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In silico Identification of New Antiproliferative Compounds, Inhibitors of Matrix Metalloproteinases

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Abstract. Tenascin C (TNC) is a glycoprotein of the extracellular matrix and its overexpression induced by matrix metalloproteinases (MMPs) is associated with the amplification of the proliferative response to growth factors, promotion of cancer cell invasion and tumoral growth. This paper looks forward to find better inhibitors of MMPs, based on structure of batimastat (BAT) – a synthetic inhibitor of MMPs and an experimental drug against matrix metalloproteinase-16 (MMP16). To reach this aim, at first it was built a complete virtual library using 12 scaffolds (SCs) from BAT, concatenated via a dummy linker with 40 building blocks (BBs) found in 14 antineoplastic drugs. Second, all the derivatives from the virtual library were filtered, through mass-computation, for "drug-likeness", undesirable moieties and checked if they are PAINS (Pan Assay Interference Compounds). At last, a virtual screening of "drug-like" virtual derivatives, free of undesirable moieties (except of the thiophene moiety) and BAT against the homologue model of MMP16 was made, using docking software in order to identify the compounds that specifically bind the enzyme. In the same time it was determinate the smallest binding energy required to bind MMP-16 to reveal the best ligands. Results show that 86 virtual derivates (1% of the 6720 derivates from the virtual library) are "drug-like" compounds. However, only 23 of the "drug-like" virtual derivates have better metrics than BAT, are free of undesirable moieties and are not PAINS. In addition, 15 of them are better ligands for MMP16 than BAT.

Keywords: tenascin C, matrix metalloproteinases, virtual screening, docking.

INTRODUCTION

TNC is a complex multifunctional protein, which has been shown to promote cell migration, inhibit focal contact formation, promote angiogenesis and, in some systems, act as a cell survival factor. Cell migration, a key factor for tumor invasion, metastasis and angiogenesis, is not possible without destruction of extracellular matrix (ECM) (Jones *and* Jones, 2000). ECM destruction is up regulated by proteolytic enzymes, particularly members of the matrix metalloproteinase (MMP) family. Overexpression of several MMPs has been described in many cancer types; several studies have indicated a role for TNC in regulating MMP gene expression (Tremble *et al.*, 1994).

Batimastat inhibits MMPs activity by binding the zinc ion from the active site of this class of metalloproteinases. BAT binds the MMPs with its thiophene moiety deeply inserted in the active site of this class of enzymes (Botos *et al.*, 1996). In present, BAT is registered as

experimental drug against matrix metalloproteinase-16 (MMP16) in the public domain of DrugBank (http://www.drugbank.ca) with the accession number DB03880 (Knox *et al.*, 2011; Wishart *et al.*, 2006, 2008). The thiophene moiety, which gives BAT its specific binding ability to the active site of MMPs, is a flagged or intermediate substructure (substructure/compound that could be problematic in drug development process).

Due to the enormous costs required be the development of new drugs, computational drug design became necessary owing its capability for handling large amounts of data and its multidimensional approach. Moreover, computer simulations are less costly per compound than any laboratory test, so the large compounds collections can be tested *in silico* with low expenses. The "drug-likeness" indicators, compulsory used for new drug development, do not indicate that a compound will be a good drug for a certain disease, but compounds that do not meet "drug-likeness" criteria frequently fail to be good drugs due to weak bioavailability, toxicity or ADME (administration, distribution, metabolism and excretion) problems (Young, 2009). Binding properties play a major role over compound efficacy (the qualitative property of a compound having the desired effect) and its activity (the quantitative measure of how much of that compound is required to have a measured effect).

The aim of this work, carried out entirely *in silico*, is to improve the "drug-likeness" metrics and to increase the binding properties of compounds from a full virtual library build on the backbone structure of BAT – due to its binding properties the thiophene moiety was conserved in the structure of all virtual derivates; even it is a flagged substructure. To avoid ADME and toxicity related issues of virtual derivates a very strict filtering for "drug-likeness" indicators was used. In addition, "drug-like" virtual derivates were checked for presence of undesirable moieties and if they can be PAINS. Finally, "drug-like" virtual derivates free of undesirable moieties (except of thiophene moiety, present in the backbone structure of all compounds from virtual library), classified as "intermediate compounds", and together with BAT were submitted for virtual screening against the homologue model of MMP16.

MATERIALS AND METHODS

Entire work was made computationally. MarvinSketch was used for drawing of the chemical structures, generation of SMILES (Simplified Molecular Input Line Entry System) strings for SCs and BBs, tridimensional (3D) optimization of chemical structures for BAT and "intermediate compounds" and displaying of the chemical structures, MarvinSketch 5.5.0.1, 2011, ChemAxon (http://www.chemaxon.com). MarvinView was used for displaying of all compound libraries and export as stand-alone file in MOL2 (Tripos Mol2) file format of each chemical structure from "intermediate compounds" library, MarvinView 5.5.0.1, 2011, ChemAxon (http://www.chemaxon.com). Based on SMILES strings for SCs and BBs and using a dummy linker as connector between SC and BB, SmiLib v2.0 (Schüller *et al.*, 2003) generated the full virtual library (written as SMILES strings). Web-based software tool FAF-Drugs2 (Lagorce *et al.*, 2008), hosted on the public domain of The Ressource Parisienne en Bioinformatique Structurale (http://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py), was used to filter for "drug-likeness", undesirable moieties and PAINS, the entire virtual library and BAT. As input data for FAF-Drugs2 were used SMILES strings. FAF-Drugs2 exported "drug-like" virtual derivates free of undesirable moieties (except of thiophene moiety) as compound library of "intermediate compounds" in SDF (structure data file) file format. PyRx – Python Prescription 0.8 (Wolf, 2009) has been used to screen "intermediate compounds" and BAT against the homologue model of MMP16. PyRx – Python Prescription 0.8 used AutoDock Vina (Trott and Olson, 2010) as docking software.

Building the full virtual library: SmiLib v2.0 concatenates SCs (Markush structures of molecules that contain sites of variability or R-groups) with BBs (small Markush molecules) via linkers (connectors between scaffold molecules and building blocks) using virtual reactions. MarvinSketch was used to draw the structures for SCs and BBs and to generate the input data for SmiLib v2.0 (SMILES strings for SCs and BBs).

The thiophene moiety was conserved in the chemical structure of all twelve SCs (Fig. 1) and was imposed one or two positions for sites of variability (R_1 and R_2) located in various positions. Eight scaffolds have only one site of variability (SC1, 3, 5, 7-10 and 12), meanwhile four scaffolds have two sites of variability (SC2, 4, 6 and 11).

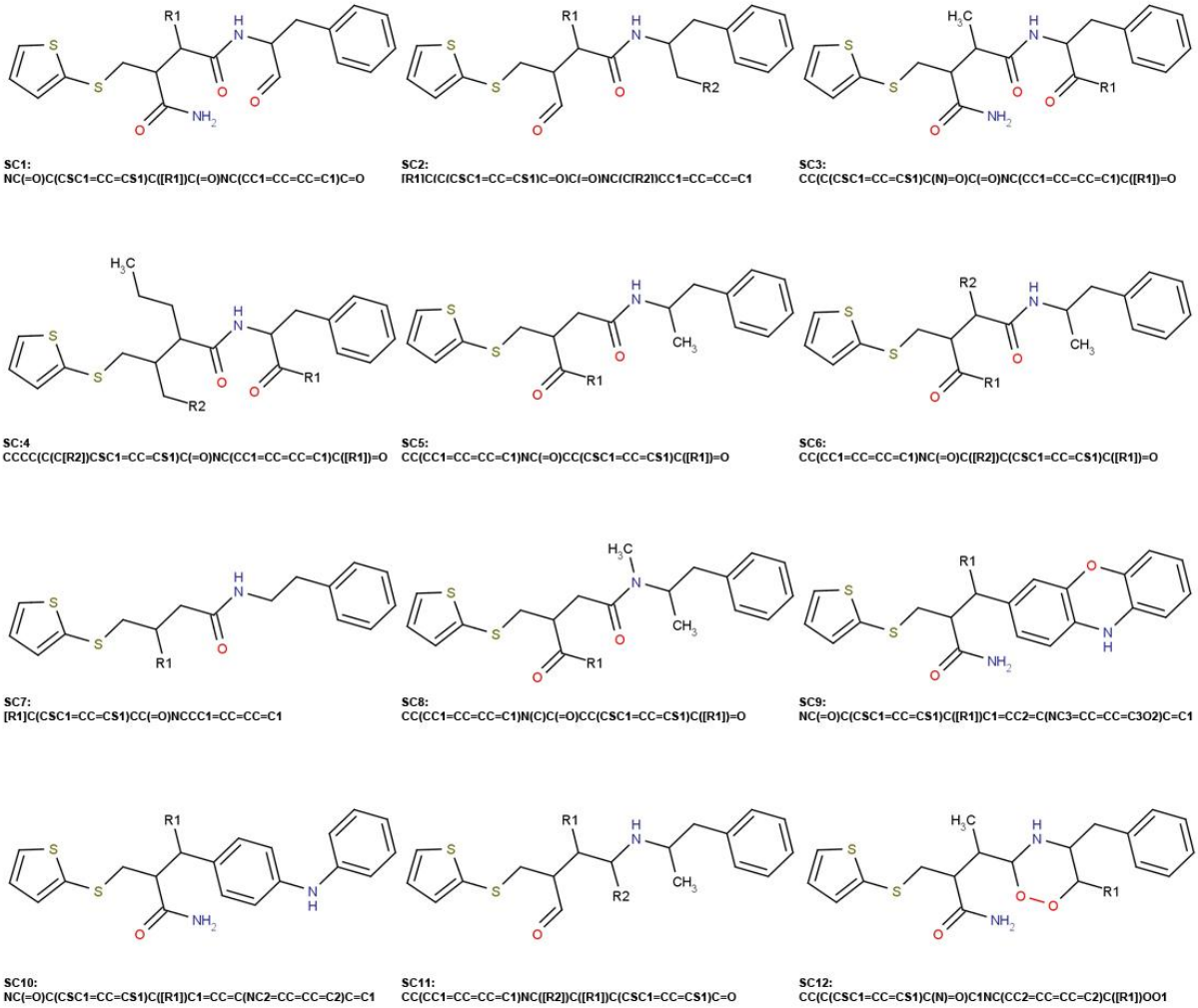

Fig. 1. The scaffolds based of backbone structure of BAT

All BBs used to construct the virtual library are based of structural motifs most frequently found in some antineoplastic drugs (cyclophosphamide, mechlorethamine, uramustine, melphalan, carmustine, lomustine, streptozotocin, busulfan, procarbazine, mercaptopurine, thioguanine, methotrexate, pemetrexed and raltitrexed), which were previously used to improve the permeability properties of anticancer anthracyclines through structural modification (Tamaian *et al.*, 2010).

To reduce the size and molecular weight of the virtual derivates it was used a dummy (empty) linker, which contains only the instructions regarding the attachment site [A] and the site of variability [R] and have the notation: [A][R].

SmiLib v2.0 constructed the full virtual library using virtual reactions and assigned the identifiers (IDs) for each virtual derivate using the following general definition: *scaffold.linker1_bblock1.linker2_bblock2...*

Computational filtering to indentify the "drug-like" virtual derivates, undesirable moieties and PAINS has been done using exclusively with FAF-Drugs2. First, the entire virtual library and BAT was filtered for "drug-likeness" according to the preset specifications of FAF-Drugs2:

- molecular weight (MW) between 150 Da and 600 Da;
- number of the hydrogen bond donors (HBD) between 0 and 5;
- number of the hydrogen bond acceptors (HBA) between 0 and 10;
- octanol/water partition coefficient (Log *P*) between -4 and 5, calculated according XLOGP3 method developed by Cheng *et al.*, 2007;
- maximum one violation of the Lipinski rule of fives (vRO5) for oral bioavailability regarding values of MW, HBD, HBA and Log *P* as stated by Lipinski *et al.* (1997, 2001);
- number of the heavy atoms (HVA) between 0 and 37;
- number of the rotatable bonds (RotB) between 0 and 15;
- number of the rigid bonds (RB) between 0 and 30;
- number of the chemical rings (CR) between 0 and 5;
- maximum size of the largest ring (MxCR) = 12;
- topological polar surface area (TPSA) between 0.00 Å and 160.00 Å, calculated according to the method developed by Ertl *et al.*, 2000;
- number of the formal charges (FC) between 0 and 3;
- sum of the formal charges (SFC) between -2 and 2.

In addition to those filters, it was calculated solubility (mg/l) and bioavailability was expressed as "good" or "bad" according both to rules of Egan *et al*., (2000) and Veber *et al.*, (2002).

"Drug-like" virtual derivates and BAT were checked for undesirable moieties and if they are PAINS. The undesirable moieties and substructures involved in toxicity issues and searched by FAF-Drugs2 are warheads according to Rishton (2003), frequent hitters according to Roche *et al.* (2002), promiscuous inhibitors according to McGovern *et al.* (2002), flagged or intermediate substructure according to medicinal chemistry knowledge (substructures or compounds that can be problematic) and other diversity moieties (excluding PAINS). PAINS, according to Baell and Holloway (2010), are promiscuous compounds that appear as frequent hitters in many biochemical high throughput screens and are increasingly prevalent, as promising starting points for further exploration, whereas they may not be.

Virtual screening: prior to virtual screening and docking the individual files of each intermediate "drug-like" virtual derivates and BAT were 3D optimized with MarvinSketch. The screening software, PyRx – Python Prescription 0.8, operates with two types of molecules: ligands and receptors. Ligands are small molecules (test compounds) and receptors are macromolecules, which represent de therapeutic target of small molecules.

As receptor it was used a homologue model of MM16 for *Homo sapiens* (UniProtKB/Swiss-Prot ID: P51512) built-up on the base of the crystal structure of the catalytic domain of MMP16 (PDB ID: 1rm8) using the public domain of SWISS-MODEL

Repository (http://swissmodel.expasy.org/repository/), (Kiefer et al., 2009; Kopp and Schwede, 2004).

PyRx – Python Prescription 0.8 use AutoDock Vina as docking software and AutoDock Vina in order de operate requires structures of the molecules being docked and grid-boxes to define the binding site (search space). AutoDock Vina do not need to calculate the grid maps and to assign atom charges. The predicted binding affinity of bound structures is given in kcal/mol. To compare the accuracy of the predictions of the experimental structure, AutoDock Vina use a measure of distance between the experimental and predicted structures – RMSD (root-mean-square deviation). RMSD metrics are expressed as RMSD lower bound (rmsd/lb) and RMSD upper bound (rmsd/ub), differing in how the atoms are matched in the distance calculation. For scoring, AutoDock Vina uses a united-atom function, which involves only the heavy atoms.

To start virtual screening, all ligands and the receptor were loaded in PyRx – Python Prescription 0.8 graphic user interface (GUI) and search space was set as following:

- center coordinates: X = 9.5101; Y = 12.0527; Z = 46.1456;
- dimensions (Å): X = 25.0000; Y = 25.0000; Z = 25.0000.

After Autodock Vina calculated de lowest binding affinities and RMSD metrics, docking results were displayed and analyzed in the GUI of PyRx – Python Prescription 0.8.

RESULTS AND DISCUSSIONS

Using virtual reactions, SmiLib v2.0 concatenated the twelve SCs (eight with one site of variability and four of them with two sites of variability) and forty BBs using a dummy linker as connector between them. The full virtual library contains 6720 virtual derivates displayed as SMILES strings (Fig. 2).

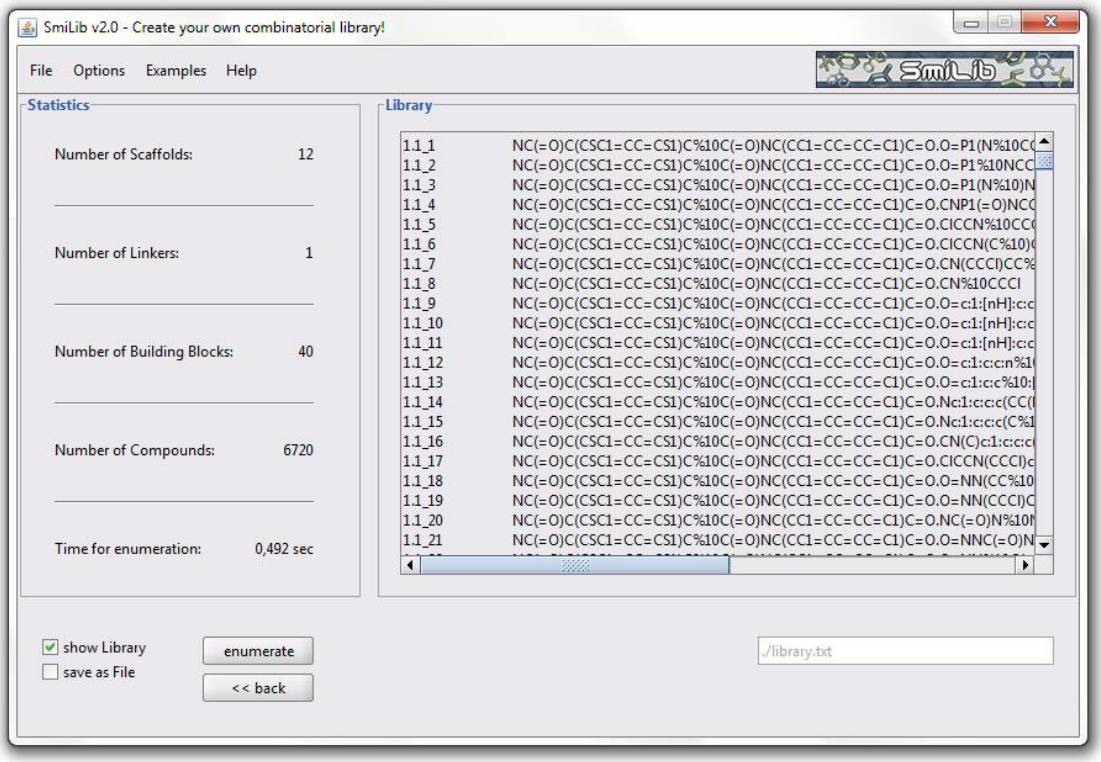

Fig. 2. GUI of SmiLib v2.0 with enumeration of virtual library (6720 virtual derivates)

Each of the eight SCs with one site of variability (SC1, 3, 5, 7-10 and 12) generated 40 virtual derivates with 40 BBs (1x40=40). Each of the scaffolds with two sites of variability (SC2, 4, 6 and 11) generated 1600 virtual derivates with 40 BBs (1x40x40=1600). The resulted virtual library contains 320 (5%) compounds from SCs with one site of variability and 6400 (95%) compounds from SCs with two sites of variability.

Computational filtering done with FAF-Drugs2 qualified as "drug-like" virtual derivates only 86 (1%) compounds, meanwhile 6634 (99%) compounds were rejected (data not shown).

Filtering the "drug-like" virtual derivates for undesirable moieties revealed that 62 compounds were rejected due to severe risk to heath caused especially by warhead moieties. The rest of 24 virtual derivates are considered intermediate compounds due their thiophene moiety (data not shown).

Filtering the "drug-like" virtual derivates for PAINS reveal that only 5.1_16 can be a such as promiscuous compound (data not shown) and cannot be considered an intermediate compound .

Tab. 1.

The result of FAF-Drugs filtering for intermediate compounds

ID	MW (Da)	HBD	HBA	Log *P*	vRO5	HVA	RotB	RB	CR	MxCR	TPSA (Å)	FC	SCF	Solubility (mg/l)
BAT	477.64	4	7	3.55	0	32	12	17	2	6	161.07	0	0	6083.19
5.1_2	466.55	2	6	2.93	0	30	10	21	3	6	147.85	0	0	8141.83
5.1_3	481.57	3	7	2.66	0	31	10	22	3	6	159.88	0	0	9116.63
5.1_4	495.60	3	7	2.25	0	32	11	21	3	6	159.88	0	0	11967.74
5.1_12	457.57	2	7	2.82	0	31	9	23	3	6	154.57	0	0	7745.82
7.1_2	424.52	2	5	2.81	0	27	9	20	3	6	130.78	0	0	9586.33
7.1_3	439.53	3	6	3.13	0	28	10	20	3	6	142.81	0	0	7938.12
7.1_4	453.56	3	6	2.67	0	29	10	20	3	6	142.81	0	0	10106.08
7.1_11	415.53	3	6	2.51	0	28	9	21	3	6	148.36	0	0	10851.90
7.1_12	415.53	2	6	2.70	0	28	9	21	3	6	137.50	0	0	9627.66
7.1_13	415.53	3	6	2.51	0	28	9	21	3	6	148.36	0	0	10851.90
7.1_20	392.50	3	7	2.90	0	26	9	17	2	6	158.40	0	0	10026.01
7.1_21	392.50	3	7	3.12	0	26	10	17	2	6	153.20	0	0	9323.92
7.1_29	455.62	1	6	4.16	0	30	9	24	3	9	159.21	0	0	3614.38
7.1_35	539.71	3	7	5.00	1	37	14	22	3	6	152.28	1	-1	2033.36
7.1_36	539.71	3	7	5.00	1	37	14	22	3	6	152.28	1	-1	2033.36
7.1_38	506.68	2	6	4.02	1	35	11	25	3	10	131.63	1	1	3360.74
8.1_2	480.58	1	6	3.11	0	31	10	21	3	6	139.06	0	0	6908.57
8.1_3	495.60	2	7	2.84	0	32	10	22	3	6	151.09	0	0	7725.41
8.1_4	509.62	2	7	2.43	1	33	11	21	3	6	151.09	0	0	10127.64
8.1_11	471.59	2	7	2.93	0	32	10	22	3	6	156.64	0	0	7378.89
8.1_12	471.59	1	7	3.00	0	32	9	23	3	6	145.78	0	0	6609.60
8.1_13	471.59	2	7	2.93	0	32	10	22	3	6	156.64	0	0	7378.89
10.1_2	487.57	4	6	3.50	0	32	8	26	4	6	156.80	0	0	4243.31

Note: Underlined values represent violations of RO5 according with the specifications of the authors (Lipinski *et al.*, 1997, 2001) for a good bioavailability: MW ≤ 500 Da, HBD ≤ 5, HBA ≤ 10 and CLog *P* ≤ 5. Violation of a single rule does not disqualify a compound for being bioavailable.

In Tab. 1 are summarized the results of FAF-Drugs filtering for "drug-like" compounds, selectively presented only for BAT and "drug-like" virtual derivates free of for undesirable moieties and definitely not PAINS.

Based on result from Tab. 1, FAF-Drugs qualified BAT and all "drug-like" virtual derivates free of for undesirable moieties and definitely not PAINS as having a "good" bioavailability according both to rules of Egan *et al*., (2000) and Veber *et al.*, (2002). However, BAT presents a value of TPSA higher than the upper limit, which can affects the human intestinal absorption, Caco-2 monolayer permeability, and blood-brain barrier penetration of this compound. The 23 "drug-like" virtual derivates free of for undesirable moieties and definitely not PAINS qualified as intermediate compounds don not overpass the TPSA value.

Analyzing the origin of intermediate compounds from Tab. 1 it can be observed that not even a single virtual derivate based on any of SCs with two sites of variability has been qualified as intermediate compound according the imposed criteria, even those derivates represents 95% of entire virtual library.

The virtual screening results show that all intermediate compounds are strong inhibitors of MMP16 (Tab. 2).

Tab. 2

Docking results (only the lowest energy for each compound corresponding to the best bound structure prediction are showed)

Receptor - Ligand	Binding affinity (kcal/mol)	rmsd/ub (Å)	rmsd/lb (Å)
MM16 - 7.1_38	-9.50	0.00	0.00
MM16 - 10.1_2	-9.10	0.00	0.00
MM16 - 8.1_13	-9.00	0.00	0.00
MM16 - 5.1_12	-8.90	0.00	0.00
MM16 - 7.1_13	-8.90	0.00	0.00
MM16 - 5.1_4	-8.80	0.00	0.00
MM16 - 7.1_21	-8.80	0.00	0.00
MM16 - 7.1_12	-8.70	0.00	0.00
MM16 - 8.1_3	-8.70	0.00	0.00
MM16 - 8.1_11	-8.70	0.00	0.00
MM16 - 7.1_11	-8.60	0.00	0.00
MM16 - 7.1_20	-8.60	0.00	0.00
MM16 - 7.1_29	-8.60	0.00	0.00
MM16 - 7.1_36	-8.60	0.00	0.00
MM16 - 7.1_4	-8.50	0.00	0.00
MM16 - 7.1_35	-8.50	0.00	0.00
MMP16 - BAT	-8.40	0.00	0.00
MMP16 - 5.1_3	-8.30	0.00	0.00
MMP16 - 7.1_2	-8.30	0.00	0.00
MMP16 - 5.1_2	-8.20	0.00	0.00
MMP16 - 8.1_4	-8.20	0.00	0.00
MMP16 - 7.1_3	-8.10	0.00	0.00
MMP16 - 8.1_2	-7.70	0.00	0.00
MMP16 - 8.1_12	-7.70	0.00	0.00

Note: Results are showed arranged from the strongest ligand (top) to the weaker ligand (bottom).

The RMSD cutoff of 2Å is usually used as criteria of the correct bound structure prediction (Bursulaya *et al.*, 2003). Using the same cutoff value, the two metrics used for RMSD (summarized in Tab. 2) indicate that all predictions for tested compounds are very accurate. Results from Tab. 2 indicate that 15 intermediate compounds are better ligands of MMP16 than BAT because they require a lesser energy for biding; one compound presents the same binding affinity as BAT and 7 intermediate compounds are weaker ligands than BAT.

From the Fig. 3 it can be observed that all tested compounds bind in the active site of MMP16, having various spatial orientations correlated with their spatial conformation.

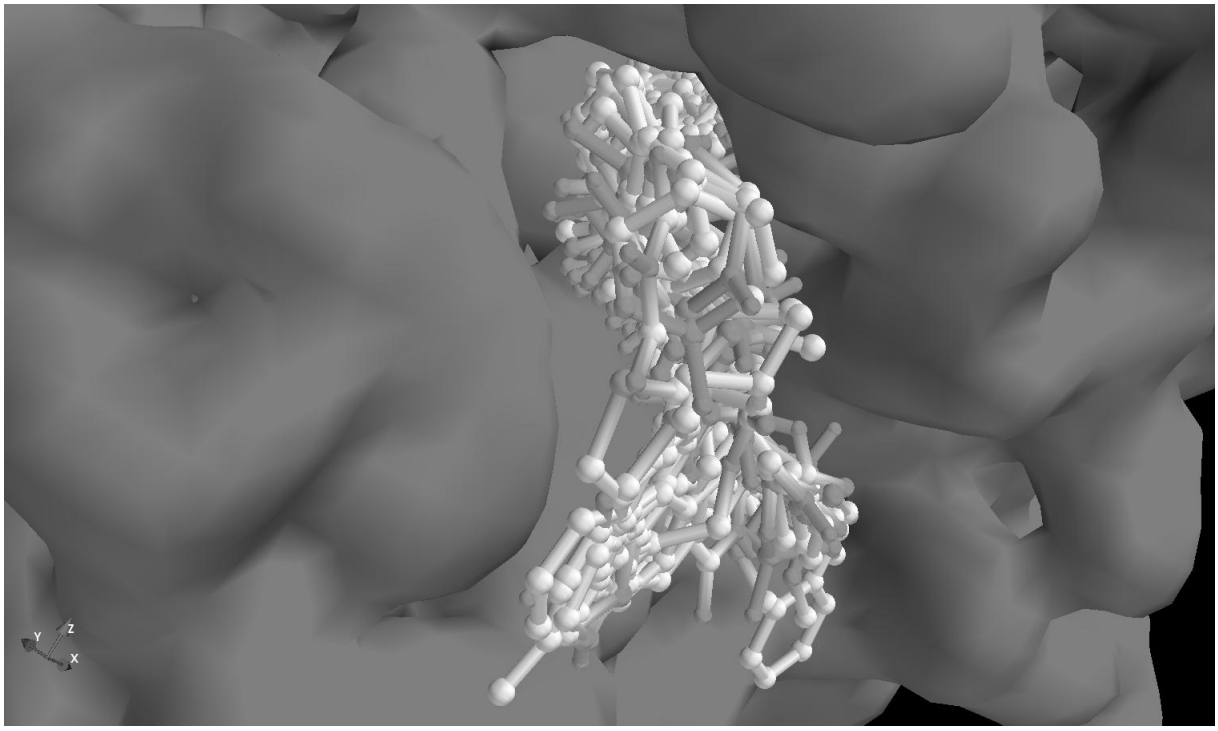

Fig. 3. Image of binding conformations of docked compounds in the active site of MMP16

CONCLUSIONS

In this work, a computational approach was used to identify safer and better inhibitors of MMPs aimed to reduce the amplification of the proliferative response to growth factors, promotion of cancer cell invasion and tumoral growth. Using a large virtual library based on backbone structure of BAT, a severe selection of the safer "drug-like" virtual derivatives has been made using mass-computation methods. The virtual screening and molecular docking revealed 16 "drug-like" virtual derivatives, which are not only safer than BAT, but they are also much stronger ligands. Computational techniques represent a preliminary work in drug development process, which help to prioritize the organic syntheses and biochemical screening of most promising candidates.

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