Discovery of Novel, Non-Peptide HIV-1 Protease Inhibitors by Pharmacophore Searching

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Fifteen novel non-peptide HIV-1 protease inhibitors were identified by flexible 3D database pharmacophore searching of the NCI DIS 3D database. The pharmacophore query used in the search was derived directly from the X-ray determined structures of protease/inhibitor complexes. These 15 inhibitors, belonging to nine different chemical classes, are promising leads for further development. The two best inhibitors found, NSC 32180, a "dimer" of 4-hydroxycoumarin, and NSC 117027, a "tetramer" of 2-hydroxy quinone, had ID_{50} values of 0.32 and 0.75 μM for HIV-1 protease inhibition, respectively, and two other inhibitors had ID_{50} values close to 1 μM. Among the potent inhibitors, NSC 158393 not only demonstrated activity against HIV-1 protease (ID_{50} 1.7 μM) but also exhibited promising antiviral activity in HIV-1-infected CEM-SS cells (EC_{50} = 11.5 μM). Validation of the pharmacophore used in the search was accomplished by conformational analysis. The binding modes of the most potent inhibitor found in our studies, NSC 32180, were predicted employing docking and molecular dynamics techniques.

Introduction

Human immunodeficiency virus type-1 (HIV-1) is the causative agent of acquired immunodeficiency syndrome (AIDS).[1] HIV-1 gene-encoded protease is a member of the aspartic protease family and mediates the post-translational processing of viral structural and replicative enzymes encoded by the *gag-pol* genes.[2] HIV-1 protease (HIVPR) is essential for the replication and maturation of HIV-1 since the inactivation of HIV-1 protease by either mutation or chemical inhibition leads to the production of immature, noninfectious viral particles.[3] HIV-1 protease has therefore emerged as one of the most targeted viral elements for the development of new anti-AIDS drugs.[4] Although a number of potent and selective HIV-1 protease inhibitors have been developed, most of these are peptidic in nature.[5] These peptide-based HIV-1 protease inhibitors have liabilities with respect to metabolism and compromised bioavailability.[6] Efforts have been made in several laboratories to develop non-peptide-based HIV-1 protease inhibitors,[7] but to date such compounds have not reached the same level of potency of the best peptidomimetic inhibitors. There is, as a result, a continuing need for lead compounds for the development of non-peptide HIV-1 protease inhibitors.

Three-dimensional searching of large databases has recently gained attention for its ability to discover new leads in drug development programs.[8] This approach has been utilized by several groups to identify novel HIV-1 protease inhibitor leads[9,10] or provide synthetic frameworks[11] for the design of non-peptide inhibitors.

We have recently built a searchable three-dimensional (3D) database[12] of a total of ca. 407 000 structures from the 2D structures of the National Cancer Institute (NCI) Drug Information System (DIS) database[13] using the program Chem-X.[14] Previous use of this 3D database has led us to the discovery of a number of novel protein kinase C agonists.[15] In this paper, we report the application of this technique to HIV-1 protease and the resulting discovery of 15 non-peptide HIV-1 protease inhibitors, comprising nine separate chemotypes.

Methods and Materials

NCI 3D Database and Database Search Software. The details of the NCI 3D database and the Chem-X program used in both the 3D database build and search processes have been described elsewhere.[12] The current version of the NCI 3D database consists of 206 876 "open" and 201 036 "discreet" (proprietary) structures, for a total of 407 912 structures. The conformationally flexible search algorithm implemented in Chem-X (July 1994 version, running on a Silicon Graphics workstation) was employed: for flexible compounds, multiple conformations are generated and analyzed during both building and searching of the database.[12,14] Chem-X does not explicitly calculate the conformational energy for each conformation generated in the search, and as a result, some compounds may be found with conformations that meet the pharmacophore requirements, but have very high conformational energy. This results in some false positives, as will be discussed later in more detail.

Molecular Modeling. All molecular modeling studies were performed using the QUANTA molecular modeling package[16] (version 4.0) with its associated molecular mechanics program CHARMm,[17] using the version 22 MSI parameter set, running on a SG IRIS Indigo, or a stand-alone version of CHARMm running on a Convex supercomputer. Template charges were used to assign the atomic charges for all the molecules in the calculations. Energy minimization was typically computed with 30 000 iterations or until convergence (defined as an energy gradient of 0.001 kcal mol^{-1} $Å^{-1}$ or less), using an Adjusted Basis Newton-Raphson algorithm as implemented in CHARMm. A distance dependent dielectric constant was used throughout the calculations.

The structures of the compounds studied were built using the ChemNote module within QUANTA and were energy-

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minimized using CHARMm. Conformational searches were conducted using either the systematic or the Monte Carlo random search algorithms implemented in QUANTA.

Molecular dynamics simulations were carried out by heating the system over 10 ps from 0 to 300 K, with a time step of 0.001 ps. The system was then equilibrated for 30 ps, and a constant-temperature dynamics simulation was then performed for 50 ps. The simulation trajectory was recorded every 0.1 ps. A SHAKE algorithm was used to constrain bonds to hydrogens.

The X-ray structure of HIV-1 protease complexed with A-767928, an HIVPR inhibitor developed by Abbott Laboratories,[18] was used as the initial structure in our docking and molecular dynamics simulations. All crystallographic waters were removed, including H_2O 301, which was tightly bound to residues Ile 50 and Ile 50′ in the complex. All titratable residues were modeled in their charged state except for one of the two aspartic acid groups (Asp 25 and Asp 25′) in the active site since previous studies[19] have shown that at least one of these two aspartic acids is protonated.

***In Vitro* HIV Protease Assays.** The analytical procedure employed to determine the reagent-induced inhibition of HIV-1 protease activity has been previously described.[20] Recombinant HIV-1 protease (Bachem BioScience Inc., King of Prussia, PA) and the substrate (Val-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-NH2, Multiple Peptide Systems, San Diego, CA) were utilized to determine the concentration of test compound required to inhibit protease activity by 50% (ID_{50}). Briefly, HIV-1 protease (14.2 nM final) was mixed with various concentrations of the test compounds in 250 mM potassium phosphate buffer, pH 6.5, 2.5% (v/v) glycerol, 0.5 mM dithiothreitol, 0.5 mM EDTA, and 375 mM ammonium sulfate, after which the substrate was added (30 nmol) and the reaction incubated at 37 °C for 30 min. Reactions were terminated by the addition of 20 μL of a mixture of 8 M guanidine hydrochloride to 10% trifluoroacetic acid (8:1), and the reaction products were separated by reversed-phase HPLC on a Nova-Pak C-18 column. Absorbance was measured at 206 nm, peak areas were quantitated, and the percentage conversion to product was used to calculate the percentage of control cleavage in the presence of inhibitors.

***In Vitro* Anti-HIV Assay.** Anti-HIV screening of test compounds was performed using the XTT cytoprotection assay as previously described.[21] This cell-based microtiter assay quantitates the drug-induced protection from the cytopathic effect of HIV-1_{RF} on CEM-SS (CD4+ T-cell line) cells. Data are presented as the percent control of XTT values for the uninfected, drug-free control. The EC_{50} values reflect the drug concentration that provides 50% protection from the cytopathic effect of HIV-1 in infected cultures, while the IC_{50} reflects the concentration of drug that causes 50% cell death in the uninfected cultures. The XTT-based results were confirmed by measurement of cell-free supernatant reverse transcriptase and p24 levels.

Mechanism of Action Assays. Binding of HIV-1_{RF} to PBLs was measured by a p24-based assay.[20] Briefly, 2×10^5 PBLs cells were incubated with a concentrated stock of virus for 30 min at 37 °C in the absence or presence of various concentrations of inhibitor, the unbound virus was washed away, and the cell-associated virus was solubilized in 1% Triton X-100 and 1% BSA and analyzed by the p24 antigen capture assay as previously described. The effects of inhibitors on the *in vitro* activity of purified reverse transcriptase (RT) were determined by measurement of incorporation of [^{3}H]TTP onto the poly(rA):oligo(dT) (rAdT) homopolymer template/primer system.[22] Samples (5 μL) were blotted onto DE81 paper, washed with 5% dibasic sodium phosphate as previously described,[23] and then quantitated on a Packard Matrix 9600 direct beta counter. The positive control for inhibition of RT was 3′-azido-2′,3′-dideoxythymidine 5′-triphosphate.

To determine if compounds affected the HIV-1 p7 nucleocapsid protein zinc fingers, fluorescence measurements of the Trp[37] residue in the C-terminal zinc finger of the HIV-1 p7 nucleocapsid protein were performed as previously described.[24] The p7NC protein was prepared at 20 μg/mL in 10 mM sodium phosphate buffer (pH 7.0) and treated with 25 μM of test compound, and then after indicated time intervals the samples were diluted 1/10 in 10 mM sodium phosphate buffer (pH 7.0) and the fluorescence intensity was measured. The excitation and emission wavelengths utilized with the Shimadzu RF5000 spectrofluorimeter were 280 and 351 nm, respectively.

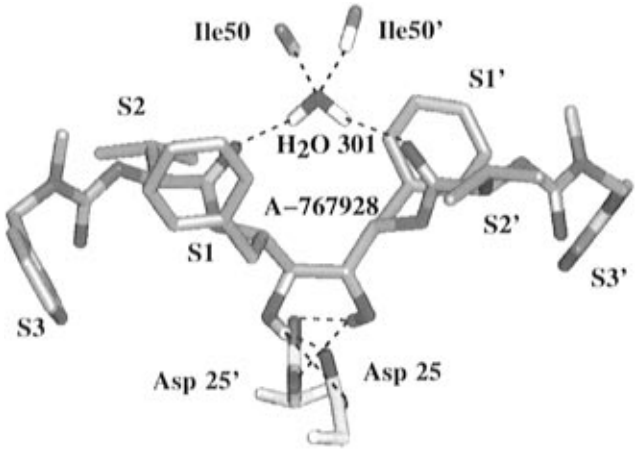


Figure 1. Interaction between HIV protease and the inhibitor A-767928.

Results and Discussion

X-ray Structures of HIV-1 Protease/Inhibitor Complexes and 3D Pharmacophore. The active structure of HIV-1 protease is a *C*2-symmetric homodimer; each monomer is a 99-mer. A large number of HIV-1 protease/inhibitor complex structures have been determined by X-ray crystallography and used to guide the rational design of HIVPR inhibitors.[25] Structures of the complexes formed by HIVPR with high-affinity inhibitors[26] showed that a number of hydrogen bonds (primarily in the P1 and P1′ regions) and hydrophobic interactions at the S1, S1′, S2, and S2′ sites seem to be essential if an inhibitor is to have binding affinity in the nanomolar range. The schematic representation of the interactions between HIVPR and an inhibitor with high bonding affinity, A-767928, is shown in Figure 1.

As can be seen from Figure 1, A-767928, like many other peptide-based HIVPR inhibitors, forms hydrogen bonds to the backbone amido groups of isoleucine 50 (Ile 50) and isoleucine 50′ (Ile 50′) of the HIV protease *via* a water molecule (designated as H_2O 301). It also forms hydrogen bonds with aspartate 25 (Asp 25) and aspartate 25′ (Asp 25′). These hydrogen bonds represent critical specific interactions between the inhibitor and HIVPR, and they were found to be optimal or nearly optimal for inhibitors with nanomolar or sub-nanomolar binding affinities. The two hydroxyl and two carbonyl groups in A-767928 are located in ideal positions and orientations, maximizing the hydrogen bond interactions with HIVPR.

Two variants of a pharmacophore model can readily be derived from these analyses. The first pharmacophore model incorporates two carbonyl groups (hydrogen bond acceptors) and two hydroxyl groups (hydrogen bond donors) in A-767928. In the second pharmacophore model (Figure 2), the two carbonyl groups are replaced by a single hydrogen bond acceptor. This hydrogen bond acceptor is in the position originally occupied by H_2O 301, presumably interacting with the amido groups of Ile 50 and Ile 50′. The displacement of this water molecule by an inhibitor is of advantage to the binding since entropy can be gained by releasing

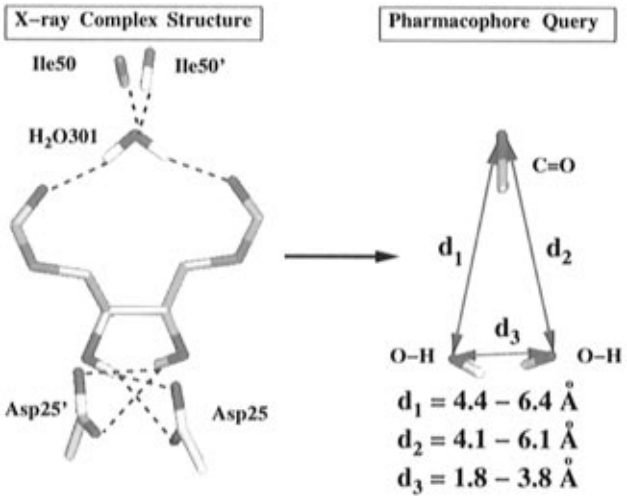


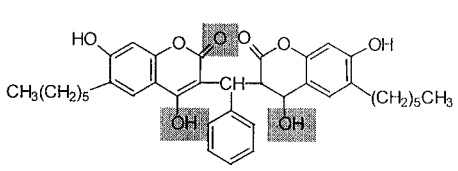
Figure 2. HIV protease pharmacophore.

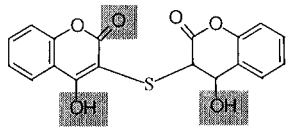
the water molecule. Indeed, this concept has been successfully used by Lam *et al.*[11] in their design of a class of potent, seven-membered ring cyclic-urea HIVPR inhibitors.

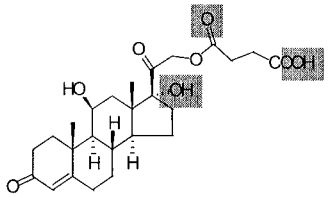
The second pharmacophore was used exclusively in the search in our present studies. As to chemical substructure constraints in the pharmacophore, only hydroxyl groups were admitted as hydrogen bond donors and only carbonyl groups as hydrogen bond acceptors. The pharmacophore query used in the search, incorporating all these chemical and geometrical constraints, is shown in Figure 2.

3D Database Search Results. The search of the open NCI database of 206 876 compounds using the pharmacophore query shown in Figure 2 yielded 2368 hits. Of these, some 1200 were available in the NCI inventory. Since hydrophobic interactions are known to be important in the binding of inhibitors to HIVPR,

Chart 1. Structures and Activities of HIV Protease Inhibitors Discovered in a 3D Search[a]


NSC 32180, ID_{50} = 0.32μM


NSC 41234, ID_{50} = 21.0μM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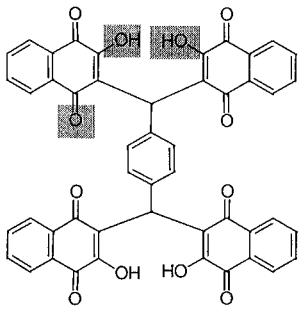
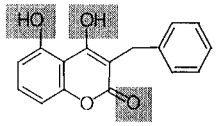
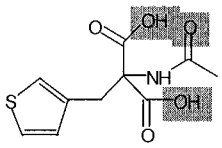
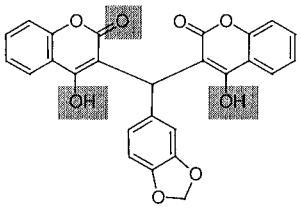

NSC 117027,ID_{50} = 0.75μM


NSC 251156, ID_{50} = 6.0 μM


NSC 109412, ID_{50} = 23.0μM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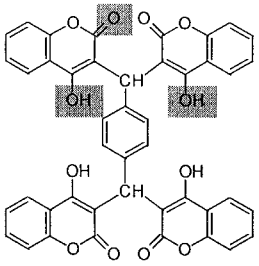
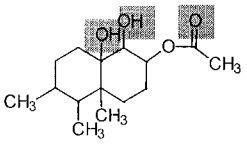

NSC 158393, ID_{50} = 1.7μM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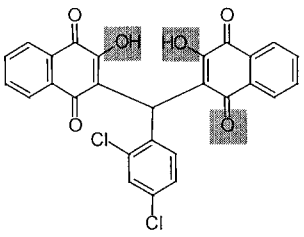
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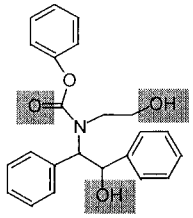
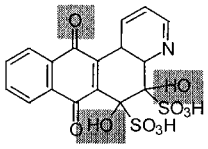

NSC 40328 ID_{50} =32.0μM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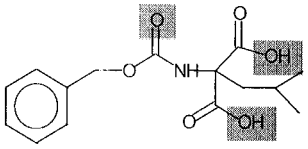
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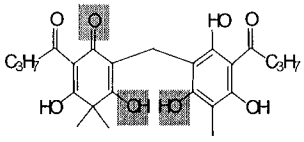
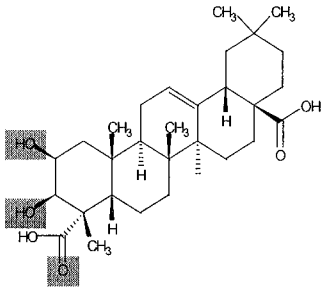

NSC 115497, ID_{50} = 26.0μM


NSC 382025, ID_{50} = 35.0 μM

[a] The most probable pharmacophore embedded in each compound is shown with shaded squares.

compounds with no apparent hydrophobic moiety were excluded from further consideration. Finally, since our goal is to discover novel and diverse new leads, chemical diversity and novelty were important factors in selecting compounds for evaluation. Using these additional criteria, a total of 50 compounds were ultimately submitted for testing in the *in vitro* HIV protease assay.

***In Vitro* HIV Protease Bioassay Evaluations.** Fifteen of the 50 candidate compounds were found to have ID_{50} values below 50 μM (Chart 1) with the individual ID_{50} values ranging from 0.32 to 35 μM. The two most inhibitory compounds, NSC 32180 and 117027, had ID_{50} values below 1 μM, and two other compounds (NSC 9152 and 158393) were found to have ID_{50} values close to 1 μM. As can be seen from Chart 1, these 15 HIV protease inhibitors are indeed chemically diverse, being roughly classifiable into nine different chemical classes. Ten out of the fifteen compounds in Chart 1 are achiral.

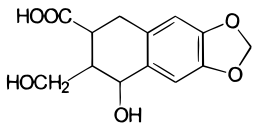
Thirty-five compounds did not show significant binding affinity at a concentration of 50 μM, despite the fact that they all possessed the pharmacophore defined in Figure 2. Possible reasons for their inactivity will be addressed later in the following section (Molecular Modeling Studies), where 10 randomly selected inactive compounds (Chart 2) are analyzed in greater detail.

Among these 15 inhibitors, NSC 32180, 41234, 158393, 251156, and 373937 all contained a 4-hydroxycoumarin substructure. Recently, compounds containing a single 4-hydroxycoumarin fragment have been reported to possess HIV-1 protease inhibitory activity.[7] It was therefore of interest to evaluate compounds containing this substructure. Accordingly, we carried out a substructure search of the NCI 2D database which identified 11 compounds containing a single 4-hydroxycoumarin moiety. All of these 11 compounds were evaluated in the *in vitro* HIV-1 protease assay. Three of them, NSC 251150, 251152, and 647086 (Chart 3), showed significant activity, with ID_{50} values of 13, 42, and 31 μM, respectively. These results suggest that, while compounds containing a single 4-hydroxycoumarin moiety are capable of binding to HIV-1 protease, compounds such as NSC 32180 (ID_{50} = 0.32 μM) and 158393 (ID_{50} = 1.7 μM), which contain a "dimer" or "tetramer" of 4-hydroxycoumarin, may be superior leads for further development.

Antiviral Activity and Inhibition of Other HIV-1 Enzymes. All 15 HIV-1 protease inhibitors discovered through the 3D pharmacophore search and the three other inhibitors discovered in the 2D substructure search were tested in cells for their antiviral activity against HIV-1. The assay used was the XTT-based cytoprotection anti-HIV assay of the NCI program. Of the 15 compounds (Chart 1) which showed enzyme inhibition, NSC 158393 was the only compound showing antiviral activity, with an EC_{50} value of 11.5 μM (mean of 8.9 and 14.2 μM) in a cellular assay against HIV-1 virus, the results of which are shown in Figure 3. Its cytotoxicity (IC_{50}) was found to be 55 μM (mean of 57.6 and 52.0 μM). NSC 158393 is not the most potent HIV-1 protease inhibitor found in our present study, but has shown the most promising antiviral activity against HIV-1. One of the possibilities to account for its favorable antiviral activity is that NSC 158393 may exert its activity through more than one possible pathway.

Chart 2. Ten Inactive Compounds Possessing the HIV-1 Protease Pharmacophore

NSC 34278



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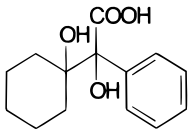

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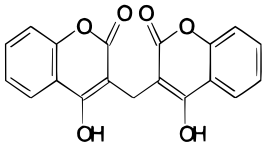

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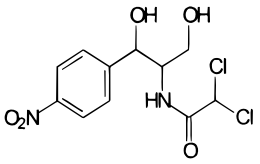
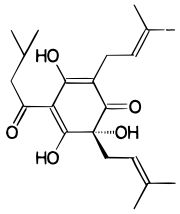


NSC 56351



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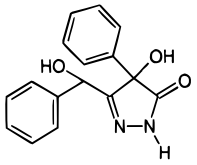

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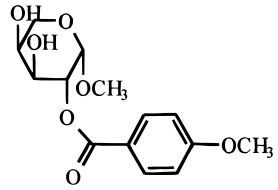

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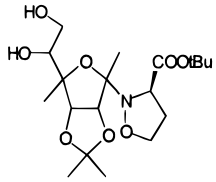

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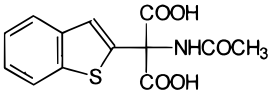

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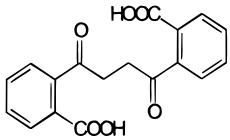
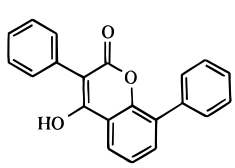


Indeed, our further investigations on this compound, summarized in Table 1, have identified NSC 158393 as an inhibitor of HIV-1 integrase[27] and HIV-1 reverse transcriptase. Virion attachment to target cells and to the HIV-1 p7 nucleocapsid protein zinc fingers were unaffected by NSC 158393. This compound therefore represents a lead for the development of potential anti-AIDS drugs that may target concurrently the protease, RT, and integrase enzymes, each of which is important to the replication of HIV.

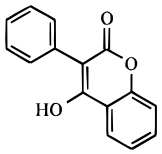
Molecular Modeling Studies

Conformational Analysis and Pharmacophore Mapping. Characterization of the active conformation of each of these 15 active compounds was accomplished through first selecting the low-energy conformations from the conformational search results and then fitting these conformations to the pharmacophore shown in Figure 2. Each of these inhibitors indeed satisfies, with a low-energy conformation, the 3D requirements defined

Chart 3. Coumarins with Inhibitory Activity against HIV-1 Protease



NSC 251150, ID_{50} = 13 μM



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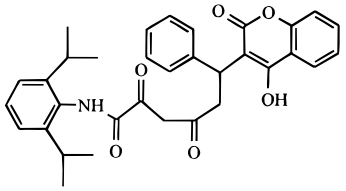

NSC 647086, ID_{50} = 31 μM

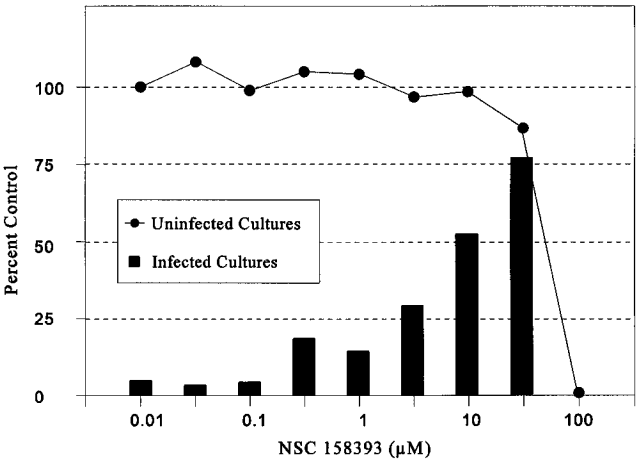

Figure 3. Cellular anti-HIV assay of NSC 158393.

Table 1. Activity of NSC 158393 against Various HIV Targets

parameter	observed inhibitory concentration of NSC 158393 (μM)
infection[a]	
antiviral activity (EC_{50})	11.5
cytotoxicity (IC_{50})	54.8
virion attachment (ID_{50})[b]	NI[c]
reverse transcriptase enzymatic activity (ID_{50})	7.1
protease enzymatic activity (ID_{50})	1.7
HIV-1 integrase enzymatic activity	1.5, 0.7[d]
p7 nucleocapsid protein zinc fingers ($RFU_{\%}$)[e]	NI

[a] XTT cytopathicity assay. [b] Attachment of HIV-1 to fresh human PBLs and the effects of compounds on HIV-1 RT and HIV-1 protease were quantitated as described in the Materials and Methods Section. ID_{50} values were derived from graphs in which each point represented the mean of three replicates. As controls, AZT triphosphate inhibited RT activity with an ID_{50} = 54 nM (non-phosphorylated AZT was not inhibitory), the KNI-272 protease inhibitor reduced protease activity with an ID_{50} = 4.7 nM, and dextran sulfate inhibited virion binding with an ID_{50} = 0.9 μg/mL. [c] No inhibition. [d] 3′ processing and strand transfer, respectively. [e] The $RFU_{\%}$ value represents the (percentage) decrease in the relative fluorescence intensity of the zinc finger after treatment of the p7 nucleocapsid protein with 25 μM of NSC 158393 for 10 min (NI = no inhibition).

there, thus providing support for the pharmacophore hypothesis. It was also found that for most compounds the global energy minimum calculated *in vacuo* from the conformational search indeed contains the 3D pharmacophore. However, for NSC 20143, 40328, 41234, 109412, 117027, and 112500, the global minimum conformation *in vacuo* did not contain the 3D pharmacophore. It is necessary to include conformations with conformational energy a few kcal/mol above the global minimum, in order to find an alternative conformation that does satisfy the geometric pharmacophore requirement. Such behavior has been shown to be quite common in small, flexible molecules binding to enzymes.[28]

Additional Factors Important to the HIV-1 Protease Activity. All 50 compounds tested in the HIV protease assay possessed the pharmacophore defined in Figure 2. However, only 15 out of the 50 compounds showed ID_{50} values below 50 μM. Clearly, the pharmacophore defined in Figure 2 was not sufficient for effective binding.

At least two additional factors are important in the binding of a compound to HIVPR. The first is the conformational energy penalty exacted for a compound to achieve the desired conformation. If this energy penalty is too large, the compound will have difficulty adopting the desired conformation and will bind much less effectively, if at all. The second factor concerns hydrophobic interactions between an inhibitor and HIVPR. HIV-1 protease has four hydrophobic pockets near its active sites, and it has been shown that favorable hydrophobic interactions with these pockets are desirable for an inhibitor to achieve nanomolar potency.[26]

The conformational energy penalty problem arises from one of the caveats in the search software, the Chem-X program. In the 3D database pharmacophore search, Chem-X does not explicitly take the conformational energy into account, and energy data such as that provided by CHARMm in the previous section are not available. Consequently, some compounds were found by Chem-X to be hits (*i.e.*, contain the pharmacophore), even though only high energy conformations fit the pharmacophore. To investigate this point, conformational search and pharmacophore mapping were carried out for 10 inactive compounds (Chart 2), randomly selected from the set of 35 inactive compounds. It was found that the lack of binding affinity for four compounds (NSC 130958, 353740, 357584, and 518963) may indeed be due, at least in part, to this conformational energy problem. Both NSC 130958 and 353740 were unable to meet the pharmacophore requirements even within 20 kcal/mol above the global minimum. NSC 518963 was able to meet the pharmacophore requirement, but with a 10 kcal/mol conformational energy penalty. For NSC 357584, a conformation 3.7 kcal/mol above the global minimum was found to contain the pharmacophore. However, a close examination of this conformation revealed the carbonyl group in this compound to be oriented in such a way that it was unable to interact effectively with HIVPR. The other six inactive compounds in Chart 2 were, however, found to contain the pharmacophore in a low-energy conformation. Their inactivity therefore cannot be explained simply in terms of a conformational energy penalty, and are probably due to the lack of favorable hydrophobic

interactions between these compounds and HIV-1 protease.

On the basis of the structure−activity relationships of NSC 32180, 158393, and 373937 (Chart 1) and NSC 41834 (Chart 2), it is evident that hydrophobicity plays an important role for their anti-HIV protease activity. Indeed, although all four compounds contain a dimer of 4-hydroxycoumarins and each of them satisfies the pharmacophore defined in Figure 2 with low-energy conformations, their ID_{50} values range from 0.32 μM for NSC 32180 and to >50 μM for NSC 41834.

In order to gain a better understanding on the relationship between the inhibitory activity of a compound and its hydrophobic and polar interactions with the HIV-1 protease, as well as to provide structural information for the design of more potent HIV-1 protease inhibitors, we have evaluated the binding modes of the most potent inhibitor found in our study, NSC 32180, by means of docking and molecular dynamics techniques, as discussed in the following section.

Predictions of Binding Modes. The binding modes of the most potent inhibitor found, NSC 32180, were studied using docking and molecular dynamics techniques. Conformations of this compound were sampled using a Monte Carlo random search approach. Each conformation was minimized, and the results were analyzed to obtain distinct low-energy conformations. These distinct low-energy conformations were then manually docked into the active site of HIVPR with the aid of computer graphics. These initial ligand/HIVPR complexes were fully minimized using CHARMm, and the minimized complex structures were used as starting points for molecular dynamics simulations. Snapshots were taken at 0.1 ps intervals during the simulation period and were then subsequently analyzed to obtain the total interaction energies between the inhibitor and the enzyme.

The total interaction energy (E_{int}) was defined as

$$E_{int} = E_{complex} - (E_{enzyme} + E_{inhibitor}) \qquad (1)$$

where $E_{complex}$ is CHARMm calculated potential energy of a dynamics simulation snapshot for the complex, E_{enzyme} and $E_{inhibitor}$ are CHARMm-calculated potential energies for the enzyme and inhibitor, respectively. E_{int} is the sum of the van der Waals and the electrostatic interactions. A recent study[29] has shown that E_{int} is a good predictor for the relative binding affinities of HIV-1 protease inhibitors. Therefore, this value was used as the basis for the predictions of the binding modes. The intermolecular hydrogen bond energy (E_{HB}) between the inhibitor and HIVPR was also calculated for each complex, since our experience has shown that the hydrogen bond energy, although a small portion of the total energy of interaction, plays a very important role in determining the binding affinity of a ligand.

Four distinct minimized conformations (A−D) for NSC 32180 were obtained from the conformational analysis, differing in the two torsional angles identified in Figure 4. These two torsional angles determine the relative orientations of the two coumarin rings, and therefore define the pharmacophore geometry in this compound. The results of the docking and the subsequent molecular dynamics simulation for each of the four complex structures are summarized in Table 2, which lists the interaction energy (E_{int}) and the individual energy terms, averaging a total of 500 molecular dynamics frames recorded from the final 50 ps simulation run. These data suggest that conformation D, with the lowest value of E_{int}, forms the most energetically favored complex with HIVPR. The specific hydrogen bonds formed between HIVPR and NSC 32180 in this binding mode are shown in Figure 5, and the hydrophobic interactions between the inhibitor and the enzyme are shown in Figure 6. As can be seen from Figure 5, a number of hydrogen bonds form between NSC 32180 and residues Asp 25, Asp 25′, Ile 50, Ile 50′, Gly 48, Gly 48′, Arg 8, Asp 30′, and Lys 45′. It was also noted that some of the hydrogen bonds, such as that formed between the amido group of Ile 50′ and the

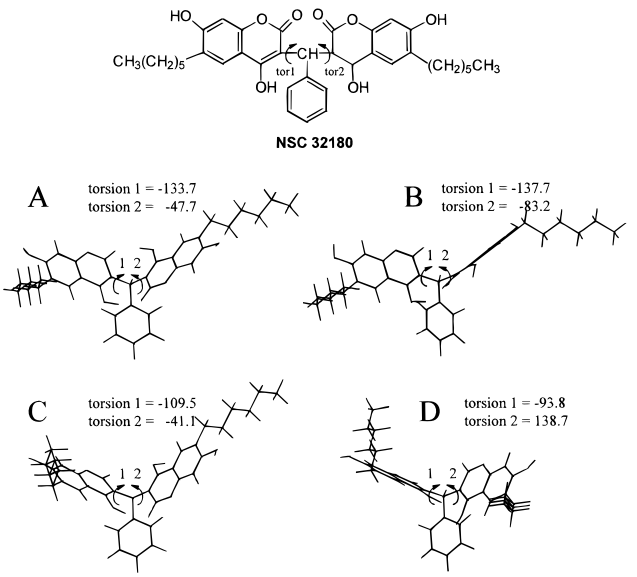

Figure 4. Four distinct low-energy conformations of NSC 32180.

Table 2. Results of 50 ps Dynamics Simulations of the Complex Structures of HIV PR/NSC 32180

complex of NSC 32180/HIVPR	E_{int} (kcal/mol)	E_{vdw} (kcal/mol)	E_{elec} (kcal/mol)	E_{HB} (kcal/mol)
A	−106.5 ± 6.6	−71.0 ± 4.4	−35.5 ± 5.8	−12.8
B	−102.3 ± 5.1	−57.7 ± 5.1	−44.6 ± 5.4	−9.9
C	−92.4 ± 4.5	−56.5 ± 4.5	−35.9 ± 4.7	−12.1
D	−124.6 ± 5.9	−63.2 ± 4.9	−61.4 ± 5.8	−16.3

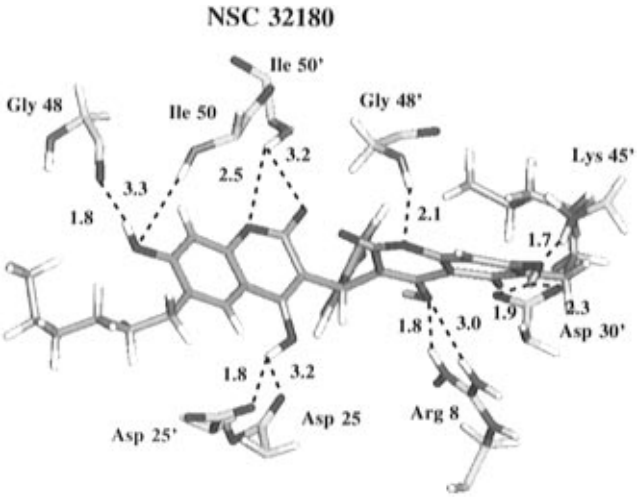

Figure 5. Hydrogen bonds between NSC 32180 and HIVPR in the predicted binding mode.

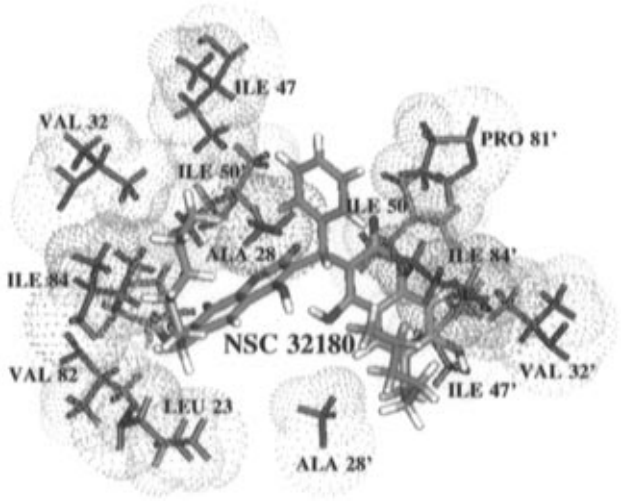

Figure 6. Hydrophobic interactions between NSC 32180 and HIVPR in the predicted binding mode.

coumarin ring are not optimal, suggesting room for further improvement of its binding affinity. As can be seen from Figure 6, the hydrophobic groups of NSC 32180, i.e., the two aromatic rings and the two *n*-hexyl groups interact well with a number of hydrophobic residues, such as Leu 23, Val 82, Ile 84, Val 32, Ile 47, Ala 28, Ile 50′, Pro 81′, Ile 84′, Ile 50, Val 32′, Ile 47′, and Ala 28′. These good hydrogen bonds and hydrophobic interactions are presumably responsible for its potent inhibitory activity against HIV-1 protease. Although, up to date, the binding mode for NSC 32180 predicted through molecular modeling studies has not been validated by X-ray crystallography, our prediction nevertheless provides valuable information for the design of improved HIV-1 protease inhibitors based upon this lead compound.

Conclusions

We have reported 15 structurally diverse, non-peptide HIV protease inhibitors. Their discovery, as described in this paper, shows that conformationally flexible 3D database pharmacophore searching is effective in generating new leads.

Several factors played together in the discovery of these novel HIV protease inhibitors. The availability of X-ray determined complex structures of HIVPR and some of its inhibitors provided an understanding of the interactions between the enzyme and the inhibitor. Although both nonspecific hydrophobic and specific hydrogen bond interactions were found to be important for an inhibitor to achieve good binding affinity, our experience suggests that one should focus, at least initially, on the specific hydrogen bond interaction sites when developing a pharamacophore for a 3D database search. Hydrophobicity and other considerations, such as conformational energy and chemical novelty, are more useful in the second stage in which compounds are selected for testing in a bioassay.

Molecular mechanics calculations and molecular dynamics simulations were used to predict the binding modes of the most potent inhibitor found, NSC 32180. These binding modes are being used in the structure-based drug design efforts underway in our laboratory. Each of the novel HIV inhibitors reported here could be used as a lead for the design and development of new classes of potent inhibitors. Among these, NSC 32180, with the best ID_{50} (0.32 μM), would seem to be the primary candidate. However, NSC 158393 may be a more attractive lead for the development of potential anti-AIDS drugs, since it not only shows fairly potent inhibitory action against three HIV-1 enzymes (protease, reverse transcriptase, and integrase[27]) but also has promising antiviral activity.

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