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Synthesis, biological activity and modelling studies of two novel anti HIV PR inhibitors with a thiophene containing hydroxyethylamino core

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Abstract—An efficient method has been developed for the synthesis of a versatile intermediate bearing azido, hydroxyl and ester functions, a useful precursor for peptidomimetic compounds. The two main features for this synthesis were the use of the Sharpless asymmetric dihydroxylation on thiophene acrylate and the subsequent regioselective ring opening by sodium azide of the cyclic sulfite. Highly chemoselective reduction of the azido alcohol led to a key compound which was utilized for the synthesis of two analogues of commercial anti HIV PR such as nelfinavir and saquinavir. The biological activity and molecular modelling study on these two new potential drugs have been evaluated.
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1. Introduction

More recent therapies for AIDS[1,2] are essentially directed against the most important enzymes which regulate HIV: reverse transcriptase (RT) and aspartyl protease (HIV PR).[3] In particular, significant results have been achieved in the development of drugs as HIV PR inhibitors, based upon early recognition of the central role of this enzyme in viral maturation.

HIV PR is an aspartyl protease acting as a C_2 symmetric homodimer with a single active site in the free form and with 99 residues for each monomer. The C_2 axis correlates the catalytic aspartate (Asp-25 and Asp-25′) in the active site of the enzyme. Using the standard nomenclature, the S_1 and $S_{1'}$ subsites are structurally equivalent.

Although peptide-derived compounds could be potent HIV PR inhibitors, they are typically not suitable drug candidates.[4] Their use is limited by some undesirable factors such as poor solubility, low metabolic stability towards degradative enzymes and poor bioavailability after oral ingestion.[5]

All the commercially available anti HIV PR drugs possess a peptidomimetic structure based on the tetrahedral transition state mimetic concept, in which a non-hydrolysable hydroxyethylene or dihydroxyethylene or hydroxyethylamine moiety is used as central core of the molecule (Fig. 1).[1,2]

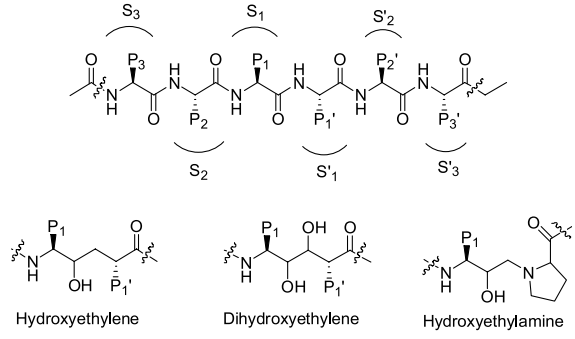

Figure 1.

In fact, the central core in this class of anti HIV PR inhibitors is an isostere replacement at the scissile peptidic bond involved in the proteolytic reaction of the precursor polypeptide and therefore, it has been found to be essential in all the drugs with anti HIV PR activity. The development of such a new class of inhibitors has been also well

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supported by the improvement in the field of peptidomimetic synthesis.[6]

Currently six drugs are commercially available as HIV PR inhibitors: these compounds are indeed furnishing good results in AIDS therapy (together in combination with other drugs)[3b] but viral resistance is often increasing due to mutations in the protease.[1,7]

The development of drug resistance and the consequent lack of a final decisive therapy for the HIV infection, makes the elaboration and the synthesis of innovative anti HIV PR drugs urgent.

To this aim we have been exploring the possibility of introducing a thienyl ring in the 'core' of some anti HIV PR drugs. It is known that the thienyl ring mimics the phenyl group of phenylalanine in peptidomimetics[6] in many drugs.[8–10] It was also observed that the biological half-life of antidepressant drugs such as viloxazine became longer when a benzene ring is replaced by thiophene. Furthermore, a recent study on C_2-symmetric diol-based HIV PR inhibitors,[11] has clearly shown that the introduction of a thienyl ring instead of a phenyl group, greatly enhances antiviral activity of the compound.

We have focused our attention on the structures of two of the commercially available drugs namely nelfinavir (**1**)[12] and saquinavir (**2**)[13] (Fig. 2) and in this paper, we report a short and highly stereocontrolled synthesis of modified nelfinavir and saquinavir compounds starting from a single thiophene containing chiral precursor, as well as the preliminary results in the evaluation of biological activities backed by a molecular modelling study.

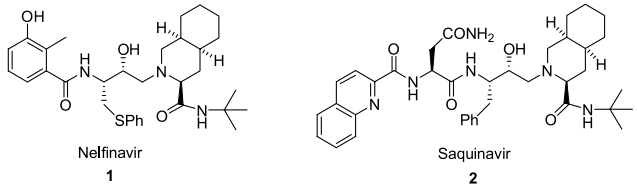


Figure 2.

2. Results and discussion

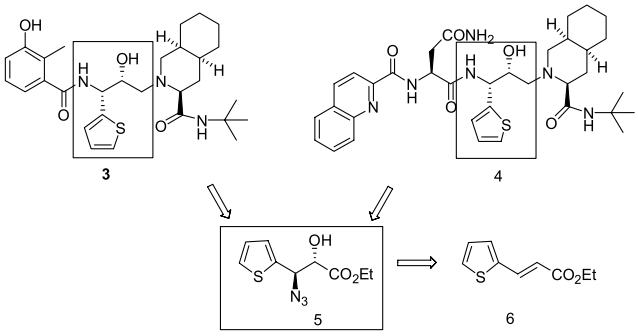
The chemical structures of nelfinavir (**1**) and saquinavir (**2**) (Fig. 2) show the presence of a perhydroisoquinoline (PHIQ) residue as $P_{2'}$–$P_{n'}$ unit which seems of particular importance for a good interaction with the corresponding subsites of the enzyme.[4]

On the other hand, the P_2–P_n termini of the two structures are very different: in nelfinavir a 2-methyl-3-hydroxybenzoic acid residue is present at this position, while in saquinavir a dipeptide unit (asparagine–quinaldic acid) extends the structure toward the S_3 subsite of the enzyme.[4]

Also the crucial core is slightly different for the two drugs: in nelfinavir there is a phenyl moiety, while in saquinavir a phenylalanine residue. However, they posses the same (*R*) absolute configuration at the carbon bearing the central hydroxyl[3,4] and the same relative *anti* configuration at the two vicinal stereocentres.

We therefore, found interesting a modification in the core of both the drug candidates, with the introduction of a simple thiophene moiety in this class of HIV PR inhibitors. With the intention of testing the influence of a thiophene ring on the activity of potential drugs we have therefore, defined the first goal of our synthetic approach as the building of the novel amino alcohol core in nelfinavir and saquinavir structures.

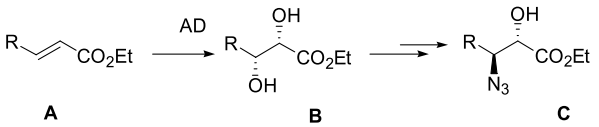
To achieve this objective the two designed targets (compounds **3** and **4** in Scheme 1) could be derived, in a retrosynthetic analysis, from the same chiral thienyl derivative **5** which possesses the correct stereochemistry and the appropriate functionalizations for the subsequent elaboration to the final products.



Scheme 1. Retrosynthetic analysis of compounds **3** and **4**.

This key chiral synthon **5** could be derived from the easily available 3-thiophen-2-yl-acrylic acid ethyl ester **6**.[14] With the crucial goal of introducing the correct chirality in the core structure we hypothesized a new strategy never before utilised for the synthesis of this class of HIV PR inhibitors.[1,12,13]

To this end, a suitable acrylic derivative, such as **A**, can be easily transformed into the chiral diol **B** via Sharpless AD.[15] The introduction of the nitrogen function through inversion of configuration and high regioselectivity represents, in the chosen strategy, the key step in order to obtain the highly functionalized chiral compound **C**, ready for the transformation into different potential analogues of known HIV PR inhibitors (Scheme 2).



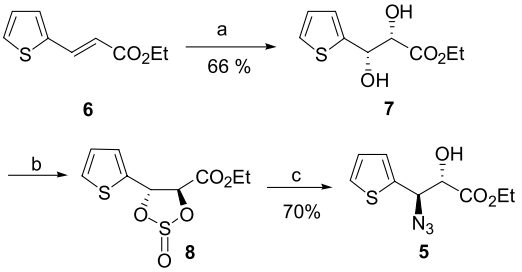
Scheme 2.

This general synthetic strategy was first applied to the synthesis of thiophene containing nelfinavir and saquinavir analogues.[16]

3. Synthesis of the thiophene azido alcohol 5

The known (*E*) olefin **6**[14] (Scheme 3), prepared from

commercially available 2-thiophencarboxaldehyde, was subjected to the Sharpless dihydroxylation following a recently modified procedure.[17] Further, improvements of overall yields of the purified diol **7** have been achieved by adding 20% more AD mix-β and ligand (66% yield and ee > 98).



Scheme 3. (a) AD mix-β, $[DHQD]_2PHAL$, $MeSO_2NH_2$, H_2O/*t*-BuOH, 0–15 °C, 15 h; (b) $SOCl_2$, Pyr, CH_2Cl_2, 0 °C, 15 h; (c) NaN_3, DMF/CH_3CN, 15 °C, 15 h.

Having built the diol moiety the subsequent step was the introduction of the nitrogen with inversion of configuration, that could be, in principle, better achieved from the corresponding cyclic sulfate,[18] obtained via oxidation of the corresponding cyclic sulfite. However, the possibility to oxidize the thienyl ring during the conversion of the sulfite into the sulfate prompted us to directly perform the ring opening reaction with sodium azide, using sulfite **8** as crude material. Pleasingly, the reaction on compound **8** furnished the isolated and purified azido alcohol **5** in 70% yield starting from diol **7** (two steps) (Scheme 3). The reaction was totally regio- and stereoselective as demonstrated in comparison with known compounds.[19]

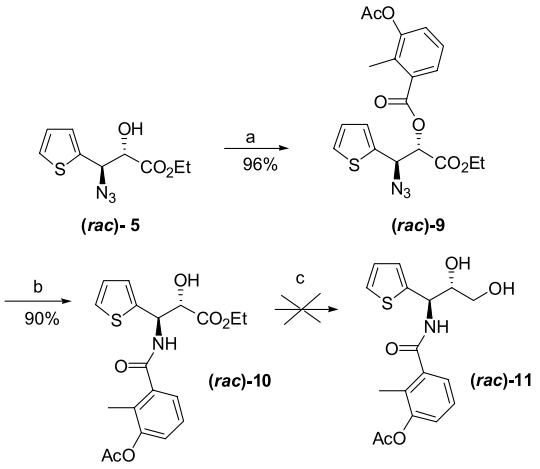
Azido alcohol **5** is a very interesting chiral synthon due to the presence of three different functions on vicinal carbons: an amino precursor as an azido group, a secondary hydroxyl function and a carboxylic group.

4. Synthesis of nelfinavir analogue 3

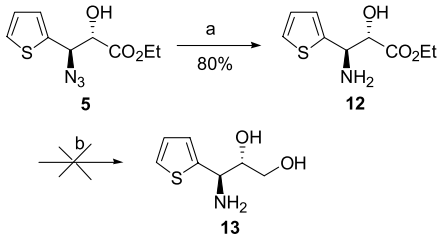
With this key compound **5** in hand we next turned our attention to the synthesis of the nelfinavir analogue **3**, with the introduction on the hydroxyl group of a benzoic acid derivative to obtain compound (*rac*)-**9** (Scheme 4). The proposed sequence was firstly achieved on racemic compound **5**. As expected, the esterification step[20] proceeded smoothly in high yield (96% yield): the subsequent reduction of azido group to the corresponding amino alcohol with the rearrangement shift of carboxylic substituent on the amino group[21] was then achieved in good yield (90%).

However, quite disappointingly, in the last step of the sequence to the target diol (*rac*)-**11** all attempts to chemoselectively reduce the ester function (by $BH_3 \cdot SMe_2$/$NaBH_4$ and similar reagents)[22] gave nearly no reaction. Therefore, we had to change our synthetic strategy in order to reduce the ester function.

In a second approach (Scheme 5) the selective hydrogenation of the azido alcohol **5** provided the amino alcohol **12** in



Scheme 4. (a) 3-Acetoxy-2-methyl benzoic acid, DCC, DMAP, CH_2Cl_2, rt, 3 h; (b) (i) Pd/C, H_2, MeOH, rt, 7 h; (ii) MeOH, rt, 72 h; (c) $BH_3 \cdot SMe_2$, $NaBH_4$, THF, rt, 2 h.



Scheme 5. (a) EtOAc, Pd/C, rt, 14 h. (b) (i) $NaBH_4$, MeOH, rt, 24 h or (ii) $LiAlH_4$, THF, rt, 21 h or (iii) DIBAL, toluene, rt, 6 h.

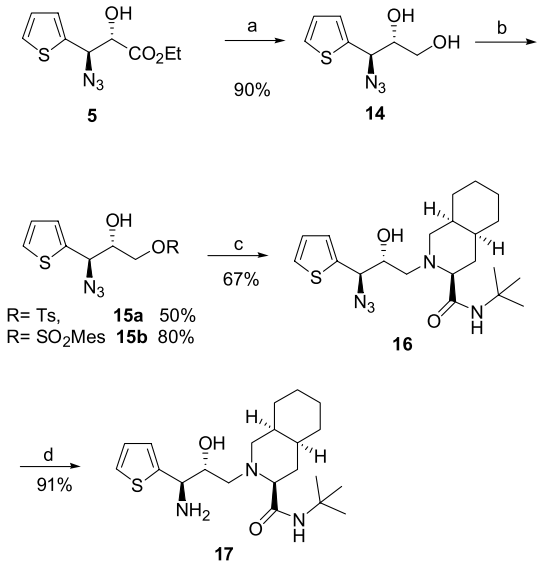
good yield. However, different reaction conditions ($NaBH_4$, $LiAlH_4$, DIBAL) for reduction of the carboxylic ester only afforded degradation products or starting amino alcohol **12**.

Best results were finally obtained by planning a reduction of the carboxylic function on the azido alcohol **5**. The use of $BH_3 \cdot SMe_2$, a known reagent for reduction of α-hydroxy esters,[23] afforded quite nicely the expected azido diol **14** in 90% yield with complete chemoselectivity (Scheme 6). This reaction is noteworthy since it has never been applied to such particularly functionalized compounds.

To complete the functional group differentiation on compound **14** it was necessary to regioselectively activate the primary hydroxyl group for the subsequent introduction of the PHIQ unit. Both activations with tosyl or mesitylenesulfonyl ($MesSO_2$, 2,4,6-trimethylbenzenesulfonyl) chloride were tested, but much better result (80% yield) were obtained with the more hindered mesitylene derivative **15b**.

The first amino side chain was easily introduced by treating the azido alcohols **15**[24] with commercially available PHIQ thus, obtaining the product **16** in good chemical yield (67%). The azido alcohol was quantitatively transformed into amino alcohol **17** by hydrogenation with 5% Pd/C at rt.

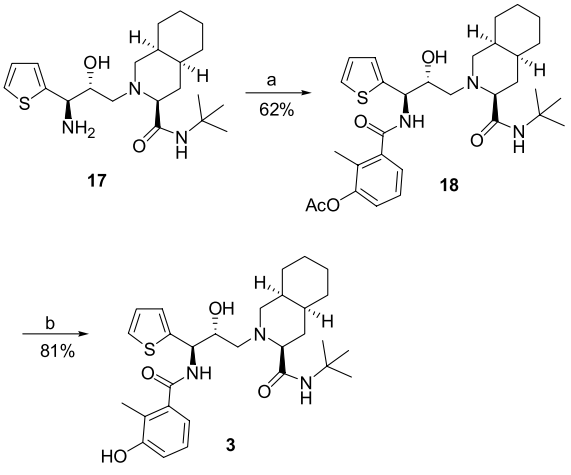
This compound **17** was purified either by column chromatography or by crystallization and represents an important intermediate for the preparation of possible new HIV protease inhibitors containing a thiophene ring. In fact,



Scheme 6. (a) $BH_3\,SMe_2$, $NaBH_4$, THF, MeOH, rt, 3 h; (b) (i) R = Ts, TsCl, Pyr, 50 °C, 4 h; (ii) R = SO_2Mes, $MesSO_2Cl$, Pyr, CH_2Cl_2, rt, 22 h; (c) K_2CO_3, PHIQ, *i*-PrOH, 50 °C, 21 h; (d) H_2, Pd/C, MeOH, rt, 3 h. Mes = 2,4,6-trimethylbenzene.

starting from compound **17**, nelfinavir and saquinavir analogues (see below) were finally prepared.

For the synthesis of thienyl nelfinavir **3**, two additional steps were required (Scheme 7). The peptide coupling of **17** with 3-acetoxy-2-methyl benzoic acid furnished compound **18**. Then final deacetylation of the phenolic group with Na/MeOH afforded the target compound **3** in 50% overall yield (two steps).



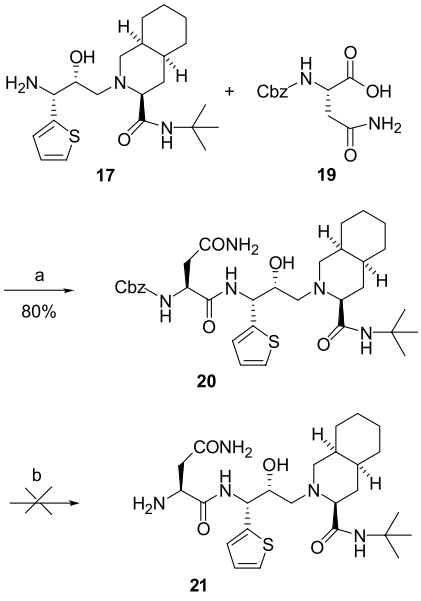
Scheme 7. (a) 3-Acetoxy-2-methyl benzoic acid, DCC, DMAP, CH_2Cl_2, rt, 2 h; (b) Na, MeOH, rt, 1 h.

5. Synthesis of saquinavir analogue 4

Key compound **17** has also been utilized for the synthesis of the thienyl saquinavir **4**, where a dipeptide unit containing asparagine N-bonded to quinaldic acid has to be introduced to the P_2–P_n end.

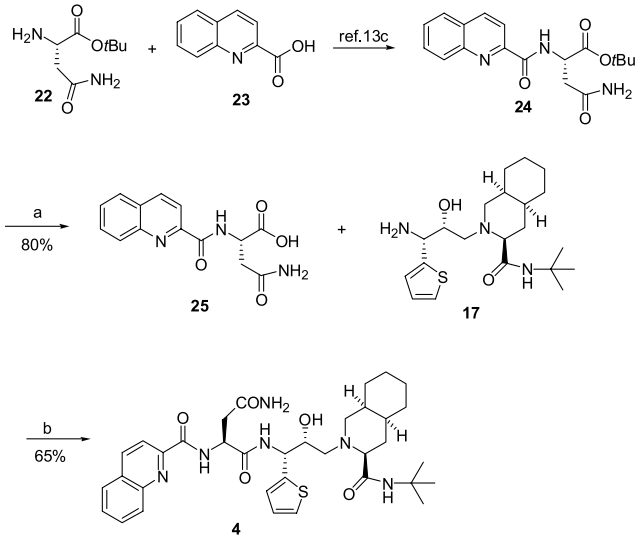
Two possible approaches could be followed for the synthesis of saquinavir analogue **4** according to the protocol used in the preparation of saquinavir **2**.[1,13] The first strategy provides initial coupling between the commercially available N-Cbz asparagine **19** and fragment **17**, N-Cbz through hydrogenation with Pd/C, and final coupling with quinaldic acid.

Using this procedure we obtained, as expected, compound **20** (Scheme 8), but all subsequent attempts to deprotect the amino group in **21** under various conditions failed.



Scheme 8. (a) DCC, DMAP, CH_2Cl_2, rt, 6 h; (b) H_2, Pd/C, EtOH, rt, 48 h.

Therefore, an alternative synthetic sequence was followed starting with the preparation of dipeptide **24**, obtained by coupling of asparagine *t*-butyl ester **22** and quinaldic acid **23**. Then dipeptide **24**[13c] was hydrolyzed to acid **25**,[25] and coupling with fragment **17** (Scheme 9) afforded the desired product **4** in 65% yield.



Scheme 9. (a) CH_2Cl_2, TFA, rt, 6 h; (b) DCC, DMF, DMAP, CH_2Cl_2, rt, 7 h.

6. Biological activity and molecular modelling studies

The activity of compounds **3** and **4** was evaluated on the inhibition of the catalytic activity of a recombinant wild-type HIV PR. IC_{50} values were obtained by measuring the initial velocities of hydrolysis of the fluorogenic substrate Abz-Thr-Ile-Nle-Phe(NO_2)-Gln-Arg, and are reported in Table 1, column 2, together with the IC_{50} values measured in the same experiment for the reference inhibitors nelfinavir **1** and saquinavir **2**.

Table 1

Compound	IC_{50} (μM)	$\Delta E_{compl,rel}$[a] (Kcal/mol)
1	0.0019	2.6
2	0.0004	0.0
3	30.0	64.9
4	5.0	87.6

[a] $\Delta E_{compl,rel}$=(EPL-E°L)–(EPsaq-E°saq), where EPL, E°L are the Amber energies for the optimized geometries of the protein–ligand complexes and for the ground state conformations of the free ligands, respectively. EPsaq and E°saq are the corresponding values for the reference ligand saquinavir.

Both candidates, **3** and **4**, show a clear inhibitory activity, with IC_{50} values in the micromolar range (30 and 5 μM, respectively, Table 1), but they are much less efficient than nelfinavir and saquinavir, which show, under our experimental conditions, IC_{50} values in the low nanomolar range.

A preliminary molecular modelling study was carried out by docking compound **3** onto the structure of the nelfinavir -HIV-PR complex, while the complex of **4** was modelled over the structure of the saquinavir-HIV-PR complex. After a first optimization, molecular dynamics runs were carried out for all the complexes and went to equilibration, indicating a good fit of the inhibitors into the active site. Final post-dynamics optimizations gave the relative complexation energies reported in Table 1, column 3, and the final geometries of the complexes. The energy differences are largely overestimated at this stage, mainly due to the lack of accounting for solvation of the free ligands, but the trend of such relative complexation energies, which are commonly used in the first ligand screening stages,[26] are qualitatively in agreement with the trend of the measured IC_{50}.

Both the geometries of the **3** and **4** complexes, when compared with nelfinavir and saquinavir, respectively, suggest the same explanation for the difference of activity. **3** and nelfinavir are almost perfectly superimposable as to the core hydroxyethylene unit and the residues at P2, P3, P2′ and P3′ (Fig. 3 a), and the same occurs for **4** and saquinavir (Fig 3 b).

However, in both the complexes the thiophene moiety at P1 enters the S1 protease subsite less deeply, due to the lack of the phenylalanine benzylic carbon.

7. Conclusions

In conclusion, a general strategy for the synthesis of thiophene containing HIV PR inhibitor analogues has been developed, with the preparation of key chiral compounds which can be utilized for the synthesis of different potential HIV PR inhibitors. The two target compounds **3** and **4** have been obtained in nine and eight steps starting from acrylate **6** with 10 and 13% in overall yields, respectively.

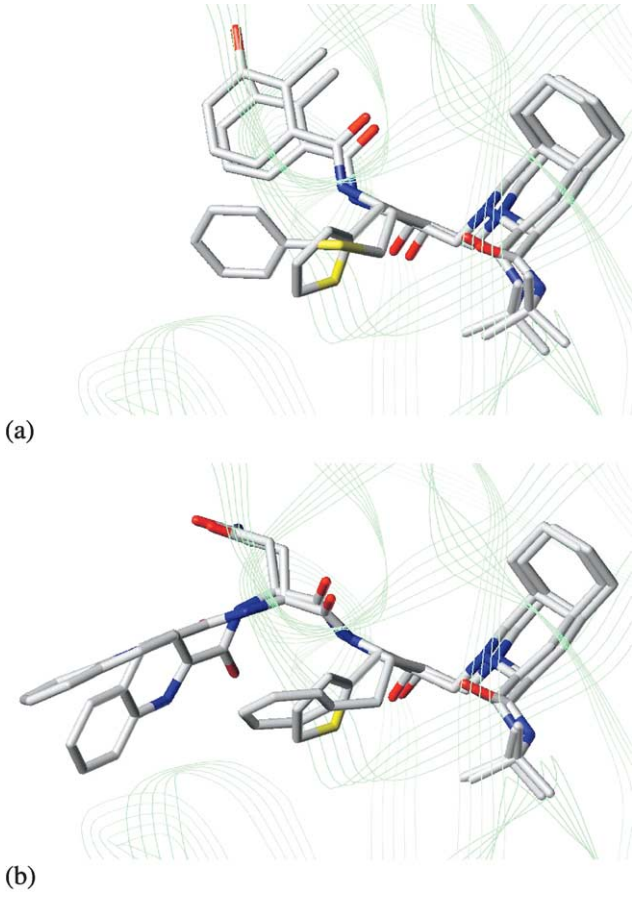


Figure 3. RMS overlay of the optimized complexes of HIV PR with nelfinavir and **3** (a), and saquinavir with and **4** (b).

The biological experimental and the theoretical data furnished information of the low activity of **3** and **4** with respect to the unmodified molecules **1** and **2**. Further studies are in progress for improving the activity of these drugs containing a thiophene unit.

8. Experimental

8.1. General

Unless otherwise specified, materials were purchased from commercial suppliers and used without further purification. THF, toluene, diethyl ether were distilled from sodium/benzophenone ketyl immediately before use. Dichloromethane was distilled from P_2O_5. DMF was freshly distilled and stored over 4 Å sieves. Moisture-sensitive reactions were conducted in oven- or flame-dried glassware under an argon atmosphere. All reactions were magnetically stirred and monitored by thin-layer chromatography using pre-coated silica gel (60 F_{254}) plates. Column chromatography was performed with the indicated solvents using silica gel-60 Å. Mass spectra were obtained by GC/MS with electron

impact ionization. NMR spectra were recorded in $CDCl_3$ solution at rt (^1H at 300 and 500 MHz and ^{13}C at 75 and 125 MHz, respectively). Chemical shifts (δ) were expressed in ppm and coupling constant (J) in Hz. Optical rotations were determined operating at the sodium D line at 25 °C. HPLC analyses were conducted using Chiralcel OJ-H column with UV detection at 235 nm.

8.1.1. (+)-(2*S*,3*S*)-3-(2-Thienyl)-2,3-dihydroxy ethyl propanoate (7). Prepared according to the standard procedure for the AD,[15] using 1.68 g of AD-mix β and adding 9.6 mg of $(DHQD)_2PHAL$ for 1 mmol of olefin **6**. The reaction residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH 98:2) to give the pure diol **7** (142 mg, 66%) as a yellow oil: R_f=0.2 ($CHCl_3$/MeOH 98:2); $[\alpha]_D^{20}$ −2.7 (*c* 4, MeOH); ee=99.87 (Chiralcel OJ-H, Hexane/*i*-PrOH 95:5, 0.5 mL/min, λ=235 nm, t_R=59.76, t_R=65.16); ^1H NMR (500 MHz, $CDCl_3$) δ 7.32 (d, J=4.8 Hz, 1H), 7.11 (d, J=3.4 Hz, 1H), 7 (m, 1H), 5.27 (d, J=1.8 Hz, 1H), 4.44 (d, J=2.4 Hz, 1H), 4.31 (q, J=7 Hz, 2H), 1.32 (t, J=7 Hz, 3H), 2.85 (br s, 1H), 3.3 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 143.8, 126.8, 125.8, 125.4, 74.6, 71.1, 62.5, 14.3. EI-MS *m/z*: M^+, 216, (100), 113. Anal. Calcd for $C_9H_{12}O_4S$: C, 49.99; H, 5.59. Found: C, 50.01; H, 6.02.

8.1.2. 2-Oxo-5-thiophen-2-yl-2λ4-[1,3,2]dioxathiolane-4-carboxylic acid ethyl ester (8). To a cold (0 °C) stirred solution of diol **7** (21.4 mg, 0.1 mmol) in dry CH_2Cl_2 (0.28 mL) under nitrogen atmosphere, pyridine (22.7 mg, 0.27 mmol) and $SOCl_2$ (0.01 mL, 0.13 mmol) were added. After 15 h few millilitres of Hexane/EtOAc 2:1 were added, the mixture was filtered and the residue was washed with EtOAc. After solvent removal the brown crude product was used in the next step without purification for the preparation of compound **5**. ^1H NMR (300 MHz, $CDCl_3$) δ 7.45–6.95 (m, 6H), 6.43 (d, J=7.6 Hz, 1H), 5.85 (d, J=7.6 Hz, 1H), 5.35 (d, J=7.6 Hz, 1H), 4.91 (d, J=7.6 Hz, 1H), 4.40–4.25 (m, 4H), 1.45–1.30 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0, 165.0, 138.0–125.0, 82.5, 80.9, 79.7, 82.6, 82.3, 14.1, 13.9. Anal. Calcd for $C_9H_{10}O_5S_2$: C, 41.21; H, 3.84. Found: C, 41.19; H, 3.86.

8.1.3. (+)-(2*S*,3*R*)-3-Azido-2-hydroxy-3-thiophen-2-yl-propionic acid ethyl ester (5). The crude sulfite **8** (26 mg, 0.1 mmol) in CH_3CN (1.5 mL)/DMF (1.5 mL) was treated with NaN_3 (0.32 g, 0.5 mmol) and stirred at 15 °C for 16 h. Then a solution of H_2SO_4 20% (0.3 mL) was added and the solid residue was extracted with EtOAc. The organic layer was washed with 10% H_2SO_4, water, saturated aqueous solution of $NaHCO_3$ and brine. After drying over Na_2SO_4 and solvent removal the crude product was purified by column chromatography (hexane/EtOAc 8:2) affording the pure azido alcohol **5** as a brown oil (17 mg, 70%): R_f=0.4 (hexane/EtOAc 8:2); $[\alpha]_D^{20}$ +36.0 (*c* 2, MeOH); ^1H NMR (500 MHz, $CDCl_3$) δ 7.35 (d, J=5.5 Hz, 1H), 7.13 (d, J=3.5 Hz, 1H), 7.04 (m, 1H), 5.1 (d, J=3 Hz, 1H), 4.6 (d, J=3 Hz, 1H), 4.207 (q, J=8 Hz, 2H), 3.22 (s, 1H, OH), 1.21 (t, J=8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 135.1, 127.6, 127.0, 127.0, 73.6, 73.4, 62.4, 14.0. EI-MS *m/z*: M^+-N_2, 213, (100), 110. Anal. Calcd for $C_9H_{11}N_3O_3S$: C, 44.80; H, 4.60. Found: C, 44.82; H, 4.61.

8.1.4. 3-Acetoxy-2-methyl-benzoic acid 2-azido-1-ethoxycarbonyl-2-thiophen-2-yl-ethyl ester [(*rac*)-9]. To a mixture of azido alcohol (*rac*)-**5** (200 mg, 0.83 mmol), 3-acetoxy-2-methylbenzoic acid (308 mg, 1.66 mmol) and DMAP (32 mg, 0.29 mmol) in dry CH_2Cl_2 (4 mL) a solution of DCC (320 mg, 1.66 mmol) in dry CH_2Cl_2 (1.2 mL) was slowly added. The reaction, monitored by TLC, was quenched after 3 h by adding EtOH (2.4 mL) and the solid was filtered on Buckner funnel, washed with petroleum ether and the filtrated was treated with 10% HCl. The organic layer was washed with saturated aqueous solution of $NaHCO_3$, brine and after solvent removal the crude product was purified by column chromatography (petroleum ether/EtOAc 8:2) affording ester (*rac*)-**9** (333 mg, 96% yield) as a yellow oil: R_f=0.4 (petroleum ether/EtOAc 8:2); ^1H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, J=8.3 Hz, 1H), 7.34 (m, 1H), 7.31 (m, 1H), 7.24 (d, J=8.3 Hz, 1H), 7.20 (m, 1H), 7.00 (m, 1H), 5.66 (d, J=15 Hz, 1H), 5.42 (d, J=15 Hz, 1H), 4.17 (q, J=10 Hz, 2H), 2.42 (s, 3H), 2.33 (s, 3H), 1.18 (t, J=10 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.3, 166.8, 165.9, 150.2, 135.6, 133.3, 130.6, 128.8, 128.6, 127.6, 127.0, 126.8, 126.7, 74.5, 62.3, 60.7, 20.9, 14.2, 13.6. Anal. Calcd for $C_{19}H_{19}N_3O_6S$: C, 54.67; H, 4.59. Found: C, 54.63; H, 4.61.

8.1.5. 3-(3-Acetoxy-2-methyl-benzoylamino)-2-hydroxy-3-thiophen-2-yl-propionic acid ethyl ester [(*rac*)-10]. To a solution of 10% Pd/C (67 mg) in methanol (5 mL) previously activated in hydrogen atmosphere for 30 min, a solution of substrate (*rac*)-**9** (94 mg, 0.224 mmol) in MeOH (15 mL) was added. The reaction was monitored by TLC (petroleum ether/EtOAc 7:3) until substrate disappearance (7 h). After hydrogen removal, the mixture was filtered and the solution was stirred for 72 h. The solvent was evaporated affording the pure amide (*rac*)-**10** (285 mg, 91%) as a yellow oil that was used without any purification: R_f=0.3 (petroleum ether/EtOAc 7:3); ^1H NMR (500 MHz, $CDCl_3$) δ 9.24 (br s, 1H), 8.19 (m, 1H), 7.24 (m, 4H), 6.86 (m, 1H), 6.20 (d, J=3.5 Hz, 1H), 5.30 (d, J=3.5 Hz, 1H), 4.22 (q, J=7 Hz, 2H), 2.36 (s, 6H), 1.13 (t, J=7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.9, 172.0, 158.4, 136.2, 130.8, 129.0, 127.4, 125.7, 123.3, 122.5, 121.7, 112.9, 80.5, 61.5, 59.4, 57.9, 14.0, 13.6. Anal. Calcd for $C_{19}H_{21}NO_6S$: C, 58.30; H, 5.41. Found: C, 58.32; H, 5.45.

8.1.6. (−)-(2*S*,3*R*)-3-Amino-2-hydroxy-3-thiophen-2-yl-propionic acid ethyl ester (12). To a solution of ethanol (1.5 mL) of 10% Pd/C (15 mg), previously activated in hydrogen atmosphere for 30 min a solution of substrate **5** (55 mg, 0.23 mmol) in EtOH (3.5 mL) was added. The reaction was monitored by TLC (petroleum ether/EtOAc 8:2) until substrate disappearance (21 h). After hydrogen removal, the mixture was filtered and the solvent was evaporated in vacuo. The crude product was purified by column chromatography ($CHCl_3$/MeOH 9:1) affording the amino alcohol **12** (40 mg, 80%) as a yellow solid: R_f=0.69 ($CHCl_3$/MeOH 9:1); $[\alpha]_D^{20}$ −6.8 (*c* 1, MeOH); mp 50–53 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.22 (d, J=5.5 Hz, 1H), 6.95 (m, 2H), 4.60 (d, J=3 Hz, 1H), 4.48 (d, J=3 Hz, 1H), 4.15 (q, J=7 Hz, 2H), 1.2 (t, J=7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 144.1, 126.7, 125.0, 124.9, 74.7, 62.0, 54.7, 14.3. EI-MS *m/z*: M^+-H^+, 214, 112,

(100), 113. Anal. Calcd for $C_9H_{13}N_3O_3S$: C, 50.21; H, 6.09. Found: C, 50.24; H, 6.10.

8.1.7. (+)-(2*R*,3*R*)-3-Azido-3-thiophen-2-yl-propane-1,2-diol (14). To a cold (0 °C) stirred solution of azido alcohol **5** (400 mg, 1.6 mmol) in dry THF (8 mL), a solution of $BH_3 \cdot SMe_2$ (399 mg, 3.2 mmol) in dry THF (0.32 mL) was slowly added under argon atmosphere and then the temperature was allowed to warm to rt. After 45 min, $NaBH_4$ (3.2 mg, 0.08 mmol) was added and the mixture was stirred for 3 h. The reaction was slowly quenched adding MeOH (1 mL) at 0 °C and stirred for 45 min. After solvent removal the crude material was purified by column chromatography ($CHCl_3$/MeOH 95:5) affording the azido diol **14** (297 mg, 90%) as a deep oil: R_f=0.3 ($CHCl_3$/MeOH 95:5); $[\alpha]_D^{20}$ +4.9 (*c* 1.5, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.4 (d, J=5 Hz, 1H), 7.14 (d, J=3.5 Hz, 1H), 7.08–7.06 (m, 1H), 4.91 (d, J=6.5 Hz, 1H), 3.91 (dd, J=6.5, 3.5 Hz, 1H), 3.77–3.69 (m, 2H); 2.40 (s, 1H, OH); 2.06 (s, 1H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4, 127.8, 127.7, 127.1, 74.3, 63.1, 62.87. EI-MS *m*/*z*: $M^+ - H^+$, 199, 110, (100). Anal. Calcd for $C_7H_9N_3O_2S$: C, 42.20; H, 4.55. Found: C, 42.19; H, 4.57.

8.1.8. (+)-(2*R*,3*R*)-Toluene-4-sulfonic acid 3-azido-2-hydroxy-3-thiophen-2-yl-propyl ester (15a). To a solution of azido diol **14** (150 mg, 0.75 mmol) and pyridine (1.5 mL) TsCl (173 mg, 0.91 mmol) in portions was added at 0 °C under argon atmosphere, and the solution was stirred at 50 °C for 4 h. After pyridine elimination in vacuo, the crude mixture was dissolved in EtOAc and washed with cold 2 M HCl, water, saturated aqueous solution of $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, concentrated and the mixture was purified by column chromatography (petroleum ether/EtOAc 8:2) affording product **15a** as a colourless oil (132 mg, 50%): R_f=0.3 (petroleum ether/EtOAc 8:2); $[\alpha]_D^{20}$ +93.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81–7.01 (m, 7H), 4.87 (d, J=6.5 Hz, 1H), 4.11–4.08 (m, 2H), 4.08–4.06 (m, 1H), 2.46 (s, 3H), 2.6 (s, 1H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.6, 137.2, 132.5, 130.3, 130.2, 128.4, 128.2, 127.4, 127.2, 72.2, 70.1, 62.4, 62.2, 22.0. Anal. Calcd for $C_{14}H_{15}N_3O_4S_2$: C, 47.58; H, 4.28. Found: C, 47.60; H, 4.26.

8.1.9. (+)-(2*R*,3*R*)-2,4,6-Trimethyl-benzenesulfonic acid 3-azido-2-hydroxy-3-thiophen-2-yl-propyl ester (15b). To a cold (0 °C) stirred solution of azido diol **14** (280 mg, 1.43 mmol) in CH_2Cl_2 (3.7 mL), pyridine (236 mg, 2.86 mmol) and mesitylenesulfonyl chloride (344 mg, 1.57 mmol) were added. The reaction mixture was stirred at 0 °C for 4 h and then was warmed at 20 °C for 20 h. The solvent was eliminated in vacuo, the crude mixture was dissolved in EtOAc and washed with cold 2 M HCl (5 mL), water, saturated aqueous solution of $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude product was purified by column chromatography (petroleum ether/EtOAc 8:2) affording the pure compound **15b** (430 mg, 80%) as a deep yellow oil: R_f=0.5 (petroleum ether/EtOAc 8:2); $[\alpha]_D^{20}$ +81.3 (*c* 0.5, EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (d, J=4.5 Hz, 1H), 7.10 (d, J=3.5 Hz, 1H), 7.04–7.03 (m, 1H), 6.99 (s, 2H), 4.89 (d, J=6.5 Hz, 1H), 4.06–4.04 (m, 3H), 2.75 (br s, 1H), 2.63 (s, 6H), 2.33 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.0, 137.2, 132.1, 130.3, 130.9, 128.2, 128.1, 127.4, 127.2, 72.2, 69.2, 62.6, 62.3, 23.1, 22.8, 21.2. Anal. Calcd for $C_{16}H_{19}N_3O_4S_2$: C, 50.38; H, 5.02. Found: C, 50.41; H, 5.05.

8.1.10. (−)-(3*S*,4*aS*,8*aS*,2′*R*,3′*R*)-[(3′-Azido-2′-hydroxy-3′-(thiophen-2-yl)-propyl]-decahydro-isoquinoline-3-carboxylic acid *tert*-butylamide (16). The same procedure was followed for **15a** and **15b**. To a solution of **15a** (200 mg, 0.57 mmol) or **15b** (217 mg, 0.57 mmol) in *i*-PrOH (10.8 mL) PHIQ (231 mg, 0.97 mmol) and K_2CO_3 (157 mg, 1.14 mmol) were added. The mixture was stirred at 50 °C for 21 h, then *i*-PrOH was evaporated in vacuo and the residue dissolved in EtOAc (20 mL). The organic layer was washed with saturated solution of NH_4Cl and brine then was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by chromatography (petroleum ether/EtOAc 7:3) to give amine **16** as a pale yellow oil (160 mg, 67%): R_f=0.37 (petroleum ether/EtOAc 7:3); $[\alpha]_D^{20}$ −10.2 (*c* 0.5, EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–6.98 (m, 3H), 6.07–6.04 (br s, 1H), 4.76–4.74 (m, 1H), 3.97–3.93 (m, 1H), 3.42–3.61 (br s, 1H), 2.99–2.95 (m, 1H), 2.72–2.70 (m, 2H), 2.40–2.31 (m, 2H), 1.93–1.27 (m, 12H), 1.35 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4, 135.0, 127.4, 126.7, 126.6, 72.9, 70.0, 64.4, 60.1, 58.1, 50.9, 35.8, 33.2, 30.8, 30.5, 29.7, 26.0, 25.9, 20.8. EI-MS *m*/*z*: $M^+ - Th–CHN_3^+$, 280, 182, (100). Anal. Calcd for $C_{21}H_{33}N_5O_2S$: C, 60.11; H, 7.93. Found: C, 60.13; H, 7.95.

8.1.11. (−)-(3*S*,4*aS*,8*aS*,2′*R*,3′*R*)-[(3′-Amino-2′-hydroxy-3′-(thiophen-2-yl)-propyl]-decahydro-isoquinoline-3-carboxylic acid *tert*-butylamide (17). Prepared as described for **12**, starting from **16** using methanol instead of ethanol. After column chromatography ($CHCl_3$/MeOH 9:1) compound **17** (91%) was obtained as a deep brown oil: R_f=0.3 ($CHCl_3$/MeOH 9:1); $[\alpha]_D^{20}$ −76.9 (*c* 0.55, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25–7.24 (m, 1H), 6.98–6.97 (m, 2H), 6.34–6.32 (br s, 1H, NH), 4.32–4.29 (m, 1H), 3.93–3.89 (m, 1H), 3.07–3.00 (m, 1H), 2.70–2.20 (m, 4H), 2.10–0.90 (m, 15H), 1.36 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4, 145.4, 126.5, 124.8, 124.8, 73.1, 69.8, 59.4, 59.1, 56.1, 50.7, 35.6, 33.2, 30.5, 30.5, 29.7, 25.9, 25.9, 20.8. Anal. Calcd for $C_{21}H_{35}N_3O_2S$: C, 64.08; H, 8.96. Found: C, 64.10; H, 8.94.

8.1.12. (−)-(3*S*,4*aS*,8*aS*,1′*R*,2′*R*)-[Acetic acid 3-[3′-(3-*tert*-butylcarbamoyl-decahydro-isoquinolin-2-yl)-2′-hydroxy-1′-thiophen-2-yl-propylcarbamoyl]-2-methyl-phenyl ester (18). Prepared as described for (*rac*)-**9**, using alcohol **17**, 1.01 equiv of acid, 1.3 equiv of DCC. The crude product was purified by column chromatography (petroleum ether/EtOAc 7:3) affording amide **18** (62% yield) as a viscous white oil: R_f=0.4; $[\alpha]_D^{20}$ −27.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.33 (m, 1H), 7.26–7.20 (m, 2H), 7.16–7.13 (m, 1H, NH), 7.12–7.11 (m, 1H), 7.08–7.06 (m, 1H), 6.99–6.97 (m, 1H), 6.00 (br s, 1H, NH), 5.49–5.46 (m, 1H), 4.22–4.19 (m, 1H), 2.75–2.60 (m, 3H), 2.30 (s, 3H), 2.2 (s, 3H), 1.9–1.00 (m, 15H), 1.33 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.6, 168.9, 168.3, 149.6, 140.7, 139.2, 128.6, 126.7, 126.5, 126.4, 125.2, 124.6, 123.5, 71.1, 69.7, 59.3, 59.1, 53.0, 49.9, 35.5, 33.2, 30.6, 30.3, 28.5, 25.7, 25.5, 24.8, 20.8, 12.8. Anal. Calcd for $C_{31}H_{43}N_3O_5S$: C, 65.35; H, 7.61. Found: C, 65.37; H, 7.59.

8.1.13. (−)-(3*S*,4*aS*,8*aS*,2′*R*,3′*R*)-2-[2′-Hydroxy-3′-(3-hydroxy-2-methyl-benzoylamino)-3′-(thiophen-2-yl)-propyl]-decahydro-isoquinoline-3-carboxylic acid *tert*-butylamide (3). To a solution of amino alcohol **18** (30 mg, 0.053 mmol) in MeOH (1 mL) a catalytic amount of sodium was added and after 45 min the reaction was stopped by solvent removal. The crude mixture dissolved in EtOAc (10 mL) was washed with water and the aqueous layers extracted twice with EtOAc (10 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The mixture was purified by column chromatography (EtOAc/petroleum ether 7:3) affording amide **3** (22 mg 81%): R_f=0.46; $[\alpha]_D^{20}$ −27.0 (*c* 0.6, EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26–7.24 (m, 1H), 7.13–7.12 (m, 1H), 7.05–7.02 (m, 1H, NH), 6.99–6.98 (m, 2H), 6.90–6.85 (m, 2H), 6.05 (br s, 1H, NH), 5.47–5.43 (m, 1H), 4.22–4.19 (m, 1H), 2.68–1.20 (m, 19H), 2.14 (s, 3H), 1.34 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.0, 169.8, 155.1, 140.2, 137.9, 127.3, 126.8, 125.8, 122.6, 119.0, 116.9, 71.2, 70.1, 60.0, 59.4, 53.1, 51.5, 35.9, 33.4, 30.9, 30.7, 29.9, 28.8, 26.2, 25.9, 21.3, 21.1, 12.6. Anal. Calcd for $C_{29}H_{41}N_3O_4S$: C, 66.00; H, 7.83. Found: C, 66.03; H, 7.82.

8.1.14. (−)-(3*S*,4*aS*,8*aS*,2′*R*,3′*R*)-{1-[3-(3-*tert*-Butyl-carbamoyl-decahydro-isoquinolin-2-yl)-2-hydroxy-1-thiophen-2-yl-propylcarbamoyl]-2-carbamoyl-ethyl}-carbamic acid benzyl ester (20). Prepared as described for **18** starting from **19** affording after purification by column chromatography ($CHCl_3$/MeOH 98:2) compound **20** (68 mg, 80%) as a yellow oil: R_f=0.2 ($CHCl_3$/MeOH 98:2); $[\alpha]_D^{20}$ −41.0 (*c* 1.5, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (m, 1H), 7.36 (br s, 5H), 7.19 (m, 1H), 7.00 (m, 1H), 6.91 (m, 1H), 6.27 (m, 2H), 6.09 (s, 1H), 5.65 (s, 1H), 5.23 (m, 1H), 5.08 (s, 2H), 4.61 (m, 1H), 4.16 (m, 1H), 2.81 (m, 2H), 2.55 (m, 3H), 2.33 (m, 2H), 1.55 (m, 12H), 1.34 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0, 173.5, 170.0, 156.4, 139.6, 136.3, 128.7, 128.6, 128.2, 128.1, 127.5, 126.8, 126.6, 125.8, 71.3, 69.6, 67.2, 58.9, 53.4, 51.8, 49.2, 38.2, 35.5, 34.0, 33.2, 30.6, 30.5, 28.7, 26.4, 25.9, 25.6, 25.0, 21.4. Anal. Calcd for $C_{33}H_{47}N_5O_3S$: C, 61.75; H, 7.38; Found C, 61.78; H, 7.40.

8.1.15. (2*S*)-2-[(Quinoline-2-carbonyl)-amino]-succinamic acid (25). To a stirred cold (0 °C) solution of **24** (100 mg, 0.29 mmol) in CH_2Cl_2 (1 mL) TFA (0.4 mL) dropwise was added. The solution was warmed to 25 °C and after 6 h the solvent was evaporated in vacuo. The product **25** was obtained after crystallization by MeOH/EtOAc (66 mg, 80%). The compound shows the same spectroscopic data as reported in the literature.[24]

8.1.16. (+)-*N*1-(3*S*,4*aS*,8*aS*,1′*R*,2′*R*)-[3-(3-*tert*-Butyl-carbamoyl-decahydro-isoquinolin-2-yl)-2-hydroxy-1-thiophen-2-yl-propyl]-(2*S*)-2-[(quinoline-2-carbonyl)-amino]-succinamide (4). To a stirred solution of **17** (40 mg, 0.1 mmol) and DMAP (4.4 mg, 0.035 mmol) in CH_2Cl_2 (1.5 mL), a solution of **25** (34 mg, 0.12 mmol) in DMF (0.1 mL) and a solution of DCC (27 mg, 0.13 mmol) in CH_2Cl_2 (0.2 mL) were added. The solution was stirred at rt for 7 h, diluted with CH_2Cl_2 and washed with 10% HCl, water, saturated aquoeus solution of $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude mixture was purified by column chromatography on silica gel ($CHCl_3$/MeOH 95:5) affording compound **4** (43 mg, 65%) as a paled yellow oil: R_f=0.35 ($CHCl_3$/MeOH 95:5); $[\alpha]_D^{20}$ +21.4 (*c* 0.65, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD) δ 9.26 (d, *J*=7 Hz, 1H), 8.26 (d, *J*=8 Hz, 1H), 8.19 (d, *J*=8.5 Hz, 1H), 8.15 (d, *J*=9 Hz, 1H), 7.85 (d, *J*=8 Hz, 1H), 7.79–7.74 (m, 2H), 7.64–7.61 (m, 1H), 7.16 (d, *J*=5 Hz, 1H), 7.08 (d, *J*=3 Hz, 1H), 6.90 (t, *J*=4 Hz, 1H), 6.41 (s, 1H), 6.19 (s, 1H), 5.82 (s, 1H), 5.31–5.30 (m, 1H), 5.06–5.04 (m, 1H), 4.26–4.24 (m, 1H), 3.04–2.90 (m, 3H), 2.68–2.59 (m, 2H), 2.32–2.22 (m, 2H), 1.85–1.66 (m, 6H), 1.62–1.58 (m, 1H), 1.48–1.37 (m, 6H), 1.33 (s, 9H); ^{13}C NMR (125 MHz, CD_3OD) δ 174.0, 173.2, 169.9, 165.0, 149.1, 146.8, 139.6, 137.7, 130.3, 130.3, 129.6, 128.4, 127.8, 127.1, 126.8, 125.9, 118.9, 71.4, 69.6, 59.9, 53.9, 53.8, 50.9, 50.7, 50.6, 38.5, 35.6, 33.4, 30.8, 30.7, 30.0, 28.9, 28.9, 26.2, 21.2. Anal. Calcd for $C_{35}H_{46}N_6O_5S$: C, 63.42; H, 6.99. Found: C, 63.40; H, 6.95.

8.1.17. Enzyme assays. Recombinant HIV-1 protease was purchased from Bioczech (Prague, Czech Republic); the fluorogenic substrate Abz-Thr-Ile-Nle-Phe(NO_2)-Gln-Arg was purchased from Bachem (Bubendorf, Switzerland). The extent of inhibition of the activity of the aspartic protease was evaluated as previously reported for other series of mono- and dihydroxy pseudopeptide HIV-1-PR inhibitors.[27] The assays were performed at 25 °C in 400 mM NaCl, 1 mM EDTA, 1 mM DTT, 100 mM MES-NaOH buffer at pH 5.5, and 30 mM fluorogenic substrate. Titration of HIV PR was carried out with two reference inhibitors structurally related to our series, namely nelfinavir and saquinavir, using the equation $IC_{50}=[E]/2+K_i(1+[S]/K_m)^2$, where K_i are the literature values of the inhibition constants for nelfinavir and saquinavir,[28] and K_m was 25 μM, as determined under our conditions. The active enzyme concentration in the assay, [E], was estimated to be 3.6±2.3 nM.

8.1.18. Molecular modelling. Calculations were carried out on two Silicon Graphics Octane 1 R12000 workstations and on a Pentium4 2.53GHz/Red Hat Linux machine. The Cornell version of the Amber force field[29] as implemented in Sybyl6.8 (Tripos Inc.) was used in all energy minimizations and dynamics runs. New Amber parameters for the heterocyclic systems of the ligands were developed according to Geremia and Calligaris[30] or following the original Amber protocol. The required ab initio calculations on the ligands were carried out with Gaussian03.[31] All the energy minimizations were carried out until a convergence criterion of 0.001 Kcal/mol/Å was achieved for all the energy gradients. The conjugate gradient minimisation algorithm was always used after running 20 initial steps of Simplex linear minimization. All the calculations were carried out in a continuum dielectric of relative permittivity ε=4rij.[32]

The structures of the ligands were built with standard bond lengths and angles, and optimized first at the RHF-6.31G* level, in order to obtain a charge distribution comparable to that used in the AMBER parameterization. The ligands were then submitted to an extensive conformational search driven by a genetic algorithm operating on all the routable bonds.[33] The AMBER energies of the absolute conformational

minima thus, obtained were taken as reference energies of the free ligands.

The crystallographic coordinates of two reference complexes of HIV PR with saquinavir and nelfinavir were obtained from the Protein Data Bank, Brookhaven National Laboratory (Saquinavir: Pdb id 1C6Z;[34] nelfinavir: Pdb id 1OHR).[35] All the hydrogen atoms were added, assuming an environment pH of 5.5, and a single proton to be shared between the two catalytic aspartic residues.[36] All the crystallization water molecules were removed, with the exception of the essential water always present inside the HIV PR binding site complexed with non ureidic inhibitors, and hydrogen bonded to Ile50 and 50′ residues. The reference complexes were then allowed to relax by the multistage minimization procedure described by Levit and Lifson.[37]

The initial geometries of the ligand-HIV PR complexes were built by docking the ligand structures onto the structures of nelfinavir (for compound **3**) and saquinavir (for compound **4**) bound to HIV PR as in the optimized reference complexes. The structure were first optimized by running an energy minimisation to a 0.05 Kcal/mol/Å energy gradient. The models were then subjected to a molecular dynamics run in the NTV ensemble; the systems were gradually heated to 300 K, in three steps, allowing a 25 psec interval per each 100 K, then equilibrated for 25 psec at 300 K, and finally, submitted to a 400 psec collection run at 300 K. The lowest potential energy equilibrium geometries were finally re optimized.

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References and notes

1. Ghosh, A. K.; Bilcer, G.; Schiltz, G. *Synthesis* **2001**, *15*, 2203.
2. Brik, A.; Wong, C.-H. *Org. Biomol. Chem.* **2003**, *1*, 5.
3. (a) Wlodawer, A.; Gustchina, A. *Biochim. Biophys. Acta* **2000**, *1477*, 16. (b) Tomasselli, A. G.; Heinrikson, R. L. *Biochim. Biophys. Acta* **2000**, *1477*, 189.
4. (a) Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1367. (b) Ripka, A. S.; Satyshur, K. A.; Bohacek, R. S.; Rich, D. H. *Org. Lett.* **2001**, *3*, 2309.
5. Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244.
6. Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699.
7. Leug, D.; Abbenante, G.; Schiltz, G. *J. Med. Chem.* **2000**, *43*, 305.
8. Corral, C.; Lissavetzky, J.; Manzanares, I.; Darias, V.; Exposito-Orta, M. A.; Conde, J. A. M.; Sanchez-Mateo, C. C. *Bioorg. Med. Chem.* **1999**, *7*, 1349.
9. Zhang, A.; Zhou, G.; Rong, S.-B.; Johnson, K. M.; Zang, M.; Kozikowski, A. P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 993.
10. Uckun, F. M.; Pendergrass, S.; Maher, D.; Zhu, D.; Tuel-Ahlgren, L.; Mao, C.; Venkatachalam, T. K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3411.
11. In this paper the authors described the effects on the substitution of the oxyalkyl and oxyaryl P1/P1′ groups with thioalkyl and thioaryl groups. Muhlman, A.; Classon, B.; Haliberg, A.; Samuelsson, B. *J. Med. Chem.* **2001**, *44*, 3402.
12. For reported syntheses of Nelfinavir see: (a) Kaldor, S. W.; Kalish, V. J.; Davies, J. F., II; Shetty, B. V.; Fritz, J. E.; Appelt, K.; Burgess, J. A.; Campanale, K. M.; Chirgadze, N. Y.; Clawson, D. K.; Dressman, B. A.; Hatch, S. D.; Khalil, D. A.; Kosa, M. B.; Lubbehusen, P. P.; Muesing, M. A.; Patick, A. K.; Reich, S. H.; Su, K. S.; Tatlock, J. H. *J. Med. Chem.* **1997**, *40*, 3979. (b) Rieger, D. L. *J. Org. Chem.* **1997**, *62*, 8546. (c) Inaba, T.; Birchler, A. G.; Yamada, Y.; Sagawa, S.; Yokota, K.; Ando, K.; Uchida, I. *J. Org. Chem.* **1998**, *63*, 7582. (d) Zook, S. E.; Busse, J. K.; Borer, B. C. *Tetrahedron Lett.* **2000**, *41*, 7017. (e) Inaba, T.; Yamada, Y.; Abe, H.; Sagawa, S.; Cho, H. *J. Org. Chem.* **2000**, *65*, 1623. (f) Albizati, K. F.; Babu, S.; Birchler, A.; Busse, J. K.; Fugett, M.; Grubbs, A.; Haddach, A.; Pagan, M.; Potts, B.; Remarchuk, T.; Rieger, D.; Rodriguez, R.; Shanley, J.; Szendroi, R.; Tibbetts, T.; Whitten, K.; Borer, B. V. *Tetrahedron Lett.* **2001**, *42*, 6481. (g) Ma, D.; Zou, B.; Zhu, W.; Xu, H. *Tetrahedron Lett.* **2002**, *43*, 8511. (h) Ikunaka, M.; Matsumoto, J.; Fujima, Y.; Hirayama, Y. *Org. Proc. Res. Dev.* **2002**, *6*, 49.
13. For reported syntheses of Saquinavir see: (a) Thompson, W. J.; Ghosh, A. K.; Holloway, M. K.; Lee, H. Y.; Munson, P. M.; Schwering, J. E.; Wai, J.; Darke, P. L.; Zugay, J.; Emini, E. A.; Schleif, W. A.; Huff, J. R.; Anderson, P. S. *J. Am. Chem. Soc.* **1993**, *115*, 801. (b) Parkes, K. E. B.; Bushnell, D. J.; Crackett, P. H.; Dunsdon, S. J.; Freeman, A. C.; Gunn, M. P.; Hopkins, R. A.; Lambert, R. W.; Martin, J. A.; Merrett, J. H.; Redshaw, S.; Spurden, W. C.; Thomas, G. J. *J. Org. Chem.* **1994**, *59*, 3656. (c) Fassler, A.; Bold, G.; Capraro, H.-G.; Cozens, R.; Mestan, J.; Poncioni, B.; Rosel, J.; Tintelnot-Blomley, M.; Lang, M. *J. Med. Chem.* **1996**, *39*, 3203. (d) Gohring, W.; Gokhale, S.; Hilpert, H.; Roessler, F.; Schlageter, M.; Vogt, P. *Chimia* **1996**, *50*, 532. (e) Ghosh, A. K.; Hussain, K. A.; Fidanze, S. *J. Org. Chem.* **1997**, *62*, 6080.
14. (a) Tay, M. K.; About-Joudet, E.; Callignon, N.; Teulade, M. P.; Savignac, P. *Synth. Commun.* **1988**, *18*, 1349. (b) Kosakara, A.; Izumi, T.; Ogihara, T. *J. Heterocycl. Chem.* **1989**, *26*, 597. (c) Rossi, R.; Carpita, A.; Ciofalo, M.; Lippolis, V. *Tetrahedron* **1991**, *47*, 8443. (d) Guncheal, K.; Don Gyu, L.; Sukbok, C. *Bull. Korean Chem. Soc.* **2001**, *22*, 943.
15. Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483.
16. For preliminary communication of part of this work see: Bonini, C.; Chiummiento, L.; De Bonis, M.; Funicello, M.; Lupattelli, P. *Tetrahedron Lett.* **2004**, *45*, 2797.
17. Bonini, C.; D'Auria, M.; Fedeli, P. *Tetrahedron Lett.* **2002**, *43*, 3813.
18. (a) Byun, H. S.; He, L.; Bittman, R. *Tetrahedron* **2000**, *56*, 7051. (b) Xiong, C.; Wang, W.; Cai, C. J.; Hruby, V. J. *J. Org. Chem.* **2002**, *67*, 1399.
19. Solladié-Cavallo, A.; Lupattelli, P.; Bonini, C.; De Bonis, M. *Tetrahedron Lett.* **2003**, *44*, 5075.
20. Superchi, S.; Contursi, M.; Rosini, C. *Tetrahedron* **1998**, *54*, 11247.

21. Denis, J.-N.; Green, A. E.; Serra, A. A.; Luche, M.-J. *J. Org. Chem.* **1986**, *51*, 11247.
22. (a) Hamada, Y.; Shibata, M.; Sugiura, T.; Kato, S.; Shioiri, T. *J. Org. Chem.* **1987**, *52*, 1252. (b) Kozikowski, A. P.; Sato, K.; Basu, A.; Lazo, J. S. *J. Am. Chem. Soc.* **1989**, *111*, 6228. (c) Saito, S.; Ishikawa, T.; Kuroda, A.; Koga, K.; Moriwake, T. *Tetrahedron* **1992**, *48*, 4067.
23. (a) Bellamy, F. D.; Bondoux, M.; Dodey, P. *Tetrahedron Lett.* **1990**, *31*, 7323. (b) Pommier, A.; Pons, J. M.; Kocienski, P. J. *J. Org. Chem.* **1995**, *60*, 7334.
24. The compound **16** was obtained with same yield (67%) starting from both compounds **15a** and **15b** (see Section 8).
25. (a) Varney, M. D.; Appelt, K.; Kalish, V.; Rami Reddy, M.; Tatlock, J.; Palmer, C. L.; Romines, W. H.; Wu, B.-W.; Musick, L. *J. Med. Chem.* **1994**, *37*, 2274. (b) Abell, A. D.; Foulds, G. J. *J. Chem. Soc., Perkin Trans 1* **1997**, 2475. (c) May, B. C. H.; Abell, A. D. *J. Chem. Soc., Perkin Trans 1* **2002**, 172.
26. (a) Tossi, A.; Benedetti, F.; Norbedo, S.; Skrbec, D.; Berti, F.; Romeo, D. *Bioorg. Med. Chem.* **2003**, *11*, 4719–4727. (b) Skrbec, D.; Romeo, D. *Biochem. Biophys. Res. Commun.* **2002**, *297*, 1350. (c) Tossi, A.; Bonin, I.; Antcheva, N.; Norbedo, S.; Benedetti, F.; Miertus, S.; Nair, A. C.; Maliar, T.; Dal Bello, F.; Palù, G.; Romeo, D. *Eur. J. Biochem.* **2000**, *267*, 1715.
27. Cha, S. *Biochem. Pharmacol.* **1975**, *24*, 2177.
28. Markgren, P. O.; Hämäläinen, M.; Danielson, U. H. *Anal. Biochem.* **2000**, *279*, 71.
29. Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M., Jr.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179.
30. Geremia, S.; Calligaris, M. *J. Chem. Soc., Dalton Trans.* **1997**, 1541.
31. *Gaussian 03*, *Revision A.1*, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, Jr., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; and Pople, J. A.; Gaussian, Pittsburgh PA, 2003.
32. Orozoco, M.; Laughton, C. A.; Herzyk, P.; Neidle, S. *J. Biomol. Struct. Dyn.* **1990**, *8*, 359.
33. Judson, R. S.; Rabitz, H. *Phys. Rev. Lett.* **1992**, *68*, 1500.
34. Chen, Z.; Yan, Y.; Li, Y.; Olsen;, D. B.; Schock, H. B.; Galvin, B. B.; Dorsey, B.; Kuo, L. C. *Acta Crystallogr. D* **2000**, *56*, 38.
35. Kaldor, S. W.; Kalish, V. J.; Davies, J. F., II; Shetty, B. V.; Fritz, J. E.; Appelt, K.; Burgess, J. A.; Campanale, K. M.; Chirgadze, N. Y.; Clawson, D. K.; Dressman, B. A.; Hatch, S. D.; Khalil, D. A.; Kosa, M. B.; Lubbehusen, P. P.; Muesing, M. A.; Patick, A. K.; Reich, S. H.; Su, K. S.; Tatlock, J. H. *J. Med Chem.* **1997**, *40*, 3979.
36. (a) Carloni, P.; Rothlisberger, U.; Parrinello, M. *Acc. Chem. Res.* **2002**, *35*, 455. (b) Piana, S.; Sebastiani, D.; Carloni, P.; Parrinello, M. *J. Am. Chem. Soc.* **2001**, *123*, 8730.
37. Levit, M.; Lifson, S. *J. Mol. Biol.* **1969**, *46*, 269.