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Design and Synthesis of Pseudo-Symmetric HIV Protease Inhibitors Containing a Novel Hydroxymethylcarbonyl (HMC)-Hydrazide Isostere

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Abstract—Pseudo-symmetric HIV-1 protease inhibitors containing a novel HMC-hydrazide isostere as the transition-state mimic were designed and synthesized. Most of the synthetic compounds with varied structures at the P and P′ sites around this core unit showed potent inhibitory activity against HIV-1 protease with nanomolar K_i values.
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The human immunodeficiency virus type-1 (HIV-1) protease inhibitors have proven to be highly efficacious therapeutic agents when used in combination for the treatment of HIV infection. The activity of these drugs results from potent inhibition of the essential *gag* and *gag-pol* polyprotein processing steps that occur during viral maturation into infectious virions. However, in spite of the impressive potency of currently used HIV-1 protease inhibitors, the emergence of drug resistant viruses against these inhibitors reminds us of the importance of continuous research for the development of newer classes of compounds directed against this important target.[1,2]

In our previous research in this field, we developed substrate-based peptidomimetic inhibitors containing allophenylnorstatine [Apns; (2*S*, 3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid] which has a hydroxymethylcarbonyl (HMC) isostere as an ideal transition-state mimic.[3,4] One of the best compounds, KNI-764, exhibited excellent drug-profiles with low toxicity, high bioavailability, broad inhibitory spectrum against mutant proteases, and low emergence of drug resistant viruses.[1b,3] These results suggested to us the potential utility of the HMC structure as a core for a novel class of HIV-1 protease inhibitors that may solve the present problems of clinically used drugs, including the emergence of drug resistant viruses. Therefore, we have expanded our investigation of HIV-1 protease inhibitors based on the HMC structure and introduce herein a novel isostere named 'HMC-hydrazide', which leads to a new series of pseudo-symmetric HIV-1 protease inhibitors. These compounds showed potent HIV-1 protease inhibitory and promising antiviral activities.

Design

HIV protease has been shown to exist as a C_2-symmetric homodimer in its active form. Hence, several dipeptide isosteres such as diaminoalcohol, diaminodiol and hydroxyethylhydrazine have been previously employed in the development of pseudo-symmetric inhibitors (Fig. 1).[5–8] To introduce our HMC structure into this type of inhibitors, KNI-577[9] as a representative compound was used for molecular design. Using a strategy similar to that reported by Kempf et al.,[6] KNI-577 was inverted around a hypothetical axis of symmetry defined between the carbonyl- and α-carbons of the Apns residue, and the resultant structure was superposed over the original molecule. Next, the P′ region of the structures was deleted. And finally, to realize a putative fused molecule

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with both the P sites, an HMC-hydrazide structure was employed as shown in compound **7a** (Fig. 2). Based on this design, we decided to carry out the modification of both wings of the molecule by the introduction of the P2 or P2–P3 units which were found in the previously developed potent inhibitors such as KNI-727 and KNI-272 shown in Figure 3.[3,4,9]

Chemistry

The syntheses of these pseudo-symmetric inhibitors are shown in Schemes 1 and 2. Acylhydrazides **4** were prepared from benzylhydrazine **1** by coupling to the carboxylic acids, 3-acetoxy-2-methylbenzoic acid **2** and 2,6-dimethylphenoxyacetic acid **3**, with the BOP reagent. These hydrazides **4** were next coupled to Boc-Apns-OH by the mixed-anhydride method using isobutyl chloroformate (IBCF). This reaction afforded the compound **5** in which the α-hydroxyl group of Apns was modified with an isobutyloxycarbonyl group derived from IBCF. However, **5** was used for further reactions without the removal of the carbonate. The Boc group of **5** was removed with 4N HCl-dioxane, and subsequent coupling to three different kinds of acyl components, that is **2**, **3** and 4-(*tert*-butoxycarbonylamino)-2,6-dimethylphenoxyacetic acid **6**, with the BOP reagent, and the removal of protecting and/or modified groups on the hydroxyl groups with 1N NaOH, afforded the desired compounds **7a–d** (Scheme 1). In the case of **7e**, further treatment with 4 N HCl-dioxane was performed to remove the Boc group from its 4-amino-2,6-dimethylphenoxyacetyl group.

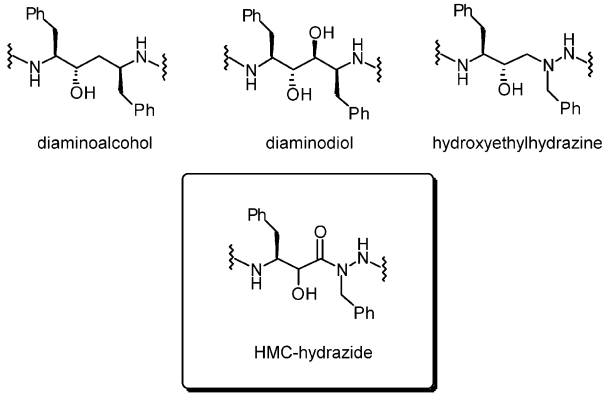


Figure 1. Pseudo-symmetric dipeptide isosteres.

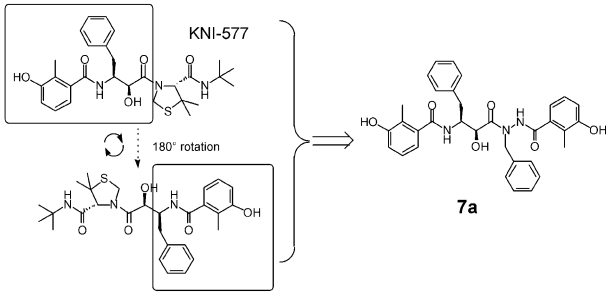


Figure 2. Design of pseudo-symmetric inhibitors containing Apns.

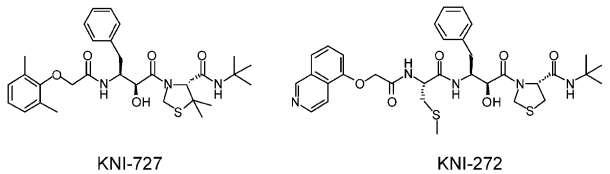
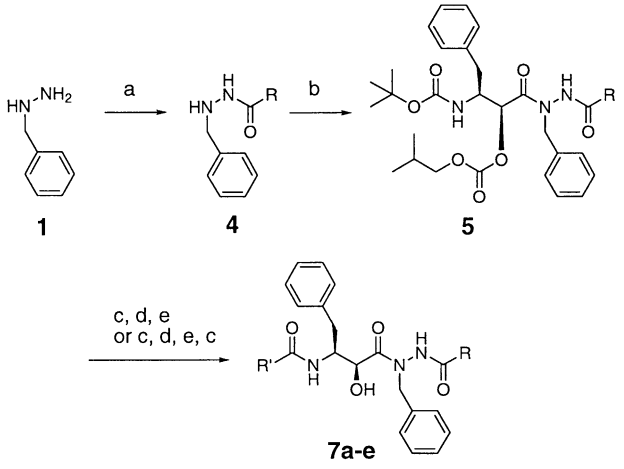


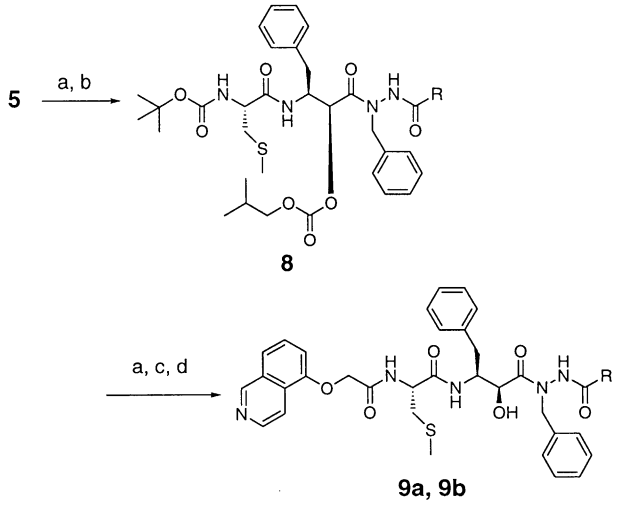
Figure 3. Structures of KNI-727 and KNI-272.

Compound **8** was obtained from **5** by the cleavage of Boc and subsequent coupling to Boc-L-methylthioalanine with BOP, and then **8** was converted to **9a** and **9b** in three steps with the deprotection, introduction of 5-isoquinolyloxyacetic acid and saponification (Scheme 2).

Compound **10** was synthesized by the same method with **7a** using Boc-Pns-OH [Pns = phenylnorstatine: (2*R*, 3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid] instead of Boc-Apns-OH.



Scheme 1. Reagents and conditions: (a) RCOOH(**2**, **3**), Et_3N, BOP, DMF; (b) Boc-Apns-OH, IBCF, NMM, DMF; (c) 4N HCl-dioxane; (d) R′COOH(**2**, **3**, **6**), Et_3N, BOP, DMF; (e) 1 N NaOH.

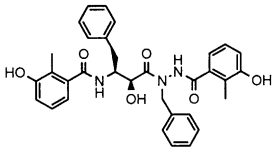
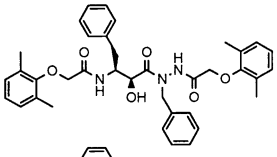
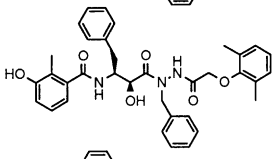
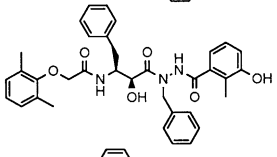
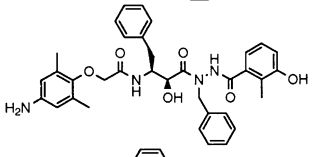
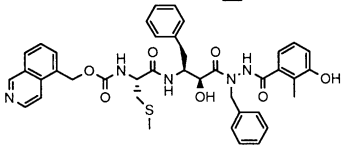
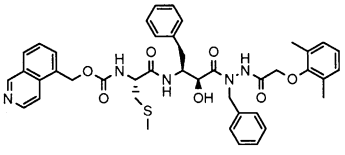
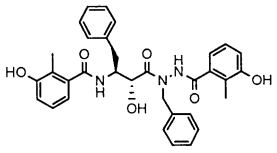


Scheme 2. Reagents and conditions: (a) 4 N HCl-dioxane, anisole; (b) Boc-L-methylthioalanine, Et_3N, BOP, DMF; (c) 5-isoquinolyloxyacetic acid, Et_3N, BOP, DMF; (d) 1 N NaOH.

Results and Discussion

The inhibitory activity of the synthetic compounds against HIV-1 protease was examined as described previously.[10,11] As Table 1 shows, compound **7a**, which was designed from KNI-577, showed potent inhibitory activity with the K_i value of 15 nM, suggesting that our HMC-hydrazide served as an effective pseudo-symmetric isostere for HIV-1 protease inhibition. Next, the P2 and P2′ sites of **7a** were modified to yield compound **7b**, which has two 2,6-dimethylphenoxyacetyl groups derived from the P2 unit of a potent dipeptide-based inhibitor KNI-727.[3a,9] However **7b** only showed modest activity with an estimated K_i of 5 μM. This result may be due to the loss of affinity for the active site of the enzyme by the introduction of two bulky groups. Thus, we synthesized **7c** and **7d** which have one 2,6-dimethylphenoxyacetyl group at either the P2 or P2′ site and one smaller 3-hydroxy-2-methylbenzoyl group at the other site. Both **7c** and **7d** were equipotent as **7a**. Furthermore, in combination with P2–P3 units of KNI-272 and a P2 unit derived from either KNI-577 or -727 at the P2′ site, respectively, the corresponding compounds **9a** and **9b** showed increased enzyme inhibitory activity. Next, the Apns residue was replaced with a diastereomeric Pns residue to examine a stereochemical preference of the

Table 1. Inhibitory activity against HIV-1 protease, anti-HIV-1 IIIB activity, and cytotoxicity

Compd	Structure	K_i[a] (nM)	HIV-1 IIIB/MT-4[b]		SI (TD_{50}/EC_{50})
			EC_{50} (μM)	TD_{50} (μM)	
7a (KNI-1276)		15	1.1	73	66
7b (KNI-1278)		5000[c,d]	nd[c]	nd[c]	—
7c (KNI-1279)		5.8	1.8	12	6.7
7d (KNI-1277)		3.0	1.1	12	11
7e (KNI-1166)		5.0	0.48	35	73
9a (KNI-1224)		0.47	0.40	23	58
9b (KNI-1289)		0.25	1.9	>100	>53
10 (KNI-1167)		0.16	1.6	91	57

[a]Reference 10.
[b]Reference 11.
[c]Not determined.
[d]IC_{50} was determined to be 5 μM. In this concentration range IC_{50} approximates K_i under the conditions of the assay.

α-hydroxyl group of HMC-hydrazide inhibitors. The corresponding compound **10** showed 100-fold higher activity than original **7a**. This is consistent with previously published results.[12,13]

In spite of the potent enzyme inhibitory activity observed, most of the derivatives showed moderate antiviral activity with the EC_{50} values of about 1 μM against HIV-1 IIIB strain. This large difference is probably related to the physicochemical features of these compounds such as solubility and cell-penetration. A similar observation has been reported for KNI-727. This molecule, which has a dimethylphenoxyacetyl group, exhibits a high binding affinity towards the protease, but low antiviral activity due to its hydrophobicity.[3,9] In an effort to increase the hydrophilicity of KNI-727, a 4-amino group was introduced into the 2,6-dimethylphenoxyacetyl group.[14] The same approach was used in synthesizing compound **7e**. Intoduction of a *p*-amino-2,6-dimethylphenoxyacetyl group showed a two-fold improvement in antiviral activity. The cytotoxicity of synthetic compounds against MT-4 cells was >10 μM (TD_{50}) and the values of selectivity index (SI) were >50 in compounds **7a**, **7e**, **9a**, **9b** and **10**. More extensive modification of the structure around the HMC-hydrazide core will be explored to achieve increased antiviral potency and SI values. Additional work is underway to assess the inhibitory potency of this new series against resistant viruses.

In conclusion, we have designed and synthesized novel pseudo-symmetric HIV-1 protease inhibitors containing HMC-hydrazide isostere, which effectively inhibited HIV-1 protease and viral replication in vitro. This HMC-hydrazide isostere promises to be a useful template for the development of novel agents for the treatment of HIV and for the design of inhibitors against other aspartic proteases.

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