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Synthesis, characterization, *in vitro* antimicrobial and DNA cleavage studies of Co(II), Ni(II) and Cu(II) complexes with ONOO donor coumarin Schiff bases

Sangamesh A. Patil [a,*], Shrishila N. Unki [a], Ajaykumar D. Kulkarni [a], Vinod H. Naik [a], Prema S. Badami [b]

[a] P.G. Department of Studies in Chemistry, Karnataka University, Dharwad, 580 003 Karnataka, India
[b] Department of Chemistry, Shri Sharanabasaveshwar College of Science, Gulbarga 585 102, India

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ABSTRACT

A series of Co(II), Ni(II) and Cu(II) complexes have been synthesized with Schiff bases derived from 2-hydroxy-1-naphthaldehyde and 2-oxo-2H-chromene-3-carbohydrazide/6-bromo-2-oxo-2H-chromene-3-carbohydrazide. The chelation of the complexes has been proposed in the light of analytical, spectral (IR, UV–Vis, ^{1}H NMR, ESR, FAB-mass and fluorescence), magnetic and thermal studies. The measured molar conductance values indicate that, the complexes are non-electrolytic in nature. The redox behavior of the complexes was investigated with electrochemical method by using cyclic voltammetry. The Schiff bases and their metal complexes have been screened for their *in vitro* antibacterial (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Salmonella typhi*) and antifungal activities (*Candida albicans*, *Cladosporium* and *Aspergillus niger*) by MIC method. The DNA cleavage is studied by agarose gel electrophoresis method.

1. Introduction

Coumarin is structurally the least complex member of a large class of compounds known as benzopyrones [1]. Coumarin derivatives possess wide range of biological activities viz. antithrombotic [2], antimicrobial [3], antiallergic [4], antiinflammatory [5], antitumour [6] and anticoagulants [7], etc. Recently, coumarin derivatives have been evaluated in the treatment of human immunodeficiency virus, due to their ability to inhibit human immunodeficiency virus integrase [8,9]. *In vitro* and *in vivo* studies have suggested the possible use of coumarins in the treatment of cancer [10]. The coumarin nucleus is also present in the novobiocin, clorobiocin and coumermycin A_1 antibiotics. These antibiotics are active against methicillin-resistant *Staphylococcus aureus* [11]. The interaction of transition metal complexes with DNA have been extensively studied for their usage as probes for DNA structure and their potential application in chemotherapy and are potent catalytic inhibitors of DNA gyrase [12–14]. One of the important DNA related activity of the transition metal complexes is that some of the complexes show the ability to cleave DNA. Very recently Cu(II) complexes have been reported to be active in DNA strand scission [15–17].

Such a wide spectrum of biological application of coumarins prompted us to synthesize some of the transition metal complexes with coumarin Schiff bases.

Hence, in continuation with our earlier work on coumarin Schiff bases [18–22], here we report the synthesis, antimicrobial activity and DNA cleavage property of Co(II), Ni(II) and Cu(II) metal complexes with newly synthesized Schiff bases derived from 2-hydroxy-1-naphthaldehyde and 2-oxo-2H-chromene-3-carbohydrazide/6-bromo-2-oxo-2H-chromene-3-carbohydrazide (Scheme 1). The Schiff bases and their metal complexes have been characterized by various spectral and analytical techniques.

2. Experimental

2.1. Analysis and physical measurements

Carbon, hydrogen and nitrogen were estimated by using Elemental Analyzer Truspec (Leco Corporation USA). The IR spectra of the Schiff bases and their Co(II), Ni(II) and Cu(II) complexes were recorded on a HITACHI-270 IR spectrophotometer in the 4000–250 cm^{-1} region in KBr disc. The electronic spectra of the complexes were recorded in HPLC grade DMF and DMSO solvent on a VARIAN CARY 50-BIO UV-spectrophotometer in the region of 200–1100 nm. The ^{1}H NMR spectra of ligands were recorded in DMSO-d_6 on a BRUKER 300 MHz spectrometer at room temperature using TMS as an internal reference. The electrochemistry of the metal complexes was recorded on CHI1110A-electrochemical analyzer (Made in USA) in dimethyl formamide (DMF) containing 0.05 M n-Bu_4NClO_4 as the supporting electrolyte. The ESR spectrum was recorded on Varian-E-4X-band EPR spectrometer and the field set is 3000 G at modulation frequency of 100 K Hz under

* Corresponding author.
E-mail address: patil1956@rediffmail.com (S.A. Patil).

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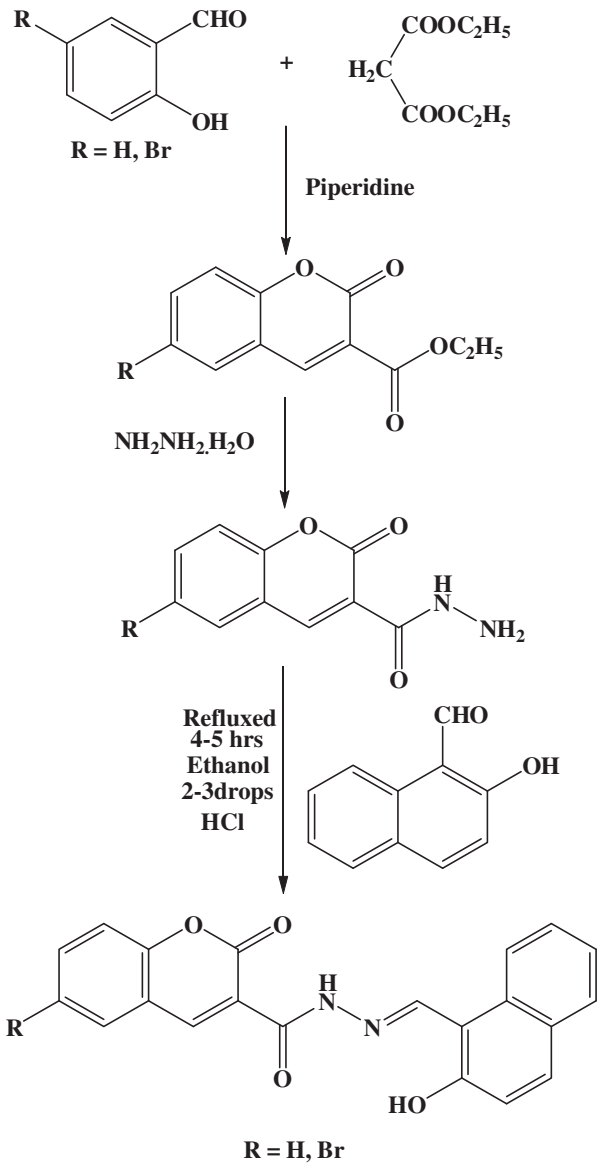

Scheme 1. Synthesis of Schiff bases.

liquid nitrogen temperature using TCNE as '*g*' marker. Thermogravimetric analyses data were measured from room temperature to 1000 °C at a heating rate of 10 °C/min. The data were obtained by using TA Instruments Water LLC, New Castle, Delware, USA. Model; DCS Q 20, 2009. FAB-mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6 kV, 10A) as the FAB gas. Molar conductivity measurements were recorded on ELICO-CM-82 T Conductivity Bridge with a cell having cell constant 0.51 and magnetic moment of the complexes was carried out by using Faraday balance.

2.2. Synthesis

All chemicals and solvents used were of AR grade. All metal salts were used as their chlorides. 2-Oxo-2H-chromene-3-carbohydrazide was synthesized by reported method [23,24].

2.2.1. Synthesis of ethyl 6-bromo-2-oxo-2H-chromene-3-carboxylate

5-Bromo-2-hydroxybenzaldehyde (0.01 mol) and diethylmalonate (0.01 mol) were dissolved in ethanol to give a clear solution. Catalytic amount of piperidine was added and mixture was refluxed for 5 h. The content was concentrated to a small volume. The product was poured onto crushed ice, filtered out and crystallized from ethanol to give yellowish shiny crystals. Yield: 68%; M.p: 134 °C.

2.2.2. Synthesis of 6-bromo-2-oxo-2H-chromene-3-carbohydrazide

Ethyl 6-bromo-2-oxo-2H-chromene-3-carboxylate (0.01 mol) and hydrazine hydrate 99% (0.01 mol) were dissolved in ethanol (50 ml) to give a clear solution and refluxed for 10 h. The content was concentrated to half of the volume and allowed to cool. The solid mass of product, which separated out on cooling was retained by filtering and washed with small amount of ice-cooled ethanol. Yield: 62%; M.p: 230 °C.

2.2.3. Synthesis of Schiff bases [I–II]

A mixture of 2-hydroxy-1-naphthaldehyde (0.01 mol) and 2-oxo-2H-chromene-3-carbohydrazide/6-bromo-2-oxo-2H-chromene-3-carbohydrazide (0.01 mol) in 30 ml alcoholic medium containing few drops of concentrated HCl was refluxed for 3–4 h. The product separated on evaporation of the solvent was filtered, washed with alcohol and then finely recrystallized from EtOH. Yield (m.p.): 77% (216 °C) and 78% (214 °C) of Schiff bases I and II respectively.

Schiff base I: FAB MS: *m/z* 358 M^+. ^{1}H NMR (d_6-DMSO) (ppm): 12.97 (s, 1H, OH), 11.41 (s, 1H, NH), 9.66 (s, 1H, HC=N), 7.0–8.64 (m, 11H, Ar-H). ^{13}C NMR (DMSO) (ppm): 117.4–153.7 (Aromatic Carbon), 145.2 (C=N), 160.0 (C=O), 169.7 (amide C=O).

Schiff base II: FAB MS: *m/z* 437 M^+. ^{1}H NMR (d_6-DMSO) (ppm): 13.05 (s, 1H, OH), 11.50 (s, 1H, NH), 9.56 (s, 1H, HC=N), 7.2–8.54 (m, 10H, Ar-H). ^{13}C NMR (DMSO) (ppm): 117.6–156.7 (Aromatic Carbon), 144.2 (C=N), 162.2 (C=O), 168.9 (amide C=O).

2.2.4. Synthesis of Co(II), Ni(II) and Cu(II) complexes [1–6]

A general method has been followed for the preparation of complexes using reaction of metal salts and the corresponding Schiff bases in molar ratio (M:L = 1:1). An alcoholic solution (30 ml) of Schiff bases (1 mmol) was refluxed with an alcoholic solution (30 ml) of 1 mmol of $CoCl_2{\cdot}6H_2O$/$NiCl_2{\cdot}6H_2O$/$CuCl_2{\cdot}2H_2O$ on steam bath for 1 h. Then, to the reaction mixture 2 mmol of sodium acetate was added, refluxed for another 3 h. The separated complexes were filtered off, washed several times with 50% (v/v) ethanol–water mixture to remove any traces of unreacted starting materials, then washed with diethyl ether and dried in a vacuum desiccator over $CaCl_2$.

3. Pharmacology

3.1. DNA cleavage experiment

Preparation of culture media and DNA isolation of *A. niger* microbial strains were done according to the literature procedure [22,25]. Potato dextrose broth [potato, 250; dextrose, 20; in (g/l)] was used for the culture of *A. niger*.

3.2. Agarose gel electrophoresis

Cleavage products were analyzed by agarose gel electrophoresis method [25]. Test samples (1 mg/ml) were prepared in DMF. The samples (25 μg) were added to the isolated DNA of *A niger*. The samples were incubated for 2 h at 37 °C and then 20 μl of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 l) and finally loaded on agarose gel and passed the constant 50 V of electricity for around 30 min. Removed the