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Structure-based design of non-peptide HIV protease inhibitors

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Abstract

A number of structurally novel P_2-ligands have been designed and synthesized. Incorporation of these ligands in the (*R*)-(hydroxyethyl)sulfonamide isostere provided a series of potent non-peptidyl HIV protease inhibitors.

Keywords: Protease; Inhibitor; Ligand; Design; Non-peptide

1. Introduction

The clinical effectiveness of the HIV protease inhibitors in combination with reverse transcriptase inhibitors for the treatment of AIDS has been well established [1]. These combination therapies have significantly reduced plasma viral loads, increased CD_4 cell counts and halted the progression of AIDS. Despite this remarkable progress, there are major limitations in the current therapies. These include low oral bioavailability, presence of a substantial 'peptide-like' character as well as emergence of resistance to these protease inhibitors [2]. To alleviate these problems, the current emphasis has been to design and synthesize non-peptidyl protease inhibitors and optimize their potency against mutant strains resistant to the currently approved protease inhibitors. We have recently designed and developed a number of structurally novel non-peptidyl ligands for the HIV protease inhibitors based upon X-ray structures of the protein-ligand complexes [3–5]. Incorporation of these designed ligands in the saquinavir derived (*R*)-hydroxyethylamine isostere and (*R*)-(hydroxyethyl)sulfonamide isostere resulted in a series of very potent and non-peptidal HIV protease inhibitors [6,7]. Herein we report further design of novel ligands based upon our structure–activity studies and the structural information obtained from the reported inhibitor bound X-ray structures of HIV-1 protease.

2. Results and discussion

We recently reported the structure-based design of (*R*)-1,1-dioxotetrahydro-2H-thiopyran-3-carboxamides as high affinity P_2-ligands for the HIV protease substrate binding site [3]. As illustrated in Fig. 1, replacement of P_2-asparagine and P_3-quinoline of the saquinavir (**1**, K_i value 1.4 nM and antiviral ID_{50} of 16 nM)[1] by a 1,1-dioxotetrahydro-2H-thiopyran-3-carboxamide derivative provided the protease inhibitor **2** with an enzyme inhibitory potency of 15 nM (K_i value) and antiviral potency of 200 nM in MT_4 human T-lymphoid cells infected with IIIB isolate (ID_{50} value). Incorporation of the same (3*R*)-cyclic sulfone carboxamide as the P_2-ligand in (*R*)-(hydroxyethyl)-sulfonamide isostere afforded a very potent inhibitor **3** with an enzyme K_i of 1.4 nM and antiviral ID_{50} value of 17 nM. Inhibitor **4** with an acyclic sulfone derivative as P_2-ligand has also exhibited potent enzyme inhibitory potency (K_i value of 1.4 nM).

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[1] In-house prepared saquinavir (**1**) exhibited enzyme $K_i = 1.4$ nM in assay developed in our laboratory and antiviral ID_{50} value of 18 nM in Dr Hollands' assay.

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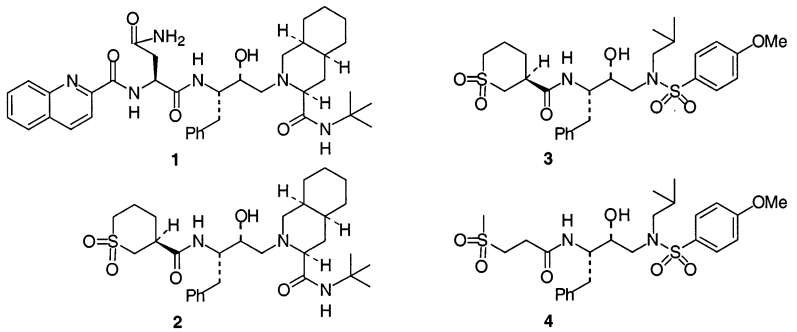

Fig. 1.

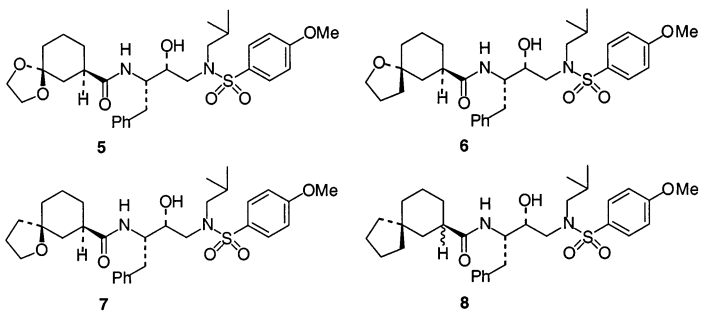

Fig. 2.

The X-ray structure of saquinavir-bound HIV-1 protease and model structures of inhibitors **2** and **3** indicated that the sulfone oxygen *trans* to the carboxamide functionality forms hydrogen bonds to the backbone NH of the Asp 29 and 30 residues in the active site. Based upon possible ligand-binding site interactions, we have further speculated that a 1,3-dioxolane derivative or a stereochemically defined spirocyclic ether may interact effectively with the same Asp 29 and 30 residues. As depicted in Fig. 2, inhibitor **5** incorporating a 1,3-dioxolane derivative as the P_2-ligand exhibited an enzymatic K_i value of 14 nM and an antiviral potency of 110 nM in MT_4 cells.

Presumably, one of the ketal oxygens is involved in hydrogen bonding with the backbone NH of the Asp 29 and 30 residues. As mentioned earlier, it appears that the *trans* oxygen with respect to the carboxamide functionality is within proximity to these residues. This assumption is supported by the fact that the removal of the top ketal oxygen of inhibitor **5** resulted in inhibitor **6** with a spirocyclic ether as the P_2-ligand. This inhibitor has shown nearly 7-fold enhancement of its enzyme inhibitory potency (K_i value of 2.9 nM) compared to 1,3-dioxolane derivative **5**. Inhibitor **6** exhibited improved antiviral potency (75 nM in MT_4 cells) as well. Consistent with our speculation, removal of the bottom ketal oxygen of inhibitor **5** afforded the inhibitor **7** which has shown a substantially lower K_i value of 260 nM. The removal of both ketal oxygens resulted in inhibitor **8** (1:1 mixture of diastereomers) with further loss of protease inhibitory potency ($K_i =$ 685 nM). These results indicated the stereochemical importance of the spiro tetrahydrofuranyl oxygen in inhibitor **6** as well as its specific involvement in hydrogen bonding interactions with the residues in the active site of HIV protease.

On the basis of stereochemical relevance and the position of oxygen in the spirocyclic ligand in inhibitor **6**, we further designed and investigated the abilities of spirocyclic ketals to function as novel P_2-ligands for the protease inhibitors. As depicted in Fig. 3, consistent with the stereochemistry of the spirotetrahydrofuran in **6**, the corresponding spiroketal with (4*R*)-configuration was synthesized and incorporated in the (*R*)-(hydroxyethyl)sulfonamide isostere. In the resulting inhibitor **9**, because of the anomeric effect, the spiro ketal conformation is expected to be as shown, with axial orientation of the tetrahydrofuranyl oxygen [8]. Indeed, inhibitor **9** ($K_i = 3.9$ nM) with a spiro ketal derivative has shown comparable enzyme inhibitory potency to inhibitor **6**. Incorporation of the enantiomeric ligand (4*S*-configuration) in the (*R*)-(hydroxyethyl)sulfonamide isostere provided the inhibitor **10** with a K_i value of 7.6 nM. The removal of the tetrahydrofuranyl oxygen afforded the inhibitor **11** ($K_i = 16$ nM, 1:1 diastereomeric mixture) with at least 4-fold loss of potency compared to inhibitor **9**. The above results demonstrate the importance of the ligand stereochemistry as well as the ring oxygen position in the ligand. While these ligands are structurally unique, the in-

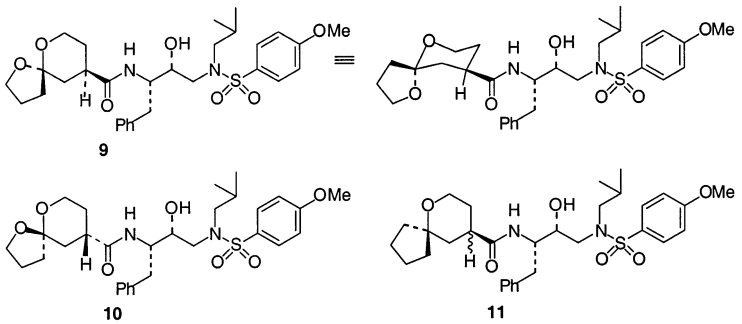

Fig. 3.

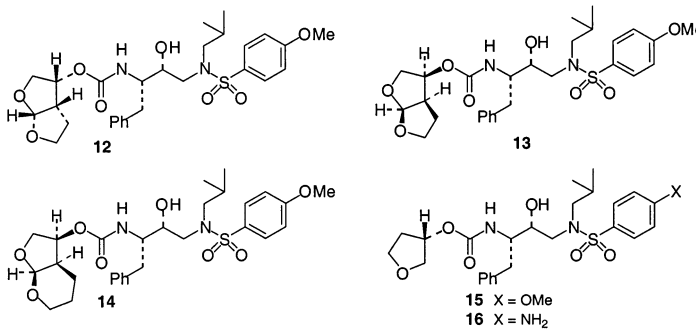

Fig. 4.

hibitors (**9** and **10**) incorporating these ligands, however, exhibited antiviral potency in the micromolar range (ID_{50} values of 1.2 μM for **9** and 2.7 μM for **10**).

On the basis of previous structure–activity studies and with the aim of further improving antiviral activities and drug-resistance profiles of inhibitors derived from the (*R*)-(hydroxyethyl)sulfonamide isostere, we have incorporated various structure-based designed fused tetrahydrofuranyl urethanes as P_2-ligands. As shown in Fig. 4, 3(*R*), 3a(*S*), 6a(*R*)-bis-tetrahydrofuranyl (bis-THF) urethane (**12**) exhibited remarkable enzyme inhibitory ($K_i = 1.1$ nM) and antiviral potencies ($ID_{50} = 1.4$ nM) [7,9][2]. Incorporation of the enantiomeric 3(*S*), 3a(*R*), 6a(*S*)-bis-THF ligand also provided the very potent inhibitor **13** ($K_i = 1.4$ nM and antiviral $ID_{50} = 4.2$ nM). Inhibitor **14** ($K_i = 2.2$ nM and antiviral $ID_{50} = 4.5$ nM) incorporating 3(*S*), 3a(*R*), 7a(*S*)-hexahydrofuropyranyl urethane exhibited inhibitory properties comparable to inhibitor **13**. Incorporation of our structure-based designed 3(*S*)-tetrahydrofuranyl urethane as the P_2-ligand provided inhibitor **15** with an enzyme inhibitory potency of 1.5 nM and an antiviral potency of 12 nM [10]. Amprenavir (**16**), incorporating 3(*S*)-tetrahydrofuranyl urethane as the P_2-ligand but 4-aminobenzenesulfonamide as the P_2'-ligand, has shown similar inhibitory properties ($K_i =$ 1.6 nM, antiviral $ID_{50} = 15$ nM) [11]. The potency enhancing effect of a stereochemically defined bis-THF ligand is thus evident in inhibitor **12**. A preliminary X-ray structure of inhibitor **12** bound to HIV-1 protease indicated that both oxygen atoms of the bis-THF ligands are within hydrogen bonding distance to the backbone NH of Asp 29 and Asp 30. Furthermore, the 4-methoxyl oxygen of inhibitor **12** is within hydrogen-bonding distance to the Asp 29′ and Asp 30′ residues [12].

In conclusion, we have reported the development of a series of non-peptidyl ligands designed through structure-based design strategies. Incorporation of these ligands in the (*R*)-(hydroxyethyl)sulfonamide isostere resulted in a number of structurally diverse protease inhibitors. Compound **12** is the most potent inhibitor in this series. Drug-resistance studies of inhibitor **12** are being evaluated [13]. Chemical modifications of these inhibitors and further design of novel inhibitors are underway.

Acknowledgements

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[2] Inhibitor **12** has exhibited enzyme inhibitory $K_i = 0.016$ nM and antiviral cell RNAIC = 0.71 nM in the assay protocol developed at the Dupont Company.

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