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Indolylarylsulfones, a fascinating story of highly potent human immunodeficiency virus type 1 non-nucleoside reverse transcriptase inhibitors

Valeria Famiglini and Romano Silvestri

Abstract

Indolylarylsulfones are a potent class of human immunodeficiency virus type 1 non-nucleoside reverse transcriptase inhibitors. In this review, the structure activity relationship (SAR) studies to improve the profile of sulfone L-737,126 discovered by Merck AG have been analysed with focus on introduction of the 3′,5′-dimethyl groups at the 3-phenylsulfonyl moiety, the 2-hydroxyethyl tail at the indole-2-carboxamide nitrogen, coupling of the carboxamide nitrogen with one or two glycinamide and alaninamide units, a fluorine atom at position 4 of the indole ring and correlation between configuration of the asymmetric centre and linker length. IAS derivatives look like promising drug candidates for the treatment of AIDS and related infections in combination with other antiretroviral agents.

Keywords

Human immunodeficiency virus type 1, non-nucleoside reverse transcriptase inhibitor, indolylarylsulfone

Introduction

Human immunodeficiency virus type 1 (HIV-1) is the causative agent of HIV infection and acquired immunodeficiency syndrome (AIDS). HIV remains a major global public health issue, having claimed more than 35 million lives so far. AIDS and HIV-related infection caused some 1.1 million deaths in 2015 with more than two million HIV newly infected people.[1] Current combination antiretroviral therapies (cART) combine drugs targeting different steps of the HIV life cycle: combination of three or four antiretroviral drugs has proven to inhibit effectively the infection in HIV-1-infected people adhering to a cART regimen.[1] One cART pill a day remarkably reduces the risk of acquiring the infection in pre- and post-exposure prophylaxis to HIV uninfected people.[2] Thus far, there are no safe and effective vaccines available for HIV. A National Institutes of Health funded study suggested that HIV preventive vaccination could reduce the number of HIV-infected people by 36% globally over a period of 15 years.[3]

The approved anti-HIV-1 drugs can be viewed as falling in five main classes: nucleoside reverse transcriptase inhibitors (NRTIs) and nucleotide reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors which prevent the maturation step, viral entry inhibitors (fusion inhibitors and co-receptor antagonists) and integration inhibitors.[4–8] These antiretroviral drugs are taken singly or combined into multidrug pills.[9] The cART regimens have proven to be effective inhibitors of the HIV replication and prevent the breakthrough of the infection in early stages of the disease. After starting treatment with cART, the levels of plasma viraemia fall below the limit of detection within 24 weeks and remain for at least six months in most treated patients.[10,11] Despite its great initial effectiveness, long-term cART treatments cause the development of

Department of Drug Chemistry and Technologies, Sapienza University of Rome, Laboratory affiliated to Istituto Pasteur Italia – Fondazione Cenci Bolognetti, Roma, Italy

Corresponding author:
Romano Silvestri, Department of Drug Chemistry and Technologies, Sapienza University of Rome, Laboratory affiliated to Istituto Pasteur Italia – Fondazione Cenci Bolognetti, Piazzale Aldo Moro 5, Roma I-00185, Italy.
Email: romano.silvestri@uniroma1.it

drug resistance[12] and cross resistance among compounds of the same class,[13–15] toxicological problem and adverse side reaction, with associated compliance failure to the prescribed cART regimens.[16] There is still a need of new antiretroviral drugs with improved resistance profiles and better tolerability.

Reverse transcriptase (RT) is a heterodimeric enzyme which is composed of two subunits, p66 and p51.[17,18] The RT catalyses the transformation of the RNA retroviral genome into proviral DNA[19] The catalytic core of the RT is placed in the p66 subunit and resembles a right hand with fingers, palm, thumb and connection subdomains. The p51 subunit does not exhibit any catalytic activity and shows structural capacity only.[20,21] The NNRTIs behave as non-competitive allosteric inhibitors at the non-nucleoside binding site (NNBS) located a 10 Å distance from the catalytic site in the p66 palm subdomain.[22,23]

On 21 June 1996, the U.S. Food and Drug Administration (FDA) approved the first HIV-1 NNRTI, nevirapine (**[1]**, NVP) (Viramune, Boehringer Ingelheim), for use in combination with NRTIs in HIV-1-infected adults. On 4 April 1997, delavirdine mesylate (**[2]**, DLV) (Rescriptor, Pharmacia & Upjohn) and on 17 September 1998 efavirenz (**[3]**, EFV) (Sustiva, DuPont) were approved for the treatment of HIV-1 infection. Etravirine (**[4]**, ETR) (TMC-125, Intelence, Tibotec) and rilpivirine (**[5]**, RPV) (Edurant, Tibotec) were approved by the FDA on 18 January 2008 and 20 May 2011, respectively, for treatment in drug combination of HIV-1-infected people for whom NNRTI-based therapies have failed (Chart 1).

First sulfone HIV-1 NNRTIs

In 1993, McMahon et al.[24] at the National Cancer Institute (NCI) of Bethesda started a drug-screening programme to identify new synthetic compounds and natural products with anti-HIV-1 activity. This study led to the selection of 2-nitrophenyl phenyl sulfone (**[6]**, NPPS) (Chart 2), a diarylsulfone which showed appreciable anti-HIV-1 activity at micromolar concentration. Studies on diarylsulfone congeners led to the discovery of potent classes, such as 2-carboxamido-3-arylsulfonylthiophene **[7]**,[25] 2-amino-6-arylsulfonylbenzonitrile **[8]**[26] and diarylsulfone[27] derivatives.

At the time of the diarylsulfone project at the NCI, new pyrryl aryl sulfones (PASs) structurally related to NPPS, for example 2-nitro-PAS **[9]**, were synthesized. First-generation PASs were characterized by a nitro group at position 2 of the benzene ring and an ethoxycarbonyl group at position 2 of the pyrrole nucleus.[28] An important progress in PAS development was achieved by replacing the 2-nitrobenzene group with a 4-chloroanilino moiety to obtain 2-amino-PASs, for example **[10]**, with potent antiviral activity at micromolar concentrations.[29–32] The 4-chloroaniline was also a key pharmacophore for the antiretroviral activity of pyrrolo[1,2-*b*][1,2,5]benzothiadiazepines (PBTDs, for example **[11]**).[32–34] PBTD NNRTIs, structurally related

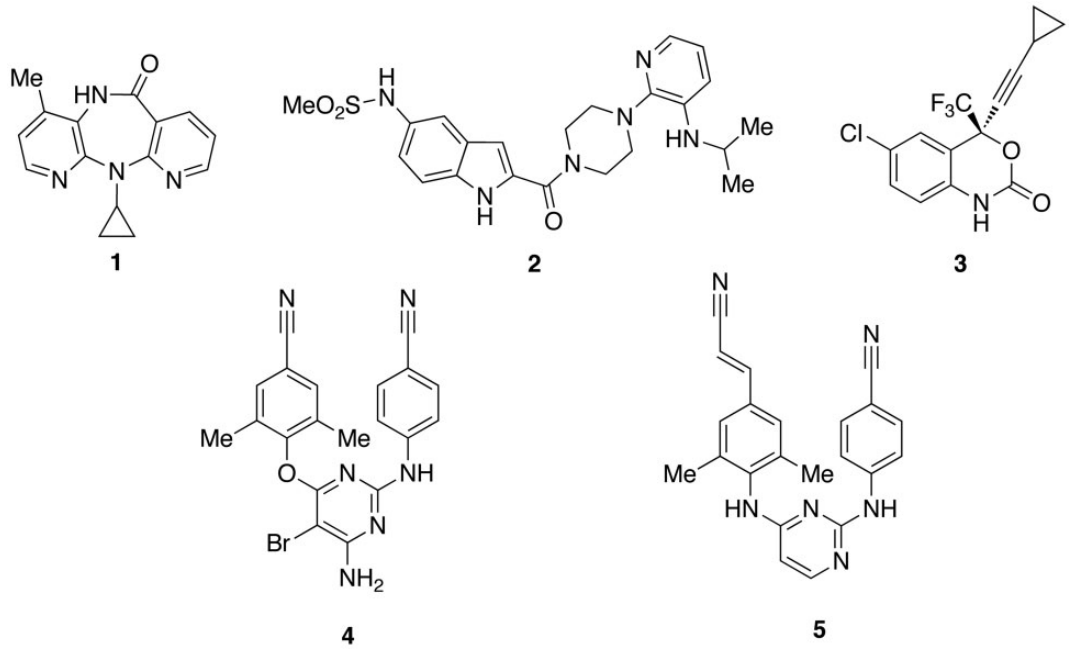


Chart 1. HIV-1 NNRTIs in clinical practice.

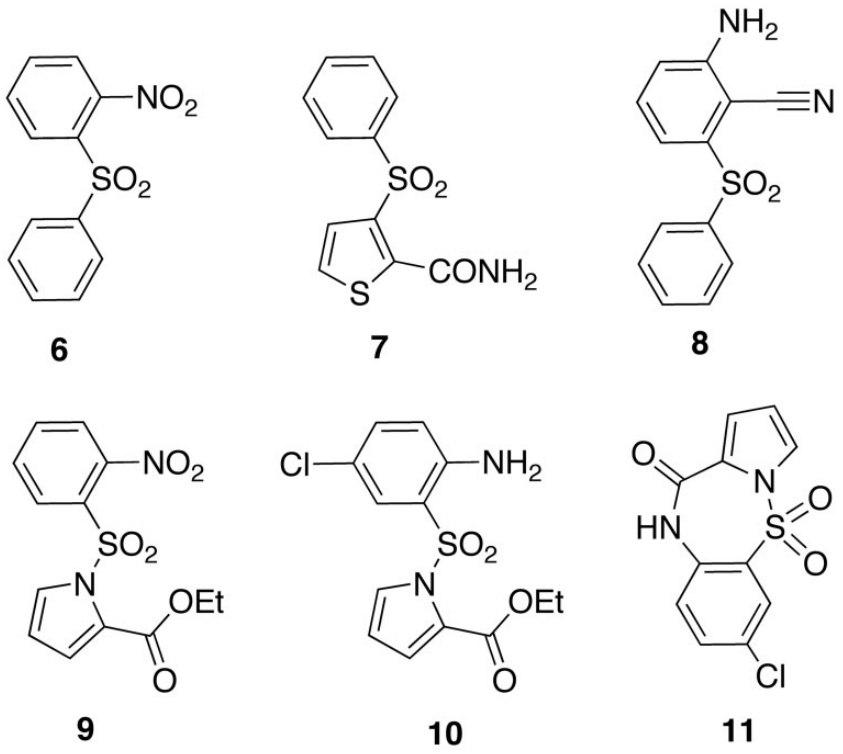


Chart 2. Diarylsulfones **[6]–[8]**, pyrrylarylsulfone **[9]** and **[10]**, and pyrrolo[1,2-*b*][1,2,5]benzothiadiazepine **[11]**.

to NVP, were obtained by intramolecular cyclization of PASs.[33]

Indolylarylsulfone (IAS) HIV-1 NNRTIs

In 1993, Merck Research Laboratories reported the discovery of 5-chloro-3-(benzenesulfonyl)indole-2-carboxyamide (L-737,126; **[12]**) that showed potent and selective inhibition of HIV-1 WT_{IIIB} strain with EC_{50} of 1 nM.[35–37] Carboxamide **[12]** proved to be highly potent as inhibitor of the HIV-1 WT strain (EC_{50} = 1 nM).[38] Introduction of a methyl group at position 2′, 3′ or 4′ of the 3-phenylsulfonyl ring led to IAS derivatives less cytotoxic than **[12]**. IAS derivatives bearing the 2′,4′- or 3′,5′-dimethyl **[13]** substitution pattern at the 3-phenylsulfonyl group were more cytotoxic than the monomethyl derivatives. Most importantly, the two methyl groups at positions 3′ and 5′ of the 3-phenylsulfonyl moiety proved to be a key structural requirement for an effective inhibition of the HIV-1 mutant strains[38] (Chart 3). IAS derivative **[13]** exhibited potent activity against the HIV-1 WT (EC_{50} = 4 nM) and the HIV-1 mutant strains Y181C (EC_{50} = 30 nM), K103N-Y181C (EC_{50} = 650 nM) in MT-4 cells (MTT method) and EFV^R (EC_{90} = 80 nM) in 8166 cell (p24 method) (EFV^R: EFV-resistant HIV-1 strain carrying K103R, V179D and P225H mutations; EFV EC_{90} = 1800 nM). These studies highlighted the role of the 3′,5′-dimethyls against the drug-resistant HIV-1 mutant strains. In summary, compound **[13]** was comparable with **[12]** and EFV against HIV-1 WT and Y181C mutant, but it was more potent than **[12]** against the HIV-1 K103N-Y181C mutant, and superior to **[12]** and EFV against the EFV^R mutant strain.

Hydroxyethyl derivatives

Efforts to improve water solubility and bioavailability of **[12]** led Merck to synthesize a series of bioisosteres by replacing the 2-carboxamide with a nitrogen-containing heterocycle.[39] Interestingly, among these new compounds 3-(benzenesulfonyl)-5-chloro-2-(5-methylimidazol-2-yl)indole **[14]** was found to be 11-fold superior to **[12]** against the HIV-1 K103N mutant strain. Molecular modelling studies were performed to gain insight of the binding mode of **[12]** into the NNBS of 14 RTs. A 3D quantitative structure activity relationship (SAR) model was obtained using a training set of 70 IAS derivatives.[40] The structure of the diarylsulfone 739W9424 in complex with the HIV-1 RT (PDB code 1jlq) was used to model the training set.[27] These findings prompted the synthesis of IAS derivatives bearing a 2-hydroxyethyl tail at the indole-2-carboxamide or indole-2-carboxydrazide nitrogen.[41,42] Carboxamide **[15]** and carboxhydrazide **[16]** were the most potent inhibitors of the HIV-1

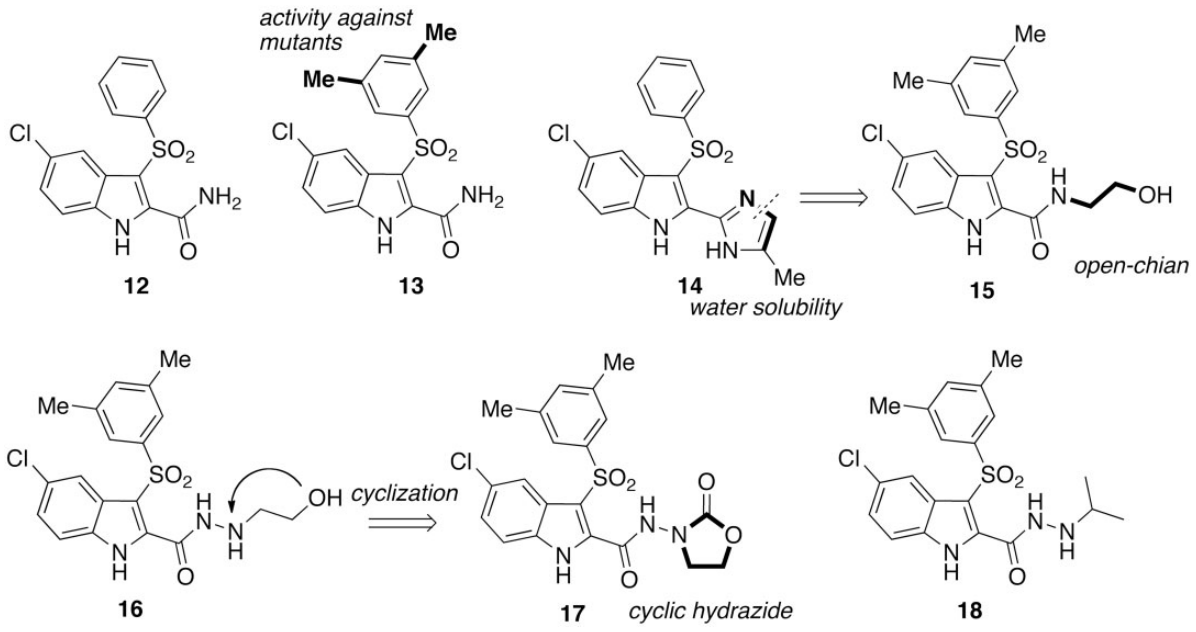


Chart 3. IAS carboxamides **[12]**, **[13]** and **[15];** carboxamide bioisostere **[14]** and carbohydrazides **[16]–[18]**.

WT_{IIIB} strain ($EC_{50} = 1$ nM) in MT-4 cells (MTT method). IAS **[15]** was superior to **[12]** against the HIV-1 Y181C and K103N-Y181C mutants and was more potent than **[12]** and EFV against the EFV^R mutant strain. IASs **[15]** and **[16]** were as potent as EFV against the HIV-1 WT RT, superior to EFV against the HIV-1 K103N mutant, but slightly inferior as inhibitor of the HIV-1 KY181I mutation (i.e. comparable with the Y181C in terms of drug resistance).

N′-carbohydrazide derivatives

The training set of the 3D quantitative SAR model[40] was enlarged from 70 to 101 compounds to improve the predictive capability.[43] Docking simulations and 3D quantitative SAR models led to design new potent carbohydrazide derivatives. IASs **[17]** and **[18]** showed strong inhibition of the HIV-1 WT_{IIIB} strain in MT-4 cells with EC_{50} of 3 and 0.7 nM, respectively, and high selectivity indexes. Compound **[17]** inhibited the HIV-1 K103N-Y181C double mutant with $EC_{50} = 900$ nM. Compounds **[17]** and **[18]** were evaluated against the primary isolates HIV-1 WT_{IIB}, HIV-112 and HIV-AB1 carrying K103N–V108I–M184V and L100I–V108I mutations, respectively, from two HIV-1-Ab seropositive patients who developed treatment failure after an initial response to the cART therapy. In lymphocytes compound **[18]** inhibited the primary isolates with subnano- (IIIB) or low nanomolar (112, AB1) EC_{50} values; in macrophages, **[17]** inhibited the HIV-1 $IIIB_{Ba\text{-}L}$ with EC_{50} of 2 nM and showed selectivity index >10.000.

IAS containing peptide units

Efforts to improve the interaction of IAS NNRTIs with the NNBS of the HIV-1 RT prompted the design of IAS derivatives bearing 1–3 glycine/alanine unit(s) at the 2-carboxamide nitrogen. The chemical manipulations included, for example, transposition of N-acetylamino to 2-acetamido, or replacement of semicarbazide with glycine carbohydrazide (Chart 4).[44] IAS derivatives bearing the glycine **[20]**, alanine **[21]** or glycine–glycine **[22]** unit strongly inhibited the HIV-1 WT_{IIIB} strain with EC_{50}s of 3, 6 and 0.7 nM, respectively. As inhibitors of the HIV-1 Y181C mutant strain, IASs **[21]** and **[22]** showed EC_{50} of 10 and 5 nM, respectively. IASs **[20]** and **[22]** inhibited the HIV-1 K103N–Y181C double mutant with EC50s of 800 and 700 nM. IASs **[20]–[22]** inhibited the EFV-resistant HIV-1 K103R–V179D–P225H strain with EC50s of 100, 40 and 100 nM, respectively[38] (Chart 4).

These results prompted the synthesis of new IAS derivatives bearing natural and unnatural amino acid units at the indole-2-carboxamide, for example **[23]** and **[24]**.[45] As inhibitors of the HIV-1 WT_{IIIB} strain in CEM cells the new IASs yielded nanomolar EC_{50} concentrations (**[23]**: $EC_{50} = 1.4$ nM; **[24]**: $EC_{50} = 2.3$ nM) and were comparable with EFV. The new compounds inhibited the Coxsackie B4 viruses with EC50s of 2–9 μm. These agents showed the

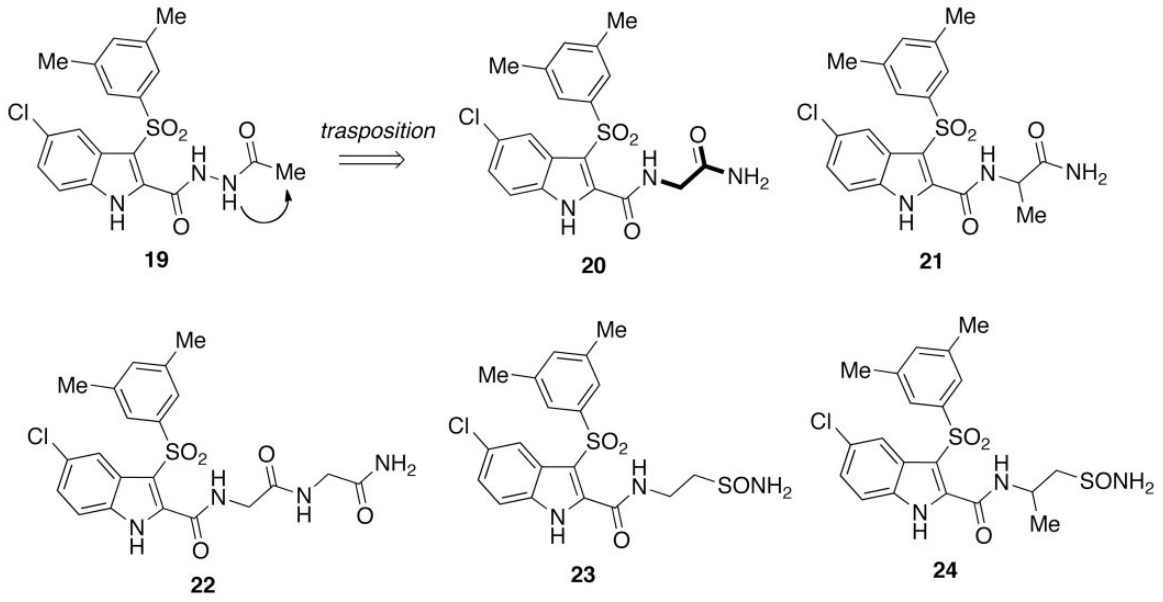


Chart 4. IAS acetylhydrazide **[19]** and IASs containing peptide units **[20]–[24]**.

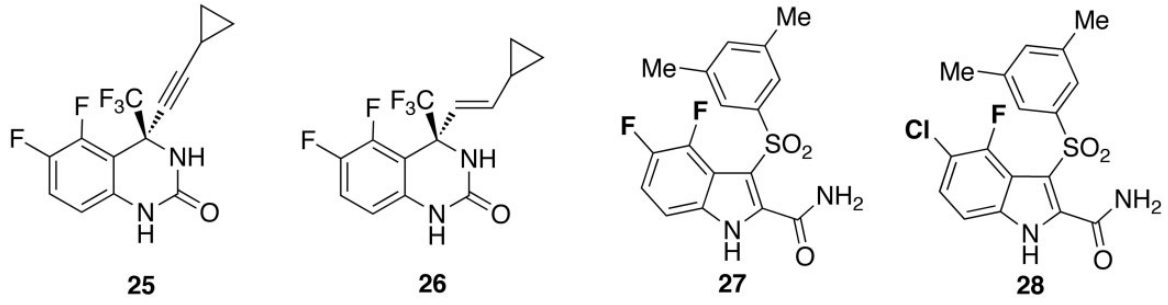


Chart 5. Di-halo IASs **[27]** and **[28]** correlated to quinazolinones **[25]** and **[26]**.

potential as new drugs to treat both the HIV-1 and Coxsackie B4 infection.[45]

IASs containing two halogen atoms

The HIV-1 K103N mutant strain is the most frequent mutation in >90% EFV-treated patients whose viral load rebounded after an initial response to the drug. The K103 mutation is frequently followed by the HIV-1 K103N–V108N and K103N–P225H double mutations.[46] Moreover, drug expedited clearance may result as a consequence of upregulation of P450 liver isoenzyme CYP3A4.[47] Efforts to improve the activity of EFV[48] against the drug-resistant mutants led DuPont Pharmaceuticals to synthesize quinazolinone analogues with two halogen atoms[49,50] (Chart 5). Compounds with the halogen atoms at positions 5 and 6 of the quinazolinone ring, for example **[25]** and **[26]**, were superior to the corresponding 5- and 6-mono-halogenated counterparts, partly due to the weaker binding with the plasma proteins.[51]

New IAS derivatives bearing two halogen atoms at positions 4–7 of the indole ring were synthesized based on these findings.[52] Introduction of chlorine and fluorine atoms at positions 4 and 5 of the indole provided highly potent HIV-1 NNRTIs. The di-halo IASs **[27]** and **[28]** exhibited potent inhibition of HIV-1 WT_{IIIB} strain in MT-4 cells with EC_{50}s of 1.0 and 0.5 nM, respectively. As inhibitor of the HIV-1 Y181C and K103N–Y181C mutant strains, **[28]** was comparable with EFV. Compounds **[27]** and **[28]** effectively inhibited the primary isolates 112 and the AB1 strains in lymphocytes and the HIV-1 WT_{IIIB} Ba-L strain in macrophages (p24 method).

Di-halo-IASs showed selectivity for the enzyme–substrate complex because of a different dissociation rate of the drug from the enzymatic form along the reaction pathway.[53] By comparing the HIV-1 WT RT

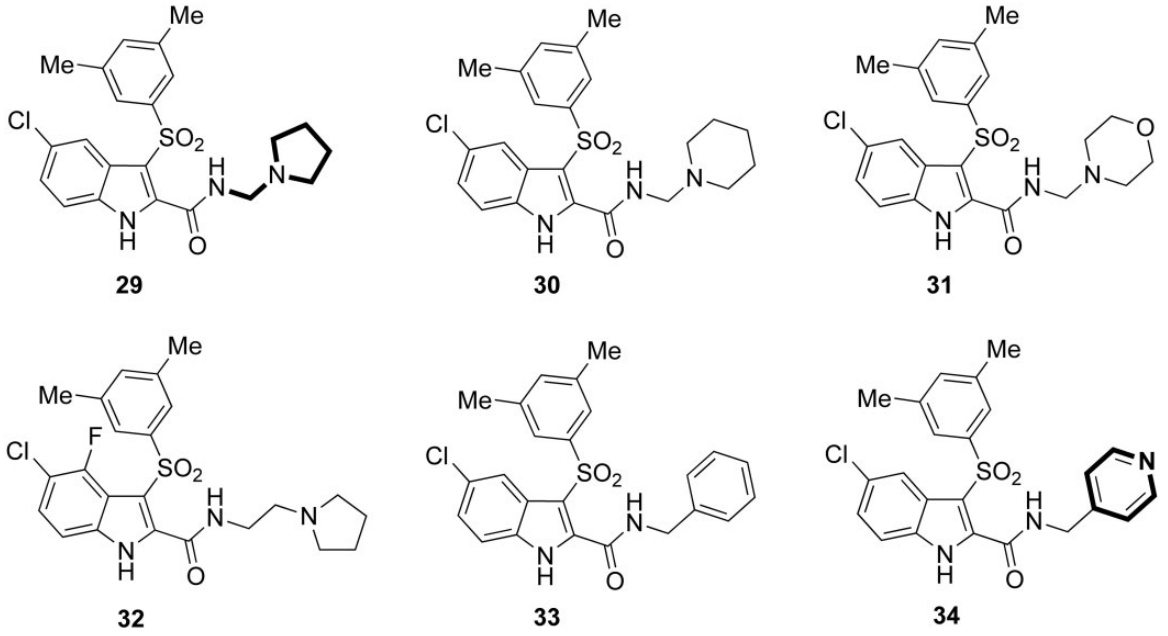


Chart 6. IASs **[29]–[34]** containing a third cyclic ring.

and drug-resistant K103N-, L100I- and Y181I-mutated RTs, IAS derivatives were characterized by highly dynamic interaction with the viral RT.[53,54] A greater flexibility of IAS **[28]** to form stable interactions with the amino acid residues of the NNBS of the RT may correlate with the potent anti-HIV-1 activity.[55]

IASs with a third ring

HIV-1 NNRTIs containing three aromatic rings, for example diaryltriazine (Janssen, NJ, USA),[56] dipyridodiazepinone (Boehringer Ingelheim, CT, USA)[57,58] and pyrrolidin-1-ylsulfone (Merck, PA, USA)[59] derivatives, showed potent and broad spectrum anti-HIV-1 activity. Previous docking studies of IAS derivatives containing a short peptide unit[44,45] highlighted that a tail at the 2-carboxamide nitrogen could form effective binding interactions inside the NNBS of the RT surrounded by the R172, I180, V179 and E138:B, and T139:B amino acid residues. These computational studies served as basis for the design of new IAS derivatives characterized by the presence of a pyrrolidine/piperidine/morpholine heterocyclic ring linked to the carboxamide nitrogen through a short 1–2 carbon spacer, for example **[29]–[32]** (Chart 6).[60] The new IAS bearing the third nucleus showed potent inhibition of the HIV-1 WT_{IIIB} strain in CEM cells with EC_{50} values at nanomolar concentrations (**[29]**: $EC_{50} = 3.3$ nM; **[30]**: $EC_{50} = 1.3$ nM; **[31]**: $EC_{50} = 1.9$ nM; **[32]**: $EC_{50} = 6.5$ nM). As inhibitors of the HIV-1 L100I and K103N mutant strains, **[31]** ($EC_{50} = 8.0$ and 11 nM) and **[32]** ($EC_{50} = 11$ and 15 nM) were superior to EFV ($EC_{50} = 22$ and 130 nM). Dissociation rates showed that compounds **[29]** and **[30]** formed stronger binding interactions with the L100I- and K103N-mutated enzymes than the WT enzyme.[61] IAS derivatives **[29]**, **[31]** and **[32]** inhibited the HIV-1 clade A in peripheral blood mononuclear cells (PBMCs) with EC_{50} values of 0.1, 0.1 and 2.1 nM, respectively.

Benzyl derivative **[33]** showed appreciable inhibition of the HIV-1 WT_{IIIB} strain in CEM cells and the HIV-1 mutant strains in MT-4 cells. These findings prompted the synthesis of new IAS derivatives containing nitrogen heterocycles at the 2-carboxamide nitrogen.[62] Among them, compound **[34]** showed consistent inhibition of the HIV-1 WT_{NL4-3} strain ($EC_{50} = 2.0$ nM) in MT-4 cells, the HIV-1 K103N ($EC_{50} = 8.8$ nM), Y181C ($EC_{50} = 2.2$ nM) and Y188L ($EC_{50} = 22$ nM) mutant strains and the HIV-1 IRLL98 multidrug-resistant strain ($EC_{50} = 1$ nM) containing the K101Q, Y181C and G190A mutations conferring resistance to NVP, DLV and EFV.[63] Compound **[34]** was more active against the IRLL98 mutant strain than the WT NL4-3 strain, and it proved to be a potent inhibitor of HIV-1 clades A, B, C, D, A/E, F and G in PBMCs in the higher picomolar range, except clade G. Compound **[34]** inhibited the HIV-1 K103N RT, the major mutation emerging in EFV-treated patients,[46] with EC_{50} of 45 nM, and exhibited EC_{50} of 11 nM against the HIV-1 L100I RT. These new IASs shared a typical feature of ETR, a state-of-art HIV-1 NNRTI, that is the presence of a pendant (third) aromatic ring.[55]

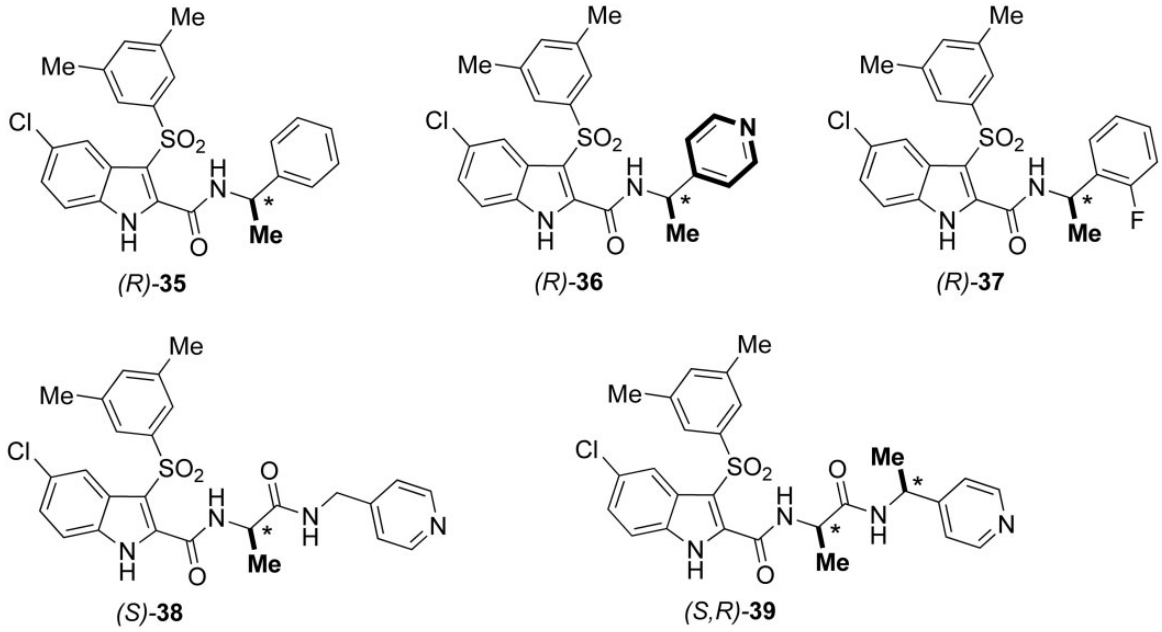


Chart 7. Chiral IASs **[35]–[39]**.

These results underline the potential of IAS **[34]** as a new agent for the treatment of EFV-treated patients who show the L100I and K103N mutations.

Focus on chirality of IASs

Chirality considerably affects the pharmacological profile of the drugs due to the high specific interaction of the ligand to the recognition site. Accordingly, the enantioselectivity plays an important role for the binding of antiviral agents to HIV-1 RT.[64] New IAS HIV-1 NNRTIs were synthesized to evaluate unexplored substitutions of the benzyl/phenylethyl group linked at the indole-2-carboxamide.[65] In recent studies, the enantiomers of IASs bearing chiral centres demonstrated significant differences in terms of antiretroviral activity. Several IAS derivatives were superior to NVP and EFV against the HIV-1 NL4-3 WT strain and inhibited the HIV-1 K103N mutant strain at nanomolar concentration. Some derivatives were superior to EFV against the HIV-1 Y181C and L100I mutant strains. The racemate *(R,S)*-**[35]** was separated into the enantiomers *(R)*-**[35]** and *(S)*-**[35]** by HPLC on the cellulose derived coated Chiralcel OD chiral stationary phase (CSP) using the binary mixture *n*-hexane–ethanol 1:1 as a mobile phase at both analytical and semipreparative level. Assignment of the absolute configuration of *(R)*-**[35]** and *(S)*-**[35]** was achieved by (i) synthesis of the enantiomer *(S)*-**[35]** starting from the amine of known stereochemistry *(S)*-(−)-α-methylbenzylamine, and (ii) comparison of the enantiomeric peaks of *(R)*-**[35]** and *(S)*-**[35]** obtained from the enantioseparation under the same enantioselective HPLC conditions. Against the NL4-3 HIV-1 strain, the enantiomers *(R)*-**[35]** and *(S)*-**[35]** showed small differences of activity. In contrast, *(R)*-**[35]** was found to be significantly more potent than *(S)*-**[35]** against the HIV-1 mutant strains (*(R)*-**[35]** EC_{50}, *(S)*/*(R)* ratio: K103N (4.3 nM, 30-fold), Y181C (86 nM, 40-fold), Y188L (193 nM, >189-fold) and K103N-Y181C (1670 nM, >22-fold)) (Chart 7).

Docking studies of *(R)*-**[35]** and *(S)*-**[35]** in the HIV-1 WT RT gave similar results. However, into the HIV-1 K103N-mutated RT the methyl group of the *(R)*-**[35]** pointed towards the entrance channel of the NNRTI NNBS, while the corresponding group of the *(S)*-**[35]** pointed towards the bottom of the cleft, leaving the binding pocket more exposed to the aqueous environment. The difference in the observed biological activity of *(R)*-**[35]** and *(S)*-**[35]** could be due to a different binding kinetics rather than affinity.[66] *(R)*-**[35]** was able to seal the binding pocket, while *(S)*-**[35]** left the site accessible to water with a consequent negative impact on the binding kinetic of this inhibitor.

Further step of this research project was the synthesis of IAS derivatives carrying a heterocyclic tail at the indole-2-carboxamide nitrogen.[67] Several new IASs inhibited the HIV-1 WT and mutant strains in MT-4 cells with EC_{50} values less than 1.0 nM. Replacement of the phenyl group of **[35]** with a pyridinyl ring to obtain **[36]** resulted in a general improvement of antiviral

activity. Racemate **[36]** was found to be three-fold more potent than **[35]** as inhibitor of the NL4-3 WT strain ($EC_{50} = 0.2$ nM,). Against the K103N ($EC_{50} = 9.4$ nM), Y181C ($EC_{50} = 87$ nM) and K103N-Y181C ($EC_{50} = 1111$ nM) mutant strains, IAS **[36]** was, respectively, three, eight and three times superior to **[35]**.

The racemate *(R,S)*-**[36]** was separated into the enantiomers *(R)*-**[36]** and *(S)*-**[36]** by enantioselective HPLC. The CD spectra of enantiomers *(R)*-**[36]** and *(S)*-**[36]** were compared with those of *(R)*-**[35]** and *(S)*-**[35]**.[65] Similar to the enantiomers of **[35]**, *(R)*-**[36]** and *(S)*-**[36]** ($EC_{50} = 0.2$ nM) were equipotent against the HIV-1 WT_{NL4-3} strain; on the contrary, *(R)*-**[36]** showed higher inhibition than *(S)*-**[36]** of the HIV-1 mutant strains (*(R)*-**[36]** EC_{50}, *(S)*/*(R)* ratio: K103N (0.2 nM, 22-fold), Y181C (2.1 nM, 61-fold) and K103N-Y181C (150 nM, 27-fold)) in the cellular assay. Compound *(R)*-**[36]** was superior to NVP, EFV and AZT reference drugs, except AZT, against the K103N–Y181C mutant strain. In addition, compounds (*S*)-**[36]** and (*R*)-**[36]** were evaluated against various HIV-1 group M clinical isolates in PBMC. The antiviral activity was potent and consistent and varied only between 0.7 and 5.2 nM when evaluated against all different virus isolates. Compound *(R)*-**[36]** showed excellent plasma and metabolic stability and did not behave as a prodrug. It showed good membrane permeability and low water solubility.[67] Shifting the 4-nitrogen of the pyridine to either position 3 or 2 resulted in a decreased anti-HIV-1 activity against the mutant strains.

From docking simulations in the HIV-1 WT RT, the PLANTS proposed binding mode of *(R,S)*-**[36]** was consistent with the previously reported binding poses for the IAS family[41–45,52,55,60,62,65] featuring these pharmacophoric interactions: (i) the indole NH established a H-bond with the K101 carbonyl oxygen; (ii) the chlorine atom fitted into a hydrophobic cavity surrounded by V106 and L234; (iii) the 3′,5′-dimethylphenyl moiety laid in the aromatic cleft formed by the side chains of Y181, Y188 and W229 residues establishing a network of hydrophobic interactions; (iv) the pyridyl moiety formed hydrophobic interactions with the side chains of V179 and E138:B (Figure 1).

The *(R)*-**[36]** and *(S)*-**[36]** enantiomers showed some significant differences in their binding modes: the methyl group of *(R)*-**[36]** pointed towards the cleft created by the K103N mutation, sealing the binding pocket and reducing the solvent-accessible surface,

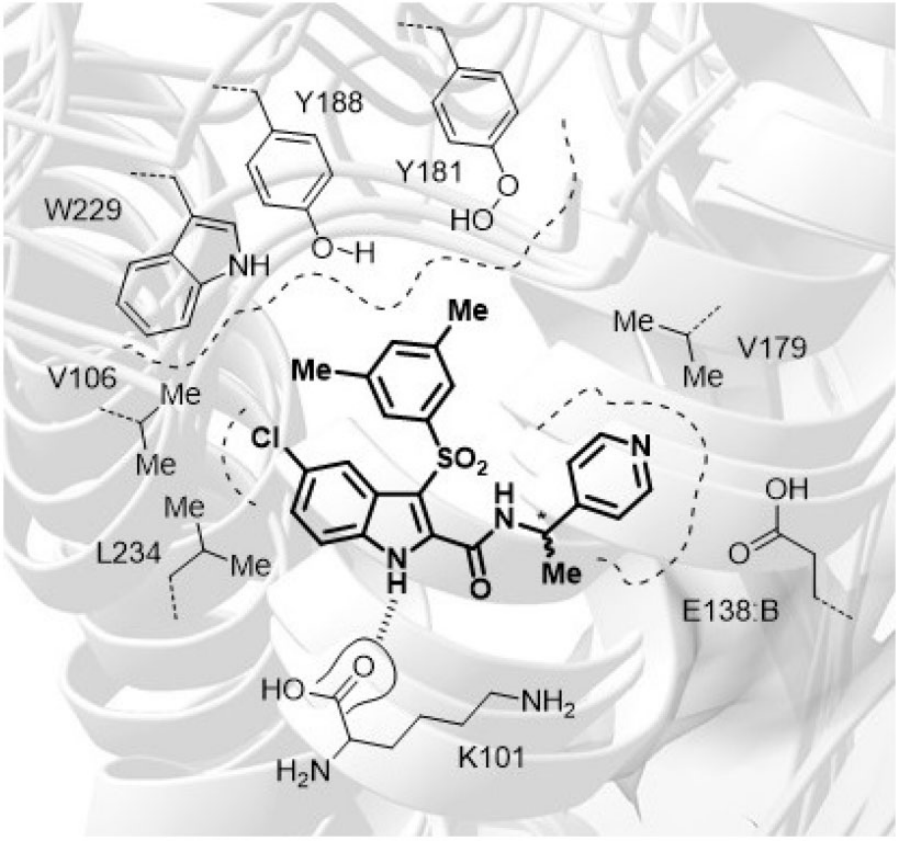


Figure 1. Sketch of amino acid residues within 5 Å of IAS *(R,S)*-**[36]** bounded in the NNBS of the HIV-1 WT RT.

the corresponding group of *(S)*-**[36]** left the pocket more exposed to solvent, in agreement with the previously reported mechanism of resistance to the HIV-1 K103N mutation based on a binding kinetic effect.[66,68] Molecular dynamics simulations showed that trajectory analyses of both K103N RT/(*(R)*-**[36]** and K103N RT/*(S)*-**[36]** complexes were stable during the whole simulation time. Solvent-accessible surface area[69] performed on the entire binding site of the receptor in complex with *(S)*-**[36]** (235.64 Å^2) was greater than the corresponding one for the *(R)*-**[36]**, (210.20 Å^2). *(S)*-**[36]** showed a number of water molecules surrounding the methyl cleft, 3.5 times greater than the number of solvent molecules observed for the *(R)*

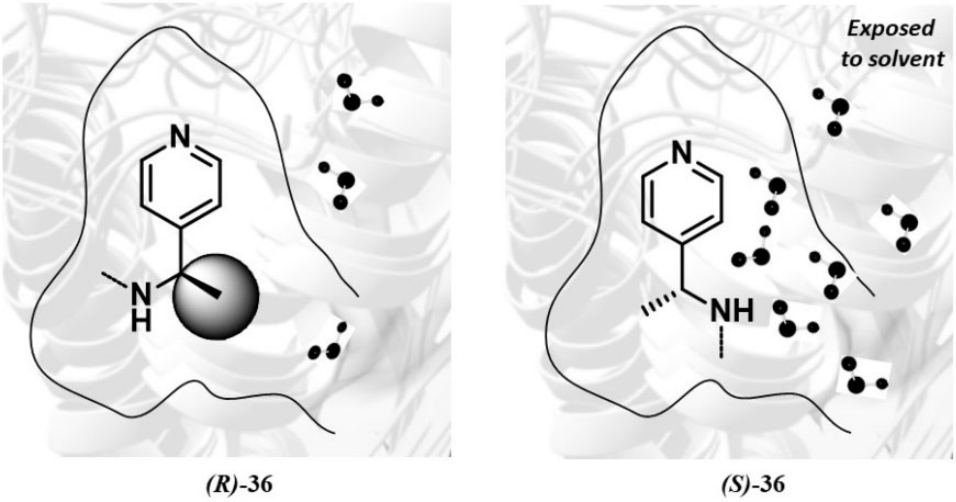


Figure 2. Sketch the 1-(pyridin-4-yl)ethyl group of derivatives *(R)*-**[36]** and *(S)*-**[36]** into the NNBS of the K103N RT. The image shows a different shape and position of the methyl group.

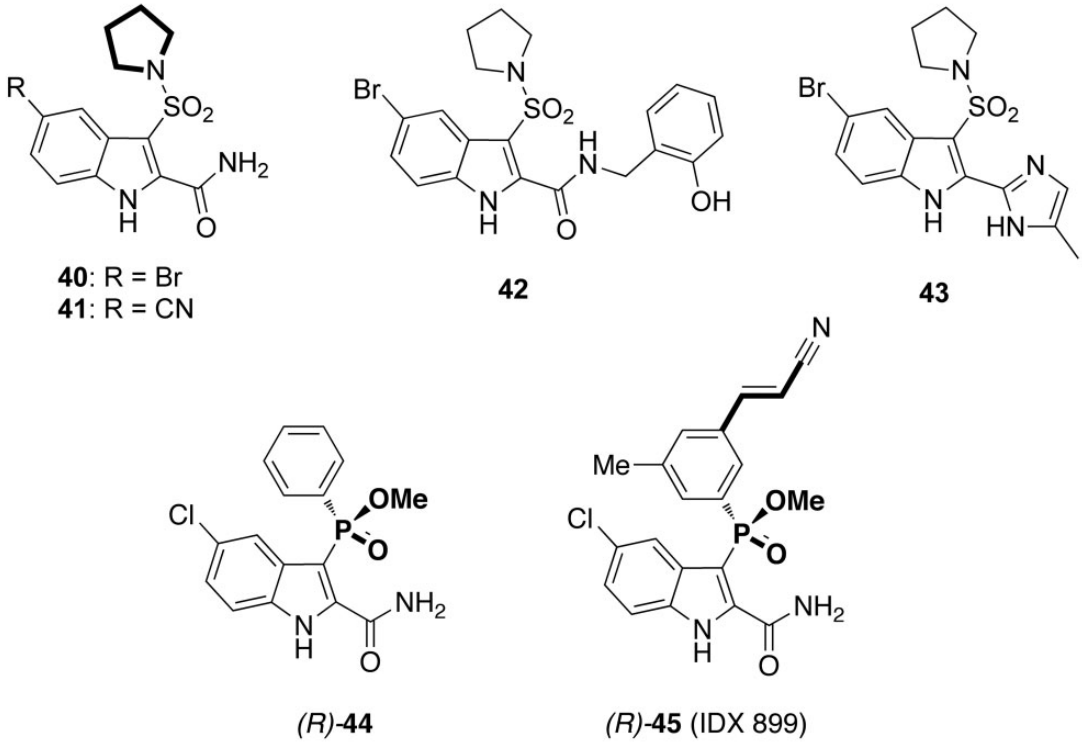
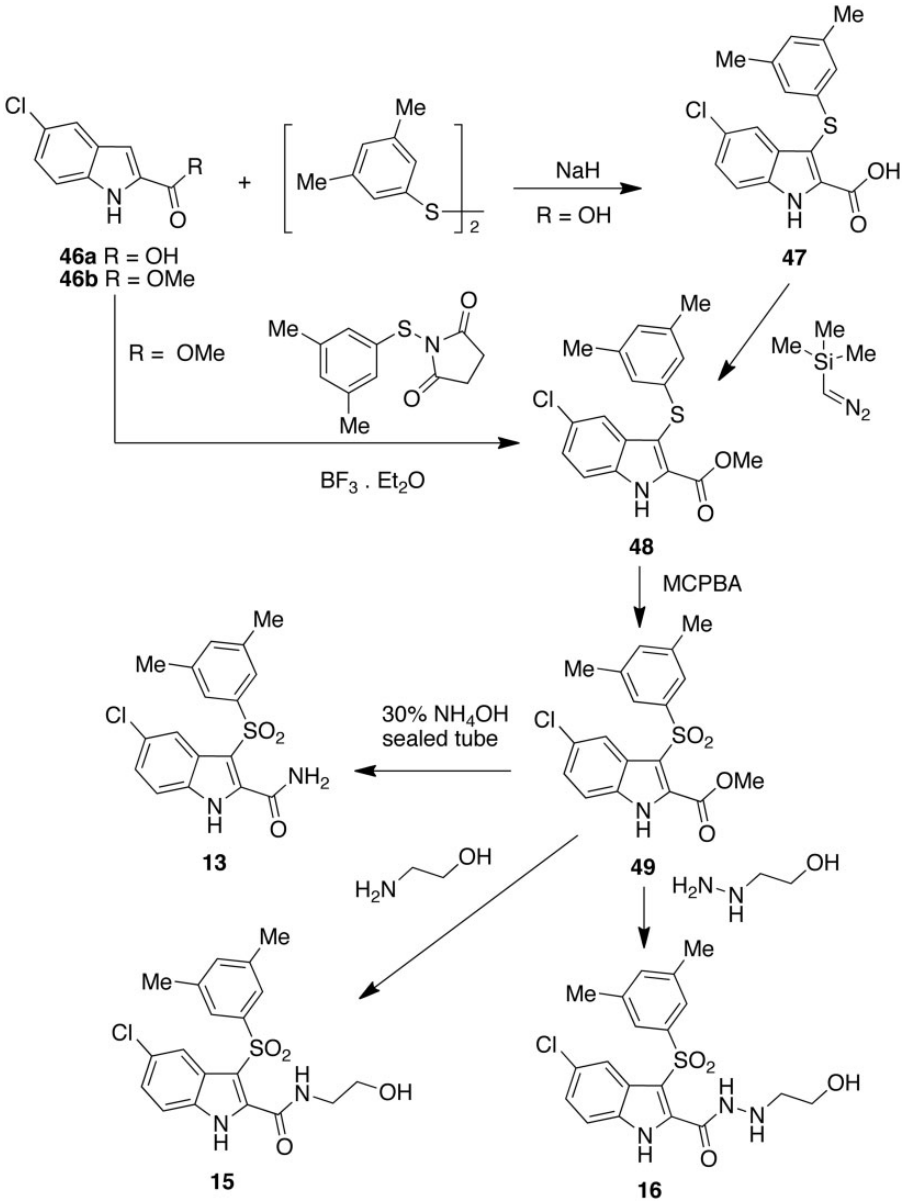


Chart 8. Indolylsulfonamides **[40]–[43]** and API **[44]** and **[45]**.

enantiomer. These results were taken into account to explain the different biological activity observed between *(R)*-**[36]** and *(S)*-**[36]** enantiomers (Figure 2).

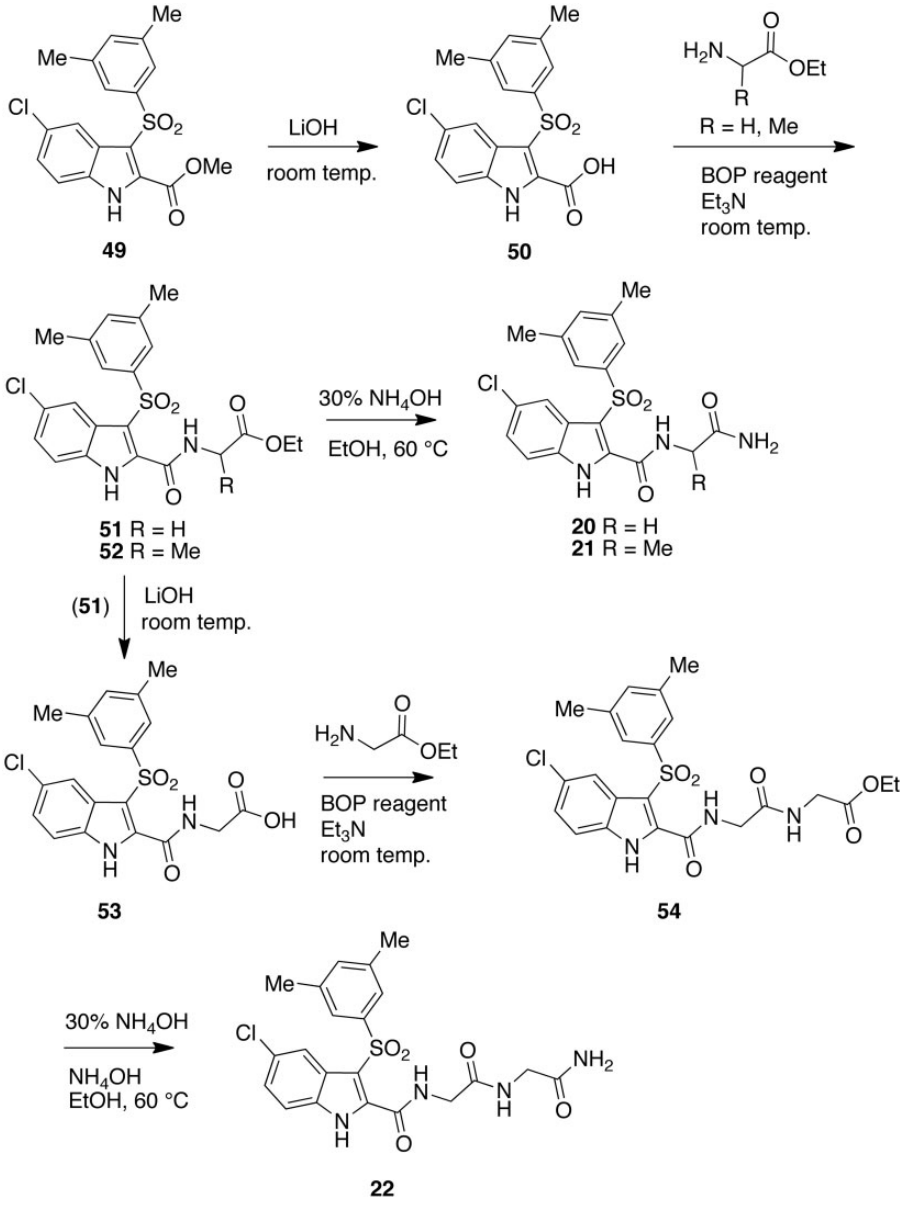
A new series of chiral IASs showed potent inhibition of the HIV-1 WT NL4-3 strain and of the HIV-1 K103N, Y181C, Y188L and K103N-Y181C HIV-1 mutant strains.[70] Six racemic mixtures were separated at the semipreparative level by enantioselective HPLC into their pure enantiomers. The *(R)*-enantiomers of IAS derivatives bearing the chiral α-methylbenzyl were superior to the *(S)*-counterparts. IAS *(R)*-**[37]** inhibited the HIV-1 WT strain with EC_{50} of 0.7 nM, and K103N ($EC_{50} = 0.7$ nM), Y181C ($EC_{50} = 0.7$ nM), Y188L ($EC_{50} = 165$ nM) and K103N–Y181C ($EC_{50} = 2486$ nM) in the cellular assay. In previous studies, the antiretroviral activity of IAS **[21]** appeared only weakly affected by the chirality of the alanine unit.[45] On the contrary, coupling of alanine with pyridin-4-ylmethanamine provided IAS derivatives with marked stereospecific activity: *(S)*-**[38]** was superior to the corresponding *(R)*-enantiomer, and *(S,R)*-**[39]** and *(S,S)*-**[39]** were superior to the corresponding *(R,S)*-**[39]** and *(R,R)*-**[39]** enantiomers. IASs *(R)*-**[38]** and *(S)*-**[38]** were equally active against the HIV-1 WT_{NL4-3} with $EC_{50} = 0.7$ nM. Otherwise, *(S)*-**[38]** was really more potent than *(R)*-**[38]** against



Scheme 1. Synthesis of IASs **[13]**, **[15]** and **[16]**.

the HIV-1 mutant strains (EC_{50}, *(S)/(R)* ratio) K103N (0.7 nM, 162-fold), Y181C (0.7 nM, 258-fold), Y188L (666 nM, >35-fold) and K103N–Y181C (857 nM, eight-fold) in MT-4 cells. The cellular data correlated with the enzymatic results. The diastereomeric mixture **[39]** was separated into the four stereoisomers *(R,S)*-**[39]**, *(S,R)*-**[39]**, *(R,R)*-**[39]** and *(S,S)*-**[39]**. IASs *(S,R)*-**[39]** and *(R,S)*-**[39]** showed marked differences against the HIV strains (EC_{50}, *(S)/(R)* ratio) WT_{NL4-3} (0.6 nM, three-fold), K103N (0.6 nM, 340-fold), Y181C (0.6 nM, 1113-fold), Y188L (742 nM, 38-fold) and K103N–Y181C (1261 nM, 25-fold). The biological results highlighted a correlation between configuration of the asymmetric centre and linker length: The *(R)*-enantiomers were superior to the *(S)*-counterparts when associated to a short linker unit, and the *(S)*-enantiomers were more potent than the *(R)*-enantiomers in the presence of a long linker.

Despite the significant increase of life expectancy in HIV-infected people,[71] cART treatments cause neurological problems to nearly half of HIV cases.[72] The neurocognitive damage often accelerates during cART treatment[73] and continues even after the peripheral viral infection has ceased.[74] Compound *(R,S)*-**[38]** protected hippocampal neuronal cells from the

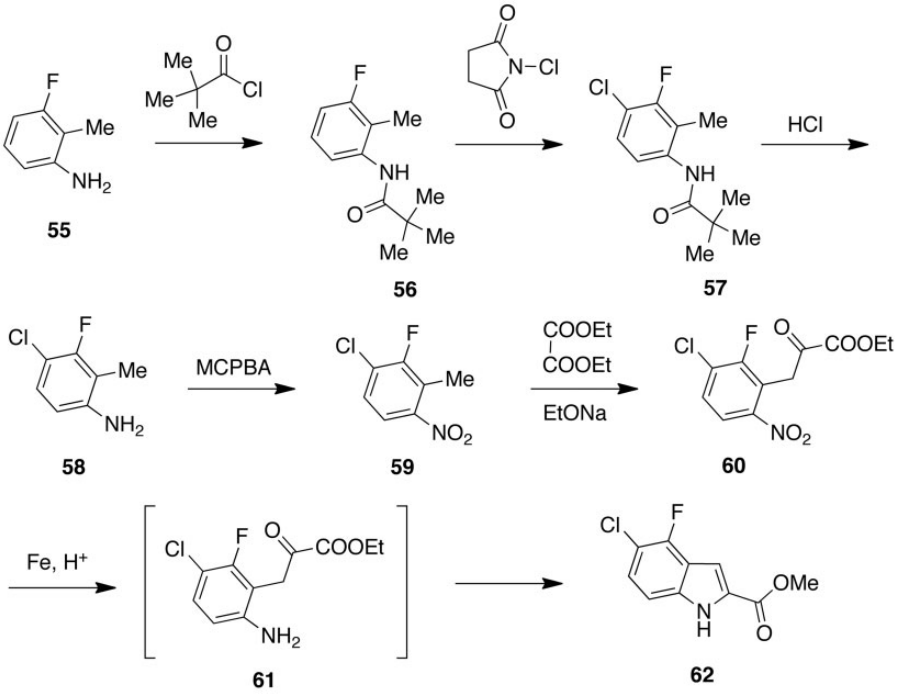


Scheme 2. Synthesis of IASs **[20]–[22]**.

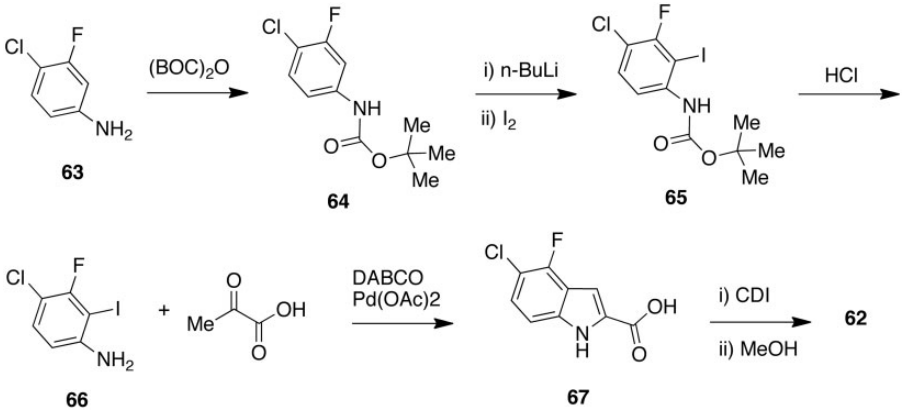
excitotoxic insult, while EFV did not contrast the neurotoxic effect of glutamate. The new IASs showed improved resistance profile against the mutant HIV-1 strains and reduced neurotoxic effects.

Indole-3-sulfonamides, Merck & Co., Inc

Fifteen years after the discovery of compound **[12]**,[35] Merck & Co., Inc. reported the synthesis of a series of indolylsulfonamides bearing a linear or cyclic alkylamine linked at the sulfone group at position 3 of the indole.[59] Preliminary docking studies suggested that a 3-alkylsulfonamide and a halogen atom at position 5 of the indole could increase the activity against the HIV-1 Y181C mutant strain. Both secondary and tertiary sulfonamides inhibited the HIV-1 RT with IC_{50} values at nanomolar concentration. In HIV-1 WT-infected cells, only pyrrolidine **[40]**, [**41]** and piperidine tertiary sulfonamides showed inhibitory concentrations (Spread CIC_{95}) comparable to the enzymatic assay. X-ray co-crystal structure of **[40]** bound to HIV-1 WT RT revealed that it assumed a 'butterfly-like' orientation, similarly to **[12]** and other NNRTIs.[55] However, **[40]** and **[41]** showed weak inhibition of the HIV-1 K103N



Scheme 3. Synthesis of ethyl 5-chloro-4-fluoro-1H-indole-2-carboxylate **[62]**.



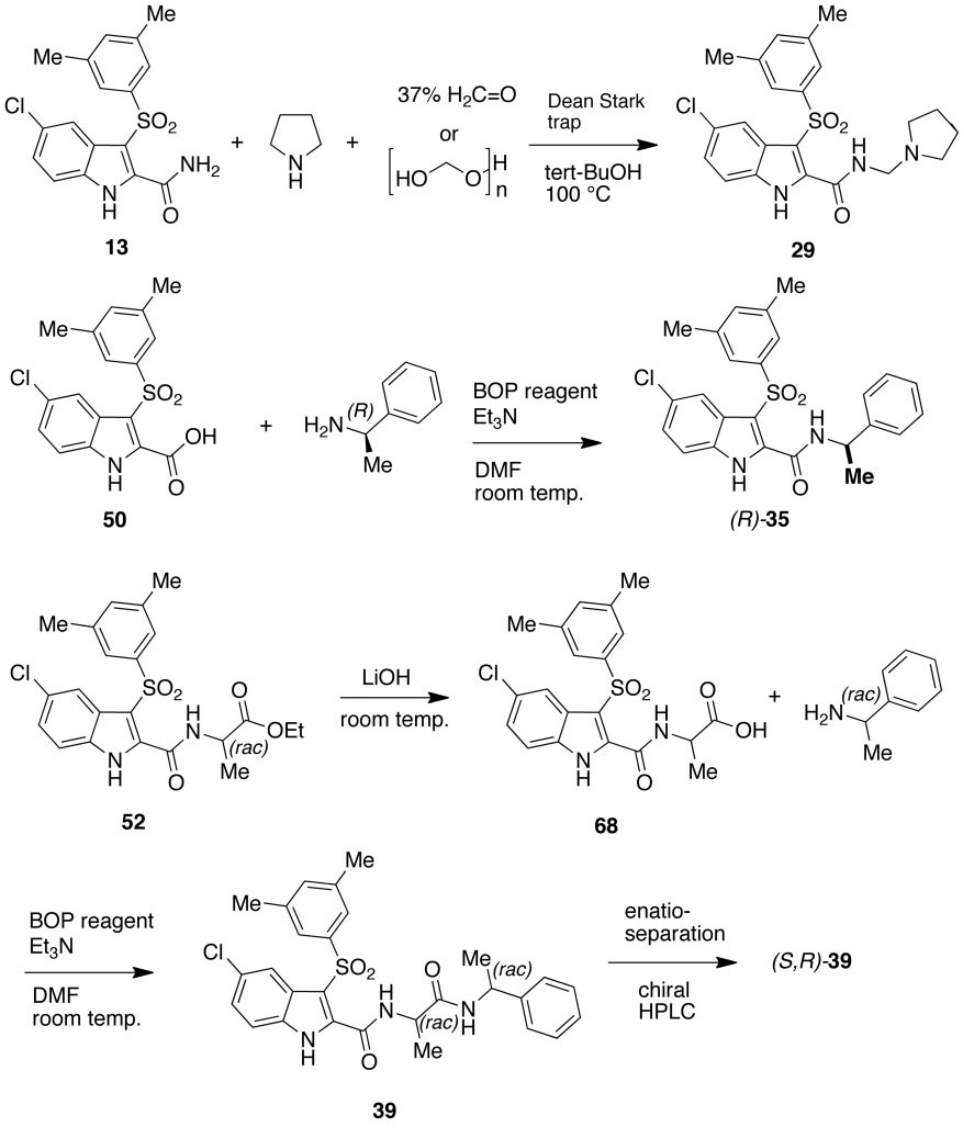
Scheme 4. Synthesis of ethyl 5-chloro-4-fluoro-1H-indole-2-carboxylate **[56]**.

and Y181C mutant strains. Efforts to improve the activity against the mutant strains led to modification of the 2-carboxmide function, as previously reported by Young et al.,[39] with introduction of a benzyl group at the carboxamide nitrogen (e.g. **[42]**), or replacement of the carboxamide with an imidazole ring (e.g. **[43]**). Compounds **[42]** and **[43]** showed inhibition of the HIV-1 K103N and Y181C mutants comparable to the WT in both enzymatic and cellular assays (Chart 8).

Arylphosphoindoles, Idenix Laboratories

Idenix Laboratories synthesized bioisosteres of the IAS derivatives by replacing the 3-sulfonyl bridging group with a phosphinic acid methyl ester one.[75] Arylphosphoindole (API) derivative **[44]** inhibited the HIV-1 WT and the K103N, Y181C mutant strains at low nanomolar concentration. The racemic mixture **[44]** was separated into the corresponding enantiomers by supercritical fluid chromatography. In MT-4-infected cells, the enantiomer (*R*)-**[44]** inhibited the HIV-1 WT with EC_{50} of 0.1 nM and the HIV-1 mutant strains K103N, Y181C and K103N-Y81C with EC_{50} values of 1.2, 3.6 and 137.4 nM, respectively. The stereochemistry of (*R*)-**[44]** was assigned on the basis of binding energy calculation (−23 kcal/mol) and was confirmed by X-ray crystallography diffraction.

Idenix introduced the typical *Z*-cyanovinyl arm of RPV at position 3′ of the 3-phenyl phosphinic group to obtain HIV-1 NNRTIs with broad spectrum of activity against the drug-resistant mutant strains.



Scheme 5. Synthesis of IASs **[29]**, *(R)*-**[35]** and *(S,R)*-**[39]**.

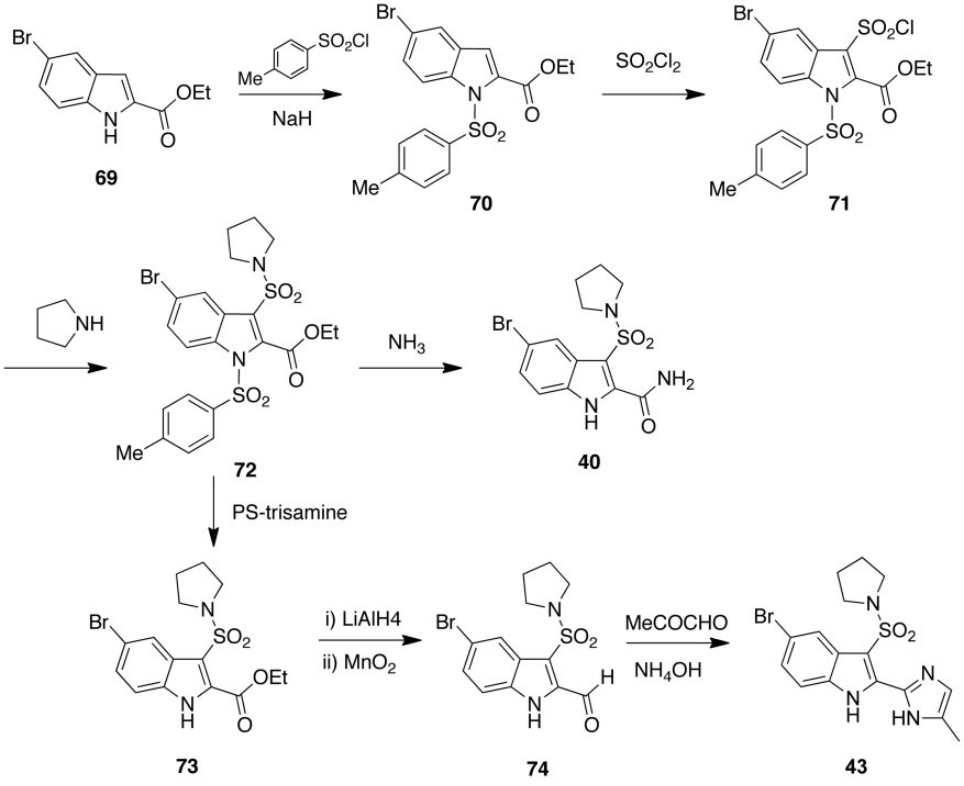
The enantiomer *(R)*-**[45]** (IDX 899) showed effective inhibition of the HIV-1 WT and of the HIV-1 mutant strains resistant to other NNRTIs, reduced HIV-1 RNA levels and exhibited barrier to resistance superior to EFV. *(R)*-**[45]** was selected as highly potent second-generation NNRTI drug candidate and was evaluated in a phase II clinical trial. In February 2009 it was licensed to GlaxoSmithKline and its name was changed to fosdevirine/GSK2248761.[76] In phase I clinical trials increased CD4+ cell counts in treatment-naïve patients infected with HIV-1[77,78] (Chart 8).

Synthetic aspects

Compound **[13]** was synthesized starting from acid **[46a]** which transformed into the 3-arylthio intermediate **[47]** by Atkinson reaction[79] with an appropriate arylthiodisulfide in the presence of sodium hydride and then transformed in the corresponding methyl ester **[48]** with trimethylsilyl diazomethane. Alternatively, **[48]** was obtained from ester **[46b]** and *N*-(3,5-dimethylphenylthio)succinimide in the presence of boron trifluoride diethyl etherate. Compound **[48]** was oxidized to sulfone **[49]** with 3-chloroperoxybenzoic acid. Finally, **[49]** was heated with ammonium hydroxide in closed vessel to give **[13]**. Heating of **[49]** in ethanolamine or 2-hydrazinoethanol furnished IAS **[15]** or **[16]**, respectively (Scheme 1).

IAS derivatives **[20]–[22]** were synthesized starting from ester **[49]**. LiOH hydrolysis of **[49]** at room temperature yielded the carboxylic acid **[50].** The acid was treated with glycine or alanine in the presence of BOP reagent (benzotriazol-l-yl-oxy-*tris*-(dimethylamino) phosphonium hexafluorophosphate) and triethylamine as coupling reagents to provide esters **[51]** or **[52]**. These compounds were transformed into IASs **[20]** or **[21]** with concentrated ammonium hydroxide in ethanol at 60 °C. Glycine ester **[51]** underwent alkaline hydrolysis with LiOH to give acid **[53]**. Elongation of the chain was performed by coupling **[53]** with a glycine unit in the presence of BOP reagent and triethylamine. Finally, ester **[54]** was transformed into IAS **[22]** by heating at 60 °C with ammonium hydroxide (Scheme 2).

Di-halo-IASs **[27]** and **[28]** were synthesized by following the above reported procedure starting from an appropriate ethyl di-halo-*1H*-indole-2-carboxylate. The required esters were prepared from the corresponding phenylhydrazones[80] by Fisher indole synthesis in polyphosphoric acid at 100 °C.[81] The isomeric esters were separated with difficulty by column chromatography after repeated passages providing **[62]** in 5% yield.[82] To improve yield, **[62]** was synthesized starting from 3-fluoro-2-methylaniline

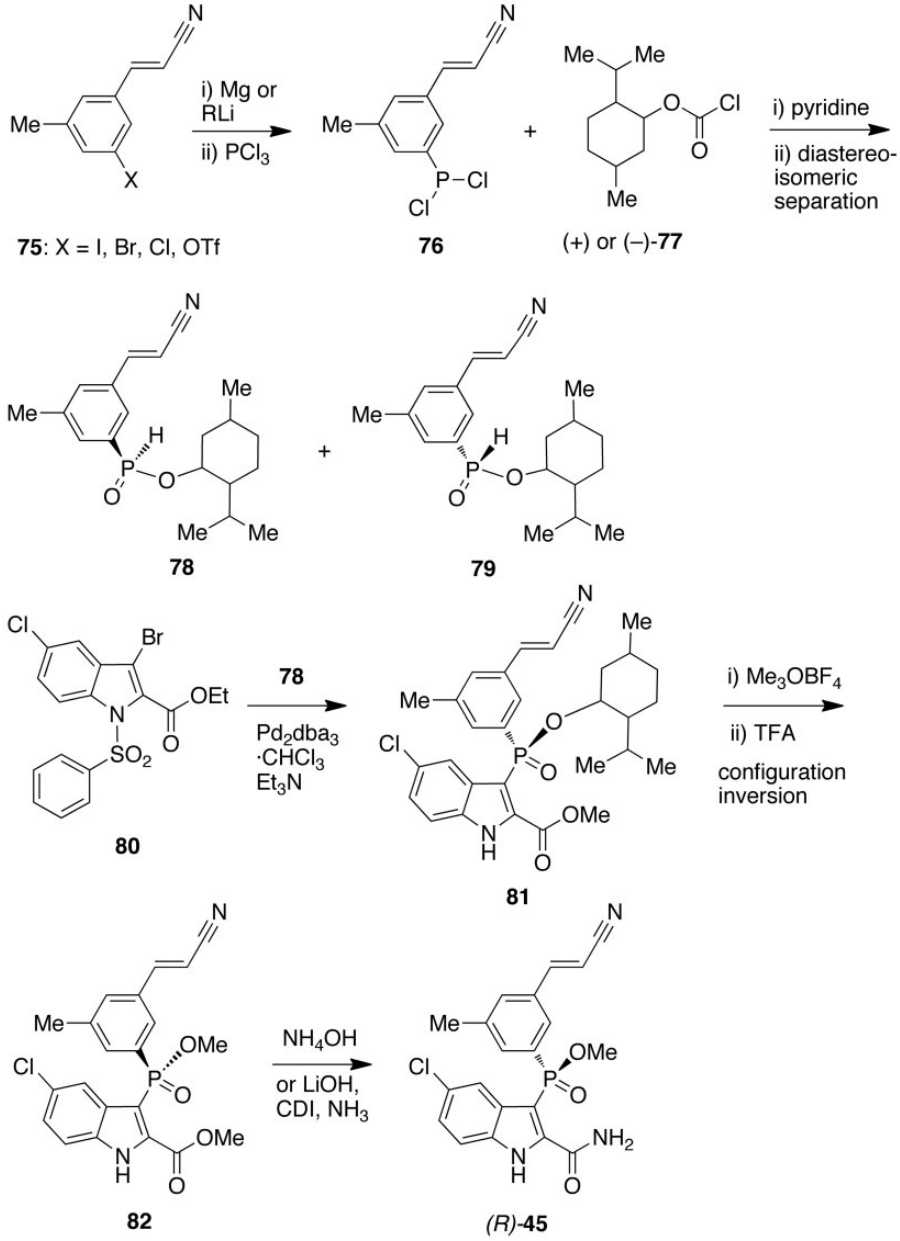


Scheme 6. Synthesis of indolylsulfonamides **[40]** and **[43]**.

[55]. The *N*-pivaloyl derivative **[56]** was treated with *N*-chlorosuccinimide to give the 4-chloro derivative **[57]**. After hydrolysis of **[57]** with hydrochloric acid, the aniline **[58]** was oxidized to nitro **[59]** with 3-chloroperoxybenzoic acid. Treatment of the latter compound with diethyl oxalate in the presence of sodium ethoxide gave ethyl 3-(3-chloro-2-fluoro-6-nitrophenyl)-2-oxopropanoate **[60]**. Iron powder reduction of **[60]** followed by intramolecular cyclization provided **[62]** in six steps and 37% overall yield[83] (Scheme 3).

Idenix Pharm. (MA, USA) synthesized **[62]** starting from 4-chloro-3-fluoroaniline **[63]**.[84] C2 iodination of BOC-protected **[64]** afforded **[65]**. After deprotection of **[65]** with hydrochloric acid, the aniline **[66]** was reacted with pyruvic acid in the presence of palladium(II) acetate and 1,4-diazabicyclo[2.2.2]octane to afford 5-chloro-4-fluoro-indole-2-carboxylic acid **[67]**. Esterification of **[61]** to **[62]** was easily achieved by treatment with 1,1′-carbonyldiimidazolole and then with methanol (Scheme 4).

Compound **[29]** was synthesized according to the Mannich reaction by refluxing **[13]** in *tert*-butanol at 100 °C with pyrrolidine in the presence of 37% formaldehyde or paraformaldehyde using a Dean–Stark trap.[60] Reaction of *(S)*-(−)-α-methylbenzylamine with acid **[50]** in the presence of BOP reagent and



Scheme 7. Synthesis of API (*R*)-**[45]**.

triethylamine in DMF at room temperature furnished the chiral IAS *(R)*-**[35]**. This compound was used as a reference compound to compare the peaks of enantiomers *(R)*-**[35]** and *(S)*-**[35]** obtained by enantioseparation of the racemate **[35]**.[65] Acid **[68]** was obtained by LiOH hydrolysis of the racemic ester **[52]**. Treatment of **[52]** with the racemic α-methylbenzylamine in the presence of BOP reagent and triethylamine to furnish the diasteromeric compound **[39]**. HPLC separation of the enantiomers was performed using a polysaccharide-based CSP Chiralpak IC and *n*-hexane–ethanol–dichloromethane–DEA 40:15:45:0.3 as eluent[70] (Scheme 5).

Ethyl 5-bromo-1*H*-indole-2-caboxylate **[69]** was protected with 4-toluensulfonyl chloride in the presence of NaH to give **[70]** and converted to sulfonyl chloride **[71]** with sulfuryl chloride. The sulfonyl chloride was treated with pyrrolidine to afford **[72]**. Aminolysis of the 2-ethoxycarbonyl group with ammonia underwent concomitant deprotection of position 1 of the indole to provide **[40]**. Deprotection of **[72]** with tris-(2-aminoethyl)aminomethyl polystyrene (PS-trisamine) gave **[73].** The ester was transformed to aldehyde **[74]** by a two-step procedure with lithium aluminium hydride and subsequent oxidation with manganese(IV) oxide. The latter compound was cyclized to imidazole **[43]** with acetaldehyde and ammonium hydroxide under microwave irradiation (Scheme 6).

Compound *(R)*-**[45]** (IDX899)[85] was synthesized starting from an aryl halide or triflate **[75]** in the presence of Mg or an alkyl Li and PCl_3 to provide dichlorophosphite **[76]**. Compound **[76]** was treated with (+)- or (−)-menthylchloroformate **[77]** in pyridine (Hewitt reaction)[86] to give the mixture of diastereoisomers **[78]** and **[79]** which were separated by crystallization at low temperature in *n*-hexane. Compound **[78]** was treated with ethyl 3-bromo-5-chloro-1-phenylsulfonyl-1*H*-indole-2-carboxylate **[80]** in the presence of tris(dibenzylideneacetone) dipalladium(0)-chloroform adduct ($Pd_2(dba)_3{\cdot}CHCl_3$) and triethylamine to give **[81]**. Formation of **[82]** from **[80]** and trimethyloxonium tetrafluoroborate (Meerwein salt) and then trifluoroacetic acid underwent with inversion of configuration. Finally, *(R)*-**[45]** was obtained by reaction of **[82]** with ammonium hydroxide, or, alternatively, by hydrolysis of the ester with lithium hydroxide and subsequent treatment with ammonia by displacement of the intermediate imidazolide (Scheme 7).

Conclusions

IASs are a potent class of HIV-1 NNRTIs. SAR studies led to improve remarkably the profile of L-737,126 discovered by Merck AG. Introduction of the 3′,5′-dimethyl groups at the 3-phenylsulfonyl moiety furnished IAS derivatives with potent and selective activity against HIV-1 mutants carrying NNRTI resistance mutations at positions 103 and 181 of the RT. The presence of a 2-hydroxyethyl tail at the indole-2-carboxamide nitrogen improved the activity against the HIV-1 K103N–Y181C double mutant. Coupling of the carboxamide nitrogen with one or two glycinamide and alaninamide units moieties produced short peptides with potent activity and selectivity against the HIV-1 WT and the mutant strains Y181C, K103N-Y181C and EFV^R carrying K103R, V179D and P225H mutations. Introduction of a fluorine atom at position 4 of the indole ring improved the antiviral potency against the HIV-1 WT and the HIV-1 drug-resistant mutants. IASs bearing the third nucleus linked at the carboxamide nitrogen showed potent antiretroviral activity. Chiral derivatives having *(R)* configuration were significantly more potent than *(S)* counterparts against the HIV-1 mutant strains. The alanine spacer between the carboxamide nitrogen and the α-methylbenzyl brought attention to a correlation between configuration of the asymmetric centre and linker length: the *(R)*-enantiomers were superior to the *(S)*-counterparts when associated to a short linker unit, and the *(S)*-enantiomers were more potent than the *(R)*-enantiomers in the presence of a long linker. IAS derivatives are promising drug candidates for the treatment of AIDS/HIV-1 infection in combination with other HIV-1 agents. This review provides some key SARs for the design and synthesis of new potent HIV-1 NNRTIs.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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