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 D2/D3 receptor agonists may be a better choice for the treatment of Parkinson’s disease (PD), and the stimulation of 5-HT1A 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 D2/D3/5-HT1A receptors were further tested. The results showed that several compounds exhibited a multitarget combination of D2/5-HT1A agonism. Compounds 7b and 34c showed agonistic activities on D2/D3/5-HT1A receptor. The EC50 value of 7b for D2/D3/5-HT1A receptor were 0.9/19/2.3 nmol/L, respectively; and the EC50 value of 34c for D2/D3/5-HT1A receptor were 3.3/10/1.4 nmol/L, respectively. In addition, 34c exhibited good metabolic stability (the half-life T1/2 = 159.7 minutes) in vitro, which is of great significance for the further exploration of multitarget anti-PD drugs.

Keywords
► Parkinson’s disease
► D2/D3 receptor partial agonist
► 5-HT1A 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. 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. 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. The nonmotor symptoms are related to specific dysfunction of cholinergic, noradrenergic, and serotonergic pathways in the brain, together with the dopaminergic pathways.

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). Despite clear symptomatic benefits, long-term use of L-DOPA often caused motor fluctuations (on-off phenomena of L-DOPA efficacy) and dyskinesias. 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. In the advanced stages, those agents are combined with levodopa to reduce “off” time. DAs can also relieve several bothersome nonmotor symptoms. For example, D2/D3 receptor agonists pramipexole can effectively treat PD depressive symptoms and ropinirole is beneficial for sleep, anxiety, and depression. 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. In contrast, the use of partial DA D2/D3 receptor agonists may be a better choice. First, D2/D3 receptor partial agonists were also able to elevate locomotion significantly, implying its application in PD therapy. Second, such compounds would hypothetically balance the dopaminergic tone by stimulating DA D2/D3 receptors and counteracting excessive activation of them, thereby reducing the occurrence of side effects.

The 5-HT1A receptor also plays an important role in PD pharmacotherapy, mainly reflected in three aspects. First, activation of 5-HT1A receptors can improve L-DOPA-induced dyskinesia (e.g., eltoprazine and NLX-112). Second, they are expected to improve cognitive impairments (e.g., aripiprazole) and relieve symptoms of anxiety and depression. Further, 5-HT1A receptor agonists have also shown neuroprotective effects (e.g., BAY-639044). Miyazaki et al demonstrated that activation of 5-HT1A receptor can induce proliferation of astrocytes and increase the level of antioxidant molecules in the striatum, which seems to prevent progressive dopaminergic neurodegeneration.

Stimulation of 5-HT1A receptors alleviates motor and nonmotor disorders mainly via nondopaminergic mechanisms, implying that the multitarget approach combining the therapeutic effects of dopaminergic and serotonergic receptors may provide a double bonus for the treatment of PD. Ligands endowed with such a multitarget feature have shown clinical effectiveness. The D2/D3/5-HT1A receptor agonist Pardoprunox (SLV-308) displays an anti-PD effect, along with antidepressant and anxiolytic efficacy. 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.

Therefore, D2/D3/5-HT1A receptor agonists may be of great significance to develop novel potential anti-Parkinson’s drugs at present. This work aims at identifying compounds with D2/D3R partial agonism and 5-HT1A R agonism to develop novel anti-Parkinson’s active molecules with a lower propensity for side effects. Arylpiprazine is a privileged motif for aminergic receptor ligands. Compounds targeting both DA and seroton receptors are characterized by an arylpiprazine, comprising a flexible aliphatic spacer and an additional lipophilic moiety serving as secondary pharmacophore. Many studies selected this flexible system as the basic scaffold to achieving a fine balance of D2R/D3R and 5-HT1A 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 (D2/D3 and 5-HT1A agonist, Fig. 1). Xu et al identified pyridinecarboxamide derivatives III based on bioisosterism of compound I, which showed improved anagonism for D2R and agonism for 5-HT1A R (Fig. 2).

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-HT1A R, and its EC50 values are comparable to that of compound II in terms of activities to the target receptors. Surprisingly, in comparison to brexpiprazole, a partial D2/D3/5-HT1A agonist, 7a not only retained partial agonism on D2R but also exhibited full agonism on 5-HT1A 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 D2R, D3R and 5-HT1A 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).
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**Fig. 1** Structures of D2/D3/5-HT1A agents.

**Fig. 2** Design of pyridinecarboxamide derivatives from benzamide derivatives.

**Fig. 3** Design of 1-(3-((6-fluoropyridin-3-yl)oxy)propyl)piperazine derivatives.
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$_2$CO$_3$ and KI in CH$_3$CN to obtain the target compounds 7a–7l, respectively.
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-toluene-sulfonyl 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 Cs₂CO₃ 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 mono-hydrolysis 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 SN₂ mechanism to yield target compounds 34a–34d.

### Biological Activity

The functional activities of the obtained pyridine derivatives on D₂L/D₅/5-HT₁A receptors were further evaluated using cAMP Gi assay. DA and serotonin served as control agents. Cells used in this assay included (1) D₂L, human recombinant (HEK293 cells, genscript); (2) D₅L, human recombinant (CHO cells, Jiangsu Enhua Pharmaceutical Co., Ltd.); (3) 5-HT₁ARs, human recombinant (HEK-293 cells, Jiangsu Enhua Pharmaceutical Co., Ltd.). The concentration of the target compounds was 10 μM/L. and the test results are shown in Table 1.

The receptor functional activity test in vitro showed that 10 compounds have D₂L/5-HT₁A dual agonism activities, and two compounds having D₂L/5-HT₁A 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 Tₗ/₂ values were 110.8, 85.5, and 159.7 minutes) displayed better metabolic stability than 7b (Tₗ/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 7i, 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 arylpiperazines.

### 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 D₂R, and were approximately threefold to 10-fold more potent than 7b for 5-HT₁AR agonism activity. Conversely, introducing bromine or trifluoromethyl to the position, the activities of compounds 7d and 7f for D₂R or 5-HT₁AR were dramatically decreased.
- Changing the relative position between the pyridine N atom and fluorine group (7g) led to the absence of agonism for D₂L. Compounds 7h, 7i, 7j, and 7k without
Table 1  Functional activity assays of target compounds for D2L/D3/5-HT1A receptors

| Compd. | Structure | 5-HT1A (agonist mode) | D2L (agonist mode) | D3 (agonist mode) |
|--------|-----------|-----------------------|--------------------|-------------------|
|        |           | EC50 (nmol/L) | E\text{\textsubscript{max}} (%) | EC50 (nmol/L) | E\text{\textsubscript{max}} (%) | EC50 (nmol/L) | E\text{\textsubscript{max}} (%) |
| 7a     | ![Structure](image1.png) | 23.7 | 97.1 | 0.8 | 27.1 | >1,000 | – |
| 7b     | ![Structure](image2.png) | 2.3 | 96.1 | 0.9 | 54.3 | 19 | 81.0 |
| 7c     | ![Structure](image3.png) | 0.8 | 102.6 | 22.4 | 44.8 | >1,000 | – |
| 7d     | ![Structure](image4.png) | 0.6 | 102.4 | >1,000 | – | NT | NT |
| 7e     | ![Structure](image5.png) | 1.1 | 96.5 | 15.8 | 40.1 | >1,000 | – |
| 7f     | ![Structure](image6.png) | 498.5 | 56.7 | 32.7 | 76.5 | NT | NT |
| 7g     | ![Structure](image7.png) | 0.2 | 118.9 | >1,000 | – | NT | NT |
| 7h\textsuperscript{b} | ![Structure](image8.png) | 19.1 | 97.8 | >1,000 | – | NT | NT |
| 7i\textsuperscript{b} | ![Structure](image9.png) | >1,000 | 60.4 | 281 | 31.4 | NT | NT |
| 7j     | ![Structure](image10.png) | 0.2 | 123.6 | >1,000 | – | NT | NT |
| Compd. | Structure | 5-HT<sub>1A</sub> (agonist mode) | D<sub>2L</sub> (agonist mode) | D<sub>3</sub> (agonist mode) |
|--------|-----------|-------------------------------|-------------------------------|-------------------------------|
|        |           | EC<sub>50</sub> (nmol/L)<sup>a</sup> | E<sub>max</sub> (%)<sup>a</sup> | EC<sub>50</sub> (nmol/L)<sup>a</sup> | E<sub>max</sub> (%)<sup>a</sup> | EC<sub>50</sub> (nmol/L)<sup>a</sup> | E<sub>max</sub> (%)<sup>a</sup> |
| 7k     | ![Structure](image1) | 16.4 | 97.9 | >1,000 | – | NT | NT |
| 7l     | ![Structure](image2) | 373.9 | 73.5 | >1,000 | – | NT | NT |
| 11     | ![Structure](image3) | 4.1 | 97.4 | 1.00 | 29.4 | NT | NT |
| 16     | ![Structure](image4) | 18.2 | 102.8 | 7.2 | 37.4 | >1,000 | – |
| 21     | ![Structure](image5) | 170.5 | 64.4 | >1,000 | – | NT | NT |
| 26     | ![Structure](image6) | 216.8 | 94.4 | >1,000 | – | NT | NT |
| 34a    | ![Structure](image7) | 28.8 | 95.4 | >1,000 | – | NT | NT |
| 34b    | ![Structure](image8) | >1,000 | 23.7 | >1,000 | 8.2 | NT | NT |
| 34c    | ![Structure](image9) | 1.4 | 81.7 | 3.3 | 46.8 | 10.0 | 54.2 |

(Continued)
one of pre-existing substitutions or with an additional substitution on the 3-position also showed no agonistic activities on D2L. These data suggested that the pyridine-2-fluorine fragment might be important to the agonism on D2R.

### SAR of the Linker

Results from the SAR analysis of the linker showed that:

- Linker shortening (7l) led to a loss of efficacy for D2 and 5-HT1A 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-HT1A 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 D2L 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 D2LR and 5-HT1AR.
- Replacement of the 1-(benzo[b]thiophen-4-yl)piperazine (7b) with 4-(piperazin-1-yl)-1H-indole: the derivative 34c exhibited high efficacies for the three receptors (D2, EC50 = 3.3 nmol/L; D3, EC50 = 10.0 nmol/L; 5-HT1A, EC50 = 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 D2/D3 and 5-HT1A receptors. Most of these derivatives were D2/5-HT1A receptor agonists, and compounds 7b and 34c behaved as partial D2/D3R agonists and potent full 5-HT1AR 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-d6 or D2O 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 conditions include mobile phase A (water with 0.05% TFA) and B (CH3CN); detection at 220 nm; flow rate: 1.0 mL/min; temperature: 25°C.

Synthesis of Intermediates

Procedures for the Preparation of Compound 4
The Pd3(dbta)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 1 hour (TLC showed no starting). THF (30 mL) was carefully added DIPEA (2.30 g, 1.75 mmol). To a mixture of 4-chlorothieno[2,3-d]pyrimidine (32, 1.00 g, 5.86 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). The residue was purified by silica gel column chromatography to provide 31 (0.80 g, 53% yield) as oil.

Procedures for the Preparation of Compound 31
To a solution of anhydrous piperoxide (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.

Procedures for the Preparation of Compounds 10
The 6-fluoropyridine-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 CH2Cl2 (75 mL), the solution of 9a (3.00 g, 25.6 mmol) in dry CH2Cl2 (25 mL) was added slowly at 0°C. The reaction mixture was stirred for 1 hour at r.t., washed with water and salinic acid, and dried over anhydrous Na2SO4, 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 (K₂CO₃, 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 H₂O, 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-hydroxypropyridin-2-yl)acetoacetamide (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-(2-Thiophen-4-yl)piperazin-1-yl)propoxy)pyridin-3-yl)oxy)propyl)piperazine hydrochloride (7a): HPLC: 98.97%. Mp: 259.8–261.1°C. ESI-MS (m/z): calcd. for C₂₀H₂₂ClN₃OS [M + H]⁺ 388.1172; found 388.10. ¹H NMR (400 MHz, DMF-d₅) 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-Benz[b]thiophen-4-yl)-4-((3-(6-bromopyridin-3-yl)oxy)propyl)piperazine hydrochloride (7b): HPLC: 100.0%. Mp: 259.0–261.4°C. ESI-MS (m/z): calcd. for C₂₀H₂₂BrN₃OS [M + H]⁺ 432.0667; found 434.00. ¹H NMR (400 MHz, D₂O) δ 8.07–7.98 (m, 1H), 7.73 (d, J = 8.2 Hz, 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).

1-Benz[b]thiophen-4-yl)-4-((3-(6-bromopyridin-3-yl)oxy)propyl)piperazine hydrochloride (7d): HPLC: 99.00%. Mp: 257.7–259.8°C. ESI-MS (m/z): calcd. for C₂₀H₂₂ClN₃OS [M + H]⁺ 379.1514; found 379.30. ¹H NMR (400 MHz, DMSO-d₆) 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-Benz[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 C₂₀H₂₂F₃N₃OS [M + H]⁺ 422.1436; found 422.10. ¹H NMR (400 MHz, DMSO-d₆) δ 10.79 (s, 1H), 8.62 (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-Benz[b]thiophen-4-yl)-4-((3-(5-fluoropyridin-2-yl)oxy)propyl)piperazine hydrochloride (7g): HPLC: 99.95%. Mp: 240.1–242.3°C. ESI-MS (m/z): calcd. for C₂₀H₂₂FN₂N₃OS [M + H]⁺ 372.1468; found 372.20. ¹H NMR (400 MHz, DMSO-d₆) δ 8.88 (d, J = 3.1 Hz, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.72 (d, J = 8.1 Hz, 1H), 7.62 (d, J = 5.6 Hz, 1H), 7.55 (dd, 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 (d, 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-Benz[b]thiophen-4-yl)-4-((3-(3-fluorophenyl)propoxy)propyl)piperazine hydrochloride (7h): HPLC: 99.50%. Mp: 180.3–180.9°C. ESI-MS (m/z): calcd. for C₂₃H₂₅F₂N₃OS [M + H]⁺ 354.1562; found 354.00. ¹H NMR (400 MHz, DMSO-d₆) 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, 6H), 6.90 (m, 1H), 5.70 (m, 1H), 3.90 (m, 2H), 3.50 (m, 2H), 3.20 (m, 2H), 2.23 (m, 2H).
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-chloroprimidine hydrochloride (7): HPLC: 97.79%. Mp: 262.6−263.8°C. ESI-MS (m/z): calcd. for C_{19}H_{21}ClN_{4}O_{5} [M + H]^+ 389.1125; found 389.10. 1H NMR (400 MHz, DMSO-d_6) 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-(6-difluoropyridin-3-yl)oxy)propyl)piperazine hydrochloride (8k): HPLC: 99.75%. Mp: 259−259.7°C. ESI-MS (m/z): calcd. for C_{20}H_{21}F_{2}N_{3}O_{5} [M + H]^+ 390.1373; found 389.30. 1H NMR (600 MHz, DMSO-d_6) 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 = 11.1 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 (7h): HPLC: 100%. Mp: 194.5−195.7°C. ESI-MS (m/z): calcd. for C_{19}H_{20}F_{2}N_{3}O [M + H]^+ 358.1311; found 358.10. 1H 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_{2}CO_{3} (12.00 g, 37.50 mmol), Pd(OAc)$_2$ (1.60 g, 10.11 mmol) in dry THF (30 mL) at 0°C. To this mixture was added amine (5.00 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 purified by silica gel column chromatography to give 19 (31.1 g, 88.6% yield) as a colorless oil.

To a stirred solution of 18 (1.60 g, 10.11 mmol) in DCM (20 mL) was added amine (5.00 g, 10.23 mmol), HOBr (1.60 g, 11.84 mmol) at r.t. The mixture was stirred for 2 hours, quenched by H$_2$O (15 mL), and extracted by DCM (15 mL x 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 (31.1 g, 88.6% yield) as a colorless oil.

To a stirred solution of 19 (31.0 g, 8.60 mmol) in dry THF (20 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 Ph$_3$P (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 warmed to 50°C 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 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).

**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$_2$Cl$_2$ (20 mL x 2). The aqueous layer was acidified with 3 mol/L HCl (aq) until pH = 0.7, and continuously extracted with CH$_2$Cl$_2$. The new organic phase was dried with Na$_2$SO$_4$ and concentrated to give 18 (2.50 g, 74% yield) as oil.
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 (dd, 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 K2CO3 (3.51 mmol) in CH3CN (30 mL) 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 hydrochlorides were obtained.

1-(Benzo[b]thiophen-4-yl)-4-(4-(6-fluoropyridin-3-yl)oxy)piperazine hydrochloride (11): HPLC: 99.63%. Mp: 204.4–205.7°C. ESI-MS (m/z): calcd. for C19H21FN4OS [M + H]+ 373.1420; found 373.2011H NMR (400 MHz, DMSO) δ 6.1 Hz, 1H), 7.71 (d, J = 6.1 Hz, 1H), 7.63 (dd, J = 9.4, 6.6, 3.2 Hz, 1H), 7.16 (d, 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).

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. 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 µmol/L glucose 6 phosphate dehydrogenase. The mixtures were incubated in a 37°C water bath for 10, 30, and 90 minutes before quenching with acetonitrile containing tobutamide 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 (T1/2) 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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