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Design, synthesis and biological evaluation of dipeptides as novel non-covalent 20S proteasome inhibitors

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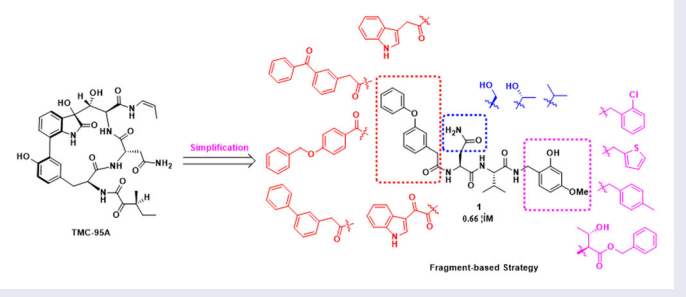
ABSTRACT

Based on the interaction modes of the natural 20S proteasome inhibitors **TMC-95A**, we have previously discovered a dipeptide **1**. To explore the SAR around compound **1**, we designed and synthesized a series of dipeptides (**8–38**) with a fragment-based strategy. Among them, nine compounds showed significant inhibitory activities against the chymotrypsin-like activity of human 20S proteasome with IC_{50} values at the submicromolar level, which were comparable or even superior to the parent compound **1**. Meanwhile, they displayed no significant inhibition against trypsin-like and caspase-like activities of 20S proteasome. The results suggested the feasibility to design dipeptides as novel and potent 20S proteasome inhibitors.



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KEYWORDS
20S proteasome inhibitors; non-covalent; dipeptides

1. Introduction

The ubiquitin-proteasome system (UPS) is a proteolytic system responsible for the precise control of cellular protein homeostasis, and it participates in a great variety of biological processes [1, 2]. As the main component of UPS, the 26S proteasome is a

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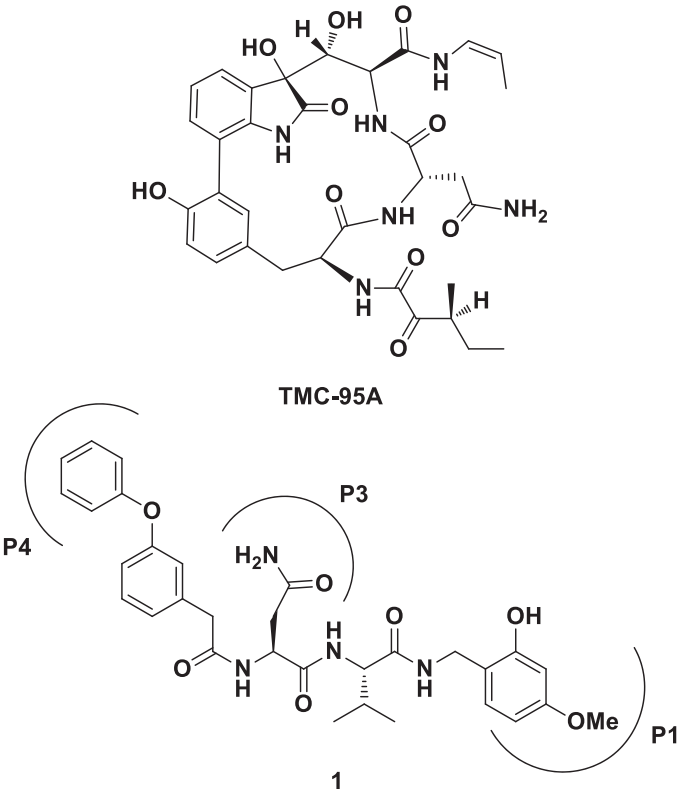
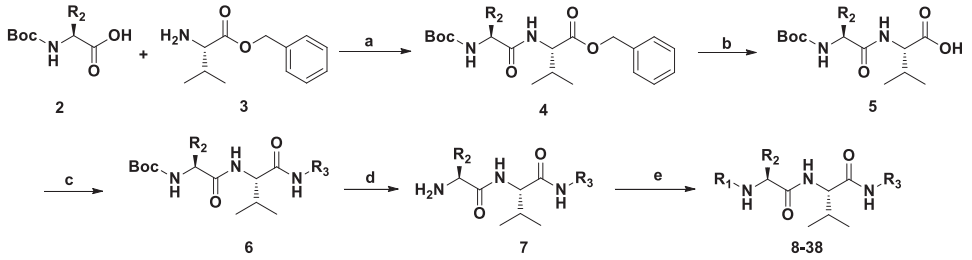


Figure 1. The structures of **TMC-95A** and compound **1**.

proteolytic machine with multiple functions. In eukaryotes, the 26S proteasome is composed of the regulatory 19S and the catalytic 20S particles [3]. The cylinder-shaped 20S proteasome is the catalytic core of 26S proteasome and is formed by four stacked rings with twenty-eight subunits [4]. Among them, only β1, β2 and β5 subunits are proteolytically active, which are associated with the caspase-like (C-L), trypsin-like (T-L) and chymotrypsin-like (CT-L) activities, respectively [5, 6]. The approval of the 20S proteasome inhibitors, bortezomib, carfilzomib and ixazomib, has validated 20S proteasome as a therapeutic target for cancer treatment [7]. However, as covalent and peptidic inhibitors, their clinical application is hindered by issues of severe side effects, acquired drug resistance, and unsatisfactory pharmacokinetic profiles [8–10]. Non-covalent proteasome inhibitors are generally assumed to have potential benefits over their covalent peers [11–12]. Therefore, the development of novel non-covalent 20S proteasome inhibitors has drawn extensive research interests in recent years.

TMC-95A (Figure 1) is a potent non-covalent 20S proteasome inhibitor isolated from the fermentation broth of the ascomycete fungus *Apiospora montagnei* [13]. Although the complex structure of **TMC-95A** impedes a fully SAR exploration of the chemical space around it, some simplified mimics, including cyclic tripeptides [14–16], linear tripeptides and dipeptides [17–19], were rationally designed based on its binding mode [20]. Inspired by the structure of **TMC-95A**, we designed a series of



Scheme 1. Synthetic route of compounds **8–38**. Reagents and conditions: (a) HATU, DIEA, DMF (*N*,*N*-dimethyllformamide), rt, overnight; (b) 10% Pd/C, MeOH (methanol), H_2, rt, overnight; (c) corresponding amines, HATU, DIEA, DMF, rt, overnight;(d)TFA (trifluoroacetic acid), DCM (dichloromethane), rt, 4 h; (e) corresponding acids, HATU, DIEA, DMF, rt, overnight.

linear dipeptides previously, and compound **1** (Figure 1) showed apparent inhibition against the CT-L activity of human 20S proteasome with an IC_{50} value of 0.66 μM. As revealed by molecular docking, molecular areas of P4, P3 and P1 in compound **1** protrude into the S4, S3 and S1 pockets, respectively, and the dipeptide backbone could form extensive hydrogen bonds with the key residues of the active site [21]. The results encouraged us to further develop more potent linear dipeptides as novel non-covalent 20S proteasome inhibitors. Based on a fragment-based strategy, we approach the optimization of compound **1** by a stepwise evolution of the P4, P3 and P1 moieties to explore the SAR of the chemical space around compound **1**, and hopefully to further improve the activity profile of this compound class.

2. Results and discussion

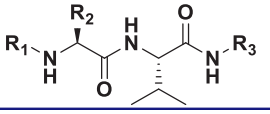
2.1. Chemistry

The synthetic route of target compounds was shown in Scheme 1. First, the corresponding Boc-protected amino acids **2** and H-*L*-Val-OBn **3** were coupled in the presence of HATU (2-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate) and DIEA (*N*,*N*-diisopropylethylamine) to give dipeptides **4**. The dipeptides **4** were subjected to hydrogenation using 10% Pd/C to liberate the carboxylic group and produce acids **5**. Then acids **5** were coupled with corresponding amines to afford compounds **6**, which were treated with trifluoroacetic acid to free the amino group and give compounds **7**. Finally, compounds **7** were coupled with corresponding acids to provide target compounds **8–38**.

2.2. Biological evaluation

The inhibitory profiles of the synthesized dipeptides were initially assayed against the CT-L activity of human 20S proteasome as described previously [14]. As shown in Table 1, most compounds displayed moderate to potent inhibitory activities, and nine of them showed IC_{50} values at the submicromolar level, which are comparable or even superior to the parent compound **1**.

Table 1. Inhibitory activities of compounds **8–38** against CT-L, T-L and C-L activities of human 20S proteasome.



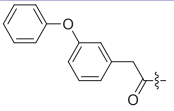
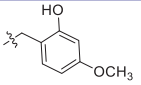
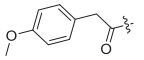
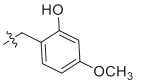
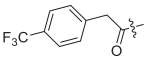
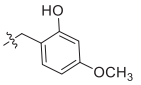
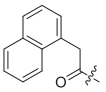
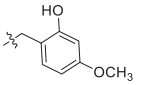
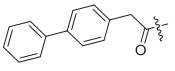
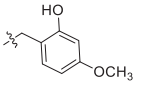
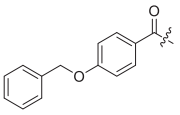
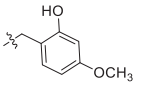
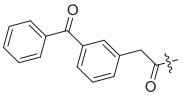
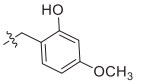
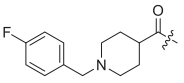
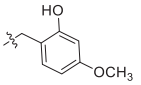
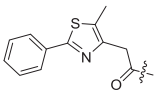
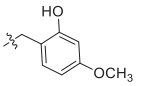
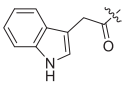
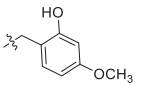
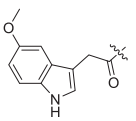
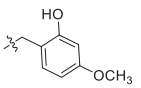
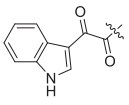
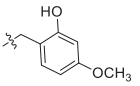
Comp.	R_1	R_2	R_3	IC_{50} (μM) CT-L	T-L	C-L
1				0.66	ND	ND
8				5.10	ND	ND
9				17.2	ND	ND
10				1.40	ND	ND
11				0.84	>100	>100
12				0.17	>100	>100
13				0.53	>100	>100
14				5.10	ND	ND
15				2.06	ND	ND
16				0.57	>100	>100
17				0.87	>100	>100
18				0.20	>100	>100

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Table 1. Continued.

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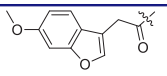
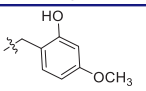
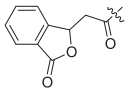
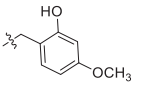
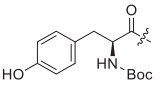
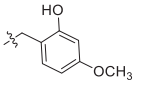
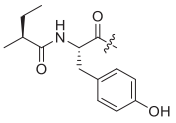
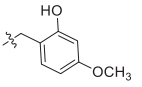
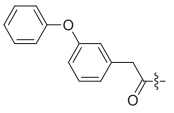
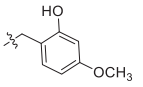
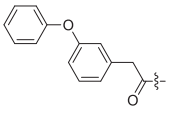
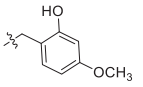
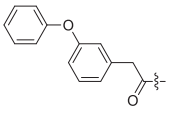
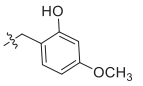
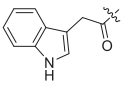
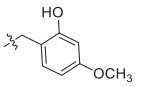
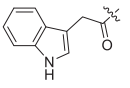
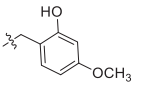
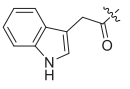
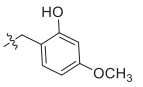
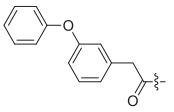
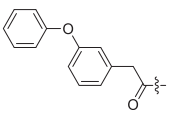
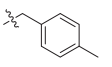
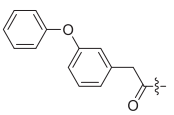
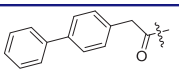
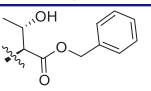
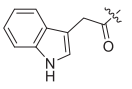
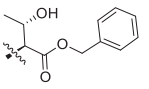
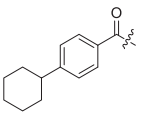
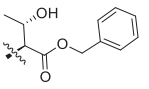
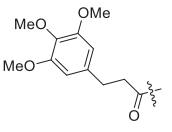
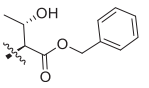
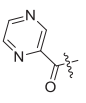
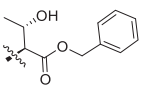
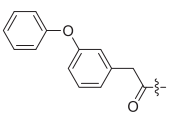
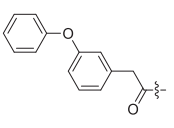
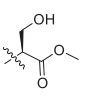
19				4.50	ND[#]	ND
20				0.89	>100	>100
21				3.50	ND	ND
22				3.70	ND	ND
23				11.8	ND	ND
24				5.50	ND	ND
25				0.89	>100	>100
26				9.06	ND	ND
27				5.68	ND	ND
28				3.54	ND	ND
29				6.90	ND	ND
30				1.65	ND	ND
31				2.98	ND	ND

(*continued*)

Table 1. Continued.

Comp.	R_1	R_2	R_3	IC_{50} (μM) CT-L	T-L	C-L
32				0.36	>100	>100
33				5.70	ND	ND
34				15.4	ND	ND
35				NA*	ND	ND
36				NA	ND	ND
37				NA	ND	ND
38				NA	ND	ND

*NA: The percentage inhibition was less than 50% under the concentration of 10 μM.
#ND: not determined.

Preliminary SAR could be deduced from Table 1. For compounds **8**-**22**, we have the P1 and P3 moieties of compound **1** retained to preserve the interactions with S1 and S3 pockets, while have its P4 group replaced with various hydrophobic substituents to explore SAR in this molecular area. The introduction of phenyl or naphthyl groups (**8**-**10**) led to an apparent drop of activity. Groups with two phenyl rings, including 4-phenylbenzene (**11**), 4-benzyloxybenzene (**12**) and 3-benzoylbenzene (**13**), seemed to be favored for this position. Compound **12** was the most potent derivative in this series, which had a nearly 4-fold improvement in the IC_{50} value as compared to compound **1**. The insertion of piperidine (**14**) or thiazole (**15**) between phenyl and carbonyl resulted in a decrease of activity. When the 3-indole groups (**16**-**18**) were introduced at P4, the inhibitory activity against the CT-L activity was generally maintained. Interestingly, although the replacement of the indole ring with benzofuran (**19**) resulted in a loss of inhibitory activity, further change of the furan ring into γ-lactone (**20**) significantly restored the activity. The incorporation of tyrosine-derived moiety at P4 (**21** and **22**) led to a reduction of the activity. The structural variation on P4 suggested that two aromatic rings with appropriate distance and orientation

might be important for the preservation of inhibitory activities against the CT-L activity of human 20S proteasome. This is in consistent with the previous docking results, which underscored π-π stacking and π-cation interactions with residues Tyr 96 and His 98, respectively [21].

Different amino acids were then introduced at P3 (**23**-**28**) with the P4 and P1 moieties of compound **1** intact. However, all the amino acids except valine (**25**) resulted in significant decline in activity. It was suggested that the asparagine residue might be optimal for the P3 area.

According to previous docking studies, the P1 area of compound **1** forms a hydrogen bond network with Gly47 and Thr1 [21]. Consistently, compounds **29**-**31**, which were deprived of the hydrogen bond forming atoms, showed reduced inhibitory activity against CT-L activity. Instead, when *L*-allo-Thr-OBn was introduced at P1, the inhibitory activity significantly varied with different substituents at P4 (**32–36**), and a biphenyl group was obviously favored at P4 (**32**). D-Thr-OMe and D-Ser-OMe were also introduced at P1 (**37** and **38**), however, such a structural variation led to a complete loss in activity.

The nine most active compounds (**11**-**13**, **16**-**18**, **20**, **25** and **32**) were further evaluated for their inhibition on T-L and C-L activities of human 20S proteasome. As shown in Table 1, all these compounds selectively inhibited the CT-L activity, while displayed no significant inhibition against the T-L and C-L activities.

2.3. Molecular modeling

Compound **18** was selected as a representative compound for molecular docking to investigate the interaction mode of this compound class with the β5 subunit of the 20S proteasome. As depicted in Figure 2, compound **18** could situate in the active site of the 20S proteasome with the 3-indole group extending to the hydrophobic region of the active site. Extensive hydrogen bonds were monitored between compound **18** and residues Gly47, Thr1, Ala49, Thr21 and Ala50 (β5) and Asp114 (β6).

Based on the interaction modes of non-covalent 20S proteasome inhibitors **TMC-95A** and compound **1,** we designed and synthesized a series of linear dipeptides (**8–38**) via a fragment-based strategy. Most compounds exhibited moderate to potent inhibition against the CT-L activity of 20S proteasome. Among them, nine compounds showed significant inhibitory activities with IC_{50} values at submicromolar levels, and two most active compounds, **12** and **18**, had IC_{50} values of 0.17 and 0.20 μM, respectively. Furthermore, no obvious inhibition against T-L and C-L activities was observed for nine most active compounds. Molecular docking on compound **18** revealed the interaction modes of this compound class. The results suggested that these dipeptides might represent potential leads for the development of novel 20S proteasome inhibitors.

3. Experimental

3.1. Chemistry

All chemical reagents and solvents were obtained commercially and were used without further purification. An SGW X-4A microscopic apparatus was used to determine

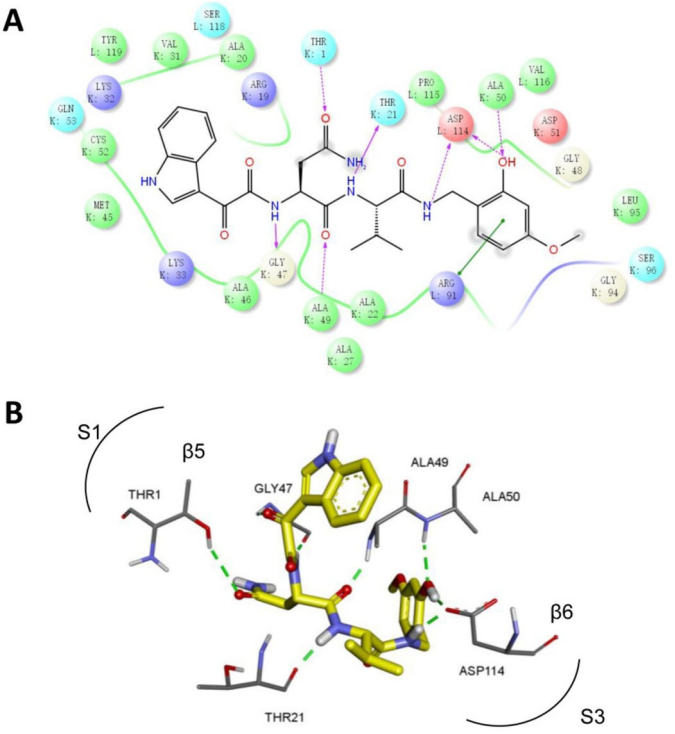


Figure 2. The binding mode of compound **18** proposed by docking study. (a) 2 D-plots: Residues from β5 and β6 subunits of the 20S proteasome were represented with letters K and L, respectively. Hydrogen bonding and π-cation interactions were shown in purple and green, respectively. (b) 3 D-plots: Hydrogen bonding interactions were shown as green dotted lines.

melting points which are uncorrected. ^{1}H-NMR spectra were recorded on Varian Mercury 300 or 400 MHz spectrometers with tetramethylsilane (TMS) as internal standard. Mass spectra with HR-ESI mode were recorded on a LC/MSD TOF instrument from Agilent Technologies.

Compounds **4**-**38** were synthesized with reported methods [9]. Briefly, **2** (11 mmol), **3** (10 mmol), HATU (12 mmol), DMF (50 ml) and DIEA (36 mmol) were added to a 200 ml round bottom flask. The mixture was stirred overnight at room temperature. After removal of the solvent under reduced pressure, 100 ml ethyl acetate was added and the organic phase was washed by water, saturated sodium hydrocarbonate and brine sequentially. The removal of the solvent gave **4** which were used in the next step without further purification. **4** were suspended with 10% Pd/C (100 mg) in methanol (40 ml) under hydrogen atmosphere. The mixture was stirred overnight at room temperature. After filtration, the filtrate was concentrated to give **5**. Then, DMF (30 ml), amine (9 mmol), HATU (12 mmol) and DIEA (36 mmol) were added to **5**. The mixture was stirred overnight at room temperature. After the same process for **4**, **6** was afforded by column chromatography. **6** (5 mmol) was dissolved in TFA/DCM (10 ml/10 ml), the mixture was stirred for 4 hours at room temperature. Then the solvent was removed to give **7** without further purification. **7** (0.5 mmol), acids (0.5 mmol), HATU (0.6 mmol) and DIEA (1.8 mmol) were dissolved in DMF

(10 ml), and the mixture was then stirred overnight at room temperature. The reaction mixture was then concentrated, and the desired compounds **8–38** were obtained by column chromatography.

3.1.1. N-2-(4-Methoxyphenyl) acetyl-L-Asn-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (8)

Yield: 71.7%; mp 217-218 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.63 (s, 1H), 8.38 (d, J = 7.6 Hz, 2H), 7.67 (s, J = 8.1 Hz, 1H), 7.43 (s, 1H), 7.16 (d, J = 7.7 Hz, 2H), 6.97 (d, J = 8.2 Hz, 1H), 6.91 (s, 1H), 6.82 (d, J = 7.9 Hz, 2H), 6.39(s, 1H), 6.32 (d, J = 8.1 Hz, 1H), 4.58-4.63 (m, 1H), 4.11-4.15 (m, 4H), 3.71 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 3.38 (s, 2H), 2.54-2.58 (m, 1H), 1.95-2.00 (m, 1H), 0.70-0.75 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 515.2491 $[M+H]^+$ (calcd for $C_{26}H_{35}N_4O_7$, 515.2506).

3.1.2. N-2-(4-Trifluoromethylphenyl)acetyl-L-Asn-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (9)

Yield: 36.6%; mp 227-228 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.65 (s, 1H), 8.63 (d, J = 8.1 Hz, 1H), 8.35 (brs, 1H), 7.59-7.69 (m, 3H), 7.46-7.48 (m, 3H), 6.85-7.01 (m, 2H), 6.39 (s, 1H), 6.29 (d, J = 7.6 Hz, 1H), 4.60 (d, J = 7.0 Hz, 1H), 4.09 (brs, 3H), 3.64 (s, 3H, OCH_3), 3.57 (s, 2H), 2.54-2.59 (m, 1H), 2.38-2.43 (m, 1H), 1.91-1.97 (m, 1H), 0.65-0.71 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 553.2247 $[M+H]^+$ (calcd for $C_{26}H_{32}F_3N_4O_6$, 553.2274).

3.1.3. N-2-(1-Naphthyl)acetyl-L-Asn-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (10)

Yield: 42.6%; mp 235-236 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.54 (s, 1H), 8.52 (d, J = 7.2 Hz, 1H), 8.32-8.34 (m, 1H), 8.03-8.07 (m, 1H), 7.87-7.90 (m, 1H), 7.77-7.80 (m, 1H), 7.70 (d, J = 7.2 Hz, 1H), 7.47-7.50 (m, 2H), 7.40-7.41 (m, 3H), 6.92-6.97 (m, 2H), 6.29-6.34 (m, 2H), 4.61-4.67 (m, 1H), 4.09-4.16 (m, 3H), 3.93 (s, 2H), 3.64 (s, 3H, OCH_3), 2.54-2.61 (m, 1H), 2.41-2.47 (m, 1H), 1.91-1.97 (m, 1H), 0.65-0.72 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 535.2568 $[M+H]^+$ (calcd for $C_{29}H_{35}N_4O_6$, 535.2557).

3.1.4. N-2-(1-Biphenyl)acetyl-L-Asn-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (11)

Yield: 43.6%; mp 231-232 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.57 (s, 1H), 8.44 (d, J = 6.7 Hz, 1H), 8.34 (s, 1H), 7.50-7.75 (m, 5H), 7.40-7.46 (m, 3H), 7.32-7.35 (m, 3H), 6.93-6.98 (m, 2H), 6.29-6.34 (m, 2H), 4.63 (d, J = 6.9 Hz, 1H), 4.12 (brs, 3H), 3.65 (s, 3H, OCH_3), 3.50 (s, 2H), 2.55-2.62 (m, 1H), 2.36-2.44 (m, 1H), 1.97 (d, J = 6.5 Hz, 1H), 0.70-0.75 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 561.2697 $[M+H]^+$ (calcd for $C_{31}H_{37}N_4O_6$, 561.2713).

3.1.5. N-2-(4-Benzyloxyphenyl)acyl-L-Asn-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (12)

Yield: 54.2%; mp 217-218 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.57 (s, 1H), 8.54 (d, J = 7.4 Hz, 1H), 8.34-8.36 (m, 1H), 7.82 (d, J = 8.7 Hz, 2H), 7.73 (d, J = 8.8 Hz, 1H), 7.34-7.48 (m, 6H), 7.09 (d, J = 8.7 Hz, 2H), 6.98 (d, J = 8.2 Hz, 2H), 6.92 (s, 1H), 6.32-6.36 (m, 2H), 5.18 (s, 2H), 4.77-4.83 (m, 1H), 4.11-4.21 (m, 3H), 3.67 (s, 3H, OCH_3), 2.56-2.69 (m, 2H), 1.95-2.02 (m, 1H), 0.75-0.81 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 577.2662 $[M+H]^+$ (calcd for $C_{31}H_{37}N_4O_7$, 577.2662).

3.1.6. N-2-(4-Benzoylphenyl)acetyl-L-Asn-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (13)

Yield: 64.4%; mp 197-199 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.53 (s, 1H), 8.46 (d, J = 7.9 Hz, 1H), 8.30-8.34 (m, 1H), 7.64-7.73 (m, 5H), 7.52-7.57 (m, 4H), 7.45 (t, J = 7.6 Hz, 1H), 7.37 (s, 1H), 6.95 (d, J = 8.2 Hz, 1H), 6.91 (s, 1H), 6.29-6.34 (m, 2H), 4.58-4.65 (m, 1H), 4.08-4.13 (m, 3H), 3.64 (s, 3H, OCH_3), 3.55 (s, 2H), 2.52-2.60 (m, 1H), 2.33-2.40 (m, 1H), 1.90-1.97 (m, 1H), 0.64-0.69 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 589.2642 $[M+H]^+$ (calcd for $C_{32}H_{37}N_4O_7$, 589.2662).

3.1.7. N-4-(4-Fluorobenzylpiperidinyl)acyl-L-Asn-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (14)

Yield: 50%; mp 224-225 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.89 (s, 1H), 8.20-8.32 (m, 2H), 7.63-7.74 (m, 2H), 7.29-7.40 (m, 2H), 7.08-7.12 (m, 2H), 6.86-6.91 (m, 2H), 6.22-6.33 (m, 2H), 4.53-4.57 (m, 1H), 4.02-4.10 (m, 3H), 3.63 (s, 3H, OCH_3), 3.39 (s, 2H), 2.74-2.77 (m, 1H), 2.37-2.55 (m, 2H), 1.82-1.97 (m, 5H), 1.50-1.60 (m, 4H), 0.76-0.78 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 586.3036 $[M+H]^+$ (calcd for $C_{30}H_{41}FN_5O_6$, 586.3041).

3.1.8. N-2-(5-Methyl-2-phenylthiazolyl)acetyl-L-Asn-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (15)

Yield: 50.4%; mp 215-216 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.69 (s, 1H), 8.44 (d, J = 7.5 Hz, 1H), 8.34-8.38 (m, 1H), 7.83-7.85 (m, 2H), 7.69 (d, J = 8.7 Hz, 1H), 7.44-7.46 (m, 4H), 6.94-6.98 (m, 2H), 6.36 (s, 1H), 6.32 (d, J = 8.3 Hz, 1H), 4.61-4.68 (m, 1H), 4.10-4.17 (m, 3H), 3.64-3.71 (m, 2H), 3.66 (s, 3H, OCH_3), 2.55-2.62 (m, 1H), 2.43-2.50 (m, 1H), 2.41 (s, 3H), 1.94-2.00 (m, 1H), 0.70-0.75 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 582.2373 $[M+H]^+$ (calcd for $C_{29}H_{36}N_5O_6S$, 582.2386).

3.1.9. N-2-(3-Indolyl)acetyl-L-Asn-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (16)

Yield: 63%; mp 214-215 °C; ^{1}H-NMR (300 MHz, CD_3OD): δ 8.27 (s, 1H), 8.02 (d, J = 7.8 Hz, 1H), 7.48 (d, J = 9.0 Hz, 1H), 7.27 (d, J = 9.0 Hz, 1H), 7.14 (s, 1H), 6.92-7.06 (m, 3H), 6.28-6.30 (m, 2H), 4.72 (t, J = 6.0 Hz, 1H), 4.18 (s, 2H), 4.01-4.08 (m, 1H), 3.70 (s, 3H, OCH_3), 3.65-3.69 (m, 2H), 2.51-2.67 (m, 3H), 0.66-0.72 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 524.2512 $[M+H]^+$ (calcd for $C_{27}H_{34}N_5O_6$, 524.2509).

3.1.10. N-2-(3-(5-Methoxyindolyl))acetyl-L-Asn-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (17)

Yield: 35.7%; mp 206-208 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.70 (s, 1H), 9.57 (s, 1H), 8.36 (s, 1H), 8.28 (d, J = 7.3 Hz, 1H), 7.73 (d, J = 8.8 Hz, 1H), 7.44 (s, 1H), 7.20 (d, J = 8.8 Hz, 1H), 7.14 (s, 1H), 7.03 (s, 1H), 6.96 (d, J = 8.1 Hz, 1H), 6.90 (s, 1H), 6.69 (d, J = 6.9 Hz, 1H), 6.35 (s, 1H), 6.30 (d, J = 8.2 Hz, 1H), 4.61-4.65 (m, 1H), 4.12 (brs, 3H), 3.75 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 3.51 (s, 2H), 2.50-2.60 (m, 1H), 2.37-2.45 (m, 1H), 1.93-1.98 (m, 1H), 0.68-0.73 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 554.2605 $[M+H]^+$ (calcd for $C_{28}H_{36}N_5O_7$, 554.2615).

3.1.11. N-2-(3-Indolyl)-2-ketone-acetyl-L-Asn-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (18)

Yield: 50.2%; mp 215-216 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.91 (d, J = 7.9 Hz, 1H), 8.73 (s, 1H), 8.37 (t, J = 5.6 Hz, 1H), 8.22-8.25 (m, 1H), 7.87 (d, J = 8.6 Hz, 1H), 7.53-7.56 (m, 1H), 7.43 (s, 1H), 7.25-7.28 (m, 2H), 6.98 (d, J = 8.2 Hz, 2H), 6.38 (d, J = 2.2 Hz, 1H), 6.31 (dd, J = 8.4 Hz, 2.2 Hz, 1H), 4.68-4.75 (m, 1H), 4.09-4.21 (m, 3H), 3.63 (s, 3H, OCH_3), 3.33 (s, 2H), 2.60-2.67 (m, 2H), 1.94-2.02 (m, 1H), 0.79-0.81 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 538.2294 $[M+H]^+$ (calcd for $C_{27}H_{32}N_5O_7$, 538.2302).

3.1.12. N-2-(6-Methoxybenzofuranyl)acetyl-L-Asn-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (19)

Yield: 40%; mp 204-206 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.54 (s, 1H), 8.40-8.46 (m, 1H), 8.28-8.34 (m, 1H), 7.69-7.73 (m, 1H), 7.67 (s, 1H), 7.47 (d, J = 9.0 Hz, 1H), 7.36-7.39 (m,1H), 7.11-7.12 (m, 1H), 6.92-6.98 (m, 2H), 6.80-6.84 (m, 1H), 6.26-6.34 (m, 2H), 4.62-4.66 (m, 1H), 4.09-4.16 (m, 3H), 3.76 (s, 3H, OCH_3), 3.64 (s, 3H, OCH_3), 3.50 (s, 2H), 2.52-2.59 (m, 1H), 2.37-2.44 (m, 1H), 1.93-1.99 (m, 1H), 0.68-0.77 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 555.2458 $[M+H]^+$ (calcd for $C_{28}H_{35}N_4O_8$, 555.2455).

3.1.13. N-2-(3-Isobenzofuran-1-one)acetyl-L-Asn-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (20)

Yield: 30%; mp 262-263 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.55 (d, J = 2.0 Hz, 1H), 8.35-8.41 (m, 2H), 7.81 (d, J = 7.6 Hz, 1H), 7.74 (d, J = 7.5 Hz, 1H), 7.65-7.70 (m, 1H), 7.58 (t, J = 7.6 Hz, 1H), 7.43 (d, J = 9.0 Hz, 1H), 6.96-7.01 (m, 2H), 6.31-6.36 (m, 2H), 5.86-5.92 (m, 1H), 4.71-4.75 (m, 1H), 4.12-4.16 (m, 3H), 3.73(m, 1H), 3.66 (s, 3H, OCH_3), 2.81-2.88 (m, 1H), 2.67-2.75 (m, 1H), 2.54-2.62 (m, 1H), 2.37-2.45 (m, 1H), 2.03-2.09 (m, 1H), 0.83-0.85 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 541.2307 $[M+H]^+$ (calcd for $C_{27}H_{33}N_4O_8$, 541.2298).

3.1.14. N-(tert-butyl (S)-(1-(4-Hydroxyphenyl)-3-oxobutanyl))acyl-L-Asn-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (21)

Yield: 64.7%; mp 162-163 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.53 (s, 1H), 9.11 (s, 1H), 8.32-8.34 (m, 1H), 8.19 (d, J = 7.5 Hz, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.40 (s, 1H), 6.94-7.02 (m, 4H), 6.81 (d, J = 8.5 Hz, 1H), 6.61 (d, J = 8.3 Hz, 2H), 6.34 (d, J = 2.3 Hz, 1H), 6.30 (dd, J = 8.3 Hz, 2.3 Hz, 1H), 4.57-4.63 (m, 1H), 4.10-4.17 (m, 4H), 3.64 (s, 3H, OCH_3), 2.80-2.83 (m, 1H), 2.54-2.60 (m, 2H), 2.41-2.45 (m, 1H), 1.97-2.02 (m, 1H), 1.28 (s, 9H), 0.77-0.80 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 630.3111 $[M+H]^+$ (calcd for $C_{31}H_{44}N_5O_9$, 630.3139).

3.1.15. N-((S)-N-((S)-1-(4-Hydroxyphenyl)-3-oxobutan-2-yl))acyl-L-Asn-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (22)

Yield: 13.7%; mp 220-221 °C; ^{1}H-NMR (300 MHz, CD_3OD): δ 6.96-7.02 (m, 3H), 6.62 (d, J = 8.4 Hz, 2H), 6.26-6.31 (m, 2H), 4.62-4.67 (m, 1H), 4.48-4.53 (m, 1H), 4.21 (s, 2H), 4.13 (d, J = 6.0 Hz, 1H), 3.66 (s, 3H, OCH_3), 2.98-3.02 (m, 1H), 2.62-2.75 (m,

3H), 2.04-2.18 (m, 2H), 1.13-1.30 (m, 2H), 0.98 (d, $J = 6.0$ Hz, 2H), 0.82-0.86 (m, 6H, $C(CH_3)_2$), 0.72-0.76 (m, 3H), 0.54-0.69 (m, 1H); HR-ESI-MS: m/z 614.3188 $[M + H]^+$ (calcd for $C_{31}H_{44}N_5O_8$, 614.3190).

3.1.16. N-2-(3-Phenoxyphenyl)acetyl-L-Ser-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (23)

Yield: 52.6%; mp 145-146 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.54 (s, 1H), 8.18-8.25 (m, 2H), 7.73 (d, $J = 8.8$ Hz, 1H), 7.36 (t, $J = 7.5$ Hz, 2H), 7.27 (t, $J = 7.5$ Hz, 1H), 7.11 (t, $J = 7.5$ Hz, 1H), 6.94-7.03 (m, 5H), 6.82 (dd, $J = 7.8$ Hz, 1.9 Hz, 1H), 6.34 (d, $J = 2.3$ Hz, 1H), 6.30 (dd, $J = 8.3$ Hz, 2.4 Hz, 1H), 4.99 (t, $J = 5.1$ Hz, 1H), 4.35-4.41 (m, 1H), 4.08-4.19 (m, 3H), 3.64 (s, 3H, OCH_3), 3.50-3.55 (m, 2H), 3.48 (s, 2H), 1.93-2.0 (m, 1H), 0.74-0.78 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 550.2549 $[M + H]^+$ (calcd for $C_{30}H_{36}N_3O_7$, 550.2553).

3.1.17. N-2-(3-Phenoxyphenyl)acetyl-L-Thr-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (24)

Yield: 20%; mp 135-136 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.56 (s, 1H), 8.27-8.31 (m, 1H), 8.06 (d, $J = 8.4$ Hz, 1H), 7.66 (d, $J = 8.4$ Hz, 1H), 7.38 (t, $J = 8.0$ Hz, 2H), 7.29 (t, $J = 8.0$ Hz, 1H), 7.13 (t, $J = 7.4$ Hz, 1H), 7.06 (d, $J = 7.8$ Hz, 1H), 6.97-7.01 (m, 4H), 6.85 (d, $J = 7.8$ Hz, 1H), 6.36 (d, $J = 2.4$ Hz, 1H), 6.31 (dd, $J = 8.3$ Hz, 2.4 Hz, 1H), 4.95 (d, $J = 4.8$ Hz, 1H), 4.35-4.41 (m, 1H), 4.28-4.32 (m, 1H), 4.1-4.2 (m, 2H), 3.92-3.98 (m, 1H), 3.66 (s, 3H, OCH_3), 3.52-3.56 (m, 2H), 1.91-2.00 (m, 1H), 0.95 (d, $J = 6.0$ Hz, 3H), 0.78-0.82 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 564.2716 $[M + H]^+$ (calcd for $C_{31}H_{38}N_3O_7$, 564.2710).

3.1.18. N-2-(3-Phenoxyphenyl)acetyl-L-Val-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (25)

Yield: 22%; mp 140-141 °C; ^{1}H-NMR (300 MHz, CD_3OD): δ 7.24-7.35 (m, 3H), 7.02-7.10 (m, 3H), 6.94-6.99 (m, 3H), 6.85 (d, $J = 8.1$ Hz, 1H), 6.31-6.35 (m, 2H), 4.24(d, $J = 6.0$ Hz, 2H), 4.08-4.19 (m, 2H), 3.71 (s, 3H, OCH_3), 3.52 (d, $J = 6.0$ Hz, 2H), 1.96-2.05 (m, 2H), 0.84-0.87 (m, 12H, $C(CH_3)_2$); HR-ESI-MS: m/z 562.2902 $[M + H]^+$ (calcd for $C_{32}H_{40}N_3O_6$, 562.2917).

3.1.19. N-2-(3-Indolyl)acetyl-L-Ser-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (26)

Yield: 59%; mp 198-200 °C; ^{1}H-NMR (300 MHz, CD_3OD): δ 7.56 (d, $J = 8.1$ Hz, 1H), 7.34 (d, $J = 8.1$ Hz, 1H), 7.20 (s, 1H), 7.12-7.00 (m, 3H), 6.31-6.35 (m, 2H), 4.45-4.52 (m, 1H), 4.15-4.24 (m, 3H), 3.75-3.81 (m, 1H), 3.73 (s, 2H), 3.67 (s, 3H, OCH_3), 2.69 (m, 1H), 1.98-2.04 (m,1H), 0.77-0.84 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 497.2403 $[M + H]^+$ (calcd for $C_{26}H_{33}N_4O_6$, 497.2400).

3.1.20. N-2-(3-Indolyl)acetyl-L-Thr-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (27)

Yield: 49%; mp 94-95 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.84 (brs, 1H), 9.54 (s, 1H), 8.25-8.29 (m, 1H), 7.77 (d, $J = 7.9$ Hz, 1H), 7.64(d, $J = 8.7$ Hz, 1H), 7.53 (d, $J = 7.8$ Hz, 1H), 7.31 (d, $J = 8.1$ Hz, 1H), 7.20 (s, 1H), 7.04 (t, $J = 7.5$ Hz, 1H), 6.91-6.98 (m, 2H), 6.34 (d, $J = 2.3$ Hz, 1H), 6.30 (dd, $J = 8.4$ Hz, 2.2 Hz, 1H), 4.92 (d,

$J = 4.8$ Hz, 1H), 4.28-4.32 (m, 1H), 4.08-4.20 (m, 3H), 3.92-3.97 (m, 1H), 3.60-3.64 (m, 2H), 3.55 (s, 3H, OCH_3), 1.91-1.97 (m, 1H), 0.95 (d, $J = 6.0$ Hz, 3H), 0.76-0.80 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: *m/z* 511.2555 $[M + H]^+$ (calcd for $C_{27}H_{35}N_4O_6$, 511.2557).

3.1.21. N-2-(3-Indolyl)acetyl-L-Val-L-Val-NH-(2-hydroxy-4-methoxybenzyl) (28)

Yield: 29%; mp 145-146 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.81 (brs, 1H), 9.54 (s, 1H), 8.19-8.23 (m, 1H), 7.87 (d, $J = 8.9$ Hz, 1H), 7.80 (d, $J = 8.8$ Hz, 1H), 7.52 (d, $J = 7.9$ Hz, 1H), 7.30 (d, $J = 8.1$ Hz 1H), 7.16 (s, 1H), 6.89-7.05 (m, 3H), 6.34 (d, $J = 2.3$ Hz, 1H), 6.28 (dd, $J = 8.3$ Hz, 2.3 Hz, 1H), 4.18-4.23 (m, 1H), 4.09-4.14 (m, 3H), 3.64 (s, 3H, OCH_3), 3.50-3.55 (m, 2H), 1.86-1.97 (m, 2H), 0.74-0.78 (m, 12H, $C(CH_3)_2$); HR-ESI-MS: *m/z* 509.2762 $[M + H]^+$ (calcd for $C_{28}H_{37}N_4O_5$, 509.2764).

3.1.22. N-2-(3-Phenoxyphenyl)acetyl-L-Asn-L-Val-NH-(2-chlorobenzyl) (29)

Yield: 73.4%; mp 252-253 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.52 (t, $J = 5.8$ Hz, 1H), 8.41 (d, $J = 7.5$ Hz, 1H), 7.81 (d, $J = 8.1$ Hz, 1H), 7.36-7.41 (m, 4H), 7.25-7.32 (m, 4H), 7.13 (t, $J = 7.2$ Hz, 1H), 6.95-7.05 (m, 5H), 6.84 (dd, $J = 8.1$ Hz, 1.8 Hz, 1H), 4.62-4.68 (m, 1H), 4.31-4.33 (m, 2H), 4.14-4.19 (m, 1H), 3.46 (s, 2H), 2.55-2.62 (m, 1H), 2.36-2.43 (m, 1H), 2.01-2.08 (m, 1H), 0.74-0.80 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: *m/z* 565.2232 $[M + H]^+$ (calcd for $C_{30}H_{34}ClN_4O_5$, 565.2218).

3.1.23. N-2-(3-Phenoxyphenyl)acetyl-L-Asn-L-Val-NH-(4-methylbenzyl) (30)

Yield: 79%; mp 249-250 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.36-8.41 (m, 2H), 7.67 (d, $J = 8.7$ Hz, 1H), 7.34-7.38 (m, 3H), 7.26 (t, $J = 7.8$ Hz, 1H), 7.09-7.13 (m, 5H), 6.93-7.02 (m, 5H), 6.82 (d, $J = 8.0$ Hz, 1H), 4.58-4.64 (m, 1H), 4.07-4.19 (m, 3H), 3.44 (s, 2H), 2.52-2.59 (m, 1H), 2.32-2.40 (m, 1H), 2.24 (s, 3H, CH_3), 1.96-2.00 (m, 1H), 0.68-0.78 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: *m/z* 545.2761 $[M + H]^+$ (calcd for $C_{31}H_{37}N_4O_5$, 545.2764).

3.1.24. N-2-(3-Phenoxyphenyl)acetyl-L-Asn-L-Val-NH-(2-thienyl) (31)

Yield: 73.2%; mp 240-241 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.51-8.58 (m, 1H), 8.32-8.39 (m, 1H), 7.67(d, $J = 8.9$ Hz, 1H), 7.34-7.39 (m, 4H), 7.26 (t, $J = 7.5$ Hz, 1H), 7.11 (t, $J = 7.5$ Hz, 1H), 6.96-7.02 (m, 3H), 6.89-6.92 (m, 4H), 6.80-6.84 (m, 1H), 4.58-4.64 (m, 1H), 4.36-4.40 (m, 2H), 4.06-4.13 (m, 1H), 3.44 (s, 2H), 2.51-2.58 (m,1H), 2.32-2.40 (m, 1H), 1.96-2.00 (m, 1H), 0.69-0.78 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: *m/z* 537.2166 $[M + H]^+$ (calcd for $C_{28}H_{33}N_4O_5S$, 537.2172).

3.1.25. N-2-(1-Biphenyl)acetyl-L-Asn-L-Val-L-allo-Thr-OBn (32)

Yield: 30%; mp 242-243 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.44 (d, $J = 7.4$ Hz, 1H), 8.19 (d, $J = 6.8$ Hz, 1H), 7.62 (d, $J = 7.3$ Hz, 4H), 7.55 (d, $J = 8.0$ Hz, 4H), 7.44 (t, $J = 7.3$ Hz, 3H), 7.33 (t, $J = 7.4$ Hz, 5H), 6.90 (s, 1H), 5.01 (d, $J = 5.1$ Hz, 1H), 4.60-4.63 (m, 1H), 4.14-4.26 (m, $J = 6.0$ Hz, 2H), 3.85-3.90 (m, 1H), 3.58 (s, 3H), 3.50 (s, 2H), 2.53-2.62 (m, 1H), 2.34-2.42 (m, 1H), 1.88-1.93 (m, 1H), 1.09 (d, $J = 6.0$ Hz, 3H, CH_3), 0.76 (d, $J = 6.3$ Hz, 3H, CH_3), 0.69 (d, $J = 6.4$ Hz, 3H, CH_3); HR-ESI-MS: *m/z* 617.2977 $[M + H]^+$ (calcd for $C_{34}H_{41}N_4O_7$, 617.2975).

3.1.26. N-2-(3-Indolyl)acetyl-L-Asn-L-Val-L-allo-Thr-OBn (33)

Yield: 44%; mp 234-235 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 10.85 (brs, 1H), 8.26 (d, J = 7.7 Hz, 2H), 7.51-7.58 (m, 2H), 7.34 (brs, 7H), 7.19 (s, 1H), 7.05 (t, J = 7.2 Hz, 1H), 6.93 (t, J = 7.2 Hz, 1H), 6.87 (s, 1H), 5.10 (s, 2H), 5.06 (d, J = 5.5 Hz, 1H), 4.54-4.71 (m, 1H), 4.24 (t, J = 7.0 Hz, 2H), 3.90-3.96 (m, 1H), 3.55 (s, 2H), 2.50-2.58 (m, 1H), 2.34-2.42 (m, 1H), 1.82-1.92 (m, 1H), 1.10 (d, J = 6.3 Hz, 3H, CH_3), 0.72 (d, J = 6.7 Hz, 3H, CH_3), 0.65 (d, J = 6.7 Hz, 3H, CH_3); HR-ESI-MS: m/z 580.2732 $[M+H]^+$ (calcd for $C_{30}H_{38}N_5O_7$, 580.2771).

3.1.27. N-2-(4-Cyclohexylphenyl)acyl-L-Asn-L-Val-L-allo-Thr-OBn (34)

Yield: 43%; mp 219-220 °C; ^{1}H-NMR (300 MHz, CD_3OD): δ 7.77-7.82 (m, 2H), 7.33-7.37 (m, 7H), 5.19 (s, 2H), 4.94-4.97 (m, 1H), 4.46-4.48 (m, 1H), 4.30-4.32 (m, 1H), 4.11-4.13 (m, 1H), 2.77-2.83 (m, 2H), 2.54-2.60 (m, 1H), 2.07-2.09 (m, 1H), 1.75-1.85 (m, 5H), 1.42-1.47 (m, 3H), 1.20-1.23 (m, 3H), 0.88-0.90 (m, 6H, $C(CH_3)_2$); HR-ESI-MS: m/z 609.3225 $[M+H]^+$ (calcd for $C_{33}H_{45}N_4O_7$, 609.3288).

3.1.28. N-2-(3,4,5-Trimethoxylphenyl)propionyl -L-Asn-L-Val-L-allo-Thr-OBn (35)

Yield: 51.7%; mp 241-242 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.35 (d, J = 7.3 Hz, 1H), 8.27 (d, J = 7.8 Hz, 1H), 7.62 (d, J = 9.2 Hz, 1H), 7.42 (s, 1H), 6.89 (s, 5H), 6.88 (s, 1H), 6.51 (s, 2H), 5.14 (d, J = 5.5 Hz, 1H), 5.10 (s, 2H), 4.60-4.64 (m, 1H), 4.22-4.28 (m, 2H), 3.92-3.98 (m, 1H), 3.74 (s, 6H, OCH_3), 3.61 (s, 3H, OCH_3), 2.71-2.76 (m, 2H), 2.36-2.45 (m, 4H), 1.92-1.98 (m, 1H), 1.11 (d, J = 6.0 Hz, 3H), 0.79 (d, J = 6.0 Hz, 3H, CH_3), 0.74 (d, J = 6.0 Hz, 3H, CH_3); HR-ESI-MS: m/z 645.3112 $[M+H]^+$ (calcd for $C_{32}H_{45}N_4O_{10}$, 645.3136).

3.1.29. N-2-(2-Pyrazinyl)acyl-L-Asn-L-Val-L-allo-Thr-OBn (36)

Yield: 51.5%; mp 205-206 °C; ^{1}H-NMR (300 MHz, CD_3OD): δ 9.23 (s, 1H), 8.79 (s, 1H), 8.69 (s, 1H), 7.31-7.36 (m, 5H), 5.16 (s, 2H), 4.96-5.00 (m, 1H), 4.44 (d, J = 6.0 Hz, 1H), 4.27 (d, J = 6.0 Hz, 1H), 4.08 (t, J = 6.0 Hz, 1H), 2.83 (t, J = 6.0 Hz, 2H), 2.05-2.11 (m, 4H), 1.21(m, 4H), 0.89 (m, 8H); HR-ESI-MS: m/z 529.2405 $[M+H]^+$ (calcd for $C_{25}H_{33}N_6O_7$, 529.2411).

3.1.30. N-2-(3-Phenoxyphenyl)acetyl-L-Asn-L-Val-D-allo-Thr-OMe (37)

Yield: 71.4%; mp 215-217 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.38 (d, J = 7.2 Hz, 1H), 8.00 (d, J = 7.2 Hz, 1H), 7.59 (d, J = 9.0 Hz, 1H), 7.34-7.39 (m, 3H), 7.26 (t, J = 7.5 Hz, 1H), 7.11 (t, J = 7.2 Hz, 1H), 6.96-7.02 (m, 2H), 6.88-6.92 (m, 2H), 6.81-6.83 (m, 2H), 4.93-4.95 (m, 1H), 4.55-4.62 (m, 2H), 4.20-4.25 (m, 2H), 4.04-4.12 (m, 1H), 3.59 (s, 3H, OCH_3), 3.43 (s, 2H), 2.52-2.60 (m, 2H), 2.30-2.38 (m, 1H), 1.94-1.99 (m, 1H), 1.04 (d, J = 6.3 Hz, 1H), 0.77 (d, J = 6.8 Hz, 3H, CH_3), 0.70 (d, J = 6.8 Hz, 3H, CH_3); HR-ESI-MS: m/z 557.2605 $[M+H]^+$ (calcd for $C_{28}H_{37}N_4O_8$, 557.2611).

3.1.31. N-2-(3-Phenoxyphenyl)acetyl-L-Asn-L-Val-D-Ser-OMe (38)

Yield: 58%; mp 165-166 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.41 (d, J = 8.1 Hz, 1H), 8.28 (d, J = 7.2 Hz, 1H), 7.55 (d, J = 9.0 Hz, 1H), 7.36-7.41 (m, 3H), 7.28 (t,

$J = 7.8$ Hz, 1H), 7.13 (t, $J = 7.2$ Hz, 1H), 6.98-7.04 (m, 2H), 6.90-6.94 (m, 2H), 6.82-6.85 (m, 2H), 4.56-4.62 (m, 1H), 4.23-4.30 (m, 2H), 3.63-3.69 (m, 2H), 3.61 (s, 3H, OCH_3), 3.45 (s, 2H), 2.53-2.60 (m, 2H), 2.32-2.39 (m, 1H), 1.92-1.98 (m, 1H), 0.73 (d, $J = 6.8$ Hz, 3H, CH_3), 0.71 (d, $J = 6.8$ Hz, 3H, CH_3); HR-ESI-MS: *m/z* 543.2468 $[M + H]^+$ (calcd for $C_{27}H_{35}N_4O_8$, 543.2455).

3.2. Biological evaluation

The chymotrypsin-like, trypsin-like and caspase-like activities of the human 20S proteasome activity were determined by monitoring the decrease in hydrolysis of the fluorogenic substrates Suc-LLVY-AMC, Bz-VGR-AMC and (Z)-LLE-bNA, respectively. The rate of the cleavage reaction was measured with the excitation wavelength of 365 nm and the emission wavelength of 465 nm [14].

3.3. Molecular modeling

The structure of compound **18** was generated and molecular docking was performed with Glide (version 2007, Schrödinger, LLC, New York). The structure of the 20S proteasome (β5 and β6 subunits) was obtained from the Protein Data Bank (PDB code: 3SDK). Prior to docking for **18**, the co-crystal ligand was redocked into the active site successfully within a RMSD value of 2.00 Å. All docking calculations were run in the Standard Precision (SP) mode of Glide with default settings. The best scored pose was kept with a SP docking score of −10.956 kcal/mol.

Disclosure statement

No potential conflict of interest was reported by the authors.

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