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Understanding DP receptor antagonism using a CoMSIA approach

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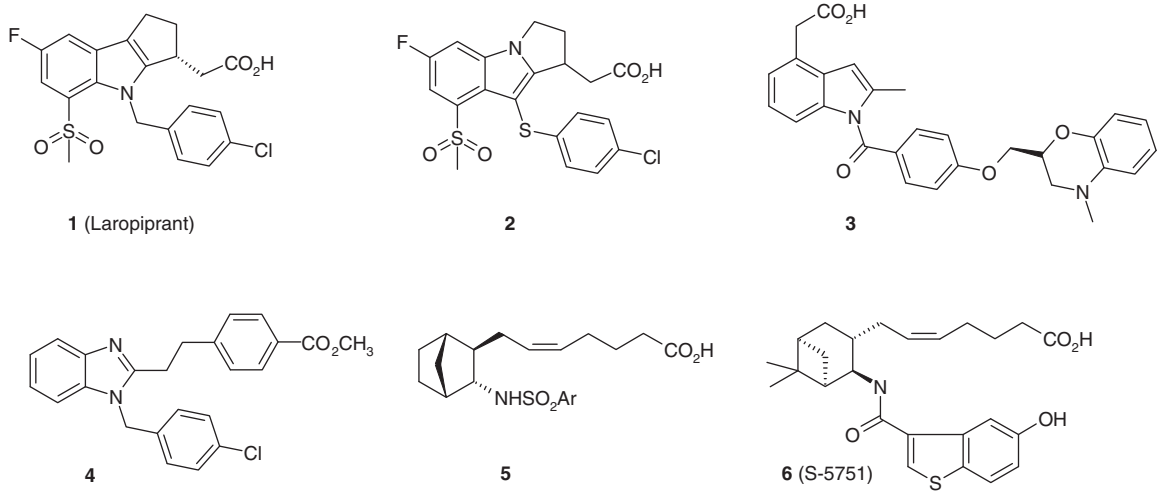
ABSTRACT

A Comparative Molecular Similarity Indices Analysis (CoMSIA) was performed for 2,6-substituted-4-monosubstituted aminopyrimidine antagonists of prostaglandin D_2 receptor (DP). Both two-component ($Q^2 = 0.63$, $R^2 = 0.82$, SEE = 0.47 pIC_{50}) and three-component ($Q^2 = 0.70$, $R^2 = 0.91$, SEE = 0.36 pIC_{50}) CoMSIA models were established. Two hydrogen-bond acceptors with spatial separation of about 8 Å are shown as optimal for binding. A large hydrophobic center that separates the two acceptors confers to the potency of the 2,6-substituted-4-monosubstituted aminopyrimidine. The models were used to predict IC_{50} values for compounds which had functional groups different from those in the training set.

Prostaglandin D_2 (PGD_2) is a major inflammatory mediator implicated in asthma and allergic rhinitis. It is largely produced as the major cyclooxygenase metabolite upon allergen-provoked degranulation from mast cells.[1,2] Intranasal instillation of PGD_2 causes a dose dependent increase in upper airway obstruction.[3] Thus, discovering antagonists of the DP receptor is actively



Scheme 1. DP antagonists.

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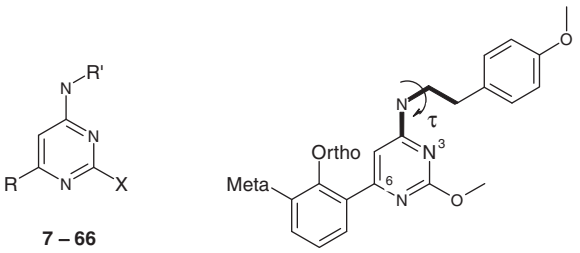
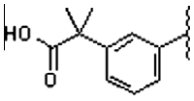
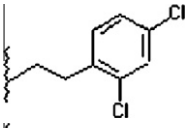
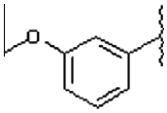
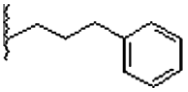
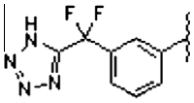
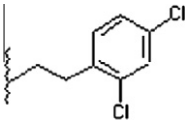
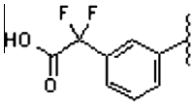
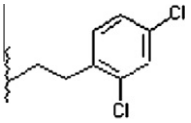
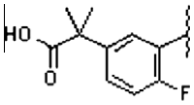
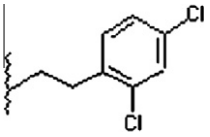
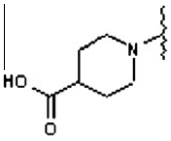
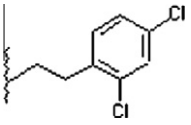
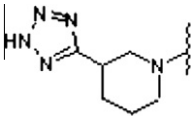
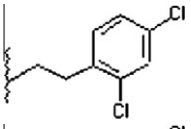
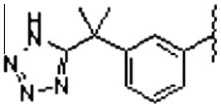
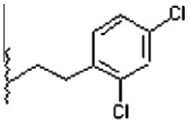
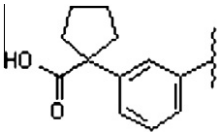
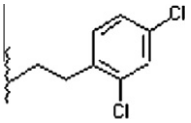
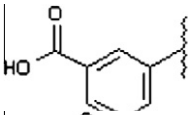
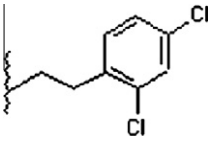
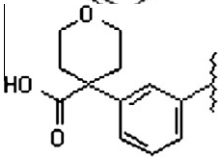
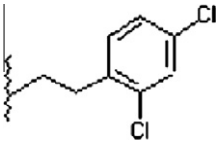


Scheme 2.

pursued as asthma and allergic rhinitis therapy,[4] and the structure–activity relationships of several series of DP antagonists (Scheme 1) have been reported.[5–9]

PGD_2 exerts its actions by activating two G-protein-coupled receptors.[2,4] One is the prostaglandin D_2 receptor (DP, DP1), and the other is CRHT2 (DP2), both of which are members of the prostanoids receptor subfamily. Blockage of PGD_2 using DP antagonists have been shown to be effective at alleviating the symptoms of allergic rhinitis in multiple species,[10,11] and more specifically have been shown to inhibit the antigen-induced nasal congestion.

Table 1
Training compound set[15]

No.	R	X	R′	pIC_{50} (exptl)	Calc_pIC_{50} (2 comp)	Calc_pIC_{50} (3 comp)
7		CH_2OMe		8.57	8.69	8.73
8		OMe		5.77	6.00	5.89
9		OMe		10.00	9.37	9.28
10		OMe		10.70	10.00	10.12
11		OMe		9.30	8.93	9.17
12		OMe		8.15	8.07	8.87
13		OMe		8.30	8.57	8.60
14		OMe		9.40	9.52	9.35
15		OMe		9.30	10.01	9.61
16		OMe		9.52	9.01	9.68
17		OMe		10.30	10.39	9.68

(*continued on next page*)

Table 1 (*continued*)

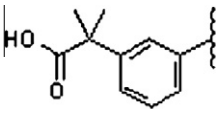
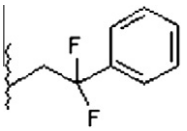
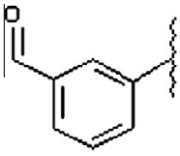
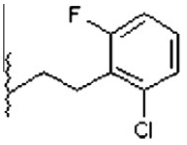
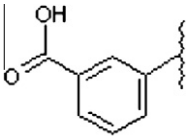
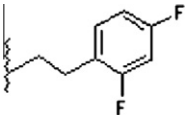
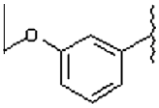
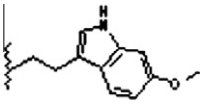
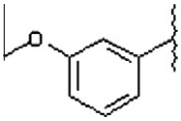
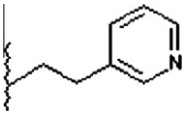
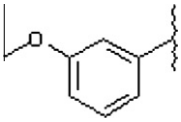
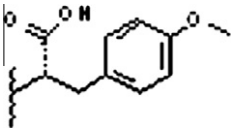
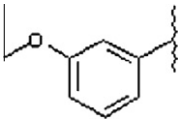
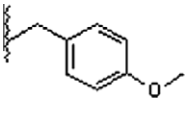
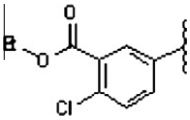
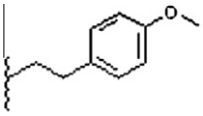
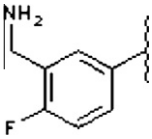
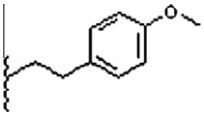
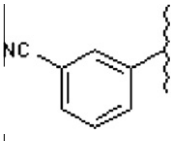
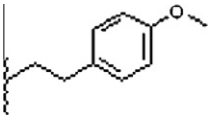
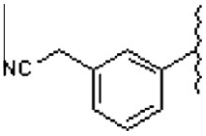
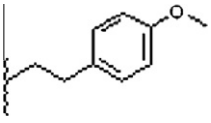
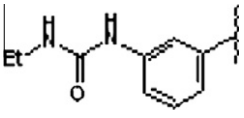
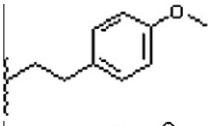
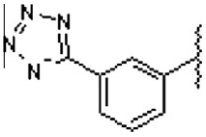
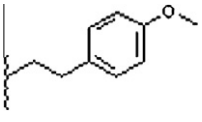
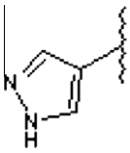
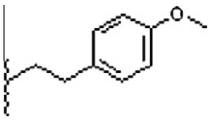
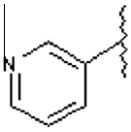
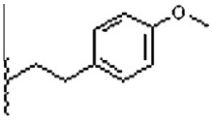
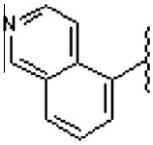
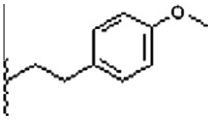
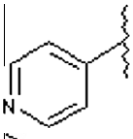
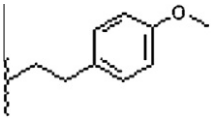
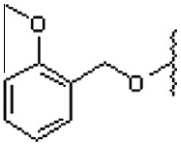
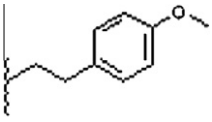
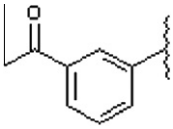
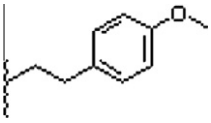
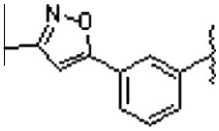
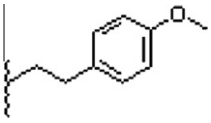
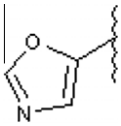
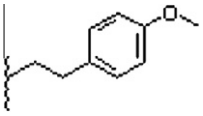
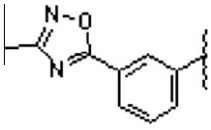
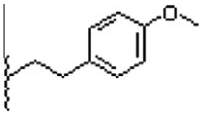
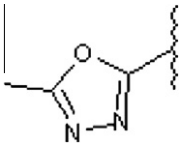
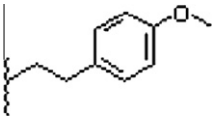
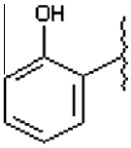
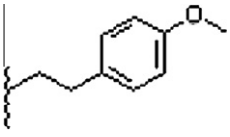
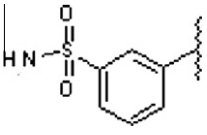
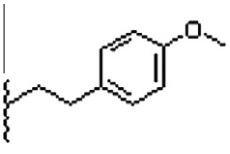
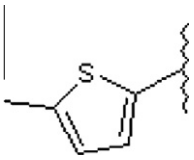
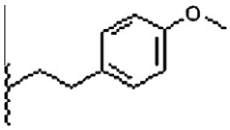
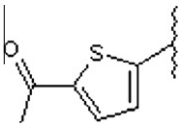
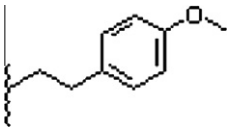
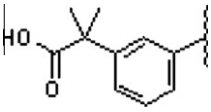
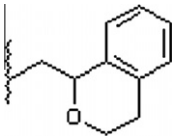
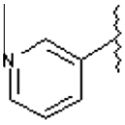
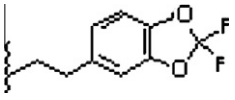
No.	R	X	R′	pIC_{50} (exptl)	Calc_pIC_{50} (2 comp)	Calc_pIC_{50} (3 comp)
18		OMe		7.42	8.17	7.83
19		OMe		8.44	8.58	8.45
20		OMe		9.10	8.95	9.60
21		OMe		6.98	6.80	6.83
22		OMe		6.61	6.75	6.69
23		OMe		6.26	6.52	6.18
24		OMe		5.97	5.75	5.75
25		OMe		7.49	7.92	7.79
26		OMe		7.39	7.99	7.63
27		OMe		9.05	8.43	8.80
28		OMe		8.22	8.51	8.33
29		OMe		8.12	8.56	7.92
30		OMe		9.40	8.14	8.85

Table 1 (*continued*)

No.	R	X	R′	pIC_{50} (exptl)	Calc_pIC_{50} (2 comp)	Calc_pIC_{50} (3 comp)
31		OMe		7.59	7.99	7.97
32		OMe		8.00	7.22	7.31
33		OMe		7.19	7.63	7.34
34		OMe		8.10	7.50	7.68
35		OMe		7.92	7.82	7.93
36		OMe		8.30	8.84	8.60
37		OMe		8.22	8.65	8.44
38		OMe		8.11	7.85	7.88
39		OMe		8.59	8.48	8.61
40		OMe		7.26	8.02	7.93
41		OMe		7.77	7.64	7.70
42		OMe		8.54	8.55	8.43

(*continued on next page*)

Table 1 (*continued*)

No.	R	X	R′	pIC_{50} (exptl)	Calc_pIC_{50} (2 comp)	Calc_pIC_{50} (3 comp)
43		OMe		8.09	7.85	7.84
44		OMe		8.42	8.10	8.13
45		OMe		7.64	7.32	7.22
46		OMe		6.67	7.07	7.13

The observed and predicted DP receptor potency values are listed.

Table 2
Validation compound set

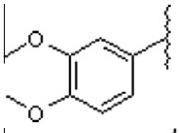
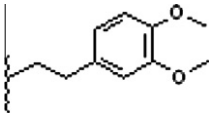
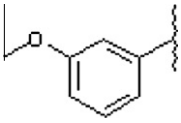
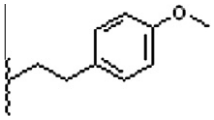
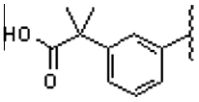
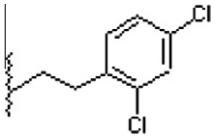
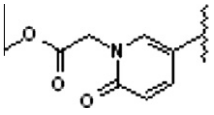
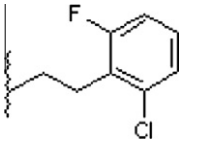
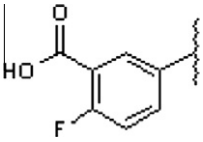
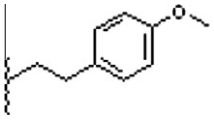
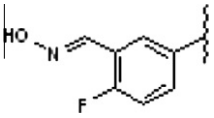
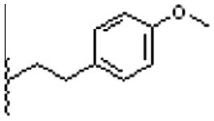
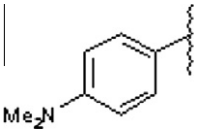
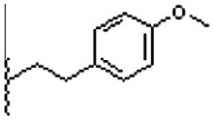
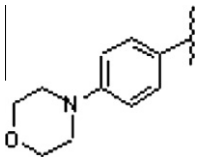
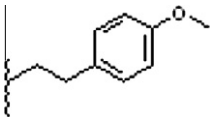
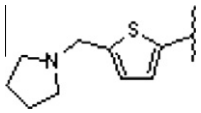
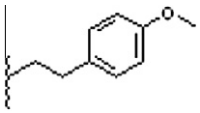
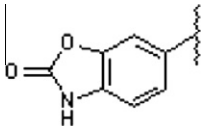
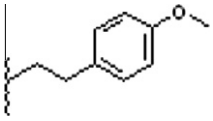
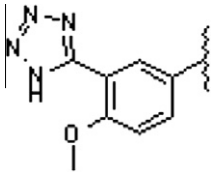
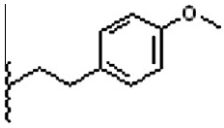
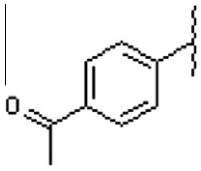
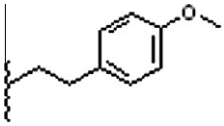
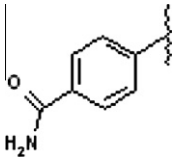
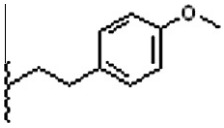
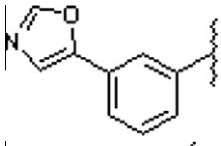
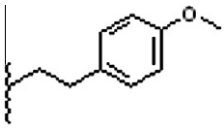
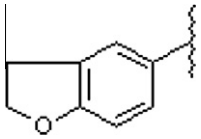
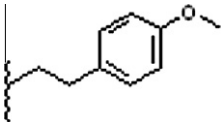
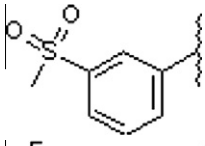
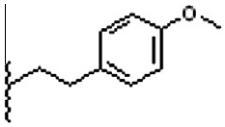
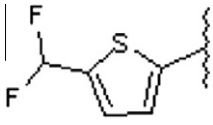
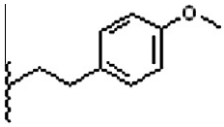
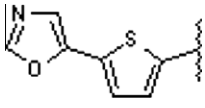
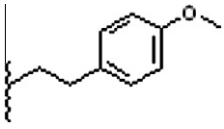
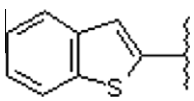
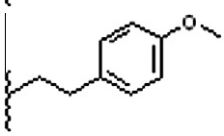
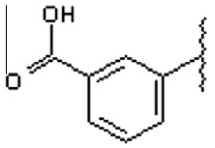
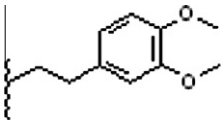
No.	R	X	R′	pIC_{50} (exptl)	Calc_pIC_{50} (2 comp)	Calc_pIC_{50} (3 comp)
47		Ethoxy		6.18	7.28	7.45
48		Ethyl		6.66	7.28	7.34
49		OMe		9.52	8.72	8.73
50		OMe		7.85	8.31	8.25
51		OMe		9.40	8.99	9.68
52		OMe		8.35	8.35	8.30
53		OMe		7.38	7.61	7.55

Table 2 (*continued*)

No.	R	X	R′	pIC_{50} (exptl)	Calc_pIC_{50} (2 comp)	Calc_pIC_{50} (3 comp)
54		OMe		7.89	7.73	7.71
55		OMe		7.36	8.03	7.96
56		OMe		8.15	8.03	8.19
57		OMe		9.30	8.90	8.72
58		OMe		8.20	7.73	8.09
59		OMe		8.64	7.78	8.14
60		OMe		8.59	8.04	7.95
61		OMe		7.89	7.82	7.57
62		OMe		8.14	8.78	8.76
63		OMe		7.96	8.20	8.05
64		OMe		8.64	8.05	8.16
65		OMe		8.29	7.61	7.67

(*continued on next page*)

Table 2 (*continued*)

No.	R	X	R′	pIC_{50} (exptl)	Calc_pIC_{50} (2 comp)	Calc_pIC_{50} (3 comp)
66		SMe		6.61	7.93	7.89

The observed and predicted DP receptor potency values are given.

A recent report shows that DP antagonists are also effective in suppression of nicotinic acid-induced flushing.[12]

We report here quantitative structure-activity relationship CoMSIA models[13,14] for the aminopyrimidine class of DP antagonists (Scheme 2). The models were developed and used to evaluate the functional antagonism of the compounds determined by cAMP assays in human LS174T cells.[15] The CoMSIA models provided detailed pharmacophore hypothesis on how, and where steric bulk or positive/negative charged groups would influence compound activity. In silico predictions were made based on the CoMSIA models, and have been used successfully for prioritization of the compounds proposed for synthesis.

CoMSIA models were constructed using the potency data of 40 DP receptor antagonists shown in Table 1. These are structurally diverse 2,6-substituted-4-monosubstituted aminopyrimidines, with pIC_{50} ($-\log IC_{50}$) activities ranging from 6 to 10. Subsequently synthesized 20 compounds shown in Table 2 served as the external prediction set for model validation.

CoMSIA calculations were performed using default settings in the SYBYL program (version 7.2, available from Tripos, Inc., St. Louis, MO). Initial 3D structures were generated by Concord within SYBYL 7.2, followed by molecular mechanics minimizations using the MMFF94 force field with a convergence criterion of RMS (root mean square) less than 0.01 kcal/(mol Å) at conjugate gradient steps. The conformations of 2,6-substituted-4-monosubstituted aminopyrimidine were generated from conformational searching following a Grid Search, as implemented in SYBYL 7.2 using the MMFF94 force field in vacuum. The candidate active conformations were selected from among those within 3 kcal/mol of the lowest minimum energy conformation (which also gave the best correlation in the CoMSIA model). For conformations of the ethyl aminopyrimidine scaffold, it was found the C–N–C–C torsion angle (τ) shown in Scheme 2 varies between 77° and 179° by searching Cambridge structure database. Different torsion angles used to generate the CoMSIA model do not significantly affect the overall results.

All compounds were aligned to the methyl aminopyrimidine cores that were derived from the minimum energy conformer of the most potent compound (**10**). The pariwise least-squares alignments were performed using SYBYL 7.2 Match utility.

CoMSIA indices were calculated using electrostatic and steric probes. The 3D grid that contained the aligned molecules was generated to have 470 points with 2 Å grid spacing and a minimum sigma of 2.0 kcal.

The details of the synthetic route for the 2,6-substituted-4-monosubstituted aminopyrimidine analogs listed in Tables 1 and 2 had been reported elsewhere by Lim et al.[15]

LS174T cells, which endogenously expressed the human DP receptor, were obtained from ATCC (American Type Culture Collection) and used for PGD_2-induced cAMP assays.[15] Cells were plated at 40,000 cells per well of a 96 well plate. After overnight incubation at 37 °C, medium was replaced and cells were stimulated with defined concentrations of PGD_2, or other agonists, for 15 min. cAMP accumulation was measured in the stimulated cells using the cAMP SPA (scintillation proximity assay) Direct Screening Assay System (Amersham) according to procedures specified by the manufacturer. For IC_{50} determination, compounds were pre-incubated with the DP transfected cells for 15 minutes, prior to stimulation with 15 nM PGD_2.

The indices of steric and electrostatic fields contribute 22% and 78%, respectively, to the overall model. The statistics of the model are shown in Figure 1. For the two-component model, the Q^2 of the validation set was 0.63 and the R^2 of the training set was 0.82. Adding one more component improved the Q^2 and R^2 to 0.70 and 0.91, respectively, and reduced the standard error of prediction (SEP) from 0.69 to 0.64 log units. However, both Q^2 and R^2 reached plateau with three components in the model.

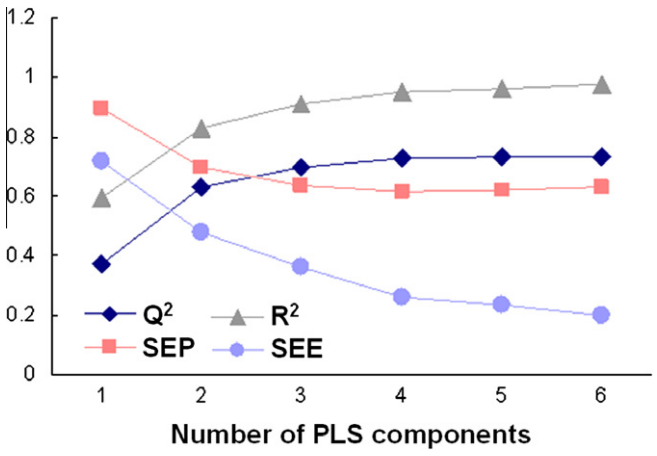


Figure 1. Statistical parameters Q^2, R^2, Standard Error of Prediction (SEP) of the validation set, and Standard Error of Estimate (SEE) from the PLS model.

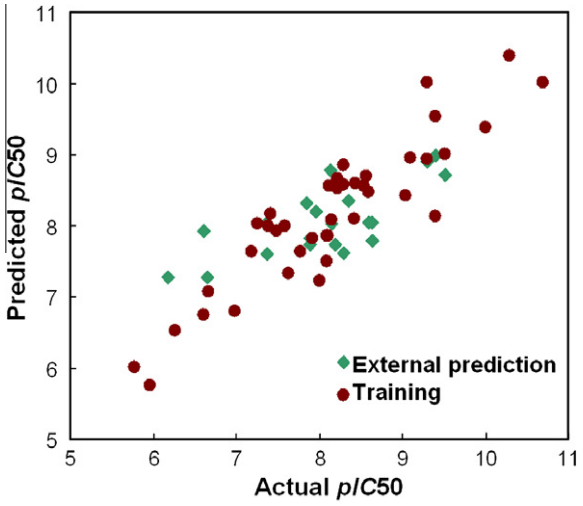


Figure 2. Predicted vs. observed pIC_{50} values of the test and validation data sets.

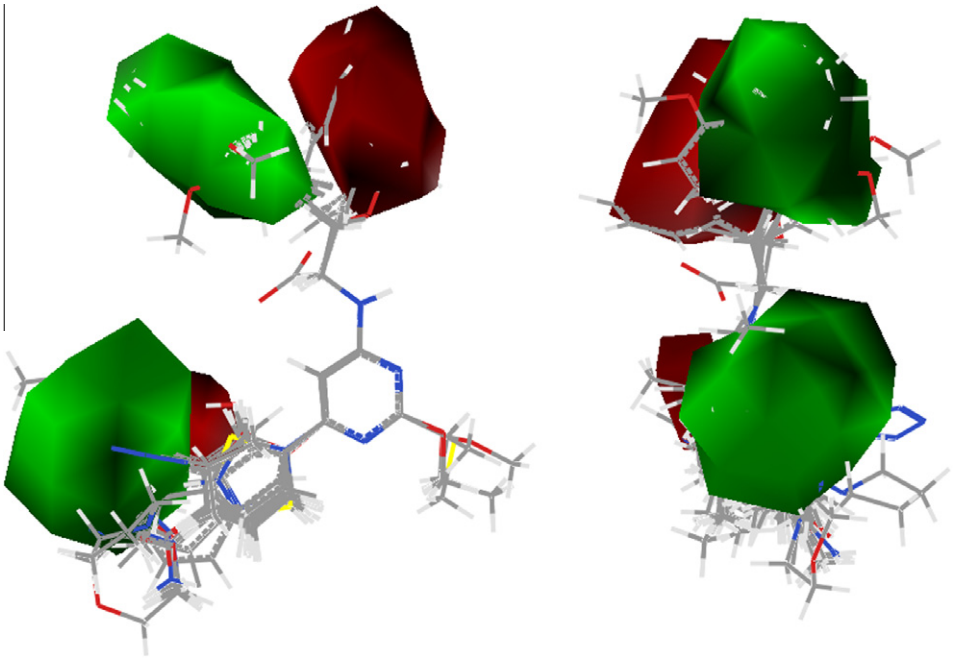


Figure 3. Steric CoMSIA coefficients of the two-component model. The contour plots correspond to the standard deviation coefficients at +0.005 (green) and −0.005 (red).

Both experimental and predicted pIC_{50} using two- and three-component models are listed in Tables 1 and 2 and the correlation plot is shown in Figure 2. The red and green dots represent compounds from the training and the external sets, respectively. Compound **30** is a significant outlier in the training set of the two-component model. This compound is a sodium salt that had high solubility.

The predicted IC_{50} values of two compounds, **47** and **66** in the validation set, were at least 10-fold greater than the corresponding observed values. Both models over-predicted the pIC_{50} for these compounds, with the three-component model being more pronounced. Compound **66** is the most obvious outlier in the validation set. Replacing the methoxy group at the 2-position by an ethyl (compound **47**) or methylsulfanyl (compound **66**) group resulted in a considerable loss of activity. The 2-methoxy and the pyrimidine sp^2-N at the 3-position are hypothesized to interact with Lys-76 in the 2nd TM (trans-membrane helix) of the DP receptor. In compound **66**, the sulfur could shield access to the sp^2-N at the 3-position thereby decreasing the activity of compound **66**. Unfortunately only one compound (**7**) in the training set had a different group (methoxymethyl instead of methoxy) at the 2-position. This limited variation at 2-position in the training set may have caused over prediction of the activity for compounds **47** and **66**.

Contour plots correspond to the standard deviation coefficients of +0.005 and −0.005 of the two-component model were shown in

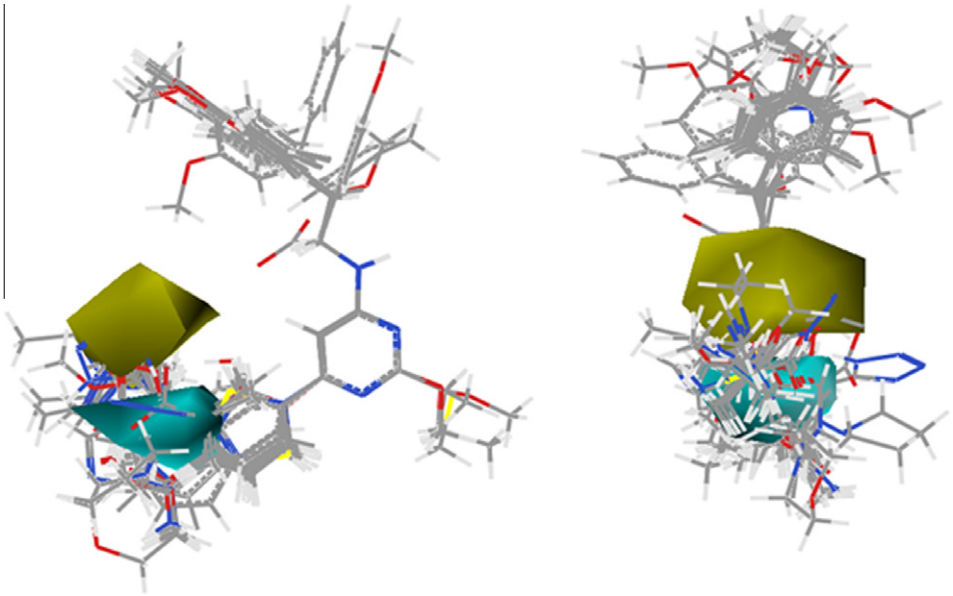


Figure 4. The electrostatic CoMSIA coefficients of the two-component model. The contour plots correspond to the standard deviation coefficients at +0.005 (cyan) and −0.005 (yellow).

Figures 3 and 4. The negative steric contour shown in red (Fig. 3) depicts regions that are unfavorable for steric bulk. Compounds of less activity such as **8** and **24**, with phenyl-propylamino and methoxybenzylamino substitutions, respectively, at the 4-position have their phenyl groups projected in the sterically unfavorable red zone, which are consistent with contours of the model. Another small red contour region is near the *meta*-position of the phenyl at of the 6-position. It reflects minor steric hindrance usually accompanied by a larger torsion angle between the core pyrimidine and an aryl substituent at the 6-position, such as **33**.

The positive steric contours shown in green in Figure 3 correspond to two regions that are favorable for increased steric bulk. One encompasses the phenyl portion of the phenylethylamino at the 4-position. Compounds with substituted phenyl in this region demonstrated good DP potency. The other green contour is located near the *meta*- of the phenyl at the 6-position, slightly opposite to the afore mentioned minor region of steric hindrance. Considerable variation of substitutions at the 6-position was tolerated, which may correspond to binding interactions with solvent exposed surface of the receptor.

The negative electrostatic contour in yellow as shown in Figure 4 encompasses the *meta*-carboxylate group on the phenyl substituent at 6-position. This carboxylate group is likely to interact inter-molecularly with an H-bond donor/salt-bridge partner on the receptor, presumably Arg-310 in the 7th TM domain of the human DP receptor (Fig. 5). Amino acid Arg-310 is conserved across

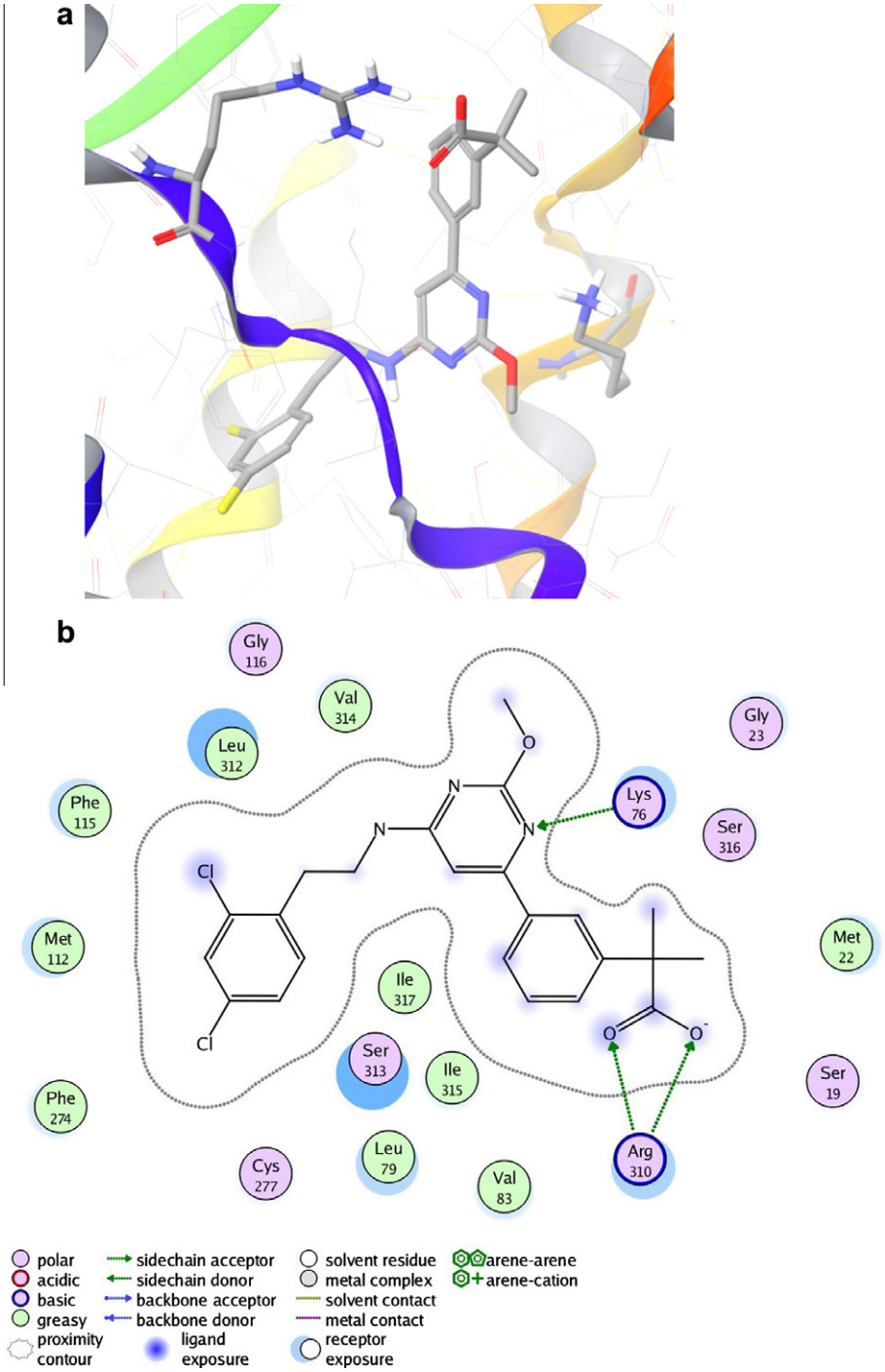


Figure 5. The predicted binding mode of the compound **49** in human DP homology model.

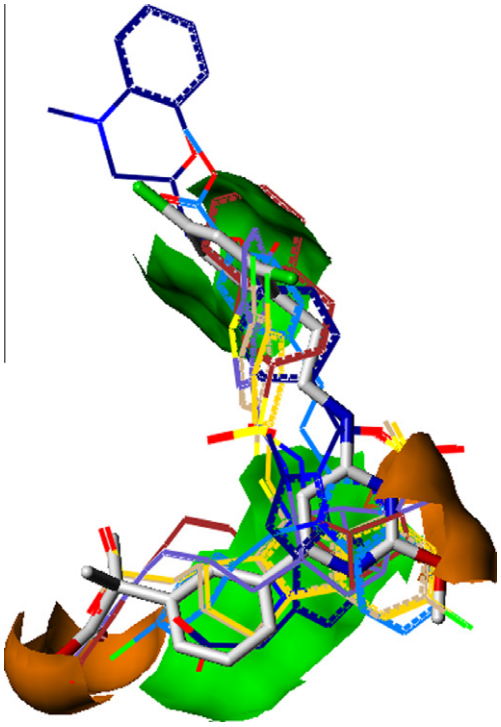


Figure 6. Overlay of known DP antagonists. Orange: H-bond acceptors; green: hydrophilic centers.

the prostanoids subfamily. All but one published DP receptor antagonists shown on Scheme 1[5–9] as well as the endogenous ligand PGD_2 possesses a carboxylate group. There appears to be an optimal distance between this carboxylate group (or its bioisostere) and another H-bond acceptor approximately 8 Å away so that they can interact simultaneously with Arg-310 and Lys-76 of the DP receptor, respectively. Specifically, the distal carboxylate, methoxy, methyl esters, tetrazole, sulfonamide substituents on the *meta*-substituted phenyl at the 6-position served as one of the acceptor, whereas the 2-methoxy group along with the sp^2-nitrogen at 3-position on the pyrimidine ring acted as the other acceptor.

The positive electrostatic contour shown in Figure 4 in cyan reflects a region in favor of a partial positive charged moiety. However, its contribution to the antagonist potency was deemed trivial as compared to other features.In summary, our CoMSiA model revealed several key pharmacophore features of DP antagonists:

(1) Two H-bond acceptors with spatial separation of about 8 Å. One of the acceptors is preferably negatively charged (to engage in salt-bridge with presumably Arg-310 in the seventh TM domain[16]). The negatively charged acceptor appears to be important for high affinity binding not only for our compounds but also for other known DP antagonists (Fig. 6).
(2) An H-bond donor adjacent to the main hydrophobic center of aminopyrimidine DP antagonists plays an important role in the binding interaction. Without such a donor for the compounds described here (data not shown), the functional antagonism diminishes.
(3) A large hydrophobic center that separates the two acceptors. This hydrophobic center spans at least 4 Å in space. Additional hydrophobic centers 8 Å away from the main hydrophobic center enhance antagonist potency (a hydrophobic rich interface among TM domains II, III and IV may be involved in binding.[16])

The pharmacophore features defined by the CoMSIA model are not only consistent with various known DP receptor antagonists, but they are also in good agreement with binding hypothesis derived from DP structural models[16] and mutation studies.[17,18]

Acknowledgments

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