RESEARCH ARTICLE

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Design, synthesis, and bioactivities of tasiamide B derivatives as cathepsin D inhibitors

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Cathepsin D (Cath D) is overexpressed and hypersecreted by malignant tumors and involved in the progress of tumor invasion, proliferation, metastasis, and apoptosis. Cath D has been considered as a potential target to treat cancer. Our previous studies revealed that tasiamide B derivatives **TB-9** and **TB-11** exhibited high potent inhibition against Cath D and other aspartic proteases, but their molecular weights are still high, and the role of each residue is unknown yet. Based on this, two series of tasiamide B derivatives have been designed, synthesized, and evaluated for their inhibitory activity against Cath D/Cath E/BACE1. Enzymatic assays revealed that the target compound **1** with lower molecule weight showed good inhibitory activity against Cath D with IC_{50} of 3.29 nM and satisfactory selectivity over Cath E (72-fold) and BACE1 (295-fold), which could be a valuable template for the design of highly potent and selective Cath D inhibitors.

KEYWORDS

Ahppa, cathepsin D, selective inhibitor, tasiamide B derivatives

1 | INTRODUCTION

Cathepsin D (Cath D) is one of the aspartyl endoproteinase, involved in different physiological processes and related with numerous pathological conditions.[1-6] The main physiological functions of Cath D consist of metabolic degradation of intracellular proteins, activation, and degradation of polypeptide hormones and growth factors, activation of enzymatic precursors, processing of enzyme activators and inhibitors, brain antigen processing, and regulation of programmed cell death.[7-10] However, it is overexpressed and hypersecreted by malignant tumors, involved in the progress of tumor invasion, proliferation, metastasis, and apoptosis.[11-14] Cath D has been considered as a potential target in various types of diseases[15-20] and as an independent marker of prognostic in cancer, including breast and ovarian cancers.[12,13,19] Therefore, inhibition of Cath D has been considered as an attractive pathway for the development of novel anticancer drugs.

Over the past 20 years, a large number of nonpeptidic and peptidic Cath D inhibitors have been reported.[21-26] Some typical nonpeptidic Cath D inhibitors that discovered by computer-aided drug design or random screening have been documented previously.[27] On the other hand, most of the potent peptidic inhibitors contained a statine unit (γ-amino-β-hydroxy acid) or a statine-like unit (4-amino-3-hydroxy-5-phenylpentanoic acid, Ahppa), which formed tetrahedron transition-state with two aspartic acids in the catalytic site. Pepstatin A (Figure 1) is a typical peptidic inhibitor bearing a statine unit with IC_{50}s at subnanomolar level for Cath D and other aspartic proteases.[28-32] Tasiamide B (Figure 1), a linear peptide isolated from the marine cyanobacteria *Symploca* sp.,[33] was proved as a good template for the development of aspartic proteases inhibitors.[32,34] Our research group finished the total synthesis and stereochemical reassignment of tasiamide B and then prepared series of its derivatives.[27,32,35] **TB-9** and **TB-11**, two respective compounds among them, showed highly potent inhibitory activity against Cath D. As part of the ongoing research, herein, we would like to report the design, synthesis, and bioactivities of two novel series of Cath D inhibitors based on **TB-9** and **TB-11** (Figure 1).
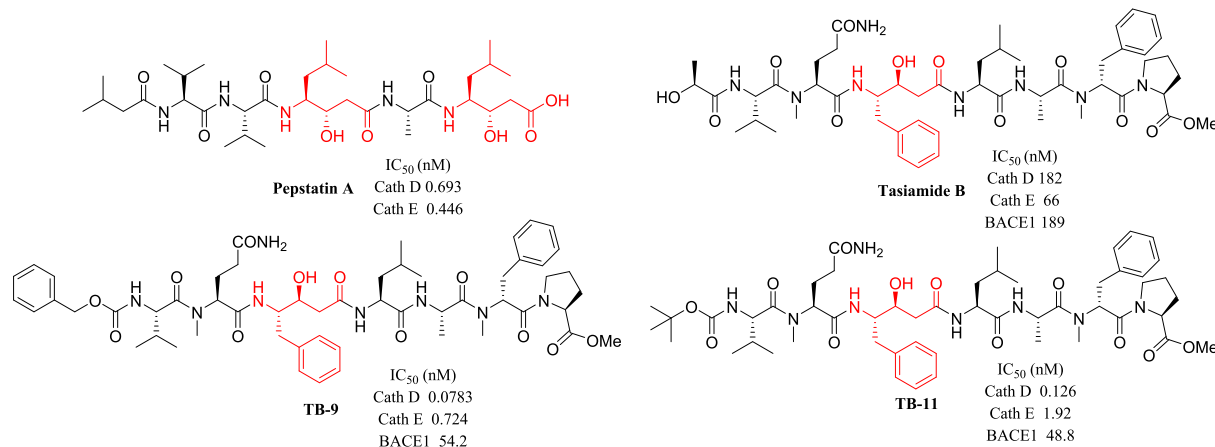
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FIGURE 1 The structures of pepstatin A, tasiamide B, **TB-9**, and **TB-11**

2 | RESULTS AND DISCUSSION

2.1 | Compounds design

In our previous studies,[32] the lactic acid of tasiamide B was truncated, a Cbz and Boc group were introduced to the N-terminus yielding **TB-9** and **TB-11**, respectively. The IC$_{50}$s of **TB-9** were 0.0783, 0.724, and 54.2 nM against Cath D, Cath E, and BACE1. For **TB-11**, the IC$_{50}$s against these three aspartic proteases were 0.126, 1.92, and 48.8 nM, respectively. Although these two compounds exhibited highly potent inhibition against Cath D, their molecular weights are

still high, and the role of each residue is unknown yet. Based on this, the amino acid units of **TB-9** and **TB-11** were truncated at the C-terminus in sequence to design two series derivatives (Figure 2), in which Val-Me-Gln-Ahppa fragment was retained while other residues were truncated one-by-one from the C-terminus.

2.2 | Chemical synthesis

These newly designed compounds were prepared by standard solution-phase peptide synthesis procedures (Schemes 1 and 2). Compounds **1** to **4** and **8a** were prepared as previously
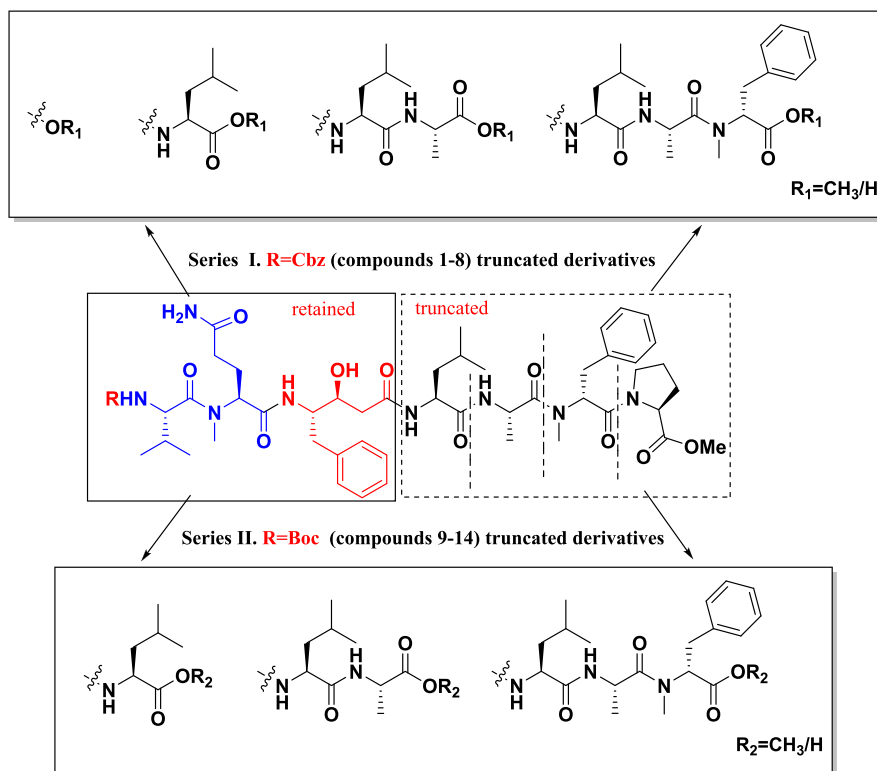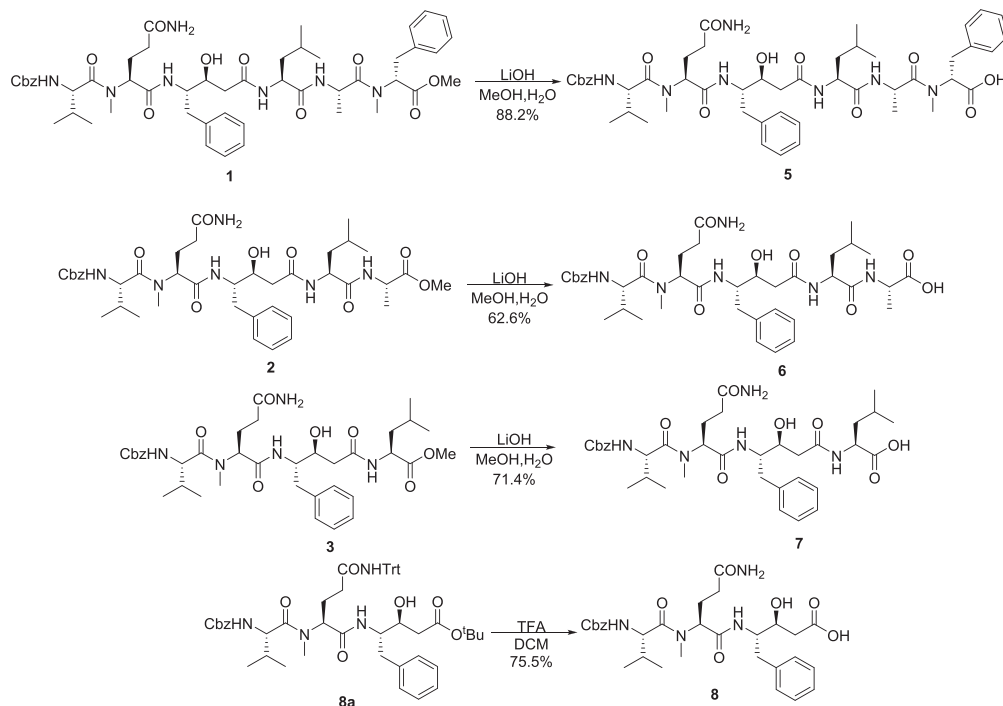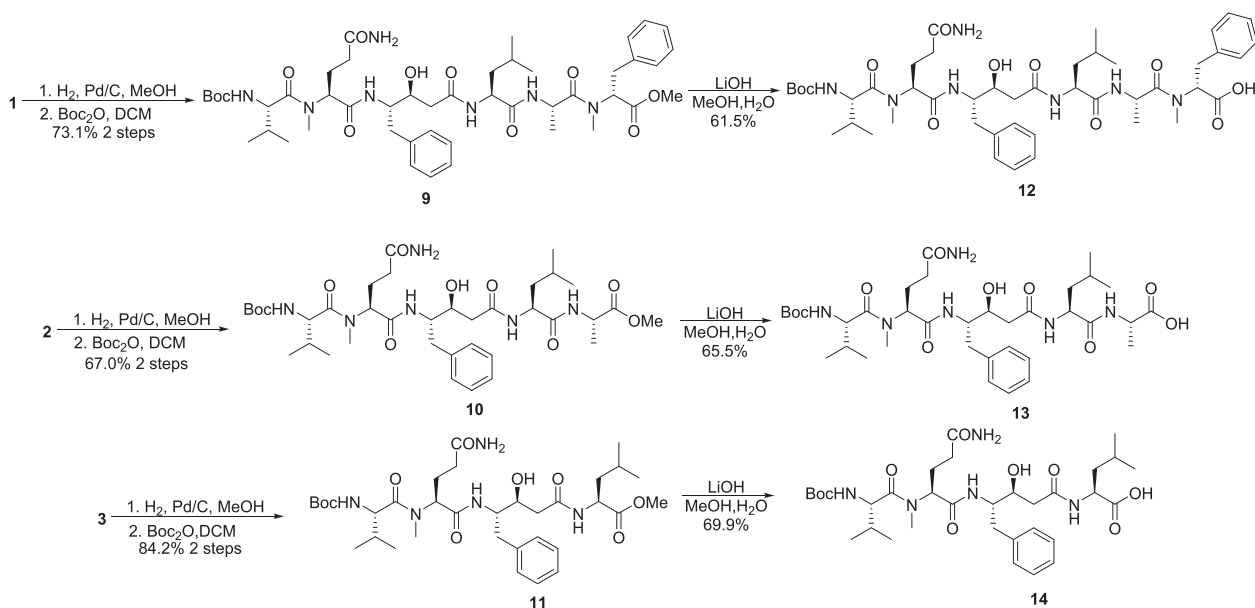

FIGURE 2 The structures of two series tasiamide B derivatives

SCHEME 1 Synthesis of series I compounds

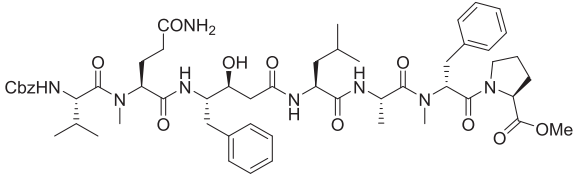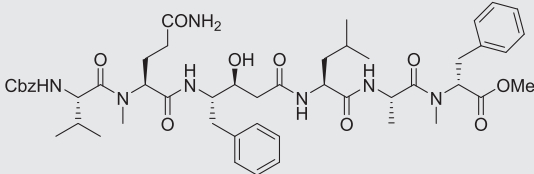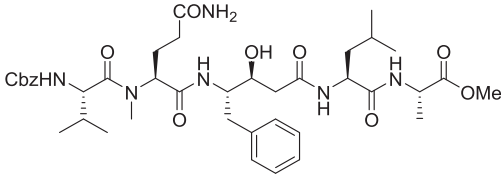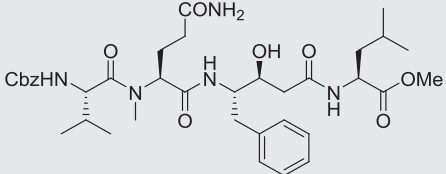**SCHEME 2** Synthesis of series II compounds
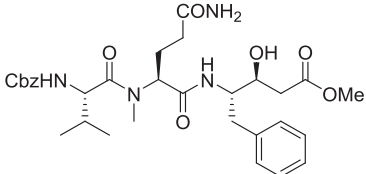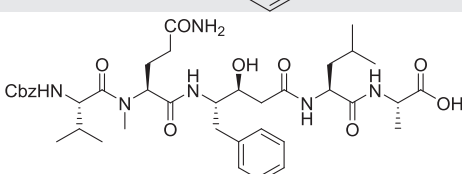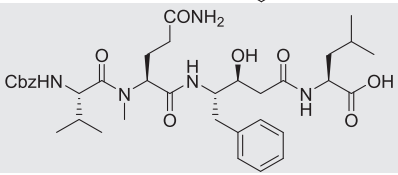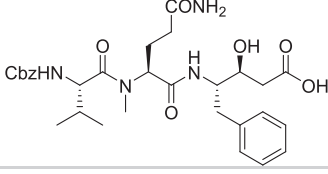
described.[32,35] Methyl esters **1**, **2**, and **3** were hydrolyzed with lithium hydroxide (LiOH) to afford compounds **5**, **6**, and **7**, respectively. Treatment of **8a** with trifluoroacetic acid (TFA) in dichloromethane (DCM) gave compound **8**. Deprotection of the Cbz group of methyl esters **1**, **2**, and **3** afforded the free amine, which were then masked with Boc group yielding compounds **9**, **10**, and **11**, respectively. Other target compounds **12**, **13**, and **14** were prepared in similar way by hydrolyzing methyl esters **9**, **10**, and **11**, respectively. The structures of **5** to **14** were confirmed by nuclear magnetic resonance

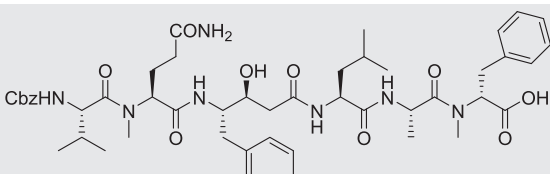(¹H NMR), carbon NMR (¹³C NMR), and high-resolution mass spectra (HRMS).

2.3 | In vitro activity assays

These two series tasiamide B derivatives (**1-14**) were assessed for their ability to inhibit the activity of Cath D, Cath E, and BACE1 (Tables 1 and 2). Enzymatic assays revealed that the inhibitory ability

TABLE 1 IC_{50}s of TB-9 analogs **1** to **8** against three aspartic proteases

Compound	Structure	In Vitro IC_{50}, nM[a]		
		Cath D[b]	Cath E[b]	BACE1[b]
TB-9		0.0783	0.724	54.2
1		3.29	236	972
2		102	243	642
3		196	2740	5599
4		NA[c]	2105	NA
5		27.0	100	502
6		108	168	1330
7		6384	1199	more than 10 000
8		more than 10 000	more than 10 000	more than 10 000

[a]All inhibitory values are means of at least two separate experiments.

[b]IC_{50} of positive control (pepstatin A): 0.59 nM for Cath D; 0.57 nM for Cath E; 92.4 nM for BACE1.
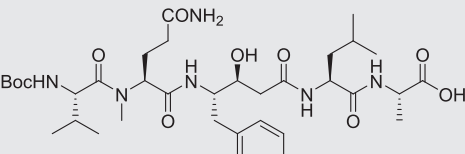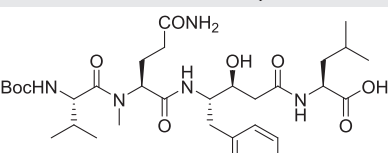
[c]"NA" means not active at 10 μM.

of three aspartic proteases had a sharp downtrend as the removal of amino acid units at the *C*-terminus (**1-4**, **5-8**, **9-11**, **12-14**). To be specific, the inhibition against Cath D was almost completely lost with the lack of proline, phenylalanine, alanine, and leucine residues even at the concentration of 10 μM (**4** and **8**). In series I (**1-8**), the methyl ester derivatives had slightly improved in activity and selectivity against Cath D than corresponding carboxylic acid (**1** vs **5**, **2** vs **6**, **3** vs **7**). What is more, the results indicated that compounds with aromatic ring at the

N-terminus were crucial for the inhibitory potency against Cath D (**1** vs **9**, **2** vs **10**, **3** vs **11**, **5** vs **12**, **6** vs **12**, **7** vs **13**), as same as **TB-9** vs **TB-11**.

Among the tested compounds, **1**, **2**, **3**, **5**, **6**, **9**, and **12** showed moderate-to-good inhibition against Cath D. Most notably in that regard, we found that compound **1** is extremely potent against Cath D with IC_{50} of 3.29 nM. This compound, with the lack of proline unit compared with **TB-9**, showed improved selectivity for Cath D with 72-fold over Cath E.

TABLE 2 IC_{50}s of TB-11 analogs **9** to **14** against three aspartic proteases

Compound	Structure	In Vitro IC_{50}, nM[a]		
		Cath D[b]	Cath E[b]	BACE1[b]
TB-11		0.126	1.92	48.8
9		190	233	1117
10		407	67.3	806
11		more than 10 000	494	3718
12		131	53.4	181
13		903	217	91.4
14		more than 5000	more than 5000	NA[c]

[a]All inhibitory values are means of at least two separate experiments.

[b]IC_{50} of positive control (pepstain A): 0.59 nM for Cath D; 0.57 nM for Cath E; 92.4 nM for BACE1.
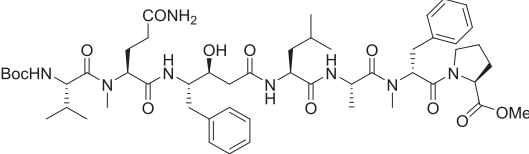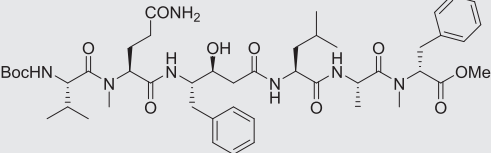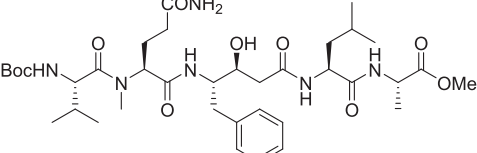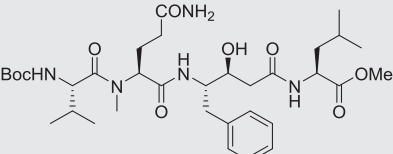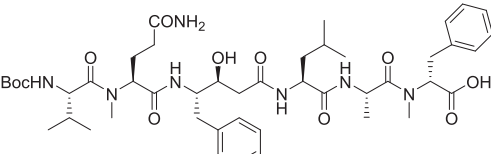
[c]"NA" means not active at 10 μM.

3 | CONCLUSIONS

In conclusion, two series of tasiamide B derivatives have been designed, synthesized, and evaluated as selective Cath D inhibitors, in which Val-*Me*-Gln-Ahppa fragment was retained, while the amino acid units of **TB-9** and **TB-11** were truncated at the *C*-terminus in sequence. The target compound **1** with lower molecule weight showed highly potent inhibitory activity against Cath D with IC_{50} of 3.29 nM and satisfactory selectivity over Cath E (72-fold) and BACE1 (295-fold). These results could provide a new and good template for the development of selective Cath D inhibitors. Further studies on the structural optimization of tasiamide B derivatives are currently undergoing in our laboratory and will be reported in due course.

4 | EXPERIMENTAL SECTION

4.1 | Chemistry

All reactions were carried out in oven-dried or flame-dried glassware. All commercial reagents were used without further purification unless otherwise noted. Anhydrous DCM, methanol (MeOH), and tetrahydrofuran (THF) were obtained by Solvent Purification System (PS-MD-5, Innovation Technology, USA). Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with silica gel plates (60F-254) and visualized by UV irradiation or by staining with iodine absorbed on silica gel, phosphomolybdic acid/ethanol solution, or aqueous acidic ammonium molybdate solution as appropriate. Flash column chromatography was performed on silica gel (200-300 meshes) with the indicated solvent system, and preparative TLC was performed on silica gel F254 glass plates (layer thick 0.4-0.5 mm). Yields refer to chromatographically and spectroscopically pure compounds. NMR spectra were recorded on a Bruker 400 MHz or Bruker Avance III 600 as indicated in the data list. Chemical shifts for ^{1}H NMR spectra are reported in parts per million relative to the signal residual tetramethylsilane (TMS) at 0 ppm. Chemicals shifts for carbon ^{13}C NMR spectra are reported in parts per million relative to the center line of the $CDCl_3$ triplet at 77.16 ppm and the CD_3OD heptet at 49.00 ppm. The abbreviations s, d, dd, t, q, br, and m stand for the resonance multiplicity singlet, doublet, doublet of doublets, triplet, quartet, broad, and multiplet, respectively. Optical rotation was measured on an AUTOPOL V (Na D line) by using a microcell of 1-dm path length. HRMS were obtained by using a quadrupole time-of-flight mass spectrometer. Copies of ^{1}H and ^{13}C NMR spectra for compounds **5-14** may be found in the Supporting Information.

4.1.1 | Compound 8

To the cold solution (ice bath) of compound **8a** (30.0 mg, 0.034 mmoL) in DCM (2 mL) was added TFA (2 mL) and stirred for 30 minutes. The solution was warmed to room temperature and stirred for another 1.5 hours. When TLC analysis showed no starting material remained the solution was concentrated *in vacuo*. The residue was redissolved

in DCM (5 mL), concentrated *in vacuo* again and purified by preparative TLC with DCM/MeOH (10/1) to give **8** as white solid (15.0 mg, 75.5%). $[\alpha]_D^{25}$ = −26.3 (*c* = 2.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.09 (m, 10H), 6.66 (s, 1H), 6.55 (s, 1H), 5.99 and 5.71 (both d, *J* = 8.1 Hz, total 1H), 5.13-4.91 (m, 3H), 4.41-4.17 (m, 2H), 4.15-3.99 (m, 1H), 2.97-2.83 (m, 1H), 2.77 (d, *J* = 9.0 Hz, 1H), 2.70 and 2.29 (both s, total 3H), 2.45 (s, 2H), 2.19-2.02 (m, 3H), 1.86-1.73 (m, 2H), 0.99-0.77 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.3, 175.8, 173.5, 169.7, 156.8, 138.0, 136.2, 129.1, 128.6, 128.4, 128.3, 128.1, 128.0, 126.4, 68.7, 67.0, 56.3, 55.6, 53.7, 39.3, 37.8, 31.4, 30.6, 30.5, 23.7, 19.6, 17.5. HRESIMS calcd for $C_{30}H_{40}N_4O_8$ $[M + Na]^+$ 607.2738, found 607.2742.

4.2 | General method for preparation of compounds 9 to 11

(exemplified by **9**)

4.2.1 | Compound 9

Compound **1** (93.0 mg, 0.099 mmoL) in MeOH (10 mL) was hydrogenated for 3 hours at room temperature in the presence of a catalytic amount of Pd/C (10 wt%). When TLC analysis showed no starting material remained, Pd/C was removed by filtration, and the resulting filtrate was concentrated to give the free amine as colorless oil, which was used without further purification.

To the cold solution (ice bath) of the amine obtained above in DCM (5 mL) was added di-*tert*-butyl dicarbonate (Boc$_2$O, 26 mg, 0.12 mmoL). The mixture was stirred at room temperature for 2 hours. When TLC analysis showed no starting material remained, and then the solution was concentrated *in vacuo*. The residue was purified by flash column chromatography with DCM/MeOH (50:1-20:1) to give compound **9** as white solid (65.5 mg, 73.1%). $[\alpha]_D^{25}$ = −34.4 (*c* = 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 7.6 Hz, 1H), 7.50-7.32 (m, 1H), 7.30-7.05 (m, 10H), 6.32 (s, 1H), 6.27 (s, 1H), 5.47-5.31 (m, 1H), 5.25 (d, *J* = 8.2 Hz, 1H), 5.10-4.94 (m, 1H), 4.86-4.71 (m, 1H), 4.73-4.61 (m, 1H), 4.50-4.35 (m, 1H), 4.36-4.24 (m, 1H), 4.07 (s, 1H), 3.74 (s, 3H), 3.45-3.26 (m, 1H), 3.08-2.90 (m, 2H), 2.89-2.63 (m, 7H), 2.53-2.40 (m, 1H), 2.38-2.20 (m, 2H), 2.20-2.01 (m, 2H), 2.00-1.77 (m, 2H), 1.74-1.59 (m, 1H), 1.59-1.50 (m, 2H), 1.45 (s, 9H), 1.10-0.64 (m, 15H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.1, 173.9, 173.3, 172.3, 171.8, 171.0, 170.9, 156.1, 138.4, 136.5, 129.2, 129.0, 128.8, 128.7, 128.4, 127.1, 126.4, 79.9, 69.5, 58.1, 56.3, 55.7, 54.5, 52.6, 52.1, 45.6, 41.1, 41.0, 37.1, 34.8, 32.5, 31.9, 30.8, 28.4, 24.9, 24.3, 23.2, 21.8, 19.7, 17.6, 17.5; HRESIMS calcd for $C_{47}H_{71}N_7O_{11}$ $[M + Na]^+$ 932.5104, found 932.5108.

4.2.2 | Compound 10

Compound **10** was obtained from **2** according to the similar procedure. White solid; yield 67.0%; $[\alpha]_D^{25}$ = −67.0 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (brs, 1H), 7.50 (d, *J* = 6.7 Hz, 1H), 7.33 (d, *J* = 5.1 Hz, 1H), 7.29-7.09 (m, 5H), 6.50 (s, 1H), 6.15 (s, 1H),

5.14 (d, J = 7.9 Hz, 1H), 4.98 (s, 1H), 4.54-4.38 (m, 2H), 4.33-4.20 (m, 1H), 4.17-4.01 (m, 2H), 3.71 (s, 3H), 2.95 (d, J = 6.7 Hz, 2H), 2.86 (s, 3H), 2.61-2.41 (m, 1H), 2.38-2.19 (m, 2H), 2.08 (s, 2H), 1.94 (brs, 1H), 1.88–1.78 (m, 1H), 1.77-1.52 (m, 3H), 1.46 (s, 9H), 1.38 (d, J = 6.9 Hz, 3H), 0.97-0.84 (m, 12H); ^{13}C NMR (151 MHz, CDCl$_3$) δ 175.2, 173.7, 173.2, 173.0, 171.9, 170.9, 156.1, 138.3, 129.1, 128.4, 126.4, 79.9, 69.9, 55.9, 55.6, 54.3, 52.4, 52.0, 48.3, 41.1, 40.8, 37.1, 30.7, 28.4, 24.8, 24.4, 23.0, 21.8, 19.6, 17.7, 17.4; HRESIMS calcd for C$_{37}$H$_{60}$N$_6$O$_{10}$ [M + Na]$^+$ 771.4263, found 771.4256.

4.2.3 | Compound 11

Compound **11** was obtained from **3** according to the similar procedure. White solid; yield 84.2%; $[\alpha]_D^{25}$ = −64.7 (c = 0.6, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.43 (d, J = 6.9 Hz, 1H), 7.30-7.10 (m, 5H), 6.20 (s, 1H), 6.11 (s, 1H), 5.16 (d, J = 7.7 Hz, 1H), 5.02 (s, 1H), 4.63-4.50 (m, 1H), 4.38-4.23 (m, 1H), 4.18 (d, J = 6.9 Hz, 1H), 4.07 (s, 1H), 3.71 (s, 3H), 3.01-2.89 (m, 2H), 2.83 (s, 3H), 2.55-2.42 (m, 1H), 2.35-2.25 (m, 2H), 2.18-2.06 (m, 2H), 1.96 (s, 1H), 1.90-1.79(m, 1H), 1.77-1.66 (m, 1H), 1.65-1.57 (m, 2H), 1.46 (s, 9H), 1.11-0.75 (m, 12H); ^{13}C NMR (151 MHz, CDCl$_3$) δ 174.9, 174.1, 173.9, 171.9, 170.7, 156.1, 138.2, 129.2, 128.4, 126.4, 80.0, 69.4, 56.0, 55.6, 54.1, 52.3, 50.8, 40.7, 37.2, 31.6, 30.6, 28.4, 24.9, 24.0, 22.9, 21.7, 19.7, 17.4; HRESIMS calcd for C$_{34}$H$_{55}$N$_5$O$_9$ [M + Na]$^+$ 700.3892, found 700.3896.

4.3 | General method for preparation of compounds 5 to 7 and 12 to 14

(exemplified by **5**)

4.3.1 | Compound 5

Compound **1** (55.0 mg, 0.058 mmoL) in MeOH (2 mL) was treated with a solution of LiOH monohydrate (8.0 mg, 0.19 mmoL) in water (0.5 mL). The mixture was stirred at room temperature for 3 hours. When TLC analysis showed no starting material remained, the mixture was adjusted to pH = 4 with 1 M HCl and extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine (10 mL × 3), dried over Na$_2$SO$_4$, and then concentrated *in vacuo*. The residue was purified by preparative TLC with DCM/MeOH (10/1) to give **5** as white solid (47.8 mg, 88.2%). $[\alpha]_D^{25}$ = −2.8 (c = 0.1, CHCl$_3$); ^{1}H NMR (400 MHz, CD$_3$OD, two rotamers, *cat*. 1:1) δ 7.44-7.08 (m, 15H), 7.03 (d, J = 7.0 Hz, 1H), 5.28-5.14 (m, 2H), 5.11-4.99 (m, 2H), 4.65 (dd, J = 6.8, 3.0 Hz, 1H), 4.44-4.03 (m, 4H), 3.45-3.33 (m, 1H), 3.15-2.98 (m, 1H), 2.91 (s, 3H), 2.84 (d, J = 3.9 Hz, 1H), 2.79 and 2.18 (both s, total 3H), 2.75-2.58 (m, 1H), 2.52-2.26 (m, 3H), 2.24-2.06 (m, 2H), 2.07-1.85 (m, 2H), 1.74-1.63 (m, 1H), 1.62-1.44 (m, 2H), 1.08-0.80 (m, 15H); ^{13}C NMR (151 MHz, CD$_3$OD, two rotamers, *cat*. 1:1) δ 177.6, 175.2, 174.8, 174.7, 174.6, 174.2, 174.1, 173.8, 172.0, 170.4, 159.2, 158.7, 139.9, 139.7, 139., 138.2, 138.2, 130.3, 130.3, 130.1, 129.6, 129.5, 129.5, 129.1, 129.1, 128.9, 128.8, 127.6,

127.4, 127.2, 79.3, 71.2, 70.0, 68.1, 67.8, 60.7, 60.3, 58.1, 57.8, 57.6, 56.9, 55.7, 53.2, 49.6, 46.8, 41.9, 41.8, 41.5, 38.4, 37.8, 35.9, 33.4, 32.7, 32.3, 31.9, 31.7, 31.5, 29.1, 26.3, 25.8, 25.3, 23.6, 21.9, 21.9, 20.1, 20.0, 19.2, 18.4, 17.4; HRESIMS calcd for C$_{49}$H$_{67}$N$_7$O$_{11}$ [M + H]$^+$ 930.4971, found 930.5011.

4.3.2 | Compound 6

Compound **6** was obtained from **2** according to the similar procedure. White solid; yield 62.6%; $[\alpha]_D^{25}$ = −34.2 (c = 1.3, CHCl$_3$); ^{1}H NMR (400 MHz, CD$_3$OD, two rotamers, *cat*. 1:1) δ 7.45-7.08 (m, 10H), 7.04 (d, J = 7.1 Hz, 1H), 5.23 (q, J = 12.6 Hz, 1H), 5.07 (s, 1H), 5.06-4.98 (m, 1H), 4.48-4.06 (m, 5H), 3.00-2.85 (m, 1H), 2.77 and 2.17 (both s, total 3H), 2.83-2.66 (m, 1H), 2.51-2.20 (m, 3H), 2.13 (s, 2H), 2.06-1.87 (m, 2H), 1.80-1.66 (m, 1H), 1.67-1.52 (m, 2H), 1.38 (d, J = 7.0 Hz, 3H), 1.07-0.90 (m, 12H); ^{13}C NMR (151 MHz, CD$_3$OD, two rotamers, *cat*. 1:1) δ 177.7, 177.6, 176.6, 175.2, 174.6, 174.5, 173.9, 173.7, 172.0, 170.4, 159.2, 158.7, 139.9, 139.7, 138.2, 130.3, 130.3, 129.5, 129.5, 129.4, 129.1, 129.0, 128.9, 128.8, 127.4, 127.1, 71.3, 70.2, 68.1, 67.7, 60.3, 58.1, 57.8, 57.6, 56.7, 55.3, 53.3, 50.0, 42.1, 42.0, 41.8, 41.5, 38.6, 38.0, 32.7, 32.3, 31.9, 31.6, 31.4, 29.0, 26.4, 25.8, 25.3, 23.5, 22.0, 21.9, 20.1, 19.9, 19.1, 18.3, 18.0, 17.9; HRESIMS calcd for C$_{39}$H$_{56}$N$_6$O$_{10}$ [M + H]$^+$ 769.4131, found 769.4150.

4.3.3 | Compound 7

Compound **7** was obtained from **3** according to the similar procedure. White solid; yield 71.4%; $[\alpha]_D^{25}$ = −74.2 (c = 0.6, CHCl$_3$); ^{1}H NMR (400 MHz, CD$_3$OD, two rotamers, *cat*. 1:1) δ 7.45-7.09 (m, 10H), 7.05 (d, J = 7.5 Hz, 1H), 5.23 (q, J = 12.5 Hz, 1H), 5.07 (s, 1H), 5.03 (d, J = 9.0 Hz, 1H), 4.49-4.21 (m, 3H), 4.17-4.04 (m, 1H), 2.98-2.88 (m, 1H), 2.81 and 2.17 (both s, total 3H), 2.77-2.60 (m, 1H), 2.48-2.26 (m, 3H), 2.12 (s, 2H), 2.07-1.88 (m, 2H), 1.84-1.70 (m, 1H), 1.68-1.59 (m, 2H), 1.07-0.90 (m, 12H); ^{13}C NMR (151 MHz, CD$_3$OD, two rotamers, *cat*. 1:1) δ 177.7, 177.6, 176.6, 175.3, 174.6, 174.0, 173.7, 172.1, 170.5, 159.2, 158.7, 139.9, 139.7, 138.1, 130.3, 130.2, 129.5, 129.5, 129.4, 129.1, 129.0, 128.9, 128.8, 127.4, 127.1, 71.3, 70.1, 68.1, 67.7, 60.2, 58.0, 57.7, 57.6, 56.9, 55.7, 52.5, 49.4, 49.3, 49.1, 49.0, 48.9, 48.7, 48.6, 41.8, 41.7, 41.5, 38.4, 37.8, 32.6, 32.2, 31.9, 31.6, 31.4, 29.0, 26.3, 26.0, 25.3, 23.5, 21.9, 21.9, 20.1, 19.9, 19.2, 18.4; HRESIMS calcd for C$_{36}$H$_{51}$N$_5$O$_9$ [M + H]$^+$ 698.3760, found 698.3766.

4.3.4 | Compound 12

Compound **12** was obtained from **9** according to the similar procedure. White solid; yield 61.5%; $[\alpha]_D^{25}$ = −2.8 (c = 1, MeOH); ^{1}H NMR (400 MHz, CD$_3$OD, two rotamers, *cat*. 4:5) δ 7.41-7.03 (m, 10H), 5.21 (d, J = 8.7 Hz, 1H), 5.05 (s, 1H), 4.66 (d, J = 5.8 Hz, 1H), 4.45-3.97 (m, 4H), 3.42-3.34 (m, 1H), 3.15-3.00 (m, 1H), 2.93 (s, 3H), 2.84 (s, 1H), 2.79 (s, 3H), 2.45-2.24 (m, 3H), 2.22-2.06 (m, 3H), 2.04-1.84 (m, 2H), 1.79-1.54 (m, 3H), 1.52 and 1.42 (both s, total 9), 1.08-0.71 (m, 15H); ^{13}C NMR (151 MHz, CD$_3$OD, two rotamers, *cat*. 4:5) δ 177.8, 177.6, 175.5, 175.0, 174.8, 174.7, 174.2, 174.1, 173.8, 173.4,

172.0, 170.5, 158.6, 158.1, 140.0, 139.7, 139.0, 130.4, 130.2, 130.1, 129.7, 129.6, 129.5, 127.6, 127.4, 127.2, 81.0, 80.6, 71.3, 70.0, 60.6, 60.2, 57.6, 57.5, 57.2, 56.9, 55.6, 53.2, 46.7, 41.9, 41.8, 38.4, 37.8, 35.8, 33.4, 32.7, 32.3, 31.9, 31.5, 29.1, 28.7, 26.3, 25.8, 25.8, 25.4, 23.6, 21.9, 21.8, 20.1, 20.0, 19.1, 18.3, 17.4, 17.4; HRESIMS calcd for $C_{46}H_{69}N_7O_{11}$ $[M + Na]^+$ 918.4947, found 918.4974.

4.3.5 | Compound 13

Compound **13** was obtained from **10** according to the similar procedure. White solid; yield 65.5%; $[\alpha]_D^{25} = -31.7$ (c = 0.6, MeOH); ^1H NMR (400 MHz, CD$_3$OD, two rotamers, *cat*. 4:5) δ 7.27-7.00 (m, 6H), 5.03-4.86 (m, 1H), 4.40-3.95 (m, 5H), 2.87-2.79 (m, 1H), 2.78-2.67 (m, 1H), 2.65 and 2.01 (both s, total 3H), 2.41-2.18 (m, 3H), 2.05-1.98 (m, 2H), 1.95-1.76 (m, 2H), 1.69-1.58 (m, 1H), 1.57-1.47 (m, 2H), 1.43 and 1.33 (both s, total 9H), 1.31-1.25 (m, 3H), 0.97-0.78 (m, 12H); ^{13}C NMR (151 MHz, CD$_3$OD, two rotamers, *cat*. 4:5) δ 177.2, 176.9, 175.7, 175.5, 173.4, 173.0, 171.9, 171.6, 170.0, 168.5, 156.6, 156.0, 138.1, 137.7, 128.3, 128.2, 127.5, 127.4, 125.4, 125.1, 79.0, 78.6, 69.4, 68.0, 58.2, 55.6, 55.4, 55.1, 54.0, 51.5, 49.8, 40.0, 39.9, 36.3, 35.8, 30.8, 30.2, 29.9, 29.5, 29.4, 27.0, 26.7, 24.2, 23.9, 23.8, 23.4, 21.6, 19.9, 19.8, 18.1, 18.0, 17.5, 17.1, 16.3; HRESIMS calcd for $C_{36}H_{58}N_6O_{10}$ $[M + Na]^+$ 757.4107, found 757.4125.

4.3.6 | Compound 14

Compound **14** was obtained from **11** according to the similar procedure. White solid; yield 69.9%; $[\alpha]_D^{25} = -7.7$ (c = 0.7, MeOH); ^1H NMR (400 MHz, CDCl$_3$) δ 7.79 (s, 1H), 7.41-7.30 (m, 1H), 7.29-7.06 (m, 5H), 6.83 (s, 1H), 6.55 (s, 1H), 5.29 (d, J = 7.1 Hz, 1H), 4.98 (s, 1H), 4.43 (d, J = 6.2 Hz, 1H), 4.34-3.96 (m, 3H), 3.01-2.86 (m, 2H), 2.72 (s, 3H), 2.58-2.42 (m, 1H), 2.44-2.22 (m, 2H), 2.08 (s, 2H), 1.98-1.77 (m, 2H), 1.77-1.53 (m, 3H), 1.45 (s, 9H), 1.02-0.78 (m, 12H); ^{13}C NMR (151 MHz, CDCl$_3$) δ 176.6, 176.1, 173.8, 172.4, 170.9, 156.2, 138.3, 129.1, 128.4, 126.4, 80.0, 69.7, 55.9, 55.7, 54.5, 51.4, 40.4, 36.9, 31.6, 30.6, 28.4, 24.9, 24.1, 23.0, 21.6, 19.6, 17.4; HRESIMS calcd for $C_{33}H_{53}N_5O_9$ $[M + Na]^+$ 686.3736, found 686.3735.

4.4 | Biological assays

Aspartic protease enzymatic assays to determine inhibitory activity against Cath D, Cath E, and BACE1 were carried out as described previously.[27] Enzymes BACE1 (R&D Systems), cathepsin D (Enzo Life Sciences), and cathepsin E (R&D systems) and corresponding substrates were prepared freshly in their respective reaction buffers. The reaction buffer for BACE1 contained 100 mM NaOAc (pH 4.0), and the reaction buffer for cathepsins D and E contained 100 mM NaOAc/100 mM NaCl (pH 3.5). Enzymes were added (final concentrations are 30 μg/mL for BACE1, 1.0 μg/mL for cathepsin D, and 0.05 μg/mL for cathepsin E) followed by compounds dissolved in DMSO at six different concentrations (10, 5, 1, 0.5, 0.1, 0.01 μM final

assay concentration, up to less than or equal to 2% DMSO). The enzyme activities were detected as a time-course measurement of the increase in fluorescence signal (Ex 320 nM, Em 405 nM) from fluorescently labeled peptide substrate for 120 minutes at room temperature. Then, we determined IC$_{50}$ values with nonlinear regression in GraphPad Prism 5.

The inhibitory activities of tasiamide B derivatives **1**–**14** against these three aspartic proteases are summarized in Tables 1 and 2.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.