International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(6): 1858-1868

Received: 25-09-2018
Accepted: 30-10-2018

Om Prakash
Veterinary Polytechnic, Surajpur, Chhattisgarh Kamdhenu Vishwavidyalaya, Durg, Chhattisgarh, India

Shankarlingam Gomathinayagam
Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Science University, Chennai, Tamil Nadu, India

Tirunelveli Jeyagopal Harikrishnan
Tamil Nadu Veterinary and Animal Science University, Chennai, Tamil Nadu, India

Muthusamy Raman
Transboundary Research Platform for Veterinary Biological, Tamil Nadu Veterinary and Animal Science University, Chennai, Tamil Nadu, India

Velayudham Pandiyan
Department of Veterinary Biochemistry, Madras Veterinary College, Chennai, Tamil Nadu, India

Samapika Sahoo
Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Science University, Chennai, Tamil Nadu, India

Correspondence
Om Prakash
Veterinary Polytechnic, Surajpur, Chhattisgarh Kamdhenu Vishwavidyalaya, Durg, Chhattisgarh, India

Identification of analogue compounds of P-glycoprotein associated anthelmintics resistance reversal agents through cheminformatics approaches

Om Prakash, Shankarlingam Gomathinayagam, Tirunelveli Jeyagopal Harikrishnan, Muthusamy Raman, Velayudham Pandiyan and Samapika Sahoo

Abstract
The use of anthelmintics in intensive farming has followed swiftly by the emergence of anthelmintics resistance (AR) which is now an emerging phenomenon in parasitic nematodes of sheep, goats, horses, and cattle. AR is defined as a genetically transmissible trait in which the sensitivity to a particular drug is lost in a population of worms over time. Cheminformatics studies were carried out to find out the P glycoprotein modulators/inhibitors from plant compounds/chemicals sources. Three dimensional structure of Pgp was designed using the sequences from NCBI. Five models of Pgp were received. Based on I-TASSER modeling and RAMPAGE score Pgp model 1 was selected and molecular docking was done to interact with 36 plant compounds/chemicals using Accelrys Discovery Studio client 2.5. Based on the best molecular docking score (more than 90 interactions), Curcumin, Quercetin, Kaempferol, Phloretin, Verapamil and Loperamide with molecular docking scores of 98.324, 96.073, 95.47, 94.895,93.453 and 92.807 were selected.

Keywords: anthelmintic resistance, cheminformatics, haemonchus, p- glycoprotein, docking

Introduction
In the continued absence of commercial vaccines, the use of broad-spectrum anthelmintics has been the primary method to control the pathogens in cattle and sheep for over 50 years [1, 2]. The use of anthelmintics in intensive farming has followed swiftly by the emergence of anthelmintic resistance (AR) which is now an emerging phenomenon in parasitic nematodes of sheep, goats, horses, and cattle [3]. AR is defined as a genetically transmissible trait in which the sensitivity to a particular drug is lost in a population of worms over time [4]. Resistance to the majority of the anthelmintics including the Benzimidazole [5, 6], Salicylanilides [6], Macrocyclic lactones [5, 7] were reported. The key to control anthelmintic resistance in *H. contortus* is to understand various mechanisms that may be involved, for each class of anthelmintics has a known different target. There are three main groups of mechanisms: those that change the binding sites of drugs, those that detoxify, and those that involve the active efflux of drugs by membrane transporters [8, 9]. The mechanism that is primarily considered to be involved in resistance to macrocyclic lactones is the detoxification process of P-glycoproteins. P-glycoproteins (Pgps) are efflux transporters which actively transport compounds, including drugs, across membranes [10]. The primary function of Pgp is to protect the organism by actively pumping toxic substances out of its cells [11]. P-glycoproteins have been identified in *H. contortus* and the full cDNA sequence has been obtained [12]. The mechanism believed to be associated with anthelmintic resistance in *H. contortus* is the overexpression of Pgp. Both benzimidazole and ivermectin-resistant strains of *H. contortus* have been found to possess Pgp alleles in higher frequency than susceptible strains. Pgp may modulate benzimidazole concentration at the target site [9]. A relationship between Pgp and benzimidazole resistance was indirectly demonstrated through the use of the Pgp inhibitor Verapamil [13, 14]. Verapamil is a calcium channel blocker, which actively inhibits the Pgp drug-binding domain. In the presence of Verapamil, the toxicity of the drug increased and the benzimidazole resistance could be partially reversed [12]. A role for P-glycoprotein (P-gp)

drug efflux pumps in ML resistance in *H. contortus* and other parasitic nematodes [15]. The expression of P-gps has been increased in IVM resistant isolates [11, 16, 14]. A number of P-gp inhibitors/modulators have been shown to reverse BZ and ML resistance in *H. contortus*, both *in vitro* and *in vivo.* Valspodar increased the sensitivity of resistant and susceptible strains of *H. contortus* and *Teladorsagia circumcincta* to IVM [17]. A potentiated efficacy of IVM against field IVM resistant isolate of *H. placei* by multidrug resistant inhibitors (MDRIs), Verapamil and Cyclosporine had been demonstrated resulting in higher efficacy and lower IVM $EC_{50.}$ [18]. Third generation Pgp inhibitors including tariquidar, zosuquidar and elacridar increased the efficacy of IVM, levamisole (LEV) and thiabendazole [19].

Cheminformatics is a field of information technology that uses computers and computer programs to facilitate the collection, storage, analysis, and manipulation of large quantities of chemical data. Cheminformatics approaches had been attempted to identify the MDRIs analogue compounds. The herbal compounds curcumin, baicailin and dronabinol have been identified as novel monoamine oxidase inhibitors for treatment of Parkinson disease in human [20]. The specificity and strong binding affinity of curcumin to major inflammatory mediators such as, cytokines/chemokines, signaling proteins and transcription factors using molecular docking [21]. *In silico* study of enzyme-inhibitor binding simulation between eight phytochemicals and Janus kinase enzymes (JAK 1, 2 and 3) using the Patchdock docking server and Curcumin was found to have strongest binding potential when compared with other chemicals and suggested curcumin can be utilized as natural JAK inhibitors [22]. Molecular docking to determine the orientation of Stigmasterol β – D Glucoside bound in the active site of tubulin as target for anthelmintic activity [23]. Recently studied interaction of Curcumin with the involvement of higher number of amino acids as compared to thymoquinone using i*n silico* molecular docking and concluded that curcumin could be more effective in inhibiting the antioxidant enzymes of *F. gigantica* [24]. Even though several herbal and synthetic chemicals were found to alter the resistance to BZ/IVM in *H. contortus,* some of these compounds are quite toxic and cannot be used in food animals. Hence, it is imperative that safe ecofriendly compounds, whether synthetic or herbal with strong affinity to interact with Pgp, need to be identified to potentiate the efficacy of the fewer anthelmintics available for animals. Perusals of literature indicate that such studies are at infancy and there is enough scope to identify new compounds which are safer through cheminformatics approaches.

Materials and Methods

The Permissible glycoprotein (Pgp), a member of group of integral membrane proteins that contain the ATP- binding cassette, widely represented in animal kingdom. In nematodes, possible functions include transport of lipophilic peptides and hormone as well as exclusion of toxin across cell membranes [11]. A Pgp mediated modulation of drug concentration at target site, is another potential mechanism of anthelmintic resistance [11, 9, 18, 8]. Identification of analogue compounds of anthelmintic resistance reversal agents through cheminformatics approach was performed as follows:

Retrieval of P-glycoprotein

The sequences of P glycoprotein (Pgp A) m RNA of *H. contortus* was retrieved from the National Centre for Biotechnology Information (NCBI, USA, 1988) as FASTA Format files. P glycoprotein Gen Bank accession number was AF003908 [12].

Modelling of P-glycoprotein

Iterative Threading Assemmbly Refinement (I-TASSER) was a bioinformatics tool for predicting three-dimensional structure model of protein molecules, developed at Yang Zhang Lab, University of Michigan, Ann Arbor, USA. The retrieved P glycoprotein sequences were submitted to the "I-TASSER" database, for modelling of the P glycoprotein 3D Structures.

Evaluation and analysis of residues

The modelled 3D structure of P glycoprotein of *H. contortus* was online submitted to RAMPAGE [25].

Ligand preparation

Plantcompounds and certain chemicals had been identified as Pgp modulators/inhibitors in multidrug resistance [25]. The 3 D structure of 36 plant compounds /chemicals known to be Pgp modulator/inhibitors in cancer drug resistance, anti-diabetics resistance and antibiotics resistance was downloaded from the Chemical Database "PubChem" (NCBI, USA, 2004) which was the repository of the chemicals. Using these compounds**,** identification of compounds in the reversal of Pgp associated anthelmintics resistance was carried out *in silico* using software Accelrys Discovery Studio Client 2.5 (Biovia, USA, 2009).

Molecular docking

Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand-protein docking was to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. The molecular docking of 36 plant compounds/chemicals (Ligands) with P-glycoprotein (Protein) was carried out with Accelrys Discovery Studio client 2.5 (Biovia, USA, 2009) to predict the interaction of ligands with various sites of P glycoprotein of *H. contortus.*

Selection of plant compounds/chemical for reversal of anthelmintic resistance

Based on highest molecular docking score**,** plant compounds/chemicals were selected to find out the efficacy of these compounds in the reversal of anthelmintics resistance.

Results

Identification of analogue compounds of anthelmintic resistance reversal agents (Chemosensitizers) through cheminformatics approach is performed as follows:

Retrieval of P-glycoprotein

The retrieved P glycoprotein constituted with 1275 amino acids.The modelling of Pgp2 of *H. contortus* was done to get the three dimensional structure of Pgp2.

Modelling of P-glycoprotein

Five three dimensional Pgp2 models were received from I-TASSER (**Plate 1**). I – TASSER P gp model score for all five models were presented in **Table.1** [27]. Confidence score (C score) of the model-I was 2 and Topology score (TM score) was more than 0.5; hence model 1 was selected for further studies.

Evaluation and analysis of residues

The modelled 3D structure of Pgp2 of *H. contortus* was validated by submitting them to RAMPAGE [25] to visualize energetically allowed regions and amino acid residues in protein structure. Number of residues in favoured region (~98.0% expected), number of residues in allowed region (~2.0% expected) and number of residues in outlier region for all the five models are depicted (Table 2.0). Besides this ray diagrams for validation of all the five models were presented in Plate 2. Based on the RAMPAGE, (Ramachandran Plot) assessment of the Pgp2, model I was selected for molecular docking interaction with ligands.

Ligand preparation

The 3 D structure of 36 compounds (both chemicals as well as compounds of plant origin) known to be Pgp modulator/inhibitors in cancer drug resistance, anti-diabetics resistance and antibiotics resistance was downloaded and compounds are listed in Table 3.0. Majority of the compounds were from plant origin. The three dimensional structure of the downloaded compounds were presented in Plate 3a and Plate 3b. These compounds were individually interacted with all the binding sites of Pgp2.

Molecular docking

P-glycoprotein 2 of *H. contortus*had overall 47 binding sites. In this study, out of 47 sites, 13 binding sites viz., 1, 2, 3, 9, 15, 16, 28, 32, 33, 35, 38, 41 and 45 did not interact with any compounds. A total of 2151 interactions with all the thirty six compounds were observed in 34 sites of Pgp2. At the binding site 26, the highest interaction between ligands and protein i.e., 240 posses was found. Molecular docking score of some compounds (Ligands) are shown in Table 4.0.

Selection of plant compounds/chemicals for reversal of anthelmintic resistance

Based on highest molecular docking score (more than 90 interactions)**,** Curcumin, Quercetin, Kaempferol, Phloretin, Verapamil and Loperamide with molecular docking scores of 98.324, 96.073, 95.472, 94.895, 93.453 and 92.807 were selected. Molecular docking (intraction between ligands and Pgp) of the selected compounds are depicted in Plate 4.

Discussion

P-glycoproteins (Pgp) are efflux transporters which actively transport compounds, including drugs across membranes [10]. The primary role of Pgp is to protect the organism by actively pumping toxic substances out of its cells [11]. Many researchers have reported higher levels of Pgp in resistant parasites [28, 9, 29, 30, 31]. and being over expressed in response to chemotherapy in tumor cells [32, 33]. But increased Pgp did not appear to be a primary mechanism of drug resistance in L3 of *H. contortus* [34]. Many findings after 2007 confirmed the involvement of several Pgps in the drug resistance [32, 35] found enhancement of Pgp2 and had shown The ability of Pgp2 anf Pgp-11 to modulate ivermectin susceptibility by its expression [29]. All these results have clearly shown that there was involvement of Pgps in MDR. Hence, the objective of the study was to find out compounds that could alter or reduce the efflux of the drugs by modulating or inhibiting Pgp. Many researchers used *in silico* approaches or cheminformatics approaches to find out new compounds/drugs that can interact with the target sites/compound to bring about the desired effect in human diseases [20, 22, 36]. and to find out anti-inflammatory drug [37].

In this study, cheminformatics has been used to identify compounds which will interact with Pgp to modulate or inhibit Pgp, thereby decreasing the efflux of the anthelmintics drug from cell that is attributed to be the main cause of drug resistance. Hence, toxicity of the drug is increased or potentiated. Otherwise resistance to the drug is reversed or reduced. There are many computer software used to identify compounds /design new drugs like AUTODOCK 4 Programme [21, 37, 38, 39, 40], Patchdock docking server [22], and Hex 6.3 tool. In this present, Accelrys Discovery Studio client 2.5 (Biovia, USA, 2009) was used to carry out docking study.

One of the foremost requirements for the cheminformatics studies is the availability of three dimensional structures of the target as well as binding compounds. In present study the three dimensional structure of Pgp 2 or Pgp A from the database was not obtained and it had to be modelled from the protein sequence of Pgp downloaded from the PDB.P-glycoprotein A/Pgp2 was modeled through I- TASSER and five models were received. Model-I was selected for further study based on the highest C-score (2 for model-I) and TM score (0.99±0.04). C-score is a confidence score to evaluate the quality of predicted models by I-TASSER. It was based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations. The range of C-score is -5 to 2, where a higher value of C-score signifies a model with a high confidence and vice-versa. TM-score and RMSD are known standards for measuring structural similarity between two structures which are usually used to measure the accuracy of structure modeling when the native structure is known. In case where the native structure is not known, it becomes necessary to predict the quality of the modelling prediction. C-score is highly correlated with TM-score and RMSD. In the table, the quality prediction (TM-score and RMSD) for the first model is presented, because the correlation between C-score and TM-score was weak for other models. TM-score is a recently proposed scale for measuring the structural similarity between two structures. The purpose of proposing TM-score is to solve the problem of RMSD which is sensitive to the local error. A TM-score more than 0.5 indicates a model of correct topology and a TM-score less than 0.17 means a random similarity [41]. In this study the model-I has a C score and TM score of 2 and 0.91 respectively. Hence, with high C and TM scores, model-I was selected.

The modelled 3D structure of P glycoprotein of *H. contortus* was validated by RAMPAGE, Ramchandran Plot and based on less number of residues in outlier region model 1 was selected for molecular docking with legends [42]. Molecular docking is a tool in structural molecular biology and computer-assisted drug design. The goal of ligand-protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. In present study the molecular docking was performed with 36 plant compounds/chemicals (Ligands) having Pgp modulation and/or inhibitory properties as listed [26]. P-glycoprotein of *H. contortus* by using Accelrys Discovery Studio client 2.5 (Biovia, USA). Based on highest dock score (>90) Verapamil, Loperamide, Quercetin, Kaempferol, Phloretin and Curcumin were identified

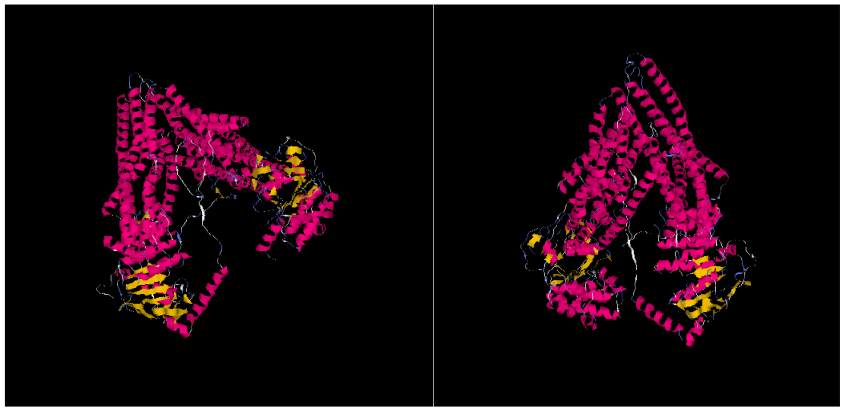
Previously many scientists used cheminformatics approaches like molecular docking to identify compounds such as curcumin for use in obesity [44]; Curcumin, Baicailin and Dronabinol in Parkinson‟s disease [20]; Curcumin on MRP1 in retinoblastoma cells [45]; Curcumin as natural JAK inhibitors [22]; Stigmasterol β – D glucoside isolated from the methanol

extract of rhizomes of *Hedychium spicatum* as anthelmintic against adult Indian earthworm [23]; interaction of ML and other anthelmintic drugs to Cel-Pgp-1 [40] and Thymoquinone, Curcumin in inhibiting the antioxidant enzymes of *F. gigantica* and Cathepsin L to prevent virulence [24].

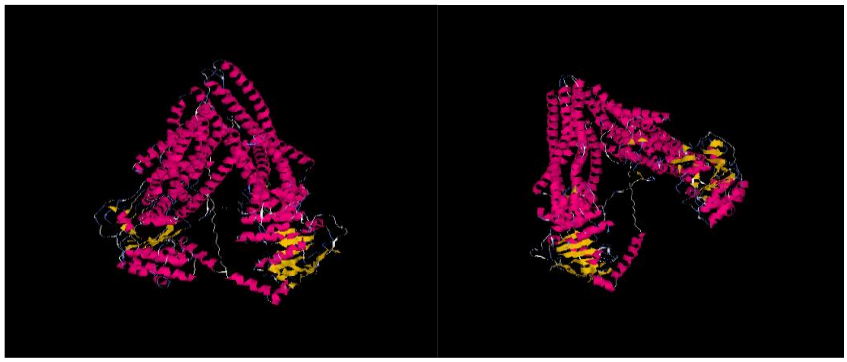
Perusal of literature indicated that no or limited researchers have so far used cheminformatics approaches to find out interaction of Pgp modulators/chemosentizers with Pgps of *H. contortus* and application of this *in silico* technology for identification of compounds useful for reversal of anthelmintic resistance. In this study, all the 36 compounds already listed have been individually docked with modeled Pgp and finally four plant compounds and two chemicals had been shortlisted.

Conclusion

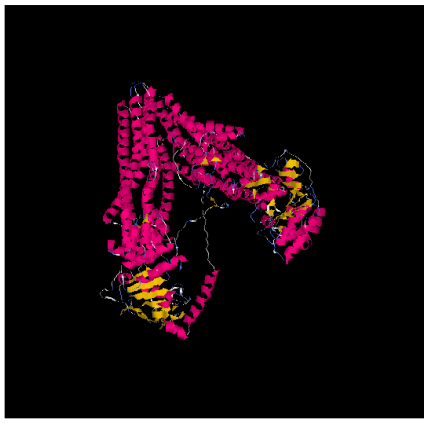
Cheminformatics studies were carried out to find out the P glycoprotein modulators/inhibitors from plant compounds/ chemicals sources. Three dimensional structure of Pgp is not known and it was designed using the sequences from NCBI. Five models of Pgp were received. Based on I-TASSER modeling and RAMPAGE score Pgp model 1 was selected and molecular docking was done to interact with 36 plant compounds/chemicals using Accelrys Discovery Studio client 2.5. Based on the best molecular docking score (more than 90 interactions)**,** Curcumin, Quercetin, Kaempferol, Phloretin, Verapamil and Loperamide with molecular docking scores of 98.324, 96.073, 95.47, 94.895, 93.453 and 92.807 were selected.



Model-I **Model-II**

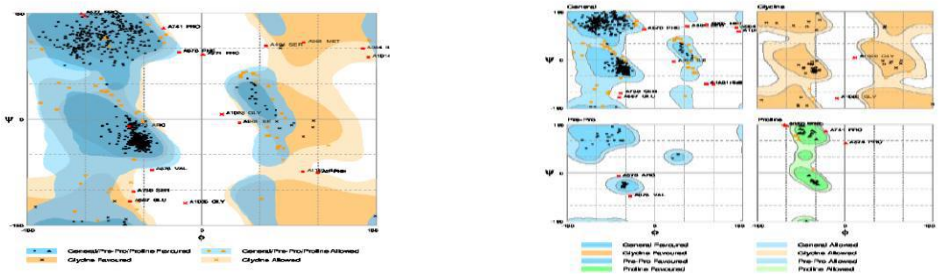


Model-III **Model-ModeIV**

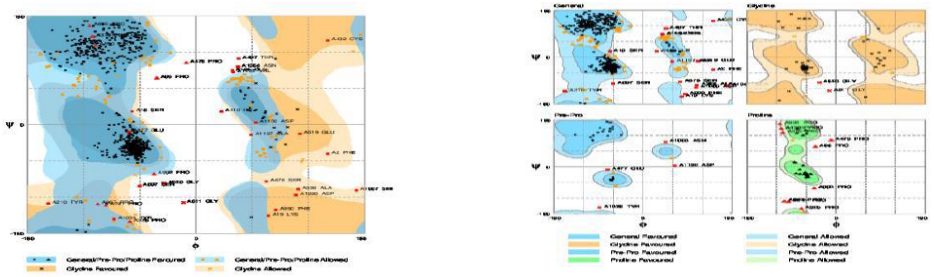


Model -V

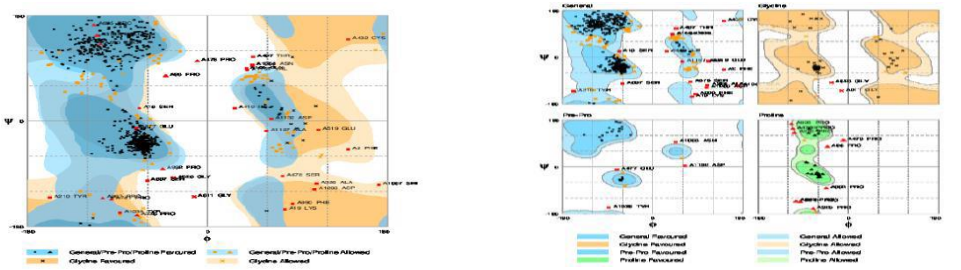
Plate 1.0: Five 3D Models of P- Glycoprotein of *H. Contortus* by I- TASSER



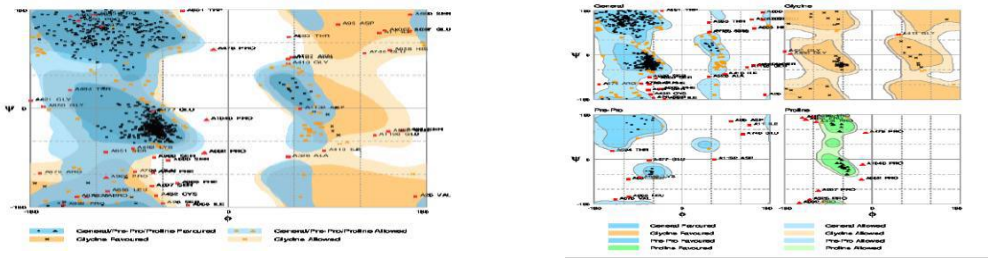
Model-I



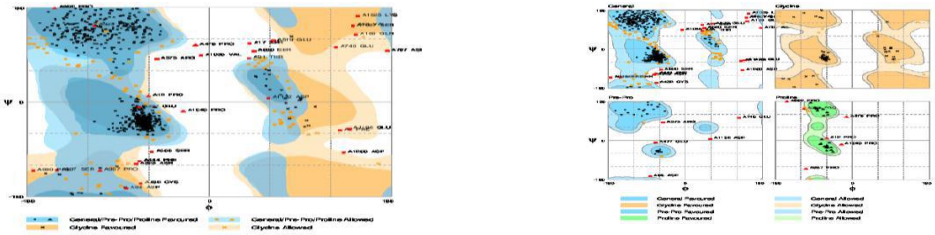
Model –II



Model –III

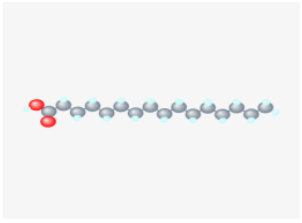


Model –IV

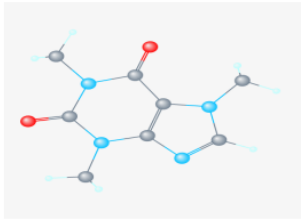


Model-V

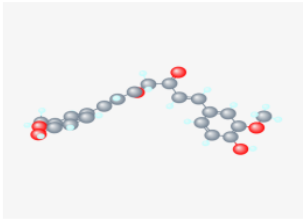
Plate 2.0: Ray Diagrams for Validation of P gp Models



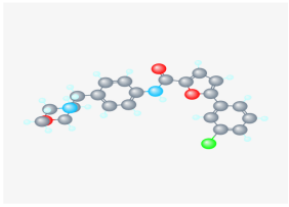
Palmitic acid (CID 985)



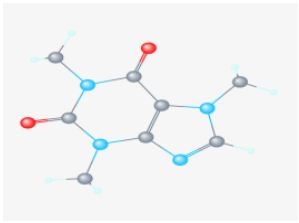
Caffeine (CID 2519)



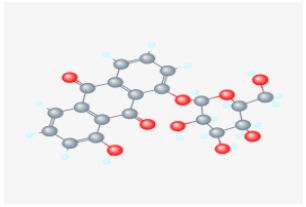
Curcumin (CID2889)



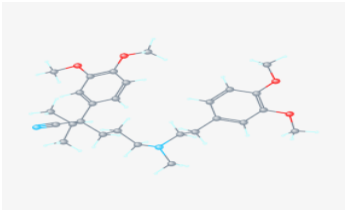
Phloretin
(CID 4788)



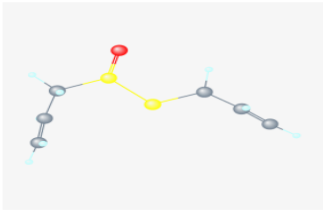
Caffeine citrate
(CID 6241)



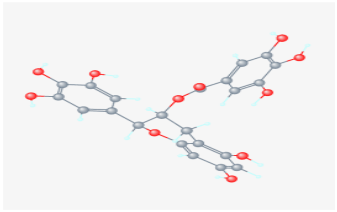
9,10-anthraquinone
(CID: 3042554



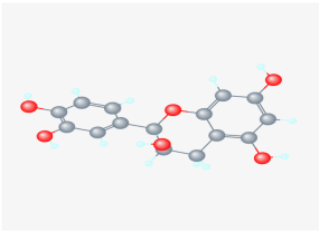
Verapamil
(CID 62969)



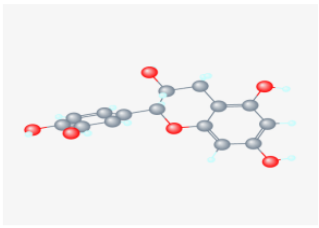
Allicin
(CID65036)



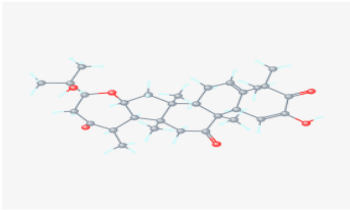
Epigallocatechingallate
(CID 65064)



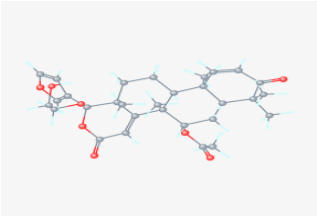
Epicatechin
(CID 72276)



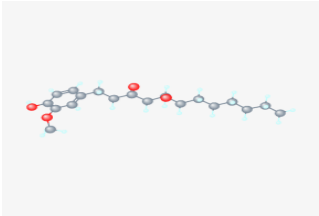
(-)-Catechin
(CID 73160)



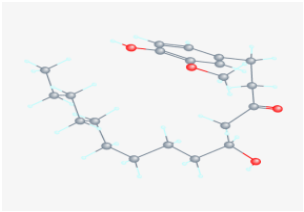
Cucurbitacins
(CID 119287)



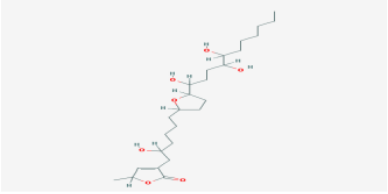
Mahmoodinm
(CID 126566)



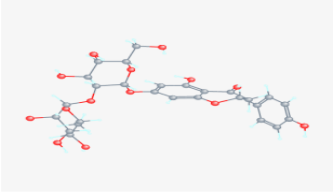
8-gingerol
(CID 168114)



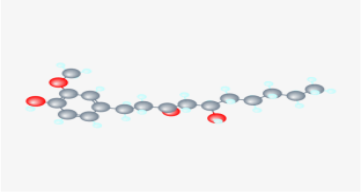
10-Gingerol
(CID 168115)



Acetogenins
(CID 393472)

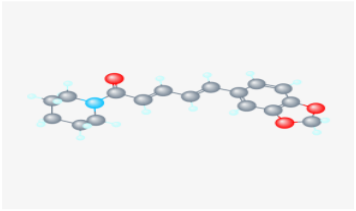


Naringin
(CID 442428)

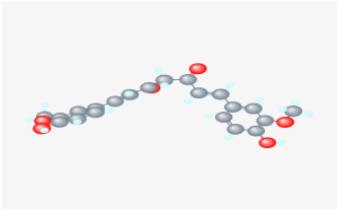


6-Gingerol
(CID 442793)

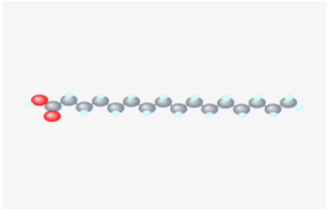
Plate 3a: Three dimensional structure of PGP inhibitors/modulators as ligands



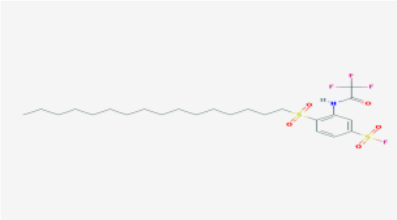
Piperine
(CID 638024)



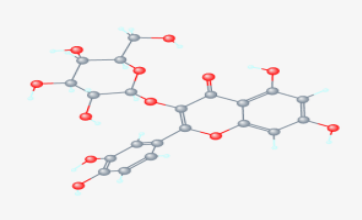
Curcumin
(CID 969516)



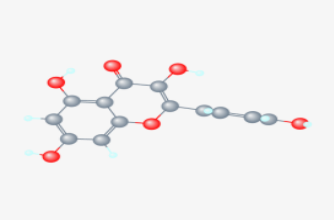
Palmitic Acid
(CID 2735111)



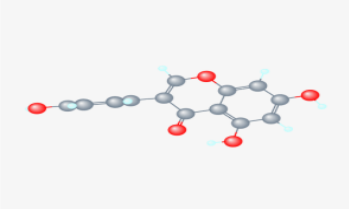
NSC122336
(CID 275467)



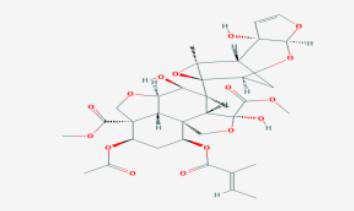
Quercetin
(CID 5280343)



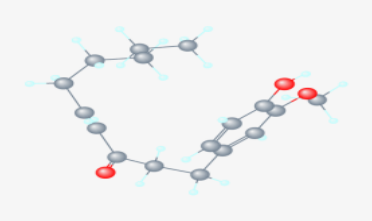
Kaempferol
(CID 5280863)



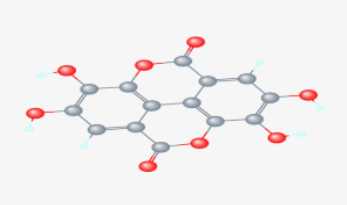
Genistein
(CID 5280961)



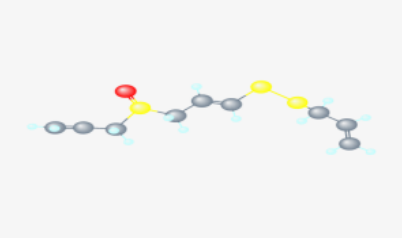
Azadirachtin
(CID 5281303)



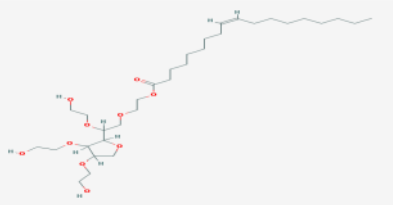
Shogaol
(CID 5281794)



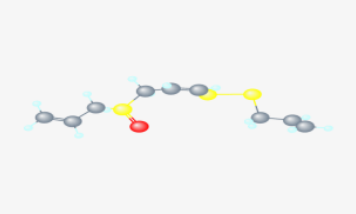
Ellagic Acid
(CID 5281855)



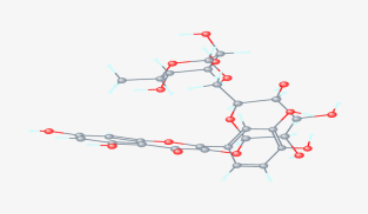
Ajoene
(CID 5386591)



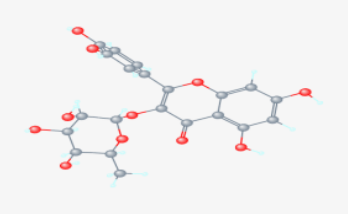
Polysorbates
(CID 6364656)



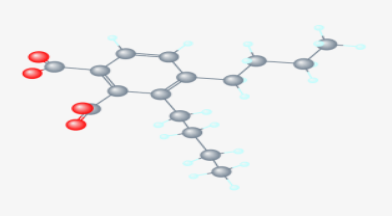
(Z)-Ajoene
(CID 9881148)

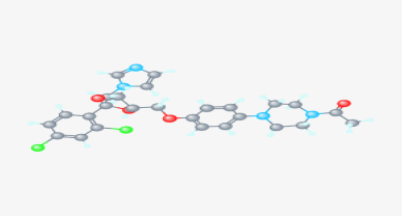


Rutintrihydrate
(CID16218542)



Quercitrin hydrate
(CID 6219923)





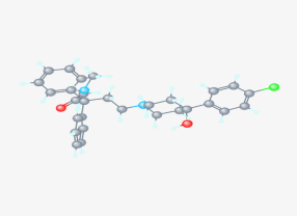
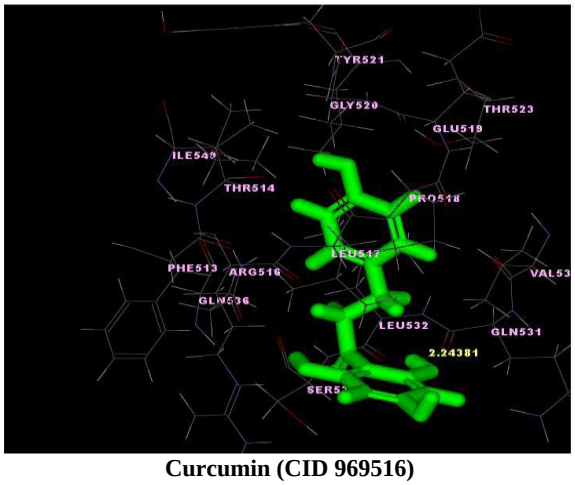
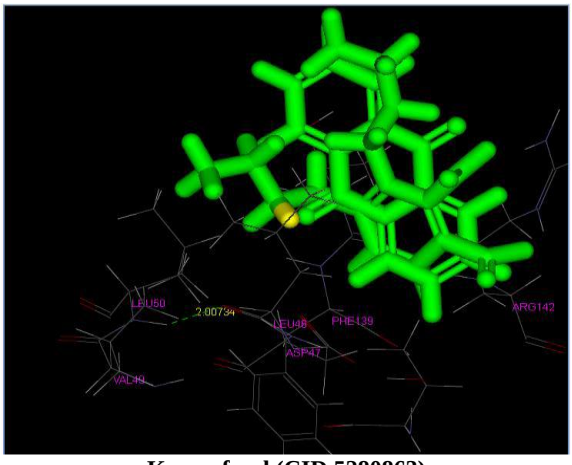


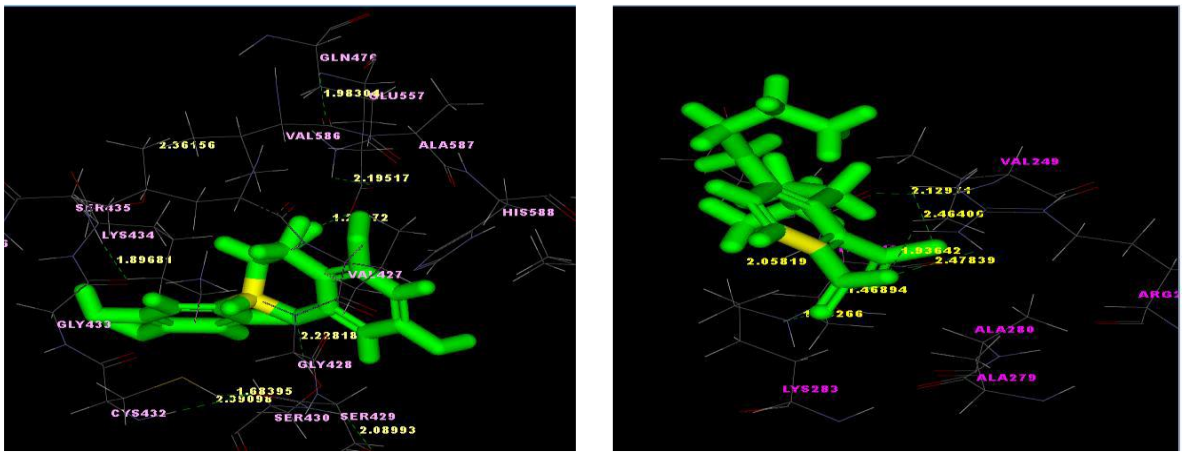
Plate 3b: Three dimensional structure of PGP inhibitors/modulators as ligands



Curcumin (CID 969516)



Kaempferol (CID 5280863)



Phloretin (CID 4788) **Quercetin (CID 6219923)**

Verapamil (CID 62969) **Loperamide (CID71420)**

Plate 4.0: Molecular docking (intraction between ligands and Pgp) of the selected compounds

Table 1: I-TASSER modelling scores of Pgp of *H. contortus*

Model	C-score	Exp. TM-score	EXP. RMS	No. of decays	Cluster density
Model-1	2.00	0.99±0.04	4.6±3.0	2307	0.8576
Model-2	-0.05	-	-	202	0.0901
Model-3	-1.16	-	-	133	0.0299
Model-4	-1.45	-	-	84	0.0224
Model-5	-2.16	-	-	61	0.0110

Table 2: 6. Evaluation and analysis of residues by RAMPAGE

Pgp Model	Residues in favored region	Residues in allowed region	Residues in outer region
Model-1	1192 (93.6%)	63 (4.9%)	18 (1.4%)
Model-2	1159 (91.0%)	81 (6.4%)	33 (2.6%)
Model-3	1157 (90.7%	86 (6.8%)	30 (2.4%)
Model-4	1191 (93.6%)	59 (4.6%)	23 (1.8%)
Model-5	1123 (88.07%)	103 (8.1%)	47 (3.7%)

Table 3: Compounds (both chemicals as well as compounds of plant origin) known to be Pgp modulator/inhibitors in cancer drug resistance, anti-diabetics resistance and antibiotics resistance

S.No	Pub Chem ID	Active compound	Molecular formula	Molecular weight/gmol
1	CID 985	Palmitic acid	C16H32O2	256.42
2	CID 2519	Caffeine	C8H10N4O2	194.19
3	CID2889	Curcumin	C21H20O6	368.37
4	CID 4788	Phloretin	C15H14O5	274.26
5	CID 6241	caffeine citrate	C14H18N4O9	386.31
6	CID 3042554	9,10-anthraquinone	C14H8O2	208.21
7	CID 62969	Verapamil	C27H39ClN2O4	491.06
8	CID65036	Allicin	C6H10OS2	162.27

9	CID 65064	Epigallocatechingallate	C22H18O11	458.37
10	CID 72276	Epicatechin	C15H14O6	290.26
11	CID 73160	(-)-Catechin	C15H14O6	290.26
12	CID 119287	Cucurbitacins	C30H42O6	498.65
13	CID 126566	Mahmoodin	C30H38O8	526.6
14	CID 168114	8-gingerol	C19H30O4	322.43
15	CID 168115	10-Gingerol	C21H34O4	350.49
16	CID 393472	Acetogenins	C26H46O7	470.63
17	CID 442428	Naringin	C27H32O14	580.53
18	CID 442793	6-Gingerol	C17H26O4	294.38
19	CID 638024	Piperine	C17H19NO3	285.33
20	CID 969516	Curcumin	C21H20O6	368.37
21	CID 2735111	Palmitic Acid	C16H31NaO2	278.40
22	CID 275467	NSC122336	C24H37F4NO5S2	559.67
23	CID 5280343	Quercetin	C15H10O7	302.23
24	CID 5280863	Kaempferol	C15H10O6	286.236
25	CID 5280961	Genistein	C15H10O5	270.23
26	CID 5281303	Azadirachtin	C35H44O16	720.71
27	CID 5281794	Shogaol	C17H24O3	276.37
28	CID 5281855	Ellagic Acid	C14H6O8	302.19
29	CID 5386591	Ajoene	C9H14OS3	234.40
30	CID 6364656	Polysorbates	C32H60O10	604.81
31	CID 9881148	(Z)-Ajoene	C9H14OS3	234.40
32	CID16218542	Rutintrihydrate	C27H36O19	664.56
33	CID 6219923	Quercitrin hydrate	C21H22O12	466.39
34	CID 2227427	Dibutylphthalate	C16H20O4-2	276.32
35	CID 9775382	Allicin& Ketoconazole	C32H38Cl2N4O5S2	693.70
36	CID 71420	Loperamide	C29H34Cl2N2O2	513.50

Table 4: Molecular docking (intrecrion between ligands and Pgp of H. contorus) scores of compounds (ligands)

Site	PubChem ID	Ligands	Dock Score	Ligands intrection energy	PMF
21	4788	Phloretin	98.324	207.07	75.83
21	969516	Curcumin	96.073	98.324	12.93
31	22227427	Quercetin	95.47	19.483	37.97
34	5280863	Kaempferol	94.895	60.005	56.38
21	71420	Loperamide	93.453	56.338	18.09
21	62969	Verapamil	92.807	543.325	70.25
34	CID 5281794	Shogaol	84.031	352.121	68.09
17	CID 168115	10-Gingerol	77.251	208.102	78.781

Acknowledgement
The Dean, Madras Veterinary College, Tamil Nadu Veterinary and animal Science University, Chennai, India, 600 007 for providing facilities to conduct research work and we also acknowledge Dr. Kumarasamy, P., and Dr. Sureshkannan, S. to help and provded facilities for molecular docking study.

References

1. McKellar QA, Jackson F. Veterinary Anthelmintics: old and new. Trends in Parasitol. 2004; 20:456–461.
2. Wolstenholme AJ, Fairweather I, Prichard RK, von Samson Himmelstjerna G, Sangster NC.Drug resistance in veterinary helminths. Trends Parasitol. 2004; 20:469-476.
3. Prichard RK, von Samson-Himmelstjerna G, Blackhall WJ, Geary TG. Foreword: towards markers for anthelmintic resistance in helminths of importance in animal and human health. Parasitol. 2007; 134:1073-1076.
4. Kohler P. The biochemical basis of anthelmintic action and resistance. Int. J. Parasitol. 2001; 31:336-345.
5. Lakshmipriya. Assessment and in vitro reversal of anthelmintic resistance in haemonchus contortus. M.V.Sc. Thesis, Submitted to Tamil Nadu Veterinary and Animal Sciences University, Chennai, India, 2012.
6. Om Prakash, Gomathinayagam S, Harikrishnan TJ, Raman M, Pandiyan V, Saho S. Haemonchus contortusload and anthelmintics resistance in Sheep and Goats in Chennai, Tamil Nadu, India. Int. J Chem. Studies. 2018; 6(5):2712-2716.
7. Van Wyk JA, Gerber HM. A field strain of Haemonchus contortus showing slightresistance to rafoxanide. Onderstepoort J. Vet. Res. 1980; 47:137-142.
8. Om Prakash, Gomathinayagam S, Harikrishnan TJ, Raman M, Pandiyan V, Saho S. Potentiation of efficacy of ivermectin in Haemonchus contortus by P-glycoprotein modulator and/or inhibitors. J. Ent. Zoolog. Studies (*In Press*), 2018a.
9. Kerboeuf D, Blackhall W, Kaminsky R, von Samson-Himmelstjerna G. p-glycoprotein in helminths: function and perspectives for anthelmintic treatment and reversal of resistance. Int. J Antimic. Agents. 2003; 22:332-346.
10. Sangster NC, Dobson RJ. Anthelmintic resistance. In: Lee, D.L. (Ed.), The Biology of Nematodes. Taylor & Francis, London, 2002, 531-567.
11. Sangster NC. P-glycoproteins in nematodes. Parasitol. Today. 1994; 10(8):319-322.
12. Xu M, Molento M, Blackhall W, Ribeiro P, Beech R, Prichard RK. Ivermectin resistance in nematodes may be caused by alteration of p-glycoprotein homolog. Mol. Biochem. Parasitol. 1998; 91:327-335.

13. Beugnet F, Gauthey M, Kerboeuf D. Partial in vitro reversal of benzimidazole resistance by the free-living stages of Haemonchus contortus with verapamil. Vet. Rec. 1997; 141:575-576.
14. Om Prakash. Evaluation of the efficacy of rational compounds in the reversal of anthelmintic resistance in *Haemonchus contortus.* Ph.D. Thesis, Submitted to Tamil Nadu Veterinary and Animal Sciences University, Chennai, India, 2017.
15. Lespine A, Menez C, Bourguinat V, Prichard RK. P-glycoproteins and othermultidrug resistance transporters in the pharmacology of anthelmintics: prospects forreversing transport-dependent anthelmintic resistance. Int. J Parasitol. Drugs Drug Resist. 2012; 2:58-75.
16. Sarai RS, Kopp SR, Coleman GT, Kotze AC. Drug-efflux and target-site geneexpression patterns in Haemonchus contortus larvae able to survive increasing concentrations of levamisole in vitro. Int. J Parasitol. Drugs Drug Resist. 2014; 4:77-84.
17. Bartley DJ, Mc Allister H, Bartley Y, Dupuy J, Menez C, Alvinerie M, Jackson F, Lespine A. P-glycoprotein interfering agents potentiate ivermectin susceptibility in ivermectin sensitive and resistant isolates of Teladorsagia circumcinata and Haemonchus contortus. Parasitol. 2009a; 136:1081-1088.
18. Heckler RP, Almeida GD, Santos LB, Borges DGL, Neves JPL, Onizuka MKV, Borges FA. P-gp modulating drugs greatly potentiate the in vitro effect ofivermectin against resistant larvae of *Haemonchus placei*, Vet. Parasitol. 2014; 205:638-645.
19. Raza, A, Kopp SR, Jabbar A, Kotze AC. Effects of third generation P-glycoproteininhibitors on the sensitivity of drug-resistant and -susceptible isolates of Haemonchus contortus to anthelmintics in vitro. Vet. Parasitol. 2015; 211:80-88.
20. Singh R, Chaturvedi N, Singh VK. In-silico study of herbal compounds (baicalin, curcumin and dronabinol) as novel mao inhibitors for parkinson‟s disease treatment. Int. J. Life Sci. and Phar. Res. 2012; 2(3):2250-0480.
21. Venkata M, Sripathy R, Anjana D, Somashekara N, Krishnaraju A, Krishanu S, Murali M, *et al*. In Silico, In Vitroand In Vivo assessment of safety and anti-inflammatory activity of curcumin, American J. Infec. Dis. 2012; 8(1):26-33.
22. Nag A, Mukherjee A. In silico docking analysis of Janus kinase enzymes and phytochemicals, J. Chem. Pharm. Res. 2014; 6(11):493-499.
23. Sravani, T, Paarakh PM, Shruthi SD. In silico and in vitro anthelmintic activity of stigmasterol β -d-glucoside isolated from rhizomes of Hedychium spicatum Buch. ham. World J. Pharm. Pharm. Sci. 2014; 3:664-672
24. Ullah R, Rehman A, Zafeer MF, Rehman L, Khan YA, Khan MAH, Khan SN, *et al*. Anthelmintic potential of thymoquinone and curcumin on *Fasciola gigantica*. Plos One. 2017; 12(2): e0171267.
25. Ramachandran GNC, Ramakrishnan, Sasisekharan V. Stereochemistry of polypeptide chain configurations. J. Mol. Bio. 1963; 7**:**95-99.
26. Bansal,T, Jaggi M, Khar RK, Talegaonkar S. Emerging significance of flavonoids as P-Glycoprotein inhibitors in cancer chemotherapy. J. Pharm. Pharmaceut. Sci. 2009; 12(1):46-78.
27. Zhang Y. I-TASSER server for protein 3D structure prediction. BMC Bioinformatics. 2008; 23:9:40.
28. Smith JM, Prichard RK. Localization of p-glycoprotein mRNA in the tissues of *Haemonchus contortus* adult worms and its relative abundance in drug selected and susceptible strains.*J. Parasitol*. 2002; 88:612-620.
29. Blackhall WJ, Prichard RK, Beech RN. P-glycoprotein selection in strains of *Haemonchus contortus* resistant to benzimidazoles. *Vet. Parasitol*. 2008; 152:101–107.
30. Lloberas M, Alvarez L, Entrocasso C, Virkel G, Ballent M, Mate L, Lanusse C, *et al*. Comparative tissue pharmacokinetics and efficacy of moxidectin, abamectin and ivermectin in lambs infected with resistant nematodes: Impact of drug treatments on parasite P-glycoprotein expression. Int. J. Parasitol: Drugs and Drug Resis. 2013; 3:20-27.
31. Jana I, Janssen I, Krucken J, Demeler J, von Samson-Himmelstjerna G. Transgenically expressed *Parascaris* P-glycoprotein-11 can modulateivermectin susceptibility in *Caenorhabditis elegans.* Int. J Parasitol: Drugs and Drug Resis**.** 2015; 5:44–47.
32. Naito M. Therapeutic approach to drug resistant tumors. Ther. Drug. Monit. 1998; 20(5)**:**577-580**.**
33. Lespine A, Menez C, Bourguinat C, Prichard RK. P-glycoproteins and other multidrug resistance transporters in the pharmacology of anthelmintics: prospects forreversing transport-dependent anthelmintic resistance. Int. J. Parasitol. Drugs Drug Resist. 2012; 2**:**58-75.
34. Garretson PD. Role of p-glycoprotein in *Haemonchus contortus* anthelmintic resistance. Thesis submitted to Colorado State University, 2007.
35. Bygarski EE, Prichard RK, Ardelli BF. Resistance to the macrocyclic lactone moxidectin is mediated in partby membrane transporter P- glycoproteins: Implications for controlof drug resistant parasitic nematodes. Int. J. Parasitol: Drugs and Drug Resis. 2014; 4:143–151.
36. Shetty P, Palve A, Pimpliskar M, Jadhavand RN, Shinde P. In-silicoDocking analysis of sterculia lychnophora compounds against proteins causing alzheimer‟s disease. Int. Eng. Sci. Inno. Tech. 2014; 3(4):158-164.
37. Kumar DR, Seetha Lakshmi P, Saravanic N, Marimuthud S. In silico molecular docking studies on porcine pancreatic phospholipase A2 against plant extracts of phenolic inhibitors. Int. J Res. Biomedi. Biotech. 2012; 2(3):8-16.
38. Venkata M, Sripathy R, Anjana D, Somashekara N, Krishnaraju A, Krishanu S, Murali M, *et al*. In Silico, In Vitroand *In Vivo* assessment of safety and anti-inflammatory activity of curcumin, American J Infec. Dis. 2012; 8(1):26-33.
39. Yang C, Zhang T, Li Z, Xu L, Liu F, Ruan J, Liu K *et al*. Toxicol. app. Pharmacol. 2013; 273:561-568.
40. David MA, Orlowski S, Prichard RK, Hashem S, Andre F, Lespine A. In silico analysis of the binding of anthelmintics to Caenorhabditis elegans P-glycoprotein 1. Int. J. Parasitol: Drugs and Drug Res. 2016; 6:299-313.
41. Zhang Y. I-TASSER server for protein 3D structure prediction. BMC Bioinformatics. 2008; 23(9):40.
42. Lovell SC, Davis IW, Arendall WB, de Bakker PIW, Word JM, Prisant MG, Richardson JS *et al*. Structure Validation by Cα Geometry: φ,ψ and C^{β} Deviation. Proteins: Structure, Function, and Genetics. 2003; 50:437-450.
43. Molnar J, Engi H, Hohmann J, Molnar P, Deli J, Wesolowska O, Michalak K, *et al*. Reversal of multidrug resistance by natural substances from plants. Curr Top Med Chem. 2010; 10:1757-1768.

44. Archana P, Sathishkumar N, Bharathi N. In silico docking analysis of curcumin-An inhibitor for obesity. Int. J Pharma. Bio. Sci. 2010; 1(4):224-235.
45. Sreenivasan S, Ravichandran S, Vetrivel U, Krishnakumar S. *In vitro* and In silico studies on inhibitory effects of curcumin on multi drug resistance associated protein (MRP1) in retinoblastoma cells. Bio information. 2012; 8(1):013-019.