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Novel mononuclear zinc complexes with 2,2′-dimethyl-4,4′-bithiazole: Synthesis, crystal structure and DNA-binding studies

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ABSTRACT

Two neutral mononuclear zinc complexes with 2,2′-dimethyl-4,4′-bithiazole (**dm4bt**), [Zn(dm4bt)Br_2] (**1**) and [Zn(dm4bt)I_2] (**2**) were synthesized and characterized by elemental analysis, IR, UV–Vis and NMR spectroscopy and their structures were studied by single-crystal diffraction. These complexes have a bidentate nitrogenous ligand with two halide anions attached to a zinc metal in a distorted tetrahedral geometry. The interaction ability of the two complexes with native calf thymus DNA (CT-DNA) has been monitored as a function of the metal complex–DNA molar ratio by UV–Vis absorption spectrophotometry, fluorescence spectroscopy, circular dichroism (CD) and thermal denaturation studies. The intrinsic binding constants K_b of complexes **1** and **2**, with CT-DNA obtained from UV–Vis absorption studies were $3.47 \pm 0.02 \times 10^4\ M^{-1}$ and $3.19 \pm 0.02 \times 10^4\ M^{-1}$, respectively. Both complexes exhibit luminescent properties in the absence and presence of CT-DNA and the fluorescence study ascertain the interaction of **1** and **2** with CT-DNA. Moreover the addition of the complexes to CT-DNA (1:2) led to an increase of the melting temperature of DNA up to around 2.7 °C. Further fluorimetric studies were performed using methylene blue (MB) as a fluorescence probe, indicating low intercalative interaction of the complexes with CT-DNA. The CD study also points to groove binding mode in the complexes rather than intercalation mode.

1. Introduction

There has been substantial interest in metal-based small molecules because of their relevance in the development of new reagents for biotechnology and medicine [1–3]. The metal complexes containing multidentate aromatic ligands are very important due to their capacity for binding and cleaving DNA under physiological conditions [4,5]. These efforts stem from the development of novel chemotherapeutics and highly sensitive diagnostic agents [6]. Metal complexes are known to bind to DNA in either a non-covalent or a covalent fashion. In covalent binding, the labile ligands of the complexes are replaced by a nitrogen base of DNA such as guanine N7 [7]. Non-covalent DNA interactions include three binding modes: intercalation, groove (surface) binding and external static electronic effects, along the outside of the DNA helix [8].

Zinc is one of the most abundant trace elements present in biological systems as well as biological processes and several metallo-proteins contain this element [9–11]. Zn(II) plays a central key in zinc fingers, it presents unique abilities to facilitate rewinding of melted DNA, it induces hydrolysis of DNA and RNA and it can promote B–Z transitions of DNA, suggest binding ability to DNA from the nucleo-bases and the phosphate groups [12] where the theoretical studies demanded the stabilization energy of zinc with base-N to be further than other bioactive metals such as copper and nickel [13]. Therefore the biological activity, especially DNA interaction ability of zinc complexes have been the subject of a large number of studies [14–16].

On the other hand, bithiazole is a moiety of bleomycins (BLMs), a natural antibiotic, used clinically in combination chemotherapy in the treatment of germ cell tumors, lymphomas, Kaposi's sarcoma, cervical cancers, and squamous cell carcinomas of the head and neck [17–19] where the bithiazole tail of bleomycin plays an important role in the interaction of bleomycin with DNA [20,21]. The bithiazole domain is responsible for multiple modes of DNA binding including partial intercalation and binding within the minor groove [22].

As part of our continuing research on metal complexes with bithiazole [23–25], in the present work, our experimental approach to the design of the proposed anticancer zinc complexes with bithiazole derivatives, 2,2′-dimethyl-4,4′-bithiazole, involves three stages: (i) synthesis and identifying of the complexes by means of elemental analysis, IR, UV–Vis, NMR and X-ray diffraction methods, (ii) improving their ability to interact with DNA using UV–Vis and

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fluorescence spectroscopy, and (iii) the investigation of their interaction mechanism with DNA by thermal denaturation, fluorescence study of MB–DNA and circular dichroism (CD) spectra. Reports on zinc complexes with bithiazole are limited [23,26–29] and to our knowledge, there is no report on the biological investigation of zinc-bithiazole complexes.

2. Experimental

2.1. Materials

The reagents and chemicals were purchased from commercial sources and used as received without further purification. The ligand 2,2′-dimethyl-4,4′-bithiazole (**dm4bt**) was prepared according to our previous report [23]. Calf thymus DNA (CT-DNA) was obtained from Sigma. The stock solution of CT-DNA gave a ratio 1.8–1.9 in UV absorbance at 260 and 280 nm (A260/A280) to check DNA purity, indicating that the DNA was sufficiently free of protein contamination [30]. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (ε = 6600 M^{-1} cm^{-1} at 260 nm) [31,32]. The stock solutions were stored at 5 °C and were consumed within 4 days. All the experiments involving interactions of the compounds with DNA were carried out in doubly distilled water buffer containing 5 mM Tris–HCl [Tris(hydroxymethyl)-aminomethane] and 50 mM NaCl, and adjusted to pH 7.4 with hydrochloric acid.

2.2. Physical measurements

The UV–Vis spectra were recorded on a Varian Cary 100 UV–Vis spectrophotometer using a 1 cm path length cell. The fluorescence spectra were recorded on a Varian Cary eclipse spectrofluorometer. Infrared spectra (4000–250 cm^{-1}) of solid samples were taken as 1% dispersions in KBr pellets using a Shimadzu-470 spectrometer. ^{1}H NMR spectra were acquired on a Bruker AC-300 MHz spectrometer at ambient temperature in DMSO-d_6. The melting points are uncorrected and were obtained by a Kofler Heizbank Rechart type 7841 melting point apparatus. Elemental analyses were performed using a Heraeus CHN–O Rapid analyzer. The T_m spectra were recorded on a Varian BioCary-100 UV–Vis spectrophotometer using a 1 cm path length cell. Circular dichroism measurements were carried out on a Jasco-810 spectropolarimeter at room temperature with a rectangular quartz cell of 1 cm path.

2.3. Synthesis of [Zn(dm4bt)Br$_2$] (**1**)

$ZnBr_2$ (0.080 g, 0.355 mmol) was dissolved in water (10 ml), mixed with methanol (30 ml) and reacted with 2,2′-dimethyl-4,4′-bithiazole (0.070 g, 0.356 mmol) [23] dissolved in acetonitrile (30 ml). The resulting mixture was stirred at 50 °C for 2 h and then filtered and left at room temperature. After a week, it began to produce pale yellow prismatic crystals of **1** (yield 0.102 g, 68%). ^{1}H NMR δ_H (DMSO-d_6): 2.69 (s, 3H, Me) and 7.75 (s, 1H, Ar). ^{13}C NMR δ_C (DMSO-d_6): 19.3 (Me), 115.6, 149.8 and 166.7 (Ar). IR (KBr, cm^{-1}): 3149, 3090 (νC–H, Ar), 2983 (νC–H, Me), 2914, 1559 (νC=C), 1527(νC=N), 1436 (νC–C), 1212 (νC–N), 1148, 977, 795 (νS–C), 639, 575 (νZn–N) and 251 (νZn–Br). UV–Vis (DMF) λ_{max}: 252 nm. *Anal.* Calc.: C, 22.80; H, 1.90; N, 6.64. Found: C, 22.63; H, 1.88; N, 6.59%.

2.4. Synthesis of [Zn(dm4bt)I$_2$] (**2**)

Complex **2** was prepared according to the procedure described for complex **1**. ZnI_2 (0.080 g, 0.250 mmol) was dissolved in water (10 ml), mixed with methanol (30 ml) and reacted with 2,2′-dimethyl-4,4′-bithiazole (0.050 g, 0.255 mmol) [23] dissolved in acetonitrile (30 ml). The resulting solution was stirred at 50 °C for 2 h, and was then filtered and left at room temperature. After a week, it began to produce colorless prismatic crystals of **2** (yield 0.094 g, 72%). ^{1}H NMR δ_H (DMSO-d_6): 2.68 (s, 3H, Me) and 7.75 (s, 1H, Ar). ^{13}C NMR δ_C (DMSO-d_6): 19.3 (Me), 115.6, 149.8 and 166.8 (Ar). IR (KBr, cm^{-1}): 3080 (νC–H, Ar), 2907 (νC–H, Me), (νC=C), 2825, 1563 (νC=C), 1528 (νC=N), 1431 (νC–C), 1374, 1287, 1210 (νC–N), 1155, 977, 770 (νS–C), 637, 586 (νZn–N) and 259 (νZn–I). UV–Vis (DMF) λ_{max}: 232 nm. *Anal.* Calc.: C, 18.64; H, 1.55; N, 5.43. Found: C, 18.51; H, 1.53; N, 5.39%.

2.5. Crystal structure determination and refinement

The X-ray diffraction measurements were made on a Bruker APEX II CCD area detector diffractometer (Mo Kα radiation, graphite monochromator, λ = 0.71073 Å). For [Zn(dm4bt)Br$_2$] (**1**) and [Zn(dm4bt)I$_2$] (**2**), colorless prismatic crystals with dimensions of 0.35 × 0.35 × 0.30 mm^3, and 0.21 × 0.14 × 0.08 mm^3, were respectively chosen and mounted on a glass fiber and used for data collection. Cell constants and an orientation matrix for data collection were obtained by least square refinements of diffraction data from 3425 unique reflections for **1** and 3144 for **2**. Data were collected at a temperature of −173(2) °C [100(2) K] to a maximum 2θ value of 57.98° for **1** and 54.00° for **2**, in a series of ω scans in 1° oscillations. Semi-empirical absorption corrections were carried out using the program SADABS [33]. The structures were solved by direct methods using the program SHELXS-97 [34]. The refinement and all further calculations were carried out using SHELXL-97 [34]. The C-bound H-atoms were included in calculated positions and treated as riding atoms using SHELXL-97 default parameters. The non-H atoms were refined anisotropically, using weighted full-matrix least-squares on F^2. Software packages APEX2 (data collection), SAINT (cell refinement and data reduction) and SHELXTL (molecular graphics and publication material) were also used [34,35]. A summary of the crystal data, experimental details and refinement results has been given in Table 1.

Table 1
Crystallographic and structure refinement data for compounds **1** and **2**.

	1	**2**
Formula	$C_8H_8Br_2N_2S_2Zn$	$C_8H_8I_2N_2S_2Zn$
Formula weight	421.47	515.45
T (K)	100(2)	100(2)
λ (Å)	0.71073	0.71073
Crystal system	monoclinic	monoclinic
Space group	$P2_1/n$	$P2_1/n$
Crystal size (mm)	0.35 × 0.35 × 0.30	0.21 × 0.14 × 0.08
a (Å)	8.8711(8)	9.0969(11)
b (Å)	12.4900(12)	12.7098(13)
c (Å)	12.1499(12)	12.9660(12)
β (°)	106.236(2)	105.388(7)
V (Å^3)	1292.5(2)	1445.4(3)
Z	4	4
D_{calc} (g cm^{-3})	2.166	2.369
θ range for data collection	2.39–28.99	2.29–27.00
F(000)	808	952
Absorption coefficient (mm)	8.374	6.232
Index ranges	$-12 \leqslant h \leqslant 12$ $-17 \leqslant k \leqslant 17$ $-16 \leqslant l \leqslant 16$	$-11 \leqslant h \leqslant 11$ $-11 \leqslant k \leqslant 16$ $-16 \leqslant l \leqslant 14$
Data collected	15089	9628
Unique data (R_{int})	3425 (0.0378)	3144 (0.0185)
Parameters, restraints	138, 0	138, 0
Final R_1, wR_2[a] (observed data)	0.0213, 0.0481	0.0241, 0.0671
Final R_1, wR_2[a] (all data)	0.0288, 0.0494	0.0253, 0.0678
Goodness-of-fit on F^2 (S)	1.006	1.005
Largest difference in peak and hole (e Å^{-3})	0.500, −0.695	0.537, −2.333

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma(w(F_o^2 - F_c^2)^2)/\Sigma w(F_o^2)^2]^{1/2}$.