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Impact of Molecular Hydrophobic Field on Passive Diffusion, P–Glycoprotein Active Efflux, and P–Glycoprotein Modulation of Steroids

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Abstract

Motivation. Passive diffusion is the most common process that drugs undergo when penetrating the bilayer membrane. However, the extra involvement of P–glycoprotein (P–gp) always results in the active efflux of drugs out of the cells. Steroids are a class of compounds that possess various pharmacological functions that are essential to human health, and some steroids can be either P–gp substrates and/or inhibitors. To determine the main structural features of steroid–based drugs affecting their permeation effects by simply passive diffusion, or by at the mean time P–gp–mediated active efflux, as well as the permeation effect when steroids are P–gp inhibitors, three different datasets were studied by QSAR methods respectively. And the contributions and distributions of molecular hydrophobic field, an important hydrophobicity descriptor, to the three different permeation processes were analyzed and compared with those of ClogP, another hydrophobicity descriptor.

Method. Comparative molecular similarity index analysis (CoMSIA) was applied to the three datasets.

Results. All developed models exhibited statistically satisfactory results, with their predictability validated by test sets independent of training ones.

Conclusions. Our findings are that the contributions of hydrophobic field and ClogP to three different processes are totally distinct, and the hydrophobic field in 3D–space distribution correlates better with the potency of a steroid molecule to passively diffuse or be actively transported by P–gp, or modulate P–gp–mediated drug efflux. The comparison of different hydrophobic field contour plots in different models was also conducted, which is useful for further steroid–based drug design.

Keywords. Steroid; P–glycoprotein; passive diffusion; active efflux; hydrophobic field; comparative molecular similarity index analysis (CoMSIA).

Abbreviations and notations

CoMSIA, comparative molecular similarity index analysis	PLS, partial least squares
F, F–test value	QSAR, quantitative structure–activity relationships
OPN, the optimal number of components	*SEE*, standard error of estimation
P–gp, P–glycoprotein	*SEP*, standard error of prediction

Dedicated on the occasion of the 65th birthday to Danail Bonchev.

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1 INTRODUCTION

Steroid hormones are a class of compounds possessing a great variety of pharmacological functions that are essential to human health [1]. They are so important that probably every organ in the mammalian body is under their influence, whether in growing, developing, aging or dying stages. Interestingly, though steroids play so crucial roles to human health, their interaction mechanisms are widely divergent and, mostly, unclear due to different sites. For instance, estrogen therapy is efficient for preventing people from the risk of osteoporosis and cardiovascular diseases, but unopposed estrogen is always associated with an increased risk of endometrial cancer, though continuing debate on its role existed [2–4]. On the other hand, proper administration of progesterone, another steroid molecule, might reduce or eliminate the risk of endometrial cancer development [5]. This indicates that different steroids with similar structures might possess distinct pharmacological functions only because of subtle structural difference or different interaction sites. A clear structure–activity relationship (SAR) analysis of these compounds is, consequently, necessary to obtain optimal steroid drug candidates with favorable physicochemical properties.

Thus it can be imaged how important the absorption and disposition of steroids in various organs are due to their significant roles. However, as an endogenous substance, most of the intracellular steroids are self–generated, secreted by various glands and tissues. To exhibit proposed physiological functions, they should first transfer across the cell membrane to be further effectively delivered to the sites of action with suitable concentrations. Without the involvement of certain carriers or transporters, usually this delivery occurs by passive diffusion, in which no direct energy supply is required. In this process, depending on the molecular size and electrostatic condition a steroid compound moves across a lipid bilayer directly down the concentration gradient. Passive diffusion is also a process that all steroids, whether endogenous or exogenous, will undergo when penetrating the cell membrane.

Whereas, the structure of human including especially several important organs like the brain is very delicate, and evolution built very efficient ways to protect it. Reports have demonstrated that on most conditions, only a small quantity of steroids penetrate across the biomembranes simply by way of passive diffusion [6]. Without the help of certain carriers, most of the steroids that have penetrated into the cell by passive diffusion are expelled out of the cell spontaneously with involvement of diverse active transporters, in which P–glycoprotein (P–gp) is probably the most important one. P–gp is a 170–kDa transmembrane protein highly expressed at the interface of many important tissues like gut, kidneys, liver, as well as capillary endothelial cells of brain, testis and placenta [7]. It belongs to the ABC (ATP–binding cassette) transporter family and serves to pump exogenous substances out of the cells. In this active efflux process, energy originating from adenosinetriphosphate (ATP) hydrolysis is directly consumed. Since the function of P–gp always results in lack of intracellular levels of the drug necessary for effective therapy, the overexpression

of P–gp in certain malignant cells is always associated with the multidrug resistance (MDR) phenotype [8].

Therefore, the involvement, as well as the modulation of P–gp in transport always frustrates the absorption of steroids. The final disposition of steroid is actually a result of balance of all possible transport mechanisms, including passive diffusion, P–gp–mediated active efflux, and modulation of P–gp etc. And it is the same for steroid molecules as drug candidates. Their absorption effects also depend on the specific transport mechanisms. Up to now, many steroids, whatever the natural or synthetic compounds like aldosterone and cortisol, have been proven to be transported (effluxed) by P–gp as substrates [9,10]. Other studies, however, demonstrate that some, but not all, steroid compounds can perform as a specific kind of P–gp inhibitors lack of characteristics of nitrogen atoms [11,12]. Meanwhile, evidence also exists that some steroids such as dexamethasone can either act as P–gp substrates and inhibitors [10,12,13]. Therefore, the interaction mechanism between steroids and P–gp always attracts many interests, which yet still remains unclear.

It is well known that the lipophilicity property of a molecule and its hydrophobic interaction with surrounding environment always plays crucial roles when the molecule penetrates across the biomembranes to get to its target organism. However, up to now the impacts of these molecular properties of steroids on entering a cell with or without P–gp are still not intensively studied. The aim of the present work is to investigate and compare the individual impacts of hydrophobic interactions of steroids on their permeation effects in different permeation processes, including passive diffusion, active transport by P–gp and their inhibition effects on P–gp by computational approaches. In present work, three groups of steroid derivatives collected from literatures were used as datasets. Comparative Molecular Similarity Index Analysis (CoMSIA) was employed for three–dimensional quantitative structure–activity relationship (3D–QSAR) analysis on the datasets, resulting in three models with proper predictability validated by test sets independent of the training sets.

2 MATERIALS AND METHODS

2.1 Dataset Building

For P–gp–steroid active transport study, 13 steroids were used as dataset (Appendix 1, Table A1, compounds **1**–**13**). Ten of the molecules were used as training set to derive a model, and the rest 3 ones, *i.e.*, medroxyprogesterone acetate, aldosterone, dehydrotestosterone were used as test set to validate the predictivity of the model.

For P–gp–steroid inhibition investigation, 20 steroids (Appendix 1, Table A1, all compounds) were used. Eighteen molecules in the dataset were used for training and the rest 2 ones including 6,16α–methylpregnenolone and corticosterone were used for testing.

Both of above datasets were collected from the paper of Barnes *et al*. [9], where the PDA (the percent decrease in steroid accumulation) and VA (the vinblastine accumulation in presence of various steroids) in MDR SW620 Ad300 cells were used as biological activity for steroid active transport by P–gp and steroid inhibition on P–gp function studies, respectively.

For steroid passive diffusion research, 18 steroids were used (Appendix 1, Table A2). In the original paper referenced [14], the steroid passive diffusion data were obtained from the study of permeability of steroids penetrating across Caco–2 cell. The authors have demonstrated that the permeability coefficients of the steroids in their work were only results of passive diffusion and no involvement of P–gp–mediated transport was observed. These molecules were divided into two sets, 15 compounds for training and the rest 3 ones, *i.e*., norethisterone, Org32540 and Org4325 for testing. The logarithm form of apparent permeability coefficient (P_{app}) of the molecules [14], namely $\log_{10}P_{app}$, in HTB37 (human colon adenocarcinoma) Caco–2 cells was used as biological activity.

As can be seen from the structure of steroids (Appendix 1), all of the molecules in this study possess a same skeleton composed of three hexagonal (rings A, B and C) and one pentagonal rings (ring D) and are structurally similar, therefore we think it is helpful to analyze the structure–activity relationship of these compounds though they are collected from two papers.

2.2 Molecular Modeling

3D–structure generating and molecular modeling were performed using SYBYL 6.9 installed on a Dell precision 650 workstation running LINUX RedHat 8.0 operating system. As no X–ray crystal structures of compounds were available, all 2D–structures were obtained from a commercial available MDL–ISIS database. Conformational search and energy minimization were carried on by Powell conjugate gradient algorithm using a Tripos force field [15], with an energy gradient limit set to 0.05 kcal/mol·Å to get the most stable conformation. The minimization was terminated when the energy gradient convergence criterion of 0.001 kcal/mol was reached. Partial atomic charges were assigned to each atom with Gasteiger–Huckel Charge method [16].

An appropriate structural alignment of the molecules in a dataset is a critical step in 3D–QSAR modeling. Generally, one low energy conformation of the molecule in a dataset who has relatively high biological activity and fairly fixed conformation was chosen as a reference. In this way, cortisol, pregnanedione and nandrolone with low energy conformation by using SYBYL random search option are taken as the template molecules for steroid passive diffusion, P–gp steroid substrate, P–gp steroid inhibitor datasets, respectively. Then atom fit molecular alignment methods were employed in the present study, which involves atom based fitting (RMS fitting) of the ligands. After all other molecules in a dataset being fitted to their template molecule, a minimization was carried on by using maximum 30 iterations so that there was no big change in conformation.

2.2.1 CoMSIA

CoMSIA was performed to evaluate the gradual changes in observed biologic properties of a molecule by employing the standard option of SYBYL. It assumes that a suitable sampling of the steric (van der Waals interactions), electrostatic (Coulombic interactions), hydrogen–bond interactions (including H–bond donor and acceptor) and hydrophobic fields generated around a set of aligned molecules with a probe atom might provide all the important features for understanding their biological activities, and that the changes in binding affinities of ligands are related to changes in molecular properties. All field descriptors were calculated within a lattice box with a grid spacing of 2 Å using an sp^3–carbon (+ 1 charge) as probe atom. CoMSIA similarity indices (A_F) for a molecule *j* with atom *i* at a grid point *q* are calculated by equation 1 as follows:

$$A_{F,k}^{q}(j) = \sum \omega_{probe,k}\omega_{ik}e^{-\alpha r_{iq}^2} \tag{1}$$

where $\omega_{probe,k}$ is the probe atom with radius 1 Å, charge +1, hydrophobicity +1, H–bond donating +1 and hydrogen bond accepting +1, ω_{ik} is the actual value of the physicochemical property *k* of atom *i*, r_{iq} is the mutual distance between the probe atom at grid point *q* and item *i* of the test molecule [17]. In present study, only three physicochemical properties *k* including hydrophobic, H–bond donor and H–bond acceptor were involved.

2.2.2 PLS

Partial least square (PLS) [18] was used to correlate the field descriptors with biologic activities. The optimum number of components (OPN) used to derive the non–validated model was defined as the number of components leading to the highest cross–validated Q^2 and the lowest standard error of prediction (SEP). The predictive value of the models was evaluated first by leave–*n*–out technique. The cross–validated coefficient, Q^2, was calculated using Eq. (2).

$$Q^2 = 1 - \frac{\sum_{Y}(Y_{predicted} - Y_{observed})^2}{\sum_{Y}(Y_{observed} - Y_{mean})^2} \tag{2}$$

where $Y_{predicted}$, $Y_{observed}$ and Y_{mean} are predicted, actual and mean values of the target property, respectively. $\sum(Y_{predicted} - Y_{observed})^2$ is the predictive sum of squares (*PRESS*). The non–cross–validated models were then assessed by the explained variance R^2, standard error of estimate (*SEE*) and F test–ratio. The non–cross–validated analyses were used to make predictions of the percent decrease data of the test set and to display coefficient contour maps.

3 RESULTS

Altogether three CoMSIA models for steroid passive diffusion, active efflux by P–gp and inhibition on P–gp studies were derived respectively. All models exhibited satisfactory results with cross–validated $Q^2 > 0.500$ and conventional $R^2 > 0.970$.

3.1 Steroid Passive Diffusion

Employing ClogP and hydrophobic field descriptors, a model with statistical results of $Q^2 = 0.520$, $R^2 = 0.992$, $SEE = 0.017$ and $F = 117.834$ was obtained, suggesting a good correlation of passive diffusion ability of steroids with the two descriptors (Table 1). When validating the model by the test set independent of the training one, a $SEP = 0.043$ was obtained, indicating a good predictability. The actual and predicted activity values of the model are shown in Figure 1.

Table 1. The Actual, Calculated and Residual LgP_{app} Values of Passive Diffusion Model

No	Steroid	Actual	Calculated	Residual
1	aldosterone	–0.064	–0.066	0.002
2	cortisone	–0.063	–0.072	0.009
3	dexamethasone	0.319	0.319	–8.72e–5
4	estradiol	–0.217	–0.220	0.003
5	etonogestrel	0.132	0.146	–0.014
6	gestodene	0.097	0.100	–0.003
7	nandrolone	–0.017	0.017	–0.034
8	norethisterone	0.057	0.082	–0.025
9	norgestrel	0.111	0.114	–0.003
10	Org30659	0.127	0.111	0.016
11	Org34694	0.080	0.081	–0.001
12	Org36410	0.261	0.258	0.003
13	Org4060	0.160	0.162	–0.002
14	OrgOM08	–0.005	–0.006	0.001
15	prednisolone	0.110	0.113	–0.003
16	progesterone	0.058	0.055	0.003
17	spironolactone	0.225	0.155	0.070
18	testosterone	0.148	0.129	0.019

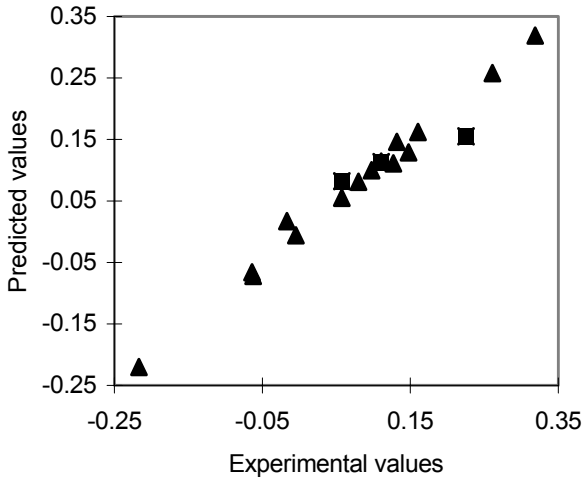

Figure 1. Predicted versus experimental $Log_{10}P_{app}$ values for the training set of 15 steroids (▲) and the test set of 3 steroids (■) obtained from steroid passive diffusion model.

All of the relative contribution results of various molecular descriptors employed in QSAR models are summarized in Table 2. For passive diffusion model, the relative field contributions of the two descriptors, *i.e.*, 92.2% for hydrophobic field and 7.8% for ClogP, indicate that hydrophobic field contributes much more than ClogP to steroid passive diffusion.

Table 2. Relative field contributions of all CoMSIA models

CoMSIA models	Relative contribution		
	H	ClogP	HB
Passive diffusion	0.922	0.078	–
P–gp active efflux	0.642	0.358	–
P–gp modulation	0.169	0.126	0.704

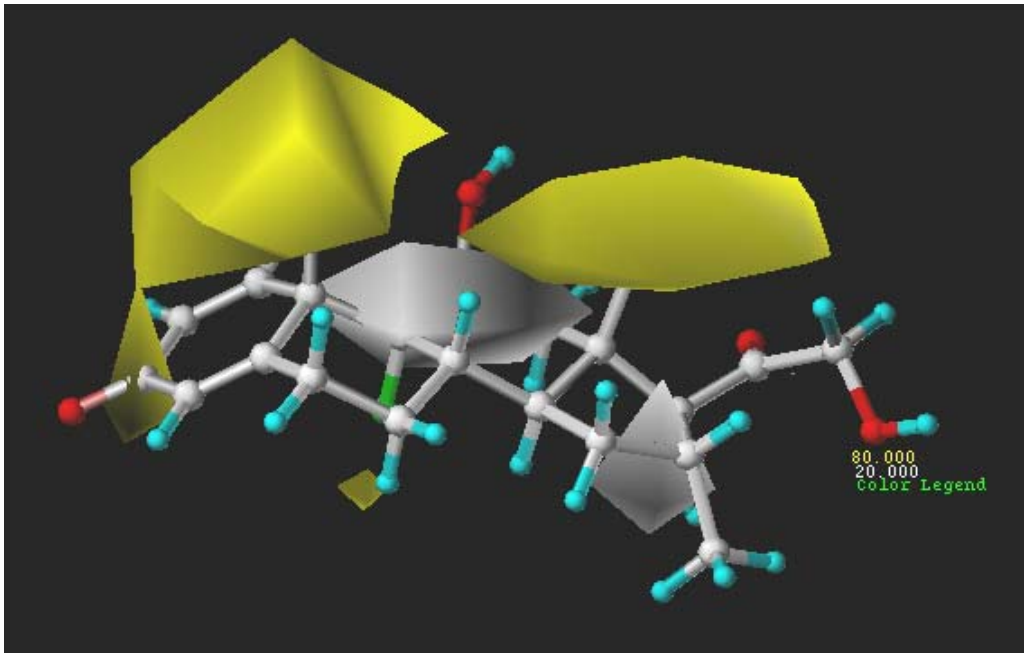

Figure 2. Hydrophobic contour plots of steroids for passive diffusion study. The yellow contours indicate regions where hydrophobic groups increases activity, whereas gray contours indicates regions where hydrophobic group decreases activity. The model is based on pharmacophore alignment.

Figure 2 displays the hydrophobic contour map of the model, in which the yellow contours indicate regions where hydrophobic groups increases activity, whereas gray contours indicates regions where hydrophobic group decreases activity. In this contour map hydrophobic group favored yellow regions are found near C–3, C–6α positions and the upside of ring A and ring D. Hydrophobic group unfavorable gray region is found around C–9, C–10 as well as C–17α positions.

3.2 Steroid Active Transport by P–gp

For steroid active efflux by P–gp, a model with $Q^2 = 0.573$, $R^2 = 0.973$, $SEE = 0.034$, $F = 71.957$ and $SEP = 0.039$ was obtained using ClogP and hydrophobic descriptors (Table 3), suggesting proper reliability and predictability of the model. Figure 3 shows the actual and predicted activity values of the model. The relative field contributions of descriptors in the model are 64.2% for hydrophobic field and 35.8% for ClogP (Table 2) respectively. Figure 4 depicts the hydrophobic contour plot of the active transport model. C–17β and C–21 positions are found to favor the hydrophobic interaction, where bulky yellow contour exists. The C–3 position is found to disfavor the hydrophobic interaction, where gray contour appears. In a word, one end side of the molecule favors hydrophobic substituents, whereas the other end side disfavors hydrophobic substituents.

Table 3. The Experimental, Calculated and Residual PDA Values of P–gp Active Efflux Model

No	Steroid	Experimental	Calculated	Residual
1	11–deoxycortisol	0.36	0.322	0.038
2	17α–hydroxyprogesterone	0.23	0.247	–0.017
3	aldosterone	0.44	0.450	–0.010
4	androstenedione	0.02	0.019	0.001
5	corticosterone	0.33	0.275	0.055
6	cortisol	0.46	0.478	–0.018
7	dehydroepiandrosterone	0.18	0.159	0.021
8	dexamethasone	0.41	0.437	–0.027
9	dihydrotestosterone	0.07	0.079	–0.009
10	medroxyprogesterone acetate	0.03	0.094	–0.064
11	pregnenolone	0.14	0.130	0.010
12	progesterone	0.01	0.012	–0.002
13	testosterone	0.08	0.111	–0.031

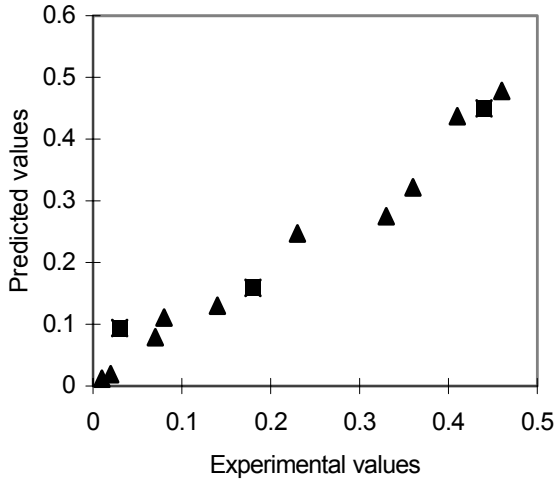

Figure 3. Predicted versus experimental PDA values for the training set of 10 steroids (▲) and the test set of 3 steroids (■) obtained from P–gp–mediated active transport model.

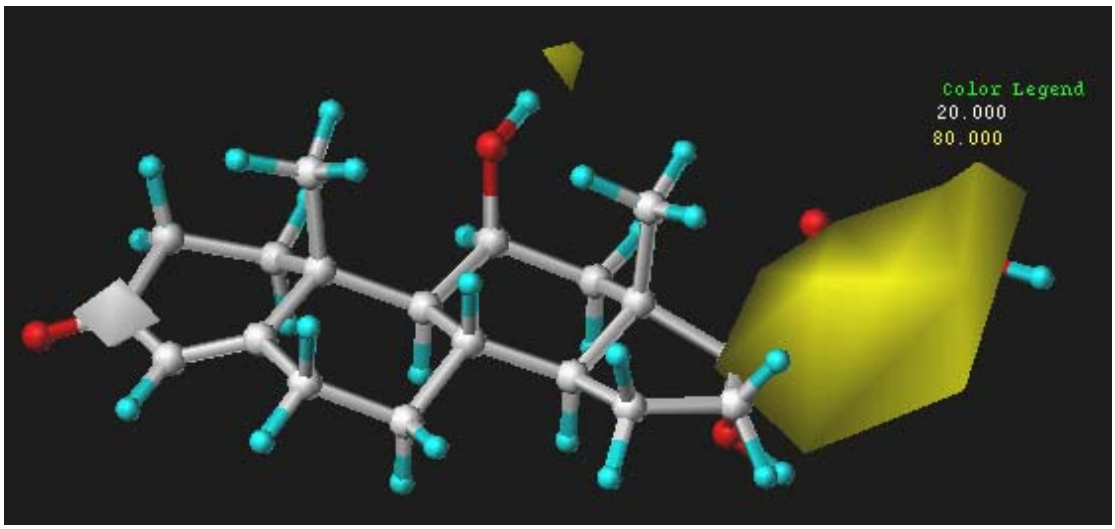

Figure 4. Hydrophobic contour plots of P–gp–mediated active transport model. The yellow contours indicate regions where hydrophobic groups increases activity, whereas gray contours indicates regions where hydrophobic group decreases activity.

Table 4. The Actual, Calculated and Residual VA Values of P–gp Inhibition Model

No	Steroid	Actual	Calculated	Residual
1	11–deoxycortisol	5.74	4.078	1.662
2	16α–methylprogesterone	15.64	18.095	–2.455
3	17–hydroxyprogesterone	4.03	4.545	–0.515
4	17α–hydroxypregnenolone	1.61	1.174	0.436
5	6,16α–methylpregnenolone	18.72	18.343	0.377
6	aldosterone	1.50	1.098	0.402
7	androstenedione	3.92	4.904	–0.984
8	androsterone	3.28	3.825	–0.545
9	corticosterone	2.4	4.387	–1.987
10	cortisol	2.46	3.562	–1.102
11	dehydroepiandrosterone	2.03	1.798	0.232
12	deoxycorticosterone	5.49	6.365	–0.875
13	dexamethasone	1.74	1.422	0.318
14	dihydrotestosterone	2.60	2.314	0.286
15	medroxyprogesterone acetate	21.27	22.276	–1.006
16	medroxyprogesterone	6.41	6.886	–0.476
17	pregnanedione	15.87	16.481	–0.611
18	pregnenolone	2.89	3.314	–0.424
19	progesterone	17.29	14.588	2.702
20	testosterone	2.64	2.517	0.123

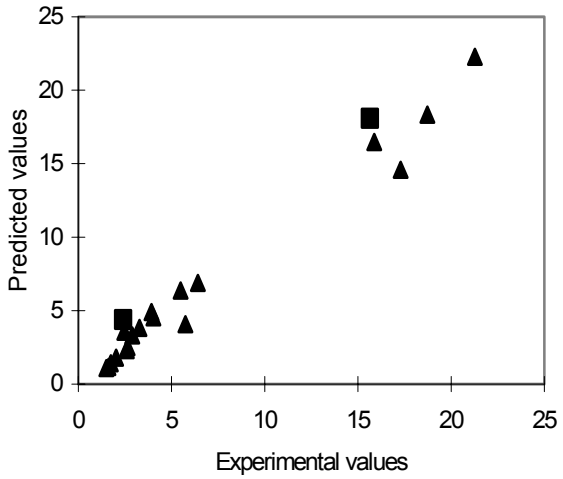

Figure 5. Predicted versus experimental VA values for the training set of 18 steroids (▲) and the test set of 2 steroids (■) obtained from steroid inhibition model.

3.3 Steroid Inhibition on P–gp

For steroid inhibition investigation, a model employing hydrophobic field, ClogP and H–bond descriptors was obtained, with the statistical results of Q^2 = 0.536, R^2 = 0.978, *SEE* = 1.338, F = 51.050 and *SEP* = 2.233 (Table 4). The actual and predicted activity values of the model are shown in Figure 5.

The relative field contributions of the three descriptors indicate that, for this time, the contribution of the hydrophobic field (16.9%) exhibits still a little larger than that of ClogP (12.6%), though both of them contributes little compared with that of the H–bond interactions (70.4%).

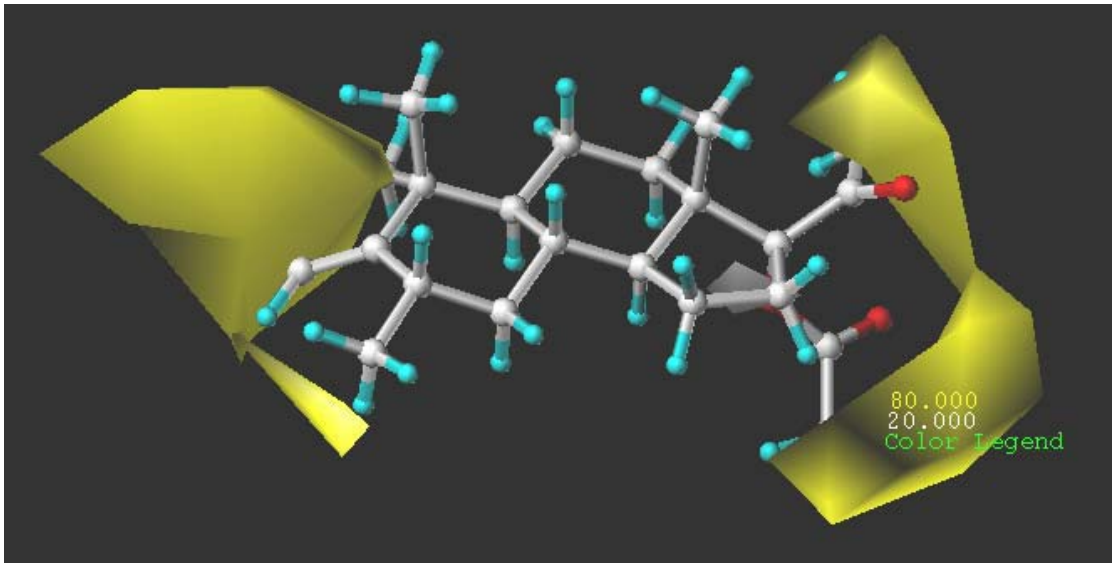

Figure 6. Hydrophobic contour plots of steroid inhibition on P–gp study. The yellow contours indicate regions where hydrophobic groups increases activity, whereas gray contours indicates regions where hydrophobic group decreases activity.

Figure 6 displays the hydrophobic contour plot for steroid inhibition model. Around C–2, C–3 positions and ring D, especially the regions near C–20 and C–21, large yellow contours are observed favoring the hydrophobic interaction. Whereas, around C–17α position small gray contours are found disfavoring the hydrophobic interactions. Generally speaking, most, but not all, regions of steroid molecules favor hydrophobic substituents for inhibition effects on P–gp.

4 DISCUSSION

Whatever mechanism the permeation of steroids into the enterocyte involves, it is accepted that lipophilicity of the molecule and the hydrophobic interactions between steroid and P–gp when P–gp exists in the bilayer play crucial roles. Therefore, a clear study of these impacts on three different transmembrane processes, *i.e.*, passive diffusion and the interaction of steroids with P–gp as substrates or inhibitors, is always valuable and needed.

4.1 Contribution of Hydrophobic Field and ClogP

Hydrophobicity is one of the most important properties related to biomolecular interactions, which can be interpreted in terms of the association of non–polar groups or molecules in an aqueous environment which arises from the tendency of water to exclude non–polar molecule [19]. Abraham terms it as 'hydrophobic bonding' [20]. The presence of a hydrophobic part in the structure of a molecule presumes such a hydrophobic bonding within an appropriate hydrophobic environment. To quantitatively depict the hydrophobicity of a molecule, various molecular descriptors are used, in which lipophilicity represented by logP (or ClogP, the calculated logP) is a mostly used one.

Traditionally, the lipophilicity of a molecule is expressed as logP, the logarithm of its partition coefficient in a lipidic phase and an aqueous phase, representing the tendency of the drug to prefer a lipidic environment to an aqueous one. The larger logP/ClogP a molecule possesses, the higher lipophilicity the molecule exhibits and larger hydrophobicity it has. Whereas, hydrophobic field is another popularly employed hydrophobic index that though just arise recently. Being considered as a 3D–molecular hydrophobic descriptor, hydrophobic field describes the specific distribution of the hydrophobic property in 3D–space or surface area of a molecule. Though these two concepts are both hydrophobicity descriptors and widely applied in many studies, they have different meanings and should be carefully discriminated.

From the statistical results of our study, the different contributions of lipophilicity (ClogP) to three transport mechanisms are clearly displayed. For steroid active transport by P–gp, apparently, lipophilicity appears as a general requirement. The correlation between lipophilicity (ClogP) and the substrate active transport effects are remarkable, with a 35.8% relative contribution of ClogP. However, for passive diffusion and steroid inhibition effect models, the relative contributions of ClogP are much less, only accounting for 7.8% and 12.6% respectively. What are the reasons for the difference in ClogP contributions for different transmembrane models?

First of all, we have to admit that to whatever transbilayer mechanisms, certain degree of lipophilicity is prerequisite to a drug molecule. Since P–gp recognizes it substrates within the lipid bilayer and its transmembrane domain has been demonstrated as the substrate–interaction sites [21,22], a substrate has to initially possess certain ability to cross biomembranes, after which it can bind to P–gp to be actively expelled out of the cell. The same is for a P–gp modulator, no matter what mechanisms it involves, it has to transfer first across the membrane and then interact with P–gp. For passive diffusion, it is also that the more lipophilicity a drug bears, the much easily it partitions into the bilayer despite the influence of molecular size and charge. Moreover, it is almost impossible for a too hydrophilic molecule to distribute into the membrane by passive diffusion [23]. Therefore, lipophilicity is definitely a necessary structural requirement for all steroid transport mechanisms.

However, our statistical results demonstrate that lipophilicity is, though important, but not the most crucial one governing the transmembrane processes. The statistical results not only display relatively small ClogP contributions but also revealed primary contributions from another molecular descriptor, namely the molecular hydrophobic field, to the different transport models. For passive diffusion model, hydrophobic field contributes as much as 11.8 times of ClogP, suggesting that the hydrophobic field can better correlate with the passive diffusion ability of the molecule. For both P–

gp active efflux and P–gp inhibition models, hydrophobic field also accounts more importance than the molecule macroscopic feature ClogP. The contribution ratios of hydrophobic field/ClogP are 1.8 and 1.3 for active efflux and steroid inhibition models, respectively. In a word, the analysis of the models suggests a larger potency of hydrophobic field than the classical partition coefficient in the correlation studies. The different contributions of ClogP and hydrophobic field are tightly connected with their specific meanings.

As molecular indices, ClogP is a useful parameter for finding the optimal hydrophobicity of a series of compounds. It is usually calculated from the sum of partition coefficients of the chemical fragments composing the molecule. However, since in ClogP calculation no hydrophobic dipoles could be defined which is essential to estimate the space directionality of hydrophobicity, ClogP value of a free ligand does not necessarily equal to the hydrophobic contribution to the interactions between the ligand and its receptor. ClogP reflects only the overall lipophilicity of a molecule and consequently is insufficient when topochemical or stereochemical features are required to describe intermolecular interactions between a ligand and its receptor [24]. The conformational change of certain groups of a molecule for interacting with the target site in an active 'mode' might be expected to influence the lipophilicity of other unchanged groups, and in this way perturbs the integral hydrophobicity of the system. The strategies to increase a drug's lipid solubility by attachment of hydrophobic substituents or deletion of hydrophilic ones from the drug molecule do not definitely enhance the hydrophobic interactions between the drug and its acceptor. Sometimes it may actually result in drug inactivation via altered pharmacokinetics or structural inability to bind to the required receptor due to wrong structural modification atom places of the drug. Under this condition, obviously, it is improper to only adopt lipophilicity as molecular property to correlate with the molecule's biological activities.

In contrast, the 3D distribution of lipophilicity in space or molecular surfaces, *i.e*., the molecular hydrophobic field, can reflect the degree of lipophilicity of the different parts of a molecule as well as the tendency of hydrophobic interactions between the molecule and its ligand, thus overcoming the disadvantages of ClogP in correlation studies. The limitation of ClogP in accounting for the complex hydrophobic interactions in bio–systems has been expatiated in Kellogg's work [25].

It is well known that passive diffusion, to a large extent, depends not only on lipophilicity, but also on two other physicochemical properties, *i.e*., polarity (charge, hydrogen bonding) and molecular size [26]. All of these three properties are connected with the distribution of hydrophobic fields in space as well as the molecule–membrane interactions, which cannot be simply represented by the lipophilicity of the molecule. Indeed, Liu *et al*. [27] reported that no sufficient correlation

between ClogP and the penetration ability of 28 structurally diverse drug–like compounds across the blood–brain barrier simply by passive diffusion could be found. Rubas *et al*. [28] demonstrated that even apparent partition coefficient (logD), another term better expressing lipophilicity than logP, is not a good predictor of the permeability coefficient and oral absorption. Actually when penetrating into the membrane the amphiphilic molecule aims to expose the maximum hydrophobic surface, therefore the hydrophobic interactions might be more important for drug–membrane interactions. The hydrophobic field as space–distributed 3D characteristics should be more correlated with the compound passive diffusion as shown in our results, although a much profound investigation of the mechanism is still needed.

The same might is also true for a molecule interacting with hydrophobic areas at a protein binding site like P–gp. No matter what patterns steroids interact with P–gp, the interaction belongs to molecule–protein interactions, where hydrophobic field in the same way displays its superiority to ClogP in correlation studies. Berger *et al*. [29] observed no clear trend correlating the lipophilicity of a series of 59 tetrahydroisoquinolines and isoindoline derivatives with their MDR reversal effects. Even in those studies where moderate correlations between logP and inhibition effects of series of drugs were conditionally observed, the insufficiency of logP parameter to explain the differences in MDR reversing activities are indicated [30–32]. Pajeva *et al*. [33, 34] not only observed that the molecular profile of hydrophobicity plays an essential role in membrane–mediated mechanisms of MDR modulation, but also demonstrated that describing hydrophobicity as a space–directed molecular property, *i.e*., a field, is preferable to the use of logP representation of hydrophobicity. According to Wiese, even for structurally related subclasses of molecules, the lipophilicity (logP) remains no longer the main structural determinant for the observed MDR reversal effect after certain structural changes [19], whereas other factors including hydrophobic field, H–bond or others should also be considered in correlating with MDR activities, which is associated with our results. In conclusion, all above results have proven that ClogP values or the degree of molecular lipophilicity, although important, is not the sole determinant of potency for their biological activity [33,34]. Hydrophobic field, another molecule property, may play more critical roles in correlating with the biological activity, as well as mimicking the complex interactions between a molecule and membrane or protein.

4.2 3D–space Distribution of Hydrophobic Field

As shown in Figure 2, the favorable distribution of hydrophobic contours for steroid passive diffusion is a space–interval distributed hydrophobic fields, *i.e*., the hydrophobic and hydrophilic

groups substitute the steroid skeleton at intervals. With such kind of structure, a steroid molecule is proposed be able to traverse cell membrane easily by passive diffusion. The reason might be that this structure favors the interaction of steroids with the cell lipid bilayer, which also possesses a hydrophobic–hydrophilic interleaving structure.

For P–gp active efflux of steroids seen from Figure 4, the favorable hydrophobic field distribution is, apparently, a one end–hydrophobic and another end–hydrophilic structure. In other words, the region near C–3 favors but the region near C–17 disfavors hydrophobic substituents. This is might be related to the interaction of the hydrophobic part of steroids with the inside–pocket of P–gp. The hydrophobic end of a steroid molecule may be attracted by P–gp and binds to its inside pocket which is also, mostly, a hydrophobic environment. Thereafter, another hydrophilic end may favor the departure of the molecule from P–gp after being transferred out of the cell, which hypothesis needs further experimental demonstrations.

For steroids interacting with P–gp as inhibitor, Figure 6 indicates that most regions in the molecule structure are under the cover of hydrophobic contours, especially near the two end sides though small region disfavoring hydrophobic groups exists. Since a steroid molecule possessing this structure more or less satisfies the hydrophobic field characteristics of steroid–based P–gp substrates discussed above (one end hydrophobic and the other end hydrophilic), it can bind to P–gp (This, however, doesn't mean that that a P–gp steroid inhibitor has to be firstly a P–gp substrate). But because its wider–spread hydrophobicity distribution the molecule may bind too tight to the inside–pocket of P–gp to depart from it, and in this way, block the outward transport of P–gp.

Based on above results, it is clearly seen that subtle difference in hydrophobic molecular field distribution and logP may result in quite distinct performances for structurally similar steroid molecules. The study of Wiese and Pajeva [33,35] on a series of potent thioxanthene–based modulators like the *trans*– and *cis*–flupentixol, *trans*– and *cis*–clopenthixol is a good example. The experiments demonstrated that though these pairs of isomers possess same ClogP values they exhibit 3–7–fold difference in MDR reversing activity, the reason might due to differences in their hydrophobic fields as well as other factors like electrostatic interactions causing different orientations of the molecules in the membrane lipid environment.

Though due to the limited amount of datasets, the applicability of above results still needs further experimental investigations, they do reflect some virtual conditions in the transmembrane process of steroids in cells expressing or not expressing P–gp, and are helpful for further steroid–related drug design. However, the plasma membrane is a complex lively fluctuating system, and many

other events may contribute to the transmembrane process except for those we studied in present work. Other factors, like the involvement of specific carrier in transport, the dependence of transmembrane rate on initial donor (substrate or inhibitor) concentration, pH and temperature, together with the contribution of encytosis should also be considered. In a word, the search for models for drug transmembrane process that are characterized by either a partition coefficient or an overall distribution of hydrophobic fields may not be very fruitful if the process involves many mechanisms. In addition, when dealing with biomembrane transport systems, the clarification of respective contributions of various transbilayer mechanisms is of importance. Lampidis *et al*. [36] have reported the role of anthracyclines lipophilicity on circumvention of P–gp MDR. In his work, despite the interaction of anthracyclines with P–gp, the resistance index decreased with increasing lipophilicity, the reason might be due to a possible higher drug influx rate owing to lipophilicity. Eytan *et al*. [37] also reported in his experiments that the success of P–gp in lowering the intracellular concentration of an MDR–drug is determined by a limited passive transbilayer movement of the drug. Therefore, for different purposes like to improve the bioavailability, or to improve the inhibition effects of a drug, different structural modification measurements should be carefully conducted to find an optimal balance of all possible conditions, based on a careful study of the transbilayer process and evaluation of specific contributions of each mechanism to the permeation.

5 CONCLUSIONS

Structurally similar drugs may possess distinct bioavailability due to the difference in absorption, and finally result in distinct therapeutic effects in vivo. Therefore, a profound understanding of the permeation process in biomembranes of a drug and related influencing factors is of value for improving the bioavailability, disposition, as well as therapeutic efficacy of the molecule. In present work, the impacts of two important factors in steroid transmembrane process, *i.e*., the molecular lipophilicity and distribution of hydrophobic molecular field were analyzed respectively. Their influences on three different transmembrane processes including passive diffusion, active transport (efflux) by P–gp and inhibition effect on P–gp of steroids were analyzed and compared. Our results indicate that hydrophobic field, compared with ClogP, better correlates with the biological activity of steroids penetrating across the bilayer. In addition, the specific 3D–space distribution of hydrophobic field of a molecule determines its specific interaction mechanism with membrane or P–gp.

Acknowledgment

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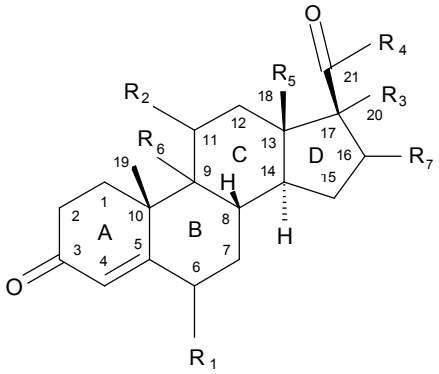
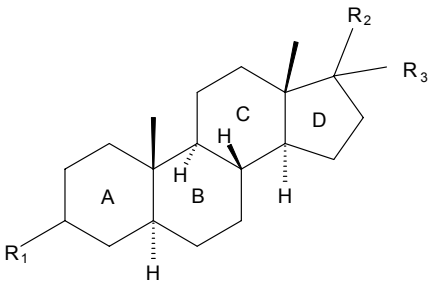
Appendix 1

Table A1. Structure of steroids used in P–gp–mediated active transport and P–gp modulation studies. Backbone numbering is according to IUPAC.



Structural type: SA

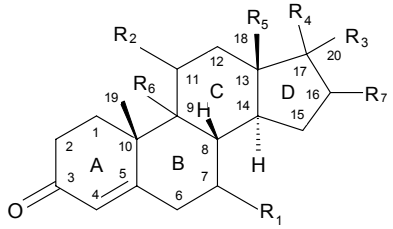


Structural type: SB

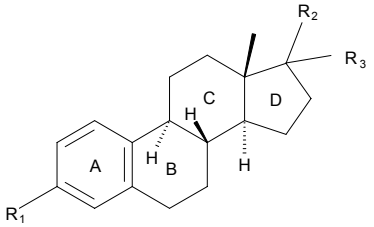
No	Steroid compound		R_1	R_2	R_3	R_4	R_5	R_6	R_7
1	deoxycorticosterone	SA	$-H_2$	$-H_2$	–H	$-CH_2OH$	◄ CH_3	⁞ H	$-H_2$
2	medroxyprogesterone	SA	◄ CH_3	$-H_2$	⁞ OH	$-CH_3$	◄ CH_3	⁞ H	$-H_2$
3	16α–methylprogesterone	SA	$-H_2$	$-H_2$	–H	$-CH_3$	◄ CH_3	⁞ H	⁞ CH_3
4	cortisol	SA	$-H_2$	◄ OH	⁞ OH	$-CH_2OH$	◄ CH_3	⁞ H	$-H_2$
5	17α–hydroxyprogesterone	SA	$-H_2$	$-H_2$	⁞ OH	$-CH_3$	◄ CH_3	⁞ H	$-H_2$
6	progesterone	SA	$-H_2$	–H2	–H	–CH3	◄ CH_3	⁞ H	$-H_2$
7	corticosterone	SA	$-H_2$	◄ OH	–H	$-CH_2OH$	◄ CH_3	⁞ H	$-H_2$
8	11–deoxycortisol	SA	$-H_2$	$-H_2$	⁞ OH	$-CH_2OH$	◄ CH_3	⁞ H	$-H_2$
9	medroxyprogesterone actetate	SA	⁞ CH_3	$-H_2$	⁞ $O-(CO)CH_3$	$-CH_3$	◄ CH_3	⁞ H	$-H_2$
10	aldosterone	SA	$-H_2$	◄ OH	–H	$-CH_2OH$	◄ CHO	⁞ H	$-H_2$
11	dexamethasone ¶	SA	$-H_2$	◄ OH	⁞ OH	$-CH_2OH$	◄ CH_3	⁞ F	⁞ CH_3
12	dehydroepiandrosterone †	SB	◄ OH	=O					
13	pregnenolone †	SB	◄ OH	$-(CO)CH_3$	–H				
14	testosterone ‡	SB	=O	◄ OH	–H				
15	androstenedione ‡	SB	=O	=O					
16	dihydrotestosterone	SB	=O	◄ OH	–H				
17	17α–hydroxypregnenolone†	SB	◄ O	$-(CO)CH_3$	⁞ OH				
18	androsterone	SB	⁞ OH	=O					
19	pregnanedione	SB	=O	$-(CO)CH_3$	–H				
20	6,16α–methylpregnenolone†	SB	◄ OH	$-(CO)CH_3$	–H				

¶ 1, 2–Double bond, † 5, 6–Double bond, ‡ 4, 5–Double bond.

Table A2. Structure of steroids used in passive diffusion study. Backbone numbering is according to IUPAC



Structure type: SA



Structure type: SB

No			R_1	R_2	R_3	R_4	R_5	R_6	R_7
1	hydrocortisone	SA	$-H_2$	◄ OH	┤ OH	◄ $C(O)CH_2OH$	◄ CH_3	┤ H	$-H_2$
2	testosterone	SA	$-H_2$	$-H_2$	–H	◄ OH	◄ CH_3	┤ H	$-H_2$
3	progesterone	SA	$-H_2$	$-H_2$	–H	◄ $C(O)CH_3$	◄ CH_3	┤ H	$-H_2$
4	nandrolone	SA	$-H_2$	$-H_2$	–H	◄ OH	◄ CH_3	┤ H	$-H_2$
5	cortisone	SA	$-H_2$	=O	┤ OH	◄ $C(O)CH_2OH$	◄ CH_3	┤ H	$-H_2$
6	aldosterone	SA	$-H_2$	◄ OH	┤ H	◄ $C(O)CH_2OH$	◄ CHO	┤ H	$-H_2$
7	norethisterone	SA	$-H_2$	$-H_2$	┤ C≡CH	◄ OH	◄ CH_3	┤ H	$-H_2$
8	dexamethasone [a]	SA	$-H_2$	◄ OH	┤ OH	◄ $C(O)CH_2OH$	◄ CH_3	┤ F	┤ CH_3
9	prednisolone [a]	SA	$-H_2$	◄ OH	┤ OH	◄ $C(O)CH_2OH$	◄ CH_3	┤ H	$-H_2$
10	gestodene [b]	SA	$-H_2$	$-H_2$	┤ C≡CH	◄ OH	◄ C_2H_5	┤ H	–H
11	etonogestrel	SA	$-H_2$	$=CH_2$	┤ C≡CH	◄ OH	◄ C_2H_5	┤ H	$-H_2$
12	estradiol	SB	–OH	◄ OH	–H				
13	Org 32540	SA	$-H_2$	$=CH_2$	┤ CH_2–N=N–NH_2	◄ OH	◄ C_2H_5	┤ H	$-H_2$
14	Org 34694	SA	┤ CH_3	=CH–CH_3	┤ C≡CH	◄ OH	◄ CH_3	┤ H	$-H_2$
15	Org 36410 [c]	SA	$-H_2$	◄ C_6H_5–$C(CH_3)_3$	┤ C≡CH	◄ OH	◄ CH_3		┤ OH
16	Org 30659 [b]	SA	$-H_2$	$=CH_2$	┤ C≡CH	◄ OH	◄ CH_3	┤ H	–H
17	Org 4325	SA	$-H_2$	◄ $CH=CH_2$	┤ C≡CH	◄ OH	◄ CH_3	┤ H	$-H_2$
18	Org 4060	SA	$-H_2$	◄ C_2H_5	┤ C≡CH	◄ OH	◄ CH_3	┤ H	$-H_2$

[a] 1, 2–Double bond
[b] 15, 16–Double bond
[c] 9, 10–Double bond; In its R_2 substitution, the $C(CH_3)_3$ group is in the contrapuntal position of the phenyl ring.

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