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Original article

Anticancer activity of ruthenium(II) arene complexes bearing 1,2,3,4-tetrahydroisoquinoline amino alcohol ligands

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ABSTRACT

Ruthenium complexes offer potential reduced toxicity compared to current platinum anticancer drugs. 1,2,3,4-tetrahydrisoquinoline amino alcohol ligands were synthesised, characterised and coordinated to an organometallic Ru(II) centre. These complexes were evaluated for activity against the cancer cell lines MCF-7, A549 and MDA-MB-231 as well as for toxicity in the normal cell line MDBK. They were observed to be moderately active against only the MCF-7 cells with the best IC_{50} value of 34 μM for the *cis*-diastereomeric complex **C4**. They also displayed excellent selectivity by being relatively inactive against the normal MDBK cell line with SI values ranging from 2.3 to 7.4.

1. Introduction

Interest in the design of organometallic Ru(II) complexes as anticancer agents has increased in recent years as these species have exhibited promising activity in both *in vivo* and *in vitro* studies [1,2]. These complexes show evidence of low toxicity compared to traditional cisplatin agents, alternative mechanisms of action [3] and a versatile spectrum of activity amongst cancer types [1,4]. Aird et al. have reported organometallic Ru(II) complexes of the type [(η^6-arene)Ru(*N*,*N*)Cl], where *N*,*N* is a series of chelating diamine ligands, that exhibited non-cross-resistance with cisplatin-resistant cells [1]. Additional features of these complexes include their air stability and water solubility [2].

The presence of a chelating ligand in these "piano-stool" Ru(II) complexes offers structural stability and the opportunity to "tune" the electronics of the ruthenium centre. Different donor elements such as phosphorus, nitrogen and oxygen have also been studied in terms of their anticancer activity when coordinated to ruthenium [5]. Sadler and co-workers demonstrated that a change of donor ligand has a profound effect on the electronic properties of the Ru(II) complex. For example, the rate of hydrolysis of the Ru–Cl bond is greater with an anionic *O*,*O*-chelating ligand than with a neutral *N*,*N*-ligand [6]. This tuning of the ligand also resulted in a changed preference of the targeted nucleobases. Subsequent studies to establish SARs on Ru(II) complexes with various chelating donor sites, were performed on ligands such as *N*,*N*-(diamines and bipyridine), *N*,*O*-(amino acidates) and *O*,*O*-(acetylacetonate) [7]. In that study complexes with *N*,*N*- ligands possessed superior activity to the *O*,*O* chelates and the *N*,*O*-complexes were inactive [7]. The *N*,*N* ligands have been studied more extensively in the literature and are thus far the preferred chelate donor heteroatom combination [7,8].

According to the rules concerning structure–activity relationships (SARs) for an effective Pt anticancer drug, it has been stated that the two non-leaving *cis*-coordinated amine ligands are crucial for anticancer activity [9]. This rule is based on the observation for cisplatin where non-leaving N-donor amine ligands are considered vital for its anticancer properties [10]. Numerous metal complexes (including Ru) with aromatic N-donor ligands have exhibited promising anticancer properties. Such ligands include derivatives

Abbreviations: A549, human lung epithelial adenocarcinoma; atm, atmosphere; ATR, attenuated total reflectance; Cbz-Cl, benzyl chloroformate; DIPEA, *N*,*N*-diisopropylethylamine; DBU, 1,8- diazabicycloundec-7-ene; ESI-QTOF, electrospray ionization quadrupole time of flight; HRMS, high resolution mass spectrometry; FA, formic acid; IC_{50}, half maximal inhibitory concentration; $LiAlH_4$, lithium aluminium hydride; Me_2SO_4, dimethyl sulfate; MCF-7, Michigan cancer foundation-7; MDA-MB-231, human breast epithelial adenocarcinoma; MDBK, Madin–Darby bovine kidney; MDR, multidrug resistance; $NaBH_4$, sodium borohydride; Na_2SO_4, sodium sulfate; *N*,*O*, amino alcohol; *N*,*N*, diamine; N-donor, nitrogen donor; Pd/C, palladium on carbon; R_f, retention factor; Ru(II), ruthenium(II); SAR, structure activity relationship; SI, selectivity index; $SOCl_2$, thionyl chloride; TIQ, 1,2,3,4-tetrahydrisoquinoline; TBDMS, *tert*-butyl dimethyl silyl; TEA, triethylamine; μM, micro molar; ppm, part per million.

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of phenanthroline, pyridine and imidazole [10]. Ligands featuring at least one NH moiety in Ru(II) anticancer complexes facilitate an effective interaction with DNA through hydrogen bonding [11,12]. These complexes have different DNA-binding modes to that of cisplatin and have exhibited excellent activity in cisplatin-resistant cancer systems both *in vitro* and *in vivo*. Given the pharmaceutical properties, of TIQ ligands, the N-donor properties as well as the effectiveness of Ru(II) complexes, it is therefore hypothesised that Ru(II)- amino alcohol TIQ ligands could potentially display interesting anticancer activity.

TIQ compounds isolated from natural sources possess a basic heterocyclic nitrogen structure and are classified as alkaloids [13]. Saframycin, naphthyridinomycin and quinocarcin are examples of this family [13]. The isolation of naphthyridinomycin alkaloid TIQ's lead to the discovery that they rendered antitumor activities [14]. Due to this recognition medicinal chemists have been inspired to synthesize further TIQ compounds in order to obtain an increased number of novel medicinal agents. Synthetic TIQ derivatives have been found to exhibit interesting biological activities [15] including histidine H3 antagonism [16], antidiabetic activity [17], and multidrug resistance (MDR) reversal for certain cancers [18]. Several other studies have shown the outstanding antitumor activity of novel synthetic TIQ derivatives [15,19]. These remarkable properties suggested that incorporation of the TIQ moiety as a backbone in metal complexes could be a viable anticancer drug discovery strategy. This has indeed already been reported by Steglich and co-workers who employed a range of racemic platinum *N*,*N* TIQ complexes in 1999 against L1210 murine leukemia cells showing increased activity *versus* cisplatin [20]. Further to that Kuo *et al*., used a similar array of racemic platinum *N*,*N* TIQ derivatives and demonstrated good activity against the tumour cell lines MCF, Hepa59T, WiDr and HeLa [21]. More recently Liu and co-workers demonstrated a SAR with diastereomerically pure platinum *N*,*N* TIQ complexes that were active against MCF-7, HCT-8, BEL-7402, A2780, HeLa, A549 and BGC-823 [22]. None of these reports included any toxicity experiments with normal cells.

As far as we can ascertain no equivalent ruthenium TIQ based complexes have been reported. Bearing that in mind, with the paucity of *N*,O ligands and the fact that the synthesis of these molecules would be facile compared to the *N*,*N* species we decided to investigate this family of molecules as potential ruthenium centred active agents. Based on the understanding that ruthenium complexes have been reported to offer a different mechanism compared platinum examples we undertook to synthesise both diastereomers of the envisaged TIQ ligands.

2. Materials and methods

Dulbecco's minimum essential medium (DMEM), Roswell Park Memorial Institute (RPMI 1640), penicillin/streptomycin mixture, trypsin-versene mixture and phosphate buffer saline (PBS) were purchased from Lonza. Heat-inactivated foetal bovine serum (FBS) was obtained from Invitrogen. Tissue culture treated flasks (25 mL and 75 mL) were purchased from Corning Costar. Cell star 96-well, flat bottom tissue culture plates were bought from Greiner Bio-one. Cryopreservation of cells was performed using a Nalgene cryo-freezing container using 2 mL cryovials obtained from Greiner Bio-one. Cell counting was done on Invitrogen Countess automated cell counter. A Bright-Line hemacytometer from Hausser Scientific and an Olympus CKX41 microscope were used for manual cell counting. Cytotoxicity was assessed using the CellTiter 96 one solution cell proliferation assay from Promega and absorbance readings for the MTS assay was performed using an Automated Microplate Reader (ELx800) from Bio-Tek Instruments.

2.1. Tissue culture

Details provided in the Supplementary information section.

2.2. MTS assay

This procedure was adapted from the manufacture's instruction [23], as well as from literature [24,25]. Fully constituted RPMI 1640 supplemented with 10% (v/v) FCS was used for the MTS assay and shall be referred to as RPMI 1640 in the rest of the paper. The cells were trypsinized as described above and resuspended in RPMI 1640.

The cells were counted and plated into 96-well tissue culture plated at density of 5×10^4 and incubated at 37 °C for 6 h to allow attachment of the cells to the tissue culture wells.

After the incubation period, the cells were treated with respective concentrations of prepared samples from 5000 M stock solution as per protocol. Dilutions were performed with sterile water and media. The treated cells were incubated for 42 h at 37 °C. After this period, 15 μL of the MTS solution was added to each well and the plate incubated at 37 °C for 3 h. The optical density (OD) was measured at 490 nm. Each sample concentration was run in four replicates, of which the average and standard deviations were calculated.

To ensure that the test protocol and technique was efficient, the sensitivity of the cells to cadmium chloride was determined, and used as a positive control in all assays.

3. Results and discussion

3.1. Synthesis of TIQ ligands and coordination to Ru metal

Currently there are numerous routes available for preparing these compounds but the basic principle in most examples involves intramolecular cyclisation, since TIQ compounds are heterocyclic [15]. Classical routes for assembling TIQ compounds are the Bischler–Napieralski, the Pomeranz–Fritsch or Pictet–Spengler reaction [26]. Chiral TIQs are of interest in pharmaceutical industry due to their application as intermediates in the manufacturing of numerous alkaloids [26]. The synthesis of enantio- or diastereomerically pure TIQs involves the use of amino acids or a chiral starting material with known stereochemistry.

The synthesis was initiated with the use of commercially available phenylalanine (**1**) and L-dopamine (**2**) (Scheme 1). Potential racemisation of this position was avoided through the choice of conditions and reagents used for each subsequent reaction. A Pictet–Spengler reaction [27] was carried out to cyclise the compounds into the TIQ backbone, using an aldehyde in an acidic solution. An imine intermediate is formed from the condensation of formaldehyde with the amine of phenylalanine. This intermediate then undergoes nucleophilic attack from the aromatic ring to give the cyclised product **3** under acidic conditions. The ester group was formed simply by treating the carboxylic group with $SOCl_2$ in methanol to give solid **4a** in quantitative yields (Scheme 1).

To make **4b**, starting material L-dopamine (**2**) was cyclised to give **5** through a Pictet–Spengler reaction. The dimethoxy and ester groups in **6** were introduced by first protecting the secondary amine with Cbz-Cl followed by subsequent *in situ* conversions of the free alcohol and acid with Me_2SO_4 in acetone under reflux conditions (Scheme 1). The TIQ ester **4b** was finally obtained after deprotection of the Cbz group with Pd/C under 1 atm of H_2 gas.

The preparation of the third ligand **4c** involved the protection of the phenolic groups. The starting material **7** was obtained from **5** after esterification using $SOCl_2$. TBDMS was chosen as a suitable protection group [28]. The attachment of this group to the starting