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Studies on inclusion complex of 4-methyl 3-phenyl 2-thiocarbamoyl -3,3a dihydro pyrazolo[3,4c] 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 and its inclusion complex has been prepared with β-cyclodextrin so as to increase its solubility and bio-accessibility in polar medium . The formation of inclusion complex has been ascertained by studying the changes in physical and spectral properties (UV, FT-IR, XRD, NMR etc). The thermodynamic stability constant and free energy of activation are studied to know whether the inclusion complex formation is thermodynamically allowed. Finally, antibacterial and antioxidant activity of the compound and its inclusion complex are studied. It is found that inclusion complex of 4-methyl 3-phenyl 2-thiocarbamoyl -3,3a dihydropyrazolo [3,4c] pyrazole with β-cyclodextrin is stable and increases antibacterial and antioxidant activities significantly.*

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

I. Introduction

Pyrazole moeity 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 bio-accessibility. The solubility and hence, bio-accessibility can be enhanced by forming inclusion complexes with β-cyclodextrin [8-12]. The inclusion complex formation ability of cyclodextrins with various guest molecules of suitable polarity, dimension and compatibility is due to their special molecular structure like hydrophobic internal cavity and hydrophilic external surface.[23]

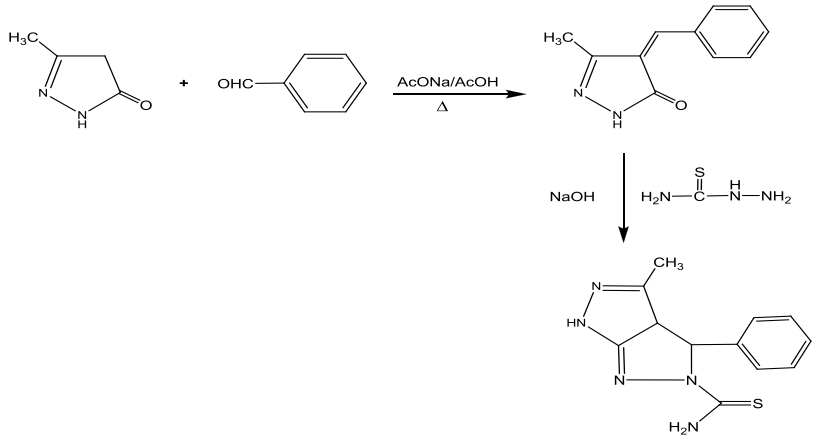
In this paper, an attempt has been made to synthesize 4-methyl 3-phenyl 2- thiocarbamoyl -3,3a dihydro pyrazolo[3,4c] pyrazole in its purest form by incorporating an active pharmacophore, pyrazoline into a pyrazole unit. The inclusion complex of the compound has been prepared with a non-toxic oligosaccharide, β-cyclodextrin and characterized by the study of spectral and thermodynamic properties .The compound and its inclusion complex are also screened for antibacterial and antioxidant activity .

II. Materials And Methods

2.1 Apparatus and Materials

All the chemicals of acceptable standards have been procured from local market. Double distilled water to be used as solvent is prepared in the laboratory. Electronic spectra are recorded on Shimadzu UV-1700 Spectrophotometer and IR spectra have recorded in KBr pellets in Shimadzu8400 FTIR Spectrophotometer. ^{1}H NMR spectra (DMSO-d_6) are scanned on a DRX-300 (300MHz) spectrophotometer using TMS as internal standard and chemical shifts are expressed in δ, ppm. X-ray powder diffraction patterns are recorded using a X'pert PROPANlytical Diffractometer. Purity of synthesized compounds has been checked by elemental analysis and homogeneity has been checked by TLC using silica gel-G, as adsorbent. Melting points are recorded by open capillary method.

The compound, 4-methyl 3-phenyl 2- thiocarbamoyl -3,3a dihydro pyrazolo[3,4c] pyrazole has been synthesised in the following steps as shown in Scheme -1.



Scheme 1 Synthetic pathway of compound

2.2 Synthesis of 4-methyl 3-phenyl 2- thiocarbamoyl -3,3a dihydro pyrazolo[3,4c] pyrazole. The compound, 4-methyl 3-phenyl 2- thiocarbamoyl -3,3a dihydro pyrazolo[3,4c] pyrazole has been synthesized as per the following steps

2.2.1 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) is added drop wise to it with stirring. The temperature was raised during this addition and it is maintained at 60˚C when a crystalline solid separated. The reaction mixture is further stirred for 1 hr at room temperature then cooled in an ice bath to complete the crystallization. The separated solid is washed with ice cold ethanol.

2.2.1 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.

2.2.2 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 is filtered, dried and recrystallized from DMF.

2.3 Phase Solubility Measurements:-

The aqueous phase solubility of the compound at various concentrations of β-cyclodextrin (0-10mMl) is studied as per Higuchi-Corner method[15]. Accurately weighed amount of the compound is taken in different concentrations of β -cyclodextrin (0-10mMl) and these are 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 are filtered through Whatmann-42 filter paper and are analyzed in a UV-visible spectrophotometer. The various values of absorbance at λ-max are plotted against different concentrations of β – cyclodextrin.

2. 4 Synthesis of inclusion complex:-

The inclusion complex of the compound with β–cyclodextrin are prepared as per co-precipitation method [13,14]. The solution of the compound in required concentration is added drop by drop to β–cyclodextrin solution of the required concentration. The mixture is stirred for a period of 48 hours and filtered. The filtrate is cooled for 24 hours in refrigerator. The precipitate obtained is filtered through G-4 crucible, washed with water and dried in air for 24 hours

2.5 Study of thermodynamic properties:-

The thermodynamic stability constant of the complexes was calculated using Benesi-Hilderband relation.[16]

The value of ΔG was calculated at 298 K using the equation:

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

2.6 Evaluation of Antibacterial activity.

The antibacterial activity of compounds is studied as per cup-plate method [20]. The solutions of the test compounds were prepared in dimethyl sulphoxide (DMSO) at 500µg/ml. The bacterial strains are inoculated into 100ml of the sterile nutrient broth and incubated at 37±1 ^{0}C for 24 hours. The density of the bacterial suspension is standardized by McFarland method. Well of uniform diameter (6mm) are made on agar plates, after inoculating them separately with the test organisms aseptically. The drug, control and the test compounds are introduced with the help of micropipette and the plates are placed in the refrigerator at 8- 10^{o}C for proper diffusion of drug into the media. After two hours of cold incubation, the Petri plates are transferred to incubator and maintained at 37±2^{o}C for 18-24 hours. Then the Petri plates are observed for zone of inhibition by using vernire scale. The results are reported by comparing the zone of inhibition shown by the test compounds with standard drug Tetracycline. The results are the mean value of zone of inhibition of three sets measured in millimetre.

2.7 Evaluation of Antioxidant activity

In the present study DPPH (2, 2-Diphenyl-1-picrylhydrazyl) scavenging assay method is used for screening the antioxidant activity of the synthesized compounds as suggested by Tagashira and Ohtake. [19] Test sample solution is prepared in 100µg/ml concentration in ethanolic DPPH. After vortexing, the mixture is incubated for 10 minutes at room temperature. The absorbances of the samples are measured at 517 nm. The activity of the sample is calculated by finding the difference of absorbance between a test sample and a control. Butylated Hydroxyl Toluene (BHT) is used as reference substance.

III. Result 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) .

Table 1 Physical and Spectral properties of compound and inclusion complex

Sl no.	Compound/ complex	Melting point	colour	Elemental analysis (finding value) calculated value				λmax (nm)	IR(KBR) (cm-^{1})	NMR
1	Compound	168°C	Brownish	C (42.28) 42.30	H (34.42) 34.41	N (9.12) 19.23	S (3.74) 3.84	303	3415, 3375 (N-H str.) ,3052 (C-H str. in Ar-H 2980 (C-H str. CH_3), 1180 (C=S str.	^{1}H NMR (DMSO): δ 7.60-7.80 (m, 4H, Ar-H), 6.64 (s, 2H, NH_2), 4.22 (d, 2H, C*H*-CH), 4.26 (d, 2H, CH-CH), 2.04 (s, 3H,)
2	Inclusion complex	278°C	White					305	3395, (N-H str.), 2931 (C-H str. in Ar-H) ; 2980 (C-H str. CH_3), 1168 (C=S str),	

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 give a prominent peak at 303 nm which undergoes a shift towards higher wavelength of 305 nm after the formation of inclusion complex.

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 be attributed to encapsulation of the compound in the hydrophobic core of β –cyclodextrin and development of weak interactions in between guest and host. [13]

Similarly the NMR peaks of the compound and its inclusion complex are shown in the fig.3 and fig.4. Comparisons of both the figures suggest the prominent PMR signals are shifted towards upfield in the inclusion complex which could be attributed to encapsulation induced shielding within the cavity of β-cyclodextrin.

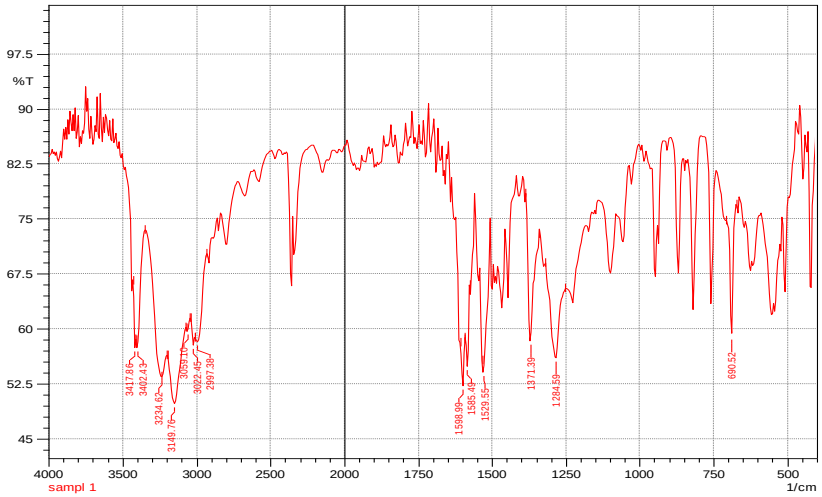


Fig.1 IR spectra of the compound

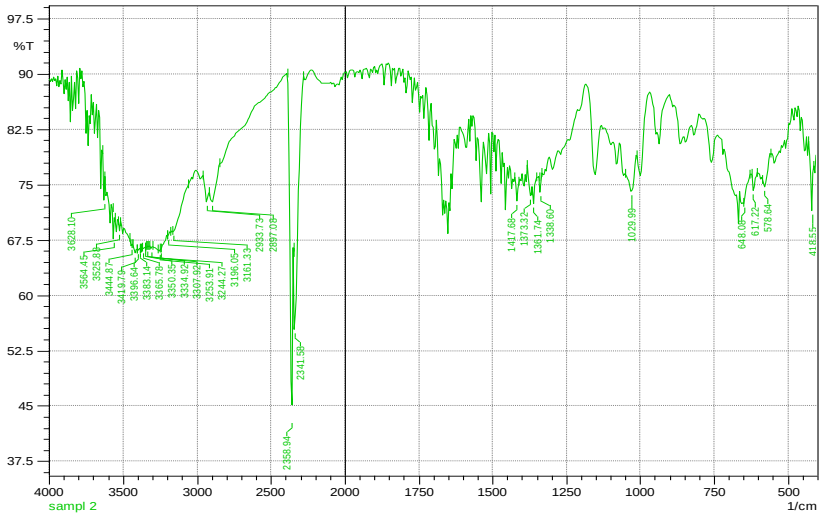


Fig.2 IR spectra of the Inclusion Copmlex

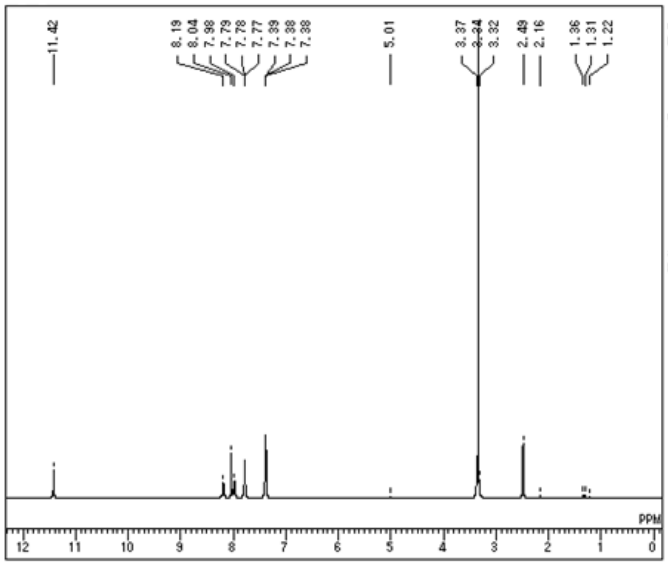


Fig.3 NMR spectra of the compound

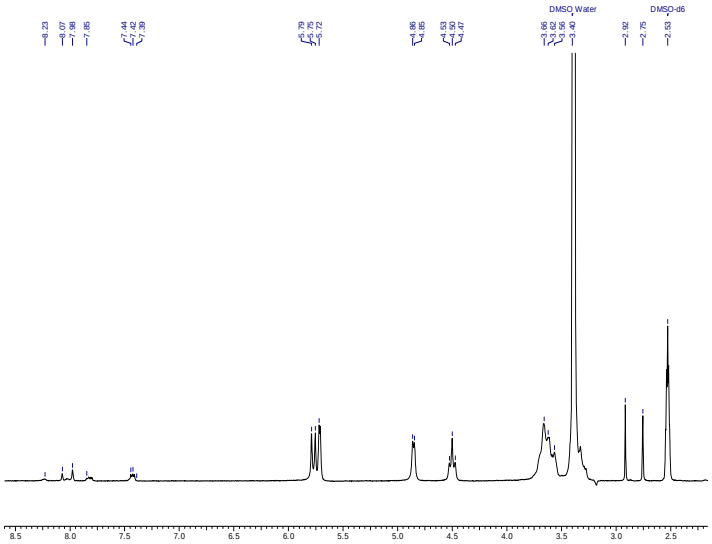


Fig.4 NMR Spectra of the Inclusion complex

The formation of inclusion complex can be further supported by X-ray diffractometry[18,21]. The comparative powder X-ray diffractometric pictures of β –cyclodextrin, compound and inclusion complex are shown in Fig.5. The X-ray diffractometric picture of inclusion complex is different from that of pure β –cyclodextrin and synthesized compound. This difference in the XRD spectrum is due to the encapsulation of compound with β –cyclodextrin cavity resulting in a new crystal structure i.e. inclusion complex.

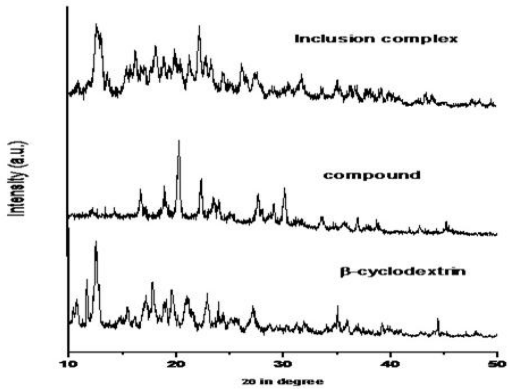


Figure 5 XRD picture of β-CD, Compound and Inclusion complex

It is seen that there is a linear increase in solubility with increasing concentration of β –cyclodextrin as shown in fig 6. Since the slope of the plot is less than unity, the stoichiometry of the inclusion complex may be 1: 1.[17]

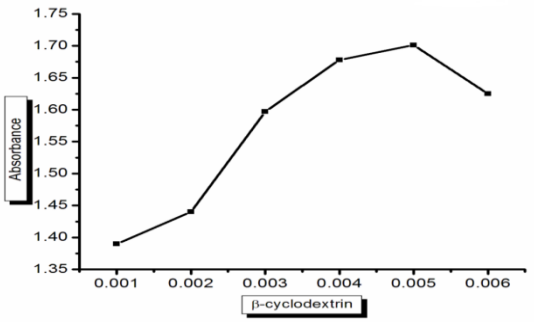


Figure 6. Phase solubility study of Compound with β-CD.

The thermodynamic stability of the inclusion complex has been calculated by Benesi –Hildebrand relation. [16]

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

Where $\Delta\varepsilon$ 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 $1/. [\beta\text{-CD}]_o$ as shown in fig 7.

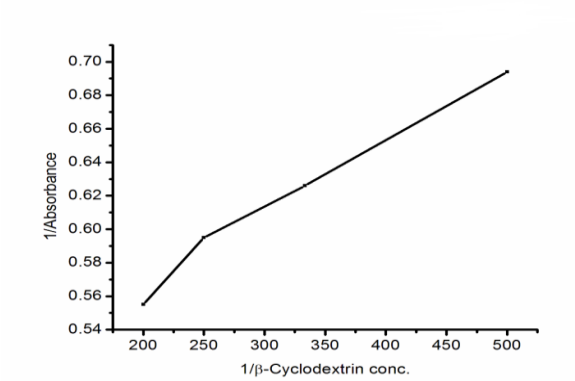


Figure 7. Plot of 1/OD verses 1/ conc. of β –cyclodextrin

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 165 indicating appreciable stability of inclusion complex because the value remains within the ideal range of 100-1000[22]. 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 [13]. The antioxidant activity of the compound and its inclusion complex is shown in fig.8.

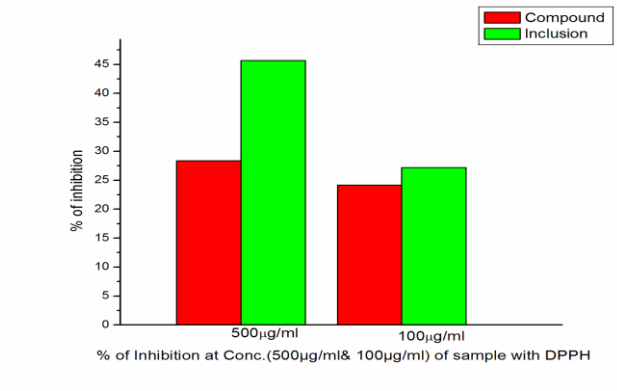


Figure 8. Antioxidant activity of the compound and inclusion complex

The radical scavenging activity of the compound increases significantly after the formation of inclusion complex. This can be correlated to the higher solubility of the compound due to inclusion complex formation there by increasing the bioaccessibility. Higher the bioaccessibility of the compounds, higher becomes the ability of compounds to trap the reactive oxygen species or free radicals, thereby increasing antioxidant activity of the compounds[24]. The antibacterial activities of the compound and its inclusion complex against S.aureus , E.coli, S. pyogenes are shown in fig 9.

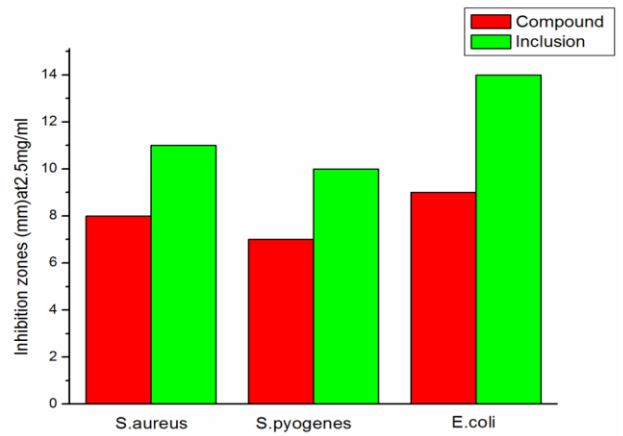


Figure 8. Antibacterial activity of the compound and inclusion complex

Both the compound and its inclusion complex are susceptible to the bacteria. It is clear from the figure that the inclusion complex has higher antibacterial activity as compared to its compound. This is due to the enhanced solubility of the inclusion complex which becomes more available to specific tissues leading to increased antibacterial activity [1,20].

IV. 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 increases the antibacterial and antioxidant activity of the compound significantly.

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