The inhibitors of DNA repair enzymes based on usnic acid

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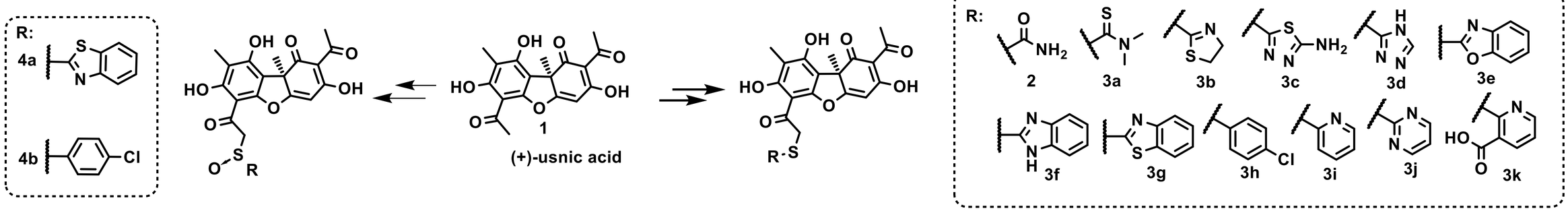
The search of new DNA repair enzymes inhibitors is a promising approach of modern pharmacology and one of the ways to increase cancer therapy effectiveness, especially for drug resistant tumors. At the same time the development of new compounds that effectively inhibit activity of various DNA repair enzymes is not an easy task. In the present work an attempt was made to synthesize dual or triple inhibitors of Tyrosyl-DNA-phosphodiesterase 1 and 2 (Tdp1 and 2) and Poly(ADP-ribose)polymerase 1 (PARP1).

The natural compound usnic acid **1** is a widespread secondary metabolite of lichens, the chemical transformation of which previously produced compounds with inhibitory activity against the DNA repair enzyme - Tdp1.[1]

In this work, we have synthesized a number of usnic acid thioesters **2,3** and sulfoxides **4** that differ in the structure of the sulfur atom substituent and have studied their inhibitory activity to the repair enzymes Tdp1, Tdp2 and PARP1.



The usnic acid thioether and sulfoxide derivatives efficiently suppressed TDP1 activity with IC_{50} values in 1.4-25.2 mM range. The structure of the heterocyclic substituent affects the TDP1 inhibitory efficiency of these compounds. Derivatives containing a five-membered heterocyclic fragment itself or fused to benzene ring (**3b,c,e-g** and **4a**) inhibit TDP1 in the low micromolar concentration range (IC_{50} 1.4–4.4 μM). Compounds containing a six-membered heterocycle (**3i-k**) inhibit TDP1 at higher concentrations (IC_{50} > 11 μM). The presence of a halogen in *para*-position of the benzene substituent enhances the inhibitory properties of the compounds. For the most effective inhibitors of TDP1 **3g** and **3h** and their sulfoxide analogs **4a** and **4b** we observed the uncompetitive type of inhibition. The uncompetitive inhibitors prevent the second step of the reaction stabilizing the enzyme-DNA covalent complex. Thus, uncompetitive TDP1 inhibitors could lead to the accumulation of the single-strand breaks in the cancer cells.

Anticancer effect of TOP1 inhibitors can be significantly enhanced by the simultaneous inhibition of PARP1, TDP1 and TDP2. We tested the ability of the synthesized compounds to inhibit TDP1, TDP2 and PARP1 activities. We found the compounds act as dual or triple inhibitors of TDP1, TDP2, and PARP1. Some of the compounds inhibited not only TDP1 but also TDP2 and PARP1, but at significantly higher concentration ranges than TDP1.

	IC_{50} (TDP1), μM	HEK293A CC_{50}, μM	HEK293FT CC_{50}, μM	HeLa CC_{50}, μM	PARP1, 1 mM	TDP2, 1 mM
UA	> 50	ND	ND	20±10	-	-
2	6.6 ± 1.0	>50	7 ± 2	13 ± 1	+	+
3a	5.4 ± 2.9	>50	>50	13.0 ± 0.5	+	+
3b	4.4 ± 1.0	>50	16 ± 5	11.0 ± 0.5	+	+
3c	4.3 ± 0.5	>50	35 ± 2	33 ± 2	+	+
3d	25.2 ± 6.5	ND	ND	ND	+	+
3e	3.2 ± 0.5	26 ± 2	4.5 ± 1.0	2.9 ± 0.2	-	+
3f	2.4 ± 1.0	>50	10 ± 2	13 ±	-	+
3g	1.7 ± 0.6	3.5 ± 0.3	3 ± 1	2.0 ± 0.6	-	+
4a	2.1 ± 0.2	20 ± 2	15 ± 2	15 ± 1	-	+
3h	2.2 ± 0.5	10 ± 2	4 ± 1	2.5 ± 0.5	+	+
4b	1.4 ± 0.2	>50	20 ± 3	27 ± 2	-	+
3i	11.9 ± 0.4	ND	ND	ND	-	+
3j	16.9 ± 2.4	ND	ND	ND	+	+
3k	19.6 ± 2.8	ND	ND	ND	+	+

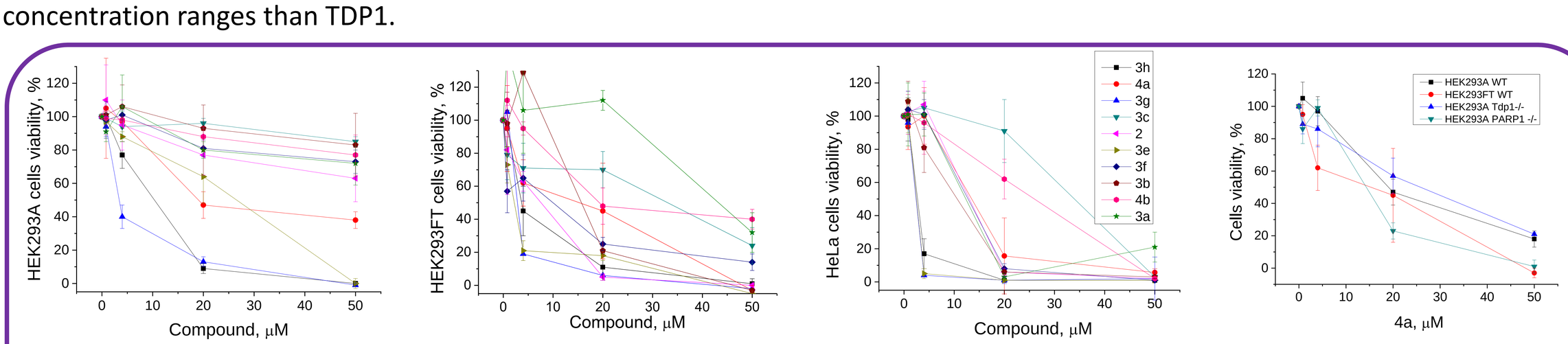


Figure 1. TDP1 inhibitors' intrinsic cytotoxicity on HEK293A, HEK293FT and HeLa cells, dose-dependent action of the most effective compounds. Intrinsic cytotoxicity of **10a** on HEK293FT, HEK293A cells, and Tdp1 and PARP1 knockout HEK293A cell lines (HEK293A Tdp1-/- and HEK293A PARP1-/-), dose-dependent action.

This study was funded by Russian Foundation for Basic Research 19-415-540002

References

[1] A.L. Zakharenko et al. *Eur. J. Med. Chem.*, **2019**, *161*, 581-93; A.S. Filimonov et al., *Molecules*. **2019**, *24(20)*, 3711