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Dual-target-directed 1,3-diphenylurea derivatives: BACE 1 inhibitor and metal chelator against Alzheimer's disease

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ABSTRACT

Dual-target-directed 1,3-diphenylurea derivatives were designed by hybridizing BACE 1 inhibitor **1** with metal chelator LR-90. A database consisted of 1,3-diphenylurea derivatives was built and screened by the pharmacophore model (Hypo 1) of BACE 1 inhibitor. Based on the predicted results, 11 compounds (**6a–d**, **9a–g**) with favorable Fitvalues were selected, synthesized and evaluated for their BACE 1 inhibitory activities, which showed that the predicted results were in good agreement with the experimental values. Besides, the synthesized compounds also displayed the ability to chelate metal ions. The most effective BACE 1 inhibitor **9f** (27.85 ± 2.46 μmol/L) was selected for further receptor-binding studies, the result of which indicated that an essential hydrogen bonds was formed between the urea group of **9f** and the catalytic aspartate Asp228.

1. Introduction

Alzheimer's disease (AD), the leading cause of dementia in the elderly, is a complex neurodegenerative disorder. Until now, multiple factors, such as amyloid-β (Aβ) aggregation,[1,2] metal dyshomeostasis, oxidative stress, mitochondrial dysfunction and reduced acetylcholine (ACh) level, have been considered to play important roles in the pathogenesis of AD.[3] The complexity and multiple etiologies of AD make single-target strategy difficult to shed desirable therapeutic effect. Thus multi-target-directed ligand (MTDL) raises as a potentially more effective strategy for AD treatment.[4] MTDL, the goal of which is to enhance efficacy and improve safety, is rationally designed to hit multiple targets for a particular disease. In the past two years, many MTDLs have been reported with improved pharmacological profiles, such as AChE inhibitors with metal chelate property and AchE-induced Aβ aggregation inhibitory activity,[5] BACE1 inhibitors bearing acetylcholinesterase (AChE) inhibitory activity,[6] and cholinesterase inhibitors with both antioxidant and neuroprotective properties.[7]

Amyloid-β (Aβ), formed by the continuously proteolytic processing of β-amyloid precursor protein (APP) by β and γ-secretase,[8–10] is thought to play a central role in the pathogenesis of AD. Since β-secretase (BACE 1) mediated cleavage of APP is the first step of the amyloidogenic pathway, inhibition of BACE 1 is likely to reduce the production of Aβ and thereby delay or halt the progression of AD.[11,12]

Recent evidence indicated that dyshomeostasis of biometals (Fe, Cu, Zn) in the brain may contribute to AD pathology.[13] Experiments also found that the level of metal ions in AD patients is 3–7-folds higher than that of healthy individuals.[14] Therefore, decreasing the level of metal ions in brain by using metal chelator represents another rational therapeutic approach for the treating of AD.

Previously, we reported the establishment of pharmacophore model for BACE 1 inhibitor, Hypo 1, which was validated by Enrichment and ROC method (EF at 2%, 5% and 10% are 30.6, 12.2 and 7.7; AUC of the ROC curve is 0.93).[15] Analysis based on established pharmacophore model and BACE 1 inhibitory activity revealed that compound **1** displayed the highest BACE 1 activity among two series synthesized *N*-phenyl-1-arylamide and *N*-phenylbenzenesulfonamide derivatives.

Considering the crucial roles of BACE 1 and metal ions in AD pathology, we focused on dual-target-directed ligands that can simultaneously impact on BACE 1 and metal ions in this study. Based on the previous work, a series of novel 1,3-diphenylurea derivatives were designed by hybridizing metal chelator LR-90 with BACE 1 inhibitor **1** (Fig. 1). A database consisted of 1,3-diphenylurea derivatives was built and screened by Hypo 1. Based on Fitvalues, 11 1,3-diphenylurea derivatives were picked out, synthesized and subsequently evaluated for their BACE 1 inhibitory activity, as well as the ability to chelate metal ions. Docking

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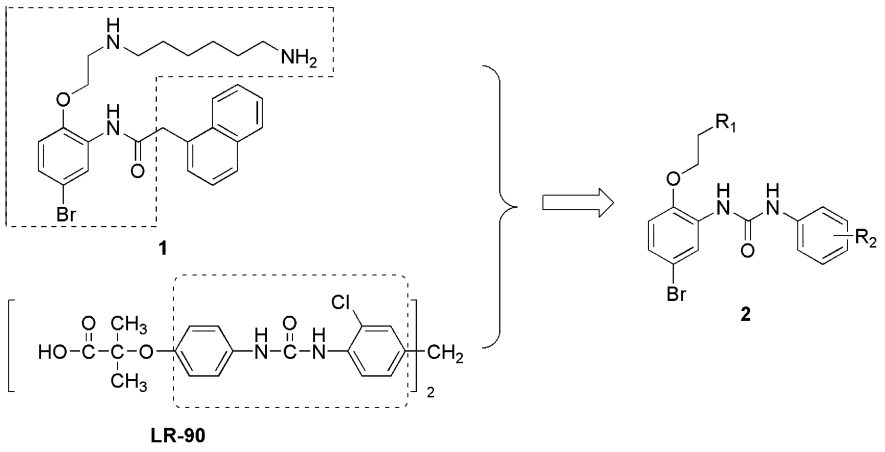


Figure 1. Structure of parent compound (**1**), metal chelator LR-90 and 1,3-diphenylurea derivatives (**2**).

analysis of **9f** with corresponding receptor protein was carried out to study its binding pattern.

2. Results and discussion

2.1. Build designed database and virtual screening

The designed database consisted of 1,3-diphenylurea derivatives was built using the Build 3D database protocol within the Pipeline Pilot software. Enumerate stereoisomers protocol was used to expend the database, which contains 206,259 compounds. Followed Lipinski filtration (*AlogP* was changed as $\geq$3, with the remaining parameters were set as default) led to a reduced set of 99,494 derivatives. All compounds were then optimized by the Discovery Studio 2.1 software package.

The pharmacophore model of BACE 1 inhibitors (Hypo 1) was built in previous work.[15] Discovery Studio 2.1/Ligand Pharmacophore Mapping protocol was used to screen the designed database using Hypo 1 as the template. Compounds **6a–d**, **9a–g** with high Fitvalues (3.25–4.25, the maximum of the Fitvalue is 5.00) were picked out and synthesized.

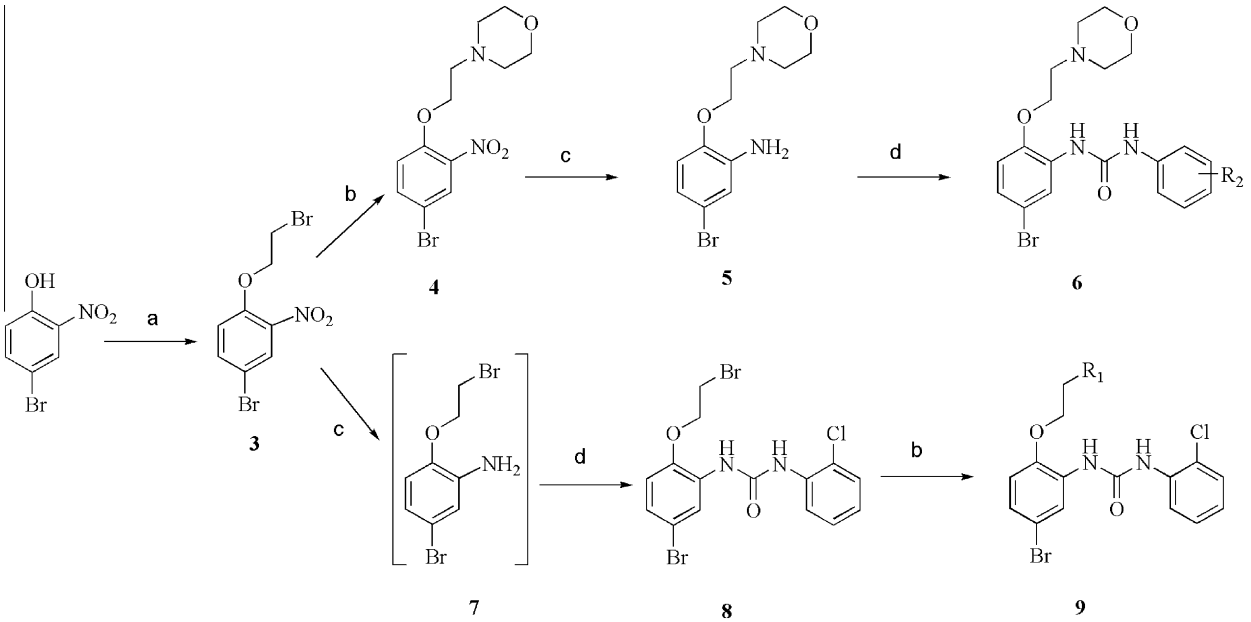
2.2. Chemistry

The synthetic route for designed 1,3-diphenylurea derivatives **6a–d** and **9a–g** are illustrated in Scheme 1. Reaction of 4-bromo-2-nitrophenol with 1,2-dibromoethane afforded bromide **3**, which was reacted with morpholine in refluxing acetonitrile to yield compound **4**. Reduction of **4** with stannous chloride afforded **5** according to the known methods.[15] Target compounds **6a–d** were obtained in good yields by the reaction compound **5** with substituted isocyanatobenzenes.

Bromide **3** was reduced with stannous chloride, and then reacted with substituted isocyanatobenzenes to yield **8a–g** in good yields. Followed reaction of **8a–g** with various amines obtained target compounds **9a–g**.

2.3. BACE 1 inhibitory activity

All synthesized compounds were evaluated for their BACE 1 inhibitory activities using a fluorescence resonance energy transfer (FRET) assay, with potent peptidomimetic inhibitor OM99-2 as the positive control. Compounds with BACE 1 inhibitory rates at



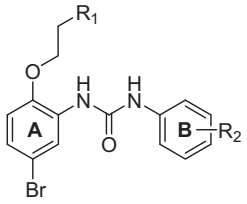
Scheme 1. Reagents and conditions: (a) $BrCH_2CH_2Br$, 40%NaOH, reflux; (b) morpholine, CH_2Cl_2, rt; (c) $SnCl_2{\cdot}2H_2O$, EtOH/THF, rt; (d) isocyanatobenzene, CH_2Cl_2, reflux.

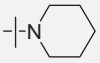
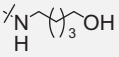
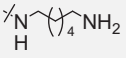
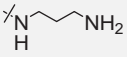
20 μg/mL not less than 50% were tested for IC_{50} values. The results are summarized in Table 1.

As shown in Table 1, compounds **9a**–**g** showed more potent activity than compounds **6a**–**d**. Compounds **9c** and **9f** displayed equivalent or more potent BACE 1 inhibitory activities in comparison with compound **1**. Compound **9f**, R_1 is 1,6-diaminohexane, exhibited the most potent BACE 1 inhibitory activity with an IC_{50} value of 27.85 ± 2.46 μmol/L.

In series **6**, compound **6d** with a chlorine atom at the 2-position of ring B, showed potent activity with an inhibitory rate of 36.8%. Replacement the 2-chlorine atom of ring B with either a 3-nitro group or a 4-methoxy group (**6b**, **6c**) decreased activity.

Table 1
The BACE 1 activity of 1,3-diphenylurea derivatives



Compd	R_1	R_2	Inhibition at 20 μg/mL[a](%)	IC_{50}[d] (μmol/L)	Fitvalue
OM99-2			102.1 ± 2.9[b]	0.25 ± 0.03	
1			83.7 ± 1.3	—	3.96
6a	Morpholine	H	24.5 ± 1.6	—	3.92
6b	Morpholine	*m*-NO_2	20.3 ± 9.7	—	3.95
6c	Morpholine	*p*-OCH_3	20.9 ± 1.4	—	3.81
6d	Morpholine	*o*-Cl	36.8 ± 10.8	—	4.20
9a		*o*-Cl	61.9 ± 3.3	>44.25	3.99
9b		*o*-Cl	87.7 ± 0.5	>42.82	3.93
9c		*o*-Cl	79.4 ± 4.0	29.40 ± 6.92	3.53
9d		*o*-Cl	67.5 ± 4.1	>42.46	4.13
9e		*o*-Cl	77.1 ± 2.7	37.63 ± 8.92	3.25
9f		*o*-Cl	NA[c]	27.85 ± 2.46	4.25
9g		*o*-Cl	77.9 ± 0.5	>45.25	4.12

[a] Data are means ± standard deviation of three independent experiments.
[b] OM99-2 was tested at 2 μg/mL.
[c] There is an interference of compound with fluorescence resonance energy transfer (FRET) assay.
[d] IC_{50} value only determined for compounds attaining over 50% BACE 1 inhibition at 20 μg/mL.

On the other hand, the BACE 1 inhibitory activities of tested compounds were highly dependent on the structures of 2-aminoethoxy side chains on ring A. Compounds, whose R_1 were secondary amines, exhibited more potent inhibition (e.g., **9b–g**). The result implied that introduction of 2-tertiary amines side chains to ring A failed to enhance BACE 1 inhibitory activity. Comparing the IC_{50} values of compounds **9b** and **9e** revealed that a small cyclic amine R_1 (**9b**) was beneficial for BACE 1 inhibitory potency, while compound with a long chain amine on ring A (**9f**) showed more effective BACE 1 activity than compound with a small chain amine (**9g**).

2.4. Metal chelating effect

All compounds were tested for their metal chelating effect using difference UV–vis spectra recorded in methanol at 298 K with wavelength ranging from 210 to 400 nm. Numerical subtraction of the spectra of the copper/iron ion alone and the compound alone from the spectra of the mixture obtained the difference UV–vis spectra due to complex formation.[5] Results showed that all 1,3-diphenylurea derivatives were potent metal chelators. The UV–vis spectra (Fig. 2a) and the difference UV–vis spectra between **9f** and copper ion (Fig. 2b) were shown as an example. In difference UV–vis spectra, the absorption of **9f** (25 μmol/L) decreased along with the increased concentration of copper ion from 1.25 to 50 μmol/L. The result using iron ion was similar with that of copper ion. These observations indicated that there was an interaction between **9f** and copper/iron ion. On the other hand, compound **1** produced no significant spectral change when mixed with up to 20-fold $FeSO_4$ or $CuCl_2$ (Fig. 2c), indicating that the observed metal ion binding was attributed to specific interactions between metal ions and the urea moiety of 1,3-diphenylurea derivatives.

In order to investigate the ratio of ligand/metal ion in the complex, a fixed amount of compound **9f** (25 μmol/L) was mixed with growing amounts of copper ion (2.5–50 μmol/L) and tested the difference UV–vis spectra. It was observed that the maximum intensity of difference spectra reached at 2:1 ratio, demonstrating that the ratio of ligand/metal ion (Cu^{2+}) in the complex was 2:1.

2.5. Molecular docking study

In an attempt to understand the molecular interaction between **9f** and BACE 1, a molecular docking study was performed using the Discovery Studio 2.1/CDOCKER protocol. The crystal structure of OM99-2/BACE 1 complex (PDB ID: 2ZHR) was used as the template. Docking and subsequent scoring studies were performed using default parameters. The result disclosed that compound **9f** made several important interactions along the active-site of BACE 1 with different mode in comparison with compound **1**.[15] As shown in Figure 3a, the bromo substituted benzene ring of **9f** made hydrophobic contacts with the S_1 pocket, and formed classical π–π stacking with the benzene ring of Tyr71. Hydrogen bonding interactions were observed between urea

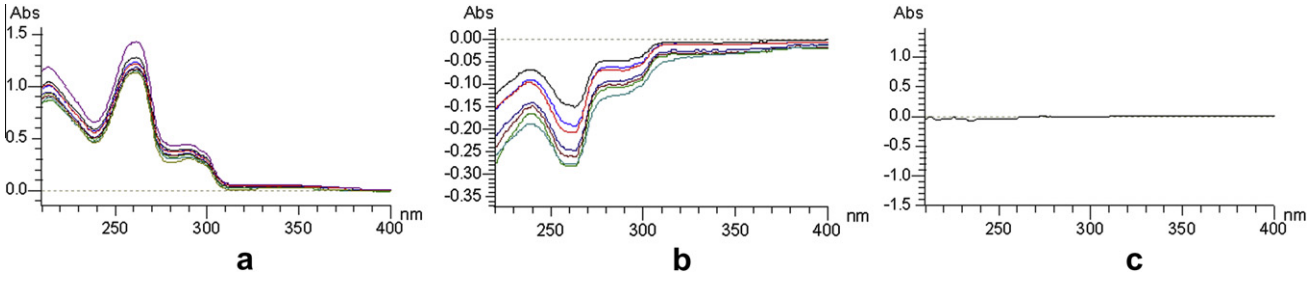


Figure 2. (a) The UV–vis spectra of **9f**. (The purple curve is the UV–vis spectra of compound alone. Curves with other colors are the UV–vis spectra of compound mixed with different concentrations of copper ion.); (b) The difference UV–vis spectra between **9f** and copper ion. (Curves with other colors are the difference UV–vis spectra between compound **9f** mixed with different concentrations of copper ion.); (c) The difference UV–vis spectra between compound **1** and copper ion.

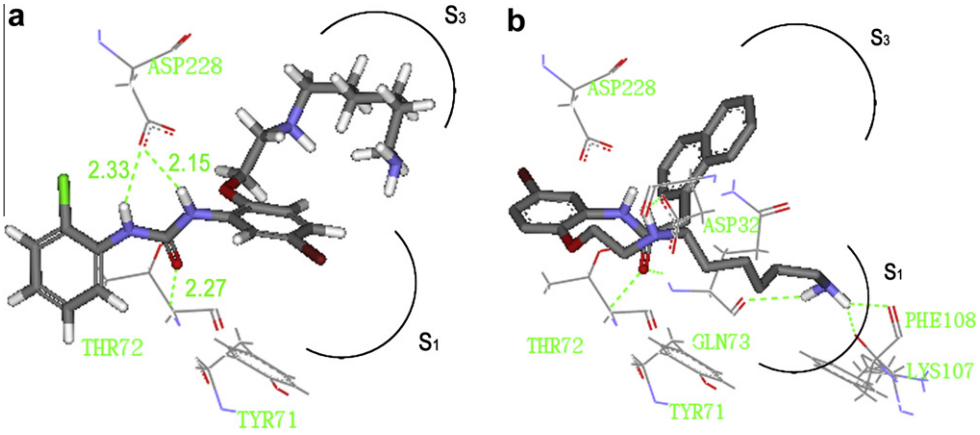


Figure 3. (a) The binding pattern of **9f** into the BACE 1; (b) The binding pattern of compound **1** into the BACE 1.

group and Thr72 with a distance of 2.27 Å. The urea group formed two hydrogen bonds with catalytic aspartate Asp228, with distances of 2.33 and 2.15 Å, respectively. Besides, hydrophobic interactions with the S_3 pocket and hexanamine group further increased the affinity of **9f**. The different binding modes of **9f** and **1** may explain the fact that compound **9f** showed more potent activity than compound **1**.

3. Conclusions

In this work, the strategy of hybridizing metal chelator LR-90 with BACE 1 inhibitor **1** was utilized to design 1,3-diphenylurea derivatives as Dual-Target-Directed ligands. The designed database of 1,3-diphenylurea derivatives was built and screened by a previous built and validated BACE 1 inhibitor pharmacophore model. Eleven compounds (**6a–d**, **9a–g**) with high Fitvalues were selected, synthesized and evaluated for their biological activities, which revealed that the theoretical results were in good agreement with the experimental values. Compound **9f** (27.85 ± 2.46 μmol/L) was tested as the most effective BACE 1 inhibitor. Docking study of **9f** indicated that the urea group made central hydrogen bonds with the catalytic aspartate Asp228. All synthesized compounds also showed the ability to chelate metal ions. The MTDL design strategy used in this study may thus shed instructive light on the treatment of AD in the near future.

4. Experimental

4.1. General

All solvents used were of analytical grade. Melting points were recorded on a Buchi apparatus and uncorrected, IR spectra were recorded on a Bruker VECTOR 22 FTIR spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker Avance III 500 M instrument (chemical shifts are expressed as δ values relative to TMS as internal standard). Mass spectra (MS) were recorded on an Esquire-LC-00075 spectrometer. The designed database of 1,3-diphenylurea derivatives was built by Pipeline Pilot software. The pharmacophore model (Hypo 1) of BACE 1 inhibitors was built in previous work.[15] Molecular model studies were performed using the Discovery Studio 2.1.

4.2. Chemistry

Compound **5** was synthesized using 4-bromo-2-nitrophenol as the starting material according to the known methods.[15]

4.2.1. General procedure for the preparation of compounds 6a–d

To a solution of **5** (0.22 mmol) in CH_2Cl_2 (5 mL), isocyanatobenzene (0.2 mmol) was added, and the mixture was refluxed for 1 h. After removal of the solvent, the residue was purified by silica gel column chromatography (PE/EtOAc/TEA = 1:1:0.1) to afford **6**.

4.2.1.1. 1-(5-Bromo-2-(2-morpholinoethoxy)phenyl)-3-phenylurea 6a. White solid (79%), mp 170–173 °C. IR (KBr): 2810, 1602, 1496, 1384 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.51–2.53 (t, 4H, J = 4.4 Hz, morpholine), 2.74–2.77 (t, 2H, J = 5.2 Hz, –CH_2N), 3.74–3.76 (t, 4H, J = 4.4 Hz, morpholine), 4.06–4.09 (t, 2H, J = 5.2 Hz, –Ar-OCH_2), 6.66–6.68 (d, 1H, J = 8.4 Hz, ArH), 7.00–7.03 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.0 Hz, ArH), 7.06–7.09 (t, 1H, J = 7.6 Hz, ArH), 7.29–7.33 (t, 2H, J = 8.0 Hz, ArH), 8.40–8.42 (d, 1H, J = 7.6 Hz, ArH), 7.48 (s, 1H, –CONH), 8.19 (s, 1H, –CONH), 8.47–8.48 (d, 1H, J = 2.0 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 53.2, 55.1, 65.3, 66.6, 112.8, 114.4, 121.5, 122.3, 122.5, 123.1, 124.3, 124.6, 127.7, 129.1, 136.1, 146.2, 152.4; MS (ESI): m/z = 420 [M+H]$^+$.

4.2.1.2. 1-(5-Bromo-2-(2-morpholinoethoxy)phenyl)-3-(3-nitrophenyl)urea 6b. Yellow solid (84%), mp 211–213 °C. IR (KBr): 2807, 1647, 1599, 1346 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.55–2.57 (m, 4H, morpholine), 2.81–2.84 (t, 2H, J = 5.2 Hz, –CH_2N), 3.77–3.80 (t, 4H, J = 4.4 Hz, morpholine), 4.07–4.10 (t, 2H, J = 5.2 Hz, –ArOCH_2), 6.65–6.67 (d, 1H, J = 8.4 Hz, ArH), 7.00–7.03 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz, ArH), 7.37–7.41 (t, 1H, J = 8.4 Hz, ArH), 7.79–7.81 (dd, 1H, J_1 = 8.0 Hz, J_2 = 1.6 Hz, ArH), 7.89–7.91 (dd, 1H, J_1 = 8.0 Hz, J_2 = 1.6 Hz, ArH), 8.24–8.25 (m, 1H, ArH), 8.46–8.47 (d, 1H, J = 3.2 Hz, ArH), 8.68 (s, 1H, –CONH), 8.83 (s, 1H, –CONH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 53.2, 55.7, 65.7, 66.8, 112.6, 114.8, 121.5, 122.5, 123.1, 123.6, 124.3, 129.2, 131.6, 136.1, 145.8, 147.4, 152.8; MS (ESI): m/z = 465 [M+H]$^+$.

4.2.1.3. 1-(5-Bromo-2-(2-morpholinoethoxy)phenyl)-3-(4-methoxyphenyl)urea 6c. White solid (70%), mp 178–182 °C. IR (KBr):2965, 2936, 2804, 1628, 1597, 1351 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.50–2.55 (m, 4H, morpholine), 2.70–2.73 (m, 2H, –CH_2N), 3.74–3.76 (m, 4H, morpholine), 3.81 (s, 3H, –OCH_3), 4.04–4.07 (t, 2H, J = 5.6 Hz, –ArOCH_2), 6.65–6.67 (d, 1H, J = 8.0 Hz, ArH), 6.88–6.90 (d, 2H, J = 8.8 Hz, ArH), 7.01–7.04 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.0 Hz, ArH), 7.08 (s, 1H, –CONH), 7.30–7.32 (d, 2H, J = 8.8 Hz, ArH), 7.88 (s, 1H, –CONH), 8.47–8.48 (d, 1H, J = 2.0 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 53.1, 55.1, 55.4, 65.5, 66.2, 113.1, 115.2, 121.4, 123.0, 123.5, 124.4, 127.6, 129.0, 131.8, 135.8, 146.2, 152.1, 159.1; MS (ESI): m/z = 450 [M+H]$^+$.

4.2.1.4. 1-(5-Bromo-2-(2-morpholinoethoxy)phenyl)-3-(2-chlorophenyl)urea 6d. White solid (76%), mp 94–100 °C. IR (KBr): 2801, 1633, 1599, 1350 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.64–2.66 (t, 4H, J = 4.4 Hz, morpholine), 2.89–2.92 (t, 2H, J = 5.2 Hz, –CH_2N), 3.82–3.84 (t, 4H, J = 4.4 Hz, morpholine), 4.09–4.12 (t, 2H, J = 5.2 Hz, –$ArOCH_2$), 6.66–6.68 (d, 1H, J = 8.0 Hz, ArH), 6.99–7.04 (m, 2H, ArH), 7.18–7.22 (t, 1H, J = 8.0 Hz, ArH), 7.28–7.31 (m, 1H, ArH), 7.58–7.59 (t, 1H, J = 2.0 Hz, –ArH), 8.22 (s, 1H, –CONH), 8.48 (s, 1H, –CONH), 8.50–8.51 (d, 1H, J = 1.2 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 53.2, 55.1, 65.3, 66.1, 112.3, 114.1, 121.5, 122.6, 122.9, 123.2, 124.5, 127.6, 129.1, 130.8, 136.3, 146.1, 153.5; MS (ESI): m/z = 454 $[M+H]^+$.

4.2.1.5. 1-(5-Bromo-2-(2-bromoethoxy)phenyl)-3-(2-chlorophenyl)urea 8. To a solution of **3** (0.975 g, 3 mmol) in ethanol (3 mL) and THF (3 mL), $SnCl_2{\cdot}2H_2O$ (2.03 g, 9 mmol) was added in a portion and then stirred at room temperature for 4 h. The solvent was evaporated under reduced pressure to afford a white solid, to which 15% NaOH (3 mL) was added, stirred at room temperature for 0.5 h. The reaction mixture was extracted with ether and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum to afford compound **7** as a red oil, which was then mixed with 1-chloro-2-isocyanatobenzene (0.51 g, 3.3 mmol) and 10 mL CH_2Cl_2, and refluxed for 1 h. The solvent was removed, and the remained solid was purified by silica gel column chromatography (PE/EtOAc = 2:1) to afford **8** as yellow oil (29%), IR (KBr): 2807, 1647, 1599, 1346 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.66–3.69 (t, 2H, J = 6.4 Hz, –CH_2Br), 4.31–4.34 (t, 2H, J = 6.4 Hz, –CH_2O), 6.74–6.77 (d, 1H, J = 11.2 Hz, ArH), 7.02–7.12 (m, 3H, ArH), 7.27–7.31 (t, 1H, J = 10.0 Hz, ArH), 7.37–7.39 (dd, 1H, J_1 = 10.0 Hz, J_2 = 1.2 Hz, ArH), 7.48 (s, 1H, –CONH), 8.10–8.15 (t, 1H, –ArH), 8.42–8.43 (d, 1H, J = 2.0 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 33.2, 70.1, 115.1, 116.3, 121.3, 121.4, 122.2, 123.0, 124.4, 127.4, 129.3, 130.7, 136.2, 146.2, 153.2; MS (ESI): m/z = 449 $[M+H]^+$.

4.2.2. General procedure for the preparation of compounds 9a–g

To a solution of **8** (0.045 g, 0.1 mmol) in 3 mL anhydrous acetonitrile, substituted amine (0.12 mmol) was added and the mixture was refluxed for 3 h. The mixture was evaporated under reduced pressure to dryness and the residue was purified by silica gel column chromatography (PE/EtOAc/TEA = 3:1:0.1) to give **9**.

4.2.2.1. 1-(5-Bromo-2-(2-(piperidin-1-yl)ethoxy)phenyl)-3-(2-chlorophenyl)urea 9a. White solid (82%), mp 140–142 °C. IR (KBr): 2936, 2800, 1694, 1594, 1538, 1442, 1409 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.51 (m, 2H, piperidine), 1.66–1.72 (m, 4H, piperidine), 2.43–2.61 (m, 4H, piperidine), 2.85–2.88 (t, 2H, J = 5.2 Hz, –CH_2N), 4.05–4.08 (2H, t, J = 5.2 Hz, –$ArOCH_2$), 6.64–6.66 (d, 1H, J = 8.8 Hz, ArH), 6.92–6.97 (m, 1H, ArH), 6.99–7.03 (m, 1H, ArH), 7.22–7.26 (t, 1H, J = 8.8 Hz, ArH), 7.31–7.34 (dd, 1H, J_1 = 8.0 Hz, J_2 = 1.6 Hz, ArH), 8.29–8.31 (dd, 1H, J_1 = 8.0 Hz, J_2 = 1.6 Hz, –ArH), 8.44 (s, 1H, –CONH), 8.55–8.56 (d, 1H, J = 2.0 Hz, ArH), 9.32 (s, 1H, –CONH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.9, 25.1, 53.7, 57.0, 63.8, 112.2, 114.2, 121.5, 121.9, 122.5, 123.1, 124.3, 127.5, 129.1, 130.8, 136.0, 146.1, 152.6; MS (ESI): m/z = 452 $[M+H]^+$.

4.2.2.2. 1-(5-Bromo-2-(2-(cyclohexylamino)ethoxy)phenyl)-3-(2-chlorophenyl)urea 9b. White solid (62%), mp 108–109 °C. IR (KBr): 3306, 3265, 2927, 2851, 1691, 1593, 1541, 1444, 1408 cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 0.96–1.23 (m, 6H, –$NCHCH_2CH_2CH_2CH_2CH_2$), 1.51–1.54 (m, 1H, –NCH), 1.62–1.83 (m, 4H, –$NCHCH_2CH_2CH_2CH_2CH_2$), 2.39–2.44 (m, 1H, –NH), 2.92–2.95 (t, 2H, J = 5.6 Hz, –CH_2NH), 4.11–4.14 (2H, t, J = 5.6 Hz, –$ArOCH_2$), 7.01–7.13 (m, 3H, ArH), 7.28–7.32 (t, 1H, J = 8.4 Hz, ArH), 7.45–7.47 (d, 1H, J = 8.0 Hz, ArH), 8.00–8.02 (d, 1H, J = 8.0 Hz, ArH), 8.27–8.28 (d, 1H, J = 2.4 Hz, ArH), 8.95 (s, 1H, –CONH), 9.02 (s, 1H, –CONH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.1, 27.8, 35.1, 55.4, 58.1, 64.3, 112.1, 114.2, 121.3, 121.5, 122.3, 123.1, 124.2, 127.6, 129.2, 130.5, 135.9, 146.2, 153.2; MS (ESI): m/z = 466 $[M+H]^+$.

4.2.2.3. 1-(5-Bromo-2-(2-(hexylamino)ethoxy)phenyl)-3-(2-chlorophenyl)urea 9c. Red oil (45%), IR (KBr): 3304, 3261, 2955, 2851, 1692, 1594, 1542, 1441, 1403 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.22–1.35 (m, 9H, –$NHCH_2CH_2CH_2CH_2CH_2CH_3$), 1.80–1.82 (m, 2H, –$NHCH_2CH_2CH_2$–), 2.93–2.97 (t, 1H, J = 10.0 Hz, –$NHCH_2CH_2CH_2$–), 3.35–3.37 (t, 2H, J = 5.2 Hz, –$NHCH_2CH_2O$), 4.28–4.30 (t, 2H, J = 5.2 Hz, –$NHCH_2CH_2O$), 6.59–6.61 (d, 1H, J = 11.0 Hz, ArH), 6.96–7.02 (m, 2H, ArH), 7.21–7.25 (t, 1H, J = 10.0 Hz, ArH), 7.31–7.33 (d, 2H, J = 10.0 Hz, ArH), 8.04–8.06 (d, 1H, J = 10.0 Hz, ArH), 8.47 (d, 1H, J = 2.4 Hz, ArH), 8.93 (s, 1H, –NHCO), 9.25 (s, 1H, –NHCO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 21.3, 25.9, 29.9, 30.6, 52.3, 53.5, 65.2, 111.2, 114.2, 121.5, 121.6, 122.1, 122.9, 124.4, 127.4, 129.1, 130.4, 135.9, 146.1, 153.1; MS (ESI): m/z = 468 $[M+H]^+$.

4.2.2.4. 1-(5-Bromo-2-(2-(5-hydroxypentylamino)ethoxy)phenyl)-3-(2-chlorophenyl)urea 9d. Yellow oil (49%), IR (KBr): 3304, 3261, 2934, 2859, 1701, 1646, 1593, 1477, 1410 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.52–1.69 (m, 6H, –$NHCH_2CH_2CH_2CH_2$), 2.82–2.85 (m, 2H, –$NHCH_2$ CH_2CH_2), 3.15–3.18 (t, 2H, J = 6.0 Hz, –OCH_2CH_2NH), 3.53 (m, 2H, –OH), 3.63–3.65 (m, 1H, –CH_2NHCH_2), 3.66–3.69 (m, 2H, –CH_2OH), 4.10–4.12 (t, 2H, J = 6.0 Hz, –$ArOCH_2$), 6.65–6.67 (d, 1H, J = 10.0 Hz, ArH), 6.94–6.98 (t, 1H, J = 9.2 Hz, ArH), 7.01–7.04 (dd, 1H, J_1 = 10.0 Hz, J_1 = 3.2 Hz, ArH), 7.22–7.24 (d, 1H, J = 10.0 Hz, ArH), 7.31–7.33 (d, 1H, J = 10.0 Hz, ArH), 8.20–8.22 (d, 1H, J = 9.2 Hz, ArH), 8.41 (s, 1H, –NHCO), 8.51–8.52 (d, 1H, J = 3.2 Hz, ArH), 9.21 (s, 1H, –NHCO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.4, 28.9, 29.1, 51.3, 53.6, 65.2, 65.8, 110.9, 113.9, 121.3, 121.8, 122.3, 123.0, 124.7, 128.0, 129.3, 131.0, 136.2, 145.9, 152.8; MS (ESI): m/z = 470 $[M+H]^+$.

4.2.2.5. 1-(5-Bromo-2-(2-(cyclopropylamino)ethoxy)phenyl)-3-(2-chlorophenyl)urea 9e. Yellow oil (71%). IR (KBr): 3442, 3319, 2926, 2856, 1701, 1653, 1529, 1475, 1446, 1409 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.54–0.57 (m, 2H, –$NHCHCH_2CH_2$), 0.82–0.87 (m, 2H, –$NHCHCH_2CH_2$), 2.23–2.24 (m, 1H, –$NHCH$), 3.14–3.16 (t, 2H, J = 6.0 Hz, –$NHCH_2$), 4.13–4.16 (t, 2H, J = 6.0 Hz, –$ArOCH_2$), 6.73–6.75 (d, 1H, J = 10.0 Hz, ArH), 6.97–7.00 (t, 1H, J = 9.2 Hz, ArH), 7.28–7.30 (t, 1H, J = 9.2 Hz, ArH), 7.32–7.35 (d, 2H, J = 9.2 Hz, ArH), 7.79 (s, 1H, –NHCO), 8.18–8.20 (d, 1H, J = 10.0 Hz, ArH), 8.47 (d, 1H, J = 2.4 Hz, ArH), 8.67 (s, 1H, –NHCO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.4, 29.1, 52.9, 66.1, 111.2, 114.2, 121.4, 121.9, 123.1, 123.3, 124.8, 128.7, 129.2, 131.1, 137.1, 146.1, 153.0; MS (ESI): m/z = 424 $[M+H]^+$.

4.2.2.6. 1-(2-(2-(6-Aminohexylamino)ethoxy)-5-bromophenyl)-3-(2-chlorophenyl)urea 9f. Red oil (52%). IR (KBr): 3318, 2938, 1697, 1591, 1531, 1476, 1409 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.31–1.32 (m, 4H, –$NH_2CH_2CH_2CH_2CH_2$), 1.41–1.45 (m, 4H, –$NH_2CH_2CH_2$ $CH_2CH_2CH_2$), 2.76–2.79 (m, 3H, –$NHCH_2$, –NH_2), 3.13–3.15 (t, 2H, J = 5.6 Hz, –CH_2NH), 3.18–3.29 (m, 4H, –$NHCH_2$, NH_2CH_2–), 4.13–4.15 (t, 2H, J = 5.6 Hz, –$ArOCH_2$), 6.67–6.69 (d, 1H, J = 11.2 Hz, ArH), 6.92–6.96 (t, 1H, J = 10.0 Hz, ArH), 6.99–7.02 (dd, 1H, J_1 = 11.2 Hz, J_1 = 3.0 Hz, ArH), 7.19–7.24 (t, 1H, J = 10.0 Hz, ArH), 7.29–7.31 (d, 1H, J = 10.0 Hz, ArH), 8.16–8.18 (d, 1H, J = 10.0 Hz, ArH), 8.48–8.49 (d, 1H, J = 3.2 Hz, ArH), 8.52 (s, 1H, –NHCO), 9.23 (s, 1H, –NHCO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.7, 23.9, 33.1, 33.4, 47.1, 54.3, 55.6, 65.3, 66.6, 111.8, 115.0, 121.6, 121.9, 123.3, 123.8, 124.9, 128.8, 130.1, 131.4, 137.6, 145.8, 153.4; MS (ESI): m/z = 483 $[M+H]^+$.

4.2.2.7. 1-(2-(2-(3-Aminopropylamino)ethoxy)-5-bromophenyl) -3-(2-chlorophenyl)urea 9g. Red oil (44%). IR (KBr): 3323, 2938, 1698, 1592, 1534, 1479, 1411 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.72–1.75 (m, 2H, $-NHCH_2CH_2CH_2NH_2$), 2.81–2.89 (m, 4H, $-NHCH_2CH_2CH_2NH_2$), 3.09–3.11 (t, 2H, J = 5.6 Hz, $-OCH_2CH_2NH$), 4.07–4.09 (t, 2H, J = 5.6 Hz, $-ArOCH_2$), 6.68–6.70 (d, 1H, J = 10.4 Hz, ArH), 6.95–6.99 (t, 1H, J = 8.4 Hz, ArH), 7.03–7.06 (dd, 1H, J_1 = 10.4 Hz, J_1 = 3.0 Hz, ArH), 7.25–7.26 (d, 1H, J = 8.4 Hz, ArH), 7.33–7.35 (d, 1H, J = 8.4 Hz, ArH), 8.28–8.30 (d, 1H, J = 10.4 Hz, ArH), 8.48 (s, 1H, –NHCO), 8.56–8.57 (d, 1H, J = 3.2 Hz, ArH), 9.03 (s, 1H, –NHCO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 33.1, 41.1, 47.1, 50.3, 67.8, 112.5, 115.9, 120.6, 121.4, 123.7, 124.8, 125.0, 128.6, 130.6, 131.3, 136.9, 146.9, 152.3; MS (ESI): m/z = 441 $[M+H]^+$.

4.3. In vitro BACE 1 inhibit activity screening

All compounds were tested as BACE 1 inhibitors, using a fluorescence resonance energy transfer (FRET) assay, which used purified insect-expressed BACE 1 and a specific substrate. An excitation wavelength of 355 nm and an emission wavelength of 460 nm were used to monitor the hydrolysis of substrate. Compounds with inhibitory rates at 20 μg/mL upon 50% were tested for IC_{50} values.

4.4. Spectrophotometric measurement of complex with Cu and Fe[5]

All compounds were tested as metal chelators, using difference UV–vis spectra recorded in methanol at 298 K with wavelength ranging from 210 to 400 nm. Numerical subtraction of the spectra of the metal alone and the compound alone from the spectra of the mixture obtained the difference UV–vis spectra due to complex formation. A fixed amount of **9f** (25 μmol/L) was mixed with growing amounts of copper ion (1.25–50 μmol/L) and tested the difference UV–vis spectra to investigate the ratio of ligand/metal in the complex.

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