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Research Article

SYNTHESIS AND EVALUATION OF ANTHELMINTIC AND INSECTICIDAL ACTIVITIES OF 4-AMINO-ANTIPYRINE DERIVATIVES OF AMINO ACIDS AND PEPTIDES

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ABSTRACT
Antipyrine has been drawn as promising structural units in the field of medicinal and agricultural chemistry. The enzymatic synthesis of Z-L-aminoacyl-antipyrine amides from Z-protected amino acid esters and 4-aminoantipyrine (AAP) was accomplished by papain in aqueous-organic and biphasic media as well as in suspension. Hence, an attempt is made towards the synthesis of 4-Aminoantipyrine derivatives of amino acid and peptides i.e. 3-(4-antipyryl)benzoyl-amino acids/peptides) using solution phase technique of peptide synthesis. 4-Amino antipyrine is diazotized and then reacted with benzoic acid in presence of $CuCl_2$ giving 3-(4-antipyryl)benzoic acid which is the precursor for the synthesis of desired compounds. The structures of the new compounds synthesized by the above method were confirmed by FTIR, ^{1}H NMR and mass spectral analysis and were tested for their biological activities against various micro-organisms. All the compounds showed potent insecticidal and anthelmintic activities.
Keywords: Antipyrine, amino acid, benzoic acid, Insecticidal activity, Anthelmintic activity.

INTRODUCTION

Innovation of newer and more potent analogs of molecules with already established activities form a key part of research in the pharmaceutical field. Bringing about modifications by manipulating the parent compound structures serves to enhance the activity of the compound and also in most cases, eliminates adverse effects or toxicity associated with the parent drug.

An exceptionally large number of drugs are heterocyclic compounds, mostly are of synthetic origin[1], few have been obtained from natural resources, plants and animals which include alkaloids, xanthenes, cardiac glycosides, vitamins and several antibiotics. Heterocyclic derivatives having two nitrogen atoms oriented in, 1-3 position are endowed with wide spectrum of biological activities[2]. Number of organo-sulphur and nitrogen containing compounds are present in living and non living system. Among the heterocyclic compounds containing sulphur and nitrogen, the six and five membered heterocyclic compounds containing sulphur and nitrogen have maximum attention, as they have many biological and industrial application[3].

4-aminoantipyrine and its derivatives are known for their variety of clinical applications. New kinds of chemotherapeutic agents containing Schiff bases have gained significant attention among biochemists and of those aminopyrines are commonly administered intravenously to detect liver disease in clinical treatment. An attempt has been made in this project to couple peptide chain to the 3-(4-antipyryl)benzoic acid and warranted for biological activities.

The main goal of this project work was to synthesize and to carry out biological evaluation of 3-(4-antipyryl)benzoyl amino acids and dipeptides. The synthesis of 4-aminoantipyrine derivatives has attracted the attention of several research groups due to their potential biological activities. In this context, broad spectra of bioactive 4-aminoantipyrine derivatives and their metal complexes have been investigated and diversities of bioactivities such as analgesic, anti-inflammatory, antimicrobial, and anticancer activity have been reported. The antibacterial activity caught our attention because antimicrobial resistance developed by important pathogens has increased in the last decade. Besides, emerging and re-emerging bacterial infectious diseases still causes death and disability worldwide. As part of our continuing interest on the syntheses of potential bioactive compounds, we undertook the syntheses of 4-aminoantipyrine derivatives in search for enhanced biological activities[4-7]. In this work, we describe our results concerning the synthesis, IR, ^{1}H NMR, GC-MS structural analysis.

Therefore, an attempt is made to synthesize a new bioactive series of 3-(4-antipyryl)benzoic acid derivatives of amino acids and peptides. Biological activity studies like anthelmintic and insecticidal activities were performed on these synthetic compounds and gave good results.

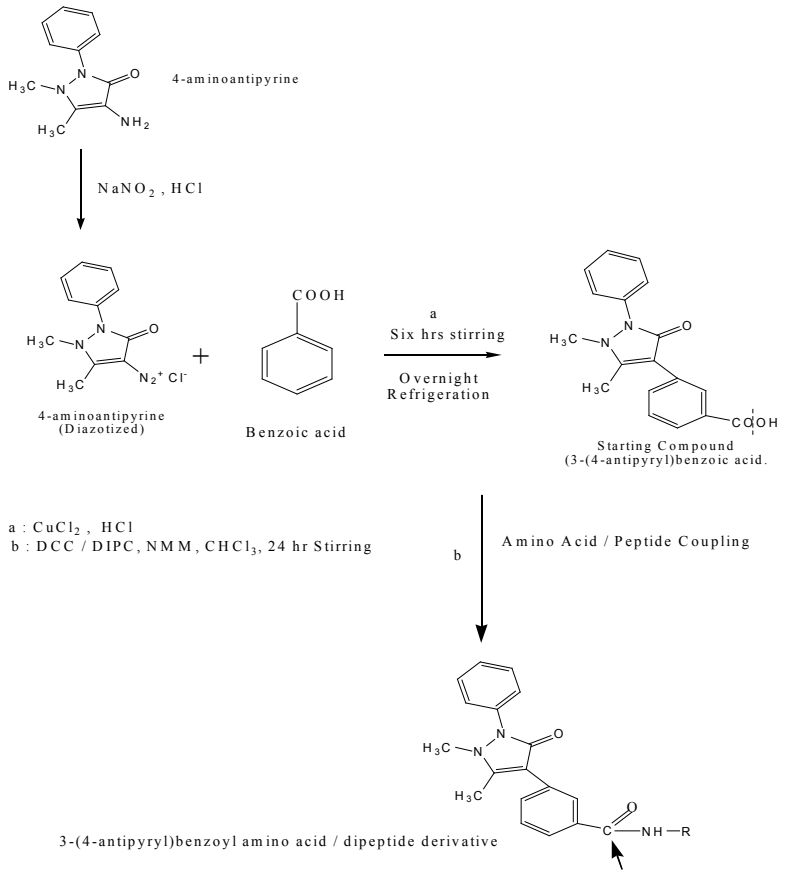
EXPERIMENTAL

All the experiments pertaining to this work has been done in the laboratory, under normal conditions of temperature and pressure, unless otherwise stated. The reactions requiring anhydrous conditions were conducted in flame dried apparatus. Solvents and reagents were prepared and purified by standard methods. All the reactions were magnetically stirred unless otherwise stated. Organic extracts were dried over anhydrous sodium sulphate. Melting points were determined by capillary method and were uncorrected.

IR characterizations of synthesized compounds were done at the Technology Business Incubation (TBI), VIT University, using a thin film supported on KBr pellets for solids and chloroform as a solvent for semisolids. The values are reported as nmax (cm^{-1}).

^{1}H NMR characterization of synthesized compounds was done at IISc, Bangalore. The spectra were obtained in $CDCl_3$ and the chemical shift values are reported as values in ppm relative to TMS (d = 0) as internal standard. Multiplicities were described using the abbreviations: s=singlet, d = doublet, q=quartet, m=multiplet and br = broad. Processing speed of 400.134 MHz. The GC-MS characterizations of synthesized compounds were done at IIT-Madras, Chennai. Instrument JOEL GMate, Inlet: Direct Probe.

SCHEME-I



Preparation of 3-(4-antipyryl)benzoic acid (SC):
A mixture of 4-amino antipyrine (6.1 gms, 30 mmol), dilute hydrochloric acid (15%, 15ml) and water (18ml) was heated to get a clear solution. The solution was cooled to RT and diazotized by the addition of sodium nitrite solution (30%,5.76ml) added slowly while stirring. The diazonium salt solution was filtered and to the filtrate, dilute HCl (12ml) and Benzoic acid (3.36 gm, 30 mmol) and aqueous cupric chloride (0.6 gms in 2.4 ml 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 3-(4-antipyryl)benzoic acid, which is the starting compound for further synthesis.

Preparation of 3-(4-antipyryl)benzoyl-amino acids/dipeptides:
To the amino acid methylester/peptide methyl ester (5.0 mM) DCC(1.1g), was added to 3-(4-antipyryl)benzoic acid (1.54gms, 5.0 mM), NMM (1.15 ml) , chloroform (30 ml) and stirred at room temperature for 24 hours in magnetic stirrer**.** 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), washed with water 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**.** Using the above procedure the following compounds were prepared[8-12]**.**

Table 1: Physical data of 3-(4-antipyryl)benzoyl-amino acids / dipeptides

Sl.No	Compound	Molecular Formula	Molecular Weight	Physical State		Melting Point	% Yield
				Colour	State		
01	SC	$C_{18}H_{16}N_2O_3$	308.36	Brown	Crystalline	99	82.23
02	C1	$C_{21}H_{21}N_3O_4$	379.45	Brown	Crystalline	90	89.75
03	C2	$C_{30}H_{30}N_4O_6$	542.64	Brown	Pasty Mass	92	76.00
04	C3	$C_{29}H_{34}N_4O_5$	518.66	Brown	Solid	97	91.56
05	C4	$C_{27}H_{32}N_4O_5$	492.62	Brown	Solid	118.00	80.88
06	C5	$C_{20}H_{19}N_3O_4$	365.43	Brown	Solid	115.00	56.12

Legend of Compounds

1. 3-(4-antipyryl)benzoic acid (SC)
2. 3-(4-antipyryl)benzoyl-alanine (C1)
3. 3-(4-antipyryl)benzoyl-alanyl-tyrosine (C2)
4. 3-(4-antipyryl)benzoyl-prolyl-isoleucine (C3)
5. 3-(4-antipyryl)benzoyl-alanyl-isoleucine (C4)
6. 3-(4-antipyryl)benzoyl-glycine (C5)

SPECTRAL DATA:

1. 3-(4-antipyryl)benzoic acid (SC)

IR ($CHCl_3$): 3327 (-OH str), 3069 (Arom –CH str), 2930 (aliph –CH str), 1697(C=O amide str). **GC-MS**: (m/z): 308.09.

2. 3-(4-antipyryl)benzoyl-alanine (C1)

IR ($CHCl_3$): 3325 (-OH str), 3068 (Arom –CH str), 2929 (aliph –CH str), 1698(C=O amide str). **GC-MS**: (m/z): 379.67.

3. 3-(4-antipyryl)benzoyl-alanyl-tyrosine (C2)

IR ($CHCl_3$): 3434 (-OH str), 3072 (Arom –CH str), 2928 (aliph –CH str), 1689(C=O amide str). **^{1}H NMR(400MHz, $CDCl_3$):** δ 8.2 (2H, s, NH), 7.8-7.1 (13H, m, Arom-H), 4.2-4.0 (2H, m, ά-H), 3.4 (3H, s, Ar-CH_3), 2.2 (3H, ά', N-CH_3) 1.8 (5H,m, β-H). **GC-MS**: (m/z): 542.21.

4. 3-(4-antipyryl)benzoyl-alanyl-tyrosine (C3)

IR ($CHCl_3$): 3411 (-OH str), 3029 (Arom –CH str), 2948 (aliph –CH str), 1658(C=O amide str). **^{1}H NMR(400MHz, $CDCl_3$):** δ 8.2-7.2 (10H, m, NH, Ar-H), 4.6-4.2 (2H, m, ά-H), 3.7 (3H, s, OCH_3), 3.4 (3H, Pro, s, Ar-CH_3), 2.2 (3H, m, N-CH_3), 2.0-1.0 (14H, β,γ, δ protons of Pro, Ile). **GC-MS**: (m/z): 518.28.

4. 3-(4-antipyryl)benzoyl-alanine (C4)

IR ($CHCl_3$): 3433 (-OH str), 3030 (Arom –CH str), 2929 (aliph –CH str), 1652(C=O amide str). **GC-MS**: (m/z): 492.17.

BIOLOGICAL EVALUATION

a) 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 Eugenia)* by Garg's method[13]. 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 20ml of the test samples. Standard drug, Mebendazole was also prepared with the same concentration in a similar way. Earthworm was placed in a beaker containing 20ml of suspension of the test standard drugs (Mebendazole) at RT. Another set of earth worm was kept as control in 20ml suspension of distilled water and 15% Tween 80. 20ml each of the suspensions of the test compounds were added into separate beaker containing one earthworm 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. The results of Anthelmintic Activity of the newly synthesized compounds are given in Table 2 .

Table 2. Data of Anthelmintic Activity

Sl No	Compounds	Concentration (100 mg/20ml)	Duration in Minutes	
			Paralysis	Death
1	C1	100mg	17	35
2	C2	100mg	15	40
3	C3	100mg	4	25
4	C4	100mg	8	48
5	C5	100mg	25	72
6	Mebendazole	100mg	2	10
7	Control	……..	------	------

b) Evaluation of Insecticidal Activity:

Insecticides are pesticides used against insects. A suitable *invitro* test can be considered as a useful screening method.

General Procedure: Insecticidal studies of the synthesized compounds were carried out against termites (*Coptotermis formasanus*) by Morita et. al. method[14].

The insecticidal potential of the synthesized compounds were carried on local wood termites. 6 clean, oven dried petri plates were taken. Whatmann filter papers were cut to the size of petri plates and fitted. 100g of compounds, C1, C2, C3, C4, and C5 were dissolved in chloroform and poured in the Petri-plates with filter paper fitted in. The solvent evaporates while the respective compounds forms even layer on the filter paper. The subject, termites (about 5 no's) were kept on each of these Petri-dishes and covered with the lid attached with water soaked cotton attached to the ceiling of

the lid. One control was also prepared without compound. The set up was kept undisturbed while time was noted. The results of Insecticidal Activity of the newly synthesized compounds are given in Table 3.

Table 3. Data of Insecticidal Activity

Sl No	Compounds	Concentration (100mg/2ml)	Death Duration
1	C1	100mg	4hrs
2	C2	100mg	4hrs 19mins
3	C3	100mg	4hrs 2mins
4	C4	100mg	4hrs 3mins
5	C5	100mg	5hrs 3mins
6	Chloropyrifos	100mg	2hrs 30 mins
7	Control	----------	No Effect

RESULTS AND DISCUSSION

All the title compounds could be conveniently and efficiently synthesized by prescribed Scheme I with good yields (Table 1). The newly synthesized compounds were characterized by IR, ^{1}HNMR and GC-MS. The spectral data revealed the formation of title compounds. All the synthesized compounds were subjected to anthelmintic activity by Garg et.al. method and insecticidal activity by Morita et. al. The compounds exhibited potent anthelmintic activity against *Eudrillus Eugenia* at the concentration of 100mg/20ml of test samples, similar to the standard drug, Mebendazole. The synthesized compounds showed high activity against *Coptotermis Formasanus* at the concentration of 100mg/2ml of test samples, as compared to the standard Chloropyrifos.

CONCLUSION

Using the above mentioned scheme series of amino acid and peptide derivates of 3-(4-antipyryl)benzoic acid could be conveniently and efficiently synthesized in the laboratory, with reasonable yield. Amino acids and dipeptides could be easily coupled with the 3-(4-antipyryl)benzoic giving desired compounds of type 3-(4-antipyral)benzoyl-amino acid/peptide with potential anthelmintic and insecticidal activities. Methyl ester could be efficiently synthesized by using microwave irradiation of about 50 watt for 85 seconds, with significant yield. The synthesized compounds showed relatively high anthelmintic potential of which dipeptides were more active. The synthesized compounds also relatively higher insecticidal potential of which amino acid derivatives were more active. All these compounds can be prepared in the ordinary laboratories, under normal conditions of temperature and pressure.

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