Research paper

Discovery of imidazopyridines containing isoindoline-1,3-dione framework as a new class of BACE1 inhibitors: Design, synthesis and SAR analysis

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1. Introduction

Alzheimer’s disease is characterized by chronic neurodegeneration leading to dementia. The main cause of neurodegeneration is considered to be the accumulation of amyloid-β. Inhibiting BACE1 is a well-studied approach to lower the burden of amyloid-β aggregates. We designed a series of imidazopyridines-based compounds bearing phthalimide moieties as inhibitors of BACE1. The compounds 8a- o were synthesized by the Groebke–Blackburn–Bienaymé three-component reaction of heteroaromatic amidines, aldehydes and isocyanides. Evaluating the BACE1 inhibitory effects of the synthesized compounds revealed that introducing an aminocyclohexyl moiety in the imidazopyridine core resulted in a significant improvement in its BACE1 inhibitory potential. In this regard, compound 8e was the most potent against BACE1 with an IC50 value of 2.84 (±0.95) μM. Molecular docking revealed that the nitrogen atom of imidazopyridines and the oxygen atom of the phenoxypropyl linker were involved in hydrogen bond interactions with Asp228 and Asp32 of BACE1 active site, respectively. The phthalimide moiety oriented toward the flap pocket and interacted with phe108, lle110, Trp115, lle118 through van der Waal’s and hydrophobic interactions. These findings demonstrate that imidazopyridines-based compounds bearing phthalimide moiety have the potential to decrease amyloid-β levels and ameliorate the symptoms of Alzheimer’s disease.

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Article info

Article history:
Received 6 March 2017
Received in revised form 31 May 2017
Accepted 22 June 2017
Available online 24 June 2017

Dedicated to Professor Abbas Shafiee (1937 –2016) for his lifetime achievement in pharmaceutical sciences research and education

Keywords:
Alzheimer’s disease
β-Secretase inhibitor
Groebke–Blackburn–Bienaymé reaction
Imidazopyridines
Phthalimide
Molecular docking

A B S T R A C T

Alzheimer’s disease is characterized by chronic neurodegeneration leading to dementia. The main cause of neurodegeneration is considered to be the accumulation of amyloid-β. Inhibiting BACE1 is a well-studied approach to lower the burden of amyloid-β aggregates. We designed a series of imidazopyridines-based compounds bearing phthalimide moieties as inhibitors of BACE1. The compounds 8a- o were synthesized by the Groebke–Blackburn–Bienaymé three-component reaction of heteroaromatic amidines, aldehydes and isocyanides. Evaluating the BACE1 inhibitory effects of the synthesized compounds revealed that introducing an aminocyclohexyl moiety in the imidazopyridine core resulted in a significant improvement in its BACE1 inhibitory potential. In this regard, compound 8e was the most potent against BACE1 with an IC50 value of 2.84 (±0.95) μM. Molecular docking revealed that the nitrogen atom of imidazopyridines and the oxygen atom of the phenoxypropyl linker were involved in hydrogen bond interactions with Asp228 and Asp32 of BACE1 active site, respectively. The phthalimide moiety oriented toward the flap pocket and interacted with phe108, lle110, Trp115, lle118 through van der Waal’s and hydrophobic interactions. These findings demonstrate that imidazopyridines-based compounds bearing phthalimide moiety have the potential to decrease amyloid-β levels and ameliorate the symptoms of Alzheimer’s disease.

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Hence, developing non-peptidic BACE1 inhibitors is of particular interest.

Among various non-peptidic scaffolds, amidine- or guanidine-containing heterocycles were found to be suitable inhibitors due to the formation of a hydrogen-bond network with the catalytic aspartyl dyad of BACE1. Recently, Merck has introduced a guanidine-based drug for Alzheimer’s disease known as Verubecestat (MK-8931) currently in phase II/III trials. Verubecestat is a potent inhibitor of BACE1 with an IC50 value of 0.4 nM. The X-ray cocrystal structure confirmed hydrogen-binding interactions between the amidine moiety and the BACE1 catalytic dyad. High-affinity binding toward the relatively hydrophobic S1 and S3 subsites results from a diaryl amide substituent that occupies the above-mentioned subsites in BACE1. MK-8931 has favorable physicochemical properties including stability at physiological pH, oral absorption, high cellular permeability and high BBB penetration. The results suggest that the presence of pyridine and guanidine moieties results in satisfactory curative effects.

In addition, other studies reported isoindoline-1,3-dione (phthalimide) derivatives (compound C) as cholinesterase and Aβ aggregation inhibitors with neuroprotective effects. These compounds did not show cytotoxicity and had therapeutic potential for treating Alzheimer's disease. Our previous study on phenyliminochromene carboxamide derivatives bearing bromophenyl piperazine moieties (compound B, Fig. 1) suggested that the introduction of an isoindoline-1,3-dione moiety into the piperazine pendant results in a significant improvement in BACE1 inhibitory activity (IC50 = 0.098 μM) and suppression of Aβ production in N2a-APPswe cells (% Inhibition of Aβ1-40 production = 39.4 at 10 μM). Phthalimide moiety could be considered as a non-peptidyl framework with low-molecular weight involved in hydrophobic interaction with hydrophobic residue of the S2 sub-pocket of active site and the network of hydrogen bonding interactions with Arg235, Thr23 and Gly230. This observation might partially explain the significant inhibitory potential resulted from the incorporation of phthalimide moiety into the BACE1 inhibitor scaffold.

As part of our ongoing research to design and synthesize novel anti-Alzheimer’s agents, we focused on an imidazopyridine core as a promising scaffold for inhibiting BACE1. To this end, we employed molecular hybridization and bioisosterism replacement approaches to identify novel non-peptidic inhibitors with aspartyl binding motifs as new entities for the inhibition of this enzyme (Fig. 1). Our design is based on bioisosteric replacement of the phenyl linker with a phenoxypropyl one to increase the flexibility of the linker and to allow the inhibitor to properly access and orient itself within the active site of BACE1. Different secondary amines were incorporated into the structure to investigate the importance of substituted groups at the 3-position of the imidazopyridines. Our previous results prompted us to replace the benzimidazole group of compound A with a phthalimide pendant to improve the anti-BACE1 effects and enhancing the accessibility to the S2-sub pocket of the active site.

2. Results and discussion

2.1. Chemistry

The synthetic procedure for the preparation of imidazopyridines bearing phthalimide moiety is depicted in Scheme 1. Reaction of phthalimide 1 and 1,3-dibromopropane 2 in the presence of K2CO3 in refluxing acetone gave 2-(3-bromopropyl)isoindoline-1,3-dione 3. Next, the reaction of compound 3 and 4-hydroxyaldehyde derivative 4 in the presence of K2CO3 in DMF at 80 °C gave the desired aldehyde 5.

The target compounds were prepared through the reaction of...
aldehyde 5, 2-aminopyridins 6, and isocyanides 7 in refluxing toluene in the presence of ammonium chloride (NH₄Cl).

2.2. Determination of BACE1 inhibition

BACE1 inhibitory effects of the synthesized imidazopyridines bearing a phthalimide moiety via the phenoxypropyl linker were determined using a FRET-based assay kit. The concentration of compounds that produced 50% maximum inhibition of BACE1 activity (IC₅₀) and enzyme inhibition percentages at 50 and 10 μM concentrations of the test compounds were assessed and summarized in Table 1. Experiments were repeated three to four times for each derivative and mean percent of enzymatic inhibition at 50 and 10 μM were used for comparing the potencies of the test compounds. Compounds 8e and 8c bearing amino cyclohexyl pendent and a methyl substitute on imidazo pyridine core were the most potent derivatives against BACE1 (IC₅₀ = 2.84 μM and 5.93 μM; respectively). Investigation of the structure activity relationship of synthesized derivatives resulted in the following observations:

- Assessment of aminoalkyl substitute (NHR₂) at imidazopyridine core:
  Aminocyclohexyl containing derivatives (R₂ = cyclohexyl) were more potent than their amino t-butyl counterparts in most cases. Compound 8i bearing methoxy, amino cyclohexyl and 6-chloro moieties at R₁, R₂ and R₃ respectively, demonstrated a higher BACE1 inhibitory potential (inhibition at 50 μM = 88.10%) over its amino t-butyl containing counterpart 8k (inhibition at 50 μM = 37.90%). Similar results were observed in the case of the aminocyclohexyl derivative 8e (R₁ = H and R₃ = 7-CH₃, 100% BACE1 inhibition at 50 μM; IC₅₀ = 2.84 μM) and its amino t-butyl counterpart (inhibition at 50 μM = 18.32%). These findings indicate the important influence of the amino cyclohexyl moiety on BACE1 inhibition. Furthermore, the inappropriate orientation of t-butyl derivatives may hinder access and prevent interaction between important functional groups of the ligand and the catalytic residues of the enzyme’s active site.

- Investigation of substituted moiety (R₃) on amino cyclohexyl imidazopyridine core.
  The effect of type of substituted group R₃ on the amino cyclohexyl imidazopyridine core suggested that the introduction of methyl substitutes (R₂ = CH₃) at different positions of imidazo pyridine ring increases the inhibitory potency against BACE1 and the preference order for the position of methyl substitute is 7 > 5 > 6,8. The most potent compound of this series, 7-methylimidazopyridine derivative 8e (100% inhibition at 50 μM) demonstrated superior potency over its 5-methylimidazopyridine (8c), 6-methylimidazopyridine (8d) and 8-methylimidazopyridine (8g) counterparts with 87.94, 63.70 and 69.43% BACE1 inhibition at 50 μM, respectively. Furthermore, the introduction of a small lipophilic electron-donating moiety such as a methyl group into the 3-aminocyclohexyl imidazopyridine derivatives increased the potency of compounds against BACE1. Finally, no significant difference in potency was observed between bromine and chlorine derivatives.

- Effect of substituted group R₁ into the phenoxypropyl linker
  Introduction of a methoxy substitute into the phenoxypropyl linker of the designed scaffold did not significantly alter the potency of compounds against BACE1. The exception was in the case of the amino cyclohexyl imidazopyridine bearing chloride group at R₁ position of imidazopyridine ring and methoxy group in linker part (compound 8i); 8i was four times more potent than its non-methoxylated counterpart in inhibiting BACE1 at 50 μM (inhibition was 88.10 and 19.6%, respectively). We attribute this to the structural and conformational changes imposed by the spatial hindrance between the methoxy and cyclohexyl substitutes resulting in proper orientation of functional groups into the key residues and pockets of BACE1 active site.

2.3. Molecular docking study

We performed molecular docking to better understand the Structure-activity relationship (SAR) of all of the synthesized...
compounds. To validate and optimize our docking protocol, redocking of the co-crystallized conformation of a native ligand into the 4ACU active site was performed [22]. The binding pose of the top rank cognate ligand was superimposed over the X-ray crystallographic structure. The best-docked and the experimental conformations of the inhibitor correlate well with an RMSD of 0.601 Å and binding free energies of −12.934 kcal/mol. This validated docking procedure was performed on all the compounds under investigation. Estimated free binding energies (ΔGbinding) and Kᵢ for synthesized molecules are summarized in Table 2. All the ligand/receptor interactions were analyzed using Chimera 1.11 and Viewer lite 4.2 software and following results were obtained:

- In almost all docked structures containing an amino cyclohexyl pendant at R₂ and a different motif at R₁ (compounds 8a, 8b, 8c, 8d, 8e, 8f, 8g, 8h, 8i, 8j, 8k, 8l, 8m, 8n, 8o, OM99-2, and 8p) a key hydrogen bond interaction between the nitrogen atom of imidazopyridine and Asp228 of the catalytic dyad residues was observed. The Cyclohexyl moiety in these structures occupies the P₂ pocket and phthalimide oriented toward flap pocket. The substituted imidazopyridines was surrounded by Thr231, Thr232, and Asn233.

- As mentioned previously, replacing the cyclohexyl pendant with a t-butyl group reduced the inhibitory activity of the compounds. To investigate this observation, we performed computational analysis on of one of the most potent compounds, compound 8e, (aminocyclohexyl derivative) and its amino t-butyl counterpart 8f. As it is depicted in Fig. 2, 8e and 8f demonstrated different binding orientations in the active site of BACE1. As it is shown, the phthalimide moiety of 8e was directed toward the flap pocket of the active site surrounded by Phe108, Phe109 and Ileu118. Furthermore, the methyl imidazopyridine core was oriented toward the P₂ pocket and amino cyclohexyl pendant occupied the P₂ pocket surrounded by Thr231, Thr232, Asn233 and Arg235. Moreover, Asp32 and Asp228 are involved in H-bond interaction with the oxygen atom of phenoxypropyl and the nitrogen atom of the imidazopyridine, respectively (Table 2). Surprisingly, the amino t-butyl derivative 8f is inversely directed in the active site; the phthalimide is oriented toward P₂ pocket, amino t-butyl pendant and imidazopyridine are oriented toward P₂ and flap pockets, respectively. Such an orientation partly hindered H-bond interaction of 8c with key catalytic Asp32 and partly justifies its poor BACE1 inhibitory potential. We conclude that the introduction of an amino t-butyl pendant into the imidazopyridine core imposes an unfavorable binding mode that hinders proper ligand-receptor interaction.

Values represent mean (±) standard error of mean (S.E.M.) of three or four independent experiments.

It is worth noting that the nitrogen atom of the imidazopyridine, respectively.

### Table 1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
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<tr>
<td>8a</td>
<td>H</td>
<td>cyclohexyl</td>
<td>–</td>
</tr>
<tr>
<td>8b</td>
<td>OCH₃</td>
<td>cyclohexyl</td>
<td>–</td>
</tr>
<tr>
<td>8c</td>
<td>H</td>
<td>cyclohexyl</td>
<td>5-CH₃</td>
</tr>
<tr>
<td>8d</td>
<td>H</td>
<td>cyclohexyl</td>
<td>6-CH₃</td>
</tr>
<tr>
<td>8e</td>
<td>H</td>
<td>cyclohexyl</td>
<td>7-CH₃</td>
</tr>
<tr>
<td>8f</td>
<td>H</td>
<td>t-Butyl</td>
<td>7-CH₃</td>
</tr>
<tr>
<td>8g</td>
<td>H</td>
<td>cyclohexyl</td>
<td>8-CH₃</td>
</tr>
<tr>
<td>8h</td>
<td>H</td>
<td>cyclohexyl</td>
<td>6-Cl</td>
</tr>
<tr>
<td>8i</td>
<td>OCH₃</td>
<td>cyclohexyl</td>
<td>6-Cl</td>
</tr>
<tr>
<td>8j</td>
<td>H</td>
<td>t-Butyl</td>
<td>6-Cl</td>
</tr>
<tr>
<td>8k</td>
<td>OCH₃</td>
<td>t-Butyl</td>
<td>6-Cl</td>
</tr>
<tr>
<td>8l</td>
<td>H</td>
<td>cyclohexyl</td>
<td>6-Br</td>
</tr>
<tr>
<td>8m</td>
<td>OCH₃</td>
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<td>6-Br</td>
</tr>
<tr>
<td>8n</td>
<td>H</td>
<td>t-Butyl</td>
<td>6-Br</td>
</tr>
<tr>
<td>8o</td>
<td>OCH₃</td>
<td>t-Butyl</td>
<td>6-Br</td>
</tr>
<tr>
<td>OM99-2</td>
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### Table 2

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R₁</th>
<th>R₂</th>
<th>% Inhibition at 50 µM</th>
<th>% Inhibition at 10 µM</th>
<th>IC₅₀ (µM)</th>
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</thead>
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<tr>
<td>8a</td>
<td>H</td>
<td>cyclohexyl</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>–</td>
</tr>
<tr>
<td>8b</td>
<td>OCH₃</td>
<td>cyclohexyl</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8c</td>
<td>H</td>
<td>cyclohexyl</td>
<td>69.49 (±7.52)</td>
<td>29.30 (±6.12)</td>
<td>–</td>
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<tr>
<td>8d</td>
<td>H</td>
<td>cyclohexyl</td>
<td>61.54 (±7.16)</td>
<td>5.93 (±1.93)</td>
<td>–</td>
</tr>
<tr>
<td>8e</td>
<td>H</td>
<td>cyclohexyl</td>
<td>61.32 (±6.14)</td>
<td>2.84 (±0.95)</td>
<td>–</td>
</tr>
<tr>
<td>8f</td>
<td>H</td>
<td>t-Butyl</td>
<td>18.32 (±10.89)</td>
<td>24.24 (±8.33)</td>
<td>–</td>
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<tr>
<td>8g</td>
<td>H</td>
<td>cyclohexyl</td>
<td>69.43 (±8.71)</td>
<td>44.42 (±5.04)</td>
<td>–</td>
</tr>
<tr>
<td>8h</td>
<td>H</td>
<td>cyclohexyl</td>
<td>19.60 (±6.86)</td>
<td>11.50 (±13.19)</td>
<td>–</td>
</tr>
<tr>
<td>8i</td>
<td>OCH₃</td>
<td>cyclohexyl</td>
<td>88.097 (±7.53)</td>
<td>26.86 (±8.82)</td>
<td>14.90 (±8.98)</td>
</tr>
<tr>
<td>8j</td>
<td>H</td>
<td>t-Butyl</td>
<td>32.95 (±11.30)</td>
<td>17.67 (±11.01)</td>
<td>–</td>
</tr>
<tr>
<td>8k</td>
<td>OCH₃</td>
<td>t-Butyl</td>
<td>37.90 (±13.18)</td>
<td>19.41 (±5.85)</td>
<td>–</td>
</tr>
<tr>
<td>8l</td>
<td>H</td>
<td>cyclohexyl</td>
<td>45.30 (±5.83)</td>
<td>32.16 (±10.05)</td>
<td>–</td>
</tr>
<tr>
<td>8m</td>
<td>OCH₃</td>
<td>cyclohexyl</td>
<td>43.17 (±1.18)</td>
<td>36.71 (±2.71)</td>
<td>–</td>
</tr>
<tr>
<td>8n</td>
<td>H</td>
<td>t-Butyl</td>
<td>34.28 (±9.20)</td>
<td>7.87 (±0.02)</td>
<td>–</td>
</tr>
<tr>
<td>8o</td>
<td>OCH₃</td>
<td>t-Butyl</td>
<td>48.72 (±12.23)</td>
<td>33.19 (±11.12)</td>
<td>–</td>
</tr>
<tr>
<td>OM99-2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.014 (±0.0028)</td>
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</tbody>
</table>

2.3.1. Conclusion

In this study, we synthesized and evaluated the anti-BACE1 activity of fifteen hybrid imidazopyridines containing phthalimide moieties. The goal was to discover novel small compounds with potentially improved BACE1 inhibitory properties. Our preliminary BACE1 inhibitory results suggested that a cyclohexyl substitution at R₂ was a key structure for improving the inhibitory activity. In the case of aminocyclohexyl containing derivatives, introducing a methyl substituent at 6 or 7 positions of the imidazopyridine core (as in 8d and 8e) resulted in considerable improvement of BACE1 inhibitory potential. Besides, molecular docking simulation indicated that the amino t-butyl derivatives have different binding...
orientations in the active site of BACE1 in comparison with aminocyclohexyl derivatives. Moreover, all aminocyclohexyl derivatives showed favorable binding interactions with P2, P’2 and stacking pockets; imidazopyridines and phenoxypyropyl linkers were involved in hydrogen bond interaction with Asp228 and Asp32 of BACE1 active site, respectively. The Phthalimide portion was oriented toward the flap pocket and demonstrated van der Waals’ and hydrophobic interactions with phe108, ile110, Trp115, Ile118. The results of the present study could guide the rational design of more potent BACE1 blocking agents.

3. Experimental section

3.1. Chemistry

All chemicals were purchased from commercial sources (Merck and Aldrich) and used without further purification. Melting points were taken on a Kofler hot stage apparatus and were uncorrected. $^1$H and $^{13}$C NMR spectra were recorded on Bruker FT-500 (Germany), using TMS as an internal standard. The IR spectra were obtained on a Nicolet MagnaFTIR 550 spectrometer (KBr disks). The elemental analysis was performed with an Elementar Analysensystem GmbH VarioEL CHNS mode (Germany).
3.1. Synthesis of aldehydes

A mixture of phthalimide (1 mmol), 1,3-dibromopropane (1 mmol), and KO2CO3 (1.3 mmol) in acetone (15 mL) was heated at reflux for 8 h. After completion of the reaction (checked by TLC), the mixture was poured into crushed ice and the precipitate was filtered and dried to give 2-(3-bromopropyl)isoindoline-1,3-dione (8c). Next, mixture of compound 3 (1 mmol), 4-hydroxyaldehyde derivative (1 mmol), and KO2CO3 (1.3 mmol) in DMF (10 mL) was heated at 80 °C for 8 h. Upon completion of the following reaction, the mixture was poured into crushed ice and the precipitate was filtered and dried affording aldehydes 5.

3.2. Synthesis of imidazopyridine derivatives 8a

A mixture of aldehyde 5 (1 mmol), 2-aminopyridines (1 mmol), isocyanides (7.2 mmol), NH4Cl (1 mmol) and toluene (10 mL) was heated under reflux conditions for 8–12 h. After completion of the reaction (checked by TLC), the solvent was evaporated under vacuum and the residue was recrystallized from ethanol to afford pure imidazopyridines 8a.

3.3. 2-(3-(4-((3-Cyclohexylamino)imidazo[1,2-a]pyridin-2-yl)phenoxo)propyl)isoindoline-1,3-dione (8b)

Yield: 75%, Mp: 182–184 °C. IR (KBr): 3425, 2931, 2853, 1771, 1705, 1610, 1523 cm⁻¹; MS: m/z (%) 525 (M⁺, 30), 415 (4), 248 (5), 228 (7), 188 (100), 160 (45), 135 (10), 84 (10), 79 (57), 75 (10); 1H NMR (CDCl3, 500 MHz): δH (ppm) = 8.47–8.49 (m, 3H, H5, H7, H8), 7.86–7.84 (m, 2H, H1', H4'), 7.76–7.77 (m, 1H, H3'), 7.73–7.72 (m, 2H, H2', H3'), 6.75 (d, J = 8.0 Hz, 1H, H5), 6.90 (m, 2H, H6, H8), 4.14 (t, J = 5.0 Hz, 2H, CH2), 3.95 (t, J = 5.0 Hz, 2H, CH2), 3.79 (s, 3H, OCH3), 3.48 (bs, 1H, NH), 3.00–2.99 (m, 1H, CH), 2.28 (p, J = 6.5 Hz, 2H, CH2), 1.81–1.16 (m, 10H, H1-H10 (cyclohexyl)); 13C NMR (CDCl3, 125 MHz): δC (ppm) = 168.4, 165.7, 163.0, 150.0, 149.5, 133.8, 132.2, 131.6, 130.4, 127.5, 125.1, 122.8, 119.5, 117.8, 115.9, 112.7, 111.1, 66.9, 56.8, 35.6, 34.1, 28.4, 25.6, 24.7. Anal. Cald. for C30H30N4O3: C, 70.57; H, 5.92; N, 10.97. Found: C, 70.34; H, 6.18; N, 11.23.

3.3. 2-(3-(4-((3-Cyclohexylamino)imidazo[1,2-a]pyridin-2-yl)phenoxo)propyl)isoindoline-1,3-dione (8b)

Yield: 71%, Mp: 181–183 °C. IR (KBr): 3228, 2923, 2849, 1743, 1708, 1611, 1503 cm⁻¹; MS: m/z (%) 525 (M⁺, 30), 415 (4), 248 (5), 228 (7), 188 (100), 160 (45), 135 (10), 84 (10), 79 (57), 75 (10); 1H NMR (CDCl3, 500 MHz): δH (ppm) = 8.47–8.49 (m, 3H, H5, H7, H8), 7.86–7.84 (m, 2H, H1', H4'), 7.76–7.77 (m, 1H, H3'), 7.73–7.72 (m, 2H, H2', H3'), 6.75 (d, J = 8.0 Hz, 1H, H5), 6.90 (m, 2H, H6, H8), 4.14 (t, J = 5.0 Hz, 2H, CH2), 3.95 (t, J = 5.0 Hz, 2H, CH2), 3.79 (s, 3H, OCH3), 3.48 (bs, 1H, NH), 3.00–2.99 (m, 1H, CH), 2.28 (p, J = 6.5 Hz, 2H, CH2), 1.81–1.16 (m, 10H, H1-H10 (cyclohexyl)); 13C NMR (CDCl3, 125 MHz): δC (ppm) = 168.4, 165.7, 163.0, 150.0, 149.5, 133.8, 132.2, 131.6, 130.4, 127.5, 125.1, 122.8, 119.5, 117.8, 115.9, 112.7, 111.1, 66.9, 56.8, 35.6, 34.1, 28.4, 25.6, 24.7. Anal. Cald. for C30H30N4O3: C, 70.57; H, 5.92; N, 10.97. Found: C, 70.34; H, 6.18; N, 11.23.

3.1.2. Synthesis of pyridine derivatives 8d

Yield: 81%, Mp: 175–177 °C. IR (KBr): 3462, 2931, 2853, 1771, 1708, 1600, 1561 cm⁻¹; MS: m/z (%) 508 (M⁺, 1), 482 (4), 374 (10), 212 (7), 188 (100), 160 (40), 130 (12), 104 (3), 83 (4), 55 (14). 1H NMR (CDCl3, 500 MHz): δH (ppm) = 8.57 (d, J = 9.0 Hz, 2H, H3', H5'), 7.97 (s, 1H, H5), 7.86–7.84 (m, 2H, H1', H4'), 7.74–7.72 (m, 2H, H2', H3'), 7.27–7.26 (m, 2H, H6, H7), 6.87 (d, J = 9.0 Hz, 2H, H2', H6'), 4.61 (s, 3H, OCH3), 1.43 (t, J = 6.5 Hz, 2H, CH2), 2.33 (p, J = 6.5 Hz, 2H, CH2), 2.27 (p, J = 6.5 Hz, 2H, CH2), 2.24 (s, 3H, CH3), 2.26–2.22 (m, 1H, CH), 1.91–1.25 (m, 10H, H1-H10 (cyclohexyl)); 13C NMR (CDCl3, 125 MHz): δC (ppm) = 170.7, 169.3, 165.8, 163.4, 157.0, 151.9, 140.1, 138.2, 134.0, 133.0, 132.4, 133.3, 117.8, 114.7, 65.9, 60.8, 35.2, 33.0, 28.4, 28.1, 25.5, 23.9. Anal. Cald. for C30H29N4O3: C, 72.85; H, 6.11; N, 11.33. Found: C, 73.11; H, 6.21; N, 11.12.

\[ \text{N, 11.67.} \]


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123.5, 123.2, 122.7, 121.5, 120.5, 116.6, 112.7, 111.7, 66.9, 56.0, 55.9, 35.5, 30.3, 28.2. Anal. Calcd for C29H27ClN4O3: C, 68.40; H, 5.24; N, 10.80. Found: C, 68.49; H, 5.51; N, 10.71.

185.8, 158.0, 157.2, 144.8, 143.2, 136.3, 135.1, 133.9, 132.1, 128.3, 127.0, 143.2, 123.1, 114.6, 66.5, 56.6, 35.4, 40.0, 28.3, 25.5, 24.7. Anal. Calcd for C29H27ClN4O3: C, 68.40; H, 5.24; N, 10.80. Found: C, 68.49; H, 5.51; N, 10.71.

123.5, 123.2, 122.7, 121.5, 120.5, 116.6, 112.7, 111.7, 66.9, 56.0, 55.9, 35.5, 30.3, 28.2. Anal. Calcd for C29H27ClN4O3: C, 68.40; H, 5.24; N, 10.80. Found: C, 68.49; H, 5.51; N, 10.71.

123.5, 123.2, 122.7, 121.5, 120.5, 116.6, 112.7, 111.7, 66.9, 56.0, 55.9, 35.5, 30.3, 28.2. Anal. Calcd for C29H27ClN4O3: C, 68.40; H, 5.24; N, 10.80. Found: C, 68.49; H, 5.51; N, 10.71.

123.5, 123.2, 122.7, 121.5, 120.5, 116.6, 112.7, 111.7, 66.9, 56.0, 55.9, 35.5, 30.3, 28.2. Anal. Calcd for C29H27ClN4O3: C, 68.40; H, 5.24; N, 10.80. Found: C, 68.49; H, 5.51; N, 10.71.

123.5, 123.2, 122.7, 121.5, 120.5, 116.6, 112.7, 111.7, 66.9, 56.0, 55.9, 35.5, 30.3, 28.2. Anal. Calcd for C29H27ClN4O3: C, 68.40; H, 5.24; N, 10.80. Found: C, 68.49; H, 5.51; N, 10.71.
3.2. BACE1 enzymatic assay

Enzyme inhibition assay was carried out using BACE1 (β-secretase) FRET assay kit, from Invitrogen (former Pan Vera, Madison, USA) for thorough editing of this manuscript.

**Appendix A. Supplementary data**

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.06.040

**References**


Invitrogen http://tools.invitrogen.com/content/sfs/manuals/L0724.pdf.