Sabadinine: A Potential Non-Peptide Anti-Severe Acute-Respiratory-Syndrome Agent Identified Using Structure-Aided Design

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Abstract: A novel human coronavirus has been reported to be the causative agent of severe acute respiratory syndrome (SARS). Since replication of HcoVs depends on extensive proteolytic processing, the main proteinase, 3CL^{pro}, is an attractive drug target for anti-SARS agents. We have employed molecular docking of a chemical database into the active site of 3CL^{pro} to search for non-peptidyl inhibitors. One compound was identified to be the natural product sabadinine, isolated from a historical herbal remedy.

The recent spread of severe acute respiratory syndrome (SARS) has caused concern because of its relatively high fatality rate, particularly among the elderly.[1] A novel human coronavirus (HCoV) is reported to be the causative agent of SARS.[2] The viral main proteinase (3CL^{pro} or M^{pro}) encoded by HCoV has been identified as an attractive drug target, and the X-ray crystal structure has been determined recently.[3] The structural relationships between proteinases HCoV 3CL^{pro}, porcine coronavirus (transmissible gastroenteritis virus (TGEV)) M^{pro} or 3CL^{pro}, and human rhinovirus (HRV) serotype 2 3C^{pro} have been used to identify AG7088 as a starting point for structure-aided design of anti-SARS drugs.[3] AG7088 is an HRV 3C^{pro} inhibitor currently in clinical trials for the common cold. Since AG7088 is a peptidyl molecule, it was considered to be of great interest to search for non-peptidyl inhibitors of 3CL^{pro}, a cysteine protease.

We have employed the 3CL^{pro} crystal structure in an automated docking computer software of flexible ligands to macromolecules called AutoDock[4] to screen a large public database of small molecules as a first step toward the selection of compounds for testing in antiviral assays.

Results. We chose the National Cancer Institute (NCI) diversity set that is representative of a collection of approximately 140 000 chemicals as a potential source for biologically relevant chemicals predicted to be 3CL^{pro} inhibitors. This set of 1853 compounds was selected by the NCI as three-point pharmacophores and includes both synthetic compounds and natural products. AutoDock performs ligand docking on precomputed grid maps representing different atom types in the receptor. The docking grid was centered on Cys^{144}, a member of the catalytic dyad of 3CL^{pro}. The algorithm calculates the conformation of each compound with the lowest energy, and the chemical database was rank-ordered. These energies were then used as a guide to predict potential binding to the enzyme active site. The algorithm performed 10 docking optimizations per ligand, each starting from a different random initial location of the compound. Calculations for the same ligand with a root-mean-square deviation of <0.5 Å from each other were considered to be a cluster of solutions, since they represent reproducible results. The top 5% of the compounds ranked according to docking energy were then sorted by the size of the cluster. The resulting statistics for all compounds are provided in Figure 1. Within the lowest energy dockings, 10 compounds (∼0.5%) showed clustering of 5 or greater out of the 10 independent docking studies.

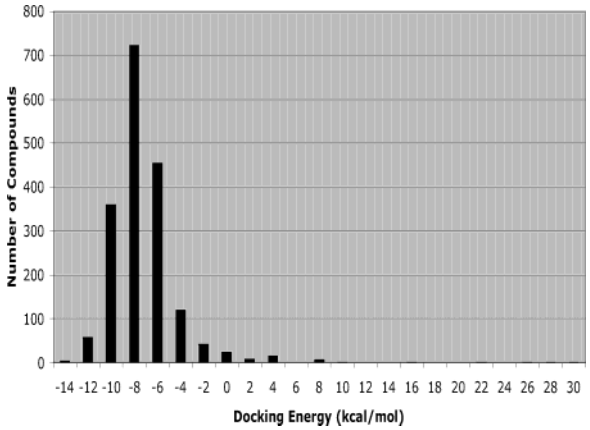
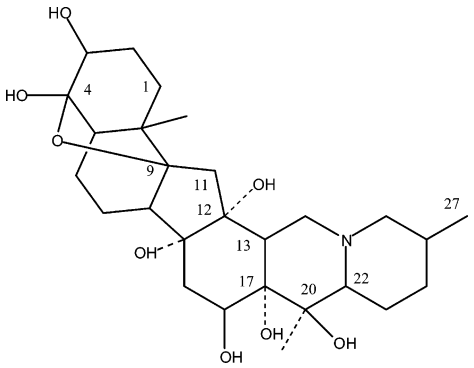


Figure 1. Histogram of the lowest docking energy for each conformer using the National Cancer Institute diversity set.

Scheme 1



The 10 compounds selected using the criteria described above were then evaluated for desirable chemical properties as therapeutics. While most compounds within this group did not have desirable physicochemical properties, sabadinine (diversity 1043) was exceptional (Scheme 1). Figure 2 shows sabadinine docked into the active site of 3CL^{pro} having a docking energy of −11.6 kcal/mol and a clustering of 9 out of 10 conformers. The lowest energy conformer of sabadinine is characterized by both steric factors and hydrogen bonding within the active site of 3CL^{pro}. Sabadinine is

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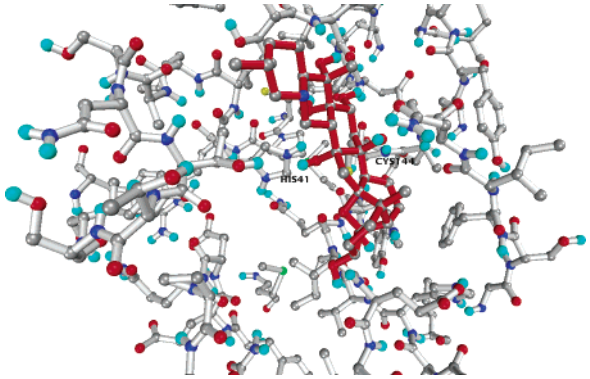

Figure 2. Molecular docking of sabadinine in the active site of $3CL^{pro}$. Atom types in the protein are colored according to the following scheme: oxygen (red), nitrogen (blue), carbon (grey), sulfur (yellow), and hydrogen (cyan). Sabadinine is shown using bond connectivity in red.

situated near the residues comprising the catalytic dyad of the enzyme, His^{44} and Cys^{144}. By use of the lowest energy conformer, the distance between the hydroxyl O at C(20) of sabadinine and the H bound to N(ϵ) of His^{44} is calculated to be 2.93 Å, consistent with a hydrogen bond.

To determine whether sabadinine exhibits any activity in a cellular assay, viral infection was measured using the prototype of group II coronaviruses, mouse hepatitis virus (murine hepatitus virus (MHV) strain A59).[5,6] Infections were performed as described previously.[5] Neither sabadinine nor the vehicle control caused cellular toxicity up to 100 μM. To evaluate whether sabadinine affects coronavirus replication, cells were observed for syncytium formation and cytopathic effects at 4, 6, 8, 10, 12, and 24 h postinfection (hpi). Syncytium formation began at 4 hpi, peaking at 12 hpi, in both untreated and treated infected cells irrespective of the sabadinine concentration and viral multiplicity of infection. Thus, sabadinine did not affect murine coronavirus replication under the conditions tested.

Discussion. The availability of the X-ray crystal structure of $3CL^{pro}$ offers the possibility of structure-aided design of inhibitors of this enzyme as a first step toward the identification of anti-SARS agents. Recently, a similar docking approach was used to identify potential proteinase inhibitors of $3CL^{pro}$.[7] However, these authors carried out a docking study prior to the availability of the X-ray structure of $3CL^{pro}$ and relied on a homology model using the X-ray structure of TGEV M^{pro}. In addition, the best compound identified among a set of 73 known proteinase inhibitors was reported to have a docking energy of −7.66 kcal/mol,[7] representing a significantly higher value than that observed with sabadinine. After this manuscript had been submitted, a more recent study reported docking studies of known antiviral compounds into the active site of $3CL^{pro}$ and identified calanolide A as having the lowest docking energy.[8]

In the current study, we have used criteria of lowest energy conformers docked into the active site of $3CL^{pro}$, clustering of multiple calculations, and desirable physicochemical properties for therapeutics to reveal the natural product sabadinine from the NCI diversity set as a potential non-peptidyl inhibitor. Sabadinine is a natural product isolated originally from the Lily plant *Veratrum sabadilla* (family *N. O. Liliaceae*) and has been used as an herbal remedy.[9] While this compound did not exhibit any significant antiviral activity against the murine hepatitis virus (MHV strain A59), we will be testing sabadinine against other coronaviruses, as well as isolated 3CLpro. That sabadinine is a natural product with known medicinal properties is a promising first step toward structure-aided design of non-peptidyl anti-SARS agents.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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