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Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF 4-[2-(5-NITRO) IMIDAZOLYL] BENZOYL AMINO ACIDS AND PEPTIDES

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ABSTRACT

A new series of 4-[2-(5-nitro) imidazolyl] benzoylamino acids and peptides were synthesized by solution phase technique. The synthesized compounds were confirmed by FTIR, ^{1}H NMR and mass spectral analysis and evaluated for Antimicrobial activity and Anthelmintic activity. Evaluation of the Anthelmintic activity for nitro-imidazole derivatives was carried out. All the compounds were found to possess Anthelmintic activity. Compound 14 showed better activity than the standard drug. All the compounds showed moderate antibacterial activity. The cyclic pentapeptides showed moderate antifungal activity, whereas all the nitro-imidazole derivatives showed potent antifungal activity as compared to the standard drug and compound 1 showed potent antifungal activity, much higher than the standard drug.

Keywords: Peptides, Amino acids, Imidazoles, Antimicrobial activity, Anthelmintic activity.

INTRODUCTION

Imidazole derivatives have occupied a unique place in the field of medicinal chemistry[1-3]. The incorporation of the imidazole nucleus is an important synthetic strategy in drug discovery. The high therapeutic properties of the imidazole related drugs have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents. Imidazole drugs have broadened scope in remedying various dispositions in clinical medicines. Numerous methods for the synthesis of imidazole and also their various structure reactions offer enormous scope in the field of medicinal chemistry. This articles aims to review the work reported, their chemistry and biological activities of imidazole during past years[4-6].

In the field of five membered heterocyclic structures imidazole nucleus shows various properties. The high therapeutic properties of imidazole related drugs have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents. Imidazole drugs have broadened scope in remedying various dispositions in clinical medicines. Medicinal properties of imidazole include anticancer, carboxypeptidase inhibitors, antiaging agents, anticoagulants, anti-inflammatory, antibacterial, antifungal, antiviral, antitubercular, antidiabetic and antimalarial[7-10].

A review of chemical literature on the synthesis of heterocyclic derivatives of amino acids and peptides reveals a few examples of condensation of heterocyclic moieties with amino acids resulting in compounds with potent antibacterial and antifungal activities. It is noticed that not of much work has been carried out on the condensation of heterocyclic moiety with peptide units[11-13].

Nitro-imidazoles have a wide spectrum of chemotherapeutic properties along with various side effects[14]. Hence, an attempt was made towards the synthesis of a new series of 4-[2-(5-nitro)imidazolyl] imidazolyl]benzoylamino acids and peptides with an anticipation of obtaining more potent agents wit least side effects[15].

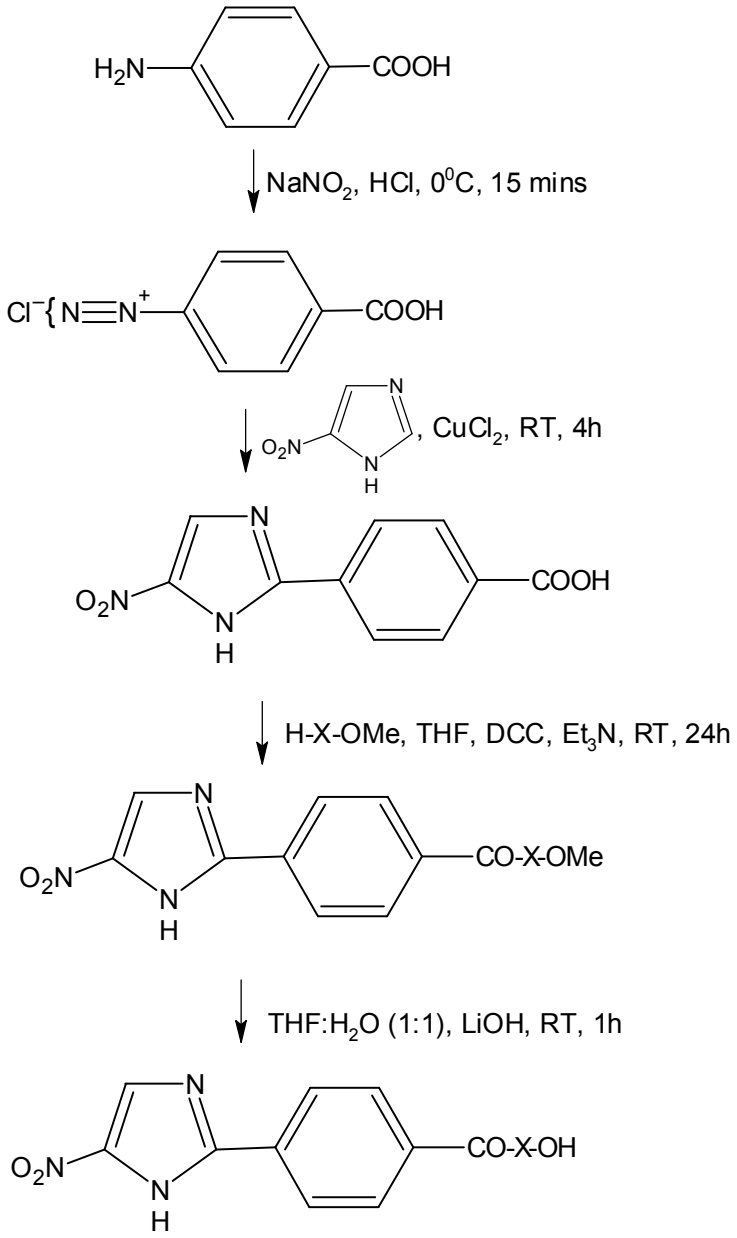
MATERIALS AND METHODS

Synthesis of 4-[2-(5-nitro)imidazolyl]benzoylamino acids and peptides:

p-Aminobenzoic acid was diazotised and subsequently coupled with nitro-imidazole in presence of cupric chloride to get 4-[2-(5-nitro)imidazolyl]benzoic acid (1). The amino acid methyl esters and the dipeptide L-Try-L-Leu-OMe were prepared as per the procedure mentioned in chapter-2 and coupled with compound (1) using DCC and triethylamine (TEA) to get a series of 4-[2-(5-nitro)imidazolyl]benzoyl-

amino acid and peptide methyl esters. The esters were hydrolysed with LiOH to get the free acids: 4-[2-(5-nitro)imidazolyl]benzoylamino acids and peptides (Scheme-1).

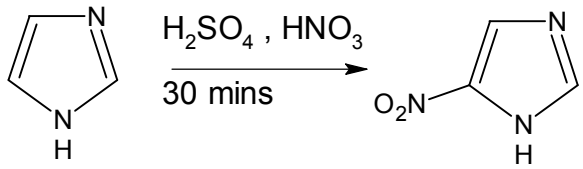
Scheme-1



Where, X = L-Phe, L-Leu, L-Tyr, L-Thr, L-Pro, L-Val, L-Trp-L-Leu

Preparation of 5-nitro-imidazole:

Imidazole is nitrated with nitrating mixture (Conc HNO_3: Conc H_2SO_4, 1:1) by the standard procedure to get nitro-imidazole.



Preparation of 4-2-(5-nitro)imidazolyl]benzoic acid:
P-amino benzoic acid was diazotised with sodium nitrite and dilute HCl at 0^0C. The resulting diazonium salt was then stirred with 5-nitro-imidazole in presence of an aqueous solution of cupric chloride for 4 hours at RT to get the title compound (1).

Synthesis of 4-2-(5-nitro)imidazolyl]benzoylamino acids and peptides:
4-2-(5-nitro)imidazolyl]benzoylamino acids and peptides were prepared by coupling 4-2-(5-nitro)imidazolyl] benzoic acid with corresponding amino acid methyl ester hydrochloride or peptide methyl ester (Scheme 1).

Table-1 Physical Data of Compounds

Compound No.	Physical state	M.P. (^{0}C)	% yield
1	Pale brown solid	256	21.49
2	Reddish brown sticky mass	–	95.5
3	Pale yellow solid	102	23.17
4	Pale brown solid	145	27.41
5	Brown sticky mass	–	35.94
6	Brown sticky mass	–	72.39
7	Reddish brown sticky mass	–	68.74
8	Brown sticky mass	–	99.37
9	Reddish brown crystals	93-95	77.78
10	Yellow crystals	64	72.43
11	Brown crystals	95	48.98
12	Brown solid	82	55.54
13	Brown solid	86	53.31
14	Reddish brown solid	76	93.51
15	Brown sticky mass	–	74.53

The structure of the compounds was confirmed by spectral and elemental analysis.

Evaluation of Antimicrobial Activity
All the plates were kept in the refrigerator for 30 minutes to allow the diffusion of the sample into the surrounding agar medium. Then the plates inoculated with bacterial cultures were incubated at 37 ^{0}C for 18 hours and those with fungi were incubated at 25^0C for 48 hours. Diameter of the zones of inhibition wherever produced were measured and the average diameter for each sample was calculated. The diameters obtained for the test samples were compared with that produced by the standard antibiotics, benzyl penicillin for antibacterial activity and griseofulvin for antifungal activity[16]. The results are given in Table 4.3.

RESULTS AND DISCUSSION

Evaluation of antibacterial and antifungal activity was carried out for all the synthesized cyclic pentapeptides and nitro-imidazole derivatives of amino acids and peptides. All the compounds showed moderate antibacterial activity. The cyclic pentapeptides showed moderate antifungal activity, whereas all the nitro-imidazole derivatives showed potent antifungal activity as compared to the standard drug and compound 39 showed potent antifungal activity, much higher than the standard drug. This compound can be subjected to further studies for the toxicity.

Evaluation of Anthelmintic Activity:
Anthelmintics are therapeutic agents used to destroy parasitic worms or remove them from the infected host**.** The ultimate test of anthelmintic activity is the ability of a chemical agent to eliminate the worms from a specifically parasitized animal with a minimum of toxic effect to the host**.** A suitable *in vitro* test can be considered as a useful screening method, although *in vivo* screening methods provide a natural environment for the studies**.**

General Procedure:
Anthelmintic activity studies were carried out against earth worms (*Portoscoplex corethrusus*) by Garg's method[17]**.**
Suspensions of the samples were prepared by triturating the samples with 0**.**5% tween 80 and distilled water and the resultant mixtures were stirred using a mechanical stirrer for 30 minutes**.** The resulting suspensions were used for the activity studies**.** The suspensions were diluted to contain 200 mg in 5 ml of the test samples**.** Standard drug, piperazine citrate was also prepared with the same concentration in a similar way**.**

Five earth worms of similar sizes were placed in a petri plate of 4 inches diameter containing 50ml of suspension of the test standard drug (piperazine citrate) at room temperature. Another set of five earthworms was kept as control in 50ml suspension of distilled water and 0.5% tween 80.

50ml each of the suspensions of the test compounds were added into separate petri plates containing five earthworms in each. The time required for the paralysis and death of the worms were noted. The death time was ascertained by placing the earthworms in warm water at 50^0C, which stimulated the movement if the worm was alive (Table 4.4).

Table 2: Data of Antimicrobial Activity

Sl. No.	Compound No.	Diameter of Zone of Inhibition (in mm)					
		B. sub.	***S. aur.***	***E. coli***	***P. aer.***	***C. alb.***	***A. niger***
1	1	10	–	10	–	20	11
2	2	9	–	–	9	10	13
3	3	9	–	10	9	11	10
4	4	10	10	9	10	13	14
5	5	10	11	10	11	15	11
6	6	10	8	11	8	10	9
7	7	11	10	10	9	13	14
8	8	10	10	9	10	16	15
9	9	9	–	–	9	8	14
10	10	10	–	9	8	10	10
11	11	10	9	9	10	9	15
12	12	10	10	11	9	10	11
13	13	10	–	11	–	11	11
14	14	10	10	11	9	12	14
15	15	10	–	9	–	9	10
16	Benzyl penicillin	15	16	14	16	–	–
17	Griseofulvin	–	–	–	–	15	16

Table 3: Data of the Anthelmintic Activity

Sl no	Compound no	Conc. of the compound (mg)	Mean paralysing time (min.)± S.E	Mean death time (min.)± S.E
1	Control	–	N.E.	N.E.
2	Piperazine citrate	200	30.26±1.22	52.02±1.5
3	9	200	32.66±1.18	54.42±1.1
4	10	200	36.38±0.54	58.42±1.26
5	11	200	32.34±0.28	50.46±1.20
6	12	200	32.32±1.22	52.68±2.18
7	13	200	34.42±2.24	52.32±1.14
8	14	200	30.46±1.16	50.22±2.28
9	15	200	34.26±0.24	54.56±1.16

CONCLUSION

A new series of 5-nitro-imidazole derivatives of amino acids and peptides were synthesized with good yields and the structure was confirmed by spectral analysis. The compounds showed moderate antibacterial activity, good Anthelmintic activity and remarkable antifungal activity. Compound 14 showed better Anthelmintic activity than the standard drug. On passing the toxicity tests, these compounds may be good candidates for clinical studies and can be added into new antifungal drugs.

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