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Research Article

# SYNTHESIS OF 4-[(4)5- IMIDAZOLYL] BENZOYL DERIVATIVES OF AMINO ACIDS AND PEPTIDES AS POTENT ANTHELMINTIC AGENTS

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**ABSTRACT**
A series of 4-[(4)5-imidazolyl] benzoic acids derivatives of amino acids and peptides were synthesized by reacting 4-[(4)5-imidazoyl benzoic acid with amino acids and dipeptides. The structures of the synthesized compounds were characterized by FTIR, $^{1}$H NMR, $^{13}$C NMR, and GC-MS data and the synthesized compounds were screened for their anthelmintic activity and the results showed that the compounds containing Ala-Ile and Pro-Val exhibited potent anthelmintic activity against *Eudrillus Eugenia* at that of standard Mebendazole a concentration of 100mg/50ml. The synthesized compounds were also studied for their insecticidal activity and they showed moderate to less insecticidal activity as compared to the standard drug Chloropyrifos.

**Key words:** Benzimidazole, Peptides, solution phase peptide synthesis, anthelmintic activity and insecticidal activity.

## INTRODUCTION

Imidazoline containing compounds are known to be the ligands of imidazoline-receptores[1] and reveal pharmacological activity towards metabolism, secretion, ion exchange, intraocular pressure and to the cardiovascular system. imidazole-nitroge donor atoms are common binding site for metal ions in various metalloenzymes[2]. Imidazole act as antituberculosis agent[3] and it also possesses anticonvulsant activity[4]. Peptides are the important class of organic compounds with potent biological activities. Peptides function as hormones, enzymes, enzyme inhibitors or substrates, growth promoters or inhibitors, neurotransmitters and immunomodulators[5-10]. Therefore incorporation of imidazole in to amino acids and peptides was expected to result in compounds with high medicinal values. Thus an attempt has been made to synthesize a new bioactive series of imidazole derivatives of amino acids and peptides and biological studies were performed on these synthetic compounds.

## MATERIALS AND METHODS

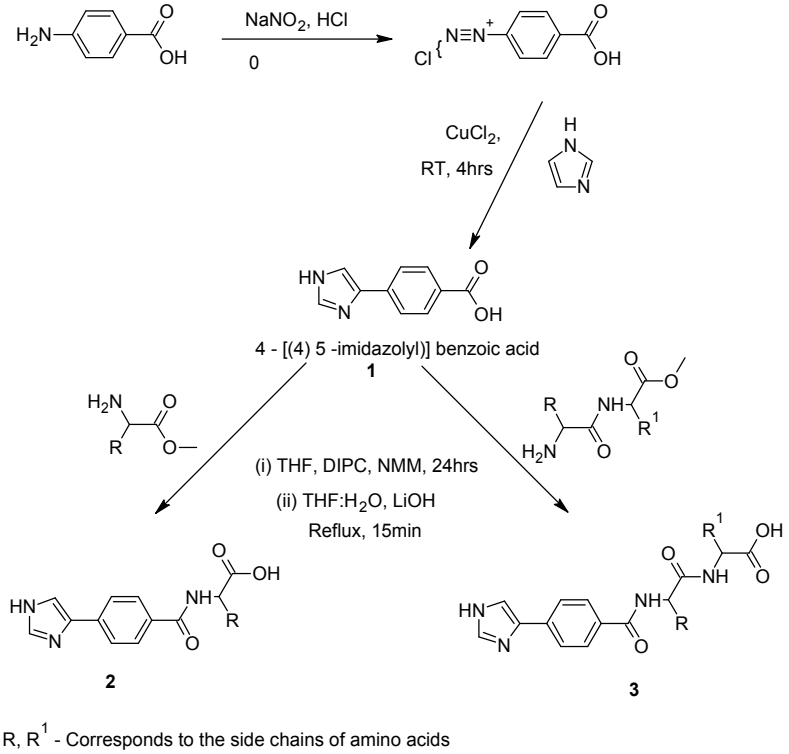
Amino acids, di-tert-butylpyrocarbonate, trifluoroacetic acid, diisopropyl carbodimide were obtained from Spectrochem Ltd. Mumbai and used as such. All the reactions requiring anhydrous conditions were conducted in dried apparatus and were magnetically stirred unless otherwise stated. Organic extracts were dried over anhydrous sodium sulphate. The melting points were determined by capillary method and were uncorrected. FTIR characterization for the synthesized compounds were done with Avatar Thermo Nicolett-350 FT/IR-spectrometer using a thin film support on KBr pellets for solids and chloroform as a solvent for semisolids. The values are reported as Vmax(cm-$^{1}$). $^{I}$H NMR spectra were recorded on GEOL-JMS D-300 (MHz) NMR spectrometer. The spectra were obtained in CDCl3 and the chemical shift values are reported as values in ppm relative to TMS ( as internal standard. Multiplicities were described using the abbreviations: s=singlet, d=doublet, q=quartet, m=multiplet and br=broad. The ES-MASS spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer using Argon/Xenon as the carrier gas. The spectra were recorded at room temperature using m-nitrobenzyl alcohol as the matrix.

**Preparation of 4-[(4)5-imidazolyl] benzoic acid**

A mixture of p-amino benzoic acid (3.425 gms, 25 mmol), dilute hydrochloric acid (15%, 12ml) and water (15ml) was heated to get a clear solution. The solution was cooled to RT and diazotized by the addition of sodium nitrite solution (30%, 4.8ml). The diazonium salt solutions were filtered and to the filtrate, dilute HCl (10ml) and imidazole (25 mmol) and aqueous cupric chloride (0.5gms in 2ml of water) were added with stirring. Stirring was continued for 6 hrs and kept overnight in the refrigerator. The separated solid was collected by filtration and washed with water to obtain pure 4-[(4)5-imidazolyl] benzoic acid.Preparation of 4-[(4)5-imidazolyl]benzoyl amino acid and Peptide methyl esters

To the amino acid methyl ester / peptide methyl ester (7.0 mmol.), THF (30ml), added 4-[(4)5-imidazolyl]benzoic acid (7.0 mmol.), DiisopropylCarbodiimides (DIC)(1.1ml), NMM (1.6ml) and stirred at room temperature for 24 hours. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure, residue was dissolved in CHCl3, washed with 10% NaHCO3 (10ml) and 5% HCl (10ml), dried over anhydrous Na2SO4 and evaporated under vacuum to get the title compounds. The crude product was recrystallized from CHCl3. Using the above procedure the following compounds were prepared[11-14].

**SCHEME 1**




R, $R^1$ - Corresponds to the side chains of amino acids

**SPECTRAL DATA:**

**4-[(4)5-imidazolyl] benzoic acid (1):**

FTIR (KBr $cm^{-1}$) 3339(-NH Stretch), 3038(Arom-CH Stretch), 1712(CO Stretch), 2933(-OH of COOH Stretch). $^1H$ NMR ($CDCl_3$): 7.8-7.1 (m, 6H, Ar-H), 7.33 (s, 1H, -NH), 4.2(s, 1H-OH). GC-MS: m/z: 189 (M+1).

**4-[(4)5-imidazolyl] benzoyl Phenyalanine (2a):**

FTIR (KBr $cm^{-1}$) 3342(-NH Stretch), 3030(Arom-CH Stretch), 2978(-CH Stretch), 1709(CO Stretch), 2928(-OH Stretch). $^1H$ NMR ($CDCl_3$): 8.1-7.1 (m, 11H, Ar-H), 7.28 (s, 1H, -NH), 4.7(s, 1H, -OH), 3.2(m, 2H, $CH_2$). GC-MS: m/z: 336 (M+1).

**4-[(4)5-imidazolyl] benzoyl Tyrosine (2b):**

FTIR (KBr $cm^{-1}$) 3330(-NH Stretch), 3080(Arom-CH Stretch), 2920(-CH Stretch), 1724(CO Stretch), 2910(-OH Stretch). $^1H$ NMR ($CDCl_3$): 7.9-7.4 (m, 10H, Ar-H), 6.88 (s, 1H, -NH), 4.3(s, 1H, -OH), 3.5(m, 2H, $CH_2$). GC-MS: m/z: 352 (M+1).

**Evaluation of Anthelmintic Activity**

Anthelmintics are therapeutic that act either locally to expel or destroy parasitic worms from the gastrointestinal tract or systematically to eradicate adult helminthes or development forms form that invade organs and tissues[15]. The in vitro screening by a suitable method was performed, although in vivo screening methods provide a natural environment for the studies.

**General Procedure**

Anthelmintic activity studies of all synthesized compounds were carried out by Garg's method[16]. The sample

**4-[(4)5-imidazolyl] benzoyl β-Alanyl-Phenylalanine (3a):**

FTIR (KBr $cm^{-1}$) 3392(-NH Stretch), 3068(Arom-CH Stretch), 2974(-CH Stretch), 1691(CO of ester group),1633(CO Stretch), 2928(-OH Stretch). $^1H$ NMR ($CDCl_3$): 8.4-7.2 (m, 11H, Ar-H), 7.12 (s, 1H, -NH), 4.1(s, 1H, -OH), 3.2(m, 2H, $CH_2$). GC-MS: m/z: 407 (M+1).

**4-[(4)5-imidazolyl] benzoyl L-Alanyl-Isoleucine(3b):**

FTIR (KBr $cm^{-1}$) 3345(-NH Stretch), 3036(Arom-CH Stretch), 2962(-CH Stretch), 1746(CO Stretch), 2940(-OH Stretch). $^1H$ NMR ($CDCl_3$): 7.7-7.2 (m, 6H, Ar-H), 7.12 (s, 1H, -NH), 4.5(s, 1H, -OH), 3.8(m, 2H, $CH_2$). GC-MS: m/z: 374 (M+1).

**4-[(4)5-imidazolyl] benzoyl Prolyl-Valine(3c):**

FTIR (KBr $cm^{-1}$) 3353(-NH Stretch), 3046(Arom-CH Stretch), 2952(-CH Stretch), 1736(CO Stretch), 2930(-OH Stretch). $^1H$ NMR ($CDCl_3$): 8.0-7.5 (m, 6H, Ar-H), 7.22 (s, 1H, -NH), 4.3(s, 1H, -OH), 3.5(m, 2H, $CH_2$). GC-MS: m/z: 398 (M+1).

suspension of the synthesized compounds were prepared by stirring the sample mixture with 15%of tween 80 titutated with water for 30min. the test samples and standard drug Mebendazole were prepared at aconcentration of 100mg in 50ml. to the beaker containing 50ml suspension of test sample, standard *Eudrilus Eugeniea* (earthworms) were placed at room temperature. A control suspension of 15% Tween 80 in 50ml distilled water was also taken. The time required for the paralysis and death of the earthworm in the standard as well as test sample suspension were noted. The results of the anthelmintic study are shown in Table 2.

**Table 1: Physical data of 4-[(4)5-imidazoyl amino acid/ dipeptides**

| Code | Compound | Molcecular Formula | Molecular Weight | Physical State | | Melting Point | % Yield |
|---|---|---|---|---|---|---|---|
| | | | | Colour | State | | |
| 1. | 4-[(4)5-imidazolyl] benzoic acid | $C_{10}H_8N_2O_2$ | 188.18 | Brown | Solid | 212 | 23.1 |
| 2a. | 4-[(4)5-imidazolyl] benzoyl Phenyalanine | $C_{19}H_{17}N_3O_3$ | 335.35 | Brown | Semi Solid | - | 71.3 |
| 2b. | 4-[(4)5-imidazolyl] benzoyl Tyrosine | $C_{19}H_{17}N_3O_4$ | 351.35 | Brown | Semi Solid | - | 63.81 |
| 3a. | 4-[(4)5-imidazolyl] benzoyl β-Ala-Phenylalanine | $C_{22}H_{22}N_4O_4$ | 406.4 | Brown | Semi Solid | - | 78.3 |
| 3b. | 4-[(4)5-imidazolyl] benzoyl L-Ala-Isoleucine | $C_{19}H_{24}N_4O_4$ | 372.42 | Brown | Semi Solid | - | 86.9 |
| 3c. | 4-[(4)5-imidazolyl] benzoyl Pro-Valine | $C_{20}H_{24}N_4O_4$ | 384.42 | Brown | Semi Solid | - | 73.4 |

**Table 2: Data of Antihelmintic Activity**

| SI.No | Compounds | Concentration (100mg/50ml) | Duration in Minutes | |
|---|---|---|---|---|
| | | | Paralysis Time | Death Time |
| 1 | 2a | 100 | 1:45 | 1:50 |
| 2 | 2b | 100 | 1:55 | 2:05 |
| 3 | 3a | 100 | 1:50 | 2:00 |
| 4 | 3b | 100 | 2:20 | 2:25 |
| 5 | 3c | 100 | 2:15 | 2:20 |
| 6 | Mebendazole | 100 | 2.20 | 2.30 |
| 7 | Control | - | No effect | No effect |

**Table 3: Data of Insecticidal Activity**

| SI.No | Compounds | Concentration (100mg/2ml) | Death Time Hrs:min |
|---|---|---|---|
| 1 | 2a | 100mg | 4:30 |
| 2 | 2b | 100mg | 3:10 |
| 3 | 3a | 100mg | 4:20 |
| 4 | 3b | 100mg | 6:00 |
| 5 | 3c | 100mg | 4:10 |
| 6 | Chloropyrifas | 100mg | 2.45 |
| 7 | Control | - | No effect |

**Evaluation of insecticidal activity**

Insecticides are the chemical agents used to kill insects. Insecticidal activity studies of the synthesized compounds were carried out by the following method [17].

**General Procedure**

The insecticidal activity studies were carried against *Coptotermes Formosanus* a termite by Morita el al., method[12]. The test samples were prepared by dissolving 100mg of the synthesized compounds in chloroform. The Whatmann filter papers were cut to the inner diameters of the petri plates and fitted in all plates. The test samples of (100mg/2ml) concentration were poured on to the filter papers. The standard, chloropyrifos were also prepared in similar steps and control containing only the solvent was poured on to the petri plate fitted with filter paper. Termites of 5 nos. were placed on each plate and covered with a lid, with wet cotton placed on the upper lid. The death time was observed and the results were tabulated in Table 3.

## RESULTS AND DISCUSSION

A series of 4-[(4)5-imidazolyl] benzoic acids derivatives of amino acids and peptides were synthesized by reacting 4-[(4)5-imidazoyl benzoic acid with amino acids and dipeptides as illustrated in scheme 1. The structures of the synthesized compounds were characterized by FTIR, $^1$H NMR and GC-MS data and the synthesized compounds were screened for their anthelmintic activity and the results showed that the compounds **3b** and **3c** exhibited potent anthelmintic activity against *Eudrillus Eugenia* at that of standard Mebendazole a concentration of 100mg/50ml. The synthesized compounds were also studied for their insecticidal activity and they showed moderate to less insecticidal activity as compared to the standard drug Chloropyrifas.

## CONCLUSION

A series of 4-[(4)5-imidazolyl] benzoic acids derivatives of amino acids and peptides were conventionally synthesized with significant yield. The synthesized compounds showed potent anthelmintic activity with that of standard Mebendazole. The synthesized compounds expected to exhibit anthelmintic activity probably by inhibiting microtubule polymerization which is similar to that of Benzimidazole (Thiabendazole,Mebendazole, Albendazole). The compounds were also showed to possess moderate to less insecticidal activity.

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