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Synthesis, characterization and anthelmintic activity of 4-[2'-(5'-nitro) imidazolyl] benzoyl (n-methyl) amino acid derivatives

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Abstract

Compound containing the imidazole ring are very important in living system, such as vitamin B_{12} and several pilocarpin alkaloids. Condensation of heterocyclic moieties viz nicotinic acid, thiazole, coumarin, quinoline, 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, insecticidal, anti-inflammatory, tyrosinase inhibitory and melanin production inhibitory activities. Imidazoles have been drawn as promising structural units in the field of medicinal chemistry. 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 sythesis of 5-nitroimidazolyl-benzoic acid derivative of N-methyl amino acids and peptide using solution phase technique of peptide synthesis. The synthesized amino acid derivatives studied for anthelmintic activity.

Key-Words: Thiazole, Tyrosine, Quinoline, Imidazole, Valine, Threonine, Alanine

Introduction

A number of synthetic imidazoles such as priscal, privine and antihistamine have physiological properties. The antihistamine azamycine is a 2-nitroimidazole. Amongst synthetic imidazoles in use as therapeutic agents are cimetidine for the treatment of peptic ulcer, metronidazole an antibacterial and an antiprotozoal used in the treatment of amoebic dysentry. Miconazole, clotrimazole, ketaconzole are some currently used antifungal imidazole derivatives. They act by inhibiting ergosterol synthesis which is a primary cellular sterol of fungi cell wall. Ketaconazole was the first successful orally active imidazole.

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. N-methylation was done by treating with methyl iodide and sodium hydride.

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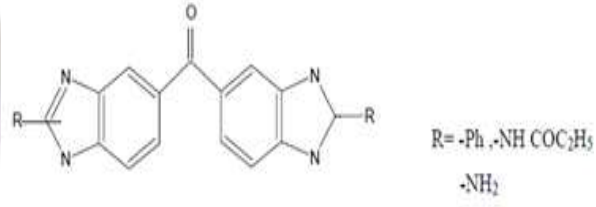
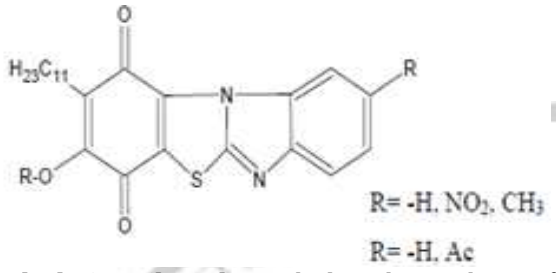
The ester group was then removed by lithium hydroxide. The Boc(N-methyl) amino acids or Boc(N-methyl)dipeptide were coupled to amino acids or Boc(N-methyl)dipeptide were coupled to 4-2')5'-nitro imindazolyl] benzoic acids. The synthesized compounds will be tested for their biological activitie as anthelmintic activitie[1,2].

Some noteworthy works done are mentioned below:

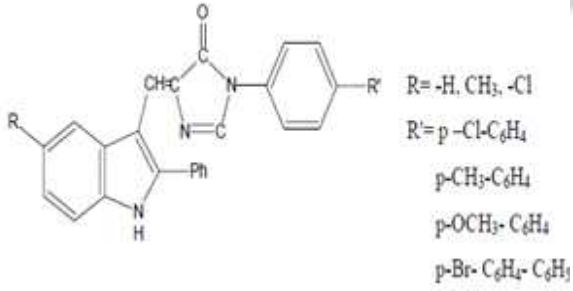
Srivastava R. et al,.Worked on 2,5-disubstituted benzimidazole pyrido[1,2-a]benzimida zoles and some tetrasubstituted benzophenones as probenzimidazoles. This compound showed anti- filarial activity against *L.carinii and B.malayi[5].*



Usha Rani T. et al,. Worked on the synthesis of the substituted-9- hydroxy-10-undecyl [2',3',4,5]-thiazol-[2,3-b] benzimidazole-8,11-diones. These compounds where found to possess fungicidal activity against *F.oxysporum, Lanata and bactericidal activity B.substilis E.coli and P.vulgaris[6]*



Renukadevi Patil et.al,. Worked on the synthesis of 1,2-di-substituted-4-[(5'- substituted-2'-phenylindol-3'-yl)methylene]iminolin-5-(4H)-ones.The compounds were found to exhibit antibacterial activity[7].



Material and Methods

General Method of Amino acid Synthesis:

The method involved in the synthesis of 4-[2' - (5'-nitro) imidazolyl] benzoyl(N-Me) amino acids is the coupling reaction between amino acid and 4-[2'-(5'-nitro) imidazolyl]benzoic acid

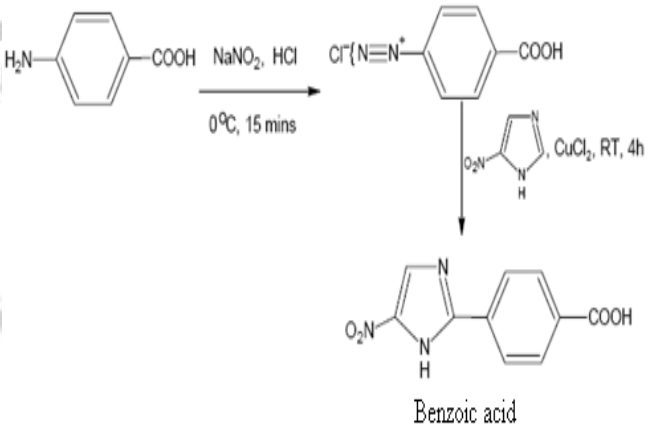
Synthesis of N-methyl amino acids methyl ester:

Amino acids were converted into the corresponding methyl ester hydrochloride using thionyl chloride and methanol. The amino end was then protected by introducing Boc-group using ditertiary butylpyrocarbonate and triethylamine to get Boc-L-amino methyl ester. N-methylation of this compound was done by treating with methyl iodide and sodium hydride (Benoition method) to get Boc-(N-Me) amino methyl ester [1, 5].

Preparation of4-[2-(5-nitro)imidzolyl] benzoic acid :

A mixture of p-amino benzoic acid (34.25 gms, 250 mmol), dilute hydrochloric acid (15%, 120ml) and water (150ml) was heated to get a clear solution**.** The solution was cooled to RT and diazotized by the addition of sodium nitrite solution (30%, 48ml)**.** The diazonium salt solution was filtered and to the filtrate, dilutes HCl (100ml) and nitroimidazole (250 mmol) and aqueous cupric chloride (5gms in 20ml 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**.** The crude compound was crystallized from acetone to obtain pure of4 [2-(5-nitro)imidazolyl]benzoic acid[3,6].

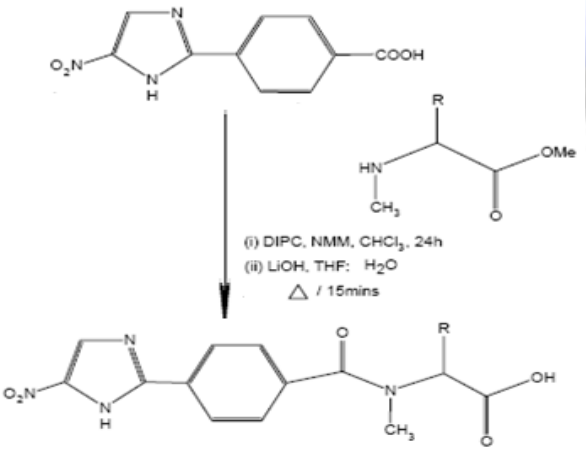
SCHEME-1



Benzoic acid

Preparation of 4-[2'-(5'-nitro) imidazolyl]benzoyl(N-Me)-aminoacid

To the (N-Me) amino acid methylester(7**.**0 mmol.) THF (20ml), added 4-[2'-(5'-nitro) imidazolyl] benzoic acid acid (1.631gms, 7**.**0 mmol.), DIPC, Et_3N (2.8ml) and stirred at room temp.for 24hr. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure, residue was dissolved in $CHCl_3$, washed with 10% $NaHCO_3$ (10ml) and 5% HCl (10ml), dried over anhydrous Na_2SO_4 and evaporated under vacuum to get the title compounds**.** The crude product was recrystallized from $CHCl_3$ and n-hexane [8]**.**



R = Valine, Phenyl alanine, Tyrodine, Threonine, Leucine

Preliminary Analysis of the sample

Thin-layer chromatography (TLC) was commonly used in the qualitative description of the complexity and composition of chemical mixtures.

Application of sample on TLC plates

The sample was applied to the chromatogram by repeated "spotting" above 1-2 cm from one end of the plate with a capillary tube.

The most important precaution was not to apply spots below the level of the top of the solvent system in developing chamber

Developing solvent systems

Development chamber was used for developing chromatogram. Chloroform: Methanol: Water 5:3:2 was the solvent system used for running TLC of these compounds.

Visualization of chromatogram

After developing, the TLC plates were dried and then exposed to iodine vapours in a chamber, since chromalograms of many synthetic products were frequently observed by iodine vapors. Rf value was noted down. Purity of all the synthesized compounds including intermediates was checked by TLC on silica gel G plates. All compounds have shown only single spot indicating the completion of the reaction and thepurity of the product obtained.

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 earthworms *(Eudrilus eugeniea)* by Garg's method.[41]Suspensions of the samples were prepared by triturating the samples with 15% tween 80 and distilled water and the resultant mixtures were stirred using a mechanical stirrer for 30 mins. The resulting suspensions were used for the activity studies. The suspensions were diluted to contain 100 mg in 5 ml of the test samples. Standard drug, Mebendazole was also prepared with the same concentration in a similar way.Five earthworms of similar sizes were placed in a petri plate of 4 inches diameter containing 50 ml of suspension the test standard drugs (Mebendazole) at RT. Another set of five earth worms was kept as control in 50ml suspension of distilled water and 15% tween 80.50 ml 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 was noted. The death time was ascertained by placing the earthworms in warm water at 50°C, which stimulated the movement if the worm was alive. Physical data of this activity is shown in table no.7.

Results and Discussion

The new synthesized amino derivatives were screened for anthelmintic activity; the results of anthelmintic activity against *Eudrilus eugeniea* are shown in Table 7

Conclusion

The new 4-[2'-(5'-nitro) imidazolyl] benzoyl (N-Me) amino acid derivatives was synthesized and characterized by physicochemical analytical data. Bythis studies find the structure-activity relationship and to optimize the structure. The synthesized amino acid derivative i.e., 4-[2'-(5'-Nitro) imidazolyl] benzoyl(N-Me) amino acid derivatives was confirmed by physicochemical & spectral analysis and further screened for anthelmintic activity, all derivatives shows that good activity.

Acknowledgement

In the first place we would like to record my gratitude to my parents and my sister for their inspiration and encouragement given to me during this work with deep appreciation for their determination and enthusiasm at each and every front of my life to transform my dreams into reality.I am very thankful and prevail age to my deep sense of gratitude to Abdul Mohammed Bari, M.pharm, Ph.D, Director of Bright laboratories, Hyderabad.

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Physicochemical analysis

Table 1

S/No.	4-[2'-(5'-nitro)imidazolyl] benzoyl(N-Me)Tyrosine	
1.	Mol. Formula	$C_{20}O_6N_4H_{18}$
2.	Mol. Weight	410
3.	Melting Point	295°C
4.	Physical state	Brown Solid
5.	Rf.Value	0.55
6.	Solvent System	$CHCl_3$: CH_3OH : H_2O (5:3:2)

Table 2

S/No.	4-[2'-(5'-nitro)imidazolyl]benzoyl(N-Me)Phenyl alanine	
1.	Mol. Formula	$C_{20}O_5N_4H_{18}$
2.	Mol. Weight	394
3.	Melting Point	173°C
4.	Physical state	Sticky Brown Mass
5.	Rf.Value	0.64
6.	Solvent System	$CHCl_3$: CH_3OH : H_2O (5:3:2)

Table 3

S/No.	4-[2'-(5'-nitro)imidazolyl] benzoyl(N-Me)threonine	
1.	Mol. Formula	$C_{15}O_6N_4H_{18}$
2.	Mol. Weight	350
3.	Melting Point	173°C
4.	Physical state	Brown Solid
5.	Rf.Value	0.57
6.	Solvent System	$CHCl_3$: CH_3OH : H_2O (5:3:2)

Table 4: Physical data of 4-[2'-(5' nitro) imidazolyl] benzoic acid

S/No.	Product Name	Physical state	M.P(^{0}C)	%yield
1.	4-[2'-(5'-nitro) imidazolyl]benzoic acid	Pale brown solid	256	21.49

Table 5: Physicochemical analysis of4-[2'-(5'-nitro) imidazolyl] benzoyl(N-Me)valine

S/No.	Physicochemical analysis of4-[2'-(5'-nitro) imidazolyl] benzoyl(N-Me)valine	
1.	Mol. Formula	$C_{16}O_5N_4H_{18}$
2.	Mol. Weight	346
3.	Melting Point	120°C
4.	Physical state	Reddish Brown solid
5.	Rf.Value	0.53
6.	Solvent System	$CHCl_3$: CH_3OH : H_2O (5:3:2)

Table 6

S/No.	Physicochemical analysis of4-[2'-(5'-nitro) imidazolyl] benzoyl(N-Me)leuline	
1.	Mol. Formula	$C_{17}O_5N_4H_{20}$
2.	Mol. Weight	360
3.	Melting Point	120°C
4.	Physical state	Light Brown Solid
5.	R_f.Value	0.55
6.	Solvent System	$CHCl_3$: CH_3OH : H_2O (5:3:2)

Table 7: Data of anthelmentic activity

S/No.	Compound	Concentrations (mg)	Mean paralying time (min) + S.E		Mean death time (min) +S.E
1.	Control	-	N.E		
2.	Mebendazole	100	6.00	± 0.34	7.40
3.	1	100	6.90	± 0.31	7.35
4.	2	100	6.12	± 0.35	7.34
5.	3	100	6.10	± 0.51	6.90
6.	4	100	7.04	± 0.32	8.29
7.	5	100	6.10	± 0.31	7.40

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