In silico Evaluation of the Antiproliferative Mithocondrial Targeted Mechanism of Action of Some Pentacyclic Triterpene Derivatives

IOANA MACASOI[1#], MARIUS MIOC[1#], DELIA BERCEANU VADUVA[2#], ROXANA GHIULAI[1]*, ALEXANDRA MIOC[1], CODRUTA SOICA[1], DANINA MUNTEAN[2], VICTOR DUMITRASCU[2]

[1]Victor Babes University of Medicine and Pharmacy, Faculty of Pharmacy, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania
[2]Victor Babes University of Medicine and Pharmacy, Faculty of Medicine, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

Mitochondria play an important role in regulating cell viability. Mitochondrial dysfunction has been associated with many known pathologies including cancer. Mitocans are a class of compounds that alter important mitochondrial functions in cancer cells thus inducing cellular death. New pentacyclic triterpene derivatives are constantly developed with the aim of obtaining highly active antiproliferative agents. In this study a set of previously synthesized rhodamine B triterpene conjugates, designed as mitocans, were in silico evaluated with the purpose of elucidating their targeted mithocondrial mechanism of action. Molecular docking revealed that the compounds would predominantly interact with proteins that are part of the mithocondrial electron transport chain (ETC), such as NAD(P)H-quinone oxidoreductase (NDH) and succinate dehydrogenase (SDH).

Keywords: mitochondrial targeting, Rhodamine B, pentacyclic triterpene, molecular docking.

In spite of the efforts made to develop new anti-proliferative agents, cancer remains one of the leading causes of global mortality. Finding effective therapeutic measures remains a challenge, considering that tumor cells undergo continuous mutations that provide them with resistance against the current chemotherapy [1]. Given the fact that mitochondria is *the power house* of the cell, playing an important role in cell survival, recent studies have focused on finding new compounds that target mitochondria, thereby causing cancer cell death [2, 3]. Mitochondrial dysfunction has been associated with many pathologies such as cardiovascular and neurodegenerative diseases, cancer, infertility and increased drug toxicity, such as anti-HIV therapy [4,5]. The agents which target mitochondria in the cancer cells are generically named "mitocans". This class of substances act by altering important mitochondrial functions, inducing cell apoptosis or inhibiting their growth [6]. Important mitochondrial proteins used as targets for the discovery of new anticancer agents include: hexokinase type II (HK II), apoptosis regulator Bcl-2 (Bcl-2), ADP/ATP translocase (ANT) or proteins that are part of the mithocondrial electron transport chain (ETC) such as NAD(P)H-quinone oxidoreductase (NDH), succinate dehydrogenase (SDH), coenzyme Q - cytochrome c oxidoreductase (Qcrc), and ATP synthase [7].

Pentacyclic triterpenes are a class of naturally occurring compounds which are extensively researched for their diverse therapeutic effects including their antiproliferative activity [8,9]. So far a large body of work has been put on developping new pentacyclic triterpene derivatives with enhanced therapeutic effects [10-12].

In a recent study a set of triterpene derivatives containing a carboxyl function were conjugated with rhodamine B (figure 1) in order to penetrate the mitochondrial membrane aiming to achieve superior antiproliferativ effects by means of a mitochondria targeted mechanism of action [13]. In this study the intramitochondrial presence of the compounds was reported but the mechanism by which these compounds exerted their antiproliferative effect remains unexplored [13].

Therefore the aim of our current study was to elucidate, by means of molecular docking, the mithocondrial targeted mechanism of action of some rhodamine-conjugated pentacyclic tritepene derivatives.

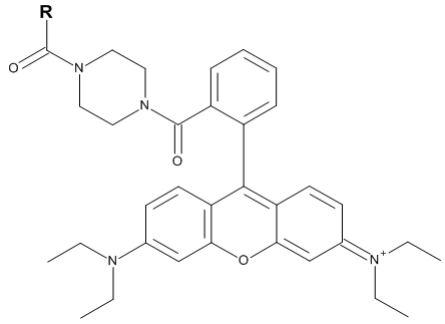

Fig. 1. General structure of the rhodamine B conjugated triterpenes, where R represents a triterpene structure corresponding to ursolic (UA), oleanolic (OA), glycyrrhetinic (GA), platanic (PA), betulinic (BA) or maslinic acid (MA), respectively.

Experimental part

Materials and methods

The protein structures used in the study were available from the RCSB Protein Data Bank [14]. For the purpose of this study, the protein structures employed, 5HG1 (HK II), 4G73 (NDH), 4IEH (Bcl-2), 2JIZ (ATP synthase), 1OKC (ANT), 1NTZ (Qcrc), 1NEN (SDH), were prepared as suitable targets for molecular docking using Autodock Tools 1.5.6. From each target structure, water molecules, undesired protein chains, metallic atoms and the co-crystalized ligand (if present) were removed, after which polar hydrogen atoms and Kollman charges were added for each protein. Targets were saved as a suitable file format (*.pdbqt). Ligand molecules corresponding to the rhodamine B conjugates of the six above mentioned triterpenes, abbreviated here as BA_rod, GA_rod, MA_rod, OA_rod, PA_rod and UA_rod, were drawn using Biovia Draw (Dassault Systemes Biovia), saved as mol files, after which were converted into 3D structures using PyRx's Open Babel

*email: roxana.ghiulai@umft.ro

[#] *Authors with equal contribution jointly sharing the position as first author.*

module. Geometry optimization was also carried out with Open Babel, using the Uff force field. Ligand molecule structures were then converted to the .pdbqt format. The co-crystalized ligands, present within each protein target structure, were prepared as suitable .pdbqt ligands and re-docked in their respective binding sites, for the purpose of comparing obtained binding energies of the pentacyclic triterpene derivatives with values obtained for the known inhibitors and for the validation of the employed method.

Molecular docking was carried out with the GUI software, PyRx (version 0.8) using Vina's scoring function [15]. Molecules were docked in specific binding domains of each protein structure, using default docking parameters, as follows:

i.for structures 4G73, 1NTZ, 1NEN, that correspond to proteins active in the ETC, molecules were docked in the ubiquinone (UQ) binding site;

ii.for 5HG1 structures were docked in the region corresponding to the glucose binding site;

iii.in the case of Bcl-2 the BH3 domain was used, for molecular docking

iv.for 1OKC docking was employed using the central cavity domain, corresponding to the carboxyatractyloside binding pocket;

v.in the case o 2JIZ structures were docked in the resveratrol binding site

Recorded scores for docked molecules were given as free binding energy (ÄG) values (kcal/mol). Ligand-protein binding paterns were analyzed using Accelerys Discovery Studio 4.1 (Dassault Systemes Biovia).

Results and discussions

Obtained binding energy values of the six triterpene derivatives for each protein are presented in table 1. First observations reveal very high binding energy values (low inhibition probability) for 5HG1, 2JIZ, 1NTZ as well as a relative large difference between the mean energy values,of the docked compounds and the binding energy calculated for the respective co-crystalized ligands (table 1).

In other cases, for proteins like 4IEH (Bcl-2) and 1OKC (ANT) good energy values were recorded, comparable or in some cases even lower then ÄG values obtained from re-docking the co-crystalized ligands. Most notable values were recorded for OA_rod (4IEH) and MA_rod (1OKC). However the best obtained values for our compounds were in the case of protein targets such as 4G73 (NDH) and 1NEN (SDH). In the first case, by rank, the lowest ÄG value was recorded for UA_rod. Interactions formed in the NDH binding site are depicted in figure 2.

Binding analysis show that the large hydrophobic triterpene moiety is well stabilized by hydrophobic interactions and occupies mainly the UQ I binding site, whereas the rhodamine B structure is linked in the UQ II binding site by a hydrogen bond (HB) and an electrostatic interaction formed with His397 (fig. 2). The interaction pattern is somewhat similar to the bound ubiquinone which is mainly stabilized in the binding pocket by its large hydrophobic side chain [16].

In the case of 1 NEN (SDH) the best obtained binding energy values were recorded for the same UA derivative. Binding analysis of the UA_rod compound in the SDH protein is depicted in figure 3.

Unlike the inhibitor, 2-[1-methylhexyl]-4,6-dinitrophenol, which holds a centered position in the UQ binding pocket forming HBs with the B chain's Trp164 [17], UA_rod occupies a larger space being linked with the C and D chain through 4 HBs (Ile18, Gln16, Leu15 and Lys85), the rest of the molecule being stabilized by multiple hydrophobic interactions (fig. 3).

These results can be correlated with the findings of Somerwerk et al.; according to this study, the mechanism by which the effect of triterpene derivatives modifid cell viability is not based on extrinsic pathway mediated cellular apoptosis and the presence of these compounds was observed at the mitochondrial level [13]. If we heat map our obtained ΔG by comparison with the energy values calculated for the co-crystalize ligands, used as reference (first row) and sort these values in descending order, a clear tendency emerges (table 2). Our tested compounds recorded notable energy values (by comparison with the reference) in the case of proteins such as 4G73 (NDH) and 1NEN (SDH) which are in fact complex I and II of the ETC. Thus we can conclude that, presumably, these compounds may alter cancer cell viability by targeting the ETC thus

Table 1

BINDING ENERGY VALUES (kcal/mol) OF THE BEST DOCKED CONFORMATION, CORRESPONDING TO THE SIX TRITERPENE DERIVATIVES AND EACH DOCKED CO-CRYSTALIZED LIGAND

	ΔG (kcal/mol)							
	Prot ID *Prot co-crystalized ligand*	*5HG1* *-9.3*	*4G73* *-6.6*	*4IEH* *-10.8*	*2JIZ* *-6.7*	*1OKC* *-8.9*	*1NTZ* *-8.1*	*1NEN* *-8*
Compound	BA_rod	-0.3	-8	-9.1	-5.5	-9.3	-4.6	-9.1
	GA_rod	1.5	-9.2	-9.3	-9.3	-9.5	-4.6	-8.7
	MA_rod	-0.9	-9.2	-10.5	-4.9	-10.8	-1	-8
	OA_rod	-4	-8.8	-11.3	-6.6	-10.6	-1.7	-8.1
	PA_rod	4.2	-7.6	-9.3	-5.1	-9.3	-6.8	-8.1
	UA_rod	-4.6	-10.1	-10.1	-6.5	-9.2	0	-9.7

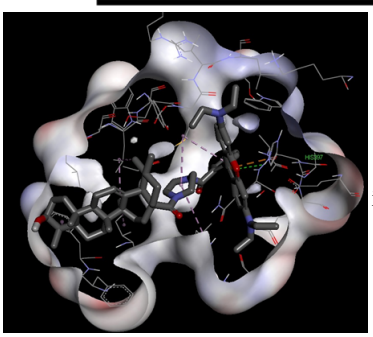

Fig. 2. NDH (4G73) in complex with docked compound UA_rod; HB (green) and electrostatic interaction (orange) formed with His397

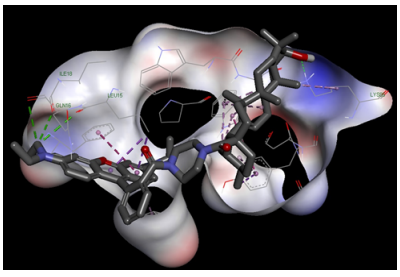

Fig. 3. SDH (1NEN) in complex with docked compound UA_rod; HB (green) formed with Lys85 and three carbon-HBs formed with Ile18, Gln16, Leu15

5HG1	4G73	4IEH	2JIZ	1OKC	1NTZ	1NEN
-9.3	-6.6	-10.8	-8.7	-8.9	-8.1	-8
4.2	-7.6	-9.1	-4.9	-9.2	0	-8
1.5	-8	-9.3	-5.1	-9.3	-1	-8.1
-0.3	-8.8	-9.3	-5.5	-9.3	-1.7	-8.1
-0.9	-9.2	-10.1	-6.3	-9.5	-4.6	-8.7
-4	-9.2	-10.5	-6.5	-10.6	-4.6	-9.1
-4.6	-10.1	-11.3	-6.6	-10.8	-6.8	-9.7

Table 2
BINDING ENERGY VALUES HEAT MAP; ΔG VALUES OF DOCKED COMPOUNDS WAS SORTED FROM HIGHEST TO LOWEST TO BETTER EXPRESS THE BINDING TENDENCY COMPARED WITH CO-CRYSTALIZED LIGANDS VALUES (FIRST ROW)

subsequently inducing ROS generation due to the inhibition of ETC components.

Conclusions

In the present study we aimed to propose a mitochondrial targeted mechanism of action for a set of rhodamine B-triterpene conjugates by means of molecular docking. Our results showed that the most notable binding energy values were obtained for targets such as NDH and SDH that act on the electron transport chain. Binding analysis revealed that these compounds are well accommodated in the UQ binding sites. However the fact that these compound would act as *mitocans* that alter the ETC and induce cell death by ROS production remains to be experimentally validated.

References

1.PANDA V, KHAMBAT P, PATIL S., Int J Clin Med., **02,** 2011, p. 515.
2.ARMSTRONG JS., Br J Pharmacol, **151**, 2007, p. 1154.
3.WEINBERG SE, CHANDEL NS., Nat Chem Biol, **11,** 2015, p. 9.
4.EDEAS M, WEISSIG V., Mitochondrion, **13,** 2013, p. 389.
5.PETRUS A, DUICU OM, STURZA A, NOVEANU L, KISS L, DA M, et al., Can J Physiol Pharmacol, **93**, 2015, p. 818.
6.NEUZIL J, DYASON JC, FREEMAN R, DONG L-F, PROCHAZKA L, WANG X-F, et al., J Bioenerg Biomembr, **39,** 2007, p. 65.
7.NEUZIL J, DONG L, ROHLENA J, TRUKSA J, RALPH SJ. Mitochondrion, **13**, 2012, p. 199.
8.PISHA E, CHAI H, S LEE I, E. CHAGWEDERA T, R. FARNSWORTH N, A. CORDELL G, et al. Nat Med, **1**, 1995, p. 1046.
9.SOICA CM, DEHELEAN CA, PEEV C, ALUAS M, ZUPKO I, KASA P, et al., Nat Prod Res, **26**, 2012, p. 968.
10.PETRONELLI A, PANNITTERI G, TESTA U., Anticancer Drugs **20**, 2009, p. 880.
11.LEDETI I, AVRAM S, BERCEAN V, VLASE G, VLASE T, LEDETI A, et al., Molecules **20**, 2015 p. 811.
12.PINZARU, I., TRANDAFIRESCU, C., SZABADAI,Z., MIOC, M., LEDETI, I., CORICOVAC, D., Rev Chim. (Bucharest), **65**, 2014, p. 848.
13.SOMMERWERK S, HELLER L, KERZIG C, KRAMELL AE., Eur J Med Chem, **127**, 2017, p. 1.
14.BERMAN HM, WESTBROOK J, FENG Z, GILLILAND G, BHAT TN, WEISSIG H, et al., Nucleic Acids Res, **28**, 2000, p. 235.
15.TROTT O, OLSON A., J Comput Chem, **31**, 2010, p. 455.
16.FENG Y, LI W, LI J, WANG J, GE J, XU D, et al., Nature, **491**, 2012, p. 478.
17.YANKOVSKAYA V, HORSEFIELD R, TORNROTH S, LUNA-CHAVEZ C, MIYOSHI H, LEGER C, et al., Science, **299**, 2003, p. 700.

Manuscript received: 31.08.2018