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Design, synthesis and biological evaluation of novel tripeptidyl epoxyketone derivatives constructed from β-amino acid as proteasome inhibitors

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ABSTRACT

A series of novel tripeptidyl epoxyketone derivatives constructed from β-amino acid were designed, synthesized and evaluated as proteasome inhibitors. All target compounds were tested for their proteasome inhibitory activities and selected compounds were tested for their anti-proliferation activities against two multiple myeloma (MM) cell lines RPMI 8226 and NCI-H929. Among them, eleven compounds exhibited proteasome inhibitory rates of more than 50% at the concentration of 1 μg/mL and nine compounds showed anti-proliferation activities with IC_{50} values at low micromolar level. Compound **20h** displayed the most potent proteasome inhibitory activities (IC_{50}: 0.11 ± 0.01 μM) and anti-proliferation activities with IC_{50} values at 0.23 ± 0.01 and 0.17 ± 0.02 μM against two tested cell lines. Additionally, the polyubiquitin accumulation in the western blot analysis supported that proteasome inhibition in a cellular system was induced by compound **20h**. All these experimental results confirmed that β-amino acid can be introduced as a building block for the development of proteasome inhibitors.

1. Introduction

The functionally active proteasome is a proteolytic complex that is responsible for digestion of about 80–90% intracellular proteins including misfolded and abnormal proteins as well as various regulatory proteins associated with cell growth, differentiation, and apoptosis into short peptides and amino acids.[1–5] The prominent roles of proteasome in protein degradation has made it a promising anti-cancer drug target.[6–8] To date, various proteasome inhibitors have been identified and developed, and a few of them are extensively evaluated in clinical trials with possibilities for clinical applications.[9]

The approval of proteasome inhibitors bortezomib and carfilzomib (Fig. 1) for the treatment of multiple myeloma (MM) validates the close correlation between this proteolytic particle and tumorigenesis, maintenance and progression.[10,11] Compared to bortezomib, the peptidyl epoxyketone compound carfilzomib causes fewer side effects, especially low rates of peripheral neuropathy.[12,13] In addition, carfilzomib was proved to be able to overcome bortezomib resistance in some cancer models due to its longer and irreversible inhibition of the proteasome.[14] Herein, development of carfilzomib analogue with epoxyketone fragment may lead to proteasome inhibitors with enhanced safety profiles.

To best of our knowledge, most proteasome inhibitors approved or under clinical trials are short peptides sharing a similar α-peptide skeleton. It is reported that adding an extra backbone carbon by introducing a β-amino acid building block could improve the biological activity and enzymatic stability of the compound.[15] Besides, Zhu and colleagues reported some β-amino acid contained dipeptidyl boronic acid compounds with similar proteasome inhibitory activities and even better pharmacokinetic profiles in comparison with bortezomib, which validated the remarkable value of this building block.[16,17] In this manuscript, 33 tripeptidyl epoxyketone proteasome inhibitors (Fig. 2) constructed from β-amino acid (i.e. 3-amino-3-phenylpropionic acid in this paper) were synthesized and evaluated, and structure–activity relationships (SARs) were discussed in detail.

2. Results and discussion

2.1. Chemistry

The epoxyketone fragment **7** was synthesized following the method described in the literature with modifications,[18,19] and

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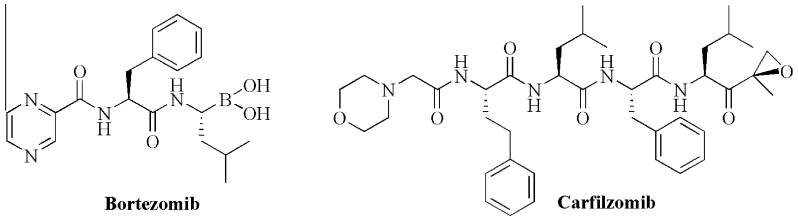


Figure 1. Structures of approved proteasome inhibitors bortezomib and carfilzomib.

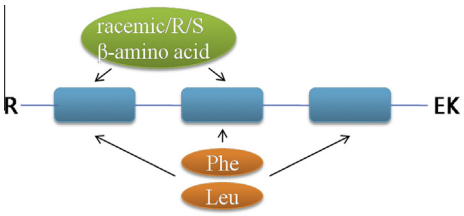


Figure 2. Epoxyketone tripeptidyl compounds constructed from β-amino acid. R: various substitutions at terminal positions of the tripeptides EK: epoxyketone.

the synthetic routes are summarized in Scheme 1. Reaction of *N*-Boc-protected amino acid **1** with *N,O*-dimethylhydroxylamine hydrochloride gave Weinreb amide **2**, which was then treated with isopropenylmagnesium bromide at 0 °C to form the desired α,β-unsaturated ketone **3**.[18] Subsequent reduction of **3** with sodium borohydride and cerium chloride afforded allylic alcohol **4**, which was oxidized into epoxide **5** in the presence of mCPBA.[18,19] The epoxide **5** was not stable enough and was thereby oxidized into epoxyketone **6** directly with Dess–Martin reagent.[18] Afterwards, deprotection of **6** with trifluoroacetic acid resulted in compound **7**.

The synthetic routes for tripeptidyl epoxyketone derivatives are summarized in Schemes 2 and 3. The racemic and enantiomeric *N*-Boc-protected β-amino acids were purchased from commercial sources. Reaction of epoxyketone **7a** or **7b** with corresponding *N*-Boc-protected amino acid furnished dipeptides **8**, **11** or **16**, which were deprotected and treated with various *N*-Boc-protected amino acids again to afford tripeptides **9**, **12**, **14**, **17**, **19** or **21**. Finally, the Boc-protecting group of the tripeptides were removed and the generated products were subjected to reaction with corresponding acid to obtain target compounds **10a–e**, **13a–e**, **15a–e**, **18a–n**, **20f**, **20h**, **22f** and **22h**.

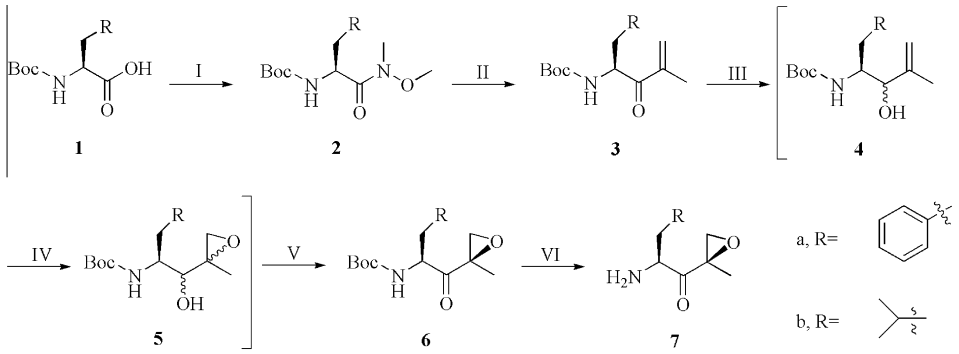
2.2. Proteasome inhibitory activities

The synthesized target compounds were evaluated for their 20S proteasome chymotrypsin-like inhibitory activities in vitro. Bortezomib and carfilzomib were employed as the positive controls. The results are summarized in Tables 1–3.

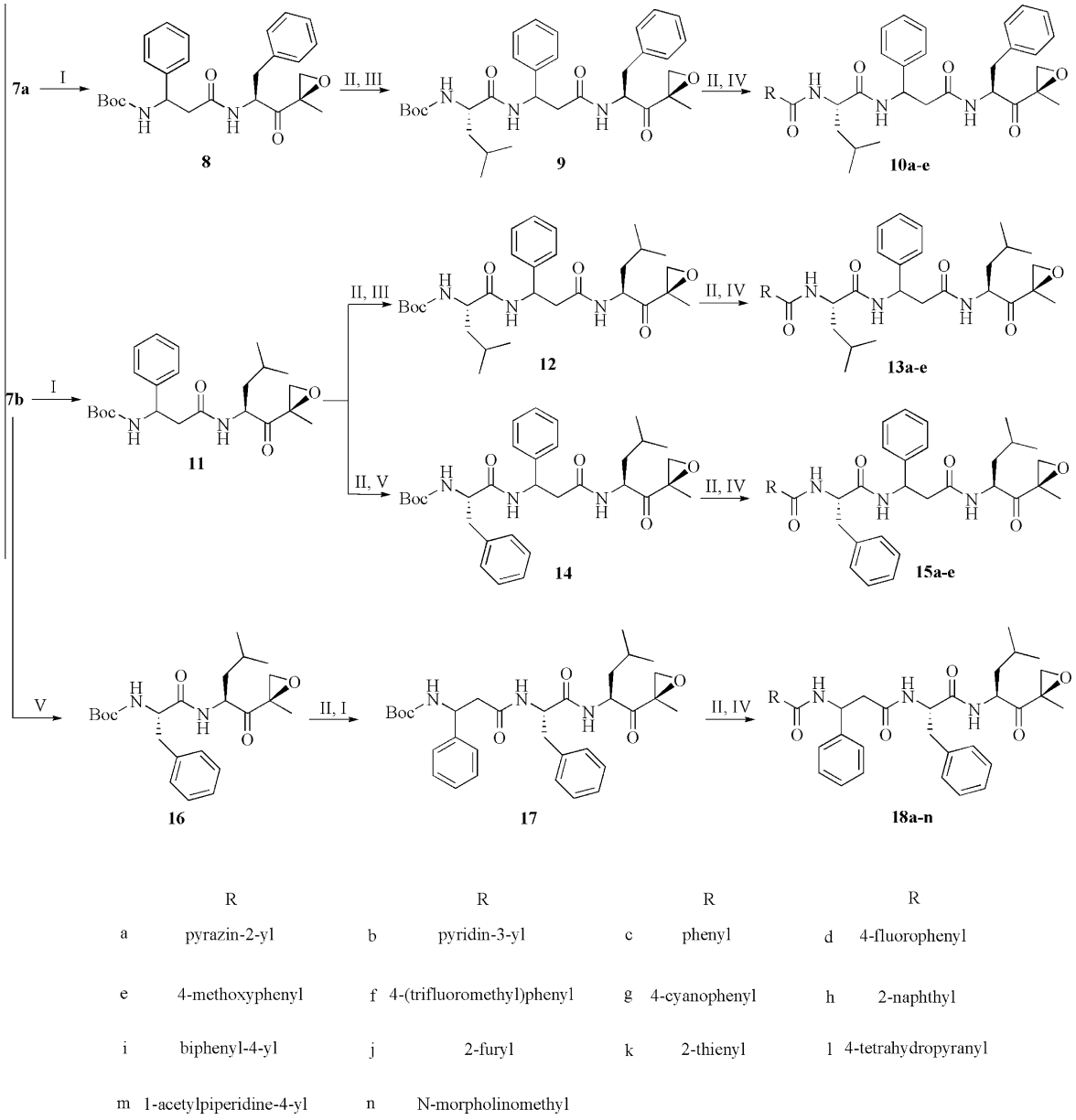
As shown in Table 1, compounds (**18a–e**) with β-amino acid-Phe-Leu skeleton exhibited the best proteasome inhibitory activities, and four compounds (**18b–e**) showed inhibitory rates of more than 80% at the concentration of 1 μg/mL, with IC_{50} values of 0.34 ± 0.03, 0.44 ± 0.04, 0.38 ± 0.03, and 0.34 ± 0.04 μM, respectively. However, compounds (**10a–e**, **13a–e** and **15a–e**) with β-amino acid in the middle of the tripeptidyl skeleton (Leu-β-amino acid-Phe, Leu-β-amino acid-Leu and Phe-β-amino acid-Leu) displayed weak proteasome inhibitory activities. Therefore, the peptidyl skeleton played important roles in maintaining the activities of this series of compounds.

In order to intensively evaluate the influences of substituents at the end of carboxamide in compounds **18a–e** on proteasome inhibitory activity, compounds **18f–n** with various substituents at the end of carboxamide were synthesized. According to the data of inhibitory activities of compounds **18a–n** (Tables 1 and 2), compounds **18b–h** with phenyl or pyridyl moieties at the end of carboxamide showed more potent activities than that of compounds with five-membered aromatic heterocyclic rings (**18j–k**) and aliphatic heterocyclic rings (**18l–n**) at the end of carboxamide. In addition, various replacements on the phenyl ring (**18c–g**) only have a slight impact on activity.

Further study of the impact of stereo configuration of the β-amino acid on activity was performed with selected two stereo isomers (**20f** vs **22f**; **20h** vs **22h**) of compound **18f** and **18h**. As



Scheme 1. Synthesis of epoxyketone fragments (**7a** and **7b**). Reagents and conditions: (I) HOBt, EDCI, *N,O*-dimethylhydroxylamine hydrochloride, diisopropylethylamine, DCM, 0 °C; (II) isopropenylmagnesium bromide, THF, 0 °C; (III) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, THF, 0 °C–rt; (IV) *m*CPBA, DCM, 0 °C; (V) Dess–Martin periodinane, DCM, 0 °C–rt; (VI) trifluoroacetic acid, DCM, 0 °C–rt.



Scheme 2. Synthesis of tripeptidyl target compounds (**10a–e**, **13a–e**, **15a–e**, and **18a–n**). Reagents and conditions: (I) Boc-3-amino-3-phenylpropionic acid, HOBt, EDCI, diisopropylethylamine, DCM, rt; (II) trifluoroacetic acid, DCM, 0 °C–rt; (III) Boc-Leu, HOBt, EDCI, diisopropylethylamine, DCM, rt; (IV) RCOOH, HOBt, EDCI, diisopropylethylamine, DCM, rt; (V) Boc-Phe, HOBt, EDCI, diisopropylethylamine, DCM, rt.

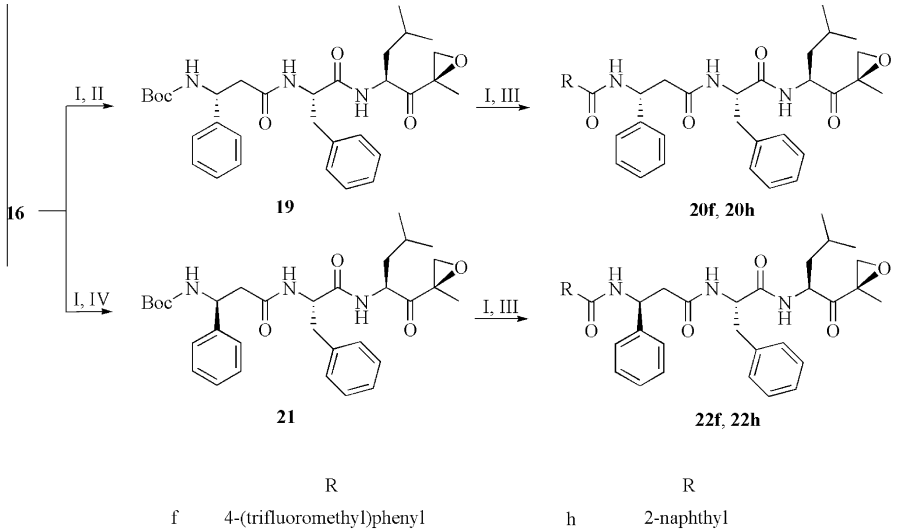
shown in Table 3, the *R*-isomers **20f** (84.44 ± 1.93% inhibition at 1 μg/mL) and **20h** (86.08 ± 1.48% inhibition at 1 μg/mL) exhibited much more potent inhibitory activities than that of corresponding *S*-isomers **22f** (25.63 ± 1.49% inhibition at 1 μg/mL) and **22h** (11.76 ± 6.86% inhibition at 1 μg/mL). The results suggested that the stereo configuration of the β-amino acid was also a critical factor for the activities of these compounds.

2.3. Tumor cell growth inhibition

Based on the evaluation of proteasome chymotrypsin-like inhibitory activities, selected compounds (**18b–h**, **20f**, and **20h**) were further tested for their cytotoxic activities in vitro against two MM cell lines (RPMI 8226 and NCI-H929) by MTS assay with bortezomib and carfilzomib employed as the positive controls. All the tested compounds showed potent cytotoxic activities against two MM cell lines, with IC_{50} values in low micromolar range, and two *R*-configuration compounds **20f** and **20h** exhibited the most potent cytotoxic activities (Table 4).

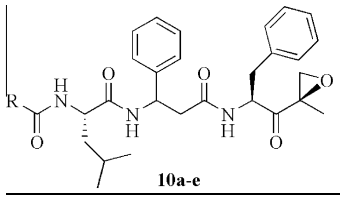
2.4. Western blot analysis

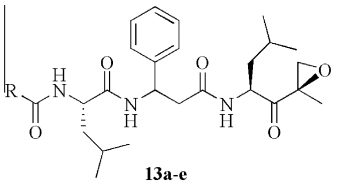
Subsequently, the proteasome inhibitory effect of selected compound **20h** was tested in a cellular system by Western blot analysis. Carfilzomib and compound **15e** were employed as the positive and negative control, respectively. As illustrated in Figure 3, poly-ubiquitin is clearly accumulated in RPMI 8226 cells treated with compound **20h**, which indicated that the compound induced proteasomal dysfunction in the cells. Additionally, cleavage of poly (ADP-ribose) polymerase (PARP), a critical marker of apoptosis,[20] was observed, suggesting that the apoptotic pathway was activated by **20h**. On the contrary, the negative compound **15e** was unable to accumulate poly-ubiquitin and induce PARP cleavage, which was mainly attributed to its poor proteasome inhibitory activity.



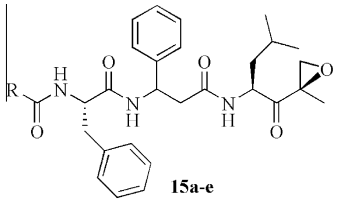
Scheme 3. Synthesis of tripeptidyl target compounds (**20f**, **20h**, **22f**, and **22h**). Reagents and conditions: (I) trifluoroacetic acid, DCM, 0 °C–rt; (II) Boc-(*R*)-3-amino-3-phenylpropionic acid, HOBt, EDCI, diisopropylethylamine, DCM, rt; (III) RCOOH, HOBt, EDCI, diisopropylethylamine, DCM, rt; (IV) Boc-(*S*)-3-amino-3-phenylpropionic acid, HOBt, EDCI, diisopropylethylamine, DCM, rt.

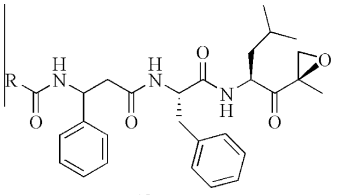
Table 1
20S proteasome chymotrypsin-like inhibitory activities of tripeptidyl target compounds (**10a–e**, **13a–e**, **15a–e**, and **18a–e**)





Compound	R	Inhibitory rate at 1 μg/mL[a] (%)	IC_{50}[a] (μM)	Compound	R	Inhibitory rate at 1 μg/mL[a] (%)	IC_{50}[a] (μM)
10a	Pyrazin-2-yl	8.70 ± 0.36	NT[b]	**13a**	Pyrazin-2-yl	9.01 ± 0.01	NT[b]
10b	Pyridin-3-yl	3.16 ± 0.39	NT[b]	**13b**	Pyridin-3-yl	0.84 ± 0.22	NT[b]
10c	Phenyl	1.56 ± 0.32	NT[b]	**13c**	Phenyl	6.42 ± 1.96	NT[b]
10d	4-Fluorophenyl	1.43 ± 0.19	NT[b]	**13d**	4-Fluorophenyl	5.46 ± 0.92	NT[b]
10e	4-Methoxyphenyl	0.39 ± 0.03	NT[b]	**13e**	4-Methoxyphenyl	0.56 ± 0.71	NT[b]





15a	Pyrazin-2-yl	1.51 ± 0.10	NT[b]	**18a**	Pyrazin-2-yl	48.61 ± 1.16	NT[b]
15b	Pyridin-3-yl	0.48 ± 0.04	NT[b]	**18b**	Pyridin-3-yl	85.76 ± 4.61	0.34 ± 0.03
15c	Phenyl	0.30 ± 0.87	NT[b]	**18c**	Phenyl	88.55 ± 7.10	0.44 ± 0.04
15d	4-Fluorophenyl	0.37 ± 0.33	NT[b]	**18d**	4-Fluorophenyl	87.72 ± 3.56	0.38 ± 0.03
15e	4-Methoxyphenyl	0.30 ± 0.69	NT[b]	**18e**	4-Methoxyphenyl	84.12 ± 5.55	0.34 ± 0.04
Bortezomib	—	—	9.77 ± 0.89 (nM)	Carfilzomib	—	—	8.60 ± 1.40 (nM)

[a] The inhibitory rate and IC_{50} values are an average of three independent determinations.
[b] NT = not tested.

3. Conclusion

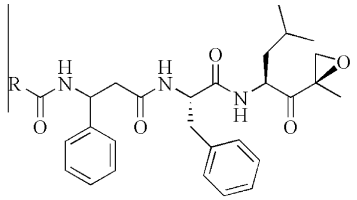
A series of novel tripeptidyl epoxyketone derivatives were synthesized and evaluated for their proteasome inhibitory activities. Among which, eleven compounds displayed potent proteasome inhibitory activities, with IC_{50} values lower than 1 μM, which were consistent with the cytotoxic activities against two MM cell lines RPMI 8226 and NCI-H929. In addition, the western blot analysis of selected compound **20h** suggested that proteasome inhibition caused an accumulation of poly-ubiquitin and cleavage of PARP, which resulted in cell apoptosis, thus explaining the cell growth inhibitory effects. This study validated the potential of developing β-amino acid constructed peptides as potent proteasome inhibitors, which might be helpful in further studies.

4. Experimental procedures

4.1. Chemistry

Melting points were determined on a Büchi B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Brüker 500 MHz spectrom-

Table 2
20S proteasome chymotrypsin-like inhibitory activities of tripeptidyl target compounds (**18f–n**)

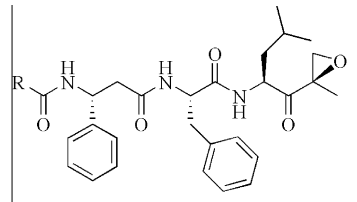


18f-n

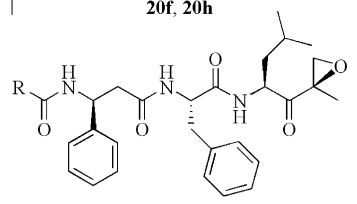
Compound	R	Inhibitory rate at 1 μg/mL[a] (%)	IC_{50}[a] (μM)
18f	4-(Trifluoromethyl)phenyl	76.05 ± 0.06	0.22 ± 0.01
18g	4-Cyanophenyl	72.00 ± 2.64	0.36 ± 0.02
18h	2-Naphthyl	74.83 ± 0.25	0.19 ± 0.01
18i	Biphenyl-4-yl	47.28 ± 1.59	NT[b]
18j	2-Furyl	42.27 ± 0.47	NT[b]
18k	2-Thienyl	62.46 ± 3.92	0.95 ± 0.07
18l	4-Tetrahydropyranyl	69.55 ± 19.22	0.63 ± 0.03
18m	1-Acetylpiperidine-4-yl	48.48 ± 2.41	NT[b]
18n	*N*-Morpholinomethyl	39.97 ± 4.23	NT[b]
Bortezomib	—	—	8.96 ± 0.56 (nM)

[a] The inhibitory rate and IC_{50} values are an average of three independent determinations.
[b] NT = not tested.

Table 3
20S proteasome chymotrypsin-like inhibitory activities of tripeptidyl target compounds constructed from enantiomeric β-amino acid (**20f**, **20h**, **22f**, and **22h**)



20f, **20h**



22f, **22h**

Compound	R	Inhibitory rate at 1 μg/mL[a] (%)	IC_{50}[a] (μM)
20f	4-(Trifluoromethyl)phenyl	84.44 ± 1.93	0.17 ± 0.01
20h	2-Naphthyl	86.08 ± 1.48	0.11 ± 0.01
22f	4-(Trifluoromethyl)phenyl	25.63 ± 1.49	NT[b]
22h	2-Naphthyl	11.76 ± 6.86	NT[b]
Bortezomib	—	—	7.14 ± 0.36 (nM)

[a] The inhibitory rate and IC_{50} values are an average of three independent determinations.
[b] NT = not tested.

eter (Brüker Bioscience, Billerica, MA, USA) with $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts (δ) were reported in partsper million (ppm) relative to internal TMS, and coupling constants (J) were reported in Hertz (Hz). Splitting patterns were designated as

Table 4
In vitro cytotoxic activities of selected compounds (**18b-h**, **20f**, and **20h**) against two MM cell lines

Compound	Cytotoxicity (IC_{50}, μM)[a]	
	RPMI 8226	NCI-H929
18b	4.73 ± 0.81	8.70 ± 0.27
18c	1.68 ± 0.08	2.69 ± 0.07
18d	1.55 ± 0.12	2.37 ± 0.13
18e	1.16 ± 0.04	1.35 ± 0.01
18f	0.32 ± 0.08	0.96 ± 0.06
18g	0.41 ± 0.09	0.61 ± 0.03
18h	0.42 ± 0.01	0.70 ± 0.04
20f	0.34 ± 0.01	0.23 ± 0.01
20h	0.23 ± 0.01	0.17 ± 0.02
Bortezomib	7.96 ± 0.11 (nM)	10.85 ± 0.51 (nM)
Carfilzomib	13.19 ± 0.56 (nM)	21.32 ± 0.83 (nM)

[a] The IC_{50} values are an average of three independent determinations.

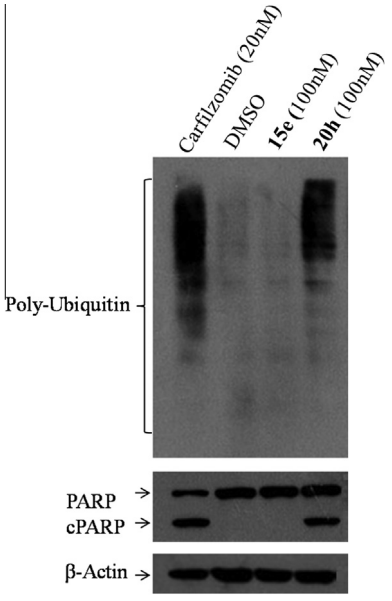


Figure 3. Immunoblotting analysis of poly-ubiquitin, PARP, and cPARP in RPMI 8226 cells treated with carfilzomib, DMSO (control), or selected β-amino acid derivatives (**15e** and **20h**).

singlet (s), broad singlet (brs), doublet (d), triplet (t), quartet (q) and multiplet (m). Mass spectral data were obtained by Esquire-LC-00075 spectrometer (Brüker Bioscience). Reagents and solvents were purchased from common commercial suppliers and were used without further purification unless stated otherwise. Column chromatography was performed using silica gel (300–400 mesh). All yields are unoptimized and generally represent the result of a single experiment.

4.1.1. General procedure for the synthesis of α,β-unsaturated ketone (3)

To a suspension of Boc-protected amino acid **1** (10.0 mmol) in DCM (45.0 mL), HOBt (10.0 mmol) and EDCI (15.0 mmol) were added at 0 °C. The reaction mixture was kept at the same temperature and stirred for 30 min. Then *N*,*O*-dimethylhydroxylamine hydrochloride (10.0 mmol) and diisopropylethylamine (25.0 mmol) were added. After stirring at 0 °C for 3 h, the resulting mixture was washed with aqueous $NaHCO_3$ solution (2×20 mL), brine (1×20 mL), and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the residue obtained was used in the next step without further purification.

To a solution of isopropenylmagnesium bromide (15 mmol, 0.5 M in THF) was added a solution of Weinreb amide **2** (10 mmol) in dry THF (50 mL) dropwise at 0 °C under an atmosphere of nitrogen. The reaction mixture was stirred at the same temperature overnight and then poured into 100 mL of saturated NH_4Cl solution. The solution was acidified to pH 2.0 with 6 N HCl, and the mixture was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with aqueous $NaHCO_3$ (1 × 30 mL), brine (1 × 30 mL), and dried over Na_2SO_4. The solvent was evaporated in vacuo and the crude product was purified by flash column chromatography (ethyl acetate–petroleum ether = 1:20) to give compound **3**.

4.1.1.1. *S*-*tert*-Butyl 4-methyl-3-oxo-1-phenylpent-4-en-2-ylcarbamate (3a). White solid; yield: 75% (2 steps); mp: 92–93 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.26 (m, 3H, Ar-H), 7.06 (m, 2H, Ar-H), 6.01 (d, 1H, J = 1.5 Hz, =CH_2), 5.85 (d, 1H, J = 1.5 Hz, =CH_2), 5.27 (m, 2H, NH+CH), 3.02 (m, 2H, CH_2), 1.86 (s, 3H, CH_3), 1.41 (s, 9H, CH_3); ESI-MS: m/z = 290 $[M+H]^+$.

4.1.1.2. *tert*-Butyl [(1*S*)-3-methyl-1-(2-methylpropyl)-2-oxobut-3-en-1-yl]carbamate (3b). Colorless oil; yield: 72% (2 steps); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.07 (s, 1H, =CH_2), 5.87 (s, 1H, =CH_2), 5.14 (d, 1H, J = 8.0 Hz, NH), 5.05 (m, 1H, CH), 1.89 (s, 3H, CH_3), 1.72 (m, 1H, CH), 1.46 (m, 1H, CH_2), 1.42 (s, 9H, CH_3), 1.32 (m, 1H, CH_2), 0.98 (d, 3H, J = 6.5 Hz, CH_3), 0.90 (d, 3H, J = 6.5 Hz, CH_3); ESI-MS: m/z = 256 $[M+H]^+$.

4.1.2. General procedure for the synthesis of Boc-protected epoxyketone (6)

To a solution of compound **3** (5.0 mmol) in MeOH (10.0 mL) and THF (10.0 mL) was added $CeCl_3{\cdot}7H_2O$ (7.0 mmol) under an atmosphere of nitrogen at 0 °C. Then $NaBH_4$ (7.0 mmol) was added. The reaction mixture was kept at 0 °C for 2 h and quenched with glacial acetic acid (4.0 mL). The volatiles were evaporated under reduced pressure, and the residue was diluted with H_2O (20.0 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (1 × 30 mL), brine (1 × 30 mL), and dried over Na_2SO_4. The solvent was evaporated in vacuo and the crude product **4** was used in the next step without further purification.

To a solution of alcohol **4** (5.0 mmol) in DCM (20.0 mL) was added *m*CPBA (5.5 mmol) at 0 °C. The reaction mixture was stirred at the same temperature for 3 h, and then warmed to room temperature. The solution was washed with aqueous $NaHCO_3$ (2 × 30 mL), brine (1 × 30 mL), and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the crude product **5** was put in the next step without further purification.

To a solution of abovementioned compound **5** (5.0 mmol) in DCM (20.0 mL) was added Dess–Martin periodinane (7.5 mmol) in DCM (15.0 mL) at 0 °C under an atmosphere of nitrogen. The reaction was stirred at 0 °C overnight. The resulting mixture was poured into aqueous $NaHCO_3$ solution (30 mL), and filtrated. The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with H_2O (2 × 20 mL), brine (1 × 20 mL), and dried over Na_2SO_4. The solvent was evaporated under reduced pressure. Purification by flash column chromatography (ethyl acetate–petroleum ether = 1:15–1:10) gave epoxyketone **6**.

4.1.2.1. *tert*-Butyl *S*-1-(*R*-2-methyloxiran-2-yl)-1-oxo-3-phenylpropan-2-ylcarbamate (6a). White solid; yield: 39% (3 steps); mp: 86–87 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.28 (m, 3H, Ar-H), 7.16 (m, 2H, Ar-H), 4.93 (d, 1H, J = 8.0 Hz, NH), 4.58 (m, 1H, CH), 3.28 (d, 1H, J = 5.0 Hz, OCH_2), 3.10 (dd, 1H, J = 13.5, 5.0 Hz, CH_2), 2.90 (d, 1H, J = 5.0 Hz, OCH_2), 2.73 (dd, 1H, J = 13.5, 7.5 Hz, CH_2), 1.50 (s, 3H, CH_3), 1.36 (s, 9H, CH_3); ESI-MS: m/z = 306 $[M+H]^+$.

4.1.2.2. *tert*-Butyl [(1*S*)-3-methyl-1-{[(2*R*)-2-methyloxiran-2-yl]carbonyl}butyl]carbamate (6b). Colorless oil; yield: 52% (3 steps); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.84 (d, 1H, J = 8.5 Hz, NH), 4.31 (m, 1H, CH), 3.28 (d, 1H, J = 4.5 Hz, OCH_2), 2.87 (d, 1H, J = 4.5 Hz, OCH_2), 1.71 (m, 1H, CH), 1.51 (s, 3H, CH_3), 1.46 (m, 1H, CH_2), 1.40 (s, 9H, CH_3), 1.16 (m, 1H, CH_2), 0.94 (dd, 6H, J = 16.5, 6.5 Hz, CH_3); ESI-MS: m/z = 272 $[M+H]^+$.

4.1.3. General procedure for the synthesis of deprotected epoxyketone (7)

To a solution of epoxyketone fragment **6** (5.0 mmol) in DCM (20.0 mL) was added trifluoroacetic acid (5.0 mL) dropwise at 0 °C. The reaction mixture was then warmed to room temperature and stirred for 1 h. The volatiles were evaporated and the crude **7** salt was put in the next step without further purification.

4.1.4. General procedure for the synthesis of dipeptidyl intermediates 8, 11, and 16

To a suspension of Boc-protected β-amino acid or Phe (2.0 mmol) in DCM (8.0 mL), HOBt (2.0 mmol) and EDCI (3.0 mmol) were added. After stirring at room temperature for 30 min, corresponding amine **7a** or **7b** (2.0 mmol) and diisopropylethylamine (4.0 mmol) were added. The reaction mixture was stirred for another 3 h and washed with aqueous $NaHCO_3$ solution (2 × 10 mL), brine (1 × 10 mL), and dried over Na_2SO_4. The solvent was evaporated in vacuo and the obtained residue was purified by flash column chromatography (ethyl acetate–petroleum ether = 1:6–1:4).

4.1.4.1. *tert*-Butyl 3-[*S*-1-(*R*-2-methyloxiran-2-yl)-1-oxo-3-phenylpropan-2-ylamino]-3-oxo-1-phenylpropylcarbamate (8). White solid; yield: 91%; mp: 144–146 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.29 (m, 8H, Ar-H), 6.99 (m, 2H, Ar-H), 6.08 and 5.99 (br s, 50:50, 1H, NH), 5.90 (m, 1H, CH), 4.99 (br s, 1H, NH), 4.85 (m, 1H, CH), 2.93 and 2.82 (m, 50:50, 1H, CH_2), 2.68 (m, 4H, CH_2 + OCH_2), 2.60 and 2.47 (d, 50:50, 1H, J = 4.5 Hz, OCH_2), 1.43 (s, 3H, CH_3), 1.41 (s, 9H, CH_3); ESI-MS: m/z = 453 $[M+H]^+$.

4.1.4.2. *tert*-Butyl 3-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-3-oxo-1-phenylpropylcarbamate (11). White solid; yield: 92%; mp: 62–64 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.27 (m, 5H, Ar-H), 6.19 and 5.80 (m, 60:40, 1H, NH), 5.79 (m, 1H, NH), 5.06 (m, 1H, CH), 4.55 (m, 1H, CH), 3.25 and 3.20 (d, 60:40, 1H, J = 5.0 Hz, OCH_2), 2.89 and 2.87 (d, 60:40, 1H, J = 5.0 Hz, OCH_2), 2.70 (m, 2H, CH_2), 1.49 and 1.48 (s, 60:40, 3H, CH_3), 1.43 and 1.41 (s, 60:40, 9H, CH_3), 1.38 (m, 1H, CH_2), 1.11 (m, 2H, CH + CH_2), 0.89 and 0.81 (m, 60:40, 6H, CH_3); ESI-MS: m/z = 419 $[M+H]^+$.

4.1.4.3. *tert*-Butyl (*S*)-1-{*S*-4-methyl-1-[*R*-2-methyloxiran-2-yl]-1-oxopentan-2-ylamino}-1-oxo-3-phenylpropan-2-ylcarbamate (16). White solid; yield: 87%; mp: 151–153 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.20 (m, 5H, Ar-H), 6.18 (d, 1H, J = 6.5 Hz, NH), 4.95 (d, 1H, J = 6.5 Hz, NH), 4.57 (m, 1H, CH), 4.32 (m, 1H, CH), 3.24 (d, 1H, J = 4.5 Hz, OCH_2), 3.03 (m, 2H, CH_2), 2.88 (d, 1H, J = 4.5 Hz, OCH_2), 1.63 (m, 1H, CH), 1.49 (s, 3H, CH_3), 1.46 (m, 1H, CH_2), 1.43 (s, 9H, CH_3), 1.17 (m, 1H, CH_2), 0.92 (d, 3H, J = 6.5 Hz, CH_3), 0.87 (d, 3H, J = 6.5 Hz, CH_3); ESI-MS: m/z = 419 $[M+H]^+$.

4.1.5. General procedure for the synthesis of tripeptidyl intermediates 9, 12, 14, 17, 19, and 21

To a solution of dipeptidyl fragment **8**, **11**, or **16** (5.0 mmol) in DCM (20.0 mL) was added trifluoroacetic acid (5.0 mL) dropwise

at 0 °C. The reaction mixture was then warmed to room temperature and stirred for 1 h. The volatiles were evaporated and the crude amine salt was put in the next step without further purification.

To a suspension of corresponding Boc-protected amino acid (2.0 mmol) in DCM (8.0 mL), HOBt (2.0 mmol) and EDCI (3.0 mmol) were added. After stirring at room temperature for 30 min, aforementioned amine salt (2.0 mmol) and diisopropylethylamine (4.0 mmol) were added. The reaction mixture was stirred for another 3 h and was washed with aqueous $NaHCO_3$ solution (2 × 10 mL), brine (1 × 10 mL), and dried over Na_2SO_4. The solvent was evaporated in vacuo and the obtained residue was purified by flash column chromatography (ethyl acetate–petroleum ether = 1:3–2:1).

4.1.5.1. *tert*-Butyl (*S*)-4-methyl-1-{3-[*S*-1-(*R*-2-methyloxiran-2-yl)-1-oxo-3-phenylpropan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxopentan-2-ylcarbamate (9). White solid; yield: 80%; mp: 158–160 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.67 and 7.62 (d, 50:50, 1H, J = 8.0 Hz, NH), 7.25 (m, 8H, Ar-H), 7.01 (m, 2H, Ar-H), 6.48 and 5.89 (d, 50:50, 1H, J = 7.0 Hz, NH), 5.38 and 5.27 (m, 50:50, 1H, NH), 5.02 and 4.90 (m, 50:50, 1H, CH), 4.87 (m, 1H, CH), 4.15 (br s, 1H, CH), 2.93 (m, 1H, CH_2), 2.66 (m, 4H, CH_2 + OCH_2), 2.55 and 2.40 (d, 50:50, 1H, J = 4.5 Hz, OCH_2), 1.69 (m, 2H, CH_2), 1.45 (m, 13H, CH + CH_3), 0.95 (m, 6H, CH_3); ESI-MS: m/z = 566 $[M+H]^+$.

4.1.5.2. *tert*-Butyl (*S*)-4-methyl-1-{3-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxopentan-2-ylcarbamate (12). White solid; yield: 88%; mp: 185–187 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.99 and 7.50 (d, 50:50, 1H, J = 8.5 Hz, NH), 7.28 (m, 5H, Ar-H), 6.64 and 5.90 (d, 50:50, 1H, J = 6.5 Hz, NH), 5.50 and 5.26 (m, 50:50, 1H, NH), 4.95 (m, 1H, CH), 4.53 (m, 1H, CH), 4.19 (m, 1H, CH), 3.27 and 3.25 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.87 (m, 1H, OCH_2), 2.68 (m, 2H, CH_2), 1.71 (m, 2H, CH_2), 1.48 (m, 14H, CH_2 + CH_3), 1.22 (m, 2H, CH_2), 0.95 (m, 6H, CH_3), 0.87 (m, 6H, CH_3); ESI-MS: m/z = 532 $[M+H]^+$.

4.1.5.3. *tert*-Butyl (*S*)-1-{3-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxo-3-phenylpropan-2-ylcarbamate (14). White solid; yield: 82%; mp: 167–169 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.69 and 6.96 (d, 50:50, 1H, J = 8.5 Hz, NH), 7.23 (m, 10H, Ar-H), 6.36 and 5.80 (br s, 50:50, 1H, NH), 5.15 (m, 2H, NH + CH), 4.50 (m, 2H, CH + CH), 3.23 and 3.17 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 3.06 (m, 2H, CH_2), 2.85 and 2.83 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.63 (m, 2H, CH_2), 1.65 (m, 1H, CH), 1.50 and 1.46 (s, 50:50, 3H, CH_3), 1.40 (m, 11H, CH_2 + CH_3), 0.86 (m, 6H, CH_3); ESI-MS: m/z = 566 $[M+H]^+$.

4.1.5.4. *tert*-Butyl 3-{*S*-1-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropylcarbamate (17). White solid; yield: 73%; mp: 95–97 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.28 (m, 8H, Ar-H), 7.12 (m, 1H, Ar-H), 7.02 (m, 1H, Ar-H), 6.13 (m, 1H, NH), 5.86 (m, 2H, NH), 4.98 (m, 1H, CH), 4.48 (m, 2H, CH), 3.19 and 3.16 (d, 45:55, 1H, J = 5.0 Hz, OCH_2), 2.87 (m, 5H, OCH_2 + CH_2), 1.58 (s, 9H, CH_3), 1.49 and 1.48 (s, 3H, CH_3), 1.42 (m, 3H, CH + CH_2), 0.87 (m, 6H, CH_3); ESI-MS: m/z = 566 $[M+H]^+$.

4.1.5.5. *tert*-Butyl (*R*)-3-{*S*-1-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropylcarbamate (19). White solid; yield: 65%; mp: 139–141 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.28 (m, 8H, Ar-H), 7.11 (d, 2H, J = 7.5 Hz, Ar-H), 6.23 (br s, 1H, NH), 5.91 (m, 2H, NH), 4.99 (br s, 1H, CH), 4.48 (m, 2H, CH), 3.17 (d, 1H, J = 5.0 Hz, OCH_2), 2.87 (m, 3H, OCH_2 + CH_2), 2.69 (m, 2H, CH_2), 1.49 (s, 3H, CH_3), 1.41 (m, 11H, CH_2 + CH_3), 1.12 (m, 1H, CH), 0.86 (m, 6H, CH_3); ESI-MS: m/z = 566 $[M+H]^+$.

4.1.5.6. *tert*-Butyl (*S*)-3-{*S*-1-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropylcarbamate (21). White solid; yield: 73%; mp: 135–137 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.26 (m, 8H, Ar-H), 7.01 (d, 2H, J = 7.5 Hz, Ar-H), 6.24 (br s, 1H, NH), 5.98 (m, 2H, NH), 5.00 (br s, 1H, CH), 4.51 (m, 1H, CH), 4.46 (m, 1H, CH), 3.15 (d, 1H, J = 5.0 Hz, OCH_2), 2.87 (m, 2H, OCH_2 + CH_2), 2.63 (m, 3H, CH_2), 1.48 (s, 3H, CH_3), 1.42 (m, 11H, CH_2 + CH_3), 1.11 (m, 1H, CH), 0.86 (dd, 6H, J = 13.5, 6.0 Hz, CH_3); ESI-MS: m/z = 566 $[M+H]^+$.

4.1.6. General procedure for the synthesis of target compounds 10a–e, 13a–e, 15a–e, 18a–n, 20f, 20h, 22f, and 22h

To a solution of tripeptidyl fragment **9**, **12**, **14**, **17**, **19**, or **21** (5.0 mmol) in DCM (20.0 mL) was added trifluoroacetic acid (5.0 mL) dropwise at 0 °C. The reaction mixture was then warmed to room temperature and stirred for 1 h. The volatiles were evaporated and the crude amine salt was put in the next step without further purification.

To a suspension of corresponding acid (2.0 mmol) in DCM (8.0 mL), HOBt (2.0 mmol) and EDCI (3.0 mmol) were added. After stirring at room temperature for 30 min, aforementioned amine salt (2.0 mmol) and diisopropylethylamine (4.0 mmol) were added. The reaction mixture was stirred for another 3 h and was washed with aqueous $NaHCO_3$ solution (2 × 10 mL), brine (1 × 10 mL), and dried over Na_2SO_4. The solvent was evaporated in vacuo and the obtained residue was purified by flash column chromatography (ethyl acetate–petroleum ether = 1:3–10:1).

4.1.6.1. *N*-{*S*-4-Methyl-1-{3-[*S*-1-(*R*-2-methyloxiran-2-yl)-1-oxo-3-phenylpropan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxopentan-2-yl}pyrazine-2-carboxamide (10a). White solid; yield: 75%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.36 (s, 1H, pyrazine-H), 8.75 and 8.73 (d, 50:50, 1H, J = 2.0 Hz, pyrazine-H), 8.53 and 8.46 (d, 50:50, 1H, J = 2.0 Hz, pyrazine-H), 8.24 and 8.17 (d, 50:50, 1H, J = 7.5 Hz, NH), 7.86 and 7.78 (d, 50:50, 1H, J = 8.0 Hz, NH), 7.25 (m, 8H, Ar-H), 7.00 (m, 2H, Ar-H), 6.44 and 5.93 (d, 50:50, 1H, J = 7.5 Hz, NH), 5.39 and 5.29 (m, 50:50, 1H, CH), 4.85 and 4.79 (q, 50:50, 1H, J = 7.0 Hz, CH), 4.68 (m, 1H, CH), 2.91 (m, 1H, CH_2), 2.65 (m, 4H, CH_2 + OCH_2), 2.52 and 2.40 (d, 50:50, 1H, J = 4.5 Hz, OCH_2), 1.80 (m, 3H, CH + CH_2), 1.43 and 1.40 (s, 50:50, 3H, CH_3), 0.98 (m, 6H, CH_3); ESI-MS: m/z = 572 $[M+H]^+$.

4.1.6.2. *N*-{*S*-4-Methyl-1-{3-[*S*-1-(*R*-2-methyloxiran-2-yl)-1-oxo-3-phenylpropan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxopentan-2-yl}nicotinamide (10b). White solid; yield: 56%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.13 and 9.10 (s, 50:50, 1H, pyridine-H), 8.69 (s, 1H, pyridine-H), 8.21 (m, 2H, pyridine-H + NH), 7.75 (d, 1H, J = 2.5 Hz, pyridine-H), 7.41 and 7.37 (m, 50:50, 1H, NH), 7.26 (m, 8H, Ar-H), 7.02 (m, 2H, Ar-H), 6.83 and 6.41 (br s, 50:50, 1H, NH), 5.43 and 5.27 (q, 50:50, 1H, J = 6.5 Hz, CH), 4.78 (m, 2H, CH + CH), 2.76 (m, 5H, CH_2 + OCH_2), 2.43 and 2.39 (d, 50:50, 1H, J = 4.5 Hz, OCH_2), 1.74 (m, 3H, CH + CH_2), 1.42 and 1.38 (s, 50:50, 3H, CH_3), 0.95 (m, 6H, CH_3); ESI-MS: m/z = 571 $[M+H]^+$.

4.1.6.3. *N*-{*S*-4-Methyl-1-{3-[*S*-1-(*R*-2-methyloxiran-2-yl)-1-oxo-3-phenylpropan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxopentan-2-yl}benzamide (10c). White solid; yield: 49%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.81 (m, 2H, Ar-H), 7.45 (m, 3H, Ar-H), 7.32 (m, 8H, Ar-H), 7.26 (m, 8H, Ar-H), 7.03 (m, 2H, Ar-H),

6.85 (m, 1H, NH), 6.69 and 6.62 (d, 40:60, 1H, J = 7.5 Hz, NH), 6.02 and 5.95 (d, 40:60, 1H, J = 8.0 Hz, NH), 5.42 and 5.28 (m, 40:60, 1H, CH), 4.77 (m, 2H, CH + CH), 2.89 (m, 1H, CH_2), 2.74 (m, 4H, CH_2 + -OCH_2), 2.53 and 2.42 (d, 60:40, 1H, J = 4.5 Hz, OCH_2), 1.71 (m, 3H, CH + CH_2), 1.44 and 1.41 (s, 60:40, 3H, CH_3), 0.99 (m, 6H, CH_3); ESI-MS: m/z = 570 $[M+H]^+$.

4.1.6.4. 4-Fluoro-*N*-{*S*-4-methyl-1-{3-[*S*-1-(*R*-2-methyloxiran-2-yl)-1-oxo-3-phenylpropan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxopentan-2-yl}benzamide (10d). White solid; yield: 56%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.82 (m, 2H, Ar-H), 7.24 (m, 8H, Ar-H), 7.26 (m, 8H, Ar-H), 7.07 (m, 5H, Ar-H + NH), 6.89 and 6.73 (d, 50:50, 1H, J = 7.0 Hz, NH), 6.57 and 5.95 (d, 50:50, 1H, J = 7.0 Hz, NH), 5.41 and 5.26 (q, 50:50, 1H, J = 7.0 Hz, CH), 4.79 (m, 2H, CH + CH), 2.91 (m, 1H, CH_2), 2.68 (m, 4H, CH_2 + -OCH_2), 2.50 and 2.42 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 1.70 (m, 3H, CH + CH_2), 1.44 and 1.41 (s, 50:50, 3H, CH_3), 0.98 (m, 6H, CH_3); ESI-MS: m/z = 588 $[M+H]^+$.

4.1.6.5. 4-Methoxy-*N*-{*S*-4-methyl-1-{3-[*S*-1-(*R*-2-methyloxiran-2-yl)-1-oxo-3-phenylpropan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxopentan-2-yl}benzamide (10e). White solid; yield: 46%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.78 (m, 3H, Ar-H + NH), 7.28 (m, 8H, Ar-H), 7.08 (m, 2H, Ar-H), 6.90 (t, 2H, J = 9.0 Hz, Ar-H), 6.78 and 6.74 (d, 50:50, 1H, J = 6.5 Hz, NH), 6.66 and 6.05 (br s, 50:50, 1H, NH), 5.42 and 5.27 (q, 50:50, 1H, J = 6.5 Hz, CH), 4.77 (m, 2H, CH + CH), 3.84 and 3.83 (s, 50:50, 3H, CH_3), 2.89 (m, 1H, CH_2), 2.71 (m, 4H, CH_2 + OCH_2), 2.51 and 2.42 (d, 50:50, 1H, J = 4.5 Hz, OCH_2), 1.75 (m, 3H, CH + CH_2), 1.44 and 1.41 (s, 50:50, 3H, CH_3), 0.97 (m, 6H, CH_3); ESI-MS: m/z = 600 $[M+H]^+$.

4.1.6.6. *N*-{*S*-4-Methyl-1-{3-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxopentan-2-yl}pyrazine-2-carboxamide (13a). White solid; yield: 73%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.37 (m, 1H, pyrazine-H), 8.77 and 8.75 (d, 60:40, 1H, J = 2.5 Hz, pyrazine-H), 8.56 and 8.53 (m, 60:40, 1H, pyrazine-H), 8.20 (m, 1H, NH), 8.12 and 7.62 (d, 60:40, 1H, J = 8.0 Hz, NH), 7.25 (m, 5H, Ar-H), 6.70 and 5.93 (d, 60:40, 1H, J = 8.0 Hz, NH), 5.49 and 5.28 (q, 60:40, 1H, J = 6.0 Hz, CH), 4.74 and 4.66 (q, 40:60, 1H, J = 7.5 Hz, CH), 4.51 (m, 1H, CH), 3.24 and 3.22 (d, 60:40, 1H, J = 5.0 Hz, OCH_2), 2.86 (m, 1H, OCH_2), 2.70 (m, 2H, CH_2), 1.77 (m, 2H, CH_2), 1.47 (m, 5H, CH_2 + CH_3), 1.25 (m, 2H, CH_2), 1.01 (m, 6H, CH_3), 0.88 (m, 6H, CH_3); ESI-MS: m/z = 538 $[M+H]^+$.

4.1.6.7. *N*-{*S*-4-Methyl-1-{3-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxopentan-2-yl}nicotinamide (13b). White solid; yield: 78%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.02 (d, 1H, J = 11.0 Hz, pyridine-H), 8.70 (m, 1H, pyridine-H), 8.10 (m, 1H, pyridine-H), 8.06 and 7.85 (d, 50:50, 1H, J = 8.0 Hz, NH), 7.49 and 7.30 (d, 50:50, 1H, J = 7.5 Hz, NH), 7.40 (m, 1H, pyridine-H), 7.25 (m, 5H, Ar-H), 6.95 and 6.26 (d, 50:50, 1H, J = 7.5 Hz, NH), 5.48 and 5.21 (q, 50:50, 1H, J = 7.0 Hz, CH), 4.72 (m, 1H, CH), 4.49 (m, 1H, CH), 3.24 (m, 1H, OCH_2), 2.84 (m, 1H, OCH_2), 2.70 (m, 2H, CH_2), 1.74 (m, 2H, CH_2), 1.46 (m, 5H, CH_2 + CH_3), 1.24 (m, 2H, CH_2), 0.98 (m, 6H, CH_3), 0.83 (m, 6H, CH_3); ESI-MS: m/z = 537 $[M+H]^+$.

4.1.6.8. *N*-{*S*-4-Methyl-1-{3-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxopentan-2-yl}benzamide (13c). White solid; yield: 75%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.11 (m, 1H, NH), 7.80 (m, 2H, Ar-H), 7.45 (m, 3H, Ar-H), 7.30 (m, 5H, Ar-H), 7.03 and 6.94 (d, 50:50, 1H, J = 7.5 Hz, NH), 7.00 and 6.22 (br s, 50:50, 1H, NH), 5.47 and 5.23 (q, 50:50, 1H, J = 5.5 Hz, CH), 4.82 and 4.71 (q, 50:50, 1H, J = 6.5 Hz, CH), 4.50 (m, 1H, CH), 3.26 and 3.24 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.85 and 2.82 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.71 (m, 2H, CH_2), 1.74 (m, 2H, CH_2), 1.47 (m, 5H, CH_2 + CH_3), 1.19 (m, 2H, CH_2), 0.99 (m, 6H, CH_3), 0.86 (m, 6H, CH_3); ESI-MS: m/z = 536 $[M+H]^+$.

4.1.6.9. 4-Fluoro-*N*-{*S*-4-methyl-1-{3-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxopentan-2-yl}benzamide (13d). White solid; yield: 96%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.11 and 7.83 (d, 50:50, 1H, J = 9.0 Hz, NH), 7.78 (m, 2H, Ar-H), 7.23 (m, 6H, Ar-H + NH), 7.02 (m, 2H, Ar-H), 6.96 and 6.27 (m, 50:50, 1H, NH), 5.46 and 5.23 (m, 50:50, 1H, CH), 4.77 and 4.69 (m, 50:50, 1H, CH), 4.50 (m, 1H, CH), 3.26 and 3.24 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.85 and 2.83 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.70 (m, 2H, CH_2), 1.73 (m, 2H, CH_2), 1.47 (m, 5H, CH_2 + CH_3), 1.25 (m, 2H, CH_2), 0.96 (m, 6H, CH_3), 0.84 (m, 6H, CH_3); ESI-MS: m/z = 554 $[M+H]^+$.

4.1.6.10. 4-Methoxy-*N*-{*S*-4-methyl-1-{3-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxopentan-2-yl}benzamide (13e). White solid; yield: 69%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.06 and 7.31 (d, 50:50, 1H, J = 8.5 Hz, NH), 7.77 (m, 2H, Ar-H), 7.26 (m, 5H, Ar-H), 6.94 and 6.08 (d, 50:50, 1H, J = 8.0 Hz, NH), 6.91 (m, 2H, Ar-H), 6.75 (m, 1H, NH), 5.49 and 5.21 (q, 50:50, 1H, J = 7.0 Hz, CH), 4.78 and 4.67 (q, 50:50, 1H, J = 8.0 Hz, CH), 4.50 (m, 1H, CH), 3.84 and 3.83 (s, 3H, CH_3), 3.25 (m, 1H, OCH_2), 2.77 (m, 3H, OCH_2 + CH_2), 1.75 (m, 2H, CH_2), 1.48 (m, 5H, CH_2 + CH_3), 1.22 (m, 2H, CH_2), 0.96 (m, 6H, CH_3), 0.85 (m, 6H, CH_3); ESI-MS: m/z = 566 $[M+H]^+$.

4.1.6.11. *N*-{*S*-1-{3-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxo-3-phenylpropan-2-yl}pyrazine-2-carboxamide (15a). White solid; yield: 79%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.33 (s, 1H, pyrazine-H), 8.74 and 8.72 (d, 50:50, 1H, J = 2.5 Hz, pyrazine-H), 8.53 and 8.50 (d, 50:50, 1H, J = 2.5 Hz, pyrazine-H), 8.36 (m, 1H, NH), 7.77 and 7.29 (d, 50:50, 1H, J = 9.0 Hz, NH), 7.23 (m, 9H, Ar-H), 6.93 (m, 1H, Ar-H), 6.46 and 5.82 (d, 50:50, 1H, J = 8.0 Hz, NH), 5.39 and 5.15 (q, 50:50, 1H, J = 6.0 Hz, CH), 4.87 (m, 1H, CH), 4.45 (m, 1H, CH), 3.21 (m, 3H, CH_2 + OCH_2), 2.82 (m, 1H, OCH_2), 2.67 (m, 2H, CH_2), 1.46 and 1.44 (s, 50:50, 3H, CH_3), 1.39 (m, 2H, CH_2), 1.18 (m, 1H, CH), 0.83 (m, 6H, CH_3); ESI-MS: m/z = 572 $[M+H]^+$.

4.1.6.12. *N*-{*S*-1-{3-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxo-3-phenylpropan-2-yl}nicotinamide (15b). White solid; yield: 92%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.96 and 8.93 (d, 50:50, 1H, J = 1.5 Hz, pyridine-H), 8.68 (m, 1H, pyridine-H), 8.04 (m, 1H, pyridine-H), 7.84 and 7.52 (d, 50:50, 1H, J = 9.0 Hz, NH), 7.51 and 7.42 (d, 50:50, 1H, J = 7.0 Hz, NH), 7.34 (m, 1H, pyridine-H), 7.24 (m, 9H, Ar-H), 7.00 (m, 1H, Ar-H), 6.67 and 6.07 (d, 50:50, 1H, J = 8.0 Hz, NH), 5.41 and 5.18 (q, 50:50, 1H, J = 7.0 Hz, CH), 4.94 (m, 1H, CH), 4.46 (m, 1H, CH), 3.21 (m, 3H, CH_2 + OCH_2), 2.83 and 2.80 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.73 (m, 2H, CH_2), 1.45 and 1.42 (s, 50:50, 3H, CH_3), 1.39 (m, 2H, CH_2), 1.22 (m, 1H, CH), 0.84 (m, 6H, CH_3); ESI-MS: m/z = 571 $[M+H]^+$.

4.1.6.13. *N*-{*S*-1-{3-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxo-3-phenylpropan-2-yl}benzamide (15c). White solid; yield: 88%; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.78 and 7.47 (d, 50:50, 1H, J = 8.5 Hz, NH), 7.73 (m, 2H, Ar-H), 7.37 (m, 3H, Ar-H), 7.25 (m, 9H, Ar-H), 6.99 (m, 2H, Ar-H + NH), 6.55 and 5.95 (d, 50:50, 1H, J = 8.0 Hz, NH), 5.39 and 5.17 (q, 50:50, 1H, J = 7.5 Hz, CH), 4.91 (m, 1H, CH), 4.46 (m, 1H, CH), 3.23 (m, 3H, CH_2 + OCH_2), 2.79 (m,

1H, OCH_2), 2.59 (m, 2H, CH_2), 1.45 and 1.43 (s, 50:50, 3H, CH_3), 1.39 (m, 2H, CH_2), 1.23 (m, 1H, CH), 0.84 (m, 6H, CH_3); ESI-MS: m/z = 570 $[M+H]^+$.

4.1.6.14. 4-Fluoro-*N*-{*S*-1-{3-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxo-3-phenylpropan-2-yl}benzamide (15d). White solid; yield: 68%; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.81 and 7.50 (d, 50:50, 1H, J = 8.5 Hz, NH), 7.73 (m, 2H, Ar-H), 7.22 (m, 13H, Ar-H + NH), 6.61 and 6.00 (d, 50:50, 1H, J = 8.0 Hz, NH), 5.39 and 5.18 (q, 50:50, 1H, J = 6.5 Hz, CH), 4.91 (m, 1H, CH), 4.46 (m, 1H, CH), 3.20 (m, 3H, CH_2 + OCH_2), 2.80 (m, 1H, OCH_2), 2.60 (m, 2H, CH_2), 1.45 and 1.43 (s, 50:50, 3H, CH_3), 1.38 (m, 2H, CH_2), 1.19 (m, 1H, CH), 0.83 (m, 6H, CH_3); ESI-MS: m/z = 588 $[M+H]^+$.

4.1.6.15. 4-Methoxy-*N*-{*S*-1-{3-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-3-oxo-1-phenylpropylamino}-1-oxo-3-phenylpropan-2-yl}benzamide (15e). White solid; yield: 84%; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.77 and 7.42 (d, 50:50, 1H, J = 9.0 Hz, NH), 7.66 (m, 2H, Ar-H), 7.24 (m, 9H, Ar-H + NH), 6.87 (m, 4H, Ar-H), 6.62 and 5.96 (d, 50:50, 1H, J = 7.5 Hz, NH), 5.39 and 5.16 (q, 50:50, 1H, J = 6.0 Hz, CH), 4.93 and 4.86 (q, 50:50, 1H, J = 7.5 Hz, CH), 4.46 (m, 1H, CH), 3.83 and 3.82 (s, 50:50, 3H, CH_3), 3.21 (m, 3H, CH_2 + OCH_2), 2.80 (m, 1H, OCH_2), 2.59 (m, 2H, CH_2), 1.47 and 1.45 (s, 50:50, 3H, CH_3), 1.42 (m, 2H, CH_2), 1.23 (m, 1H, CH), 0.84 (m, 6H, CH_3); ESI-MS: m/z = 600 $[M+H]^+$.

4.1.6.16. *N*-{3-{*S*-1-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}pyrazine-2-carboxamide (18a). White solid; yield: 92%; IR (KBr): 3294 cm^{-1}, 3063 cm^{-1}, 2959 cm^{-1}, 1721 cm^{-1}, 1645 cm^{-1}, 1519 cm^{-1}, 746 cm^{-1}, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 9.44 and 9.06 (d, 55:45, 1H, J = 8.0 Hz, NH), 9.37 and 9.35 (d, 55:45, J = 1.0 Hz, pyrazine-H), 8.74 (m, 1H, pyrazine-H), 8.60 and 8.57 (m, 55:45, 1H, pyrazine-H), 7.33 (m, 4H, Ar-H), 7.28 (m, 1H, Ar-H), 7.18 (m, 3H, Ar-H), 7.11 (m, 1H, Ar-H), 6.95 (m, 1H, Ar-H), 6.27 (m, 1H, NH), 5.89 and 5.84 (d, 55:45, 1H, J = 8.0 Hz, NH), 5.56 (m, 1H, CH), 4.59 (m, 1H, CH), 4.45 (m, 1H, CH), 3.15 and 3.13 (d, 45:55, 1H, J = 5.0 Hz, OCH_2), 2.85 (m, 5H, OCH_2 + CH_2), 1.48 (s, 3H, CH_3), 1.41 (m, 2H, CH_2), 1.11 (m, 1H, CH), 0.86 (m, 6H, CH_3); ESI-MS: m/z = 572 $[M+H]^+$.

4.1.6.17. N-{3-{S-1-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}nicotinamide (18b). White solid; yield: 67%; IR (KBr): 3294 cm^{-1}, 3063 cm^{-1}, 2958 cm^{-1}, 1719 cm^{-1}, 1648 cm^{-1}, 1541 cm^{-1}, 749 cm^{-1}, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 9.30 and 9.20 (s, 50:50, 1H, pyridine-H), 8.89 and 8.83 (d, 50:50, 1H, J = 7.5 Hz, NH), 8.72 (m, 1H, pyridine-H), 8.35 and 8.27 (d, 50:50, 1H, J = 7.5 Hz, pyridine-H), 7.46 (m, 1H, pyridine-H), 7.31 (m, 4H, Ar-H), 7.15 (m, 5H, Ar-H), 6.95 (m, 1H, Ar-H), 6.57 and 6.10 (d, 50:50, 1H, J = 7.5 Hz, NH), 6.38 (m, 1H, NH), 5.56 (m, 1H, CH), 4.57 (m, 1H, CH), 4.40 (m, 1H, CH), 3.12 and 3.08 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.84 (m, 5H, OCH_2 + CH_2), 1.47 (m, 5H, CH_2 + CH_3), 1.10 (m, 1H, CH), 0.83 (m, 6H, CH_3); ESI-MS: m/z = 571 $[M+H]^+$.

4.1.6.18. N-{3-{S-1-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}benzamide (18c). White solid; yield: 65%; IR (KBr): 3287, 3066^{-1}, 2959, 1724, 1644, 1538, 737, 701 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.53 and 8.17 (d, 50:50, 1H, J = 8.0 Hz, NH), 7.89 (m, 2H, Ar-H), 7.43 (m, 3H, Ar-H), 7.30 (m, 4H, Ar-H), 7.20 (m, 4H, Ar-H), 7.11 (m, 1H, Ar-H), 6.92 (m, 1H, Ar-H), 6.32 (m, 1H, NH), 5.92 (m, 1H, NH), 5.54 (m, 1H, CH), 4.55 (m, 1H, CH), 4.46 (m, 1H, CH), 3.16 and 3.13 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.86 (m, 5H, OCH_2 + CH_2), 1.48 and 1.47 (s, 3H, CH_3), 1.42 (m, 2H, CH_2), 1.08 (m, 1H, CH), 0.86 (m, 6H, CH_3); ESI-MS: m/z = 570 $[M+H]^+$.

1.1.6.19. 4-Fluoro-*N*-{3-{*S*-1-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}benzamide (18d). White solid; yield: 66%; IR (KBr): 3291, 3065, 2959, 1722, 1643, 1543, 738, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.58 and 8.23 (d, 55:45, 1H, J = 7.5 Hz, NH), 7.88 (m, 2H, Ar-H), 7.29 (m, 4H, Ar-H), 7.23 (m, 4H, Ar-H), 7.10 (m, 3H, Ar-H), 6.92 (m, 1H, Ar-H), 6.33 (m, 1H, NH), 5.93 (m, 1H, NH), 5.50 (m, 1H, CH), 4.54 (m, 1H, CH), 4.46 (m, 1H, CH), 3.15 and 3.12 (d, 45:55, 1H, J = 5.0 Hz, OCH_2), 2.87 (m, 5H, OCH_2 + CH_2), 1.48 and 1.47 (s, 3H, CH_3), 1.43 (m, 2H, CH_2), 1.08 (m, 1H, CH), 0.85 (m, 6H, CH_3); ESI-MS: m/z = 588 $[M+H]^+$.

4.1.6.20. 4-Methoxy-N-{3-{S-1-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}benzamide (18e). White solid; yield: 79%; IR (KBr): 3292, 3068, 2960, 1725, 1644, 1545, 748, 701 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.38 and 8.04 (d, 50:50, 1H, J = 7.5 Hz, NH), 7.83 (m, 2H, Ar-H), 7.29 (m, 4H, Ar-H), 7.22 (m, 4H, Ar-H), 7.10 (m, 1H, Ar-H), 6.93 (m, 3H, Ar-H), 6.33 (m, 1H, NH), 5.96 (m, 1H, NH), 5.51 (m, 1H, CH), 4.54 (m, 1H, CH), 4.47 (m, 1H, CH), 3.85 and 3.84 (s, 3H, CH_3), 3.16 and 3.13 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.85 (m, 5H, OCH_2 + CH_2), 1.48 and 1.47 (s, 3H, CH_3), 1.42 (m, 2H, CH_2), 1.08 (m, 1H, CH), 0.85 (m, 6H, CH_3); ESI-MS: m/z = 600 $[M+H]^+$.

4.1.6.21. *N*-{3-{*S*-1-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}-4-(trifluoromethyl)benzamide (18f). White solid; yield: 71%; IR (KBr): 3303, 3067, 2962, 1724, 1646, 1544, 743, 702 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.77 and 8.43 (d, 50:50, 1H, J = 7.5 Hz, NH), 7.98 (m, 2H, Ar-H), 7.70 (m, 2H, Ar-H), 7.28 (m, 8H, Ar-H), 7.11 (m, 1H, Ar-H), 6.93 (m, 1H, Ar-H), 6.34 (m, 1H, NH), 5.87 (d, 1H, J = 8.0 Hz, NH), 5.52 (m, 1H, CH), 4.56 (m, 1H, CH), 4.46 (m, 1H, CH), 3.13 and 3.11 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.86 (m, 5H, OCH_2 + CH_2), 1.48 (s, 3H, CH_3), 1.42 (m, 2H, CH_2), 1.09 (m, 1H, CH), 0.83 (m, 6H, CH_3); ESI-MS: m/z = 638 $[M+H]^+$.

4.1.6.22. 4-Isocyano-*N*-{3-{*S*-1-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}benzamide (18g). White solid; yield: 57%; IR (KBr): 3296, 3064, 2958, 1721, 1645, 1541, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.87 and 8.54 (d, 50:50, 1H, J = 8.0 Hz, NH), 7.99 (dd, 2H, J = 17.0, 8.5 Hz, Ar-H), 7.75 (t, 2H, J = 8.0 Hz, Ar-H), 7.29 (m, 8H, Ar-H), 7.11 (d, 1H, J = 7.0 Hz, Ar-H), 6.92 (m, 1H, Ar-H), 6.24 and 6.21 (d, 50:50, 1H, J = 7.5 Hz, NH), 5.78 (m, 1H, NH), 5.52 (m, 1H, CH), 4.51 (m, 2H, CH + CH), 3.13 and 3.10 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.89 (m, 5H, OCH_2 + -CH_2), 1.49 (s, 3H, CH_3), 1.44 (m, 2H, CH_2), 1.09 (m, 1H, CH), 0.84 (m, 6H, CH_3); ESI-MS: m/z = 595 $[M+H]^+$.

4.1.6.23. *N*-{3-{*S*-1-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}-2-naphthamide (18h). White solid; yield: 54%; IR (KBr): 3289, 3063, 2957, 1721, 1643, 1535, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.62 and 8.26 (d, 50:50, 1H, J = 7.5 Hz, NH), 7.90 (m, 4H, Ar-H), 7.55 (m, 2H, Ar-H), 7.35 (m, 5H, Ar-H), 7.23 (m, 5H, Ar-H), 6.96 (m, 1H, Ar-H), 6.32 (m, 1H, NH), 5.91 (m, 1H, NH), 5.61 (m, 1H, CH), 4.57 (m, 1H, CH), 4.45 (m, 1H, CH), 3.13 (m, 1H, OCH_2), 2.91 (m, 5H, OCH_2 + CH_2), 1.47

(s, 3H, CH_3), 1.40 (m, 2H, CH_2), 1.07 (m, 1H, CH), 0.81 (m, 6H, CH_3); ESI-MS: m/z = 620 $[M+H]^+$.

4.1.6.24. *N*-{3-{*S*-1-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}biphenyl-4-carboxamide (18i). White solid; yield: 47%; IR (KBr): 3287, 3061, 2954, 1721, 1647, 1538, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.56 and 8.21 (d, 50:50, 1H, J = 7.5 Hz, NH), 7.96 (m, 2H, Ar-H), 7.62 (m, 4H, Ar-H), 7.45 (m, 2H, Ar-H), 7.39 (m, 9H, Ar-H), 7.12 (m, 1H, Ar-H), 6.95 (m, 1H, Ar-H), 6.29 (m, 1H, NH), 5.88 (m, 1H, NH), 5.56 (m, 1H, CH), 4.57 (m, 1H, CH), 4.47 (m, 1H, CH), 3.16 and 3.13 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.90 (m, 5H, OCH_2 + CH_2), 1.49 and 1.47 (s, 50:50, 3H, CH_3), 1.41 (m, 2H, CH_2), 1.09 (m, 1H, CH), 0.83 (m, 6H, CH_3); ESI-MS: m/z = 646 $[M+H]^+$.

4.1.6.25. *N*-{3-{*S*-1-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}furan-2-carboxamide (18j). White solid; yield: 48%; IR (KBr): 3288, 3067, 2957, 1724, 1645, 1532, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.32 and 7.94 (d, 50:50, 1H, J = 8.0 Hz, NH), 7.48 and 7.45 (d, 50:50, 1H, J = 1.5 Hz, furan-H), 7.29 (m, 10H, Ar-H+furan-H), 6.95 (m, 1H, Ar-H), 6.49 (m, 1H, furan-H), 6.32 (m, 1H, NH), 5.96 (m, 1H, NH), 5.49 (m, 1H, CH), 4.58 (m, 1H, CH), 4.46 (m, 1H, CH), 3.17 and 3.14 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.89 (m, 5H, OCH_2 + CH_2), 1.48 (s, 3H, CH_3), 1.41 (m, 2H, CH_2), 1.09 (m, 1H, CH), 0.85 (m, 6H, CH_3); ESI-MS: m/z = 560 $[M+H]^+$.

4.1.6.26. *N*-{3-{*S*-1-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}thiophene-2-carboxamide (18k). White solid; yield: 56%; IR (KBr): 3296, 3064, 2957, 1721, 1644, 1538, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.37 and 8.04 (d, 50:50, 1H, J = 8.0 Hz, NH), 7.62 (d, 1H, J = 3.0 Hz, thiophene-H), 7.48 (t, 1H, J = 5.0 Hz, thiophene-H), 7.24 (m, 10H, Ar-H + thiophene-H), 6.94 (m, 1H, Ar-H), 6.29 (m, 1H, NH), 5.89 (m, 1H, NH), 5.49 (m, 1H, CH), 4.53 (m, 2H, CH + CH), 3.16 and 3.13 (d, 50:50, 1H, J = 4.5 Hz, OCH_2), 2.86 (m, 5H, OCH_2 + CH_2), 1.48 (s, 3H, CH_3), 1.43 (m, 2H, CH_2), 1.09 (m, 1H, CH), 0.85 (m, 6H, CH_3); ESI-MS: m/z = 576 $[M+H]^+$.

4.1.6.27. *N*-{3-{*S*-1-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}-tetrahydro-2H-pyran-4-carboxamide (18l). White solid; yield: 66%; IR (KBr): 3293, 3064, 2957, 1721, 1647, 1541, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.71 and 7.40 (d, 50:50, 1H, J = 7.5 Hz, NH), 7.29 (m, 8H, Ar-H), 7.11 (m, 1H, Ar-H), 6.93 (m, 1H, Ar-H), 6.21 (d, 1H, J = 7.0 Hz, NH), 5.84 (m, 1H, NH), 5.34 (m, 1H, CH), 4.49 (m, 2H, CH + CH), 4.01 (m, 2H, CH_2), 3.42 (m, 2H, CH_2), 3.17 and 3.14 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.88 (m, 5H, OCH_2 + CH_2), 2.42 (m, 1H, CH), 1.81 (m, 4H, CH_2), 1.49 and 1.48 (s, 50:50, 3H, CH_3), 1.44 (m, 2H, CH_2), 1.15 (m, 1H, CH), 0.87 (m, 6H, CH_3); ESI-MS: m/z = 578 $[M+H]^+$.

4.1.6.28. 1-Acetyl-*N*-{3-{*S*-1-[*S*-4-methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}piperidine-4-carboxamide (18m). White solid; yield: 73%; IR (KBr): 3285, 3061, 2958, 1721, 1642, 1525, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.85 and 7.54 (d, 50:50, 1H, J = 7.5 Hz, NH), 7.28 (m, 8H, Ar-H), 7.09 (m, 1H, Ar-H), 6.92 (m, 1H, Ar-H), 6.39 (m, 1H, NH), 6.06 (m, 1H, NH), 5.32 (m, 1H, CH), 4.50 (m, 3H, CH + CH + CH_2), 3.83 (m, 1H, CH_2), 3.15 (m, 1H, OCH_2), 3.08 (m, 1H, CH_2), 2.84 (m, 6H, OCH_2 + CH_2), 2.40 (m, 1H, CH), 2.07 (s, 3H, CH_3), 1.89 (m, 2H, CH_2), 1.63 (m, 2H, CH_2), 1.49 and 1.48 (s, 50:50, 3H, CH_3), 1.42 (m, 2H, CH_2), 1.11 (m, 1H, CH), 0.85 (m, 6H, CH_3); ESI-MS: m/z = 619 $[M+H]^+$.

4.1.6.29. (*S*)-*N*-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-yl]-2-[3-(2-morpholinoacetamido)-3-phenylpropanamido]-3-phenylpropanamide (18n). White solid; yield: 63%; IR (KBr): 3285, 3067, 2951, 1721, 1647, 1541, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.57 and 7.33 (d, 50:50, 1H, J = 7.5 Hz, NH), 7.28 (m, 8H, Ar-H), 7.13 (m, 1H, Ar-H), 7.02 (m, 1H, Ar-H), 6.40 (m, 1H, NH), 6.29 and 6.02 (d, 50:50, 1H, J = 7.5 Hz, NH), 5.33 (m, 1H, CH), 4.57 (m, 1H, CH), 4.49 (m, 1H, CH), 3.76 (m, 4H, CH_2), 3.20 and 3.18 (d, 50:50, 1H, J = 5.0 Hz, OCH_2), 2.92 (m, 5H, OCH_2 + -CH_2), 2.70 (m, 2H, CH_2), 2.58 (m, 4H, CH_2), 1.48 (s, 3H, CH_3), 1.42 (m, 2H, CH_2), 1.15 (m, 1H, CH), 0.86 (m, 6H, CH_3); ESI-MS: m/z = 593 $[M+H]^+$.

4.1.6.30. *N*-{*R*-3-{*S*-1-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}-4-(trifluoromethyl) benzamide (20f). White solid; yield: 63%; IR (KBr): 3308, 3065, 2961, 1721, 1645, 1538, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.42 (d, 1H, J = 7.5 Hz, NH), 7.96 (d, 2H, J = 8.5 Hz, Ar-H), 7.68 (d, 2H, J = 9.0 Hz, Ar-H), 7.28 (m, 8H, Ar-H), 7.09 (d, 2H, J = 7.0 Hz, Ar-H), 6.31 (d, 1H, J = 6.0 Hz, NH), 5.90 (d, 1H, J = 8.0 Hz, NH), 5.51 (m, 1H, CH), 4.53 (q, 1H, J = 7.0 Hz, CH), 4.45 (m, 1H, CH), 3.13 (d, 1H, J = 5.0 Hz, OCH_2), 2.94 (m, 2H, CH_2), 2.85 (d, 1H, J = 5.0 Hz, OCH_2), 2.78 (m, 2H, CH_2), 1.48 (s, 3H, CH_3), 1.42 (m, 2H, CH_2), 1.08 (m, 1H, CH), 0.83 (dd, 6H, J = 10.0, 6.0 Hz, CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 207.82, 170.71, 170.23, 165.28, 140.64, 137.50, 135.99, 129.21, 128.82, 128.70, 127.72, 127.67, 127.21, 126.13, 125.58, 124.81, 122.64, 58.90, 54.44, 52.22, 50.89, 50.21, 41.30, 40.17, 38.36, 25.02, 23.22, 21.26, 16.61; ESI-MS: m/z = 638 $[M+H]^+$.

4.1.6.31. *N*-{*R*-3-{*S*-1-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}-2-naphthamide (20h). White solid; yield: 54%; IR (KBr): 3292, 3061, 2958, 1720, 1643, 1533, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.37 (s, 1H, Ar-H), 8.28 (d, 1H, J = 7.5 Hz, NH), 7.88 (m, 4H, Ar-H), 7.54 (m, 2H, Ar-H), 7.37 (d, 2H, J = 7.5 Hz, Ar-H), 7.30 (t, 2H, J = 7.5 Hz, Ar-H), 7.22 (m, 4H, Ar-H), 7.10 (d, 2H, J = 7.0 Hz, Ar-H), 6.38 (d, 1H, J = 6.0 Hz, NH), 5.95 (d, 1H, J = 7.0 Hz, NH), 5.57 (m, 1H, CH), 4.57 (q, 1H, J = 7.0 Hz, CH), 4.45 (m, 1H, CH), 3.13 (d, 1H, J = 5.0 Hz, OCH_2), 2.95 (m, 2H, CH_2), 2.84 (m, 3H, OCH_2 + CH_2), 1.47 (s, 3H, CH_3), 1.40 (m, 2H, CH_2), 1.05 (m, 1H, CH), 0.81 (dd, 6H, J = 15.0, 6.0 Hz, CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 207.83, 170.73, 170.30, 166.76, 140.97, 136.12, 134.85, 132.67, 131.40, 129.24, 129.10, 128.78, 128.66, 128.39, 127.82, 127.72, 127.65, 127.56, 127.14, 126.66, 126.25, 123.76, 58.90, 54.42, 52.24, 50.91, 50.15, 41.62, 40.15, 38.30, 25.01, 23.21, 21.29, 16.62; ESI-MS: m/z = 620 $[M+H]^+$.

4.1.6.32. *N*-{*S*-3-{*S*-1-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}-4-(trifluoromethyl) benzamide (22f). White solid; yield: 68%; IR (KBr): 3307, 3064, 2961, 1721, 1645, 1539, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.77 (d, 1H, J = 7.5 Hz, NH), 7.99 (d, 2H, J = 8.0 Hz, Ar-H), 7.70 (d, 2H, J = 8.0 Hz, Ar-H), 7.27 (m, 8H, Ar-H), 6.92 (d, 2H, J = 7.0 Hz, Ar-H), 6.34 (d, 1H, J = 6.0 Hz, NH), 5.93 (d, 1H, J = 8.0 Hz, NH), 5.53 (br s, 1H, CH), 4.57 (m, 1H, CH), 4.45 (m, 1H, CH), 3.10 (d, 1H, J = 5.0 Hz, OCH_2), 2.91 (m, 3H, OCH_2 + CH_2), 2.69 (m, 2H, CH_2), 1.48 (s, 3H, CH_3), 1.42 (m, 2H, CH_2), 1.08 (m, 1H, CH), 0.84 (dd, 6H, J = 21.0, 5.5 Hz, CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 207.81, 170.86, 170.02, 165.09, 140.44, 137.45, 135.77,

129.26, 128.79, 128.65, 127.76, 127.68, 127.17, 126.06, 125.63, 124.82, 122.65, 58.88, 54.02, 52.19, 50.82, 50.22, 41.37, 40.29, 38.27, 25.05, 23.21, 21.28, 16.59; ESI-MS: m/z = 638 $[M+H]^+$.

4.1.6.33. *N*-{*S*-3-{*S*-1-[*S*-4-Methyl-1-(*R*-2-methyloxiran-2-yl)-1-oxopentan-2-ylamino]-1-oxo-3-phenylpropan-2-ylamino}-3-oxo-1-phenylpropyl}-2-naphthamide (22h). White solid; yield: 46%; IR (KBr): 3283, 3062, 2957, 1722, 1643, 1536, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.64 (d, 1H, J = 7.0 Hz, NH), 8.42 (s, 1H, Ar-H), 7.90 (m, 4H, Ar-H), 7.55 (m, 2H, Ar-H), 7.34 (m, 4H, Ar-H), 7.18 (m, 4H, Ar-H), 6.94 (d, 2H, J = 7.0 Hz, Ar-H), 6.41 (d, 1H, J = 6.0 Hz, NH), 6.02 (d, 1H, J = 7.5 Hz, NH), 5.61 (m, 1H, CH), 4.61(q, 1H, J = 6.0 Hz, CH), 4.48 (m, 1H, CH), 3.12 (d, 1H, J = 4.5 Hz, OCH_2), 2.91 (m, 2H, CH_2), 2.82 (d, 1H, J = 5.0 Hz, OCH_2), 2.73 (m, 2H, CH_2), 1.47 (s, 3H, CH_3), 1.40 (m, 2H, CH_2), 1.08 (m, 1H, CH), 0.82 (dd, 6H, J = 25.0, 6.0 Hz, CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 207.85, 170.90, 170.17, 166.92, 140.77, 135.91, 134.87, 132.70, 131.34, 129.29, 129.13, 128.76, 128.62, 128.44, 127.72, 127.68, 127.63, 127.43, 127.10, 126.66, 126.19, 123.73, 58.88, 54.08, 52.21, 50.77, 50.16, 44.35, 40.28, 38.20, 25.04, 23.22, 21.29, 16.61; ESI-MS: m/z = 620 $[M+H]^+$.

4.2. Biological evaluation

4.2.1. 20S proteasome chymotrypsin-like inhibition assay

Chymotrypsin-like enzyme activity assay was carried out in 50 μL volume. One microliter compound was added into 10 μL purified human proteasome (25 μg/mL), a gift from Dr. Jiang-ping Wu (Notre-Dame Hospital, Montreal, Quebec, Canada), incubated for 15 min, and then added with 39 μL synthesized substrate Suc-Leu-Leu-Val-Tyr-AMC (50 μM) (GL Biochem Ltd., Shanghai, P.R. China) as the reference reported.[21] And the AMC of probe was detected by monitoring the increase of fluorescence with Envision, at 355 nm excitation and 460 nm Emission. The IC_{50} data was calculated using the software GraphPad Prism, and chosen the equation 'sigmoidal dose–response (variable slope)' for curve fitting.

4.2.2. Tumor cell anti-proliferation assay

3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) was purchased from Promega (Madison, WI, USA). Human multiple myeloma (MM) cell lines NCI-H929, RPMI 8226, were gift from Professor Jian Hou (Chang zheng hospital, Shanghai, P.R. China), and were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and penicillin-Streptomycin from Invitrogen (Grand Island, NY, USA) at 37 °C in a 5% CO_2 humidified atmosphere.

A 100 μL NCI-H929 or RPMI 8226 cells (10^4/well) were seeded into 96-well plates. After treated with tested compounds for 72 h, cells were added with MTS at a final concentration of 0.5 mg/mL for 2–4 h. Optical density was determined at 490 nm (background subtraction at 690 nm) by SpectraMax 340 microplate reader (Molecular Devices, Sunnyvale, CA, USA). The growth inhibitory ratio was calculated as follows: Growth inhibitory ratio = $(A_{control} - A_{sample})/A_{control}$. IC_{50} values were derived from a nonlinear regression model (curvefit) based on sigmoidal dose response curve (variable slope) and computed using Graghpad Prism version 5.02, Graphpad Software.

4.2.3. Western blot analysis

Poly ADP-ribose polymerase (PARP), and Ubiquitin antibody were purchased from Cell Signaling Technology (Boston, MA, USA); β-actin antibody was obtained from Sigma. All antibodies were used as recommended by the manufacturers.

For experiments, cells were grown in 6-well plates incubated with Compounds for 12 h. Cells were rinsed twice with ice-cold PBS and lysed with 1× SDS loading buffer. Samples were electrophoresed on 10% SDS–polyacrylamide gels, and transferred to PVDF membranes. The membranes were blocked for 1 h with 5% w/v milk, incubated with indicated antibodies for 2 h, washed 3 times with PBS, incubated with the anti-rabbit or anti-mouse secondary antibody for 1 h, washed 3 times with PBS, and detected with the ECL Kit.

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