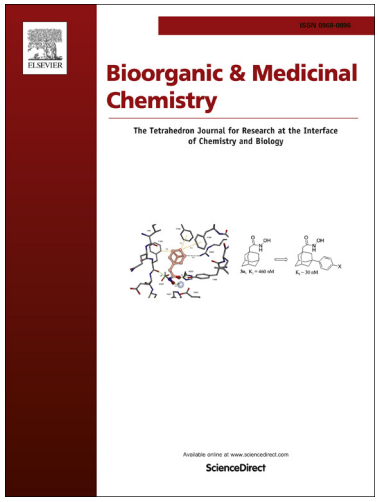

Synthesis and biological evaluation of new simple indolic non peptidic HIV Protease inhibitors: the effect of different substitution patterns

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Synthesis and biological evaluation of new simple indolic non peptidic HIV Protease inhibitors: the effect of different substitution patterns

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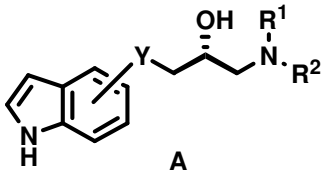
Abstract

New structurally simple indolic non peptidic HIV Protease inhibitors were synthesized from (*S*)-glycidol by regioselective methods. Following the concept of targeting the protein backbone, different substitution patterns were introduced onto the common stereodefined isopropanolamine *core* modifying the type of functional group on the indole, the position of the functional group on the indole and the type of the nitrogen containing group (sulfonamides or perhydroisoquinoline), alternatively. The systematic study on *in vitro* inhibition activity of such compounds confirmed the general beneficial effect of the 5-indolyl substituents in presence of arylsulfonamide moieties, which furnished activities in the micromolar range. Preliminary docking analysis allowed to identify several key features of the binding mode of such compounds to the protease.

Introduction

The devastating effect of the AIDS epidemic is still a reality. However, since the highly active antiretroviral therapy (HAART) has been employed to combat the illness, HIV infection has definitely become more manageable.[1] During the last 20 years an unprecedented success has been achieved in discovering anti-HIV drugs as reflected by the fact that there are now more drugs approved for the treatment of HIV than for all other viral infections taken together.

The currently FDA approved anti-HIV drugs can be divided into seven groups: nucleoside reverse transcriptase inhibitors, nucleotide reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors (PIs), fusion inhibitors, co-receptor inhibitors, and integrase inhibitors.[2] Detailed knowledge of the structure of HIV protease and its substrate has led to the preparation of specific PIs, whose arrival was a pivotal moment in the development of antiretroviral therapy and made possible the dual class triple combination therapy.[3] Despite the already marketed PIs have an evident crucial role into HAART regimen, their clinical utility can be limited by low bioavailability and reduced long-term viral inhibition, with multiple protease resistance mutations being observed. Thus, novel PIs with high potency against the known HIV protease variants have been designed. We recently demonstrated the beneficial effect of a heteroaromatic group in a series of new thienyl ring containing analogues of nelfinavir and saquinavir, which showed to maintain or even increase their activity against either wild type or mutant HIV protease.[4] Recently the concept of targeting the protein backbone in structure-based drug design was introduced. Thus new non-peptidic templates, which can maximize interactions in the HIV-protease active site, particularly with the enzyme backbone atoms, were developed.[5] Both extensive hydrogen bonding and hydrophobic interactions with enzyme subsites can limit the protease ability to acquire drug resistance as the geometry of the catalytic site must be conserved to maintain functionality.[6] This new concept allowed to design different compounds of very simple structure and to focus the interest more toward their easy synthetic availability and less to structural similarity. Our preliminary investigation showed the beneficial effect of indolyl ring on a simple substituted stereodefined isopropanolamine core.[7] In this respect, with the aim of finding new easily accessible non-peptidic PIs, we started a systematic study on the synthesis and inhibition activity of new indolyl derivatives with general structure **A** (Fig. 1), modifying the following parameters:



A

Figure 1

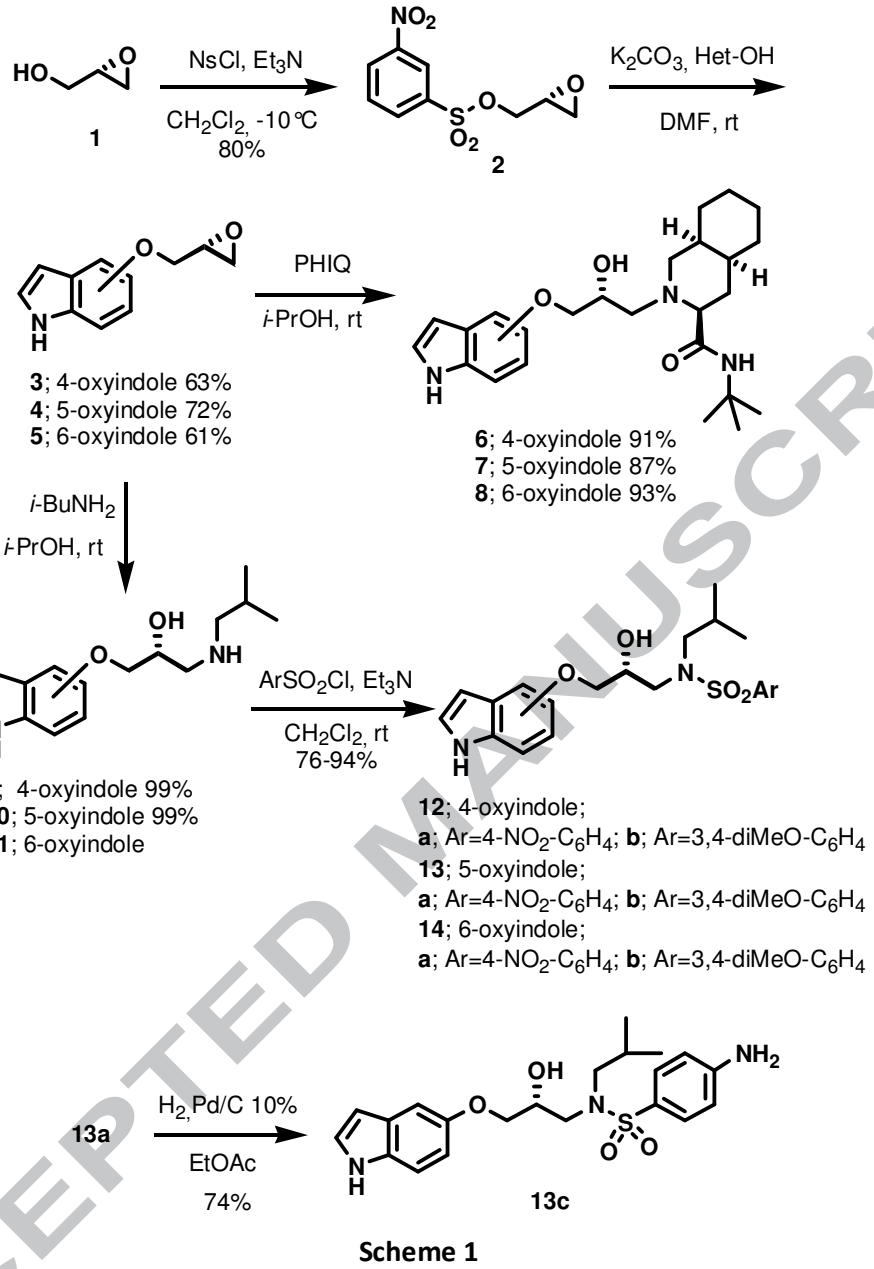
1) type of functional group on the indole

2) position of the functional group on the indole

3) type of the nitrogen containing group R^1 and R^2 (sulfonamides or PHIQ)

Results and discussion

Although the synthetic work can appear tedious at a first sight, we took advantage of the commercially available bidentate electrophile (*S*)-glycidol **1** for the generation of the core (scheme 1). The glycidol was first activated by the reaction with *m*-nosyl chloride to obtain compound **2**. The subsequent nucleophilic displacement of the nosyl group was performed by the commercially available 4-, 5-, or 6-hydroxyindoles affording the corresponding oxyindoles **3**, **4** and **5** in good yields and mild reaction conditions, without any competitive epoxide ring opening.[8] Each oxyindole represents the common precursor for the preparation of either perhydroisoquinyl- or sulfonamidyl derivatives. Indeed, perhydroisoquinoline (PHIQ) was introduced by direct regioselective oxiranyl ring opening, obtaining the corresponding **6**, **7** and **8** in good yield and as single diastereoisomers (> 57% overall yield in 3 steps).

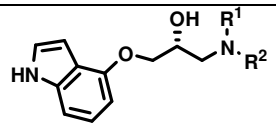
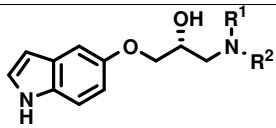
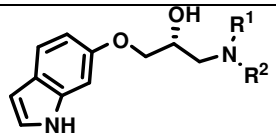


Scheme 1

For the preparation of arylsulfonamidyl compounds, the oxiranyl-oxyindoles **3**, **4** and **5** were reacted with isobutylamine, affording the amino-derivatives **9**, **10** and **11** in excellent yield. Starting from this triad, arylsulfonyl fragments with different electronic properties were introduced by employing suitable chlorides, affording compounds **12a,b**, **13a,b** and **14a,b** (> 46% overall yield in 4 steps). In particular, electron releasing 3,4-dimethoxyphenyl- and electron withdrawing 4-nitrophenyl groups were introduced to evaluate their potentially different effect on inhibitory activity. Compound **13a** was also transformed into 4-aminophenyl derivative **13c** by Pd-catalyzed hydrogenation.

All these oxyindoles were tested *in vitro* for anti HIV-PR activity and the results are reported in Table 1.

Table 1.

Regioisomer	PHIQ (IC_{50}, μM)	4-$NO_2C_6H_4SO_2NR$ (4-$NH_2C_6H_4SO_2NR$) (IC_{50}, μM)	3,4-di$MeOC_6H_4SO_2NR$ (IC_{50}, μM)
 4-oxyindole	**6** (24)	**12a** (346)	**12b** (336)
 5-oxyindole	**7** (14)	**13a** (10) (**13c** (12))	**13b** (1)
 6-oxyindole	**8** (138)	**14a** (46)	**14b** (346)

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.

This systematic study confirms the trend observed in our preliminary results on oxyindoles.[7b] In general 5-oxyindoles show better activity than the 4- and 6-substituted ones and this difference is highly amplified by the presence of sulfonamidyl groups, no matter which substituent is placed on the aryl ring. Promising IC_{50} value of 1.04 μM was obtained for compound **13b**, suggesting a beneficial effect of the two methoxy groups on the aryl ring. This is in line with the reported data for derivatives of Darunavir in which the aniline group is replaced by 1,3-dioxolane group.[9] The two oxygen atoms are thought to interact with the protease by two direct hydrogen bonds and a water-mediated interaction with Gly 48, which may stabilize the flexible flap region.

Next step of our study was to change the functionality on the indole ring, so we focused our attention on amino indoles. PHIQ derivatives **15** and **16** were recently prepared starting from available 4- and 5-aminoindoles and (*S*)-glycidol, by a slight modification of our synthetic approach (figure 2).[7b] Their potency was disappointing, the IC_{50} values ranging between 150 and 180 μM.

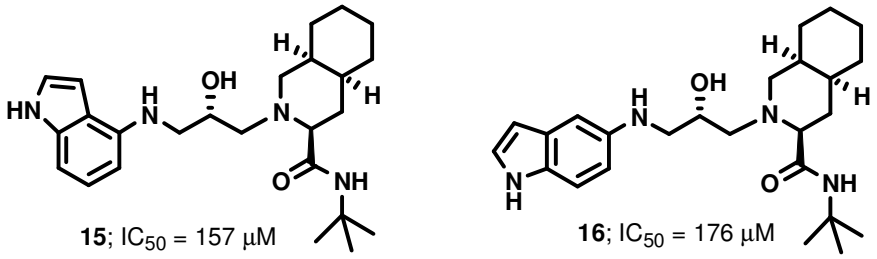
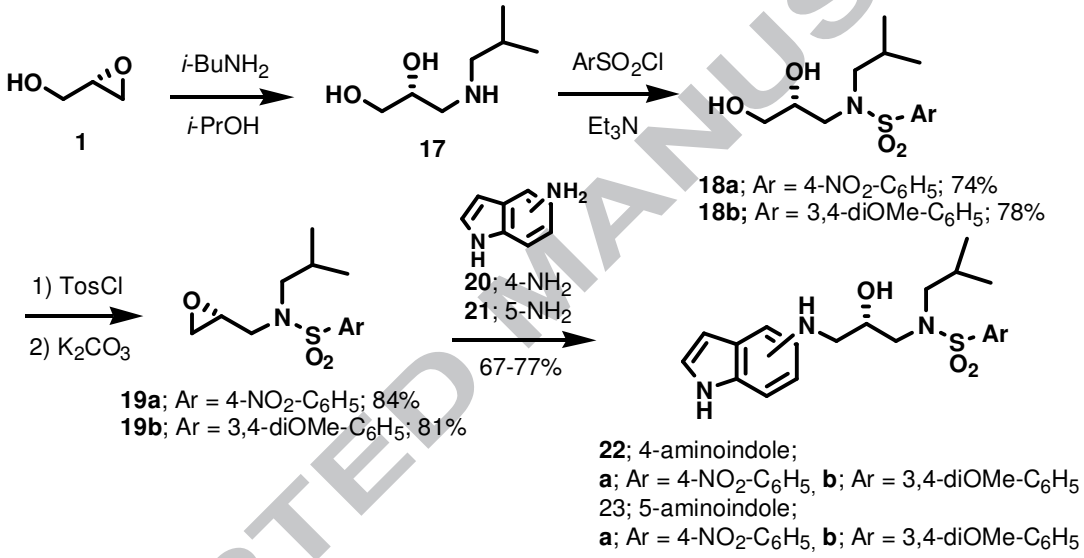


Figure 2

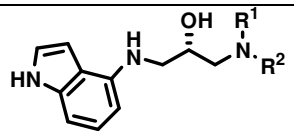
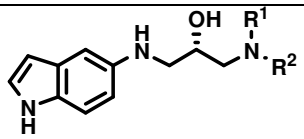
Thus, we wanted to prepare and test the corresponding 4-nitro- and 3,4-dimethoxyphenyl-sulphonamidyl derivatives. (*S*)-glycidol was regioselectively opened with *i*-$BuNH_2$, affording the aminodiol **17** (scheme 2).



Scheme 2

Then the suitable arylsulfonyl moiety was introduced, namely 4-nitro- and 3,4-dimethoxyphenylsulfonyl groups, in high yield giving **18a** and **18b**. After regioselective tosylation of primary hydroxyl group, the two epoxides **19a** and **19b** were alternatively prepared, and they were finally opened with 4- and 5-aminoindoles, affording the four new derivatives **22a,b** and **23a,b** (> 41% overall yield in 5 steps). *In vitro* tests of these aminoindolyl compounds against wild type HIV-1 PR are reported in Table 2.

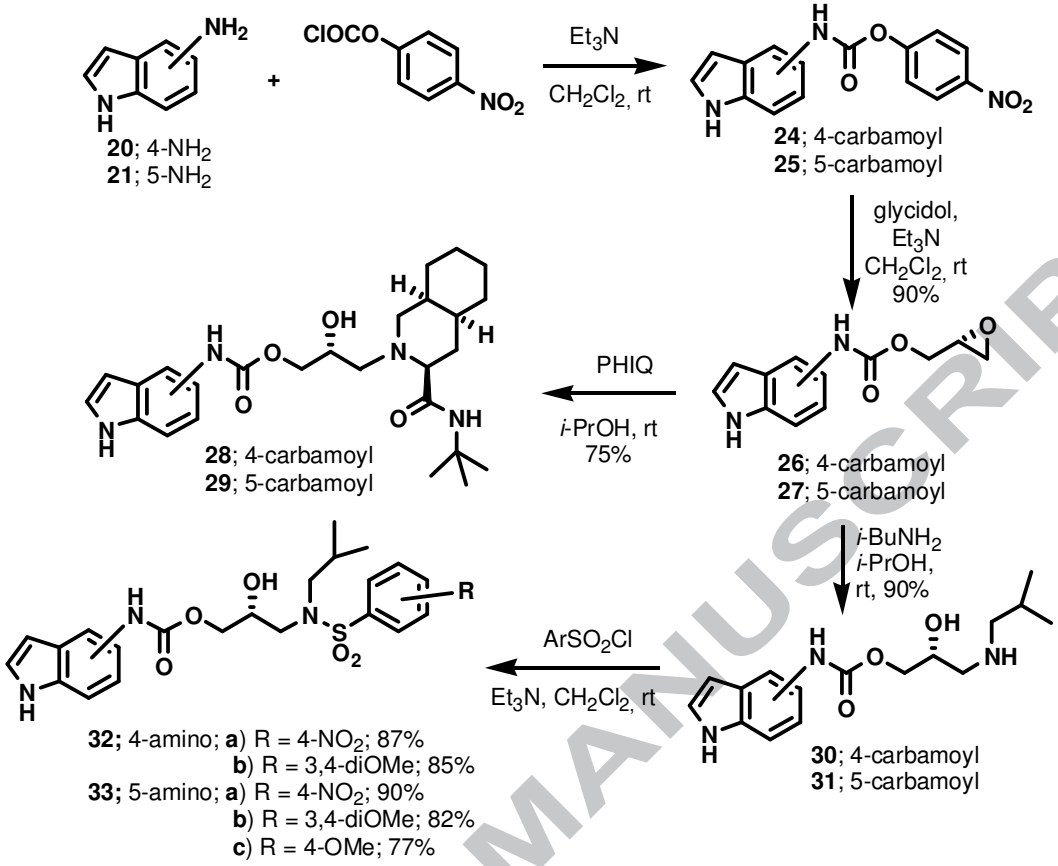
Table 2

Regioisomer	4-$NO_2C_6H_4SO_2NR$ (IC_{50}, μM)	3,4-di$MeOC_6H_4SO_2NR$ (IC_{50}, μM)
 4-aminoindole	**22a** (2630)	**22b** (336)
 5-aminoindole	**23a** (inactive)	**23b** (4400)

These results pointed out the general negative effect of amino group on the inhibition activity of indole derivatives, whatever the position of the nitrogen on the heterocycle. The introduction of sulfonamidyl groups instead of PHIQ even reduced the activity of the resulting compounds.

Finally, we wanted to investigate the effect of carbamoyl moiety, instead of amino group, considering its beneficial effect in certain non peptidic inhibitors, as Darunavir.[10]

The preparation of the corresponding carbamoyl derivatives of 4- and 5-indoles was straightforward. 4- and 5-aminoindoles **20** and **21** were first reacted with *p*-nitrophenylchlorocarbonate, to afford the activated carbamates **24** and **25** (scheme 3). Glycidol was then introduced by substitution reaction, and the common intermediates oxiranyl carbamates **26** and **27** were obtained in good yield.

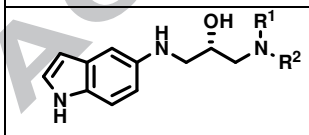
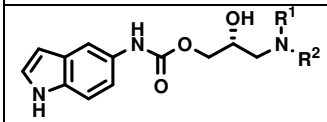


Scheme 3

These intermediates were alternatively transformed into PHIQ derivatives **28**, **29** (> 67% overall yield in 3 steps) and sulfonamides **32a,b**, **33a,b,c** (> 63% overall yield in 4 steps) by oxiranyl ring opening with PHIQ and *iso*-butylamine followed by sulfonylation, respectively.

All these carbamates were tested and their inhibition activities are listed in Table 3.

Table 3

Regioisomer	PHIQ (IC_{50}, μM)	4-$NO_2C_6H_4SO_2NR$ (IC_{50}, μM)	3,4-di$MeOC_6H_4SO_2NR$ (IC_{50}, μM)
 4-carbamoyl	**28** (50)	**32a** (72)	**32b** (615)
 5-carbamoyl	**29** (606)	**33a** (2)	**33b** (1400) **33c** (4-OMe) (8)

Although the general positive effect on the activity of the substitution of amino group with carbamoyl one, usually ranging in the micromolar values, the results appear difficult to rationalize. PHIQ moiety contributes better on 4-indole derivative, whether arylsulphonamide group matches with 5-indole one. Such a structure is very sensitive to the overall length of the molecule, depending on the linkage between the indole ring and the core of the inhibitor, and requires a careful selection of the substituents on the aryl system. Thus, the 3,4-dimethoxy aryl system is favourable in compounds as **13b**, but fails entirely in the corresponding carbamate **33b**, with an extremely high IC_{50} of 1.4 mM. Conversely, the nitroaryl system is effective in both the series of inhibitors. To address this point, we have carried out a preliminary computational analysis by docking inhibitors **13**, **23** and **33** inside the proteases. Analysis of the docked poses shows that the activities of the inhibitors are determined by the distance between the hydrophobic part of the indole ring and the central carbon atom. For one atom linkers, -O- or -N-, the indole ring lies mostly in sub-site S1, rather than in sub-site S2. For a longer linker as the carbamate one, the indole ring will lie in S2 but this forces the arylsulphonamide side of the molecules to a deeper extent inside S2' subsite (Figure 3).

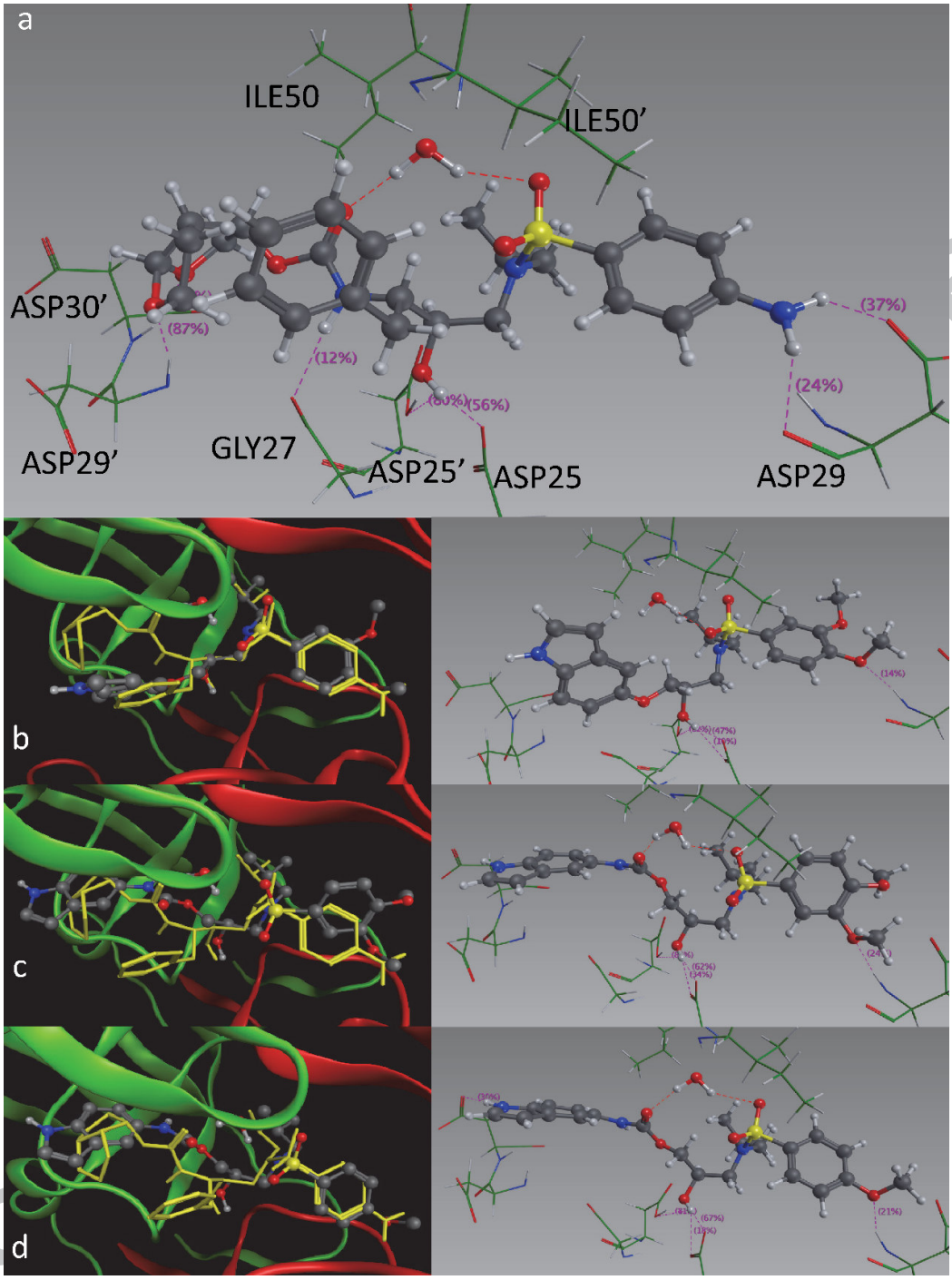


Figure 3[11]**.** Binding poses of some active compounds; HIV PR were presented with red/green ribbons or sticks. Darunavir was used as the reference and is presented as yellow sticks. Ligands are shown as ball and sticks, coloured by atom types. **a**) crystal structure of darunavir with HIV PR. b) binding mode of **13b**. c) binding mode of **33b**. d) binding mode of **33c**.

The nitro- (**13a**) or one of the two methoxy- groups (**13b**, Figure 3b) could establish favourable hydrogen bonding with the backbone amide proton of Asp30'. However, the second methoxy-group in the larger **33b** structure, clearly leads to unfavourable steric clash (Figure 3c). This prompted us to remove such a group as in **33c** (Figure 3c), and this led to a three orders of magnitude gain in activity (see Table 3).

Conclusion

In conclusion, our systematic study confirmed the possibility of introducing indole ring in anti-HIV-1 PR inhibitors which are active in the micromolar range. Although the net contribution of the individual substituents cannot be fully rationalized, the following general insights may be found:

a) oxyindoles and carbamoyl indoles showed general good activity, whereas simple aminoindoles are much less active,

b) 5-substituted indoles appear to be the best regioisomers, apparently they fit better into the S_1 or S_2 site of the enzyme, depending on the linker length and distance from the *core*,

c) arylsulfonamidyl moieties are in general beneficial for the inhibition activity, but their effect is compound type-dependent: 5-oxyindole matches with 3,4-diOMe-phenylsulfonamide group at best, whilst 5-carbamoyl indole matches well with both 4-NO_2- and 4-OMe-phenylsulfonamide moiety, with a slight preference for the former,

d) a preliminary docking analysis allowed to identify several key features of the binding mode of our compounds to the protease, prompting further developments of inhibitors with improved activity.

Experimental section

Chemistry

Preparative chromatography was carried out on Merck silica gel (0.063–0.200 mm particle size) by progressive elution with opportune solvent mixtures. ^{1}H and ^{13}C NMR spectra were normally carried out in $CDCl_3$ solutions on a VARIAN INOVA 500 MHz or Bruker 400 MHz and referenced to Me_4Si. Mass spectra were obtained with a Hewlett–Packard 5971 mass-selective detector on a Hewlett–Packard 5890 gas chromatograph [(OV-1 capillary column between 70 and 250 °C (20 °C $min^{-}1$)]. The optical purity was evaluated by using a polarimeter JASCO Mod Dip-370. Dichloromethane was dried by distillation over anhydrous $CaCl_2$ in inert atmosphere. Dry dimethylformamide was commercially available. Compound **2** was prepared according to the literature[8a, 12] starting from the commercially available (*S*)-(-)-glycidol **1**.

Nosyl displacement with hydroxyindoles: general procedure

K_2CO_3 (0.1433 g, 1.04 mmol) was added to a stirred solution of hydroxyindole (0.0461 g, 0.35 mmol) in dry DMF (4 mL) at room temperature under argon atmosphere; after 1 h a DMF solution (3 mL) of compound **2** (0.0815 g, 0.31 mmol) was added and the mixture was stirred overnight. After 14 h (TLC control, $CHCl_3$/ CH_3OH 99:1) the reaction mixture was quenched by adding ammonium chloride (saturated aqueous solution), then was extracted with diethyl ether and the organic layer washed with brine. After drying over Na_2SO_4, the organic layer was concentrated *in vacuo* and the crude was purified by column chromatography on silica gel (eluent: CH_2Cl_2/EtOAc 99:1)

*(-)-(R)-4-Oxiranylmethoxy-1H-indole (**3**)*

Compound **3** was isolated as a brown thick oil (0.038 g, 63%). $[\alpha]_D^{20}$ -6.4 (c 1.6, $CHCl_3$); Rf 0.5 (CH_2Cl_2/EtOAc 99:1); δ_H (500 MHz, $CDCl_3$) 8.25 (1H, s), 7.14–7.05 (3H, m), 6.71 (1H, t, *J* = 2.5 Hz), 6.55 (1H, d, *J* = 8.0 Hz), 4.38 (1H, dd, *J* = 3.0 and 11.0 Hz), 4.17 (1H, dd, *J* = 6.0 and 11.0 Hz), 3.49–3.47 (1H, m), 2.96 (1H, t, *J* = 5.0 Hz), 2.85 (1H, dd, *J* = 2.5 and 5.0 Hz); δ_C (125 MHz, $CDCl_3$) 152.1, 133.2, 127.1, 120.5, 117.6, 112.3, 103.4, 102.7, 70.5, 50.9, 44.3. MS (EI)m/z: 189 (M^+) (100), 132 (63), 104 (50). Anal. Calcd for $C_{11}H_{11}NO_2$: C, 69.83; H, 5.86; N, 7.40. Found: C, 69.85; H, 5.84; N, 7.45.

*(-)-(R)-5-Oxiranylmethoxy-1H-indole (**4**)*

Compound **4** was isolated as a brown thick oil (0.045 g, 72%). $[\alpha]_D^{20}$ -2.2 (c 1.2, $CHCl_3$); Rf 0.5 (CH_2Cl_2/EtOAc 99:1); δH (500 MHz, $CDCl_3$) 8.16 (1H, s), 7.31–7.14 (3H, m), 6.92 (1H, dd, *J* = 1.5 and 8.5 Hz), 6.5 (1H, s), 4.27 (1H, dd, *J* = 3.5 and 12.0 Hz), 4.04 (1H, dd, *J* = 6 and 11.0 Hz), 3.43–3.42 (1H, m), 2.94 (1H, t, *J* = 4.5 Hz), 2.81 (1H, dd, *J* 3.0 and 5.5 Hz); δ_C (125 MHz, $CDCl_3$) 153.0, 131.2, 128.1, 125.0, 112.8, 111.7, 103.8, 102.3, 69.6, 50.4, 44.9; MS (EI) m/z: 189 (M^+) (100), 132 (80), 104 (54). Anal. Calcd for $C_{11}H_{11}NO_2$: C, 69.83; H, 5.86, N, 7.40. Found: C, 69.82; H, 5.87; N, 7.35.

*(-)-(R)-6-Oxiranylmethoxy-1H-indole (**5**)*

Compound **5** was isolated as a yellow solid (0.036 g, 61%). Mp 105 °C; $[\alpha]_D^{20}$ -3.4 (c 1, $CHCl_3$); Rf 0.4 (CH_2Cl_2/EtOAc 99:1); δ_H (500 MHz, $CDCl_3$) 8.15 (1H, s), 7.54 (1H, d, *J* = 11.0 Hz), 7.09 (1H, s), 6.85 (2H, d, *J* = 6.5 Hz), 6.50 (1H, d, *J* = 1.0 Hz), 4.24 (1H, dd, *J* = 1.5 and 13.5 Hz), 3.96 (1H, dd, *J* = 7.0 and 14.0 Hz), 3.39 (1H, m), 2.93–2.78 (2H, m); δ_C (125 MHz, $CDCl_3$) 155.1, 136.3, 123.4, 122.5, 121.2, 110.2, 102.2, 95.9, 69.3, 50.3, 44.7; MS (EI) m/z: 189 (M^+) (100), 132 (80), 104 (54). Anal. Calcd for $C_{11}H_{11}NO_2$: C, 69.83; H, 5.86; N, 7.40. Found: C, 69.82; H, 5.87; N, 7.38.

Ring opening of the epoxides with PHIQ: general procedure

PHIQ (0.043 g, 0.18 mmol) was added to a stirred solution of suitable epoxide (0.15 mmol) in *i*-PrOH (2 mL) at room temperature. After 20 h the solvent was removed under reduced pressure and the crude purified by column chromatography on silica gel ($CHCl_3$/CH_3OH 95:5).

*(-)-(3S,4aS,8aS)-2-[(2R)-Hydroxy-3-(1H-indol-4-yloxy)-propyl]-decahydro-isoquinoline-3-carboxylic acid tert-butylamide (**6**)*

Compound **6** was isolated as a pink solid (0.061 g, 91%); mp 85 °C; $[\alpha]_D^{20}$ -61.2 (c 1.5, $CHCl_3$); Rf 0.4 ($CHCl_3$/CH_3OH 95:5); δ_H (500 MHz, $CDCl_3$) 8.51 (1H, s), 7.14–7.06 (3H, m), 6.65 (1H, d, *J* = 2.5 Hz), 6.53 (1H, d, *J* = 7.5 Hz), 6.25 (1H, s, NH), 4.42 (1H, d, *J* = 9.0 Hz), 4.07 (1H, t, *J* = 8.0 Hz), 3.12 (1H, s, OH), 2.99 (1H, d, *J* = 7.5 Hz), 2.78 (1H, d, *J* = 8.0 Hz), 2.66 (1H, d, *J* = 8.0 Hz), 2.44 (1H, d, *J* = 7.5 Hz), 2.31 (1H, d, *J* = 8.0 Hz), 1.92–1.39 (9H, m), 1.34 (9H, s), 1.28–1.16 (4H, m); δ_C (125 MHz, $CDCl_3$) 171.5, 152.1, 137.3, 122.8, 122.5, 118.6, 105.1, 100.7, 99.5, 70.4, 68.2, 59.5, 58.4, 50.6, 35.7, 33.1, 30.8, 30.6, 28.6, 26.1, 25.7, 20.4. IR (cm^{-1}) 3311, 2925, 1652, 1365. Anal. Calcd for $C_{25}H_{37}N_3O_3$: C, 70.22; H, 8.72; N, 9.83. Found: C, 70.20; H, 8.73; N, 9.80.

*(-)-(3S,4aS,8aS)-2-[(2R)-Hydroxy-3-(1H-indol-5-yloxy)-propyl]-decahydro-isoquinoline-3-carboxylic acid tert-butylamide (**7**)*

Compound **7** was isolated as a yellow thick oil (0.063 g, 87%); $[\alpha]_D^{20}$ -70.3 (c 2, $CHCl_3$); Rf 0.4 ($CHCl_3$/CH_3OH 95:5); δ_H (500 MHz, $CDCl_3$) 8.45 (1H, s), 7.29 (1H, t, *J* = 9.0 Hz), 7.21 (1H, t, *J* = 2.5 Hz), 7.11 (1H, d, *J* = 2.5 Hz), 6.87 (1H, dd, *J* = 2.5 and 9.0 Hz), 6.47 (1H, t, *J* = 2.5 Hz), 6.29 (1H, s, NH), 4.19 (1H, s,OH), 4.11 (1H, dd, *J* = 4.0 and 9.5 Hz), 3.95 (1H, dd, *J* = 7.0 and 9.0 Hz), 3.01–2.22 (5H, m), 1.91–139 (10H, m), 1.35 (9H, s), 1.22–1.18 (3H, m); δ_C (125 MHz, $CDCl_3$) 173.9, 152.9, 131.3, 128.2, 125.1, 112.6, 111.8, 103.7, 102.7, 71.2, 70.3, 68.3, 59.4, 58.6, 50.7, 35.7, 35.7, 33.1, 30.8, 30.6, 29.6, 28.6, 26.1, 25.7, 20.5. IR (cm^{-1}) 3311, 2925, 1652, 1365. Anal. Calcd for $C_{25}H_{37}N_3O_3$: C, 70.22; H, 8.72; N, 9.83. Found: C, 70.19; H, 8.76; N, 9.85.

*(-)-(3S,4aS,8aS)-2-[(2R)-Hydroxy-3-(1H-indol-6-yloxy)-propyl]-decahydro-isoquinoline-3-carboxylic acid tert-butylamide (**8**)*

Compound **8** was isolated as a brown thick oil (0.063 g, 93%); $[\alpha]_D^{20}$ -66.0 (c 1.3, CH_3OH); Rf 0.6 ($CHCl_3$/CH_3OH 95:5); δ_H (500 MHz, $CDCl_3$) 8.53 (1H, s), 7.51 (1H, d, *J* = 8.5 Hz), 7.21 (1H, t, *J* = 2.5

Hz), 6.90 (1H, d, *J* = 2.0 Hz), 6.79 (1H, dd, *J* = 2.5 and 9.0 Hz), 6.48 (1H, t, *J* = 2.0 Hz), 6.24 (1H, s, NH), 4.16 (1H, t, *J* = 5.0 Hz), 4.08 (1H, dd, *J* = 4.5 and 9.0 Hz), 3.95 (1H, dd, *J* 6.5 and 9.5 Hz), 2.95 (1H, dd, *J* = 2.0 and 11.5 Hz), 2.76–2.64 (2H, m), 2.4 (1H, dd, *J* = 5.0 and 13.5 Hz), 2.28 (1H, dd, *J* = 3.0 and 12.0 Hz), 1.92–1.40 (11H, m), 1.37 (9H, s), 1.34–1.21 (3H, m); δ_C (125 MHz, $CDCl_3$) 174.0, 155.0, 136.4, 123.4, 122.5, 121.1, 110.1, 102.1, 95.9, 70.9, 70.2, 68.3, 59.5, 58.5, 50.7, 35.7, 33.1, 30.8, 30.6, 28.6, 26.1, 25.7, 20.5. IR (cm^{-1}) 3311, 2925, 1652, 1365. Anal. Calcd for $C_{25}H_{37}N_3O_3$: C, 70.22; H, 8.72; N, 9.83. Found: C, 70.20; H, 8.71; N, 9.86.

*(-)-(3S,4aS,8aS)-(1H-Indol-4-yl)-carbamic acid 3-(3-tert-butylcarbamoyl-octahydro-isoquinolin-2-yl)-(2R)-hydroxy-propyl ester (**28**)*

Compound **28** was isolated as a brown powder (0.050 g, 80%) starting from **27**. Mp 130 °C; $[\alpha]_D^{20}$ +36.0 (c 1, CH_3OH); Rf 0.8 ($CHCl_3/CH_3OH$ 9:1); δ_H (500 MHz, $CDCl_3$) 8.68 (1H, s), 7.11 (1H, t, *J* =2.5 Hz), 6.85 (1H, d, *J* 8.0 Hz), 6.48–6.35 (3H, m), 4.37 (1H, d, *J* = 8.0 Hz), 4.06–4.11 (2H, m), 2.90 (1H, d, *J* = 11.5 Hz), 2.65 (1H, t, *J* = 5.0 Hz), 2.26 (2H, t, *J* = 12.0 Hz), 2.23–1.38 (11H, m), 1.36 (9H, s), 1.21–1.18 (3H, m); δ_C (125 MHz, $CDCl_3$) 173.7, 138.6, 136.7, 129.7, 128.1, 125.2, 115.7, 111.3, 106.4, 102.6, 70.1, 68.1, 59.4, 58.8, 55.7, 50.8, 35.7, 33.0, 31.9, 30.7, 30.6, 29.7, 29.3, 25.7. IR (cm^{-1}) 3411, 3282, 3042, 2937, 1731, 1642, 1312, 1238, 1124. Anal. Calcd for $C_{26}H_{38}N_4O_4$: C, 66.36; H, 8.14; N, 11.91 Found: C, 66.33; H, 8.18; N, 11.88.

*(-)-(3S,4aS,8aS)-(1H-Indol-5-yl)-carbamic acid 3-(3-tert-butylcarbamoyl-octahydro-isoquinolin-2-yl)-(2R)-hydroxy-propyl ester (**29**)*

Compound **29** was isolated as a brown powder (0.052 g, 75%) starting from **27**. Mp 139 °C; $[\alpha]_D^{20}$ +42.0 (c 1.2, CH_3OH); Rf 0.6 ($CHCl_3/CH_3OH$ 9:1); δ_H (500 MHz, $CDCl_3$) 8.31 (1H, s), 7.68 (1H, s), 7.31 (1H, d, *J* = 9.0 Hz), 7.21 (1H, t, *J* = 2.5 Hz), 7.11 (1H, d, *J* = 8.0 Hz), 6.85 (1H, br s), 6.47 (1H, t, *J* = 2.5 Hz), 6.50 (1H, s), 6.16 (1H, s), 4.39 (1H, d, *J* = 8.0 Hz), 4.08–4.12 (2H, m), 2.92 (1H, d, *J* = 11.5 Hz), 2.63 (1H, t, *J* = 5.0 Hz), 2.28 (2H, t, *J* = 12.0 Hz), 2.22–1.39 (11H, m), 1.35 (9H, s), 1.22–1.18 (3H, m); δ_C (125 MHz, $CDCl_3$) 173.7, 154.4, 133.1, 130.0, 129.7, 128.1, 125.2, 115.7, 111.3, 102.6, 70.1, 68.1, 59.4, 58.8, 50.8, 35.7, 33.0, 31.9, 30.7, 30.6, 29.7, 29.3, 28.7, 26.1, 25.7, 20.6. IR (cm^{-1}) 3410, 3280, 3046, 2939, 1730, 1642, 1308, 1245, 1128. Anal. Calcd for $C_{26}H_{38}N_4O_4$: C, 66.36; H, 8.14; N, 11.91. Found: C, 66.39; H, 7.15; N, 11.93.

Ring opening of the epoxides with *iso*-butylamine: general procedure

i-ButNH_2 (1.12 g, 1.48 mmol) was added to a stirred solution of suitable epoxide (1.38 mmol) in *i*-PrOH (30 mL) at room temperature for 26 h. Then the solvent was removed under reduced

pressure. The crudes of compounds **11**, **17** and **30** were used in the subsequent reaction without any purification. Compounds **9**, **10** and **31** were purified by column chromatography on silica gel.

*(+)-(R)-1-(1H-Indol-4-yloxy)-3-isobutylamino-propan-2-ol (**9**)*

Compound **9** was obtained as a colourless thick oil (0.400 g, 99%). $[\alpha]_D^{20}$ +6.8 (c 1.2, $CHCl_3$); Rf 0.5 ($CHCl_3$/CH_3OH 9:1); δ_H (500 MHz, $CDCl_3$) 8.30 (1H, s), 7.14–7.05 (3H, m), 6.68 (1H, d, *J* = 2.5 Hz), 6.55 (1H, d, *J*= 7.5 Hz), 4.20–4.15 (1H, m), 2.95–2.85 (2H, m), 3.72–3.68 (2H, m), 2.87 (2H, d, *J* = 7.0 Hz), 1.85–1.80 (1H, m), 0.94 (3H, d, *J* = 3.5 Hz), 0.93 (3H, d, *J* = 3.5 Hz); δ_C (125 MHz, $CDCl_3$) 152.9, 131.2, 128.2, 125.1, 112.4, 111.7, 103.6, 101.9, 71.3, 67.9, 57.4, 51.8, 27.7, 20.4. IR (cm^{-1}) 3402, 3313, 3050, 2958, 1455, 1159. Anal. Calcd for $C_{15}H_{22}N_2O_2$: C, 68.67; H, 8.45; N, 10.68. Found: C, 68.69; H, 8.43; N, 10.72.

*(+)-(R)-1-(1H-Indol-5-yloxy)-3-isobutylamino-propan-2-ol (**10**)*

Compound **10** was obtained as a colourless thick oil (0.358 g, 99%). $[\alpha]_D^{20}$ +6.5 (c 1.3, CH_3OH); Rf 0.5 ($CHCl_3$/CH_3OH 9:1); δ_H (500 MHz, $CDCl_3$) 8.66 (1H, s), 7.25–7.10 (3H, m), 6.85 (1H, dd, *J* = 1.5 and 8.5 Hz), 6.45 (1H, s), 4.22–4.18 (1H, m), 4.02–3.97 (2H, m), 3.72–3.68 (2H, m), 2.87 (2H, d, *J* = 7.0 Hz), 1.85–1.80 (1H, m), 0.94 (3H, d, *J* = 3.5 Hz), 0.93 (3H, d, *J* = 3.5 Hz); δ_C (125 MHz, $CDCl_3$) 152.9, 131.2, 128.2, 125.1, 112.4, 111.7, 103.6, 101.9, 71.3, 67.9, 57.4, 51.8, 27.7, 20.4. IR (cm^{-1}) 3402, 3313, 3050, 2958, 1455, 1159. Anal. Calcd for $C_{15}H_{22}N_2O_2$: C, 68.67; H, 8.45; N, 10.68. Found: C, 68.69; H, 8.43; N, 10.64.

*(+)-(1H-Indol-5-yl)-carbamic acid (2R)-hydroxy-3-isobutylamino-propyl ester (**31**)*

Compound **31** was obtained as yellow thick oil (0.270 g, 92%) from **21** (0.24 g, 0.96 mmol), *i*-$BuNH_2$ (0.3 mL, 2.9 mmol), after 34 h. $[\alpha]_D^{20}$ +4.9 (c 1.4, CH_3OH); Rf 0.6 ($CHCl_3$/CH_3OH 7:3); δ_H (500 MHz, $CDCl_3$) 8.45 (1H, s), 7.66 (1H, s), 7.27–7.04 (3H, m), 6.46 (1H, s), 4.28–4.25 (1H, m), 4.13–3.94 (2H, m), 2.75–2.41 (4H, m), 1.75–1.70 (1H, m), 0.91 (3H, d, *J* = 3.0 Hz), 0.89 (3H, d, *J* = 3.0 Hz); δ_C (125 MHz, $CDCl_3$) 154.4, 133.0, 130.1, 128.0, 125.3, 125.1, 115.5, 111.3, 102.6, 67.9, 67.2, 57.6, 51.3, 28.3, 20.5. IR (cm^{-1}) 3316, 2958, 1704, 1557, 1481, 1237, 1063. Anal. Calcd for $C_{16}H_{23}N_3O_3$: C, 62.93; H, 7.59; N, 13.76. Found: C, 62.89; H, 7.55; N, 13.68.

Synthesis of arylsulfonamides: general procedure

Dry triethylamine (0.14 mL, 1.01 mmol) and the suitable arylsulfonyl chloride (0.93 mmol) were added to a stirred solution of the substrate (0.78 mmol) in dry CH_2Cl_2 (40 mL) at room temperature under argon atmosphere. After 24 h (TLC control in $CHCl_3$/CH_3OH 99:1) the reaction

mixture was quenched by adding a 5% solution of H_2SO_4 and extracted by CH_2Cl_2. The organic layer was washed with a $NaHCO_3$ (saturated aqueous solution) and brine, then it was dried over Na_2SO_4 and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel ($CHCl_3$/CH_3OH 99:1).

*(+)--N-[(2R)-Hydroxy-3-(1H-indol-4-yloxy)-propyl]-N-isobutyl-4-nitro-benzenesulfonamide (**12a**)*

Compound **12a** was isolated as a yellow solid (0.285 g, 82%). Mp 142 °C; $[\alpha]_D^{20}$ +24.0 (c 1.0, $CHCl_3$); Rf 0.6 ($CHCl_3$/CH_3OH 99:1); δ_H (500 MHz, $CDCl_3$) 8.25 (1H, s), 8.18 (2H, d, *J* = 8.5 Hz), 7.90 (2H, d, *J* = 8.5 Hz), 7.06 (1H, s), 6.98-7.01 (2H, m),), 6.42 (1H, s), 6.39 (1H, d, *J* = 8.0 Hz), 4.18–4.09 (1H, m), 3.41–3.37 (1H, m), 3.13–2.95 (5H, m), 1.98–1.94 (1H, m), 0.96-0.84 (6H, m); δ_C (125 MHz, $CDCl_3$) 151.3, 149.1, 145.8, 133.3, 128.4, 128.2, 124.5, 124.2, 119.3, 107.8, 102.6, 98.5, 74.2, 65.3, 57.7, 52.2, 25.7, 20.9. IR (cm^{-1}) 3408, 3123, 2898, 1489, 1353, 1224. Anal. Calcd for $C_{21}H_{25}N_3O_6S$: C, 56.36; H, 5.63; N, 9.39; S, 7.17. Found: C, 56.38; H, 5.61; N, 9.35; S, 7.20.

*(+)-N-[(2R)-Hydroxy-3-(1H-indol-4-yloxy)-propyl]-N-isobutyl-3,4-dimethoxy-benzenesulfonamide (**12b**)*

Compound **12b** was isolated as a white solid (0.340 g, 94%). Mp 118 °C; $[\alpha]_D^{20}$ +4.1 (c 1.6, $CHCl_3$); Rf 0.6 ($CHCl_3$/CH_3OH 99:1); δ_H (500 MHz, $CDCl_3$) 8.31 (1H, s), 7.47 (1H, dd, *J* = 2.0 and 8.5 Hz), 7.29 (1H, d, *J* = 2.0 Hz), 7.14-7.06 (3H, m), 6.95 (1H, d, *J* = 8.5 Hz), 6.58-6.53 (2H, m), 4.39 (1H, bs), 4.22–4.00 (2H, m), 3.85 (3H, s), 3.82 (3H, s), 3.29 (2H, d, *J* = 5.5 Hz), 3.02–2.97 (2H, m), 2.87-2.83 (1H, m), 1.95–1.90 (1H, m), 1.32-1.29 (2H, m), 0.87 (3H, d, *J* = 2.5 Hz), 0.81 (3H, d, *J* = 2.5 Hz); δ_C (125 MHz, $CDCl_3$) 153.0, 150.1, 148.5, 132.9, 129.1, 128.4, 124.1, 118.5, 118.2, 115.8, 112.4, 107.5, 102.5, 99.0, 74.1, 66.7, 57.1, 56.2, 55.3, 26.5, 20.9. IR (cm^{-1}) 3421, 3118, 2954, 1565, 1320, 1148. Anal. Calcd for $C_{23}H_{30}N_2O_6S$: C, 59.72; H, 6.54; N, 6.06; S, 6.93. Found: C, 59.68; H, 6.52; N, 6.11; S, 6.90.

*(+)-N-[(2R)-Hydroxy-3-(1H-indol-5-yloxy)-propyl]-N-isobutyl-4-nitro-benzenesulfonamide (**13a**)*

Compound **13a** was isolated as a yellow solid (0.279 g, 80%). Mp 135 °C; $[\alpha]_D^{20}$ +18.0 (c 0.8, $CHCl_3$); Rf 0.7 ($CHCl_3$/CH_3OH 99:1); δ_H (500 MHz, $CDCl_3$) 8.29 (1H, d, *J* = 8.5 Hz), 8.16 (1H, s), 7.99 (2H, d, *J* = 8.5 Hz), 7.27 (1H, d, *J* = 7.0 Hz), 7.20 (1H, d, *J* = 2.5 Hz), 7.07 (1H, s), 6.80 (1H, dd, *J* = 1.5 and 8.5 Hz), 6.47 (1H, s), 4.22–4.18 (1H, m), 4.00–3.99 (2H, m), 3.42–3.38 (2H, m), 3.09–3.05 (2H, m), 2.86 (2H, d, *J* = 4.5 Hz), 2.01–1.98 (1H,m), 0.92 (3H, d, *J* = 2.5 Hz), 0.90 (3H, d, *J* = 2.5 Hz); δ_C (125 MHz, $CDCl_3$) 152.6, 149.9, 145.1, 131.3, 128.5, 128.3, 124.0, 112.3, 111.8, 103.8, 102.4, 70.2, 68.8, 57.4, 51.8, 26.7, 19.9. IR (cm^{-1}) 3417, 3102, 2961, 1529, 1349, 1159. Anal. Calcd for $C_{21}H_{25}N_3O_6S$: C, 56.36; H, 5.63; N, 9.39; S, 7.17. Found: C, 56.38; H, 5.61; N, 9.34; S, 7.15.

*(+)-N-[(2R)-Hydroxy-3-(1H-indol-5-yloxy)-propyl]-N-isobutyl-3,4-dimethoxy-benzenesulfonamide (**13b**)*

Compound **13b** was isolated as a white solid (0.339 g, 94%). Mp 118 °C; $[\alpha]_D^{20}$ +4.1 (c 1.6, $CHCl_3$); Rf 0.6 ($CHCl_3$/CH_3OH 99:1); δ_H (500 MHz, $CDCl_3$) 8.14 (1H, s), 7.45 (1H, dd, *J* 1.5 and 8.0 Hz), 7.29–7.26 (2H, m), 7.19 (1H, d, *J* = 2.5 Hz), 7.09 (1H, s), 6.93 (1H, d, *J* = 8.5 Hz), 6.83 (1H,dd, *J* 2.0 and 8.5 Hz), 6.47 (1H, t, *J* 1.0 Hz), 4.26–4.22 (1H, m), 4.06–3.99 (2H, m), 3.93 (3H, s), 3.91 (3H, s), 3.33–3.21 (2H, m), 3.04–2.92 (2H,m), 1.98–1.96 (1H, m), 0.94 (3H, d, *J* = 8.5 Hz), 0.90 (3H, d, *J* 8.5 Hz); δ_C (125 MHz, $CDCl_3$) 152.8, 152.6, 149.1, 131.3, 130.5, 128.3, 125.1, 121.3, 112.4, 111.7, 110.6, 109.9, 103.7, 102.4, 70.3, 69.2, 58.3, 56.2, 56.0, 52.7, 27.0, 20.0. IR (cm^{-1}) 3390, 2954, 1509, 1262, 1137. Anal. Calcd for $C_{23}H_{30}N_2O_6S$: C, 59.72; H, 6.54; N, 6.06; S, 6.93. Found: C, 59.70; H, 6.51; N, 6.03; S, 6.90.

*(+)-N-[(2R)-Hydroxy-3-(1H-indol-6-yloxy)-propyl]-N-isobutyl-4-nitro-benzenesulfonamide (**14a**)*

Compound **14a** was isolated as a yellow solid (0.265 g, 76%). Mp 131 °C; $[\alpha]_D^{20}$ +10.8 (c 0.5, $CHCl_3$); Rf 0.7 ($CHCl_3$/CH_3OH 99:1); δ_H (500 MHz, $CDCl_3$) 8.35 (2H, dd, *J*= 2.0 and 7.0 Hz), 8.10 (1H, s), 8.03 (2H, dd, *J*= 2.0 and 7.0 Hz), 7.52 (1H, d, *J* = 8.5 Hz), 7.15 (1H, m), 7.15 (1H, d, *J*= 2.0 Hz), 6.77 (1H, dd, *J*= 2.0 and 8.5 Hz), 6.51 (1H, m), 4.25 (1H, bs), 4.03 (2H, d, *J*= 5.0 Hz), 3.43–3.38 (2H, m), 3.09–3.06 (2H, m), 2.88 (1H, bs), 2.03–2.00 (1H, m), 0.94 (3H, d, *J* = 2.5 Hz), 0.91 (3H, d, *J* = 2.5 Hz); δ_C (125 MHz, $CDCl_3$) 154.8, 150.0, 144.9, 136.3, 128.5, 124.3, 123.4, 122.9, 121.5, 110.0, 102.4, 95.4, 69.9, 68.8, 57.7, 51.9, 29.5, 19.9. IR (cm^{-1}) 3430, 3156, 2889, 1565, 1332, 1104. Anal. Calcd for $C_{21}H_{25}N_3O_6S$: C, 56.36; H, 5.63; N, 9.39; S, 7.17. Found: C, 56.40; H, 5.66; N, 9.40; S, 7.20.

*(+)-N-[(2R)-Hydroxy-3-(1H-indol-6-yloxy)-propyl]-N-isobutyl-3,4-dimethoxy-benzenesulfonamide (**14b**)*

Compound **14b** was isolated as a white solid (0.339 g, 94%). Mp 126 °C; $[\alpha]_D^{20}$ +2.2 (c 1.0, $CHCl_3$); Rf 0.6 ($CHCl_3$/CH_3OH 99:1); δ_H (500 MHz, $CDCl_3$) 8.23 (1H, bs), 7.51 (1H, d, *J* = 8.5 Hz), 7.46 (1H, dd, *J* = 2.0 and 8.5 Hz), 7.30 (1H, d, *J*= 2.0 Hz), 7.14-7.13 (1H, m), 6.94 (1H, d, *J* = 8.5 Hz), 6.83 (1H, s), 6.76 (1H, dd, *J* = 2.0 and 8.5 Hz), 6.49 (1H, s), 4.25 (1H, bs), 4.03–3.96 (2H, m), 3.94 (3H, s), 3.93 (3H, s), 3.38-3.29 (2H, m), 3.22 (1H, d, *J* = 4.0 Hz), 3.09–2.93 (2H, m), 2.40-1.96 (1H, m), 0.95 (3H, d, *J* = 2.5 Hz), 0.92 (3H, d, *J* = 2.5 Hz); δ_C (125 MHz, $CDCl_3$) 155.2, 152.8, 149.3, 136.6, 130.7, 123.6, 122.7, 121.5, 110.9, 110.5, 110.0, 102.9, 102.5, 95.5, 70.2, 69.3, 58.4, 56.6, 56.4, 52.6, 29.9, 20.3. IR (cm^{-1}) 3402, 3098, 2922, 1560, 1280, 1142. Anal. Calcd for $C_{23}H_{30}N_2O_6S$: C, 59.72; H, 6.54; N, 6.06; S, 6.93. Found: C, 59.68; H, 6.52; N, 6.10; S, 6.90.

*(+)-N-(2R,3-Dihydroxy-propyl)-N-isobutyl-4-nitro-benzenesulfonamide (**18a**)*

Compound **18a** was isolated as a thick oil (0.192 g, 74%). $[\alpha]_D^{20}$ +2.8 (c 0.8, $CHCl_3$); Rf 0.4 ($CHCl_3/CH_3OH$ 95:5); δ_H (500 MHz, $CDCl_3$) 8.38 (2H, d, *J*= 7.5 Hz), 8.03 (2H, d, *J*= 7.0 Hz), 3.93-3.89 (1H, m), 3.73-3.65 (1H, m), 3.23 (2H, d, *J*= 6.5 Hz), 3.04-3.00 (3H, m), 1.98-1.92 (1H, m), 0.91 (3H, d, *J*= 2.5 Hz), 0.90 (3H, d, *J*= 2.5 Hz); δ_C (125 MHz, $CDCl_3$) 150.1, 144.6, 128.5, 124.4, 70.1, 58.2, 51.8, 27.1, 19.9. IR (cm^{-1}) 3410, 3071, 2957, 1709, 1535, 1346, 1161. Anal. Calcd for $C_{13}H_{20}N_2O_6S$: C, 46.98; H, 6.07; N, 8.43; S, 9.65. Found: C, 46,95; H, 6.09; N, 8.46; S, 9.62.

*(+)-N-(2R,3-Dihydroxy-propyl)-N-isobutyl-3,4-dimethoxy-benzenesulfonamide (**18b**)*

Compound **18b** was isolated as a thick oil (0.211 g, 78%). $[\alpha]_D^{20}$ +1.5 (c 1.5, $CHCl_3$); Rf 0.3 ($CHCl_3/CH_3OH$ 97:3); δ_H (500 MHz, $CDCl_3$) 7.44 (1H, d, *J* = 8.5 Hz), 7.23 (1H, s), 6.96 (1H, d, *J* = 8.5 Hz), 3.93 (3H, s), 3.91 (3H, s), 3.70-3.51 (3H, m), 2.73-2.58 (2H, m), 2.40-2.31 (2H, m), 1.96-1.89 (1H, m), 0.90 (3H, d, *J* = 2.5 Hz), 0.89 (3H, d, *J* = 2.5 Hz); δ_C (125 MHz, $CDCl_3$) 153.0, 150.1, 129.2, 118.4, 115.6, 112.2, 68.0, 64.9, 57.3, 56.2, 51.8, 25.3, 20.2. IR (cm^{-1}) 3391, 3172, 2808, 1523, 1354, 1187. Anal. Calcd for $C_{15}H_{25}NO_6S$: C, 51.86; H, 7.25; N, 4.03; S, 9.23. Found: C, 51.84; H, 7.22; N, 4.06; S, 9.22.

*(+)-(1H-indol-4-yl)-carbamic acid (2R)-hydroxy-3-[isobutyl-(4-nitro-benzenesulfonyl)-amino]-propyl ester (**32a**)*

Compound **32a** was isolated as a yellow thick oil (0.332 g, 87%). $[\alpha]_D^{20}$ +10.2 (c 1.2, $CHCl_3$); Rf 0.6 ($CHCl_3/CH_3OH$ 99:1); δ_H (500 MHz, $CDCl_3$) 8.36 (bs, 1H), 8.16 (2H, d, *J* = 8.5 Hz), 7.87 (2H, d, *J* = 8.5 Hz), 7.18-7.20 (m, 4H), 6.96 (bs, 1H), 6.50-6.51 (m, 1H), 4.26 (1H, dd, *J* = 5.0 and 11.5 Hz), 4.16–4.08 (2H, m), 3.25–3.02 (4H, m), 1.95–1.90 (1H, m), 0.91 (3H, d, *J* = 3.0 Hz), 0.89 (3H, d, *J* = 3.0 Hz); δ_C (125 MHz, $CDCl_3$) 153.7, 151.2, 145.9, 136.1, 130.3, 128.4, 127.3, 124.4, 124.1, 114.9, 112.5, 102.6, 66.3, 64.5, 57.3, 52.2, 25.8, 19.9. IR (cm^{-1}) 3420, 3073, 2952, 1721, 1513, 1354, 1168. Anal. Calcd for $C_{22}H_{26}N_4O_7S$: C, 53.87; H, 5.34; N, 11.42; S, 6.54. Found: C, 53.83; H, 5.38; N, 11.46; S, 6.52.

*(+)-(1H-indol-4-yl)-carbamic acid (2R)-hydroxy-3-[isobutyl-(3,4-dimethoxy-benzenesulfonyl)-amino]-propyl ester (**32b**)*

Compound **32b** was isolated as a thick oil (0.335 g, 85%). $[\alpha]_D^{20}$ +2.4 (c 1.0, $CHCl_3$); Rf 0.7 (CH_2Cl_2/EtOAc 98:2); δ_H (500 MHz, $CDCl_3$) 8.36 (1H, bs), 7.47 (1H, m), 7.33 (1H, d, *J* = 8.5 Hz), 7.18 (1H, d, *J*= 5.5 Hz), 7.10-7.06 (3H, m), 6.9 (1H, m), 6.80 (1H, d, *J* = 8.5 Hz), 6.4 (1H, s), 4.26-4.17 (2H, m), 4.08 (1H, bs), 3.82 (3H, s), 3.79 (3H, s), 3.41 (1H, bs), 3.18-3.05 (2H, m), 2.93-2.80 (2H, m), 1.85-1.78 (1H, m), 0.85 (3H, d, *J* = 2.5 Hz), 0.82 (3H, d, *J* = 2.5 Hz); δ_C (125 MHz, $CDCl_3$) 152.7, 149.1, 136.5, 130.0, 129.5, 123.9, 122.6, 121.3, 110.6, 109.9, 107.8, 98.4, 69.2, 66.9, 58.4, 56.2, 56.1,

52.5, 27.1, 20.1, 19.9. IR (cm^{-1}) 3392, 3106, 2955, 1548, 1193, 1178. Anal. Calcd for $C_{24}H_{31}N_3O_7S$: C, 57.01; H, 6.18; N, 8.31; S, 6.34. Found: C, 57.04; H, 6.15; N, 8.33; S, 6.37.

*(+)-(1H-indol-5-yl)-carbamic acid (2R)-hydroxy-3-[isobutyl-(4-nitro-benzenesulfonyl)-amino]-propyl ester (**33a**)*

Compound **33a** was isolated as a yellow thick oil (0.344 g, 90%). $[\alpha]_D^{20}$ +8.8 (c 0.8, $CHCl_3$); Rf 0.6 ($CHCl_3$/CH_3OH 99:1); δ_H (500 MHz, $CDCl_3$) 8.38 (1H, s), 8.24 (2H, br s), 7.95 (2H, br s), 7.63 (1H, s), 7.28–7.00 (4H, m), 6.46 (1H, s), 4.27 (1H, dd, *J* = 5.0 and 11.5 Hz), 4.18–4.09 (2H, m), 3.27–3.01 (4H, m), 1.95–1.91 (1H, m), 0.92 (3H, d, *J* = 3.0 Hz), 0.89 (3H, d, *J* = 3.0 Hz); δ_C (125 MHz, $CDCl_3$) 154.2, 149.9, 144.8, 133.1, 129.8, 128.6, 128.4, 128.1, 125.5, 124.3, 115.6, 111.5, 102.4, 68.8, 66.8, 57.4, 51.6, 26.8, 19.9. IR (cm^{-1}) 3410, 3071, 2957, 1709, 1535, 1346, 1161. Anal. Calcd for $C_{22}H_{26}N_4O_7S$: C, 53.87; H, 5.34; N, 11.42; S, 6.54. Found: C, 53.82; H, 5.36; N, 11.45; S, 6.51.

*(+)-(1H-indol-5-yl)-carbamic acid (2R)-hydroxy-3-[isobutyl-(3,4-dimethoxy-benzenesulfonyl)-amino]-propyl ester (**33b**)*

Compound **33b** was isolated as a thick oil (0.323 g, 82%). $[\alpha]_D^{20}$ +1.8 (c 1.0, $CHCl_3$); Rf 0.7 (CH_2Cl_2/EtOAc 98:2); δ_H (500 MHz, $CDCl_3$) 8.38 (1H, bs), 7.72-7.68 (1H, m), 7.43-7.39 (1H, m), 7.32-7.24 (2H, m), 7.19 (1H, s), 7.11-7.07 (1H, m), 6.95-6.85 (1H, m), 6.44 (1H, s), 4.28-4.14 (2H, m), 4.07 (1H, bs), 3.84 (3H, s), 3.83 (3H, s), 3.58 (1H, bs), 3.25-3.12 (2H, m), 3.03-2.85 (2H, m), 1.91-1.82 (1H, m), 0.88 (3H, d, *J* = 2.5 Hz), 0.83 (3H, d, *J* = 2.5 Hz); δ_C (125 MHz, $CDCl_3$) 154.3, 152.7, 149.1, 133.0, 130.1, 129.9, 128.0, 125.3, 121.3, 115.6, 111.6, 111.3, 110.6, 109.9, 102.6, 69.2, 66.8, 58.3, 56.2, 56.1, 52.5, 27.1, 20.1, 19.9. IR (cm^{-1}) 3445, 3108, 2918, 1498, 1265, 1123. Anal. Calcd for $C_{24}H_{31}N_3O_7S$: C, 57.01; H, 6.18; N, 8.31; S, 6.34. Found: C, 57.04; H, 6.22; N, 8.28; S, 6.32.

*(+)-(1H-indol-5-yl)-carbamic acid (2R)-hydroxy-3-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-propyl ester (**33c**)*

Compound **33c** was isolated as a brown solid (0.285 g, 77%). Mp 96 °C; $[\alpha]_D^{20}$ +3.2 (c 1.0, $CHCl_3$); Rf 0.6 ($CHCl_3$/MeOH 95:5); δ_H (500 MHz, $CDCl_3$) 8.44 (1H, s), 7.69-7.50 (3H, m), 7.23 (1H, d, *J* = 9.5 Hz), 7.13 (1H, s), 7.04 (1H, d, *J* = 9.0 Hz), 6.96-6.88 (3H, m), 6.42 (1H, s), 4.24-4.09 (3H, m), 3.77 (3H, s), 3.60 (1H, s), 3.46 (1H, d, *J* = 8.5 Hz), 3.13 (2H, bs), 2.91 (2H, d, *J* = 8.5 Hz), 1.24-1.17 (6H, m); δ_C (125 MHz, $CDCl_3$) 162.9, 154.4, 133.0, 130.9, 129.9, 129.5, 128.7, 128.0, 125.3, 115.6, 114.3, 111.3, 102.4, 69.1, 66.7, 58.3, 55.5, 52.4, 27.1, 19.9. IR (cm^{-1}) 3486, 3122, 2866, 1423, 1196, 1141. Anal. Calcd for $C_{23}H_{29}N_3O_6S$: C, 58.09; H, 6.15; N, 8.84; S, 6.74. Found: C, 58.11; H, 6.18; N, 8.82; S, 6.75.

*(+)-(R)-4-Amino-N-[3-(1H-indol-5-yloxy)-2-hydroxypropyl]-N-isobutyl-benzenesulfonamide (**13c**)*

Compound **13a** (0.104 g, 0.23 mmol) was added to a suspension of 10% Pd/C (14 mg) in ethyl acetate (20 mL) under hydrogen atmosphere. After 28 h the reaction mixture was filtered on a plate of Celite, concentrated under *vacuo* and purified by column chromatography on silica gel ($CHCl_3$/CH_3OH 95:5) to afford compound **13c** as a violet thick oil (0.071 g, 74%). $[\alpha]_D^{20}$ +12.0 (c 1, CH_3OH); Rf 0.3 ($CHCl_3$/CH_3OH 95:5); δ_H (500 MHz, CD_3OD) 7.64 (2H, d, *J* = 9.0 Hz), 7.26 (1H, d, *J* = 8.5 Hz), 7.18 (1H, d, *J* = 3.0 Hz), 7.06 (1H, d, *J* = 2.5 Hz), 6.99 (1H, d, *J* = 9.0 Hz), 6.79 (1H, dd, *J* = 2.0 and 9.0 Hz), 6.36 (1H, d, *J* = 2.5 Hz), 4.18–4.14 (1H, m), 4.00 (1H, dd, *J* = 4.0 and 10.0 Hz), 3.94 (1H, dd, *J* = 5.0 and 9.5 Hz), 3.42 (1H, dd, *J* = 4.5 and 15.0 Hz), 3.11 (1H, dd, *J* 7.0 and 14.5 Hz), 2.96 (1H, dd, *J* 7.5 and 13.5 Hz), 2.88 (1H, dd, *J* = 7.5 and 13.5 Hz), 2.01–1.97 (1H, m), 0.93 (3H, d, = 3.0 Hz), 0.91 (3H, d, *J* 3.0 Hz); δ_C (125 MHz, CD_3OD) 157.1, 154.1, 133.1, 129.8, 126.2, 114.5, 113.2, 112.9, 112.7, 104.4, 102.2, 71.9, 70.3, 59.1, 53.1, 28.0, 20.5. IR (cm^{-1}) 3465, 3312, 3065, 2954, 1509, 1262, 1137. Anal. Calcd for $C_{21}H_{27}N_3O_4S$: C, 60.41; H, 6.52; N, 10.06; S, 7.68. Found: C, 60.39; H, 6.53; N, 10.10; S, 7.65.

Toluene-4-sulfonic acid (2R)-hydroxy-3-[isobutyl-(4-nitro-benzenesulfonyl)-amino]-propyl ester and *Toluene-4-sulfonic acid (2R)-hydroxy-3-[isobutyl-(3,4-dimethoxy-benzenesulfonyl)-amino]-propyl ester*

Dry pyridine (0.095 mL, 1.16 mmol) and tosyl chloride (0.222 g, 1.16 mmol) were added to a stirred solution of compound **18a** (0.351 g, 1.06 mmol) (or **18b**, 0.368 g, 1.06 mmol) in dry CH_2Cl_2 (15 mL) at room temperature. The mixture was stirred in argon atmosphere for 20 h, then it was washed with diluted hydrochloric acid (0.1 M, 10 mL), with a saturated aqueous solution of $NaHCO_3$ and finally with brine. The organic layer was dried and evaporated under reduced pressure. *Toluene-4-sulfonic acid (2R)-hydroxy-3-[isobutyl-(4-nitro-benzenesulfonyl)-amino]-propyl ester* and *Toluene-4-sulfonic acid (2R)-hydroxy-3-[isobutyl-(3,4-dimethoxy-benzenesulfonyl)-amino]-propyl ester* were obtained as yellow oil and used in the subsequent reaction without any purification.

*N-Isobutyl-4-nitro-N-(R)-oxiranylmethyl-benzenesulfonamide (**19a**)* and *N-Isobutyl-3,4-dimethoxy-N-(R)-oxiranylmethyl-benzenesulfonamide (**19b**)*

Potassium carbonate was added to a stirred solution of the crude tosyl derivative of **18a** (or **18b**) in methanol (26 mL) at room temperature.

After disappearance of the starting material, the reaction was quenched by adding ammonium chloride (saturated aqueous solution), then extracted with diethyl ether and washed with brine.

The combined extracts were dried over Na_2SO_4 and evaporated under reduced pressure to give the crude epoxide.

*N-Isobutyl-4-nitro-N-(R)-oxiranylmethyl-benzenesulfonamide (**19a**)* was isolated as thick oil (0.280 g, 84% in two steps) $[\alpha]_D^{20}$ +2.4 (c 1.2, $CHCl_3$); Rf 0.6 (EP/EtOAc 7:3); δ_H (500 MHz, $CDCl_3$) 8.38 (2H, d, *J* = 8.5 Hz), 8.04 (2H, d, *J* = 8.5 Hz), 3.70 (1H, dd, *J*= 15.0 and 3.0 Hz), 3.13 (1H, dd, *J*= 14.0 and 8.0 Hz), 3.06-2.90 (3H, m), 2.79-2.81 (1H, dd, *J*= 5.0 and 3.0 Hz), 2.53 (1H, dd, *J*= 5.0 and 3.0 Hz), 2.06-1.97 (1 H, m), 0.97 (3H, d, *J* = 7.0 Hz), 0.92 (3H, d, *J* = 6.5 Hz); δ_C (125 MHz, $CDCl_3$) 151.3, 145.9, 128.4, 124.3, 58.1, 51.6, 50.2, 45.4, 25.7, 20.2. IR (cm^{-1}) 3389, 3135, 2885, 1537, 1328, 1137. Anal. Calcd for $C_{13}H_{18}N_2O_5S$: C, 49.67; H, 5.77; N, 8.91; S, 10.20. Found: C, 49.64; H, 5.79; N, 8.88; S, 10.23.

*N-Isobutyl-3,4-dimethoxy-N-(R)-oxiranylmethyl-benzenesulfonamide (**19b**)* was isolated as thick oil (0.282 g, 81% in two steps) $[\alpha]_D^{20}$ +1.6 (c 0.8, $CHCl_3$); Rf 0.7 (EP/EtOAc 6:4); δ_H (500 MHz, $CDCl_3$) 7.42 (1H, d, *J* = 8.5 Hz), 7.25 (1H, s), 6.95 (1H, d, *J* = 8.5 Hz), 4.05-3.97 (1H, m), 3.95 (3H, s), 3.93 (3H, s), 3.56-3.50 (3H, m), 3.20-2-97 (3H, m), 1.98-1.92 (1H, m), 0.97-0.89 (6H, m); δ_C (125 MHz, $CDCl_3$) 153.2, 150.3, 129.1, 118.5, 115.4, 112.2, 56.6, 56.1, 51.7, 50.2, 45.6, 25.7, 20.1. IR (cm^{-1}) 3492, 3202, 2796, 1540, 1365, 1204. Anal. Calcd for $C_{15}H_{23}NO_5S$: C, 54.69; H, 7.04; N, 7.25; S, 9.73.

Ring opening of epoxides with aminoindoles: general procedure

4-Aminoindole **20** (or 5-aminoindole **21**) (0.145 g, 1.10 mmol) was added to a stirred solution of the epoxide **19a** (0.283 g, 0.9 mmol) (or epoxide **19b**, 0.296 g, 0.9 mmol) in *i*-PrOH (15 mL). The mixture was heated at reflux temperature until disappearance of epoxide (about 10 h, TLC control, $CHCl_3/CH_3OH$ 9:1). After cooling, the solvent was removed under reduced pressure and the crude was purified by column chromatography on silica gel (eluent: $CHCl_3/CH_3OH$ 9:1).

*N-[(2R)-Hydroxy-3-(1H-indol-4-ylamino)-propyl]-N-isobutyl-4-nitro-benzenesulfonamide (**22a**)* was isolated as a brown thick oil (0.309 g, 77%). $[\alpha]_D^{20}$ +18.0 (c 0.8, $CHCl_3$); Rf 0.6 ($CHCl_3/CH_3OH$ 99:1); δ_H (500 MHz, $CDCl_3$) 8.18 (2H, d, *J* = 8.5 Hz), 8.15 (1H, s), 7.85 (2H, d, *J* = 8.5 Hz), 7.06 (1H, s), 6.98-6.93 (1H, m), 6.80 (1H, d, *J* = 8.0 Hz), 6.42 (1H, s), 6.19 (1H, d, *J* = 7.5 Hz), 4.15–4.04 (1H, m), 3.39–3.35 (1H, m), 3.24–3.19 (3H, m), 2.96 (2H, d, *J* = 7.5 Hz), 1.91–1.84 (1H, m), 0.85-0.82 (6H, m); δ_C (125 MHz, $CDCl_3$) 151.5, 146.1, 135.8, 135.3, 128.3, 127.4, 124.3, 124.2, 107.2, 104.3, 102.1, 99.4, 68.2, 57.3, 53.2, 51.2, 25.9, 20.1. IR (cm^{-1}) 3422, 3112, 2945, 1587, 1298, 1087. Anal. Calcd for $C_{21}H_{26}N_4O_5S$: C, 56.49; H, 5.87; N, 12.55; S, 7.18. Found: C, 56.51; H, 5.88; N, 12.52; S, 7.20.

*N-[(2R)-Hydroxy-3-(1H-indol-4-ylamino)-propyl]-N-isobutyl-3,4-dimethoxy-benzenesulfonamide (**22b**)* was isolated as a brown thick oil (0.278 g, 67%). $[\alpha]_D^{20}$ +3.5 (c 1.2, $CHCl_3$); Rf 0.5 ($CHCl_3/CH_3OH$ 99:1); δ_H (500 MHz, $CDCl_3$) 8.18 (1H, s), 7.35 (1H, dd, *J* = 2.0 and 8.5 Hz), 7.02-6.94 (2H, m), 7.83-7.78 (2H, m), 6.47-6.40 (1H, m), 6.24 (1H, d, *J* = 8.5 Hz), 4.18 (1H, bs), 3.85 (3H, s), 3.80 (3H, s), 3.40–3.36 (1H, m), 3.29-3.18 (2H, m), 3.15–2.82 (4H, m), 1.90-1.81 (1H, m), 0.87 (3H, d, *J* = 2.5 Hz), 0.81 (3H, d, *J* = 2.5 Hz); δ_C (125 MHz, $CDCl_3$) 152.7, 149.2, 141.3, 136.5, 130.2, 123.4, 122.1, 121.3, 117.2, 110.7, 110.0, 101.9, 99.8, 98.8, 69.3, 58.6, 56.3, 56.2, 54.0, 47.4, 27.3, 20.1, 20.0. IR (cm^{-1}) 3408, 3145, 2814, 1505, 1290, 1121. Anal. Calcd for $C_{23}H_{31}N_3O_5S$: C, 59.85; H, 6.77; N, 9.10; S, 6.95. Found: C, 59.88; H, 6.80; N, 9.07; S, 6.99.

*N-[(2R)-Hydroxy-3-(1H-indol-5-ylamino)-propyl]-N-isobutyl-4-nitro-benzenesulfonamide (**23a**)* was isolated as a brown thick oil (0.297 g, 74%). $[\alpha]_D^{20}$ +21.0 (c 1.0, $CHCl_3$); Rf 0.6 ($CHCl_3/CH_3OH$ 99:1); δ_H (500 MHz, $CDCl_3$) 8.18 (2H, d, *J* = 8.5 Hz), 8.16 (1H, s), 7.85 (2H, d, *J* = 8.5 Hz), 7.06 (1H, s), 6.98-6.93 (1H, m), 6.80 (1H, d, *J* = 8.0 Hz), 6.42 (1H, s), 4.19 (1H, d, *J* = 7.5 Hz), 4.12–4.05 (1H, m), 3.41–3.38 (1H, m), 3.25–3.21 (3H, m), 2.95 (2H, d, *J* = 7.5 Hz), 1.91–1.82 (1H, m), 0.85-0.82 (6H, m); δ_C (125 MHz, $CDCl_3$) 151.5, 146.2, 137.1, 129.3, 128.3, 124.5, 124.2, 123.8, 113.6, 104.8, 102.5, 101.7, 68.4, 57.1, 53.7, 51.4, 26.2, 20.1. IR (cm^{-1}) 3465, 3112, 2944, 1517, 1323, 1178. Anal. Calcd for $C_{21}H_{25}N_4O_5S$: C, 56.49; H, 5.87; N, 12.55; S, 7.18. Found: C, 56.51; H, 5.89; N, 12.51; S, 7.15.

*N-[(2R)-Hydroxy-3-(1H-indol-5-ylamino)-propyl]-N-isobutyl-3,4-dimethoxy-benzenesulfonamide (**23b**)* was isolated as a brown thick oil (0.286 g, 69%). $[\alpha]_D^{20}$ +4.1 (c 1.0, $CHCl_3$); Rf 0.6 ($CHCl_3/CH_3OH$ 98:2); δ_H (500 MHz, $CDCl_3$) 8.06 (1H, s), 7.42 (1H, dd, *J* = 2.0 and 8.5 Hz), 7.22-7.14 (2H, m), 6.95-6.91 (2H, m), 6.71 (1H, d, *J* = 8.5 Hz), 6.41 (1H, s), 4.15-4.13 (1H, m), 3.94 (3H, s), 3.91 (3H, s), 3.36–3.16 (4H, m), 3.02-2.91 (2H, m), 1.94-1.89 (1H, m), 0.94 (3H, d, *J* = 2.5 Hz), 0.92 (3H, d, *J* = 2.5 Hz); δ_C (125 MHz, $CDCl_3$) 152.9, 149.4, 141.4, 131.1, 130.5, 129.0, 125.0, 121.6, 113.2, 112.0, 110.9, 110.2, 104.3, 102.2, 69.2, 58.7, 56.5, 56.4, 54.1, 50.1, 27.5, 20.4, 20.2. IR (cm^{-1}) 3422, 3202, 2774, 1445, 1288, 1091. Anal. Calcd for $C_{23}H_{31}N_3O_5S$: C, 59.85; H, 6.77; N, 9.10; S, 6.95. Found: C, 59.84; H, 6.79; N, 9.09; S, 6.94.

*(1H-Indol-4-yl)-carbamic acid 4-nitro-phenyl ester (**24**) and (1H-Indol-5-yl)-carbamic acid 4-nitro-phenyl ester (**25**).*

Dry triethylamine (0.54 mL, 3.9 mmol) and *p*-nitrophenylchlorocarbonate (0.786 g, 3.9 mmol) were added to a stirred solution of 4-aminoindole **22** (or 5-aminoindole **23**) (0.396 g, 3.0 mmol) in dry CH_2Cl_2 (30 mL) at room temperature and in argon atmosphere. After 2 h a TLC control (CH_2Cl_2/EtOAc 99:1) showed the disappearance of the aminoindole and the reaction was

quenched by adding water (15 mL). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in *vacuo* affording compound **24** (or **25**) as a yellow solid. This compound was used as crude for the following reaction.

*(+)-(1H-Indol-4-yl)-carbamic acid (R)-oxiranylmethyl ester (**26**)* and *(+)-(1H-Indol-5-yl)-carbamic acid (R)-oxiranylmethyl ester (**27**)*

Dry triethylamine (0.7 mL, 5 mmol) and *S*-glycidol 1 (0.279 g, 3.8 mmol) were added to a stirred solution of the crude compound **24** (or **25**) in dry CH_2Cl_2 (30 mL) at room temperature and under argon atmosphere. After 10 h and the disappearance of glycidol (by TLC monitoring, CH_2Cl_2/EtOAc 8:2) the mixture was diluted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in *vacuo*. The crude was purified by column chromatography (CH_2Cl_2/EtOAc 8:2).

*(+)-(1H-Indol-4-yl)-carbamic acid (R)-oxiranylmethyl ester (**26**)* was obtained as a yellow solid (0.612 g, 88% in two steps). Mp 113 °C; $[\alpha]_D^{20}$ +9.2 (c 1.2, $CHCl_3$); Rf 0.4 (CH_2Cl_2/EtOAc 8:2); δ_H (500 MHz, $CDCl_3$) 8.36 (1H, bs,) 7.18-7.20 (4H, m), 6.96 (1H, bs), 6.50-6.51 (1H, m), 4.60-4.63 (1H, m), 4.03-4.10 (1H, m), 3.32-3.45 (1H, m), 2.90-2.92 (1H, m), 2.71-2.75 (1H, m); δ_C (125 MHz, $CDCl_3$) 153.8, 134.7, 130.3, 127.8, 124.5, 114.9, 112.4, 106.9, 102.6, 64.6, 50.3, 44.2. IR (cm^{-1}) 3465, 3218, 2804, 1425, 1308, 1121. Anal. Calcd for $C_{12}H_{12}N_2O_3$: C, 62.06; H, 5.21; N, 12.06. Found: C, 62.09; H, 5.24; N, 12.08.

*(+)-(1H-Indol-5-yl)-carbamic acid (R)-oxiranylmethyl ester (**27**)* was obtained as a yellow solid (0.626 g, 90% in two steps). Mp 109 °C; $[\alpha]_D^{20}$ +7.0 (c 1, $CHCl_3$); Rf 0.3 (CH_2Cl_2/EtOAc 8:2); δ_H (500 MHz, $CDCl_3$) 8.18 (1H s), 7.69 (1H, s), 7.33–7.13 (3H, m), 6.70 (1H, s), 6.51 (1H, d, *J* = 2.5 Hz), 4.56 (1H, dd, *J* = 3.0 and 12.5 Hz), 4.01 (1H, dd, *J* = 5.5 and 11.0 Hz), 3.30–2.71 (3H, m); δ_C (125 MHz, $CDCl_3$) 153.7, 133.0, 130.0, 128.1, 125.3, 115.7, 111.3, 111.1, 102.7, 65.5, 49.8, 44.7. IR (cm^{-1}) 3456, 3264, 2708, 1418, 1292, 1065. Anal. Calcd for $C_{12}H_{12}N_2O_3$: C, 62.06; H, 5.21; N, 12.06. Found: C, 62.09; H, 5.24; N, 12.09.

Protease inhibition assay

Biological assays were performed by measuring the increase in the fluorescence due to the Abz-NF*-6 (K_m=37±8 µM; V_{max}=690±90 nmol min^{-1} (mg protease)$^{-1}$; K_{cat}=0.29±0.03 S^{-1}; K_{cat}/K_m=7.8±0.3 mM^{-1}) substrate's hydrolysis by a commercially available HIV-PR, at a λ_{exc} and at a λ_{em} of 325 nm and 420 nm, respectively; 114 µL of the fluorogenic substrate (with a concentration of 53 µM, obtained by diluting 10 µL of a stock solution containing 10 mg mL^{-1} of substrate in DMSO with

1.99 mL of pH 5.5 MES buffer) were put in a thermostated cuvette (25 °C) with 75 µL of MES buffer (containing 100 mM 2-[*N*-morpholino]ethansulfonic acid (MES)/NaOH, pH 5.5; 400 mM NaCl; 1 mM ethylendiaminotetracetic acid (EDTA); 1 mM dithiotreitol (DTT)), obtaining a final concentration of 30 µM and starting measuring the fluorescence. After 1.5 min the HIV-PR was added (11 µL of a solution obtained diluting 1:100, with a MES/BSA buffer, a stock solution of 0.4 mg mL^{-1} of HIV-PR in a 10 mM sodium phosphate pH=6.5, 1 mM EDTA/10% glycerol/0.05% mercaptoethanol/50–100 mM NaCl), obtaining an enzymatic concentration of 10 nm. The increase in the fluorescence was then measured; after 1 min the inhibitor containing solution was added (2 µL) and the fluorescence measured for other additional 10 min. For each inhibitor, a stock solution in DMSO was prepared by weight, then diluted it with DMSO or MES buffer. The amount of the inhibition was evaluated comparing the initial rates, extrapolated from the linear parts of the curves obtained by plotting fluorescence versus time, of the catalyzed reaction in the presence of different inhibitor's concentration. IC_{50} values were obtained simply by plotting the different slopes versus inhibitor's concentrations (expressed using a logarithmical scale) and interpolating the value corresponding to the 50% of inhibition.

Molecular modelling

To inspect the binding mode of the synthesized inhibitors to the HIV-PR, conformation searching, docking and binding pose refining were carried out for each of the molecules using MOE program (Chemical Computing Group, Montreal, QC, Canada. Version 2012.10). First, crystal structures of Darunavir (ligand 017 from the *Protein Data Bank* entry 2IEO) and Nelfinavir (ligand 1UN from the PDB entry 2Q63) were taken as references for the sulfonamide derivatives and decahydro-isoquinoline derivatives, respectively. Then, possible conformations of the ligands were generated by a grid searching algorithm considering the linkers to be the flexible part of the molecules. After molecular mechanics geometry optimization, each of the conformers were inserted into the binding site by superimposing the ligand to the reference inhibitor structure. A multi-step in situ energy minimization was carefully performed and the complexes were refined with help of the MOE LigX module. For each compound, over 30 reasonable ligand poses (out of many generated ligand conformations) were further refined and only the pose with the lowest energy of the enzyme-inhibitor complex was kept.

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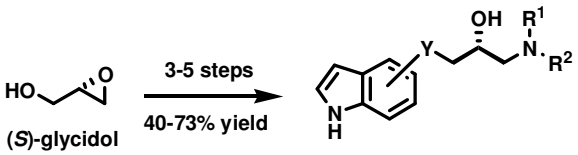
Graphical Abstract



Y = 4-, 5- or 6- O, NH, NHCOO
R^1, R^2 = PHIQ or R^1 = *i*-but, R^2 = $ArSO_2$
IC_{50} up to 1 μM