	± 0.25	7.45	± 0.26
10	43	100	6.05	± 0.28	7.95	± 0.32

S.E represent Standard Error and N.E. indicates No Effect

DISCUSSION AND CONCLUSION

All the synthesized compounds were subjected to antimicrobial activity and anthelmintic activity. N-methylated analog has shown significant anti bacterial activity. The dipeptides showed good activity against gram positive as well as gram negative *E.coli* and *P.aereginosa*. All the compounds showed potent antifungal activity against *C.albicans*.

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