Research Article

Design, Synthesis, and Acetylcholinesterase Inhibition Assay of Novel 9-(1-(Substituted-benzyl)piperidin-4-yl)-2-chloro-9H-purin-6-amine Derivatives

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A new series of 9-(1-(substituted-benzyl)piperidin-4-yl)-2-chloro-9H-purin-6-amine derivatives were designed, synthesized, and characterized on the basis of ¹H-NMR, ¹³C-NMR, and mass spectra. The newly synthesized compounds were evaluated for the inhibition activity against acetylcholinesterase (AChE). Biological results revealed that four compounds among them showed moderate activities against AChE with inhibitory percentage more than 10% at 100 μ M. Further pharmacology investigation towards other pathological routes in AD is currently underway.

1. Introduction

Since a cholinergic hypothesis has been postulated as a crucial element in Alzheimer's disease (AD) symptoms etiology, catalytic acetylcholinesterase (AChE) inhibitors have constituted until today the main drugs used against AD [1]. Reversible inhibition of brain AChE by anti-AD drugs such as tacrine [2], donepezil (Figure 1) [3], and huperzine [4] increases the AChE levels and improves neurotransmission in cholinergic synapses [5]. Since AChE plays a proaggregating (noncatalytic) role to accelerating β -amyloid peptide (A β) aggregation and deposition into the fibrils, inhibition of AChE is still the most successful therapeutic strategy for the symptomatic treatment of AD and its progression [6]. The search for novel AChE inhibitors with improved biological profiles continues to be of great interest to medicinal chemists.

In the past few years, a group of 2,4-disubstituted pyrimidine derivatives were reported as cholinesterase inhibitors and agents targeting multiple pathological routes in AD. Particularly, compound **PY-1** was identified as the lead

candidate with a dual ChE (AChE IC_{50} = 9.9 μ M; BuChE IC_{50} = 11.4 μ M), A β -aggregation (AChE-induced = 59.3%; self-induced = 17.4% at 100 μ M), and BACE-1 (34% inhibition at 10 μ M) inhibitory profile along with good cell viability (81.0% neuroblastoma cell viability at 40 μ M) [7–10]. Meanwhile, *o*-fluorophenyl methyl derived triazole **PU2012051296**, effectively suppressed A β -induced neurotoxicity in hippocampal slice cultures. Importantly, the neuroprotective effect of compound **PU2012051296** is comparable to those of flavopiridol and roscovitine, state of the art pharmaceuticals [11].

The refinement of the central core in bioactive molecules combined with introducing privileged substituents is a common practice in medicinal chemistry to find proprietary and novel hits. Based on the considerable similarities of these lead compounds, a fragment-based design was employed to identify alternative chemotypes (Figure 2). Meanwhile, in current drug research field, substituted purines have attracted considerable attention from the medicinal chemists in recent years, owing to the high number of positive hits encountered with this heterocycle. In this paper, a novel

FIGURE 1: Structure of donepezil, 2,4-disubstituted pyrimidine AChE inhibitors, and purine derivative PU2012051296.

FIGURE 2: The general structure formula of newly designed 9-(1-(substituted-benzyl)piperidin-4-yl)-2-chloro-9H-purin-6-amine derivatives.

series of 9-(1-(substituted-benzyl)piperidin-4-yl)-2-chloro-9H-purin-6-amine derivatives were designed, synthesized, and evaluated for the inhibition activity against AChE.

2. Results and Discussion

2.1. Chemistry. The synthetic route of the target compounds is outlined in Scheme 1, which was started from commercially available materials and completed using well established methods. Initially, N-(3,4-dimethoxybenzyl)-2-chloro-9H-purin-6-amine (**2a**) and N-(benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9H-purin-6-amine (**2b**) intermediates were synthesized from the starting material 2,6-dichloro-9H-purine (**1**) by a nucleophilic aromatic substitution reaction at the C-6 position using either (3,4-dimethoxyphenyl) methanamine or benzo[d][1,3]dioxol-5-ylmethanamine in the presence of triethylamine (TEA). The reaction was run in *t*-BuOH at 60°C for 30 min. Intermediates **6** and **7** were obtained in good yields ranging (90.5% and 89.7%)

(Scheme 1) [7–10]. In the meantime, *tert*-butyl 4-hydroxypiperidine-1-carboxylate (**4**) was synthesized according to the reported procedure [12]. Then treatment of intermediate **4** with compound **2a** or **2b** in the presence of NaH afforded *tert*-butyl 4-(6-(3,4-dimethoxybenzylamino)-2-chloro-9H-purin-9-yl)piperidine-1-carboxylate (**5a**) or *tert*-butyl 4-(6-(benzo[d][1,3]dioxol-5-ylmethylamino)-2-chloro-9H-purin-9-yl)piperidine-1-carboxylate (**5b**), respectively. The deprotection of the *tert*-butoxycarbonyl (*t*-Boc) group of **5a** and **5b** was accomplished using trifluoroacetic acid (TFA) in dichloromethane (DCM) to yield N-(3,4-dimethoxybenzyl)-2-chloro-9-(piperidin-4-yl)-9H-purin-6-amine (**6a**) and N-(benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9-(piperidin-4-yl)-9H-purin-6-amine (**6b**), respectively, in good yield (91% and 87%). In the last step, the alkylation reaction of piperidine at NH position with various substituted benzyl chloride (bromine) afforded the target compounds. The newly synthesized compounds were characterized by physico-chemical and spectral means, and the MS and NMR spectral

2a, 5a, 6a, 7a1–a13: Ar = 3,4-dimethoxyphenyl

2b, 5b, 6b, 7b1–b13: Ar = benzo[d][1,3]dioxol-5-yl

SCHEME 1: *Reagents and Conditions.* (i) *t*-BuOH, Et₃N; (ii) methanesulfonyl chloride (MsCl), DCM, Et₃N, and 4-DMAP; (iii) NaH, DMF; (iv) TFA, DCM; (v) substituted benzyl chloride (bromine, fluorine), DMF, and K₂CO₃.

data are found in agreement with the assigned molecular structures.

2.2. Cholinesterase Inhibition. All the newly synthesized compounds (**7a1–a13**, **7b1–b13**) were evaluated for their inhibitory activities toward AChE using an in vitro assay based on the reported protocol [7–10], in comparison with donepezil as standard drug. The anticholinesterase activities are summarized in Table 1. Preliminary results in Table 1 revealed that compound **7b9** with a 3-cyanobenzyl group in the benzo[d][1,3]dioxol-5-yl series displayed the highest AChE inhibitory activity (15.42% at 100 μ M) among all the tested compounds. Besides, compounds **7a1**, **7a7**, and **7b10** with inhibitory percentage more than 10% at 100 μ M showed similar activities with compound **7b9** against AChE. Generally, all the compounds in both 3,4-dimethoxyphenyl and benzo[d][1,3]dioxol-5-yl series demonstrated much lower inhibitory activity toward AChE than the reference drug donepezil (80.07% at 100 μ M). Obviously, the activity of the compounds depends upon the nature of the substituents attached with purine ring. Even small modifications in every series can decrease and even lost their activity against AChE. Therefore, the coherent structure-activity relationship will be afforded by further pharmacology investigation, which is currently underway in our lab.

3. Conclusion

In summary, a novel series of 9-(1-(substituted-benzyl) piperidin-4-yl)-2-chloro-9H-purin-6-amine derivatives were designed and synthesized, and their AChE inhibitory activities were preliminary examined. Biological assay demonstrated that four compounds showed moderate activities against AChE with inhibitory percentage more than 10% at 100 μ M. Further investigation of other bioactivities associated with AD of this series is ongoing work within our group and will be reported in due course.

4. Experimental Section

4.1. Chemistry. All melting points were determined on a micromelting point apparatus and are uncorrected. ¹H-NMR and ¹³C-NMR spectra were obtained on a Bruker Avance-400 NMR-spectrometer in the indicated solvents. Chemical shifts are expressed in δ units and TMS as internal reference. Mass spectra were taken on an LC Autosampler Device: Standard G1313A instrument. TLC was performed on Silica Gel GF254 for TLC (Merck) and spots were visualized by iodine evapours or by irradiation with UV light ($\lambda = 254$ nm). Flash column chromatography was performed on column packed with Silica Gel60 (230–400 mesh). Solvents were reagent grade and when necessary were purified and dried by standard methods. Concentration of the reaction solutions involved the use of rotary evaporator at reduced pressure.

4.1.1. General Procedure for the Preparation of 2a and 2b. 2,6-Dichloro-9H-purine (1.89 g, 10 mmol) and Et₃N (1.6 mL, 11 mmol) was dissolved in *t*-BuOH (50 mL), and the mixed solution of 3,4-dimethoxybenzylamine (**1a**, 1.50 mL, 10 mmol) and *t*-BuOH (20 mL) or benzo[d][1,3]dioxol-5-ylmethanamine (**1b**, 1.27 mL, 10 mmol) and *t*-BuOH (20 mL) was slowly added to the previous solution in 20 min at 60°C. The resulting mixture was stirred at 60°C for 30 min. Then the reaction mixture was poured into cold H₂O (100 mL), the resulting precipitate was collected by filtration under reduced pressure and washed sequentially with H₂O then dried to give the corresponding product **2a** as a white power with yield of 90.5% or **2b** as a white power with yield of 89.7%.

4.1.2. General Procedure for the Preparation of 4. *N*-Boc-4-hydroxypiperidine (**3**, 8.05 g, 40 mmol) was dissolved in DCM (50 mL), then methanesulfonyl chloride (3.4 mL, 44 mmol), Et₃N (6.7 mL, 48 mmol) and 4-DMAP (0.05 g, 4.0 mmol) were added to the above solution slowly in turn at 0°C. The mixed solution of methanesulfonyl chloride (3.4 mL,

TABLE 1: Cholinesterase inhibition activity of 9-(1-(substituted-benzyl)piperidin-4-yl)-2-chloro-9H-purin-6-amine derivatives^a.

Compd.	Ar	R ₁	Cholinesterase inhibition % at 100 μM
7a1	3,4-Dimethoxyphenyl	3-Chlorobenzyl	11.03
7a2	3,4-Dimethoxyphenyl	3-bromobenzyl	0.24
7a3	3,4-Dimethoxyphenyl	4-Cyanobenzyl	0.47
7a4	3,4-Dimethoxyphenyl	4-Nitrobenzyl	3.84
7a5	3,4-Dimethoxyphenyl	3-Fluorobenzyl	3.48
7a6	3,4-Dimethoxyphenyl	Pyridine-4-yl-methyl	NA ^b
7a7	3,4-Dimethoxyphenyl	Benzyl	14.57
7a8	3,4-Dimethoxyphenyl	4-Bromobenzyl	NA
7a9	3,4-Dimethoxyphenyl	3-Cyanobenzyl	6.10
7a10	3,4-Dimethoxyphenyl	4-Methoxybenzyl	NA
7a11	3,4-Dimethoxyphenyl	2,6-Dicholobenzyl	NA
7a12	3,4-Dimethoxyphenyl	2,4,6-Trimethylbenzyl	NA
7a13	3,4-Dimethoxyphenyl	2-Fluorobenzyl	4.02
7b1	Benzo[d][1,3]dioxol-5-yl	3-Chlorobenzyl	NA
7b2	Benzo[d][1,3]dioxol-5-yl	3-Bromobenzyl	0.09
7b3	Benzo[d][1,3]dioxol-5-yl	4-Cyanobenzyl	NA
7b4	Benzo[d][1,3]dioxol-5-yl	4-Nitrobenzyl	NA
7b5	Benzo[d][1,3]dioxol-5-yl	3-Fluorobenzyl	0.14
7b6	Benzo[d][1,3]dioxol-5-yl	Pyridine-4-yl-methyl	NA
7b7	Benzo[d][1,3]dioxol-5-yl	Benzyl	7.55
7b8	Benzo[d][1,3]dioxol-5-yl	4-Bromobenzyl	7.29
7b9	Benzo[d][1,3]dioxol-5-yl	3-Cyanobenzyl	15.42
7b10	Benzo[d][1,3]dioxol-5-yl	4-Methoxybenzyl	11.21
7b11	Benzo[d][1,3]dioxol-5-yl	2,6-Dicholobenzyl	3.80
7b12	Benzo[d][1,3]dioxol-5-yl	2,4,6-Trimethylbenzyl	1.51
7b13	Benzo[d][1,3]dioxol-5-yl	2-Fluorobenzyl	6.14
Donepezil			80.07

^aSee the experimental section for assay details. ^bNot active.

44 mmol) and DCM (20 mL) was slowly added to the mixed solution in 30 min at 60°C. The mixtures were stirred at 0°C overnight. Then the reaction solution was alkalized to PH = 9 with NaHCO₃. The solution was washed by water (50 mL) then extracted with DCM (3 × 15 mL). Combined organic phase dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to give the product **4** as white solid. Yield: 94.3%.

4.1.3. General Procedure for the Preparation of **5a** and **5b**.

2-Chloro-*N*-(3,4-dimethoxybenzyl)-9H-purin-6-amine (**2a**, 3.20 g, 10 mmol) or *N*-(benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9-(piperidin-4-yl)-9H-purin-6-amine (**2b**, 3.03 g, 10 mmol) was dissolved in DMF (60 mL) and added 60% dispersion NaH (0.48 g, 12 mmol) to the solution at room temperature. Then it slowly added the intermediate **4** (3.10 g,

11 mmol) that dissolved in DMF (20 mL) to the previous mixed solution in 20 min at 60°C. The reaction mixture was rapid stirred at 100°C for 16 h (monitored by TLC). After removal of the solvent under reduced pressure, water (30 mL) was added and extracted with ethyl acetate (3 × 10 mL). Combined organic phase was washed with brine and dried over anhydrous Na₂SO₄ to give the corresponding crude product, which was purified by flash column chromatography to afford compounds **5a** as a pink crystal with yield of 31.5% or **5b** as a pink crystal with yield of 29.7%.

4.1.4. General Procedure for the Preparation of **6a** and **6b**.

To a solution of **5a** (600 mg, 1.19 mmol) or **5b** (600 mg, 1.23 mmol) in DCM (20 mL) TFA was added (3 mL) at room temperature and the solution was stirred for 5 h. Then the reaction solution was alkalized to PH = 9 with NaHCO₃. The solution was

washed by water (50 mL) and then extracted with DCM (3 × 15 mL). Combined organic phase dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to give the product **6a** or **6b**.

2-Chloro-N-(3,4-dimethoxybenzyl)-9-(piperidin-4-yl)-9H-purin-6-amine (6a). White crystal, yield 91%, mp: 157–160°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.73 (1H, s), 8.25 (1H, s), 7.07 (1H, s), 6.87 (2H, d, *J* = 4.04 Hz), 5.08 (1H, s, NH), 4.55 (2H, d, *J* = 5.36 Hz), 4.36 (1H, dt, *J*₁ = 10.80 Hz, *J*₂ = 5.12 Hz), 3.74 (3H, s, OCH₃), 3.71 (3H, s, OCH₃), 3.08 (2H, d, *J* = 8.88 Hz), 2.65 (2H, dt, *J*₁ = 14.28 Hz, *J*₂ = 3.52 Hz), 1.92–1.94 (4H, m). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.3, 153.2, 149.8, 149.0, 148.3, 140.0, 132.1, 120.1, 118.9, 112.4, 112.1, 56.0, 55.8, 53.3, 45.6 (2C), 43.5, 33.0 (2C). ESI-MS: *m/z* 403.5 (M + 1), 405.5 (M + 3), 525.3 (M + Na). C₁₉H₂₃ClN₆O₂ (402.16).

N-(Benzo[d][1,3]Dioxol-5-ylmethyl)-2-chloro-9-(piperidin-4-yl)-9H-purin-6-amine (6b). White crystal, yield 87%, mp: 205–209°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.73 (1H, s), 8.26 (1H, s), 6.93 (1H, s), 6.83 (2H, d, *J* = 7.68 Hz), 5.97 (2H, s, OCH₂O), 5.05 (1H, s, NH), 4.54 (2H, d, *J* = 4.92 Hz), 4.35 (1H, dt, *J*₁ = 10.48 Hz, *J*₂ = 5.40 Hz), 3.05 (2H, d, *J* = 12.32 Hz), 2.60 (2H, dt, *J*₁ = 14.76 Hz, *J*₂ = 3.60 Hz), 1.96 (4H, dd, *J*₁ = 16.64 Hz, *J*₂ = 2.80 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.3, 153.2, 149.9, 147.6, 146.6, 140.1, 133.7, 121.1, 118.9, 101.3, 55.3, 53.4, 45.7 (2C), 43.4, 33.2 (2C). ESI-MS: *m/z* 387.3 (M + 1), 389.3 (M + 3), 409.4 (M + Na). C₁₈H₁₉ClN₆O₂ (386.13).

4.1.5. General Procedure for the Target Compounds 7a and 7b. Compound **6a** (or **6b**) was dissolved in anhydrous DMF (10 mL) in the presence of anhydrous K₂CO₃ (1.2 eq), followed by addition of appropriate substituted benzyl chloride (bromine, fluorine) (1.1 eq). The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, and then water (20 mL) was added. Extracted with ethyl acetate (3 × 10 mL), and the organic phase was washed with saturated sodium chloride (10 mL) then dried over anhydrous Na₂SO₄ to give the corresponding crude product, which was purified by flash column chromatography to afford compounds **7a1–13** (or **7b1–13**).

2-Chloro-9-(1-(3-chlorobenzyl)piperidin-4-yl)-N-(3,4-dimethoxybenzyl)-9H-purin-6-amine (7a1). Recrystallized from EA/PE as a white crystal, yield 62.3%, mp: 176–180°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.30 (1H, s), 7.29–7.39 (4H, m), 7.06 (1H, s), 6.86 (2H, d, *J* = 4.32 Hz), 5.07 (1H, s, NH), 4.55 (2H, d, *J* = 7.24 Hz), 4.30 (1H, dt, *J*₁ = 8.32 Hz, *J*₂ = 3.27 Hz), 3.73 (3H, s, OCH₃), 3.71 (3H, s, OCH₃), 3.54 (2H, s), 2.92 (2H, d, *J* = 7.80 Hz), 2.11–2.19 (4H, m), 1.93 (2H, d, *J* = 8.80 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.3, 153.2, 150.0, 149.0, 148.3, 141.7, 140.1, 133.4, 132.2, 130.5, 128.9, 127.9, 127.4, 120.1, 118.9, 112.5, 112.2, 61.4, 56.0, 55.9, 52.9, 52.5 (2C), 43.5, 31.9 (2C). ESI-MS: *m/z* 527.3 (M + 1), 529.2 (M + 3), 549.3 (M + Na). C₂₆H₂₈Cl₂N₆O₂ (526.17).

2-Chloro-9-(1-(3-bromobenzyl)piperidin-4-yl)-N-(3,4-dimethoxybenzyl)-9H-purin-6-amine (7a2). Recrystallized from

EA/PE as a white crystal, yield 57.2%, mp: 187–190°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.31 (1H, s), 7.61 (1H, dd, *J*₁ = 7.96, *J*₂ = 1.0 Hz), 7.52 (1H, dd, *J*₁ = 7.64 Hz, *J*₂ = 1.52 Hz), 7.40 (1H, dt, *J*₁ = 7.48 Hz, *J*₂ = 1.0 Hz), 7.23 (1H, dt, *J*₁ = 7.76 Hz, *J*₂ = 1.68 Hz), 7.06 (1H, s), 6.86 (2H, d, *J* = 8.26 Hz), 5.07 (1H, s, NH), 4.54 (2H, d, *J* = 5.44 Hz), 4.34 (1H, dt, *J*₁ = 11.72 Hz, *J*₂ = 3.84 Hz), 3.73 (3H, s, OCH₃), 3.71 (3H, s, OCH₃), 3.60 (2H, s), 2.96 (2H, d, *J* = 11.52 Hz), 2.27 (2H, t, *J* = 11.68 Hz), 2.08–2.17 (2H, m), 1.95–1.99 (2H, d, *J* = 7.28 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.3, 153.2, 150.0, 149.0, 148.3, 140.0, 138.0, 133.0, 132.2, 131.3, 129.4, 128.0, 124.4, 120.1, 119.0, 112.5, 112.2, 61.4, 56.0, 55.9, 52.8, 52.7 (2C), 43.5, 31.9 (2C). ESI-MS: *m/z* 573.2 (M + 3), 574.4 (M + 4), 576.2 (M + 6). C₂₆H₂₈BrClN₆O₂ (570.11).

2-Chloro-9-(1-(4-cyanobenzyl)piperidin-4-yl)-N-(3,4-dimethoxybenzyl)-9H-purin-6-amine (7a3). Recrystallized from EA/PE as a white crystal, yield 66.5%, mp: 193–196°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.30 (1H, s), 7.82 (2H, d, *J* = 4.10 Hz), 7.54 (2H, d, *J* = 4.08 Hz), 7.06 (1H, s), 6.87 (2H, dd, *J*₁ = 8.64 Hz, *J*₂ = 3.26 Hz), 5.07 (1H, s, NH), 4.54 (2H, d, *J* = 5.20 Hz), 4.32 (1H, dt, *J*₁ = 8.62 Hz, *J*₂ = 3.56 Hz), 3.74 (3H, s, OCH₃), 3.71 (3H, s, OCH₃), 3.62 (2H, s), 2.90 (2H, d, *J* = 9.28 Hz), 2.17 (4H, dd, *J*₁ = 16.60, *J*₂ = 8.88 Hz), 1.95 (2H, d, *J* = 11.56 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.3, 153.2, 149.9, 149.0, 148.3, 145.1, 140.1, 132.6 (2C), 132.1, 130.0 (2C), 120.1, 119.3, 118.9, 112.4, 112.1, 110.2, 61.6, 56.0, 55.8, 52.8, 52.6 (2C), 43.5, 31.8 (2C). ESI-MS: *m/z* 518.4 (M + 1), 540.4 (M + Na). C₂₇H₂₈ClN₇O₂ (517.20).

2-Chloro-9-(1-(4-nitrobenzyl)piperidin-4-yl)-N-(3,4-dimethoxybenzyl)-9H-purin-6-amine (7a4). Recrystallized from EA/PE as a yellow crystal, yield: 53.4%, mp: 209–212°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.29 (1H, s), 8.21 (2H, d, *J* = 8.12 Hz), 7.62 (2H, d, *J* = 8.08 Hz), 7.06 (1H, s), 6.89 (2H, d, *J* = 4.68 Hz), 5.07 (1H, s, NH), 4.55 (2H, d, *J* = 6.32 Hz), 4.32 (1H, dt, *J*₁ = 11.32, *J*₂ = 3.28 Hz), 3.74 (3H, s, OCH₃), 3.71 (3H, s, OCH₃), 3.67 (2H, s), 2.92 (2H, t, *J* = 9.08 Hz), 2.14–2.25 (4H, m), 1.97 (2H, d, *J* = 4.58 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.3, 153.2, 150.0, 149.0, 148.3, 147.3, 147.1, 140.1, 132.1, 130.2 (2C), 124.0 (2C), 120.1, 118.9, 112.4, 112.1, 61.3, 56.0, 55.8, 52.8, 52.6 (2C), 43.5, 31.8 (2C). ESI-MS: *m/z* 538.4 (M + 1), 540.4 (M + 3), 560.3 (M + Na). C₂₆H₂₈ClN₇O₄ (537.19).

2-Chloro-9-(1-(3-fluorobenzyl)piperidin-4-yl)-N-(3,4-dimethoxybenzyl)-9H-purin-6-amine (7a5). Recrystallized from EA/PE as a white crystal, yield: 49.7%, mp: 183–185°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.30 (1H, s), 7.38 (1H, dt, *J*₁ = 8.04 Hz, *J*₂ = 6.16 Hz), 7.17 (2H, dd, *J*₁ = 9.36 Hz, *J*₂ = 6.28 Hz), 7.09 (2H, s), 6.87 (2H, d, *J* = 3.26 Hz), 5.07 (1H, s, NH), 4.55 (2H, d, *J* = 5.56 Hz), 4.31 (1H, dt, *J*₁ = 10.28 Hz, *J*₂ = 3.68 Hz), 3.74 (3H, s, OCH₃), 3.71 (3H, s, OCH₃), 3.55 (2H, s), 2.93 (2H, d, *J* = 7.16 Hz), 2.11–2.17 (4H, m), 1.93 (2H, d, *J* = 8.88 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 163.9, 161.5, 155.3, 153.2, 149.9, 149.0, 148.3, 142.0, 140.0, 132.1, 130.5, 125.2, 120.1, 118.9, 115.7, 114.3, 112.4, 61.5, 56.0, 55.8, 52.8, 52.5 (2C), 43.5, 31.8 (2C). ESI-MS: *m/z* 511.5 (M + 1), 513.4 (M + 3), 515.4 (M + 5), 533.3 (M + Na). C₂₆H₂₈ClFN₆O₂ (510.19).

2-Chloro-9-(1-(pyridine-4-yl-methyl)piperidin-4-yl)-N-(3,4-dimethoxybenzyl)-9H-purin-6-amine (7a6). Recrystallized from EA/PE as a yellow crystal, yield: 61.2%, mp: 158–161°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.53 (2H, dd, *J*₁ = 4.56 Hz, *J*₂ = 1.36 Hz), 8.30 (1H, s), 7.35 (2H, d, *J* = 5.80 Hz), 7.06 (1H, s), 6.86 (2H, d, *J* = 2.28 Hz), 5.06 (1H, s, NH), 4.53 (2H, d, *J* = 5.44 Hz), 4.32 (1H, dt, *J*₁ = 7.28 Hz, *J*₂ = 3.76 Hz), 3.73 (3H, s, OCH₃), 3.70 (3H, s, OCH₃), 3.57 (2H, s), 2.92 (2H, d, *J* = 9.40 Hz), 2.13–2.20 (4H, m), 1.94 (2H, d, *J* = 11.2 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.3, 153.2, 150.3 (2C), 149.0, 148.3, 148.0, 140.1, 132.2, 124.2 (2C), 120.2, 119.3, 118.9, 112.5, 112.2, 60.9, 56.0, 55.9, 52.7, 52.6 (2C), 43.5, 31.8 (2C). ESI-MS: *m/z* 494.4 (M + 1), 496.4 (M + 3), 516.5 (M + Na). C₂₅H₂₈ClN₇O₂ (493.20).

2-Chloro-9-(1-benzylpiperidin-4-yl)-N-(3,4-dimethoxybenzyl)-9H-purin-6-amine (7a7). Recrystallized from EA/PE as a white crystal, yield: 55.7%, mp: 179–182°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.29 (1H, s), 7.33 (4H, t, *J* = 4.68 Hz), 7.27 (1H, t, *J* = 4.24 Hz), 7.06 (1H, s), 6.87 (2H, d, *J* = 5.06 Hz), 5.07 (1H, s, NH), 4.55 (2H, d, *J* = 5.16 Hz), 4.31 (1H, dt, *J*₁ = 9.26 Hz, *J*₂ = 4.04 Hz), 3.73 (3H, s, OCH₃), 3.70 (3H, s, OCH₃), 3.52 (2H, s), 2.93 (2H, d, *J* = 6.24 Hz), 2.12–2.14 (4H, m), 1.94 (2H, d, *J* = 6.43 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.3, 153.2, 150.0, 149.0, 148.3, 140.1, 138.9, 132.1, 129.3 (2C), 128.7 (2C), 127.4, 120.1, 118.9, 112.3, 112.1, 62.3, 56.0, 55.8, 52.9, 52.6 (2C), 43.5, 31.8 (2C). ESI-MS: *m/z* 493.4 (M + 1), 495.3 (M + 3), 515.4 (M + Na). C₂₆H₂₉ClN₆O₂ (492.20).

2-Chloro-9-(1-(4-bromobenzyl)piperidin-4-yl)-N-(3,4-dimethoxybenzyl)-9H-purin-6-amine (7a8). Recrystallized from EA/PE as a white crystal, yield 52.5%, mp: 209–212°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.30 (1H, s), 7.52 (2H, d, *J* = 1.60 Hz), 7.29 (2H, d, *J* = 8.36 Hz), 7.07 (1H, s), 6.87 (2H, d, *J* = 4.38 Hz), 5.07 (1H, s, NH), 4.55 (2H, d, *J* = 5.76 Hz), 4.29 (1H, dt, *J*₁ = 8.37 Hz, *J*₂ = 5.76 Hz), 3.74 (3H, s, OCH₃), 3.71 (3H, s, OCH₃), 3.49 (2H, s), 2.91 (2H, d, *J* = 6.36 Hz), 1.99–2.14 (4H, m), 1.93 (2H, d, *J* = 6.24 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.3, 153.2, 150.0, 149.0, 148.3, 140.1, 138.4, 132.1, 131.5 (2C), 131.4 (2C), 120.4, 120.1, 118.9, 112.4, 112.1, 61.4, 56.0, 55.8, 52.8, 52.5 (2C), 43.5, 31.8 (2C). ESI-MS: *m/z* 571.3 (M + 1), 573.2 (M + 3), 575.3 (M + 5), 595.3 (M + Na). C₂₆H₂₈BrClN₆O₂ (570.11).

2-Chloro-9-(1-(3-cyanobenzyl)piperidin-4-yl)-N-(3,4-dimethoxybenzyl)-9H-purin-6-amine (7a9). Recrystallized from EA/PE as a white crystal, yield 62.1%, mp: 209–210°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.30 (1H, s), 7.77 (1H, dd, *J*₁ = 25.48 Hz, *J*₂ = 7.60 Hz), 7.68 (2H, d, *J* = 7.80 Hz), 7.56 (1H, t, *J* = 7.68 Hz), 7.06 (1H, s), 6.87 (2H, d, *J* = 9.84 Hz), 5.07 (1H, s, NH), 4.55 (2H, d, *J* = 5.16 Hz), 4.33 (1H, dt, *J*₁ = 10.84, *J*₂ = 3.26 Hz), 3.73 (3H, s, OCH₃), 3.71 (3H, s, OCH₃), 3.59 (2H, s), 2.93 (2H, d, *J* = 9.16 Hz), 2.11–2.18 (4H, m), 1.93 (2H, d, *J* = 12.80 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.3, 153.2, 150.0, 149.0, 148.3, 140.8, 140.1, 134.1, 132.6, 132.1, 131.3, 129.9, 120.1, 119.4, 118.9, 112.4, 111.7, 61.1, 56.0, 55.8, 52.7, 52.5 (2C), 43.5, 31.8 (2C). ESI-MS: *m/z* 518.4 (M + 1), 520.3 (M + 3), 522.6 (M + 5), 540.3 (M + Na). C₂₇H₂₈ClN₇O₂ (517.20).

2-Chloro-9-(1-(4-methoxybenzyl)piperidin-4-yl)-N-(3,4-dimethoxybenzyl)-9H-purin-6-amine (7a10). Recrystallized from EA/PE as a white crystal, yield 56.3%, mp: 202–205°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.28 (1H, s), 7.24 (2H, d, *J* = 8.56 Hz), 7.06 (1H, s), 6.90 (2H, d, *J* = 8.60 Hz), 6.86 (2H, d, *J* = 4.56 Hz), 5.07 (1H, s, NH), 4.54 (2H, d, *J* = 5.48 Hz), 4.27 (1H, dt, *J*₁ = 8.62 Hz, *J*₂ = 3.64 Hz), 3.74 (3H, s, OCH₃), 3.73 (3H, s, OCH₃), 3.70 (3H, s), 3.44 (2H, s), 2.92 (2H, d, *J* = 6.32 Hz), 2.07–2.09 (4H, m), 1.92 (2H, s). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 158.8, 155.3, 153.2, 149.9, 149.0, 148.3, 140.1, 132.1, 130.6, 130.5 (2C), 120.1, 118.9, 114.0 (2C), 112.4, 112.1, 61.7, 56.0, 55.8, 55.5, 53.0, 52.4 (2C), 43.5, 31.8 (2C). ESI-MS: *m/z* 523.5 (M + 1), 525.4 (M + 3), 545.3 (M + Na). C₂₇H₃₁ClN₆O₃ (522.21).

2-Chloro-9-(1-(2,6-dicholobenzyl)piperidin-4-yl)-N-(3,4-dimethoxybenzyl)-9H-purin-6-amine (7a11). Recrystallized from EA/PE as a white crystal, yield 58.3%, mp: 196–198°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.28 (1H, s), 7.34 (2H, dt, *J*₁ = 17.22 Hz, *J*₂ = 7.20 Hz), 7.34 (1H, dd, *J*₁ = 8.56 Hz, *J*₂ = 7.48 Hz), 7.06 (1H, s), 6.86 (2H, d, *J* = 3.82 Hz), 5.06 (1H, s, NH), 4.55 (2H, d, *J* = 4.56 Hz), 4.29 (1H, dt, *J*₁ = 10.26, *J*₂ = 4.32 Hz), 3.74 (3H, s, OCH₃), 3.72 (2H, s), 3.70 (3H, s, OCH₃), 2.93 (2H, d, *J* = 11.68 Hz), 2.40 (2H, dt, *J*₁ = 9.92 Hz, *J*₂ = 1.80 Hz), 1.93–2.06 (4H, m). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.3, 153.2, 150.0, 149.0, 148.3, 140.1, 136.5 (2C), 134.3, 132.0, 130.4, 129.2 (2C), 120.1, 118.9, 112.4, 112.1, 62.3, 56.3, 56.0, 55.8, 52.5 (2C), 43.2, 31.8 (2C). ESI-MS: *m/z* 561.2 (M + 1), 563.2 (M + 3), 565.3 (M + 5). C₂₆H₂₇Cl₃N₆O₂ (560.13).

2-Chloro-9-(1-(2,4,6-trimethylbenzyl)piperidin-4-yl)-N-(3,4-dimethoxybenzyl)-9H-purin-6-amine (7a12). Recrystallized from EA/PE as a white crystal, yield: 63.3%, mp: 203–206°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.27 (1H, s), 7.06 (1H, s), 6.86 (2H, s), 6.80 (2H, s), 5.07 (1H, s, NH), 4.54 (2H, d, *J* = 5.48 Hz), 4.40 (1H, dt, *J*₁ = 11.24 Hz, *J*₂ = 5.48 Hz), 3.73 (3H, s, OCH₃), 3.70 (3H, s, OCH₃), 3.43 (2H, s), 2.86 (2H, d, *J* = 11.4 Hz), 2.32–2.33 (6H, m), 2.19–2.21 (5H, m), 1.92–1.94 (4H, m). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.3, 153.2, 149.9, 149.0, 148.3, 139.9, 137.9 (2C), 136.0, 132.2, 132.1, 129.1 (2C), 120.1, 118.9, 112.4, 112.1, 56.0, 55.8, 55.4, 53.0, 52.3 (2C), 43.5, 32.1 (2C), 21.0, 20.2 (2C). ESI-MS: *m/z* 535.3 (M + 1), 537.4 (M + 3). C₂₉H₃₅ClN₆O₂ (534.25).

2-Chloro-9-(1-(2-fluorobenzyl)piperidin-4-yl)-N-(3,4-dimethoxybenzyl)-9H-purin-6-amine (7a13). Recrystallized from EA/PE as a white crystal, yield 54.3%, mp: 188–192°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.27 (1H, s), 7.44 (1H, dt, *J*₁ = 8.04 Hz, *J*₂ = 1.80 Hz), 7.34 (1H, dt, *J*₁ = 5.48 Hz, *J*₂ = 1.72 Hz), 7.15–7.22 (2H, m), 7.06 (1H, s), 6.86 (2H, d, *J* = 4.32 Hz), 5.07 (1H, s, NH), 4.55 (2H, d, *J* = 4.96 Hz), 4.29 (1H, dt, *J*₁ = 11.26 Hz, *J*₂ = 7.40 Hz), 3.73 (3H, s, OCH₃), 3.71 (3H, s, OCH₃), 3.58 (2H, s), 2.95 (2H, d, *J* = 10.52 Hz), 2.09–2.22 (4H, m), 1.93 (2H, d, *J* = 8.28 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 162.5, 160.1, 155.3, 153.2, 149.9, 149.0, 148.3, 140.1, 132.1, 129.6, 125.1, 124.6, 120.1, 118.9, 115.7, 115.5, 112.3, 56.0, 55.8, 54.9, 52.8, 52.4 (2C), 43.5, 31.8 (2C). ESI-MS: *m/z* 511.4 (M + 1), 513.4 (M + 3), 533.3 (M + Na). C₂₆H₂₈ClFN₆O₂ (510.19).

N-(Benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9-(1-(3-chlorobenzyl)piperidin-4-yl)-9H-purin-6-amine (**7b1**). Recrystallized from EA/PE as a white crystal, yield 55.9%, mp: 146–150°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.32 (1H, s), 7.38 (1H, d, *J* = 10.44 Hz), 7.33 (3H, dd, *J* = 9.96 Hz, 8.28 Hz), 6.93 (1H, s), 6.83 (2H, dd, *J*₁ = 8.38 Hz, *J*₂ = 2.83 Hz), 5.96 (2H, s, OCH₂O), 5.04 (1H, s, NH), 4.53 (2H, d, *J* = 5.20 Hz), 4.32 (1H, dt, *J*₁ = 9.24, *J*₂ = 3.64 Hz), 3.54 (2H, s), 2.92 (2H, d, *J* = 7.92 Hz), 2.15 (4H, t, *J* = 9.72 Hz), 1.96 (2H, t, *J* = 8.88 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.3, 153.2, 150.0, 147.6, 146.6, 141.5, 140.0, 133.6, 133.4, 130.5, 128.9, 127.9, 127.4, 126.7, 121.1, 118.9, 108.5, 101.3, 61.4, 52.8, 52.5 (2C), 43.4, 31.8 (2C). ESI-MS: *m/z* 511.3 (M + 1), 513.4 (M + 3), 533.2 (M + Na). C₂₅H₂₄Cl₂N₆O₂ (510.13).

N-(Benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9-(1-(3-bromobenzyl)piperidin-4-yl)-9H-purin-6-amine (**7b2**). Recrystallized from EA/PE as a white crystal, yield 62.1%, mp: 174–178°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.31 (1H, s), 7.61 (1H, d, *J* = 7.92 Hz), 7.52 (1H, d, *J* = 7.48 Hz), 7.39 (1H, t, *J* = 7.36 Hz), 7.21 (1H, t, *J* = 7.44 Hz), 6.92 (1H, s), 6.83 (2H, dd, *J*₁ = 12.16, *J*₂ = 4.28 Hz), 5.97 (2H, s, OCH₂O), 5.04 (1H, s, NH), 4.53 (2H, d, *J* = 5.28 Hz), 4.32 (1H, dt, *J*₁ = 7.92 Hz, *J*₂ = 4.48 Hz), 3.61 (2H, s), 2.96 (2H, d, *J* = 11.40 Hz), 2.27 (2H, t, *J* = 11.44 Hz), 2.14 (2H, dd, *J*₁ = 21.84 Hz, *J*₂ = 11.72 Hz), 1.96 (2H, d, *J* = 10.24 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.2, 153.2, 150.0, 147.6, 146.6, 140.1, 137.9, 133.6, 133.0, 131.3, 129.4, 128.1, 124.4, 121.1, 120.3, 118.9, 108.5, 101.3, 61.4, 55.3, 52.6 (2C), 43.4, 31.8 (2C). ESI-MS: *m/z* 555.2 (M + 1), 558.2 (M + 4), 579.1 (M + Na). C₂₅H₂₄BrClN₆O₂ (554.08).

N-(Benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9-(1-(4-cyanobenzyl)piperidin-4-yl)-9H-purin-6-amine (**7b3**). Recrystallized from EA/PE as a white crystal, yield 58.4%, mp: 221–225°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.30 (1H, s), 7.80 (2H, d, *J* = 8.04 Hz), 7.54 (2H, d, *J* = 8.06 Hz), 6.83 (2H, dd, *J*₁ = 10.88 Hz, *J*₂ = 7.84 Hz), 6.93 (1H, s), 5.97 (2H, s, –OCH₂O–), 5.03 (1H, s, NH), 4.53 (2H, d, *J* = 4.68 Hz), 4.32 (1H, dt, *J*₁ = 10.88 Hz, *J*₂ = 4.26 Hz), 3.62 (2H, s), 2.92 (2H, d, *J* = 9.20 Hz), 2.17 (4H, t, *J* = 9.44 Hz), 1.97 (2H, t, *J* = 11.60 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.3, 153.2, 150.0, 147.6, 146.6, 145.1, 143.2, 140.1, 133.6 (2C), 132.6, 130.0 (2C), 128.4, 121.1, 119.4, 110.2, 108.5, 101.3, 65.9, 61.6, 52.5 (2C), 43.3, 31.8 (2C). ESI-MS: *m/z* 502.3 (M + 1), 504.3 (M + 3), 506.3 (M + 5), 524.4 (M + Na). C₂₆H₂₄ClN₇O₂ (501.17).

N-(Benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9-(1-(4-nitrobenzyl)piperidin-4-yl)-9H-purin-6-amine (**7b4**). Recrystallized from EA/PE as a yellow crystal, yield 49.5%, mp: 137–141°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.30 (1H, s), 8.22 (2H, dd, *J*₁ = 7.08 Hz, *J*₂ = 1.68 Hz), 7.62 (2H, d, *J* = 8.68 Hz), 6.92 (1H, s), 6.83 (2H, dd, *J*₁ = 8.38 Hz, *J*₂ = 2.83 Hz), 5.96 (2H, s, –OCH₂O–), 5.04 (1H, s, NH), 4.53 (2H, d, *J* = 5.20 Hz), 4.32 (1H, dt, *J*₁ = 11.40, *J*₂ = 3.88 Hz), 3.67 (2H, s), 2.93 (2H, d, *J* = 10.44 Hz), 2.17 (4H, t, *J* = 10.44 Hz), 1.96 (2H, t, *J* = 3.84 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.2, 153.2, 149.7, 147.6, 147.3, 147.1, 146.6, 140.2, 133.6, 130.2 (2C), 128.6, 124.1 (2C), 121.1, 118.9, 108.5, 101.3, 61.3, 52.8, 52.6 (2C),

43.3, 31.8 (2C). ESI-MS: *m/z* 522.3 (M + 1), 524.4 (M + 3), 544.4 (M + Na). C₂₅H₂₄ClN₇O₄ (521.16).

N-(Benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9-(1-(3-fluorobenzyl)piperidin-4-yl)-9H-purin-6-amine (**7b5**). Recrystallized from EA/PE as a white crystal, yield 64.1%, mp: 144–148°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.31 (1H, s), 7.38 (1H, dt, *J*₁ = 8.04 Hz, *J*₂ = 6.48 Hz), 7.17 (2H, dt, *J*₁ = 7.96 Hz, *J*₂ = 2.12 Hz), 7.08 (1H, dt, *J*₁ = 8.64 Hz, *J*₂ = 2.23 Hz), 6.93 (1H, s), 6.83 (2H, t, *J* = 7.72 Hz), 5.97 (2H, s, –OCH₂O–), 5.05 (1H, s, NH), 4.54 (2H, d, *J* = 5.72 Hz), 4.32 (1H, dt, *J*₁ = 7.24 Hz, *J*₂ = 4.26 Hz), 3.55 (2H, s), 2.93 (2H, d, *J* = 6.92 Hz), 2.16 (4H, t, *J* = 8.96 Hz), 1.94 (2H, d, *J* = 7.72 Hz). ¹³C NMR (400 MHz, DMSO-d₆, ppm) δ: 163.9, 161.5, 155.3, 153.2, 150.0, 147.6, 146.6, 142.1, 140.1, 133.6, 130.6, 125.1, 121.1, 118.9, 115.7, 114.2, 108.5, 101.2, 61.5, 52.8, 52.5 (2C), 43.4, 31.8 (2C). ESI-MS: *m/z* 495.3 (M + 1), 497.4 (M + 3), 517.4 (M + Na). C₂₅H₂₄ClFN₆O₂ (494.16).

N-(Benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9-(1-(pyridine-4-yl-methyl)piperidin-4-yl)-9H-purin-6-amine (**7b6**). Recrystallized from EA/PE as a yellow crystal, yield: 62.3%, mp: 154–157°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.53 (2H, dd, *J*₁ = 4.48 Hz, *J*₂ = 1.44 Hz), 8.31 (1H, s), 7.35 (2H, d, *J* = 5.84 Hz), 6.92 (1H, s), 6.83 (2H, dd, *J*₁ = 10.12 Hz, *J*₂ = 7.80 Hz), 5.97 (2H, s, –OCH₂O–), 5.04 (1H, s, NH), 4.53 (2H, d, *J* = 5.76 Hz), 4.32 (1H, dt, *J*₁ = 10.48 Hz, *J*₂ = 3.62 Hz), 3.57 (2H, s), 2.93 (2H, d, *J* = 9.20 Hz), 2.17 (4H, t, *J* = 12.16 Hz), 1.95 (2H, d, *J* = 10.64 Hz). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.2, 153.2, 150.0 (2C), 148.0, 147.6, 147.0, 146.6, 140.1, 133.6, 124.2 (2C), 121.1, 120.3, 118.9, 108.5, 101.3, 60.9, 52.6, 49.1 (2C), 43.4, 31.8 (2C). ESI-MS: *m/z* 478.3 (M + 1), 480.3 (M + 3), 482.4 (M + 5), 500.2 (M + Na). C₂₄H₂₄ClN₇O₂ (477.17).

N-(Benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9-(1-benzylpiperidin-4-yl)-9H-purin-6-amine (**7b7**). Recrystallized from EA/PE as a white crystal, yield 58.5%, mp: 169–172°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.30 (1H, s), 7.33 (4H, t, *J* = 4.60 Hz), 7.25 (1H, dd, *J*₁ = 8.52 Hz, *J*₂ = 4.28 Hz), 6.92 (1H, s), 6.83 (2H, dd, *J*₁ = 10.0 Hz, *J*₂ = 7.80 Hz), 5.96 (2H, s, –OCH₂O–), 5.04 (1H, s, NH), 4.52 (2H, d, *J* = 5.68 Hz), 4.29 (1H, dt, *J*₁ = 7.81, *J*₂ = 3.88 Hz), 3.52 (2H, s), 2.92 (2H, d, *J* = 6.36 Hz), 2.13 (4H, dt, *J*₁ = 11.36, *J*₂ = 7.40 Hz), 1.94 (2H, s). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.2, 153.1, 150.0, 147.6, 146.6, 140.2, 138.9, 133.6, 129.3 (2C), 128.6 (2C), 127.4, 121.1, 120.3, 118.9, 108.5, 101.3, 62.3, 52.9, 52.6 (2C), 43.4, 31.8 (2C). ESI-MS: *m/z* 477.3 (M + 1), 479.3 (M + 3), 581.4 (M + 5), 599.3 (M + Na). C₂₅H₂₅ClN₆O₂ (476.17).

N-(Benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9-(1-(4-bromobenzyl)piperidin-4-yl)-9H-purin-6-amine (**7b8**). Recrystallized from EA/PE as a white crystal, yield 54.3%, mp: 204–208°C. ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 8.30 (1H, s), 7.54 (2H, d, *J* = 1.60 Hz), 7.28 (2H, d, *J* = 8.32 Hz), 6.93 (1H, s), 6.83 (2H, dd, *J*₁ = 7.26 Hz, *J*₂ = 1.84 Hz), 5.97 (2H, s, –OCH₂O–), 5.04 (1H, s, NH), 4.54 (2H, d, *J* = 5.80 Hz), 4.30 (1H, dt, *J*₁ = 7.28 Hz, *J*₂ = 4.28 Hz), 3.50 (2H, s), 2.92 (2H, d, *J* = 6.6 Hz), 2.20 (4H, dt, *J*₁ = 12.36, *J*₂ = 8.60 Hz), 1.94 (2H, s). ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 155.2, 153.2, 150.0, 147.6, 146.7, 140.2, 138.4, 133.6, 131.5 (2C), 131.4 (2C), 121.1, 120.9,

120.4, 118.9, 108.5, 101.2, 61.4, 52.9, 52.5 (2C), 43.4, 31.8 (2C). ESI-MS: m/z 557.1 (M + 3), 559.1 (M + 5). $C_{25}H_{24}BrClN_6O_2$ (554.08).

N-(Benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9-(1-(3-cyanobenzyl)piperidin-4-yl)-9H-purin-6-amine (**7b9**). Recrystallized from EA/PE as a white crystal, yield 48.4%, mp: 200–204°C. 1H NMR (400 MHz, DMSO- d_6 , ppm) δ : 8.31 (1H, s), 7.76 (1H, dd, $J_1 = 10.84$ Hz, $J_2 = 7.64$ Hz), 7.69 (2H, d, $J = 7.84$ Hz), 6.57 (1H, t, $J = 7.68$ Hz), 6.93 (1H, s), 6.83 (2H, dd, $J_1 = 9.76$, $J_2 = 7.80$ Hz), 5.97 (2H, s, $-OCH_2O-$), 5.04 (1H, s, NH), 4.53 (2H, d, $J = 5.40$ Hz), 4.31 (1H, dt, $J_1 = 8.12$, $J_2 = 3.84$ Hz), 3.60 (2H, s), 2.93 (2H, d, $J = 9.28$ Hz), 2.17 (4H, dt, $J_1 = 12.04$ Hz, $J_2 = 9.68$ Hz), 1.94 (2H, d, $J = 11.04$ Hz). ^{13}C NMR (400 MHz, DMSO- d_6 , ppm) δ : 155.2, 153.2, 150.0, 147.6, 146.6, 140.8, 140.1, 134.1, 133.6, 132.5, 131.3, 129.9, 121.1, 119.4, 118.8, 111.7, 108.5, 101.3, 61.1, 55.4, 52.7, 52.5 (2C), 43.4, 31.8 (2C). ESI-MS: m/z 502.3 (M + 1), 504.3 (M + 3), 506.3 (M + 5), 524.4 (M + Na). $C_{26}H_{24}ClN_7O_2$ (501.17).

N-(Benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9-(1-(4-methoxybenzyl)piperidin-4-yl)-9H-purin-6-amine (**7b10**). Recrystallized from EA/PE as a white crystal, yield 59.4%, mp: 182–185°C. 1H NMR (400 MHz, DMSO- d_6 , ppm) δ : 8.30 (1H, s), 7.24 (2H, d, $J = 8.52$ Hz), 6.90 (3H, t, $J = 6.92$ Hz), 6.83 (2H, dd, $J_1 = 7.52$ Hz, $J_2 = 3.68$ Hz), 5.97 (2H, s, $-OCH_2O-$), 5.04 (1H, s, NH), 4.54 (2H, d, $J = 5.92$ Hz), 4.28 (1H, dt, $J_1 = 8.25$, $J_2 = 3.32$ Hz), 3.74 (3H, s), 3.44 (2H, s), 2.92 (2H, d, $J = 6.24$ Hz), 2.07 (4H, t, $J = 7.88$ Hz), 1.93 (2H, s). ^{13}C NMR (100 MHz, DMSO- d_6 , ppm) δ : 158.8, 155.3, 153.2, 150.0, 147.6, 146.6, 140.1, 134.5, 133.6, 130.7, 130.5 (2C), 121.1, 118.9, 114.0 (2C), 108.5, 101.3, 61.7, 55.5, 53.0, 52.4 (2C), 43.4, 31.8 (2C). ESI-MS: m/z 507.3 (M + 1), 509.3 (M + 3), 529.3 (M + Na). $C_{26}H_{27}ClN_6O_3$ (506.18).

N-(Benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9-(1-(2,6-dichlorobenzyl)piperidin-4-yl)-9H-purin-6-amine (**7b11**). Recrystallized from EA/PE as a white crystal, yield: 56.8%, mp: 199–203°C. 1H NMR (400 MHz, DMSO- d_6 , ppm) δ : 8.28 (1H, s), 7.48 (2H, dt, $J_1 = 8.40$ Hz, $J_2 = 0.76$ Hz), 7.34 (1H, dd, $J_1 = 7.54$ Hz, $J_1 = 8.62$ Hz), 6.92 (1H, s), 6.83 (2H, dd, $J_1 = 11.74$, $J_1 = 7.9$ Hz), 5.97 (2H, s, $-OCH_2O-$), 5.04 (1H, s, NH), 4.54 (2H, d, $J = 5.76$ Hz), 4.54 (2H, d, $J = 6.04$ Hz), 4.32 (1H, dt, $J_1 = 7.82$ Hz, $J_2 = 4.26$ Hz), 3.74 (2H, s), 2.95 (2H, d, $J = 11.6$ Hz), 2.39 (2H, t, $J = 1.6$ Hz), 1.99 (4H, dt, $J_1 = 11.51$ Hz, $J_2 = 9.62$ Hz). ^{13}C NMR (100 MHz, DMSO- d_6 , ppm) δ : 155.2, 153.2, 150.0, 147.6, 146.6, 140.1, 136.6 (2C), 134.3, 133.6, 131.9, 130.4, 129.1 (2C), 121.1, 118.9, 108.5, 101.3, 62.3, 56.3, 52.7 (2C), 43.3, 31.8 (2C). ESI-MS: m/z 547.2 (M + 3), 549.2 (M + 5), 551.2 (M + 7). $C_{25}H_{23}Cl_3N_6O_2$ (544.09).

N-(Benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9-(1-(2,4,6-trimethylbenzyl)piperidin-4-yl)-9H-purin-6-amine (**7b12**). Recrystallized from EA/PE as a white crystal, yield 63.4%, mp: 164–167°C. 1H NMR (400 MHz, DMSO- d_6 , ppm) δ : 8.28 (1H, s), 6.92 (1H, s), 6.80–6.85 (4H, m), 5.96 (2H, s, $-OCH_2O-$), 5.03 (1H, s, NH), 4.53 (2H, d, $J = 5.20$ Hz), 4.29 (1H, dt, $J_1 = 10.28$ Hz, $J_2 = 4.80$ Hz), 3.44 (2H, s), 2.86 (2H, d, $J = 11.40$ Hz), 2.32 (6H, s), 2.19–2.21 (5H, m), 1.94–1.96 (4H, m). ^{13}C NMR (100 MHz, DMSO- d_6 , ppm) δ : 155.2, 153.2,

150.0, 147.6, 146.6, 140.0, 138.1, 137.9, 136.0, 133.6, 132.2, 129.1 (2C), 121.0, 118.8, 108.5, 101.2, 61.9, 55.4, 52.3 (2C), 43.3, 32.1 (2C), 21.0, 20.2, 19.7. ESI-MS: m/z 519.4 (M + 1), 521.3 (M + 3), 523.5 (M + 5), 541.4 (M + Na). $C_{28}H_{31}ClN_6O_2$ (518.22).

N-(Benzo[d][1,3]dioxol-5-ylmethyl)-2-chloro-9-(1-(2-fluorobenzyl)piperidin-4-yl)-9H-purin-6-amine (**7b13**). Recrystallized from EA/PE as a white crystal, yield 64.8%, mp: 192–195°C. 1H NMR (400 MHz, DMSO- d_6 , ppm) δ : 8.29 (1H, s), 7.44 (1H, dt, $J_1 = 8.0$, $J_2 = 1.56$ Hz), 7.33 (1H, dt, $J_1 = 5.64$, $J_2 = 1.92$ Hz), 7.18 (2H, dd, $J_1 = 14.2$ Hz, $J_2 = 6.4$ Hz), 6.92 (1H, s), 6.85 (2H, t, $J = 7.8$ Hz), 5.97 (2H, s, $-OCH_2O-$), 5.04 (1H, s, NH), 4.54 (2H, d, $J = 5.76$ Hz), 4.30 (1H, dt, $J_1 = 11.52$ Hz, $J_2 = 4.16$ Hz), 3.59 (2H, s), 2.95 (2H, d, $J = 10.2$ Hz), 2.15 (4H, dt, $J_1 = 23.2$ Hz, $J_2 = 11.72$ Hz), 1.94 (2H, t, $J = 12.88$ Hz). ^{13}C NMR (100 MHz, DMSO- d_6 , ppm) δ : 162.4, 160.0, 155.2, 153.2, 149.9, 147.6, 146.7, 140.2, 133.6, 132.1, 129.6, 125.2, 124.6, 121.1, 118.9, 115.7, 108.5, 101.3, 54.9, 52.8, 52.4 (2C), 43.4, 31.8 (2C). ESI-MS: m/z 495.3 (M + 1), 497.4 (M + 3), 517.4 (M + Na). $C_{25}H_{24}ClFN_6O_2$ (494.16).

4.2. Acetylcholinesterase Inhibition Assay. The ability of derivatives **7a1–7a13** and **7b1–7b13** to inhibit human acetylcholinesterase (hAChE) (Sigma-Aldrich, USA) was examined using the 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) method (to generate a yellow chromophore (5-mercapto-2-nitrobenzoic acid) detectable at the 405–412 nm range) with the acetylcholinesterase assay kit (Keygen, China). The assay was carried out as a previously described protocol [10] using donepezil as controls ($n = 4$). Stock solutions of test compounds were dissolved in a minimum volume of DMSO (1%) and were diluted using saline. In 96-well plates, 50 μ L of AChE (0.22 U/mL diluted using saline) was incubated with 10 μ L of various concentrations of test compounds (1.0, 10.0, 100 μ M) at room temperature for 10 min followed by the addition of relative agents according to the kit instruction. The absorbance was measured at a wavelength of 405 nm with Thermo MK3 microplate reader. Percent inhibition was calculated by the comparison of compound-treated to various control incubations.

Conflict of Interests

The authors declare no conflict of interests.

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