Natural Product Research

Supplementary Material associated with the paper

Anticancer potential of some *β*-diketonates: DNA interactions, protein binding properties, and molecular docking study

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Abstract: With the goal to discover a new antitumor drug with the better or similar effects to existing, a small series of *β*-diketonate was tested on a cisplatin-resistant MDA-MB-231 and HeLa tumor cell lines, and nontumor MRC-5 cell line. All compounds showed notable cytotoxicity against both tumor cell lines and good selectivity. Importantly, *β*-diketonates displayed greater selectivity than cisplatin, which is the crucial factor for a new antitumor drug candidate. Further, investigations with biomacromolecules such as DNA and serum albumin were performed. Investigations showed that tested compounds bind to DNA through intercalation and have appropriate affinity for binding to bovine serum albumin. In addition, the molecular docking study was performed to investigate more specifically the sites and binding mode of tested *β*-diketonate to DNA or bovine serum albumin. In conclusion, all results indicated the big potential of these compounds for application in clinical practice in future.

EXPERIMENTAL

Cell lines

Human cervix adenocarcinoma cells (HeLa), breast tumor cell line (MDA-MB 231) and human fetal lung fibroblasts (MRC-5) were obtained from the American Type Culture Collection (ATCC). Cells were maintained in DMEM (Dulbecco's modified Eagle's medium) medium suppemented with 10% heath-inactivated FBS (Fetal Bovine Serum), L-glutamine (2mM), non-essencial amino acids (0,1mM), penicillin (100 IU/mL) and streptomycin (100 μg/mL)(Sigma, Germany). Cells were cultivated at 37°C in an 5% CO_2 atmosphere, and absolute humidity.

MTT assay

Cytotoxicity of compounds **A-E** against human cell lines was performed by tetrazolium colorimetric MTT assay (Sigma, Germany). The assay is based on the transformation of the tetrazolium salt 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), to formazan by mitochondrial dehydrogenase in viable cells. Briefly, cells were harvested and plated in 96-well microtiter plates (Thermo Fisher Scientific, United States) at an optimal seeding density of $5x10^3$ cells per well and incubated overnight for adherence. After overnight incubation, the medium was replaced with medium containing a range concentration of tested compounds (100, 30, 10, 3, 1 and 0.3μM) or with fresh medium as a control. Cisplatin was used as reference compound. Cells were incubated at 37°C in a 5% CO_2atmosphere and absolute humidity for 24 and 48 hours. After incubation, media was removed and 100μL of MTT (0.5 mg/mL PBS) was added to each well. After 4h incubation under culture conditions MTT solution was removed and 150μL of DMSO was added to dissolve the formazan crystals. Absorbance was measured at 550 nm with a multiplate reader (Zenith 3100, Anthos Labtec Instruments GmbH, Austria). Experiments were performed in triplicates and repeated in three independent series. Cytotoxicity was calculated according to the formula: $[(A_{CONTROL}-A_{TEST})/A_{CONTROL}]x100$. The IC_{50} value (50% inhibitory concentration) was calculated using ED50plus v1.0 software.

DNA fluorescence binding study

DNA fluorescence binding study was done according to the previously described method with minor corrections (Joksimović et. al. 2019.c). The binding mode of compounds **C** and **E** with DNA was studied using the fluorescence spectroscopy method. CT-DNA-EB complex was prepared by adding 2.5 mM EB and 2.5 mM CT-DNA in 0.01 M PBS buffer solution. A series of **C** or **E**–DNA solutions were prepared by combining the DNA-EB with different concentrations of **C** or **E**. The final concentration of DNA was 50 μM, while for **C** or **E** varied from 0 μM to 100 μM. The molar ratios of EB-DNA : **C** and **E** followed the order: 1 : 0 (control), 1 : 0.2, 1 : 0.4, 1 : 0.6, 1 : 0.8, 1 : 1, 1 : 1.2, 1 : 1.4, 1 : 1.6, 1 : 1.8, 1 : 2, in a total volume of 5.0 mL, pH 7.4, at 25 °C with an incubation time of 6 h. The fluorescence intensities of the series of the solutions were measured with the excitation wavelength and the fluorescence emission set at 527 and 612 nm, respectively. The emission intensity was recorded at the wavelength range of 550-700 nm.

BSA fluorescence binding study

BSA fluorescence binding study was done according to the previously described method with minor corrections (Joksimović et. al. 2019.c). 100 μM BSA solution was prepared in 10 mM PBS buffer (pH = 7.4) and stored in the darkroom at 5 °C for 6 h. The **C** or **E**–BSA complexes were prepared by mixing an even amount of BSA and increasing amounts of **C** or **E**. The molar ratios of BSA: **C** or **E** followed the order: 1: 0 (control), 1:0.5, 1:1, 1:1.5, 1:2, 1:2.5, 1:3. The samples were prepared in a total volume of 5.0 ml, pH 7.4, at 25°C with an incubation time of 6h. The final concentration of BSA was 10 μM, while **C** or **E** concentrations were 0-30 μM. The fluorescence emission spectra were obtained upon excitation at 280 nm and recorded immediately after the incubation. The emission intensity was recorded at the wavelength range of 300-500 nm. Data such as binding constant (K_a) and a number of binding sites per BSA molecule (n) were calculated using the following equation (Strekowski and Wilson 2007.):

$$\log(F_0\text{-}F/F) = \log K_a + n \log[Q]$$

Where F_0 is the emission intensity in the absence of **C** and **E**, F is the emission intensity for the complexes **C** or **E**–BSA. [Q] is the concentration of **C** and **E**.

Docking studies

For the purpose of the docking studies, structures of examined molecules were generated in DS Visualizer and optimized using MOPAC PM7 method (Stewart 1990.). Structures of target DNA and BSA molecules were obtained from crystal structures downloaded from the rscb.org site (Berman et al. 2000.). Used structures are 1Z3F (Canals et al. 2005.), structure of 6 base pair DNA in complex with ellipticine, 4F5S, (Bujacz 2012.) structure of BSA, and 4OR0, (Bujacz et al. 2014.) structure of BSA in complex with naproxen. Structures were further prepared using AutoDockTools 1.5.6 (Morris et al. 2009.) by removing co-crystalized substrates and water molecules, calculating Gasteiger charges, and merging non-polar hydrogens. For the docking experiments targeting DNA, grid box was set to cover cavity produced by intercalated molecule that was removed from crystal structure. Two tryptophane residues were targeted in docking experiments with BSA. Grid boxes were set to cover cavities in the vicinity of TRP134 and TRP213 residues. AutoDock 4.2.6 was used to run docking simulations. For each simulation 10 genetic algorithm runs were initialized with 25 million energy evaluations per run, while rests of the settings were left at default values.

Table S1. The bimolecular quenching rate constant (k_q), Stern–Volmer constant (K_{sv}), and correlation coefficient (R) for compounds **C** and **E**.

Compound	k_q [M^{-1} s^{-1}]	K_{sv} [M^{-1}]	R
C	$(4.8 \pm 0.1) \times 10^{11}$	$(4.8 \pm 0.1) \times 10^{3}$	0.989
E	$(4.1 \pm 0.1) \times 10^{11}$	$(4.1 \pm 0.1) \times 10^{3}$	0.995

Table S2. Binding parameters (K_a and n) and the correlation coefficient (R) for interactions of **C** and **E** with BSA.

compound	K_a [M^{-1}]	n	R
C	$(1.2 \pm 0.2) \times 10^{6}$	1.6	0.998
E	$(1.1 \pm 0.2) \times 10^{6}$	1.4	0.997

Table S3. Energies of binding from docking of compounds **C** and **E** to DNA

compound	E_b [kcal mol^{-1}]
C	-7.36
E	-6.44

Table S4. Energies of binding derived from docking of the compounds **C** and **E** to two structures of BSA. On each structure two residues were targeted. Included are energies derived from re-docking of naproxen to its parent structure.

Compound	E_b [kcal mol^{-1}]			
	4F5S TRP134	4F5S TRP213	4OR0 TRP134	4OR0 TRP213
C	-8.94	-9.04	-8.10	-8.49
E	-8.67	-8.51	-7.92	-8.70
naproxen			-6.61	-8.49

Figure S1. Fluorimetric titration of DNA (**a**) and BSA (**b**) with compounds **C** and **E**

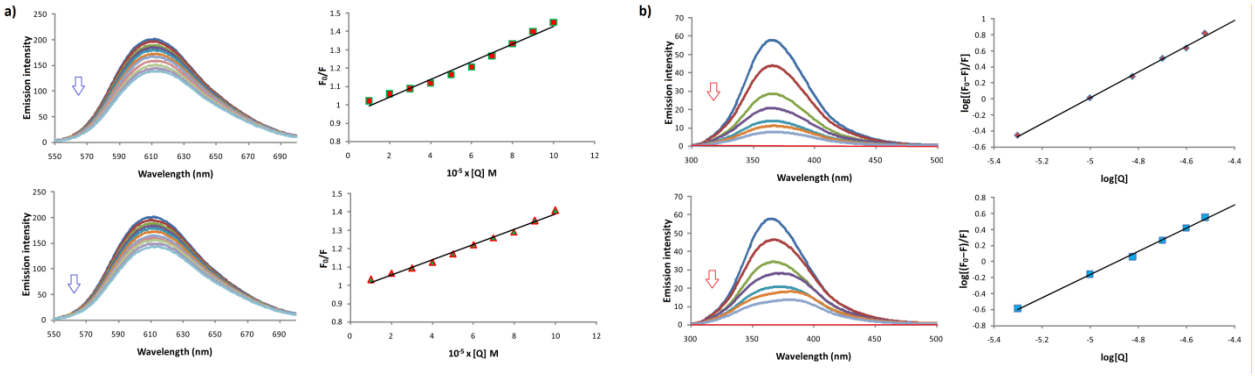

Figure S2. a) Binding modes of compound **C** (left) and compound **E** (right) with DNA. b) Compound **C** bound to the BSA in the vicinity of TRP134 and TRP213 (left). Actualized interactions of compound **C** with the residues in the vicinity of TRP213 (right).