ChemComm

Cite this: *Chem. Commun.*, 2012, **48**, 4839–4841

www.rsc.org/chemcomm

COMMUNICATION

Targeting the DNA-topoisomerase complex in a double-strike approach with a topoisomerase inhibiting moiety and covalent DNA binder†

Andrea Kurzwernhart,[ab] Wolfgang Kandioller,[a] Caroline Bartel,[a] Simone Bächler,[c] Robert Trondl,[a] Gerhard Mühlgassner,[a] Michael A. Jakupec,[ab] Vladimir B. Arion,[ab] Doris Marko,[c] Bernhard K. Keppler[ab] and Christian G. Hartinger*[abd]

Received 13th February 2012, Accepted 20th March 2012
DOI: 10.1039/c2cc31040f

Ru^{II}(arene)–flavonoids with high *in vitro* antitumour activity were synthesised. These compounds are capable of inhibiting human topoisomerase IIα and binding covalently to DNA.

Tumourigenic diseases are one of the major burdens of mankind and many patients are still not treatable or do not respond to standard drugs. This is often related to acquired or intrinsic resistance which hampers the success of chemotherapy with organic and inorganic anticancer agents, such as cisplatin. In order to overcome this drawback, several approaches have been used. The concept of multi-targeted anticancer agents (Fig. 1), *i.e.*, components of a molecule impact multiple separate targets,[1] has been shown to offer several advantages over "classic" chemotherapeutics, *e.g.*, altered pharmacological properties, metabolism and resistance development, tuneable antitumour activity, "intramolecular" combination therapy, and also selective targeted properties.[1]

Among the metal complexes developed as anticancer agents, Ru(III) compounds are considered the most promising drug candidates, and KP1019 and NAMI-A are currently undergoing clinical trials. More recently, Ru^{II}(arene) organometallics have attracted considerable interest, and especially the RAPTA family and ethylene-1,2-diamine complexes are at an advanced preclinical development stage.[2]

One way to prepare biologically active molecules with multi-targeted properties is to link metal fragments to bioactive ligand systems. This strategy has already resulted in promising approaches with compounds exhibiting novel modes of action.[3–5] Especially the use of ligand systems derived from natural compounds appears attractive due to the often advantageous toxicity profile.[6] Flavonoids as secondary metabolites of plants are such a compound class with a rich variety of functions.[7] Importantly, flavonoids are known to exhibit properties such as antiradical and antioxidant, anti-inflammatory, estrogenic, antimicrobial and also anticancer activity.[8]

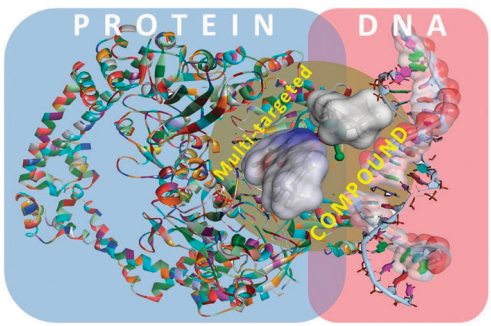


Fig. 1 The concept of a multi-targeted small molecule. We aimed to prepare a compound which is capable of binding *via* its ligand system into the active site of a protein, whereas the metal fragment can form a covalent bond to DNA.

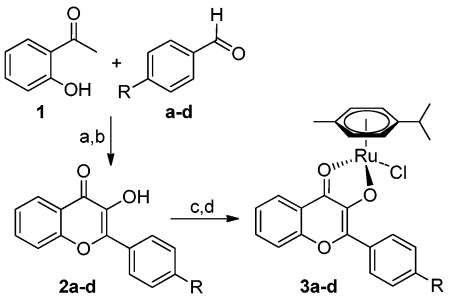
We decided to link flavonoids to Ru^{II}(arene) moieties (Scheme 1), since a few examples of metal–flavonoid complexes are known to exhibit promising biological properties.[9] However, these studies often did not aim to correlate the biological activity with the inhibition of particular targets.[10,11] Flavonoids act primarily through the inhibition of several enzymes, and they have also been shown to interact with human topoisomerases.[7,12]

The flavonol ligands **2a–d** were prepared in two steps by a Claisen–Schmidt condensation and subsequent Algar–Flynn–Oyamada reaction (Scheme 1, ESI†).[13–15] Ligands **2a–d** were converted in good yields into **3a–d** with bis[dichlorido(η^6-*p*-cymene)-ruthenium(II)] under alkaline conditions (Scheme 1).[16] The complexes only show minor signs of hydrolysis in aqueous solution within 6 days.

In addition to characterisation by standard analytical methods (Supporting Information†), single crystals of **3b**·CH_3OH were analysed by X-ray diffraction (Fig. 2).‡ **3b** features a pseudo-octahedral "piano-stool" configuration.[17] The 3-hydroxyflavone upon coordination to Ru acts as a bidentate ligand, forming an envelope-like five-membered cycle, and the two Ru–O bonds

[a] *University of Vienna, Institute of Inorganic Chemistry, Währinger Str. 42, 1090 Vienna, Austria. E-mail: christian.hartinger@univie.ac.at; Fax: 43 1 4277 9526; Tel: 43 1 4277 52609*
[b] *University of Vienna, Research Platform "Translational Cancer Therapy Research", Währinger Str. 42, A-1090 Vienna, Austria*
[c] *University of Vienna, Institute of Food Chemistry and Toxicology, Währinger Str. 38, 1090 Vienna, Austria*
[d] *The University of Auckland, School of Chemical Sciences, Private Bag 92019, Auckland 1142, New Zealand*

† Electronic supplementary information (ESI) available: Materials and methods, synthetic procedures, experimental setup. CCDC 826085. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2cc31040f



Scheme 1 Synthesis of ligands (**2a–d**) and $Ru^{II}(\eta^6$-*p*-cymene) complexes (**3a–d**): (a) NaOH; (b) H_2O_2; (c) NaOMe; (d) $[Ru(\eta^6$-*p*-cymene$)Cl_2]_2$. **2a/3a**: R = H, **2b/3b**: R = CH_3, **2c/3c**: R = F, **2d/3d**: R = Cl.

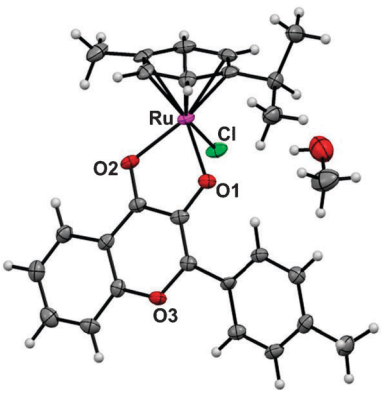


Fig. 2 Molecular structure of the $Ru^{II}(\eta^6$-*p*-cymene) complex **3b**·CH_3OH.

are slightly different with 2.076(2) and 2.099(3) Å, as observed in structurally related compounds.[17] The phenyl substituent of the ligand is twisted with a torsion angle of 15.11°.

The stability of the complexes in aqueous solution has been studied by ^{1}H NMR spectroscopy.

The *in vitro* anticancer activity of ligands **2a–d** and complexes **3a–d** was determined in the human cancer cell lines CH1 (ovarian carcinoma), SW480 (colon carcinoma) and A549 (non-small cell lung carcinoma) by means of the colorimetric MTT assay (Table 1). Notably, the IC_{50} values of **3a–d** were found to be in the low μM range, and only a few examples with similar *in vitro* anticancer activity have been reported.[1,18–20] The substituent in *para* position of the phenyl ring has a significant influence on the *in vitro* activity, with IC_{50} values of the unsubstituted compound **3a** being 2–3 times higher than those of the most active chloro derivative **3d**. Compared to cisplatin, **3a–c** are only 2–3 times less active and **3d** even exhibits the same activity in the SW480 cell line. In the CH1 and A549 cell lines **3a–d** are about one order of magnitude less active than cisplatin (Table 1), but significantly more active than the majority of known tumour-inhibiting organoruthenium compounds.

In order to demonstrate the multi-targeted character of Ru^{II}(flavone) complexes, we studied the inhibition of human topoisomerase IIα activity and the binding ability to DNA models. Topoisomerase IIα is over-expressed in many types of cancer and inhibitors, such as doxorubicin, etoposide and mitoxantrone, are routinely used in the clinic,[21] but only a small number of Ru complexes with topoisomerase inhibitory

Table 1 *In vitro* anticancer activity[a] (IC_{50} values in μM) of **2a–d** and **3a–d** in ovarian (CH1), colon (SW480) and non-small cell lung carcinoma (A549) compared to cisplatin and topoisomerase IIα inhibition of **2a–d** and **3a–d**[b]

	Topoisomerase inhibition[b]	IC_{50}/μM		
		CH1	SW480	A549
2a	+	1.9 ± 0.2	11 ± 3	25 ± 10
3a	++	2.1 ± 0.2	9.6 ± 1.5	20 ± 2
2b	+	1.1 ± 0.1	6.3 ± 1.1	81 ± 9
3b	++	1.8 ± 0.2	7.2 ± 0.5	17 ± 2
2c	+	1.56 ± 0.04	7.0 ± 0.9	37 ± 10
3c	++	1.7 ± 0.4	7.9 ± 2.1	18 ± 1
2d	++	0.60 ± 0.10	3.7 ± 0.4	7.9 ± 1.2
3d	+++	0.86 ± 0.06	3.8 ± 0.5	9.5 ± 0.5
cisplatin[c]	—	0.14 ± 0.03	3.3 ± 0.4	1.3 ± 0.4

[a] 96 h exposure. [b] Estimated 50% inhibitory activity: + >40 μM, ++ ≈ 20–40 μM, +++ <20 μM inhibitor. [c] Taken from ref. 25.

activity have been reported. The major part of them are polypyridyl- and related Ru^{II} complexes with DNA intercalating ligands (for a review see ref. 22) and only a few Ru^{II}(arene) complexes are known which inhibit topoisomerases.[23,24] In this study, human topoisomerase IIα catalytic activity was determined by means of the decatenation assay (Fig. 3; Supporting information†).

Catenated kinetoplast DNA (kDNA) was incubated with topoisomerase IIα in the presence of different concentrations of the flavone complexes **3a–d** and their ligands **2a–d**. Depending on the substituent in *para* position of the phenyl ring, differing potential to inhibit topoisomerase IIα was observed. The chloro compound **3d** is the most potent inhibitor, and in general the extent of inhibition correlates well with the *in vitro* anticancer activity (Table 1). The complexes were generally more active than the ligands, which could however at least be related to a partial release of the ligand. The role of the metal centre in topoisomerase inhibition was recently shown for Cu-thiosemicarbazonato complexes, where the Cu compounds were about an order of magnitude more potent than the respective ligands.[26] However, Ru^{II}(arene) complexes *per se* inhibit the enzyme only to a minor extent, as demonstrated for $[Ru(\eta^6$-benzene$)(DMSO)Cl_2]$.[27] To the best of our knowledge, this is the first example of metal compounds that show topoisomerase inhibitory potency correlating to their antiproliferative activity.

The altered topoisomerase IIα inhibitory activity of the complexes as compared to the ligands may be explained by the multi-targeted character of the complexes. In order to demonstrate the potential of the compounds to interact covalently with DNA as the second target molecule, the reactions of **3a–d**

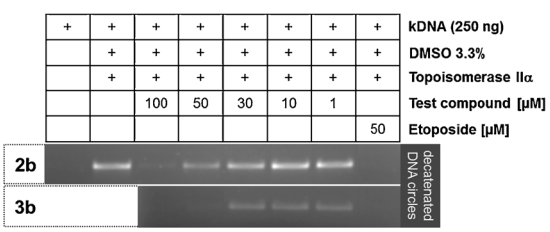


Fig. 3 Effect of complex **3b** and ligand **2b** on the catalytic activity of topoisomerase IIα, as determined by the decatenation assay.

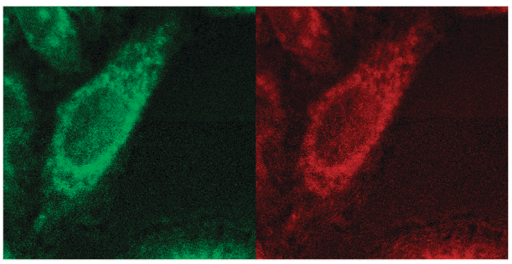


Fig. 4 Live cell imaging with confocal fluorescence microscopy in SW480 cells of **3c** (left) and co-stained with ER-Tracker™ Red (right).

with the DNA model compound 5′-GMP were studied by ^{1}H NMR spectroscopy. Complexes **3a–d** reacted quickly with the *N*7 atom of 5′-GMP (H8 shift from δ = 8.1 to approximately 7.6 ppm). Notably, the flavone ligand remains attached to the Ru centre to interact with topoisomerase IIα. However, the simultaneous interaction of the compounds with DNA and the protein is difficult to prove and will be subject of a separate study.

The flavonoids and their $Ru^{II}(\eta^6$-*p*-cymene) complexes are fluorescent, with an emission maximum at *ca.* 520 nm (**3c**; λ_{ex} = 458 nm). This intrinsic property was used to localise **3c** in SW480 cells in co-staining experiments with fluorescence confocal laser scanning microscopy (Fig. 4). **3c** and the endoplasmic reticulum (ER) marker ER-Tracker™ Red (λ_{ex} = 587 nm, λ_{em} = 615 nm) give largely overlapping signals, and therefore we conclude that the ER is the primarily targeted organelle. This observation is common for lipophilic compounds,[28] and the ER might act as a reservoir for the cytotoxic species.

In conclusion, the Ru^{II}(arene)-flavonoid system offers access to multi-targeted anticancer drugs consisting of a DNA binding metal centre and a biologically active ligand system inhibiting topoisomerase IIα. With the accumulation in the endoplasmic reticulum as a reservoir for the anticancer active moiety and the covalent binding to DNA accompanied by increased topoisomerase IIα inhibitory activity as compared to its ligand **2d** and the high *in vitro* antitumour activity, the $Ru^{II}(\eta^6$-*p*-cymene) complex **3d** is a promising development candidate for an anticancer drug following a double-strike approach.

We thank the University of Vienna, the Austrian Science Fund (FWF), the Johanna Mahlke geb. Obermann Foundation, and COST D39 for financial support. We gratefully acknowledge Alexander Roller for collecting the X-ray diffraction data.

References

‡ Crystallographic details: **3b**·CH_3OH: $C_{27}H_{29}ClO_4Ru$, M_r = 554.02, 0.12 × 0.05 × 0.01 mm, triclinic, $P\bar{1}$, a = 7.8882(5) Å, b = 11.9690(9) Å, c = 13.5794(11) Å, α = 74.879(5)°, β = 73.366(4)°, γ = 89.101(4)°, V = 1183.49(15) Å^3, Z = 2, ρ_{calcd} = 1.555 mg m^{-3}, μ = 0.807 mm^{-1}, Mo-Kα, λ = 0.71073 Å, T = 100(2)K, $2\theta_{max}$ = 27.50°, 5420 measured independent reflections, R_{int} = 0.0972, R_1 = 0.0466, wR_2 = 0.1020; description of data collection and refinement see supporting information; CCDC 826085 contains the supplementary crystallographic data for this paper (The Cambridge Crystallographic Data Centre, www.ccdc.cam.ac.uk/data_request/cif).

1 G. R. Zimmermann, J. Lehár and C. T. Keith, *Drug Discovery Today*, 2007, **12**, 34.
2 W. H. Ang, A. Casini, G. Sava and P. J. Dyson, *J. Organomet. Chem.*, 2011, **696**, 989.
3 D. Hamels, P. M. Dansette, E. A. Hillard, S. Top, A. Vessieres, P. Herson, G. Jaouen and D. Mansuy, *Angew. Chem., Int. Ed.*, 2009, **48**, 9124.
4 W. H. Ang, L. J. Parker, A. De Luca, L. Juillerat-Jeanneret, C. J. Morton, M. Lo Bello, M. W. Parker and P. J. Dyson, *Angew. Chem., Int. Ed.*, 2009, **48**, 3854.
5 I. Ott, B. Kircher, C. P. Bagowski, D. H. W. Vlecken, E. B. Ott, J. Will, K. Bensdorf, W. S. Sheldrick and R. Gust, *Angew. Chem., Int. Ed.*, 2009, **48**, 1160.
6 J. É. Debreczeni, A. N. Bullock, G. E. Atilla, D. S. Williams, H. Bregman, S. Knapp and E. Meggers, *Angew. Chem., Int. Ed.*, 2006, **45**, 1580.
7 R. J. Nijveldt, E. van Nood, D. E. van Hoorn, P. G. Boelens, K. van Norren and P. A. van Leeuwen, *Am. J. Clin. Nutr.*, 2001, **74**, 418.
8 E. Middleton, Jr., C. Kandaswami and T. C. Theoharides, *Pharmacol. Rev.*, 2000, **52**, 673.
9 M. Grazul and E. Budzisz, *Coord. Chem. Rev.*, 2009, **253**, 2588.
10 X. Chen, L.-J. Tang, Y.-N. Sun, P.-H. Qiu and G. Liang, *J. Inorg. Biochem.*, 2010, **104**, 379.
11 J. Ochocki, M. Kasprzak, L. Checinska, A. Erxleben, E. Zyner, L. Szmigiero, A. Garza-Ortiz and J. Reedijk, *Dalton Trans.*, 2010, **39**, 9711.
12 O. J. Bandele and N. Osheroff, *Biochemistry*, 2007, **46**, 6097.
13 T. A. Geissman and D. K. Fukushima, *J. Am. Chem. Soc.*, 1948, **70**, 1686.
14 M. Bennett, A. J. Burke and W. I. O'Sullivan, *Tetrahedron*, 1996, **52**, 7163.
15 C. X. Qin, X. Chen, R. A. Hughes, S. J. Williams and O. L. Woodman, *J. Med. Chem.*, 2008, **51**, 1874.
16 W. Kandioller, C. G. Hartinger, A. A. Nazarov, J. Kasser, R. John, M. A. Jakupec, V. B. Arion, P. J. Dyson and B. K. Keppler, *J. Organomet. Chem.*, 2009, **694**, 922.
17 W. Kandioller, C. G. Hartinger, A. A. Nazarov, M. L. Kuznetsov, R. O. John, C. Bartel, M. A. Jakupec, V. B. Arion and B. K. Keppler, *Organometallics*, 2009, **28**, 4249.
18 M.G. Mendoza-Ferri, C. G. Hartinger, R. E. Eichinger, N. Stolyarova, K. Severin, M. A. Jakupec, A. A. Nazarov and B. K. Keppler, *Organometallics*, 2008, **27**, 2405.
19 R. E. Aird, J. Cummings, A. A. Ritchie, M. Muir, R. E. Morris, H. Chen, P. J. Sadler and D. I. Jodrell, *Br. J. Cancer*, 2002, **86**, 1652.
20 M. G. Mendoza-Ferri, C. G. Hartinger, A. A. Nazarov, R. E. Eichinger, M. A. Jakupec, K. Severin and B. K. Keppler, *Organometallics*, 2009, **28**, 6260.
21 A. K. Larsen, A. E. Escargueil and A. Skladanowski, *Pharmacol. Ther.*, 2003, **99**, 167.
22 F. Gao, H. Chao and L.-N. Ji, *Chem. Biodiversity*, 2008, **5**, 1962.
23 S. K. Singh, S. Joshi, A. R. Singh, J. K. Saxena and D. S. Pandey, *Inorg. Chem.*, 2007, **46**, 10869.
24 F. Beckford, D. Dourth, M. Shaloski, J. Didion, J. Thessing, J. Woods, V. Crowell, N. Gerasimchuk, A. Gonzalez-Sarrias and N. P. Seeram, *J. Inorg. Biochem.*, 2011, **105**, 1019.
25 Y. Y. Scaffidi-Domianello, A. A. Legin, M. A. Jakupec, V. B. Arion, V. Y. Kukushkin, M. Galanski and B. K. Keppler, *Inorg. Chem.*, 2011, **50**, 10673.
26 B. M. Zeglis, V. Divilov and J. S. Lewis, *J. Med. Chem.*, 2011, **54**, 2391.
27 Y. N. V. Gopal, D. Jayaraju and A. K. Kondapi, *Biochemistry*, 1999, **38**, 4382.
28 D. Marko, K.-H. Merz, C. Kunz, A. Müller, N. Tarasova and G. Eisenbrand, *Biochem. Pharmacol.*, 2002, **63**, 669.