Inorganica Chimica Acta 393 (2012) 252–260

Contents lists available at SciVerse ScienceDirect

Inorganica Chimica Acta

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

Ruthenium- and osmium-arene complexes of 8-substituted indolo[3,2-*c*]quinolines: Synthesis, X-ray diffraction structures, spectroscopic properties, and antiproliferative activity

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ARTICLE INFO

Article history:
Available online 15 June 2012

Metals in Medicine Special Issue

Keywords:
Indolo[3,2-*c*]quinoline
Ruthenium(II)
Osmium(II)
Anticancer compound

ABSTRACT

Six novel ruthenium(II)- and osmium(II)-arene complexes with indoloquinoline modified ligands containing methyl and halo substituents in position 8 of the molecule backbone have been synthesised and comprehensively characterised by spectroscopic methods (^{1}H, ^{13}C NMR, UV–Vis), ESI mass spectrometry and X-ray crystallography. Binding of indoloquinolines to a metal-arene scaffold makes the products soluble enough in biological media to allow for assaying their antiproliferative activity. The complexes were tested in three human cancer cell lines, namely A549 (non-small cell lung cancer), SW480 (colon carcinoma) and CH1 (ovarian carcinoma), yielding IC_{50} values in the 10^{-6}–10^{-7} M concentration range after continuous exposure for 96 h. Compounds with halo substituents in position 8 are more effective cytotoxic agents *in vitro* than the previously reported species halogenated in position 2 of the indoloquinoline backbone. High antiproliferative activity of both series of substances may be due at least in part to their potential to act as DNA intercalators.

1. Introduction

The fight against cancer has made considerable progress by the introduction of targeted therapies in recent years. This treatment modality takes advantage of certain features of malignant tumours to selectively inhibit their growth, ideally associated with low side effects for patients. The numerous concepts that are currently being explored to achieve tumour targeting in bioinorganic medicinal chemistry include 'activation by reduction' in hypoxic media, as well as 'activation by ring opening' in the solid tumour environment with lowered pH value [1–4]. Activation by reduction is believed to be the critical step in converting a prodrug into its active form [5]. Well-known examples supporting this hypothesis are satraplatin, a Pt^{IV} compound that reached a clinical phase III study [6], NAMI-A [7], as well as KP1019 [8], the first ruthenium(III) coordination compounds in clinical studies. Another way to gain selectivity for malignant cells over healthy tissue is targeting enzymes or receptors that are overexpressed in certain tumour types, e.g. thioredoxin reductase [9], ribonucleotide reductase [10,11], DNA topoisomerase [12] or glutathione S-transferase [13]. Another example are ferrocifen derivatives [14], which are based on hydroxytamoxifen, an oestrogen receptor antagonist used in hormone-positive breast cancer therapy [15]. In ferrocifen, one of the phenyl rings is replaced by a ferrocenyl unit, combining the hormone-antagonistic ligand with a metal–organic redox active moiety. Similar attempts combining the benefits of organometallic core with biologically active ligands were undertaken with indolobenzazepines, also referred to as paullones. The paullones were originally predicted to possess cyclin dependent kinase (CDK)-inhibitory properties by a COMPARE analysis [16]. CDKs together with their corresponding cyclins act as cell cycle triggers, controlling cell division [17]. By interference with this highly balanced regulatory system, cell proliferation can be controlled. *In vitro* models confirmed the CDK-inhibitory properties of the paullones [18], and up to date a broad range of paullone derivatives has been evaluated for biological activity [19,20]. For some paullones, other intracellular targets such as glycogen synthase kinase 3β (GSK3β) and mitochondrial malate dehydrogenase (mMDH) could be identified [21].

Indoloquinolines also attracted interest during the last few years [22–26] due to the development of convenient preparation routes [27]. In contrast to paullones with a folded seven-membered azepine ring, indoloquinolines are flat heteroaromatic ring systems, in which the paullone azepine ring was replaced by a six-membered pyridine ring. We anticipated that this transformation will alter significantly the physico-chemical and biological properties compared to the reference (paullone) compounds.

In order to overcome their limited solubility in biocompatible media, paullones were complexed to metal ions. Ga(III) [28], Ru(II)

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http://dx.doi.org/10.1016/j.ica.2012.06.004

[29] and Cu(II) [30] coordination compounds, as well as a series of Ru(II)- and Os(II)-arene complexes of modified paullone ligands [31–33] are well-documented in the literature. Interestingly, CDK inhibition by metal-based paullones does not necessarily parallel their *in vitro* antiproliferative activity, making other intracellular targets likely to be involved in their mechanism of action [34].

Novel SAR studies showed that some ruthenium- and osmium-arene complexes of indoloquinolines are by a factor of 10 more active than corresponding paullone complexes in human cancer cell lines. It is worth noting, however, that the indoloquinoline-based complexes with a bidentate ethylenediamine binding site are less stable than their paullone counterparts, dissociating in aqueous media with release of the ligand [34]. Remarkably, other ethylenediamine based ruthenium-arene complexes do not show propensity for dissociation under similar conditions [35–37]. To increase the thermodynamic stability and kinetic inertness of the complexes, sp^2-hybridised N-donor atoms were introduced by condensation of an indoloquinoline azine with 2-formyl- or 2-acetylpyridine [38]. This modification led to complexes with increased stability in biocompatible media, while retaining the *in vitro* antiproliferative activity. Further studies on modified indoloquinolines containing different substituents in position 2 of the molecular backbone showed that electron-withdrawing substituents are unfavourable for cytotoxicity, whereas an electron-donating methyl group has no influence on antiproliferative activity. The effect of substituents in position 8 of the indoloquinoline backbone was studied on copper(II) complexes which were found highly cytotoxic with IC_{50} values in the nanomolar concentration range [39]. Synthesis of those ligands is depicted in Scheme 1.

Herein we report on the synthesis of six novel ruthenium- and osmium-arene complexes with indoloquinoline-based ligands (**1a**,**b**–**3a**,**b**) containing substituents with different electronic properties in position 8 of the indoloquinoline backbone (Scheme 2). Their antiproliferative activity in three human cancer cell lines, namely A549 (non-small cell lung cancer), SW480 (colon carcinoma) and CH1 (ovarian carcinoma) has been studied and compared to that of chemically related complexes (**4a**,**b**–**6a**,**b** and others).

2. Experimental

2.1. Chemicals

Ethanol and THF were dried using standard procedures. α-Terpinene, 2-amino-5-chlorobenzonitrile were purchased from Acros Fisher, ruthenium trichloride and osmium tetroxide from Johnson Matthey, KBr from Merck, while 2-acetylpyridine, hydrazine dihydrochloride, hydrazine hydrate, phosphorus oxychloride, isatin, glacial acetic acid, borane in THF, 2-aminobenzonitrile, 2-amino-5-methylbenzonitrile, sodium perborate tetrahydrate were from Sigma–Aldrich. All these chemicals were used as received.

2.2. Synthesis

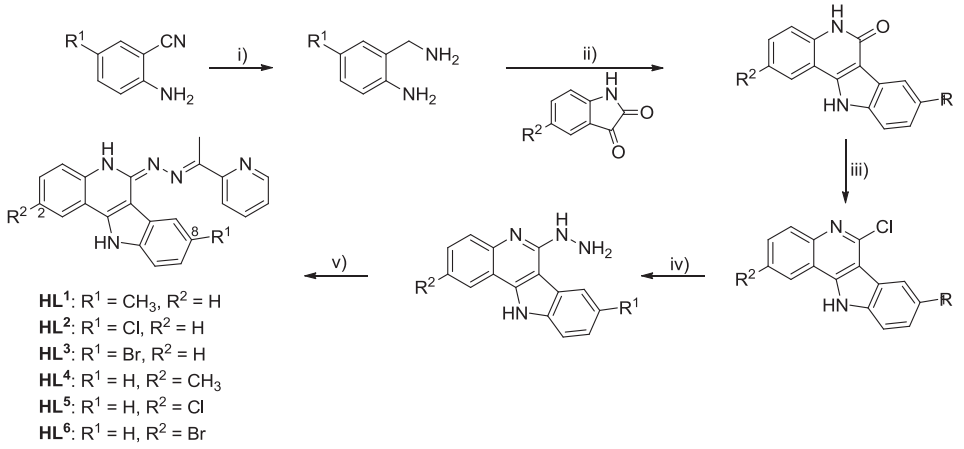
The ligands $\mathbf{HL^{1-3}}$ were synthesized by following the literature protocols [38,39]. Ruthenium- and osmium-arene starting compounds $[M(p\text{-cymene})(Cl)(\mu\text{-}Cl)]_2$, where $M = Ru^{II}$ and Os^{II}, were prepared as described previously [40,41]. For preparation of $[Os(p\text{-cymene})(Cl)(\mu\text{-}Cl)]_2$ OsO_4 was reduced first to $H_2[OsCl_6]$ by $N_2H_4{\cdot}2HCl$ in conc. HCl [42], and then reacted with α-terpinene.

General procedure A for the complexation of $\mathbf{HL^{1-3}}$ *to the metal*-arene *scaffold:* The corresponding ligand $\mathbf{HL^{1-3}}$ in a Schlenk tube was flushed with argon and suspended in dry ethanol. The corresponding metal-arene dimer was dissolved in chloroform and added to the ethanolic ligand suspension. The reaction mixture was stirred at room temperature under argon atmosphere (in the case of the Os complexes, light protection was also applied). The reaction mixture was filtered through a GF3 filter paper, and slowly added to diethyl ether previously dried over sodium sulfate. The precipitate formed was separated by filtration and dried *in vacuo* at 50 °C.

2.2.1. *[Ru(p-cymene)(HL^1)Cl]Cl,* ***1a***

General procedure A: *N*-(8-Methyl-5,11-dihydroindolo [3,2-*c*]—quinolin-6-ylidene)-*N*′-(1-pyridin-2-yl-ethylidene)azine (**HL^1**, 120 mg, 0.33 mmol), bis((η^6-*p*-cymene)(chlorido)(μ-chlorido) ruthenium(II)) (101 mg, 0.16 mmol), EtOH abs. (4 mL), $CHCl_3$ (0.3 mL), diethyl ether dried over Na_2SO_4 (100 mL), stirring for 22.5 h. To remove traces of the unreacted ruthenium dimer the red precipitate was dissolved in a minimal amount of EtOH and filtered through a GF3 filter paper. After addition of $CHCl_3$ (1 mL), the filtrate was added dropwise to diethyl ether previously dried over Na_2SO_4 (100 mL). The resulting precipitate was filtered off and dried in vacuo at 50 °C. Yield 154 mg, 69%. *Anal.* Calc. for $C_{33}H_{33}Cl_2N_5Ru{\cdot}1.5H_2O$ (M_r 698.65): C, 56.73; H, 5.19; N, 10.02. Found: C, 56.64; H, 5.01; N, 9.94%. ESI-MS (methanol), positive: *m*/*z* 636 $[M-Cl]^+$.

^{1}H NMR (500 MHz, DMSO-d_6): 12.99 (s, 1H, H^{11}), 10.36 (s, 1H, H^5), 9.60 (d, 1H, ^3J = 6 Hz, H^{17}), 8.36 (d, 1H, ^3J = 8 Hz, H^1), 8.31–8.26 (m, 1H, H^{19}), 8.23–8.19 (m, 2H, $H^7 + H^{20}$), 7.83–7.79 (m, 1H, H^{18}), 7.63 (d, 1H, ^3J = 8.3 Hz, H^{10}), 7.61–7.57 (m, 2H, $H^3 + H^4$), 7.42 (ddd, 1H, ^3J = 8 Hz, ^3J = 6 Hz, ^4J = 2 Hz, H^2), 7.31 (dd, 1H,



Scheme 1. Synthesis of the indoloquinoline modified ligands [38,39]. Reagents and conditions: (i) $BH_3{\cdot}THF$, THF, Ar, r.t., 24–72 h; (ii) glacial HOAc, reflux, 3–4 h; (iii) $POCl_3$, Ar, reflux, 26 h; (iv) $N_2H_4{\cdot}H_2O$, Ar, 100 °C, 24 h; (v) 2-acetylpyridine, EtOH, Ar, 65 °C, 18 h.