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Design and synthesis of several small-size HTLV-I protease inhibitors with different hydrophilicity profiles

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ABSTRACT

The human T cell leukemia/lymphotropic virus type 1 (HTLV-I) is clinically associated with adult T cell leukemia/lymphoma, HTLV-I associated myelopathy/tropical spastic paraparesis, and a number of other chronic inflammatory diseases. To stop the replication of the virus, we developed highly potent tetrapeptidic HTLV-I protease inhibitors. In a recent X-ray crystallography study, several of our inhibitors could not form co-crystal complexes with the protease due to their high hydrophobicity. In the current study, we designed, synthesized and evaluated the HTLV-I protease inhibition potency of compounds with hydrophilic end-capping moieties with the aim of improving pharmaceutic and pharmacokinetic properties.

The human T cell leukemia/lymphotropic virus type 1 (HTLV-I) is the first identified human retrovirus[1] and the only known retrovirus to cause human cancer, adult T cell leukemia/lymphoma (ATL).[2] HTLV-I infection may also lead to a chronic progressive myelopathy[3] and several inflammatory diseases as well as opportunistic infections.[4] There is currently no cure for the estimated 20–30 million HTLV-I infected patients[5] living in endemic regions around the world.[6] To develop a cure for HTLV-I infection, we are targeting HTLV-I protease, which is an enzyme responsible for processing precursor proteins to new viral components.[7] HTLV-I protease assumes a C2 symmetry homodimer fold consisting of 125-residue per chain, and grossly resembling the HIV-1 protease. The narrow tunnel-like active site where the precursor protein, substrate or inhibitor is accommodated spans from the symmetrical S_5 to S'_5 subsites (Fig. 1). Tucked between the S_1 and S'_1 subsites, our central P_1 inhibitory unit, a hydroxymethylcarbonyl isostere, is based on the transition state formed during hydrolysis of peptide bonds.

In a recent work, the X-ray diffraction crystallography structures of several of our tetrapeptidic HTLV-I protease inhibitors in complex with a des-(117-125)-[Ile^{40}]HTLV-I protease were solved.[8] In the study, only six out of 11 inhibitors formed co-crystals with the protease, because significant precipitation occurred in the preparation mixtures despite extensive screening for other crystallization conditions. The co-crystallized inhibitors were by and large more hydrophilic than those precipitated out of solution. The aim of the current study is to discover small-size HTLV-I protease inhibitors with different hydrophilicity profiles without jeopardizing the inhibition potency against HTLV-I protease. We selected KNI-10562 (1) as the reference compound, because of its small size and potent inhibition profile against HTLV-I protease (Table 1).

HTLV-I protease has a high degree of specificity for substrates over that of HIV-1 protease[9] and bovine leukemia virus (BLV) protease.[10] Similar to the substrate studies, we reported HTLV-I protease has a high degree of specificity for inhibitors.[7] This means structural requirements at subsites are more stringent for HTLV-I protease binding and inhibition than for HIV-1 protease and BLV protease. The HTLV-I[11] and HIV-1[12] protease inhibition profiles in the current study support this hypothesis, because although the synthesized compounds ranged from low to high HTLV-I protease inhibition (8–93% inhibition at 50 nM), all exhibited potent HIV-1 protease inhibition (72–100% inhibition at 50 nM). Of the subsites, general observations on substrate specificity studies on retroviral aspartic proteases[13] along with our experience with HTLV-I protease inhibitor specificity[14] suggest distant subsites from the catalytic S_1–S'_1 subsites have lower specificity. This means modifying the two ends of reference inhibitor **1**, namely the P'_1-cap and P_3-cap moieties, has a lower risk for negatively impacting the HTLV-I protease inhibition potency.

In the current study, using computer-assisted molecular modeling,[15] we designed novel analogues of reference HTLV-I protease inhibitor **1** with different hydrophilic groups to the P'_1-cap and P_3-cap moieties. We foresaw the hydrophilic group could either have a direct or water-mediated hydrogen bond interaction with the protease, or otherwise a hydration shell could form at the

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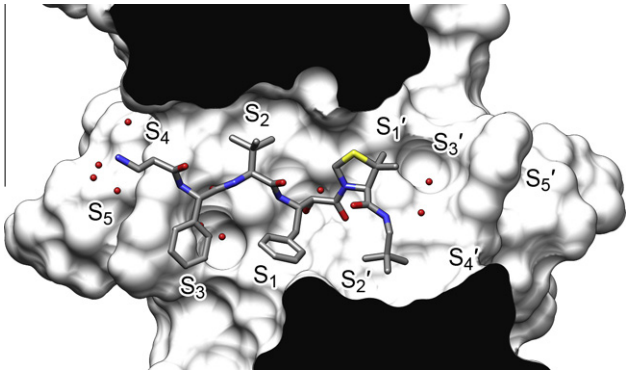


Figure 1. Cross-section view with a black slice plane of the active site of HTLV-I protease in complex with inhibitor **5**. Red spheres represent proximal water molecules. Hydrogen bond interactions with the backbone of the inhibitor occur above and below the plane of the page.

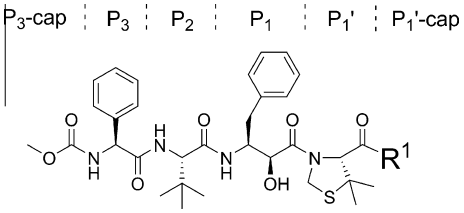
modified end of the inhibitor and trap the compound inside the active site. In our computer models, we observed backbone hydrogen bond interactions from the P'_1-cap to the P_3-cap moiety were similar in all inhibitors with differences where applicable (Fig. 2). We synthesized the designed compounds (Fig. 3)[16] and screened for greater than 50% HTLV-I protease inhibition at 50 nM. The IC_{50} value was determined for any inhibitor passing the screening assay. Lastly, the relative hydrophilicity was evaluated for highly potent inhibitors.

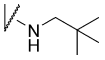
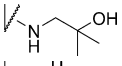
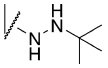
Using reference inhibitor **1** as a starting point, the P'_1-cap neopentylamine moiety was replaced by more hydrophilic analogues in compounds **2** and **3**. Inhibitor **2** was designed for a hydrogen bond between its distal hydroxyl group and the oxygen atom of Met^{37B}. Compared with reference inhibitor **1**, the lower HTLV-I protease inhibition in inhibitor **2** suggests the S'_2 pocket prefers hydrophobic groups. The distal protonated hydrazine nitrogen of the P'_1-cap *t*-butylhydrazide moiety in inhibitor **3** was designed to form a hydrogen bond with Leu^{57B}'s oxygen atom, across from the S'_2 pocket. Inhibitor **3** exhibited slightly lower HTLV-I protease inhibition than reference inhibitor **1**.

At the P_3-cap region of reference inhibitor **1**, X-ray diffraction crystallography data[8] indicate the nitrogen of the P_3-cap amide forms a hydrogen bond with the side-chain of Asp^{36A}, and the oxygen of the amide has a hydrogen bond interaction with the amide NH of Leu^{57A}. Our past study reveals the S_4 subsite can accommodate for several hydrophobic moieties of different length and bulk.[17] Considering the P_3-cap and P_4 moieties sit at the entrance to the active site, a hydration shell should form with any hydrophilic group introduced in the vicinity. In the current study, the P_3-cap methyl carbamate moiety in reference inhibitor **1** was first substituted with P_4 glycine as exemplified by inhibitor **4** that was significantly less potent than reference inhibitor **1** (Table 2). Extending the P_4 residue to β-alanine afforded a slightly more potent HTLV-I protease inhibitor (**5**). Further elongation to γ-aminobutyric acid (GABA) led to inhibitor **6** exhibiting lower HTLV-I protease inhibition potency than reference inhibitor **1**. Hence, the potency order from least to most potent for the P_4-residue is glycine, GABA and β-alanine. Reference inhibitor **7**[17] with a P_3-cap *n*-butyramide moiety is more structurally similar to P_4 β-alanine inhibitor **5** than P_3-cap methylcarbamate reference inhibitor **1**. Inhibitor **5** is slightly more potent or equipotent to reference inhibitor **7**, which suggests hydration shell formation is more likely than direct hydrogen bond interaction with the protease.

To explore the effect of two hydrophilic moieties at the P_4 position on HTLV-I protease inhibition, a L-2,3-diaminopropionic acid residue was introduced in compound **8** to compare with the

Table 1
HTLV-I protease inhibition potency of P'_1 analogues



	Compounds	R^1	HTLV-I protease inhibition[11] (IC_{50}, nM)	HIV-1 protease inhibition at 50 nM[12](%)
1	KNI-10562		7.2 ± 0.6	99
2	KNI-10608		39.1 ± 5.1	99
3	KNI-10605		10.4 ± 0.6	99

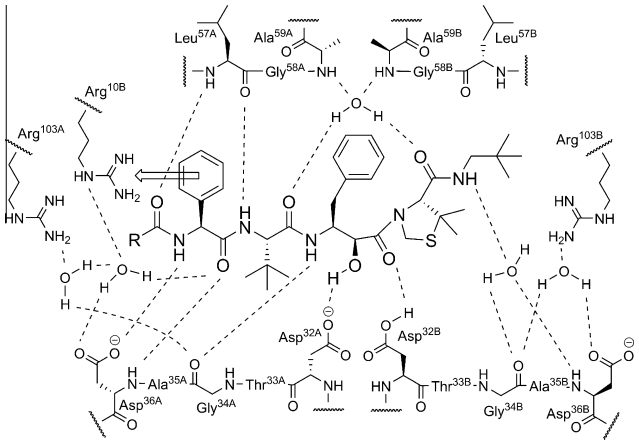


Figure 2. A typical hydrogen bond interaction pattern observed in inhibitors **1**, **4**–**9** and HTLV-I protease. A cation-π interaction is present between the inhibitor's P_3 phenyl group and the protease's Arg^{10B} (arrow). For simplicity, intra- and intermolecular hydrogen bond interactions between the two protease chains are not depicted.

possible hydrogen bond interactions found in inhibitors **4** and **5**. Compound **8** exhibited low inhibitory potency against HTLV-I protease. Compound **9** was designed with similar interactions in mind and it too presented with low HTLV-I protease inhibition. Steric hindrance is not likely a problem because the protease can accommodate different hydrophobic bulk in the S_4 subsite.[17,18] It is conceivable hydrogen bond interactions at the S_4 subsite disrupt the favorable interactions elsewhere in the inhibitor. Indeed, our recent X-ray diffraction study on HTLV-I protease inhibitors indicates stronger interactions in the peripheral subsites can pull the tips of the two flaps apart to the extent that two water molecules are bound between the flaps.[8]

Computer-assisted modeling experiments were performed with compounds **4–9** in extended pose to correlate the P_3-cap/P_4 structure with HTLV-I protease inhibition potency (Fig. 4). The protonated amino group of inhibitor **5**'s P_4 β-alanine is maximally hydrated by three water molecules (Fig. 4D). One of the water molecules mediates a hydrogen bond interaction with Ser^{55A}. Another water molecule bridged by a secondary water molecule links inhibitor **5**'s amino group with Asp^{36A} to the hydrogen bond network. Thus, inhibitor **5**'s potent inhibition against HTLV-I protease could