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New 1,2,3,4-tetrahydroisoquinoline derivatives as modulators of proteolytic cleavage of amyloid precursor proteins

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Abstract—A type of new 1,2,3,4-tetrahydroisoquinoline derivatives was synthesized via concise procedure from commercially available tetrahydroisoquinoline. These derivatives were delicately designed to possess propargyl-related pharmacophores simulated with a monoamine oxidase inhibitor rasagiline. We investigated the effect of these synthetic tetrahydroisoquinoline derivatives on the regulation of proteolytic processing of amyloid precursor protein (APP) by an ERK-dependent signaling pathway. Additionally, these compounds were also evaluated on the prevention of the proteolytic processing of C99 as γ-secretase inhibitors by using a highly efficient cell-based reporter gene assay for γ-secretase. The results suggested that certain compounds might be explored to possess both sAPPα-releasing stimulation and γ-secretase inhibitory potency, which may reflect the synergetic potential of neuroprotective activities for the treatment of Alzheimer's disease as they possessed both ERK activation and inhibition of amyloidogenic Aβ release.

1. Introduction

One of the pathological hallmarks of Alzheimer's disease (AD) is the cerebral accumulation of amyloid plaques,[1] and the major constituents of these plaques are the 40–42 residue amyloid-β peptides (Aβs), which are generated through the proteolysis of amyloid precursor protein (APP) by sequential actions of a set of membrane-bound proteases termed β- and γ-secretases.[2] Mounting contributions from cellular and pharmacological studies, affinity labeling, and biochemical approaches suggest that γ-secretase is an aspartyl protease and has thus been regarded as a pivotal role for therapeutic target for AD.[3,4] Recently, alternative investigations have also shown that the proteolytic processing of APP was regulated by mitogen-activated protein kinase (MAPK) pathway involving sequential activation of mitogen-activated protein kinase (MEK) and extracellular signal-regulated protein kinase (ERK).[5] Concerning the diverse pathway of APP processing, another secretase named α-secretase can mediate alternative processing of APP known as non-amyloidogenic pathway to produce the soluble form of amyloid precursor protein α (sAPPα), thus preclude Aβ production. It was therefore interesting to know how the respective secretase-catalyzed cleavages of APP can act in response to the control by this signaling cascade.

The soluble form of APPα was found to possess potent neurotropic and neuroprotective activities against oxidative and excitotoxic insults.[6,7] In vitro studies have established the involvement of protein kinase C (PKC) and PKC-coupled receptors in the non-amyloidogenic α-secretase pathway of APP cleavage.[5] Further investigations indicated that rasagiline (**1a**, Fig. 1), a MAO-B inhibitor, and its carbamyl-containing derivative, TV-3326 (**1b**), regulate PKC- and MEK-dependent APP processing in SH-SY5Y neuroblastoma and PC12 cells,[8]

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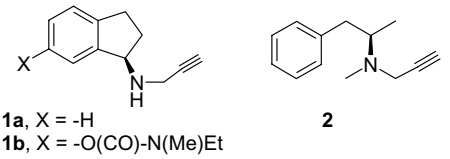


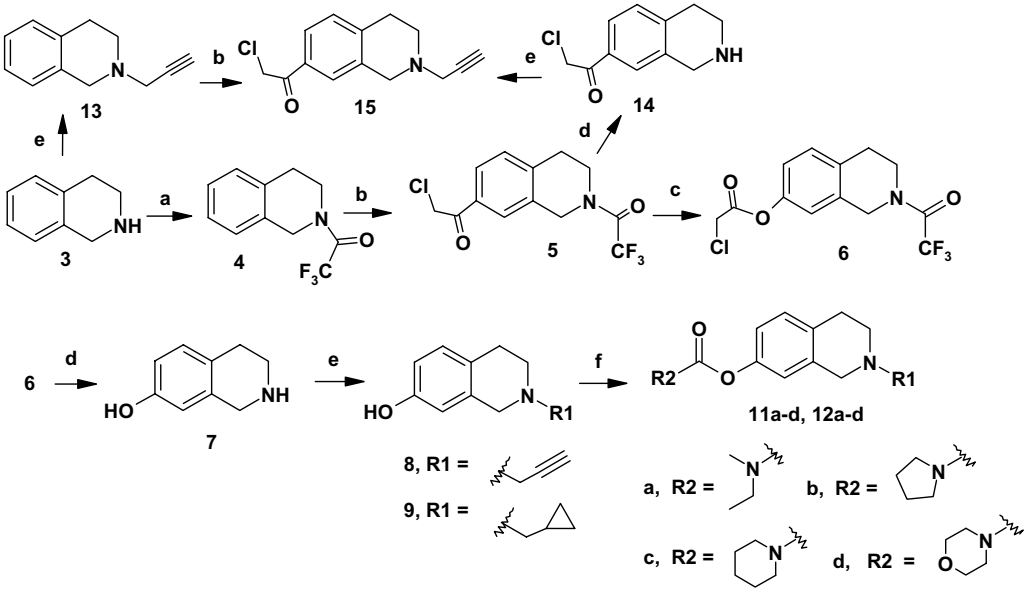
Figure 1. Chemical structures of rasagiline (**1a**), its derivative, TV-3326 (**1b**), and selegiline (**2**).

which resulted in the stimulation of release of the neuroprotective sAPPα. Nevertheless, selegiline, a well-known selective MAO-B inhibitor for increasing the efficacy of levodopa therapy in the treatment of Parkinson's disease, also exerted neuroprotective effects in various preclinical models. These results suggested that a crucial role for PKC-, MEK-dependent pathways may be involved in the enhancement of α-APP release by selegiline and rasagiline. Taken together, the secretase-mediated proteolysis of APP can be subject to multiple levels of regulation by intracellular pathways and the ERK activation could play a pivotal role in shifting the APP processing to the α-secretase-initiated non-amyloidogenic pathway synergistically blocking γ-secretase-dependent Aβ production. Conversely, reduction of formidable Aβ production by interfering γ-secretase activity with enzyme-targeted inhibitors might shift the APP processing to the α-secretase-mediated pathways.[10] Thus, control of Aβ production by direct or indirect modulation of γ-secretase activity on APP opens the approaches toward the treatment of Alzheimer's disease.

Based on the light of these findings, we here manipulated a new type of propargyl-related tetrahydroisoquinoline derivatives, simulated as congeners of rasagiline and selegiline, and made exploitation on the possible relations between the effects on the γ-secretase-mediated processing and on the modulation on MEK-dependent signaling of APP.

2. Chemistry

The synthesis of 7-carbamoyl-1,2,3,4-tetrahydroisoquioline derivatives **11a–d** and **12a–d** is depicted in Scheme 1, in which 7-hydroxy-tetrahydroisoquinoline **7** was the critical intermediate for the preparation of these 1,2,3,4-tetrahydroisoquinoline derivatives. First, trifluoroacetyl-protection of secondary amine of the starting material 1,2,3,4-tetrahydroisoquinoline was accomplished with trifluoroacetic anhydride and potassium hydroxide in 72% yields. The following acylation at 7-position of the *N*-protected 1,2,3,4-tetraisoquinoline has been considered a critical step for the synthesis of the desired products. The acetamide **4** was slowly added to 1,2-dichloroethane solution with chloroacetyl chloride and aluminum chloride over 3 h at ambient temperature to provide the desired 7-chloroacetyl derivative **5** with over 90% yields. No 6-chloroacetyl isomer appeared in this reaction. As characterized with ^{1}H NMR, the H-8 proton of **5** appeared at $\delta = 7.77$ as small doublet splitting, which was shifted downfield as compared to that of compound **4**. On the other hand, the protons H-5 and H-6 were unambiguously identified at $\delta = 7.81$ and $\delta = 7.32$ with splitting constants around 7.2 Hz, respectively. For further demonstration of the regioselective acylation at 7-position of 1,2,3,4-tetrahydroisoqunoline ring, an *N*-propargyl-7-chloroacetyl analogue **15** was synthesized either through *N*-propargylation of **3** followed by acylation of the *N*-propargyl intermediate **13** or via removal of *N*-trifluoroacetyl protection under sodium thiomethoxide in methanol and sequential *N*-propargylation. The crystallized form of **15** was obtained to provide an X-ray crystal structure as depicted in Figure 2. The chemical shifts of H-5, H-6, and H-8, and their splitting patterns in both **5** and the *N*-propargyl analogue **15** are similar to each other, indicating the right regioselective acylation at 7-position of tetraisoquinoline ring system under $AlCl_3$-catalytic conditions.



Scheme 1. Reagents and conditions: (a) $(CF_3CO)_2O$, KOH (b) $ClCH_2COCl$, $AlCl_3$ (c) *m*CPBA (d) $NaSCH_3$ (e) propargyl bromide or (bromomethyl)cyclopropane, K_2CO_3 (f) R2–CO–Cl (**10a–d**), NaH.

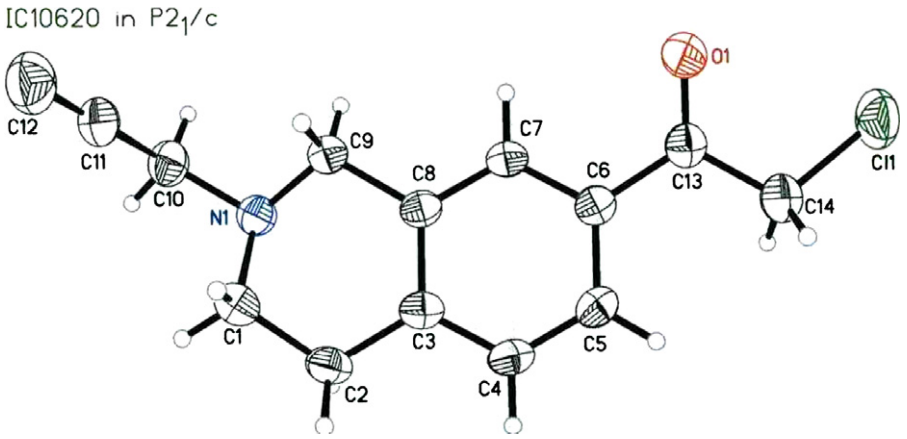


Figure 2. Crystal structure of 7-chloroacetyl-*N*-propargyl-1,2,3,4-tetrahydroisoquinoline (**15**) determined by s single-crystal X-ray crystallography.

For the introduction of an oxygen function into the 7-position of **5**, Baeyer-Villiger oxidation[11] was performed by treatment with *meta*-chloroperbenzoic acid (*m*CPBA) in the presence of trifluoroacetic acid at dark conditions for 3 days to afford the desired 7-chloroacetoxy analogue **6** in 68% yield. The following step for the removal of the acylated protections was accomplished with sodium thiomethoxide in methanol to remove both acetyl groups at compound **6** simultaneously. Thus, the resulting 7-hydroxy-tetrahydroquinoline **7** was obtained as hydrochloride salt in 54% yields.

At penultimate step, *N*-alkylation of **7** with propargyl or cyclopropylmethyl bromides was readily carried out in basic conditions to give the corresponding derivatives **8** and **9**, respectively. Following the method previously reported for the preparation of certain carbamoyl chlorides,[12] commercially unavailable *N*-ethyl-*N*-methyl-carbamoyl chloride (**10a**) and morpholinecarbonyl chloride (**10d**) were synthesized from the corresponding secondary amines by using a solution of phosgene in toluene. Finally, *N*-ethyl-*N*-methyl-carbamoyl moiety and its congeners were successfully incorporated to **8** and **9** in the presence of 60% sodium hydride to provide a series of 1,2,3,4-tetrahydroisoquinoline derivatives **11a**–**d** and **12a**–**d** in 50–82% yields.

3. Biological results and discussion

First, we investigated the effects of these synthetic tetrahydroisoquinolines on the inhibition of γ-secretase-mediated cleavage of APP. Here, a quantitative cell-based assay specifically measuring the γ-cleavage of APP-C99 was employed. The generation and validation of the cell-based assay was described previously.[13] According to this method, the fold of activation of luciferase, expressed in cell nucleus, was measured to determine the ability of γ-secretase on APP processing reversely. Thus, the effects of a series of propargyl-related tetrahydroisoquinoline derivatives are shown in Figure 3. These tetrahydroisoquinoline derivatives were structurally related to rasagiline, a known MAO-B inhibitor. The screening of these synthetic tetrahydroisoquinolines showed that

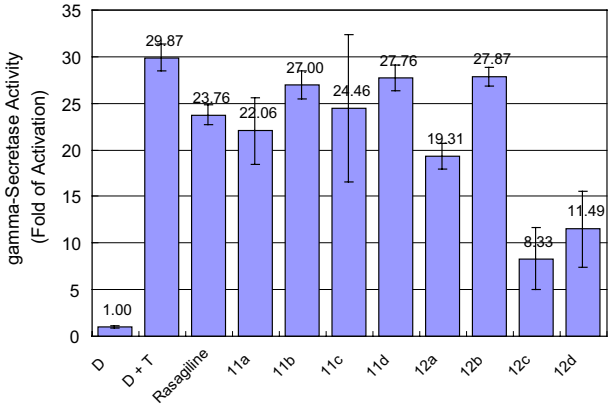


Figure 3. The γ-secretase inhibitory activity of 1,2,3,4-tetrahydroisoquinoline derivatives **11a**–**d** and **12a**–**d**. T20 cells were treated with 10 μM of each tested compounds (dissolved in DMSO, Abbr. D) in culture medium containing 1 μg/ml tetracycline (Abbr. T) and incubated at 37 °C for 24 h. The detailed procedures for luciferase reporter assays for γ-secretase inhibition were followed as previously reported. Each value represents the mean ± SD of three experiments.

compounds **12a**, **c**, **d** at 10 μM decreased 35–72% of γ-secretase activity, while rasagiline and most propargyl analogues were 5-fold less potent as only 6–15% of γ-secretase activity were decreased. The relative potency of these tetrahydroisoquinoline derivatives suggested that compounds **12a**, **c**, **d** could target γ-secretase. All of the tetrahydroisoquinoline derivatives presented no significant cytotoxicity on the HEK293 cells at this concentration as shown in Figure 4. This was the first ever report for the inhibitory potency of the tetrahydroisoquinoline derivatives on the γ-secretase activity.

Recent evidence was pointing that certain MAO-B inhibitors including rasagiline, its carbamate derivative, TV-3326, and selegiline modulated APP processing through ERK-related signaling pathways to ultimately reduce the formidable amyloid-β peptide formation.[14] The outcome of ERK stimulation was contributed to indirectly modulate the release of sAPPα. Thus these contributions possibly hint an alternate approach benefit to the treatment of Alzheimer's disease. Although the common propargylamine moiety of these compounds

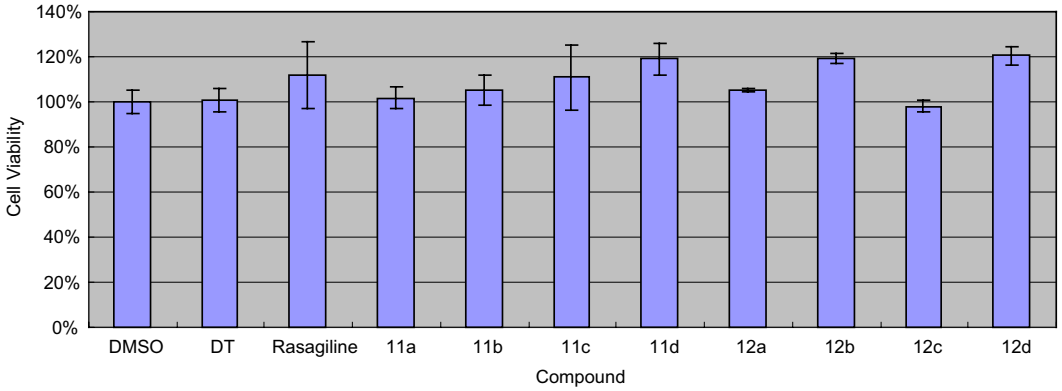


Figure 4. Effects of **11a–d** and **12a–d** on cell viability of HEK293 cells with a dose of 10 μM. Values are means ± SD, $n = 3$.

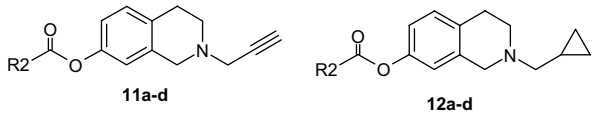
was found to be critical to the activation of ERK signaling, it was found to be independent of their MAO inhibitory activity.[15] Alternatively, it has been well established that conformationally restricted modifications on the biological function-related structures have been one of the approaches toward new compounds with improving activities.[16] Thus, we chose tetrahydroisoquinoline as a rigid skeleton to replace the flexible alkylamine moiety of selegiline or rasagiline and investigated the possible role in the proteolytic processing of APP.

Previous investigations have indicated that the propargylamine moiety of rasagiline or selegiline was shown to be essential for MAO inhibition.[14] Here, all the synthetic propargyl-containing tetrahydroisoquinolines **11a–d** showed marginal MAO-B inhibitory activity with IC_{50}s in the range of 10–20 μM, though they were 10-fold less potent than rasagiline as shown in Table 1. On the other hand, the change of the critical propargyl moiety of **11a–d** to cyclopropylmethyl group as in the analogues **12a–d** still maintained the similar activity against MAO-B. The maintenance of potency for compounds **12a–d** was partly attributed to the similar size and lipophilicity (only an increment of 0.45 of the logP value) between the cyclopropylmethyl group and propargyl group. Another factor concerning the ability of the cyclopropylmethyl group of **12a–d** to inhibit the MAO-B might be the ring strain of cyclopropyl group, which would contribute to the binding and the radical-mediated ring-opening to the targeted MAO-B at the active site.

Taken together, both propargyl- and cyclopropylmethyl-containing tetrahydroisoquinoline derivatives were shown to keep moderate MAO-B inhibition. The well-established pharmacophore could be replaced with the highly strained cyclopropylmethyl moiety as critical requirement for MAO inhibition, while only cyclopropylmethyl derivatives **12a**, **c**, **d** displayed moderate γ-secretase inhibition as determined on the cell-based assays. The α-secretase-mediated processing of APP was directly or indirectly enhanced by activation of MEK–ERK pathway by, for instance, rasagiline or selegiline, as previously described. Therefore, in the next step, we seek to determine whether these cyclopropylmethyl-containing compounds can significantly modulate the MEK–ERK signal pathway.

The relative levels of ERK activation (*p*-ERK/ERK, %) of moderate γ-secretase inhibitors **12a**, **c**, **d** were further examined in γ-30 cells and found that **12c** displayed 1.2-fold stronger ERK activation properties than rasagiline at concentration of 10 μM, while **12a** and **12d** showed comparable potency to rasagiline as shown in Table 2. Furthermore, the determination of these compounds' potential to enhance sAPPa secretion using γ-30 cells is summarized in Figure 5. Unlike T20 cells which overexpress APP-C99 as the direct substrate of γ-secretase, γ-30 cells constitutively expressed full-length APPs

Table 1. MAO-B inhibitory activity and log *P* values of tetrahydroisoquinoline derivatives **11a–d** and **12a–d**



11a-d

12a-d

Compound	R2	IC_{50} (μM)	log *P*[a]
Rasagiline		2.1 ± 0.6	2.295
11a	*N*-Ethyl-*N*-methylamino	21.5 ± 1.0	2.252
11b	1-Pyrrolidyl	13.9 ± 0.5	2.235
11c	1-Piperidinyl	15.7 ± 2.2	2.632
11d	1-Morpholinyl	18.7 ± 1.6	1.567
12a	*N*-Ethyl-*N*-methylamino	12.8 ± 2.0	2.688
12b	1-Pyrrolidyl	14.9 ± 1.1	2.671
12c	1-Piperidinyl	11.6 ± 0.7	3.088
12d	1-Morpholinyl	18.5 ± 0.8	2.033

[a] Estimated lipophilicity by CAChe v. 6.1.

Table 2. The relative levels of ERK activation of certain tetrahydroisoquinoline derivatives in treated cells compared with nontreated cells

Compound	Activation of ERK1/2 (*p*-ERK/ERK, %)
D + T	100
Rasagiline	131 ± 14
12a	131 ± 10
12c	155 ± 28*
12d	138 ± 39

Each value represents the mean ± SD of three experiments. *$P < 0.05$, significantly different compared with the control.

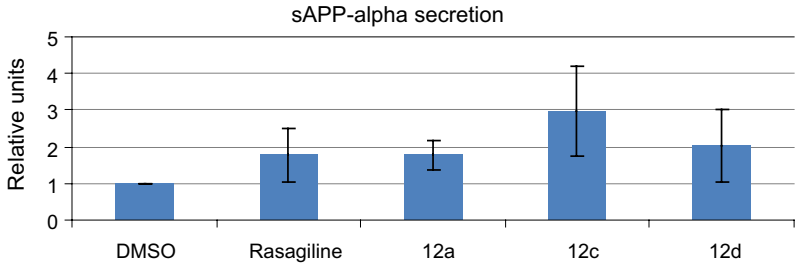


Figure 5. The secretion of sAPPα was determined by analysis of the immunoblots. The units obtained are relative to the signal from cells treated with solvent (DMSO). Each value indicates the mean ± SD of three experiments.

along with human PS1, Aph-1α2, and Pen-2, and were an ideal cell model for the detection of α-cleavage of APP.[18] The production of sAPPα in the conditioned media of γ-30 cells would be defined as the α-secretase activity. Substantial effects of these compounds, **12a**, **12c**, and **12d**, on α-secretase activity at 10 μM were observed. The effect on sAPPα secretion correlates nicely with the ERK activation and also showed modest improvement as compared to rasagiline, suggesting that the inhibition of γ-secretase and the activation of sAPPα secretion could be simultaneously mediated by ERK activation in γ-30 and T20 cells. Recently, Kim and coworkers found that the inhibition of ERK1/2 activity dramatically increased γ-secretase activity in several different cell types, while raising ERK1/2 activity by adding purified active ERK1/2 significantly reduced γ-secretase activity,[17] suggesting that ERK1/2 is an endogenous negative regulator of the γ-secretase activity. Consistent with this notion, we also found that γ-secretase activity in T20 cells treated with the MEK inhibitor PD98059 was dramatically increased, concomitant with the increased levels of secreted Aβ40 in the conditioned media (Liao, Y. F. et al. unpublished data).

4. Conclusion

In conclusion, we have developed a type of new 1,2,3,4-tetrahydroisoquinoline derivatives in order to evaluate its modulation on the proteolytic processing of amyloid precursor protein. The results presented above indicate that these *N*-cyclopropylmethyl derivatives possess sAPPα-releasing stimulation, which correlates with their effects on ERK activation, and also display γ-secretase inhibitory potency. These results may reflect the synergetic potential of neuroprotective activities for the treatment of Alzheimer's disease as they possessed both sAPPα secretion through ERK activation and simultaneous inhibition of amyloidogenic Aβ release.

5. Experimental

5.1. Chemistry

All reagents were commercial materials and were used directly unless otherwise noted. DMF was dehydrated over 4 Å molecular sieves. NMR spectra were recorded on a Varian Gemini at 300 MHz for ^{1}H and at 75 MHz for ^{13}C. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane. The deuterated NMR solvents contained 99.8–99.9% deuterium in the indicated position and were obtained from CIL, Inc. (Massachusetts, USA). ^{1}H NMR coupling constants (J values) are listed in hertz (Hz) and spin multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (b). Elemental analyses were determined using a Perkin-Elmer 240 EA analyzer. Fast atom bombardment mass spectra (FABMS) were acquired on a Finnigan Mat 95S mass spectrometer. Specific rotations were measured with an Optical Activity AA-5 Polarimeter. Chromatography refers to flash chromatography on silica gel (silica gel 60, 230–400 mesh ASTM, E. Merck). Melting points were recorded on a Thomas Hoover capillary melting point apparatus in open capillary tubes and are uncorrected.

5.1.1. *N*-Trifluoroacetyl-1,2,3,4-tetrahydroisoquinoline (4). To an ice-cooled solution of trifluoroacetic anhydride (9 ml) in CH_2Cl_2 (32.4 ml) was added dropwise a solution of 1,2,3,4-tetrahydroisoquinoline (8 ml, 60 mmol) in CH_2Cl_2 and stirred under ice-cooling for 3.5 h. A solution of KOH (4.74 g, 85 mmol) in water (72 ml) was then added, and the reaction mixture was stirred for further 2 h at room temperature. The resulting mixture was extracted with CH_2Cl_2 and the organic layer was dried over $MgSO_4$. The residue was taken on a silica gel column (*n*-hexane/EtOAc = 3:1 as eluents) to give a yellow oil (9.92 g, 72%) of the title compound; TLC $R_f = 0.58$ (*n*-hexane/EtOAc = 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29–7.19 (m, 4H, Aryl *Hs*); 4.80 (s, 2H, Ph-CH_2–N); 3.91–3.86 (m, 2H, Ph-CH_2–CH_2–N); 3.00 (br s, 2H, Ph-CH_2–CH_2–N). FABMS *m*/*z*: 230 (M + 1) ($C_{11}H_{10}NOF_3$).

5.1.2. 7-Chloroacetyl-*N*-trifluoroacetyl-1,2,3,4-tetrahydroisoquinoline (5). To a suspension of $AlCl_3$ (24.16 g) in CH_2Cl_2 (162 ml) was added chloroacetyl chloride (15 ml) dropwise at 0–5 °C under argon for 20 min and left to warm to room temperature. To this mixture was added 4 (9.29 g, 40 mmol) over a period of 30 min at room temperature. The resulting mixture was then stirred for an additional 3 h and poured onto a mixture of ice-cold water (407 ml). The mixture was stirred for 5 min, and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 200 ml). The combined organic layers were washed with water (2 × 240 ml) and 5% aqueous $NaHCO_3$ (3 × 240 ml). The organic layer was dried and the solvent evaporated to give a white solid (10.47 g, 88%). TLC $R_f = 0.4$ (*n*-hexane/

EtOAc = 3:1). mp: 120–122 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78 (q, J = 4 Hz, 1H, Aryl H), 7.34–7.26 (m, 2H, Aryl *Hs*), 4.87 (s, 2H, Ph-CH_2–N), 4.67 (s, 2H, –CH_2–C═O), 3.95–3.87 (m, 2H, –CH_2–N), 3.06–3.01 (m, 2H, Ph-CH_2–). FABMS *m*/*z*: 306 (M + 1) ($C_{13}H_{11}NO_2ClF_3$).

5.1.3. 7-Chloroacetoxy-*N*-trifluoroacetyl-1,2,3,4-tetrahydroisoquinoline (6). Compound 5 (8.4 g, 27 mmol) was dissolved in anhydrous CH_2Cl_2 (58 ml) and 3-chloroperoxybenzoic acid (12.3 g) was added in one portion. The suspension was cooled to 0 °C, and trifluoroacetic acid (2.0 ml) was added dropwise over 5–10 min. The reaction flask was protected from light, and the mixture was stirred for 3–5 days at room temperature, poured onto water (100 ml), and neutralized with ammonium hydroxide solution. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (60 ml). After purification on a silica gel column (*n*-hexane/EtOAc = 2:1) the title compound was obtained as a yellow oil (4.24 g, 48%); TLC R_f = 0.5 (*n*-hexane/EtOAc = 3:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.20 (t, J = 7 Hz, 1H, Aryl H), 7.01–6.93 (m, 2H, 2H, Aryl Hs), 4.75 (s, 2H, Ph-CH_2–N), 4.33 (s, 2H, CO–CH_2–), 3.90–3.82 (m, 2H, N–CH_2–), 2.97–2.92 (m, 2H, Ph-CH_2–). FABMS *m*/*z*: 322 (M + 1) ($C_{13}H_{11}NO_3ClF_3$).

5.1.4. 7-Hydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride salt (7). To a solution of 6 (3.6 g, 11 mmol) in MeOH (100 ml) was added 15% of aqueous NaSMe (10 ml, 107 mmol) at 20 °C. After 2 h, the mixture was acidified with 3 N HCl to pH 1. The resulting precipitate was collected as a white solid: R_f = 0.2 (DCM/MeOH = 5:1), ^{1}H NMR (300 MHz, D_2O): δ 7.15–7.00 (m, 3H, 2H, Aryl Hs), 4.22 (d, J = 6 Hz, 2H, Ph-CH_2–N), 3.80–3.58 (m, 2H, Ph-CH_2–CH_2–N), 2.65 (t, J = 5.4 Hz, 2H, Ph-CH_2–CH_2–N); FABMS *m*/*z*: 186 (M + 1) ($C_9H_{11}NO$·HCl).

5.1.5. 7-Hydroxy-*N*-propargyl-1,2,3,4-tetrahydroisoquinoline (8). To a stirred mixture of hydrochloride salt 7 (1.7 g, 11 mmol) and K_2CO_3 (3 g, 22 mmol) in CH_3CN (33 ml) was added a solution of 80% propargyl bromide (1.3 ml, 11 mmol). The reaction mixture was continued to stir at room temperature under nitrogen for 25 h and filtered. The filtrate was washed with water and the aqueous layer was extracted with EtOAc (2 × 40 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resulting residue was taken on a silica gel column (DCM/MeOH = 9:1 as eluents) to give a brown solid (110 mg, 50%) of the title compound: R_f = 0.4 (DCM/MeOH = 9:1); FABMS: *m*/*z* 188 $[M + H]^+$; mp: 82–83 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.91 (d, J = 8.1 Hz, 1H, Aryl H), 6.60 (d, J = 8.1 Hz, 1H, Aryl H), 6.38 (s, 1H, Aryl H), 3.69 (d, J = 23 Hz,2H, Ph-CH_2–N), 3.51 (d, J = 1.8 Hz, 2H, N–CH_2–C≡C–), 2.84 (t, J = 4 Hz, 4H, Ph-CH_2–CH_2–N), 2.31 (t, J = 2 Hz, 1H, C≡CH). FABMS *m*/*z*: 188 (M + 1) ($C_{12}H_{13}NO$).

5.1.6. 7-Hydroxy-*N*-cyclopropylmethyl-1,2,3,4-tetrahydroisoquinoline (9). To a stirred mixture of hydrochloride salt 7 (0.9 g, 6 mmol) and potassium carbonate (2.0 g, 12 mmol) in CH_3CN (18 ml) was added a solution of 96% (bromomethyl)cyclopro- pane (0.6 ml, 6.3 mmol). The reaction mixture was stirred at room temperature under nitrogen for 25 h and filtered. The filtrate was washed with water and the aqueous layer was extracted with ethyl acetate (20 ml x 2). The organic layer was dried over MgSO4. The residue was taken on a silica gel column (DCM/methanol = 9:1 as eluents) to give a yellow solid (0.37 g, 57%) of the title compound; TLC R_f = 0.3 (DCM/MeOH = 9:1). mp: 118–119 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.87 (d, J = 8.1 Hz, 1H, Aryl H), 6.58 (dd, J = 8.1 Hz, 2.1 Hz, 1H, Aryl H); 6.24 (d, J = 2.1 Hz, 1H, Aryl H), 3.58 (s, 2H, Ph-CH_2–N), 2.87 (dd, J = 5 Hz, 4H, Ph-CH_2–CH_2–N), 2.48 (d, J = 7 Hz, 2H, N–CH_2); 1.13–1.10 (m, 1H); 0.61–0.57 (m, 2H), 0.21 (q, J = 4.8 Hz, 2H). FABMS *m*/*z*: 204 (M + 1) ($C_{13}H_{17}NO$).

5.1.7. General procedure for the preparation of 11a–d and 12a–d. To a stirred and ice-cooled solution of 8 or 9 (1.0 equiv) in CH_3CN was added each of *N*,*N*-dialkylcarbamyl chloride (**10a**–**d**), followed by dropwise addition of NaH (60% in mineral oil, 1.3 equiv). The reaction mixture was stirred for 2 h at room temperature under argon. After evaporation of the solvent in vacuo, water was added and extracted with EtOAc (3×). The organic layers were combined, dried over $MgSO_4$, and evaporated to dryness in vacuo. The residue was purified by column chromatography to afford title compound.

5.1.7.1. 7-(*N*-Methyl-*N*-ethylcarbamoyloxy)-*N*-propargyl-1,2,3,4-tetrahydroisoquinoline (11a). Purified on a silica gel column (EtOAc/*n*-hexane = 2:1); Yield 50%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.08 (d, J = 8 Hz, 1H, Aryl H); 6.88 (d, J = 7 Hz, 1H, Aryl H), 6.80 (s, 1H, Aryl H), 3.76 (s, 2H, Ph-CH_2–N), 3.50 (d, J = 2 Hz, 2H, N–CH_2–CCH), 3.42(q, J = 7 Hz, 2H, N–CH_2–CH_3), 3.04–2.81 (m, 7H, N–CH_3, Ph-CH_2–CH_2–N), 2.28–2.26 (m, 1H, C≡CH), 1.25–1.15 (m, 3H, N–CH_2–CH_3); IR (KBr): ν cm^{-1} = 3300, 2923, 1715. FABMS *m*/*z*: 273 (M + 1) ($C_{16}H_{20}N_2O_2$).

5.1.7.2. 7-(1-Pyrrolidinecarbamoyloxy)-*N*-propargyl-1,2,3,4-tetrahydroisoquinoline (11b). Purified on a silica gel column (EtOAc/*n*-hexane = 2:1); Yield 53%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.08 (d, J = 8 Hz, 1H, Aryl H), δ 6.91 (dd, J = 8 Hz, 2 Hz, 1H, Aryl H), 6.83 (d, J = 2 Hz, 1H, Aryl H), 3.81 (s, 2H, Ph-CH_2), 3.55–3.44 (m, 6H, 2 N–CH_2–CCH), 2.94–2.90 (m, 4H, N–CH_2–CH_2), 2.30 (t, J = 3 Hz, 1H, C≡C*H*), 1.98–1.89 (m, 4H); IR (KBr): ν cm^{-1} 3375, 2951, 1715. FABMS *m*/*z*: 285 (M + 1) ($C_{17}H_{20}N_2O_2$).

5.1.7.3. 7-(1-Piperidinecarbamoyloxy)-*N*-propargyl-1,2,3,4-tetrahydroisoquinoline (11c). Purified on a silica gel column (EtOAc/*n*-hexane = 2:1); Yield 50%; mp: 72–73 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.08 (d, J = 8 Hz, 1H, Aryl H), 6.87 (dd, J = 8 Hz, 2 Hz, 1H, Aryl H), 6.80 (d, J = 2 Hz, 1H, Aryl H), 3.77 (s, 2H, Ph-CH_2–N), 3.52 (t, J = 15 Hz, 4H, N–CH_2–), 2.94–2.85 (m, 4H, Ph-CH_2–CH_2–N); 2.28 (t, J = 2 Hz, 1H, C≡CH), 1.63 (s, 6H,); IR (KBr): ν cm^{-1} = 3255, 2926, 1717. FABMS *m*/*z*: 299 (M + 1) ($C_{18}H_{22}N_2O_2$).

5.1.7.4. 7-(1-Morpholinecarbamoyloxy)-*N*-propargyl-1,2,3,4-tetrahydroisoquinoline (11d). Purified on a silica gel column (EtOAc/*n*-hexane = 1:2); Yield 82%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.10 (d, J = 8.4 Hz, 1H, Aryl H), 6.90 (dd, J = 8.4 Hz, 2.4 Hz, 1H, Aryl H), 6.81 (d, J = 2.4 Hz, 1H, Aryl H), 3.83–3.56 (m, 12H, 2–OCH_2CH_2N–, Ph-CH_2–N, N–CH_2–C≡CH), 2.96–2.92 (m, 4H, Ph-CH_2–CH_2–N), 2.32 (t, J = 2.4 Hz, 1H, –C≡CH); IR (KBr): ν cm^{-1} = 3232), 2951, 1706. FABMS *m*/*z*: 301 (M + 1) ($C_{17}H_{20}N_2O_3$).

5.1.7.5. 7-(*N*-Methyl-*N*-ethylcarbamoyloxy)-*N*-cyclopropylmethyl-1,2,3,4-tetrahydroisoquinoline (12a). Purified on a silica gel column (DCM/MeOH = 10:1); Yield 50%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.06 (d, J = 8 Hz, 1H, Aryl H); 6.84 (s, 1H, Aryl H); 6.81 (s, 1H, Aryl H); 3.71 (s, 2H, Ph-CH_2–N); δ 3.44, 3.39 (dd, J = 7 Hz, 2H, CH_3–N–CH_2CH_3); 3.03–2.75 (m, 7H, CH_3–N–CH_2CH_3, Ph-CH_2–CH_2–N); 2.42 (d, J = 6 Hz, 2H, N–CH_2); 1.24–1.17 (m, 3H, -CH_3); 1.14–0.94 (q, J = 7 Hz, 1H); 0.58–0.52 (m, 2H); 0.19–0.14 (m, 2H); IR (KBr): ν cm^{-1} = 2924, 1715. FABMS *m*/*z*: 289 (M + 1) ($C_{17}H_{24}N_2O_2$).

5.1.7.6. 7-(1-Pyrrolidinecarbamoyloxy)-*N*-cyclopropylmethyl-1,2,3,4-tetra-hydroisoquinoline (12b). Purified on a silica gel column (DCM/MeOH = 20:1); Yield 50%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.08 (d, J = 8.1 Hz, 1H, Aryl H), 6.90 (d, J = 8.1 Hz, 1H, Aryl H); δ 6.84 (s, 1H, Aryl H), 3.80 (s, 2H, Ph-CH_2–N), 3.54 (t, J = 5.1 Hz, 2H, N–CH_2–), 3.46 (t, J = 5.1 Hz, 2H, N–CH_2–), 2.92 (br s, 4H, Ph-CH_2–CH_2–N); δ 2.51 (d, J = 4.8 Hz, 2H, N–CH_2–C≡C–); 1.92 (br s, 4H, –CH_2–CH_2–); δ 1.03 (br s, 1H); 0.58 (dd, J = 6.3 Hz, 1.5 Hz, 2H); 0.20 (d, J = 1.5 Hz, 2H); IR (KBr): ν cm^{-1} = ,2876, 1715. FABMS *m*/*z*: 301 (M + 1)($C_{18}H_{24}N_2O_2$).

5.1.7.7. 7-(1-Piperidinecarbamoyloxy)-*N*-cyclopropylmethyl-1,2,3,4-tetrahydroisoquinoline (12c). Purified on a silica gel column (DCM/MeOH = 12:1); Yield 50%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.06 (d, J = 8.4 Hz, 1H, Aryl H); 6.86 (dd, J = 8.4 Hz, 2.4 Hz, 1H, Aryl H), 6.80 (d, J = 2.4 Hz, 1H, Aryl H), 3.71 (s, 2H, Ph-CH_2–N), 3.57 (br s, 2H, N–CH_2–), 3.50 (br s, 2H, N–CH_2–), 2.92–2.81 (m, 4H, Ph-CH_2–CH_2–N), 2.44 (d, J = 6.9 Hz, 2H, N–CH_2), 1.62 (br s, 6H); 1.00–0.95 (m, 1H); 0.59–0.53 (m, 2H); 0.20–0.15 (m, 2H); IR (KBr): ν cm^{-1} = 2924, 1717. FABMS *m*/*z*: 315 (M + 1)($C_{19}H_{26}N_2O_2$).

5.1.7.8. 7-(1-Morpholinecarbamoyloxy)-*N*-cyclopropylmethyl-1,2,3,4-tetrahydroisoquinoline (12d). Purified on a silica gel column (DCM/MeOH = 10:1); Yield 51%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.08 (d, J = 8.2 Hz, 1H, Aryl H), 6.86 (d, J = 8.2 Hz, 1H, Aryl H), 6.81 (s, 1H, Aryl H), 3.75–3.57 (m, 10H, O-(CH_2–CH_2)$_2$–N-, Ph-CH_2–N), 2.90 (t, J = 5.7 Hz, 4H, –CH_2–N), 2.80 (t, J = 5.7 Hz, 4H, Ph-CH_2–), 2.42 (d, J = 6.6 Hz, N–CH_2–), 0.98–0.92 (m, 1H), 0.59–0.53 (m, 2H), 0.19–0.15 (m, 2H); IR (KBr): ν cm^{-1} = 2924, 1717. FABMS *m*/*z*: 317 (M + 1) ($C_{18}H_{24}N_2O_3$).

5.1.8. *N*-Propargyl-1,2,3,4-tetrahydroisoquinoline (13). To a stirred mixture of 3 (2 ml, 15 mmol) and K_2CO_3 (2.07 g, 15 mmol) in CH_3CN (30 ml) was added a solution of 80% propargyl bromide (1.7 ml, 15 mmol). The reaction mixture was continued to stir at room temperature under nitrogen for 25 h and filtered. The filtrate was washed with water and the aqueous layer was extracted with EtOAc (2 × 40 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resulting residue was taken on a silica gel column (DCM/MeOH = 9:1) to give a brown solid (1.28 g, 75%) of the title compound: R_f = 0.3 (DCM/MeOH = 9:1); mp: 102–104 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38–7.16 (m, 4H, Aryl Hs), 3.92 (s, 2H, Ar-CH_2–N), 3.65 (d, J = 2.4 Hz, 2H, N–CH_2–C≡C–), 3.08 (t, J = 5.4 Hz, 4H, Ph-CH_2–CH_2–N), 2.97 (t, J = 5.2 Hz, 4H, Ph-CH_2–CH_2–N), 2.41 (t, J = 2.4 Hz, 1H, C≡CH). FABMS *m*/*z*: 172 (M + 1) ($C_{12}H_{13}N$).

5.1.9. 7-Chloroacetyl-1,2,3,4-tetrahydroisoquinoline (14). To a solution of 5 (4.4 g, 14 mmol) in MeOH (50 ml) was added 15% of aqueous NaSMe (5 ml, 53 mmol) at 20 °C. After 2 h, the reaction mixture was concentrated in vacuo, diluted with EtOAc (50 ml), and washed with 10% aqueous sodium thiosulfate (2 × 20 ml) and brine. The organic layer was evaporated and taken on a silica gel column (DCM/MeOH = 9:1) to give a white solid (1.98, 68%): R_f = 0.4 (DCM/MeOH = 8:1), ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (d, J = 4.2 Hz, 1H, Aryl H), 7.24–7.18 (m, 2H, Aryl Hs), 4.66 (s, 2H, Ph-CH_2–N), 4.44 (s, 2H, –CH_2–C═O), 3.76–4.65 (m, 2H, –CH_2–N), 2.98–2.86 (m, 2H, Ph-CH_2–). FABMS *m*/*z*: 210 (M + 1) ($C_{11}H_{12}NOCl$).

5.1.10. 7-Chloroacetyl-*N*-propargyl-1,2,3,4-tetrahydroisoquinoline (15). *Via acetylation of* **13**. According to the reaction procedure for preparing 5, 13 (1.28 g, 7.5 mmol) was acetylated to give **15** as a white solid (0.85 g, 46%). *Via propargylation of* **14**: A mixture of **14** (0.8 g, 3.8 mmol) and K_2CO_3in CH_3CN was treated with a solution of 80% propargyl bromide (0.45 ml, 3.9 mmol) to give **15** as a white solid (0.38 g, 40%); R_f = 0.35 (*n*-hexane/EtOAc = 4:1); mp: 108–110 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75 (q, J = 5.2 Hz, 1H, Aryl H), 7.70 (s, 1H, Aryl H), 7.28 (d, J = 5.0 Hz, 1H, Aryl H), 4.67 (s, 2H, –CH_2–C═O), 4.06 (s, 2H, Ph-CH_2–N), 3.73 (br s, 2H, N–CH_2≡CH), 3.16–3.10 (m, 2H, Ph-CH_2–), 2.45 (br s, 1H, C≡CH),. FABMS *m*/*z*: 248 (M + 1) ($C_{14}H_{14}ClNO_2$).

5.2. X-ray crystal structure determination of 7-chloroacetyl-*N*-propargyl-1,2,3,4-tetrahydroisoquinoline (15)

A selected single crystal (dimensions 0.30 mm × 0.25 mm × 0.20 mm) of **15** was mounted onto a glass fiber and set up on a Nonius Kappa-CCD diffractometer. Diffraction data were collected at room-temperature Mo Kα radiation (k = 0.71073 Å). Unit cell parameter determination, data collection strategy, and integration were carried out with the Nonius EVAL-14 suite of programs. The data were corrected from absorption by a multi-scan method. The structure was solved by direct methods with SHELXS-86, refined by full matrix

least-squares on F^2. Graphics were carried out with DIAMOND. All non-H atoms were refined with anisotropic displacement parameters and H atoms were simply introduced at calculated positions (riding model). Crystallographic data: $C_{14}H_{14}ClNO$, MW, 247.70; monoclinic, $P\bar{1}$ (No. 2); *a*, 13.275(2) Å; *b*, 14.472(4) Å; *c*, 6.529(2) Å; *V*, 1250.54(9) Å^3; *Z*, 4; D_{calc}, 1.310 mg/m^3; *F*(000), 516; μ, 0.288 mm^{-1}; 2864 observed data, with $I > 2\sigma$ (*I*).

5.3. Pharmacological evaluation

5.3.1. Reagents. Anti-ERK1/2 MAP kinase antibody and anti-MEK antibody were purchased from Cell Signaling Technology, Inc. Horseradish peroxidase-conjugated anti-rabbit IgG was from Santa Cruz Biotechnology, Inc. Horseradish peroxidase-conjugated anti-mouse IgG and ECL Western Blotting detection reagents were from Amersham Biosciences. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were from Invitrogen and Biological Industries Ltd (Kibbtuz Beit Haemek, Israel), respectively. All other reagents were of at least reagent grade and obtained from standard suppliers.

5.3.2. Cell-based γ-secretase assays. The generation of T20 cells has been reported previously.[9] T20 cells were routinely maintained in DMEM supplemented with 10% FBS, 200 μg/ml hygromycin, 5 μg/ml blasticidin, and 250 μg/ml zeocin (DMEM-HZB). The stably transfected T20 cells were detached from culture dishes by trypsinization, washed with PBS, and resuspended in DMEM-HZB, followed by plating onto 96-well microplates (2×10^4 cells/50 μl/well) and incubating at 37 °C for 24 h. Preliminarily, compounds diluted in DMEM-HZB were added to a final concentration of 10 μM in the presence of tetracycline (1 μg/ml). Treatments were terminated after incubation at 37 °C for 24 h by directly adding an equivalent volume of the Steady-Glo luciferase assay reagent (Promega), and luciferase signals from each well were performed immediately with the luminescence plate reader available in our Institute. Triplicates of each compound treatment were assayed. Luciferase signal from the stable line without tetracycline induction and compound treatment was referred to as one-fold of activation. Parallel testing with a cell line constitutively expressing only the luciferase reporter gene (e.g., under control of a CMV promoter) was performed as a control.

5.3.3. Cell viability assay. T20 cells (5×10^4/100 μl/well) were seeded onto the wells of 96-well microplates in culture medium containing 10 μM of respective compounds and incubated at 37 °C for 24 h. Viable cells were determined using the CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay (Promega) as specified in the manufacturer's instructions. Briefly, following the addition of the combined MTS/PMS solution (20 μl/well) microplates were incubated for 3 h at 37 °C. The conversion of MTS into formazan in viable cells was quantitated by the absorbance at 490 nm using a Synergy HT ELISA plate reader (BioTek). The number of living cells in culture was directly proportional to the absorbance at 490 nm. Viable cells in culture medium containing vehicle alone (1% DMSO, Control) were referred to as 100% viability. The background absorbance shown at 0 cells/well was subtracted from these data.

5.3.4. Cell culture and cell lines. Human embryonic kidney cells (HEK293) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 0.1 mg/ml penicillin and streptomycin. T-REx293 cells were purchased from Invitrogen and cultured in DMEM supplemented with 10% FBS and 5 mg/ml blasticidin. The generation of stably transfected cell lines, T20 and γ-30, has been described previously.[13] Cells were incubated in a humidified incubator at 37 °C in 5% CO_2.

5.3.5. MAO-B assays.

5.3.5.1. Fluorometric assay of rat brain protein content. MAO-B enzyme preparations were prepared from brain cortex of decapitalized male F344/N rats. The brain cortex in PBS solution was frozen with liquid nitrogen and kept at −80 °C for 3 days. The homogenate was then obtained by centrifugation (14,000 rpm, 10 min) of the frontal cortex in 0.1 M potassium phosphate buffer (pH 7.4) at 4 °C and the supernatant was taken as the enzyme source for MAO. A standard solution containing 0.1 M potassium phosphate buffer and albumin were prepared in different concentration grades. Ten microliters of each solution was taken and added with 200 μl of BCA kit. Following the similar method, the above supernatant from brain cortex was diluted and 10 μl of stock solution taken and added with 200 μl of BCA kit. Both standard and sample solutions were incubated at 37 for 30 min. Each of 200 μl of the solutions was taken to 96-well microplate and the protein contents were measured with a fluorescence microplate reader by determination of optical density at 595 nm.

5.3.5.2. MAO-B inhibitory assays. The supernatant (protein content, 80 μg/ml) in 96-well microplate was added with 1 U/ml HRP, 1 mM benzylamine, and 50 μM Amplex red in 0.1 M potassium phosphate buffer followed by the tested sample, which was prepared in 200 μg/ml in DMSO as stock solution. These mixtures were incubated at room temperature for 60 min and optical density determined by a fluorometric reader.

$$\text{Inhibition } (\%) = 1 - [(\text{OD}_{595} \text{ of sample/protein} - \text{OD}_{595} \text{ of sample})/\text{OD}_{595} \text{ of blank}] \times 100\%$$

5.3.6. Assay for extracellular signal-regulated kinase (ERK) activity. The activation of the ERKs was measured by using anti-phospho-ERK1/2 and anti-ERK1/2 antibodies (Cell Signaling Technology). In brief, T20 cells were grown in six-well plates at 5×10^5 cells/well and incubated with culture medium at 37 °C for 18 h, followed by an additional incubation with culture medium containing 1 μg/ml tetracycline at 37 °C for 18 h. Before the experiments, we replaced the medium with

DMEM containing 0.5% FBS and treated it with the tested compounds (10 μM each). After treatment, reactions were stopped by placing cells on ice and aspirating the medium. Cells were harvested and lysed in 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM EDTA, 1 mM sodium orthovanadate, 1% Triton X-100, and protease- and phosphatase-inhibitor cocktails. Protein concentration was determined by the BCA assay (Pierce). Each cell lysate, which contained 50 μg proteins, was separated on 12% SDS–polyacrylamide electrophoresis gels, immunoblotted, and identified using anti-phospho-ERK1/2 or anti-ERK1/2 antibody.

5.3.7. Determination of soluble APPα (sAPPα) fragment. Conditioned media of γ-30 cells,[18] standardized to the protein content in the corresponding cell lysates, were immunoprecipitated with a rabbit polyclonal anti-Aβ1–17 antibody (clone 02-1493), resolved by SDS–PAGE, and analyzed by Western blotting with a mouse monoclonal anti-Aβ1–17 antibody (6E10).

5.3.8. Statistical analysis. The data were presented as means ± SD and triplicates of each compound treatment were measured in the experiments. The effects of the various synthesized compounds on the biological outcome were statistically examined using a one-way analysis of variance. Dunnets's test was used to compare individual compounds. In all cases, $P < 0.05$ was accepted to denote significance.

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