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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

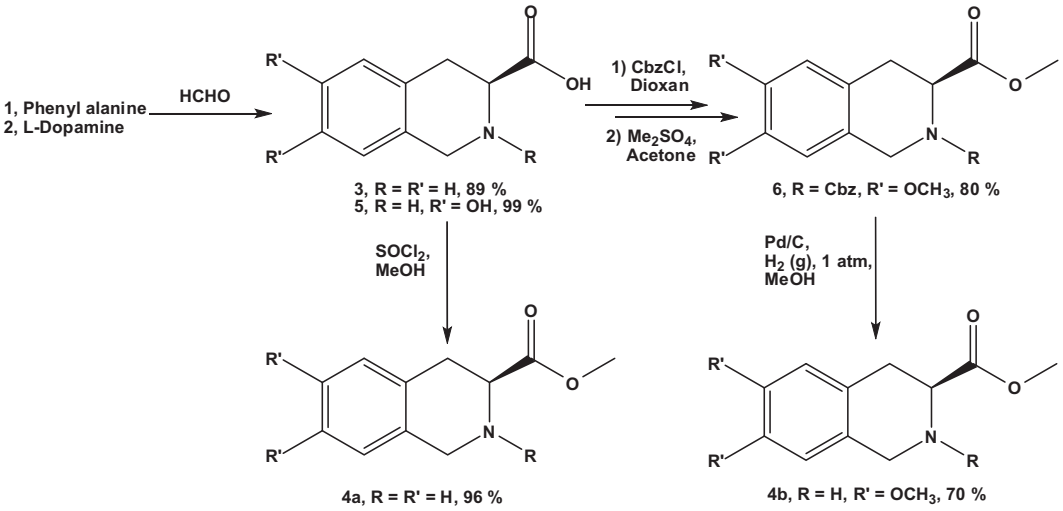
3.1. Synthesis of TIQ ligands and coordination to Ru metal

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



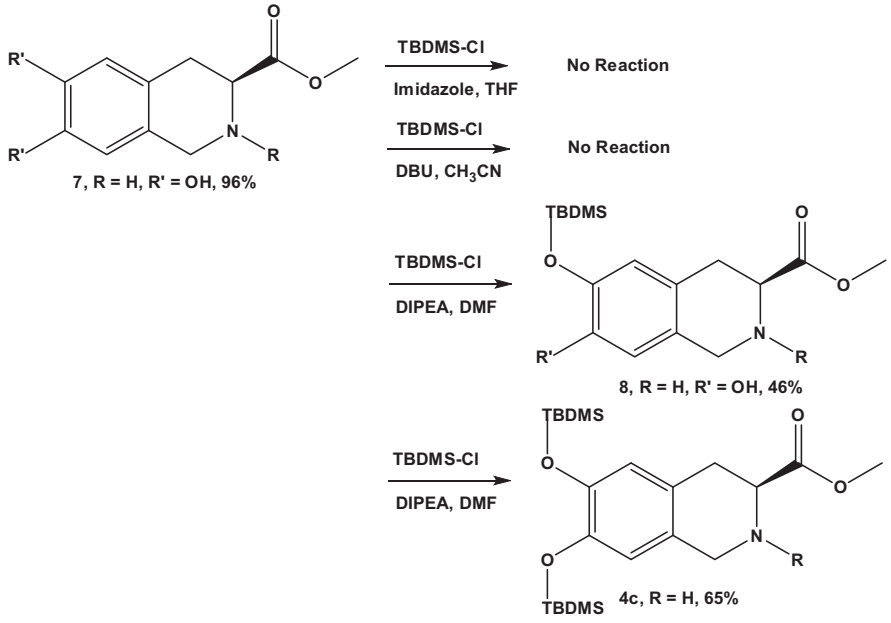
Scheme 1. Synthesis of the TIQ amino ester **4a** and **4b**.

material was complicated and numerous reaction conditions were attempted to ensure optimum results (Scheme 2). Bases such as imidazole and DBU did not lead to the desired product. The reaction was only successful with DIPEA and DMF as the solvent under dry conditions. Initially only the monosubstituted TBDMS derivative **8** was obtained. An increased concentration of DIPEA led to the desired disubstituted TBDMS TIQ ester **4c** with a small fraction of **8**. The desired product **4c** was obtained after purification with column chromatography.

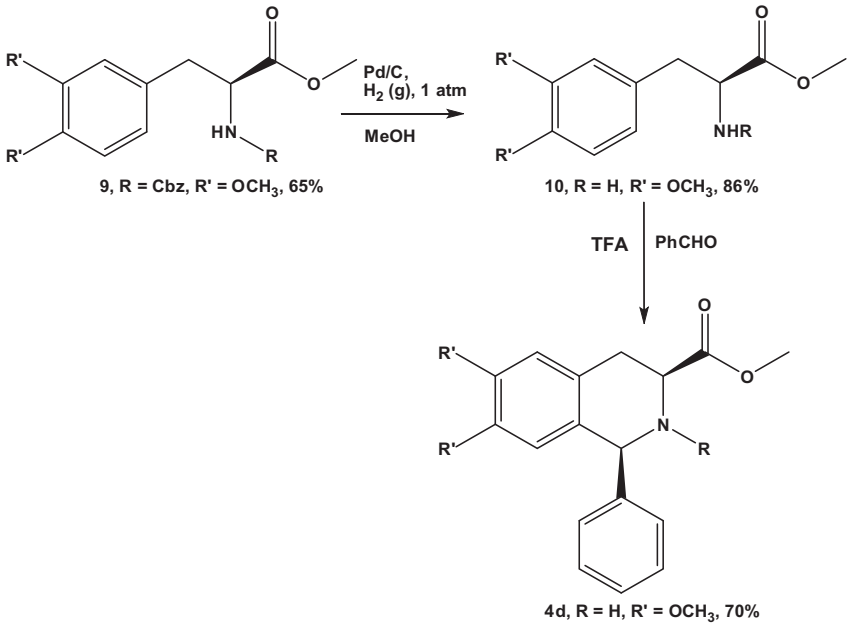
As mentioned earlier Liu and co-workers synthesised and tested diastereomeric platinum *N,N* TIQ complexes. This was achieved with the introduction of a phenyl group at C-1 creating a second chiral centre [22]. We decided to synthesise both diastereomers. Therefore, efficient methods were required to, in turn, obtain each diastereomer in excess. The polarity of the solvent has been reported to have an effect on the selectivity of either the *cis*- or *trans*-phenyl substituent in Pictet–Spengler cyclisation reactions [29]. The approach for preparing the *cis*-isomer **4d** was adjusted from literature in order to obtain higher yields (Scheme 3) [30]. The synthesis involved the preliminary Cbz protection of the secondary amine group and methylation of the phenolic groups on the L-dopamine (**2**) precursor to give **9**. Subsequently the Cbz group was removed leading to **10**. Cyclisation in TFA resulted in excess formation of the *cis*-isomer (75%).

Subsequently the synthesis of **4e** was carried out similar to the preparation of **4b** (Scheme 4). L-dopamine was treated with benzaldehyde in the presence of K_2CO_3 and aqueous ethanol to afford the *trans* substituted derivative **11**. Compound **11** was *N*-protected with benzyl chloroformate in dioxane. Methylation at phenolic and carboxylic acid position with Me_2SO_4 and $KHCO_3$ in acetone produced **12**. Deprotection of Cbz group of **12** furnished amino ester **4e**.

In order to obtain the final complexes the ruthenium dimer [(η^6-*p*-cymene)$_2$Ru$_2$(Cl$_2$)$_2$], was reacted with two mole equivalents of the TIQ amino alcohols in dry DCM and TEA, required for the



Scheme 2. Synthesis of the TIQ amino ester **4c**.



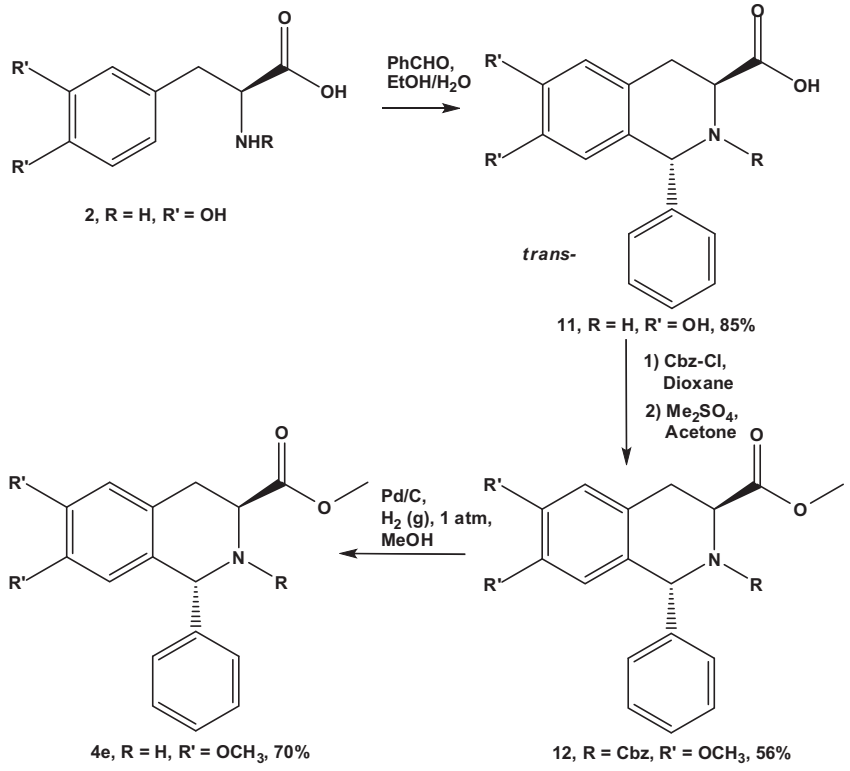
Scheme 3. Preparation of the *cis*-isomer TIQ amino ester **4d**.

deprotonation of the hydroxyl group to give neutral complexes, **C1**–**C5** (Scheme 5) [31,32]. These crude complexes were tested for anticancer activity after showing to be pure by mass spectrometry.

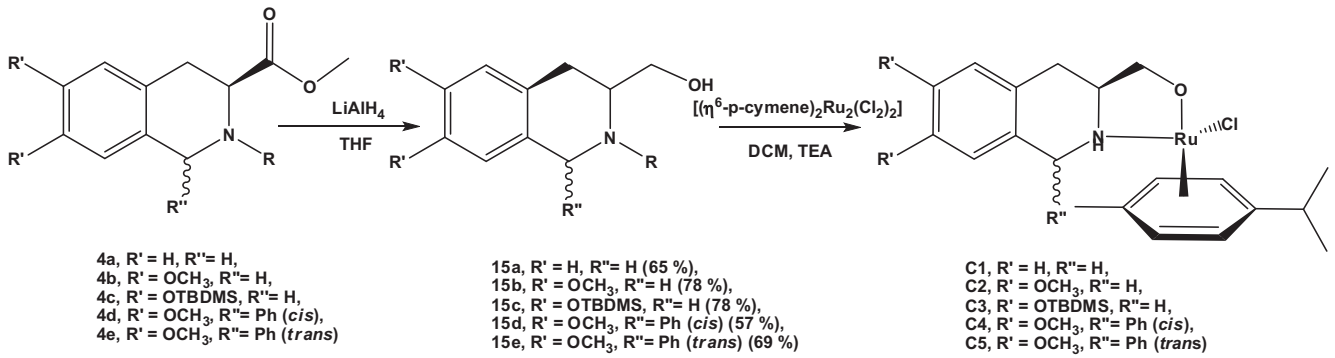
3.2. Characterization of the Ru complexes

The final complexes were characterized using, LC-MS and HRMS. NMR was used to characterise complex **C4**. The complexes **C1**–**C5** were air stable, bright orange to reddish solids and were water soluble, with the exception of the TBDMS derivative **C3** which was only slightly water soluble and was a dark brown solid.

The NMR spectroscopic data indicates the coordination of the ligand **C4** to the metal precursor in a 1:1 ratio (see the TIQ and aromatic integration). The *p*-cymene ring protons appear as broad signals in the region of 5.1–5.6 ppm (a slight downfield shift when compared to the free metal precursor), which integrate, to four



Scheme 4. Preparation of the *trans*-isomer TIQ amino ester **4e**.



Scheme 5. Synthetic route of the TIQ amino alcohols through $LiAlH_4$ reduction and coordination of it to ruthenium centre to give neutral complexes.

protons. The remaining protons that also exhibit a downfield shift in the region of (5.0–7.6 ppm) correspond to the H1, H4, H5 and the aromatic protons of one equivalent of the TIQ ligand based on integration (Fig. 1). The *HC*(Me)$_2$ and the isopropyl protons (HC(C*H*$_3$)$_2$) of the *p*-cymene ring occur at the expected 3.0 ppm and 1.3 ppm respectively. The methyl group of the *p*-cymene occurs at 2.2 ppm and integrates to three protons. In the ^{13}C spectrum the *p*-cymene signals are observed at 18.4, 22.3, 30.1, 79.6, 81.6, 94.2 and 104.6 ppm. All of which appear to be shifted from the uncomplexed precursor.

3.3. Biological testing of Ru(II)–TIQ complexes

C1–**C5** were screened for anticancer activity against the cancerous MCF-7 cell line [33] (human breast epithelial adenocarcinoma) and normal cell line MDBK [34] (bovine kidney epithelial). In addition, **C4** was screened against other cancerous cells lines, namely A549 [35] (human lung epithelial adenocarcinoma) and invasive MDA-MB-231 [36] (human breast epithelial adenocarcinoma) cell lines. The cells were exposed to varying concentrations of these novel ruthenium complexes for 42 h. Thereafter cytotoxicity was evaluated using the MTS antiproliferate assay [23]. The IC_{50} values were estimated by extrapolation from the profiles and are listed in Table 1. Cadmium chloride ($CdCl_2$) was used as a positive control.

Generally all complexes displayed dose dependent cytotoxic activities against the MCF-7 breast cancer cell line. This series of complexes would be thus considered in general moderately active against the MCF-7 cells. The unsubstituted TIQ complex **C1** gave an IC_{50} value of approximately 54 μM. Complexes **C2** and **C3** (ether derivatives) exhibited values of 60 and 68 μM respectively.

The incorporation of a phenyl ring at C-1 of the TIQ skeleton in the *trans*-phenyl ligand (**C5**) essentially negated any of the previous activity of the complexes (218 μM). Importantly however for the *cis* phenyl compound (**C4**) an increase in activity was observed giving an IC_{50} of 34 μM. This appears to be the first example of a ruthenium based complex whereby the diastereomeric nature of the ligand has such a profound effect on the activity of the complex. In contrast to the MCF-7, all of the complexes were inactive against the A549 and MDA-MB-231 cancer cell lines. The inactivity of similar ruthenium complexes against these cell lines has been observed before [37].

The selective nature of these complexes towards the cancerous cell line (MCF-7) was established by performing comparative cytotoxic assays with the normal MDBK cell line. From the study relatively low activity (independent of dosage) was evident against the MDBK cells. Although these complexes presented moderate activity against the MCF-7 cells, it was interesting to note that the majority displayed much lower activity against the MDBK cells (Table 1) [38].

An SI value of greater than three has been considered by recent reports extremely selective [38]. In general this group of complexes display the same trend of activity with an SI greater than 3 with few exceptions; therefore they seem to show promise as selective anticancer agents.

It has been reported that the activities for metal complexes are derived from the unique properties of the complexes themselves and not the individual components [39]. To ensure that this is also true for our species, the activity of the free ligand from the most active complexes (**C4**) and the ruthenium precursor were individually screened for activity against the MCF-7 and MDBK cell lines. Both candidates were inactive against the MCF-7 and MDBK cell lines at a 250 μM concentration.

The activity of these Ru-TIQ complexes against the MCF-7 could be potentially important as a large percentage of the woman diagnosed annually for cancer have this type of the disease [40]. Previous studies have reported that the MCF-7 cell line is relatively resistant to chemotherapeutic agents [41].

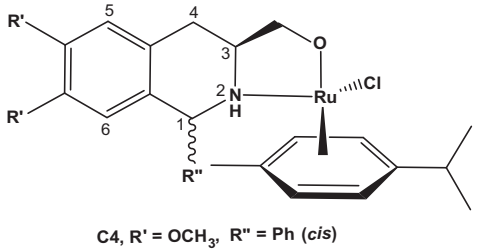


Fig. 1. Structure of complex C4.

Table 1
IC_{50} (μM) activities and SI values for complexes **C1**–**5** against MCF-7 and MDBK cell lines.

Ru complex	MCF-7 (μM)[a]	MDBK (μM)	SI[b]
C1	54 ± 5.36	>250	>5.0
C2	60 ± 4.56	>250	>4.2
C3	68 ± 3.33	>250	>3.6
C4	34 ± 0.29	>500	>2.3
C5	218 ± 2.15	>250	>7.4
Cisplatin	67 ± 5.00	41 ± 3.00	

[a] Standard deviation calculated from triplicate measurements.
[b] SI = MDBK IC_{50} ÷ MCF-7 IC_{50}.

4. Conclusions

Novel organometallic Ru(II) complexes bearing amino alcohol TIQ ligands were successfully prepared and tested for anticancer activity. These complexes exhibited moderate activity against MCF-7 cancer cells with IC_{50} values ranging from 34 to 218 μM. Complex **C5** gave the lowest activity and **C4** the highest. This was surprising as the compounds differ by only one diastereomeric centre. The complexes were inactive against the A549 and MDA-MB-231 cell lines. The most interesting result observed was the remarkable selectivity displayed for MCF-7 cells in comparison to normal MDBK cells. Our results indicate that ruthenium *N,O*-complexes are capable of effective activity and are more selective than reported platinum based drugs.

5. Experimental

All reagents and solvents were purchased from Sigma–Aldrich, Merck and Fluka, except if stated otherwise. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 400 MHz instrument, the ^{1}H NMR spectrum was recorded at 400.222 MHz and the ^{13}C NMR spectrum was recorded at 100.635 MHz. Chemical shifts are reported in ppm, referenced to the solvent used, $CDCl_3$, MeOD, D_2O and D_6-DMSO [42]. Coupling constants are expressed in Hz. NMR spectra were obtained at room temperature. Infrared (IR) spectra were obtained on a Perkin Elmer Spectrum 100 instrument with an ATR attachment. Optical rotations were carried out on a Perkin Elmer 341 polarimeter. Liquid chromatography mass spectroscopic LC-MS data were obtained from a Shimadzu LC-MS 2020 with solvent A [0.1% FA in H_2O] and solvent B (0.1% FA in acetonitrile). HRMS were determined using a Bruker ESI-QTOF mass spectrometer in positive mode. Microwave reactions were performed with a Discovery CEM Liberty automated microwave synthesizer. Some of the diamine ligands were purified using a C-18 reverse phase semi-preparative HPLC on LC-8A Shimadzu with 0.1% FA in H_2O as solvent A and 0.1% FA in methanol as solvent B as eluents. Thin layer chromatography (TLC) was carried out using Merck Kieselgel 60 F254 and all crude compounds were purified via column chromatography using silica gel 60–200 mesh. Solvents were dried using standard procedures from Vogel [43].

5.1. Experimental procedures for preparation of TIQ amino esters

Synthesis of the known intermediates (**3**–**14**) and TIQ ester (**4a**,**b**,**d**,**e**) can be found in the Supplementary information section.

5.1.1. (S)-Methyl-6,7-bis(tert-butyldimethylsilyloxy)-1,2,3,4-tetrahydroisoquinoline **4c**

The experimental procedure was adapted from literature [44]. Dry DMF (21 mL) was added to dissolve **7** (3.0 g, 13.00 mmol) and crystalline solid of TBDMS chloride (5.1 g, 33.40 mmol) under argon atmosphere. Dry DIPEA (11.7 mL, 67.52 mmol) was added to the stirred solution (in portions) over a period of 5 min, and the reaction mixture was allowed to stir at room temperature for 4 h. The reaction was monitored by TLC and it was quenched by adding H_2O (30 mL). The reaction mixture was extracted with portions of EtOAc (3 × 30 mL). The organic phase was washed consecutively with 10% $NaHCO_3$ (20 mL) and H_2O (20 mL). The organic phase was dried over K_2CO_3 and the solvent was evaporated *in vacuo* to give mixtures of crude **4c** and **8** mixtures. The product was purified by column chromatography using 20% EtOH in toluene to give **4c** as a viscous brown material. Compound **4c** was obtained in 65% yield; R_f = 0.51 (8:2 toluene:ethanol); $[\alpha]_D^{20}$ − 29.50 (c = 1.00, in $CHCl_3$); ν_{max} (neat)/cm^{-1} 2929, 2857, 1740, 1512, 1252, 835 and 779 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) 0.19 (s, 12H), 0.97 (s, 18H), 2.85 (s, 1.5H), 2.98 (s, 3H), 3.20 (dd, J = 5.0, 17.0 Hz, 1H), 3.29 (d, J = 3.2 Hz, 1H), 3.59 (q, J = 7.0 Hz, 1H), 3.62 (dd, J = 5.0, 11.5 Hz, 1H), 4.22 (s, 2H); 6.49 (d, J = 13.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) −4.05, 18.37, 25.96, 29.61, 31.64, 41.27, 45.01, 46.88, 50.22, 52.09, 55.40–55.95, 112.15, 115.36, 118.22, 121.13, 124.40–129.00, 145.25, 162.83, 173.56; HRMS m/z = 452.2714 $[M + H]^+$ for $C_{23}H_{41}NO_4Si_2$.

5.1.2. (S)-Methyl 6-(tert-butyldimethylsilyloxy)-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate **8**

Compound **8** was obtained in 27% yield; R_f = 0.4 (8:2 toluene:ethanol)); ^{1}H NMR (400 MHz, $CDCl_3$) 0.15 (d, J = 1.75 Hz, 6H), 0.91 (s, 9), 2.72 (d, J = 10.2 Hz, 1H), 2.84-2.89 (m, 2H), 3.59 (dd, J = 4.64, 10.2 Hz, 1H), 3.67 (s, 3H), 3.89 (d, J = 8.2 Hz, 2H); 6.45 (q, J = 19.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) −4.48, 18.12, 25.86, 30.95, 47.04, 51.93, 56.00, 112.00, 115.06, 118.11, 124.42, 126.58, 128.89, 145.60, HRMS m/z = 338.1787 $[M + H]^+$ for: $C_{17}H_{27}NO_4Si$.

5.2. General procedure for the preparation of ***15a–e***

The experimental procedure was adapted from literature [45,46]. A solution of TIQ ester **4a**–**e** (2.0 g) was added dropwise to a stirred suspension of $LiAlH_4$ (4 mol equiv) in cooled (0 °C in ice) dry THF (80 mL) under argon gas flow. The reaction mixture was stirred at 0 °C for 1.5 h and then at room temperature for 2 h. The reaction was monitored with TLC (7:3 hexane:ethyl acetate) to confirm the completion of the reaction. After that, THF (20 mL) was added to dilute the reaction mixture and excess $LiAlH_4$ was decomposed by the dropwise addition of saturated Na_2SO_4 at 0 °C. The inorganic salts were filtered and washed with portions of EtOAc (3 × 20 mL). The organic filtrate was dried over $MgSO_4$ and concentrated *in vacuo* to give product as a yellow solid.

5.2.1. (S)-1,2,3,4-Tetrahydroisoquin-3-yl methanol **15a** [47]

The compound **15a** was prepared from **4a**, according to general procedure **B**, in 65% yield, mp 113–115 °C (93–96 °C) [47]; ν_{max} (neat)/cm^{-1} 3280, 3245, 3047, 2801 1499, 1451, 1058, 1001 and 736 cm^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$) 2.56 (dd, J = 10.6, 26.7 Hz, 1H), 2.69 (dd, J = 4.3, 21.3 Hz, 1H), 3.08 (m, 1H), 3.52 (dd, J = 7.9, 10.9 Hz, 1H), 3.78 (dd, J = 3.8, 10.9 Hz, 1H), 4.04 (s, 2H), 6.91-7.01 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) 31.95, 56.55, 66.32, 126.95, 127,29, 127.41, 130.23, 135.09, 135.91; HRMS m/z = 164. $[M + H]^+$ for: $C_{10}H_{13}NO$.

5.2.2. (S)-6,7-Dimethoxy-1,2,3,4-tetrahydroisoquin-3-yl methanol **15b** [48]

The compound **15b** was prepared from **4b**, according to general procedure **B**, in 78% yield, mp 109–113 °C (130–134 °C) [48]; $[\alpha]_D^{20}$ + 21.85 (c = 1.00, in $CHCl_3$), ν_{max} (neat)/cm^{-1} 3258, 3130, 2937, 2834, 1610, 1518, 1463, 1227, 1075 and 853 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) 2.53 (dd, J = 11.1 Hz, 1H), 2.65 (dd, J = 4.1 Hz, 1H, 2.96-3.00 (m, 1H), 3.54 (dd, J = 7.5, 10.9 Hz, 1H), 3.70 (dd, J = 4.2, 10.9 Hz, 1H), 3.81 (d, J = 2.2 Hz, 6H), 3.83 (d, J = 2.2 Hz, 1H), 3.97 (d, J = 4.0 Hz, 2H), 6.61 (d, J = 18.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 30.02, 46.90, 55.11–55.31, 64.86, 109.39, 112.12, 125.77, 126.48, 131.01, 147.41; HRMS m/z = 224.1341 $[M + H]^+$ for $C_{12}H_{17}NO_3$.

5.2.3. (S)-6,7-Bis(tert-butyldimethylsilyloxy)-1,2,3,4-tetrahydroisoquin-3-yl methanol **15c**

The compound **15c** was prepared from **4c**, according to general procedure **B**, in 78% yield, mp 111–115 °C; $[\alpha]_D^{20}$ − 43.85 (c = 0.13, in $CHCl_3$) ν_{max} (neat)/cm^{-1} 3394, 3184, 2929, 2856, 1513, 1317, 1249, 879, 833, 778; ^{1}H NMR (400 MHz, $CDCl_3$) 0.17 (s, 12H), 0.97 (s, 18H), 2.42–2.45 (m, 1H), 2.57 (dd, J = 4.43, 7.01 Hz, 1H), 3.01–3.04 (m, 1H), 3.48 (q, J = 7.8 Hz, 1H), 3.69 (q, J = 3.4 Hz, 1H), 3.90 (s, 2H), 6.50 (d, J = 22.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$)−4.01, 18.48, 25.97, 30.30, 47.49, 55.20, 65.96, 118.19, 121.37, 126.25, 128.28, 145.23; HRMS m/z = 424.2574 $[M + H]^+$ for $C_{22}H_{41}NO_3Si_2$.

5.2.4. [(1S,3S)-6,7-Dimethoxy-1-phenyl-1,2,3,4-tetrahydroisoquinolin-3-yl]methanol **15d**

The compound **15d** was prepared from **4d**, according to general procedure **B**, in 69% yield, mp decompose > 164 °C (172–175 °C) [48]; $[\alpha]_D^{20}$ − 15.82 (c = 0.95, in MeOH), ν_{max} (neat)/cm^{-1} 3255, 2918, 1609, 1257, 1074, 1028, 700; ^{1}H NMR (400 MHz, $CDCl_3$) 2.99 (d, J = 4.4 Hz, d, J = 11.4 Hz, 1H), 3.03-3.14 (m, 1H), 3.52 (s, 3H), 3.68-3.71 (m, 1H), 3.76 (q, J = 6.72 Hz, 1H), 3.84 (s, 3H), 3.89 (2 × d, J = 3.9 Hz, 1H), 5.57 (s, 1H), 6.16 (s, 1H), 6.84 (s, 1H), 7.48 (broad s, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 29.73, 56.35, 58.53, 63.31, 63.58, 112.08, 112.87, 126.43, 130.24, 130.72, 131.00, 138.94, 149.40, 150.65; HRMS m/z = 300.1613 [M + H] $^+$ for: $C_{18}H_{21}NO_3$.

5.2.5. [(1R,3S)-6,7-Dimethoxy-1-phenyl-1,2,3,4-tetrahydroisoquinolin-3-yl]methanol **15e** [29]

The experimental procedure was adapted from literature [49]. A stirred solution of **4e** (3.1 g, 9.54 mmol) in dry THF (85 mL) $NaBH_4$ (4.4 g, 116.40 mmol) added under argon gas flow. The temperature was increased to 65 °C and dry methanol (40 mL) was added dropwise. The reaction was stirred for 5 h, monitored by LC-MS. After cooling, the reaction was quenched with 2N HCl (35 mL), the precipitate formed was filtered and the filtrated dried over $MgSO_4$ and concentrated *in vacuo* to yield **15e** in 57% yield; mp 113–115 °C (113–117 °C) [29]; $[\alpha]_D^{20}$ − 40.47 (c = 0.92, in MeOH) ^{1}H NMR (400 MHz, MeOD) 2.48 (dd, J = 10.6, 16.2 Hz, 1H), 2.71 (dd, J = 4.2, 16.2 Hz, 1H), 2.99–3.02 (m, 1H), 3.36 (dd, J = 8.0, 10.6 Hz, 1H), 3.52 (dd, J = 4.5, 10.9 Hz, 1.1H), 5.14 (s,1H), 6.72 (s, 1H), 7.07 (d, J = 7.2 Hz, 2H), 7.18 (d, J = 7.2 Hz, 1H), 7.22 (d, J = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, MeOD) 31.57, 49.83, 56.40, 56.46, 60.28, 66.22, 112.71, 113.10, 128.31–129.91, 145.81, 148.93, 149.70; HRMS m/z = 300.1598 [M + H]$^+$ for: $C_{18}H_{21}NO_3$.

5.3. Coordination of TIQ ligands to Ru(II) centre

5.3.1. General procedure for preparing Ru(II) complexes with amino alcohol ligands, ***C1–C5***

The experimental procedure was adapted from literature [31]. TEA (1 mol equiv) was added dropwise to a solution of TIQ amino alcohol **15a–e** (1 mol equiv) in dry DCM (5 mL). The reaction mixture was stirred at room temperature for 15 min [(η^6-*p*-cymene)–$RuCl_2$]$_2$ (0.50 mol equiv) was added to the reaction mixture which was then allowed to stir for 3 h at room temperature under argon gas flow. The reaction was monitored using LC-MS the solvent evaporated under reduced pressure and residual solid was protected from light using aluminium foil. These crude complexes were screened for anticancer activity.

C1. Bright orange solid. HRMS m/z = 398.1029 [M − Cl]$^+$ for $C_{20}H_{27}ClNORu$.
C2. Reddish orange solid. HRMS m/z = 458.2941 [M − Cl]$^+$ for $C_{22}H_{31}ClNO_3Ru$.
C3. Dark brown solid. HRMS m/z = 658.3840 [M − Cl]$^+$ for $C_{32}H_{55}ClNO_3RuSi_2$.
C4. Bright orange solid. HRMS m/z = 534.3315 [M − Cl]$^+$ for $C_{28}H_{35}ClNO_3Ru$.
C5. Reddish orange solid. HRMS m/z = 534.2584 [M − Cl]$^+$ for $C_{28}H_{35}ClNO_3Ru$.

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Appendix A. Supplementary material

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