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Identification of pharmacophore model, synthesis and biological evaluation of *N*-phenyl-1-arylamide and *N*-phenylbenzenesulfonamide derivatives as BACE 1 inhibitors

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ABSTRACT

The pharmacophore model of arylpiperazine amide derivatives was built using Discovery Studio 2.0 software package and the best pharmacophore model (Hypo 1) 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). According to the best pharmacophore model, 11 *N*-phenyl-1-arylamide, *N*-phenylbenzenesulfonamide derivatives, compounds **26–28**, and **33a–g**, were designed to be synthesized and their BACE 1 inhibitory activities were determined experimentally. Their theoretical results were in good agreement with the experimental values. Compound **33d**, which displayed the highest BACE 1 activity (18.33 ± 2.80 μmol/L) among these two series, was chosen to study the protein binding pattern and the result showed that it was in close contact with two essential catalytic aspartates (Asp32 and Asp228) of the BACE 1.

1. Introduction

Alzheimer's disease (AD) is one kind of the most common dementia. It has been characterized by the progressive formation of insoluble amyloid plaques (Aβ) and fibrillary tangles.[1,2] Plaquesare extracellular aggregations of a peptide fragment Aβ42,[3,4] which is formed by the sequential proteolytic processing of β-amyloid precursor protein (APP) by two enzymes, β- and γ-secretase.[5–7] Therefore, the inhibition of the two enzymes is likely to reduce the production of Aβ and thereby delay or halt the progression of AD. The idea, which γ-secretase inhibitors could affect the Notch signal path related to the growth of neural cells, is supported by experimental results, such as the fact that γ-secretase gene knockout mice can not survive.[8] Taking into account of these observations, developing β-secretase inhibitors appears to be more encouraging.[9,10]

Due to low oral bioavailability, metabolic unstability, poor ability to penetrate central nervous system (CNS) and susceptibility to P-glycoprotein transport of existing peptidomimetic inhibitors,[11–13] researchers are focus on the identification of the non-peptide inhibitors in recent years. Several series of non-peptide inhibitors, including 1,3,5-trisubstituted aromatic derivatives,[14] acylguanidine derivatives,[15] 2-amino aromatic heterocyclic derivatives[16] and arylpiperazine amide derivatives,[17–19] have been reported. Among them, the arylpiperazine amide scaffold has the benefits of its simple structure and high β-secretase inhibitory activity (IC_{50} values from 0.046 to 20 μmol/L) (Fig. 1**a**). According to the X-ray crystal structure data for piperidine derivative complexed to renin, the protonated piperidine nitrogen was positioned between the two catalytic aspartic acid residues Asp32 and Asp228. Kraus and coworkers incorporated various substituents on the N4-position of the piperazine ring, replacing the naphthyl ring with various heterocyclic moieties. These studies confirmed that the substituent on the N4-position of the piperazine ring is another important feature of the arylpiperazine amide derivatives to binding on BACE 1.

In the present studies, it was to identify the key pharmacophoric features of the arylpiperazine amide derivatives and corresponding pharmacophore models for arylpiperazine amide derivatives using Discovery Studio 2.0 software package. The validation of the best pharmacophore model (Hypo 1) was ascertained by Enrichment and receiver operating curve (ROC) method. The Hypo 1 was used to in silico screen a designed database (Fig. 1**b**). As a result, 11 novel *N*-phenyl-1-arylamide, *N*-phenylbenzenesulfonamide derivatives **26–28**, **33a–g** were selected and synthesized considering effects of different skeletons (*N*-phenyl-1-arylamide, *N*-phenylbenzenesulfonamide) and different substituents on the nitrogen of the phenoxyethylamine. BACE 1 inhibitory activities of these compounds were subsequently evaluated experimentally. The result showed that the bioactivity data was consistent with Fit values and *c*Log*P* values. In an attempt to understand the protein binding pattern of these two series, a molecular docking of compound **33d** has also been studied.

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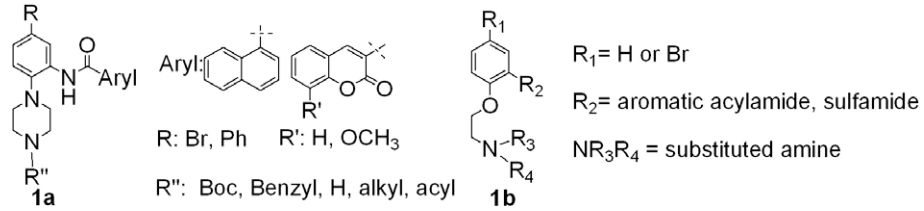


Figure 1. The structures of arylpiperazine amide derivatives **1a** and *N*-phenyl-1-arylamide and *N*-phenylbenzenesulfonamide derivatives **1b**.

2. Materials and methods

2.1. Data preparation

Twenty-one positives for the pharmacophore model study were taken from the literatures (Fig. 2),[17,18] and one thousand two hundred negatives were retrieved from Advanced Chemical Directory (ACD) database using Random Percent Filter protocol by Pipeline Pilot software. The training set was consisted of three positives and the test set was consisted of 18 positives and 1200 negatives. All compounds were optimized by Discovery Studio 2.0 software package.

2.2. Common feature-based pharmacophore model

We employed the Discovery Studio 2.0/HipHop approach to evaluate the common features required for binding. A training set consisting of three arylpiperazine amide derivatives was submitted for pharmacophore model generation based on common chemical features. All the parameters were kept at the default setting except the Best method for the Conformation Generation and Fitting.

2.3. Model validation

The validation of any pharmacophore model should be ascertained and there are a number of approaches to quantitate models.

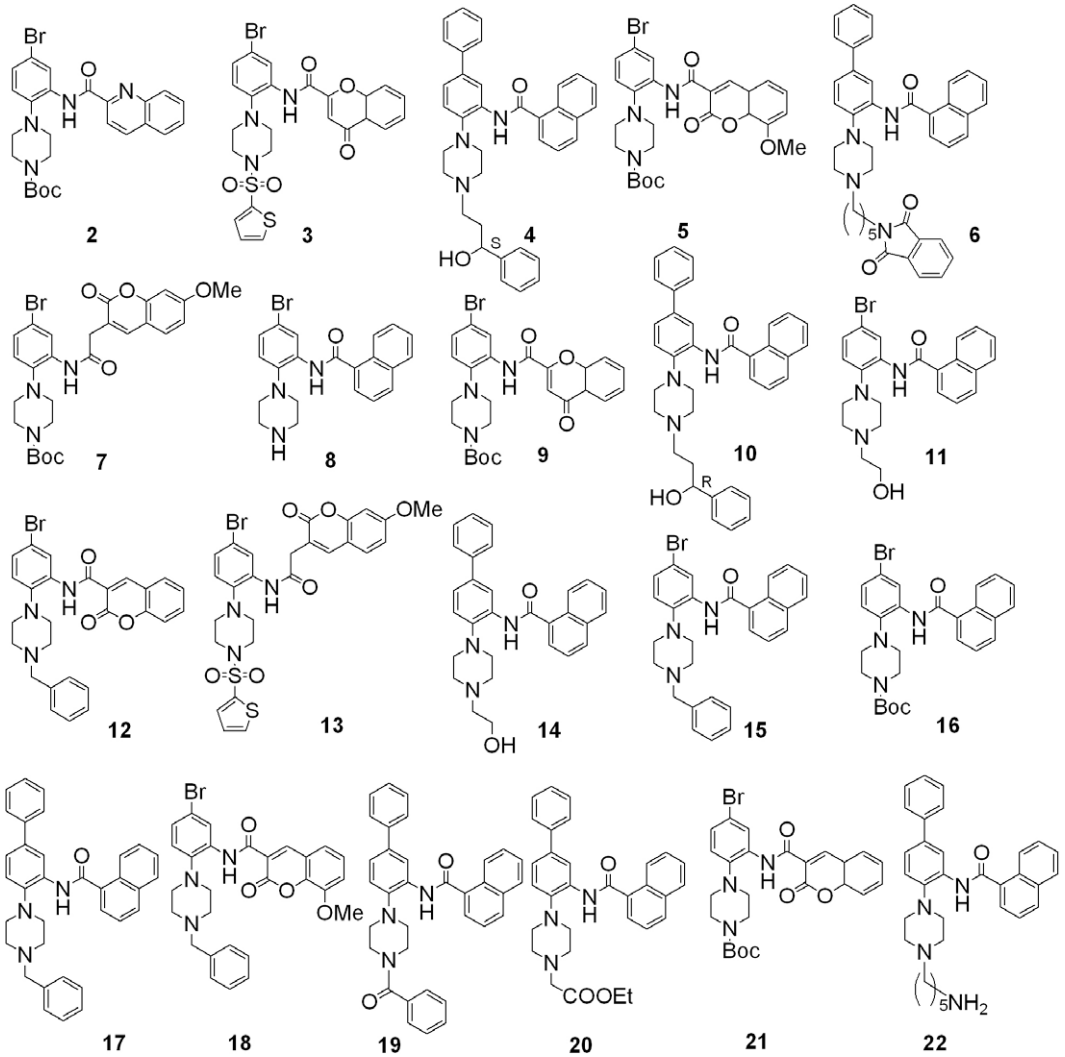


Figure 2. The structure of three positives used in training set (**2**–**4**) and 18 positives used in test set (**5**–**22**) for the pharmacophore model study.

The simplest and most often used method is Enrichment (EF) at a given percentage. EF is defined as:

$$EF = \frac{TP}{TP + FP}\frac{N}{n}$$

where TP is the number of true positives, FP is the number of false positives, N is the number of the total compounds and n is the number of the total positive compounds.[20]

In addition, the receiver operating curve (ROC) metric, allowed quick calculation of sensitivity and specificity from a comparison between in vitro and in silico, had also been applied to evaluate the validation of the model.[21]

(a) Sensitivity (Se): the likelihood that an event will be detected if that event is present. Se is defined as:

$$Se = \frac{TP}{TP + FN}$$

where FN is the number of false negatives.

(b) Specificity (Sp): the likelihood that the absence of an event will be detected. Sp is defined as:

$$Sp = \frac{TN}{TN + FP}$$

where TN is the number of true negatives.

(c) The ROC curve is a function of $(1 - Sp)$ versus the Se, and the area under the ROC curve (AUC) is the important way of measuring the performance of the test.

$$AUC = \sum_{x=2}^{N} Se(x)[(1 - Sp)(x) - (1 - Sp)(x - 1)]$$

Here, $Se(x)$ is the percent of the true positives versus the total positives at rank position x, $(1 - Sp)(x)$ is the percent of the false positives versus the total negatives at rank position x.

3. Results and discussion

3.1. Pharmacophore model and validation

For the HipHop pharmacophore analysis, three highly active arylpiperazine amide derivatives were chosen as a training set to generate the pharmacophore model based on common chemical features. Ten pharmacophore models were obtained and the best pharmacophore model (Hypo 1) with the highest score 38.78 contained five chemical features: one pos-ionizable (PI), two ring aromatic (RA1 and RA2), one hydrophobic (HP), and one hydrogen bond acceptor (HBA) (Fig. 3, 3a).

Every model should be ascertained, and the most often used and simplest is enrichment (EF) at a given percentage of the database. The EF of test set consisting of one thousand two hundred and eighteen compounds screened by Hypo 1 at 2%, 5% and 10% are 30.6, 12.2 and 7.7, respectively. In addition, the ROC curve of the test set screening by Hypo 1 shows in Figure 4 and AUC is 0.93 which means that a selected randomly active compound has a higher score than a randomly selected inactive compound 9.3 times out of 10.

The results of the EF and ROC metric suggested that the Hypo 1 would be valuable and reliable in identifying the compounds for BACE 1 inhibitory activity.

3.2. Chemistry

Discovery Studio 2.0/Ligand Pharmacophore Mapping protocol was used with Hypo 1 as pharmacophore model to screen the designed database. Compounds **26–28a**, **33a–f** with high Fit values (2.80–4.06, the maximum of the Fit value is 5.00) were picked out and synthesized. In order to investigate the predictive accuracy of the Hypo 1 for inactive compounds, **28b** and **33g** with low Fit values (1.70 and 1.89) were also prepared.

N-Phenyl-1-arylamide and *N*-phenylbenzenesulfonamide derivatives **26–28** were prepared according to the procedure depicted in Scheme 1. Reaction of 4-bromo-2-nitrophenol with 1,2-dibromoethane afforded compound **23**, which was refluxed with morpholine in acetonitrile to yield **24**, followed by reduction of nitro group with stannous chloride to get **25**, a key intermediate which was subsequently reacted with substituted aryl chloride to yield target compounds **26–28** in good yields.

Compounds **33a–g** were synthesized according to the procedure depicted in Scheme 2. The hydroxyl of 4-bromo-2-nitrophenol was protected with methoxy methyl (MOM) group to get compound **29**, which was reduced to **30a** with stannous chloride, while catalytic hydrogenation with Pd/C led to the loss of the bromine atom to get **30b**. The aniline derivatives **30a,b** were subsequently acylated with 2-naphthyl acetyl chloride, followed by treatment with diluted hydrochloric acid to afford intermediates **31a–b**. The resulting compounds were reacted with 1,2-dibromoethane to get compounds **32a–b**, which were subsequently reacted with various amine derivatives to obtain target compounds **33a–g**.

3.3. BACE 1 activity study

Eleven compounds were evaluated for their BACE 1 activities using a fluorescence resonance energy transfer (FRET) assay, with potent peptidomimetic inhibitor OM99-2 as positive control. As shown in Table 1, at 20 μg/mL, **33d** and **33e** were the most active compounds, and their IC_{50} values were 18.33 ± 2.80 μmol/L and 47.09 ± 3.37 μmol/L, respectively. Unfortunately, the activity of

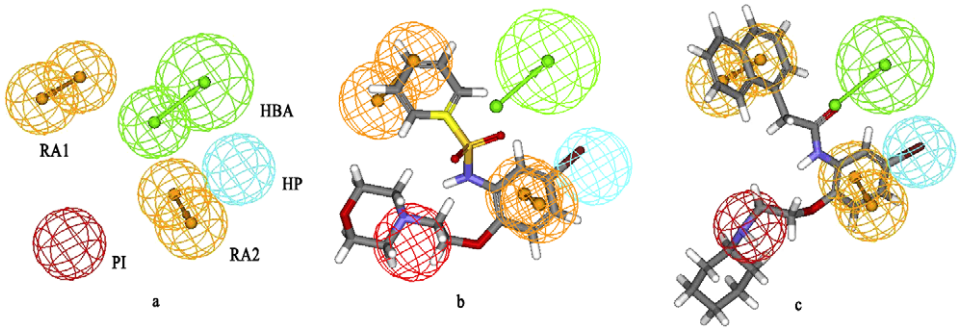


Figure 3. (a) The best pharmacophore model (Hypo 1); (b) Hypo 1 aligned to compound **28b**; (c) Hypo 1 aligned to compound **33e**.

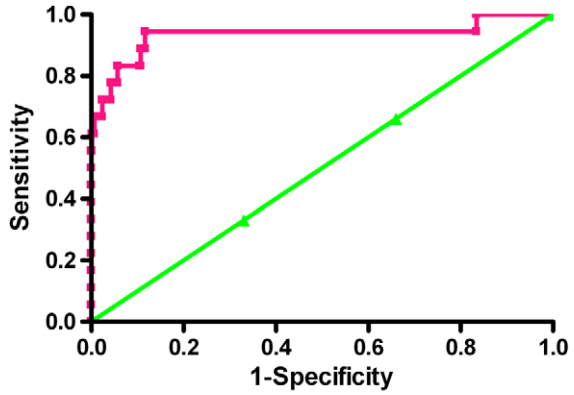


Figure 4. The red curve is the ROC curve of the test set; The green curve is the ROC curve of the random classification of the compounds.

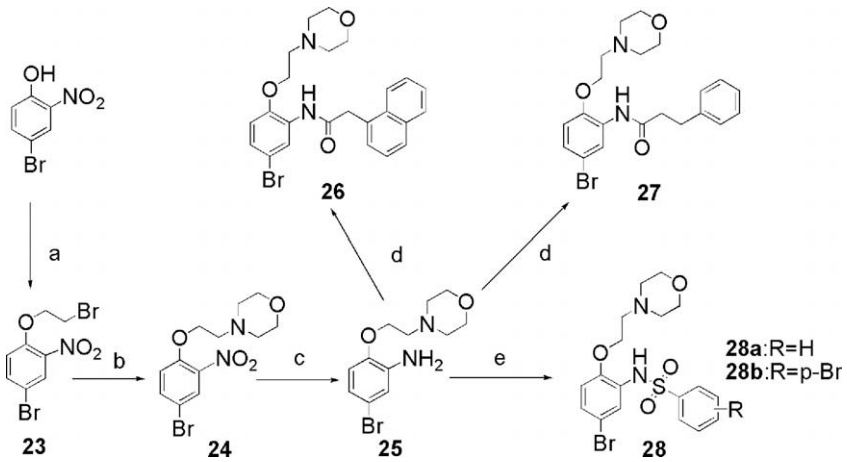
the synthesized compounds did not increase compared to the lead compound. However, we can still find some SAR of the compounds.

A correlation between the Fit values comparing the best pharmacophore model (Hypo 1) and the inhibitory activities has been observed. Nine compounds **26–28b, 33a–f** (such as compound **33e** in Fig. 3, **3c**), which were predicted with high Fit values by the Hypo 1, showed medium to good activities, while **7b** and **12g** with low Fit values (such as compound **28b** in Fig. 3, **3b**) showed weak BACE 1 inhibitory activities.

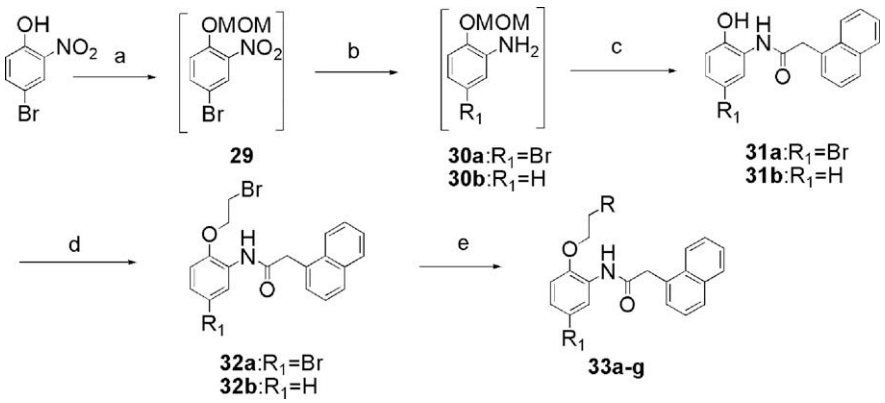
Insight into the observed effects of different skeletons (*N*-phenyl-1-arylamide, *N*-phenylbenzenesulfonamide) and different substituents on the nitrogen of the phenoxyethylamine revealed that compounds with 1-naphthalenyl acetyl group as aromatic ring moiety (**26**, *c*Log*P* = 4.53), hydrophobic group and secondary amine as substitutes on the nitrogen of the phenoxyethylamine (**33d**, *c*Log*P* = 4.68 and **33e**, *c*Log*P* = 5.95) demonstrated more potent inhibitory activity against BACE 1. Compared with the structure and the activity of the compounds **33a** and **33g**, it was worth noting that the *c*Log*P* value of the more potent compound **33a** (*c*Log*P* = 5.22) was greater than compound **33g** (*c*Log*P* = 4.19) which only differed in structure by the presence of a bromine atom. These bioactivity data were also consistent with *c*Log*P* values.

3.4. Molecular docking study

In an attempt to understand the molecular interaction between **33d** and BACE 1, a molecular docking study was performed using the AUTODOCK 3.0 with the crystal structure of OM99-2/BACE 1 complex (PDB ID: 1W51) as model. The docking and subsequent scoring were performed using default parameters. The result disclosed that the binding pattern of **33d** into the BACE 1 was similar to the crystal structure of Vertex in the patent application WO02088101 (Fig. 5).[19] Hydrogen bonding interactions were observed between acylamide groups and Thr72, Gln73, Thr231. Hydrophobic interactions between S1 pocket which lined with aromatic side chain (Try71) and naphthyl group provided a component of the affinity of **33d**. The nitrogen of the phenoxyethylamine formed hydrogen bonds with catalytic aspartates, Asp32 and Asp228, with distance of 2.73 Å and 2.66 Å,

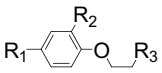


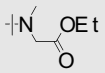
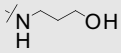
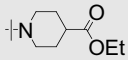
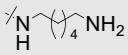
Scheme 1. Syntheses of compounds **23–28**. Reagents and condition: (a) $BrCH_2CH_2Br$, 40%NaOH, reflux; (b) morpholine, CH_2Cl_2, rt; (c) $SnCl_2{\cdot}2H_2O$, EtOH/THF, rt; (d) acyl chloride, CH_2Cl_2, rt; (e) substituted phenyl sulfonyl chloride, CH_2Cl_2, rt.



Scheme 2. Syntheses of compounds **33a–g**. Reagents and condition: (a) MOMCl, K_2CO_3, acetone, rt; (b) $SnCl_2{\cdot}2H_2O$, EtOH/THF, rt; or Pd-C, EtOH, rt; (c) naphthalenyl acetyl chloride, CH_2Cl_2, rt; (d) $BrCH_2CH_2Br$, 40%NaOH, reflux; (e) amine, CH_2Cl_2, rt.

Table 1
The BACE 1 activity of *N*-phenyl-1-arylamide and *N*-phenylbenzenesulfonamide derivatives.



Compound	R_1	R_2	R_3	Inhibition at 20 μg/mL (%)	Fit value	*c*Log*P*
OM99-2				102.09 ± 2.87[a]		
26	Br	1-Naphthalenylacetamide		39.35 ± 6.64	3.94	4.53
27	Br	Phenylpropanamide		13.84 ± 7.30	3.25	3.92
28a	Br	4-Bromobenzenesulfonamide		14.27 ± 5.96	2.80	4.77
28b	Br	Benzenesulfonamide		ND	1.70	3.76
33a	Br	1-Naphthalenylacetamide		24.74 ± 3.15	3.92	5.22
33b	Br	1-Naphthalenylacetamide		15.08 ± 9.02	3.86	3.75
33c	Br	1-Naphthalenylacetamide		34.77 ± 7.93	4.06	5.00
33d	Br	1-Naphthalenylacetamide		83.70 ± 1.30	3.96	4.68
33e	Br	1-Naphthalenylacetamide		64.46 ± 3.47	4.00	5.95
33f	Br	1-Naphthalenylacetamide		41.81 ± 9.91	3.68	6.37
33g	H	1-Naphthalenylacetamide		ND	1.89	4.16

[a] OM99-2 was tested at 2 μg/mL.

respectively, consistent with the known binding mode of arylpiperazine amide derivatives.[18] Moreover, amino group at the end of the alkyl chain had hydrogen bonding interactions with Lys-107, Phe-108 as designed, which may explain the high activity of the compound.

4. Conclusions

In this work, we built pharmacophore model with good predictive ability (EF at 2%, 5% and 10% are 30.6, 12.2 and 7.7; AUC of the ROC curve is 0.93) to predict the activity of designed database consisting of *N*-phenyl-1-arylamide and *N*-phenylbenzenesulfonamide derivatives. Nine compounds (**26–28a**, **33a–f)** with high Fit values comparing Hypo 1 and 2 compounds (**28b, 33g**) with low Fit values were selected and synthesized. The theoretical results of them were in good agreement with the experimental values. Compound **33d** (IC_{50} = 18.33 ± 2.80 μmol/L) was the most effective inhibitor against BACE 1 and as a typical example of refined structure. Docking study of **33d** indicated that substituents on the nitrogen of the phenoxyethylamine could make some additional hydrogen bonds with certain residues. These observations may direct our design in the further.

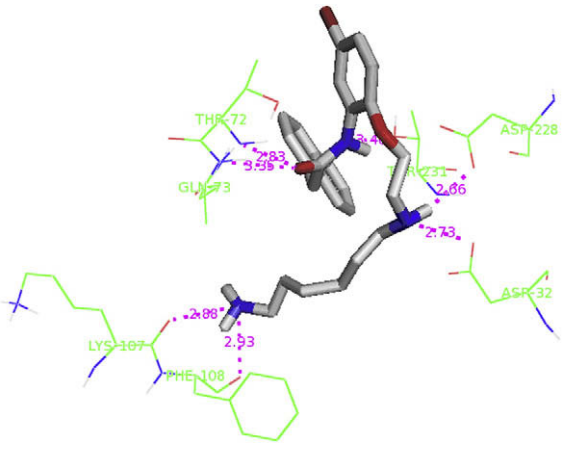


Figure 5. The docking result of inhibitor **33d** in complex with BACE 1.

5. Experimental

5.1. Chemistry

All solvents used were of analytical grade. Melting points were recorded on a Buchi apparatus and are uncorrected, IR spectra were recorded on a Bruker VECTOR 22 FTIR spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker AM 400 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.

5.1.1. 4-Bromo-1-(2-bromoethoxy)-2-nitrobenzene (23)

To a solution of 4-bromo-2-nitrophenol (1.09 g, 5 mmol) in 1,2-dibromoethane (5 mL), 40% NaOH solution (5 mL) and tetrabutyl ammonium bromide (0.05 g) were added, the mixture was refluxed for 4 h, cooled to room temperature, the organic phase

was separated, and the aqueous phase was extracted with CH_2Cl_2 (15 mL). The combined organic extract was washed with brine, dried over anhydrous Na_2SO_4, evaporated under reduced pressure, the crude solid was purified by silica-gel column chromatography (PE/EtOAc 10:1) to get a yellow solid (1.3 g, 80% yield), mp 76–78 ℃. IR (KBr) cm^{-1}: 2932, 1604, 1521. ^1H NMR ($CDCl_3$) δ: 3.65 (2H, t, J = 6.4 Hz, $-CH_2Br$), 4.38 (2H, t, J = 6.4 Hz, $-OCH_2$), 6.98 (1H, d, J = 9.2 Hz, ArH), 7.62 (1H, dd, J_1 = 9.2 Hz, J_2 = 2.4Hz, ArH), 7.97 (1H, d, J = 2.4 Hz, ArH).

5.1.2. 4-[2-(4-Bromo-2-nitrophenoxy)ethyl]morpholine (24)

A solution of **23** (0.975 g, 3 mmol) and morpholine (0.518 mL, 6 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 3 h, the mixture was evaporated under reduced pressure to afford yellow oil, which was then purified by silica-gel column chromatography (PE/EtOAc/TEA 10:1:0.1) to give a yellow oil (0.914 g, 92% yield). IR (KBr) cm^{-1}: 2925, 2863, 1599, 1511. ^1H NMR ($CDCl_3$) δ: 2.57 (t, 4H, J = 4.4 Hz, morpholine), 2.82 (2H, t, J = 5.6 Hz, $-CH_2N$), 3.69 (4H, t, J = 4.4 Hz, morpholine), 4.20 (2H, t, J = 5.6 Hz, $-OCH_2$), 6.97 (1H, d, J = 9.2 Hz, ArH),7.59 (1H, dd, J_1 = 9.2 Hz, J_2 = 3.2 Hz, ArH), 7.95 (2H, d, J = 3.2 Hz, ArH).

5.1.3. 5-Bromo-2-(2-morpholinoethoxy)benzenamine (25)

To a solution of **24** (0.993 g, 3 mmol) in the mixture of ethanol (5 mL) and 5 mL THF (5 mL), $SnCl_2{\cdot}2H_2O$ (3.39 g, 15 mmol) was added in a portion, the mixture was stirred at room temperature for 4 h. Volatiles were removed and remained solid was added the 15% NaOH (5 mL), stirred for 0.5 h in ice bath. The reaction mixture was extracted with ether, separated the organic phase which was then removed to get crude solid. The solid was purified by silica-gel column chromatography (PE/EtOAc/TEA 10:1:0.1) to give a yellow oil (0.452 g, 50% yield), mp 66–67 ℃. IR (KBr) cm^{-1}: 3380, 3311, 3196, 2923, 2877, 1591, 1503, 1458. ^1H NMR ($CDCl_3$) δ: 2.56–2.58 (4H, m, morpholine), 2.77 (2H, t, J = 5.2 Hz, $-CH_2N$), 3.72 (4H, t, J = 4.8 Hz, morpholine), 3.99–4.01 (2H, m, $-NH_2$), 4.08 (2H, t, J = 5.2 Hz, $-OCH_2$), 6.65 (1H, d, J = 8 Hz, ArH), 6.77 (1H, dd, J_1 = 8 Hz, J_2 = 2.4 Hz, ArH), 6.83 (2H, d, J = 2.4 Hz, ArH).

5.1.4. General procedure for the preparation of compounds 26–28

To a solution of **25** (0.25 mmol) in CH_2Cl_2 (5 mL), acyl chloride or the substituted phenyl sulfonyl chloride (0.3 mmol) was added, and the mixture was stirred at room temperature for 1 h. Volatiles were removed and the residue was purified by a silica-gel column chromatography (PE/EtOAc/TEA 3:1:0.1 or 2:1:0.1).

5.1.5. *N*-[5-Bromo-2-(2-morpholinoethoxy)phenyl]-1-naphthalenylacetamide (26)

Compound **26** was obtained (0.094 g, 80% yield) as a white solid, mp 77–79 ℃. IR (KBr) cm^{-1}: 2930, 2851, 1681, 1593, 1525, 788. ^1H NMR ($CDCl_3$) δ: 2.11–2.23 (6H, m, $-CH_2N$, morpholine), 3.60–3.64 (4H, m, morpholine), 3.73–3.76 (2H, m, $-CH_2CO$), 4.21(2H, s, $-CH_2O$), 6.52 (1H, d, J = 7.6Hz, ArH), 7.03–7.05 (1H, m, ArH), 7.51–7.54 (4H, m, ArH), 7.75 (1H, bs, –NHCO), 7.90 (2H, s, ArH), 8.01 (1H, s, ArH), 8.58 (1H, s, ArH). ESI-MS m/z: 469 (M^+).

5.1.6. *N*-[5-Bromo-2-(2-morpholinoethoxy)phenyl]-3-phenylpropanamide (27)

Compound **27** was obtained (0.074 g, 68% yield) as a yellow oil. IR (KBr) cm^{-1}: 2925, 2855, 1687, 1593, 1519, 753, 699. ^1H NMR ($CDCl_3$) δ: 2.51–2.53 (4H, m, morpholine), 2.70–2.74 (4H, m, $-COCH_2CH_2$, $-OCH_2CH_2$), 3.04 (2H, t, J = 8 Hz, $-COCH_2$), 3.69 (4H, t, J = 4.8 Hz, morpholine), 4.09 (2H, t, J = 5.6 Hz, $-OCH_2$), 6.73 (1H, d, J = 8.8 Hz, ArH), 7.10–7.32 (6H, m, ArH), 8.20 (1H, bs, –NHCO), 8.59 (1H, s, ArH). ESI-MS m/z: 433 (M^+).

5.1.7. 4-Bromo-*N*-[5-bromo-2-(2-morpholinoethoxy)phenyl]-benzenesulfonamide (28a)

Compound **28a** was obtained (0.013 g, 10% yield) as a yellow oil, mp 127–130 ℃. IR (KBr) cm^{-1}: 2935, 2845, 1599, 843. ^1H NMR ($CDCl_3$) δ: 2.52–2.60 (6H, m, $-CH_2N$, morpholine), 3.95 (4H, m, morpholine), 4.05 (2H, t, J = 5.6 Hz, $-ArOCH_2$), 6.78 (1H, d, J = 9.2 Hz, ArH), 7.21 (1H, dd, J_1 = 8 Hz, J_2 = 2.4 Hz, ArH), 7.56–7.62 (4H, m, ArH), 7.77 (1H, m, ArH). ESI-MS m/z: 471 (M^+).

5.1.8. *N*-[5-Bromo-2-(2-morpholinoethoxy)phenyl]benzene sulfonamide (28b)

Compound **28b** was obtained (0.094 g, 85% yield) as a white solid, mp 103–105 ℃. IR (KBr) cm^{-1}: 2929, 1595, 1497, 761, 689. ^1H NMR ($CDCl_3$) δ: 2.50–2.58 (6H, m, $-CH_2N$, morpholine), 3.92 (4H, t, J = 4 Hz, morpholine), 4.01 (2H, t, J = 4.8 Hz, $-ArOCH_2$), 6.74 (1H, d, J = 8.4 Hz, ArH), 7.16 (1H, dd, J_1 = 8 Hz, J_2 = 2 Hz, ArH), 7.40–7.44 (2H, m, ArH), 7.50–7.52 (1H, m, ArH), 7.74–7.76 (3H, m, ArH). ESI-MS m/z: 441 (M^+).

5.1.9. *N*-(5-Bromo-2-hydroxyphenyl)-1-naphthalenylacetamide (31a)

To a solution of 4-bromo-2-nitrophenol (1.09 g, 5 mmol) in acetone (25 mL), K_2CO_3 (2.76 g, 20 mol) and MOMCl (0.49 mL, 6.5 mmol) was added, the mixture was stirred at room temperature for 2 h. The organic phase was separated and the solvent was removed under reduced pressure to get yellow oil (**29**), to which ethanol (5 mL) and THF (5 mL) were added to get a homogenous solution. The reaction mixture was added $SnCl_2{\cdot}2H_2O$ (3.39 g, 15 mmol) in a portion and then stirred at room temperature for 4 h, evaporated under reduced pressure to afford a white solid, to which 15% NaOH (5 mL) was added, stirred for 0.5 h, extracted with ether and dried by Na_2SO_4. Then removed the ether under reduced pressure to afford an oil (**30a**), which was mixed with 1-naphthalenylacetyl chloride (0.74 g, 3.6 mmol) and 15 mL CH_2Cl_2, stirred at room temperature for 1 h. The solvent was removed and 3 N HCl (3 mL), isopropanol (3 mL) and THF (3 mL) was added, stirred for 36 h. Volatiles were removed and remained solid was purified by a silica-gel column chromatography (PE/EtOAc 1:1) to afford a white solid (0.57 g, 32% yield), mp 207–209℃. IR (KBr) cm^{-1}: 3365, 1668, 1588, 1532, 735, 674. ^1H NMR (DMSO-d_6) δ: 4.26 (2H, s, $-COCH_2$), 6.79 (1H, d, J = 8 Hz, ArH), 7.04–7.07 (1H, m, ArH), 7.48–7.56 (4H, m, ArH), 7.84 (1H, d, J = 8 Hz, ArH), 7.92–7.95 (1H, m, ArH), 8.10–8.13 (2H, m, ArH), 9.43 (1H, bs, –NHCO), 10.24 (1H, s, –OH).

5.1.10. *N*-(2-Hydroxyphenyl)-1-naphthalenylacetamide (31b)

Using the same method as 4-bromo-2-nitrophenol (1.09 g, 5 mmol) to get **29**, which was then dissolved in the ethanol (5 mL), 10% Pd–C (0.24 g) was added. The mixture was stirred at room temperature for 4 h under hydrogen balloon, filtered to remove Pd/C. The filtrate was evaporated under reduced pressure to get an oil (**30b**). 0.776 g of **31b** was prepared as described for **31a** but starting from **30b** (56% yield). IR (KBr) cm^{-1}: 3359, 2924, 2851, 1653, 1591, 1509, 741, 698. ^1H NMR ($CDCl_3$) δ: 4.26 (2H, s, $-NHCOCH_2-$), 5.32 (1H, s, –OH), 6.54 (1H, d, J = 8Hz, ArH), 6.70–6.74 (1H, m, ArH), 6.97–7.09 (2H, m, ArH), 7.53–7.63 (4H, m, ArH), 7.92–7.99 (3H, m, ArH), 8.69 (1H, bs, –NHCO–).

5.1.11. *N*-[5-Bromo-2-(2-bromoethoxy)phenyl]-1-naphthalenyl acetamide (32a)

Prepared as described for **23** but starting from **31a** to get the yellow oil (25% yield). IR (KBr) cm^{-1}: 3381, 2926, 1682, 1592, 1520, 1463, 711, 674. ^1H NMR ($CDCl_3$) δ: 3.02 (2H, t, J = 5.6 Hz, $-CH_2Br$), 3.91 (2H, t, J = 5.6 Hz, $-OCH_2$), 4.23 (1H, s, $-COCH_2$), 6.51 (1H, d, J = 8 Hz, ArH), 7.04–7.07 (1H, m, ArH), 7.55–7.60 (4H, m, ArH), 7.78 (1H, bs, –NHCO), 7.89–7.94 (2H, m, ArH), 8.03 (1H, d, J = 7.6 Hz, ArH), 8.59–8.60 (1H, d, J = 2 Hz, ArH).

5.1.12. *N*-[2-(2-Bromoethoxy)phenyl]-1-naphthalenyl acetamide (32b)

Prepared as described for **32a** but starting from **31b** to get the yellow oil (80% yield). IR (KBr) cm^{-1}: 3382, 2925, 2867, 1668, 1593, 1510, 1456, 762, 699. ^1H NMR (CDCl3) δ: 2.99 (2H, t, J = 5.6 Hz, $-CH_2Br$), 3.90 (2H, t, J = 5.6 Hz, $-OCH_2$), 4.21 (1H, s, $-COCH_2$), 6.63 (1H, d, J = 8 Hz, ArH), 6.92–6.94 (2H, m, ArH), 7.50–7.54 (4H, m, ArH), 7.78 (1H, bs, –NHCO), 7.87–7.92 (2H, m, ArH), 8.03–8.05 (1H, m, ArH), 8.35–8.37 (1H, m, ArH).

5.1.13. General procedure for the preparation of compounds 33a–g

To a solution of **32a** (0.115 g, 0.25 mmol) or **32b** (0.096 g, 0.25 mmol) in 3 mL anhydrous acetonitrile, substituted amine (0.3 mmol) was added and the mixture was stirred at room temperature for one day. The mixture was evaporated under reduced pressure to dryness and the residue was purified by a silica-gel column chromatography (PE/EtOAc/TEA 3:1:0.1 or PE/EtOAc/MeOH 3:1:0.1).

5.1.14. Ethyl 2-{2-[4-bromo-2-(2-naphthalen-1-yl-acetamido)phenoxy]ethyl-methyl-amino}acetate (33a)

Compound **33a** was obtained (0.097 g, 78% yield) as a white solid, mp 68–70 °C. IR (KBr) cm^{-1}: 3389, 2976, 2925, 1739, 1683, 1598, 1527, 1450, 751. ^1H NMR ($CDCl_3$) δ: 1.18–1.21 (3H, m, $-CH_2CH_3$), 2.27 (3H, s, $-NCH_3$), 2.49 (2H, t, $-ArOCH_2CH_2$), 3.10 (2H, s, NCH_2CO-), 3.75 (2H, t, J = 6.4 Hz, $-ArOCH_2$), 4.05 (2H, q, J = 7.2 Hz, $-COOCH_2$), 4.20 (2H, s, $-NHCOCH_2$), 6.54 (1H, d, J = 8.8 Hz, ArH), 6.99–7.02 (1H, m, J_1 = 8.8Hz, J_2 = 2.4Hz, ArH), 7.45–7.50 (4H, m, ArH), 7.80–7.86 (2H, m, ArH), 7.99 (1H, d, J = 8.0Hz, ArH), 8.10 (1H, bs, –NHCO-), 8.54 (1H, d, J = 2.0Hz, ArH). ESI-MS m/z: 499 (M^+).

5.1.15. *N*-{5-Bromo-2-[2-(3-hydroxypropylamino)ethoxy] phenyl}-2-(naphthalen-1-yl) acetamide (33b)

Compound **33b** was obtained (0.097 g, 85% yield) as a yellow oil. IR (KBr) cm^{-1}: 3380, 2923, 2856, 1683, 1593, 1511,762, 689. ^1H NMR ($CDCl_3$) δ: 1.66–1.69 (2H, m, $-CH_2CH_2OH$), 2.66–2.84 (4H, m, $-CH_2NHCH_2$), 3.76–3.81 (4H, m, $-ArOCH_2,CH_2OH$), 4.26 (2H, s, $-COCH_2$), 6.52 (1H, d, J = 8.8 Hz, ArH), 7.03 (1H, dd, J_1 = 8.4 Hz, J_2 = 1.6 Hz, ArH), 7.48–7.55 (4H, m, ArH), 7.85–7.91 (2H, m, 2ArH), 8.05 (1H, d, J = 7.6 Hz, ArH), 8.22 (1H, s, –NHCO), 8.52 (1 H, d, J = 1.6 Hz, ArH). ESI-MS m/z: 457(M^+).

5.1.16. Ethyl-1-{2-[4-bromo-2-2-(naphthalen-1-yl)acetamid-ophenoxy]ethyl}piperidine-4-carboxylate (33c)

Compound **33c** was obtained (0.114 g, 85% yield) as a white solid, mp 94–96 °C. IR (KBr) cm^{-1}: 2926, 2854, 1730, 1629, 1595, 1523, 1484, 764, 689. ^1H NMR ($CDCl_3$) δ: 1.66–1.69 (3H, m, $-CH_2CH_3$), 1.81–2.17 (9H, m, piperidine CH_2), 2.64–2.67 (2H, m, $-CH_2N$), 3.73 (2H, t, J = 6.4Hz, $-OCH_2$), 4.12 (2H, q, J = 7.6Hz, $-CH_2CH_3$), 4.21 (2H, s, $-COCH_2$), 6.53 (1H, d, J = 9.2 Hz, ArH), 7.02 (1H, dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, ArH), 7.50–7.56 (4H, m, ArH), 7.86–7.92 (3H, m, 2ArH, –NHCO), 8.00 (1H, d, J = 6.8 Hz, ArH), 8.57 (1H, d, J = 2 Hz, ArH). ESI-MS m/z: 539 (M^+).

5.1.17. *N*-{2-[2-(6-Aminohexylamino)ethoxy]-5-bromo-phenyl}-1-naphthalenylacetamide (33d)

Compound **33d** was obtained (0.083 g, 67% yield) as a yellow oil. IR (KBr) cm^{-1}: 3382, 2929, 2854, 1649, 1595, 1522, 1481, 762, 699. ^1H NMR ($CDCl_3$) δ: 1.29–1.43 (8H, m, cyclohexane CH_2, H-2, H-3, H-4, H-5), 2.43–2.46 (4H, m, cyclohexane CH_2, H-1, H-6), 2.64 (2H, t, J = 6.8 Hz, $-ArOCH_2CH_2$), 3.66 (2H, t, J = 6.8 Hz, $-ArOCH_2$), 4.16 (2H, s, $-COCH_2$), 6.51 (1H, d, J = 8.8 Hz, ArH), 6.98 (1H, dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, ArH), 7.46–7.52 (4H, m, ArH), 7.83–7.98 (4H, m, 3ArH, 1-NHCO), 8.52 (1H, d, J = 2.4Hz, ArH). ESI-MS m/z: 498 (M^+).

5.1.18. *N*-{5-Bromo-2-[2-(cyclohexylamino)ethoxy]phenyl}-1-naphthalenylacetamide (33e)

Compound **33e** was obtained (0.078 g, 65% yield) as a yellow oil. IR (KBr) cm^{-1}: 2956, 2925, 2854, 1630, 1459, 762, 689. ^1H NMR ($CDCl_3$) δ: 1.53–1.81 (10H, m, $-NCHCH_2CH_2CH_2CH_2CH_2$), 2.06 (1H, m, –NCH), 2.53 (2H, t, J = 5.2 Hz, $-ArOCH_2CH_2$), 3.73 (2H, t, J = 5.2 Hz, $-ArOCH_2$), 4.23 (2H, s, $-COCH_2$), 6.56 (1H, d, J = 8.8 Hz, ArH), 7.04 (1H, dd, J_1 = 8 Hz, J_2 = 2 Hz, ArH), 7.50–7.57 (4H, m, ArH), 7.87–8.05 (4H, m, 3ArH, –NHCO), 8.55 (1H, d, J = 2 Hz, ArH). ESI-MS m/z: 481 (M^+).

5.1.19. *N*-{2-[2-(Benzyl-methylamino)ethoxy]-5-bromopheny}-1-naphthalenylacetamide (33f)

Compound **33f** was obtained (0.07 g, 56% yield) as a white solid, mp 113–115 °C. IR (KBr) cm^{-1}: 2925, 1643, 1594, 1526, 1483, 735, 696. ^1H NMR ($CDCl_3$) δ: 2.12 (3H, s, $-NCH_3$), 2.25–2.28 (2H, m, $-ArOCH_2CH_2$), 3.44 (2H, s, $-NCH_2Ar$), 3.76 (2H, t, J = 6 Hz, $-ArOCH_2$), 4.18 (2H, s, $-COCH_2$), 6.54 (1H, d, J = 9.2 Hz, ArH), 7.04 (1H, dd, J_1 = 8.4Hz, J_2 = 2.4Hz, ArH), 7.27–7.37 (9H, m, ArH, –NHCO), 7.83–8.02 (4H, m, ArH), 8.59 (1H, d, J = 2.4Hz, ArH). ESI-MS m/z: 503 (M^+).

5.1.20. Ethyl 2-{methyl 2-[2-(2-naphthalen-1-yl-acetamido)phenoxy]ethyl-amino}acetate (33g)

Compound **33g** was obtained (0.089 g, 85% yield) as a yellow oil. IR (KBr) cm^{-1}: 2926, 1733, 1678, 1593, 1453, 733, 698. ^1H NMR ($CDCl_3$) δ: 1.18 (3H, t, J = 7.2 Hz, $-CH_2CH_3$), 2.25 (3H, s, $-NCH_3$), 2.37 (2H, t, J = 5.6 Hz, $-ArOCH_2CH_2$), 3.07 (2H, s, $-NCH_2CO$), 3.75 (2H, t, J = 5.6 Hz, $-ArOCH_2$), 4.05 (2H, q, J = 6.4 Hz, $-OCH_2CH_3$), 4.19 (2H, s, $-NHCOCH_2$), 6.60 (1H, d, J = 9.2 Hz, ArH), 6.87–6.90 (2H, m, ArH), 7.43–7.54 (4H, m, ArH), 7.80–7.86 (2H, m, ArH), 8.00–8.03 (2H, m, 1ArH, –NHCO), 8.32–8.34 (1H, dd, J_1 = 6.8 Hz, J_2 = 2.4 Hz, ArH). ESI-MS m/z: 420 (M^+).

5.2. In vitro BACE 1 inhibit activity screening

All the compounds were assayed 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. The compound of which the inhibitory activity at 20 μg/mL was upon 50% had been tested the IC_{50}.

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