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Design, Synthesis and Preliminary Biological Evaluation of Purine-2,6-diamine Derivatives as Cyclin-dependent Kinase (CDK) Inhibitors

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Novel purine-2,6-diamine derivatives were designed and synthesized as cyclin-dependent kinase (CDK) inhibitors. According to the preliminary biological evaluation, most of the compounds show good inhibitory activities in CDK1 enzyme assay and potent antiproliferative activities in some tumor cell lines. Especially, compound **11a** (IC_{50} = 0.35 μmol/L for CDK1/cyclin B and IC_{50} = 0.023 μmol/L for CDK2/cyclin A) possessed better inhibitory effect compared with Roscovitine (IC_{50} = 2.54 μmol/L for CDK1/cyclin B and IC_{50} = 0.092 μmol/L for CDK2/cyclin A).

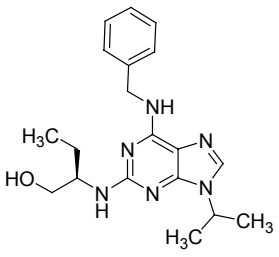
Keywords purine derivatives, CDK inhibitors, antitumor agents, regulation of cell cycle

Introduction

A variety of genetic and/or epigenetic events in human cancer are either direct or indirect consequence of deregulated cell cycle. However, the deregulation of cell cycle is often related to abnormal of cyclin-dependent kinases (CDKs) activities which frequently associated with many types of cancer.[1] CDKs, which consist of a catalytic CDK subunit and a positive regulatory cyclin subunit, belong to serine/threonine kinase family. Only a certain subset of CDKs-cyclins involved in cell cycle directly, including three interphase CDKs (CDK2, CDK4 and CDK6), a mitotic CDK (CDK1) and ten cyclins.[2] For the subtype CDK1, it can form the CDK1/cyclin A complex and CDK1/cyclin B complex, which can control cell cycle to entry into mitosis and maintenance of the mitotic state.[3] More recently, genetic studies in mice showed that the knockout of the mitotic kinase CDK1 was lethal and facilitated to the cell cycle arrest, indicating that CDK1 is essential for the cell cycle progression and executes all the events for cell division.[4-7]

Regulation of CDK1 levels has been regarded as a good way to block cell cycle or induce apoptosis in higher eukaryotes.[8] Recently, a number of literatures have illustrated that CDK1 inhibitors could block the cell cycle progression through mitosis and hold promise for treating cancer due to their abilities to control cell proliferation or to inhibit tumor growth.[3,9] Along this line, many efforts have been devoted to develop novel CDK1 inhibitors in recent years.

During the past decade many small molecule CDK inhibitors have been developed and identified with various structures.[3,10,11] Among them, purine heterocycles are one of the most investigated structural skeletons for CDK inhibitors.[12-17] For example, *R*-roscovitine (CYC202, seliciclib), a 2,6,9-trisubstituted purine CDKs inhibitor, has been studied in phase II clinical trial.[18,19] However, the moderate inhibitory activities against CDKs is still a problem for *R*-roscovitine and limited this agent in therapeutic application. Therefore, further structural modification should be carried out on purine derivatives so as to develop more potent CDK inhibitors.



R-Roscovitine

In our studies, the structural modification was performed based on the structure of Roscovitine. Different

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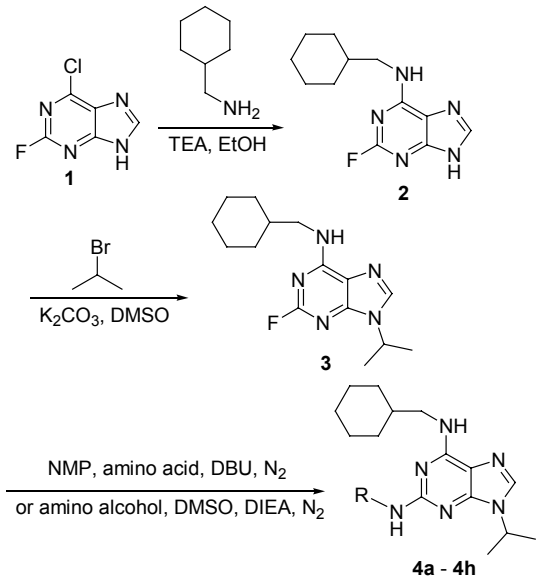
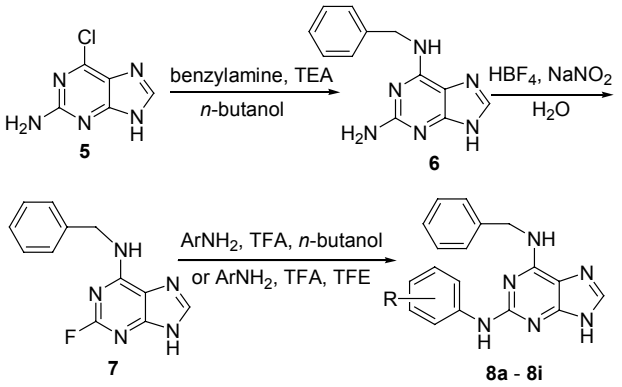
hydroxyalkylamines and amino acids were introduced to C-2 position and the cyclohexylmethyl was introduced to N-6 position at the same time. According to literature,[20] deletion of 9-isopropyl can increase the hydrogen bond interaction with the target. Therefore, other three series of N^2,N^6-disubstituted 9*H*-purine-2,6-diamine were designed to increase the CDK1 binding affinities. The synthetic routes of all the target compounds were shown in Schemes 1—4.

Scheme 1 General synthetic route of the target compounds **4a**—**4h**



Scheme 2 General synthetic route of the target compounds **8a**—**8i**

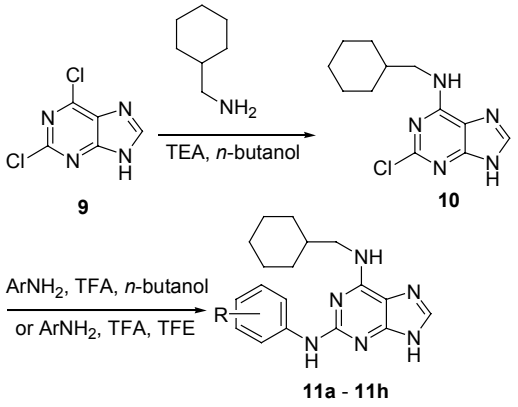


Results and Discussion

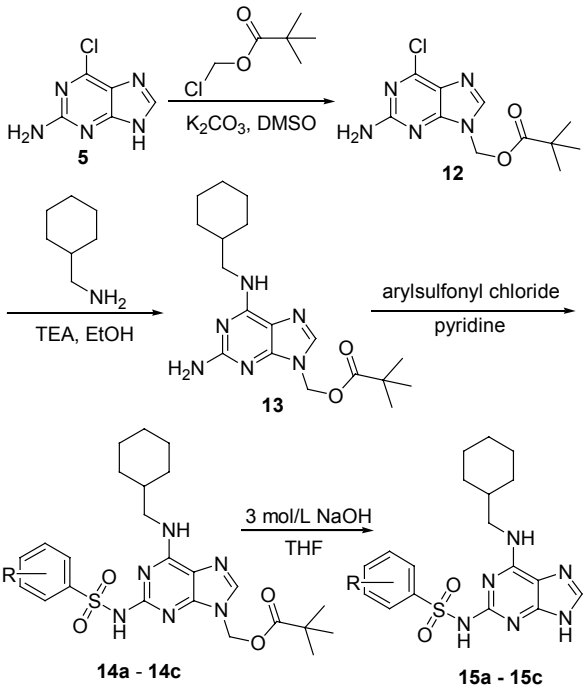
Totally twenty eight purine-2,6-diamine derivatives were synthesized. The preliminary biological evaluations include their enzyme inhibition against CDK1/cyclin B, CDK2/cyclin A and antiproliferative activities against human colon carcinoma (HCT116), human breast carcinoma (MDA-MB231) and human prostate carcinoma (PC3) cell lines.

According to the result of CDK1 inhibition, target compounds **4a**—**4h** with hydroxyalkylamine or amino acids substitution at C-2 position showed poor binding

Scheme 3 General synthetic route of the target compounds **11a**—**11h**



Scheme 4 General synthetic route of the target compounds **15a**—**15c**



affinities to CDK1. Only compounds **4e** and **4f** have the IC_{50} value about 5 μmol/L, but still are not good as Roscovitine (Table 1).

Literature reported that the derivatives without isopropyl substitution at N-9 position would be helpful to increase the inhibition to CDK. In our studies, these two series of compounds were synthesized without isopropyl in N-9 position. The difference between these two series compounds is the R^1 substitution, one is benzyl group and the other is cyclohexylmethylene fragment. For the target compounds with benzyl group in R^1 position (**8a**—**8i**), the binding affinities to CDK1 did not show significantly increase and only two compounds (**8e**, **8g**) possess the similar inhibition compared with Roscovitine. For the target compounds with cyclohexylmethylene substitution in R^1 position, introducing benzenesulfonamide fragment in C-2 position (**15a**—**15c**)

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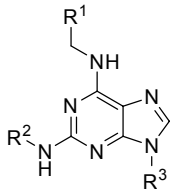
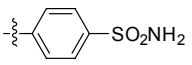
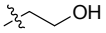
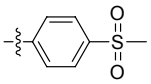
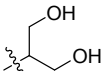
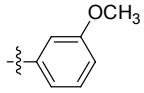
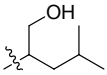
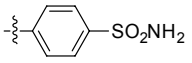
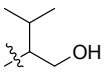
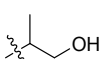
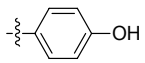
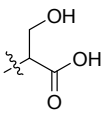
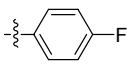
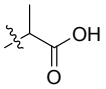
Table 1 CDK1 inhibitory activity of the target compounds



Compd	R^1	R^2	R^3	IC_{50}[a]/(μmol•L^{-1})	Compd	R^1	R^2	R^3	IC_{50}[a]/(μmol•L^{-1})
4a				9.54	**8g**			H	2.74
4b				>10	**8h**			H	>10
4c				>10	**8i**			H	>10
4d				>10	**11a**			H	0.35
4e				5.13	**11b**			H	>10
4f				5.45	**11c**			H	1.05
4g				>10	**11d**			H	5.37
4h				>10	**11e**			H	>10
8a			H	>10	**11f**			H	>10
8b			H	>10	**11g**			H	>10
8c			H	>10	**11h**			H	1.06
8d			H	>10	**15a**			H	>10
8e			H	3.34	**15b**			H	>10
8f			H	>10	**15c**			H	>10
(*R*,*S*)-Roscovitine				2.54					

[a] IC_{50} values were the mean values of duplicate experiments.

will significantly lower the binding affinities to CDK1 compared with the corresponding aniline derivatives. According to the data in Table 1, most of the target compounds with aniline substitution in C-2 position (**11a**—**11h**) exhibit similar or even better inhibition compared with Roscovitine. The substituent in the aniline also has the important impact on the inhibitory activities. For example, compounds **11e**, **11f** and **11g** with methyl, methoxy and bromo group substituted respectively showed very poor inhibition. But the compounds

11a (IC_{50}=0.35 μmol/L) and **11c** (IC_{50}=1.05 μmol/L) with sulfonamide and hydroxyl substitution exhibit more potent activities to CDK1/cyclin B than Roscovitine (IC_{50}=2.54 μmol/L).

Then MTT assay was used to investigate the antiproliferative activities of the target compounds. The preliminary evaluation was performed using HCT116 cell line (Table 2) and the results suggest that compounds **15a**—**15c** and most of the compounds **4a**—**4h** show poor antiproliferative activities. Only compounds **4a**, **4e** and **4f** exhibit better inhibition compared with Roscovitine. Most of the compounds **8a**—**8i** and all the compounds of **11a**—**11h** have better antiproliferative activities than Roscovitine. Especially compounds **11a**, **11c** and **11h** not only showed strong inhibition to HCT116 tumor cell line, but also exhibit best binding affinities to CDK1. Therefore, these three compounds were chosen to perform further biological evaluation.

To check the effect of the active compounds **11a**, **11c** and **11h** against the other subtype of CDKs, the three compounds were tested the inhibitory activity against CDK2/cyclin A. The results suggest that the compounds also show good inhibition to CDK2/cyclin A compared with Roscovitine, especially **11a** exhibits more potent binding affinity (Table 3). It also indicated that the growth of the tumor cell lines (HCT116, MDA-MB231 and PC3) could be significantly inhibited by the active compounds **11a**, **11c** and **11h**. Moreover, these three compounds possessed better growth inhibition towards the HCT116 cell than that of MDA-MB231 and PC3.

Conclusions

In summary, we report the synthesis and biological evaluation of new purine-2,6-diamine derivatives as potential antitumor agents. The active compounds **11a**, **11c** and **11h** not only showed significant inhibitory activities against CDK1/cyclin B enzyme, CDK2/cyclin A enzyme, but also had obvious inhibition to HCT116, MDA-MB231 and PC3 tumor cell lines. These results suggest that these series of purine-2,6-diamine derivatives could be used as lead compounds to investigate new potent CDK inhibitors.

Experimental

Chemistry: general procedures

All the materials used were commercially available

Table 2 Antiproliferative activities of the target compounds to HCT116

Compd	$IC_{50}^{a,b}$/(μmol•L^{-1})	Compd	$IC_{50}^{a,b}$/(μmol•L^{-1})
4a	5.38±1.8	**8g**	19.7±4.6
4b	23.2±4.1	**8h**	12.2±3.2
4c	24.6±8.1	**8i**	27.9±6.6
4d	16.4±8.7	**11a**	7.79±2.4
4e	8.44±2.7	**11b**	9.58±3.0
4f	7.74±2.4	**11c**	4.99±2.3
4g	>100	**11d**	8.06±2.7
4h	>100	**11e**	15.0±3.8
8a	6.37±2.2	**11f**	4.95±1.2
8b	12.0±4.6	**11g**	6.37±1.7
8c	45.9±34.3	**11h**	6.96±1.6
8d	9.94±2.3	**15a**	>100
8e	8.60±3.2	**15b**	>100
8f	8.96±2.9	**15c**	>100
(*R*,*S*)-Roscovitine	15.7±1.7		

[a] IC_{50} values were the mean values of three repeated experiments. [b] IC_{50} values represent the concentration which results in 50% decrease in cell growth after 48 h incubation.

Table 3 Biological activities of compounds **11a**, **11c** and **11h** against CDKs (Cyclin-dependent kinases): CDK1/cyclin B, CDK2/cyclin A and against the tumor cell lines HCT116, PC-3, MDA-MB-231

Compd	Enzyme activities IC_{50}/(μmol•L^{-1})		Anti-proliferative $IC_{50}^{a,b}$/(μmol•L^{-1})		
	CDK1/Cyclin B	CDK2/Cyclin A	HCT116	MDA-MB231	PC3
11a	0.35	0.023	7.79±2.4	21.3±6.4	13.3±2.7
11c	1.05	0.19	4.99±2.3	27.4±5.6	13.4±2.1
11h	1.06	0.35	6.96±1.6	23.6±6.8	14.6±2.8
(*R*,*S*)-Roscovitine	2.54	0.092	15.7±1.7	49.4±9.0	42.8±4.5

[a] IC_{50} values were the mean values of three repeated experiments. [b] IC_{50} values represent the concentration which results in 50% decrease in cell growth after 48 h incubation.

and used without further purification. Solvents were distilled prior to use and flash chromatography was performed using silica gel (60 Å, 200－300 mesh). All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (GF-254) and visualized with UV light, or iodine vapour. Melting points were determined on an electrothermal melting point apparatus and thermometer is uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Brucker Avance-300, Brucker Avance-400 or Varian Inova-600 spectrometers using trimethylsilane as an internal standard. High-resolution mass spectral (HRMS) data were reported as *m/z* (relative intensity). All tested compounds are＞95% pure by HPLC analysis, performed on an Agilent 1100 HPLC instrument using a Luna 5μ C18 (2) column (150 mm×4.60 mm), eluted with different ratios of acetonitrile or methanol/water (containing 0.1% formic acid) over 15 min, with detection at 259 nm and a flow rate of 1.0 mL/min.

N-(Cyclohexylmethyl)-2-fluoro-9*H*-purine-6-amine (2)

Cyclohexymethanamine (4.5 mL, 34.9 mmol) and triethylamine (TEA, 5.9 mL, 43.6 mmol) were added to a stirred solution of 6-chloro-2-fluoro purine (**1**, 5.0 g, 29 mmol) in 30 mL ethanol successively. The mixture was refluxed for 5 h. Then the solution was allowed to cool to room temperature and solvent was concentrated in vaccum. The residue was purified by column chromatography to afford **2** as white solid (4.8 g, 66%). m.p. 164－168 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.88－0.99 (m, 2H), 1.14－1.16 (m, 3H), 1.61－1.72 (m, 6H), 3.25 (s, 2H), 8.06 (s, 1H), 8.17 (s, 1H), 12.98 (s, 1H); ESI-MS *m/z*: 250.4 $[M+1]^+$.

N-(Cyclohexylmethyl)-2-fluoro-9-isopropyl-9*H*-purine-6-amine (3)

To a solution of **2** (4.80 g, 19.3 mmol) in DMSO (15 mL) were added 2-bromopropane (4.5 mL, 48.1 mmol) and K_2CO_3 (8.0 g, 57.8 mmol). The mixture stayed at 80 ℃ overnight. After addition of H_2O, the mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over $MgSO_4$, concentrated and chromatographed on silica gel to give **3** (4.0 g, 72%) as a white solid. m.p. 112－115 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.92－0.96 (m, 2H), 1.14－1.16 (m, 3H), 1.49 (d, J=6.8 Hz, 6H), 1.64－1.67 (m, 6H), 3.26 (br s, 2H), 4.59－4.65 (m, 1H), 8.20 (s, 1H), 8.29 (s, 1H); ESI-MS *m/z*: 292.4 $[M+1]^+$.

General synthesis of target compounds 4a－4c

A mixture of **3** (0.3 g, 1 mmol) and different amino alcohol (6.5 mmol) in 5 mL DMSO was stirred under N_2 at 160 ℃ till the reaction was completed. Then the mixture was cooled, water was added and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$ and then purified by column chromatography to afford the target compounds.

General synthesis of target compounds 4d－4f

A mixture of **3** (0.3 g, 1 mmol), *L*-Leucinol/*L*-Valinol/*L*-Alaninol (6.7 mmol) and *N*,*N*-diisopropyl ethylamine (1.7 mL, 10 mmol) in 5 mL DMSO was stirred at 160 ℃. After cooling, H_2O was added and the solution was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography and then conversed to the corresponding HCl salt with saturated HCl/EtOAc.

General synthesis of target compounds 4g－4h

3 (0.5 g, 1.7 mmol) was dissolved in 10 mL *N*-methyl-2-pyrrolidinone, *L*-Ser/*L*-Thr/*L*-Ala (34 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 5.3 mL, 34 mmol) were then added successively and the mixture was stirred under N_2 at 160 ℃. After cooling, the mixture was diluted with 10% critic acid and CH_2Cl_2. The organic phase was separated and washed with brine, then dried over $MgSO_4$ and purified by column chromatography to afford corresponding compounds.

2-(6-(Cyclohexyl-methylamino)-9-isopropyl-9*H*-purine-2-ylamino)butan-1-ol (4a)

Yield 32%. m.p. 116－118 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.97－1.01 (m, 2H), 1.03 (t, J=7.8 Hz, 3H), 1.13－1.26 (m, 3H), 1.53 (d, J=6 Hz, 6H), 1.56－1.60 (m, 2H), 1.61－1.67 (m, 2H), 1.71－1.73 (m, 2H), 1.80－1.82 (m, 2H), 3.38 (s, 2H), 3.63 (dd, J=10.8 Hz and 7.2 Hz, 1H), 3.82－3.89 (m, 2H), 4.57－4.61 (m, 1H), 4.88 (s, 1H), 5.45－5.67 (m, 2H), 7.49 (s, 1H); HRMS calcd for $C_{19}H_{32}N_6O$ 361.2716, found 361.2711. Anal. RP-HPLC t_R = 5.8 min [methanol ∶ water (containing 0.1% formic acid) = 60 ∶ 40; purity = 96.8%].

2-(6-(Cyclohexyl-methylamino)-9-isopropyl-9*H*-purine-2-ylamino)ethanol (4b)

Yield 32%. m.p. 95－98 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.94－1.03 (m, 2H), 1.18－1.27 (m, 3H) 1.53 (d, J=6.8 Hz, 6H), 1.55－1.61 (m, 1H), 1.62－1.64 (m, 1H), 1.69－1.72 (m, 2H), 1.78－1.81 (m, 2H), 3.38 (s, 2H), 3.55－3.58 (m, 2H), 3.83 (t, J=4.4 Hz, 2H), 4.57－4.64 (m, 1H), 5.02 (br s, 1H), 5.37 (t, J=5.4 Hz, 1H), 5.87 (s, 1H), 7.50 (s, 1H); HRMS calcd for $C_{17}H_{28}N_6O$ 333.2401, found 333.2418. Anal. RP-HPLC t_R=7.8 min [methanol∶water (containing 0.1% formic acid)=50∶50; purity=99.4%].

2-(6-(Cyclohexylmethylamino)-9-isopropyl-9*H*-purine-2-ylamino)propane-1,3-diol (4c)

Yield 41%. m.p. 166－168 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.93－0.99 (m, 2H), 1.09－1.27 (m, 3H), 1.50 (d, J=7.2 Hz, 6H), 1.53－1.60 (m, 1H), 1.63－1.65 (m, 1H), 1.69－1.71 (m, 2H), 1.77－1.79 (m, 2H), 3.32 (s, 2H), 3.82－3.90 (m, 4H), 4.08 (t, J=3 Hz, 1H), 4.56 (hept., J=7.2 Hz, 1H), 5.12 (s, 2H), 5.70 (d, J=5.4 Hz, 1H), 5.96 (s, 1H), 7.50 (s, 1H); HRMS calcd for

$C_{18}H_{30}N_6O_2$: 363.2508, found 363.2536. Anal. RP-HPLC t_R=5.2 min [methanol∶water (containing 0.1% formic acid)=50∶50; purity=99.7%].

2-(6-(Cyclohexylmethylamino)-9-isopropyl-9*H*-purine-2-ylamino)-4-methylpentane-1-ol (4d)

Yield 18%. m.p. 145−149 ℃; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.02 (t, J=6.4 Hz, 6H), 1.07−1.15 (m, 2H), 1.25−1.36 (m, 3H), 1.51−1.59 (m, 2H), 1.62−1.64 (m, 6H), 1.73−1.81 (m, 4H), 1.89−1.92 (m, 2H), 3.43−3.47 (m, 2H), 3.64−3.72 (m, 2H), 4.24−4.27 (m, 1H), 4.74−4.80 (m, 1H), 8.51 (s, 1H); HRMS calcd for $C_{21}H_{36}N_6O$ 389.3029, found 389.3007. Anal. RP-HPLC t_R = 14.3 min [methanol∶water (containing 0.1% formic acid)=60∶40; purity=98.3%].

2-(6-(Cyclohexylmethylamino)-9-isopropyl-9*H*-purine-2-ylamino)-3-methylbutan-1-ol (4e)

Yield 18%. m.p. 75−79 ℃; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.03−1.07 (m, 6H), 1.11−1.13 (m, 2H), 1.24−1.35 (m, 3H), 1.59 (dd, J=6.5 Hz and 2.8 Hz, 6H), 1.72−1.81 (m, 4H), 1.86−1.89 (m, 2H), 2.07 (t, J=5.7 Hz, 1H), 3.41 (s, 2H), 3.75 (d, J=4.8 Hz, 2H), 4.02 (s, 1H), 4.67−4.73 (m, 1H), 8.04 (s, 1H); HRMS calcd for $C_{20}H_{34}N_6O$ 375.2872, found 375.2884. Anal. RP-HPLC t_R=8.0 min [methanol∶water (containing 0.1% formic acid)=60∶40; purity=97.2%].

2-(6-(Cyclohexylmethylamino)-9-isopropyl-9*H*-purine-2-ylamino)propane-1-ol (4f)

Yield 71%. m.p. 184−188 ℃; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.09−1.17 (m, 2H), 1.24−1.30 (m, 3H), 1.34 (d, J=6.6 Hz, 3H), 1.67 (d, J=6.7 Hz, 6H), 1.72−1.74 (m, 2H), 1.79−1.82 (m, 2H), 1.92−1.95 (m, 2H), 3.52 (d, J=6.8 Hz, 2H), 3.63−3.74 (m, 2H), 4.20−4.24 (m, 1H), 4.84−4.90 (m, 1H), 9.00 (s, 1H); HRMS calcd for $C_{18}H_{30}N_6O$ 347.2559, found 347.2556. Anal. RP-HPLC t_R = 7.1 min [acetonitrile∶water (containing 0.1% formic acid) = 28∶72; purity = 99.1%].

2-(6-(Cyclohexylmethylamino)-9-isopropyl-9*H*-purine-2-ylamino)-3-hydroxypropanoic acid (4g)

Yield 17%. m.p. 158−160 ℃; ^{1}H NMR (300 MHz, CD_3OD) δ: 0.98−1.12 (m, 2H), 1.29−1.40 (m, 3H), 1.58 (d, J=6.6 Hz, 6H), 1.65−1.73 (m, 2H), 1.78−1.89 (m, 4H), 3.38 (s, 2H), 4.03 (d, J=4.2 Hz, 2H), 4.61 (t, J=7.2 Hz, 1H), 4.66−4.72 (m, 1H), 7.86 (s, 1H); HRMS calcd for $C_{18}H_{28}N_6O_3$ 377.2301, found 377.2319. Anal. RP-HPLC t_R=6.5 min [acetonitrile∶water (containing 0.1% formic acid)=25∶75; purity=96.7%].

2-(6-(Cyclohexylmethylamino)-9-isopropyl-9*H*-purine-2-ylamino) propanoic acid (4h)

Yield 28%. m.p. 84−90 ℃; ^{1}H NMR (400 MHz, CD_3OD) δ: 0.98−1.07 (m, 2H), 1.19−1.33 (m, 3H), 1.51 (d, J=7.2 Hz, 3H), 1.56 (dd, J=6.8 and 1.6 Hz, 6H), 1.68−1.70 (m, 2H), 1.74−1.77 (m, 2H), 1.82−1.85 (m, 2H), 3.38 (s, 2H), 4.43−4.48 (m, 1H), 4.61−4.68 (m, 1H), 7.82 (s, 1H); HRMS calcd for $C_{18}H_{28}N_6O_2$ 361.2352, found 361.2354. Anal. RP-HPLC t_R=9.1 min [acetonitrile∶water (containing 0.1% formic acid)=28∶72; purity=98.4%].

N^6-Benzyl-9*H*-purine-2,6-diamine (6)

To a stirred solution of 2-amino-6-chloro purine (**5**, 10.0 g, 59.0 mmol) in *n*-butanol (150 mL) at room temperature, benzylamine (9.7 mL, 88.5 mmol) and TEA (20 mL, 147.5 mmol) were added successively. The reaction mixture was refluxed at 130 ℃ for 6 h and then cooled to room temperature. The solvent was removed under low pressure and the residue was washed with *n*-propanol to afford **6** as a white solid (13.9 g, 98%) after drying under vacuum, m.p. 234−238 ℃. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 4.64 (s, 2H), 5.75 (s, 2H), 7.20 (t, J=7.2 Hz, 2H), 7.28 (t, J=7.8 Hz, 2H), 7.34 (d, J=7.2 Hz, 2H), 7.68 (s, 1H), 12.15 (s, 1H); HRMS calcd for $C_{12}H_{12}N_6$ 241.1201, found 241.1218.

***N*-Benzyl-2-fluoro-9*H*-purin-6-amine (7)**

An aqueous solution of sodium nitrite (1.4 g, 200 mmol) was added with stirring to a solution of **6** (2.4 g, 10 mmol) in 48% fluoroboric acid (30 mL) at –15 ℃. After the reaction mixture was stirred at –15 ℃ for 30 min, solution was neutralized to pH=7. The resulting precipitate was collected and extracted with ethyl acetate. The organic layer was concentrated in vaccum and then purified by chromatography to yield **7** (0.83 g, 34%). m.p. 225−227 ℃; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 4.64 (d, J=6 Hz, 2H), 7.24 (d, J=6 Hz, 1H), 7.31−7.36 (m, 4H), 8.11 (s, 1H), 8.80 (s, 1H), 13.05 (s, 1H); HRMS calcd for $C_{12}H_{10}FN_5$ 244.0998, found 244.0997.

General synthesis of N^6-benzyl-N^2-(substituted phenyl)-9*H*-purine-2,6-diamine (8a−8f)

A mixture of **7** (0.25 g, 1 mmol), substituted aniline (7 mmol) and trifluoroacetic acid (TFA, 0.1 mL, 1.3 mmol) in *n*-butanol was heated to reflux at 130 ℃ overnight. The reaction mixture was cooled and the precipitate was collected, then purified by column chromatography to give the corresponding compounds.

General synthesis of N^6-benzyl-N^2-(substituted phenyl)-9*H*-purine-2,6-diamine (8g, 8h)

A mixture of **7** (0.25 g, 1 mmol), substituted aninlines (3 mmol), TFA (0.74 mL, 10 mmol), and 2,2,2-trifluoroethanol (10 mL) was refluxed at 85 ℃ for 8 h. The solvent was concentrated in vaccum after cooling. The residue was purified by column chromatography on silica gel to afford target compounds.

N^6-Benzyl-N^2-(4-bromophenyl)-9*H*-purine-2,6-diamine (8a)

Yield 23%. m.p. 264−268 ℃; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 4.74 (t, J=5.4 Hz, 2H), 7.21 (t, J=7.2 Hz, 1H), 7.31 (t, J=7.8 Hz, 4H), 7.38 (d, J=7.8 Hz, 2H), 7.71 (d, J=4.8 Hz, 2H), 7.84 (s, 1H), 8.12 (s, 1H), 9.02

(s, 1H), 12.49 (s, 1H); HRMS calcd for $C_{18}H_{15}BrN_6$ 395.0620, found 395.0614. Anal. RP-HPLC t_R=12.7 min [acetonitrile∶water (containing 0.1% formic acid) =35∶65; purity=99.5%].

N^6-benzyl-N^2-(4-methoxyphenyl)-9*H*-purine-2,6-diamine (8b)

Yield 32%. m.p. 229−232 ℃; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 3.69 (s, 3H), 4.69 (s, 2H), 6.77 (d, J=8.4 Hz, 2H), 7.20 (t, J=7.8 Hz, 1H), 7.30 (t, J=7.8 Hz, 2H), 7.37 (d, J=7.8 Hz, 2H), 7.61 (d, J=8.4 Hz, 2H), 7.78 (s, 1H), 7.96 (s, 1H), 8.60 (s, 1H), 12.37 (s, 1H); HRMS calcd for $C_{19}H_{18}N_6O$ 347.1620, found 347.1610. Anal. RP-HPLC t_R = 13.3 min [methanol∶water (containing 0.1% formic acid) = 50∶50; purity = 98.9%].

N^6-Benzyl-N^2-(*p*-tolyl)-9*H*-purine-2,6-diamine (8c)

Yield 48%. m.p. 277−279 ℃; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 2.21 (s, 3H), 4.70 (s, 2H), 6.97 (d, J=8.4 Hz, 2H), 7.20 (t, J=7.2 Hz, 1H), 7.30 (t, J=7.2 Hz, 2H), 7.38 (d, J=7.2 Hz, 2H), 7.61 (d, J=6.6 Hz, 2H), 7.79 (s, 1H), 8.01 (s, 1H), 8.69 (s, 1H), 12.40 (s, 1H); HRMS calcd for $C_{19}H_{18}N_6$ 331.1671, found 331.1668. Anal. RP-HPLC t_R = 16.9 min [methanol∶water (containing 0.1% formic acid) = 50∶50; purity = 95.1%].

N^6-Benzyl-N^2-phenyl-9*H*-purine-2,6-diamine (8d)

Yield 83%. m.p. 261−262 ℃; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 4.74 (s, 2H), 6.88 (t, J=7.2 Hz, 1H), 7.20 (t, J=7.8 Hz, 2H), 7.25 (d, J=7.2 Hz, 1H), 7.33 (t, J=7.2 Hz, 2H), 7.39 (d, J=7.8 Hz, 2H), 7.70 (d, J=7.2 Hz, 2H), 8.11 (s, 1H), 8.32 (s, 1H), 9.10 (s, 1H), 12.81 (s, 1H); HRMS calcd for $C_{18}H_{16}N_6$ 317.1514, found 317.1521. Anal. RP-HPLC t_R=12.1 min [methanol∶water (containing 0.1% formic acid)=50∶50; purity=99.8%].

N^6-Benzyl-N^2-(4-hydroxyphenyl)-9*H*-purine-2,6-diamine (8e)

Yield 41%. m.p. 258−260 ℃; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 4.71 (t, J=6.6 Hz, 2H), 6.60 (d, J=8.4 Hz, 2H,), 7.20 (t, J=7.8 Hz, 1H), 7.29 (t, J=7.2 Hz, 2H), 7.37 (d, J=7.2 Hz, 2H), 7.46 (t, J=7.8 Hz, 2H), 7.75 (s, 1H), 7.94 (s, 1H), 8.45 (s, 1H), 8.83 (s, 1H), 12.32 (s, 1H); HRMS calcd for $C_{18}H_{16}N_6O$ 333.1464, found 333.1462. Anal. RP-HPLC t_R = 6.4 min [acetonitrile∶water (containing 0.1% formic acid)= 20∶80; purity=97.6%].

N^6-Benzyl-N^2-(4-fluorophenyl)-9*H*-purine-2,6-diamine (8f)

Yield 21%. m.p. 247−250 ℃; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 4.71 (s, 2H), 7.04 (t, J=9 Hz, 2H), 7.24 (t, J=7.2 Hz, 1H), 7.33 (t, J=7.8 Hz, 2H), 7.38 (d, J=7.8 Hz, 2H), 7.68 (s, 2H), 8.14 (s, 1H), 8.32 (s, 1H), 9.16 (s, 1H), 12.93 (s, 1H); HRMS calcd for $C_{18}H_{15}FN_6$ 335.1420, found 335.1350. Anal. RP-HPLC t_R=14.0 min [methanol∶water (containing 0.1% formic acid)= 50∶50; purity=100%].

4-(6-(Benzylamino)-9*H*-purine-2-ylamino)benzenesulfonamide (8g)

Yield 8%. m.p. 215−218 ℃; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 4.72 (s, 2H), 7.10 (s, 2H), 7.22 (s, 1H), 7.32 (s, 2H), 7.39 (d, J=6 Hz, 2H), 7.60 (d, J=7.2 Hz, 2H), 7.86 (s, 2H), 7.90 (s, 1H), 8.19 (s, 1H), 9.32 (s, 1H), 12.55 (s, 1H); HRMS calcd for $C_{18}H_{17}N_7O_2S$ 396.1242, found 396.1238. Anal. RP-HPLC t_R = 5.6 min [acetonitrile∶water (containing 0.1% formic acid)= 25∶75; purity=98.6%].

N^6-Benzyl-N^2-(4-(methylsulfonyl)phenyl)-9*H*-purine-2,6-diamine (8h)

Yield 4%. m.p. 241−244 ℃; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 3.14 (s, 3H), 4.78 (s, 2H), 7.25 (s, 1H), 7.35 (s, 2H), 7.42 (d, J=5.4 Hz, 2H), 7.73 (d, J=7.8 Hz, 2H), 7.94 (s, 2H), 8.52 (s, 1H), 8.74 (s, 1H), 9.81 (s, 1H), 13.27 (s, 1H); HRMS calcd for $C_{18}H_{17}N_7O_2S$ 395.1290, found 395.1305. Anal. RP-HPLC t_R=8.0 min [acetonitrile∶water (containing 0.1% formic acid)= 28∶72; purity=100%].

N^6-Benzyl-N^2-(3-methoxyphenyl)-9*H*-purine-2,6-diamine (8i)

The mixture of compound **7** (0.25 g, 1 mmol), 3-methoxyaniline (0.86 g, 7 mmol), TFA (0.1 mL, 1.3 mmol) and *n*-butanol (7 mL) was refluxed at 130 ℃ for 6 h. The reaction mixture was cooled to room temperature and diluted with water. The solution was neutralized to pH=7 with $NaHCO_3$ and extracted with ethyl acetate. The combined organic layer was dried with $MgSO_4$, concentrated under reduced pressure, and purified by column chromatography to obtain **8i**. Yield 42%. m.p. 236 − 238 ℃; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 3.69 (s, 3H), 4.72 (s, 2H), 7.06 (t, J=7.8 Hz, 1H), 7.20 (t, J=7.8 Hz, 1H), 7.29−7.32 (m, 3H), 7.39 (d, J=7.8 Hz, 2H), 7.57 (t, J=8.4 Hz, 2H), 7.82 (s, 1H), 8.04 (s, 1H), 8.81 (s, 1H), 12.45 (s, 1H); HRMS calcd for $C_{19}H_{18}N_6O$ 347.1620, found 347.1614. Anal. RP-HPLC t_R=7.0 min [acetonitrile∶water (containing 0.1% formic acid)=28∶72; purity=100%].

2-Chloro-*N*-(cyclohexylmethyl)-9*H*-purine-6-amine (10)

To a stirred solution of 2,6-dichloropurine (**9**, 5.0 g, 26.5 mmol) in *n*-butanol (30 mL) at room temperature, aminomethylcyclohexane (4.2 mL, 31.7 mmol) and TEA (5.4 mL, 40 mmol) were added successively. The reacting mixture was refluxed at 130 ℃ for 5 h. Then the solution was allowed to cool to room temperature. The precipitate was collected and washed with *n*-propanol to afford **10** (6.4 g, 91%) after drying. m.p. 208−210 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.92 −1.00 (m, 2H), 1.57−1.21 (m, 3H), 1.61−1.73 (m, 6H), 3.23 (s, 2H), 7.94 (s, 1H), 8.08 (s, 1H), 12.85 (s, 1H); HRMS: m/z calcd for $C_{12}H_{16}ClN_5$ $[M+1]^+$

266.1172, found 266.1177.

General synthesis of N^6-(cyclohexylmethyl)-N^2-substituted phenyl-9*H*-purine-2,6-diamine (11a, 11h)

A mixture of sulfanilamide (1.56 g, 9 mmol), compound **10** (0.6 g, 2.25 mmol), TFA (1.7 mL, 22.6 mmol) and 2,2,2-trifluoroethanol (15 mL) was heated to reflux for 15 h. After cooling to room temperature, solvent was removed in vaccum and the residue was purified with chromatography to afford the target compounds.

General synthesis of N^6-(cyclohexylmethyl)-N^2-substituted phenyl-9*H*-purine-2,6-diamine (11b－11g)

To a stirred suspension of **10** (0.6 g, 2.25 mmol) in *n*-butanol (15 mL), various substituted aniline (15.8 mmol) and TFA (0.1 mL) were added successively. The reaction mixture was heated to reflux at 130 ℃ for 6 h. After cooling to room temperature, the precipitate was filtered and washed with CH_2Cl_2 to afford corresponding compounds.

4-(6-(Cyclohexylmethyl-amino)-9*H*-purine-2-yl-amino)benzenesulfonamide (11a)

Yield 11%. m.p. 258 ℃ (decomposition point); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.00－1.05 (m, 2H), 1.18－1.23 (m, 3H), 1.64 (s, 1H), 1.70 (s, 3H), 1.79－1.82 (m, 2H), 3.39 (s, 2H), 7.16 (s, 2H), 7.70 (d, J=8.4 Hz, 2H), 7.96 (d, J=8.4 Hz, 2H), 8.22 (s, 1H), 8.39 (s, 1H), 9.65 (s, 1H), 13.32 (s, 1H); HRMS calcd for $C_{18}H_{23}N_7O_2S$ 402.1712, found 402.1688. Anal. RP-HPLC t_R=12.4 min [acetonitrile∶water (containing 0.1% formic acid)=25∶75; purity=99.2%].

N^6-(Cyclohexylmethyl)-N^2-phenyl-9*H*-purine-2,6-diamine (11b)

Yield 65%. m.p.＞300 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.95－1.03 (m, 2H), 1.13－1.21 (m, 3H), 1.63－1.80 (m, 6H), 3.36 (s, 2H), 6.94 (t, J=7.2 Hz, 1H), 7.26 (t, J=8 Hz, 2H), 7.75 (d, J=8 Hz, 2H), 8.20 (s, 2H), 9.27 (s, 1H), 13.13 (s, 1H); HRMS calcd for $C_{18}H_{22}N_6$ 323.1984, found 323.1987. Anal. RP-HPLC t_R=16.7 min [acetonitrile∶water (containing 0.1% formic acid)=30∶70; purity=96.9%].

N^6-(Cyclohexylmethyl)-N^2-(4-hydroxyphenyl)-9*H*-purine-2,6-diamine (11c)

Yield 51%. m.p. 285 ℃ (decomposition point); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.93－0.99 (m, 2H), 1.15－1.20 (m, 3H), 1.63－1.77 (m, 6H), 3.31 (s, 2H), 6.66 (d, J=8.8 Hz, 2H), 7.52 (d, J=8.8 Hz, 2H), 7.63 (br s, 1H), 7.84 (s, 1H), 8.60 (s, 1H), 8.90 (s, 1H), 12.48 (s, 1H); HRMS calcd for $C_{18}H_{22}N_6O$ 339.1933, found 339.1936. Anal. RP-HPLC t_R=6.6 min [acetonitrile∶water (containing 0.1% formic acid)=25∶75; purity=100%].

N^6-(Cyclohexylmethyl)-N^2-(4-fluorophenyl)-9*H*-purine-2,6-diamine (11d)

Yield 65 %. m.p.＞300 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.93－1.02 (m, 2H), 1.13－1.24 (m, 3H), 1.63－1.78 (m, 6H), 3.34 (s, 2H), 7.10 (t, J=8.8 Hz, 2H), 7.73－7.77 (m, 2H), 8.23 (s, 2H), 9.30 (s, 1H), 12.32 (s, 1H); HRMS calcd for $C_{18}H_{21}FN_6$ 341.1890, found 341.1876. Anal. RP-HPLC t_R = 8.1 min [acetonitrile∶water (containing 0.1% formic acid)=35∶65; purity=99.4%].

N^6-(Cyclohexylmethyl)-N^2-*p*-tolyl-9*H*-purine-2,6-diamine (11e)

Yield 60%. m.p.＞300 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.96－1.02 (m, 2H), 1.13－1.21 (m, 3H), 1.63－1.78 (m, 6H), 2.25 (s, 3H), 3.36 (s, 2H), 7.07 (d, J=8 Hz, 2H), 7.62 (d, J=8 Hz, 2H), 8.15 (s, 1H), 8.24 (s, 1H), 9.20 (s, 1H), 13.13 (s, 1H); HRMS calcd for $C_{19}H_{24}N_6$ 337.2140, found 337.2139. Anal. RP-HPLC t_R =9.3 min [acetonitrile∶water (containing 0.1% formic acid)=35∶65; purity=100%].

N^6-(Cyclohexylmethyl)-N^2-(4-methoxyphenyl)-9*H*-purine-2,6-diamine (11f)

Yield 61%. m.p.＞300 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.93－1.02 (m, 2H), 1.13－1.24 (m, 3H), 1.63－1.77 (m, 6H), 3.35 (s, 2H), 3.73 (s, 3H), 6.86 (d, J=8.8 Hz, 2H), 7.60 (d, J=8.8 Hz, 2H), 8.08 (s, 1H), 8.17 (s, 1H), 9.06 (s, 1H), 13.01 (s, 1H); HRMS calcd for $C_{19}H_{24}N_6O$ 353.2090, found 353.2078. Anal. RP-HPLC t_R=17.7 min [acetonitrile∶water (containing 0.1% formic acid)=28∶72; purity=99.3%].

N^2-(4-Bromophenyl)-N^6-(cyclohexylmethyl)-9*H*-purine-2,6-diamine (11g)

Yield 59%. m.p.＞300 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.00－1.03 (m, 2H), 1.16－1.21 (m, 3H), 1.63－1.79 (m, 6H), 3.36 (s, 2H), 7.42 (d, J=8.8 Hz, 2H), 7.76 (d, J=8.8 Hz, 2H), 8.20 (s, 1H), 8.32 (s, 1H), 9.41 (s, 1H), 13.35 (s, 1H); HRMS calcd for $C_{18}H_{21}BrN_6$ 401.1089, found 401.1114. Anal. RP-HPLC t_R = 45.7 min [methanol∶water (containing 0.1% formic acid)=60∶40; purity=95.4%].

N^6-(Cyclohexylmethyl)-N^2-(4-(methylsulfonyl)phenyl)-9*H*-purine-2,6-diamine (11h)

Yield 6%. m.p. 250－253 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.01－1.03 (m, 2H), 1.17－1.22 (m, 3H), 1.63－1.83 (m, 6H), 3.14 (s, 3H), 3.41 (s, 2H), 7.78 (d, J=8.8 Hz, 2H), 8.05 (d, J=8.8 Hz, 2H), 8.34 (s, 1H), 8.41 (s, 1H), 9.81 (s, 1H), 13.16 (s, 1H); HRMS calcd for $C_{19}H_{24}N_6O_2S$ 401.1759, found 401.1762. Anal. RP-HPLC t_R=6.5 min [acetonitrile∶water (containing 0.1% formic acid)=35∶65; purity=97.3%].

(2-Amino-6-chloro-9*H*-purine-9-yl)methylpivalate (12)

6-Chloro-9*H*-purine-2-amine (**5**, 8.5 g, 50 mmol), K_2CO_3 (20.7 g, 150 mmol) and chloromethyl pivalate (Pom-Cl, 8.7 mL, 60 mmol) were disloved in 180 mL DMSO and the mixture was reacted at room temperature overnight. Then the mixture was diluted with ethyl acetate and washed with brine, then dried

over $MgSO_4$ and concentrated under reduced pressure. The residue was purified with recrystallization from ethanol to obtain target compound **12** (3.7 g, 68%). m.p. 160－162 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.18 (s, 9H), 5.18 (s, 2H), 6.00 (s, 2H), 8.01 (s, 1H); ESI-MS *m*/*z*: 284.3 $[M+1]^+$.

(2-Amino-6-((cyclohexylmethyl)amino)-9*H*-purine-9-yl)methyl pivalate (13)

A mixture of **12** (5.7 g, 20 mmol), aminomethylcyclohexane (3.2 mL, 24 mmol) and TEA (4.2 mL, 30 mmol) in 50 mL EtOH was refluxed for 7 h. The reaction was cooled down. The precipitate was collected and washed with EtOH, then dried *in vacuo* to obtain **13** (4.9 g, 67%). m.p. 159－161 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.98－1.06 (m, 2H), 1.17 (s, 9H), 1.20－1.27 (m, 3H), 1.57－1.82 (m, 6H), 3.48 (br s, 2H), 4.81 (s, 2H), 5.75 (br s, 1H), 5.96 (s, 2H), 7.69 (s, 1H); ESI-MS *m*/*z*: 361.5 $[M+1]^+$.

General synthesis of (6-((cyclohexylmethyl)amino)-2-(substitued phenylsulfonamido)-9*H*-purine-9-yl)-methyl pivalate (14a－14c)

To the solution of **13** (0.36 g, 1 mmol) in 4 mL anhydrous pyridine, substituted benzenesulfonyl chloride (1.5 mmol) was added. Then the mixture stayed for at least 24 h till completion of the reaction. The mixture was diluted with ethyl acetate and washed with 10% citric acid, then dried over $MgSO_4$, and then purified by chromatography to get corresponding compounds.

(6-((Cyclohexylmethyl)amino)-2-(4-fluorophenylsulfonamido)-9*H*-purine-9-yl)methyl pivalate (14a)

Yield 71%. m.p. 182－183 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.92－0.98 (m, 2H), 1.17 (s, 9H), 1.19－1.31 (m, 3H), 1.66－1.75 (m, 6H), 3.28 (br s, 2H), 5.93－6.03 (m, 3H), 7.17 (t, *J*＝8.4 Hz, 2H), 7.87 (s, 1H), 8.16－8.18 (m, 2H); ESI-MS *m*/*z*: 519.4 $[M+1]^+$.

(6-((Cyclohexylmethyl)amino)-2-(4-nitrophenylsulfonamido)-9*H*-purine-9-yl)methyl pivalate (14b)

Yield 45%. m.p. 168－169 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.93－1.00 (m, 2H), 1.16 (s, 9H), 1.21－1.30 (m, 3H), 1.69－1.78 (m, 6H), 3.28 (br s, 2H), 5.82 (br s, 1H), 6.00 (s, 2H), 8.02 (s, 1H), 8.35－8.38 (m, 4H); ESI-MS *m*/*z*: 546.4 $[M+1]^+$.

(6-((Cyclohexylmethyl)amino)-2-(4-(trifluoromethoxy)phenylsulfonamido)-9*H*-purine-9-yl)methyl pivalate (14c)

Yield 38%. m.p. 126－127 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.95－0.98 (m, 2H), 1.17 (s, 9H), 1.22－1.31 (m, 3H), 1.68－1.74 (m, 6H), 3.29 (br s, 2H), 5.97 (s, 2H), 7.25 (br s, 1H), 7.33 (d, *J*＝8.4Hz, 2H), 7.88 (s, 1H), 8.22 (d, *J*＝8.4 Hz, 2H); ESI-MS *m*/*z*: 585.4 $[M+1]^+$.

General synthesis of *N*-(6-((cyclohexylmethyl)-amino)-9*H*-purine-2-yl)-substituted benzenesulfonamide (15a－15c)

The material **14** (0.3 mmol) was dissloved in 3 mL THF, then 3 mol/L NaOH (0.3 mL, 0.9 mmol) was added. After vigorous stirring for at least 1 h, the solvent was removed under reduced pressure. Then 2 mol/L HCl was used to adjust pH＝4. The precipitate was collected and washed with methanol to get target compounds.

***N*-(6-((Cyclohexylmethyl)amino)-9*H*-purine-2-yl)-4-fluorobenzenesulfonamide (15a)**

Yield 78%. m.p. 256 ℃ (decomposition point); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.75－0.77 (m, 2H), 1.11－1.16 (m, 3H), 1.46－1.63 (m, 6H), 3.03－3.05 (m, 2H), 7.26－7.45 (m, 2H), 7.74－8.14 (m, 4H), 11.17 (br s, 1H), 12.70 (br s, 1H); HRMS calcd for $C_{18}H_{21}FN_6O_2S$ 405.1509, found 405.1502. Anal. RP-HPLC t_R＝8.1 min [acetonitrile：water (containing 0.1% formic acid)＝40：60; purity＝98.7%].

***N*-(6-((Cyclohexylmethyl)amino)-9*H*-purine-2-yl)-4-nitrobenzenesulfonamide (15b)**

Yield 85%. m.p. 246 ℃ (decomposition point); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.67－0.73 (m, 2H), 1.00－1.08 (m, 3H), 1.51－1.59 (m, 6H), 2.95 (br s, 2H), 8.08 (d, *J*＝7.8 Hz, 2H), 8.17－8.21 (m, 2H), 8.37 (d, *J*＝7.8 Hz, 2H); HRMS calcd for $C_{18}H_{21}N_7O_4S$ 432.1454, found 432.1447. Anal. RP-HPLC t_R＝6.4 min [acetonitrile：water (containing 0.1% formic acid)＝40：60; purity＝95.5%].

***N*-(6-((Cyclohexylmethyl)amino)-9*H*-purine-2-yl)-4-(trifluoromethoxy)benzenesulfonamide (15c)**

Yield 82%. m.p. 261 ℃ (decomposition point); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 0.76－0.78 (m, 2H), 1.09－1.16 (m, 3H), 1.45－1.65 (m, 6H), 2.98－3.01 (m, 2H), 7.53 (d, *J*＝6.6 Hz, 2H), 7.79 (s, 1H), 7.99－8.20 (m, 3H); HRMS calcd for $C_{19}H_{21}F_3N_6O_3S$ 471.1426, found 471.1425. Anal. RP-HPLC t_R＝34.0 min [methanol：water (containing 0.1% formic acid)＝60：40; purity＝96.2%].

Biological assay

Biological activities of the target compounds include two parts: enzyme inhibition assays and cell inhibition assays.

CDK1/cyclin B enzyme inhibition assays

The IC_{50} values of tested compounds in inhibiting CDK1/cyclin B enzyme activities were measured by a homogeneous time-resolved fluorescence (HTRF) assay in a 384-well format. The enzymatic reaction buffer contained 250 mmol/L Hepes, 0.5 mmol/L Orthovanadate, 5 mmol/L $MgCl_2$, 1 mmol/L DTT, 0.1% NaN_3 and 0.05% BSA. HTRF detection buffer contained 50 mmol/L Hepes, 0.8 mol/L KF, 20 mmol/L EDTA and 0.1% BSA. ATP as follows: 10 μmol/L. For all test compounds, 100 μmol/L was used as the starting point. Three folds, ten serial dilutions, the concentrations tested were from 100 to 5.08 nmol/L. Positive control was subtracted and activities were expressed in % of the

maximal activity, *i.e.*, in the absence of inhibitors. Negative controls experiments were performed with no enzyme in the plate and the inhibition was 100%. The reaction was started by the addition of 4 μL compound to the reaction well first, then 2 μL CDK1 enzyme (8 ng/ well, Carna Biosciences) was added and incubated with compound for 15 min at room temperature, later 2 μL substrate Rb (Ser780; Cell Signaling Technology, 1 mmol/L) biotinylated peptide and 2 μL ATP were added. The reaction plate was incubated at room temperature for 70 min. At last, Streptavidin-XL665 (0.0625 mol/L) and Eu-anti-P-Rb (30 to 50 K cpm per well) (each 5 μL) were added, the plate was sealed and incubated for 1 h at room temperature. Finally, read the plate on an Infinite® F500 instrument and do data analysis. The IC_{50} values were calculated from inhibition% which was determined based on the ratio of readings at 665 nm and normalized for Europium readings at 620 nm. Ratio was calculated according to the equation: Ratio＝[665 nm counts/620 nm counts]×10000. Then inhibition was calculated by the equation based on ratio above: Inhibition %＝[(positive control ratio−compound ratio)/(positive control ratio−negative control ratio)]×100.

CDK2/cyclin A enzyme inhibition assays

A Kinase-Glo Luminescent Kinase Assay was used to test the IC_{50} values of the target compounds in inhibiting CDK2/cyclin A enzyme activities. Assay reagent included: assay buffer (25 mmol/L HEPES, 10 mmol/L $MgCl_2$, 0.01% Triton X-100, 100 μg/mL BSA, 2.5 mmol/L DTT, pH7.4), kinase-glo reagent (Promega, Cat#V6711), CDK2/Cyclin A2 (Signalchem, Cat#C22-18G), substrate (Histone H derived peptide: HDB, H09-19T) and ATP (Sigma, Cat#A7699) final concentration 10 μmol/L.

For all test compounds, 20 μmol/L was used as the starting point. Five folds, six serial dilutions, the concentrations tested were from 20 μmol/L to 6.4 nmol/L. HPE (hundred percent effect): No compound, no enzyme, but containing ATP and substrate; ZPE (zero percent effect): No compound, but containing ATP, substrate and kinase. The reaction was started by the addition of 1 μL compound to the reaction well first, then 2 μL CDK2/cyclin A2 enzyme was added and incubated with compound for 5 min at room temperature, later 2 μL substrate and 5 μL ATP were added. The reaction plate was incubated at 30 ℃ for 1 h. To the reaction mixture, 10 μL of the appropriate kinase-glo reagent was added and incubated at 27 ℃ for 20 min. Read the assay plate on Envision at last. The IC_{50} values were calculated from inhibition%, which was calculated according to the equation: Compound inhibition rate% ＝[(“compound reading”–ZPE)/(HPE– ZPE)]×100.

Cell inhibition assays

HCT116, MDA-MB-231 and PC3 cell lines were plated on 96-well plates at a density of 5000 per well and incubated in RPMI1640 medium containing 10% FBS overnight at 37 ℃ in a 5% CO_2 humidified incubator. The cells were treated with compounds and Roscovitine at final concentrations ranging from 1.5625 to 100 μmol/L, while control cells were treated with equal volume DMSO. After 48 h treatment, 0.5% MTT (Amresco, USA) solution was added to each well, and further incubated for 4 h, then cells were centrifuged at 2500 r/min for 12 min, the culture medium was removed, and 100 μL DMSO was added to dissolve the formazan. After mixing for 2 min, optical density was detected at 570 nm on a microplate reader (Thermo, USA), and the IC_{50} values were calculated according to the inhibition ratios.

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