Inorganica Chimica Acta 463 (2017) 102–111

Contents lists available at ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Research paper

Ferrocene-based Schiff bases copper (II) complexes: Synthesis, characterization, biological and electrochemical analysis

Muhammad Shabbir [a], Zareen Akhter [a,*], Iqbal Ahmad [b], Safeer Ahmed [a], Michael Bolte [c], Hammad Ismail [d], Bushra Mirza [e]

[a] Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan
[b] Department of Chemistry, Allama Iqbal Open University, Islamabad 44000, Pakistan
[c] Institut für Anorganische Chemie, J.W.Goethe-Universität Frankfurt, Max-von-Laue-Strasse 7, Frankfurt/Main 60438, Germany
[d] Department of Biochemistry & Molecular Biology, University of Gujrat, 50700 Gujrat, Pakistan
[e] Department of Biochemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan

ARTICLE INFO

Article history:
Received 21 October 2016
Received in revised form 8 March 2017
Accepted 18 April 2017
Available online 19 April 2017

Keywords:
Ferrocene
Schiff bases
Electrochemistry
Biological studies
Drug-DNA interaction

ABSTRACT

Three ferrocene based Schiff bases copper (II) complexes i.e. bis(N-(2-hydroxybenzylidene)-4-ferrocenylaniline)copper(II)($Cu(L_1)_2$), bis(N-(2,3-dihydroxybenzylidene)-4-ferrocenylaniline)copper(II)($Cu(L_2)_2$), and bis(N-(5-chloro-2-hydroxybenzylidene)-4-ferrocenylaniline)copper(II)($Cu(L_3)_2$), were prepared from Schiff bases N-(2-hydroxybenzylidene)-4-ferrocenylaniline(HL_1), N-(2,3-dihydroxybenzylidene)-4-ferrocenylaniline (HL_2) and N-(5-chloro-2-hydroxybenzylidene)-4-ferrocenylaniline (HL_3). Schiff bases and their corresponding copper complexes were characterized by various spectroscopic, analytical and electro-analytical techniques. Single crystal X-ray diffraction analysis of Schiff base (HL_2) revealed three molecules in the asymmetric unit having same molecular conformation. The molecules are connected by intermolecular O—H. . .O as well as intramolecular N—H. . .O hydrogen bonds. All the synthesized compounds were evaluated for their anti-bacterial, cytotoxicity, antitumor, DPPH, DNA protection and DNA-drug interaction studies. The copper complexes exhibited antibacterial activity against all tested strains. All the compounds showed significant activity in brine shrimps cytotoxicity and antitumor assays with IC_{50} values ranging from 2.32–69.61 μg/ml. The copper complexes were found to be more active than Schiff bases with lower IC_{50} values. The DNA-drug interaction study through voltammetry revealed their binding nature which complemented antitumor behaviour evaluated from biological studies. Moreover, Schiff bases showed prominent antioxidant activity in DPPH assay along with DNA protection activity against hydroxyl free radicals.

1. Introduction

Organometallics and their metal ion complexes are considered as privileged class of compounds due to their biochemical synthesis, electrochemical analysis, antifungal, antimicrobial as well as catalytic activities [1–4]. Different types of organometallics forming metal complexes have been extensively studied, exhibiting wide range of applications, especially in biological systems [5,6]. The study of mono, di, tridentate metal ion complexes reveal their interesting spectral properties which are helpful in understanding the various aspects of coordination chemistry of metals [7].The presence of nitrogen and oxygen donor atoms in such compounds make them structurally similar to the neutral biological systems and are utilized in elucidating the mechanism of transformation of racemization reactions. Various biological activities (antitumour, antibacterial, antifungal and herbicidal) are considered due to the presence of azomethine linkage (>C=N—) present in living systems [8].

Ferrocene based organometallics and metal complexes possess unique properties like stability, aromaticity, low toxicity, lipophilicity, redox activity, different membrane permeation properties and anomalous metabolism [9–11]. Ferrocene containing metal-chelate complexes can be regarded as multinuclear molecules possessing both the features of organometallics and of coordination chemistry [12]. Mutual interaction between the ligated metal in different coordination environments and variable oxidation states with the ferrocenyl systems may lead to interesting electron transfer processes. Cyclopentadienyl metal moiety in ferrocene may cause electronic effects on the coordination behaviour

* Corresponding author.
E-mail addresses: zareenakhter@yahoo.com, zareen_a@qau.edu.pk (Z. Akhter).

http://dx.doi.org/10.1016/j.ica.2017.04.034
0020-1693/

of the donor centers of these ligands that may influence the *in vitro* antibacterial, antifungal and cytotoxic properties of organometallic based compounds. Ferrocenium salts exhibit antitumor activity against number of tumors which is related to the oxidation state of the central iron atom of the ferrocene moiety .Ferrocenyl Schiff bases derived from aryl amines and their metal complexes exhibit potent antitumor, antioxidant and DNA protecting activity due their interesting redox properties thus making them attractive pharmacophore for drug design [13].

Copper is the third most abundant element in human body after iron and zinc. It plays a vital role in organisms ranging from bacteria to mammals. Copper complexes are important bioactive compounds *in vitro* and *in vivo* with an ever-increasing interest as potential drugs against various diseases. Copper synthetic compounds are able to promote nucleic acid (especially DNA) cleavage and are therefore worth investigating both *in vitro* and *in vivo* against cancer cells [14].

Keeping in view the worth of ferrocene based copper complexes, a synthetic strategy was developed to attach ferrocenyl moiety by forming Schiff-bases of substituted aromatic aldehydes and 4-ferrocenyl aniline. These Schiff-bases were further used to produce copper complexes. The synthesized and characterized compounds i.e. bis(N-(2-hydroxybenzylidene)-4-ferrocenylaniline)copper(II) ($Cu(L_1)_2$), bis(N-(2,3-dihydroxybenzylidene)-4-ferrocenylaniline)copper(II)($Cu(L_2)_2$), bis(N-(5-chloro-2-hydroxybenzylidene)-4-ferrocenylaniline)copper(II)($Cu(L_3)_2$) prepared from Schiff bases N-(2-hydroxybenzylidene)-4-ferrocenylaniline (HL_1), N-(2,3-dihydroxybenzylidene)-4-ferrocenylaniline (HL_2), N-(5-chloro-2-hydroxybenzylidene)-4-ferrocenylaniline (HL_3) respectively were evaluated for comparative electro-biological and DNA binding study. As literature studies have shown that ferrocene based Schiff bases and their copper complexes are immensely bioactive compounds therefore detailed biological (brine shrimp cytotoxicity, potato disc antitumor & hydroxyl free radical (·OH) scavenging assays) and electrochemical studies were carried out to determine their mode of interaction with DNA. The results were found quite convincing towards the potential use of these compounds as antioxidant and anticancer agents.

2. Experimental

2.1. Materials and methods

All reactions were performed under nitrogen atmosphere using standard Schlenk line techniques. Solvents were purified and distilled prior to use by standard procedure [15]. Ferrocene was procured from sigma-aldrich and used without further purification. The ferrocenyl Schiff bases (HL_1 –HL_3) were prepared by condensation reactions of corresponding aromatic aldehydes and ferrocenylaniline in ethanol following the reported method [16].

The copper (II) complexes ($Cu(L_1)_2$-$Cu(L_3)_2$) were prepared by the reaction of copper (II) acetate and corresponding ferrocenyl Schiff bases(HL_1–HL_3) by using the literature method[17].The progress and purity of the products were checked by thin layer chromatography on pre-coated Kieselgel 60HF TLC plates. The elemental analysis was performed on a CHNS 932 (Leco-USA) instrument. Melting temperatures were determined, using open capillary tubes on MPD Mitamura Riken Kogyo (Japan) apparatus. The solid-state Fourier transform infrared spectra were recorded on a Thermoscientific (USA) Nicolet 6700 spectrometer in the frequency range of 4000–400 cm^{-1}.^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker NMR spectrophotometer in $CDCl_3$ using tetramethyl silane as internal reference.

The molecular structure of Schiff base (HL_2) was determined on a STOE IPDS II diffractometer using MoK_α radiation (λ = 0.71073 Å) at 173(2) K. The structure was solved by direct methods and refined on F^2 using all the reflections [18].Parameters for data collection and refinement are summarized in Table 1.

UV–visible spectra were recorded in UV–visible Spectrometer Lambda 35(PerkinElmer) in the range 260–700 nm. Solutions were prepared in UV-grade ethanol and data was collected at room temperature.

The synthesized Schiff bases and their copper complexes were assessed for various biological studies. For antibacterial assay the disc diffusion method [19] was performed to check the antibacterial activity of compounds with six bacterial strains including three gram positive, *Micrococcus Luteus* (ATCC 10240), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538) and three gram negative, *Bordetella bronchiseptica* (ATCC 4617), *Escherichia coli* (ATCC 15224) and *Salmonella typhimurium* (ATCC 14028). Nutrient agar (6.4%) was prepared, autoclaved, cooled (45 °C) and was inoculated with bacterial culture (1%) in LFH. Then media was poured in petri plate and allowed to solidify. Sterile paper discs (5 mm) containing 5 μl of each test compound were placed on the petri plate with appropriate distance. These plates were incubated at 37 °C and results were noted in the form of zone of inhibition (mm) after 24 h with the help of vernier caliper.

Cytotoxicity of newly synthesized compounds was determined by previously reported method [20]. Briefly, brine shrimp (*Artemia salina*) eggs were hatched in shallow rectangular dish (22 × 32 cm) filled with commercial seawater (34 g/l). For experiment 15 shrimps were transferred to glass vial using a Pasteur pipette and 25 μl of each compound was added respectively. Then sea water was poured in each vial to raise the final volume up to

Table 1
Crystal data and structure refinement for HL_2.

Empirical formula	$C_{23}H_{19}FeNO_2$	
Formula weight	397.24	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 11.1180(10) Å	α = 85.081 (7)°
	b = 12.5318(11) Å	β = 84.936 (7)°
	c = 19.1897(17) Å	γ = 85.648 (7)°.
Volume	2647.1(4) Å^3	
Z	6	
Density (calculated)	1.495 Mg/m^3	
Absorption coefficient	0.873 mm^{-1}	
F(000)	1236	
Crystal size	0.22 × 0.21 × 0.08 mm^3	
Theta range for data collection	1.84 to 25.35°	
Index ranges	$-13\leq h\leq 13$, $-14\leq k\leq 15$, $-23\leq l\leq 23$	
Reflections collected	28621	
Independent reflections	9675 [R(int) = 0.0771]	
Completeness to theta = 28.32°	99.8%	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9935 and 0.8312	
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/parameters	9675/1/748	
Goodness-of-fit on F^2	0.984	
Final R indices [I > 2sigma(I)]	R_1 = 0.0544, wR_2 = 0.1320	
R indices (all data)	R_1 = 0.0817, wR_2 = 0.1447	
Largest diff. peak and hole	0.236 and −0.657 e.Å^{-3}	

K: Kelvin temperature; Å: Angstrom; Å^3: volume; Z: number of chemical formula units per unit cell; D: density; F: structure factor; R: reliability factor