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Synthesis, characterization and pharmacological studies of copper complexes of flavone derivatives as potential anti-tuberculosis agents

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ABSTRACT

Novel series of different hydroxyflavone derivatives and their copper complexes were synthesized. They were characterized using analytical and spectral techniques. The superoxide dismutase (SOD) mimetic activity of the synthesized complexes demonstrated that copper complex of $\mathbf{L^{10}}$ has promising SOD-mimetic activity than other ligands & complexes. The *in vitro* antimicrobial activities of the synthesized compounds were tested against the bacterial species and fungal species. The DNA binding properties of copper complexes were studied using cyclic voltametry and electronic absorption techniques. Anti-tuberculosis activity was also performed. The effective complexes was subjected to antimycobacterial activity using MABA method and summarized. The antimycobacterial activity of copper complexes have been evaluated and discussed.

1. Introduction

Microorganisms of Mycobacterium species caused serious infectious diseases to human beings, like tuberculosis (TB) [1]. In recent years, an increase in the incidence of TB disease has been observed, not only in developing countries and also in developed countries [2]. Tuberculosis (TB) remains a worldwide problem [3]. Due to the rapid spread of tuberculosis, the strains continuously developed resistance towards all first-line antituberculosis drugs such as isoniazid, rifamcin and ethionamide and second-line drugs, such as aminosalicylic acid, cycloserine, amikacin, kanamycin and capreomycin, showed side effects in clinical use. The discovery of new antituberculosis agents with potential activity, less toxicity, broader spectrum and safer therapeutic profiles, is an urgent need. Higher antituberculosis activity of metal complexes than the parent drug may be due to complexation with metal imparts some biochemical characteristics to the drug [4]. Metal coordination to biologically active molecules can be used as a strategy to enhance their pharmacological activity and overcome side effects of drugs.

The heterocyclic molecules have received much attention in the field of bioinorganic chemistry and medicinal chemistry due to their versatile coordination behavior and wide range of pharmacological profiles [5–7]. Synthesis of flavones and their derivatives have received much attention due to their significant pharmaceutical applications. In light of this fact, planarity of the C ring in flavonoids may be important for binding interaction with proteins, as the molecules with saturated C2—C3 bonds (flavanones and certain others) permit more twisting of the B-ring with reference to the C ring. A double bond at C2—C3 position increases the π-conjugation of the bond linking the B and C rings, which favors near-planarity of the two rings [8]. Molecules have near-planar structure is easier to enter into the hydrophobic pockets in proteins and base pairs of DNA through intercalation mode. Schiff bases have appeared to be important intermediates in a number of enzymatic reactions involving interaction of an enzyme with an amino or a carbonyl group of the substrate [9].

In the past few decades, a renewed interest in metal based drug therapy has been invoked based on coordination, not only bioactive ligands might improve their bioactivity profiles, but also inactive ligands may acquire pharmacological properties [10–13]. In addition, metal-coordination is one of the most efficient strategies in the design of new drug profile, repository, slow-release or long-acting drugs. Metal complexes of Schiff base ligands have been investigated as models for active sites of enzymes [14,15] including DNA cleavage systems [16, 17], and as antibacterial [18–20] and anticancer [21] drugs. Metal complexes of S-, N-, and O-chelating ligands have attracted the considerable attention because of their interesting physicochemical properties, pronounced biological activities, and as models of metalloenzyme active sites [22,23]. In particular, Cu(II) complexes of heterocyclic compounds exhibited wide range of biological and pharmaceutical activities that includes DNA binding and cleavage, antimicrobial and antioxidant behavior [24–27].

We have recently reported that copper complexes derived from 3-hydroxyflavone derivatives exhibited higher biochemical activities such as antimicrobial, SOD and anti-inflammatory activities [28]. The structural modifications of promising lead compounds are still a major

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line of approach to develop new therapeutic agents. It involves an intensive effort to condense the different pharmacophoric groups of bioactive active moieties into one compound and it may behave as chemotherapeutic agents.

The present investigation focused on the synthesis of different substituted (4-arylimino)-1-alkyl-1,4-dihydroquinoline derivatives from derivatives of hydroxyl flavones[3-hydroxyflavone/5-hydroxyflavone/3-hydroxy-2-(4-hydroxyphenyl) flavone/5-hydroxy-2-(4-hydroxyphenyl) flavone with 1-aminopentane/1-aminopropane] with aromatic primary amines, [2-nitroaniline/2-aminophenol/2-aminobenzenethiol/2-chloroaniline/o-phenelyenediamine] and characterized. They were subjected to biochemical activities of copper complexes with hydroxyflavone derivatives. It is immune interest to performed electrochemical investigations on title compounds to find out how a ligand environment could affect the redox properties of copper central metal atom and modulate SOD activity. To understand the selectivity and efficiency of DNA binding by different structures copper complexes of hydroxyflavone derivatives. The antioxidant efficiency of copper complexes were also studied and compared with their standards. The effective complexes were tested for *in vitro* antituberculosis activity against *Mycobacterium tuberculosis*.

2. Experimental

2.1. Reagents & Instrumentation

The chemicals of Analar grade were used. Copper(II) chloride was obtained from Merck. The amount of copper present in the copper complexes was estimated using ammonium oxalate method. The NMR spectra of the ligands were recorded using TMS as internal standard. Chemical shifts (δ) are expressed in units of parts per million relative to TMS. The FAB mass spectrum of the ligands and their complexes were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using argon/xenon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature using *m*-nitrobenzylalcohol (NBA) as the matrix. Molar conductance of the copper complexes was measured in DMSO solution using a coronation digital conductivity meter. The IR spectra of the ligands and their copper complexes were recorded on a Perkin-Elmer 783 spectrophotometer in 4000–200 cm^{-1} range using KBr disc. Thin-layer chromatography was carried out on aluminum-backed silica gel plates (Merck 60 F254), with visualization of components by UV light (254 nm) or exposure to I_2 to determine the progress of a reaction. Column chromatography was carried out on silica gel (Merck 230–400 mesh) for purification. Electronic spectra were recorded with a Systronics 2201 Double beam UV–Vis, spectrophotometer in the 200–1100 nm region. Thermal analysis was performed with PerkinElmer TGA 4000. The magnetic susceptibility values were calculated using the relation $\mu_{eff} = 2.83\ (\chi_m \cdot T)^{1/2}$ BM. The diamagnetic corrections were made by Pascal's constant and $Hg[Co(SCN)_4]$ was used as a calibrant. SEM images were recorded. The ESR spectra of the copper complexes were recorded at 300 and 77 K on a Varian E112 X-band spectrometer. Cyclic voltammetric measurements were performed using three electrode systems with glassy carbon working electrode, Pt wire auxiliary electrode and an Ag/AgCl reference electrode. Tetrabutylammoniumperchlorate (TBAP) was used as the supporting electrolyte. All solutions were purged with N_2 for 30 min prior to each set of experiments. Solutions of CT DNA in 50 mM NaCl/5 mM Tris–HCl (pH = 7.0) gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280} of *ca*. 1.8–1.9, indicating that the DNA was sufficiently free of protein contamination. The DNA concentration was determined by the UV absorbance at 260 nm after 1:100 dilutions. The molar absorption coefficient was taken as 6600 $M^{-1}\ cm^{-1}$. Stock solutions were kept at 4 °C and used after not >4 days.

2.2. General Procedure

The synthesis of compounds 2a − 2d was prepared by refluxing 1a-1d (different hydroxyflavone(s)) with n-propyl amine/n-pentyl amine in toluene at 120 °C whose intermediates was condensed with different aromatic amines [2-nitroaniline/2-aminophenol/2-aminobenzenethiol/2-chloroaniline/o-phenlyenediamine] in ethanolic medium is depicted in Scheme 1. The complexes were obtained by refluxing the equimolar solutions of copper chloride and ligand(s). The copper complexes (Scheme 2) were obtained as solids in different yields. The purity of synthesized compounds was confirmed by TLC and elemental analysis. C, N and H analysis were carried out micro analytically. Magnetic moments were determined at room temperature. They are insoluble in acetone, ethanol, benzene and chloroform, but their considerable solubility has been noticed in DMF and DMSO. The complexes are stable at room temperature. They are non-hygroscopic and can be stored for a long length of period without decomposition. The structures of the synthesized compounds were confirmed by analytical and spectral data (IR, ^1H & ^{13}C NMR, ESR and FAB mass).

L^{2a}: Yield: 70%. Anal.Calcd for Chemical Formula: $C_{18}H_{17}NO_2$. C, 77.40; H, 6.13; N, 5.01. Found: C, 77.42; H, 6.14; N, 4.99. FAB mass spectrometry (FAB–MS): *m/z* 280 [M + 1]. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 6.67–7.71 (9H, m, Ar—H), 4.12 (2H, t, J = 2.1 Hz, CH_2), 5.59 (1H, s, —OH, D_2O exchangeable), 1.70 (2H, m, CH_2), 1.02 (3H, t, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$, ppm): 162.2 (C-2), 97.2 (C-3), 182.1 (C-4), 162.6 (C-5), 106.6 (C-6), 136.7 (C-7), 100.7 (C-8), 145.3 (C-9), 119.3 (C-10), 134.2 (C-11), 128.3 (C-12), 128.6 (C-13), 127.9 (C-14), 128.6 (C-15), 128.3 (C-16), 48.7 (C-17), 21.4 (C-18), 11.9 (C-19). IR (KBr, cm^{-1}): 3500–3700, bs (OH); 1669, (C═O)st; 1132, (—CN—C)st.

L^{2b}: Yield: 56%. Anal.Calcd for Chemical Formula: $C_{18}H_{17}NO_3$. C, 73.20; H, 5.80; N, 4.74. Found: C, 73.21; H, 5.79; N, 4.73. FAB mass spectrometry (FAB-MS): *m/z* 296 [M + 1]. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 6.24–7.49 (8H, m, Ar—H), 6.17 (1H, s, —OH, D_2O exchangeable), 5.32 (1H, s, —OH, D_2O exchangeable), 4.09 (2H, t, CH_2), 1.72 (2H, m, CH_2), 1.01 (3H, t, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$, ppm): 162.2 (C-2), 97.2 (C-3), 182.1 (C-4), 162.6 (C-5), 106.6 (C-6), 136.7 (C-7), 100.7 (C-8), 145.3 (C-9), 119.3 (C-10), 126.8 (C-11), 130.1 (C-12), 115.8 (C-13), 157.7 (C-14), 115.8 (C-15), 130.1 s (C-16), 48.7 (C-17), 21.4 (C-18), 11.9 (C-19). IR (KBr, cm^{-1}): 3500–3700, bs (OH); 1732, (C═O)st; 1152, (—C—N—C)st.

L^{2c}: Yield: 65%. Anal.Calcd for Chemical Formula: $C_{18}H_{17}NO_3$. C, 73.20; H, 5.80; N, 4.74. Found: C, 73.21; H, 5.79; N, 4.73. FAB mass spectrometry (FAB-MS): *m/z* 296 [M + 1]. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 12.12 (1H, s, —OH, D_2O exchangeable), 6.68–7.81 (8H, m, Ar—H), 6.17 (1H, s, —OH, D_2O exchangeable), 4.09 (2H, t, CH_2), 1.72 (2H, m, CH_2), 1.01 (3H, t, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$, ppm): 123.9 (C-2), 146.0 (C-3), 178.2 (C-4), 126.3 (C-5), 122.7 (C-6), 135.3 (C-7), 108.2 (C-8), 143.9 (C-9), 127.1 (C-10), 126.8 (C-11), 130.1 (C-12), 115.8 (C-13), 157.7 (C-14), 115.8 (C-15), 130.1 s (C-16), 48.7 (C-17), 21.4 (C-18), 11.9 (C-19). IR (KBr, cm^{-1}): 3500–3700, bs (OH); 1625, (C═O)st; 1143, (—C—N—C)st.

L^{2d}: Yield: 76%. Anal.Calcd for Chemical Formula: $C_{18}H_{17}NO_2$. C, 77.40; H, 6.13; N, 5.01. Found: C, 77.42; H, 6.14; N, 4.99. FAB mass spectrometry (FAB-MS): *m/z* 280 [M + 1]. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 12.73 (1H, s, —OH, D_2O exchangeable), 6.67–7.71 (9H, m, Ar—H), 4.12 (2H, t, CH_2), 1.70 (2H, m, CH_2), 1.02 (3H, t, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$, ppm):123.8 (C-2), 146.2 (C-3), 178.2 (C-4), 126.3 (C-5), 122.7 (C-6), 135.3 (C-7), 108.2 (C-8), 143.9 (C-9), 127.1 (C-10), 134.2 (C-11), 128.3 (C-12), 128.6 (C-13), 127.9 (C-14), 128.6 (C-15), 128.3 (C-16), 48.7 (C-17), 21.4 (C-18), 11.9 (C-19). IR (KBr, cm^{-1}): 3500–3700, bs (OH); 1748, (C═O)st; 1140, (—C—N—C)st.

L^{2e}: Yield: 64%. Anal.Calcd for Chemical Formula: $C_{20}H_{21}NO_2$. C, 78.15; H, 6.89; N, 4.56; O, 10.41. Found: C, 78.16; H, 6.86; N, 4.57; O, 10.42. FAB mass spectrometry (FAB-MS): *m/z* 308 [M + 1]. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 6.67–7.71 (9H, m, Ar—H), 5.59 (1H, s, —OH, D_2O exchangeable), 4.12 (2H, t, CH_2), 1.46 (2H, m, CH_2), 1.31