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Optimization of antiviral potency and lipophilicity of halogenated 2,6-diarylpyridinamines (DAPAs) as a novel class of HIV-1 NNRTIs

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Abstract

Nineteen new halogenated diarylpyridinamine (DAPA) analogues (**6a-n** and **8a-e**) modified on the phenoxy C-ring were synthesized and evaluated for anti-HIV activity and certain drug-like properties. Ten compounds showed high anti-HIV activity (EC_{50} < 10 nM). Particularly, (*E*)-6-(2”-bromo-4”-cyanovinyl-6“-methoxy)phenoxy-N^2-(4′-cyanophenyl)pyridin-2,3-diamine (**8c**) displayed low nanomolar antiviral potency (3–7 nM) against wild-type and resistant viral strains with E138K or K101E mutation, associated with resistance to rilvipirine (**1b**). Compound **8c** exhibited much lower resistance fold changes (RFC 1.1–2.1) than **1b** (RFC 11.8–13.0). Compound **8c** also exhibited better metabolic stability (in vitro half-life) than **1b** in human liver microsomes (HLM), possessed low lipophilicity (clog D: 3.29; measured log P: 3.31), and had desirable lipophilic efficiency indices (LE > 0.3, LLE >5, LELP <10). With balanced potency and drug-like properties, **8c** merits further development as an anti-HIV drug candidate.

Keywords

anti-HIV activity; antiviral agents; diarylpyridinamine; drug-like properties; lipophiliicity

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Appendix A. Supplementary data

HPLC conditions and purity data for target compounds. This material is available free of charge via the Internet at

Introduction

Non-nucleoside HIV-1 reverse transcriptase inhibitors (NNRTIs) play an important role in antiretroviral therapy (ART)[1,2] and prevention of HIV infection due to their advantages of high potency and low toxicity. Five NNRTI drugs, including nevirapine, delavirdine, efavirenz, etravirine (**TMC125**, **1a**), and rilpivirine (**TMC278**, **1b**), have been approved by the US FDA and are currently available for the treatment of AIDS and HIV infection. Diarylpyrimidine derivatives (DAPYs) **1a** and **1b** (Figure 1) are new-generation NNRTI drugs that possess extremely high potency against wild-type and a broad spectrum of mutated NNRTI-resistant strains.[3] It has been reported that HIV-1 has a higher genetic barrier to evolve resistance to **1a** and **1b**;[4] thus, this compound type might have a better chance to overcome the deficiency of rapid drug-resistance found with earlier NNRTI drugs. However, resistance mutations to new-generation NNRTI drug **1b** have also appeared in patients.[5,6] Therefore, efforts still continue to develop additional new-generation NNRTI drugs with high efficacy and different structural scaffolds that will provide more clinical treatment options.

In our prior studies on novel NNRTI agents, we discovered diarylpyridinamines (DAPAs)[7,8] with extremely high potency against HIV-1 wild-type and drug-resistant viral strains. As an example, DAPA lead compounds **2a** and **2b** (Figure 2) exhibited subnanomolar potencies against HIV-1 wild-type (EC_{50} 0.63 and 0.71 nM) and RT multidrug-resistant (RTMDR) viral strains (EC_{50} 0.96 and 0.59 nM), which were better or comparable to those of new-generation NNRTI drugs **1a** (EC_{50} 1.4 and 1.0 nM) and **1b** (EC_{50} 0.51 and 0.49 nM), respectively, in the same assays. While DAPA and DAPY analogues have similar molecular flexibility and topology, DAPA compounds have a new chemotype scaffold and likely can adopt conformation changes (“wiggle”) as well as relocate and reorient within the NNRTI binding pocket (“jiggle”),[9,10] leading to high potency against drug-resistant viral replication. In our continued study, we have now designed and synthesized a new series of halogenated DAPA derivatives, as well as evaluated their anti-HIV activity and essential drug-like properties. Our aim was to develop potential new drug candidates with both good in vitro antiviral potency and acceptable drug-like properties. Based on prior SAR results,[11,12] we know that the *para*-cyanophenyl moiety (A-ring), the central pyridine (B-ring) with a 3-amino group and the NH linker between these two rings are necessary pharmacophores of DAPAs as HIV-1 NNRTIs. Especially, the presence of the 3-amino group on the central pyridine ring is crucial for interaction with a key amino acid K101 on the NNRTI binding site, acting as both H-bond donor and acceptor to form multiple H-bonds.[13] While the known pharmacophores of DAPAs were maintained in our new series, current modifications focused on substituents X and R on the phenoxy ring (C-ring) (see Fig. 2) to extent our knowledge of structure-activity (SAR) and structure-property (SPR) relationships. Initially, we replaced the 2,6-dimethyl groups, which are known major metabolic site(s) in drugs **1a** and **1b**,[14] with halogens (X) in order to increase metabolic stability. In addition, we introduced polar or ionizable groups or hetero atoms as *para*-substituents (R) on the C-ring to investigate how physicochemical properties affect drug-like properties. Herein, we report chemical synthesis of a new series of halogenated diarylpyridinamines (DAPAs), their antiviral activity against HIV-1 wild-

type and drug resistant viral strains, and certain in vitro drug-like properties, such as log P values, metabolic stability, aqueous solubility, and lipophilicity.

Results and discussion

Chemistry

DAPAs are available at low cost being readily synthesized from commercial reagents via short and efficient routes. Modifications on the phenol ring (C-ring) were performed as shown in Schemes 1 and 3 to produce new DAPA series **6a-n** and **8a-e**. 6-Chloro-2-(4-cyanophenyl)amino-3-nitropyridine (**3**), prepared from commercially available 2,6-dichloro-3-nitropyridine and 4-cyano phenol as described in our previous publication,[8] was coupled with various halogenated phenols in DMF in the presence of cesium carbonate or potassium carbonate under microwave irradiation at 90-100 °C for 10-15 min to afford corresponding halogenated 2,6-diaryl-3-nitropyridines **4a-e**, **5a-b**, and **7a**, respectively. The aldehyde group on the C-ring in **4a** was reduced with $NaBH_4$ to produce hydroxymethyl compound **5c**, followed by esterification with acetyl chloride to afford **5d**. Treatment of **4a** with hydroxylamine hydrochloride yielded oxime compound **5e**, which, following reflux in acetic anhydride overnight, was converted to *para*-cyano compound **5f**. The formyl group in **4a** was condensed with nucleophilic reagents, including diethyl cyanomethylphosphonate $[(EtO)_2P(O)CH_2CN]$, 2-cyanoacetamide ($CNCH_2CONH_2$), $Ph_3PCHCOOCH_3$, or acetone, under basic conditions to produce corresponding compounds **5g**, **5h**, **5i**, or **5j**, respectively, containing different *para*-groups (R) on the C-ring. Compound **5j** yielded carboxamide compound **5k** upon hydrolysis under basic conditions and treatment with ammonia. Similarly to **5g**, a series of *para*-cyanovinyl intermediates **7b-e** were prepared by condensation of the formyl group in **4b-e** with diethyl cyanomethylphosphonate. Finally, the 3-nitro group in series **5** and **7** compounds was reduced to an amino group by using sodium hydrosulfite or catalytic hydrogenation with Pd/C (5-10%) to obtain the corresponding halogenated series **6** and **8** diarylpyridinamines, respectively. Catalytic hydrogenation simultaneously reduced the double bond in the *para*-substituents of **5i**, **5j**, **5g**, and **5h**, giving **6i**, **6j**, **6l**, and **6m**, respectively. The ester group in **6j** was further reduced with $LiBH_4$ to afford **6n** with a *para*-hydroxypropyl group on the C-ring.

Evaluation of anti-HIV activity

The newly synthesized halogenated DAPA compounds were first evaluated against wild-type HIV-1 virus (NL4-3) infection of TZM-bl cells, and data are summarized in Table 1. In the fluoride DAPA series (**6**), six analogues, **6a**, **6b**, **6f**, **6g**, **6i**, and **6l,** showed extremely high potency against HIV-1 infection with low nanomolar EC_{50} values ranging from 0.75 to 7.43 nM. The *para*-R substituents on the C-ring in these six compounds were relatively nonpolar (e.g., hydrogen, bromo, cyano, cyanovinyl); however, a *para*-R substituent with more polar groups or heteroatoms (see **6c**, **6d**, **6e**, **6h**, **6j**, **6k**, **6m**, and **6n**) resulted in substantially lower antiviral activity (EC_{50} 48-350 nM). These results indicated that 2,6-halogenation on the C-ring is a beneficial approach to obtain new DAPA derivatives with high antiviral activity, but the identity of the *para*-R group also affects molecular potency. These findings are consistent with our previous postulation that the “west wing” on the NNRTI binding pocket might be a very hydrophobic narrow tunnel [6] (refer to PDB 3mec

and 3bgr). Consequently, the presence of hetero atoms, polar, or ionizable groups on the *para*-position of the phenoxy ring might impede insertion of the R-moiety in the tunnel and binding pocket, in turn compromising antiviral potency. Subsequently, four additional halogenated DAPA analogues (**8a-d**) with either *para*-cyano or *para*-cyanovinyl R groups on the C-ring, were synthesized. The *para*-cyanovinyl dibromo compound **8b** displayed the highest potency with a subnanomolar EC_{50} value of 0.33 nM, more potent than **1b** in the same assay. Dibromo **8a** with a *para*-cyano R group was also highly potent (EC_{50} 0.99 nM) against NL4-3. Both **8a** and **8b** were more potent than the corresponding difluoro **6f** and **6g** and comparably potent to dimethyl leads **2a** and **2b**. Furthermore, *para*-cyanovinyl compound **8c** with different X groups, bromo and methoxy (Br/OMe), as well as dichloro **8d** also exhibited high antiviral activity with EC_{50} values of 3.25 and 8.14 nM, respectively, only slightly less potent than *para*-cyanovinyl DAPAs **2b**, **6g**, and **8b**. These results suggested that: (1) favored order of halogenation (X) for antiviral activity is dibromide > difluoride > monobromide > dichloride and (2) a *para*-cyanovinyl substituent (R) on the C-ring is preferable over other substituents for enhancing antiviral potency. Non-halogenated *para*-cyanovinyl DAPA **8e** (X = H/MeO) remained active, again indicating that other modification of the X substituents could be acceptable, but was less potent (EC_{50} 16.7 nM) than other *para*-cyanovinyl DAPAs.

Subsequently, potent compounds **6a**, **6g**, and **8a-c** were selected for further testing in parallel with **1b** against wild-type viral strain IIIB and NNRTI-resistant HIV-1 mutants, including RT-multidrug-resistant **(**HIV-1_{RTMDR1}), K101E, E138K, and A17 (Table 2). K101E and E138K are the most important mutations associated with resistance against new-generation NNRTI drug **1b**,[6] and A17 is a NNRTI resistant strain with double mutations of K103N and Y181C. More interestingly, we found that **8c** displayed very similar potency against two wild-type (NL4-3 and III-B) and resistant viral strains with RTMDR, K101E or E138K mutation, resulting in lower resistance fold change (RFC) values (1.1–2.1) than **1b**. Resistance fold change value of **8c** against RT resistant strain A17 was 18.6, similar to that of **1b** (18.4) and lower than those of other compounds tested in the same assays. As shown in Table 2, other halogenated DAPAs also exhibited almost single-digit nanomolar potency against drug-resistant viral strains, except A17, thus indicating that modification of the 2,6-substituents on the phenoxy ring (C-ring) is acceptable and might be beneficial against **1b**-associated drug-resistance.

In general, biologic potency is often accompanied by increased molecular lipophilicity. However, high lipophilicity can result in undesirable ADME properties and toxicity, which hinder further drug development. Therefore, molecular lipophilicity has recently been considered as a major factor in the quality of a drug candidate. To balance potency and lipophilicity related to multiple drug-like properties, the concepts of ligand efficiency (LE), lipophilic ligand efficiency (LLE),[15] and ligand efficiency dependent lipophilicity (LELP)[16] have been accepted and applied [17-19] as usefully predictive tools to aid in decision making. Accordingly, we further assessed drug-like properties and predicted parameters for these new active DAPAs in parallel with **2b** and **1b** as shown in Table 3. Metabolic stability of active DAPAs was evaluated by a human liver microsome (HLM) incubation assay. Compounds **6a**, **6g**, **8a** and **8c** displayed comparable or better HLM

stability ($t_{1/2}$ 41-76 min) than **1b** ($t_{1/2}$ 39 min); however, the most potent dibromo compound **8b** appeared to be less stable ($t_{1/2}$ 21.87 min) than **1b** in the same assay. Our experimental data indicated that compounds **6g** and **8a-c** have log P values of 3.30–3.55 (at pH 7.4) similar to dimethyl-DAPA **2b** and improved compared with **1b**, implying reduced lipophilicity, even though their solubility was not noticeably enhanced (0.50-8.42 μg/mL at both pH 7.4 and 2.0). We postulated that the presence of halogen and oxygen atoms with stronger electronegativity, i.e., fluoride (F 4.0), methoxy (O 3.5) and bromide (Br 2.8), could contribute to the decreased lipophilicity. Next, lipophilic efficiency indices (LE, LLE, and LELP) of these compounds were predicted. Compliant with several desired thresholds, such as LE > 0.3, LLE >5, and LELP <10),[20] **6g** and **8c** have potential for further development as drug candidates. In contrast, compounds **6a**, **8a-b**, and **8d** did not reach all desired property indices, having either LLE values less than 5 or LELP values more than 10 as shown in Table 3. Moreover, molecular topological polar surface area (tPSA) values were calculated by using ChemDraw Ultra 12.0 as a prediction of oral bioavailability. All compounds in Table 3 had calculated *t*PSA values within the criterion of <140 Å.[2, 21] To balance favorable activity against drug-resistant viral strains and reasonable drug-like properties, compound **8c** is considered as the most promising drug candidate among the current DAPA compounds. Its structure with two different X groups (Br and OMe) might be critical in influencing antiviral potency against these drug-resistant viral strains, especially for E138K and K101E, two major mutations associated with next-generation NNRTI drugs **1a** and **1b**.

Conclusions

Synthetic modifications of X and R substituents on the phenoxy ring (C-ring) of DAPA lead **2** led to a new series of halogenated DAPA analogues (**6** and **8** series). Among them, compound **8c** was identified as a potential drug candidate with favorable physicochemical properties and good balance between potency and lipophilicity. Compound **8c** exhibited high anti-HIV potency against wild-type and resistant viral strains with E138 or K101E mutation (EC_{50} 4–7 nM), lower resistance fold change values (RFC: 1.1-2.1) than those of DAPY drug **1b** (RFC: 11.8-13) in the same assays. It also exhibited desirable lipophilicity (low clog D 3.29 and measured log P 3.31), moderate metabolic stability ($t_{1/2}$ 54.7 min) in HLM assay, and desirable ligand lipophilic-efficiency indices (LE > 0.3, LLE >5, LELP <10, and tPSA <140). The current modifications also revealed new SAR and SPR findings. (1) Introduction of halogens (X) at the 2-and/or 6-positions on the C-ring is an effective approach to improve molecular drug-like properties without loss of potency. (2) The presence of heteroatoms with stronger electronegativity at the 2,6-positions on the C-ring might reduce molecular lipophilicity and improve ADME properties, despite having less impact on aqueous solubility. (3) Dibromo DAPAs appeared to have lower metabolic stability than corresponding difluoro and dichloro compounds (see **8b**, **6g**, and **8d**).

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 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 a percent (%).The purities of target compounds were ≥95%, measured by HPLC, performed on Agilent 1200 HPLC system with UV detector and Grace Alltima HP C18 column (100 × 2.1 mm, 3 μm), eluting with a mixture of solvents A and B (condition 1: acetonitrile/water 95:5, flow rate 1.0 mL/min; condition 2: MeOH/water 80:20, flow rate 0.8 mL/min, UV 254 nm). Microwave reactions were performed on a microwave reactor from Biotage, Inc. Thin-layer chromatography (TLC) and preparative TLC plates used silica gel GF254 (200-300 mesh) purchased from Qingdao Haiyang Chemical Company. Medium-pressure column chromatography was performed using a CombiFlash companion purification system. All chemical reagents and solvents were obtained from Beijing Chemical Works or Sigma-Aldrich, Inc. NADPH, $MgCl_2$, KH_2PO_4, K_2HPO_4, and reference compound propranolol were purchased from Sigma-Aldrich. HPLC-grade acetonitrile for LC-MS analysis was purchased from VWR. Pooled human liver microsomes (Lot No# 28831) were purchased from BD Biosciences (Woburn, MA).

General coupling reaction procedure for preparation of 1,6-diaryl-3-nitro pyridines (4, 5a-b, 7a)

A mixture of 6-chloro-N^2-(4-cyanophenyl)amino-3-nitropyridine (**3**, 1 equiv) and 2,4,6-trisubstituted phenol (1.2 equiv) in DMF (1–2 mL) in the presence of Cs_2CO_3 or K_2CO_3 (3.6 equiv) was irradiated under microwave conditions with stirring at 90 °C for 15 min or at 100 °C for 10 min. The mixture was poured into ice-water, pH adjusted to 2–3 with aq HCl (5%), extracted with CH_2Cl_2 three times, and dried over anhydrous Na_2SO_4. After removal of organic solvent under reduced pressure, crude product was purified by PTLC or flash column chromatography (gradual elution) to produce the corresponding product.

N^2-(4′-Cyanophenyl))amino-6-(2”,6”-difluoro-4”-formyl)phenoxy-3-nitropyridine (4a)

Starting with 6-chloro-N^2-(4-cyanophenyl)amino-3-nitro pyridine (**3**) (274 mg, 1.0 mmol) and 2,6-difluoro-4-formylphenol (190 mg, 1.2 mmol) in DMF (1-2 mL) in the presence of Cs_2CO_3 (3.6 equiv) irradiated at 90 °C for 15 min to afford **4a** as a yellow solid (277 mg, 65%), mp: 217-219 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.34 (1H, s, NH), 10.05 (1H, s, CHO), 8.73 (1H, d, J = 8.8 Hz, PyH-4), 7.92 (2H, d, J = 8.8 Hz, ArH-3′,5′), 7.47 (2H, d, J_F = 8.8 Hz, ArH-3”,5”), 7.35 (2H, d, J = 8.8 Hz, ArH-2′,6′), 6.95 (1H, d, J = 8.8 Hz, PyH-5) ppm; MS *m/z* (%): 394.9 (M − 1, 100).

N^2-(4′-Cyanophenyl))amino-6-(2”,6”-dibromo-4”-formyl)phenoxy-3-nitropyridin e (4b)

Starting with **3** (567 mg, 2.0 mmol), 2,6-dibromo-4-formyl phenol (693 mg, 2.48 mmol), and K_2CO_3 (3.6 equiv) at 100 °C for 10 min to afford **4b** as **a** yellow solid (518 mg, 50%), mp: 227-229 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=10.58 (1H, br s, NH), 10.03 (1H, s, CHO), 8.71 (1H, d, *J* = 9.2 Hz, PyH-4), 8.18 (2H, s, ArH-3”,5”), 7.25 (4H, s, ArH-2′,3′, 5′,6′), 6.76 (1H, d, *J* = 9.2 Hz, PyH-5) ppm; MS *m/z* (%): 517.0 (M + 1, 20), 519.0 (M + 3, 100), 521.1 (M + 5, 40).

6-(2”-Bromo-4”-formyl-6”-methoxy)phenoxy-*N*^{2}-(4′-cyanophenyl))amino-3-nitro pyridine (4c)

Starting with **3** (274 mg, 1.0 mmol), 2-bromo-4-formyl-6- methoxyphenol (277 mg, 1.2 mmol), and Cs_2CO_3 (3.6 equiv) at 90 °C for 15 min to afford **4c** as a yellow solid (281 mg, 59%), mp: 222-224 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.34 (1H, s, NH), 10.06 (1H, s, CHO), 8.69 (1H, d, *J* = 8.8 Hz, PyH-4), 7.96 (1H, d, *J* = 1.6 Hz, ArH-3”), 7.73 (1H, d, *J* = 1.6 Hz, ArH-5”), 7.43 (2H, d, *J* = 8.4 Hz, ArH-3′,5′), 7.35 (2H, d, *J* = 8.8 Hz, ArH-2′,6′), 6.90 (1H, d, *J* = 8.8 Hz, PyH-5), 3.82 (3H, s, OMe) ppm; MS *m/z* (%): 467 (M − 1, 100), 469 (M + 1, 90).

N^2-(4′-Cyanophenyl))amino-6-(2”,6”-dichloro-4”-formyl)phenoxy-3-nitropyridin e (4d)

Starting with **3** (274 mg, 1.0 mmol), 2,6-dichloro-4-formylphenol (225 mg, 1.2 mmol), and K_2CO_3 (3.6 equiv) at 100 °C for 10 min to afford **4d** as a yellow solid (233 mg, 55%), mp: 232-234 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.34 (1H, s, NH), 10.09 (1H, s, CHO), 8.75 (1H, d, *J* = 8.8 Hz, PyH-4), 8.22 (2H, s, ArH-3”,5”), 7.45 (2H, d, *J* = 8.8 Hz, ArH-3′, 5′), 7.32 (2H, d, *J* = 8.8 Hz, ArH-2′,6′), 6.98 (1H, d, *J* = 8.8 Hz, PyH-5) ppm; MS *m/z* (%): 427 (M − 1, 80), 391.1 (100).

N^2-(4′-Cyanophenyl)amino-6-(4”-formyl-2”-methoxy)phenoxy-3-nitropyridine (4e)

Starting with **3** (274 mg, 1.0 mmol), 4-formyl-2-methoxy phenol (182 mg,1.2 mmol), and Cs_2CO_3 (3.6 equiv) at 90 °C for 15 min to afford **4e** as a yellow solid (227 mg, 58), mp: 213-215 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.34 (1H, s, NH), 10.09 (1H, s, CHO), 8.65 (1H, d, *J* = 8.8 Hz, PyH-4), 7.73 (1H, s, ArH-3”), 7.67 (1H, d, *J* = 8.0 Hz, ArH-5”), 7.48 (1H, d, *J* = 8.0 Hz, ArH-6”), 7.39 (4H, s, ArH-2′,3′,5′,6′), 6.80 (1H, d, *J* = 8.8 Hz, PyH-5), 3.78 (3H, s, OMe) ppm; MS *m/z* (%): 389.3 (M − 1, 100).

N^2-(4′-Cyanophenyl))amino-6-(2”,6”-difluoro)phenoxy-3-nitropyridine (5a)

Starting with **3** (2.74 g, 10 mmol), 2,6-difluoro-phenol (1.56 g,12 mmol), and K_2CO_3 (3.6 equiv) at 100 °C for 10 min to afford pure **5a** as a yellow solid (1.62 g, 44%) by flash column chromatography (gradual elution: CH_2Cl_2/MeOH 0–3%), mp: 234-236 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.64 (1H, s, NH), 8.66 (1H, d, *J* = 8.8 Hz, PyH-4), 7.26-7.31 (5H, m, ArH), 7.11 (2H, m, ArH), 6.73 (1H, d, *J* = 8.8 Hz, PyH-5) ppm; MS *m/z* (%): 369.1 (M + 1, 100).

6-(4”-Bromo-2”,6”-difluoro)phenoxy- *N*^{2}-(4′-cyanophenyl)amino-3-nitropyridine (5b)

Starting with **3** (274 mg, 1.0 mmol), 4-bromo-2,6- difluorophenol (251 mg, 1.2 mmol), and K_2CO_3 (3.6 equiv) at 100 °C for 10 min to afford **5b** as a yellow solid (290 mg, 70%), mp: 213-215 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.43 (1H, s, NH), 8.71 (1H, d, *J* = 8.8 Hz, PyH-4), 7.80 (2H, d, *J* = 8.8 Hz, ArH-3′,5′), 7.55 (2H, d, J_F = 12 Hz, ArH-3”,5”), 7.39 (2H, d, *J* = 8.8 Hz, ArH-2′,6′), 6.92 (1H, d, *J* = 8.8 Hz, PyH-5) ppm; MS *m/z* (%): 445.2 (M−1, 98), 447.0 (M + 1, 100).

N^2-(4′-Cyanophenyl))amino-6-(2”,4”,6”-tribromo)phenoxy-3-nitropyridine (7a)

Starting with **3** (2.75 mg, 10 mmol), 2,4,6-tribromophenol (3.97 mg, 12 mmol), and K_2CO_3 (3.6 equiv) at 100 °C for 10 min to afford **7a** as a yellow solid (3.60 g, 67%), mp: 192-194 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.57 (1H, s, NH), 8.68 (1H, d, *J* = 8.8 Hz, PyH-4), 7.83 (2H, s, ArH-3”,5”), 7.38 (2H, d, *J* = 9.2 Hz, ArH-3′,5′), 7.26 (2H, d, *J* = 9.2 Hz, ArH-2′,6′), 6.72 (1H, d, *J* = 8.8 Hz, PyH-5) ppm; MS *m/z* (%): 567.2 (M + 1, 20), 569.0 (M + 3, 100), 571.1 (M + 5, 90).

N^2-(4′-Cyanophenyl))amino-6-(2”,6”-difluoro-4”-hydroxymethyl)phenoxy-3-nitro pyridine (5c)

To a solution of **4a** (396 mg, 1.0 mmol) in THF (15 mL) and MeOH (5 mL) was added $NaBH_4$ (191 mg, 5 mmol) slowly in an ice-bath. The mixture was stirred for another 30 min and then poured into ice-water, pH adjusted to 4–5 with 5% aqHCl, extracted with EtOAc three times, and dried over anhydrous Na_2SO_4. After removal of organic solvent *in vacuo*, crude product was purified by silica flash column (elution: CH_2Cl_2/MeOH = 100/1) to afford pure **5c** as a yellow solid (276 mg, 70%), mp: 237–239 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.38 (1H, br. s, NH), 8.71 (1H, d, *J* = 8.8 Hz, PyH-4), 7.52 (2H, d, *J* = 8.4 Hz, ArH-3′,5′), 7.40 (2H, d, *J* = 8.4 Hz, ArH-2′,6′), 7.30 (2H, d, $J_{H\text{-}F}$ = 9.2 Hz, ArH-3”,5”), 6.92 (1H, d, *J* = 8.8 Hz, PyH-5), 5.67 (1H, t, *J* = 5.6 Hz, OH), 4.60 (2H, d, *J* = 5.6 Hz, CH_2) ppm; MS *m/z* (%): 397.0 (M − 1, 100).

N^2-(4′-Cyanophenyl))amino-6-(2”,6”-difluoro-4”-acetoxymethyl)phenoxy-3-nitro pyridine (5d)

To a solution of **5c** (598 mg, 1 mmol) in THF (15 mL) was added dropwise acetyl chloride (2 mL) and triethylamine (2 mL) simultaneously in an ice-bath. Then the mixture was stirred at room temperature for another 30 min, poured into ice-water, pH adjusted to 4-5 with aq HCl, extracted with EtOAc three times, and dried over anhydrous Na_2SO_4. After removal of solvent, crude product was purified by a silica column eluted with CH_2Cl_2 to afford **5d** as a yellow solid (412 mg, 94%), mp: 159–161 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.39 (1H, br. s, NH), 8.72 (1H, d, *J* = 8.8 Hz, PyH-4), 7.52 (2H, d, *J* = 8.8 Hz, ArH-3′,5′), 7.44 (2H, d, $J_{H\text{-}F}$ = 8.8 Hz, ArH-3”,5”), 7.40 (2H, d, *J* = 8.8 Hz, ArH-2′,6′), 6.93 (1H, d, *J* = 8.8 Hz, PyH-5), 5.17 (2H, s, CH_2), 2.19 (3H, s, CH_3) ppm; MS *m/z* (%): 439.6 (M − 1, 100).

N^2-(4′-Cyanophenyl))amino-6-[2”,6”-difluoro-4”-(hydroxyimino)methyl]phenoxy-3-nitropyridine (5e)

To a solution of **4a** (396 mg, 1.0 mmol) in THF (15 mL) was added hydroxylamine hydrochloride (175 mg, 25 mmol) in an ice-bath. The mixture was stirred for 2 h at room temperature and then poured into ice-water, pH adjusted to 7–8 with 5% aq NaOH. The resulting solid was collected and washed with water to neutral. The dried solid was then purified by silica flash column (gradual elution: MeOH/CH_2Cl_2 0-1%) to afford **5e** as a yellow solid (312 mg, 76%), mp: 240–242 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=11.77 (1H, s, OH), 10.37 (1H, br. s, NH), 8.72 (1H, d, J = 8.8 Hz, PyH-4), 8.27 (1H, s, CH=), 7.57 (2H, d, $J_{H\text{-}F}$ = 8.8 Hz, ArH-3”,5”), 7.44 (4H, m, ArH-2′,6′ and ArH-3′,5′), 6.94 (1H, d, J = 8.8 Hz, PyH-5) ppm; MS *m/z* (%): 410.2 (M − 1, 100).

6-(4”-Cyano-2”,6”-difluoro)phenoxy-*N*^{2}-(4′-cyanophenyl))amino-3-nitropyridine (5f)

5e (150 mg, 0.36 mmol) in acetic anhydride was refluxed overnight and monitored with TLC (EtOAc/petroleum ether) until reaction was completed. After removal of most acetic anhydride under reduced pressure, the residue was added to water and stirred for about 1 h. The yellow solid produced was filtered, washed with water to neutral, and dried. The crude product was purified by a silica flash column (gradual elution: CH_2Cl_2/petroleum ether) to afford solid **5f** (130 mg, 90%), mp: 282–284 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.34 (1H, s, NH), 8.73 (1H, d, J = 8.8 Hz, ArH-4), 8.12 (2H, d, $J_{F\text{-}H}$ = 8.0 Hz, ArH-3”, 5”), 7.56 (2H, d, J = 8.8 Hz, ArH-3′, 5′), 7.36 (2H, d, J = 8.8 Hz, ArH-2′, 6′), 6.95 (1H, d, J = 8.8 Hz, ArH-5) ppm; MS m/z (%): 394 (M + 1, 100).

(*E*)-*N*^{2}-(4′-Cyanophenyl))amino-6-(2”,6”-difluoro-4”-(2-carbamoyl-2-cyano)vinyl) phenoxy-3-nitropyridine (5h)

A mixture of **4a** (396 mg, 1.0 mmol) in DMF (1.5 mL), $ZnCl_2$ (27 mg, 0.2 mmol), and 2-cyanoacetamide (excess) was irradiated under microwave conditions with stirring at 100 °C for 10 min. The mixture was poured into ice-water, pH adjusted to 2-3 with aq HCl (5%), extracted with CH_2Cl_2 three times, and dried over anhydrous Na_2SO_4. After removal of solvent, crude product was purified by a silica column (gradual elution: MeOH/CH_2Cl_2 0-5%) to afford **5h** as a yellow solid (415 mg, 90%), mp: 265-267 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.36 (1H, br. s, NH), 8.73 (1H, d, J = 8.8 Hz, PyH-4), 8.29 (1H, s, CH=), 8.00 and 7.96 (each 1H, br s, NH_2), 7.89 (2H, d, $J_{H\text{-}F}$ = 8.8 Hz, ArH-3”,5”), 7.50 (2H, d, J = 8.8 Hz, ArH-3′,5′), 7.39 (2H, d, J = 8.8 Hz, ArH-2′,6′), 6.95 (1H, d, J = 8.8 Hz, PyH-5) ppm; MS *m/z* (%): 461.4 (M − 1, 25), 398.4 (100).

(*E*)-*N*^{2}-(4′-Cyanophenyl))amino-6-(2”,6”-difluoro-4”-(3-oxobut-1-en-1-yl)phenox-3-nitropyridine (5i)

To a solution of **4a** (396 mg, 1 mmol) in acetone (20 mL) was added aq NaOH (10%, 2 mL) slowly in ice-water bath and stirred for 10 min. The mixture was then stirred at room temperature for 1 h, poured into ice-water, and pH was adjusted to 4-5 with 5% aq HCl. The solid was filtered, washed with water, and purified on a silica gel column (gradient elution: EtOAc/petroleum ether 0–50%) to produce **5i** as a yellow solid (226 mg, 52%), mp: 275–277 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ=10.33 (1H, br. s, NH), 8.71 (1H, d, J = 8.8 Hz,

PyH-4), 7.76 (2H, J_{F-H} = 8.8 Hz, ArH-3",5"), 7.68 (1H, d, *J* = 16.4 Hz, ArCH=), 7.46 (2H, d, *J* = 8.8 Hz, ArH-3′, 5′), 7.36 (2H, d, *J* = 8.8 Hz, ArH-2′, 6′), 7.03 (1H, d, *J* = 16.4 Hz, =CHCO), 6.92 (1H, d, *J* = 8.8 Hz, PyH-5), 2.38 (3H, s, $COCH_3$) ppm; MS *m/z* (%): 435.2 (M − 1, 100).

(*E*)-N^2-(4′-Cyanophenyl))amino-6-(2",6"-difluoro-4"-(2-carbomethoxy)vinyl) phenoxy-3-nitropyridine (5j)

A mixture of **4a** (396 mg, 1 mmol) and $Ph_3PCHCOOCH_3$ (334 mg, 1 mmol) in $CHCl_3$ (20 mL) was heated to reflux under nitrogen overnight. After removal of solvent, the residue was purified on a silica gel column (elution: CH_2Cl_2) to give **5j** as a yellow solid (380 mg, 83%), mp: 263–265 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.34 (1H, br. s, NH), 8.71 (1H, d, *J* = 9.2 Hz, PyH-4), 7.84 (2H, J_{F-H} = 8.8 Hz, ArH-3",5"), 7.73 (1H, d, *J* = 16.0 Hz, ArCH=), 7.46 (2H, d, *J* = 8.8 Hz, ArH-3′, 5′), 7.38 (2H, d, *J* = 8.8 Hz, ArH-2′, 6′), 6.93 (1H, d, *J* = 8.8 Hz, PyH-5), 6.90 (1H, d, *J* = 16.4 Hz, =CHCO), 3.76 (3H, s, OCH_3) ppm; MS *m/z* (%): 451.2 (M − 1, 100).

(*E*)-N^2-(4′-Cyanophenyl))amino-6-(2",6"-difluoro-4"-(2-carbamoyl)vinyl) phenoxy-3-nitropyridine (5k)

A solution of **5j** (452 mg, 1 mmol) in THF (50 mL) was added dropwise to aq NaOH (10%, 1 mL) at room temperature and stirred for 36 h. The mixture was poured into water and pH was adjusted to 2-3 with aq HCl. The precipitated solid was collected and washed with water to neutral. After drying, the solid was dissolved in CH_2Cl_2, *N*,*N*′-carbonyl diimidazole (CDI, 194 mg, 1.2 mmol) was added in several portions, and stirring continued for 2 h. Then, the mixture was added into ammonia (30 mL, excess) and stirred for 2 h. The mixture was added to water and extracted with CH_2Cl_2 three times. The combined organic phase was washed with water, aq $NaHCO_3$, and brine successively and dried over Na_2SO_4. The solvent was removed to obtain crude **5k**, used without purification in the next step.

General procedure for preparation of cyanovinyl compounds

To a solution of diethylcyanomethylphosphonate [$(EtO)_2P(O)CH_2CN$, 266 mg, 1.5 mmol] in THF (15 mL) was added *t*-BuOK (1.5 mmol) at 0 °C (ice-water bath) with stirring for 30 min and then for another 30 min at room temperature. The solution of aldehyde (**4**, 1 mmol) in THF (15 mL) was quickly added dropwise into the above mixture at room temperature and stirred until the reaction was completed (1–48 h) monitored by TLC. The mixture was poured into water, extracted with EtOAc three times, and dried over anhydrous Na_2SO_4. After removal of solvent under reduced pressure, crude product was purified by a silica gel column using medium-pressure system of CombiFlash companion (gradual elution: CH_2Cl_2/petroleum ether) to give the corresponding cyanovinyl product (**5g**, **7b**-**e**).

(*E*)-N^2-(4′-Cyanophenyl))amino-6-(4"-cyanovinyl-2",6"-difluoro)phenoxy-3-nitropyri dine (5g)

Starting with **4a** (396 mg, 1 mmol) to provide **5g** as yellow solid (396 mg, 81%), mp: 262-264 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=10.61 (1H, br. s, NH), 8.68 (1H, d, *J* = 9.2 Hz, PyH-4), 7.40 (1H, d, *J* = 16.4 Hz, ArCH=), 7.34-7.26 (4H, m, ArH), 7.20 and &.18 (each

1H, s, ArH), 6.95 (1H, d, *J* = 8.8 Hz, PyH-5), 5.98 (1H, d, *J* = 16.4 Hz, =CHCN) ppm; MS *m/z* (%): 418.2 (M − 1, 100).

(*E*)-N^2-(4′-Cyanophenyl))amino-6-(4”-cyanovinyl-2”,6”-dibromo)phenoxy-3-nitro pyridine (7b)

Starting with **4b** (518 mg) to give pure **7b** as light yellow solid (363 mg, 67%), mp: 224-226 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.31 (1H, s, NH), 8.72 (1H, d, *J* = 8.8 Hz, PyH-4), 8.17 (2H, s, ArH-3”, 5”), 7.70 (1H, d, *J* = 16.0 Hz, CH=), 7.44 (2H, d, *J* = 8.4 Hz, ArH-3′, 5′), 7.33 (2H, d, *J* = 8.4 Hz, ArH-2′,6′), 6.94 (1H, d, *J* = 8.8 Hz, PyH-5), 6.76 (1H, d, *J* = 16.0 Hz, CH=) ppm; MS *m/z* (%): 538 (M − 1, 30), 540 (M + 1, 100), 542 (M + 3, 20).

(*E*)-6-(2”-Bromo-4”-cyanovinyl-6”-methoxy)phenoxy-N^2-(4′-cyanophenyl))amin o-3-nitropyridine (7c)

Starting with **4c** (469 mg) to give **7c** as yellow solid (418 mg, 85%), mp: 243-245 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.32 (1H, br, NH), 8.67 (1H, d, *J* = 8.8 Hz, PyH-4), 7.72 (1H, d, *J* = 16.4 Hz, ArCH=), 7.65 (2H, m, ArH-3”,5”), 7.44 and 7.35 (each 2H, d, *J* = 8.8 Hz, ArH-2′, 3′, 5′, 6′), 6.87 (1H, d, *J* = 8.8 Hz, PyH-5), 6.75 (1H, d, *J* = 16.4 Hz, =CHCN), 3.76 (3H, s, OCH_3) ppm; MS *m/z* (%): 490.2 (M − 1, 100).

(*E*)-N^2-(4′-Cyanophenyl))amino-6-(4”-cyanovinyl-2”,6”-dichloro)phenoxy-3-nitro pyridine (7d)

Starting with **4d** (389 mg) to obtain **7d** as yellow solid (339 mg, 88%), mp: 210-212 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.30 (1H, br. s, NH), 8.72 (1H, d, *J* = 8.8 Hz, PyH-4), 8.02 (2H, s, ArH-3”,5”), 7.71 (1H, d, *J* = 16.4 Hz, ArCH=), 7.45 (2H, d, *J* = 8.4 Hz, ArH-3′, 5′), 7.33 (2H, d, *J* = 8.4 Hz, ArH-2′, 6′), 6.94 (1H, d, *J* = 8.8 Hz, PyH-5), 6.76 (1H, d, *J* = 16.4 Hz, =CHCN) ppm; MS *m/z* (%): 450.3 (M − 1, 100).

(*E*)-N^2-(4′-Cyanophenyl)amino-6-(4”-cyanovinyl-2”-methoxy)phenoxy-3-nitro pyridine (7e)

Starting with **4e** (389 mg) to obtain **7e** as yellow solid (343 mg, 83%), mp: 228-230 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.33 (1H, br. s, NH), 8.63 (1H, d, *J* = 9.2 Hz, PyH-4), 7.75 (1H, d, *J* = 16.4 Hz, ArCH=), 7.59 (1H, d, *J* =1.6 Hz, ArH-3”), 7.41 (4H, m, ArH), 7.33 (1H, d, *J* = 8.4 and 1.6 Hz, ArH-5”), 7.29 (1H, d, *J* = 8.4 Hz, ArH-6”), 6.77 (1H, d, *J* = 8.8 Hz, PyH-5), 6.64 (1H, d, *J* = 16.4 Hz, =CHCN), 3.73 (3H, s, OCH_3) ppm; MS *m/z* (%): 412.3 (M − 1, 100).

General reduction procedures of nitro group to amine

Method 1 (reduction with sodium hydrosulfite, $Na_2S_2O_4$): To a solution of a 2,6-diaryl-3-nitropyridine (1 equiv, **5** or **7**) in THF and water (v/v 1:1) was added NH_3H_2O solution (25%, 0.5 mL) and sodium hydrosulfite (90% $Na_2S_2O_4$, 10 equiv), successively, at room temperature with stirring for 2 h monitored by TLC (CH_2Cl_2/MeOH 100:1) until reaction was completed. The mixture was then poured into ice-water and extracted with EtOAc three times. After removal of solvent *in vacuo*, the residue was purified on a silica gel flash column (gradual elution: MeOH/CH_2Cl_2 0–1%) to obtain pure corresponding diarylpyridinamines **6** (**a-c, f-h, k**) and **8**, respectively. **Method 2** (catalytic hydrogenation,

H_2 on Pd/C): A solution of a diarylnitrobenzene (1 equiv, **5**) in a solvent mixture of anhydrous EtOH and THF (15:15 mL) with hydrogen gas in the presence of excess Pd/C (10%) under 15 psi was shaken until hydrogen was no longer absorbed (*ca* 2 h) and also monitored by TLC (elution: CH_2Cl_2/MeOH 100:1) until the reaction was completed. The catalyst was filtered out from the solution and washed with EtOH several times. After removal of solvent, the residue was purified by flash column chromatography (gradual elution: MeOH/CH_2Cl_2, 0-1%) to obtain required product **6** (**d-e**, **i-j**, or **l-m**).

N^2-(4′-Cyanophenyl)amino-6-(2”,6”-difluoro)phenoxypyridin-3-amine (6a)

Method 1. Starting with **5a** (57 mg, 0.154 mmol) reduced by $Na_2S_2O_4$ to afford **6a** as a yellow solid (15 mg, 29%), mp: 161–163 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.35 (1H, s, NH), 7.38 (1H, m, ArH-4′), 7.33 (4H, m, ArH-2′,3′,5′,6′), 7.30 (2H,d, $J_{F,\,H}$ = 12 Hz, ArH-3”,5”), 7.16 (1H, d, *J* = 8.0 Hz, PyH-4), 6.56 (1H, d, *J* = 8.0 Hz, PyH-5), 4.92 (2H, s, NH_2) ppm; MS *m/z* (%): 339.2 (M + 1, 100); HPLC purity 95.18%.

6-(4”-Bromo-2”,6”-difluoro)phenoxy-*N*^{2}-(4′-cyanophenyl)amino)pyridin-3-amine (6b)

Method 1. Starting with **5b** (447 mg) reduced by$Na_2S_2O_4$ to produce **6b** as a light gray solid (218 mg, 52%), mp: 208–210 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.46 (1H, s, NH), 7.75 (2H, d, $J_{F\text{-}H}$ = 8.4 Hz, ArH-3”,5”), 7.39 (2H, d, *J* = 8.8 Hz, ArH-3′,5′), 7.31 (2H, d, *J* = 8.8 Hz, ArH-2′,6′), 7.16 (1H, d, *J* = 8.4 Hz, PyH-4), 6.58 (1H, d, *J* = 8.4 Hz, PyH-5), 4.96 (2H, br, NH_2) ppm; MS *m/z* (%): 417 (M + 1, 60), 415.3 (M − 1, 70), 397 (90), 395.1 (100); HPLC purity 95.41%.

N^2-(4′-Cyanophenyl)amino-6-(2”,6”-difluoro-4”-hydroxymethyl)phenoxypyridin e-3-amine (6c)

Starting with **5c (**398 mg) reduced by $Na_2S_2O_4$ to give **6c** as a gray solid (179 mg, 49%), mp: 210–212 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.38 (1H, br., NH), 7.36 (4H, m, ArH on A-ring), 7.24 (2H, d, *J* = 8.8 Hz, ArH-3”,5”), 7.15 (1H, d, *J* = 8.4 Hz, PyH-4), 6.55 (1H, d, *J* = 8.4 Hz, PyH-5), 5.56 (1H, t, *J* = 5.6 Hz, OH), 4.91 (2H, s, NH_2), 4.57 (2H, d, *J* = 5.6 Hz, CH_2) ppm; MS *m/z* (%): 367.2 (M − 1, 60), 320.1 (100); HPLC purity 95.22%.

N^2-(4′-Cyanophenyl)amino-6-(2”,6”-difluoro-4”-acetoxymethyl)phenoxypyridin-3-amine (6d)

Method 2. Starting with **5d** (440 mg) reduced by catalytic hydrogenation to furnish **6d** as a gray solid (209 mg, 51%), mp: 173–175 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.40 (1H, s, NH), 7.36 (6H, m, ArH), 7.15 (1H, d, *J* = 8.0 Hz, PyH-4), 6.57 (1H, d, *J* = 8.0 Hz, PyH-5), 5.14 (2H, s, CH_2), 4.93 (2H, br s, NH_2), 2.16 (3H, s, CH_3) ppm; MS *m/z* (%): 409.2 (M − 1, 70), 329.3 (100); HPLC purity 95.11%.

N^2-(4′-Cyanophenyl)amino-6-(2”,6”-difluoro-4”-(hydroxyimino)methyl)phenoxy pyridin-3-amine (6e)

Starting with **5e (**381 mg) reduced by catalytic hydrogenation to afford **6e** as a gray solid (313 mg, 53%), mp: 214–216 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=11.63 (1H, s, OH), 8.42 (1H, br., NH), 8.25 (1H, s, CH=N), 7.51 (2H, d, $J_{H\text{-}F}$ = 9.2 Hz, ArH-3”,5”), 7.34 (4H,

m, ArH-2′,6′,3′,5′), 7.16 (1H, d, *J* = 8.4 Hz, PyH-4), 6.58 (1H, d, *J* = 8.4 Hz, PyH-5); 4.94 (2H, s, NH_2) ppm; MS *m/z* (%): 380.1 (M − 1, 20), 342.2 (100); HPLC purity 96.17%.

N^2-(4′-Cyanophenyl)amino-6-(2”,6”-difluoro-4”-cyano)phenoxypyridin-3-amine (6f) Method 1

Starting with **5f** (150 mg) in the presence of $NaHCO_3$ (328 mg, 3.9 mmol) reduced by $Na_2S_2O_4$ to give **6f** (85 mg, 60%), mp: 265 °C (dec.), ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.46 (1H, s, NH), 8.08 (2H, d, $J_{H\text{-}F}$ = 8.0 Hz, ArH-3”, 5”), 7.41 (2H, d, *J* = 8.8 Hz, ArH-3′, 5′), 7.26 (2H, d, *J* = 8.8 Hz, ArH-2′, 6′), 7.16 (1H, d, *J* = 8.4 Hz, ArH-4), 6.62(1H, d, *J* = 8.4 Hz, ArH-5), 5.00 (2H, s, NH_2) ppm; MS m/z (%): 364 (M + 1,100). HPLC purity 98.51% (CH_3OH/H_2O = 70:30).

(*E*)-*N*^{2}-(4′-Cyanophenyl)amino-6-(4”-cyanovinyl-2”,6”-difluoro)phenoxypyridin-3-amine (6g) Method 1

Starting with **5g** (419 mg, 1 mmol) was reduced by $Na_2S_2O_4$ to afford **6g** as an off-white solid (254 mg, 65%), mp: 210–211 °C; ^{1}H NMR (400 MHz, CD_3COCD_3): δ=7.80 (1H, br., NH), 7.62 (1H, d, *J* = 16.8 Hz, ArCH=), 7.59 (2H, $J_{F\text{-}H}$ = 8.8 Hz, ArH-3”,5”), 7.39 (2H, d, *J* = 8.8 Hz, ArH- 3′, 5′), 7.29 (3H, m, ArH- 2′, 6′ and PyH-4), 6.57 (1H, d, *J* = 8.0 Hz, PyH-5), 6.48 (1H, d, *J* = 16.8 Hz, CH=), 4.39 (2H, br, NH_2) ppm; MS *m/z* (%): 387.9 (M − 1, 25), 368.2 (100); HPLC purity 96.37%.

(*E*)-6-[4”-(3-Amino-2-cyano-3-oxoprop-1-enyl)-2”,6”-difluoro]phenoxy-*N*^{2}-(4′-cyanophenyl)pyridin-2,3-diamine (6h). Method 1

Starting with of **5h (**462 mg) reduced by $Na_2S_2O_4$ to give **6h** as a gray solid (273 mg, 64%), mp: 227–229 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.46 (1H, br. s, NH), 8.28 (1H, s, CH=), 8.02 and 7.93 (each 1H, br, $CONH_2$), 7.89 and 7.87 (each 1H, s, ArH-3”,5”), 7.35 (4H, m, ArH), 7.18 (1H, d, *J* = 8.0 Hz, PyH-4), 6.63 (1H, d, *J* = 8.0 Hz, PyH-5), 5.02 (2H, br, NH_2) ppm; MS *m/z* (%): 431.1 (M − 1, 20), 411.3 (100); HPLC purity 96.19%.

N^2-(4′-Cyanophenyl)amino-6-(2”,6”-difluoro-4”-(3-oxobutyl)phenoxy)pyridin-3-amine (6i). Method 2

Starting with **5i (**436 mg) reduced by catalytic hydrogenation to furnish **6i** as a gray solid (167 mg, 43%), mp: 142–144 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.42 (1H, br., NH), 7.40 (2H, d, *J* = 8.8 Hz, ArH-3′, 5′), 7.35 (2H, d, *J* = 8.8 Hz, ArH-2′, 6′), 7.14-7.19 (3H, m, ArH and PyH-4), 6.54 (1H, d, *J* = 8.0 Hz, PyH-5), 4.92 (2H, br, NH_2), 4.85 (4H, m, CH_2 × 2); 2.14 (3H, s, $COCH_3$) ppm; MS *m/z* (%): 407.4 (M − 1, 70), 360.3 (100); HPLC purity 95.20%.

N^2-(4′-Cyanophenyl)amino-6-(2”,6”-difluoro-4”-propylcarbmethoxy)phenoxypyr idin-3-amine (6j). Method 2

Starting with **5j** (452 mg) reduced by catalytic hydrogenation to produce **6j** as a gray solid (158 mg, 40%), mp: 203–205 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.39 (1H, br., NH), 7.35 (4H, m, ArH-2′, 3′, 5′,6′), 7.22 (2H, $J_{F\text{-}H}$ = 8.8 Hz, ArH-3”,5”), 7.14 (1H, d, *J* = 8.0 Hz, PyH-4), 6.54 (1H, d, *J* = 8.0 Hz, PyH-5), 4.90 (2H, br s, NH_2), 3.60 (3H, s, OCH_3), 2.93 and

2.72 (each 2H, t, *J* = 7.6 Hz, CH_2) ppm; MS *m/z* (%): 423.3 (M − 1, 40), 376.3 (100); HPLC purity 97.38%.

(*E*)-*N*^{2}-(4′-Cyanophenyl)amino-6-(2”,6”-difluoro-4”-(2-carbamoyl)vinyl)phenoxy pyridin-3-amine (6k). Method 1

Starting with crude **5k** (452 mg, 1 mmol) reduced by $Na_2S_2O_4$ to furnish **6k** as an off-white solid (123 mg, 32%), mp: 239–241 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.43 (1H, br., NH), 7.56 (3H, m, ArH-3”,5” and NH), 7.48 (1H, d, *J* = 16.0 Hz, ArCH=), 7.32 (4H, m, ArH- 2′,3′,5′, 6′), 7.22 (1H, br, NH), 7.16 (1H, d, *J* = 8.0 Hz, PyH-4), 6.71 (1H, d, *J* = 16.0 Hz, =CHCN), 6.58 (1H, d, *J* = 8.0 Hz, PyH-5), 4.95 (2H, br, NH_2) ppm; MS *m/z* (%): 406.2 (M − 1, 35), 386.2 (100); HPLC purity 99.84%.

6-(4”-(2-Cyanoethyl)-2”,6”-difluoro)phenoxy-*N*^{2}-(4′-cyanophenyl)aminopyridin-3-amine (6l). Method 2

Starting with **5g** (396 mg,0.94 mmol) reduced by catalytic hydrogenation to afford **6l** as an off-white solid (198 mg, 52%), mp: 202–204 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.39 (1H, br., NH), 7.38 (2H, d, *J* = 8.8 Hz, ArH- 3′, 5′), 7.36 (2H, d, *J* = 8.8 Hz, ArH-2′, 6′), 7.30 (2H, $J_{F\text{-}H}$ = 8.4 Hz, ArH-3”,5”), 7.15 (1H, d, *J* = 8.4 Hz, PyH-4), 6.55 (1H, d, *J* = 8.0 Hz, PyH-5), 4.91 (2H, br s, NH_2), 2.96 and 2.89 (each 2H, m, CH_2) ppm; MS *m/z* (%): 390.1 (M − 1, 65), 370.0 (100); HPLC purity 95.25%.

N^2-(4′-Cyanophenyl)amino-6-(4”-(2-cyano)propylcarbamoyl-2”,6”-difluoro)phenoxypyridin-3-amine (6m). Method 2

Starting with **5h** (462 mg) reduced by catalytic hydrogenation to produce **6m** as a gray solid (233 mg, 60%), mp: 222-224 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.40 (1H, br., NH), 7.83 and 7.59 (each 1H, br, $CONH_2$), 7.42 (2H, d, *J* = 8.8 Hz, ArH-3′, 5′), 7.36 (2H, d, *J* = 8.8 Hz, ArH-2′, 6′), 7.28 (2H, d, $J_{F\text{-}H}$ = 9.2 Hz, ArH-3”,5”), 7.15 (1H, d, *J* = 8.0 Hz, PyH-4), 6.56 (1H, d, *J* = 8.0 Hz, PyH-5), 4.95 (2H, br s, NH_2), 4.08 (1H, t, *J* = 6.4 Hz, CH), 3.24 (2H, m, CH_2) ppm; MS *m/z* (%): 433.5 (M − 1, 10), 343.1 (100); HPLC purity 99.84%.

N^2-(4′-Cyanophenyl)amino-6-(2”,6”-difluoro-4”-(3-hydroxypropyl)phenoxy) pyridin-3-amine (6n)

The crude **6j** prepared from **5j** (452 mg, 1 mmol) was further reduced by $LiBH_4$ (280 mg, 10 mmol) in ice-bath with stirring for 30 min. The mixture was poured into ice-water, adjusted pH to 4–5 with aq HCl, extracted with EtOAc three times, and dried over Na_2SO_4. After removal of solvent, the residue was purified by a silica gel flash column (gradual elution: MeOH/CH_2Cl_2, 0-1%) to give **6n** as a light gray solid (264 mg, 67%), mp: 160-162 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.40 (1H, br. s, NH), 7.36 (4H, s, ArH-2′, 3′, 5′,6′), 7.15 (3H, m, ArH-3”,5” and PyH-4), 6.54 (1H, d, *J* = 8.4 Hz, PyH-4), 6.54 (1H, d, *J* = 8.0 Hz, PyH-5), 4.90 (2H, br s, NH_2), 4.52 (1H, br s, OH), 3.43 (2H, t, *J* = 7.6 Hz, OCH_2), 2.70 (2H, t, *J* = 7.6 Hz, $ArCH_2$), 1.76 (2H, m, CH_2) ppm; MS *m/z* (%): 395 (M − 1, 100); HPLC purity 96.78%.

6-(4”-Cyano-2”,6”-dibromo)phenoxy-*N*^{2}-(4′-cyanophenyl)pyridin-2,3-diamine (8a). Method 1

Starting with **7a** (515 mg, 1 mmol) reduced by $Na_2S_2O_4$ to afford **8a** as an off-white solid (309 mg, 69%), mp: 218–220 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.46 (2H, s, ArH-3”, 5”), 8.40 (1H, br. s, NH), 7.38 (2H, d, *J* = 8.8 Hz, ArH- 3′,5′), 7.26 (2H, d, *J* = 8.8 Hz, ArH-2′,6′), 7.18 (1H, d, *J* = 8.4 Hz, PyH-4), 6.59 (1H, d, *J* = 8.4 Hz, PyH-5), 4.94 (2H, s, NH_2) ppm; MS *m/z* (%): 481.6 (M − 1, .40), 483.7 (M + 1, 100); HPLC purity 99.85%.

(*E*)-*N*^{2}-(4′-Cyanophenyl)amino-6-(4”-cyanovinyl-2”,6”-dibromo)phenoxypyridin-3-diamine (8b). Method 1

Starting with **7b** (500 mg, 0.92 mmol) reduced by $Na_2S_2O_4$ to produce **8b** as a white solid (200 mg, 43%), mp: 231–233 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.39 (1H, br. s, NH), 8.15 (2H, s, ArH-3”,5”), 7.70 (1H, d, *J* = 16.8 Hz, ArCH=), 7.30 (4H, br s, ArH-2′,3′,5′,6′), 7.16 (1H, d, *J* = 8.0 Hz, PyH-4), 6.71 (1H, d, *J* = 16.8 Hz, =CHCN), 6.56 (1H, d, *J* = 8.0 Hz, PyH-5), 4.90 (2H, s, NH_2) ppm; MS *m/z* (%): 510.3 (M + 1, 100), 512.2 (M + 3, 50); HPLC purity 97.11%.

(*E*)-6-(2”-Bromo-4”-cyanovinyl-6”-methoxy)phenoxy-*N*^{2}-(4′-cyanophenyl) pyridin-2,3-diamine (8c). Method 1

Starting with **7c** (492 mg) reduced by $Na_2S_2O_4$ to afford **8c** as a white solid (285 mg, 61%), 219–220 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.35 (1H, s, NH), 7.69 (1H, d, *J* = 16.4 Hz, ArCH=), 7.65 (1H, s, ArH-3”), 7.55 (1H, s, ArH-5”), 7.31 (4H, br s, ArH-2′, 3′, 5′, 6′), 7.13 (1H, d, *J* = 8.8 Hz, PyH-4), 6.68 (1H, d, *J* = 16.4 Hz, =CHCN), 6.48 (1H, d, *J* = 8.8 Hz, PyH-5), 4.83 (2H, s, NH_2), 3.75 (3H, s, OCH_3) ppm; MS *m/z* (%): 460.3 (M − 1, 100), 462.1 (M + 1, 60); HPLC purity 95.07%.

(*E*)-*N*^{2}-(4′-Cyanophenyl)amino-6-(4”-cyanovinyl-2”,6”-dichloro)phenoxypyridin-3-amine (8d). Method 1

Starting with **7d** (452 mg) reduced by$Na_2S_2O_4$ to produce **8d** as a white solid (219 mg, 52%), mp: 216–218 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.39 (1H, br. s, NH), 8.00 (2H, s, ArH-3”,5”), 7.71 (1H, d, *J* = 16.8 Hz, ArCH=), 7.30 (2H, d, *J* = 8.8 Hz, ArH-3′,5′), 7.27 (2H, d, *J* = 8.8 Hz, ArH-2′,6′), 7.17 (1H, d, *J* = 8.4 Hz, PyH-4), 6.72 (1H, d, *J* = 16.8 Hz, =CHCN), 6.58 (1H, d, *J* = 8.4 Hz, PyH-5), 4.91 (2H, s, NH_2) ppm; MS *m/z* (%): 420.0 (M − 1, 60), 422.3 (M + 1, 100); HPLC purity 96.11%.

(*E*)-*N*^{2}-(4′-Cyanophenyl)amino-6-(2”-methoxy-4”-cyanovinyl)phenoxypyridin-3-amine (8e). Method 1

Starting with **7e** (413 mg) reduced by $Na_2S_2O_4$ to furnish **8e** as a white solid (198 mg, 52%), 82–84 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=8.35 (1H, s, NH), 7.69 (1H, d, *J* = 16.4 Hz, ArCH=), 7.53 (1H, s, ArH-3”), 7.35 (4H, m, ArH-2′, 3′, 5′, 6′), 7.28 (1H, *J* = 8.8 Hz, ArH-5”), 7.13 (1H, d, *J* = 8.8 Hz, PyH-4), 7.10 (1H, *J* = 8.8 Hz, ArH-6”), 6.56 (1H, d, *J* = 16.4 Hz, =CHCN), 6.43 (1H, d, *J* = 8.8 Hz, PyH-5), 4.86 (2H, s, NH_2), 3.74 (3H, s, OCH_3) ppm; MS *m/z* (%): 382.2 (M − 1, 100); HPLC purity 96.24%.

Bioassays

Assay for measuring the inhibitory activity of compounds on HIV-1 infection of TZM-bl 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, 800 TCID50 of virus (NL4-3 or drug resistant variants) was used to infect TZM-bl cells in the presence of various concentrations of compounds. One day after infection, the culture medium was removed from each well, and 100 μL of Bright Glo reagent (Promega, San Luis Obispo, CA) was added to the cells for measurement of luminescence using a Victor 2 luminometer. The effective concentration (EC_{50}) against HIV-1 strains was defined as the concentration that caused a 50% reduction of luciferase activity (Relative Light Units) compared to virus control wells.

HIV-1 infection assay using MT-2 cells

Inhibitory activity of compounds against infection by HIV-1 IIIB and its variant A17, which is resistant to multiple NNRTIs, was determined as previously described.[23] Briefly, MT-2 cells (10^4 well) were infected with an HIV-1 strain (100 $TCID_{50}$) in 200 μL of 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.[23] The effective concentrations for 50% inhibition (EC50) were calculated using a computer program CalcuSyn.[24]

Cytotoxicity assay

A CytoTox-Glo™ cytotoxicity assay (Promega) was used to determine the cytotoxicity of the synthesized compounds. Parallel to the anti-viral assays, TZM-bl cells were cultured in the presence of various concentrations of the compounds for one day. Percent of viable cells was determined by following the protocol provided by the manufacturer. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration that caused a 50% reduction of cell viability.

Aqueous solubility measurement

Solubility was measured separately at pH 7.4 and pH 2.0 by using an HPLC-UV method. Test compounds were initially dissolved in DMSO at 10 mg/mL. Ten microliters of this stock solution was spiked into either pH 7.4 phosphate buffer (1.0 mL) or 0.01 M HCl (approximately pH 2.0, 1 mL) with the final DMSO concentration being 1%. The mixture was stirred for 4 h at rt, and then concentrated at 3000 rpm for 10 min. The saturated supernatants were transferred to other vials for analysis by HPLC-UV. Each sample was performed in triplicate. For quantification, a model 1200 HPLC-UV (Agilent) system was used with an Agilent TC-C18 column (250 × 4.6 mm, 5 μm) and gradient elution of

acetonitrile (ACN) in water, starting with 0% of ACN, which was linearly increased up to 70% over 10 min, then slowly increased up to 98% over 15 min. The flow rate was 1.0 mL/min and injection volume was 15 μL. Aqueous concentration was determined by comparison of the peak area of the saturated solution with a standard curve plotted peak area versus known concentrations, which were prepared by solutions of test compound in ACN at 50 μg/mL, 12.4 μg/mL, 3.125 μg/mL, 0.781μg/mL, and 0.195 μg/mL,

Log P measurement

Using the above DMSO stock solution (1 mg/mL), 20 μL of this solution were added into *n*-octane (1 mL) and water (1 mL). The mixture was stirred at room temperature for 24 h and left to sit overnight. Each solution (~0.5 mL) was transferred from two phases, respectively, into other vials for HPLC analysis. The instrument and conditions were the same as those for solubility determination. The log P was calculated by the peak area ratios in *n*-octane and in water.

Determination of predictive physicochemical properties.[20]

Ligand efficiency (LE) values were calculated by normalizing binding free energy of a ligand for the number of heavy atoms, as shown in the formula $-\Delta G/HA_{(\text{non-hydrogen atom})}$. Free binding energy calculation was carried out as $\Delta G = -RT \ln K_d$, presuming $EC_{50} \approx K_d$, a temperature of 310K, and given in kcal per heavy atom (non-hydrogen atom). The pEC_{50} values, negative logarithm of the molar effective concentration of compound that causes 50% inhibition of wild-type virus, were converted from experimental data. Lipophilic ligand efficiency (LLE) was calculated by the formula pEC_{50} − clog D, in which clog D values were calculated using ACD software. LELP was defined as the ratio of clog D and LE. The topological polar surface area (tPSA) was calculated by using ChemDraw Ultra 12.0.

Microsomal stability assay

Stock solutions of testing compounds (1 mg/mL) were prepared by dissolving the pure compound in DMSO and storing at 4 °C. Before the assay, the stock solution was diluted with acetonitrile (ACN) to 0.1 mM concentration. For measurement of metabolic stability, all 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 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 the 0 min time point were prepared by adding 600 μL ice-cold ACN first, followed by 60 μL NADPH. Incubation of all samples was 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 propranolol; 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 log

percentage 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 CLint (in units of ml/min/mg protein) was calculated by the following formula[15]: CL_{int} = (0.693/in vitro $t_{1/2}$) × (ml incubation/mg microsomes). HPLC-MS analysis was carried out on a Shimadzu LCMS-2010 with an electrospray ionization source (ESI). An Alltima C18 column (5 μm, 150 mm × 2.1 mm) was used for HPLC with a gradient elution at a flow rate of 0.3 mL/min. The elution condition was ACN (B) in water (A) at 30% for 0-2 min, 85% for 2–6 min, 100% for 6–9 min, and 30% for 9–12 min. The MS conditions were optimized to detector voltage +1.6 kV, acquisition mode selected ion monitoring (SIM) of the appropriate molecular weights of the testing compounds. The CDL and heat block temperature was 200 °C, and neutralizing gas flow was 1.5 L/min. Samples were injected by auto-sampler. Electrospray ionization was operated in the positive and negative mode.

Acknowledgments

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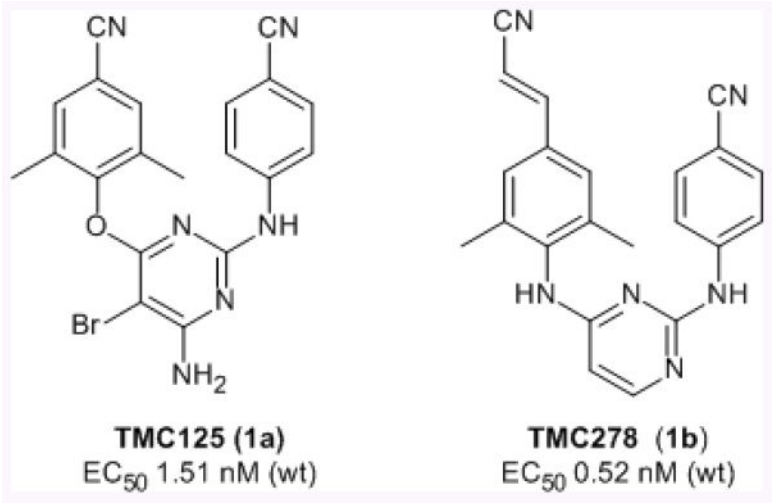


Figure 1.
Next-generation NNRTI drugs TMC125 and TMC278.

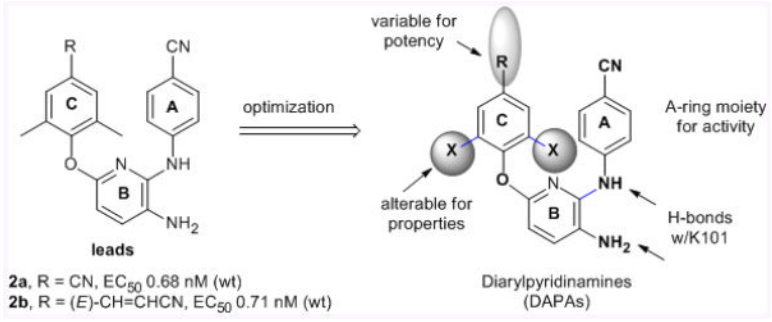
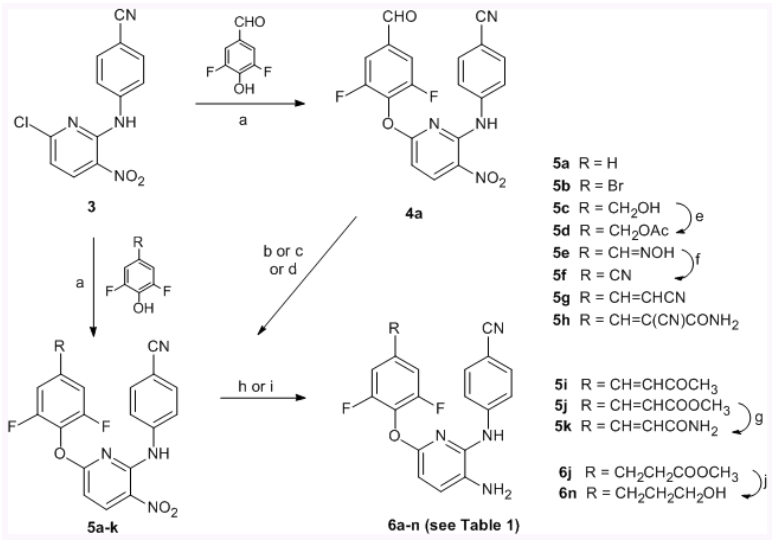
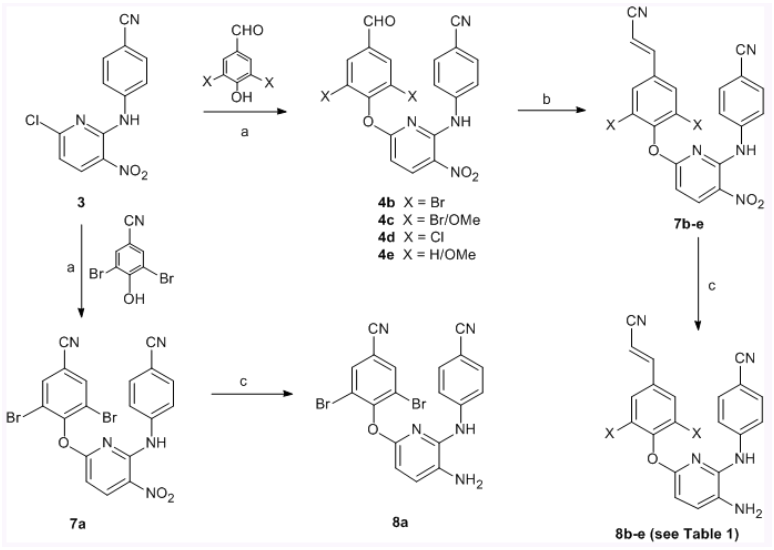


Figure 2.
Leads, new DAPAs, and optimization strategy



Scheme 1.

a) Cs_2CO_3 or K_2CO_3 in DMF, 90–100 °C, 10–15 min, microwave; b) $NaBH_4$, THF/CH_3OH, rt, 30 min for **5c**; c) $NH_2OH{\cdot}HCl$, rt, 2 h for **5e**; d) $(EtO)_2P(O)CH_2CN$, *t*-BuOK, THF, 0 °C–rt, 1 h, for **5g**; or $CNCH_2CONH_2$, $ZnCl_2$, 100 °C, microwave, 10 min, for **5h**; or CH_3COCH_3, NaOH, 2 h, rt, for **5i**; or $Ph_3PCHCOOCH_3$, $CHCl_3$, reflux, overnight, for **5j**; e) CH_3COCl, Et_3N, THF, rt, 30 min; f) Ac_2O, reflux, overnight, for **5f**; g) 1. aq NaOH, rt, 36 h; 2. CDI, NH_3H_2O, rt, 2 h; h) $Na_2S_2O_4$, NH_3 H_2O, THF/H_2O (v/v 1:1), rt, 3 h; i) Pd/C H_2, EtOH, rt, 6 h, 65–78%; j) $LiBH_4$, THF, CH_3OH, 0 °C–rt, 78%.

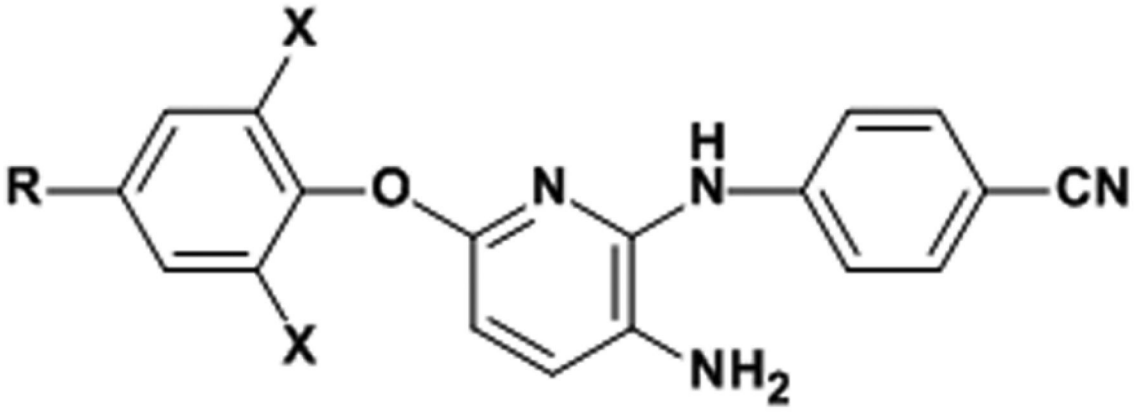


Scheme 2.

a) Cs_2CO_3 or K_2CO_3 in DMF, 90–100 °C, 10–5 min, microwave; b) *t*-BuOK, $(EtO)_2P(O)CH_2CN$, THF, 0 °C–rt, 1 h; c) $Na_2S\ O_4$, $NH_3\ H_2O$, THF/H_2O (v/v 1:1), rt, 3 h.

Table 1

Anti-HIV activity of new DAPA series **6** and **8** analogues



	Structures		NL4-3 infection of TZM-bl cells[a]		
	X	**R**	**EC_{50}[b] (nM ±SD)**	**CC_{50}[c] (μM)**	**SI[d]**
6a	F	H	6.78 ± 1.18	19.2	2,832
6b	F	Br	7.43 ± 1.1	>9.59	>1291
6c	F	CH_2OH	57.1 ± 9.2	>27.2	>476
6d	F	CH_2OAc	290 ± 46	1.58	544
6e	F	CH=NOH	261 ± 55.1	>26.2	>100
6f	F	CN	5.16 ± 1.57	>9.39	>1820
6g	F	CH=CHCN	0.75 ± 0.18	11.0	14,667
6h	F	$CH=C(CN)CONH_2$	370 ± 59	15.8	43
6i	F	$CH_2CH_2COCH_3$	4.66 ± 0.95	>9.8	>2103
6j	F	$CH_2CH_2COOCH_3$	99 ± 19	15.3	155
6k	F	$CH=CHCONH_2$	614 ± 118	>24.6	>40
6l	F	CH_2CH_2CN	2.81 ± 0.59	>10.2	>3630
6m	F	$CH_2CH(CN)CONH_2$	350 ± 52	15.1	43
6n	F	$CH_2CH_2CH_2OH$	47.98 ± 9.8	>25.2	>525
8a	Br	CN	0.93 ± 0.27	13.2	13,333
8b	Br	CH=CHCN	0.33 ± 0.09	8.22	24,909
8c	Br/OMe	CH=CHCN	3.25 ± 0.89	21.0	6462
8d	Cl	CH=CHCN	8.14 ± 2.49	10.7	1310
8e	H/OMe	CH=CHCN	16.7 ± 4.33	>20.6	>1234
2a	Me	CN	0.68 ± 0.03	8.98	13,206
2b	Me	CH=CHCN	0.71 ± 0.58	9.75	13,732
1b [e]			0.44 ± 0.11	19.4	44,091

[a] Against HIV-1 NL3-4 (wild-type) virus in TZM-bl cell lines.

[b] Concentration of compound that causes 50% inhibition of viral infection, presented as mean ± standard deviation (SD) from at least three independent experiments.

[c] A CytoTox-Glo™ cytotoxicity assay (Promega) was used to determine CC_{50} value that caused cytotoxicity to 50% of cells. CC_{50} values in the Table were averaged from two independent tests.

[d] SI (Selectivity Index) is ratio of CC_{50}/EC_{50}.

[e] Reference drug used for comparison.

Table 2

Antiviral activity of new DAPAs against mutated viral strains[[a]]

	EC_{50} (nM) (Resistance fold change)					
	TZM-bl cells				MT-2 cells	
	NL4-3(wt)	RTMDR[[b]]	K101E[[c]]	E138K[[c]]	IIIB (wt)	A17[[d]]
6a	6.78	3.55 (0.52)	53 (7.82)	>29 (4.3)	—	—
6g	0.75	34.7 (46.3)	7.20 (9.6)	7.71 (10.3)	1.11	>500 (>450)
8a	0.93	2.47 (2.6)	3.09 (3.3)	5.44 (5.8)	12.45	384 (30.8)
8b	0.33	0.74 (2.2)	7.63 (23.1)	4.11 (12.4)	3.04	199.8 (65.7)
8c	3.25	3.46 (1.1)	3.90 (1.2)	6.93 (2.1)	4.53	84.4 (18.6)
1b	0.44	0.68 (1.5)	5.74 (13.0)	5.19 (11.8)	0.49	9.03 (18.4)

[a] Each compound was tested in at least three independent experiments.

[b] 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.

[c] Mutated amino acids in NL4-3 NNRTI binding pocket confer resistance to **1b**.

[d] Multi-NNRTI-resistant strain A17 from NIH with mutations at amino acids K103N and Y181C in the viral RT.

Table 3

Drug-like properties and parameters of selected potent DAPAs

	HLM[[a]]	Aqueous solubility(μg/mL)[[b]]		log P[[c]]	clogD[[d]]	Lipophilic Efficiency Indices			tPSA[[h]]
	$t_{1/2}$ min	pH 2.0	pH 7.4			LE[[e]]	LLE [[f]]	LELP[[g]]	
6a	44.01	8.42	1.58	>5	3.56	0.45	4.61	7.95	83.43
6g	76.46	1.27	0.72	3.48	3.81	0.43	5.31	8.84	107.22
8a	41.66	0.79	0.72	3.30	4.13	0.46	4.87	9.04	107.22
8b	21.87	0.63	0.62	3.55	5.15	0.45	4.33	11.50	107.22
8c	54.71	0.82	0.50	3.31	3.29	0.39	5.20	8.49	116.45
8d	39.61	0.81	0.70	>5	4.39	0.38	3.70	11.49	107.22
2b	62.69	1.04	0.09	3.56	4.52	0.43	4.63	10.46	107.22
1b	39.52	86.8	0.24	>5	3.62	0.45	5.66	7.87	96.36

[a] Human liver microsome incubation assay data from at least two experiments in parallel with propranolol as a reference.

[b] Measured by HPLC method in triplicate.

[c] The log P values were measured at pH7.4 by HPLC method in triplicate.

[d] Predicted by using ACD software.

[e] LE values were calculated by the formula $-\Delta G/HA_{(\text{non-hydrogen atom})}$, in which normalizing binding energy $\Delta G = -RT \ln K_d$, presuming $EC_{50} \approx K_d$, HA is Heavy atoms (non-hydrogen atom) count in a compound.

[f] Calculated by formula pEC_{50} − clog D The pEC50 values, negative logarithm of the molar effective concentration of compound that causes 50% inhibition of wild-type virus, were converted from experimental data.

[g] Defined as ratio of clog D and LE.

[h] Topological polar surface area was predicted by using Chemdraw Ultra 12.0.