Comparative Molecular Field Analysis (CoMFA) of a Series of Symmetrical Bis-Benzamide Cyclic Urea Derivatives as HIV-1 Protease Inhibitors†

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Received February 11, 1998

A 3D-QSAR study using CoMFA methodology was conducted on a series of 29 symmetrical bis-benzamide cyclic urea derivatives having anti-HIV-1-protease activities. Active site minimization of the ligands was used to exclude conformations which are not sterically accessible within the active site. A significant cross-validated correlation coefficient q^2 (0.724) was obtained indicating the predictive potential of the model for untested compounds of this class. A significant non-cross-validated correlation coefficient (r^2) of 0.971 with a low standard error estimate (S) of 0.119 was obtained indicating that the model reliably predicted the anti-protease activities of poorly to highly active compounds. The model was used to predict the anti-protease activities of eight test-set compounds, and the predicted values were in good agreement with the experimental values. The CoMFA coefficient contour plots identified several key features which explain the wide range of activities. The already reported 2D-QSAR along with the CoMFA model presented here may help in designing effective HIV-1 protease inhibitors.

INTRODUCTION

HIV protease encoded by the genome of the human immunodeficiency viruses (HIV), a causative agent of the acquired immunodeficiency syndrome (AIDS), is an aspartyl protease essential for the maturation of infectious virions.[1] Since the discovery of HIV, this enzyme has become one of the important targets for designing antiretroviral therapeutics.[2−8] There are 11 anti-HIV-1 drugs approved by the Food and Drug Administration (FDA) of which four are protease inhibitors. Combination therapies with reverse transcriptase inhibitors showed clinical promise.[9] Despite these successes, continuous efforts are being made to improve the efficacy and reduce the adverse effects of the protease inhibitors. The emergence of HIV-1 strains resistant to protease inhibitors is becoming a serious problem and one of the driving forces to continue the effort to identify better inhibitors.[10,11]

Knowledge of the HIV-1 protease structure is the foundation of the structure-based drug design effort to target this enzyme. More than 150 structures have been solved most of which are from pharmaceutical companies[12] and not available in the public domain. However, a considerable number of structures (68 as of January 28, 1998) are available from the Brookhaven Protein Data Bank (PDB).[13] A number of structure−activity analyses using both the classical quantitative structure−activity relationship (QSAR)[14,15] and 3D-QSAR approaches[16−19] enhanced our knowledge of ligand-HIV-1-protease interactions involving different classes of inhibitors. Due to the limitations of every single structure−activity approach, a combination of several methods is important to understand complex phenomena contributing to anti-protease activity. Toward this goal, we have developed a Comparative Molecular Field Analysis (CoMFA) based on 3D-QSAR of a series of bis-benzamide cyclic ureas[14] which in combination with the recently reported QSAR[14] on those compounds might provide valuable insight for designing more effective inhibitors of this class. Here we report the systematic development of the 3D-QSAR for bis-benzamide cyclic ureas as HIV-1 protease inhibitors.

METHODS

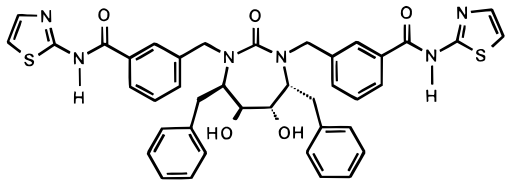
A. Computational and Modeling Tools. Computer modeling was done using a Silicon Graphics Indigo2 Extreme (R4400) computer. The modeling study including the CoMFA study was done using Sybyl 6.4 software.[20] The systematic search routine within Sybyl was used for all conformational searches. Maximin2 minimizer was used to minimize the structures. The biopolymer module within Sybyl was used to retrieve and analyze all protein structures. The CoMFA feature as a part of the QSAR module was used to derive all QSAR models.

B. Anti-HIV-1 Activity. The HIV-1 protease inhibitory activity data was selected from a recently published report.[14] This data set was intentionally selected to analyze a comparative QSAR study since in this report the physicochemical parameter-based QSAR[14] was also used. The log $1/K_i$ values for protease inhibitors were used to derive the 3D-QSAR model. The reported training (29 compounds) and test-sets (eight compounds) were used to derive the model and to predict the activities of the test compounds from the model.

C. Molecular Modeling Methods. 1. Conformational Study: Three X-ray crystal structures of HIV-1 protease complexed with the bis-benzamide cyclic urea type compounds are available in the PDB. One of them (1qbr) containing the ligand [4*R*-(4α,5α,6β,7β)]-3,3′-[[tetrahydro-5,6-dihydroxy-2-oxo-4,7-bis(phenylmethyl)-1*H*-1,3-diazepine-1,3(2*H*)-diyl)bis(methylene)]bis[*N*-2-thiazolylbenzamide] (XV638) was used as template molecule (Figure 1) to

† This manuscript has been dedicated to Professor Corwin Hansch of Pomona College, California, on his 80th birthday.

S0095-2338(98)00014-6 CCC: $15.00
Published on Web 06/06/1998



XV638

Figure 1. Chemical structure of XV638.

construct all the compounds in the training set and in the test-set, except for compounds Test-4 and Test-8. Test-4 is the template compound and Test-8 is another ligand of this type, designated as SD146, the X-ray crystal structure of which, complexed with HIV-1 protease, is also available from the PDB (1qbt). These ligands were extracted from the crystal structures, and their bound conformations were used "as is" for further analyses. The orientations of all the substituents were determined based on the steric compatibility of the ligand-HIV-1-protease complex. Stereographic visualization using stereo glasses was always used to determine these orientations.

A systematic conformational search was initiated using the "systematic search" option in Sybyl 6.4 for all the flexible molecules within the binding site of the HIV-1 protease X-ray crystal structure (1qbr). The entire protease X-ray crystal structure and the portion of the ligand common to all these molecules were kept rigid, and only the flexible substituents were subjected to the systematic conformational search. The Kollman all-atom charges were loaded from the dictionary for the entire protein portion, whereas Gasteiger−Marsili charges were used for the ligand molecules. All the flexible bonds in the substituents were initially searched with 30° torsion angle increments in the torsional space. As 2-pyridyl derivatives represent the majority of the compounds in the training set, compound 17 was first used for the conformational search. It yielded no other conformations than the starting conformation with the default van der Waals radius scale factors (General:0.95; 1−4: 0.87 and H-bond; 0.65) used in the systematic conformational search in Sybyl. Another attempt was made with 10° torsion angle increments, but it again yielded no other conformation. Compounds **6**−**13** yielded a number of conformations within the binding site.

When the systematic conformational search yielded a number of conformations, the lowest energy conformation was selected and further minimized within the active site using the Maximin2 minimization routine within Sybyl with a gradient change termination criterion of 0.05 kcal/mol*Å. During the minimization, only the cyclic urea portion of the antiviral compounds and the entire protein portions of the complex were kept rigid, as in the X-ray crystal structure, but the rest of the molecule was allowed free movement within the active site to maximize the interactions. Compounds **14**−**29** and the test compounds (Test-1 to Test-8) were constructed from the template molecule and used for complete minimization within the active site as described before. The minimization also removed any steric clashes that might have been present initially. The ligands were extracted from the complex, and the partial charges were calculated using MOPAC 6.0 with the AM1 Hamiltonian[21] adopted in the Sybyl 6.4 program. No geometry optimization was made, rather the keywords 1SCF and MMOK were used to calculate the charges and for the molecular mechanics corrections of the amidic bonds, respectively, in the ligands.

2. Alignment Rule. All the molecules in this study belong to the same structural class, bis-benzamide cyclic urea, and they were all constructed from a single template X-ray crystal structure. Since the minimization within the active site was used as the constraint to find the best possible geometry of the ligands, alignment of the C-α atoms of the minimized compound-protease complex on the C-α atoms of the template-protease structure was used as the most logical alignment rule in this particular study. This ensured the alignment of the cyclic urea portion of all the extracted ligands on the template cyclic urea atoms, as the cyclic urea portion of each compound was also kept rigid during minimization. This alignment rule also ensured the formation of four hydrogen bonds by the two diol oxygens of the cyclic urea moiety with the two catalytic aspartic acid residues (Asp25 and Asp25′) and two hydrogen bonds between the carbonyl oxygen of the cyclic urea moiety and the isoleucine residues (Ile50 and Ile50′) of the protease active site. The positioning of this carbonyl oxygen is one of the strategies used to displace the water molecule (Wat301) from the active site to design this class (and many other classes) of HIV-1 protease inhibitors. Figure 2 shows the stereoview of the aligned molecules (including the test-set) within the grid box used to generate the CoMFA column.

D. CoMFA Study. 1. Interaction Energy Calculation: The steric and electrostatic field energies of each molecule were initially calculated at all intersections of a regularly spaced (2 Å) grid in a grid box of 22.5 Å × 29 Å × 55.8 Å surrounding the molecule using the Lennard-Jones 6−12 potential and Coloumbic potential functions, respectively, within the Tripos force field[22] using an sp^3 carbon probe atom with a +1 charge and a distance-dependent dielectric constant. The grid box dimensions for CoMFA analyses were determined by using the "create automatically" feature of the Sybyl/CoMFA program. This ensured that region boundaries were extended beyond 4 Å in each direction from the coordinates of every molecule. An energy cutoff of 30 kcal/mol for both steric and electrostatic contributions was set, and the electrostatic terms were dropped within region of steric maximum, i.e., 30 kcal/mol. The effect of grid point spacing on the CoMFA analysis was also investigated at 1.5 and 1.0 Å spacing. The results are shown in Table 1.

2. Regression Analyses. The regression analyses were done using the Partial Least Squares (PLS)[23] algorithm within Sybyl. Five orthogonal principal components were first extracted by the PLS technique using the leave-one-out cross-validation technique. The minimum σ value of 2.0 kcal/mol was set as column filter to reduce the noise in the PLS analyses, and CoMFA scaling was set to CoMFA-Standard. The optimal number of components for the best predictive correlation model from non-cross-validated run was selected based on the cross-validated analyses which yielded the lowest values in the standard error of predictions (s). The best correlation model (fitted model) was derived using no-*cross*-validation with the optimum number of components. A minimum σ value of 0.00 and CoMFA scaling of CoMFA-Standard were used.

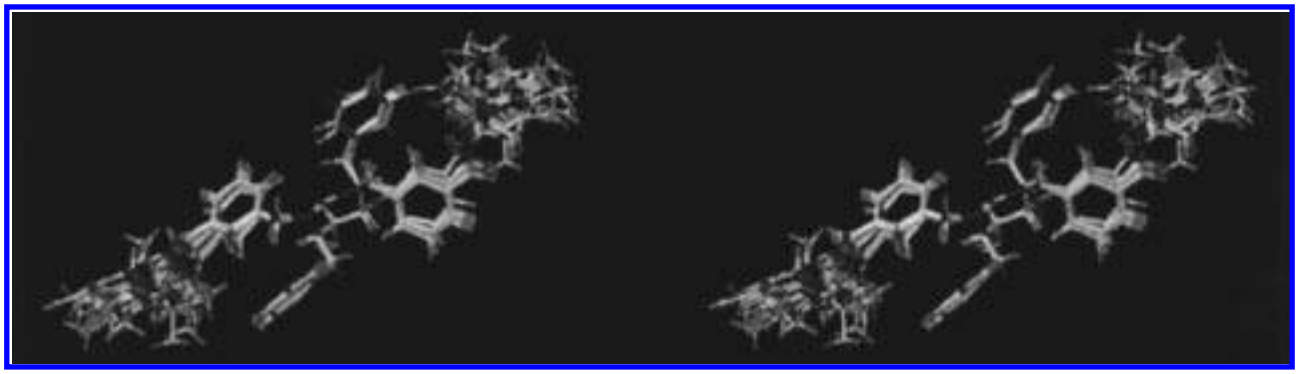


Figure 2. Stereoview of all the aligned compounds in the training and test sets.

Table 1. Results of the CoMFA PLS Analyses[a] of the Training Set

statistics	lattice space		
	2.0 Å	**1.5 Å**	1.0 Å
optimum no. of components	5	**5**	5
q^2_{cross}[b]	0.728	**0.724**	0.720
std error of predictions (s)	0.360	**0.364**	0.366
r^2	0.971	**0.971**	0.971
std error of estimate (S)	0.118	**0.119**	0.118
F test	154.0	**151.3**	152.5
contributions:			
steric	0.477	**0.527**	0.496
electrostatic	0.523	**0.473**	0.504
energy cut-off (kcal/mol):			
steric	30	**30**	30
electrostatic	30	**30**	30

[a] CoMFA-standard was used as scaling method in all CoMFA PLS analyses. [b] Minimum $\sigma = 2.0$ was used for leave-one-out cross-validated runs, whereas minimum $\sigma = 0.0$ was used for non-cross-validated runs. The model in bold was used as the predictive model.

Table 2. Statistics of Cross-Validated PLS−COMFA Using 1.5 Å Lattice Space

statistics	components				
	1	2	3	4	5[a]
standard error of predictions (s)	0.580	0.420	0.387	0.368	0.364
q^2	0.175	0.583	0.660	0.705	0.724

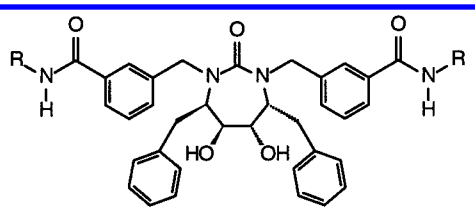
[a] Selected components.

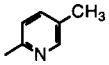
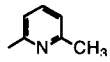
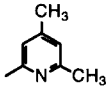
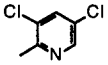
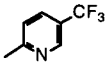
RESULTS

Three different grid boxes with 2.0, 1.5, and 1.0 Å spacings, respectively, were used for PLS analyses. Though the statistics of all three PLS analyses yielded similar and consistent results, the model with the grid box spacing of 1.5 Å was selected as the best predictive model judged by the predicted protease inhibitory activities of the test compounds, Test-1 to Test-8. At a higher grid spacing (2.0 Å), some information for H-bonding and Lennard-Jones potentials may be lost as they are dependent on distance.[24] A lower spacing (1.0 Å) may generate more noise in the PLS calculations and require a greater computational effort. The following discussion will only refer to the model generated from 1.5 Å lattice space. The number of components that produced the lowest standard error of predictions (*s*) for cross-validation studies were used to derive the non-cross-validated model (Table 2). The leave-one-out cross-validated PLS analysis resulted in a q^2 of 0.724 using five principal components. The non-cross-validated PLS run with the same number of principal components yielded a high r^2 of 0.971 with a low standard error of estimate (*S*) of 0.119. The model explained 97% of the variance in protease inhibitory activities of the compounds used in the training set. The contributions of steric and electrostatic interactions to the protease inhibitory activities are almost equal, 53% and 47% respectively. The HIV-1 protease inhibitory activity (K_i), expressed in nanomolar concentrations (nM) of the symmetrical bis-benzamide cyclic ureas, log $1/K_i$, the substituent groups (R), the calculated activities, and the deviations from the observed values are all shown in Table 3. Figure 3 shows a plot of observed vs calculated protease inhibitory activities.

The greatest advantage of CoMFA derived 3D-QSAR is that the effect of the steric and electrostatic fields on the target property can be viewed as 3D coefficient contour plots. The CoMFA steric and electrostatic fields based on the PLS analyses are shown as 3D contour plots in Figures 4 and 5, respectively. One of the most active compounds, compound **20**, is shown in the background. The coefficient contour plots may help to identify important regions where any change in the steric and/or electrostatic fields may affect the biological activity, and they may also help in identifying important regions of interactions with the ligands in the receptor site. The steric CoMFA field contour plot in Figure 4 shows that positive contours represented by the green polyhedra have a favorable steric effect. Increasing the bulk in those areas may help to increase the protease inhibitory activity. The negative contours, represented by yellow polyhedra, indicate that increasing bulk in those areas may have detrimental effects on activity. A close inspection of the steric contour plots revealed that the sterically favorable regions indicated by green contours were located in the region of either the hydrophobic pocket created by Ile47, Val56, and Leu76 or close to the phenyl ring of Phe53. Another interesting observation from this CoMFA study is that aliphatic chain substituents in compounds **6**−**13** are all located very close to the prohibitive region indicated by yellow polyhedra; these compounds have the least protease inhibitory activities. These substituents are very close to the phenyl groups in the P_1, $P_{1'}$ site in the inhibitor structures. They are all influenced by the negative steric effects from substituents on the same molecule but do not contribute hydrophobically to the receptor−ligand binding. The 2-pyridyl compounds with hydrophobic substituents (CH_3, Cl, Br, etc.) are all located in the favorable green regions and the

Table 3. Observed and Calculated Anti-HIV-1-Protease activities of Bis-Benzamide Cyclic Ureas in the Training Set



Cmpd	R	K_i[a] (nM)	log 1/K_i[a] Obs	Calcd	Diff	Cmpd	R	K_i[a] (nM)	log 1/K_i[a] Obs	Calcd	Diff
1	H	0.039	1.41	1.40	0.01	16		0.290	0.54	0.64	-0.10
2	NH_2	0.018	1.74	1.68	0.06	17		0.043	1.37	1.45	-0.08
3	OH	0.020	1.70	1.83	-0.13	18		0.260	0.59	0.61	-0.02
4	OCH_3	0.045	1.35	1.33	0.02	19		0.027	1.57	1.56	0.01
5	CH_3	0.066	1.18	1.14	0.04	20		0.011	1.96	1.93	0.03
6	CH_2CH_3	0.210	0.68	0.66	0.02	21		0.020	1.70	1.72	-0.02
7	$CH(CH_3)_2$	0.579	0.24	0.22	0.02	22		0.016	1.80	1.78	0.02
8	$CH_2CH_2CH_3$	0.359	0.44	0.44	0.00	23		0.012	1.92	1.48	0.44
9	$CH_2CH_2CH_2CH_3$	0.424	0.37	0.32	0.05	24		0.245	0.61	0.63	-0.02
10	$C(CH_3)_3$	2.400	-0.38	-0.40	0.02	25		0.035	1.46	1.65	-0.19
11	CH_2-C_3H_5	0.741	0.13	0.19	-0.06	26		0.115	0.94	0.98	-0.04
12	CH_2CF_3	0.210	0.68	0.68	0.00	27		0.085	1.07	1.10	-0.03
13	CH_2CN	0.063	1.20	1.22	-0.02	28		0.018	1.74	1.76	-0.02
14		0.430	0.37	0.52	-0.15	29		0.152	0.82	0.82	0.00
15		0.410	0.39	0.23	0.16						

[a] K_i SD < ±40%.

corresponding compounds (**20**, **23**, **25**, and **27** in Table 3) have high inhibitory activity. The substituents, *tert*-butyl and cyclopropylmethyl, in two of the least active compounds, **10** and **11**, respectively, penetrated directly to the forbidden yellow regions. These critical analyses of the steric contour plots revealed several key features which need to be considered when designing better inhibitors of this class. The effect of positive steric contours may be interpreted as steric and/or hydrophobic effect, i.e., bulkier groups, especially hydrocarbons or phenyl rings, in those regions may increase the protease inhibitory activity. The electrostatic field contours are shown in Figure 5. The contour shows that more negative charge in the red regions increases potency, whereas more negative charge in the blue region decreases potency. At least two blue contours are located near the carboxylic acid moiety of Asp29 indicating that any negative charges in those regions will be detrimental to the anti-protease activity.

A region focusing technique, available in the Advance CoMFA module in Sybyl, which refines a model by increasing the weight on those lattice points most important for explaining the target properties, was also used on the

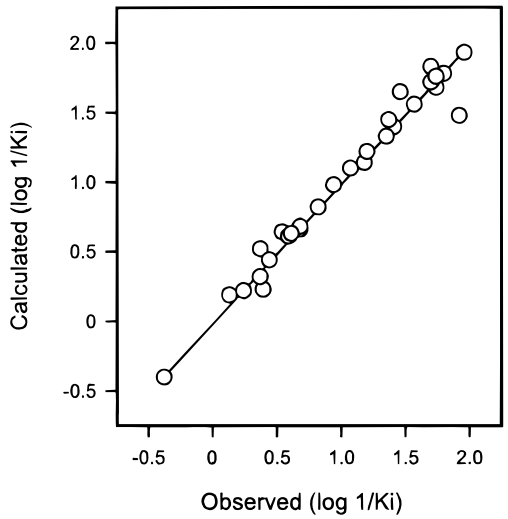


Figure 3. Plot of observed vs calculated anti-protease activities of the compounds in the training set.

selected model (data not shown) but did not improve either the predictive or the fitted model.

DISCUSSION

A 3D-QSAR model has been developed using the CoMFA methodology[25] for a set of 29 symmetrical bis-benzamide cyclic urea derivatives having anti-HIV-1 protease activity, for which a 2D-QSAR has been recently reported. The major objective of this study was to show that CoMFA-derived 3D-QSARs can be of great value, as shown in many publications[26−32] for other compounds (only selected ones are listed here), in delineating the structure−activity relationships if used judiciously in conjunction with 2D-QSAR. The CoMFA results through the 3D contour plots of the steric and electrostatic fields, in combination with the knowledge of the X-ray crystallographic coordinates of the ligand-HIV-protease complexes, are expected to help in designing better HIV-1 protease inhibitors. This analysis revealed that steric/hydrophobic and electrostatic interactions play almost equal roles in explaining the anti-protease activity of symmetrical bis-benzamide cyclic urea derivatives. The model yielded statistically significant results and resulted in accurately predicted activities of the least active to the most active compounds. Although the above analyses pointed out the effect of either steric or electrostatic interactions separately, the anti-protease activities may be due to the combined effect of these two interactions along with many other interactions e.g., hydrogen bond formation, hydrophobic effect, etc.

The systematic conformational search within the active site pocket of HIV-1 protease was extensively used by Marshall's group before.[17] It became evident during our study that a number of low energy conformations were obtained when the ligands were subjected to conformational search in isolation from the protease. However, when they were superimposed on the common atoms of the template ligand molecule in the complex, the substituent groups occupied the sterically forbidden areas in the active site. The active site geometry was used as the constraint to find the acceptable conformations within the active site. The geometry of the active site (and of the protein portion of the complex) was kept rigid during the search process to restrict the computation time to a manageable level, although admittedly the structure might be flexible in reality. The active site minimization technique, in the absence of any X-ray crystal structure of the ligand-HIV-1-protease complex, in the training set was essential for defining the best possible conformation of the ligands. It is advisable to use the receptor information, if available, to determine the 3D geometry of the ligands, as pointed out by Marshall's group.[17]

The reported QSAR using log $1/K_i$ as the activity parameter for the same training set, used the ionization potential (IP), ClogP, molar volume (as MV/100), and an indicator variable (I) to derive the model. A parabolic model with ClogP was used in that study. This parabolic model has notable significance in that it unfolded the ideal ClogP value ($ClogP_0 = 5.697$) for maximum anti-protease activity for these dataset. The current CoMFA model will be useful in selecting areas of the molecules to be utilized for adjusting the lipophilicity for improved in vivo activity. The directionality from the 3D contour plots will assist in identifying these positions and help to avoid any possible steric clashes with the receptor site. The QSAR reported in the literature also found some role of the ionization potential as an electronic parameter for explaining biological activity. The CoMFA QSAR reported here revealed electrostatic contributions of about 43%. 3D contour plots will also be useful in

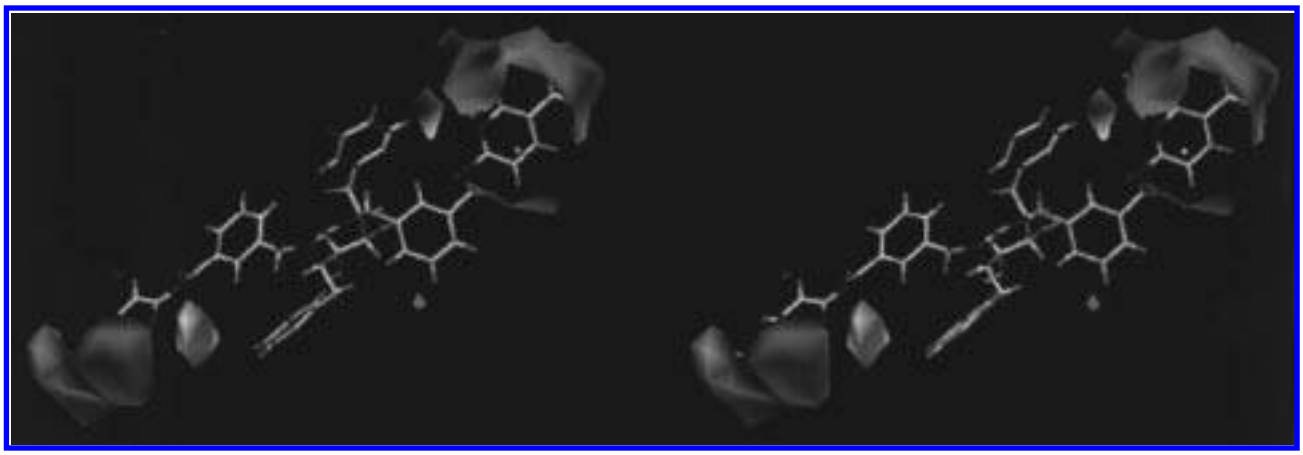


Figure 4. Stereoview of the contour plots of the CoMFA steric fields (stdev*coeff). The favored steric areas with more bulk are indicated by green polyhedra (80% contribution), whereas the disfavored steric areas are shown by yellow polyhedra (20% contribution). The most active compound (**20**) in terms of anti-protease and anti-HIV-1 activities is shown as the reference compound.

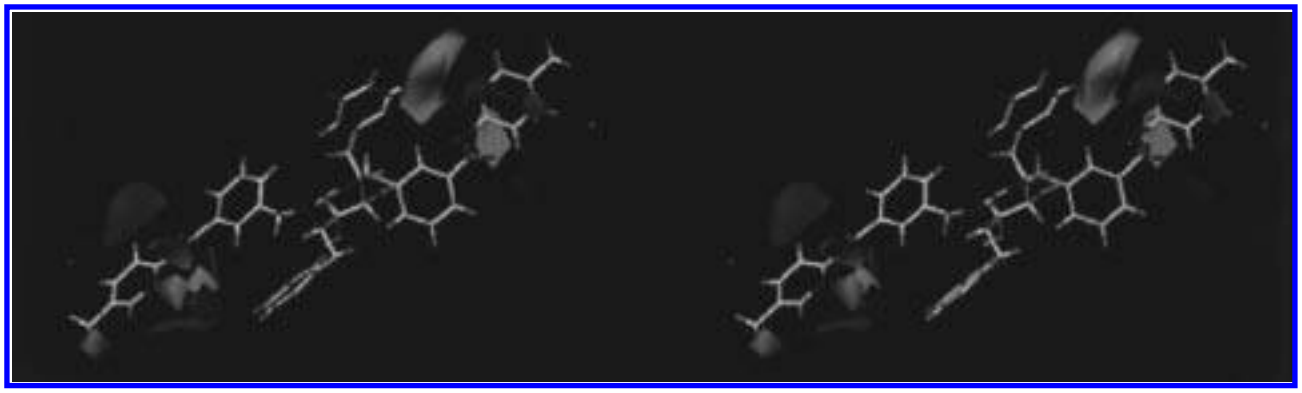
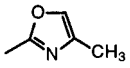
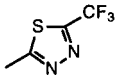
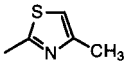
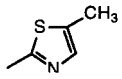
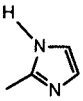
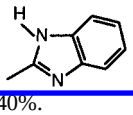


Figure 5. Stereoview of the contour plots of the CoMFA electrostatic fields (stdev*coeff). The favored electrostatic areas with positive charges are indicated by blue polyhedra (80% contribution), whereas the favored electrostatic areas with negative charges are shown by red polyhedra (20% contribution). The most active compound (**20**) in terms of anti-protease and anti-HIV-1 activities is shown as the reference compound.

Table 4. Observed and Predicted Anti-HIV-1-Protease Activities of Bis-Benzamide Cyclic Ureas in the Test Set

Cmpd	R	K_i[a] (nM)	log 1/K_i[a] Obs	Pred	Diff
Test-1		0.064	1.19	1.16	0.03
Test-2		0.180	0.74	0.75	-0.01
Test-3		0.110	0.96	0.73	0.23
Test-4		0.027	1.57	1.41	0.16
Test-5		0.025	1.60	1.09	0.51
Test-6		0.014	1.85	1.26	0.59
Test-7		0.014	1.85	0.94	0.91
Test-8		0.024	1.62	1.35	0.27

[a] K_i SD < ±40%.

identifying the positions where more negative charge will increase the activity (red regions) or decrease the activity (blue regions).

The reported QSAR resulted in a marginal correlation of 0.746 (r^2) which explained only 74% of the total variance in activity for the same training set used here, whereas the CoMFA non-cross-validated PLS analysis reported here yielded a significantly high correlation coefficient of 0.971 (r^2) with a standard error of estimate (S) of 0.119. The calculated protease inhibitory activity of one of the least active compounds (**10**) in the training set was in much better agreement with the observed activity (cald:2.51 nM vs obs: 2.60 nM) using the CoMFA model than the calculated value (0.744 nM) resulting from the reported QSAR.[14] Both methods performed equally well for the most active compounds in the training set.

It is remarkable that despite the relatively poor correlation obtained in the previously reported QSAR, the activities of the test-set compounds (total eight) were predicted quite well. The non-cross-validated CoMFA QSAR also predicted those test-set compounds equally well (Table 4). This confirms the applicability of the CoMFA model to predicting the protease inhibitory activities of untested compounds of this class.

This report in combination with the previously published QSAR studies of the symmetrical bis-benzamide cyclic urea derivatives are expected to provide significant information for designing potent anti-HIV-1-protease inhibitors.

ACKNOWLEDGMENT

This study was supported by a Grant on AIDS research by Philip Morris Companies, Inc. I am indebted to Dr. A. Robert Neurath for his encouragement to undertake this project and for reading the manuscript. I also thank J. Ng Pack for preparing some of the tables and figures.

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CI980014C