Design, Synthesis, and Evaluation of Diarylpyridines and Diarylanilines as Potent Non-nucleoside HIV-1 Reverse Transcriptase Inhibitors

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Abstract

Based on the structures and activities of our previously identified non-nucleoside reverse transcriptase inhibitors (NNRTIs), we designed and synthesized two sets of derivatives, diarylpyridines (**A**) and diarylanilines (**B**), and tested their anti-HIV-1 activity against infection by HIV-1 NL4-3 and IIIB in TZM-bl and MT-2 cells, respectively. The results showed that most compounds exhibited potent anti-HIV-1 activity with low nanomolar EC_{50} values, and some of them, such as **13m**, **14c**, and **14e**, displayed high potency with subnanomolar EC_{50} values, which were more potent than etravirine (TMC125, **1**) in the same assays. Notably, these compounds were also highly effective against infection by multi-RTI-resistant strains, suggesting a high potential to further develop these compounds as a novel class of NNRTIs with improved antiviral efficacy and resistance profile.

Introduction

Acquired immunodeficiency syndrome (AIDS), caused by human immunodeficiency virus (HIV), threatens human health and life, spreading rapidly worldwide and resulting in more than 60 million people infected by HIV and about 25 million patients dying of AIDS (www.unaids.org). Because there is no effective vaccine to prevent HIV infection, development of anti-HIV therapeutics is critical to improve the quality and save the lives of HIV infected individuals. To date, 26 anti-HIV drugs have been approved for the clinical treatment of HIV infection and AIDS (www.fda.gov/oashi/aids/virals.html), including reverse transcriptase inhibitors (RTIs), protease inhibitors (PIs), integrase inhibitors, fusion inhibitor, and entry inhibitor (CCR5 co-receptor antagonist). Highly active antiretroviral therapies (HAART), which use a combination of three to four drugs, can significantly

reduce the morbidity and mortality of AIDS. However, as a result of emerging drug-resistant HIV mutants, increasing numbers of HIV-infected patients cannot use or fail to respond to HAART. Therefore, the development of new anti-HIV drugs is urgently required.

HIV-1 reverse transcriptase (RT) is one of the most important viral enzymes and plays a unique role in the HIV-1 life cycle. It has two known drug-target sites, the substrate catalytic site and an allosteric site that is distinct from, but located closely to, the substrate site[1,2]. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) interact with the allosteric site in a noncompetitive manner to distort the enzyme's active conformation and thus disrupt the function of the enzyme. The first-generation NNRTI drugs (nevirapine, delavirdine, and efavirenz) exhibit very potent anti-HIV-1 activity and low toxicity. However, rapid drug-resistance emergence, due to single point mutations (especially K103 mutant)[3] in the NNRTI binding site, compromises their clinical usefulness. Etravirine (TMC125, **1**)[4], a diarylpyrimidine (DAPY, Figure 1), was recently approved as a next-generation NNRTI for AIDS therapy. It exhibits high potency against wild-type and a number of mutated viral strains with nanomolar EC_{50} values and has a higher genetic barrier[5] to delay the emergence of drug-resistance. The success of **1** greatly encouraged further research to explore additional novel NNRTIs.[6,7,8] The most advanced NNRTI in development is another DAPY derivative rilpivirine (TMC278, **2**)[9] in Phase III, which showed better potency and pharmacological profiles than **1**, such as once-daily administration.[10]

By using an isosteric replacement strategy on the central pyrimidine ring of DAPYs, our prior studies discovered two series of active compounds, diarylpyridine (**A**, DAPD)[11] with one pyrimidine nitrogen replaced by carbon and diarylaniline (**B**, DAAN)[12] with both pyrimidine nitrogens replaced by carbon. Exemplary di-*para*-cyanophenylpyridine compound **3** and di-*para*-cyanophenylaniline compound **4** (Figure 1) exhibited high potency against HIV-1 wild-type and RT-resistant viral strains. Previous SAR studies also indicated that the *para*-cyanoaniline moiety (A-ring) is necessary and the amino group on the central ring, either pyridine or benzene (B-ring), *ortho* to the A-ring is quite crucial for enhancing anti-HIV activity in both the **A** and **B** series. On the other hand, the *para*-substituent on the phenoxy ring (C-ring) was modifiable and could greatly affect the anti-HIV potency. We hypothesized that the amino group on the central ring might form additional H-bonds with the key amino acid K101 on the NNRTI binding site, and thus, compensate for the loss of the H-bond between K101 and the nitrogen on the pyrimidine ring in DAPYs.[13] Our previous molecular modeling results provided rational support for our hypothesis.[12] In our continuing studies, we have made further structural modifications on the C-ring moiety of **A** and **B** series of derivatives. The aims are to investigate how the C-ring moiety will affect anti-HIV activity and develop novel classes of NNRTIs with improved antiviral efficacy. Thus, we report herein the design, synthesis, and anti-HIV activity of two sets of new target **A** and **B** compounds (Figure 1).

The crystal structures of complexes HIV-1 RT/**2** [protein database (PDB) code: 2zd1] and K103N/Y181C HIV-1 RT/**2** (PDB: 3bgr)[14] reveal that the linear cyanovinyl in **2** is embedded deep into a cylindrical hydrophobic tunnel, which connects the NNRTI-binding pocket to the nucleic acid-binding cleft, and is favored to interact with the more conservable amino acids W229 and Y183. Thus, the *para*-cyanovinyl group on the C-ring of **2** is thought to be an important moiety for enhancing activity. On the basis of the information about the binding site and our previous SAR results, we designed new target compounds in the **A** and **B** series with diverse *para*-substituents (R_2) on the phenoxy ring (C-ring). The R_2 groups included cyanovinyl and other linear substituents, which could likely insert into the cylindrical hydrophobic tunnel of the binding site and reach the conserved amino acid W229. The R_1 and R_3 substituents on the central pyridine or benzene ring were limited to NH_2, NO_2, and H.

Chemistry

Totally, 34 target compounds (**A** and **B** series, Figure 1) were synthesized via the short routes detailed in Schemes 1–4. Six of them (**11a**, **11d**, **11f**, **13a**, **13d**, and **13f**) have been previously described,[11] but without the full experimental details presented herein. The starting materials 4-aminobenzonitrile, 2,6-dichloro-3-nitro pyridine (**5**), 2,4-dichloronitrobenzene (**6**), and 2,4-dichloro-1,5-dinitrobenzene (**7**) are inexpensive and commercially available. Coupling of 4-aminobenzonitrile and **5** was performed by heating at 140 °C for 4 h without solvent under nitrogen to provide intermediate 4-(6-chloro-3-nitropyridin-2-ylamino)benzonitrile (**8**) in 73% yield. The same reaction of dichloronitrobenzene **6** or **7** with 4-aminobenzonitrile took place in DMF in the presence of triethylamine or potassium *tert*-butoxide (*t*-BuOK) at room temperature to produce corresponding intermediates N-(4-cyanophenyl)-5-chloro-2-nitroaniline (**9**) or N-(4-cyanophenyl)-5-chloro-2,4-dinitroaniline (**10**) in 64% and 89% yields, respectively. Subsequently, intermediate **8** was reacted with a trisubstituted phenol in DMF in the presence of potassium carbonate by traditional heating at 130–140 °C for 6–8 h to afford corresponding 2,6-diaryl-3-nitrophyridines **11a-11c** and **11g** with yields ranging from 56 to 82%. Alternatively, N^1,5-diarylnitroanilines **12a** and **12b** were prepared from **9** or **10** by coupling with a substituted phenol under microwave irradiation in DMF in the presence of potassium carbonate[12]. As shown in Scheme 2, a Sonogashira coupling reaction between **11a** and 2-methyl-3-butyn-2-ol or ethynylcyclopropane took place in the presence of triethylamine and palladium-copper catalyst to give corresponding **11d** and **11e**, respectively, with different elongated *para*-ethynyl substituents on the C-ring. Next, the *para*-substituent on the C-ring of **11d** was removed to convert into **11f** by refluxing in dry toluene in the presence of powdered sodium hydroxide in a 69% yield. The nitro-containing compounds **11a-11f** were reduced by using sodium hydrosulfite dehydrate[15] or nickel(II) chloride hydrate and sodium borohydride to afford corresponding amino-compounds **13a–13f** with yields ranging from 43 to 82%. Moreover, the synthesis of other compounds with different elongated R_2 substituents is indicated in Scheme 3. The condensation of **11g** with nitromethane, malonic acid, or acetone under basic condition afforded **11i**, **11j**, or **11k**, respectively, with a *para*-linear side chain on the phenoxy ring (Scheme 3). The cyanovinyl compounds **11m**, **12c** and **12e** were prepared by the condensation of diethyl cyanomethyl phosphonate with aldehyde-compounds **11g**, **12a** or **12b**, respectively, in the presence of potassium terbutoxide[16] in 53–91% yields. Subsequently, diaryl-nitropyridines **11i-11m** and diaryl-mononitroaniline **12c** were reduced by sodium hydrosulfite dehydrate to provide corresponding diary-pyridinamines **13i**, **13j**, and **13m,** diarylaniline **14c,** and an unexpected reduction product **13k**, in which both the nitro group and the *para*-conjugated double bond on the phenoxy ring were reduced, albeit in low yield. To improve the yield, **13k** was then prepared from **11k** by using catalytic hydrogenation in THF in 79% yield. Alternatively, selective reduction of diaryl-dinitroanilines **12d** and **12e** was achieved by using formic acid in the presence of Pd-C (10%) and triethylamine to give corresponding diaryl-4-nitrobenzene-1,2-diamines **14d** and **14e** respectively, in which the reduced amino group was identified as ortho to the NH-linked aniline.[12] Additionally, **12e** was reduced completely by using sodium borohydride in the presence of antimony (III) chloride ($SbCl_3$) to provide diamino compound **14f**. Scheme 4 shows the syntheses of compounds **15-20** with a 6-cyanonaphthoxy moiety as the C-ring. The coupling of **8** and **10** with 6-cyano-2-naphthol or 1-bromo-6-cyano-2-naphthol by using microwave irradiation followed by the nitro reduction or selective reduction afforded corresponding diarylpyridines **15-16** and diarylanilines **17-20**, respectively, in which the *para*-cyanovinyl group was incorporated into a fused ring system.

Results and discussion

The synthesized diarylpyridines **11**, **13**, **15**, **16** (A series) and diarylanilines **12c-12e, 14, 17-20** (B series) were first tested against infection by wild-type HIV-1 strains NL4-3 and IIIB of TZM-bl and MT-2 cells, respectively, with **1** tested in parallel. HIV-1 was generally quite sensitive to the compounds (low EC_{50} values), and both assays provided similar patterns of antiviral activity. As shown in Table 1, most compounds, except **11d**, **11e**, and **11j** (EC_{50} >3 μM), were highly potent in both assays. Notably, most amino-compounds (R_3 = NH_2), including **13a**–**13f**, **13i-13m**, and **14c-14f**, were much more potent (at least tenfold) than the corresponding nitropyridine and nitrobenzene compounds (**11** and **12**, R_3 = NO_2). The most potent compounds were **13m, 14c**, and **14e** with EC_{50} values 0.7 nM against HIV-1 NL4-3 infection of TZM-bl cells and <14 nM against HIV-1 IIIB replication in MT-2 cells. These three compounds exhibited great potency than **1** (EC_{50} = 1.4 and 41 nM against NL4-2 and IIIB, respectively) and comparable potency to **3**. These results further support our previous hypothesis that the amino group on the central ring at the *ortho*-position to the 4-cyanoaniline A-ring is crucial for enhancing anti-HIV activity.

Like the DAPY analog **2**, newly synthesized DAPD compounds **13m, 14c**, **14e** all contain a cyanovinyl group as the R_2 substituent at the *para*-position on the C-ring. This common feature suggests that the *para*-cyanovinyl moiety may be a favorable structural moiety for anti-HIV activity. This postulate was further confirmed by the high potency of cyanovinyl-compound **11m** relative to corresponding compounds with the same R_1 and R_3 groups but many different R_2 substituents (**11a-11f**, **11i**, **11j**, and **11k**) and even our prior active lead **3**. However, incorporation of the cyanovinyl group into a fused ring system, such as the 6-cyanonaphthol moiety in **15-20**, resulted in obviously reduced anti-HIV activity (EC_{50} > 0.1 μM in both assays), much less than most compounds in Table 1. These results suggest that flexibility of the R_2 group might also be necessary. Further analysis of different R_2 groups indicated that hydrophobic and more linear substituents, such as bromo (Br, **13b**), allyl ($CH_2CH=CH_2$, **13c**), ethynyl (C≡CH, **13f**), and conjugated α,β-unsaturated ketone (CH=CHCOMe, **14d**), were favorable for anti-HIV activity, irregardless of substituent length. Compound **13k** with a more flexible *para*-substituent (-$CH_2CH_2COCH_3$) on the C-ring exhibited potent anti-HIV-1 activity comparable to that of **1**, but was slightly less active than **13m, 14c**, and **14e**. In contrast, R_2 substituents with increased bulk (**11d**, **11e**, **13d**, **13e**) or polarity (**11g**, **11i**, **11j**, **13i**, **13j**) led to reduced antiviral potency. Thus, the hydrophobic tunnel on the western wing of the NNRTI binding site might be very narrow and only allow a straight linear, more flexible, hydrophobic substituent to enter and adjust to an appropriate conformation.

Furthermore, 15 active compounds from the **A** and **B** series were tested against two HIV-1 strains resistant to multiple NRTI/NNRTIs, including RTMDR and A17. The data in Table 2 indicate that, in regard to the R_3 substituent, the amino-compounds (**13** and **14**) were again more effective than the nitro-compounds (**11** and **12**) against the multi-RTI-resistant HIV-1 strains, as shown by comparing the following compound pairs: **11a** vs **13a**, **11b** vs **13b**, **11f** vs **13f**, **11m** vs **13m**, and **12c** vs **14c**. Comparison of **14e** with **14c** and **14f** indicated that an additional nitro-group (R_1) on the central ring was obviously preferable to NH_2 or H at the same position against multi-RTI-resistant HIV-1 strains. Additionally, **14e** was also the least cytotoxic of the three compounds (see Table 1). The most potent compounds **13m** and **14e** against wild-type HIV still exhibited high potency against multi-drug-resistant viral strains HIV-1$_{RTMDR1}$ and A17 with low EC_{50} values (0.59 and 0.87 nM; 0.20 and 0.095 μM, respectively) and were more potent than our prior lead **3** (EC_{50} 0.96 nM and 0.39 μM, respectively) and **1** (EC_{50} 1.0 nM and 0.33 μM, respectively). Notably, **14e** showed a low resistant fold (RF) of 6.79 against viral strain A17 with double mutant K103N/Y181C. This

value is similar to that of **1** (RF = 6) in the same assay and in the literature (RF = 5)17, thus suggesting that **14e** would be a promising new lead for further development.

To understand the binding mode of the newly active compounds, molecular modeling was conducted by using the software Discovery Studio 2.5 and crystal structures of the wild-type RT/**1** (PDB code: 3mec) complex18 and the K103N/Y181C RT/**2** (PDB: 3bgr) complex. As shown in Figure 2, the most active compound (**14e**) and **1** were superposed into both wild-type and mutant NNRTI binding sites. The compounds showed similar binding orientations, horseshoe conformational shapes, and hydrogen bonds between the secondary amine linker and the main-chain carbonyl of K101 (1.88 Å and 1.85 Å respectively). However, an additional hydrogen bond was observed between the amino group on the central ring of **14e** and the K101 carbonyl (2.31 Å) in both sites, which is consistent with our previous results. 12 When compared to **1** bound with the wild-type RT structure, the cyanovinyl group of **14e** is protruded deeply into a cylindrical open hydrophobic tunnel, which is formed by the side chains of amino acid residues Y188, F227, W229, and L234 and connected to the nucleic acid-binding cleft. Accordingly, the cyanovinyl group might disrupt the viral transcription from RNA to DNA more effectively. As observed in Figure 2B and reported about **2**,14 the interactions of the cyanovinyl with the hydrophobic tunnel were conserved despite rearrangements in mutated RT. The cyanovinyl also is likely involved in supplementary interaction with the shifted Y183 (distance 3.53 Å) to compensate the affinity loss of Y181C mutation. Therefore, the extensive interactions of the cyanovinyl group and additional H-bond may explain why **14e**, as well as **13m** and **14c**, is more potent than **1**. On the other hand, modeling results indicated that the nitro group was superposed very well with the bromine atom of **1**, although no interaction of these groups (NO_2 or Br) with the binding site was observed, implying that this position on the central ring would be modifiable for exploring more SAR and new compounds with high potency against both wild-type and mutant viral strains.

Conclusion

Thirty-five target compounds of A and B series (**11-20**) were newly synthesized and evaluated for anti-HIV activity. Most of them showed potent anti-HIV activity (EC_{50} <1 μM), but amino-compounds, 2,6-diarylpyridin-3-amine (**13**) and 2,4-diarylbenzen-1,2-diamine (**14**) derivatives, were much more potent against wild-type and multi-drug-resistant viral strains than corresponding nitro-compounds. The most promising compounds **13m** and **14e** exhibited high potency against wild-type and multi-drug-resistant viral strains with EC_{50} values at subnanomolar level, and were more potent than **1** in the same assays. The modifications of the C-ring and substituents on the central B-ring provided more structure-activity relationship (SAR) correlations as follows: (1) both 2-pyridinamine and aniline could serve as the central moieties in our compounds; (2) an appropriately positioned amino (R_3) group on the central B-ring is crucial for achieving high potency; (3) *para*-cyanovinyl (R_2) on the phenoxy C-ring is a beneficial substituent; (4) a favorable *para*-R_2 substituent on the C-ring should be hydrophobic, flexible, and more linear to insert into the narrow tunnel on the NNRTI binding site; and (5) an additional nitro group (R_1) on the central ring *orthro* to the C-ring could benefit inhibitory activity and reduce cytotoxity. Therefore, 2,6-diarylpyridin-3-amines and 2,4-diarybenzen-1,2-diamines have been discovered as novel NNRTI agents highly potent against wild-type and multi-drug resistant viral strains. The promising data results and readily accessible synthetic methods greatly encourage us to perform further studies for developing and identifying new drug candidates with desirable drug-like properties.

Experimental Section

Chemistry

Melting points were measured with an RY-1 melting apparatus without correction. The proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a JNM-ECA-400 (400 MHz) spectrometer using tetramethylsilane (TMS) as the internal standard. The solvent used was DMSO-d_6, unless otherwise indicated. Mass spectra (MS) were measured on an ABI Perkin-Elmer Sciex API-150 mass spectrometer with electrospray ionization and the relative intensity of each ion peak is presented as percent (%). The purities of target compounds were 95%, measured by HPLC analyses, which were performed by Agilent 1100 HPLC system with a UV detector, using an Agilent TC-C18 column (250 mm × 4.6 mm, 5 μm) eluting with a mixture of solvents A and B (condition 1: acetonitrile/water with 0.1% formic acid 80:20, flow rate 1.2 mL/min; condition 2: MeOH/water with 0.1% formic acid 80:20, flow rate 1.0 mL/min; UV 254 nm). The microwave reactions were performed on a microwave apparatus from Biotage, Inc. Thin-layer chromatography (TLC) and preparative TLC were performed on silica gel GF$_{254}$ plates. Silica gel (200–300 mesh) from Qingdao Haiyang Chemical Company was used for column chromatography. Medium-pressure column chromatography was performed using a CombiFlash Companion purification system. All chemicals were obtained from Beijing Chemical Works or Sigma-Aldrich, Inc.

6-Chloro-2-(4′-cyanoaniline)-3-nitropyridine (8)—A mixture of 2,6-dichloro-3-nitropyridine (**5**, 1.93 g, 10 mmol) and 4-aminobenzonitrile (1.42 g, 12 mmol) was heated at 140 °C without solvent under N$_2$ protection for 4 h. After cooling to room temperature, DMF (ca. 15 mL) was added into the flask to dissolve the mixture. The solution was then poured into ice water and the pH adjusted to 2–3 with aq HCl (5%). The resulting solid was collected and recrystallized from 95% EtOH to give pure **8** (2.01 g, 73% yield), yellow solid, mp 175–178 °C. ^{1}H-NMR δ ppm 10.47 (1H, br s, NH), 8.53 (1H, d, *J* = 8.4 Hz, ArH-4), 7.86 (2H, d, *J* = 8.8 Hz, ArH-2′, 6′), 7.70 (2H, d, *J* = 8.8 Hz, ArH-3′, 5′), 6.96 (1H, d, *J* = 8.4 Hz, ArH-5). MS m/z (%) 275 (M + 1, 100), 276 (M + 2, 28).

5-Chloro-N^1-(4′-cyanophenyl)-2-nitroaniline (9)—To a mixture of 2,4-dichloronitrobenzene (**6**, 1.92 g, 10 mmols) and 4-aminobenzonitrile (1.30 g, 11 mmols) in DMF (20 mL) was added *t*-BuOK (2.24 g, 20 mmols) slowly in ice-bath, then stirred at room temperature for 24 h and monitored by TLC. The mixture was poured into ice-water and pH was adjusted to 2–3 with 5% aq HCl. The red solid was collected, washed to neutral, and dried to afford 1.75 g of **9** in 64% yield, mp 221–224 °C. ^{1}H-NMR δ ppm 9.56 (1H, br, NH), 8.20 (1H, d, *J* = 9.0 Hz, ArH-3), 7.72 (2H, d, *J* = 9.0 Hz, ArH-2′, 6′), 7.38 (1H, d, *J* = 2.0 Hz, ArH-6), 7.36 (2H, d, *J* = 9.0 Hz, ArH-3′, 5′), 6.91 (1H, d, *J* = 8.4 Hz, ArH-4). MS m/z (%) 272 (M − 1, 100), 274 (M + 1, 28).

5-Chloro-N^1-(4′-cyanophenyl)-2,4-dinitroaniline (10)—To a solution of 1,3-dichloro-4,6-dinitrobenzene (**7,** 237 mg, 1 mmol) and 4-cyanoaniline (130 mg, 1.1 mmol) in DMF (3 mL) was slowly added potassium *tert*-butoxide (2 equiv) at ice-water bath temperature with stirring and then at room temperature for about 1 h monitored by TLC until the reaction was completed. The mixture was poured into ice-water, pH was adjusted to 6 with 5% aq HCl, and solid was collected and washed with water, The crude product was purified by recrystallization from EtOH to afford 284 mg of **10** in an 89% yield, yellow solid, mp 174 – 176 °C. ^{1}H NMR (CDCl$_3$) δ ppm 7.41 (1H, s, ArH-6), 7.57 (2H, d, *J* = 8.8 Hz, ArH-2′, 6′), 7.91 (2H, d, *J* = 8.8 Hz, ArH-3′, 5′), 8.90 (1H, s, ArH-3), 10.07 (1H, s, NH). MS *m/z* (%) 319 (M + 1, 100), 321 (M + 3, 23).

General procedure for the preparation of N^2-(4′-cyanophenyl)-3-nitro-6-(4″-substituted-2″, 6″-dimethyl)phenoxypyridin-2-amines (11a-11c, 11g)

A mixture of **8** (1 equiv) and a *para*-substituted 2,6-dimethylphenol (1.2 equiv) in DMF in the presence of anhydrous K_2CO_3 (excess, 3.5 equiv) was heated at 130–140 °C for 6–8 h. The mixture was poured into ice-water and pH was adjusted to 2–3 with 5% aq HCl. Crude solid product was collected and washed to neutral, followed by silica gel column purification (eluant: CH_2Cl_2) to give corresponding pure target compounds.

N^2-(4′-Cyanophenyl)-6-(2″,6″-dimethyl-4″-iodo)phenoxy-3-nitropyridin-2-amine (11a)—Yield 82%, starting with 275 mg (1.0 mmol) of **8** and 297 mg (1.2 mmol) of 2,6-dimethyl-4-iodophenol to afford 399 mg of **11a**, yellow solid, mp 162–165 °C. ^{1}H-NMR δ ppm 10.65 (1H, br, NH), 8.63 (1H, d, *J* = 8.8 Hz, ArH-4), 7.54 (2H, s, ArH-3″, 5″), 7.38 (2H, d, *J* = 8.8 Hz, ArH), 7.21 (2H, d, *J* = 8.8 Hz, ArH), 6.65 (1 H, d, *J* = 8.8 Hz, ArH-5), 2.06 (6H, s, CH$_3$·2). MS *m/z* (%) 487 (M + 1, 100). HPLC purity: 98.63%.

6-(4″-Bromo-2″,6″-dimethyl)phenoxy-N^2-(4′-cyanophenyl)-3-nitropyridin-2-amine (11b)—Yield 64%, starting with 275 mg (1.0 mmol) of **8** and 241 mg (1.2 mmol) of 2,6-dimethyl-4-bromophenol to afford 280 mg of **11b**, yellow solid, mp 193–194 °C. ^{1}H-NMR δ ppm 10.65 (1H, br, NH), 8.63 (1H, d, *J* = 9.0 Hz, ArH-4), 7.37 (4H, m, ArH), 7.21 (2H, d, *J* = 8.8 Hz, ArH), 6.65 (1H, d, *J* = 9.0 Hz, ArH-5), 2.08 (6H, s, CH$_3$·2). MS *m/z* (%) 439 (M + 1, 100), 441 (M + 3, 90). HPLC purity: 97.24%.

6-(4″-Allyl-2″,6″-dimethyl)phenoxy-N-(4′-cyanophenyl)-3-nitropyridin-2-amine (11c)—Yield 56%, starting with 275 mg (1.0 mmol) of **8** and 194 mg (1.2 mmol) of 4-allyl-2,6-dimethylphenol to afford 225 mg of **11c**, yellow solid, mp 193–195 °C. ^{1}H-NMR δ ppm 10.67 (1H, br, NH), 8.61 (1H, d, *J* = 8.8 Hz, ArH-4), 7.27 (4H, m, ArH), 7.01 (2H, s, ArH), 6.63 (1H, d, *J* = 8.8 Hz, ArH-5), 6.05 (1H, m, CH=), 5.24 (2H, m, CH$_2$=), 3.44 (2H, d, *J* = 3.2 Hz, CH$_2$), 2.08 (6H, s, CH$_3$·2). MS *m/z* (%) 401 (M + 1, 100). HPLC purity: 95.22%.

N-(4′-Cyanophenyl)-6-(2″,6″-dimethyl-4″-formyl)phenoxy-3-nitropyridin-2-amine (11g)—Yield 66%, starting with 275 mg (1.0 mmol) of **8** and 180 mg (1.2 mmol) of 4-hydroxy-3,5-dimethylbenzaldehyde to afford 257 mg of **11g**, yellow solid, mp 226–228 °C. ^{1}H-NMR δ ppm 10.68 (1H, br, NH), 10.08 (1H, s, CHO), 8.66 (1H, d, *J* = 8.8 Hz, ArH-4), 7.75 (2H, s, ArH), 7.20 (4H, s, ArH), 6.69 (1H, d, *J* = 8.8 Hz, ArH-5), 2.21 (6H, s, CH$_3$·2). MS *m/z* (%) 389 (M + 1, 100). HPLC purity: 97.90%.

N-(4′-Cyanophenyl)-6-[2″,6″-dimethyl-4″-(3-methylbut-3-ol-1-yn)phenoxy]-3-nitropyridin-2-amine (11d)—To a mixture of **11a** (486 mg, 1 mmol), Pd(PPh$_3$)$_2$Cl$_2$ (69 mg, 0.1 mmol), and cuprous iodide (19 mg, 0.1 mmol) in anhydrous DMF (5 mL) in the presence of triethylamine (0.6 mL) was added 2-methylbut-3-yn-2-ol (420 mg, 5 mmol) slowly under N$_2$ protection at room temperature with stirring for 7 h. The mixture was poured into water and extracted with CH$_2$Cl$_2$ three times. After removal of solvent under reduced pressure, residue was purified by flash silica chromatography (gradient eluent: EtOAc/petroleum ether 0–40%) to provide 362 mg of pure **11d**, 78% yield, yellow solid, mp 114–116 °C. ^{1}H NMR δ ppm 10.37 (1H, br, NH), 8.67 (1H, d, *J* = 8.8 Hz, ArH-4), 7.40 and 7.34 (each 2H, d, *J* = 8.8 Hz, ArH), 7.31 (2H, s, ArH), 6.83 (1 H, d, *J* = 8.8 Hz, ArH-5), 5.47 (1H, s, OH), 2.00 (6H, s, ArCH$_3$·2), 1.53 (6H, s, CH$_3$·2). MS *m/z* (%) 465 (M + Na, 25), 425 (M – OH, 100). HPLC purity: 98.25%.

N-(4′-Cyanophenyl)-6-[4″-(2-cyclopropylethynyl)-2″,6″-dimethyl]phenoxy-3-nitro- pyridin-2-amine (11e)—The procedure was the same as for synthesis of **11d**,

starting with **11a** (486 mg, 1 mmol) and ethynylcyclopropane (330 mg, 5 mmol) to provide 290 mg of **11e**, in a 68% yield, yellow solid, mp 188–192 °C. ^{1}H-NMR (CDCl$_3$) δ ppm 10.65 (1H, br, NH), 8.61 (1H, d, J = 9.2 Hz, ArH-4), 7.36 (2H, d, J = 8.8 Hz, ArH), 7.22 (2H, s, ArH), 7.19 (2H, d, J = 8.8 Hz, ArH), 6.64 (1H, d, J = 9.2 Hz, ArH-5), 2.05 (6H, s, CH$_3$×2), 1.52 (1H, m, CH), 0.89 (4H, m, CH$_2$ × 2). MS m/z (%) 425 (M + 1, 100). HPLC purity: 96.95%.

N^2-(4′-Cyanophenyl)-6-(2″,6″-dimethyl-4″-ethynyl)phenoxy-3-nitropyridin-2-amine (11f)—A solution of **11d** (422 mg, 1 mmol) in toluene (20 mL) in the presence of powdered NaOH (16 mg, 0.4 mmol) was refluxed for 24 h under nitrogen protection. After cooling to room temperature, 2 drops of HOAc was dropped into the flask. The organic phase was washed with water and solvent was evaporated. The residue was purified on a flash silica column (gradient eluent: EtOAc/petroleum ether 0–40%) to provide 265 mg of **11f** in 69% yield, yellow solid, mp 186–188 °C. ^{1}HNMR (CDCl$_3$) δ ppm 10.66 (1H, br, NH), 8.63 (1H, d, J = 8.8 Hz, ArH-4), 7.35 (2H, d, J = 8.8 Hz, ArH), 7.34 (2H, s, ArH), 7.19 (2H, d, J = 8.8 Hz, ArH), 6.66 (1 H, d, J = 8.8 Hz, ArH-5), 3.17 (1H, s, ≡CH), 2.09 (6H, s, CH$_3$ · 2). MS m/z (%) 385 (M + 1, 100). HPLC purity: 96.63%.

N-(4′-Cyanophenyl)-6-[2″,6″-dimethyl-4″-(2-nitroethan-l-o1)]phenoxy-3-nitropyridin-2- amine (11i)—To a solution of **11g** (388 mg, 1 mmol) and CH$_3$NO$_2$ (2 mL, excess) in THF (20 mL) was added dropwise 33% aq NaOH (1 mL) at ice-water bath and stirred for additional 1 h, then at room temperature for 12 h. The mixture was poured into ice-water, pH was adjusted with 5% aq HCl to neutral, and the mixture extracted with CH$_2$Cl$_2$. After removal of organic solvent *in vacuo*, residue was purified on a silica gel column (eluent: CH$_2$Cl$_2$/MeOH = 60/1) to afford 306 mg of **11i** in a 68% yield, yellow solid, mp 230–234 °C. ^{1}HNMR δ ppm 10.43 (1H, br, NH), 8.67 (1H, d, J = 8.8 Hz, ArH-4), 7.49 and 7.40 (each 2H, d, J = 8.4 Hz, ArH on A-ring), 7.33 (2H, s, ArH on C-ring), 6.81 (2H, d, J = 8.8 Hz, ArH-5), 6.32 (1H, d, J = 5.6 Hz, OH), 5.33 and 4.94 (each 1H, m, CH$_2$NO$_2$), 4.66 (1H, m, <u>CH</u>OH), 2.06 (6H, s, CH$_3$ · 2). MS m/z (%) 450 (M + 1, 100), 472 (M + Na, 95). HPLC purity: 97.39%.

(E)-6-(4″-(2-Carboxyvinyl)-2″,6″-dimethyl)phenoxy-N-(4′-cyanophenyl)-3-nitropyridin-2-amine (11j)—A mixture of **11g** (388 mg, 1 mmol) in pyridine (5 mL) and malonic acid (416 mg, 4 mmol) was refluxed in the presence of piperidine (0.15 mL) for 2 h. Then the mixture was poured into inc-water and pH was adjusted to 2–3 with 5% aq HCl. After standing overnight, the solid was collected and washed with cold water to afford 377 mg of **11j** in 88% yield, yellow solid, mp 258–260 °C. ^{1}HNMR δ ppm 12.43 (1H, br, COOH), 10.38 (1H, br, NH), 8.68 (1H, d, J = 8.8 Hz, ArH-4), 7.65 (1H, d, J = 16.0 Hz, =<u>CH</u>COOH), 7.62 (2H, s, ArH on C-ring), 7.37 and 7.32 (each 2H, d, J = 8.8 Hz, ArH on A-ring), 6.70 (1H, d, J = 8.8 Hz, ArH-5), 6.60 (1H, d, J = 16.0 Hz, ArCH=), 2.07 (6H, s, CH$_3$ · 2); MS m/z (%) 431 (M + 1, 30), 453 (M + Na, 70), 413 (M – OH, 100). HPLC purity: 98.73%.

(E)-N^2-(4′-Cyanophenyl)-6-(4″-(α,β-unsaturated-but-3-)one-2″,6″-dimethyl)phenoxy-3-nitropyridin-2-amine (11k)—To a solution of **11g** (388 mg, 1 mmol) in acetone (20 mL) and MeOH (5 mL) was added 10% aq NaOH (2 mL) slowly at ice-water bath temperature. Then the mixture was stirred at room temperature for 1 h, poured into ice-water and pH adjusted to 4–5 with 5% aq HCl. The solid was filtered, washed with water, and purified on a silica gel column (gradient eluent: EtOAc/petroleum ether 0–50%) to obtain 155 mg of **11k** in 36% yield, yellow solid, mp 194–197 °C. ^{1}H NMR δ ppm (CDCl$_3$) 10.66 (1H, br, NH), 8.64 (1H, d, J = 9.2 Hz, ArH-4), 7.56 (1H, d, J = 16.0 Hz, ArCH=), 7.39 (2H, s, ArH on C-ring), 7.22 (4H, s, ArH on A-ring) 6.78 (2H, 1H, d, J =

16.0 Hz, =CHCO), 6.66 (1H, d, *J* = 9.2 Hz, ArH-5), 2.46 (3H, s, COCH$_3$), 2.14 (6H, s, CH$_3$ · 2). MS *m/z* (%) 429 (M + 1, 100), 387 (M − 43, 50). HPLC purity: 97.99%.

(*E*)-*N*-(4′-Cyanophenyl)-6-(4″-cyanovinyl-2″,6″-dimethyl)phenoxy-3-nitropyridin-2-amine (11m)—

To a solution of diethyl cyanomethyl phosphonate (266 mg, 1.5 mmol) in THF (15 mL) was added *t*-BuOK (168 mg, 1.5 mmol) at ice-water bath temperature with stirring for 30 min. Compound **11g** (388 mg, 1 mmol) in THF (15 mL) was added dropwise into the above mixture at room temperature and then stirring continued for 2 days. The reaction was then quenched with water and extracted with EtOAc. After removal of solvent under reduced pressure, the residue was purified by silica gel column chromatography (eluent: CH$_2$Cl$_2$/MeOH = 30/1) to afford 333 mg of **11m** in an 81% yield, yellow solid, mp 231–233 °C. ¹HNMR δ ppm 10.37 (1H, br, NH), 8.68 (1H, d, *J* = 8.8 Hz, ArH-4), 7.72 (1H, d, *J* = 16.8 Hz, ArCH=C), 7.56 (2H, s, ArH on C-ring), 7.34 (4H, s, ArH on A-ring), 6.84 (1H, d, *J* = 8.8 Hz, ArH-5), 6.53 (1H, d, *J* = 16.8 Hz, =CHCN), 2.06 (6H, s, CH$_3$·2). MS *m/z* (%) 412 (M + 1, 100), 434 (M + Na, 30). HPLC purity: 98.08%.

(*E*)-*N²*-(4′-Cyanophenyl)-4-(4″-cyanovinyl-2″,6″-dimethyl)phenoxy-1-nitroaniline (12c)—

The same method as the preparation of **11m**, starting with 387 mg (1 mmol) of **12a** and 266 mg (1.5 mmol) of (EtO)$_2$P(O)CH$_2$CN to afford 372 mg of **12c**, yield 91%, yellow solid, mp 178–181 °C. ¹HNMR δ ppm 9.55 (1H, br, NH), 8.18 (1H, d, *J* = 9.2 Hz, ArH-6), 7.73 (1H, d, *J* = 8.8 Hz, ArH-3′,5′), 7.60 (1H, d, *J* = 16.8 Hz, ArCH=), 7.51 (2H, s, ArH-3″, 5″), 7.32 (2H, d, *J* = 8.8 Hz, ArH-2′, 6′), 6.62 (1H, d, *J* = 2.8 Hz, ArH-3), 6.51 (1H, dd, *J* = 9.2 and 2.8 Hz, ArH-4), 6.44 (1H, d, *J* = 16.8 Hz, =CHCN), 2.08 (6H, s, CH$_3$ · 2). MS *m/z* (%) 411 (M + 1, 100), 433 (M + Na, 85). HPLC purity: 95.40%

(*E*)-*N¹*-(4′-Cyanophenyl)-5-[2″,6″-dimethyl-4″-(3-oxobut-1-enyl)]phenoxy-2,4-dinitro-aniline (12d)—

The same method as the preparation of **11k**, starting with 432 mg of **12b** to afford 152 mg of **12d**, yield 32%, brown solid, mp 213–215 °C. ¹H NMR δ ppm (CDCl$_3$) 9.94 (1H, br, NH), 9.18 (1H, s, ArH-3), 7.53 (2H, d, *J* = 8.4 Hz, ArH-3′,5′), 7.43 (1H, d, *J* = 16.4 Hz, ArCH=), 7.31 (2H, s, ArH-3″, 5″), 7.11 (2H, d, *J* = 8.4 Hz, ArH-2′,6′), 6.68 (1H, d, *J* = 16.4 Hz, =CHCN), 6.25 (1H, s, ArH-6), 2.42 (3H, s, COCH$_3$), 2.16 (6H, s, CH$_3$ · 2). MS *m/z* (%) 473 (M + 1, 100), 495 (M + Na, 80). HPLC purity: 99.47%.

(*E*)-*N¹*-(4′-Cyanophenyl)-5-(4″-cyanovinyl-2″,6″-dimethyl)phenoxy-2,4-dinitroaniline (12e)—

The same method as the preparation of **11m**, starting with 432 mg (1 mmol) of **12b** and 266 mg (1.5 mmol) of (EtO)$_2$P(O)CH$_2$CN to afford 240 mg of **12e**, yield 53%, yellow solid, mp 242–244 °C. ¹HNMR (CDCl$_3$) δ ppm 9.97 (1H, br, NH), 9.19 (1H, d, *J* = 9.2 Hz, ArH-3), 7.55 (1H, d, *J* = 8.8 Hz, ArH-3′,5′), 7.32 (1H, d, *J* = 16.4 Hz, ArCH=), 7.21 (2H, s, ArH-3″, 5″), 7.12 (2H, d, *J* = 8.8 Hz, ArH-2′, 6′), 6.26 (1H, d, s, ArH-6), 5.86 (1H, d, *J* = 16.4 Hz, =CHCN), 2.18 (6H, s, CH$_3$ · 2). MS *m/z* (%) 456 (M + 1, 100). HPLC purity: 97.15%.

General reduction methods for nitro group(s) on the central ring (pyridine or benzene) to prepare 13a-13f, 13i, 13j, 13m, and 14c

Method A: To a solution of a diaryl-nitropyridine (1 mmol) in THF and water (40 mL, v/v 1:1) was added ammonia (0.5 mL) and Na$_2$S$_2$O$_4$ (90%, 10 mmol) successively. The mixture was stirred at room temperature for 2 h monitored by TLC (CH$_2$Cl$_2$/MeOH: 15/1) until reaction was completed. The mixture was then poured into ice-water and extracted with EtOAc. After removal of solvent *in vacuo*, the residue was purified on a silica gel column (eluent: CH$_2$Cl$_2$/MeOH = 30/1) to obtain corresponding diarylpyridinamines. **Method B**: To a solution of a diaryl-nitropyridine or diaryl-nitrobenzene (1 eq) in THF and MeOH (30 mL, v/v 1:1) was added NiCl$_2$·6H$_2$O (0.3 eq) and NaBH$_4$ (4 eq.) successively at ice-water bath

temperature. Then the mixture was stirred in the ice-water bath for another 30 min until the starting material disappeared, as monitored by TLC. The mixture was poured into water, pH adjusted to 4–5, warmed to 40–50 °C for 10 min, and then extracted with EtOAc. After removal of solvent *in vacuo*, crude product was purified on a silica gel column (eluent: CH_2Cl_2/MeOH = 30/1) to provide corresponding products.

N²-(4′-Cyanophenyl)-6-(2″,6″-dimethyl-4″-iodo)phenoxypyridin-2,3-diamine (13a)

Method A, yield 49%, starting with 486 mg (1.0 mmol) of **11a** to afford 225 mg of **13a**, brown solid, mp 186–188 °C. ^{1}HNMR (CDCl$_3$) δ ppm 7.50 (2H, s, NH$_2$), 7.33 (2H, d, *J* = 8.8 Hz, ArH), 7.22 (2H, d, *J* = 8.8 Hz, ArH), 6.98 (1H, d, *J* = 8.4 Hz, ArH-4), 6.44 (1H, d, *J* = 8.4 Hz, ArH-5), 2.09 (6H, s, CH$_3$·2). MS *m/z* (%) 457 (M + 1, 100). HPLC purity: 95.72%.

6-(4″-Bromo-2″,6″-dimethyl)phenoxy-*N²*-(4′-cyanophenyl)pyridin-2,3-diamine (13b)

Method B, yield 82%, starting with 439 mg (1.0 mmol) of **11b** to afford 336 mg of **13b**, brown solid, mp 190–193 °C. ^{1}H-NMR δ ppm 8.35 (1H, br s, NH), 7.42 (2H, s, ArH), 7.32 (4H, s, ArH), 7.12 (1H, d, *J* = 8.4 Hz, ArH-4), 6.44 (1H, d, *J* = 8.4 Hz, ArH-5), 4.84 (2H, br, NH$_2$), 2.03 (6H, s, CH$_3$ · 2). MS *m/z* (%) 409 (M + 1, 100), 411 (M + 3, 90). HPLC purity: 97.36%.

6-(4″-Allyl-2″,6″-dimethyl)phenoxy-*N²*-(4′-cyanophenyl)pyridin-2,3-diamine (13c)

Method A, yield 43%, starting with 400 mg (1.0 mmol) of **11c** to afford 160 mg of **13c**, brown solid, mp 150–152 °C. ^{1}HNMR δ ppm 8.30 (1H, br, NH), 7.36 and 7.29 (each 2H, d, *J* = 8.8 Hz, ArH), 7.11 (1H, d, *J* = 8.0 Hz, ArH-4), 6.98 (2H, s, ArH), 6.39 (1H, d, *J* = 8.0 Hz, ArH-5), 6.03 (1H, m, CH=), 5.14 (2H, m, CH$_2$=), 4.76 (2H, br, NH$_2$), 3.36 (2H, d, *J* = 3.6 Hz, CH$_2$), 2.01 (6H, s, CH$_3$ · 2). MS *m/z* 371 (M + 1, 100). HPLC purity: 97.40%.

N²-(4′-Cyanophenyl)-6-[2″,6″-dimethyl-4″-(3-methylbut-3-ol-1-yn)phenoxy]pyridin-2,3-di amine (13d)

Method A, yield 48%, starting with 442 mg (1.0 mmol) of **11d** to afford 198 mg of **13d**, brown solid, mp 155–157 °C. ^{1}HNMR δ ppm 8.33 (1H, br, NH), 8.01 (1H, br, OH), 7.41 (1H, d, *J* = 8.8 Hz, ArH-4), 7.29 (4H, m, ArH), 7.23 (2H, s, ArH), 6.55 (1 H, d, *J* = 8.8 Hz, ArH-5), 4.83 (2H, br, NH$_2$), 2.08 (6H, s, Ar-CH$_3$·2), 1.51 (6H, s, CH$_3$·2). MS *m/z* (%) 413 (M + 1, 100). HPLC purity: 97.16%.

N²-(4′-Cyanophenyl)-6-[4″-(2-cyclopropylethynyl)-2″,6″-dimethylphenoxy]pyridin-2,3-diamine (13e)

Method A, yield 49%, starting with 424 mg (1.0 mmol) of **11e** to afford 193 mg of **13e**, brown solid, mp 82–85 °C. ^{1}HNMR (CDCl$_3$) δ ppm 7.33 (2H, d, *J* = 8.4 Hz, ArH-3′, 5′), 7.22 (2H, s, ArH), 7.18 (3H, m, ArH-2′, 6′ and ArH-4), 6.36 (1H, d, *J* = 8.0 Hz, ArH-5), 2.06 (6H, s, CH$_3$×2), 1.51 (1H, m, CH), 0.89 (4H, m, CH$_2$ × 2). MS *m/z* (%) 395 (M + 1, 100). HPLC purity: 96.99%.

N²-(4′-Cyanophenyl)-6-(2″,6″-dimethyl-4″-ethynyl)phenoxypyridin-2,3-diamine (13f)

Method A, yield 61%, starting with 384 mg (1.0 mmol) of **11f** to afford 217 mg of **13f**, brown solid, mp 79–82 °C. ^{1}HNMR δ ppm 8.34 (1H, br, NH), 7.37 (2H, s, ArH on C-ring), 7.03 (4H, m, ArH on A-ring), 6.56 (1H, d, *J* = 8.4 Hz, ArH-4), 6.44 (1H, d, *J* = 8.4 Hz, ArH-5), 4.84 (2H, br, NH$_2$), 3.36 (1H, s, CH≡), 2.02 (6H, s, CH$_3$ · 2). MS *m/z* (%) 355 (M + 1, 100). HPLC purity: 98.03%.

N²-(4′-Cyanophenyl)-6-[2″,6″-dimethyl-4″-(2-nitroethan-l-o1)]phenoxypyridin-2,3-diamine hydrochloride (13i)

Method A, yield 42%, starting with 90 mg (0.2 mmol) of **11i** to provide amino-coumpound, which was treated with 20% HCl-ether solution in acetone to afford 38 mg of **13i**, white solid, dec. >230

°C. ¹HNMR δ ppm 9.28 (1H, br, NH), 7.58 (1H, d, J = 8.4 Hz, ArH-4), 7.43 and 7.38 (each 2H, d, J = 9.2 Hz, ArH on A-ring), 7.27 (2H, s, ArH on C-ring), 6.59 (1H, d, J = 8.4 Hz, ArH-5), 5.29 (1H, dd, J = 3.6 & 9.8 Hz, $\underline{CH_2}NO_2$), 4.90 (1H, dd, J = 3.6 & 12.4 Hz, $\underline{CH_2}NO_2$), 4.62 (1H, dd, J = 9.8 & 12.4 Hz, CH), 3.57 (2H, br. NH_2), 2.05 (6H, s, $CH_3 \times 2$). MS m/z (%) 420 (M + 1, 100). HPLC purity: 99.44%.

(*E*)-6-[4″-(2-Acrylic)-2″,6″-dimethyl)phenoxy]-*N²*-(4′-cyanophenyl)pyridin-2,3-diamine (13j)—Method A, yield 80%, starting with 86 mg (0.2 mmol) of **11j** to afford 64 mg of **13j**, deep brown solid, mp 245–7 °C. ¹HNMR δ ppm 8.41 (1H, s, NH), 7.38 (2H, d, J = 9.2 Hz, ArH), 7.37 (2H, s, ArH-3″, 5″), 7.28 (1H, d, J = 15.6 Hz, ArCH=), 7.24 (2H, d, J = 9.2 Hz, ArH-2′, 6′), 7.11 (1H, d, J = 8.4 Hz, ArH-4), 6.43 (1H, d, J = 15.6 Hz, =CHCN), 6.39 (1H, d, J = 8.4 Hz, ArH-5), 4.83 (2H, br. NH_2), 2.05 (6H, s, $CH_3 \times 2$). MS m/z (%) 401 (M + 1, 100), 383 (M − OH, 93). HPLC purity: 99.48%.

(*E*)-*N²*-(4′-Cyanophenyl)-6-(4″-cyanovinyl-2″,6″-dimethyl)phenoxypyridin-2,3-diamine (13m)—Method A, with a 50% yield, starting with 411 mg (1 mmol) of **11m** to afford 192 mg of **13m**, brown solid, mp 162–164 °C. ¹HNMR δ ppm 8.35 (1H, br, NH), 7.68 (1H, d, J = 16.8 Hz, ArCH=C), 7.50 (2H, s, ArH on C-ring), 7.37 and 7.25 (each 2H, d, J = 8.8 Hz, ArH on A-ring), 7.13 (1H, d, J = 8.0 Hz, ArH-4), 6.45 (1H, d, J = 8.0 Hz, ArH-5), 6.45 (1H, d, J = 16.8 Hz, =CHCN), 4.84 (2H, br. s, NH_2), 2.06 (6H, s, $CH_3 \cdot 2$). MS *m/z* (%) 382 (M + 1, 100). HPLC purity: 98.44%.

(*E*)-*N²*-(4′-Cyanophenyl)-4-(4″-cyanovinyl-2″,6″-dimethyl)phenoxybenzene-1,2-diamine (14c)—Method A, starting with **12c** (410 mg, 1 mmol) to afford 226 mg of **14c**, yield 59%, brown solid, mp 215–218 °C. ¹HNMR ($CDCl_3$) δ ppm 7.46 (2H, d, J = 8.8 Hz, ArH-3′,5′), 7.32 (1H, d, J = 16.8 Hz, ArCH=), 7.18 (2H, s, ArH-3″, 5″), 6.74 (1H, d, J = 9.2 Hz, ArH-6), 6.64 (2H, d, J = 8.8 Hz, ArH-2′,6′), 6.58 (1H, d, J = 2.8 Hz, ArH-3), 6.51 (1H, dd, J = 9.2 and 2.8 Hz, ArH-5), 5.80 (1H, d, J = 16.8 Hz, =CHCN), 3.52 (2H, br, NH_2), 2.15 (6H, s, $CH_3 \cdot 2$). MS *m/z* (%) 381 (M + 1, 100). HPLC purity: 97.94%.

6-[(4″-Butan-2-one)-2″,6″-dimethyl]phenoxy-*N²*-(4′-cyanophenyl)pyridin-2,3-diamine (13k)—A mixture of **11k** (50 mg, 0.11 mmol) in THF (20 mL) and excess Pd-C (10%) was hydrogenated at 65 psi for 6 h. After removal of Pd-C and solvent successively, the residue was purified by flash silica gel column chromatography (eluent: CH_2Cl_2/MeOH = 100:1) to provide 37 mg of pure **13k**, yield 79%, gray solid, mp 84–86 °C. ¹H NMR δ ppm 8.32 (1H, br, NH), 7.38 and 7.32 (each 2H, d, J = 9.2 Hz, ArH), 7.10 (1H, d, J = 8.0 Hz, ArH-4), 6.99 (2H, s, ArH), 6.37 (1H, d, J = 8.0 Hz, ArH-5), 4.80 (2H, br, NH_2), 4.79 (4H, m, CH_2CH_2), 2.13 (3H, s, $COCH_3$), 2.00 (6H, s, $CH_3 \cdot 2$). MS *m/z* (%) 401 (M + 1, 100). HPLC purity: 97.35%.

(*E*)-*N¹*-(4′-Cyanophenyl)-5-[2″,6″-dimethyl-4″-(3-oxobut-1-enyl)]phenoxy-4-nitrobenzene-1,2-diamine (14d)—To a solution of **12d** (165 mg, 0.35 mmol) in acetonitrile (3 mL) and triethylamine (3 mL) in the presence of Pd/C (10%, 30 mg) was added formic acid (85%, 2 mL) in acetonitrile (2 mL) keeping the temperature under −15 °C and then stirred for another 1 h. Next it was heated to reflux for 1 h. After filtration of Pd/C, the mixture was poured into ice-water, pH adjusted to neutral, and red solid was collected. The crude product was purified on a silica gel column (eluent: $CHCl_3$/MeOH = 40:1) to yield 81 mg of **14d**, yield 53%, red solid, mp 210 – 212 °C; ¹H NMR ($CDCl_3$) δ ppm 9.94 (1H, br, NH), 9.18 (1H, s, ArH-3), 7.31 (2H, s, ArH-3″, 5″), 7.53 (2H, d, J = 8.8 Hz, ArH-3′, 5′), 7.44 (1H, d, J = 16.4 Hz, ArCH=), 7.27 (2H, d, J = 8.8 Hz, ArH-2′, 6′), 6.68 (1H, d, J = 16.4 Hz, =CHCO), 6.25 (1H, s, ArH-6), 2.39 (3H, s, $COCH_3$), 2.16 (6H, s, $CH_3 \times 2$). MS *m/z* (%) 443 (M + 1, 100). HPLC purity: 98.21%.

(*E*)-*N*[1]-(4′-Cyanophenyl)-5-(4″-cyanovinyl-2″,6″-dimethyl)phenoxybenzene-4-nitro-1,2-dia mine (14e)—Using the same method as preparation of **14d**, starting with 150 mg (0.35 mmol) of **12e** to afford 120 mg of **14e** as red solid, yield 81%, mp 252–254 °C. ^{1}HNMR (CDCl$_3$) δ ppm 7.63 (1H, s, ArH-3), 7.47 (2H, d, *J* = 8.8 Hz, ArH-3′,5′), 7.32 (1H, d, *J* = 16.8 Hz, ArCH=), 7.20 (2H, s, ArH-3″, 5″), 6.76 (2H, d, *J* = 8.8 Hz, ArH-2′,6′), 6.31 (1H, s, ArH-6), 5.82 (1H, d, *J* = 16.8 Hz, =CHCN), 3.55 (2H, br. NH), 2.18 (6H, s, CH$_3$ · 2). MS *m/z* (%) 426 (M + 1, 100), 448 (M + Na, 20). HPLC purity: 97.10%

(*E*)-*N*[1]-(4′-Cyanophenyl)-5-(4″-cyanovinyl-2″,6″-dimethyl)phenoxybenzene-1,2,4-triamine (14f)—To the solution of **12e** (100 mg, 0.22 mmol) in EtOH (15 mL) in the presence of SbCl$_3$ (201 mg, 0.33 mmol) was added NaBH$_4$ (168 mg, 4.4 mmol) in portions at ice-bath temperature, and then stirring continued at room temperature for 40 min. The mixture was then poured into ice-water, pH adjusted to 4 with 5% aq HCl, and stirring continued for an additional 1 h. After the pH was adjusted to 9 with Na$_2$CO$_3$, the mixture was extracted with EtOAc three times. After removal of solvent under reduced pressure, the residue was purified on a silica gel column (eluent: CHCl$_3$/CH$_3$OH = 40:1) to afford 56 mg of **14f**, yield 64%, red solid, mp 160–162 °C. ^{1}H NMR (CDCl$_3$) δ ppm 7.37 (2H, d, *J* = 8.4 Hz, ArH-3′,5′), 7.28 (1H, d, *J* = 16.4 Hz, ArCH=), 7.16 (2H, s, ArH-3″, 5″), 6.47 (2H, d, *J* = 8.4 Hz, ArH-2′,6′), 6.30 (1H, s, ArH-3), 5.78 (1H, d, *J* = 16.4 Hz, =CHCN), 5.32 (1H, s, ArH-6), 4.01 and 3.51 (each 2H, br, NH$_2$), 2.18 (6H, s, CH$_3$ × 2). MS *m/z* (%) 396 (M + 1, 100). HPLC purity: 100.0%.

6-(6-Cyano-2-naphthoxy)-*N*[2]-(4-cyanophenylamino)-3-nitropyridine (15)—A mixture of **8** (54.9 mg, 0.2 mmol), 6-hydroxy-2-naphthonitrile (50.8 mg, 0.3 mmol), and Cs$_2$CO$_3$ (227.5 mg, 0.7 mmol) in DMF (1 mL) was heated at 100 °C for 10 min under microwave irradiation. Then the mixture was poured into water and pH was adjusted to 2–3 with aq HCl (1N). The solid was collected and purified on a silica gel column (gradient eluent: petroleum ether/CH$_2$Cl$_2$ 0–60%) to afford 51 mg of **15**, yield 63%, yellow solid, mp 264–6 °C. ^{1}HNMR δ ppm 10.27 (1H, s, NH), 8.67 (1H, s, ArH-5″), 8.61 (1H, d, *J* = 8.8 Hz, ArH-8″), 8.17 (1H, d, *J* = 8.8 Hz, ArH-4), 8.04 (1H, d, *J* = 8.8 Hz, ArH-7″), 7.87 (1H, d, *J* = 2.4Hz, ArH-1″), 7.81 (1H, d, *J* = 8.8 Hz, ArH-4″), 7.55 (1H, dd, *J* = 2.4 & 8.8 Hz, ArH-3″), 7.26 (2H, d, *J* = 8.8 Hz, ArH-3′, 5′), 6.93 (2H, d, *J* = 8.8 Hz, ArH-2′, 6′), 6.78 (1H, d, *J* = 8.8 Hz, ArH-5). MS m/z (%) 408 (M + 1, 100). HPLC purity: 99.60%.

6-(6-Cyano-2-naphthoxy)-*N*[2]-(4-cyanophenylamino)pyridin-3-amine (16)—To a solution of **15** (51 mg, 0.125 mmol) in 1,4-dioxane (10 mL) was added ammonia (0.5 mL) in water (10 mL) with stirring for 30 min at rt. Then Na$_2$S$_2$O$_4$ (386 mg, excess) was added and stirring continued for an additional 2 h, monitored by TLC until reaction was completed. The mixture was poured into ice-water and extracted with diethyl ether. After removal of solvent *in vacuo*, the residue was purified by PTLC to obtain 27 mg of **16** in 57% yield, gray solid, mp 204–6 °C. ^{1}HNMR δ ppm 8.66 (1H, s, ArH-5″), 8.50 (1H, s, NH), 8.18 (1H, d, *J* = 8.8 Hz, ArH-8″), 8.09 (1H, d, *J* = 8.8 Hz, ArH-7″), 7.82 (1H, dd, *J* = 1.6 & 8.8 Hz, ArH-3″), 7.66 (1H, d, *J* = 2.0 Hz, ArH-1″), 7.51 – 7.54 (3H, m, ArH-3′, 5′, 4″), 7.30 (2H, d, *J* = 8.8 Hz, ArH-2′, 6′), 7.24 (1H, d, *J* = 8.4 Hz, ArH-4), 6.64 (1H, d, *J* = 8.4 Hz, ArH-5), 5.11 (2H, s, NH$_2$). MS m/z (%) 378 (M + 1, 100). HPLC purity: 97.53%

5-(6-Cyano-2-naphthoxy)-*N*[1]-(4′-cyanophenyl)-2,4-dinitroaniline (17)—Starting 160 mg (0.5 mmol) of **10** and 102 mg (0.75 mmol) of 6-hydroxy-2-naphthonitrile in DMF under microwave irradiation at 150 °C for 20 min to obtain 147 mg of **17** as yellow solid with a 65% yield, mp 230 – 232 °C, ^{1}H NMR (CDCl$_3$) δ ppm 10.00 (1H, s, NH), 9.22 (1H, s, ArH-3), 8.27 (1H, s, ArH-5″), 7.98, 7.82, 7.68 (each 1H, d, *J* = 8.8 Hz, ArH on C-ring), 7.55 (2 H, d, *J* = 8.8 Hz, ArH on A-ring), 7.39 (1H, s, ArH-1″), 7.37 (1H, d, *J* = 8.8 Hz,

ArH-3″), 7.25 (2H, d, J = 8.8 Hz, ArH on A-ring), 6.80 (1H, s, ArH-6). MS m/z (%) 450 (M – 1, 100). HPLC purity: 97.28%.

5-(1-Bromo-6-cyano-2-naphthoxy)-*N*-(4′-cyanophenyl)-2,4-dinitroaniline (18)— The same method as the preparation of **17.** Starting with **10** (319 mg, 1.0 mmol) and 5-bromo-6-hydroxy-2-naphthonitrile (248 mg, 1.0 mmol) to afford 390 mg of **18** as yellow solid in a 74% yield, mp 254 – 256 °C. ^{1}H NMR (CDCl$_3$) δ ppm 9.95 (1H, s, NH), 9.24 (1H, s, ArH-3), 8.40 (1H, d, J = 8.8 Hz, ArH on C-ring), 8.29 (1H, s, ArH-5″), 7.95 and 7.86 (each 1H, d, J = 8.8 Hz, ArH on C-ring), 7.44 (2 H, d, J = 8.4 Hz, ArH-3′,5′), 7.34 (1H, d, J = 8.8 Hz, ArH on C-ring), 7.17 (2H, d, J = 8.4 Hz, ArH-2′,6′), 6.45 (1H, s, ArH-6). MS m/z (%) 528 (M – 1, 100), 530 (M+1, 98). HPLC purity: 96.45%.

4-(6-Cyano-2-naphthoxy)-*N*^{2}-(4′-cyanophenyl)-5-nitrobenzene-1,2-diamine (19) —The method was the same as preparation of **14d**. Starting with **17** (188 mg, 0.35 mmol) to obtain 88 mg of **19** as red solid with a 60% yield, mp 196 – 198 °C. ^{1}H NMR (CD$_3$COCD$_3$) δ ppm 10.43 (1H, s, NH), 8.39 (1H, s, ArH-3), 8.06 and 7.94 (each 1H, d, J = 8.8 Hz, ArH on C-ring), 7.72 (1H, s, ArH-5″), 7.64 (1H, dd, J = 2.0 and 8.8 Hz, ArH on C-ring), 7.54 (2 H, d, J = 8.8 Hz, ArH on A-ring), 7.45 (1H, dd, J = 2.0 and 8.8 Hz, ArH on C-ring),7.35 (1H, d, J = 2.0 Hz, ArH on C-ring),7.19 (1H, s, ArH-6), 7.15 (2H, d, J = 8.8 Hz, ArH on A-ring), 5.18 (2H, s, NH$_2$). MS m/z (%) 422 (M + 1, 100). HPLC purity: 98.87%.

4-(1-Bromo-6-cyano-2-naphthoxy)-*N*^{1}-(4′-cyanophenyl)-5-nitrobenzene-1,2-diamine (20)—The method was the same as preparation of **14d**. Starting with 186 mg (0.35 mmol) of **18** to provide 120 mg of **20** as red solid, yield 60%, mp 190 – 192 °C. ^{1}H NMR (CDCl$_3$) δ ppm 8.38 (1H, d, J = 8.8 Hz, ArH on C-ring), 8.19 (1H, s, ArH on C-ring), 7.79 and 7.75 (each 1H, d, J = 8.8 Hz, ArH on C-ring), 7.67 (1H, s, ArH-3), 7.52 (2 H, d, J = 8.8 Hz, ArH on A-ring), 7.09 (1H, d, J = 8.8 Hz, ArH on C-ring), 6.97 (2H, d, J = 8.8 Hz, ArH on A-ring), 6.95 (1H, s, ArH-6). MS m/z (%) 500 (M + 1, 100), 502 (M + 3, 99). HPLC purity: 100.0%.

Assay for measuring the inhibitory activity of compounds on HIV-1 IIIB replication in MT-2 cells—Inhibitory activity of compounds against infection by HIV-1 IIIB and its variant A17, which is resistant to multiple RTIs, was determined as previously described.[20] Briefly, MT-2 cells (10^4 well) were infected with an HIV-1 strain (100 TCID$_{50}$) in 200 μL RPMI 1640 medium containing 10% FBS in the presence or absence of a test compound at graded concentrations overnight. Then, the culture supernatants were removed and fresh media containing no test compounds were added. On the fourth day post-infection, 100 μL of culture supernatants were collected from each well, mixed with equal volumes of 5% Triton X-100 and assayed for p24 antigen, which was quantitated by ELISA and the percentage of inhibition of p24 production was calculated as previously described.[20] The effective concentrations for 50% inhibition (EC$_{50}$) were calculated using a computer program CalcuSyn.[21]

HIV-1 infection assay using TZM-bl as reporter cells—Inhibition of HIV-1 infection was measured as reduction in luciferase gene expression after a single round of virus infection of TZM-bl cells as described previously.[22] Briefly, 200 TCID50 of virus (NL4-3) was used to infect TZM-bl cells in the presence of various concentrations of compounds. Two days after infection, the culture medium was removed from each well and 100 μl of Bright Glo reagent (Promega, Luis Obispo, CA) was added to the cells for measurement of luminescence using a Victor 2 luminometer. The 50% inhibitory concentration (IC50) was defined as the concentration that caused a 50% reduction of luciferase activity (Relative Light Units) compared to virus control wells.

Assessment of *in vitro* cytotoxicity in MT-2 cells—The *in vitro* cytotoxicity of compounds on MT-2 cells was measured using a colorimetric XTT assay.23 Briefly, 100 μL of the test compound at graded concentrations were added to equal volumes of cells ($5 \cdot 10^5$/mL) in wells of a 96-well plate. After incubation at 37 °C for 4 days, 50 μL of XTT solution (1 mg/mL) containing 0.02 μM of phenazine methosulphate (PMS) was added. After 4 h, the absorbance at 450 nm was measured with an ELISA reader. The CC_{50} (concentration for 50% cytotoxicity) values were calculated using the CalcuSyn computer program as described above.

Molecular modeling—The docking studies were conducted by using software Discovery Studio 2.5 (DS 2.5) and two crystal structures of wild-type RT/**1** complex (PDB: 3mec) and K103N/Y181C mutant RT/**2** complex (PDB: 3bgr). Following the "Guide-Docking ligands" in the software tool, the proteins and ligands were prepared, the dockings were performed by the CDOCK module, and results were analyzed in the DS 2.5 Base with relevant modules. The conformation energies of ligands, **1** and **14e**, were minimized with the modified parameters algorithm (smart minimizer), max steps (2000), and RMS gradient (0.05). The radius of the binding site sphere was defined as 10 Å. Comparing to crystal structure, the root mean squared deviation (RMSD) of the modeling was 0.29, thus ensuring the docking method was reliable.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

AIDS	acquired immunodeficiency syndrome
CC$_{50}$	concentration for 50% cytotoxicity
DAPYs	diarylpyrimidines
DAPDs	diarylpyridines
DAANs	diarylanilines
EC$_{50}$	effective concentration for 50% inhibition
HAART	highly active antiretroviral therapies
HIV	human immunodeficiency virus, NNRTI, non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
PDB	protein data base
RF	resistant fold
RT	reverse transcriptase
RTI	reverse transcriptase inhibitor
SAR	structure-activity relationship

SI   selective index (ratio of CC_{50}/EC_{50})

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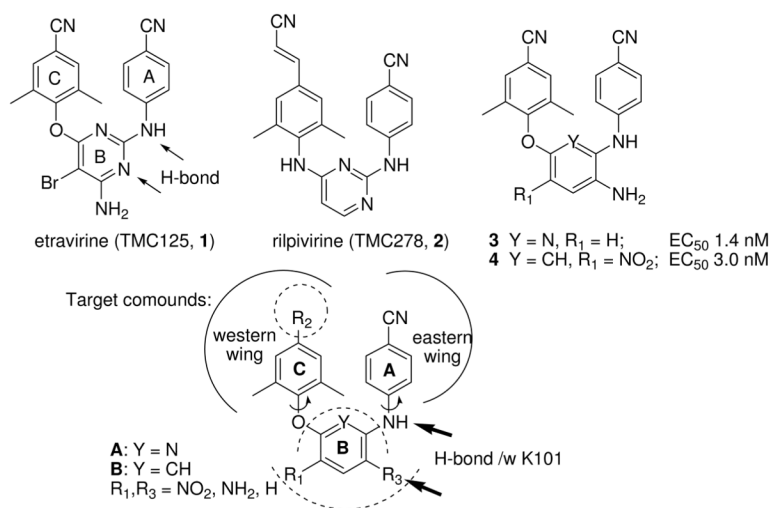
Figure 1.
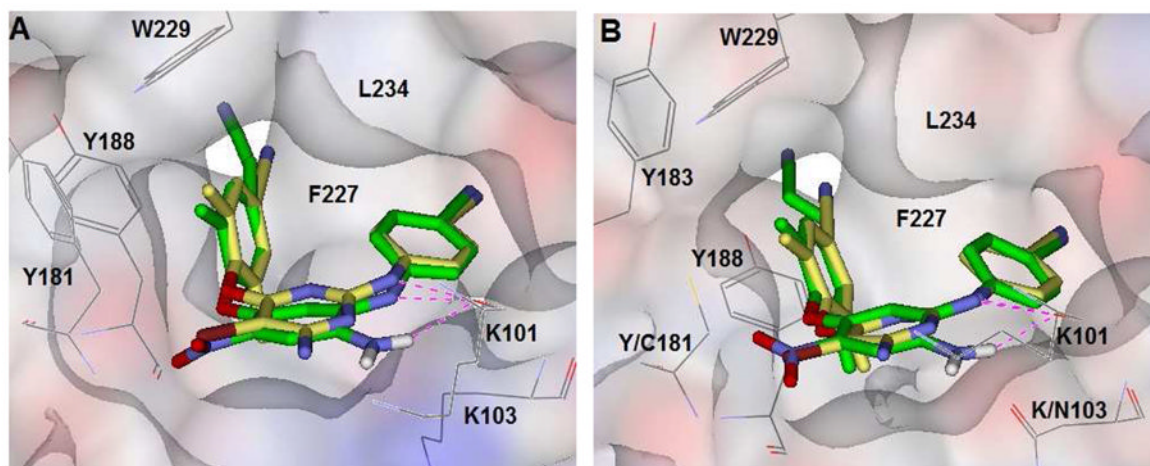Drugs (**1** and **2**), leads (**3** and **4**), and new target compounds

Figure 2.
Binding mode of **14e** (green) with (A) HIV-1 wild-type RT (PDB: 3mec) and (B) K103N/ Y181C mutant RT (PDB: 3bgr) respectively. Drug **1** (yellow) was superposed into the same pocket for comparison. The hydrogen bonds between the inhibitors and K101 are illustrated with dashed lines (pink). The side chains of key amino acids on the binding site surface are indicated with solid lines (gray).
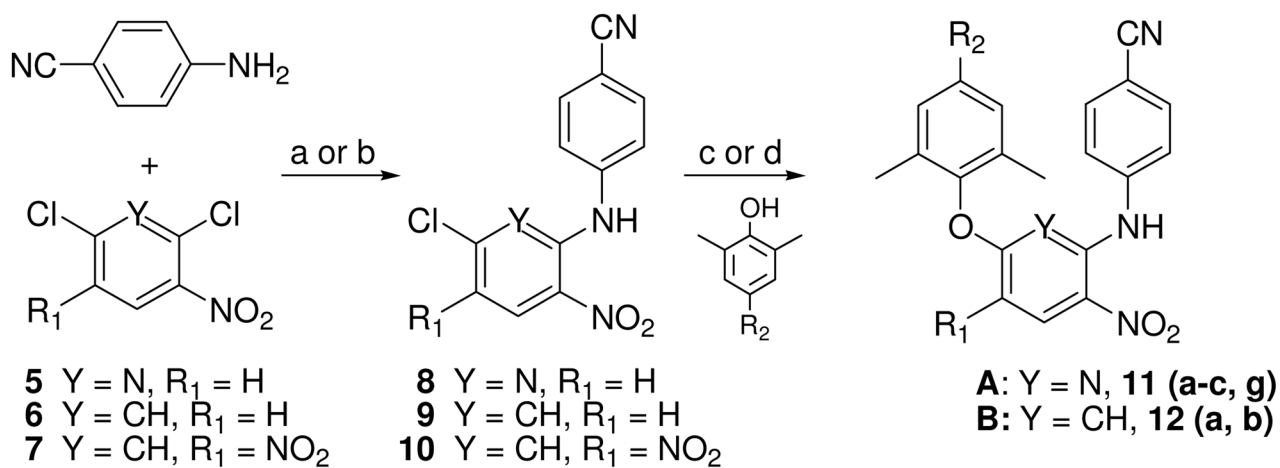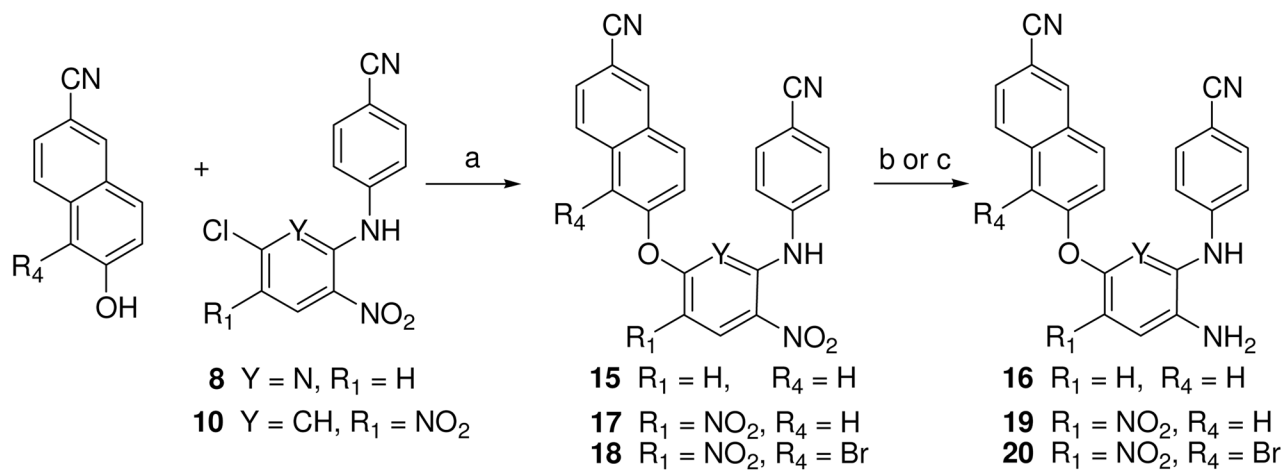
Scheme 1.
(a) heating 140 °C without solvent, 4 h; (b) *t*-BuOK/DMF, rt, 24 h.; (c) K₂CO₃/DMF, 130–
140 °C, 6–8 h; (d) K₂CO₃/DMF, 190 °C, MW, 15 min.
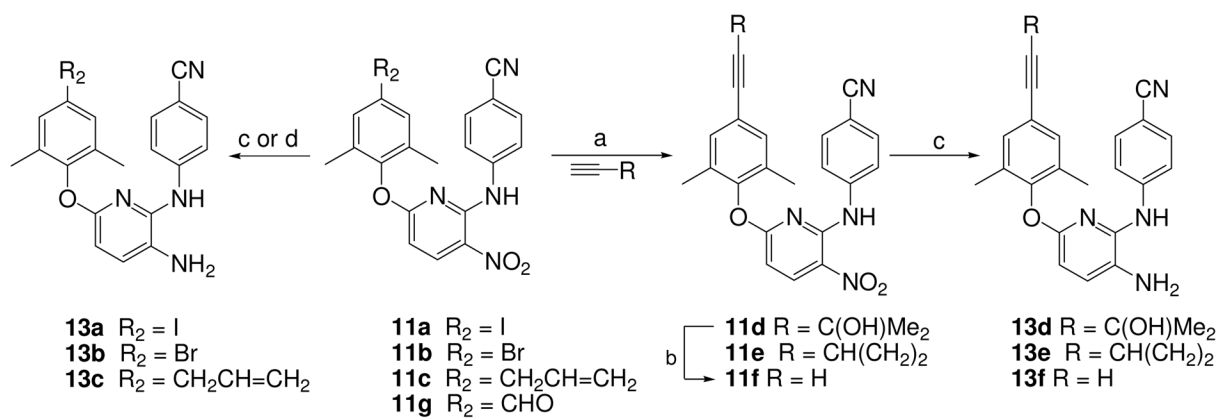
Scheme 2.
(a) $Pd(PPh_3)Cl_2$, CuI, DMF/Et_3N, N_2 protection; (b) NaOH, toluene, reflux, 16 h; (c) $Na_2S_2O_4$, $NH_3 \cdot H_2O$, THF/H_2O (v/v 1:1), rt; (d) $NiCl_2 \cdot 6H_2O/NaBH_4$, THF/MeOH (v/v 1:1).
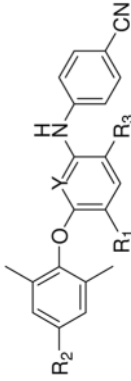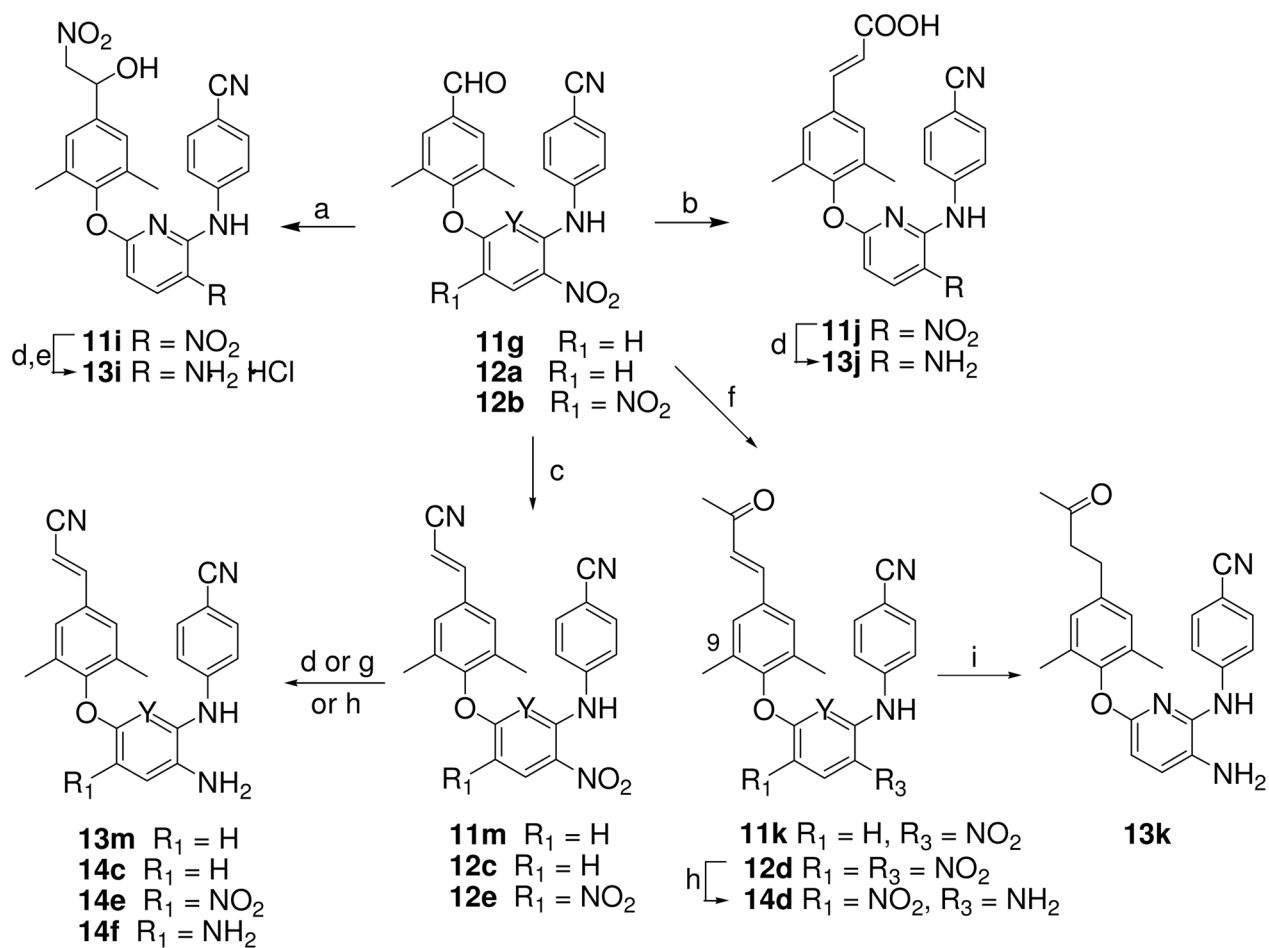
Scheme 3.
(a) CH₃NO₂, NaOH/THF, rt; (b) CH₂(COOH)₂, piperidine/Py, 2 h, reflux; (c)
(EtO)₂P(O)CH₂CN, *t*-BuOK/THF, 0 °C – rt, 48 h; (d) Na₂S₂O₄, NH₃·H₂O, THF/H₂O (1:1),
rt, 3 h; (e) 20% HCl/ether in acetone; (f) acetone, NaOH, rt; (g) NaBH₄, SbCl₃; (h)
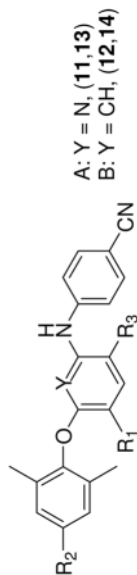HCOOH/Pd-C, Et₃N/CH₃CN, reflux, 1–3 h; (i) H₂, Pd-C/THF, 6 h.

Scheme 4.
(a) MW, 100–150 °C, 10–20 min; (b) $Na_2S_2O_4$, $NH_3 \cdot H_2O$, THF or 1,4-dioxane/H_2O (1:1), r.t. 3 h; (c) HCOOH/Pd-C, Et_3N/CH_3CN, reflux, 1–3 h.

Table 1

Data of target compounds **11-14** against HIV-1 wild-type strain replication

A: Y = N, (**11,13**)
B: Y = CH, (**12,14**)

	R_1	R_2	R_3	NL4-3 in TZM-bl cell line			IIIB in MT-2 cell line		
				$EC_{50}{}^{a}$ (μM)	$CC_{50}{}^{b}$ (μM)	SI^{c}	EC_{50} (μM)	CC_{50} (μM)	SI
11a[*]	H	I	NO_2	0.0607 ± 0.024	>41.15	>675	0.079 ± 0.002	22.93	283
13a[*]	H	I	NH_2	0.0076 ± 0.0023	16.72	2200	0.054 ± 0.004	7.24	134
11b	H	Br	NO_2	0.0502 ± 0.0068	>45.66	>909	0.045 ± 0.016	65.11	1447
13b	H	Br	NH_2	0.00729 ± 0.0020	15.54	2132	0.033 ± 0.01	9.61	291
11c	H	$CH_2CH{=}CH_2$	NO_2	NA	8.46		1.34 ± 0.70	39.68	27.37
13c	H	$CH_2CH{=}CH_2$	NH_2	0.00689 ± 0.0014	8.46	1228	0.067 ± 0.003	14.02	209
11d[*]	H	$C{\equiv}CC(OH)Me_2$	NO_2	41.35	45.25	1.09	8.91 ± 0.00	23.28	2.61
13d[*]	H	$C{\equiv}CC(OH)Me_2$	NH_2	0.241 ± 0.0147	8.73	36	1.58 ± 0.097	3.54	2.25
11e	H	$C{\equiv}CCH(CH_2)_2$	NO_2	>47.17	—		NA		
13e	H	$C{\equiv}CCH(CH_2)_2$	NH_2	0.0912 ± 0.037	7.45	82	0.41 ± 0.05	5.71	14
11f[*]	H	$C{\equiv}CH$	NO_2	0.111 ± 0.086	>52.08	>469	0.247 ± 0.040	>100	>405
13f[*]	H	$C{\equiv}CH$	NH_2	0.0067 ± 0.0010	9.94	1484	0.042 ± 0.004	9.28	221
11i	H	$CH(OH)CH_2NO_2$	NO_2	0.308 ± 0.098	>44.54	>145	1.71 ± 0.134	27.06	16
13i	H	$CH(OH)CH_2NO_2{\cdot}HCl$	NH_2	0.0101 ± 0.0026	7.02	695	0.068 ± 0.015	20.57	303
11j	H	$CH{=}CHCOOH$	NO_2	14.92	27.84	1.87	8.12 ± 0.42	34.79	4
13j	H	$CH{=}CHCOOH$	NH_2	0.60 ± 0.043	18.0	30	0.63 ± 0.22	15.69	25
11k	H	$CH{=}CHCOMe$	NO_2	0.0244 ± 0.020	>46.73	>1915	0.608 ± 0.267	>47.90	>79
13k	H	CH_2CH_2COMe	NH_2	0.00195 ± 0.00053	9.25	4744	0.055 ± 0.000	18.35	1835
11m	H	$CH{=}CHCN$	NO_2	0.0080 ± 0.0020	>48.66	>6082	0.049 ± 0.01	>100	>2041
13m	H	$CH{=}CHCN$	NH_2	0.00071 ± 0.00058	9.75	13,732	<0.014 ± 0.004	5.63	>402

A: Y = N, (11,13)
B: Y = CH, (12,14)

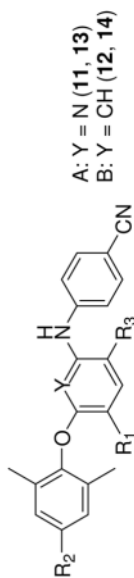|	R_1	R_2	R_3	NL4-3 in TZM-bl cell line			IIIB in MT-2 cell line		
				EC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]	EC_{50} (μM)	CC_{50} (μM)	SI
12c	H	CH=CHCN	NO_2	0.0104 ± 0.00063	>48.78	4690	0.037 ± 0.021	>100	>2702
14c	H	CH=CHCN	NH_2	0.00055 ± 0.00008	25.0	45,455	<0.014 ± 0.002	8.83	>631
12d	NO_2	CH=CHCOMe	NO_2	0.146 ± 0.040	24.36	167	0.35 ± 0.17	>100	>286
14d	NO_2	CH=CHCOMe	NH_2	0.00633 ± 0.0018	5.88	929	0.047 ± 0.023	22.63	481
12e	NO_2	CH=CHCN	NO_2	0.0971 ± 0.0178	>43.96	>453	0.37 ± 0.04	21.69	59.35
14e	NO_2	CH=CHCN	NH_2	0.00038 ± 0.00007	>47.06	>123,842	<0.014 ± 0.002	88.17	>6298
14f	NH_2	CH=CHCN	NH_2	0.0043 ± 0.0005	14.43	3,356	<0.014 ± 0.002	18.94	>1353
3	H	C≡N	NH_2	0.00068 ± 0.00003	8.98	13,206	0.015 ± 0.001	>100	>6667
1				0.0014 ± 0.0004	8.96	6400	0.041 ± 0.002	50.73	1237

[*] Data were reported in Ref 11.

[a] p24 ELISA was used to determine 50% effective concentration (EC_{50}) against HIV-1 strains.

[b] XTT assay was used to determine the CC_{50} value that causes cytotoxicity to 50% cells.

[c] SI (selective index) = CC_{50}/EC_{50}. NA = not active at the highest concentration tested.

Table 2

Data against HIV drug-resistant viral strain replication

A: Y = N (**11, 13**)
B: Y = CH (**12, 14**)

	R_1	R_2	R_3	HIV-1 RTMDR[a] in TZM-bl cell line		A17 [b] in MT-2 cell line	
				EC_{50} (µM)	RF[c]	EC_{50}(µM)	RF
11a	H	I	NO_2	0.0943 ± 0.009	1.55	>10	>127
13a	H	I	NH_2	0.00513 ± 0.00115	0.67	1.93 ± 0.06	36
11b	H	Br	NO_2	0.0776 ± 0.0137	1.55	>10	>222
13b	H	Br	NH_2	0.0068 ± 0.0030	0.93	3.49 ± 0.46	106
11f	H	C≡CH	NO_2	0.215 ± 0.033	1.94	>10	>40
13f	H	C≡CH	NH_2	0.0090 ± 0.002	1.34	1.43 ± 0.26	34
11m	H	CH=CHCN	NO_2	0.0148 ± 0.0024	1.85	>10	>204
13m	H	CH=CHCN	NH_2	0.00059 ± 0.00009	0.83	0.20 ± 0.01	14
13c	H	$CH_2CH=CH_2$	NH_2	0.0154 ± 0.0016	2.23	>10	>149
13e	H	C≡CCH(CH$_2$)$_2$	NH_2	0.121 ± 0.0177	1.33	>10	>24
13k	H	CH_2CH_2COMe	NH_2	0.0032 ± 0.0008	1.64	0.73 ± 0.058	13
12c	H	CH=CHCN	NO_2	0.0170 ± 0.0011	1.63	>10	>270
14c	H	CH=CHCN	NH_2	0.00179 ± 0.0013	3.25	0.60 ± 0.09	43
14e	NO_2	CH=CHCN	NH_2	0.00087 ± 0.0001	2.29	0.095 ± 0.001	6.79
14f	NH_2	CH=CHCN	NH_2	0.0020 ± 0.0003	0.47	3.05 ± 0.03	128
3	H	C≡N	NH_2	0.00096 ± 0.0003	1.41	0.39 ± 0.065	26
1				0.0010 ± 0.0004	0.71	0.33 ± 0.02	6.00

Each compound was tested at least in triplicate.

[a]HIV-1 RTMDR (obtained from AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH), which contains mutations in RT amino acid residues L74V, M41L, V106A, and T215Y, is resistant to AZT, ddI, nevirapine, and other non-nucleoside RT inhibitors.

[b] The multi-NRTI-resistant strain A17 from NIH with mutations at amino acids K103N and Y181C in the viral RT domain is highly resistant to NRTIs.[19]

[c] Resistant fold.