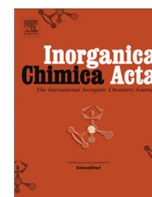
Inorganica Chimica Acta 453 (2016) 538–546

Contents lists available at ScienceDirect

Inorganica Chimica Acta

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



Research paper

A new complex of copper-phosphole. Synthesis, characterization and evaluation of biological activity

Samuel Alfonso [a,b], Sorenlis González [b], Angel R. Higuera-Padilla [b], Alba Vidal [b], Mercedes Fernández [c], Peter Taylor [c], Izaskun Urdanibia [c], Andreas Reiber [d], Yomaira Otero [b,*], William Castro [b,*]

[a] Universidad de los Andes, Facultad de Ciencias, Departamento de Química, Mérida, Venezuela
[b] Centro de Química, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas 1020-A, Venezuela
[c] Medicina Experimental, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas 1020-A, Venezuela
[d] Universidad de los Andes, Facultad de Ciencias, Departamento de Química, Bogotá, Colombia

ARTICLE INFO

Article history:
Received 16 May 2016
Received in revised form 31 August 2016
Accepted 8 September 2016
Available online 10 September 2016

Keywords:
Phosphole
Metal–phosphole complexes
DNA
Thioredoxin
Cytotoxicity

ABSTRACT

As part of the search for effective cancer chemotherapy, we describe here the synthesis, characterization, structural determination and biological activity of a new copper complex [Cu{1-phenyl-2,5-bis(2-thienyl) phosphole}$_2$Cl] (**1**), and evaluated its biological activity in terms of binding to calf thymus (CT) DNA, distribution coefficient, interaction with thioredoxin reductase (TrxR) and inhibition of tumour cell lines. Spectroscopic titration and viscosity results suggested a noncovalent interaction with DNA, probably by π–π stacking or electrostatic, while electrophoresis and competitive studies showed a weak interaction with CT-DNA. On the other hand, the TrxR system does not seem to be a target of action and the lipophilicity of the compound indicates that it may penetrate the cell membrane more effectively than the ligand or cisplatin. Finally, the compound showed little inhibitory effect on the tumour cell lines. With these results, we proposed that the low activity of the compound is due to only weak interaction with specific targets such as DNA.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Phospholes are compounds that can change their structure with relative ease due to their nucleophilic phosphorus atom. Phosphole rings have attracted growing interest for the development of complexes used in specific applications since they have the ability to act as σ-ligands due to their nucleophilic phosphorus atom [1,2], which allows relatively easy structural changes and which potentially offers the possibility of tuning the HOMO and the LUMO levels, promoting delocalisation of the π-system. These properties are important in medicinal inorganic chemistry as they permit modulation of the biological activity (increased affinity and/or reduction of side effects) of such complexes against specific targets such as DNA or human disulphide reductases [3–5].

Exceptional biological properties have been reported for phosphole complexes [3]. Specifically, gold phosphole complexes are potent inhibitors of TrxR and the related glutathione reductase, which are associated with many cellular processes such as antioxidant defence, redox balance, regulation of various proteins, and nucleotide metabolism [6]. Becker, studying the interaction of diverse M-phosphole complexes (M = Au or Pt) with TrxR and DNA [7], showed [1-phenyl-2,5-bis(2-pyridyl)phosphole]AuCl to be the more potent TrxR inhibitor, complemented by a high affinity for DNA, resulting in toxicity towards glioblastoma cells. Moreover, a sugar-modified gold phosphole complex was more cytotoxic than auranofin, with the authors suggesting a relationship between TrxR inhibition *in vitro* and its biological activity [8].

Side effects and the development of drug resistance against anti-cancer drugs are major setbacks in the treatment of this disease [9]. New structures are urgently required which may act through mechanisms different from those involved in traditional treatments, such as cisplatin [10] and other metallocenes [11]. Here we report a new copper(I)-phosphole complex and examined its interaction with CT-DNA and TrxR through diverse physical and spectroscopy techniques. Due to the interesting biological activities reported for this family of complexes, we evaluated its activity against six tumour cell lines.

* Corresponding authors.
E-mail addresses: yomaira@gmail.com (Y. Otero), wcastro10@gmail.com (W. Castro).

http://dx.doi.org/10.1016/j.ica.2016.09.012
0020-1693/© 2016 Elsevier B.V. All rights reserved.

2. Experimental

2.1. Materials and methods

All experiments were performed under an atmosphere of dry argon in the dark using a standard Schlenk technique. The solvents were previously dried and distilled following standard methods prior to use [12]. 1-Phenyl-2,5-bis(2-thienyl)phosphole (**L**) was synthesized according to published procedures [2,13,14]. ^{31}P, ^{1}H and ^{13}C NMR spectra were recorded using a Bruker Advance AM500 spectrometer. IR spectra were recorded on a Perkin Elmer 100/100 N spectrometer using KBr disks. Mass spectra were acquired on a Thermo Scientific TSQ Quantum Ultra AM Triple Quadrupole mass spectrometer employing the Heated Electrospray Ionization (HESI) technique. IR spectra were recorded on a Nicolet 5DXC UV–vis spectra were recorded in an HP-8450A spectrophotometer in cells with a 1 cm optical pathway at 298 K, using the corresponding solvent. Elemental analyses were performed with an EA 1108 and single-crystal X-ray data collection was performed at room temperature on a SuperNova diffractometer (Dual, Cu at zero, Atlas detector) with Mo-Kα radiation at λ = 0.71073 Å. Fluorescence measurements were carried out using a Perkin Elmer LS45 spectrophotometer with a pulse xenon lamp.

2.2. Synthesis of [Cu{1-phenyl-2,5-bis(2-thienyl)phosphole}$_2$Cl] (**1**)

A solution of ligand **L** (0.051 g, 0.13 mmol) and CuCl (0.053 g, 0.54 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature under argon for 2 days. The solvent was removed under vacuum and the remaining solid purified by thin-layer chromatography (SiO_2, eluant: CH_2Cl_2/hexane, 8:2 v/v) to give **1** as an orange solid (57.3 mg. Yield: 55%). Mp: 288 °C. Elemental analysis Calc. for $C_{44}H_{38}ClCuP_2S_4$: C, 61.74; H, 4.47; S, 14.98. Found: C, 61.77; H, 4.52; S, 14.97%. NMR-^{1}H (500 MHz, CD_2Cl_2, δ ppm): 7.81 (2H; d; J(H_o, H_m) = 6.75 Hz; H_o); 7.27 (5H; m; H_p, H_m y H_3); 7.17 (2H; dd; J(H_1, H_3) = 0.92 Hz, J(H_1,H_2) = 5.12 Hz; H_1); 6.80 (2H; dd; J(H_2,H_3) = 3.77 Hz; H_2); 2.75 (2H; m; H_{7a}); 2.50 (2H; m; H_{7b}); 1.71 (4H; m; H_8). NMR-^{13}C (500 MHz, CD_2Cl_2, δ ppm): 133.5 (s; C_o); 130.9 (s; C_p); 128.9 (d; J(C_m,P) = 23.32 Hz; C_m); 127.3 (s; C_2); 126.8 (s; C_3); 125.6 (s; C_1); 30.6 (s; C_7); 22.5 (s; C_8). NMR-^{31}P (500 MHz, CD_2Cl_2, δ ppm): 5.10 (s). IR-TF (υ_{max} cm^{-1}): 3111 and 3043 (ArC–H, st); 2959 and 2936 (–CH_2–, st); 1950 and 1650 (ArC–C); 1571, 1450, 1483, 1435 and 1416 (–C=C–); 1222, 1094 and 1024 (Ar–H); 749 and 688 (Ar–H). UV–Visible (CH_2Cl_2) [λ (nm), ε (M^{-1} cm^{-1})]: 230 (35951); 262 (24214); 412 (25046). MS (acetone, ESI): *m/z*: 820.07 $[M-Cl]^+$; 819.06 $[C_{44}H_{38}S_4P_2Cu]^+$; 441.06 $[M-(C_{22}H_{19}S_2P)]^+$. Molar conductivity in DMF, Λ_M = 11 ± 2 O^{-1} cm^2 mol^{-1}.

2.3. Computational methods

All structures were optimized using DMol [3,15–17]. This DFT-based program permits determination of the relative stability of all studied species based on their electronic structure. The calculations were performed using the Kohn–Sham Hamiltonian equation with the Perdew–Wang 1991 gradient correction [18] and the double-zeta plus (DNP) numerical basic set [15–17], which provides good accuracy at a relatively low computational cost. The All Electron core treatment was used for all the atoms. Frequency calculations of the structures showed that all frequencies were positive indicating that all structures are real minima.

2.4. Interaction with DNA

Spectrophotometric titrations were carried out by stepwise additions of a CT-DNA solution (1 mM, in 5 mM Tris–HCl, pH 7.2 and 50 mM NaCl buffer) to a solution of each complex (~70 μM) in DMSO, then recording the UV–vis spectra after each addition. Native DNA absorption was subtracted by adding the same amounts of CT-DNA to the blank cell. The binding affinity (K_b) was calculated from the spectrophotometric data according to the equation [19]

$$\frac{[DNA]}{(\varepsilon_a-\varepsilon_f)}=\frac{[DNA]}{(\varepsilon_0-\varepsilon_f)}+\frac{1}{K_b(\varepsilon_0-\varepsilon_f)}$$

where [DNA] is the concentration of DNA in base pairs, ε_a is the extinction coefficient of the observed absorption band at the given DNA concentration (corresponding to A_{obs}/[compound]), ε_f is the extinction coefficient of the complex free in solution, and ε_b is the extinction coefficient of the complex when fully bound to DNA. A plot of [DNA]/[$\varepsilon_a-\varepsilon_f$] versus [DNA] gave a slope 1/[$\varepsilon_a-\varepsilon_f$] and a Y intercept equal to 1/K_b[$\varepsilon_b-\varepsilon_f$]. K_b is the ratio of the slope to the intercept.

Viscosity measurements were carried out using an Ostwald viscometer immersed in a water bath maintained at 25 °C. The DNA concentration (75 μM in 5 mM Tris–HCl, 50 mM NaCl) was kept constant in all samples, while the complex concentration was increased from 0 to 70 μM. The mean flow time was calculated from at least 6 times measurements. Data are presented as $(\eta/\eta^0)^{1/3}$ versus the ratio [complex]/[DNA], where η and η^0 are the specific viscosity of DNA in the presence and absence of the complex, respectively. The values of η and η^0 were calculated from the expression (t - tb)/tb, where t is the observed flow time and tb is the flow time of buffer alone. The relative viscosity of the DNA was calculated from η/η^0 [20], using ethidium bromide as a classical intercalator control.

For the DNA electrophoresis assays, 10 μL samples of the pBR322 plasmid (20 μg/mL) were combined with the complex at different ratios (molar ratios of compound/DNA (Ri) 1–4) and then incubated for 18 h at 37 °C. The reaction was then quenched by the addition of NaCl (1 M) to give a final chloride concentration of 0.2 M. Five microliters of each sample were run (100 V for 0.5 h) on a 0.75% agarose gel with TBE 1× (0.45 M Tris-HCl, 0.45 M boric acid, 10 mM EDTA) and stained with ethidium bromide. The bands were then viewed with a transluminator and the image captured using a video camera [21].

To compare quantitatively the affinity of the compounds bound to DNA, the intrinsic binding constants (K_b) of the compounds to DNA were obtained by the luminescence titration method. Fixed amounts of compound were titrated with increasing amounts of DNA, over a range of DNA concentrations up to 20 μM. The scan speed was 480 nm/min with a slit width of 5 nm. All experiments were conducted at constant room temperature in a buffer solution containing 5 mM Tris-HCl (pH 7.1) and 50 mM NaCl. The concentration of the bound compound was calculated using the equation

$$\frac{r}{Cf}=K_b x(n-r)$$

$C_b = C_t[(F-F_0)/(F_{max}-F_0)]$; where C_t is the total compound concentration, *F* is the observed fluorescence emission intensity at a given DNA concentration, F_0 is the intensity in the absence of DNA, and F_{max} is the fluorescence of the totally bound compound. Binding data were graphed as a Scatchard plot of r/C_f versus *r*, where *r* is the binding ratio $C_b/[DNA]_t$, C_f is the free compound concentration and n is the number of binding sites.

2.5. Distribution coefficient (D)

The water/*n*-octanol distribution coefficients were determined by the stir-flask method [22,23]; water-saturated *n*-octanol and *n*-octanol-saturated water were prepared by shaking equal volumes of *n*-octanol and water for one week and allowing the

Download English Version:

https://daneshyari.com/en/article/7751042

Download Persian Version:

https://daneshyari.com/article/7751042

Daneshyari.com