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Synthesis and biological evaluation of novel small non-peptidic HIV-1 PIs: The benzothiophene ring as an effective moiety

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ABSTRACT

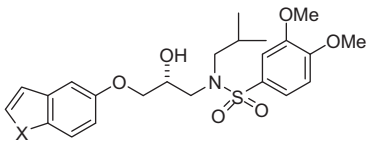
Synthesis and biological evaluation of a new series of potential HIV-1 protease inhibitors incorporating different heterocycles are described. The variation of heteroatom in such molecules has displayed totally different biological activities and a benzothiophene containing inhibitor has shown high potency against wild type HIV-1 protease with IC_{50} = 60 nM, thanks to the lower desolvation penalty to be payed by such hydrophobic moiety.

The devastating effect of the AIDS epidemic is a reality we are currently still dealing with.[1] However, since the highly active antiretroviral therapy (HAART), protease inhibitors (PIs) in combination with reverse transcriptase inhibitors (RTIs), has been employed to combat the illness, HIV infection has definitely become more manageable.[2] Hence HIV protease has been a very attractive target to develop new HIV drugs[3] but despite the already marketed PIs have an evident crucial role into HAART regimen, some complex issues associated with these drugs remain unsolved.[4] These include poor bioavailability, side effects, treatment cost and the most worrying problem, drug resistance. This emergency has led scientists to seek novel structures able to overcome such problems.[5]

Working on this direction, we recently demonstrated the beneficial effect of a heteroaromatic group in a series of new thienyl ring containing analogues of two approved PIs, nelfinavir and saquinavir.[6] Indeed such molecules were able to maintain or even increase the activity of the original drugs against either wild type or mutant HIV protease. Moreover, we also reported the facile synthesis and biological evaluation of new non-peptidic heteroaromatic molecules as PIs, which displayed modest activities.[7] Among them the best one (**1**) showed an IC_{50} of 1 μM. Despite the moderate potency, this small molecule represented a reference structure, considering the ease of its preparation. Therein we determined that 5-hydroxyindolic ring and 3,4-dimethoxy-benzenesulfonamide were suitable moieties to be linked to a stereochemically defined isopropanolic core, to obtain biological activity.

On the basis of this result, we have therefore been really intrigued by understanding the actual importance of the indole *NH* function regarding the efficiency of **1**. Thus, our initial intention was to protect the nitrogen with a methyl or a benzyl group and then to switch the heteroatom to oxygen or sulfur in order to check any variation of antiviral activity with the resulting structures **2**–**5** (Fig. 1).

The synthesis of molecules **2**–**5** is depicted in Scheme 1. Commercially available (*S*)-glycidol (98% ee) was reacted with 3-nitrobenzenesulfonyl chloride (NsCl) and triethylamine (Et_3N) in dry dichloromethane (DCM) at −10 °C to afford **6** in 80% yield. Glycidyl nosylate **6**, which represents a versatile building block in total synthesis,[8] was subjected to a regioselective displacement of the nosylate with the appropriate 5-hydroxyheteroarene and K_2CO_3 in dry dimethylformamide (DMF) at room temperature (rt) to provide the corresponding epoxides **7**, **10** and **11** in 70%, 78% and 82%



1 X= NH **2** X= NMe
3 X= NBn
4 X= O
5 X= S

Figure 1. Inhibitors **1**–**5**.

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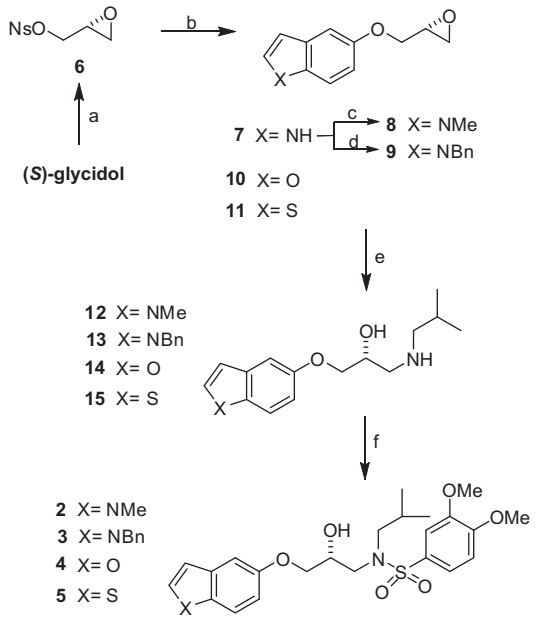
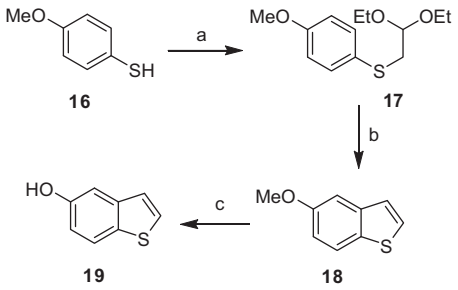


Scheme 1. Reagents and conditions: (a) NsCl, Et_3N, DCM, −10 °C, 5 h, 80%; (b) Het-OH, K_2CO_3, DMF, rt, 14 h, 70–82%; (c) MeI, NaH, DMF, rt, 1 h, 90%; (d) BnCl, NaH, DMF, rt, 1 h, 86%; (e) *i*-$BuNH_2$, *i*-PrOH, rt, 24 h, quantitative; (f) 3,4-diMeO–C_6H_3–SO_2Cl, Et_3N, DCM, rt, 24 h, 89–92%.

yields, respectively.[9] Indole derivative **7** was alkylated with methyl iodide (MeI) and benzyl chloride (BnCl) in the presence of NaH in dry DMF to furnish **8** and **9** in 90% and 86% yields. Oxiranyl ring opening reaction on **8–11** with *i*-$BuNH_2$ in *i*-PrOH at rt provided aminoalcohols **12–15** in quantitative yields. These compounds were subsequently reacted with 3,4-dimethoxybenzenesulfonyl chloride and Et_3N in dry DCM to afford target compounds **2–5** in 89%, 90%, 92% and 90% yields, respectively.

5-Hydroxybenzothiophene was prepared according to our reported procedure for the synthesis of 5-hydroxybenzofuran[10] (Scheme 2). Thus, 4-methoxythiophenol **16** was reacted with bromoacetaldehyde diethyl acetal and K_2CO_3 in dry DMF at reflux to furnish **17** in 86% yield which was directly used in the next reaction without further purification. Compound **17** was converted to 5-methoxybenzothiophene[11] **18** by treatment with polyphosphoric acid (PPA) in toluene at reflux in 24% yield. Demethylation of **18** with $BBr_3{\cdot}SMe_2$ in dry chlorobenzene at reflux provided 5-hydroxybenzothiophene (**19**) in 81% yield.

With these new potential inhibitors in hand, we could finally evaluate their potency against wild type HIV-1 protease: these results are shown in Table 1. Alkylation of the indole *NH* function with a methyl (**2**) or benzyl (**3**) group led to a complete loss of



Scheme 2. Reagents and conditions: (a) $BrCH_2CH(OEt)_2$, K_2CO_3, DMF. reflux, 3 h, 86%; (b) PPA, toluene, reflux, 2 h, 24%; (c) $BBr_3{\cdot}SMe_2$, chlorobenzene, reflux, 8 h, 81%.

Table 1
Anti-HIV activity against wild type protease

Entry	Inhibitor	IC_{50}[a] (μM)
1	**1**	1
2	**2**	>1000
3	**3**	>1000
4	**4**	130
5	**5**	0.06
6	Nelfinavir	0.009[b]

[a] IC_{50} values were obtained by measuring the initial rates of hydrolysis of the fluorogenic substrate Abz-Thr-Ile-Nle-Phe(NO_2)-Gln-Arg. Results are the mean of at least three independent experiments.
[b] The IC_{50} for nelfinavir has been measured as a reference in the same assay.

activity. The benzofuran containing compound **4** was significantly less potent than **1**, but in contrast compound **5**, bearing a benzothiophene, exhibited a 16-fold improvement, reaching a 60 nM IC_{50}.[12]

Alkylation at indole nitrogen leads to inactive compounds, most likely due to steric reasons, and the replacement of nitrogen with oxygen is not effective. However, the more hydrophobic benzothiophene containing compound is significantly more active than the parent indole molecule, and the enhanced activity is consistent with a reduced desolvation penalty. We have calculated the free energy of solvation of compounds **1** and **5** with the IEF polarizable continuum model[13] with the b3lyp density functional at the 6-3111++g(3d,3p) level of theory (Table 2), and we have found that solvation of the indole derivative is more favourable by about 5 kcal/mol. This difference fully explains the nanomolar activity of **5** in comparison with the micromolar activity of **1**, as the more polar compound is going to lose solvent stabilization upon entering the hydrophobic catalytic site, while **5** is already lacking of such stabilization.

We have carried out several attempts to obtain a crystal structure of the HIV protease–**5** complex, but the crystallization trials were not successful.

Table 2
Free energy of solvation of **1** and **5**

Inhibitor	ΔG_{sol}[a] (Kcal/mol)
1	−17.69
5	−12.84

[a] b3lyp/6-3111++g(3d,3p)/IEFPCM free energy of solvation: $\Delta G_{sol} = \Delta G_{sol(polarized\ solute\ -\ solvent)} + \Delta G_{(cavitation)} + \Delta G_{(dispersion)} + \Delta G_{(repulsion)}$ solvent = water.

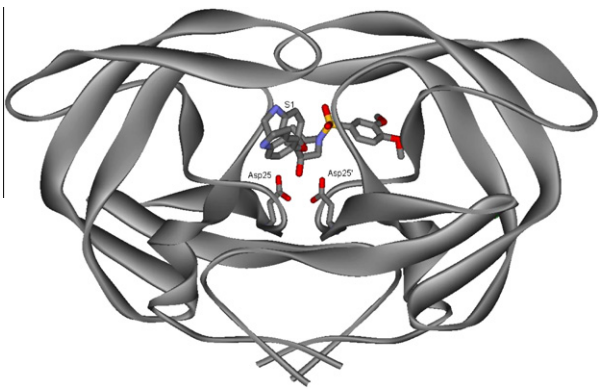


Figure 2. Overlay of the two most favourable docking poses of inhibitor **1** inside wt HIV protease. The indole system is placed in S1 subsite in both cases, with different orientation.

Docking of the inhibitors, carried out with Autodock Vina,[14] suggests a S1 placement for the heterocyclic moiety of the molecule (Fig. 2), and the system is in contact with Ile50, Pro81 and Val82, in an overall hydrophobic environment.

Pleased to have found a really promising inhibitory activity for compound **5** we also evaluated its in vitro cytotoxicity which resulted minimal, indeed the concentration that reduced the metabolic activity of target cells by 50% (CC_{50}) was greater than 90 μM.

In summary we have reported synthesis and biological evaluation of a series of novel compounds bearing a heteroarene moiety. We have discussed about the relation between heteroatom nature and inhibitory effectiveness of corresponding molecules. The benzothiophene containing compound **5** has displayed a potent antiviral activity which is worth to be underlined especially in view of its small structure with resulting short and cheap synthesis. We anticipate that further elaborations of this molecule are currently ongoing in our laboratory.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.02.046.

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