Organometallic anticancer agents that interfere with cellular energy processes: a subtle approach to inducing cancer cell death.

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Supplementary Material

Synthesis

All solvents were purified and degassed prior to use.[1] ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded on a Bruker Avance II 400 spectrometer at room temperature and were referenced to the residual ^{1}H signal of the NMR solvent. ESI-mass spectra of the compounds were obtained in MeOH on a ThermoFinnigan LCQ Deca XP Plus quadrupole ion-trap instrument operated in positive ion mode over a mass range of m/z 150-1000. The ionization energy was set at 3.5 kV and the capillary temperature at 150 °C. Melting points were determined with a Stuart Scientific SMP3 apparatus and are uncorrected. The Varian 971-FP flash chromatography system was used for compound purification. Elemental analyses were carried out by the microanalytical laboratory at the EPFL.

Synthesis of N-(3-(1H-imidazol-1-yl)propyl)-1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamide, ***1***

A solution of 1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylic acid (2.0 g, 6.22 mmol), oxalyl chloride (10.7 mL) and a catalytic amount of DMF (0.1 mL) in CH_2Cl_2 (100 mL) was heated under reflux for 2 h. Unreacted oxalyl chloride and solvent were removed under reduced pressure to yield 1-(2,4-dichlorobenzyl)-1H-indazole-3-carbonyl chloride as a yellow solid. N-(aminopropyl)imidazole (2.6 ml, 21.44 mmol) was added to a solution of the acid chloride in CH_2Cl_2 (100 mL) and the reaction mixture was stirred for 6 h at room temperature. A solution of $NaHCO_3$ (5%, 120 mL) was added to quench the reaction and the aqueous phase was extracted with CH_2Cl_2 (3 x 100 mL). The organic phases were combined and washed with brine (2 x 100 mL), dried over Na_2SO_4, and solvent was removed. The product was isolated by column chromatography on silica gel with EtOH/CH_2Cl_2 2:8 as eluent. Yield 1.98g (74 %), m.p. 121-122 °C, elem. anal. calcd (%) for $C_{21}H_{19}Cl_2N_5O$: C 58.89, H 4.47, N 16.35, found: C 58.55,

H 4.57, N 16.06. ^{1}H NMR (400.13 MHz, $CDCl_3$) δ 8.41 (d, 1H, *J* = 8.1 Hz, H_{Ar}), 7.68 (brs, 1H; N-C*H*=N), 7.46 (d, 1H; *J* = 2.1 Hz, H_{Ar}), 7.31-7.45 (m, 3H; H_{Ar}),7.12 (brs, 1H, NH), 7.12 (dd, 1H, *J* = 8.5, 2.0 Hz, H_{Ar}), 7.10 (brs, 1H, C*H*=CH), 6.95 (brs, 1H, CH=C*H*), 6.66 (d, 1H, *J* = 8.5 Hz; H_{Ar}), 5.67 (s, 2H; CH_2), 4.11 (t, 2H, *J* = 6.5 Hz; NHCH_2-CH_2-CH_2-N), 3.58 (q, 2H, *J* = 6.5 Hz; NHCH_2-CH_2-CH_2-N), 2.11 (m, 2H, NHCH_2-CH_2-CH_2-N). ^{13}C{^{1}H} NMR (100.63 MHz, $CDCl_3$) δ 162.8 (CO), 141.2 (N=*C*-C(O)), 138.2 (C_{Ar}), 137.1 (N-*C*H=N), 134.5 (C_{Ar}), 133.2 (C_{Ar}), 132.3 (C_{Ar}), 129.6 (C_{Ar}), 129.5 (C_{Ar}), 129.4 (*C*H=CH), 127.6 (C_{Ar}), 127.5 (C_{Ar}), 123.1 (C_{Ar}), 122.9 (C_{Ar}), 118.90 (CH=*C*H), 109.3 (C_{Ar}), 50.0 (CH_2), 44.6 (NH-CH_2-CH_2-*C*H_2-N), 36.2 (NH-*C*H_2-CH_2-CH_2-N), 31.5 (NH-CH_2-*C*H_2-CH_2-N); ESI-MS: m/z: 428 [M+H^{+}]$^{+}$.

Synthesis of dichlorido(η^{6}-toluene)(N-(3-(1H-imidazol-1-yl)propyl)-1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamide) ruthenium(II), ***2***

To a solution of N-(3-(1H-Imidazol-1-yl)propyl)-1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamide (**1**) (814 mg, 1.9 mmol) in CH_2Cl_2 (30 mL), a solution of [(η^{6}-toluene)RuCl(μ-Cl)]$_2$ (483 mg, 0.91 mmol) in CH_2Cl_2 (50 mL) was added and the mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated to ~10 mL and pentane (150 mL) was added to precipitate the product. The orange solid was washed with pentane (2 x 20 mL) and ether (2 x 10 mL) and dried in vacuum. Yield 805 mg, (61 %). m.p. 174-175 °C decomp., elem. anal. calcd (%) for $C_{28}H_{27}Cl_4N_5ORu$: C 48.57, H 3.93, N 10.11, found: C 48.88, H 4.01, N 10.45.^{1}H NMR(400.13 MHz, $CDCl_3$) δ 8.35 (d, 1H, *J* = 8.1 Hz; H_{Ar}), 7.96 (brs, 1H; N-C*H*=*N*), 7.46-7.25 (m, 5 H; H_{Ar}, NH), 7.19 (brs, 1H; CH=C*H*), 6.75 (d, 1H, *J* = 8.2 Hz; H_{Ar}), 5.67 (s, 2H; CH_2), 5.56 (d, 2H; *J* = 5.0 Hz, H_{Ar}), 5.36 (d, 2H, *J* = 5.0 Hz; H_{Ar}), 4.02 (tr, 2H, *J* = 6.5 Hz; NHCH_2-CH_2-C*H*$_2$-N), 3.50 (q, 2 H, *J* = 6.5 Hz; NHC*H*$_2$-CH_2-CH_2-N), 2.23 (s, 3H; CH_3), 2.11 (m, 2H; NHCH_2-C*H*$_2$-CH_2-N); ^{13}C{^{1}H} NMR (100.63 MHz, $CDCl_3$) δ 162.9 (C(O)), 141.2 (N=*C*-C(O)), 140.4 (N-*C*H=N), 138.0 (C_{Ar}), 134.6 (C_{Ar}), 133.3 (C_{Ar}), 132.3 (C_{Ar}), 132.1 (C_{Ar}), 129.8 (C_{Ar}), 129.5 (CH=*C*H), 127.8 (C_{Ar}), 127.5 (C_{Ar}), 123.1 (C_{Ar}), 122.9 (C_{Ar}), 122.8 (C_{Ar}), 119.6 (*C*H=CH), 109.5 (C_{Ar}), 99.5 (C_{Ar}), 86.2 (C_{Ar}), 81.2 (C_{Ar}), 79.6 (C_{Ar}), 77.3 (C_{Ar}), 50.1 (CH_2), 45.5 (NH-CH_2-CH_2-*C*H_2-N), 35.7 (NH-*C*H_2-CH_2-CH_2-N), 31.0 (NH-CH_2-*C*H_2-CH_2-N), 19.1 (CH_3); ESI-MS: m/z: 658 [M-Cl^{-}]$^{+}$.

Synthesis of dichlorido(η^6-p-cymene)(N-(3-(1H-imidazol-1-yl)propyl)-1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamide) ruthenium(II), ***3***

To a solution of N-(3-(1H-Imidazol-1-yl)propyl)-1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxamide (**1**) (1.0 g, 2.34 mmol) in CH_2Cl_2 (30 mL), a solution of [(η^6-p-cymene)RuCl(μ-Cl)]$_2$ (685 mg, 1.12 mmol) in CH_2Cl_2 (50 mL) was added and the mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated to ~10 mL and pentane (150 mL) was added to precipitate the product. The orange solid was washed with pentane (2 x 20 mL) and ether (2 x 10 mL) and dried in vacuum. Yield 875 mg (51 %). m.p. 144-145 °C decomp., elem. anal. calcd (%) for $C_{31}H_{33}Cl_4N_5ORu$: C 50.69, H 4.53, N 9.53, found: C 50.38, H 4.52, N 9.26. ^{1}H NMR (400.13 MHz, CDCl3) δ 8.38 (d, 1H, *J* = 7.8 Hz; H_{Ar}), 7.96 (s, 1H; N-CH=N), 7.45 (d, 1H; *J* = 2.5 Hz; H_{Ar}), 7.44-7.28 (m, 4H; H_{Ar}, NH), 7.22 (brs, 1H; CH=C*H*), 7.16(dd, 1H, *J* = 8.7, 2.5 Hz, H_{Ar}), 6.97 (brs, 1H; C*H*=CH), 6.77 (d, 1H, *J* = 8.7 Hz; H_{Ar}), 5.69 (s, 2H; CH_2), 5.46 (d, 2H, *J* = 6.0 Hz; H_{Ar}), 5.23 (d, 2H, *J* = 6.0 Hz; H_{Ar}), 3.96 (tr, 2H, *J* = 6.8 Hz; NHCH$_2$-CH$_2$-C*H*$_2$-N), 3.47 (q, 2H, *J* = 6.8 Hz; NHC*H*$_2$-CH$_2$-CH$_2$-N), 2.97 (m, 1H; CH), 2.17 (s, 3H; CH_3), 2.06 (m, 2H, NHCH$_2$-C*H*$_2$-CH$_2$-N), 1.27 (d, 6H, *J* = 7.0 Hz; CH3); ^{13}C{^{1}H} NMR (100.63 MHz, $CDCl_3$) δ 162.9 (C(O)), 141.2 (N=*C*-C(O)), 140.2 (N-*C*H=N*)*, 138.0 (C_{Ar}), 134.9 (C_{Ar}), 133.4 (C_{Ar}), 132.3 (C_{Ar}), 131.9 (C_{Ar}), 129.8 (CH=*C*H), 129.6 (C_{Ar}), 127.9 (C_{Ar}), 127.1 (C_{Ar}), 123.0 (C_{Ar}), 119.5 (CH=*C*H), 109.4 (C_{Ar}), 102.6 (C_{Ar}), 97.7 (C_{Ar}), 82.8 (C_{Ar}), 81.4 (C_{Ar}), 53.5 (C_{Ar}), 50.1 (CH_2), 45.5 (NH-CH$_2$-CH$_2$-*C*H$_2$-N), 35.7 (NH-*C*H$_2$-CH$_2$-CH$_2$-N), 31.0 (CH), 30.7 (NH-CH$_2$-*C*H$_2$-CH$_2$-N), 22.3 (CH_3), 18.5(CH_3); ESI-MS: m/z: 700 [M-Cl$^-$]$^+$.

X-ray crystallography of 2 and 3

Data for **2** were collected at low temperature [140(2) K] using Mo K_α radiation on a mar345dtb system in combination with a Genix Hi-Flux small focus generator **(***mar**µ**X* system). The data reduction was carried out by *Automar*.[2] The diffraction data of **3** were measured at low temperature [100(2) K] using Mo K_α radiation on a Bruker APEX II CCD diffractometer equipped with a kappa geometry goniometer. The dataset was reduced by EvalCCD[3] and then corrected for absorption [4]. The solutions and refinement were performed by SHELX.[5] The crystal

structures were refined using full-matrix least-squares based on F^2 with all non hydrogen atoms anisotropically defined. Hydrogen atoms were placed in calculated positions by means of the "riding" model.

Crystal data and structure refinement for **2** and **3**.

	2	**3**
Empirical formula	$C_{30}H_{29}Cl_{10}N_5ORu$	$C_{34}H_{36}Cl_{13}N_5ORu$
Formula weight	931.15	1092.60
Temperature, K	140(2)	100(2)
Wavelength, Å	0.71073	0.71073
Crystal system	Triclinic	Triclinic
Space group	*P*-1	*P*-1
Unit cell dimensions	a = 10.029(2) Å α= 82.687(7)°. b = 10.7100(10) Å β= 87.155(10)° c = 17.939(2) Å γ = 78.307(9)°	a = 17.4680(9) Å α = 90.501(8)° b = 20.4319(15) Å β= 105.712(8) c = 21.6099(16) Å γ = 112.689(9)
Volume, $Å^3$	1870.9(5)	6794.0(8)
Z	2	6
Density (calculated), mg/m^3	1.653	1.602
Absorption coefficient, mm^{-1}	1.167	1.148
F(000)	932	3288
Crystal size, mm^3	0.36 x 0.21 x 0.17	0.36 x 0.18 x 0.14
Θ range for data collection	2.29 to 27.67°	3.01 to 25.00°
Index ranges	-12<=h<=12, -13<=k<=13, -23<=l<=23	-20<=h<=20, -24<=k<=24, -25<=l<=25
Reflections collected	14011	103178
Independent reflections	7900 [R(int) = 0.0211]	23373 [R(int) = 0.1045]
Completeness to Θ 25.00°	92.5 %	97.6 %
Absorption correction	None	Semi-empirical from equivalents
Max. and min. transmission		0.7452 and 0.6476
Refinement method	Full-matrix least-squares on F2	Full-matrix least-squares on F2
Data / restraints / parameters	7900 / 0 / 462	23373 / 60 / 1459
Goodness-of-fit on F^2	1.083	1.105
Final R indices [I > 2σ(I)]	R_1 = 0.0365, wR_2 = 0.0928	R_1 = 0.0622, wR_2 = 0.0817
R indices (all data)	R_1 = 0.0393, wR_2 = 0.0949	R_1 = 0.1202, wR_2 = 0.0967
Largest diff. peak and hole, e $Å^{-3}$	0.537 and -0.661 ·	1.089 and -1.014

Cell Culture and Inhibition of Cell Growth

Human A2780 and A2780cisR ovarian carcinoma cells were obtained from the European Centre of Cell Cultures (ECACC, Salisbury, UK) and maintained in culture as described by the provider.

Human LN229 glioblastoma cells are available from the ATCC (American Tissue Culture Collection, Manassas, VA, USA). Human LN18 and LNZ308 glioblastoma cells were a kind gift from AC Diserens, CHUV, Lausanne). Primary neuronal cultures from the cerebral cortex were obtained from embryos (E-18) of Wistar rats. Cultures were prepared according to a literature method.[6] Cultures were treated after the eight DIV with lonidamine or **1-3** at concentrations of 3 and 30 μM (single application without feeding for 72 h). Cell damage was assessed using the MTT assay. The cells were grown in Dulbecco's Modified Eagle Medium (DMEM) medium containing 4.5 g/L glucose, 5% heat-inactivated fetal calf serum (FCS) and penicillin/streptomycin (all cell culture reagents were obtained from Invitrogen, Basel, Switzerland). Unless otherwise specified, cells were grown for 24 h in 48-well plates (Costar, Corning, NY, USA), then the compounds (stock solution in DMSO) were added for the indicated times and concentrations. DMSO final concentration never exceeded 1%; at concentrations below 1% DMSO has no effect on cell survival (results not shown). Following exposure to the compounds, cell viability was assessed using the MTT assay (3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich, 200 μg/ml final concentration. Absorbance at 540 nm was measured in a multi-well plate reader (iEMS Reader, Labsystems, Bioconcept, Allschwil, Switzerland) and the absorbance values of treated cells were compared to the absorbance values of untreated cells. Experiments were conducted in duplicate wells and repeated at least twice.

References

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