Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

"Novel Metal Chelators Thiosemicarbazones with Activity at the σ_2 Receptors and P-glycoprotein: an Innovative Strategy for Resistant Tumors Treatment".

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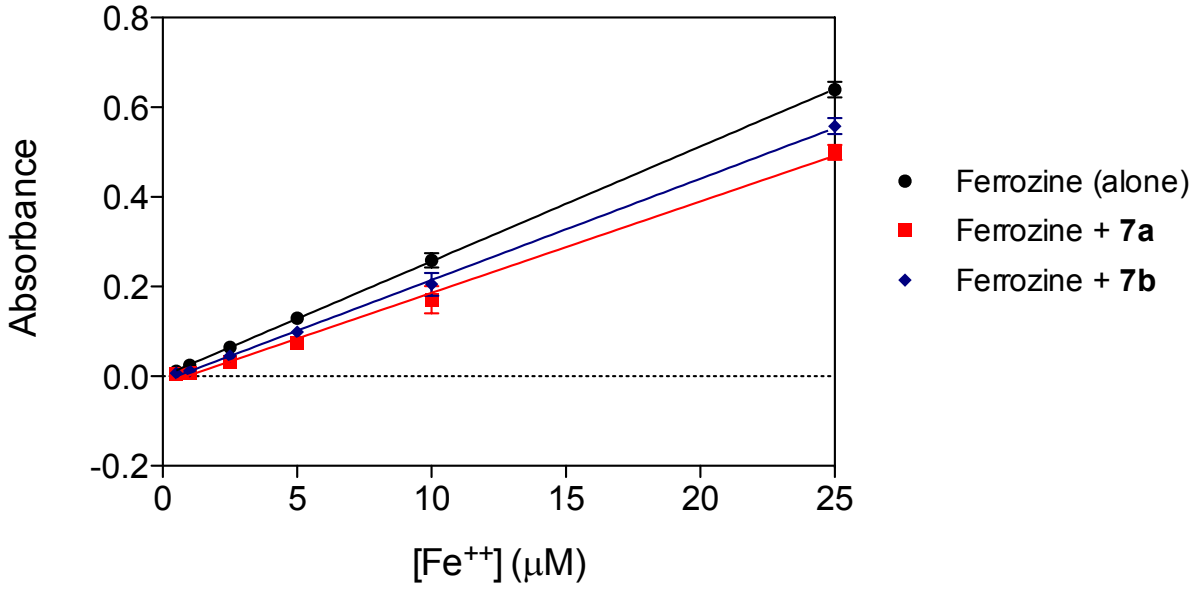
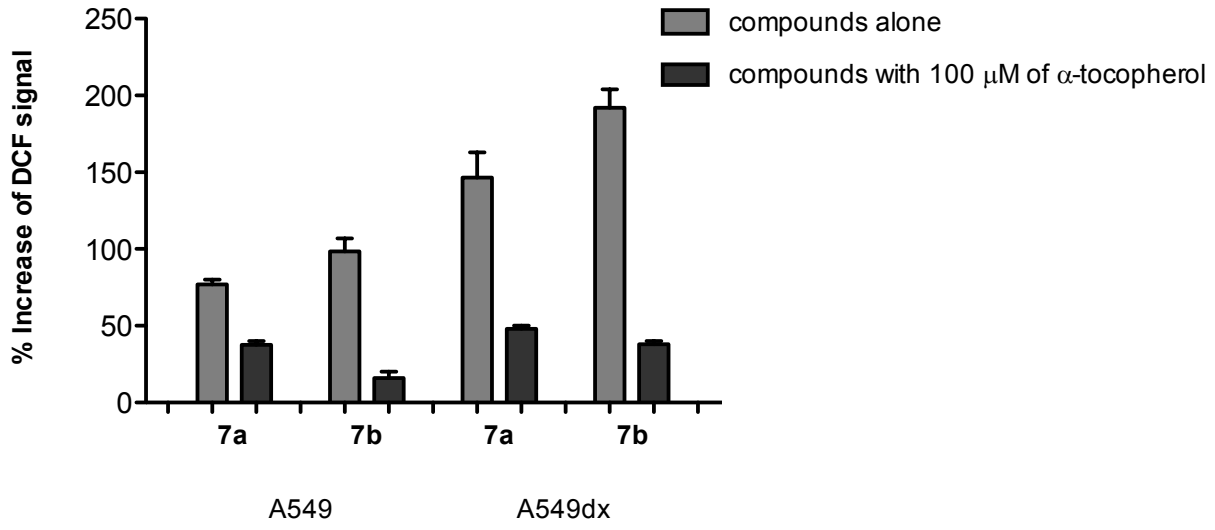
1-(4-chlorobutyl)indoline-2,3-dione (3) To a solution of Isatin (2.0 g, 13.59 mmol) in CH_3CN (20 mL), K_2CO_3 (3.76 g, 27.18 mmol) and 1-bromo-4-chlorobutane (1.72 mL, 14.95 mmol) were added and the mixture was refluxed for 4h. After the removal of the solvent under reduced pressure the residue was taken up with H_2O and extracted with AcOEt (3 × 15 mL). The collected organic layers were dried (Na_2SO_4) and evaporated under reduced pressure. The resulting crude residue was obtained as an oil which was purified by column chromatography (CH_2Cl_2/AcOEt 95:5) to give the title compound as thick red oil (2.95 g, 90% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25-1.86 (m, 4H, $NCH_2CH_2CH_2$), 3.59 (t, 2H, J = 6.0 Hz, CH_2Cl), 3.76 (t, 2H, J = 6.6 Hz, NCH_2), 6.92 (d, 1H, J = 8.0 Hz, aromatic), 7.12 (t, 1H, J = 7.5 Hz, aromatic), 7.56-7.61 (m, 2H, aromatic); GC-MS *m/z* 239 (M^+ + 2, 10), 237 (M^+, 30), 132 (100).



Supplemental Figure 1. $FeSO_4$ curve (0.5 μM – 25 μM) in the presence of ferrozine (100 μM) alone or with tested compound (100 μM) at UV/Vis spectrophotometer. Absorbance at λ = 562 nm is reported for representative compounds **7a** and **7b**.

Detection of ROS by DCF assay.[1] On day 1, 25,000 cells/well were seeded into 96-well plates in a volume of 100 μL. On day 2, 10 μM of compounds, alone or in combination with 100 μM of α-tocopherol, was added. In all the experiments, the various drug-solvents (EtOH, DMSO) were added in each control to evaluate a possible solvent cytotoxicity. After the established incubation time with drugs (24 h), 5-(and-6)-carboxy-2',7'-dichlorodihydro-fluorescein diacetate (carboxy-H_2DCFDA) (25 μM) was added to each well, and after 30 min incubation at 37 °C, the supernatant was removed and wells were washed once with PBS. The oxidative product of carboxy-H_2DCFDA to 2',7'-dichloro-fluorescein (DCF) was determined on the microplate reader Victor 3 from PerkinElmer Life Sciences (485 nm excitation and 530 nm emission).



Supplemental Figure 2. Increase of DCF signal upon treatment of A549 cells with compounds **7a** and **7b** (10 μM) alone or in the presence of α-tocopherol (100 μM).

1) J. R. Hornick, J. Xu, S. Vangveravong, Z. Tu, J. B. Mitchem, D. Spitzer, P. Goedegebuure, R. H. Mach, W. G. Hawkins, *Mol. Cancer*, 2010, **9,** 298.