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Supplementary Information

Fluorescence Properties and Cellular Distribution of the Investigational Anti-cancer Drug Triapine (3-Aminopyridine-2-carboxaldehyde Thiosemicarbazone) and its Zinc(II) Complex

Christian R. Kowol,[‡] *Robert Trondl,*[‡] *Vladimir B. Arion,*[‡,*] *Michael A. Jakupec,*[‡] *Irene Lichtscheidl,*[†] *Bernhard K. Keppler*[‡,*]

[‡]University of Vienna, Institute of Inorganic Chemistry, Währingerstr. 42, A-1090 Vienna, Austria

[†]University of Vienna, Institution of Cell Imaging and Ultrastructure Research, Althanstr. 14, A-1090 Vienna, Austria

Experimental Section

All solvents and reagents were obtained from commercial suppliers and used without further purification. Triapine was synthesized by a three-step synthesis in 64% overall yield.[1] Elemental analyses were carried out on a Carlo Erba microanalyzer at the Microanalytical Laboratory of the University of Vienna. Electrospray ionization mass spectrometry was carried out with a Bruker Esquire 3000 instrument (Bruker Daltonic, Bremen, Germany). Expected and experimental isotope distributions were compared. Infrared spectra were obtained from KBr pellets with a Perkin-Elmer FT-IR 2000 instrument (4000–400 cm^{-1}). UV–vis spectra were recorded on a Perkin Elmer Lambda 650 UV–vis spectrophotometer. Fluorescence emission spectra were recorded on a Perkin-Elmer LS 55 fluorescence spectrophotometer. Emission spectra were corrected using the published data from NIST for quinine sulfate dihydrate in 0.1 M $HClO_4$.[2] The fluorescence quantum yields were determined using the formula:

$$\phi_{\text{sample}} = \frac{A_{\text{ref}} \cdot I \cdot n^2}{A \cdot I_{\text{ref}} \cdot n_{\text{ref}}^2} \phi_{\text{ref}}$$

where quinine sulfate dihydrate in 0.1 M $HClO_4$ is the reference ($\phi_{\text{ref}} = 0.59$)[2], A the integrated area under the emission spectrum, I the absorbance at the excitation wavelength and n the refractive index of the solvent. The highest concentration of the samples was 5 µM so that the absorbance at the excitation wavelength (360 nm) did not exceed 0.1. ^{1}H and ^{13}C one- and two-dimensional NMR spectra were recorded in DMSO-d_6 with a Bruker Avance III 500 MHz FT-NMR spectrometer. The residual ^{1}H and ^{13}C present in DMSO-d_6 were used as internal references. Abbreviations for NMR data: py = pyridine, $C_{q,\ py}$ = quaternary carbon of pyridine.

Crystallographic Structure Determination. X-ray diffraction measurements were performed on a Bruker X8 APPEXII CCD diffractometer. A single crystal of suitable size was coated with Paratone-N oil, mounted at room temperature on the tip of a glass fiber and cooled under a stream of cold N_2 maintained by a KRYOFLEX low-temperature apparatus. The crystal was positioned at 40 mm from the

detector, and 2667 frames were measured, each for 20 s over 1° scan width. The data were processed using SAINT software.[3] The structures were solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were placed at calculated positions and refined as riding atoms in the subsequent least squares model refinements. The isotropic thermal parameters were estimated to be 1.2 times the values of the equivalent isotropic thermal parameters of the atoms to which hydrogens were bound. The following computer programs were used: structure solution, SHELXS-97;[4] refinement, SHELXL-97;[5] molecular diagrams, ORTEP;[6] computer: Pentium IV; scattering factors.[7] Crystal data and structure refinement details for **[Zn(Triapine)Cl$_2$]·EtOH** are given in Table S1.

Table S1. Crystal Data for **[Zn(Triapine)Cl$_2$]·EtOH**

	[Zn(Triapine)Cl$_2$]·EtOH
Empirical formula	$C_9H_{15}Cl_2N_5OSZn$
Formula weight	377.61
Space group	$P2_1/c$
a [Å]	11.4639(7)
b [Å]	10.2716(5)
c [Å]	12.7509(7)
α [deg]	
β [deg]	99.422(4)
γ [deg]	
V [Å^3]	1481.20(14)
Z	4
λ [Å]	0.71073
ρ_{calcd} [g cm^{-3}]	1.693
Crystal size [mm]	0.20 × 0.10 × 0.03
T [K]	100
μ [mm^{-1}]	2.158
R_1[a]	0.0274
wR_2[b]	0.0642
GOF[c]	1.005

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$. [c] GOF $= \{\Sigma[w(F_o^2 - F_c^2)^2]/(n - p)\}^{1/2}$, where n is the number of reflections and p is the total number of parameters refined.

Cell Culture. Human 41M (ovarian carcinoma) and SW480 (colon carcinoma) cells were kindly provided by Lloyd R. Kelland (CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, UK) and Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria), respectively. Cells were grown in 75 cm² culture flasks (Iwaki/Asahi Technoglass, Gyouda, Japan) as adherent monolayer cultures in complete culture medium, i. e. Minimal Essential Medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 4 mM L-glutamine and 1% non-essential amino acids (100×) (all purchased from Sigma-Aldrich, Vienna, Austria) without antibiotics. For fluorescence microscopy, cells were grown in 6-well plates on sterile cover slips. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air.

Cytotoxicity Tests in Cancer Cell Lines. Antiproliferative effects were determined by means of a colorimetric microculture assay (MTT assay, MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide). Cells were harvested from culture flasks by trypsinization and seeded in 100 µL aliquots into 96-well microculture plates (Iwaki/Asahi Technoglass, Gyouda, Japan) in densities of 4×10^3 cells/well (41M) and 2.5×10^3 cells/well (SW480), respectively in order to ensure exponential growth of untreated controls throughout the experiment. After a 24 h pre-incubation, dilutions of the test compounds in 100 µL/well complete culture medium were added. Because of low aqueous solubility, the test compounds were dissolved in DMSO first and then serially diluted in complete culture medium such that the effective DMSO content did not exceed 0.5%. After exposure for 96 h, all media were replaced by 100 µL/well RPMI 1640 medium (supplemented with 10% heat-inactivated fetal bovine serum and 2 mM L-glutamine) plus 20 µL/well MTT solution in phosphate-buffered saline (5 mg/ml). After incubation for 4 h, the medium/MTT mixtures were removed, and the formazan crystals formed by vital 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 vital cells was expressed in terms of T/C values by comparison to untreated control microcultures, 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.

Fluorescence Microscopy. Fluorescence properties of Triapine and $[Zn(Triapine)Cl_2]{\cdot}HCl$ were observed under UV irradiation (360–370 nm) with an Olympus BX41 microscope equipped with 60x and 100x planapochromate oil-immersion objectives and a ColorViewIII camera. Images ware taken with several concentrations, resulting always in the same distribution within the cells. Exposure time was adjusted so that almost all auto-fluorescence of cells could be excluded, and excitation time was kept as short as possible to minimize irradiation impact on cells.

Immunocytochemistry. Cells were cultured on cover slides and fixed with 4% formaldehyde. After fixation, cells were permeabilized with 0.3% Triton X-100 solution and then treated for 1 h with 5% serum diluted in PBS to block non-specific binding. The primary antibody against fibrillarin (1:400, monoclonal Rabbit, New England Biolabs GmbH, Frankfurt am Main, Germany) bound overnight at 4 °C. After washing with PBS, cells were incubated with the secondary fluorochrome conjugated antibody (1:500, Alexa Fluor 594 goat anti-rat IgG, Invitrogen, Karlsruhe, Germany) for 1 h at room temperature. Cells were washed, and the secondary antibody was localized by fluorescence microscopy (Olympus CKX41, Tokyo, Japan).

UV/vis kinetics

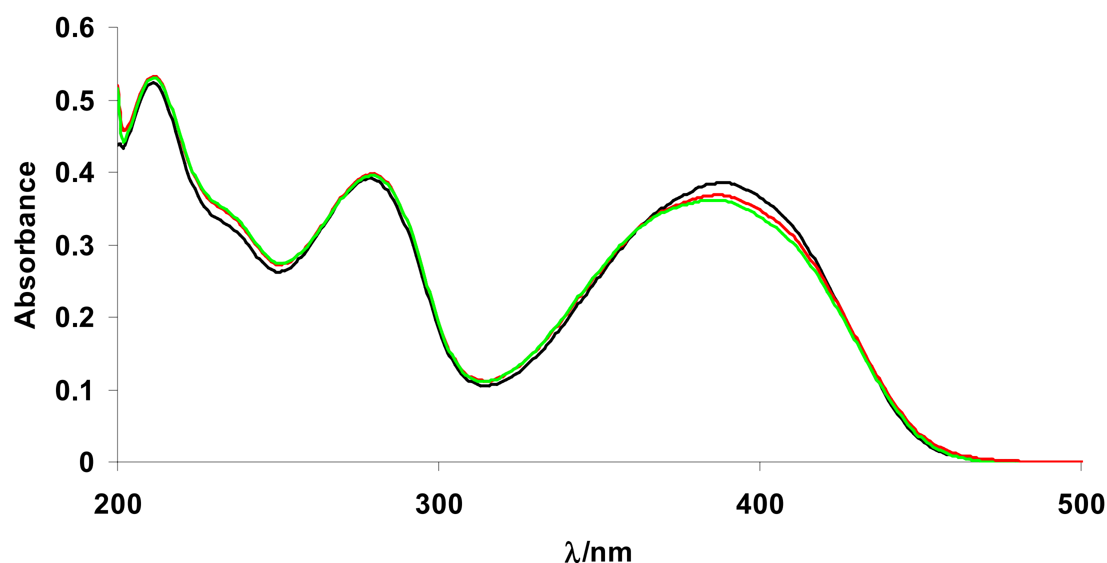

Figure S1. UV/vis spectroscopy data of **1** in phosphate buffered solution at pH 7.4 containing 0.9% NaCl. First measurement (black), after 5 h (red) and after 15 h (green).

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