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Design, synthesis, and evaluation of Leu*Ala hydroxyethylene-based non-peptide β-secretase (BACE) inhibitors

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Abstract—With the aim of developing small molecular non-peptide β-secretase (BACE) inhibitors, Leu*Ala hydroxyethylene (HE) was investigated as a scaffold to design and synthesize a series of compounds. Taking advantage of efficient combinatorial synthesis approaches and molecular modeling, extensive structure–activity relationship (SAR) studies were carried out on the N- and C-terminal residues of the Leu*Ala HE scaffold. Isobutyl amine was found to be an optimal C-cap, and suitable hydroxylalkylamines at the 3-position and nitro or methyl(methylsulfonyl)amine at the 5-position of isophthalamide as the N-terminus could form additional hydrogen bonds with BACE active sites and help improve potency. Many new potent non-peptide BACE inhibitors were identified in this study. Among them, compounds **37** and **44** exhibited excellent enzyme-inhibiting potency, comparable to that of OM99-2, and obvious inhibitory effects in cell-based assay with low molecular weights (<600).

1. Introduction

Alzheimer's disease (AD) is the most common dementia in the elderly characterized by an impairment of intellectual capacity.[1] It is reported that over 12 million persons in the world are afflicted with the disease,[2] and as the average life expectancy increases, the number of AD sufferers is growing quickly, since the elderly become more susceptible to AD as they age. Moreover, AD is a debilitating disease with patients suffering for several years or even longer, the cost of treatment can be substantial. It involves a heavy social and financial burden for both society and families. Therefore, the development of effective drugs for the prevention and treatment of AD is of significant importance.

Nowadays acetylcholinesterase (AChE) inhibitors are major approved drugs for AD treatment. These drugs have been proved to show an obvious effect on reducing symptoms, but they cannot cure the disease.[3] In search of more effective AD drugs, extensive research has been carried out, including the study of AD pathogenesis and the identification of new therapeutic targets.[4]

Recently, a novel aspartyl protease, β-secretase (BACE), as a promising new target for AD, has received considerable attention.[5] BACE has been demonstrated to catalyze the initial rate-limiting step in the generation of β-amyloid peptide (Aβ),[6] a key event in the progression of AD.[7] Moreover, the further studies demonstrated that BACE gene knockout mice showed no Aβ production and appeared healthy, which offered powerful in vivo evidence for the opinion that BACE is an attractive AD target and inhibition of the protease can effectively reduce Aβ formation and, thereby, stop the progression of AD.[8] Therefore, great efforts have been made to design and synthesize BACE inhibitors. Since first discovered in 1999,[9] a large number of BACE inhibitors have been reported. However, the majority are peptide-based analogues,[10] which, if used as drug agents, will face formidable difficulties, since their peptidic

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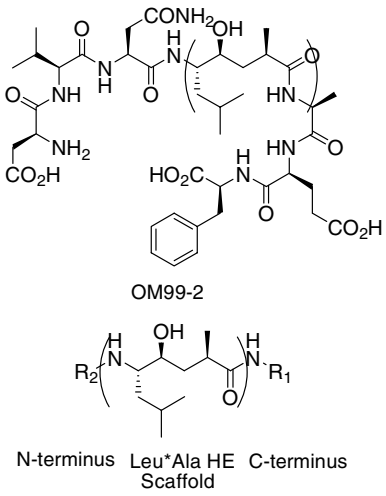


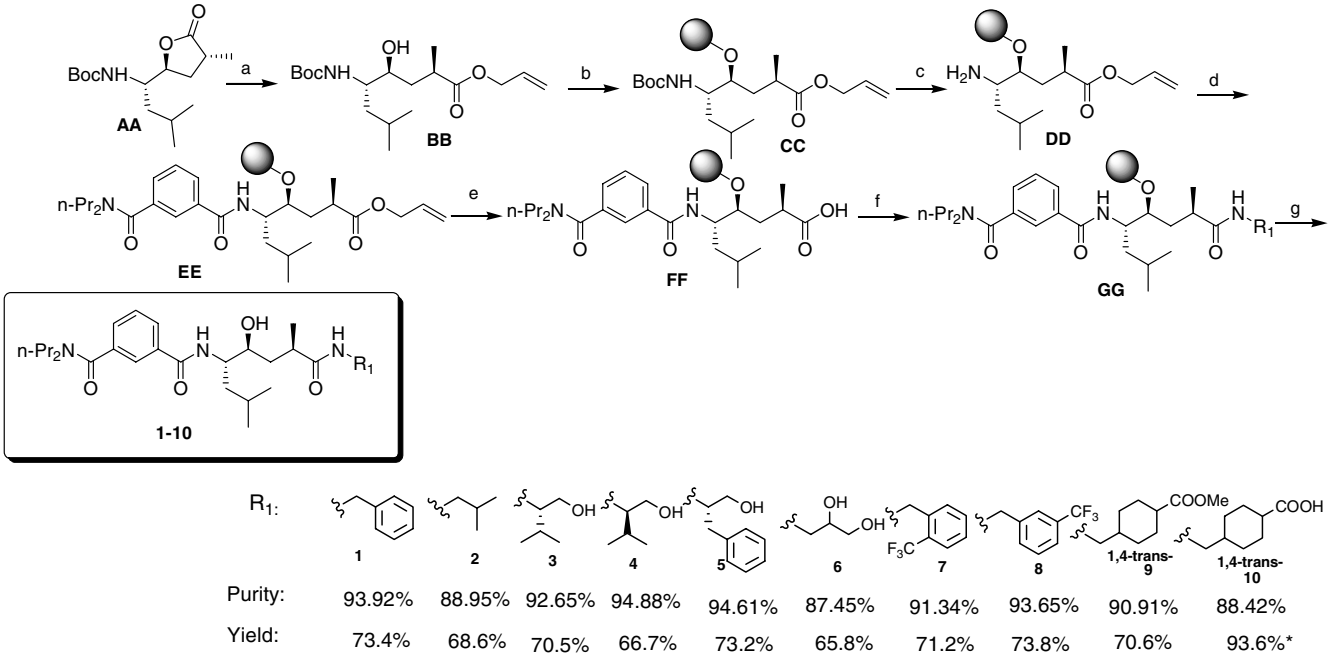
Figure 1. Structure of OM99-2 and design of non-peptide Leu*Ala HE-based inhibitors.

character is closely associated with vulnerability to degradative enzymes, rapid biliary clearance, and poor oral absorption.[11] The development of more therapeutically promising low molecular weight potent non-peptide BACE inhibitors is urgently needed.

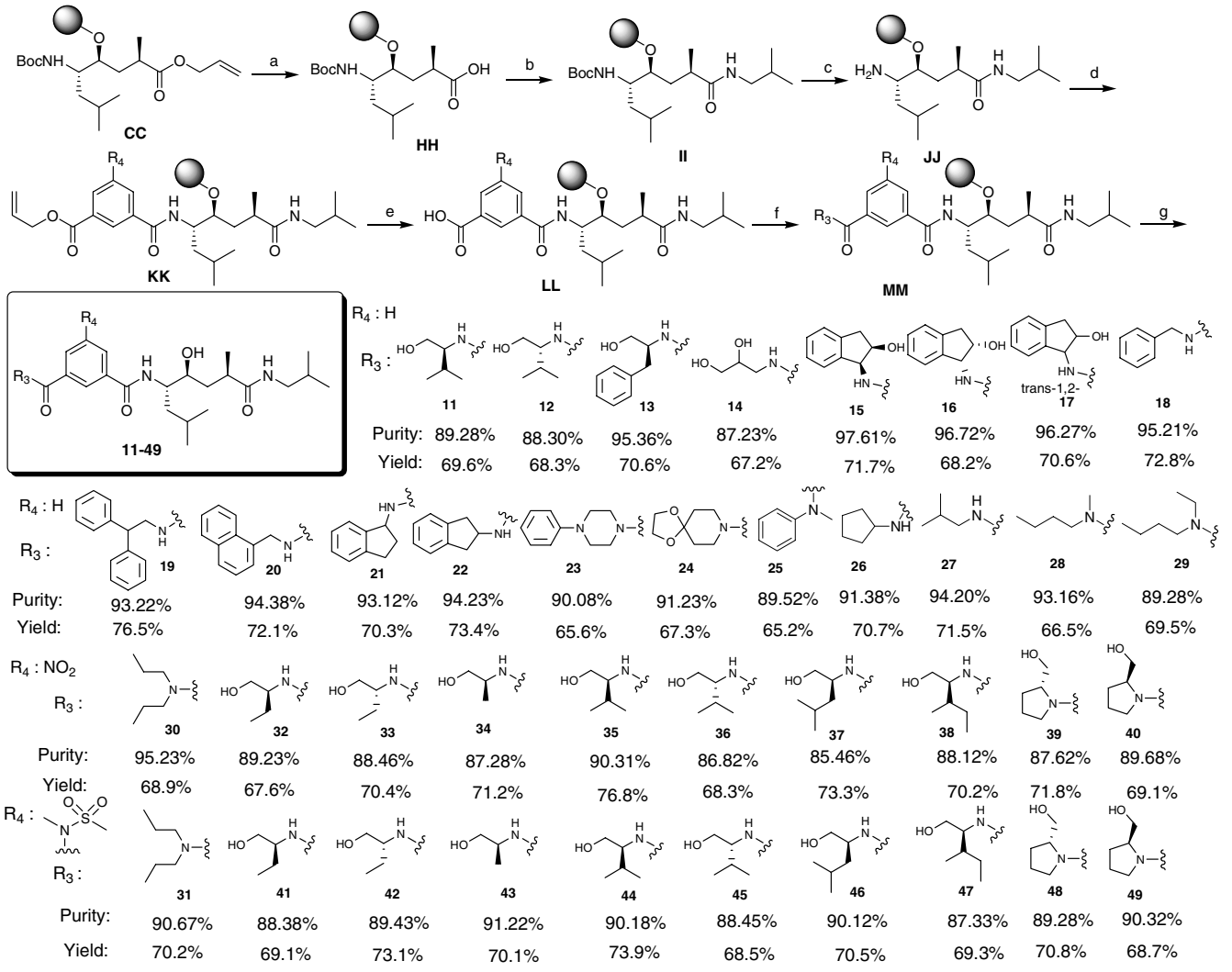
Incorporating non-hydrolyzable transition state-mimic isosteres into the molecules has been proved to be an effective method in designing potent non-peptide inhibitors of aspartyl proteases.[12] Scientists adopting this strategy have successfully developed several clinically approved drugs for AIDS.[13] In the search for potent non-peptide BACE inhibitors, we are very much interested in Leu*Ala hydroxyethylene (HE) isostere, since it has previously been used in peptide-based inhibitors and displayed excellent enzyme inhibitory activity.[10a,b,j] The crystal structure of BACE complexed with OM99-2 (Fig. 1), one of Leu*Ala HE-containing potent peptide inhibitors, has also been disclosed by Tang's group,[14] which could provide great assistance to the rational design of new Leu*Ala HE-based BACE inhibitors. Moreover, it can be synthesized from much cheaper and more readily available natural amino acid starting material than the reported (3,5-difluo-phe)*Ala HE.[15] Therefore, we attempted to use it as a scaffold to explore non-peptide BACE inhibitors (Fig. 1). Through efforts, a series of novel Leu*Ala HE-based non-peptide potent BACE inhibitors were identified. Among them, **37** and **44** showed excellent inhibiting activity on the enzyme, comparable to that of OM99-2, and exhibited obvious inhibitory effects in a cell-based assay with low molecular weights (<600). In this report, the design, synthesis, and biological evaluation of these inhibitors are described.

2. Chemistry

Owing to instability of the hydroxyl group of the HE isostere, the reported methods for preparation of Leu*Ala HE-bearing compounds in solution are usually troublesome and less efficient,[10b,j] especially in the introduction of chemical diverse ligands, which, however, are unavoidable in structure–activity relationship (SAR) studies. Referring to the literature,[16] we adopted a solid-phase strategy and used TentaGel S COOH resin as hydroxyl protection to develop mild and efficient combinatorial approaches for synthesis of these designed inhibitors **1**–**49** (Schemes 1 and 2).



Scheme 1. Syntheses of compounds **1**–**10**. Reagents and conditions: (a) i—LiOH, H_2O, CH_3OH, rt; ii—$NaHCO_3$, allyl bromide, DMF, 30 °C; (b) TentaGel S COOH resin, EDCI, DMAP, DMF/CH_2Cl_2, rt; (c) 30% TFA/CH_2Cl_2, rt; (d) *N*,*N*-dipropylisophthalamic acid, PyBOP, HOBt, DIPEA, rt; (e) $Pd(PPh_3)_4$, 1,3-dimethyl barbituric acid (DMBA), CH_2Cl_2, rt; (f) R_1NH_2, EDCI, HOBt, DIPEA, DMF, rt; (g) 10% Et_3N/CH_3OH, 55 °C. (*Yield of **10** was calculated on the basis of **9**.)



Scheme 2. Syntheses of compounds **11**–**49**. Reagents and conditions: (a) $Pd(PPh_3)_4$, DMBA, CH_2Cl_2, rt; (b) isobutylamine, EDCI, HOBt, DIPEA, DMF, rt; (c) 30% TFA/CH_2Cl_2, rt; (d) allyl mono-isophthalic ester, PyBOP, HOBt, DIPEA, rt; (e) $Pd(PPh_3)_4$, DMBA, CH_2Cl_2, rt; (f) R_3H, HBTU, HOBt, DIPEA, DMF, rt; (g) 10% Et_3N/CH_3OH, 55 °C.

The general synthesis of compounds **1**–**10** is outlined in Scheme 1. Hydrolysis of the known γ-lactone **AA**,[10a,b] employing lithium hydroxide as base, followed by reaction with allyl bromide, provided allyl ester HE analogue **BB**. The esterification of **BB** with TentaGel S COOH resin, using 1-(3-dimethylamino)propyl-3-ethylcarbodiimide hydrochloride (EDCI) and *N*,*N*-dimethylaminopyridine (DMAP), provided solid supported product **CC**. Subsequently, removal of Boc group at the N-terminus and coupling with *N*,*N*-dipropyl isophthalic acid in the presence of benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluoro phosphate (PyBOP), 1-hydroxy-benzotriazole (HOBt), and diisopropyl-ethylamine (DIPEA) in dimethylformamide (DMF) furnished **EE**. Finally, deprotection of allyl ester, employing $Pd(PPh_3)_4$, and reacting with various amines, using EDCI, HOBt as coupling agents, led to **GG**, which released the desired products **1**–**9** with 10% triethylamine in methanol. Compound **10** was prepared by saponification of **9** promoted with lithium hydroxide in a mixture of water and methanol. All crude compounds were obtained in >65% total yields and >85% purity without further purification (Scheme 1).

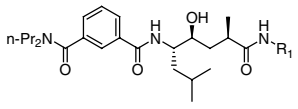
According to Scheme 2, compounds **11**–**49** were prepared under similar reaction conditions in Scheme 1. To introduce diverse N-terminal residues successfully, the reaction sequence was changed. The allyl-protecting group of C-terminus was removed first, and the resulting acid was coupled with isobutylamine in the presence of EDCI and HOBt to give **II**. After removing the *N*-Boc group of **II** in the presence of 30% CF_3COOH in CH_2Cl_2, the resulting amine was subsequently reacted with monoallyl isophthalic ester derivatives using PyBOP and HOBt as condensation agents to yield **KK**. The allyl group of **KK** was removed to form expected isophthalic acid derivatives, which were coupled with various amines to afford **MM**, respectively. The cleavage reaction was performed with 10% triethylamine in methanol, and the compounds **11**–**49** were obtained in >65% total yields and in >85% purity without further purification (Scheme 2).

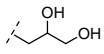
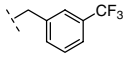
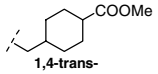
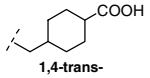
3. Results and discussion

To identify a lead for developing non-peptide BACE inhibitors, initially, an asparagine replacement found in HIV-1 inhibitor study,[17] *N*,*N*-dipropylisophthalamic acid, was employed as a temporary N-terminus of Leu*Ala HE in our study, since it has been used as N-terminal residue of previously reported other isostere-bearing BACE inhibitors,[15,18] and furthermore, in our previous study, Leu*Ala HE analogues displayed moderate enzyme inhibitory activity (IC_{50}: several μM) when it was incorporated as N-cap and optimal amino acid residues were at C-terminals.[19]

Combining *N*,*N*-dipropylisophthalamic acid as N-terminus, a series of compounds with various C-terminal residues R_1 were synthesized and investigated (Table 1). Some of R_1 were selected from C-caps of (3,5-difluo-phe)* Ala HE inhibitors.[15] From Table 1, it can be seen that compound **2** with isobutylamine and compound **9** with *trans*-*C*-(4-methoxycarbonyl-cyclohexyl)-methyl amine at the C-terminus demonstrated obvious enzyme-inhibiting activity. Surprisingly, compound **10** with *trans*-*C*-(4-carboxy-cyclohexyl)-methylamine, an excellent C-terminus of the (3,5-difluo-phe)*Ala HE inhibitors,[15] showed no obvious enzyme inhibition up to 30 μM. According to the results in Table 1, it is obvious that polar function group (like carboxylic acid and hydroxyl)-bearing ligands (**3**–**6** and **10**) and large group (like benzyl)-bearing ligands (**1**, **7**, and **8**) are not optimal C-terminal substituents. Since isobutyl is more simple and more lipophilic than *trans*-*C*-(4-methoxycarbonyl-cyclohexyl)-methyl, which may favor the penetration of the blood–brain barrier (BBB),[5] it was considered a more preferable C-terminal substitution. Furthermore, compound **2** showed moderate activity, comparable to those of the optimal peptide C-terminal analogues mentioned above. Therefore, no further elaborations were made and isobutyl was adopted as C-terminus in our study.

Table 1. Enzyme-inhibiting activities of **1**–**10** based on the C-terminal modifications of HE scaffold



Compound[a]	R_1	IC_{50}[b] (μM)
1		>30
2		1.972
3		>30
4		>30
5		>30
6		10.342
7		>30
8		>30
9	 1,4-trans-	2.047
10	 1,4-trans-	>30

[a] The compounds were further purified by preparative thin-layer chromatography and showed >98% purity determined on HPLC before biological evaluation.

[b] Values are means of three experiments.

Although it displayed only moderate activity, compound **2** was considered as a good lead to further develop potent BACE inhibitors since it possesses low molecular weight, has no amino acid residues, and easy to undergo structural modifications. To rationally make modifications on **2**, the modeled overlay structure of **2** and OM99-2 bound to BACE was constructed. As shown by Figure 2, compound **2** was accommodated in the active sites defined by the crystal structure of OM99-2 complexed with BACE. Leu*Ala HE scaffold of **2** showed to be nearly identical with that of OM99-

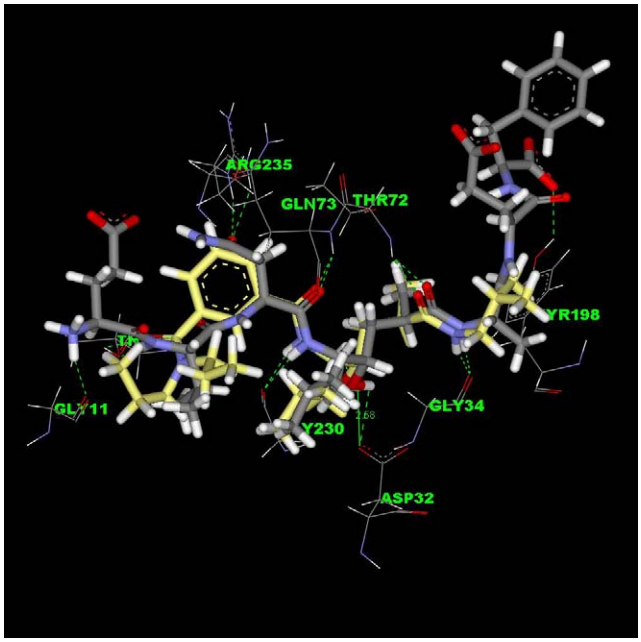
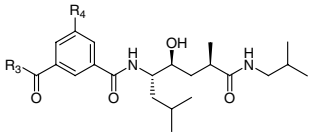


Figure 2. Modeled structure of inhibitor **2** (yellow) bound to BACE. OM99-2 (gray) is also shown for comparison. The model was constructed using Discovery Studio™ Modeling 1. 2 developed by Accelrys on the basis of the crystal structure of OM99-2 complexed BACE (PDB entry 1FKN).[14] Green dashes indicate hydrogen bonds. Red atoms represent oxygen, blue atoms represent nitrogen, and white atoms represent hydrogen.

Table 2. Enzyme-inhibiting activities of inhibitors **11**–**31** based on the modifications of the 3- or 5-position at the N-terminal isophthalamide of HE scaffold



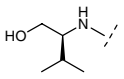
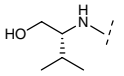
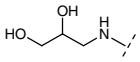
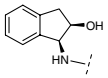
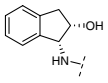
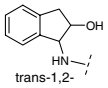
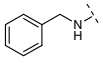
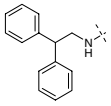
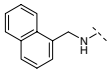
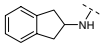
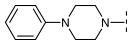
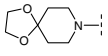
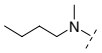
Compound[a]	R_4	R_3	IC_{50}[b] (μM)
11	H		0.359
12	H		8.561
13	H		15.672
14	H		15.010
15	H		14.721
16	H		4.174
17	H	 trans-1,2-	24.469
18	H		>30
19	H		>30
20	H		>30
21	H		17.339
22	H		>30
23	H		>30
24	H		>30
25	H		>30
26	H		1.146
27	H		3.267

Table 2 (*continued*)

Compound[a]	R_4	R_3	IC_{50}[b] (μM)
28	H		1.684
29	H		2.836
30	NO_2		0.673
31			0.404

[a] The compounds were further purified by preparative thin-layer chromatography and showed >98% purity determined on HPLC before biological evaluation.

[b] Values are means of three experiments.

2 and fits well in the S_1 and S_1' pockets. C-terminal isobutyl and the N-terminal isophthalamide occupied the S_2' and S_2 sites of BACE, respectively. *N*,*N*-Dipropylamine group in **2** was tolerated in the S_3 position. It was also observed that compared with OM99-2, the N-terminus of compound **2** lacked several important hydrogen-bond interactions with BACE, such as those between OM99-2 and Gly11 and Arg235 of BACE. We supposed that it may be the main reason for its relatively weak activity, and by introducing some groups into **2** that can form additional hydrogen bonds with BACE active sites, potent inhibitors would be achieved. To prove the hypothesis and identify more potent non-peptide inhibitors, we determined to make modifications on the N-terminus of **2**. Considering the space orientation of **2** in BACE active pocket and the convenience of chemical modifications, our attention was focused on investigating the SAR of the substituents at the 3- and 5-positions of N-terminal isophthalamide.

The effect of substituents at the 3-position (R_3) of N-terminal isophthalamide was explored using a series of diverse hydroxyl-bearing amines instead of *N*,*N*-dipropylamine (Table 2). These amines included monohydroxyalkylamines (**11**, **12**), dihydroxyalkyamine (**14**), hydroxylamine-bearing aromatic residue (**13**), and conformationally constrained hydroxylamines (**15**, **16**, and **17**). Moreover, the corresponding isomers were also synthesized for comparative purposes (**11**, **12** and **15**, **16**, and **17**). Encouragingly, compound **11**, with (*S*)-1-hydroxy-3-methylbutane-2-amine as R_3, demonstrated a greater than five times increase over **2**, although the majority of analogues still showed only moderate activity, even weaker than lead compound **2**. It was also observed that the stereo-configuration of R_3 effected the inhibiting activity. Compound **12** with (*R*)-1-hydroxy-3-methyl-butane-2-amine showed >20 less potent inhibition activity than **11** with the corresponding (*S*)-isomer at R_3. Compounds **15**, **16**, and **17** also reflected stereospecific preference. Preliminary molecular modeling on **11** and **12** revealed that the hydroxyl of (*S*)-1-hydroxy-3-methylbutan-2-amine at R_3 of compound **11** formed an additional strong hydrogen bond with Gly11 of BACE, the interaction also appearing in OM99-2 with BACE, while the corresponding isomer **12** did not have

this interaction due to a space orientation difference. The modeling results gave an insight into the causes of activity enhancement of **11** over **2** and the specific stereochemistry preference for BACE inhibitors. Meanwhile, we speculated that the relative low activity displayed by most of the prepared hydroxyl-bearing analogues was likely due to the unsuitable sizes of R_3, which could not effectively fit in the S_3 site, in other words, the aromatic rings in **13**, **15**, **16**, and **17** may be too large for the S_3 pocket. To further prove the assumption, another series of compounds with various non-hydroxy-containing R_3 was synthesized (**18**–**29**). From Table 2, it can be observed that most of the large R_3-bearing compounds (**18**–**25**) are inactive, while the compounds with simple alkylamines as R_3 (**26**–**29**) showed moderate activity, which clearly demonstrated that a suitable size of R_3 plays an important role in SAR. The results from **26** to **29** also suggested that the inhibitory activities of the compounds showed no obvious difference when R_3 was selected from primary amines or secondary amines. Furthermore, by comparing **18**–**29** with **11**–**17**, it could be concluded that the introduced hydroxyl group might help to increase activity. On the basis of the above observations, it was reasoned that hydroxyalkylamines, possessing suitable sizes and specific stereo-configuration, would be good replacements of *N*,*N*-dipropylamine for improving potency of **2**.

Parallel with the 3-position investigations, modifications of substituents at the 5-position of the N-terminal isophthalamide on **2** were performed. From space distance calculation for the modeled **2** complexed with BACE, it was speculated that nitro or methyl(methyl-sulfonyl)amine, introduced at 5-position, may form additional hydrogen bonds with BACE active sites. Compounds **30** and **31** were prepared. As shown by the bio-evaluation results, compared with **2**, compounds **30** and **31** clearly reflected an activity-improving trend.

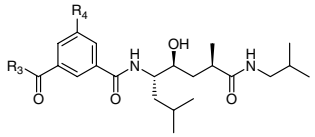
Based on the above results, a new series of inhibitors with N-terminal isophthalamide, bearing both hydroalkylamines at R_3 and nitrogen or methyl (methylsulfonyl)amine at R_4, were designed and synthesized (Table 3). Considering the availability and diversity of hydroalkylamines, R_3 was selected from amino acid-derived aminoalcohols, which can be readily purchased or prepared by lithium aluminum hydride ($LiAlH_4$) reduction of the corresponding amino acids in tetrahydrofuran (THF).[20] As expected, many such compounds showed potent activity. When R_4 is nitro and R_3 is, respectively, (*S*)-1-hydroxy-3-methylbutan-2-amine, (*S*)-1-hydroxy-4-methylpentan-2-amine or (2*S*)-1-hydroxy-3-methylpentan-2-amine, inhibitors **35**, **37**, and **38** showed potent inhibiting activity and, among them, compound **37** displayed even comparable activity to OM99-2 (IC_{50}: 0.061 vs 0.071 μM). When R_4 is methyl(methylsulfonyl) amine and R_3 is, respectively, (*S*)-1-hydroxybutan-2-amine, (*R*)-1-hydroxy-butan-2-amine, (*S*)-1-hydroxy-3-methylbutane-2-amine, (*S*)-1-hydroxy-4-methylpentan-2-amine, (2*S*)-1-hydroxy-3-methylpentan-2-amine or (*R*)-2-hydroxy-methyl pyrrolidin-1-yl, the inhibitors **41**, **42**, **44**, **46**, **47**, and **48** also showed submicromolar inhibiting activity and **44** with (*S*)-1-hydroxy-3-methylbutane-2-amine as R_3 displayed the greatest potency(IC_{50}: 0.143 μM). It is not surprising that the optimal R_3 substituents are not exactly the same when R_4 are hydrogen, nitrogen, and methyl (methylsulfonyl)amine because the sizes of R_4 are different, which may lead to subtle changes in binding orientation and cause small differences in R_3 size requirement suitable for S_3 pocket of BACE. Consistent with earlier observation, the stereo-configuration of R_3 is still an important factor impacting potency. In most cases, (*S*)-configuration of hydroxyl at R_3 is better than the corresponding (*R*)-isomers (**32** vs **33**, **35** vs **36**, **41** vs **42**, and **44** vs **45**) and, interestingly, **39** and **48** with conformationally constrained (*R*)-hydroxymethyl pyrrolidin-1-yl were more potent than **40** and **49** with the (*S*)-isomer at R_3. The modeled overlay structures of **37** and **2** were constructed and were displayed in Figure 3. From the image, four additional hydrogen bonds can be observed between **37** and Ser10, Gly11 and Arg235 of BACE which, undoubtedly, greatly helped the activity boosting of **37**.

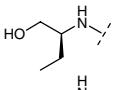
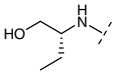
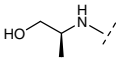
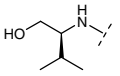
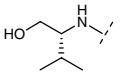
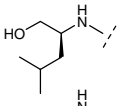
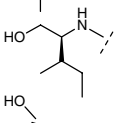
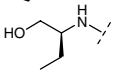
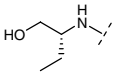
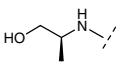
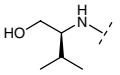
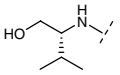
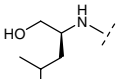
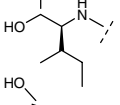
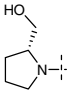
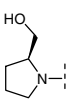
Finally, the most potent compounds **37** and **44** were selected to examine the effects on intracellular inhibition of endogenous BACE activity in CHO2B7 cells transfected with human βAPP695wt. Whole cell Aβ lowering (sandwich ELISA) was used to detect inhibitory effect of the compounds on BACE activity (Table 4). From the results, it can be seen that **37** and **44** displayed obvious inhibitory effects in cell-based assay on Aβ production and, especially, **44** displayed 56.15% inhibiting at 10^{-6} M and 47.11% at 10^{-7} M, respectively. Moreover, these potent inhibitors possessed no amino acids and low molecular weight (<600), which suggested their potential to develop novel AD drugs. Further in vivo evaluation and modifications are in progress.

4. Conclusion

As a goal to develop small molecular non-peptide BACE inhibitors, a series of Leu*Ala hydroxyethylene (HE)-based inhibitors were designed and synthesized. With the aid of efficient combinatorial synthesis approaches and molecular modeling, extensive SAR studies were carried out. It was found that isobutyl amine was an optimal C-cap of Leu*Ala HE scaffold, and the hydroxylalkylamino groups possessing suitable sizes and specific stereo-configuration, introduced at the 3-position of N-terminal isophthalamide, could form additional hydrogen bonds with BACE active sites and helped to improve potency. It was also observed that at the 5-position of isophthalamide introducing nitro or methyl(methyl-sulfonyl)amine contributed to improving their activities. Many new potent non-peptide BACE inhibitors were identified from this investigation. Among them, compounds **37** and **44** exhibited excellent enzyme-inhibiting potency, comparable to that of OM99-2, and obvious inhibitory effect in cell-based assay with low molecular weights (<600). Further studies are in progress and will be reported in due course.

Table 3. Enzyme-inhibiting activities of inhibitors **32–49** combining both hydroxyalkylamines at the 3-position and nitrogen or methyl (methylsulfonyl)amine at the 5-position of the N-terminal isophthalamide of HE scaffold



Compound[a]	R_4	R_3	IC_{50}[b] (μM)
32	NO_2		1.069
33	NO_2		4.404
34	NO_2		15.442
35	NO_2		0.372
36	NO_2		6.437
37	NO_2		0.061
38	NO_2		0.289
39	NO_2		1.016
40	NO_2		7.820
41			0.389
42			0.509
43			4.437
44			0.143
45			3.217
46			0.539
47			0.572
48			0.220
49			>30
OM99-2	/	/	0.071

[a] The compounds were further purified by preparative thin-layer chromatography and showed >98% purity determined on HPLC before biological evaluation.

[b] Values are means of three experiments.

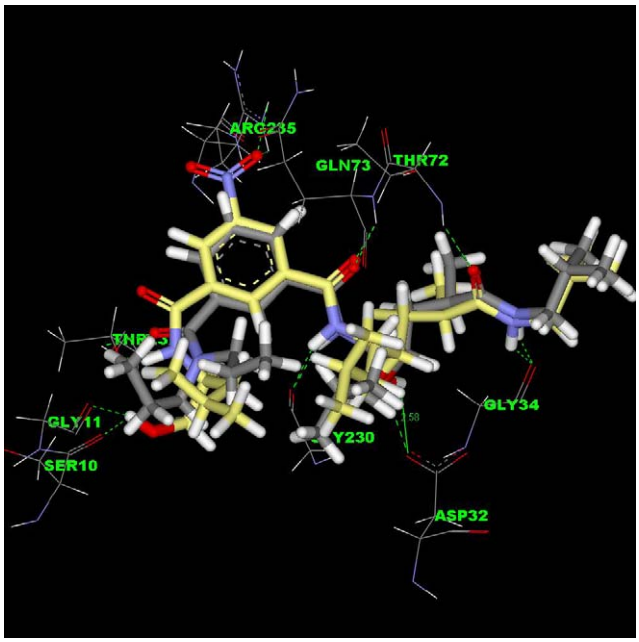


Figure 3. Overlay structures of inhibitors **2** (gray) and **37** (yellow) bound to BACE. The model was constructed using Discovery Studio™ Modeling 1. 2 developed by Accelrys on the basis of the crystal structure of OM99-2 complexed BACE (PDB entry 1FKN).[14] Green dashes indicate hydrogen bonds. Red atoms represent oxygen, blue atoms represent nitrogen, and white atoms represent hydrogen.

Table 4. Inhibitory effects in the cell-based assay of **37** and **44**

Compound	Inhibiting rate (10^{-6} M) (%)	Inhibiting rate (10^{-7} M) (%)
37	29.72	14.95
44	56.15	47.11

5. Experimental

The ^{1}H NMR (300 or 400 MHz) spectra were recorded on Varian Mercury-300 or 400 High Performance Digital FT-NMR with TMS as internal standard and the ^{13}C NMR (100 MHz) spectra were determined with Varian Mercury-400 High Performance Digital FT-NMR. The LC–MS were carried out on Thermo Finnigan LCQDECAxP and HRMS were performed with Finnigan MAT 95, EI: 70 eV, R: 10000. Purity was recorded on Gilson high-performance liquid chromatography (HPLC) (306 pump, UV/Vis-156 Detector, 215 liquid handle). The optical rotation value was determined with Perkin-Elmer-341(589 nm). TentaGel S COOH resin was purchased from Acros. All reagents are of analytical grade pure and used without further purification.

5.1. Preparation of *tert*-butyl(4*S*,5*S*,7*R*)-7-((allyloxy)-carbonyl)-5-hydroxy-2-methyloctan-4-yl-carbamate (BB)

To a solution of the known lactone **AA** (0.638 g, 2.239 mmol) in 5 mL THF was added 11.2 mL of 1N aqueous LiOH solution. The mixture was stirred at room temperature overnight. Then THF was evaporated and 5 mL water added. The aqueous solution was cooled to 0 °C and acidified with 25% citric acid solution to pH 3–4. The resulting suspension was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with water, saturated brine, dried with sodium sulfate, and concentrated to give the corresponding hydroxy acid as a white foam, which was used directly in the following reaction without any purification.

To the resulting hydroxy acid in 20 mL of anhydrous DMF were added sodium dicarboxylate (3.761 g, 44.78 mmol) and allyl bromide (1.95 mL, 22.39 mmol). The resulting mixture was stirred at 30 °C for 30 h and then extracted with ethyl acetate (3 × 60 mL). The combined organic layers were washed with water, saturated brine and dried with sodium sulfate. The oil residue was obtained after evaporating the solvent and purified on silica gel chromatography (effluent: ethyl acetate/petroleum ether ~ 1/7) to afford 0.543 g of **BB** (clear oil, yield: 70.7%). (It should be mentioned that **BB** is not very stable. It may convert to **AA** very slowly when stored at room temperature and the rate accelerates rapidly when exposed to some acids or amines, which we considered a transesterification reaction.) ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.20 (d, J = 9.2 Hz, 1H), 5.88 (m, 1H), 5.25 (ddd, J = 17.3, 3.4, 1.7 Hz, 1H), 5.17 (ddd, J = 10.4, 3.0, 1.5 Hz, 1H), 4.50 (m, 2H), 3.40–3.23 (m, 2H), 2.65 (m, 1H), 1.68 (m, 1H), 1.50 (m, 1H), 1.33 (s, 9H), 1.28–1.13 (m, 3H), 1.10 (d, J = 7.0, 3H), 0.85 (d, J = 6.6 Hz, 3H), 0.80 (d, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): 175.568, 155.621, 132.787, 117.412, 77.431, 69.881, 64.101, 52.434, 38.976, 38.767, 2× 36.758, 35.820, 28.206, 24.412, 23.292, 21.816, 18.005; LC–MS: *m*/*z* 366.4 $[M+Na]^+$; HRMS: calcd for $C_{18}H_{33}NO_5Na$ $[M+Na]^+$, 366.2244, found 366.2256; $[\alpha]_D^{20}$ − 37 (*c* 0.2100, CH_2Cl_2).

5.2. General method for preparation of compounds 1–10

To 1.5 g TentaGel S COOH resin (loading: ~0.25 mmol/g) in 30 mL mixture of CH_2Cl_2 and DMF (4:1) were added **BB** (0.643 g, 1.875 mmol), EDCI (0.359 g, 1.875 mmol), and DMAP (0.092 g, 0.75 mmol), and reacted overnight. The mixture was filtered and 0.508 g of **BB** was recovered from the filtrate by chromatography. The resin was washed three times each with DMF, 2-propanol, and CH_2Cl_2. The resulting resin **CC** was treated with 30 mL of 30% trifluoroacetate in CH_2Cl_2 for an hour and then quickly washed three times each with CH_2Cl_2, 10% triethylamine in CH_2Cl_2, CH_2Cl_2, and DMF. A mixture of 0.15 mol/L 3-(dipropylcarbamoyl)benzoic acid, 0.15 mol/L PyBOP, 0.15 mol/L HOBt, and 0.45 mol/L DIPEA in 30 mL DMF was added to **DD**, reacted overnight, and washed three times each with DMF, 2-propanol, and CH_2Cl_2. Subsequently, **EE** was mixed with $Pd(PPh_3)_4$ (0.087 g, 0.075 mmol) in 0.25 mol/L 1,3-dimethyl barbituric acid (DMBA) in 30 mL CH_2Cl_2 under Ar atmosphere for 6 h and washed three times each with 20% acetic acid in DMF, DMF, 2-propanol, and CH_2Cl_2. Then the resin was divided into 10 portions. Eight of 10 groups of resins each were treated with a mixture of amine (0.375 mmol), EDCI (0.107 g, 0.562 mmol), HOBt (0.076 g, 0.562 mmol),

and DIPEA (0.065 mL, 0.375 mmol) in anhydrous 3 mL DMF, and the residual two groups were combined and reacted with methyl *trans*-4-(aminomethyl)cyclohexane carboxylate hydrochloride (0.156 g, 0.75 mmol), EDCI (0.214 g, 1.125 mmol), HOBt (0.152 g, 1.125 mmol), and DIPEA (0.26 mL, 1.5 mmol) in anhydrous 6 mL DMF reacting for a day. The mixtures each were washed three times each with DMF, 2-isopropanol, and CH_2Cl_2. Each of **GG** reacted with 8 mL of 10% triethylamine in methanol at 55 °C for 16 h, the resin filtered and washed three times with a mixture of methanol and dichloromethane (1:1). The combined filtrates were concentrated to yield **1**–**9**. To a solution of **9** (10 mg, 0.017 mmol) in 1 mL THF was added 0.5 mL of 1 N aqueous LiOH solution and reacted overnight. Then the mixture was acidified to pH 3–4 with 25% citric acid aqueous solution and extracted with ethyl acetate. Compound **10** was obtained by evaporating the solvent. All crude compounds were obtained in >65% yields (based on theoretical loading value of resin) and showed >85% purity which were further purified by preparative thin-layer chromatography (TLC) and showed >98% purity determined on HPLC before biological evaluation.

5.2.1. N^1-((4*S*,5*S*,7*R*)-7-(Benzylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3,N^3-dipropylisophthalamide (1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (m, 2H), 7.42 (m, 2H), 7.30–7.20 (m, 5H), 6.92 (br, 1H), 6.85 (br, 1H), 4.40 (m, 2H), 4.10 (m, 1H), 3.73 (m, 1H), 3.40 (m, 2H), 3.20 (m, 2H), 2.80 (br, 1H), 2.60 (m, 1H), 1.70 (m, 4H), 1.60 (m, 2H), 1.40 (m, 2H), 1.28 (m, 1H), 1.20 (d, J = 6.9 Hz, 3H), 1.03 (m, 3H), 1.00 (d, J = 6.9 Hz, 3H), 0.95 (d, J = 7.0 Hz, 3H), 0.70 (m, 3H); LC–MS: m/z 524.3 $[M+H]^+$; $[\alpha]_D^{20}$ − 5 (c 0.1650, acetone).

5.2.2. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3,N^3-dipropylisophthalamide (2). ^{1}H NMR (300 MHz, CD_3OD): δ 7.94 (m, 1H), 7.84 (s, 1H), 7.55 (m, 2H), 4.20 (m, 1H), 3.60 (dt, J = 9.7, 3.2 Hz, 1H), 3.50 (t, J = 7.4 Hz, 2H), 3.24 (t, J = 7.8 Hz, 2H), 2.98 (d, J = 6.9 Hz, 2H), 2.65 (m, 1H), 1.80 (m, 1H), 1.70–1.60 (m, 2H), 1.60 (m, 2H), 1.40 (m, 1H), 1.30 (m, 4H), 1.15 (d, J = 6.8 Hz, 3H), 1.02 (t, J = 7.1 Hz, 3H), 0.96 (d, J = 6.2 Hz, 6H), 0.90 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.7 Hz, 3H), 0.75 (t, J = 7.3 Hz, 3H); LC–MS: m/z 490.5 $[M+H]^+$; HRMS: calcd for $C_{28}H_{47}N_3O_4$ 489.3567, found 489.3554; $[\alpha]_D^{20}$ − 28 (c 0.0250, acetone).

5.2.3. N^1-((4*S*,5*S*,7*R*)-7-((*S*)-1-Hydroxy-3-methylbutan-2-ylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3,N^3-dipropylisophthalamide (3). ^{1}H NMR (300 MHz, CD_3OD): δ 7.94 (m, 1H), 7.84 (s, 1H), 7.55 (m, 2H), 4.20 (m, 1H), 3.60 (m, 2H), 3.56 (m, 2H), 3.48 (t, J = 7.3 Hz, 2H), 3.20 (t, J = 7.3 Hz, 2H), 2.65 (m, 1H), 1.90 (m, 2H), 1.80 (m, 2H), 1.60 (m, 2H), 1.40 (m, 2H), 1.30 (m, 2H), 1.16 (d, J = 6.9 Hz, 3H), 1.02 (t, J = 7.4 Hz, 3H), 0.96 (d, J = 5.3 Hz, 6H), 0.90 (d, J = 6.7 Hz, 3H), 0.84 (d, J = 7.1 Hz, 3H), 0.75 (t, J = 7.2 Hz, 3H); LC–MS: m/z 520.5 $[M+H]^+$; $[\alpha]_D^{20}$ − 30.0 (c 0.2000, acetone).

5.2.4. N^1-((4*S*,5*S*,7*R*)-7-((*R*)-1-Hydroxy-3-methylbutan-2-ylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3,N^3-dipropylisophthalamide (4). ^{1}H NMR (300 MHz, CD_3OD): δ 7.94 (dt, J = 7.2, 1.8 Hz, 1H), 7.82 (s, 1H), 7.55 (m, 2H), 4.18 (dt, J = 10.0, 3.6 Hz, 1H), 3.70 (m, 1H), 3.60 (m, 2H), 3.50 (m, 1H), 3.46 (t, J = 6.6 Hz, 2H), 3.32 (t, J = 7.6 Hz, 2H), 2.70 (m, 1H), 1.82 (m, 2H), 1.63 (m, 4H), 1.40 (m, 2H), 1.30 (m, 2H), 1.18 (d, J = 7.0 Hz, 3H), 1.02 (t, J = 7.5 Hz, 3H), 0.96 (d, J = 6.3 Hz, 6H), 0.94 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 7.1 Hz, 3H), 0.75 (t, J = 7.4 Hz, 3H); LC–MS: m/z 520.4 $[M+H]^+$; $[\alpha]_D^{20}$ − 9 (c 0.1400, acetone).

5.2.5. N^1-((4*S*,5*S*,7*R*)-7-((*S*)-1-Hydroxy-3-phenylpropan-2-ylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3,N^3-dipropylisophthalamide (5). ^{1}H NMR (300 MHz, CD_3OD): δ 7.95 (m, 2H), 7.85 (s, 1H), 7.55 (m, 2H), 7.20 (m, 4H), 4.20 (m, 2H), 3.52 (d, J = 5.3 Hz, 2H), 3.48 (m, 3H), 3.20 (t, J = 7.5 Hz, 2H), 2.90 (m, 1H), 2.70 (m, 2H), 1.80 (m, 3H), 1.60 (m, 2H), 1.40 (m, 2H), 1.30 (m, 2H), 1.10 (d, J = 6.9 Hz, 3H), 1.00 (t, J = 7.3 Hz, 3H), 0.97 (d, J = 6.8 Hz, 3H), 0.95 (d, J = 6.5 Hz, 3H), 0.72 (t, J = 7.2 Hz, 3H); LC–MS: m/z 568.4 $[M+H]^+$; HRMS: calcd for $C_{33}H_{49}N_3O_5$ 567.3672, found 567.3677; $[\alpha]_D^{20}$ − 16.8 (c 0.4100, acetone).

5.2.6. N^1-((4*S*,5*S*,7*R*)-7-(2,3-Dihydroxypropylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3,N^3-dipropylisophthalamide (6). ^{1}H NMR (300 MHz, CD_3OD): δ 7.95 (m, 1H), 7.85 (s, 1H), 7.55 (m, 2H), 4.20 (m, 1H), 3.65 (m, 2H), 3.60 (m, 1H), 3.50 (m, 4H), 3.20 (m, 3H), 2.60 (m, 1H), 1.80 (m, 2H), 1.60 (m, 3H), 1.40 (m, 2H), 1.30 (m, 2H), 1.15 (d, J = 7.0 Hz, 3H), 1.02 (t, J = 7.0 Hz, 3H), 0.96 (d, J = 6.1 Hz, 6H), 0.75 (t, J = 7.2 Hz, 3H); LC–MS: m/z 508.3 $[M+H]^+$; $[\alpha]_D^{20}$ − 36 (c 0.1250, CH_2Cl_2).

5.2.7. N^1-((4*S*,5*S*,7*R*)-7-(2-(Trifluoromethyl)benzylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3,N^3-dipropylisophthalamide (7). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (m, 2H), 7.60 (d, J = 7.8 Hz, 1H), 7.45 (m, 4H), 7.35 (m, 1H), 6.75 (br, 1H), 6.70 (br, 1H), 4.55 (m, 2H), 4.15 (m, 1H), 3.70 (m, 1H), 3.45 (m, 2H), 3.10 (m, 2H), 2.90 (br, 1H), 2.60 (m, 1H), 1.80 (m, 4H), 1.60 (m, 2H), 1.40 (m, 1H), 1.30 (m, 2H), 1.18 (d, J = 7.1 Hz, 3H), 0.98 (t, J = 6.5 Hz, 3H), 0.94 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H), 0.73 (t, J = 6.1 Hz, 3H); LC–MS: m/z 592.3 $[M+H]^+$; $[\alpha]_D^{20}$ − 4.4 (c 0.4500, acetone).

5.2.8. N^1-((4*S*,5*S*,7*R*)-7-(3-(Trifluoromethyl)benzylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3,N^3-dipropylisophthalamide (8). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (s, 1H), 7.70 (m, 1H), 7.48 (m, 2H), 7.40 (m, 4H), 6.90 (m, 1H), 6.70 (d, J = 9.4 Hz, 1H), 4.40 (m, 2H), 4.15 (m, 1H), 3.70 (m, 1H), 3.40 (m, 2H), 3.10 (m, 2H), 2.65 (m, 1H), 1.70 (m, 4H), 1.60 (m, 2H), 1.40 (m, 1H), 1.30 (m, 2H), 1.18 (d, J = 6.8 Hz, 3H), 1.00 (t, J = 7.1 Hz, 3H), 0.98 (d, J = 6.2 Hz, 3H), 0.95 (d, J = 6.1 Hz, 3H), 0.70 (t, J = 7.1 Hz, 3H); LC–MS: m/z 592.2 $[M+H]^+$; $[\alpha]_D^{20}$ − 14 (c 0.2600, acetone).

5.2.9. (1,4-*trans*)-4-(((2*R*,4*S*,5*S*)-4-Hydroxy-5-(N^3,N^3-dipropylisophthalamido)-2,7-dimethyloctanoylamino)methyl)cyclohexanecarboxylic acid methyl ester (9). ^{1}H NMR (300 MHz, CD_3OD): δ 7.92 (m, 1H), 7.85 (s, 1H), 7.55 (m, 2H), 4.18 (m, 1H), 3.65 (s, 3H), 3.60 (dt, J = 9.6, 3.2 Hz, 1H), 3.50 (t, J = 7.6 Hz, 2H), 3.23 (t, J = 7.5 Hz, 2H), 3.00 (m, 2H), 2.60 (m, 1H), 2.20 (m, 1H), 1.95 (m, 2H), 1.80 (m, 5H), 1.60 (m, 4H), 1.40 (m, 5H), 1.30 (m, 2H), 1.13 (d, J = 6.9 Hz, 3H), 1.02 (t, J = 7.2 Hz, 3H), 0.96 (d, J = 5.2 Hz, 6H), 0.75 (t, J = 7.2 Hz, 3H); LC–MS: m/z 588.4 $[M+H]^+$; $[\alpha]_D^{20}$ − 9.1 (c 0.3200, acetone).

5.2.10. (1,4-*trans*)-4-(((2*R*,4*S*,5*S*)-4-Hydroxy-5-(N^3,N^3-dipropylisophthalamido)-2,7-dimethyloctanoylamino)methyl)cyclohexanecarboxylic acid (10). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (m, 2H), 7.43 (d, J = 4.7 Hz, 2H), 7.08 (d, J = 8.9 Hz, 1H), 6.70 (s, 1H), 4.15 (m, 1H), 3.70 (m, 1H), 3.45 (m, 2H), 3.20 (m, 2H), 3.00 (m, 2H), 2.60 (m, 1H), 2.10 (m, 1H), 1.90 (m, 2H), 1.70 (m, 5H), 1.60 (m, 4H), 1.40 (m, 4H), 1.30 (m, 3H), 1.18 (d, J = 6.7 Hz, 3H), 0.98 (m, 3H), 0.93 (d, J = 6.4 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.75 (t, J = 7.3 Hz, 3H); LC–MS: m/z 574.5 $[M+H]^+$; $[\alpha]_D^{20}$ − 10.9 (c 0.3300, acetone).

5.3. General method for preparation of compounds 11–17 and 30–31

To 1.35 g TentaGel S COOH resin (loading: ~0.25 mmol/g) in 27 mL of mixture of CH_2Cl_2 and DMF (4:1) were added **BB** (0.579 g, 1.688 mmol), EDCI (0.323 g, 1.688 mmol), and DMAP (0.082 g, 0.675 mmol), and reacted overnight. The mixture was filtered and 0.454 g **BB** was recovered from the filtrate by chromatography. The resin was washed three times each with DMF, 2-propanol, and CH_2Cl_2. **CC** was mixed with $Pd(PPh_3)_4$ (0.078 g, 0.0675 mmol) in 0.25 mol/L DMBA in 27 mL of CH_2Cl_2 under Ar atmosphere for 6 h and washed three times each with 20% acetic acid in DMF, DMF, 2-isopropanol, and CH_2Cl_2. Then the resulting resin was treated with a mixture of isobutylamine (0.34 mL, 3.38 mmol), EDCI (0.970 g, 5.063 mmol), HOBt (0.684 g, 5.063 mmol), and DIPEA (0.589 mL, 3.38 mmol) in anhydrous 27 mL DMF reacting for a day. The resin **II** was washed three times each with DMF, 2-propanol, and CH_2Cl_2, and treated with 27 mL of 30% trifluoroacetate in CH_2Cl_2 for an hour which was then quickly washed three times each with CH_2Cl_2, 10% triethylamine in CH_2Cl_2, CH_2Cl_2, and DMF. The resulting resin **JJ** was subdivided into nine portions. Two portions were each reacted with 3 mL mixture of 0.15 mol/L 3-((allyloxy)carbonyl)-5-nitrobenzoic acid or 3-((allyloxy)carbonyl)-5-(methyl(methylsulfonyl)amine)benzoic acid, 0.15 mol/L PyBOP, 0.15 mol/L HOBt, and 0.45 mol/L DIPEA in DMF, and reacted overnight. Meanwhile, the residual seven portions combined were treated with 21 mL mixture of 0.15 mol/L 3-((allyloxy)carbonyl)benzoic acid, 0.15 mol/L PyBOP, 0.15 mol/L HOBt, and 0.45 mol/L DIPEA in DMF, and reacted overnight. The resulting resins were washed three times each with DMF, 2-propanol, and CH_2Cl_2. Subsequently, **KK** was separately treated with $Pd(PPh_3)_4$ in 0.25 mol/L DMBA under Ar atmosphere for 6 h and washed three times each with 20% acetic acid in DMF, DMF, 2-propanol, and CH_2Cl_2. Then the resins were subdivided into nine groups. Each group was treated with a mixture of the corresponding amine (0.094 mmol), HBTU (0.036 g, 0.094 mmol), HOBt (0.019 g, 0.14 mmol), and DIPEA (0.033 mL, 0.188 mmol) in anhydrous 3 mL DMF. The mixtures each were washed three times each with DMF, 2-propanol, and CH_2Cl_2. Each of **MM** reacted with 8 mL of 10% triethylamine in methanol at 55 °C for 16 h, the resin filtered and washed three times with a mixture of methanol and dichloromethane (1:1). The combined filtrates were concentrated to yield **11**–**17**, **30**, and **31**. All crude compounds were obtained in >65% yields (based on theoretical loading value of resin) and showed >85% purity which were further purified by preparative TLC and showed >98% purity determined on HPLC before biological evaluation.

5.3.1. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*S*)-1-hydroxy-3-methylbutan-2-yl) isophthalamide (11). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.35 (t, J = 7.8 Hz, 1H), 7.25 (d, J = 9.1 Hz, 1H), 7.13 (d, J = 9.1 Hz, 1H), 6.40 (br, 1H), 4.20 (m, 1H), 4.00 (m, 1H), 3.85 (m, 1H), 3.78 (m, 1H), 3.70 (m, 1H), 3.05 (m, 1H), 2.82 (m, 1H), 2.60 (m, 1H), 1.95 (m, 1H), 1.78–1.50 (m, 2H), 1.40 (m, 1H), 1.35–1.20 (m, 3H), 1.20 (d, J = 7.2 Hz, 3H), 0.99 (d, J = 6.7 Hz, 3H), 0.97 (d, J = 6.8 Hz, 3H), 0.94 (d, J = 4.0 Hz, 3H), 0.92 (d, J = 4.1 Hz, 3H), 0.82 (d, J = 6.6 Hz, 3H), 0.80 (d, J = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 177.600, 2× 167.872, 2× 134.855, 130.374, 129.918, 128.557, 125.055, 71.052, 62.809, 57.627, 51.734, 46.898, 40.044, 37.676, 29.679, 29.624, 28.353, 25.029, 23.262, 22.032, 20.042, 20.006, 19.532, 19.163, 17.355; LC–MS: m/z 492.4 $[M+H]^+$; HRMS: calcd for $C_{27}H_{45}N_3O_5$ 491.3359, found 491.3359; $[\alpha]_D^{20}$ − 51.5 (c 0.5050, acetone).

5.3.2. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*R*)-1-hydroxy-3-methylbutan-2-yl)isophthalamide (12). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (s, 1H), 7.90 (m, 2H), 7.41 (t, J = 7.7 Hz, 1H), 7.06 (s, 1H), 7.03 (s, 1H), 6.40 (m, 1H), 4.16 (m, 1H), 3.90 (m, 1H), 3.80–3.70 (m, 3H), 3.05–2.90 (m, 2H), 2.60 (m, 1H), 1.95 (m, 1H), 1.78–1.52 (m, 1H), 1.40 (m, 1H), 1.38–1.23 (m, 4H), 1.15 (d, J = 7.0 Hz, 3H), 0.98 (d, J = 5.4 Hz, 3H), 0.96 (d, J = 5.5 Hz, 3H), 0.92 (d, J = 6.5 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.83 (d, J = 6.0 Hz, 3H), 0.81 (d, J = 6.0 Hz, 3H); LC–MS m/z 492.5 $[M+H]^+$; $[\alpha]_D^{20}$ − 22 (c 0.4500; acetone).

5.3.3. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*S*)-1-hydroxy-3-phenylprop an-2-yl)isophthalamide (13). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.80 (m, 1H), 7.70 (m, 1H), 7.36–7.18 (m, 6H), 7.05 (m, 1H), 6.40 (br, 1H), 4.40 (m, 1H), 4.18 (m, 1H), 3.80 (m, 1H), 3.72 (m, 1H), 3.65 (m, 1H), 2.98 (m, 3H), 2.85 (m, 1H), 2.60 (m, 1H), 1.60 (m, 1H), 1.40 (m, 1H), 1.38–1.20 (m, 4H), 1.20 (d, J = 6.9 Hz, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.92 (d,

J = 6.6 Hz, 3H), 0.81 (d, J = 6.8 Hz, 3H), 0.79 (d, J = 7.6 Hz, 3H); LC–MS: m/z 540.7 $[M+H]^+$; $[\alpha]_D^{20}$ − 65.3 (c 0.3400, acetone).

5.3.4. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-(2,3-dihydroxypropyl)isophthalamide (14). ^{1}H NMR (400 MHz, CD_3OD): δ 8.30 (m, 1H), 8.00 (m, 2H), 7.58 (m, 1H), 4.20 (m, 1H), 3.83 (m, 1H), 3.60 (m, 1H), 3.56 (m, 3H), 3.45 (m, 1H), 2.95 (d, J = 6.8 Hz, 2H), 2.65 (m, 1H), 1.75–1.60 (m, 1H), 1.40 (m, 2H), 1.28–1.20 (m, 3H), 1.15 (d, J = 7.1 Hz, 3H), 0.95 (d, J = 6.4 Hz, 6H), 0.87 (d, J = 6.0 Hz, 3H), 0.85 (d, J = 6.3 Hz, 3H); LC–MS: m/z 480.5 $[M+H]^+$; $[\alpha]_D^{20}$ − 22 (c 0.1850, acetone).

5.3.5. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((1*S*,2*R*)-2,3-dihydro-2-hydroxy-1*H*-inden-1-yl)isophthalamide (15). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 (s, 1H), 7.95 (d, J = 7.6 Hz, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.48 (m, 1H), 7.35–7.22 (m, 4H), 6.85 (d, J = 9.6 Hz, 1H), 6.12 (m, 1H), 5.63 (dd, J = 8.3, 5.1 Hz, 1H), 4.78 (dt, J = 5.0, 2.1 Hz, 1H), 4.15 (m, 1H), 3.73 (m, 1H), 3.25 (dd, J = 16.4, 5.2 Hz, 1H), 3.03–2.82 (m, 3H), 2.60 (m, 1H), 1.73–1.63 (m, 4H), 1.40 (m, 1H), 1.23 (m, 1H), 1.18 (d, J = 7.0 Hz, 3H), 0.94 (d, J = 5.4 Hz, 3H), 0.92 (d, J = 6.2 Hz, 3H), 0.81 (d, J = 6.6 Hz, 3H), 0.80 (d, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 177.377, 167.313, 167.090, 143.260, 140.614, 140.149, 134.634, 134.480, 2× 128.942, 128.245, 127.171, 2× 125.281, 124.725, 73.657, 70.397, 58.101, 46.808, 39.867, 37.927, 31.912, 29.685, 29.352, 24.967, 23.268, 22.089, 19.994, 19.357, 18.141, 17.335; LC–MS: m/z 538.4 $[M+H]^+$; HRMS: calcd for $C_{31}H_{43}N_3O_5$ 537.3203, found 537.3214; $[\alpha]_D^{20}$ − 15 (c 0.2600, acetone).

5.3.6. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((1*R*,2*S*)-2,3-dihydro-2-hydroxy-1*H*-inden-1-yl)isophthalamide (16). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (s, 1H), 8.02 (d, J = 7.9 Hz, 1H), 7.43 (d, J = 7.7 Hz, 1H), 7.49 (m, 1H), 7.33–7.22 (m, 4H), 6.85 (d, J = 9.3 Hz, 1H), 6.18 (m, 1H), 5.60 (m, 1H), 4.73 (m, 1H), 4.20 (m, 1H), 3.78 (m, 1H), 3.23 (m, 1H), 3.05–2.90 (m, 3H), 2.60 (m, 1H), 1.73–1.62 (m, 4H), 1.40 (m, 1H), 1.23 (m, 1H), 1.20 (d, J = 7.2 Hz, 3H), 0.94 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.4 Hz, 3H), 0.83 (d, J = 6.7 Hz, 3H), 0.81 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 177.432, 2× 167.281, 140.550, 140.163, 134.566, 134.284, 130.422, 130.340, 128.769, 128.145, 127.007, 125.718, 125.217, 124.484, 73.320, 70.861, 57.974, 51.821, 46.808, 40.974, 39.785, 38.392, 37.668, 29.662, 28.328, 24.935, 23.209, 22.080, 19.999, 17.348. LC–MS: m/z 538.3 $[M+H]^+$; HRMS: calcd for $C_{31}H_{43}N_3O_5$, 537.3203, found 537.3207; $[\alpha]_D^{20}$ − 17.2 (c 0.3950, acetone).

5.3.7. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-(1,2-*trans*-2,3-dihydro-2-hydroxy-1*H*-inden-1-yl)isophthalamide (17). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (d, J = 11.7 Hz, 1H), 8.05–7.92 (m, 2H), 7.52 (dd, J = 15.5, 7.8 Hz, 1H), 7.33–7.23 (m, 4H), 7.10 (m, 1H), 6.80 (t, J = 10.2 Hz, 1H), 6.00 (m, 1H), 5.38 (m, 1H), 4.55 (m, 1H), 4.16 (m, 1H), 3.78 (m, 1H), 3.35 (ddd, J = 15.7, 7.7, 1.7 Hz, 1H), 3.03–2.90 (m, 3H), 2.60 (m, 1H), 1.75–1.63 (m, 4H), 1.50 (m, 1H), 1.35 (m, 1H), 1.20 (d, J = 6.9 Hz, 3H), 0.95 (d, J = 6.4 Hz, 3H), 0.93 (d, J = 6.7 Hz, 3H), 0.83 (dd, J = 6.8, 1.0 Hz, 3H), 0.82 (d, J = 6.8 Hz, 3H); LC–MS: m/z 538.2 $[M+H]^+$; $[\alpha]_D^{20}$ − 24 (c 0.4800, acetone).

5.3.8. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-5-nitro-N^3,N^3-dipropylbenzene-1,3-diamide (30). ^{1}H NMR (400 MHz, CD_3OD): δ 8.78 (dd, J = 2.0, 1.8 Hz, 1H), 8.35 (dd, J = 1.8, 1.6 Hz, 1H), 8.22 (app s, 1H), 4.18 (m, 1H), 3.58 (dt, J = 10.0, 3.5 Hz, 1H), 3.50 (t, J = 7.4 Hz, 2H), 3.22 (t, J = 7.2 Hz, 2H), 2.97 (d, J = 6.3 Hz, 1H), 2.95 (d, J = 6.7 Hz, 1H), 2.64 (m, 1H), 1.80 (m, 1H), 1.62 (m, 3H), 1.55 (m, 3H), 1.45 (m, 1H), 1.30 (m, 2H), 1.13 (d, J = 7.0 Hz, 3H), 1.00 (t, J = 7.4 Hz, 3H), 0.95 (d, J = 6.5 Hz, 3H), 0.93 (d, J = 6.5 Hz, 3H), 0.88 (d, J = 6.9 Hz, 3H), 0.86 (d, J = 6.8 Hz, 3H), 0.76 (t, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 177.277, 168.342, 164.590, 148.068, 139.175, 136.893, 131.292, 123.855, 122.612, 69.932, 52.577, 50.983, 2× 46.908, 41.821, 38.141, 29.680, 28.442, 25.003, 23.186, 22.184, 21.835, 20.682, 2× 20.035, 17.243, 11.446, 11.059; LC–MS: m/z 557.6 $[M+Na]^+$; HRMS: calcd $C_{28}H_{46}N_4O_6$, 534.3418, found 534.3426; $[\alpha]_D^{20}$ − 8 (c 0.1800, acetone).

5.3.9. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-5-(methyl(methylsulfonyl)amine)-N^3,N^3-dipropylbenzene-1,3-diamide (31). ^{1}H NMR (300 MHz, CD_3OD): δ 7.92 (dd, J = 2.0, 1.8 Hz, 1H), 7.72 (dd, J = 1.8, 1.2 Hz, 1H), 7.55 (dd, J = 2.2, 1.2 Hz, 1H), 4.15 (m, 1H), 3.53 (dt, J = 9.9, 3.2 Hz, 1H), 3.45 (m, 2H), 3.35 (s, 3H), 3.20 (t, J = 7.7 Hz, 2H), 2.95–2.90 (m, 2H), 2.93 (s, 3H), 2.60 (m, 1H), 1.80 (m, 1H), 1.70 (m, 3H), 1.60 (m, 2H), 1.40 (m, 2H), 1.26 (m, 2H), 1.10 (d, J = 7.2 Hz, 3H), 0.96 (m, 3H), 0.92 (d, J = 7.1 Hz, 6H), 0.84 (d, J = 6.6 Hz, 3H), 0.83 (d, J = 6.7 Hz, 3H), 0.72 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 177.159, 169.672, 165.933, 141.861, 138.405, 136.260, 127.844, 124.784, 123.678, 70.133, 52.395, 50.965, 46.862, 41.789, 38.387, 38.000, 37.959, 35.782, 29.343, 28.442, 24.931, 23.227, 22.194, 21.925, 20.695, 20.081, 17.366, 14.115, 11.496, 11.005; LC–MS: m/z 597.4 $[M+H]^+$; HRMS: calcd for $C_{30}H_{52}N_4O_6S$ 596.3608, found 596.3583; $[\alpha]_D^{20}$ − 12 (c 0.2350, acetone).

5.4. General method for preparation of compounds 18–29

To 1.8 g TentaGel S COOH resin (loading: ~0.25 mmol/g) in 36 mL of mixture of CH_2Cl_2 and DMF (4:1) were added **BB** (0.772 g, 2.251 mmol), EDCI (0.431 g, 2.251 mmol), and DMAP (0.109 g, 0.9 mmol), and reacted overnight. The mixture was filtered and 0.608 g **BB** was recovered from the filtrate by chromatography. The resin was washed three times each with DMF, 2-propanol, and CH_2Cl_2. **CC** was mixed with $Pd(PPh_3)_4$ (0.104 g, 0.09 mmol) in 0.25 mol/L DMBA in 36 mL CH_2Cl_2 under Ar atmosphere for 6 h and washed three times each with 20% acetic acid in DMF, DMF, 2-isopropanol, and CH_2Cl_2. Then the resulting resin was treated with a mixture of isobutylamine (0.453 mL,

4.51 mmol), EDCI (1.293 g, 6.751 mmol), HOBt (0.912 g, 6.751 mmol), and DIPEA (0.785 mL, 4.51 mmol) in anhydrous 36 mL DMF reacting for a day. The resin **II** was washed three times each with DMF, 2-propanol, and CH_2Cl_2, and treated with 36 mL of 30% trifluoroacetate in CH_2Cl_2 for an hour which was then quickly washed three times each with CH_2Cl_2, 10% triethylamine in CH_2Cl_2, CH_2Cl_2, and DMF. The resulting resin **JJ** was treated with 36 mL mixture of 0.15 mol/L 3-((allyloxy)carbonyl)benzoic acid, 0.15 mol/L PyBOP, 0.15 mol/L HOBt, and 0.45 mol/L DIPEA in DMF and reacted overnight. The resulting resins were washed three times each with DMF, 2-propanol, and CH_2Cl_2. Subsequently, **KK** was treated with $Pd(PPh_3)_4$ in 0.25 mol/L DMBA under Ar atmosphere for 6 h and washed three times each with 20% acetic acid in DMF, DMF, 2-propanol, and CH_2Cl_2. Then the resins were subdivided into 12 groups. Each group was treated with a mixture of the corresponding amine (0.094 mmol), HBTU (0.036 g, 0.094 mmol), HOBt (0.019 g, 0.14 mmol), and DIPEA (0.033 mL, 0.188 mmol) in anhydrous 3 mL DMF. The mixtures each were washed three times each with DMF, 2-propanol, and CH_2Cl_2. Each of **MM** reacted with 8 mL of 10% triethylamine in methanol at 55 °C for 16 h, the resin filtered and washed three times with a mixture of methanol and dichloromethane (1:1). The combined filtrates were concentrated to yield **18**–**29**. All crude compounds were obtained in >65% yields (based on theoretical loading value of resin) and showed >85% purity which were further purified by preparative TLC and showed >98% purity determined on HPLC before biological evaluation.

5.4.1. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-benzylisophthalamide (18). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1 H), 7.95 (m, 2H), 7.45 (t, J = 7.70 Hz, 1H), 7.33–7.27 (m, 5H), 7.05 (t, J = 5.4 Hz, 1H), 6.80 (d, J = 9.5 Hz, 1H), 6.18 (br, 1H), 4.60 (m, 2H), 4.18 (m, 1H), 3.75 (m, 1H), 2.98 (m, 2H), 2.60 (m, 1H), 1.75–1.60 (m, 1H), 1.40 (m, 1H), 1.35–1.20 (m, 4H), 1.20 (d, J = 6.9 Hz, 3H), 0.93 (d, J = 6.5 Hz, 3H), 0.90 (d, J = 6.4 Hz, 3H), 0.82 (d, J = 5.1 Hz, 3H), 0.80 (d, J = 5.0 Hz, 3H); LC–MS: m/z 496.4 $[M+H]^+$; $[\alpha]_D^{20}$ − 27 (c 0.2000, acetone).

5.4.2. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-benzhydrylisophthalamide (19). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (s, 1H), 8.02 (d, J = 7.8 Hz, 1H), 7.92 (d, J = 7.9 Hz, 1H), 7.50 (t, J = 7.8 Hz, 1H), 7.38–7.23 (m, 10H), 7.10 (d, J = 7.7 Hz, 1H), 6.75 (d, J = 7.7 Hz, 1H), 6.43 (d, J = 7.8 Hz, 1H), 6.05 (br, 1H), 4.17 (m, 1H), 3.75 (m, 1H), 2.95 (m, 2H), 2.60 (m, 1H), 1.75–1.60 (m, 1H), 1.40 (m, 1H), 1.38–1.20 (m, 4H), 1.20 (d, J = 7.0 Hz, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.91 (d, J = 6.5 Hz, 3H), 0.83 (d, J = 4.5 Hz, 3H), 0.81 (d, J = 4.5 Hz, 3H); LC–MS: m/z 572.5 $[M+H]^+$; $[\alpha]_D^{20}$ − 25 (c 0.2500, acetone).

5.4.3. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((naphthalen-1-yl)methyl)isophthalamide (20). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H), 8.06 (d, J = 8.2 Hz, 1H), 7.90 (m, 3H), 7.80 (d, J = 7.8 Hz, 1H), 7.58–7.38 (m, 5H), 6.98 (m, 1H), 6.80 (d, J = 9.4 Hz, 1H), 6.15 (br, 1H), 5.05 (m, 2H), 4.15 (m, 1H), 3.70 (m, 1H), 2.90 (m, 2H), 2.58 (m, 1H), 1.75–1.55 (m, 2H), 1.40 (m, 1H), 1.40–1.18 (m, 3H), 1.18 (d, J = 7.0 Hz, 3H), 0.91 (d, J = 6.5 Hz, 3H), 0.88 (d, J = 6.5 Hz, 3H), 0.79 (d, J = 5.3 Hz, 3H), 0.76 (d, J = 5.3 Hz, 3H); LC–MS: m/z 546.5 $[M+H]^+$; $[\alpha]_D^{20}$ − 23.5 (c 0.6850, acetone).

5.4.4. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-(2,3-dihydro-1*H*-inden-1-yl)isophthalamide (21). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H), 7.98 (d, J = 7.6 Hz, 1H), 7.93 (d, J = 7.6 Hz, 1H), 7.49 (t, J = 7.7 Hz, 1H), 7.32 (d, J = 7.3 Hz, 1H), 7.28–7.19 (m, 3H), 6.80 (m, 2H), 6.10 (br, 1H), 5.65 (dd, J = 15.3, 7.7 Hz, 1H), 4.15 (m, 1H), 3.75 (m, 1H), 3.10–2.88 (m, 4H), 2.60 (m, 2H), 1.96 (m, 1H), 1.75–1.60 (m, 2H), 1.40 (m, 1H), 1.38–1.20 (m, 4H), 1.20 (m, 3H), 0.90 (m, 6H), 0.80 (m, 6H); LC–MS: m/z 522.4 $[M+H]^+$; $[\alpha]_D^{20}$ − 28 (c 0.2500, acetone).

5.4.5. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-(2,3-dihydro-1*H*-inden-2-yl)isophthalamide (22). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (s, 1H), 7.95 (d, J = 8.1 Hz, 1H), 7.91 (d, J = 7.5 Hz, 1H), 7.48 (t, J = 7.7 Hz, 1H), 7.21–7.16 (m, 4H), 6.93 (d, J = 7.1 Hz, 1H), 6.79 (d, J = 9.5 Hz, 1H), 6.10 (br, 1H), 4.90 (m, 1H), 4.20 (m, 1H), 3.75 (m, 1H), 3.40 (m, 2H), 3.02–2.90 (m, 4H), 2.60 (m, 1H), 1.73–1.60 (m, 1H), 1.40 (m, 1H), 1.40–1.20 (m, 4H), 1.20 (d, J = 7.1 Hz, 3H), 0.94 (d, J = 6.3 Hz, 3H), 0.91 (d, J = 6.5 Hz, 3H), 0.83 (d, J = 6.0 Hz, 3H), 0.81 (d, J = 6.4 Hz, 3H); LC–MS: m/z 522.5 $[M+H]^+$; $[\alpha]_D^{20}$ − 36 (c 0.1850, acetone).

5.4.6. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((4-phenylpiperazin)-1-yl)isophthalamide (23). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (m, 2H), 7.52 (m, 2H), 7.30 (m, 2H), 6.95 (m, 3H), 6.68 (d, J = 9.4 Hz, 1H), 6.03 (br, 1H), 4.16 (m, 1H), 3.98 (m, 2H), 3.78 (m, 1H), 3.60 (m, 2H), 3.30 (m, 2H), 3.13 (m, 2H), 3.00 (m, 2H), 2.60 (m, 1H), 1.78–1.63 (m, 1H), 1.63 (m, 1H), 1.38–1.20 (m, 4H), 1.20 (d, J = 6.6 Hz, 3H), 0.96 (d, J = 6.5 Hz, 3H), 0.93 (d, J = 6.4 Hz, 3H), 0.86 (d, J = 6.7 Hz, 3H), 0.85 (d, J = 6.6 Hz, 3H); LC–MS: m/z 551.3 $[M+H]^+$; $[\alpha]_D^{20}$ − 16 (c 0.2650, acetone).

5.4.7. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((1,4-dioxa)-8-aza-spiro[4.5]dec-8-yl)isophthalamide (24). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (m, 2H), 7.50 (m, 2H), 6.65 (d, J = 9.4 Hz, 1H), 6.10 (m, 1H), 4.15 (m, 1H), 3.98 (m, 4H), 3.85 (m, 2H), 3.76 (m, 1H), 3.45 (br, 2H), 3.10–2.95 (m, 2H), 2.60 (m, 1H), 1.80 (m, 2H), 1.58–1.20 (m, 3H), 1.40 (m, 1H), 1.40–1.20 (m, 4H), 1.19 (d, J = 6.9Hz, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.7 Hz, 3H), 0.85 (d, J = 6.7 Hz, 3H); LC–MS: m/z 532.4 $[M+H]^+$; $[\alpha]_D^{20}$ − 12.7 (c 0.3000, acetone).

5.4.8. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-methyl-N^3-phenylisophthalamide (25). ^{1}H NMR (300 MHz, CD_3OD): δ 7.90 (m, 1H), 7.75 (m, 3H), 7.40 (m, 1H), 7.25 (m, 3H), 7.15 (m, 3H), 4.10

(m, 1H), 3.53 (m, 1H), 3.48 (s, 3H), 2.96 (m, 2H), 2.62 (m, 1H), 1.60 (m, 4H), 1.40 (m, 2H), 1.12 (d, J = 6.8Hz, 3H), 0.94 (d, J = 6.4 Hz, 3H), 0.91 (d, J = 6.8 Hz, 3H), 0.88 (d, J = 6.4 Hz, 3H), 0.86 (d, J = 6.9 Hz, 3H); LC–MS: m/z 496.6 $[M+H]^+$; $[\alpha]_D^{20}$ − 18.1 (c 0.7450, acetone).

5.4.9. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-cyclopentylisophthalamide (26). ^{1}H NMR (300 MHz, CD_3OD): δ 8.25 (t, J = 1.6 Hz, 1H), 7.95 (dd, J = 7.6, 1.7 Hz, 2H), 7.85 (t, J = 5.8 Hz, 1H), 7.52 (t, J = 7.7 Hz, 1H), 4.35 (m, 1H), 4.20 (m, 1H), 3.58 (m, 1H), 2.95 (m, 2H), 2.60 (m, 1H), 2.00 (m, 2H), 1.80 (m, 3H), 1.60 (m, 6H), 1.40 (m, 3H), 1.14 (d, J = 6.7 Hz, 3H), 0.95 (d, J = 6.6 Hz, 6H), 0.86 (d, J = 6.6 Hz, 3H), 0.84 (d, J = 6.6 Hz, 3H); LC–MS: m/z 474.5 $[M+H]^+$; $[\alpha]_D^{20}$ − 41.5 (c 0.7350, acetone).

5.4.10. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-isobutylisophthalamide (27). ^{1}H NMR (300 MHz, CD_3OD): δ 8.28 (t, J = 1.7 Hz, 1H), 7.95 (dd, J = 7.8, 1.8 Hz, 2H), 7.85 (t, J = 5.6 Hz, 1H), 7.55 (t, J = 7.8 Hz, 1H), 4.20 (m, 1H), 3.55 (m, 1H), 3.22 (m, 2H), 2.95 (m, 2H), 2.60 (m, 1H), 1.90 (m, 2H), 1.60 (m, 3H), 1.40 (m, 2H), 1.13 (d, J = 6.8 Hz, 3H), 0.97 (d, J = 6.8 Hz, 6H), 0.95 (d, J = 6.0 Hz, 6H), 0.85 (d, J = 6.9 Hz, 3H), 0.84 (d, J = 6.4 Hz, 3H); LC–MS: m/z 462.5 $[M+H]^+$; $[\alpha]_D^{20}$ − 37.1 (c 0.9550, acetone).

5.4.11. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-methyl-N^3-butylisophthalamide (28). ^{1}H NMR (300 MHz, CD_3OD): δ 8.02 (m, 1H), 7.88 (m, 3H), 7.55 (d, J = 7.6 Hz, 2H), 4.18 (m, 1H), 3.51 (m, 2H), 3.23 (m, 1H), 3.08 (s, 1.5H), 2.95 (s, 1.5H), 2.94 (m, 2H), 2.60 (m, 1H), 1.70 (m, 6H), 1.40 (m, 2H), 1.13 (d, J = 7.2Hz, 3H), 1.01 (m, 3H), 0.94 (d, J = 6.3 Hz, 6H), 0.88 (d, J = 6.5 Hz, 3H), 0.86 (d, J = 6.5 Hz, 3H), 0.80 (m, 2H); LC–MS: m/z 476.5 $[M+H]^+$; $[\alpha]_D^{20}$ − 20.7 (c 0.9000, acetone).

5.4.12. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-ethyl-N^3-butylisophthalamide (29). ^{1}H NMR (300 MHz, CD_3OD): δ 8.03 (m, 1H), 7.90 (m, 2H), 7.83 (s, 1H), 7.55 (m, 2H), 4.20 (m, 1H),3.50 (m, 3H), 3.23 (m, 2H), 2.95 (m, 2H), 2.60 (m, 1H), 1.70 (m, 4H), 1.40 (m, 2H), 1.23 (m, 2H), 1.12 (d, J = 7.1 Hz, 3H), 1.10 (m, 3H), 1.02 (m, 3H), 0.94 (d, J = 6.5 Hz, 6H), 0.87 (d, J = 6.8 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.78 (m, 2H); LC–MS: m/z 490.5 $[M+H]^+$; $[\alpha]_D^{20}$ − 19.4 (c 0.7700, acetone).

5.5. General method for preparation of compounds 32–49

To 2.5 g TentaGel S COOH resin (loading: ~0.25 mmol/g) in 50 mL of mixture of CH_2Cl_2 and DMF (4:1) were added **BB** (1.072 g, 3.125 mmol), EDCI (0.598 g, 3.125 mmol), and DMAP (0.153 g, 1.25 mmol), and reacted overnight. The mixture was filtered and 0.840 g **BB** was recovered from the filtrate by chromatography. The resin was washed three times each with DMF, 2-propanol, and CH_2Cl_2. **CC** was mixed with $Pd(PPh_3)_4$ (0.145 g, 0.125 mmol) in 0.25 mol/L DMBA in 50 mL CH_2Cl_2 under Ar atmosphere for 6 h and washed three times each with 20% acetic acid in DMF, DMF, 2-isopropanol, and CH_2Cl_2. Then the resulting resin was treated with a mixture of isobutylamine (0.63 mL, 6.25 mmol), EDCI (1.797 g, 9.375 mmol), HOBt (1.267 g, 9.375 mmol), and DIPEA (1.092 mL, 6.25 mmol) in anhydrous 50 mL DMF reacting for a day. The resin **II** was washed three times each with DMF, 2-propanol, and CH_2Cl_2, and treated with 50 mL of 30% trifluoroacetate in CH_2Cl_2 for an hour which was then quickly washed three times each with CH_2Cl_2, 10% triethylamine in CH_2Cl_2, CH_2Cl_2, and DMF. The resulting resin **JJ** was subdivided into two portions. Each portion was reacted with 25 mL mixture of 0.15 mol/L 3-((allyloxy)carbonyl)-5-nitrobenzoic acid or 3-((allyloxy)carbonyl)-5-(methyl(methylsulfonyl)amine)benzoic acid, 0.15 mol/L PyBOP, 0.15 mol/L HOBt, and 0.45 mol/L DIPEA in DMF, and reacted overnight. The resulting resins were washed three times each with DMF, 2-propanol, and CH_2Cl_2. Subsequently, **KK** was separately treated with $Pd(PPh_3)_4$ in 0.25 mol/L DMBA under Ar atmosphere for 6 h and washed three times each with 20% acetic acid in DMF, DMF, 2-propanol, and CH_2Cl_2. Then the resins was subdivided into 18 groups. Each group was treated with a mixture of the corresponding amine (0.094 mmol), HBTU (0.036 g, 0.094 mmol), HOBt (0.019 g, 0.14 mmol), and DIPEA (0.033 mL, 0.188 mmol) in anhydrous 3 mL DMF. The mixtures each were washed three times each with DMF, 2-propanol, and CH_2Cl_2. **MM** reacted with 5 mL of 10% triethylamine in methanol at 55 °C for 18 h, the resin filtered and washed three times with a mixture of methanol and dichloromethane (1:1). The combined filtrate was concentrated to yield **32**–**49**. All crude compounds were obtained in >65% yield (based on theoretical loading value of resin) and showed >85% purity which were further purified by preparative TLC and showed >98% purity determined on HPLC before biological evaluation.

5.5.1. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*S*)-1-hydroxybutan-2-yl)-5-nitrobenzene-1,3-diamide (32). ^{1}H NMR (300 MHz, CD_3OD): δ 8.82 (dd, J = 2.4, 1.3 Hz, 1H), 8.79 (dd, J = 2.4, 1.9 Hz, 1H), 8.67 (dd, J = 1.9, 1.5 Hz, 1H), 4.20 (m, 1H), 4.05 (m, 1H), 3.65 (m, 2H), 3.55 (dt, J = 10.0, 3.1 Hz, 1H), 2.95 (m, 2H), 2.60 (m, 1H), 1.80 (m, 1H), 1.60 (m, 5H), 1.40–1.30 (m, 2H), 1.12 (d, J = 7.1 Hz, 3H), 0.98 (t, J = 7.6 Hz, 3H), 0.94 (d, J = 6.4 Hz, 6H), 0.85 (d, J = 6.9 Hz, 3H), 0.84 (d, J = 6.4 Hz, 3H); LC–MS: m/z 523.2 $[M+H]^+$; $[\alpha]_D^{20}$ − 40 (c 0.2900, acetone).

5.5.2. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*R*)-1-hydroxybutan-2-yl)-5-nitrobenzene-1,3-diamide (33). ^{1}H NMR (300 MHz, CD_3OD): δ 8.82 (dd, J = 2.0, 1.6 Hz, 1H), 8.79 (dd, J = 2.2, 1.2 Hz, 1H), 8.67 (dd, J = 1.8, 1.3 Hz, 1H), 4.20 (m, 1H), 4.05 (m, 1H), 3.63 (m, 2H), 3.55 (m, 1H), 2.95 (d, J = 6.9 Hz, 1H), 2.92 (d, J = 6.5 Hz, 1H), 2.60 (m, 1H), 1.80 (m, 1H), 1.60 (m, 3H), 1.40 (m, 2H), 1.20 (m, 2H), 1.12 (d, J = 7.0 Hz, 3H), 0.99 (t, J = 7.3 Hz, 3H), 0.93 (d, J = 6.1 Hz, 6H), 0.84 (d, J = 7.1 Hz, 3H), 0.83 (d, J = 6.7 Hz, 3H); LC–MS: m/z 523.3 $[M+H]^+$; $[\alpha]_D^{20}$ − 14 (c 0.1150, acetone).

5.5.3. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*S*)-1-hydroxypropan-2-yl)-5-nitrobenzene-1,3-diamide (34). ^{1}H NMR (300 MHz, CD_3OD): δ 8.83 (dd, J = 2.1, 1.8 Hz, 1H), 8.82 (dd, J = 2.1, 1.6 Hz, 1H), 8.69 (t, J = 1.6 Hz, 1H), 4.22 (m, 2H), 3.62 (d, J = 6.0 Hz, 2H), 3.58 (dt, J = 9.9, 3.0 Hz, 1H), 2.95 (d, J = 6.6 Hz, 2H), 2.62 (m, 1H), 1.80 (m, 1H), 1.60 (m, 2H), 1.40 (m, 1H), 1.30 (m, 2H), 1.27 (d, J = 6.8 Hz, 3H), 1.14 (d, J = 7.1 Hz, 3H), 0.96 (d, J = 6.5 Hz, 6H), 0.88 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.7 Hz, 3H); LC–MS: m/z 509.4 $[M+H]^+$; $[\alpha]_D^{20}$ − 29 (c 0.1400, acetone).

5.5.4. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*S*)-1-hydroxy-3-methylbutan-2-yl)-5-nitrobenzene-1,3-diamide (35). ^{1}H NMR (300 MHz, CD_3OD): δ 8.77 (s, 1H), 8.75 (s, 1H), 8.63 (s, 1H), 4.15 (m, 1H), 3.90 (m, 1H), 3.70 (m, 2H), 3.52 (m, 1H), 2.90 (d, J = 6.9 Hz, 2H), 2.60 (m, 1H), 1.82 (m, 2H), 1.60 (m, 1H), 1.40 (m, 2H), 1.30 (m, 2H), 1.08 (d, J = 6.9 Hz, 3H), 0.97 (d, J = 6.5 Hz, 3H), 0.94 (d, J = 7.0 Hz, 3H), 0.90 (d, J = 6.2 Hz, 6H), 0.82 (d, J = 6.2 Hz, 3H), 0.78 (d, J = 5.7 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): 176.963, 165.395, 165.190, 147.643, 136.112, 135.930, 130.920, 123.652, 123.556, 70.378, 60.992, 57.312, 52.635, 52.038, 45.726, 38.244, 36.823, 28.585, 28.229, 27.546, 24.108, 21.658, 20.096, 18.361, 17.933, 17.277, 16.999. LC–MS: m/z 537.5 $[M+H]^+$; HRMS: calcd for $C_{27}H_{44}N_4O_7$ 536.3210, found 536.3214; $[\alpha]_D^{20}$ − 47 (c 0.1800, acetone).

5.5.5. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*R*)-1-hydroxy-3-methylbutan-2-yl)-5-nitrobenzene-1,3-diamide (36). ^{1}H NMR (300 MHz, CD_3OD): δ 8.81 (dd, J = 2.0, 1.4 Hz, 1H), 8.79 (dd, J = 1.8, 1.4 Hz, 1H), 6.67 (t, J = 1.4 Hz, 1H), 4.19 (dt, J = 9.4, 3.4 Hz, 1H), 3.95 (m, 1H), 3.70 (m, 2H), 3.55 (dt, J = 9.8, 2.9 Hz, 1H), 2.93 (d, J = 6.9 Hz, 1H), 2.91 (d, J = 7.1 Hz, 1H), 2.61 (m, 1H), 1.95 (m, 2H), 1.60 (m, 2H), 1.40 (m, 2H), 1.30 (m, 2H), 1.12 (d, J = 7.0 Hz, 3H), 1.02 (d, J = 7.0 Hz, 3H), 0.98 (d, J = 7.1 Hz, 3H), 0.94 (d, J = 6.2 Hz, 6H), 0.85 (d, J = 6.4 Hz, 3H), 0.82 (d, J = 6.7 Hz, 3H). LC–MS: m/z 537.4 $[M+H]^+$; $[\alpha]_D^{20}$ − 20 (c 0.1350, acetone).

5.5.6. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*S*)-1-hydroxy-4-methylpentan-2-yl)-5-nitrobenzene-1,3-diamide (37). ^{1}H NMR (300 MHz, CD_3OD): δ 8.82 (dd, J = 2.2, 1.8 Hz, 1H), 8.80 (dd, J = 2.2, 1.7 Hz, 1H), 8.67 (t, J = 1.6 Hz, 1H), 4.20 (m, 2H), 3.62 (d, J = 5.4 Hz, 1H), 3.59 (d, J = 5.7 Hz, 1H), 3.56 (dt, J = 9.8, 3.0 Hz, 1H), 2.95 (d, J = 6.9 Hz, 1H), 2.93 (d, J = 6.8 Hz, 1H), 2.65 (m, 1H), 1.80 (m, 1H), 1.60 (m, 3H), 1.50 (m, 1H), 1.40 (m, 2H), 1.30 (m, 2H), 1.14 (d, J = 6.8 Hz, 3H), 0.98 (d, J = 6.4 Hz, 3H), 0.96 (d, J = 6.8 Hz, 3H), 0.94 (d, J = 7.5 Hz, 6H), 0.86 (d, J = 6.2 Hz, 3H), 0.84 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 177.660, 165.392, 165.032, 148.177, 136.801, 136.313, 130.329, 124.782, 124.627, 71.030, 65.059, 52.462, 50.754, 46.974, 40.303, 38.044, 37.534, 31.909, 29.678, 28.371, 24.937, 23.279, 23.011, 22.259, 21.986, 20.000, 17.700,14.107; LC–MS: m/z 551.4 $[M+H]^+$; HRMS: calcd for $C_{28}H_{46}N_4O_7$ 550.3366, found 550.3371; $[\alpha]_D^{20}$ − 42 (c 0.2350, methanol).

5.5.7. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((2*S*)-1-hydroxy-3-methylpen tan-2-yl)-5-nitrobenzene-1,3-diamide (38). ^{1}H NMR (300 MHz, CD_3OD): δ 8.80 (dd, J = 2.2, 1.5 Hz, 1H), 8.78 (dd, J = 2.4, 1.4 Hz, 1H), 8.65 (t, J = 1.4 Hz, 1H), 4.18 (dt, J = 10.2, 3.6 Hz, 1H), 4.00 (m, 1H), 3.70 (m, 2H), 3.55 (dt, J = 9.8, 3.1 Hz, 1H), 2.93 (d, J = 6.9 Hz, 1H), 2.90 (d, J = 6.9 Hz, 1H), 2.60 (m, 1H), 1.80 (m, 1H), 1.60 (m, 4H), 1.40 (m, 2H), 1.30 (m, 2H), 1.10 (d, J = 7.2 Hz, 3H), 0.97 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.3 Hz, 6H), 0.91 (t, J = 6.9 Hz, 3H), 0.83 (d, J = 6.4 Hz, 3H), 0.81 (d, J = 6.6 Hz, 3H); LC–MS: m/z 551.3 $[M+H]^+$; HRMS: calcd for $C_{28}H_{46}N_4O_7$, 550.3366, found 550.3377. $[\alpha]_D^{20}$ − 28 (c 0.1200, methanol).

5.5.8. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*R*)-2-(hydroxymethyl)pyrrolidin-1-yl)-5-nitrobenzene-1,3-diamide (39). ^{1}H NMR (300 MHz, CD_3OD): δ 8.75 (dd, J = 2.0, 1.7 Hz, 1H), 8.55 (s, 1H), 8.35 (s, 1H), 4.30 (m, 1H), 4.18 (dt, J = 10.5, 3.6 Hz, 1H), 3.84 (dd, J = 11.2, 5.0 Hz, 1H), 3.72 (dd, J = 11.1, 3.3Hz, 1H), 3.53 (m, 2H), 3.40 (m, 1H), 2.93 (d, J = 6.9 Hz, 2H), 2.63 (m, 1H), 2.00 (m, 2H), 1.80 (m, 2H), 1.60 (m, 2H), 1.40 (m, 2H), 1.30 (m, 2H), 1.10 (d, J = 6.9 Hz, 3H), 0.93 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 6.3 Hz, 3H), 0.86 (d, J = 6.8 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H); LC–MS: m/z 535.5 $[M+H]^+$; $[\alpha]_D^{20}$ + 28 (c 0.1200, acetone).

5.5.9. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*S*)-2-(hydroxymethyl)pyrro lidin-1-yl)-5-nitrobenzene-1,3-diamide (40). ^{1}H NMR (300 MHz, CD_3OD): δ 8.78 (s, 1H), 8.55 (s, 1H), 8.35 (s, 1H), 4.30 (m, 1H), 4.20 (m, 1H), 3.90 (dd, J = 11.1, 5.1 Hz, 1H), 3.75 (dd, J = 11.1, 3.4 Hz, 1H), 3.58 (m, 2H), 3.42 (m, 1H), 2.95 (m, 2H), 2.65 (m, 1H), 2.05 (m, 3H), 1.80 (m, 2H), 1.60 (m, 2H), 1.40 (m, 3H), 1.15 (d, J = 7.0 Hz, 3H), 0.95 (d, J = 5.9 Hz, 6H), 0.88 (d, J = 6.8 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H); LC–MS: m/z 535.4 $[M+H]^+$; $[\alpha]_D^{20}$ − 99.0 (c 0.6950, acetone).

5.5.10. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*S*)-1-hydroxybutan-2-yl)-5-(methyl(methylsulfonyl)amine)benzene-1,3-diamide (41). ^{1}H NMR (300 MHz, CD_3OD): δ 8.20 (t, J = 1.5 Hz, 1H), 8.02 (dd, J = 2.1, 1.5 Hz, 1H), 7.98 (dd, J = 2.0, 1.5 Hz, 1H), 4.20 (m, 1H), 4.00 (m, 1H), 3.62 (d, J = 6.6 Hz, 2H), 3.55 (dt, J = 10.0, 3.0 Hz, 1H), 3.35 (s, 3H), 2.95 (s, 3H), 2.92 (d, J = 6.4 Hz, 2H), 2.60 (m, 1H), 1.80 (m, 1H), 1.60 (m, 3H), 1.40 (m, 2H), 1.30 (m, 2H), 1.12(d, J = 6.9 Hz, 3H), 0.98 (t, J = 7.2 Hz, 3H), 0.94 (d, J = 6.8 Hz, 6H), 0.84 (d, J = 6.6 Hz, 3H), 0.82 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 177.565, 2× 166.352, 142.088, 135.940, 135.858, 128.016, 127.824, 123.698, 70.984, 64.203, 54.088, 52.503, 46.961, 40.553, 2× 37.939, 35.876, 29.678, 28.375, 24.964, 24.108, 23.270, 22.063, 2× 20.023, 17.714, 10.705; LC–MS: m/z 585.3 $[M+H]^+$; HRMS: calcd for $C_{28}H_{48}N_4O_7S$ 584.3244, found 584.3234; $[\alpha]_D^{20}$ − 46.1 (c 0.3800, CH_2Cl_2).

5.5.11. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*R*)-1-hydroxybutan-2-yl)-5-(methyl(methylsulfonyl)amine)benzene-1,3-diamide (42). ^{1}H NMR (300 MHz, CD_3OD): δ 8.22 (s, 1H), 8.00 (m, 2H), 4.20 (m, 1H), 4.00 (m, 1H), 3.60 (d, J = 5.8 Hz, 2H), 3.55 (m, 1H), 3.35 (s, 3H), 2.96 (s, 3H), 2.93 (d, J = 6.9 Hz, 2H), 2.60 (m, 1H), 1.80 (m, 1H), 1.60 (m, 3H), 1.40 (m, 2H), 1.20 (m, 2H), 1.12 (d, J = 6.9 Hz, 3H), 0.98 (t, J = 7.5 Hz, 3H), 0.92 (d, J = 6.5 Hz, 6H), 0.84 (d, J = 6.7 Hz, 3H), 0.82 (d, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 177.336, 166.184, 166.043, 142.135, 135.814, 135.723, 2× 128.154, 123.691, 71.012, 64.281, 54.185, 52.514, 46.876, 38.310, 38.000, 36.101, 29.676, 28.414, 24.935, 24.020, 23.250, 22.672, 22.048, 20.053, 17.722, 14.106, 10.740; LC–MS: *m*/*z* 585.4 (M+H). HRMS: calcd for $C_{28}H_{48}N_4O_7S$ 584.3244, found 584.3251; $[\alpha]_D^{20}$ − 13 (*c* 0.2800, CH_2Cl_2).

5.5.12. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*S*)-1-hydroxypropan-2-yl)-5-(methyl(methylsulfonyl)amine)benzene-1,3-diamide (43). ^{1}H NMR (300 MHz, CD_3OD): δ 8.21 (t, J = 1.6 Hz, 1H), 8.01 (dd, J = 2.1, 1.8 Hz, 1H), 7.90 (dd, J = 2.1, 1.5 Hz, 1H), 4.18 (m, 2H), 3.60 (m, 2H), 3.55 (dt, J = 9.4, 2.8 Hz, 1H), 3.35 (s, 3H), 2.95 (s, 3H), 2.92 (d, J = 6.9 Hz, 2H), 2.62 (m, 1H), 1.80 (m, 1H), 1.60 (m, 2H), 1.40 (m, 1H), 1.20 (m, 2H), 1.24 (d, J = 6.7 Hz, 3H), 1.12 (d, J = 7.0 Hz, 3H), 0.92 (d, J = 6.5 Hz, 6H), 0.84 (d, J = 6.6 Hz, 3H), 0.82 (d, J = 6.7 Hz, 3H); LC–MS: *m*/*z* 571.2 $[M+H]^+$; $[\alpha]_D^{20}$ − 26 (*c* 0.2100, acetone).

5.5.13. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*S*)-1-hydroxy-3-methylbutan-2-yl)-5-(methyl(methylsulfonyl)amine)benzene-1,3-diamide (44). ^{1}H NMR (300 MHz, CD_3OD): δ 8.20 (t, J = 1.6 Hz, 1H), 7.99 (m, 2H), 4.20 (dt, J = 10.2, 3.5 Hz, 1H), 3.90 (m, 1H), 3.70 (m, 2H), 3.52 (dt, J = 9.5, 3.0 Hz, 1H), 3.35 (s, 3H), 2.90 (s, 3H), 2.80 (d, J = 6.6 Hz, 2H), 2.60 (m, 1H), 1.90 (m, 1H), 1.60 (m, 2H), 1.40 (m, 2H), 1.23 (m, 2H), 1.10 (d, J = 6.2 Hz, 3H), 0.98 (d, J = 6.5 Hz, 3H), 0.96 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 6.4 Hz, 6H), 0.83 (d, J = 6.4 Hz, 3H), 0.81 (d, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 177.496, 2× 166.421, 141.971, 136.028, 135.950, 127.671, 127.571, 123.550, 70.925, 62.796, 57.951, 52.550, 46.880, 40.437, 37.964, 37.877, 35.805, 29.676, 29.361, 28.378, 24.958, 23.300, 22.039, 20.044, 20.012, 19.507, 19.320, 17.754; LC–MS: *m*/*z*599.2 $[M+H]^+$; HRMS: calcd for $C_{29}H_{52}N_4O_7S$ $[M+H]^+$ 599.3495, found 599.3478; $[\alpha]_D^{20}$ − 37.2 (*c* 0.3900, acetone).

5.5.14. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*R*)-1-hydroxy-3-methylbutan-2-yl)-5-(methyl(methylsulfonyl)amine)benzene-1,3-diamide (45). ^{1}H NMR (300 MHz, CD_3OD): δ 8.20 (t, J = 1.5 Hz, 1H), 7.99 (m, 2H), 4.15 (dt, J = 10.1, 3.7 Hz, 1H), 3.88 (m, 1H), 3.68 (m, 2H), 3.50 (dt, J = 10.0, 2.5 Hz, 1H), 3.34 (s, 3H), 2.93 (s, 3H), 2.90 (d, J = 7.4 Hz, 2H), 2.60 (m, 1H), 1.90 (m, 2H), 1.60 (m, 2H), 1.40 (m, 1H), 1.22 (m, 2H), 1.09 (d, J = 7.0 Hz, 3H), 0.98 (d, J = 6.9 Hz, 3H), 0.94 (d, J = 6.9 Hz, 3H), 0.90 (d, J = 6.4 Hz, 6H), 0.82 (d, J = 6.7 Hz, 3H), 0.78 (d, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 177.300, 166.302, 166.047, 142.098, 135.859, 130.914, 128.227, 127.926, 123.933, 70.921, 62.837, 57.978, 52.441, 46.716, 40.928, 38.009, 36.010, 30.933, 29.106, 28.396, 27.686, 24.921, 23.232, 22.672, 22.062, 20.035, 19.539, 19.357, 17.667; LC–MS: *m*/*z* 599.2 $[M+H]^+$; HRMS: calcd for $C_{29}H_{50}N_4O_7S$, 598.3400, found 598.3426; $[\alpha]_D^{20}$ − 10.3 (*c* 0.4850, acetone).

5.5.15. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*S*)-1-hydroxy-4-methylpentan-2-yl)-5-(methyl(methylsulfonyl)amine)benzene-1,3-diamide (46). ^{1}H NMR (300 MHz, CD_3OD): δ 8.22 (dd, J = 1.6, 1.8 Hz, 1H), 8.02 (dd, J = 2.0, 1.6 Hz, 1H), 8.00 (dd, J = 2.2, 1.8 Hz, 1H), 4.25 (m, 1H), 4.20 (m, 1H), 3.65–3.53 (m, 3H), 3.38 (s, 3H), 2.98 (s, 3H), 2.95 (d, J = 6.9 Hz, 1H), 2.93 (d, J = 6.7 Hz, 1H), 2.65 (m, 1H), 1.90 (m, 1H), 1.75 (m, 2H), 1.48 (m, 4H), 1.40–1.30 (m, 2H), 1.14 (d, J = 7.0 Hz, 3H), 0.97 (d, J = 5.8 Hz, 6H), 0.94 (d, J = 6.1 Hz, 6H), 0.86 (d, J = 6.7 Hz, 3H), 0.83 (d, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 177.152, 2× 166.143, 142.106, 135.967, 135.885, 127.911, 127.806, 123.762, 70.879, 65.350, 52.603, 50.704, 46.924, 40.731, 40.175, 38.021, 36.008, 31.905, 29.678, 28.421, 24.982, 23.311, 22.983, 22.674, 22.355, 22.086, 20.064, 17.732, 14.103. LC–MS: *m*/*z* 613.3 $[M+H]^+$; HRMS: calcd for $C_{30}H_{52}N_4O_7S$, 612.3557, found 612.3512; $[\alpha]_D^{20}$ − 31 (*c* 0.1500, acetone).

5.5.16. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((2*S*)-1-hydroxy-3-methylpentan-2-yl)-5-(methyl(methylsulfonyl)amine)benzene-1,3-diamide (47). ^{1}H NMR (300 MHz, CD_3OD): δ8.22 (t, J = 1.6 Hz, 1H), 8.02 (dd, J = 2.0, 1.7 Hz, 1H), 8.00 (d, J = 1.9, 1.7 Hz, 1H), 4.20 (dt, J = 10.3, 3.4 Hz, 1H), 3.98 (dt, J = 10.7, 3.6 Hz, 1H), 3.65 (m, 2H), 3.58 (dt, J = 10.1, 3.0 Hz, 1H), 3.40 (s, 3H), 3.00 (s, 3H), 2.95 (d, J = 7.0 Hz, 1H), 2.93 (d, J = 6.7 Hz, 1H), 2.62 (m, 1H), 1.90 (m, 1H), 1.80–1.58 (m, 4H), 1.40 (m, 2H), 1.30 (m, 2H), 1.12 (d, J = 7.0 Hz, 3H), 0.99 (d, J = 7.3 Hz, 3H), 0.95 (d, J = 6.4 Hz, 6H), 0.94 (m, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.83 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 177.446, 2× 166.343, 142.056, 2× 136.013, 2× 127.774, 123.753, 70.952, 62.682, 56.584, 52.576, 46.893, 40.617, 37.985, 35.940, 35.767, 29.669, 28.403, 25.866, 24.973, 23.297, 22.669, 22.081, 20.041, 17.782, 15.460, 14.094, 11.288. LC–MS: *m*/*z* 613.2 $[M+H]^+$; HRMS: calcd for $C_{30}H_{52}N_4O_7S$ 612.3556, found 612.3559; $[\alpha]_D^{20}$ − 54 (*c* 0.1400, acetone).

5.5.17. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*R*)-2-(hydroxymethyl)pyrrolidin-1-yl)-5-(methyl(methylsulfonyl)amine)benzene-1,3-diamide (48). ^{1}H NMR (300 MHz, CD_3OD): δ 7.92 (s, 1H), 7.85 (s, 1H), 7.70 (s, 1H), 4.22 (m, 1H), 4.13 (dt, J = 10.3, 3.5 Hz, 1H), 3.80 (dd, J = 10.8, 5.0 Hz, 1H), 3.70 (dd, J = 11.0, 3.8 Hz, 1H), 3.50 (m, 2H), 3.40 (m, 1H), 3.33 (s, 3H), 2.90 (s, 3H), 2.88 (d, J = 4.9 Hz, 2H), 2.60 (m, 1H), 2.00 (m, 3H), 1.80 (m, 2H), 1.60 (m, 2H), 1.40 (m, 2H), 1.35 (m, 1H), 1.08 (d, J = 7.0 Hz, 3H), 0.90 (d, J = 6.0 Hz, 6H), 0.83 (d, J = 6.4 Hz, 3H), 0.81 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 177.204, 169.945, 165.719, 141.757, 137.654, 136.051, 128.696, 125.476, 124.088, 70.383, 66.166, 61.626,

52.354, 51.248, 46.835, 41.634, 38.360, 37.955, 37.850, 35.673, 29.671, 28.432, 28.228, 25.040, 24.912, 23.227, 22.148, 20.053, 17.467; LC–MS: *m/z* 597.3 $[M+H]^+$; HRMS: calcd for $C_{29}H_{48}N_4O_7S$, 596.3243, found 596.3278; $[\alpha]_D^{20} + 31.3$ (*c* 0.3200, acetone).

5.5.18. N^1-((4*S*,5*S*,7*R*)-7-(Isobutylcarbamoyl)-5-hydroxy-2-methyloctan-4-yl)-N^3-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-5-(methyl(methylsulfonyl)amine)benzene-1,3-diamide (49). ^{1}H NMR (300 MHz, CD_3OD): δ 7.96 (t, *J* = 1.8 Hz, 1H), 7.90 (dd, *J* = 1.7, 1.5 Hz, 1H), 7.76 (dd, *J* = 1.9, 1.6 Hz, 1H), 4.30 (m, 1H), 4.18 (dt, *J* = 10.3, 3.5 Hz, 1H), 3.85 (dd, *J* = 11.0, 5.2 Hz, 1H), 3.75 (dd, *J* = 11.4, 3.5 Hz, 1H), 3.55 (dt, *J* = 10.0, 3.1 Hz, 2H), 3.42 (m, 1H), 3.38 (s, 3H), 2.95 (s, 3H), 2.94 (d, *J* = 6.4 Hz, 2H), 2.63 (m, 1H), 2.00 (m, 3H), 1.80 (m, 2H), 1.60 (m, 2H), 1.40 (m, 2H), 1.35 (m, 1H), 1.13 (d, *J* = 7.0 Hz, 3H), 0.94 (d, *J* = 6.1 Hz, 6H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 177.182, 169.968, 165.774, 141.801, 137.661, 136.127, 128.676, 125.520, 124.090, 70.383, 66.280, 61.634, 52.449, 51.246, 46.861, 41.628, 38.435, 37.884, 35.744, 30.926, 29.673, 28.444, 28.271, 25.046, 24.923, 23.238, 22.173, 20.082, 17.518. LC–MS: *m/z* 597.4 $[M+H]^+$; HRMS: calcd for $C_{29}H_{48}N_4O_7S$, 596.3244, found 596.3248; $[\alpha]_D^{20} - 65$ (*c* 0.2750, acetone).

5.6. Enzyme-based assay

The BACE activity was determined at room temperature by monitoring the hydrolysis of FRET sub-strate DABCYL-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-EDANS. In a typical 100 μL assay mixture containing 100 mM ammonium acetate, pH 4.0, 20 μM substrate, and 50 nM purified recombinant human BACE1/Fc, the enzyme activity was continuously monitored with excitation 355 nm/emission 460 nm filter set for 20 min and the initial rate of the hydrolysis was determined using the early linear region of the enzymatic reaction kinetic curve.

5.7. Cell-based assay

The reduction of Aβ production in cultured medium was examined to determine the effects of the compounds on BACE activity in CHO2B7 cells transfected with human βAPP695wt. CHO2B7 cells were cultured in Hams F-12 medium (Gibco) with 10% fetal bovine serum, penicillin/streptomycin and maintained in 200 μg/mL Zeocin (Invitrogen). A total of 1.5 mL of this cell suspension was seeded into a 6-well plate. In the meantime, the compounds were added to the cultures and incubated for 24 h at 37 °C and 5% CO_2. The conditioned media were removed from the culture wells and $A\beta_{1-40}$ peptide levels in the media were analyzed by sandwich ELISA assay (Biosource) after 24 h.

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