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THE DESIGN, MODELING AND EVALUATION OF POTENTIAL HIV PROTEASE INHIBITORS USING BLITZ, AN INTERACTIVE COMPUTER GRAPHICS WORKING TOOL

M. Mahmoudian, A. Laczkowski*, A. Karrer*, S. M. Swanson* and E. F. Meyer, Jr*

Department of Pharmacology, University of Medical Sciences, P.O. Box 14155-6183, Tehran, Islamic Republic of Iran
**Biographic Laboratory, Department of Biochemistry and Biophysics, Texas A & M University, College Station, TX 7743, USA*

Abstract

Several nonpeptide small molecules were designed as potential inhibitors of HIV protease and their structures were constructed by computer-aided molecular modeling and docked into the active site of HIV protease. Models of the complexes of inhibitors and the HIV protease were refined using non-bonded and H-bonding terms. The refined energy of selected complexes showed that the designed inhibitors fitted tightly into the active site of receptor cavity. The structure of the designed inhibitor (HI-082) was superimposed on the molecule of haloperidol (which has been reported to have anti-HIV protease activity) and it was found that they share a number of common structural features. These results showed that these small nonpeptide molecules interact strongly with the HIV protease and may therefore inhibit its action in which case they would be potential anti-AIDS agents.

Introduction

The spread of the HIV virus and the resulting AIDS pandemic has stimulated the search for new anti-viral agents [1-3]. One of the essential proteins which could serve as a target for anti-viral agents is the HIV protease. This aspartyl protease is the key enzyme in the maturation of gag and gag-pol precursor proteins of the HIV-1 virus [4,5]. The inhibition of the HIV protease has resulted in the accumulation of unprocessed precursor proteins and suppression of viral maturation [6-9]. The structure of HIV protease was recently determined by several groups up to 3 A° resolution [10-12]. This structure will provide a basis for rational design of potential anti-AIDS drugs. Therefore, the present study was carried out to design several nonpeptide molecules which could be fitted into the active site of HIV protease as its potential inhibitors and anti-viral agents.

Keywords: Drug design; HIV protease inhibitors; Molecular modeling

Methods

Molecular Modeling

Program ***FRODO*** [13] is a mature, interactive graphics program widely used for model building and fitting protein structure to crystallographic electron density maps. It has provision for regulation of backbone torsion angles and many other crystallographically useful features. However, it does

not have the capability of minimizing side chain-side chain interactions. As we were developing such a capability, we learned of program ***TOM*** [14], a derivative of ***FRODO***, which offered the possibility of refining the nonbonded and H-bonded interactions of ligand and receptor atoms. The initial version of program ***FRODO:TOM*** was further developed and its "*hooks*" functions were debugged by two of the authors (A. Karrer and A. Laczkowski). The "*hooks*" functions are essential for defining an inter-atom contact distance (e.g. covalent linkage, electrostatic interaction, H-bond, etc.) as part of the energy minimization procedure. These and additional features are incorporated into program ***BLITZ*** now under development in the Biographic Laboratory of Texas A&M University. The resulting energy terms are semi-quantitative rather than absolute, but, together with interactive modeling, they present a powerful contribution for creating, testing and modifying a ligand, all interactively; thus, a variety of complexes may be screened and improved during a modeling session.

The availability of increased computational speed and functionality in the new workstations make this method a highly productive approach to the design of novel inhibitors and potential drugs. On the basis of the coordinate file of a novel ligand (i.e., molecule), ***BLITZ*** can now build the necessary Z matrix and incorporate it into the library for subsequent calculation. User control of default options is given; especially important is the ability to define "*hooks*", which place a weighted (W') distance(d') constraint on designated pairs of atoms (protein:ligand pair = p'-l'). Any one such constraint causes atom 1' (with all bonded ligand atoms) to remain on the surface of a sphere of radius d' about the center atom, r' (Fig. 1). Up to 10 such '*hooks*" may be specified and conveniently used to represent covalent attachment (stong weight, w') or electrostatic or H-bonded attachment (weakweight, w'). The van der Waals (non-bonded) interaction of all sidechains and ligand atoms within a defined region is then calculated and the sum of all the forces is calculated; torsional atoms are allowed to flex; the complex is then optimized and will be displayed. A typical refinement sequence (ca. 20 ligand atoms, 8A refinement radius, 3-4 "*hooks*", 10 cycles of refinement) requires ca. 20-30 minutes on a VAX750 computer (ca. 0.9 MIPS) and was used as the first optimization/screening pass of the inhibitors presented here. This procedure now requires ca. 1 minute on an Evans and Sutherland ESV workstation. Therefore, one can consider this refinement to be functionally interactive. The optimized complex was

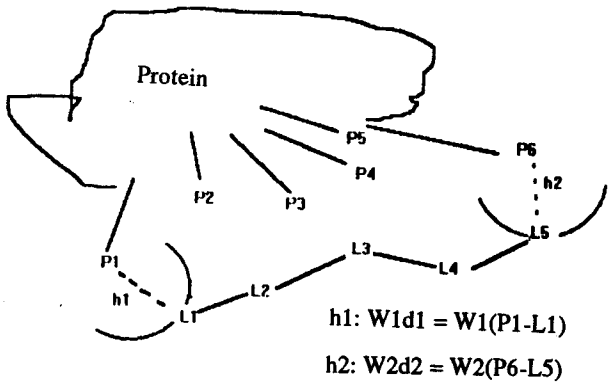
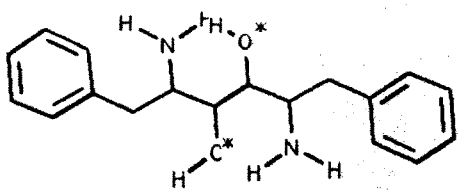


Figure 1. Schematic representation of protein-ligand interaction. Protein active site atoms are labeled P_i; the ligand atoms are labeled L_i. Hooks are drawn as dashed lines, with contact cricles of radii r_1, r_2, etc. The contact distance, d, is a vector difference of atom positions (P-L).

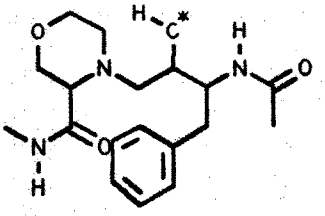
inspected; contact regions that would benefit from electrostatic, polar, or H-bonding interaction were detected visually. The "*INTERFACE*" option derived from ***FRODO*** is especially powerful in defining regions where contact is lost, i.e., where room exists for additional atoms to enhance polar or non-bonded interactions. This option was used for immediate visual inspection of the trial models; local modifications to the inhibitor were made, as needed. The modified molecule was inspected and individual torsion angles flexed interactively. Atomic bulk can be modified (e.g. a methyl group is too short, a propyl group is too large, so an ethyl group is used) and a refinement process initiated. Such features are not especially novel, rather their virtue is that both collectively and interactively they enhance an existing program. This is especially appealing because, at this stage of our work we are porting the program to the PEX standard on an Evans and Sutherland ESV workstation and our goal of having access to an interactive refinement is now attainable. The aim of the present work was to design nonpeptide small molecules which could be fit into the active site of this enzyme as potential HIV protease inhibitors and anti-HIV agents.

Design of HIV Protease Inhibitors

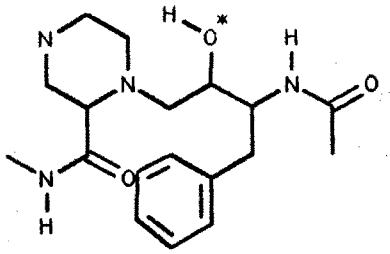
The crystal structure of HIV protease complexed with its substrate and containing 95 crystallographically-resolved water molecules [11] has been used as a coordinated base to generate models of enzyme-inhibitor complexes. Several nonpeptide small molecules (Fig. 2) which mimic the conformation of P1-P1' subunit of the substrate at active site [12] were constructed using molecular modeling package



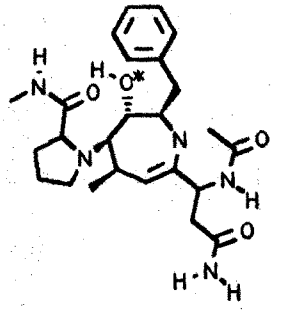
HI-010 (E = -363.59 Kcal/mol)



HI-020 (E = -451.27 Kcal/mol)



HI-030 (E = -491.90 Kcal/mol)



HI-082 (E = -259.70 Kcal/mol)

Figure 2. The structure of the designed inhibitors of HIV protease. In all molecules the oxygen atom marked with '*' can make H-binding to the OD1 and OD2 atoms of Asp-25. R is $COCH_3$ and R' is $NHCH_3$.

(Alchemy II). The structures of these molecules were energy minimized using Tripos force field parameters. Then the inhibitor molecules were fitted into the active site of the enzyme and refined visually as described above. Graphically-suggested modifications were made. A total of four trial inhibitor molecules was thus developed and evaluated qualitatively using ***BLITZ*** package.

Comparison of the Designed Inhibitor and Haloperidol

It has been reported that small molecules such as haloperidol could inhibit HIV protease [6]. Therefore, the structure of this compound was constructed and energy minimized using Alchemy package. The structure of haloperidol and HI-082 were superimposed using FIT option of Alchemy package and common features were determined.

Results and Discussion

The small nonpeptide molecules shown in Figure 2 can be fitted to the active site of the enzyme; their structure closely mimic the conformation of the substrate at active site [12]. Because these compounds may be superimposed onto the Phe-Pro residues of the crystal structure of substrate, the peptide bond was replaced with glycol or [CH_2-CH-OH] group which is resistant to the action of the enzyme. These groups are capable of H-bonding to the carbonyl groups of either ASP-25 or ASP-125 or both. It is believed that these groups are involved in the catalytic action of HIV protease. Thus, the complex would competitively render the enzyme inactive.

The refinement of the complexes of these inhibitors and HIV protease showed that the energy of the complexes are in the range of -250.7 to -441.9 Kcal/mole (Fig. 2). These values show that these complexes are readily formed and stable. Inspection of the complexes of inhibitors with enzyme visually show that the inhibitors fit tightly into the active site region of the enzyme. The model of the complex of one of these inhibitors (HI-082) with the enzyme is shown in Figure 3. The seven-membered ring of this molecule produces a relatively rigid structure which keeps the imidazoline and phenyl rings in the same conformation as found in the substrate. The hydroxy group is in the vicinity of the carboxyl groups of Asp-25 or Asp-125 (two active site aspartic residues) and could form a crucial hydrogen bond with these groups. The methyl group, which is attached to the main ring, is in a hydrophobic pocket, thus enhancing binding affinity.

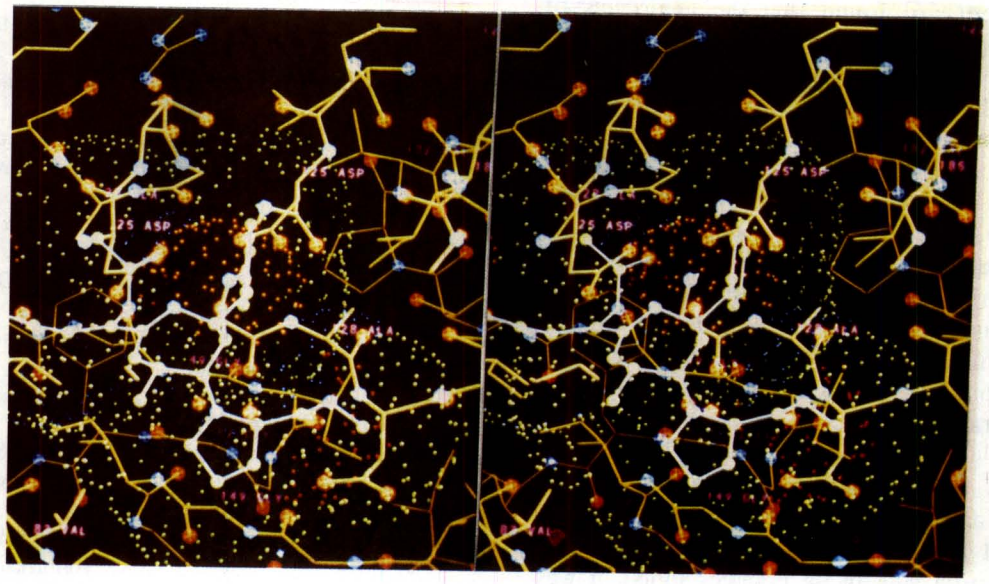


Figure 3. The structure of HI-082 in the active site of HIV protease. The dotted spheres are the contact points between the enzyme and inhibitor.

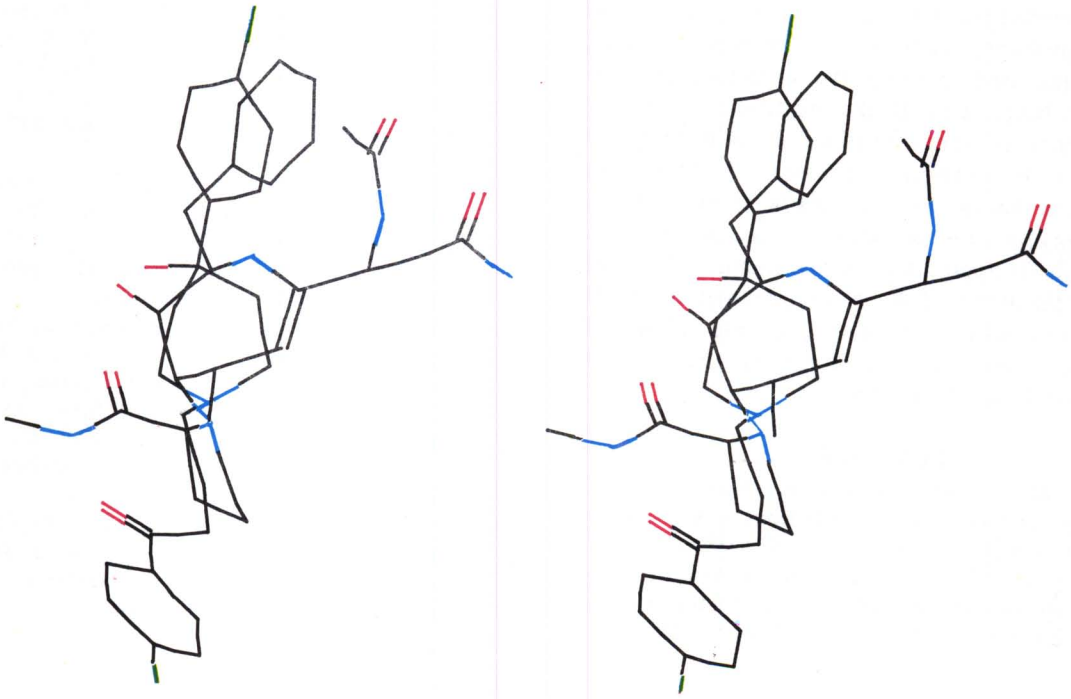


Figure 4. The structure of HI-082 superimposed on the haloperidol molecule

Using a structure-based computer assisted search, DesJarlais *et al.* [6] found that the analogues of haloperidol could be fitted into the negative image of the active site of HIV protease. Further experiments proved that this compound could inhibit the HIV protease and prevent maturation of viral polypeptides in a cell assay system. Super-imposition of the HI-082 molecule (Fig. 2) on the haloperidol molecule showed that these two molecules share a number of structural features. These are: a benzene ring, a hydroxy group, a nitrogen in a ring system, and a carboxy group (Fig. 4). It is concluded that these compounds may bind to the active site of the enzyme and are therefore potential inhibitors. They have, therefore, been given to a collaborating synthetic chemist for possible synthesis.

This brief communication may be taken as an indication of a facile approach to drug design. With three essential ingredients in place (a creative chemist, a crystal structure, and facile graphics tools), it was possible in approximately one month, from start to finish, to undertake the work reported here. As our porting of the ***BLITZ*** program proceeds, we see the beginnings of a new day in rational, targeted drug design; computational and graphical tools are being added as needed and the system is being tested with practical problems such as the one reported here. Workstations used in molecular modeling are now opening multiple vistas to the creative chemist. The main feature of these systems is that they are interactive. A working definition of interactive computing is that the answer must be received before the user forgets what the question was. A functional "upper limit" is approximately one minute. This does not render the ability of the user ineffective, rather the creative user will proceed to generate additional questions, superseding or engulfing the original question as a function of time.

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