Full Paper

Difluorophenylglycinols as New Modulators of Proteolytic Processing of Amyloid Precursor Proteins

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Synthesis and evaluation of difluorophenylglycinols as new modulators of proteolytic processing of the amyloid-β precursor proteins for Alzheimer's therapies were described. A range of *N*-substituted (*R*)- and (*S*)-difluorophenylglycinols, structured on the amino alcohol framework, were explored by incorporating the arylsulfonyl moieties and various *N*-substituents. Evans' chiral auxiliary strategy was employed for the asymmetric synthesis of these enantiomeric difluorophenylglycinols. Compounds with effects on the γ-secretase inhibition and ERK-mediated signaling pathways were evaluated on cell-based assays. Among them, *N*-cyclopropylmethyl derivatives *R*-**12c** and *R*-**13c** showed modest γ-secretase inhibition as well as ERK-dependent activation.

Keywords: Alzheimer's disease / Asymmetric synthesis / ERK-mediated signaling / γ-Secretase

Received: July 28, 2013; Revised: September 7, 2013; Accepted: September 17, 2013

DOI 10.1002/ardp.201300283

Introduction

The amyloid-β (Aβ) peptide and its aggregated plaques in the brain are considered the major hallmarks in the pathogenesis of Alzheimer's disease (AD). The Aβ peptides (mainly $A\beta_{40}$ and $A\beta_{42}$) originate from amyloid-β precursor protein (APP) by the proteolytic cleavage steps involving enzymes β-secretase and γ-secretase [1, 2], whereas γ-secretase drives the final step to generate the more fibrillogenic $A\beta_{42}$, and it has thus been regarded as a prime therapeutic target for AD therapy [3]. In addition to the known substrate APP, γ-secretase also processes Notch and the resulting Notch intracellular domain (NICD) plays a vital role in the developmental signaling that controls a cell's fate during embryonic stage. Therefore, the identity of γ-secretase inhibitors with selectivity for APP over Notch to minimize the impact on Notch signaling was thus demanded in the drug discovery strategy. Contrary to the sequential cleavage of APP by β- and γ-secretases in the generation of the fibrillogenic Aβ, α-secretase is thought to mediate a beneficial processing of APP and produces a neuroprotective, soluble amyloid precursor protein α (sAPPα), which precludes the Aβ production and contributes to the neuronal protection [4]. One of the testimonies for the beneficial mechanism was proven by certain *N*-propargyl-containing compounds such as rasagiline and selegiline, which improved the α-secretase-mediated cleavage of APP through activating mitogen-activated protein kinases (MAPKs)-dependent pathways under *in vivo* conditions [5, 6]. This process alternatively not only attenuates the β- and γ-secretase-mediated cleavage but also produces neuroprotective sAPPα. As far as how much Aβ lowering was required to restore cognitive deficits was still in debate, a recent study in the Tg2576 mouse model disclosed that partial inhibition of γ-secretase activity would lower Aβ adequately to reverse cognitive impairment while avoiding Notch-related side effects by allowing certain proteolytic processing of Notch [7]. This partial inhibition would be accessed by the γ-secretase inhibitors with Notch-sparing selectivity as well as decreasing the β-/γ-secretase-mediated proteolytic processing of APP through augmenting ERK-mediated α-secretase activity [8, 9]. From the mechanism-of-action point of view, we planned to develop new dual-acting modulators of proteolytic processing of APP as potential agents for Alzheimer's therapy.

Aiming at developing dual-acting small molecules toward AD therapies, we previously reported a type of *N*-propargyl tetrahydroisoqunoline analogs that exhibited both γ-secretase

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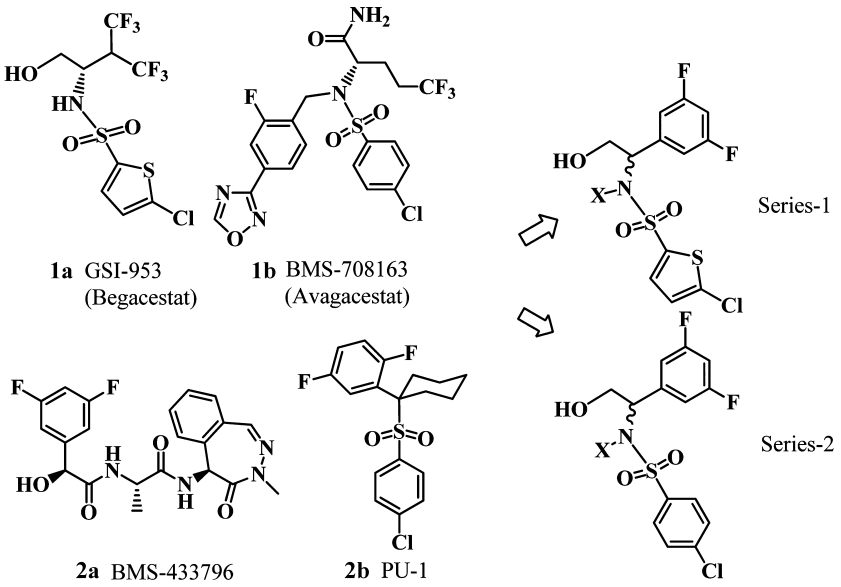


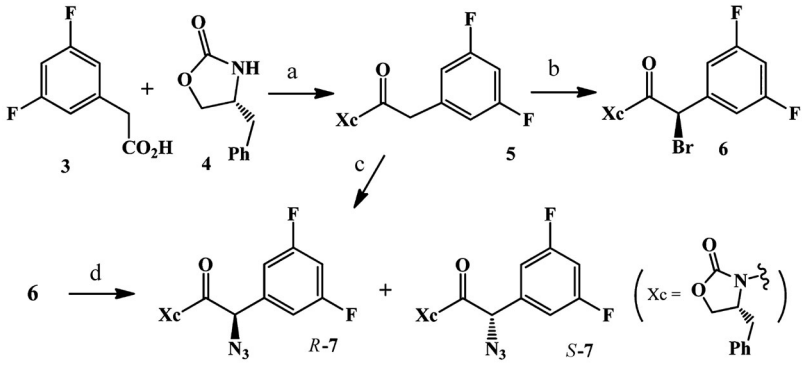
Figure 1. Potent γ-secretase inhibitors and the designed structures.

inhibition and ERK-dependent sAPPα release [10]. These molecules would act synergistically as they provide both decreased Aβ generation and neuronal protection. Mount of investigations demonstrated that certain arylsulfonamide-based small molecules are potent and selective γ-secretase inhibitors, such as GSI-953 (**1a**, begacestat, Fig. 1) and BMS-708163 (**1b**, avagacestat) [11, 12]. Structurally, begacestat is a simple amino alcohol and avagacestat is an amide derivative. They have been undergoing clinical investigations in AD patients, while avagacestat was recently terminated after phase II trials due to shortage of supported profiles for advanced development [13]. However, they are valuable to be models for further development of novel Notch-sparing γ-secretase inhibitors with chemical modifications, based upon their structural features and pharmaceutical properties. Other small molecules such as BMS-433796 (**2a**), an alaninamide derivative, and a cyclohexyl-based derivative, 1-(2,5-difluorobenzyl),1-(*p*-chlorobenzenesulfonyl)-cyclohexane (**2b**, named PU-1 here) are also potent γ-secretase inhibitors ($A\beta_{40}$ $IC_{50} = 0.3$ and 3 nM, respectively) [14, 15]. Taken together, we discovered that the difluorophenyl and chloro-substituted arylsulfonyl groups appear to be important as the core structures to exhibit γ-secretase inhibition. In fact, the 3,5-difluorophenyl moiety of BMS-433796 is also shared with another well-known inhibitor DAPT [16]. Therefore, the 3,5-difluorophenyl moiety can be a good starting point to explore new modulators of APP processing. As a beginning, we choose the difluorophenyl moiety of BMS-433796 to replace the side chain hexafluoro-isopropyl group of the amino alcohol GSI-953 followed by elaborating small *N*-propargyl-relative substituents to provide a type of *N*-substituted amino alcohols as compounds series-1, and secondly, the *p*-chlorobenzenesulfonyl group of avagacestat or **2b** is chosen to be an alternative of the 5-chlorothiophene-sulfonyl moiety of the synthesized amino alcohols to provide the analogous compounds series-2. The synthesized difluoro-phenylglycinols would directly exhibit γ-secretase inhibition as well as activation of ERK-signaling pathways, by which they may decrease the generation of amyloidogenic Aβ and simultaneously increase the neuroprotective features.

Results and discussion

Chemistry

The synthesis of these difluorophenylglycinols was accomplished by using modified methods of Evans' chiral auxiliary strategy for the asymmetric synthesis of unusual amino acids [17, 18]. As described in Scheme 1, chiral oxazolidinone **4** was condensed with difluorophenylacetic acid **3** to give amide **5** via mixed anhydride intermediate. The next diastereoselective bromination of amide **5** was achieved by enolating with dibutylboryl triflate *in situ* followed by treating the established metal enolate with *N*-bromosuccinimide (NBS), to give a highly diastereoselective β-bromocarboxamide **6**, which was readily separated by column chromatography from minor α-isomer in 90% yield. By employing sodium azide in the following azide substitution reaction, intriguingly equal amounts of diastereomeric azidocarboxamides *R*-**7** and *S*-**7** were obtained in 60% total yields after silica gel chromatography. The assignment of the absolute stereochemistry for both azidocarboxamides was confirmed by the spectroscopic characterization for one of the diastereomers identical to the exclusive product α-azidocarboxamide *R*-**7**, which was prepared from the potassium enolate of amide **5** and the hindered 2,4,6-triisopropylbenzenesulfonyl azide at



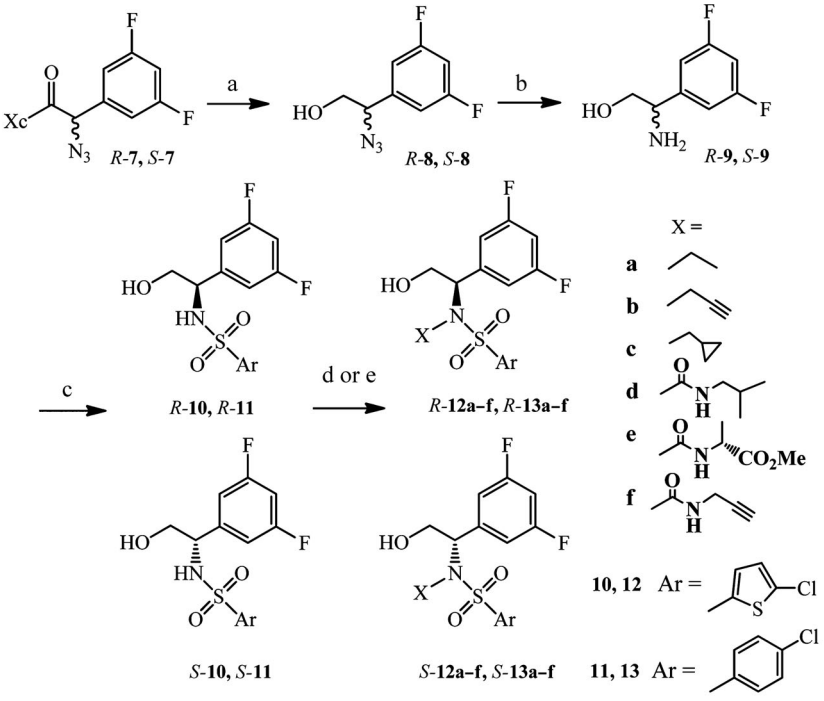
Reagents and conditions: a) pivaloyl-Cl, TEA, *n*-BuLi, −78°C; b) *n*-Bu_2BOTf, NBS; c) 2,4,6-triisopropylbenzenesulfonyl azide, KHMDS, −78°C; d) NaN_3, acetonitrile, r.t.

Scheme 1. Synthesis of the diastereomeric α-azidocarboxamides *R*-**7** and *S*-**7**.

the kinetic control conditions (step c, Scheme 1) according the method reported by Evans et al. [18].

Reductive aminolysis of each azidocarboxamide with lithium borohydride followed by heterogeneous hydrogenation (10% Pd-C, $H_{2(g)}$) of the resulting azido alcohols *R*-**8** and *S*-**8** gave the key difluorophenylglycinols *R*-**9** and *S*-**9** (Scheme 2). The enantiomers *R*-**9** and *S*-**9** were each treated with arylsulfonyl chlorides to afford the corresponding arylsulfonamides *R*-**10**, *R*-**11**, and *S*-**10**, *S*-**11** in satisfactory yields.

In order to gain an understanding of the requirement of the simple, *N*-propargyl-relative substituents for the ERK-mediated activation for these difluorophenylglycinols, various *N*-substituted derivatives *R*-**12a–f** and *S*-**12a–f** were synthesized by either alkylation or carbonylation *in situ* followed by



Reagents and conditions: a) $LiBH_4$, THF; b) 10% Pd/C, $H_{2(g)}$, MeOH; c) arylsulfonyl chloride, TEA; d) K_2CO_3, alkyl halide; e) triphosgene, alkyl amine, DIEA, r.t.

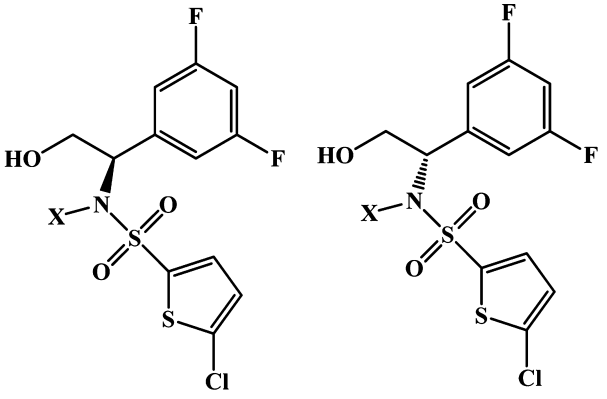
Scheme 2. Synthesis of enantiomeric *N*-substituted *N*-arylsulfonyldifluorophenylglycinols *R*-**12a–f**, *S*-**12a–f**, *R*-**13a–f**, and *S*-**13a–f**.

amidation on the sulfonamide nitrogen under basic conditions.

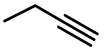
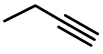
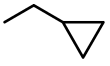
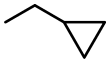
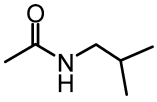
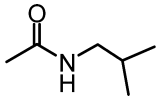
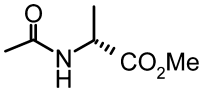
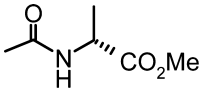
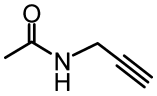
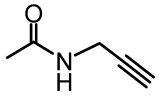
Biological evaluation

The results of initial investigation on the γ-secretase inhibition of these synthesized difluorophenylglycinols through luciferase reporter gene assays are listed in Table 1. Surprisingly, most of the (*R*)-difluorophenylglycinols lacked significant γ-secretase inhibition with <30% activity of PU-1 at 10 μM. The *N*-cyclopropylmethyl derivative *R*-**12c** had about 28% of potency, whereas the *N*-propargyl compound *R*-**12b** had only 17% activity, compared to PU-1. For the (*S*)-enantiomeric analogs, only *N*-ethyl derivative *S*-**12a** showed about 40% activity of PU-1, while others were inferior. These results suggested that the replacement of the 3,5-difluorophenyl group for the hexafluoroisopropyl moiety of GSI-953 was not an effective approach to improve γ-secretase inhibition, postulating that a bulky amino alcoholic side-chain similar to hexafluoroisopropyl moiety might be necessary for fitting to the binding pocket of the γ-secretase to exert the inhibitory activity, and the plane structure of 3,5-difluorophenyl group of compounds series-1 did not fully match the requisite binding space. The results also indicated that the difluorophenylglycinol derivatives did not show preferred

Table 1. Inhibition of γ-secretase-mediated C99 cleavage by *N*-substituted (*R*)- and (*S*)-*N*-(5-chlorothiophen-2-yl)sulfonyl phenylglycinols using luciferase reporter assay.



R-**12** *S*-**12**

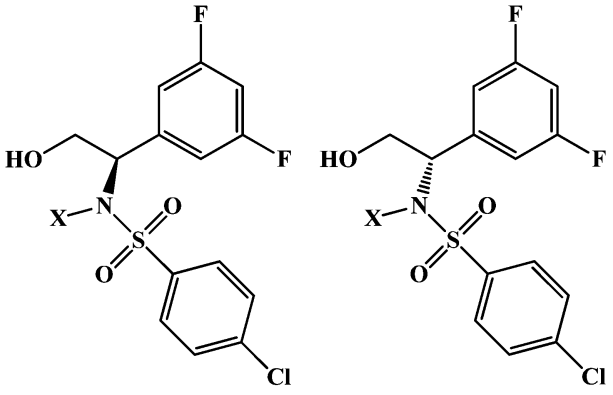
Compound	X	Reduction of C99 cleavage (%)	Compound	X	Reduction of C99 cleavage (%)
R-**12a**		0.0	*S*-**12a**		38.9
R-**12b**		16.7	*S*-**12b**		16.7
R-**12c**		27.8	*S*-**12c**		0.0
R-**12d**		5.6	*S*-**12d**		11.1
R-**12e**		11.1	*S*-**12e**		22.2
R-**12f**		11.1	*S*-**12f**		11.1
PU-1	–	100.0			

stereochemistry at the β-center of the amino alcohols, with chirality of this center exhibiting little preference for both enantiomer pairs on the γ-secretase inhibition. The loss of stereoselectivity of these difluorophenylglycinol analogs for the γ-secretase inhibition may be attributed to the worse binding ability to the active site of γ-secretase.

Next, our attention turned to the replacement of the *p*-chlorobenzenesulfonyl group for the 5-chlorothiophenesulfonyl moiety of these (*R*)- and (*S*)-3,5-difluorophenylglycinols. Thus, these chlorobenzenesulfonamides *R*-**13a–f** and *S*-**13a–f** were synthesized (Scheme 2) and the results of γ-secretase inhibition are listed in Table 2. Among them, (*R*)-*N*-cyclopropylmethyl derivative *R*-**13c** exhibited the substantial potency with 78% activities of PU-1 and about threefold improvement compared to *R*-**12c**, while *S*-**13c** is about fourfold less active than *R*-**13c** but showed a small increase in potency compared to the corresponding *S*-**12c** (Table 2). (*S*)-*N*-Alkylureido derivatives *S*-**13d** and *S*-**13f** still showed 33.3% reduction of C99 cleavage, while the corresponding (*R*)-analogs had virtually no potency. Similar to the above thiophenesulfonamide analogs, the chirality of the β-center of these amino alcohols is also not a mandatory requisite for γ-secretase inhibition as *R*-**13c**, *S*-**13d**, and *S*-**13f** all showed modest potency.

From the structure–activity relationship point of view, the *p*-chlorobenzenesulfonyl group of the compounds series-2 was

Table 2. Inhibition of γ-secretase-mediated C99 cleavage by *N*-substituted (*R*)- and (*S*)-*N*-(*p*-chlorobenzenesulfonyl)difluorophenylglycinols using luciferase reporter assay.



R-**13** *S*-**13**

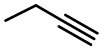
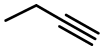
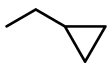
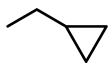
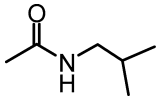
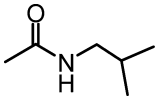
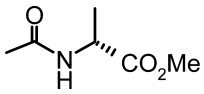
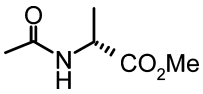
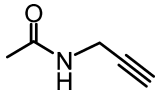
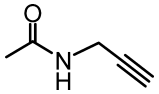
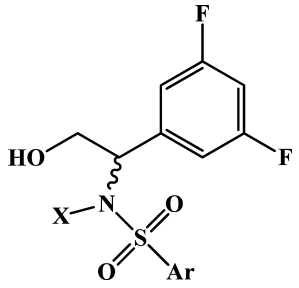
Compound	X	Reduction of C99 cleavage (%)	Compound	X	Reduction of C99 cleavage (%)
R-**13a**		11.1	*S*-**13a**		−5.6
R-**13b**		16.7	*S*-**13b**		5.6
R-**13c**		77.8	*S*-**13c**		22.2
R-**13d**		0.0	*S*-**13d**		33.3
R-**13e**		11.1	*S*-**13e**		5.6
R-**13f**		5.6	*S*-**13f**		33.3
PU-1	–	100.0			

Table 3. IC_{50} values for selected difluorophenylglycinols on γ-secretase-mediated inhibition.



Compound	Ar	X	IC_{50} (μM)	
			S3 cleavage	C99 cleavage
R-**12c**	5-Chlorothiophen-2-yl	*c*-Propylmethyl	16.5	11.7
S-**12a**	5-Chlorothiophen-2-yl	Ethyl	47.9	43.6
R-**13c**	*p*-Chlorophenyl	*c*-Propylmethyl	8.3	8.7
S-**13c**	*p*-Chlorophenyl	*c*-Propylmethyl	30.2	29.3

a little more favorable than the chlorothiophenesulfonyl moiety for the γ-secretase inhibition, and led to modest potency. When the X group is *N*-cyclopropyomethyl, both (*R*)-difluorophenylglycinols maintained 28–78% inhibitory activity; propargyl analogs were a little worse in potency. Other (*R*)-difluorophenylglycinols with X = *N*-alkylureido such as **12d–f** and **13d–f** were detrimental to the inhibitory activity. However, regarding the (*S*)-difluorophenylglycinols, *N*-alkylureido analogs such as **13d** and **13f** were favorable with 33% inhibitory activity of PU-1, while *N*-alkyl analogs were inferior except the ethyl analog **12a** with 39% activity.

We further examined whether these arylsulfonylphenylglycinols with modest γ-secretase inhibition exhibited selective Notch-sparing effects. Thus, their capability to process γ-secretase-mediated S3 cleavage of Notch was examined by using a stable cell line (N7) constitutively expressing NΔE as previously reported [19]. As shown in Table 3, the IC_{50} values of the selected compounds *R*-**12c**, *S*-**12a**, *R*-**13c**, and *S*-**13c** were quite close to those values against γ-secretase-mediated C99 cleavage of APP. The results indicated that these arylsulfonyl analogs did not exhibit substantial selectivity for the γ-secretase-mediated proteolytic processing of APP over Notch.

In the following step, we investigate whether these compounds can significantly modulate the MEK–ERK signal pathway because the α-secretase-mediated processing of APP would be enhanced directly or indirectly through the activation of MEK pathway by certain propargylamine-related compounds. Thus, the moderate γ-secretase inhibitors *R*-**12c**, *S*-**12a**, *R*-**13c**, and *S*-**13c**, which possessed propargyl-related substitution, were chosen for further investigation of the relative levels of ERK activation (*p*-ERK/ERK, %) in γ-30 cells as previously described [10, 20]. The results are described in Table 4 and showed that *N*-cyclopropylmethyl derivative *R*-**13c** was 1.1-fold more potent than rasagiline on ERK-mediated activation at 10 μM, while others exhibited similar potency. The results revealed that the introduction of an *N*-cyclopropylmethyl substituent to the difluorophenylglycinols **10** and **11** led to the activation effects on the ERK-mediated signaling. Structurally, the molecular size and chemical reactivity of the *N*-cyclopropylmethyl group in the compounds *R*-**12c** and *R*-**13c** are similar to the prevailing propargylamine moiety, which is responsible for stimulating the processing of amyloid precursor protein to sAPPα through MAP kinase-dependent activation of α-secretase as previously described [5, 20, 21]. Thus, the activation of the ERK-mediated signaling of *R*-**12c** and *R*-**13c** was considerably attributed to the *N*-cyclopropylmethyl moiety and would evoke additional

Table 4. The relative levels of ERK activation of certain *N*-arylsulfonyl-difluorophenylglycinols in treated cells compared with nontreated cells.

Compound	Activation of ERK1/2 (*p*-ERK/ERK, %)[a]
D + T[b]	100
Rasagiline	119 ± 22
R-**12c**	117 ± 23
S-**12a**	124 ± 11
R-**13c**	132 ± 23
S-**13c**	117 ± 13

[a] Each value represents the mean ± SD of three experiments.
[b] D, DMSO; T, tetracycline.

α-secretase stimulation. These effects would subsequently enhance sAPPα release and eventually provide neuronal protection accompanied by decreased Aβ production.

Conclusion

In this study, we developed a type of difluorophenylglycinol derivatives and investigated their effects on the modulation of the proteolytic processing of APP, including γ-secretase-mediated C99 cleavage and ERK-mediated activation. We presented the identification of the *N*-cyclopropylmethyl derivatives *R*-**12c** and *R*-**13c** that exhibited dual-acting activities with modest γ-secretase inhibition as well as substantial ERK activation, while they were compromised by poor selectivity of APP processing over Notch. The dual actions of the *N*-cyclopropylmethyl derivative would eventually avoid the generation of Aβ and thus provide the beneficial effects from neuronal protection. From the chemical feature for the biological results point of view, development of a type of optimized bulky alkyl amino alcohols as new modulators of APP processing will be the next course to explore selective γ-secretase inhibition with dual-acting activities for AD therapies.

Experimental

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 solvent contained 99.8–99.9% deuterium in the indicated position and was 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 (br). 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, Darmstadt, Germany). Melting points were recorded on a Thomas Hoover capillary melting point apparatus in open capillary tubes and are uncorrected.

*(R)-4-Benzyl-3-(2-(3,5-difluorophenyl)acetyl)oxazolidin-2-one (**5**)*

To a solution of **3** (5.1 g, 29.6 mmol) and triethylamine (3.6 g, 35.6 mmol) in 250 mL of THF, trimethylacetyl chloride (3.76 g, 31.2 mmol) was added, and stirred at −78°C under $Ar_{(g)}$. A solution of metallated oxazolidinone prepared by the addition of *n*-butyllithium (17.5 mL, 28.2 mmol) to a solution of 4-(*R*)-4-phenylmethyl-2-oxazolidinone (**4**, 5.0 g, 28.2 mmol) in THF at −78°C was added via cannula, and stirred for 30 min at 0°C then quenched by saturated aqueous ammonium chloride. The mixture was extracted with CH_2Cl_2, washed with NaOH and $KHSO_4$, and dried over $MgSO_4$. The residue was purified by silica gel column chromatography to give **5** (4.67 g, 50%) as yellow oil; TLC, R_f 0.3 (EtOAc/*n*-hexane = 1:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.78 (1H, dd, J = 9.6, 11.8 Hz, C<u>H</u>HAr), 3.24 (1H, dd, J = 3.3, 12.4 Hz, CH<u>H</u>Ar), 4.16–4.29 (4H, m, C<u>H</u>$_2$O and C<u>H</u>$_2$), 4.64–4.71 (1H, m, C<u>H</u>CH$_2$O), 6.69–6.76 (1H, m, Ar–<u>H</u>), 6.80–6.91 (2H, m, Ar–<u>H</u>), 7.11–7.32 (5H, m, Ar–<u>H</u>); FABMS: *m*/*z* $[M+H]^+$ 332.

*(R)-4-Benzyl-3-((R)-2-bromo-2-(3,5-difluorophenyl)acetyl)-oxazolidin-2-one (**6**)*

To a solution of **5** (3.96 g, 12 mmol) in CH_2Cl_2 (50 mL), DIEA (2.36 mL, 14.3 mmol) was added followed by dropwise addition of *n*-Bu_2BOTf (12.6 mL, 12.6 mmol) at −78°C under $Ar_{(g)}$. The pale yellow solution was stirred at −78°C for 15 min and then at 0°C for 1 h. The resulting boron enolate solution was then precooled to −78°C and added by cannula to a slurry solution of NBS (2.3 g, 13 mmol) in CH_2Cl_2 at −78°C under $Ar_{(g)}$. The mixture was stirred at −78°C for 2 h and quenched by aqueous $KHSO_4$–brine solution. The resulting mixture was extracted with EtOAc, washed with $Na_2S_2O_3$ and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give **6** (1.97 g, 40%) as brown oil. TLC, R_f 0.4 (EtOAc/*n*-hexane = 1:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.70–2.91 (1H, m, C<u>H</u>HAr), 3.21–3.34 (1H, m, CH<u>H</u>Ar), 4.27–4.30 (2H, m, C<u>H</u>$_2$O), 4.67–4.79 (1H, m, C<u>H</u>CH$_2$O), 6.76–6.86 (3H, m, Ar–<u>H</u>), 7.04–7.08 (1H, m, C<u>H</u>Br), 7.17–7.39 (5H, m, Ar–<u>H</u>); FABMS: *m*/*z* $[M+H]^+$ 411.

*(R)-3-((S)-2-Azido-2-(3,5-difluorophenyl)acetyl)-4-benzyloxazolidin-2-one (R-**7**)*

To a solution of **5** (3.96 g, 14.4 mmol) in THF (50 mL), potassium hexamethyldisilamide (KHMDS, 31.6 mL, 15.8 mmol) was added at −78°C under $Ar_{(g)}$ for 30 min. The resulting potassium enolate solution was added via cannulation to a precooled solution of 2,4,6-triisopropylbenzenesulfonyl azide (5.7 g, 18.7 mmol) in THF (50 mL) at −78°C under $Ar_{(g)}$. After 10 min, the reaction was quenched with glacial acetic acid (3.7 mL), and stirred at room temperature for 12 h. The solution was partitioned with CH_2Cl_2. The organic phases was washed with $NaHCO_3$, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give *R*-**7** (2.68 g, 50%) as white solid. TLC, R_f = 0.6 (CH_2Cl_2/*n*-hexane = 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.87 (1H, dd, J = 9.6, 13.5 Hz, C<u>H</u>HAr), 3.38 (1H, dd, J = 3, 13.5 Hz, CH<u>H</u>Ar), 4.14–4.24 (2H, m, C<u>H</u>$_2$O), 4.64–4.72 (1H, m, C<u>H</u>CH$_2$O), 6.13 (1H, s, C<u>H</u>N$_3$), 6.81–6.88 (1H, m, Ar–<u>H</u>), 6.97–7.04 (2H, m, Ar–<u>H</u>), 7.23–7.39 (5H, m, Ar–<u>H</u>); FABMS: *m*/*z* $[M+H]^+$ 373.

*(R)-3-((S)-2-Azido-2-(3,5-difluorophenyl)acetyl)-4-benzyloxazolidin-2-one (R-**7**); (S)-3-((S)-2-azido-2-(3,5-difluorophenyl)acetyl)-4-benzyloxazolidin-2-one (S-**7**)*

To a solution of **6** (0.62 g, 1.5 mmol) in acetonitrile (20 mL), NaN_3 (0.39 mg, 6 mmol) was added in an ice bath. The reaction mixture was stirred for 1 h at 0°C, and then at room temperature for 6 h and quenched by saturated aqueous $NaHCO_3$. The resulting mixture was extracted with CH_2Cl_2, dried over $MgSO_4$, and purified by silica gel column chromatography to give *R*-**7** (168 mg, 30%) that is identical to the above product as white solid; *S*-7 (168 mg, 30%) as yellow oil. TLC, R_f = 0.4 (CH_2Cl_2/*n*-hexane = 2:1);

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.68 (1H, dd, $J = 9.3$, 12.9 Hz, CHHAr), 3.21 (1H, dd, $J = 3.6$, 12.9 Hz, CHHAr), 4.16–4.31 (2H, m, CH_2O), 4.76–4.82 (1H, m, $CHCH_2O$), 6.12 (1H, s, CHN_3), 6.85–6.93 (1H, m, Ar–H), 7.03–7.09 (4H, m, Ar–H), 7.23–7.26 (3H, m, Ar–H); FABMS: m/z $[M+H]^+$ 373.

*(R)-2-Azido-2-(3,5-difluorophenyl)ethanol (R-**8**)*

To a solution of *R*-**7** (1.7 g, 4.5 mmol) and water (0.08 mL, 4.5 mmol) in THF (20 mL) at 0°C, a solution of lithium borohydride (2 M in THF, 9 mmol) was added. The reaction mixture stood to warm for 30 min and was stirred for 1 day at room temperature. The mixture was quenched with methanol and sodium hydroxide was added. The resulting mixture was extracted with CH_2Cl_2, dried over $MgSO_4$, and then purified by silica gel column chromatography to give *R*-**8** (0.63 g, 70%) as yellow oil. TLC, R_f 0.4 (CH_2Cl_2/*n*-hexane = 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.98 (1H, br, OH), 3.68 (1H, dd, $J = 7.8$, 11.7 Hz, CHHOH), 3.76 (1H, dd, $J = 4.2$, 11.7 Hz, CHHOH), 4.62 (1H, dd, $J = 4.5$, 7.9 Hz, CHN_3), 6.75–6.90 (3H, m, Ar–H); FABMS: m/z $[M+H]^+$ 200.

*(S)-2-Azido-2-(3,5-difluorophenyl)ethanol (S-**8**)*

According to the above method, azide *S*-**7** (1.7 g, 4.5 mmol) was converted to *S*-**8** (0.62 g, 70%) as yellow oil. TLC, R_f 0.4 (CH_2Cl_2/*n*-hexane = 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.36 (1H, t, $J = 5.7$ Hz, OH), 3.69–3.77 (2H, m, CH_2OH), 4.64 (1H, dd, $J = 4.2$, 7.8 Hz, CHN_3), 6.76–6.92 (3H, m, Ar–H); FABMS: m/z $[M+H]^+$ 200.

*(R)-2-Amino-2-(3,5-difluorophenyl)ethanol (R-**9**)*

A solution of *R*-**8** (0.19 g, 1.1 mmol) in 15 mL of methanol was flushed with inert gas and treated with 10% Pd on carbon (53 mg). The mixture was placed under an atmosphere of $H_{2\ (g)}$, stirred for 4 h, then filtered through a pad of celite, and concentrated under reduced pressure to give *R*-**9** (143 mg, 75%) as pale yellow powder. TLC, R_f 0.4 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.13 (3H, br, OH and NH_2), 3.51 (1H, dd, $J = 7.8$, 10.8 Hz, CHHOH), 3.73 (1H, dd, $J = 3.9$, 10.5 Hz, CHHOH), 4.04 (1H, dd, $J = 4.2$, 7.5 Hz, $CHNH_2$), 6.67–6.73 (2H, m, Ar–H), 6.86–6.90 (1H, m, Ar–H); FABMS: m/z $[M+H]^+$ 173.

*(S)-2-Amino-2-(3,5-difluorophenyl)ethanol (S-**9**)*

According to the above method, azide *S*-**8** (0.19 g, 1.1 mmol) was converted to amine *S*-**9** (143 mg, 75%) as pale powder. TLC, R_f 0.4 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.11 (3H, br, OH and NH_2), 3.50 (1H, dd, $J = 7.8$, 10.8 Hz, CHHOH), 3.72 (1H, dd, $J = 3.9$, 10.5 Hz, CHHOH), 4.04 (1H, dd, $J = 4.2$, 7.5 Hz, $CHNH_2$), 6.66–6.73 (2H, m, Ar–H), 6.85–6.90 (1H, m, Ar–H); FABMS: (m/z) $C_8H_{10}F_2NO$ $[M+H]^+$ 173.16.

*(R)-5-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-thiophene-2-sulfonamide (R-**10**)*

To a solution of *R*-**9** (0.36 g, 2.1 mmol) in CH_2Cl_2 (6 mL) 0.5 mL of pyridine was added. The mixture was stirred for 15 min at room temperature and then added 5-chlorothiophene-2-sulfonyl chloride (0.2 mL, 1.7 mmol). The reaction mixture was stirred for 1 day, the resulting mixture was extracted with CH_2Cl_2, and dried over $MgSO_4$. The residue was purified by silica gel column chromatography to give *R*-**10** (0.29 g, 40%) as white powder. TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:1); m.p. 118–120°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02 (1H, br, OH), 3.72 (1H, dd, $J = 6$, 11.4 Hz, CHHOH), 3.85 (1H, dd, $J = 3.9$, 11.4 Hz, CHHOH), 4.46–4.51 (1H, m, CHN), 5.74 (1H, d, $J = 6$ Hz, NH), 6.68–6.78 (3H, m, Ar–H), 6.81 (1H, d, $J = 4.2$ Hz, thiophene-H), 7.25 (1H, d, $J = 4.2$ Hz, thiophene-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 61.52, 62.36, 103.83, 111.01, 126.45, 131.64, 137.34, 139.43, 140.28, 163.06; $[\alpha]_D$ −37° (c 0.006, MeOH); FABMS: m/z $[M+H]^+$ 354; HRFABMS: calcd. for $C_{12}H_{11}ClF_2NO_3S_2$ $[M+H]^+$ 353.9838, found 353.9839.

*(S)-5-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-thiophene-2-sulfonamide (S-**10**)*

According to the above method, *S*-**9** (0.59 g, 3.4 mmol) was converted to *S*-**10** (0.54 g, 45%) as white powder. TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:1); m.p. 118–120°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.05 (1H, br, OH), 3.72 (1H, dd, $J = 6$, 11.1 Hz, CHHOH), 3.85 (1H, dd, $J = 3.9$, 12 Hz, CHHOH), 4.46–4.51 (1H, m, CHN), 5.75 (1H, d, $J = 6.3$ Hz, NH), 6.69–6.78 (3H, m, Ar–H), 6.81 (1H, d, $J = 4.2$ Hz, thiophene-H), 7.25 (1H, d, $J = 4.2$ Hz, thiophene-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 61.52, 62.36, 103.85, 111.03, 126.42, 131.65, 137.33, 139.42, 140.35, 163.07; $[\alpha]_D$ +37°C (c 0.006, MeOH); FABMS: m/z $[M+H]^+$ 354; HRFABMS: calcd. for $C_{12}H_{11}ClF_2NO_3S_2$ $[M+H]^+$ 353.9838, found 353.9836.

*(R)-4-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-benzenesulfonamide (R-**11**)*

According to the above method, *R*-**9** (86.6 mg, 0.5 mmol) was converted to *R*-**11** (75 mg, 43%) as white powder. TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:1); m.p. 129–131°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.95 (1H, t, $J = 5.1$ Hz, OH), 3.67–3.81 (2H, m, CH_2OH), 4.44 (1H, dd, $J = 4.2$, 6.3 Hz, CHN), 5.60 (1H, d, $J = 6.3$ Hz, NH), 6.65–6.70 (3H, m, Ar–H), 7.39 (2H, d, $J = 9$ Hz, Ar–H), 7.65 (2H, d, $J = 9.3$ Hz, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 61.36, 62.24, 103.70, 110.92, 128.63, 129.36, 139.22, 139.33, 140.55, 163.04; $[\alpha]_D$ −56.0°C (c 0.005, MeOH); FABMS: m/z $[M+H]^+$ 348; HRFABMS: calcd. for $C_{14}H_{13}ClF_2NO_3S$ $[M+H]^+$ 348.0274, found 348.0275.

*(S)-4-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-benzenesulfonamide (S-**11**)*

According to the above method, amine *S*-**9** (86.6 mg, 0.5 mmol) was converted to give *S*-**11** (86 mg, 50%) as white powder. TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:1); m.p. 128–130°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.27 (1H, t, $J = 5.1$ Hz, OH), 3.67–3.83 (2H, m, CH_2OH), 4.45 (1H, dd, $J = 6.3$, 10.8 Hz, CHN), 5.82 (1H, d, $J = 6.6$ Hz, NH), 6.63–6.70 (3H, m, Ar–H), 7.37 (2H, d, $J = 8.7$ Hz, Ar–H), 7.66 (2H, d, $J = 9$ Hz, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 61.33, 62.04, 103.69, 110.92, 128.64, 129.37, 139.27, 139.32, 140.58, 163.04; $[\alpha]_D$ +57.5°C (c 0.0065, MeOH); FABMS: m/z $[M+H]^+$ 348; HRFABMS: calcd. for $C_{14}H_{13}ClF_2NO_3S$ $[M+H]^+$ 348.0274, found 348.0272.

*(R)-5-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-ethylthiophene-2-sulfonamide (R-**12a**)*

To a mixture of *R*-**10** (0.08 g, 0.23 mmol) in DMF (6 mL), potassium carbonate (0.188 g, 1.36 mmol) and iodoethane (0.018 mL, 0.23 mmol) were added at room temperature. The mixture was stirred for 12 h, poured into brine, then extracted with EtOAc. The organic layer was separated, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography to give *R*-**12a** (40 mg, 46%) as pale yellow powder. TLC, R_f 0.4 (EtOAc/*n*-hexane = 1:2); m.p. 58.0–60.0°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (3H, t, $J = 7.2$ Hz, CH_3), 2.01 (1H, br, OH), 3.17 (1H, sextet,

$J = 7.2$ Hz, C$\underline{H}_2$CH$_3$), 3.32 (1H, sextet, $J = 7.2$ Hz, C$\underline{H}_2$CH$_3$), 4.04–4.10 (2H, m, C$\underline{H}_2$OH), 5.00 (1H, t, $J = 6.6$ Hz, C$\underline{H}$N), 6.72–6.79 (3H, m, Ar–$\underline{H}$), 6.92 (1H, d, $J = 4.2$ Hz, thiophene-$\underline{H}$), 7.38 (1H, d, $J = 3.9$ Hz, thiophene-$\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.13, 40.83, 61.66, 62.07, 103.82, 111.03, 126.58, 131.63, 137.30, 139.40, 140.29, 163.01; UV λ_{max} (MeOH) nm: 287.6 (0.626); $[\alpha]_D$ −38°C (c 0.005, MeOH); *c* log *p* = 3.65; FABMS: *m*/*z* 382 $[M+H]^+$; HRFABMS: calcd. for $C_{14}H_{15}ClF_2NO_3S_2$ $[M+H]^+$ 382.0151, found 382.0152.

*(S)-5-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-ethylthiophene-2-sulfonamide (S-**12a**)*

According to the above method, *S*-**10** (0.08 g, 0.23 mmol) was converted to *S*-**12a** (42%) as pale yellow powder. TLC, R_f 0.4 (EtOAc/*n*-hexane = 1:2); m.p. 55.0–57.0°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12 (3H, t, $J = 7.2$ Hz, C$\underline{H}_3$), 2.10 (1H, br, O$\underline{H}$), 3.19 (1H, sextet, $J = 7.2$ Hz, C$\underline{H}_2$CH$_3$), 3.31 (1H, sextet, $J = 7.2$ Hz, C$\underline{H}_2$CH$_3$), 4.04–4.09 (2H, m, C$\underline{H}_2$OH), 5.00 (1H, t, $J = 6.9$ Hz, C$\underline{H}$N), 6.72–6.78 (3H, m, Ar–$\underline{H}$), 6.92 (1H, d, $J = 4.2$ Hz, thiophene-$\underline{H}$), 7.37 (1H, d, $J = 3.9$ Hz, thiophene-$\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.13, 40.83, 61.66, 62.07, 103.82, 111.03, 126.58, 131.63, 137.30, 139.41, 140.29, 163.02; UV λ_{max} (MeOH) nm: 287 (0.459); $[\alpha]_D$ +38°C (c 0.005, MeOH); *c* log *p* = 3.65; FABMS: *m*/*z* 382 $[M+H]^+$; HRFABMS: calcd. for $C_{14}H_{15}ClF_2NO_3S_2$ $[M+H]^+$ 382.0151, found 382.0153.

*(R)-5-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-(prop-2-ynyl)thiophene-2-sulfonamide (R-**12b**)*

According to the above method, *R*-**10** (0.2 g, 0.56 mmol) was converted to *R*-**12b** (50%) as dark yellow oil. TLC, R_f 0.4 (EtOAc/*n*-hexane = 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.07 (1H, t, $J = 5.4$ Hz, O$\underline{H}$), 2.31 (1H, t, $J = 2.7$ Hz, CC$\underline{H}$), 3.78 (1H, dd, $J = 2.4$, 18.9 Hz, C$\underline{H}$HOH), 4.11–4.19 (2H, m, NC$\underline{H}_2$CCH), 4.36 (1H, dd, $J = 2.7$, 18.9 Hz, CH$\underline{H}$OH), 5.14 (1H, t, $J = 5.4$ Hz, C$\underline{H}$N), 6.78–6.89 (3H, m, Ar–$\underline{H}$), 6.94 (1H, d, $J = 4.2$ Hz, thiophene-$\underline{H}$), 7.49 (1H, d, $J = 4.2$ Hz, thiophene-$\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 33.78, 61.87, 73.69, 78.53, 104.02, 111.03, 126.70, 132.57, 137.92, 138.56, 139.46; 163.16; UV λ_{max} (MeOH) nm: 287.4 (0.673); $[\alpha]_D$ −38.8°C (c 0.0067, MeOH); *c* log *p* = 3.6; FABMS: *m*/*z* 392 $[M+H]^+$; HRFABMS: calcd. for $C_{15}H_{13}ClF_2NO_3S_2$ $[M+H]^+$ 391.9994, found 391.9995.

*(S)-5-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-(prop-2-ynyl)thiophene-2-sulfonamide (S-**12b**)*

According to the above method, *S*-**10** (0.2 g, 0.56 mmol) was converted to *S*-**12b** (62%) as yellow oil. TLC, R_f 0.4 (EtOAc/*n*-hexane = 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (1H, t, $J = 6$ Hz, O$\underline{H}$), 2.31 (1H, t, $J = 2.4$ Hz, CC$\underline{H}$), 3.79 (1H, dd, $J = 2.4$, 18.6 Hz, C$\underline{H}$HOH), 4.11–4.19 (2H, m, NC$\underline{H}_2$CCH), 4.36 (1H, dd, $J = 2.7$, 18.9 Hz, CH$\underline{H}$OH), 5.14 (1H, t, $J = 5.7$ Hz, C$\underline{H}$N), 6.75–6.88 (3H, m, Ar–$\underline{H}$), 6.94 (1H, d, $J = 4.2$ Hz, thiophene-$\underline{H}$), 7.49 (1H, d, $J = 3.9$ Hz, thiophene-$\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 33.78, 61.63, 61.87, 73.69, 78.53, 104.02, 111.03, 126.70, 132.57, 137.92, 138.56, 139.48; 163.17; UV λ_{max} (MeOH) nm: 287.2 (0.516); $[\alpha]_D$ +26°C (c 0.005, MeOH); *c* log *p* = 3.6; FABMS: *m*/*z* 392 $[M+H]^+$; HRFABMS: calcd. for $C_{15}H_{13}ClF_2NO_3S_2$ $[M+H]^+$ 391.9994, found 391.9990.

*(R)-5-Chloro-N-(cyclopropylmethyl)-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)thiophene-2-sulfonamide (R-**12c**)*

According to the above method, *R*-**10** (0.1 g, 0.3 mmol) was converted to *R*-**12c** (50 mg, 41%) as pale yellow oil. TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.15–0.20 (2H, m, cyclopropane-$\underline{H}$), 0.48–0.53 (2H, m, cyclopropane-$\underline{H}$), 0.87–0.89 (1H, m, cyclopropane-$\underline{H}$), 2.10 (1H, br, O$\underline{H}$), 2.93 (1H, dd, $J = 6.9$, 15 Hz, NC$\underline{H}_2$), 3.20 (1H, dd, $J = 6.6$, 15.3 Hz, NC$\underline{H}_2$), 4.10–4.12 (2H, m, C$\underline{H}_2$OH), 5.03 (1H, t, $J = 6.9$ Hz, C$\underline{H}$N), 6.72–6.77 (3H, m, Ar–$\underline{H}$), 6.91 (1H, d, $J = 3.9$ Hz, thiophene-$\underline{H}$), 7.38 (1H, d, $J = 3.9$ Hz, thiophene-$\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 4.94, 5.25, 11.42, 29.67, 50.60, 61.58, 62.22, 103.73, 111.07, 126.50, 131.70, 137.33, 139.58, 140.30, 163.03; UV λ_{max} (MeOH) nm: 297 (1.58); $[\alpha]_D$ −22.2°C (c 0.0054, MeOH); *c* log *p* = 4.04; FABMS: *m*/*z* 408 $[M+H]^+$; HRFABMS: calcd. for $C_{16}H_{17}ClF_2NO_3S_2$ $[M+H]^+$ 408.0308, found 408.0307.

*(S)-5-Chloro-N-(cyclopropylmethyl)-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)thiophene-2-sulfonamide (S-**12c**)*

According to the above method, *S*-**10** (0.1 g, 0.3 mmol) was converted to *S*-**12c** (71 mg, 58%) as yellow oil. TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.16–0.21 (2H, m, cyclopropane-$\underline{H}$), 0.49–0.54 (2H, m, cyclopropane-$\underline{H}$), 0.87–0.90 (1H, m, cyclopropane-$\underline{H}$), 1.99 (1H, t, $J = 5.7$ Hz, O$\underline{H}$), 2.93 (1H, dd, $J = 6.9$, 15.3 Hz, NC$\underline{H}_2$), 3.21 (1H, dd, $J = 6.6$, 15.3 Hz, NC$\underline{H}_2$), 4.10–4.16 (2H, m, C$\underline{H}_2$OH), 5.03 (1H, t, $J = 7.2$ Hz, C$\underline{H}$N), 6.72–6.78 (3H, m, Ar–$\underline{H}$), 6.92 (1H, d, $J = 3.9$ Hz, thiophene-$\underline{H}$), 7.39 (1H, d, $J = 4.2$ Hz, thiophene-$\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 4.94, 5.25, 11.42, 29.67, 50.60, 61.58, 62.22, 103.73, 111.07, 126.50, 131.70, 137.33, 139.58, 140.30, 163.03; UV λ_{max} (MeOH) nm: 297.0 (1.58); $[\alpha]_D$ +16°C (c 0.005, MeOH); *c* log *p* = 4.04; FABMS: *m*/*z* 408 $[M+H]^+$; HRFABMS: calcd. for $C_{16}H_{17}ClF_2NO_3S_2$ $[M+H]^+$ 408.0308, found 408.0310.

*(R)-5-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-(isobutylcarbamoyl)thiophene-2-sulfonamide (R-**12d**)*

To a stirred solution of triphosgene (78.3 mg, 0.26 mmol) in CH_2Cl_2 a solution of isobutylamine (0.033 mL, 0.33 mmol) and DIEA (0.05 mL) in CH_2Cl_2 was slowly added over a period of 20 min using a syringe pump. After a further 30 min of stirring, a solution of *R*-**10** (118 mg, 0.33 mmol) and DIEA (0.11 mL, 0.66 mmol) in CH_2Cl_2 (10 mL) was added in one portion. The mixture was stirred for 12 h, poured into water, then extracted with CH_2Cl_2. The organic layer was separated, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography to give *R*-**12d** (15 mg, 10%) as white powder. TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:2); m.p. 102.0–103.5°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (6H, d, $J = 6.6$ Hz, CH(C$\underline{H}_3$)$_2$), 1.75 (1H, septet, $J = 6.3$ Hz, C$\underline{H}$(CH$_3$)$_2$), 2.99 (2H, t, $J = 6.6$ Hz, C$\underline{H}_2$), 4.10 (1H, dd, $J = 3.6$, 12 Hz, C$\underline{H}$HOH), 4.23 (1H, dd, $J = 8.1$, 12 Hz, CH$\underline{H}$OH), 4.61 (1H, br, C$\underline{H}$N), 4.80 (1H, br, O$\underline{H}$), 6.23 (1H, br, N$\underline{H}$), 6.70–6.80 (3H, m, Ar–$\underline{H}$), 6.82 (1H, d, $J = 3.9$ Hz, thiophene-$\underline{H}$), 7.24 (1H, d, $J = 3.9$ Hz, thiophene-$\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.86, 28.63, 29.69, 48.66, 57.71, 66.46, 103.71, 110.07, 126.45, 131.87, 132.19, 139.13, 141.03, 156.54, 163.03; UV λ_{max} (MeOH) nm (A): 286.4 (0.413); $[\alpha]_D$ −15°C (c 0.004, MeOH); *c* log *p* = 3.86; FABMS: *m*/*z* 453 $[M+H]^+$; HRFABMS: calcd. for $C_{17}H_{20}ClF_2N_2O_4S_2$ $[M+H]^+$ 453.0523, found 453.0523.

*(S)-5-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-(isobutylcarbamoyl)thiophene-2-sulfonamide (S-**12d**)*

According to the above method, *S*-**10** (140 mg, 0.39 mmol) was converted to *S*-**12d** (23 mg, 13%) as pale yellow powder. TLC, R_f 0.5

(EtOAc/*n*-hexane = 1:2); m.p. 104.0–106.0°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (6H, d, J = 6.6 Hz, CH(C<u>H</u>$_3$)$_2$), 1.74 (1H, septet, J = 6.9 Hz, C<u>H</u>(CH$_3$)$_2$), 2.98 (2H, t, J = 6.6 Hz, C<u>H</u>$_2$), 4.12 (1H, dd, J = 3.9, 12 Hz, C<u>H</u>HOH), 4.21–4.30 (1H, m, CH<u>H</u>OH), 4.64 (1H, br, O<u>H</u>), 4.90 (1H, br, C<u>H</u>N), 6.46 (1H, br, N<u>H</u>), 6.69–7.23 (4H, m, Ar–<u>H</u>), 7.25 (1H, d, J = 3.9 Hz, thiophene-<u>H</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.86, 28.63, 29.69, 48.66, 57.71, 66.46, 103.71, 110.06, 126.60, 131.87, 132.19, 139.13, 141.03, 156.54, 163.04; UV λ_{max} (MeOH) nm: 285.6 (0.143); $[\alpha]_D$ +11.7°C (c 0.006, MeOH); $c\log p$ = 3.86; HRFABMS: calcd. for $C_{17}H_{20}ClF_2N_2O_4S_2$ $[M+H]^+$ 453.0523, found 453.0523.

*(S)-Methyl-2-(3-(5-chlorothiophen-2-ylsulfonyl)-3-((R)-1-(3,5-difluorophenyl)-2-hydroxy-ethyl)ureido)propanoate (R-**12e**)*

According to the above method, *R*-**10** (140 mg, 0.39 mmol) was converted to *R*-**12e** (92 mg, 49%) as yellow oil. TLC, R_f 0.3 (EtOAc/*n*-hexane = 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24–1.43 (3H, m, C<u>H</u>$_3$), 2.94 (1H, br, O<u>H</u>), 3.76 (3H, s, OC<u>H</u>$_3$), 4.13–4.33 (2H, C<u>H</u>$_2$OH), 4.60–4.66 (1H, m, C<u>H</u>CH$_3$), 5.62 (1H, d, J = 7.8 Hz, C<u>H</u>N), 6.33 (1H, d, J = 6.9 Hz, N<u>H</u>), 6.65–6.80 (4H, m, Ar–<u>H</u>), 7.23 (1H, d, J = 4.2 Hz, thiophene-<u>H</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.25, 36.78, 51.25, 57.40, 66.44, 103.71, 110.07, 126.45, 131.87, 132.19, 139.13, 141.03, 156.54, 163.06, 173.25; UV λ_{max} (MeOH) nm: 287.0 (0.666); $[\alpha]_D$ −7.9°C (c 0.0076, MeOH); $c\log p$ = 2.65; FABMS: m/z 483 $[M+H]^+$; HRFABMS: calcd. for $C_{17}H_{18}ClF_2N_2O_6S_2$ $[M+H]^+$ 483.0265, found 408.0263.

*(S)-Methyl 2-(3-(5-chlorothiophen-2-ylsulfonyl)-3-((S)-1-(3,5-difluorophenyl)-2-hydroxyethyl)ureido)propanoate (S-**12e**)*

According to the above method, *S*-**10** (140 mg, 0.39 mmol) was converted to *S*-**12e** (109 mg, 58%) as yellow oil. TLC, R_f 0.3 (EtOAc/*n*-hexane = 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (3H, d, J = 7.2 Hz, C<u>H</u>$_3$), 2.86 (1H, br, O<u>H</u>), 3.77 (3H, s, OC<u>H</u>$_3$), 4.17–4.33 (2H, C<u>H</u>$_2$OH), 4.63–4.66 (1H, m, C<u>H</u>CH$_3$), 5.55 (1H, d, J = 7.8 Hz, C<u>H</u>N), 6.28 (1H, d, J = 6.3 Hz, N<u>H</u>), 6.66–6.80 (4H, m, Ar–<u>H</u>), 7.23–7.27 (1H, m, thiophene-<u>H</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.25, 36.78, 51.25, 57.40, 66.44, 103.71, 110.03, 126.60, 131.87, 132.19, 139.13, 141.03, 156.54, 163.05, 173.24; UV λ_{max} (MeOH) nm: 287.0 (0.166); $[\alpha]_D$ +21.1°C (c 0.009, MeOH); $c\log p$ = 2.65; FABMS: m/z 483 $[M+H]^+$; HRFABMS: calcd. for $C_{17}H_{18}ClF_2N_2O_6S_2$ $[M+H]^+$ 483.0265, found 483.0261.

*(R)-5-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-(prop-2-ynylcarbamoyl)-thiophene-2-sulfonamide (R-**12f**)*

According to the above method, *R*-**10** (210 mg, 0.59 mmol) was converted to *R*-**12f** (110 mg, 43%) as yellow oil. TLC, R_f 0.3 (EtOAc/*n*-hexane = 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.28 (1H, br, CC<u>H</u>), 3.93–3.96 (2H, m, NC<u>H</u>$_2$CCH), 4.19–4.26 (2H, m, C<u>H</u>$_2$OH), 4.67 (1H, br, O<u>H</u>), 5.27 (1H, t, J = 5.4 Hz, C<u>H</u>N), 6.55 (1H, br, N<u>H</u>), 6.69–6.75 (3H, m, Ar–<u>H</u>), 6.81 (1H, d, J = 3.9 Hz, thiophene-<u>H</u>), 7.25 (1H, d, J = 3.9 Hz, thiophene-<u>H</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.58, 57.00, 66.63, 71.95, 79.11, 103.60, 110.01, 137.53, 138.90, 140.80, 155.68, 162.91; UV λ_{max} (MeOH) nm: 288 (0.85); $[\alpha]_D$ −16°C (c 0.0075, MeOH); $c\log p$ = 2.85; FABMS: m/z 435 $[M+H]^+$; HRFABMS: calcd. for $C_{16}H_{14}ClF_2N_2O_4S_2$ $[M+H]^+$ 435.0053, found 435.0056.

*(S)-5-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-(prop-2-ynylcarbamoyl)-thiophene-2-sulfonamide (S-**12f**)*

According to the above method, *S*-**10** (108 mg, 0.3 mmol) was converted to *S*-**12f** (50 mg, 38%) as yellow oil. TLC, R_f 0.3 (EtOAc/*n*-hexane = 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.27 (1H, br, CC<u>H</u>), 3.92–3.95 (2H, m, NC<u>H</u>$_2$CCH), 4.15–4.25 (2H, m, C<u>H</u>$_2$OH), 4.65 (1H, br, O<u>H</u>), 5.20 (1H, br, C<u>H</u>N), 6.44 (1H, d, J = 6 Hz, N<u>H</u>), 6.68–6.75 (3H, m, Ar–<u>H</u>), 6.80 (1H, d, J = 3.9 Hz, thiophene-<u>H</u>), 7.24 (1H, d, J = 4.2 Hz, thiophene-<u>H</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.58, 57.00, 66.63, 71.95, 79.11, 103.60, 110.00, 126.57, 131.90, 137.53, 138.90, 140.80, 155.68, 162.90; UV λ_{max} (MeOH) nm: 288.0 (0.775); $[\alpha]_D$ +20°C (c 0.009, MeOH); $c\log p$ = 2.85; FABMS: m/z 435 $[M+H]^+$. HRFABMS: calcd. for $C_{16}H_{14}ClF_2N_2O_4S_2$ $[M+H]^+$ 435.0053, found 435.0051.

*(R)-4-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-ethylbenzenesulfonamide (R-**13a**)*

According to the method for the preparation of *R*-**12a**, *R*-**11** (82 mg, 0.24 mmol) was converted to *R*-**13a** (43 mg, 47%) as white powder. TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:2); m.p. 66.0–67.5°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (3H, t, J = 7.2 Hz, C<u>H</u>$_3$), 2.03 (1H, br, O<u>H</u>), 3.15 (1H, sextet, J = 7.2 Hz, C<u>H</u>$_2$CH$_3$), 3.32 (1H, sextet, J = 7.2 Hz, C<u>H</u>$_2$CH$_3$), 4.01–4.08 (2H, m, C<u>H</u>$_2$OH), 5.02 (1H, t, J = 6.9 Hz, C<u>H</u>N), 6.65–6.76 (3H, m, Ar–<u>H</u>), 7.48 (2H, d, J = 9 Hz, Ar–<u>H</u>), 7.79 (2H, d, J = 8.7 Hz, Ar–<u>H</u>); TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.20, 40.44, 61.33, 62.04, 103.69, 110.90, 128.64, 129.37, 139.26, 139.32, 140.58, 163.04; UV λ_{max} (MeOH) nm: 286.6 (0.114); $[\alpha]_D$ −37.8°C (c 0.0045, MeOH); $c\log p$ = 3.72; FABMS: m/z 376 $[M+H]^+$; HRFABMS: calcd. for $C_{16}H_{17}ClF_2NO_3S$ $[M+H]^+$ 376.0587, found 376.0588.

*(S)-4-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-ethylbenzenesulfonamide (S-**13a**)*

According to the above method, *S*-**11** (82 mg, 0.24 mmol) was converted to *S*-**13a** (56 mg, 62%) as yellow oil. TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:2); m.p. 63.0–65.0°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (3H, t, J = 7.2 Hz, C<u>H</u>$_3$), 1.93 (1H, br, O<u>H</u>), 3.16 (1H, sextet, J = 7.2 Hz, C<u>H</u>$_2$CH$_3$), 3.31 (1H, sextet, J = 7.2 Hz, C<u>H</u>$_2$CH$_3$), 4.01–4.11 (2H, m, C<u>H</u>$_2$OH), 5.02 (1H, t, J = 6.9 Hz, C<u>H</u>N), 6.67–6.76 (3H, m, Ar–<u>H</u>), 7.49 (2H, d, J = 8.7 Hz, Ar–<u>H</u>), 7.78 (2H, d, J = 8.7 Hz, Ar–<u>H</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.20, 40.44, 61.33, 62.04, 103.69, 110.92, 128.64, 129.37, 139.27, 139.31, 140.58, 163.03; UV λ_{max} (MeOH) nm: 288.4 (0.150); $[\alpha]_D$ +36°C (c 0.005, MeOH); $c\log p$ = 3.72; FABMS: m/z 376 $[M+H]^+$; HRFABMS: calcd. for $C_{16}H_{17}ClF_2NO_3S$ $[M+H]^+$ 376.0587, found 376.0589.

*(R)-4-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-(prop-2-ynyl)benzenesulfonamide (R-**13b**)*

According to the above method, *R*-**11** (0.130 g, 0.375 mmol) was converted to *R*-**13b** (82 mg, 57%) as pale yellow powder. TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:2); m.p.: 82.5–84.0°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.93 (1H, t, J = 6 Hz, O<u>H</u>), 2.22 (1H, t, J = 2.4 Hz, CC<u>H</u>), 3.75 (1H, dd, J = 2.4, 16.5 Hz, C<u>H</u>HOH), 4.05–4.13 (2H, m, NC<u>H</u>$_2$CCH), 4.40 (1H, dd, J = 2.4, 18 Hz, CH<u>H</u>OH), 5.15 (1H, t, J = 5.7 Hz, C<u>H</u>N), 6.73–6.88 (3H, m, Ar–<u>H</u>), 7.49 (2H, d, J = 9 Hz, Ar–<u>H</u>), 7.88 (2H, d, J = 8.7 Hz, Ar–<u>H</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 39.37, 61.48, 61.63, 73.26, 78.85, 103.94, 110.92, 129.15, 138.70, 139.63, 163.16; UV λ_{max} (MeOH) nm: 300.4 (0.253); $[\alpha]_D$ −47.3°C (c 0.0055, MeOH); $c\log p$ = 3.9; FABMS: m/z 386 $[M+H]^+$; HRFABMS: calcd. for $C_{17}H_{15}ClF_2NO_3S$ $[M+H]^+$ 386.0430, found 386.0426.

*(S)-4-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-(prop-2-ynyl)benzenesulfonamide (S-**13b**)*

According to the above method, *S*-**11** (0.130 g, 0.375 mmol) was converted to give *S*-**13b** (92 mg, 64%) as pale yellow powder. TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:2); m.p. 80.5–82.0°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.92 (1H, t, J = 7.5 Hz, OH), 2.22 (1H, t, J = 2.7 Hz, CCH), 3.75 (1H, dd, J = 2.4, 16.5 Hz, CHHOH), 4.05–4.13 (2H, m, NCH_2CCH), 4.40 (1H, dd, J = 2.4, 17.1 Hz, CHHOH), 5.15 (1H, t, J = 5.7 Hz, CHN), 6.73–6.87 (3H, m, Ar–H), 7.48 (2H, d, J = 8.7 Hz, Ar–H), 7.88 (2H, d, J = 9 Hz, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 39.37, 61.48, 61.63, 73.26, 78.85, 103.94, 110.93, 129.15, 138.70, 139.63, 163.15; UV λ_{max} (MeOH) nm: 278.6 (0.253); $[\alpha]_D$ +40°C (c 0.005, MeOH); *c* log *p* = 3.9; FABMS: *m*/*z* 402 $[M+H]^+$; HRFABMS: calcd. for $C_{18}H_{19}ClF_2NO_3S$ $[M+H]^+$ 402.0744, found 402.0744.

*(R)-4-Chloro-N-(cyclopropylmethyl)-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)benzenesulfonamide (R-**13c**)*

According to the above method, *R*-**11** (0.11 g, 0.32 mmol) was converted to *R*-**13c** (37 mg, 29%) as pale yellow oil. TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.11–0.15 (2H, m, cyclopropane-H), 0.42–0.48 (2H, m, cyclopropane-H), 0.79–0.84 (1H, m, cyclopropane-H), 2.20 (1H, br, OH), 2.90 (1H, dd, J = 6.9, 15.3 Hz, NCH_2), 3.19 (1H, dd, J = 6.6, 15.3 Hz, NCH_2), 4.07–4.14 (2H, m, CH_2OH), 5.05 (1H, t, J = 6.9 Hz, CHN), 6.69–6.75 (3H, m, Ar–H), 7.46 (2H, d, J = 8.7 Hz, Ar–H), 7.80 (2H, d, J = 8.7 Hz, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 4.88, 5.23, 11.49, 50.31, 61.26, 62.22, 103.87, 110.97, 126.11, 128.68, 131.25, 139.44, 162.94; UV λ_{max} (MeOH) nm: 296 (1.326); $[\alpha]_D$ −34.6°C (c 0.0052, MeOH); *c* log *p* = 4.11; FABMS: *m*/*z* 402 $[M+H]^+$; HRFABMS: calcd. for $C_{18}H_{19}ClF_2NO_3S$ $[M+H]^+$ 402.0744, found 402.0744.

*(S)-4-Chloro-N-(cyclopropylmethyl)-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)benzenesulfonamide (S-**13c**)*

According to the above method, *S*-**11** (0.11 g, 0.32 mmol) was converted to *S*-**13c** (60 mg, 47%) as pale yellow oil. TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08–0.17 (2H, m, cyclopropane-H), 0.43–0.48 (2H, m, cyclopropane-H), 0.80–0.85 (1H, m, cyclopropane-H), 2.11 (1H, br, OH), 2.90 (1H, dd, J = 7.2, 15.1 Hz, NCH_2), 3.20 (1H, dd, J = 6.6, 15.1 Hz, NCH_2), 4.07–4.15 (2H, m, CH_2OH), 5.05 (1H, t, J = 7.2 Hz, CHN), 6.69–6.75 (3H, m, Ar–H), 7.47 (2H, d, J = 8.7 Hz, Ar–H), 7.80 (2H, d, J = 8.7 Hz, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 4.88, 5.23, 11.49, 50.31, 61.26, 62.22, 103.87, 110.97, 126.11, 128.68, 131.25, 139.25, 162.96; UV λ_{max} (MeOH) nm: 296.2 (1.322); $[\alpha]_D$ +24.3°C (c 0.0074, MeOH); *c* log *p* = 4.11; FABMS: *m*/*z* 402 $[M+H]^+$; HRFABMS: calcd. for $C_{18}H_{19}ClF_2NO_3S$ $[M+H]^+$ 402.0744, found 402.0740.

*(R)-4-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-(isobutylcarbamoyl)benzenesulfon amide (R-**13d**)*

According to the method for the preparation of *R*-**12d**, *R*-**11** (95 mg, 0.27 mmol) was converted to *R*-**13d** (29 mg, 24%) as white powder. TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:2); m.p. 111–113°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (6H, d, J = 6.6 Hz, $CH(CH_3)_2$), 1.71 (1H, septet, J = 6.6 Hz, $CH(CH_3)_2$), 2.94 (2H, t, J = 6 Hz, CH_2), 4.07 (1H, dd, J = 4.2, 12 Hz, CHHOH), 4.20 (1H, dd, J = 7.8, 11.7 Hz, CHHOH), 4.57–4.63 (1H, m, CHN), 4.84 (1H, t, J = 5.4 Hz, OH), 6.37 (1H, d, J = 6.3 Hz, NH), 6.63–6.73 (3H, m, Ar–H), 7.36 (2H, d, J = 8.7 Hz, Ar–H), 7.65 (2H, d, J = 8.7 Hz, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.81, 28.60, 29.66, 48.60, 57.38, 66.42, 103.55, 110.04, 128.41, 129.07, 138.84, 139.57, 141.02, 156.41, 162.96; UV λ_{max} (MeOH) nm: 290.6 (0.155); $[\alpha]_D$ −28.3°C (c 0.006, MeOH); *c* log *p* = 3.93; FABMS: *m*/*z* 447 $[M+H]^+$; HRFABMS: calcd. for $C_{19}H_{22}ClF_2N_2O_4S$ $[M+H]^+$ 447.0959, found 447.0956.

*(S)-4-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-(isobutylcarbamoyl)benzenesulfonamide (S-**13d**)*

According to the above method, *S*-**11** (95 mg, 0.27 mmol) was transferred to *S*-**13d** (15 mg, 12%) as white powder. TLC, R_f 0.5 (EtOAc/*n*-hexane = 1:2); m.p. 110–111.5°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (6H, d, J = 6.6 Hz, $CH(CH_3)_2$), 1.71 (1H, m, $CH(CH_3)_2$), 2.95 (2H, t, J = 6.6 Hz, CH_2), 4.06 (1H, dd, J = 3.9, 12 Hz, CHHOH), 4.19 (1H, dd, J = 8.1, 11.7 Hz, CHHOH), 4.59 (1H, br, CHN), 4.78 (1H, br, OH), 6.24 (1H, br, NH), 6.64–6.73 (3H, m, Ar–H), 7.37 (2H, d, J = 8.7 Hz, Ar–H), 7.65 (2H, d, J = 8.7 Hz, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.81, 28.60, 29.66, 48.60, 57.38, 66.42, 103.55, 110.04, 128.41, 129.07, 138.84, 139.16, 140.92, 156.41, 162.96; UV λ_{max} (MeOH) nm: 290.6 (0.173); $[\alpha]_D$ +26.7°C (c 0.006, MeOH); *c* log *p* = 3.93; FABMS: *m*/*z* 447 $[M+H]^+$; HRFABMS: calcd. for $C_{19}H_{22}ClF_2N_2O_4S$ $[M+H]^+$ 447.0959, found 447.0959.

*(S)-Methyl-2-(3-(4-chlorophenylsulfonyl)-3-((R)-1-(3,5-difluorophenyl)-2-hydroxyethyl)-ureido)propanoate (R-**13e**)*

According to the above method, *R*-**11** (107 mg, 0.31 mmol) was converted to *R*-**13e** (66 mg, 45%) as white powder. TLC, R_f 0.3 (EtOAc/*n*-hexane = 1:2); mp 122.5–124.0°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (3H, d, J = 7.2 Hz, CH_3), 2.24 (1H, br, OH), 3.78 (3H, s, OCH_3), 4.11–4.31 (2H, m, CH_2OH), 4.47–4.62 (1H, m, $CHCH_3$), 5.41 (1H, d, J = 7.5 Hz, CHN), 6.13 (1H, d, J = 6.9 Hz, NH), 6.64–6.72 (3H, m, Ar–H), 7.37 (2H, d, J = 9 Hz, Ar–H), 7.66 (2H, d, J = 9 Hz, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.25, 36.78, 51.25, 57.40, 66.44, 103.55, 110.08, 128.43, 129.07, 139.16, 141.00, 156.41, 163.05, 173.22; UV λ_{max} (MeOH) nm (A): 289.8 (0.178); $[\alpha]_D$ −29.6°C (c 0.004, MeOH); *c* log *p* = 2.73; HRFABMS: calcd. for $C_{19}H_{20}ClF_2N_2O_6S$ $[M+H]^+$ 477.0701, found 477.0697.

*(S)-Methyl-2-(3-(4-chlorophenylsulfonyl)-3-((S)-1-(3,5-difluorophenyl)-2-hydroxyethyl)ureido)propanoate (S-**13e**)*

According to the above method, *S*-**11** (105 mg, 0.3 mmol) was transferred to *S*-**13e** (57 mg, 40%) as white powder. TLC, R_f 0.3 (EtOAc/*n*-hexane = 1:2); m.p. 122.0–123.5°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (3H, d, J = 7.2 Hz, CH_3), 2.62 (1H, br, OH), 3.77 (3H, s, OCH_3), 4.11–4.28 (2H, m, CH_2OH), 4.43–4.47 (2H, m, $CHCH_3$ and CHN), 6.05 (1H, d, J = 6.9 Hz, NH), 6.61–6.72 (3H, m, Ar–H), 7.34–7.39 (2H, m, Ar–H), 7.63–7.68 (2H, m, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.25, 36.78, 51.25, 57.40, 66.44, 103.55, 110.09, 128.43, 129.07, 138.85, 140.93, 156.41, 163.03, 173.22; UV λ_{max} (MeOH) nm: 289.8 (0.170); $[\alpha]_D$ +33.3°C (c 0.0057, MeOH); *c* log *p* = 2.73; FABMS: *m*/*z* 477 $[M+H]^+$; HRFABMS: calcd. for $C_{19}H_{20}ClF_2N_2O_6S$ $[M+H]^+$ 477.0701, found 477.0698.

*(R)-4-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-(prop-2-ynylcarbamoyl)benzenesulfonamide (R-**13f**)*

According to the above method, *R*-**11** (127 mg, 0.36 mmol) was converted to *R*-**13f** (53 mg, 69%) as pale yellow powder. TLC, R_f 0.35 (EtOAc/*n*-hexane = 1:2); m.p. 85.0–86.5°C; ^{1}H NMR (300 MHz,

$CDCl_3$) δ 2.28 (1H, t, $J = 2.1$ Hz, CCH), 3.91–3.93 (2H, m, NCH_2CCH), 4.10–4.22 (2H, m, CH_2OH), 4.59–4.61 (1H, m, CHN), 5.17 (1H, t, $J = 5.4$ Hz, OH), 6.37 (1H, d, $J = 6.3$ Hz, NH), 6.64–6.73 (3H, m, Ar–H), 7.37 (2H, d, $J = 8.7$ Hz, Ar–H), 7.65 (2H, d, $J = 8.7$ Hz, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.80, 56.87, 66.69, 71.97, 79.11, 103.55, 110.09, 128.46, 129.13, 138.60, 139.23, 140.76, 155.67, 162.86; UV λ_{max} (MeOH) nm: 288.0 (0.310); $[\alpha]_D$ −24°C (c 0.005, MeOH); *c* log *p* = 2.92; FABMS: *m/z* 429 $[M+H]^+$; HRFABMS: calcd. for $C_{18}H_{16}ClF_2N_2O_4S$ $[M+H]^+$ 429.0489, found 429.0485.

*(S)-4-Chloro-N-(1-(3,5-difluorophenyl)-2-hydroxyethyl)-N-(prop-2-ynylcarbamoyl)benzenesulfonamide (S-**13f**)*

According to the above method, *S*-**11** (127 mg, 0.36 mmol) was transferred to *S*-**13f** (83 mg, 54%) as yellow powder. TLC, R_f 0.35 (EtOAc/*n*-hexane = 1:2); m.p. 85.0–87.0°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.28 (1H, t, $J = 2.1$ Hz, CCH), 3.90–3.93 (2H, m, NCH_2CCH), 4.12 (1H, dd, $J = 3.9$, 11.7 Hz, CHHOH), 4.22 (1H, dd, $J = 7.8$, 11.7 Hz, CHHOH), 4.59–4.61 (1H, m, CHN), 5.17 (1H, t, $J = 5.4$ Hz, OH), 6.36 (1H, d, $J = 6.9$ Hz, NH), 6.64–6.73 (3H, m, Ar–H), 7.37 (2H, d, $J = 8.7$ Hz, Ar–H), 7.65 (2H, d, $J = 8.7$ Hz, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.80, 56.87, 66.69, 71.97, 79.11, 103.55, 110.06, 128.40, 129.08, 138.54, 139.24, 140.76, 155.67, 162.83; UV λ_{max} (MeOH) nm: 288.2 (0.301); $[\alpha]_D$ +22.2°C (c 0.0054, MeOH); *c* log *p* = 2.92; FABMS: *m/z* 429 $[M+H]^+$; HRFABMS: calcd. for $C_{18}H_{16}ClF_2N_2O_4S$ $[M+H]^+$ 429.0489, found 429.0485.

Pharmacological evaluation

Reagents

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

Cell-based γ-secretase assays

The production of T20 cells has been reported previously [22]. 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 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, 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 were referred to as onefold 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 [22]. N7 cells were plated onto 12-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 13200*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–polyacrylamide electrophoresis gels (SDS–PAGE) and analyzed by Western blotting using an anti-Notch (Val1744) polyclonal antibody.

Cell culture and cell lines

Human embryonic kidney cells (HEK293) were incubated in DMEM supplemented with 10% 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 [23]. Cells were incubated in a humidified incubator at 37°C in 5% CO_2.

Assay for extracellular signal-regulated kinase (ERK) activity

The stimulation of the ERKs was measured by using anti-phospho-ERK1/2 and anti-ERK1/2 antibodies (Cell Signaling Technology, Danvers, MA, USA). 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–PAGE, immunoblotted, and identified using anti-phospho-ERK1/2 or anti-ERK1/2 antibody.

Data analysis

The data presented are 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.

This work was supported by the National Science Council, Taiwan, of the Republic of China (NSC 98-2320-B-016-004-MY3 to M.-K. Hu and NSC 98-2320-B-001-014-MY2 to Y.-F. Liao). High-resolution mass spectra analyses performed by the National Science Council Regional

Instruments Center at National Chiao Tung University are also gratefully acknowledged.

The authors have declared no conflict of interest.

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