Bioorganic & Medicinal Chemistry Letters 22 (2012) 2376–2379

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Optimization of 2,4-diarylanilines as non-nucleoside HIV-1 reverse transcriptase inhibitors

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ARTICLE INFO

Article history:
Received 23 December 2011
Revised 16 February 2012
Accepted 17 February 2012
Available online 24 February 2012

Keywords:
Diarylaniline
NNRTIs
Lead optimization
Anti-HIV agents

ABSTRACT

The current optimization of 2,4-diarylaniline analogs (DAANs) on the central phenyl ring provided a series of new active DAAN derivatives **9a**–**9e**, indicating an accessible modification approach that could improve anti-HIV potency against wild-type and resistant strains, aqueous solubility, and metabolic stability. A new compound **9e** not only exhibited extremely high potency against wild-type virus (EC_{50} 0.53 nM) and several resistant viral strains (EC_{50} 0.36–3.9 nM), but also showed desirable aqueous solubility and metabolic stability, which were comparable or better than those of the anti-HIV-1 drug TMC278 (2). Thus, new compound **9e** might be a potential drug candidate for further development of novel next-generation NNRTIs.

Since the first case of acquired immunodeficiency syndrome (AIDS) was reported by the US in 1981, the scientific progress in HIV/AIDS research has been extraordinary, especially in the development of antiretroviral therapy (ART) that has proven to be life-saving to millions of people. Recent scientific evidence has demonstrated that ART is also effective at preventing infection[1,2]; thus it offers an unprecedented opportunity to control the AIDS pandemic. Therefore, the discovery and development of novel highly potent anti-HIV drugs is imperative.

Among current anti-HIV drugs, non-nucleoside reverse transcriptase inhibitors (NNRTIs) are used in combination with other drugs as key components in the highly active antiretroviral therapy (HAART).[3,4] NNRTIs target an allosteric binding pocket on HIV-1 reverse transcriptase (RT) in a noncompetitive manner to cause distortion of the three-dimensional structure of the enzyme to inhibit RT catalytic function.[5] Currently, five NNRTIs drugs have been approved by FDA and are marketed (Fig 1) with the advantages of high potency and low toxicity. Despite the clinical high efficiency, the first-generation NNRTIs drugs (nevirapine, delavirdine, efavirenz) are limited in clinical use due to rapid emergence of drug-resistance. However, the second-generation NNRTI drugs etravirine (TMC125, **1**)[6] and rilpivirine (TMC278, **2**)[7] greatly overcome this deficiency. Both **1** and **2** possess potent antiviral activity with subnano- or low nano-molar EC_{50} values against wild-type and a broad spectrum of mutated viral strains. There is also a higher genetic barrier[8,9] to delay the emergence of drug-resistance toward etravirine and rilpivirine.[10] Although both drugs are poorly water soluble over a wide pH range, **2** has the advantage of better bioavailability when compared to **1**, resulting in a once-daily oral dosing. The success of etravirine and rilpivirine greatly encouraged more research to explore additional novel next-generation NNRTI agents with new scaffolds, high potencies, improved resistant profiles, and better pharmacokinetic profiles than **1** and **2** for more efficacious therapy and potential AIDS prevention.

In prior studies toward the design and synthesis of novel next-generation NNRTIs, we discovered a series of new diarylanilines (DAANs, Fig 2) with nano- to subnano-molar anti-HIV potencies against wild-type and multi-RT-resistant viral strains,[11,12] as exemplified by compound **3** with higher potency against wild-type (EC_{50} 0.38 nM) and RT multi-resistant viral strains (EC_{50} 0.87 nM) than **1** in the same assays. Previous SAR results also defined the key pharmacophores of DAANs as NNRTIs: (1) a *para*-cyanoaniline moiety (A-ring), (2) a crucial amino group on the central phenyl ring (B-ring) *ortho* to the A-ring position, (3) a trisubstituted phenoxy ring (C-ring) with a *para*-linear hydrophobic substituent, and (4) similar molecular flexibility to **1** and **2**. Their high potency and straightforward synthesis prompted us to develop DAANs as new anti-AIDS drug candidates. For orally anti-HIV drug candidates, aqueous solubility is one of the most critical physicochemical properties to be considered in the process of lead optimization, because low aqueous solubility would limit molecular absorption

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doi:10.1016/j.bmcl.2012.02.055

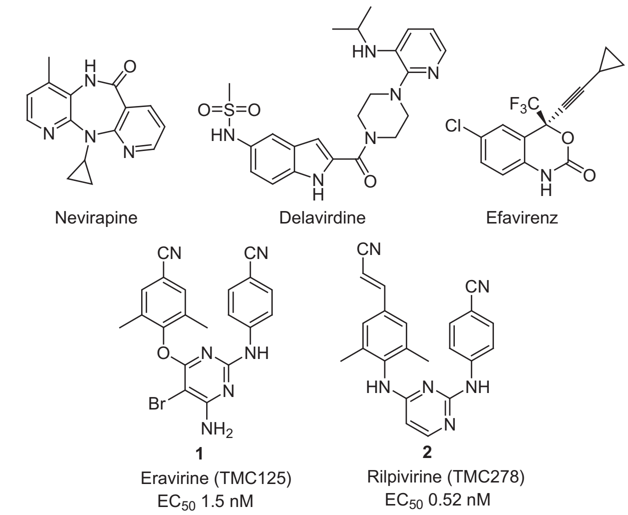


Figure 1. Current marketed NNRTIs.

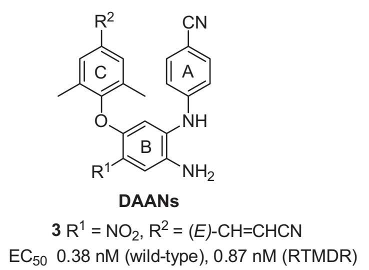
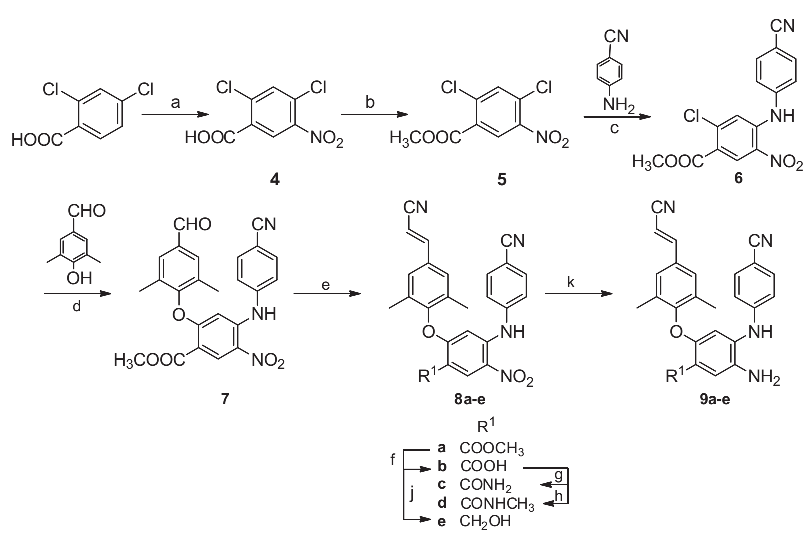


Figure 2. 2,4-Diarylaniline leads.

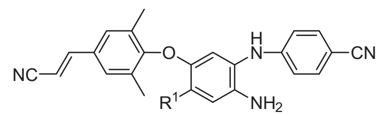
and bioavailability in vivo. On the other hand, most small organic molecules generally have poor water solubility. Therefore, our current optimization of DAANs must generate active DAANs with desirable molecular aqueous solubility. Our early results indicated that the nitro group (R^1) on the central phenyl ring (B-ring) of DAANs was associated with antiviral potency by creating a small electrostatic interaction with the positively charged amino acid K172 on the NNRTI binding site.[11] Thus, we proposed that other polar or ionizable groups, similar to the nitro group, on the B-ring might provide comparable or additional interaction point(s) with the NNRTI binding site via a 'salt bridge' and/or H-bonds to enhance antiviral potency. Furthermore, the presence of R^1 substituent(s) to serve as a H-bond acceptor or donor is expected to be favorable for improving aqueous solubility. Therefore, a series of new DAAN derivatives with different R^1 groups on the central phenyl ring are reported herein, including chemical synthesis, antiviral activity, aqueous solubility, and metabolic stability in vitro.

As shown in Scheme 1, the nitration of 2,4-dichlorobenzoic acid, a commercially available and inexpensive reagent, was performed with concentrated HNO_3 in H_2SO_4 at low temperature (0–5 °C) to produce 2,4-dichloro-5-nitrobenzoic acid (**4**) with a 91% yield. Compound **4** was esterified with MeOH in the presence of H_2SO_4 to afford methyl 2,4-dichloro-5-nitrobenzoate (**5**). Coupling of **5** and 4-aminobenzo-nitrile was performed in DMF in the presence of excess cesium carbonate at 100 °C for about 10 h to provide methyl 2-chloro-4-(4-cyanophenyl amino)-5-nitrobenzoate (**6**), which was characterized by an NH signal at δ 9.76 ppm in the ^{1}H NMR spectrum, ascribable to the chelated nitro group and consistent with previous results that the chlorine *ortho* to the nitro group has higher reactivity for nucleophilic substitution with an aromatic amine. Next, intermediate **6** was reacted with 4-hydroxy-3,5-dimethylbenzaldehyde under microwave irradiation in DMF in the presence of potassium carbonate with stirring at 190 °C for about 15 min to afford **7** with a three-phenyl ring skeleton in a 67% yield. Subsequently, the aldehyde group in **7** was converted into a cyanovinyl moiety by condensation with diethyl cyanomethyl phosphonate in the presence of potassium *tert*-butoxide to afford compound **8a**. Then, the ester group in **8a** was hydrolyzed under basic conditions to afford the corresponding benzoic acid compound **8b**. The carboxylic acid group was then converted into an amide or *N*-methyl amide by treatment with $SOCl_2$, followed with ammonia or methylamine to afford corresponding compounds **8c** and **8d** respectively. In addition, the ester group in **8a** was reduced with $LiBH_4$ to provide compound **8e** with a hydroxymethyl substituent. Finally, the nitro group on the central ring in compounds **8a–8e** was reduced by using sodium hydrosulfite



Scheme 1. Reagent and conditions: (a) HNO_3/H_2SO_4, 0–5 °C to rt, 2 h; (b) H_2SO_4/MeOH, reflux, 2 h; (c) Cs_2CO_3/DMF, 100 °C, 10 h; (d) K_2CO_3/DMF, 190 °C, microwave, 10–15 min; (e) $(EtO)_2P(O)CH_2CN$, *t*-BuOK/THF, 0 °C to rt, 3 h; (f) THF/MeOH, aq NaOH, 0.5 h; (g) (i) $CH_2Cl_2/SOCl_2$, reflux, 3 h; (ii) ammonia in THF, 0 °C, 0.5 h; (h) (i) $SOCl_2/CH_2Cl_2$, reflux, 3 h; (ii) methylamine in THF, 0 °C, 0.5 h; (j) $LiBH_4$, THF/MeOH, 1 h; (k) $Na_2S_2O_4$, ammonia, THF/H_2O (1:1, v/v), rt, 2 h.

Table 1
Anti-HIV activities of **9a–9e** in cellular assay and aqueous solubility [a]



	R^1	EC_{50} (nM) [b]	CC_{50} (μM) [c]	SI[d]	Aqueous solubility (μg/mL)	
					pH 7.4	pH 2.0
9a	$COOCH_3$	2.74 ± 0.71	>22.8	>8465	1.30	1.75
9b	COOH	230 ± 38	14.9	65	117	4.40
9c	$CONH_2$	0.87 ± 0.28	>23.6	>27126	0.63	2.10
9d	$CONHCH_3$	5.72 ± 0.94	>9.2	>1606	1.32	8.63
9e	CH_2OH	0.53 ± 0.13	16.3	30754	3.23	21.0
3[e]	NO_2	0.38 ± 0.07	>47.1	>123947	0.29	0.29
2 (TMC278)		0.52 ± 0.14	19.4	37305	0.24	156

[a] HIV-1 NL3-4 (wild-type) virus in TZM-bl cell lines.
[b] Concentration of compound that causes 50% inhibition of viral replication and presented as mean ± standard deviation (SD) in at least triplicate.
[c] Concentration of compound that causes cytotoxicity to 50% of cells.
[d] Selectivity index (SI) is the ratio of CC_{50}/EC_{50}.
[e] Compound and activity data were published previously.[12]

Table 2
Data for **9c** and **9e** against resistant viral strains and metabolic stability

	EC_{50} (nM) [a]			Human liver microsome[b]		Rat liver microsome[b]	
	HIV-1_{RTMDR1}	K101E	E138 K	$t_{1/2}$ min	CL mL/min/mg	$t_{1/2}$ min	CL mL/min/mg
9c	0.31 ± 0.10	8.8 ± 3.3	9.0 ± 2.6	35	0.20	122	0.057
9e	0.36 ± 0.10	3.4± 1.05	3.9 ± 0.90	30	0.23	84	0.083
3	0.87 ± 0.33	10.8 ± 3.53	11.3 ± 3.76	47	0.15	139	0.050
2	0.49 ± 0.10	5.7 ± 1.4	5.2 ± 1.6	34	0.20	120	0.057

[a] Experiments were performed at least in triplicate and data presented as mean ± SD.
[b] Data from at least two experiments and presented as means.

dehydrate to produce corresponding target compounds **9a–9e**, a series of 5-substituted 2-(4-cyanophenylamino)-4-(4-cyanovinyl-2,6-dimethylphenoxy)anilines.[13]

New compounds **9a–9e** were first tested against wild-type HIV-1 NL4-3 infection of TZM-bl cells in parallel with TMC278 and **3**. The data are summarized in Table 1. Compounds **9c** (R^1 = $CONH_2$) and **9e** (R^1 = CH_2OH) exhibited extremely high potency with EC_{50} values of 0.87 and 0.53 nM, respectively, and both showed high selective indexes (SIs) of >27,126, comparable to those of new drug **2** (TMC278) and **3** in the same assay. Compounds **9a** (R^1 = $COOCH_3$) and **9d** (R^1 = $CONHCH_3$) were also potent with EC_{50} values of 2.7 and 5.7 nM, respectively, and SI values of >8464 and >1606, respectively. On the other hand, **9b** with a carboxylic acid group (R^1 = COOH) was significantly less potent (EC_{50} 230 nM). Thus, the current results, even with the limited data set, demonstrated that the R^1 substituent on the central phenyl ring was modifiable and could greatly affect antiviral potency. The aqueous solubility of new compounds **9a–9e** at pH 7.4 and pH 2.0 were measured by a HPLC/UV method in parallel with **2** and **3**, and the data are also shown in Table 1. Compared to **2** and **3**, all new **9** series compounds had improved aqueous solubility at pH 7.4 and were generally more soluble at pH 2.0, likely due to the presence of a free amino group. The most active new compound **9e** (R^1 = CH_2OH) showed better aqueous solubility (3.23 μg/mL at pH 7.4 and 21.0 μg/mL at pH 2.0) than the remaining four compounds in the series. Subsequently, the most active compounds **9c** and **9e** were selected for testing against NNRTI resistant HIV-1 mutants RT-multi-drug-resistant (RTMDR), K101E, and E138K. As shown in Table 2, new compounds **9c** and **9e** exhibited improved antiviral activity compared to **3** against the drug resistant viral strains, and a little better or comparable to those of **2**. Compounds **9c** and **9e** were further evaluated in rat and human liver microsome (RLM and HLM, respectively) assays in vitro,[14,15] respectively, to predict their metabolic stability in vivo. The two data sets in Table 2 indicated a similar metabolic stability pattern. Compounds **9c** and **9e** had moderate stability with half-lives of 35 and 30 min in HLM and 122 and 84 min in RLM assays, respectively. Compound **9c** had a longer maintaining time (was more stable) than **9e** in both assays, and both compounds showed comparable metabolic stability to that of **2** ($t_{1/2}$ 34 min) in the HLM assay.

In conclusion, our optimization focused on the central phenyl ring of DAANs provided a series of new active compounds **9a–9e** and also verified our previous hypothesis that the R^1 substitution could optimize the antiviral potency and drug-like properties. This study resulted in the discovery of a new compound **9e** that not only exhibited extremely high potency against wild-type virus (EC_{50} 0.53 nM) and several resistant viral strains (EC_{50} 0.36–3.9 nM), but also showed desirable aqueous solubility and metabolic stability, which were better than those of drug **2**. Thus, compound **9e** could serve as a potential drug candidate for further development.

Acknowledgments

This investigation was supported by grants 30930106 and 81120108022 from the Natural Science Foundation of China (NSFC) awarded to L. Xie and U.S. NIH grants awarded to C. H. Chen (AI65310) and K. H. Lee (AI33066).

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13. Synthetic procedure for 4-substituted 1,5-diarylbenzene-1,2-diamines (**9a**–**9e**). To a solution of a diaryl-nitrobenzene (**8**, 1 equiv) in THF and water (30 mL, v/v 1:1) was added ammonia aqueous solution (25%) and sodium hydrosulfite (10 equiv) successively with stirring at room temperature for 2 h. The reaction was monitored by TLC (CH_2Cl_2/MeOH 60:1) until completed. The mixture was poured into ice-water and extracted with EtOAc three times. After removal of organic solvent under reduced pressure, crude product (**9**) was purified by a flash silica gel column chromatograph (eluent: CH_2Cl_2/MeOH = 30/1) with the CombiFlash Flash chromatography system, ISCO company, Inc. to obtain pure target compounds **9a**–**9e** respectively. HPLC analyses for purities of **9a**–**9e** were performed on an Agilent 1200 HPLC system with UV detector and a Grace Alltima HP C18 column (100 × 2.1 mm, 3 μm) eluting with a mixture of solvents A and B in two conditions: (1) acetonitrile (ACN)/water 70:30, flow rate 1.0 mL/min; (2) MeOH/water 70:30, flow rate 0.8 mL/min. The samples were detected under UV wavelength at 254 nm and an injection volume of 3 μL. Compounds: **9a**, yield 64%, white solid, mp 240–242 °C; ^{1}H NMR ($CDCl_3$) δ ppm 2.16 (6H, s, CH_3 × 2), 3.93 (3H, s, OCH_3), 5.78 (1H, s, NH), 5.80 (1H, d, *J* = 16.8 Hz, =CH), 6.20 (1H, s, ArH-6), 6.67 (2H, d, *J* = 8.8 Hz, ArH), 7.17 (2H, s, ArH), 7.31 (1H, d, *J* = 16.8 Hz, CH=), 7.42 (2H, d, *J* = 8.8 Hz, ArH), 7.45 (1H, s, ArH-3); MS *m*/*z* (%) 439.3 (M+1, 100); HPLC-purity 96.1%. **9b**: yield 35%, brown solid, mp 226–228 °C. ^{1}H NMR ($CDCl_3$) δ ppm 2.19 (6H, s, CH_3 × 2), 5.84 (1H, d, *J* = 16.8 Hz, =CH), 6.04 (1H, s, NH), 6.27 (1H, s, ArH-6), 6.75 (2H, d, *J* = 8.8 Hz, ArH-2′,6′), 7.22 (2H, s, ArH-3″,5″), 7.32 (1H, d, *J* = 16.8 Hz, CH=), 7.44 (2H, d, *J* = 8.8 Hz, ArH-3′,5′), 7.72 (1H, s, ArH-3); MS *m*/*z* (%) 423.2 (M-1, 100); HPLC purity 100.0%. **9c**: yield 63%, white solid, mp 290–292 °C; ^{1}H NMR (DMSO-d_6) δ ppm 2.10 (6H, s, CH_3 × 2), 4.75 (2H, s, NH_2), 6.01 (1H, s, ArH-6), 6.39 (1H, d, *J* = 16.8 Hz, =CH), 6.63 (2H, d, *J* = 8.8 Hz, ArH-2′,6′), 7.45 (2H, d, *J* = 8.8 Hz, ArH-3′,5′), 7.47 (2H, s, ArH-3′,5′), 7.57 (1H, d, *J* = 16.8 Hz, CH=), 7.61 (1H, s, ArH-3), 8.20 (1H, s, NH); MS *m*/*z* (%) 424.2 (M+1, 100); purity (HPLC) 98.2%. **9d**: yield 31%, white solid, mp 112–114 °C; ^{1}H NMR ($CDCl_3$) δ ppm 2.16 (6H, s, CH_3 × 2), 3.07 (3H, d, NCH3), 5.80 (1H, s, NH), 5.83 (1H, d, *J* = 16.8 Hz, =CH), 6.18 (1H, s, ArH-6), 6.65 (2H, d, *J* = 8.8 Hz, ArH-2′,6′), 7.21 (2H, s, ArH-3″,5″), 7.32 (1H, d, *J* = 16.8 Hz, CH=), 7.41 (2H, d, *J* = 8.8 Hz, ArH-3′,5′), 7.81 (1H, s, ArH-3); MS *m*/*z* (%) 438.4 (M+1, 100); HPLC-purity 100.0%. **9e**: yield 81%, white solid, mp 186–188 °C; ^{1}H NMR ($CDCl_3$) δ ppm 2.13 (6H, s, CH_3 × 2), 4.87 (2H, s, CH_2), 5.50 (1H, s, NH), 5.79 (1H, d, *J* = 16.8 Hz, CH=), 6.03 (1H, s, ArH-6), 6.55 (2H, d, *J* = 8.8 Hz, ArH-2′,6′), 6.94 (1H, s, ArH-3), 7.17 (2H, s, ArH-3″,5″), 7.30 (1H, d, *J* = 16.8 Hz, CH=), 7.40 (2H, d, *J* = 8.8 Hz, ArH-3′,5′); MS *m*/*z* (%) 411.3 (M+1, 100); HPLC-purity 99.9%.
14. Microsomal stability assay. Stock solutions of test compounds (1 mg/mL) were prepared by dissolving the pure compound in DMSO and stored at 4 °C. Before assay, the stock solution was diluted with ACN to 0.1 mM concentration. For measurement of metabolic stability, all test compounds were brought to a final concentration of 1 μM with 0.1 M potassium phosphate buffer at pH 7.4, which contained 0.1 mg/mL human liver microsomes and 5 mM $MgCl_2$. The incubation volumes were 300 μL, and reaction temperature was 37 °C. Reactions were started by adding 60 μL of NADPH (final concentration of 1.0 mM) and quenched by adding 600 μL of ice-cold ACN to stop the reaction at 5, 15, 30, 60 min time points. Samples at 0 min time point were prepared by adding 600 μL ice-cold ACN first, followed by 60 μL NADPH. Incubations of all samples were conducted in duplicate. After quenching, all samples were centrifuged at 12,000 rpm for 5 min at 0 °C. The supernatant was collected, and 20 μL of the supernatant was directly injected onto a Shimadzu LC-MS-2010 system with an electrospray ionization source (ESI) for further analysis. The following controls were also conducted: (1) positive control incubation containing liver microsomes, NADPH, and reference compound; (2) negative control incubation omitting NADPH; and (3) baseline control containing only liver microsomes and NADPH. The peak heights of test compounds at different time points were converted to percentage of remaining, and the peak height values at initial time (0 min) served as 100%. The slope of the linear regression from log percentage remaining versus incubation time relationships ($-k$) was used to calculate in vitro half-life ($t_{1/2}$) value by the formula of in vitro $t_{1/2}$ = 0.693/k, regarded as first-order kinetics. Conversion to in vitro CL_{int} (in units of ml/min/mg protein) was calculated by the formula15: CL_{int} = (0.693/in vitro $t_{1/2}$) × (ml incubation/mg microsomes).
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