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3D-QSAR Studies of Latrunculin-Based Actin Polymerization Inhibitors Using CoMFA and CoMSIA Approaches

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Abstract

The marine-derived macrolide latrunculins A and B, from the Red Sea sponge *Negombata magnifica*, are known to reversibly bind actin monomers, forming 1:1 complex with G-actin, disrupting its polymerization. Latrunculins have remarkable physiological properties and widely used as biochemical markers. Nevertheless, no QSAR studies have been developed for any kind of actin disruptors. In the present study, Comparative Molecular Field Analysis (CoMFA) and Comparative Molecular Similarity Indices Analysis (CoMSIA) implemented in the SYBYL software packages were used to develop predictive 3D-QSAR models. Two alignment strategies were used to fit analyzed molecules to a suitable conformational template. By means of the SYBYL multifit alignment function, the best CoMFA and CoMSIA results presented cross-validated correlation coefficient values (q^2) of 0.621 and 0.659, and non-cross-validated values (r^2) of 0.938 and 0.965, respectively. Comparable to multifit-derived models, CoMFA and CoMSIA 3D-QSAR models were also derived using a molecular alignment obtained by docking latrunculin derivatives into the ATP active site of actin. In addition to q^2 , the predictive ability was validated using external test set of five compounds. The results of this study suggest that the established model has a strong predictive ability and can be prospectively used in the molecular design and action mechanism analysis of this kind of cytotoxic compounds.

Keywords

Latrunculin; Actin; CoMFA; CoMSIA; Contour maps; QSAR

1. Introduction

Actin is a cytoskeleton protein that forms versatile dynamic polymers, which can define cell polarity, control cell shape and promote stable cell-cell and cell-matrix adhesions [1]. Actin forms versatile dynamic polymers that can define cell polarity, organize cytoplasmic organelles, control cell shape and promote stable cell-cell and cell-matrix adhesions, and

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generate protrusive forces required for migration [1]. These functions usually fail and become abnormal in cancer cells [1].

The marine-derived macrolides latrunculins A and B were first reported by Kashman and coworkers from the Red Sea sponge *Negombata magnifica* [1,2]. Latrunculin A (**1**) was the first marine macrolide known to contain 16-membered ring and the unique 2-thiazolidinone moiety connected via a tetrahydropyran ring (THP) [2,3]. Latrunculins A and B and derivatives showed antiangiogenic, antiproliferative, antimicrobial, and anti-metastatic activities [4–7]. The most important biological effect of latrunculins is their ability to disrupt microfilament organization, inhibit microfilament-mediated processes without affecting the organization of microtubular system [6–15]. Latrunculins A and B reversibly bind the cytoskeleton actin monomers, forming a 1:1 complex with G-actin and disrupting its polymerization [6–14]. It has striking selectivity, rapid onset of action, and remarkable potency that exceed those of cytochalasin D by 1-2-fold order of magnitude [10]. Latrunculin A was also reported to decrease intraocular pressure and increase outflow facility without corneal effects in monkeys [9,10]. Latrunculin A also showed antiviral and antibacterial activities, inhibited stress-activated MAP kinase (SAPK) pathway [13], and suppressed hypoxia-induced HIF-1 activation in breast cancer cells [5].

The detailed binding of **1** to G-actin was identified as follows and depicted in Figure 1: C-1 carbonyl oxygen-through water-to glutamate 214 carboxy, C-17 lactol hydroxyl to arginine 210 NH (major binding), C-17 pyran oxygen to tyrosine 69 hydroxy, thiazolidinone NH to aspartate 157 carboxy, and thiazolidinone C-20 carbonyl oxygen to threonine 186 hydroxy [14,15].

To date, no QSAR studies have been established for any actin inhibitors. Since actin is essential for cellular proliferation, this activity was quantified by MTT proliferation assay using MCF-7 breast cancer cell lines. Therefore, the antiproliferative activities of latrunculin derivatives (Table 1) semisynthesized by the authors [4–6] were utilized to develop a three-dimensional quantitative structure-activity relationship (3D-QSAR) models to correlate their biological activities with three-dimensional structures. This was accomplished with the use of the comparative molecular field analysis (CoMFA) [16,17] and the comparative molecular similarity indices analysis (CoMSIA) [18–20]. These studies provided a better understanding of the latrunculin structural elements that are essential for actin binding. CoMFA and CoMSIA provided contour maps that should guide the future structural modifications for design of latrunculin-related actin disruptors with enhanced activity and to assist *de novo* design of small molecules that fit ATP binding cleft of actin with potent activities.

2. Results and Discussion

2.1 3D-QSAR Modeling

Since there is no previous report of any QSAR model for latrunculins or other actin disruptors in general, it is very crucial to build such model because it will enable the prediction and optimization of the properties and activities of untested latrunculins and determine the key structural requirements for high binding affinity and actin disruption. Therefore, 3D-QSAR models for latrunculins were designed using the most widely used computer-based methodologies, CoMFA and CoMSIA.

Good correlation between antiproliferative activities against MCF and actin polymerization inhibition in derivatives **1–14** was established [6] with good correlation coefficient ($R^2 = 0.8797$, Figure 2). Therefore, latrunculins **1–25** were tested for their antiproliferative activities against MCF7, and the calculated IC_{50} were then used in the CoMFA and

CoMSIA QSAR analysis (Tables 1 and 2). The actual and predicted (by CoMFA and CoMSIA) pIC₅₀ for latrunculins are listed in Table 2.

PLS, the statistical method used in deriving the 3D-QSAR models, is a variation of principal component regression in which the original variables are replaced by a small set of linear combinations thereof [21]. The latent variables so generated were used for multivariate regression, maximizing the communality of predictor and response variable blocks. The advantages of PLS include: 1- the ability to handle multivariate regression analysis in cases where the number of independent variables is greater than the number of samples as found in CoMFA and CoMSIA 3D-QSAR analysis; 2- the ability to perform well even when inter-descriptor correlations exist, which would pose a problem for traditional multiple linear regression [22]; and 3- the reduction of the risk of chance correlations [33]. Initial leave-one-out (LOO) cross-validated PLS analysis were used to determine the optimum number of components to be used in the final QSAR models. PLS results are summarized in Table 3 while Figures 3 and 4 show the prediction curves obtained with the final CoMFA and CoMSIA 3D-QSAR models.

The LOO cross-validation method might lead to high q^2 values, which do not necessarily reflect a general predictiveness of the models. Therefore, cross-validation was performed using two groups (leave-half-out) in CoMFA study. In this method, 50% of the compounds were randomly selected and a model was generated, which was then used to predict the activity of the rest of compounds. The random formation of the cross-validation groups may have an effect on the results and therefore cross-validation was performed 100 times for all the analysis. The mean q^2 value was 0.588 which is slightly lower compared to the values obtained with the LOO method. In no case were q^2 values negative. The obtained results suggest that there is a good internal consistency in the underlying data set.

2.2 3D-QSAR Studies with Multifit Alignment

2.2.1 CoMFA 3D-QSAR Models—PLS analysis of the multifit alignment of all of the compounds in the training set using default parameters resulted in a CoMFA QSAR model with a good q^2 value of 0.621. Examination of the residuals from the LOO cross-validated predictions indicated that compound **7** might be outliers. Omission of this compound resulted in an increase in the q^2 value to 0.694 for the remaining 24 compounds. The outlier status of **7** could stem from the structural uniqueness of cyclopentane attached to thiazolidinone nitrogen. One would expect a lower potency for **7** than it exhibited based upon the presence of the *N*-cyclopentyl moiety, which possibly act like methyl or ethyl substituents.

Lower energy cutoff values, e.g., 25, 20, and 15 kcal/mol, were investigated, but all led to a decrease in the q^2 value (data not shown). The PLS stdev*coefficient contour maps for the CoMFA model are shown in Figure 5. Green regions indicate areas where steric bulk is predicted to enhance the actin-disrupting activity, whereas yellow contours indicate regions where steric bulk is predicted to be detrimental to this activity. Blue-colored regions indicate areas where electropositive groups are predicted to enhance the actin-binding affinity, while red regions represent areas where electronegative groups are predicted to favor the activity. There is major green contour in the frontward area of C-17 hydroxyl and thiazolidinone nitrogen. This is immediately flanked by yellow contours suggesting that limited bulk right close to the molecules will be favorable but cannot be extended beyond that (Figure 5B). This is obvious in **3** and **18** (the most potent compounds) where aromatic group with specific a distance, as described in SAR, will improve the activity of **1**, or recover the activity of **2** as in **9**, **10**, and **13**. These aromatic rings are accommodated in Gly 156, Gly 182, Arg 206, and Arg 210 pocket (Figure 1). However, extra-steric effect is unfavorable as illustrated in the suppressed antiproliferative activities in **11**, **15**, **16**, **17**, **23**, and **24**.

There are prominent red contours almost at the same area covered by sterically favored green contour (Figure 5A). This electronegative favored area is generated by the electronic clouds of benzene rings of the active compounds **3** and **18**, and it complements with the electropositive red area of Arg 210 for the electrostatic potential surface map of the binding site generated by MOLCAD (Figure 1). This mapping provides optimum electrostatic interaction between the electron cloud of the aromatic ring and the positively-charged guanidine moiety of Arg 210. A blue contour resides in the front of thiazolidinone nitrogen, bracing the hydroxyl-hydrogen atoms of *N*-hydroxymethylene moiety in **14** and **25**, indicating that electropositive groups are predicted to increase activity in this area (Figure 5A). This also further indicates that HBD groups will be favored in this location and complements with the electronegative blue area created by carboxylate group of Asp 157 (Figures 1 and 5A).

2.2.2 CoMSIA 3D-QSAR Models—CoMSIA is less affected by changes in molecular alignment and provides smoother and interpretable contour maps as a result of employing Gaussian type distance dependence with the molecular similarity indices it uses. Furthermore, in addition to the steric and electrostatic fields, CoMSIA defines explicit hydrophobic and HBD and HBA descriptor fields, which are not available with standard CoMFA.

Using the default SYBYL CoMSIA parameters and all compounds in the training set with the multifit molecular alignment resulted in a PLS model with a better q^2 value of 0.659 (as compared to a q^2 value of 0.621 for CoMFA) and an r^2 value of 0.965 for 4 PLS components. CoMSIA 3D-QSAR models performed well in the prediction of the inhibitory activities of the test compounds and much better than the CoMFA 3D-QSAR models. This can be seen from the prediction curves for the test compounds (Figure 4). The superior performance of CoMSIA relative to CoMFA, with this data set, may be attributed to the higher contributions from the hydrophobic and HBD and HBA fields to the CoMSIA QSAR models (Table 3). Unlike CoMSIA, CoMFA does not have explicit hydrophobic and hydrogen-bonding descriptors, which are assumed to be implicitly treated in the CoMFA steric and electrostatic fields, respectively.

The CoMSIA steric and electrostatic PLS contours (not shown) were similarly placed as those of the CoMFA model. The additional hydrophobic, HBD and HBA contours of CoMSIA are displayed in Figures 6A and B, respectively. The hydrophobic fields (yellow, hydrophobic group favored; white, hydrophobic disfavored) and the HBD (cyan, favored; purple, disfavored) and HBA (magenta, favored; red, disfavored) fields indicate areas around the molecules where changes increased or decreased activity. The HBD fields made the highest contribution to the CoMSIA QSAR models (Table 3), which suggests that among the descriptors considered, the HBD is the most important factor influencing the actin-disrupting activity of the latrunculins in the training set. This indicates that the presence of free hydroxyl groups at C-17 (**1**), C-15 (**20**), and *N*-hydroxymethylenes (**14** and **25**) were important for binding at the active site.

Similar to steric contour in CoMFA, hydrophobic-favored yellow contour is flanked by hydrophilic favored white contours (Figure 6A), suggesting that limited hydrophobic substitution right close to the molecules will be tolerable but cannot be extended to the hydrophilic surface of Arg 206 and Arg 210. The HBD-favored cyan contours overlapped with HBA-disfavored contour are located at the area between C-17 and the thiazolidinone nitrogen (Figure 6). This model explains the dramatic activity reduction of the C-17 and C-15 latrunculin A and B methyl ethers **2** and **22**, respectively. A large cyan HBD contour is facing the thiazolidinone nitrogen, necessary to engage H-bonding with Asp 157 for optimum orientation inside the active site (Figure 6B). Acceptor-favored magenta contour is

found against C-1 carbonyl oxygen to form H-bond bridged by water molecule to Glu 214 carboxy (Figure 6B).

2.3 3D-QSAR Studies with Docked Alignment

In addition to the 3D-QSAR studies using the multifit alignment described above, CoMFA and CoMSIA studies with molecules aligned by docking at the ATP binding cleft of actin monomer were also conducted. The docked alignment was quite different as the molecules were more staggered, and compounds **7** and **21** were outliers from the start as they were docked differently from the rest of the compounds. A CoMFA 3D-QSAR model with a modest q^2 value (0.418 with 2 PLS components, Table 3) was obtained using 25 of the compounds in the docked alignment. It is interesting to note that compound **7** was indicated outliers in the CoMFA model using the multifit alignment. Elimination of the outliers (**7** and **21**) in this CoMFA model resulted in a substantial increase in the q^2 value of the model to 0.502 with 3 PLS components for the remaining 23 compounds and an r^2 value of 0.988. The steric and electrostatic field contributions to this model were 0.569 and 0.431, respectively. In the multifit alignment CoMFA, the corresponding field contributions were 0.674 and 0.326, respectively (Table 3), indicating that the steric fields dominated in both CoMFA models. The CoMFA PLS steric and electrostatic field coefficient map obtained for the docked alignment is shown in Figure 7. It shows similarity to the map obtained for the multifit alignment CoMFA (Figure 5). This indicates a general agreement between both CoMFA models.

Similarly, CoMSIA 3D-QSAR study was conducted on the molecules in the docked alignment. CoMSIA PLS analysis afforded a q^2 value of 0.586 with 3 PLS components for the 25 compounds in the alignment (Table 3). CoMSIA provided a better QSAR model than CoMFA with this alignment as indicated by the improved q^2 value and the number of compounds that could be accommodated. The superiority of CoMSIA over CoMFA found with the multifit alignment QSAR models was magnified with the docked alignment. This can be justified via two factors; 1- CoMSIA model is less affected by alignment heterogeneity. 2- The predominant contribution of H-bonding fields in CoMSIA models which is absent in CoMFA models (Table 3). The relative order of importance of the various CoMSIA descriptor fields, i.e., HBD, steric, hydrophobic, HBA, and electrostatic is the same as the order obtained by the multifit alignment CoMSIA (Table 3). The HBD field is shown to be the most important, once again. The CoMSIA PLS field coefficient contour maps obtained with the docked alignment are shown in Figures 7C and D. The hydrophobic and hydrogen bond field contour maps are very similar to the corresponding maps of the multifit alignment CoMSIA (Figure 6), which further document the close agreement between both CoMSIA models.

3. Conclusions

Latrunculins are potent and selective inhibitors of actin polymerization. 3D-QSAR analyses characterized the key structural elements of latrunculins required for efficient actin binding. 3D-QSAR models have good cross-validated q^2 values, suggesting a good predictive ability. The characteristics of the 3D contour plots derived in these study assess the understanding of the underlying mechanism of receptor—drug interaction. The 3D-QSAR study of the latrunculins provides the basis for future rational design of new direct actin disruptors for possible experimental and therapeutic use as anti-motility, antiproliferative, and anti-invasive entities. Figure 8 presents five examples that were designed according to our current understanding of SAR and the 3D-QSAR models with potent predicted activities.

4. Materials and Methods

4.1 3D-QSAR Data Setting

Twenty-five latrunculin analogues (natural and semisynthetic, Table 1) [4–6] were used to build CoMFA and CoMSIA 3D-QSAR models. An attractive feature of these compounds is their relative conformational rigidity, which makes them more amenable to meaningful CoMFA and CoMSIA analysis than flexible molecules. Furthermore, X-ray crystallographic structural of **1** [14] is available, which provided a template for modeling other latrunculins. To maintain the active conformation, actin-bounded crystallographic structure of **1** was used as template to sketch other compounds using SYBYL 8.0. Confort conformational analysis was conducted for each derivative and the lowest energy conformer was used later in the alignment procedure. The IC₅₀ (concentration causing 50% inhibitory effect in the MCF7 proliferation) values were converted to pIC₅₀ (–log IC₅₀) values and used as dependent variables in the CoMFA and CoMSIA QSAR analysis. It was imperative to evaluate the predictivity of the 3D-QSAR models generated. The molecules were divided into training set and test set. Selection of the training set and the test set molecules was done by considering the fact that test set molecules represent a range of biological activity similar to that of the training set. Thus, the test set was the true representative of the training set. This was achieved by arbitrarily setting aside 5 compounds as a test set with a regularly distributed biological data. Table 2 shows the actual and predicted activity for both training and test sets.

4.2 Molecular Modeling and Alignment

Three-dimensional structure building and all modeling were performed using the SYBYL program package [24], version 8.0, installed on DELL desktop workstations equipped with a dual 2.0 GHz Intel® Xeon® processor running the Red Hat Enterprise Linux (version 5) operating system. Conformations of each compound in the training set were generated using Confort™ conformational analysis. Energy minimizations were performed using the Tripos force field [25] with a distance-dependent dielectric and the Powell conjugate gradient algorithm with a convergence criterion of 0.01 kcal/(mol Å). Partial atomic charges were calculated using the semiempirical program MOPAC 6.0 and applying the AM1 [26].

CoMFA and CoMSIA studies required that the 3D structures of the analyzed molecules to be aligned according to a suitable conformational template, which was assumed to be a “bioactive” conformation [17]. The molecular alignments used for the studies were obtained by means of the SYBYL “multifit” alignment function or SurFlex-docked alignment. For the multifit alignment, the most active compound (**3**) was used as the alignment template. Molecular alignment using molecular docking was achieved by SurFlex dock as described in Molecular Docking.

4.3 CoMFA and CoMSIA 3D-QSAR Models

To derive the CoMFA and CoMSIA descriptor fields, a 3D cubic lattice with a grid spacing of 2 Å and extending 4 Å units beyond the aligned molecules in all directions was created to encompass the aligned molecules. CoMFA Descriptors were calculated using sp³ carbon probe atom with a van der Waals radius of 1.52 Å and a charge of +1.0 to generate steric (Lennard-Jones 6–12 potential) field energies and electrostatic (Coulombic potential) fields with a distance-dependent dielectric at each lattice point. The SYBYL default energy cutoff of 30 kcal/mol was used as well as cutoffs of 25, 20, and 15 kcal/mol. The CoMFA steric and electrostatic fields generated were scaled by the CoMFA-STD method in SYBYL.

CoMSIA similarity indices descriptors were derived as previously described¹⁸ with the same lattice box as was used for the CoMFA calculations, with a grid spacing of 2 Å employing a

C¹⁺ probe atom with a radius of 1.0 Å as implemented in SYBYL. Five physicochemical properties (steric, electrostatic, hydrophobic, HBD, and HBA) were evaluated using the probe atom. In CoMSIA, the steric indices were related to the third power of the atomic radii, the electrostatic descriptors were derived from atomic partial charges, the hydrophobic fields were derived from atom-based parameters developed by Viswanadhan et al. [19], and the HBD and HBA indices were obtained by a rule-based method derived from experimental values [20].

The CoMFA and CoMSIA descriptors were used as independent variables, and pIC₅₀ values were used as dependent variables in partial least squares (PLS) regression analysis to derive 3D-QSAR models using the standard implementation in the SYBYL package. The predictive value of the models was evaluated first by LOO cross-validation. The cross-validated coefficient, q^2 , is defined as $q^2 = 1 - \text{PRESS} / \sum (Y - Y_{\text{mean}})^2$ where $\text{PRESS} = \sum (Y - Y_{\text{Pred}})^2$. To maintain the optimum number of PLS components and minimize the tendency to over fit the data, the number of components corresponding to the lowest PRESS value was used for deriving the final PLS regression models. In addition to the q^2 the number of components, the conventional correlation coefficient r^2 and its standard error s were also computed. CoMFA and CoMSIA coefficient maps were generated by interpolation of the pair-wise products between the PLS coefficients and the standard deviations of the corresponding CoMFA or CoMSIA descriptor values.

4.4 Molecular Docking

Surflex-Dock program version 2.0 interfaced with SYBYL 8.0 was used to dock the compounds to the active site of actin [27,28]. SurFlex-Dock employs an idealized active site ligand (protomol) as a target to generate putative poses of molecules or molecular fragments [29]. These putative poses were scored using the Hammerhead scoring function [30]. The program was used to dock the training set molecules into the active site of G-actin. The 3D structure was taken from the Brookhaven Protein Databank (PDB code: 1esv) [14].

4.5 MTT Proliferation Assay

The growth of MCF7 breast cancer cell lines was measured using MTT kit (TACSTM, TREVIGEN[®], Inc.) [31,32]. Exponentially growing cells were plated in a 96-well plate at a density of 8×10^3 cells per well, and allowed to attach for 24 h. Complete growth medium was then replaced with 100 µL of RPMI of serum free medium (GIBCO-Invitrogen, NY) containing various doses (50, 10, 1, 0.5, 0.1 µM) of the specific tested compound and culture continued at 37 °C under 5% CO₂. After 72 h of culture, the cells were treated with MTT solution (10 µL/ well) at 37 °C for 4 h. The color reaction was stopped by the addition of solubilization/stop solution (100 µL/well), and the incubation at 37 °C continued to completely dissolve the formazan product. Absorbance of the samples was determined at 570 nm with an ELISA plate reader (BioTek, VT). The number of cells per well was calculated against a standard curve prepared by plating various concentration of cells, as determined by hemocytometer, at the start of each experiment. IC₅₀ for each compound was calculated using nonlinear regression (curve fit) of log concentration versus the number of cells/well implemented using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA).

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Figure 1. MOLCAD visualization of **1** into the ATP binding site of actin showing the interacting amino acids.

Figure 2.
Correlation of actin polymerization inhibition of compounds **1–14** with their antiproliferative activities against MCF7 breast cancer cell line.

Figure 3.

CoMFA predicted versus experimental pIC_{50} values. (a) Fitted predictions for the training set. Correlation coefficient (r^2) = 0.938. (b) Fitted predictions for the test set. Correlation coefficient (r^2) = 0.8442.

Figure 4.

CoMSIA predicted versus experimental pIC_{50} values. (a) Fitted predictions of the training set. Correlation coefficient (r^2) = 0.965. (b) Fitted predictions for the test set. Correlation coefficient (r^2) = 0.8616.

Figure 5. Graphic presentation of CoMFA model generated by multfit-alignment with compound **11** displayed. (a) CoMFA stdev*coeff steric contour plots. (b) CoMFA stdev*coeff electrostatic contours plots.

Figure 6. Graphic presentation of CoMSIA model generated by multifit-alignment with compound **11** displayed. (a) CoMSIA stdev*coeff hydrophobic contour plots. (b) CoMSIA stdev*coeff HBD and HBA contour plots.

Figure 7. Graphic presentation of CoMFA and CoMSIA models generated by docked-alignment with compound **11** displayed. (a) CoMFA stdev*coeff electrostatic contour plots. (b) CoMFA stdev*coeff steric contour plots. (c) CoMSIA stdev*coeff HBD and HBA contour plots. (d) CoMSIA stdev*coeff hydrophobic contour plots.

Figure 8. Proposed latrunculin A derivatives designed according to multfit-aligned 3D-QSAR studies. The predicted activities present the average CoMFA and CoMSIA predictions.

Table 1

Structure of Latrunculin Analogues.^a

Compound	R ₁	R ₂	Compound	R ₃	R ₄
1	H	H	20	H	H
2	CH ₃	H	21	H	COCH ₃
3	a	H	22	CH ₃	H
4	b	H	23	(CH ₂) ₃ CH ₃	H
5	CH ₃	CH ₃	24	(CH ₂) ₇ CH ₃	H
6	CH ₃	CH ₂ CH ₃	25	H	CH ₂ OH
7	CH ₃	c			
8	CH ₃	CH ₂ CH ₂ CH ₂ OH			
9	CH ₃	d			
10	CH ₃	b			
11	CH ₃	e			
12	H	CH ₂ CH ₃			
13	H	d			
14	H	CH ₂ OH			
15	CONHCH ₂ CH ₂ Cl	H			
16	CONHCH ₂ CH ₂ Br	H			
17	CONH(CH ₂) ₃ Cl	H			
18	CONHC ₆ H ₅	H			
19	CONHCH ₂ C ₆ H ₅	H			

^aIn bold face, the reference atoms used in multifit alignment protocol of the SYBYL program.

Table 2Experimental and Predicted pIC₅₀ of The Training and Test Sets.

compound	actual pIC ₅₀ ^a	pred. pIC ₅₀ ^b	pred. pIC ₅₀ ^c	sets
1	0.319	0.536	0.328	training
2	-1.008	-1.231	-0.800	training
3	1.000	0.891	0.946	training
4	0.284	0.249	0.391	training
5	-1.116	-1.416	-1.241	training
6	-1.315	-1.047	-1.338	training
7	-0.716	-1.717	-1.605	training
8	-1.283	-1.379	-1.119	training
9	0.108	0.092	0.009	training
10	0.260	0.278	0.292	training
11	-1.188	-0.812	-0.012	training
12	0.022	0.185	0.069	training
13	0.201	0.176	0.597	test
14	0.921	1.205	1.246	training
15	-1.238	-1.078	-1.119	test
16	-1.288	-1.398	-1.153	training
17	-1.410	-1.505	-1.625	training
18	0.886	1.124	0.826	test
19	-0.544	-0.667	-0.761	test
20	-0.281	-0.327	-0.338	training
21	-0.633	-0.859	0.850	training
22	-1.286	-1.115	-1.307	training
23	-1.700	-1.999	-1.845	training
24	-1.700	-1.532	-1.826	test
25	-0.371	-0.359	-0.463	training

^apIC₅₀ (-logIC₅₀) values were experimentally tested in the proliferation assay in MCF7 cells.^bPredicted pIC₅₀ from the multifit-CoMFA 3D-QSAR model.^cPredicted pIC₅₀ from the multifit-CoMSIA 3D-QSAR model.

Table 3

PLS Statistics of CoMFA and CoMSIA 3D-QSAR Models.

PLS Statistics	Multifit alignment		SurFlex-docked alignment	
	CoMFA	CoMSIA	CoMFA	CoMSIA
q^2	0.621	0.659	0.418	0.586
r^2	0.938	0.965	0.825	0.947
s	0.224	0.095	0.370	0.373
F	80.905	141.67	77.61	36.917
PLS component	3	4	2	3
Field contribution				
Steric	0.674	0.201	0.569	0.199
Electrostatic	0.326	0.114	0.431	0.121
Hydrophobic		0.186		0.196
Donor		0.336		0.305
Acceptor		0.163		0.179