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Studies on Inclusion Complex of 3-(2-Chloro Phenyl) 4-Methyl 2- Thiocarbamoyl -3,3a Dihydro Pyrazolo[3,4c] Pyrazole

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ABSTRACT

The inclusion complex of 3-(2-chloro phenyl) 4-methyl 2-thiocarbamoyl-3,3a dihydropyrazolo[3,4c] pyrazole has been synthesized with β-cyclodextrin so as to improve its solubility and bio accessibility. The formation of inclusion complex has been ascertained by changes in melting point and spectral data (UV, IR, XRD, NMR etc). The determination of thermodynamic stability constant suggests that the inclusion complex has appreciable stability and its formation is thermodynamically allowed.The study of the antibacterial activity of the compound and its inclusion complex suggests that inclusion formation increases antibacterial activity significantly.

Keywords: Bis-pyrazole, Inclusion-complex, β-Cyclodextrin, Aqueous phase solubility, Antibacterial activity.

INTRODUCTION

Pyrazole framework is an important pharmacophore and has been extensively used for the synthesis 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.[8-14] In this paper, the compound 3-(2-chloro phenyl) 4-methyl 2-thiocarbamoyl-3,3a dihydropyrazolo [3,4c] pyrazole has been synthesized in its purest form by incorporating pyrazoline into a pyrazole unit and its inclusion complex has been prepared with β-cyclodextrin.Finally an attempt has been made to study the spectral, thermodynamic and antibacterial

properties of the compound and its inclusion complex.

EXPERIMENTAL SECTION

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) were 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 were recorded by open capillary method.

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

Ethyl acetoacetate (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 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-(2-chlorobenzylidene)-5-methyl-2,4-dihydro-3H-pyrazol-3-one(B)

The compound (A) (0.01 mole), 2-chloro 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 filtered and recrystallized from ethanol.

Step-3: Synthesisof 3-(2-chloro phenyl) 4-methyl 2-thiocarbamoyl-3, 3a dihydro-pyrazolo [3, 4c] pyrazole

To the mixture of above synthesized compound (B) (0.01mole) and thiosemicar-bazide (0.01mole) in 50ml of ethanol, a solution of NaOH 0.02 mole in 5ml of water was added and refluxed for 10hrs. The product was poured into crushed ice. The solid obtained was filtered and recrystallized from DMF. (C)

Phase Solubility Measurements

The aqueous phase solubility of the compound at various concentrations of β-cyclodextrin (0-10mMl) was studied as per Higuchi-Corner method.[15]

Synthesis of inclusion complexes

The inclusion complex of the compound with β–cyclodextrin was prepared as per co-precipitation method.[12,14]

Evaluation of Antibacterial activity

The antibacterial activity of compounds was studied as per cup-plate method.[16]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 (6 mm) were made on agar plates, after inoculating them separately with the test organisms aseptically. The drug, control and the test compounds were introduced with the help of micropipette and the plates are placed in the refrigerator at 8-10^{0}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^{0}C for 18-24 hours. Then

the Petri plates are observed for zone of inhibition by using vernier scale. The results were 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.

RESULTS AND DISCUSSION

The structure of the compound has been confirmed from elemental analysis and study of spectral characteristics. The elemental composition matches with theoretical data. The IR and ^{1}H NMR data of the compound and the corresponding inclusion complex indicate 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).

The formation of inclusion complex can be further supported by X-ray diffractometry.[17] The powder X-ray diffractometric pictures of β –cyclodextrin, compound and inclusion complex are shown in Fig.1.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 in β–cyclodextrin cavity resulting in a new crystal structure i.e. inclusion complex.

It has been 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.[14,18]

The thermodynamic stability of the inclusion complex has been calculated by Benesi–Hilderbrand 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,

$$K_T = \text{Intercept/slope}$$

The K_T value of the inclusion complex is found to be 211.34 indicating appreciable stability of inclusion complex because the value remains within the ideal range of 100-1000.[18,19]

The antibacterial activities of the compound and its inclusion complex against *S.aureus, E. coli, S. pyogenes* and *P. vulgaris* were shown in Fig.4. 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.[20, 21]

CONCLUSION

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

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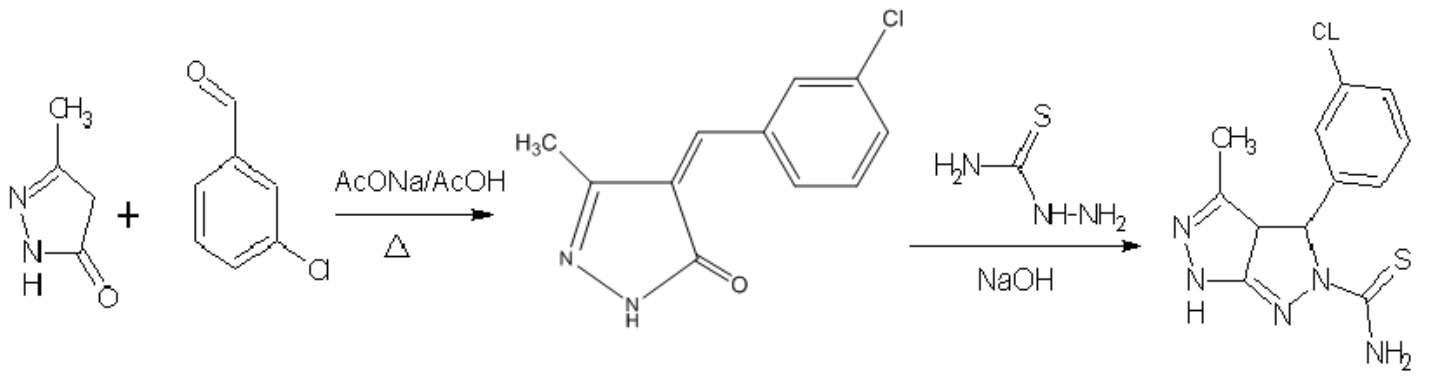
Table 1. Physical and spectral properties of the compound with and without inclusion complex

Comp./ Complex	Melting point^{0}C	Colour	Elemental analysis First line indicates the finding value Second line indicates calculated value					λmax nm	IR(KBr) cm^{-1}	NMR Data
Comp.	185	Brownish	N 16.01 16.12	S 3.11 3.22	C 38.24 38.70	H 38.21 38.70	Cl 3.14 3.22	306	3375, (N-H str.),3122 (C-H str. in Ar-H); 2912 (C-H str. CH_3), 1180 (C=S str,703(C-Clstr)	δ 7.24-8.07(m,4H, Ar-H), 6.78(s, 2H,NH_2),4.25(d,2H,CH-CH),2.06(s,3H, CH_3)
Inclusion complex	261	White						308	3395, (N-H str.),2931 (C-H str. in Ar-H) ; 2980 (C-H str. CH_3), 1168 (C=S str), 706(C-Clstr)	δ 5.1-6.2(m,4H,Ar-H),7.31(s, 2H,NH_2), 4.41(d,2H,CH-CH),2.72(s,3H,CH_3)



SCHEME-1 (completely re-drawn)

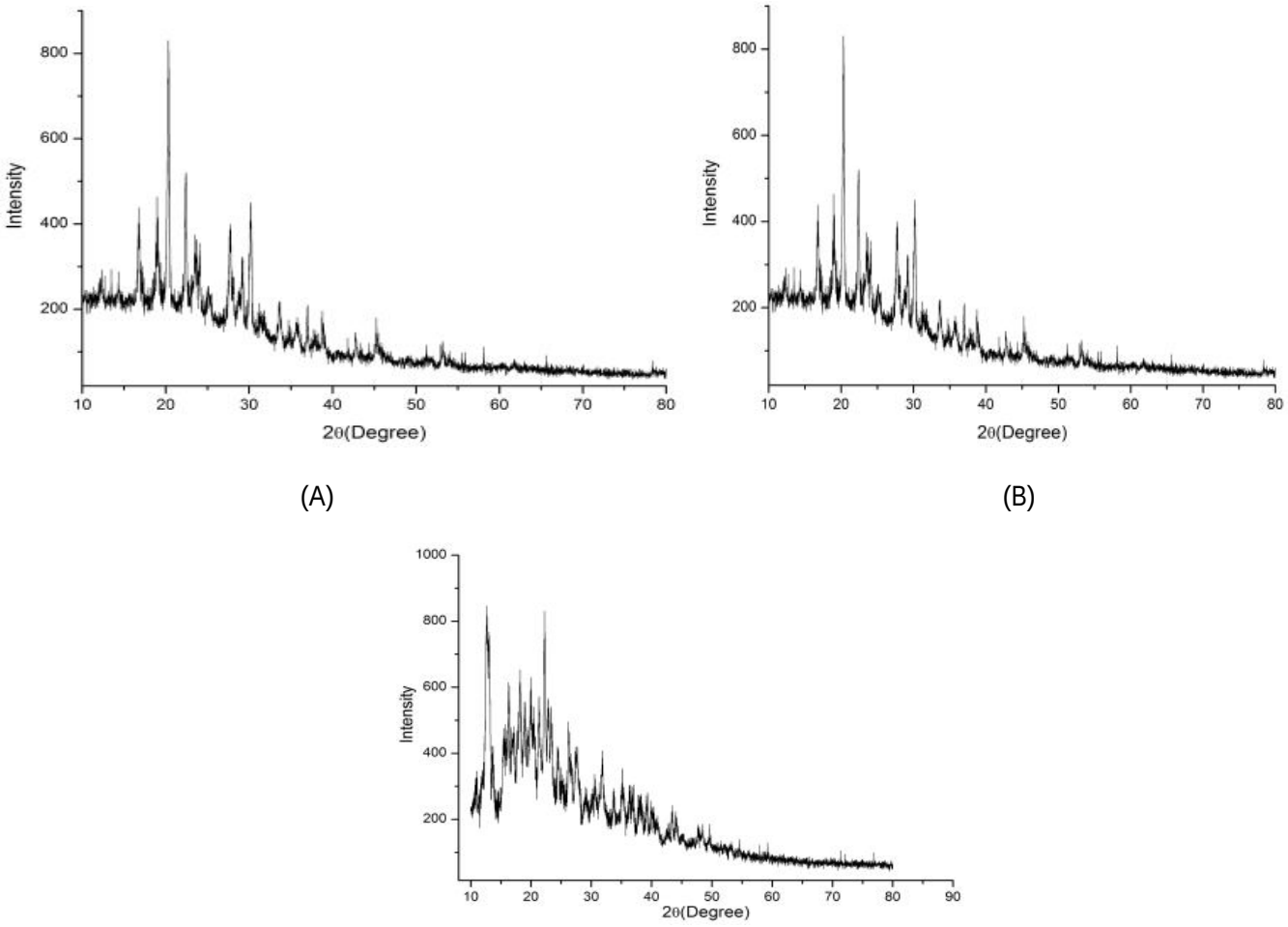


Figure 1. XRD pictures of A) β-cyclodextrin, B) Compound, C) Inclusion complex

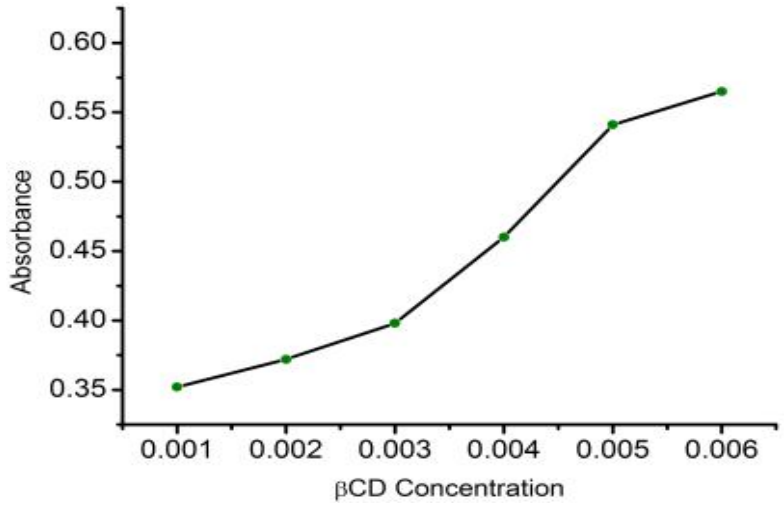


Figure 2. Aqueous phase solubility plot of the compound with β–cyclodextrin

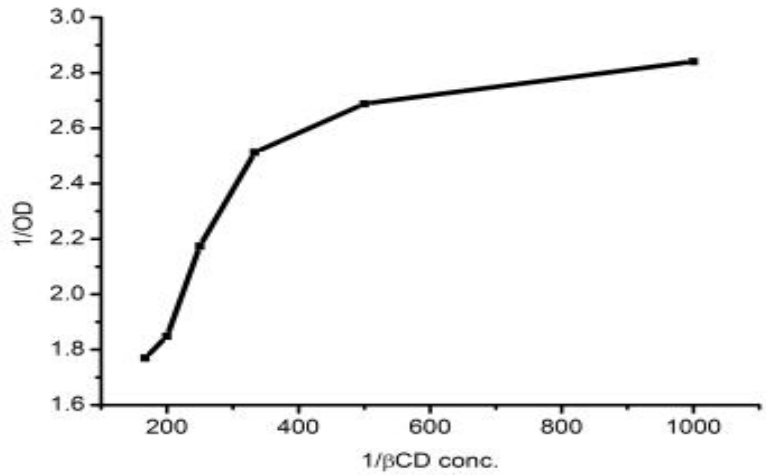


Figure 3. Plot of 1/OD vs 1/β-CD concentration

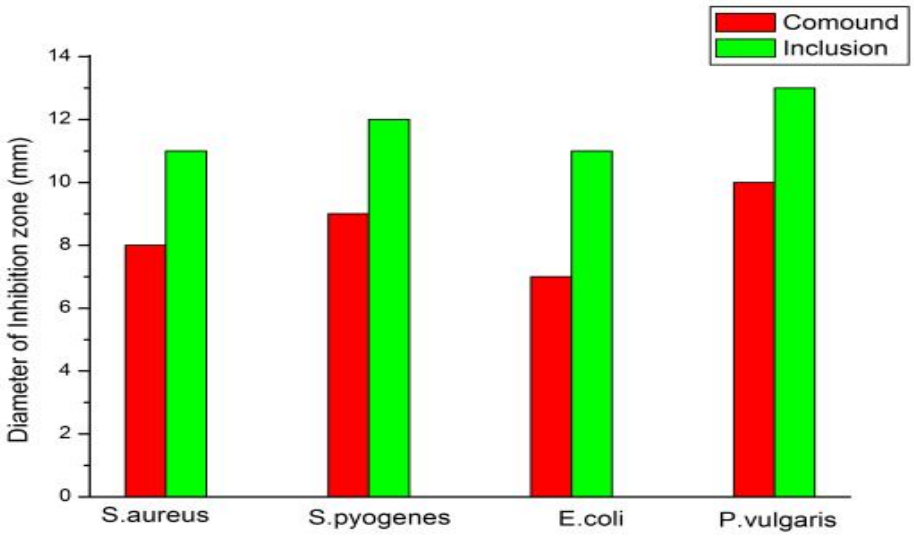


Figure 4. Antibacterial activity of the compound and inclusion complex