Variations of the P2 group in HIV-1 protease inhibitors containing a tertiary alcohol in the transition-state mimicking scaffold†

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A short synthetic protocol leading to HIV-1 protease inhibitors with a tertiary alcohol based transition-state mimicking unit and different P2 side chains has been developed.

HIV-1 protease inhibitors (PIs) are important in the most frequently used regimen for the treatment of HIV/AIDS, the highly active antiretroviral therapy (HAART), and have improved the quality of life and prolonged the lifetimes of infected patients.[1] Seven PIs have reached the market so far,[2,3] and in addition, tipranavir has been granted accelerated approval by the FDA.[4] All PIs currently available for therapy are peptide-like and suffer from shortcomings related to their pharmacokinetic properties. Low membrane permeability, high protein binding and rapid metabolism have been reported for almost all approved inhibitors.[3,5] In addition, considering the increasing numbers of HIV-1 strains resistant to the present PI generation,[6,7] the need for new structural themes and unique chemical entities to combat HIV/AIDS is obvious.[8]

We recently described a new class of HIV-1 PIs containing a tertiary alcohol, a component which has rarely been used in transition-state analogs.[9,10] These inhibitors, exemplified by **1** in Fig. 1, exhibited low K_i values and, notably, excellent membrane permeation properties in a Caco-2 assay. The latter observation was accounted for by anticipating that participation in intramolecular hydrogen bonding could mask the tertiary hydroxyl group. The stereochemistry of the tertiary alcohol was critical for inhibition, with the (*S*)-isomer being considerably more potent than the corresponding (*R*)-isomer. The transition-state mimicking unit in these inhibitors was synthesized by ring opening of a 2,2-disubstituted epoxide comprising an amido-indanol moiety, present in the approved PI indinavir as shown in Fig. 1.[11,12] Unfortunately, results from the first two series of inhibitors indicated high intrinsic clearance (Cl_{int}) in liver microsomes. This could be attributed to degradation of the P2 indanol group, known to be metabolized by CYP enzymes *via* benzylic oxidation in related systems.[13,14] We were therefore encouraged to develop an alternative synthetic route that would allow the incorporation of other P2 groups in a straightforward manner. Herein we present a new protocol and enzyme inhibition data from a series of P2-varied compounds.

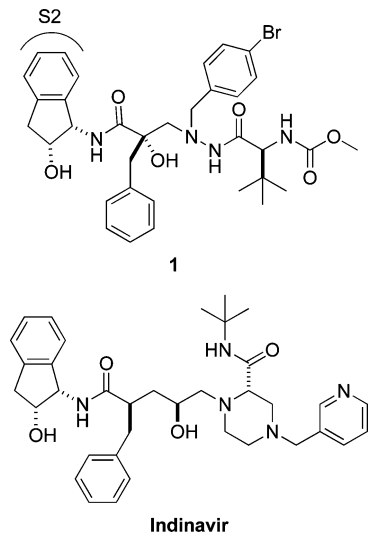
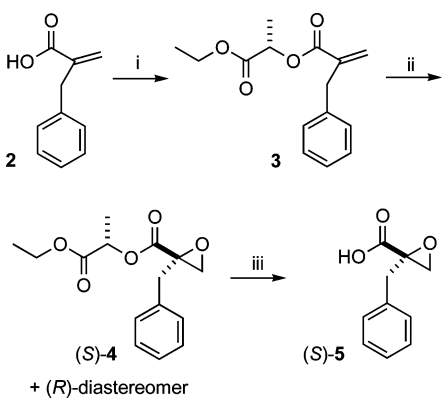


Fig. 1 Structures of HIV-1 protease inhibitor **1**, containing a tertiary alcohol-derived transition-state mimic, and indinavir.

We were interested in using (2*S*)-2-benzyloxirane-2-carboxylic acid ((*S*)-**5**) as a key intermediate in the synthesis of the new HIV-1 protease inhibitors (Scheme 1). Amide coupling of (*S*)-**5** to different amines followed by epoxide ring opening would afford inhibitors encompassing various P2 groups. Thus, a synthetic protocol starting from 2-benzylacrylic acid (**2**), prepared according to a literature procedure,[15] was developed (Scheme 1). Compound **2** was activated by $SOCl_2$ and then coupled with ethyl-(*S*)-lactate producing ester **3** in 75% isolated yield. Epoxidation by 3-chloroperoxybenzoic acid (mCPBA) followed by flash chromatography gave the pure diastereomers (*S*)-**4** and (*R*)-**4**, in



Scheme 1 *Reagents and conditions:* (i) $SOCl_2$, room temp and then ethyl-(*S*)-lactate, DMAP, CH_2Cl_2, room temp, 75%; (ii) mCPBA, CH_2Cl_2, reflux, 77%; (iii) NaOH, THF, room temp, quantitative yield.

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addition to a mixed fraction (in a 4 : 5 : 1 ratio) in a total yield of 77%. The lactate unit was cleaved from the epoxide part using NaOH in water–THF, providing the free acid in quantitative yield. The absolute configuration of (*S*)-**5** was confirmed by coupling with (1*S*,2*R*)-2-aminoindanol followed by comparison of NMR data and optical rotation of the isolated compound with the reported values.[9]

Initially, (*S*)-**5** was coupled with different amines using EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride), NMM (*N*-methylmorpholine) and HOBT (1-hydroxybenzotriazole), resulting in the corresponding amides at low to moderate yields (**6a**, **b**, **f–h**, Scheme 2). For the more sluggish aromatic amines this system did not promote any coupling and instead PyBOP ((benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate) and (*i*-Pr)$_2$NH were used, which resulted in compounds **6c** and **6d** in 46% and 39% isolated yields, respectively (Scheme 2). With L-valine-methylamide, HATU (*O*-(7-azabenzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) together with (*i*-Pr)$_2$EtN provided the most efficient coupling reaction in this series and amide **6e** was generated in 60% yield (Scheme 2). The amino acid α-carbon partly racemized during the amide couplings and mixtures of amides **6e–h** with minor amounts of the corresponding (*R*)-isomers (as determined by NMR) were isolated. These impurities were removed by reverse-phase LC-MS of the final products. Coupling between **2** and L-*tert*-leucine-methylamide, followed by epoxidation yielded a 50 : 50 mixture of (*S*)-**8** and (*R*)-**8**, which was separated by flash chromatography (Scheme 2). The absolute configuration at the quaternary carbon atom in (*S*)- and (*R*)-**8** was determined using X-ray crystallographic data from the corresponding (*S*)-inhibitor **18**, obtained by ring opening of (*S*)-**8** (Fig. 2, Table 1).[16] Interestingly, in this X-ray structure, a hydrogen bond was revealed between the tertiary alcohol and the prime side hydrazide carbonyl group (distance 1.8 Å). This observation further supports our hypothesis that intramolecular hydrogen bonding to the tertiary alcohol in the transition-state mimic is present in these molecules, which, in turn, could contribute to efficient membrane permeation.

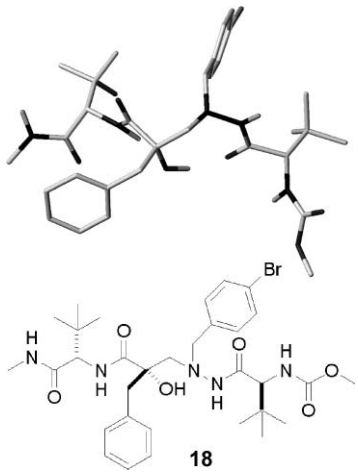
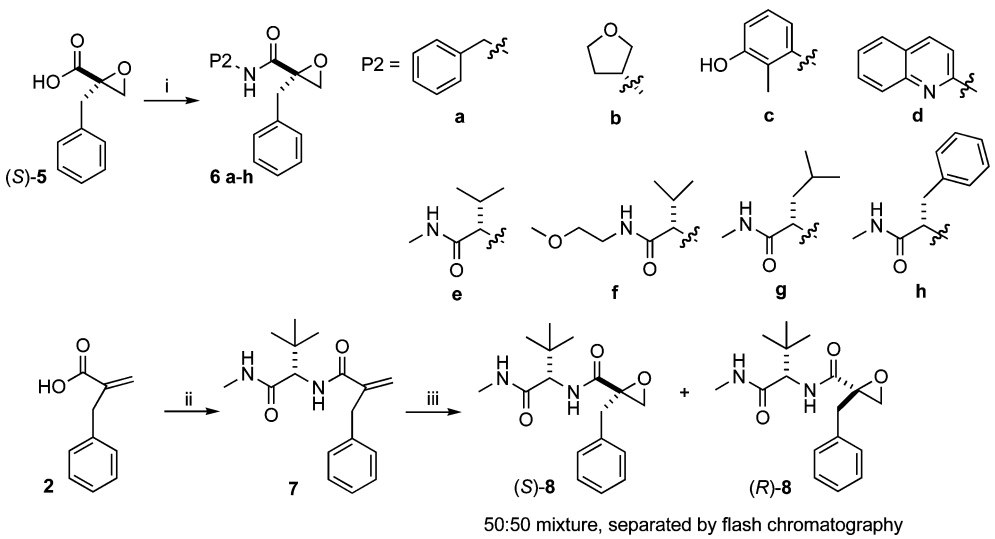


Fig. 2 X-Ray crystal structure of compound **18**.

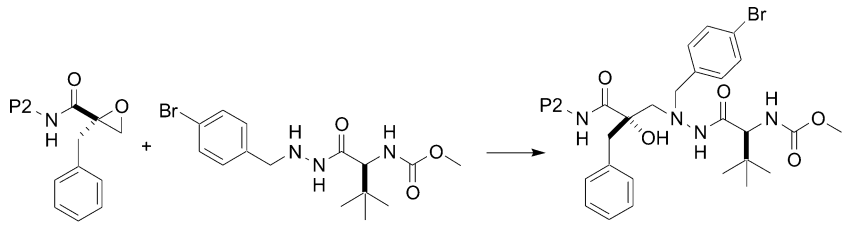
Hydrazide **9**, prepared as previously reported[9] was used to open disubstituted epoxides **6a–h** and **8** (Table 1). Surprisingly, attempts to use Ti(O*i*-Pr)$_4$ as a catalyst, successfully applied in the earlier studies using indanol-amide epoxides,[9,10] rendered the extensive formation of by-products. An uncatalyzed protocol provided clean product patterns, but required longer reaction times (4–8 days). Purification by reverse-phase LC-MS resulted in moderate to good yields of most target compounds. However, the products derived from epoxides **6c** and **d** comprising aromatic amides were recovered in very poor yields (Table 1).

Enzyme inhibition data and anti-HIV activity in cell culture for structures **10–19** are summarized as K_i and EC_{50} values in Table 1. The amido-indanol derivative, **1**, is included as a reference compound. Evaluation of inhibitors **10–13**, with cyclic P2 groups, resulted in poor to moderate inhibitory potencies,

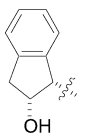
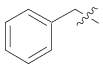
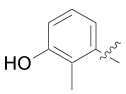
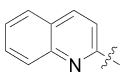
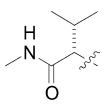
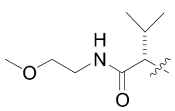
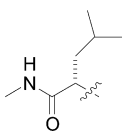
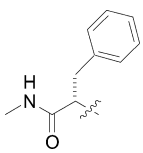
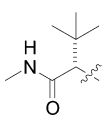
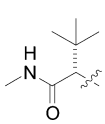


Scheme 2 *Reagents and conditions:* (i) **6a**, **b**, **f–h**: P2–NH_2, EDC, HOBT, NMM, EtOAc, room temp, 17–45%; **6c**, **d**: P2–NH_2, PyBOP, (*i*-Pr)$_2$NH, CH_2Cl_2, room temp, 46%, 39%; **6e**: P2–NH_2, HATU, (*i*-Pr)$_2$EtN, CH_2Cl_2, room temp, 60%; (ii) L-*tert*-leucine-methylamide, EDC, HOBT, NMM, EtOAc, room temp, 54%; (iii) mCPBA, AIBN (2,2'-azobis(2-methylpropionitrile), $ClCH_2CH_2Cl$, reflux, 76%.

Table 1 Synthesis and enzyme inhibition data for compounds **10–19**[a]



6a-h, 8 **9** **10-19**

Compound	P2 group	From epoxide	Yield (%)	K_i (nM)	EC_{50} (μM)
1		—	—	2.4	1.1
10		**6a**	54	88	>10
11		**6b**	52	110	>10
12		**6c**	7	480	>10
13		**6d**	11	1140	>10
14[b]		**6e**	46	7.4	7.3
15[c]		**6f**	73[d]	5.1	3.1
16		**6g**	35	81	>10
17		**6h**	39	860	>10
18		(*S*)-**8**	49	180	>10
19		(*R*)-**8**	69	>5000	>10

[a] Conditions: *i*-PrOH, 80 °C, 4–8 days. [b] $Cl_{int} = 160$ μL min^{-1} mg^{-1}. [c] $Cl_{int} = 230$ μL min^{-1} mg^{-1}. [d] Based on the amount of converted epoxide.

$K_i = 88$–1140 nM (Table 1). Compounds **14–18**, comprising amino acid-derived P2 units, displayed large variability of the K_i values depending on the size of the amino acid side chain (Table 1). The *iso*-propyl group in compounds **14** and **15** seemed to be well tolerated in the enzyme S2 pocket affording highly potent HIV-1 protease inhibitors with $K_i = 7.4$ and 5.1 nM, respectively. On the other hand, the *iso*-butyl, *tert*-butyl and benzyl side chains afforded several times less potent inhibitors as deduced from the corresponding K_i values (**16–18**, Table 1). As expected, the (*R*)-analogue **19** was not active in the enzyme assay at concentrations below 5000 nM. Surprisingly, most of the inhibitors did not exhibit any cellular antiviral activity (Table 1). Only the best enzyme inhibitors, **14** and **15**, were active in this assay with $EC_{50} = 7.3$ and 3.1 μM, respectively. Compound **14** and **15** were further evaluated for stability in the presence of liver microsomes (Table 1). Slightly lower intrinsic clearance was observed for these two compounds ($Cl_{int} = 160$ and 230 μL min^{-1} mg^{-1}, respectively), lacking the metabolically unstable indanol-amide P2 group compared to inhibitor **1** ($Cl_{int} = 266$ μL min^{-1} mg^{-1}).

Several of the approved HIV-1 protease inhibitors comprise relatively small, cyclic P2 structural elements. This is the case for example with amprenavir, containing tetrahydrofuran as the P2 group and for nelfinavir with a phenol-related structure in this position.[3] On the other hand, the most recently launched inhibitor atazanavir, carries N-derivatized amino acid residues in the P2/P3 and P2′/P3′ positions.[17] We were encouraged to evaluate structural units representing both these types of substituents as potential P2 groups in our new inhibitors. The HIV-1 protease inhibition data summarized in Table 1 suggest that the size and polarity of the P2 substituent are crucial to allow proper accommodation in the S2 sub-site. Small P2 groups, unable to reach the enzyme S3 pocket, furnish poor to moderate inhibitory potencies (**10–13**). Furthermore, the distance between the transition-state mimicking tertiary hydroxyl group and P2 aromatic ring structures in **10**, **12** and **13** proved to be of importance. A methylene spacer between the amide bond and the P2 aryl group as in compound **10** afforded a 5 to 13 times more potent inhibitor than **12** and **13** (Table 1). The amino acid-derived P2 substituents in compounds **14–18** have the potential of reaching both the enzyme S2 and S3 pockets, which could be beneficial for efficient binding. However, the bulkiness of the P2 side chain strongly affected inhibition and only the *iso*-propyl group present in compounds **14** and **15** provided highly potent inhibitors (Table 1). The fact that compound **18** was devoid of activity in the cellular assay was somewhat surprising and is difficult to rationalize since a similar *tert*-leucine-derived P2/P3 group present in the approved inhibitor atazanavir has been reported to afford both excellent potency in cell culture and high oral bioavailability.[17]

In summary, an enantiomerically pure epoxy carboxylic acid was identified as the key building block in a novel synthetic strategy delivering HIV-1 protease inhibitors with a tertiary alcohol in the transition-state mimicking scaffold and comprising various P2 groups. The inhibitors were prepared applying four or five synthetic steps and no protecting groups were required. Compound **15** exhibited the lowest K_i value (5.1 nM) in the series and also demonstrated the highest activity in cell culture ($EC_{50} = 3.1$ μM).

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Notes and references

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16 **Crystal data**. $C_{32}H_{46}BrN_5O_6xC_2H_5OH$, $M = 722.72$, monoclinic, $a = 10.6340(3)$ Å, $b = 12.1440(4)$ Å, $c = 15.9560(8)$ Å, $\beta = 108.102(1)°$, $U = 1958.6(1)$ Å^3, $T = 293(2)$ K, space group $P2_1$, $Z = 2$, $\mu = 1.098$ mm^{-1}, 8473 reflections measured, 8463 unique ($R_{int} = 0.0252$). Final $R_1 = 0.0830$ (for 4316 reflections with $I > \sigma(I)$), wR_2 (on F^2) = 0.2307 (for all data). CCDC reference number 607406. For crystallographic data in CIF format see DOI: 10.1039/b606859f.
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