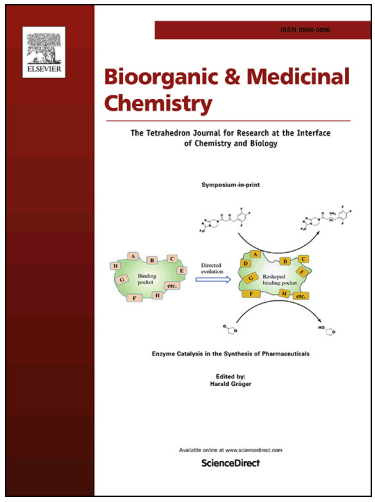
Accepted Manuscript



Design, synthesis and evaluation of vilazodone-tacrine hybrids as multitarget-directed ligands against depression with cognitive impairment

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Graphical Abstract

Design, synthesis and evaluation of vilazodone-tacrine hybrids as multitarget-directed ligands against depression with cognitive impairment

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[b] CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, China

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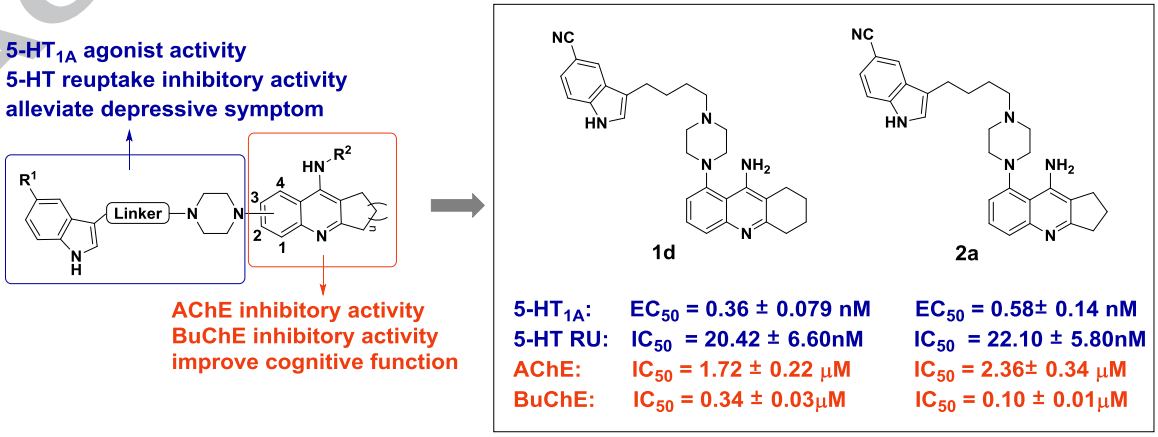

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[d] University of Chinese Academy of Science, No.19A Yuquan Road, Beijing 100049, China
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ABSTRACT

Depression, a severe mental disease, is greatly difficult to treat and easy to induce other neuropsychiatric symptoms, the most frequent one is cognitive impairment. In this study, a series of novel vilazodone-tacrine hybrids were designed, synthesized and evaluated as multitarget agents against depression with cognitive impairment. Most compounds exhibited good multitarget activities and appropriate blood-brain barrier permeability. Specifically, compounds **1d** and **2a** exhibited excellent 5-HT_{1A} agonist activities (**1d**, EC_{50} = 0.36 ± 0.08 nM; **2a**, EC_{50} = 0.58 ± 0.14 nM) and 5-HT reuptake inhibitory activities (**1d**, IC_{50} = 20.42 ± 6.60 nM; **2a**, IC_{50} = 22.10 ± 5.80 nM). In addition, they showed moderate ChE inhibitory activities (**1d**, AChE IC_{50} = 1.72 ± 0.217 μM, BuChE IC_{50} = 0.34 ± 0.03 μM; **2a**, AChE IC_{50} = 2.36 ± 0.34 μM, BuChE IC_{50} = 0.10 ± 0.01 μM). Good multitarget activities with goodt blood-brain barrier permeability of **1d** and **2a** make them good lead compounds for the further study of depression with cognitive impairment.

1. Introduction

Depression, a common, severe mental disorder, is the primary cause of disability, suicide, diminished productivity and dependent care, with approximate 350 million people affected worldwide.[1] It is also a chronic and recurrent affective disease with multifarious etiology and symptoms.[2-4] Depressed people always suffer from weak social skills, chronic stress, low emotion, decreased energy and cognitive impairment.[5] Currently, most antidepressants take effect by noradrenergic-serotonergic transmitter systems or inhibiting monoamine oxidase (MAO) to reduce the degradation of serotonin (5-HT) and norepinephrine (NE).[6] It has been proved that the co-morbidity with other neuro-affected diseases is a significant characteristic of depression.[7] Along with the in-depth research of depression, antidepressant drugs based on different strategies have been conducted.[8-11]

It is recognized that depression, especially late-onset depression, is associated with cognitive impairment, such as poor concentration, information processing and memory impairment, executive dysfunction.[12-15] Antidepressants exhibited deficiency in cognitive impairment despite of the successful depression remission. Also, depressive patients with concurrent cognitive impairment, in particular cognitive impairement, undergo a lower antidepressant therapy response and higher recurrence.[16,17] Besides, depressive patients with cognition decline are a high risk group of Alzheimer's disease (AD) and it also brings negative consequences in patients and heavy burden in caregivers.[18-21] Such impaired cognition and memory processes in depressive disorder may attribute to the dysfunction of the cholinergic system and it could be alleviated by acetylcholinesterase inhibitors (AChEIs).[22-25] AChEIs, reducing the hydrolysis of acetylcholine, are commonly accepted as first-line drugs in treatment of moderate cognitive impairment.[26-28] A study has indicated that donepezil, a cholinesterase inhibitor, improved cognition in depressed people and may delay conversion to a diagnosis of AD.[29] Therefore, therapeutic strategies which target at cholinesterase may improve cognitive

function in depressive people and prevent depression from recurrencing by improving executive function.[28,30]

Above all, aiming at complexed pathological mechanisms of neuropsychiatric and neurodegenerative diseases, design of multi-target ligands is a promising research direction.[31] Several studies have been successful in developing multi-target ligands which acted on central nervous system (CNS), and multi-target ligands could exhibit multiple pharmacological activities in *vivo*.[32,33] It is therefore understandable that the attempts to develop a kind of drug candidate which can alleviate depressive symptoms and cognitive impairment simultaneously based on the multi-target design strategy have been in pressing need. Our group devoted to the research of drug repurposing and the secondary development of conventional drugs. Vilazodone, an antidepressant, was found possessing moderate acetylcholinesterase (AChE) inhibitory activity (IC_{50} = 21.3 ± 3.0 μM) in a high throughput screening (HTS) test with an in-house old drug library. As reported, vilazodone is a selective serotonin reuptake inhibitor (SSRI) and 5-HT_{1A} receptor agonist,[34] which can increase serotonin concentration of synaptic cleft. Serotonin, a significant neurotransmitter existing in the brain extensively, plays a pivotal role in normal brain function.[35,36] 5-HT_{1A} receptor agonists play a significant role in the therapeutics of major depressive disorder (MDD).[37-40] Moreover, SSRI, could increase the amount of available synaptic 5-HT, also were a remarkable characteristic of antidepressants.[41-43]

In order to meet our goal in developing novel drug candidate which can alleviate depressive symptoms and cognitive impairment simultaneously, we attempt to enhance the AChE inhibitory potency of vilazodone while keeping its effect on 5-HT system by two molecular design strategies. Firstly, traditional medicinal chemistry structural modification strategy was employed to modify the chemical structure of vilazodone. We had obtained several vilazodone derivatives in previous work, but their AChE inhibitory activities were not significantly ehnhanced. Alternatively, pharmacophore grafting is also considered as an efficient method to enhance AChE inhibitory potency. Hence, grafting an AChE inhibitory pharmacophore of known AChE inhibitors (AChEIs) into vilazodone was employed in the current study. In view of the simple structure and structure-modifying accessibility, tacrine was selected as the grafting pharmacophore of AChEI.

According to structure-activity relationships (SARs) of vilazodone, the benzofuran-2-carboxamide fragment is an appropriate site for structural modification and can tolerate a diversity of substituents.[44,45] Therefore, benzofuran-2-carboxamide fragment of vilazodone can be substituted by tacrine derivatives. In previous work of our group, Li *et al*[46] have designed and synthesized the first class of vilazodone-tacrine hybrids targeting at ChE, 5-HT_{1A} and 5-HT transport. In his design strategy, piperazine ring of vilazodone was linked to amino of tacrine. Although his design improved ChE inhibitory activity of vilazodone, the antidepressive activity was decreased severely. In order to enhance ChE inhibitory activity while the 5-HT_{1A} agonist and 5-HT reuptake inhibitory activities are remained, a novel design strategy was conducted: piperazine ring of vilazodone was linked to benzene ring of tacrine (Figure 1). We aim at developing a series multifunctional compounds with optimal antidepressive activity and adjuvant cognition promotion.

Based on the strategy mentioned above, a series of novel vilazodone-tacrine hybrids were designed, synthesized and evaluated in this article. Their AChE, BuChE, 5-HT reuptake inhibitory activities and 5-HT_{1A} agonist activity were evaluated in vitro. Besides, the parallel artificial membrane permeability assay (PAMPA) was performed to provide preliminary predictions of the blood-brain barrier (BBB) penetration, expecting to provide evidence for optimized selection.

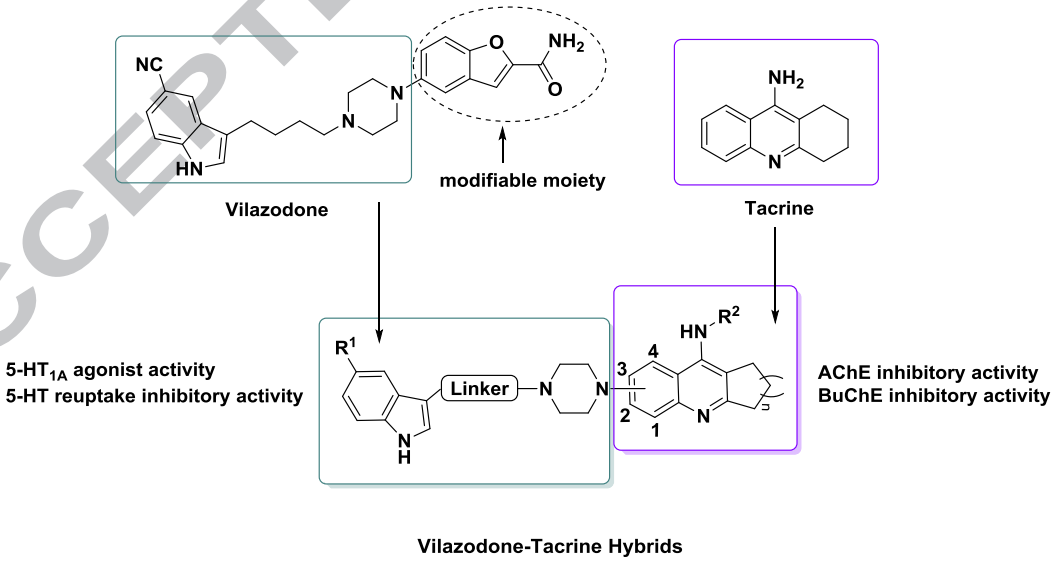


Figure 1. Design strategy of novel vilazodone-tacrine hybrids.

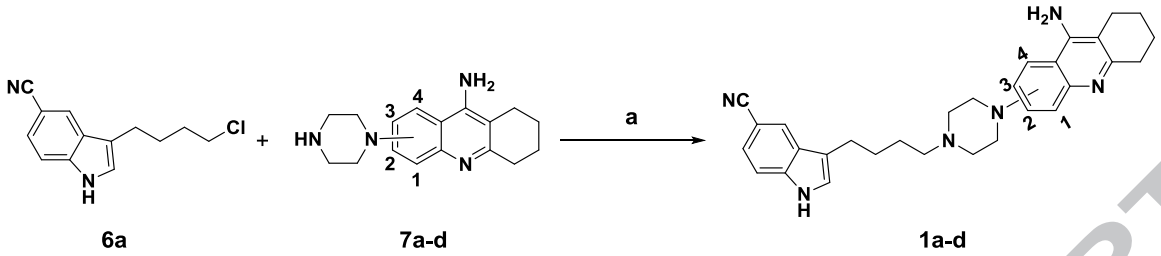
2. Results and discussion

2.1. Chemistry

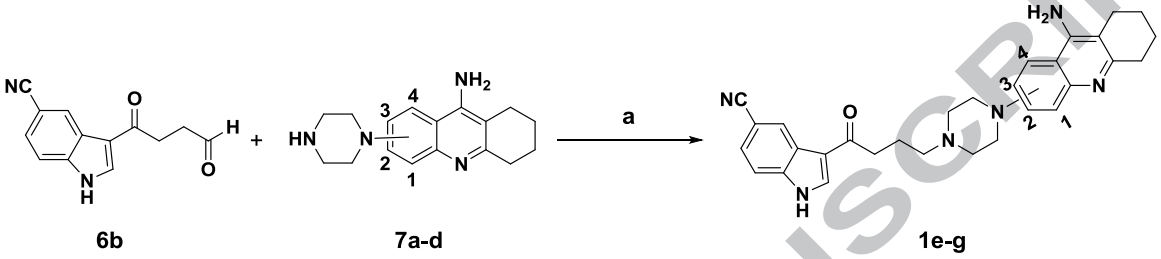
The synthesis of the target compounds was accomplished according to the route shown in Schemes 1-3. In Scheme 1-2, intermediate **6a** (commercial available) and **6b** reacted with tacrine derivatives respectively in the presence of Et_3N to obtain **1a-h**. In tacrine derivatives, piperazine connected to 9-amino-1,2,3,4-tetrahydroacridine in four different points, was depicted in **7a-d**.

In Scheme 3, derivatives were synthesized from indole intermediate and different tacrine derivatives respectively. The synthesis of key intermediates **6b-k** and **7a-g** were described in supporting information. The detailed structures of compounds

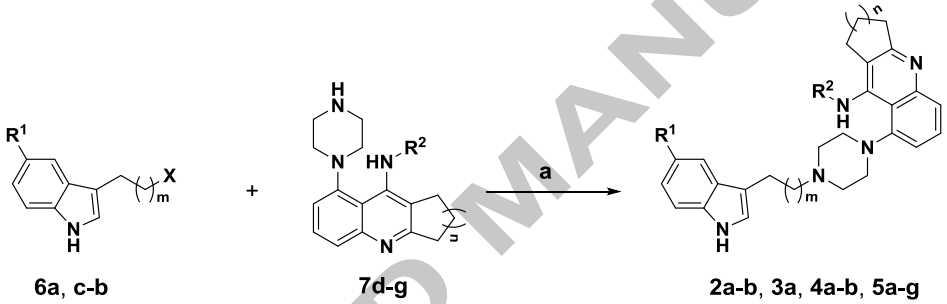
were illustrated in Table 1, and the synthesis of intermediates were showed in supporting information.



Scheme 1. Synthesis of compounds **1a-d**. Reagents and conditions: a) MeCN, KI, Et_3N, reflux, 36h.



Scheme 2. Synthesis of compounds **1e-g**. Reagents and conditions: a) $NaBH_3CN$, MeOH:$C_2H_4Cl_2$ = 1:1 (v:v), rt, 12 h.



6a: R^1 = CN; X = Cl; m = 3
6c: R^1 = CN; X = Br; m = 1
6d: R^1 = CN; X = Cl; m = 2
6e: R^1 = $COOCH_3$; X = Cl; m = 3
6f: R^1 = F; X = Cl; m = 3
6g: R^1 = Cl; X = Cl; m = 3
6h: R^1 = Br; X = Cl; m = 3
6i: R^1 = H; X = Cl; m = 3
6j: R^1 = OCH_3; X = Cl; m = 3
6k: R^1 = CH_3; X = Cl; m = 3

7d: R^2 = H; n = 2
7e: R^2 = H; n = 1
7f: R^2 = H; n = 3
7g: R^2 = CH_3CO; n = 2

2a: R^1 = CN; R^2 = H m = 3; n = 1
2b: R^1 = CN; R^2 = H; m = 3; n = 3
3a: R^1 = CN; R^2 = CH_3CO; m = 3; n = 2
4a: R^1 = CN; R^2 = H; m = 1; n = 2
4b: R^1 = CN; R^2 = H; m = 2; n = 2
5a: R^1 = $COOCH_3$; R^2 = H; m = 3; n = 2
5b: R^1 = F; R^2 = H; m = 3; n = 2
5c: R^1 = Cl; R^2 = H; m = 3; n = 2
5d: R^1 = Br; R^2 = H; m = 3; n = 2
5e: R^1 = H; R^2 = H; m = 3; n = 2
5f: R^1 = OCH_3; R^2 = H; m = 3; n = 2
5g: R^1 = CH_3; R^2 = H; m = 3; n = 2

Scheme 3. Synthesis of compounds **2a-b**, **3a**,**4a-b**, **5a-g**. Reagents and conditions: a) MeCN, KI, Et_3N, reflux, 36h.

2.2. 5-HT Agonist Activity

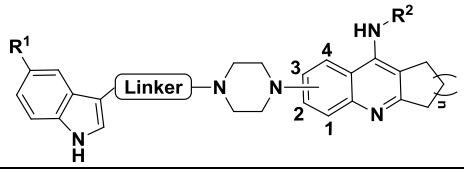
In order to evaluate the antidepressant-like activity of newly designed and synthesized vilazodone-tacrine hybrids, $5HT_{1A}$ agonism assay was conducted by using Human Embryonic Kidney 293 (HEK293) cells expressing correlated human 5-HT_{1A} receptors. The results, together with vilazodone and 8-OH-DPAT as references, are expressed as half-maximal effective concentration (EC_{50}) values (Table 1).

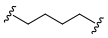
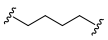
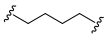
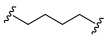
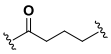
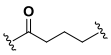
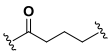
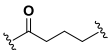
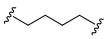
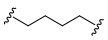
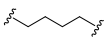
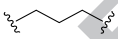
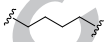
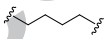
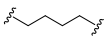
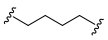
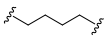
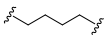
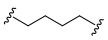
To judge the optimal chain position and linker type of vilazodone fragments, derivatives **1a-h** were provided to the biological assay. From results of 5-HT_{1A} agonistic activity, piperazinyl on the position 4 is the optimal mode. Compound **1d** (EC_{50} = 0.36 ± 0.079 nM) turned out to be a potential derivative. Considering that the optimal chain position is 4, derivatives **2a**, **2b**, **3a**, **4a** and **4b** were tested focusing on different size of naphthenic ring in tacrine, the length of carbon chain and substituent in amino of tacrine. Test results explain that 5-HT_{1A} agonistic activity is related with the size of naphthenic ring: cycloheptane cycle (**2b**) < cyclopentane cycle (**2a**) < cyclohexanone cycle (**1d**). Derivatives with short carbon chain show poor activities (**4a** and **4b**) and derivative with substituent in amino (**3a**) also displays common activity.

Besides, series **5** were prepared for studying 5-substituent on indole. Derivatives **5a-g** were newly synthesized in order to improve both ChE inhibition activity and 5-HT_{1A} agonist activity. In biological assay, although this series of compounds display moderate ChE inhibitory activities, a large part of them show excellent 5-HT_{1A} agonist activities (IC_{50} < 1 nM). Among them, compounds **1d**, **2a**, **5a** and **5f** exhibit great potency at 5-HT_{1A} agonist activity. Structures, 5-HT_{1A} agonist activities, ChE

inhibitory activities of compounds **1a-h**, **2a-b**, **3a**, **4a-b**, **5a-g** was displayed in Table 1.

Table 1. Structures, 5-HT_{1A} agonist activities, ChE inhibitory activities of compounds **1a**–**h**, **2a**–**b**, **3a**, **4a-b**, **5a-g**.



Compd	Linker	R^1	R^2	n	Chain position	EC_{50} ± SD[a] (nM) for $5HT_{1A}$ agonist activity	IC_{50} ± SD[b] (μM) for mAChE inhibitory activity	IC_{50} ± SD[b] (μM) for mBuChE inhibitory activity
1a		CN	H	2	1	1.55 ± 0.17	2.33 ± 0.35	0.31 ± 0.02
1b		CN	H	2	2	28.80 ± 1.76	5.10 ± 0.88	0.25 ± 0.02
1c		CN	H	2	3	14.93 ± 0.29	10.24 ± 1.57	0.64 ± 0.13
1d		CN	H	2	4	0.36 ± 0.079	1.72 ± 0.22	0.34 ± 0.03
1e		CN	H	2	1	34.98 ± 5.60	3.31 ± 0.22	1.85 ± 0.26
1f		CN	H	2	2	567.00 ± 64.00	1.98 ± 0.26	0.37 ± 0.03
1g		CN	H	2	3	1563.21 ± 324.30	2.91 ± 0.32	0.25 ± 0.02
1h		CN	H	2	4	62.43 ± 4.31	1.36 ± 0.07	0.39 ± 0.02
2a		CN	H	1	4	0.58 ± 0.14	2.36 ± 0.34	0.10 ± 0.01
2b		CN	H	3	4	146.01 ± 36.00	18.30 ± 1.46	0.23 ± 0.02
3a		CN	CH_3CO	2	4	93.37 ± 15.20	5.61 ± 1.35	2.92±0.18
4a		CN	H	2	4	>10000	2.10 ± 0.23	1.13 ± 0.07
4b		CN	H	2	4	52.00 ± 0.13	1.82 ± 0.20	0.42 ± 0.02
5a		$COOCH_3$	H	2	4	< 0.14	2.22 ± 0.27	0.41 ±0.03
5b		F	H	2	4	0.72 ± 0.03	NS[c]	NS[c]
5c		Cl	H	2	4	0.75 ± 0.05	NS[c]	NS[c]
5d		Br	H	2	4	0.35 ± 0.09	NS[c]	NS[c]
5e		H	H	2	4	1.20 ± 0.21	1.44 ± 0.17	0.39 ± 0.04
5f		OCH_3	H	2	4	< 0.14	1.62 ± 0.29	0.40 ±0.02
5g		CH_3	H	2	4	0.60 ± 0.01	1.12 ± 0.13	0.36 ± 0.06
Vilazodone	—	—	—	—	—	0.12 ± 0.01	21.30 ± 3.00	NT[d]
Tacrine	—	—	—	—	—	NT[d]	0.13 ± 0.02	0.08 ± 0.01

Hup A	—	—	—	—	—	NT[d]	0.12 ± 0.02	36.4 ± 1.838

—Not applicable.

[a]Results are expressed as the mean of at least two experiments.

[b]Results are expressed as the mean of at least three experiments.

[c]Not soluble at the given concentration.

[d]No test.

2.3. ChE Inhibitory Activity

To evaluate the ChE inhibitory activities of the newly synthesized vilazodone-tacrine hybrids against cognitive impairment in depressive patients, the ChE inhibitory activities were tested through the method of Ellman *et al*[47] by using AChE from mice cortex and BuChE from mice serum. Tacrine and huperzine A (Hup A) were used as reference. Results are presented as half-maximal inhibitory concentration (IC_{50}) values (Table 1**)**.

From the whole test results as showed in Table 1, most of these compounds have moderate AChE inhibitory activities and considerable BuChE inhibitory activities. The IC_{50} values for mAChE inhibitory activity ranged from 1.12 μM to 18.30 μM. In initial stage, we changed different chain positions of vilazodone-like indolebutylamine fragments, and then we found that the optimal position is 4 (derivatives **1d** and **1h**). Considering balanced AChE inhibitory activity and 5-HT_{1A} agonist activity, derivative **1d** (IC_{50} = 1.72 ± 0.22 μM) is regarded as the relatively optimal compound in the first round. Next, the change of naphthenic ring in tacrine, the length of carbon chain and substituent in amino of tacrine were conducted. Derivatives **2a-4b** do not show any improvements on AChE inhibitory activity. Especially, activities of derivatives **2b** declined to a great extent and the substituted one (**3a**) by acetyl also shows weak activity. In series **5**, derivatives **5a**-**5g** were synthesized aiming at improving AChE inhibitory activities. However, derivatives **5b**, **5c** and **5d** do not soluble at the given concentration. Derivatives **5e** (IC_{50} = 1.44 ± 0.17 μM) and **5g** (IC_{50} = 1.12 ± 0.13 μM) showed slight better activities than **1d**.

The IC_{50} values of these hybrids for mBuChE inhibition ranged from 0.10 ± 0.01 μM to 2.92 ± 0.18 μM. Most of these compounds show good activities at nanomolar. From series **1**, we cann't find obvious structure activity relationship (SAR) in BuChE inhibitory activities. In series **2**, compound **2a** (cyclopentane ring, IC_{50} = 0.10 ± 0.01 μM) shows good BuChE inhibitory activity than **1d** (cyclohexane ring) and **2b** (cycloheptane ring). Then, in series **3**, **4** and **5**, BuChE inhibitory activities don't exhibit any improvement. In general, compound **1d** and **2a** showed better inhibitory activities in AChE and BuChE. According to the experimental data of ChE inhibitory activity, compared with vilazodone, derivatives have showed improved activity.

2.4. 5-HT Reuptake Inhibitory Activity

In order to evaluate whether these hybrids can inhibit the process of 5-HT transporter reuptake and show antidepressant function, 5-HT transporter reuptake inhibition (RUI) assay was conducted. Experiment were performed by using HEK293 cell line stably expressing the serotonin transporter protein, vilazodone and citalopram were used as control simultaneously. Considering the coordination of AChE inhibitory activity, BuChE inhibitory activity and 5-HT_{1A} agonist activity, compounds **1d**, **2a**, **5a** and **5f** were selected to be tested in this assay (Table 2**)**. From the results, we can find that different substituents in indole significantly influence the 5-HT reuptake activities. 5-HT reuptake inhibitory activities are better when the substituent is cyano group (**1d** and **2a**) than ester group (**5a**) and methoxy group (**5f**). Then, Compounds **1d** (cyclohexane ring) and **2a** (cyclopentane ring) displayed almost equally good activities. In summary, this finding explains that compounds **1d** and **2a** perform well not only in 5-HT_{1A} agonist activity and ChE inhibitory activity but also 5-HT reuptake inhibitory activity.

Table 2. The 5-HT reuptake inhibitory activities of selected compounds.

Compd	IC_{50} ± SD (nM) for RUI	Compd	IC_{50} ± SD (nM) for RUI
1d	20.42 ± 6.60	**5f**	229.30 ± 13.01
2a	22.10 ± 5.80	**Vilazodone**	0.40 ± 0.10
5a	314.10 ± 77.00	**Citalopram**	6.27 ± 4.51

2.5. In Vitro Blood-Brain Barrier Permeation Assay

Both anti-depression and anti-AD drugs which react on therapeutic targets in the brain, need to cross the blood-brain barrier. Considering the property of our derivatives, a parallel artificial membrane permeability assay was conducted to predict the penetration of vilazodone-tacrine hybrids preliminarily. The assay method was conducted according to Di *et al*.[48] In vitro permeability (P_e) of derivatives **1a-5g** was tested dissolving in PBS/ethanol (70:30) through a brain lipid porcine membrane. From the results (Table 3), we can find that most of the hybrids ($P_e > 3.08 \times 10^{-6}$ cm s^{-1}, CNS+) show the capacity of crossing the BBB. Especially, when the chain position of vilazodone-like indolebutylamine fragments is 4, they almost show good permeability. Therefore, P_e values of the tested compounds can be considered as reliable references in the screening process of potential drug candidates.

Table 3. Prediction of BBB penetration of compounds **1a–h**, **2a–b**, **3a**, **4a-b**, **5a-g**.

Compd	P_e (10^{-6}cm s^{-1})[a]	Prediction[b]	Compd	P_e (10^{-6} cm s^{-1})[a]	Prediction[b]
1a	4.43 ± 0.56	CNS+	**3a**	1.92 ± 0.09	CNS±
1b	2.99 ± 0.62	CNS±	**4a**	4.48 ± 0.07	CNS+
1c	3.55 ± 0.32	CNS+	**4b**	5.46 ± 0.11	CNS+
1d	5.11 ± 0.47	CNS+	**5a**	6.22 ± 0.46	CNS+
1e	1.61 ± 0.38	CNS±	**5b**	3.76 ± 0.37	CNS+
1f	1.68 ± 0.54	CNS±	**5c**	2.74 ± 0.29	CNS±
1g	1.04 ± 0.58	CNS−	**5d**	3.05 ± 0.45	CNS±
1h	1.33 ± 0.55	CNS±	**5e**	3.52 ± 0.45	CNS+
2a	4.38 ± 0.41	CNS+	**5f**	4.53 ± 0.44	CNS+
2b	3.97 ± 0.36	CNS+	**5g**	3.64 ± 0.40	CNS+

[a]Values are expressed as the mean ± SD of at least three independent experiments.
[b]Compounds with permeabilities $P_e > 3.08 \times 10^{-6}$ cm s^{-1} could cross the BBB by passive diffusion (CNS+), $P_e < 1.13 \times 10^{-6}$ cm s^{-1} could not cross the BBB (CNS−), 1.13×10^{-6} cm $s^{-1} < P_e < 3.08 \times 10^{-6}$ cm s^{-1} show uncertain BBB permeation (CNS±).

3. Conclusion

By a rational design strategy, a series of multifunctional vilazodone-tacrine hybrids against depression and cognitive impairment were designed, synthesized, and evaluated in *vitro*. Most of derivatives exhibited considerable inhibitory activities toward AChE and BuChE. Besides, most of them showed great 5-HT agonist activity, 5-HT reuptake inhibitory activity and excellent blood-brain barrier permeability. Compounds **1d** and **2a** have a similar structure, being evaluated to be the most efficient ones. The two compounds exhibited remarkable 5-HT reuptake inhibitory activities and 5-HT agonist activities. **2a** shows stronger BuChE inhibitory activity than **1d**, and the two compounds have almost the same inhibition efficacy in AChE. In blood-brain barrier permeation assay, which was considered as reliable references in the screening process of potential drug candidates, **1d** and **2a** all performed good penetration of BBB. Overall, vilazodone-tacrine hybrids provide a novel thought for drug discovery on depression with cognitive impairment and are worthy to be further explored.

4. Material and Methods

4.1. Chemistry

Reagents and solvents were purchased from J&K Scientific, Energy Chemical, Macklin, Bide Pharmatech Ltd, Adamas-beta Target Molecule, TCI, Alfa Aesar and Acros, and they were used without further purification. Reactions were followed by thin-layer chromatography (TLC) on silica gel HSGF254 plates (0.2 ± 0.03 mm thickness; Yantai JiangYou silicone development co., LTD, Yantai, China), and the spots were visualized with UV lamp (λ = 254 nm). Yields were not optimized. Melting points were measured in capillary tubes on a SGW X-4 melting point apparatus without correction. Nuclear magnetic resonance (NMR) spectroscopy was performed on a Bruker AMX-400 NMR. Chemical shifts were reported in parts per million (ppm, δ) downfield from tetramethylsilane. Proton coupling patterns were described as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), multiplet (m). High-resolution mass spectra (HRMS) were obtained by electric ionization (EI) and electrospray ionization (ESI) using a Waters GCT Premie and Waters LCT. HPLC data analysis of compounds **1a-5g** was conducted on an Agilent 1100 with a quaternary pump and diode-array detector (DAD). The peak purity was verified by UV spectra. All final compounds showed ≥ 95% purity. (Table S1, supporting information).

*4.1.1. 3-(4-(4-(9-Amino-5,6,7,8-tetrahydroacridin-4-yl)piperazin-1-yl)butyl)-1H-indole-5-carbonitrile (**1a**)*

A mixture of 3-(4-chlorobutyl)-1*H*-indole-5-carbonitrile (**6a**, 116 mg, 0.5 mmol), corresponding intermediates **7a** (0.5 mmol) and KI (0.05 mmol) was added Et_3N (1 mmol). The reaction mixture was stirred at reflux temperature for 24 h. Then removal the solvent under vacuum and the residue was purified by column chromatography (CH_3OH:CH_2Cl_2 = 1:20 to 1:10, *v*:*v*) to afford compound **1a**. Compound **1a** was isolated as a yellow powder, 41% yield, mp 214–216 °C. ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.19–8.10 (m, 1H), 8.02 (s, 1H), 7.77 (d, *J* = 7.4 Hz, 1H), 7.64–7.56 (m, 1H), 7.50–7.44 (m, 1H), 7.40–7.34 (m, 1H), 7.29–7.24 (m, 1H), 3.27–2.96 (m, 8H), 2.86 (t, *J* = 12.7 Hz, 4H), 2.75–2.44 (m, 4H), 2.21–1.96 (m, 4H), 1.96–1.86 (m, 2H), 1.83–1.78 (m, 2H). HRMS (EI) m/z calcd $C_{30}H_{33}N_5$ (M^+) 463.2736, found 463.2737.

*4.1.2. 3-(4-(4-(9-Amino-5,6,7,8-tetrahydroacridin-3-yl)piperazin-1-yl)butyl)-1H-indole-5-carbonitrile (**1b**)*

1b was synthesized by the general procedure of **1a**, and **7a** was replaced by **7b**. Compound **1b** was isolated as a yellow powder, 45% yield, mp 225–227 °C. ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.14 (d, *J* = 9.5 Hz, 1H), 8.03 (d, *J* = 0.8 Hz, 1H), 7.51–7.47 (m, 1H), 7.40– 7.34 (m, 2H), 7.26 (s, 1H), 6.87 (d, *J* = 2.4 Hz, 1H), 3.58–3.45 (m, 4H), 2.99–2.73 (m, 8H), 2.71–2.62 (m, 2H), 2.59 (t, *J* = 5.6 Hz, 2H), 2.01– 1.92 (m, 4H), 1.85–1.77 (m, 2H), 1.76–1.67 (m, 2H). HRMS (EI) m/z calcd $C_{30}H_{34}N_6$ (M^+) 478.2845, found 478.2844.

*4.1.3. 3-(4-(4-(9-Amino-5,6,7,8-tetrahydroacridin-2-yl)piperazin-1-yl)butyl)-1H-indole-5-carbonitrile (**1c**)*

1c was synthesized by the general procedure of **1a**, and **7a** was replaced by **7c**. Compound **1c** was isolated as a yellow powder, 39% yield, mp 208–210 °C. ^{1}H NMR (400 MHz, CD_3OD) *δ*: 7.99 (d, *J* = 0.9 Hz, 1H), 7.70 (dd, *J* = 9.3, 2.4 Hz, 1H), 7.65 (s, 1H), 7.54 (d, *J* = 2.3 Hz, 1H), 7.46 (d, *J* = 8.4 Hz,

1H), 7.35 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.26 (s, 1H), 3.64–3.40 (m, 4H), 3.25–3.01 (m, 4H), 3.01–2.88 (m, 4H), 2.85 (dd, *J* = 14.4, 7.7 Hz, 2H), 2.60 (t, *J* = 5.9 Hz, 2H), 2.03–1.86 (m, 4H), 1.84–1.73 (m, 4H). HRMS (ESI) m/z calcd $C_{30}H_{34}N_6$ $[M+H]^+$ 479.2923, found 479.2922.

*4.1.4. 3-(4-(4-(9-Amino-5,6,7,8-tetrahydroacridin-1-yl)piperazin-1-yl)butyl)-1H-indole-5-carbonitrile (**1d**)*

1d was synthesized by the general procedure of **1a**, and **7a** was replaced by **7d**.Compound **1d** was isolated as a yellow powder, 48% yield, mp 202–205 °C.^{1}H NMR (400 MHz, CD_3OD) *δ*: 7.96 (s, 1H), 7.74 (t, *J* = 7.8 Hz, 1H), 7.54–7.39 (m, 3H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.22 (s, 1H), 3.16 (m, 4H), 3.03 (t, *J* = 11.0 Hz, 2H), 2.92 (m, 2H), 2.81 (t, *J* = 6.4 Hz, 2H), 2.63 (m, 2H), 2.54 (m, 4H), 1.94 (m, 4H), 1.76 (m, 2H), 1.65 (m, 2H). HRMS (ESI) m/z calcd $C_{30}H_{34}N_6$ $[M+H]^+$ 479.2923, found 479.2922.

*4.1.5. 3-(4-(4-(9-Amino-5,6,7,8-tetrahydroacridin-4-yl)piperazin-1-yl)butanoyl)-1H-indole-5-carbonitrile (**1e**)*

A mixture of 3-(4-oxobutanoyl)-1*H*-indole-5-carbonitrile (**6b**, 113 mg, 0.5 mmol) and intermediates **7a** (0.5 mmol) in CH_3OH:$C_2H_4Cl_2$ = 1:1 (*v*:*v*) was added $NaBH_3(CN)$ (62.8 mg, 1 mmol). The mixture was stirred at room temperature for 24 h, and solvent was evaporated to dryness under reduced pressure. The residue was purified on a silica gel column using mixtures of CH_3OH:CH_2Cl_2 as eluent, obtaining the target compounds **1e**. Compound **1e** was isolated as a yellow powder, 50% yield, mp > 300 °C. ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.16 (dd, *J* = 11.5, 6.7 Hz, 1H), 7.84 (t, *J* = 8.1 Hz, 1H), 7.67–7.54 (m, 2H), 7.48 (d, *J* = 7.4 Hz, 1H), 7.37 (t, *J* = 7.5 Hz, 1H), 7.09–6.99 (m, 1H), 3.47 (t, *J* = 10.8 Hz, 4H), 3.30– 3.23 (m, 4H), 3.22–2.89 (m, 6H), 2.65 (t, *J* = 5.1 Hz, 2H), 2.32–2.16 (m, 2H), 2.04–1.94 (m, 4H). HRMS (ESI) m/z calcd $C_{30}H_{32}N_6O$ $[M+H]^+$ 493.2716, found 493.2717.

*4.1.6. 3-(4-(4-(9-Amino-5,6,7,8-tetrahydroacridin-3-yl)piperazin-1-yl)butanoyl)-1H-indole-5-carbonitrile (**1f**)*

1f was synthesized by the general procedure of **1e**, and **7a** was replaced by **7b**. Compound **1f** was isolated as a yellow powder, 48% yield, mp > 300 °C. ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.60 (s, 1H), 8.36 (s, 1H), 8.05 (d, *J* = 9.4 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.45 (d, *J* = 8.2 Hz, 1H), 7.26 (d, *J* = 8.7 Hz, 1H), 6.77 (s, 1H), 3.45 –3.34 (m, 4H), 3.01 (t, J = 6.6 Hz, 2H), 2.88 (m, 2H), 2.83–2.71 (m, 4H), 2.70–2.60 (m, 2H), 2.57–2.49 (m, 2H), 2.11–1.99 (m, 2H), 1.92 (m, 4H). HRMS (ESI) m/z calcd $C_{30}H_{32}N_6O$ $[M+H]^+$ 493.2716, found 493.2715.

*4.1.7. 3-(4-(4-(9-Amino-5,6,7,8-tetrahydroacridin-2-yl)piperazin-1-yl)butanoyl)-1H-indole-5-carbonitrile (**1g**)*

1g was synthesized by the general procedure of **1e**, and **7a** was replaced by **7c**. Compound **1g** was isolated as a yellow powder, 51% yield, mp > 300 °C. ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.64 (s, 1H), 8.41 (s, 1H), 7.68–7.59 (m, 3H), 7.52–7.46 (m, 2H), 3.43–3.36 (m, 4H), 3.06 (t, *J* = 7.1 Hz, 2H), 3.01–2.87 (m, 6H), 2.82 –2.71 (m, 2H), 2.62 (t, *J* = 4.5 Hz, 2H), 2.15–2.06 (m, 2H), 2.00–1.95 (m, 4H). HRMS (ESI) m/z calcd $C_{30}H_{32}N_6O$ $[M+H]^+$ 493.2716, found 493.2715.

*4.1.8. 3-(4-(4-(9-Amino-5,6,7,8-tetrahydroacridin-1-yl)piperazin-1-yl)butanoyl)-1H-indole-5-carbonitrile (**1h**)*

1h was synthesized by the general procedure of **1e**, and **7a** was replaced by **7d**. Compound **1h** was isolated as a yellow powder, 45% yield, mp >300 °C. ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.61 (s, 1H), 8.37 (s, 1H), 7.61 (dd, *J* = 20.3, 7.6 Hz, 2H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 7.7 Hz, 1H), 7.03 (d, *J* = 7.2 Hz, 1H), 3.08–2.83 (m, 8H), 2.70 (t, *J* = 10.3 Hz, 2H), 2.61–2.44 (m, 4H), 2.41–2.30 (m, 2H), 2.08–1.83 (m, 6H). HRMS (ESI) m/z calcd $C_{30}H_{32}N_6O$ $[M+H]^+$ 493.2716, found 493.2715.

*4.1.9. 3-(4-(4-(9-Amino-2,3-dihydro-1H-cyclopenta[b]quinolin-8-yl)piperazin-1-yl)butyl)-1H-indole-5-carbonitrile (**2a**)*

A mixture of 3-(4-chlorobutyl)-1*H*-indole-5-carbonitrile (**6a**, 116 mg, 0.5 mmol), corresponding intermediates **7e** (0.5 mmol) and KI (0.05 mmol) was added Et_3N (1 mmol). The reaction mixture was stirred at reflux temperature for 24 h. Then removal the solvent under vacuum and the residue was purified by column chromatography (CH_3OH:CH_2Cl_2 = 1:20 to 1:10, *v*:*v*) to afford compound **2a**. Compound **2a** was isolated as a yellow powder, 40% yield, mp 170–171 °C. ^{1}H NMR (400 MHz, CD_3OD) *δ*: 7.99 (d, *J* = 0.8 Hz, 1H), 7.81–7.74 (m, 1H), 7.54–7.49 (m, 2H), 7.45 (dd, *J* = 8.5, 0.6 Hz, 1H), 7.37–7.32 (m, 1H), 7.24 (s, 1H), 3.20 (t, *J* = 7.8 Hz, 6H), 3.12–3.02 (m, 2H), 2.97–2.91 (m, 2H), 2.84 (t, *J* = 7.2 Hz, 2H), 2.77–2.55 (m, 4H), 2.39–2.26 (m, 2H), 1.83–1.75 (m, 2H), 1.74–1.63 (m, 2H). HRMS (EI) m/z calcd $C_{29}H_{32}N_6$ (M^+) 464.2688, found 464.2689.

*4.1.10. 3-(4-(4-(11-Amino-7,8,9,10-tetrahydro-6H-cyclohepta[b]quinolin-1-yl)piperazin-1-yl)butyl)-1H-indole-5-carbonitrile (**2b**)*

2b was synthesized by the general procedure of **2a**, and **7e** was replaced by **7f**. Compound **2b** was isolated as a yellow powder, 45% yield, mp 181–183 °C. ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.00 (s, 1H), 7.78 (t, *J* = 8.1 Hz, 1H), 7.52 (dd, *J* = 12.0, 7.8 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.35 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.24 (s, 1H), 3.23–3.13 (m, 4H), 3.11–3.03 (m, 4H), 2.84 (t, *J* = 7.2 Hz, 4H), 2.72–2.50 (m, 4H), 1.99–1.89 (m, 2H), 1.85–1.75 (m, 4H), 1.73– 1.66 (m, 4H). HRMS (EI) m/z calcd $C_{31}H_{36}N_6$ (M^+) 492.3001, found 492.3002.

*4.1.11. N-(8-(4-(4-(5-cyano-1H-indol-3-yl)butyl)piperazin-1-yl)-1,2,3,4-tetrahydroacridin-9-yl)acetamide (**3a**)*

3a was synthesized by the general procedure of **2a**, and **7e** was replaced by **7g**. Compound **3a** was isolated as a yellow powder, 38% yield, mp 207–209 °C. ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.01 (s, 1H), 7.70 (d, *J* = 8.4 Hz, 1H), 7.60 (t, *J* = 7.9 Hz, 1H), 7.45 (t, *J* = 6.9 Hz, 1H), 7.36 (dd, *J* = 8.5, 1.5 Hz, 2H), 7.25 (s, 1H), 3.24–3.16 (m, 6H), 3.07 (t, *J* = 6.1 Hz, 2H), 2.91–2.76 (m, 6H), 2.30–2.20 (m, 2H), 2.01–1.94 (m, 2H), 1.87 (s, 3H), 1.83–1.72 (m, 4H), 1.64–1.48 (m, 2H). HRMS (EI) m/z calcd $C_{32}H_{36}N_6O$ (M^+) 520.2951, found 520.2949.

*4.1.12. 3-(2-(4-(9-Amino-5,6,7,8-tetrahydroacridin-1-yl)piperazin-1-yl)ethyl)-1H-indole-5-carbonitrile (**4a**)*

A mixture of 8-(piperazin-1-yl)-1,2,3,4-tetrahydroacridin-9-amine (**7d**, 141 mg, 0.5 mmol), corresponding intermediates **6c** (0.5 mmol) and KI (0.05 mmol) was added Et_3N (1 mmol). The reaction mixture was stirred at reflux temperature for 24 h. Then removal the solvent under vacuum and the residue was purified by column chromatography (CH_3OH:CH_2Cl_2 = 1:20 to 1:10, *v*:*v*) to afford compound **4a**. Compound **4a** was isolated as a yellow powder, 40% yield, mp 132–135 °C. ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.08 (d, *J* = 16.1 Hz, 1H), 7.75 (t, *J* = 8.1 Hz, 1H), 7.56–7.44 (m, 3H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.32 (s, 1H), 3.29–3.16 (m, 4H), 3.16–3.00 (m, 4H), 2.95 (t, *J* = 5.5 Hz, 2H), 2.86–2.79 (m, 2H), 2.65–2.47 (m, 4H), 2.06– 1.86 (m, 4H). HRMS (EI) m/z calcd $C_{28}H_{30}N_6$ (M^+) 450.2532, found 450.2533.

*4.1.13. 3-(3-(4-(9-Amino-5,6,7,8-tetrahydroacridin-1-yl)piperazin-1-yl)propyl)-1H-indole-5-carbonitrile (**4b**)*

4b was synthesized by the general procedure of **4a**, and **6c** was replaced by **6d**. Compound **4b** was isolated as a yellow powder, 42% yield, mp 94–96 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 8.04 (s, 1H), 7.73 (t, J = 8.1 Hz, 1H), 7.54–7.43 (m, 3H), 7.37 (dd, J = 8.4, 1.3 Hz, 1H), 7.29–7.20 (m, 1H), 3.20–3.00 (m, 6H), 2.93 (t, J = 5.7 Hz, 2H), 2.86 (t, J = 7.3 Hz, 2H), 2.55 (t, J = 6.1 Hz, 4H), 2.40 (t, J = 10.7 Hz, 2H), 2.01–1.91 (m, 6H). HRMS (EI) m/z calcd $C_{29}H_{32}N_6$ (M^+) 464.2688, found 464.2690.

*4.1.14. Methyl 3-(4-(4-(9-amino-5,6,7,8-tetrahydroacridin-1-yl)piperazin-1-yl)butyl)-1H- indole-5-carboxylate (**5a**)*

5a was synthesized by the general procedure of **4a**, and **6c** was replaced by **6e**. Compound **5a** was isolated as a yellow powder, 45% yield, mp 144–146 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 8.31 (s, 1H), 7.76 (dd, J = 8.3, 5.2 Hz, 2H), 7.49 (dd, J = 15.0, 8.1 Hz, 2H), 7.36 (d, J = 8.6 Hz, 1H), 7.14 (s, 1H), 3.89 (s, 3H), 3.21–3.09 (m, 4H), 3.05 (t, J = 11.1 Hz, 2H), 2.94 (t, J = 5.4 Hz, 2H), 2.85 (t, J = 7.1 Hz, 2H), 2.68–2.60 (m, 2H), 2.59–2.44 (m, 4H), 2.02–1.89 (m, 4H), 1.86– 1.78 (m, 2H), 1.74–1.63 (m, 2H). HRMS (EI) m/z calcd $C_{31}H_{37}N_5O_2$ (M^+) 511.2947, found 511.2946.

*4.1.15. 8-(4-(4-(5-Fluoro-1H-indol-3-yl)butyl)piperazin-1-yl)-1,2,3,4-tetrahydroacridin-9-amine (**5b**)*

5b was synthesized by the general procedure of **4a**, and **6c** was replaced by **6f**. Compound **5b** was isolated as a yellow powder, 43% yield, mp 148–150 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.82–7.75 (m, 1H), 7.56–7.46 (m, 2H), 7.28 (dd, J = 8.8, 4.4 Hz, 1H), 7.23–7.17 (m, 1H), 7.10 (s, 1H), 6.84 (td, J = 9.1, 2.5 Hz, 1H), 3.21– 3.01 (m, 6H), 2.96 (t, J = 5.8 Hz, 2H), 2.78 (t, J = 7.2 Hz, 2H), 2.58 (t, J = 6.0 Hz, 4H), 2.45 (t, J = 10.3 Hz, 2H), 2.06–1.91 (m, 4H), 1.83–1.73 (m, 2H), 1.71–1.62 (m, 2H). HRMS (EI) m/z calcd $C_{29}H_{34}FN_5$ (M^+) 471.2798, found 471.2802.

*4.1.16. 8-(4-(4-(5-Chloro-1H-indol-3-yl)butyl)piperazin-1-yl)-1,2,3,4-tetrahydroacridin-9-amine (**5c**)*

5c was synthesized by the general procedure of **4a**, and **6c** was replaced by **6g**. Compound **5c** was isolated as a yellow powder, 43% yield, mp 149–151 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.77 (t, J = 7.8 Hz, 1H), 7.61–7.43 (m, 3H), 7.28 (d, J = 8.6 Hz, 1H), 7.09 (s, 1H), 7.02 (d, J = 8.5 Hz, 1H), 3.23–2.86 (m, 8H), 2.78 (t, J = 6.7 Hz, 2H), 2.69–2.39 (m, 6H), 1.96 (m, 4H), 1.83–1.72 (m, 2H), 1.71–1.59 (m, 2H). HRMS (EI) m/z calcd $C_{29}H_{34}ClN_5$ (M^+) 487.2503, found 487.2507.

*4.1.17. 8-(4-(4-(5-Bromo-1H-indol-3-yl)butyl)piperazin-1-yl)-1,2,3,4-tetrahydroacridin-9-amine (**5d**)*

5d was synthesized by the general procedure of **4a**, and **6c** was replaced by **6h**. Compound **5d** was isolated as a yellow powder, 39% yield, mp 151–153 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.80–7.74 (m, 1H), 7.66 (d, J = 1.7 Hz, 1H), 7.54–7.46 (m, 2H), 7.24 (d, J = 8.6 Hz, 1H), 7.15 (dd, J = 8.6, 1.9 Hz, 1H), 7.07 (s, 1H), 3.21– 3.00 (m, 6H), 2.95 (t, J = 5.9 Hz, 2H), 2.78 (t, J = 7.3 Hz, 2H), 2.56 (t, J = 6.1 Hz, 4H), 2.47 (t, J = 10.5 Hz, 2H), 2.03–1.89 (m, 4H), 1.83–1.72 (m, 2H), 1.70–1.61 (m, 2H). HRMS (EI) m/z calcd $C_{29}H_{34}BrN_5$ (M^+) 531.1998, found 531.1997.

*4.1.18. 8-(4-(4-(1H-indol-3-yl)butyl)piperazin-1-yl)-1,2,3,4-tetrahydroacridin-9-amine (**5e**)*

5e was synthesized by the general procedure of **4a**, and **6c** was replaced by **6i**. Compound **5e** was isolated as a yellow powder, 45% yield, mp 112–114 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.77 (t, J = 8.1 Hz, 1H), 7.52 (dd, J = 8.1, 2.9 Hz, 2H), 7.47 (d, J = 7.7 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.05 (dd, J = 14.7, 7.5 Hz, 2H), 6.98 (t, J = 7.4 Hz, 1H), 3.14 (d, J = 5.9 Hz, 4H), 3.05 (t, J = 10.9 Hz, 2H), 2.95 (t, J = 5.8 Hz, 2H), 2.82 (t, J = 7.1 Hz, 2H), 2.66–2.59 (m, 2H), 2.59–2.42 (m, 4H), 2.04–1.89 (m, 4H), 1.84–1.77 (m, 2H), 1.72–1.63 (m, 2H). HRMS (EI) m/z calcd $C_{29}H_{35}N_5$ (M^+) 453.2892, found 453.2893.

*4.1.19. 8-(4-(4-(5-Methoxy-1H-indol-3-yl)butyl)piperazin-1-yl)-1,2,3,4-tetrahydroacridin-9-amine (**5f**)*

5b was synthesized by the general procedure of **4a**, and **6c** was replaced by **6j**. Compound **5f** was isolated as a yellow powder, 43% yield, mp 102–104 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.76 (t, J = 8.1 Hz, 1H), 7.48 (dd, J = 19.5, 8.1 Hz, 2H), 7.19 (d, J = 8.7 Hz, 1H), 6.99 (s, 2H), 6.72 (dd, J = 8.7, 2.2 Hz, 1H), 3.80 (s, 3H), 3.13 (t, J = 10.3 Hz, 4H), 3.03 (t, J = 11.0 Hz, 2H), 2.93 (t, J = 5.6 Hz, 2H), 2.77 (t, J = 7.1 Hz, 2H), 2.64–2.43 (m, 6H), 2.02–1.88 (m, 4H), 1.81–1.74 (m, 2H), 1.71–1.62 (m, 2H). HRMS (EI) m/z calcd $C_{30}H_{37}N_5O$ (M^+) 483.2998, found 483.2999.

*4.1.20. 8-(4-(4-(5-Methyl-1H-indol-3-yl)butyl)piperazin-1-yl)-1,2,3,4-tetrahydroacridin-9-amine (**5g**)*

5g was synthesized by the general procedure of **4a**, and **6c** was replaced by **6k**. Compound **5g** was isolated as a yellow powder, 46% yield, mp 95–97 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.76 (t, J = 8.1 Hz, 1H), 7.48 (dd, J = 22.3, 8.0 Hz, 2H), 7.29 (s, 1H), 7.18 (d, J = 8.3 Hz, 1H), 6.96 (s, 1H), 6.88 (d, J = 8.3 Hz, 1H), 3.22–3.11 (m, 4H), 3.04 (t, J = 10.9 Hz, 2H), 2.93 (t, J = 5.8 Hz, 2H), 2.77 (t, J = 7.0 Hz, 2H), 2.68–2.61 (m, 2H), 2.60–2.49 (m, 4H), 2.39 (s, 3H), 2.01–1.88 (m, 4H), 1.80–1.73 (m, 2H), 1.70–1.61 (m, 2H). HRMS (EI) m/z calcd $C_{30}H_{37}N_5$ (M^+) 467.3049, found 467.3050.

4.2 ChE Inhibitory Activity

The assay of ChE was conducted by using the method of Ellman *et al*[45]. with slight modification. The rat cortex was homogenized in cold 75 mM sodium phosphate buffer (pH = 7.4) with 0.4 mM Tetraisopropylpyrophosphoramide (iso-OMPA) as the AChE source. And the rat serum was collected as the BuChE source. The assay solution was made of 20 mM phosphate buffer, 1 mM 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB, Sigma), 240 μM acetylthiocholine iodide (Sigma) or 320 μM butyrylthiocholine (Sigma), and 10 μL mice cortex homogenate or 20 μL mice serum, respectively. Compounds were concentrated appropriately and added into the assay solution and incubated for 20 min at room temperature. Then, the reactions were stopped by sodium-dodecyl sulfate (SDS) and the absorption values were measured by a microplate reader (DTX 880, Beckman Coulter) at 450 nm. Last, the inhibition ratio caused by the presence of test compounds was calculated,, and the IC_{50} was defined as half-maximal inhibitory concentration values.

4.3 In vitro Blood-Brain Barrier Permeation Assay

In order to evaluate the BBB penetrability of compounds **1a-5g,** a parallel artificial membrane permeation assay was conducted. Commercial drugs were purchased from Sigma and Alfa Aesar. The porcine brain lipid (PBL) was bought from Avanti Polar Lipids. The donor microplate (PVDF membrane, pore size 0.45 mm) and the acceptor microplate were bought from Millipore. The 96-well UV plate was from Corning Incorporated. Compounds were dissolved in DMSO at 5 mg/mL and diluted 50-fold in PBS/EtOH (7:3, *v*:*v*) to get the concentration of 100 μg/mL. The filter membrane of the donor 96-well microplate was impregnated with 4 μL of PBL in

dodecane (20 mg mL^{-1}). It was added 200 μL of the tested compounds to the donor wells and then carefully placed on the acceptor 96-well microplate with 300 μL of PBS/EtOH (7:3, *v*:*v*) to form a sandwich, which was left undisturbed for 12 h at 25 °C. After incubation, the donor plate was carefully removed and the concentration of compounds in the acceptor wells was determined using a UV plate reader (SpectraMax i3). Each sample was analyzed at eight wavelengths, three wells and three independent experiments, and the results are given as the mean ± standard deviation. P_e was calculated using the following equation.

$$P_e = -\left(\frac{(V_D \times V_A)}{(V_D + V_A)\text{area} \times \text{time}}\right) \times \ln\left(1 - \frac{[\text{drug}]_{\text{acceptor}}}{[\text{drug}]_{\text{equilibrium}}}\right)$$

V_D represents the volume in the donor well, and V_A represents the volume in the acceptor well. Area represents the filter area, and time is the permeation time. $[\text{drug}]_{\text{acceptor}}$ means the absorbance of the compound in the acceptor well, and $[\text{drug}]_{\text{equilibrium}}$ means the theoretical equilibrium absorbance.

There were 13 quality control standards of known BBB permeability were included to validate the analysis set (Table S2, Supporting Information). The experimental data versus reference values gave a strong linear correlation, P_e (exp.) = 0.974P_e (lit.) – 0.8152 (R_2 = 0.9651) (Figure S1, Supporting Information). From this equation and the limit established by Di *et al*. for blood−brain barrier permeation, we can get the conclusion that compounds with P_e values greater than 3.08×10^{-6} cm s^{-1} could cross the blood-brain barrier (CNS+), P_e values from 1.13×10^{-6} cm s^{-1} to 3.08×10^{-6} cm s^{-1} were classified as "CNS±" (BBB permeation uncertain) and P_e values less than 1.13×10^{-6} cm s^{-1} could not cross the BBB (CNS−) (Table S3, supporting information).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/.

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