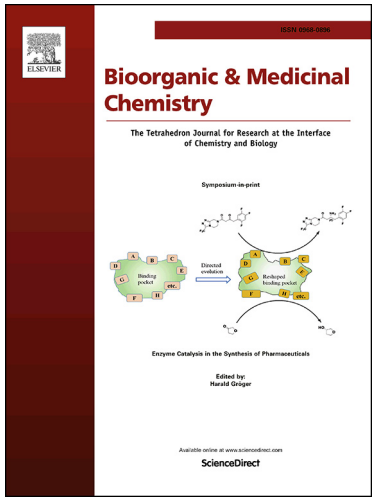


Preparation and Biological Evaluation of Soluble Tetrapeptide Epoxyketone Proteasome Inhibitors

Meng Lei, Haoyang Zhang, Hang Miao, Xiao Du, Hui Zhou, Jia Wang, Xueyuan Wang, Huayun Feng, Jingmiao Shi, Zhaogang Liu, Jian Shen, Yongqiang Zhu

PII: S0968-0896(19)31074-0
DOI: https://doi.org/10.1016/j.bmc.2019.07.044
Reference: BMC 15028

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 27 June 2019
Revised Date: 23 July 2019
Accepted Date: 26 July 2019

Please cite this article as: Lei, M., Zhang, H., Miao, H., Du, X., Zhou, H., Wang, J., Wang, X., Feng, H., Shi, J., Liu, Z., Shen, J., Zhu, Y., Preparation and Biological Evaluation of Soluble Tetrapeptide Epoxyketone Proteasome Inhibitors, *Bioorganic & Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.bmc.2019.07.044

Preparation and Biological Evaluation of Soluble Tetrapeptide Epoxyketone Proteasome Inhibitors

Meng Lei[a, *], Haoyang Zhang[b], Hang Miao[a], Xiao Du[b], Hui Zhou[b], Jia Wang[c], Xueyuan Wang[b], Huayun Feng[a], Jingmiao Shi[c], Zhaogang Liu[c], Jian Shen[d], Yongqiang Zhu[b, c, *]

[a] College of Science, Nanjing Forestry University, No. 159 Longpan Road, Nanjing 210037, PR China

[b] College of Life Science, Nanjing Normal University, No. 1 Wenyuan Road, Nanjing 210037, PR China

[c] Jiangsu Chia Tai Fenghai Pharmaceutical Co. Ltd., No. 9 Weidi Road, Nanjing 210046, PR China

[d] *College of Chemistry and Materials Science, Nanjing Normal University, No. 1 Wenyuan Road, Nanjing 210046, PR China*

*Corresponding authors.

Tel.:+86 25 85891591.

E-mail address: zhyqscu@hotmail.com (Y.Q. Zhu)

Tel.:+86 25 85427621.

E-mail address: hk-lm@163.com (M. Lei)

ABSTRACT: A series of novel tetrapeptidyl epoxyketone inhibitors of 20S proteasome was designed and synthesized. To fully understand the SAR, various groups at R^1, R^2, R^3, R^4 and R^5 positions, including aromatic, aliphatic substituents and isomers were designed, synthesized and biologically assayed. Based on the enzymatic results, seven compounds were selected to evaluate their cellular activities and soluble compound **36** showed strong potency against human multiple myeloma (MM) cell lines. Microsomal stability results indicated that compound **36** was more stable in mice, rat and human microsomes than marketed carfilzomib. The in vivo activities of this compound were evaluated with the xenograft mice models of MM cell lines ARH77 and RPMI-8226 with luciferase expression and the T/C value of the two models were 49.5% and 37.6%, respectively. To evaluate the potential cardiovascular toxicity, inhibition of hERG ion channel in HEK293 cells by compound **36** and carfilzomib was carried out. The results indicated that **36** had no binding affinity for the hERG ion channel while carfilzomib could bind it with IC_{50} of 92.1 μM.

1. Introduction

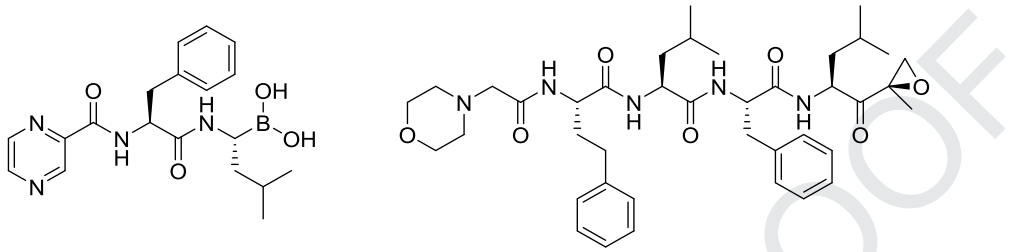
The ubiquitin-proteasome system (UPS) plays a central role in maintaining cellular homeostasis, such as cell-cycle regulation, activation of transcription factors, DNA repair, obsolete proteins and apoptosis induction[1]. The disorder of UPS could cause a variety of diseases including malignancies, neurodegenerative diseases and lead to systematic autoimmunity[2]. As the heart of this system, 26S proteasome functions the nonlysosomal degradation in eukaryote, which consists of one barrel-shaped 20S proteolytic core particle capped with two 19S regulatory subunits[3]. Two 19S caps are located at the two outer sides of the 20S proteolytic core and responsible for recognizing the ubiquitinated proteins, which are degraded into compounds consisting of small amino acids or peptides by the 20S proteasome[4]. The catalytic core 20S proteasome is composed of four stacked rings covered with two outer α-rings and two inner *β*-rings and shows three different types of catalytic activities including chymotrypsin-like (CT-L, *β*5 subunit), trypsin-like (T-L, *β*2

subunit) and caspase-like (PGPH, β1 subunit)[5]. And the CT-L activity is the rate-limiting step in the degradation of intracellular proteins, including those that determine tumor growth and survival[6]. The inhibitors that primarily target the CT-L activity of the proteasome have been found to arrest cell cycle progression and to induce apoptosis in a variety of human neoplastic cells derived from hematological malignancies and solid tumors under experimental conditions[7].

Over the past years, the academic institutes and pharmaceutical companies around the world showed great interests in discovering and developing proteasome inhibitors based on 20S proteasome. The first generation proteasome inhibitor bortezomib (Velcade®, Fig. 1) was approved by the FDA in 2003 and was used for the treatment of relapsed and refractory multiple myeloma (MM) [8, 9]. The structure of this drug was a dipeptide with the carbon terminal substituted by boronic acid as a warhead. However, it showed serious side effects in clinical uses after several years, especially in the peripheral nerve[10, 11]. In 2015, an oral dipeptidyl boronic acid proteasome inhibitor, ixazomib was approved by the FDA. To overcome the reported side effects of bortezomib, the second generation proteasome inhibitor carfilzomib (Kyprolis®, Fig. 1) was developed and approved by FDA in 2012 for the treatment of relapsed MM patients who had received at least two prior therapies including bortezomib[12, 13]. The unique pharmacophore epoxyketone of carfilzomib could reduce the serious peripheral neurotoxicity of this drug in a certain extent[14, 15]. However, the solubility of this drug was too poor and additional 3000 mg of sulfobutylether beta-cyclodextrin had to be added to help solve 60 mg of this drug in clinical usage, which had the potential to lead to acute renal failure of patients treated with this drug for a long time. Furthermore, during the clinical trials of carfilzomib, the most common grade 3 or higher treatment-emergent adverse events were thrombocytopenia, anemia, lymphoenia, neutropenia, pneumonia, fatigue and hyponatremia, especially cardiovascular events[16]. The above-mentioned drawbacks limited its usage in clinic.

Thus, there is a great need to design and develop new proteasome inhibitors with less side effects to meet the clinical need. Our group previously carried out many studies in this area[17-19]. As a continuance of our work, 39 tetrapeptidyl epoxyketones

proteasome inhibitors were synthesized and their biological activities were evaluated in vitro and in vivo in this manuscript. The structure-activity relationships (SARs) were discussed in detail. From the screened compounds, a structurally novel and soluble inhibitor was selected as a clinical candidate.



bortezomib carfilzomib

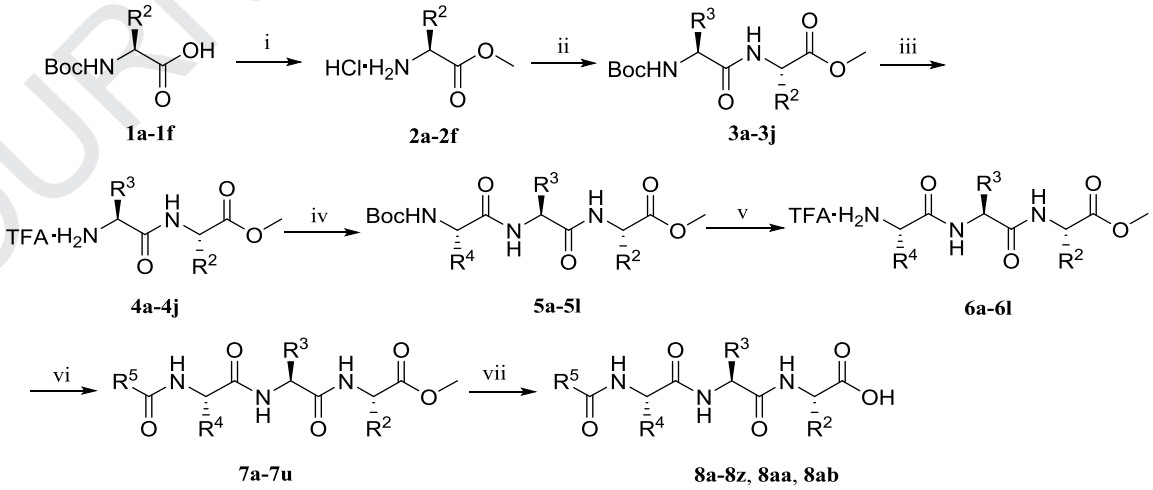
Fig. 1. Chemical structures of proteasome inhibitors bortezomib and carfilzomib.

2. Results and discussion

2.1.Chemical synthesis

The synthesis of intermediate tripeptide acids **8a-8z**, **8aa** and **8ab** was illustrated in Scheme 1 (Detailed procedure was showed in SI file). The key epoxyketone fragments **16a-16e** were prepared in moderate to high yields according to our reported method[20] and showed in Scheme 2. With tripeptide acids **8a-8z**, **8aa**, **8ab** and epoxyketone fragments **16a-16e** in hand, the tetrapeptide epoxyketone **17-47** were obtained by coupling reaction in the presence of HOBt and EDCI (Scheme 3).

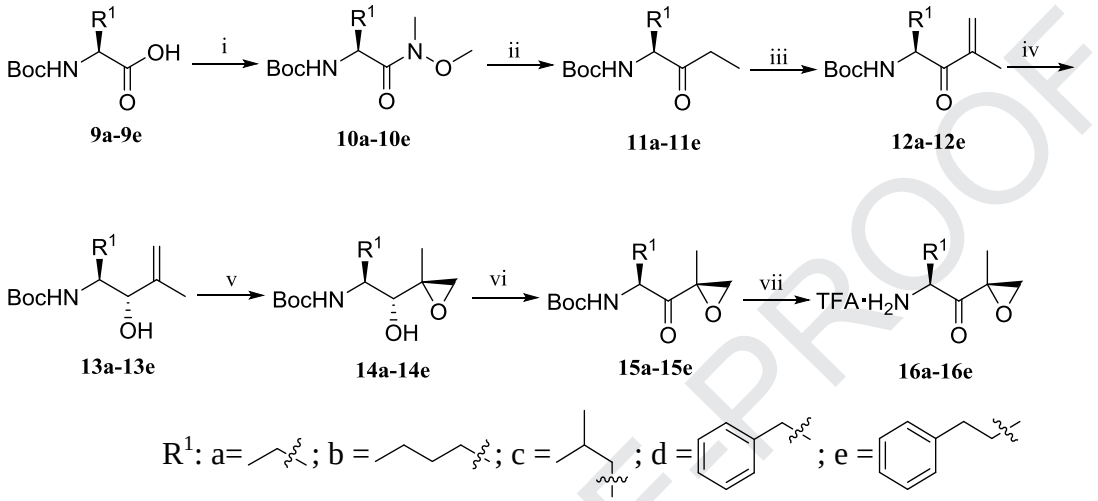
Scheme 1. General synthesis of tripeptide acids **8a-8z**, **8aa** and **8ab**. [a]



[a]Reagents and conditions: (i) MeOH, $SOCl_2$, 0 ℃ to rt, 2 h, 90.5-98.9%; (ii) BocNHCH(R^3)COOH, EDCI, HOBt, DIPEA, DCM, 0 ℃ to rt, 12 h, 50.4-80.7%; (iii) 2 N TFA, DCM, 0 ℃ to rt, 3 h, 85.6-93.5%; (iv) BocNHCH(R^4)COOH, EDCI, HOBt,

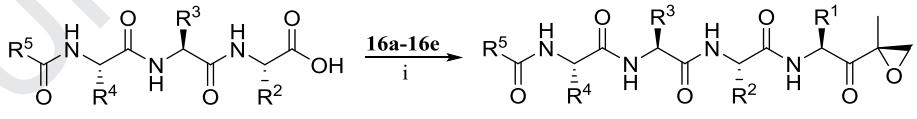
DIPEA, DCM, 0 ℃ to rt, 12 h, 40.4-80.9%; (v) 2 N TFA in DCM, 0 ℃ to rt, 3 h, 80.3-90.8%; (vi) R^5COOH, EDCI, HOBt, DIPEA, DCM, 0 ℃ to rt, 12 h, 50.6-70.2%; (vii) MeOH, 3N LiOH·H_2O, 0 ℃ to rt, 3 h, 70.9-90.1%.

Scheme 2. General synthesis of key epoxyketone fragments **16a-16e**. [a]



[a]Reagents and conditions: (i) HOBt, EDCI, N,O-dimethylhyclroxylamine hydrochloride, DIPEA, DCM, 0 °C, 12 h, 70.4-90.8%; (ii) THF, C_2H_5BrMg, -20 °C, 6 h, 95.4-97.6%; (iii) piperidinium acetate, piperidine, paraformaldehyde, THF, 68 °C, 12 h, 30.1-50.6%; (iv) aluminium isopropoxide, isopropanol, toluene, 50 °C, 3 h, 60.2-80.7%; (v) tert-butylhydroperoxide solution, vanadium(III) acetylacetonate, DCM, 0 ℃ to rt, 12 h, 40.3-50.8%; (vi) pyridine sulfur trioxide, DIPEA, dimethyl sulfoxide, 6 h, 51.6-70.3%; (vii) 2N TFA in DCM, 0 ℃ to rt, 2 h, 85.2-90.7%.

Scheme 3. General synthesis of target compounds **17-47**. [a]



[a]Reagents and condition: (i) HOBt, EDCI, DIPEA, DCM, 0 ℃ to rt, 12 h, 45.2-70.7%.

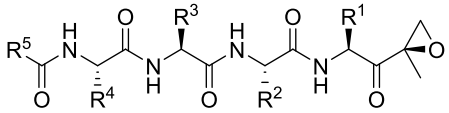
2.2. Biological evaluation

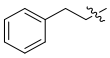
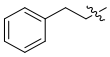
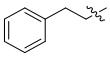
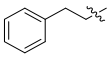
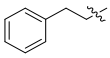
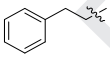
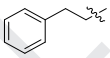
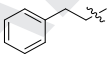
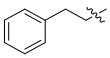
Inhibition of chymotrypsin-like threonine proteasome.

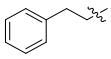
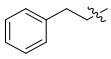
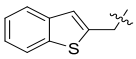
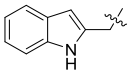
The target compounds were screened for their 20S proteasome chymotrypsin-like inhibitory activities in vitro. Carfilzomib was employed as the standard. To fully understand SAR of tetrapeptide epoxyketone proteasome inhibitors,

various substituents on the R^1, R^2, R^3, R^4 and R^5 positions were investigated. Table 1 summarized the biological results.

Table 1. Structures of compounds **17-47** and inhibitory activities against β5 subunit.



Compd.	R^5	R^4	R^3	R^2	R^1	IC_{50} (nM)[a]
17						77.1±8.1
18						52.2±9.1
19						139.6±13.9
20						729.0±34.2
21				H		NA
22						98.2±10.5
23						162.2±12.1
24						26.8±4.0
25						37.5±2.1
26						50.9±3.4
27						48.3±1.1
28						36.8±0.8
29						32.0±5.3
30						36.6±8.4
31						30.8±7.6

32						NA
33						NA
34						44.9±6.9
35						34.1±3.4
36						36.8±3.6
37						20.6±4.1
38						60.7±8.0
39						25.5±3.3
40		H				56.5±5.5
41						30.6±7.4
42						41.5±3.3
43						43.1±9.1
44						38.8±8.2
45						64.8±12.9
46						52.1±8.3
47			H			NA
carfilzomib						23.6±8.7

[a] All assays were repeated three times.

[b] NA, no activity.

R^1 modification. To investigate the influence of substituents at the R^1 position on potency, the corresponding substituents at R^5, R^4, R^3 and R^2 positions of carfilzomib were employed in the newly designed compounds **17**-**20** (Table 1). The

biological data revealed that aliphatic groups, such as ethyl (**17**, 77.1 nM) and butyl (**18**, 52.2 nM) were more beneficial to the potency compared with aromatics groups, such as phenyl (**19**, 139.6 nM) and benzyl (**20**, 729.0 nM). Moreover, among the aliphatic groups, more steric substituents were more beneficial to the activity than the less steric ones, which was proved by the comparison between isobutyl (carfilzomib, 23.6 nM) and **17**, **18**. These results indicated that isobutyl group played important roles in maintaining the activities of this series of compounds. Therefore, this substituent was employed in the design of other series of compounds.

R^2 modification. Several aromatics and aliphatic groups were designed at R^2 position (compounds **21-25**, Table 1). The biological results showed that H atom at this position led to the inactivity of compound **21**, which suggested that substituent at R^2 position was essential to potency. Aliphatic groups at R^2 position such as ethyl (**22**, 98.2 nM) and n-butyl (**23**, 162.2 nM) increased the binding affinity of molecules to β5 subunit. However, aromatic fragments such as thiazole (**24**, 26.8 nM), benzothiazole (**25**, 37.5 nM) and phenyl (carfilzomib, 23.6 nM) were more beneficial to the activity than aliphatic ones, which suggested that π-π interactions might occur between inhibitors and amino acid residues of β5 subunit.

R^3 modification. Modifications of the R^3 position indicated that aliphatic fragments were more beneficial to the activity than heteroaromatic ones. Methoxymethylene (**26**, 50.9 nM), 2-hydroxyethyl (**27**, 48.3 nM), ethyl (**28**, 36.8 nM), cyclopropyl (**29**, 32.0 nM), cyclopentylmethyl (**30**, 36.6 nM), cyclohexyl (**31**, 30.8 nM) all showed good inhibition of β5 subunit. However, substitutions with

heteroaromatic groups (**32** and **33**) led to the loss of potency and the same case for H atom substitution (**47**, NA vs **41**, 30.6 nM). Furthermore, hydrophobic groups (compounds **28** and **31**) slightly increased the potency compared with the hydrophilic ones (**26** and **27**).

R^4 modification. Several aromatics and aliphatic groups were chosen for R^4 modification with either morpholinylmethyl or 5-methyl-3-isoxazole as the R^5 substituent and the biological results were outlined in Table 1. As for morpholinemethyl group at R^5 position, compounds with the aromatic groups at R^4 position, such as thiophene (**34**, 44.9 nM), benzothiophene (**35**, 34.1 nM), thiazole (**36**, 36.8 nM) showed similar potency. While for the 5-methyl-3-isoxazole as the R^5 substituent, aromatic 2-pyridyl (**38**, 60.7 nM), 2-indolyl (**39**, 25.5 nM) and aliphatic methoxymethylene (**41**, 30.6 nM), isobutyl (**42**, 41.5 nM), cyclopropyl (**43**, 43.1 nM) demonstrated the similar potency. Furthermore, potency of H atom at R^4 position (**40**, 56.5 nM) also maintained, which suggested that the substituents at this position had no crucial interactions with the residues of the active site.

R^5 modification. A series of aromatic and aliphatic carboxylic acids were attached to R^5 positions and the enzymatic activities of resulting molecules were listed in Table 1. Introduction of morpholinylmethylene (**36**, 36.8 nM), 5-methyl-3-isoxazole (**37**, 20.6 nM), pyrazinyl (**44**, 38.8 nM), 2,5-dichlorophenyl (**45**, 64.8 nM) and hydroxymethyl (**46**, 52.1 nM) resulted in similar potency to inhibit the activity of β5 subunit, which suggested that this position had no obvious influence on the potency and could be used to optimize the physicochemical properties of the

compounds.

Cellular activities.

Based on the evaluation of proteasome chymotrypsin-like inhibitory activities, seven compounds were selected to evaluate their cytotoxic activities against two MM cell lines RPMI 8226 and U266B1 by CCK8 assay with carfilzomib as the positive control. The biological results showed that all compounds showed potent cytotoxic activities against two MM cell lines with IC_{50} values less than 0.1 μM (Table 2). Among them, compound **36** exhibited potent cytotoxic activities against two MM cell lines. So this compound was selected for further biological investigations.

Table 2. The cellular activities of compounds against MM cell lines (IC_{50}, nM). [a]

Compd. / Cell lines	**24**	**25**	**28**	**31**	**34**	**35**	**36**	carfilzomib
RPMI8226	76.9 ±6.5	86.6 ±9.0	47.0 ±4.3	14.7 ±2.4	34.6 ±4.5	53.8 ±7.8	27.6 ±3.2	39.1 ±4.7
U226B1	56.5 ±4.5	80.5 ±8.6	45.5 ±5.7	23.2 ±3.2	23.8 ±3.5	33.2 ±5.1	24.5 ±2.4	35.7 ±4.8

[a]All assays were repeated three times.

In vitro microsomal stability evaluation.

The microsomal stabilities of compound **36** was determined with various species of liver microsomes, such as rat, mice, dog, monkey and human. And the results were illustrated in Table 3. The marketed drug carfilzomib was selected as the standard. The half-life ($T_{1/2}$) and intrinsic clearance (CL_{int}) data were used to evaluate the metabolic stabilities. The results indicated that the stabilities of compound **36** and carfilzomib were quite different in various species. Furthermore, compound **36** demonstrated better metabolic stability profiles in mice, rat and human microsomes than carfilzomib. Especially in human microsome stability, the half-life $t_{1/2}$ of compound **36** was nearly 4 fold as that of carfilzomib, which showed compound **36** might have improved in vivo stability compared with carfilzomib.

Table 3. The microsomal stability profiles of compound **36** and carfilzomib.

Compd.	Parameters	Species				
		Rat	Mice	Dog	Monkey	Human
36	$T_{1/2}$ (min)	1.36	1.10	0.243	0.211	1.07
	CL_{int} (mL/min/kg)	1021	1254	5712	6558	1301
carfilzomib	$T_{1/2}$ (min)	0.62	0.34	0.244	<1.00	0.286
	CL_{int} (mL/min/kg)	2231	4104	5673	>1386	4847

Due to its excellent in vitro data, compound **36** was further evaluated in vivo antitumor efficacy against MM xenograft models.

In vivo efficacy of the luciferase expression of human MM cell RPMI8226 xenograft models.

To evaluate the inhibition of hematologic tumor, xenograft NOD/SCID mice models inoculated with luciferase expression of RPMI8226 MM cell lines were built and the tumor was detected by the optical in vivo imaging equipment IVIS Spectrum (PerkinElmer life Science, Hopkinton, MA, USA). The average fluorescence intensity and distribution were measured via Living Image 4.4 software using a region of interest (ROI) centered of tumor tissue after mice were received D-luciferin solution (150 mg/kg body weight) in 3 min. Once the tumor volume reached 100-150 mm^3, mice were intravenously injected with 2 mg/kg of compound **36** QD for 21 days. The tumor was checked once a week. After 21 days, the treatment group exhibited sustained relative tumor average radiance [$p/s/cm^2/sr$] (Fig. 2A and 2B) and more significant in vivo antitumor efficacy than the control group. The inhibition of tumor growth (T/C%) for the treatment group was 49.5%.

In vivo efficacy of human MM cell ARH77 xenograft models.

To further investigate the in vivo efficacy of compound **36**, human MM cell ARH77 xenograft models were successfully developed with nude mice and the mice were dosed with 5 mg/kg of compound **36** on day 1, 4, 8, 11, 15 and 18, and the tumor was checked by caliper every two days. After 21 days treatment, the mice were sacrificed with euthanasia. The results showed that compound **36** displayed significant in vivo antitumor efficacy compared with control group and was nearly as

active as carfilzomib (Fig. 2C), and the inhibitions of tumor growth (T/C%) for the two compounds were 37.6% and 34.6%, respectively.

Inhibition of the human ether-a-go-go related gene (hERG) ion channel.

It was reported that carfilzomib showed cardiovascular side-effect in clinical trial[21]. Generally, the human ether-a-go-go related gene (hERG) ion channel inhibition, which may result in a concomitant risk of sudden death, must be avoided during drug development. The inhibition of the hERG channel by compound **36** and carfilzomib was evaluated in HEK293 cells using an in vitro Ion Works Quattro study. The results indicated that candidate **36** had no binding with the hERG ion channel while carfilzomib could bind the ion channel with IC_{50} of 92.1 μM, which suggested that compound **36** might have less probabilities to induce QT interval prolongation than carfilzomib for the patients.

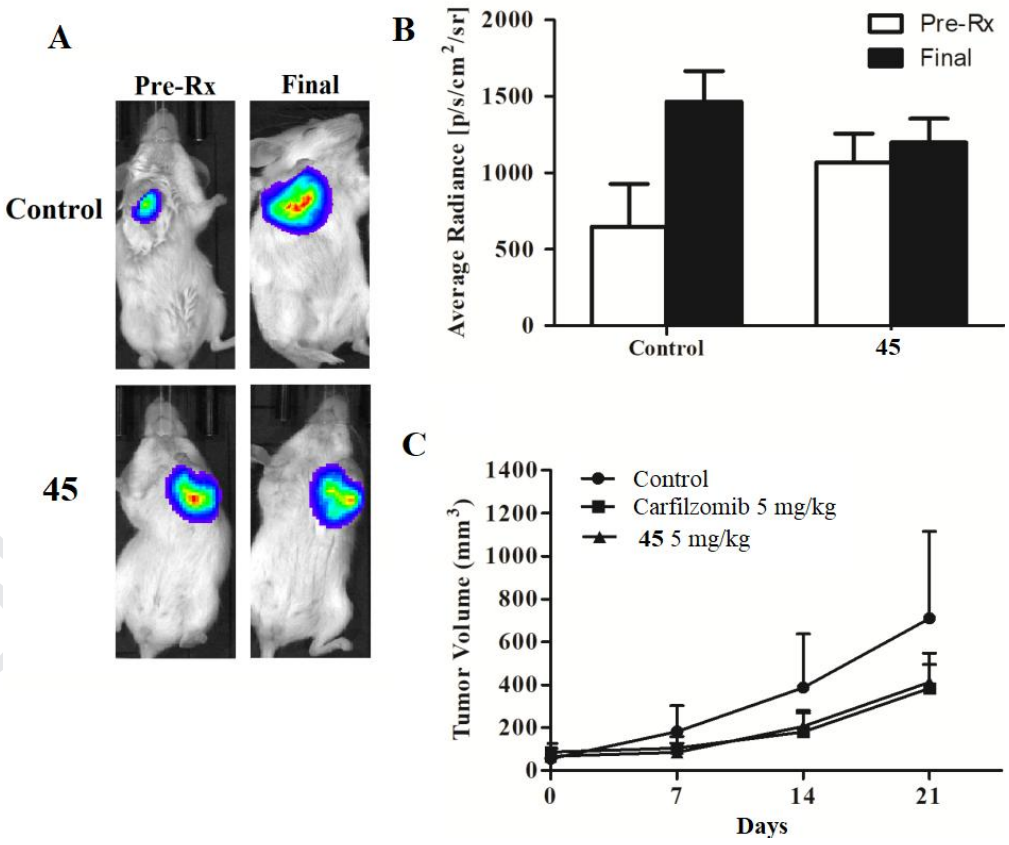


Fig. 2. In vivo efficacy evaluation of compound **36** with human tumor cell xenograft models: (A) Representative live animal imaging demonstrating luciferase expression of human MM cell RPMI 8226 xenografts models in NOD/SCID mice before initiation of treatment and at the end of experiment; (B) Effect of compound **36** on the

luciferase expression of human MM cell RPMI-8226 xenograft models in NOD/SCID mice after 2 mg/kg dose treatment QD; (C) Effect of compound **36** on the volume of xenograft models in nude mice with human MM cell ARH77 after 5 mg/kg dose treatment.

3. Conclusion

Thirty-two tetrapeptide epoxyketone proteasome inhibitors with variations of R^1, R^2, R^3, R^4 and R^5 substituents were synthesized and biologically investigated. Comprehensive SAR analysis for such kind of compounds were performed in detail. It showed that groups at R^4 and R^5 positions had no effects on the inhibition of enzymatic activity while those at R^1, R^2 and R^3 positions greatly affected the activity. The cellular activity results showed that compound **36** was active against human multiple myeloma (MM) cell lines with IC_{50} less than 25 nM. Microsomal stability results indicated that compound **36** was more stable in mice, rat and human microsomes than carfilzomib. Especially in human microsome, the half-life $t_{1/2}$ of compound **36** was nearly 4 fold as that of carfilzomib, which showed compound **36** might have improved in vivo stability compared with carfilzomib. The in vivo xenograft mice models of human cancer cells ARH77 and luciferase expression of RPMI-8226 showed that compound **36** effectively inhibited the tumor growth and the T/C value of the two models were 49.5% and 37.6%, respectively. Results of inhibiting hERG ion channel in HEK293 cells indicated that **36** did not bind the hERG ion channel while carfilzomib could bind the ion channel with IC_{50} of 92.1 μM, which suggested that compound **36** might have less probabilities to induce QT interval prolongation than carfilzomib in clinic. Compound **36** is now being developed as a new candidate for the treatment of MM.

4. Experimental section

4.1. General methods

Unless otherwise indicated，chemicals, solvents and reagents were purchased from commercial suppliers and they were used without any purification. Absolutely anhydrous solvents (CH_2Cl_2, THF, DMF, etc.) were purchased from Energy packaged under nitrogen in Sure/Seal bottles. All reactions involving air or moisture-sensitive

reagents were performed under an argon atmosphere. All reactions were detected by thin layer chromatography on silica gel 60 plate coated with 0.25 mm layer and spotted with UV light or iodine. All final products were purified to >95% purity. The purity of the final products was determined by HPLC (Thermo) on an Agilent Poroshell 120 EC-C18 column (50 mm × 4.6mm, 2.7μm) at 0.3 mL/min flow rate and 254 nm detector wavelength. ^{1}H and ^{13}C spectra were acquired at room temperature on a Bruker Avance 400 spectrometer with chemical shift (δ, ppm) reported relative to TMS as an internal standard. High-resolution mass spectra (HRMS) were recorded on a ZAB-HS instrument using an electrospray ion source (ESI).

4.2 Chemistry

The traditional liquid peptide synthesis procedures were employed to prepare key intermediates **8a-8z**, **8aa** and **8ab**. A typical procedure for preparation of tripeptide epoxyketone proteasome inhibitors of **17-47** was exemplified by the synthesis of candidate **36**.

(S)-4-methyl-N-((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((S)-2-(2-morpholinoacetamido)-3-(thiazol-4-yl)propanamido)pentanamide (**36**). To a cooled solution of **8q** (0.7 g, 1.0 mmol) dissolved in anhydrous CH_2Cl_2 (50 mL) was added HOBt (0.2 g, 1.5 mmol). After 20 min, the temperature of the reaction system was cooled to -15 ℃ and EDC·HCl (0.3 g, 1.5 mmol) was added. Finally, a solution of **16c** (0.3 g, 1.0 mmol) and DIPEA (0.5 ml, 3.0 mmol) in anhydrous CH_2Cl_2 (5 mL) was added. The mixture was stirred at -15 ℃ for 30 min and at room temperature for 12 h, finally quenched with water. The organic phase was washed with 10% of citric acid, 5% of $NaHCO_3$ and saturated brine, respectively, and dried over anhydrous Na_2SO_4, filtered and evaporated to provide crude product. Column chromatography using dichloromethane/methanol (50:1) afforded 0.3 g of pure **36**. Yield 65%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 – 0.80 (m, 6H, CH_3), 0.98 – 0.90 (m, 6H, CH_3), 1.25 (s, 3H, CH_3), 1.33 – 1.28 (m, 2H, CH), 1.64 – 1.50 (m, 4H, CH_2), 2.87 – 2.48 (m, 4H, CH_2), 3.05 – 2.97 (m, 2H, CH_2), 3.10 (s, 2H, CH_2), 3.26 – 3.07 (m, 2H, CH_2), 3.37 – 3.27 (m, 4H, CH_2), 3.72 – 3.64

(m, 4H, CH_2), 4.31 – 4.20 (m, 1H, CH), 4.74 – 4.49 (m, 3H, CH), 6.42 (d, *J* = 7.1 Hz, 1H, CONH), 6.57 (d, *J* = 8.1 Hz, 1H, CONH), 7.12 (d, *J* = 1.9 Hz, 1H, CONH), 7.15 (s, 1H, CONH), 7.17 (d, *J* = 1.4 Hz, 1H, Ph), 7.25 – 7.19 (m, 3H, Ph), 7.27 (d, *J* = 3.1 Hz, 1H, Ph), 8.36 (d, *J* = 6.4 Hz, 1H, 4-H of thiazole), 8.78 (d, *J* = 2.0 Hz, 1H, 2-H of thiazole);^{13}C NMR (100 MHz, $CDCl_3$) δ 16.63, 21.20, 21.54, 22.89, 23.29, 24.65, 24.97, 29.61, 32.59, 36.93, 39.88, 40.32, 49.98, 52.19, 52.25, 52.75, 53.74, 53.88, 58.96, 61.70, 66.84, 116.18, 126.71, 128.39, 129.12, 136.96, 152.36, 153.42, 170.66, 170.73, 170.88, 171.78, 207.87; MS (ESI) m/z: 714.0 $[M+H]^+$; HRMS calcd for $C_{36}H_{52}N_6NaO_7S$, $[M+Na]^+$ 735.3550, found 735.3510.

(S)-4-methyl-N-((S)-1-(((S)-1-((R)-2-methyloxiran-2-yl)-1-oxobutan-2-yl) amino)-1-oxo-3-phenylpropan-2-yl)-2-((S)-2-(2-morpholinoacetamido)-4-phenyl butanamido)pentanamide ***(17)***

Compound **17** was synthesized from **8a** and **16a** according to the general procedure for preparing **36**. Yield 73%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.98 – 0.79 (m, 9H, CH_3), 1.41 (s, 3H, CH_3), 1.48 – 1.41 (m, 1H, CH), 1.70 – 1.48 (m, 4H, CH_2), 1.97 – 1.74 (m, 2H, CH_2), 2.50 – 2.41 (m, 4H, CH_2), 2.84 – 2.71 (m, 2H, CH_2), 2.97 – 2.86 (m, 2H, CH_2), 3.05 – 3.01 (m, 2H, CH_2), 3.22 – 3.06 (m, 2H, CH_2), 3.62 – 3.45 (m, 4H, CH_2), 4.32 – 4.11 (m, 2H, CH), 4.36 – 4.33 (m, 1H, CH), 4.62 – 4.50 (m, 1H, CH), 7.32 – 7.03 (m, 10H, Ph), 7.99 – 7.91 (m, 2H, CONH), 8.11 (d, *J* = 7.9 Hz, 1H, CONH), 8.23 (d, *J* = 6.9 Hz, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 10.39, 16.26, 21.58, 22.96, 23.34, 24.10, 31.40, 37.44, 40.72, 51.15, 51.41, 51.87, 52.24, 53.03, 58.88, 60.84, 65.83, 125.78, 126.14, 127.88, 128.24, 128.26, 129.08, 137.44, 141.49, 170.92, 170.96, 171.08, 171.54, 208.03; MS (ESI) m/z: 692.5 $[M+H]^+$; HRMS calcd for $C_{38}H_{53}N_5O_7Na$, $[M+Na]^+$ 714.3837, found 714.3828.

(S)-4-methyl-N-((S)-1-(((S)-1-((R)-2-methyloxiran-2-yl)-1-oxohexan-2-yl) amino)-1-oxo-3-phenylpropan-2-yl)-2-((S)-2-(2-morpholinoacetamido)-4-phenyl butanamido)pentanamide ***(18)***

Compound **18** was synthesized from **8a** and **16b** according to the general procedure for preparing **36**. Yield 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.79 (d, *J* = 6.3 Hz, 3H, CH_3), 0.85 (d, *J* = 6.0 Hz, 6H, CH_3), 1.24 – 1.18 (m, 2H, CH_2), 1.35 –

1.29 (m, 2H, CH_2), 1.38 – 1.32 (m, 2H, CH_2), 1.39 (s, 3H, CH_3), 1.63 – 1.45 (m, 2H, CH_2), 1.96 – 1.71 (m, 2H, CH_2), 2.46 – 2.31 (m, 4H, CH_2), 2.83 – 2.70 (m, 1H, CH_2), 2.95 (d, J = 4.8 Hz, 2H, CH_2), 2.98 (d, J = 3.9 Hz, 1H, CH_2), 3.21 – 3.06 (m, 2H, CH_2), 3.83 – 3.61 (m, 4H, CH_2), 4.31 – 4.15 (m, 2H, CH), 4.40 – 4.32 (m, 1H, CH), 4.54 – 4.51 (m, 1H, CH), 7.31 – 7.03 (m, 10H, Ph), 7.94 (d, J = 7.9 Hz, 1H, CONH), 8.11 (d, J = 7.9 Hz, 1H, CONH), 8.23 (d, J = 6.9 Hz, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.68, 16.32, 21.57, 21.71, 24.09, 27.56, 29.52, 31.39, 34.32, 37.45, 40.74, 50.75, 51.13, 51.44, 51.82, 52.98, 53.13, 53.38, 58.84, 66.06, 125.76, 126.13, 127.88, 127.94, 128.24, 129.08, 137.44, 141.50, 170.84, 170.94, 171.03, 171.51, 208.03; MS (ESI) m/z: 720.6 $[M+H]^+$， 743.6 $[M+Na]^+$; HRMS calcd for $C_{40}H_{57}N_5O_7Na$, $[M+Na]^+$ 742.4150, found 742.4159.

(S)-4-methyl-N-((S)-1-(((S)-1-((R)-2-methyloxiran-2-yl)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido)pentanamide ***(19)***

Compound **19** was synthesized from **8a** and **16d** according to the general procedure for preparing **36**. Yield 80%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.79 (d, J = 6.2 Hz, 3H, CH_3), 0.84 (d, J = 6.5 Hz, 3H, CH_3), 1.24 (s, 3H, CH_3), 1.39 – 1.32 (m, 1H, CH), 1.56 – 1.49 (m, 2H, CH_2), 1.95 – 1.74 (m, 2H, CH_2), 2.46 (s, 4H, CH_2), 2.56 (dd, J = 17.0, 6.8 Hz, 1H, CH_2), 2.78 – 2.60 (m, 2H, CH_2), 2.93 (d, J = 4.6 Hz, 2H, CH_2), 2.98 (d, J = 11.0 Hz, 1H, CH_2), 3.18 – 3.01 (m, 2H, CH_2), 3.61 (s, 4H, CH_2), 4.28 (d, J = 4.2 Hz, 1H, CH), 4.37 (dd, J = 13.0, 7.7 Hz, 1H, CH), 4.64 – 4.55 (m, 2H, CH), 7.32 – 6.89 (m, 15H, Ph), 7.89 (s, 1H, CONH), 7.92 (s, 1H, CONH), 8.08 (s, 1H, CONH), 8.59 (s, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.19, 21.49, 23.02, 29.02, 31.39, 35.55, 37.59, 40.59, 51.02, 51.55, 51.79, 52.11, 52.57, 53.11, 58.89, 58.94, 65.99, 125.76, 126.14, 126.56, 127.82, 127.88, 128.25, 128.99, 129.06, 129.08, 137.00, 137.34, 141.52, 170.75, 170.98, 171.05, 171.47, 207.80; MS (ESI) m/z: 754.6 $[M+H]^+$; HRMS calcd for $C_{43}H_{55}N_5O_7Na$, $[M+Na]^+$ 776.3993, found 776.4001.

(S)-4-methyl-N-((S)-1-(((S)-1-((R)-2-methyloxiran-2-yl)-1-oxo-4-phenylbutan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido)pentanamide ***(20)***

Compound **20** was synthesized from **8a** and **16e** according to the general procedure for preparing **36**. Yield 76%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (dd, *J* = 10.8, 5.7 Hz, 6H, CH_3), 1.33 (s, 3H, CH_3), 1.47 – 1.39 (m, 1H, CH), 1.62 – 1.49 (m, 2H, CH_2), 1.71 (dd, *J* = 40.3, 30.2 Hz, 2H, CH_2), 2.04 (dd, *J* = 11.7, 7.1 Hz, 4H, CH_2), 2.38 – 2.18 (m, 1H, CH_2), 2.74 – 2.39 (m, 6H, CH_2), 2.82 (dd, *J* = 14.8, 4.9 Hz, 1H, CH_2), 3.10 – 3.02 (m, 2H, CH_2), 3.35 – 3.11 (m, 2H, CH_2), 3.73 (s, 4H, CH_2), 4.67 – 4.41 (m, 3H, CH), 4.75 (d, *J* = 43.0 Hz, 1H, CH), 5.34 (s, 1H, CONH), 6.69 (s, 1H, CONH), 7.30 – 7.06 (m, 15H, Ph), 7.35 (s, 1H, CONH), 7.54 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.69, 22.94, 24.84, 29.81, 30.32, 32.04, 32.90, 38.38, 41.67, 51.57, 52.14, 52.34, 52.56, 52.81, 53.45, 54.20, 59.12, 66.50, 126.24, 126.33, 127.07, 128.39, 128.45, 128.50, 128.56, 128.66, 129.38, 136.48, 140.73, 140.82, 171.18, 171.46, 171.71, 172.10, 207.50; MS (ESI) m/z: 768.8 $[M+H]^+$; HRMS calcd for $C_{44}H_{57}N_5O_7Na$, $[M+Na]^+$ 790.4150, found 790.4139.

(S)-4-methyl-N-(2-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-2-oxoethyl)-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido)-pentanamide ***(21)***

Compound **21** was synthesized from **8b** and **16c** according to the general procedure for preparing **36**. Yield 35%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 – 0.84 (m, 12H, CH_3), 1.23 (s, 3H, CH_3), 1.68 – 1.52 (m, 2H, CH), 1.86 – 1.69 (m, 2H, CH_2), 2.05 – 1.93 (m, 2H, CH_2), 2.08 (dd, *J* = 13.8, 6.7 Hz, 1H, CH_2), 2.56 (dd, *J* = 19.0, 10.9 Hz, 1H, CH_2), 2.88 (d, *J* = 5.0 Hz, 2H, CH_2), 3.01 (d, *J* = 9.6 Hz, 2H, CH_2), 3.31 (d, *J* = 5.0 Hz, 2H, CH_2), 3.73 (d, *J* = 9.6 Hz, 2H, CH_2), 4.68 – 4.45 (m, 2H, CH), 4.76 – 4.71 (m, 1H, CH), 6.62 (s, 1H, CONH), 6.99 (s, 1H, CONH), 7.24 – 7.10 (m, 5H, Ph), 7.63 (s, 1H, CONH), 7.72 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.75, 16.74, 21.21, 22.24, 22.28, 22.77, 23.28, 24.69, 25.15, 27.33, 29.64, 31.81, 32.67, 35.07, 39.39, 42.13, 50.26, 51.54, 52.19, 52.37, 52.66, 53.56, 59.12, 66.68, 125.98, 128.04, 128.38, 140.79, 171.17, 171.75, 208.31; MS (ESI) m/z: 630.8 $[M+H]^+$; HRMS calcd for $C_{34}H_{51}N_5O_7$, $[M+H]^+$ 630.3861, found 630.3842.

(S)-4-methyl-N-((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-

2-yl)amino)-1-oxobutan-2-yl)-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutan-amido) pentanamide ***(22)***

Compound **22** was synthesized from **8c** and **16c** according to the general procedure for preparing **36**. Yield 35%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (dd, *J* = 12.3, 6.3 Hz, 12H, CH_3), 1.26 (d, *J* = 12.5 Hz, 3H, CH_3), 1.49 – 1.35 (m, 2H, CH), 1.65 – 1.57 (m, 2H, CH_2), 1.89 – 1.71 (m, 4H, CH_2), 2.22 – 2.00 (m, 4H, CH_2), 2.53 (d, *J* = 3.8 Hz, 2H, CH_2), 2.71 – 2.61 (m, 2H, CH_2), 3.02 (s, 2H, CH_2), 3.77 – 3.68 (m, 4H, CH_2), 4.06 – 3.98 (m, 2H, CH), 4.52 – 4.39 (m, 2H, CH), 6.68 (s, 1H, CONH), 7.23 – 7.13 (m, 5H, Ph), 7.29 (s, 1H, CONH), 7.60 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.86, 22.86, 24.69, 31.85, 33.78, 40.52, 41.10, 51.65, 52.32, 52.50, 53.71, 61.67, 66.79, 126.20, 128.31, 128.52, 140.57, 170.05, 171.50, 171.94; MS (ESI) m/z: 658.6 $[M+H]^+$; HRMS calcd for $C_{35}H_{56}N_5O_7$, $[M+H]^+$ 658.4174, found 658.4198.

(S)-N-((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)-2-((S)-4-methyl-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido)pentanamido)hexan-amide ***(23)***

Compound **23** was synthesized from **8d** and **16c** according to the general procedure for preparing **36**. Yield 51%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 – 0.84 (m, 15H, CH_3), 1.27 (t, *J* = 8.4 Hz, 3H, CH_3), 1.37 – 1.30 (m, 4H, CH_2), 1.53 – 1.46 (m, 2H, CH), 1.69 – 1.54 (m, 4H, CH_2), 1.99 – 1.73 (m, 2H, CH_2), 2.14 – 2.02 (m, 2H, CH_2), 2.54 (dd, *J* = 19.6, 11.4 Hz, 2H, CH_2), 2.88 (d, *J* = 5.0 Hz, 2H, CH_2), 2.99 – 2.87 (m, 4H, CH_2), 3.33 (t, *J* = 7.3 Hz, 2H, CH_2), 3.73 (t, *J* = 6.0 Hz, 2H, CH_2), 4.56 – 4.43(m, 2H, CH), 4.68 (d, *J* = 7.0 Hz, 1H, CH), 4.82 (d, *J* = 7.1 Hz, 1H, CH), 6.72 (s, 1H, CONH), 7.03 (t, *J* = 10.2 Hz, 2H, Ph), 7.21 – 7.14 (m, 2H, Ph), 7.38 (s, 2H, CONH), 7.73 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.71, 16.73, 21.19, 22.22, 22.78, 23.29, 24.69, 25.14, 26.33, 31.82, 35.11, 39.41, 42.14, 50.29, 51.52, 52.21, 52.37, 53.63, 59.13, 66.74, 125.96, 128.04, 128.37, 140.83, 171.19, 171.61, 171.82, 208.49; MS (ESI) m/z: 686.9 $[M+H]^+$; HRMS calcd for $C_{38}H_{59}N_5O_7$, $[M+H]^+$ 686.4487, found 686.4516.

(S)-4-methyl-N-((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-

2-yl)amino)-1-oxo-3-(thiazol-4-yl)propan-2-yl)-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido)pentanamide ***(24)***

Compound **24** was synthesized from **8e** and **16c** according to the general procedure for preparing **36**. Yield 55%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.76 (d, *J* = 6.4 Hz, 3H, CH_3), 0.80 (s, 3H, CH_3), 0.86 (d, *J* = 5.9 Hz, 3H, CH_3), 0.91 (d, *J* = 5.9 Hz, 3H, CH_3), 1.24 (s, 3H, CH_3), 1.42 – 1.33 (m, 2H, CH), 1.62 (dd, *J* = 16.8, 9.5 Hz, 2H, CH_2), 2.02 (dd, *J* = 12.4, 5.5 Hz, 4H, CH_2), 2.71 – 2.57 (m, *J* = 21.4, 13.2, 6.7 Hz, 6H, CH_2), 3.13 (d, *J* = 13.0 Hz, 2H, CH_2), 3.22 (t, *J* = 12.0 Hz, 2H, CH_2), 3.34 (dd, *J* = 14.7, 5.7 Hz, 2H, CH_2), 3.75 (s, 2H, CH_2), 4.30 (dd, *J* = 10.0, 4.4 Hz, 1H, CH), 4.47 (d, *J* = 7.9 Hz, 1H, CH), 4.55 (s, 1H, CH), 4.75 (dd, *J* = 13.0, 5.5 Hz, 1H, CH), 7.06 (s, 1H, CONH), 7.13 (d, *J* = 7.3 Hz, 3H, Ph), 7.21 (t, *J* = 7.4 Hz, 2H, Ph), 7.28 (s, 1H, CONH), 7.29 (s, 1H, CONH), 7.31 (d, *J* = 4.2 Hz, 1H, CONH), 7.94 (d, *J* = 7.2 Hz, 1H, 4-H of thiazole), 8.19 (s, 1H, 2-H of thiazole); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.83, 21.13, 21.54, 23.11, 23.56, 24.93, 24.99, 29.81, 31.93, 32.36, 39.54, 40.61, 50.03, 52.41, 52.99, 53.09, 53.87, 59.14, 60.53, 66.52, 66.60, 116.31, 126.60, 128.68, 128.86, 140.67, 152.91, 153.29, 170.56, 171.67, 172.17, 207.85; MS (ESI) m/z: 727.5 $[M+H]^+$; HRMS calcd for $C_{37}H_{54}N_6O_7SH$, $[M+H]^+$ 727.3847, found 727.3870.

(S)-N-((S)-3-(benzo[b]thiophen-2-yl)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxopropan-2-yl)-4-methyl-2-((S)-2-(2-morpholino acetamido)-4-phenylbutanamido)pentanamide ***(25)***

Compound **25** was synthesized from **8f** and **16c** according to the general procedure for preparing **36**. Yield 51%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 – 0.77 (m, 6H, CH_3), 0.93 – 0.86 (m, 6H, CH_3), 1.25 (s, 3H, CH_3), 1.41 – 1.36 (m, 2H, CH), 1.52 – 1.49 (m, 2H, CH_2), 1.67 – 1.56 (m, 2H, CH_2), 2.03 – 1.90 (m, 2H, CH_2), 2.59 – 2.39 (m, 6H, CH_2), 2.78 (d, *J* = 52.3 Hz, 2H, CH_2), 2.99 (s, 2H, CH_2), 3.24 (d, *J* = 28.1, 2H, CH_2), 3.75 (s, 2H, CH_2), 4.50 (s, 1H, CH), 4.58 (d, *J* = 6.8 Hz, 1H, CH), 4.77 – 4.65 (m, 2H, CH), 6.01 (s, 2H, CONH), 6.93 (s, 1H, CONH), 7.04 (s, 1H, CONH), 7.18 – 7.09 (m, 5H, Ph) 7.23 (m, 1H, 3-H of benzothiophene), 7.35 – 7.29 (m, 3H, Ph), 7.83 – 7.68 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.82, 21.45, 22.98, 23.54, 24.88, 25.17, 29.85, 37.80, 39.34, 40.11, 40.74, 52.43, 52.53, 53.59, 53.67, 54.11, 55.98,

57.37, 58.51, 59.12, 66.01, 121.69, 123.22, 124.60, 124.90, 127.07, 128.64, 129.41, 130.92, 136.68, 138.67, 140.52, 170.72, 171.21, 207.96; MS (ESI) m/z: 776.4 $[M+H]^+$; HRMS calcd for $C_{42}H_{57}N_5O_7SH$, $[M+H]^+$ 776.4051, found 776.4143.

N-((S)-1-(((S)-3-methoxy-1-(((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopropan-2-yl) amino)-1-oxo-4-phenylbutan-2-yl)-5-methylisoxazole-3-carboxamide ***(26)***

Compound **26** was synthesized from **8g** and **16c** according to the general procedure for preparing **36**. Yield 30%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.43 – 1.40 (m, 1H, CH), 1.50 – 1.47 (m, 2H, CH_2), 2.49 (s, 3H, 5-CH_3 of isoxazole), 2.87 (d, J = 4.7 Hz, 1H, CH_2), 2.90 – 2.87 (m, 2H, CH_2), 3.01 (d, J = 19.2 Hz, 2H, CH_2), 3.21 – 3.15 (m, 2H, CH_2), 3.31 (s, 3H, OCH_3), 4.27 – 4.21 (m, 1H, CH), 4.55 – 4.45 (m, 1H, CH), 4.63 – 4.58 (m, 1H, CH), 4.81 – 4.76 (m, 1H, CH), 6.38 (s, 1H, 4-H of isoxazole), 6.66 (s, 1H, CONH), 7.11 (s, 1H, CONH), 7.19 – 7.13 (m, 4H, Ph), 7.23 – 7.19 (m, 3H, Ph), 7.34 (s, 1H, Ph), 7.52 (d, J = 8.2 Hz, 2H, Ph), 7.74 (s, 1H, CONH), 8.52 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.29, 16.87, 21.44, 21.53, 22.85, 23.13, 24.76, 36.48 , 38.56, 39.99, 40.66, 50.21, 52.47, 53.77, 54.14, 54.30, 59.22, 101.38, 126.61, 126.85, 128.60, 129.29, 129.34, 135.39, 135.50, 147.19, 151.69, 159.17, 162.48, 167.86, 171.07, 171.68, 172.26, 208.10; MS (ESI) m/z: 711.6 $[M+Na]^+$; HRMS calcd for $C_{37}H_{48}N_6O_7Na$, $[M+Na]^+$ 711.3476 found 711.3405.

N-((S)-1-(((2S,3S)-3-hydroxy-1-(((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxobutan-2-yl) amino)-1-oxo-4-phenylbutan-2-yl)-5-methylisoxazole-3-carboxamide ***(27)***

Compound **27** was synthesized from **8h** and **16c** according to the general procedure for preparing **36**. Yield 80%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.91 – 0.73 (m, 6H, CH_3), 1.23 – 1.18 (m, 1H, CH), 1.30 (s, 3H, CH_3), 1.39 (d, J = 6.9 Hz, 3H, CH_3), 1.97 (d, J = 7.2 Hz, 2H, CH_2), 2.47 (s, 3H, 5-CH_3 of isoxazole), 2.60 (m, 2H, CH_2), 2.90 – 2.70 (m, 2H, CH_2), 3.04 – 2.90 (m, 2H, CH_2), 3.23 (d, J = 5.2 Hz, 3H, CH_2), 3.45 (d, J = 5.5 Hz, 1H, CH_2), 4.42 – 4.24 (m, 3H, CH), 4.54 – 4.50 (m, 2H,

CH), 5.37 (s, 1H, OH), 6.57 (d, *J* = 6.8 Hz, 1H, 4-H of isoxazole), 7.32 – 7.02 (m, 10H, Ph), 8.15 (s, 1H, CONH), 8.40 (s, 2H, CONH), 8.77 (s, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 11.83, 16.38, 21.04, 23.11, 31.61, 33.65, 37.43, 38.58, 49.17, 51.48, 52.52, 52.76, 53.25, 58.24, 58.80, 71.86, 101.40, 125.78, 126.17, 127.91, 128.29, 129.07, 129.21, 137.31, 141.37, 158.65, 162.28, 168.96, 170.76, 170.90, 171.22, 208.18; MS (ESI) m/z: 690.5 $[M+H]^+$, 713.5 $[M+Na]^+$; HRMS calcd for $C_{37}H_{47}N_5O_8Na$, $[M+Na]^+$ 712.3316, found 712.3320.

5-methyl-N-((S)-1-(((S)-1-(((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxobutan-2-yl)amino)-1-oxo-4-phenylbutan-2-yl)isoxazole-3-carboxamide ***(28)***

Compound **28** was synthesized from **8i** and **16c** according to the general procedure for preparing **36**. Yield 77%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 – 0.71 (m, 9H, CH_3), 1.31 – 1.22 (m, 1H, CH), 1.39 (d, *J* = 7.8 Hz, 3H, CH_3), 1.70 – 1.44 (m, 2H, CH_2), 2.04 – 1.92 (m, 2H, CH_2), 2.47 (s, 3H, 5-CH_3 of isoxazole), 2.60 – 2.56 (m, 2H, CH_2), 2.92 – 2.70 (m, 2H, CH_2), 2.95 – 2.84 (m, 1H, CH_2), 3.16 – 3.07 (m, 1H, CH_2), 4.22 – 4.19 (m, 1H, CH), 4.42 – 4.28 (m, 1H, CH), 4.48 (m, 1H, CH), 4.64 – 4.54 (m, 1H, CH), 6.58 (s, 1H, 4-H of isoxazole), 7.32 – 7.01 (m, 10H, Ph), 8.07 – 7.97 (m, 2H, CONH), 8.29 (s, 2H, CONH), 8.71 (d, *J* = 8.0 Hz, 2H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 9.95, 11.82, 16.40, 23.12, 24.48, 31.64, 33.65, 37.54, 38.42, 49.26, 51.54, 52.87, 53.70, 58.80, 59.74, 101.41, 125.79, 126.17, 127.91, 128.24, 128.30, 129.05, 137.43, 141.40, 158.64, 170.29, 170.73, 170.88, 171.08, 171.21, 208.18; MS (ESI) m/z: 674.6 $[M+H]^+$, 696.6 $[M+Na]^+$; HRMS calcd for $C_{37}H_{47}N_5O_7Na$, $[M+Na]^+$ 696.3367, found 696.3374.

N-((S)-1-(((S)-1-cyclopropyl-2-(((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)amino)-1-oxo-4-phenylbutan-2-yl)-5-methylisoxazole-3-carboxamide ***(29)***

Compound **29** was synthesized from **8j** and **16c** according to the general procedure for preparing **45**. Yield 54%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.69 – 0.40 (m,

4H, CH_2), 1.09 – 0.92 (m, 6H, CH_3), 1.25 – 1.19 (m, 1H, CH), 1.41 (d, *J* = 7.8 Hz, 3H, CH_3), 1.49 – 1.45 (m, 1H, CH), 1.76 – 1.61 (m, 2H, CH_2), 2.25 – 2.19 (m, 2H, CH_2), 2.64 (s, 3H, 5-CH_3 of isoxazole), 3.00 (d, *J* = 4.9 Hz, 1H, CH_2), 3.18 – 3.09 (m, 2H, CH_2), 3.44 (d, *J* = 4.9 Hz, 1H, CH_2), 4.34 – 4.28 (m, 1H, CH), 4.80 – 4.72 (m, 1H, CH), 5.18 – 5.08 (m, 2H, CH), 6.70 (s, 1H, 4-H of isoxazole), 7.39 – 7.02 (m, 10H, Ph), 7.55 – 7.40 (s, 2H, CONH), 8.02 (s, 2H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 3.36, 3.61, 12.60, 14.39, 14.74, 16.87, 21.79, 22.97, 23.41, 25.43, 29.64, 29.98, 32.12, 32.21, 35.05, 38.84, 40.39, 49.96, 52.53, 53.16,54.18, 56.72, 59.17, 77.00, 77.32, 77.52, 77.63, 101.89, 126.30, 127.10, 128.61, 128.65, 128.70, 129.55, 136.57, 141.08, 158.67, 159.64, 171.05, 171.09, 171.39, 171.43, 208.61; MS (ESI) m/z: 686.8 $[M+H]^+$, 708.8 $[M+Na]^+$; HRMS calcd for C_{38} H_{48} N_5O_7, $[M+H]^+$ 686.3586, found 686.3548.

N-((S)-1-(((S)-3-cyclopentyl-1-(((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopropan-2-yl) amino)-1-oxo-4-phenylbutan-2-yl)-5-methylisoxazole-3-carboxamide ***(30)***

Compound **30** was synthesized from **8k** and **16c** according to the general procedure for preparing **36**. Yield 76%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 – 0.88 (m, 6H, CH_3), 1.25 (s, 3H, CH_3), 1.31 – 1.28 (m, 1H, CH), 1.32 – 1.90 (m, 10H, CH_2), 2.49 (s, *J* = 12.2 Hz, 3H, 5-CH_3 of isoxazole), 2.90 – 2.80 (m, 2H, CH_2), 3.15 – 3.08 (m, 1H, CH_2), 3.37 – 3.19 (m, 3H, CH_2), 4.44 – 4.31 (m, 1H, CH), 4.65 – 4.52 (m, 2H, CH), 4.80 – 4.72 (m, 1H, CH), 6.25 (s, 1H, CONH), 6.41 (s, 1H, 4-H of isoxazole), 6.56 (s, 1H, CONH), 6.70 (s, 1H, CONH), 7.02 – 6.97 (m, 2H, Ph), 7.21 – 7.11 (m, 5H, Ph), 7.37 (m, 2H, Ph), 7.52 (s, 1H, Ph), 7.71 (s, 1H, CONH), 8.46 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.30, 14.08, 16.69, 21.30, 21.72, 22.68, 23.32, 24.51, 25.08, 29.67, 31.41, 31.90, 37.11, 39.90, 40.45, 52.33, 54.15, 59.03, 101.30, 109.89, 111.49, 118.71, 119.96, 122.55, 123.45, 123.95, 124.45, 126.84, 126.97, 128.49, 129.12, 136.31, 136.76, 158.08, 159.59,170.87, 170.96, 171.52, 171.58, 207.93; MS (ESI) m/z: 728.0 $[M+H]^+$, 750.1 $[M+Na]^+$; HRMS calcd for $C_{40}H_{51}N_6O_7$, $[M+H]^+$ 727.3855, found 727.3813.

N-((S)-1-(((S)-1-cyclohexyl-2-(((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)amino)-1-oxo-4-phenylbutan-2-yl)-5-methylisoxazole-3-carboxamide ***(31)***

Compound **31** was synthesized from **8l** and **16c** according to the general procedure for preparing **36**. Yield 76%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89 – 0.69 (m, 6H, CH_3), 1.38 (d, J = 5.4 Hz, 3H, CH_3), 1.44 – 1.39 (m, 1H, CH), 1.68 – 1.50 (m, 10H, CH_2), 2.04 – 1.87 (m, 2H, CH_2), 2.47 (s, 3H, 5-CH_3 of isoxazole), 2.66 – 2.52 (m, 2H, CH_2), 2.80 – 2.69 (m, 1H, CH_2), 2.92 – 2.82 (m, 1H, CH_2), 3.07 – 2.95 (m, 1H, CH_2), 3.33 – 3.15 (m, 1H, CH_2), 4.18 (t, J = 6.5 Hz, 1H, CH), 4.42 – 4.27 (m, 1H, CH), 4.48 (d, J = 4.7 Hz, 1H, CH), 4.57 – 4.49 (m, 1H, CH), 6.57 (s, 1H, 4-H of isoxazole), 7.30 – 6.97 (m, 10H, Ph), 7.89 (s, 1H, CONH), 8.08 (s, 1H, CONH), 8.22 (s, 1H, CONH), 8.73 (s, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 11.84, 16.37, 20.95, 23.15, 24.42, 25.59, 27.99, 28.97, 31.69, 33.72, 37.52, 38.58, 49.10, 51.39, 52.92, 53.41, 57.08, 58.75, 101.41, 125.80, 126.11, 127.88, 128.26, 128.29, 128.94, 137.41, 141.38, 158.63, 170.28, 170.54, 170.79, 171.00, 171.24, 208.01; MS (ESI) m/z: 728.8 $[M+H]^+$, 750.9 $[M+Na]^+$; HRMS calcd for $C_{41}H_{53}N_5O_7Na$, $[M+Na]^+$ 750.3837, found 750.3839.

5-methyl-N-((S)-1-(((S)-1-(((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-(thiazol-4-yl) propan-2-yl)amino)-1-oxo-4-phenylbutan-2-yl)isoxazole-3-carboxamide ***(32)***

Compound **32** was synthesized from **8m** and **16c** according to the general procedure for preparing **36**. Yield 39%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 – 0.88 (m, 6H, CH_3), 1.25 (s, 3H, CH_3), 1.47 – 1.41 (m, 1H, CH), 1.71 – 1.69 (m, 2H, CH_2), 2.12 – 2.03 (m, 2H, CH_2), 2.34 – 2.23 (m, 2H, CH_2), 2.47 (s, 3H, 5-CH_3 of isoxazole), 2.93 (d, J = 4.9 Hz, 2H, CH_2), 3.35 (d, J = 4.9 Hz, 2H, CH_2), 3.49 – 3.39 (m, 2H, CH_2), 4.89 – 4.66 (m, 4H, CH), 6.37 (s, 1H, 4-H of isoxazole), 7.00 (s, 1H, CONH), 7.04 (d, J = 33.5 Hz, 10H, Ph), 7.35 (s, 2H, CONH), 7.52 (s, 1H, CONH), 8.79 (s, 1H, 4-H of thiazol), 8.98 (s, 1H, 2-H of thiazol); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.31, 16.69, 22.67, 22.92, 23.31, 24.60, 24.70, 25.05, 29.09, 29.24, 29.34, 29.44, 29.58, 29.63,

29.67, 31.91, 33.88, 36.98, 39.90, 40.13, 50.11, 52.30, 52.33, 53.66, 54.14, 59.03, 101.32, 116.42, 126.73, 128.45, 129.19, 137.10, 151.92, 153.66, 158.13, 170.30, 171.02, 171.53, 171.69, 178.29, 207.84; MS (ESI) m/z: 743.3; HRMS calcd for $C_{39}H_{46}N_6O_7SNa$, $[M+Na]^+$ 766.3073, found 766.3462.

5-methyl-N-((S)-1-(((S)-1-(((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-(pyridin-2-yl) propan-2-yl)amino)-1-oxo-4-phenylbutan-2-yl)isoxazole-3-carboxamide ***(33)***

Compound **33** was synthesized from **8n** and **16c** according to the general procedure for preparing **36**. Yield 42%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (dd, *J* = 8.2, 6.5 Hz, 3H, CH_3), 0.99 (dd, *J* = 6.3, 3.5 Hz, 3H, CH_3), 1.44 – 1.41 (m, 1H, CH), 1.28 (s, 3H, CH_3), 1.65 – 1.63 (m, 2H, CH_2), 1.67 – 1.65 (m, 2H, CH_2), 2.06 – 1.94 (m, 2H, CH_2), 2.39 – 2.16 (m, 2H, CH_2), 2.45 (s, 3H, 5-CH_3 of isoxazole), 2.94 – 2.87 (m, 2H, CH_2), 3.08 (d, *J* = 4.7 Hz, 1H, CH_2), 3.21 (d, *J* = 4.7 Hz, 1H, CH_2), 4.68 – 4.57 (m, 1H, CH), 4.98 – 4.86 (m, 2H, CH), 5.36 – 5.21 (m, 1H, CH), 6.20 (s, 1H, 4-H of isoxazole), 6.72 (s, 1H, CONH), 7.38 – 7.26 (m, 4H, Ph), 7.50 – 7.38 (m, 4H, Ph), 7.62 – 7.53 (m, 4H, Ph), 7.71 (d, *J* = 26.2, 8.4 Hz, 1H, 4-H of pyridin), 8.04 (d, *J* = 8.5 Hz, 2H, 6-H of pyridin), 8.07 (s, 3H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.61, 16.52, 21.14, 21.68, 24.05, 29.46, 32.51, 35.26, 37.51, 39.12, 51.40, 54.55, 55.35, 56.79, 57.05, 57.73, 58.14, 100.35, 121.74, 122.47, 125.10, 126.07, 127.00, 127.97, 128.30, 128.67, 128.80, 129.14, 129.58, 136.10, 136.47, 142.05, 148.22, 150.08, 159.13, 160.76, 169.51, 170.09, 171.61, 171.92, 210.71; MS (ESI) m/z: 737.4 $[M+H]^+$; HRMS calcd for $C_{41}H_{49}N_6O_7$, $[M+H]^+$ 737.3657, found 737.3640.

(S)-4-methyl-N-((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((S)-2-(2-morpholinoacetamido)-3-(thiophen-3-yl)propanamido)pentanamide ***(34)***

Compound **34** was synthesized from **8o** and **16c** according to the general procedure for preparing **36**. Yield 60%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.67 (s, 3H, CH_3), 0.77 (s, 3H, CH_3), 0.83 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.43

– 1.37 (m, 1H, CH), 1.51 – 1.44 (m, 2H, CH_2), 2.59 – 2.46 (m, 2H, CH_2), 2.88 – 2.77 (m, 4H, CH_2), 3.05 – 2.93 (m, 4H, CH_2), 3.15 – 3.10 (m, 2H, CH_2), 3.31 – 3.20 (m, 2H, CH_2), 3.75 – 3.64 (m, 4H, CH_2), 4.59 – 4.35 (m, 3H, CH), 5.12 – 5.09 (s, 1H, CH), 6.18 (s, 2H, CONH), 6.87 (s, 1H, CONH), 7.00-7.07 (m, 3H, 2, 4, 5-H of thiophen), 7.23 – 7.14 (m, 5H, Ph), 8.41 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.77, 21.19, 21.38, 22.34, 22.98, 23.35, 24.81, 29.83, 31.91, 39.81, 42.04, 51.85, 52.44, 54.30, 54.51, 56.08, 57.08, 59.13, 59.28, 61.50, 66.01, 66.02, 123.17, 126.14, 126.16, 128.34, 128.55, 140.78, 140.88, 169.62, 171.28, 205.99; MS (ESI) m/z: 712.5 $[M+H]^+$; HRMS calcd for $C_{37}H_{53}N_5O_7SNa$, $[M+Na]^+$ 734.3557, found 734.3683.

(S)-2-((S)-3-(benzo[b]thiophen-2-yl)-2-(2-morpholinoacetamido)propanamido)-4-methyl-N-((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl) amino)-1-oxo-3-phenylpropan-2-yl)pentanamide ***(35)***

Compound **35** was synthesized from **8p** and **16c** according to the general procedure for preparing **36**. Yield 65%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (d, *J* = 10.9, 6H, CH_3) 0.90 – 0.86 (m, 6H, CH_3), 1.25 (s, 3H, CH_3), 1.49 – 1.43 (m, 2H, CH), 1.86 –1.71 (m, 4H, CH_2), 2.08 – 1.95 (m, 2H, CH_2), 2.25 – 2.12 (m, 2H, CH_2), 2.39 – 2.26 (m, 2H, CH_2), 2.52 – 2.42 (m, 2H, CH_2), 2.88 – 2.79 (m, 2H, CH_2), 3.05 (d, *J* = 5.3 Hz, 2H, CH_2), 3.28 – 3.17 (m, 2H, CH_2), 3.48 – 3.31 (m, 2H, CH_2), 4.32 – 4.25 (m, 1H, CH), 4.56 – 4.49 (m, 1H, CH), 4.84 – 4.63 (m, 2H, CH), 6.62 (d, *J* = 26.0 Hz, 4H, CONH), 7.20 – 7.11 (m, 2H, Ph), 7.38 –7.22 (m, 5H, Ph), 7.59 (s, 1H, 3-H of benzothiophene), 7.83 – 7.65 (m, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.82, 21.45, 22.98, 23.54, 24.88, 25.17, 29.85, 37.80, 39.34, 40.11, 40.74, 52.43, 52.53, 53.59, 53.67, 54.11, 55.98, 57.37, 58.51, 59.12, 66.01, 121.69, 123.22, 124.60, 124.90, 127.07, 128.64, 129.41, 130.92, 136.68, 138.67, 140.52, 170.72, 171.21, 207.96; MS (ESI) m/z: 762.4 $[M+H]^+$; HRMS calcd for $C_{41}H_{55}N_5O_7SNa$, $[M+Na]^+$ 784.3714, found 784.3858.

5-methyl-N-((S)-1-(((S)-4-methyl-1-(((S)-1-(((S)-4-methyl-1-((R)-2-methyl oxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo pentan-2-yl)amino)-1-oxo-3-(thiazol-4-yl)propan-2-yl)isoxazole-3-carboxamide ***(37)***

Compound **37** was synthesized from **8r** and **16c** according to the general procedure for preparing **36**. Yield 39%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 – 0.98 (m, 6H, CH_3), 1.25 (s, 3H, CH_3), 1.47 – 1.41 (m, 2H, CH), 1.54 – 1.48 (m, 2H, CH_2), 1.59 – 1.55 (m, 2H, CH_2), 2.47 (s, 3H, 5-CH_3 of isoxazole), 2.93 (d, *J* = 4.9 Hz, 2H, CH_2), 3.35 (d, *J* = 4.9 Hz, 2H, CH_2), 3.49 – 3.42 (m, 2H, CH_2) , 4.89 – 4.66 (m, 4H, CH), 6.37 (s, 1H, 4-H of isoxazole), 7.00 (s, 1H, CONH), 7.04 – 7.35 (m, 5H, Ph), 7.35 (s, 3H, CONH), 7.52 (s, 1H, 4-H of thiazol), 8.79 (s, 1H, 2-H of thiazol); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.31, 16.69, 22.67, 22.92, 23.31, 24.60, 24.70, 25.05, 29.67, 36.98, 39.90, 40.13, 50.11, 52.30, 52.33, 53.66, 54.14, 59.03, 101.32, 116.42, 126.73, 128.45, 129.19, 137.10, 151.92, 153.66, 158.13, 170.30, 171.02, 171.53, 171.69, 178.29, 207.84; MS (ESI) m/z: 695.0; HRMS calcd for $C_{35}H_{46}N_6O_7SNa$, $[M+Na]^+$ 717.3050, found 717.3040.

5-methyl-N-((S)-1-(((S)-4-methyl-1-(((S)-1-(((S)-4-methyl-1-((R)-2-methyl oxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-pentan-2-yl)amino)-1-oxo-3-(pyridin-2-yl)propan-2-yl)isoxazole-3-carboxamide ***(38)***

Compound **38** was synthesized from **8s** and **16c** according to the general procedure for preparing **36**. Yield 30%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.73 (s, 3H，CH_3), 0.82 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.43 – 1.40 (m, 2H, CH), 1.50 – 1.47 (m, 4H, CH_2), 2.49 (s, 3H, 5-CH_3 of isoxazole), 2.87 (d, *J* = 4.7 Hz, 1H, CH_2), 3.01 (d, *J* = 19.2 Hz, 2H, CH_2), 3.21 –3.15 (m, 2H, CH_2), 3.31 – 3.25 (m, 1H, CH_2), 4.27 – 4.21 (m, 1H, CH), 4.55 – 4.48 (m, 1H, CH), 4.63 – 4.58 (m, 1H, CH), 4.81 – 4.76 (m, 1H, CH), 6.38 (s, 1H, 4-H of isoxazole), 6.66 (s, 1H, CONH), 7.19 – 7.11 (m, 5H, Ph), 7.23 – 7.19 (m, 3H, Ph), 7.34 (s, 1H, CONH), 7.52 (d, *J* = 8.2 Hz, 1H, 5-H of pyridin), 7.74 (s, 1H, CONH), 8.52 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.29, 16.87, 21.44, 21.53, 22.85, 23.13, 24.76, 36.48 , 38.56, 39.99, 40.66, 50.21, 52.47, 53.77, 54.14, 54.30, 59.22, 101.38, 126.61, 126.85, 128.60, 129.29, 129.34, 135.39, 135.50, 147.19, 151.69, 159.17, 162.48, 167.86, 171.07, 171.68, 172.26, 208.10; MS (ESI) m/z: 688.6 $[M+H]^+$, 711.6 $[M+Na]^+$; HRMS calcd for $C_{37}H_{48}N_6O_7Na$, $[M+Na]^+$ 711.3476, found 711.3605.

N-((S)-3-(1H-indol-2-yl)-1-(((S)-4-methyl-1-(((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopentan-2-yl)amino)-1-oxopropan-2-yl)-5-methylisoxazole-3-carboxamide ***(39)***

Compound **39** was synthesized from **8t** and **16c** according to the general procedure for preparing **36**. Yield 44%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 – 0.72 (m, 12H, CH_3), 1.25 (s, 3H, CH_3), 1.28 – 1.32 (m, 2H, CH_3), 1.42 – 1.39 (m, 1H, CH), 1.51 – 1.47 (m, 1H, CH_2), 1.63 – 1.55 (m, 4H, CH_2), 2.49 (s, 3H, 5-CH_3 of isoxazole), 2.90 – 2.80 (m, 2H, CH_2), 3.08 (dd, *J* = 14.0, 5.9 Hz, 1H, CH_2), 3.37 – 3.19 (m, 3H, CH_2), 4.31 (m, 1H, CH), 4.65 – 4.52 (m, 2H, CH), 4.80 – 4.74 (m, 1H, CH), 6.25 (s, 1H, 3-H of indol), 6.41 (s, 1H, 4-H of isoxazole), 6.56 (s, 1H, CONH), 6.70 (s, 1H, CONH), 7.02 – 6.97 (m, 3H, Ph), 7.21 – 7.11 (m, 5H, Ph), 7.37 (d, *J* = 8.1 Hz, 1H, Ph), 7.52 (s, 2H, CONH), 7.71 (d, *J* = 7.7 Hz, 1H, Ph), 8.46 (s, 1H, 1-H of indol); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.30, 14.08, 16.69, 21.30, 21.72, 22.68, 23.32, 24.51, 25.08, 29.67, 31.41, 31.90, 37.11, 39.90, 40.45, 52.33, 54.15, 59.03, 101.30, 109.89, 111.49, 118.71, 119.96, 122.55, 123.45, 123.95, 124.45, 126.84, 126.97, 128.49, 129.12, 136.31, 136.76, 158.08, 159.59, 170.87, 170.96, 171.52, 171.58, 207.93; MS (ESI) m/z: 728.0 $[M+H]^+$, 750.1 $[M+Na]^+$; HRMS calcd for $C_{40}H_{51}N_6O_7$, $[M+H]^+$ 727.3855, found 727.3814.

5-methyl-N-(2-(((S)-4-methyl-1-(((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopentan-2-yl)amino)-2-oxoethyl)isoxazole-3-carboxamide ***(40)***

Compound **40** was synthesized from **8u** and **16c** according to the general procedure for preparing **36**. yield 35%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (d, *J* = 4.8 Hz, 12H, CH_3). 1.33 (s, 3H, CH_3), 1.45 – 1.40 (m, 2H, CH), 1.58 – 1.53 (m, 4H, CH_2), 2.48 (s, 3H, 5-CH_3 of isoxazole), 2.86 (d, *J* = 3.8 Hz, 1H, CH_2), 3.15 – 2.91 (m, 2H, CH_3), 3.26 (d, *J* = 4.2 Hz, 1H, CH_2), 3.85 (s, 2H, CH_2), 4.08 (s, 1H, CH), 4.61 – 4.42 (m, 2H, CH), 4.83 (d, *J* = 7.2 Hz, 1H, CH), 6.43 (s, 1H, 4-H of isoxazole), 7.16 – 7.01 (m, 5H, Ph), 7.18 (d, *J* = 7.0 Hz, 3H, CONH), 7.66 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.30, 16.60, 22.13, 22.67, 22.74, 23.20, 24.71, 25.08, 38.13, 40.06,

41.29, 43.05, 49.88, 51.92, 52.26, 54.17, 58.93, 101.36, 123.96, 126.86, 128.42, 129.28, 136.45, 158.09, 159.76, 168.13, 170.88, 171.39, 171.69, 208.15; MS (ESI) m/z: 598.8 $[M+H]^+$; HRMS calcd for $C_{31}H_{44}N_5O_7$, $[M+H]^+$ 598.3282, found 598.3235.

N-((4S,7S,10S,13S)-10-benzyl-7-isobutyl-15-methyl-13-((R)-2-methyloxirane-2-carbonyl)-5,8,11-trioxo-2-oxa-6,9,12-triazahexadecan-4-yl)-5-methylisoxazole-3-carboxamide ***(41)***

Compound **41** was synthesized from **8v** and **16c** according to the general procedure for preparing **36**. Yield 82%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.91 – 0.77 (m, 12H, CH_3), 1.31 – 1.25 (m, 2H, CH), 1.40 (d, J = 3.6 Hz, 3H, CH_3), 1.76 –1.58 (m, 4H, CH_2), 2.46 (s, 3H, 5-CH_3 of isoxazole), 2.84 – 2.71 (m, 1H, CH_2), 2.98 – 2.85 (m, 1H, CH_2), 3.04 – 2.99 (m, 1H, CH_2), 3.17 – 3.09 (m, 1H, CH_2), 3.23 (s, 3H, CH_3), 3.68 – 3.51 (m, 2H, CH_2), 4.29 – 4.22 (m, 1H, CH), 4.43 – 4.30 (m, 1H, CH), 4.61 – 4.46 (m, 1H, CH), 4.73 – 4.62 (m, 1H, CH), 6.58 (d, J = 6.6 Hz, 1H, CH_2, 4-H of isoxazole), 7.25 – 7.12 (m, 5H, Ph), 8.05 (s, 1H, CONH), 8.14 (s, 1H, CONH), 8.34 – 8.20 (s, 1H, CONH), 8.63 (s, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 11.83, 16.40, 22.96, 23.15, 24.06, 24.43, 37.19, 38.52, 40.84, 49.23, 51.25, 51.51, 52.76, 53.37, 58.19, 58.80, 71.56, 101.36, 126.20, 127.98, 129.07, 137.64, 158.49, 168.75, 170.94, 171.05, 171.36, 171.47, 208.03, 208.12; MS (ESI) m/z: 642.2 $[M+H]^+$, 664.2 $[M+Na]^+$; HRMS calcd for $C_{33}H_{47}N_5O_8Na$, $[M+Na]^+$ 664.3316, found 664.3315.

5-methyl-N-((S)-4-methyl-1-(((S)-4-methyl-1-(((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopentan-2-yl)amino)-1-oxopentan-2-yl)isoxazole-3-carboxamide ***(42)***

Compound **42** was synthesized from **8w** and **16c** according to the general procedure for preparing **36**. Yield 60%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 – 0.81 (m, 18H, CH_3),1.25 (s, 3H, CH_3), 1.47 – 1.42 (m, 3H, CH), 1.68 – 1.56 (m, 6H, CH_3), 2.49 (s, 3H, 5-CH_3 of isoxazole), 2.86 (d, J = 4.9 Hz, 1H, CH_2), 3.05 – 2.98 (m, 2H,

CH_2), 3.27 (d, *J* = 4.9 Hz, 1H, CH_2), 4.39 – 4.30 (m, 1H, CH), 4.58 – 4.51 (m, 2H, CH), 4.76 (dd, *J* = 14.7, 7.3 Hz, 1H, CH), 6.47 (s, 1H, 4-H of isoxazole), 6.69 (s, 1H, CONH), 6.83 (s, 3H, CONH), 7.23 – 7.11 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.32, 16.62, 21.33, 21.93, 22.77, 22.95, 23.29, 24.78, 25.00, 29.68, 40.03, 40.64, 40.91, 49.86, 52.14, 52.22, 54.03, 58.92, 101.42, 126.88, 128.43, 129.22, 136.60, 158.06, 159.65, 170.66, 171.41, 171.47, 171.60, 207.88; MS (ESI) m/z: 654.9 $[M+H]^+$, 677.0 $[M+Na]^+$; HRMS calcd for $C_{35}H_{58}N_6O_7$, $[M+NH]^+$ 668.4052, found 668.4089.

N-((S)-1-cyclopropyl-2-(((S)-4-methyl-1-(((S)-1-(((S)-4-methyl-1-((R)-2-methyl oxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo pentan-2-yl)amino)-2-oxoethyl)-5-methylisoxazole-3-carboxamide ***(43)***

Compound **43** was synthesized from **8x** and **16c** according to the general procedure for preparing **36**. Yield 60%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.55 – 0.44 (m, 2H, CH_2), 0.72 – 0.62 (m, 2H, CH_2), 0.88 – 0.81 (m, 12H, CH_3), 1.11 – 1.08 (m, 1H, CH), 1.27 (s, 3H, CH_3), 1.51 – 1.47 (m, 2H, CH), 1.65 – 1.63 (m, 4H, CH_2), 2.50 (s, 3H, 5-CH_3 of isoxazole), 2.88 (d, *J* = 4.9 Hz, 2H, CH_2), 3.08 – 2.98 (m, 2H, CH_2), 4.34 – 4.25 (m, 1H, CH), 4.52 – 4.46 (m, 1H, CH), 4.69 – 4.54 (m, 1H, CH), 5.33 – 5.27 (m, 1H, CH_2), 6.43 (s, 1H, 4-H of isoxazole), 7.11 (s, 1H, CONH), 7.14 (s, 1H, CONH), 7.24 – 7.18 (m, 5H, Ph), 7.46 (s, 1H, CONH), 7.53 (s, 1H, CONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 3.36, 3.61, 12.21, 12.68, 18.45, 21.47, 21.69, 22.17, 22.88, 24.36, 24.47, 36.37, 39.31, 41.03, 50.89, 54.01, 56.42, 57.69, 58.84, 62.20, 101.03, 125.93, 126.88, 127.34, 128.03, 128.98, 137.00, 149.91, 159.81, 169.22, 170.62, 171.5, 174.38, 202.11; MS (ESI) m/z: 638.5 $[M+H]^+$; HRMS calcd for $C_{34}H_{47}N_5O_7Na$, $[M+Na]^+$ 660.3132, found 660.3211.

N-((S)-1-(((S)-4-methyl-1-(((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopentan-2-yl)amino)-1-oxo-3-(thiazol-4-yl)propan-2-yl)pyrazine-2-carboxamide ***(44)***

Compound **44** was synthesized from **8y** and **16c** according to the general procedure for preparing **36**. Yield 60%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.77 (d, *J* = 6.3 Hz, 3H, CH_3), 0.81 (d, *J* = 6.4 Hz, 3H, CH_3), 0.89 – 0.85 (m, 3H, CH_3), 1.32 – 1.26 (m, 2H, CH), 1.50 (s, 3H, CH_3), 1.63 – 1.55 (m, 2H, CH_2), 2.07 – 1.94 (m, 2H, CH_2), 2.88 (d, *J* = 5.0 Hz, 1H, CH_2), 2.97 – 2.89 (m, 1H, CH_2), 3.19 – 3.12 (m, 1H, CH_2), 3.32 (d, *J* = 4.9 Hz, 1H, CH_2), 3.36 – 3.28 (m, 1H, CH_2), 3.44 – 3.39 (m, 1H, CH_2), 4.45 – 4.34 (m, 1H, CH), 4.61 – 4.52 (m, 1H, CH), 4.70 – 4.62 (m, 1H, CH), 4.90 – 4.86 (m, 1H, CH), 6.85 (s, 1H, 4-H of thiazol), 7.03 (d, *J* = 6.0 Hz, 2H, CONH), 7.20 – 7.07 (m, 4H, Ph), 7.48 (d, *J* = 8.3 Hz, 1H, Ph), 8.50 (s, 1H, CONH), 8.76 (d, *J* = 4.7 Hz. 2H, pyrazine), 9.03 (s, 1H, 2-H of thiazo), 9.28 (s, 1H, pyrazine); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.70, 21.23, 21.49, 22.95, 23.29, 24.60, 25.07, 32.96, 36.88, 39.71, 40.36, 50.09, 52.21, 52.32, 53.48, 53.92, 59.06, 116.38, 126.72, 128.38, 128.93, 136.88, 142.97, 143.61, 144.21, 147.70, 151.88, 153.88, 163.43, 170.33, 171.03, 171.94, 207.97; MS (ESI) m/z: 692.7 $[M+H]^+$; HRMS calcd for $C_{35}H_{45}N_7O_6SNa$, $[M+Na]^+$ 714.3040, found 714.3042.

2,5-dichloro-N-((S)-1-(((S)-4-methyl-1-(((S)-1-(((S)-4-methyl-1-((R)-2-methyl oxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo pentan-2-yl)amino)-1-oxo-3-(thiazol-4-yl)propan-2-yl)benzamide ***(45)***

Compound **45** was synthesized from **8z** and **16c** according to the general procedure for preparing **36**. Yield 60%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.71 (d, *J* = 6.4 Hz, 3H, CH_3), 0.76 (d, *J* = 6.4 Hz, 3H, CH_3), 0.92 (d, *J* = 6.6 Hz, 6H, CH_3), 1.15 – 1.04 (m, 2H, CH), 1.41 – 1.32 (m, 2H, CH_2), 1.51 (s, 3H, CH_3), 1.67 – 1.58 (m, 2H, CH_2), 2.88 (m, 1H, CH_2), 2.94 (m, 1H, CH_2), 3.24 (m, 1H, CH_2), 3.38 – 3.32 (m, 2H, CH_2), 3.44 (m, 1H, CH_2), 4.25 – 4.18 (m, 1H, CH), 4.57 (m, 1H, CH), 4.71 – 4.65 (m, 1H, CH), 4.75 (m, 1H, CH), 6.46 (d, *J* = 6.9 Hz, 1H, CONH), 6.73 (d, *J* = 8.0 Hz, 1H, CONH), 7.08 – 7.04 (m, 2H, Ph), 7.16 – 7.12 (m, 4H, Ph), 7.18 (d, *J* = 1.9 Hz, 1H, Ph), 7.41 (s, 1H, Ph), 7.62 (s, 1H, Ph), 8.19 (s, 1H, CONH), 8.79 (s, 1H, 2-H of thiazol); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.73, 21.10, 21.30, 22.99, 23.39, 24.60, 25.04, 31.69, 36.67, 39.77, 39.83, 50.04, 52.36, 52.43, 53.95, 54.85, 59.10, 76.68,

77.00, 77.20, 77.32, 116.84, 126.52, 128.28, 128.57, 128.99, 130.04, 131.37, 131.84, 133.65, 135.61, 137.22, 151.66, 153.86, 165.88, 170.25, 171.06, 171.52, 207.82; MS (ESI) m/z: 758.6 $[M+H]^+$; HRMS calcd for $C_{37}H_{45}Cl_2N_5O_6SNa$, $[M+Na]^+$ 780.2340, found 780.2355.

(S)-2-((S)-2-(2-hydroxyacetamido)-3-(thiazol-4-yl)propanamido)-4-methyl-N-((S)-1-(((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)pentanamide ***(46)***

Compound **46** was synthesized from **8aa** and **16c** according to the general procedure for preparing **36**. Yield 61%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 – 0.77 (m, 3H, CH_3), 0.89 – 0.84 (m, 3H, CH_3), 0.98 – 0.89 (m, 6H, CH_3), 1.27 (s, 3H, CH_3), 1.39 – 1.32 (m, 2H, CH), 1.48 – 1.43 (m, 4H, CH_3), 2.62 – 2.58 (m, 1H, CH_2), 3.00 – 2.87 (m, 2H, CH_2), 3.26 – 3.17 (m, 3H, CH_2), 4.05 – 3.93 (m, 1H, CH), 4.27 (s, 1H, OH), 4.51– 4.49 (m, 1H, CH), 4.66 – 4.58 (m, 2H, CH), 7.19 –7.04 (m, 4H, Ph), 7.24 (s, 1H, 4-H of thiazol), 7.58 (s, 1H, Ph), 7.79 (s, 1H, CONH), 7.79 (s, 1H, CONH), 7.88 (s, 1H, CONH), 8.06 – 7.98 (m, 2H, CONH), 8.83 (s, 1H, 2-H of thiazol); ^{13}C NMR(100 MHz, $CDCl_3$) δ 14.13, 14.65, 21.56, 21.77, 22.40, 22.69, 23.23, 29.35, 29.52, 29.69, 31.41, 37.62, 39.75, 42.35, 50.93, 52.24, 53.24, 54.23, 57.44, 58.83, 60.60, 76.68, 77.00, 77.20, 77.32, 79.42, 116.95, 128.29, 128.39, 129.47, 138.88, 152.08, 159.99, 171.74, 171.90, 171.92, 203.54; MS (ESI) m/z: 644.6 $[M+H]^+$; HRMS calcd for $C_{33}H_{45}N_5O_7S$, $[M+H]^+$ 644.3112, found 644.3110.

N-((4S,10S,13S)-10-benzyl-15-methyl-13-((R)-2-methyloxirane-2-carbonyl)-5,8,11-trioxo-2-oxa-6,9,12-triazahexadecan-4-yl)-5-methylisoxazole-3-carboxamide ***(47)***

Compound **47** was synthesized from **8ab** and **16c** according to the general procedure for preparing **36**. Yield 89%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92 – 0.72 (m, 6H, CH_3), 1.27 – 1.21 (m, 1H, CH), 1.37 – 1.28 (m, 2H, CH_2), 1.40 (d, J = 5.1 Hz, 3H, CH_3), 2.46 (s, 3H, 5-CH_3 of isoxazole), 2.81 – 2.66 (m, 1H, CH_2), 2.98 – 2.83 (m, 1H, CH_2), 3.00 (d, J = 5.2 Hz, 1H, CH_2), 3.23 – 3.16 (m, 1H, CH_2), 3.25 (s, 3H, OCH_3), 3.68 – 3.53 (m, 3H, CH_2), 3.72 – 3.67 (m, 1H, CH_2), 4.40 – 4.24 (m, 1H, CH),

4.71 – 4.49 (m, 2H, CH), 6.63 (s, 1H, 4-H of isoxazole), 7.28 – 7.13 (m, 5H, Ph), 7.95 (s, 1H, CONH), 8.13 (s, 1H, CONH), 8.35 (s, 2H, CONH), 8.51 (s, 1H, 2-H of isoxazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ 11.83, 16.44, 20.90, 23.15, 26.82, 38.40, 42.05, 48.72, 49.49, 51.65, 52.85, 58.20, 58.92, 71.50, 101.39, 126.27, 128.03, 129.15, 137.63, 158.50, 158.67, 168.13, 169.10, 170.75, 171.32, 208.23; MS (ESI) m/z: 586.1 $[M+H]^+$; HRMS calcd for $C_{29}H_{39}N_5O_8Na$, $[M+Na]^+$ 608.2690, found 608.2682.

Enzyme and inhibition assay

Suc-Leu-Leu-Val-Tyr-AMC (Suc represents succinyl and AMC represents 7-amido-4-methyicoumarin, gained from Enzo) for chymotryptic-like (CT-L) activity was used to analyze the enzymatic activities of the proteasome. Four microliter of human 20S proteasome (0.2 nM) was developed with 4 μl of various concentrations of compounds. After 10 min, 8 μl of fluorogenic peptides (50 μM) was added and incubated at 37 ℃ for 1 h. The fluorescence of released AMC reagents was measured by a spectrofluorimeter (CLARIOstar, BMG Germany) at excitation and emission wavelengths of 360/460 nm. 1% DMSO was served as control. The inhibition rate was calculated and then the IC_{50} value was obtained.

Cell culture and cytotoxicity assays

The cytotoxicity of compounds was detected using CellTiter 96 Aqueous One Solution Cell Proliferation assay (Promega). Suspension cells (RPMI8226, U266B1) growing in log phase were plated at 5000-10000 cells per well. U266B1 cells were plated at 10000 cells per well and collected by centrifugation, while RPMI8226 cells at 5000 cells. Twenty-four hours after plating, media including the test compounds were added to each well to afford the intended final concentration. After 72 h, the cell viability was detected using the assay protocol recommended by the manufacturers. The resulting signals were quantified using a CLARIOstar Microplate Reader (BMG LABTECH).

Microsomal stability assay

The metabolic stability profiles of compound **36** and reference compound

carfilzomib were assessed by monitoring the disappearance of the test compounds in the presence of liver microsomes. A typical incubation mixture (100 μL of total volume) for metabolic stability studies contained 1 μM of test compounds, 0.5 mg/mL of microsomal protein (pooled Balb/c mouse liver microsomes prepared in-house or BD UltraPool human liver microsomes), 100 mM of Tris-HCl buffer (pH 7.4) and NADPH generating system (5 mM of isocitric acid, 0.2 unit/mL of isocitric acid dehydrogenase, 5 mM of magnesium chloride, 1 mM of NADP+). After preincubation at 37 °C for 5 min, the reactions were started by addition of NADP+ and further incubated for another 0, 5, 10, 20 min. For control experiments, NADPH and/or liver microsomes were omitted from these incubations. The reactions were terminated by adding 100 μL of ice-cold acetonitrile containing phenytoin (1 μM) as internal standard and keeping on ice for 30 min, followed by centrifugation at 16100g for 15 min to obtain the supernatant. Aliquots (5 μL) were then analyzed for substrate disappearance using liquid chromatography−tandem mass spectrometry (Agilent 1200 HPLC instrument interfaced with Applied Biosystems Qtrap 3200) equipped with an electrospray ion source.

In vivo efficacy of mice xenograft models

Human ARH77 MM cell xenograft model was established by subcutaneously inoculating 1×10^7 cells in nude mice. Once the average tumor volume reached 100-150 mm^3, animals were randomized into treatment and control groups (n = 5). Each group was dosed by intravenous administration (i.v.) day 1, 4, 8, 11, 15 and 18 for 3 weeks with compound **36** and carfilzomib (5 mg/kg). The size of tumors was measured once a week with caliper. Human RPMI-8226 MM cell xenograft model was established by subcutaneously inoculating 5×10^6 cells in NOD/SCID mice. Once the average tumor volume reached 100-150 mm^3, animals were randomized into treatment and control groups (n = 3). Each group was dosed by intravenous administration (i.v.) QD for 3 weeks with compound **36** (2 mg/kg). The size of tumors was measured once a week with caliper. Tumor volume (TV) was calculated as: $V = (\text{length} * \text{width}^2)/2$. The individual relative tumor volume (RTV) was calculated as follows: $RTV = V_t/V_0$, where V_t is the volume on each day of measurement and V_0 is

the volume on the day of initial treatment. Therapeutic effect of compound was expressed in terms of T/C% and the calculation formula is T/C (%) = (mean RTV of the treated group)/(mean RTV of the control group)×100%.

Supporting information

Detailed synthesis and characterization data of the intermediates, see supporting information.

Acknowledgments

This work was supported by the following grants: National Natural Science Foundation of China (21877061), Natural Science Foundation of Jiangsu Province (BK20171448), National and Local Joint Engineering Research Center of Biomedical Functional Materials. Dr. Hailong Liu carried out the Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS).

References

1. Teicher BA, Tomaszewski JE. Proteasome inhibitors. *Biochem Pharmacol.* 2015;96(1):1-9.

2. Nalepa G, Rolfe M, Harper JW. Drug discovery in the ubiquitin-proteasome system. *Nat rev Drug Discov.* 2006;5(7):596-613.

3. Moreau P, Richardson PG, Cavo M. Proteasome inhibitors in multiple myeloma: 10 years later. *Blood*. 2012;120(5):947-959.

4. Gonzalez F, Delahodde A, Kodadek T, et al. Recruitment of a 19S proteasome subcomplex to an activated promoter. *Science*. 2002;296(5567):548-550.

5. Groll M, Ditzel L, Löwe J, et al. Structure of 20S proteasome from yeast at 2.4 Å resolution. *Nature.* 1997;386:463-471.

6. Hempel D, Wojtukiewicz Z, Kozlowski L, et al. Increased plasma proteasome chymotrypsin-like activity in patients with advanced solid tumors. *Tumor Bio.* 2011;32(4):753-759.

7. Kisselev AF, Goldberg AL. Proteasome inhibitors: from research tools to drug candidates. *Chem. Biol.* 2001;8(8):739-758.

8. Penna F, Bonetto A, Aversa Z, et al. Effect of the specific proteasome inhibitor bortezomib on cancer-related muscle wasting. *J Cachexia Sarcopenia.* 2016;7(3):345-354.

9. Maseda D, Meister S, Neubert K. Proteasome inhibition drastically but reversibly impairs murine lymphocyte development. *Cell Death Differ.* 2008;15(3):600-612.

10. Kaplan GS, Torcun CC, Grune T, et al. Proteasome inhibitors in cancer therapy: treatment regimen and peripheral neuropathy as a side effect. *Free Radical Bio Med.* 2017;103:1-13.

11. Orlowski RZ, Zeger EL. Targeting the proteasome as a therapeutic strategy against haematological malignancies. *Expert Opin Inv Drug*. 2006;15(2):117-130.

12. Redic K. Carfilzomib: a novel agent for multiple myeloma. *J Pharm Pharmacol.* 2013;65(8):1095-1106.

13. O'Connor A, Stewart K, Vallone M, et al. A phase 1 dose escalation study of the safety and pharmacokinetics of the novel proteasome inhibitor carfilzomib (PR-171) in patients with hematologic malignancies. *Clin Cancer Res.* 2009;15(22):7085-7091.

14. Alé A, Bruna J, Navarro X, et al. Neurotoxicity induced by antineoplastic proteasome inhibitors. *Neurotoxicology.* 2014;43:28-35.

15. Kisselev AF, Linden WA, Overkleeft, HS. Proteasome inhibitors: an expanding army attacking a unique target. *Chem Biol.* 2012;19(1):99-115.

16. Watanabe T, Tobinai K, Matsumoto M. A phase 1/2 study of carfilzomib in Japanese patients with relapsed and/or refractory multiple myeloma. *Brit J Haematol.* 2016;172(5):745-756.

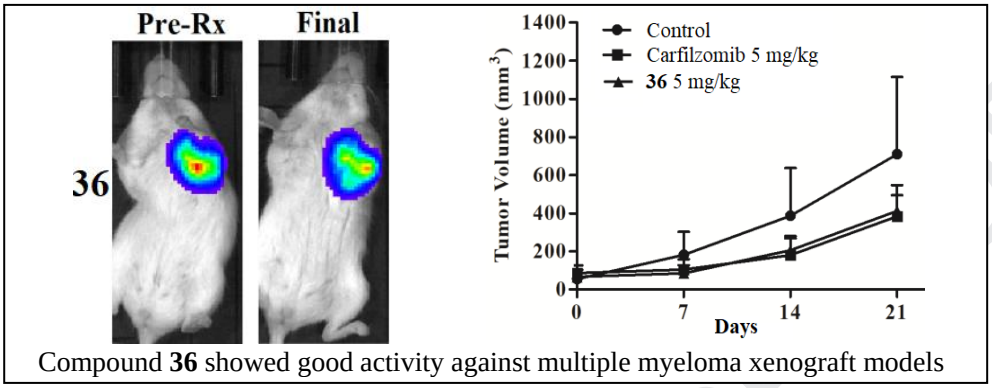
17. Zhu YQ, Zhao X, Zhu XR, et al. Design, synthesis, biological evaluation, and structure-activity relationship (SAR) discussion of dipeptidyl boronate proteasome inhibitors, part I: comprehensive understanding of the SAR of *α*-amino acid boronates. *J Med Chem.* 2009;52:4192-4199.

18. Lei M, Feng HY, Wang C, et al. 3D-QSAR-aided design, synthesis, in vitro and in vivo evaluation of dipeptidyl boronic acid proteasome inhibitors and mechanism studies. *Bioorgan Med Chem.* 2016;24:2576-2588.

19. Lei M, Feng HY, Bai EH, et al. Design, synthesis, in vitro and in vivo evaluation, and structure-activity relationship (SAR) discussion of novel dipeptidyl boronic acid proteasome inhibitors as orally available anti-cancer agents for the treatment of multiple myeloma and mechanism studies. *Bioorgan Med Chem.* 2018;26:3975-3981.

20. Du X, Zhang HY, Lei M, et al. An efficient preparation of novel epoxyketone intermediates for the synthesis of carfilzomib and its derivatives. *J Chem Res.* 2016;40:82-86.

21. Bringhen S, Milan A, Ferri C, et al. Cardiovascular adverse events in modern myeloma therapy-incidence and risks. A review from the European Myeloma Network (EMN) and Italian Society of Arterial Hypertension (ASIIA). *Haematologica*. 2018;103(9):1422-1432.



Compound **36** showed good activity against multiple myeloma xenograft models