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3D-Quantitative Structure Metabolism Relationship (QSMR) Studies of CYP3A4 Substrates

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ABSTRACT

3D-Quantitative structure metabolism relationship studies were conducted on the substrates of CYP3A4 based on CoMFA. The CoMFA model was found to be successful in the prediction of catalytic activity of CYP3A4 substrates, with a cross-validated correlation co-efficient (q^2) of 0.593 and a non-cross validated correlation co-efficient (r^2) of 0.994. The CoMFA model was also validated with the training and test set of CYP3A4 substrates. The model helped to understand the structural features that are involved in CYP3A4 mediated drug metabolism. Therefore, the model can be subjected to the initial stage of the drug discovery processes for developing the therapeutic analogs with the improved CYP3A4 mediated metabolic profiles.

Keywords: 3D-QSMR, CoMFA, Drug metabolism, CYP3A4

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INTRODUCTION

Cytochromes P450 (CYPs) are the family of catalytic enzymes involved in drug metabolism. CYPs constitute heme at the active site and are mostly present in the liver cells of the human body. CYP3A4 is a predominant drug metabolizing enzyme and metabolizes over 50% of clinically administered drugs.^{1,2} The active site of CYP3A4 constitutes hydrophobic amino acid residues. The substrates which are undergoing CYP3A4 mediated metabolism are hydrophobic in nature.² CYP3A4 catalyzes the oxidation of endogenous as well as exogenous substrates during the metabolism. The oxidative reactions of CYP3A4 includes (i) C-oxidation (ii) N-demethylation (iii) O-demethylation (iv) S-oxidation, etc. These reactions are grouped as a phase-I metabolic reactions.^{1,3} The phase-I metabolic reactions generate various metabolites which are therapeutically inactive in most of the cases. In few cases; the metabolites are converted into reactive species and these reactive metabolites are toxic.⁴

3D-Quantitative structure metabolism relationship (QSMR) is a useful approach in quantifying the bio-activity and this approach can be employed in the absence of target information. Comparative molecular field analysis (CoMFA) is one of the types of 3D-QSMR and is used to describe the impact of structural features on bio-activity. CoMFA relates the 3D-steric and electrostatic properties of small organic molecules against bio-activity by means of mathematical equations. The approach assumes that the therapeutic and metabolic activity of molecules is due to changes in the size, shape or substituent of the core moiety while binding to the macromolecule. These changes are determined using a reference molecule and are computed in the form of steric and electrostatic field properties. Then, these field properties are correlated with the metabolic activity using partial least squares analysis (PLS) to generate a CoMFA model.

A few 3D-QSAR studies were reported for CYP3A4 in the literature. Roy *et al.*⁵ reported QSAR model for the inhibitors of CYP3A4 by employing twenty-eight structurally diverse compounds. The model was developed to investigate the electronic, spatial, topological and thermodynamic descriptors using Cerius 2 software.⁵ The techniques like stepwise multiple linear regression (MLR), PLS, artificial neural networks (ANN), etc. were employed. All the models indicated the importance of partition co-efficient ($\log P$), electronic and topological parameters. Further, the model was also validated with a test set. In another study, Ekins *et al.*⁶ constructed a 3D-hypothesis consisting of two hydrogen bond acceptors, one hydrogen bond donor and one hydrophobic region. The inter-feature distance of hydrogen bond acceptor-hydrogen bond acceptor, hydrophobe-hydrogen bond acceptor, and hydrogen bond donor-hydrogen bond acceptor was found to be

respectively 7.7, 6.6 and 6.4 Å. The significance of the model was validated and subjected to predict the K_m values. Further, the common feature hypothesis model was also generated for the activators of CYP3A4. The model suggested that the activators of CYP3A4 possess multiple hydrophobic regions which are away from the metabolic site.⁶

The reported QSMR studies on CYP3A4 were mostly focused on descriptors or pharmacophoric features of the inhibitors. These studies help independently to understand the specific role of the enzyme on drug metabolism. However, the metabolic process of CYP3A4 varies from one substrate/inhibitor to another. The influence of steric and electrostatic properties of substrates on the catalytic activity has not been established. This prompts to conduct a study with a diverse dataset of molecules and such types of studies are necessary to identify the primary metabolic reactions of new chemical entity (NCE). In this work, the development of the CoMFA model was attempted for the substrates of CYP3A4 and this study will be useful to identify the primary metabolic reaction in a quantitative manner.

MATERIALS AND METHOD

All the computational studies were performed on SYBYL6.9 software installed on Silicon Graphics Octane2 workstation, running under the IRIX 6.5 operating system.⁷

Dataset selection

Training set and a test set of CYP3A4 substrates were selected from the literature where the information regarding the site of metabolism (SOM), metabolites and catalytic activity (K_m values) were reported. The substrates included in this study constitute structural as well as therapeutic diversity since our focus was emphasized to study the metabolic activity of therapeutic drugs. Training set and a test set of CYP3A4 substrates respectively constitute 34, 15 metabolic reaction pathways (Table 2 and Table 3).

Ligand pre-processing

The 3D-structure of CYP3A4 substrates was built using *sketch molecule* panel implemented in SYBYL software. The *in-built* structures were energetically minimized by Powell method with a convergence criterion of 0.05 kcal/mol using Tripos force field. Gasteiger charge was assigned for all the substrates to calculate the partial atomic charges. The optimized 3D-structures were stored in SYBYL database as .mol2 files.

Molecular alignment

The accuracy of the CoMFA model and the prediction of the stereoelectronic region directly depends upon the molecular alignment. The relative alignment of all the dataset of compounds to

the template molecule was carried out using Atom-based alignment. The molecules were aligned against the template based on experimentally reported SOMs. To perform molecular alignment, the most active molecule of the data set, tofisopam (**1**) was used as a template. The alignment of a dataset of molecules with the template molecule was carried out such that SOM of template molecule matched with the SOM of dataset of other molecules.⁸ This alignment reflects the native binding mode of CYP substrates since SOM of substrates moves close to the heme at the active site of CYPs during the process of drug metabolism.⁸⁻⁹

Comparative molecular field analysis

CoMFA calculates the steric fields using a Lennard-Jones potential and electrostatic fields using a Coulomb potential.¹⁰ The field properties were calculated with a combination of steric and electrostatic molecular fields. The fields were sampled at each point of regularly spaced grids of 2.0 Å. A sp³ carbon atom with a charge of +1 was used as a probe to calculate the field properties. The CoMFA cut off value (i.e., 30 kcal/mol) was set to steric and electrostatic fields. The CoMFA-STD method in SYBYL was used to scale the field properties.

Partial least square analysis

The calculated field properties were correlated with the catalytic activity (pK_m) using PLS approach. The optimum number of components was determined by leave-one-out (LOO) cross-validation using a maximum of 6 principal components. To avoid the over fitted 3D-QSMR, the optimum number of components were derived from analysis with a highest q^2 value of the training set. This procedure speeds up the analysis and reduces the noise to generate a robust CoMFA model. During the PLS analysis, the CoMFA field properties were used as the independent variables and metabolic activity was used as the dependent variable. The best CoMFA model was chosen out of several models on the basis of a cross-validation approach.

Statistical significance

The statistical significance of CoMFA model was determined by computing (i) cross-validated leave-one-out correlation co-efficient (q^2) (ii) non-cross validated correlation co-efficient (r^2) (iii) optimum number of components (iv) boots trapping runs (R^2 bs^k). Several CoMFA models were generated and only the robust model was chosen for further analysis. The CoMFA model was considered to be robust when ($q^2 > 0.5$) and ($r^2 > 0.9$).

RESULTS AND DISCUSSION

Statistical significance of comparative molecular field analysis

Several CoMFA models were generated for the substrates of CYP3A4 on the basis of QSMR approach. However, only the robust model was chosen for further analysis. The representative CoMFA model of CYP3A4 was found to be statistically significant with a cross-validated correlation co-efficient (q^2) of 0.593. In addition, a non-cross validated correlation co-efficient (r^2) value was computed and was also found to be significant ($r^2=0.994$). The statistical summary of the CoMFA model was depicted in Table 1. Further, the CoMFA model was subjected to predict the metabolic/catalytic activity of training set molecules and the model was found to be predictive of catalytic activity of CYP3A4 substrates (Figure 1, Table-2).

Table 1. The statistical significance of the CoMFA model for the substrates of CYP3A4

No.	Validation parameters	Statistical significance
1.	Cross validated correlation co-efficient (q^2)	0.593
2.	Non-cross validated correlation co-efficient (r^2)	0.994
3.	Optimum number of components (Noc)	6
4.	Standard error of estimate (SEE)	0.062
5.	Standard error of prediction (SEP)	0.598
6.	F value	751.452
7.	Contribution of steric	70.10
8.	Contribution of electrostatic	29.90
9.	Boots trapping runs (R^2 bs ^k)	0.998

Table 2: Prediction of metabolic activity of CYP3A4 substrates (Training set) using CoMFA

No.	CYP3A4 substrates	Types of metabolic reactions	Observed Activity (pK _m)	Predicted Activity (pK _m)	Residuals (observed-predicted)	References
1.	adinazolam	N-dealkylation	4.68	4.67	0.01	Ref. ¹¹
2.	albendazole	S-oxidation	5.00	5.00	0.00	Ref. ¹²
3.	amiodarone	N-demethylation	4.27	4.26	0.01	Ref. ¹³
4.	amitriptyline	N-demethylation	4.04	3.93	0.11	Ref. ¹⁴
5.	astemizole	6-hydroxylation	5.01	5.04	-0.03	Ref. ¹⁵
6.	celecoxib	Hydroxylation	4.74	4.73	0.01	Ref. ¹⁶
7.	(S)-chloroquine	N-desethylation	5.30	5.29	0.01	Ref. ¹⁷
8.	cilostazol (OPC13217)	Hydroxylation	5.27	5.23	0.04	Ref. ¹⁸
9.	cilostazol (OPC13226)	Hydroxylation	5.25	5.26	-0.01	Ref. ¹⁸
10.	(S)-citalopram	N-dealkylation	3.23	3.26	-0.03	Ref. ¹⁹
11.	clozapine	N-demethylation	3.64	3.59	0.05	Ref. ²⁰
12.	dasatinib (M20)	Hydroxylation	5.22	5.23	-0.01	Ref. ²¹
13.	(R)-dihydrobromperidol	Oxidation	4.29	4.19	0.10	Ref. ²²
14.	(S)-dihydrobromperidol	Oxidation	4.47	4.36	0.11	Ref. ²²
15.	(R)-dihydrohaloperidol	Oxidation	4.04	4.20	-0.16	Ref. ²²
16.	(S)-dihydrohaloperidol	Oxidation	4.40	4.36	0.04	Ref. ²²
17.	erlotinib	O-dealkylation	5.76	5.77	-0.01	Ref. ²³
18.	(R)-fluoxetine	N-demethylation	4.47	4.44	0.03	Ref. ²⁴
19.	(S)-fluoxetine	N-demethylation	4.47	4.46	0.01	Ref. ²⁴
20.	(R)-ketamine	N-demethylation	3.15	3.29	-0.14	Ref. ²⁵
21.	(S)-ketamine	N-demethylation	3.40	3.35	0.05	Ref. ²⁵
22.	(R)-lansoprazole	Hydroxylation	4.57	4.58	-0.01	Ref. ²⁶
23.	(R)-lansoprazole	S-oxidation	4.42	4.46	-0.04	Ref. ²⁶
24.	(S)-lansoprazole	Hydroxylation	4.49	4.49	0.00	Ref. ²⁶
25.	(S)-lansoprazole	S-oxidation	4.82	4.77	0.05	Ref. ²⁶
26.	laquinimod	Hydroxylation	5.77	5.77	0.00	Ref. ²⁷
27.	(R)-omeprazole	Hydroxylation	3.50	3.50	0.00	Ref. ²⁸
28.	(R)-omeprazole	S-Oxidation	4.08	4.09	-0.01	Ref. ²⁸
29.	(S)-omeprazole	Hydroxylation	3.44	3.49	-0.05	Ref. ²⁸

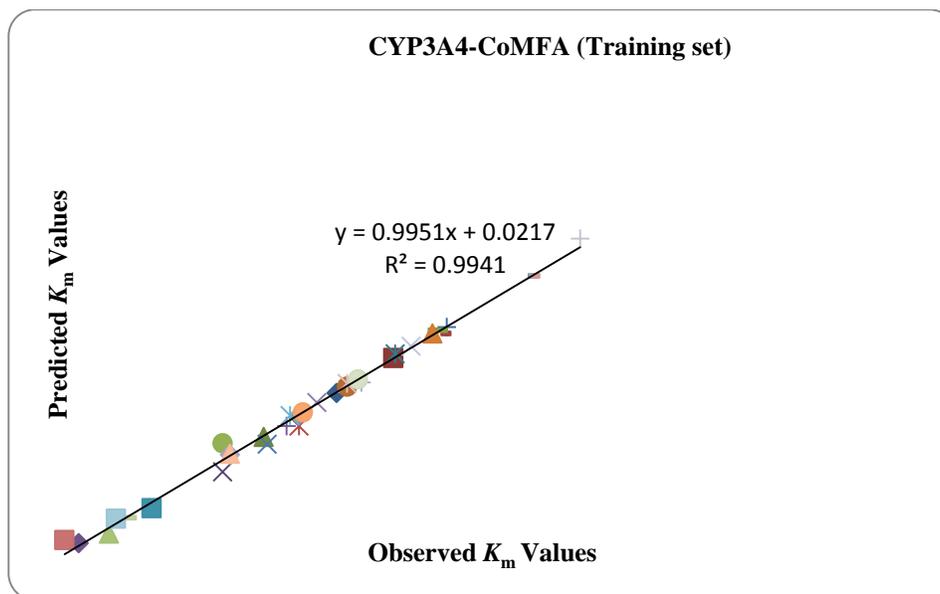
30.	(S)-omeprazole	S-oxidation	4.08	4.10	-0.02	Ref. ²⁸
31.	perphenazine	N-dealkylation	5.10	5.11	-0.01	Ref. ²⁹
32.	(R,R)-reboxetine	O-dealkylation	4.74	4.76	-0.02	Ref. ³⁰
33.	(S,S)-reboxetine	O-dealkylation	4.80	4.80	0.00	Ref. ³⁰
34.	(R)-tofisopam (Template) (1)	O-dealkylation	6.05	6.12	-0.07	Ref. ³¹

Validation of the CoMFA model with the test set

A test set was created to validate the CoMFA model. The test set comprises a structurally diverse class of therapeutic drugs which were reported as CYP3A4 substrates. Test set molecules were assigned to Gasteiger charge and aligned with respect to the template molecule. The alignment was carried out such that the reported SOM of the test set molecules matched with the template molecule. These test set molecules were stored separately in SYBYL database and the catalytic activity was predicted using the representative CoMFA model. The model predicted the catalytic activity of the test set successfully (Table 3).

Table 3: Prediction of metabolic activity of CYP3A4 substrates (Test set) using CoMFA

No.	CYP3A4 substrates	Types of metabolic reactions	Observed activity (pK _m)	Predicted activity (pK _m)	Residuals (observed-predicted)	References
1.	alfentanil	N-dealkylation	4.85	4.52	0.33	Ref. ³²
2.	clobazam	N-demethylation	4.53	4.20	0.33	Ref. ³³
3.	dextromethorphan	N-demethylation	4.25	4.31	-0.06	Ref. ³⁴
4.	ezlopitant alkene (CJ-12458)	Hydroxylation (CP-611781)	4.66	4.50	0.16	Ref. ³⁵
5.	ezlopitant alkene (CJ-12458)	Hydroxylation (CP-616762)	4.62	4.50	0.12	Ref. ³⁵
6.	ezlopitant	Oxidation (CJ-12458)	5.04	5.03	0.01	Ref. ³⁶
7.	ezlopitant	Oxidation (CJ-12764)	4.95	5.03	-0.08	Ref. ³⁶
8.	felodipine	Aromatization	4.57	4.64	-0.07	Ref. ³⁷
9.	midazolam (1-OH)	Hydroxylation	5.05	5.06	-0.01	Ref. ³⁸
10.	midazolam (4-OH)	Hydroxylation	4.53	4.44	0.09	Ref. ^{37,38,39}
11.	nifedipine	Aromatization	4.67	4.59	0.08	Ref. ³⁷
12.	propofol	Hydroxylation	4.37	4.68	-0.31	Ref. ⁴⁰
13.	sildenafil	N-dealkylation	3.65	3.80	-0.15	Ref. ⁴¹
14.	voriconazole	Hydroxylation	4.95	4.73	0.22	Ref. ⁴²
15.	voriconazole	N-oxidation	3.63	3.77	-0.14	Ref. ⁴²



(c)

Figure 1: Observed and predicted K_m values (pK_m values) for the substrates of CYP3A4 (Training set)

Contour map analysis

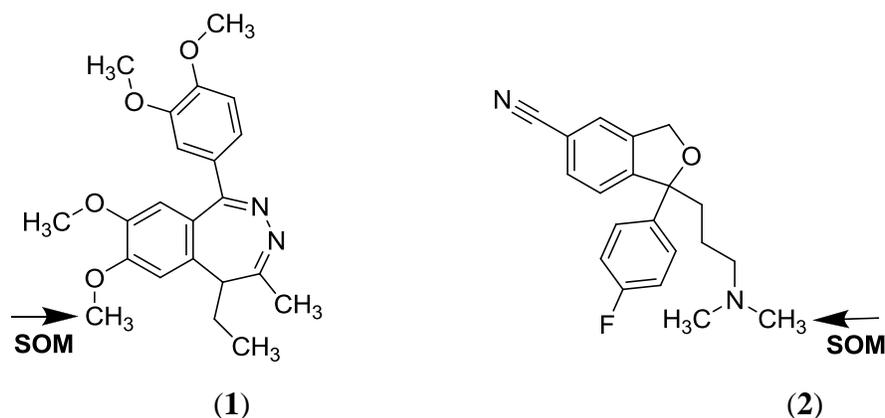


Figure 2: CYP3A4 substrates: (R)-tofisopam (1) and (S)-citalopram (2)

In the contour maps, the compound with the highest metabolic activity ((R)-tofisopam) and lowest metabolic activity ((S)-citalopram) among the training set molecules were investigated (Figure 2). The fused nucleus of (R)-tofisopam occupied the sterically favored green region (Figure 3a). This describes the highest metabolic activity of (R)-tofisopam (Table 1). Inversely, N-methyl structural unit of ((S)-citalopram) occupied the sterically disfavored yellow region of contour maps and this demonstrates the observed lower metabolic activity of (S)-citalopram (Table 1; Figure 3b). The visual inspection of the steric and electrostatic contour maps of the CoMFA models towards CYP substrates may guide the medicinal chemists to make a substitution pattern in those positions of the molecular skeleton which occupies favored / disfavored region. This further leads to develop a

therapeutic molecule with an altered metabolic activity. Finally, the work presented here enabled us to propose the stereoelectronic regions which are affecting the CYP metabolic activity. This can be relevant to develop the therapeutic analogs with the efficient metabolic profiles.

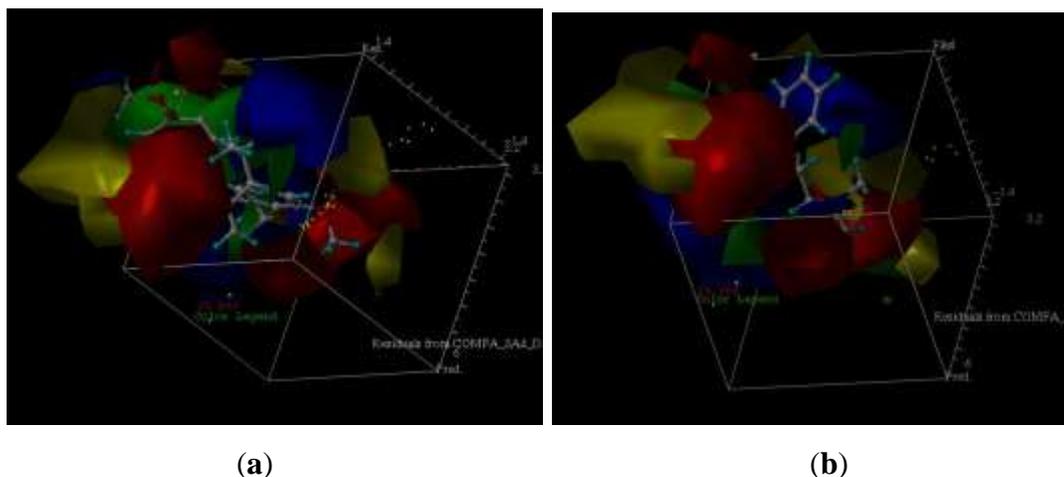


Figure 3: Contour map analysis of CYP3A4 substrates: (a) (R)-tofisopam (1) and (b) (S)-citalopram (2)

CONCLUSION

CoMFA was performed for the substrates of CYP3A4. The CoMFA model was found to be statistically significant in predicting the catalytic activity. Further, the model was also validated with the training and test set of molecules. The obtained model helped to explain the stereoelectronic structural features that are responsible for the CYP3A4 mediated metabolism. The information obtained from the contour map analysis of CYP3A4 substrates could be useful to design the therapeutic analogs with efficient metabolic profiles.

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