



SDI Review Form 1.6

PART 1:

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	MS: 2013_IJBcRR_3268
Title of the Manuscript:	Structural Mapping of Inhibitor Binding Sites on P-glycoprotein: Mechanism of Inhibition of P-glycoprotein by Herbal Isoflavones.

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- This form has total 9 parts. Kindly note that you should use all the parts of this review form.



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PART 2: Review Comments

	Reviewer's comment	Author's comment <i>(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)</i>
<u>Compulsory</u> REVISION comments	This manuscript by Arora et al (IJBRR_3268) reports on docking calculations for binding of a series of plant isoflavones on the mouse P-glycoprotein, whose structure has been recently solved with medium resolution after crystallization. The main outcome is the in-silico determination of two separate binding sites located in the inner chamber of the protein. However, this manuscript suffers from many weaknesses. The first non-addressed question is the relevance of the pose determined in-silico for each ligand tested: what are the respective docking scores for them, and overall what are, for each ligand, the scores obtained for the following poses that are not shown. In other words, how is validated the binding stoichiometry of 1 which is presented for each of them ? Second, what are the validations of the calculated data regarding correlations with in-vitro experiments ? For example, what are the respective affinities of the tested isoflavones, and are they correlated with their docking scores ? Third, there are enough differences between the sequences of mouse and human P-gp to make questionable or even dubious the use of docking data on mouse P-gp to draw some conclusions about the binding of ligands of possible therapeutic interest.	Thank you very much for the comments. 1.more docking information has been incorporated in table 1 and section 3.3. The correct pose and orientation was determined by checking docking score and energy of the complex formation. 2. Pgp inhibitors interactions of isoflavones and flavonoids have been extensively reported in literature (ref 48-58). Although the nature of inhibition of all the isoflavones is still not clear. The current study has shown binding pattern of the ligands, which is essential in recognizing key residues in Pgp, which can be exploited for designing of compounds with higher affinity and specificity. Some of the isoflavones have been reported to have micromolar affinity to the efflux transporters. The literature has been updated. Docking scores are given in Table 1. 3.The overall topology of transporter family is different but the interior of these proteins is very much similar, which is responsible for their binding to common ligands. Mouse and Human Pgp have 85% overall similarity and 100% similarity with the hydrophobic cavity amino acids (ref 24), which are good enough for mouse Pgp to be used as model for human Pgp. Recently, the structure of human Pgp has been modelled using mouse Pgp template structure (ref 69)



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Minor REVISION comments	In addition, there are along the manuscript a number of awkwardnesses that render its reading unsatisfying: - in keywords, avoid too general terms such as "Cancer" and "Ligand", and rather include P-glycoprotein. - in Introduction, MDR phenotype is also due to the expression of MRP1 as well as P-gp or BCRP ; BCRP is ABCG2 (from subfamily G, not "gene" (ln 67)) ; by the way, there are numerous mentions of this transporter while the work presented does not address it at all. - ln 75: "hydrophobic vacuum cleaner" is a functioning model of P-gp that refers to the capacity of P-gp to exclude its substrates out of the membrane phase, as opposed to the floppase model where it translocates its substrates from the cytosolic leaflet to the exoplasmic leaflet of the cell membrane ; this does not refer to the capacity of P-gp to efflux substrates out of the cell. - paragraph 2.1 is not a "method", it should rather be included in Introduction. - in paragraph 2.2, ln 123 and ln 129 seem quite the same. Please, give references for the softwares used (LIGPREP, IMPACT...). - ln 131-33 are already given in Introduction, ln 85-87. - in Methods section, there should be presented how is calculated the hydrophobic / hydrophilic potential used in Fig.3. - ln 159: since P-gp is not a "channel", it is better to say that "it effluxes drugs". - ln 164: the "dimer" seen in crystals has no actual structural (and functional) relevance. - ln 168: a TMD is formed by 6 helices (a full transporter has 2 TMDs and 2 NBDs). - ln 171: P-gp is not a dimer, it is a "tandem" protein, whose two halves display high homology. - ln 181 (and elsewhere): the inner chamber of P-gp is not a channel since it is never open at its two extremities simultaneously (a strict requirement for its active transport function !). - ln 194: "the outer face" rather generally refers to "the exoplasmic portion" of the protein, so it would be better to say "the peripheral face". - ln 195-6: remaining ten, not nine, helices ($12 - 2 = 10$). - ln 205: please give the values for docking scores and IC50 ! - ln 207-8: this generalization is rather speculative, since things are probably not so simple... - ln 221: there are 8 ligands that are presented to bind on Site 2.	-Included -corrected, new references have been added. -term removed -section 2.1 shifted to introduction - changed. reference added -removed -added in method section -corrected -crystallographic dimer is used -corrected -term tandem is used -channel is replaced by cavity -corrected -corrected -added -changed -Corrected
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	- In 239-42: already said a page before. - In 246-8 and In 267-9: hydrophobicity is likely not the only parameter involved. - legend to Fig.1 and 3: precise the orientation of the view. - legend to Fig.3: precise the color code for blue and red.	-deleted -specified -changed - colour code added in legend.
<u>Optional/General</u> comments		