

A Study on Docking Mode of HIV Protease and Their Inhibitors

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The capability to propose feasible ways of binding a putative ligand inhibitor to a known receptor site is crucial to the successful structure-based drug design. A computer docking approach is to position or “dock” ligand and receptor molecules together in many different ways and then score each orientation by applying a reasonable evaluation function. AutoDock3.0 is an unbiased type docking program in which a user does not have to direct a ligand to an active site, but the system finds an optimal position after a ligand is placed in a random manner. Synthesized derivatives of the intact inhibitor (inh1) of HIV protease were investigated for their docking modes as compared with their K_i values. Among the derivatives, inh3trans and inh6H were found to be more powerful inhibitors of HIV protease than the others. Gibbs free energy calculated by applying molecular mechanics interaction energies was compared with the one obtained by using experimental inhibitory potencies for a series of HIV protease inhibitors, and a fairly good correlation was found between the two. Based on this favorable relationship between the computational and the experimental results, the computational experiments were pursued for the compounds drawn by Sybyl taking into consideration the fact that unexploited carbon affinity regions (or hydrophobic regions) with sizable volume were detected on the docking study of inh1 and inh8 against HIV protease. Those were compounds with a t-butyl substituted by various hydrophobic side chains. Among those a compound with a benzyl group exhibited the lowest docking energy. Since one of the goals of this paper was to perform the computational drug-design experiment to investigate potential HIV protease inhibitors, the authors would like to leave the clinical investigational work for the expertise of those areas.

Keywords: Computer docking, Drug design, HIV protease, Gibbs free energy, Ligand inhibitor

1 Introduction

Structural information of biological macromolecules complexed with ligands is being used to the extended degree in the discovery of novel pharmaceutical compounds. This information is crucial, and during the drug design cycle, experimental structures for a target protein complexed to ligand(s) can afford researchers to examine the detailed picture of the binding site and to design more active analogues [1]. A number of computer docking softwares have been utilized successfully to fulfill the purpose of this computer-based drug design [2]. This type of softwares is useful in evaluating the binding mode of candidate ligands or drug molecules to target macromolecules such as DNA, RNA, or enzyme. During the drug design cycle, experimental structures for a target protein complexed to ligand can allow scholars to examine the details of the binding site and to design de novo compounds [2].

There are two types of computer docking systems. The first is the direct type in which a user moves a ligand to a known active site and lets a docking program find a position of the one with the least Energy. In those positions hydrogen bonding and/or steric complementarity usually take place [3,4]. The second is an unbiased type in which a user does not have to direct a ligand to an active site, and can place a ligand in a random manner. The system finds an optimal position in which a ligand and a macromolecule bind.

AutoDock3.0 is an example of unbiased type and its version 3.0 has a ligand mobilized by a generic algorithm method and evaluates a rapid grid-based energy. Previous applications of AutoDock 3.0 include the prediction of substrate binding to enzymes [5], computer-aided drug design of non-peptide inhibitors of HIV protease [6], and docking of two proteins [7]. Considerable efforts have been made to investigate an active inhibitor against HIV protease [8–10]. To date none of them are powerful enough to be successfully utilized therapeutically. Candidate inhibitors have been synthesized and the K_i was measured for each of 12 different inhibitors. Computer dockings between inhibitors and HIV protease by using AutoDock3.0 were performed on UNIX machines. Upon analyzing docking results of candidate inhibitors, the other preferred candidate compounds were proposed. It is said that the level of involvement in the elucidation of structures of HIV protease and its complexes with various inhibitors is enormous and numerous inhibitors have been reported [9].

The human immunodeficiency virus protease (HIV protease) has been a target for anti-HIV protease drug development in recent years. In other words, the world-wide AIDS epidemic has stimulated a research effort directed toward identifying therapeutic strategy effective for control of the disease and its causative agent. One approach of intense scrutiny over the past few years has been inhibition of the virally encoded protease, an enzyme essential for mutation of viral particles to their infectious stage [11]. Attempts have been made to investigate various inhibitors such as nonpeptide sulfonamides, and fullerene [12,13].

There have been varied attempts in the HIV protease related inhibitor studies such as that of the monocyte to chemokine through CD4 signal dependent chemokine receptor down-regulation by HIV protease -1 envelope gp20 [14], of chemoattractant receptor expression by HIV protease -1 envelope gp41 [15], and of HIV protease -1 expression by orphan upload receptor oligonucleotides [16]. This indicates that a variety of approaches should be taken into consideration in the inhibition of HIV protease infection.

2 Methodology

Complex structure of HIV protease with inhibitor 1 (inh1) was taken from the Internet (URL* <http://www.rcsb.org/pdb/index.html>). Various derivatives (first inhibitor group) of ih1 were synthesized and their K_i values against HIV protease were determined in vitro. For the docking of other inhibitors, the mode of interaction of inh1 against HIV protease was used as a standard docked model, the one used for RMSD calculation.

AutoDock3.0 was run by feeding an inhibitor and HIV protease. Since AutoDock3.0 consists of three major components, AutoGrid, AutoTors, and AutoDock3.0, the grid map was created by running AutoGrid as the first step. AutoTors was then run by assigning the root position and the number of torsions. Finally AutoDock3.0 was run in order to investigate mode of interaction between the inhibitor and HIV protease. Innovative inhibitors were proposed based on the docked result of the first inhibitor group.

3 Background

In order to compare the Gibbs free energy calculated from the experimental value with the AutoDock 3.0 result, the following steps were applied to obtain the total Gibbs AutoDock3.0 free energy:

1) The torsional entropy was obtained by multiplying the number of torsions by the degree of freedom. This scoring function is based on the principles of QSAR (quantitative structure-activity relationships) and was parameterized using a large number of protein-inhibitor complexes for which both their structure and inhibition constants, or K_i , were known [17].

$$\text{torsional entropy} = \text{number of torsions} \times \text{degree of freedom} \dots\dots\dots(1)$$

2) The total Gibbs free energy was then obtained by taking into consideration the three factors, torsional entropy, lowest AutoDock3.0 energy obtained, and internal energy of inhibitor

$$\text{Total AutoDock3.0 Gibbs free energy} = \text{torsional entropy} + \text{lowest AutoDock3.0 energy} - \text{internal energy of inhibitor} \dots\dots\dots(2)$$

On the other hand, experimental Gibbs free energy(ΔG) was obtained as follows:

$$\Delta G = -RT \times \text{Ln } K_i \dots\dots\dots(3)$$

where,

$$R = (8.314/4.184 \times 1000) \text{ J/Kmol}$$

$$T = 298.2 \text{ }^\circ\text{K}$$

K_i in Mol

4 Results and Discussion

Figure 1 shows inhibitors synthesized [18] and tested for docking against HIV protease. Inh1 is a standard docking model ligand and when it is compared with the docking of other inhibitors one

can tell how closer to the reference inhibitor the other inhibitors are docked. Upon the examination of docking feature between inh1 and HIV protease it was found that two types of hydrogen bonds were formed. As shown in Figure 2, one of the hydrogen bonds was found between gem-idol and an aspartate (residue number B25) moiety of HIV protease and the other between amide oxygen attached to proline ring and the other aspartate (residue number A25) moiety of HIV protease. Since 13 torsion angles were detected in inh1 the torsional entropy was obtained to be 4.05 Kcal/mol by multiplying it by the degree of freedom that was 0.3113 Kcal/mol (Table 1). Since the lowest total interaction energy of the complex and the internal energy for inh1 were -11.74 Kcal/mol and 4.03 Kcal/mol respectively, $\Delta G_{AutoDock}$ was determined to be -11.99 Kcal/mol taking into account the torsional entropy by the use of equation (2).

Table 1. Comparison of experimental and computed Gibbs free energies of tested HIV protease inhibitors.

inhibitor	number of torsions	torsional entropy (a)	total interaction energy of complex(b)	internal energy of inhibitor (c)	$\Delta G_{AutoDock}$ (a+b-c)	$\Delta G_{experimental}$
inh1	13	4.05	-11.74	4.03	-11.99	-9.12
inh3cis	16	4.98	-12.1	5.11	-12.13	-8.86
inh3trans	16	4.98	-10.27	5.95	-11.24	-9.8
inh4	14	4.36	-11.19	4.71	-11.54	-9.08
inh6H	20	6.23	-7.63	8.36	-9.76	-9.29
inh8	17	5.29	-9.6	3.79	-8.1	-6.41
inh12	29	9.03	-5.01	9.15	-5.13	-7.36

Note 1 Entropy, ΔG , and energy are given in Kcal/mol.

Note 2 Except for inh12, the number of AutoDock runs was 20. Since AutoDock job of a compound with large number of torsions is apt to be endless, the AutoDock run number for inh12 was reduced to 10.

Note 3 Torsional entropy was obtained by multiplying number of torsions by the degree of freedom which is 0.3113 Kcal/mol.

Note 4 $\Delta G_{experimental}$ was obtained by the equation given in the Background section of the text.

Table 1 shows Gibbs free energies obtained by both the AutoDock3.0 runs and the laboratory experiment for tested inhibitors. The Gibbs free energy of inhibitors obtained by the experiment can be grouped into two classes; the one with lower Gibbs free energies which includes inh1, inh3cis, inh3trans, inh4 and inh6H, and the one with higher Gibbs free energies which includes inh8 and inh12. The Gibbs free energy of inhibitors obtained by the AutoDock3.0 runs can also be grouped into two classes in a similar manner. This indicates that there is a fairly good correlation between the AutoDock3.0 runs and the laboratory experimental results as is stated later in this text.

Figure 3 shows dockings of inh1 and inh8 against HIV protease and the unexploited carbon affinity region (or hydrophobic region) were observed. Thus, additional inhibitors with hydrocarbon side chains extended in that region were created by Sybyl software as inhibitor candidate compounds (shown in Figure 4). Those are the compounds in which various types of hydrocarbon side chains were attached to an amide functional group on the proline ring. The extended side chains for exten0,

inhibitor	structure	molecular formula	molecular weight	Ki
inh1		$C_{27}H_{33}N_3O_5$	479	Ki= 214nM
inh3cis inh3trans		(cis) $C_{34}H_{39}N_3O_6$ (trans)	586	Ki= 318nM Ki= 65nM
inh4		$C_{28}H_{35}N_3O_6$	509	Ki= 220nM
inh6H		R=H $C_{38}H_{51}N_5O_9$ R=OBn $C_{45}H_{56}N_5O_{10}$	722 826	Ki= 154nM
inh8		$C_{33}H_{45}N_3O_9$	627	Ki= 20 micro M
inh12		$C_{43}H_{64}N_{11}O_{12}$	926	Ki = 4 micro M

Cbz =

Figure 1. HIV protease inhibitors and their properties tested for AutoDock3.0 runs.

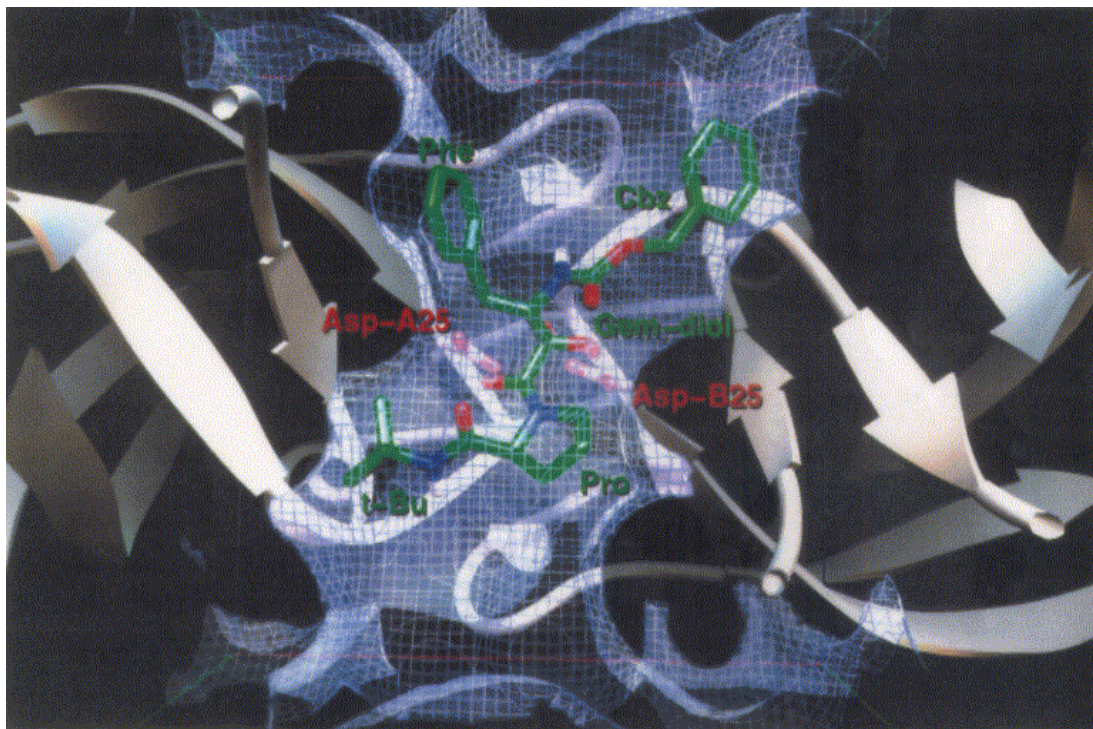


Figure 2. Docking mode of inhibitor 1 (inh1) as a reference inhibitor against HIV protease as obtained by AutoDock3.0. Atoms in inh1 are shown by different colors (red, oxygen; blue, nitrogen; and green, carbon).

exten1, exten2, and exten5 consist of saturated alkyl groups, and exten3 and exten4 contain phenyl groups additionally. Those candidate compounds were tested for docking study and the results were shown in Table 2. Exten4 showed lower ΔG , which was -12.73 Kcal/mol. This was lower than the inh1 ΔG , which was -11.99 Kcal/mol indicating that exten4 would be a stronger inhibitor of HIV protease. Figure 5 shows the docked mode between exten4 and HIV protease where exten4 was docked fairly superimposingly to inh1. The unexploited carbon affinity region (or hydrophobic region) around the carboxybenzyl (Cbz) side chain was fairly well exploited by the phenyl ring of exten4, and the other unexploited carbon affinity region (or hydrophobic region) around the t-butyl side chain was exploited by the phenyl side chain attached to an amide functional group on the proline ring.

Correlation between Gibbs free energies obtained by AutoDock runs and experimental results were plotted in Figure 6. The straight line was drawn by the least square method. The slope of the line was 0.348, which was a definite positive value and it shows that there is a fairly good correlation between the Gibbs free energies obtained by AutoDock runs and experimental results previously stated. Although the slope value of one would show the best correlation between the two, the authors believe that the value 0.348 was not so discouraging to state the correlation between the two because of the limitation of comparison between the biological system and computational chemistry due to the fact that there are lots of unsolved areas such as incorporations of the effects of solvation and the protein movements into the system and so on at this time.

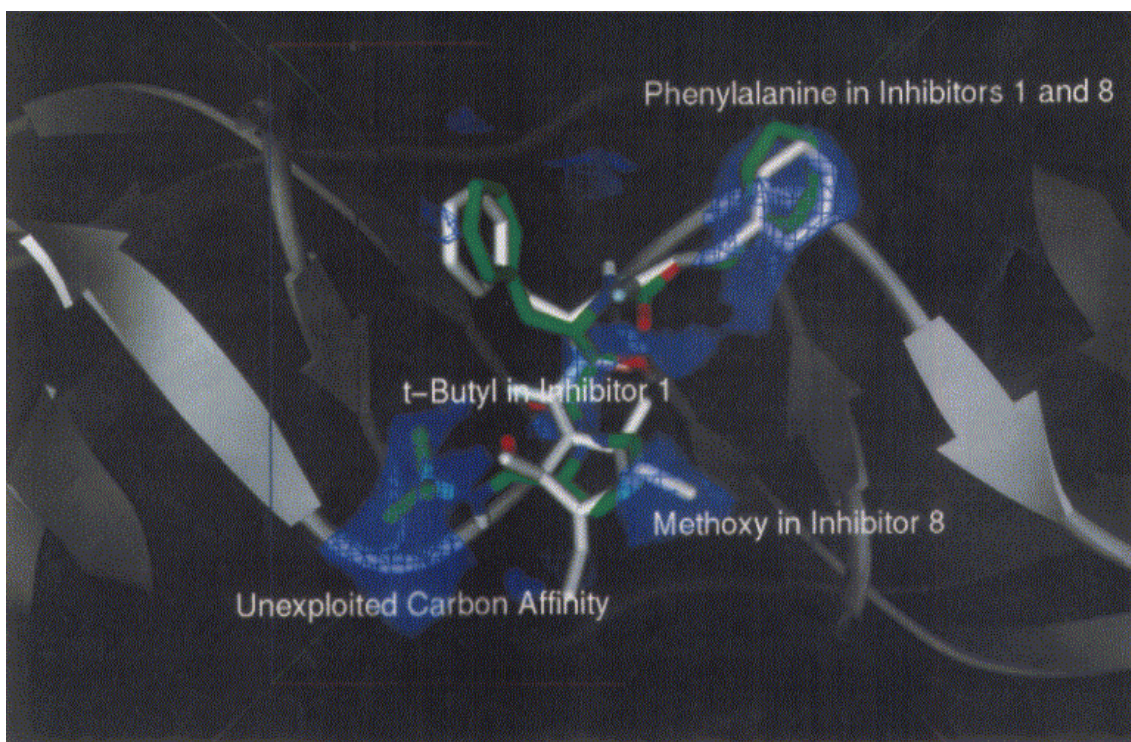


Figure 3. Docking mode of inhibitor 1 (inh1, green) and inhibitor 8 (inh8, white) against HIV protease as obtained by AutoDock3.0. Phenylalanines in inh1 and inh8 are superimposed. Bluish netted areas represent carbon affinity region. Atoms in inh1 are shown by different colors (red, oxygen; blue, nitrogen; and green, carbon).

Table 2. Gibbs free energy obtained for HIV protease inhibitors predicted by the application of AutoDock3.0 runs.

inhibitor	number of torsions	torsional entropy (a)	total interaction energy of complex (b)	internal energy of inhibitor (c)	$\Delta G_{AutoDock}$ (a+b-c)
inh1	13	4.05	-11.74	4.3	-7.69
exten0	14	4.36	-6.66	7.75	-2.3
exten1	14	4.36	-11.16	4.25	-6.8
exten2	15	4.67	-10.16	5.48	-5.49
exten3	13	4.05	-11.74	3.33	-7.69
exten4	14	4.36	-14.38	2.71	-7.07
exten5	14	4.36	-14.81	1.54	-10.45

Note 1 Entropy, ΔG , and energy are given in Kcal/mol.

Note 2 Torsional entropy was obtained by multiplying number of torsions by the degree of freedom which was 0.3113 Kcal/mol.

Note 3 Structures of inhibitors are shown in Figure 1.

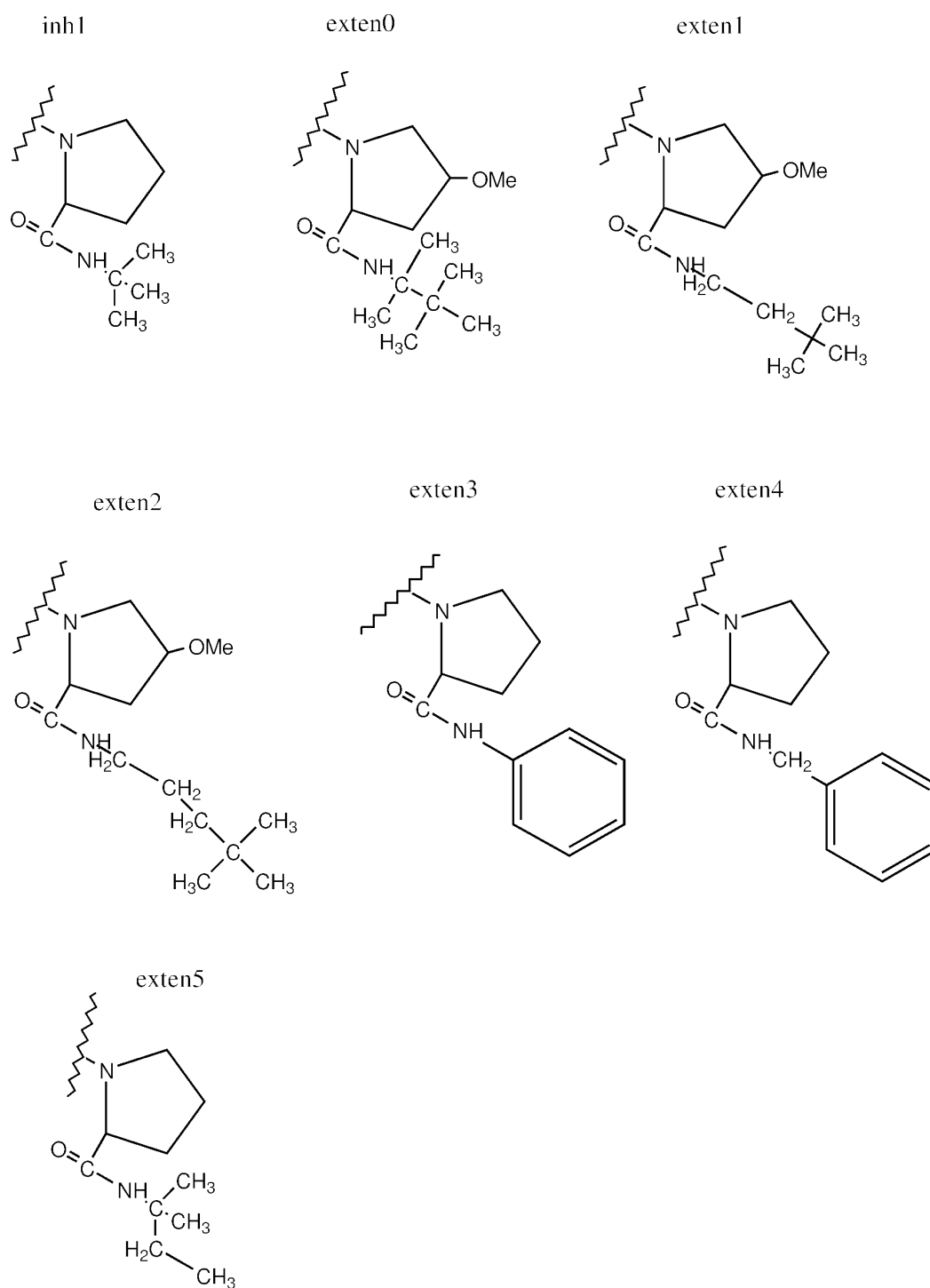


Figure 4. HIV inhibitors tested for AutoDock runs.

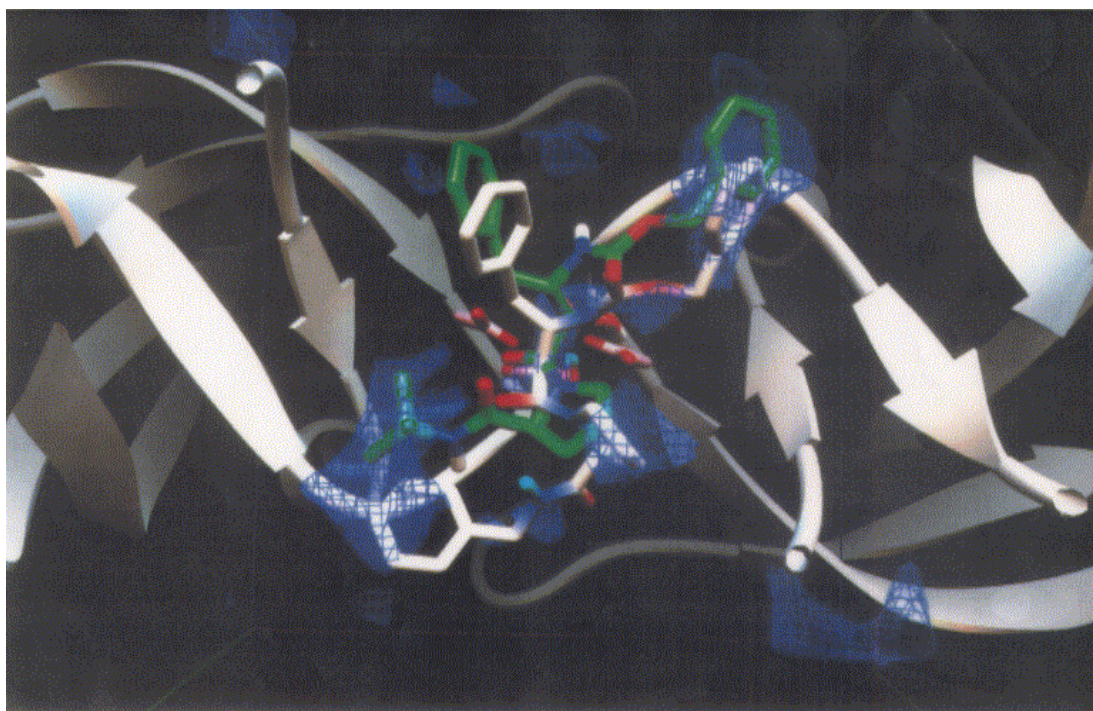


Figure 5. Docking mode of the inhibitor candidate (exten4, white) against HIV protease as obtained by AutoDock3.0. The reference inhibitor 1 (inh1, green) are also shown. Atoms in exten4 and inh1 are shown by different colors (red, oxygen; blue, nitrogen; and white or green, carbon).

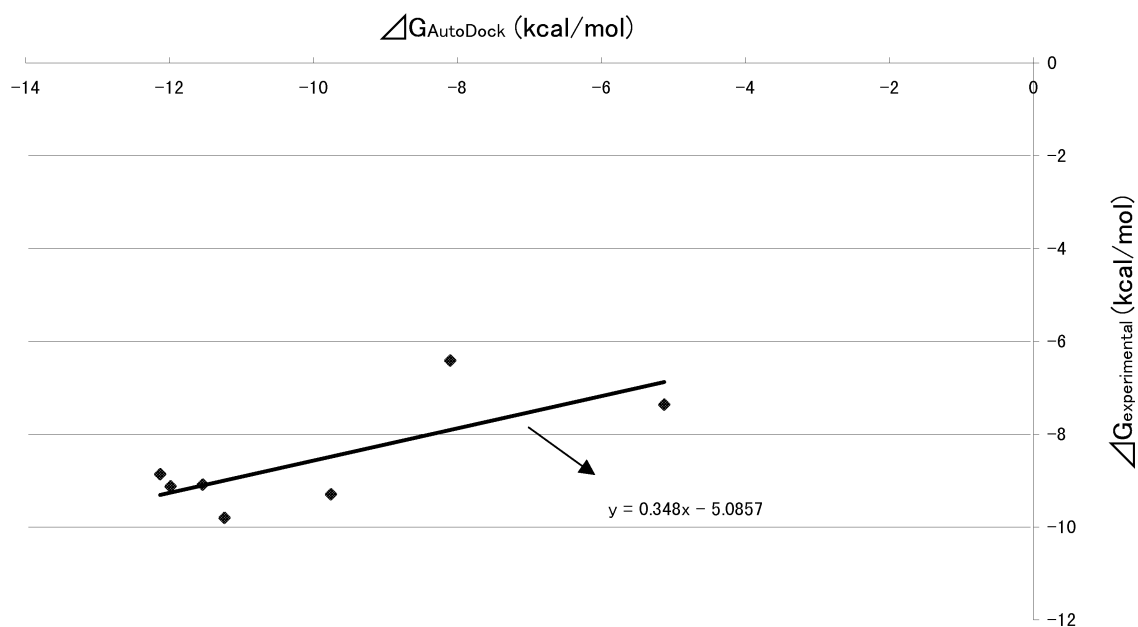


Figure 6. Correlation between AutoDock and experimental Gibb's free energies of HIV and inhibitor docking

5 Conclusions

It was found that compounds inh3trans and inh6H were more powerful inhibitors of HIV protease than the others. Furthermore, there wasn't a big discrepancy between the free energies obtained by AutoDock3.0 and by experimental method. It is feasible to state that the fairly good correlation was found between calculated molecular mechanics interaction energies and experimental inhibitory potencies for a series of HIV protease inhibitors. Based on this favorable relationship between the computational result and the experimental result, the computational experiment was performed for the Sybyl-drawn potential inhibitors taking into consideration the fact that an unexploited carbon affinity region (or hydrophobic region) with sizable volume was observed in the docking mode for inh1 and inh8 against HIV protease. Those were compounds with t-butyl substituted by hydrophobic side chains to fill the unexploited carbon affinity region. Among those the proposed inhibitor with t-butyl substituted by benzyl group exhibited the lowest docking energy. Since one of the goals of this paper was to perform the computational drug-design experiment to investigate potential HIV protease inhibitors, the authors would like to leave the clinical investigational work for the expertise of those areas.

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HIV プロテアーゼとその阻害剤に対する ドッキング・モードに関する研究

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リガンド阻害物質が受容体に結合する様式を同定できるかどうかは、化学構造を基にした薬物設計を成功させる鍵になる。コンピュータドッキングは、リガンドと受容体分子をいろいろな方法で、一定方向に位置付け(ドッキング)し、それに対して適当な評価式を用いて得点を与えることである。AutoDock3.0はリガンドを活性部位にユーザーが誘導する必要はなく、システムが、自動的に最適な位置を定めるものである。HIV protease に元々組み込まれているリガンド (inh1) を元にして、各種の誘導体を合成しそのドッキング・モードを研究し K_i 値と比較した。その結果 inh3trans と inh6H が他の阻害物質より、より強力であることがわかった。一連の HIV protease 阻害物質に対し分子間相互作用エネルギーからギブスの自由エネルギーを計算し、実験により求めた阻害強度から計算した値と比較したところ比較的良好な相関性が見られた。この事実に基づき、inh1 と inh8 のドッキングにおいて、プロリン環の t-butyl の周辺の疎水性領域が空白となっていることに着目し、その領域を満たす化合物をコンピュータ上で作成した。それらの化合物のドッキングを行ったところ、benzyl 基を持つものが最も低い、ドッキングエネルギーを呈した。この論文の目的のひとつは、計算機実験を行って、HIV protease 阻害物質に対する薬物設計を行うことであつたので、著者はこれらの化合物に対する臨床上の探索をその分野の専門家に委ねる。

キーワード: Computer docking, Drug design, HIV protease, Gibbs free energy, Ligand inhibitor