Design, synthesis and evaluation of $N$-arylmethylamide derivatives as cholinesterase inhibitors

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Research Article

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Abstract

A series of N-arylmethylamide derivatives were designed and synthesized as cholinesterase inhibitors (ChEIs) for the treatment of Alzheimer's disease (AD). Furthermore, the compounds were assayed for their inhibitory activity to cholinesterase in vitro, and the results indicated that most of the compounds had moderate inhibitory activity to cholinesterase. Among them, compound 22j showed the best inhibitory activity against BuChE (IC\textsubscript{50} = 0.46 µM) and moderate inhibitory activity against AChE (IC\textsubscript{50} = 6.52 µM); remarkably, compound 22c was found to be a dual inhibitor of AChE (IC\textsubscript{50} = 1.11 µM) and BuChE (IC\textsubscript{50} = 1.14 µM). In addition, the results of molecular docking studies exhibited that 22j could simultaneously bind to both CAS and PAS of BuChE, which was consistent with the mixed mode of inhibition shown by enzyme kinetic studies of 22j. Moreover, the molecular properties of all compounds were predicted by the molinspiration server, and the compounds 22j and 22c matched the most properties of orally administered drugs. All these suggested that 22j and 22c could be considered as a lead compound for the development of AD drugs.

Introduction

Alzheimer's disease (AD) is a severe and irreversible progressive neurodegenerative disease of the central nervous system characterized by memory loss, disorientation, cognitive deficits and behavioral abnormalities [1]. Currently, AD affects more than 50 million people worldwide, and this number will continue to increase with the aging population growing [2]. In addition, the annual healthcare costs associated with AD amount to $820 billion, which will place a huge social and economic burden on patients and their families [3].

Researchers have made many efforts to treat AD, and various hypotheses have been proposed by researchers, such as the cholinergic hypothesis [4], the amyloid (Aβ) toxicity hypothesis [5], the Tau protein abnormality hypothesis [6], the neuroinflammatory hypothesis [7], the free radical damage hypothesis [8] and so on. Of these, the cholinergic hypothesis is one of the widely accepted hypotheses by researchers [9]. This hypothesis suggests that AD patients lose acetylcholine (ACh) in the brain during the onset of the disease, which in turn affects the patient's ability to focus and memory [10]. It has been confirmed that the decreasing of ACh in the brain is mainly caused by acetylcholinesterase (AChE) hydrolyzing. Therefore, the classical treatment of AD is to increase the concentration of ACh by inhibiting AChE activity, thereby improving the patient's cognitive level [11, 12]. Up to now, most of the drugs approved by the U.S. Food and Drug Administration (FDA) for the treatment of AD are acetylcholinesterase inhibitors (AChEIs), including tacrine, rivastigmine, donepezil, and galantamine [13] (Fig. 1). Currently, AChEIs are still one of the research hotspots, and many new AChEIs for the treatment of AD have been reported, such as compounds 5–7 [14–16] (Fig. 2).

With the in-depth study on AD, it was found that AChE is the main enzyme responsible for ACh metabolism in the normal brain and in the early stages of AD [17], but the AChE is 67% lower than normal level in stages of advanced AD, which lead to lose efficacy of AChEIs for treating advanced AD. However,
butyrylcholinesterase (BuChE) in the brain of advanced AD was found to be 165% higher than normal level [18, 19], which can also hydrolyze ACh [20]. Thereby, butyrylcholinesterase inhibitors (BuChEIs) may be used to treat advanced AD, and by now, many novel BuChEIs for the treatment of AD have been reported, such as compounds 8–10 [22–24] (Fig. 2).

Therefore, simultaneous inhibition of AChE and BuChE could be a beneficial treatment approach for AD. Based on this, compounds with AChE and BuChE inhibitory activities were designed for the treatment of AD in this paper, and their design philosophy was as follows: in a previous study, it was shown that compound 11 (Fig. 3) could inhibit AChE and BuChE with IC$_{50}$ value of 4.61 µM and 0.94 µM separately [25]. And structural analysis showed that the A region could enhance the inhibitory activity against BuChE. Therefore, the A region of compound 11 is the core molecule of its BuChE inhibitory activity. In addition, it was indicated that compound 12 (Fig. 3) possessed good ChE inhibitory activity (AChE: IC$_{50}$ = 0.22 µM; BuChE: IC$_{50}$ = 0.0016 µM) [26]. Moreover, molecular docking analysis showed that the B region could be associated with hydrogen bonding as well as π-π stacking with the PAS site of BuChE. Meanwhile, the B region could also produce hydrogen bonding as well as π-π stacking with the PAS site of AChE. Therefore, the A region of compound 11 was combined with the B region of compound 12 through an amide chain to produce a new compound 13 for inhibiting AChE and BuChE simultaneously; then under the guidance of molecular docking evaluation, the compound 13 was optimized and the target compounds were designed (Fig. 3).

Results and discussion

Chemical synthesis

The synthetic strategy for target compounds 22a-22v was depicted in Scheme 1. Commercially available compound 14a-14d was reacted with diamines protected by a Boc group to afford the intermediates 15a-15i. Then, compounds 15a-15i were reacted with different acyl chlorides to give compounds 16a-16n. After purification by column chromatography, Boc removal was achieved by dissolving 16a–16n in MeOH solution of HCl gas at 25°C for 6 hours, then the resulted free amines were immediately reacted with the monomethyl terephthalate to give target compounds 19a. After hydrolysis, compounds 20a were converted into compounds 22a-22i by reacting with different amines in the presence of O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium tetrafluoroborate (TBTU) and N,N-Diisopropylethylamine (DIEA). In addition, monomethyl terephthalate was reacted with 3-(trifluoromethyl)-benzylamine, which was hydrolyzed to afford compound 21a. The condensation reactions of compound 21a with compounds 17b-17n in the presence of TBTU and DIEA yielded the target compounds 22j-22v. The structures of new compounds confirmed by the $^1$H NMR, $^{13}$C NMR, HRMS and IR spectra, and the purity of all the target compounds was determined to be over 95.0% by high-performance liquid chromatography (HPLC) analysis.
AChE and BuChE assay

To evaluate the eeAChE and eqBuChE inhibitory activity of the target compounds, Ellman's method [27] was performed using Huperzine-A and tacrine as reference compounds, and the results were shown in Table 1. In general, all of the target compounds 22a-22v showed a certain inhibitory activity against eeAChE and eqBuChE with IC$_{50}$ values ranging from 0.46 to 38.79 µM. Among them, compound 22j (eeAChE, IC$_{50}$ = 0.46 µM; eqBuChE, IC$_{50}$ = 6.52 µM) was found to have the best inhibitory activity to eqBuChE and moderate inhibitory activity to eeAChE. In addition, it was found that compound 22c (eeAChE, IC$_{50}$ = 1.11 µM; eqBuChE, IC$_{50}$ = 1.14 µM) showed good inhibitory activity to both eeAChE and eqBuChE. For eqBuChE, when the substituent on benzene ring of R$_3$ is an electron-withdrawing group, the activity is significantly higher than that of the electron-donating group. For example, the activity of compound 22c with trifluoromethyl on benzene ring of R$_3$ is significantly higher than that of compound 22d with methoxy on benzene ring of R$_3$. However, the inhibitory activity against eqBuChE was significantly reduced when the R$_3$ group was changed to pyrazin-2-methyl and 2-(pyrrolidin-1-yl)ethyl. Moreover, when R$_2$ was propyl, the inhibitory effect on eqBuChE was the best; and the inhibitory effect on eqBuChE decreased with the increasing of n value. Furthermore, the inhibitory activity of eqBuChE was significantly reduced when the R$_1$ group was changed from the naphthalene to the biphenyl, anthracene and indole. For eeAChE, when the R$_3$ was benzyl or benzyl with substituents, the inhibitory activity is better than that of pyrazin-2-methyl or 2-(pyrrolidin-1-yl)ethyl. In addition, when R$_2$ was isopropyl and n is 1, the inhibitory effect on eeAChE was the best.

Kinetic analysis

In order to understand the inhibition mechanism of BuChE, kinetic analysis of BuChE inhibition was carried out for compound 22j. Lineweaver-Burk plots were plotted for three different concentrations of compound 22j with six different concentrations of substrates. As shown in Fig. 4, double reciprocal curves for different concentrations of 22j intersected in the second quadrant, which indicates that compound 22j inhibited BuChE in competitive and non-competitive manner simultaneously.

ADMET prediction

The properties of the synthesized compounds 22a-22v were predicted online using SwissADME (http://www.swissadme.ch). As shown in Table 3, the synthesized compounds were found to match the most properties of orally administered drugs.

Unfortunately, the most of the tested compounds could not cross the blood-brain barrier, thus they could not be used directly as oral drugs and needed optimizing further.

Molecular docking study

In order to explore the potential binding modes of compound 22j with AChE (PDB code: 4EY7) and BuChE (PDB code: 5K5E), molecular docking studies were performed using Autodock 4.2, and 3D docking results
were outputted using the software PyMOL (https://www.pymol.org/pymol.html). As shown in Fig. 5, the benzene ring of the F region of compound 22j could interact \( \pi-\pi \) with Tyr-341 and the benzene ring of the \( R_3 \) group could interact \( \pi-\pi \) with Trp-86. At the same time, hydrogen bonding between the amide bond in the E region of the benzene ring and Phe-295 was also observed. Therefore, compound 22j was tightly bound to the optimal conformation of AChE and stabilized in the cavity. As shown in Fig. 6, the F region of the benzene ring of compound 22j could interact \( \pi-\pi \) with Tyr-332. Meanwhile, the naphthalene ring of \( R_1 \) group could interact with Phe-329 and Trp-231. In addition, a hydrogen bond was observed between the amide bond in the G region and Pro-285. Thus, compound 22j was tightly bound to the optimal conformation of BuChE and stabilized in the cavity.

**Conclusion**

In summary, a series of \( N \)-arylmethylamide derivatives were designed, synthesized, and evaluated as potential ChEIs against AD. Biological assays demonstrated that all synthesized compounds possessed a certain inhibitory activity to AChE and BuChE. Among them, compound 22j was the most potent BuChE inhibitor (IC\(_{50}\) = 0.46 \( \mu \)M) and possessed AChE inhibitory activity (IC\(_{50}\) = 6.52 \( \mu \)M). In addition, the inhibitory activity of compound 22c to AChE (IC\(_{50}\) = 1.11 \( \mu \)M) and BuChE (IC\(_{50}\) = 1.14 \( \mu \)M) was good and equal. Furthermore, molecular docking studies showed that 22j was able to bind to the CAS and PAS of BuChE, which is consistent with the mixed mode of inhibition shown by enzyme kinetic studies. Moreover, the predicted ADMET results indicate that the designed compounds conform to most of the drug properties. Taken together, compounds 22j and 22c as the dual inhibitors of AChE and BuChE may be a promising lead compounds for the development of new anti-AD drugs.

**Experimental Section**

**Chemistry**

All experiments were carried out under air atmosphere, 2-naphthaldehyde, tert-butyl \( N \)-(2-aminoethyl)carbamate, mono-methyl terephthalate, 3-(trifluoromethyl)benzy-lamine 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 Bruker Maxis 4G 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. eeAChE and eqBuChE were purchased from Sigma, Huperzine-A and tacrine was purchased from Shanghai Yuanye Biotechnology Co., Ltd.
General procedure for the synthesis of target compounds 22a–22i

To a solution of compound 20a (2.0 mmol, 0.836 g) and 3, 5-bis(trifluoromethyl)benzylamine (2.0 mmol, 0.486 g) in CH₂Cl₂ (10 mL) at 0 °C were added TBTU (3.0 mmol, 0.963 g) and DIEA (3.0 mmol, 0.387 g). The reaction mixture was stirred at 25 °C for 4h. The completion of the reaction was assessed by TLC. Then washed with 5% aqueous HCl (15.0 mL), 5% aqueous NaOH (20.0 mL) and brine (20.0 mL). The organics was dried over Na₂SO₄, filtered, and concentrated in vacuo to give crude compound 22a. The crude compound 22a was purified by column chromatography on silica gel and recrystallized from CH₂Cl₂/MeOH (1:50) to afford target compound 22a (1.080 g, 84%). Compounds 22b-22i were prepared by the same method produces.

\[ \text{N}^1-(3,4\text{-bis(trifluoromethyl)benzyl)}-\text{N}^4-(2-(N-(naphthalen-2-ylmethyl) isobutyramido)ethyl)terephthalamide} \ (22a) \]

Chemical Formula: C₃₄H₃₁F₆N₃O₃; white solid; Yield: 84%; mp 167.1-167.6 °C; Purity: 95.15% (HPLC); \(^1\)H NMR (600 MHz, CDCl₃) \( \delta \) 7.86 (d, J = 5.9 Hz, -ArH, 5H), 7.82 (s, -ArH, 4H), 7.81 – 7.74 (m, -ArH, 3H), 7.57 (s, -NH- 1H), 7.53 – 7.42 (m, -CH₂, 2H), 7.14 (t, J = 6.0 Hz, -NH, 1H), 4.77 (s, -CH₂, 2H), 4.74 (d, J = 5.8 Hz, -CH₂, 2H), 3.76 – 3.72 (m, -CH₂, 2H), 3.62 – 3.58 (m, -CH₂, 2H), 2.89 – 2.80 (m, -CH, 1H), 1.11 (s, -CH₃, 3H), 1.10 (s, -CH₃, 3H); \(^13\)C NMR (151 MHz, CDCl₃) \( \delta \) 180.73, 167.02, 166.51, 141.03, 136.96, 136.31, 133.60, 133.38, 132.92, 132.36, 132.14, 131.92, 131.70, 129.12, 127.97, 127.79, 127.69, 127.44, 127.38, 126.75, 126.34, 124.82, 124.10, 124.02, 122.30, 121.56, 53.40, 51.47, 44.93, 43.26, 40.42, 30.70, 19.75; ESI-MS cacld for 644.2349 [M+H]⁺, found 644.2340 [M+H]⁺; IR (KBr), \( \nu \) (cm⁻¹): 3292 (-CONH-), 1643 (C=O), 1633 (C=O).

\[ \text{N}^1-(2-(N-(naphthalen-2-ylmethyl)isobutyramido)ethyl)-\text{N}^4-(3-(trifluoromethyl)benzyl)terephthalamide(22b) \]

Chemical Formula: C₃₃H₃₂F₃N₃O₃; white solid; Yield: 79%; mp 109.5-110.4 °C; Purity: 96.41% (HPLC); \(^1\)H NMR (600 MHz, CDCl₃) \( \delta \) 7.86 (d, J = 4.2 Hz, -ArH, 4H), 7.84 (d, J = 11.5 Hz, -ArH, 2H), 7.77 (s, -ArH, 2H), 7.60 (s, -ArH, 1H), 7.57 (s, -ArH, 1H), 7.56 (s, -ArH, 1H), 7.54 (s, -NH-, 1H), 7.52 – 7.49 (m, -ArH, 2H), 7.47 (s, -ArH, 1H), 7.46 (s, -ArH, 1H), 6.82 (t, J = 6.0 Hz, -NH-, 1H), 4.77 (s, -CH₂, 2H), 4.70 (d, J = 5.8 Hz, -CH₂, 2H), 3.79 – 3.71 (m, -CH₂, 2H), 3.65 – 3.56 (m, -CH₂, 2H), 2.89 – 2.80 (m, -CH, 1H), 1.10 (s, -CH₃, 3H), 1.09 (s, -CH₃, 3H); \(^13\)C NMR (151 MHz, CDCl₃) \( \delta \) 180.67, 166.81, 166.49, 139.22, 136.82, 136.62, 136.65, 133.39, 132.92, 131.28, 131.22, 131.01, 129.30, 129.10, 127.70, 127.41, 127.35, 127.30, 127.16, 126.31, 124.81, 124.58, 124.55, 124.48, 124.04, 51.41, 44.89, 43.67, 40.35, 34.70, 30.69, 19.76; ESI-MS cacld for 576.2475 [M+H]⁺, found 576.2480 [M+H]⁺; IR (KBr), \( \nu \) (cm⁻¹): 3318 (-CONH-), 1644 (C=O), 1639 (C=O).

\[ \text{N}^1-(2-(N-(naphthalen-2-ylmethyl)isobutyramido)ethyl)-\text{N}^4-(4-(trifluoromethyl)benzyl)terephthalamide(22c) \]
Chemical Formula: C$_{33}$H$_{32}$F$_3$N$_3$O$_3$; white solid; Yield: 87%; mp 168.5-169.1 °C; Purity: 96.19% (HPLC); $^1$H NMR (600 MHz, CDCl$_3$) δ 7.87 (d, J = 5.2 Hz, -ArH, 4H), 7.86 - 7.83 (m, -ArH, 2H), 7.78 (d, J = 2.7 Hz, -ArH, 2H), 7.62 - 7.56 (m, -ArH, 3H), 7.51 - 7.47 (m, -ArH, 4H), 7.28 (s, -NH-, 1H), 6.76 (t, J = 6.1 Hz, -NH-, 1H), 4.78 (s, -CH$_2$-2H), 4.71 (d, J = 5.8 Hz, -CH$_2$-, 2H), 3.78 - 3.72 (m, -CH$_2$-, 2H), 3.63 (d, J = 5.5 Hz, -CH$_2$-, 2H), 2.90 - 2.79 (m, -CH-, 1H), 1.11 (s, -CH$_3$, 3H), 1.10 (s, -CH$_3$, 3H); $^{13}$C NMR (151 MHz, CDCl$_3$) δ 180.70, 166.79, 166.45, 142.21, 136.85, 136.58, 133.63, 133.39, 132.93, 130.05, 129.84, 129.11, 128.06, 127.44, 127.28, 126.75, 126.32, 125.77, 125.74, 125.72, 125.69, 124.96, 124.82, 124.04, 123.16, 51.43, 44.89, 43.64, 40.38, 30.70, 26.92, 19.76; ESI-MS calcd for 576.2475 [M+H]$^+$, found 576.2482 [M+H]$^+$; IR (KBr), υ (cm$^{-1}$): 3318 (-CONH-), 1662 (C=O), 1642 (C=O).

$N^4$-(3,4-dimethoxybenzyl)-$N^4$-(2-(N-(naphthalen-2-ylmethyl)isobutyramido)-ethyl)terephthalamide(22d)

Chemical Formula: C$_{34}$H$_{37}$N$_3$O$_5$; white solid; Yield: 75%; mp 118.3-118.9 °C; Purity: 99.42% (HPLC); $^1$H NMR (600 MHz, CDCl$_3$) δ 7.86 (d, J = 3.2 Hz, -ArH, 1H), 7.85 (d, J = 3.4 Hz, -ArH, 2H), 7.84 (s, -ArH, 2H), 7.83 (s, -ArH, 1H), 7.83 - 7.76 (m, -ArH, 2H), 7.76 - 7.71 (m, -ArH, 1H), 7.57 (s, -ArH, 1H), 7.53 - 7.47 (m, -ArH, 2H), 6.90 (d, J = 8.3 Hz, -ArH, 2H), 6.84 (d, J = 7.9 Hz, -NH-, 1H), 6.56 (t, J = 5.6 Hz, -NH-, 1H), 4.77 (s, -CH$_2$-, 2H), 4.58 (d, J = 5.5 Hz, -CH$_2$-, 2H), 3.87 (s, -CH$_3$, 3H), 3.86 (s, -CH$_3$, 3H), 3.77 - 3.72 (m, -CH$_2$-, 2H), 3.65 - 3.61 (m, -CH$_2$-, 2H), 2.87 - 2.80 (m, -CH-, 1H), 1.10 (s, -CH$_3$, 3H), 1.09 (s, -CH$_3$, 3H); $^{13}$C NMR (151 MHz, CDCl$_3$) δ 180.61, 166.55, 166.52, 149.31, 148.72, 136.98, 136.65, 133.69, 133.40, 130.61, 127.36, 127.22, 126.72, 124.81, 124.05, 120.35, 111.50, 111.41, 55.98, 55.96, 53.40, 51.38, 44.88, 44.13, 40.28, 30.69, 19.76; ESI-MS calcd for 568.2812 [M+H]$^+$, found 568.2815 [M+H]$^+$; IR (KBr), υ (cm$^{-1}$): 3316 (-CONH-), 1657 (C=O), 1636 (C=O).

$N^4$-(4-methoxybenzyl)-$N^4$-(2-(N-(naphthalen-2-ylmethyl)isobutyramido)-ethyl)terephthalamide(22e)

Chemical Formula: C$_{33}$H$_{35}$N$_3$O$_4$; white solid; Yield: 77%; mp 181.4-181.9 °C; Purity: 99.16% (HPLC); $^1$H NMR (600 MHz, CDCl$_3$) δ 7.87 (d, J = 3.3 Hz, -ArH, 1H), 7.85 (d, J = 6.6 Hz, -ArH, 4H), 7.83 (d, J = 2.3 Hz, -ArH, 1H), 7.79 (d, J = 2.6 Hz, -ArH, 1H), 7.73 (s, -ArH, 1H), 7.57 (s, -ArH, 1H), 7.52 - 7.47 (m, -ArH, 2H), 7.30 - 7.26 (m, -ArH, 2H), 6.95 (d, J = 7.6 Hz, -ArH, 1H), 6.91 (s, -NH-, 1H), 6.86 - 6.82 (m, -ArH, 1H), 6.57 (d, J = 5.6 Hz, -NH-, 1H), 4.78 (s, -CH$_2$-, 2H), 4.63 (d, J = 5.6 Hz, -CH$_2$-, 2H), 3.80 (s, -CH$_3$, 3H), 3.77 - 3.72 (m, -CH$_2$-, 2H), 3.66 - 3.60 (m, -CH$_2$-, 2H), 2.88 - 2.79 (m, -CH-, 1H), 1.11 (s, -CH$_3$, 3H), 1.10 (s, -CH$_3$, 3H); $^{13}$C NMR (151 MHz, CDCl$_3$) δ 180.63, 166.61, 166.52, 160.01, 139.58, 136.92, 136.65, 133.70, 133.40, 132.92, 129.89, 129.09, 127.78, 127.37, 127.29, 127.11, 126.73, 124.81, 124.06, 120.17, 113.63, 113.12, 113.02, 55.27, 51.37, 44.86, 44.22, 40.28, 30.69, 26.92, 19.77; ESI-MS calcd for 538.2707 [M+H]$^+$, found 538.2719 [M+H]$^+$; IR (KBr), υ (cm$^{-1}$): 3301 (-CONH-), 1656 (C=O), 1628 (C=O).

$N^4$-benzyl-$N^4$-(2-(N-(naphthalen-2-ylmethyl)isobutyramido)ethyl)terephthalamide(22f)
Chemical Formula: $C_{32}H_{33}N_3O_3$; white solid; Yield: 76%; mp 130.5-131.1 °C; Purity: 96.06% (HPLC); $^1$H NMR (600 MHz, CDCl$_3$) δ 7.87 (d, J = 2.7 Hz, -ArH, 1H), 7.86 - 7.85 (m, -ArH, 2H), 7.84 (s, -ArH, 2H), 7.83 (d, J = 2.3 Hz, -ArH, 1H), 7.78 (d, J = 2.8 Hz, -ArH, 1H), 7.73 (d, J = 6.2 Hz, -ArH, 1H), 7.57 (s, -NH$_2$, 1H), 7.53 - 7.47 (m, -ArH, 2H), 7.37 (s, -ArH, 2H), 7.36 (d, J = 1.6 Hz, -ArH, 2H), 7.30 (d, J = 2.7 Hz, -ArH, 2H), 6.60 (t, J = 5.7 Hz, -NH$_2$, 1H), 4.77 (s, -CH$_2$-2H), 4.65 (d, J = 5.6 Hz, -CH$_2$-2H), 3.77 - 3.72 (m, -CH$_2$-2H), 3.66 - 3.60 (m, -CH$_2$-2H), 2.88 - 2.79 (m, -CH$_3$, 1H), 1.10 (s, -CH$_3$, 3H), 1.09 (s, -CH$_3$, 3H); $^{13}$C NMR (151 MHz, CDCl$_3$) δ 180.62, 166.63, 166.54, 138.01, 136.94, 136.64, 133.69, 133.40, 132.92, 129.09, 128.84, 127.97, 127.78, 127.71, 127.36, 127.28, 127.24, 126.73, 126.30, 124.81, 124.06, 51.37, 44.86, 44.25, 40.26, 30.69, 29.69, 19.76; ESI-MS calcd for 508.2601 [M+H]+, found 508.2593 [M+H]+; IR (KBr), ν (cm$^{-1}$): 3318 (-CONH-), 1662 (C=O), 1634 (C=O).

$^N$-benzhydryl-$^N$-(2-(N-(naphthalen-2-ylmethyl)isobutyramido)ethyl)terephthalamide(22g)

Chemical Formula: $C_{38}H_{37}N_3O_3$; white solid; Yield: 77%; mp 145.3-145.8 °C; Purity: 99.22% (HPLC); $^1$H NMR (600 MHz, CDCl$_3$) δ 7.86 (d, J = 2.8 Hz, -ArH, 4H), 7.85 (d, J = 3.2 Hz, -ArH, 2H), 7.78 (d, J = 2.6 Hz, -ArH, 1H), 7.74 (s, -ArH, 1H), 7.58 (s, -ArH, 1H), 7.50 (t, J = 2.8 Hz, -ArH, 2H), 7.37 (d, J = 2.0 Hz, -ArH, 1H), 7.36 (s, -ArH, 2H), 7.34 (s, -ArH, 2H), 7.32 (d, J = 1.7 Hz, -ArH, 2H), 7.31 - 7.30 (m, -ArH, 2H), 7.29 (s, -NH$_2$, 1H), 7.28 (d, J = 1.7 Hz, -ArH, 1H), 6.84 (d, J = 7.9 Hz, -ArH, 1H), 6.46 (d, J = 7.8 Hz, -CH$_3$, 1H), 4.77 (s, -CH$_2$-2H), 3.79 - 3.71 (m, -CH$_2$-2H), 3.67 - 3.59 (m, -CH$_2$-2H), 2.89 - 2.77 (m, -CH$_3$, 1H), 1.11 (s, -CH$_3$, 3H), 1.10 (s, -CH$_3$, 3H); $^{13}$C NMR (151 MHz, CDCl$_3$) δ 180.62, 166.47, 165.81, 141.31, 136.78, 133.70, 133.41, 132.92, 129.09, 128.79, 128.62, 127.79, 127.71, 127.65, 127.53, 127.39, 127.33, 127.14, 126.73, 126.30, 124.81, 124.06, 57.58, 53.40, 51.42, 44.92, 40.32, 30.70, 19.78; ESI-MS calcd for 584.2914 [M+H]+, found 584.2925 [M+H]+; IR (KBr), ν (cm$^{-1}$): 3302 (-CONH-), 1652 (C=O), 1634 (C=O).

$^N$-(2-(N-(naphthalen-2-ylmethyl)isobutyramido)ethyl)-$^N$-(pyrazin-2-ylmeth-yl)terephthalamide(22h)

Chemical Formula: $C_{30}H_{31}N_3O_3$; white solid; Yield: 78%; mp 123.8-124.3 °C; Purity: 96.35% (HPLC); $^1$H NMR (600 MHz, CDCl$_3$) δ 8.70 (s, -ArH, 1H), 8.59 - 8.50 (m, -ArH, 2H), 7.90 (d, J = 2.3 Hz, -ArH, 4H), 7.85 (d, J = 3.1 Hz, -ArH, 2H), 7.78 (s, -ArH, 1H), 7.76 (d, J = 4.5 Hz, -ArH, 1H), 7.58 (s, -NH$_2$, 1H), 7.50 (t, J = 3.7 Hz, -ArH, 2H), 7.45 (t, J = 5.2 Hz, -ArH, 1H), 7.29 (d, J = 1.8 Hz, -NH$_2$, 1H), 4.83 (d, J = 5.1 Hz, -CH$_2$-2H), 4.79 (s, -CH$_2$-2H), 3.82 - 3.71 (m, -CH$_2$-2H), 3.69 - 3.61 (m, -CH$_2$-2H), 2.93 - 2.77 (m, -CH$_3$, 1H), 1.11 (s, -CH$_3$, 3H), 1.10 (s, -CH$_3$, 3H); $^{13}$C NMR (151 MHz, CDCl$_3$) δ 201.56, 180.62, 166.77, 166.50, 152.14, 144.22, 143.67, 143.63, 136.87, 136.48, 133.70, 133.40, 132.92, 129.09, 127.78, 127.71, 127.40, 127.35, 127.16, 126.73, 126.30, 124.82, 124.06, 51.39, 44.89, 42.71, 40.28, 30.69, 29.68, 19.76; ESI-MS calcd for 510.2506 [M+H]+, found 510.2511 [M+H]+; IR (KBr), ν (cm$^{-1}$): 3318 (-CONH-), 1672 (C=O), 1645 (C=O).

$^N$-(2-(N-(naphthalen-2-ylmethyl)isobutyramido)ethyl)-$^N$-(2-(pyrrolidin-1-yl)-ethyl)terephthalamide(22i)
Chemical Formula: C_{31}H_{38}N_{4}O_{3}; white solid; Yield: 80%; mp 119.5-121.3 °C; Purity: 97.71% (HPLC); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 8.71 (s, -ArH, 1H), 8.03 (s, -NH-, 1H), 8.01 (s, -ArH, 1H), 7.84 (d, \(J = 8.7\) Hz, -ArH, 2H), 7.81 (d, \(J = 8.2\) Hz, -ArH, 2H), 7.78 (d, \(J = 9.0\) Hz, -ArH, 2H), 7.64 (s, -ArH, 1H), 7.57 (s, -NH-, 1H), 7.48 (t, \(J = 3.2\) Hz, -ArH, 2H), 4.78 (s, -CH\(_2\), 2H), 3.87 (t, \(J = 5.6\) Hz, -CH\(_2\), 2H), 3.77 − 3.71 (m, -CH\(_2\), 2H), 3.67 − 3.60 (m, -CH\(_2\), 2H), 3.37 − 3.26 (m, -CH\(_2\), 6H), 2.87 − 2.78 (m, \(J = 6.1, 5.5\) Hz, -CH\(_2\), 1H), 2.09 − 2.07 (m, -CH\(_2\), 4H), 1.09 (s, -CH\(_3\), 3H), 1.07 (s, -CH\(_3\), 3H); \(^13\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 180.29, 167.18, 166.74, 136.82, 135.91, 133.90, 133.40, 132.89, 129.02, 127.76, 127.72, 127.12, 126.22, 125.90, 124.80, 124.12, 118.88, 55.81, 54.49, 51.34, 44.91, 39.92, 36.61, 31.91, 30.66, 29.67, 23.30, 19.75, 14.08; ESI-MS calcd for 515.3023 [M+H]+, found 515.3015 [M+H]+; IR (KBr), \(\nu\) (cm\(^{-1}\)): 3336 (-CONH), 1662 (C=O), 1547 (C=O).

**General procedure for the synthesis of target compounds 22j–22v**

To a solution of compound 21a (2.0 mmol, 0.646 g) and compound 17b (2.0 mmol, 0.540 g) in CH\(_2\)Cl\(_2\) (10.0 mL) at 0 °C were added TBTU (3.0 mmol, 0.963 g) and DIEA (3.0 mmol, 0.387 g). The reaction mixture was stirred at 25 °C for 4h. The completion of the reaction was assessed by TLC. Then washed with 5% aqueous HCl (15.0 mL), 5% aqueous NaOH (20.0 mL) and brine (20.0 mL). The organics was dried over Na\(_2\)SO\(_4\), filtered, and concentrated in vacuo to give crude compound 22j. The crude compound 22j was purified by column chromatography on silica gel and recrystallized from CH\(_2\)Cl\(_2\)/MeOH (1:45) to afford target compound 22j (1.007 g, 77%). Compounds 22k–22v were prepared by the same method produces.

\(N^1\)-(2-((N-(naphthalen-2-ylmethyl)butyramido)ethyl)-\(N^4\)-(3-(trifluoromethyl)-benzyl)terephthalamide(22j)

Chemical Formula: C\(_{33}\)H\(_{32}\)F\(_3\)N\(_2\)O\(_3\); white solid; Yield: 77%; mp 159.0-159.7 °C; Purity: 97.00% (HPLC); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 7.82 (d, \(J = 8.0\) Hz, -ArH, 2H), 7.79 (d, \(J = 9.7\) Hz, -ArH, 3H), 7.59 (s, -ArH, 1H), 7.56 (s, -ArH, 1H), 7.53 (s, -ArH, 1H), 7.52 (d, \(J = 1.7\) Hz, -ArH, 2H), 7.51 − 7.50 (m, -ArH, 1H), 7.50 (s, -ArH, 1H), 7.49 − 7.48 (m, -ArH, 1H), 7.45 (d, \(J = 7.7\) Hz, -ArH, 1H), 7.18 (d, \(J = 8.4\) Hz, -ArH, 1H), 6.67 (s, -NH-, 1H), 6.34 (s, -NH-, 1H), 4.69 − 4.66 (m, -CH\(_2\), 4H), 3.71 (t, \(J = 5.7\) Hz, -CH\(_2\), 2H), 3.57 − 3.49 (m, -CH\(_2\), 2H), 2.16 (t, \(J = 7.6\) Hz, -CH\(_2\), 2H), 1.67 − 1.62 (m, -CH\(_2\), 2H), 0.94 (t, \(J = 7.3\) Hz, -CH\(_3\), 3H); \(^13\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 174.44, 172.58, 166.55, 139.10, 138.96, 135.38, 133.31, 132.91, 131.25, 131.01, 129.30, 129.05, 127.77, 127.73, 127.44, 126.97, 126.70, 126.36, 125.70, 124.51, 124.43, 52.84, 44.08, 43.64, 38.70, 37.96, 19.08, 13.84; ESI-MS calcd for 576.2475 [M+H]+, found 576.2469 [M+H]+; IR (KBr), \(\nu\) (cm\(^{-1}\)): 3297 (-CONH), 1665 (C=O), 1626 (C=O).

\(N^1\)-(2-((N-(naphthalen-2-ylmethyl)pentanamido)ethyl)-\(N^4\)-(3-(trifluoromethyl)-benzyl)terephthalamide(22k)

Chemical Formula: C\(_{34}\)H\(_{34}\)F\(_3\)N\(_2\)O\(_3\); white solid; Yield: 80%; mp 131.7-132.3 °C; Purity: 99.23% (HPLC); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 7.86 (d, \(J = 6.9\) Hz, -ArH, 5H), 7.83 (d, \(J = 2.4\) Hz, -NH-, 1H), 7.79 (d, \(J = 2.8\) Hz, -ArH, 2H), 7.60 (s, -ArH, 1H), 7.58 (s, -NH-, 1H), 7.56 (s, 2H), 7.55 (s, -ArH, 1H), 7.50 (dd, \(J = 6.4, 3.4\) Hz, -ArH, 2H), 7.47 (s, -ArH, 1H), 6.80 (s, -ArH, 1H), 4.75 (s, -CH\(_2\), 2H), 4.71 (d, \(J = 5.1\) Hz, -CH\(_2\), 2H), 3.74 (s, -CH\(_2\),
(s, -CH₂, 2H), 2.42 (t, J = 7.5 Hz, -CH₂, 2H), 1.61 (t, J = 7.4 Hz, -CH₂, 2H), 1.28 (q, J = 7.5 Hz, -CH₂, 2H), 0.82 (t, J = 7.3 Hz, -CH₃, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 176.57, 166.79, 166.50, 139.21, 136.87, 136.61, 133.40, 132.94, 131.27, 131.03, 129.30, 129.13, 127.78, 127.72, 127.45, 127.30, 126.74, 126.32, 124.96, 124.56, 124.54, 124.51, 124.48, 124.15, 123.09, 51.95, 45.10, 43.68, 40.52, 33.14, 27.53, 22.44, 13.76; ESI-MS calcd for 590.2631 [M+H]⁺, found 590.2639 [M+H]⁺; IR (KBr), v (cm⁻¹): 3310 (-CONH), 1652 (C=O), 1636 (C=O).

**N¹-(2-(3-methyl-N-(naphthalen-2-ylmethyl)butanamido)ethyl)-N⁴-(3-(trifluoromethyl)benzyl)terephthalamide(22l)**

Chemical Formula: C₃₄H₃₄F₃N₃O₃; white solid; Yield: 81%; mp 167.5-168.3 °C; Purity: 99.51% (HPLC); ¹H NMR (600 MHz, CDCl₃) δ 7.88 (d, J = 8.1 Hz, -ArH, 3H), 7.85 (d, J = 8.3 Hz, -ArH, 4H), 7.81 – 7.77 (m, -NH, 1H), 7.61 (s, -ArH, 2H), 7.58 – 7.54 (m, -ArH, 3H), 7.52 – 7.45 (m, -ArH, 3H), 6.80 (s, -NH, 1H), 4.75 (s, -CH₂, 2H), 4.70 (d, J = 5.6 Hz, -CH₂, 2H), 3.76 – 3.72 (m, -CH₂, 2H), 3.59 (t, J = 5.2 Hz, -CH₂, 2H), 2.31 (d, J = 7.0 Hz, -CH₂, 2H), 2.25 – 2.17 (m, -CH, 1H), 0.91 (s, -CH₃, 3H), 0.90 (s, -CH₃, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 175.93, 166.79, 166.43, 139.23, 136.82, 136.61, 133.39, 133.35, 132.95, 131.27, 129.30, 129.12, 127.79, 127.33, 127.28, 126.74, 126.33, 124.96, 124.57, 124.55, 124.50, 124.47, 124.45, 124.13, 123.09, 51.90, 45.01, 43.67, 42.14, 40.69, 26.92, 25.87, 22.57; ESI-MS calcd for 590.2631 [M+H]⁺, found 590.2623 [M+H]⁺; IR (KBr), v (cm⁻¹): 3310 (-CONH), 1668 (C=O), 1636 (C=O).

**N¹-(2-(N-(naphthalen-2-ylmethyl)hexanamido)ethyl)-N⁴-(3-(trifluoromethyl)benzyl)terephthalamide(22m)**

Chemical Formula: C₃₅H₃₅F₃N₃O₃; white solid; Yield: 78%; mp 155.4-155.8 °C; Purity: 98.11% (HPLC); ¹H NMR (600 MHz, CDCl₃) δ 7.99 – 7.81 (m, -ArH, 5H), 7.81 – 7.71 (m, -ArH, 2H), 7.60 (s, -ArH, 2H), 7.55 (d, J = 7.6 Hz, -ArH, 2H), 7.51 (s, -NH, 1H), 7.50 (d, J = 6.1 Hz, -ArH, 4H), 6.83 (s, -NH, 1H), 5.11 – 4.43 (m, -CH₂, 4H), 4.01 – 2.99 (m, -CH₂, 4H), 2.52 – 2.10 (m, -CH₂, 2H), 1.64 (s, -CH₂, 2H), 1.30 (s, -CH₂, 2H), 1.23 (s, -CH₂, 2H), 0.78 (d, J = 6.1 Hz, -CH₃, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 177.15, 166.83, 166.59, 139.28, 136.84, 136.64, 133.46, 133.40, 132.94, 131.33, 131.19, 130.98, 129.31, 129.16, 127.80, 127.77, 127.48, 126.76, 126.33, 124.90, 124.58, 124.46, 124.26, 123.10, 43.72, 40.42, 33.57, 33.48, 31.54, 26.79, 25.19, 22.43, 13.87; ESI-MS calcd for 604.2788 [M+H]⁺, found 604.2779 [M+H]⁺; IR (KBr), v (cm⁻¹): 3305 (-CONH), 1663 (C=O), 1636 (C=O).

**N¹-(2-(N-(naphthalen-2-ylmethyl)heptanamido)ethyl)-N⁴-(3-(trifluoromethyl)benzyl)terephthalamide(22n)**

Chemical Formula: C₃₆H₃₆F₃N₃O₃; white solid; Yield: 79%; mp 163.4-163.9 °C; Purity: 96.48% (HPLC); ¹H NMR (600 MHz, CDCl₃) δ 7.85 (d, J = 9.5 Hz, -ArH, 6H), 7.80 (s, -ArH, 1H), 7.60 (s, -ArH, 1H), 7.57 (d, J = 9.0 Hz, -ArH, 2H), 7.55 (d, J = 8.3 Hz, -ArH, 2H), 7.51 – 7.48 (m, -ArH, 3H), 7.45 (d, J = 7.7 Hz, -NH, 1H), 6.89 (s, -NH, 1H), 4.74 (s, -CH₂, 2H), 4.70 (s, -CH₂, 2H), 3.74 (s, -CH₂, 2H), 3.59 (s, -CH₂, 2H), 2.41 (t, J = 7.5 Hz, -CH₂, 2H), 1.77 (s, -CH₂, 2H), 1.61 (d, J = 7.4 Hz, -CH₂, 2H), 1.27 – 1.25 (m, -CH₂, 2H), 1.17 (t, J = 3.8 Hz, -
CH₂−, 2H), 0.81 − 0.74 (m, -CH₃, 3H);¹³C NMR (151 MHz, CDCl₃) δ 176.56, 166.79, 166.52, 139.26, 136.83, 136.60, 133.45, 133.39, 133.33, 132.93, 131.29, 130.99, 129.30, 129.28, 129.13, 127.79, 127.35, 126.74, 126.32, 124.96, 124.54, 124.48, 124.46, 124.19, 123.09, 45.14, 43.67, 40.57, 33.45, 31.52, 29.69, 29.03, 25.44, 22.43, 13.94; ESI-MS calcd for 618.2944 [M+H]+, found 618.2933 [M+H]+; IR (KBr), ν (cm⁻¹): 3305 (-CONH), 1647 (C=O), 1634 (C=O).

N¹-(2-(N-(naphthalen-2-ylmethyl)octanamido)ethyl)-N¹-(3-( trifluoromethyl)-benzyl)terephthalamide(22o)

Chemical Formula: C₃₇H₄₆F₉N₃O₃; white solid; Yield: 80%; mp 132.4-132.6 °C; Purity: 97.35% (HPLC);¹H NMR (600 MHz, CDCl₃) δ 7.88 − 7.80 (m, -ArH, 3H), 7.79 (d, J = 7.1 Hz, -ArH, 2H), 7.59 (s, -NH, 1H), 7.56 (s, -ArH, 1H), 7.55 − 7.48 (m, -ArH, 5H), 7.44 (t, J = 7.6 Hz, -ArH, 2H), 7.17 (d, J = 8.3 Hz, -ArH, 1H), 6.68 (s, -NH, 1H), 6.32 (s, -ArH, 1H), 4.67 (d, J = 6.6 Hz, -CH₂⁻, 4H), 3.80 − 3.19 (m, -CH₂⁻, 4H), 2.16 (d, J = 7.8 Hz, -CH₂, 2H), 1.61 (t, J = 7.3 Hz, -CH₂, 2H), 1.35 − 1.15 (m, -CH₂, 8H), 0.85 (t, J = 6.9 Hz, -CH₃, 3H);¹³C NMR (151 MHz, CDCl₃) δ 173.88, 172.54, 166.54, 139.11, 138.96, 135.38, 133.32, 132.90, 131.24, 131.22, 131.01, 129.29, 129.04, 127.77, 127.43, 127.00, 126.70, 126.35, 125.71, 124.50, 52.87, 44.13, 43.64, 37.95, 36.81, 31.67, 29.29, 28.99, 25.70, 22.59, 14.04; ESI-MS calcd for 632.3101 [M+H]+, found 632.3110 [M+H]+; IR (KBr), ν (cm⁻¹): 3307 (-CONH), 1642 (C=O), 1627 (C=O).

N¹-(3-(N-(naphthalen-2-ylmethyl)butyramido)propyl)-N¹-(3-( trifluoromethyl)-benzyl)terephthalamide(22p)

Chemical Formula: C₃₄H₃₄F₉N₃O₃; white solid; Yield: 76%; mp 145.1-145.7 °C; Purity: 98.19% (HPLC);¹H NMR (600 MHz, CDCl₃) δ 8.08 (t, J = 6.1 Hz, -NH, 1H), 7.96 (d, J = 8.0 Hz, -ArH, 2H), 7.86 (d, J = 8.1 Hz, -ArH, 4H), 7.80 (d, J = 2.2 Hz, -ArH, 1H), 7.61 (s, -NH, 1H), 7.59 − 7.53 (m, -ArH, 3H), 7.50 (t, J = 2.8 Hz, -ArH, 2H), 7.46 (t, J = 7.8 Hz, -ArH, 2H), 6.92 (t, J = 5.9 Hz, -ArH, 1H), 4.70 (d, J = 4.8 Hz, -CH₂⁻, 4H), 3.59 (t, J = 6.0 Hz, -CH₂⁻, 2H), 3.44 − 3.37 (m, -CH₂⁻, 2H), 2.42 (t, J = 7.5 Hz, -CH₂⁻, 2H), 1.77 − 1.68 (m, -CH₂⁻, 4H), 0.94 (t, J = 7.4 Hz, -CH₃, 3H);¹³C NMR (151 MHz, CDCl₃) δ 175.17, 166.92, 166.16, 139.16, 137.50, 136.50, 133.56, 133.40, 132.90, 131.24, 131.20, 129.27, 129.03, 127.69, 127.47, 127.28, 126.71, 126.27, 124.91, 124.76, 124.48, 124.44, 123.11, 51.03, 43.64, 42.35, 35.93, 35.32, 26.94, 19.04, 13.93; ESI-MS calcd for 590.2631 [M+H]+, found 590.2627 [M+H]+; IR (KBr), ν (cm⁻¹): 3302 (-CONH), 1665 (C=O), 1639 (C=O).

N¹-(4-(N-(naphthalen-2-ylmethyl)butyramido)butyl)-N¹-(3-( trifluoromethyl)-benzyl)terephthalamide(22q)

Chemical Formula: C₃₅H₃₆F₉N₃O₃; white solid; Yield: 78%; mp 116.5-117.2 °C; Purity: 98.87% (HPLC);¹H NMR (600 MHz, CDCl₃) δ 7.86 (d, J = 8.1 Hz, -ArH, 2H), 7.83 (d, J = 5.1 Hz, -ArH, 2H), 7.80 (d, J = 8.0 Hz, -ArH, 4H), 7.74 (d, J = 2.5 Hz, -NH, 1H), 7.61 (s, -ArH, 1H), 7.55 (s, -ArH, 2H), 7.53 (s, -ArH, 1H), 7.48 (t, J = 2.3 Hz, -NH, 1H), 7.45 (d, J = 3.5 Hz, -ArH, 1H), 7.39 (s, -ArH, 1H), 7.11 (s, -ArH, 1H), 4.70 (s, -CH₂⁻, 2H), 4.67 (s, -CH₂⁻, 2H), 3.50 − 3.41 (m, -CH₂⁻, 4H), 2.36 (t, J = 7.5 Hz, -CH₂⁻, 2H), 1.71 − 1.66 (m, -CH₂⁻, 2H), 1.66 − 1.63 (m, -CH₂⁻, 2H), 1.59 − 1.56 (m, -CH₂⁻, 2H), 0.91 (t, J = 7.4 Hz, -CH₃, 3H);¹³C NMR (151 MHz, CDCl₃) δ 173.96, 173.23, 166.65, 139.34, 139.24, 137.39, 136.54, 135.37, 134.12, 133.37, 132.83, 131.25, 129.25,
N^1-(5-(N-(naphthalen-2-ylmethyl)butyramido)pentyl)-N^4-(3-(trifluoromethyl)-benzyl)terephthalamide (22r)

Chemical NMR (600 MHz, CDCl₃) δ 7.82 (d, J = 6.8 Hz, -ArH, 3H), 7.80 (d, J = 2.1 Hz, -ArH, 3H), 7.75 (d, J = 5.3 Hz, -ArH, 1H), 7.6 (s, -NH, 1H), 7.55 (s, -ArH, 1H), 7.54 (s, -ArH, 1H), 7.52 (s, -ArH, 1H), 7.49 (t, J = 2.5 Hz, -ArH, 2H), 7.43 (d, J = 3.4 Hz, -ArH, 2H), 7.13 (d, J = 6.1 Hz, -ArH, 1H), 6.88 (d, J = 5.8 Hz, -NH, 1H), 4.69 (s, -CH₂, 2H), 4.67 (s, -CH₂, 2H), 3.44 (t, J = 7.0 Hz, -CH₂, 2H), 3.40 – 3.37 (m, -CH₂, 2H), 2.31 (t, J = 7.5 Hz, -CH₂, 2H), 1.85 (s, -CH₂, 2H), 1.64 (t, J = 7.9 Hz, -CH₂, 2H), 1.60 – 1.58 (m, -CH₂, 2H), 1.33 (t, J = 7.8 Hz, -CH₂, 2H), 0.84 (t, J = 7.4 Hz, -CH₃, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 174.07, 173.22, 166.91, 139.33, 137.53, 136.55, 135.42, 134.21, 133.39, 132.80, 131.25, 129.23, 128.85, 128.43, 127.75, 127.69, 127.42, 127.21, 126.60, 126.18, 126.13, 124.66, 124.54, 124.26, 123.11, 51.11, 45.28, 43.61, 40.11, 35.29, 28.45, 27.15, 23.95, 18.85, 13.88; ESI-MS caclcd for 618.2944 [M+H]⁺, found 618.2949 [M+H]⁺; IR (KBr), ν (cm⁻¹): 3304 (-CONH-), 1642 (C=O), 1632 (C=O).

N^1-(6-(N-(naphthalen-2-ylmethyl)butyramido)hexyl)-N^4-(3-(trifluoromethyl)-benzyl)terephthalamide (22s)

Chemical NMR (600 MHz, CDCl₃) δ 7.83 (d, J = 2.9 Hz, -ArH, 2H), 7.80 (d, J = 2.6 Hz, -ArH, 4H), 7.75 (s, -ArH, 1H), 7.72 (d, J = 7.8 Hz, -ArH, 1H), 7.59 (s, -ArH, 2H), 7.53 (d, J = 5.8 Hz, -ArH, 2H), 7.48 (d, J = 1.9 Hz, -ArH, 1H), 7.43 (d, J = 2.7 Hz, -ArH, 2H), 7.32 (d, J = 1.6 Hz, -NH, 1H), 7.07 (d, J = 6.7 Hz, -NH, 1H), 4.72 (s, -CH₂, 2H), 4.66 (d, J = 4.8 Hz, -CH₂, 2H), 3.42 (t, J = 7.2 Hz, -CH₂, 2H), 3.36 (d, J = 6.3 Hz, -CH₂, 2H), 2.33 (t, J = 7.5 Hz, -CH₂, 2H), 1.64 (q, J = 7.4 Hz, -CH₂, 2H), 1.56 (d, J = 7.2 Hz, -CH₂, 2H), 1.54 (d, J = 7.3 Hz, -CH₂, 2H), 1.37 (t, J = 7.5 Hz, -CH₂, 2H), 1.27 – 1.24 (m, -CH₂, 2H), 0.88 (t, J = 7.4 Hz, -CH₃, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 173.87, 173.30, 166.82, 139.38, 136.54, 135.43, 134.28, 133.39, 132.80, 131.24, 129.22, 129.20, 128.84, 128.42, 127.75, 127.70, 127.67, 127.38, 127.28, 126.59, 126.11, 124.67, 124.50, 124.29, 123.13, 51.08, 48.39, 45.39, 43.60, 40.04, 39.51, 35.27, 29.09, 27.22, 18.82, 13.90; ESI-MS caclcd for 632.3101 [M+H]⁺, found 632.3107 [M+H]⁺; IR (KBr), ν (cm⁻¹): 3302 (-CONH-), 1648 (C=O), 1620 (C=O).

N^1-(2-(N-(1H-indol-4-yl)methyl)butyramido)ethyl)-N^4-(3-(trifluoromethyl)-benzyl)terephthalamide (22t)

Chemical NMR (600 MHz, CDCl₃) δ 8.54 (s, -NH, 1H), 7.89 (s, -ArH, 1H), 7.83 (s, -ArH, 3H), 7.76 (s, -ArH, 1H), 7.69 (s, -ArH, 1H), 7.56 (t, J = 7.4 Hz, -ArH, 3H), 7.48 (d, J = 7.8 Hz, -NH, 1H), 7.37 (d, J = 8.2 Hz, -ArH, 1H), 7.18 (t, J = 7.7 Hz, -ArH, 1H), 6.86 (d, J = 7.2 Hz, -ArH, 1H), 6.79 (d, J = 6.3 Hz, -ArH, 1H), 6.50 (t, J = 2.5 Hz, -NH, 1H), 4.87 (s, -CH₂, 2H), 4.70 (d, J = 5.8 Hz, -CH₂, 2H), 3.74 (d, J = 5.0 Hz, -CH₂, 2H), 3.58 – 3.51 (m, -CH₂, 2H),
2.42 (t, J = 7.4 Hz, -CH₂, 2H), 1.68 (s, -CH₂, 2H), 0.89 (t, J = 7.3 Hz, -CH₂, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 176.57, 166.90, 166.48, 139.22, 136.88, 136.49, 135.93, 131.26, 129.29, 127.46, 127.36, 127.26, 125.70, 124.83, 124.46, 122.22, 116.57, 110.97, 99.73, 50.14, 45.26, 43.64, 40.82, 35.20, 18.83, 13.87; ESI-MS calcd for 565.2427 [M+H]+, found 565.2420 [M+H]+; IR (KBr), υ (cm⁻¹): 3287 (-CONH-), 1655 (C=O), 1624 (C=O).

N¹-(2-(N-(anthracen-9-ylmethyl)butyramido)ethyl)-N⁴-(3-(trifuoromethyl)-benzyl)terephthalamide (22u)

Chemical Formula: C₃₇H₃₄F₃N₃O₃; white solid; Yield: 84%; mp 118.6-119.3 °C; Purity: 99.63% (HPLC); ¹H NMR (600 MHz, CDCl₃) δ 8.41 (s, -NH-, 1H), 8.39 – 8.30 (m, -ArH, 1H), 8.03 (s, -ArH, 2H), 7.96 (s, -ArH, 1H), 7.85 (s, -ArH, 1H), 7.60 (s, -ArH, 1H), 7.59 (s, -NH-, 1H), 7.57 (s, -ArH, 1H), 7.53 (d, J = 7.8 Hz, -ArH, 1H), 7.51 – 7.47 (m, -ArH, 1H), 7.47 – 7.41 (m, -ArH, 3H), 7.38 (s, -ArH, 1H), 6.98 (s, -ArH, 1H), 5.91 (s, -CH₂, 1H), 5.50 (s, -CH₂, 2H), 3.35 – 2.77 (m, -CH₂, 4H), 2.24 – 2.00 (m, -CH₂, 2H), 1.61 (s, -CH₂, 2H), 0.87 (t, J = 6.9 Hz, -CH₃, 3H); ¹³C NMR (151 MHz, DMSO-d₆) δ 172.28, 170.10, 166.56, 141.02, 131.40, 130.99, 130.71, 129.36, 129.30, 129.28, 129.09, 128.88, 127.47, 126.72, 126.56, 125.26, 125.12, 123.75, 123.54, 123.32, 68.15, 54.85, 45.57, 42.29, 36.41, 18.40, 13.62; ESI-MS calcd for 626.2631 [M+H]+, found 626.2628 [M+H]+; IR (KBr), υ (cm⁻¹): 3302 (-CONH-), 1662 (C=O), 1637 (C=O).

N¹-(2-(N-([1,1'-biphenyl]-4-ylmethyl)butyramido)ethyl)-N⁴-(3-(trifuoromethyl)-benzyl)terephthalamide (22v)

Chemical Formula: C₃₅H₃₄F₃N₃O₃; white solid; Yield: 82%; mp 123.9-124.3 °C; Purity: 95.05% (HPLC); ¹H NMR (600 MHz, CDCl₃) δ 7.86 (s, -ArH, 2H), 7.85 (s, -ArH, 3H), 7.60 (d, J = 1.8 Hz, -ArH, 1H), 7.59 (s, -NH-, 1H), 7.57 (d, J = 1.1 Hz, -ArH, 4H), 7.55 – 7.54 (m, -ArH, 2H), 7.53 (d, J = 6.1 Hz, -ArH, 2H), 7.36 (d, J = 2.1 Hz, -ArH, 1H), 7.23 (s, -ArH, 1H), 6.79 (t, J = 6.0 Hz, -NH-, 1H), 4.70 (d, J = 5.9 Hz, -CH₂, 2H), 4.63 (s, -CH₂, 2H), 3.76 – 3.66 (m, -CH₂, 2H), 3.64 – 3.56 (m, -CH₂, 2H), 2.38 (t, J = 7.5 Hz, -CH₂, 2H), 1.67 (q, J = 7.4 Hz, -CH₂, 2H), 0.91 (t, J = 7.4 Hz, -CH₃, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 176.29, 166.80, 166.49, 141.01, 140.31, 139.20, 136.85, 136.58, 134.94, 131.29, 131.00, 129.31, 129.29, 128.88, 128.83, 127.82, 127.72, 127.58, 127.49, 127.41, 127.30, 127.05, 127.03, 126.74, 124.57, 124.55, 124.51, 77.23, 77.02, 76.81, 51.40, 43.67, 40.52, 35.24, 18.94, 18.82, 13.88; ESI-MS calcd for 602.2631 [M+H]+, found 602.2634 [M+H]+; IR (KBr), υ (cm⁻¹): 3302 (-CONH-), 1662 (C=O), 1637 (C=O).

AChE and BuChE Inhibition Assay

The assay was performed according to our previous reports [28] based on the Ellman's method.

Kinetic analysis of 22j

To determine the inhibition type of these compounds against electroporation BuChE, a kinetic study was carried out with inhibitor 22j as the representative BuChEI. In the process, the used concentrations of inhibitor 0.5 × IC₅₀, IC₅₀, and 2 × IC₅₀ were 0.23, 0.46, and 0.92μM, respectively. The type of inhibition was established from the analysis of Lineweaver-Burk reciprocal plots.
Molecular docking study

Molecular docking studies were performed using AutoDock 4.2. X-ray structures of AChE (PDB ID: 4EY7) and BuChE (PDB ID: 5K5E) were downloaded from the Protein Data Bank (https://www.rcsb.org/). The downloaded proteins were then prepared by adding hydrogen atoms, removing water, and assigning Kollman atomic charges, and the prepared proteins were converted to pdbqt files using AutoDock, while the ligands were prepared in the pdbqt files. The processed proteins and ligands were then docked using AutoDock. Finally, the docking results are prepared by PyMOL.

Declarations

Acknowledgements

This work was supported by Hebei Natural Science Foundation (S&T Program of Hebei, NO. B2020201056, H2023201020).

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

References


Tables

Table 1 is available in the Supplementary Files section.
Table 3
ADMET prediction of the compound 22a-22v.

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MW: 150g/mol < MW < 500g/mol, LIPO(Lipophilicity): -0.7 < LOGP < + 5.0, HBA(H-bond acceptors): 0 < Num. H-bond acceptors < 10, HBD(H-bond donors): 0 < Num. H-bond donors < 5, POLAR(Polarity): 20 Å² < TPSA < 130 Å², \(n_{\text{violations}}\)(number violations from Lipinski’s rule): \(n_{\text{violations}}\) ≤ 1, BBB: The ability of crossing BBB blood brain barrier.
Schemes

Scheme 1 is available in the Supplementary Files section

Figures

Figure 1

Marketed AChE inhibitors
Figure 2

The structure of AChEIs and BuChEIs

Figure 3

Design strategy for target compounds
Figure 4

Lineweaver-Burk plot of inhibition kinetics of compound 22j
Figure 5

3D model of AChE (4EY7) docked with compound 22j
Figure 6

3D model of BuChE (5K5E) docked with compound 22j

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.docx
- Scheme1.png
- Supportinginformation.doc