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[Ru(η⁶-*p*-cymene)Cl$_2$(pta)] (pta = 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane): a water soluble compound that exhibits pH dependent DNA binding providing selectivity for diseased cells

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The water soluble complex [Ru(η⁶-*p*-cymene)Cl$_2$(pta)] (pta = 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane), exhibits pH dependent DNA damage; the pH at which damage is greatest correlates well to the pH environment of cancer cells.

Current inorganic drugs such as cisplatin are successfully used in the treatment of many cancers, including testicular, ovarian, oropharyngeal, bronchogenic, cervical and bladder carcinomas, lymphoma, osteosarcoma, melanoma and neuroblastoma.[1] However there are problems associated with their use including general toxicity (leading to side effects) and drug resistance. The general toxicity of cisplatin has been reduced by the development of special drug-dosing protocols,[2] but the need for further improvements remains. In contrast, the ruthenium based anticancer drug, recently launched in the clinic ImH[*trans*-RuCl$_4$(DMSO)Im] (NAMI-A), shows a remarkably low general toxicity.[3] Since ruthenium complexes have been shown to specifically accumulate in tumour cells,[4] the reduced general toxicity of NAMI-A compared to platinum drugs could be due to ruthenium selectivity. In this paper we describe a new ruthenium compound that exhibits pH dependent DNA damage, which could show increased selectivity towards cancer cells and reduce toxic side effects in healthy cells.

The reaction of [Ru(η⁶-*p*-cymene)Cl$_2$]$_2$ with two equivalents of 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane, pta,[5] under reflux in methanol, for 24 h affords [Ru(η⁶-*p*-cymene)Cl$_2$(pta)] **1** in high yield.[6] Characterisation of **1** was achieved using mass spectrometry and NMR spectroscopy.[7]

The molecular structure of **1** (Fig. 1) has been determined by single crystal X-ray diffraction [8] and contains two independent molecules in the asymmetric unit. The C$_6$-ring is coordinated to the ruthenium(II) centre with an average Ru–C bond length of 2.20 and 2.21 Å in each molecule. Two chlorine ligands (mean Ru–Cl 2.42 Å and 2.43 Å in each molecule) and the pta group [Ru–P 2.296(2) and 2.298(3) Å in each molecule] make up the rest of the coordination sphere. All parameters are in keeping with previously determined structures related to **1**, which vary in the nature of the arene and phosphine ligands.[9]

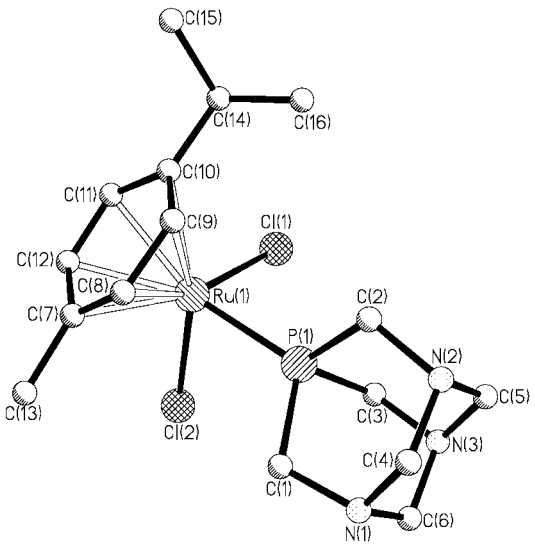

Fig. 1 The molecular structure of one of the independent molecules of **1**.

The presence of the pta ligand provides **1** with versatile soluble properties. For example, **1** and **1** + H$^+$ are soluble in water and polar organic solvents such as $CHCl_3$, CH_2Cl_2 and $(CH_3)_2CO$. Protonation of the pta ligand influences the solubility properties, with the deprotonated species having a higher solubility in organic solvents. The pK_a of **1** was estimated as 6.5 by monitoring the change in absorbance at 455 nm. We recognised that the pH dependant solubility of phosphaamine ligands like pta could also be exploited in biological systems, with the possibility of providing clinical uses. At physiological pH the predominant species carries no charge and hence can diffuse through lipid membranes and move freely into and within cells. In some diseased tissues the pH is reduced due to the associated changes in metabolism and in this environment the pta ligand is protonated, trapping **1** in the cell. In addition, we have shown that the protonated species induces DNA damage more readily than unprotonated **1** (see below).

The DNA substrate used in agarose gel electrophoresis[10] is 95% supercoiled (SC) and 5% open circular (OC), and their positions can be distinguished on the gel (Fig. 2). The results show that when DNA is incubated with **1** at pH 7.5 or above, the DNA migrates similarly to the DNA substrate. These results are in accordance with independent studies using a ruthenium(II)–arene compound, with a DMSO ligand in place of the pta in **1**, that did not detect an interaction with DNA.[11] However, at pH 7.0, the SC form of DNA incubated with **1** is slightly retarded compared to the substrate DNA and the retardation is progressively increased as the pH is reduced. At all pH values the position of OC DNA in the gel remains the same, indicating that the pH dependent retardation of SC DNA by **1** is not due to charge neutralisation.

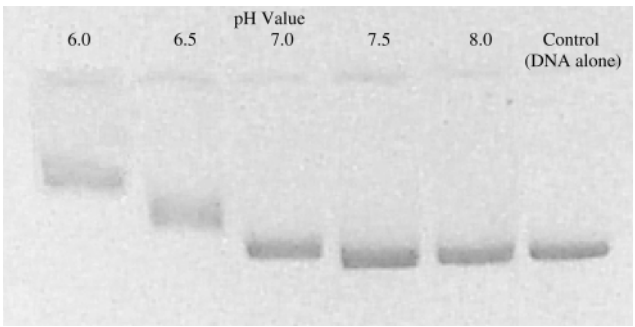

Fig. 2 Comparison of DNA damage induced by **1** incubated at different pH values; visualised by electrophoretic DNA migration in an agarose gel.

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The pH range over which **1** retards DNA migration closely matches the pK_a of the pta ligand, and as DNA is negatively charged, it would be expected that the interaction between these two species would be promoted if they each carried opposite charges. The importance of this result is that DNA binding is not favoured at physiological pH. Many diseased cells have a reduced pH, due to metabolic changes in part associated with the accelerated cell division.[12] Thus, **1** would have a higher affinity for DNA in diseased cells, compared to healthy cells, providing a means of selectivity.

Further studies are currently in progress to delineate the way in which **1** with interacts with DNA. We are also comparing the effect that the type of arene ligand has on DNA and the results obtained from these studies will be reported in due course.

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Notes and references

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2 J. Reedijk, *Chem. Rev.*, 1999, **99**, 2499 and references therein.

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4 S. C. Srivastava, P. Richard, G. E. Meinken, S. M. Larson and Z. Grunbaum, in *Radiopharmaceuticals–Structure–Activity relationships*, ed. R. P. Spencer, Grune & Stratton Inc., New York, 1981, pp. 207–223.

5 *Synthesis* of pta: D. J. Daigle, A. B. Pepperman Jr. and S. L. Vail, *J. Heterocycl. Chem.*, 1974, **17**, 407.

6 *Synthesis* of Ru(η^6-*p*-cymene)Cl_2(pta) **1**: a methanolic solution of [Ru(η^6-*p*-cymene)Cl_2]$_2$ (200 mg, 0.33 mmol) and pta (1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane), 103 mg, 0.66 mmol) was refluxed for 24 h. The solution was allowed to cool to room temperature and filtered. Removal of the solvent affords a red microcrystalline product Ru(η^6-*p*-cymene)Cl_2(pta) **1** (281 mg, 92%).

7 *Spectroscopic data* for **1**: Positive ion electrospray mass spectrum (H_2O): 486 (rel. int. 13) [Ru(η^6-*p*-cymene)Cl_2(pta) + Na]$^+$ (calc. 486.358), 464 (rel. int. 4) [Ru(η^6-*p*-cymene)Cl_2(pta) + H]$^+$ (calc. 464.376), 428 (rel. int. 100) [Ru(η^6-*p*-cymene)Cl(pta)]$^+$ (calc. 427.918). ^{31}P NMR ($CDCl_3$) δ −36.63 (s). ^{1}H NMR ($CDCl_3$) δ 5.46 (q, *J* 19.73 Hz, 4 *p*-cymeme), 4.53 (s, 6 NCH_2N), 4.32 (s, 6 PCH_2N), 2.78 (septet, *J* 41.37 Hz, CH), 2.08 (s, 3 CH_3), 1.22 (d, *J* 6.93 Hz, 6 CH_3). Anal. Found: (calc.) C, 41.14 (41.47); H, 5.66 (5.66)%.

8 Structural details of **1**: single crystals of **1**·CH_2Cl_2 suitable for crystallography were grown from a dichloromethane–hexane solution at 4 °C. Data were collected on a Bruker SMART-CCD equipped with an Oxford Cryostreams low temperature device. *Crystal data* for **1:** [$RuCl_2(PC_6H_{12}N_3)(C_{10}H_{14})$]·$CH_2Cl_2$, red needle of dimensions 0.38 × 0.12 × 0.10, M = 548.26, orthorhombic, space group *Pna*21, Z = 8, a = 13.2180 (11), b = 15.8201(13), c = 20.8468(18), U = 4354.3(6) Å^3, D_c = 1.673 g cm^{-3}, T = 150(2) K, F(000) = 2224, Mo-Kα radiation (λ = 0.71073), μ = 1.292 mm^{-1}, reflections measured in the range 1.62 ≤ θ ≤ 23.32°, 5356 unique (R_{int} = 0.0744). The structure was solved by direct methods and refined by full-matrix least squares on F^2 [SHELXTL NT (G. M. Sheldrick, SHELXL97, an integrated system for solving and refining crystal structures from diffraction data, University of Göttingen, Germany, 1997)] to $R1$ = 0.0476, $wR2$ = 0.1255, S = 1.060, for 4825 reflections with $F > 4\sigma(F)$ and 476 refined parameters with allowance for the thermal anisotropy of all non-hydrogen atoms. A semi-empirical absorption correction was applied based on symmetry equivalent and repeated reflections (minimum and maximum transmission coefficients 0.657 and 0.928). Minimum and maximum final electron density −1.089 and 1.607 e Å^{-3}. The largest residual electron density peak 1.61 e$^-$ lies close to a dichloromethane of crystallisation and no sensible crystallographic or chemical model for this peak could be found. CCDC reference number 161466. See http://www.rsc.org/suppdata/cc/b1/b104021a/ for crystallographic data in CIF or other electronic format.

9 E. A. V. Ebsworh, R. O. Gould, R. A. Mayo and M. Walkinshaw, *J. Chem. Soc., Dalton Trans.*, 1987, 2831; W. Keim, P. Kraneburg, G. Dahmen, G. Deckers, U. Englert, K. Linn, T. P. Spaniol, G. Raabe and C. Krüger, *Organometallics*, 1994, **13**, 3085; M. R. J. Elsegood and D. A. Tocher, *Polyhedron*, 1995, **14**, 3147; I. Moldes, E. de la Encarnación, J. Ros, Á. Alvarez-Larena and J. F. Piniella, *J. Organomet. Chem.*, 1998, **566**, 165.

10 In this assay 2.5 mM **1** in 10 mM phosphate buffer, pH 5.5–8.0 in 0.5 step increments, was incubated for 4 h with 0.05 mg ml^{-1} pBR322 DNA. The DNA damage was assessed by comparing the electrophoretic migration of the species in a 1% agarose gel, prepared in TAE buffer [40 mM tris(hydroxymethyl)aminomethane acetate and 1 mM EDTA] with similar migrations of the control incubations: control 1 tested the activity of **1** under similar assay conditions known to result in DNA retardation (as above but with no phosphate buffer) and control 2 was DNA under the same conditions without **1**. From each assay, 8 μl were mixed with 1 μl dye (0.025 mg bromophenol blue, 1 ml glycerol and 1 ml distilled water) and pipetted into wells on the horizontal gel. A potential difference of 30 mV was applied over the gel for 4 h and the bands visualised by staining with ethidium bromide. The effect of pH alone on DNA was assessed by performing a similar experiment in the absence of **1**.

11 Y. N. A. Gopal, D. Jayaraju and A. K. Kondapi, *Biochemistry*, 1999, **38**, 4382.

12 For example, see: G. R. Martin and R. K. Jain, *Cancer Res.*, 1994, **54**, 5670.