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Lead Optimization of Diarylpyrimidines as Non-nucleoside Inhibitors of HIV-1 Reverse Transcriptase

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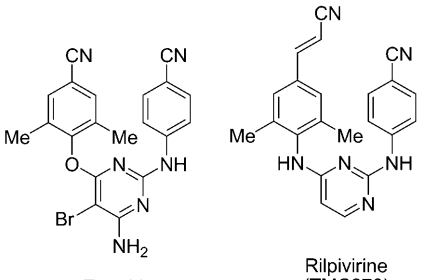
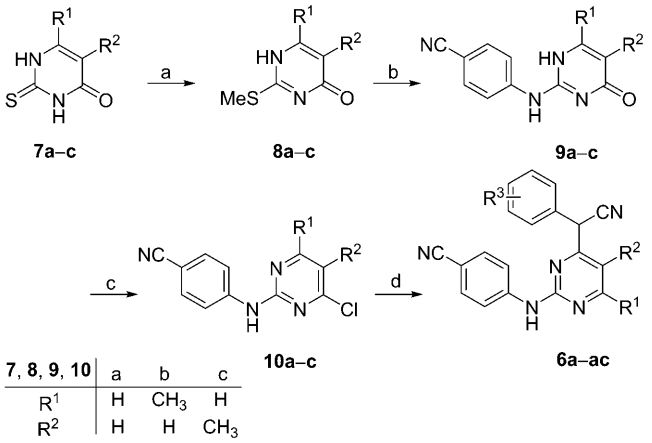
Over the past few years, considerable efforts have been devoted to the structural modification of diarylpyrimidines (DAPYs), a family of non-nucleoside reverse transcriptase inhibitors (NNRTIs) with remarkable anti-HIV-1 activity, leading to the development of etravirine (**1**), rilpivirine (TMC278, **2**) and other highly potent compounds against both wild type and mutant strains of HIV-1 reverse transcriptase (RT).[1–12]



These investigations incorporated a linker between the pyrimidine moiety and the aryl group to the left, and substitution of the same aryl ring: *NH*-DAPYs (**3**), *O*-DAPYs (**4**), *CH*$_2$-DAPYs (**5**). However, there are still unexplored possibilities in diversifying the CH_2 linker between the pyrimidine core and aryl moiety of *CH*$_2$-DAPYs. As part of our ongoing program to develop new DAPYs with improved activity against wild-type and mutant strains of RT,[12] we became interested in the influence of a cyano substituent, the most strongly electron withdrawing group,[13] on the CH_2 linkage of *CH*$_2$-DAPYs. In the present work, the synthesis of the CH_2-linker-modified DAPYs (**6a**–**ac**) and their anti-HIV activity are described.

The key intermediates 4-(4-chloro-pyrimidin-2-ylamino)benzonitriles (**10a**–**c**) were prepared from the commercially available thiouracils according to our previously reported procedures.[11] All of the cyano-*CH*$_2$-DAPYs **6a**–**ac** were synthesized in 29–81% overall yields (four steps) via the condensation of **10a**–**c** with the appropriately substituted aryl acetonitrile in the presence of sodium hydride. The synthetic route is depicted in Scheme 1.



Scheme 1. Synthetic route to new cyano-*CH*$_2$-DAPYs (**6a**–**ac**) *Reagents and conditions*: a) MeI, NaOH, H_2O, RT, 24 h; b) 4-cyanoaniline, 180–190 °C, 8 h; c) $POCl_3$, reflux, 30 min; d) R^3-aryl acetonitrile, 60% NaH, Ar, anhyd THF, 48–72 h.

Cyano-*CH*$_2$-DAPYs **6a**–**ac** were tested in MT-4 cells to evaluate their cytotoxicity and their ability to inhibit the wild-type HIV-1 (LAI strain, IIIB), HIV-1 double mutant K103N + Y181C and HIV-2 strain ROD. Three FDA-approved drugs nevirapine (NEV), delavirdine (DEV) and efavirenz (EFV) were also tested as reference drugs. As seen from the results listed in Table 1, most cyano-*CH*$_2$-DAPYs (**6b**, **6e**, **6i**–**m**, **6o**–**y**, **6aa**–**ac**) showed potent inhibition of wild-type HIV-1 replication with EC_{50} values ranging from 195 to 1.8 nM, which were more potent than those of nevirapine and delavirdine. In particular, compound **6r** displayed the highest potency with an EC_{50} value of 1.8 nM against HIV-1 and the greatest selectivity for the viral target (SI = 118595). It proved more active by twofold, 113-fold

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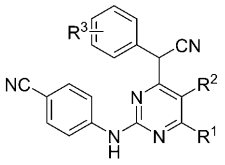
Table 1. Anti-HIV activity and cytotoxicity of compounds **6a**–**ac** in MT-4 cells.[a]



Compd	R^1	R^2	R^3	EC_{50} [nM][b] wild type (IIIB)	HIV-2	103N + 181C	CC_{50} [μM][c]	SI[d]
6a	Me	H	4-F	4869 ± 1050	> 34.8	> 34.8	34.8 ± 4.2	23
6b	Me	H	2-F	34.9 ± 14.5	< 35.5	> 35.5	35.5 ± 6.1	983
6c	Me	H	4-Me	218 ± 23.6	> 34.4	> 34.4	34.4 ± 15.9	157
6d	Me	H	3-Me	442 ± 295	> 35.7	> 35.7	35.7 ± 15.2	83
6e	Me	H	2-Me	9.4 ± 1.2	> 36.6	> 36.6	36.6 ± 1.1	3818
6f	Me	H	4-Cl	1250 ± 361	> 25.6	> 25.6	25.6 ± 3.6	20
6g	Me	H	4-Br	1138 ± 148	> 26	> 26	26 ± 3.2	23
6h	Me	H	4-MeO	873 ± 507	> 82.3	> 82.3	82.3 ± 43.7	93
6i	Me	H	2,3-CH=CH-CH=CH-	123 ± 88	> 25.1	> 25.1	25.1 ± 9.6	202
6j	Me	H	H	800 ± 431	> 398	> 398	> 398 ± 4.2	> 392
6k	Me	H	3,5-dimethyl	760 ± 535	> 33.1	> 33.1	33.1 ± 5.2	43
6l	H	H	4-Me	52.3 ± 21.5	≥ 38.5	≥ 38.5	≥ 38.5	749
6m	H	H	2-Me	18.5 ± 13.9	> 35.9	> 35.9	35.9 ± 5.1	1940
6n	H	H	4-MeO	469 ± 293	≥ 29.6	≥ 29.6	≥ 29.6	≥ 63
6o	H	H	H	118 ± 6.4	> 44.6	≥ 11.4	44.6 ± 2.8	378
6p	H	H	3,5-dimethyl	174 ± 100	> 238.6	> 238.6	≥ 238.6	≥ 1371
6q	H	Me	4-F	195.3 ± 99	> 287.3	> 287.3	287.3 ± 43.9	1467
6r	H	Me	2-F	1.8 ± 0.29	≥ 216.6	≥ 41.1	≥ 216.6	≥ 118595
6s	H	Me	4-Me	35.4 ± 23.6	> 3.4	> 3.4	3.4 ± 2.1	93
6t	H	Me	3-Me	8.6 ± 5.6	> 37.9	> 37.9	37.9 ± 5.5	4441
6u	H	Me	2-Me	7.1 ± 4.7	> 249.9	> 249.9	≥ 249.9	≥ 35524
6v	H	Me	4-Cl	172.2 ± 116.7	> 30.6	> 30.6	30.6 ± 10.1	177
6w	H	Me	4-Br	59.4 ± 19.8	> 49.3	> 49.3	49.3 ± 53.6	832
6x	H	Me	4-MeO	180.2 ± 106.8	> 4.1	> 4.1	4.1 ± 1.48	27
6y	H	Me	2,3-CH=CH-CH=CH-	69.3 ± 21.3	> 18.7	> 18.7	18.7 ± 3.9	273
6z	H	Me	3,4-CH=CH-CH=CH-	594 ± 396	> 32.16	> 32.16	32.16 ± 5.7	51
6aa	H	Me	H	33.8 ± 24.6	≥ 37.2	≥ 139.7	139.7 ± 149.8	4044
6ab	H	Me	3,5-dimethyl	14.4 ± 3.7	> 50.8	> 50.8	50.8 ± 16.5	3548
6ac	H	Me	2-Cl	26.3 ± 14.4	> 58.1	≥ 32.2	≥ 58.1	2189
NEV				203 ± 112.8	–	> 15	> 15	> 75
EFV				3.5 ± 0.6	–	0.18	> 6.3	> 1843
DEV				719 ± 328	–	> 43.6	> 43.6	> 62

[a] Data represent mean values of at least three separate experiments. [b] EC_{50}: compound concentration (nM or μM) required to protect against viral cytopathogenicity by 50% in MT-4 cells. [c] CC_{50}: concentration of compound that reduces the normal uninfected MT-4 cell viability by 50%. [d] SI: selectivity index: ratio CC_{50}/EC_{50} (wild type).

and 399-fold than efavirenz, nevirapine and delavirdine, respectively.

In terms of structure–activity relationships within these new cyano-*CH*$_2$-DAPYs, a methyl group at the C5-position (R^2) of the pyrimidine core increased the anti-HIV-1 activity; for example, compounds **6q**–**ac** were more active against HIV-1 RT with EC_{50} values ranging from 1.8 to 594 nM than the corresponding unsubstituted compounds **6l**–**p** or C6-methyl substitution as in **6a**–**k**. These results suggest that the steric properties of the methyl group in the C5-position of the pyrimidine moiety might decrease the molecular flexibility to a small extent, and this would be beneficial to keep the inhibitors in low-energy conformations.

Keeping the 5-methylpyrimidine motif as the optimum subunit, a number of compounds with R^3 substitutions to the left-hand aryl ring were prepared (**6q**–**ac**) and evaluated for their antiviral activity. The substitution on the aryl ring plays a significant role in the antiviral activity. The unsubstituted compound **6aa** exhibited anti-HIV-1 activity with an EC_{50} value of 33.8 nM. When the *para* position of the phenyl was substituted by a methyl group (**6c**), potency was nearly the same as for **6aa** (EC_{50} = 35.4 nM). Other *para*-substituents, such as fluoro, chloro, bromo and methoxy, all resulted in a decrease in potency compared with **6aa**. These data suggested that a group with suitable steric bulk in the *para* position of the phenyl ring is required for a significant level of anti-HIV-1 potency.

In two of the three possible direct comparisons, a clear positional preference on the phenyl ring was observed. The inhibitory potency was higher for the *ortho*-substituted derivative **6u** than for the *meta*-substituted analogue (**6t**), which in turn was more active than the *para*-substituted congener (**6s**). Optimal activity was obtained with compound **6r** with a 2-fluoro-substituted aromatic ring. It is also worthwhile to mention that two isomers **6y** and **6z**, with a naphthyl ring at the C4-position of the pyrimidine ring, gave markedly different anti-HIV-1 activities: **6y** exhibited an EC_{50} value of 69.3 nM, while the activity of **6z** was ninefold less active.

The activities of these compounds against the double mutant HIV-1 strain K103N + Y181C and HIV-2 ROD virus were also evaluated. Compounds **6o**, **6r** and **6ac** showed weak activity against the double mutant strains, and compound **6aa** exhibited low potency against the HIV-2 ROD virus. Other analogues were found to be inactive (Table 1).

With the aim to investigate the binding mode of our newly synthesized compounds, five compounds were docked into the HIV-1 RT non-nucleoside binding site (NNBS) using Molegro Virtual Docker (demo version).[14] Coordinates of the NNBS were taken from the crystal structure of the RT/4-(4-(mesitylamino)pyrimidin-2-ylamino)benzonitrile (TMC120) complex (PDB code: 1S6Q)[15] due to the high degree of structural similarity between TMC120 and cyano-*CH*$_2$-DAPYs.

Figure 1 shows the theoretical binding mode of **6r** to the NNBS, and the overlay of the predicted binding modes of com-

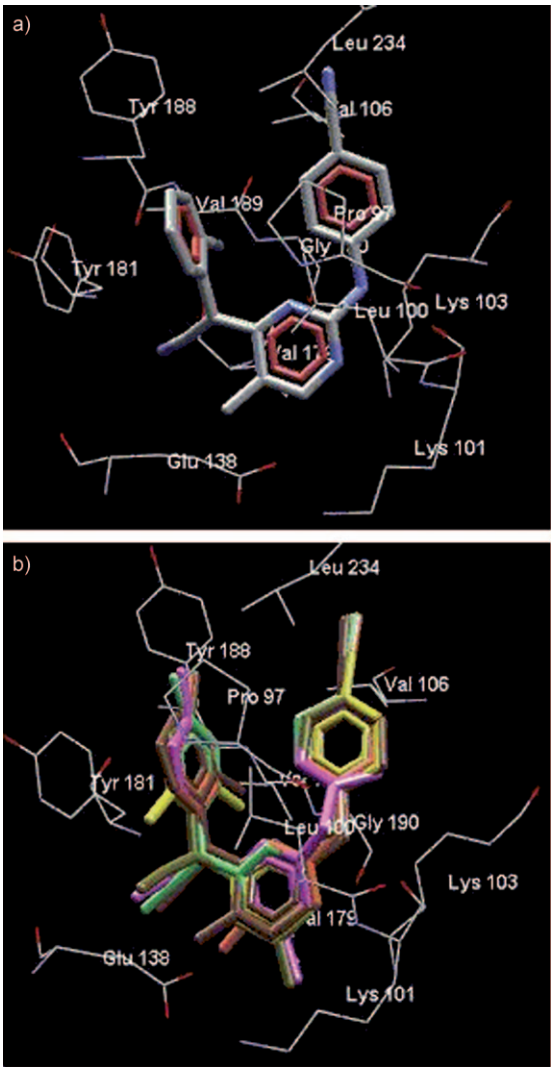


Figure 1. a) Predicted binding mode of **6r** to the NNBS of HIV-1 RT (PDB code: 1S6Q); b) Overlay of predicted binding modes for compounds **6a** (gray), **6c** (purple), **6L** (cyan), **6q** (brown), **6r** (nut-brown) and the experimental binding mode of TMC120 (yellow) in the NNBS of HIV-1 RT.

pounds **6a**, **6c**, **6L**, **6q**, **6r** with the experimental binding mode of TMC120 in the HIV-1 RT NNBS. These molecular modeling studies suggest that compounds **6a**, **6c**, **6L**, **6q**, **6r** probably fit the NNRTI site in a very similar manner to TMC120. All analogues adopt a horseshoe mode-of-binding similar to that seen for most of the DAPY compounds.[15] As such, the critical interactions between inhibitor and binding site were retained: namely the hydrogen bonds between the NH group linking the pyrimidine and 4-cyanophenyl group and amino acid residues Lys 101 and Lys 103, and the π–π interactions between the left-hand aromatic rings and aromatic amino acids Tyr 181, Tyr 188, Phe 227, Tyr 318, and Trp 229. In addition, the cyano group is proximate to the aromatic amino acid residues Tyr 181 and Tyr 188, and this might add further van der Waals and π–π-stacking interactions within the binding pocket of RT. These additional interactions would lead to the improved binding affinity and increased anti-HIV-1 activity of these inhibitors.

In summary, a series of 4-(4-(cyano(alkyl)methyl)pyrimidin-2-ylamino)benzonitriles was discovered as novel NNRTIs, and their preliminary structure–activity relationships were established through chemical modifications. In addition, modeling studies were employed to better understand the interactions between these inhibitors and the NNBS of HIV-1 RT and to guide the structure–activity relationship studies. This investigation led to the identification of highly potent compounds against wild type HIV-1 RT. In particular, compound **6r** showed the most potent activity against with an EC_{50} value of 1.80 nM and a selectivity index of 118595.

Experimental Section

General procedure for the synthesis of 6a–ac: A solution of **10a–c** (2 mmol) in anhydrous THF (30 mL) was treated with the appropriate aryl acetonitrile (2.2 mmol) at RT and stirred for 0.5 h. NaH (0.2 g, 4.8 mmol; 60% dispersion in mineral oil) was added portion wise at RT under Ar and the reaction was stirred at RT for 48–72 h. The resulting mixture was poured into water and extracted with EtOAc. The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash chromatography (silica gel; EtOAc/petroleum ether, 1:3) gave the final products **6a–ac**. Full protocols and characterization data for intermediates and all final compounds can be found in the Supporting Information.

4-(4-(Cyano-(2-fluorophenyl)methyl)-5-methylpyrimidin-2-ylamino)benzonitrile (6r): (57%); mp: 203.9–204.6 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 2.16$ (s, 3H, CH_3), 6.23 (s, 1H, CH), 7.30–7.51 (m, 4H, ArH), 7.60 (d, 2H, $J = 8.8$ Hz, $ArH_{2,6}$), 7.83 (d, 2H, $J = 8.8$ Hz, $ArH_{3,5}$), 8.45 (s, 1H, H_6), 10.26 (br s, 1H, NH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 14.2$, 36.3, 102.8, 116.5, 118.2, 118.5 (2C), 120.0, 120.4, 120.9, 121.1, 125.9, 130.6, 131.7, 133.3 (2C), 145.2, 158.3, 160.9, 161.3; MS (ESI+): *m/z* 344 $[M+H]^+$; Anal. calcd for $C_{20}H_{14}N_5F$: C 69.96, H 4.11, F 5.53, N 20.40, found: C 69.92, H 3.98, F 5.71, N 20.39.

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Keywords: HIV-1 · inhibitors · NNRTI · reverse transcriptase · structure–activity relationships

[1] D. W. Ludovici, R. W. Kavash, M. J. Kukla, C; Y. Ho, H. Ye, B. L. De Corte, K.; Andries, M.-P. de Béthune, H. Azijn, R. Pauwels, H. E. Moereels, J. Heeres, L. M. Koymans, M. R. de Jonge, K. J. Van Aken, F. F. Daeyaert, P. J. Lewi, K. Das, E. Arnold, P. A. J. Janssen, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2229–2234; Y. Ho, H. Ye, B. L. De Corte, K.; Andries, M.-P. de Béthune, H. Azijn, R. Pauwels, H. E. Moereels, J. Heeres, L. M. Koymans, M. R. de Jonge, K. J. Van Aken, F. F. Daeyaert, P. J. Lewi, K. Das, E. Arnold, P. A. J. Janssen, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2229–2234.

[2] S. G. Sarafianos, K. Das, S. H. Hughes, E. Arnold, *Curr. Opin. Struct. Biol.* **2004**, *14*, 716–730.

[3] K. Das, P. J. Lewi, S. H. Hughes, E. Arnold, *Prog. Biophys. Mol. Biol.* **2005**, *88*, 209–231.

[4] B. L. De Corte, *J. Med. Chem.* **2005**, *48*, 1689–1696.

[5] P. A. J. Janssen, P. J. Lewi, E. Arnold, F. Daeyaert, M. de Jonge, J. Heeres, L. Koymans, M. Vinkers, J. Guillemont, E. Pasquier, M. Kukla, D. Ludovici, K. Andries, M.-P. de Bethune, R. Pauwels, K. Das, A. D. C. Jr, Y. V. Frenkel,

S. H. Hughes, B. Medaer, F. D. Knaep, H. Bohets, F. D. Clerck, A. Lampo, P. Williams, P. Stoffelsb, *J. Med. Chem.* **2005**, *48*, 1901 – 1909.
[6] J. Heeres, M. R. de Jonge, L. M. H. Koymans, F. F. D. Daeyaert, M. Vinkers, K. J. A. Van Aken, E. Arnold, K. Das, A. Kilonda, G. J. Hoornaert, F. Compernolle, M. Cegla, R. A. Azzam, K. Andries, M.-P. de Béthune, H. Azijn, R. Pauwels, P. J. Lewi, P. A. J. Janssen, *J. Med. Chem.* **2005**, *48*, 1910 – 1918.
[7] J. Guillemont, E. Pasquier, P. Palandjian, D. Vernier, S. Gaurrand, P. J. Lewi, J. Heeres, M. R. de Jonge, L. M. H. Koymans, F. F. D. Daeyaert, M. H. Vinkers, E. Arnold, K. Das, R. Pauwels, K. Andries, M.-P. de Béthune, E. Bettens, K. Hertogs, P. Wigerinck, P. Timmerman, P. A. J. Janssen, *J. Med. Chem.* **2005**, *48*, 2072 – 2079.
[8] C. Mordant, B. Schmitt, E. Pasquier, C. Demestre, L. Queguiner, C. Masungi, A. Peeters, L. Smeulders, E. Bettens, K. Hertogs, J. Heeres, P. Lewi, J. Guillemont, *Eur. J. Med. Chem.* **2007**, *42*, 567 – 579.
[9] J. Heeres, P. J. Lewi, *Adv. Antiviral Drug Des.* **2007**, *5*, 213 – 242.
[10] E. De Clercq, *Nat. Rev. Drug Discovery* **2007**, *6*, 1001 – 1018.
[11] X. Q. Feng, Y. H. Liang, Z. S. Zeng, F. E. Chen, J. Balzarini, C. Pannecouque, E. De Clercq, *ChemMedChem* **2009**, *4*, 219 – 224.
[12] Y. H. Liang, X. Q. Feng, Z. S. Zeng, F. E. Chen, J. Balzarini, C. Pannecouque, E. De Clercq, *ChemMedChem* **2009**, *4*, 1537 – 1545.
[13] M. O. Sinnokrot, C. D. Shrill, *J. Am. Chem. Soc.* **2004**, *126*, 7690 – 7697.
[14] R. Thomsen, M. H. Christensen, *J. Med. Chem.* **2006**, *49*, 3315 – 3321.
[15] K. Das, Clark, A. D., Jr.; Jr. , P. J. Lewi, J. Heeres, M. R. De Jonge, L. M. H. Koymans, M. Vinkers, F. Daeyaert, D. W. Ludovici, M. J. Kukla, B. De Corte, R. W. Kavash, C; Y. Ho, H. Ye, M. A. Lichtenstein, K. Andries, R. Pauwels, M.-P. de Béthune, P. L. Boyer, P. Clark, S. H. Hughes, P. A. J. Janssen, E. Arnold, *J. Med. Chem.* **2004**, *47*, 2550 – 2560; Y. Ho, H. Ye, M. A. Lichtenstein, K. Andries, R. Pauwels, M.-P. de Béthune, P. L. Boyer, P. Clark, S. H. Hughes, P. A. J. Janssen, E. Arnold, *J. Med. Chem.* **2004**, *47*, 2550 – 2560.

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