



## SDI FINAL EVALUATION FORM 1.1

### PART 1:

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| Journal Name:            | <a href="#">International Journal of Biochemistry Research &amp; Review</a>                                                              |
| Manuscript Number:       | <b>MS: 2013_IJBcRR_3268</b>                                                                                                              |
| Title of the Manuscript: | <b>Structural Mapping of Inhibitor Binding Sites on P-glycoprotein: Mechanism of Inhibition of P-glycoprotein by Herbal Isoflavones.</b> |

### PART 2:

| FINAL EVALUATOR'S comments on revised paper (if any)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Authors' response to final evaluator's comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
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| <p>The manuscript (IJBRR_3268) has been significantly improved in its presentation. There remains few scores:</p> <ul style="list-style-type: none"> <li>- line 26: MRP1 is ABCC1, so shift the bracket</li> <li>- ln 33: fluorescent</li> <li>- ln 43-49: I don't see ref 30</li> <li>- ln 59, 98 and 158: transporter, not receptor</li> <li>- ln 131, 143 and 229: cavity, not channel</li> <li>- ln 173 and 175, and Tab1: kcal</li> <li>- ln 208: of (of)</li> <li>- ln 224: exotransport</li> <li>- ln 226: og/of</li> </ul> <p>However, the main question that is opened by this manuscript is : how is it possible that so subtle differences in the chemical structures of the tested isoflavones can induce a "shift" in their binding between Site 1 and Site 2 (see Table 1) ? This shows the importance to assess the validity of the presented dockings. Indeed, the 3 basic points quoted in the previous review are still not fully addressed, although they are worth being:</p> <p>1- For each ligand with different poses obtained after clustering the calculated docking positions (see ln 179 and 190), does the second (and/or the third) one fit to the same Site (1 or 2) that the first one that is shown ? Or alternatively, does it "shift" to the other Site ? In other words, do these two Sites actually reflect perfectly selective binding, or possibly are they two alternative binding modes for all the tested ligands that can be obtained when considering the few relevant docking positions of rather comparable energies ?</p> <p>2- The docking calculations show that the glycosides isoflavones have systematically better binding scores than the aglycones counterparts, but do they indeed display better affinities in experimentally collected data reported in the literature ? If this was the case, even for few examples of isoflavones (or even related molecules), this would be a fair validation of the presented in silico data.</p> <p>3- Even if sequence similarity level between the murine and the human isoforms is rather high, it is not total, and a single residue difference could make a sensitive difference in binding modes for a given ligand. Actually, there are few examples in literature showing species specificities for P-gp handling of drugs or dyes. So, docking data performed on a murine structural model can hardly be taken for (human) therapeutical perspectives with good confidence, and this requires, at least, very carefull statements.</p> | <p>-corrected<br/>-corrected<br/>-corrected<br/>-corrected<br/>-corrected<br/>-corrected<br/>-corrected<br/>-corrected</p> <p><b>1- Both the sites are located close by and in fact contain overlapping regions. All of the optimal poses of ligands (having multiple poses) were observed in the single site for respective ligands. There was no shift observed for various orientations. In other words the optimal poses were site specific in binding pattern.</b></p> <p><b>2- Higher affinity of glycosides is understandable by their more polar nature which makes them capable of forming stronger attractive polar interactions with Pgp. Number of attractive interactions and buried surface area calculations also indicated better bindings of glycoside isoflavones. Glycosides have been found to bind this transporter family more strongly than their aglycosides counter parts (ref51).</b></p> <p><b>3- It indeed true that even slight structural change can render change in affinity and specificity of ligands. Unfortunately the crystal structure of human Pgp is not yet available in literature, to best of our knowledge. Therefore, using murine model was the only option available to perform docking studies. The precise differences in binding pattern and affinity of isoflavones to human Pgp can only be answered, when we have the structure of human form available. Moreover, we only propose to have similar binding pattern of isoflavones to human form as the residues in ligand binding pocket are identical except one change in mSer725/hAla729 in both the proteins (ref 26).</b></p> |