Crown Ether Proselytized Conglomeration and Anthelmintic Evaluation of BIS-(3-Phenyl, 5-Amino Pyrazolyl)-18-Crown-6 Composite

Karpakavalli M[1]*, Sasikala M[2], Sangilimuthu AY[3], Komala M[4] and Mohan S[5]

[1]Department of Pharmaceutical Chemistry, Karpagam College of Pharmacy, Coimbatore-641032, Tamil Nadu

[2]Department of Pharmaceutical Analysis, Karpagam College of Pharmacy, Coimbatore-641032, Tamil Nadu

[3]Deptartment of Biotechnology, Karpagam University, Coimbatore-641021, Tamil Nadu

[4]Deptartment of Pharmaceutics, Mohamed Sathak A.J College of Pharmacy, Chennai-600119, Tamil Nadu

[5]Department of Pharmaceutical Biotechnology, Karpagam College of Pharmacy, Coimbatore-641032, Tamil Nadu

***Corresponding author: E-Mail: sreemenakag@gmail.com**

ABSTRACT

There is much evidence that herbal insecticides allowed in organic farming are just as toxic as synthetic pesticides. Now a day's recognition for the implement of green synthetic protocols is increasing. Many heterocyclic compounds have often proved as the very assuring part of a drug molecule's profound biological activity by being a vital intermediate and in particular pyrazole plays a significant and versatile role as a valid, potent precursor nucleus for many of the bioactive agents. The field of complexation chemistry has extended to a major domain of organic chemistry. Crown ethers are of massive engrossment and significant in general chemistry, biochemistry, medicinal chemistry, synthetic chemistry, materials science, catalysis, resolution, encapsulated processes, as well as in the design of many novel leads with particular characteristics, manifold capabilities and programmable functions. Helminth sepsis is among the most prevalent infections in humans and mostly the GI helminthes turn to be resistant to forthwith available anthelmintic drugs which thus directs to ailments alleviating crisis. As there are no reports on systematic and scientific study of anthelmintic activity of a complexed pyrazole derivative, we plan to synthesize and evaluate the same in one such compound. The contemporary project is a crown ether proselytized fabrication of Bis-(3-phenyl, 5-amino pyrazolyl)-18-crown-6 compound. The assignment of research also indulges the determination of anthelmintic activity for the same.

KEY WORDS: Anthelmintic activity, Crown Ether Promoted Synthesis, 18-Crown-6, Characterization, Pyrazole derivatives.

1. INTRODUCTION

Economic planning and leveling of energy utilization have aggressively been recognized as a new paradigm in deciding the innovation of fresh lead molecules being targeted in a more sustainable way and stringent to government regulatory policies. Some of the existing misinterpretations are synthetic chemicals are more toxic, organically grown edible is better and synthetic copies of herbal composites are not as good. In the field of chemistry and its related technology, numerous investigations now routinely use non-traditional synthetic methodologies such as cleaner ways as solvent-free reactions or various other alternative activation techniques to give energy to the substrate, say microwave radiation, ultrasound, the replacement of volatile organic solvents by water, acetonitrile, DMSO, ionic liquids, active catalysts or supercritical CO_2, etc. Limitations in the form of drastic reaction conditions, low product yields, tedious work-up procedures or the use of toxic metal salts as catalysts or relatively expensive reagents has resulted in the reduced commercial attractiveness. In view of the above said disadvantages, there emerges an expectation for the organization of simplistic and green technique for the creation of the organic composites or derivatives or intermediates.

Medicinal chemistry literates, researchers and pharmaceutical manufacturing industries pay much attention to the development of new, energy saving cost effective, environmentally safe technologies for the synthesis of heterocyclic compounds like azoles, pyridine, azepine, pyrimidine, thiadiazole, tetrazole, quinoline etc., as they have often been demonstrated as a most routinely used multifaceted intermediates for many pharmacologically active compounds. In particular, azoles and their derivatives have attracted the elevating scopes. The fascinating group of pyrazole has multiple activities such as antimicrobial, anti-inflammatory, anticancer, analgesic, anticonvulsant, anthelmintic, antioxidant and herbicidal.

Complexation chemistry is the science of the designed recognition and complexation of chosen ions or molecules. Thousands of surplus and inspiring complexation molecules have been created and these results have been found to be of critical importance in areas as distinctive as biochemistry, physical organic chemistry and bioinorganic chemistry. Classical crown ethers are macrocyclic polyethers $[(OCH_2CH_2)_n]$ that contain 3-20 oxygen atoms separated from each other by two or more carbon atoms and able to dissolve ionic compounds in organic solvents. Ions like $K^{+,}$ Li+ etc are usually accommodated in the central cavity of crown ethers, solvating cations inside a hydrophilic cavity and possessing the hydrophobic C-H bonds in the outer shell. The availability of crown ethers with cavities of different sizes allows specific cations to be solvated with a high degree of selectivity. Like crown ethers, cryptands can be used to enhance the solubility of ionic compounds.

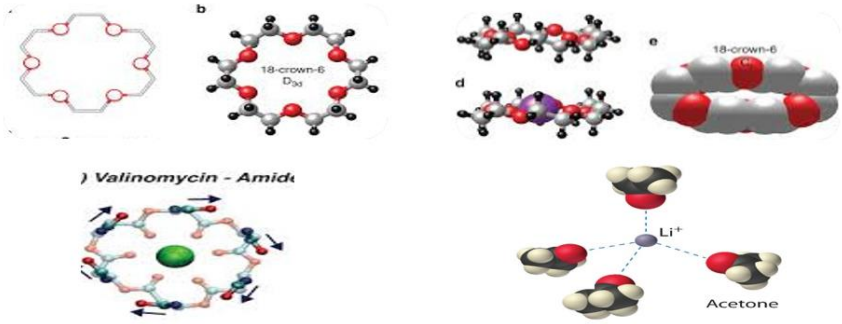
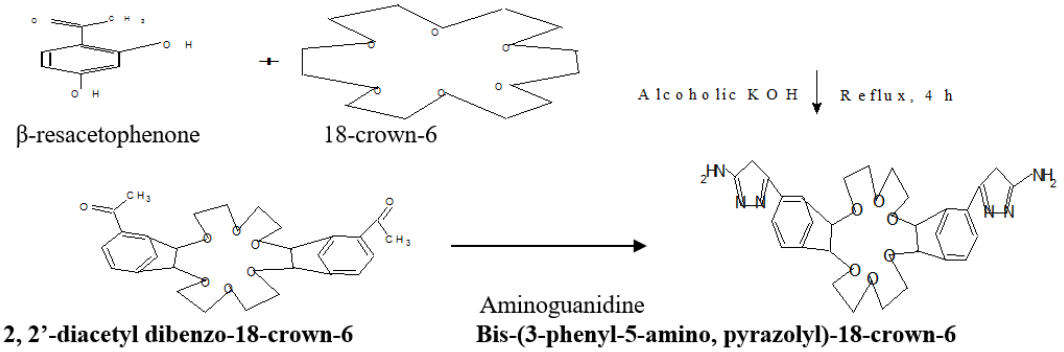


Figure.1. Ion–Dipole Interactions in the salvation of Li^+ Ions by Acetone, a Polar Solvent

The compounds having nitrogen atoms at their crown rings, via the introduction of sidearm by reaction with electrophiles, can be transformed in to rounded crown ethers exhibiting new functionality. It has additional applications i.e. mechanically networked chemical features are attractive to chemists owing to their aesthetic winsome and to their intensive potential applications in molecular machines and smart materials, for example in benzo crown ethers substitution is easy-to-achieve and are exceptionally multi-skilled in selectively binding a range of metal ions and a variety of organic neutral and ionic species.

The most hazardous, pervasive and a foremost degenerative disease distressing a large population is Helminthes, repeatedly entitled helminthiasis, restricted in tropical regions. The helminthes pests are mainly seen in human body in intestinal tract and tissues. It causes enormous danger to the body and becomes responsible for the prevalence of malnutrition, undernourishment, pneumonia, anaemia, eosinophilia. Most diseases caused by helminths are of a chronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. The GI helminthes turn to be resistant to forthwith available anthelmintic drugs which thus directs to ailments alleviating crisis and therefore it becomes necessary to meet out the increasing demand for alternative strategies against gastrointestinal nematodes.

We thus plan to synthesize a new macro molecule containing pyrazole moiety involving a reaction between β-resacetophenone (3, 4-dihydroxy acetophenone) and crown ether 18-crown 6 ($C_{12}H_{24}O_6$); resultant targeted to be reacting with 1-amino guanidine bicarbonate to give the target molecule of crown ether containing a pyrazole moiety.



Scheme

2. EXPERIMENTAL WORK

Chemicals and Instruments: All chemicals and solvents used were of analytical grade and obtained from SD fine chemicals, Chennai, India. The purity of the compounds was checked by melting point using capillary tubes, TLC-using Silica gel-G (E-Merck). U.V. spectral studies were done on Schimadzu UV spectrophotometer (Model No. UV- 2400 PC). I.R. spectra were recorded in KBr on Schimadzu spectrophotometer.

Procedure for the preparation of 2, 2'-diacetyl dibenzo-18-crown-6: To potassium hydroxide (3.0 g) in ethanol (10 ml) taken in round bottom flask, crown ether 18-crown 6 (1.2 g; 0.5 mole) was added and the reaction mixture was stirred at room temperature. Then β-resacetophenone (1.5 g; 1mmole) was added and agitated the resultant for 4-5 h. The completion of reaction was visualized by TLC. After completion of the reaction, the mixture was poured into ice water and neutralized with dilute hydrochloric acid. The separated crude product was filtered, dried and re-crystallized with ethanol. The yield and all the other physical data of the synthesized compounds are given in Table.1 & 2, Figure.1(a).

Procedure for the preparation of Bis-(3-phenyl-5-amino, pyrazolyl)-18-crown-6: A mixture of 2, 2'-diacetyl dibenzo-18-crown-6 (0.5 g; 1mmole), 1-amino guanidine bicarbonate (0.34 g; 2.5 mmole), sodium methoxide (0.20 g; 4mmole) in methanol (20 ml) was refluxed on water bath for 6-7 h. The completion of reaction was noticed by TLC. After completion of the reaction, the resultant was poured into ice water and neutralized with dilute hydrochloric acid. The separated crude product was filtered, dried and re-crystallized with ethanol. The yield and all the other physical data of the synthesized compounds are given in Table.1 & 2, Figure.1(b).

Preliminary physical data and spectral characterization: (a) dissolved the sample solution (0.5 ml) in concentrated acid (2.0 m) and distilled water (3 ml) and cooled the solution to 0 - 5°C in an ice-bath for 5 minutes. A cold solution (ice-bath) of sodium nitrite (0.5 g) was added in distilled water (2.0 ml) from a dropper, with swirling of the test tube and keeping in the ice-bath. b) Placed the compound (0.5 g), sodium hydroxide (5 %, 15 - 10 ml) and benzene sulphonyl chloride (1 ml) in a test tube, stoppered the tube and shaken until the odor of the sulphonyl chloride had disappeared. The solution must be kept alkaline. No reaction found to be occurring in the precipitate formation and thus the substance is a tertiary amine. A UV spectrum of compound (20 µg / ml) was recorded using spectral grade distilled water. UV spectral data of the compound is given in Table-2. The infrared spectral study was done on Schimadzu IR spectrophotometer by KBr disc method and the relevant data of the compound is given in Table-2.

Pharmacological Work: Adult earthworms (*Pheretima posthuma*) and Tapeworm (*Raillietina spiralis*) were made use to determine the anthelmintic activity *in vitro*. They were collected from moist soil of local place, near the swampy water along Malappura area, Kerala and washed with water to remove soil and other fecal matters. All earthworms were of approximately equal size. The average size of earthworm was 5-7 cm and was identified in Dept. of Zoology, Avinasilingam College, Coimbatore, and by services of veterinary practitioners.

Dose: Albendazole (Intas pharmaceuticals Ltd, 10 mg/ml) was prepared by using 0.5 % w/v of CMC as a suspending agent and final volume was made up to 10 ml for respective concentration; Test compounds (20 mg /ml; 50 mg /ml) were prepared in distilled water.

Experimental procedure: The anthelmintic activity was carried out as per the method of Narguna et al., This *in vitro* method involves the usage of adult earthworm (*Pheretima posthuma*) and Tapeworm (*Raillietina spiralis*) owing to the physio-structural similarity with the intestinal roundworm parasites of human beings. Eighteen earthworms were divided into six groups (3 each) and groups of approximately equal size worms were released into in each 20 ml of desired concentration of standard and test in the petridish. First group I, served as normal control which received distilled water only; Second group II, received the standard drug, Albendazole (10 mg /ml); Groups III & IV received doses of synthetic compounds (20 mg /ml; 50 mg /ml) respectively. Observations were made for the time taken to cause paralysis and death of individual worms for two hours. Mean time (min) for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time (min) for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C) or when given external stimuli. Results were shown in Table -3 and expressed as a mean ± SEM of three worms in each group.

Statistical analysis: The data on biological studies were reported as mean ± Standard deviation (n = 3). For determining the statistical significance, standard error mean and analysis of variance (ANOVA) at 5 % level significance was employed. $P < 0.05$ was considered significant.

Table.1. Physical data of the synthesized compounds

Name of the Compound	Molecular formula	Mol. Wt.	Melting Point ºC	Practical Yield (g)	% yield
2, 2'-diacetyl dibenzo-18-crown-6	$C_{27}H_{29}O_7$	465	180	6.40	92.0
Bis (3-phenyl-5-amino, pyrazolyl)-18-crown-6	$C_{29}H_{33}O_6N6$	561	214	0.85	78.0

Table.2. Spectral details of the synthesized compounds

Name of the compound	Rf	λmax, nm	IR spectral data
2, 2'-diacetyl dibenzo-18-crown-6	0.42	245	-
Bis (3-phenyl-5-amino, pyrazolyl)-18-crown-6	0.92	220	3000-2900 cm^{-1} C-H stretching; 1600 cm^{-1}; C-C stretching; 1581 cm^{-1} aromatic C=C conjugation; 1550-1510 cm^{-1} benzene nucleus; 1200-1100 cm^{-1} C-O stretching. 829 cm^{-1} C-H out of plane bending, 1,3-disubstituted benzene ring; 730-665 cm^{-1} C-H bending

Table.3. Anthelmintic activity of synthesized compound Bis (3-phenyl-5-amino, pyrazolyl)-18-crown-6

Groups	Conc. (mg/ml)	Earthworm (*Pheretima posthuma*)		Tapeworm (*Raillietina spiralis*)	
		Time taken for Paralysis (P) in min	Time taken for Death (D) in min	Time taken for Paralysis (P) in min	Time taken for Death (D) in min
Control	-	-	-	-	-
Standard (Albendazole)	10	32 ± 0.7	56 ± 0.9	24 ± 1.0	51 ± 1.1
Test compound I	20	55 ± 0.5	96 ± 1.5	48 ± 1.1	76 ± 1.8
Test compound II	50	37 ± 0.8	61 ± 2.0	30 ± 0.7	62 ± 1.2

Each value represents mean ± SEM (N=3)

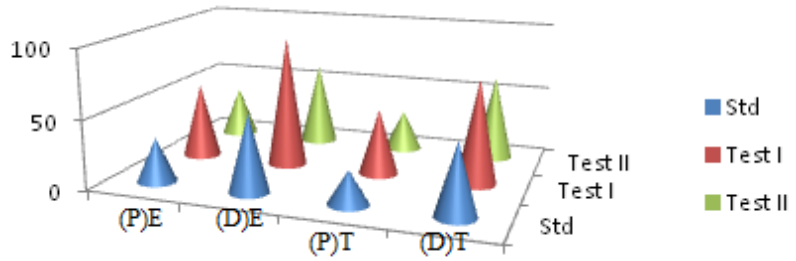


Figure.2. Bar diagram representing the anthelmintic activity of the synthesized compound Bis (3-phenyl-5-amino, pyrazolyl)-18-crown-6

3. RESULTS AND DISCUSSION

A compound of an intermediate 2, 2'-diacetyl dibenzo-18-crown-6 was synthesized from 18-crown 6 and β-resacetophenone, which in turn was obtained by acetylating resorcinol with glacial acetic acid upon reflux at 150 °C. Next, the title compound Bis-(3-phenyl, 5-amino pyrazolyl)-18-crown-6 was synthesized from 2, 2'-diacetyl dibenzo-18-crown-6 and 1-amino guanidine bicarbonate using a traditional refluxing method for 6-8 hours. The compounds synthesized by crown ether promotion were checked for their purity and completeness of the reaction by melting point determination and Co-TLC methods (Figures 1 & 2.) TLC, UV and IR spectral studies were carried out in order to characterize the synthesized composites (Tables-2). The fabricated title compound was tested for a qualitative reaction of sodium nitrite and was found to be showing a green color indicated the presence of a tertiary amine; and also found to be not responding to alkaline solution of benzene sulphonyl chloride, confirming the presence of nitrogen in the nucleus as in the form a tertiary amine.

The synthesized compounds melting point values are given in the Table-1. Thin layer chromatography techniques were performed for the designed molecules and the reactants to have a comparing Co-TLC method. Detection is done by using Iodine vapour and UV light. All synthesized compounds were observed in a single spot whose Rf values are different from their reactants. It ultimately shows that the compund's purity and completion of the reaction. The Rf values are given in Table.2. The absorption maxima, λmax (Table.2) of the synthesized compounds were recorded by using distilled water as solvent. It proves further confirmation of the synthesized compounds. IR spectra were taken for the synthesized compounds. The characteristic absorption peaks were observed for all relevant groups (Table.2).

Anthelmintic activity has been carried out for the synthesized compound of Bis-(3-phenyl, 5-amino pyrazolyl)-18-crown-6 using the adult earthworms (*Pheretima posthuma*) and Tapeworm (*Raillietina spiralis*). Mean time for Paralysis and Death of both the organisms in the standard albendazole (10 mg/ml) and in test solutions of various concentrations (20 and 50 mg/ml) had been noted so as to analyze the effect in comparison. Data showed that the title compound of concentration (50 mg/ml) produced an almost equal effect as that of the standard for paralysis and death. Mean paralysis and death time, when compared between the two organisms, it was found that tapeworm responded faster to the effect of drug solution and were affected so earlier.

Since crown ethers are typically highly flexible, frustrating efforts to rigidify them for many uses that demand higher binding affinity and selectivity have to be encouraged. Therefore, we planned to present a research work in synthesizing a compound with high molecular weight and hoping that this arrangement constrains the crown ethers to be rigid and planar. Moreover, the compounds can be focused in future to have a part of it only, instead of a 'bis' type molecule, for getting attached to a pharmacophore of drug molecules, preferably an anti-cancer substance. Instead of a routine traditional method of refluxing, the above mentioned procedure could be tuned to depend on any modern techniques like UV or microwave irradiation methods thereby, to conserve time, expenses and energy. Thus our project has been aimed clearly to obtain a rational molecular design by using a refined synthetic method in order to develop a new specific host molecule.

4. CONCLUSION

In conclusion, we have provided a cost effective and industrially scalable process for synthesizing a crown ether promoted Bis-(3-phenyl, 5-amino pyrazolyl)-18-crown-6, an assuring anti-cancer intermediate/precursor molecule. The main advantages of the use of crown ether in synthesis are evident when compared with classical methodologies i.e., improving the solubility factor for pharmacophores. From these features present methods can be correlated for safer and efficient synthesis of precursors/leads for anti-cancerous activities. However, these efforts do not mean that everything is known, and there is still a lot to learn about this fascinating and useful reaction. We recommend for a further research in this research work, as crown ether compounds could either induce toxicities that are different from those of conventional antitumor drugs, or complement drugs in current use, thereby providing a valuable adjunct to therapy.

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