Journal of Inorganic Biochemistry 160 (2016) 275–286

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

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

Anthracene-terpyridine metal complexes as new G-quadruplex DNA binders

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ARTICLE INFO

Article history:
Received 2 November 2015
Received in revised form 15 March 2016
Accepted 3 April 2016
Available online 9 April 2016

Keywords:
G-quadruplex
Terpyridine derivatives
Platinum complexes
Copper complexes
DNA interaction
c-myc

ABSTRACT

The formation of quadruple-stranded DNA induced by planar metal complexes has particular interest in the development of novel anticancer drugs. This is especially relevant for the inhibition of telomerase, which plays an essential role in cancer cell immortalization and is overexpressed in ca. 85–90% of cancer cells. Moreover, G-quadruplexes also exist in other locations in the human genome, namely oncogene promoter regions, and it has been hypothesized that they play a regulatory role in gene transcription. Herein we report a series of new anthracene-containing terpyridine ligands and the corresponding Cu(II) and Pt(II) complexes, with different linkers between the anthracenyl moiety and the terpyridine chelating unit. The interaction of these ligands and metal complexes with different topologies of DNA was studied by several biophysical techniques. The Pt(II) and Cu(II) complexes tested showed affinity for quadruplex-forming sequences with a good selectivity over duplex DNA. Importantly, the free ligands do not have significant affinity for any of the DNA sequences used, which shows that the presence of the metal is essential for high affinity (and selectivity). This effect is more evident in the case of the Pt(II) complexes. Moreover, the presence of a longer linker between the chelating terpyridine unit and the anthracene moiety enhances the interaction with G-quadruplex-forming sequences. We further evaluated the ability of the Cu(II) complexes to interact with, and stabilize G-quadruplex containing regions in oncogene promoters via a polymerase stop assay. These studies indicated that the metal complexes are able to induce G-quadruplex formation and stop polymerase activity.

1. Introduction

G-quadruplex DNA is a thermodynamically stable topology of DNA which has been proposed to be functionally relevant to a number of essential biological processes. In particular, it has been suggested that quadruplexes play a role in regulation of gene expression, translation, telomere-end maintenance, recombination and replication [1–5]. Therefore, G-quadruplexes have emerged as novel and distinctive targets for the development of anticancer drugs [6,7]. G-quadruplexes are four-stranded DNA structures formed from stacked tetrads of hydrogen-bonded guanine bases. These stable, non-classical, secondary structures are formed in vitro at physiological salt concentration and pH, from DNA sequences containing stretches of adjacent guanine residues [8,9]. There is mounting experimental evidence indicating that these non-canonical DNA structures are also present in vivo [10–12]. Interestingly, stabilization of intramolecular telomeric DNA in a G-quadruplex conformation has been shown to inhibit its elongation by the enzyme telomerase [4]. As telomerase is expressed in most cancer cells at substantially higher levels than in normal somatic cells, the enzyme is a potentially attractive target for selective anti-cancer therapy and drug development [13]. Telomerase itself is not an oncogene, but its repression and tight regulation in humans can be a tumour suppressor, and its targeting has advantages such as relative universality and specificity for cancer cells [14–18].

In addition to telomeric DNA, an intramolecular-quadruplex forming sequence has also been identified in the promoter region of the *c-myc* oncogene, amongst other gene promoters [6], and stabilization of this quadruplex structure has been implicated in the downregulation of the transcription of the gene [19,20]. All these facts suggest that a deeper understanding of quadruplex recognition by synthetic molecules could provide the basis of a novel anticancer drug.

Thus, researchers have taken up the challenge of synthesizing ligands that stabilize the quadruplex folded conformation focusing on the fact that a desired molecule should bind a human G-quadruplex structure with high affinity and with specificity over duplex DNA [21–23]. The number of known G-quadruplex ligands has increased steadily and several classes of quadruplex-targeting ligands have been reported that selectively bind to and stabilize quadruplexes [24–30]. Many of these ligands present a large planar aromatic surface, cationic

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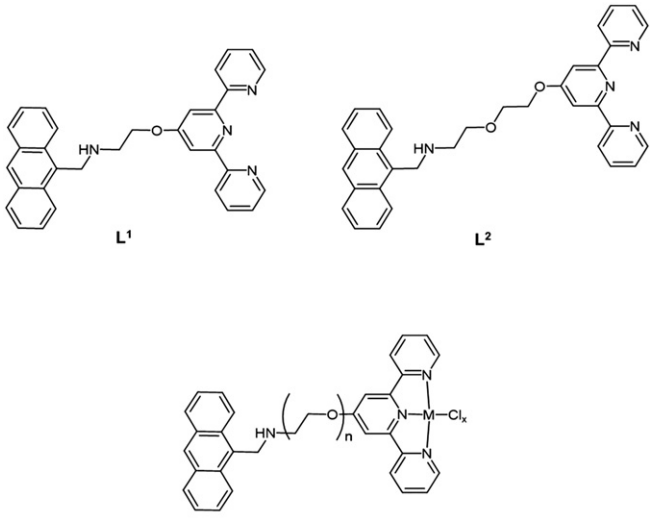
http://dx.doi.org/10.1016/j.jinorgbio.2016.04.002
0162-0134/

charges, and ability to adopt a terminal stacking mode [22,23]. In this context, the use of metal complexes as G-quadruplex binders is becoming very attractive due to the often easier synthetic access and their promising DNA binding properties [22]. The cationic or highly polarized nature of metal complexes is expected to promote the association with the negatively charged G-quadruplex-DNA [22,23]. Therefore, this approach assumes that the central metal centre can be positioned over the cationic channel of the quadruplex, optimizing the stacking interactions of the surrounding chelating agent with the accessible G-quartet. For instance, it has been reported that some Schiff base metal complexes are highly effective stabilizers of the human telomeric DNA, in particular square planar complexes of copper(II) and nickel(II) that are very prone to stack with the G-quartet [31–33]. Other types of ligands, namely derived from porphyrins and terpyridines, have been also tested and the corresponding Cu(II) and Pt(II) complexes were shown to act as G-quadruplex binders with high affinity and selectivity [34].

One of the features that quadruplex DNA binders should have if they are to be leads in drug discovery, is high selectivity over duplex DNA to minimise potential side effects. A successful strategy to improve such selectivity relies on the use of structures containing more than one interlinked planar polyaromatic moieties (e.g. anthracene, naphthalene, bipyridine or pyrene). As reported by Teulade-Fichou et al. [35], this type of strategy was successfully applied for macrocyclic organic compounds of the bis-intercalator or tris-intercalator type. This success inspired us to explore a similar approach to improve the quadruplex-selectivity of M(II)-terpyridine (M = Cu, Pt) complexes, known to interact with G-quadruplex sequences [34,36–40], by combining these terpyridine metal cores with a planar intercalating anthracenyl group.

Herein we report on the synthesis of a series of new anthracene-containing terpyridine ligands and the corresponding Cu(II) and Pt(II) complexes (Scheme 1) and on the biophysical characterization of their interaction with DNA of different topologies. In this work, the terpyridine chelating unit and the anthracenyl moiety were assembled using linker chains with different length and polarity, as we had anticipated that the different structural motifs (metal geometry, linker and pendant DNA intercalator) could influence the selectivity of the compounds' interaction with quadruplex DNA over duplex DNA. The interactions between ligands and metal complexes with the different topologies of DNA were studied by a variety of biophysical and spectroscopic techniques including FRET melting assays, fluorescence and CD spectroscopy. A polymerase stop assay was also performed to assess the ability of the compounds to interact and stabilize G-quadruplex containing regions in an oncogene promoter.



Scheme 1. Structural formulae of the new anthracene-containing terpyridine ligands (L^1 and L^2) and the corresponding Cu(II) and Pt(II) complexes (CuL^1, CuL^2, PtL^1 and PtL^2).

2. Experimental section

All chemicals used were analytical grade and used as received. The chemical reactions were followed by thin-layer chromatography (TLC), which was performed on precoated silica plates 60 F_{254} (Merck). Visualization of the plates was carried out using Ultraviolet (UV) light (254 nm). Gravity column chromatography was carried out on silica gel (Merck, 70–230 mesh).The multinuclear NMR spectra were measured on a Bruker 500 MHz nuclear magnetic resonance (NMR) Spectrometer at 500 MHz (^{1}H), 100.5 MHz (^{13}C), and 85.9 MHz (^{195}Pt), or in a Varian Unity 300 MHz spectrometer, and chemical shifts are given in ppm, referenced with the residual solvent resonances relative to $SiMe_4$. ^{1}H and ^{13}C NMR chemical shifts were reported in parts per million referenced to solvent resonances. ^{195}Pt NMR spectra were recorded using a solution of $K_2[PtCl_4]$ in saturated aqueous KCl as external reference. The shift for $K_2[PtCl_4]$ was adjusted to −1628 ppm relative to $Na_2[PtCl_6]$ ($\delta = 0$ ppm). For the Pt(II) complexes, Reversed Phase-High-performance liquid chromatography (RP-HPLC) and mass analysis were performed using a Waters LC-MS (Liquid chromatography-mass spectrometry) instrument equipped with Alliance 2695 separations module, 2487 dual lambda absorbance detector, and 3100 mass detector. Electrospray ionisation mass spectrometry (ESI-MS) was performed with a Micromass ZMD mass spectrometer (Micromass, Manchester, UK). Typically, a diluted solution of the compound in methanol (with 2% of the suitable co-solvent) was delivered directly to the spectrometer source at 0.01 mL min^{-1} by Hamilton microsyringe controlled by a single-syringe infusion pump. The nebulizer tip operated at 3000–3500 V and 150 °C, with nitrogen used both as a drying and a nebulizing gas. The cone voltage was usually 30 V. For the chelators and Pt(II) complexes, ESI-MS was performed on a QITMS (Quadrupole ion trap mass spectrometry) instrument in positive and negative ionisation mode. Peaks were assigned on the basis of the *m/z* values and of the simulated isotope distribution patterns. Elemental analysis (EA) was performed on an EA 110 CE Instruments automatic analyzer.

2.1. Synthesis

2.1.1. Synthesis of 2-([2.2′:6′,2″-terpyridin]-4′-yloxy)-N-(anthracen-9-ylmethyl)ethan-1-amine (*AntTerp*, L^1)

To a solution of anthracene-9-carbaldehyde (2.062 g; 10 mmol) in a 25% CH_3OH/CH_2Cl_2 solution (10 mL) 2-aminoethanol (0.72 mL, 12 mmol) was added. The reaction mixture was let to react overnight, at room temperature and under N_2. After vacuum removal of solvent, the solid residue was redissolved in 50% CH_3OH/CH_2Cl_2 solution (20 mL) and $NaBH_4$ (1.1349 g, 30 mmol) was added, at 0 °C. After 18 h reaction at room temperature, the solvent was removed and the residue dissolved in CH_2Cl_2 (50 mL) and washed with a solution of Na_2CO_3 (pH 10, 3 × 50 mL). The organic layer was dried over Na_2SO_4 and the solvent removed under vacuum to obtain 2-(anthracen-9-ylmethylamino)ethanol (**1, AntOH**). Yield: 2.238 g (89%). ^{1}H NMR ($CDCl_3$-d_3) δ: 8.42 (1H, s, H_{28}), 8.32 (2H, d, $H_{23\ +\ 33}$), 8.01 (2H, d, $H_{26\ +\ 30}$), 7.51 (4H, ddt, $H_{25\ +\ 24}$, $H_{31\ +\ 32}$), 4.75 (2H, d, H_{20}), 3.70 (2H, td, H_{16}), 3.2 (2H, m, H_{19}), 2.17 (1H, s, NH) ppm.

Then, 2-(anthracen-9-ylmethylamino)ethanol (0.251 g, 1 mmol) was let to react with 4′-chloro-2.2′:6′,2″-terpyridine (0.250 g, 0.9 mmol) and KOH (0.281 g, 5 mmol) in dimethyl sulfoxide (DMSO) for 4 h at 60 °C. The reaction was extracted with CH_2Cl_2 (3 × 40 mL) and the organic layer washed with water (3 × 30 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under vacuum. The solid residue was purified by column chromatography (100% CH_2Cl_2 to 95/5 CH_2Cl_2 /CH_3OH) to afford 2-([2.2′:6′,2″-terpyridin]-4′-yloxy)-*N*-(anthracen-9-ylmethyl)ethan-1-amine (**AntTerp, L^1**), which