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Use of 1,2,4-triazolo[1,5-*a*]pyrimidines to design new "piano-stool" ruthenium(II) compounds

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ABSTRACT

A series of neutral half-sandwich organometallic ruthenium(II) complexes of the general formula $[(\eta^6\text{-p-cym})Ru(L)Cl_2]$, where η^6-p-cym – p-cymene, was prepared by the direct reaction of a dichloride p-cymene ruthenium(II) dimer, $[\{(\eta^6\text{-p-cym})Ru(\mu\text{-Cl})\}_2Cl_2]$, with corresponding 1,2,4-triazolo[1,5-*a*]pyrimidine ligands (L), namely, L-1,2,4-triazolo[1,5-*a*]pyrimidine (tp) (**1**), 5,7-dimethyl-1,2,4-triazolo[1,5-*a*]pyrimidine (dmtp) (**2**), 5,7-ditertbutyl-1,2,4-triazolo[1,5-*a*]pyrimidine (dbtp) (**3**), 7-isobutyl-5-methyl-1,2,4-triazolo[1,5-*a*]pyrimidine (ibmtp) (**4**), and 5,7-diphenyl-1,2,4-triazolo[1,5-*a*]pyrimidine (dptp) (**5**). The complexes were prepared in molar ratios of 1:2 (except (**1**), for which an excess of tp was used). The structures of the complexes were determined by multinuclear magnetic resonance (^1H, ^{13}C, ^{15}N) spectroscopy and X-ray diffraction. According to all the spectroscopic data, the molecules adopted the usual "piano-stool" geometry with a monodentate N3 triazolopyrimidine ligand. Studies on these lipophilic ruthenium(II) complexes revealed rather low *in vitro* antiproliferative activities against two human cell lines.

1. Introduction

Over the past 25 years, ruthenium complexes have received much attention from researchers due to their interesting chemical and biological properties. Ruthenium complexes are particularly attractive in the cancer therapy field due to three main properties: (i) rates of ligand exchange often comparable to those of platinum complexes, which can be tuned by coordinating appropriate ancillary ligands; (ii) the ability of ruthenium ions to mimic the behavior of iron when binding with certain (main) biological molecules, including serum transferrin and albumin [1–5], and (iii) under physiological conditions, the accessibility of these complexes in several oxidation states (Ru(II), Ru(III), Ru(IV)). Additionally, in biological systems, glutathione, ascorbic acid and single electron transfer proteins are able to reduce Ru(III) to Ru(II) [6].

Two ruthenium(III) complexes, NAMI-A ([ImH][*trans*-Ru(dmso)(Im)Cl_4], where Im – imidazole) and KP1019 ([IndH][trans-Ru(Ind)$_2Cl_4$], Ind – indazole) [7,8], are currently approved for phase II clinical trials. Other interesting ruthenium compounds that have attracted tremendous amounts of attention in recent years are ruthenium–arene complexes [9–12], which show considerable promise and tend to be somewhat more stable than the ruthenium(III) complexes that are currently undergoing clinical evaluations [13]. Indeed, the unique properties of organometallic compounds, with behaviors between those of classical inorganic and organic materials, provide new opportunities in applications for medicinal chemistry. Additionally, organometallic chemistry offers a potentially rich field for the development of new medicinal agents that act through novel mechanisms. Therefore, organoruthenium(II) mononuclear "piano-stool" complexes are the subject of interest in the design of metal-based complexes for use as anticancer agents [13–17].

Here, we report for the first time the formation of five p-cymene (η^6-p-cym) ruthenium(II) complexes with triazolopyrimidine of the following formulas: $[(\eta^6\text{-p-cym})Ru(tp)Cl_2]$ (**1**), $[(\eta^6\text{-p-cym})Ru(dmtp)Cl_2]$ (**2**), $[(\eta^6\text{-p-cym})Ru(dbtp)Cl_2]$ (**3**), $[(\eta^6\text{-p-cym})Ru(ibmtp)Cl_2]$ (**4**), and $[(\eta^6\text{-p-cym})Ru(dptp)Cl_2]$ (**5**) (Fig. 1).

Triazolopyrimidine derivatives are versatile heterocyclic ligands that are well known for bonding metal ions because they

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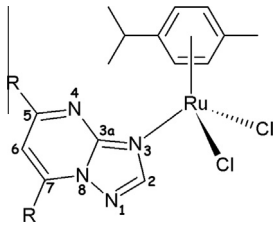

Fig. 1. General structures of [(η^6-p-cym)Ru(L)Cl$_2$] compounds with 1,2,4-triazolo [1,5-*a*]pyrimidine (L); R_1, R_2 = H (tp) (**1**); CH_3 (dmtp) (**2**); $C(CH_3)_3$ (dbtp) (**3**); C_6H_5 (dptp) (**4**); R_1 = CH_3, R_2 = C_3H_7 (ibmtp) (**5**).

contain several nitrogen atoms with accessible lone pairs [18–21]. Moreover, their versatility can be increased through ring-substitutions with functional groups that contain donor atoms such as nitrogen, sulfur or oxygen, leading to a wider range of coordination modes and structural topologies in their metal complexes [22–26]. Triazolopyrimidines have gained great attention as ligands due to their suitability as building blocks for the syntheses of novel metal-organic multidimensional systems [27–30]; in some cases, triazolopyrimidines show interesting magnetic, luminescent and biological properties [18,31,32].

2. Experimental

2.1. Starting materials

3-Amino-1,2,4-triazole (⩾95%), 1,2,4-triazolo[1,5-*a*]pyrimidine (99%), 2,4-pentanedione (⩾99%), 2,2,6,6,-tetramethyl-3,5-heptanedione (⩾98%), 1,3-diphenyl-1,3-propanedione (98%), 6-methyl-2,4-heptanedione (⩾98%), ruthenium(III) chloride hydrate and α-terpinene (85%) were purchased from Aldrich. Analytical-grade solvents were purchased from Avantor.

The key intermediates were prepared following previously published methods. A series of 5,7-disubstituted-1,2,4-triazolo[1,5-*a*] pyrimidines were synthesized by the condensation of 3-amino-1,2,4-triazole with corresponding diketones. [33]. A dimeric ruthenium(II)-arene precursor, [{(η^6-p-cym)Ru(μ-Cl)}$_2$Cl$_2$], was prepared according to the method reported by Bennett through the reaction of ruthenium(III) chloride with α-terpinene [34].

2.2. Syntheses of complexes

2.2.1. [(η^6-p-cym)Ru(tp)Cl$_2$] (**1**)

To a suspension of [{(η^6-p-cym)Ru(μ-Cl)}$_2$Cl$_2$] (0.067 g, 0.11 mmol) in isopropanol (10 mL), tp (0.053 g, 0.44 mmol) was added, and the mixture was heated to 50 °C and stirred for 4 h. After evaporation to of the reaction mixture to 5 mL, 20 mL of ethyl acetate was added. An orange precipitate formed after 1 h in the refrigerator. The product was filtered, washed with diethyl ether (2 × 10 mL) and dried under a vacuum. Yield: 0.055 g, 61%. Analysis: calc/found for (**1**) $C_{15}H_{18}N_4Cl_2Ru$ (M 426.31): C, 42.26/42.48; H, 4.26/4.25; N, 13.14/13.22.

2.2.2. [(η^6-p-cym)Ru(dstp)Cl$_2$] (**2**–**5**)

All novel Ru(II) complexes with 5,7-disubtituted-1,2,4-triazolo [1,5-*a*]pyrimidines (dstp) were obtained through reactions between [{(η^6-p-cym)Ru(μ-Cl)}$_2$Cl$_2$] and corresponding dstp ligands (dstp – dmtp (**2**), dbtp (**3**), ibmtp (**4**), dptp (**5**)) in molar ratios of 1:2 in isopropanol. The mixtures were stirred and heated at 50–60 °C for 14 (**2**), 16 (**3**), 38 (**4**), and 8 (**5**) h. Precipitates formed directly in the reaction mixtures after several hours in the refrigerator, which were then filtered, washed with diethyl ether (2 × 10 mL) and dried under a vacuum. Yield: 92% (**2**), 71% (**3**), 86% (**4**), 82% (**5**). Analysis: calc/found for (**2**) $C_{17}H_{22}N_4Cl_2Ru$ (M 454.35): C, 44.90/45.08; H, 4.80/4.75; N, 12.33/12.39, (**3**) $C_{23}H_{34}N_4Cl_2Ru$ (M 538.51): C, 51.30/51.73; H, 6.36/6.17; N, 10.40/9.97, (**4**) $C_{20}H_{28}N_4Cl_2Ru$ (M 496.43): C, 48.40/48.93; H, 5.70/5.86; N, 11.28/10.82, (**5**) $C_{27}H_{26}N_4Cl_2Ru$ (M 578.49): C, 51.30/51.73; H, 6.36/6.17; N, 10.40/9.97.

2.3. Instrumentation

The contents of C, H, N were determined on an ELEMENTAR Analysensysteme GmbH Vario MACRO CHN analyzer. The NMR spectra were recorded at 298 K in $CDCl_3$ on a Varian INOVA 500 spectrometer, operated at 499.8, 125.7 and 50.6 MHz for ^{1}H, ^{13}C and ^{15}N, respectively. The external reference standard was TMS for ^{1}H and ^{13}C and CH_3NO_2 for ^{15}N. The ^{15}N NMR spectra were recorded in inverse gated decoupling mode using a 30 flip angle, a spectral range of 18 kHz, an acquisition time of 0.2 s, a relaxation delay of 5 s and 60 k data points. The ^{1}H–^{15}N correlation spectra (HMBC) were optimized for a coupling constant of 8 or 1 Hz with the following experimental conditions: an acquisition time of 0.3 s, spectral windows of 5000 (F2) and 15 000 Hz (F1), 4096 data points, 512 time increments (zero filled to 2048), a 1.4 s relaxation delay and 64 transients per increment.

2.4. X-ray structure determination

Brown crystals of [(η^6-p-cym)Ru(dmtp)Cl$_2$] (**2**) and [(η^6-p-cym)Ru(dptp)Cl$_2$] (**5**) were obtained from a methylene chloride:methanol 1:1 (v/v) solution. Complex [(η^6-p-cym)Ru(ibmtp)Cl$_2$] (**4**) was crystallized from a methylene chloride:ethanol 1:1 (v/v) solution. All reported complexes crystallized in the triclinic $P\bar{1}$ space group. Diffraction data were collected on an Oxford Sapphire CCD diffractometer with Mo Kα radiation. The structures were solved by direct methods and refined with the full-matrix least-squares method on F^2 using a SHELX-97 program package [35]. An analytical absorption correction was applied [36] with maximum and minimum transmissions of 0.8843 and 0.5257, 0.9617 and 0.7310, as well as 0.8584 and 0.7316 for (**2**), (**4**) and (**5**), respectively. The hydrogen atoms were located based on the differences in electron density maps and were constrained during refinement. The structural data have been deposited to the Cambridge Crystallographic Data Centre, with CCDC deposition numbers of 1404279 (**2**), 1404280 (**4**) and 1404281 (**5**). The crystal structure data for the three reported complexes are summarized in Table 1.

2.5. Cell growth inhibition assay

The *in vitro* antiproliferative activities of the heterocycle ligands and their ruthenium(II) complexes against two human tumor cell lines, A549 (non-small cell lung carcinoma) and T47D (breast cancer), were investigated. Cells were plated in 96-well sterile plates at a density of 10^4 cells per well (in 100 μL of culture medium) and incubated for 24 h. A suitable amount of each compounds was added to generate concentrations ranging from 0.1 to 100 μg/mL. The samples were then incubated for an additional 72 h. Cisplatin was used as a reference material. The *in vitro* cytotoxic activities of the complexes are expressed as IC_{50} values (the dose of compound (μM) that inhibits the proliferation rate of the tumor cells by 50%) compared with untreated control cells. The reported IC_{50} values are the average of 3–4 independent determinations. The details of this technique have been previously described by Skehan [37]. Analyses of the *in vitro* antiproliferative activities of the evaluated compounds against the adherent cell lines were performed using sulforothamine B (SRB) assays and an