Design, synthesis and assay of 2-(4-phenylpiperazin-1-yl)pyrimidine-5-carboxamide derivatives as acetylcholinesterase inhibitors

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Abstract
A series of 2-(4-phenylpiperazin-1-yl)pyrimidine-5-carboxamide derivatives as acetylcholinesterase inhibitors (AChEIs) were designed and synthesized for the treatment of Alzheimer’s disease (AD). Their bioactivities were evaluated by the Ellman’s method, and the results showed that most of synthesized compounds displayed moderate acetylcholinesterase inhibitory activities in vitro. Among them, compound 6g exhibited the most potent inhibitory activity against AChE with IC50 of 0.90 μM, and poor inhibitory activity against butyrylcholinesterase (BuChE) with IC50 of 7.53 μM, which indicated that compound 6g was a selective AChE inhibitor, and compound 6g as a selective AChE inhibitor was confirmed by the molecular docking studies of compound 6g with AChE and BuChE. Furthermore, the mechanism of inhibition of compound 6g against AChE was analyzed by the kinetic study, and the result indicated that compound 6g was the mixed-type inhibitor of competitive inhibition and non-competitive inhibition. All the above showed that compound 6g could be considered as a lead compound for the development of AD drugs.

Graphical abstract

Keywords Alzheimer’s disease · Acetylcholinesterase inhibitor · Kinetic study · Molecular docking study

Introduction
Alzheimer’s disease (AD) is a neurodegenerative disease which is clinically characterized as progressive cognitive decline, memory impairment, language dysfunction, and inability to perform daily life tasks as usual [1–4]. Globally, more than 50 million people are suffering from AD, which is predicted to increase up to 152 million by 2050 [5, 6]. Currently, AD treatment is based on reducing the symptomatology, not on stopping or slowing down the progression of the disease. Donepezil, Rivastigmine, Galantamine and Huperzine-A as acetylcholinesterase inhibitors (AChEIs) are the most popular AD treatments, and they can temporarily relieve symptoms and reduce memory impairment. Nevertheless, these drugs offer limited ability to prevent or reverse the progression of the disease [7, 8].
The pathogenic mechanism of AD is highly complicated, and the exact etiology remains unknown. The cholinergic deficiency hypothesis, which is the basis of the main therapeutic approach in the treatment of AD, suggests that the low level of acetylcholine (ACh) is the main cause of memory and cognitive impairment in AD patients [9, 10]. The increasing studies suggest cholinergic neurotransmitters (ACh, BuCh) play an important role in learning and memory. Acetylcholinesterase (AChE) is selectively responsible for hydrolyzing ACh in the healthy brain, while butyrylcholinesterase (BuChE), possessing wider substrate specificity than AChE, can take over AChE to some extent to modulate acetylcholine, enhancing cognition functions [11–13].

Heterocyclic compounds play a wide role in drug discovery processes and possess considerable chemical significance and biological activities. Among them, pyrimidine was widely utilized for the development of AD drugs because of its diverse biological potential [14–16] (Fig. 1), and piperazine is also an important scaffold for AD drug design [17–19] (Fig. 1).

It is worth mentioning that the 2-(piperazin-1-yl)pyrimidine fragments have been found to possess excellent anti-acetylcholinesterase and have recently been reported for the design of AD drugs. A new series of phenyl sulfonyl-pyrimidine carboxylate derivatives were synthesized and evaluated as the potential multi-target drugs with effective anti-Alzheimer’s action [20]. Among them, compound BS-10 (Fig. 2) exhibited the best AChE inhibitory activity (AChE: IC50 = 47.33 nM) and could be regarded as a promising lead candidate for AD therapy. Meanwhile, the compound S-1 (Fig. 2) designed by S. Montanari et al. displayed promising AChE inhibition with IC50 value of 37.4 nM and hence was reported effective therapeutic option for the treatment of AD [21]. Its O-phenyl group and alkyl chain containing N and O atoms caught our attention. Herein a series of 2-(4-phenylpiperazin-1-yl)pyrimidine-5-carbo-xamide as AChE inhibitors had been designed and synthesized through inspiring of compound BS-10 and compound S-1. The 2-(piperazin-1-yl)pyrimidine fragments of compound BS-10 was preserved, and O-phenyl group of compound S-1 was linked to C-5 of pyrimidine ring with amide chain to design the target compounds 1 (Fig. 2).

Results and discussion

Chemical synthesis

The synthetic strategy for 6a–6n and 7 was depicted in Scheme 1. Commercially available compound 1 was reacted with Boc protected diamines to afford the intermediates 2a–2c in excellent yields. After hydrolysis, compounds 3a–3c were converted into amides 4a–4c in 40-45% yield by reacting with the N-methyl-3-(4-nitro-(4-nitrophenoxy)propan-1-amine in the presence of O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium tetrafluoroborate (TBTU) and N,N-Diisopropylethylamine (DIEA). After purification by column chromatography, Boc removal was achieved by dissolving 4a–4c in HCl/MeOH at room temperature for 2 h. The resulting unprotected free amines were immediately reacted with the 4-chloro-3-nitrobenzamide

![](image)

Fig. 1 The AChE inhibitors containing pyrimidine and piperazine fragments
Fig. 2 Design strategy for target compounds

Scheme 1 The synthesis of target compounds 6a–6n and 7
derivatives or 4-(morpholine-4-carbonyl)benzoic acid to give target compounds 6a–6n or 7 in 27-50% yield. The synthetic strategy for target compounds 12a–12d and 13a–13d was outlined in Scheme 2. Firstly, compound 8 was treated with piperazine at 80 °C to form compound 9, which was reacted with compound 1 to yield compound 10 in 65% yield. After hydrolysis, compound 11 was converted into amides 12a–12d and 13a–13d in 38-65% yield by reacting with the N-methyl-3-phenoxypropan-1-amine derivatives or N-(2-aminoethyl)benzamide derivatives in the presence of TBTU and DIEA. The structures of the target compounds were characterized by 1H NMR, 13C NMR, HRMS and IR, and the purity of all the target compounds was determined by high-performance liquid chromatography (HPLC) analysis to be over 95.0%.

**AChE and BuChE assay**

To evaluate the AChE and BuChE inhibitory activities of target compounds, the Ellman’s method [22] was performed with Huperzine-A as reference compound. The results were shown in Tables 1 and 2. Visibly, most of the synthesized target compounds exhibited moderate inhibitory activities against AChE with IC_{50} values ranging from 0.90 to 42.2 μM. Among them, compound 6k and 12d displayed good AChE inhibitory activities, and their IC_{50} values for inhibiting AChE both reached 1.00 μM. Compound 6g was found to be the most potent AChE inhibitors, with IC_{50} value of 0.90 μM. Meanwhile, the synthesized compounds exhibited a certain ability to inhibit BuChE, and several compounds inhibited BuChE with IC_{50} value less than 5 μM, for example, compounds 6e, 6h and 6n exhibited the better BuChE inhibitory activities with the IC_{50} values of 3.42 μM, 3.87 μM and 3.96 μM, respectively. Notably, compound 6g inhibited AChE with IC_{30} value of 0.90 μM, while it inhibited BuChE with IC_{50} value of 7.53 μM, which revealed 8.3 times selectivity for AChE over BuChE. Through the analysis and comparison of structure and activity data (Table 1), the following structure-activity relationships could be found: (1) The inhibitory activity of AChE from compounds with Q group as piperazine ring was significantly better than that of compounds with Q group as ethylenediamine and propanediamine, for example,

![Scheme 2](image-url)
| Compounds | Q | R₁ | AChE IC₅₀ (μM) ± SD | BuChE IC₅₀ (μM) ± SD |
|-----------|---|----|---------------------|---------------------|
| 6a        |   |    | 42.2 ± 0.54         | 5.08 ± 0.89         |
| 6b        |   |    | 8.88 ± 0.25         | 27.0 ± 1.41         |
| 6c        |   |    | 30.3 ± 0.95         | 5.83 ± 0.53         |
| 6d        |   |    | 24.8 ± 0.58         | 7.55 ± 1.55         |
| 6e        |   |    | 5.84 ± 0.91         | 3.42 ± 1.37         |
| 6f        |   |    | 2.99 ± 0.81         | 42.0 ± 1.38         |
| 6g        |   |    | 0.90 ± 0.64         | 7.53 ± 1.18         |
| 6h        |   |    | 38.2 ± 0.75         | 3.87 ± 0.96         |
| 6i        |   |    | 25.1 ± 0.71         | 46.0 ± 1.15         |
| 6j        |   |    | 7.75 ± 1.27         | 7.11 ± 1.01         |
compound 6g (piperazine ring, IC50 = 0.90 μM) was 9.5 fold more active than 6m (ethylenediamine chain, IC50 = 8.52 μM) and 6.1 fold more active than 6n (propylenediamine chain, IC50 = 5.50 μM). However, the inhibition of BuChE by compounds whose Q group was a piperazine ring or a diaminoalkyl chain was similar. (BuChE: 6g piperazine ring, IC50 = 7.52 μM; 6m ethylenediamine chain, IC50 = 7.98 μM; 6n propylenediamine chain, IC50 = 3.96 μM). (2) The R1 substitution as morpholine resulted in the best AChE inhibitory activity (6g: IC50 = 0.90 μM), whereas the AChE inhibitory activity was worst when the R1 substituent was N, O-dimethylhydroxylamine (6a: IC50 = 42.2 μM), and the AChE inhibitory activity of 6g was 46.9 fold more active than that of 6a. (3) After connecting the piperazine ring and the phenyl ring A by amide bond, and removing the nitro group on the phenyl ring A, the AChE inhibitory activity would decline significantly, for example, the IC50 value of 6g for inhibiting AChE was 0.90 μM, which was 5.1 times more active than that of compound 7 (IC50 = 4.61 μM). (4) The AChE inhibitory activity decreased when the nitro group on the phenyl ring B was replaced by other groups, but the least decrease in activity occurred when all hydrogens on the phenyl ring B were replaced by fluorine atoms. Furthermore, the position of the substituent also had some influence on AChE inhibitory activity with para-substituted compounds having better AChE inhibitory activity than that of meta-substituted compounds, such as 12b (IC50 = 5.36 μM) and 12a (IC50 = 25.0 μM). (5) The inhibitory activity was not significantly improved by connecting pyrimidine ring to the aromatic ring B through two amide bonds, but it was found that extending the length of one carbon chain resulted in the decrease of the inhibitory activity against AChE, such as 13d (IC50 = 10.0 μM, n = 2) and 13b (IC50 = 6.85 μM, n = 1). Moreover, when R3 was

Table 1 (continued)

| Compounds       | Q                  | R1                  | AChE IC50(μM) ± SD | BuChE IC50(μM) ± SD |
|-----------------|--------------------|---------------------|--------------------|---------------------|
| 6a              | –                  | –                   | 5.28 ± 1.20        | >10000.00           |

SD indicates standard deviation
substituted with different groups, the order of AChE inhibitory activity was $13b$ (4-NO$_2$) $> 13a$ (4-CF$_3$) $> 13c$ (4-CN), and compound $13b$ (4-NO$_2$) with IC$_{50}$ value of 6.85 μM is more favorable than others.

**Kinetic analysis**

In order to know the mechanism of AChE inhibition, compound $6g$ was conducted for kinetic analysis of AChE inhibition. The Lineweaver-Burk plot was constructed for three varied concentration of compound $6g$ against six different concentrations of substrate (acetylthiocholine iodide, ATCl). According to the characteristics of enzymatic kinetics, the double-reciprocal curve of the competitive inhibition type is on the Y-axis and the double-reciprocal curve of the non-competitive inhibition type is on the negative semi-axis of the X-axis. the double-reciprocal curve of the mixed inhibition type is assigned to the second quadrant. As shown in Fig. 3, the straight lines of different concentrations of $6g$ intersected in the second quadrant (Fig. 3). As the concentration of $6g$ increases, the slope and vertical axis intercept increased, which indicated that compound $6g$ displayed both competitive and non-competitive inhibitory effects on AChE.

**Molecular docking study**

The potential binding mode of compound $6g$ with AChE (PDB: 4EY7) and BuChE (PDB: 5K5E) were investigated by the molecular docking study, which was performed by using Autodock 4.2 with structure images created by Pymol 1.5. The protein structure of AChE (PDB code: 4EY7) and BuChE (PDB code: 5K5E) was downloaded from the Protein Data Bank (https://www.rcsb.org/). Then the downloaded protein was prepared by adding hydrogen atoms, removing water and assigning the Kollman atomic charges to the protein. AutoDock was used to switch the prepared protein into pdbqt file and prepare the ligand in pdbqt file. A grid box spacing of 0.375 Å was constructed over the docking area and docking procedure was carried out. Finally, Pymol (https://www.pymol.org/pymol.html) and BIOVIA Discovery Studio (Free Download: BIOVIA Discovery Studio Visualizer - Dassault Systèmes (3ds.com)) software was used to visualize the 3D and 2D interaction of docking study. As shown in Fig. 4, The nitro group on the phenyl ring B of $6g$ can participate in hydrogen bonds with Asp-74 and Trp-86 simultaneously. The oxygen atom of the amide bond at C-5 of the pyrimidine ring involved in a hydrogen bond with Tyr-124. Meanwhile, hydrogen bonds were observed between the N atom of the pyrimidine ring and Phe-295, and the nitro group on the phenyl.

### Table 2 Inhibition of AChE and BuChE of compounds 12a–12d and 13a–13d

| Compounds | R$_2$ | R$_3$ | n | AChE IC$_{50}$(μM) ± SD | BuChE IC$_{50}$(μM) ± SD |
|-----------|------|------|---|----------------|-------------------|
| 12a       | 3-OCH$_3$ | –    | – | 25.0 ± 0.36 | 5.94 ± 1.05 |
| 12b       | 4-OCH$_3$ | –    | – | 5.36 ± 0.72 | 4.83 ± 1.72 |
| 12c       | 4-CF$_3$ | –    | – | 15.0 ± 0.35 | 4.66 ± 1.66 |
| 12d       | 2,3,4,5,6-F | – | – | 1.00 ± 0.70 | 3.10 ± 1.47 |
| 13a       | –    | 4-CF$_3$ | 1 | 10.2 ± 0.56 | 19.4 ± 1.33 |
| 13b       | –    | 4-NO$_2$ | 1 | 6.85 ± 1.40 | 5.50 ± 1.57 |
| 13c       | –    | 4-CN  | 1 | 36.3 ± 0.92 | 6.56 ± 1.80 |
| 13d       | –    | 4-NO$_2$ | 2 | 10.0 ± 1.12 | 4.48 ± 1.86 |
| Huperzine-A | – | – | – | 5.28 ± 1.20 | >10000.00 |

SD indicates standard deviation
ring A involved in two hydrogen bonds with Tyr-341 and Ser-293, respectively. Additionally, the pyrimidine ring formed π-π interaction with Trp-286, and the phenyl ring B of compound 6g interacted with Trp-86 through π-π stacking effect. Therefore, 6g was strongly bound with the optimal conformation of AChE and stabilized in the cavity. Moreover, as shown in Fig. 4, the phenyl ring A of 6g interacted with Gly-116 through π-Amide. The oxygen atom of the amide bond at C-5 of the pyrimidine ring involved in a hydrogen bond with Asn-289 Fig. 5.

As shown in Fig. 6, through the conformational overlap of compounds 6g, BS-10, and S-1, it was found that the docking model of the lead compound BS-10, S-1 has an obvious fold relative to compound 6g. This may be the reason why the inhibitory activity of compound 6g is lower than that of BS-10, S-1. Adding several rotatable carbon bonds between the piperazine ring and the benzene ring may give the compound better activity.

**Conclusion**

In summary, a series of 2-(4-phenylpiperazin-1-yl)pyrimidine-5-carboxamide derivatives were synthesized and evaluated as potential acetylcholinesterase inhibitors (AChEIs) against AD. Among them, compound 6g emerged as the most potent AChE inhibitor (IC50 = 0.90 μM) with mixed type of inhibitory pattern, and 6g also exhibited 8.3 times AChE selectivity over BuChE (IC50 = 7.53 μM), which indicated that compound 6g was a good selective AChE inhibitor. The molecular docking studies displayed that compound 6g was bound up with AChE and BuChE through hydrogen bond and π-π interaction. Taken together, the 2-(4-phenylpiperazin-1-yl)pyrimidine-5-carboxamide derivatives provided a useful template, and compound 6g might be a promising lead compound for the development of new anti-AD drugs.

**Experimental Section**

**Chemistry**

All experiments were carried out under air atmosphere, 4-chloro-3-nitrobenzamide derivatives, N-methyl-3-phenoxypyropan-1-amine derivatives, N-(2-aminoethyl)benzamide derivatives, 4-(morpholine-4-carbonyl)benz-oic acid and other reagent materials were commercially available analytically pure or chemically pure, unless stated otherwise. Reaction progress was observed by thin layer chromatography on the glass-backed silica gelsheets (Silica Gel 60 GF254) and visualized under UV light (254 nm). High Resolution Mass Spectrometry was determined by TSQ Quantum Ultra Mass Spectrometer, and the nuclear magnetic resonance spectrum was measured by Bruker Avance III 600 MHz nuclear magnetic resonance spectrometer. The infrared (IR) spectra were run as KBr disk on FTIR-850 spectrophotometer (Tianjin Gangdong Sci. & Tech Co., Ltd.). The purity of the target compounds was determined by LC-3000 HPLC system (Beijing Chuangxin tongheng Technology Co., Ltd.). The melting point was determined by SGW X-4 micro melting point apparatus (Shanghai...
Precision Scientific Instrument Co., Ltd.). The 96 plate was read by 1420 Victor Microplate Reader. Acetylcholinesterase and Butyrylcholinesterase were purchased from Sigma, Huperzine-A was purchased from Shanghai Yuanye Biotechnology Co., Ltd.

**General procedure for the synthesis of target compounds 6a–6n**

To a solution of compounds 5a–5c (1.00 mmol) and 4-chloro-3-nitrobenzamide derivatives (2.00 mmol) in DMF (5 mL) were added DIEA (195 mg, 3.0 mmol). The reaction mixture was stirred at 120 °C overnight with stirring. After the consumption of the starting material (monitored by TLC), the H2O (15 mL) was added and the aqueous layer was extracted twice with ethyl acetate (15 mL). Then, the organics was washed with brine and dried over Na2SO4, filtered, and concentrated in vacuo to give crude compounds 6a–6n. The crude compounds 6a–6n were purified by column chromatography on silica gel to afford compounds 6a–6n.

2-(4-(4-(methoxy(methyl)carbamoyl)-2-nitrophenyl)piperazin-1-yl)-N-methyl-N-(3-(4-nitrophenoxy)propyl)pyrimidine-5-carboxamide (6a)

Chemical Formula: C28H32N8O8; yellow solid; Yield: 31%; mp 71.0-72.9 °C; Purity: 97.83% (HPLC); 1H NMR (600 MHz, CDCl3) δ 8.46 (s, pyrimidine ring, 2H), 8.33 (d, J = 8.7 Hz, -ArH, 2H), 7.94 (dd, J = 8.6, 2.1 Hz, -ArH, 1H), 7.14 (d, J = 8.7 Hz, -ArH, 1H), 7.02-6.83 (m, -ArH, 2H), 4.19–4.08 (m, -CH2–, 2H), 3.76-3.66 (m, -CH2–, 2H), 3.61 (s, -OCH3, 3H), 3.38 (s, -NCH3, 3H), 3.25 (t, J = 5.1 Hz, piperazine ring, 4H), 3.14 (s, -NCH3, 3H), 2.24–2.14 (m, -CH2–, 2H); 13C NMR (151 MHz, CDCl3) δ 166.78, 161.14, 157.45, 147.23, 141.59, 140.81, 134.18, 127.59, 126.00, 125.96, 125.89, 119.62, 118.19, 114.43, 114.38, 66.61, 63.76, 61.24, 50.73, 47.19, 44.77, 43.51, 38.58, 36.54, 33.48, 27.04; ESI-MS calcd for 609.2416 [M + H]+, found 609.2421 [M + H]+; IR (KBr), v (cm⁻¹): 1629 (C=O), 1594 (C=O).

**N-methyl-2-(4-(2-nitro-4-(piperidine-1-carbonyl) phenyl)piperazin-1-yl)-N-(3-(4-nitrophenoxy)propyl)pyrimidine-5-carboxamide (6b)**

Chemical Formula: C31H36N8O7; yellow solid; Yield: 27%; mp 77.8–79.1 °C; Purity: 98.07% (HPLC); 1H NMR (600 MHz, CDCl3) δ 8.45 (s, pyrimidine ring, 2H), 8.19 (d, J = 8.5 Hz, -ArH, 2H), 7.92 (s, -ArH, 1H), 7.58 (s, -ArH, 1H), 7.39 (d, J = 8.5 Hz, -ArH, 2H), 7.21 (t, J = 7.7 Hz, -ArH, 1H), 7.02-6.79 (m, -ArH, 2H), 4.19–4.04 (m, -CH2–, 2H), 3.76-3.66 (m, -CH2–, 2H), 3.61 (s, -OCH3, 3H), 3.38 (s, -NCH3, 3H), 3.25 (t, J = 5.1 Hz, piperazine ring, 4H), 3.14 (s, -NCH3, 3H), 2.24–2.14 (m, -CH2–, 2H); 13C NMR (151 MHz, CDCl3) δ 166.78, 161.14, 157.45, 147.23, 141.59, 140.81, 134.18, 127.59, 126.00, 125.96, 125.89, 119.62, 118.19, 114.43, 114.38, 66.61, 63.76, 61.24, 50.73, 47.19, 44.77, 43.51, 38.58, 36.54, 33.48, 27.04; ESI-MS calcd for 668.2715 [M + H]+, found 668.2723 [M + H]+; IR (KBr), v (cm⁻¹): 1629 (C=O), 1594 (C=O).
N-methyl-2-(4-(2-nitro-4-(pyrrolidine-1-carbonyl) phenyl)piperazin-1-yl)-N-(3-(4-nitrophyridopyrimidine-5-carboxamidem(6c)
Chemical Formula: C36H40N8O9; yellow solid; Yield: 50%; mp 168.5–168.7 °C; Purity: 99.68% (HPLC); 1H NMR (600 MHz, CDCl3) δ 8.44 (s, pyrimidine ring, 2H), 8.27 (d, J = 2.2 Hz, -CONH-, 1H), 8.18 (d, J = 8.9 Hz, -ArH, 2H), 7.97 (dd, J = 8.7, 2.2 Hz, -ArH, 1H), 7.24 (dd, J = 5.1, 1.2 Hz, thiophene ring, 1H), 7.13 (d, J = 8.7 Hz, -ArH, 1H), 7.01–7.03 (m, -ArH, 1H), 6.96 (dd, J = 5.1, 3.5 Hz, thiophene ring, 1H), 6.91 (s, -ArH, 2H), 6.80 (s, thiophene ring, 1H), 4.80 (d, J = 5.5 Hz, -CH2, 2H), 4.12 (s, -CH2, 2H), 4.04 (t, J = 5.1 Hz, piperazine ring, 4H), 3.69 (t, J = 7.2 Hz, -CH2, 2H), 3.23 (t, J = 5.1 Hz, piperazine ring, 4H), 3.13 (s, -NCH3, 3H), 2.18 (s, -CH2, 2H), 1.3C NMR (151 MHz, CDCl3) δ 164.64, 160.96, 157.45, 147.85, 141.69, 140.86, 140.50, 132.56, 126.99, 126.48, 126.42, 125.96, 125.53, 125.46, 120.19, 118.20, 114.36, 50.72, 43.54, 38.89; ESI-MS calcd for 661.2187 [M + H]+, found 661.2194 [M + H]+; IR (KBr), ν (cm−1): 1616 (C=O), 1595 (C=O).

2-(4-(4-(3,4-dimethylcarbamoyl)phenyl)piperazin-1-yl)-N-methyl-2-(4-(4-(dimethylcarbamoyl)-2-nitrophenyl)piperyridine-5-carboxamide(6f)
Chemical Formula: C28H32N8O7; yellow solid; Yield: 35%; mp 163.2-165.4 °C; Purity: 99.77% (HPLC); 1H NMR (600 MHz, CDCl3) δ 8.45 (s, pyrimidine ring, 2H), 8.19 (d, J = 8.8 Hz, -ArH, 2H), 7.95 (dd, J = 8.5, 2.1 Hz, -ArH, 1H), 7.62 (dd, J = 8.5, 2.1 Hz, -ArH, 1H), 7.17 (d, J = 8.5 Hz, -ArH, 1H), 6.93 (s, -ArH, 2H), 4.17-4.07 (m, -CH2, 2H), 4.05 (t, J = 5.0 Hz, piperazine ring, 4H), 3.30 (t, J = 5.0 Hz, piperazine ring, 4H), 3.14 (s, -NCH3, 3H), 3.10 (d, J = 12.0 Hz, -CH2, 6H), 2.19 (s, -CH2, 2H), 1.3C NMR (151 MHz, CDCl3) δ 164.90, 161.18, 157.47, 146.62, 141.66, 132.81, 129.27, 125.93, 125.79, 120.62, 118.15, 114.37, 51.09, 43.63; ESI-MS calcd for 593.2467 [M + H]+, found 593.2471 [M + H]+; IR (KBr), ν (cm−1): 1627 (C=O), 1593 (C=O).
morpholine ring, 8H), 3.65 (s, –CH2–, 2H), 3.21 (t, J = 5.1 Hz, piperazine ring, 4H), 3.14 (s, –NCH3, 3H), 2.19 (s, –CH2–, 2H); 13C NMR (151 MHz, CDCl3) δ = 167.95, 161.15, 157.49, 146.86, 141.68, 141.56, 132.83, 127.97, 125.98, 125.94, 120.69, 118.16, 114.37, 66.78, 50.99, 43.58, 38.61, 31.86, 22.67; ESI-MS calcd for 635.2572 [M + H]+, found 635.2571 [M + H]+; IR (KBr), ν (cm−1): 1633 (C=O), 1596 (C=O).

2-(4-(4-acetylpiperazine-1-carbonyl)-2-nitrophenyl) piperazin-1-yl)-N-methyl-N-(3-(4-nitrophenoxy) propyl) pyrimidine-5-carboxamide(6h)

Chemical Formula: C33H38N8O9; yellow solid; Yield: 36%; mp 88.9–90.5 °C; Purity: 99.26% (HPLC); 1H NMR (600 MHz, CDCl3) δ = 8.45 (s, pyrimidine ring, 2H), 8.19 (d, J = 8.8 Hz, -ArH, 2H), 7.95 (d, J = 2.1 Hz, -ArH, 1H), 7.60 (dd, J = 8.5, 2.1 Hz, -ArH, 1H), 7.18 (d, J = 8.5 Hz, -ArH, 1H), 7.01-6.79 (m, -ArH, 2H), 4.21-4.07 (m, –CH2–, 2H), 4.05 (t, J = 5.0 Hz, piperazine ring, 4H), 3.79-3.58 (m, piperazine ring, 8H), 3.54 (s, –CH2–, 2H), 3.23 (t, J = 5.1 Hz, piperazine ring, 4H), 3.14 (s, –NCH3, 3H), 2.19 (s, –CH2–, 2H), 2.14 (s, –COCH3, 3H); 13C NMR (151 MHz, CDCl3) δ = 169.20, 168.21, 161.17, 157.49, 147.01, 141.68, 141.40, 132.80, 127.62, 126.12, 125.94, 120.66, 118.24, 114.37, 50.94, 46.07, 43.55, 41.39, 21.34; ESI-MS calced for 676.2838 [M + H]+, found 676.2844 [M + H]+; IR (KBr), ν (cm−1): 1632 (C=O), 1596 (C=O).

N-methyl-2-(4-(2-nitro-4-(thiazol-2-ylcarbamoyl)phenyl) piperazin-1-yl)-N-(3-(4-nitrophenoxy) propyl) pyrimidine-5-carboxamide(6j)

Chemical Formula: C32H29N9O8S; yellow solid; Yield: 39%; mp 242.1–244.0 °C; Purity: 95.02% (HPLC); 1H NMR (600 MHz, DMSO-d6) δ = 12.72 (s, –CONH–, 1H), 8.68 (d, J = 2.3 Hz, thiazole ring, 1H), 8.48 (s, pyrimidine ring, 2H), 8.28 (dd, J = 8.8, 2.3 Hz, thiazole ring, 1H), 8.20 (d, J = 8.7 Hz, -ArH, 2H), 7.57 (d, J = 3.6 Hz, -ArH, 1H), 7.43 (d, J = 8.9 Hz, -ArH, 1H), 7.28 (d, J = 3.6 Hz, -ArH, 1H), 7.11 (s, -ArH, 2H), 4.31-4.00 (m, –CH2–, 2H), 3.93 (s, piperazine ring, 4H), 3.57 (s, –CH2–, 2H), 3.34 (d, J = 3.8 Hz, piperazine ring, 4H), 3.02 (s, –NCH3, 3H), 2.07 (t, J = 6.4 Hz, –CH2–, 2H); 13C NMR (151 MHz, DMSO-d6) δ = 160.49, 157.19, 147.48, 140.77, 138.96, 133.17, 126.93, 125.83, 119.91, 118.57, 114.89, 113.77, 49.52, 42.88; ESI-MS calced for 648.1983 [M + H]+, found 648.1981 [M + H]+; IR (KBr), ν (cm−1): 1609 (C=O), 1596 (C=O).

2-(4-(4-acetylpiperazine-1-carbonyl)-2-nitrophenyl) piperazin-1-yl)-N-methyl-N-(3-(4-nitrophenoxy) propyl) pyrimidine-5-carboxamide(6l)

Chemical Formula: C33H38N8O9; yellow solid; Yield: 36%; mp 72.2–74.1 °C; Purity: 97.71% (HPLC); 1H NMR (600 MHz, CDCl3) δ = 8.45 (s, pyrimidine ring, 2H), 8.27-8.11 (m, -ArH, 4H), 7.91 (s, -ArH, 1H), 7.55 (s, -ArH, 1H), 7.21-7.05 (m, -ArH, 1H), 6.92 (s, -ArH, 4H), 4.09 (s, –CH2–, 2H), 4.05 (s, piperazine ring, 4H), 3.70 (t, J = 7.2 Hz, -CH2–, 4H), 3.19 (d, J = 6.3 Hz, piperazine ring, 4H), 3.14 (s, –NCH3, 3H), 3.10 (s, –NCH3, 3H), 2.19 (s, –CH2–, 2H); 13C NMR (151 MHz, CDCl3) δ = 161.15, 157.49, 146.68, 141.72, 141.46, 132.70, 129.04, 125.96, 120.62, 118.20, 114.36, 51.06, 43.62; ESI-MS calced for 758.2893 [M + H]+, found 758.2904 [M + H]+; IR (KBr), ν (cm−1): 1621 (C=O), 1592 (C=O).

N-methyl-2-(4-(3-(4-nitrophenoxy)propyl) carbamoyl)-2-nitrophenyl)piperazin-1-yl)-N-(3-(4-nitrophenoxy)propyl)pyrimidine-5-carboxamide(6k)

Chemical Formula: C33H34N8O9; yellow solid; Yield: 41%; mp 150.4–152.3 °C; Purity: 98.97% (HPLC); 1H NMR (600 MHz, CDCl3) δ = 8.46 (s, pyrimidine ring, 2H), 8.42 (d, J = 2.2 Hz, -ArH, 1H), 8.21-8.16 (m, -ArH, 2H), 8.07 (dd, J = 8.7, 2.2 Hz, -ArH, 1H), 7.15 (d, J = 8.8 Hz, -ArH, 1H), 6.93 (s, -ArH, 2H), 4.19-4.08 (m, –CH2–, 2H), 4.06 (d, J = 5.3 Hz, piperazine ring, 4H), 3.70 (t, J = 7.2 Hz, -CH2–, 2H), 3.32 (dd, J = 6.3, 3.9 Hz, piperazine ring, 4H), 3.14 (s, –NCH3, 3H), 2.59 (s, –COCH3, 3H), 2.19 (s, –CH2–, 2H); 13C NMR (151 MHz, CDCl3) δ = 194.82, 160.99, 157.47, 148.56, 141.68, 140.11, 133.14, 129.14, 127.66, 125.94,
N-methyl-2-((2-((4-morpholine-4-carbonyl)-2-nitrophenyl)amino)ethylamino)-N-(3-(4-nitrophenoxy)propyl)pyrimidine-5-carboxamide (6m)

Chemical Formula: C_{28}H_{32}N_{8}O_{8}; yellow solid; Yield: 33%; mp 72.9–74.3 °C; Purity: 99.69% (HPLC); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 8.65 (s, -NH-, 1H), 8.50 (s, pyrimidine ring, 2H), 8.27 (s, -ArH, 1H), 8.18 (s, -ArH, 2H), 7.57 (d, \(J = 8.6\) Hz, -ArH, 1H), 6.98–6.89 (m, -ArH, 3H), 3.77-3.55 (m, -CH\(_2-\) and morpholine ring, 12H), 3.16 (s, -NCH\(_3\), 3H), 2.20 (s, -CH\(_2-\), 2H); \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 168.59, 162.46, 158.75, 158.42, 150.87, 138.62, 138.34, 136.89, 136.81, 131.18, 131.05, 125.88, 125.11, 119.76, 113.40, 113.30, 66.80, 40.15, 38.72, 30.74; ESI-MS calcd for 618.2671 [M\(^+\), found 619.2416 [M\(^+\)]; IR (KBr), \(\nu\) (cm\(^{-1}\)): 3370 (-NH-) 1625 (-CH\(_2\)-); 1HNMR \((600\) MHz, CDCl\(_3\)) \(\delta\) 7.68 (m, -ArH, 3H), 6.74 (s, -OCH\(_3\), 3H), 6.28 (s, -ArH, 2H), 4.30-4.22 (m, -CH\(_2-\), 2H), 3.75-3.65 (m, piperazine ring, 4H), 3.13 (s, -NCH\(_3\), 3H), 2.23-2.11 (m, -CH\(_2-\) and morpholine ring, 12H), 3.34 (m, piperazine ring, 4H), 3.04-3.00 (m, -CH\(_2-\), 2H); \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 168.54, 162.49, 158.98, 157.64, 151.02, 146.89, 141.58, 132.76, 130.99, 129.91, 129.80, 127.92, 125.92, 120.65, 118.51, 106.70, 106.54, 106.40, 106.26, 101.16, 99.81, 99.69, 98.15, 70.48, 68.70, 68.54, 67.64, 50.35, 45.96, 43.09, 42.19, 26.56; ESI-MS calcd for 564.2205 [M\(^+\)]; IR (KBr), \(\nu\) (cm\(^{-1}\)): 1670 (C=O), 1599 (C=O).

N-methyl-2-((3-((4-(morpholine-4-carbonyl)-2-nitrophenyl)piperazin-1-yl)-N-(3-(4-nitrophenoxy)propyl)pyrimidine-5-carboxamide (6n)

Chemical Formula: C_{31}H_{35}N_{7}O_{7}; yellow solid; Yield: 41%; mp 40.2–42.3 °C; Purity: 99.15% (HPLC); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 8.44 (s, pyrimidine ring, 2H), 8.18 (d, \(J = 8.8\) Hz, -ArH, 2H), 7.54-7.45 (m, -ArH, 4H), 6.92 (s, -ArH, 2H), 4.11 (s, -CH\(_2-\), 2H), 4.02-3.68 (m, morpholine ring and piperazine ring, 12H), 3.66-3.58 (m, -CH\(_2-\), 2H), 3.56-3.34 (m, piperazine ring, 4H), 3.13 (s, -NCH\(_3\), 3H), 2.25-2.11 (m, -CH\(_2-\), 2H); \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 169.70, 164.96, 161.10, 157.45, 141.72, 136.99, 136.97, 127.42, 127.39, 125.91, 118.53, 113.46, 66.82, 63.72, 43.79, 29.65, 21.96; ESI-MS calcd for 618.2671 [M\(^+\)], found 618.2680 [M\(^+\)]; IR (KBr), \(\nu\) (cm\(^{-1}\)): 1638 (C=O), 1591 (C=O).

General procedure for the synthesis of target compounds 12a–12d

To a solution of compound 11 (400 mg, 0.91 mmol) in CH\(_2\)Cl\(_2\) (10 mL) at 0 °C were added TBTU (440 mg, 1.65 mmol) and DIEA (213 mg, 1.65 mmol). The solution was stirred at room temperature for 12 h, then washed with 5% aqueous HCl (10.0 mL), 5% aqueous NaOH (10.0 mL) and brine (20.0 mL). The organics were dried over Na\(_2\)SO\(_4\), filtered, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel to afford target compounds 12a–12d.

N-(3-(3-methoxyphenoxy)propyl)-N-methyl-2-(4-(4-(morpholine-4-carbonyl)-2-nitrophenyl)piperazin-1-yl)pyrimidine-5-carboxamide (12a)

Chemical Formula: C_{31}H_{37}N_{7}O_{7}; yellow solid; Yield: 40%; mp 89.5–91.0 °C; Purity: 99.09% (HPLC); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 8.46 (s, pyrimidine ring, 2H), 7.93 (d, \(J = 2.1\) Hz, -ArH, 1H), 7.58 (dd, \(J = 8.5, 2.1\) Hz, -ArH, 1H), 7.20–7.11 (m, -ArH, 2H), 6.52-6.40 (m, -ArH, 3H), 4.05 (t, \(J = 5.1\) Hz, piperazine ring, 4H), 4.02-3.90 (m, -CH\(_2-\), 2H), 3.78 (s, -OCH\(_3\), 3H), 3.71 (s, morpholine ring, 6H), 3.66 (s, morpholine ring, 6H), 3.23-3.18 (m, -CH\(_2-\) and morpholine ring, 12H), 3.13 (s, -NCH\(_3\), 3H), 2.25-2.11 (m, -CH\(_2-\), 2H); \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 167.95, 161.17, 160.88, 157.41, 146.89, 141.58, 132.76, 129.91, 129.80, 127.92, 125.92, 120.65, 118.51, 106.70, 106.54, 106.40, 106.26, 101.16.
N-(3-(4-methoxyphenoxy)propyl) -N-methyl-2-(4-(morpholine-4-carbonyl)-2-nitrophenyl)piperazine-1-yl) pyrimidine-5-carboxamide(12b)

Chemical Formula: C_{31}H_{37}N_{7}O_{7}; yellow solid; Yield: 41%; mp 77.2–79.1 °C; Purity: 95.58% (HPLC); 1H NMR (600 MHz, CDCl_3) δ 8.47 (s, pyrimidine ring, 2H), 7.92 (s, -ArH, 1H), 7.62–7.58 (m, -ArH, 1H), 7.19–7.16 (m, -ArH, 1H), 6.81 (s, -ArH, 4H), 4.10–4.01 (m, piperazine ring, 4H), 3.93 (s, -CH_2-, 2H), 3.79–3.63 (m, morpholine ring and -OCH_3, 13H), 3.29–3.17 (m, piperazine ring, 4H), 3.10 (s, -NCH_3, 3H), 2.09 (s, -CH_2-, 2H); 13C NMR (151 MHz, CDCl_3) δ 167.94, 161.17, 157.40, 153.99, 146.86, 141.55, 132.74, 127.89, 125.90, 120.64, 118.51, 115.34, 114.69, 66.75, 63.71, 55.72, 50.98, 43.55, 29.63; ESI-MS calc for 620.2827 [M+H]⁺, found 620.2836 [M+H]⁺; IR (KBr), ν (cm⁻¹): 1615 (C=O), 1594 (C=O).

N-methyl-2-(4-(4-(morpholine-4-carbonyl)-2-nitrophenyl)piperazine-1-yl)-N-(3-(4-(trifluoromethyl)phenoxy)propyl) pyrimidine-5-carboxamide(12c)

Chemical Formula: C_{31}H_{37}F_3N_8O_6; white solid; Yield: 38%; mp 40.2–42.3 °C; Purity: 95.09% (HPLC); 1H NMR (600 MHz, CDCl_3) δ 8.46 (s, pyrimidine ring, 2H), 7.93 (d, J = 2.1 Hz, -ArH, 1H), 7.59 (dd, J = 8.5, 2.1 Hz, -ArH, 1H), 7.53 (d, J = 8.4 Hz, -ArH, 2H), 7.17 (d, J = 8.5 Hz, -ArH, 1H), 6.93 (s, -ArH, 2H), 4.06 (q, J = 6.8, 5.1 Hz, piperazine ring and -CH_2-, 6H), 3.80–3.48 (m, morpholine ring and -OCH_3, 10H), 3.27–3.18 (m, piperazine ring, 4H), 3.12 (s, -NCH_3, 3H), 2.16 (s, -CH_2-, 2H); 13C NMR (151 MHz, CDCl_3) δ 167.94, 161.17, 157.44, 146.85, 141.60, 132.76, 127.98, 127.08, 126.95, 126.93, 126.90, 126.88, 125.92, 125.28, 123.48, 120.67, 118.35, 114.37, 66.76, 63.73, 50.99, 43.58, 38.58, 29.65, 21.95; ESI-MS calc for 658.2595 [M+H]⁺, found 658.2605 [M+H]⁺; IR (KBr), ν (cm⁻¹): 1616 (C=O), 1595 (C=O).

General procedure for the synthesis of target compounds 13a-13d

To a solution of compound 11 (400 mg, 0.91 mmol) in CH_2Cl_2 (10 mL) at 0 °C were added TBTU (440 mg, 1.37 mmol), DIEA (177 mg, 1.37 mmol) and N-(2-aminooethyl)benzamide derivatives (1.40 mmol). The reaction mixture was stirred at room temperature for 10 h, then washed with 5% aqueous HCl (10.0 mL), 5% aqueous NaOH (10.0 mL) and brine (20.0 mL). The organics were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel to afford target compounds 13a–13d.

2-(4-(4-(moropliine-4-carbonyl)-2-nitrophenyl)piperazine-1-yl)-N-(2-(4-(trifluoromethyl)benzamido)ethyl)pyrimidine-5-carboxamide(13a)

Chemical Formula: C_{32}H_{31}F_3N_8O_6; white solid; Yield: 58%; mp 165.4–167.9 °C; Purity: 98.64% (HPLC); 1H NMR (600 MHz, DMSO-d_6) δ 8.82 (d, J = 5.1 Hz, -NH-, 1H), 8.80 (s, pyrimidine ring, 2H), 8.52 (s, -NH-, 1H), 8.04 (d, J = 8.0 Hz, -ArH, 2H), 7.92 (d, J = 2.1 Hz, -ArH, 1H), 7.85 (d, J = 8.0 Hz, -ArH, 2H), 7.64 (dd, J = 8.6, 2.1 Hz, -ArH, 1H), 7.37 (d, J = 8.6 Hz, -ArH, 1H), 3.96 (t, J = 5.0 Hz, piperazine ring, 4H), 3.65–3.42 (m, -CH_2- and morpholine ring, 12H), 3.20 (t, J = 5.1 Hz, piperazine ring, 4H); 13C NMR (151 MHz, DMSO-d_6) δ 166.97, 165.33, 163.86, 161.37, 157.59, 145.80, 140.86, 138.32, 132.60, 128.08, 127.77, 125.24, 125.22, 120.93, 116.56, 79.14, 78.92, 78.70, 65.97, 62.79, 54.82, 50.25, 43.26, 38.61; ESI-MS calc for 657.2391 [M+H]⁺, found 657.2398 [M+H]⁺; IR (KBr), ν (cm⁻¹): 3246 (-CONH-), 1622 (C=O), 1594 (C=O).

2-(4-(4-(morpholine-4-carbonyl)-2-nitrophenyl)piperazin-1-yl)-N-(2-(4-nitrobenzamido)ethyl)pyrimidine-5-carboxamide(13b)

Chemical Formula: C_{32}H_{31}N_8O_6; white solid; Yield: 62%; mp 40.2–42.3 °C; Purity: 97.36% (HPLC); 1H NMR (600 MHz, DMSO-d_6) δ 8.91 (d, J = 5.6 Hz, -NH-, 1H), 8.8.0 (s, pyrimidine ring, 2H), 8.55–8.48 (m, -NH-, 1H), 8.34–8.28 (m, -ArH, 2H), 8.11–8.04 (m, -ArH, 2H), 7.92 (d, J = 2.0 Hz, -ArH, 1H), 7.65 (dd, J = 8.6, 2.1 Hz, -ArH, 1H), 7.38 (d, J = 8.6 Hz, -ArH, 1H), 3.96
(t, J = 5.1 Hz, piperazine ring, 4H), 3.66-3.39 (m, -CH2- and morpholine ring, 12H), 3.20 (t, J = 5.1 Hz, piperazine ring, 4H); 13C NMR (151 MHz, DMSO-d6) δ 166.96, 164.87, 163.86, 161.36, 157.60, 148.96, 145.80, 140.84, 140.21, 132.61, 128.68, 127.77, 125.23, 123.44, 120.93, 116.55, 65.97, 54.83, 50.24, 43.26, 38.56; ESI-MS calcd for 127.77, 125.22, 120.93, 118.30, 116.55, 113.50, 65.96, 157.59, 145.80, 140.85, 138.56, 132.61, 132.34, 128.05, 127.77, 126.22, 120.93, 118.30, 116.56, 113.50, 65.96, 54.84, 50.24, 43.26, 40.08, 38.56; ESI-MS calcd for 634.2368 [M + H]+; found 634.2377 [M + H]+; IR (KBr), ν (cm⁻¹): 3295 (-CONH-), 1635 (C=O), 1632 (C=O), 1596 (C=O).

N-(2-(4-cyanobenzoamido)ethyl)-2-(4-(4-morpholine-4-carbonyl)-2-nitrophenyl)piperazin-1-yl)pyrimidine-5-carboxamide(13c)

Chemical Formula: C30H33N9O8; white solid; Yield: 58%; mp 150.3–152.1 °C; Purity: 99.59% (HPLC); 1H NMR (600 MHz, DMSO-d6) δ 8.83 (d, J = 5.5 Hz, -NH-, 1H), 8.80 (s, pyrimidine ring, 2H), 8.51 (d, J = 5.5 Hz, -NH-, 1H), 8.00 (d, J = 8.5 Hz, -ArH, 2H), 7.96 (d, J = 8.4 Hz, -ArH, 2H), 7.92 (d, J = 2.1 Hz, -ArH, 1H), 7.65 (dd, J = 8.6, 2.1 Hz, -ArH, 1H), 7.38 (d, J = 8.6 Hz, -ArH, 1H), 3.96 (dd, J = 6.6, 3.7 Hz, piperazine ring, 4H), 3.68-3.41 (m, -CH2- and morpholine ring, 12H), 3.20 (t, J = 5.1 Hz, piperazine ring, 4H); 13C NMR (151 MHz, DMSO-d6) δ 166.97, 165.12, 163.86, 161.36, 157.59, 145.80, 140.85, 138.56, 132.61, 132.34, 128.05, 127.77, 125.22, 120.93, 118.30, 116.56, 113.50, 65.96, 54.84, 50.24, 43.26, 40.08, 38.58; ESI-MS calcd for 614.2470 [M + H]+, found 614.2478 [M + H]+; IR (KBr), ν (cm⁻¹): 3293 (-CONH-), 1632 (C=O), 1632 (C=O), 1591 (C=O).

2-(4-(4-morpholine-4-carbonyl)-2-nitrophenyl)piperazin-1-yl)-N-(3-(4-nitrobenzamido)propyl)pyrimidine-5-carboxamide(13d)

Chemical Formula: C30H33N9O8; white solid; Yield: 58%; mp 128.7–130.4 °C; Purity: 98.80% (HPLC); 1H NMR (600 MHz, DMSO-d6) δ 8.81 (d, J = 5.6 Hz, -NH-, 1H), 8.80 (s, pyrimidine ring, 2H), 8.39 (t, J = 5.6 Hz, -NH-, 1H), 8.34-8.27 (m, -ArH, 2H), 8.11-8.04 (m, -ArH, 2H), 7.92 (d, J = 2.1 Hz, -ArH, 1H), 7.65 (dd, J = 8.6, 2.1 Hz, -ArH, 1H), 7.38 (d, J = 8.6 Hz, -ArH, 1H), 3.96 (t, J = 5.1 Hz, piperazine ring, 4H), 3.61 (s, morpholine ring, 4H), 3.52 (s, -CH2-, 3H), 3.39-3.31 (m, -CH2- and morpholine ring, 7H), 3.20 (t, J = 5.1 Hz, piperazine ring, 4H); 13C NMR (151 MHz, DMSO-d6) δ 166.97, 164.55, 163.54, 161.35, 157.49, 148.91, 145.81, 140.85, 140.20, 132.60, 128.58, 127.76, 125.24, 123.42, 120.90, 116.54, 79.13, 78.92, 78.70, 65.97, 54.81, 50.25, 43.25, 40.09, 37.40, 36.85, 29.01; ESI-MS calcd for 648.2525 [M + H]+, found 648.2536 [M + H]+; IR (KBr), ν (cm⁻¹): 3299 (-CONH-), 1635 (C=O), 1596 (C=O).

AChE and BuChE Inhibition Assay

The assay was performed according to our previous reports [23, 24] based on the Ellman’s method.

Kinetic analysis of 6g

To determine the inhibition type of these compounds against electroporation AChE, a kinetic study was carried out with inhibitor 6g as the representative AChEI. In the process, the used concentrations of inhibitor 0.5×IC50, IC50, and 2×IC50 were 0.45, 0.90, and 1.80 μM, respectively. The type of inhibition was established from the analysis of Lineweaver-Burk reciprocal plots.

Molecular docking study

Molecular docking study of the most potent compound 6g in the active site of AChE and BuChE, respectively, was performed by Auto docking Tools using previously described method [24]. The center of the grid box was placed at the bottom of the active site gorge (AChE [-14.11 -43.83 -27.67]; BuChE [3.36 9.53 14.4]). The dimensions of the active site box were set at 36 × 36 × 36 Å.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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