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3D-QSAR studies on sildenafil analogues, selective phosphodiesterase 5 inhibitors

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Abstract—Sildenafil, one of selective phosphodiesterase 5 (PDE5) inhibitors, is a widely used oral agent for the treatment of erectile dysfunction. To develop new PDE5 inhibitors with improved therapeutic efficacy, a series of sildenafil analogues have been prepared and their in vitro PDE5 inhibitory activities were evaluated. Their IC_{50} values ranged from 423 to 0.05 nM. Herein, the results of 3D-QSAR (CoMFA and CoMSIA) analyses on these inhibitors are reported. Both CoMFA and CoMSIA gave reliable models with q^2 values >0.75 and r^2 values >0.99. The resulting CoMFA and CoMSIA models reveal a good correlation between the contour maps and the active site residues critical for the interaction with inhibitor, and nicely predict the key structural features of new analogues with improved activity and selectivity.
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The cGMP-specific PDE5 carries out the principal cGMP-hydrolyzing activity in human corpus cavernosum tissue. Sildenafil (Viagra®), a known selective and potent inhibitor of PDE5, was the first drug for the treatment of male erectile dysfunction in 1998.[1] By an action of blocking the PDE5 activity, cGMP accumulates in the corpus cavernosum and eventually leads to penile erection. PDE5 is a therapeutic target of considerable research interest as can be seen by numerous publications regarding the design, synthesis, and optimization of new PDE5 inhibitors such as vardenafil (Levitra® FDA approved on Aug. 2003), tadalafil (Cialis® FDA approved on Nov. 2003), and other compounds under clinical trials.[2,3]

To develop new PDE5 inhibitors with improved therapeutic efficacy based on the structure of sildenafil, we have prepared a series of sildenafil analogues and evaluated their in vitro PDE5 inhibitory activities (Fig. 1 and Table 1).[4] Many of these compounds were shown to have more potent inhibitory activities than sildenafil.

In this study, we have conducted a 3D-QSAR/CoMFA (comparative molecular field analysis) and CoMSIA (comparative molecular similarity indices analysis) on the compounds listed in Table 1 with respect to their PDE5 inhibition activity. The results would decide the substituent properties of these compounds and provide a rationale for the design of more selective and potent analogues.

CoMFA and CoMSIA. 3D-QSAR analyses[5] were performed using CoMFA and CoMSIA for training set of 26 novel compounds including sildenafil and testing set of 7 compounds listed in Table 1.

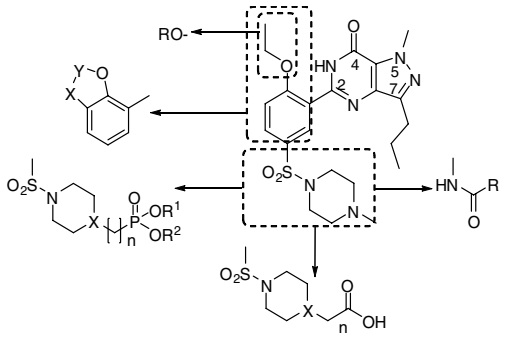

Figure 1. Modification of sildenafil structure.

Keywords: PDE5 inhibitors; 3D-QSAR modeling; SK3530.
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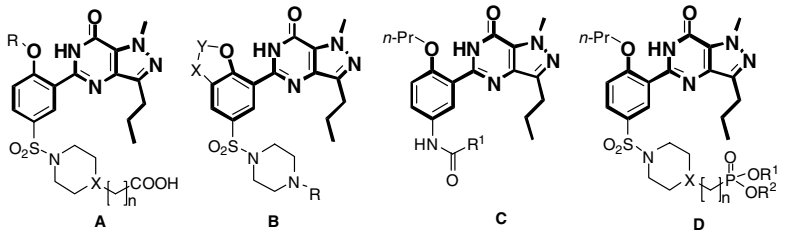
Table 1. Structure of the compounds in the training and test set



Entry	Group	R	*n*	X	Y	$-\log IC_{50}$ (μM)	Entry	Group	X	*n*	R^1	R^2	$-\log IC_{50}$ (μM)
1	A	Et	0	CH	—	3.29	17	C	—	—	Me	—	3.09
2	A	Et	1	CH	—	3.48	18	C	—	—	Et	—	3.42
3	A	Et	2	CH	—	3.82	19	C	—	—	*n*-Pr	—	3.57
4[a]	A	Et	3	CH	—	3.66	20[a]	C	—	—	*i*-Pr	—	3.46
5	A	Et	2	N	—	3.39	21[a]	C	—	—	*c*-Hex	—	3.38
6[a]	A	*n*-Pr	0	CH	—	3.82	22	D	N	1	Et	H	3.74
7	A	*n*-Pr	1	CH	—	3.96	23	D	N	2	Et	H	3.55
8	A	*n*-Pr	2	CH	—	4.3	24	D	CH	0	Et	H	3.52
9[a]	A	*n*-Pr	3	CH	—	3.3	25	D	CH	1	Et	H	3.77
10	B	Me	—	CH_2	CH_2	0.62	26	D	N	1	Et	Et	3.24
11	B	Me	—	O	CH_2	0.37	27	D	N	2	Et	Et	2.98
12[a]	B	Me	—	O	$(CH_2)_2$	2.1	28	D	CH	0	Et	Et	3.39
13	B	$(CH_2)_2OH$	—	CH_2	CH_2	0.69	29	D	CH	1	Et	Et	3.47
14	B	$(CH_2)_2OH$	—	CH_2	$(CH_2)_2$	1.9	30	D	N	2	H	H	3.03
15	B	$(CH_2)_2OH$	—	O	CH_2	0.48	31	D	CH	0	H	H	3.15
16	B	$(CH_2)_2OH$	—	O	$(CH_2)_2$	2.2	32[a]	D	CH	1	H	H	3.49
Sildenafil						2.75							

[a] Compounds in test set.

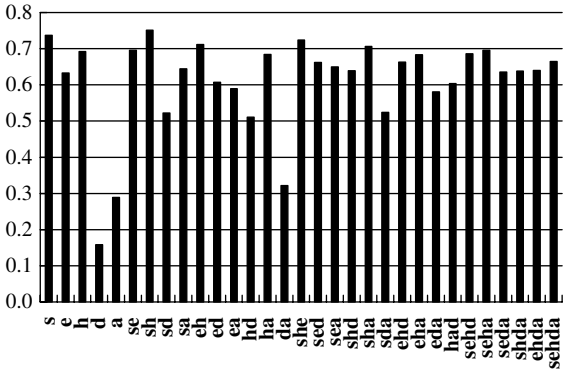


Figure 2. Results of cross-validated q^2 for different field combinations. (s, steric; e, electrostatic; h, hydrophobic; d/a, H-bond donor/acceptor).

The 3D-QSAR on CoMFA model was carried out using standard option in Sybyl.[6] Statistically significant values of CoMFA gave cross-validated $q^2 = 0.819$ with five optimized components. The ratio of contribution from steric and electrostatic is 0.446:0.554.

For CoMSIA modeling,[5] an evaluation of the five CoMSIA fields which are actually needed for a predictive model was initially performed by calculating all possible combinations of fields, and the results are shown in Figure 2. The first five models, using a single CoMSIA field, indicated that steric and hydrophobic fields were more important than the other three fields. The combination of steric and hydrophobic field gave the best model ($q^2 = 0.751$).

As the best CoMFA and CoMSIA model, a correlation coefficient of $r^2 = 0.995$ and a cross-validated coefficient of $q^2 = 0.819$ and 0.751 were obtained, respectively (Table 2). The final CoMFA and CoMSIA models were satisfactory by giving the statistical significance and the predictive ability of the training set and test set (see the Supplementary Fig. 1).

Binding mode of sildenafil. The X-ray crystal structure of PDE5 complexed with sildenafil (pdb entry = 1UDT)[7a] was retrieved from the Protein Data Bank (PDB). The active site region was defined as a sphere within the 6.5 Å from the reference ligand, sildenafil. Surface hydrophobicity (lipophilicity) potential physicochemical property map of the PDE5 active site was generated on the solvent assessible (connolly) surface using the MOLCAD module in SYBYL.[6]

The resulting lipophilic potential surface map of the binding site shows a general complementarity as depicted in Figure 3. Brown regions on the binding site surface map represent lipophilic potential (hydrophobic). The ethoxyphenyl group of sildenafils fit into the hydrophobic pocket formed by Phe786, Ala783, Leu804, and Val782, and the pyrazolopyrimidinone ring also forms hydrophobic interaction with the side chains of Val782, Tyr612, and Phe820 in the binding pocket.[7]

CoMFA and CoMSIA contour maps. To visualize the information content of the derived 3D-QSAR models, CoMFA and CoMSIA contour maps were generated. The field energies at each lattice point were calculated as the scalar results of the coefficient and the standard

Table 2. Summary of 3D-QSAR analysis results

	N^a	q^{2b}	r^{2c}	F value	SE[d]
CoMFA (steric, electrostatic)	5	0.819	0.995	759.0	0.092
CoMSIA (steric, hydrophobic)	5	0.751	0.995	726.4	0.094

[a] Optimum number of component.
[b] Cross-validated.
[c] Conventional.
[d] Standard error.

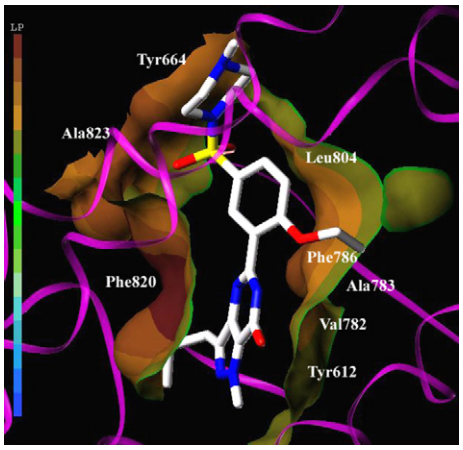


Figure 3. Surface representation of the sildenafil-bindig pocket of PDE5 in the X-ray structure. Color ramp (left): lipophilic potential (LP), magenta (ribbon): PDE5 secondary structure.

deviation associated with a particular column of data table, which was plotted as the percentage of the contribution to the CoMFA or CoMSIA equation. The contour plots around the substitutions could reflect the properties of the corresponding region in the active site of PDE5 and could guide the rational optimization of the sildenafil in the data set.

CoMFA and CoMSIA contour maps of steric fields revealed similar results (Fig. 4). The green contours represent the regions of high steric tolerance, while yellow contours represent regions of unfavorable steric effects. The sterically favored regions and electrostatic field are around the methylpiperazine group of sildenafil (Fig. 4A), which indicates that compounds with larger substitutions or charged are essential for high inhibitory activity. For example, the groups A and D that introduce a carboxylic acid group and phosphonate group to the 5′-sulfonamide moiety of the phenyl ring greatly enhanced PDE5 inhibitory activity.

As shown in Figure 4B and C, cyan regions in CoMSIA contour map indicate areas where hydrophobic substitutions are preferred. The hydrophobic favored regions are around the alkoxy group of the phenyl ring and methylpiperazine group of sildenafil, which is similar to the steric favored regions in CoMFA countour map. It was observed that the PDE5 inhibitory activity of group C was enhanced as the chain length of R^1 group increased. A comparison of group A and group B shows that the addition of an ether ring fused into the phenyl moiety results in the decrease of the inhibitory activity.

The open chain 2′-alkoxy group of the phenyl ring in compound **8**, although less effective for inducing the co-planarity, seemed to act as a much better hydrophobic requirement than the cyclic alkoxy moiety in compound **11** (Fig. 4B and C).

Validation of 3D-QSAR models. In the present study, we have established predictive CoMFA and CoMSIA 3D-QSAR models for a series of novel sildenafil analogues.[4] 3D-QSAR analyses showed that predicted activities correlate well with experimental IC_{50} values, suggesting that the 3D-QSAR models are reliable.

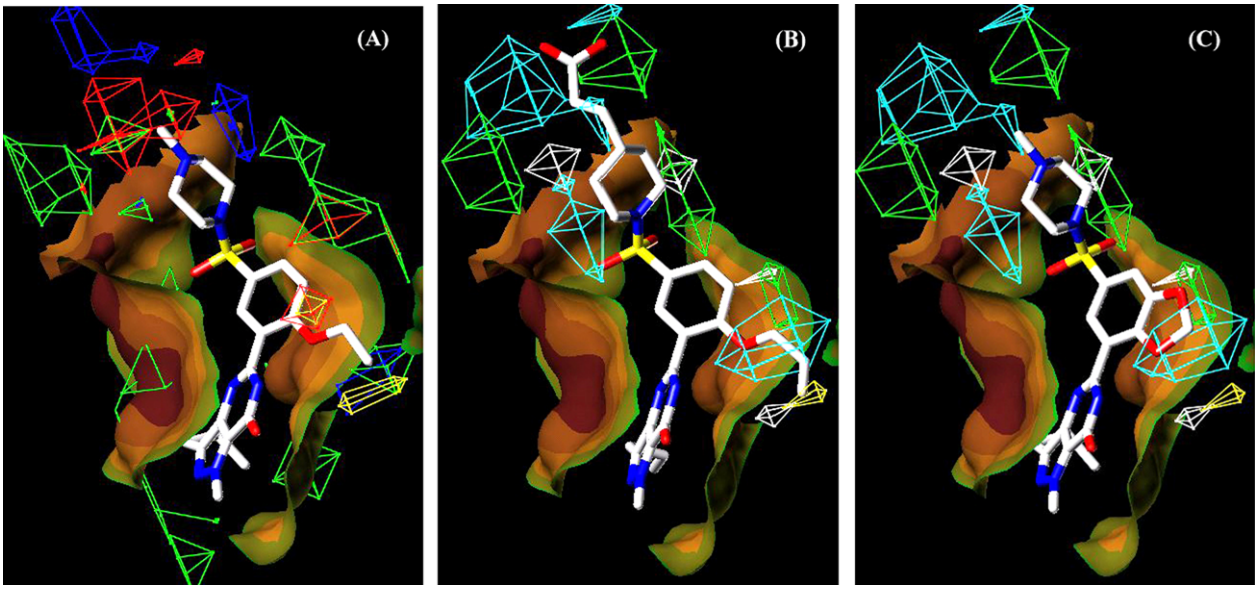


Figure 4. Contour plots of CoMFA model with sildenafil (A), and CoMSIA model with the most active compound **8** (B) and the least active compound **11** (C). Steric fields (green, bulky substitution favored; yellow, bulky substitution disfavored); electrostatic fields (blue, electropositive group favored; red, electronegative group favored); hydrophobic fields (cyan, favored; white, disfavored).

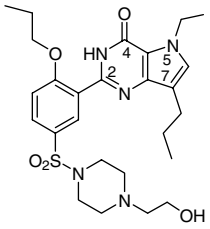


Figure 5. Structure of SK3530, 5-ethyl-2-{5-[4-(2-hydroxyethyl)-piperazine-1-sulfonyl]-2-propoxyphenyl}-7-propyl-3,5-dihydropyrrolo [3,2-*d*]-pyrimidin-4-one.

The resulting CoMFA-steric and CoMSIA-hydrophobic field maps well represent the sildenafil-binding pocket in PDE5 and suggest where to modify a molecular structure in order to improve the binding affinity.

Interestingly, the predictive ability of these models can be validated by the structure of SK3530 shown in Figure 5. SK3530 is a new analogue derived from series **B** and **C** (Table 1) having more improved activity and selectivity over other PDEs,[8] and is currently under clinical evaluation.[9]

The noticeable structural features of SK3530 are *N*-ethyl group of pyrrolopyrimidinone ring (N5 position) and propoxyphenyl group, which bear a substituent ;one methylene unit longer, respectively, in comparison with the corresponding positions of sildenafil. These alkyl substituents well conform to the features shown both in the CoMFA steric and CoMSIA hydrophobicity contour maps shown in Figure 4, which predict the positions for bulky and hydrophobic substituents to increase the activity.

In summary, the CoMFA and CoMSIA models obtained from this study offer crucial information about the three-dimensional interaction of various sildenafil analogues with PDE5. Especially, the steric and hydrophobic requirements for recognition of the active site of receptor are well described. This study provides structural insight into the design of analogous PDE5 inhibitor with improved activity and selectivity.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.05.064.

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5. Three-dimensional structures of the compounds in a dataset were prepared in MOL2 format using the sketcher module of Tripos Sybyl 7.0 software package based on Red-Hat Linux 3.0.5. The geometry of molecule was optimized until the energy gradient fell below 0.001 kcal/mol Å using the conjugate gradient method with standard Tripos force field and Gasteiger–Huckel charge. The PDE5-bound conformation of sildenafil in the X-ray structure (PDB entry = 1UDT) was used as a template, and common atoms of analogues listed (Table 1, bold bond) were defined as an anchor fragment in shape-based screening of FlexS technique. The minimum volume overlap was set at 0.6, and the number of alignments per ligand was 30. The alignment of top-ranked conformer of each compound was utilized in CoMFA and CoMSIA modeling. The CoMFA descriptors, steric and electrostatic field energies, were calculated by the following parameters: an sp3 carbon probe atom (+1 charge and 1.52 Å van der Waals radius) on a 2 Å spaced lattice, and energy cutoff of 30 kcal/mol. CoMSIA similarity indices were calculated on a rectangular grid containing the aligned molecules using steric, electrostatic, hydrophobic, H-bond donor, and H-bond acceptor fields. The attenuation factor was set to the default value of 0.3. The CoMFA and CoMSIA descriptors were used as independent variables, and $-\log IC_{50}$ values were used as dependent variables in partial least-squares (PLS) regression analyses to derive 3D-QSAR models. The optimum number of principal components is obtained by the leave-one-out (LOO) cross-validation procedure. Using the optimal number of principal components, the final PLS analysis was carried out without cross-validation to generate the predictive QSAR model with a conventional correlation coefficient r^2. To graphically interpret the 3D-QSAR results in terms of field contributions, isocontour maps were generated using the field type 'stdev*coeff' and the contour levels were set to default values.
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8. SK3530 shows IC50 values of 0.34, 16 400, 86 500, 10.2, and 3750 nM against PDE5, PDE1, PDE3, PDE6 (rodent), and PDE11A, respectively. In contrast, the IC_{50} values of sildenafil against PDE5, PDE1, PDE3, PDE6 (rodent), and PDE11A are 3.50, 281, 16 200, 37, and 2730 nM, respectively.
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