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Brief Article

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New Nitrogen Containing Substituents at the Indole-2-carboxamide Yield High Potent and Broad Spectrum Indolylarylsulfone HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors

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Supporting Information

ABSTRACT: New indolylarylsulfone (IAS) derivatives bearing nitrogen containing substituents at the indole-2-carboxamide inhibited the HIV-1 WT in MT-4 cells at low nanomolar concentrations. In particular, compound **9** was uniformly effective against the mutant Y181C, Y188L, and K103N HIV-1 strains; it was highly active against the multidrug resistant mutant IRLL98 HIV-1 strain bearing the K101Q, Y181C, and G190A mutations conferring resistance to NVP, DLV, and EFV and several HIV-1 clades A in PBMC.

INTRODUCTION

Acquired immune deficiency syndrome (AIDS) pandemic and AIDS-related diseases remain among the leading causes of death worldwide. At the end of 2010, an estimated 34 million people were living with HIV globally, including 3.4 million children less than 15 years, and there were 2.7 million new HIV infections.[1] Combination therapies based on three (recommended) or more different antiretroviral drugs designated as highly active antiretroviral therapy (HAART) slow down the viral reproduction with marked reduction of plasma viremia below the detection level in most patients undergoing treatment for at least six months.[2] Despite major improvement achieved with HAART regimens, the management of associated drug resistance and adverse effects remain unsolved problems of chronic long-term treatments.[3]

HAART regimens containing first-generation non-nucleoside reverse transcriptase inhibitors (NNRTIs), namely Nevirapine (NVP), Delavirdine (DLV), or Efavirenz (EFV), may lead to development of drug resistance. In particular, the K103N and Y181C were the most prevalent mutations in clinical HIV-1 isolates. To overcome the drug resistance, new molecules with better resistance profile are desirable.[4] Among them, Etravirine (ETV) and Rilpivirine (RPV) were approved for use in combination with other antiretroviral agents for the management of HIV-1 infection in treatment-experienced and naïve adult patients, respectively.[5]

The activity against mutant HIV-1 strains of indolylarylsulfone (IAS) NNRTIs was significantly improved by introduction of two methyl groups at positions 3′ and 5′ of the 3-phenylsulfonyl moiety (**1**, **2**).[6] Coupling of the indole-2-carboxamide functionality with amino acid units (e.g., **3**) resulted in potent IAS derivatives.[7] Similarly, a hydroxyethyl moiety at the indole-2-carboxamide led to highly potent IAS derivatives.[8] The addition of a third heterocyclic nucleus to the parent compound resulted in NNRTIs with broad spectrum of activity against the mutant HIV-1 strains (e.g., DATAs, BIRL 355BS).[9] Recently, we found that IAS derivatives bearing a cyclic moiety at the 2-carboxamide nitrogen linked through a short spacer group (e.g., **4**–**6**) were endowed with potent antiretroviral activity.[10] Here, we have expanded the SAR studies by the introduction of a pyridinyl or 4-cyanophenyl moiety at the 2-carboxamide nitrogen through a methylene/ethylene bridge. The new IAS derivatives **7**–**17** showed potent HIV-1 inhibitory activity in the low nanomolar range (Chart 1) (Table 1).

CHEMISTRY

Carboxamides **7**–**13** and **17** were synthesized by coupling reaction of 5-chloro-3-((3,5-dimethylphenyl)sulfonyl)-1*H*-indole-2-carboxylic acid[6] (**18**) with the appropriate amine in the

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Chart 1. New IASs 7−17 and Reference Compounds 1−6

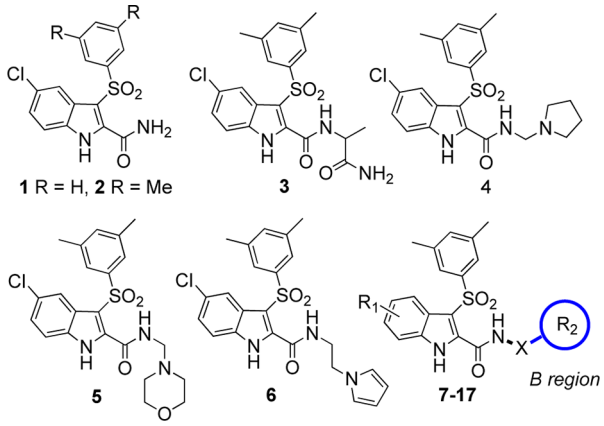
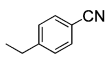


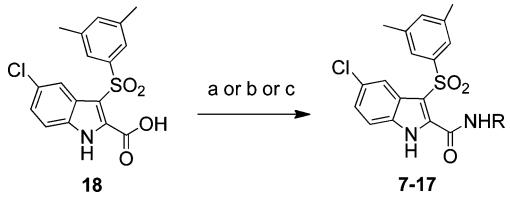
Table 1. Anti-HIV-1 Activity of 7-17 in MT-4 Cells[a]

		HIV-1 (NL4-3)		
Compd	R	EC_{50} (nM)[a]	CC_{50} (nM)[b]	SI[c]
7		2.0± 0.06	>11015	>5508
8		2.2 ± 0.2	>11015	>5007
9		2.0 ± 0.2	>11015	>5508
10		12 ± 2	>9921	>827
11		2 ± 0.4	>10684	>5342
12		2.1 ± 0.7	>10684	>5088
13		2 ± 1	>10684	>5342
14		2 ± 2	>10777	>5389
15		2.2 ± 0.02	>10777	>4899
16		11 ± 6.6	>10777	>980
17		1.5 ± 0.002	>10461	>6974
3[d]	-	2 ± 0.2	6038	3019
AZT	-	7.8 ± 7.8	>18710	>2399
NVP	-	1502 ± 939	>18776	>13
EFV	-	6.3 ± 3.2	>15839	>2514

[a]EC_{50}: effective concentration (nM) to inhibit by 50% HIV-1 (NL4−3 strain) induced cell death, as evaluated with the MTT method in MT-4 cells. [b]CC_{50}: cytotoxic concentration (nM) to induce 50% death of noninfected cells, as evaluated with the MTT method in MT-4 cells. [c]SI: selectivity index calculated as CC_{50}/EC_{50} ratio. [d]Data of D,L racemic mixture.

presence of (benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluorophosphate (BOP reagent) and triethylamine in anhydrous DMF at 25 °C for 12 h. Alternatively, the acid **18** was transformed into acid chloride by heating at reflux with thionyl chloride for 1.5 h or into carboxylic acid imidazolide with 1,1′-carbonyldiimidazole in anhydrous THF at 25 °C for 3 h. Subsequent treatment of the activated acid with the appropriate amine provided the carboxamides **14**−**16** (Scheme 1).

Scheme 1. Synthesis of Compounds 7−17[a]



[a]Reagents and reaction conditions: (a) (**7**−**13** and **17**) amine, BOP reagent, triethylamine, anhydrous DMF, 25 °C, 12 h, 35−80%; (b) (**14** and **16**) (i) thionyl chloride, reflux temperature, 1.5 h, (ii) amine, triethylamine, anhydrous THF, 25 °C, 1 h, 98%; (c) (**15**) (i) 1,1′-carbonyldiimidazole, anhydrous THF, 25 °C, 3 h, (ii) 3-amino-benzonitrile, 25 °C, 2 h, 45%.

RESULTS AND DISCUSSION

Initially, we synthesized new IAS derivatives **7**−**9**. Independently of the position of the nitrogen atom of the pirimidinylmethyl moiety, derivatives **7**−**9** inhibited the NL4−3 HIV-1 strain in the low nanomolar range of concentration (EC_{50} values: **7** and **9** = 2.0 nM; **8** = 2.2 nM; MTT method). Compounds **7**−**9** were all superior to the reference drugs AZT, NVP, and EFV, being ≥3.5-, ≥368-, and ≥2.8-fold more potent, respectively (Table 1). When tested for their cytotoxicity (CC_{50} values), **7**−**9** showed selectivity indexes (SI values) ≥5007, which were higher than those of all of the reference compounds, including IAS **3**. The molecular docking studies carried out following our previously reported methodology[10] suggested the possible binding mode of **7**−**9** into the WT RT NNBS: (i) the indole NH established a H-bond with the carbonyl oxygen of Lys101, (ii) the chlorine atom at position 5 of the indole acceded to a hydrophobic pocket surrounded by Val106 and Leu234, and (iii) the 3,5-dimethylphenyl moiety lay in a hydrophobic cleft formed by Tyr181, Tyr188, Trp229, and Pro95 residues establishing hydrophobic interactions. Focusing on the p51/p66 interface cleft, the pyridinyl ring of **7**−**9** was stabilized by hydrophobic interactions with the side chains of Val179 (p66), Glu138, and Thr139 (p51) (Figure 1S, Supporting Information).

In the case of the quinolin-4-ylmethyl compound **10**, the molecular docking experiments showed that, although the quinoline ring could form the same hydrophobic interactions as the pyridine analogue, part of the heterocycle was placed outside the cleft and exposed to the solvent. This in silico observation might justify the biological results obtained, as derivative **10** showed an EC_{50} of 12 nM and was 6-fold less potent than **7** and **9**.

We synthesized the pirimidinylethyl derivatives **11**−**13**, which joined structural characteristics of highly potent IASs: (i) the pirimidinyl group of **7**−**10** and (ii) the ethylene linker of **6**. In this case, the modeling predictions did not show any significant differences in comparison with **7**−**9** and were in good agreement with the biological results. Indeed, IAS derivatives **11**−**13** (EC_{50}s: **11** and **13** = 2 nM; **12** = 2.1 nM) were potent inhibitors of the NL4−3 HIV-1 strain, with inhibitory concentrations absolutely comparable to those of the corresponding derivatives **7**−**10**.

Finally, we synthesized the cyanophenyl derivatives **14**−**17**. We envisaged compounds **14**−**17** by pushing out the nitrogen atom from the pirimidinyl ring while its distance from the carboxamide nitrogen was kept fixed. The molecular modeling suggested again that the aromatic ring would be stabilized by hydrophobic interactions, independently on the position of the

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cyano group. Compounds **14**, **15**, and **17** potently inhibited the NL4−3 HIV-1 strain at EC_{50} values of 2 nM (**14**), 2.2 nM (**15**), and 1.5 nM (**17**) and were equipotent to pyridinyl compounds **7**−**9** and **11**−**13**.

Among the most potent compounds, IAS derivatives **8**, **9**, **12**, **15**, and **17**, representing significant structural differences within this series, were evaluated in MT4 cells against mutant HIV-1 strains harboring Y181C, Y188L, and K103N single amino acid mutations in the RT (Table 2). Compounds **8**, **9**, **12**, **15**, and **17** were potent inhibitors of the mutant Y181C HIV-1 strain, with EC_{50}s ranging from 2.2 nM (**9**) to 44 nM (**15**). Against this mutant, all the compounds were superior to EFV, and derivatives **8**, **9**, and **12** displayed comparable results to AZT. Against the Y188L mutant strain, compounds **8** and **9** were 7- and 14-fold respectively more potent than EFV and were comparable to AZT.

Table 2. Anti-HIV-1 Activity of Compounds 8, 9, 12, 15, and 17 against Mutant HIV-1 Strains in MT-4 Cell Cultures[a]

compd	Y181C EC_{50} (nM)[a] FC[b]	Y188L EC_{50} (nM) FC	K103N EC_{50} (nM) FC
8	8.8 ± 2.9	44 ± 15	37.8 ± 22
	4.4	20.0	17.2
9	2.2 ± 2.2	22 ± 15	8.8 ± 2.2
	1.1	10.0	4.4
12	6.3 ± 0.0	504 ± 273	27.3 ± 4.2
	3.0	240.0	13.0
15	44 ± 8.8	1386 ± 1144	19.8 ± 15.4
	20	630	9.0
17	32.1 ± 4.2	>10714	117.9 ± 64.3
	21.4	>7142	78.6
AZT	1.6 ± 0.4	11.7 ± 7.8	15.6 ± 11.7
	0.2	1.5	2.0
NVP	>3756	>3756	>3756
	>2.5	>2.5	>2.5
EFV	157.5 ± 157.5	315.0 ± 189.0	69.3 ± 31.5
	25	50	11.0

[a]EC_{50}: effective concentration (nM) to inhibit by 50% cell death induced by the indicated mutant HIV-1 strain, as evaluated with the MTT method in MT-4 cells. [b]FC: fold change obtained as ratio between EC_{50}s of drug resistant mutant HIV-1 strain and WT HIV-1 strain.

With the exception of **17**, these inhibitors were more effective than EFV against the K103N mutant, and two compounds, **9** and **15**, were equipotent to AZT. The activity against the K103N mutation is of particular therapeutic value because it is the major mutation emerging in patients treated with EFV whose viral loads rebounds after initial response to the drug.[11] Compound **9** was uniformly potent against all the mutant HIV-1 strains, with inhibitory concentrations in the low nanomolar range of concentration. In particular, **9** was 71-, 14-, and 8-fold superior to EFV against the Y181C, Y188L, and K103N mutations, respectively. It is worth noting that against these mutants **9** was absolutely comparable to AZT.

Compound **9** was evaluated against the multiresistant IRLL98[12] HIV-1 strain bearing the K101Q, Y181C, and G190A mutations conferring resistance to NVP, DLV, and EFV (Table 3). Compounds **9** was more active against the mutant IRLL98 HIV-1 strain than the WT NL4−3 strain. In contrast, NVP and EFV showed the typical drug resistance differential while AZT was slightly less active against this strain.

Table 3. Anti-HIV-1 Activity of Compound 9 against Wild Type HIV-1 NL4-3 and Mutant IRLL98 Strains in MT-4 Cells[a]

compd	EC_{50} (nM) HIV-1 WT (NL4−3)	EC_{50} (nM) HIV-1 mutant (IRLL98)
9	2 ± 0.2	1 ± 0.05
AZT	3.5 ± 1.8	3.8 ± 0.8
NVP	227 ± 141	>18916
EFV	1.8 ± 0.9	980 ± 300

[a]EC_{50}: effective concentration (nM) required to inhibit by 50% the cell death induced by the indicated mutant HIV-1 strain, as evaluated with the MTT method in MT-4 cells.

To ascertain that the test compounds were also inhibitory in primary T-lymphocyte cells using virus strains that belong to different clades of HIV-1, the most potent, **9**, was included in this study (Table 4). Compound **9** proved to inhibit potently HIV-1 clades A, B, C, D, A/E, F, and G. With the exception of clade G, the EC_{50} mean concentrations in two different PBMC donors were in the higher picomolar range. Most of the clinical isolates, in contrast to HIV-1 (NL4−3) (CXCR4-using), were CCR5-using or CCR5-tropic. As reference compounds, Maraviroc (CCR5 antagonist) or AMD3100 (CXCR4 antagonist) were used and were inhibitory in their expected nM (1−20 nM) range. We also evaluated HIV-1 NL4.3 strain in PBMC, and here we obtained an EC_{50} of 0.4−0.8 nM. No antiviral activity was observed against an HIV-2 isolate (>200 nM). These results point to a broad spectrum of anti-HIV-1 activity of this class of compounds.

Table 4. Inhibitory Activity of Compound 9 against Various HIV-1 Clades in Peripheral Blood Mononuclear Cells (PBMC)

HIV-1 clade	EC_{50}[a,b] range (nM)
clade A (UG273)	0.6−1.2
clade B (BaL)	0.1−0.4
clade C (DJ259)	0.4−0.8
clade C (SM145)	0.3−0.4
clade D[c] (UG270)	0.4−0.6
clade A/E (ID12)	0.6−0.9
clade F (BZ162)	0.6−0.8
clade G (BCF-DIOUM)	1.7−1.8

[a]Compound concentration required to inhibit HIV-1 p24 production in virus-infected PBMC; mean values of two independent experiments in two different PBMC donors. [b]The clinical HIV-1 isolates were sensitive to maraviroc (CCR5 antagonist) or AMD3100 (CXCR4 antagonist) in the 1−20 nM range. [c]CXCR4-using clade.

As inhibitors of the RT of HIV-1 WT, compounds **8** and **9** showed inhibitory concentrations comparable to the reference ATI **3**, were four and 20 times superior to NVP and EFV, respectively, and two times inferior to ETV (Table 5). Compounds **8** and **9** inhibited in the nanomolar range the RT of K103N HIV-1, the major mutation emerging in patients treated with EFV.[11] Against the K103N RT, the most active compounds **8** and **9** were notably more active than NVP and EFV and the reference ATI **3**. Compounds **8** and **9** were also potent inhibitors of the L100I RT. Compound **8** was 140 and >1000 times more potent than the reference ATI **3** and NVP, respectively.

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Table 5. HIV-1 RT Inhibitory Activity of Compounds 8, 9, 12, 15, and 17 against the WT and Mutant RTs Carrying Single Amino Acid Substitutions[a]

	IC_{50}[b] (nM)		
compd	WT	K103N	L1001
8	20	32	8
9	21	45	11
12	40	162	24
15	506	>40	287
17	>10	>10	>4
3[c]	23	190	112
NVP	400	7000	9000
EFV	80	400	nd[d]
ETV	10	20	10

[a]Data represent mean values of at least three separate experiments. [b]Compound concentration (IC_{50}, nM) required to inhibit by 50% the RT activity of the indicated strain. [c]Literature[7] [d]nd: no data.

The binding modes of IASs into WT and mutated RTs are discussed in Supporting Information

CONCLUSIONS

We synthesized new IAS derivatives **7**−**17** bearing nitrogen containing substituents at the indole-2-carboxamide linked through a methylene/ethylene spacer. The IASs **7**−**17** proved to be potent inhibitors of the HIV-1 WT (NL4−3 strain) in MT-4 cells in the low nanomolar concentrations and weakly cytotoxic. Several compounds were potent inhibitors of the mutant HIV-1 strains. Compound **9** was uniformly effective against the mutant Y181C, Y188L, and K103N HIV-1 strains, superior to EFV and equipotent to AZT. IAS **9** was highly active against the multiresistant mutant IRLL98 HIV-1 strain bearing the K101Q, Y181C, and G190A mutations conferring resistance to NVP, DLV, and EFV. Compound **9** also potently inhibited in the higher picomolar range various HIV-1 clades, independently of their coreceptor use, in PBMC. ATI **9** emerged as a useful lead compound for the development of new therapeutic tools for EFV-based HIV-1 therapies which show the emergence of the L100I and K103N mutations. We succeeded in the development of new three-cycles containing ATIs as hybrides between two-wings and horseshoe-conformation NNRTIs.[9] Such results provide useful information for further development of this class of HIV-1 NNRTIs.

EXPERIMENTAL SECTION

Chemistry. Combustion analysis was used as a method for establishing compound purity. Purity of the tested compounds was ≥95%. See Supporting Information for details.

General Procedure for the Preparation of Derivatives 7−13 and 17. Example: 5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)-*N*-(pyridin-2-ylmethyl)-1*H*-indole-2-carboxamide (7). A mixture of **18** (300 mg, 0.82 mmol), 2-picolylamine (130 mg, 0.13 mL, 1.24 mmol), BOP reagent (360 mg, 0.82 mmol), and triethylamine (250 mg, 0.35 mL, 2.48 mmol) in anhydrous DMF (10 mL) was stirred at 25 °C for 12 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine and dried. Removal of the solvent gave a residue that was purified by column chromatography (silica gel, ethyl acetate as eluent) to furnish **7** (190 mg, 60%) as a white solid, mp 229−231 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.99 (s, 6H), 4.69 (d, J = 5.9 Hz, 2H), 7.26 (s, 1H), 7.30−7.36 (m, 2H), 7.54−7.58 (m, 2H), 7.66 (s, 2H), 7.82 (t, J = 7.7 Hz, 1H), 7.97 (s, 1H), 8.57 (d, J = 3.9 Hz, 1H), 9.59 (br t, J = 4.9 Hz, 1H, disappeared on treatment with D_2O), 13.1 ppm (br s, 1H, disappeared on treatment with D_2O). IR: ν 1636, 3220, 3297 cm^{-1}. Anal. ($C_{23}H_{20}ClN_3O_3S$ (453.94)) C, H, Cl, N, S.

General Procedure for the Preparation of Derivatives 14 and 16. Example: 5-Chloro-*N*-(2-cyanophenyl)-3-((3,5-dimethylphenyl)sulfonyl)-1*H*-indole-2-carboxamide (14). A mixture of **18** (150 mg, 0.41 mmol) and thionyl chloride (5 mL) was heated at reflux temperature for 1.5 h. After evaporation to dryness, the residue was dissolved in anhydrous THF (5 mL). Triethylamine (0.41 mg, 0.06 mL, 0.41 mmol) and 2-aminobenzonitrile (72 mg, 0.61 mmol) were added, and the mixture was stirred at 25 °C for 1 h. Water and ethyl acetate were added while shaking. The organic layer was washed with brine and dried. Removal of the solvent gave a residue that was purified by column chromatography (silica gel, ethyl acetate as eluent) to furnish **14** (186 mg, 98%), mp 282−285 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.29 (s, 6H), 7.25 (s, 1H), 7.38 (d, J = 8.3 Hz, 1H), 7.47 (t, J = 8.1 Hz, 1H), 7.59 (d, J = 8.6 Hz, 1H), 7.67 (s, 2H), 7.74−7.79 (m, 2H), 7.91−7.94 (m, 2H), 11.30 (br s, 1H, disappeared on treatment with D_2O), 13.31 ppm (br s, 1H, disappeared on treatment with D_2O). IR: ν 1656, 2224, 3230 cm^{-1}. Anal. ($C_{24}H_{18}ClN_3O_3S$ (463.94)) C, H, Cl, N, S.

5-Chloro-*N*-(3-cyanophenyl)-3-((3,5-dimethylphenyl)sulfonyl)-1*H*-indole-2-carboxamide (15). 1,1′-Carbonyldiimidazole (120 mg, 0.75 mmol) was added to a suspension of **18** (250 mg, 0.69 mmol) in anhydrous THF (15 mL). The reaction mixture was stirred at 25 °C for 3 h, and then 3-aminobenzonitrile (150 mg, 1.25 mmol) was added. The reaction mixture was stirred at 25 °C for 2 h and diluted with water and extracted with ethyl acetate. The organic layer was washed with brine and dried. Removal of the solvent gave a residue that was purified by column chromatography (silica gel, ethyl acetate:*n*-hexane = 1:1 as eluent) to furnish **15** (110 mg, 45%), mp 270 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 6H), 7.27 (s, 1H), 7.36 (dd, J = 2.3 and 8.3 Hz, 1H), 7.58 (t, J = 8.2 Hz, 1H), 7.66−7.67 (m,4H), 7.94 (d, J = 2.1 Hz, 1H), 7.95−7.82 (m, 1H), 8.20 (s, 1H), 11.23 (br s, 1H, disappeared on treatment with D_2O), 13.31 ppm (br s, 1H, disappeared on treatment with D_2O). IR: ν 1678, 2232, 3235 cm^{-1}. Anal. ($C_{24}H_{18}ClN_3O_3S$ (463.94)) C, H, Cl, N, S.

ASSOCIATED CONTENT

Supporting Information

Additional chemical and biological information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

IAS, indolylarylsulfone; HIV-1, human immunodeficiency virus type 1; AIDS, acquired immune deficiency syndrome; RT, reverse transcriptase; NNRTI, non-nucleoside reverse transcriptase inhibitor; HAART, highly active antiretroviral therapy; WT, wild type; NVP, nevirapine; EFV, efavirenz; ETV, etravirine; RPV, rilpivirine; PBMC, peripheral blood mononuclear cells

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