IN SILICO CHARACTERIZATION AND PRELIMINARY ANTICANCER ASSESSMENT OF SOME 1,3,4-THIADIAZOLES

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The aim of this study was to evaluate antiproliferation potency of three 1,3,4-thidiazole derivatives previously synthetized and characterized, by molecular docking approach. In this context, possible medicinal applications by accelerated in silico assessment using computational tools were achieved by identification of intra-molecular interactions and binding affinity related to Human Topo IIα ATPase/AMP-PNP, known as target for anticancer agents.

Additionally, oral bioavailability and key physical chemical properties for druglikeness assessment were evaluated using dedicated software. Results of docking simulations reveal lower docking score for investigated structures than for the native ligand. Forward structural optimization by increasing hydrophilicity is required.

Keywords: molecular docking, 1,3,4-thiadiazoles, oral bioavailability, antiproliferative potency

1. Introduction

1,3,4-Thiadiazoles are aromatic heterocycles containing nitrogen and sulfur atoms, recognized as promising scaffold associated with potential biological and pharmacological properties [1 - 3]. Several of their derivatives have reveal either antimicrobial (*e.g.* 2-amino-1,3,4-thiadiazole) [4 - 7], anti-inflammatory [8], anticonvulsant [9, 10], antituberculosis [11] or antiproliferative activity [12, 13]. Starting from computational concept of analog series-based scaffolds [14] premises, this study aims to virtually screen some 1,3,4–thiadiazole

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derivatives, recently synthetized and characterized [15] for antiproliferative potency. Targeting Human DNA topoisomerase II and other cancer-related targets represent attractive approach for design new anticancer therapeutic agents [16].

2. Computational procedure

The molecular docking simulations were carried out using CLC Drug Discovery Workbench (Qiagen) on Human Topo IIα ATPase/AMP-PNP (PDB ID: 1ZXM) at 1.87 Å resolution [17].

The structures of the investigated ligands 2-phenyl-5-((4,6,8-trimethylazulen-1-yl)diazenyl)-1,3,4-thiadiazole (**T1**), 2-(azulen-1-yld)-5-phenyl-1,3,4-thiadiazole (**T2**), and 2-(azulen-1-yldiazenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazole (**T3**) were generated with Spartan 16 Software, Wavefunction Inc, USA [18], and optimized by molecular mechanics simulations [19] leading to the lowest energy conformers. The structures are given in Fig. 1 as 2D representation (up) and 3D tube representation (down) as minimized geometries with their atom labeling arbitrary chosen by the software.

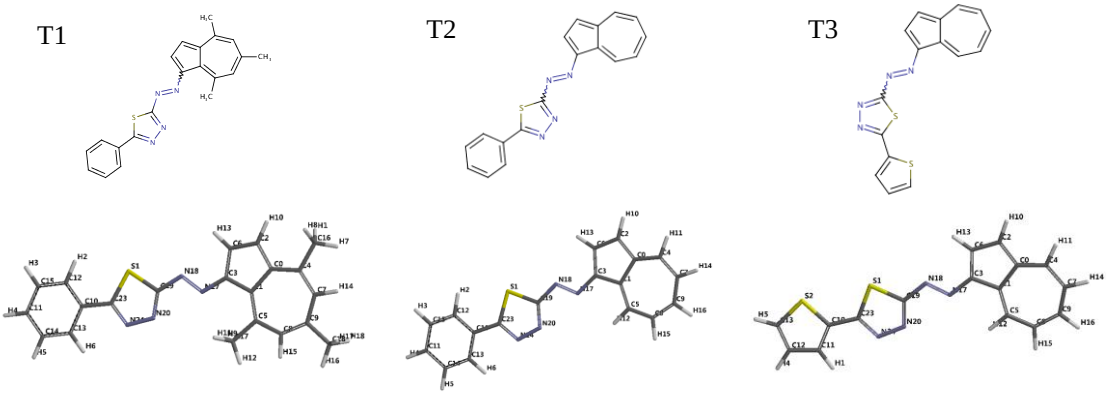

Fig. 1. 2D structures (up) and 3D optimized geometries (down) of **T1**, **T2** and **T3**

The water molecules and existing co-factor (magnesium ion) were removed before docking. The co-crystallized phosphoaminophosphonic acid-adenylate ester (ANP) was re-docked. Similar docking pose with the native ligand was obtained. Thus, the docking protocol was validated. The binding pocket was setup up on chain A at 159.23 $Å^2$. The results are given as docking score function and root mean square deviation (RMSD).

Property computations were performed using *Density Functional Theory* (DFT) and hybrid B3LYP functional [20], 6-31G* polarization basis set [21] for equilibrium geometry at ground state. Physical chemical descriptors were calculated with 16 Software, Wavefunction Inc, USA [18] and SwissADME online platform (http://www.swissadme.ch) [22].

3. Results and discussion

Table 1

Results of docking simulations for the native ligand ANP on 1ZXM

Ligand	Interacting group	Hydrogen bonds/Length (Å)	Score/RMSD
co-crystalized ANP A 901	LYS378, GLN376, ASN163, ARG162, GLY150, GLY161, ASN150, GLY150, GLY164, TYR165, GLY166, GLU87, SER148, SER149, ASP94, THR147, LYS168, ALA167, ILE88, ILE141, PHE142, ILE141, PHE142, ILE217, ASN120, THR215, ILE125, GLY124, LYS123, ALA92, ASN95, ARG98 (**chain A**)	O2A sp^2 – N sp^2 ALA167 / 2.960 O2A sp^2 – N sp^3 LYS168 / 2.882 O2A sp^2 – N sp^2 GLY166 / 3.292 O1A sp^2 – N sp^2 ALA167 / 3.383 O1A sp^2 – N sp^2 ASP91 / 2.909 O5' sp^3 – N sp^3 LYS168 / 3.204 O2B sp^2 – O sp^3 SER148 / 2.758 O2B sp^2 – N sp^2 ASN150 / 3.137 O3' sp^3 – N sp^2 SER149 / 3.315 O2' sp^3 – O sp^3 SER149 / 2.706 N6 sp^2 – O sp^2 ASN120 / 2.824 O3G sp^2 – N sp^2 GLY164 / 2.987 O3G sp^2 – N sp^2 TYR165 / 2.682 O3G sp^2 – N sp^2 GLY166 / 2.878 O3G sp^2 – N sp^2 GLN376 / 3.310 N3B sp^3 – N sp^2 GLY164 / 3.089 N3B sp^3 – N sp^2 ASN163/ 3.290 N3B sp^3 – N sp^2 ARG162 / 2.815 O2G sp^2 – N sp^2 ASN163 / 3.160 O2G sp^2 – N sp^2 ARG162 / 2.826 O2G sp^2 – N sp^3 LYS378 / 3.057	-77.87 / 0.82

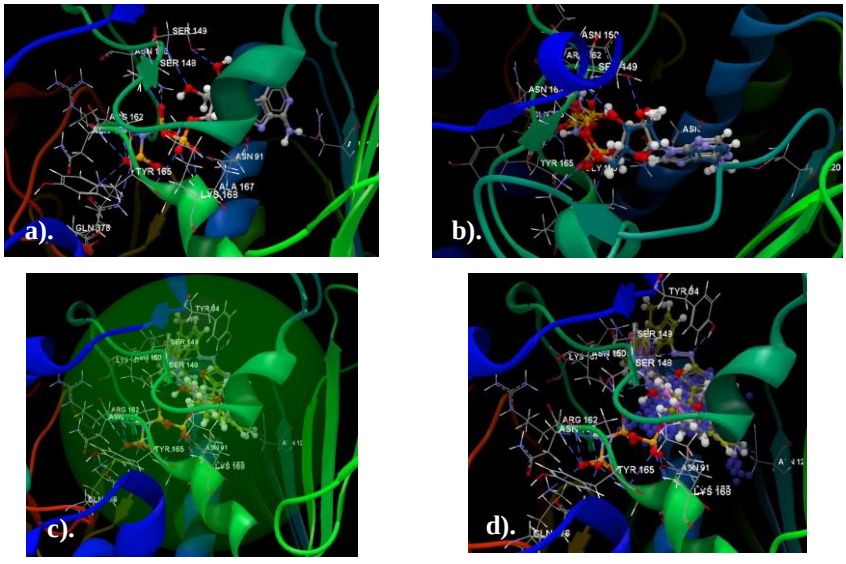

Fig. 2. Intramolecular interactions between the native ligand ANP and 1ZXM (a); superposition of the docked ANP (b); superposition of all docked ligands in the active binding site (transparent green sphere) of 1ZXM (c) and representation of binding pocket 159.23 $Å^2$ (blue spheres) (d)

ANP was initially docked into the crystal structure of Human Topo IIa ATPase, in order to validate the docking protocol, and its interactions with the target protein were analyzed. As can be seen Fig. 2a, the natural ligand forms 21 hydrogen bonding interactions with amino acid residues from chain A: LYS378, GLN376, ASN163, ARG162, GLY150, GLY161, ASN150, GLY150, GLY164, TYR165, GLY166, GLU87, SER148, SER149, ASP94, THR147, LYS168, ALA167, ILE88, ILE141, PHE142, ILE141, PHE142, ILE217, ASN120, THR215, ILE125, GLY124, LYS123, ALA92, ASN95, ARG98. In Fig. 2b, the superposition of the binding pose of ANP, obtained by re-docking, is shown. As illustrated in Fig. 2c, all investigated structures were found to have similar binding poses to the natural ligand, thus validating the used docking methodology. The binding pocket is displayed in blue dots in Fig. 2d. As listed in Table 1, the co-crystallized ANP interacts through conventional hydrogen bonds by its numerous Osp^3 from libre hydroxyls attached to tetrahydrofuran ring with SER149, and its numerous Osp^2 of its phosphoaminophosphonic acid residue with ALA167, LYS168, GLY166, ASP91, SER148, ASN150, GLY164, TYR165, GLN376, ASN163, ARG162, LYS378. The nitrogen atom of amino group (N6) of the purine derivative (adenine) forms hydrogen bond with ASN120 and the nitrogen included in the phosphoaminophosphonic acid group (N3B) is involved in the realization of three hydrogen bonds with Nsp^2 of GLY164, ASN163 and ARG162. The resulting docking score for ANP is -77.87 (RMSD: 0.82), suggesting a very string binding affinity and stability of ANP-DNA topoisomerase II, alpha isozyme complex.

As it can be seen in Table 2, the investigated ligands are poorly interacting, forming two (**T1**) or three (**T2** and **T3**) hydrogen bonds, with the same amino acid residue, SER149. **T1** interferes with chain B too, by TYR34. **T1** ligand interacts by the two nitrogen atoms of the azo bond linking the azulene ring and thiadiazole (N17 and N18). **T2** forms three hydrogen bonds with SER149 and ASN150 by its two nitrogen (N20 and N24) of the thiadiazole heterocycle. Although, apparently more interacting than its analogue tri-methyl substituted on azulene, **T2** docking simulations result in a lower score (-58.26 *vs* -61.42). Regarding **T1** compound, it forms hydrogen bonding with SER149 by the nitrogen of azo bond near to the azulene (N17) and with LYS157 by one nitrogen of the thiadiazole, resulting a docking score of -55.31. Fig. 3 depicts the intramolecular interactions in the resulted complex of DNA topoisomerase II, alpha isozyme and investigated ligands **T1**-**T3**.

Considering all above-mentioned findings, it can be assumed that, among the screened 1,3,4 thiadiazole derivatives, **T1** ligand exhibits the greater docking score, and consequently the stronger binding affinity related to Human Topo II ATPase. It has higher antiproliferative potency from the investigated ligands. Substituting the thiadiazole with a phenyl ring (**T2**) with a thiophen in position 5

(**T3**), leads to increased score (-58.26 *vs* -55.31). All these observations can be useful for further optimization, by designing hybrid structural analogues and enhancing the binding affinity by favor hydrophilic interactions.

Table 2

Results of docking simulations for T1-T3 ligands on 1ZXM

Target / Ligand Score/RMSD	Interacting group	Hydrogen bonds/Length (Å)
1ZXM / T1 -61.42 / 0.06	**Chain A**: PHE142, ILE125, ILE141, LYS168, ALA167, GLY166, ARG98, ASN91, ASN95, ASP94, THR159, GLN97, VAL158, LYS157, GLU155, ASN150, SER149, GLY164, SER148, THR147 **Chain B**: ILE33, TYR34	N17 sp^2 – O sp^3 SER149:A /2.561 N18 sp^2 – O sp^3 TYR34:B / 3.064
1ZXM / T2 -58.26 / 0.02	**Chain A:** ARG98, GLN97, ASN95, THR215, ASN120, ALA92, ASP94, ASN91, ILE217, THR159, VAL158, ALA167, PHE142, ILE141, ASN150, SER149, LYS157, ILE125	N20 sp^2 – N sp^2 ASN150/ 3.111 N20 sp^2 – O sp^3 SER149 / 2.762 N24 sp^2 – O sp^3 SER 149 / 3.015
1ZXM T3 -55.31 / 0.15	**Chain A**: THR147, SER149, SER148, ASN150, LYS157, VAL158, THR159, GLN97, ASP94, ASN91, ASN95, ALA167, ARG98, LYS168, PHE142, ILE141 **Chain B**: TYR34	N17 sp^2 – O sp^3 SER149 / 3.142 N20 sp^2 – O sp^3 SER149 / 2.578 N24 sp^2 – N sp^3 LYS157 / 3.078

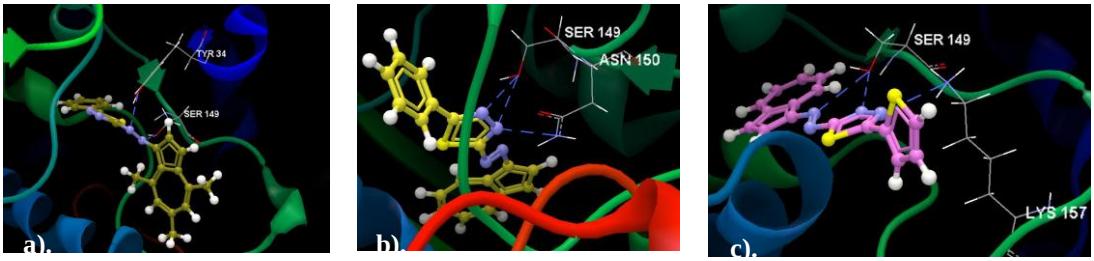

Fig. 3. Hydrogen bonding interactions (blue dashed lines) of **T1** (a), **T2** (b) and **T3** (c) with amino acid residues form the active binding site of 1ZXM

Lipinski C.A. et al. [23, 24] established restrictions for molecular weight (MW ≤ 500 Da), number of hydrogen bond donors (HBD ≤5), number of hydrogen bond acceptor (HBA≤10) and the octanol-water partition coefficient (logP ≤5) in order to evaluate the oral availability of drug candidates and to assess their pharmacokinetics.

Veber and co-workers [25] added supplementary limitations for polar surface area (PSA ≤140 $Å^2$) and flexibility of the molecules (no more than 10 rotatable bonds) to improve the predictions about drug-likeness.

In Table 3, are listed the key molecular descriptors, calculated to evaluated such properties of pharmacological interest: molecular mass (MW), polar surface area (PSA), calculated with Spartan software, TPSA – topological polar surface area, calculated with SwissADME online platform, counts of hydrogen-bond donors (HBD) and acceptors (HBA), the water-octanol partition coefficient calculated with Spartan (logP) and with SwissADME tools (WLOGP), number of rotatable bonds (rb) and number of Lipinski's rule of five violations (RO5). In Spartan procedures, logP is estimated according to the method of Ghose, Pritchett and Crippen, 1988 [26]. SwissADME tools uses the atomistic method implemented by Wildman S.A. and Crippen G.M., 1999 [27] to calculate logP (WLOPG). Topological surface area (TPSA) values are calculated from Ertl P. et al 2000 [28] method, with SwissADME prediction tools.

Table 3

Calculated molecular descriptors for oral bioavailability prediction

ligand	MW*	PSA*	TPSA*	HBD	HBA	logP	WLOGP	rb	RO5
ANP	**502.16**	-	311.36	5	**18**	-7.56	-2.37	8	2
T1	358.46	34.289	78.74	0	4	**5.24**	6.65	3	**1**
T2	316.38	36.099	78.74	0	4	**5.49**	5.73	3	**1**
T3	322.41	36.404	106.98	0	4	**5.21**	5.79	3	**1**

* Units: MW – mol·kg^{-1}; PSA and TPSA - $Å^2$

As observed form Table 3, the investigated 1,3,4-thiadiazole derivatives respect all properties restrictions, except logP, that is greater than 5, suggesting highly lipophilic behavior, with poor aqueous solubility. Generally, this parameter servs to categorization of the compounds by water-solubility and membrane-permeability; values of logP over 5 suggest poor absorption or permeation. This aspect can be improved by structure optimization of such ligands containing together azulene and thiadiazole moieties, to increase the hydrophilicity and to enhance their interacting capacity; thus, the possibility to become biologically active, can be successfully achieved. The co-crystallized ANP reveals 2 Lipinski's violations, due to its molecular mass, greater than 500 mol·kg^{-1} and too many hydrogen bond acceptors (18>10). Its structure is highly hydrophilic (negative logP value).

In drug design and development, an intuitive and rapid method to assess the passive gastrointestinal absorption and brain access of small is *the Brain Or IntestinaL EstimateD permeation method* (BOILED-Egg) by computing the lipophilicity and polarity [29].

These relevant physicochemical parameters are taken into consideration for the biopharmaceutical evaluation of new drug candidates, allowing to rationalize their selection or to enhance drug solubilization by using oral lipid-based drug delivery systems [30, 31].

Fig. 4 represents the plot of two physicochemical descriptors, WLOGP-*versus*-TPSA, as BOILED-Egg representation, allowing the evaluation of passive human gastrointestinal absorption (HIA) and blood-brain penetration (BBB) in function of the position of the molecules in the graph.

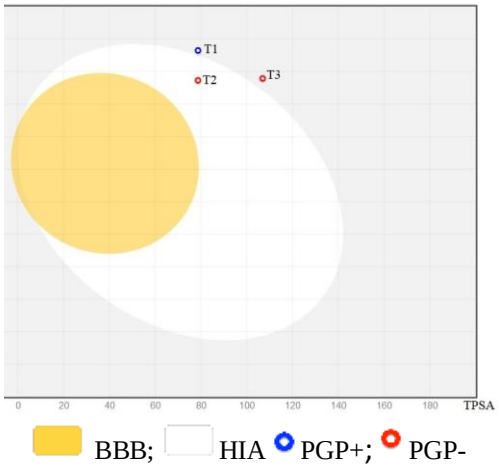

Fig. 4. BOILED-Egg representation, calculated with SwissADME software platform

From the BOILED-Egg permeation predictive model diagram, it can be observed that all investigated compounds have no BBB permeability, none of them being located in the yellow area. **T2** is in the withe ellipse, indicating the probability to be passively absorbed by the gastrointestinal tract. **T1** and **T3** are represented in the grey zone, suggesting that these molecules are not well absorbed, not blood-brain barrier (BBB) permeant. **T1** (point colored in blue) is predicted to be substrate of the P-glycoprotein (PGP+) and hence actively pumped up from the brain or to the gastrointestinal lumen. **T2** and **T3** (points colored in red) are predicted as non-substrate of the P-glycoprotein (PGP-). Although very hydrophilic (WLOGP = -2.37), the native ligand, ANP is out of range in the BOILED-Egg plot, due to very high value of the polar surface area, greater than the maximal required value (311.36 >> 140 $Å^2$).

4. Conclusions

The attempt to evaluate three hybrid compounds containing azulene and thiadiazole moieties coupled by azo bond in regard with their potential as drug candidates with anti-proliferative activity showed poor hydrophilicity of investigated molecules. It has as consequence the probability of poor or passive absorption through the gastrointestinal tract. 2-phenyl-5-((4,6,8-trimethylazulen-1-yl)diazenyl)-1,3,4-thiadiazole and 2-(azulen-1-yldiazenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazole are expected not to be blood-brain barrier permeant.

Lower docking score for all calculated structures than for the native ligand were observed. However, 2-phenyl-5-((4,6,8-trimethylazulen-1-yl)diazenyl)-1,3,4-thiadiazole exhibits a moderate docking score and stronger binding affinity related with Human Topo IIα ATPase.

Forward structural optimization is mandatory by adding hydrophilic groups such as hydroxyl, amino, sulphonyl, etc. in order to increase the hydrophilic-lipophilic balance. Starting from this accelerated computational screening optimized hybrid structural analogues with antiproliferative potency can be designed for further pre-clinical assays.

Such ligand-based interactions approach should be trained and tested for larger number of compounds for which activity against Human Topo IIα ATPase has been experimentally noticed.

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R E F E R E N C E S

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