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Supporting Information

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Supporting Information

for

Enhanced Cellular Uptake and Cytotoxicity Studies of Organometallic Bioconjugates with the NLS Peptide in HepG2 Cells

Fozia Noor, Ralf Kinscherf, Gabriel A. Bonaterra, Steffen Walczak, Stefan Wölfl, and Nils Metzler-Nolte*

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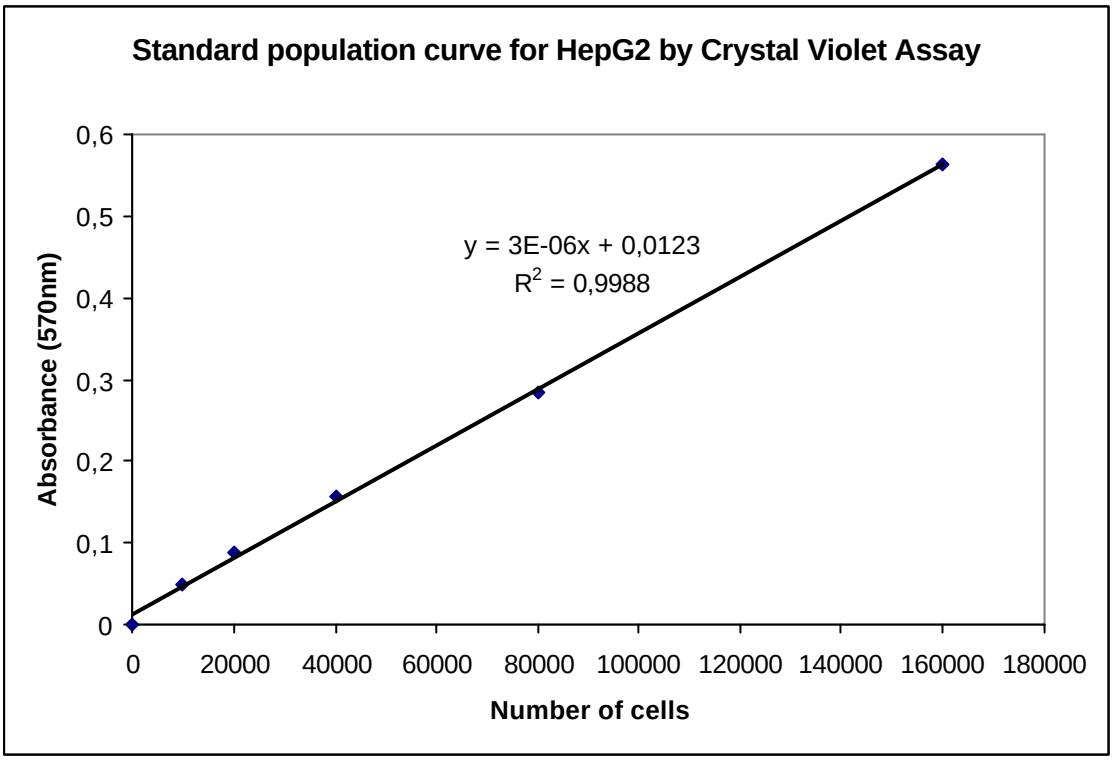

Figure S1. Standard population curve for Hep G2 cells as determined by crystal violet cell quantification method for determination of optimal number of cells for the assay.

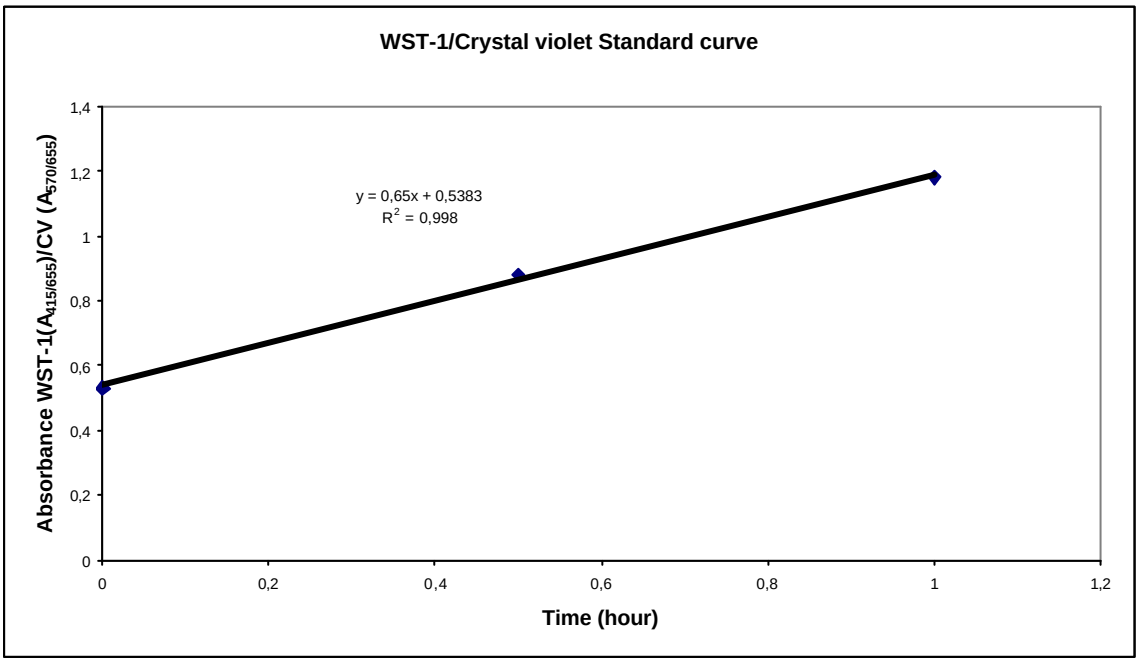

Figure S2. Standard curve for optimal time of incubation with WST-1 for Hep G2 cells.

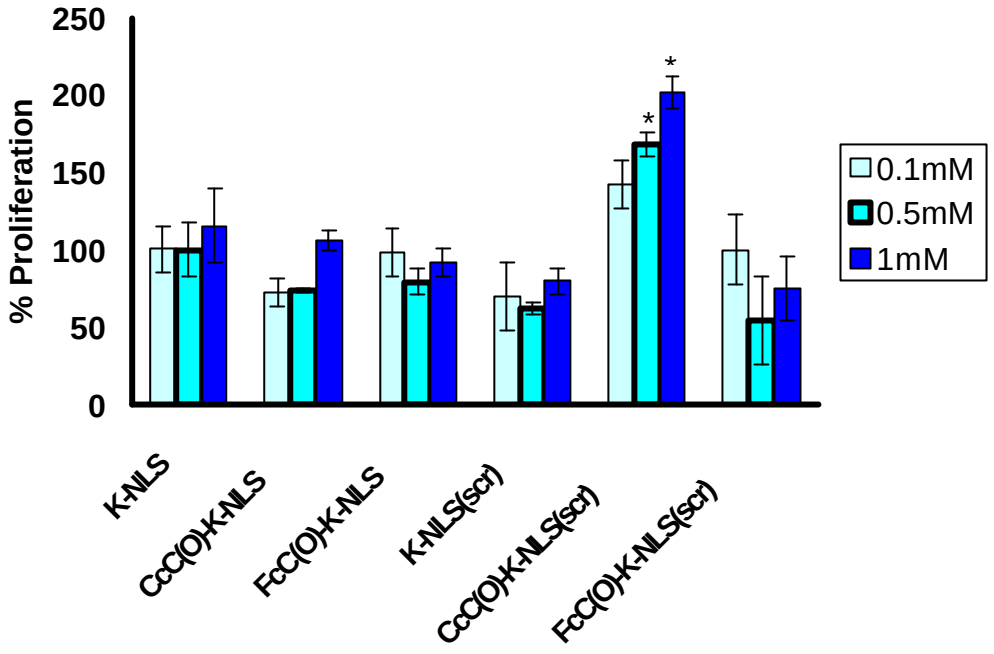

Figure S3. Effects of tested compounds on the proliferation rate of Hep G2 cells. The results are presented as % of the control (absorbance of control taken as 100 %; l = 0.23287 nm). The tests were done in quadruplet. Student *t*-test or the Mann-Whitney Rank Sum test was used to calculate significance. The results are represented as Mean +/- SEM and are considered significant when ($P < 0.005$).

Table S1

	RNR2		RAD54	
Compound No.	Cytotox	Genotox	Cytotox	Genotox
14 (K-NLS)	weak pos. / LEC = 1 mM	negative	weak pos. / LEC = 1 mM	negative
15 (CcC(O)-K-NLS)	negative	negative	negative	negative
5 (FcC(O)-K-NLS)	weak pos. / LEC = 1 mM	weak pos. / LEC = 1 mM	negative	negative
Ferrocene	negative	negative	negative	negative
Cobaltocenium hexafluorophosphate	negative	negative	negative	pos. / LEC = 116,5 µg/ml
FcC(O)-NLS	weak pos. / LEC = 1 mM	negative	weak pos. / LEC = 1 mM	negative
CcC(O)-NLS	pos. / LEC = 250 µM	negative	negative	negative

Experimental Section for the Yeast Assay (Table S1)

Reporter strains are obtained by transfection of yeast cells with reporter plasmids, containing the green fluorescent protein yEGFP under the control of the promoters for RNR2 and RAD54 respectively. The control strain is transfected with a reporter plasmid containing a frame shift mutation in the green fluorescent protein, which leads to the expression of a nonfunctional truncated protein (Afanassiev, Cahill). In preparation of the assay, reporter and control strains were grown until mid log phase in selective F1 (F1-URA). Assay plate with serial dilutions of test compounds (twofold f.c.) in assay medium were prepared and cells of the respective reporter strains were added (twofold final OD_{600}) using an automated pipetting robot system (Tecan Genesis) so that cells were present at a final optical density (OD_{600}) of 0.1 and the test substances in serial dilutions of the indicated concentration range (1 mM to 2 µM). Assay plates were incubated at 30 °C for 16h. Fluorescence intensity and absorption were measured with a Tecan Ultra plate reader at time points 0 h and 16 h to monitor growth and reporter gene expression. Changes in optical density are a direct measure for cytotoxicity when compared with methanol treatement in the same assay system as cytotoxicity reference standard; genotoxicity is assessed as described based on changes in normalized fluorescence values relative to the response to methyl methane sulfonate (MMS) as genotoxic reference standard (Afanassiev). A detailed description of plasmids, media etc. is given in the two references below.

References:

[1] "Application of Yeast Cells Transformed with GFP Expression Constructs Containing the RAD54 or RNR2 Promoter as a Test for the Genotoxic Potential of Chemical Substances", V. Afanassiev, M. Sefton, T. Anatachaiyong, G. Barker, R. Walmsley, S. Wölfl, *Mutation Res.* **2000**, *464*, 297-308

[2] "The GreenScreen Genotoxicity Assay: A Screening Validation Programme", P. A. Cahill, A. W. Knight, N. Billinton, M. G. Barker, L. Walsh, P. O. Keenan, C. V.Williams, D. J. Tweats, R. M. Walmsley, *Mutagenesis* **2004**, *19*, 105-119.