Antifungal Activity of Metal Complexes of Thiosemicarbazones

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Introduction

Thiosemicarbazones of substituted aromatic aldehydes are known to possess antifungal, antibacterial [1–3], antiviral [4–6], antitubercular [7, 8] and anticarcinogenic [9] properties when they are complexed with metal ions. With the increasing use of antifungal agents, demonstration of de novo resistance to 5-Fluoco-cytosin (5FC), as well as the development of resistant strains of *Cryptococcus* species, *Candida albicans*, *Torulopsis glabrata* [10, 11] and *Aspergillus* species [12, 13], which are some of the commonest causative agents of fungal infection, the need for new or chemically modified antifungal compounds is shown.

In the present study we have chosen to study the antifungal activity of two thiosemicarbazones namely, pyridine-3-aldehyde thiosemicarbazone and pyridine-2-aldehyde thiosemicarbazone (hereinafter abbreviated as HNAT and HPAT respectively) and several of their complexes.

Experimental

HNAT: was obtained commercially from Fluka AG, Chemische Fabrik, CH-9470, Buchs SG, Switzerland. HPAT: was prepared by heating a mixture of α-picoline (93.8 ml) and sulphur (64.0 g) to a temperature of 135 °C. Thiosemicarbazide (91.8 g) was then added and the temperature maintained at 120–130 °C for 3–4 h. The reaction mixture was allowed to stand overnight to yield a solid product which was dissolved in 0.5 N sodium hydroxide to give a brown solution. This was filtered and the filtrate was neutralized with dilute hydrochloric acid after which the ligand separated out as a pale yellow solid. This was washed with water and recrystallised from methanol (m.p. 0.203–4 °C).

Preparation of Complexes

The complexes, $[Mn(HNAT)_2Cl_2]$, $[Zn(HNAT)_2Cl_2]$, $[Fe(HNAT)_2Cl_2]Cl$, $[Zn(HPAT)Cl_2]\cdot C_2H_5OH$ and $[Fe(PAT)_2]Cl$ were prepared by mixing a hot solution of the respective anhydrous metal salt (0.01 M) in absolute ethanol with a hot saturated solution of the ligand (0.02 M) in the same solvent. Both the HPAT complexes separated out immediately, but for the complete separation of the Fe(III) complex with HNAT, the reaction mixture had to be stirred for 2 h at room temperature. For the Zn(II) and Mn(II) chloride complexes of HNAT, the reaction mixture was refluxed for half an hour. The complexes were filtered, washed with ethanol, dried under vacuum and analysed.

Antifungal Activity

(a) Strains

Antifungal activity of HNAT, HPAT and their complexes was carried out by the broth dilution method for *Candida albicans* and by the agar diffusion method for *Aspergillus fumigatus*. *Candida albicans* was grown in Sabourauds Dextrose Broth (SDB) at 37 °C for 48 h. The inoculum was prepared by suspending the cells in SDB medium so as to obtain a final concentration of (8.8×10^6 CFU/ml) as determined by spectrophotometric method (O.D. = 0.2, 530 nm). Stock solutions (5000 μg/ml) of HNAT, HPAT and their complexes were prepared by dissolving 25 mg of each compound in 1 ml of dimethylsulphoxide (DMSO) and diluting this solution to 5 ml with SDB of pH 6.8.

(b) Susceptibility test

Six different concentrations of each compound (100, 200, 400, 600, 800 and 1000 μg/ml) were prepared in SDB medium from the stock solution. 0.05 ml of the inoculum was added in each tube containing 4.95 ml of the compound containing medium. The tubes were incubated at 37 °C for 48 h. The lowest concentration of the compound at which there was no visible growth was considered

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as the minimum inhibitory concentration (MIC). The minimum fungicidal concentration (MFC) was determined by subculturing the tubes showing no visible growth on to the sabourauds dextrose agar in petridishes. These were also incubated at 37 °C for 48 h. The concentration of the compound in the inoculum tube producing no colonies was considered as MFC.

For testing the susceptibility of *Aspergillus fumigatus* inoculum was prepared on Czepec's medium. Three to four millilitres of spore suspension of *Aspergillus fumigatus* (95% transmission at 530 nm) was spread uniformly on a sabouraud dextrose agar medium in plates. The excess of fluid was drained off. After drying the plates, wells of 6 mm size were cut in the medium. To each one of these wells 0.2 ml of a solution of different concentration of the compound was added. The zone of inhibition of the growth of *Aspergillus fumigatus* in the presence of these compounds with different concentrations, was measured after 48 and 72 h of incubation at 37 °C.

Results and Discussion

Our attempts at structural characterization of the HPAT and HNAT complexes under study have shown [14] that the three HNAT complexes are dimeric or polymeric having six coordinate metal ion and with HNAT behaving as a bidentate ligand and coordinating through pyridine ring nitrogen and the sulphur atom. Of the two HPAT complexes, the zinc complex is octahedral and dimeric with chlorine bridges while the iron(III) complex is monomeric and octahedral. In both the complexes the ligand coordinates through the nitrogen of the pyridine ring, nitrogen of the hydrazine residue and the sulphur atom but whereas in the zinc complex it coordinates as a neutral tridentate molecule, in the iron complex it does so as a monobasic tridentate molecule.

Antifungal Activity

(a) With Candida albicans

HNAT, HPAT and the complex $[Mn(HNAT)_2Cl_2]$ did not show any inhibition of *Candida albicans* (8.8×10^6 CFU/ml). However, in the presence of $[Fe(HNAT)_2Cl_2]Cl$ (1000 μg/ml), an eight-fold decrease in the concentration of *Candida albicans* (1×10^6 CFU/ml) was seen. The MIF and minimum fungicidal concentration (MFC) for $[Zn(HNAT)_2Cl_2]$ was found to be 800 μg/ml and for $[Zn(HPAT)Cl_2]\cdot C_2H_5OH$ and $[Fe(PAT)_2]Cl$ both it was found to be 1000 μg/ml.

(b) With Aspergillus fumigatus

Since the filamentous growth does not give uniform turbid growth in liquid medium, only the disk diffusion method was used. In the presence of HNAT and its complex $[Mn(HNAT)_2Cl_2]$, there was no inhibition of *Aspergillus fumigatus*. However, in the presence of $[Zn(HNAT)_2Cl_2]$ an inhibition zone of 30.0 mm was found. Similarly, at a concentration of 1000 μg/ml and 750 μg/ml of $[Fe(PAT)_2]Cl$ and 1000 μg/ml of $[Zn(HPAT)Cl_2]\cdot C_2H_5OH$ the inhibition zone was found to be 20, 15 and 24 mm respectively. Although, a very small inhibition zone (10 mm) was seen at the end of 48 h incubation for HPAT, it increased to 15 mm after 72 h. With the other compounds used in the study the *Aspergillus fumigatus* could not be inhibited after 48 h.

It is concluded from these results (Fig. 1) that at a concentration of 1000 μg/ml $[Zn(HNAT)_2Cl_2]$ shows maximum activity. HNAT as such was found to be inactive against *Aspergillus fumigatus* but its zinc complex was highly active.

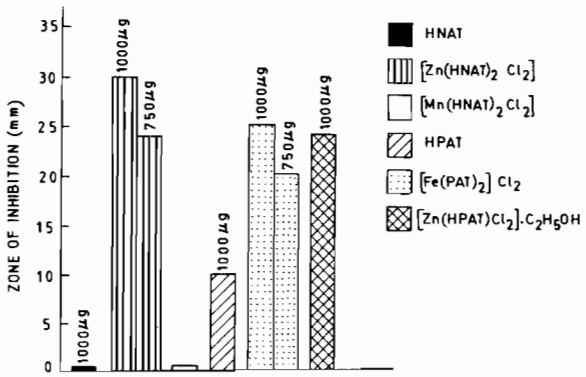

Fig. 1. Zone of inhibition of *Aspergillus fumigatus* in the presence of HNAT, HPAT and their complexes after 48 h of incubation at 37 °C on Sabrauds Dextrose Agar.

Conclusion

The present study clearly shows that all thiosemicarbazones are not active. HPAT was active against *Aspergillus fumigatus* whereas HNAT was found to be inactive. In general the activity of thiosemicarbazones increases on complexing with transition metals. Change in the type of metal seems to play an important role in the interaction with fungus by changing the spatial configuration of ligands and their electrostatic bonding. Detailed study, however, is required to elucidate the mechanism behind the variation in the fungicidal property of different HPAT and HNAT compounds. Thus the present work has potential bearings on the use of such compounds in

the treatment of some of the chronic fungal diseases, primarily or secondarily produced by *Candida albicans* and *Aspergillus fumigatus*.

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