Computational Studies on HIV-1 Protease Inhibitors: Influence of Calculated Inhibitor−Enzyme Binding Affinities on the Statistical Quality of 3D-QSAR CoMFA Models

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A theoretical study was performed on a set of 38 human immunodeficiency type 1 (HIV-1) protease inhibitors that are structurally similar to the AIDS drug Indinavir. Comparison between the computed binding energies and experimental activity data (pIC_{50}) found a high degree of correlation ($r^2 = 0.82$). Three-dimensional quantitative structure−activity relationship (3D-QSAR) models using comparative molecular field analysis (CoMFA) yielded predicted activities that were in excellent agreement with the corresponding experimentally determined values. Inclusion of the calculated enzyme−inhibitor binding energy as an additional descriptor in the CoMFA model yielded a significant improvement in the internal predictive ability of our model ($q^2 = 0.45$ to $q^2 = 0.69$). Separate CoMFA models were constructed to evaluate the influence of different alignment schemes (*Atom Fit* and *Field Fit*) and different partial atomic charge assignment schemes (Discover CVFF, Gasteiger−Marsili, and AM1-ESP) on the statistical quality of the models.

Introduction

HIV-1 protease plays a vital role in the replication cycle of the AIDS virus. Its function is to cleave a key polypeptide that is essential for the successful assembly of infectious daughter virions.[1] The relatively small size of this enzyme and the availability of good crystal structures have made HIV-1 a major target for anti-AIDS drugs. A large number of inhibitors have been designed, synthesized, and assayed, and several HIV-1 protease inhibitors are now utilized in the treatment of AIDS.[2,3] However, the search continues for new inhibitors that exhibit increased potency, lower toxicity, and, critically, effectiveness against the growing population of mutants.[1]

The availability of computational techniques based on structure−activity relationships (SARs) has accelerated the drug design process. Nevertheless, large databases of candidate inhibitors exist that have yet to be evaluated against HIV-1 protease or its resistant variants. This backlog has exerted pressure to develop faster and more effective strategies for the "virtual screening" of candidate inhibitors. For compound databases of moderate size (<1000 entries), "docking and scoring" protocols which sacrifice computational rigor for speed can be judiciously dismissed in favor of more reliable and discriminating schemes. Regardless of which computational scheme is selected, it should be capable of ranking the inhibitor candidates according to activity so that those compounds predicted as most active are chosen first for further evaluation. The accurate prediction of enzyme−inhibitor binding affinities provides a basis both for the rational design of new candidates and for the screening of moderately large data sets of structurally related compounds.[4] Molecular dynamics and Monte Carlo simulations can be used to provide quantitative estimates of binding energies. Although these methods are conceptually rigorous, they are also time-consuming and computationally demanding and are therefore inappropriate for screening large numbers of compounds. Molecular mechanics methods, on the other hand, are computationally less intensive and thus suitable for the rapid calculations required for larger databases of compounds. Differences in entropy terms and solvation energies can be tacitly assumed to cancel out for structurally related molecules, thus ensuring that the relative accuracy of the calculated energies is not compromised. Enzyme−inhibitor binding energies computed by molecular mechanics will often correlate quite well with experimentally determined in vitro biological activities, thus yielding a predictive model useful for screening new derivatives.

Comparative molecular field analysis (CoMFA) is a versatile and powerful tool in rational drug design and related applications. CoMFA samples the steric and electrostatic fields surrounding a set of ligands and constructs a 3D-QSAR model by correlating these 3D steric and electrostatic fields with the corresponding observed activities. In the present study, calculated binding energies were included as an additional descriptor to determine if the statistical quality of the standard CoMFA model could be improved and, if so, to what degree. Such an "enhanced" CoMFA model can be used to predict the activity of new inhibitors, and the CoMFA contour maps are extremely useful as visual guides in the rational design of new inhibitors. To maximize the predictive ability of our 3D-QSAR model, we explored several variations in developing the CoMFA models with respect to the alignment scheme, the

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partial charge formalism, the inclusion of bioavailability factors, and the inclusion of calculated binding affinities.

Materials and Methods

Data Set and Modeling. All molecular modeling techniques used in this research were performed on Silicon Graphics, Inc. (SGI) workstations. The original data set consisted of 38 HIV-1 protease inhibitors for which published biological activity data (pIC_{50}) were available (Table 1).[5,6] Perez et al.[6] kindly provided an Insight II Delphi file for molecule **1** docked inside HIV-1 protease obtained from a crystallographic structure.[7] The structure included the active-site structural water molecule, and in agreement with experimental evidence, Asp25_A was protonated whereas Asp25_B was not. The docking and binding energy calculations were performed using Insight II 97.2,[8] and the CoMFA studies were performed using the Sybyl 6.4 molecular modeling software.[9]

Calculation of Binding Energies. Adopting eq 1 to describe the reaction equilibrium for the reversible binding of HIV-1 protease (PR) with an inhibitor (I), the complexation energy E_{compl} can be defined by eq 2:

$$PR + I \rightleftharpoons PR{:}I \tag{1}$$

$$E_{compl} = E_{PR:I} - E_{PR} - E_{I} \tag{2}$$

where the energies E_{PR}, E_I, and $E_{PR:I}$ correspond to the free enzyme, the free inhibitor, and the enzyme−inhibitor complex, respectively. To calculate the complexation energy of each inhibitor with HIV-1 protease, a series of molecular mechanics calculations were performed on the free enzyme, each of the free inhibitors, and each of the enzyme−inhibitor complexes.[10,11]

The inhibitor compounds were geometry optimized inside the active site of the HIV-1 protease using the consistent valence force field (CVFF) with the default partial atomic charges available in Discover version 2.98.[8] The cutoff for nonbonded interaction energies was set to ∞ (i.e., no cutoff). To avoid unrealistic movements of the enzyme caused by computational artifacts, the structures were relaxed gradually with the dielectric constant set at $\epsilon = 4$ to account for the dielectric shielding found in proteins. Each minimization was carried out in two steps, first using steepest descent minimization for 200 cycles and then using conjugate gradient minimization until the average gradient fell below 0.01 kcal/mol. All atoms within 3.0 Å of the inhibitor were allowed to relax during the minimization, whereas those atoms beyond 3.0 Å were held rigid. In addition to making the calculations less CPU intensive, this procedure prevented unrealistic movement of the protein atoms. Each energy-minimized inhibitor was extracted from the complex, after which the energies of the free inhibitor and free enzyme were calculated by energy minimization while applying the same convergence criterion.

CoMFA Study: Alignment and Calculation of Field Energies. Of the numerous formalisms available for assigning atomic partial charges, none is generally accepted as the standard or "correct" scheme. To assess the influence of different assignment schemes, a comparison of empirical (CVFF[8] and Gasteiger−Marsili[9]) and semiempirical (AM1-ESP[8]) procedures was performed for the data set. The Gasteiger−Marsili method calculates atomic charges based on information about the atoms and their connectivity within the molecule. The charge on each atom is initially assigned its formal charge. Then an iterative procedure is employed to calculate the electronegativities using a polynomial relationship between the orbital electronegativity of an atom and the charge of that atom. The magnitude of charge transferred between bonded atoms decreases at each step. The objective is to achieve partial equalization of the orbital electronegativity.[12]

To obtain a more realistic conformation for the inhibitors during the alignment procedure in CoMFA, each inhibitor was extracted from the active site in its geometry-optimized form within the HIV-1 protease complex. Two methods were used to align the inhibitor molecules for the calculation of the CoMFA fields. The *Atom Fit* method uses the core backbone atoms of inhibitor **1** as a template to align the remaining inhibitor molecules. In contrast, the *Field Fit* method aligns the molecules by their fields. *Field Fit* adjusts the geometry of the molecules to maximize the similarity of the steric and electrostatic fields between the template molecule and the other molecules in the data set.

The CoMFA region was defined to extend 5 Å beyond the van der Waals radii of the assembly of superimposed molecules, along each of the principal axes of a Cartesian coordinate system. The grid spacing was set to 2 Å units in all *x*, *y*, and *z* directions, and a C_{sp^3} atom with a formal charge of +1 and a van der Waals radius of 1.52 Å served as the probe. The steric term represents the van der Waals (dispersion-like) interactions, while the Coulombic term represents the electrostatic interactions for which a distance-dependent dielectric expression $\epsilon = \epsilon_0 R_{ij}$ with $\epsilon_0 = 1.0$ was adopted. The maximum field values were truncated to 30 kcal/mol for the steric field energies and ±30.0 kcal/mol for the electrostatic field energies. To improve efficiency and to reduce "noise", a column filter was employed to exclude those columns with a variance smaller than 2.0 kcal/mol. The CoMFA standard relative weights were applied from Sybyl 6.4 for E_{steric} (5.50×10^{-3}), $E_{electro}$ (2.22×10^{-2}), and E_{compl} (1.34×10^{-1}).

Partial Least-Squares Analysis. Partial least-squares (PLS) regression was used to construct the 3D-QSAR model. The PLS method is applied to rationalize the structural features relevant to biological activity. PLS analysis converts the steric and electrostatic field descriptors to principal components that are linear combinations of the original descriptor variables. This procedure produces a linear relationship between the principal components and the biological activity. To find the optimum number of principal components corresponding to the smallest error of prediction, a "leave-one-out" cross-validation procedure was performed yielding the cross-validated correlation coefficient (q^2) defined as:

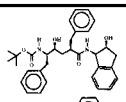
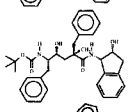
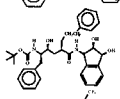
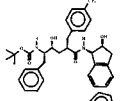
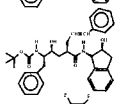
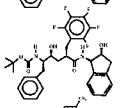
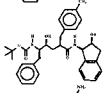
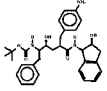
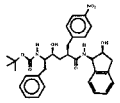
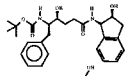
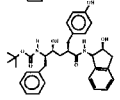
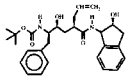
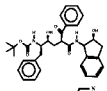
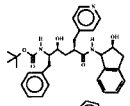
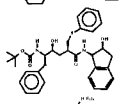
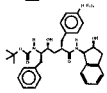
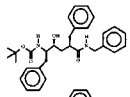
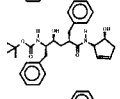
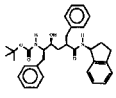
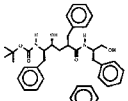
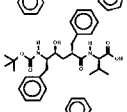
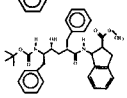
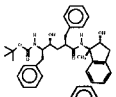
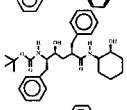
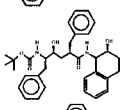
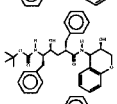
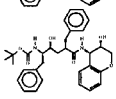
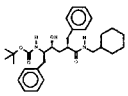
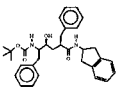
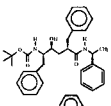
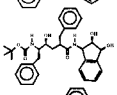
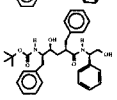
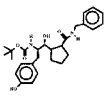
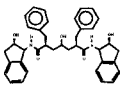
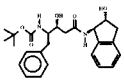
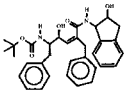
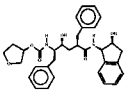
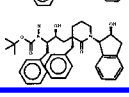
$$q^2 = \frac{SD - PRESS}{SD}$$

where SD is the variance of the biological activities of the molecules around the mean value and PRESS represents the sum of the squared differences between the predicted and actual target property values for every compound. The "leave-one-out" technique provides a convenient way to quantify the internal predictive ability of the model. Each compound is omitted one at a time, after which its activity is predicted by the model constructed from the remaining compounds in the data set. The model is generally considered internally predictive if $q^2 > 0.5$ (where q^2 can vary from −∞ to 1.0). The optimal number of components corresponds to that which yields either the smallest rms error or the largest q^2 value. A final PLS analysis was performed inclusive of all compounds in the data set, yielding a conventional correlation coefficient (r^2) which provides a measure of the internal consistency (goodness of fit) of the model.

Results and Discussion

Correlation of Binding Energy with Activity. The binding energy of each inhibitor with HIV-1 protease was calculated as described above. The results are reported in Table 1 along with the corresponding experimentally determined activities (pIC_{50} values).[6] A least-squares-fit plot of pIC_{50} versus our calculated binding energy (Figure 1) yielded a strong linear correlation ($r^2 = 0.82$; $q^2 = 0.64$). This degree of correlation is similar to that obtained by Perez et al.[6] ($r^2 = 0.85$) using the AMBER force field. The strong positive correlation validates the use of binding energy as a fast and reliable method for predicting activity for this application.

Table 1. Comparison of Experimentally Observed and CoMFA-Predicted Activities (pIC_{50}) for 38 HIV-1 Protease Inhibitors

	Inhibitor Structure	Calculated Binding Energy (kcal/mol)	Exp. Activity pIC_{50}	CoMFA Model 6 Predicted Activity	Residual
1		-93.9	9.60	9.04	0.56
2		-95.4	8.11	8.29	-0.18
3		-99.2	9.72	9.67	-0.08
4		-97.5	9.59	9.89	-0.30
5		-101.9	9.64	9.53	-0.11
6		-92.0	9.22	9.27	-0.05
7		-96.0	9.54	9.44	0.10
8		-95.6	9.51	9.46	0.05
9		-98.4	9.57	9.85	-0.28
10		-83.2	5.53	5.97	-0.44
11		-94.6	9.80	9.67	0.13
12		-90.9	7.56	7.49	0.07
13		-100.1	8.27	8.62	-0.35
14		-93.8	9.28	9.28	0.00
15		-95.4	9.60	8.88	0.72
16		-98.7	9.77	9.85	-0.08
17		-84.4	6.94	6.95	-0.01
18		-82.1	8.02	8.31	-0.29
19		-91.2	7.47	7.73	-0.26
20		-76.4	6.16	5.95	0.21
21		-81.1	6.79	6.59	0.20
22		-96.6	7.18	6.96	0.22
23		-88.6	6.67	6.51	0.16
24		-82.2	6.91	7.07	-0.16
25		-90.9	9.16	8.86	0.30
26		-95.6	9.75	9.76	-0.01
27		-88.3	7.39	7.18	0.21
28		-77.9	4.52	4.44	0.08
29		-89.9	6.89	7.57	-0.68
30		-84.3	6.84	6.59	0.25
31		-96.0	10.00	10.03	-0.03
32		-87.6	7.41	7.53	-0.12
33		-80.2	6.23	6.22	0.01
34		-105.4	9.16	9.14	0.02
35		-79.8	6.25	6.28	-0.03
36		-95.7	8.89	8.94	-0.05
37		-96.9	10.22	10.25	-0.03
38		-87.4	5.90	5.98	-0.08

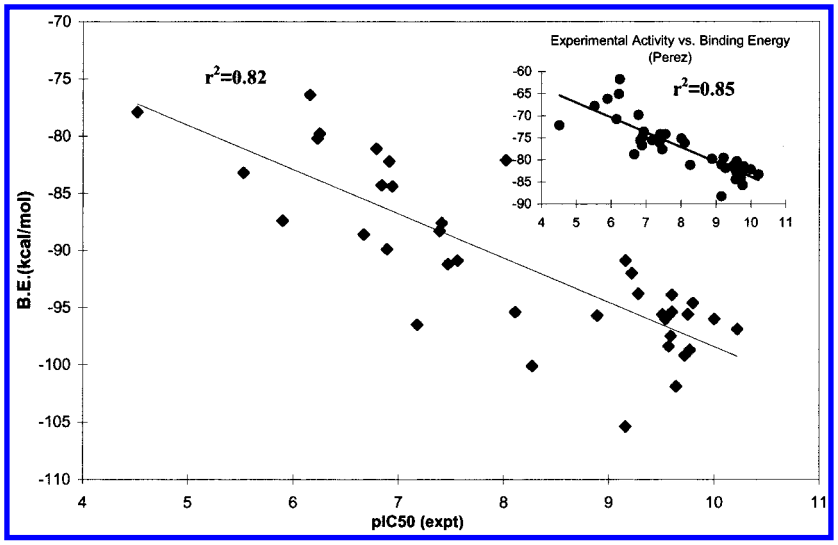


Figure 1. Least-squares-fit plot of experimental activity (pIC_{50}) versus the calculated binding energy (kcal/mol). Inset shows a similar plot based on Perez et al. who calculated binding energies using the Amber force field.

Table 2. Influence of Molecular Alignment (*Atom Fit* vs *Field Fit*), Partial Atomic Charge Scheme (CVFF, GM, and AM1-ESP), and Inclusion of Calculated Binding Energy on CoMFA Models

	binding energy	CoMFA alone *Atom Fit*			CoMFA plus binding energy *Atom Fit*			CoMFA alone *Field Fit*	CoMFA *Field Fit* plus binding energy
model	0	1	2	3	4	5	6	7	8
	CVFF	CVFF	GM	AM1-ESP	CVFF	GM	AM1-ESP	AM1-ESP	AM1-ESP
PCs		5	5	5	5	5	5	3	3
r^2	0.82	0.98	0.85	0.98	0.98	0.92	0.97	0.98	0.97
q^2	0.64	0.54	0.53	0.45	0.64	0.66	0.69	0.50	0.64

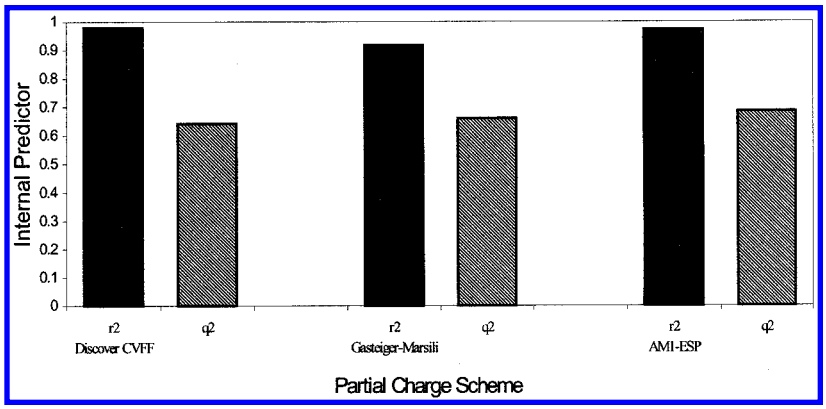
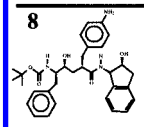
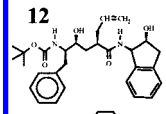
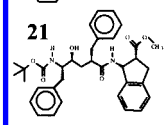


Figure 2. Comparison of r^2 and q^2 values for CoMFA models in which different partial atomic charge formalisms are applied.

CoMFA Models. To explore the effect of different variables on the predictive ability of the CoMFA model, separate CoMFA models were constructed that varied in terms of their alignment scheme (*Atom Fit* vs *Field Fit*) and partial-charge assignment schemes (CVFF, Gasteiger−Marsili, and AM1-ESP). Results from the various CoMFA models are reported in Table 2**.** Considering variations in partial-charge schemes, the inhibitors were assigned partial charges using three different schemes: CVFF (model 1), Gasteiger−Marsili using Sybyl version 6.4[9] (model 2), and AM1-ESP using Cerius[2] version 2.3[8] (model 3). In all three cases, *Atom Fit* was employed for molecular alignment. All three models exhibited good statistical quality between the predicted and experimentally determined values of pIC_{50}: model 1 ($r^2 = 0.98$; $q^2 = 0.54$), model 2 ($r^2 = 0.85$; $q^2 = 0.53$), and model 3 ($r^2 = 0.98$; $q^2 = 0.45$). A visual comparison of the results from these three partial-charge assignment schemes is given in Figure 2. To examine the influence of including the calculated enzyme−inhibitor binding energies on the statistical quality of these CoMFA models, models 1−3 were reconstructed after adding the binding energy as an independent descriptor to yield models 4−6, respectively. The improvement in the predictive ability of the models was quite impressive; specifically, q^2 improved 15% for model 4, 20% for model 5, and 35% for model 6. Considering that binding energy is only one descriptor out of the thousands of CoMFA-generated steric−electrostatic field descriptors, its contribution to the resulting model is substantial. This is not surprising from a physical point of view in that the binding energy would be expected to capture the true nature of the enzyme−inhibitor interactions. On the other hand, the steric−electrostatic fields from CoMFA are hypothetical in the sense that

Table 3. Comparison of CoMFA-Predicted vs Experimentally Observed Values of pIC_{50} for Test-Set Compounds

Compound	Binding Energy	Exp. Activity pIC_{50}	Model 9 Predicted Activity	Model 10 Predicted Activity (B.E)
8 	-95.6	9.51	9.38	9.55
12 	-90.9	7.56	7.04	7.67
21 	-81.1	6.79	8.20	6.73

they are derived from the ligands themselves and not from any explicit information about the enzyme.

As a final variation, models 3 and 6 were reconstructed to yield models 7 and 8, respectively, where *Field Fit* was used instead of *Atom Fit* for molecular alignment. Each of these two new CoMFA models exhibited good internal consistency ($r^2 = 0.98$ for model 7 and $r^2 = 0.97$ for model 8) and good internal predictive ability ($q^2 = 0.50$ for model 7 and $q^2 = 0.64$ for model 8).

Test Set. Among the parameter variations considered above, the combination of AM1-ESP partial charges and *Atom Fit* molecular alignment yielded the best CoMFA model whether the calculated binding energies were included (model 6) or not included (model 3). Using this combination, two new CoMFA models were constructed using only 35 compounds (instead of the original 38 compounds) for the training set and setting aside 3 compounds for testing the models (Table 3). These three compounds were selected as representative of the full range of activity across the data set. The new CoMFA models (models 9 and 10) were prepared using the 35-compound training set, respectively with and without inclusion of binding energy as an additional descriptor. Whereas model 10 was the same as model 9 in terms of internal consistency ($r^2 = 0.98$), it was clearly superior in terms of predictive ability ($q^2 = 0.65$ vs $q^2 = 0.42$). This improvement in predictive ability can be seen clearly for each of the test-set compounds (Figure 3).

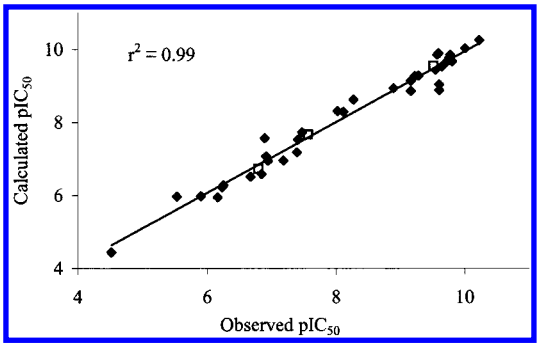


Figure 3. Plot of experimentally observed vs fitted values of pIC_{50} from CoMFA model 6 for the training-set compounds (■) and the predicted values for the 3 test-set compounds (□) from model 10.

Conclusions

Enzyme−inhibitor binding affinities computed using molecular mechanics exhibited a high correlation ($r^2 = 0.82$) with the experimental activity data (pIC_{50}). Taking into account the reliability and reasonable speed of calculation, this approach is more than adequate for the rapid screening of moderately sized compound libraries and even for the prediction of biological activity. The present 3D-QSAR models exhibited good to excellent agreement between the CoMFA-predicted and experimentally observed pIC_{50} values. Although model 6 yielded the best results among the models considered, all the models yielded acceptable statistical quality (high r^2 and q^2 values). Model 6 was constructed using the *Atom Fit* alignment scheme and AM1-ESP partial charges together with the computed binding energies. The significance of this is clearly demonstrated by the results of our test set where the accuracy of the predicted activity improved considerably when binding energy was added as an additional descriptor. In fact, inclusion of the calculated inhibitor−enzyme binding energy as an additional descriptor resulted in a significant improvement in the predictive ability of all our models. Including the CoMFA fields does not improve the internal predictive ability of the binding energy model (model 0: $q^2 = 0.64$, model 4: $q^2 = 0.64$). However, the dramatic improvement in the CoMFA models provides convincing evidence of the utility of including the calculated binding energy to predict activity. Hence, such predictions can form the basis for a rapid, first-pass, virtual screening of candidate inhibitors prior to synthesis.

The primary objective of this study was to evaluate an efficient scheme for reliable prediction of biological activity by combining the essential features of ligand-based approaches (i.e., 3D-QSAR models) and receptor-based approaches (i.e., enzyme−inhibitor binding energies). This objective was achieved in that the inclusion of this single descriptor (i.e., calculated binding energy) led to substantial improvement in the predictive ability of the resulting CoMFA models as reflected in both q^2 and the test-set molecules.

As demonstrated in the present study, these two approaches can be employed separately to yield reasonably predictive models. The statistical quality and predictive ability of the 3D-QSAR models constructed using CoMFA alone are more than adequate, and likewise for the model developed using the calculated binding energies alone. However, it is imperative in drug discovery efforts to develop and apply QSAR models of the highest statistical quality and predictive ability. This can be achieved by using the full extent of information provided for the system under study. As in the present case, this information may include knowledge of the three-dimensional structure of the receptor and/or receptor−ligand binary complex. The availability of such structural data is indispensable to exploiting the vast array of receptor-based approaches in rational drug design. This includes the docking of known ligands (e.g., inhibitors) inside the receptor's binding site and calculation of their binding energies. The present study has demonstrated that calculated binding energies can serve as a reasonably predictive tool for screening a series of compounds in terms of their relative binding affinity.

At the same time, this study has demonstrated that the utility of calculated binding energies can be extended even further by including them as an additional descriptor in 3D-QSAR models. This straightforward integration of information from ligand-based design and receptor-based design approaches yields "enhanced" 3D-QSAR models that exceed the CoMFA approach taken alone in terms of predictive ability.

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