Full paper

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Synthesis, characterization and catalytic, cytotoxic and antimicrobial activities of two novel cyclotriphosphazene-based multisite ligands and their Ru(II) complexes

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Two novel cyclotriphosphazene ligands (2 and 3) bearing 3-oxypyridine groups and their corresponding Ru(II) complexes (4 and 5) were synthesized and their structures were characterized using Fourier transform infrared, ^{1}H NMR and ^{31}P NMR spectroscopic data and elemental analysis. The Ru(II) complexes were used as catalysts for catalytic transfer hydrogenation of *p*-substituted acetophenone derivatives in the presence of KOH. Additionally, the cytotoxic activities of compounds 2–5 were evaluated against PC3 (human prostate cancer), DLD-1 (human colorectal cancer), HeLa (human cervical cancer) and PNT1A (normal human prostate) cell lines. Finally the antimicrobial activities of compounds 2–5 were evaluated against a panel of Gram-positive and Gram-negative bacteria and yeast cultures. The complexes showed efficient catalytic activity towards transfer hydrogenation of acetophenone derivatives, especially those bearing electron-withdrawing substituents on the *para*-position of the aryl ring. The compounds were found to have moderate to high cytotoxic and antimicrobial activities, and Ru(II) complexation enhanced both cytotoxic and antimicrobial activities in comparison with the parent compounds. Copyright © 2015 John Wiley & Sons, Ltd.

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Keywords: cyclotriphosphazene; Ru(II) complex; transfer hydrogenation; cytotoxicity; antimicrobial activity

Introduction

The use of cyclophosphazenes as ligands to transition metals has been attracting the attention of researchers due to their reactive periphery and robust skeleton which provide a perfect platform to build various types of multisite coordination ligands. Their versatile structure and coordination ability allow the construction of new macrocyclic ligands. Pyridyloxycyclophosphazenes are multisite coordination ligands where the coordinating pyridine ligand is attached to the phosphorus atom of the cyclophosphazene ring.[1–5] When these ligands are combined with transition metals or main group metals, a number of different complexes, which exhibit a rich diversity and potential chemical and biological activities, can be readily prepared.

Ru(II) metal-based catalytic conversion of primary and secondary alcohols into their corresponding aldehydes and ketones is an essential reaction in organic synthesis.[6–9] Much research has been aimed at the design of good Ru(II) catalysts for the transfer hydrogenation of ketones.[10–15] To the best of our knowledge, no study has so far dealt with the catalytic activities of Ru(II) complexes of cyclotriphosphazene ligands in transfer hydrogenation of ketones. Recently, we have shown that pyridyloxy-substituted phosphazene derivatives and their Ru(II) complexes have good catalytic activities.[16,17] In the study reported here, we synthesized two novel cyclotriphosphazene ligands (**2** and **3**) containing 3-oxypyridine groups and their corresponding Ru(II) complexes (**4** and **5**) and evaluated the catalytic activity of the complexes in transfer hydrogenation of ketones.

Having more than 200 types, each with different causes, symptoms and treatments, cancer is one of the major causes of global deaths. According to the World Health Organization, cancer caused 7.6 million deaths in 2008[18] and the death toll reached 8.2 million in 2012.[19] Despite rapid technological and medical developments to fight cancer, the number of deaths caused by cancer keeps increasing each year. Even though various chemotherapeutics are employed in the treatment of cancer patients, almost all of these drugs cause severe side effects and their intended effectiveness either fails or greatly diminishes as a consequence of continued

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administration of the drug. This keeps the door open for the development of new drugs with improved efficiency and minimum side effects. Cyclotriphosphazene derivatives have long been attracting attention as potential anti-cancer agents.[20–23] Cyclotriphosphazenes are a unique group of inorganic ring systems and their chemical, physical and biological properties vary depending on the substituents they bear. With this fact in mind, we also investigated the cytotoxic activities of the cyclotriphosphazene ligands **2** and **3** and their Ru(II) complexes **4** and **5** against PC3 (human prostate cancer), DLD-1 (human colorectal cancer), HeLa (human cervical cancer) and PNT1A (normal human prostate) cell lines. Finally the antimicrobial activities of compounds **2–5** were evaluated against a panel of Gram-positive and Gram-negative bacteria and yeast cultures.

Experimental

Materials and methods

All chemicals for the synthesis and the solvents used were of analytical grade quality from commercial sources and were used without further purification.

FT-IR spectra were recorded as pressed KBr discs, using a PerkinElmer FTIR 1000 series spectrophotometer in the range 400–4000 cm^{-1}. Melting points were determined with an Electro Thermal IA 9100 apparatus using a capillary tube. The ^{1}H NMR spectra were recorded in DMSO with tetramethylsilane as internal reference using a Bruker AVANCE spectrometer at 300 MHz. The ^{31}P NMR spectra were recorded with an Agilent spectrometer at 202.5 MHz. Column chromatography was performed on silica gel (60-mesh, Merck). TLC was carried out on Merck 0.2 mm silica gel 60 F254 analytical aluminium plates. Elemental analyses of the compounds were carried out using a LECO CHNS-932 analyser.

Synthesis and characterization

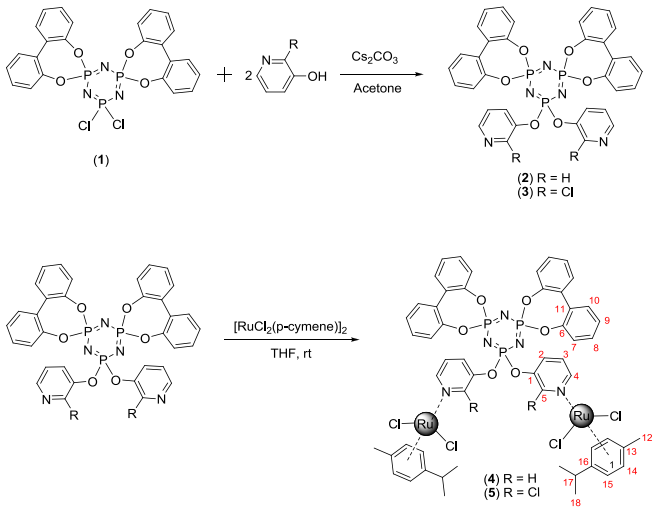
*Synthesis of spiro-$N_3P_3[(O_2C_{12}H_8)_2(OC_5H_4N\text{-}3)_2$ (**2**)*

To a solution of $[N_3P_3Cl_2(O_2C_{12}H_8)_2]$ (**1**; 0.518 g, 1.115 mmol) in acetone (80 ml) were added 3-hydroxypyridine (0.285 g, 3 mmol) and Cs_2CO_3 (2.1 g, 6.5 mmol). After stirring under argon atmosphere for 24 h, evaporation of the solvent gave a white solid, which was purified using TLC on a silica plate. Ligand **2** was separated as a pure white solid (Scheme 1) using a 5:1 ratio of $CHCl_3$–THF as eluent.

Yield 0.632 g (82%). IR (KBr, ν_{max}, cm^{-1}): ν(P=N) 1231–1167, ν(P–O–C) 1091, ν(C=C) 1604, ν(Ar–H) 3061. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.63 (s, 2H, H-5), 8.56 (d, J = 4.7 Hz, 2H, H-4), 7.82 (d, J = 8.4 Hz, 2H, H-2), 7.66 (d, J = 7.6 Hz, 4H, H-10), 7.61 (dd, J_1 = 8.4 Hz, J_2 = 4.7 Hz, 2H, H-3), 7.52 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 4H, H-8), 7.43 (dd, J_1 = 7.6 Hz, J_2 = 7.6 Hz, 4H, H-9), 7.17 (d, J = 8.0 Hz. 4H, H-7). ^{13}C NMR (DMSO-d_6, δ, ppm): 147.5 (C-6), 147.0 (C-1), 143.1 (C-4), 139.6 (C-5), 130.8 (C-10), 130.5 (C-8), 129.2 (C-11), 128.2 (C-7), 127.3 (C-3), 125.4 (C-9), 122.0 (C-2). ^{31}P NMR (DMSO-d_6, δ, ppm): 24.90 (d, P(–$O_2C_{12}H_8$)$_2$), 12.00 (t, P(–OC_5H_4N-3)$_2$). Anal. Calcd for $C_{34}H_{24}N_5O_6P_3$ (%): C, 59.05; H, 3.50; N, 10.13. Found (%): C, 58.85; H, 3.62; N, 10.18.

*Synthesis of spiro-$N_3P_3[(O_2C_{12}H_8)_2(OC_5H_3ClN\text{-}3)_2$ (**3**)*

To a solution of $[N_3P_3Cl_2(O_2C_{12}H_8)_2]$ (**1**; 0.3 g, 0.65 mmol) in acetone (80 ml) were added 2-chloro-3-hydroxypyridine (0.1675 g, 1.3 mmol) and Cs_2CO_3 (0.85 g, 2.61 mmol). After stirring under argon atmosphere for 24 h, evaporation of the solvent gave a white solid, which was purified using TLC on a silica plate. Ligand **3** was separated as a pure white solid (Scheme 1), using a 5:1 ratio of $CHCl_3$–THF as eluent.



Scheme 1. Synthesis of ligands **2** and **3** and Ru(II) complexes **4** and **5**.

Yield 0.385 g (78%). IR (KBr, ν_{max}, cm^{-1}): ν(P=N) 1173–1200, ν(P–O–C) 1072, ν(C=C) 1719, ν(Ar–H) 3065. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.41 (d, J = 4.7 Hz, 2H, H-4), 7.96 (d, J = 8.1 Hz, 2H, H-2), 7.70–7.61 (m, 6H, H-10, H-3), 7.52 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 4H, H-8), 7.43 (dd, J_1 = 7.6 Hz, J_2 = 7.6 Hz, 4H, H-9), 7.15 (d, J = 8.00 Hz, 4H, H-7). ^{13}C NMR (DMSO-d_6, δ, ppm): 147.4 (C-6), 147.1 (C-1), 143.5 (C-4), 143.3 (C-5), 131.3 (C-11), 130.8 (C-10), 130.5 (C-8), 128.1 (C-7), 127.3 (C-3), 125.2 (C-9), 122.0 (C-2). ^{31}P NMR (DMSO-d_6, δ, ppm): 24.38 (d, P(–$O_2C_{12}H_8$)), 11.92 (t, P(–OC_5H_3ClN-3)$_2$). Anal. Calcd for $C_{36}H_{28}N_5O_6P_3$ (%): C, 53.70; H, 2.92; N, 9.21. Found (%): C, 53.52; H, 2.87; N, 9.25.

General procedure for synthesis of Ru(II) complexes

Complexes **4** and **5** were prepared according to the following general method. $[RuCl_2(p\text{-cymene})]_2$ (0.05 mmol) in 5 ml of THF was added to the ligand (0.05 mmol) in 10 ml of THF. The reaction mixture was stirred at room temperature for 12 h. After evaporation of THF, the resulting residue was washed with diethyl ether (20 ml) and recrystallized from MeOH.

Compound **4**. Yield 0.045 g (65%). IR (KBr, ν_{max}, cm^{-1}): ν(P=N) 1219–1166, ν(P–O–C) 1092, ν(C=C) 1770, ν(Ar–H) 3051. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.63 (s, 2H, H-5), 8.57 (d, J = 4.6 Hz, 2H, H-4), 7.82 (d, J = 8.4 Hz, 2H, H-2), 7.66 (d, J = 7.6 Hz, 4H, H-10), 7.61 (dd, J_1 = 8.4 Hz, J_2 = 4.6 Hz, 2H, H-3), 7.52 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 4H, H-8), 7.43 (dd, J_1 = 7.6 Hz, J_2 = 7.6 Hz, 4H, H-9), 7.17 (d, J = 8.0 Hz. 4H, H-7), 5.78 (dd, J_1 = 17.0 Hz, J_2 = 6.2 Hz, 8H, H-14,H-15), 2.81 (m, 2H, H-17), 2.07 (s, 6H, H-12), 1.17 (d, J = 6.9 Hz, 12H, H-18). ^{13}C NMR (DMSO-d_6, δ, ppm): 147.5 (C-6), 147.0 (C-1), 143.1 (C-4), 130.8 (C-10), 130.5 (C-8), 129.2 (C-11), 128.2 (C-7), 127.2 (C-3), 126.5 (C-5), 125.4 (C-9), 122.0 (C-2), 106.8 (C-16), 100.5 (C-13), 86.8 (C-14), 8519 (C-15), 30.4 (C-17), 22.0 (C-18), 18.3 (C-12). ^{31}P-NMR (DMSO-d_6, δ, ppm): 24.87 (d, P(–$O_2C_{12}H_8$)$_2$), 11.95 (t, P(–OC_5H_4N-3)$_2$). Anal. Calcd for $C_{56}H_{56}$ $Cl_4N_5O_6P_3Ru_2$ (%): C, 50.50; H, 4.24; N, 5.26. Found (%): C, 50.55; H, 4.36; N, 5.15.

Compound **5**. Yield 0.040 g (56%). IR (KBr, ν_{max}, cm^{-1}): ν(P=N) 1224–1172, ν(P–O–C) 1094, ν(C=C) 1704, ν(Ar–H) 3058. ^{1}H NMR

(400 MHz, DMSO-d_6, δ, ppm): 8.41 (d, J = 4.5 Hz, 2H, H-4), 7.96 (d, J = 8.1 Hz, 2H, H-2), 7.67 (m, 6H, H-3, H-10), 7.52 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 4H, H-8), 7.43 (dd, J_1 = 7.6 Hz, J_2 = 7.6 Hz, 4H, H-9), 7.15 (d, J = 8.0 Hz, 4H, H-7), 5.78 (dd, J_1 = 17.0 Hz, J_2 = 6.2 Hz, 8H, H-14,H-15), 2.81 (m, 2H, H-17), 2.06 (s, 6H, H-12),1.17 (d, J = 6.9 Hz, 12H, H-18). ^{13}C NMR (DMSO-d_6, δ, ppm): 147.4 (C-6), 147.2 (C-1), 143.4 (C-4), 143.2 (C-5), 131.4 (C-11), 130.8 (C-10), 130.5 (C-8), 128.1 (C-7), 127.3 (C-3), 125.2 (C-9), 121.9 (C-2), 106.8 (C-16), 100.5 (C-13), 86.8 (C-14), 85.9 (C-15), 30.4 (C-17), 21.9 (C-18), 18.3 (C-12). ^{31}P NMR (DMSO-d_6, δ, ppm): 24.32 (d, P(–$O_2C_{12}H_8$)), 11.87 (t, P(–OC_5H_3ClN-3)$_2$). Anal. Calcd for $C_{56}H_{54}Cl_6N_5O_6P_3Ru_2$ (%): C, 48.01; H, 3.89; N, 5.00. Found (%): C, 48.10; H, 3.85; N, 5.04.

General method for transfer hydrogenation of ketones

A mixture of Ru(II) complex **4** or **5** (0.085 mmol), acetophenone derivative (0.85 mmol) and KOH (3.4 mmol) was refluxed at 80°C in 2-propanol (4 ml) for 2 h. The reaction was monitored using gas chromatography. At the end of this time, the mixture was cooled to room temperature and diluted with diethyl ether (5 ml) and filtered from a mini-column filled with silicagel. The yields were related to the residual unreacted acetophenone.

In vitro cytotoxic activity

Cell cultures

The human prostate cancer (PC3), colorectal adenocarcinoma (DLD-1), cervix carcinoma (HeLa) and normal prostate epithelium (PNT1A) cells were obtained from American Type Culture Collection (ATCC Manassas) and propagated as recommended in DMEM/F12 and RPMI-1640 medium supplemented with 5% foetal bovine serum, L-glutamine (2 mM), penicillin (100 U ml^{-1}) and streptomycin (100 mg ml^{-1}) in humidified atmosphere with 5% CO_2 at 37°C. Cells were harvested using 0.25% trypsin (Hyclone) when they were 70–80% confluent in culture.

Cytotoxicity assay and determination of IC_{50}

Cellular viability upon exposure to compounds **2–5** was determined using the colorimetric 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide (MTT) assay. This assay is based on the conversion of yellow MTT into purple formazan crystals by living cells, which determines mitochondrial activity. The total mitochondrial activity is related to the number of viable cells. Basically, PC3, DLD-1, HeLa and PNT1A cells (1×10^5 cells per well) were seeded in 96-well microtitre plates (Nunc, Denmark). The cultured cells were exposed to different concentrations of compounds **2–5** for 24 h and then washed once with phosphate-buffered saline. After that, 100 µl of serum-free medium containing 5 mg ml^{-1} of MTT (Sigma, Missouri) was added to each well. After incubation for 4 h, the supernatant was removed and the formazan crystals obtained were dissolved in 100 µl of DMSO (Sigma). The mixture was stirred for 20 min on a microtitre plate shaker and the absorbance was read at 570 nm. Cell viability was expressed as the percentage of untreated cells that served as the control group and was designated as 100%.

Cytotoxicity was expressed as mean percentage increase relative to the unexposed control ± standard deviation. Control values were set at 0% cytotoxicity. Cytotoxicity data (where appropriate) were fitted to a sigmoidal curve and a four-parameter logistic model was used to calculate the IC_{50}, which is the concentration of material causing 50% inhibition in comparison to the untreated controls. The mean IC_{50} is the concentration of material that reduces cell growth by 50% under the experimental conditions and is the average from at least three independent measurements that were reproducible and statistically significant. The IC_{50} values were reported at ±95% confidence intervals (±95% CI). This analysis was performed with Graph Pad Prism (San Diego, CA, USA).

Antibacterial assay

Microorganisms

Antimicrobial activities of compounds **2–5** were evaluated against Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 7064, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* La 2971), Gram-negative bacteria (*Escherichia coli* ATCC 11230, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Enterobacter aerogenes* ATCC 13048) and yeast cultures (*Candida albicans* ATCC 10231, *Kluyveromyces fragilis* NRRL 2415, *Rhodotorula rubra* DSM 70403) using both disc diffusion and dilution methods. Lyophilized pure strains of bacteria and yeast were obtained from the Basic and Industrial Microbiology Research Laboratory in Canakkale Onsekiz Mart University, Faculty of Science and Arts, Department of Biology.

Agar disc diffusion method

Antimicrobial susceptibility testing was performed using the disc diffusion method according to the protocol described by the Clinical and Laboratory Standards Institute.[24] Fresh stock solutions of compounds **2–5** were prepared in DMSO (2 mg ml^{-1}). To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO using the same procedure as used in the experiments. The bacteria were incubated at 30°C for 24 h in nutrient broth. The yeasts were incubated in malt extract broth for 48 h. Sterile antibiotic discs (6 mm, Schleicher & Schull no.2668, Germany) were impregnated with 20 µl of solutions. After that the discs were placed on the agar inoculated with test microorganisms and then the plates were incubated at 35°C for 24 h for bacteria and at 25°C for 72 h for yeast. All experiments were done in triplicate and the average was taken as the final reading. The antibacterial and antifungal activities of compounds **2–5** were compared with known antibiotics: ampicillin, cefotaxime, tetracycline, nystatin, ketoconazole and clotrimazole.

Dilution method

Minimum inhibitory concentrations (MICs) of the ligands and their Ru(II) complexes against the test microorganisms were determined using a standard method.[25] Briefly, tested bacteria were activated in nutrient broth after incubation at 30°C for 24 h while the yeasts were activated in malt extract broth for 48 h. The compounds were dissolved in DMSO and then diluted using Müller–Hinton broth. Two-fold serial concentrations of the compounds were employed to determine the MIC ranging from 200 to 1.56 µg ml^{-1}. In each case triplicate tests were performed and the average was taken as the final reading. MICs of compounds **2–5** were compared with those of gentamycin antibiotic and nystatin antifungal drug.

Results and discussion

Synthesis and characterization

Compound **1** was synthesized by adapting a known synthetic procedure.[26] Compounds **2** and **3** were prepared by the reaction of **1** with 3-hydroxypyridine and 2-chloro-3-hydroxypyridine,

respectively. Ru(II) complexes **4** and **5** were obtained from the reaction of ligands **2** and -**3** with $[RuCl_2(p\text{-cymene})]_2$ in THF. All compounds were characterized using ^{1}H NMR, ^{31}P NMR and FT-IR spectroscopies, giving results that are consistent with their proposed compositions.

The absorption bands assignable to the stretching of –P=N– and P–O–C are, respectively, 1231–1167 and 1091 cm^{-1} for **2**; 1173–1200 and 1072 cm^{-1} for **3**. Also, the characteristic –P=N– and P–O–C bands are seen, respectively, at 1219–1166 and 1092 cm^{-1} for complex **3**; 1224–1172 and 1094 cm^{-1} for complex **4**.

According to ^{1}H NMR spectra, the aromatic proton signals of ligands are observed in the region 8.63–7.17 ppm for **2**; 8.41–7.15 ppm for **3**. In the ^{1}H NMR spectra of complexes, the signals due to the methyl protons relating to *p*-cymene are easily distinguishable and they are observed at 2.07 ppm for **4** and 2.06 ppm for **5** as singlets. In addition, –CH protons of isopropyl groups are observed at 2.81 ppm for both **4** and **5**.

The ^{31}P NMR spectra of ligands **2** and **3** and complexes **4** and **5** are of the AB_2 type. In the ^{31}P NMR spectra of the ligands, the spiro cyclic phosphorus atoms ($P(O_2C_{12}H_8)$) resonate at 24.90 and 24.38 ppm (doublet) while signals of other phosphorus atoms are seen at 12.00 and 11.92 ppm (triplet). The Ru(II) complexes show triplets at 11.95 ppm (**4**) and 11.87 ppm (**5**) and doublets at 24.87 ppm (**4**) and 24.32 ppm (**5**).

Catalytic studies

To determine the most suitable reaction conditions for catalytic transfer hydrogenation, we first examined the influence of time and various bases on the reactivity of acetophenone (0.85 mmol) in the presence of compound **4** (0.0085 mmol) as catalyst at 82°C. The base variation experiments were performed using a catalyst-to-substrate ratio of 1:400 in 4 ml of 2-propanol with Cs_2CO_3, $NaHCO_3$, K_2CO_3, NaOH and KOH bases. In addition, optimization studies showed that better activities were obtained with a base-to-ketone ratio of 4:1. The conversion rate results after 1 and 2 h are shown in Fig. 1. When the reaction is done without any base, no reaction is observed even after 12 h. The best results are obtained with KOH for 2 h. The question about whether ligand-free moieties contribute to the catalysis is eliminated by carrying out the reaction with only $[RuCl_2(p\text{-cymene})]_2$ as catalyst under the same conditions: a conversion rate of 20% is observed.

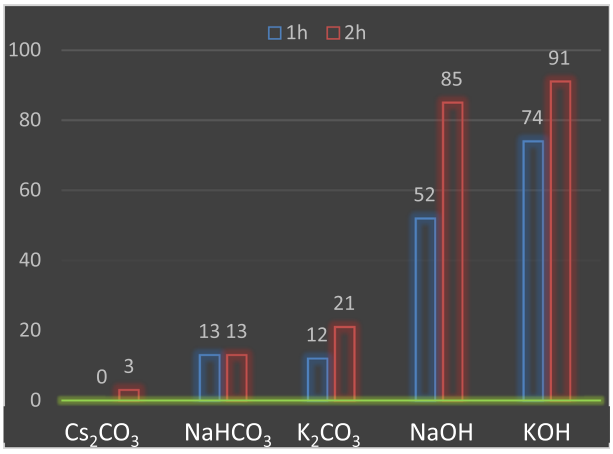


Figure 1. Base variation study. (Experimental conditions: acetophenone (0.85 mmol), catalyst **4** (0.0085 mmol), base (3.4 mmol), i-PrOH (4 ml), reflux, under argon atmosphere at 82°C.

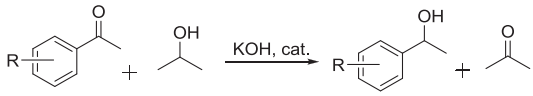
Under these optimum conditions, the catalytic activity of compounds **4** and **5** was investigated and the results are given in Table 1. Complex **5**, which has a chloro substituent on the pyridyl ring, shows lower conversions in comparison with complex **4**. The difference in the catalytic activities of these complexes can be explained on the basis of the electron densities on the metal centre. The presence of the electron-withdrawing group of chloride on the aromatic ring of compound **5** possibly decreases the electron density on its metal centre and in turn the transfer hydrogenation rate.[9]

The complexes exhibit more efficient catalytic activity towards transfer hydrogenation of acetophenone derivatives that have electron-withdrawing substituents, chloride and bromide, at the *para* position compared to acetophenone derivatives that have electron-donating groups, methoxy and methyl, instead. Introduction of multiple methyl groups at the *para* and *ortho* positions decreases the catalytic activity even more, even though they are weak electron-donating groups. This is because the introduction of electron-withdrawing substituents to the *para* position of the aryl ring of the ketone decreases the electron density on the C=O bond and this leads to an improved catalytic activity giving rise to easier hydrogenation. Introduction of electron-donating groups has just the opposite effect.

In vitro cytotoxicity studies

Cytotoxic activity of the newly synthesized compounds **2–5** against PC3, DLD-1 and HeLa tumour cells and healthy PNT1A cells was determined using MTT assay. Normal PNT1A cells were used as a control group. All test cells were exposed to compounds **2–5** at concentrations of 10, 25, 50, 100 and 200 μM for 24 h. The results were analysed using cell viability curves in a concentration range

Table 1. Catalytic activity for transfer hydrogenation of ketones catalysed by Ru(II) complexes[a]



Entry	Catalyst	R	Conversion (%)
1	**4**	H	86
2	**5**	H	81
3	**4**	*p*-NO_2	93
4	**5**	*p*-NO_2	91
5	**4**	*p*-CN	91
6	**5**	*p*-CN	87
7	**4**	*p*-Br	72
8	**5**	*p*-Br	69
9	**4**	*p*-Cl	89
10	**5**	*p*-Cl	83
11	**4**	*p*-Me	35
12	**5**	*p*-Me	31
13	**4**	*p*-OMe	10
14	**5**	*p*-OMe	5
15	**4**	*p*-OH	7
16	**5**	*p*-OH	<5
17	**4**	2,4,6-Me	<5
18	**5**	2,4,6-Me	<5

[a]Reaction conditions: acetophenone (0.85 mmol), catalyst (0.0085 mmol), KOH (3.4 mmol), i-PrOH (4 ml), 2 h, reflux, under argon atmosphere at 82°C.

from 0 to 200 μM. The relationship between cell viability percentage and drug concentration was plotted (Fig. 2) to determine the growth inhibition curve for each cell line.

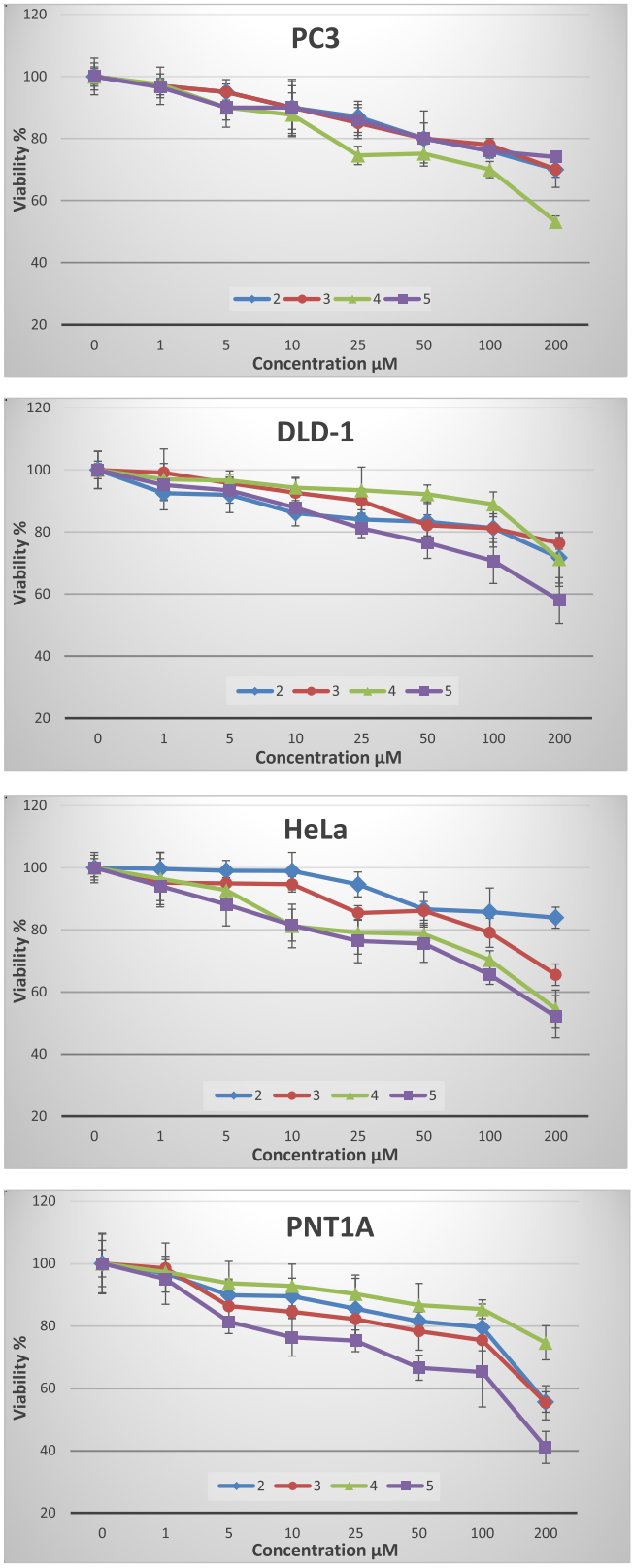


Figure 2. Plot of viable cells at various concentrations of **2**, **3**, **4** and **5** against (a) PC3, (b) DLD-1 and (c) HeLa tumour cells and (d) PNT1A normal cell line.

The results show that exposure to the compounds decreases the cell viability for all tested cells in a dose-dependent manner. A comparison of the cell viability results reveals that Ru(II) complexes **4** and **5** show better cytotoxic effects than their corresponding metal-free cyclotriphosphazene ligands **2** and **3** in general, but there is not a specific pattern. In other words, cytotoxic response to compounds differs from cell to cell and does not follow a certain trend. Compound **4** shows the highest antiproliferative effect against PC3 tumour cells and decreases cell viability by 47% at 200 μM, while compound **5** has the highest cytotoxic activity against DLD-1 cancer cell line and causes 42% cell viability decrease at 200 μM. Other compounds show around 30% reduction in cell viability for both PC3 and DLD-1 cells at 200 μM. Compounds **2** and **3** cause 16 and 35% cell viability reduction in HeLa cells, respectively. Ruthenium complexation seems to increase the cytotoxic effect of compounds **2** and **3** and a significant decrease in the number of viable cells is observed against HeLa cells with compounds **4** and **5**, reaching 46 and 48% reduction, respectively. Taken together, the data indicate that compounds **4** and **5** show the highest cytotoxic activity against the tested cancer cells. However, compound **5** also shows severe toxic effects on normal PNT1A cells. A 59% reduction in the number of viable PNT1A cells is observed upon treatment with compound **5**. This significant decrease in viable cells can be contrasted with the much lower 25% decrease in cell viability upon treatment with compound **4**. The non-toxic nature of compound **4** to host cells may render it a candidate for potential drugs.

In the literature, it has been reported that some cyclotriphosphazene derivatives are not very effective against HeLa cells,[27] and our results for cyclotriphosphazene ligands **2** and **3** also support that finding. However, Ru(II) complexes of these ligands (**4** and **5**) increase the effectiveness of their parent compounds (**2** and **3**) at inhibiting proliferation of HeLa cells by up to almost three times. This effect may be explained in view of the chelation theory described by Tweedy[28] and the cell permeability concept described by Overtone.[29] According to Tweedy, the polarity of the metal ion will be lowered on complexation because its positive charge will be shared with donor groups. Moreover, it will increase the delocalization of π-electrons over the entire chelate ring. Consequently, this enhances the lipophilicity of the

Table 2. *In vitro* antimicrobial activities of **2–5** and standard antimicrobials[a] according to disc diffusion assay

Microorganism	Inhibition zone (mm)[b]									
	2	**3**	**4**	**5**	AM	CT	TE	NY	KE	CL
Bacteria										
M. luteus	12	13	13	12	30	34	20	—	—	—
S. aureus	16	13	16	14	15	14	26	—	—	—
K. pneumoniae	13	16	14	14	15	16	30	—	—	—
P. vulgaris	13	11	12	11	18	20	24	—	—	—
L. monocytogenes	10	10	10	10	14	14	28	—	—	—
E. coli	12	12	12	15	14	12	25	—	—	—
B. cereus	10	10	13	11	14	14	22	—	—	—
Fungi										
R. rubra	10	9	10	9	—	—	—	23	22	24
C. albicans	9	12	9	10	—	—	—	20	22	16
K. fragilis	18	13	13	15	—	—	—	16	15	18

[a]AM, ampicillin 10 μg; CT, cefotaxime 30 μg; TE, tetracycline 30 μg; NY, nystatin 100 μg; KE, ketaconazole 20 μg; CL, clotrimazole 10 μg.
[b]Includes filter paper disc diameter (6 mm).

complexes. Overtone's concept of cell permeability states that entry of any molecule into a cell is governed by its lipophilicity because the lipid membrane surrounding the cell favours the passage of materials that are soluble in lipids. Thus, the increased lipophilicity upon complexation enhances the penetration of the complexes into cells and blocks the metal binding sites of receptors.

Antimicrobial screening

The results for *in vitro* antimicrobial activities of compounds **2–5** together with inhibition zones of standard drugs are summarized in Table 2. Disc diffusion assay results depend not only on the potential antimicrobial potency but also on the diffusion ability of the components. If the compounds do not diffuse well in the polar agar medium, poor results might be obtained due to the low diameter of inhibition even if the compounds are actually good antimicrobials. Disc diffusion is good for qualitative analysis but may not be so applicable for evaluating the potency of large compounds and complexes which diffuse slowly into the culture medium. Therefore, we also determined MIC values of compounds **2–5** and standard drugs (Table 3). The antimicrobial data reveal that the ligands and Ru complexes show moderate to high antimicrobial activities against all the tested microorganisms.

The ligands and complexes show higher antibacterial activity against *M. luteus* and *S. aureus* than the commercial antibiotic gentamycin. Compounds exhibit about the same antibacterial activity against *K. pneumoniae* and moderate activity against the other bacteria in comparison with the standard drug. The results also show that Ru(II) complexes have generally better antimicrobial activities than metal-free ligands. This trend can especially be discernible against fungal strains. This can be attributed to the Tweedy chelation theory of reduced metal polarity upon complexation due to the partial sharing of positive charge with donor group and possible π-electron delocalization over the whole ring. This increased lipophilicity of the metal chelate amplifies its permeation through lipid layers of bacterial membranes and blocks the metal binding sites in the enzymes of microorganisms. The complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of organisms.[30] The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the cells of the microbes or differences in ribosomes of microbial cells.[31] These results suggest that the compounds which exhibit good antimicrobial activities can be further developed for application as effective antimicrobial agents.

Table 3. Minimum inhibitory concentrations (MICs) of compounds **2–5** and standard drugs[a]

	MIC (μg ml^{-1})					
Microorganism	**2**	**3**	**4**	**5**	GEN	NY
Bacteria						
M. luteus	12.5	12.5	12.5	12.5	50	—
S. aureus	6.25	12.5	6.25	12.5	25	
K. pneumoniae	12.5	6.25	6.25	12.5	6.25	
P. vulgaris	12.5	12.5	12.5	12.5	6.25	—
L. monocytogenes	25	25	25	25	12.5	
E. coli	50	12.5	25	6.25	6.25	—
B. cereus	25	25	6.25	25	6.25	—
Fungi						
R. rubra	6.25	3.125	3.125	3.125	—	3.125
C. albicans	6.25	6.25	3.125	3.125	—	3.125
K. fragilis	12.5	6.25	3.125	3.125	—	6.25

[a]GEN, gentamycin; NY, nystatin. Light red, low activity; yellow, moderate activity; green, high activity.

Conclusions

Two novel cyclotriphosphazene ligands (**2** and **3**) and their ruthenium(II) complexes (**4** and **5**) were prepared and characterized using spectral and analytical methods, and their catalytic, cytotoxic and antimicrobial activities were investigated. Catalytic activity studies of Ru(II) complexes **4** and **5** in catalytic transfer hydrogenation of *para*-substituted acetophenone derivatives in the presence of KOH revealed that complex **4** showed better catalytic activity than complex **5**. Presence of a chloro substituent on the pyridyl ring most likely reduced the electron density on the metal centre of complex **5** and caused a lower transfer hydrogenation rate compared to complex **4**. Ruthenium complexation also enhanced cytotoxic and antimicrobial activities of metal-free ligands, and complexes **4** and **5** showed higher cytotoxic and antimicrobial activities compared to their parent ligands (**2** and **3**). This enhancing effect is most likely due to the increased lipophilicity of the complexes resulting from the dissipation of positive charge on the metal by donor groups. However, unlike complex **4**, complex **5** showed detrimental effects on healthy PNT1A cells. The non-toxicity of compound **4** to host cells make it a candidate for potential chemotherapeutics considering that most of the drugs used today also impair healthy cells along with tumour cells.

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