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Novel broad-spectrum metal-based antifungal agents

Correlations amongst the structural and biological properties of copper(II) 2-acetylpyridine N^4-dialkylthiosemicarbazones

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Summary. Copper(II) complexes of the type [Cu(L)X], where L = tridentate anion of 2-acetylpyridine N^4-diethyl thiosemicarbazone and X = Cl or Br, were screened against seven fungal strains pathogenic to man viz. *Aspergillus niger, Aspergillus fumigatus, Candida albicans, Cryptococcus neoformans, Tricophyton rubrum, Epidermophyton floccosum* and *Microsporum canis*. The greater growth inhibition exhibited by the bromo complex can be explained on the basis of its lower Cu(II)/Cu(I) redox couple and greater covalent bonding. These compounds represent a novel class of metal-based antifungal agents which provide opportunities for a large number of synthetic variations for modulation of the activities.

Key words: Antifungal agents – Cyclic voltammetry – Copper thiosemicarbazone complexes

Introduction

Thiosemicarbazones possess a wide range of biological activities including antitumor (French and Blanz 1966; Moore et al. 1970), antiviral (Logan et al. 1975), antimalarial (Klayman et al. 1979) and antileprotic (Hooper and Purohit 1983; Collins et al. 1982). Studies have shown that their metal complexes, especially those containing copper(II) and iron(II), are more active than the uncoordinated thiosemicarbazone molecules (Scovill et al. 1982). This enhanced biological activity of metal thiosemicarbazones has been under investigation for some time; studies on the antifungal properties of heterocyclic thiosemicarbazones were published a few years ago (Parwana et al. 1985). The electrochemical potentials of copper(II) complexes of 2-acetylpyridine N^4-dialkylthiosemicarbazones have been indicated to play a significant role in the antifungal properties against common subcutaneous fungi, viz. *Aspergillus niger* and *Paecilomyces variotti* (Kumbhar et al. (1991)). Thus, we were motivated to extend these studies to include other classes of fungi and to examine the influence of redox potentials (either metal- or ligand-based) on the antifungal properties of this class of compounds. The present work indicates that the compounds possess a broad-spectrum antifungal activity against a variety of fungi including *Tricophyton rubrum, Epidermophyton floccosum, Candida albicans, Aspergillus fumigatus, Cryptococcus neoformans* and *Microsporum canis* which can be correlated with the Cu(II)/Cu(I) redox couple of these compounds.

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Experimental procedures

Syntheses, as well as the spectral and magnetic properties, of [Cu(L)Cl] and [Cu(L)Br] where L = tridentate anion of 2-acetylpyridine N^4-diethylthiosemicarbazone were reported previously (West et al. 1990).

The cyclic voltammetric experiments were performed on a BAS-CV-27 assembly in conjunction with an *X/Y* recorder. Measurements were made on the degassed (N_2 bubbled for 15 min) 1 mM solutions in dimethylsulfoxide containing 0.1 M tetraethylammonium perchlorate as supporting electrolyte. The working electrode was a platinum inlay electrode referenced with an Ag/AgCl electrode and platinum wire as auxillary electrode.

The antifungal activities of the test complexes, along with the standard antifungal compound, viz. mycostatin, were determined by the well-diffusion method (Copper 1964). Inhibition was determined as the diameter of the inhibitory zone. The minimum inhibitory concentrations (MIC) were determined by the serial dilution method (Reevs et al. 1978).

Results and discussion

Structural studies on the copper(II) complexes have shown that the overall geometry of these compounds is planar with a general formula of [M(L)X], where L = anion of 2-acetylpyridine N^4-diethylthiosemicarbazone and X = Cl or Br. The structural parmameters derived from the optical and ESR spectra of these compounds (Table 1) are in agreement with such an assignment.

Table 1. Structural parameters of the copper(II) complexes of 2-acetylpyridine N^4-diethylthiosemicarbazone

Parameter	Value for compound	
	[Cu(L)Cl]	[Cu(L)Br]
μ^a	1.7	1.6
$^2B_{1g} \rightarrow {}^2B_{2g}$ (cm^{-1})	14430	14490
g_{11}	2.149	2.126
k_{11}	0.57	0.52
$^2B_{2g} \rightarrow {}^2E_g$ (cm^{-1})	17180	17360
$g_\perp$	2.024	2.038
$k_\perp$	0.48	0.61
A (cm^{-1})	0.0187	0.0171
α^2	0.67	0.61

[a] The value is given in Bohr magneton, μ/μ_B

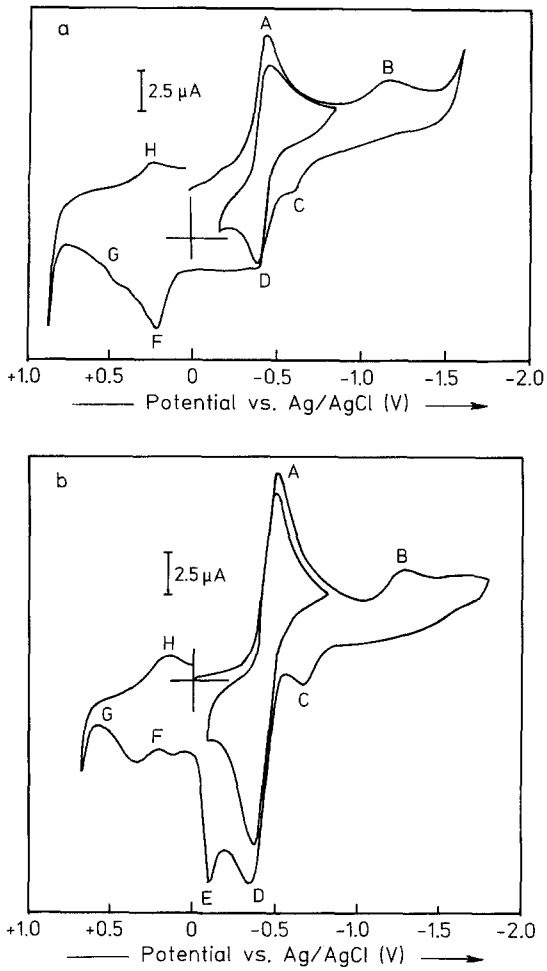

Fig. 1. Cyclic voltammograms (scan rate 100 mV s^{-1}) of 1 mM solutions in dimethylsulfoxide, 0.1 M tetraethylammonium perchlorate at 25° C. (**a**) [Cu(L)Cl], (**b**) [Cu(L)Br] where L = tridentate anion of 2-acetylpyridine N^4-diethylthiosemicarbazone

The cyclic voltammetric profiles of the two complexes (Fig. 1) indicate three reduction peaks (A, B and H) and several oxidation peaks (C, D, E, F and G). Repeated scans show that the main reduction peak, denoted by A, and its oxidation counterpart, D, are reversible. These two peaks represent reduction of copper(II) to copper(I) and oxidation of copper(I) to copper(II) within the scan rates employed, the separation between them, 60–80 mV, is characteristic of a quasi-reversible one-electron process arising probably from the relaxation process involved in a stereochemical change from planar copper(II) to tetrahedral copper(I). The metal center in the bromo complex ($E^0 = -0.37$ V) is easier to reduce than its chloro counterpart ($E^0 = -0.42$ V). However the values of the redox potentials for both the compounds are indicative of considerable 'hard-acid' character for the heterocyclic thiosemicarbazone ligand, making it comparable to the ethylenediamine type of ligands ($E^0 = -0.35$ V).

The second reduction peak, B, for these compounds corresponds to the reduction of the azomethine double bond of the coordinated thiosemicarbazone ligand and its value -1.22 V is comparable with those observed for many aromatic thiosemicarbazone ligands (-1.31 V at pH 6.0; Eisner and Eisner 1970). This reduction is followed by three oxidation peaks C, E and F which are due to coupled chemical reactions. Peaks G and H correspond to the oxidation reduction peaks ot the halide ligands.

The inhibitory activities of the copper(II) complexes were examined against all four types of fungi, i.e. Dermatophytes (*Tricophyton rubrum, Epidermyphyton floccosum*); subcutaneous and systemic (*Aspergillus fumigatus, Microsporum canis*); Candidiasis (*Candida albicans*) and opportunistic (*Cryptococus neoformans*). From the results summarized in Table 2, it is evident that both compounds possess a broad-spectrum antifungal activity. Since the inhibitory activities of the metal complexes are found to be significantly higher than the free ligands, it may be concluded that complexation is able to modulate or alter a ligand property crucial for the biological activity of the thiosemicarbazone compounds. In an earlier communication (Kumbhar et al.

Table 2. Electrochemical parameters and antifungal activity of copper(II) 2-acetylpyridine N^4-diethylthiosemicarbazones

Parameter	Value for compound		
	[Cu(L)Cl]	[Cu(L)Br]	mycostatin
E^0 (A–D) [V]	−0.46	−0.38	—
E^p (B) [V]	−1.22	−1.22	—
Inhibition zone [mm] (MIC [μg/ml]) for:			
A. niger	10 (62.5)	13 (31.5)	— (31.5)
A. fumigatus	12 (62.5)	14 (62.5)	— (62.5)
C. albicans	12 (125)	12 (62.5)	— (31.5)
C. neoformans	9 (125)	17 (62.5)	— (62.5)
T. rubrum	9 (125)	11 (125)	— (62.5)
E. floccosum	10 (125)	12 (62.5)	— (231.5)
M. canis	12 (125)	14 (62.5)	— (62.5)

Concentration of the compounds was 200 mg/ml in dimethylsulfoxide. Redox potentials were measured vs Ag/AgCl. Inhibition was measured as the diameter of the growth inhibition zone (6.0 mm = no inhibition). MIC = minimum inhibitory concentration

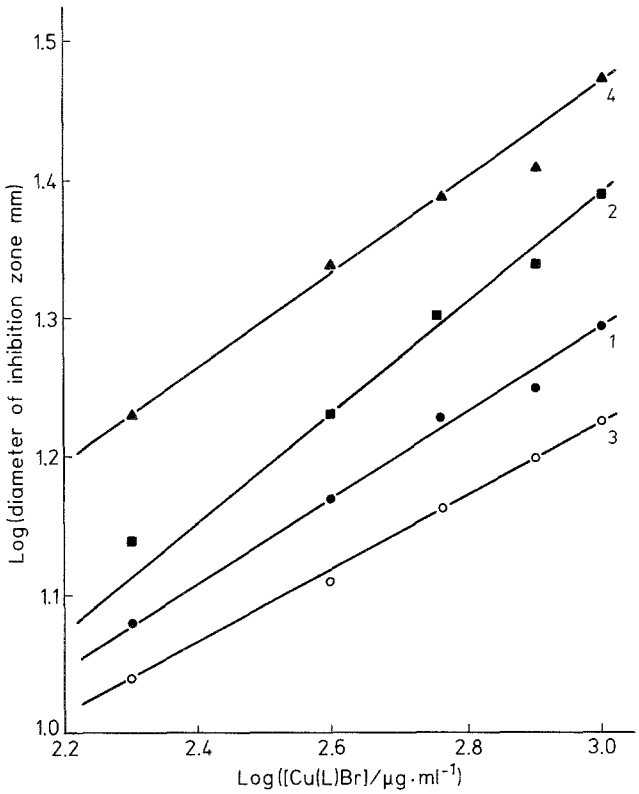

Fig. 2. Log(dose)/log (response) curves for [Cu(L)Br] against four classes of fungi: *A. niger* (**1**), *C. albicans* (**2**), *T. rubrum* (**3**) and *C. neoformans* (**4**)

1991), we have shown that substitution on the thiosemicarbazone side chain at the terminal N^4 center affects the second reduction peak, B, in the cyclic voltammograms of the copper(II) compounds and the corresponding ligand reduction potentials can be correlated with their antifungal properties. In the present case variation in the auxillary halo ligand results in a change in the metal-based redox couple (from -0.46 V for the chloro compound to -0.38 V for the bromo compound). This shift towards more positive potential is reflected in the increased antifungal activity of the bromo complex against all of the organisms examined. The observation suggests that coarse and fine tuning of antifungal activities of these copper complexes of thiosemicarbazone ligands may be possible with the help of both the auxillary ligand and the N^4 substituents.

Studies on the concentration dependence of the antifungal activities of the present compounds indicate linear relationships (Fig. 2) while the minimum inhibitory concentrations (Table 2) indicate that these compounds possess activity comparable to the standard antifungal compounds like mycostatin, at least for *Cryptococus neoformans, Microsporum canis, Aspergillus niger* and *Aspergillus fumigatus,* which are the most common systemic and opportunistic fungi. It has been shown that the antifungal activity of mycostatin is related to its disruptive action on the membrane structure, while the thiosemicarbazone compounds are known to block DNA synthesis through inhibition of ribonucleotide diphosphate reductase enzyme.

This work has not only shown a broad-spectrum antifungal activity for copper(II)-thiosemicarbazone complexes, but also ways in which reduction potentials of these compounds can be controlled for effective biological activities.

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