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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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PART 2: Review Comments

	Reviewer's comment	Author's comment (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)
Compulsory REVISION comments	This paper describes the docking of some isoflavones, reported as BCRP inhibitors (cf. ref. 30), within the crystal structure of mouse P-glycoprotein, with the aim of elucidating the inhibition mechanism of P-glycoprotein by isoflavones, and designing a new generation of P-glycoprotein inhibitors. Such a rationale is not acceptable for the following reasons: 1) it is well known that all BCRP inhibitors do not inhibit P-glycoprotein (i.e. FTC, Ko143, novobiocin, methoxy-stilbenes, chalcones, chromones, ...) as, reciprocally, all P-glycoprotein inhibitors do not inhibit BCRP (i.e. zosuquidar, lathyranes, jatrophanes, ...). Therefore, binding to P-glycoprotein, through docking of BCRP inhibitors such as isoflavones, does not mean that these ligands will indeed inhibit. A first mandatory experimental verification should have consisted in evaluating the true inhibitory capacity of these isoflavones on P-glycoprotein-mediated drug efflux activity. Despite displaying widely overlapping substrate patterns, P-glycoprotein/ABCB1 and BCRP/ABCG2 belong to different classes of ABC transporters and then have distinct topologies and 3D-structures. 2) Such a P-glycoprotein inhibition by isoflavones is expected to be low because isoflavones are known to be poor inhibitors by comparison to flavonols, such as quercetin and derivatives, the effects of which have been reported in the old ref. 21, 22 and 24. The single reference (n° 23) related to isoflavones (genistein) only reported binding to isolated NBD, not inhibition of drug efflux. The literature about inhibition of P-glycoprotein by flavonoids and derivatives or analogues is not updated, since the most recent references are from 1997, and many studies are lacking (such as flavones, methoxy-stilbenes, chalcones and chromones, as mentioned above). 3) Nothing is indicated about the mechanism of such inhibitors: are they competitive, and then probably transported, or are they non-competitive, and then not transported ? Competitive and non-	Thank you very much for the comments. 1. The discussion and comparison with BCRP has been removed as suggested. Pgp inhibitors interactions of isoflavones and flavonoids have been extensively reported in literature (ref 48-58). More than potency of inhibition of Pgp, the current study has shown binding pattern of the ligands, which is essential in identifying key residues in Pgp, which can be exploited for designing of higher affinity and specificity compounds. All the members of these transporter family varies in topologies, but the interior of these protein has similar stereochemical environment such as presence of cluster of aromatic amino acids in MRP1 (ref 64). 2. Some of the isoflavones have been reported to have micromolar affinity to the efflux transporters. Flavanols being more polar should have higher affinity as we also have suggested that by adding polar groups on a hydrophobic nucleus, a ligand should be stronger inhibitor to Pgp. The literature has been updated. 3. In vivo and in vitro studies have shown that isoflavones and flavonoids are inhibitor to Pgp mediated exotransport. The conformational change in binding of



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	competitive inhibitors would not be expected to bind to the same sites, which then should be possibly overlapping but distinct. 4) It is difficult to imagine that such loose, if indeed, inhibitors could help in designing better inhibitors than the 3rd generation inhibitors already known for P-glycoprotein, such as Zosuquidar, Tariquidar, Elacridar, Valspodar, Dofequidar, CBT-1, HM-30181A, W-198 and Ontogen, which were found to be highly potent and submitted to clinical trials.	these compounds to Pgp has not been understood as the three dimensional structure of the complexes of Pgp are not available. Although we have observed two overlapping binding sites but it is very difficult to categorise them at this time. 4. So far there is no information available about interacting residues to isoflavones due to unavailability of three dimensional structures of the complexes of Pgp. The binding pattern and knowledge about interacting residues of Pgp is important in guiding rational designing of potent and specific compounds against Pgp.
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments		