



## **Structural Mapping of Inhibitors Binding Sites on P-glycoprotein: Mechanism of Inhibition of P-Glycoprotein by Herbal Isoflavones**

**Neha Arora<sup>1</sup>, Shakti Sahi<sup>1</sup> and Nagendra Singh<sup>1\*</sup>**

<sup>1</sup>*School of Biotechnology, Gautam Buddha University, Greater Noida, U.P.201308, India.*

### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author NA performed experiments. Author SS supervised experiments and assisted in manuscript preparation. Author NS conceived the idea, supervised and managed analysis the results and wrote the manuscript. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** Understanding the pattern of inhibitors binding to p-glycoprotein (Pgp).  
**Study Design:** Pgp is an ATP dependent transporter protein, responsible for multi-drug resistance in metastatic tumors. It removes toxins by exporting a variety of structurally unrelated compounds outside the cells, which make Pgp a promising target for designing anti cancer supplementary therapeutic molecules. Isoflavones are present in soyabean and other herbal extracts. The idea was to explore inhibitor binding sites on Pgp to find hotspots which eventually may prove useful in designing compounds with higher specificity and affinity.  
**Place and Duration of Study:** School of Biotechnology, Gautam Buddha University, Greater Noida, between February 2012 and December 2012.  
**Methodology:** The biochemical nature of binding of isoflavones to Pgp has been extensively studied, but the atomic details of their interactions were not understood. Therefore, we have used *in silico* methods to study binding of eleven isoflavones to Pgp. The docking studies were performed using grid-based ligand docking with energetic (GLIDE).  
**Results:** Isoflavones binds at two slightly distinct sites perpendicular to each other, present in the large hydrophobic cavity of Pgp. Three isoflavones bind to site 1, whereas

\*Corresponding author: Email: [nagendratomar@gmail.com](mailto:nagendratomar@gmail.com);

eight isoflavones bind to site 2 by forming van der Waals and H-bonded interactions. Both the sites are highly hydrophobic in nature and are contributed mainly by side chain of non polar residues present on twelve transmembrane  $\alpha$ -helices. Site 1 has minimum dimension of 7.5Å and maximum as 22Å whereas, site 2 is wider and deeper than site 1. One sidewall of the site 2 is formed by polar amino acid residues of helix H12, which makes several hydrogen bonds with ligands.

**Conclusion:** Structure analysis revealed that addition of polar group to hydrophobic ligand may enhance its binding affinity for Pgp, which may be used for designing potent inhibitors to find lead compounds for drug design.

*Keywords: Multidrug resistance; P-glycoprotein; isoflavones; molecular docking.*

## 1. INTRODUCTION

Toxic chemotherapy is used for treatment of cancer. Multi drug resistance (MDR) is the most common phenomena occurring in metastatic cancerous cells. Therefore, MDR has been major thrust area for cancer biologist since long time. MDR is generally conferred by efflux of drug compounds by a group of transmembrane proteins in an ATP dependent manner [1]. These proteins get overexpressed in cancerous cells. A variety of such ATP dependent transporters have been identified, such as P-glycoprotein (ABCB1) [2] MRP1 (multi drug resistance associated protein 1, ABCC1) [3], MRP2 (ABCC2), MRP3 (ABCC3) [4] and BCRP/MXR1 (ABCG2) [5,6]. These are expressed in various tissues of the human body including intestinal lumen, bile canaliculus in liver hepatocytes and capillaries in the blood brain barrier [5]. Along with other members, Pgp has also been reported to be involved in the exotransport of anticancer drugs [7,8]. It has a very broad specificity to compounds ranging from 330Da to 4000Da molecular weights [9,10]. It binds mainly to hydrophobic compounds and expels them outside the cell [11].

Effects of a variety of compounds on Pgp, such as oroxylin A [12], fluorescent compounds [13], selective serotonin reuptake inhibitors [14], mollugin [15], antipsychotic drugs [16] etc. have been reported. Structures of several bacterial ABC transporters have been determined [17-22], although only low resolution structure of mouse Pgp is available [23-25]. Mouse Pgp serves as a good model as it has a significant similarity with human Pgp [26]. It is a 170 kDa transmembrane protein, expressed in various resistant tumour cell membranes. As it is involved in MDR in tumour cells, designing of inhibitors to Pgp may have a great therapeutic potential.

Isoflavones and flavonoids are groups of polyphenolic compounds derived from plant extracts. These are derived from flowers, fruits, vegetables, nuts and stems and form an abundant component of human food [27-30]. Isoflavones are the largest group of natural isoflavonoids, present in mostly leguminous plants. There are two major categories of isoflavones known as aglycones and glycosides, depending on presence of a glucose substitution in their chemical structures. These compounds have a wide biochemical and pharmacological effect on human health such as anti carcinogenic, antiviral and anti-inflammatory. These active plant compounds have been found inhibiting Pgp and contributing to decrease MDR in tumour cells [31-35]. Several other molecules such as rosmarinic acid [36], 5-bromotetrandrine [37], bromoditerpenes [38], benzopyranes and benzopyrano oxazines [39], macrolides [40], chemosensitizing agents [41], flavonoids [42],

modified peptides [43-44], furanocoumarin [45], lactones [46] and detergents [47] have also been reported as inhibitors of Pgp.

Pgp has similar topology as other members of the ABC transporter family including MRP1, MRP2, MRP3, BCRP proteins and plays similar role in xenobiotic metabolism. *In vivo* and *in vitro* studies have indicated that flavones and isoflavones inhibit Pgp and other ATP transporters mediated exotransport of anticancer drugs and other xenobiotic compounds. These transporters were over expressed and effects of flavones and isoflavones were investigated by monitoring exotransport of various known substrates and compounds [48-51]. Although interactions of a large number of natural compounds have been extensively studied with Pgp and MRP1 [52-56], the details of the binding pattern of isoflavones to Pgp was not understood so far. Since the three-dimensional crystal structure is available only for mouse Pgp and the sequence of mouse Pgp share 87% identity with the human form, and almost 100% identity with residues present in binding cavity [26], therefore we performed *in silico* studies to identify interactions between isoflavones and mouse Pgp.

Such knowledge can provide further scope of designing potent and specific inhibitors of Pgp, which may have a good therapeutic potential. Isoflavones binding studies may play a vital role in finding hotspots in Pgp and eventually may be proved useful in designing compounds with high affinity and specificity to the protein, which can be used along with anti cancer chemotherapeutic drugs. This manuscript provides details of interactions between eleven isoflavones varying in their chemical nature and affinity with Pgp, which has helped in understanding the precise sites and the functional groups involved in inhibitor recognition.

## 2. MATERIALS AND METHODS

### 2.1 Docking Isoflavones on Pgp

The atomic coordinates of 11 isoflavones (diadzein, daidzin, formononetin, ononin, genistein, genistin, biochanin A, sissotrin, coumestrol, coumestrin, glycitein) were generated using structures described earlier [51]. 3-D atomic coordinates for isoflavone structures were generated using LIGPREP [57] with the correct chiralities of atoms. The resulting geometries were optimized by molecular mechanics using IMPACT [57] in a dynamic environment using standard TIP4P water model. Energy minimization was done using Polak-Ribier conjugate gradient algorithm using Optimized Potentials for Liquid Simulations 2005 (OPLS 2005) force field. RMS gradient of 0.01 was used as the convergence threshold. Conformational models of all the ligands were generated. The ligands were prepared using LIGPREP [57]. The structures with correct bond length, bond angles, and conformation, stereochemistry and ionization states were selected for docking studies. All the isoflavones except coumestrol and coumestrin have identical basic structure, which have variations in groups substitute at C7, C8, C9 and C14 positions on the nucleus structure (Table 1).

**Table 1. Structure and docking calculations of binding of isoflavones to Pgp**

Isoflavone	Substitution position				Docking score	H-bonded residues	Residues involved in van der Waals interactions	Buried surface area (Å <sup>2</sup> )	Energy (kcal / mol)
	7	8	9	14					
Genistein	OH	H	OH	OH	-7.3	Gly 222 (NH)	Leu 221, Phe 299, Ile 302, Phe 303, Ala 338, Phe 339	239.2	-48.09
Genistin	OH	H	Glc	OH	-7.5	Ser 989 (OH) Gly 985 (O) Gln 986 (NH <sub>2</sub> )	Leu 221, Phe 299, Ile 302, Phe 303, Ala 338, Phe 339	337.1	-62.03
Biochanin A	OH	H	OH	OCH <sub>3</sub>	-7.2	Gly 222 (NH)	Leu 221, Phe 299, Ile 302, Phe 303, Ala 338, Phe 339	252.5	-48.57
Sissotrin	OH	H	Glc	OCH <sub>3</sub>	-7.6	Gln 721 NH <sub>2</sub> Met 982 (O) Ser 989 (OH) Asn 717 (CO, NH <sub>2</sub> )	Leu 300, Tyr 303, Phe 724, Phe 332, Phe 766, Phe 833, Phe 974, Val 978, Phe 990	373.1	-59.05
Glycitein	H	OCH <sub>3</sub>	OH	OH	-7.4	-	Leu 221, Phe 299, Ile 302, Phe 303, Ala 338, Phe 339	267.1	-48.05
Daidzein	H	H	OH	OH	-7.3	-	Leu 221, Phe 299, Ile 302, Phe 303, Ala 338, Phe 339	237.4	-45.43
Daidzin	H	H	Glc	OH	-7.9	Met 982 (O), Gln 986 (NH <sub>2</sub> ) Ile 302 (O)	Ala 225, Phe 299, Leu 300, Tyr 303, Leu 335, Ala 338, Phe 339,	331.3	-59.2
Formononetin	H	H	OH	OCH <sub>3</sub>	-4.9	Tyr 303 (OH)	Leu 300, Tyr 303, Phe 724, Phe 766, Phe 833, Phe 990	270.1	-39.99
Ononin	H	H	Glc	OCH <sub>3</sub>	-7.7	Gly 222 (NH) Ser 989 (OG) Ser 989 (OG), Gly 985 (O)	Leu 221, Phe 299, Ile 302, Phe 303, Ala 338, Phe 339	342.2	-64.93
Coumestrol	-	-	OH	OH	-5.8	Ser 989 (OH) Asn 717 (NH <sub>2</sub> )	Leu 300, Tyr 303, Leu 758, Phe 766, Phe 833,	214.6	-36.41
Coumestrin	-	-	Glc	OH	-7.7	Ser 989 (OH), Gly 985 (O) Gln 717(NH <sub>2</sub> )	Leu 300, Tyr 303, Phe 724, Phe 332, Phe 766, Phe 833, Phe 974, Val 978, Phe 990	371.8	-55.83

Docking studies were performed using GLIDE [58]. Atomic coordinates of the native receptor were extracted from protein data bank (PDB: 3G5U). The submitted coordinates were corresponding to a homo dimer of Pgp. Monomer (Chain A) of the Pgp was used for study. Each monomer contained 1271 amino acid residues and 18521 non hydrogen atoms. The structure of Pgp complex with a cyclic peptide is also available as PDB code 3G60 in protein databank. Pre-processing of the native transporter structure was done by assigning correct bond order, addition of hydrogen atoms, capping uncapped termini, adjusting bonds and formal charges for groups and correcting mislabeled elements. All docking studies were carried out using extra precision (XP) method of GLIDE (Grid-Based Ligand Docking with Energetic) which examines the complementarities of ligand-receptor interactions using a grid-based method based on the empirical Chem Score function for flexible ligand docking. The centroid of selected active site residues (using PDB: 3G60) were used for receptor grid generation for docking as well as blind docking. 1000 docked poses per compound were generated for the different conformations of the compounds. The affinity grid maps centered on the peptide binding site had dimensions of  $80 \times 80 \times 80 \text{ \AA}$  with  $0.375 \text{ \AA}$  spacing between grid points. The binding free energy of the receptor-ligand binding ( $\Delta G_{\text{bind}}$ ) is calculated by taking the difference between the free energies of the receptor-ligand complex ( $\Delta G_{\text{complex}}$ ) and the unbound receptor ( $\Delta G_{\text{receptor}}$ ) and ligand ( $\Delta G_{\text{ligand}}$ ).

The structures of docked complexes of Pgp with isoflavones were analyzed using "O" program [59] and COOT [60]. The surface potential of the Pgp was calculated using PyMol [61].

### **3. RESULTS AND DISCUSSION**

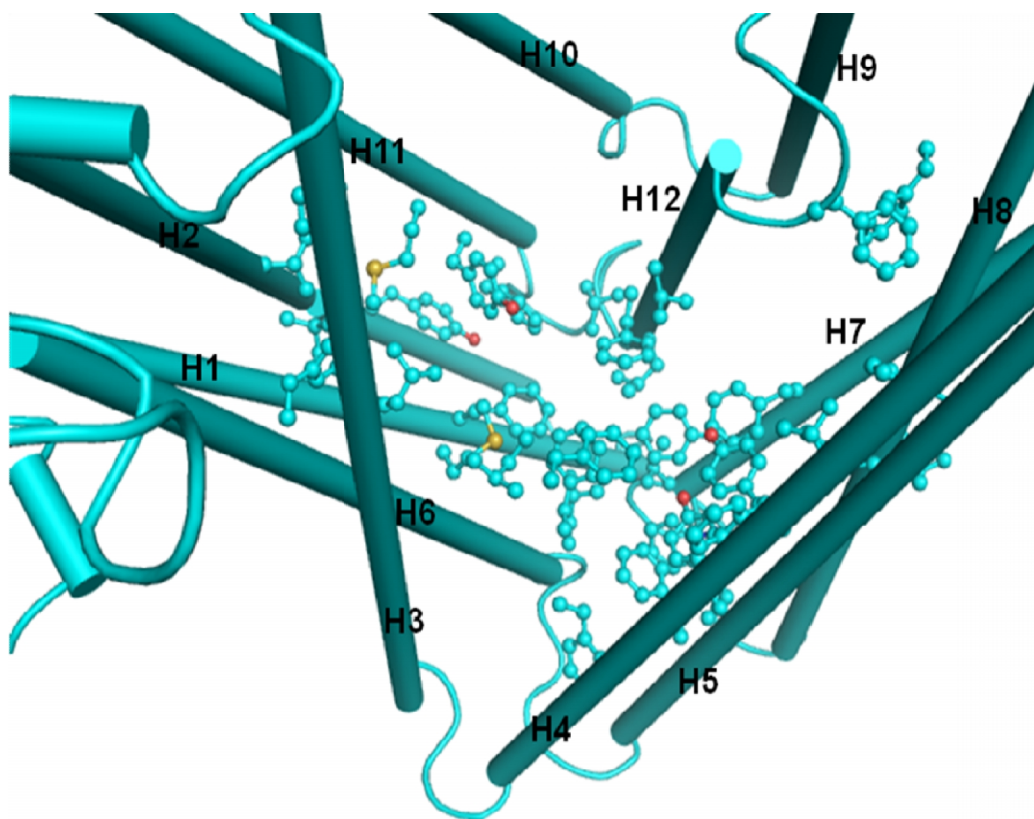
#### **3.1 Structure of the Pgp Model**

Pgp is a transmembrane pump which effluxes drugs and other xenobiotic compounds outside the cell and is responsible for MDR. A crystal structure was reported for mouse Pgp [25]. The PDB coordinates were available for native Pgp (PDB id: 3G5U) and complex with a cyclic hexapeptide inhibitor (PDB id: 3G61) in protein data bank (PDB). The native structure of Pgp was determined at  $3.8 \text{ \AA}$  resolution. Pgp has a helical bundle rich fold with 56%  $\alpha$ -helical and 7%  $\beta$ -strand secondary structure content. The structure shows a crystallographic dimer having 1284 amino acid in each monomeric unit. Structure of Pgp can be divided into two highly homologous parts, each half of the structure contains six membrane spanning  $\alpha$ -helices in form of transmembrane domain (TMD), followed by a nucleotide binding domain (NBD). So the complete molecule contains twelve long  $\alpha$ -helices in form of TMD and two NBD regions.

#### **3.2 The Ligand Binding Cavity**

Pgp is a tandem protein, comprising of two identical homologous halves, containing total 1271 amino acids. The structure is V shaped, having nucleotide binding domain on both termini. The membrane spanning region is made up of 12 long alpha helices (H1: 44-87, H2: 93-158, H3: 166-205, H4:210-249, H5: 266-317, H6: 327-365, H7: 708-736, H8: 743-794, H9: 807-847, H10: 852-904, H11:909-957 and H12: 968-1009). Each consecutive  $\alpha$ -helix is connected through flexible loop regions which are capable of making the helices move apart while performing its functions. The wall of the ligand binding site is formed by the contribution of residues from these 12  $\alpha$ -helices. Furthermore, hydrophobic stacking of these  $\alpha$ -helices makes them more amenable to opening and closing of the interstitial space. The

ligand binding site is present in form of a wide and long hydrophobic cavity. The cavity is predominantly formed by hydrophobic amino acids. Aromatic residues are most abundant in the cavity and form a layer of aromatic amino acids. Such a layer has also been reported in modeled structure of MRP1 [62]. Indeed, some compounds have also been found to be common inhibitors of both Pgp and MRP proteins [63]. The predominant amino acids involved in the formation of ligand binding hydrophobic cavity are Leu 64, Met 68, Phe 71, Tyr 110, Tyr 111, Tyr 113, Ile 117, Val 121, Phe 190, Phe 299, Leu 300, Tyr 303, Tyr 306, Phe 310, Trp 311, Leu 328, Phe 331, Phe 332, Ile 336, Phe 339, Phe 724, Phe 728, Val 731, Val 732, Leu 757, Leu 758, Phe 766, Phe 833, Phe 938, Met 945, Tyr 949, Phe 953, Val 970, Leu 971, Phe 974, Val 978, Phe 990 (Fig. 1). The remaining part of sequence forms two ATP binding domains which are  $\alpha$ - $\beta$  bundle. ATP binding domains are located from 378-626 and 1018-1271 amino acid residues. The cavity formed is very big in size and has a total volume of approximately  $6000\text{\AA}^3$  [25].



**Fig. 1. Interior of the hydrophobic cavity of Pgp. Hydrophobic residues forming ligand binding sites are shown in ball and stick model. The figure was drawn using PyMol [61]**

### 3.3 Binding of Isoflavones

The binding affinity of isoflavones and Pgp was measured in terms of docking score and the energy of the complex formation. Docking scores distinguish between molecules that bind strongly in their optimal placement from those that bind weakly to the transporter. The

extensive ligand binding cavity of Pgp is responsible for binding of a wide variety of hydrophobic ligands. H3 and H9 helices are present on the peripheral face of the molecule and seem to be playing no direct role in ligand binding, whereas remaining ten helices have protruding-in side chains of amino acids, which are responsible for formation of hydrophobic cavity. In present study, due to structural and chemical variations isoflavones occupy two different but overlapping sites in the hydrophobic cavity, which are formed by six  $\alpha$ -helices, H4, H5, H6, H7, H8 and H12. These sites are designated as site 1 and site 2 for the ease of description in the manuscript. Sissotrin, coumestrin and formononetin bind to site 1 whereas biochanin A, genistein, genistin, daidzein, daidzin, glycitein, coumestrol and ononin bind to site 2 in the wide hydrophobic cavity. Glycosides isoflavones have higher binding affinity to Pgp than the aglycones, as indicated by their docking scores, which can be attributed to their polar nature of glycosides by presence of glucose moiety, as indicated by relative energy, docking scores and buried surface area of the ligands (Table 1). The glycosides isoflavones have docking score and energy of the complex ranging from -7.9 to -7.6 and -64.9 to -55.8 kcal/mol respectively, whereas the docking score and energy of complex in case of aglycones ranged from -7.3 to -4.8 and -48.0 to -36.4 kcal/mol respectively.

### **3.3.1 Site 1: Binding of sissotrin, coumestrin and formononetin**

These three compounds bind to Pgp with almost identical manner at a common site. In case of sissotrin and coumestrin 30 poses were obtained (docking score ranging from -7.35 to -6.34 for different poses in case of both molecules). The analysis of complexes revealed that their binding site is formed mainly by side chains of residues Leu 300, Tyr 303, Phe 332, Leu 335, Phe 339, Phe 724, Phe 766, Phe 974, Leu 978 and Phe 990. Sissotrin, coumestrin and formononetin are stacked in this hydrophobic cavity through van der Waals interactions. Along with these hydrophobic forces, sissotrin and coumestrin shared common hydrogen bonds with the amide side group of Asn 717 and hydroxyl side group of Ser 989 whereas, formononetin made a hydrogen bond with the side chain of Tyr 303. In addition coumestrin also makes one hydrogen bond with the side amide group of Asn 721 (Fig. 2a).

### **3.3.2 Site 2: Binding of biochanin A, genistein, daidzein, daidzin, genistin, glycitein, coumestrol and ononin**

Only one optimal pose was obtained in this category of isoflavones except for genistin (50 poses) and ononin (30 poses). The average docking scores for these are shown in Table 1. The docking score of different poses for genistin ranged from -7.5 to -6.5 and for ononin, it ranged from -7.7 to -6.7. Site 2 is located slightly overlapping and almost perpendicular to the site 1. It recognizes the above mentioned eight isoflavones in the hydrophobic cavity. This site is formed mainly by residues Phe 299, Tyr 303, Tyr 306, Phe 310, Leu 335, Ala 338, Phe 339, Phe 766. Residues Phe 299 and Phe 339 provide stacking interactions from two sides of the aromatic rings of ligands. One of the walls of the site 2 is made up of polar face of the helix H12 consisting of exposed side chain of Gln 721, carbonyl groups of Met 982 and Gly 985, hydroxyl side chain of Ser 989 and amide side group of Gln 986. OH groups of ononin, daidzein and genistin interacted with the polar surface of the site 2 through hydrogen bonding interactions. Daidzein and genistin interacted with the side chain of Gln 986 through H-bonding whereas ononin makes hydrogen bonds with hydroxyl group of Ser 989 and carbonyl oxygen of Gly 985 residues (Fig. 2b).

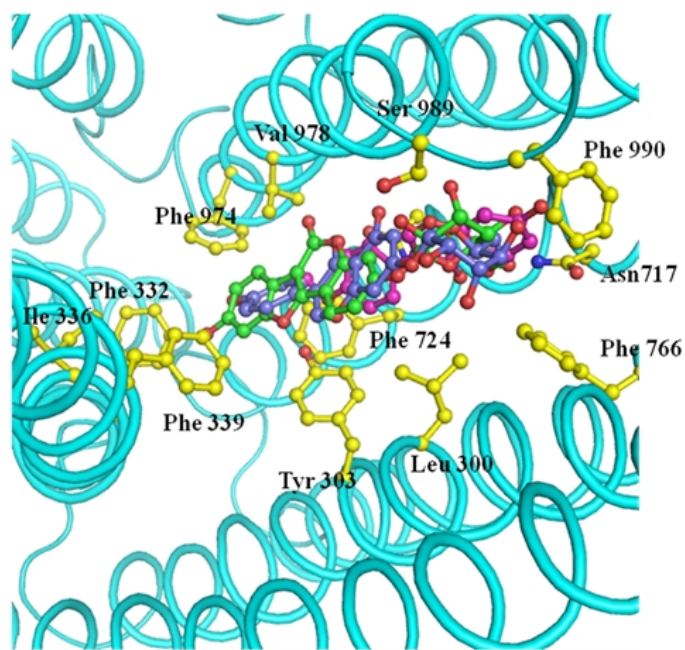


Figure 2a

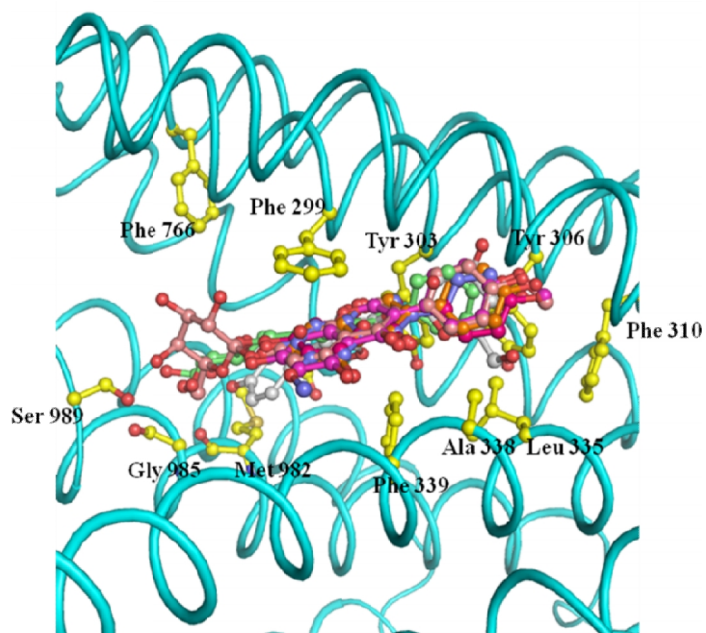


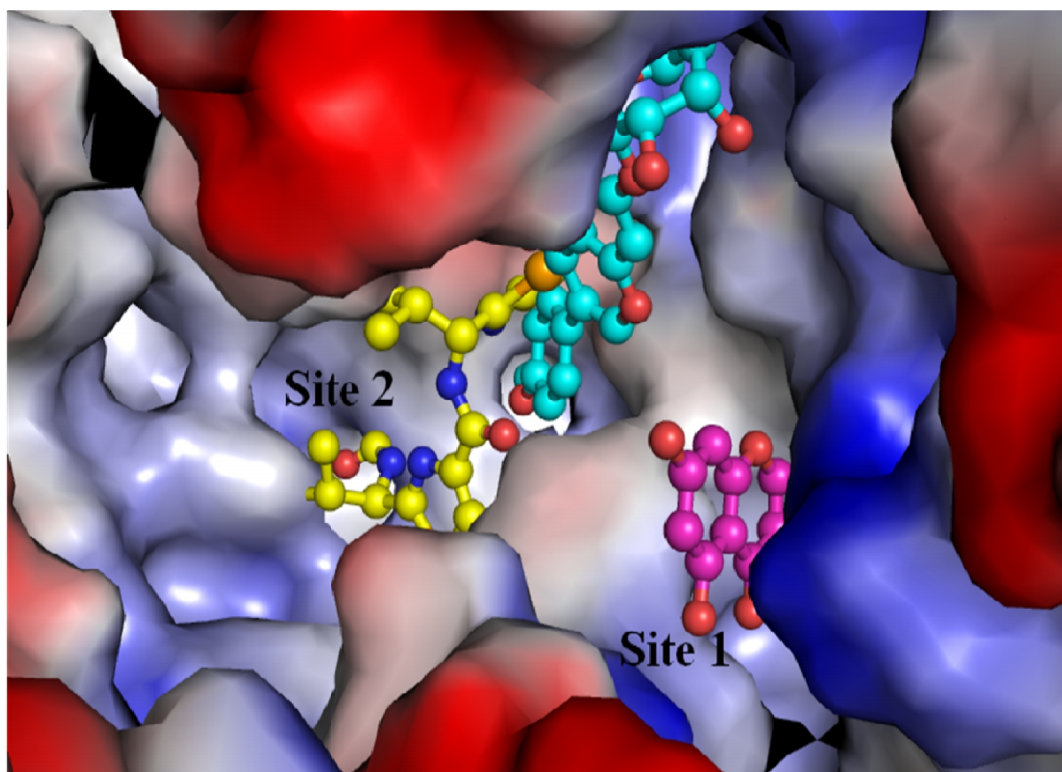
Figure 2b

**Fig. 2. Ball and stick representation of ligand interacting residues of hydrophobic cavity. Sissoterin (blue), coumestrin (green) and formentin (magenta) are also drawn in site 1 (a) and biochanin A (magenta), genistein (green), daidzein (pink), daidzein (grey), genistin (dark yellow), glycitein (blue), coumestrol and ononine (pink) are drawn in site 2 (b). The figure was drawn using PyMol [61]**



### 3.4 Comparison with the Binding of Cyclic Peptide Inhibitor

Pgp complex with a cyclic peptide has also been determined and deposited to the PDB database with accession code 3G60 [25]. The inside of hydrophobic cavity has a gradient of polarity and hydrophobicity. The cytosolic face (towards NBD) has higher occurrence of charge and polar residues, whereas the relative number of hydrophobic residues increases as we go deeper in the cavity towards extracellular side (Fig. 3). Isoflavones occupies two distinct but overlapping sites on Pgp structure as discussed in previous section. The cyclic hexa peptide (AV)<sub>3</sub> binds at site 2 of Pgp and therefore it share binding space with biochanin A, genistein, daidzein, daidzin, genistin, glycitein, coumestrol and ononin isoflavones. The peptide has bound slightly deeper in the site 2 of the hydrophobic cavity which may be attributed to its higher hydrophobic nature and easier diffusion in the cavity, as the cavity has relatively more hydrophobic residues towards extracellular side, to prefer similar compounds (Fig. 3).



**Fig. 3. Top view of the hydrophobic cavity showing electrostatic potential of the inner side of the cavity. Ligands binding to site 1 (magenta) and site 2 (cyan) are also shown. Cyclic peptide inhibitor [25] is also drawn in yellow colour ball and stick model. Carbon atoms are shown in grey, nitrogen in blue and oxygen in red color. The figure was drawn using PyMol [61]**

### **3.5 Comparison with the Binding of Flavones and other Ligands**

It was observed that phenothiazines interact with drug binding sites in TM region, whereas flavones bind to NBD sites of Pgp and MRP1 proteins [54]. Recently, docking studies of flavones on Pgp has also been reported [64]. In contrast to isoflavones, flavones have been found to be indeed interacting to NBD of Pgp. In the current study, isoflavones bind to the hydrophobic cavity in TM region whereas, flavones could be modulating Pgp mediated exotransport of drugs by inhibiting the nucleotide binding. One more study has also shown the substrate and inhibitor binding sites in Pgp [65] by docking of berberine (BBR) as substrate and HIV protease inhibitors (HIVPI) as inhibitors of Pgp. BBR binds to a deeper site in comparison to HIVPIs to Pgp, so that binding of BBR get competitively inhibited by HIVPIs. When compared, it was observed that isoflavones also bind to upper part of the binding cavity like HIVPIs, which is close to the cyclic peptide inhibitor binding site as well [25]. Cytotoxic drugs and other inhibitor molecules also have been found to be interacting at two distinct sites although for modulating ATPase activity [66] whereas isoflavones inhibit ligands binding activity of Pgp.

### **4. CONCLUSIONS**

Pgp contains a wide cavity for binding to relatively hydrophobic and aromatic ligand molecules. The modeled human Pgp structure has shown very good similarity with the mouse Pgp [67]. The hydrophobic cavity is formed by contribution of side chains of mainly Phe, Leu and Ile residues from six  $\alpha$ -helices in the Pgp structure. Isoflavones recognizes two slightly distinct but overlapping sites on Pgp called site 1 and site 2. Sissotrin, coumestrin and formononetin recognize interacting groups in site 1 whereas biochanin A, genistein, daidzein, daidzin, genistin glycitein, coumestrol and ononin bind to site 2 in the wide hydrophobic cavity present in Pgp. Site 1 and site 2 are located differently in the hydrophobic cavity and also contain an overlapping space as well (Fig. 4). Both the sites have hydrophobic amino acids to form van der Waals interactions with the ligands. In addition to hydrophobic residues, site 2 also contains one polar face from helix H2, which is responsible for forming several hydrogen bonded interactions with the ligands. A gradient of polar and hydrophobic amino acid residues is present inside the ligand binding cavity of Pgp. The cytosolic side of the cavity is very polar and charged whereas the hydrophobic environment starts increasing towards extracellular side of the cavity (Fig. 3). Site 1 has minimum dimension as small as 7.5Å and maximum as 22Å whereas site 2 is wider and deeper than site 1. Therefore, Pgp is capable of binding a variety of substrates, although hydrophobic compounds bind deeper in the cavity whereas relatively polar compounds bind towards cytosolic side, which is also evident from deeper binding of BBR to Pgp [65]. Hence, the cyclic (AV)<sub>3</sub> peptide being highly hydrophobic binds in the deeper region of site 2 in comparison to the isoflavones. These features may be utilized to construct compounds which have hydrophobic and polar groups and can occupy both the sites to form stronger attractive interactions. As the movement of helices are responsible for transporting drugs outside the cell, bigger compounds which can occupy most of the area of the cavity including site 1 and 2 may form an effective inhibitor of Pgp. In addition, presence of polar groups in the aromatic/hydrophobic ligand may enhance its binding affinity for Pgp.

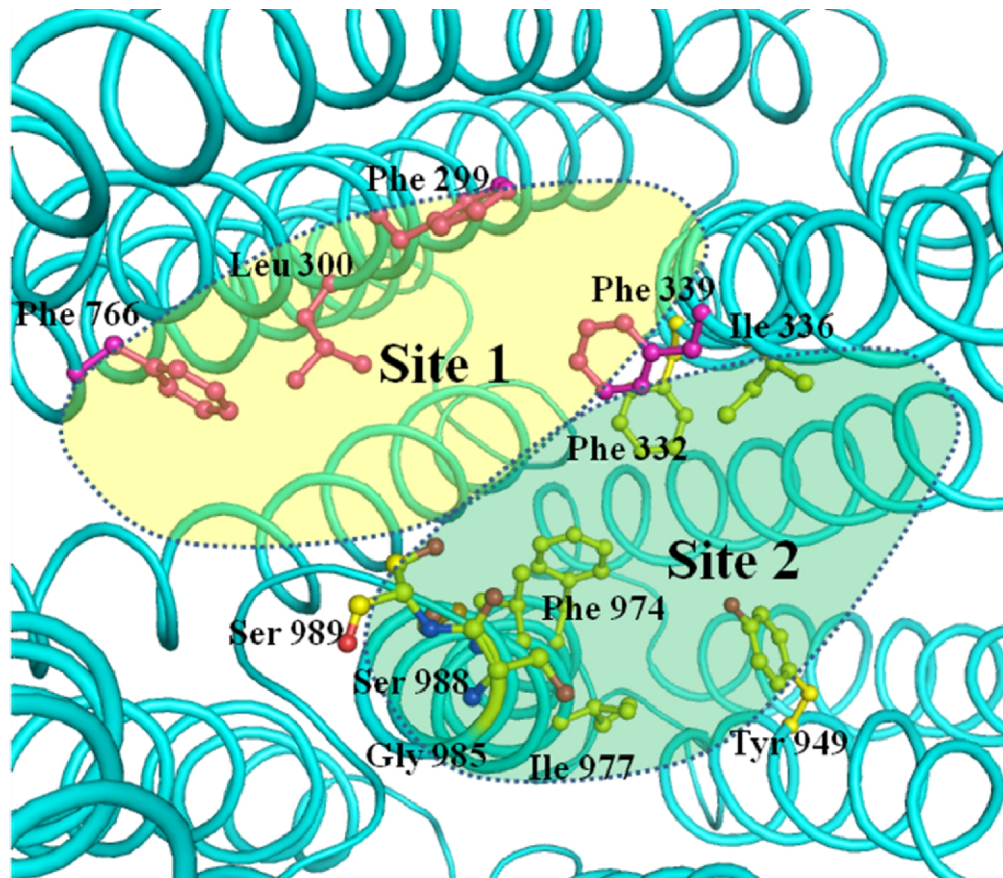


Fig. 4. Site 1 and site 2 are shown. Key residues interacting with ligands at respective sites are also drawn in ball and stick model. The figure was drawn using PyMol [61]

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Szakacs G, Paterson JK, Ludwig JA, Genthe CB, Gottesman MM. Multidrug resistance in Cancer. *Nature Reviews Drug Discovery*. 2006;5(3):219-34.
2. Leveille-Webster CR, Arias IM. The biology of the P-glycoproteins. *J Membr Biol*. 1995;143(2):89-102.
3. Cole SP, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, et al. Over expression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science*. 1992;258(5088):1650-54.
4. Borst P, Elferink RO. Mammalian ABC Transporters in Health and Disease. *Annu Rev Biochem*. 2002;71:537-92.
5. Doyle LA, Ross DD. Multidrug resistance mediated by the breast cancer resistance protein BCRP (ABCG2). *Oncogene*. 2003;22(47):7340-58.

6. Miyake K, Mickley L, Litman T, Zhan Z, Robey R, Cristensen B et al. Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes. *Cancer Res.* 1999;59(1):8-13.
7. Jovelet C, Deroussent A, Broutin S, Paci A, Farinotti R, Bidart JM, et al. Influence of the multidrug transporter P-glycoprotein on the intracellular pharmacokinetics of vandetanib. *Eur J Drug Metab Pharmacokinet.* 2013; (in press).
8. Wang SY, Duan KM, Li Y, Mei Y, Sheng H, et al. Effect of quercetin on P-glycoprotein transport ability in Chinese healthy subjects. *Eur J Clin Nutr;* 2013 (in press).
9. Ramachandra M, Ambudkar SV, Chen D, Hrycyna CA, Dey S, Gottesman MM et al. Human P-glycoprotein exhibits reduced affinity for substrates during a catalytic transition state. *Biochemistry.* 1998;37(14):5010-19.
10. Lam FC, Liu R, Lu P, Shapiro AB, Renoir JM, Sharom FJ, et al. Beta-Amyloid efflux mediated by p-glycoprotein. *Journal of Neurochemistry.* 2001;76(4):1121-28.
11. Raviv Y, Pollard HB, Bruggemann EP, Pastan I, Gottesman MM. Photosensitized labeling of a functional multidrug transporter in living drug-resistant tumor cells. *Journal of Biological Chemistry.* 1990;265(7):3975-3980.
12. Zhu L, Zhao L, Wang H, Wang Y, Pan D, Yao J, et al. Oroxylin A reverses P-glycoprotein-mediated multidrug resistance of MCF7/ADR cells by G2/M arrest; 2013. (in press).
13. Jacob Strouse J, Ivnitski-Steele I, Waller A, Young SM, Perez D, Evangelisti AM, et al. Fluorescent Substrates for Flow Cytometric Evaluation of Efflux Inhibition in ABCB1, ABCC1, and ABCG2 transporters. *Anal Biochem;* 2013 (in press).
14. Kapoor A, Iqbal M, Petropoulos S, Ho HL, Gibb W, Matthews SG. Effects of sertraline and fluoxetine on p-glycoprotein at barrier sites: in vivo and in vitro approaches. *PLoS One.* 2013;8(2):e56525.
15. Tran TP, Kim HG, Choi JH, Na MK, Jeong HG. Reversal of P-glycoprotein-mediated multidrug resistance is induced by mollugin in MCF-7/adriamycin cells. *Phytomedicine.* 2013;S0944-7113(13)00045-7.
16. Reed A, Huie K, Perloff ES, Cassella JV, Takahashi LH. Loxapine P-glycoprotein interactions in vitro. *Drug Metab Lett.* 2012;6(1):26-32.
17. Hollenstein K, Frei DC, Locher KP. Structure of an ABC transporter in complex with its binding protein. *Nature.* 2007;446(7132):213-16.
18. Pinkett HW, Lee AT, Lum P, Locher KP, Rees DC. An inward-facing conformation of a putative metal-chelate-type ABC transporter. *Science.* 2007;315(5810):373-77.
19. Ward A, Reyes CL, Yu J, Roth CB, Chang G. Flexibility in the ABC transporter MsbA: Alternating access with a twist. *Proc Natl Acad Sci USA.* 2007;104(48):19005-10.
20. Oldham ML, Khare D, Quioco FA, Davidson AL, Chen J. Crystal structure of a catalytic intermediate of the maltose transporter. *Nature.* 2007;450(169):515-21.
21. Locher KP, Lee AT, Rees DC. The E. coli BtuCD structure: a framework for ABC transporter architecture and mechanism. *Science.* 2002;296(5570):1091-98.
22. Dawson RJ, Locher KP. Structure of a bacterial multidrug ABC transporter. *Nature.* 2006;443(7108):180-85.
23. Rosenberg MF, Kamis AB, Callaghan R, Higgins CF, Ford RC. Three-dimensional structures of the mammalian multidrug resistance P-glycoprotein demonstrate major conformational changes in the transmembrane domains upon nucleotide binding. *J Biol Chem.* 2002;278(10):8294-99.
24. Lee JY, Urbatsch IL, Senior AE, Wilkens S. Nucleotide-induced structural changes in P-glycoprotein observed by electron microscopy. *J Biol Chem.* 2008;283(9):5769-79.
25. Aller SG, Yu J, Ward A, Weng Y, Chittaboina S, Zhuo R et al. Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science.* 2009;323(5922):1718-22.

26. Dolgih E, Bryant C, Renslo AR, Jacobson MP. Predicting binding to P-glycoprotein by flexible receptor docking. *PLoS computational Biology*. 2011;7(6):e1002083.
27. Barron D, Ibrahim RK. Isoprenylated flavonoids a survey. *Phytochemistry* 1996;43(5):921–82.
28. Benavente-Garcia O, Castillo J, Marin FR, Ortuno A, Del Rio JA. Uses and properties of citrus flavonoids. *J Agric Food Chem*. 1997;45(12):4505–15.
29. Harborne JB, Williams CA. Advances in flavonoid research since 1992. *Phytochemistry*. 2000;55(6):481–504.
30. Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. *J. Nutr*. 2000;130(8):2073S–2085S.
31. Scambia G, Ranelletti FO, Panici PB, Vincenzo R De, Bonanno G, Ferrandina G et al. Quercetin potentiates the effect of adriamycin in a multidrug-resistant MCF-7 human breast-cancer cell line: P-glycoprotein as a possible target. *Cancer Chemother Pharmacol*. 1994;34(6):459–64.
32. Chieli E, Romiti N, Cervelli F, Tongiani R. Effects of flavonols on P-glycoprotein activity in cultured rat hepatocytes. *Life Science*. 1995;57(19):1741–1751.
33. Castro AF, Altenberg GA. Inhibition of drug transport by genistein in multidrug-resistant cells expressing P-glycoprotein. *Biochem Pharmacol*. 1997;53(1):89-93.
34. Shapiro AB, Ling V. Effect of quercetin on Hoechst 33342 transport by purified and reconstituted P-glycoprotein. *Biochem Pharmacol*. 1997;53(4):587-96.
35. Jaganathan SK. Can flavonoids from honey alter multidrug resistance? *Med Hypotheses*. 2011;76(4):535-37.
36. Li FR, Fu YY, Jiang DH, Wu Z, Zhou YJ, Guo L et al. Reversal effect of rosmarinic acid on multidrug resistance in SGC7901/Adr cell. *J Asian Nat Prod Res*; 2013 (in press).
37. Zhang W, Chen BA, Jin JF, He YJ, Niu YQ. Involvement of c-Jun N-terminal Kinase in the Reversal of Multidrug Resistance Human Leukemia Cells in Hypoxia by 5-bromotetrandrine. *Leuk Lymphoma*; 2013 (in press).
38. Huang XC, Sun YL, Salim AA, Chen ZS, Capon RJ. Parguerenes: Marine red alga bromoditerpenes as inhibitors of P-glycoprotein (ABCB1) in multidrug resistant human cancer cells. *Biochem Pharmacol*; 2013 (in press).
39. Jabeen I, Wetwitayaklung P, Chiba P, Pastor M, Ecker GF. 2D- and 3D-QSAR studies of a series of benzopyranes and benzopyrano[3,4b][1,4]-oxazines as inhibitors of the multidrug transporter P-glycoprotein. *J Comput Aided Mol Des*. 2013;27(2):161-71.
40. Eberl S, Renner B, Neubert A, Reisig M, Bachmakov I, König J, et al. Role of p-glycoprotein inhibition for drug interactions: evidence from in vitro and pharmacoepidemiological studies. *Clin Pharmacokinet*. 2007;46(12):1039-49.
41. Angelini A, Di Pietro R, Centurione L, Castellani ML, Conti P, Porreca E, Cuccurullo F. Inhibition of P-glycoprotein-mediated transport by S-adenosylmethionine and cynarin in multidrug-resistant human uterine sarcoma MES-SA/Dx5 cells. *J Biol Regul Homeost Agents*. 2012;26(3):495-504.
42. Chan KF, Wong IL, Kan JW, Yan CS, Chow LM, Chan TH. Amine linked flavonoid dimers as modulators for P-glycoprotein-based multidrug resistance: structure-activity relationship and mechanism of modulation. *J Med Chem*. 2012;55(5):1999-2014.
43. Ma B, Chai S, Li N, To KK, Kan WL, Yang D, Lin G. Reversal of P-glycoprotein-mediated multidrug resistance by a synthetic  $\alpha$ -aminoxy peptidomimetic. *Int J Pharm*. 2012;424(1-2):33-39.
44. Arnaud O, Koubeissi A, Ettouati L, Terreux R, Alamé G, Grenot C, et al. Potent and fully noncompetitive peptidomimetic inhibitor of multidrug resistance P-glycoprotein. *J Med Chem*. 2010;53(18):6720-29.

45. Iwanaga K, Yoneda S, Hamahata Y, Miyazaki M, Shibano M, Taniguchi M, et al. Inhibitory effects of furanocoumarin derivatives in Kampo extract medicines on P-glycoprotein at the blood-brain barrier. *Biol Pharm Bull*. 2011;34(8):1246-51.
46. Lespine A, Martin S, Dupuy J, Roulet A, Pineau T, Orłowski S, et al. Interaction of macrocyclic lactones with P-glycoprotein: structure-affinity relationship. *Eur J Pharm Sci*. 2007;30:84-94.
47. Orłowski S, Selosse MA, Boudon C, Micoud C, Mir LM, Belehradek J Jr, et al. Effects of detergents on P-glycoprotein ATPase activity: differences in perturbations of basal and verapamil-dependent activities. *Cancer Biochem Biophys*. 1998;16(1-2):85-110.
48. Alvarez AI, Real R, Pérez M, Mendoza G, Prieto JG, Merino G. Modulation of the activity of ABC transporters (P-glycoprotein, MRP2, BCRP) by flavonoids and drug response. *J. Pharm. Sci*. 2010;99(2):598-617.
49. Merino G, Perez M, Real R, Egido E, Prieto JG, Alvarez AI. In vivo inhibition of BCRP/ABCG2 mediated transport of nitrofurantoin by the isoflavones genistein and daidzein: a comparative study in *Bcrp1 (-/-)* mice. *Pharm Res*. 2010;27(10):2098-2105.
50. Zhang S, Sagawa K, Arnold RD, Tseng E, Wang X, Morris ME. Interactions between the flavonoid biochanin A and P-glycoprotein substrates in rats: in vitro and in vivo. *J Pharm Sci*. 2010;99(1):430-41.
51. Tamaki H, Satoh H, Hori S, Ohtani H, Sawada Y. Inhibitory effects of herbal extracts on breast cancer resistance protein (BCRP) and structure-inhibitory potency relationship of isoflavonoids. *Drug Metab Pharmacokinetics*. 2010;25(2):170-79.
52. Wesołowska O, Wiśniewski J, Sroda-Pomianek K, Bielawska-Pohl A, Paprocka M, Duś D et al. Multidrug Resistance Reversal and Apoptosis Induction in Human Colon Cancer Cells by Some Flavonoids Present in Citrus Plants. *J Nat Prod*; 2012 (in press).
53. Michalak K, Wesołowska O. Polyphenols counteract tumor cell chemoresistance conferred by multidrug resistance proteins. *Anticancer Agents Med Chem*. 2012;12(8):880-90.
54. Wesołowska O. Interaction of phenothiazines, stilbenes and flavonoids with multidrug resistance-associated transporters, P-glycoprotein and MRP1. *Acta Biochim Pol*. 2011;58(4):433-48.
55. Wesołowska O, Wisniewski J, Bielawska-Pohl A, Paprocka M, Duarte N, Ferreira MJ, Dus D, Michalak K. Stilbenes as multidrug resistance modulators and apoptosis inducers in human adenocarcinoma cells. *Anticancer Res*. 2010;30(11):4587-93.
56. Wesołowska O, Wiśniewski J, Sroda K, Krawczenko A, Bielawska-Pohl A, Paprocka M, et al. 8-Prenylnaringenin is an inhibitor of multidrug resistance-associated transporters, P-glycoprotein and MRP1. *Eur J Pharmacol*. 2010;644(1-3):32-40.
57. Schrödinger LLC. LigPrep, version 2.3. New York; 2009.
58. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, et al. Glide: a new approach for rapid, accurate docking and scoring method and assessment of docking accuracy. *J Med Chem*. 2004;47(7):1739-49.
59. Jones TA, Zou JY, Cowan SW, Kjeldgaard M. Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr Sect A*. 1991;47(2):110-119
60. Emsley P, Cowtan K. Coot: model-building tools for molecular graphics. *Acta Crystallogr. D Biol. Crystallogr*. 2004;60(12):2126-2132.
61. De Lano WL. The PyMol molecular Graphics System, DeLano Scientific, San Carlos, CA, 2002. Available: <http://www.pymol.org>.



62. Campbell JD, Koike K, Moreau C, Sansom MS, Deeley RG, Cole SP. Molecular modeling correctly predicts the functional importance of Phe594 in transmembrane helix 11 of the multidrug resistance protein, MRP1 (ABCC1). *J Biol Chem*. 2004;279(1):463-8.
63. Lespine A, Dupuy J, Orlowski S, Nagy T, Glavinas H, Krajcsi P et al. Interaction of ivermectin with multidrug resistance proteins (MRP1, 2 and 3). *Chem Biol Interact*. 2006;159(3):169-79.
64. Kothandan G, Gadhe CG, Madhavan T, Choi CH, Cho SJ. Docking and 3D-QSAR (quantitative structure activity relationship) studies of flavones, the potent inhibitors of p-glycoprotein targeting the nucleotide binding domain. *Eur J Med Chem*. 2011;46(9):4078-88.
65. Zha W, Wang G, Xu W, Liu X, Wang Y, Zha BS, et al. Inhibition of P-Glycoprotein by HIV Protease Inhibitors Increases Intracellular Accumulation of Berberine in Murine and Human Macrophages. *PLoS One*. 2013;8(1):e54349.
66. Pascaud C, Garrigos M, Orlowski S. Multidrug resistance transporter P-glycoprotein has distinct but interacting binding sites for cytotoxic drugs and reversing agents. *Biochem J*. 1998;333(2):351-58.
67. Yamaguchi H, Kidachi Y, Kamiie K, Noshita T, Umetsu H. Homology modeling and structural analysis of human P-glycoprotein. *Bioinformation*. 2012;8(22):1066–74.

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