International Journal of Pharmaceutical Archive-1(3), 2012, 60-62
Available online through www.ijpaonline.info ISSN 2319-7226

Research Article

SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTHELMINTIC ACTIVITY OF N-[2-(BENZOYLAMINO)-3-(PHENYL)-1-OXO-2-PROPENYL] ARGININE

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(Received on: 30-11-12; Revised & Accepted on: 21-12-12)

ABSTRACT

A synthetic arginine analogue N-[2-(benzoylamino)-3-(phenyl)-1-oxo-2-propenyl]arginine was synthesized using Azlactones,also called as 4-benzylidene-2-phenyl oxazol-5-ones which serves as an intermediate. Pheretima posthuma (Indian adult earth worms) were used for evaluating the anthelminthic activity. The purity of the synthesized compounds was evaluated by analytical methods such as TLC, UV and FT-IR. Time required for the paralysis and death of the earth worms was noted for each sample, by taking Piperazine citrate as the standard drug for comparing the results.

Key words: *Azlactones, Arginine analogue, Piperazine citrate, Pheretima posthuma, Anthelmintic activity.*

INTRODUCTION

An anthelmintic is a substance that expels or destroys gastro-intestinal worms. The more common name is dewormer or wormer. Anthelmintics are also called as parasiticides, enterocides, nematocides, parasitic, antiparasitics and drenches. All anthelmintics essentially kill worms by either starving them to death or paralyzing them. Because worms have no means of storing energy, they must eat almost continuously to meet their metabolic needs. Any disruption in this process results in energy depletion. Interfering with feeding for 24 hrs or less is sufficient to kill most of the adult parasites. Parasites will also die if they become paralyzed and temporarily lose their ability to maintain their position in the gut.

L-Arginine, the most basic naturally occurring amino acid plays a major role in number of pathophysiological processes [1]. Past studies reveal that L-Arginine and its Analogues possess activity such as relaxation of smooth muscles. Acute and chronic administration of L-Arginine showed to improve the endothelial function in animal models of hypercholesteremia and atherosclerosis [2, 3].

MATERIALS AND METHODS

Synthetic chemistry

Present study was undertaken to synthesize N-[2-(benzoylamino)-3-(phenyl)-1-oxo-2-propenyl] arginine and evaluate the anthelmintic activity. Target compound was obtained in two steps:

Step I: Synthesis of 4-benzylidene-2-phenyl oxazol-5-ones

A mixture of benzoylglycine, redistilled benzaldehyde, acetic acid and anhydrous sodium acetate was heated on an electric hot plate with stirring. On liquefaction, it was heated for 2 hours and ethanol was added slowly and the mixture was allowed to stand overnight. The product obtained is washed with boiling water and dried at 100^{o}C. The product obtained in step I was used in step II for further synthesis.

Step II: Synthesis of N-[2-(benzoylamino)-3-(phenyl)-1-oxo-2-propenyl] arginine

The product obtained in step I was reacted with L-Arginine in alkali like sodium hydroxide and acetone which results in clear solution after two to three hours of reaction. The solution thus obtained was acidified by the addition of HCl.

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The product separated is N-[2-(benzoylamino)-3-(phenyl)-1-oxo-2-propenyl] arginine which is washed with cold water and dried. The compound thus obtained is used for screening the anthelmintic activity after purification and characterization.

STRUCTURES WITH NAMES

Characterization of the synthesized compound

Melting points was determined in an open capillary tube using an electro thermal digital melting point apparatus and are uncorrected. UV absorption peaks for the compound are observed in the region of 221 nm and 460 nm.FT-IRspectrophotometer Schimadzu displayed bands at 3220-3430cm^{-1} due to N-H stretching and at 2920-3240 cm-1 due to O-H stretching. A band of 1690-1760cm^{-1} is observed due to C=O stretching of CONH and at 1640-1655 cm^{-1} due to C=O stretching of carbonyl group. A band of 1600-1610cm^{-1} was observed due to C=C (styryl) stretching. Another band at 1558-1581 cm^{-1} was also observed due to C=N stretching. Compound was checked for its purity by TLC on silica gel plates and the spots were visualized in iodine vapours.

Preparation of the test sample

The synthesized compound N-[2-(benzoylamino)-3-(phenyl)-1-oxo-2-propenyl] arginine was insoluble in water. So, an organic solvent like 10%V/V Dimethylsulphoxide (DMSO) in distilled water was prepared. The test compound was dissolved in 1 ml of 10% V/V DMSO and diluted to prepare four concentrations i.e.,0.1%W/V, 0.2%W/V,0.5%W/Vand 1.0%W/V.

Standard drug

Piperazine citrate was taken as the standard drug so as to compare the anthelmintic activity of the test compound. Piperazine citrate is dissolved in 1 ml of DMSO and diluted upto 10 ml to prepare concentrations i.e.,0.1%W/V, 0.2%W/V,0.5%W/V and 1.0%W/V. 10%V/V in distilled water served as control.

Animals

Indian adult earth worms (Pheretima posthuma) are collected from moist soil and washed with normal saline to remove all the faecal matter. Worms of 3-5 cm il length and 0.1-0.2 cm in width were used for the study of anthelmintic activity.

Anthelmintic activity

Indian adult earth worms were used for the screening of in-vitro anthelmintic activity of the synthesized test compound. The worms of equal size were divided into the respective groups containing six earth worms in each group. The test and standard compounds were prepared in the concentrations of 0.1%W/V, 0.2%W/V,0.5%W/V and 1.0%W/V. Six earth worms approximately of equal size were placed in each Petri dish containing 10 ml of above test solution and standard drug solution and one group was treated as control with 10% V/V DMSO at room temperature [4]. The time taken for complete paralysis and death was recorded. To ascertain death of the worms, each worm was frequently applied an external stimuli like pricking with a ball pin, which stimulates and induces movement in the worms, if alive [5].Paralysis was said to occur when the worms do not revive in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body color. The test results were compared with standard drug piperazine citrate.

Table I: Anthelmintic activity of N-[2-(benzoyl amino)-3-(phenyl)-1-oxo-2-propenyl]arginine

Compound	Concentration (%)	Time of paralysis (min)	Time of death (min)
Test sample	0.1%	2.3020 ± 0.120	4.420 ± 0.136
	0.2%	2.512 ± 0.132	3.863 ± 0.198
	0.5%	2.371 ± 0.170	3.626 ± 0.176
	1.0%	1.876 ± 0.092	2.134 ± 0.154
Standard drug	0.1%	3.120 ± 0.115	4.417 ± 0.189
	0.2%	2.986 ± 0.102	3.916 ± 0.176
	0.5%	1.962 ± 0.127	3.424 ± 0.153
	1.0%	1.425 ± 0.071	1.742 ± 0.120

Results are expressed as Mean±SEM from 6 observations.

RESULTS AND DISCUSSION

As the literature on L-Arginine revealed that it produces smooth muscle relaxation, an attempt was made to assess whether the synthesized compound i.e., N-[2-(benzoyl amino)-3-(phenyl)-1-oxo-2-propenyl]arginine has anthelmintic activity, because relaxation is followed by paralytic effect for most of the compounds. The anthelmintic activity was

carried out as per the method of SP. Thievendren et.al.[6] There are various species of worms available such as Ascaris, Earth worms, Nippostrongylus and Heterakis. Of all the species earth worms have been widely used for the evaluation of Anthelmintic activity in-vitro because they resemble intestinal worms in their reaction to Anthelmintics and are easily available. It has been demonstrated that all Anthelmintics are toxic to earth worms and a substance toxic to earth worms is worthy for investigation as an Anthelmintic. The results in table: 1indicates that the compounds synthesized i.e. L-Arginine analogue is active against the earth worm. Further Anthelmintic activity of the test compound can be carried out against other species of Helminthes so as to assess its activity on a broader scale, which is our future plan of research work.

ACKNOWLEDGEMENTS

Authors are thankful to the manager of vermi culture plant, who has provided us the earth worms. Authors also express their sinscere gratitude to the Management and Directors of Sree Dattha Institutions, Sheriguda, Ibrahimpatnam, Hyderabad for providing us the facilities to carry out the research work.

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Source of support: Nil, Conflict of interest: None Declared

