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Review Article

STRUCTURE-BASED DRUG DESIGN APPROACHES FOR PREDICTING BINDING AFFINITIES OF HIV1 PROTEASE INHIBITORS

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Computational assessment of the binding affinity of enzyme inhibitors prior to synthesis is an important component of computer-assisted drug design (CADD) paradigms. The free energy perturbation (FEP) methodology is the most accurate means of estimating relative binding affinities between two inhibitors. However, due to its complexity and computation-intensive nature, practical applications are restricted to analysis of structurally-related inhibitors. Accordingly, there is a need for methods that enable rapid assessment of large number of structurally-unrelated molecules in a suitably accurate manner. In this review, the FEP method is compared with regression-based methods that employ multivariate models to assess the advantages of each in the estimation of relative binding affinities of inhibitors to an enzyme. Semi-quantitative predictions of relative binding free energies of human immunodeficiency virus 1 (HIV1) protease inhibitors are also presented and compared with the corresponding FEP results. The results indicate that the regression-based methods and the FEP method are useful in the semi-quantitative and quantitative assessment of relative binding affinities of enzyme inhibitors, respectively, prior to synthesis.

Keywords: Free energy perturbation calculation; Regression methods; HIV1 protease; Molecular dynamics simulations; Minimization calculations; Hydrophobicity

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1. INTRODUCTION

The human immunodeficiency virus 1 (HIV1) is a member of the retrovirus family and is the cause of the debilitating and fatal disease acquired immune deficiency syndrome (AIDS).[1] As part of an overall effort to develop an effective treatment for AIDS, researchers have mounted an intense campaign to understand and exploit the critical pathways in the life-cycle of HIV1.[2] Inhibition of HIV1 protease has emerged as one of the most promising drug targets identified to date.[3] The efforts to discover HIV1 protease inhibitors, include the screening of chemical libraries,[4] designing substrate analogs,[5] and more recently using the X-ray structure of HIV1 protease in structure-based drug design approaches.[6] To complement the structural information, a variety of computational tools[7] have been developed and used as part of an overall strategy of computer-assisted drug design (CADD) strategy.

Advances in protein crystallography and molecular simulations have greatly aided CADD paradigms and the accuracy of their binding affinity predictions.[8] Methods of inhibitor design range from graphical visualization of the ligand in the binding site to calculation of relative binding affinities using molecular dynamics simulations in conjunction with the free energy perturbation (FEP) approach.[6,9] Figure 1 shows a typical flow chart employed by drug discovery groups for structure-based drug design. The process begins by generating a working computational model from crystallographic data. This step usually entails developing molecular mechanics parameters for non-standard residues, assigning the protonation states of histidines, and orientating carbonyl and amide groups of asparagine and glutamine amino acid residues based upon neighboring donor/acceptor groups. Characterization of the active site is then aided by a variety of visualization tools. For example, hydrophobic and hydrophilic regions of the active site are readily identified by calculating the electrostatic potential at different surface grid points. The information gained by graphical analysis of the active-site aids new lead design and optimization of the lead through analog design.

2. LEAD GENERATION

The holy grail of structure-based drug design is to use the structural information gained through X-ray crystallography in the *de novo* design of a new

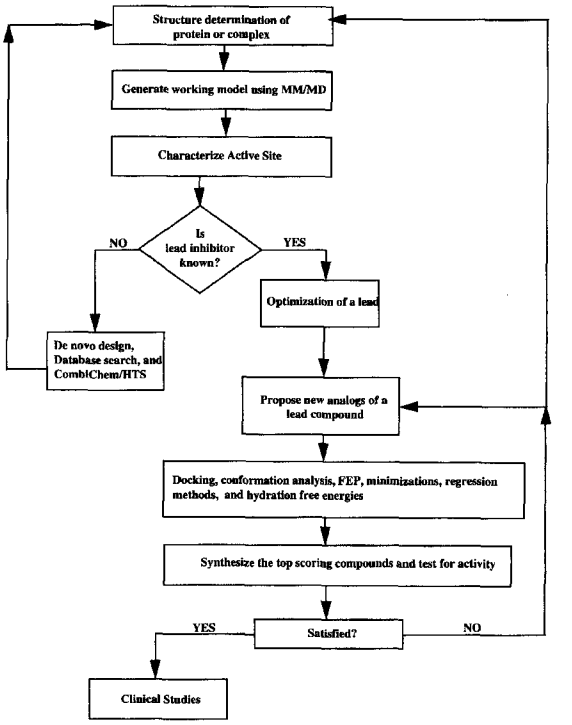

FIGURE 1 Structure-based drug design flowchart.

lead inhibitor. A few successes are reported but overall *de novo* design represents a goal and not a reality. In some cases new lead compounds were identified by searching databases of known[10] chemicals for particular structural features. *De novo* molecular design methods have also been used to design new structures by sequentially adding molecular fragments to a growing structure, by adding functionality to an appropriately-sized molecular scaffold, or by adding fragments building toward the center of a molecule starting from distant sites thought to interact with the target.[11] In addition, database search methods have been developed that search databases for compounds that have particular molecular functionality separated by a specified number of bonds or distance ranges. More chemically intuitive database search methods search for chemicals with particular steric and electrostatic fields.[12] And also a growing number of drug leads are being generated by combinatorial methods in combination with high-throughput screening.[13]

3. OPTIMIZATION OF LEAD COMPOUNDS

Optimization of lead compounds is often a step-wise process using computational methods in combination with SAR information to determine areas on the molecule to expand, contract, or modify. Accordingly, the challenge is, based on their structural properties, to prioritize a large diverse set of molecules to a small set of compounds that have the highest likelihood to bind. Methods that rapidly and accurately predict absolute binding affinities represent the long term goals. Currently, the methods can either provide qualitative rank ordering of a large number of molecules in a relatively short period of time[14] or generate quantitatively accurate predictions of relative binding affinities for structurally-related molecules by using significant computing power.[15]

A large percentage of the proposed analogs can usually be eliminated by evaluating their expected binding affinities based on docking,[16] graphical analysis and conformational analysis. The remaining analogs are prioritized using one or all of the following methods, depending on the availability of computational power, time and resources: (1) FEP calculations, which provide accurate predictions, but are computationally very expensive,[6,15] (2) molecular mechanics calculations, which provide rapid qualitative predictions,[17] (3) regression methods[14,18] that incorporate interaction variables and ligand properties, which provide semi-quantitative predictions and are much faster than FEP calculations, and (4) relative hydration free energies.[19] Then, top scoring compounds are synthesized and tested for activity. Depending on the convergence criteria of the biological activity, the flow chart is iterated. This review focuses on lead inhibitor optimization strategies using the free energy perturbation approach and regression methods and evaluates the merits of each method for predicting relative binding affinities of inhibitors to HIV1 protease enzyme.

4. FREE ENERGY PERTURBATION METHOD

Application of the FEP methodology[6,9] to the design of HIV1 protease inhibitors began following analysis of accuracy of the method using the HIV1 protease crystal structure complexed with known inhibitors,[3f] namely JG365 and JG365A (Figure 3(a)).

(a) Methodology

The thermodynamic cycle-perturbation (TCP) approach is a method for computing the relative changes of binding free energy using non-physical

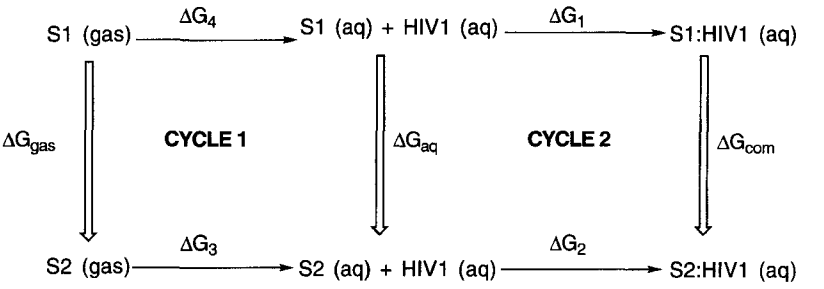

FIGURE 2 Schematic solvation and binding free energy changes for related inhibitors S1 and S2 and enzyme HIV1 protease. The horizontal free energies correspond to experimental measurements, while the non-physical vertical ones are calculated.

paths connecting the desired initial and terminal states. This approach enables calculation of the relative change in binding free energy difference ($\Delta\Delta G_{\text{bind}}$) between two related compounds, by computationally simulating the 'mutation' of one to the other. The relative solvation free energy change for two substrates is computed using the first cycle shown in Figure 2, as represented in the following equation:

$$\Delta G_3 - \Delta G_4 = \Delta G_{\text{aq}} - \Delta G_{\text{gas}} = \Delta\Delta G_{\text{sol}}. \quad (1)$$

The relative binding free energy change for the two substrates is computed using the second cycle (Figure 2), which is represented by the following equation:

$$-k_{\text{B}}T \ln(k2/k1) = \Delta G_2 - \Delta G_1 = \Delta G_{\text{com}} - \Delta G_{\text{aq}} = \Delta\Delta G_{\text{bind}}, \quad (2)$$

where k_{B} is the Boltzmann constant and T is the absolute temperature, $k1$ and $k2$ refer to experimentally measured binding constants for the inhibitors $S1$ and $S2$, respectively.

(b) Computational Details

All molecular dynamics, mechanics and FEP calculations were carried out with the AMBER program using an all atom force field[20] and the SPC/E water model potential[21] to describe water interactions. Partial charges for non-standard solute atoms were obtained by fitting *ab initio* 6-31G*//3-21G* wave functions calculated using Gaussian92[22] with CHELP.[23] All equilibrium bond lengths, bond angles, and dihedral angles for non-standard

residues were used from *ab initio* optimized geometries at 6-31G*//3-21G* basis set level. Missing force field parameters were estimated using parameters reported for similar chemical species within the AMBER database.

Solvation free energy calculations entailed solvating the solute with SPC/E water followed by molecular dynamics (MD) simulations using periodic boundary conditions and constant temperature and pressure (N, P and T). For the protein complex simulations, the HIV1 protease structure complexed with JG365 inhibitor was used[3b] to generate the computer model. In all simulations the Asp124 was protonated and the total charge was $+5e$. The entire system was immersed in a 25.0 Å radius sphere of solvent centered around the mutating groups, which was subject to a half-harmonic restraint near the boundary to prevent evaporation. During the simulation, all atoms of the protein were fixed beyond 25.0 Å. The algorithm for the complex simulation (N, V and T) was identical to the solvent simulation, except for the presence of periodic boundary conditions.[6a]

(c) Structural Comparison

The X-ray structure of HIV1 protease complexed with JG365 (shown in Figure 3) inhibitor was used as a starting model for the FEP calculations. Initially, an energy minimization (500 steps of steepest descent followed by 2000 steps of conjugate gradient optimization) of the HIV1 protease complex was performed. This was followed by a 20 ps MD simulation equilibration stage. The average 'dynamical' structure of the complex was computed

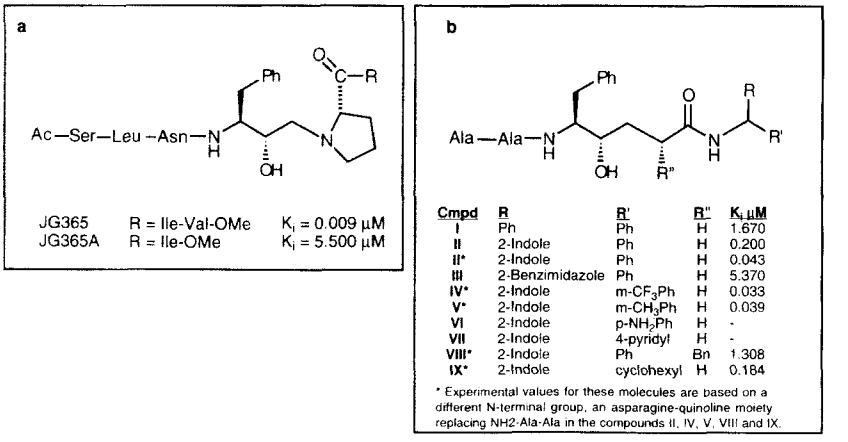

FIGURE 3 Structures of the HIV1 protease inhibitors considered in this work.

from the MD simulation. For time steps of 1 and 2 fs in MD simulations, the RMS deviations from the crystal structure were 1.03 and 1.10 Å for backbone atoms and 1.55 and 1.65 Å for side chain atoms, respectively. Overall RMS deviations are within the range typically observed for MD simulations of protein-inhibitor complexes. These results support the validity of the force field parameters and MD time step. Since both time steps (1 and 2 fs) yielded good agreement with the X-ray structure, the larger time step of 2 fs was used for all free energy calculations, in order to save computer time.

(d) Results and Discussion

(i) *Validation of FEP Methodology*

The relative solvation free energy difference ($\Delta\Delta G_{sol}$) between inhibitors JG365 and JG365A was computed using the FEP method and the first cycle of Figure 2. The results indicate that removing the valine residue results in a difference of about 8 kcal/mol. This large relative difference was attributed to three good hydrogen bonds between valine backbone atoms of JG365 inhibitors and solvent water. The calculated relative solvation free energy (8 kcal/mol) agreed with the experimental result obtained for isolated valine (8.2 kcal/mol[24]). This agreement indicates that the force field parameters and FEP methodology are very good for calculating relative solvation free energies between these inhibitors.

The relative binding free energy difference for these inhibitors complexed to HIV1 protease was calculated using the second cycle shown in Figure 2. The calculated result (3.25 ± 11.06 kcal/mol) is in good agreement with the experimental value of 3.81 ± 1.3 kcal/mol.[3f] The calculated results indicate that, even though JG365 costs about 8 kcal/mol more to desolvate, it is a better inhibitor of HIV1 protease than JG365A. A comparison of the HIV1 protease–inhibitor complexes suggests that the high binding preference for JG365 is due to a good hydrogen bond and strong electrostatic interactions between the carbonyl oxygen of valine and Arg-8 as well as good hydrophobic interactions between the valine side chain (JG365) and other protein residues. These interactions dominate over an opposing contribution arising from the larger desolvation penalty of JG365 compared to JG365A. These results, along with the results from other research groups[6] who used the AMBER program for calculating relative binding affinities of inhibitors to HIV1 protease, suggest that this method could be used for screening proposed analogs of a lead inhibitor of HIV1 protease prior to synthesis.

TABLE I Relative differences in the binding free energies (kcal/mol)

Compounds	$\Delta\Delta G_{bind}$ (*expt*)	$\Delta\Delta G_{bind}$ (*TCP*)	ΔE_{com} (*inter*)	$\Delta\Delta E_{bind}$ (*intra*)	$\Delta\Delta E_{bind}$ (*inter*)	$\Delta\Delta E_{bind}$ (*tot*)	ΔP_{com} (*inter*)
JG365A–JG365	3.8	3.3	23.6	0.4	18.6	19.2	0.22
I–II	1.30	1.9	10.91	0.3	2.0	2.3	0.13
III–II	1.95	1.3	−4.47	2.5	2.4	4.9	0.03
IV*–II*	−0.16	0.2	−1.93	1.5	−2.3	−0.8	−0.25
V*–II*	−0.06	0.4	−1.20	2.0	−0.7	1.3	−0.33
VI–II	—	1.1	−2.9	5.0	−2.0	3.0	0.10
VII–II	—	0.8	−2.1	2.4	−1.6	0.8	0.15
VIII*–II*	2.03	—	−3.20	3.5	2.1	5.6	−0.25
IX*–II*	0.86	—	2.65	4.8	−1.9	2.9	−0.42

*Experimental values for these molecules are based on a different N-terminal group, an asparagine–quinoline moiety replacing H_2N-Ala-Ala- in the compounds **II**, **IV**, **V**, **VIII** and **IX**.

(ii) *Binding Affinity Predictions using FEP Method*

The final list of HIV1 protease inhibitors considered for FEP predictions is shown in Figure 3(b). These are the analogs of a lead compound (molecule **II** in Figure 3(b)) for which the X-ray crystal structure of the HIV1 protease enzyme complex was solved at 2.5 Å resolution.[25] Initially, more than 20 analogs of the lead compound were proposed. Based on graphical/conformational and desolvation costs, 7 molecules (**I–VII**) shown in Figure 3(b) were identified for relative free energy calculation.

The relative solvation free energies ($\Delta\Delta G_{sol}$) and binding free energies ($\Delta\Delta G_{bind}$) were calculated for the pairs of inhibitors shown in Figure 3(b). Using the X-ray structure of HIV1 protease complexed with compound **II**, binding affinities were predicted[25] prior to synthesis. Later, some of the compounds were synthesized and K_i's were measured.[25] The predicted relative binding free energies and experimental results are shown in Table I. The comparison of predicted relative binding affinities with available experimental results showed very good agreement. This was the first study, which involved a large set of molecules, whose relative binding affinities were predicted using the FEP method prior to synthesis. Confirmation of the predictions with experimental measurements suggests that the method was useful for the design of novel inhibitors for the HIV1 protease.

5. REGRESSION METHODS

Although the FEP method enables accurate binding affinity predictions between two structurally similar inhibitors, it is not practical for the

evaluation of a large set of structurally-diverse molecules. Therefore, efforts have been on-going to develop faster methods that can accommodate structural diversity without compromising accuracy. Increased structural diversity, requires accurate accounting of a multitude of factors that significantly impact the compound's binding affinity, including solvation, hydrophobic effects, and conformational flexibility. Understanding the magnitude of each contribution is key to an accurate prediction. Incorporation of each factor into a regression equation will increase the accuracy of relative binding affinity predictions. Recently, efforts have been attempted to derive this information from the X-ray structures of protein–inhibitor complexes for incorporating into the parameters used with a traditional 3D-QSAR approaches.[26] The study by Marshall and coworkers[27] showed that the accuracy of binding affinity predictions of HIV1 protease inhibitors was enhanced with the use of crystal structure information. However, even these methods do not include scoring functions that incorporate energy variables derived using known X-ray structure information. Another study by Holloway and coworkers[14] used the intermolecular interaction energies of molecular mechanics calculations on protein complexes with other molecular properties in regression-based approaches and predicted relative binding affinities of several inhibitors to HIV1 protease. However, these methods do not include molecular properties such as solvation and entropy contributions, which are factors known to be important to binding affinity.

Similarly, we developed a regression-based method for semi-quantitative prediction of relative binding affinities for a set of HIV1 protease inhibitors[17c] (Figure 3). In this approach,[17c] the energy variables (intra and inter) were calculated by performing molecular mechanics calculations both in complexed and solvated states using an explicit solvent water model. The strength of the hydrophobic interactions was calculated using minimized structures of complexes. The results of both calculations were used to derive a regression equation for predicting relative binding free energies.

(a) Computational Details and Selection of Variables

HIV1 protease inhibitors considered for this study are the same as inhibitors used in the FEP calculations discussed earlier (Figure 3). Molecular mechanics calculations (energy minimizations) on all the structures were performed using the BORN module of the AMBER program. A four-stage protocol was followed for energy minimizations of the protein–inhibitor complexes as well as the solvated inhibitors. All the technical details are described in our earlier paper.[17c] Minimized structures in the complexed

and solvated states were used for calculating the following energy variables:

$$\Delta E_{\text{bind}}(\text{intra}) = E_{\text{com}}(\text{intra}) - E_{\text{sol}}(\text{intra}) \quad (3)$$

$$\Delta E_{\text{bind}}(\text{inter}) = E_{\text{com}}(\text{inter}) - E_{\text{sol}}(\text{inter}) \quad (4)$$

where, ΔE_{bind}(intra) and ΔE_{bind}(inter) are relative intra and intermolecular interaction energies of a ligand in the complexed and solvated states, respectively, and where E_{com}(intra), E_{com}(inter), E_{sol}(intra), and E_{sol}(inter) are intra and intermolecular interaction energies of a ligand in the complexed and solvated states, respectively. Relative differences in intra and intermolecular interaction energies for a pair of ligands L1 and L2 are given by,

$$\Delta E_{\text{com}}(\text{inter: L1} \rightarrow \text{L2}) = E_{\text{com}}(\text{inter: L2}) - E_{\text{com}}(\text{inter: L1}) \quad (5)$$

$$\Delta\Delta E_{\text{bind}}(\text{intra: L1} \rightarrow \text{L2}) = \Delta E_{\text{bind}}(\text{intra: L2}) - \Delta E_{\text{bind}}(\text{intra: L1}) \quad (6)$$

$$\Delta\Delta E_{\text{bind}}(\text{inter: L1} \rightarrow \text{L2}) = \Delta E_{\text{bind}}(\text{inter: L2}) - \Delta E_{\text{bind}}(\text{inter: L1}) \quad (7)$$

$$\Delta\Delta E_{\text{bind}}(\text{tot: L1} \rightarrow \text{L2}) = \Delta\Delta E_{\text{bind}}(\text{intra: L1} \rightarrow \text{L2}) + \Delta\Delta E_{\text{bind}}(\text{inter: L1} \rightarrow \text{L2}) \quad (8)$$

where $\Delta\Delta E_{\text{bind}}$(tot: L1 → L2) is the total relative difference in the binding energies of L1 and L2. Similarly, the relative hydrophobic interaction variable between ligands L1 and L2 is given by,

$$\Delta P_{\text{com}}(\text{inter: L1} \rightarrow \text{L2}) = P_{\text{com}}(\text{inter: L2}) - P_{\text{com}}(\text{inter: L1}), \quad (9)$$

where P_{com}(inter: L2) and P_{com}(inter: L1) are the scores for hydrophobic interactions for ligands L2 and L1, respectively. In Table I, the relative differences in the binding free energies measured experimentally ($\Delta\Delta G_{\text{bind}}$ (expt)) and calculated using TCP method ($\Delta\Delta G_{\text{bind}}$(TCP)) are compared with the scores of relative energy differences. The relative differences in the energy scores (Table I): ΔE_{com}(inter), $\Delta\Delta E_{\text{bind}}$(intra), $\Delta\Delta E_{\text{bind}}$(inter), $\Delta\Delta E_{\text{bind}}$(tot), P_{com}(inter) are calculated using the Eqs. (5)–(9), respectively. All the technical details of the method used for calculating the hydrophobic interaction variables are discussed elsewhere.[17c]

(b) Results and Discussion

A total of five variables were used to build regression models for evaluation of their utility in predicting relative binding affinities of two ligands L1 and L2 to HIV1 protease. The variables were: (1) relative intermolecular

interaction energy of ligands in the complex ignoring solvent contributions (Eq. (5)), (2) relative intramolecular interaction energy (Eq. (6)), (3) relative intermolecular interaction energy (Eq. (7)), (4) total relative binding energy (Eq. (8)), and (5) hydrophobic interaction energy (Eq. (9)). These variables (Table I) were used in different combinations in developing the regression equations and calculating correlations. Initially, the intermolecular interaction energy of the inhibitors to the protein was used (column 4 in Table I) as the only regression variable:

$$\Delta\Delta G_{\text{bind}}(\text{expt}) = 0.09\Delta E_{\text{bind}}(\text{inter}) + 1.06$$
$$n = 7, \quad r = 0.65, \quad \text{RMS} = 1.14. \tag{10}$$

The regression model obtained using relative intermolecular interaction energies of ligands with protein, neglecting solvent contributions, produced unsatisfactory correlation ($r = 0.65$). Addition of solvent contributions (column 6) to the regression model improved correlation significantly from 0.65 to 0.89:

$$\Delta\Delta G_{\text{bind}}(\text{expt}) = 0.17\Delta\Delta E_{\text{bind}}(\text{inter}) + 0.90$$
$$n = 7, \quad r = 0.89, \quad \text{RMS} = 0.68 \tag{11}$$

which was further improved by inclusion of ligand strain (column 7):

$$\Delta\Delta G_{\text{bind}}(\text{expt}) = 0.19\Delta\Delta E_{\text{bind}}(\text{tot}) + 0.41$$
$$n = 7, \quad r = 0.92, \quad \text{RMS} = 0.57. \tag{12}$$

By using inter (column 6) and intramolecular (column 5) interaction energies as independent variables in a multiple linear regression (MLR) model, the correlation further improved slightly:

$$\Delta\Delta G_{\text{bind}}(\text{expt}) = 0.20\Delta\Delta E_{\text{bind}}(\text{inter}) + 0.24\Delta\Delta E_{\text{bind}}(\text{intra}) + 0.29$$
$$n = 7, \quad r = 0.93, \quad \text{RMS} = 0.64. \tag{13}$$

Finally, by using $\Delta\Delta E_{\text{bind}}(\text{tot})$ (column 7) and $\Delta P_{\text{com}}(\text{inter})$ (column 8) as independent variables a very good correlation was obtained:

$$\Delta\Delta G_{\text{bind}}(\text{expt}) = 0.16\Delta\Delta E_{\text{bind}}(\text{tot}) + 1.20\Delta P_{\text{com}}(\text{inter}) + 0.71$$
$$n = 7, \quad r = 0.94, \quad \text{RMS} = 0.58. \tag{14}$$

For this model, a leave-one-out cross validation gave $r = 0.84$ and RMS = 0.81, indicating satisfactory predictive power. The predicted relative

binding affinities of HIV1 protease inhibitors using Eq. (11) showed that the regression equation with energy and hydrophobic variables provide semi-quantitative agreement with experimental results. These results suggest that regression models offer a fast way of semi-quantitatively predicting relative binding affinities of inhibitors within a series, and therefore a possible alternative to the FEP method. Recently, a similar procedure was used, to develop a multivariable regression equation ($r = 0.92$ and leave-one-out cross validation gave $r = 0.81$) for a large set of ($N = 25$) of fructose-1,6-bisphosphatase inhibitors.[28]

6. CONCLUSION

A comparison of the calculated and experimental relative binding affinities for structurally similar inhibitors to HIV1 protease indicates that the FEP method is more accurate, but it suffers from practical limitations due to its relative complexity and computation-intensive nature. Accordingly, there is a need for methods that enable rapid assessment of a large number of structurally-unrelated molecules in a semi-quantitative manner. Based on these results, energies calculated for inhibitors in the complexed and solvated states as well as the strength of hydrophobic interactions calculated using energy minimized structures of complexes are sufficient to estimate the relative binding free energy differences between two inhibitors. As shown with HIV1 protease inhibitors, multivariate models that account for these properties are useful as a rapid computational alternative to FEP calculations. These models will continue to evolve and become more accurate as force fields are optimized, and as other important variables for binding affinity are included in the regression models.

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