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Research paper

New insight into the structural, electrochemical and biological aspects of macroacyclic Cu(II) complexes derived from S-substituted dithiocarbazate schiff bases

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ABSTRACT

Copper (II) complexes synthesized from the products of condensation of S-methyl- and S-benzyldithiocarbazate with 2,5-hexanedione (SMHDH2 and SBHDH2 respectively) have been characterized using various physicochemical (elemental analysis, molar conductivity, magnetic susceptibility) and spectroscopic (infrared, electronic) methods. The structures of SMHDH2, its copper (II) complex, CuSMHD, and the related CuSBHD complex as well as a pyrrole byproduct, SBPY, have been determined by single crystal X-ray diffraction. In order to provide more insight into the behaviour of the complexes in solution, electron paramagnetic resonance (EPR) and electrochemical experiments were performed. Antibacterial activity and cytotoxicity were evaluated. The compounds, dissolved in 0.5% and 5% DMSO, showed a wide range of antibacterial activity against 10 strains of Gram-positive and Gram-negative bacteria. Investigations of the effects of efflux pumps and membrane penetration on antibacterial activity are reported herein. Antiproliferation activity was observed to be enhanced by complexation with copper. Preliminary screening showed Cu complexes are strongly active against human breast adenocarcinoma cancer cell lines MDA-MB-231 and MCF-7.

1. Introduction

Effective treatment of multi-drug resistant (MDR) bacterial infections has become increasingly challenging as the efficiency of the available antibiotic arsenal is reduced, resulting in increased frequency of therapeutic failure [1,2]. Over-expression of efflux pumps can contribute to resistance of bacteria by expulsion of structurally unrelated compounds causing a decrease in the

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intracellular concentration of antibiotics [3,4]. It is essential to understand efflux-mediated resistance in bacterial pathogens to develop efficient antibacterial agents circumventing this mechanism. In addition, parallel concerns relating to acquired drug resistance as well as the serious side-effects of anticancer drugs in the midst of the increasing rate of cancer diagnoses drives the effort to develop better alternatives [5,6]. Due to their many tunable functionalities, dithiocarbazate compounds are exciting candidates for exploration and potential development as antimicrobial and cytotoxic agents.

Sulphur-nitrogen chelating agents derived from S-alkyl/aryl esters of dithiocarbazic acid have been extensively investigated in recent years for their cytotoxicity [7,8], antibacterial [9], antiamoebic [10], anti-*Trypanosoma cruzi* [11] and anti-*Mycobacterium tuberculosis* [12] activities. Considerable attention continues to be given to these and related Schiff bases [13–16], since their properties can be modulated by introducing different substituents through condensation of various S-substituted dithiocarbazate esters with a wide array of aldehydes and ketones. In many cases, the bioactivities of various dithiocarbazate derivatives have been shown to differ widely although there may be only slight modifications in their molecular structures [8]. Since these ligands possess both hard nitrogen and soft sulfur donor atoms they are capable of coordinating with a wide range of transition and non-transition metal ions forming metal complexes with interesting physicochemical and enhanced biological properties [17–19]. The wide diversity of structures displayed by macrocyclic and macroacyclic Schiff bases [20] results in various coordination abilities that could potentially lead to applications ranging from diagnostics to therapeutics [21,22]. As part of our ongoing exploration of these interesting properties, we investigated the synthesis and characterization of some macroacyclic bis(dithiocarbazato) Schiff bases and their Cu(II) complexes. Copper complexes derived from the analogues thiosemicarbazate have also been subjected to intensive research [23–26] and appear to be very efficient antimicrobial [27] and anticancer [28] agents. The copper (II) complexes of quadridentate NNSS donor ligands reported in the literature are also known to be neutral, stable ($K_{ass} = 10^{18}$) compounds that easily cross cellular membranes [23,29]. Thus, copper ion was a logical choice for complexation in our search for effective metallodrugs.

The main aim of the present work is to explore the biological potential of newly synthesized bis(dithiocarbazato) ligands and their Cu(II) complexes by determining their potencies against different bacterial strains expressing a multi-drug resistance phenotype and the effect of efflux pumps and membrane penetration on their antibacterial activity. In addition cytotoxicity assays against two breast cancer cell lines was carried out to determine the effect of complexation with copper upon the activity of the ligands against these cells. Whereas syntheses of many dithiocarbazate compounds have been reported in the literature, reports on the bioactivities [30], crystallography, EPR and electrochemistry [31,32] of Cu(II) bis(dithiocarbazate) complexes are limited. To develop such compounds with effective pharmacological activity, it is essential to orient effort towards correlating the biological activities of this class of compounds with their solid and solution structures as well as their physicochemical properties to identify the optimum geometry about the Cu ion. This goal can be achieved through the synthesis of a graduated series of ligands designed to reveal the mode of bioaction.

2. Results and discussion

2.1. Synthesis and characterization

The synthesis of S-substituted dithiocarbazates was performed as previously described [33,34]. Carbon disulfide and hydrazine were reacted in basic ethanol. After workup, the dithiocarbazate produced was directly reacted with methyl iodide or benzyl chloride to afford S-methyldithiocarbazate (SMDTC) and S-benzyldithiocarbazate (SBDTC), respectively. Schiff bases were then prepared by a slight variation of the method described by Ali et al. [35]. The respective S-substituted dithiocarbazates and 2,5-hexanedione were condensed in 2:1 ratio (Scheme 1). The initial attempts to synthesize the ligand SBHDH2 with prolonged heating followed by purification using column chromatography were unsuccessful. NMR, ESI, elemental analysis and single crystal X-ray diffraction confirmed cyclization to a pyrrole derivative. We postulate that bis(dithiocarbazate) indeed formed but was then hydrolyzed to mono(dithiocarbazate) and S-benzyldithiocarbazate [36,37] with subsequent cyclization of the mono(dithiocarbazate) to a pyrrole *via* the Paal–Knorr reaction. To our knowledge, this is the first pyrrole derived from a dithiocarbazate reported although there are two recent reports of formation of pyrrole byproducts upon reaction of thiosemicarbazone with 2,5-hexanedione [38,39]. Encouraged by the remarkable pharmacological properties of functionalized pyrroles [40,41], we tested the compound for its antimicrobial activity, the results of which are discussed below. The Schiff base, SBHDH2, was finally obtained using either of the following two methods: stirring the dione and SBDTC at room temperature for 30 min or heating for only 5 min after which the white precipitate formed immediately. SMHDH2 was synthesized without the complication of side-reaction occurrence. The precipitate was recrystallized to afford pure SMHDH2 (70% yield).

Cu(II) complexes with NNSS coordination were obtained from the reaction of copper (II) acetate with an equimolar amount of the respective ligand (in acetonitrile for SBHDH2 and methanol for SMHDH2). The complexes were isolated by filtration with yields of 77% and 73% for CuSMHD and CuSBHD, respectively. Black crystals were grown from acetonitrile.

2.2. Characterization of the complexes in the solid state

The characteristic infrared bands of the S-substituted dithiocarbazate ligand, ν(N–H) at *ca.* 3129 cm^{-1} and ν(C=S) at *ca.* 1050 cm^{-1} disappeared upon formation of the Cu(II) complexes. In addition, ν(C=N) of the azomethine bond shifted to lower energy (1611 cm^{-1} and 1606 cm^{-1} for CuSMHD and CuSBHD, respectively) and a second ν(N=C) band in complexes containing anionic dithiocarbazate moieties appeared [42]. The hydrazinic band, ν(N–N), at *ca.* 828 cm^{-1} in the free ligand also shifted upon complexation, to higher (CuSBHD) and lower (CuSMHD) wavenumbers. These observations confirm deprotonation of the Schiff bases with coordination through the azomethine nitrogen atom. The ν(CSS) band *ca.* 985 cm^{-1} (ligand) splits into two components at 1000-955 cm^{-1} upon complexation. The presence of this band and the absence of the C=S band in the spectra of the metal complexes provide additional evidence of the coordination of the Schiff base to the metal in its thiolate form [43,44]. To confirm the 1:1 stoichiometry, the complexes were also characterized by elemental microanalyses for which the analytical data were found to agree with the formulations proposed for the complexes. As expected for paramagnetic $3d^9$ ions, the magnetic susceptibility values measured at room temperature for the CuSMHD and CuSBHD complexes (1.66 B.M and 1.48 B.M, respectively) suggest a square-planar environment (spin-only value 1.73 B.M) [44,45]. The slightly low values observed can be attributed to interaction between Cu(II) ion centers [46,47] or distortion in the Cu(II) environment [48].

Crystals of SBPY, SMHDH2, CuSMHD and CuSBHD suitable for single crystal X-ray diffraction were obtained; crystallographic data