Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(5):2540-2544

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Studies on Inclusion complexes of 4-methyl 3-phenyl 2- thiocarbamoyl -3,3a dihydro pyrazolo[3,4-c] pyrazole

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ABSTRACT

The compound, 4-methyl 3-phenyl 2-thiocarbamoyl -3,3a dihydropyrazolo [3,4c] pyrazole has been synthesized starting from 5-methyl-2,4-dihydro-3H pyrazol-3-one and benzaldehyde in its purest form. Since the compound formed is having poor solubility in polar medium, its inclusion complex has been prepared with β-cyclodextrin so as to improve its solubility and bioaccessibility. The formation of inclusion complex has been ascertained by changes in melting point and spectral characteristics. The determination of thermodynamic stability constant and free energy of activation suggest the inclusion complex formation is the thermodynamically allowed and the complex formed is stable.

Key words: Bis-pyrazole, Inclusion complex, β-cyclodextrin, Aqueous phase solubility.

INTRODUCTION

Pyrazole framework is an important pharmacophore and has been extensively used for the preparation of a number of medicines such as antibacterial, antifungal, antiviral, anti tubercular, antiamoebic etc.[1-5]. In addition, fused bispyrazoles are also reported to be a fertile resource of medicine for treating a number of diseases like cancer, malaria etc.[6] Introducing suitable functionality into these derivatives, attempts have also been made to potentiate their pharmacological activities [7]. But poor solubility of all these compounds in polar medium may be a limiting factor for reducing their bioaccessibility. The solubility and hence bioaccessibility can be enhanced by forming inclusion complexes with β-cyclodextrin[8-12]. In this paper an attempt has been made to synthesize 4-methyl 3-phenyl 2- thiocarbamoyl -3,3a dihydro pyrazolo[3,4c] pyrazole in its purest form by incorporating an active pharmacophore, pyrazoline into a pyrazole unit. The inclusion complex of the compound has been prepared with a non-toxic oligosaccharide, β-cyclodextrin which may have higher solubility and better pharmacological activity.

EXPERIMENTAL SECTION

Apparatus and Materials

All the chemicals of acceptable standards were procured from local market. Double distilled water to be used as solvent was prepared in the laboratory. Electronic spectra were recorded on Shimadzu UV-1700 Spectrophotometer and IR spectra were recorded in KBr pellets in Shimadzu8400 FTIR Spectrophotometer. Melting points were recorded by open capillary method.

Step-1: Synthesis of 5-methyl-2,4dihydro-3H-pyrazol-3-one

Ethylacetoacetate (0.1mole) was taken in conical flask and hydrazine hydrate (0.2 mole) in ethanol (20 ml) was added drop wise to it with stirring. The temperature raised during this addition and it was maintained at 60°C when a crystalline solid separated. The reaction mixture was further stirred for 1 hr at room temperature then cooled in an ice bath to complete the crystallization. The separated solid was washed with ice cold ethanol.

Step-2: Synthesis of 4-(4-benzylidene)-5-methyl-2, 4-dihydro-3H-pyrazol-3-one
5-methyl-2, 4dihydro-3H-pyrazol-3-one (0.01 mole), benzaldehyde (0.01mole) and anhydrous sodium acetate (0.02 mole) were dissolved in acetic acid and refluxed for 10 hrs. The reaction mixture was filtered and the filtrate was poured on crushed ice. The solid obtained was recrystallized from ethanol.

Step-3: **Synthesis of 4-methyl 3-phenyl 2- thiocarbamoyl -3,3a dihydro pyrazolo[3,4c] pyrazole**
To the mixture of above synthesized compound (as in step-2) 0.01mole and thiosemicarbazide 0.01mole in 50ml of ethanol, a solution of NaOH 0.02mole in 5ml of water was added and refluxed for 10hrs. The product was poured into crushed ice which was filtered, dried and recrystallized from DMF.

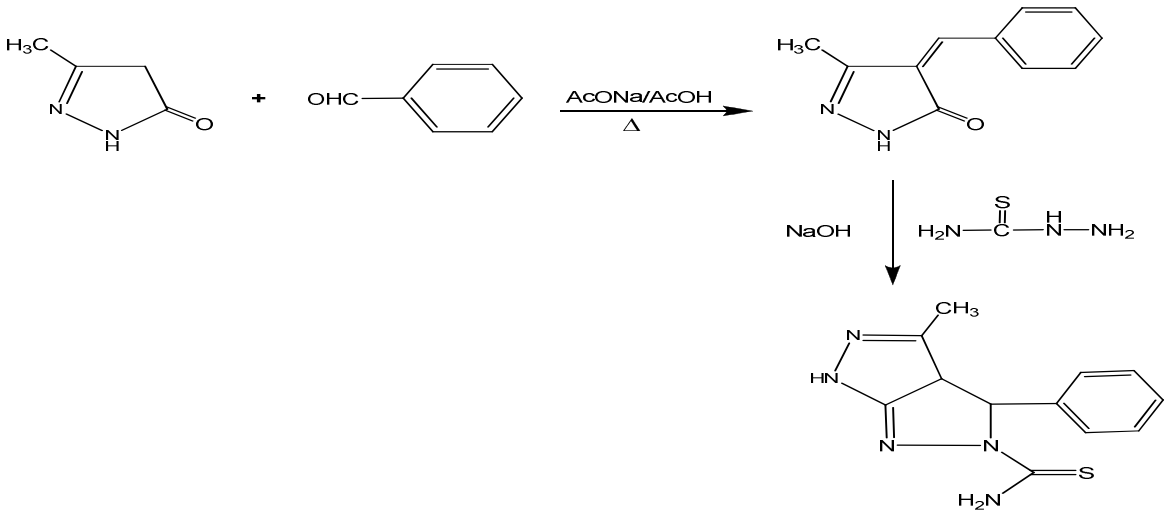
Phase Solubility Measurements:-
The aqueous phase solubility of the compound at various concentrations. β -cyclodextrin (0-10mMl) was studied by Higuchi-Corner method[13]. Accurately weighed sample of these compounds were shaken in rotary flash shaker at room temperature in a series of conical flask for a period of 48 hours till the attainment of equilibrium. The solutions were filtered through Whatmann-42 filter paper and were analyzed in a UV-visible spectrophotometer. The various Values of absorbance at λ-max were plotted against different concentrations of β – cyclodextrin.

Synthesis of inclusion complexes:-
The inclusion complexes of the compounds (I,II and III) with β –cyclodextrin were prepared as per co-precipitation method[14]. The solutions of these compounds in required concentrations were added drop by drop to β – cyclodextrin solution of the required concentration. The mixtures were stirred for a period of 48 hours and filtered. The filtrate was cooled for 24 hours in refrigerators. The precipitate obtained was filtered through G-4 crucible, washed with water and dried in air for 24 hours

Study of thermodynamic properties:-
The thermodynamic stability constant of the complexes was calculated using Benesi-Hilderband relation.
The value of ΔG was calculated at 298 K using the equation:

$$\Delta G = -2.303RT \log K$$

SCHEME-1



RESULTS AND DISCUSSION

The synthesis of the compound has been confirmed from elemental analysis and study of spectral characteristics. The elemental composition matches with theoretical data. IR data of the compound show characteristic absorption at 3415, 3375 (N-H str.) ,3052 (C-H str. in Ar-H) ; 2980 (C-H str. CH_3), 1180 (C=S str.)etc. indicating the presence of N-H, Ar-H, -CH_3,C=S etc. in the expected compound (Table-1) . The synthesis of inclusion complex of the compound has been confirmed from changes in melting point, colour and spectral characteristics (Table -1). As shown from the table, the melting point of the complex is much higher than the compound and β –cyclodextrin. The higher melting point of the inclusion complex is due to the fact that an extra amount of energy is required to bring the compound out of β –cyclodextrin. The UV spectra of the compound gives a prominent peak at 303 nm which undergoes a shift towards higher wavelength of 304 nm after the formation of inclusion complex(Fig-1) . Although IR data do not undergo much change the peaks become weaker, broader and smoother. All these changes in spectral

characteristics after inclusion complex formation may attributed to encapsulation of the compound in the hydrophobic core of β –cyclodextrin and development of weak interactions in between guest and host. [15, 16]

Sl no.	Compound/complex	Melting point	colour	Elemental analysis First line indicate the s finding value Second line indicates calculated value					λmax	IR(KBR)
				C	H	N	S			
1	Compound	168°C	Brownish	42.28 42.30	34.42 34.61	19.12 19.23	3.74 3.84	O	303	3415, 3375 (N-H str.) ,3052 (C-H str. in Ar-H) ; 2980 (C-H str. CH_3), 1180 (C=S str.
2	Inclusion complex	278°C	White						304	

Aqueous phase solubility plot of the compound in β –cyclodextrin is shown in figure -2. It is seen that there is a linear increase in solubility with increasing concentration of β –cyclodextrin. Since the slope of the plot is less than unity the stoichiometry of the inclusion complex may be 1: 1.[17]

The thermodynamic stability of the inclusion complex has been calculated by Benesi –Hildebrand relation. [18]

$1/\Delta A = 1/\Delta\varepsilon + 1/ K_T [\text{Guest}]_{o.} [\beta\text{-CD}]_o$

where Δε is change in molar extinction coefficient,ΔA is change in absorbance and K_T is thermodynamic stability constant. Good correlation has been obtained for a plot of $1/\Delta A$ verses $1/_. [\beta\text{-CD}]_{o.}$

The value of K_T has been calculated using the relation, K_T = Intercept /slope

The K_T value of the inclusion complex is found to be 107 indicating appreciable stability of inclusion complex because the value remains within the ideal range of 100-1000[18, 19]. Using van'tHoff's reaction isotherm and value of K_T , the value of free energy of activation has been calculated and found to be -11.652kJ/ mole. The negative value of free energy change indicates that the inclusion complex formation is a thermodynamically allowed process.

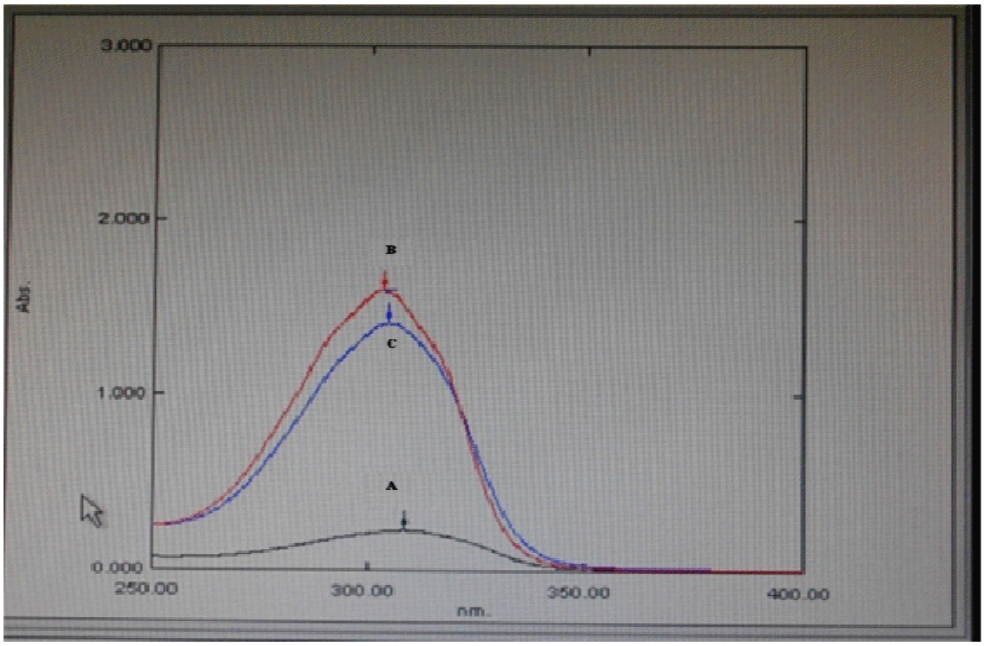


Fig-1 ; UV-visible spectra of β-CD(A), compound(B) and inclusion complex(C)

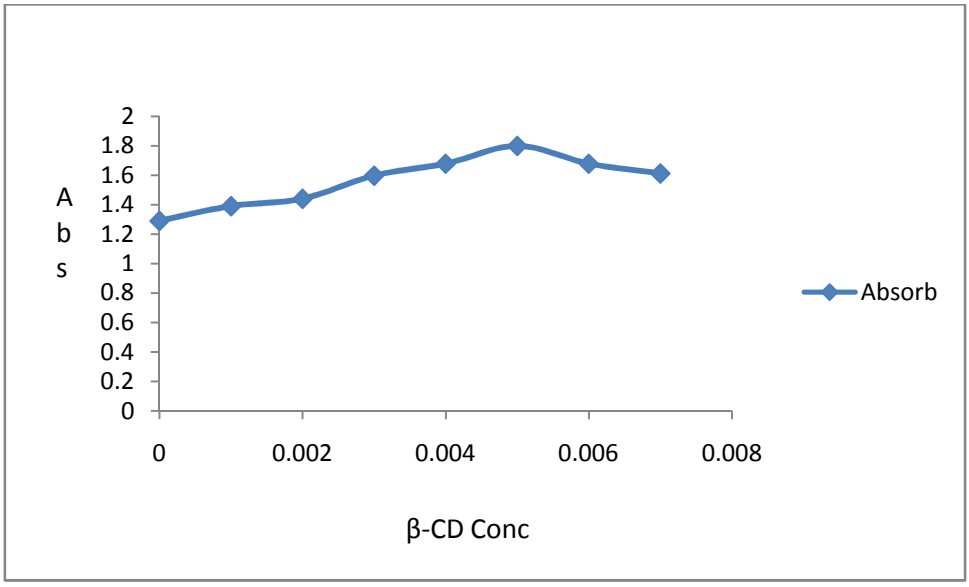


Fig -2 Aqueous phase solubility study of compound

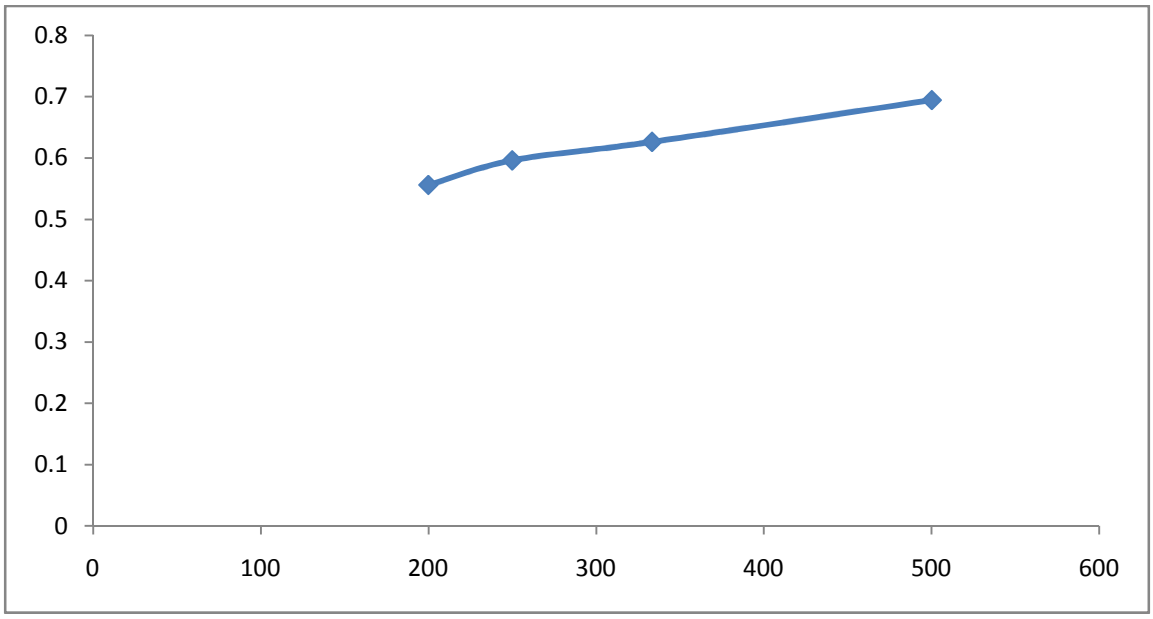


Fig-3 Plot of 1/OD verses 1/ conc. of β –cyclodextrin

CONCLUSION

From the above experimental observation and inference , it is concluded that the inclusion complex formation of the compound 4-methyl 3-phenyl 2- thiocarbamoyl -3,3a dihydro pyrazolo[3,4c] pyrazole with β –cyclodextrin is thermodynamically allowed and it can be used to enhance the bioaccessibility of the compound.

Acknowledgement
The authors thank UGC, New Delhi for providing financial assistance to carry out this piece of work.

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