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Original article

Discovery of small molecular (D)-leucinamides as potent, Notch-sparing γ-secretase modulators

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ABSTRACT

Structural optimization of the prior lead **3** led to the small molecular (D)-leucinamides with potent modulating activity and Notch-sparing selectivity on the proteolytic processing of amyloid-β precursor proteins. The *N*-(*R*)-epoxypropyl analog **10c** exhibited potent γ-secretase modulation compared to DAPT and showed substantial substrate selection for APP cleavage over Notch cleavage, while *N*-(2-fluoro) benzyl analog **10e** showed the most potent γ-secretase inhibition with dull selectivity. The exceptional suppression of ERK-mediated activation suggested that these potent γ-secretase modulators may adapt an alternative pathway to prominently induce the differential inhibition of C99 cleavage by γ-secretase.

1. Introduction

Alzheimer's disease (AD) has been characterized as a progressively neurodegenerative disorder with the deposition of amyloid plaques and intracellular neurofibrillary tangles in the brain [1,2]. Amyloid plaques are predominately composed of 40–42 amino acid amyloid-β protein (Aβ), which is produced from sequential cleavage of amyloid-β precursor protein (APP) by β- and γ-secretases [3]. According to the amyloid hypothesis of AD, the generation and oligomerization of Aβ that results in neuronal insults is the ultimate cause of the disease [4]. In addition to the APP as the known substrate, γ-secretase is also involved in controlling and differentiation in the Notch signaling pathway, a pivotal step that mediates differentiation in embryonic cells during the developmental stage. Selectivity for APP processing over Notch processing to avoid certain Notch-related side effects was thus demanded in the identity of a γ-secretase modulator targeting the chronic treatment of AD [5]. Numerous contributions demonstrated that certain small molecular arylsulfonamides are potent and selective γ-secretase inhibitors such as GSI-953 (begacestat, **1**) and BMS-708163 (avagacestat, **2**) as shown in Fig. 1 [6,7]. Both compounds have been in clinical trials in AD patients, while avagacestat was recently terminated after phase II trials due to lack of supported data for advanced investigations [8]. Intriguingly, an amino acid derivative (D)-leucinamide **3** was reported to be a nanomolar inhibitor of amyloid synthesis by an Aβ lowering cellular assay [9]. Generally, these amino acid-derived γ-secretase inhibitors contain three universal components on the basis of structural features: an aryl group, a sulfonamide moiety, a small alkyl side-chain. Therefore, attention to the structural optimization on the core fragments can be worth to pay for developing new modulators for AD therapies.

Contrary to the β- and γ-secretase-mediated proteolytic cleavage of APP, α-secretase was thought to mediate a beneficial cleavage of APP and generate a neuroprotective, soluble amyloid precursor protein α (sAPPα) in addition to the *C*-terminal fragment C83, thus preclude amyloidogenic Aβ production [10]. This α-secretase-mediated APP cleavage was thought through downstream activation from mitogen-activated protein kinases (MAPKs) signaling pathway [11,12]. Accordingly, decreased Aβ formation accompanied by neuronal protection would be accessed by selective γ-secretase modulators with ERK-mediated activation, which decrease β-/γ-secretase-mediated proteolytic processing of APP and alternatively augment sAPPα release [13,14]. From the molecular mechanism point of view, we planned to develop a type of new modulators of proteolytic processing of APP as potential agents for Alzheimer's therapy [15].

In our studies to identify new modulators on the proteolytic processing of APP for AD therapies, we have reported a type of propargylamine-related small molecules, which exhibited both

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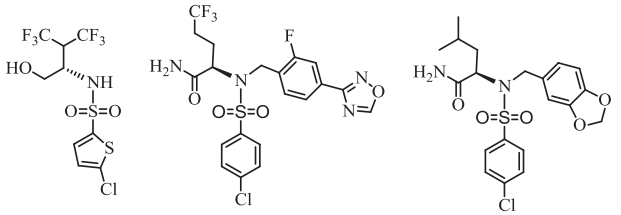
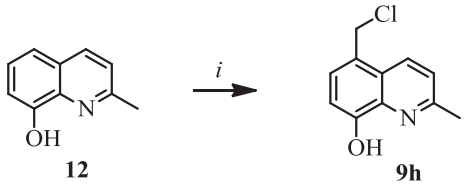


Fig. 1. Chemical structures of GSI-953 (1), BMS-708163 (2), and a (D)-leucinamide **3**.

γ-secretase inhibition activities and ERK-mediated signaling effects [16]. This stimulated our interest in designing and synthesizing of a series of small molecular *N*- arylsulfonyl-(D)-leucinamides with optimization of the additional *N*-substitution that led to the discovery of potent modulators with Notch-sparing selectivity on the proteolytic processing of APP.

2. Chemistry

The synthesis of *N*-substituted *N*-arylsulfonyl-(D)-leucinamides was described in Scheme 1. (D)-Leucinamide (**4**) was coupled to *p*-chlorobenzenesulfonyl chloride (**5**) or 5-chlorothiophene-2-sulfonyl chloride (**6**) in the presence of Hunig's base to give the corresponding arylsulfonamides **7** and **8**, which were each condensed with selected alkyl halides **9a**–**h** in basic conditions to yield a type of *N*-substituted (D)-leucinamides **3**, **10a**–**h**, and **11a**–**i** in modest yields. For the preparation of the selected alkyl halide, 5-chloromethyl-2-methyl-8-quinolinol (**9h**), a solution of 2-methyl-8-quinolinol (**12**), concentrated hydrochloride, and 37% formaldehyde at an ice bath was treated with gaseous hydrogen chloride for 6 h to afford the desired halide **9h** in 48% yield (Scheme 2).

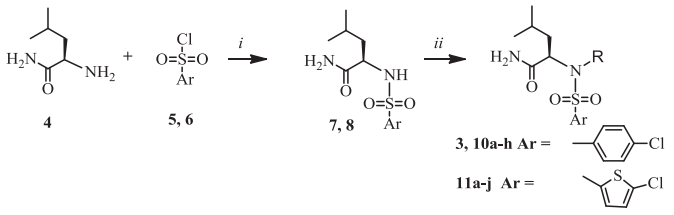


Scheme 2. *i*) 36% HCl, 37% HCHO, HCl(g), 0 °C.

3. Results and discussion

3.1. Evaluation of selective modulating activity on the γ-secretase-mediated C99 and S3 cleavage

To determinate the modulation of γ-secretase-mediated cleavage of C99 (C99-GL-T20) for the synthesized *N*-substituted (D)-leucinamides, the highly efficient and quantitative cell-based luciferase reporter gene assays as described previously were used [17]. The effects of these synthesized (D)-leucinamides containing either *p*-chlorobenzenesulfonyl or 5-chlorothiophene-2-sulfonyl moieties on the γ-secretase modulation are shown in Table 1. First, the screening showed evidence that the lead compound **3** and its thiophenesulfonyl analog **11i** bearing *N*-piperonyl substituent exhibited potent inhibition of γ-secretase, which showed almost equal potency to the prominent DAPT in modulating γ-secretase-mediated C99 cleavage [18]. Intriguingly, all the synthesized *N*-fluorobenzyl compounds **10e**–**g** and **11e**–**g** proved effective in establishing that the (D)-leucinamides with either monofluoro



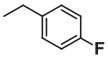
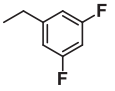
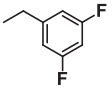
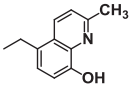
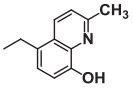
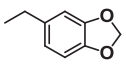
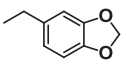
Scheme 1. *i*) diisopropylethylamine, 0 °C – r.t. *ii*) alkyl halides (**9a**–**i**), K_2CO_3, r.t.

substitution at the *ortho*- and *para*-positions, or difluoro substitution at the *meta*-position on the *N*-benzyl ring gain significant potency on γ-secretase inhibition. They showed 92–103% activity of DAPT and maintained similar potency to the lead compound **3**. These results also indicated that most of the *N*-(*p*-chlorobenzenesulfonyl) analogs showed slightly more potency than the corresponding *N*-(5-chlorothiophenesulfonyl) compounds (i.e.**10e**–**g** vs. **11e**–**g**). However, it was noticed that the *N*-(5-chlorothiophenesulfonyl) substituted compound **11i** is about 3% more potent than compound **3**. The bigger, polar substitution was clearly disfavored as seen with the *N*-(8-hydroxy-2-methyl-quinolinyl)methyl analogs **10h** and **11h**. They were around 5-fold less potent than the analogs with small, neutral *N*-benzylic substitutions, such as **10e**–**g** and **11e**–**g**. Taken together, it is clear that both *N*-arylsulfonyl moieties in these substituted (D)-leucinamides are the effective core components for exhibiting γ-secretase inhibition activity and *N*-fluorobenzyl analogs were generally equipotent as seen with **10e**–**g** and **11e**–**g**.

A type of propargylamine-containing small molecules have been proven to be effective to provide neuroprotective features through ERK-mediated signaling pathways as previously described [11,19]. Following the established features, certain *N*-propargyl-related analogs of (D)-leucinamide were envisioned that would couple some of the beneficial pharmacological actions. Accordingly, small molecular (D)-leucinamides **10a**–**d** and **11a**–**d**, which contain *N*-propargyl, -cyclopropyl, and -epoxypropyl moieties were synthesized. As expected, the initial evaluation on modulating the γ-secretase-mediated APP processing showed that the *N*-propargyl analogs **10b** and **11b** preserved potent γ-secretase inhibition with 94–99% activity of DAPT, while *N*-cyclopropyl analogs **10a** and **11a** maintained 88–98% potency. For the (*R*)- and (*S*)-epoxypropyl analogs, compounds **10c** and **10d** bearing the *N*-chlorobenzenesulfonyl substituent were clearly more favorable for γ-secretase inhibition than the corresponding chlorothiophenesulfonyl analogs **11c** and **11d**. These results revealed that *N*-arylsulfonyl-(D)-leucinamides incorporated with *N*-parpargyl-related small moieties can recapitulate activity on γ-secretase inhibition. All of the synthesized *N*-substituted (D)-leucinamides, with only the exception of **11i**, presented no significant cellular toxicity as shown in Fig. 2.

We next examined whether these *N*-arylsulfonyl-(D)-leucinamides can circumvent the γ-secretase-mediated S3 cleavage of Notch, which would reflect the selectivity for the γ-secretase-mediated proteolytic processing of APP over Notch. Accordingly, their influence on the processing of S3 cleavage of Notch was measured by using a stable cell line (NG) constitutively expressing NΔE as previously reported [17]. The relative potency of γ-secretase-mediated S3 cleavage of Notch referred as fold of activation for luciferase gene expression by the synthesized *N*-arylsulfonyl (D)-leucinamides was described in Fig. 3. The results indicated that all of the effective γ-secretase inhibitors with the exception of **10c** were shown to significantly block γ-secretase-mediated S3 cleavage of NΔE in NG cells, suggesting that these compounds were consequentially lack of selectivity for γ-mediated processing of APP over Notch. We found that 62% activity of the γ-secretase were

Table 1
Inhibition of γ-secretase-mediated C99 cleavage by the synthesized *N*-arylsulfonyl-(D)-leucinamides using a luciferase reporter gene assay. The production of T20 cells has been reported previously [20]. T20 cells were 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. Tested compounds diluted in DMEMHZB 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, Madison, WI, USA), 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 signals from the stable line without tetracycline induction and tested 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 panel.

Compound **10**	R	Reduction of C99 cleavage (%)	Compound **11**	R	Reduction of C99 cleavage (%)
a		88.5 ± 1.5	**a**		98.9 ± 1.2
b		99.2 ± 1.0	**b**		94.3 ± 1.6
c		89.7 ± 1.2	**c**		39.2 ± 3.0
d		63.4 ± 1.6	**d**		22.9 ± 4.2
e		103.1 ± 1.2	**e**		99.6 ± 3.0
f		98.9 ± 1.0	**f**		93.1 ± 2.5
g		97.3 ± 1.0	**g**		92.1 ± 1.0
h		23.7 ± 1.4	**h**		20.2 ± 1.3
3		98.9 ± 2.0	**i**		101.9 ± 1.0
DAPT	—	100.0 ± 1.0			

maintained under the treatment of 10 μM of *N*-(*R*)-epoxypropyl analog **10c** to mediate the processing of S3 cleavage, while DAPT was shown to completely block the γ-secretase-mediated proteolytic process. The (*S*)-isomer **10d** exhibited a little better selectivity than **10c**, but it was less active for the inhibition of the γ-secretase-mediated C99 cleavage. Similarly, other *N*-epoxypropyl analogs **11c** and **11d** and compounds **10h** and **11h** did not influence the γ-secretase-mediated processing of S3; they were also lack of inhibition activity on γ-secretase-mediated C99 processing as described above. Apparently, the *N*-(*R*)-epoxypropyl analog **10c** was the best potent and Notch-sparing γ-secretase modulator among the synthesized *N*-substituted (D)-leucinamides.

3.2. Modulation of ERK-mediated signaling

In the following step, we sought to determine whether these compounds can significantly modulate the MEK-ERK signal pathway because the α-secretase-mediated processing of APP would be slightly enhanced directly or indirectly through the activation of MEK-pathway by certain *N*-propargyl-related compounds as previously described. Thus, the relative ERK activation (p-ERK/ERK, %) of the selected γ-secretase inhibitors **3**, **10b**, **10c**, and **10e** were further examined in γ-30 cells as previously described [16,19]. The results were shown in Fig. 4 and indicated that all the selected γ-secretase inhibitors unexpectedly suppressed ERK activation compared to the prevailing ERK-activated rasagiline and propargylamine. The way to incorporate the *N*-propargyl-related moieties to the core (D)-leucinamides did not provide additive pharmacologic effects as we first proposed to. The results suggested that these γ-secretase modulators were lack of beneficial effects on stimulating α-secretase activity; on the contrary, they might slightly boost the overall γ-secretase activity (based on our previous data). The exceptional actions on ERK-mediated signaling possibly posed an alternative pathway for these modulators to induce the differential inhibition of C99 cleavage as they all exhibited strong γ-secretase inhibition activity. Nevertheless, only the *N*-(*R*)-epoxypropyl analog **10c** showed substantial Notch-sparing selectivity as described above, the *N*-(*R*)-epoxypropyl moiety in the compound **10c** may play crucial roles in the differential modulation and substrate selectivity on the γ-secretase-mediated processing of C99 cleavage.

4. Conclusion

In this study, we designed and synthesized a type of small molecular (D)-leucinamides and investigated their selective inhibition activities on the γ-secretase-mediated proteolytic processing between APP and Notch, and their modulation on the ERK-signaling pathway. The results demonstrated that the small molecular *N*-(*R*)-epoxypropyl-(D)-leucinamide **10c** exhibited potent γ-secretase inhibition with promising selectivity on the γ-secretase-mediated APP cleavage over Notch cleavage, while other potent analogs were

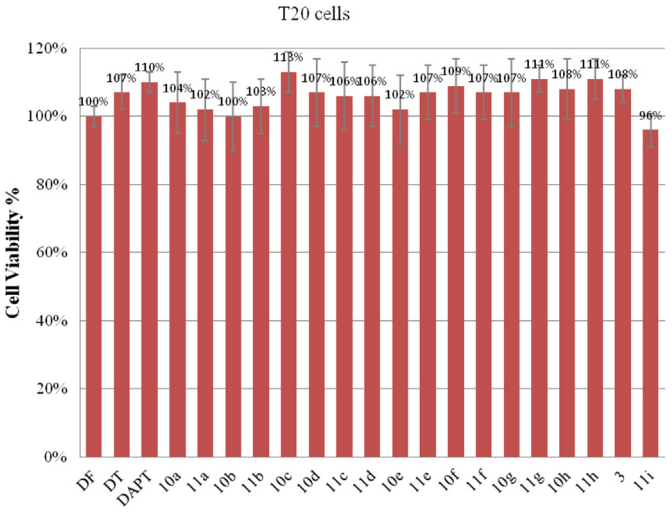


Fig. 2. Viability of host cells in the presence of the synthesized *N*-arylsulfonyl-(D)-leucinamides. T20 cells (5×10^4/100 μL/well) were seeded onto the wells of 96-well microplates in culture medium containing 10 μM of respective compounds and incubated at 37 °C for 24 h. Viable cells were determined using the CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI, USA) 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, Winooski, VT, USA). 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 devoid of cells was subtracted from these data.

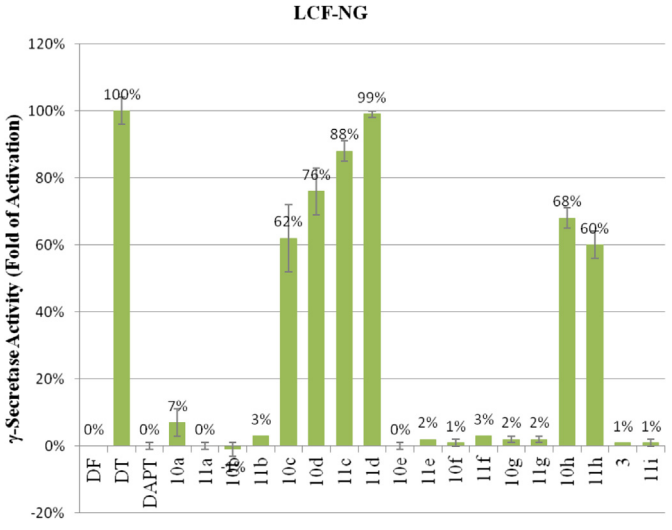


Fig. 3. Inhibition of γ-secretase-mediated S3 cleavage of Notch by the *N*-arylsulfonyl-(D)-leucinamides using a luciferase reporter gene assay. A HEK293-derived stable cell line (N7) that was constitutively expressing NΔE was used to examine the inhibition of γ-secretase-mediated S3 cleavage of Notch by the tested compounds as previously described [20]. N7 cells were plated onto12-well microplates in 1 mL/well DMEM supplemented with 10% FBS at 5×10^5 cells/well. After incubation at 37 °C overnight, cells were treated with compounds at 10 μM as described above and incubated at 37 °C for 24 h. Treated cells were harvested using PBS containing 20 mM EDTA and dissolved in 50 μL of 1× PLB, followed by centrifugation at 13,200*g* for 5 min to remove cell debris. The protein concentrations of clarified supernatants were determined using a BCA protein assay reagent kit, and cell extracts containing equivalent amounts of proteins were resolved by SDS-PAGE and analyzed by Western blotting using an anti-Notch(Val1744) polyclonal antibody.

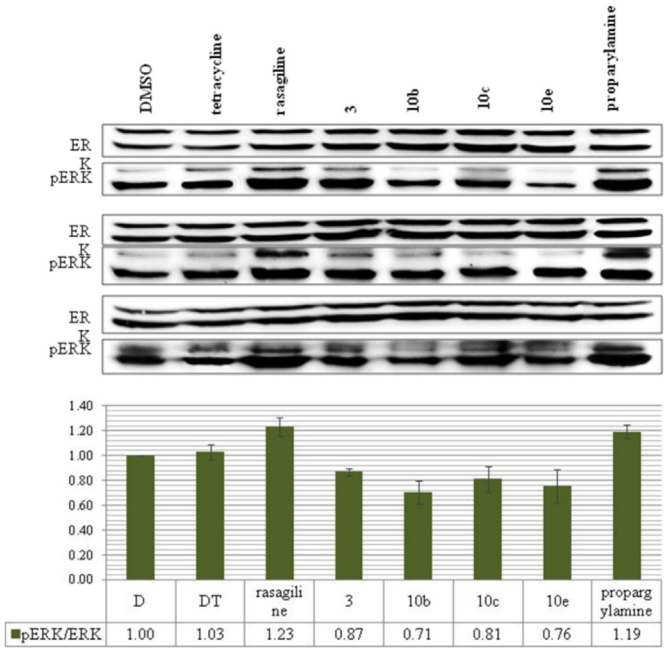


Fig. 4. The modulation of ERK-mediated signaling assay for the selected (D)-leucinamides and the western blot images from 3 independent assays. The stimulation of the ERKs was measured by using anti-phospho-ERK1/2 and anti-ERK1/2 antibodies (Cell Signaling Technology, Danvers, MA, USA) as previously described [16]. Briefly, γ-30 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, Rockford, IL, USA). 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.

obviously devoid of substrate selection. The dual actions on exhibiting potent γ-secretase inhibition and inversely suppressing ERK activation suggest that these potent γ-secretase modulators may adapt an alternative pathway to induce the differential inhibition of C99 cleavage by γ-secretase. It is worth to pursue the relative epoxypropyl-containing compounds further to see if it is a prerequisite entity for the selective modulation of C99 cleavage.

5. Experimental section

5.1. Chemistry

All chemicals and solvents were commercial materials and were used directly unless otherwise noted. DMF was dehydrated over 4 Å molecular sieves. Reactions were monitored by thin layer chromatography using Echo silica gel F254 plates visualized under UV irradiation along with staining with phosphomolybdic acid/heat, or iodine. Melting points were determined with a Thomas Hoover capillary melting point apparatus in open capillary tubes and were uncorrected. A Varian Gemini-300 spectrometer was employed for ^{1}H and ^{13}C NMR spectra with tetramethylsilane or chloroform as an internal reference. Fast atom bombardment mass spectra (FABMS) were acquired on a Finnigan Mat 95S mass spectrometer. Chromatography refers to flash chromatography on silica gel (silica gel 60, 230–400 mesh ASTM, E. Merck, Darmstadt, Germany).

*5.1.1. N-(4-Chlorobenzenesulfonyl)-(D)-leucinamide (**7**)*

To a mixture of diisopropylethylamine (DIPEA, 0.53 mL, 3.0 mmol) and (D)-leucinamide hydrochloride (**4**, 170 mg, 1.0 mmol) in CH_2Cl_2 (10 mL) was added slowly 4-chlorobenzenesulfonyl chloride (**5**, 250 mg, 1.2 mmol). The resulting mixture was stirred at room temperature overnight, then diluted with CH_2Cl_2, washed with H_2O, and dried over $MgSO_4$. The resulting residue was purified by silica gel chromatography to give **7** (258 mg, 85%) as a white powder; tlc $R_f = 0.55$ (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 256 (0.244); mp 228–229 °C; $[\alpha]_D$ +3.7° (c 0.003, MeOH); *c*Log*P* = 2.56; ^{1}H NMR (DMSO-d_6) δ 0.70 (3H, d, *J* = 6.6 Hz, C$\underline{H}_3$), 0.80 (3H, d, *J* = 6.6 Hz, C$\underline{H}_3$), 1.27–1.38 (2H, m, CHC$\underline{H}_2$), 1.55–1.50 (1H, m, $CH_3C\underline{H}CH_3$), 3.64 (1H, t, *J* = 7.2 Hz, COC$\underline{H}$), 6.88 (1H, br, N$\underline{H}$), 7.27 (1H, br, N$\underline{H}$), 7.61 (2H, d *J* = 8.7 Hz, Ar-$\underline{H}$), 7.76 (2H, d, *J* = 8.7 Hz, Ar-$\underline{H}$), 7.95 (1H, br, N$\underline{H}$); ^{13}C NMR (DMSO-d_6) δ 21.7, 23.2, 24.3, 42.2, 55.0, 128.9, 129.4, 1378.4, 140.5, 173.3; FABMS: 305 $[M + H]^+$.

*5.1.2. N-(5-Chlorothiophene-2-sulfonyl)-(D)-leucinamide (**8**)*

To a mixture of diisopropylethylamine (0.53 mL, 3. 0 mmol) and (D)-leucinamide·HCl (**4**, 0.17 g, 1.0 mmol) in CH_2Cl_2 (10 mL) was added slowly 5-chlorothiophene-2-sulfonyl chloride (**6**, 0.26 g, 1.20 mmol). The resulting mixture was stirred at room temperature overnight, and then diluted with CH_2Cl_2, washed with H_2O, and dried over $MgSO_4$. The crude residue was purified by silica gel chromatography to give **8** (267 mg, 86%) as a white powder; tlc $R_f = 0.50$ (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 288 (1.583); mp 228–229 °C; $[\alpha]_D$ +10.0° (c 0.002, MeOH); *c*Log*P* = 2.34; ^{1}H NMR (DMSO-d_6) δ 0.70 (3H, d, *J* = 6.6 Hz, C$\underline{H}_3$), 0.80 (3H, d, *J* = 6.6 Hz, C$\underline{H}_3$), 1.27–1.38 (2H, m, CHC$\underline{H}_2$), 1.50–1.55 (1H, m, $CH_3C\underline{H}CH_3$), 3.64 (1H, t, *J* = 7.2 Hz, COC$\underline{H}$), 6.88 (1H, br, N$\underline{H}$), 7.27 (1H, s, N$\underline{H}$), 7.61 (2H, d *J* = 8.7 Hz, thiophene-$\underline{H}$), 7.76 (2H, d, *J* = 8.7 Hz, thiophene-$\underline{H}$), 7.95 (1H, br, N$\underline{H}$); ^{13}C NMR (DMSO-d_6) δ 21.7, 23.2, 24.4, 42.1, 55.2, 128.0, 131.8, 134.6, 141.0, 173.2; FABMS: 311 $[M + H]^+$.

*5.1.3. N-(4-Chlorobenzenesulfonyl)-N-cyclopropylmethyl-(D)-leucinamide (**10a**)*

To a mixture of **7** (0.30 g, 1 mmol) in DMF (6 mL) was added potassium carbonate (0.83 g, 6.0 mmol), potassium iodide (0.01 g, 0.06 mmol), and (chloromethyl)cyclopropane (**9a**, 0.28 mL, 3.0 mmol) at room temperature. The mixture was stirred for 48 h, poured into brine, then extracted with CH_2Cl_2. The organic layer was separated, dried over $MgSO_4$, and concentrated. The crude residue was purified by silica gel column chromatography to give **10a** (100 mg, 28%) as a yellow oil. tlc $R_f = 0.46$ (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 288 (1.057); $[\alpha]_D$ +24.1° (c 0.003, MeOH); *c*Log*P* = 3.76; ^{1}H NMR ($CDCl_3$) δ 0.26–0.36 (2H, m, cyclopropane-$\underline{H}_2$), 0.46–0.59 (2H, m, cyclopropane-$\underline{H}_2$), 0.72 (6H, d, *J* = 6.6 Hz, C$\underline{H}_3$), 0.98–1.15 (2H, m, cyclopropane-$\underline{H}$, C$\underline{H}$H), 1.25–1.35 (1H, m, CH$\underline{H}$), 1.86–2.03 (1H, m, $CH_3C\underline{H}CH_3$), 3.16 (2H, dd, *J* = 6.6, 5.4 Hz, NC$\underline{H}_2$), 4.23 (1H, t, *J* = 6.9 Hz, COC$\underline{H}$), 5.57 (1H, br, N$\underline{H}$), 6.67 (1H, br, N$\underline{H}$), 7.49 (2H, d, *J* = 8.7 Hz, Ar-$\underline{H}$), 7.78 (2H, d, *J* = 8.7 Hz, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ 4.5, 5.7, 11.2, 22.3, 24.6, 37.2, 49.8, 57.1, 128.6, 129.3, 138.7, 139.3, 173.1; FABMS: 359 $[M + H]^+$; HRFABMS: Calcd for $C_{16}H_{24}N_2O_3SCl$ $[M + H]^+$ 359.1198; found 359.1196.

*5.1.4. N-(5-Chlorothiophene-2-sulfonyl)-N-cyclopropylmethyl-(D)-leucinamide (**11a**)*

Compound **11a** was prepared from **8** (0.31 g, 1.0 mmol) and (chloromethyl)cyclopropane (**9a**, 0.28 mL, 3.0 mmol) following the procedure for the synthesis of **10a**. Purification by silica gel column chromatography gave **11a** (29 mg, 8%) as a yellow oil; tlc $R_f = 0.30$ (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 288 (1.622); $[\alpha]_D$ +15.1° (c 0.003, MeOH); *c*Log*P* = 3.49; ^{1}H NMR ($CDCl_3$) δ 0.30–0.34 (2H, m, cyclopropane-$\underline{H}_2$), 0.50–0.56 (2H, m, cyclopropane-$\underline{H}_2$), 0.79 (3H, d, *J* = 6.6 Hz, C$\underline{H}_3$), 0.81 (3H, d, *J* = 6.6 Hz, C$\underline{H}_3$), 0.98–1.08 (1H, m, cyclopropane-$\underline{H}$), 1.17–1.26 (1H, m, C$\underline{H}$H), 1.38–1.45 (1H, m, CH$\underline{H}$), 1.87–1.97 (1H, m, $CH_3C\underline{H}CH_3$), 3.15 (2H, dd, *J* = 6.9, 1.5 Hz, NC$\underline{H}_2$), 4.28 (1H, t, *J* = 7.2 Hz, COC$\underline{H}$), 5.91 (1H, br, N$\underline{H}$), 6.51 (1H, br, N$\underline{H}$), 6.91 (2H, d, *J* = 3.9 Hz, thiophene-$\underline{H}$), 7.39 (2H, d, *J* = 3.9 Hz, thiophene-$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ 4.6, 5.6, 11.2, 22.3, 22.4, 24.8, 29.6, 37.3, 49.9, 57.5, 126.5, 131.8, 137.3, 138.8, 172.9; FABMS: 365 $[M + H]^+$; HRFABMS: Calcd for $C_{14}H_{22}N_2O_3S_2Cl$ $[M + H]^+$ 365.0763; found 365.0764.

*5.1.5. N-(4-Chlorobenzenesulfonyl)-N-propargyl-(D)-leucinamide (**10b**)*

Compound **10b** was prepared from **7** (0.15 g, 0.5 mmol) and propargyl bromide (**9b**, 0.09 mL, 1.0 mmol) following the same protocol as for **10a**. Purification by silica gel column chromatography gave **10b** (99 mg, 58%) as a yellow oil; tlc $R_f = 0.46$ (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 285 (0.445); $[\alpha]_D$ +33.3° (c 0.002, MeOH); *c*Log*P* = 3.76; ^{1}H NMR ($CDCl_3$) δ 0.75 (3H, d, *J* = 6.3 Hz, C$\underline{H}_3$), 0.78 (3H, d, *J* = 6.6 Hz, C$\underline{H}_3$), 1.31–1.50 (2H, m, C$\underline{H}_2$), 1.77–1.86 (1H, m, $CH_3C\underline{H}CH_3$), 2.22 (1H, t, *J* = 2.4 Hz, CC$\underline{H}$), 4.04 (1H, dd, *J* = 18.6, 2.4 Hz, NC$\underline{H}$H), 4.30 (1H, t, *J* = 7.5 Hz, COC$\underline{H}$), 4.38 (1H, dd, *J* = 18.6, 2.4 Hz, NCH$\underline{H}$), 5.44 (1H, br, N$\underline{H}$), 6.33 (1H, br, N$\underline{H}$), 7.50 (2H, d, *J* = 8.7 Hz, Ar-$\underline{H}$), 7.88 (2H, d, *J* = 8.7 Hz, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ 21.9, 22.3, 24.5, 33.6, 37.7, 58.0, 73.1, 78.8, 129.0, 129.2, 138.0, 139.6, 172.6; FABMS: 343 $[M + H]^+$; HRFABMS: Calcd for $C_{15}H_{20}N_2O_3SCl$ $[M + H]^+$ 343.0885; found 343.0884.

*5.1.6. N-(5-Chlorothiophene-2-sulfonyl)-N-propargyl-(D)-leucinamide (**11b**)*

Compound **11b** was prepared form **8** (0.16 g, 0.5 mmol) and propargyl bromide (**9b**, 0.09 mL, 1.0 mmol) using the procedure for the synthesis of **10a**. Purification by silica gel column chromatography gave **11b** (26 mg, 15%) as a yellow oil; tlc $R_f = 0.36$ (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 288 (1.587); $[\alpha]_D$ +33.3° (c 0.002, MeOH); *c*Log*P* = 3.12; ^{1}H NMR ($CDCl_3$) δ 0.86 (6H, d, *J* = 6.3 Hz, C$\underline{H}_3$), 1.50–1.60 (2H, m, C$\underline{H}_2$), 1.80–1.89 (1H, m, $CH_3C\underline{H}CH_3$), 2.27 (1H, t, *J* = 2.4 Hz, CC$\underline{H}$), 4.11 (1H, dd, *J* = 18.9, 2.7 Hz, NC$\underline{H}$H), 4.21 (1H, dd, *J* = 18.9, 2.7 Hz, NCH$\underline{H}$), 4.35 (1H, t, *J* = 7.8 Hz, COC$\underline{H}$), 5.49 (1H, br, N$\underline{H}$), 6.20 (1H, br, N$\underline{H}$), 6.94 (1H, d, *J* = 3.9 Hz, thiophene-$\underline{H}$), 7.49 (1H, d, *J* = 4.2 Hz, thiophene-$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ 22.1, 22.3, 24.7, 33.9, 37.7, 48.1, 58.1, 73.3, 78.4, 126.7, 132.6, 137.9, 138.0, 172.2; FABMS: 349 $[M + H]^+$; HRFABMS: Calcd for $C_{13}H_{18}N_2O_3S_2Cl$ $[M + H]^+$ 349.0449; found 349.0448.

*5.1.7. N-(4-Chlorobenzenesulfonyl)-N-((R)-2,3-epoxypropyl)-(D)-leucinamide (**10c**)*

Compound **10c** was prepared from **7** (0.61 g, 2.0 mmol) and (*R*)-epichlorhydrin (**9c**, 0.47 mL, 6. 0 mmol) using the procedure for the synthesis of **10a**. Purification by silica gel column chromatography gave **10c** (216 mg, 30%) as a yellow oil; tlc R_f = 0.37 (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 284 (0.218); $[\alpha]_D$ −4.5° (c 0.002, MeOH); *c*Log*P* = 2.69; ^{1}H NMR ($CDCl_3$) δ 0.77 (3H, d, *J* = 6.6 Hz, C$\underline{H}_3$), 0.79 (3H, d, *J* = 6.6 Hz, C$\underline{H}_3$), 1.10–1.18 (1H, m, C$\underline{H}$H), 1.33–1.42 (1H, m, CH$\underline{H}$), 1.82–1.91 (1H, m, $CH_3C\underline{H}CH_3$), 2.60 (1H, dd, *J* = 4.5, 2.4 Hz, CHOC$\underline{H}$H), 2.81 (1H, t, *J* = 4.5 Hz, C$\underline{H}$OCHH), 3.10–3.16 (1H, m, CHOCH$\underline{H}$), 3.31 (1H, dd, *J* = 15.6, 6.3 Hz, NC$\underline{H}$H), 3.54 (1H, dd, *J* = 15.6, 4.2 Hz, NCH$\underline{H}$), 4.32 (1H, t, *J* = 7.5 Hz, COC$\underline{H}$), 5.64 (1H, br, N$\underline{H}$), 6.50 (1H, br, N$\underline{H}$), 7.50 (2H, d, *J* = 8.7 Hz, Ar-$\underline{H}$), 7.83 (2H, d, *J* = 8.7 Hz, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ 22.2, 22.3, 24.6, 37.7, 46.8, 47.3, 50.7, 57.8, 128.8, 129.3, 138.1, 139.7, 172.2; FABMS: 361 $[M + H]^+$; HRFABMS: Calcd for $C_{15}H_{22}N_2O_4SCl$ $[M + H]^+$ 361.0991; found 361.0990.

*5.1.8. N-(4-Chlorobenzenesulfonyl)-N-((S)-2,3-epoxypropyl)-(D)-leucinamide (**10d**)*

Compound **10d** was prepared from **7** (0.61 g, 2.0 mmol) and (*S*)-epichlorhydrin (**9d**, 0.47 mL, 6.0 mmol) following the same protocol as for **10a**. Purification by silica gel column chromatography gave **10d** (180 mg, 25%) as a yellow oil; tlc $R_f = 0.46$ (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 286 (0.328); $[\alpha]_D$ +4.2° (c 0.002, MeOH); *c*Log*P* = 2.69; ^{1}H NMR ($CDCl_3$) δ 0.80 (3H, d, J = 6.3 Hz, C$\underline{H}_3$), 0.81 (3H, d, J = 6.6 Hz, C$\underline{H}_3$), 1.27–1.36 (1H, m, C$\underline{H}$H), 1.43–1.49 (1H, m, CH$\underline{H}$), 1.70–1.79 (1H, m, $CH_3C\underline{H}CH_3$), 2.58 (1H, dd, J = 4.5, 2.4 Hz, CHOC$\underline{H}$H), 2.81 (1H, t, J = 4.5 Hz, C$\underline{H}$OCHH), 3.15–3.19 (1H, m, CHOCH$\underline{H}$), 3.30 (1H, dd, J = 15.9, 6.3 Hz, NC$\underline{H}$H), 3.41 (1H, dd, J = 15.9, 4.2 Hz, NCH$\underline{H}$), 4.30 (1H, t, J = 7.5 Hz, COC$\underline{H}$), 5.45 (1H, br, N$\underline{H}$), 6.31 (1H, br, N$\underline{H}$), 7.51 (2H, d, J = 8.7 Hz, Ar-$\underline{H}$), 7.80 (2H, d, J = 8.7 Hz, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ 22.1, 22.4, 24.6, 38.0, 46.9, 47.6, 50.5, 57.5, 128.8, 129.5, 137.9, 139.8, 172.3; FABMS: 361 $[M + H]^+$; HRFABMS: Calcd for $C_{15}H_{22}N_2O_4SCl$ $[M + H]^+$ 361.0991; found 361.0993.

*5.1.9. N-(5-Chlorothiophene-2-sulfonyl)-N-((R)-2,3-epoxypropyl)-(D)-leucinamide (**11c**)*

Compound **11c** was prepared from **8** (0.62 g, 2.0 mmol) and (*R*)-epichlorhydrin (**9c**, 0.47 mL, 6.00 mmol) following the same protocol as for **10a**. Purification by silica gel column chromatography gave **11c** (132 mg, 18%) as a pale yellow oil; tlc $R_f = 0.36$ (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 288 (1.305); $[\alpha]_D$ −6.7° (c 0.002, MeOH); *c*Log*P* = 2.43; ^{1}H NMR ($CDCl_3$) δ 0.85 (3H, d, J = 7.2 Hz, C$\underline{H}_3$), 0.89 (3H, d, J = 6.6 Hz, C$\underline{H}_3$), 1.18–1.30 (1H, m, C$\underline{H}$H), 1.44–1.56 (1H, m, CH$\underline{H}$), 1.86–1.95 (1H, m, $CH_3C\underline{H}CH_3$), 2.62 (1H, dd, J = 4.5, 2.4 Hz, CHOC$\underline{H}$H), 2.83 (1H, t, J = 4.5 Hz, C$\underline{H}$OCHH), 3.16–3.21 (1H, m, CHOCH$\underline{H}$), 3.32 (1H, dd, J = 15.9, 6.0 Hz, NC$\underline{H}$H), 3.53 (1H, d, J = 15.9, 4.8 Hz, NCH$\underline{H}$), 4.35 (1H, dd, J = 7.8, 6.6 Hz, COC$\underline{H}$), 5.48 (1H, br, N$\underline{H}$), 6.35 (1H, br, N$\underline{H}$), 6.95 (2H, d, J = 4.2 Hz, thiophene-$\underline{H}$), 7.46 (2H, d, J = 3.9 Hz, thiophene-$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ 22.3, 22.5, 24.8, 37.7, 46.9, 47.4, 50.6, 58.1, 126.8, 132.3, 137.7, 138.2, 171.7; FABMS: 367 $[M + H]^+$; HRFABMS: Calcd for $C_{13}H_{20}N_2O_4S_2Cl$ $[M + H]^+$ 367.0555; found 367.0556.

*5.1.10. N-(5-Chlorothiophene-2-sulfonyl)-N-((S)-2,3-epoxypropyl)-(D)-leucinamide (**11d**)*

Compound **11d** was prepared from **8** (0.62 g, 2.0 mmol) and (*S*)-epichlorhydrin (**9d**, 0.47 mL, 6.00 mmol) using the procedure for the synthesis of **10a**. Purification by silica gel column chromatography gave **11d** (125 mg, 17%) as a yellow oil; tlc $R_f = 0.42$ (EtOAc/*n*-hexane); UV λ_{max} (MeOH) nm (log ε): 288 (1.430); $[\alpha]_D$ +6.2° (c 0.002, MeOH); *c*Log*P* = 2.43; ^{1}H NMR ($CDCl_3$) δ 0.89 (6H, d, J = 6.3 Hz, C$\underline{H}_3$), 1.31–1.48 (1H, m, C$\underline{H}$H), 1.52–1.58 (1H, m, CH$\underline{H}$), 1.72–1.81 (1H, m, $CH_3C\underline{H}CH_3$), 2.60 (1H, dd, J = 4.5, 2.4 Hz, CHOC$\underline{H}$H), 2.83 (1H, t, J = 4.5 Hz, C$\underline{H}$OCHH), 3.17–3.22 (1H, m, CHOCH$\underline{H}$), 3.34 (1H, dd, J = 15.9, 6.0 Hz, NC$\underline{H}$H), 3.54 (1H, dd, J = 15.9, 4.2 Hz, NCH$\underline{H}$), 4.35 (1H, t, J = 7.5 Hz, COC$\underline{H}$), 5.39 (1H, br, N$\underline{H}$), 6.14 (1H, br, N$\underline{H}$), 6.94 (2H, d, J = 3.9 Hz, thiophene-$\underline{H}$), 7.43 (2H, d, J = 3.9 Hz, thiophene-$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ 22.3, 22.6, 24.9, 38.4, 47.0, 48.0, 50.7, 58.0, 126.9, 132.3, 137.9, 138.1, 172.1; FABMS: 367 $[M + H]^+$; HRFABMS: Calcd for $C_{13}H_{20}N_2O_4S_2Cl$ $[M + H]^+$ 367.0555; found 367.0557.

*5.1.11. N-(4-Chlorobenzenesulfonyl)-N-(2-fluorobenzyl)-(D)-leucinamide (**10e**)*

Compound **10e** was prepared from **7** (0.16 g, 0.53 mmol) and 2-fluorobenzyl bromide (**9e**, 0.13 mL, 1.06 mmol) using the procedure for the synthesis of **10a**. Purification by silica gel column chromatography gave **10e** (169 mg, 77%) as a white solid; tlc R_f = 0.58 (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 286 (0.238); mp 172–173 °C; $[\alpha]_D$ +25.0° (c 0.003, MeOH); *c*Log*P* = 4.63; ^{1}H NMR ($CDCl_3$) δ 0.71 (3H, d, J = 6.6 Hz, C$\underline{H}_3$), 0.79 (3H, d, J = 6.6 Hz, C$\underline{H}_3$), 1.17–1.26 (1H, m, C$\underline{H}$H), 1.28–1.37 (1H, m, CH$\underline{H}$), 1.80–1.89 (1H, m, $CH_3C\underline{H}CH_3$), 4.33 (1H, t, J = 7.2 Hz, COC$\underline{H}$), 4.47 (1H, d, J = 15.9 Hz, NCH$\underline{H}$), 4.63 (1H, d, J = 16.5 Hz, NC$\underline{H}$H),5.18 (1H, br, N$\underline{H}$), 6.28 (1H, br, N$\underline{H}$), 6.96 (1H, t, J = 9.6 Hz, Ar-$\underline{H}$), 7.09 (1H, t, J = 7.5 Hz, Ar-$\underline{H}$), 7.46 (2H, d, J = 8.4 Hz, Ar-$\underline{H}$), 7.49 (2H, t, J = 7.8 Hz, Ar-$\underline{H}$), 7.70 (2H, d, J = 8.7 Hz, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ 22.1, 22.3, 24.7, 37.4, 42.0, 58.0, 115.4 ($^2J_{C\text{-}F}$ = 22.4 Hz, C-3), 123.3 ($^2J_{C\text{-}F}$ = 13.1 Hz, C-1), 124.2 ($^3J_{C\text{-}F}$ = 3.5 Hz, C-4), 128.8, 129.4, 129.9 ($^4J_{C\text{-}F}$ = 8.0 Hz, C-5), 131.5 ($^3J_{C\text{-}F}$ = 3.5 Hz, C-6), 138.6, 139.7, 161.0 ($^1J_{C\text{-}F}$ = 246.1 Hz, C-2), 171.6; FABMS: 414 $[M + H]^+$; HRFABMS: Calcd for $C_{19}H_{24}FN_2O_3SCl$ $[M + H]^+$ 414.1182; found 414.1184.

*5.1.12. N-(5-Chlorothiophene-2-sulfonyl)-N-(2-fluorobenzyl)-(D)-leucinamide (**11e**)*

Compound **11e** was prepared from **8** (0.16 g, 0.52 mmol) and 2-fluorobenzyl bromide (**9e**, 0.13 mL, 1.06 mmol) following the same protocol as for **10a**. Purification by silica gel column chromatography gave **11e** (53 mg, 24%) as a white solid; tlc $R_f = 0.56$ (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 289 (1.333); mp 135–136 °C; $[\alpha]_D$ +8.7° (c 0.002, MeOH); *c*Log*P* = 4.37; ^{1}H NMR ($CDCl_3$) δ (ppm) 0.74 (3H, d, J = 6.6 Hz, C$\underline{H}_3$), 0.83 (3H, d, J = 6.6 Hz, C$\underline{H}_3$), 1.23–1.32 (1H, m, C$\underline{H}$H), 1.38–1.47 (1H, m, CH$\underline{H}$), 1.82–1.91 (1H, m, $CH_3C\underline{H}CH_3$), 4.35 (1H, t, J = 7.2 Hz, COC$\underline{H}$), 4.50 (1H, d, J = 15.9 Hz, NCH$\underline{H}$), 4.66 (1H, d, J = 15.9 Hz, NC$\underline{H}$H), 5.24 (1H, br, N$\underline{H}$), 6.14 (1H, br, N$\underline{H}$), 6.91 (1H, d, J = 3.9 Hz, thiophene-$\underline{H}$), 7.00 (1H, t, J = 9.3 Hz, Ar-$\underline{H}$), 7.12 (1H, t, J = 7.5 Hz, Ar-$\underline{H}$), 7.28 (1H, t, J = 6.6 Hz, Ar-$\underline{H}$), 7.15 (1H, d, J = 4.2 Hz, thiophene-$\underline{H}$) 7.53 (1H, t, J = 7.8 Hz, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ 22.2, 22.4, 24.9, 37.4, 41.9, 42.0, 58.2, 115.3 ($^2J_{C\text{-}F}$ = 16.2 Hz, C-3), 123.2 ($^2J_{C\text{-}F}$ = 9.9 Hz, C-1), 124.2 ($^3J_{C\text{-}F}$ = 2.6 Hz, C-4), 126.6, 129.8 ($^4J_{C\text{-}F}$ = 6.2 Hz, C-5), 131.2 ($^3J_{C\text{-}F}$ = 2.6 Hz, C-6), 132.1, 137.8, 138.1, 161.0 ($^1J_{C\text{-}F}$ = 184.6, C-2), 171.4; FABMS: 420 $[M + H]^+$; HRFABMS: Calcd for $C_{17}H_{22}FN_2O_3S_2Cl$ $[M + H]^+$ 420.0747; found 420.0745.

*5.1.13. N-(4-Chlorobenzenesulfonyl)-N-(4-fluorobenzyl)-(D)-leucinamide (**10f**)*

Compound **10f** was prepared form **7** (0.11 g, 0.37 mmol) and 4-fluorobenzylbromide (**9f**, 0.19 mL, 0.75 mmol) following the same protocol as for **10a**. Purification by silica gel column chromatography gave **10f** (75 mg, 49%) as a white solid; tlc $R_f = 0.61$ (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 284 (0.376); mp 146–147 °C; $[\alpha]_D$ +26.7° (c 0.003, MeOH); *c*Log*P* = 4.63; ^{1}H NMR ($CDCl_3$) δ 0.67 (3H, d, J = 6.6 Hz, C$\underline{H}_3$), 0.76 (3H, d, J = 6.6 Hz, C$\underline{H}_3$), 1.09–1.18 (1H, m, C$\underline{H}$H), 1.26–1.37 (1H, m, CH$\underline{H}$), 1.79–1.88 (1H, m, $CH_3C\underline{H}CH_3$), 4.29 (1H, t, J = 7.2 Hz, COC$\underline{H}$), 4.36 (1H, d, J = 15.9 Hz, NCH$\underline{H}$), 4.57 (1H, d, J = 15.6 Hz, NC$\underline{H}$H),5.18 (1H, br, N$\underline{H}$), 6.21 (1H, br, N$\underline{H}$), 6.98 (2H, t, J = 8.7 Hz, Ar-$\underline{H}$), 7.33 (2H, dd, J = 8.4, 5.4 Hz, Ar-$\underline{H}$), 7.46 (2H, d, J = 8.7 Hz, Ar-$\underline{H}$), 7.66 (2H, t, J = 8.4 Hz, Ar-$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ 22.0, 22.4, 24.7, 37.9, 47.9, 57.7, 115.2 ($^2J_{C\text{-}F}$ = 16.0 Hz, C-3 & C-5), 128.6, 129.4, 130.7 ($^3J_{C\text{-}F}$ = 6.1 Hz, C-2 & C-6), 132.0 ($^4J_{C\text{-}F}$ = 2.4 Hz, C-1), 138.5, 139.5, 162.4 ($^1J_{C\text{-}F}$ = 184.3 Hz, C-4), 171.6; FABMS: 414 $[M + H]^+$; HRFABMS: Calcd for $C_{19}H_{24}FN_2O_3SCl$ $[M + H]^+$ 414.1182; found 414.1183.

*5.1.14. N-(5-Chlorothiophene-2-sulfonyl)-N-(4-fluorobenzyl)-(D)-leucinamide (**11f**)*

Compound **11f** was prepared from **8** (0.11 g, 0.37 mmol) and 4-fluorobenzylbromide (**9f**, 0.19 mL, 0.75 mmol) following the same protocol as for **10a**. Purification by silica gel column chromatography gave **11f** (105 mg, 68%) as a white solid; tlc $R_f = 0.53$ (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 289 (2.192); mp 143–144 °C; $[\alpha]_D$ +13.5° (c 0.004, MeOH); *c*Log*P* = 4.37; ^{1}H NMR ($CDCl_3$) δ 0.72 (3H, d, J = 6.6 Hz, C$\underline{H}_3$), 0.83 (3H, d, J = 6.6 Hz, C$\underline{H}_3$), 1.21–1.27 (1H, m, C$\underline{H}$H), 1.36–1.43 (1H, m, CH$\underline{H}$), 1.80–1.97 (1H, m,

CH_3CHCH_3), 4.33 (1H, t, J = 7.5 Hz, COCH), 4.40 (1H, d, J = 15.6 Hz, NCHH), 4.56 (1H, d, J = 15.6 Hz, NCHH), 5.18 (1H, br, NH), 6.38 (1H, br, NH), 6.91 (1H, d, J = 4.2 Hz, thiophene-H), 7.00 (2H, t, J = 8.7 Hz, Ar-H), 7.29 (1H, d, J = 3.9 Hz, thiophene-H) 7.37 (2H, dd, J = 8.7, 5.7 Hz, Ar-H); ^{13}C NMR ($CDCl_3$) δ 22.1, 22.4, 24.7, 37.9, 48.1, 58.0, 115.4 ($^2J_{C\text{-}F}$ = 21.2 Hz, C-3 & C-5), 126.7, 130.8 ($^3J_{C\text{-}F}$ = 8.0 Hz, C-2 & C-6), 132.0 ($^4J_{C\text{-}F}$ = 2.0 Hz, C-1), 132.1, 137.9, 138.7, 162.6 ($^1J_{C\text{-}F}$ = 245.0 Hz, C-4), 171.5; FABMS: 420 $[M + H]^+$; HRFABMS: Calcd for $C_{17}H_{22}FN_2O_3S_2Cl$ $[M + H]^+$ 420.0747; found 420.0746.

5.1.15. N-(3,5-Difluorobenzyl)-N-(4-chlorobenzenesulfonyl)-(D)-leucinamide (**10g**)

Compound **10g** was prepared from **7** (0.30 g, 1 mmol) and 3,5-difluorobenzyl bromide (**9g**, 0.26 mL, 2.0 mmol) using the procedure for the synthesis of **10a**. Purification by silica gel column chromatography gave **10g** (207 mg, 48%) as a white solid; tlc R_f = 0.63 (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 286 (0.183); mp 176–177 °C; $[\alpha]_D$ +29.0° (c 0.003, MeOH); *c*Log*P* = 4.77; ^{1}H NMR ($CDCl_3$) δ 0.70 (3H, d, J = 6.6 Hz, CH$_3$), 0.79 (3H, d, J = 6.6 Hz, CH$_3$), 1.04–1.13 (1H, m, CHH), 1.31–1.40 (1H, m, CHH), 1.74–1.84 (1H, m, CH_3CHCH_3), 4.33 (1H, dd, J = 8.1, 6.9 Hz, COCH), 4.34 (1H, d, J = 15.9 Hz, NCHH), 4.57 (1H, d, J = 16.5 Hz, NCHH), 5.27 (1H, br, NH), 6.18 (1H, br, NH), 6.71 (1H, tt, J = 8.7, 2.4 Hz, Ar-H), 6.88 (2H, d, J = 6.0 Hz, Ar-H), 7.49 (2H, d, J = 8.4 Hz, Ar-H), 7.70 (2H, d, J = 8.7 Hz, Ar-H); ^{13}C NMR ($CDCl_3$) δ 22.0, 22.4, 24.7, 37.9, 47.9, 57.7, 103.2 ($^2J_{C\text{-}F}$ = 18.8 Hz, C-4), 111.3 ($^{2,4}J_{C\text{-}F}$ = 14.0, 5.3 Hz, C-2 & C-6), 128.6, 129.5, 138.1, 139.8, 140.7 ($^3J_{C\text{-}F}$ = 6.6 Hz, C-1), 162.8 ($^{1,3}J_{C\text{-}F}$ = 186, 9.5 Hz, C-3 & C-5), 171.2; FABMS: 431 $[M + H]^+$; HRFABMS: Calcd for $C_{19}H_{22}F_2N_2O_3SCl$ $[M + H]^+$ 431.1010; found 431.1012.

5.1.16. N-(3,5-Difluorobenzyl)-N-(5-chlorothiophene-2-sulfonyl)-(D)-leucinamide (**11g**)

Compound **11g** was prepared from **8** (0.31 g, 1 mmol) and 3,5-difluorobenzyl bromide (**9g**, 0.26 mL, 2.0 mmol) using the procedure for the synthesis of **10a**. Purification by silica gel column chromatography gave **11g** (209 mg, 48%) as a white solid; tlc R_f = 0.58 (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 289 (1.347); mp 162–163 °C; $[\alpha]_D$ +13.0° (c 0.002, MeOH); *c*Log*P* = 4.51; ^{1}H NMR ($CDCl_3$) δ 0.74 (3H, d, J = 6.6 Hz, CH$_3$), 0.85 (3H, d, J = 6.6 Hz, CH$_3$), 1.14–1.23 (1H, m, CHH), 1.38–1.47 (1H, m, CHH), 1.75–1.84 (1H, m, CH_3CHCH_3), 4.36 (1H, t, J = 8.1 Hz, COCH), 4.43 (1H, d, J = 16.5 Hz, NCHH), 4.58 (1H, d, J = 16.5 Hz, NCHH), 5.33 (1H, br, NH), 6.06 (1H, br, NH), 6.72 (1H, tt, J = 8.7, 2.4 Hz, Ar-H), 6.93 (2H, d, J = 7.5 Hz, Ar-H), 6.93 (2H, d, J = 4.2 Hz, thiophene-H), 7.35 (2H, d, J = 4.2 Hz, thiophene-H); ^{13}C NMR ($CDCl_3$) δ 22.1, 22.4, 24.8, 38.1, 48.0, 58.0, 103.2 ($^2J_{C\text{-}F}$ = 19.0 Hz, C-4), 111.2 ($^{2,4}J_{C\text{-}F}$ = 14.0, 5.3 Hz, C-2 & C-6), 126.7, 132.2, 137.8, 138.1, 140.8 ($^3J_{C\text{-}F}$ = 6.5 Hz, C-1), 162.9 ($^{1,3}J_{C\text{-}F}$ = 186, 9.5 Hz, C-3 & C-5), 171.2; FABMS: 437 $[M + H]^+$; HRFABMS: Calcd for $C_{17}H_{20}F_2N_2O_3S_2Cl$ $[M + H]^+$ 437.0574; found 437.0576.

5.1.17. 5-Chloromethyl-2-methyl-8-quinolinol (**9h**)

To a mixture of 2-methyl-8-quinolinol (1.46 g, 10 mmol), 1.6 mL of 36% HCl, and 1.6 mL of 37% formaldehyde was treated with hydrogen chloride gas at an ice bath for 6 h. The solution was then allowed to stand at room temperature for 2 h without stirring. The yellow solid formed was collected on a filter, washed with 90% alcohol, dried over $MgSO_4$, and concentrated to give **9h** (0.94 g, 48%) as a yellow solid; tlc Rf = 0.25 (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 323 (0.783); mp 241–242 °C; $[\alpha]_D$ −10.0° (c 0.002, MeOH); *c*Log*P* = 3.14; ^{1}H NMR (DMSO-d_6) δ 2.97 (3H, s, CH$_3$), 5.29 (2H, s, CH$_2$),7.53 (1H, d, J = 7.8 Hz, Ar-H), 7.80 (1H, d, J = 8.4 Hz, Ar-H), 8.01 (1H, d, J = 9.0 Hz, Ar-H), 9.10 (1H, d, J = 9.0 Hz, Ar-H); ^{13}C NMR (DMSO-d_6) δ 20.8, 60.5, 115.9, 124.4, 126.5, 128.8, 129.3, 129.8, 143.1, 147.6, 157.5, 228.5; FABMS: 208 $[M + H]^+$.

5.1.18. N-(4-Chlorobenzenesulfonyl)-N-(8-hydroxy-2-methyl-quinolin-5-yl)methyl-(D)-leucinamide (**10h**)

To a mixture of **7** (0.12 g, 0.38 mmol) in $CHCl_3$ (6 mL) was added DIPEA (0.13 mL, 0.75 mmol), potassium iodide (0.01 g), and **9h** (0.16 mg, 0.75 mmol) at room temperature. The mixture was stirred overnight, poured into brine, then extracted with $CHCl_3$. The organic layer was separated, washed with 5% $NaHCO_3$, brine, and then dried over $MgSO_4$, and concentrated. The resulting residue was purified by silica gel column chromatography to give **10h** (62 mg, 34%) as a yellow solid; tlc R_f = 0.65 (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 313 (0.489); mp 166–167 °C; $[\alpha]_D$ +9.7° (c 0.003, MeOH); *c*Log*P* = 4.92; ^{1}H NMR ($CDCl_3$) δ 0.58 (3H, d, J = 6.6 Hz, CH$_3$), 0.76 (3H, d, J = 6.6 Hz, CH$_3$), 0.93–1.01 (1H, m, CHH), 1.33–1.44 (1H, m, CHH), 1.79–1.88 (1H, m, CH_3CHCH_3), 2.80 (3H, s, Ar-CH$_3$), 4.31 (1H, t, J = 8.7 Hz, COCH), 4.69 (1H, d, J = 14.7 Hz, NCHH), 5.03 (1H, d, J = 14.7 Hz, NCHH), 5.29 (1H, br, NH), 5.97 (1H, br, NH), 7.03 (1H, d, J = 8.1 Hz, Ar-H), 7.33 (2H, d, J = 7.8 Hz, Ar-H), 7.41 (1H, d, J = 8.4 Hz, Ar-H), 7.41 (2H, d, J = 8.7 Hz, Ar-H), 7.69 (2H, d, J = 8.7 Hz, Ar-H), 8.69 (1H, d, J = 8.7 Hz, Ar-H); ^{13}C NMR ($CDCl_3$) δ 21.1, 21.9, 22.9, 25.0, 29.8, 37.3, 46.8, 57.3, 109.5, 120.6, 123.2, 125.6, 128.8, 129.2, 130.1, 137.8, 139.5, 151.9, 156.9, 171.7; FABMS: 476 $[M + H]^+$; HRFABMS: Calcd for $C_{23}H_{27}N_3O_4SCl$ $[M + H]^+$ 476.1413; found 476.1412.

5.1.19. N-(5-Chlorothiophene-2-sulfonyl)-N-(8-hydroxy-2-methyl-quinolin-5-yl)methyl-(D)-leucinamide (**11h**)

Compound **11h** was prepared from **8** (0.12 g, 0.38 mmol) and **9h** (0.16 mg, 0.75 mmol) using the procedure for the synthesis of **10h**. Purification by silica gel column chromatography gave **11h** (33 mg, 18%) as a yellow solid; tlc R_f = 0.61 (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 361 (1.712); mp 164–165 °C; $[\alpha]_D$ +8.6° (c 0.004, MeOH); *c*Log*P* = 4.66; ^{1}H NMR ($CDCl_3$) δ 0.74 (3H, d, J = 6.6 Hz, CH$_3$), 0.83 (3H, d, J = 6.6 Hz, CH$_3$), 1.23–1.32 (1H, m, CHH), 1.38–1.47 (1H, m, CHH), 1.82–1.91 (1H, m, CH_3CHCH_3), 4.35 (1H, t, J = 7.2 Hz, COCH), 4.50 (1H, d, J = 15.9 Hz, NCHH), 4.66 (1H, d, J = 15.9 Hz, NCHH), 5.24 (1H, br, NH), 6.14 (1H, br, NH), 6.91 (1H, d, J = 3.9 Hz, thiophene-H), 7.00 (1H, t, J = 9.3 Hz, Ar-H), 7.12 (1H, t, J = 7.5 Hz, Ar-H), 7.28 (1H, t, J = 6.6 Hz, Ar-H), 7.15 (1H, d, J = 4.2 Hz, thiophene-H) 7.53 (1H, t, J = 7.8 Hz, Ar-H); ^{13}C NMR ($CDCl_3$) δ 22.0, 22.5, 24.8, 25.0, 37.7, 46.9, 57.8, 108.9, 120.6, 122.5, 123.1, 125.4, 126.6, 129.6, 132.3, 132.7, 137.8, 139.1, 152.3, 158.9, 171.4; FABMS: 482 $[M + H]^+$; HRFABMS: Calcd for $C_{21}H_{25}N_3O_4S_2Cl$ $[M + H]^+$ 482.0978; found 482.0977.

5.1.20. N-(4-Chlorobenzenesulfonyl)-N-piperonyl-(D)-leucinamide (**3**)

Compound **3** was prepared from **7** (0.12 g, 0.38 mmol) and piperonyl chloride (**9i**, 0.13 mg, 0.76 mmol) using the procedure for the synthesis of **10a**. Purification by silica gel column chromatography gave **3** (114 mg, 69%) as a white solid; tlc R_f = 0.40 (EtOAc/*n*-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 298 (2.010); mp 126–127 °C; $[\alpha]_D$ +20.0° (c 0.003, MeOH); *c*Log*P* = 4.45; ^{1}H NMR ($CDCl_3$) δ 0.71 (3H, d, J = 6.6 Hz, CH$_3$), 0.78 (3H, d, J = 6.6 Hz, CH$_3$), 1.17–1.24 (1H, m, CHH), 1.31–1.37 (1H, m, CHH), 1.80–1.90 (1H, m, CH_3CHCH_3), 4.29 (1H, d, J = 15.3 Hz, NCHH), 4.31 (1H, t, J = 7.2 Hz, COCH), 4.48 (1H, d, J = 15.6 Hz, NCHH), 5.26 (1H, br, NH), 5.94 (2H, s, CH$_2$), 6.25 (1H, br, NH), 6.70 (1H, t, J = 7.8 Hz, Ar-H), 7.33 (2H, dd, J = 8.4, 5.4 Hz, Ar-H), 7.46 (2H, d, J = 8.7 Hz, Ar-H), 7.66 (2H, t, J = 8.4 Hz, Ar-H); ^{13}C NMR ($CDCl_3$) δ 22.2, 22.3, 24.6, 37.9, 48.6, 57.7, 101.1, 107.9, 109.3, 122.6, 128.7, 129.2, 129.8, 138.6, 139.3, 147.3, 147.7, 172.2; FABMS: 439 $[M + H]^+$; HRFABMS: Calcd for $C_{20}H_{24}N_2O_5SCl$ $[M + H]^+$ 439.1096; found 439.1098.

*5.1.21. N-(5-Chlorothiophene-2-sulfonyl)-N-piperonyl-(D)-leucinamide (**11i**)*

Compound **11i** was prepared from **8** (0.16 g, 0.37 mmol) and piperonyl chloride (**9i**, 0.13 mg, 0.76 mmol) following the same protocol as for **10a**. Purification by silica gel column chromatography gave **11i** (115 mg, 70%) as a white solid; tlc $R_f = 0.30$ (EtOAc/n-hexane = 1 : 1); UV λ_{max} (MeOH) nm (log ε): 289 (2.241); mp 111–112 °C; $[\alpha]_D$ +11.8° (c 0.002, MeOH); cLogP = 4.19; ^{1}H NMR ($CDCl_3$) δ 0.73 (3H, d, J = 6.6 Hz, C$\underline{H}_3$), 0.82 (3H, d, J = 6.6 Hz, C$\underline{H}_3$), 1.24–1.33 (1H, m, C$\underline{H}$H), 1.37–1.45 (1H, m, CH$\underline{H}$), 1.77–1.86 (1H, m, $CH_3C\underline{H}CH_3$), 4.32 (1H, t, J = 7.2 Hz, COC$\underline{H}$), 4.47 (1H, d, J = 15.3 Hz, NCH$\underline{H}$), 4.34 (1H, d, J = 15.3 Hz, NC$\underline{H}$H), 5.64 (1H, br, N$\underline{H}$), 5.94 (2H, s, C$\underline{H}_2$), 6.15 (1H, br, N$\underline{H}$), 6.71 (1H, d, J = 8.1 Hz, Ar-$\underline{H}$), 6.81 (1H, d, J = 8.1 Hz, Ar-$\underline{H}$), 6.88 (1H, s, Ar-$\underline{H}$), 6.90 (1H, d, J = 3.9 Hz, thiophene-$\underline{H}$), 7.30 (1H, d, J = 3.9 Hz, thiophene-$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ 22.2, 22.4, 24.8, 37.8, 48.8, 59.0, 101.1, 108.2, 109.2, 122.6, 126.5, 129.7, 132.0, 137.7, 138.5, 147.4, 147.8, 171.6; FABMS: 445 $[M + H]^+$; HRFABMS: Calcd for $C_{18}H_{22}N_2O_5S_2Cl$ $[M + H]^+$ 445.0661; found 445.0663.

5.2. Pharmacological evaluation

5.2.1. Reagents

Anti-MEK antibody and anti-ERK1/2 MAP kinase antibody were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Horseradish peroxidase-conjugated anti-rabbit IgG was obtained from Santa Cruz Biotechnology (Dallas, TX, USA). Horseradish peroxidase-conjugated anti-mouse IgG and ECL Western Blotting detection reagents were obtained from Amersham Biosciences (Piscataway, NJ, USA). 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 reagent grade and obtained from standard suppliers abroad.

5.2.2. Cell-based γ-secretase assays

The production of T20 cells has been reported previously [20]. T20 cells were 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. Tested compounds diluted in DMEMHZB 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, Madison, WI, USA), 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 signals from the stable line without tetracycline induction and tested 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 panel.

To examine compound inhibition of γ-secretase-dependent S3 cleavage of Notch, we generated a HEK293-derived stable line (N7) that was constitutively expressing NΔE as previously described [20]. N7 cells were plated onto12-well microplates in 1 mL/well DMEM supplemented with 10% FBS at 5×10^5 cells/well. After incubation at 37 °C overnight, cells were treated with compounds at 10 μM as described above and incubated at 37 °C for 24 h. Treated cells were harvested using PBS containing 20 mM EDTA and dissolved in 50 μL of 1× PLB, followed by centrifugation at 13,200*g* for 5 min to remove cell debris. The protein concentrations of clarified supernatants were determined using a BCA protein assay reagent kit, and cell extracts containing equivalent amounts of proteins were resolved by SDS-PAGE and analyzed by Western blotting using an anti-Notch(Val1744) polyclonal antibody.

5.2.3. 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, Madison, WI, USA) 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, Winooski, VT, USA). 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 devoid of cells was subtracted from these data.

5.2.4. Cell culture and cell lines

Human embryonic kidney cells (HEK293) were incubated 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 [21]. Cells were incubated in a humidified incubator at 37 °C in 5% CO_2.

5.2.5. Assay for extracellular signal-regulated kinase (ERK) activation

The stimulation of the ERKs was measured by using anti-phospho-ERK1/2 and anti-ERK1/2 antibodies (Cell Signaling Technology, Danvers, MA, USA) as previously described [16]. Briefly, γ-30 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, Rockford, IL, USA). 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.

5.2.6. Data analysis

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

Author contributions

Y.-C.T. carried out the synthesis of the designed compounds. M.-Y.C. and B.-J.W. performed the pharmacological experiments. Y.-F.L. conceived and designed the pharmacological experiments. M.-K.H. conceived and designed the targeted compounds. Y.-F.L. and M.-K.H. analyzed the data.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.006.

References

[1] P. Tiraboschi, L.A. Hansen, L.J. Thal, J. Corey-Bloom, The importance of neurotic plaques and tangles to the development and evolution of AD, Neurology 62 (2004) 1984–1989.
[2] M. Goedert, M.G. Spillantini, A century of Alzheimer's disease, Science 314 (2006) 777–781.
[3] B.A. Bergmans, B. De Strooper, γ-Secretase: from cell biology to therapeutic strategies, Lancet Neurology 9 (2010) 215–226.
[4] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (2002) 353–356.
[5] B.P. Imbimbo, G.A. Giardina, γ-Secretase inhibitors and modulators for the treatment of Alzheimer's disease: disappointments and hopes, Current Topics in Medicinal Chemistry 11 (2011) 1555–1557.
[6] C.R. Hopkins, ACS chemical neuroscience molecule spotlight on begacestat (GSI-953), ACS Chemical Neuroscience 3 (2012) 3–4.
[7] K.W. Gillman, J.E. Starrett Jr., M.F. Parker, K. Xie, J.J. Bronson, L.R. Marcin, K.E. McElhone, C.P. Bergstrom, R.A. Mate, R. Williams, J.E. Meredith Jr., C.R. Burton, D.M. Barten, J.H. Toyn, S.B. Roberts, K.A. Lentz, J.G. Houston, R. Zaczek, C.F. Albright, C.P. Decicco, J.E. Macor, R.E. Olson, Discovery and evaluation of BMS-708163, a potent, selective and orally bioavailable γ-secretase inhibitor, Medicinal Chemistry Letters 1 (2010) 120–124.
[8] Bristol-Myers Squibb Company. Available online: http://www.bms.com/news/features/2012/Pages/AvagacestatDevelopmentStatus.aspx.
[9] E.A. Kitas, G. Galley, R. Jakob-Roetne, A. Flohr, W. Wostl, H. Mauser, A.M. Alker, C. Czech, L. Ozmen, P. David-Pierson, D. Reinhardtc, H. Jacobsen, Substituted 2-oxo-azepane derivatives are potent, orally active γ-secretase inhibitors, Bioorganic & Medicinal Chemistry Letters 18 (2008) 304–308.
[10] H. Meizane, J.C. Dodart, C. Mathis, S. Little, J. Clemens, S.M. Paul, A. Ungerer, Memory-enhancing effects of secreted forms of the β-amyloid precursor protein in normal and amnestic mice, Proceedings of the National Academy of Sciences of the United States of America 95 (1998) 12683–12688.
[11] M. Yogev-Falach, T. Amit, O. Bar-Am, Involvement of MAP kinase in the regulation of amyloid precursor protein processing by novel cholinesterase inhibitors derived from rasagiline, FASEB Journal 16 (2002) 1674–1676.
[12] O. Bar-Am, M. Yogev-Falach, T. Amit, Regulation of protein kinase C by the anti-Parkinson drug, MAO-B inhibitor, rasagiline and its derivatives, Journal of Neurochemistry 89 (2004) 1119–1125.
[13] T.A. Comery, R.L. Martone, S. Aschmies, K.P. Atchison, G. Diamantidis, X. Gong, H. Zhou, A.F. Kreft, M.N. Pangalos, J. Sonnenberg-Reines, J.S. Jacobsen, K.L. Marquis, Acute γ-secretase inhibition improves contextual fear conditioning in the Tg2576 mouse model of Alzheimer's disease, Journal of Neuroscience 25 (2005) 8898–8902.
[14] A.Y. Hung, C. Haass, R.M. Nitsch, W.Q. Qiu, M. Citron, R.J. Wurtman, J.H. Growdon, D.J. Selkoe, Activation of protein kinase C inhibits cellular production of the amyloid β-protein, Journal of Biological Chemistry 268 (1993) 22959–22962.
[15] J. Desdouits-Magnen, F. Desdouits, S. Takeda, Regulation of secretion of Alzheimer amyloid precursor protein by the mitogen-activated protein kinase cascade, Journal of Neurochemistry 70 (1998) 524–530.
[16] M.K. Hu, Y.F. Liao, J.F. Chen, B.J. Wang, Y.T. Tung, H.C. Lin, K.P. Lee, New 1,2,3,4-tetrahydroisoquinoline derivatives as modulators of proteolytic cleavage of amyloid precursor proteins, Bioorganic & Medicinal Chemistry 16 (2008) 1957–1965.
[17] Y.F. Liao, B.J. Wang, W.M. Hsu, H. Lee, C.Y. Liao, S.Y. Wu, H.T. Cheng, M.K. Hu, Unnatural amino acid-substituted (hydroxyethyl)urea peptidomimetics inhibit γ-secretase and promote the neuronal differentiation of neuroblastoma Cells, Molecular Pharmacology 71 (2007) 588–601.
[18] A. Czerwinski, F. Valenzuela, P. Afonine, M. Dauter, Z. Dauter, *N*-{*N*-[2-(3,5-Difluoro-phenyl)acetyl]-(*S*)-alanyl}-(*S*)-phenylglycine *tert*-butyl ester (DAPT): an inhibitor of γ-secretase, revealing fine electronic and hydrogen-bonding features, Acta Crystallographica Section C 66 (2010) 585–588.
[19] M. Yogev-Falach, T. Amit, O. Bar-Am, M.B.H. Youdim, The importance of propargylamine moiety in the anti-Parkinson drug rasagiline and its derivatives in MAPK-dependent amyloid precursor protein processing, FASEB Journal 17 (2003) 2325–2327.
[20] Y.F. Liao, B.J. Wang, H.T. Cheng, L.H. Kuo, M.S. Wolfe, Tumor necrosis factor-alpha, interleukin-1beta, and interferon-gamma stimulate gamma-secretase-mediated cleavage of amyloid precursor protein through a JNK-dependent MAPK pathway, Journal of Biological Chemistry 279 (2004) 49523–49532.
[21] P. Bakshi, Y.F. Liao, J. Gao, J. Ni, R. Stein, L.N. Yeh, M.S. Wolfe, A high-throughput screen to identify inhibitors of amyloid β-protein precursor processing, Journal of Biomolecular Screening 10 (2005) 1–12.