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Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF INDOLE-3-CARBOXYLIC ACID DERIVATIVES OF AMINO ACIDS AND PEPTIDES

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ABSTRACT
A novel series of Indole-3-carboxylic acid derivatives of amino acids and peptides were synthesized by solution phase technique. The synthesized compounds were characterized by FTIR, ^{1}H NMR and mass spectral analysis and evaluated for their antibacterial and anthelmintic activities. The compounds exhibited significant antibacterial and anthelmintic activities as compared to standard drugs Clotrimazole and mebendazole, respectively.

KEYWORDS: Indole-3-carboxylic acid, amino acids/peptides, antibacterial activity.

INTRODUCTION
Indoles and their derivatives are found to be associated with various biological activities such as anticancer[1], antibacterial[2], antifungal[3], anthelmintic[4] and antiinflammatory[5,6] activities. In view of the diverse biological activities associated with indoles, we wish to report the synthesis and antibacterial and anthelimintic activities of amino acids and peptides incorporated with Indole-3-carboxylic acid. The molecule with Indole-3-carboxylic acid derivatives of amino acids and peptides were synthesized by using DCC/Et3N mediated solution phase technique of peptide synthesis. The acid group was protected by esterification process. The Boc-amino acids were coupled with amino acid methyl ester hydrochlorides by dicyclohexylcarbodiimide (DCC) as a coupling agent and triethylamine (Et3N) as a base to get protected dipeptides. The with Indole-3-carboxylic acid was coupled with Boc-dipeptides using DCC to get with Indole-3-carboxylic acid derivatives of amino acid and dipeptide followed by hydrolysis of Boc-group with trifluoroacetic acid.

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[7]. N-methylated amino acids were prepared using NaH/CH_3I by Benoiton method[8]. 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, Triethylamine (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 Indole-3-carboxyl derivatives of Amino acids/peptides[9]

To the amino acid/peptides (10mmol) in $CHCl_3$ (25ml), Indole-3-carboxylic acid (10mmol), Et_3N (2.69 ml, 20mmol) and DIPC was added at 0^0C and stirred for 24 hours. The reaction mixture was washed with 10% $NaHCO_3$ until the byproduct 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), LiOH (0.274 g, 2.4mmol) was added, stirred for 2 hour at room temperature and washed with 10% $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4. The physical data of the synthesized compound is mentioned in the tabular column-1.

Antimicrobial activity[10]: The antimicrobial activity was determined using disc diffusion method by measuring the inhibition zone in mm. All the synthesized compounds were evaluated *in vitro* for their antibacterial and antifungal activities. The compounds were tested at a concentration of 50 µg/mL against bacterial strains (*Enterococcus faecalis* and *Escherichia coli*) diffusion method, respectively. Ampicillin and fluconazole were served as standard drugs for comparison of the results. The culture media used were nutrient agar and sabourd's medium for bacteria and fungus strains, respectively. The results are presented in Table-3.

Anthelmintic activity[11]: The synthesized compounds were evaluated for their anthelmintic activity against *Eudrilus eugenia* by Garg's and Atal method[11] using mebendazole as a standard drug. All the synthesized compounds were found to be potent anthelmintics. The results are given in Table-2.

Spectral Data

Compound-1: Indole-3-carboxyl phenylalanine methyl ester: **IR (KBr Pallets):** 3361.53(N-H stretch), 3020.41 (aromatic C-H stretch), 2934.32, 2859.61(aliphatic C-H stretch), 1665.04(C=O stretch (amide cm^{-1}. **^{1}H NMR (300MHz, $CDCl_3$):** δ7.186 - 7.819 (11 H, m, aromatic –H), δ6.491 (1H, d, -NH) δ4.407 (1H, m, αH), δ3.797 (3H, s, -OCH3), δ3.393 (2H, d, β H) **FABMass:** m/z = 323.1.

Compound-2: Indole-3-carboxyl tyrosine methyl ester: 3243.60 (N-H stretch), 2926.59, 2855.06 (aliphatic C-H stretch),1734.06 (C=O stretch(ester)), 1668.90 (C=O stretch(amide) cm^{-1}. **^{1}H NMR (300MHz, $CDCl_3$):** δ7.188 - 7.649 (10 H, m, aromatic –H), δ4.407 (1H, m, α H), δ3.797 (3H, s, -OCH3), δ3.393 (2H, d, β H).**FABMass:** m/z = 337.20.

Compound-3 : Indole-3-carboxyl glycine methyl ester: IR (KBr Pallets): 3321.03 (N-H stretch), 2931.55, 2857.37 (aliphatic C-H stretch), 1747.13 (C=O stretch(ester)), 1667.31 (C=O stretch(amide) cm^{-1}. **^{1}H NMR (300MHz, $CDCl_3$):** δ7.280 - 8.228 (6H, m, aromatic –H, -NH-), δ4.086, 4.079 (2H, d, α H), δ3.843 (3H, s, -OCH3) **FABMass:** m/z = 231.1.

Compound-4: Indole-3-carboxyl dipeptide methyl ester: IR (KBr Pallets): 3301.93 (N-H stretch),3057.41 (aromatic C-H stretch), 2926.47,2855.41 (aliphatic C-H stretch), 1651.53 (C=O stretch (amide) cm^{-1}. **^{1}H NMR (300MHz, $CDCl_3$):** δ8.896 - 6.929 (15 H, m, aromatic –H, NH-), δ6.929 (2H, m, -NH), δ4.128 (2H, m, α H), δ3.680 (3H, s, - OCH3), δ3.103 (4H, d, β H).**FABMass: m/z** 485.35.

Compound-5: Indole-3-carboxyl (N-Me) Phe methyl ester: IR (KBr Pallets): 3281.99 (N-H stretch), 2924.10, 2854.42 (aliphatic C-H stretch), 1371.69 (aliphatic C-H in plane bending), 1671.24 (C=O stretch (amide) cm^{-1}. **^{1}H NMR (300MHz, $CDCl_3$):** δ7.187 - 8.228 (11 H, m, aromatic –H, NH-), δ4.128 (1H, m, α H), δ3.680 (3H, s, -OCH_3), δ3.103 (2H, d, β H) . **FABMass:** m/z = 358.2.

RESULTS AND DISCUSSION

Structural modification of Indole-3-carboxylic acid was carried out by coupling N-methylated amino acids and dipeptides with the amino group of indole-3-carboxylic acid and the synthesized compounds were characterized by FTIR, ^{1}H NMR and Mass spectral analysis. The compounds were subjected to

antimicrobial evaluation by Disk Diffusion method. All the compounds had shown potent antibacterial activity against *Enterococcus faecalis* which can be comparable to the standard drug (Clotrimazole and mebendazole). In fact all the bacterial strains used for the study were susceptible to the synthesized compounds but *Enterococcus faecalis* was more sensitive than other strains to the synthesized compounds. However the compound 4 and 5 having (N, dipep) phe-tyr unit as a substituent showed better activity equally to the n-methylated compounds. Final conclusion was made, based on the antimicrobial activities of the newly synthesized indole-3-carboxylic acid derivatives, the methylated and dipeptide compounds had shown potent antibacterial and anthelimintic activities.

REFERENCES

1. John Porter , Simon Lumb, Richard J. Franklin, Jose M. Gascon-Simorte, Mark Calmiano, Kelly Le Riche, Benedicte Lallemand, Jean Keyaerts, Helen Edwards, Alison,MaloneyJean Delgado, Lloyd King, Anne Foley, Fabien Lecomte, James Reuberson, Christoph Meier, Mark Batchelor, Discovery of 4-azaindoles as novel inhibitors of c-Met kinase, Bioorganic & Medicinal Chemistry Letters 2009 ;19: 2780–2784.
2. Rakesh Kumar Tiwari,a Devender Singh,a Jaspal Singh,a Vibha Yadav, Ajay K. Pathak, Rajesh Dabur,Anil K. Chhillar, Rambir Singh, G. L. Sharma, Ramesh Chandraa, and Akhilesh K. Vermaa, Synthesis and antibacterial activity of substituted1,2,3,4-tetrahydropyrazino [1,2-a] indoles, Bioorganic & Medicinal Chemistry Letters 2006; 16: 413–416.
3. Chung-Kyu Ryu, Jung Yoon Lee, Rae-Eun Park, Mi-Young Ma and Ji-Hee Nho,Synthesis and antifungal activity of 1H-indole-4,7-diones, Bioorganic & Medicinal Chemistry Letters 2007 ;17: 127–131.
4. Yong Hai Nan, Jeong-Kyu Bang, Song Yub Shin, Design of novel indolicidin- derived antimicrobial peptides with enhanced cell specificity and potent anti-inflammatory activity, Peptides 2009; 30 :832–838.
5. Roman Mezencev, Melina Galizzi b, Peter Kutschy, Roberto Docampo, Trypanosoma cruzi: Antiproliferative effect of indole phytoalexins on intracellular amastigotes in vitro, Experimental Parasitology 2009; 122: 66–69.
6. Himaja M, Rajiv, Ramana MV, Synthesis of 6-nitrimidazolyl-1-acetyl amino acids and peptides as potent anthelmintic agents, Ind J Hetero Chem 2002; 12: 121-124.
7. Webb RG, Haskell MW, Stammer CH. A novel method of protection for the carboxyl group, J. Org. Chem 1994; 59: 5912.
8. Belagali SL, Mathew T, Himaja M, Kocienski P. A highly efficient method of N-methylation for amino acid derivatives, Ind. J. Chem 1995; 34B:45-47.
9. Bodanszky M, Bodanszky A, Practice of Peptide synthesis (Springer- Verlag, New York), 1984: 143.
10. Y.Morita et al, Biological activity of Propolone", Biological Pharma Bulletin, 2003; 26: 1487
11. Garg LC, Atal CK. Evaluation of anthelmintic activity, Ind J Pharmacology 1969; 31: 104

Table 1: Physical data of synthesized amino acid/dipeptide Indole-3-carboxylic acid

S.No	Dipeptides	Mol For.	Mol. Wt	Physical state	% yield
1.	Indole-3-carboxyl phenylalanine methyl ester	$C_{19}H_{18}N_2O_3$	323.1	Yellow semisolid	87.2
2.	Indole-3-carboxyl tyrosine methyl ester	$C_{19}H_{18}N_2O_4$	337.2	Orange crystals	82.4
3.	Indole-3-carboxyl glycine methyl ester	$C_{12}H_{12}N_2O_4$	231.1	Brown semisolid	78.9
4.	Indole-3-carboxyl dipeptide methyl ester	$C_{28}H_{26}N_3O_5$	485.3	Yellow crystals	84.6
5.	Indole-3-carboxyl (N-Me) Phe methyl ester	$C_{20}H_{20}N_2O_3$	358.2	Pale brown crystals	81.2

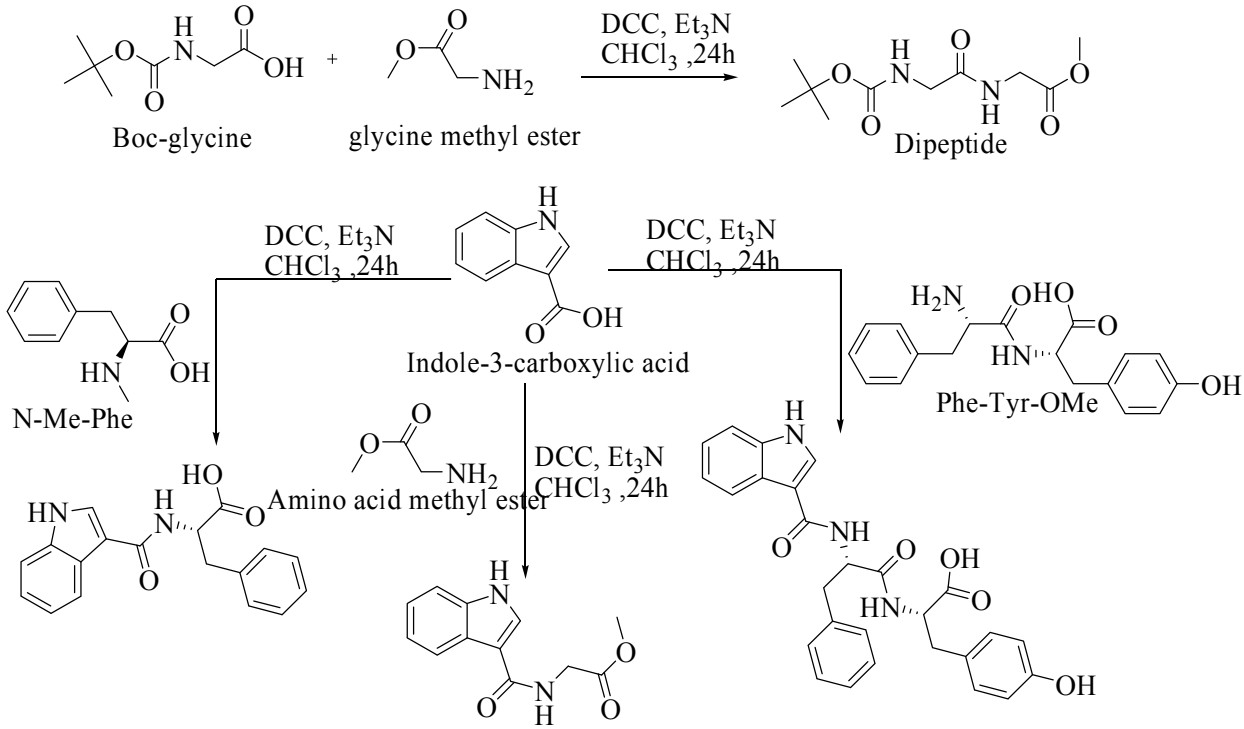
Table-2 Results of Anthelimintic activity

S.No	Synthesized compounds	Conc. (mg/50ml)	Paralyzing time	Death time
1.	Indole-3-carboxyl phenylalanine methyl ester	100	01:10	02:00
2.	Indole-3-carboxyl tyrosine methyl ester	100	01:00	02:14
3.	Indole-3-carboxyl glycine methyl ester	100	00:45	02:00
4.	Indole-3-carboxyl dipeptide methyl ester	100	04:10	07:00
5.	Indole-3-carboxyl (N-Me) Phe methyl ester	100	07:00	11:00
6.	Mebendazole	100	01:00	02:10
7.	Control	-	No effect	No effect

Table 3: Antibacterial Activity of Indole-3-carboxylic acid Derivatives

Sl. No	Compound no	Diameter of zone of Inhibition (mm)	
		Enter. fae	*E. coli*
1	Indole-3-carboxyl phenylalanine methyl ester	10	8
2	Indole-3-carboxyl tyrosine methyl ester	15	10
3	Indole-3-carboxyl glycine methyl ester	12	8
4	Indole-3-carboxyl dipeptide methyl ester	17	15
5	Indole-3-carboxyl (N-Me) Phe methyl ester	18	18
6	Ampicillin	20	22

(–) indicates no inhibition zone (no activity)



Synthesized compounds - Indole-3-carboxyl-phe/tyr/gly; Indole-3-carboxyl-phe-tyr/N-Me-Phe

Scheme-I