Identification of New 2,5-Diketopiperazine Derivatives as Simultaneous Effective Inhibitors of $\alpha\beta$ -Tubulin and BCRP Proteins: Molecular Docking, Structure–Activity Relationships and Virtual Consensus Docking Studies

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List of Abbreviations:

ABC: ATP-Binding Cassette

BCRP: Breast Cancer Resistance Protein

DKP: Diketopiperazine

NBD: Nucleotide Binding Domain

RMSD: Root Mean Square Deviation

SAR: Structure–Activity Relationships

TMD: Trans-Membrane Domain

TPS-A: Tryprostatin-A

Abstract

In the first part of this paper, docking method was employed in order to study the binding mechanism of breast cancer resistance protein (BCRP) with a group of previously synthesized TPS-A derivatives which known as potent inhibitors of this protein to get insight into drug binding site of BCRP and to explore structure–activity relationship of these compounds. Molecular docking results showed that most of these compounds bind in the binding site of BCRP at the interface between the membrane and outer environment. In the second part, a group of designed TPS-A derivatives which showed good binding energies in the binding site of $\alpha\beta$ -tubulin in the previous study were chosen to study their binding energies in the binding site of BCRP to investigate their simultaneous inhibitory effect on both $\alpha\beta$ -tubulin and BCRP. The results showed that all of these compounds bind to the binding site of BCRP with relatively suitable binding energies and therefore could be potential inhibitors of both $\alpha\beta$ -tubulin and BCRP proteins. Finally, virtual consensus docking method was utilized with the aim of design of new 2,5-diketopiperazine derivatives with significant inhibitory effect on both $\alpha\beta$ -tubulin and BCRP proteins. For this purpose binding energies of a library of 2,5-diketopiperazine derivatives in the binding sites of $\alpha\beta$ -tubulin and BCRP was investigated by using AutoDock and AutoDock vina tools. Molecular docking results revealed that a group of 36 compounds among them exhibit strong anti-tubulin and anti-BCRP activity.

Key words: 2,5-Diketopiperazine, Breast Cancer Resistance Protein, $\alpha\beta$ -tubulin, Molecular Docking, Structure–Activity Relationships, Virtual Consensus Docking.

1. Introduction

Resistance of cancer cells to anticancer drugs is a main reason of failure in cancer therapy that is often associated with increased expression of ATP-binding cassette (ABC) proteins. Membrane ABC transporters which pump drugs out of cells, consist of two hydrophobic trans-membrane domains (TMDs) with twelve α -helices and two hydrophilic nucleotide binding domains (NBDs) [1]. As seen in Fig.1 these transporters use the energy provided by hydrolysis of ATP molecules at their NBDs.

Fig. 1 Schematic illustration of an ABC transporter protein.

Breast cancer resistance protein (BCRP) is a member of ABC transporters that is highly expressed in organs important for absorption, distribution and elimination of drugs. It has been shown that this protein produce resistance to several anticancer drugs [2,3]. BCRP is a half ABC transporter with one NBD and one TMD [4], which probably needs homo-dimerization to exert its activity [5,6].

An important approach to overcome BCRP activity is identification of highly efficient inhibitors. One of the most effective and specific inhibitors of BCRP is Tryprostatin-A (TPS-A) [7]. It was

shown that TPS-A inhibited cell cycle progression of tsFT210 cells at concentration of 50 $\mu\text{g/mL}$ [8,9]. Moreover, It was found that this compound inhibit the cellular BCRP-dependent mitoxantrone growth in the human gastric carcinoma and breast cancer cell lines [10].

TPS-A ((3*S*,8*aS*)-3-[[6-methoxy-2-(3-methylbut-2-enyl)-1*H*-indol-3-yl] methyl]-2,3,6,7,8,8*a*-hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione) as an alkaloid-based compound, contains a 2,5-diketopiperazine (DKP) ring (Fig.2a). The 2,5-DKP derivatives are the smallest cyclic dipeptides that comprises of a six-membered ring containing two amide linkages where the two carbonyls and the two nitrogen atoms are at opposite positions in the ring. There are several experimental reports on synthesis and biological activities of these compounds [11–14].

It should be noted that the main targets of TPS-A are microtubules that are the key components of cell structure. Microtubules contain $\alpha\beta$ -tubulin heterodimers (Fig.2b) which cooperate in chromosome separation during cell division [15,16].

Fig. 2 a) Chemical structure of TPS-A. b) Schematic illustration of microtubules and $\alpha\beta$ -tubulins.

Since TPS-A (as a derivative of 2,5-DKPs) inhibits microtubule assembly and BCRP-dependent mitoxantrone accumulation, 2,5-DKP derivatives would be suitable candidates for design of novel anticancer drugs with simultaneous inhibitory effects on both $\alpha\beta$ -tubulin and BCRP

proteins. For this purpose, in the present work the following computational studies have been done:

1-Binding mechanism of BCRP with a group of previously synthesized TPS-A derivatives which known as potent inhibitors of this protein [10] were investigated by using molecular docking method to get insight into drug binding site of BCRP.

2-A group of designed TPS-A derivatives which showed good binding energies in the binding site of $\alpha\beta$ -tubulin in a previous study [17] were chosen to study their binding energies in the binding site of BCRP to investigate their simultaneous inhibitory effect on both $\alpha\beta$ -tubulin and BCRP.

3- By taking into account the results of the first part, virtual consensus docking method was used for screening of a library of 2,5-DKP derivatives to identify new compounds as effective inhibitors of both $\alpha\beta$ -tubulin and BCRP proteins.

2. Methods

2.1. Docking

In the present work two of the best docking programs, Autodock4.2.2 and Autodock Vina, were used for docking calculations. Both programs require the pdbqt input files and allow for flexibility of all the torsional bonds of small molecules. For AutoDock program, the implemented empirical free energy function and the Lamarckian Genetic Algorithm (LGA) were used [18]. Gastiger charges and hydrogen atoms were added to small molecules and protein structures [19]. For all docking calculations the amount of docking runs was set to 250 with 5,000,000 energy evaluations for each run. The size of the box that defines the search space was set at 4 Å around the small molecules. For each 2,5-DKP derivative, the first result (the lowest

energy conformation) of Vina and Autodock were selected as the docking result. Finally, the LigPlot⁺ program was used to do analysis of the docking results.

2.2. Preparation of receptors structures for docking

The structure of $\alpha\beta$ -tubulin was taken from the tubulin-colchicine complex (pdb code: 1SA0) [20]. During the preparation of the $\alpha\beta$ -tubulin input file, ligands and water molecules were removed.

Although, a high-resolution 3D structure of BCRP has not been available, several homology models of this transporter protein have been reported [21–23]. Rosenberg et al. for the first time demonstrated the conformational changes of BCRP in the presence of its substrate drug, mitoxantrone. They generated two open and closed homology models of BCRP in the absence of nucleotide (Fig.3).

In the present study, the inward-facing closed model of BCRP in the presence of its substrate drug that was previously reported by Rosenberg et al. and is based on the X-ray structure of mouse P-glycoprotein (pdb code: 3G60) was used to perform molecular docking calculations. This homology model of BCRP has been previously shown to be consistent with most of the existing biochemical data for this protein [21].

Fig. 3 a) Model of substrate-bound and closed inward-facing conformation of BCRP based on the mouse P-gp (pdb code: 3G60) structure. b) Model of substrate-free and open inward-facing conformation of BCRP based on MsbA (pdb code: 3B5W) structure.

3. Results

3.1. Molecular docking of previously synthesized derivatives of TPS-A

To get insight into drug binding site of BCRP, the binding mechanism of BCRP with a group of twelve previously synthesized derivatives of TPS-A (Table.1) which known as potent inhibitors of this protein with IC_{50} of equal or lower than 100 μ M [10] was investigated by using molecular docking method. The IC_{50} (half maximal inhibitory concentration) is a measure of how effective a drug is. It indicates how much of a particular inhibitor is needed to inhibit a given biological process by half.

Table. 1 Chemical structures of previously synthesized derivatives of TPS-A (according to Fig. 2a) and their inhibitory potency (IC_{50}) on tsFT210 cell proliferation [10].

Compound NO.	R ₁	R ₂	R ₃	R ₄ / R ₅	IC_{50} (μ M)
1 (TPS-A)	OMe	H	Isoprenyl	–(CH ₂) ₃ –	68
2	OMe	Benzyl	H	–(CH ₂) ₃ –	46

3	OMe	Allyl	H	-(CH ₂) ₃ -	62
4	OMe	Benzyl	Isoprenyl	-(CH ₂) ₃ -	55
5	OMe	Allyl	Isoprenyl	-(CH ₂) ₃ -	75
6	OMe	H	Allyl	-(CH ₂) ₃ -	60
7	OMe	H	Br	-(CH ₂) ₃ -	96
8	OMe	H	H	Benzyl / H	100
9	OMe	H	Isoprenyl	Isopropyl / H	19
10	OMe	H	Isoprenyl	Benzyl / H	10
11	NCS	H	Isoprenyl	-(CH ₂) ₃ -	50
12	N ₃	H	Isoprenyl	-(CH ₂) ₃ -	60

As mutational experiments showed that Arg482, Asn629 and Arg383 residues, located in TMD, and Leu241 and Gly83 residues, located in NBD, have essential contribution in BCRP activity [24,25], in order to identify the drug binding site of BCRP, five grids based on these residues as centers were generated for docking calculations.

As seen in Table.2 for most of the compounds, the grid generated using Asn629 as center was found to have the lowest binding energy, ΔG , (binding energy of docking result is the final intermolecular energy plus the ligand's torsional free energy); hence, all docking calculations in this study are based on the binding mode of small molecules at this considerable hydrophobic binding site which is at the interface between the membrane and outer environment.

Table. 2 Binding energies of 12 previously synthesized TPS-A derivatives on grids based on five important residues.

Compound	ΔG (kcalmol ⁻¹)	ΔG (kcalmol ⁻¹)	ΔG (kcalmol ⁻¹)	ΔG (kcalmol ⁻¹)	ΔG (kcalmol ⁻¹)
	on	on	on	on	on
	Arg482 grid	Asn629 grid	Arg383 grid	Leu241 grid	Gly83 grid
1	-7.07	-7.87	-8.45	-7.50	-6.85
2	-7.31	-8.26	-8.55	-8.59	-7.28
3	-7.54	-7.62	-7.16	-7.52	-6.56
4	-7.87	-8.81	-7.71	-6.75	-7.63
5	-7.96	-8.71	-7.41	-7.19	-7.35
6	-7.11	-8.05	-6.93	-7.93	-6.11
7	-7.07	-8.11	-6.67	-7.88	-6.27
8	-6.96	-8.28	-8.64	-8.37	-6.25
9	-6.88	-8.71	-8.15	-8.16	-6.54
10	-6.62	-9.05	-8.19	-8.15	-7.08
11	-6.84	-9.26	-8.48	-8.61	-6.9
12	-7.82	-7.74	-8.84	-8.44	-7.08

Molecular docking results show that all of twelve previously synthesized TPS-A derivatives bound to BCRP and showed relatively suitable binding energies ranging from -7.62 to -9.26 kcal/mol. Fig.4 shows the interactions of compound 1 (TPS-A) within the binding site of BCRP. This binding site is considerably hydrophobic and Ala580, Phe507, Leu626, His630, Trp627, Asn584, Leu581, Ala634, Val631 and Thr490 residues involved in hydrophobic contacts with TPS-A . However, in spite of the main role of hydrophobic interactions, the indole (NH) group of TPS-A was found to interact with Asn629 residue through hydrogen bond.

Fig. 4 3D structure of TPS-A in the binding site of BCRP and Two-dimensional scheme of the interactions between TPS-A and BCRP, generated by Ligplot+. Only more important residues for binding are shown.

Detailed investigation of binding modes and binding energies of previously synthesized derivatives of TPS-A in the binding site of BCRP lead us to the SAR analysis of these compounds. The SAR analysis of the docking calculation showed that:

- 1- Compounds 1, 11 and 12 have similar structures with different R_1 substitution. As seen in Fig. 5a, these compounds show similar binding modes. The comparison of binding energies between these compounds shows that thiocyanate substitution which could be contributed in both hydrophobic contacts and hydrogen bonds resulted in compound with better binding score.
- 2- Compounds 2 and 3 have similar structures with different R_2 substitutions. The superposition of the docking poses signifies the similarity of the binding modes for these two compounds (Fig. 5b). The comparison between predicted binding energies suggests that substitution of the indole moiety (NH) with more lipophilic groups resulted in compound with better binding score.

3- Compounds 6 and 7 have similar structures with different R₃ substitutions. The superposition of the docking poses shows the similarity of the binding modes for these compounds (Fig. 5c). On the basis of the docking scores of these two compounds, it can be found that substitution of lipophilic groups at this position was favorable for inhibition of BCRP.

Fig. 5 Superposition of the docking poses of a) compounds 1 (red), 11 (blue) and 12 (yellow) b) Compounds 2 (green) and 3 (red) c) Compounds 6 (black) and 7 (pink).

3.2. Molecular docking of previously designed derivatives of TPS-A

In this part of study, the binding energies, ΔG , and inhibition constants, k_i , ($\Delta G = -RT \ln I/k_i$) to the binding site of BCRP of a group of thirteen previously designed TPS-A derivatives (Table.3), featuring good binding affinity to $\alpha\beta$ -tubulin [17], were calculated using a molecular docking method. The results identified the compounds with simultaneous inhibitory effect on both $\alpha\beta$ -tubulin and BCRP proteins.

Table. 3 Chemical structures of previously designed derivatives of TPS-A [17] and their binding energies and inhibition constants in the binding site of BCRP.

Compound No.	Molecular structure	ΔG (kcalmol ⁻¹)	K_i (nM)
13		-9.41	126.17
14		-7.38	3900
15		-8.64	461.78
16		-9.90	55.69

17		-9.25	165.28
18		-10.31	27.88
19		-9.97	48.90
20		-9.15	196.95
21		-9.43	122.86
22		-9.00	251.91

23		-10.14	36.88
24		-9.71	75.85
25		-10.39	24.32

As seen in Table 3, all of these TPS-A derivatives bound to BCRP and showed relatively suitable binding energies ranging from -7.38 to -10.39 kcal/mol, interestingly, the binding energies of twelve compounds are lower than TPS-A. Therefore, all of these compounds could be potential inhibitors of both $\alpha\beta$ -tubulin and BCRP proteins.

The superposition of the docking poses for compounds 18, 23 and 25 (as best docked compounds) and TPS-A shows the similarity of their binding modes (Fig. 6a). Fig.6b shows the interactions of compound 25 (best docked compound) within the binding site of BCRP. This compound is in hydrophobic contact with the following residues: Ala580, Phe507, Leu626, His630, Trp627, Asn584 and Leu581. Moreover, the DKP ring carbonyl oxygen of this compound was found to interact with nitrogen atom of Asn629 residue through hydrogen bond.

Fig. 6 a) Superposition of the docking poses of compounds 1 (red), 18 (green), 23 (blue) and 25 (yellow) in the binding site of BCRP. b) Two-dimensional scheme of the interactions between compound 25 and BCRP, generated by Ligplot+. Only the more important residues for binding are shown.

3.3. Virtual Consensus Docking

Virtual molecular docking is a computer-aided technique that is used to inexpensive and fast identification of small compounds that bind to specific targets. Virtual docking involves the docking of large libraries of compounds in the binding site of particular targets and therefore potential ligands having high binding affinity against the targets can be selected for biological testing. Since virtual docking method has a key role in the identification of new compounds for the inhibition of protein targets, this method was used to identify novel inhibitors of both $\alpha\beta$ -tubulin and BCRP proteins. One of the limitations of the molecular docking method is that considerably different poses can give similar docking scores with no way of distinguishing which one is accurate. Combining the results of two or several programs (consensus docking) improve accuracy of docking results. For example the pose prediction success for Autodock and Vina was found to be 55% and 64%, respectively, but by using both programs together, pose prediction success is 82% [26]. If the Autodock and Vina docked modes are different, it is

impossible to identify which is the correct prediction. Only in those cases, where Autodock and Vina agree, accuracy increase [26].

Several computational architectures have been used to implement virtual docking of compound databases. The protocol used in this work for virtual consensus docking is shown in Fig. 7.

Fig. 7. Schematic representation of virtual consensus docking protocol implemented for design of new DKP derivatives as inhibitors of both $\alpha\beta$ -tubulin and BCRP proteins.

As seen in this figure, 4687 commercially available 2,5-DKP derivatives were chosen from the ZINC free repository. In the initial screening, these compounds have been filtered on basis of Lipinski's "Rule of five". This rule is the most widely accepted drug-like filter, which is based on simple analysis of four important properties of the drug molecules i.e. number of hydrogen bond donor, number of hydrogen bond acceptor, molecular weight, and solubility [27,28]. The Zinc database, which has itself the ability to choose the compounds that obey the Lipinski's rule of five, was limited to give the drug like compounds among the library.

The remaining 2977 compounds were docked in the binding site of BCRP and $\alpha\beta$ -tubulin by Vina program, which is several orders of magnitude faster than AutoDock. The compounds were ranked based on their predicted binding energies and the top 5% (~200) of compounds was selected for each protein. The inspection of these two groups led us to find the compounds with simultaneous inhibition activity on both proteins. 55 compounds with these criteria are reported in Table.4.

Table 4. Binding energies of 55 best scored derivatives of DKP in the binding sites of $\alpha\beta$ -tubulin and BCRP calculated with Vina program. The unit of reported binding energies is kcal.mol⁻¹.

Zinc code	ΔG (kcalmol ⁻¹) in the binding site of BCRP	ΔG (kcalmol ⁻¹) in the binding site of $\alpha\beta$ -tubulin	Zinc code	ΔG (kcalmol ⁻¹) in the binding site of BCRP	ΔG (kcalmol ⁻¹) in the binding site of $\alpha\beta$ -tubulin
01656473	-11.3	-11.8	15955418	-10.9	-11.2
01656475	-11.6	-11.5	15956197	-10.8	-11.1
02092933	-11.2	-11.4	15956514	-11.8	-11.3
02095428	-11.1	-11.2	16031442	-12.0	-11.3
02101594	-11.4	-11.2	16031444	-11.4	-11.3
02103383	-11.2	-11.2	16032483	-11.2	-11.5
02103428	-11.0	-11.3	16032523	-11.2	-11.4
02104482	-12.0	-11.3	16032556	-10.8	-11.3
02108366	-11.6	-11.5	16042220	-10.7	-11.1
02108366	-11.3	-12.0	16042236	-11.4	-11.9
02151835	-11.4	-11.8	16042237	-11.7	-11.6
02155992	-11.2	-11.4	16042647	-11.3	-11.9
06005212	-11.2	-11.4	16042657	-11.7	-11.5
15953490	-12.1	-12.1	16042738	-11.3	-11.6
15953491	-11.2	-11.2	16042919	-11.3	-11.2
15953648	-11.4	-11.5	16043124	-11.8	-11.1
15953810	-12.4	-12.0	16043125	-12.5	-11.6
15953811	-11.8	-11.6	15955266	-12.1	-12.0
15953907	-11.1	-11.9	26267319	-11.3	-11.8
15953908	-11.5	-11.1	26276259	-10.7	-11.1
15953984	-11.1	-11.6	26771701	-11.1	-12.0
15953996	-10.8	-11.2	26772064	-11.2	-11.8
15954373	-10.7	-11.1	26772065	-11.6	-11.3
15954374	-12.0	-11.5	26772174	-11.1	-11.5
15954641	-10.8	-11.1	26772253	-11.4	-11.4
15954798	-11.0	-11.1	37238873	-10.8	-11.1
15955209	-11.1	-11.3	38338217	-11.4	-11.3
15955267	-11.8	-11.4			

Subsequently, the top 55 highest ranked compounds were subjected to docking using Autodock program and the predicted binding poses were compared with the relevant obtained results from Vina. The calculated RMSD values between Vina and AutoDock poses are presented in Table 5.

Table 5. Calculated RMSD (Å) between Vina and Auto Dock poses in $\alpha\beta$ -tubulin and BCRP binding sites for 55 derivatives of DKP. Compounds which have RMSD lower than 2 Å in the $\alpha\beta$ -tubulin and BCRP binding sites are underlined.

Zinc code	RMSD in the binding site of BCRP (Å)	RMSD in the binding site of $\alpha\beta$ -tubulin (Å)	Zinc code	RMSD in the binding site of BCRP (Å)	RMSD in the binding site of $\alpha\beta$ -tubulin (Å)
<u>01656473</u>	0.99	0.41	<u>15955418</u>	1.39	0.38
01656475	0.36	2.52	<u>15956197</u>	0.60	0.63
02092933	3.06	3.07	<u>15956514</u>	0.64	1.82
02095428	1.81	3.55	<u>16031442</u>	1.04	0.87
<u>02101594</u>	1.85	0.53	<u>16031444</u>	1.98	0.75
02103383	3.73	0.55	<u>16032483</u>	0.60	1.35
02103428	3.99	0.96	16032523	1.50	2.47
02104482	2.99	1.86	16032556	1.25	3.10
02108366	1.17	2.21	<u>16042220</u>	1.51	0.81
02108366	0.68	2.41	<u>16042236</u>	1.33	0.81
<u>02151835</u>	1.07	1.77	<u>16042237</u>	0.48	1.79
02155992	2.51	0.56	16042647	2.97	0.59
06005212	0.78	3.29	<u>16042657</u>	0.69	0.47
<u>15953490</u>	1.78	0.63	16042738	0.79	4.03
<u>15953491</u>	1.40	0.53	<u>16042919</u>	1.03	1.57
<u>15953648</u>	1.26	0.75	<u>16043124</u>	0.92	0.71
<u>15953810</u>	1.19	0.85	<u>16043125</u>	0.76	0.90
<u>15953811</u>	1.79	0.49	<u>15955266</u>	1.07	0.90
15953907	2.66	1.38	26267319	2.95	2.30
<u>15953908</u>	1.13	1.62	26276259	2.59	1.16
<u>15953984</u>	1.71	0.95	26771701	3.12	0.98
<u>15953996</u>	1.81	0.43	26772064	2.28	1.37
<u>15954373</u>	0.60	1.97	<u>26772065</u>	1.40	1.89
<u>15954374</u>	0.71	1.86	<u>26772174</u>	0.65	0.82
<u>15954641</u>	1.01	0.54	<u>26772253</u>	1.23	0.84
<u>15954798</u>	0.51	0.65	<u>37238873</u>	1.83	0.76
<u>15955209</u>	0.76	0.53	<u>38338217</u>	0.62	0.59
<u>15955267</u>	0.74	1.14			

Of those 55 trials, 36 cases were found to have $\text{RMSD} \leq 2.0$ Å for both targets that shows the agreement of Autodock and Vina results for a particular binding mode. Chemical structures of the top 36 hits are shown in Table 6.

Table 6. Chemical structures of 36 derivatives of DKP with simultaneous remarkable inhibition activity on both $\alpha\beta$ -tubulin and BCRP proteins.

ZINC Code	Chemical Structure	ZINC Code	Chemical Structure
01656473		15955267	
15955266		15955418	
15956514		15956197	
02101594		16031442	
16032483		16031444	
16042236		16042220	
02151835		16042237	

15953490

16042657

15953491

16043124

15953648

16042919

15953810

15955209

15953811

16043125

15953908

15953984

15953996

26772065

15954373

26772174

15954374

26772253

15954641

37238873

15954798

38338217

The superposition of the docking poses of the best docked compounds (zinc codes: 15953490, 15953810 and 15955266) and TPS-A shows the similarity of their binding modes in the binding site of BCRP and $\alpha\beta$ -tubulin. Fig.8 shows the interactions of the best docked compounds within the binding site of BCRP and $\alpha\beta$ -tubulin. All of these compounds are in hydrophobic contacts with Phe489, Leu581, Leu626, Ala580, Trp627, Phe507, Tyr576, Phe511 and Ile573 residues and in hydrophilic contacts with Ser486, Asn584, Asn629 and His630 residues in the binding site of BCRP.

Residues Ala250, Tyr224, Leu248, Phe141, Ile171, Tyr172, Ala12 and Ala180 are responsible for the hydrophobic interactions in the binding site of $\alpha\beta$ -tubulin, whereas the hydrophilic interactions involve the polar residues Asn249, Lys254, Ser178, Glu183, Asn205, Ser140, Gln11 and Asn101. Moreover, formation of hydrogen bonds between nitrogen atom of Asn101 and

these compounds bind to considerable hydrophobic binding site of BCRP at the interface between the membrane and outer environment with relatively good binding energies, ranging from -7.62 to -9.26 kcal/mol. Structure–Activity Relationships (SAR) analysis of docking calculation shows that more lipophilic groups at R₂ and R₃ is favorable for inhibition of BCRP.

The second challenge was to calculation of binding energies of some previously designed derivatives of TPS-A (which showed good binding affinity to $\alpha\beta$ -tubulin in the pervious study) in the binding site of BCRP. Analysis of binding energies shows that all of these compounds could be potential inhibitors of both $\alpha\beta$ -tubulin and BCRP proteins.

Finally virtual consensus docking tools was used to identify a group of some 2,5-Diketopiperazine (2,5-DKP) derivatives which would be potent inhibitors of both $\alpha\beta$ -tubulin and BCRP proteins. Molecular docking results revealed that a group of 36 compounds exhibits simultaneous remarkable anti-tubulin and anti-BCRP activity. The results also suggest that these molecules feature a higher inhibition efficiency in comparison to TPS-A and indicate the importance of DKP ring on their inhibition activity.

The drug design strategy described in this study can be highly useful for the development of new simultaneous inhibitors of both $\alpha\beta$ -tubulin and BCRP proteins with high efficiency and selectivity.

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Diketopiperazine Derivatives

+

BCRP

$\alpha\beta$ -Tubulin

4687 of 2,5-Diketopiperazine
Derivatives from ZINC database

Drug
Like

2977 Compounds

+BCRP

Vina

+Tubulin

200 best scored

55 Compounds

200 best scored

+BCRP

AutoDock

+Tubulin

RMSD between Vina
and AutoDock poses
 ≤ 2.0 Å

36
Hits

RMSD between Vina
and AutoDock poses
 ≤ 2.0 Å