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COMPARATIVE STUDY OF PLANT DERIVED NATURAL COMPOUNDS AND ESTABLISHED INHIBITORS OF P-GLYCOPROTEIN OF MOUSE AND HUMAN USING MOLECULAR DOCKING

Susmita Roy[1^], Sujata Roy[1^], Vineet Vishal[2^], Sonia Bansal[2], Sayak Ganguli[3*] and Pratiti Ghosh[4]

Department of Nutrition, Rastraguru Surendranath College, Barrackpore, India
[1]Department of Biotechnology, Rajalakshmi Engineering College, Thandalam, Chennai - 602105, India
[2] Computational Biology Division, The Biome, Kolkata - 700064, India
[3] Theoretical and Computational Biology Division, AIIST, ABPL, Palta - 743122
[4] Department of Physiology, West Bengal State University, Berunanpukhuria, Barasat, 700126

***Corresponding Author (s) Email:** raysusmita2006@yahoo.co.in, pratiti_ghosh@yahoo.co.in

ABSTRACT

One of the key physiological barriers which is involved in preventing the intrusion of toxins and xenobiotics out of the cells, is the ATP binding cassette transporter, P-glycoprotein (P-gp). It is localised in the epithelial lining of the colon, small intestine, pancreatic ductules, bile ductules, kidney proximal tubules, and the adrenal gland. The endothelial cells of the blood brain barrier (BBB) also houses. The transporter has been reported to be overexpressed on the surface of many neoplastic cells leading to restrictions in cell entry. It functions to protect these susceptible organs from toxic compounds, preventing them to enter the cytosol and extrude them to the exterior. Thus, it also enhances the secretion of metabolites and xenobiotics into bile, urine, and the lumen of gastrointestinal tract. The dark side of this protein is its role in multidrug resistance in various diseases specially cancer. The resistance is generally conferred by mediating the ATP-dependent efflux of a large number of anticancer drugs. Direct inhibitors against P-gp has been the general strategy though competitive inhibitors which compete with general anticancer drugs have also been explored. Though considerable in vitro success has been achieved, very few compounds have actually been able to "block" P-gp–mediated resistance in the clinic. This work focusses on exploring the efficacy of interactions of a few natural compounds including curcumin against this important transporter.

KEY WORDS

P glycoprotein, Natural Compounds, Interaction sites, Curcumin

INTRODUCTION:

The first report of P-glycoprotein (P-gp) was made from tumour cells. As a result of overexertion of this protein, cytotoxic drugs failed to access these cells. This resulted in the phenomenon of multidrug resistance (MDR) and the gene for this membrane bound efflux transporter (P-gp) is known as *MDR1*. Concomitant expression of *MDR1* or P-gp along with the cytochrome P450 (CYP) family of enzymes, can be earmarked to be a very important step for adaptation against potentially toxic chemicals. It is an evolutionary important phenomenon as well. The diverse localizations of P-gp significantly contribute towards its role in pharmacokinetics. (Fojo et al, 1987). Data from knockout transgenic mice also strongly support the role of *MDR1* in drug absorption, disposition, elimination, and detoxification pathways (Schinkel, 1997; Johnson et al, 2001). Extensive research has been done on the pharmacological function of

MDR1 but only recently have new studies reported several different single-nucleotide polymorphisms (SNPs), which occur as a result of single-nucleotide substitutions, in the human *MDR1* coding region.

Improvement of the delivery of therapeutic agents is generally achieved as a result of the inhibition of P-gp. In general, three mechanisms are employed:

(i) Competitive or allosteric blockage of the interaction site

(ii) Interference of the hydrolysis of ATP and

(iii) Alteration of lipid integrity of the cell membrane.

All of the above three mechanisms results in improved drug bioavailability, targeted organ uptake of the drug and improvement in overall cancer therapy by the selective blockage of function of P-gp. Based on their specificity, affinity and toxicity, reported inhibitors of P-gp can be classified into three different generations. Among these, inhibitors such as vermeil, cyclosporin A, etc. are themselves transported through P-gp. The first generation of inhibitors are not direct inhibitors but do so as an accessory function to their original locale of treatment and target; as a result, they are non- specific and are required in dosage which exceed the threshold of toxicity often causing uncharted pharmacokinetic effects. The second generation of inhibitors are characterized by their higher P-gp affinity, lacking other pharmacological functions. However, as a pitfall they also inhibit the CYPA4 enzyme and other ATP binding cassette (ABC) transporters resulting in the decrease in the metabolic rate and resulting in an altered pharmacokinetic cascade. Following the mixed rate of success with first and second-generation inhibitors, structure activity relationships have been explored along with computer aided drug design (CADD) methods to develop specific inhibitors with lower toxicity e.g., tariquidar. Bispecific antibodies and radioisotope conjugates as well as conjugate mono clonal antibodies have also been explored to inhibit P-gp mediated transport of substrates such as calcein-AM, daunorubicin, and 99 mTc-hexakis-2-methoxybutylisonitrile. UIC2 antibody has been reported to recognize leading to the inhibition of the rest of the P-gp molecules only in the presence of certain inhibitors, including vinblastine, cyclosporine A, and PSC 833 (valspodar). So, these inhibitors can be used simultaneously along with UIC2 leading to the accumulation of certain substrates of P-gp by total inhibition of P-gp pump activity. So a variety of approaches are being experimented upon to develop P-gp inhibitors or mechanisms to bypass the efflux activity. The wonder molecule once identified should not only be able to inhibit the target protein but also lead to the increase of cellular uptake of drugs along with the increase in the transport and half-lifes. This shall result in the formulation of a cost-effective therapeutic intervention shortening the time for optimal drug delivery. Srivalli and Lakshmi (2012) have documented the use of various small molecule inhibitors of natural origin against P-gp; however, the exact binding sites have not been elucidated. It is important to document the interacting sites for selecting the next generation inhibitors of P-gp with the imminent threat of multidrug resistance. This work attempts to compare the efficacy of interactions among established P-gp inhibitors and novel natural products to identify potent lead compounds.

MATERIALS AND METHOD:

A total of 20 molecules were selected for the study (Table 1) out of which five were established P-gp inhibitors of either human or mouse, six natural compounds out of which, functions of four are not elucidate till date and the others various transport protein inhibitors with the potential to inhibit P-gp. Tertiary structure predictions of the proteins were modeled using comparative modelling approach. Template selection was done using PSI-BLAST and BLAST [Atschul et al, 1997] (https://blast.ncbi.nlm.nih.gov) for similarity against Protein Databank [Berman et al, 2000], keeping all the parameters at default. The models were produced using MODELLER 9.18 [Webb and Sali, 2014], a program for comparative structure modelling. The modeled structure was then subjected to molecular simulation for 10ns using a GROMOS96 43a2 force field in Gromacs. MOLPROBITY (http://molprobity.biochem.duke.edu/) server [Davies et al, 2007] was used to assess and validate the generated structures. Quality estimation of the models was performed using QMEAN (https://swissmodel.expasy.org/qmean/) Server [Benkert et al, 2008].

After the validation step, pocket detection and druggability assessment was performed using the tool, DOGSITE SCORER [Volkamer et al, 2012] (http://proteinsplus.zbh.uni-hamburg.de/). In order to study the interactions between the P-gp and the compound library selected for the study, docking was performed using the software Autodock. The docked complexes were analyzed using PDBSUM and LigPlot+ [Wallace et al, 1996] to study the hydrogen bonds and common interacting residues.

Table 1: List of compounds selected for the study

Serial	Name of Compound	Source / Function	References
1	Dexniguldipine	chemosensitizer in multidrug resistant cells	Hoffman J (1995)
2	Dexverapamil	R-enantiomer of the calcium channel blocker verapamil	Thürlimann B (1995)
3	Dofequidar	ATP binding casette transporter blocker	Katayama (2009)
4	Elacridar	P- Glycoprotein Inhibitor	Colabufo et al, (2008)
5	Laniquidar	P- Glycoprotein Inhibitor	Luurtsema (2009)
6	Mitotane	orphan drug for adrenocortical carcinoma	Terzolo et. al. (2007)
7	Quinidine	acts on sodium channels on the neuronal cell membrane	Sheets et al, (2010)
8	Reserpine	inhibition of the ATP/Mg2+ pump responsible for the sequestering of neurotransmitters	Mandela et al, (2010)
9	Tamoxifen	binds to estrogen receptors (ER), inducing a conformational change in the receptor	Cyrus K et al (2010)
10	Tariquidar	P- Glycoprotein Inhibitor	Weidner et.al. (2016)
11	Toremifene	binds to estrogen receptors and may exert estrogenic, antiestrogenic, or both activities	Christos Markopoulos et al. (2015)
12	Valspodar	P- Glycoprotein Inhibitor	Tomillero A, Moral MA. (2008)
13	Verapamil	inhibits voltage-dependent calcium channels	Tfelt-Hansen P, Tfelt-Hansen J (2009)
14	Yohimbine	blockade of central alpha 2-adrenergic receptors producing an increase in sympathetic drive	Laurila JM et.al. (2007)
15	Zosuquidar	P- Glycoprotein Inhibitor	Cripe et.al (2000)
16	Axillarine A	pyrrolizidine alkaloids from *Crotalaria axillaris*	Crout (1969)
17	22 AC Petuniasterone	novel ergostane-type steroids of *Petunia hybridia* Vilm.	Elliger et.al (1988)
18	Sarcovagine A	novel steroid from *Sarcococca vagans*	Yu et.al. (1997)
19	Sarsalignenone	steroidal alkaloid from *Sarcococca hookeriana*	Devkota et al, (2008)
20	Curcumin	Numerous proposed effects	Kai et.al. (2013)

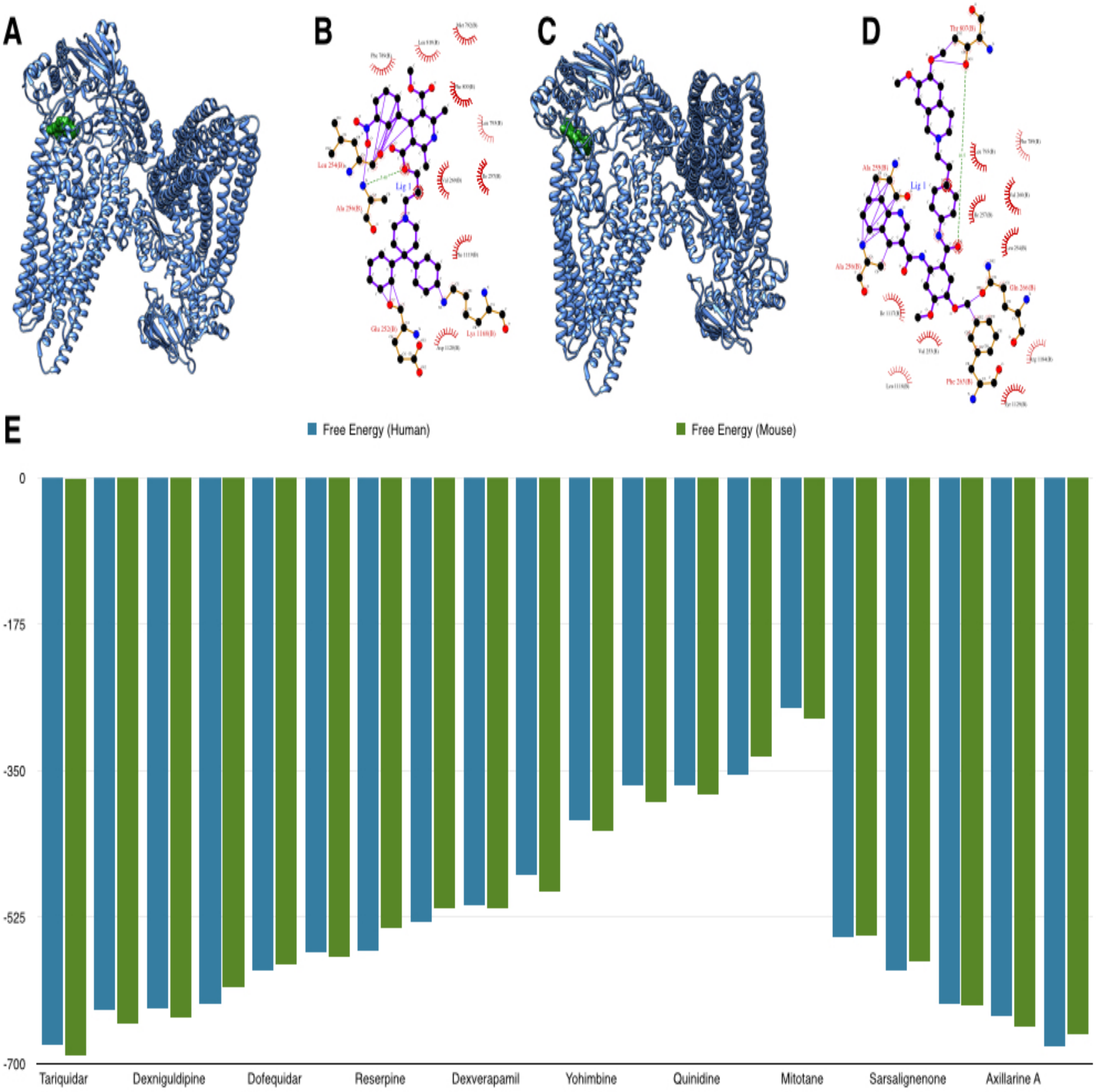
RESULTS AND DISCUSSION:

The modeled and simulated structure was found to be stable stereochemically as revealed by the Ramachandran plot analyses. The radius of gyration over the 10 nanosecond (ns) duration along with the RMSD values over time showed that there were conformational states of the structure which were further established by the analyses of B - factors and RMSD values in a residue specific manner. The predictions of Disopred had revealed that the initial 14 residues of the N terminal region of the protein had propensity binding proteins despite being in disordered state; this conformed with the above analyses as maximum fluctuations were recorded in B factors specific to that N-terminal region. Numerous binding pockets were identified in the structure and their properties were calculated (supplementary file 1).

All the molecules selected for the study exhibited interactions with both the glycoprotein structures belonging to human and mouse. In case of the interactions of mouse glycoprotein with the compounds selected for the study we observed that the lowest binding energy was obtained from the interactions of MPGP with curcumin closely followed by tariquidar.

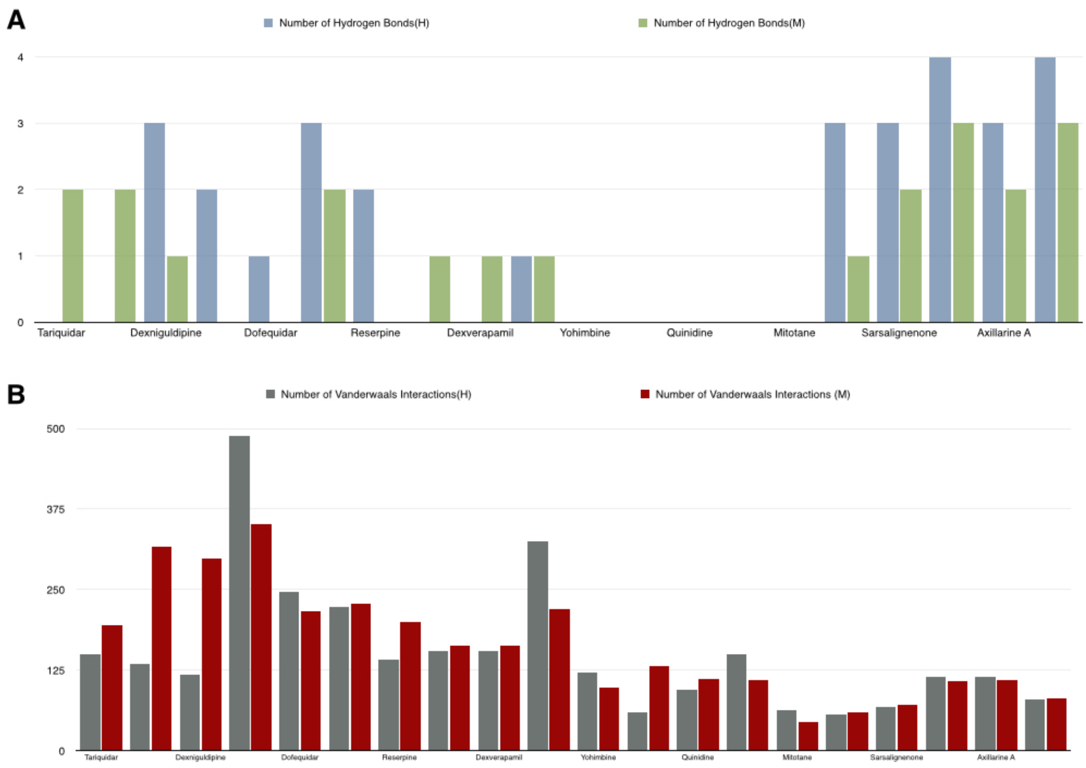
Curcumin exhibited higher number of hydrogen bonded interactions than that of tariquidar, however, the area of interaction and the number of van der waals interactions were much lower in context of curcumin (supplementary Table 1).

Fig 1: Interactions of P-glycoprotein with small molecules used in the study.



A: Representative model of Interaction of Human P-Glycoprotein with Petuniasterone -C; B: Hydrogen bonded and Van der waals interactions (Ligplot Image); C: Representative model of Interaction of Mouse P-Glycoprotein with Petuniasterone -C; D: Hydrogen bonded and Van der waals interactions (Ligplot Image); E: Comparative graph of free energy values of interactions of human and mouse P-gp.

Fig 2: Graphical representation of the different interactions of the individual small molecules with P-gp of human and mouse.



A: Number of hydrogen bonds formed during interactions; B: Number of non-bonded contacts generated during interactions.

A general trend that was observed in case of natural compound interactions with mouse P-gp was that all the compounds under study exhibited hydrogen bonded interactions along with van der waals interactions with small binding area (Fig. 1 and Fig. 2). This may be attributed to their small structure in comparison with the other small molecules and established inhibitors used in the study. This same trend was observed in case of human P-gp - small molecule interactions as well where curcumin interactions were the most stable in terms of binding energy followed very closely by tariquidar. The four experimental molecules with no established biological roles - sarcovagine A, sarsalignenone, 22AC petuniasterone and axillarine A were also found to interact with both hydrogen bonded and van der waals interactions with the human protein. Among the third generation established P-gp inhibitors used in this study the performance of tariquidar was better in comparison with the other small molecules - zosuquidar, laniquidar, mitotane, and elacridar. Hydrogen bonded interactions were observed in case of zosuquidar and elacridar along with van der waals contacts, however, the total interface area and relative entropy of complex formation were higher in comparison to tariquidar and four unreported molecules. First and second-generation small molecule inhibitors such as verampil, reserpine, yohimbine, quinidine, valspodar, and dofequidir fumarate also exhibited interactions but low binding affinity as reported in many studies.

CONCLUSION

Curcumin has already been reported to inhibit the actions of P - glycoprotein under laboratory conditions; this study confirms its mode of interactions with the protein and thus along with the four small molecules used in this study - sarcovagine A, sarsalignenone, 22AC petuniasterone and axillarine A can be proposed as potential lead molecules which can be explored further in clinical trials as they have exhibited potent interactions using bonded and non-bonded contacts as well as low binding energy and satisfactory interacting area. These molecules should be suitably modified and tested *in vitro* for their effectiveness and dosage in suitable model systems.

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***Corresponding Author:**

Susmita Roy*

*Email:***raysusmita2006@yahoo.co.in,**
pratiti_ghosh@yahoo.co.in