Inorganica Chimica Acta 434 (2015) 239–251

Contents lists available at ScienceDirect

Inorganica Chimica Acta

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

Ruthenium(II)-arene complexes with naphthalimide-tagged N,O- and N,N-chelating ligands: Synthesis and biological evaluation

Kesete Ghebreyessus [a,*], Ashley Peralta [a], Meena Katdare [b], Krishnan Prabhakaran [b], Shanthi Paranawithana [a]

[a] Department of Chemistry, Hampton University, Hampton, VA 23668, USA
[b] Skin of Color Research Institute, Hampton University, Hampton, VA 23668, USA

ARTICLE INFO

Article history:
Received 24 January 2015
Received in revised form 26 May 2015
Accepted 28 May 2015
Available online 9 June 2015

Keywords:
Ruthenium(II)-arene complexes
Antitumor activity
DNA-binding
Naphthalimide-tagged chelating ligands

ABSTRACT

A new family of ruthenium(II)-arene complexes with naphthalimide functionalized N,O- and N,N-chelating ligands of the general formula [Ru(η^6-*p*-cymene)Cl(**L**)] (**2b–4b**) (where: **L** = 4-[N-(2-((2-hydroxy-5-Br-phenyl)methyl imino)ethyl)]-N-butyl-1,8-naphthalimide (**2a**), 4-[N-(2-((2-hydroxy-5-Cl-phenyl)methyl imino)ethyl)]-N-butyl-1,8-naphthalimide (**3a**), and N-butyl-4-[N-(2-((2-hydroxy-5-NO_2-phenyl)methylimino)ethyl)]-N-butyl-1,8-naphthalimide (**4a**), and [Ru(η^6-*p*-cymene) Cl(**L′**)]Cl (**8b–9b**) (where **L′** = N-(2,2′-dipyridylaminoethyl)-1,8-naphthalimide (**8a**) and N-(2,2′-dipyridylaminopropyl)-1,8-naphthalimide (**9a**) have been synthesized and characterized. The *in vitro* cytotoxic activities of the ligands (**2a**, **9a**) and the complexes (**2b–4b**, and **8b–9b**) have been evaluated against the human melanoma skin cancer (CRL7687) and normal noncancerous (CA-M75) cell lines. All the compounds exhibit potent cytotoxic activities with IC_{50} values of ~1 μM or less but displayed variable selectivity. The compounds with N,O-ligands were found to be less selective than those containing N,N-chelating ligands. Notably, complex **9b** displayed the highest selectivity towards cancer cells over health cells. The interactions of the compounds with calf thymus DNA (CT-DNA) have also been investigated by UV–Vis and fluorescence spectra, ethidium bromide displacement assay and gel electrophoretic studies, which revealed that the compounds bind to CT-DNA moderately presumably through an intercalative mode.

1. Introduction

Ruthenium based coordination and organometallic complexes are increasingly gaining in importance as promising candidates for the design of new and more effective metal-based anticancer agents [1–6]. This interest is stimulated by the successful entry of two ruthenium(III) compounds, NAMI-A ([H_2im][*trans*-$RuCl_4$(S-dmso)-(Him)]; Him = imidazole, dmso = dimethyl sulfoxide) and KP1019 ([H_2ind][*trans*-$RuCl_4(Hind)_2$]; Hind = indazole), into clinical trials [7–10]. More recently, promising result in clinical studies of NKP-1339 ([Na][*trans*-$RuCl_4(Hind)_2$]; Hind = indazole), the sodium analogue of KP1019 has also illuminated further interest for the design and development of ruthenium(III) based coordination compounds as anticancer agents [11,12]. The most attractive profile of NKP-1339 is its high water solubility compared to KP1019. It has been established that ruthenium compounds exhibit low toxicities while maintaining high selectivity toward cancer cells *in vitro* and high efficacy against platinum-drug-resistant tumors [9–13]. The more selective activity of ruthenium compounds is believed to be due to their preferential accumulation in cancer cells and the ability of ruthenium to mimic iron in binding to biomolecules [3,7,9,11–13]. Ruthenium compounds are also believed to have a biological mode of action that is significantly different from those of platinum-based drugs [9,13–15]. Furthermore, the rich synthetic chemistry, diverse coordination geometries, redox accessible oxidation states, and favorable ligand substitution reactions of ruthenium complexes have been advantageously considered for the design of new anticancer agents [3,16].

More recently, the family of half-sandwich ruthenium(II)-arene organometallic complexes are also being actively investigated and evaluated as a potential source of new and effective metal-based anticancer agents. A favorable property of this type of complexes is the versatile pseudo-tetrahedral coordination geometry conferred by the metal center. Such a characteristic provides considerable possibilities for creating new compounds with interesting biological properties through rational ligand design and functionalization. In this context, several promising families of ruthenium(II)-arene based complexes with diverse ligand

* Corresponding author. Tel.: +1 757 727 5475; fax: +1 757 727 5604.
E-mail address: Kesete.Ghebreyessus@hamptonu.edu (K. Ghebreyessus).

http://dx.doi.org/10.1016/j.ica.2015.05.025
0020-1693/

frameworks have been synthesized, and evaluated for their antitumor activity against a broad spectrum of cancer cell lines. For example, ruthenium(II)-arene complexes of the type [(η^6-arene)Ru(en)Cl]$^+$ (en = 1,2-ethylenediamine) developed by Sadler and co-workers have shown to exhibit high antitumor activities in various cancer cell-lines [16–21]. Related compounds of the type [(η^6-arene)RuCl$_2$(pta)] (pta = 1,3,5-triaza-7-phosphaadmantane) reported by the Dyson group have also been shown to exhibit antimetastatic and antitumor activities [1,10,22–25]. The antitumor activity of many ruthenium(II)-arene complexes is generally related to their enhanced DNA binding affinity, which involves covalent coordination and/or simultaneous intercalation of extended aromatic groups and specific hydrogen bonding depending on the particular type of ligands used. In this regard, diverse ligand types are increasingly being developed and combined with the ruthenium(II)-arene moiety to enhance their DNA-binding properties, so as to achieve different biological functions and to maximize their effectiveness as therapeutic agents [21–26].

In recent years, research on targeted and multifunctional ruthenium(II)-arene complexes tethered to biologically active ligands have received increasing attention, mainly due to the potential synergism that could be achieved by combining a metal ion and a bioactive ligand [1,27–30]. It has been noted that tethering of biologically active ligands to the metal ion increases the biological potency of the complexes, through a combination of increased solubility, altered mechanisms of action, increased uptake, and improved cancer targeting properties. Dyson and co-workers have recently prepared new half-sandwich ruthenium(II)-arene complexes that incorporate the bioactive 1,8-naphthalimde-tagged arene and imidazole based ligands and,-which exhibited significantly higher anticancer activities compared to the prototype [(η^6-arene)RuCl$_2$(pta)] (pta = 1,3,5-triaza-7-phosphaadmantane) complexes. The higher cytotoxic activity of these new complexes is attributed to the incorporation of the 1,8-naphthalimide moiety onto the ruthenium(II)-arene unit, which provides multi-targeting properties including strong DNA-binding and interaction with proteins [29].

In light of these promising results, we initiated the investigations on the design and synthesis of new ruthenium(II)-arene complexes consisting of N,O- and N,N-based chelating ligands conjugated with the bioactive 1,8-naphthalimide moiety as potential anticancer agents. 1,8-naphthalimides are heterocyclic pharmacophores that are known to readily interact with DNA through intercalation, and also to act as potent topoisomerase II inhibitors [31–36]. As a result, 1,8-naphthalimide and its derivatives have been extensively investigated for their potential use as anticancer drugs, and two of these compounds (mitonafide, and amonafide) have reached clinical trials [32–34]. In addition to their broad spectrum of biological activities, 1,8-naphthalimides have also been described as strongly fluorescent agents, a property that could be useful for probing the interaction of these compounds with biomolecules [37–40].

Hence, in this article, we report the synthesis and characterization of a series of naphthalimide-tethered chelating ligands and their corresponding ruthenium(II)-arene complexes. The cytotoxic activity of the complexes toward the human skin melanoma cancer cell line (CRL7687) and normal skin melanocyte (CA-M75) was investigated by using the methylthiazolyldiphenyltetrazolium bromide (MTT) assay. The DNA-binding properties of the compounds were explored by UV–Vis and fluorescence spectroscopy, and gel electrophoretic mobility studies. The results of our investigation revealed that conjugation of the naphthalimide moiety to the ruthenium(II) center have no distinct advantages on the cytotoxic activities of the ruthenium(II)-arene complexes on the cell lines tested. The details are presented herein.

2. Experimental

2.1. Materials and methods

All synthetic procedures were performed under nitrogen. All chemicals and solvents were purchased from commercial sources and used as received. Double stranded calf thymus DNA (CT-DNA) (Sodium salt, highly polymerized type) and supercoiled pUC18 plasmid DNA were purchased from Sigma Aldrich. [(η^6-cymene)RuCl$_2$]$_2$ was prepared according literature methods [41]. ^{1}H and ^{13}C{^{1}H} NMR spectra were recorded on a JEOL Eclipse 2–400 MHz spectrometer using solvent resonances as internal references. Electrospray ionization (ESI) mass spectra were recorded on an Agilent (Varian) MS-500 series and analyzed using MS-Varian 6.9.3 software. UV–Vis absorption spectra were recorded on a Varian Cary 50 BIO spectrometer and emission spectra on a Cary Eclipse fluorospectrometer. Milli-Q H_2O (18.2 mΩ) was used as a solvent for all UV–Vis, fluorescence, and gel electrophoresis studies.

2.2. Synthesis and characterization of the ligands (**2a**–**4a**)

2.2.1. Synthesis of 4-[(2′-aminoethyl)amino]-N-butyl-1,8-naphthalimide (**1**)

The precursor compound, 4-[(2′-aminoethyl)amino]-N-butyl-1,8-naphthalimide (**1**), was synthesized following a procedures reported in the literature [42,43].

2.2.2. Synthesis of N-(2-((2-hydroxy-5-Br-phenyl)methylimino)ethyl)-N-butyl-1,8- naphthalimide (**2a**)

A stirred solution of the precursor compound 4-[(2′-aminoethyl)amino]-N-butyl-1,8-naphthalimide (**1**), (0.50 g) and 5-bromo-salicylaldehyde in ethanol (50 mL) was heated to reflux for 24 h under a nitrogen atmosphere. The volume of the reaction mixture was reduced to ~5 mL by rotary evaporation. The solution was then cooled to room temperature and placed in an ice bath to precipitate the product. The resulting yellow precipitate was isolated by filtration and washed with diethyl ether to give the pure product as a yellow microcrystalline solid (yield: 0.56 g, 71%). *Anal.* Calc. for $C_{25}H_{25}N_3O_3Br$: C, 60.61; H, 5.09; N, 8.48. Found: C, 60.43; H, 4.91; N, 8.50%. ESI-MS (CH_3CN): m/z = 494.7 [**2a**+H]$^+$. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.58 (dd, J = 7.3 Hz, J = 0.9 Hz, 1H, Ar-H), 8.48 (d, J = 8.7 Hz, 1H, Ar-H), 8.31 (s, CH═N), 8.03 (d, J = 7.8 Hz, 1H, Ar-H), 7.62 (d, J = 7.3 Hz, 1H, Ar-H), 7.40 (dd, J = 6.4 Hz, J = 2.8 Hz, 1H, Ar-H), 7.32 (d, J = 2.3 Hz, 1H, Ar-H), 6.87 (d, J = 8.7 Hz, 1H, Ar-H), 6.81 (d, J = 8.2 Hz, 1H, Ar-H), 5.47 (t, J = 6.0 Hz, 1H, NH), 4.16 (t, J = 7.8 Hz, 2H, NCH_2CH_2–, butyl), 4.00 (t, J = 5.5 Hz, 2H, NCH_2–), 3.83 (q, J = 6.0 Hz, 2H, –NCH_2CH_2), 1.71 (m, 2H, –$CH_2CH_2CH_3$, butyl), 1.44 (m, 2H, –$CH_2CH_2CH_2$–, butyl), 0.92 (t, J = 7.4 Hz, 3H, CH_3, butyl). ^{13}C ($CDCl_3$, 100 MHz) δ 166.0 (C═O), 164.6 (C═O), 164.1 (N═CH), 159.9 (Ar-C), 148.5 (Ar-C), 135.5 (Ar-C), 134.1 (Ar-C), 133.7 (Ar-C), 131.2 (Ar-C), 129.8 (Ar-C), 125.5 (Ar-C, Ph), 125.2 (Ar-C, Ph), 123.4 (Ar-C, Ph), 120.4 (Ar-C), 119.8 (Ar-C), 119.1 (Ar-C), 111.4 (Ar-C), 110.4 (Ar-C), 104.6 (Ar-C), 57.9 (NCH_2–), 43.9 (NCH_2, butyl), 40.0 (CH_2NH), 30.3 (NCH_2CH_2–, butyl), 20.4 (–$CH_2CH_2CH_2$–), 13.9 (CH_3, butyl).

2.2.3. Synthesis of N-(2-((2-hydroxy-5-Cl-phenyl)methylimino)ethyl)-N-butyl-1,8- naphthalimide (**3a**)

The same procedure as in **2a** using 5-chloro-salicylaldehyde to give **3a** (yield: 0.60 g, 83%). *Anal.* Calc. for $C_{25}H_{25}N_3O_3Cl$: C, 66.59; H, 5.59; N, 9.32. Found: C, 66.40; 5.38; N, 9.20%). ESI-MS (CH_3CN): m/z = 450.4 [**3a**]$^+$. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.58 (d, J = 7.3 Hz, 1H, Ar-H), 8.48 (d, J = 8.2 Hz, 1H, Ar-H), 8.31 (s, 1H, N═CH), 8.04 (d, J = 8.3 Hz, 1H, Ar-H), 7.61 (d, J = 7.3 Hz, 1H, Ar-H, Ph), 7.28 (d, J = 2.8 Hz, 1H, Ar-H), 7.17 (d, J = 2.8 Hz, 1H, Ar-H),