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Synthesis, characterization, cytotoxicity and antimicrobial activity of copper complexes of *N*-imine pendant derivatives of 2-(methylthio) aniline

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ABSTRACT

NS-containing ligands with the 2-thioaniline motif and imine functional group linked phenyl, 2-pyridyl and 2-thienyl pendants were synthesized by imine condensation reactions with corresponding aldehydes. 2-(methylthio)-*N*-(2-thienylmethylene)-benzenamine is a new compound characterized by NMR, FTIR and UV–vis. Single crystal X-ray crystallographic data was obtained for all three ligands. The corresponding Cu(I) complexes were obtained by addition of CuBr in toluene/acetonitrile solutions affording the *bis*-bidentate *NS*- and *SNS*-complexes. Aerobic oxidation of one compound, the Cu(I) complex of 2-(methylthio)-*N*-(2-pyridylmethylene)-benzenamine, afforded the corresponding Cu(II) complex which was characterized by single crystal X-ray crystallography and presented a pentacoordinated $CuBr_2$ adduct of 2-(methylthio)-*N*-(2-pyridylmethylene)-benzenamine with a distorted square pyramidal coordination environment. Free ligands and the Cu complexes were screened for antimicrobial activity and 2-(methylthio)-*N*-(2-pyridylmethylene)-benzenamine copper(II) dibromide showed activity against *B. cereus* and *S. aureus*. The cytotoxicity of ligands and Cu complexes on mammalian cell cultures was assessed by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay on mouse macrophages cell line (J774.A1) and human breast cancer cell line (MCF7). The free *NS*-ligands showed good biocompatibility with both cell lines. Cu(I) and Cu(II) complexes showed significant effects on cell viability with dose-response dependency.

1. Introduction

The continual evolution of antibiotic-resistant strains of bacteria poses a serious threat to public health. The development of effective antimicrobial drugs with a novel mechanism of action has become an urgent task in light of the few newly approved drugs [1]. Recent occurrences of *S. aureus* strains that are methicillin-resistant (MRSA), and vancomycin and vancomycin-intermediate resistant (VSRA and VISA) are of particular concern as vancomycin is considered one of the classified drugs of last resort [2].

N and S donor atoms containing compounds are known to exhibit antimicrobial properties due to the key role of the heteroatoms in the coordination of metals at the active sites of numerous metallo-biomolecules. The efficacy of *NS*-compounds can be attributed to their hard and soft binding ability to a variety of active centers. Coordination chemistry has an instrumental role in designing new metal-based antibacterial and antifungal compounds as a potential new class of antimicrobials [3–7]. Nevertheless, the toxicity of drugs that contain d-block metals is problematic; therefore, antimicrobial drugs based on essential metals, such as copper, zinc, and vanadium, with lower toxicity for humans have been developed. Patil and co-workers reported antibacterial activities of Co (II), Ni(II), and Cu(II) complexes with a 1,2,4-triazole Schiff base ligand containing an *OONS* motif [7]. Brodie and co-workers reported complexes of the same metals to a thiosemicarbazone ligand exhibiting efficacy against *S. aureus* and *E. coli* although a related thiosemicarbazone ligand with complexed to Cu (II) exhibited cytotoxicity with human cell line for colon cancer [8]. An *ortho*-aminothiophenol-based imine ligand was reported to be active against in its free form against a series of Gram-(+) and

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Gram-(−) bacteria and fungi [9], and its complexes with Co(II), Ni (II), Cu(II) and Zn(II) also showed antimicrobial activity [10].

Copper is the third most bio-essential d-block element, after iron and zinc, due do to its tunable coordination to ligands [11]. Typically in its +2 oxidation state, copper is essential for several biological pathways and vital for the function of many cellular enzymes, such as ceruloplasmin, albumin and transcuprein [12]. In addition, Cu complexes have demonstrated a wide range of pharmacological activity such as antibacterial [13–19], antifungal [20,21], antiviral [5,22], anticancer [6,23,24], antibiofilm [25], and anti-inflammatory [26]. It has been demonstrated that the location of substituents within the supporting ligand framework may enhance biological activity in Cu complexes [8]. One mode of antibacterial action is mediated by the reaction of Cu ions on carbonyl groups in the peptide linkages of cell wall of bacteria. One mode of antibacterial action is mediated by reaction of Cu ions on carbonyl groups in the peptide linkages of cell wall of bacteria leading to degradation of the proteins. Alternatively, copper complexes can also generate reactive oxygen species (ROS), which in turn will result in the destruction of the bacterial cell wall and other bacterial macromolecules [16]. In addition, treatment with Cu complexes has been shown to hinder bacteria replication via Cu complexation to bacterial DNA [17]. Cu complexes of *N,S*-ligands have also been a focus on mimicking electron-transferring blue copper proteins and for monooxygenase enzymes such as PHM (α-peptidylglycine hydroxylating monooxygenase) or DβM (dopamine-β-monooxygenase) [27–29].

Previously, we have reported the synthesis and characterization of *NS*-compounds containing an *ortho*-aminothiophenol motif via a convenient reaction pathway of *S*-alkylation and imine condensation reactions [30–32]. Our interest lies in ligand compounds with hard and soft donor atoms coordination as well as non-innocent ligand behavior. The coordination chemistry of *ortho*-aminothiophenol is well suited for this task allowing the generation of radical ligand ions and the stabilization of various metal oxidation states [33–35]. The relative good biocompatibility of copper in the human body makes *NS*-Cu complexes a good target for laying the foundation of new approaches to antimicrobial agents. Herein, we report the synthesis and characterization of a series of Cu complexes with *N,S*-coordination and *N*-(2-pyridyl), *S*-(2-thienyl), and phenyl pendants, their cytotoxicity as free ligands and as respective Cu complexes, as well as the X-ray crystallography structures of all ligands and one Cu(II) complex.

2. Materials and methods

2.1. Chemicals and reagents

All solvents and chemicals (reagent grade) for synthesis were purchased from commercial sources and were used without further purification. UPLC solvents were Optima® grade obtained from Fisher Scientific. NMR spectra were recorded on a 400 MHz Bruker Avance II spectrometer operating at 400.17 MHz for ^{1}H and 100.6 MHz for ^{13}C. $^{1}H/^{13}C$ NMR chemical shifts are reported in ppm and referenced to tetramethylsilane ($\delta = 0$ ppm) or residual solvent signal ($CDCl_3$ $\delta = 7.26$ ppm/77.0 ppm or DMSO-d_6 $\delta = 2.50$ ppm/39.5 ppm) as internal standard. *J* values are given in Hz. UPLC-HRMS analyses were performed on a Waters Acquity Xevo G2 QToF using a C-18 column (Waters BEH C18 1.7 μm, 2.1 mm × 50 mm) and ESI positive mode. Compounds were dissolved in CH_3CN (ligands and Cu complexes) for UPLC-HRMS analysis. MS fragmentation results are provided for major signals of isotopic pattern envelope and rounded to 0.1 *m/z* for signals greater than 10% of height of base signal. Elemental analysis (CHN) was conducted by MHW Labs, Phoenix, Arizona, US. ICP-MS analysis was performed using an PerkinElmer ICP-MS NexION 300D on Cu (mass 63 amu) and samples in the range of 300 ppb in a 1 wt% HNO_3 solution (50 mL) containing less than 1 wt% DMSO and Sc (mass 45 amu) as internal standard. Calibration was conducted with $CuBr_2$ in the range of 100–500 ppb. ICP-MS results are the averages of duplicate runs and the measurement error is estimated to ±4% based on measurement error, purity of calibration standard and sample preparation. UV–vis spectra were recorded in 1 cm quartz cuvettes on a Varian Cary 100 Bio UV–vis spectrometer with extinction coefficient ε as $L\,cm^{-1}\,mol^{-1}$. FTIR spectra were recorded on a Thermo Nicolet 6700 FTIR Spectrometer as KBr pellet (approximately 1.5 mg compound in 300 mg anhydrous KBr) in the 4000–400 cm^{-1} range with 2 cm^{-1} resolution. X-ray structures of **2a** (CCDC 1485822), **2b** (CCDC 1487673), **2c** (CCDC 1485821) and **4** (CCDC 1485823) were deposited with the Cambridge Crystallographic Data Centre.

Sterile assay discs (6 mm diameter) were obtained from VWR Whatman and antibiotic assay discs (6 mm diameter) were obtained from Becton, Dickinson and Company (USA). Dimethyl sulfoxide (DMSO) for microbial and cytotoxic experiments was BioReagent™ grade and was purchased from Sigma-Aldrich. The nutrient broth and Müller-Hinton agar was purchased from Becton, Dickinson and Company (USA). DMEM and RPMI medium, as well as fetal bovine serum (FBS) essential amino acids, sodium pyruvate, penicillin, streptomycin and L-glutamine were purchased from GIBCO. McFarland turbidity standards containing barium sulfate were obtained from bioMérieux. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), insulin, DMSO cell culture grade were purchased from Sigma.

2.2. Synthesis and characterization of compounds

2.2.1. Ligands

Synthesis of 2-(methylthio)-*N*-(phenylmethylene)-benzenamine (**2a**) and 2-(methylthio)-*N*-(2-pyridylmethylene)-benzenamine (**2b**) ligand was performed as previously published [36].

Synthesis of 2-(methylthio)-*N*-(2-thienylmethylene)-benzenamine (**2c**)

A yellow MeOH solution (10 mL) containing 609.8 mg (5.44 mmol) of 2-thiophenecarboxaldehyde was added to a light green solution of 757.2 mg (5.44 mmol) 2-methylthioaniline in MeOH (15 mL). The mixture was allowed to reflux for 12 h. After cooling to room temperature, the solvent was removed under reduced pressure. The resulting oil was washed successively with cold hexanes (2 × 5 mL) and dried under vacuum. This oil crystallized over 5 days upon standing at which point crystals were collected and washed with cold hexanes (2 × 5 mL). After drying under vacuum, 1.172 g (92%) of yellow crystals were isolated. ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 8.52$ (s, 1H), 7.52 (dd, $J = 5.2$ Hz, 14.4 Hz, 2H), 7.21 (d, $J = 4.4$ Hz, 2H), 7.15 (m, 2H), 6.99 (d, $J = 7.6$ Hz, 1H), 2.47(s, 3H) ppm. $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$) $\delta = 153.0$, 142.9, 136.1, 132.3, 130.9, 129.1, 128.6, 127.8, 126.7, 126.2, 188.1, 37.0. FTIR (KBr) 3432 (br), 3096, 3084, 3050, 2993, 2945, 2920, 2877, 1606 (s), 1571 (s), 1467, 1435, 1426, 1372, 1266, 1223, 1193, 1164, 1132, 1084, 1070, 967, 935, 914, 869, 835, 772, 754, 729, 706, 681, 647, 580, 557, 549, 469, 454 cm^{-1}. Anal. Calcd. for $C_{12}H_{11}NS_2$: C = 61.76%, H = 4.75%, N = 6.00%. Found: C = 61.05%, H = 4.98%, N = 6.44%. UV–vis (DMF, 0.025 mg/mL) λ_{max} (ε) = 270 (14.7×10^3), 375.

2.2.2. Synthesis of copper complexes

Synthesis of 2-(methylthio)-*N*-(2-phenylmethylene)-benzenamine copper(I) bromide **(3a)**

A yellow solution of 259.5 mg (1.14 mmol) of **2a** in toluene (20 mL) was added to a green CH_3CN solution (25 mL) containing 81.9 mg (0.571 mmol) CuBr with stirring at ambient temperature.