Novel 4-arylaminoquinoline-3-carbonitriles as Inhibitors of HIV-1 Reverse Transcriptase

Beatriz C. L. Melo,[a] Alice M. R. Bernardino,[a] Gustavo Polillo,[a] Helena S. Pereira,[b] Izabel C. P. Paixão,[b] Michele S. Ribeiro,[b] and Julio C. Borges[a,c]*

[a]Instituto de Química, Programa de Pós-Graduação em Química, Departamento de Química Orgânica, Campus do Valonguinho, Universidade Federal Fluminense, CEP 24020-141 Niterói, RJ, Brazil
[b]Instituto de Biologia, Programa de Pós-Graduação em Ciências e Biotecnologia, Departamento de Biologia Celular e Molecular, Campus do Valonguinho, Universidade Federal Fluminense, CEP 24210-150 Niterói, RJ, Brazil
[c]Instituto Federal de Educação Ciência e Tecnologia do Rio de Janeiro, Campus Nilópolis, CEP 26530-060 Nilópolis, RJ, Brazil
*E-mail: julio.borges@ifrj.edu.br

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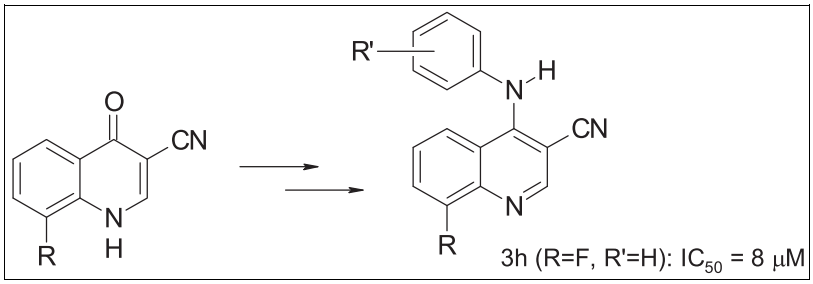

This paper describes the synthesis of several new 4-arylaminoquinoline-3-carbonitriles derivatives. These were evaluated on the activity reverse transcriptase (RT) of HIV-1. Most of the synthesized compounds showed significant *in vitro* inhibition of RT enzyme, especially derivative **3h** (IC_{50} = <8 μM). The derivatives showed low-level cytotoxicity.

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INTRODUCTION

The human immunodeficiency virus type 1 (HIV-1) was discovered in 1983 and is part of a subfamily of retroviruses called Lentivirus. Human immunodeficiency virus is the causative agent of acquired immunodeficiency syndrome (AIDS), a life-threatening global fatal disease [1,2]. An HIV particle, which is approximately 145 nm in diameter and contains a linear single-stranded RNA (ssRNA) genome encoding 15 mature viral proteins [3,4]. It destroys immune system leaving the victim vulnerable to opportunistic infections, being responsible for millions of deaths worldwide [5]. Many drugs have been developed to try to inhibit the replication of HIV. Reverse transcriptase (RT) is considered a key enzyme in the replication cycle of retroviruses and has been widely used as a tool to search compounds having antiviral potential [6,7].

The two major classes of drugs that inhibit reverse transcription are nucleoside analogues (NRTIs) and nonnucleoside RT inhibitors (NNRTIs). The nucleoside analogues (NRTIs) are compounds with a structure like natural nucleosides and interact with the dNTP binding site of the RT. All NRTI compounds are known as 2,3-dideoxynucleoside analogues, with similar mechanisms of drug action, and need to be phosphorylated to their triphosphate forms to act as competitive inhibitors of the RT. The non-nucleoside RT inhibitors (NNRTIs) are small hydrophobic molecules that bind to an allosteric pocket of RT and act as non-competitive inhibitor [8].

Quinoline moiety is present in many classes of biologically active compounds such as antimalarial [9], antibacterial [10], antifungal [11], antitumor [12], and antiviral [13] agents. Our research group reported the synthesis of various substances of pyrazolopyridine and thienopyridine systems that showed antiviral activity [14–16]. A series of 4-arylamino-1*H*-pyrazolo[3,4-*b*] pyridine (I) inhibited approximately 40% of the RT activity at 50-μM concentration [16]. Recent data show the potential of quinoline derivatives to inhibit the activity of enzyme RT of HIV-1 [17,18]. The quinolinic ring was chosen based on the isosteric relationship between it and the pyrazolo [3,4-b] pyridine ring (I), which showed the excellent inhibition results on RT shown above [16]. The arylamino group (also present in compound I) was maintained, and structural modifications were performed, such as variations among the substituents present therein, so that a more efficient screening could be performed. All 14 compounds inhibited the RT-HIV-1 activity at micromolar concentrations and showed no toxicity (Fig. 1).

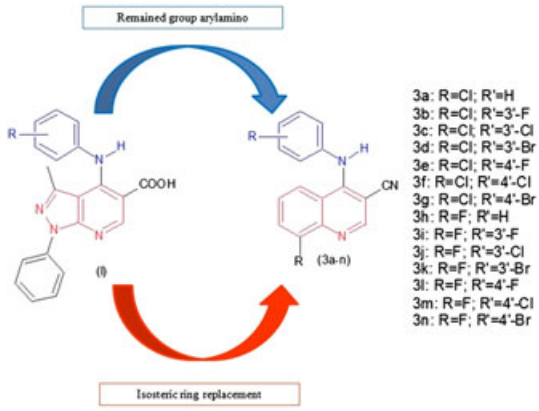
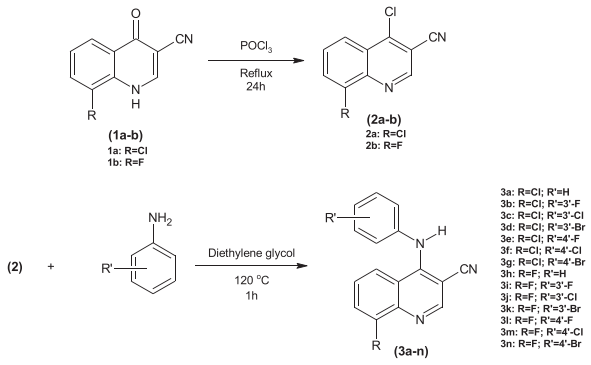


Figure 1. Rational approach to the design of compounds 3a–n. [Color figure can be viewed at wileyonlinelibrary.com]

RESULT AND DISCUSSION

The 4-arylaminoquinoline-3-carbonitrile derivatives (3a–n) were prepared through the reaction of 4-chloroquinolines-3-carbonitriles (2a, 2b) with anilines using diethylene glycol as solvent (Scheme 1) [19–21]. The chlorine atom in the position 4 can be easily replaced by nucleophiles, such as anilines in a nucleophilic aromatic substitution. For other similar reactions, we usually do not use solvent, and the reaction is completed in approximately 4 h [19,20]. However, it was not possible in the present case, due to higher melting points of the intermediates 3a (199°C) and 3b (164°C). These do not melt for the temperature required for such reaction condition (120°C), and hence, the desired product could not be obtained. Using the solvent diethylene glycol, the reagents were easily solubilized, the optimum temperature (120°C) for the reaction was reached, and the reaction time was reduced to 1 h. Another advantage of this solvent is easy removal from the reaction medium by pouring into ice water. The 4-chloroquinolines (2a, 2b) are readily prepared from 4-oxo-1,4-dihydroquinolines (1a, 1b) [21]. The 4-oxo-1,4-dihydroquinolines, in turn, can be readily made by methodology similar to the Gould-Jacobs [20]. See Scheme 1.

Scheme 1. Synthesis of 4-arylaminoquinoline-3-carbonitriles 3a–n (71–91%).



Products were generally identified by ^{1}H NMR, ^{13}C NMR, FT-IR spectroscopies, and mass spectrometry.

The 4-arylaminoquinoline-3-carbonitrile derivatives were evaluated on their ability to inhibit HIV-1 RT enzyme and the potential cytotoxic effect on human T-cell lines transformed by HTLV-1 (MT2 cells). The results are summarized in Table 1. Our results show that derivatives 3a–n displayed low cytotoxicity ($CC_{50} \geq 100$ μM), with the exception of derivative 3j that showed a higher cytotoxicity in lymphocyte (MT-2) cells ($CC_{50} = 0.09$ μM). Derivatives 3a–n were tested in RT inhibition assays and showed to be active as inhibitors of HIV-1 RT (Table 1). The results highlighted that derivative 3h ($IC_{50} < 8$ μM) turned out to be more active. The derivatives 3a–g, 3i–k, and 3 m–n have also been showing promising IC_{50} values ranging between 10 and 12 μM. In contrast, derivative 3l exhibited decreased activities towards the enzyme ($IC_{50} \geq 50$ μM). The results suggest that the 4-arylaminoquinoline-3-carbonitrile derivatives are a promising target for studies in the search for new substances with anti-HIV-1 activity, including *in vitro* studies of their action mechanisms. Also, the relative low cytotoxicity of the samples makes them potential candidates for *in vivo* studies.

In summary, we report the synthesis of 4-arylaminoquinoline-3-carbonitrile derivatives (3a–n). Fourteen new compounds showed to be active as

Table 1

Cytotoxicity and HIV-1 RT inhibitory activities of compounds **3a–n**.

Derivative	R	R′	$CC_{50} \pm SD(\mu M)^{a}$	$IC_{50}(\mu M)^{b,c}$
3a	Cl	H	140 ± 1.52	≤12
3b	Cl	3′-F	100 ± 0.50	≤10
3c	Cl	3′-Cl	100 ± 0.13	≤12
3d	Cl	3′-Br	146 ± 0.32	≤10
3e	Cl	4′-F	100 ± 2.1	≤12
3f	Cl	4′-Cl	100 ± 0.67	≤12
3g	Cl	4′-Br	142 ± 0.56	≤12
3h	F	H	142 ± 1.6	<8
3i	F	3′-F	2.216 ± 0.28	≤12
3j	F	3′-Cl	0.09 ± 1.39	≤12
3k	F	3′-Br	816 ± 0.84	≤12
3l	F	4′-F	816 ± 1.29	≥50
3m	F	4′-Cl	1.047 ± 0.76	≤12
3n	F	4′-Br	100 ± 0.21	≤10

All values are the mean of three independent experiments.
[a]Cellular toxicity of the 4-arylaminoquinoline-3-carbonitriles was determined using T-cell line MT2. CC_{50} = Cytotoxic concentration that reduced cell viability to 50%.
[b]The inhibition of recombinant HIV-1 RT activity was performed with a commercially available EnzChek Assay Kit (Molecular Probes) according to the instructions of the manufacturer. IC_{50} = effective concentration that inhibits 50% of HIV-1 RT.
[c]Efavirenz, an HIV-1 RT inhibitor used as positive control. IC50 = 0.006 μM.

inhibitors of HIV-1 RT. The derivative that exhibited the higher activity was 3h (R = F, R′ = H) with IC_{50} value of 8 μM. The compounds have low-level cytotoxicity and represent a viable source of prototype antivirals. It was identified chemical scaffold that may inform further development of synthetic HIV-1 inhibitors and provide new options for future anti-HIV therapies.

EXPERIMENTAL

Chemistry. The ^{1}H NMR and ^{13}C NMR spectra were obtained using a Varian model Unity Plus spectrometer operating at 300.00 and 75 MHz, respectively. Infrared spectra were recorded in a Perkin-Elmer Spectrum One FT-IR spectrophotometer in KBr disks. Melting points (m.p.) were determined with a Fisher-Johns apparatus. All reagents and solvents used were analytical grade. For fingerprinting ESI-MS analysis, a hyBrid high-resolution and high-accuracy (5 ppm) Micromass Q-TOF mass spectrometer (Micromass, Manchester, UK) was used. The general conditions were as follows: source temperature of 100°C, capillary voltage of 3.5 kV, and cone voltage of 30 V. For measurements in the positive ion mode ESI(+)-MS, 10.0 μL of concentrated formic acid was added giving a final concentration of 0.1%. ESI-MS was performed by direct infusion with a flow rate of 10 μL min^{-1} using a syringe pump (Harvard Apparatus). Mass spectra were acquired and accumulated over 60 s, and spectra were scanned in the range between 50 and 700 *m/z*. The equipment was calibrated with a solution of phosphoric acid, permitting a resolution of less than 20 ppm.

Preparation of the 4-arylaminoquinoline-3-carbonitrile **(3a–n)**: 1.0 mmol of 4-chloroquinolines **(2)**, and 1.5 mmol of corresponding aniline was stirred in 6.0 mL of diethyleneglycol at 120°C for 1 h. Finaly, the mixture was poured in a beaker with 100 mL of ice and water. The crystals formed were filtered and recrystallized from ethanol.

8-Chloro-4-phenylaminoquinoline-3-carbonitrile ($C_{16}H_{10}ClN_3$), 3a. Compound was obtained as a light yellow solid. Yield 91%; mp 205°C; FT-IR (KBr) ν_{max}/cm^{-1}: NH 3298, CN 2205, C═C/C═N 1583, 1559, 1531, 1494, 1482, 1408; ^{1}H NMR (DMSO, δ in ppm): 8.78 (s, 1H, H-2), 8.58 (dd, *J* = 8.4 and 1.2 Hz, 1H, H-5), 7.76 (t, *J* = 8.4 Hz, 1H, H-6), 8.15 (dd, *J* = 8.4 and 1.2 Hz, 1H, H-7), 7.57–7.37 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′), 10.08 (s, 1H, NH); ^{13}C NMR (DMSO, δ in ppm): 153.7, 88.7, 151.4, 120.9, 122.3, 126.2, 132.1, 144.7, 133.0, 139.1, 124.8, 129.1, 126.4, 129.1, 124.8, 116.4. ESI-HRMS ($M + H^+$): 280.0510.

8-Chloro-4-(3′-fluorophenylamino)quinoline-3-carbonitrile ($C_{16}H_9ClFN_3$), 3b. Compound was obtained as a light yellow solid. Yield 76%; mp 212°C; FT-IR (KBr) ν_{max}/cm^{-1}: NH 3301, CN 2209, C═C/C═N 1585, 1560, 1528, 1490, 1448, 1402. ^{1}H NMR (DMSO, δ in ppm): 8.85 (s, 1H, H-2), 8.54 (dd, *J* = 8.7 and 1.2 Hz, 1H, H-5), 7.76 (t, *J* = 8.7 Hz, 1H, H-6), 8.14 (dd, *J* = 8.7 and 1.2 Hz, 1H, H-7), 7.32–7.16 (m, 4H, H-2′, H-4′, H-5′, H-6′), 10.15 (s, 1H, NH). ^{13}C NMR (DMSO, δ in ppm): 153.5, 90.2, 151.1, 121.2, 122.4, 126.6, 132.3, 144.8, 133.1, 141.5, 110.9, 162.4, 112.2, 130.7, 119.9, 116.3. ESI-HRMS ($M + H^+$): 298.0372.

8-Chloro-4-(3′-chlorophenylamino)quinoline-3-carbonitrile ($C_{16}H_9Cl_2N_3$), 3c. Compound was obtained as a light yellow solid. Yield 71%; mp 200°C; FT-IR (KBr) ν_{max}/cm^{-1}: NH 3178, CN 2209, C═C/C═N 1580, 1559, 1522, 1475, 1402, 1286. ^{1}H NMR (DMSO, δ in ppm): 8.87 (s, 1H, H-2), 8.53 (dd, *J* = 8.7 and 0.9 Hz, 1H, H-5), 7.75 (t, 8.7 Hz, 1H, H-6), 8.16 (dd, *J* = 8.7 and 0.9 Hz, 1H, H-7), 7.57–7.40 (m, 4H, H-2′, H-4′, H-5′, H-6′), 10.28 (s, 1H, NH). ^{13}C NMR (DMSO, δ in ppm): 153.5, 90.1, 151.1, 121.3, 122.4, 126.7, 132.3, 144.8, 133.1, 141.1, 123.5, 133.4, 125.4, 130.6, 122.4, 116.4. ESI-HRMS ($M + H^+$): 315.0098.

4-(3′-Bromophenylamino)-8-chloroquinoline-3-carbonitrile ($C_{16}H_9BrClN_3$), 3d. Compound was obtained as a light yellow solid. Yield 85%; mp 203°C; FT-IR (KBr) ν_{max}/cm^{-1}: NH 3176, CN 2209, C═C/C═N 1579, 1558, 1522, 1473, 1401, 1287. ^{1}H NMR (DMSO, δ in ppm): 8.88 (s, 1H, H-2), 8.53 (dd; *J* = 8.7 and 1.2 Hz, 1H, H-5), 7.76 (t, *J* = 8.7 Hz, 1H, H-6), 8.17 (dd, *J* = 8.7 and 1.2 Hz, 1H, H-7), 7.64 (t, *J* = 1.5 Hz, 1H, H-2′); 7.56–7.42 (m, 3H, H-4′, H-5′, H-6′), 10.23 (s, 1H, NH). ^{13}C NMR (DMSO, δ in ppm): 153.5, 90.0, 151.0, 121.7, 122.8, 126.7, 132.3, 144.8, 133.1, 141.1, 122.4, 121.2, 128.3, 130.8, 126.3, 116.4. ESI-HRMS ($M + H^+$): 359.9489.

8-Chloro-4-(4′-fluorophenylamino)quinoline-3-carbonitrile ($C_{16}H_9ClFN_3$), 3e. Compound was obtained as a light yellow solid. Yield 70%; mp 215°C; FT-IR (KBr) ν_{max}/cm^{-1}: NH 3352, CN 2204, C═C/C═N 1588, 1563, 1528, 1509, 1459, 1409, 1217. ^{1}H NMR (DMSO, δ in ppm): 8.76 (s, 1H, H-1), 8.56 (dd, *J* = 8.4 and 1.2 Hz, 1H, H-5), 7.73 (t, *J* = 8.4 Hz, 1H, H-6), 8.14 (dd, *J* = 8.4 and 1.2 Hz, 1H, H-7), 7.42–7.36 (m, 2H, H-2′, H-6′), 7.55–7.51 (m, 2H, H-3′, H-5′), 10.07 (s, 1H, NH). ^{13}C NMR (DMSO, δ in ppm): 153.8, 87.7, 151.9, 120.4, 122.1, 126.4, 132.1, 144.6, 135.4, 133.1, 116.0, 128.1, 160.6, 128.1, 116.0, 116.4. ESI-HRMS ($M + H^+$): 298.0349.

8-Chloro-4-(4′-chlorophenylamino)quinoline-3-carbonitrile ($C_{16}H_9Cl_2N_3$), 3f. Compound was obtained as a light yellow solid. Yield 74%; mp 235°C; FT-IR (KBr) ν_{max}/cm^{-1}: NH 3340, CN 2204, C═C/C═N 1587, 1561, 1526, 1494, 1410, 1249. ^{1}H NMR (DMSO, δ in ppm): 8.83 (s, 1H, H-2), 8.55 (dd, *J* = 8.5 and 1.2 Hz, 1H, H-5), 7.74 (t, *J* = 8.5 Hz, 1H, H-6), 8.16 (dd, *J* = 8.5 and 1.2 Hz, 1H, H-7), 7.46 (dd, *J* = 8.7 and 2.1 Hz, 2H, H-2′, H-6′), 7.59 (dd, *J* = 8.7 and 2.1 Hz, 2H, H-3′, H-5′), 10.10 (s, 1H, NH). ^{13}C NMR (DMSO, δ in ppm): 153.6, 89.2, 151.3, 121.0, 122.3, 126.6, 132.3, 144.8, 133.1, 135.0, 126.2, 129.0, 138.4, 129.0, 126.2, 116.5. ESI-HRMS ($M + H^+$): 315.0098.

4-(4′-Bromophenylamino)-8-chloroquinoline-3-carbonitrile ($C_{16}H_9BrClN_3$), 3g. Compound was obtained as a light yellow solid. Yield 73%; mp 218°C; FT-IR (KBr) ν_{max}/cm^{-1}: NH 3214, CN 2206, C═C/C═N 1580, 1558, 1522, 1488, 1403. ^{1}H NMR (DMSO, δ in ppm): 8.85 (s, 1H, H-2), 8.55 (d, *J* = 8.5 Hz, 1H, H-5), 7.72 (t, *J* = 8.5 Hz, 1H, H-6), 8.17 (d, *J* = 8.5 Hz, 1H, H-7), 7.40 (d, *J* = 8.5 Hz, 2H, H-2′, H-6′), 7.77–7.69 (m, 2H, H-3′, H-5′), 10.09 (s, 1H, NH). ^{13}C NMR (DMSO, δ in ppm): 153.6, 89.5, 151.2, 117.9, 122.4, 126.6, 132.3, 144.8, 133.1, 138.8, 126.2, 131.9, 121.1, 131.9, 126.2, 166.4. ESI-HRMS ($M + H^+$): 359.9424.

8-Fluoro-4-phenylaminoquinoline-3-carbonitrile ($C_{16}H_{10}FN_3$), 3h. Compound was obtained as a light yellow solid. Yield 78%; mp 200°C, FT-IR (KBr) ν_{max}/cm^{-1}: NH 3359, CN 2205, C═C/C═N 1591, 1525, 1488, 1452, 1408, 1212. ^{1}H NMR (DMSO, δ in ppm): 8.72 (s, 1H, H-2), 8.42 (d, *J* = 8.1 Hz, 1H,

H-5), 7.85–7.72 (m, 2H, H-6, H-7), 7.46–7.38 (m, 3H. H-2′, H-4, H-6′), 7.58–7.52 (m, 2H, H-3′, H-5′), 10.09 (s1H, NH). ^{13}C NMR (DMSO, δ in ppm): 153.9, 89.0, 151.5, 121.6, 126.9, 119.3, 117.1, 158.0, 139.1, 139.6, 125.5, 129.6, 126.8, 129.6, 125.5, 117.0. ESI-HRMS (M + H$^+$): 264.0902.

8-Fluoro-4-(3′-fluorophenylamino)quinoline-3-carbonitrile ($C_{16}H_9F_2N_3$), 3i. Compound was obtained as a light yellow solid. Yield 73%; mp 220°C, FT-IR (KBr) ν_{max}/cm^{-1}: NH 3358, CN 2208, C═C/C═N 1595, 1526, 1489, 1438, 1406, 1263, 1145. ^{1}H NMR (DMSO, δ in ppm): 8.80 (s, 1H, H-2), 8.38 (d, *J* = 8.1 Hz, 1H, H-5), 7.87–7.73 (m, 2H, H-6, H-7), 7.31–7.26 (m, 2H, H-2′, H-6′), 7.60–7.53 (m, 1H, H-4′), 7.23–7.17 (m, 1H, H-5′), 10.11 (s, 1H, NH). ^{13}C NMR (DMSO, δ in ppm): 153.2, 89.9, 150.7, 120.2, 119.0, 116.7, 121.4, 111.1, 162.4, 112.4, 126.7, 120.1, 116.5. ESI-HRMS (M + H$^+$): 282.0826.

4-(3′-Chlorophenylamino)-8-fluoroquinoline-3-carbonitrile ($C_{16}H_9ClFN_3$), 3j. Compound was obtained as a light yellow solid. Yield 64%; mp 187°C, FT-IR (KBr) ν_{max}/cm^{-1}: NH 3448, CN 2210, C═C/C═N 1594, 1582, 1528, 1498, 1432, 1206. ^{1}H NMR (DMSO, δ in ppm): 8.81 (s, 1H, H-2); 8.38 (d, *J* = 8.4 Hz, 1H, H-5), 7.87–7.75 (m, 2H, H-6, H-7), 7.58–7.39 (m, 4H, H-2′, H-4′, H-5′, H-6′), 10.11 (s, 1H, NH). ^{13}C NMR (DMSO, δ in ppm): 153.3, 89.8, 150.7, 126.7, 119.0, 116.8, 121.4, 125.7, 133.5, 123.8, 130.8, 122.7, 116.6. ESI-HRMS (M + H$^+$): 298.0494.

4-(3′-Bromophenylamino)-8-fluoroquinoline-3-carbonitrile ($C_{16}H_9BrFN_3$), 3k. Compound was obtained as a light yellow solid. Yield 79%; mp 200°C, FT-IR (KBr) ν_{max}/cm^{-1}: NH 3211, CN 2214, C═C/C═N 1593, 1579, 1526, 1500, 1475, 1433, 1406, 1209. ^{1}H NMR (DMSO, δ in ppm): 8.81 (s, 1H, H-2), 8.37 (d, *J* = 8.1 Hz, 1H, H-5), 7.87–7.75 (m, 2H, H-6, H-7), 7.65 (t, *J* = 1.5 Hz, 1H, H-2′), 7.57–7.50 (m, 3H, H-4′, H-5′, H-6′), 10.10 (s, 1H, NH). ^{13}C NMR (DMSO, δ in ppm): 153.2, 89.8, 150.7, 121.5, 122.7, 119.0, 116.7, 157.7, 126.8, 138.8, 125.7, 131.0, 123.8, 130.7, 126.5, 116.7. ESI-HRMS (M + H$^+$): 343.0073.

8-Fluoro-4-(4′-fluorophenylamino)quinoline-3-carbonitrile ($C_{16}H_9F_2N_3$), 3l. Compound was obtained as a light yellow solid. Yield 69%; mp 200°C, FT-IR (KBr) ν_{max}/cm^{-1}: NH 3354, CN 2203, C═C/C═N 1589, 1567, 1528, 1488, 1468, 1418, 1407, 1213. ^{1}H NMR (DMSO, δ in ppm): 8.69 (s, 1H, H-2), 8.41 (d, *J* = 7.8 Hz, 1H, H-5), 7.83–7.69 (m, 2H, H-6, H-7), 7.42–7.36 (m, 2H, H-2′, H-6′), 7.56–7.51 (m, 2H, H-3′, H-5′), 10.04 (s, 1H, NH). ^{13}C NMR (DMSO, δ in ppm): 153.4, 87.4, 151.5, 120.6, 118.7, 126.3, 116.5, 157.5, 120.7, 135.2; 128.1, 115.9, 160.7, 115.9, 128.1, 116.5. ESI-HRMS (M + H$^+$): 282.0803.

4-(4′-Chlorophenylamino)-8-fluoroquinoline-3-carbonitrile ($C_{16}H_9ClFN_3$), 3m. Compound was obtained as a light yellow solid. Yield 75%; mp 248°C, FT-IR (KBr) ν_{max}/cm^{-1}: NH 3352, CN 2201, C═C/C═N 1587, 1524, 1483, 1410, 1213. ^{1}H NMR (DMSO, δ in ppm): 8.76 (s, 1H, H-2), 8.39 (d, *J* = 8.1 Hz, 1H, H-5), 7.86–7.72 (m, 2H, H-6, H-7), 7.47 (d, *J* = 8.4 Hz, 2H, H-2′, H-6′), 7.59 (d, *J* = 8.4 Hz, 2H, H-3′, H-5′), 10.08 (s, 1H, NH). ^{13}C NMR (DMSO, δ in ppm): 153.2, 88.9, 150.9, 121.1, 126.6, 118.9, 116.6, 157.5, 138.6, 138.2, 126.4, 129.0, 130.1, 129.0, 126.4, 116.6. ESI-HRMS (M + H$^+$): 298.0529.

4-(4′-Bromophenylamino)-8-fluoroquinoline-3-carbonitrile ($C_{16}H_9BrFN_3$), 3n. Compound was obtained as a light yellow solid. Yield 75%; mp 245°C, FT-IR (KBr) ν_{max}/cm^{-1}: NH 3353, CN 2200, C═C/C═N 1588, 1524, 1482, 1407, 1212. ^{1}H NMR (DMSO, δ in ppm): 8.76 (s, 1H, H-2), 8.38 (d, *J* = 8.4 Hz, 1H, H-5), 7.85–7.69 (m, 2H, H-6, H-7) 7.41 (d, *J* = 9.0 Hz, 2H, H-2′, H-6′), 7.71 (d, *J* = 9.0 Hz, 2H, H-3′, H-5′), 10.11 (s, 1H, NH). ^{13}C NMR (DMSO, δ in ppm):153.2, 89.2, 150.7, 121.2, 126.5, 118.9, 116.7, 157.5, 138.7, 118.2, 126.4, 131.9, 138.6, 131.9, 126.4, 116.5. ESI-HRMS (M + H$^+$): 343.9974.

Biological and cytotoxity assays. *Escherichia coli* strain BL21 (DE3) was used as a recipient for DNA transformations. *E. coli* cells transformed with the plasmid containing RTp66 and RTp51 HIV-1 gene were cultured in Luria-Bertani (LB) containing ampicillin (100 μg/mL) under shaking, at 220 rpm at 37°C; overnight. This overnight culture was used as inoculum for 1 L of LB medium containing 100 μg/mL of ampicillin. Cells were grown for 6 h at 37°C with vigorous shaking, then induced with isopropyl-b-D-thiogalactopyranoside (IPTG) (1 mM) for 2 h. Cells were harvested by centrifugation (5000 g, 15 min), and bacterial lysates were prepared using a lysis buffer (50 mM Tris–HCl (pH 7.9 at 4°C), 60 mM NaCl, 1 mM EDTA) and using lysozyme/DNAse I treatment. Clarified lysates were used for the isolation of the p51/p66 heterodimeric RT. The active RT heterodimer was purified by using MagneHis™ Protein Purification System according to the manufacturer's instructions.

HIV-1 RT inhibition. The effects of 4-arylaminoquinoline-3-carbonitriles derivatives on the RT were evaluated using RT EnzChek Assay Kit (Molecular Probes) according to the manufacturer's instructions. IC_{50} values were determined using an Poly(A) ribonucleotide template annealed to a Oligo d(T)16 primer. To a 96-well solid black plate for fluorescence readings was added 5 μL of increasing concentrations of derivatives, followed by 1 μL of the enzyme (15–80 ng/mL) in reaction buffer. The reaction was incubated at 25°C for 60 min, and 2 μL of 200 mM EDTA was added to each reaction. The polymerizing activity was measured using a fluorometric assay by adding 600 μL of PicoGreen in TE buffer (10 mM Tris–HCl, pH 7.5, 1 mM EDTA) to the EDTA-terminated reaction mixture. The reaction was incubated for 10 min on ice, in the dark. The sample fluorescence was measured using a microplate reader (Spectramax-M4 Molecular Devices) (ex. 480 nm, em. 520 nm). Efavirenz was used as a positive control. IC_{50} values were determined using Prism5 (GraphPad Software). All assays were performed in triplicate.

Cell-based assay: cytotoxicity assays. To evaluate the cytotoxicity of 4-arylaminoquinoline-3-carbonitriles derivatives, we used MTT assay accordingly to the manufacturer's protocol. Cytotoxicity was performed in the T-cell line MT2 (10^6 cells/well) treated with increasing concentrations of derivatives at 37°C in a humidified 5% CO_2 incubator for 72 h. After this, MTT solution (1 mg/mL) was added, and cells were incubated for 4 h. After incubation, 100-μL stop solution (0.04 N HCl) was added, and the absorbance was determined in an automatic plate reader at 545 nm according to the manufacturer's instructions. CC_{50} values were determined using Prism5 (GraphPad Software). All assays were performed in triplicate.

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