Synthesis, Structure, and Antiproliferative Activity of Ruthenium(II) Arene Complexes of Indenoisoquinoline Derivatives

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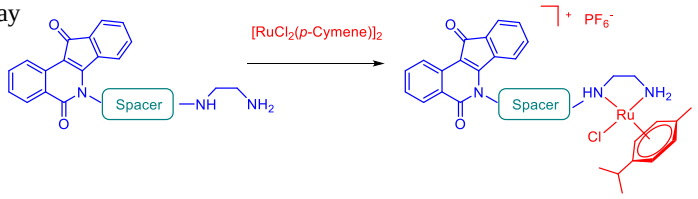
Supporting Information Placeholder

ABSTRACT: Novel ruthenium complexes of indenoisoquinoline derivatives were synthesized and characterized. The structure of the complex **9** was determined by single crystal X-ray crystallography. Ruthenium complexes displayed strong DNA interactions. The cytotoxic activity of the complexes was tested against five cancer cell lines (MDA-MB-231, MCF-7, HEK-293, HT-29 ad DU-145).



INTRODUCTION

Metal-based compounds have become the most widely used chemotherapeutic agents. In particular, platinum complexes such as cisplatin, carboplatin and oxaliplatin are widely used in the clinical treatment of a broad range spectrum of cancer.[1] Ruthenium complexes are also considered to be one of the most promising types of metal-based drugs.[2] Particularly, two Ru(III) species, KP1019 **1** and NAMI-A **2**, are presently undergoing clinical trials (Figure 1).[3] In addition, some Ru(II) complexes, such as RAPTA-C **3** and Ru(II)(arene) complexes of bidentate ethylenediamine **4**, exhibit interesting *in vitro* and *in vivo* anticancer activity and are already under preclinical evaluation (Figure 1).[4] Recently, indenoisoquinolines have been identified as novel topoisomerase I inhibitors exhibiting better pharmacokinetic features and greater chemical stability than camptothecin.[5] In particular, two anticancer agents, indotecan **5** (LMP400) and indimitecan **6** (LMP776), were promoted to a Phase I clinical trial at the National Institute of Health. Interestingly, structural modifications of the indenoisoquinolines induced good topoisomerase II inhibition properties.[6]

As part of our ongoing effort to develop metal-based indenoisoquinoline derivatives as anticancer agents,[7] we report our preliminary results on the preparation and the characterization of Ru(II)-arene complexes **8** and **9** containing diamines as ligand. Their cytotoxicities on cancer cell lines and their DNA interaction were evaluated. (Figure 2).

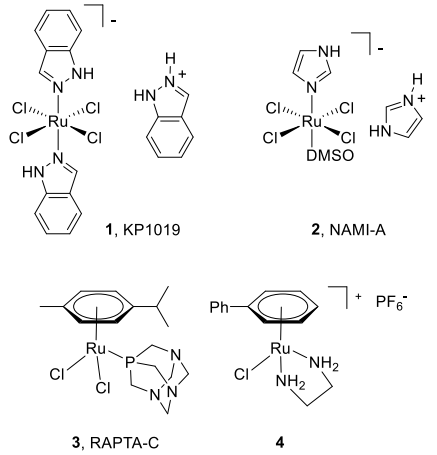


Figure 1 Ruthenium anticancer agents.

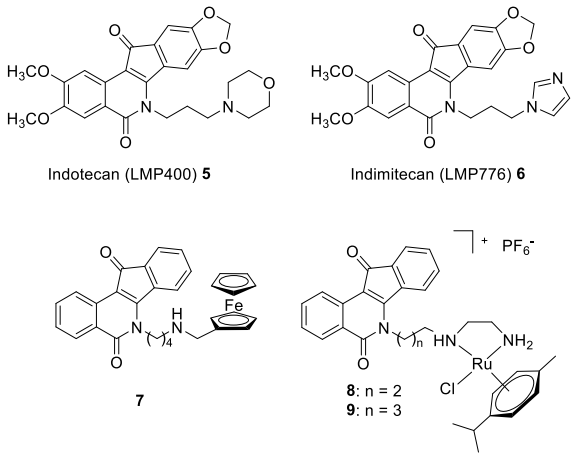


Figure 2 Indenoisoquinoline derivatives.

RESULTS AND DISCUSSION

Synthesis and characterization. The synthesis of ruthenium complexes **8** and **9** are described in Scheme 1. Indenoisoquinolines **11** and **12** were first obtained in a four step reaction. Condensation of the benzo[*d*]indeno[1,2-*b*]pyran-5,11-dione **10** with a primary aminoalcohol was followed by tosylation of the alcohol function. The substitution of the tosyl group by the protected ethylenediamine and the consecutive deprotection of the Boc group led to the indenoisoquinolines **11** and **12** in 65-68% global yield. Their complexation in the presence of [(*p*-cymene)$RuCl_2$]$_2$ in ethanol followed by an exchange of chloride by hexafluorophosphate afforded the ruthenium indenoisoquinolines **8** and **9** in 95% and 88% yields respectively.[8]

The structure of **9** was established by single crystal X-ray analysis. The drawing is displayed in Figure 3. The Ru-complex **9** crystallizes in the triclinic space group *P*Γ with two formula units per unitcell. As can be seen in Figure 3, the complex shows a mononuclear structure in which the Ru(II) atom is four coordinated by one chlorine atom, an arene ligand and two nitrogen atoms from the indenoisoquinoline-diamine ligand.

The compound **9** demonstrated good stability in water and in BPE buffer as no significant change of their UV-Vis absorption spectra over a period of 24 h was observed (Figure S3, ESI).

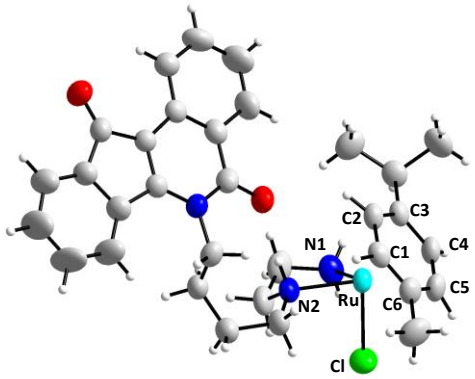


Figure 3 Molecular structure of Ru-complex **9** showing the local geometry around the Ruthenium and ligand. Selected bond lengths (Å) and angles (°): Ru–N1, 2.128 (4); Ru–N2, 2.167 (4); Ru–Cl, 2.3893 (15); Ru–C1, 2.167 (4); Ru–C2, 2.167 (5); Ru–C3, 2.191(5); Ru–C4, 2.178 (5); Ru–C5, 2.168 (5); Ru–C6, 2.209 (5); N1–Ru–N2, 79.60 (15); N1–Ru–Cl, 85.04 (13); N2–Ru–Cl, 86.88 (11).

Additionally, the ESI-MS of complex **9** has been acquired to understand its relative composition and stability. ESI-MS spectra were recorded directly after dissolution in water and after 2 and 6 h. Besides the anticipated parent mass peaks, a peak at m/z (**9**-HCl) was also observed and was attributed to the cleavage of the Ru-Cl bond. This result suggests that the chlorine atom of the Ru-complex is highly labile. An identical ESI-MS profile was obtained when the complex **9** was dissolved in acid solution at pH = 2.7. It can be noted that the cleavage of the Ru-Cl bond is more important in an acidified aqueous solution than in water (Figures S4 and S5, ESI)

Biological studies. The ability of drugs to protect calf thymus DNA (CT DNA, 42% GC bp) against thermal denaturation was used as an indicator of the relative capacity of indenoisoquinoline derivatives to bind and to stabilize the DNA double helix. ΔT_m values are reported in Table 1.[9] The ruthenium complexes **8** and **9** displayed similar ΔT_m values (21°C for **8** and for **9**, drug/DNA ratio = 1). Binding affinities were determined using a fluorescence method. Since a weak intrinsic fluorescence was observed for our compounds, an indirect method was preferred.[10]

Scheme 1. Synthesis of Ruthenium Complexes.

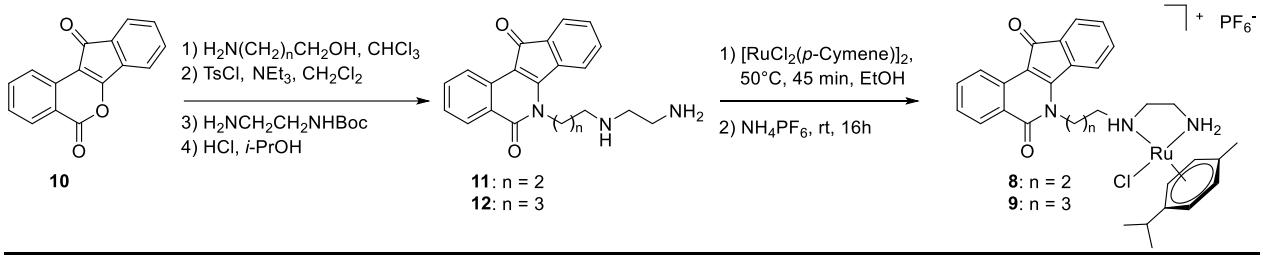


Table 1. Interaction of Ruthenium complexes with DNA and IC_{50} values (μM) against human cancer cells after 72h.[a]

Compd	ΔT_m (°C)[b]	K_{app} (10^7 M^{-1})[c]	IC_{50} (μM)				
			MDA-MB-231	MCF-7	HEK-293	HT-29	DU-145
8	21	0.76±0.07	19.16 ± 1.85	>50	18.07 ± 0.64	16.30 ± 0.10	>50
9	21	3.19±0.01	1.04 ± 0.10	1.10 ± 0.40	0.79 ± 0.09	2.02 ± 0.20	0.93 ± 0.11
Cisplatin	Nd[d]	Nd	33.80 ± 1.27	40.39 ± 11.96	16.47 ± 1.61	178 ± 14	2.30 ± 0.40

[a] Drug concentration that inhibits cell growth by 50% after incubation for 72h. Data are the mean of at least three independent experiments. [b] Variations in melting temperature ($\Delta T_m = T_m^{\text{drug-DNA complex}} - T_m^{\text{DNA alone}}$) at ratio of 1. [c] Apparent binding constant measured by fluorescence using [EB]/[DNA] = 1.26. [d] Not determined.

We used the conventional fluorescence quenching assay based on DNA binding competition between the intercalating drug ethidium bromide (EB) and the tested molecules. Indeed, EB displacement studies were preformed to have deep insight into both binding affinities with DNA and the binding mode. Intrinsic fluorescence of compounds was firstly verified.[9] The apparent DNA binding constant K_{app} values were calculated using the equation: $K_{app} = 1.26K_{app}(EB)/IC_{50}$ with $K_{app}(EB) = 10^7$ M^{-1} and IC_{50} in μM. In agreement with ΔT_m, Ru-complexes have good binding affinities with DNA. However, it should be noted that complex **9**, possessing four methylene units, exhibited a higher K_{app} value ($K_{app} = 3.19$ 10^7 M^{-1}) than the complex **8** ($K_{app} = 0.76$ 10^7 M^{-1}) (Table 1).

The antiproliferative activity of ruthenium complexes was tested on breast cancer (MDA-MB-231 and MCF-7) and human embryonic kidney (HEK-293), colon carcinoma (HT-29) and prostate cancer (DU-145) cell lines using a colorimetric cell proliferation assay. Cisplatin was used as reference compound. The ability of ruthenium complexes to inhibit the growth of cancer cells is summarized in Table 1. The Ru-complex **9** exhibited excellent antiproliferative activities with IC_{50} values ranging from 0.79 to 1.10 μM. This compound was found to be more effective than the platinum compound cisplatin with the five cancer cell lines. The complex **8**, possessing three methylene units, is less active than its analogue **9** with four methylene units.

CONCLUSION

In summary, new ruthenium complexes containing an indenoisoquinoline scaffold were synthesized. The molecular structure of complex **9** has been confirmed by single-crystal X-Ray diffraction analysis. These compounds displayed strong DNA interaction and excellent cytotoxic activities against five cancer cell lines. These preliminary results will be continued by biological studies. Particularly, the influence of the complexation of the indenoisoquinoline amines by other metals will be investigated.

EXPERIMENTAL SECTION

Chemistry. All commercial reagents and solvents were used without further purification. Melting points were determined with a Barnstead Electrothermal (BI 9300) capillary melting point apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded on a Bruker AC300 spectrometer at 300 and 75.5 MHz respectively using tetramethylsilane (TMS) as internal standard and DMSO-d6 as solvent. HRMS experiments were performed on Q Exactive Benchtop LC–MS/MS (Thermo Scientific). ESI-MS studies were realized on a Obitrap LTQ XL (ThermoFisher) in positive ionization (NanoESI, 1μL/mn, complex concentration 80 pmol/μL in water/DMSO: 95/5). Thin layer chromatography (TLC) was carried out on aluminium-baked Macherey-Nagel silica gel 60. Column chromatography was performed on silica gel (230-400 mesh). The electronic absorption spectra were acquired on a UV-Vis double beam spectrophotometer SPECORD® PLUS (Analytikjena). The molar conductance measurement was carried out using a CDRV 62 Tacussel electronic bridge, employing a calibrated 10^{-2}M KCl solution and 10^{-3} M solutions of compounds in DMSO. Purities of all tested compounds were ≥95%, as estimated by HPLC analysis. HPLC analyses were performed on a Thermo Finnigam using a 5 μM C18 Gravity Nucleodur column. Elemental analyses were performed with a varioMICRO analyser. Ligands **11** and **12** were synthesized according the literature procedure.[6b]

Ruthenium complex 8: To a solution of [(η^6-*p*-cymene)$RuCl_2$]$_2$ (56 mg, 0.09 mmol) in dry ethanol (20 mL) was added diamine **11** (63 mg, 0.18 mmol). After stirring at 50°C for 45 min, the reaction mixture was filtered and NH_4PF_6 (100 mg, 0.6 mmol) was added to the filtrate. After standing at room temperature for 16h, the solvent was removed and the powder was recrystallized in a mixture methanol/ether to give orange crystals (133 mg, 95% yield). Anal. Calc. for $C_{31}H_{35}ClF_6N_3O_2PRu.H_2O$: C, 47.67; H, 4.77; N, 5.38; Found: C, 47.69; H, 4.71; N, 5.31. M.p. 202°C. ^1H NMR (DMSO-d_6) δ8.63 (d, $^3J_{HH}$ = 7.8 Hz, 1H, ArH), 8.28 (d, $^3J_{HH}$ = 7.8 Hz, 1H, ArH), 7.87 (m, 2H, ArH), 7.63 (m, 2H, ArH), 7.57 (m, 2H, ArH), 6.71 (br s, 1H, NH), 6.48 (br s, 1H, NH), 5.63-5.54 (m, 4H, PhH), 4.58 (m, 2H, $CONCH_2$), 3.75 (br s, 1H, NH), 3.25 (m, 2H, CH_2), 3.00 (m, 1H, $CHMe_2$), 2.77 (m, 2H, CH_2), 2.26 (m, 3H, CH_2), 2.16 (s, 3H, CH_3), 1.85 (b, 1H, C*H*H), 1.20 (d, $^3J_{HH}$ = 6.9 Hz, 3H, CH_3), 1.12 (d, $^3J_{HH}$ = 6.9 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 189.9, 162.9, 156.2, 136.4, 134.4, 134.0, 133.9, 131.8, 131.4, 128.1, 127.2, 123.9, 122.8, 122.7, 122.6, 107.2, 105.6, 94.6, 82.2, 82.0, 81.2, 79.2, 51.2, 49.8, 40.3, 40.1, 30.0, 28.2, 22.3, 21.4, 16.8. Λ_m ($\Omega^{-1}.cm^2.mol^{-1}$) 26. HRMS *m/z* (ESI) calcd for $C_{31}H_{35}ClN_3O_2Ru$ $(M)^+$ 618.1461 found 618.14551.

Ruthenium complex 9: To a solution of [(η^6-*p*-cymene)$RuCl_2$]$_2$ (51 mg, 0.08 mmol) in dry ethanol (30 mL) was added diamine **12** (60 mg, 0.16 mmol). After stirring at 50°C for 45 min, the reaction mixture was filtered and NH_4PF_6 (100 mg, 0.6 mmol) was added to the filtrate. After standing at room temperature for 16h, the solvent was removed and the powder was recrystallized in a mixture methanol/ether to give orange crystals (104 mg, 88% yield). Anal. Calc. for $C_{32}H_{37}ClF_6N_3O_2PRu.H_2O$: C, 48.34; H, 4.94; N, 5.28. Found: C, 48.38; H, 4.90; N, 5.33. Orange crystals, m.p. 178°C. ^1H NMR (DMSO-d_6) δ 8.60 (d, $^3J_{HH}$ = 8.1 Hz, 1H), 8.26 (d, $^3J_{HH}$ = 7.5 Hz, 1H), 7.86 (t, $^3J_{HH}$ = 7.2 Hz, 1H, ArH), 7.80 (m, 1H, ArH), 7.63 (m, 2H, ArH), 7.56 (m, 2H, ArH), 6.57 (b, 1H), 6.44 (b, 1H), 5.66 (d, $^3J_{HH}$ = 6.0 Hz, 1H, PhH), 5.63 (d, $^3J_{HH}$ = 6.0 Hz, 1H, PhH), 5.57 (d, *J* = 6.0 Hz, 1H, PhH), 5.51 (d, $^3J_{HH}$ = 6.0 Hz, 1H, PhH), 4.6 (m, 2H), 3.77 (m, 1H, $CONCH_2$), 3.71 (br s, 1H, NH), 3.13 (m, 2H, CH_2), 2.68 (m, 1H, $CHMe_2$), 2.46 (m, 2H, CH_2), 2.15 (s, 3H, CH_3), 1.83 (m, 4H, CH_2), 1.21 (d, $^3J_{HH}$ = 6.0 Hz, 3H, CH_3), 1.19 (d, $^3J_{HH}$ = 6.0 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 189.9, 162.6, 156.1, 136.4, 134.4, 134.1, 133.9, 131.8, 131.4, 128.1, 127.2, 123.7, 122.8, 122.7, 122.6, 107.0, 105.7, 94.3, 82.2, 82.1, 81.1, 79.5, 54.4, 49.5, 45.0, 40.3, 30.1, 26.7, 25.3, 22.6, 21.1, 16.8. Λ_m ($\Omega^{-1}.cm^2.mol^{-1}$) 30. HRMS *m/z* (ESI) calcd for $C_{32}H_{37}ClN_3O_2Ru$ $(M)^+$ 632.1618 found 632.1612.

X-ray Crystallography. Suitable crystal of Ru complex **9** [$C_{32}H_{37}ClN_3O_2Ru$, F_6P, 0.5(H_2O)] with approximate dimension 0.30 × 0.05 × 0.02 mm^3 was selected under polarizing optical microscope and glued on a glass fiber for a single-crystal X-ray diffraction experiment.

X-ray intensity data were collected on a Bruker X8-APEX2 CCD area-detector diffractometer using Mo-K_α radiation (λ = 0.71073 Å) with an optical fiber as collimator. Several sets of narrow data frames were collected at different values of θ (2.302 to 26.54°) and 2 initial values of ϕ and ω, respectively, using 0.3° increments of ϕ or ω. Data reduction was accomplished using SAINT V7.53a. The substantial redundancy in data allowed a semi-empirical absorption correction (SADABS)[11] to be applied, on the basis of multiple measurements of equivalent reflections. The structures were solved by direct methods (*SHELXS*)[12] and refined by full-matrix least-squares procedures (*SHELXL*) using Olex program.[13] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in the refinement at calculated positions using a riding model. The PF_6^- anions in the crystal structure were disordered over several orientations and were taking account by two different orientations.

Aqueous stability assays. Aqueous stability of the Ru-complex **9** was monitored using UV-Vis spectroscopy. Complex **9** was dissolved in DMSO and diluted with water or with BPE buffer to give 50 µM solution. The UV-vis peak profile of the sample was monitored for 24 h at 1h intervals. The temperature was kept constant at 25°C throughout.

Biological testing assay.

DNA and Drugs Solutions. Calf thymus DNA (CT DNA, Pharmacia) was deproteinized with sodium dodecyl sulfate (SDS, protein content less than 0.2%) and extensively dialyzed against the required experimental buffer. An extinction coefficient of 6600 $M^{-1}cm^{-1}$ was used to measure the nucleotide concentration of DNA solutions.[9a] All synthesized compounds were dissolved as 10 mM solutions in DMSO. Further dilutions were made in the appropriate aqueous buffer.

Melting Temperature Studies. Melting curves were obtained using an Uvikon 943 spectrophotometer coupled to a Neslab RTE111 cryostat. To perform the melting temperature (T_m) measurements, CT DNA (20 µM) was incubated alone (control T_m) then, each compound was incubated with CT DNA at a drug/CT DNA ratio of 0.25, 0.5 and 1. Typically, 20, 10, 5µM of the various drugs were prepared in 1 mL of BPE buffer (6 mM Na_2PO_4, 2 mM NaH_2PO_4, 1 mM EDTA, pH 7.1) in the presence or absence of 20 µM of CT DNA and transferred into a quartz cuvette of 10 mm path length. The absorbance at 260 nm was measured every minute over the range 20-100°C with an increment of 1°C per minute and the spectra were recorded from 230 to 500 nm and referenced against a cuvette containing the same DNA concentration in the same buffer. The T_m values were obtained from first-derived plots.

Fluorescence measurements. Fluorescence titration data were recorded at room temperature using a SPEX Fluorolog fluorometer. Since compounds show weak fluorescence intensity variation with DNA titration, the binding studies were carried out through a competitive displacement fluorimetry assay using DNA-bound ethidium bromide.[10b] Excitation was set at 546 nm and the fluorescence emission was monitored over the range 490-630 nm. Experiments were performed with an ethidium bromide/DNA molar ratio of 12.6/10 and drug concentration ranges of 0.02-4 µM in a BPE buffer, pH 7.2. IC_{50} values for ethidium bromide displacement were calculated using a fitting function incorporated into GraphPad Prism 3.0 software, and the apparent binding constant was calculated as follows: $K_{app} = (1.26/IC_{50})K_{ethidium}$, with $K_{ethidium} = 10^7\ M^{-1}$.

Cell cultures and antiproliferative assay. Cells were grown at 37°C in a humidified atmosphere containing 5% CO_2 in DMEM medium, supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1 mM sodium pyruvate, penicillin (100 IU/mL), and streptomycin (100 µg/mL). The cytotoxicity of the tested compounds was assessed using a cell proliferation assay developed by Promega (CellTiter 96 Aqueous one solution cell proliferation assay). Briefly, 2 x 10^4 exponentially growing cells were seeded in 96-well microcultures plates with various drug concentrations in a volume of 100 µL. After 72h incubation at 37°C, 20 µL of the tetrazolium dye was added to each well and the samples were incubated for a further 1h at 37°C. Plates were analysed on a Labsystems Multiskan MS (type 352) reader at 492 nm.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

NMR of all new compounds, UV-Vis and ESI-MS spectra and crystallographic data for **9** (PDF and CIF)

AUTHOR INFORMATION

The contribution of authors was detailed as follows:
Performed the experiments: NW, NH, AM
Conceived and designed the experiments: LP, JFG.
Analyzed the data: NSF, JFG, SP, PTN.
Contributed reagents/materials/analysis tools: BB, CBM, SM, JFB
Wrote the paper: LP, TB, NSF, NW, JFG.

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REFERENCES

(1) (a) Gasser, G.; Ott, I.; Metzler-Nolte, N. *J. Med. Chem*. **2011**, *54*, 3–25. (b) Bergamo, A.; Gaiddon, C.; Schellens, J. H. M.; Beijnen, J. H.; Sava, G. *J. Inorg. Biochem*. **2012**, *106*, 90–99. (c) Hartinger, C.; Dyson, P. J. *Chem. Soc. Rev*. **2009**, *38*, 391–401. (d) Arnesano, F.; Natile, G. *Coord. Chem. Rev*. **2009**, *253*, 2070–2081. (e) Hambley, T. W. *Dalton Trans*. **2007**, *21*, 4929–4937.

(2) (a) Süss-Fink, G. *Dalton Trans*. **2010**, *39*, 1673–1688. (b) Bergamo, A.; Sava, G. *Dalton Trans*. **2011**, *40*, 7817−7823.

(3) (a) Rademaker-Lakhai, J. M.; van den Bongard, D.; Pluim, D.; Beijnen, J. H.; J. H. Schellens, J. H. *Clin. Cancer Res*. **2004**, *10*, 3717−3727. (b) Trondl, R.; Heffeter, P.; Kowol, C. R.; Jakupec, M. A.; Berger, W.; Keppler, B. K. *Chem. Sci*. **2014**, *5*, 2925–2932.

(4) (a) Grozav, A.; Balacescu, O.; Balacescu, L.; Cheminel, T.; Berindan-Neagoe, I.; Therrien, B. *J. Med. Chem*. **2015**, *58*, 8475−8490. (b) Singh, A. K.; Pandey, D. S.; Xu, Q.; Braunstein, P. *Coord. Chem. Rev*. **2014**, *270–271*, 31–56. (c) Zhang, Y.; Zheng, W.; Luo, Q.; Zhao, Y.; Zhang, E.; Liu, S.; Wang, F. *Dalton Trans*. **2015**, *44*, 13100–13111. (d) Bonfili, L.; Pettinari, R.; Cuccioloni, M.; Cecarini, V.; Mozzicafreddo, M.; Angeletti, M.; Lupidi, G.; Marchetti, F.; Pettinari, C.; Eleuteri, A. M. *ChemMedChem* **2012**, *7*, 2010–2020. (e) Wu, Q.; Chen, T.; Zhang, Z.; Liao, S.; Wu, X.; Wu, J.; Mei, W.; Chen, Y.; Wu, W.; Zeng, L.; Zheng, W. *Dalton Trans*. **2014**, *43*, 9216–9225. (f) Aird, R. E.; Cummings, J.; Ritchie, A. A.; Muir, M.; Morris, R. E.; Chen, H.; Sadler, P. J.; Jodrell, D. I. *Br. J. Cancer* **2002**, *86*, 1652–1657; (g) Adhireksan, Z.; Davey, G. E.; Campomanes, P.; Groessl, M.; Clavel, C. M.; Yu, H.; Nazarov, A. A.; Yeo, C. H.; Ang, W. H.; Dröge, P.; Rothlisberger, U.; Dyson, P. J.; Davey, C. A. *Nat. Commun*. **2014**, *5*, 3462–3474.

(5) (a) Antony, S.; Agama, K. K.; Miao, Z. H.; Takagi, K.; Wright, M. H.; Robles, A. I.; Varticovski, L.; Nagarajan, M.; Morrell, A.; Cushman, M.; Pommier, Y. *Cancer Res*. **2007**, *67*, 10397–10405; (b) Cinelli, M. A.; Reddy, P. V.; Lv, P. C.; Liang, J. H.; Chen, L.; Agama, K.; Pommier, Y.; van Breemen, R. B.; Cushman, M. *J. Med. Chem*. **2012**, *55*, 10844–10862; (c) Pommier, Y.; Cushman, M. *Mol. Cancer Ther*. **2009**, *8*, 1008–1014; (d) Khadka, D. B.; Le, Q. M.; Yang, S. H.; Van, H. T.; Le, T. N.; Cho, S. H.; Kwon, Y.; Lee, K. T.; Lee, E. S.; Cho, W. J. *Bioorg. Med. Chem.* **2011**, *19*, 1924–1929.

(6) (a) Ryckebusch, A.; Garcin, D.; Lansiaux, A.; Goossens, J. F.; Baldeyrou, B.; Houssin, R.; Bailly, C.; Hénichart, J. P. *J. Med. Chem*.

2008, *51*, 3617–3629; (b) Ahn, G.; Lansiaux, A.; Goossens, J. F.; Bailly, C.; Baldeyrou, B.; Schifano-Faux, N.; Grandclaudon, P.; Couture, A.; Ryckebusch, A. *Bioorg. Med. Chem*. **2010**, *18*, 8119–9133.
(7) Wambang, N.; Schifano-Faux, N.; Aillerie, A.; Baldeyrou, B.; Jacquet, C.; Bal-Mahieu, C.; Bousquet, T.; Pellegrini, S.; Ndifon Téké, P.; Meignan, S.; Goossens, J. F.; Lansiaux, A.; Pélinski, L. *Bioorg. Med. Chem*. **2016**, *24*, 651–660.
(8) Morris, R. E.; Aird, R. E.; Murdoch, P. S.; Chen, H.; Cummings, J.; Hughes, N. D.; Parsons, S.; Parkin, A.; Boyd, G.; Jodrell, D. I.; Sadler, P. J. *J. Med. Chem*. **2001**, *44*, 3616–3621.
(9) (a) Wells, R. D.; Larson, J. E.; Grant, R. C.; Shortle, B. E.; Cantor, C. R. *J. Mol. Biol*. **1970**, *54*, 465–497. (b) David-Cordonnier, M.-H.; Gajate, C.; Olmea, O.; Laine, W.; de la Iglesia-Vicente, J.; Perez, C.; Cuevas, C.; Otero, G.; Manzanares, I.; Bailly, C.; Mollinedo, F. *Chem. Biol*. **2005**, *12*, 1201–1210.
(10) (a) Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. *J. Med. Chem*. **1981**, *24*, 170–177. (b) Pavlov, V.; Kong Thoo Lin, P.; Rodilla, V. *Chem. Biol. Interact*. **2001**, *137*, 15–24.
(11) Sheldrick, G. M. SADABS, version 2; University of Göttingen: Göttingen, Germany, 2004.
(12) Sheldrick, G. M. *Acta Cryst*. **2008**, *A64*, 112-122.
(13) Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. *J. Appl. Cryst*. **2009**, *42*, 339-341.