Inorganica Chimica Acta 453 (2016) 284–291

Contents lists available at ScienceDirect

Inorganica Chimica Acta

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

Research paper

Synthesis, structural and *in vitro* functional characterization of arene ruthenium complexes with 1,3,5-tris(di-2-pyridylaminomethyl)benzene ligand

A. Basava Punna Rao [a], A. Uma [b], T. Chiranjeevi [b], M.S. Bethu [c], B. Yashwanth [c], J. Venkateswara Rao [c], Krishna Mohan Poluri [d], Mohan Rao Kollipara [a,*]

[a] Centre for Advanced Studies in Chemistry, North-Eastern Hill University, Shillong 793 022, India
[b] Centre for Biotechnology, IST, Jawaharlal Nehru Technological University, Kukatpally 500 085, India
[c] Biology Division, CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India
[d] Department of Biotechnology and Center for Nanotechnology, Indian Institute of Technology Roorkee, Roorkee 247667, Uttarakhand, India

ARTICLE INFO

Article history:
Received 25 June 2016
Received in revised form 13 August 2016
Accepted 16 August 2016
Available online 17 August 2016

Keywords:
Arene ruthenium
Antibacterial
Metal based drugs
Cytotoxicity
MTT assay
Cancer

ABSTRACT

Ruthenium based complexes are growing as a promising formulations for the cure of cancer with their distinct activity and less side effects. In the present study, a series of mono, di and trinuclear octahedral arene ruthenium complexes containing 1,3,5-tris(di-2-pyridylaminomethyl)benzene (**L**) as a nitrogen donor ligand have been prepared by the reaction of $[(arene)RuCl_2]_2$ dimer. These complexes are fully characterized by spectroscopic studies. The complexes **1–6** have the general compositions $[\{(benzene)RuCl\}_nL]^{n+}$ where n = 1 (**1**), n = 2 (**2**), n = 3 (**3**), $[\{(p\text{-cymene})RuCl\}_nL]^{n+}$ where n = 1 (**4**), n = 2 (**5**) and n = 3 (**6**) respectively. The X-ray structure of $[(p\text{-cymene})RuLCl]^+$ (**4**) revealed a distorted octahedral coordination around the central metal atom. Antibacterial and the cytotoxicity characteristics of these complexes have been evaluated by the zone inhibition and anti-proliferative studies. Antibacterial studies evidenced highest zone of inhibition (17 nm) for *Staphylococcus aureus* and *Escherichia coli*, and the cytotoxicity experiments revealed more selectivity towards the *Carcinoma* cell lines than the *leukemia* cell lines.

1. Introduction

World's one of the major causes of death is *cancer*, which makes the body with an uncontrollable increase of diseased cells that are distinctly different from healthy cells through their redox metabolism [1–3]. Such an abnormal growth phenomenon was controlled by one of the metal-based drug *cis*-platin in the 1960's [4,5]. Though huge number of metal complexes has been synthesized to fight against cancer, only few of them are in successful clinical trials. Among them, ruthenium based complexes (Chart 1) are one of the promising metal based drugs, as they exhibit distinct activity with less side effects among the platinum group counter parts [6–8].

Ruthenium is 8th group metal, which can mimic iron in its properties except the fact that it exists in higher oxidation state of Ru(IV), where it is stabilized as RuO_2. The other oxidation states Ru(II) and Ru(III) do possess their own activities under physiological conditions. One of the feasible coordination's of ruthenium is six either in Ru(II) or Ru(III), such a high coordination can tune the biological properties like binding to DNA, proteins and enzymes in human body [9–12]. The most abundant transport proteins; albumin at 40 mg per ml [13] and transferrin at 3.0 mg per ml [14] contained in the human blood are the most welcoming partners for these metal based drugs when they are administered [15,16]. NAMI-A is the first ruthenium compound used in clinical trials (since 1999), which exhibits good amount of activity on lung carcinoma cell lines. Such a selective activity makes NAMI-A to be used as a second line chemotherapy drug in lung metastases [17]. KP1019 is another ruthenium compound that is currently in the clinical trials (since 2003) due to its low toxicity and high degree of tumor selectivity against *cis*-platin resistant tumors.

Half sandwich arene ruthenium complexes such as [Ru(arene)(X)(L-L)] are another class of ruthenium complexes which are also active against many cancer lines with less side effects. Among them *RM 175* and series of *RAPTA* complexes (Chart 1) exhibit better antitumor and antimetastatic properties and are standing at an advanced preclinical trails [18,19]. The activity of these complexes

* Corresponding author.
E-mail address: mohanrao59@gmail.com (M.R. Kollipara).

http://dx.doi.org/10.1016/j.ica.2016.08.031
0020-1693/

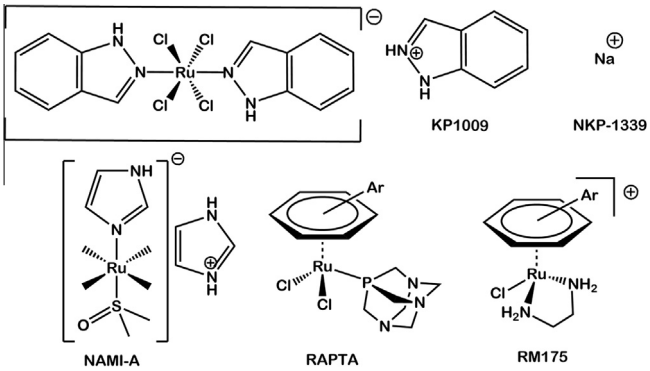

Chart 1. Clinically investigated ruthenium based anticancer agents.

depends on three structural variations; (a) arene – which occupies three coordination sites of the metal as a seat of *piano stool*, (b) X – mostly chloride ion and lastly (c) L-L either mono-dentate or bidentate as legs of the stool. Changing L-L does enhance the biological activity of the arene ruthenium complexes [20–24].

In this study, six new arene ruthenium complexes containing 1,3,5-tris (di-2-pyridylaminomethyl)benzene ligand (**L**) were synthesized. Their biological properties, such as antibacterial on four human pathogenic bacteria by agar well diffusion method and antiproliferative activity on four cancerous cell lines (three human and one mice cell lines) by MTT assay are evaluated.

2. Experimental section

2.1. Materials and methods

$RuCl_3{\cdot}H_2O$, was purchased from Arora Matthey Ltd, while 1,4-cyclohexadiene, α-terpinene, dipyridylamine and tris(bromomethyl)benzene were obtained from Sigma-Aldrich. All chemicals were used without further purification and the solvents used for synthesis were dried and distilled prior to use according to the standard procedures and stored over activated molecular sieves [25]. Precursor complexes *viz*., $[\{(benzene)RuCl_2\}_2]$ and $[\{(p\text{-cymene})RuCl_2\}_2]$ and ligand **L**, respectively were prepared according to the literature methods and ligand was purified by flash column chromatography [26–28].

2.2. General procedure for synthesis of complexes ***1–6***

2.2.1. Synthesis of complexes ***1–3***

A mixture of $[(benzene)RuCl_2]_2$, ligand **L** and NH_4BF_4 with the corresponding ratio was stirred in dry DCM and methanol (1:1) (30 ml) at room temperature for 6–12 h during which yellow precipitate was formed. Resulted precipitate was then filtered and washed with cold methanol followed by diethylether and air-dried. This precipitate was soluble in acetonitrile, DMSO, DMF, partially soluble in solvents such as DCM, acetone, methanol, and is insoluble in non-polar solvents such as hexane and diethyl ether.

*2.2.1.1. [(Benzene)RuClL]BF_4 (**1**).* Ratio of metal precursor, ligand and ammonium tetrafluoroborate (1:2:2); for 6 h; Yield: 46%; IR (KBr pellets, cm^{-1}): 1595 ($\nu_{C=C}$), 1468 ($\nu_{C=N}$), 1084 (ν_{B-F}); ^1H NMR (400 MHz, DMSO-d_6): δ 8.54 (d, J = 5.6 Hz, 2H), 8.24 (d, J = 4.4 Hz, 4H), 7.77 (t, J = 7.4 Hz, 2H), 7.44 (t, J = 7.4 Hz, 4H), 7.39 (s, 1H), 7.33 (s, 2H), 7.25 (t, J = 6.3 Hz, 2H), 7.17 (t, J = 6.3 Hz, 4H), 6.93 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.2 Hz, 4H), 6.25 (s, 6H), 5.38 (s, 4H), 5.31 (s, 2H); Molar conductivity (^$_M$, 10^{-3} M, CH_3CN): 118 S cm^2 mol^{-1}.

*2.2.1.2. $[\{(Benzene)RuCl\}_2L](BF_4)_2$ (**2**).* (1:1:2); 10 h; Yield: 84%; IR (KBr pellets, cm^{-1}): 1596 ($\nu_{C=C}$), 1467 ($\nu_{C=N}$), 1084 (ν_{B-F}); ^1H NMR (400 MHz, DMSO-d_6): δ 8.69 (d, J = 4.2 Hz, 4H), 8.59 (d, J = 5.0 Hz, 2H), 7.95 (t, J = 7.5 Hz, 2H), 7.77 (t, J = 7.4 Hz, 4H), 7.36 (d, J = 8.3 Hz, 4H), 7.30 (s, 1H), 7.17 (d, J = 6.7 Hz, 2H), 7.15 (s, 2H), 6.77 (t, J = 6.6 Hz, 4H), 6.59 (t, J = 6.6 Hz, 2H), 6.35 (s, 12H), 5.48 (s, 4H), 5.31 (s, 2H); Molar conductivity (^$_M$, 10^{-3} M, CH_3CN): 198 S cm^2 mol^{-1}.

*2.2.1.3. $[\{(Benzene)RuCl\}_3L](BF_4)_3$ (**3**).* (1.5:1:2); overnight; Yield: 91%; IR (KBr pellets, cm^{-1}): 1597 ($\nu_{C=C}$), 1465 ($\nu_{C=N}$), 1084 (ν_{B-F}); ^1H NMR (300 MHz, DMSO-d_6): δ 8.64 (d, J = 5.7 Hz, 6H), 7.89 (t, J = 7.3 Hz, 6H), 7.33 (d, J = 8.5 Hz, 6H), 7.21 (s, 3H), 7.04 (t, J = 6.6 Hz, 6H), 6.25 (s, 18H), 5.46 (s, 6H); Molar conductivity (^$_M$, 10^{-3} M, CH_3CN): 330 S cm^2 mol^{-1}.

2.2.2. Synthesis of complexes ***4–6***

A mixture of $[(p\text{-cymene})RuCl_2]_2$, ligand **L** and NH_4BF_4 in the corresponding ratio was stirred in dry DCM and methanol (1:1) (30 ml) at room temperature for 6–12 h during which the color of the solution changed from yellow to orange. The solvent was removed under vacuum and the residue was dissolved in dichloromethane (10 ml). The solution was then filtered through the bed of Celite to remove ammonium chloride and excess salts. Addition of the diethylether to the concentrated (~2–3 ml) orange solution of complexes resulted them as orange-yellow precipitate, which were separated and air-dried. They are soluble in polar organic solvents DCM, acetone, methanol and acetonitrile but are insoluble in non-polar solvents hexane and diethyl ether.

*2.2.2.1. [(p-Cymene)RuClL]BF_4 (**4**).* (1:2:2); 6 h; Yield: 47%; IR (KBr pellets, cm^{-1}): 1598 ($\nu_{C=C}$), 1468 ($\nu_{C=N}$), 1084 (ν_{B-F}); ^1H NMR (400 MHz, $CDCl_3$): δ 8.66 (d, J = 5.7 Hz, 2H), 8.63 (d, J = 4.6 Hz, 4H), 7.75 (t, J = 7.8 Hz, 2H), 7.46 (t, J = 7.8 Hz, 4H), 7.39 (s, 1H), 7.31 (s, 2H), 7.15 (t, J = 6.5 Hz, 2H), 7.07 (t, J = 6.5 Hz, 4H), 6.98 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.4 Hz, 4H), 5.61 (d, J = 5.9 Hz, 2H), 5.38 (s, 4H), 5.34 (d, J = 4.8 Hz, 2H), 5.31 (s, 2H), 2.68 (sep, 1H), 1.72 (s, 3H), 1.21 (d, J = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.01, 156.76, 156.42, 156.12, 155.83, 153.81, 153.57, 148.09, 148.02, 147.86, 141.74, 141.09, 140.88, 140.17, 139.43, 137.56, 137.39, 137.13, 135.83, 134.01, 125.87, 125.43, 123.57, 120.85, 120.31, 117.25, 117.01, 116.19, 115.82, 114.61, 114.32, 114.06, 106.00, 105.89, 100.53, 100.11, 85.76, 85.59, 84.08, 83.81, 77.42, 77.11, 76.79, 56.02, 55.21, 51.25, 50.87, 30.61, 22.27, 17.69; Molar conductivity (^$_M$, 10^{-3} M, CH_3CN): 125 S cm^2 mol^{-1}.

*2.2.2.2. $[\{(p\text{-Cymene})RuCl\}_2L](BF_4)_2$ (**5**).* (1:1:2); 10 h; Yield: 83%; IR (KBr pellets, cm^{-1}): 1599 ($\nu_{C=C}$), 1467 ($\nu_{C=N}$), 1084 (ν_{B-F}); ^1H NMR (400 MHz, $CDCl_3$): δ 8.67 (d, J = 4.4 Hz, 4H), 8.63 (d, J = 5.0 Hz, 2H), 7.92 (t, J = 7.8 Hz, 2H), 7.76 (t, J = 7.7 Hz, 4H), 7.37 (d, J = 8.6 Hz, 4H), 7.30 (s, 1H), 7.16 (d, J = 6.7 Hz, 2H), 7.14 (s, 2H), 6.75 (t, J = 6.8 Hz, 4H), 6.66 (t, J = 6.8 Hz, 2H), 5.67 (d, J = 5.6 Hz, 4H), 5.48 (s, 4H), 5.46 (d, J = 4.6 Hz, 4H), 5.31 (s, 2H), 2.72 (sep, 2H), 1.73 (s, 6H), 1.23 (d, J = 6.9 Hz, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.74, 156.40, 156.09, 155.74, 153.92, 153.78, 153.49, 148.07, 147.85, 141.76, 140.86, 140.13, 137.54, 137.37, 136.86, 135.83, 125.77, 123.54, 120.82, 120.26, 117.33, 117.23, 116.18, 115.84, 114.30, 114.03, 106.23, 105.87, 100.53, 100.31, 85.75, 85.44, 83.96, 83.79, 77.38, 77.06, 76.74, 55.23, 50.63, 30.53, 22.28, 17.87, 17.68; Molar conductivity (^$_M$, 10^{-3} M, CH_3CN): 223 S cm^2 mol^{-1}.

*2.2.2.3. $[\{(p\text{-Cymene})RuCl\}_3L](BF_4)_3$ (**6**).* (1.5:1:2); overnight; Yield: 92%; IR (KBr pellets, cm^{-1}): 1600 ($\nu_{C=C}$), 1466 ($\nu_{C=N}$), 1084 (ν_{B-F}); ^1H NMR (400 MHz, $CDCl_3$): δ 8.63 (d, J = 5.8 Hz, 6H), 7.91 (t, J = 7.2 Hz, 6H), 7.37 (d, J = 8.5 Hz, 6H), 7.20 (s, 3H), 7.16 (t,