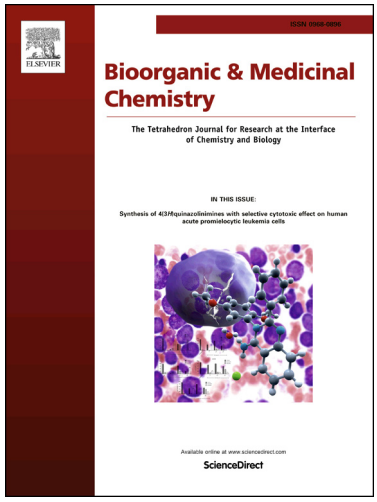

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Design and Optimization of Purine Derivatives as in-vivo Active PDE10A Inhibitors

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Abstract

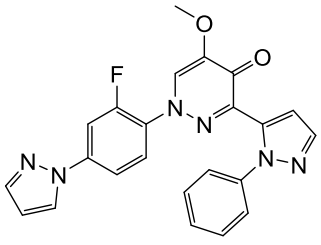
Phosphodiesterases are important enzymes regulating signal transduction mediated by second messenger molecules cAMP or cGMP. PDE10A is a unique member in the PDE family because of its selective expression in medium spiny neurons. It is recognized as anti-psychotic drug target. Based on the structural similarity between

our previous chemistry work on 8-aminoimidazo[1,2-a]pyrazines and the PDE10A inhibitors reported by Bartolome-Nebreda et al., we initialized a project for developing PDE10A inhibitors. After several rounds of optimization, we were able to obtain a few compounds with good PDE10A enzymatic activity. And after further PDE enzymatic selectivity study, metabolic stability assay and in vivo pharmacological tests we identified two inhibitors as interesting lead compounds with the potential for further PDE10A lead optimizatioin.

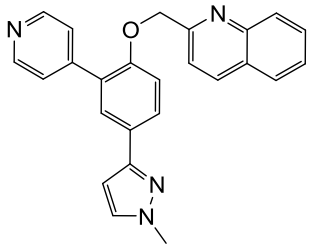
Keywords: Phosphodiesterase; PDE10A; purine; inhibitor; anti-psychotic

Introduction

Through hydrolysis of second messengers cAMP and cGMP, the cyclic nucleotide phosphodiesterases (PDE) regulate the localization, duration, and amplitude of cyclic nucleotide signaling.[1-2] Given their important roles in mediating signal transduction, PDEs catch considerable attention in drug development.[3-5] PDE10A is a unique member in the PDEs family.[6-8] Unlike the broad distribution in tissues of other members, the expression of PDE10A is limited to brain and testes.[9-12] The selective expression in the medium spiny neurons of the striatum was thought to play critical roles in regulating the response to external stimuli. Many studies and disclosed clinical data have shown that inhibiting PDE10A is a potential anti-psychotic approach.[13] A number of PDE10A inhibitors have been developed in the past few years[14-18] and several of them are now in clinical trials against schizophrenia, including TAK-063 (Takeda, Phase II), OMS-824 (Omeros, Phase II, structure unknown), PF-2545920 (Pfizer, Phase II) as shown in Figure 1 (Data from Thomson Reuters Cortellis).



TAK-063



PF-2545920

Figure 1. Representative PDE10A inhibitors

PDE10A contains two GAF domains and a catalytic domain (The GAF domain is

named after some of the proteins where it is found, including cGMP-specific phosphodiesterases, adenylyl cyclases and FhlA.). Although the GAF domains of PDE10A can bind cAMP, they merely act as regulating domains and do not show enzymatic activity in *in vitro* binding study. The co-crystal structures of inhibitors bound to PDE10A provided detailed information about different binding modes. Besides the conserved hydrogen bonds between Gln716 and small molecules, the binding site also contained several subpockets: a selectivity pocket, a hydrophobic clamp, a ribose region and a large exo-binding region.[6] The small molecular inhibitors occupying more than one of above-mentioned regions in the binding site usually exhibited potent binding affinity.

Our previous chemistry offered an efficient route to 8-aminoimidazo[1,2-a]pyrazines (Structure A in Figure 2).[19] Replacement of NHR^1 with morpholine ring (Structure B in Figure 2) yielded a structure resembling closely the PDE10A inhibitors by Bartolome-Nebreda et al (Compound C). These compounds contained the same imidazo[1,2-a]pyrazine scaffold and showed a unique binding mode that the N^1 of the imidazole made an interaction with the backbone NH of Gln716 and the morpholine group extended into a small PDE10A specific subpocket.[20-21] Based on this binding mode, the purine skeleton was also considered as a suitable scaffold.

Thus, above-mentioned two types of compounds were screened against PDE10A enzymatic assay, and showed several submicromolar PDE10A inhibitors, which were further optimized to two compounds with potent enzymatic and in vivo activities and

good metabolic stability. The new compounds could be useful for further antipsychotic drug development and the details of the study were reported herein.

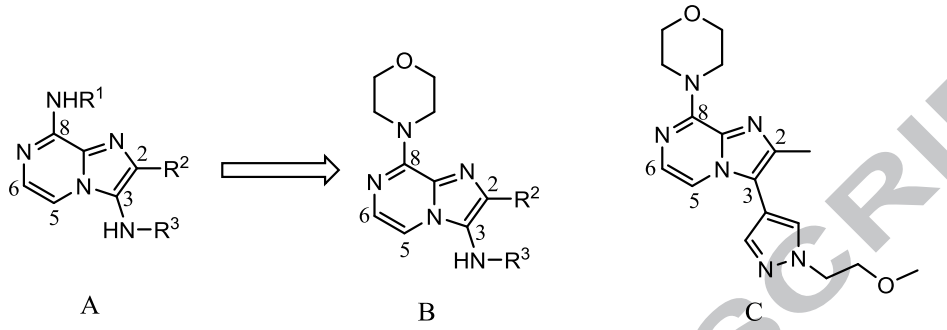
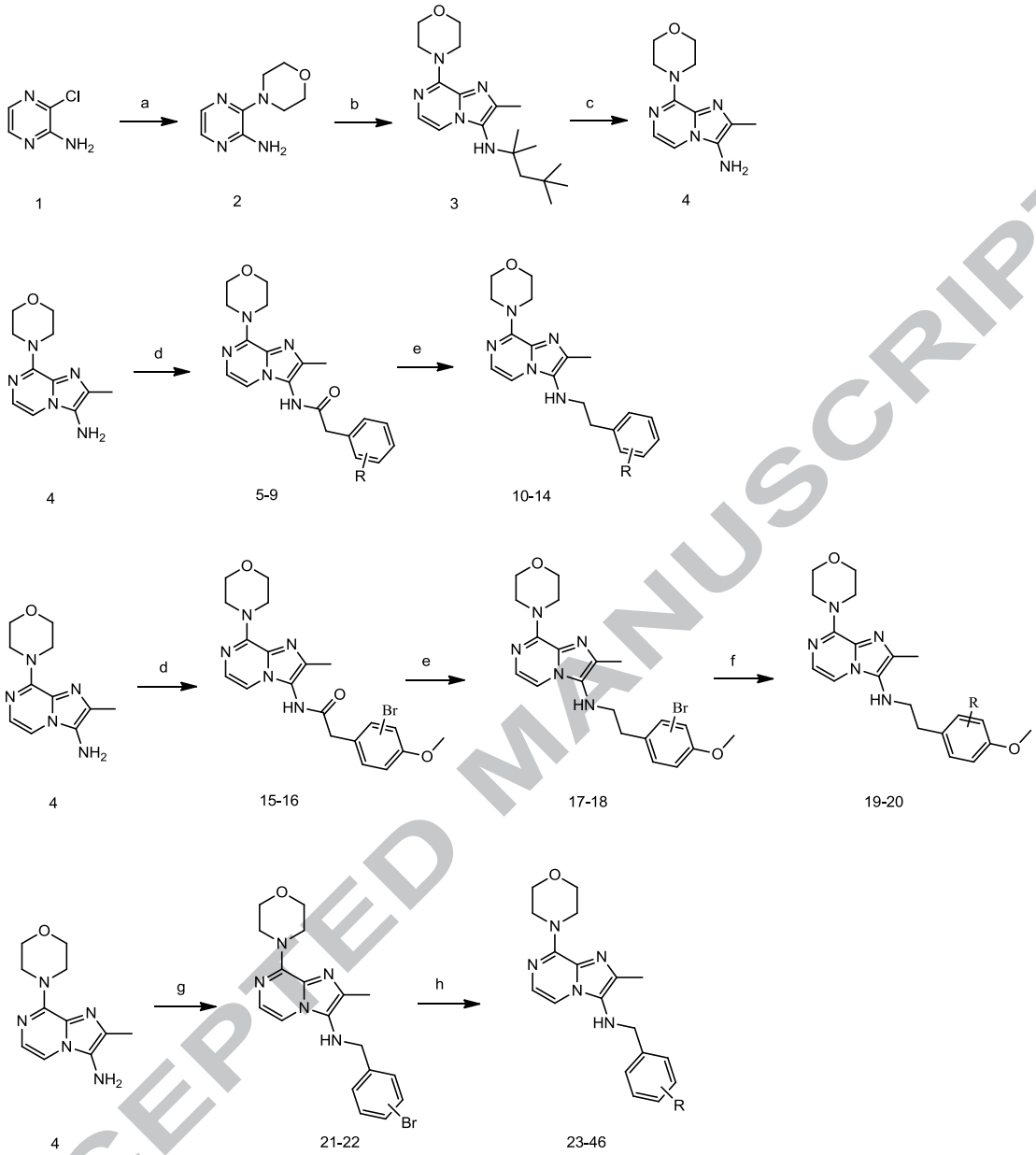


Figure 2. Design of hit compounds

Chemistry

Compound **4** was prepared by the methods we published previously.[19] Commercially available 2-Amino-3-chloropyrazine was reacted with morpholine to give compound **2** which underwent Groebke–Blackburn–Bienaymé reaction and deprotection to yield intermediate compound **4.** Compound **4** was coupled with phenylacetic acids and the carbonyl groups were reduced to yield **10-14**. Compounds 1**9-20** were obtained by Suzuki reaction from **17-18** that were prepared as described above. Compounds **23-46** were obtained from intermediate **4** through nucleophilic substitution and Suzuki reaction.

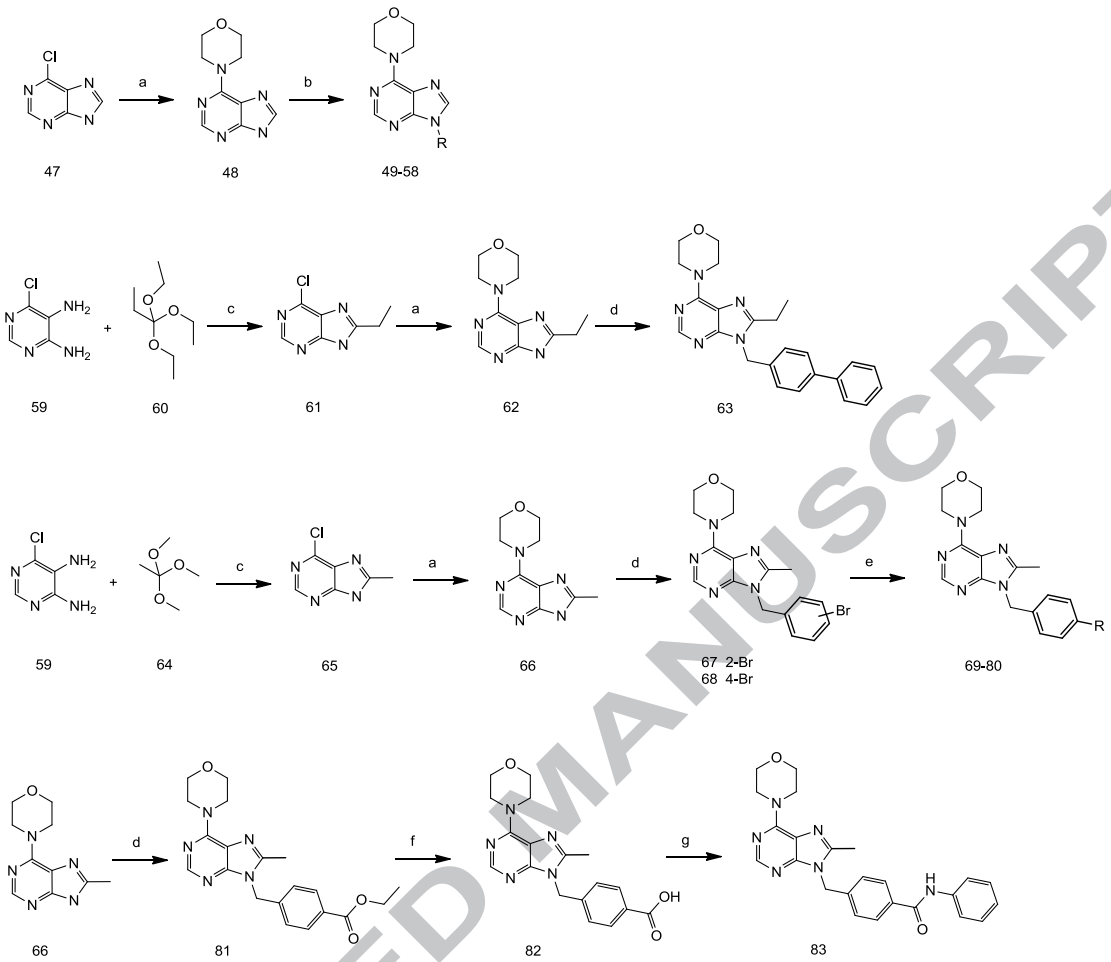
Scheme 1



Reagents and conditions: a) morpholine, DIEA, 120-130 ^{o}C, 12h; b) Yb(OTf)$_3$,2-isocyano-2,4,4-trimet-hylpentane, CH_3CHO, MeOH, 65 ^{o}C, 2h; (c) HCl/EtOAc, MeOH, 25 ^{o}C, 8h; d) phenylacetic acids, HOBt, EDC, TEA, CH_2Cl_2, rt, 8h; e) BH_3-THF complex, THF, 80 ^{o}C, 1h; f) dioxane/water or DMF, $Pd_2(dba)_3$ or $Pd(OAc)_2$, x-phos, K_2CO_3 or Cs_2CO_3, boric acids or boronic acid pinacol esters, 80 ^{o}C, overnight; g) DMF, benzyl bromides, 8h; h) dioxane/water, $Pd_2(dba)_3$, x-phos, K_2CO_3, boric acids or boronic acid pinacol esters, 100 ^{o}C, overnight.

The synthesis route of purine series was shown in scheme 2. 6-chloro-9H-purine (**47**) was reacted with morpholine and then with benzyl bromides to give compounds **49-58**. 6-Chloro-4,5-diaminopyrimidine and ethyl orthopropionate were condensed to generate compound **61**, from which compound **63** was obtained by the same route of **49-58** described above. Compounds **67-68** were achieved with the same methods as **63** using trimethyl orthoacetate to replace ethyl orthopropionate. Suzuki reaction with **67-68** provided compounds **69-80**. Compound **81** was also obtained with the methods of **63**. Then, it was hydrolyzed with LiOH and coupled with aniline to give **83**.

Scheme 2



Reagents and conditions: a) morpholine, 80 ^{o}C overnight; b) benzyl bromides, DMF, Cs_2CO_3, rt, overnight; c) TEA, acetonitrile, 120 ^{o}C, overnight; d) benzyl bromides, DMF, K_2CO_3, rt, overnight; e) boric acids, 2M Na_2CO_3, $Pd(PPh_3)_4$, DME, 100 ^{o}C, overnight; f) LiOH, MeOH/H_2O(3:1), 60 ^{o}C, 5h; g) aniline, EDC, DMAP, DMF, rt, overnight.

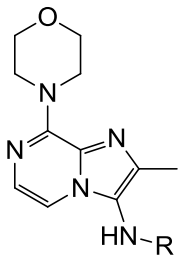
Results and Discussion

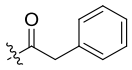
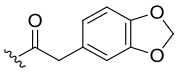
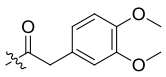
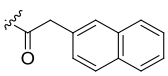
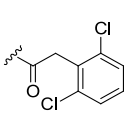
Hit identification

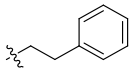
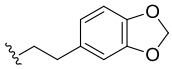
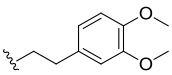
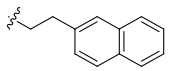
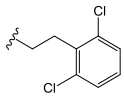
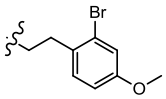
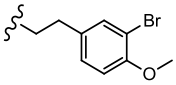
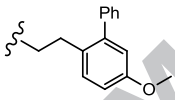
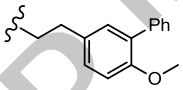
Based on our multi-component reaction and reported PDE10A inhibitors, we designed and synthesized fourteen compounds of morpholine substituted aminoimidazo[1,2-a]pyrazines as shown in Table 1 for preliminary evaluation. Since

there was an amino group left on the imidazo[1,2-a]pyrazine ring using our synthesis, modifications were made on it at the first stage. From the PDE10A enzymatic assay, these compounds all showed moderate inhibition at concentration 5 μM. In particular, the amides (**5-9**) displayed lower activities than their reduction products (**10-14**). To further explore the SAR, compounds **17-20** with different substitutions on the phenyl ring were synthesized. 2,4-Bis-substituted compounds **17** and **19** showed better inhibitory activities than that of 3,4-bis-substituted compounds **18** and **20**. And large substitution like phenyl group was also well-tolerated (**19, 20**). These indicated that 2- and 4-position might be the direction for further optimization.

Table 1. Inhibition of Compound 5-14，17-20 on PDE10A



Compd.	R	Inhibition%@5μM[a]
5		51%
6		44%
7		50%
8		24%
9		47%

10		74%
11		82%
12		83%
13		78%
14		98% IC_{50} = 447 nM
17		92% IC_{50} = 243 nM
18		81%
19		93% IC_{50} = 336 nM
20		80%

a: All assay data are reported as the average of at least two measurements.

To predict the binding conformation of this series of compounds, we selected compound **17** as representative to dock into the binding site of PDE10A catalytic domain. Initially, the crystal structure of PDE10A bound with an analogue of compound C (PDB ID: 4BBX) was downloaded from PDB data bank, and prepared with Maestro program of Schrödinger software by adding the hydrogen atoms and assigning the bond order to the complex. Then the Glide program implemented in Schrödinger software package was adopted to make the grid file and perform the SP docking with default parameters. According to the predicted binding conformation, the parts of imidazo[1,2-a]pyrazine and morpholine fit well into the binding site of

PDE10A structure and the N^1 had a potential hydrogen bond with Gln716. The part of phenylethylamine pointed to a different direction from the ligand in original crystal structure, making van der Waals interactions with residues Ser561, Met581 and Leu625. These results showed that it was a proper design for modification on our scaffold to achieve PDE10A inhibition activities.

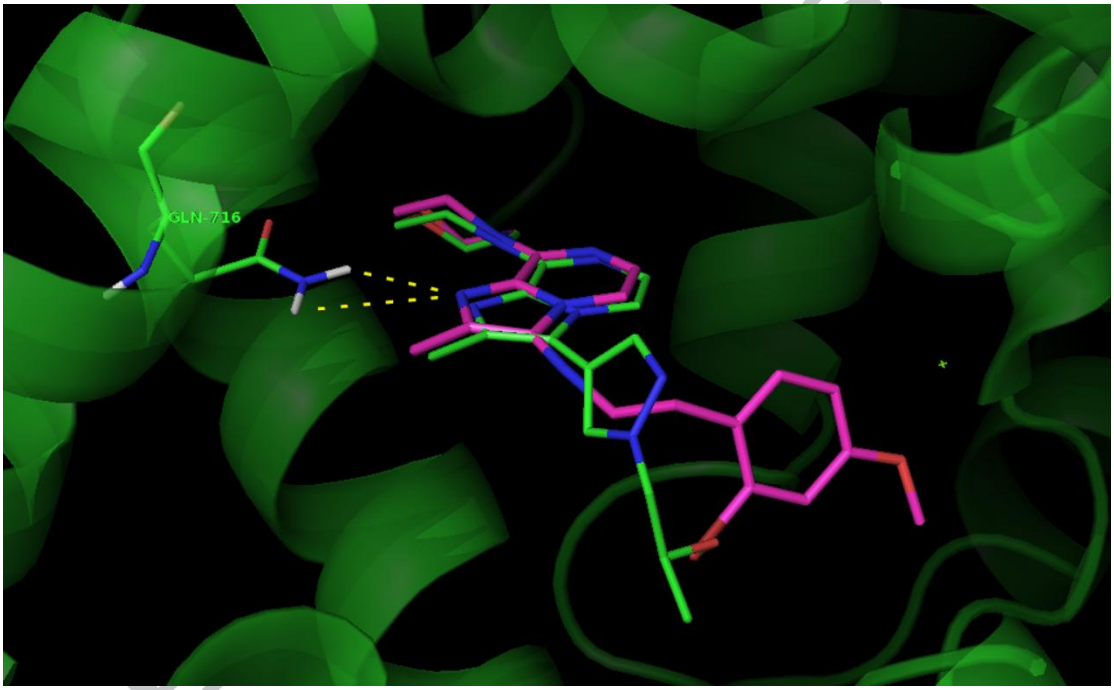


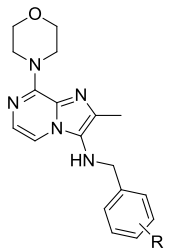
Figure 3. Superimposition of compound 17 (Red) with PDE10A crystal structure (PDB ID: 4BBX, Green).

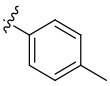
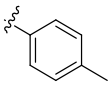
SAR of Aminoimidazo[1,2-a]pyrazines

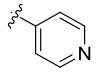
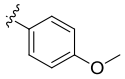
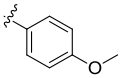
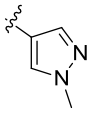
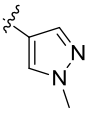
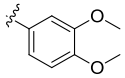
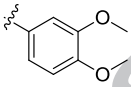
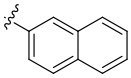
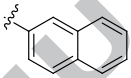
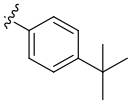
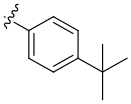
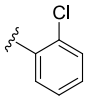
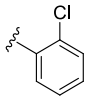
Further SAR investigation on phenyl ring was carried out. From the docking results we predicted that the phenyl ring pointed outward of the binding site, offering the possibility to introduce larger substitutions. An additional aryl group was attached to the 2- or 4-position of the phenyl ring. However, such modification would increase

the molecular weight significantly. To compensate this unwanted effect, the modification was carried out on benzylamine skeleton in Table 2, instead of on phenylethylamine skeleton in Table 1. As shown in Table 2, compounds with aryl substitutions at 4-position of the phenyl ring generally showed better inhibitory activities than those substituted at 2-position. Further, analyzing the substitution pattern on the distal phenyl ring indicated that compounds with methoxyl group (**39, 42**) showed stronger inhibition than other substituents such as methyl (**35**), tert-butyl (**45**) and chlorine atom (**46**). Heterocycles such as pyridyl (**37, 38**), isoxazolyl (**40**) and pyrazolyl (**41**) generally showed better activities than substituted phenyl rings. However all these compounds did not exhibit satisfactory inhibition against PDE10A.

Table 2. Inhibition of Compound 21-46 on PDE10A



Compd.	2-R	Inhibition %@5μM[a]	IC_{50} nM	Compd.	4-R	Inhibition %@5μM[a]	IC_{50} nM
21	Br	73%	--	22	Br	77%	--
23		57%	--	35		74%	--
24		61%	--	36		79%	--
25		76%	--	37		90%	529

26		70%	--	38		91%	391
27		60%	--	39		87%	--
28		68%	--	40		90%	518
29		64%	--	41		88%	--
30		51%	--	42		92%	452
31		63%	--	43		54%	--
32		75%	--	44		55%	--
33		39%	--	45		38%	--
34		87%	--	46		77%	--

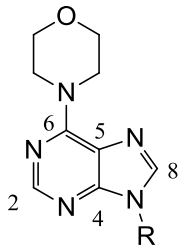
a: All assay data are reported as the average of at least two measurements.

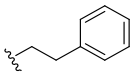
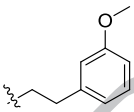
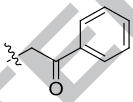
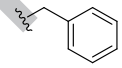
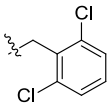
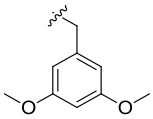
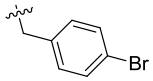
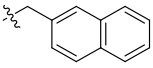
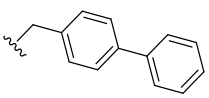
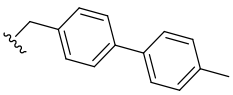
SAR of Purines

According to the solved crystal structure (PDB entry: 4BBX) the N^1 on imidazole ring (see Figure 2 for the numbering) interacted with the protein backbone and was essential to the binding activities.[12] Thus, besides the imidazo[1,2-a]pyrazine scaffold, other eligible structures were also considered. The structures of cAMP and cGMP both contain the purine ring, and moreover, the first-generation PDE inhibitors known as pan-PDE inhibitors including theophylline and xanthine also contained the purine moiety. Together with the SAR of aminoimidazo[1,2-a]pyrazines, we decided to

combine the purine and the morpholine ring to form a new series of PDE10A inhibitors.

Table 3. Inhibition of Compound 49-58 on PDE10A

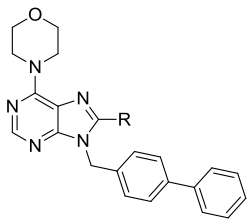


Compd.	R	Inhibition%@5μM[a]	IC_{50} nM
49		13%	--
50		20%	--
51		11%	--
52		66%	--
53		70%	--
54		85%	--
55		90%	530
56		100%	107
57		94%	103
58		97%	75

a: All assay data are reported as the average of at least two measurements.

As shown in Table 3, if substitution R was phenylethyl group the compounds (**49, 50**) showed weak inhibition on PDE10A; while if substitution R was benzyl group the compounds (**52-58**) had an obvious increase of the inhibitory activities from lower than 20% up to 100 % at 5 μM. This implied that the length between the purine and phenyl group was an important factor for binding. Large groups replacing the phenyl group could improve the activity, as demonstrated by compounds **55** and **56**. And biphenyls (**57** and **58**) exhibited better potency over phenyls, which confirmed that *para*-position of phenyl group was important for the binding. Taking together, these pointed the direction for following optimization. Another worrisome issue associated with purine scaffold was that the hydrogen atom on C8 of the purine ring was commonly considered to be metabolically unstable.[22] Therefore we prepared compounds with substitutions at this position to potentially block the metabolism and also assessed their binding affinities.

Table 4. Inhibition of Compound 63, 69 on PDE10A



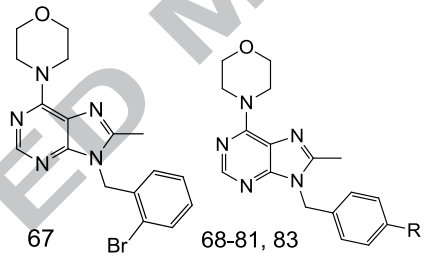
Compd.	R	Inhibition%@5μM[a]	IC_{50} nM
57	H	94	103

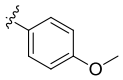
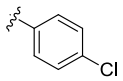
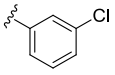
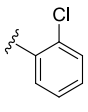
63	Et	97	168
69	Me	99	113

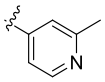
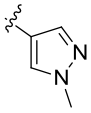
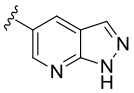
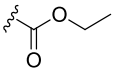
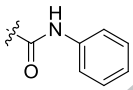
a: All assay data are reported as the average of at least two measurements.

As indicated in Table 4, the compound **69** with a methyl group had equivalent activity to compound **57**. If substitution R was the ethyl group, the activity of the compound decreased slightly (**63**). Based on the aforementioned SAR information, we chose 8-methyl-purine as the scaffold for further optimization.

Table 5. Inhibition of Compound 67-81, 83 on PDE10A



Compd.	R	Inhibition%@5μM[a]	IC_{50} nM
67	-	87	--
68	Br	93	327
69		99	118
70		94	62
71		91	168
72		93	258
73		98	38

74		96	116
75		97	61
76		98	56
77		97	76
78		96	68
79		96	119
80		98	60
81		90	--
83		96	130

a: All assay data are reported as the average of at least two measurements.

To reassess the obtained SAR information, we prepared two compounds (**67** and **68**) and found that 4-Br indeed showed higher activity than 2-Br. Then, as shown in Table 5, further optimization was focused on the 4-position to synthesize derivatives with the aryl substitution on the phenyl ring. From the enzymatic assay, the bi-aryl compounds obviously improved the inhibition on PDE10A and half of the tested compounds showed IC_{50} value lower than 100 nM. For compounds with substituted phenyl as R group (**71-73**), their inhibition activities did not show large differences (within 4 fold) when comparing with compound **70**. The 2-Cl phenyl (**73**) was the most potent compound in this series with the IC_{50} value of 38 nM. Compounds with heterocyclic rings as R group (**74-80**) showed similar activities and their IC_{50} values

ranged from 56-119 nM. Even the amide (**83**) or ester (**81**) compounds showed good activities. And these observations were consistent with our prediction that the investigated part (R group) might be located at the solvent-accessible range of the binding site, and therefore various moieties were well-tolerated. Finally, compounds **42**, **58, 73**, **76**, **77** and **78** were selected for further evaluation based on their inhibitory activities and structural diversity.

Profiling Lead Compounds

Metabolic stability is an important concern for developing successful drugs, as it will enable the molecules to prolong the effects at the functional sites in the body. We tested compounds **42**, **58, 73**, **76**, **77** and **78** on mouse and human liver microsomes respectively (MLM and HLM in Table 6), and all compounds showed good stability with reasonable half-life. In addition to the liver microsome assay, five cytochrome P450 enzymes commonly metabolizing exogenous chemicals were used to test the direct inhibition of these six compounds. The evaluation showed that these compounds had less than 50% inhibitory activity at concentration 10 μM in direct inhibition tests against selected five CYPs. The time-dependant inhibition test also clarified that the compounds had no covalent interaction issue with the five CYPs.

Table 6. Metabolic stability in mouse and human liver microsomes of compounds 42, 58, 73, 76, 77 and 78

Compd.	HLM	MLM	DI (%)[a] / TDI[b]

	$t_{1/2}$(min)	$t_{1/2}$(min)	3A4	2D6	2C9	1A2	2C19
42	18.24	12.83	42/ NI[c]	24/ NI	41/ NI	29/ NI	NI/ NI
58	22.35	49.5	NI/NI	6/NI	30/NI	12/20	44/NI
73	43.31	17.77	9/50	22/NI	52/10	15/NI	65/NI
76	18.24	23.9	12/NI	6/60	18/10	34/30	21/70
77	49.5	69.3	22/20	NI/30	NI/NI	12/10	NI/90
78	231	99	40/NI	7/20	10/40	21/NI	22/80

a. DI = direct inhibition. If the value is less than 50 that means the compound has no obvious inhibition on CYPs.

b. TDI = TDI: time-dependent inhibition of CYPs. If the value is less than 200 that means the compound has no obvious inhibition on CYPs.

c: NI = no inhibition

PDE family contains eleven members that distribute in different tissues and regulate corresponding physiological functions. Since inhibition on different subtypes of PDEs may cause adverse effects, target selectivity is an important issue to be checked when developing the PDE inhibitors. Based on the structural diversity, compounds **42**, **73** and **77** were subjected to selectivity assessment. All these three compounds showed weak inhibition on PDE2, PDE4 and PDE5 (representing PDEs that hydrolyze both cAMP and cGMP, cAMP only and cGMP only, respectively).

Table 7. Inhibition on PDEs of compounds 42, 73 and 77

Compd.	Inhibition%@1μM			PDE10A
	PDE2A1	PDE4A1A	PDE5A1	IC_{50}(nM)
42	5	14	-3	452
73	29	6	5	38
77	4	0	-3	76

It was reported that PDE10A inhibitors might increase the functionality of striatal medium spiny neurons and produce antipsychotic-like effects in rodents.[23] Compounds **73** and **77** were submitted to locomotor and prepulse inhibition (PPI) tests to evaluate their in vivo activities. As shown in Figure 4, pretreatment with **73** and **77** significantly attenuated PCP-stimulated locomotor activity. A significant decrease in total travel distance was observed in rats with 10 mg/kg dosage, however, the high dose (50 mg/kg) showed no activities. The results did not show a dose-dependent response where higher doses caused higher reductions in PCP locomotion until the maximal effect was achieved. The effect of compounds **73** and **77** on sensorimotor gating in rat was assessed by measuring the disruption of PPI, which have been widely used to evaluate the antipsychotic potential for drug development. As shown in Figure 5, pretreatment with 10 mg/kg dose of compound **77** significantly attenuated PPI disruption induced by 5 mg/kg PCP at least at one prepulse level (two-way ANOVA analysis followed by Tukey's test). It was noted that 10 mg/kg and 50 mg/kg **73** and 50 mg/kg **77** did not rescue the PCP-induced PPI deficit, indicating that the limited dose range for compound **73** to elicit effect on PPI.

It was not surprised since the optimal concentration of cAMP/cGMP was important to maintain the normal neuronal function and over-inhibition of PDE could induce the over-shoot of intracellular cAMP/cGMP that was potentially harmful to cellular function. This was in agreement with our previous observation in which we found low dose of MP-10, a well-known PDE10A inhibitor, attenuated morphine-induced conditioned place preference but not with high doses.[24-26]

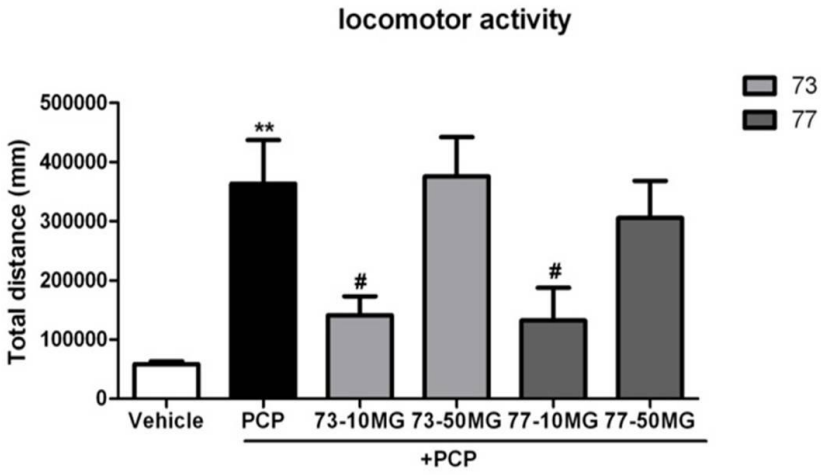


Figure 4. Compounds 73 and 77 inhibit PCP-stimulated locomotor activity in rats. Drugs were administered (p.o.) 30 min prior to 5 mg/kg PCP (phencyclidine) challenge (i.p.). The locomotor activity was measured for a 1.5 h duration after PCP administration and the total traveled distance was expressed as mean ± SEM. Statistical analysis showed 10mg/kg **73** and **77** significantly attenuated hyperlocomotor activity induced by PCP.

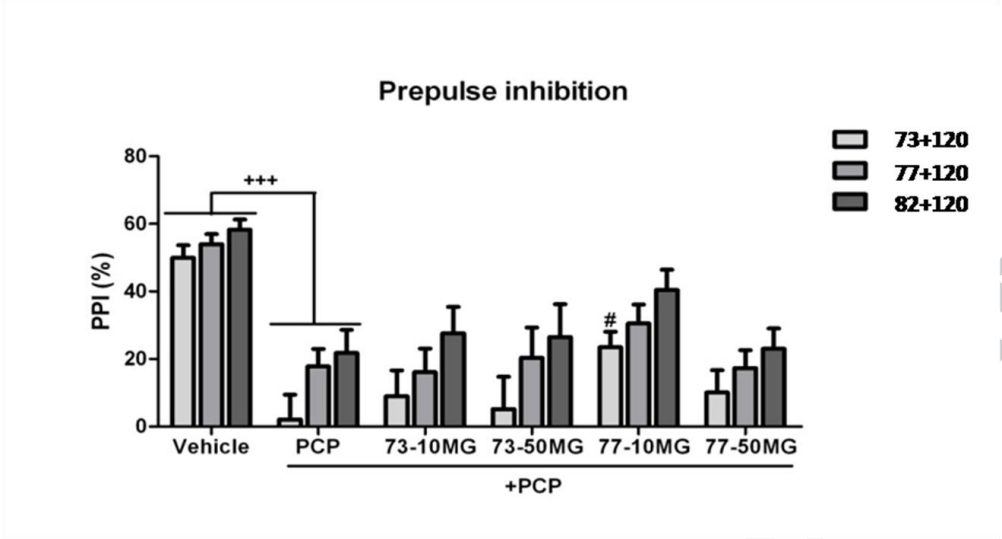


Figure 5. Effects of compounds 73 and 77 in prepulse inhibition tests. Drugs were pretreated (p.o.) 30 min prior to 5mg/kg PCP (i.p.) and the test was performed 5-10 min after PCP injection. Two-way ANOVA analysis with Tukey's test indicated that **73** (10mg/kg) significantly prevented PPI disruption induced by PCP.

Conclusion

Inspired by a series of PDE10A inhibitors reported by Bartolome-Nebreda et al, two series were designed, synthesized, and evaluated. The purine-based compounds showed good inhibitory activities, among which compounds **73** and **77** also showed good metabolic stability and PDE subtype selectivity. Following several recent reports that suggested dual cAMP and cGMP phosphodiesterase 10A inhibitors might present a novel mechanism to treat schizophrenia, we demonstrated that compounds **73** and **77** elicited potent antipsychotic effects as evidenced by attenuating the acute PCP-induced hyperlocomotive activity. However, higher dose of compound **73** and **77** failed to produce the effects. This indicating that optimal therapeutic window for PDE10A inhibitor was very important in order to achieve the expected efficacy.

Further research is now carried out.

Experiment and Methods

Chemistry

Reagents (chemicals) were purchased from Alfa-Aesar (Karlsruhe, Germany), Acros (Geel, Belgium), Aldrich (St. Louis, MO, USA), Adamas-beta (Shanghai, China) and Shanghai Chemical Reagent Company (Shanghai, China) and were used without further purification. Analytical thin-layer chromatography was performed on HSGF 254 (150–200 mm thickness; Yantai Huiyou Company, Yantai, Shandong, China). ^{1}H NMR (300 MHz or 400 MHz) spectra were recorded on a Varian Mercury-300 or 400 High Performance Digital FT-NMR with TMS as an internal standard. HPLC analysis was performed using a Gilson HPLC system with UV detection at 214 and 254 nm. LC–MS spectra were obtained on an LCQ Deca XP ion trap mass spectrometer (Thermo-Finnigan, San Jose, CA, USA). Accurate mass measurements were carried out on a Q-TOF ultima Globe mass spectrometer (Micromass, Manchester, UK).

Synthesis

3-Morpholinopyrazin-2-amine (2). 3-Chloropyrazin-2-amine (10 g, 77 mmol) was stirred with morpholine (40 mL, 463 mmol) at 120 ^{o}C for 12h. The mixture was diluted with ethyl acetate, washed with water and brine. The organic layer was dried with Na_2SO_4 and concentrated. The crude product was purified by silica gel (ethyl acetate/ petroleum 1:1) to afford 12.83 g product as white solid (yield 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J*=2.9 Hz, 1H), 7.67 (d, *J*=2.9 Hz, 1H), 4.72 (brs, 2H), 3.90-3.82 (m, 4H), 3.22-3.15 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.00, 141.78, 131.43, 127.06, 62.17, 43.83. HRMS (ESI^+) calcd for $C_8H_{12}ON_4H^+$ 181.1084, found 181.1082.

2-Methyl-8-morpholino-N-(2,4,4-trimethylpentan-2-yl)imidazo[1,2-a]pyrazin-3-amine (3). To the solution of compound **2** (9 g, 50 mmol) in 30 mL methanol was added 1,1,3,3-tetramethylbutyl Isocyanide (17.76 mL,100 mmol), CH_3CHO (14 mL,250 mmol) and $Yb(OTf)_3$ (6.2 g,10 mmol). The mixture was stirred at 65 °C for 2h, cooled to room temperature and concentrated. The residue was diluted with ethyl acetate and washed with brine. The organic layer was dried with Na_2SO_4 and concentrated. The residue was purified by silica gel (ethyl acetate/ petroleum 1:4) to afford compound **3** 14.85g as brown oil (yield 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J=4.6 Hz, 1H), 7.34 (d, *J*=4.5 Hz, 1H), 4.23-4.17 (m, 4H), 3.93-3.88 (m, 4H), 2.40 (s, 3H), 1.67 (s, 2H), 1.18 (s, 6H), 1.12 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.10, 136.01, 130.16, 126.30, 125.30, 109.32, 67.00, 60.00, 56.74, 46.86, 31.88, 31.74, 31.60, 31.42, 29.29, 29.03, 14.20. HRMS (ESI+) calcd for $C_9H_{31}ON_5H^+$ 346.2601, found 346.2599.

2-Methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (4). Compound **3** (7.94 g, 22.98 mmol) was dissolved in 50 mL methanol and added 4 M HCl in EtOAc (30 mL). The mixture was stirred at room temperature for 8h and then concentrated. The residue was neutralized by sat.$NaHCO_3$ and extracted with ethyl acetate. The organic layer was washed with brine, dried with Na_2SO_4 and concentrated. The crude product was purified by silica gel (CH_2Cl_2/ MeOH 10:1) to obtain compound **4** 5.26 g as pale brown solid (yield 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.34 (m, 2H), 4.23- 4.18 (m, 4H), 3.90-3.85 (m, 4H), 3.08 (brs, 2H), 2.37 (s, 3H). HRMS (ESI^+) calcd for $C_{11}H_{15}ON_5H^+$ 234.1349, found 234.1346.

General procedure A for preparing compound 5-9. The corresponding phenylacetic acids (2.5eq.), EDC (2 eq.) and HOBt (2 eq.) were dissolved in dichloromethane. The mixture was stirred at room temperature for 15 minutes and then compound **4** (1 eq.) and triethylamine (6 eq.) were added. After stirred at room temperature for 8h, the reaction became turbid. The mixture was filtered and the precipitate was washed with dichloromethane. The solid was dried under vacuum to get the products.

N-(2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-yl)-2-phenylacetamide (5). White solid, yield 90%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H), 7.40-7.34 (m, 5H), 7.31-7.26 (m, 2H), 4.13 (t, *J*=4.7 Hz, 4H), 3.76 (s, 2H), 3.73 (t, *J*=4.8 Hz, 4H), 2.19 (s, 3H). MS (ESI): m/z 352 (M+H) +.

2-(Benzo[d][1,3]dioxol-5-yl)-N-(2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-yl)acetamide (6). White solid, yield 81%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.00 (s, 1H), 7.35 (d, *J*=4.5 Hz, 1H), 7.29 (d, *J*=4.5 Hz, 1H), 6.95 (d, *J*=1.6 Hz, 1H), 6.90 (d, *J*=7.9 Hz, 1H), 6.84 (dd, *J*= 8.0, 1.7 Hz, 1H), 6.00 (s, 2H), 4.12 (t, *J*=4.8 Hz, 4H), 3.74- 3.70 (m, 4H), 3.66 (s, 2H), 2.18 (s, 3H). MS (ESI): m/z 396 $(M+H)^+$.

2-(3,4-Dimethoxyphenyl)-N-(2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-yl)acetamide (7). White solid, yield 88%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.03 (s, 1H), 7.35 (d, *J*=4.5 Hz, 1H), 7.28 (d, *J*=4.5 Hz, 1H), 7.01 (d, *J*=1.9 Hz, 1H), 6.96-6.88 (m, 2H), 4.13 (t, *J*=4.8 Hz, 4H), 3.76 (d, *J*=6.3 Hz, 6H), 3.73 (t, *J*=4.7 Hz, 4H), 3.67 (s, 2H), 2.19 (s, 3H). MS (ESI): m/z 412

$(M+H)^+$.

N-(2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-yl)-2-(naphthalen-2-yl)acetamide (8). White solid, yield 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.16 (s, 1H), 7.95-7.88 (m, 4H), 7.59-7.48 (m, 3H), 7.37-7.32 (m, 2H), 4.13 (t, *J*=4.8 Hz, 4H), 3.95 (s, 2H), 3.73 (dd, *J*=5.5, 4.0 Hz, 4H), 2.20 (s, 3H). MS (ESI): m/z 402 $(M+H)^+$.

2-(2,6-Dichlorophenyl)-N-(2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-yl)acetamide (9). White solid, yield 77%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.70 (s, 1H), 7.51 (d, *J*=8.1 Hz, 2H), 7.42 (d, *J*=4.4 Hz, 1H), 7.40-7.31 (m, 2H), 4.20 (s, 2H), 4.13 (t, *J*=4.8 Hz, 4H), 3.73 (t, *J*=4.8 Hz, 4H), 2.24 (s, 3H). MS (ESI): m/z 420 $(M+H)^+$.

General procedure B for preparing compound 10-14. Compound **5-9** (1 eq.) were dissolved in dry tetrahydrofuran and added 1M BH_3-THF (6 eq.) by dropwise under N_2 protection. The mixture was heated at 80 ^{o}C for 0.5-1h. The solvent was removed by vacuum. Methanol 5 mL was add to the residue and refluxed at 80 ^{o}C for another 0.5h. The solvent was removed again. The residue was dissolved with ethyl acetate and washed with sat.NaHCO3 and brine. The organic layer was dried with Na_2SO_4 and concentrated. The crude products were purified by silica gel (ethyl acetate/ petroleum) to afford the corresponding reduction products **10-14**.

2-Methyl-8-morpholino-N-phenethylimidazo[1,2-a]pyrazin-3-amine (10). Pale yellow oil, yield 68%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.30 (m, 3H), 7.30-7.23 (m, 4H), 4.22 (t, *J*=4.8 Hz,

4H), 3.92-3.86 (m, 4H), 3.30 (t, *J*=7.0 Hz, 2H), 2.89 (t, *J*=7.0 Hz, 2H), 2.33 (s, 3H). MS (ESI): m/z 338 $(M+H)^+$.

N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (11). Pale yellow oil, yield 79%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, *J* = 4.5 Hz, 1H), 7.30 (d, *J*=4.6 Hz, 1H), 6.79 (d, *J*=7.8 Hz, 1H), 6.74-6.72 (m, 1H), 6.69 (dd, *J*=7.9, 1.7 Hz, 1H), 5.97 (s, 2H), 4.22 (t, *J*=4.8 Hz, 4H), 3.89 (t, *J*=4.8 Hz, 4H), 3.24 (t, *J*=7.0 Hz, 2H), 2.79 (t, *J*=7.0 Hz, 2H), 2.34 (s, 3H). MS (ESI): m/z 382 $(M+H)^+$.

N-(3,4-dimethoxyphenethyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (12). Pale yellow oil, yield 72%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, *J*=4.5 Hz, 1H), 7.29 (d, *J*=4.6 Hz, 2H), 6.85 (d, *J*=8.1 Hz, 1H), 6.79 (dd, *J*=8.1, 1.9 Hz, 1H), 6.75 (d, *J*=1.9 Hz, 1H), 4.22 (t, *J*=4.8 Hz, 4H), 3.90-3.87 (m, 10H), 3.27 (t, *J*=7.0 Hz, 2H), 2.83 (t, *J*=7.0 Hz, 2H), 2.34 (s, 3H). MS (ESI): m/z 398 $(M+H)^+$.

2-Methyl-8-morpholino-N-(2-(naphthalen-2-yl)ethyl)imidazo[1,2-a]pyrazin-3-amine (13). Pale yellow oil, yield 64%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (m, 3H), 7.69 (d, *J*=1.6 Hz, 1H), 7.49 (tt, *J*=6.9, 5.1 Hz, 2H), 7.38 (dd, *J*=8.4, 1.7 Hz, 1H), 7.30 (s, 2H), 4.21 (t, *J*=4.8 Hz, 4H), 3.91-3.86 (m, 4H), 3.40 (t, *J*=7.0 Hz, 2H), 3.05 (t, *J*=7.0 Hz, 2H), 2.33 (s, 3H). MS (ESI): m/z 388 $(M+H)^+$.

N-(2,4-dichlorophenethyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (14). Pale

yellow oil, yield 77%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.30 (m, 4H), 7.13 (t, *J*=8.0 Hz, 1H), 4.25-4.19 (m, 4H), 3.92-3.87 (m, 4H), 3.30-3.19 (m, 4H), 2.40 (s, 3H). MS (ESI): m/z 406 $(M+H)^+$.

Compound **15-16** were prepared using general procedure A.

2-(2-Bromo-4-methoxyphenyl)-N-(2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-yl)acetamide (15). White solid, yield 82%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H), 7.43-7.36 (m, 3H), 7.22 (d, *J*=2.6 Hz, 1H), 6.97 (dd, *J*=8.5, 2.7 Hz, 1H), 4.15-4.11 (m, 4H), 3.89 (s, 2H), 3.78 (s, 3H), 3.75-3.71 (m, 4H), 2.24 (s, 3H). HRMS (ESI^+) calcd for $C_{20}H_{22}O_3N_5BrH^+$ 460.0979, found 460.0977.

2-(3-Bromo-4-methoxyphenyl)-N-(2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-yl)acetamide (16). White solid, yield 88%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (d, *J* =2.1 Hz, 1H), 7.38-7.31 (m, 3H), 7.11 (d, *J*=8.5 Hz, 1H), 4.13 (t, *J*=4.8 Hz, 4H), 3.85 (s, 3H), 3.77-3.69 (m, 6H), 2.19 (s, 3H). MS (ESI): m/z 460 $(M+H)^+$.

Compound **17-18** were prepared using general procedure B.

N-(2-bromo-4-methoxyphenethyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (17). Pale brown solid, yield 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.30 (m, 2H), 7.16-7.12 (m, 2H), 6.83 (dd, *J*=8.5, 2.7 Hz, 1H), 4.26-4.18 (m, 4H), 3.92-3.86 (m, 4H), 3.81 (s, 3H), 3.25 (dd,

J=8.1, 6.7 Hz, 2H), 2.95 (dd, *J*=8.1, 6.7 Hz, 2H), 2.38 (s, 3H). HRMS (ESI^+) calcd for $C_{20}H_{24}O_2N_5BrH^+$ 446.1186, found 446.1191.

N-(3-bromo-4-methoxyphenethyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (18). Pale brown solid, yield 78%. ^1H NMR (400 MHz, $CDCl_3$) δ 7.43 (dd, *J*=5.3, 2.4 Hz, 1H), 7.34 (t, *J*=5.0 Hz, 1H), 7.30 (q, *J*=2.0 Hz, 1H), 7.19-7.09 (m, 1H), 6.87 (dd, *J*=9.6, 4.6 Hz, 1H), 4.22 (q, *J*=5.0 Hz, 4H), 3.89 (m, 7H), 3.25 (m, 2H), 2.90 (s, 1H), 2.80 (q, *J*=6.6 Hz, 2H), 2.37-2.33 (m, 3H). MS (ESI): m/z 446 $(M+H)^+$.

General procedure C for preparing compound 19-20. Compound **17-18** (1 eq.), K_2CO_3 (2 eq.), Xphos (2 eq.) and phenylboronic acid were suspended in dioxane/H_2O(20:1) and $Pd_2(dba)_3$ (0.1 eq.) was added under N_2 protection. The reaction tube was sealed and heated at 100 ^oC for 5h. The mixture was diluted with ethyl acetate. The organic layer was washed with water and brine, dried with Na_2SO_4 and concentrated. The crude products were purified by silica gel (ethyl acetate/ petroleum) to afford the corresponding products **19-20**.

N-(2-(5-methoxy-[1,1'-biphenyl]-2-yl)ethyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (19). Pale yellow oil, yield 61%. ^1H NMR (400 MHz, $CDCl_3$) δ 7.54-7.38 (m, 9H), 7.32 (dd, *J*=8.1, 5.3 Hz, 2H), 6.72 (s, 1H), 4.22-4.18 (m, 4H), 3.89 (s, 3H), 3.87-3.83 (m, 4H), 3.21 (t, *J*=7.1 Hz, 2H), 2.79 (t, *J*=7.0 Hz, 2H), 2.22 (s, 3H). MS (ESI): m/z 444 $(M+H)^+$.

N-(2-(6-methoxy-[1,1'-biphenyl]-3-yl)ethyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-

amine (20). Brown oil, yield 58%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56-7.51 (m, 2H), 7.43 (m, 2H), 7.38-7.33 (m, 1H), 7.32-7.29 (m, 2H), 7.19 (d, *J*=7.5 Hz, 2H), 6.98-6.94 (m, 1H), 4.25-4.18 (m, 4H), 3.92-3.86 (m, 4H), 3.83 (s, 3H), 3.29 (t, *J*=7.1 Hz, 2H), 2.93 (s, 1H), 2.87 (t, *J*=7.0 Hz, 2H), 2.35 (s, 3H). MS (ESI): m/z 444 $(M+H)^+$.

N-(2-bromobenzyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (21). Compound **4** (3 g, 12.86 mmol), 2-bromobenzyl bromide (3.54 g, 14.15 mmol) and K_2CO_3 were suspended in 20 mL DMF and stirred at room temperature for 18h. The mixture was poured into 50 mL water and extracted with ethyl acetate. The organic layer was washed with brine, dried with Na_2SO_4 and concentrated. The crude product was purified by silica gel (ethyl acetate/ petroleum 1:3) to afford the product as white solid (4.45 g, yield 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61-7.57 (m, 1H), 7.43 (d, *J*=4.5 Hz, 1H), 7.33 (d, *J*=4.5 Hz, 1H), 7.22-7.14 (m, 2H), 7.05 (dd, *J*=7.2, 2.0 Hz, 1H), 4.22-4.16 (m, 6H), 3.89-3.84 (m, 4H), 3.59 (t, *J*=7.0 Hz, 1H), 2.04 (s, 3H). MS (ESI): m/z 402 $(M+H)^+$.

N-(4-bromobenzyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (22). Compound **22** was prepared using the same method as compound **21**. Brown solid 4.6 g, yield 89%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.47-7.43 (m, 2H), 7.06-7.02 (m, 2H), 4.30 (d, *J*=5.1 Hz, 4H), 3.88-3.84 (m, 4H), 2.45 (s, 3H). MS (ESI): m/z 402 $(M+H)^+$.

Compound **23-46** were prepared using general procedure C.

2-Methyl-N-((4'-methyl-[1,1'-biphenyl]-2-yl)methyl)-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (23). Brown solid, yield 65%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.34 (m, 3H), 7.28-7.24 (m, 1H), 7.20-7.15 (m, 3H), 7.14-7.10 (m, 2H), 6.96 (d, *J*=4.5 Hz, 1H), 4.22-4.17 (m, 4H), 4.10 (s, 2H), 3.91-3.86 (m, 4H), 2.40 (s, 3H), 2.00 (s, 3H). MS (ESI): m/z 414 $(M+H)^+$.

2-Methyl-8-morpholino-N-(2-(pyrimidin-5-yl)benzyl)imidazo[1,2-a]pyrazin-3-amine (24). Brown solid, yield 61%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.62 (s, 2H), 7.48 (m, 3H), 7.26-7.21 (m, 2H), 7.03 (d, *J*=4.5 Hz, 1H), 4.25-4.19 (m, 4H), 4.11 (s, 2H), 3.92-3.87 (m, 4H), 2.00 (s, 3H). MS (ESI): m/z 402 $(M+H)^+$.

2-Methyl-8-morpholino-N-(2-(pyridin-3-yl)benzyl)imidazo[1,2-a]pyrazin-3-amine (25). Brown solid, yield 59%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58-8.51 (m, 2H), 7.49-7.38 (m, 4H), 7.26-7.18 (m, 3H), 7.02 (d, *J*=4.5 Hz, 1H), 4.21 (dd, *J*=5.7, 3.9 Hz, 4H), 4.10 (s, 2H), 3.91-3.85 (m, 4H), 1.99 (s, 3H). MS (ESI): m/z 401 $(M+H)^+$.

2-Methyl-8-morpholino-N-(2-(pyridin-4-yl)benzyl)imidazo[1,2-a]pyrazin-3-amine (26). Brown solid, yield 67%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60-8.55 (m, 2H), 7.48-7.39 (m, 3H), 7.24-7.21 (m, 2H), 7.16-7.12 (m, 2H), 7.02 (d, *J*=4.5 Hz, 1H), 4.23-4.18 (m, 4H), 4.10 (s, 2H), 3.91-3.86 (m, 4H), 2.01 (s, 3H). MS (ESI): m/z 401 $(M+H)^+$.

N-((4'-methoxy-[1,1'-biphenyl]-2-yl)methyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (27). Brown solid, yield 74%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.32 (m, 3H), 7.27-7.23

(m, 1H), 7.20 (d, *J*=4.5 Hz, 1H), 7.17-7.11 (m, 2H), 7.00 (d, *J*=4.5 Hz, 1H), 6.92-6.86 (m, 2H), 4.23-4.17 (m, 4H), 4.10 (s, 2H), 3.88 (t, *J*=4.8 Hz, 4H), 3.86 (s, 3H), 2.01 (s, 3H). MS (ESI): m/z 430 $(M+H)^+$.

N-(2-(3,5-dimethylisoxazol-4-yl)benzyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (28). Brown solid, yield 68%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53-7.50 (m, 1H), 7.46-7.37(m, 2H), 7.29 (d, *J*=4.5 Hz, 1H), 7.16-7.13 (m, 1H), 7.09 (d, *J*=4.5 Hz, 1H), 4.22-4.18 (m, 4H), 3.99-3.89 (m, 2H), 3.89-3.84 (m, 4H), 2.20 (s, 3H), 2.14 (s, 3H), 2.08 (s, 3H). MS (ESI): m/z 419 $(M+H)^+$.

2-Methyl-N-(2-(1-methyl-1H-pyrazol-4-yl)benzyl)-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (29). Brown solid, yield 59%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.34 (s, 1H), 7.33-7.29 (m, 2H), 7.28-7.23 (m, 3H), 7.18 (d, *J*=4.5 Hz, 1H), 4.20 (m, 6H), 3.92 (s, 3H), 3.89-3.86 (m, 4H), 2.07 (s, 3H). MS (ESI): m/z 404 $(M+H)^+$.

N-((3',4'-dimethoxy-[1,1'-biphenyl]-2-yl)methyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (30). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.32 (m, 3H), 7.30-7.27 (m, 1H), 7.22 (d, *J*=4.5 Hz, 1H), 7.05 (d, *J*=4.5 Hz, 1H), 6.85-6.83 (m, 1H), 6.80 (m, 1H), 6.75 (m, 1H), 4.21-4.16 (m, 4H), 4.12 (s, 2H), 3.93 (s, 3H), 3.89-3.85 (m, 7H), 2.02 (s, 3H).MS (ESI): m/z 460 $(M+H)^+$.

2-Methyl-8-morpholino-N-(2-(naphthalen-2-yl)benzyl)imidazo[1,2-a]pyrazin-3-amine (31). Brown solid, yield 72%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92-7.79 (m, 3H), 7.68-7.65 (m, 1H),

7.58-7.51 (m, 2H), 7.48-7.44 (m, 1H), 7.44-7.40 (m, 2H), 7.39-7.35 (m, 2H), 6.86 (d, *J*=4.5 Hz, 1H), 6.75 (d, *J*=4.5 Hz, 1H), 4.15 (m, 6H), 3.89-3.85 (m, 4H), 2.00 (s, 3H). MS (ESI): m/z 450 $(M+H)^+$.

N-([1,1'-biphenyl]-2-ylmethyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (32). Brown solid, yield 66%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.35 (m, 6H), 7.29 (d, *J*=2.9 Hz, 1H), 7.25-7.22 (m, 2H), 7.20 (d, *J*=4.5 Hz, 1H), 6.99 (d, *J*=4.5 Hz, 1H), 4.22-4.17 (m, 4H), 4.09 (s, 2H), 3.90-3.86 (m, 4H), 2.01 (s, 3H). MS (ESI): m/z 400 $(M+H)^+$.

N-((4'-(tert-butyl)-[1,1'-biphenyl]-2-yl)methyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (33). Brown solid, yield 52%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.35 (m, 5H), 7.28-7.25 (m, 1H), 7.19 (d, *J*=4.5 Hz, 1H), 7.16-7.11 (m, 2H), 7.03 (d, *J*=4.5 Hz, 1H), 4.22-4.18 (m, 4H), 4.09 (s, 2H), 3.91-3.86 (m, 4H), 1.95 (s, 3H), 1.38 (s, 9H). MS (ESI): m/z 456 $(M+H)^+$.

N-((2'-chloro-[1,1'-biphenyl]-2-yl)methyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (34). Brown solid, yield 63%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49-7.45 (m, 2H), 7.45-7.37 (m, 2H), 7.35-7.30 (m, 1H), 7.25-7.18 (m, 3H), 7.03-6.99 (m, 2H), 4.22-4.17 (m, 4H), 3.93-3.83 (m, 6H), 2.02 (s, 3H).MS (ESI): m/z 434 $(M+H)^+$.

2-Methyl-N-((4'-methyl-[1,1'-biphenyl]-4-yl)methyl)-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (35). Brown solid, yield 51%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59-7.55 (m, 2H), 7.53-7.49 (m, 2H), 7.45 (d, *J*=4.5 Hz, 1H), 7.37-7.33 (m, 3H), 7.30-7.26 (m, 2H), 4.26-4.20 (m, 4H), 4.17 (s,

2H), 3.93-3.87 (m, 4H), 2.25 (s, 3H). MS (ESI): m/z 414 $(M+H)^+$.

2-Methyl-8-morpholino-N-(4-(pyrimidin-5-yl)benzyl)imidazo[1,2-a]pyrazin-3-amine (36). Brown solid, yield 55%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.22 (s, 1H), 8.96 (m, 2H), 7.59-7.54 (m, 2H), 7.49-7.42 (m, 3H), 7.36-7.34 (m, 1H), 4.24-4.18 (m, 6H), 3.88 (t, *J*=4.8 Hz, 4H), 2.25 (d, *J*=1.1 Hz, 3H). MS (ESI): m/z 402 $(M+H)^+$.

2-Methyl-8-morpholino-N-(4-(pyridin-3-yl)benzyl)imidazo[1,2-a]pyrazin-3-amine (37). Brown solid, yield 53%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86-8.84 (m, 1H), 8.62-8.59 (m, 1H), 7.91-7.86 (m, 1H), 7.59-7.55 (m, 2H), 7.46-7.37 (m, 4H), 7.35 (d, *J*=4.5 Hz, 1H), 4.24-4.18 (m, 6H), 3.91-3.85 (m, 4H), 2.25 (s, 3H). MS (ESI): m/z 401 $(M+H)^+$.

2-Methyl-8-morpholino-N-(4-(pyridin-4-yl)benzyl)imidazo[1,2-a]pyrazin-3-amine (38). Brown solid, yield 60%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69-8.64 (m, 2H), 7.64-7.58 (m, 2H), 7.53-7.49 (m, 2H), 7.45-7.39 (m, 3H), 7.34 (d, *J*=4.5 Hz, 1H), 4.23-4.18 (m, 6H), 3.90-3.84 (m, 4H), 2.24 (s, 3H). MS (ESI): m/z 401 $(M+H)^+$.

N-((4'-methoxy-[1,1'-biphenyl]-4-yl)methyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (39). Brown solid, yield 67%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57-7.51 (m, 4H), 7.45 (d, *J*=4.5 Hz, 1H), 7.37-7.32 (m, 3H), 7.03-6.98 (m, 2H), 4.25-4.20 (m, 4H), 4.17 (s, 2H), 3.91-3.87 (m, 7H), 2.24 (s, 3H). MS (ESI): m/z 430 $(M+H)^+$.

N-(4-(3,5-dimethylisoxazol-4-yl)benzyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (40). Brown solid, yield 73%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J*=4.5 Hz, 1H), 7.39-7.35 (m, 2H), 7.33 (d, *J*=4.5 Hz, 1H), 7.25-7.21 (m, 2H), 4.24-4.19 (m, 4H), 4.18 (s, 2H), 3.93-3.84 (m, 4H), 2.41 (s, 3H), 2.27 (s, 3H), 2.24 (s, 3H). MS (ESI): m/z 419 $(M+H)^+$.

2-Methyl-N-(4-(1-methyl-1H-pyrazol-4-yl)benzyl)-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (41). Brown solid, yield 68%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.62 (s, 1H), 7.45-7.41 (m, 3H), 7.33 (d, *J*=4.5 Hz, 1H), 7.26 (d, *J*=8.1 Hz, 2H), 4.21 (t, *J*=4.8 Hz, 4H), 4.12 (s, 2H), 3.95 (s, 3H), 3.90-3.85 (m, 4H), 2.22 (s, 3H). MS (ESI): m/z 404 $(M+H)^+$.

N-((3',4'-dimethoxy-[1,1'-biphenyl]-4-yl)methyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (42). Brown solid, yield 53%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56-7.52 (m, 2H), 7.45 (d, *J*=4.5 Hz, 1H), 7.37-7.32 (m, 3H), 7.16 (dd, *J*=8.3, 2.1 Hz, 1H), 7.11 (d, *J*=2.1 Hz, 1H), 6.97 (d, *J*=8.3 Hz, 1H), 4.22 (dd, *J*=5.6, 4.0 Hz, 4H), 4.17 (s, 2H), 3.98 (s, 3H), 3.95 (s, 3H), 3.91-3.87 (m, 4H), 2.25 (s, 3H). MS (ESI): m/z 460 $(M+H)^+$.

2-Methyl-8-morpholino-N-(4-(naphthalen-2-yl)benzyl)imidazo[1,2-a]pyrazin-3-amine (43). Brown solid, yield 62%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07-8.05 (m, 1H), 7.96-7.93 (m, 2H), 7.92-7.88 (m, 2H), 7.76-7.68 (m, 1H), 7.74-7.69 (m, 1H), 7.55-7.51 (m, 2H), 7.47 (d, *J*=4.5 Hz, 1H), 7.44-7.39 (m, 2H), 7.37 (d, *J*=4.5 Hz, 1H), 4.26-4.22 (m, 4H), 4.21 (s, 2H), 3.93-3.88 (m, 4H), 2.27 (s, 3H). MS (ESI): m/z 450 $(M+H)^+$.

N-([1,1'-biphenyl]-4-ylmethyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (44). Brown solid, yield 61%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63-7.57 (m, 4H), 7.49-7.44 (m, 3H), 7.40-7.34 (m, 4H), 4.24-4.21 (m, 4H), 4.19 (s, 2H), 3.91-3.88 (m, 4H), 2.25 (s, 3H).MS (ESI): m/z 400 $(M+H)^+$.

N-((4'-(tert-butyl)-[1,1'-biphenyl]-4-yl)methyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (45). Brown solid, yield 57%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60-7.54 (m, 4H), 7.52-7.48 (m, 2H), 7.45 (d, *J*=4.5 Hz, 1H), 7.35 (dd, *J*=6.4, 1.8 Hz, 3H), 4.25-4.21 (m, 4H), 4.17 (s, 2H), 3.92-3.87 (m, 4H), 2.24 (s, 3H), 1.40 (s, 9H). MS (ESI): m/z 456 $(M+H)^+$.

N-((2'-chloro-[1,1'-biphenyl]-4-yl)methyl)-2-methyl-8-morpholinoimidazo[1,2-a]pyrazin-3-amine (46). Brown solid, yield 63%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51-7.41 (m, 4H), 7.39-7.29 (m, 5H), 7.15-7.07 (m, 1H), 4.28-4.20 (m, 4H), 4.19 (d, *J*=3.0 Hz, 2H), 3.94-3.86 (m, 4H), 2.25 (d, *J*=1.7 Hz, 3H). MS (ESI): m/z 434 $(M+H)^+$.

4-(9H-purin-6-yl)morpholine (48). 6-Chloro-7H-purine (5 g, 32.35 mmol) was added morpholine 60 mL and heated to 80 ^{o}C overnight. The mixture was concentrated in vacuum. The residue was purified by silica gel (CH_2Cl_2/MeOH 20:1) to obtain the product **48** as pale yellow solid 6.4 g, yield 96%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (s, 1H), 8.14 (s, 1H), 3.82-3.76 (m, 4H), 3.74-3.68 (m, 4H). MS (ESI): m/z 205 $(M+H)^+$.

General procedure D for preparing compound 49-58. 4-(9H-purin-6-yl)morpholine (1eq.),

benzyl bromides (1eq.) and K_2CO_3 (1.1eq.) were stirred in DMF at room temperature overnight. The mixture was added water and extracted with EtOAc. The organic layer was washed with brine, dried with Na_2SO_4 and concentrated. The crude product was purified by silica gel chromatography to give the desired product.

4-(9-Phenethyl-9H-purin-6-yl)morpholine (49). Colorless oil, yield 79%. 1H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.37 (s, 1H), 7.33-7.23 (m, 3H), 7.10 (d, *J*=6.7 Hz, 2H), 4.45 (t, *J*=7.0 Hz, 2H), 4.35-4.29 (m, 4H), 3.88-3.83 (m, 4H), 3.19 (t, *J*=7.0 Hz, 2H). MS (ESI): m/z 310 $(M+H)^+$.

4-(9-(3-Methoxyphenethyl)-9H-purin-6-yl)morpholine (50). Brown solid, yield 88%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.39 (s, 1H), 7.22 (t, *J*=7.9 Hz, 1H), 6.8-6.78 (m, 1H), 6.69 (d, *J*=7.6 Hz, 1H), 6.63 (t, *J*=2.1 Hz, 1H), 4.45 (t, *J*=6.9 Hz, 2H), 4.32 (s, 4H), 3.88-3.83 (m, 4H), 3.77 (s, 3H), 3.16 (t, *J*=6.9 Hz, 2H). MS (ESI): m/z 340 $(M+H)^+$.

2-(6-Morpholino-9H-purin-9-yl)-1-phenylethanone (51). Pale yellow oil, yield 84%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.10-8.07 (m, 2H), 7.87 (s, 1H), 7.70 (t, *J*=7.4 Hz, 1H), 7.60-7.54 (m, 2H), 5.70 (s, 2H), 4.36 (s, 4H), 3.91-3.86 (m, 4H). MS (ESI): m/z 324 $(M+H)^+$.

4-(9-Benzyl-9H-purin-6-yl)morpholine (52). Pale yellow solid, yield 89%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.73 (s, 1H), 7.40-7.29 (m, 5H), 5.39 (s, 2H), 4.37-4.29 (m, 4H), 3.88-3.84 (m, 4H). MS (ESI): m/z 296 $(M+H)^+$.

4-**(9-(2,6-Dichlorobenzyl)-9H-purin-6-yl)morpholine (53).** Brown solid, yield 68%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.50 (s, 1H), 7.45 (s, 1H), 7.43 (s, 1H), 7.36-7.31 (m, 1H), 5.65 (s, 2H), 4.32 (s, 4H), 3.85 (t, *J*=5.5Hz, 4H). MS (ESI): m/z 364 $(M+H)^+$.

4-(9-(3,5-Dimethoxybenzyl)-9H-purin-6-yl)morpholine (54). Brown solid, yield 77%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.73 (s, 1H), 6.41 (q, *J*=2.0 Hz, 3H), 4.33 (s, 4H), 3.88-3.83 (m, 4H), 3.76 (d, *J*=0.6 Hz, 6H). MS (ESI): m/z 356 $(M+H)^+$.

4-(9-(4-Bromobenzyl)-9H-purin-6-yl)morpholine (55). Brown solid, yield 84%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.73 (s, 1H), 7.51-7.47 (m, 2H), 7.18-7.14 (m, 2H), 5.34 (s, 2H), 4.33 (s, 4H), 3.88-3.84 (m, 4H).MS (ESI): m/z 373 $(M+H)^+$.

4-(9-(Naphthalen-2-ylmethyl)-9H-purin-6-yl)morpholine (56). Brown solid, yield 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (s, 1H), 7.86-7.79 (m, 3H), 7.78 (s, 1H), 7.74-7.71 (m, 1H), 7.53-7.48(m, 2H), 7.39 (dd, *J*=8.5, 1.8 Hz, 1H), 5.55 (s, 2H), 4.34 (d, *J*=7.1 Hz, 4H), 3.86 (t, *J*=5.6Hz, 4H). MS (ESI): m/z 346 $(M+H)^+$.

4-(9-([1,1'-Biphenyl]-4-ylmethyl)-9H-purin-6-yl)morpholine (57). Brown solid, yield 68%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.78 (s, 1H), 7.61-7.55 (m, 4H), 7.49-7.42 (m, 2H), 7.40-7.34 (m, 3H), 5.44 (s, 2H), 4.34 (s, 4H), 3.88-3.84 (m, 4H). MS (ESI): m/z 374 $(M+H)^+$.

4-(9-((4'-Methyl-[1,1'-biphenyl]-4-yl)methyl)-9H-purin-6-yl)morpholine (58). Brown solid,

yield 58%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.78 (s, 1H), 7.60-7.54 (m, 3H), 7.50-7.44 (m, 3H), 7.37-7.32 (m, 2H), 7.27-7.23 (m, 2H), 5.43 (s, 2H), 4.34 (s, 4H), 3.89-3.84 (m, 4H), 2.41 (s, 3H). MS (ESI): m/z 386 $(M+H)^+$.

4-(8-Ethyl-9H-purin-6-yl)morpholine (62). 6-Chloro-4,5-diaminopyrimidine (5 g, 35 mmol), ethyl orthopropionate 49 mL and triethylamine 48 mL were dissolved in 50 mL acetonitrile and stirred at 120 ^oC overnight. The mixture was concentrated in vacuum to yield the crude product **61** (5 g). Compound **62** was prepared using the same method of compound **48**. Pale yellow solid 4.2 g was obtained. Total yield of two steps was 52%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.81 (s, 1H), 8.17 (d, *J*=0.8 Hz, 1H), 4.17 (s, 4H), 3.71 (t, *J*=4.8 Hz, 4H), 2.77 (q, *J*=7.5 Hz, 2H), 1.28 (td, *J*=7.6, 1.2 Hz, 3H). MS (ESI): m/z 234 $(M+H)^+$.

4-(9-([1,1'-Biphenyl]-4-ylmethyl)-8-ethyl-9H-purin-6-yl)morpholine (63). Compound **63** was prepared using the general procedure D. Pale yellow solid, yield 88%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.57-7.51 (m, 4H), 7.47-7.41 (m, 2H), 7.38-7.33 (m, 1H), 7.23-7.19 (m, 2H), 5.43 (s, 2H), 4.35 (s, 4H), 3.92-3.82 (m, 4H), 2.78 (q, *J*=7.5 Hz, 2H), 1.34 (t, *J*=7.5 Hz, 3H). MS (ESI): m/z 400 $(M+H)^{+\cdot}$

4-(8-Methyl-9H-purin-6-yl)morpholine (66). Compound **66** was prepared using the same method of compound **62**. Pale yellow solid, yield 61%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.79 (s, 1H), 8.16 (s, 1H), 4.20-4.12 (m, 4H), 3.71 (t, *J*=4.8 Hz, 4H), 2.44 (s, 3H). MS (ESI): m/z 220 $(M+H)^+$.

4-(9-(3-Bromobenzyl)-8-methyl-9H-purin-6-yl)morpholine (67). Compound **67** was prepared using the general procedure D. Pale yellow solid, yield 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.42 (dt, *J*=7.9, 1.4 Hz, 1H), 7.29 (t, *J*=1.9 Hz, 1H), 7.18 (t, *J*=7.8 Hz, 1H), 7.06 (ddd, *J*=7.7, 1.9, 1.0 Hz, 1H), 5.33 (s, 2H), 4.30 (s, 4H), 3.89-3.81 (m, 4H), 2.44 (s, 3H). MS (ESI): m/z 388 $(M+H)^+$.

4-(9-(4-Bromobenzyl)-8-methyl-9H-purin-6-yl)morpholine (68). Compound **68** was prepared using the general procedure D. Pale yellow solid, yield 87%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48-7.43 (m, 2H), 7.38 (d, *J*=4.5 Hz, 1H), 7.33 (d, *J*=4.5 Hz, 1H), 7.18-7.14 (m, 2H), 4.24-4.19 (m, 4H), 4.09 (d, *J*=6.2 Hz, 2H), 3.90-3.85 (m, 4H), 2.21 (s, 3H). MS (ESI): m/z 388 $(M+H)^+$.

General procedure E for preparing compound 69-80. Compound **68** (1 eq.), boronic acid (1.25 eq.) and 2M Na_2CO_3 (2.5 eq.) were suspended in DME. The mixture was added $Pd(PPh_3)_4$ (0.05 eq.) after degassed for 10 minutes. The tube was sealed and stirred at 100 ^{o}C for 5 hours. The mixture was diluted with EtOAc and washed with water and brine. The organic layer was concentrated in vacuo. The residue was purified by silica gel chromatography (CH_2Cl_2: MeOH/ 30:1) to yield the product.

4-(9-([1,1'-Biphenyl]-4-ylmethyl)-8-methyl-9H-purin-6-yl)morpholine (69). Brown solid, yield 59%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.58-7.52 (m, 4H), 7.48-7.41 (m, 2H), 7.39-7.33 (m, 1H), 7.26-7.21 (m, 2H), 5.43 (s, 2H), 4.38-4.27 (m, 4H), 3.90-3.84 (m, 4H), 2.44 (s, 3H). MS

(ESI): m/z 386 $(M+H)^+$.

4-(9-((4'-Methoxy-[1,1'-biphenyl]-4-yl)methyl)-8-methyl-9H-purin-6-yl)morpholine (70). Brown solid, yield 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.52-7.48 (m, 4H), 7.21 (d, *J*=8.2 Hz, 2H), 7.01-6.95 (m, 2H), 5.41 (s, 2H), 4.32 (s, 4H), 3.89-3.85 (m, 7H), 2.50 (s, 3H). MS (ESI): m/z 416 $(M+H)^+$.

4-(9-((4'-Chloro-[1,1'-biphenyl]-4-yl)methyl)-8-methyl-9H-purin-6-yl)morpholine (71). Brown solid, yield 51%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.52-7.46 (m, 4H), 7.42-7.38 (m, 2H), 7.22 (d, *J*=1.8 Hz, 2H), 5.42 (s, 2H), 4.37-4.28 (m, 4H), 3.89-3.84 (m, 4H), 2.49 (s, 3H). MS (ESI): m/z 420 $(M+H)^+$.

4-(9-((3'-Chloro-[1,1'-biphenyl]-4-yl)methyl)-8-methyl-9H-purin-6-yl)morpholine (72). Brown solid, yield 45%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.54-7.51 (m, 2H), 7.51-7.49 (m, 1H), 7.44-7.31 (m, 3H), 7.23 (d, *J*=6.4 Hz, 2H), 5.42 (s, 2H), 4.35-4.30 (m, 4H), 3.90-3.84 (m, 4H), 2.49 (s, 3H).MS (ESI): m/z 420 $(M+H)^+$.

4-(9-((2'-Chloro-[1,1'-biphenyl]-4-yl)methyl)-8-methyl-9H-purin-6-yl)morpholine (73). Brown solid, yield 39%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.49-7.46 (m, 1H), 7.43-7.38 (m, 2H), 7.33-7.29 (m, 3H), 7.22 (d, *J*=7.9 Hz, 2H), 5.44 (s, 2H), 4.34-4.31 (m, 4H), 3.89-3.84 (m, 4H), 2.52 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.22 , 152.42 , 151.86 , 147.71 , 139.76 , 139.09 , 135.18 , 132.42 , 131.27 , 130.02×2 , 130.01 , 128.73 , 126.90 , 126.60×2 , 119.00,

67.11×2 , 45.51 ×2, 29.73 , 14.37 .MS (ESI): m/z 420 $(M+H)^+$.HRMS (ESI+) calcd for $C_{23}H_{23}N_5OCl(M+H)^+$ 420.1586, found 420.1584.

4-(8-Methyl-9-(4-(pyridin-4-yl)benzyl)-9H-purin-6-yl)morpholine (74). Brown solid, yield 53%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (m, 2H), 8.38 (s, 1H), 7.60 (m, 2H), 7.48 (m, 2H), 7.28 (m, 2H), 5.44 (s, 2H), 4.35-4.29 (m, 4H), 3.91-3.80 (m, 4H), 2.50 (s, 3H). MS (ESI): m/z 387 $(M+H)^+$.

4-(8-Methyl-9-(4-(pyridin-3-yl)benzyl)-9H-purin-6-yl)morpholine (75). Brown solid, yield 40%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.60 (d, *J*=4.48Hz, 1H), 8.38 (s, 1H), 7.85 (d, *J*=7.85Hz, 1H), 7.54 (m, 2H), 7.41-7.35 (dd, *J*=7.67Hz, 4.70Hz, 1H), 7.27 (m, 2H), 5.44 (s, 2H), 4.35-4.28 (m, 4H), 3.93-3.81 (m, 4H), 2.50 (s, 3H). MS (ESI): m/z 387 $(M+H)^+$.

4-(8-Methyl-9-(4-(2-methylpyridin-4-yl)benzyl)-9H-purin-6-yl)morpholine (76). Brown solid, yield 37%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55-8.51 (m, 1H), 8.37 (s, 1H), 7.60-7.55 (m, 2H), 7.34-7.32 (m, 1H), 7.29-7.24 (m, 3H), 5.44 (s, 2H), 4.32 (s, 4H), 3.89-3.84 (m, 4H), 2.62 (s, 3H), 2.49 (s, 3H). MS (ESI): m/z 401 $(M+H)^+$.

4-(8-Methyl-9-(4-(pyrimidin-5-yl)benzyl)-9H-purin-6-yl)morpholine (77). Brown solid, yield 44%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.22 (s, 1H), 8.93 (s, 2H), 8.38 (s, 1H), 7.55 (d, *J*=8.0 Hz, 2H), 7.32 (d, *J*=8.0 Hz, 2H), 5.46 (s, 2H), 4.32 (s, 4H), 3.88-3.85 (m, 4H), 2.50 (s, 3H). ^{13}C NMR (126 MHz, CDCl3) δ 157.63, 154.80, 153.21, 152.34, 151.90, 147.37, 136.92, 133.99, 133.65,

127.94×2, 127.54×2, 118.96, 114.97, 77.25×2, 67.06, 45.29, 29.67, 14.23.MS (ESI): m/z 388 $(M+H)^+$. HRMS (ESI+) calcd for $C_{21}H_{22}N_7$ $(M+H)^+$388.1880, found 388.1870.

4-(8-Methyl-9-(4-(1-methyl-1H-pyrazol-4-yl)benzyl)-9H-purin-6-yl)morpholine (78). Brown solid, yield 51%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.73 (s, 1H), 7.59 (s, 1H), 7.41 (d, *J*=8.0 Hz, 2H), 7.16 (d, *J*=8.0 Hz, 2H), 5.37 (s, 2H), 4.34-4.28 (m, 4H), 3.95 (s, 3H), 3.90-3.84 (m, 4H), 2.48 (s, 3H). MS (ESI): m/z 390 $(M+H)^+$.

4-(9-(4-(3,5-Dimethylisoxazol-4-yl)benzyl)-8-methyl-9H-purin-6-yl)morpholine (79). Brown solid, yield 60%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.25-7.19 (m, 4H), 5.42 (s, 2H), 4.35-4.29 (m, 4H), 3.90-3.84 (m, 4H), 2.52 (s, 3H), 2.39 (s, 3H), 2.25 (s, 3H). MS (ESI): m/z 405 $(M+H)^+$.

4-(9-(4-(1H-pyrazolo[3,4-b]pyridin-5-yl)benzyl)-8-methyl-9H-purin-6-yl)morpholine (80). Brown solid, yield 56%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.40 (s, 1H), 8.23 (s, 1H), 8.16 (s, 1H), 7.57 (d, *J*=7.9 Hz, 2H), 7.31 (d, *J*=7.9 Hz, 2H), 5.46 (s, 2H), 4.33 (s, 4H), 3.90-3.84 (m, 4H), 2.52 (s, 3H). MS (ESI): m/z 427 $(M+H)^+$.

Ethyl 4-((8-methyl-6-morpholino-9H-purin-9-yl)methyl)benzoate (81). Compound **81** was prepared using the general procedure D. Pale yellow solid, yield 74%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37-8.36 (m, 1H), 8.02-7.99 (m, 2H), 7.20 (d, *J*=8.2 Hz, 2H), 5.43 (s, 2H), 4.41-4.35 (m, 2H),4.32 (s, 4H), 3.90-3.85 (m, 4H), 2.44 (s, 3H), 1.39 (t, *J*=7.1 Hz, 3H). MS (ESI): m/z 381

$(M+H)^+$.

4-((8-Methyl-6-morpholino-9H-purin-9-yl)methyl)-N-phenylbenzamide (83). Compound **81** (55 mg, 0.14 mmol) was suspended in mixed solvent (MeOH: H_2O/3:1) 0.7 mL and 150 mg LiOH was added. The mixture was heated at 60 ^oC for 5 hours and stirred at room temperature overnight. The reaction was diluted with water and washed with EtOAc for twice. The aqueous layer was adjusted to pH 6 with 1N HCl. White precipitate was formed. The solid was filtered, washed with water and dried to give the product compound **82** 35 mg. The mixture of compound **82** (30 mg, 0.09 mmol), aniline (21 mg, 0.23 mmmo), EDC.HCl (23 mg, 0.115 mmol), DMAP (1.5 mg, 0.01 mmol) and DMF 1 mL was stirred at room temperature overnight. The mixture was concentrated in vacuum. The residue was purified by silica gel chromatography (CH_2Cl_2: MeOH/ 15:1) to yield the product **83** as white solid (22 mg, total yield 43%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.37 (d, *J*=0.8 Hz, 1H), 7.87-7.81 (m, 2H), 7.81-7.77 (m, 1H), 7.63 (d, *J*=8.1 Hz, 2H), 7.39 (t, *J*=7.8 Hz, 2H), 7.26 (s, 1H), 7.21-7.15 (m, 1H), 5.45 (s, 2H), 4.33 (s, 4H), 3.90-3.85 (m, 4H), 2.47 (s, 3H). MS (ESI): m/z 429 $(M+H)^+$.

Enzymatic assay

The IMAP fluorescence polarization PDE assays were performed by Shanghai ChemPartner Co., Ltd. in which binding of hydrolyzed fluorescent cyclic nucleotide substrate to the IMAP reagent increases fluorescence polarization. Briefly, dose response curves or indicated concentrations of compounds were made in DMSO. A 200-nL aliquot of the compound solution was transferred into a 384-well plate by

Echo 550 in duplicate (the final fraction of DMSO was 1%). Compound was incubated with recombinant PDE2A1, PDE4A1A, PDE5A1 and PDE10A1 (BPS Biosciences) at room temperature for 15 min, followed by adding fluorescein-labeled cAMP or fluorescein-labeled cGMP (the former for PDE2A1, PDE4A1A and PDE10A1, and the latter for PDE5A1) to initiate the reaction. Reactions were incubated for 30 min at room temperature and were terminated by the addition of IMAP binding reagent. The assay plate was protected from light and incubated for 1hr at RT. FP signal was then read on Victor at Ex485 Em535(s), Em535(p).

For 100% inhibition control (Min), 1× assay buffer was used instead of PDE enzyme solution. And for no inhibition control (Max), DMSO was used instead of compound DMSO solution. The inhibition percentage in the presence of the compound was calculated according to the equation, Percent inhibition = (Max-Signal) / (Max-Min)*100%. Fit the data in GraphPad Prism V5.0 software to obtain IC_{50} values using equation, Y=Bottom + (Top – Bottom)/(1 + 10^((LogIC50 – X)*HillSlope), where Y stands for inhibition percentage and X stands for compound concentration.

In vitro metabolic stability study

Microsomes (Human microsome: RILD，Lot No. SUBK; Mouse microsome: RILD, Lot No. STOM) (0.5 mg/mL) were preincubated with 1 μM test compound for 5 min at 37 °C in 0.1 M phosphate buffer (pH 7.4) with 1 mmol EDTA, and 5 mmol $MgCl_2$. The reactions were initiated by adding cofactors (1 mmol NADPH). After 0, 5, 10, and 30 min incubations at 37 °C, the reactions were stopped by adding an equal

volume of cold acetonitrile containing tinidazole (Lot No.:074H0126) of 0.1μg/ml as internal standard. The samples were vortexed for 10 min and then centrifuged at 10,000 × g for 10 min. Supernatants were analyzed by LC/MS/MS for the amount of parent compound remaining, and the corresponding loss of parent compound also determined by LC/MS/MS.

The CYP enzymatic activities were characterized based on their probe reactions: CYP3A4 (midazolam), CYP2D6 (dextromethorphan), CYP2C9 (Diclofenac), CYP1A2 (phenacetin) and CYP2C19 (Mephenytoin). Incubation mixtures were prepared in a total volume of 100 μL as follows: 0.2 mg/mL microsome (Human microsome: Xenotech, Lot No. 1410013), 100 mmol phosphate buffer (pH 7.4), probe substrates cocktail (10 μM Midazolam, 100 μM Testosterone, 10 μM Dextromethophan, 20 μM Diclofenac, 100 μM Phenacetin, 100 μM Mephenytoin) and 10 μM tested compound or positive control cocktail (10μM ketoconazole，10 μM quinidine， 100 μM Sulfaphenazole， 10 μM Naphthoflavone, and 1000 μM Tranylcypromine) or negative control (PBS). The final concentration of organic reagent in incubation mixtures was less than 1% v/v. There was a 5 min preincubation period at 37 °C before the reaction was initiated by adding a 1 mmol/L NADPH. Reactions were conducted for 20 minutes for CYPs. For each probe drug, the percentage of metabolite conversion was less than 20% of substrate added. The inhibition rate was calculated as: (The formation of the metabolite of probe substrates with 10 μM tested compound)/ (The formation of the metabolite of probe substrates with PBS) × 100%.

***In vivo* tests**

Male SD rats (200–250 g) supplied by The Shanghai SLAC Laboratory were used for the acoustic startle and locomotor activity studies, Animals were housed under standard laboratory conditions under a 12-h light/dark cycle with food and water available ad libitum and were allowed a minimum of 1 week for acclimation before experimentation. Animals were handled and cared for in accordance with the Institute of Laboratory Animal Resources (1996), and all procedures were performed with the approval of the Soochow University Institutional Animal Care and Use Committee.

Phencyclidine hydrochloride (PCP) was synthesized by the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, and dissolved in sterile 0.9% saline. The PDE10A selective inhibitors **73** and **77** were dissolved and administered in sterile saline containing 0.5% Tween 80 (v /v) 0.2%CMC-sodium.

Locomotion test. Drugs were administered (p.o.) 30 min prior to 5 mg/kg PCP challenge (i.p.) and the test was performed 5 min after PCP injection. The locomotor activity was measured for a 1.5 h duration after PCP administration and the total traveled distance was expressed as mean ± SEM.. Rats were placed into a Plexiglas open field arena (40 × 40 × 45 cm, Jiliang Co. Ltd., Shanghai, China) with a video camera connected to a video recorder. Automated activity was recorded for 90 min and the total distance traveled was calculated by Jiliang Vision software (Jiliang Co. Ltd., Shanghai, China) as previously described.[16]

Prepulse inhibition test. Drugs were pretreated (p.o.) 30 min prior to 5mg/kg PCP (i.p.) and the test was performed 5-10 min after PCP injection. Data is expressed as mean ± SEM (+++ P<0.001 vs SAL,* P<0.05 vs PCP, n = 11–16 per group). PPI of the acoustic startle response was measured as described previously[17] in four startle chambers from Med Instruments (MED Associates, St. Albans, USA). Briefly, after a acclimation period, four respective types of trial were applied: a PULSE ALONE trial presented with a 40ms, 120dB white noise burst; three PREPULSE (72dB, 76dB or 81dB)+PULSE trials in which sounds (20ms, 3kHz) were presented for 100ms, respectively, before the onset of a 120dB pulse. All types of trial were presented 12 times in random order with an interval between 15s to 25s. To achieve a relatively stable level of startle reactivity, 3 PULSE ALONE trials were conducted at the beginning of the test session. A ventilating fan built into the chamber provided a background noise of 68dB throughout the test. PPI values were calculated as described before[18]. The acoustic startle magnitude was calculated as the average response of the PULSE ALONE trials. Results were expressed as mean±SEM and analyzed by ANOVA (one- or two-way) with appropriate post hoc tests using SPSS. The significance level was set to P<0.05.

Abbreviation

DIEA: N,N-Diisopropylethylamine; DME: Dimethoxyethane; DMF: N,N-Dimethylformamide; EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt: 1-Hydroxybenzotriazole; rt: Room temperature; TEA: Triethylamine; THF:

Tetrahydrofuran.

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Graphic Abstract