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Synthesis and Biological Evaluation of a Series of Novel 1-(3-((6-Fluoropyridin-3-yl)oxy)propyl) piperazines as Dopamine/Serotonin Receptor Agonists

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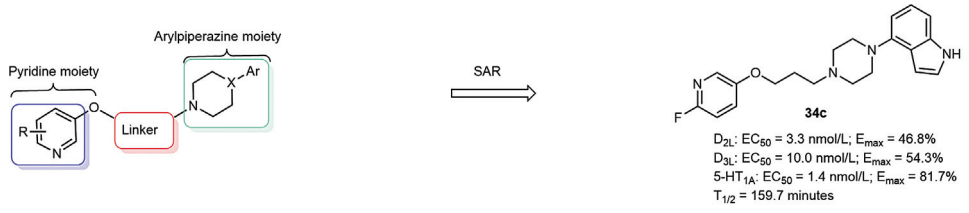

Abstract

Evidence suggested that the use of partial dopamine D_2/D_3 receptor agonists may be a better choice for the treatment of Parkinson's disease (PD), and the stimulation of 5-HT_{1A} receptors (mainly via nondopaminergic mechanisms) alleviates motor and non-motor disorders of PD, implying that the multitarget approach may provide a double bonus for the treatment of the disease. In this study, 20 novel 1-(3-((6-fluoropyridin-3-yl)oxy)propyl)piperazine derivatives were designed and synthesized using a bioisosterism approach, and their activities for D_2/D_3/5-HT_{1A} receptors were further tested. The results showed that several compounds exhibited a multitarget combination of D_2/5-HT_{1A} agonism. Compounds **7b** and **34c** showed agonistic activities on D_2/D_3/5-HT_{1A} receptor. The EC_{50} value of **7b** for D_2/D_3/5-HT_{1A} receptor were 0.9/19/2.3 nmol/L, respectively; and the EC_{50} value of **34c** for D_2/D_3/5-HT_{1A} receptor were 3.3/10/1.4 nmol/L, respectively. In addition, **34c** exhibited good metabolic stability (the half-life $T_{1/2} = 159.7$ minutes) *in vitro*, which is of great significance for the further exploration of multitarget anti-PD drugs.

Keywords

- Parkinson's disease
- D_2/D_3 receptor partial agonist
- 5-HT_{1A} agonist

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Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease in the elderly with a prevalence of approximately 2% of the population over 60 years old.[1] Patients show motor symptoms such as tremors at rest, bradykinesia, rigidity, and postural instability accompanied by nonmotor symptoms such as autonomic dysfunctions, cognitive impairment, sleep disorders, and mood disorders for the most part.[2–5] Pathologically, the cardinal motor deficits result from the gradual depletion of dopamine (DA) in the striatum caused by loss of dopaminergic neurons in the substantia nigra pars compacta, and accumulation of presynaptic neuronal protein α-synuclein known as Lewy bodies.[6] The nonmotor symptoms are related to specific dysfunction of cholinergic, noradrenergic, and serotonergic pathways in the brain, together with the dopaminergic pathways.[7,8]

Currently, pharmacologic treatments for PD mainly focus on DA-based strategies, including the DA precursor levodopa (L-DOPA), the adjunctive drugs monoamine oxidase B inhibitors, catechol-*O*-methyl transferase inhibitors, and DA agonists (DAs).[2,9] Despite clear symptomatic benefits, long-term use of L-DOPA often caused motor fluctuations (on–off phenomena of L-DOPA efficacy) and dyskinesias.[10] Although the DAs are less effective than L-DOPA, the motor symptoms of early PD are sufficiently controlled by the DA agonist monotherapy which can also delay the progression of the disease.[9] In the advanced stages, those agents are combined with levodopa to reduce "off" time.[10] DAs can also relieve several bothersome nonmotor symptoms. For example, D_2/D_3 receptor agonists pramipexole can effectively treat PD depressive symptoms and ropinirole is beneficial for sleep, anxiety, and depression.[11] Unfortunately, this strategy is not devoid of limitations. Over time, patients develop dyskinesias and psychotic-like symptoms, which might be due to the pulsatile stimulation of DA receptors.[12–14] In contrast, the use of partial DA D_2/D_3 receptor agonists may be a better choice. First, D_2/D_3 receptor partial agonists were also able to elevate locomotion significantly, implying its application in PD therapy.[15] Second, such compounds would hypothetically balance the dopaminergic tone by stimulating DA D_2/D_3 receptors and counteracting excessive activation of them,[16] thereby reducing the occurrence of side effects.

The 5-HT_{1A} receptor also plays an important role in PD pharmacotherapy, mainly reflected in three aspects. First, activation of 5-HT_{1A} receptors can improve L-DOPA-induced dyskinesia (e.g., eltoprazine and NLX-112).[17] Second, they are expected to improve cognitive impairments (e.g., aripiprazole) and relieve symptoms of anxiety and depression.[18] Further, 5-HT_{1A} receptor agonists have also shown neuroprotective effects (e.g., BAY-639044). Miyazaki et al demonstrated that activation of 5-HT_{1A} receptor can induce proliferation of astrocytes and increase the level of antioxidant molecules in the striatum, which seems to prevent progressive dopaminergic neurodegeneration.[19]

Stimulation of 5-HT_{1A} receptors alleviates motor and nonmotor disorders mainly via nondopaminergic mechanisms, implying that the multitarget approach combining the therapeutic effects of dopaminergic and serotoninergic receptors may provide a double bonus for the treatment of PD.[18,20] Ligands endowed with such a multitarget feature have shown clinical effectiveness. The D_2/D_3/5-HT_{1A} receptor agonist Pardoprunox (SLV-308) displays an anti-PD effect, along with antidepressant and anxiolytic efficacy.[21] In addition, it has a lower propensity to elicit side effects such as dyskinesia compared with other dopaminergic agents and it is now in phase III clinical trials for the treatment of PD.[22,23] Therefore, D_2/D_3/5-HT_{1A} receptor agonists may be of great significance to develop novel potential anti-Parkinson's drugs at present.

This work aims at identifying compounds with D_2/D_3R partial agonism and 5-HT_{1A}R agonism to develop novel anti-Parkinson's active molecules with a lower propensity for side effects. Arylpiperazine is a privileged motif for aminergic receptor ligands.[24] Compounds targeting both DA and serotonin receptors are characterized by an arylpiperazine, comprising a flexible aliphatic spacer and an additional lipophilic moiety serving as secondary pharmacophore.[24] Many studies selected this flexible system as the basic scaffold to achieving a fine balancing of D_2R/D_3R and 5-HT_{1A}R activities. Earlier studies showed that two fragments during I-1 and II, benzamide and phenylacetamide, were developed as new pharmacophores by opening the amide ring of aripiprazole or brexpiprazole (D_2/D_3 and 5-HT_{1A} agonist, ►**Fig. 1**).[25–30] Xu et al identified pyridinecarboxamide derivatives III based on bioisosterism of compound I, which showed improved antagonism for D_2R and agonism for 5-HT_{1A}R (►**Fig. 2**).[26]

In this article, brexpiprazole and compound II were used as the lead compounds to synthesize **7a** for the first time. As shown in ►**Fig. 3**, compound **7a** exhibited higher potency for DR/5-HT_{1A}R, and its EC_{50} values are comparable to that of compound II in terms of activities to the target receptors. Surprisingly, in comparison to brexpiprazole, a partial D_{2L}/D_3/5-HT_{1A} agonist, **7a** not only retained partial agonism on D_2R but also exhibited full agonism on 5-HT_{1A}R, which may result in stronger efficacy against PD and smaller side effects as previously mentioned.

Starting from **7a**, further structural optimizations were conducted to look for more favorable multitarget agonists. Herein, a series of pyridine derivatives were synthesized and their activities on D_2R, D_3R and 5-HT_{1A}R were evaluated. The effects of the substituents of pyridine, spacer, and arylpiperazine moieties on compound activity (structure–activity relationship [SAR]) were also explored (►**Fig. 3**). At last, compounds with better activities were selected to test for microsomal stabilities *in vitro*.

Results and Discussion

Chemistry

The synthesis of 20 target compounds is outlined in **Schemes 1** to **4**. Their structures have been confirmed by mass spectrometry (MS) and nuclear magnetic resonance (NMR), and their purities have been tested by high-performance liquid chromatography (HPLC).

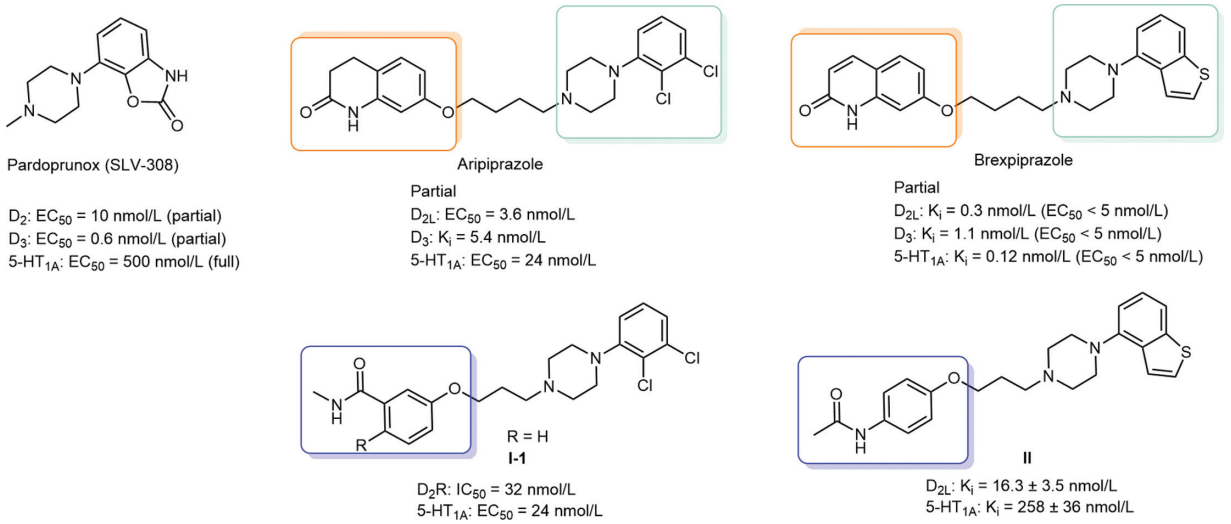


Fig. 1 Structures of $D_2/D_3/5\text{-}HT_{1A}$ agents.

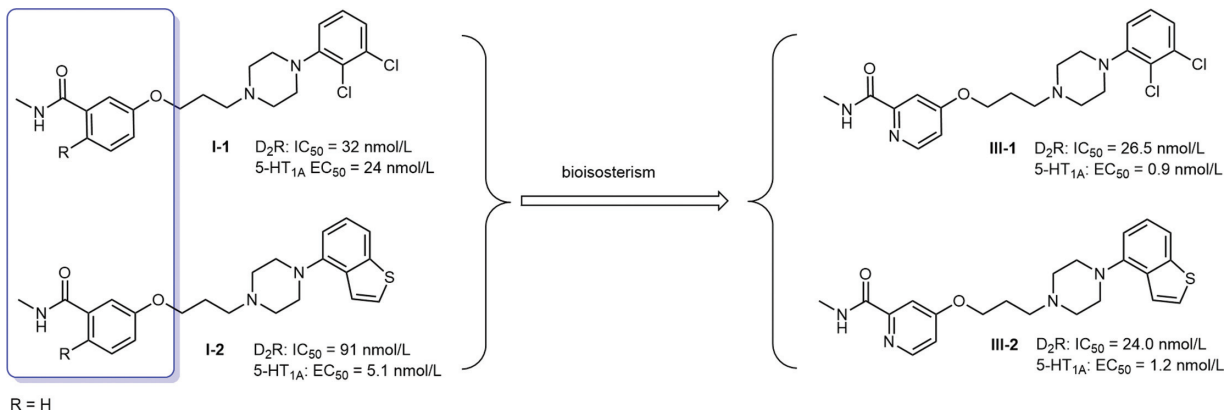


Fig. 2 Design of pyridinecarboxamide derivatives from benzamide derivatives.

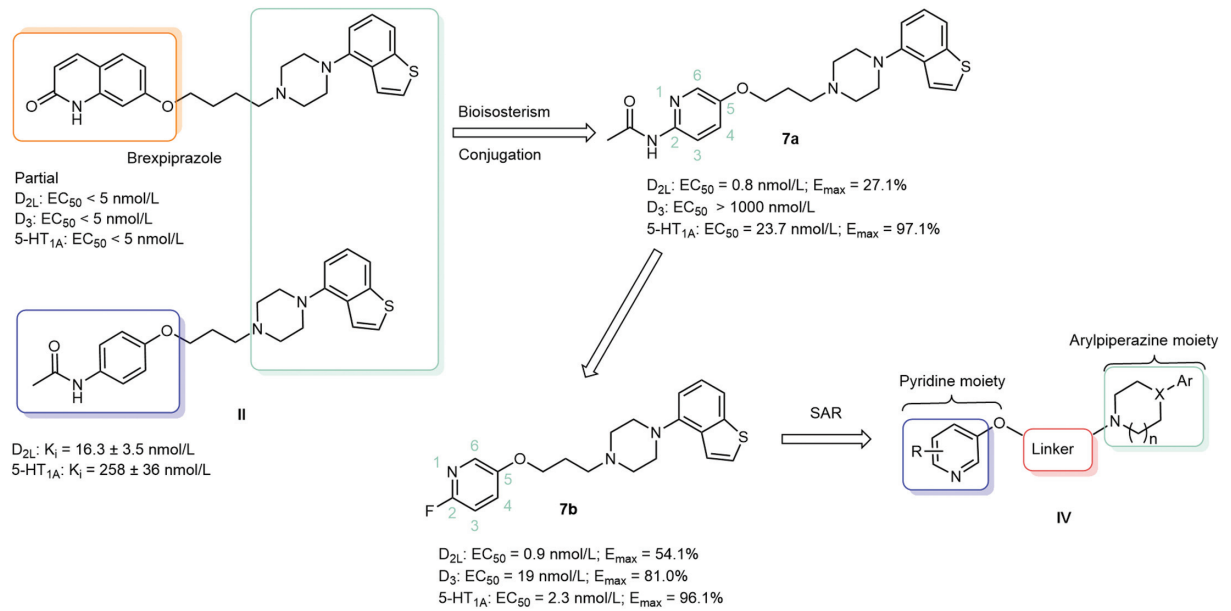
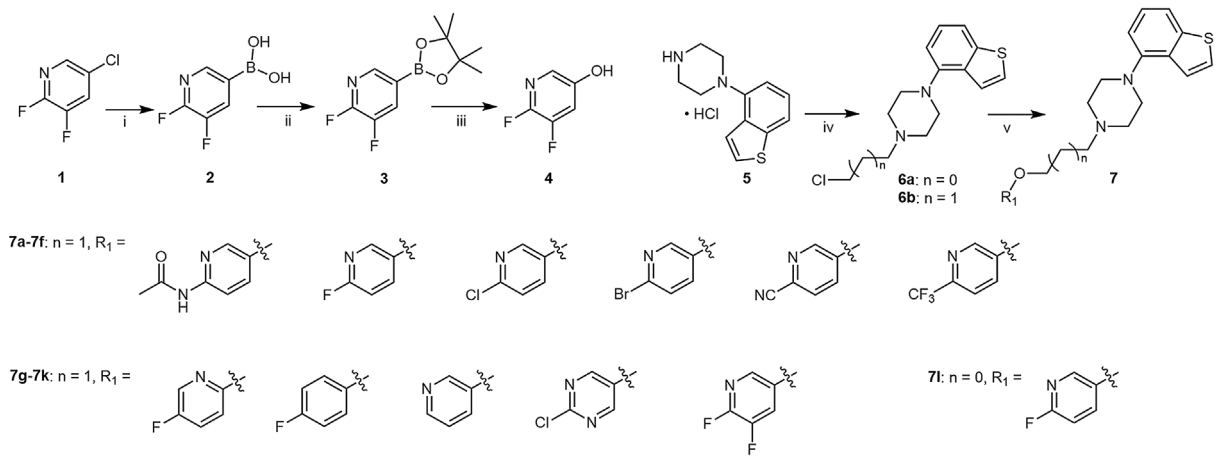
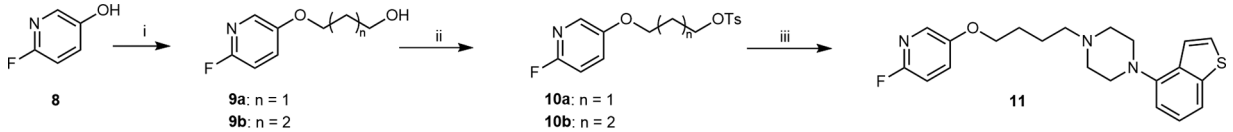


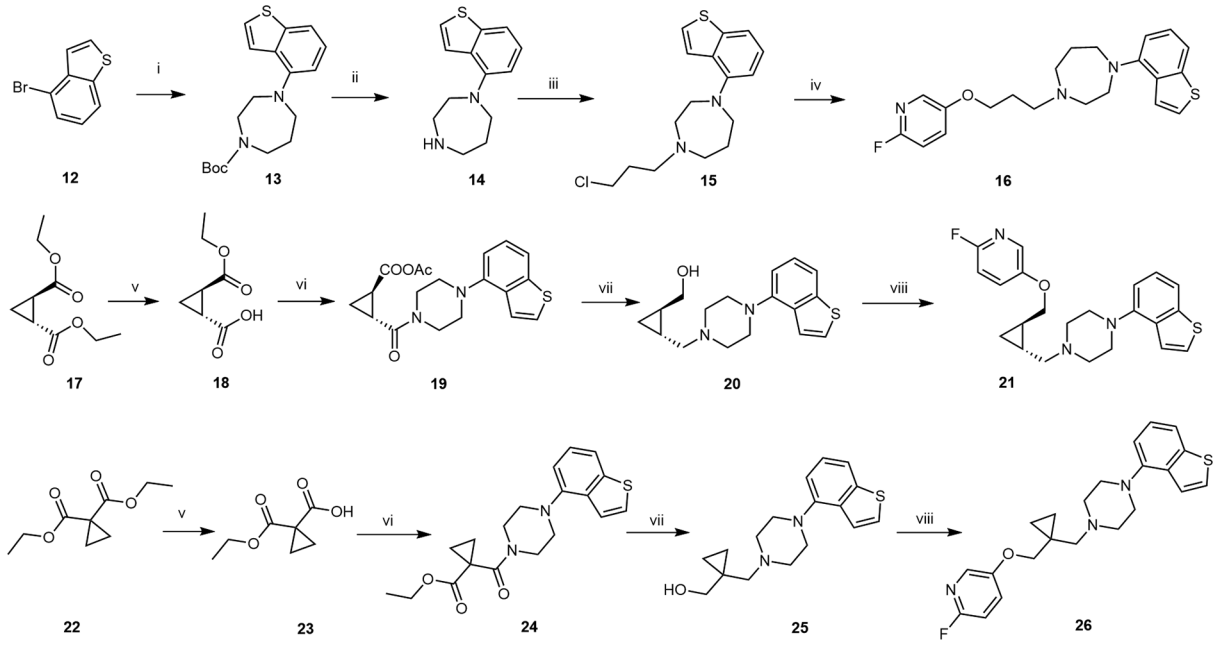
Fig. 3 Design of 1-(3-((6-fluoropyridin-3-yl)oxy)propyl)piperazine derivatives.



Scheme 1 The synthetic route for compounds **7a–7l**. Reagents and conditions: (i) $Pd_2(dpa)_3$, PCy_3, $(BPIN)_2$, CH_3COONa, 1,4-dioxane, 85°C; (ii) pinacol, $MgSO_4$, toluene, r.t.; (iii) H_2O_2, r.t.; (iv) 1-bromo-2-chloroethane or 1-bromo-3-chloropropane, K_2CO_3 or 25% NaOH, r.t. or 60°C; (v) pyridine derivatives, K_2CO_3, CH_3CN, reflux.

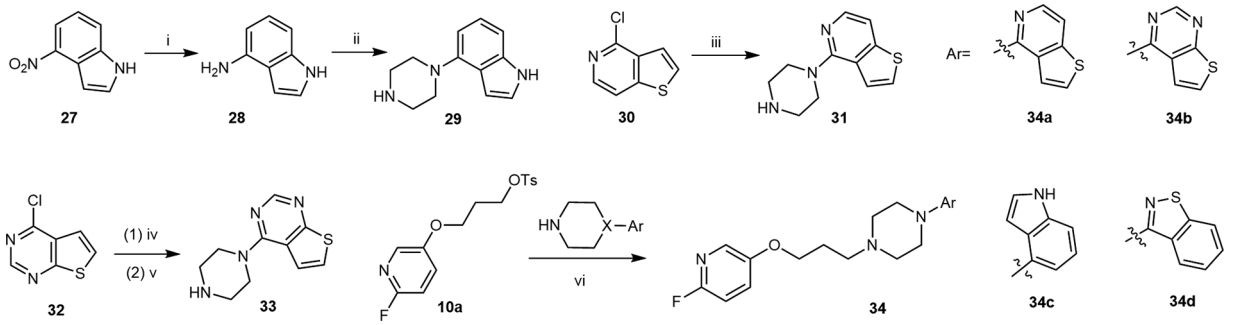


Scheme 2 The synthetic route for compound **11**. Reagents and conditions: (i) 4-bromo-1-butanol or 3-bromo-1-propanol, KI, K_2CO_3, CH_3CN, reflux; (ii) TsCl, DMAP, Et_3N, DCM, 0°C→r.t.; (iii) **5**, K_2CO_3, CH_3CN, reflux.



Scheme 3 The synthetic route for compounds **16**, **21**, and **26**. Reagents and conditions: (i) 1-boc-1,4-diazepane, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, 18 hours, 80°C; (ii) 4 N HCl (MeOH); (iii) 20% NaON, 1-bromo-3-chloropropane; (iv) K_2CO_3, CH_3CN, reflux; (v) 14 mol/L NaOH (aq), EtOH, reflux, 5 minutes; (vi) EDCI, HObt, CH_2Cl_2, Et_3N, r.t.; (vii) $LiAlH_4$,THF, r.t.; (viii) PPh_3, DIAD, THF, 0°C→r.t.

The preparations of 1-(benzo[*b*]thiophen-4-yl)piperazine derivatives (**7a–7l**) are shown in **Scheme 1**. First, 5-chloro-2,3-difluoropyridine (**1**) sequentially underwent coupling reaction, boronic esterification, and oxidization reaction to get intermediate **4**. At the same time, compound **5** was treated with 1-bromo-2-chloroethane or 1-bromo-3-chloropropane to give intermediates **6a** and **6b**, which next reacted with **4** or other corresponding pyridinol in the presence of K_2CO_3 and KI in CH_3CN to obtain the target compounds **7a–7l**, respectively.



Scheme 4 Reagents and conditions: (i) Pd/C (10 wt %), HCO_2NH_4, EtOH; (ii) 2,2′-dichloro-diethylamine hydrochloride, K_2CO_3, isopropanol, 120° C; (iii) piperazine, ethylene glycol, 140°C, 12 hours; (iv) 1-boc-piperazine, DIPEA, THF, r.t. (v) 5N HCl (aq); (vi) K_2CO_3, CH_3CN, reflux.

As shown in **Scheme 2**, compound **8** reacted with 4-bromo-1-butanol or 3-bromo-1-propanol to afford compounds **9a–9b**. Activation of alcohol **9a–9b** with 4-toluenesulfonyl chloride (TsCl) in the presence of triethylamine provided **10a–10b** at room temperature (r.t.). The nucleophilic substitution of **5** with intermediate **10b** gave the four-carbon linker compound **11**.

The preparation of derivatives with different spacers (**16**, **21**, **26**) is shown in **Scheme 3**. Buchwald–Hartwig amination of 4-bromobenzo[*b*]thiophene **12** with homopiperazine in the presence of $Pd(OAc)_2$, BINAP, and Cs_2CO_3 afforded the protected product **13**. Compound **13** was deprotected *in situ* to provide intermediate **14**, which underwent chloroalkylation and condensation reaction to obtain **16**. Basic monohydrolysis of one of the ethyl esters of **17** gave the 1,2-cyclopropanedicarboxylate monomethyl ester **18**, which was subsequently transformed into amides **19**. The simultaneous reduction of the amide and ester moieties with lithium aluminum hydride produced intermediate **20**. Finally, a Mitsunobu reaction with hydroxypyridine gave the target compound **21**. And compound **26** was achieved following a similar fashion.

Scheme 4 describes the synthesis of target compounds with variations to arylpiperazine moieties (**34a–34d**). The arylpiperazine fragments were either commercially supplied (**34d**) or synthetically prepared. In the first step, the hydrogenation reaction of the nitro group of **27** was performed in EtOH at r.t. using Pd/C as the catalyst and ammonium formate as the hydrogen source, leading to the generation of **28**. Then arylamines **28** reacted with bis(2-chloroethyl)ethylamine to obtain **29** and aromatic halohydrocarbon **30** reacted with anhydrous piperazine in ethylene glycol to furnish **31**. The intermediate **33** was obtained by substitution and the subsequent deprotection of Boc. Finally, those heterocyclic arylpiperazine intermediates were treated with **10a** via SN_2 mechanism to yield target compounds **34a–34d**.

Biological Activity

The functional activities of the obtained pyridine derivatives on $D_{2L}/D_3/5\text{-}HT_{1A}$ receptors were further evaluated using cAMP Gi assay. DA and serotonin served as control agents. Cells used in this assay included (1) D_{2L}Rs, human recombinant (HEK293 cells, genscript); (2) D_3Rs, human recombinant (CHO cells, Jiangsu Enhua Pharmaceutical Co., Ltd.); (3) $5\text{-}HT_{1A}$Rs, human recombinant (HEK-293 cells, Jiangsu Enhua Pharmaceutical Co., Ltd.). The concentration of the target compounds was 10 μmol/L and the test results are shown in ►**Table 1**.[31]

The receptor functional activity test *in vitro* showed that 10 compounds have $D_2/5\text{-}HT_{1A}$ dual agonism activities, and two compounds having $D_2/D_3/5\text{-}HT_{1A}$ triple agonism activities. Compounds **7b** and **34c** were subjected to rat/human liver microsomes (RLMs/HLMs) to assess their metabolic stability; the results are shown in ►**Table 2**. The data indicated that compound **34c** (the half-life $T_{1/2}$ values were 110.8, 85.5, and 159.7 minutes) displayed better metabolic stability than **7b** ($T_{1/2}$ values were 23.9, 11.1, and 17.6 minutes).

Preliminary Structure–Activity Relationships

In this study, **7b–7k** were synthesized to investigate the influence of pyridine N atom and R-groups on the agonism presumably by the conformation constraint that arises from potential hydrogen bond. Varying linkers were conferred on compounds **7l**, **11**, **16**, **21**, and **22** to see if the rigidity influenced the activity. In addition, compounds **34a**–**34d** containing diverse base moieties while maintaining pyridine-2-fluorine fragment as the pharmacophore in the pyridine moiety were meant to examine the effect of aryl piperazines.

SAR of the Pyridine Moiety

Results from the SAR analysis of the pyridine moiety showed that:

- Replacement of the amide group (**7a**) with fluorine atom, chlorine, and cyano group yielded compounds **7b**, **7c**, and **7e**, which had higher potency for the D_2R, and were approximately threefold to 10-fold more potent than **7b** for $5\text{-}HT_{1A}R$ agonism activity. Conversely, introducing bromine or trifluoromethyl to the position, the activities of compounds **7d** and **7f** for D_2R or $5\text{-}HT_{1A}R$ were dramatically decreased.
- Changing the relative position between the pyridine N atom and fluorine group (**7g**) led to the absence of agonism for D_{2L}. Compounds **7h**, **7i**, **7j**, and **7k** without

Table 1 Functional activity assays of target compounds for D_{2L}/D_3/5-HT1A receptors

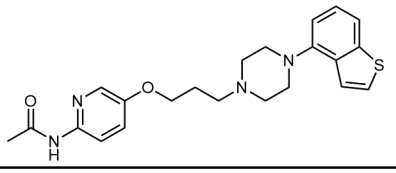
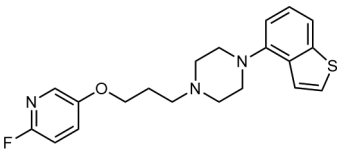
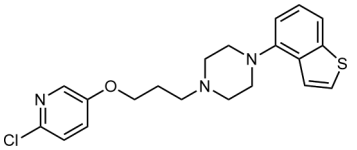
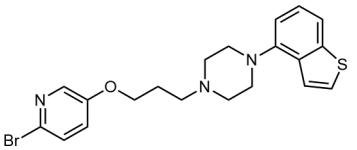
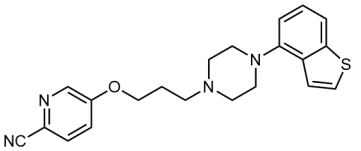
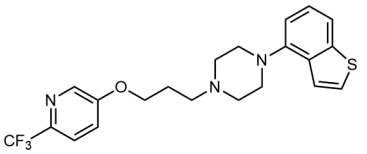
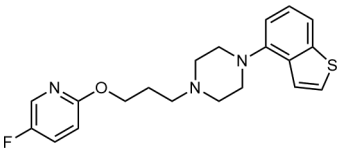
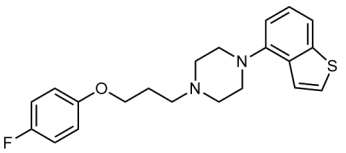
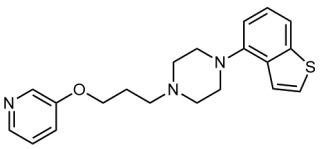
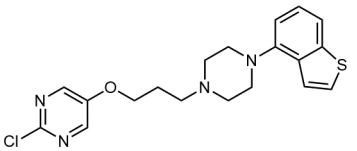
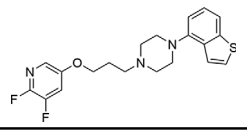
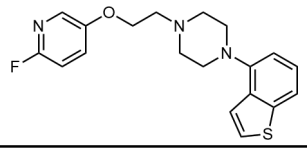
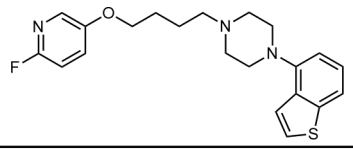
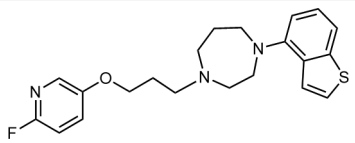
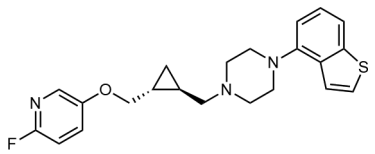
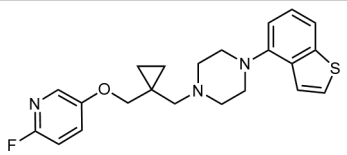
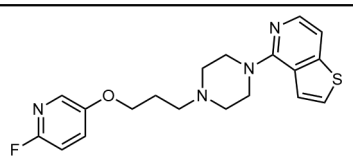
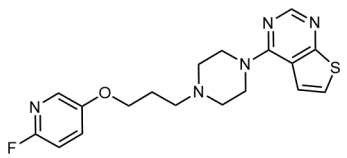
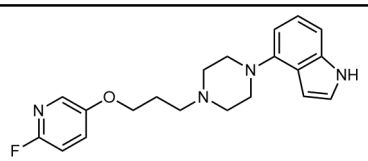
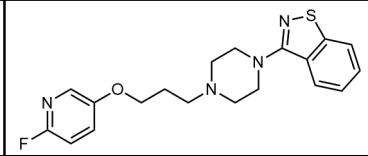
Compd.	Structure	5-HT_{1A} (agonist mode)		D_{2L} (agonist mode)		D_3 (agonist mode)	
		EC_{50} (nmol/L)[a]	E_{max} (%)[a]	EC_{50} (nmol/L)[a]	E_{max} (%)[a]	EC_{50} (nmol/L)[a]	E_{max} (%)[a]
7a		23.7	97.1	0.8	27.1	>1,000	–
7b		2.3	96.1	0.9	54.3	19	81.0
7c		0.8	102.6	22.4	44.8	>1,000	–
7d		0.6	102.4	>1,000	–	NT	NT
7e		1.1	96.5	15.8	40.1	>1,000	–
7f		498.5	56.7	32.7	76.5	NT	NT
7 g		0.2	118.9	>1,000	–	NT	NT
7h[b]		19.1	97.8	>1,000	–	NT	NT
7i[b]		>1,000	60.4	281	31.4	NT	NT
7j		0.2	123.6	>1,000	–	NT	NT

Table 1 (*Continued*)

Compd.	Structure	5-HT$_{1A}$ (agonist mode)		D_{2L} (agonist mode)		D_3 (agonist mode)	
		EC_{50} (nmol/L)[a]	E_{max} (%)[a]	EC_{50} (nmol/L)[a]	E_{max} (%)[a]	EC_{50} (nmol/L)[a]	E_{max} (%)[a]
7k		16.4	97.9	>1,000	–	NT	NT
7l		373.9	73.5	>1,000	–	NT	NT
11		4.1	97.4	1.00	29.4	NT	NT
16		18.2	102.8	7.2	37.4	>1,000	–
21		170.5	64.4	>1,000	–	NT	NT
26		216.8	94.4	>1,000	–	NT	NT
34a		28.8	95.4	>1,000	–	NT	NT
34b		>1,000	23.7	>1,000	8.2	NT	NT
34c		1.4	81.7	3.3	46.8	10.0	54.2

(*Continued*)

Table 1 (*Continued*)

Compd.	Structure	5-HT_{1A} (agonist mode)		D_{2L} (agonist mode)		D_3 (agonist mode)	
		EC_{50} (nmol/L)[a]	E_{max} (%)[a]	EC_{50} (nmol/L)[a]	E_{max} (%)[a]	EC_{50} (nmol/L)[a]	E_{max} (%)[a]
34d		3.7	80.3	>1,000	–	NT	NT
Dopamine	/			0.3	104.3	2.3	105.8
Serotonin	/	0.2	105.2	–	–	–	–

[a]EC_{50} and E_{max} values are the average of two independent experiments done in duplicate with 10 μmol/L concentration.
[b]Compound **7h** (CAS No. 928232–08–0) and compound **7i** (CAS No. 928232–24–0) were reported in the literature,[31] and served as control for the research of SAR.

Table 2 Animal and human liver microsomal metabolic stability assay

Compd.	Species	$T_{1/2}$ (min)
7b	Human	17.6
	SD Rat	11.1
	CD-1 Mouse	23.9
34c	Human	159.7
	SD Rat	85.5
	CD-1 Mouse	110.8
Testosterone	Human	16.7
	SD Rat	0.9
	CD-1 Mouse	6.7

one of pre-existing substitutions or with an additional substitution on the 3-position also showed no agonistic activities on D_{2L}. These data suggested that the pyridine-2-fluorine fragment might be important to the agonism on D_2R.

SAR of the Linker

Results from the SAR analysis of the linker showed that:

- Linker shortening (**7l**) led to a loss of efficacy for D_2 and 5-HT_{1A} receptors. Linker lengthening (**11**), replacing the piperazine group with the homopiperazine group (**16**), or modifying the classic aliphatic spacer by introducing a cyclopropyl ring (**21**, **22**) cannot maintain activities for DA and 5-HT_{1A} receptors at the same time.
- A flexible linker of three carbons may be necessary to maintain the agonistic activity of the three receptors.

SAR of the Arylpiperazine Moiety

Results from the SAR analysis of the arylpiperazine moiety showed that:

- Replacement of the 1-(benzo[*b*]thiophen-4-yl)piperazine (**7b**) with a 4-(piperazin-1-yl)thieno[3,2-*c*]pyridine or 3-(piperazin-1-yl)benzo[*d*]isothiazole: compounds **34a** and **34d** were deprived of the efficacy for D_{2L} receptor. With two carbon atoms in 1-(benzo[*b*]thiophen-4-yl)piperazine (**7b**) replaced by nitrogen atoms, the 4-(piperazin-1-yl)thieno[2,3-*d*]pyrimidine derivative (**34b**) was deprived of the efficacy both for $D_{2L}R$ and 5-$HT_{1A}R$.
- Replacement of the 1-(benzo[*b*]thiophen-4-yl)piperazine (**7b**) with 4-(piperazin-1-yl)-1*H*-indole: the derivative **34c** exhibited high efficacies for the three receptors (D_2, $EC_{50} = 3.3$ nmol/L; D_3, $EC_{50} = 10.0$ nmol/L; 5-HT_{1A}, $EC_{50} = 1.4$ nmol/L, respectively).

Conclusion

In summary, 20 new compounds of pyridyl alkylarylpiperazines were synthesized based on bioisosterism which were also biophysically evaluated for D_2/D_3 and 5-HT_{1A} receptors. Most of these derivatives were D_2/5-HT_{1A} receptor agonists, and compounds **7b** and **34c** behaved as partial D_2/D_3R agonists and potent full 5-$HT_{1A}R$ agonists. Reactive molecules with these pharmacological profiles could effectively address motor and nonmotor disorders with a lower propensity for side effects. Compound **34c** also exhibited good metabolic stability *in vitro*, so it was confirmed as the optimal compound. Besides, preliminary SAR between the designed compounds and three targets was further discussed, which could provide insights into the development of novel multi-target anti-PD molecules.

Experimental Section

Unless specified otherwise, all starting materials, reagents, and solvents were commercially available. All reactions were monitored by thin-layer chromatography (TLC) on silica gel plates (GF-254) and visualized with ultraviolet (UV) light (Shanghai Heqi Glass Instrument Co., Ltd.). Column chromatographic purification was performed using silica gel (Greagent). NMR spectra were recorded in DMSO-d_6 or D_2O on a 400 MHz or 600 MHz spectrometer (Unity Inova) with tetramethylsilane as an internal reference. All chemical

shifts are reported in parts per million (ppm). ESI-MS data were recorded on an Agilent 1946B spectrometer (Agilent). Melting points were obtained on the WRS-2A melting point apparatus (Shanghai INESA Physical Optical Instrument Co., Ltd.) and were uncorrected. The purity of compounds was evaluated by HPLC (Waters PAD 2998) with a Waters XBridge column, C18 (5 mm, 250 mm × 4.6 mm). Other HPLC condition includes mobile phase A (water with 0.05% TFA) and B (CH_3CN); detection at 220 nm; flow rate: 1.0 mL/min; temperature: 25°C.

Synthesis of Intermediates

Procedures for the Preparation of Compound 4

The $Pd_2(dba)_3$ (1.30 g, 1.40 mmol), tricyclohexyl phosphine (897 mg, 3.20 mmol), bis (pinacolato)diboron (11.60 g, 45.77 mmol), sodium acetate (7.80 g, 57.20 mmol), and 5-chloro-2,3-difluoropyridine (**1**) (5.00 g, 38.14 mmol) were dissolved in 1,4-dioxane solution (100 mL). And then the mixture was bubbled with nitrogen and stirred at 85°C for 16 hours under nitrogen. The reaction mixture was diluted with water, filtered, and extracted with ethyl acetate. The combined organic portions were washed with saturated saline water, then dried over anhydrous sodium sulfate and concentrated. The residue was purified by silica gel column chromatography to give 5,6-difluoropyridin-3-ylboronic acid (compound **2**, 5.60 g, 92% yield) as oil.

To a stirred suspension of **2** (3.00 g, 18.90 mmol) in toluene (20 mL) were added pinacol (2.20 g, 18.90 mmol) and anhydrous magnesium sulfate (15.00 g). The mixture was stirred at r.t. overnight and filtered. The filtrate was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography to give **3** (2.43 g, 54% yield) as oil.

The hydrogen peroxide water (1.2 mL, 12.10 mmol) and 2,3-difluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (2.43 g, 10.10 mmol) were dissolved in tetrahydrofuran (15 mL). The mixture was stirred at r.t. for 2 hours, then diluted with water and extracted with ethyl acetate. The combined organic portions were washed with aqueous 5% sodium thiosulfate solution and saturated saline water, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by silica gel column chromatography to give **4** (1.00 g, 75.6% yield) as oil.

Procedures for the Preparation of Compound 29

The nitro compounds **27** (2.00 g, 12.30 mmol) and Pd/C (0.30 g, 10 wt % palladium on activated carbon paste and 55% moisture) were dissolved in EtOH (30 mL) and stirred at r.t. The mixture was bubbled with nitrogen. This is followed by the addition of HCO_2NH_4 (3.2 g, 50.7 mmol) and the mixture was stirred at r.t. overnight. The reaction was finished and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by silica gel column chromatography to provide **28** (1.60 g, 99% yield) as oil.

The arylamine **28** (1.00 g, 7.60 mmol), bis(2-chloroethyl) amine hydrochloride (9.10 mmol), K_2CO_3 (9.10 mmol), and KI (9.10 mmol) were dissolved in isopropanol (30 mL), and the mixture was stirred at 120°C for 48 hours. After cooling to r.t., the solvent was evaporated, and dichloromethane (DCM) was added. The mixture was washed with water and saline water, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography to give **29** (0.80 g, 53% yield) as oil.

Procedure for the Preparation of Compound 31

To a solution of anhydrous piperazine (5.10 g, 59.00 mmol) in ethylene glycol (100 mL), 7-chlorofuro[2,3-*c*]pyridine (**30**, 1.00 g, 5.90 mmol) was added, and the mixture was stirred at 140°C for 9 hours. After cooling down, the mixture was washed with saturated aqueous sodium hydrogen carbonate solution and extracted with chloroform. The organic layer was dried over anhydrous magnesium sulfate, and the solvent was distilled off under reduced pressure. The residue was purified by silica gel column chromatography to provide **31** (0.80 g, 75% yield) as oil.

Procedure for the Preparation of Compound 33

To a mixture of 4-chlorothieno[2,3-*d*]pyrimidine (**32**, 1.00 g, 58.60 mmol) and Boc-piperazine (1.10 g, 58.60 mmol) in THF (30 mL) was carefully added DIPEA (2.30 g, 1.75 mmol). The mixture was stirred at r.t. for 6 hours (TLC showed no starting **32**), added H_2O (15 mL) and extracted with EtOAc (30 mL × 2). The organic phase was washed with brine (50 mL), dried with $MgSO_4$ and concentrated. The residue was purified by column chromatography (petroleum ether: ethyl acetate = 5: 1) to provide an oil, which was dissolved in HCl (aq) in methanol (5N, 20 mL) and stirred at r.t. overnight. After adding H_2O (10 mL), the mixture was manually extracted with CH_2Cl_2 (30 mL × 2). The aqueous layer was basified with 10% $NaHCO_3$ until pH value was 11, then extracted with CH_2Cl_2. The organic phase was dried with Na_2SO_4 and concentrated to give **33** (1.2 g, 93% yield) as white solid.

Procedures for the Preparation of Compounds 10

The 6-fluoropyridin-3-ol (4.00 g, 35.39 mmol), 3-bromo-1-propanol (6.00 g, 42.47 mmol), and potassium carbonate (14.60 g, 106.17 mmol) were dissolved in anhydrous acetonitrile (200 mL). The mixture was stirred at reflux temperature for 20 hours, cooled to r.t., filtered potassium carbonate over a funnel, and washed with acetone (50 mL). The crude filtrate was evaporated under vacuum and purified by silica gel column chromatography to afford **9a** (5.00 g, 82.6% yield) as a colorless liquid.

To a stirred solution of *p*-TsCl (6.30 g, 33.30 mmol), triethylamine (7.80 g, 76.80 mmol), and DMAP (313 mg, 2.56 mmol) in dry CH_2Cl_2 (75 mL), the solution of **9a** (3.00 g, 25.6 mmol) in dry CH_2Cl_2 (25 mL) was added slowly at 0°C. The reaction mixture was stirred for 1 hour at r.t., washed with water and saline water, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography to give **10a** (5.92 g, 71% yield) as a white solid. Following the same procedure for compound **10a**, compound **10b** was obtained with **8** and 4-bromo-1-butanol being used as the starting materials.

Synthesis of Target Compounds

General Procedures for the Preparation of Compound 7a–7i

Compound **5** (6.00 g, 27.23 mmol) was dissolved in acetone (60 mL), and potassium carbonate (K_2CO_3, 11.29 g, 81.69 mmol) was added, followed by dropwise addition of 1-bromo-2-chloroethane (7.81 g, 54.46 mmol). The reaction mixture was stirred at 60°C for 12 hours, cooled to r.t., filtered, and concentrated. The residue was purified by silica gel column chromatography to give compound **6a** (1.02 mg, 13.5% yield) as a clear liquid.

Compound **5** (3.50 mg, 13.74 mmol) was dissolved in acetone (35 mL), 1-bromo-3-chloropropane (2.81 g, 17.86 mmol) was added, followed by dropwise addition of 25% NaOH (2.2 g NaOH and 6.6 g H_2O, 54.97 mmol). The reaction mixture was stirred at r.t. for 16 hours, filtered, and concentrated. The residue was purified by silica gel column chromatography to give compound **6b** (3.10 g, 77.5%) as a clear liquid.

Compounds **6b** (484 mg, 1.64 mmol), *N*-(5-hydroxypyridin-2-yl)acetamide (250 mg, 1.64 mmol), potassium carbonate (600 mg, 4.90 mmol), and potassium iodide (272 mg, 1.64 mmol) were added to acetonitrile (20 mL), and then the reaction mixture was refluxed overnight, cooled to r.t., filtered, and concentrated. The residue was purified by silica gel column chromatography to give compound **7a** as a colorless oil. Compound **7a** was dissolved in EA (10 mL), then hydrogen chloride ethyl acetate solution (2 N, 1 mL) was added dropwise. The mixture was stirred at r.t. for 1 hour, then filtered. The residue was washed with EtOAc or EtOH, dried *in vacuo* to give **7a** hydrochloride. Following the same procedure, compounds **7b–7k** and **7l** were obtained.

***N*-(5-(3-(4-(Benzo[*b*]thiophen-4-yl)piperazin-1-yl)propoxy)pyridin-2-yl)acetamide hydrochloride (7a):** HPLC: 98.61%; Mp: 250.8–253.2°C. ESI-MS (*m*/*z*): calcd. for $C_{22}H_{26}N_4O_2S$ $[M+H]^+$ 411.1776; found 411.30. ^{1}H NMR (400 MHz, DMSO) δ 10.44 (s, 1H), 10.38 (s, 1H), 8.09 (d, *J* = 3.0 Hz, 1H), 8.06 (d, *J* = 8.9 Hz, 1H), 7.82 (d, *J* = 5.6 Hz, 1H), 7.75 (d, *J* = 8.1 Hz, 1H), 7.54 (d, *J* = 5.5 Hz, 1H), 7.48 (dd, *J* = 9.1, 3.1 Hz, 1H), 7.36 (t, *J* = 7.9 Hz, 1H), 7.02 (d, *J* = 7.6 Hz, 1H), 4.19 (t, *J* = 6.0 Hz, 2H), 3.71 (d, *J* = 11.9 Hz, 2H), 3.61 (d, *J* = 12.8 Hz, 2H), 3.41 (d, *J* = 10.3 Hz, 4H), 3.22 (t, *J* = 12.2 Hz, 2H), 2.27 (m, 2H), 2.10 (s, 3H).

1-(Benzo[*b*]thiophen-4-yl)-4-(3-((6-fluoropyridin-3-yl)oxy)propyl)piperazine hydrochloride (7b): HPLC: 98.97%. Mp: 240.8–242.4°C. ESI-MS (*m*/*z*): calcd. for $C_{20}H_{22}FN_3OS$ $[M+H]^+$ 372.1468; found 372.20. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 1H), 7.94 (dd, *J* = 3.2, 1.8 Hz, 1H), 7.77 (d, *J* = 5.5 Hz, 1H), 7.71 (d, *J* = 8.1 Hz, 1H), 7.64 (ddd, *J* = 8.9, 6.6, 3.2 Hz, 1H), 7.53–7.47 (m, 1H), 7.32 (t, *J* = 7.9 Hz, 1H), 7.16 (dd, *J* = 8.9, 3.4 Hz, 1H), 6.98 (d, *J* = 7.6 Hz, 1H), 4.19 (t, *J* = 6.0 Hz, 2H), 3.67 (d, *J* = 11.9 Hz, 2H), 3.57 (d, *J* = 12.9 Hz, 2H), 3.36 (m, 4H), 3.19 (t, *J* = 12.2 Hz, 2H), 2.24 (m, 2H).

1-(Benzo[*b*]thiophen-4-yl)-4-(3-((6-chloropyridin-3-yl)oxy)propyl)piperazine hydrochloride (7c): HPLC: 99.35%. Mp: 259.8–261.1°C. ESI-MS (*m*/*z*): calcd. for $C_{20}H_{22}ClN_3OS$ $[M+H]^+$ 388.1172; found 388.10. ^{1}H NMR (400 MHz, DMSO) δ 10.30 (s, 1H), 8.20 (d, *J* = 3.0 Hz, 1H), 7.82 (d, *J* = 5.5 Hz, 1H), 7.75 (d, *J* = 8.1 Hz, 1H), 7.59–7.51 (m, 3H), 7.36 (t, *J* = 7.9 Hz, 1H), 7.03 (d, *J* = 7.6 Hz, 1H), 4.24 (t, *J* = 6.0 Hz, 2H), 3.71 (d, *J* = 11.8 Hz, 2H), 3.61 (d, *J* = 12.9 Hz, 2H), 3.41 (d, J = 10.3 Hz, 4H), 3.20 (m, 2H), 2.27 (m, 2H).

1-(Benzo[*b*]thiophen-4-yl)-4-(3-((6-bromopyridin-3-yl)oxy)propyl)piperazine hydrochloride (7d): HPLC: 100%. Mp: 259.0–261.4°C. ESI-MS (*m*/*z*): calcd. for $C_{20}H_{22}BrN_3OS$ $[M+H]^+$ 432.0667; found 434.00. ^{1}H NMR (400 MHz, D_2O) δ 8.07–7.98 (m, 1H), 7.73 (d, *J* = 8.2Hz, 1H), 7.62 (d, *J* = 5.6 Hz, 1H), 7.51 (d, *J* = 8.8 Hz, 1H), 7.44 (d, *J* = 5.6 Hz, 1H), 7.39–7.30 (m, 2H), 7.11–7.00 (m, 1H), 4.18 (t, *J* = 5.7 Hz, 2H), 3.77 (d, *J* = 12.3 Hz, 2H), 3.68 (d, *J* = 13.3 Hz, 2H), 3.45–3.40 (m, 4H), 3.19 (m, 2H), 2.28 (m, 2H).

5-(3-(4-(Benzo[*b*]thiophen-4-yl)piperazin-1-yl)propoxy)picolinonitrile hydrochloride (7e): HPLC: 99.00%. Mp: 257.7–259.8°C. ESI-MS (*m*/*z*): calcd. for $C_{21}H_{22}N_4OS$ $[M+H]^+$ 379.1514; found 379.30. ^{1}H NMR (400 MHz, DMSO) δ 10.16 (s, 1H), 8.51 (d, *J* = 2.9 Hz, 1H), 8.10 (d, *J* = 8.7 Hz, 1H), 7.82 (d, *J* = 5.6 Hz, 1H), 7.75 (d, *J* = 8.2 Hz, 1H), 7.67 (dd, *J* = 8.8, 3.0 Hz, 1H), 7.54 (d, J = 5.6 Hz, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.03 (d, *J* = 7.6 Hz, 1H), 4.34 (t, *J* = 5.9 Hz, 2H), 3.71 (d, *J* = 11.9 Hz, 2H), 3.62 (d, *J* = 12.8 Hz, 2H), 3.42 (m, 4H), 3.19 (t, *J* = 12.2 Hz, 2H), 2.30 (t, *J* = 7.9 Hz, 2H).

1-(Benzo[*b*]thiophen-4-yl)-4-(3-((6-(trifluoromethyl)pyridin-3-yl)oxy)propyl)piperazine hydrochloride (7f): HPLC: 99.40%. Mp: 267.3–268.2°C. ESI-MS (*m*/*z*): calcd. for $C_{21}H_{22}F_3N_3OS$ $[M+H]^+$ 422.1436; found 422.10. ^{1}H NMR (400 MHz, DMSO) δ 10.79 (s, 1H), 8.52 (d, *J* = 2.8 Hz, 1H), 7.93 (d, *J* = 8.7 Hz, 1H), 7.82 (d, *J* = 5.5 Hz, 1H), 7.75 (d, *J* = 8.1 Hz, 1H), 7.68 (dd, *J* = 8.7, 2.9 Hz, 1H), 7.54 (d, *J* = 5.4 Hz, 1H), 7.36 (t, *J* = 7.9 Hz, 1H), 7.02 (d, *J* = 7.6 Hz, 1H), 4.34 (t, *J* = 6.0 Hz, 2H), 3.74–3.67 (m, 2H), 3.60 (d, *J* = 12.6 Hz, 2H), 3.41 (m, 4H), 3.26 (m, 2H), 2.33 (m, 2H).

1-(Benzo[*b*]thiophen-4-yl)-4-(3-((5-fluoropyridin-2-yl)oxy)propyl)piperazine hydrochloride (7 g): HPLC: 100%. Mp: 240.1–242.3°C. ESI-MS (*m*/*z*): calcd. for $C_{20}H_{22}FN_3OS$ $[M+H]^+$ 372.1468; found 372.20. ^{1}H NMR (400 MHz, D_2O) δ 7.94 (d, *J* = 3.1 Hz, 1H), 7.72 (d, *J* = 8.1 Hz, 1H), 7.62 (d, *J* = 5.6 Hz, 1H), 7.55 (ddd, *J* = 9.1, 7.8, 3.1 Hz, 1H), 7.43 (d, *J* = 5.6 Hz, 1H), 7.38–7.30 (m, 1H), 7.04 (d, *J* = 7.8 Hz, 1H), 6.85 (dd, *J* = 9.2, 3.7 Hz, 1H), 4.30 (t, *J* = 5.8 Hz, 2H), 3.87–3.53 (m, 5H), 3.45–3.41 (m, 2H), 3.39–3.12 (m, 3H), 2.25 (m, *J* = 7.7, 5.8 Hz, 2H).

1-(Benzo[*b*]thiophen-4-yl)-4-(3-(4-fluorophenoxy)propyl)piperazine hydrochloride (7h): HPLC: 99.55%. Mp: 248.9–251.2°C. ESI-MS (m/z): calcd. for $C_{21}H_{23}FN_2OS$ $[M+H]^+$ 371.4864; found 371.18. 1H NMR (400 MHz, D2O) δ 7.73 (d, J = 8.2 Hz, 1H), 7.62 (d, J = 5.6 Hz, 1H), 7.43 (d, J = 5.6Hz, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.10–6.99 (m, 3H), 6.99–6.89 (m, 2H), 4.12 (t, J = 5.7 Hz, 2H), 3.76 (d, J = 12.4 Hz, 2H), 3.68 (d, J = 13.4 Hz, 2H), 3.48–3.37 (m, 4H), 3.17 (m, 2H), 2.23 (m, 2H).

1-(Benzo[b]thiophen-4-yl)-4-(3-(pyridin-3-yloxy)propyl)piperazine hydrochloride (7i): HPLC: 95.00%. Mp: 180.3–180.9°C. ESI-MS (*m*/*z*): calcd. for $C_{20}H_{23}N_3OS$ $[M+H]^+$ 354.1562; found 354.00. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.35 (s, 1H), 7.77 (d, *J* = 5.5 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.49 (d, *J* = 5.5 Hz, 1H), 7.32 (t, *J* = 7.9 Hz, 1H), 7.19–7.09 (m,

2H), 6.98 (m, 3H), 4.08 (t, $J = 6.0$ Hz, 2H), 3.64 (d, $J = 10.8$ Hz, 2H), 3.54 (d, $J = 11.1$ Hz, 2H), 3.33 (m, 6H), 2.26 (m, 2H).

5-(3-(4-(Benzo[*b*]thiophen-4-yl)piperazin-1-yl)propoxy)-2-chloropyrimidine hydrochloride (7j): HPLC: 97.79%. Mp: 262.6–263.8°C. ESI-MS (*m*/*z*): calcd. for $C_{19}H_{21}ClN_4OS$ $[M+H]^+$ 389.1125; found 389.10. ^{1}H NMR (400 MHz, DMSO) δ 10.25 (s, 1H), 8.62 (s, 2H), 7.82 (d, $J = 5.4$ Hz, 1H), 7.75 (d, $J = 8.1$ Hz, 1H), 7.54 (d, $J = 5.7$ Hz, 1H), 7.37 (t, $J = 7.9$ Hz, 1H), 7.03 (d, $J = 7.6$ Hz, 1H), 4.34 (t, $J = 5.9$ Hz, 2H), 3.71 (d, $J = 12.1$ Hz, 2H), 3.41 (s, 5H), 3.19 (t, $J = 12.1$ Hz, 2H), 2.29 (m, 3H).

1-(Benzo[*b*]thiophen-4-yl)-4-(3-((5,6-difluoropyridin-3-yl)oxy)propyl)piperazine hydrochloride (7k): HPLC: 99.75%. Mp: 259.4–259.7°C. ESI-MS (*m*/*z*): calcd. for $C_{20}H_{21}F_2N_3OS$ $[M+H]^+$ 390.1373; found 390.30. ^{1}H NMR (600 MHz, DMSO) δ 10.61 (s, 1H), 7.86 (ddd, $J = 10.9$, 7.9, 2.7 Hz, 1H), 7.81 (t, $J = 2.5$ Hz, 1H), 7.78 (d, $J = 5.5$ Hz, 1H), 7.71 (d, $J = 8.0$ Hz, 1H), 7.50 (d, $J = 5.5$ Hz, 1H), 7.33 (t, $J = 7.8$ Hz, 1H), 6.99 (d, $J = 7.6$ Hz, 1H), 4.22 (t, $J = 6.0$ Hz, 2H), 3.66 (d, $J = 11.9$ Hz, 2H), 3.57 (d, $J = 12.9$ Hz, 2H), 3.43–3.33 (m, 4H), 3.21 (m, 2H), 2.25 (m, 2H).

1-(Benzo[*b*]thiophen-4-yl)-4-(2-((6-fluoropyridin-3-yl)oxy)ethyl)piperazine hydrochloride (7l): HPLC: 100%. Mp: 194.5–195.7°C. ESI-MS (*m*/*z*): calcd. for $C_{19}H_{20}FN_3OS$ $[M+H]^+$ 358.1311; found 358.10. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.87 (s, 1H), 8.02 (m, 1H), 7.77 (d, $J = 5.5$ Hz, 1H), 7.75–7.67 (m, 2H), 7.50 (d, $J = 5.5$Hz, 1H), 7.32 (t, $J = 7.8$ Hz, 1H), 7.20 (dd, $J = 8.9$, 3.4 Hz, 1H), 7.01–6.95 (m, 1H), 4.55 (t, $J = 4.8$ Hz, 2H), 3.69 (m, 4H), 3.56 (d, $J = 13.1$ Hz, 2H), 3.52–3.42 (m, 2H), 3.24 (t, $J = 11.9$ Hz, 2H).

Procedures for the Preparation of Compound 16

To an oven-dried flask, 1-boc-homopiperazine (5.00 g, 25.00 mmol), Cs_2CO_3 (12.00 g, 37.50 mmol), $Pd(OAC)_2$ (66 mg, 0.63 mmol), BINAP (1.60 g, 2.50 mmol), toluene (120 mL), and **12** (5.30 g, 25.00 mmol) were added. While stirring the reaction mixture at r.t., the air in the flask was removed and replaced by N_2. This process was repeated three times. The reaction mixture was further stirred at 80°C for 16 hours, filtered, and the filtrate was concentrated to give **13** (crude, 6.00 g).

A solution of the crude **13** (6.00 g, 18.10 mmol) was dissolved in methanol (20 mL), then a 4 mol/L hydrogen chloride methanol solution (20 mL) was added dropwise. The mixture was stirred at r.t. overnight and H_2O (20 mL) was added. The aqueous solution was extracted with CH_2Cl_2 (30 mL × 2). The aqueous layer was basified with 10% $NaHCO_3$ until pH = 11 and continuously extracted with CH_2Cl_2. The organic phase was dried (Na_2SO_4) and the solvent was removed to give **14** (0.8 g, 19% yield) as a white solid. Following the same procedure for **6b**, compound **15** was obtained. Following the same procedure for **7**, compound **16** was obtained. HPLC: 100.00%. Mp: 145.9–147.2°C. ESI-MS (*m*/*z*): calcd. for $C_{21}H_{24}FN_3OS$ $[M+H]^+$ 386.1624; found 386.20. ^{1}H NMR (400 MHz, D_2O) δ 7.65 (dt, $J = 8.2$, 0.8 Hz, 1H), 7.62 (dd, $J = 3.2$, 1.5 Hz, 1H), 7.54 (d, $J = 5.6$ Hz, 1H), 7.42–7.33 (m, 1H), 7.35–7.25 (m, 2H), 7.05 (dd, $J = 7.8$, 0.9 Hz, 1H), 6.85 (ddd, $J = 9.0$, 2.7, 0.5 Hz, 1H), 4.14 (t, $J = 5.6$ Hz, 2H), 3.68 (m, 4H), 3.47 (m, 5H), 2.24 (m, 5H).

General Procedures for the Preparation of Compounds 21 and 26

A solution of **17** (4.00 g, 21.48 mmol) in ethanol (12 mL) was heated at reflux. Aqueous 14 mol/L NaOH (1.5 mL, 21.48 mmol) was added for 2 minutes, and the mixture was continued to reflux for 5 minutes, cooled down, and added water (40 mL). The aqueous solution was extracted with CH_2Cl_2 (30 mL × 2). The aqueous layer was acidified with 3 mol/L HCl (aq) until pH = 0.7, and continuously extracted with CH_2Cl_2. The new organic phase was dried with Na_2SO_4 and concentrated to give **18** (2.50 g, 74% yield) as oil.

To a stirred solution of **18** (1.60 g, 10.11 mmol) in DCM (20 mL) was added amine **5** (2.60 g, 10.23 mmol), EDCI (2.50 g, 12.64 mmol), and HOBt (1.60 g, 11.84 mmol) at r.t. The mixture was stirred for 2 hours, quenched by H_2O (15 mL), and extracted by DCM (15 mL × 3). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography to afford **19** (3.1 g, 88.6% yield) as a colorless oil.

To a stirred solution of **19** (3.10 g, 8.60 mmol) in dry THF (30 mL) was added slowly a suspension of $LiAlH_4$ (1.96 g, 25.80 mmol) in dry THF (50 mL) at 0°C. The reaction was stirred at r.t. for 5 hours and quenched with 10% NaOH solution. The mixture was extracted with DCM and washed with water and saline water. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography to give **20** as a yellow oil (1.10 g, 42.3% yield).

A solution of **20** (1.10 g, 3.6 mmol), 6-fluoropyridin-3-ol (497 mg, 4.4 mmol), and PPh_3 (1.1 g, 4.2 mmol) was stirred in dry THF (30 mL) at 0°C under a N_2 atmosphere. To this mixture was added dropwise DIAD (0.77 g, 4.4 mmol) for 10 minutes, then the reaction was allowed to warm to r.t. and monitored by TLC. After completion of the reaction, the solvent was evaporated under reduced pressure and the resulting oil was purified by silica gel column chromatography to give **21**. The compound **21** was dissolved in ethyl acetate (10 mL), then hydrogen chloride ethyl acetate solution (2 N, 2 mL) was added dropwise. The mixture was stirred at r.t. for 1 hour, then filtered. The residue was washed with EtOAc or EtOH, dried *in vacuo* to give **21** hydrochloride (782 mg). Following the same procedure, compound **26** was obtained.

1-(Benzo[*b*]thiophen-4-yl)-4-(((1*R*,2*R*)-2-(((6-fluoropyridin-3-yl)oxy)methyl)cyclopropyl)methyl)piperazine hydrochloride (21): HPLC: 99.56%. MP: 206.0–207.4°C. ESI-MS (*m*/*z*): calcd. for $C_{22}H_{24}FN_3OS$ $[M+H]^+$ 398.1624; found 398.20. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.24 (s, 1H), 7.91 (dd, $J = 3.2$, 1.8 Hz, 1H), 7.77 (d, $J = 5.5$ Hz, 1H), 7.70 (d, $J = 8.0$ Hz, 1H), 7.61 (ddd, $J = 9.5$, 6.7, 3.2 Hz, 1H), 7.49 (d, $J = 5.5$ Hz, 1H), 7.32 (t, $J = 7.8$ Hz, 1H), 7.11 (dd, $J = 8.9$, 3.4 Hz, 1H), 6.99 (d, $J = 7.6$ Hz, 1H), 4.06 (dd, $J = 10.3$, 6.7 Hz, 1H), 3.98 (dd, $J = 10.3$, 7.3 Hz, 1H), 3.73 (d, $J = 11.2$ Hz, 1H), 3.67–3.47 (m, 3H), 3.48–3.14 (m, 5H), 3.03 (ddd, $J = 13.3$, 8.2, 5.4 Hz, 1H), 1.47 (m, 1H), 1.28 (m, 1H), 0.85–0.73 (m, 2H).

1-(Benzo[*b*]thiophen-4-yl)-4-((1-(((6-fluoropyridin-3-yl)oxy)methyl)cyclopropyl)methyl)piperazine hydrochloride (26): HPLC: 98.86%. Mp: 199.9–202.1°C. ESI-MS (*m*/*z*): calcd. for $C_{22}H_{24}FN_3OS$ $[M+H]^+$ 398.1624; found 398.20. ^{1}H

NMR (400 MHz, DMSO) δ 9.92 (s, 1H), 7.94 (dd, J = 3.2, 1.7 Hz, 1H), 7.82 (d, J = 5.5 Hz, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.64 (ddd, J = 9.5, 6.7, 3.2 Hz, 1H), 7.54 (d, J = 5.6 Hz, 1H), 7.37 (t, J = 7.9 Hz, 1H), 7.18 (dd, J = 8.9, 3.3 Hz, 1H), 7.04 (d, J = 7.6 Hz, 1H), 4.12 (s, 2H), 3.83 (s, 1H), 3.63 (d, J = 12.8 Hz, 3H), 3.46–3.37 (m, 4H), 3.28 (t, J = 12.3 Hz, 2H), 0.97–0.85 (m, 4H).

General Procedures for the Preparation of Compounds 11 and 34

A mixture of **10** (1.17 mmol), phenylpiperazine derivatives (1.17 mmol), and K_2CO_3 (3.51 mmol) in CH_3CN (30 mL) was stirred at 80°C for 12 hours. The solvent was removed under reduced pressure, then CH_2Cl_2 (30 mL) was added. The mixture was washed with water and saline water, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography to give **11**. The compound **11** was dissolved in ethyl acetate (10 mL), then hydrogen chloride ethyl acetate solution (2 N, 1 mL) was added dropwise. The mixture was stirred at r.t. for 1 hour, then filtered. The residue was washed with EtOAc or EtOH, dried *in vacuo* to give **11** hydrochloride. Following the same procedure, **34a–34d** hydrochloride were obtained.

1-(Benzo[*b*]thiophen-4-yl)-4-(4-((6-fluoropyridin-3-yl)oxy)butyl)piperazine hydrochloride (11): HPLC: 99.63%. Mp: 204.4–205.7°C. ESI-MS (m/z): calcd. for $C_{21}H_{24}FN_3OS$ $[M+H]^+$ 386.1624; found 386.28. ^{1}H NMR (400 MHz, DMSO) δ 10.59 (s, 1H), 7.96 (dd, J = 3.3, 1.8 Hz, 1H), 7.81 (d, J = 5.6 Hz, 1H), 7.74 (d, J = 8.1 Hz, 1H), 7.66 (ddd, J = 8.9, 6.6, 3.2 Hz, 1H), 7.53 (d, J = 5.6 Hz, 1H), 7.36 (t, J = 7.8 Hz, 1H), 7.18 (dd, J = 8.9, 3.4 Hz, 1H), 7.01 (d, J = 7.6 Hz, 1H), 4.14 (t, J = 6.0 Hz, 2H), 3.70–3.55 (m, 5H), 3.41–3.18 (m, 4H), 1.90 (m, 5H).

4-(4-(3-((6-Fluoropyridin-3-yl)oxy)propyl)piperazin-1-yl)thieno[3,2-*c*]pyridine hydrochloride (34a): HPLC: 97.85%. Mp: 215.7–218.9°C. ESI-MS (m/z): calcd. for $C_{19}H_{21}FN_4OS$ $[M+H]^+$ 373.1420; found 373.20. ^{1}H NMR (400 MHz, D_2O) δ 7.89 (d, J = 5.9 Hz, 1H), 7.76 (dd, J = 3.2, 1.5 Hz, 1H), 7.65 (d, J = 5.6 Hz, 1H), 7.60 (dd, J = 6.0, 0.9 Hz, 1H), 7.56–7.48 (m, 1H), 7.46 (d, J = 5.6 Hz, 1H), 6.98 (dd, J = 9.0, 2.6 Hz, 1H), 4.15 (t, J = 5.7 Hz, 2H), 3.60 (m, 8H), 3.44–3.36 (m, 2H), 2.25 (m, 2H).

4-(4-(3-((6-Fluoropyridin-3-yl)oxy)propyl)piperazin-1-yl)thieno[2,3-*d*]pyrimidine hydrochloride (34b): HPLC: 91.7%. Mp: 228.4–230.6°C. ESI-MS (m/z): calcd. for $C_{18}H_{20}FN_5OS$ $[M+H]^+$ 374.1373; found 374.00. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.69 (s, 1H), 8.53 (s, 1H), 7.93 (dd, J = 3.2, 1.7 Hz, 1H), 7.77 (d, J = 6.1 Hz, 1H), 7.71 (d, J = 6.1 Hz, 1H), 7.63 (ddd, J = 9.4, 6.6, 3.2 Hz, 1H), 7.16 (dd, J = 8.9, 3.4 Hz, 1H), 4.69 (d, J = 14.2 Hz, 2H), 4.17 (t, J = 5.9 Hz, 2H), 3.69–3.60 (m, 4H), 3.31 (dd, J = 10.1, 5.5 Hz, 2H), 3.26–3.18 (m, 2H), 2.22 (m, 2H).

4-(4-(3-((6-Fluoropyridin-3-yl)oxy)propyl)piperazin-1-yl)-1*H*-indole hydrochloride (34c): HPLC: 97.47%. Mp: 227.4–229.1°C. ESI-MS (m/z): calcd. for $C_{20}H_{23}FN_4O$ $[M+H]^+$ 355.1856; found 355.20. ^{1}H NMR (400 MHz, DMSO) δ 11.19 (s, 1H), 10.51 (s, 1H), 7.98 (dd, J = 3.2, 1.8 Hz, 1H), 7.68 (ddd, J = 9.5, 6.6, 3.2 Hz, 1H), 7.33 (t, J = 2.8 Hz, 1H), 7.20 (dd, J = 8.9, 3.4 Hz, 1H), 7.14 (d, J = 8.1 Hz, 1H), 7.04 (t, J = 7.8 Hz, 1H), 6.56 (d, J = 7.4 Hz, 1H), 6.49 (t, J = 2.7 Hz, 1H), 4.22 (t, J = 6.0 Hz, 2H), 3.76 (d, J = 12.8 Hz, 2H), 3.70 (d, J = 11.9 Hz, 2H), 3.45–3.30 (m, 4H), 3.17 (t, J = 12.3 Hz, 2H), 2.28 (m, 2H).

3-(4-(3-((6-Fluoropyridin-3-yl)oxy)propyl)piperazin-1-yl)benzo[*d*]isothiazole hydrochloride (34d): HPLC: 99.54%. Mp: 239.9–241.1°C. ESI-MS (m/z): calcd. for $C_{19}H_{21}FN_4OS$ $[M+H]^+$ 373.1420; found 373.20. ^{1}H NMR (400 MHz, D_2O) δ 7.98 (dt, J = 8.2, 1.0 Hz, 1H), 7.93 (dt, J = 8.3, 0.9 Hz, 1H), 7.78 (dd, J = 3.2, 1.5 Hz, 1H), 7.61–7.49 (m, 2H), 7.45 (ddd, J = 8.1, 7.0, 1.0 Hz, 1H), 6.99 (dd, J = 9.0, 2.6 Hz, 1H), 4.16 (t, J = 5.7 Hz, 2H), 3.89–3.06 (m, 10H), 2.26 (m, 2H).

Microsomal Metabolic Stability Assay

Microsomal metabolic stability assay was performed to determine the metabolic stability of the optimal compound using human, rat, and mouse liver microsomes *in vitro* according to a reported study.[32] Human liver microsomes were obtained from Corning Inc., Corning, New York, United States with CAS No. 452117; SD rat liver microsomes were obtained from Research Institute for Liver Diseases (Shanghai) Co. Ltd. with CAS No. LM-DS-02M; and CD-1 mouse liver microsomes were obtained from Research Institute for Liver Diseases (Shanghai) Co. Ltd. with CAS No. LM-XS-02M. The final incubation contained 0.5 mg/mL microsomal protein, 1 μmol/L test article/positive control, 1.3 mmol/L NADP, 3.3 mmol/L glucose 6 phosphate, and 0.6 U/mL glucose 6 phosphate dehydrogenase. The mixtures were incubated in a 37°C for 10, 30, and 90 minutes before quenching with acetonitrile containing tolbutamide and propranolol (serve as internal standard). LC-MS/MS was used for analysis. The aqueous mobile phase consisted of 0.1% formic acid; and the organic mobile phase consisted of 0.1% formic acid and 99.9% acetonitrile. The flow rate was set as 0.5 mL/min. The C18 trapping cartridge was a polymer-based column. A multiple reaction monitoring method was used to analyze each molecule. And the data were analyzed by Analyst 7.1 (Sciex, Framingham, Massachusetts, United States). The ratio of the peak area response of each compound to that of an internal standard was used to calculate the half-life ($T_{1/2}$) of the tested compounds, as determined by the slope of the corresponding lines.

Ethics Statement

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest

The authors declare no conflict of interest.

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