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Evolution of Anti-HIV Drug Candidates. Part 3: Diarylpyrimidine (DAPY) Analogues

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Abstract—The synthesis and anti-HIV-1 activity of a series of diarylpyrimidines (DAPYs) are described. Several members of this novel class of non-nucleoside reverse transcriptase inhibitors (NNRTIs) are extremely potent against both wild-type and a panel of clinically significant single- and double-mutant strains of HIV-1.

The use of non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as Rescriptor™ (delavirdine)[1] and Viramune™ (nevirapine)[2] for the treatment of acquired immune deficiency syndrome (AIDS) has been well established. NNRTIs have become key components in combination regimens with nucleoside reverse transcriptase inhibitors such as AZT and 3TC, and protease inhibitors.[3,4] The most recently approved NNRTI, Sustiva™ (efavirenz), is active against many strains of the virus that had evolved resistance to delavirdine and nevirapine and it has quickly become part of key combination therapies and even as a first line therapy.[5]

As indicated in the previous two papers, our program in NNRTIs led to the discovery of a series of acyclic imidoyl thioureas[6] (ITUs) and a family of triazines[7] with excellent anti-HIV activity. We report in this paper the discovery of a series of diarylpyrimidines (DAPYs) that appear to be even more effective against wild-type and various mutant strains of HIV-1.

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In the previous communication, we reported the discovery of a group of diaryltriazines (DATAs) as a novel class of NNRT inhibitors.[7] While the structure–activity relationship (SAR) of a variety of dichlorobenzyltriazines was being explored, synthesis of the three isomeric pyrimidine analogues [**2a** (R = H), **3** (R = H), and **4**] of triazine **1** was pursued in an attempt to gain insight into the importance of the central heterocycle. Synthesis of pyrimidines **2a** and **3** was accomplished as outlined in Scheme 1. 2,4,6-Trichloropyrimidine **5** was reacted with 2,6-dichlorobenzylmagnesium chloride in diethyl ether to afford **6** in excellent yield. Treatment of **6** with ammonia in isopropanol resulted in a 1:1 mixture of aminopyrimidines **7** (R = H) and **8** (R = H). The mixture was treated with 4-cyanoaniline to yield equimolar amounts of diaminopyrimidines **2a** and **3**.

The third isomer **4** was prepared by reacting amidine **9** with diethyl malonate in the presence of sodium ethoxide, resulting in the formation of pyrimidinedione **10** (Scheme 2).[8] Conversion of **10** to the dichloropyrimidine with phosphorus oxychloride, followed by consecutive treatment with ammonia and 4-cyanoaniline, led to the formation of the desired pyrimidine **4**.

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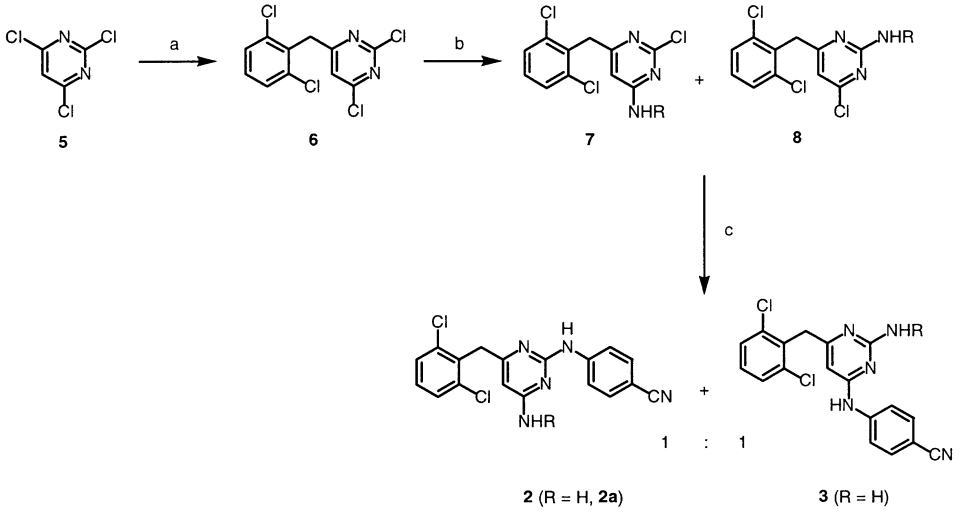
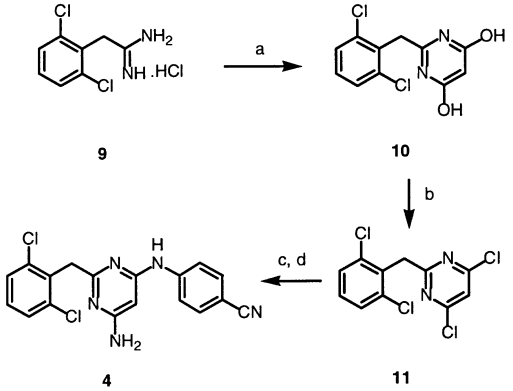


Scheme 1. (a) 2,6-Dichlorobenzylmagnesium chloride, Et_2O, 0 °C, 90%; (b) RNH_2, *i*PrOH, quant.; (c) 4-cyanoaniline, melt at 160 °C, 73%.

Of the three isomeric pyrimidines, **2a** and **3** were as effective as the triazine in inhibiting the LAI strain of HIV-1. Interestingly, among the three, only **2a** was effective in inhibiting several key mutant strains. When compared to triazine **1**, pyrimidine **2a** was more active against several clinically relevant mutant strains (Table 1).

We then embarked on an extensive SAR study of **2** with the goal of finding analogues that were active against as many clinically relevant HIV-1 mutant strains as possible, especially those that were resistant to the important treatment alternative, Sustiva™.

In our studies with the ITU and DATA series, we had found that the *para*-cyanoaniline 'eastern aromatic wing' (Wing I in the terminology used in describing HIV-1 RT/NNRTI complex structures[10]) was optimum. Consequently, we focused our efforts on varying the amino and dichlorobenzyl moieties. Keeping the 2,6-dichlorobenzyl unchanged, we introduced a variety of amine substituents at the 4-position of the pyrimidine ring as outlined in Scheme 1. Replacement of the NH_2 by a variety of bulkier amines (RNH, R variable) did not result in an improvement of activity against the wild type, and the inhibition profile for most mutant HIV-1 strains was poor (data not shown).



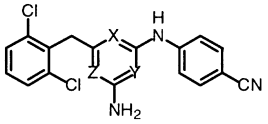
Scheme 2. (a) Diethyl malonate, NaOEt in EtOH, 76%; (b) $POCl_3$; 82%; (c) NH_3/*i*PrOH, 62%, (d) 4-cyanoaniline, 64%.

We were also interested in synthesizing the pyrimidine with hydrogen at the 6-position. Molecular modeling, based on crystal structures of HIV-1 RT complexed with molecules from the DATA series, indicated that this modification might be useful. Unfortunately, all attempts to prepare 2-(4-cyanoanilino)-4-(2,6-dichlorobenzyl)pyrimidine by reacting 2,6-dichlorobenzyl magnesium chloride with 2,4-dichloropyrimidine failed. As the introduction of the 2,4,6-trimethylanilino substituent in the triazine series had resulted in improved activity against the wild type and a number of mutant strains,[7] we decided to prepare 2-(4-cyanoanilino)-4-(2,4,6-trimethylanilino) pyrimidine **13a** (Ar-Y = 2,4,6-trimethylanilino)as outlined in Scheme 3.

We were pleasantly surprised to observe that removal of the amine (i.e., no substitution) resulted in a compound (**13a**) that was nearly as potent as **2a**. Based on this observation, compound **12** became an ideal substrate for exploration of the SAR of the 'western aromatic wing' (Wing II in HIV-1 RT/NNRTI crystal structures[10]), and a variety of aromatic nucleophiles were reacted with this intermediate (Scheme 3). Anilines were introduced by reaction with the hydrochloride salt of **12**, whereas the oxygen and sulfur analogues were prepared by reaction of **12** with sodium phenolates and sodium thiolates.

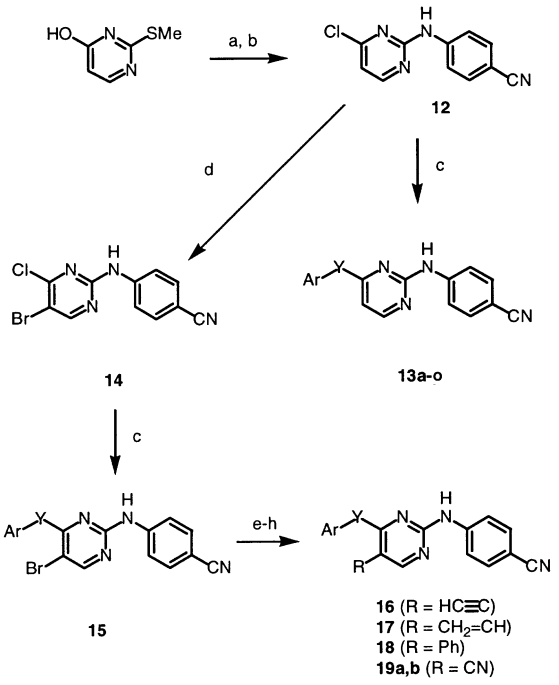
In this series, the compounds containing the 4-cyano-2,6-dimethylphenoxy and 4-cyano-2,6-dimethylanilino

Table 1. Inhibition of HIV-1 (IC_{50}, μM)[9]



Compd	X	Y	Z	LAI	100I	103N	181C	188L
1	N	N	N	0.006	0.398	0.040	0.200	0.316
2a	N	N	CH	0.001	0.299	0.012	0.175	0.071
3	CH	N	N	0.010	96.6	33.3	>100	>100
4	N	CH	N	0.452	>100	>100	>100	>100

substituents (**13b** and **13c**) consistently displayed strong potency against most single mutants tested (Table 2), and also had the best activity against the HIV-1 mutants tested. However, the activity of these compounds against the double mutants, particularly the 100I + 103N mutant, was only moderate and in the micromolar range. We then set out to explore the effect of substitution on C-5 of the pyrimidine nucleus. In this series, the 4-cyanoanilino group in the 2-position was not varied, and the substituents in the 4-position were limited to 2,4,6-trimethylanilino, 4-cyano-2,6-dimethylanilino, and 4-cyano-2,6-dimethylphenoxy. The 5-bromopyrimidine **15** was synthesized by halogenation of **12** with NBS in chloroform, followed by treatment with 4-cyanoaniline (Scheme 3). 5-Bromopyrimidines **15a** and **15c** were further converted to the corresponding 5-ethynyl (**16**),[11] 5-vinyl (**17**),[12] and 5-phenylpyrimidines (**18**)[13] using well documented transition metal catalysis chemistry. 5-Cyanopyrimidines **19a** and **19b** were obtained by treatment of **15a** and **15c** with cuprous cyanide in dimethylformamide.[14] The 5-chloropyrimidines (**20a–c**), 5-methylpyrimidines (**21a** and **21b**), and 5-nitropyrimidine (**22**) were obtained from the corresponding commercially available 2,4,5-trichloropyrimidine, thymine, and 5-nitrouracil, respectively, applying chemistry as outlined in Scheme 3. 5-Aminopyrimidine **23** was obtained by hydrogenation (H_2/10% Pd on C) of 5-nitropyrimidine **22**. The acetylated aminopyrimidine **24** was obtained by treatment of **23** with acetic anhydride in acetone.

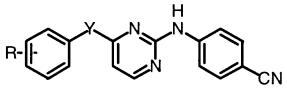


Scheme 3. (a) 4-Cyanoaniline, diglyme; (b) $POCl_3$; (c) Ar-YH (Y = NH) or Ar-Y^-Na^+ (Y = O, S), 1,4-dioxane; (d) NBS, $CHCl_3$; (e) **16**: TMS–acetylene, $Pd(Ph_3P)_2Cl_2$, CuI, Et_3N, 3%; (f) **17**: tetravinyltin, $Pd(PPh_3)_4$, 1,4-dioxane, 12%; (g) **18**: $PhSn(Bu)_3$, $Pd(PPh_3)_4$, 1,4-dioxane, 23%; (R) **19a** and **19b**: CuCN, DMF, 17 and 24%, respectively.

The introduction of a substituent in the 5-position of the pyrimidine clearly resulted in improved activity against most strains tested. With the exception of the 5-nitro analogue **22**, these compounds displayed excellent activity against HIV-1 wild-type and single mutants (Table 3). We were also delighted to see that, in particular, the 5-bromo derivatives **15a** and **15b** displayed strong potency against double mutants 100I + 103N and 103N + 181C.

The NH_2 functionality was also reintroduced in the 6-position of the pyrimidine for comparison with **15b**. The synthetic scheme followed was similar to the one described for the synthesis of **4** (Scheme 4). Base catalyzed condensation of 4-cyanophenyl guanidine **26**[15] and diethyl malonate resulted in formation of pyrimidinedione **27**. Conversion of **27** to the corresponding 5-bromo-2,4-dichloro derivative **28** was accomplished using phosphorus oxychloride, followed by bromination.[16] Reaction of **28** with 4-cyano-2,6-dimethyl phenolate and ammonia resulted in formation of target compound **25**.

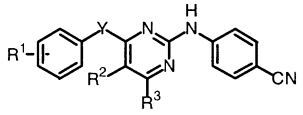
Table 2. Inhibition of HIV-1 (IC_{50}, μM)[9]



Compd	R	Y	LAI	100I	103N	181C	188L	100I + 103N	103N + 181C
13a	2,4,6-triMe	N	0.0010	0.018	0.0043	0.0075	0.048	> 10	0.044
13b	2,6-diMe-4-CN	O	0.0011	0.073	0.0027	0.037	0.019	0.798	0.094
13c	2,6-diMe-4-CN	N	0.0004	0.034	0.0019	0.0071	0.0078	1.086	0.037
13d	2,6-diMe-4-Br	O	0.0029	0.113	0.0047	0.059	0.079	2.371	0.217
13e	2,6-diMe-4-Br	S	0.0057	0.174	0.039	0.068	0.044	3.890	0.354
13f	2,6-diMe-4-(HCC)	O	0.0055	0.185	0.012	0.171	0.050	4.188	0.793
13g	2,4,6-triMe	S	0.0036	0.054	0.012	0.025	0.020	4.786	nd
13h	2,4,6-triMe	O	0.0029	0.022	0.0030	0.038	0.055	8.2	1.1
13i	2,4-diBr-6-F	N	0.0006	0.015	0.0057	0.015	0.064	> 10	nd
13j	2,4,6-triCl	N	0.0007	0.014	0.0030	0.010	0.099	> 10	nd
13k	2,6-diMe	N	0.0007	0.212	0.028	0.061	0.260	> 10	nd
13l	2,4-diCl-6-Me	N	0.0010	0.060	0.013	0.018	0.086	> 10	nd
13m	2,6-diMe-4-Cl	N	0.0021	0.327	0.013	0.019	0.115	> 10	0.401
13n	2,6-diBr-4-Me	N	0.0007	0.0030	0.0030	0.0034	> 100	> 100	0.133
13o	2,6-diMe-4-Br	N	0.0023	0.076	0.012	0.017	0.069	> 100	0.150

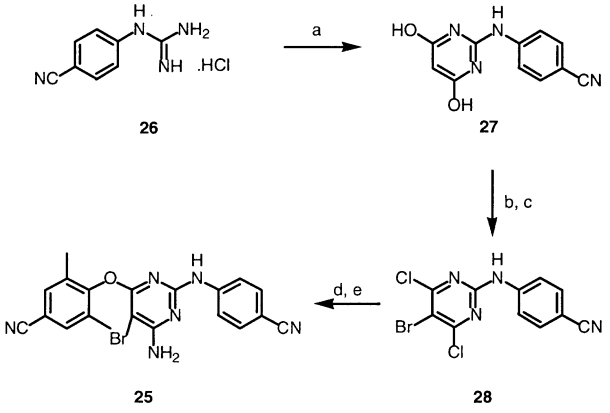
nd: not determined.

Table 3. Inhibition of HIV-1 (IC_{50}, μM)[9]



Compd	R^1	Y	R^2	R^3	LAI	100I	103N	181C	188L	100I + 103N	103N + 181C
15a	2,6-diMe-4-CN	N	Br	—	0.0004	0.0070	0.0004	0.0096	0.0033	0.037	0.032
15b	2,6-diMe-4-CN	O	Br	—	0.0014	0.0066	0.0014	0.022	0.0059	0.049	0.025
15c	2,4,6-triMe	N	Br	—	0.0055	0.011	0.0073	0.036	0.027	0.348	0.283
16	2,4,6-triMe	N	HCC	—	0.0042	0.028	0.0062	0.043	0.042	0.071	0.042
17	2,4,6-triMe	N	Vinyl	—	0.0025	0.035	0.0058	0.036	0.029	> 100	0.106
18	2,4,6-triMe	N	Ph	—	0.024	nd	nd	nd	nd	nd	nd
19a	2,6-diMe-4-CN	N	CN	—	0.0005	0.091	0.0026	0.076	0.0086	0.312	0.181
19b	2,4,6-triMe	N	CN	—	0.0010	0.063	0.0034	0.040	0.077	nd	nd
20a	2,6-diMe-4-CN	N	Cl	—	0.0012	0.0052	0.0009	0.014	0.0047	0.144	0.069
20b	2,6-diMe-4-CN	O	Cl	—	0.0014	0.0075	0.0026	0.034	0.0048	0.138	0.038
20c	2,4,6-triMe	N	Cl	—	0.0027	0.017	0.0060	0.032	0.034	0.319	0.138
21a	2,6-diMe-4-CN	N	Me	—	0.0008	0.016	0.0017	0.012	0.016	> 10	0.162
21b	2,4,6-triMe	N	Me	—	0.0017	0.012	0.0026	0.012	0.018	> 100	0.053
22	2,6-diMe-4-CN	O	NO_2	—	0.018	0.121	0.010	0.304	0.782	> 10	0.194
23	2,6-diMe-4-CN	O	NH_2	—	0.0010	0.016	0.0013	0.0093	0.0057	0.282	0.014
24	2,6-diMe-4-CN	O	NHAc	—	0.0019	0.031	0.0033	0.030	0.0084	0.205	0.030
25	2,6-diMe-4-CN	O	Br	NH_2	0.0014	0.0033	0.0012	0.0070	0.0046	0.019	0.0043
Nevirapine					0.032	0.316	6.310	10.000	> 100	nd	> 100
Delavirdine					0.063	2.512	2.512	1.995	1.259	nd	19.953
Efavirenz					0.0010	0.040	0.040	0.0020	0.158	> 10	0.040

nd: not determined.



Scheme 4. (a) Diethyl malonate, NaOEt in EtOH, 76%; (b) $POCl_3$; 86%; (c) Br_2, $NaHCO_3$, H_2O, MeOH, 78%; (d) sodium 4-cyano-2,6-dimethylphenolate, *N*-methyl-pyrrolidone, 1,4-dioxane, 45%; (e) NH_3/*i*PrOH, 41%.

Compound **25** proved to be even more potent than any other compound synthesized in this series. In fact, **25** inhibits all HIV-1 mutant strains tested with IC_{50} values less than 20 nM, including the 100I + 103N mutant, which is not affected by Sustiva™.

In summary, a variety of 2,4-disubstituted, 2,4,5-trisubstituted, and 2,4,5,6-tetrasubstituted pyrimidines were successfully synthesized as part of an ongoing anti-HIV program. Several of the 2,4-disubstituted pyrimidines **13** possessed good activity against the HIV-1 wild type and a number of clinically relevant mutants, but their activity against the double mutants was only in the micromolar range. Introduction of substitution in the 5-position, including 6-amino-5-bromopyrimidine **25**, led to compounds that displayed excellent potency against both the wild type and the single and double mutants tested. A number of compounds from this series are being considered for clinical evaluation.

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9. (a) All compounds were tested for potency (IC_{50}, μM) to achieve 50% protection of MT-4 cells from HIV-1 cytopathicity as determined by the MTT method (Pauwels, R.; Balzarini, J.; Baba, M.; Snoek, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309). Unless noted otherwise, the LAI strain of HIV-1 was the infecting virus. Other infecting mutant (in the reverse transcriptase) strains of virus are characterized in the tables by the mutated amino acid position and the one letter codes. For instance, 181C refers to replacement of tyrosine at position 181 with cysteine. All determinations are the result of multiple tests. (b) nd, not determined.
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17. Results to be part of future publications.