An Approach to Rapid Estimation of Relative Binding Affinities of Enzyme Inhibitors: Application to Peptidomimetic Inhibitors of the Human Immunodeficiency Virus Type 1 Protease

Vellarkad N. Viswanadhan,*[,†,||] M. Rami Reddy,*[,||,‡] Alexander Wlodawer,[§] Michael D. Varney,[‡] and John N. Weinstein[†]

Laboratory of Molecular Pharmacology, National Cancer Institute, Developmental Therapeutics Program, National Institutes of Health, Bethesda, Maryland 20892, Agouron Pharmaceuticals, Inc., 3565 General Atomics Court, San Diego, California 92121, and Macromolecular Structure Laboratory, National Cancer Institute-Frederick Cancer Research and Development Center, ABL-Basic Research Program, Frederick, Maryland 21702

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This report describes a method for rapid assessment of the binding affinities of a series of analogous ligands to an enzyme. This approach is based on two variables (scores), representing (i) the enthalpy of binding and (ii) the strength of hydrophobic interaction. The method is then used to evaluate the binding of 11 different peptidomimetic inhibitors to the HIV-1 protease. Three-dimensional structures of these enzyme−inhibitor complexes are modeled based on the crystal structures of HIV-1 protease complexes with the known inhibitors. These structures are minimized using the AMBER force field, and the scores of binding enthalpy for each of the ligands are calculated. A second score to represent the hydrophobic interaction between a pair of atoms uses an exponential function of distance between the atoms and the product of their atomic hydrophobicity constants. This exponential function is used to assess the hydrophobic interaction energy between an enzyme and its inhibitor and also to compute and display a 'molecular hydrophobicity map' as a 3D visualization tool. These methods are then applied to obtain trends in relative binding affinities of pairs of analogous inhibitors. Calculated scores agree well with corresponding results from thermodynamic cycle perturbation (TCP) simulations as well as experimental binding data. Since the proposed calculations are computationally cheaper and faster than TCP calculations, it is suggested that these scores can form the basis for rapid, preliminary theoretical screening of proposed derivatives of an inhibitor prior to TCP analysis, synthesis, and testing.

Introduction

The HIV-1 protease (HIV1-PR) has emerged as an important therapeutic target for the treatment of acquired immunodeficiency syndrome (AIDS) because its inhibition interrupts the life cycle of the AIDS virus.[1−3] For that reason, a large number of inhibitors of HIV1-PR[4−10] have been designed, synthesized, and biochemically tested. In many of these cases, their complexes with the enzyme have been studied crystallographically. Rational design of such protein inhibitors can be greatly expedited if one is able to predict the relative binding affinities for a set of related inhibitors.[11,12] In this regard, a free energy simulation method known as thermodynamic cycle perturbation (TCP)[13,14] has been successfully used.[15−21] A few applications of TCP approach have also focused on predictions prior to synthesis.[22,23] This emerging approach is promising, but it is by no means simple, and it is very intensive computationally. Its relative complexity makes it rather unsuitable for routine computational screening of many analogs of a lead inhibitor. Consequently, efficient (though approximate) methods to compute free energy *trends* by simulations have been advanced.[24]

The pace at which 3D structures have been determined for enzyme−inhibitor complexes of pharmacological relevance has accelerated considerably in recent years. For example, over 120 crystal structures have been solved for complexes of HIV protease alone![25] It is, therefore, a matter of some urgency to pursue methods for rapid estimation of binding affinity (or free energy) trends among analogous inhibitors, making use of known crystallographic information. In this article we seek to obtain qualitative *trends* for enthalpic changes from molecular mechanics calculations, which are computationally less demanding than TCP calculations. We also report a method to score hydrophobic interaction energy between an inhibitor and protein as a logical next step to account for significant entropic factors. An extension of this approach is also used to visualize the 3D hydrophobicity map of a molecule from an atomic hydrophobicity scale derived from 1-octanol-to-water partition coefficients.[26] We show that in the case of 11 protein−inhibitor complexes of the HIV-1 protease, these tools can be used to predict binding qualitatively. Hence, such predictions can form the basis for a rapid, preliminary screening of proposed derivatives of an inhibitor prior to synthesis and testing or more rigorous theoretical analysis.

Computational Methodology

To predict the binding affinity of an inhibitor to a protein, we need to obtain realistic estimates for enthalpic and entropic components of the binding free energy of the structure. Previously, active site geometries of various ligands bound to the HIV-1 protease were used in a 3D-QSAR (quantitative structure−activity relationship) approach to predict binding affinity.[27] In the present approach, however, we employ known

* Correspondence may be addressed to either author.
† National Cancer Institute, Developmental Therapeutics Program.
‡ Agouron Pharmaceuticals, Inc.
§ National Cancer Institute-Frederick Cancer Research Center.
|| Present address: Gensia, Inc., 9390 Towne Center Dr., San Diego, CA 92121.
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information from the crystal structures of complexes (and from models for complexes of related inhibitors obtained by docking and energy minimization) to derive a quantitative model on the basis of very few variables for each complex to represent enthalpic and hydrophobic contributions. Since crystal structures of complexes for all molecules of interest (designed, synthesized, and/or tested) are not usually available, the best way to obtain the binding modes of these inhibitors is to carefully align them with the known inhibitor structure. We then subject all of these complexes (crystal structures as well as docked complexes) to an energy minimization protocol. These minimized structures form the basis for calculating certain interaction variables that could be useful in deriving a quantitative model for predicting the binding affinity. It must, however, be emphasized that such an approach does not yield rigorous estimates of the free energy components and can only be compared with other scoring methods[28,29] in terms of prediction accuracy. More accurate scores could be derived for select complexes by using molecular dynamics/Monte Carlo (MD/MC) simulations to obtain enthalpic estimates. However, our present interest is in the development of a suitable methodology that enables rapid initial screening of proposed inhibitors of an enzyme before undertaking more computationally intensive MD/MC protocols.

Scores To Represent Enthalpic Contribution to Binding Affinity. Let E_{com}(intra) refer to the intramolecular interaction energy of the ligand in the bound state and E_{sol}(intra) refer to the intramolecular interaction energy of the ligand in the aqueous medium. A measure (or score) of the internal strain in the ligand (ΔE_{intra}) upon binding can be expressed by the following equation.

$$\Delta E_{intra} = E_{com}(\text{intra}) - E_{sol}(\text{intra}) \quad (1)$$

Similarly, ΔE_{inter} from the following equation may be used to score the strength of intermolecular interactions of the ligand in the complex relative to the solvent phase.

$$\Delta E_{inter} = E_{com}(\text{inter}) - E_{sol}(\text{inter}) \quad (2)$$

Here, E_{com}(inter) refers to the intermolecular interaction energy of the ligand in the bound state, and E_{sol}(inter) refers to the intermolecular interaction energy of the ligand in the aqueous medium. ΔE_{tot} from the following equation may then be used to score the total strength of interaction energy of a given ligand in the complex relative to the aqueous phase.

$$\Delta E_{tot} = \Delta E_{intra} + \Delta E_{inter} \quad (3)$$

Since these scores (ΔE's for a particular complex) could be subject to considerable uncertainties (depending upon the minimization protocol and the quality of parameters), a better comparison of similar molecules would be in terms of relative differences in the strength of interaction energy between pairs of related inhibitors when identical minimization protocols are employed. This score ($\Delta\Delta H_{bind}$) for a modification of the ligand (L1 → L2) may be defined as,

$$\Delta\Delta H_{bind}(\text{L1} \rightarrow \text{L2}) = \Delta E_{tot}(\text{HIV1-PR:L2}) - \Delta E_{tot}(\text{HIV1-PR:L1}) \quad (4)$$

where ΔE_{tot}(HIV1-PR:L2) and ΔE_{tot}(HIV1-PR:L1) are the respective scores (eq 3) for ligands L2 and L1 and PR denotes the enzyme, in this case the HIV-1 protease. $\Delta\Delta H_{bind}$ represents relative difference in the enthalpy of binding for related inhibitors L1 and L2. Although $\Delta\Delta H_{bind}$ is not a rigorous estimate of the relative enthalpic difference, it provides a reasonable way to rapidly score relative binding affinities of similar ligands binding to an enzyme. Rigorous estimates of enthalpy of binding would require molecular dynamics calculations of given complexes at constant pressure.

The score $\Delta\Delta H_{bind}$ includes intra- and intermolecular interaction energy scores for the pair of ligands under consideration. In contrast, in several TCP simulations and analyses, it is assumed[19,30,31] that interaction energies (ligand−protein and ligand−solvent) play the dominant role in dictating binding preferences and that the change in internal energy (i.e., internal strain upon binding) can be neglected to a first approximation when an inhibitor is mutated.

Hydrophobic Interactions and Their Contribution to Binding Affinity. A simple equation similar to the Buckingham function for van der Waals interactions is used to compute hydrophobic interactions[32] for a given ligand, L, binding to a protein.

$$P(\text{int}) = -k\sum_{i=1}^{n}\sum_{j=1}^{m} a_i b_j \exp(-D_{ij}) \quad (5)$$

Here, P(int) is the score for hydrophobic interaction, a_i represents the atomic hydrophobicity constant[26] assigned to atom 'i' in the protein with n atoms, b_j represents the atomic constants assigned to atom 'j' in the inhibitor with m atoms, D_{ij} is the distance between them, and k is a scaling constant. While this expression is suitable for evaluating hydrophobic interaction between two hydrophobic atoms or groups, computing such an interaction energy score between polar atoms or groups requires some special considerations.[28] Generally speaking, the atomic hydrophobicity constants will be negative for polar atoms. These are involved in hydrogen bonds or electrostatic interactions which are adequately represented by the corresponding ΔE_{tot} as described in the above section (eq 3). Hence, we excluded the 'hydrophobic' contribution of polar atoms. Though more research effort and experience would be necessary to determine the correct form of energy expression to compute hydrophobic interaction energy or score, we consider an exponential distance dependence of interaction energy for pairs of mutually attractive hydrophobic atom types to be a good first approximation. Alternative expressions to account for solvation (and/or hydrophobic interactions)[33−36] are discussed later.

A score for the difference in hydrophobic interaction energy for a given modification of a ligand, L1 → L2, may be expressed as:

$$\Delta P(\text{L1} \rightarrow \text{L2}) = P_{L2}(\text{int}) - P_{L1}(\text{int}) \quad (6)$$

where P_{L2}(int) and P_{L1}(int) are the scores for hydrophobic interaction (eq 5)) for ligands L2 and L1, respectively. A positive ΔP indicates that L1 has better hydrophobic contacts than L2.

Computational Models. Figure 1 shows the covalent structures of two sets of related inhibitors analyzed in the present work. These compounds have been the subject of previous TCP simulations.[22,23]

The protein, the inhibitor, and the solvent water were modeled using the AMBER all-atom force field.[37,38] The AMBER Hamiltonian[37,38] consists of energy contributions from bond length, bond angle, and dihedral angle changes from equilibrium geometry, as well as van der Waals, electrostatic, and hydrogen bond interactions.

Two sets of computational models were developed. The first set, shown in Figure 1a, was based on the X-ray structure of the HIV-1 protease complexed with Ac-Ser-Leu-Asn-(Phe-Hea-Pro)-Ile-Val-OMe (JG365).[39] The computer model of compound **II** (JG365A) was generated using the molecular editor menu option in QUANTA,[40] by modifying the terminal -Val-OMe in the 3D structure of JG365 while retaining the common part between the two molecules in the same geometry and conformation. Crystal structures were available for the complexes of AG1006 and AG1007 (**I** and **II** shown in Figure 1b).[41] Three-dimensional structures of ligands (Figure 1b) were generated by appropriately modifying the 3D structure of AG1007 using the molecular editor menu option in QUANTA[40] while retaining the common part between AG1007 and each of the other analogs during the modification.

The hydrogens of the crystallographic water, the inhibitor, and the protein dimer were added using the EDIT module of AMBER.[37,38] Electrostatic charges and parameters for the standard residues of the inhibitor model were taken from the AMBER[37,38] database. For nonstandard residues in the inhibi-

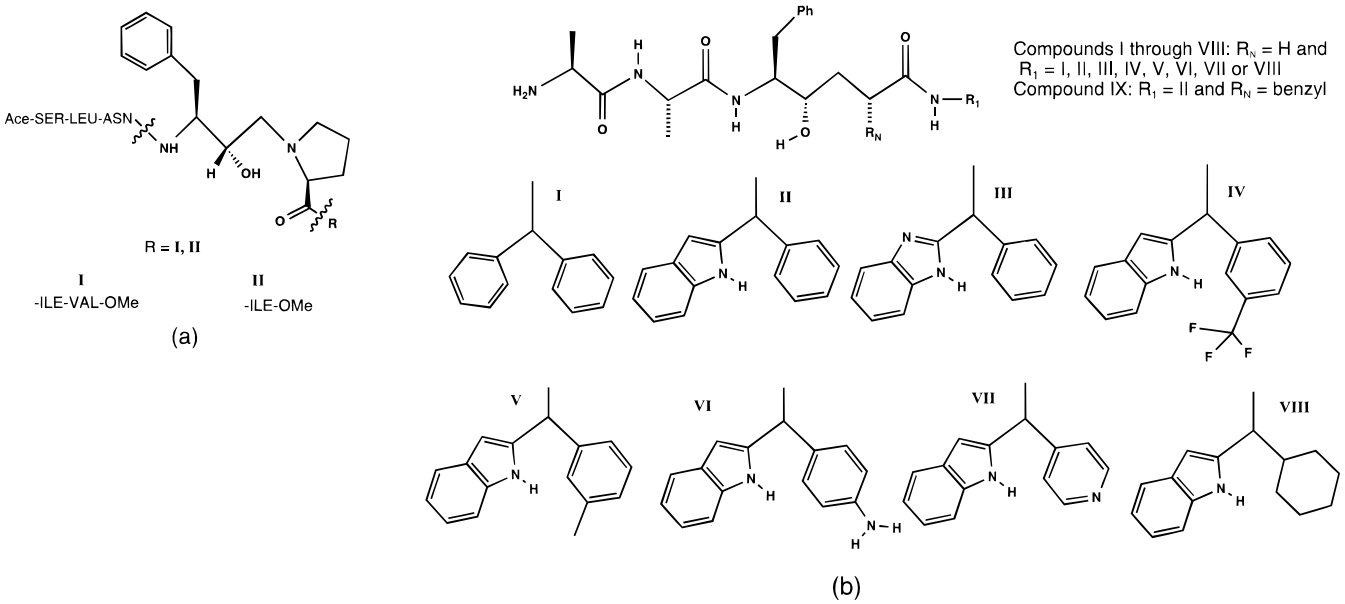

Figure 1. Structures of the HIV-1 protease inhibitors considered in this work. (a) Pair of peptidomimetic inhibitors of the HIV-1 protease. **I** is JG365, and **II** is its analog (JG365A). (b) Series of nine related inhibitors of the HIV1 protease (referred in the text as compounds **I**−**IX**). Compound **IX** is a modification of compound **II** with R_N = benzyl.

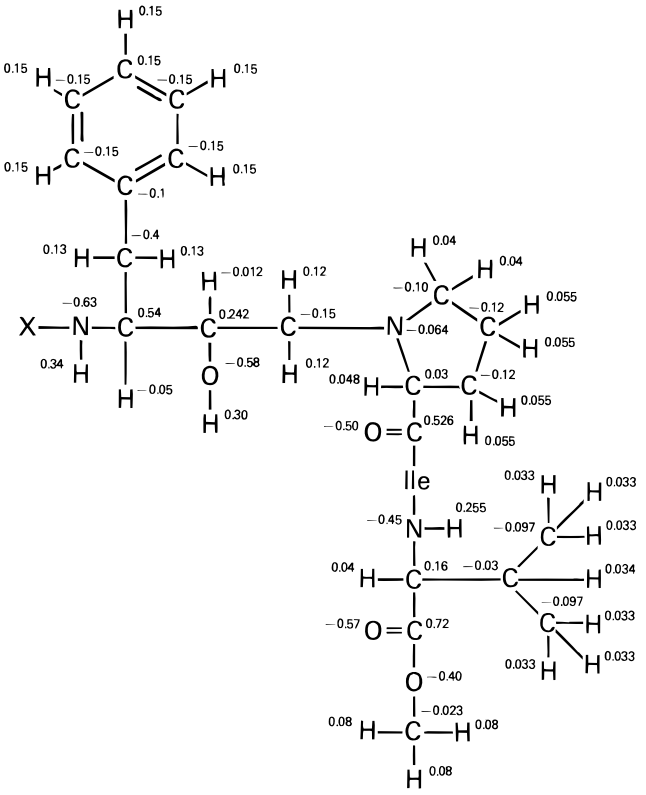

Figure 2. Partial atomic charges (in e, derived from ab initio (GAUSSIAN88) wave functions) for the nonstandard residues of JG365.

tors JG365 and JG365A, electrostatic charges were fitted with CHELP[42] from ab initio (single-point) 6-31G* wave functions, using the structures optimized at 3-21G* level calculated with GAUSSIAN88.[43] Calculated partial charges for the nonstandard residue in JG365 are shown in Figure 2. For nonstandard residues in the AG1006/AG1007 series, the partial charges were taken from a recent publication.[23] The total charge on the protease dimer is +5 e. One of the aspartic acids in the catalytic dyad (Asp 124) was protonated in all calculations. All equilibrium bond lengths, bond angles, and dihedral angles for nonstandard residues were calculated from structures optimized using ab initio quantum mechanics (GAUSSIAN88).[43] Missing force field parameters were estimated from similar chemical species in the AMBER[37,38] database. For the protein complex calculations, the solute was immersed in a large spherical water bath (of radius 25 Å) constructed from repeated cubes of SPC/E water molecules which represented a snapshot from an MD simulation of liquid water.[44] The SPC/E rigid geometry model[45] potential was used to explicitly model the solvent water. This potential reproduces pertinent properties of bulk water quite well[44]. For the solvent calculations, the solute was solvated with SPC/E water, in a rectangular box whose dimensions allowed a 10.0 Å layer of water to surround the solute atoms. Any water molecules located <2.5 Å from the solute atom were removed.

Energy Minimizations. Molecular mechanics calculations (energy minimizations) on all the structures were performed using the BORN module of the AMBER[37,38] program. A four-stage protocol was set up for energy minimizations of the protein−inhibitor complex. Minimization at each stage was performed using 100 steps of steepest descent and 1000 steps of conjugate gradient algorithms. In the first stage, only the waters were minimized (by 100 steps of steepest descent and 1000 steps of conjugate gradient methods), keeping the inhibitor and the protein (in the complex calculation) fixed. The purpose of this step is to relieve any bad contacts involving water molecules in the initially solvated system. In the second stage, only hydrogens in the system were allowed to relax by 100 steps of steepest descent and 1000 steps of conjugate gradient algorithms for minimization. This step relaxes the hydrogen atoms prior to relaxing heavy atoms. It was performed because the hydrogen locations are not specified by the X-ray structure and because adjustments in hydrogen atom locations are necessary to improve hydrogen bond geometries. The third stage was performed for all the modified ligand−protein complexes (i.e., when the ligand is modified from the original ligand in an X-ray structure complex). In this third stage, all atoms of the protein were fixed and atoms common to the ligand in the crystal structure complex (JG365 or AG1007) and the modified ligand were also fixed, while allowing the modified group in the ligand and the solvent to move during optimization. This stage allows for the relaxation of the modified group with respect to the protein, establishes the preferred interactions (e.g., hydrogen bonds), and was performed using 100 steps of steepest descent and 1000 steps of conjugate gradient algorithms for energy minimization. In the fourth and final stage, all atoms of all residues within 25 Å of any atom in the center of the modified group (waters, protein atoms, and the ligand) were allowed to relax. This

Table 1. Differences in the Interaction Energies of HIV1-PR Inhibitors (in kcal/mol) in the Complex and Solvated States[a]

system	ΔE_{intra}	ΔE_{inter}	ΔE_{tot}	P
JG365	7.6	−78.5	−70.9	−2.63
JG365A	8.2	−59.9	−51.7	−2.41
		AG1007 Series		
I (AG1006)	2.7	−63.0	−60.3	−1.40
II (AG1007)	2.4	−65.0	−62.6	−1.53
III	4.9	−62.6	−57.7	−1.50
IV	3.9	−67.3	−63.4	−1.78
V	4.4	−65.7	−61.3	−1.86
VI	7.4	−67.0	−59.6	−1.43
VII	4.8	−66.6	−61.8	−1.38
VIII	5.9	−62.9	−57.0	−1.78
IX	7.2	−66.9	−59.7	−1.95

[a] ΔE_{intra} is the difference in intramolecular interaction energy between the complexed and solvated states of an inhibitor. ΔE_{inter} is the corresponding difference in intermolecular interaction energy. The sum of the two numbers (ΔE_{tot}) represents the total interaction energy of an inhibitor in the complex state relative to the solvated state. Score for the hydrophobic interaction (P) is also shown for each ligand complex (calculated using eq 5).

Table 2. Relative Differences in the Free Energy of Binding (in kcal/mol) as Observed Experimentally ($\Delta\Delta G_{bind}$(expt)) and by TCP Simulations ($\Delta\Delta G_{bind}$(TCP)) Compared with the Scores of Relative Enthalpic Differences ($\Delta\Delta H_{bind}$(calcd)) and Changes in Hydrophobic Interaction Strength (ΔP_{bind}(calcd)) for Pairs of Analogous Inhibitors of the HIV-1 Protease

'mutation'	$\Delta\Delta G_{bind}$-(expt)	$\Delta\Delta G_{bind}$-(TCP)	$\Delta\Delta H_{bind}$-(calcd)	ΔP_{bind}-(calcd)
JG365 → JG365A	3.80	3.3	19.2	0.22
		AG1007 Series		
II → **I**	1.30	1.9	2.3	0.13
II → **III**	1.95	1.3	4.9	0.03
II[a] → **IV**[a]	−0.16	0.2	−0.8	−0.25
II[a] → **V**[a]	−0.06	0.4	1.3	−0.33
II → **VI**		1.1	3.0	0.10
II → **VII**		0.8	0.8	0.15
II[a] → **VIII**[a]	2.03		5.6	−0.25
II[a] → **IX**[a]	0.86		2.9	−0.42

[a] Experimental values for these molecules are based on a different N-terminal group, an asparagine−quinoline moiety replacing NH_2-Ala-Ala in the compounds **II, IV**, **V**, **VIII**, and **IX**.

step was performed using 100 steps of steepest descent and 2000 steps of conjugate gradient methods.

A four-stage protocol was established for energy minimization of the solvated inhibitor. These minimizations were carried out using periodic boundary conditions in all directions. In the first stage of minimization, only the waters were minimized (100 steps of steepest descent and 1000 steps of conjugate gradient methods), keeping the inhibitor (i.e., the solute) fixed. In the second stage, only hydrogens in the system were allowed to relax. This step involved 100 steps of steepest descent and 1000 steps of conjugate gradient minimization. In this third stage, atoms common to the ligand in the crystal structure complex (JG365 or AG1007) and the modified ligand were also fixed, while allowing the solvent and the modified group in the ligand to move during optimization. In the fourth stage of the solvent calculation, all water molecules and the solute (ligand) were allowed to relax. This stage of minimization was performed using 100 steps of steepest descent and 2000 steps of conjugate gradient optimization.

Results and Discussion

All 11 complexes shown in Figure 1 were subjected to the energy minimization protocol described in the previous section. Table 1 shows the results from energy minimization in the complex state and in the solution state. For each complex, the intramolecular (ΔE_{intra}) and intermolecular (ΔE_{inter}) energy contributions to ΔE_{tot} are listed separately.

Our earlier TCP simulations offered an explanation for the relative binding affinities of JG365 and JG365A.[22] The calculated difference (averaged for the two diastereomeric configurations of the hydroxyethylene group) in the binding free energy[22] (3.3 ± 1.1 kcal/mol) is in good agreement with the experimental[46] value of 3.8 ± 1.3 kcal/mol obtained from an equilibrium mixture of the two configurations. These results from TCP calculations[22] can be summarized by stating that stronger HIV1-PR:JG365 interaction dominates an opposing contribution to the relative binding affinity change arising from the larger desolvation penalty of JG365 relative to JG365A.

As shown in Table 1, the score ΔE_{tot} for JG365 is much better than the score for JG365A. Thus, a qualitative answer for the relative binding preference of JG365 is obtained from these scores. The ΔE scores shown in Table 1 are computed from two sets of minimizations. ΔE_{intra} is the difference in intramolecular interaction energies between the complex and solvated states of an inhibitor. ΔE_{inter} is the corresponding difference in intermolecular interaction energies. It is expected that such scores computed from energy minimizations are associated with uncertainties and will not match the quality of results from TCP simulations. Hence, we expect only an agreement in the overall trends between the TCP results and the energy minimization results. Nevertheless, it is noteworthy that a large mutation such as the present one is rather well explained by these scores.

An analysis of the relative binding preferences of AG1006 (compound **I** in Table 1 in the AG1007 series) and AG1007 (compound **II** in Table 1) to the HIV-1 protease has been described recently using MD simulations and the TCP approach.[23] The difference in the binding free energy $\Delta\Delta G$ calculated by TCP simulations is 1.9 kcal/mol, whereas the experimental value is 1.3 kcal/mol. Table 1 shows that the scores for compound **II** are better than the scores for compound **I**, which is in satisfactory agreement with the experimental results. The differences in the ΔH score of compound **II** from those of compounds **IV**, **V**, and **VII** are rather small indicating that one may not expect major differences in the binding affinity for these compounds, when compared with compound **II**. Compound **III** has a score of 4.9 kcal/mol higher in energy than that of compound **II**, and an even larger score is indicated for compound **VIII**. Compound **VIII**, which has a cyclohexyl group replacing a phenyl in compound **II**, does not bind with higher affinity than compound **II** partly because of the internal strain on the ligand. A similar effect is also seen in the case of compound **IX**, which has a benzyl group replacing a hydrogen in compound **II**.

Table 2 shows the experimentally measured relative free energies of binding[41,46] and the results from corresponding TCP calculations[22,23] for 11 'mutations'. Also listed in Table 2 are the corresponding scores, $\Delta\Delta H_{bind}$ and ΔP, obtained by eqs 4 and 6. The ΔP scores are discussed subsequently. In some cases, as noted in Table 2, the experimental measurements have been performed on N-terminal-modified groups. It is reasonable to expect that differences in interactions will be cancelled out between similarly modified ligands, since the site of 'mutation' is at the other end of the ligand.

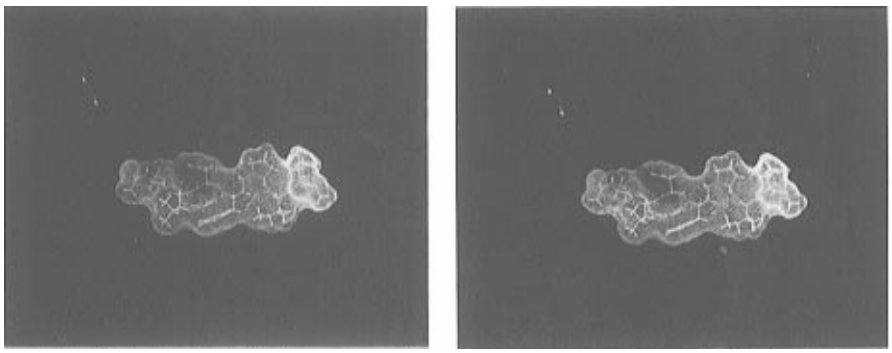

Figure 3. Three-dimensional hydrophobicity map of the JG365 inhibitor shown in stereo. The dot surface represents the lipophilicity potential of the molecule at different points along the surface accessible to a probe of radius 1.0 Å. The color code represents highly hydrophobic (red) to highly hydrophilic (blue-violet) regions.

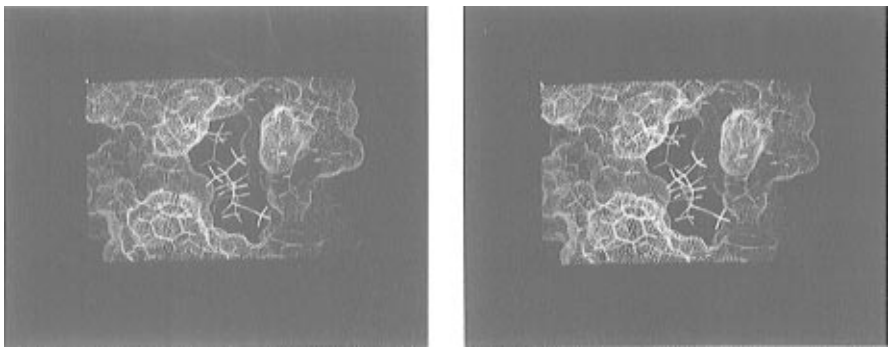

Figure 4. Three-dimensional hydrophobicity map of the HIV-1 protease at the active site area shown in stereo, along with the JG365 inhibitor (The inhibitor is not involved in the coloring of the 3D hydrophobicity map.) Note that the valine side chain (visible prominently at the front) is located rather close to the surface which is mildly hydrophobic. The color coding is the same as that of Figure 3.

It may be seen from this table that the scores of $\Delta\Delta H_{bind}$ are in qualitative agreement with experimental results. Table 2 also shows that, in general, the $\Delta\Delta H_{bind}$ scores agree qualitatively with the earlier TCP results.[23]

Molecular Hydrophobicity Maps and Hydrophobic Contribution to Relative Binding Free Energy Differences. For the analysis of ligand−protein interactions, it is of interest to compute and display a color-coded molecular hydrophobicity map,[47] which indicates the relative hydrophobic character of different parts of the molecule on a van der Waals or contact surface. We used eq 5 for a probe with unit atomic hydrophobicity constant (including all-atom−probe interactions) to compute molecular hydrophobicity maps based on (a) an extended van der Waals surface (i.e., the van der Waals radius being extended by 1 Å), (b) an accessibility surface[48] for a probe with a radius of 1.4 Å, tracing the center of the probe sphere along the surface of the molecule using Connoly's[49] molecular surface representation procedure, and (c) a molecular surface similar to that in b but using the contact points of the sphere rather than the center of the sphere. Rolling a spherical probe over the van der Waals atomic surface bridges small crevices and produces a more continuous image of molecular topography, and hence we prefer this contact surface representation. An example of this surface is shown in Figure 3for the JG365 inhibitor with a probe radius of 1.0 Å. In that figure, red dots indicate the hydrophobic region and blue-violet dots indicate the polar region of the molecule. Alternative functional forms[47] have also been used to generate surfaces, but we did not find significant differences in the surface coloring. Figure 4shows inhibitor JG365 in the active site of HIV1-PR, with the surface computed using a probe radius of 1.0 Å. The surface near the penultimate valine residue of JG365 is seen to be neither highly hydrophobic nor highly hydrophilic, whereas the extended hydrophobic region occupied by Leu, Phe, and Ile of JG365 is strongly hydrophobic (see Figure 3). From Figure 4 it may be seen that the penultimate Val has some hydrophobic interactions with the protein, but the presence of some polar groups in the proximity of that group limits the extent of these interactions.

To obtain a quantitative assessment of hydrophobic interactions in protein−ligand complexes, which could be significant in some cases, the empirical expression shown in eq 5 is used. Medicinal chemists have relied on 1-octanol-to-water partition coefficients (log *P*'s)[50] to account for hydrophobic and entropic contribution to relative free energy changes. Relative biological response (or binding constants) for a series of ligands binding to an enzyme receptor can be successfully modeled by 2D- or 3D-QSAR equations that include 'log *P*' (or the associated substituent constant) as a variable to model hydrophobic and part of the entropic effects.[51,52]

When the structure of the receptor has been determined as in the present case, a QSAR (or 3D-QSAR) approach would obviously be inadequate to take advantage of known hydrophobic interactions at the atomic level. While the TCP approach permits the estimation of differences in entropy contribution (of the ligands, including the conformational entropy differences), it is computationally intensive and time consuming. One may estimate these hydrophobic interactions in 'bulk' for each complex (or ligand) based directly on the extent of buried surface area[48,53] or use a function of surface area weighted by atomic parameters.[28,33−36,54] It is,

however, critical that the atomic parameters be derived from a substantial database that include a variety of atom types sufficient to deal with diverse organic functional groups of interest to the medicinal chemist. The parameter set of Eisenberg[33] contained only a small number of atom types, and they were derived specifically to model amino acids and proteins. Even the more recent approaches[34−36] in this regard were shown to be no more effective than the 'bulk' approach of Chothia[53] to estimate solvation energy based on the extent of total buried surface area.[54] In addition, it should be obvious that a surface area-based approach does not account for the relative hydrophobicity of *buried* nonpolar groups. Abraham and co-workers[28] developed the HINT approach to model hydrophobic and polar interactions associated with ligand binding. It employs a rather complex empirical formulation. Though our method has some similarity with the HINT approach,[28] there are significant differences, as discussed below.

(a) The HINT[28] approach is based on atomic constants derived indirectly (using a Rekker-type fragmental constant approach from the CLOGP[55] *estimates* of 'log *P*'). In contrast, the present approach is based on the atomic hydrophobicity parameters[26] (the ALOGP parameters[26]) derived directly from a substantial 'log *P*' database. An assessment of the performance of the ALOGP[26] method in predicting 'log *P*' shows that it performs quite well for nucleoside analogs[56] and neutral amino acids.[47] For these reasons, we believe that the calculation of hydrophobic interactions using the atomic hydrophobicity constants[26] derived directly from a 'log *P*' database is likely to yield better results.

(b) The HINT[28] approach uses a sign-flip function to account for polar interactions using the atomic hydrophobicity constants and an explicit Lennard−Jones term. In the present approach, since polar (or electrostatic) and van der Waals interactions are computed using the AMBER[37,38] force field as part of $\Delta\Delta H$ estimation, the interactions between polar atoms (e.g., those involving phenolic and alcoholic hydroxyls and -NH and $-NH_2$ groups) are excluded in computing the score for hydrophobic interactions.

Table 1 shows the values of the hydrophobic interaction energy score (P) for all the inhibitors in this study, and Table 2 lists corresponding ΔP values for 11 'mutations'. For the mutation JG365 → JG365A, the ΔP score is 0.22, indicating better hydrophobic contacts for JG365 than for JG365A. The side chain of the valine residue has hydrophobic interactions with the side chains of Ile 146 and Phe 152 and the nonpolar part of the Lys 144 side chain. Some of these interactions are also evident in Figure 4. Table 1 shows that AG1007 (compound **II**) has better hydrophobic interaction scores than compounds **I**, **III**, **VI**, or **VII**. The placement of the indole ring allows for better hydrophobic contacts of the six-membered part of the fused ring with the side chains of Val 92 and Leu 23 residues and the hydrophobic part of Pro 91. This orientation also makes it possible for the -NH group of the indole ring to have a water-mediated hydrogen bond with the carbonyl group of Gly 27. Due to the larger distance of the phenyl ring (relative to indole) in compound **I** with respect to the hydrophobic groups (Val 92, Leu 23), the hydrophobic interaction score of compound **I** is smaller than that of compound **II**. The main chain carbonyl of Gly 147 interacts with the -NH- group in indole (of compound **II**) through a water bridge and obviously corresponding interactions are absent in the complex with compound **I**. This bridge stabilizes the binding conformation, and the same hydrogen bond is expected to be preserved for other analogous inhibitors in the series. This provides the justification for assuming that the modified ligands (**III**−**VII**) bind in an orientation similar to **II**. Compounds **IV** and **V** are predicted to have better hydrophobic interactions than compound **II** because of the addition of hydrophobic groups on the phenyl ring. This could explain the relative binding affinity of these compounds. The ΔP score is negative for four of the mutations (**II** → **IV**, **II** → **V**, **II** → **VIII**, and **II** → **IX**). Compounds **II** and **III** differ only in the five-membered part of the fused ring system where the 3-carbon of indole in compound **II** is replaced by a nitrogen. This nitrogen, however, does not have any hydrogen bond interactions with the protein, and hence it does not compensate the cost of its desolvation; consequently the ΔE_{int} score for compound **III** is worse by 2.4 kcal/mol than that of compound **II**. Experimental results for mutations **II** → **IV** and **II** → **V** are in qualitative agreement with the corresponding ΔP scores. In the case of mutations **II** → **VIII** and **II** → **IX**, the modifications clearly enhance hydrophobicity, and the ΔP_{bind} scores correctly reflect the relative hydrophobic character of the cyclohexyl and benzyl groups. The energetic cost of internal strain (ΔE_{intra}) on the ligand in the complex and relative differences in the van der Waals and electrostatic interactions contribute to the weaker binding of compounds **VIII** and **IX**, despite their better hydrophobicity scores. The data shown in Table 2 have been used to perform regression and correlation analyses correlating the values of $\Delta\Delta G_{bind}$(expt) with the two variables $\Delta\Delta H_{bind}$(calcd) and ΔP_{bind}(calcd).

We obtain the following equation when both variables ($\Delta\Delta H_{bind}$(calcd) and ΔP_{bind}(calcd)) are used.

$$\Delta\Delta G_{bind}(\text{expt}) = 0.16\Delta\Delta H(\text{calcd}) + 1.20\Delta P(\text{calcd}) + 0.71 \quad (7)$$

$$n = 7,\ r = 0.94,\ \text{SD} = 0.58$$

With $\Delta\Delta H$ alone as a variable, we obtain the following equation,

$$\Delta\Delta G(\text{expt}) = 0.19\Delta\Delta H + 0.41 \quad (8)$$

$$n = 7,\ r = 0.92,\ \text{SD} = 0.57$$

With ΔP alone, we obtain the following equation,

$$\Delta\Delta G(\text{expt}) = 2.77\Delta P + 1.78 \quad (9)$$

$$n = 7,\ r = 0.72,\ \text{SD} = 1.05$$

These results show that $\Delta\Delta H_{bind}$(calcd) correlated well with $\Delta\Delta G$(expt) and the addition of ΔP as a variable did not produce significant improvement in r ($r = 0.92$ for the one-variable model vs 0.94 for the two-variable model) for this dataset. Additional experiments have been conducted with somewhat different minimization protocols. The overall results are fairly robust with regard to the choice of minimization protocol (e.g., a longer minimization sequence), though the contribution of ΔP to the overall correlation is seen to vary with the protocol. The data presented in Table 2 indicate that

the scores, ΔP and $\Delta\Delta H_{bind}$, can be useful in assessing the relative binding affinities of inhibitors to an enzyme analogous to a lead compound. However, due to the small size of the dataset, these deductions are subject to some uncertainities, and more experience with bigger datasets is clearly important in the assessment of the methodology. Holloway and co-workers[57] have employed a bigger database of 33 inhibitors to the HIV-1 protease to correlate ligand−protein interaction energies with corresponding binding affinities. Though their work demonstrates the importance of intermolecular interactions in the prediction of binding affinities for inhibitors of similar size, their methodology does not include the effect of conformational entropy, desolvation, and hydrophobic effects on binding affinities. Oprea et al.[58] have analyzed the binding of peptidomimetic inhibitors to the HIV-1 protease, by employing the HINT hydrophobicity field in conjunction with comparative molecular field analysis (CoMFA) steric and electrostatic fields in a 3D-QSAR study. They concluded that the inclusion of the HINT fields does not improve the predictive power of the CoMFA model, though it is useful for understanding the role of hydrophobic interactions. These earlier works[57,58] and the present work indicate the need for additional efforts in the modeling of hydrophobic interactions.

Conclusions

The present work (i) describes a new approach to rapidly estimate relative binding affinities of enzyme inhibitors and (ii) applies that approach to a set of peptidomimetic inhibitors of the HIV-1 protease. This analysis offers a rationale for the preferential binding of JG365 and AG1007 to the HIV-1 protease compared with their respective analogs. These findings are consistent with earlier TCP results. The interaction energies of JG365 and AG1007 in the protein complex are greater than the corresponding interaction energies in the solvent state, hence overcoming the larger desolvation energies. A new method of scoring hydrophobic interaction energy using atomic hydrophobicity constants predicts correctly that the hydrophobic interaction of JG365 with the protein should be stronger than that of its analog. In the case of AG1007, the indole ring has better hydrophobic contacts and stronger interaction with the HIV-1 protease than the corresponding phenyl ring of its analog, AG1006. The calculations on 11 different inhibitors of the HIV-1 protease in the complex and in the solvent demonstrate qualitative agreement of the scores, $\Delta\Delta H_{bind}$ and ΔP, with experimentally determined binding affinities and corresponding TCP results. Therefore, these scores may be used to assess qualitatively the relative binding affinities of similar inhibitors to an enzyme. However, conformational flexibility gained or lost during the modification of an inhibitor is an additional factor affecting the relative difference in free energy ($\Delta\Delta G_{bind}$). The present methodology does not take into account this factor, since the results from a single minimization sequence are used in each case. Nevertheless, such calculations offer a rapid and approximate way to screen proposed analogs of a lead inhibitor and to select candidates for further analysis by TCP simulations or for synthesis and biochemical testing.

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