The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

First-in-class, thiosemicarbazide-based, dual inhibitors of human DNA topoisomerase IIα and indoleamine-2,3-dioxygenase 1 (IDO-1) with strong anticancer properties

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals MDPI

BARBARA KAPROŃ[1,*], TOMASZ PLECH[2]

[1] Department of Clinical Genetics, Medical University of Lublin, Lublin, Poland
[2] Department of Pharmacology, Medical University of Lublin, Lublin, Poland

* Corresponding author: barbarakapron@umlub.pl

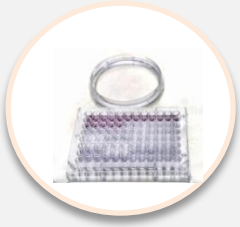
The work was supported by the National Science Centre, Poland, under grant Preludium (UMO-2018/29/N/NZ7/01726)

First-in-class, thiosemicarbazide-based, dual inhibitors of human DNA topoisomerase IIα and indoleamine-2,3-dioxygenase 1 (IDO-1) with strong anticancer properties

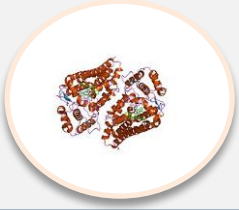
In silico molecular modelling
(3QX3/ 1ZXM)

Topo II enzymatic studies

Synthesis



- **MTT**
- **BrdU**



Screening of IDO inhibition

Abstract:

According to WHO report from 2020, cancer constitutes one of the leading causes of death worldwide. The number of cancer deaths is estimated to be approximately 10 million per year. These epidemiological data confirm that cancer is increasingly a global healthcare problem that needs urgent action. From the biological point of view, the basic feature of cancer is the uncontrolled growth and spread of abnormal cells from the place of origin to another part of the body. Inhibition of uncontrolled proliferation is one of the main goals of anticancer therapy. During our preliminary studies, we identified a group of thiosemicarbazide-based human DNA topoisomerase II inhibitors that decreased the viability of cancer cells and inhibited intracellular biosynthesis of their DNA much stronger than etoposide - i.e., clinically relevant topoisomerase II inhibitor. What is also important, the investigated compounds were recognized as topoisomerase II poisons because of their ability to stabilize DNA-topoII cleavable complex. The investigated thiosemicarbazide derivatives were examined as potential anticancer agents against a panel of ten cancer cell lines. Moreover, we have discovered and described the first-in-class dual inhibitors of human DNA topoisomerase II/indoleamine-2,3-dioxygenase 1 (IDO1) that can lead to the future use of thiosemicarbazide derivatives as relevant components of anticancer immunotherapy.

Keywords: thiosemicarbazide derivatives; human DNA topoisomerase II; anticancer drug design; IDO1

Introduction

According to WHO report (2020), cancer constitutes one of leading cause of death worldwide[1]. The number of cancer deaths is estimated to be approximately 10 million per year. Only in the USA, about 1.9 million new cases of cancer are expected to be diagnosed in 2022[2]. At the same time, the predicted number of cancer deaths in Europe is approximately 1.2 million[3]. These data confirm that cancer is increasingly a global health-care problem that needs urgent action. From the biological point of view, the basic feature of cancer is uncontrolled growth and spread of abnormal. Inhibition of the uncontrolled proliferation is one of the main goal of anticancer therapy. Such effect may be obtained, for example, through the use of chemical compounds that affect replication of DNA in cancer cells. Human DNA topoisomerases catalyze topological changes in single- or double stranded helices of DNA. These enzymes are necessary in the processes of replication, transcription, recombination, and significantly contribute to the genome stability maintenance[4]. Moreover, since human DNA topoisomerases are directly involved in DNA repair, the application of topoisomerase inhibitors as concomitant drugs during radiotherapy or chemotherapy is one of the strategies enhancing the effectiveness of cancer treatment.

During our studies, we identified a group of thiosemicarbazide-based human DNA topoisomerase II inhibitors that decreased viability of cancer cells and inhibited intracellular biosynthesis of their DNA much stronger than etoposide – i.e., clinically relevant topoisomerase II inhibitor.

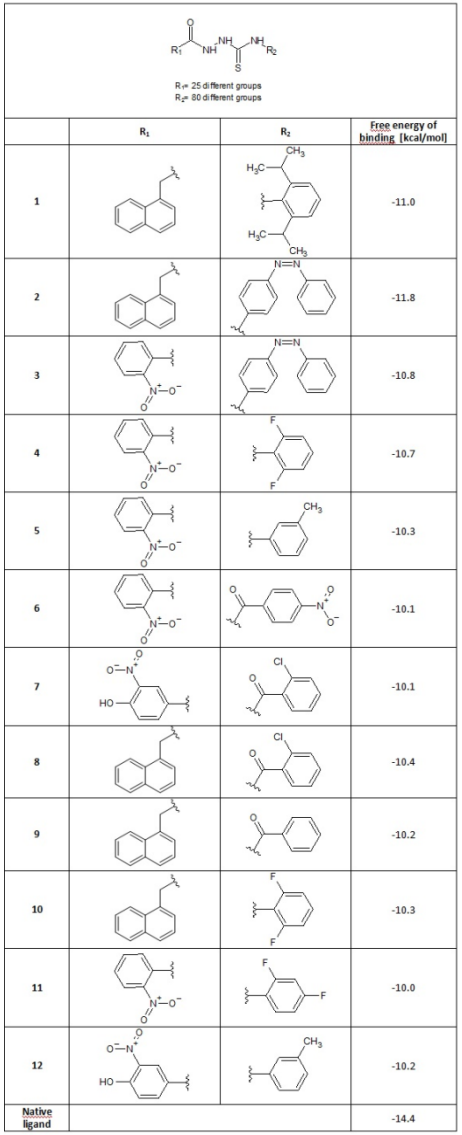
Since molecular docking simulations provides insight into the conformation of ligands within the active site of target protein, such approach is an integral part of drug design and discovery.

Combined application of *in-silico* and *in-vitro* techniques enabled us to identify new inhibitors characterized by strong cytotoxic/antiproliferative properties and high selectivity against cancer cells. Moreover, we have discovered and described the first-in-class dual inhibitors of human DNA topoisomerase II/indoleamine-2,3-dioxygenase 1 (IDO 1) that can lead to the future use of thiosemicarbazide derivatives as relevant components of anticancer immunotherapy.

Results and discussion

❑ Design and synthesis of thiosemicarbazide-based inhibitors of human DNA topoisomerase II

The analyzed dataset of nearly 2000 ligands included molecules composed of thiosemicarbazide skeleton enriched with structurally diverse R1 and R2 substituents. Designed ligands had different aryl, aroyl, alkyl (branched and unbranched), heteroaryl groups at distal positions of thiosemicarbazide core. The full set of ligands was docked to a single protein topoisomerase II structure found in the PDB database under entry: 3qx3 (X-ray resolution: 2.16Å, i.e. the highest available among topoisomerase II structures deposited in the PDB database). The aim of this stage was to identify the potentially most potent compounds, exhibiting the lowest protein-ligand binding free energy. Using this criterion, twelve compounds, characterized by the most favorable binding mode (associated with the lower binding energy), were selected for the synthesis and for further in-vitro experiments.



	R_1	R_2	Free energy of binding [kcal/mol]
1			-11.0
2			-11.8
3			-10.8
4			-10.7
5			-10.3
6			-10.1
7			-10.1
8			-10.4
9			-10.2
10			-10.3
11			-10.0
12			-10.2
Native ligand			-14.4

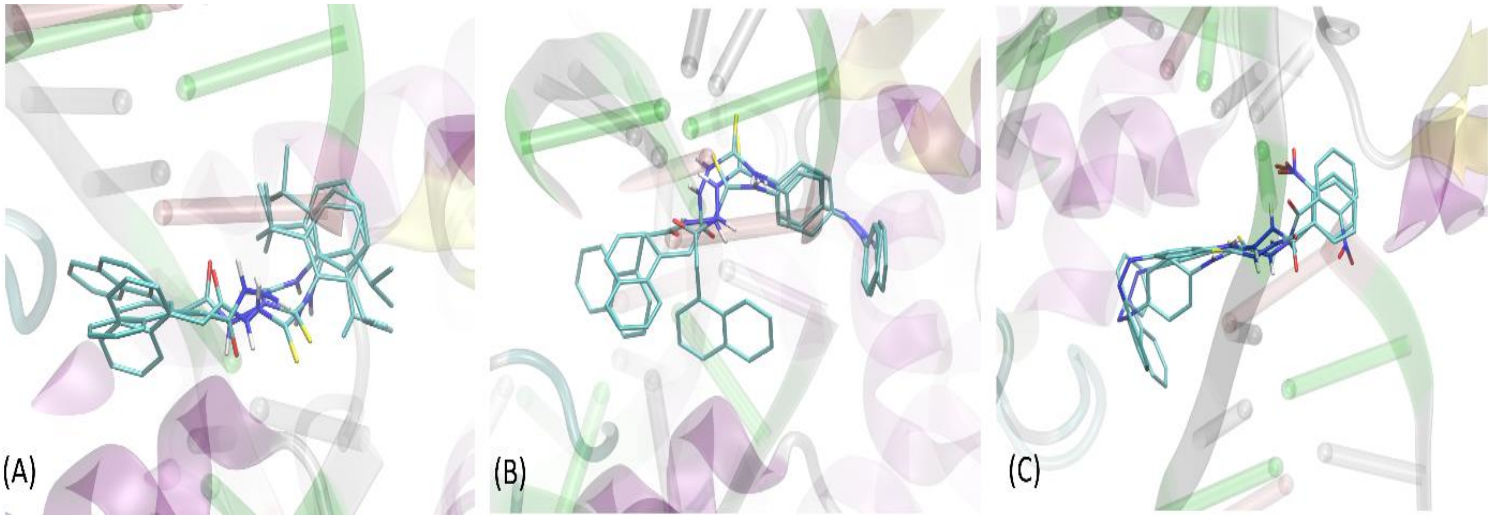


Figure 1. The exemplary, representative poses of the three ligands interacting with binding cavity of topoisomerase II. All depicted poses, characteristic of a given ligand, exhibit similar level of binding energies. (A) compound 1; (B) compound 2; (C) compound 3.

In-vitro testing of compounds*

Enzymatic studies

Anticancer activity of the investigated compounds

*Due to low solubility in culture media and buffers, compounds **11** and **12** were excluded from further in-vitro experiments.

Results and discussion

❑ Enzymatic studies

The inhibitory effect of compounds 1-10 and etoposide (positive control) was evaluated using human topoisomerase IIα relaxation assay. As relaxation of supercoiled DNA (scDNA) is one of numerous functions of topoisomerase IIα, so the appearance/disappearance of relaxed DNA bands during agarose gel electrophoresis can be used to identify compounds that inhibit the enzyme. Among the investigated thiosemicarbazide derivatives, compounds 1-3 turned out to inhibit the activity of human DNA topoisomerase IIα as indicated by the increased intensity of scDNA band and disappearance of relaxed DNA bands (Figure 2).

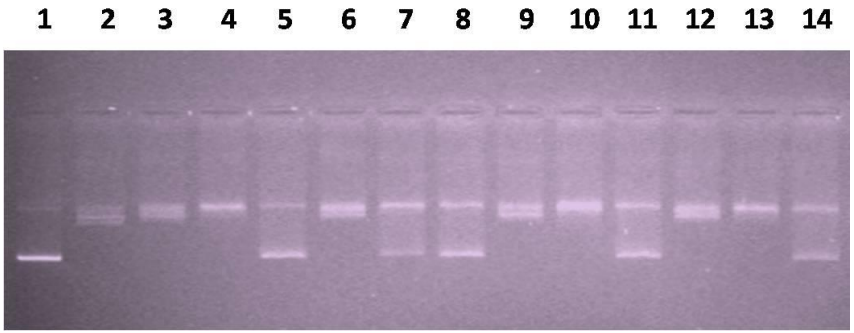


Figure 2. Determination of the inhibitory effect of the thiosemicarbazide derivatives 1-10 in Topoisomerase IIα relaxation assay (compounds 11 and 12 were excluded from enzymatic tests because of their low solubility in the buffers used).
Lane 1: scDNA; lane 2: scDNA + topoII; lane 3: scDNA + topoII + compound 1 (25μM); lane 4: scDNA + topoII + compound 1 (50μM); lane 5: scDNA + topoII + compound 1 (100μM); lane 6: scDNA + topoII + compound 2 (25μM); lane 7: scDNA + topoII + compound 2 (50μM); lane 8: scDNA + topoII + compound 2 (100μM); lane 9: scDNA + topoII + compound 3 (25μM); lane 10: scDNA + topoII + compound 3 (50μM); lane 11: scDNA + topoII + compound 3 (100μM); lane 12: scDNA + topoII + etoposide (50μM); lane 13: scDNA + topoII + etoposide (100μM); lane 14: scDNA + topoII + etoposide (150μM).

Results and discussion

❑ Anticancer activity of the investigated compounds

Since the inhibition of human DNA topoisomerase II should result in decreased proliferation of cancer cells, compounds 1-3 were tested using BrdU assay against a panel of cancer cell lines, including:
MCF-7 (estrogen-dependent human breast cancer cells),
MDA-MB-231 (estrogen-independent human breast cancer cells),
SCC-25 (squamous cell carcinoma of the tongue),
FaDu (squamous cell carcinoma of the pharynx),
A549 (human lung carcinoma),
AGS (human gastric adenocarcinoma),
LS-180, HT-29 (colon cancer cell lines),
T98G (glioblastoma cells), and
A375 (melanoma cells).
Human normal skin fibroblasts (CRL-2072) were also used as a reference cell line.

The BrdU assay allows quantification of cell proliferation since it measures incorporation of thymidine analogue (BrdU) during DNA replication. The examined compounds exhibited wide spectrum of anticancer activity and they inhibited the growth of all types of cancer cells much stronger than reference drug – etoposide (Table 1).

Table 1. Antiproliferative activity of compounds **1-3** and etoposide examined in BrdU assay

	IC_{50} [µg/mL] ± SD			
	Etoposide	**1**	**2**	**3**
MDA-MB-231	>100	26.29 ± 0.99	>100	>100
MCF-7	54.94 ± 1.82	1.19 ± 0.04	33.16 ± 1.05	32.54 ± 1.98
SCC-25	21.21 ± 0.74	1.10 ± 0.02	1.06 ± 0.04	4.89 ± 0.22
A549	2.60 ± 0.11	0.23 ± 0.01	0.83 ± 0.03	0.25 ± 0.01
AGS	45.99 ± 2.61	0.51 ± 0.02	62.34 ± 3.04	43.92 ± 1.94
T98G	29.51 ± 0.93	0.72 ± 0.02	6.19 ± 1.83	6.28 ± 0.37
LS180	>100	4.68 ± 0.17	10.39 ± 0.77	10.56 ± 0.24
FaDu	12.82 ± 0.27	1.04 ± 0.04	7.11 ± 0.34	9.04 ± 0.39
A375	9.18 ± 0.15	0.96 ± 0.03	0.85 ± 0.04	0.56 ± 0.03
HT-29	19.93 ± 0.25	0.44 ± 0.03	9.47 ± 0.51	13.14 ± 0.81
Human skin fibroblasts	55.69 ± 3.01	22.38 ± 0.94	24.17 ± 1.40	32.11 ± 1.75

BrdU incorporation was tested after 24 and 48h incubation of the cells with the investigated compounds. However, due to strong inhibition of BrdU incorporation by compounds 1-3, the results obtained after 48h of incubation (IC_{50} values tend to zero for most of the cancer cell lines) were omitted for clarity.

Results and discussion

Having in mind that different molecules are able to inhibit the growth of cancer cells through more than one molecular mechanism, it is possible that also other (i.e., other than 1-3) representatives of the set of synthesized thiosemicarbazide derivatives can exhibit anticancer activity that is unrelated to DNA topoisomerase II inhibition. Therefore, the whole set of compounds were tested by using **MTT assay** in order to check their overall cytotoxic effect. The growth of cells was monitored after 24 and 48h of incubation with the increased concentrations (1-100 μg/ml) of the thiosemicarbazide derivatives. The results of MTT assay (Table 2) proved that mainly DNA topoisomerase II inhibition contribute to the anticancer effect of the designed compounds, since compounds 1-3 were characterized by the most potent cytotoxic effect in relation to the other thiosemicarbazide derivatives examined in this study.

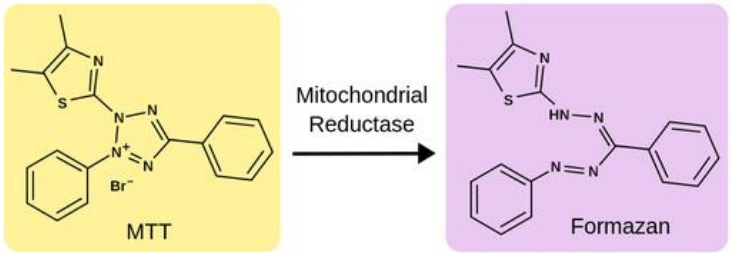


Table 2. Cytotoxicity of the investigated compounds **1-10** and etoposide against a panel of cancer cell lines measured by using MTT assay after 24 and 48h incubation.

		IC_{50} [μg/mL]*										
		Etoposide	1	2	3	4	5	6	7	8	9	10
MDA-MB-231	24h	>100	9.21	7.64	10.47	>100	>100	>100	>100	>100	>100	83.42
	48h	40.31	8.04	7.07	6.02	32.48	56.31	16.84	69.37	17.23	>100	68.12
MCF-7	24h	>100	9.82	9.18	7.67	>100	>100	>100	>100	>100	>100	64.11
	48h	6.15	8.45	8.85	5.48	19.42	28.13	15.93	24.84	12.74	16.37	20.64
SCC-25	24h	59.07	7.06	4.73	4.76	>100	>100	25.98	42.41	>100	32.19	52.41
	48h	23.72	5.22	3.12	3.87	40.86	17.32	9.78	24.32	29.47	34.48	23.86
A549	24h	>100	3.87	5.84	5.37	>100	>100	20.77	>100	>100	>100	92.63
	48h	15.59	2.27	5.78	5.13	>100	>100	23.45	>100	>100	>100	32.84
AGS	24h	50.79	5.92	5.32	5.54	>100	>100	23.46	>100	>100	>100	29.46
	48h	29.77	2.69	2.96	4.31	63.87	63.92	29.77	18.76	>100	19.66	21.06
T98G	24h	>100	6.98	5.53	3.35	55.47	>100	8.41	11.45	97.22	>100	47.23
	48h	17.06	2.27	4.85	2.94	7.26	30.08	9.78	10.66	11.82	17.39	30.63
LS180	24h	>100	4.57	2.87	3.12	>100	>100	>100	>100	>100	>100	72.43
	48h	11.71	2.54	2.24	2.70	55.62	19.84	10.41	24.68	41.15	19.82	12.55
FaDu	24h	12.54	7.26	2.75	2.49	>100	>100	12.89	33.52	>100	28.16	20.12
	48h	10.73	2.01	2.54	2.18	39.25	31.69	14.77	22.47	12.81	19.53	29.74
A375	24h	10.21	4.57	8.96	1.80	>100	89.31	>100	>100	>100	>100	47.92
	48h	6.48	1.82	2.47	0.56	14.81	38.45	7.98	13.88	9.45	8.14	30.15
HT-29	24h	94.49	8.96	8.96	7.37	25.01	45.23	22.78	47.38	>100	>100	32.94
	48h	11.30	5.33	5.33	3.77	>100	12.30	6.78	7.18	14.77	5.23	32.16
HEK-293	24h	37.85	17.60	15.59	27.54	>100	78.14	>100	67.15	>100	67.03	63.71
	48h	29.12	9.61	10.03	9.19	>100	24.59	60.13	32.70	23.42	23.03	19.50

*SD values were ommitted for clarity

Results and discussion

❑ Screening of indoleamine-2,3-dioxygenase (IDO) inhibition

The concept of use of thiosemicarbazide core as an attractive scaffold in anticancer drug design and development was enriched by the results obtained by Serra et al.[5]. Their studies demonstrated that some of the thiosemicarbazide derivatives highlighted inhibitory effect on indoleamine-2,3-dioxygenase (IDO), which is a promising target for anticancer immunotherapy. The use of IDO inhibitors may be a useful strategy to overcome tumor-induced immunosuppression. Moreover, numerous in-vitro and in-vivo clinical trials confirmed that combined application of IDO 1 inhibitors with classical chemotherapy or radiotherapy improved the outcomes of the treatment[6]. Taking into consideration that dual inhibitors, acting both on human topoisomerase II and IDO1, could possess beneficial anticancer properties, the final stage of the study aimed to check if the compounds 1-3 are able to inhibit these two enzymes simultaneously (Table 3).

Table 3. Inhibitory effect of compounds **1-3** against indoleamine-2,3-dioxygenase 1 (IDO 1)
The compounds were tested in a fixed dose of 50 µg/ml. Since compounds 1-3 were devoid of inhibitory effect towards IDO 2 and TDO (also examined during the assay), therefore these results are not included in the table.

	IDO 1 inhibition (%) ± SD
1	91.37 ± 1.82
2	41.37 ± 2.47
3	21.43 ± 1.05
Epacadostat	99.58 ± 0.43

Conclusions

Summarizing, three novel and potent dual inhibitors of human DNA topoisomerase II and IDO 1 were discovered using computer-aided drug design techniques. The obtained thiosemicarbazide derivatives exhibited a wide spectrum of antiproliferative and cytotoxic properties since they effectively inhibit the growth of numerous cancer cell lines tested with IC_{50} values even up to 90-fold lower than etoposide – clinically used chemotherapeutic agent, and selectivity index values reaching 125. Mechanistic studies showed that inhibition of human topoisomerase II by the investigated compounds is maintained through the contact of the protein with aromatic moieties located at limiting edges of ligand molecules (Figure 3) and intensive interactions of the thiosemicarbazide core with the DNA fragments present in the catalytic site of the enzyme.

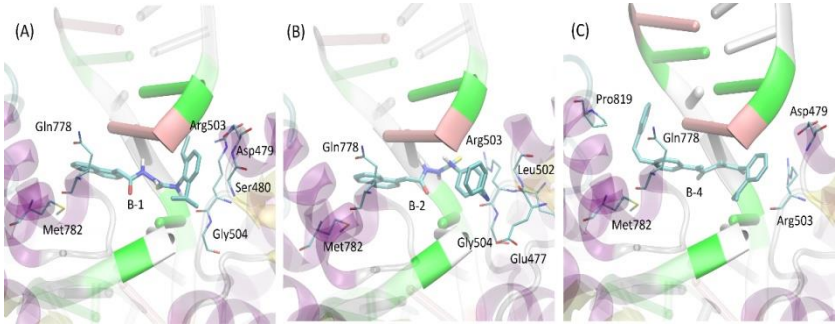


Figure 3. The exemplary, energetically-favorable poses of the three lead compounds identified during docking study: (A) compound **1 (B1)**; (B) compound **2 (B2)**; (C) compound **3(B4)s**.The ligand molecules are shown as thick sticks whereas all the closest amino-acid residues (within the distance of 0.4nm) are represented by thin sticks.

Acknowledgments

NATIONAL SCIENCE CENTRE POLAND

The work was supported by the National Science Centre, Poland, under grant Preludium (UMO-2018/29/N/NZ7/01726)

References

[1] https://www.who.int/news-room/fact-sheets/detail/cancer (accessed on 23 May 2022)
[2] https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2022/2022-cancer-facts-and-figures.pdf (accessed on 23 May 2022)
[3] Dalmartello M, La Vecchia C, Bertuccio P, et al. European cancer mortality predictions for the year 2022 with focus on ovarian cancer. Ann Oncol 2022;33:330-339.
[4] Nittis JL. Targeting DNA topoisomerase II in cancer chemotherapy. Nat Rev Cancer 2009; 9:338-350.
[5] Serra S, Moineaux L, Vancraeynest C, et al. Thiosemicarbazide, a fragment with promising indolamine-2,3-dioxygenase (IDO) inhibition properties. Eur J Med Chem 2014;82:96-105.
[6] Röhrig UF, Majjigapu SR, Vogel P, et al. Challenges in the discovery of indoleamine 2,3-dioxygenase 1 (IDO1) inhibitors. J Med Chem 2015;58:9421-9437.