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Novel PARP1 inhibitors potentiate doxorubicin antitumor activity *in vitro*

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Six novel potential PARP1 inhibitors were identified by means of substructure search and molecular docking into PAPR1 active site; one compound (STK970217) potentiated the cytotoxicity of doxorubicin in hepatocellular carcinoma HepG2 cells being non-cytotoxic as a single agent, while three other identified compounds inhibited the growth of HepG2 cells both individually and in combination with doxorubicin.

The enzyme poly(ADP-ribose) polymerase-1 (PARP1) is localized in a cell nucleus, and it catalyzes the poly-ADP-ribosylation of protein and DNA targets.[1] PARP1 is activated by single-strand breakages in DNA; upon activation, it catalyzes the formation of branched poly(ADP-ribose) chains on the DNA surface, which in turn attracts DNA-repairing enzymes. The inhibition of PARP1-mediated DNA reparation is a promising approach to a combination therapy of cancer.[2,3] Currently, six PARP1 inhibitors undergo phase III clinical trials as monotherapy, while combination use of PARP1 inhibitors with chemotherapy is substantially limited by the toxicity of such combinations.[4] Therefore, the discovery of effective and safe PARP1 inhibitors for mono and combination therapy of cancer is of great value.

Previously, we have identified the novel fragment-like PARP inhibitor 3,5,6,7-tetrahydro-4*H*-cyclopenta[4,5]thieno[2,3-*d*]-pyrimidin-4-one (**1**, IC_{50} 17±14 μM) by means of molecular docking.[5,6] Here, to identify more potent PARP1 inhibitors, we used commercially available compounds that possess a substructure of **1**, performed molecular docking into PARP1 active site and then experimentally studied the best compounds in an *in vitro* model of cytotoxicity in hepatocellular carcinoma HepG2 cells individually and in combination with doxorubicin.†

We have selected compounds **2**–**7**, which bind to PARP1 similarly to compound **1**, from the VitasM compound library using substructure search and molecular docking methods (Figure 1). The individual and combination (with doxorubicin) cytotoxicity of these compounds was assessed in HepG2 cells.

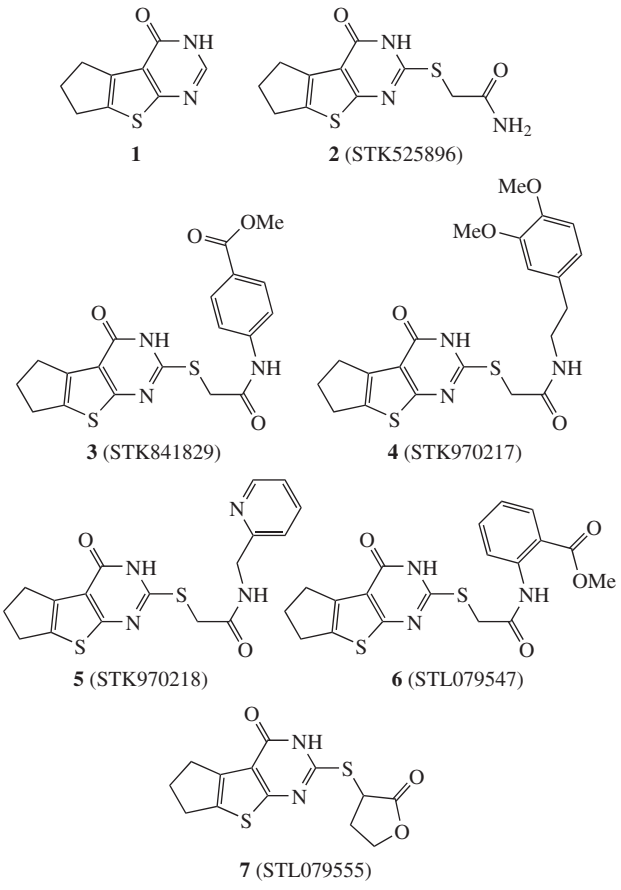

Figure 1 Potential PARP1 inhibitors.

Doxorubicin inhibited HepG2 cell growth with IC_{50} of 1.5–2 μg ml^{-1}. Isomers **3** and **6** and compound **7** also demonstrated individual cytotoxicity in the test concentration range 0–20 μM [Figure 2(*a*)]. Compounds **3**, **4**, **6** and **7** potentiated doxorubicin cytotoxicity, while compounds **2** and **5** did not affect it [Figure 2(*b*)].

† The substructure search was performed in the commercially available compounds' library VitasM Laboratories (http://www.vitasmlab.com/). Molecular docking and structural filtration was performed by the Lead Finder 1.15 software.[7]

HepG2 cells were plated in 96-well plates (3000 cells in 0.1 ml DMEM supplemented with 10% FBS) and treated with various concentrations of PARP1 inhibitors and doxorubicin alone or in combination. Each concentration was tested in quadruplicate. Cytotoxicity was measured using a standard MTT assay[8] after drug exposure for 72 h. Results were quantified using a Universal Microplate Reader (Bio-Rad) at 570 nm wavelength, and a control absorbance in wells containing DMSO was designated as 100% of cell survival.

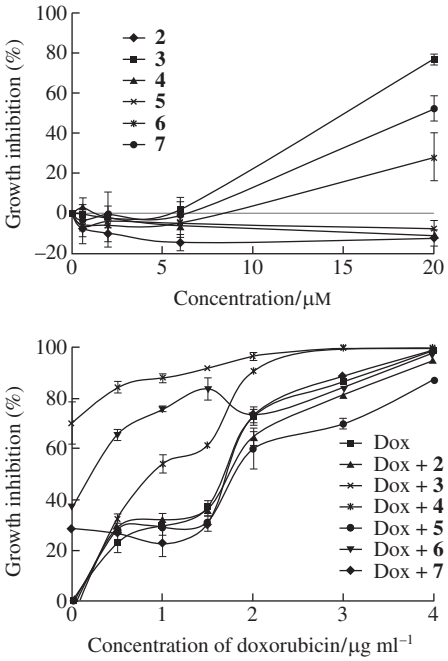

Figure 2 (*a*) Cytotoxicity of compounds **2**–**7** in HepG2 cells; (*b*) combined cytotoxicity of doxorubicin and compounds **2**–**7** (20 μM) in HepG2 cells.

These data indicate that compound **4** increases the cytotoxicity of doxorubicin while being non-cytotoxic as monotherapy. Compounds **3**, **6** and **7** also increased the cytotoxicity of doxorubicin but were also cytotoxic individually. To study the synergism of these combinations, we used a Bliss independence model.[9] According to this model, additive effects that correspond to the independent action of both components of a combination are $GI_{Add} = GI_{Dox} + (100\% - GI_{Dox})GI_{cpd}$, where GI_{Dox} is the percent of cell death caused by selected doxorubicin conentration, and GI_{cpd} is the percent of cell death caused by a selected PARP1 inhibitor concentration. If doxorubicin and PARP1 inhibitor act synergically, the observed effect will be greater than the calculated additive effect; if participants act as antagonists, the effect will be smaller than that calculated by an additivity hypothesis.

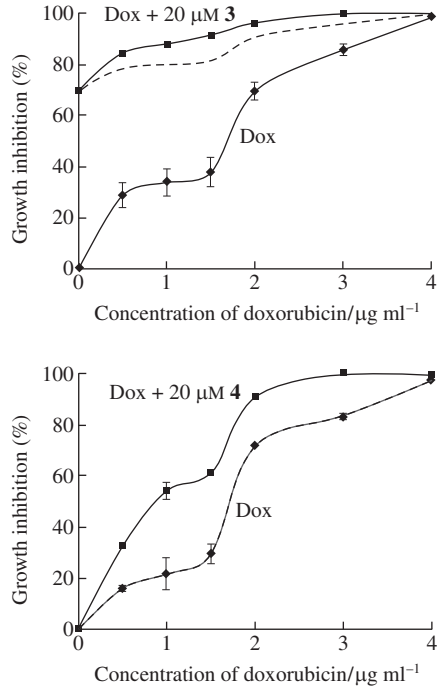

Figure 3 Combined cytotoxicity of doxorubicin and (*a*) compound **3** (20 μM) and (*b*) compound **4** (20 μM) in HepG2 cells. Dashed lines correspond to the predicted additive effect.

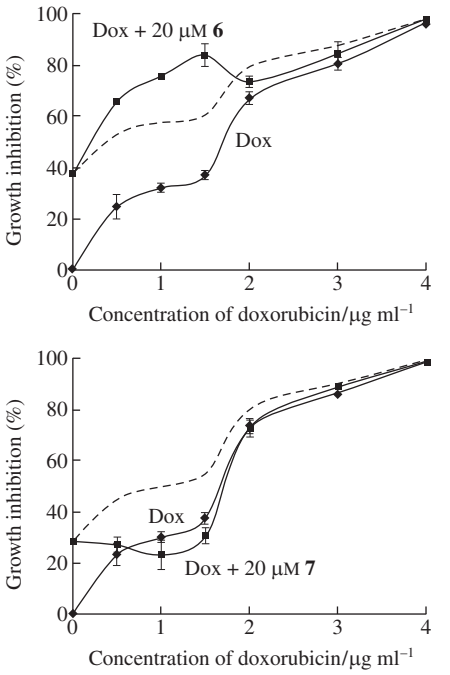

Figure 4 Combined cytotoxicity of doxorubicin and (*a*) compound **6** (20 μM) and (*b*) compound **7** (20 μM) in HepG2 cells. Dashed lines correspond to the predicted additive effect.

Experimental cytotoxicity of doxorubicin and compound **4** (20 μM) combination in the entire doxorubicin concentration range is greater than predicted additive effect, unambiguously indicating synergy [Figure 3(*b*)]. Compound **7** demonstrated slight antagonism [Figure 4(*b*)], compound **3** – slight synergy [Figure 3(*a*)], the effect of compound **6** depended on doxorubicin concentration [Figure 4(*a*)]. Negative cooperation between compound **7** and doxorubicin could be attributed to the common mechanism of action such as DNA damaging.

PARP1 inhibitors can possess cytotoxicity both individually[10] and in combination with DNA-damaging agents[11] depending on the nature and molecular biology of the tumor. Among the test compounds, compound **4** increased the efficiency of doxorubicin and did not possess cytotoxicity as a single agent, while compounds **3**, **6** and **7** inhibited the growth of HepG2 cells individually and did not synergized with doxorubicin. These results indicate that compound **4** targets PARP1, while the mechanism of cytotoxicity of compounds **3**, **6** and **7** should be additionally studied.

In conclusion, by means of substructure search and molecular docking, we identified six novel compounds that potentially inhibit PARP1, four of which increased doxorubicin cytotoxicity in HepG2 cells. Compound **4** did not possess cytotoxicity as a single agent; therefore, it is promising for the further development of a safe PARP1 inhibitor for combination with DNA-damaging agents.

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