Journal of Molecular Structure 992 (2011) 39–47

Contents lists available at ScienceDirect

Journal of Molecular Structure

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

Synthesis and characterization of mixed-ligand diimine-piperonal thiosemicarbazone complexes of ruthenium(II): Biophysical investigations and biological evaluation as anticancer and antibacterial agents

Floyd A. Beckford [a,*], Jeffrey Thessing [a], Michael Shaloski Jr. [a], P. Canisius Mbarushimana [a], Alyssa Brock [a], Jacob Didion [a], Jason Woods [a], Antonio Gonzalez-Sarrías [b], Navindra P. Seeram [b]

[a] Science Division, Lyon College, Batesville, AR 72501, USA
[b] Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, USA

ARTICLE INFO

Article history:
Received 17 January 2011
Received in revised form 15 February 2011
Accepted 16 February 2011
Available online 19 February 2011

Keywords:
Thiosemicarbazone
Diimine
DNA
Anticancer
Human serum albumin
Topoisomerase II

ABSTRACT

We have used a novel microwave-assisted method developed in our laboratories to synthesize a series of ruthenium-thiosemicarbazone complexes. The new thiosemicarbazone ligands are derived from benzo[*d*][1,3]dioxole-5-carbaldehyde (piperonal) and the complexes are formulated as $[(diimine)_2Ru(TSC)](PF_6)_2$ (where the TSC is the bidentate thiosemicarbazone ligand). The diimine in the complexes is either 2,2′-bipyridine or 1,10-phenanthroline. The complexes have been characterized by spectroscopic means (NMR, IR and UV–Vis) as well as by elemental analysis. We have studied the biophysical characteristics of the complexes by investigating their anti-oxidant ability as well as their ability to disrupt the function of the human topoisomerase II enzyme. The complexes are moderately strong binders of DNA with binding constants of 10^4 M^{-1}. They are also strong binders of human serum albumin having binding constants on the order of 10^4 M^{-1}. The complexes show good *in vitro* anticancer activity against human colon cancer cells, Caco-2 and HCT-116 and indeed show some cytotoxic selectivity for cancer cells. The IC_{50} values range from 7 to 159 μM (after 72 h drug incubation). They also have antibacterial activity against Gram-positive strains of pathogenic bacteria with IC_{50} values as low as 10 μM; little activity was seen against Gram-negative strains. It has been established that all the compounds are catalytic inhibitors of human topoisomerase II.

1. Introduction

Schiff bases are an important set of chelating ligands in main group and transition metal coordination chemistry and have become an important class of compounds in medicinal and pharmaceutical fields as well [1–3]. Metal complexes of Schiff bases have been designed and synthesized to explore their pharmacological activity, for instance finding applications as model analogs of certain metallo-enzymes. One particular set of Schiff bases that has been studied very aggressively over the past decade is the thiosemicarbazones. Thiosemicarbazones are of considerable pharmacological interest since a number of derivatives have shown a broad spectrum of chemotherapeutic properties. The wide range of biological activities possessed by substituted thiosemicarbazones includes cytotoxic, antitumor [4], antibacterial [5], and antiviral [6] properties. The biological properties of the ligands can be modified and enhanced by linkage to metal ions [7–9].

Ruthenium complexes of diimine ligands such as 2,2′-bipyridine (bpy) and 1,10-phenanthroline (phen) are widely used in bioinorganic chemistry particularly as probes for DNA. Some of these compounds also possess interesting anticancer properties and may be candidates for drugs [10–15]. While it is believed that DNA is a primary target for such complexes, since DNA replication is integral to the progression of these diseases, there is also the recognition that the observed biological activity is not always related to their DNA-binding ability. Consequently studies that seek to investigate other possible targets such as enzymes and other proteins are being undertaken [16–18]. The binding of drugs to plasma proteins is a fundamental factor, important in determining the overall pharmacological activity of the drug. Among the human serum proteins, albumin (HSA) acts as a reservoir for a long duration of action, and binding ultimately affects drug absorption, metabolism, distribution and excretion, properties that are of key importance to drug development. Since HSA serves as a transport carrier for drugs, it is important to study the interactions of potential drugs with this protein.

In this paper we report on a study of a family of mixed-ligand diimine ruthenium complexes of the type $[(bipy)_2Ru(TSC)](PF_6)_2$ and $[(phen)_2Ru(TSC)](PF_6)_2$ where TSC is a chelating

* Corresponding author. Tel.: +1 870 307 7212; fax: +1 870 307 7496.
E-mail address: floyd.beckford@lyon.edu (F.A. Beckford).

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doi:10.1016/j.molstruc.2011.02.029

thiosemicarbazone ligand derived from piperonal. We report on their biophysical reactivity (-interaction with DNA and human serum albumin), their cytotoxicity towards a number of human cancer cell lines and their antibacterial behavior towards a number of pathogenic and facultative bacteria. We also report their capability as anti-oxidants by investigating their reactivity with 2,2-diphenyl-1-picrylhydrazyl (dpph) radicals.

2. Experimental

2.1. Materials and methods

Analytical or reagent grade chemicals were used throughout. All the chemicals including solvents were obtained from Sigma–Aldrich (St. Louis, MO, USA) or other commercial vendors and used as received. The metal complexes were synthesized using a Discover S-Class microwave reactor (CEM, Matthews, USA). Microanalyses (C, H, N) were performed by Desert Analytics, Tucson, USA. Proton and carbon nuclear magnetic resonance (NMR) spectra were recorded in dimethylsulfoxide-d_6 on a Varian Mercury 300 MHz spectrometer operating at room temperature. The residual ^{1}H and ^{13}C present in DMSO-d_6 (2.50 and 39.51 ppm respectively) were used as internal references. Infrared (IR) spectra in the range 4000–500 cm^{-1} were obtained using the ATR accessory on a Nicolet 6700 FTIR spectrophotometer. The electronic spectra were recorded using quartz cuvettes on an Agilent 8453 spectrophotometer in the range 190–1100 nm using samples dissolved in DMSO. Fluorescence spectra were recorded on a Varian Cary Eclipse spectrophotometer.

2.1.1. Synthesis of compounds

2.1.1.1. Ligands. The ligands, 2-(benzo[*d*][1,3]dioxol-5-ylmethylene)hydrazinecarbothioamide, (HpTSC), 2-(benzo[*d*][1,3]dioxol-5-ylmethylene)-N-methylhydrazinecarbothioamide, (MepTSC), 2-(benzo[*d*][1,3]dioxol-5-ylmethylene)-N-ethylhydrazinecarbothioamide, (EtpTSC) and 2-(benzo[*d*][1,3]dioxol-5-ylmethylene)-N-phenylhydrazinecarbothioamide (PhpTSC), were synthesized as follows: Equimolar amounts piperonal (benzo[*d*][1,3]dioxole-5-carbaldehyde) and the appropriate N(4) alkyl-substituted thiosemicarbazide were suspended in 80 mL of absolute anhydrous ethanol containing a few drops of glacial acetic acid. The reaction mixture was heated at reflux for 3–4 h and a pale yellow suspension resulted. The reaction mixture was cooled and filtered through a glass-sintered crucible. The pale-yellow solid which was obtained was thoroughly washed with ethanol followed by ether and dried by suction.

pHTSC. Yield 80% of an off-white solid. Analysis – Calc. for $C_9H_9N_3O_2S$: C, 48.42; H, 4.06; N, 18.82. Found: C, 48.40; H, 4.02; N, 18.67. m.p.189 °C. IR (cm^{-1}): ν (NH_2, NH) 3427, 3252, 3153; ν (C=N) 1592; ν (C=S) 1260, 838. ^{1}H NMR (300.08 MHz, DMSO-d_6): δ = 6.04 (2H, s, H1), 7.63 (1H, s, H3), 7.04 (1H, d J = 8.4 Hz, H5), 6.89 (1H, d J = 7.8 Hz, H6), 7.92 (1H, s, H8), 8.01 (1H, s, N_bH), 8.04 (1H, s, N_bH), 11.3 (1H, s, N_aH). ^{13}C NMR (75.463 MHz, DMSO-d_6); δ = 102.01 (C1), 105.97 (C6), 108.81 (C3), 124.55 (C5), 129.42 (C4), 142.68 (C8), 148.70 (C2), 149.54 (C7), 178.30 (C9).

pMeTSC. Yield 96% of a pale-yellow solid. Analysis – Calc. for $C_{10}H_{11}N_3O_2S$: C, 50.62; H, 4.67; N, 17.71. Found: C, 50.62; H, 4.62; N, 17.50. m.p. 209 °C. IR (cm^{-1}): ν (NH_2, NH) 3344, 3152; ν (C=N) 1590; ν (C=S) 1283, 828. ^{1}H NMR (300.08 MHz, DMSO-d_6): δ = 6.07 (2H, s, H1), 7.66 (1H, s, H3), 7.07 (1H, d J = 8.1 Hz, H5), 6.91 (1H, d J = 7.8 Hz, H6), 8.51 (1H, s, H8), 7.94 (1H, s, N_bH), 11.38 (1H, s, N_aH), 3.00 (3H, t, N_b—CH_3). ^{13}C NMR (75.463 MHz, DMSO-d_6); δ = 30.78 (N_b—CH_3), 101.47 (C1), 105.15 (C6), 108.19 (C3), 123.76 (C5), 128.88 (C4), 141.38 (C8), 148.82 (C2), 148.06 (C7), 177.51 (C9).

pEtTSC. Yield 84% of a pale-yellow solid. Analysis – Calc. for $C_{11}H_{13}N_3O_2S$: C, 52.57; H, 5.21; N, 16.72. Found: C, 52.66; H, 5.06; N, 16.58. m.p. 169 °C. IR (cm^{-1}): ν (NH_2, NH) 3375, 3143; ν (C=N) 1589; ν (C=S) 1255, 832. ^{1}H NMR (300.08 MHz, DMSO-d_6): δ = 6.07 (2H, s, H1), 7.66 (1H, s, H3), 7.07 (1H, d J = 8.1 Hz, H5), 6.92 (1H, d J = 7.8 Hz, H6), 8.56 (1H, s, H8), 7.94 (1H, s, N_bH), 11.32 (1H, s, N_aH), 3.58 (2H, q, N_b—CH_2), 1.14 (3H, t, N_b—CH_2—**CH_3**). ^{13}C NMR (75.463 MHz, DMSO-d_6); δ = 14.72 (N_b—CH_2—**CH_3**), 38.22 (N_b—**CH_2**—CH_3), 101.47 (C1), 105.23 (C6), 108.19 (C3), 123.78 (C5), 128.84 (C4), 141.50 (C8), 148.83 (C2), 148.05 (C7), 176.46 (C9).

pPhTSC. Yield 94% of a light-yellow solid. Analysis – Calc. for $C_{15}H_{13}N_3O_2S$: C, 60.18; H, 4.38; N, 14.04. Found: C, 60.21; H, 4.50; N, 13.95. m.p. 189 °C. IR (cm^{-1}): ν (NH_2, NH) 3353, 3134; ν (C=N) 1597; ν (C=S) 1253, 869. ^{1}H NMR (300.08 MHz, DMSO-d_6): δ = 6.08 (2H, s, H1), 7.85 (1H, s, H3), 7.55 (1H, d J = 14 Hz, H5), 7.38 (1H, d J = 15 Hz, H6), 8.07 (1H, s, H8), 10.11 (1H, s, N_bH), 11.74 (1H, s, N_aH), 6.93–7.25 (5H, m, N_b—C_6H_5). ^{13}C NMR (75.463 MHz, DMSO-d_6); δ = 101.50 (C1), 105.67 (C6), 108.18 (C3), 142.67 (C8), 149.09 (C2), 148.10 (C7), 175.78 (C9). The phenyl carbons were located in a closely-spaced cluster between 120 and 130 ppm that overlapped with the signals from the aromatic ring of the benzodioxole moiety.

2.1.1.2. Metal complexes. The starting ruthenium complexes, $[(phen)_2RuCl_2]\cdot H_2O$ and $[(bpy)_2RuCl_2]$, were synthesized as described in the literature [19]. The target complexes were synthesized by the following general method: Equimolar amounts of $[(phen)_2RuCl_2]\cdot H_2O$ or $[(bpy)_2RuCl_2]$ and the appropriate ligand was suspended in 8–10 mL of ethylene glycol in a 35-mL reaction vessel. The vessel was capped and the reaction mixture saturated with argon for 15 min. The reaction vessel was then placed in the microwave reactor and heated at 150 °C for 5 min (using a dynamic method). The dark brown suspension became a dark red solution. This solution was poured onto 5–10 mL of a saturated aqueous solution of KPF_6 which resulted in the immediate precipitation of a red solid. The solid was collected by vacuum filtration, washed with water followed by ether and then dried at the vacuum pump. The product was recrystallized from dichloromethane/ether (**1** and **2**), ethanol/hexanes (**3** and **4**) or ethanol/ether (**5**).

$[(bpy)_2Ru(HpTSC)](PF_6)_2$. **1**. Red solid. Yield: 180 mg (47%). Analysis; Calc. for $C_{29}H_{25}F_{12}N_7O_2P_2RuS$: C, 37.59; H, 2.72; N, 10.58. Found: C, 37.84: H, 2.73; N, 10.75. IR (cm^{-1}): ν (NH_2, NH) 3424(w), 3373, 3152(w); ν (C=N) 1620; ν (N—N) 1036; ν (C=S) 1255, 830; λ_{max} (log ε); 294 nm (3.8); 430 nm (broad).

$[(bpy)_2Ru(EtpTSC)](PF_6)_2$. **2**. Red solid. Yield: 259 mg (66%). Analysis; Calc. for $C_{31}H_{29}F_{12}N_7O_2P_2RuS$: C, 39.00; H, 3.06; N, 10.26. Found: C, 39.29; H, 3.18; N, 10.18. IR (cm^{-1}): ν (NH_2, NH) 3421(w), 3113(w, b); ν (C=N) 1586; ν (N—N) 1034; ν (C=S) 1247, 827; λ_{max} (log ε); 303 nm (3.8); 276 nm (broad).

$[(phen)_2Ru(HpTSC)](PF_6)_2\cdot 0.25C_4H_{10}O$. **3**. Red solid. Yield: 166 mg (47%). Analysis; Calc. for $C_{34}H_{27.5}F_{12}N_7O_{2.5}P_2RuS$: C, 41.12; H, 2.79; N, 9.87. Found: C, 41.84; H, 2.42; N, 10.33. IR (cm^{-1}): ν (NH_2, NH) 3424, 3373(w), 3152(w); ν (C=N) 1620; ν (N—N) 1036; ν (C=S) 1251, 830; λ_{max} (log ε); 300 nm (sh); 430 nm (broad).

$[(phen)_2Ru(MepTSC)](PF_6)_2$. **4**. Dark-red solid. Yield: 256 mg (71%). Analysis; Calc. for $C_{34}H_{27}F_{12}N_7O_2P_2RuS$: C, 41.30; H, 2.75; N, 9.92. Found: C, 42.20; H, 2.62; N, 10.19. IR (cm^{-1}): ν (NH_2, NH) 3424(w), 3337(w); ν (C=N) 1620; ν (N—N) 1036; ν (C=S) 1256, 833; λ_{max} (log ε); 360 nm (sh); 440 nm (broad).

$[(phen)_2Ru(EtpTSC)](PF_6)_2$. **5**. Dark-red solid. Yield: 286 mg (71%). Analysis; Calc. for $C_{35}H_{29}F_{12}N_7O_2P_2RuS$: C, 41.92; H, 2.92; N, 9.78. Found: C, 41.80; H, 2.68; N, 9.88. IR (cm^{-1}): ν (NH_2, NH) 3420, 3371(w); ν (C=N) 1574; ν (N—N) 1036; ν (C=S) 826; λ_{max} (log ε); 292 nm (3.8); 400 nm (broad).

2.2. DNA-interaction studies

All experiments involving interaction of the complexes with DNA were carried out in Tris buffer (5 mM Tris, 50 mM NaCl, pH