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Synthesis and biological evaluation of purine derivatives incorporating metal chelating ligands as HIV integrase inhibitors

Xingnan Li and Robert Vince*

Center for Drug Design, Academic Health Center, and Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, 8-123A WDH, 308 Harvard St SE, Minneapolis, MN 55455, USA

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Abstract—Because of its essential role in HIV replication and lack of human counterpart, HIV integrase is an attractive target for the development of novel anti-AIDS agents. Among the recently developed integrase inhibitors, only the α,γ-diketo acid (DKA) compounds were biologically validated as potent and selective integrase inhibitors. The general structure of DKAs contains a diketo acid moiety as the Mg^{2+} chelating pharmacophore, and an adjacent aryl group to provide selectivity. Numerous structure–activity relationship (SAR) studies on DKAs have been conducted, which generally involved substituting the carboxylate group or the aryl group. Our objective was to investigate the SARs of the DKA molecule by incorporating a purine ring in the aryl moiety and replacing the labile diketo acid moiety with other divalent metal (Me^{2+}) chelating ligands. A series of amide substituted purine derivatives were synthesized via palladium-catalyzed amidation reactions, and their biological activities against HIV integrase were evaluated. These purine derivatives showed anti-integrase activity at low micromolar range. The biological results indicated that the type of Me^{2+} ligands, two-point ligand picolinamide or three-point ligand 8-hydroxy-quinoline-7-carboxamide, affected inhibitory potency depending on the substitution position of the *para*-fluorobenzyl group. The C_6-,C_8-dipicolinamide substituted purine (**32**) exhibited the best potency among this series.

1. Introduction

The acquired immunodeficiency syndrome (AIDS) has become a worldwide pandemic since its first diagnosis in the early 1980s. It has claimed the lives of more than 25 million people globally and over 40 million people are still living with HIV.[1] Currently, a standard treatment strategy for AIDS is referred to as the highly active antiretroviral therapy (HAART), which is a combination of three or more anti-HIV drugs inhibiting two viral enzymes (i.e., reverse transcriptase and protease) and virus fusion. Although HAART regimens have greatly improved the life span and life quality of AIDS patients,[2] their application and effectiveness is hampered by related toxicity issues and the ever-increasing multi-drug resistance of HIV.[3,4] New anti-HIV agents with alternative target sites, therefore, are greatly desired either as replacements or as supplements to the current HAART regimens.

HIV integrase has recently attracted attention as a promising anti-HIV target because of its necessity in viral replication and its lack of human counterpart (see reviews[5–7]). HIV integrase catalyzes the insertion of viral DNA into host genome, a critical step for viral maturation that includes a 3′-processing step and a subsequent strand transfer step.[5] Recent studies using recombinant integrase and high throughput screening assays have led to the identification of the α,γ-diketo acid (DKA) type of compounds as potent and selective HIV integrase inhibitors (Fig. 1A, **1** and **2**).[8,9] DKAs compete with host DNA for the binding sites on HIV integrase, thus selectively inhibiting the strand transfer process.[8] This inhibition is dependent on the presence of divalent magnesium ion, or manganese ion in in vitro assays.[10] It is believed that the α,γ-diketo acid moiety chelates with two divalent metal ions on the active site of the integrase to form a tertiary ligand–Me^{2+}–integrase complex, thus blocking substrate DNA binding.[10]

The general structure of DKAs contains an α,γ-diketo acid moiety as the key pharmacophore,[10] and an adjacent aryl group providing efficacy and selectivity[11–13] (Fig. 1B). Numerous structure–activity relationship (SAR) studies on DKAs have been conducted to search

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* Corresponding author. Tel.: +1 612 624 9911; fax: +1 612 624 0139; e-mail: vince001@umn.edu

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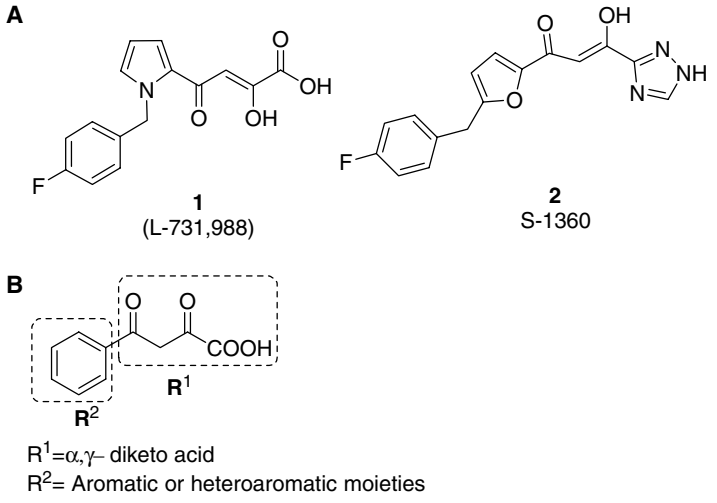


Figure 1. (A) Representative α,γ-diketo acid compounds (**1** and **2**) as selective HIV integrase inhibitors, (B) general structure of α,γ-diketo acid (DKA).

for a clinical candidate. Many of the reported SARs focused on substituting the aryl group with aromatic[12] or heteroaromatic groups.[14–18] Among these heterocycles, pyrimidine scaffolds were used to mimic the nucleobases, which have led to significant increases in anti-integrase activity.[15,19]

Because the α,γ-diketo acid group is biologically labile, we were interested in replacing it with alternative, more stable, metal chelating ligands. In this study, we describe the synthesis and biological evaluation of new DKA analogs with purine scaffolds.[20] The purine core was used to mimic the nucleobases and it was substituted with a *para*-fluorobenzyl group to improve potency.[11,12] Two types of metal chelating ligands were selected to replace the α,γ-diketo acid moiety, including the two-point ligand picolinamide (Fig. 2, a), which possesses a moderately hard amide O- and a softer pyridine N-binding site that can form a bidentate ligand–Me^{2+} complex with many divalent metal ions,[21,22] and the three-point ligand 8-hydroxy-quinoline-7-carboxamide which is known as a strong Mg^{2+} chelating ligand and mimics the diketo acid group[23] (Fig. 2, b). The spatial relationships of the purine substituents were also investigated to look for the optimal geometry of the inhibitor molecule, and the chelating ligands were substituted either at 8- (**4**)

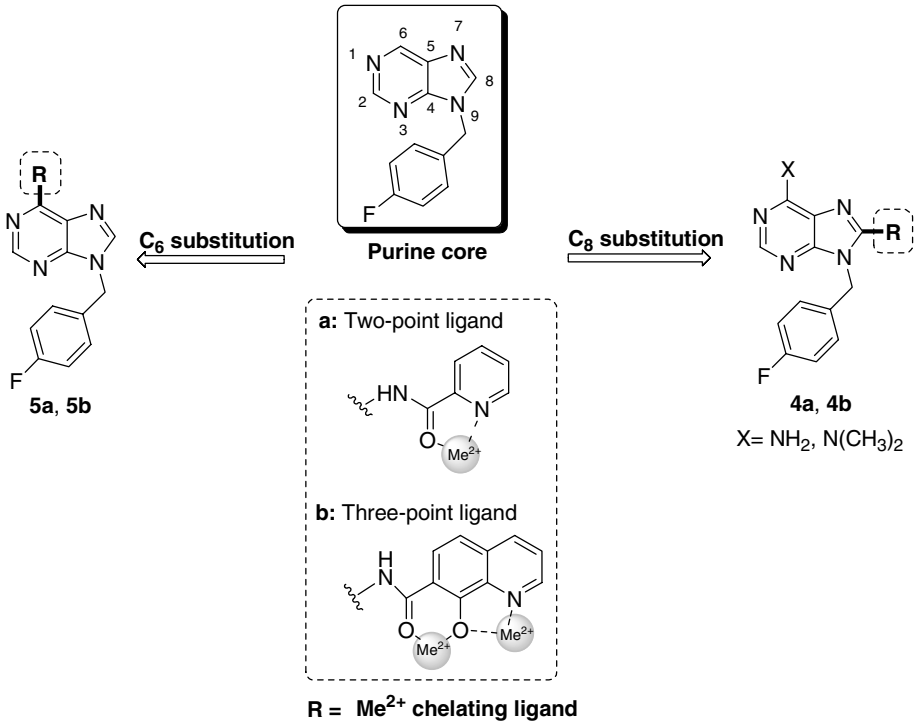


Figure 2. Design of purine derivatives incorporating metal chelating ligands as HIV integrase inhibitors.

or 6- (**5**) (Fig. 2) positions on the purine ring. Based on these considerations, a new series of DKA analogs were synthesized.

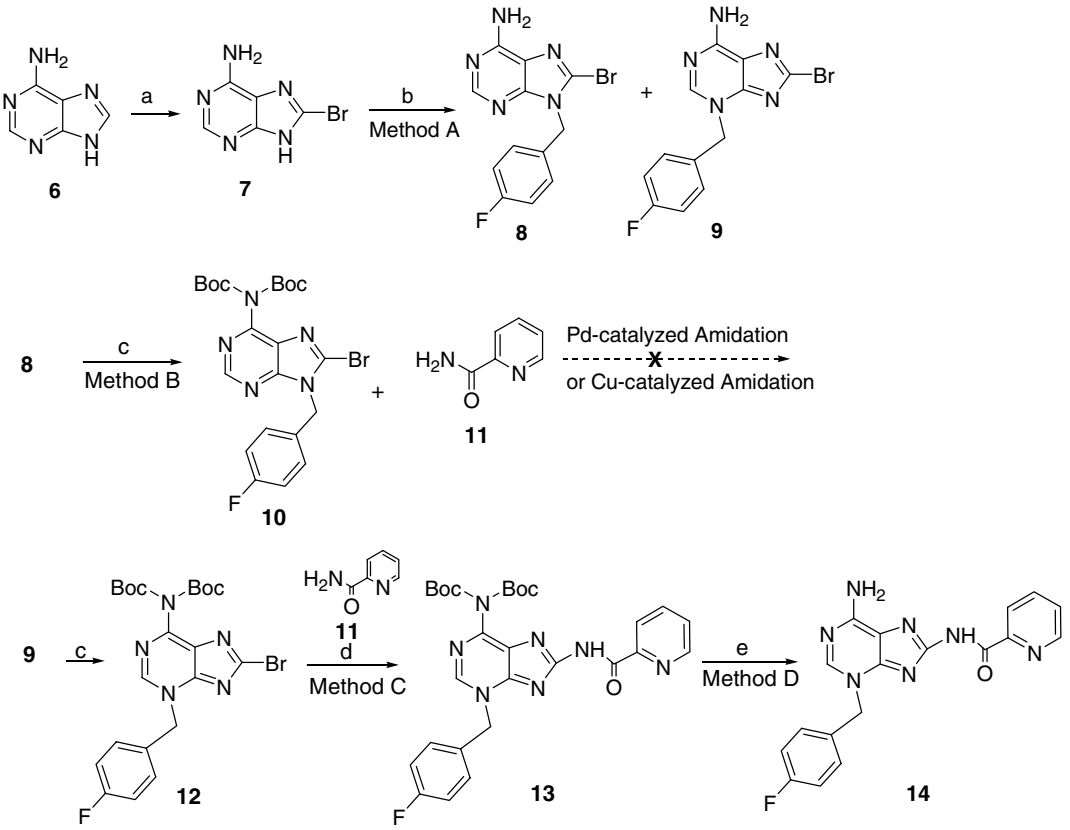
2. Results and discussion

2.1. Chemistry

Palladium-catalyzed amination methodologies for the formation of C–N bonds have been successfully applied to the synthesis of nucleosides derivatives.[24,25] More recently, amides were used as coupling substrates in the Pd-catalyzed amidations with 6-halopurines.[26] In this study, we applied this methodology for the synthesis of the target C_8- (**4**) and C_6- (**5**) amide substituted purines.

The C_8-amide substituted purine derivative **4** was synthesized from adenine **6**, which was first converted to the amidation substrate 8-bromopurine **8** by bromination[27] and subsequent alkylation with *para*-fluorobenzyl bromide (Scheme 1). Two products were isolated from the alkylation step in a 1 (**8**) to 1.3 (**9**) ratio, and they were determined as N_9- (**8**) and N_3- (**9**) benzylated purines by ^{13}C and HMBC NMR analysis (Fig. 3A). The N_9-benzylated 8-bromo adenine **8** was further double Boc protected (**10**) to improve solubility and to avoid the competition between the amino and amide nitrogens in the next amidation step. Our first attempt to couple compound **10** with picolinamide **11** under the reported amidation conditions for 6-halo or 6-arylsulfonate nucleoside substrates[25,26] using palladium catalyst was unsuccessful. Subsequent attempts using Cu-catalyzed amidation[28] or other palladium ligand[29] still could not give any products. Interestingly, changing the substrate from N_9-benzylated **10** to N_3-benzylated 8-bromopurine **12** readily afforded the amidation product **13** under Pd-catalyzed conditions. The Boc groups were subsequently removed in trifluoroacetic acid (TFA) to give the 6-amino compound **14** (Scheme 1). The difference in amidation reactivity of N_9- (**10**) and N_3- (**12**) benzylated purines could be due to the steric hindrance brought by the benzyl group.

The N_3-benzylated 8-bromopurine **12** was next coupled with the three-point ligand 8-hydroxy-quinoline-7-carboxamide **17**. The amide **17** was prepared from the carboxylic acid **15** via esterification[30] and subsequent aminolysis (Scheme 2). Under previous Pd-catalyzed conditions, however, amide **17** did not react with compound **12**. We surmised that the palladium catalyst may lose activity by chelating to the 8-hydroxy-quinoline moiety (**17b**). Therefore, the phenol group in compound **15** was protected as a benzyl ether via ester **18** (Scheme 2), and the resulting amide **19** readily reacted with compound **12** under previous conditions to give the coupled product **21** (Scheme 3). Subsequent removal of the Boc groups of **21** in TFA afforded not only 50% of de-Boc product **22**, but also 30% of de-Boc and



Scheme 1. Reagents and conditions: (a) Br_2, rt, overnight; (b) NaH, DMF, 4-fluorobenzyl bromide, 70 °C, 2 h; (c) $(Boc)_2O$, DMAP, Et_3N, DMF, rt, 2 h; (d) $Pd_2dba_3{\cdot}CHCl_3$, dppf, Cs_2CO_3, toluene, 90 °C, 4 h; (e) TFA, DCM, rt, overnight.

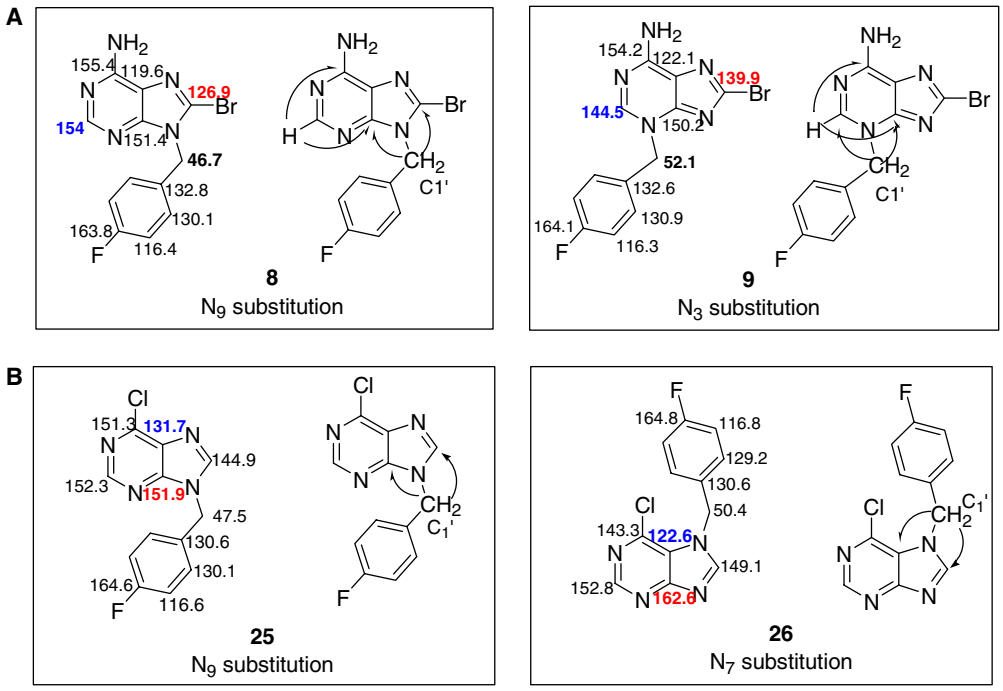
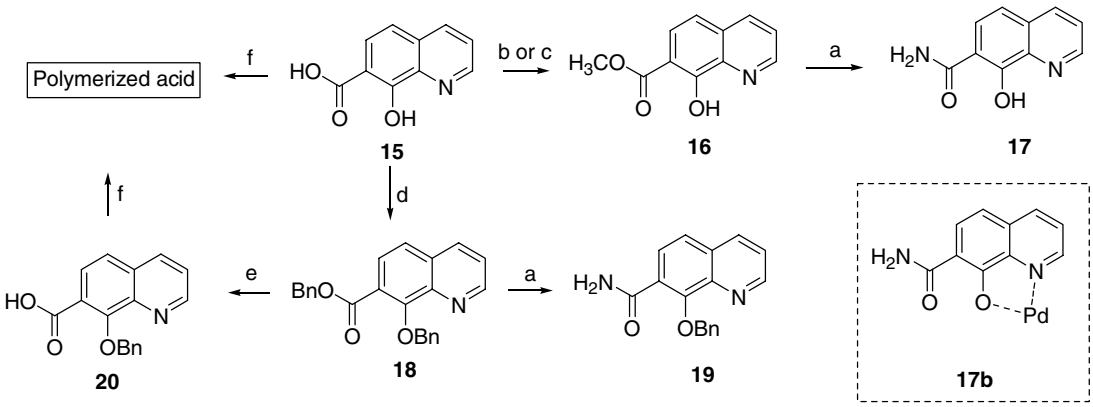


Figure 3. ^{13}C and HMBC correlation between C_1' proton and purine carbons of (A) N_3/N_9-benzylated 8-bromo adenine, (B) N_9/N_7-benzylated 6-chloropurine.

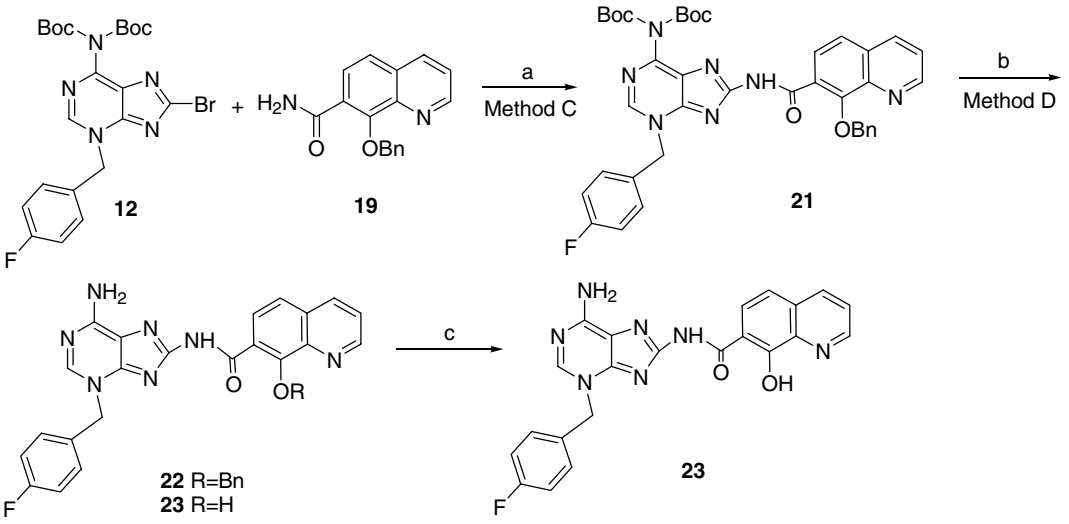


Scheme 2. Reagents and conditions: (a) liquid NH_3, bomb, 70 °C, overnight; (b) H_2SO_4, MeOH, reflux, 5%; (c) $HClSO_3$, MeOH, reflux, 10%; (d) BnBr, K_2CO_3, acetone/DMF, reflux, overnight; (e) 1 N NaOH, MeOH, H_2O, rt; (f) $SOCl_2$, heating 1 h.

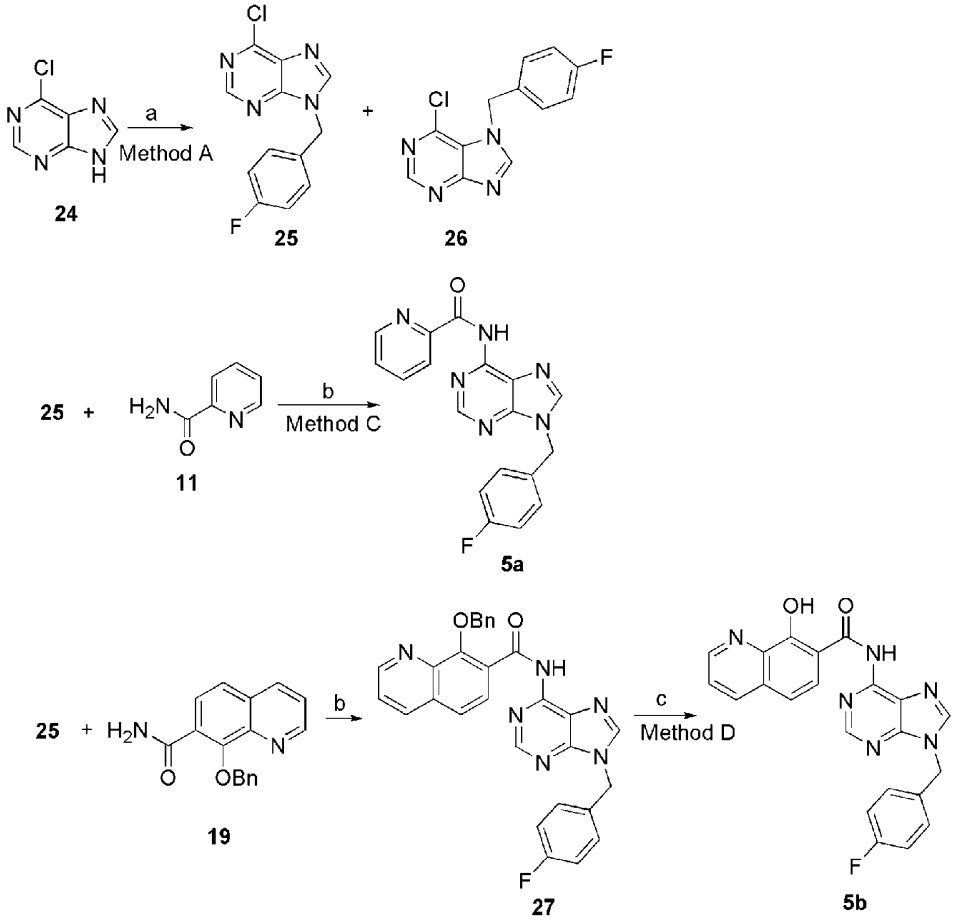
de-benzyl product **23**. Further debenzylation of compound **22** by catalytic hydrogenation only led to the reduced quinoline, therefore the benzyl group was removed in TFA to afford the final product **23** (Scheme 3).

Using the same Pd-catalyzed amidation conditions, the C_6-amide substituted purine derivatives **5a**,**b** were successfully synthesized (Scheme 4). 6-Chloropurine **24** was alkylated with *para*-fluorobenzyl bromide to give N_9- (**25**) and N_7- (**26**) alkylated purines in a 3:1 ratio (Fig. 3B). Subsequent Pd-catalyzed amidation of N_9-benzylated 6-chloropurine **25** with amide **11** and **19** afforded the corresponding coupled products **5a** and **27**. The *O*-benzyl group in compound **27** was removed in TFA to give the target compound **5b**. Amidation of the N_7-benzylated 6-chloropurine **26** with amide **11** under previous conditions did not afford any product. We further investigated these amidation conditions with palladium catalysts (Table 1). We were able to obtain the amidation product **29** in 29% yield (entry 4), but we could not get any coupled product using the bigger amide **19**.

As previously described, the N_9-benzyl 8-bromopurine **10** did not undergo amidation, therefore, we switched to amide coupling to prepare the N_9-benzyl C_8-amide purines. The coupling intermediate N_9-benzylated 8-amino adenine **30** was synthesized from 8-bromo compound **8** by azido replacement followed by reduction[31] (Scheme 5). Interestingly, the 8-amino compound **30** did not react with the carboxylic acid **15** under conventional EDC or CDI conditions, and the more reactive



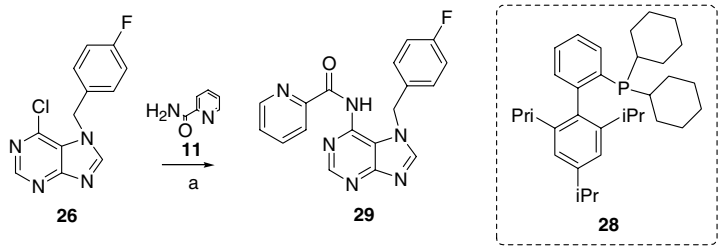
Scheme 3. Reagents and conditions: (a) $Pd_2dba_3{\cdot}CHCl_3$, dppf, Cs_2CO_3, toluene, 90 °C, 4 h; (b) TFA, DCM, rt, 2 days, **22** (50%), **23** (30%); (c) TFA, DCM, rt, 1 day.



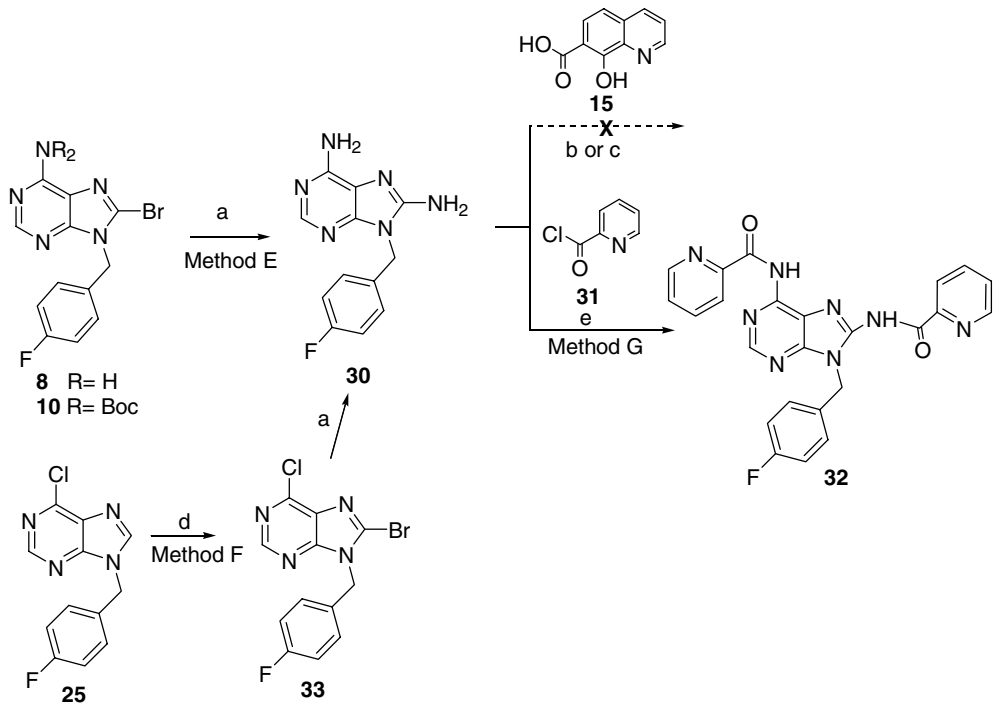
Scheme 4. Reagents and conditions: (a) NaH, DMF, 4-fluorobenzyl bromide, 70 °C, 1 h, **25** (63%), **26** (23%); (b) $Pd_2dba_3{\cdot}CHCl_3$, dppf, Cs_2CO_3, toluene, 90 °C, 5 h; (c) TFA, DCM, rt, 3 days, 84%.

picolinoyl chloride **31** readily reacted with compound **30** to give the C_6- and C_8-double amide substituted purine **32**. In order to avoid the free 6-amino group in the coupling step, the Boc protected compound **10** was subjected to the sodium azide treatment. Nonetheless, only 6,8-diaminopurine was isolated after the reduction step. Changing the substrate to 6-chloropurine **25** also resulted in the 6,8-diamino compound due to the lack of selectivity between 6-chloro and 8-bromo (**33**) toward azide replacement (Scheme 5).

Table 1. Amidation conditions of N_7-alkylated 6-chloropurine 26 with picolinamide **11**



Entry	Catalyst	Ligand	Base	Solvent	Temp (°C)	Time	Yield (%)
1	Pd_2dba_3	dppf	Cs_2CO_3	Toluene	110	Overnight	0
2	Pd_2dba_3	Ligand **28**	Cs_2CO_3	Toluene	110	18 h	0
3	$Pd(OAc)_2$	Ligand **28**	K_2CO_3	^{t}BuOH	110	Overnight	<10
4	$Pd(OAc)_2$	Ligand **28**	K_3PO_4	^{t}BuOH	110	2 days	29

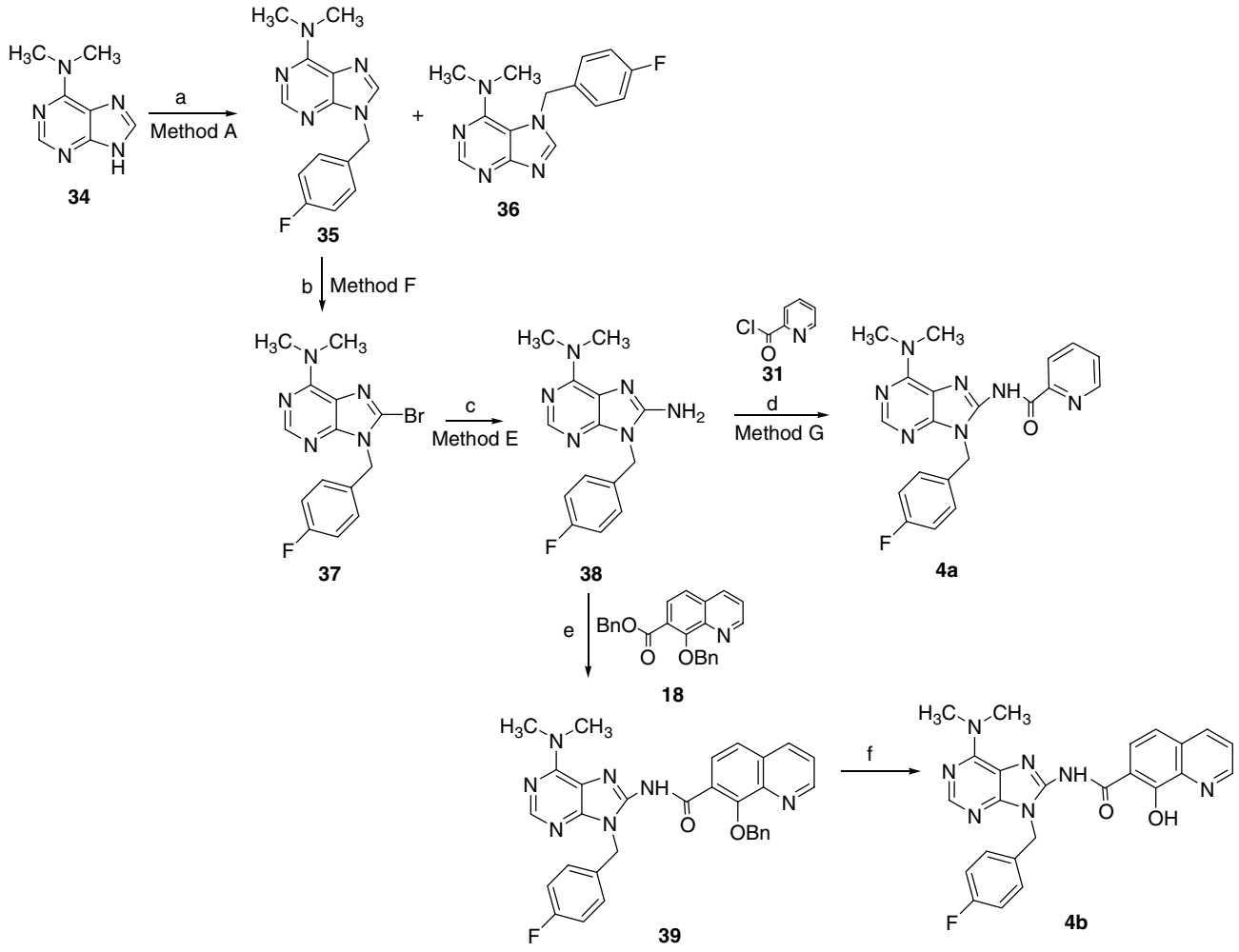


Scheme 5. Reagents and conditions: (a) i—NaN_3, DMF, 90 °C, overnight; ii—10% Pd/C, H_2 balloon, MeOH, rt, overnight; (b) EDC, HOBt, *N*-methyl morphiline, DMF, rt, overnight; (c) CDI, DMF, rt, overnight; (d) LDA, 1,2-dibromotetrachloroethane, THF, −70 °C, 2 h; (e) **31**, TEA, DMF, 60 °C, 2 h.

The *N*,*N*-dimethyl 8-amide purine derivative **4** was prepared from *N*,*N*-dimethyl adenine **34** (Scheme 6). Compound **34** was first benzylated and the N_9-benzylated compound **35** was 8-brominated[32] and subsequently converted to 8-amino compound **38**. Treatment of compound **38** with picolinoyl chloride **31** afforded the desired monoamide **4a**. Unfortunately, the acyl chloride of carboxylic acid **15** cannot be obtained by thionyl chloride or oxalyl chloride treatment, and only polymerized product was isolated. Changing to the benzyl protected acid **20** also afforded the polymerized products (Scheme 2). Unable to get the acyl chloride, the target compound **4b** was prepared by aminolysis of the benzyl ester **18** with 8-amino compound **38** in the presence of sodium hydride. The resulting coupled product **39** was debenzylated in TFA to give target compound **4b** (Scheme 6).

2.2. Structure–activity relationship studies

All the final compounds were tested for their activities against HIV integrase in microtiter and radioactive gel assays (Table 2). These DKA analogs with purine core inhibited recombinant HIV integrase at low micromolar range, but are less potent than the known α,γ-diketo acid inhibitors (Table 2, **1**). Our SAR results indicated that the two types of metal chelating ligands used in this study did not lead to significant difference in potency; the three-point Mg^{2+} ligand 8-hydroxy-quinoline-7-carboxamide, which mimics diketo acid, and the two-point Me^{2+} ligand picolinamide showed 10–45% and 20–50% inhibition at 10 μM, respectively. Our results also indicated that the spatial relationship between the two purine substituents was important for potency, purines containing the



Scheme 6. Reagents and conditions: (a) NaH, DMF, 4-fluorobenzyl bromide, rt, 2 h; (b) LDA, 1,2-dibromotetrachloroethane, THF, −70 °C; (c) i—NaN_3, DMF, 80 °C, 8 h; ii—H_2 balloon, 10% Pd/C, MeOH, overnight; (d) **31**, TEA, DMF, 60 °C, 3 h; (e) NaH, rt, DMF; (f) TFA, rt, 2 days.

three-point ligand were slightly more active than the two-point ligand containing purines when the *para*-fluorobenzyl group was N_3-substituted (**14** and **23**); but were 2-fold less active when the *para*-fluorobenzyl group was N_9-substituted (Table 2, **4a**,**b** and **5a**,**b**). The results also demonstrated that the position of *para*-fluorobenzyl group was crucial for potency, and the N_7-substituted purine (**29**) was 2- to 3-fold less active than the N_9 (**4a** and **5a**) and N_3 (**14**) substituted ones. Interestingly, the C_6,C_8-dipicolinamide substituted purine **32** showed very potent inhibitory activity against integrase at 10 μM, and is 2- to 3-fold more active than the C_8- (**4a**) or C_6- (**5a**) mono-picolinamide substituted purines. This result suggested that an additional metal chelating ligand could contribute to the increase in potency, and the spatial arrangement of the ligand could be crucial.

3. Experimental

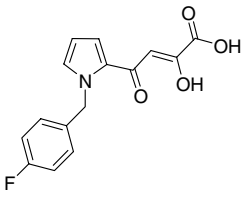
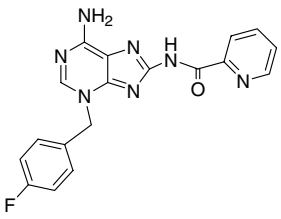
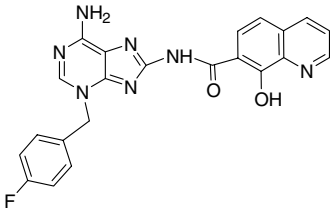
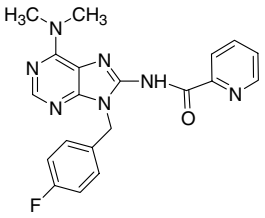
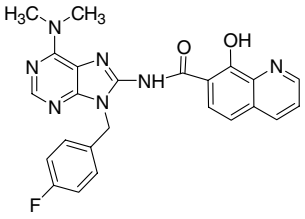
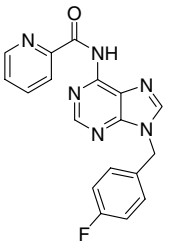
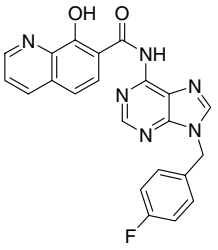
3.1. Biological assays

All the synthesized compounds were tested in a dual microtiter assay and radioactive gel assay (data provided by Southern Research Institute[33,34]). The results were shown as the combined activity of both 3′-processing and strand transfer steps given as percentage of inhibition at 10 μM. Each assay reactions was done in duplicate, and the data were given as an average of the duplicate.

3.2. Chemistry

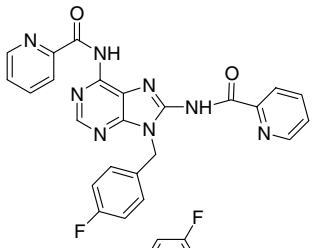
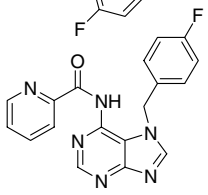
All reactions were performed in septum-sealed flasks under argon atmosphere. Progress of the reaction was followed by TLC. Organic solutions were dried over $MgSO_4$ and evaporated on a rotary evaporator under reduced pressure. Melting points were determined on a Mel-Temp II apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were obtained using Varian 300 and 600 spectrometers operating at field strength of 300 and 600 MHz. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) in hertz. Standard and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; p, pentet; br s, broad singlet; and m, multiplet. Mass spectra were determined using Bruker BioTOF II and Agilent LC-TOF spectrometers. Flash chromatography was performed using 200–300 mesh silica gel. All commercial solvents were of reagent

Table 2. Anti-HIV integrase activities of purine derivatives in microtiter and gel assays

Compound	Structure	Inhibition at 10 μM[a] (%)	
		Microtiter assay (SD)	Gel assay (SD)
1		79.0 (1.2)	61.4 (1.2)
14		27.0 (3.3)	31.0 (1.2)
23		41.4 (3.6)	44.6 (2.4)
4a		40.2 (8.9)	51.4 (9.0)
4b		26.1 (5.6)	19.3 (4.9)
5a		20.2 (4.3)	41 (12.5)
5b		11.2 (4.3)	30.1 (2.6)

(*continued on next page*)

Table 2 (*continued*)

Compound	Structure	Inhibition at 10 μM[a] (%)	
		Microtiter assay (SD)	Gel assay (SD)
32		72.2 (8.9)	83.6 (6.5)
29		13.1 (5.5)	17.1 (7.8)

[a] Mean and standard deviation (SD) were calculated from duplicate experiments.

grade or better and used directly as supplied. The vials and caps for the amidation reactions were purchased from Biotage, VA. Yields refer to purified products and were not optimized.

3.3. 8-Bromo adenine (7)

The title compound was prepared as described.[27] Yield: 70%; mp >260 °C; NMR (DMSO-d_6): δ 8.08 (1H, s), 7.39 (2H, s); ^{13}C NMR (DMSO-d_6): δ 154.5, 152.7, 150.6, 120.6; MS (ESI): *m*/*z* 214, 216 $[M+H]^+$.

3.4. Method A: Preparation of 8-bromo 9-(4-fluorobenzyl) adenine (8) and 8-bromo 3-(4-fluorobenzyl) adenine (9)

A suspension of 8-bromo adenine (**7**, 1.07 g, 5 mmol) in 25 mL anhydrous DMF was treated with sodium hydride (220 mg, 5.5 mmol) and heated at 70 °C for 30 min. 4-Fluorobenzyl bromide (1.04 g, 685 μL, 5.5 mmol) was added dropwise at the same temperature. The reaction was complete after 2 h of heating. The solid precipitate was filtered and the filtrate was concentrated in vacuo. The crude product was purified by flash chromatography ($CHCl_3$/MeOH 40:1–20:1) to give **8** as white crystal. Yield: 37%; mp 230–231 °C; ^{1}H NMR (DMSO-d_6): δ 8.14 (1 H, s), 7.44 (2H, br s), 7.27 (2H, m), 7.16 (2H, m), 5.32 (2H, s); ^{13}C NMR (DMSO-d_6): δ 163.8, 160.6, 155.4, 153.8, 151.5, 132.8 (2), 130.1 (2), 126.9, 119.6, 116.4, 116.1, 46.7; MS (ESI): *m*/*z* 322.0, 324.0 $[M+H]^+$, 344.0, 346.0 $[M+Na]^+$, and **9** as white crystal. Yield: 48%; mp 216–217 °C; ^{1}H NMR (DMSO-d_6): δ 8.53 (1H, s), 8.24 (1H, br s), 8.08 (1H, br s), 7.47 (2H, dd, J = 6.0, 9.0 Hz), 7.17 (2H, t, J = 9.0 Hz), 5.42 (2H, s); ^{13}C NMR (DMSO-d_6): δ 164.1, 160.8, 154.2, 150.2, 144.5, 140.0, 132.6 (2), 130.9 (2), 122.2, 116.3, 116.1, 52.2; MS (ESI): 322.0, 324.0 $[M+H]^+$, 344.0, 346.0 $[M+Na]^+$.

3.5. Method B: Preparation of *N*,*N*-(di-*tert*-butoxycarbonyl) 8-bromo 9-(4-fluorobenzyl) adenine (10)

8-Bromo 9-(4-fluorobenzyl) adenine (**8**, 500 mg, 1.55 mmol) and di-*tert*-butyl dicarbonate (470 mg, 3.1 mmol) were dissolved in 10 mL DMF and treated with triethylamine (650 μL, 4.65 mmol) and DMAP (20 mg). The mixture was stirred at room temperature for 2 h. After the reaction was complete, the mixture was concentrated and purified by flash chromatography (EtOAc/hexanes 1:10–1:5) to give the title compound as white crystal. Yield: 95%; mp 176–178 °C; ^{1}H NMR ($CDCl_3$): δ 8.85 (1H, s), 7.36 (2H, dd, J = 4.5, 9 Hz), 7.03 (2H, t, J = 9 Hz), 5.44 (2H, s), 1.45 (18H, s); ^{13}C NMR ($CDCl_3$): δ 164.4, 161.1, 154.5, 152.5, 150.4, 149.1, 133.5, 130.5, 130.1, 129.1, 116.3, 116.0, 84.3, 47.6, 36.8, 28.1; MS (ESI): *m*/*z* 544.09, 546.09 $[M+Na]^+$.

3.6. *N*,*N*-(di-*tert*-Butoxycarbonyl) 8-bromo 3-(*para*-fluorobenzyl) adenine (12)

The title compound was prepared from 8-bromo 3-(4-fluorobenzyl) adenine (**9**) according to method B. Yield: 80%; mp 155–157 °C; ^{1}H NMR ($CDCl_3$): δ 7.97 (1H, s), 7.08 (2H, t, J = 8 Hz), 6.74 (2H, t, J = 8 Hz), 5.33 (2H, s), 1.12 (18H, s); ^{13}C NMR ($CDCl_3$): δ 161.8, 156.9, 152.6, 150.1, 145.4, 143.7, 139.2, 135.5, 131.2, 117.0, 116.7, 110.0, 84.9, 54.1, 28.1; MS (ESI): *m*/*z* 522.1, 524.1 $[M+H]^+$.

3.7. Method C: Preparation of *N*,*N*-(di-*tert*-butoxycarbonyl)-3-(4-fluorobenzyl) 8-picolinamide adenine (13)

A 10 mL vial was charged with $Pd_2dba_3{\cdot}CHCl_3$ (20.7 mg, 0.02 mmol) and dppf (33.3 mg, 0.06 mmol). The vial was sealed with Teflon cap and filled with argon to evacuate air. A solution of *N*,*N*-(di-*tert*-butoxycarbonyl) 8-bromo 3-(*para*-fluorobenzyl) adenine (**12**, 208.8 mg, 0.4 mmol) in 8 mL dry toluene was added via cannula, followed by the addition of picolinamide (**11**, 53.7 mg, 0.44 mmol) and cesium carbonate (182.5 mg, 0.56 mmol). The air was again evacuated with argon. The resulting mixture was heated at 80 °C for 4 h. After cooling down, the mixture was concentrated and purified by flash chromatography ($CHCl_3$/MeOH 40:1) to give the title compound as white solid. Yield: 93%; mp 196–198 °C; ^{1}H NMR ($CDCl_3$): δ 11.01 (1H, s), 8.65 (1H, m), 8.36 (1H, dt, J = 0.9,

7.2 Hz), 8.25 (1H, s), 7.93 (1H, td, J = 1.5, 7.5 Hz), 7.55–7.46 (3H, m), 7.07 (2H, t, J = 6.0 Hz), 5.75 (2H, s), 1.47 (18H, s); ^{13}C NMR ($CDCl_3$): δ 165.0, 163.5, 161.6, 158.4, 150.4, 149.2, 148.4, 142.6, 137.9, 137.6, 135.1, 131.1 (2), 129.3 (2) 127.2, 123.1, 116.8 (2), 84.3, 53.7, 28.2; MS (ESI): m/z 564.3 $[M+H]^+$.

3.8. Method D: Preparation of 3-(4-fluorobenzyl) 8-picolinamide adenine (14)

A solution of *N*,*N*-(di-*tert*-butoxycarbonyl)-3-(4-fluorobenzyl) 8-(picolinamide) adenine (**13**, 210 mg, 0.37 mmol) in 15 mL DCM was treated with 1 mL TFA and stirred at room temperature. The reaction was complete after two days and the mixture was concentrated. The resulting solid was redissolved in 20 mL DCM, poured into 10 mL ice-water, and neutralized with saturated $NaHCO_3$ to pH 8–9. The aqueous layer was extracted with DCM. The organic portions were combined and dried over $MgSO_4$. After concentration, the crude product was purified by flash chromatography ($CHCl_3$/MeOH 30:1) to give the title compound as white solid. Yield: 63%; mp >160 °C (dec); ^{1}H NMR (DMSO-d_6): δ 10.46 (1H, br s), 8.71 (1H, d, J = 4.5 Hz), 8.53 (1H, s), 8.18 (1H, d, J = 7.8 Hz), 8.08 (1H, t, J = 7.8 Hz), 7.80 (2H, br s), 7.69 (1H, m), 7.56 (2H, m), 7.19 (2H, t, J = 9 Hz), 5.48 (2H, s); ^{13}C NMR (DMSO-d_6): δ 160.9, 153.6, 150.1, 149.2, 143.2, 139.0, 133.0 (2), 131.1 (2), 127.8, 122.8, 116.3, 116.0, 52.0; MS (ESI): m/z 364.2 $[M+H]^+$, 386.2 $[M+Na]^+$; HRMS (ESI): Calcd for $(C_{18}H_{14}FN_7O)H^+$: 364.1317, found: 364.1317 MH^+.

3.9. 8-Hydroxy-quinoline-7-carboxylic acid methyl ester (16)

The title compound was prepared as described.[30] Yield: 10%; ^{1}H NMR ($CDCl_3$): δ 11.92 (1H, br s), 8.96 (1H, dd, J = 1.8, 4.2 Hz), 8.09 (1H, dd, J = 1.5, 8.4 Hz), 7.87 (1H, d, J = 8.7 Hz), 7.51 (1H, dd, J = 4.2, 8.4 Hz), 7.26 (1H, d, J = 8.7 Hz), 4.01 (3H, s); ^{13}C NMR ($CDCl_3$): δ 170.9, 160.1, 149.9, 139.9, 135.9, 132.5, 125.6, 124.1, 117.9, 109.4, 52.9.

3.10. 8-Hydroxy-quinoline-7-carboxamide (17)

8-Hydroxy-quinoline-7-carboxylic acid methyl ester (**16**, 80 mg, 0.39 mmol) was treated with 10 mL liquid ammonia. The mixture was heated in a sealed steel bomb at 70 °C overnight. The excess ammonia was evaporated in the hood. The crude product was recrystallized with DCM to give the title compound as faint yellow crystal. Yield: 55%; mp 190–192 °C; ^{1}H NMR ($CDCl_3$): δ 8.86 (1H, dd, J = 1.5, 4.5 Hz), 8.19 (2H, dd, J = 1.5, 7.5 Hz), 8.15 (1H, d, J = 9 Hz), 7.7 (1H, br s), 7.55 (1H, dd, J = 4.5, 9 Hz), 7.39 (1H, d, J = 9 Hz); MS (ESI): m/z 211.04 $[M+Na]^+$.

3.11. 8-Benzyloxy-quinoline-7-carboxylic acid benzyl ester (18)

A suspension of 8-hydroxy-quinoline-7-carboxylic acid (**15**, 95 mg, 0.5 mmol) and anhydrous potassium carbonate (194 mg, 1.4 mmol) in 20 mL acetone and 0.5 mL DMSO was heated at 70 °C for 30 min. Benzyl bromide (180 μL, 1.5 mmol) was then added and the resulting mixture was heated at 70 °C overnight. After cooling down, the undissloved solid was filtered, and the filtrate was concentrated and purified by flash chromatography (hexanes/EtOAc 15:1) to give the title compound as yellow oil. Yield: 98%; ^{1}H NMR ($CDCl_3$): δ 9.03 (1H, s), 8.16 (1H, d, J = 9 Hz), 7.89 (1H, d, J = 9 Hz), 7.58 (2H, m), 7.45 (2H, m), 7.37 (8H, m), 5.54 (2H, s), 5.39 (2H, s); MS (ESI): m/z 370.14 $[M+H]^+$.

3.12. 8-Benzyloxy-quinoline-7-carboxamide (19)

8-Benzyloxy-quinoline-7-carboxylic acid benzyl ester (**18**, 800 mg) was treated with 10 mL liquid ammonia. The mixture was heated in a sealed steel bomb at 70 °C overnight. The excess ammonia was evaporated in the hood, and the crude product was purified by flash chromatography (hexanes/EtOAc 5:1–2:1) to give the title compound as cream-colored crystal. Yield: 80%; mp 128–130 °C; ^{1}H NMR ($CDCl_3$): δ 9.02 (1H, dd, J = 1.8, 4.5 Hz), 8.24 (1H, d, J = 9 Hz), 8.19 (1H, dd, J = 1.8, 9 Hz), 8.05 (1H, br s), 7.64 (1H, d, J = 9 Hz), 7.57–7.49 (3H, m), 7.42–7.37 (3H, m), 5.96 (1H, br s), 5.61 (2H, s); ^{13}C NMR ($CDCl_3$): δ 167.1, 154.6, 150.0, 142.8, 136.5 (2), 132.0, 128 (m), 127.8, 124.7, 123.6, 122.9, 78.7; MS (ESI): m/z 279.10 $[M+H]^+$.

3.13. 8-Benzyloxy-quinoline-7-carboxylic acid (20)

8-Benzyloxy-quinoline-7-carboxylic acid benzyl ester (**18**, 1.8 g, 4.8 mmol) was dissolved in 10 mL methanol and 25 mL THF. The solution was treated with 6 mL of 1 N NaOH and stirred at room temperature for 5 h. The organic solvent was removed in vacuo and the crude product was redissloved in 15 mL water. The aqueous portion was washed with DCM and subsequently acidified to pH 4–5 with 1 N HCl under ice bath. The crystal precipitate was collected by filtration and washed with DCM to give the title compound as cream-colored crystal. Yield: 73%; mp 176–180 °C; ^{1}H NMR (DMSO-d_6): δ 13.18 (1H, s), 9.01 (1H, d, J = 2.7 Hz), 8.43 (1H, d, J = 8 Hz), 7.77 (2H, s), 7.66–7.58 (3H, m), 7.41–7.32 (3H, m), 5.42 (2H, s); ^{13}C NMR (DMSO-d_6): δ 168.3, 154.4, 151.1, 143.1, 138.4, 137.1, 131.5, 128 (m), 126.4, 124.2, 123.6, 77.1; MS (ESI): m/z 280.0 $[M+H]^+$, 302.0 $[M+Na]^+$.

3.14. *N*,*N*-(di-*tert*-Butoxycarbonyl)-3-(4-fluorobenzyl)-8-(8-benzyloxy-quinoline-7-carboxamide) adenine (21)

Compound **21** was prepared from *N*,*N*-(di-*tert*-butoxycarbonyl) 8-bromo 3-(*para*-fluorobenzyl) adenine (**12**) and 8-benzyloxy-quinoline-7-carboxamide (**19**) according to method C and purified by flash chromatography (hexanes/EtOAc 5:1–1:1) to give the title compound as white solid. Yield: 74%; mp 185–187 °C; ^{1}H NMR ($CDCl_3$): δ 11.27 (1H, s), 9.07 (1H, dd, J = 1.8, 4.5 Hz), 8.35 (1H, d, J = 9 Hz), 8.23 (1H, d, J = 1.8 Hz), 8.21 (1H, s), 7.67 (1H, d, J = 9 Hz), 7.60–7.53 (3H, m), 7.47 (2H, m), 7.24 (4H, m), 7.08 (2H, m), 5.76 (2H, s), 5.70 (2H, s), 1.48 (18H, s); ^{13}C NMR ($CDCl_3$): δ 164.0, 163.6, 162.4, 162.1, 158.2, 153.7,

150.4, 150.0, 142.6, 142.2, 137.1, 136.3, 135.4, 134.8, 132.1, 130.9, 129.7, 129.3, 128.8, 128.6, 127.8, 125.1, 123.7, 122.9, 116.5, 116.3, 83.9, 78.8, 53.4, 27.8; MS (ESI): *m*/*z* 720.29 $[M+H]^+$.

3.15. 3-(4-Fluorobenzyl) 8-(8-benzyloxy-quinoline-7-carboxamide) adenine (22) and 3-(4-fluorobenzyl) 8-(8-hydroxy-quinoline-7-carboxamide) adenine (23)

The title compounds were prepared from compound **21** according to method D. After the reaction was complete, the mixture was concentrated and the resulting semi-solid was dissolved in 25 mL DCM and washed with saturated $NaHCO_3$. Some yellow solid was precipitated out while mixing with the basic solution. The solid was collected by filtration and recrystallized with methanol to give **23** as bright yellow crystal. Yield: 30%; mp >250 °C (dec); ^{1}H NMR (DMSO-d_6): δ 13.09 (1H, br s), 8.89 (3H, s), 8.82 (1H, d, J = 7.8 Hz), 8.68 (1H, s), 8.14 (1H, d, J = 9 Hz), 7.95 (1H, m), 7.57 (2H, m), 7.23 (2H, t, J = 9 Hz), 7.16 (1H, d, J = 7.8 Hz), 5.57 (2H, s); ^{13}C NMR (DMSO-d_6): δ 170.8, 168.4, 161.8, 158.5, 158.3, 148.1, 131.8, 130.0 (2), 117.0, 116.5, 116.4, 110.6, 52.0; HRMS (ESI): Calcd for $(C_{22}H_{17}FN_7O_2)^+$: 430.1422, found: 430.1435 MH^+. The concentrated organic portion was purified by flash chromatography (hexanes:EtOAc 5:1–1:2) to give compound **22** as white solid. Yield: 49%; mp 228–230 °C; ^{1}H NMR (DMSO-d_6): δ 10.82 (1H, br s), 9.02 (1H, s), 8.43 (2H, d, J = 9 Hz), 7.79 (2H, m), 7.68 (2H, m), 7.63 (1H, m), 7.57 (2H, m), 7.48 (1H, br s), 7.24 (3H, m), 7.112 (1H, br s), 5.53 (2H, br s), 5.44 (2H, br s); ^{13}C NMR (DMSO-d_6): δ 163.3, 161.7, 153.5, 150.9, 150.1, 142.8, 137.2, 130.9 (2), 129.2, 128.8, 128.6, 126.9, 123.2, 116.1, 115.9, 51.7; MS (ESI): *m*/*z* 520.2 $[M+H]^+$; 542.1 $[M+Na]^+$. Compound **22** was further hydrolyzed with TFA in DCM to give compound **23**, yield 30%.

3.16. 9-(4-Fluorobenzyl) 6-chloropurine (25) and 7-(4-fluorobenzyl) 6-chloropurine (26)

The title compounds were prepared from 6-chloropurine (**24**) according to method A. Compound **25** was obtained as white crystal. Yield: 63%; mp 131–132 °C; ^{1}H NMR ($CDCl_3$): δ 8.79 (1H, s), 8.10 (1H, s), 7.33 (2H, dd, J = 6, 9 Hz), 7.07 (2H, t, J = 9 Hz), 5.43 (2H, s); ^{13}C NMR ($CDCl_3$): δ 164.6, 161.3, 152.4, 151.9, 151.3, 144.9, 131.7, 130.6 (2), 130.0, 116.7, 116.4, 47.6; MS (ESI): *m*/*z* 285.1 $[M+Na]^+$. Compound **26** was obtained as white crystal. Yield: 23%; mp 167–168 °C; ^{1}H NMR ($CDCl_3$): δ 8.89 (1H, s), 8.26 (1H, s), 7.18 (2H, m), 7.09 (2H, m), 5.68 (2H, s); ^{13}C NMR ($CDCl_3$): δ 164.6, 162.2, 161.3, 152.8, 149.1, 143.3, 130.6 (2), 129.2 (2), 122.6, 116.8, 116.5, 50.4; MS (ESI): *m*/*z* 263.04 $[M+H]^+$.

3.17. 6-Picolinamide 9-(4-fluorobenzyl) purine (5a)

Compound **5a** was prepared from compound **25** and picolinamide **11** according to Method C. Yield: 68%; mp 222–224 °C; ^{1}H NMR ($CDCl_3$): δ 11.24 (1H, s), 8.89 (1H, s), 8.70 (1H, d, J = 4.8 Hz), 8.39 (1H, d, J = 7.8 Hz), 8.01 (1H, s), 7.95 (1H, t, J = 7.8 Hz), 7.54 (1H, dd, J = 4.8, 7.0 Hz), 7.34 (2H, dd, J = 6, 8.4 Hz), 7.06 (2H, t, J = 8.4 Hz), 5.46 (2H, s); ^{13}C NMR ($CDCl_3$): δ 163.6, 161.9, 161.4, 153.1, 152.0, 149.2, 149.0, 148.3, 142.6, 137.8, 131.0, 129.8 (2), 127.2, 123.1, 122.8, 116.3, 116.1, 46.8; MS (ESI): *m*/*z* 349.12 $[M+H]^+$; HRMS (ESI): Calcd for $(C_{18}H_{13}FN_6O)Na^+$: 371.1027, found: 371.1021 $[M+Na]^+$.

3.18. 6-(8-Benzyloxy-quinoline-7-carboxamide)-9-(4-fluorobenzyl) purine (27)

Compound **27** was prepared from compound **25** and 8-benzyloxy-quinoline-7-carboxamide (**19**) according to method C. Yield: 68%; mp 276–278 °C; ^{1}H NMR ($CDCl_3$): δ 11.42 (1H, s), 9.06 (1H, dd, J = 1.8, 4.2 Hz), 8.84 (1H, s), 8.34 (1H, d, J = 8.4 Hz), 8.21 (1H, d, J = 8.4 Hz), 7.92 (1H, s), 7.66 (1H, d, J = 9 Hz), 7.54 (1H, dd, J = 4.2, 8.4 Hz), 7.41 (2H, d, J = 6.6 Hz), 7.34 (2H, dd, J = 4.8, 8.4 Hz), 7.11–7.06 (5H, m), 5.89 (2H, s), 5.42 (2H, s); ^{13}C NMR ($CDCl_3$): δ 163.6, 162.5, 161.9, 154.0, 152.9, 151.9, 149.9, 149.5, 142.6, 142.2, 136.3, 135.6, 132.2, 131.2 (2), 129.8 (2), 129.7, 128.6, 128.2, 127.8, 124.9, 123.6, 123.2, 122.9, 116.2, 116.1, 78.7, 46.7; MS (ESI): *m*/*z* 505.1 $[M+H]^+$, 527.1 $[M+Na]^+$.

3.19. 6-(8-Hydroxy-quinoline-7-carboxamide)-9-(4-fluorobenzyl) purine (5b)

A solution of compound **27** (40 mg, 0.08 mmol) in 15 mL DCM was treated with 1 mL TFA. The mixture was stirred at room temperature for 3 days. The excess TFA was removed in vacuo, and the resulting semi-oil was crystallized with DCM to give the title compound as bright yellow crystals. Yield: 62%; mp 238–240 °C; ^{1}H NMR (DMSO-d_6): δ 8.90 (1H, d, J = 4.2 Hz), 8.76 (1H, d, J = 9 Hz), 8.72 (2H, s), 8.13 (1H, d, J = 8.4 Hz), 7.89 (1H, dd, J = 4.8, 7.8 Hz), 7.44 (2H, dd, J = 5.4, 7.8 Hz), 7.25 (1H, d, J = 8.4 Hz), 7.18 (2H, t, J = 9 Hz), 5.51 (2H, s); ^{13}C NMR (DMSO-d_6): δ 163.3, 161.7, 161.3, 151.7, 149.3, 148.7, 145.9, 144.8, 142.8, 136.9, 136.9, 133.2, 133.1, 129.6, 124.8, 123.4, 116.4, 116.2, 114.8, 46.8; MS (ESI): *m*/*z* 415.05 $[M+H]^+$; 437.03 $[M+Na]^+$, 453.00 $[M+K]^+$; HRMS (ESI): Calcd for $(C_{22}H_{15}FN_6NaO_2)^+$: 437.1133, found: 4378.1143 $[M+Na]^+$.

3.20. 6-Picolinamide-7-(4-fluorobenzyl) purine (29)

A 10 mL vial was charged with $Pd(OAc)_2$ (3.8 mg, 0.017 mmol) and ligand **28** (14.2 mg, 0.03 mmol). The vial was filled with argon to evacuate air. About 4 mL of *t*-BuOH (solid) was added and the tube was filled with argon and the air evacuated. The mixture was heated at 30–40 °C for 10 min for *t*-BuOH to melt. of the red-brownish solution were added compound **27** (105 mg, 0.4 mmol), picolinamide (**11**, 73 mg, 0.6 mmol), phenyl boronic acid (3.2 mg, 0.026 mmol), and potassium phosphate (150 mg, 0.71 mmol). The vial was sealed with Teflon cap, filled with argon, and heated at 100–110 °C. The reaction was complete after 3 days of heating (starting materials were all consumed, several new spots observed on TLC). After cooling down, the mixture was concentrated and purified by flash chromatography (hexanes/EtOAc 1:1–$CHCl_3$/MeOH 50:1) to give the title

compound. Yield: 29%; mp 201–204 °C; ^{1}H NMR ($CDCl_3$): δ 10.28 (1H, br s), 8.96 (1H, s), 8.63 (1H, dt, J = 4.2, 0.9 Hz), 8.30 (1H, dt, J = 6.9, 0.9 Hz), 8.21 (1H, s), 7.97 (1H, dt, J = 1.8, 7.5 Hz), 7.59 (1H, ddd, J = 1.2, 4.8, 7.8 Hz), 7.02 (2H, m), 6.94 (2H, t, J = 9 Hz), 5.55 (2H, s); ^{13}C NMR (DMSO-d_6): δ 162.9, 161.3, 152.5, 150.9, 149.3, 138.8, 133.4, 129.3 (2), 128.2, 123.4, 116.0, 115.9, 49.8; MS (ESI): m/z 349.2 $[M+H]^+$, 371.2 $[M+Na]^+$; HRMS (ESI): Calcd for $(C_{18}H_{13}FN_6ONa)^+$: 371.1027, found: 371.1053 $[M+Na]^+$.

3.21. Method E: Preparation of 8-amino 9-(4-fluorobenzyl) adenine (30)

8-Bromo 9-(4-fluorobenzyl) adenine (**8**, 450 mg, 1.4 mmol) and sodium azide (182 mg, 2.8 mmol) were suspended in 10 mL DMF and heated at 100 °C. The reaction was complete after 9 h of heating. The reaction mixture was concentrated in vacuo to nearly dryness and co-evaporated with toluene. The crude product was then dissolved in 40 mL methanol and treated with 200 mg 10% Pd/C. The mixture was stirred under hydrogen balloon at room temperature overnight. After the reaction was complete, the catalyst was filtered and the concentrated filtrate was purified by flash chromatography ($CHCl_3$/MeOH 20:1–10:1) to give the title compound as faint yellow solid. Yield: 70%; mp >232 °C (dec); ^{1}H NMR (DMSO-d_6): δ 7.93 (1H, s), 7.30 (2H, dd, J = 3.0, 9.0 Hz), 7.12 (2H, t, J = 9 Hz), 6.62 (2H, s), 6.59 (2H, s), 5.18 (2H, s); ^{13}C NMR (DMSO-d_6): δ 163.6, 160.4, 152.6 (2), 150.3, 149.5, 133.8, 130.0 (2), 117.6, 116.1, 457.8, 43.6; MS (ESI): m/z 259.1 $[M+H]^+$, 281.1 $[M+Na]^+$.

3.22. Method G: Preparation of 6,8-dipicolinamide 9-(4-fluorobenzyl) purine (32)

The picolinoyl chloride **31** was prepared by refluxing picolinic acid (95 mg, 0.77 mmol) in 1 mL thionyl chloride for 1 h. The excess thionyl chloride was removed in vacuo and co-evaporated with toluene. The crude product (**31**, white solid) was directly used for the next step. In a 10 mL flask were added 8-amino 9-(4-fluorobenzyl) 6-aminopurine (**30**, 80 mg, 0.3 mmol) and 2 mL DMF. To the solution were added triethylamine (100 μL) and a solution of compound **31** in 2 mL DMF. The resulting mixture was heated at 60 °C for 2 h. After concentration, the crude product was purified by flash chromatography ($CHCl_3$/MeOH 50:1) to give the title compound as faint yellow solid. Yield: 43%; mp 241–243 °C; ^{1}H NMR (DMSO-d_6, δ ppm): (hard to dissolve in DMSO) 12.9 (0.5H, br s); 11.5 (0.5H, br s); 11.1 (0.5H, br s); 10.9 (0.5H, br s); 8.7 (3H, m); 8.3 (1H, m); 8.1 (2H, m); 7.9 (1H, m); 7.7 (2H, s); 7.6 (2H, s); 7.2–7.06 (3H, m); 5.4 (2H, s); MS (ESI): m/z 469.0 $[M+H]^+$, 491.0 $[M+Na]^+$; HRMS (ESI): Calcd for $(C_{24}H_{17}FN_8O_2Na)^+$ 491.1351 $[M+Na]^+$, found: 491.1356 $[M+Na]^+$.

3.23. Method F: Preparation of 8-Bromo 9-(4-fluorobenzyl) 6-chloropurine (33)[32]

LDA solution was freshly made by stirring diisopropylamine (295 μL, 2.1 mmol) and 2.5 M n-butyl lithium solution (844 μL, 2.1 mmol) in 2 mL anhydrous THF at −70 °C under argon for 1 h. To the LDA solution was added a solution of 9-(4-fluorobenzyl) 6-chloropurine (**25**, 394 mg, 1.5 mmol) in 4.5 mL THF at −70 °C. The mixture turned dark after adding **25**. After stirring for 1 h at −70 °C, a solution of dibromotetrachloroethane (977 mg, 3 mmol) in 3 mL THF was added dropwise. The reaction was complete after stirring at −70 °C for 1 h. The mixture was gradually warmed up to room temperature, and 2 mL of saturated NH_4Cl was added. The mixture was concentrated to remove THF. The resulting residue was redissolved in DCM and washed with water. The organic layer was separated and dried over $MgSO_4$. After concentration, the crude product was purified by flash chromatography (hexanes/EtOAc 5:1) to give the title compound as white crystal. Yield: 55%; mp 143–144 °C; ^{1}H NMR ($CDCl_3$): δ 8.74 (1H, s), 7.38 (2H, dd, J = 3.0, 9 Hz), 7.01 (2H, t, J = 9 Hz), 5.45 (2H, s); ^{13}C NMR ($CDCl_3$): δ 164.5, 161.2, 153.0, 152.4 (2), 149.7, 134.2, 132.0, 130.2 (m), 116.4, 116.1, 48.0; MS (ESI): m/z 362.8, 364.8 $[M+Na]^+$.

3.24. *N*,*N*-Dimethyl 9-(4-fluorobenzyl) adenine (35) and *N*,*N*-dimethyl 7-(4-fluorobenzyl) adenine (36)

The title compounds were prepared from *N*,*N*-dimethyl adeine (**34**) at room temperature according to Method A. The reaction was complete after stirring at room temperature for 8 h. Compound **35** was obtained as white crystal. Yield: 68%; mp 129–130 °C; ^{1}H NMR ($CDCl_3$): δ 8.38 (1H, s), 7.69 (1H, s), 7.26 (2H, m), 7.02 (2H, t, J = 9 Hz), 5.32 (2H, s), 3.53 (6H, br s); ^{13}C NMR ($CDCl_3$): δ 164.2, 160.9, 155.0, 152.7, 150.6, 137.9, 131.9, 129.6, 120.1, 116.2 (2), 46.5, 38.8; MS (ESI): m/z 272.0 $[M+H]^+$, 294.0 $[M+Na]^+$, and **36** as white crystal. Yield: 30%; mp 124–126 °C; ^{1}H NMR ($CDCl_3$): δ 8.03 (1H, s), 7.95 (1H, s), 7.40 (2H, m), 7.03 (2H, m), 5.49 (2H, s), 3.94 (3H, s), 3.34 (3H, s); ^{13}C NMR ($CDCl_3$): δ 164.5, 161.3, 153.3, 152.8, 150.6, 140.8, 130.7, 130.3, 121.9, 116.4, 116.1, 52.5, 40.1, 38.3; MS (ESI): m/z 272.0 $[M+H]^+$, 294.0 $[M+Na]^+$.

3.25. *N*,*N*-Dimethyl 8-bromo 9-(4-fluorobenzyl) adenine (37)

The title compound was prepared from *N*,*N*-dimethyl 9-(4-fluorobenzyl) adenine (**35**) according to Method F. Compound **37** was obtained as white crystal. Yield: 73%; mp 154–155 °C; ^{1}H NMR ($CDCl_3$): δ 8.28 (1H, s), 7.28 (2H, dd, J = 6, 9 Hz), 6.96 (2H, t, J = 9 Hz), 5.30 (2H, s), 3.45 (6H, br s); ^{13}C NMR ($CDCl_3$): δ 164.2, 160.9, 153.8, 152.8, 152.1, 131.4, 129.8 (2), 124.5, 116.0 (2), 46.9, 38.8; MS (ESI): m/z 350.0, 352.0 $[M+H]^+$, 372.0, 374.0 $[M+Na]^+$.

3.26. *N*,*N*-Dimethyl 8-amino 9-(4-fluorobenzyl) adenine (38)

Compound **38** was prepared from *N*,*N*-dimethyl 8-bromo 9-(4-fluorobenzyl) adenine (**37**) according to method E. Compound **38** was obtained as white crys-

tal. Yield: 50%; mp 210–212 °C; ^{1}H NMR (DMSO-d_6): δ 7.97 (1H, s), 7.26 (2H, dd, J = 6, 9 Hz), 7.12 (2H, t, J = 9 Hz), 6.57 (2H, s), 5.16 (2H, s), 3.32 (6H, s); ^{13}C NMR (DMSO-d_6): δ 151.8, 151.2, 150.9, 148.9, 133.9, 130.0 (2), 118.1, 116.0 (2), 43.5, 38.5; MS (ESI): m/z 287.07 $[M+H]^+$, 309.05 $[M+Na]^+$. The reduced starting material (same as **35**) was also isolated, yield 50%.

3.27. *N*,*N*-Dimethyl 8-picolinamide-9-(4-fluorobenzyl) adenine (4a)

Compound **4a** was prepared from compound **38** (30 mg, 0.1 mmol) according to method G. Yield: 54%; mp 172–173 °C; ^{1}H NMR ($CDCl_3$): δ 10.04 (1H, s), 8.59 (1H, d, J = 4.2 Hz), 8.38 (1H, s), 8.28 (1H, d, J = 7.8 Hz), 7.94 (1H, t, J = 7.8 Hz), 7.54 (1H, dd, J = 4.8, 6.6 Hz), 7.18 (2H, m), 6.91 (2H, t, J = 8.4 Hz), 5.45 (2H, s), 3.58 (6H, br s); ^{13}C NMR ($CDCl_3$): δ 163.2, 161.6, 152.3, 150.8, 148.4, 148.1, 137.8, 131.7, 129.4 (2), 127.3, 122.8, 115.8, 115.6, 45.5, 38.5; MS (ESI): m/z 392.0 $[M+H]^+$, 414.0 $[M+Na]^+$; HRMS (ESI): Calcd for $(C_{20}H_{18}FN_7NaO)^+$ 414.1449, found: 414.1471 $[M+Na]^+$.

3.28. *N*,*N*-Dimethyl 9-(4-fluorobenzyl) 8-(8-benzyloxy-quinoline-7-carboxamide) adenine (39)

A solution of *N*,*N*-dimethyl 8-amino 9-(4-fluorobenzyl) adenine (**38**, 30 mg, 0.105 mmol) in 1 mL DMF was treated with sodium hydride (5 mg, 0.125 mmol) in a 5 mL flask at room temperature for 20 min. A solution of 8-benzyloxy-quinoline-7-carboxylic acid benzyl ester (**18**, 60 mg, 0.162 mmol) in 1 mL DMF was added to the flask and continued stirring at room temperature overnight. The excess DMF was removed in vacuo and the crude product was purified by flash chromatography ($CHCl_3$/MeOH 100:1) to give the title compound as white solid. Yield: 53%; mp 216–220 °C; ^{1}H NMR ($CDCl_3$): δ 10.42 (1H, br s), 9.07 (1H, d, J = 3.6 Hz), 8.37 (1H, s), 8.29 (1H, d, J = 9 Hz), 8.24 (1H, d, J = 8.4 Hz), 7.70 (1H, d, J = 8.4 Hz), 7.58 (1H, dd, J = 4.8, 8.4 Hz), 7.55 (2H, d, J = 4.2 Hz), 7.36 (3H, m), 7.045 (2H, m), 6.81 (2H, t, J = 8.4 Hz), 5.63 (2H, s), 5.29 (2H, s), 3.53 (6H, br s); ^{13}C NMR ($CDCl_3$): δ 164.2, 161.4, 154.5, 152.2, 150.9, 150.2, 139.9, 136.3, 135.6, 132.3, 129.4, 129.2, 129.1, 129.0, 128.7, 127.3, 123.8, 123.2, 115.6, 115.5, 78.8, 45.3, 38.4; MS (ESI): m/z 548.2 $[M+H]^+$, 570.1 $[M+Na]^+$.

3.29. *N*,*N*-Dimethyl 9-(4-fluorobenzyl) 8-(8-hydroxy-quinoline-7-carboxamide) adenine (4b)

A solution of compound **39** (25 mg, 0.045 mmol) in 5 mL DCM was treated with 1 mL TFA. The mixture was stirred at room temperature for 3 days. After concentration, the semi-solid was dissolved in 1 mL EtOAc and crystallized to give the title compound as bright yellow crystal. Yield: 50%; mp 265–267 °C; ^{1}H NMR (DMSO-d_6): δ 8.91 (1H, d, J = 4.2 Hz), 8.61 (1H, d, J = 8.1 Hz), 8.28 (1H, s), 8.02 (1H, d, J = 9 Hz), 7.80 (1H, dd, J = 8.1, 4.8 Hz), 7.31 (3H, m), 7.09 (2H, t, J = 9 Hz), 5.38 (2H, s), 4.01 (6H, br s); ^{13}C NMR (DMSO-d_6): δ 161.4, 158.7, 133.0, 132.4, 130.3, 128.6, 124.4, 116.2, 116.0, 53.7, 38.9; MS (ESI): m/z 458.2 $[M+H]^+$; HRMS (ESI): Calcd for $(C_{24}H_{21}N_7O_2F)^+$ 458.1735, found 458.1741 MH^+.

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