Electronic Supplementary Material (ESI) for Chemical Communications

SUPPORTING INFORMATION

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

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1. Materials and methods

All solvents were dried and distilled prior to use. 2-Hydroxyacetophenone **1** (Fluka, Acros Organics), benzaldehyde **a** (Fluka), 4-tolualdehyde **b** (Acros Organics), 4-fluorobenzaldehyde **c** (Fluka), 4-chlorobenzaldehyde **d** (Acros Organics), ruthenium(III) chloride (Johnson Matthey), α-terpinene (Acros Organics), sodium methoxide (Aldrich) were purchased and used without further purification. Bis[(η^6-*p*-cymene)dichloridoruthenium(II)] was synthesized as described elsewhere.[1]

Melting points were determined with a Büchi *Melting Point* B-540 apparatus. Elemental analyses were carried out with a Perkin Elmer 2400 CHN Elemental Analyser at the Microanalytical Laboratory of the University of Vienna. NMR spectra were recorded at 25 °C using a Bruker FT-NMR spectrometer Avance III^{TM} 500 MHz. ^{1}H NMR spectra were measured in d_6-DMSO or $CDCl_3$ at 500.10 MHz and $^{13}C\{^1H\}$ NMR spectra at 125.75 MHz. The 2D NMR spectra were recorded in a gradient-enhanced mode. The X-ray diffraction measurements were performed with single crystals of **3b**·CH_3OH using a Bruker X8 APEXII CCD diffractometer at 100 K. The single crystal was positioned at 40 mm from the detector, and 1541 frames were measured, each for 80 s over 1° scan width. The data were processed using the SAINT software package.[2] The structure was solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were inserted at calculated positions and refined with a riding model. The following computer programs were used: structure solution, SHELXS-97; refinement, SHELXL-97;[3] molecular diagrams, ORTEP-3;[4] scattering factors.[5]

2. Synthetic procedures

2.1. Synthesis of 3-hydroxyflavones

General procedure: NaOH (5 M, 4.3 eq) was added to a solution of 2'-hydroxyacetophenone **1** and aldehyde **a-d** in ethanol and the mixture was stirred for 18 h at room temperature. The reaction mixture was acidified by addition of acetic acid (30%) and the 2'-hydroxychalcone was isolated by filtration. The 2'-hydroxychalcone was suspended in ethanol, and NaOH (5 M, 2 eq) and H_2O_2 (30%, 2.2 eq) were added at 4 °C. The mixture was stirred for 18 h at room temperature, afterwards acidified with HCl (2 M) and poured into water (400 mL). The precipitate was collected by filtration and the pure product was obtained by recrystallization from methanol.

3-Hydroxy-2-phenyl-4H-chromen-4(1H)-one (2a): The synthesis was performed according to the general procedure using **1** (2.00 g, 14.7 mmol, 1 eq) and **a** (1.56 g, 14.7 mmol, 1 eq) to

afford **2a** as a yellow powder (2.63 g, 75%). Mp: 165–169 °C; ^{1}H NMR (500.10 MHz, d_6-DMSO): δ = 7.49–7.54 (m, 2H, H4'/H7), 7.59 (dd, ^{3}J(H,H) = 7 Hz, ^{3}J(H,H) = 7 Hz, 2H, H3'/H5'), 7.79 (d, ^{3}J(H,H) = 7 Hz, 1H, H8), 7.83–7.81 (m, 1H, H6), 8.14 (dd, ^{4}J(H,H) = 1 Hz, ^{3}J(H,H) = 8 Hz, 1H, H5), 8.24 (d, ^{3}J(H,H) = 7 Hz, 2H, H2'/H6'), 9.64 (br s, 1H, OH) ppm; ^{13}C{^{1}H} NMR (125.75 MHz, d_6-DMSO): δ = 118.9 (C8), 121.8 (C8a), 125.1 (C7), 125.3 (C5), 128.1 (C2'/C6'), 129.0 (C3'/C5'), 130.4 (C4'), 131.8 (C2), 134.2 (C6), 139.6 (C1'), 145.7 (C3), 155.1 (C4a), 173.5 (C4) ppm; elemental analysis calcd for $C_{15}H_{10}O_3$: C 75.62, H 4.23%; found: C 75.62, H 4.09%.

3-Hydroxy-2-(4-methylphenyl)-4H-chromen-4(1H)-one (2b): The synthesis was performed according to the general procedure using **1** (2.00 g, 14.7 mmol, 1 eq) and **b** (1.76 g, 14.7 mmol, 1 eq) to afford **2b** as yellow crystals (1.64 g, 44%). Mp: 191–195 °C; ^{1}H NMR (500.10 MHz, d_6-DMSO): δ = 2.41 (s, 3H, CH_3), 7.40 (d, ^{3}J(H,H) = 8 Hz, 2H, H3'/H5'), 7.47–7.50 (m, 1H, H7), 7.79 (d, ^{3}J(H,H) = 7 Hz, 1H, H8), 7.79–7.82 (m, 1H, H6), 8.14 (dd, ^{4}J(H,H) = 1 Hz, ^{3}J(H,H) = 8 Hz, 1H, H5), 8.16 (d, ^{3}J(H,H) = 8 Hz, 2H, H2'/H6'), 9.56 (br s, 1H, OH) ppm; ^{13}C{^{1}H} NMR (125.75 MHz, d_6-DMSO): δ = 21.5 (CH_3), 118.9 (C8), 121.7 (C8a), 125.0 (C7), 125.3 (C5), 128.1 (C2'/C6'), 129.1 (C2), 129.6 (C3'/C5'), 134.1 (s, C6), 139.4 (C1'), 140.3 (C4'), 145.9 (C3), 155.1 (C4a), 173.4 (C4) ppm; elemental analysis calcd for $C_{16}H_{12}O_3 \cdot 0.15H_2O$: C 75.37, H 4.86%; found: C 75.38, H 4.47%.

3-Hydroxy-2-(4-fluorophenyl)-4H-chromen-4(1H)-one (2c): The synthesis was performed according to the general procedure using **1** (2.00 g, 14.7 mmol, 1 eq) and **c** (1.17 g, 14.7 mmol, 1 eq) to afford **2c** as yellow needles (1.65 g, 44%). Mp: 151–152 °C; ^{1}H NMR (500.10 MHz, d_6-DMSO): δ = 7.42–7.45 (m, 2H, H3'/H5'), 7.47–7.50 (m, 1H, H7), 7.79 (d, ^{3}J(H,H) = 7 Hz, 1H, H8), 7.81–7.83 (m, 1H, H6), 8.13 (dd, ^{4}J(H,H) = 1 Hz, ^{3}J(H,H) = 8 Hz, 1H, H5), 8.29–8.32 (m, 2H, H2'/H6'), 9.72 (br s, 1H, OH) ppm; ^{13}C{^{1}H} NMR (125.75 MHz, d^6-DMSO): δ = 116.1 (d, ^{2}J(H,H) = 22 Hz, C3'/C5'), 118.9 (C8), 121.8 (C8a), 125.1 (C7), 125.3 (C5), 128.3 (d, ^{4}J(C,F) = 3 Hz, C1'), 130.6 (d, ^{3}J(C,F) = 8 Hz, C2'/C6'), 134.2 (C6), 139.3 (C2), 144.9 (C3), 155.0 (C4a), 163.0 (d, ^{1}J(C,F) = 249 Hz, C4'), 173.4 (C4) ppm; elemental analysis calcd for $C_{15}H_9O_3F \cdot 0.15H_2O$: C 69.58, H 3.62%; found: C 69.66, H 3.47%.

3-Hydroxy-2-(4-chlorophenyl)-4H-chromen-4(1H)-one (2d): The synthesis was performed according to the general procedure using **1** (2.00 g, 14.7 mmol, 1 eq) and **d** (2.10 g, 14.7 mmol, 1 eq) to afford **2d** as yellow powder (2.31 g, 58%). Mp: 202–204 °C; ^{1}H NMR (500.10 MHz, d_6-DMSO):): δ = 7.49 (m, 1H, H7), 7.65 (d, ^{3}J(H,H) = 8 Hz, 2H, H3'/H5'), 7.78 (d, ^{3}J(H,H) = 8 Hz, 1H, H8), 7.83–7.85 (m, 1H, H6), 8.13 (dd, ^{4}J(H,H) = 1 Hz, ^{3}J(H,H) = 8 Hz, 1H, H5), 8.26 (d, ^{3}J(H,H) = 9 Hz, 2H, H2'/H6'), 9.84 (br s, 1H, OH) ppm; ^{13}C{^{1}H} NMR (125.75 MHz, d^6-DMSO): δ = 118.9 (C8), 121.7 (C8a), 125.1 (C7), 125.3 (C5), 129.2 (C2'/C6'), 129.8 (C3'/C5'), 130.7 (C2), 134.4 (C6), 139.9 (C4'), 140.3 (C1'), 144.5 (C3), 155.0 (C4a), 173.5 (C4) ppm; elemental analysis calcd for $C_{15}H_9O_3Cl \cdot 0.05H_2O$: C 65.85, H 3.35%; found: C 65.80, H 3.17%.

2.2. Synthesis of ruthenium(II)(η^6-*p*-cymene) complexes

General procedure: A solution of [Ru(η^6-*p*-cymene)Cl_2]$_2$ in CH_2Cl_2 (15 mL) was added to a solution of the 3-hydroxyflavone ligands **2a**–**d** and sodium methoxide in methanol (15 mL). The reaction mixture was stirred at room temperature and under argon atmosphere for 18 h. The solvent was evaporated in vacuum; the residue was extracted with dichloromethane, filtered and concentrated. Pure complex was obtained by recrystallization from methanol.

Chlorido[3-(oxo-κ*O*)-2-phenyl-chromen-4(*1H*)-onato-κ*O*](*η*^{6}-*p*-cymene)ruthenium(II) (3a): The reaction was performed according to the general complexation procedure using **2a** (164 mg, 0.73 mmol, 1 eq), NaOMe (43 mg, 0.8 mmol, 1.1 eq) and [Ru(η^6-*p*-cymene)Cl_2]$_2$ (200 mg, 0.33 mmol, 0.45 eq) to afford **3a** as a deep red powder (170 mg, 51%). Mp: 229–230 °C (decomp.); ^{1}H NMR (500.10 MHz, $CDCl_3$): δ = 1.42 (m, 6H, $CH_{3,Cym}$), 2.43 (s, 3H, $CH_{3,Cym}$), 3.02 (m, 1H, CH_{Cym}), 5.39 (dd, ^3J(H,H) = 5 Hz, ^3J(H,H) = 5 Hz, 2H, H3/H5$_{Cym}$), 5.67 (dd, ^3J(H,H) = 5 Hz, ^3J(H,H) = 5 Hz, 2H, H2/H6$_{Cym}$), 7.33–7.35 (m, 1H, H7), 7.41 (dd, ^3J(H,H) = 7 Hz, ^3J(H,H) = 7 Hz, 1H, H4'), 7.48 (dd, ^3J(H,H) = 7 Hz, ^3J(H,H) = 7 Hz, 2H, H3'/H5'), 7.57 (d, ^3J(H,H) = 8 Hz, 1H, H8), 7.59–7.61 (m, 1H, H6), 8.22 (dd, ^4J(H,H) = 1 Hz, ^3J(H,H) = 8 Hz, 1H, H5), 8.61 (d, ^3J(H,H) = 7 Hz, 2H, H2'/H6') ppm; ^{13}C{^{1}H} NMR (125.75 MHz, $CDCl_3$): δ = 18.7 ($CH_{3,Cym}$), 22.5 ($CH_{3,Cym}$), 31.3 (CH_{Cym}), 78.0 (C3/C5$_{Cym}$), 81.0 (C2/C6$_{Cym}$), 95.9 (C4$_{Cym}$), 98.9 (C1$_{Cym}$), 117.9 (C8), 120.0 (C8a), 124.0 (C7), 124.6 (C5), 127.3 (C2'/C6'), 128.2 (C3'/C5'), 129.3 (C4'), 132.5 (C2), 132.6 (C6), 149.2 (C1'), 153.9 (C4a), 154.0 (C3), 183.3 (C4) ppm; elemental analysis calcd for $C_{25}H_{23}O_3ClRu$: C 59.11, H 4.56%; found: C 59.04, H 4.39%.

Chlorido[3-(oxo-κ*O*)-2-(4-methylphenyl)-chromen-4(*1H*)-onato-κ*O*](*η*^{6}-*p*-cymene)ruthenium(II) (3b): The reaction was performed according to the general complexation procedure using **2b** (184 mg, 0.73 mmol, 1 eq), NaOMe (43 mg, 0.8 mmol, 1.1 eq) and [Ru(η^6-*p*-cymene)Cl_2]$_2$ (200 mg, 0.33 mmol, 0.45 eq) to afford **3b** as a red powder (240 mg, 68%). Single crystals were grown from $CHCl_3$/*n*-hexane, suitable for X-ray diffraction analysis. Mp: 235–236 °C (decomp.); ^{1}H NMR (500.10 MHz, $CDCl_3$): δ = 1.41 (m, 6H, $CH_{3,Cym}$), 2.42 (s, 3H, $CH_{3,Cym}$), 2.44 (s, 3H, CH_3), 3.01 (m, 1H, CH_{Cym}), 5.38 (dd, ^3J(H,H) = 5 Hz, ^3J(H,H) = 5 Hz, 2H, H3/H5$_{Cym}$), 5.65 (dd, ^3J(H,H) = 6 Hz, ^3J(H,H) = 6 Hz, 2H, H2/H6$_{Cym}$), 7.28 (d, ^3J(H,H) = 9 Hz, 2H, H3'/H5'), 7.32–7.35 (m, 1H, H7), 7.55 (d, ^3J(H,H) = 8 Hz, 1H, H8), 7.58–7.61 (m, 1H, H6), 8.21 (dd, ^4J(H,H) = 1 Hz, ^3J(H,H) = 8 Hz, 1H, H5), 8.50 (d, ^3J(H,H) = 8 Hz, 2H, H2'/H6') ppm; ^{13}C{^{1}H} NMR (125.75 MHz, $CDCl_3$): δ = 18.7 ($CH_{3,Cym}$), 21.6 (CH_3), 22.5 ($CH_{3,Cym}$), 31.2 (CH_{Cym}), 77.9 (C3/C5$_{Cym}$), 80.9 (C2/C6$_{Cym}$), 95.9 (C4$_{Cym}$), 98.9 (C1$_{Cym}$), 117.8 (C8), 120.4 (C8a), 124.0 (C7), 124.5 (C5), 127.3 (C2'/C6'), 129.0 (C3'/C5'), 130.0 (C2), 132.6 (C6), 139.8 (C4'), 149.9 (C1'), 153.7 (C4a), 154.2 (C3), 183.1 (C4) ppm; elemental analysis calcd for $C_{26}H_{25}O_3ClRu$: C 59.82, H 4.83%; found: C 59.82, H 4.57%.

Chlorido[3-(oxo-κ*O*)-2-(4-fluorophenyl)-chromen-4(*1H*)-onato-κ*O*](*η*^{6}-*p*-cymene)ruthenium(II) (3c): The reaction was performed according to the general

complexation procedure using **2c** (187 mg, 0.73 mmol, 1 eq), NaOMe (43 mg, 0.8 mmol, 1.1 eq) and [Ru(η^6-*p*-cymene)Cl_2]$_2$ (200 mg, 0.33 mmol, 0.45 eq) to afford **3c** as red needles (230 mg, 66%). Mp: 235–236 °C (decomp.); ^{1}H NMR (500.10 MHz, $CDCl_3$): δ = 1.42 (m, 6H, $CH_{3,Cym}$), 2.42 (s, 3H, $CH_{3,Cym}$), 3.00 (m, 1H, CH_{Cym}), 5.40 (dd, ^3J(H,H) = 5 Hz, ^3J(H,H) = 5 Hz, 2H, H3/H5$_{Cym}$), 5.66 (dd, ^3J(H,H) = 5 Hz, ^3J(H,H) = 5 Hz, 2H, H2/H6$_{Cym}$), 7.15–7.18 (m, 2H, H3'/H5'), 7.33–7.36 (m, 1H, H7), 7.54 (d, ^3J(H,H) = 8 Hz, 1H, H8), 7.60–7.62 (m, 1H, H6), 8.22 (dd, ^4J(H,H) = 1 Hz, ^3J(H,H) = 8 Hz, 1H, H5), 8.61–8.64 (m, 2H, H2'/H6') ppm; ^{13}C{^{1}H} NMR (125.75 MHz, $CDCl_3$): δ = 18.7 ($CH_{3,Cym}$), 22.5 ($CH_{3,Cym}$), 31.3 (CH_{Cym}), 78.0 (C3/C5$_{Cym}$), 81.0 (C2/C6$_{Cym}$), 95.9 (C4$_{Cym}$), 98.9 (C1$_{Cym}$), 115.2 (d, ^2J(C,F) = 21 Hz, C3'/C5'), 117.7 (C8), 120.1 (C8a), 124.1 (C7), 124.6 (C5), 128.8 (C2), 129.4 (d, ^3J(C,F) = 8 Hz, C2'/C6'), 132.6 (C6), 148.4 (d, ^4J(C,F) = 1 Hz, C1'), 153.8 (C4a), 154.1 (C3), 163.0 (d, ^1J(C,F) = 251 Hz, C4'), 183.3 (C4) ppm; elemental analysis calcd for $C_{25}H_{22}O_3FClRu$: C 57.09, H 4.22%; found: C 56.98, H 4.06%.

Chlorido[3-(oxo-κ*O*)-2-(4-chlorophenyl)-chromen-4(*1H*)-onato-κ*O*](*η*^{6}-*p*-cymene)ruthenium(II) (3d): The reaction was performed according to the general complexation procedure using **2d** (199 mg, 0.73 mmol, 1 eq), NaOMe (43 mg, 0.8 mmol, 1.1 eq) and [Ru(η^6-*p*-cymene)Cl_2]$_2$ (200 mg, 0.33 mmol, 0.45 eq) to afford **3d** as a deep red powder (179 mg, 100%). Mp: 214–217 °C (decomp.); ^{1}H NMR (500.10 MHz, $CDCl_3$): δ = 1.42 (m, 6H, $CH_{3,Cym}$), 2.42 (s, 3H, $CH_{3,Cym}$), 3.01 (m, 1H, CH_{Cym}), 5.39 (dd, ^3J(H,H) = 5 Hz, ^3J(H,H) = 5 Hz, 2H, H3/H5$_{Cym}$), 5.66 (dd, ^3J(H,H) = 5 Hz, ^3J(H,H) = 5 Hz, 2H, H2/H6$_{Cym}$), 7.32–7.35 (m, 1H, H7), 7.44 (d, ^3J(H,H) = 9 Hz, 2H, H3'/H5'), 7.54 (d, ^3J(H,H) = 8 Hz, 1H, H8), 7.62–7.63 (m, 1H, H6), 8.21 (dd, ^4J(H,H) = 1 Hz, ^3J(H,H) = 8 Hz, 1H, H5), 8.55 (d, ^3J(H,H) = 9 Hz, 2H, H2'/H6') ppm; ^{13}C{^{1}H} NMR (125.75 MHz, $CDCl_3$): δ = 18.7 ($CH_{3,Cym}$), 22.4 ($CH_{3,Cym}$), 30.0 (CH_{Cym}), 77.9 (C3/C5$_{Cym}$), 81.0 (C2/C6$_{Cym}$), 95.9 (C4$_{Cym}$), 99.0 (C1$_{Cym}$), 117.8 (C8), 120.0 (C8a), 124.1 (C7), 124.7 (C5), 125.5 (C2'/C6'/C3'/C5'), 131.0 (C2), 132.8 (C6), 134.9 (C4'), 143.5 (C1'), 153.9 (C4a), 154.6 (C3), 183.5 (C4); elemental analysis calcd for $C_{25}H_{22}O_3Cl_2Ru$: C 55.36, H 4.09%; found: C 55.28, H 3.90%.

3. Biological and biophysical experiments

3.1. Cytotoxicity in cancer cell lines

3.1.1. Cell lines and culture conditions

CH1 cells originate from an ascites sample of a patient with a papillary cystadenocarcinoma of the ovary and were a generous gift from Lloyd R. Kelland, CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, UK. SW480 (human adenocarcinoma of the colon) and A549 (human non-small cell lung cancer) cells were kindly provided by Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria). All cell culture reagents were obtained from Sigma-Aldrich Austria. Cells were grown in 75 cm² culture flasks (Iwaki) as adherent monolayer cultures in Minimum Essential Medium (MEM) supplemented with 10% heat inactivated fetal calf serum, 1 mM sodium pyruvate, 4 mM L-glutamine and 1% nonessential amino acids (100x). Cultures were maintained at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2.

3.1.2. MTT assay

Cytotoxicity was determined by the colorimetric MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, Fluka] microculture assay. For this purpose, cells were harvested from culture flasks by trypsinization and seeded in 100 µL aliquots into 96-well microculture plates (Iwaki). Cell densities of 1.5×10^3 cells/well (CH1), 2.5×10^3 cells/well (SW480) and 4×10^3 cells/well (A549) were chosen in order to ensure exponential growth of untreated controls throughout the experiment. Cells were allowed to settle and resume exponential growth in drug-free complete culture medium for 24 h. Stock solutions of the test compounds in DMSO were appropriately diluted in complete culture medium so that the maximum DMSO content did not exceed 1%. These dilutions were added in 100 µL aliquots to the microcultures and cells were exposed to the test compounds for 96 hours. At the end of exposure, all media were replaced by 100 µL/well RPMI1640 culture medium (supplemented with 10% heat-inactivated fetal bovine serum) plus 20 µL/well MTT solution in phosphate-buffered saline (5 mg/ml). After incubation for 4 h, the supernatants were removed, and the formazan crystals formed by viable cells were dissolved in 150 µL DMSO per well. Optical densities at 550 nm were measured with a microplate reader (Tecan Spectra Classic), using a reference wavelength of 690 nm to correct for unspecific absorption. The quantity of viable cells was expressed in terms of T/C values by comparison to untreated controls, and 50% inhibitory concentrations (IC_{50}) were calculated from concentration-effect curves by interpolation. Evaluation is based on means from at least three independent experiments, each comprising at least three replicates per concentration level.

3.2. Interaction with the DNA model compound 5'-GMP

Complexes **3a–d** (~0,1 mg/mL) were dissolved in D_2O (containing 10% d_6-DMSO due to the low solubility in water), yielding the corresponding highly reactive aqua species. The aqua complexes were converted *in situ* by addition of 100 µL of 5'-GMP solution (10 mg/mL) into the respective 5'-GMP adduct and the reaction was monitored by ^{1}H NMR.

3.3. Determination of topoisomerase II activity

Effects on the catalytic activity of topoisomerase II were determined using a decatenation assay. Catenated kinetoplast DNA (kDNA) was used as a substrate. kDNA is an aggregate of interlocked DNA minicircles (mostly 2.5 kb), which can be released by topoisomerase II. kDNA (200 ng, TopoGen, OH, USA) was incubated in a final volume of 30 µL (containing 40 ng of topoisomerase IIα; 50 mM Tris, pH 7.9; 120 mM KCl; 10 mM $MgCl_2$; 1 mM ATP; 0.5 mM DTT; 0.5 mM EDTA; and 0.03 mg/mL BSA) at 37 °C for 60 min. The reaction was stopped by the addition of 1/10 volume of 1 mg/mL proteinase K in 10% (w/v) SDS and incubation at 37 °C for further 30 min. Gel electrophoresis was performed in the absence of ethidium bromide at 60 V for 3 h in 1% (w/v) agarose gels with Tris acetate/EDTA buffer (40 mM Tris; 1 mM EDTA, pH 8.5; and 20 mM acetic acid). Subsequently, the gel was stained in 10 µg/mL ethidium bromide solution for 20 min. The fluorescence of ethidium bromide was detected with the LAS-4000 system (Fujifilm, Raytest, Germany).

3.4. Live cell imaging

Live cell images of SW480 colon carcinoma cells were obtained with a confocal laser scanning microscope (CSLM, Leica) at the Institution of Cell Imaging and Ultrastructure Research, University of Vienna, Austria. Cells were pretreated with 600 µM of **3c** for 5 min at 37 °C, and pictures were taken after excitation with a 458 nm laser. Co-staining experiments were performed with ER-Tracker™ Red (Invitrogen, Paisely, UK) at a concentration of 1 µM according to the protocol given by the manufacturer. ER-Tracker™ Red was chosen, because the excitation spectrum of this dye is not interfering with spectra of compound **3c**.

4. Acknowledgement

We are grateful to Prof. Irene Lichtscheidl (Institution of Cell Imaging and Ultrastructure Research, University of Vienna, Austria) for providing access to the confocal laser scanning microscopic equipment.

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