European Journal of Medicinal Chemistry 110 (2016) 76–86

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Design, synthesis and biological evaluation of novel asperphenamate derivatives

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ARTICLE INFO

Article history:
Received 25 October 2014
Received in revised form
13 January 2016
Accepted 13 January 2016
Available online 18 January 2016

Keywords:
Asperphenamate
Dipeptide analog
Synthesis
Anticancer activity
Structure–activity relationship
Inducing autophagy

ABSTRACT

A series of novel asperphenamate derivatives were designed and synthesized, including series I (the A-phenyl group replaced with various aromatic heterocycles) and series II (the acyl group substituted by sulfonyl group). All compounds have been screened for their antiproliferative activity *in vitro* against MCF-7, HeLa, and BEL-7402 cell lines by the standard MTT method. Structure–activity relationship studies displayed the heterocycle type played an important role in activity. Six-membered ring derivatives displayed more potency than five-membered ring and the sulfonyl group in A-ring region made an important contribution to activity. Among all derivatives, tosyl derivative **8c** exhibited the greatest potency in three human cancer cell lines. Especially in MCF-7 cells, the cellular potency of **8c** was approximately 3.0-fold more potent than that of cisplatin. Firstly, the mechanism of cell death induced by **8c** in MCF-7 cells was investigated. The results showed that the cell death was induced by autophagy instead of apoptosis or cell cycle arrest. Further studies indicated that **8c** might induce autophagic cell death in HeLa and BEL-7402 cell lines.

1. Introduction

Cancer is one of the most prevalent diseases diagnosed in developed countries and one of the leading causes of mortality [1,2]. The development of cancer therapies over the past few decades has focused principally on chemotherapy combined with other treatment approaches such as surgery, radiotherapy, and targeted therapy. Although a large majority of current anti-cancer drugs exhibit their effects through the induction of apoptosis in cancer cells, many types of cancer are resistant to proapoptotic stimuli. Therefore, new types of drugs that display antiproliferative effects against such apoptosis-resistant cancers are urgently needed.

Autophagy is an important mechanism to maintain cell homeostasis and promote cell survival during starvation [3,4]. But persistent stress or prolonged starvation can result in autophagic cell death, or type II programmed cell death, which is distinct from apoptotic cell death (type I programmed cell death) [5,6]. More recently, induction of autophagy was considered as a mechanism for some anti-cancer compounds and provided a novel target for the development of chemotherapeutic agents [7–11].

Asperphenamate, a dipeptide analog, has an *N, N'*-substituted phenylalanine-phenylalaninol ester framework. It was isolated from raw malt, a traditional medicine for the treatment of mammary hyperplasia [12]. In previous work, we found both the type and position of substituent on A-ring displayed a remarkable influence on bioactivity and *ortho*-benzyloxy substituted derivative (**OBA**) showed the greatest potency. It inhibited the growth of MCF-7 cells by inducing autophagy instead of apoptosis [13]. The results suggested that A-ring region had a significant impact on anticancer effect of asperhenamate.

Heterocyclic compounds occur widely in nature and are essential for life. The widespread use of them as a scaffold in medicinal chemistry establishes this moiety as a member of the privileged structures class [14,15]. Recently, many sulfonyl derivatives have also been reported to display substantial anticancer activity *in vitro*

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http://dx.doi.org/10.1016/j.ejmech.2016.01.020
0223-5234/

and/or *in vivo* [16–19]. In our effort to discover and develop autophagy regulator as potential new anticancer agents, we further investigated the effect of the phenyl and acyl moiety in A-ring region on activity, respectively. And two series of derivatives were designed and synthesized, including series I: the A-phenyl group was replaced by various aromatic heterocycles; series II: the acyl group was substituted by sulfonyl group.

In this paper, we described the synthesis of two series of derivatives as well as their anti-proliferative activities in MCF-7, HeLa, and BEL-7402 cell lines *in vitro*. The cell death mechanisms were further explored in three human cancer cell lines.

2. Results and discussion

2.1. Chemistry

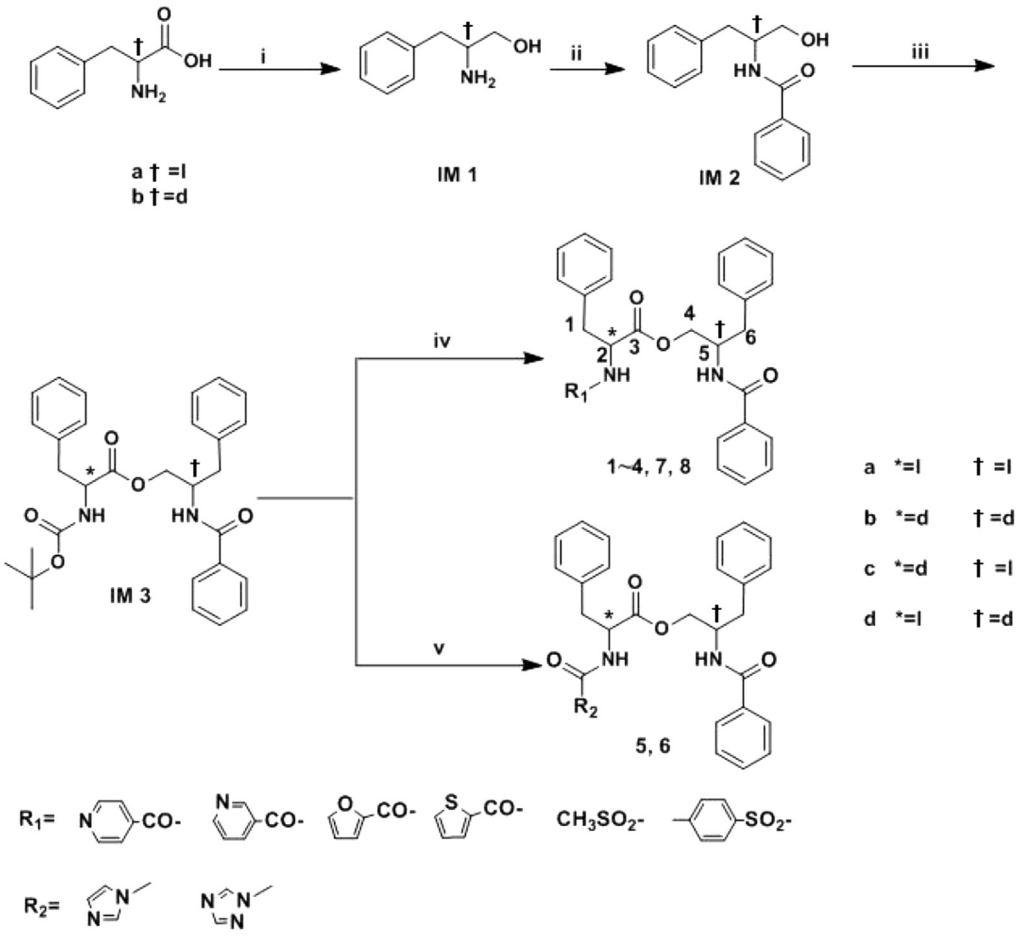
The synthesis of novel asperphenamte derivatives **1**–**8** has been accomplished as outlined in Scheme 1. Starting from optically pure phenylalanine, phenylalaninol **IM1** was prepared according to the method developed by McKennon [20]. *N*-benzoyl-phenylalaninol **IM2** was synthesized by the method of Lewanowicz [21]. Condensation of optically pure *N*-Boc-phenylalanine with **IM2** was promoted by 1, 1'-carbonyldiimidazole (CDI) to afford the key intermediate **IM3**. After the removal of the Boc group by use of 1.5 mol/L HCl (g)/ethyl acetate, **1**–**4, 7** and **8** were directly obtained through acylation reaction. Furthermore, the reaction of deprotected product with triphosgene and imidazole or 1,2,4-triazole in the presence of Et_3N afforded compounds **5** and **6**.

2.2. Biological activity and structure-activity relationships (SAR)

All of these analogs were screened *in vitro* for antitumor activities against MCF-7, HeLa and BEL-7402 cells using the standard MTT method, and cisplatin and **OBA**, the most potent compound in our previous work [13], were used as the positive control. The results were presented in Table 1.

In heterocycles derivatives, almost all *(S, S)* and *(S, R)* configuration compounds displayed no growth inhibitory effect. 2-Furyl and 2-thienyl analogs were inactive with an average IC_{50} value greater than 100 μM. **1c** and **2c** with *(R, S)* configuration, 4-pyridyl and 3-pyridyl derivatives, showed slightly more potency than cisplatin against MCF-7 and BEL-7402 cells. The cytotoxicity of **1c** and **2c** was approximately equal. Imidazolyl and 1, 2, 4-triazolyl derivatives showed similar growth inhibitory effect. And imidazolyl and 1, 2, 4-triazolyl substitution type led to a three to four fold decreased activity compared with **1c**.

In sulfonyl derivatives, with the exception of mesyl derivative with (*S, R*) configuration, other derivatives displayed moderate to excellent growth inhibitory effect. The growth inhibitory activity against three cancer cell lines for tosyl derivatives was superior to mesyl derivatives. Tosyl derivative **8c** showed the greatest potency among tested compounds against three cell lines. The cellular potency of **8c** against MCF-7 and BEL-7402 cell lines was 3.0 and 1.6-fold more potent than that of cisplatin, respectively. **8c** exhibited a similar



Scheme 1. Reagents and conditions: (i) H_2SO_4, $NaBH_4$, I_2, THF, reflux, 98%; (ii) benzoyl chloride, K_2CO_3, MeOH, rt, 95%; (iii) N-Boc-(L or D)-phenylalanine, CDI, $CHCl_3$, rt, 65%; (iv) (a) 1.5 N HCl/AcOEt, rt, 76%; (b) Different acyl chlorides and sulfonyl chlorides, Et_3N, CH_2Cl_2, rt, 92–94%; (v) (a) 1.5 N HCl/AcOEt, rt, 76%; (b) Triphosgene, imidazole or 1,2,4-triazole, Et_3N, CH_2Cl_2, rt, 67–72%.

Table 1
IC_{50} values of different compounds against three human cancer cell lines.

Compound	IC_{50}(μM)[a]		
	MCF-7	HeLa	BEL-7402
1a	>100	>100	>100
1b	53.61 ± 0.76	89.67 ± 1.03	64.71 ± 0.85
1c	15.26 ± 0.35	33.79 ± 0.46	19.33 ± 0.39
1d	64.75 ± 0.8	>100	>100
2a	>100	>100	>100
2b	>100	>100	>100
2c	16.72 ± 0.22	24.85 ± 0.31	16.62 ± 0.21
2d	>100	>100	>100
3a	>100	>100	>100
3b	>100	>100	>100
3c	>100	>100	>100
3d	>100	>100	>100
4a	>100	>100	>100
4b	>100	>100	>100
4c	>100	>100	>100
4d	>100	>100	>100
5a	>100	>100	>100
5b	87.22 ± 0.94	79.09 ± 0.81	91.11 ± 0.95
5c	66.25 ± 0.67	72.46 ± 0.72	78.38 ± 0.75
5d	>100	>100	>100
6a	>100	>100	>100
6b	89.02 ± 0.89	91.23 ± 0.90	95.66 ± 0.93
6c	67.54 ± 0.65	78.93 ± 0.77	82.09 ± 0.79
6d	>100	>100	>100
7a	76.3 ± 0.74	93.85 ± 0.86	70.42 ± 0.67
7b	59.97 ± 0.61	63.95 ± 0.62	52.21 ± 0.47
7c	21.85 ± 0.22	22.11 ± 0.23	37.03 ± 0.37
7d	>100	>100	>100
8a	61.1 ± 0.55	37.59 ± 0.38	49.97 ± 0.46
8b	36.74 ± 0.36	31.57 ± 0.30	22.19 ± 0.21
8c	**5.87 ± 0.11**	**16.75 ± 0.20**	**12.46 ± 0.16**
8d	28.59 ± 0.25	41.89 ± 0.43	54.32 ± 0.56
OBA	6.03 ± 0.97	20.99 ± 1.14	15.65 ± 1.01
Cisplatin[b]	18.9 ± 0.23	8.7 ± 0.11	20.1 ± 0.34

Bold values show the IC_{50} values of the target compounds lower than the values of the positive control.

[a] The growth inhibitory effects of all compounds in MCF-7 (human breast cancer), HeLa (human cervical cancer), BEL-7402 (human liver cancer) cell lines were measured by MTT assay after 3 days of incubation. IC_{50} values were calculated using the software of Statistical Product and Service Solutions. Data were shown as mean ± SD from three independent experiments.

[b] Used as a positive control.

activity to **OBA** against three cell lines. And **8c** against MCF-7 cells showed obvious selectivity over HeLa and BEL-7402 cells (see Fig. 1).

Taken together, the biological activity results indicated that (a) the molecular configuration had an impact on antitumor activity; (b) the heterocycle type played an important role in activity. Six-membered ring displayed more potency than five-membered ring derivatives. In pyridyl analogs, the position of nitrogen atom had no significant influence on activity; (c) the sulfonyl group in A-ring region made an important contribution to activity.

Among the tested compounds, **8c** showed the most potent inhibitory activity against MCF-7 cells, and hence, the mechanism of cell death induced by **8c** in human breast cancer MCF-7 cells was firstly investigated.

2.3. *8c did not induce cell cycle arrest and apoptosis in MCF-7 cells*

MCF-7 cells were treated with 10 μM **8c** for 24, 48 and 72 h, and then examined by flow cytometry analysis after PI staining. The positive control, 0.1 mM H_2O_2, led to a significant increase in the SubG1 cell population (subG1 populations: 38.13%) which represents cells undergoing apoptosis [22]. Compared with it, **8c** did not influence the cell cycle progression and induce the characteristic hypodiploid DNA content peak (subG1) (subG1 populations during 24, 48 and 72 h: 3.38%, 3.22% and 3.76%, respectively, Fig. 2). These results indicated that **8c** did not induce apoptosis or influence the distribution of cell cycle in MCF-7 cell lines.

2.4. *8c inducing autophagy in MCF-7*

Autophagy, type II programmed cell death, is a cell death pathway distinct from apoptosis. More recently, induction of autophagy was considered as a mechanism for some anticancer compounds and provided a novel target for the development of chemotherapeutic agents. For example, rapamycin derivatives CCI-779 and RAD001 (mTOR-specific inhibitors) induced autophagy, and inhibition of autophagy by siRNA against Beclin 1 gene attenuated their anti-cancer effects [23]. The treatment of breast carcinoma cell line MCF-7 with estrogen antagonist tamoxifen caused autophagic cell death [24].

Because **8c** did not induce apoptosis and cell cycle arrest in MCF-7 cells, we continued to investigate whether **8c** could induce autophagy. The formation of acidic vesicular organelles (AVOs) is a characteristic of autophagy. To assess the development of AVOs, MCF-7 cells were treated with **8c** (5 and 10 μM) for 72 h. After stained with AO and MDC [25,26], the morphological changes were observed through fluorescence microscopy. As shown in Fig. 3A and **B**, **8c** could apparently increase the acid vesicles accumulation compared with control cells. MDC fluorescent intensity was also measured to determine AVOs by flow cytometry. As shown in Fig. 3C, after treatment 5 and 10 μM of **8c** for 72 h, MCF-7 cells showed higher MDC associated fluorescence than that of the control cells (from 2.54% to 40.31% and 56.08%).

To exhibit a far more explicit function of **8c** on autophagy of MCF-7 cells, the conversion of LC3-I to LC3-II was investigated. It was known that LC3-I was involved in the formation of autophagosome and was incorporated into the autophagic vacuolemembrane. LC3-I must be conjugated to phosphatidylethanolamine (PE). The PE-conjugated LC3-I is designated as LC3-II. The LC3-II devise is a potential marker of activation of the autophagic pathway [27]. Cellular extracts were prepared from cells incubated with or without **8c** for a period of 48 h and the level of both forms of LC3 was assessed by western blot analysis. As shown in Fig. 3D, the conversion of LC3-I to LC3-II was observed. These results indicated that **8c** was capable of inducing autophagy in MCF-7 cell lines.

2.5. *8c inducing autophagy in HeLa and BEL-7402 cells*

Based on above results, it was important to determine the cell death mechanisms involved in HeLa and BEL-7402 cells. To detect whether **8c** could result in apoptosis, flow cytometry analysis was carried out in two cell lines treated with 20 μM compound **8c** during 48 and 72 h Fig. 4A displayed that **8c** did not influence the

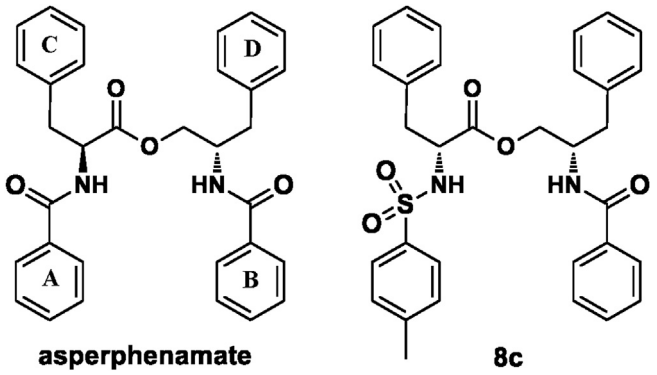


Fig. 1. The structure of asperphenamate and 8c.

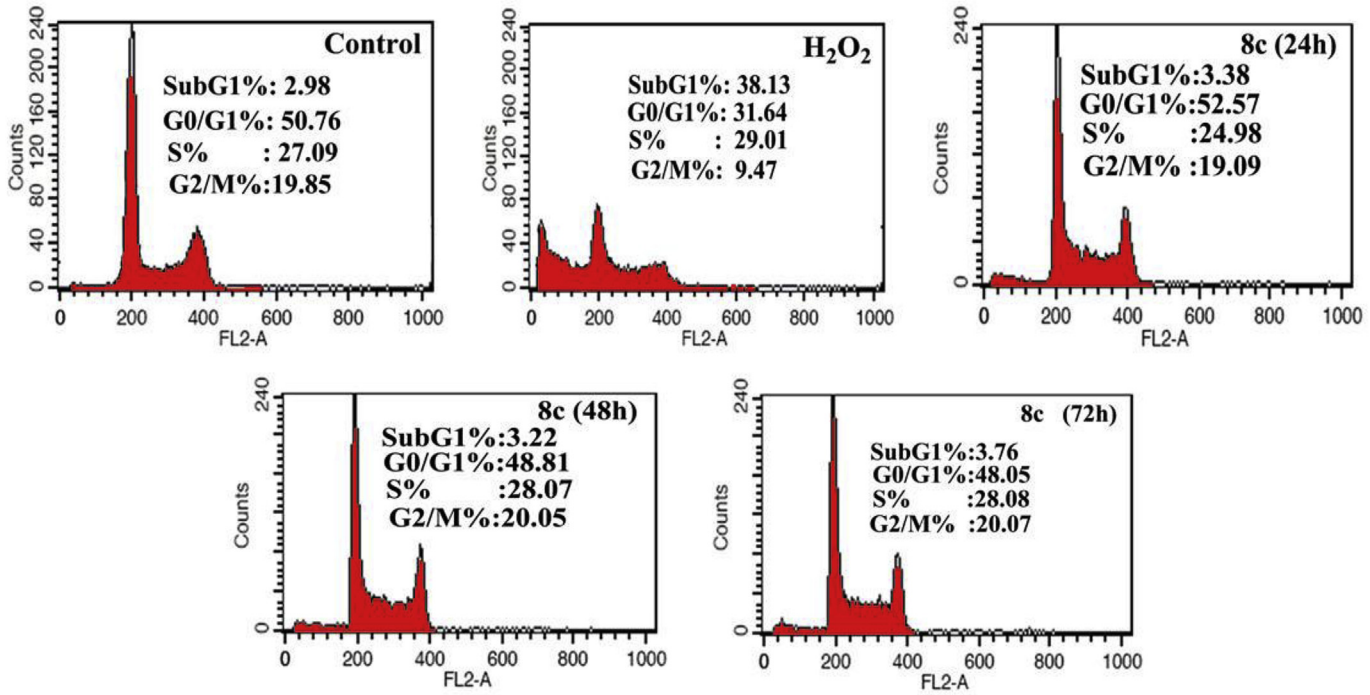


Fig. 2. Flow cytometric analysis of cell cycle distribution in MCF-7 cells treated with 10 μM 8c for 24, 48 and 72 h. The 100 μM of H_2O_2 treating for 12 h was used as a positive control.

cell cycle progression. Meanwhile, the subG1 cell population remained nearly unchanged compared with control (subG1 populations during 48 and 72 h: 2.12 and 2.43% in HeLa and 2.89% in control; 3.01 and 3.78% in BEL-7402 and 2.87% in control), indicating that **8c** did not promote apoptotic death or influence the distribution of cell cycle in two cells.

In order to confirm if the growth inhibitory effects of **8c** were due to autophagy, we examined whether AVOs accumulated in HeLa and BEL-7402 cells by AO staining assay. After treatment with **8c** (20 and 40 μM) for 72 h, acidic vesicles noticeably accumulated in HeLa and BEL-7402 cells (Fig. 4B). Further flow cytometry analysis showed the percentage of autophagic cells displayed a significant increase from 1.92% to 26.11% and 49.37% for HeLa cells; 3.94%–23.66% and 48.25% for BEL-7402, respectively (Fig. 4C). To gain further insight in **8c** inducing autophagy in two cells, we examined the protein level of LC3-II. As shown in Fig. 4D, the LC3-I was almost converted into LC3-II compared with control. This observation, together with the formation of AVOs, demonstrated that autophagy occurred in HeLa and BEL-7402 cells evidently.

3. Conclusion

In summary, two series of asperphenamate derivatives were synthesized including aromatic heterocycles and sulfonyl derivatives. The growth inhibitory activity *in vitro* for all derivatives was evaluated. Among all compounds, **8c** with tosyl moiety showed the strongest inhibitory activity. The mechanism of cell death induced by **8c** in MCF-7, HeLa and BEL-7402 cells was further investigated. The results showed that inhibition of growth was attributed to the autophagy induction.

4. Experimental

4.1. Reagents and apparatus

All melting points were determined on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. High resolution accurate mass determinations (HRMS) for all final target compounds were obtained on a Bruker Micromass Time of Flight mass spectrophotometer equipped with electrospray ionisation (ESI). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker ARX-600 (600 MHz) spectrophotometer or Bruker ARX-300 (300 MHz) spectrophotometer (Bruker Bioscience, Billerica, MA, USA), using $CDCl_3$ and DMSO-d_6 as solvent and tetramethylsilane (TMS) as an internal standard. Unless otherwise indicated, reagents were purchased from commercial suppliers and used without further purification. Organic solvents were dried by standard methods when necessary. The column chromatography was performed using silica gel (200–300 mesh) from Qingdao Ocean Chemicals (Qingdao, Shandong, China) with chloroform/acetone mixture as eluent.

Compounds **IM1** and **IM2** were synthesized in accordance with literature procedures [20,21].

4.2. General procedure for the synthesis of 2-benzamido-3-phenylpropyl 2-(tert-butoxycarbonylamino)-3-phenylpropanoate (*IM 3a-d*)

Commercially available *N-tert*-butoxycarbonyl-*L (or D)*-phenylalanine (4.24 g, 16 mmol) and CDI (3.24 g, 20 mmol) in 200 mL ethanol-free $CHCl_3$ were stirred for 20 min at room temperature. Then a solution of **IM2** (3.98 g, 15.5 mmol) in 80 mL ethanol-free $CHCl_3$ was added in 4 portions at 20 min intervals and stirred for an additional 48 h. The crude product was purified by chromatography on silica gel (chloroform/acetone, 40:1) to give the key intermediate **IM 3a-d** as a white solid.

4.3. Spectroscopy data of compounds *IM 3a-d*

4.3.1. *(S)-(S)-2-Benzamido-3-phenylpropyl 2-(tert-butoxycarbonylamino)-3-phenylpropanoate tert-butoxycarbonylamino)-3-phenylpropanoate (IM 3a)*

Yield: 65.3%; M.p.: 135–137 °C; $[\alpha]_D^{20}$ -15.1 (*c* 0.5,

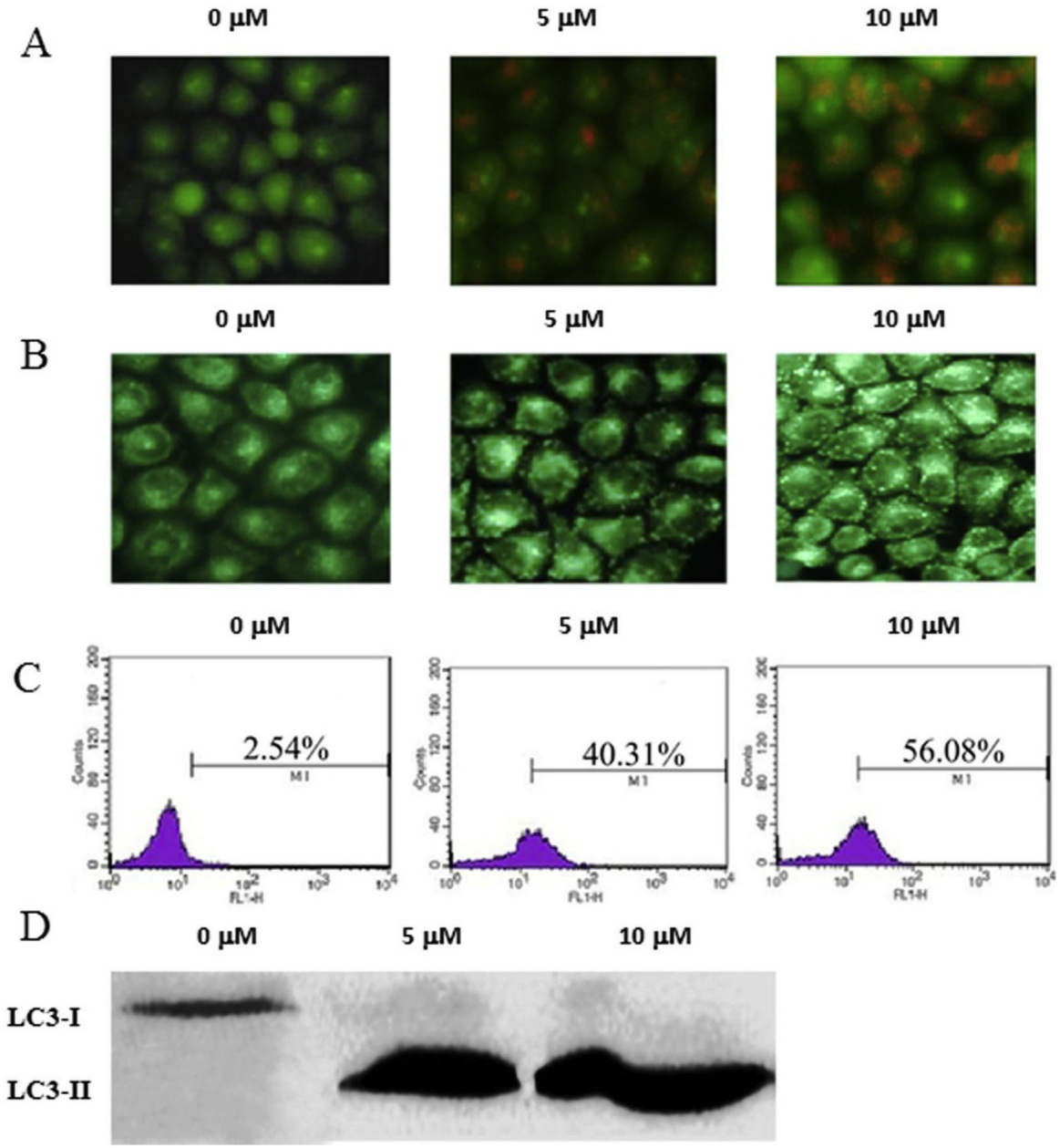


Fig. 3. 8c induced autophagy in MCF-7 cells. (A) Autophagosomes observation. MCF-7 cells treated with 8c (5 and 10 μM) for 72 h, and then the cells were stained with AO and observed by fluorescence microscope. (B) Autophagosomes observation. MCF-7 cells treated with 8c (5 and 10 μM) for 72 h, and then the cells were stained with MDC and observed by fluorescence microscope. (C) Autophagic cells detection. MCF-7 cells treated with 8c (5 and 10 μM) for 72 h, and then the cells were stained with MDC and subjected to flow cytometric analysis. (D) Western blotting analysis for autophagy. MCF-7 cells were treated with 8c (5 and 10 μM) for 48 h, and then the total proteins were extracted and detected by western blot analysis.

$CHCl_3$:MeOH = 1:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.16 (m, 15H, ArH), 6.49 (d, 1H, J = 7.8 Hz, NH), 4.99 (d, 1H, J = 7.2 Hz, NH), 4.57 (m, 1H, 2-H), 4.53 (m, 1H, 5-H), 4.35 (dd, 1H, J = 11.4, 9.6 Hz, 4H-a), 4.09 (dd, 1H, J = 11.4, 3.6 Hz, 4H-b), 3.09 (dd, 1H, J = 13.8, 6.0 Hz, 1H-a), 3.05 (dd, 1H, J = 13.8, 6.6 Hz, 1H-b), 2.95 (dd, 1H, J = 13.8, 8.4 Hz, 6H-a), 2.81 (dd, 1H, J = 13.8, 6.0 Hz, 6H-b), 1.38 (s, 9H, *t*-Bu).

*4.3.2. (R)-(R)-2-Benzamido-3-phenylpropyl 2-(tert-butoxycarbonylamino)-3-phenylpropanoate (**IM 3b**)*

Yield: 66.1%; M.p.: 132–134 °C; $[\alpha]_D^{20}$ 14.8 (*c* 0.5, $CHCl_3$:MeOH = 1:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.16 (m, 15H, ArH), 6.49 (d, 1H, J = 7.8 Hz, NH), 4.99 (d, 1H, J = 7.2 Hz, NH), 4.57 (m, 1H, 2-H), 4.53 (m, 1H, 5-H), 4.35 (dd, 1H, J = 11.4, 9.6 Hz, 4H-a), 4.09 (dd, 1H, J = 11.4, 3.6 Hz, 4H-b), 3.09 (dd, 1H, J = 13.8, 6.0 Hz, 1H-a), 3.05 (dd, 1H, J = 13.8, 6.6 Hz, 1H-b), 2.95 (dd, 1H, J = 13.8, 8.4 Hz, 6H-a), 2.81 (dd, 1H, J = 13.8, 6.0 Hz, 6H-b), 1.38 (s, 9H, *t*-Bu).

*4.3.3. **(R)-(S)-2-Benzamido-3-phenylpropyl 2-(tert-butoxycarbonylamino)-3-phenylpropanoate tert-butoxycarbonylamino)-3-phenylpropanoate (IM 3c)***

Yield: 65.5%; M.p.: 133–134 °C; $[\alpha]_D^{20}$ -8.1 (*c* 0.5, $CHCl_3$:MeOH = 1:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.14 (m, 15H, ArH), 6.49 (d, 1H, J = 7.2 Hz, NH), 4.99 (d, 1H, J = 6.6 Hz, NH), 4.57 (m, 1H, 2-H), 4.54 (m, 1H, 5-H), 4.22 (dd, 1H, J = 11.4, 4.1 Hz, 4H-a), 4.09 (dd, 1H, J = 11.4, 3.0 Hz, 4H-b), 3.07 (m, 2H, 1-H), 2.93 (dd, 1H, J = 13.8, 8.4 Hz, 6H-a), 2.77 (dd, 1H, J = 13.8, 9.0 Hz, 6H-b), 1.38 (s,

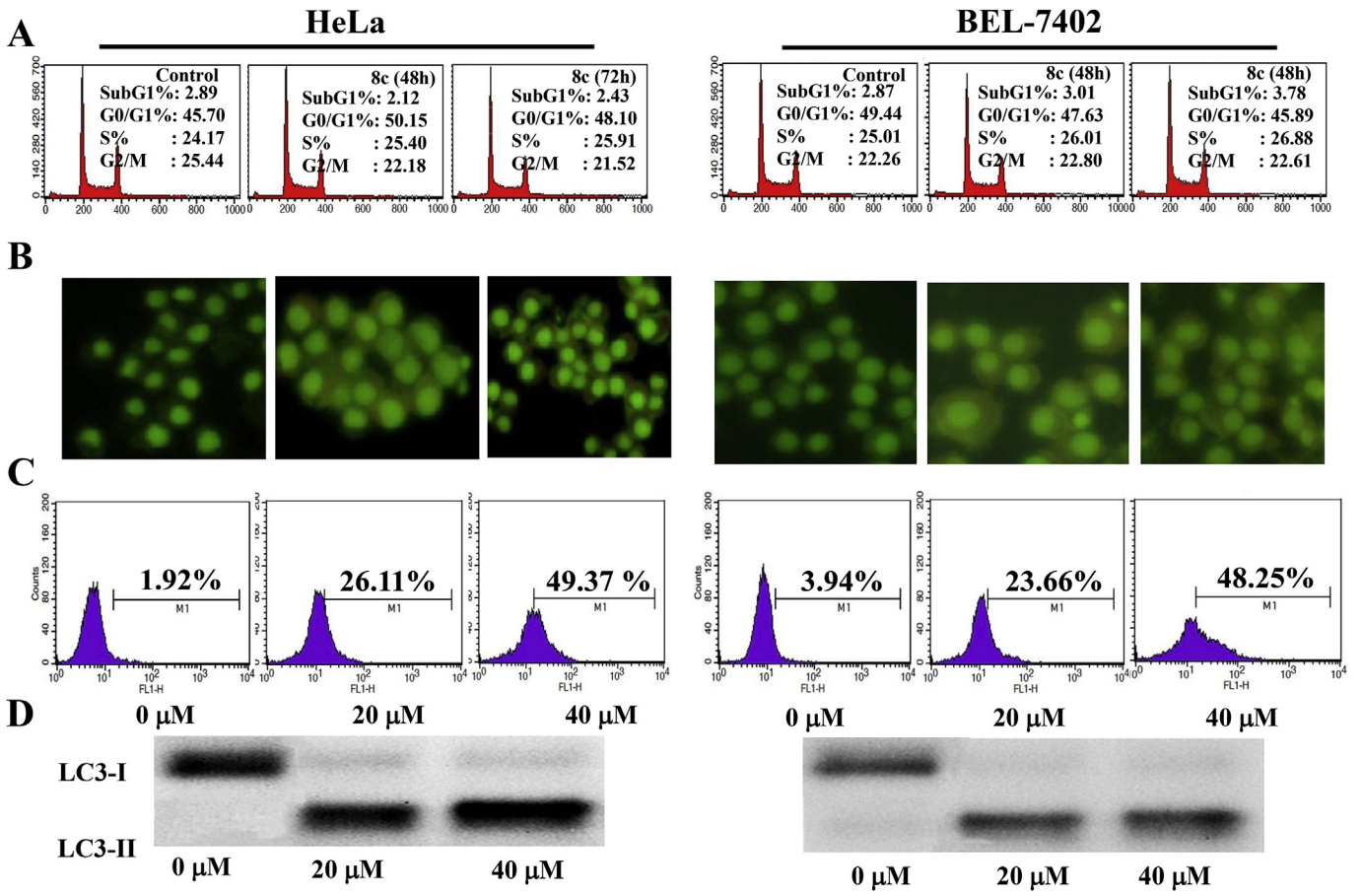


Fig. 4. 8c induced autophagy in HeLa and BEL-7402 cells. (A) Flow cytometric analysis of cell cycle distribution in HeLa and BEL-7402 cells treated with 20 μM 8c for 48 and 72 h, respectively. (B) Autophagosomes observation. HeLa and BEL-7402 cells treated with 8c (20 and 40 μM) for 72 h, and then the cells were stained with AO and observed by fluorescence microscope, respectively. (C) Autophagic cells detection. HeLa and BEL-7402 cells treated with 8c (20 and 40 μM) for 72 h, and then the cells were stained with MDC and subjected to flow cytometric analysis, respectively. (D) Western blotting analysis for autophagy. HeLa and BEL-7402 cells were treated with 8c (20 and 40 μM) for 48 h, and then the total proteins were extracted and detected by western blot analysis, respectively.

9H, *t*-Bu).

4.3.4. ***(S)-(R)-2-Benzamido-3-phenylpropyl 2-(tert-butoxycarbonylamino)-3-phenylpropanoate tert-butoxycarbonylamino)-3-phenylpropanoate (IM 3d)***

Yield: 64.2%; M.p.: 135–136 °C; $[\alpha]_D^{20}$ 7.8 (*c* 0.5, $CHCl_3$:MeOH = 1:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.13 (m, 15H, ArH), 6.49 (d, 1H, *J* = 7.2 Hz, NH), 5.01 (d, 1H, *J* = 6.6 Hz, NH), 4.57 (m, 1H, 2-H), 4.54 (m, 1H, 5-H), 4.21 (dd, 1H, *J* = 11.4, 4.1 Hz, 4H-a), 4.12 (dd, 1H, *J* = 11.4, 3.0 Hz, 4H-b), 3.06 (m, 2H, 1-H), 2.93 (dd, 1H, *J* = 13.8, 8.4 Hz, 6H-a), 2.76 (dd, 1H, *J* = 13.8, 9.0 Hz, 6H-b), 1.38 (s, 9H, *t*-Bu).

4.4. General procedure for the synthesis of compounds ***1–4, 7*** and ***8***

IM 3 (0.3 g, 0.6 mmol) was treated with a solution of dry hydrochloride gas in ethyl acetate (1.5 mol/L, 3.0 mL) for 4 h at room temperature. The solvent was removed under reduced pressure to give a white solid. To a solution of the deprotected product and triethylamine (0.5 mL) in anhydrous CH_2Cl_2 (10 mL) was added dropwise corresponding acyl chloride or sulfonyl chloride (0.8 mmol). The reaction mixture was stirred overnight at room temperature and concentrated under vacuum to give the crude product, which was purified by chromatography on silica gel (chloroform/acetone, 35:1) to yield **1–4, 7** and **8** as a white solid.

4.5. Spectroscopy data of compounds ***1–4, 7*** and ***8***

4.5.1. *(S)-(S)-2-Benzamido-3-phenylpropyl 2-isonicotinamido-3-phenylpropanoate (**1a**)*

Yield: 93.1%; M.p.: 171–175 °C; $[\alpha]_D^{20}$ -20.3 (*c* 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 530.2057 $[M+Na]^+$ (Calcd. for $C_{31}H_{29}N_3O_4Na$, 530.2056); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.18 (m, 19H, ArH), 6.79 (d, 1H, *J* = 6.8 Hz, NH), 6.43 (d, 1H, *J* = 8.3 Hz, NH), 4.94 (m, 1H, 2-H), 4.63 (m, 1H, 5-H), 4.47 (dd, 1H, *J* = 11.2, 3.4 Hz, 4H-a), 4.05 (dd, 1H, *J* = 11.2, 5.3 Hz, 4H-b), 3.27 (dd, 1H, *J* = 13.8, 6.3 Hz, 1H-a), 3.17 (dd, 1H, *J* = 13.8, 6.6 Hz, 1H-b), 2.95 (dd, 1H, *J* = 13.8, 6.4 Hz, 6H-a), 2.85 (dd, 1H, *J* = 13.8, 7.8 Hz, 6H-b); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.33, 167.46, 166.40, 150.21 (2C), 136.78, 135.59, 131.51, 129.13 (2C), 129.06 (2C), 128.76 (2C), 128.69 (2C), 128.42 (2C), 127.33, 126.94 (2C), 126.86, 120.99 (2C), 66.81, 54.44, 50.00, 37.33, 37.17.

4.5.2. *(R)-(R)-2-Benzamido-3-phenylpropyl 2-isonicotinamido-3-phenylpropanoate (**1b**)*

Yield: 93.4%; M.p.: 173–174 °C; $[\alpha]_D^{20}$ 21.1 (*c* 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 530.2057 $[M+Na]^+$ (Calcd. for $C_{31}H_{29}N_3O_4Na$, 530.2056); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.18 (m, 19H, ArH), 6.79 (d, 1H, *J* = 6.8 Hz, NH), 6.43 (d, 1H, *J* = 8.3 Hz, NH), 4.94 (m, 1H, 2-H), 4.63 (m, 1H, 5-H), 4.47 (dd, 1H, *J* = 11.2, 3.4 Hz, 4H-a), 4.05 (dd, 1H, *J* = 11.2, 5.3 Hz, 4H-b), 3.27 (dd, 1H, *J* = 13.8,

6.3 Hz, 1H-a), 3.17 (dd, 1H, J = 13.8, 6.6 Hz, 1H-b), 2.95 (dd, 1H, J = 13.8, 6.4 Hz, 6H-a), 2.85 (dd, 1H, J = 13.8, 7.8 Hz, 6H-b); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.33, 167.46, 166.40, 150.21 (2C), 136.78, 135.59, 131.51, 129.13 (2C), 129.06 (2C), 128.76 (2C), 128.69 (2C), 128.42 (2C), 127.33, 126.94 (2C), 126.86, 120.99 (2C), 66.81, 54.44, 50.00, 37.33, 37.17.

*4.5.3. (R)-(S)-2-Benzamido-3-phenylpropyl 2-isonicotinamido-3-phenylpropanoate (**1c**)*

Yield: 93.2%; M.p.: 195–197 °C; $[\alpha]_D^{20}$ -10.6 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 530.2057 $[M+Na]^+$ (Calcd. for $C_{31}H_{29}N_3O_4Na$, 530.2056); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.15 (m, 19H, ArH), 6.82 (d, 1H, J = 6.8 Hz, NH), 6.30 (d, 1H, J = 9.0 Hz, NH), 5.00 (m, 1H, 2-H), 4.71 (m, 1H, 5-H), 4.36 (dd, 1H, J = 12.0, 6.0 Hz, 4H-a), 4.17 (dd, 1H, J = 12.0, 3.0 Hz, 4H-b), 3.20 (m, 2H, 1-H), 2.91 (dd, 1H, J = 13.8, 9.0 Hz, 6H-a), 2.84 (dd, 1H, J = 13.8, 9.0 Hz, 6H-b); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.46, 166.62, 165.44, 150.63 (2C), 140.93, 138.64, 137.96, 134.82, 131.56, 129.41 (2C), 129.37 (2C), 128.65 (2C), 128.58 (4C), 127.56 (2C), 126.90, 126.59, 121.64 (2C), 66.04, 54.75, 50.09, 36.69, 36.32.

*4.5.4. (S)-(R)-2-Benzamido-3-phenylpropyl 2-isonicotinamido-3-phenylpropanoate (**1d**)*

Yield: 93.5%; M.p.: 195–197 °C; $[\alpha]_D^{20}$ 12.0 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 530.2057 $[M+Na]^+$ (Calcd. for $C_{31}H_{29}N_3O_4Na$, 530.2056); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.15 (m, 19H, ArH), 6.82 (d, 1H, J = 6.8 Hz, NH), 6.30 (d, 1H, J = 9.0 Hz, NH), 5.00 (m, 1H, 2-H), 4.71 (m, 1H, 5-H), 4.36 (dd, 1H, J = 12.0, 6.0 Hz, 4H-a), 4.17 (dd, 1H, J = 12.0, 3.0 Hz, 4H-b), 3.20 (m, 2H, 1-H), 2.91 (dd, 1H, J = 13.8, 9.0 Hz, 6H-a), 2.84 (dd, 1H, J = 13.8, 9.0 Hz, 6H-b); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.46, 166.62, 165.44, 150.63 (2C), 140.93, 138.64, 137.96, 134.82, 131.56, 129.41 (2C), 129.37 (2C), 128.65 (2C), 128.58 (4C), 127.56 (2C), 126.90, 126.59, 121.64 (2C), 66.04, 54.75, 50.09, 36.69, 36.32.

*4.5.5. (S)-(S)-2-Benzamido-3-phenylpropyl 2-nicotinamido-3-phenylpropanoate (**2a**)*

Yield: 93.1%; M.p.: 176–178 °C; $[\alpha]_D^{20}$ -34.5 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 530.2057 $[M+Na]^+$ (Calcd. for $C_{31}H_{29}N_3O_4Na$, 530.2056); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.20 (m, 19H, ArH), 6.95 (s, 1H, NH), 6.59 (d, 1H, J = 8.2 Hz, NH), 4.96 (m, 1H, 2-H), 4.64 (m, 1H, 5-H), 4.48 (dd, 1H, J = 11.4, 3.2 Hz, 4H-a), 4.05 (dd, 1H, J = 11.4, 4.9 Hz, 4H-b), 3.28 (dd, 1H, J = 13.6, 6.3 Hz, 1H-a), 3.19 (dd, 1H, J = 13.6, 6.8 Hz, 1H-b), 2.97 (dd, 1H, J = 13.8, 6.8 Hz, 6H-a), 2.85 (dd, 1H, J = 13.8, 7.8 Hz, 6H-b); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.66, 166.62, 165.59, 152.52, 148.82, 138.67, 137.99, 135.46, 134.84, 131.52, 129.56, 129.43 (2C), 129.37 (2C), 128.66 (2C), 128.59 (2C), 128.56 (2C), 127.55 (2C), 126.91, 126.59, 123.83, 66.01, 54.76, 50.20, 36.67, 36.40.

*4.5.6. (R)-(R)-2-Benzamido-3-phenylpropyl 2-nicotinamido-3-phenylpropanoate (**2b**)*

Yield: 93.2%; M.p.: 176–178 °C; $[\alpha]_D^{20}$ 33.9 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 530.2057 $[M+Na]^+$ (Calcd. for $C_{31}H_{29}N_3O_4Na$, 530.2056); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.20 (m, 19H, ArH), 6.95 (s, 1H, NH), 6.59 (d, 1H, J = 8.2 Hz, NH), 4.96 (m, 1H, 2-H), 4.64 (m, 1H, 5-H), 4.48 (dd, 1H, J = 11.4, 3.2 Hz, 4H-a), 4.05 (dd, 1H, J = 11.4, 4.9 Hz, 4H-b), 3.28 (dd, 1H, J = 13.6, 6.3 Hz, 1H-a), 3.19 (dd, 1H, J = 13.6, 6.8 Hz, 1H-b), 2.97 (dd, 1H, J = 13.8, 6.8 Hz, 6H-a), 2.85 (dd, 1H, J = 13.8, 7.8 Hz, 6H-b); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.66, 166.62, 165.59, 152.52, 148.82, 138.67, 137.99, 135.46, 134.84, 131.52, 129.56, 129.43 (2C), 129.37 (2C), 128.66 (2C), 128.59 (2C), 128.56 (2C), 127.55 (2C), 126.91, 126.59, 123.83, 66.01, 54.76, 50.20, 36.67, 36.40.

*4.5.7. (R)-(S)-2-Benzamido-3-phenylpropyl 2-nicotinamido-3-phenylpropanoate (**2c**)*

Yield: 92.8%; M.p.: 174–178 °C; $[\alpha]_D^{20}$ -16.9 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 530.2057 $[M+Na]^+$ (Calcd. for $C_{31}H_{29}N_3O_4Na$, 530.2056); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.17 (m, 19H, ArH), 6.82 (d, 1H, J = 6.8 Hz, NH), 6.30 (d, 1H, J = 9.0 Hz, NH), 5.01 (m, 1H, 2-H), 4.71 (m, 1H, 5-H), 4.36 (dd, 1H, J = 12.0, 6.0 Hz, 4H-a), 4.17 (dd, 1H, J = 12.0, 3.0 Hz, 4H-b), 3.20 (m, 2H, 1-H), 2.90 (dd, 1H, J = 13.8, 9.0 Hz, 6H-a), 2.82 (dd, 1H, J = 13.8, 9.0 Hz, 6H-b); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.58, 166.64, 165.42, 152.18, 148.52, 138.65, 137.99, 135.87, 134.80, 129.41 (2C), 129.38 (2C), 128.64 (2C), 128.58 (4C), 127.56 (2C), 126.90, 126.58, 124.01, 66.02, 54.76, 50.01, 36.88, 36.41.

*4.5.8. (S)-(R)-2-Benzamido-3-phenylpropyl 2-nicotinamido-3-phenylpropanoate (**2d**)*

Yield: 93.3%; M.p.: 174–178 °C; $[\alpha]_D^{20}$ 17.8 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 530.2057 $[M+Na]^+$ (Calcd. for $C_{31}H_{29}N_3O_4Na$, 530.2056); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.17 (m, 19H, ArH), 6.82 (d, 1H, J = 6.8 Hz, NH), 6.30 (d, 1H, J = 9.0 Hz, NH), 5.01 (m, 1H, 2-H), 4.71 (m, 1H, 5-H), 4.36 (dd, 1H, J = 12.0, 6.0 Hz, 4H-a), 4.17 (dd, 1H, J = 12.0, 3.0 Hz, 4H-b), 3.20 (m, 2H, 1-H), 2.90 (dd, 1H, J = 13.8, 9.0 Hz, 6H-a), 2.82 (dd, 1H, J = 13.8, 9.0 Hz, 6H-b); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.58, 166.64, 165.42, 152.18, 148.52, 138.65, 137.99, 135.87, 134.80, 129.41 (2C), 129.38 (2C), 128.64 (2C), 128.58 (4C), 127.56 (2C), 126.90, 126.58, 124.01, 66.02, 54.76, 50.01, 36.88, 36.41.

*4.5.9. (S)-(S)-2-Benzamido-3-phenylpropyl 2-(furan-2-carboxamido)-3-phenylpropanoate (**3a**)*

Yield: 87.2%; M.p.: 150–152 °C; $[\alpha]_D^{20}$ -41.2 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 519.1896 $[M+Na]^+$ (Calcd. for $C_{30}H_{28}N_2O_5Na$, 519.1896); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.06 (m, 18H, ArH), 6.76 (d, 1H, J = 6.6z, NH), 6.66 (d, 1H, J = 8.4Hz, NH), 4.87 (m, 1H, 2-H), 4.59 (m, 1H, 5-H), 4.51 (dd, 1H, J = 11.4, 3.0 Hz, 4H-a), 4.02 (dd, 1H, J = 11.4, 4.2 Hz, 4H-b), 3.25 (dd, 1H, J = 13.8, 6.6 Hz, 1H-a), 3.18 (dd, 1H, J = 13.8, 6.6 Hz, 1H-b), 2.97 (dd, 1H, J = 13.8, 6.6 Hz, 6H-a), 2.71 (dd, 1H, J = 13.8, 9.0 Hz, 6H-b); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.60, 167.09, 158.18, 144.41, 131.32, 129.22 (2C), 129.05 (2C), 128.78 (2C), 128.59 (2C), 128.35 (2C), 127.29, 127.09 (2C), 126.69, 115.07, 112.14, 65.26, 53.77, 50.23, 37.56, 37.15.

*4.5.10. (R)-(R)-2-Benzamido-3-phenylpropyl 2-(furan-2-carboxamido)-3-phenylpropanoate (**3b**)*

Yield: 86.5%; M.p.: 154–158 °C; $[\alpha]_D^{20}$ 40.5 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 519.1896 $[M+Na]^+$ (Calcd. for $C_{30}H_{28}N_2O_5Na$, 519.1896); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.06 (m, 18H, ArH), 6.76 (d, 1H, J = 6.6 Hz, NH), 6.66 (d, 1H, J = 8.4 Hz, NH), 4.87 (m, 1H, 2-H), 4.59 (m, 1H, 5-H), 4.51 (dd, 1H, J = 11.4, 3.0 Hz, 4H-a), 4.02 (dd, 1H, J = 11.4, 4.2 Hz, 4H-b), 3.25 (dd, 1H, J = 13.8, 6.6 Hz, 1H-a), 3.18 (dd, 1H, J = 13.8, 6.6 Hz, 1H-b), 2.97 (dd, 1H, J = 13.8, 6.6 Hz, 6H-a), 2.71 (dd, 1H, J = 13.8, 9.0 Hz, 6H-b); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.60, 167.09, 158.18, 144.41, 131.32, 129.22 (2C), 129.05 (2C), 128.78 (2C), 128.59 (2C), 128.35 (2C), 127.29, 127.09 (2C), 126.69, 115.07, 112.14, 65.26, 53.77, 50.23, 37.56, 37.15.

*4.5.11. (R)-(S)-2-Benzamido-3-phenylpropyl 2-(furan-2-carboxamido)-3-phenylpropanoate (**3c**)*

Yield: 87.5%; M.p.: 168–170 °C; $[\alpha]_D^{20}$ -19.6 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 519.1896 $[M+Na]^+$ (Calcd. for $C_{30}H_{28}N_2O_5Na$, 519.1896); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.06 (m, 18H, ArH), 6.77 (d, 1H, J = 6.6 Hz, NH), 6.56 (d, 1H, J = 8.4 Hz, NH), 4.94 (m, 1H, 2-H), 4.57 (m, 1H, 5-H), 4.45 (dd, 1H, J = 11.4, 3.0 Hz, 4H-a), 4.05 (dd, 1H, J = 11.4, 4.2 Hz, 4H-b), 3.23 (m, 2H, 1-H), 2.87 (dd, 1H, J = 13.8, 6.6 Hz, 6H-a), 2.70 (dd, 1H, J = 13.8, 8.4 Hz, 6H-b);

^{13}C NMR (150 MHz, $CDCl_3$) δ 171.10, 167.00, 158.31, 147.11, 144.47, 135.53, 131.45, 129.13 (2C), 128.99 (2C), 128.82 (2C), 128.57 (2C), 128.43 (2C), 127.34, 127.06 (2C), 126.69, 115.08, 112.22, 65.40, 53.50, 50.01, 37.60, 37.20.

*4.5.12. (S)-(R)-2-Benzamido-3-phenylpropyl 2-(furan-2-carboxamido)-3-phenylpropanoate (**3d**)*

Yield: 87.7%; M.p.: 165–166 °C; $[\alpha]_D^{20}$ 19.9 (*c* 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 519.1896 $[M+Na]^+$ (Calcd. for $C_{30}H_{28}N_2O_5Na$, 519.1896); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.06 (m, 18H, ArH), 6.77 (d, 1H, *J* = 6.6 Hz, NH), 6.56 (d, 1H, *J* = 8.4 Hz, NH), 4.94 (m, 1H, 2-H), 4.57 (m, 1H, 5-H), 4.45 (dd, 1H, *J* = 11.4, 3.0 Hz, 4H-a), 4.05 (dd, 1H, *J* = 11.4, 4.2 Hz, 4H-b), 3.23 (m, 2H, 1-H), 2.87 (dd, 1H, *J* = 13.8, 6.6 Hz, 6H-a), 2.70 (dd, 1H, *J* = 13.8, 8.4 Hz, 6H-b); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.10, 167.00, 158.31, 147.11, 144.47, 135.53, 131.45, 129.13 (2C), 128.99 (2C), 128.82 (2C), 128.57 (2C), 128.43 (2C), 127.34, 127.06 (2C), 126.69, 115.08, 112.22, 65.40, 53.50, 50.01, 37.60, 37.20.

*4.5.13. (S)-(S)-2-Benzamido-3-phenylpropyl 2-(thiophene-2-carboxamido)-3-phenyl propanoate (**4a**)*

Yield: 89.7%; M.p.: 144–145 °C; $[\alpha]_D^{20}$ -48.1 (*c* 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 535.1668 $[M+Na]^+$ (Calcd. for $C_{30}H_{28}N_2O_4SNa$, 535.1667); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.05 (m, 18H, ArH), 6.62 (d, 1H, *J* = 8.4 Hz, NH), 6.43 (d, 1H, *J* = 6.6 Hz, NH), 4.87 (m, 1H, 2-H), 4.60 (m, 1H, 5-H), 4.52 (dd, 1H, *J* = 11.4, 3.6 Hz, 4H-a), 4.01 (dd, 1H, *J* = 11.4, 4.2 Hz, 4H-b), 3.26 (dd, 1H, *J* = 13.8, 6.6 Hz, 1H-a), 3.20 (dd, 1H, *J* = 11.4, 4.2 Hz, 1H-b), 2.98 (dd, 1H, *J* = 13.8, 8.4 Hz, 6H-a), 2.86 (dd, 1H, *J* = 13.8, 6.6 Hz, 6H-b). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.60, 167.13, 161.83, 137.60, 137.08, 135.65, 134.06, 131.34, 130.66, 129.21 (2C), 129.11 (2C), 128.78 (2C), 128.73, 128.60 (2C), 128.39 (2C), 127.69, 127.29, 127.05 (2C), 126.71, 65.34, 54.38, 50.18, 37.47, 37.15.

*4.5.14. (R)-(R)-2-Benzamido-3-phenylpropyl 2-(thiophene-2-carboxamido)-3-phenyl propanoate (**4b**)*

Yield: 90.2%; M.p.: 144–147 °C; $[\alpha]_D^{20}$ 49.4 (*c* 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 535.1668 $[M+Na]^+$ (Calcd. for $C_{30}H_{28}N_2O_4SNa$, 535.1667); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.05 (m, 18H, ArH), 6.62 (d, 1H, *J* = 8.4 Hz, NH), 6.43 (d, 1H, *J* = 6.6 Hz, NH), 4.87 (m, 1H, 2-H), 4.60 (m, 1H, 5-H), 4.52 (dd, 1H, *J* = 11.4, 3.6 Hz, 4H-a), 4.01 (dd, 1H, *J* = 11.4, 4.2 Hz, 4H-b), 3.26 (dd, 1H, *J* = 13.8, 6.6 Hz, 1H-a), 3.20 (dd, 1H, *J* = 11.4, 4.2 Hz, 1H-b), 2.98 (dd, 1H, *J* = 13.8, 8.4 Hz, 6H-a), 2.86 (dd, 1H, *J* = 13.8, 6.6 Hz, 6H-b). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.60, 167.13, 161.83, 137.60, 137.08, 135.65, 134.06, 131.34, 130.66, 129.21 (2C), 129.11 (2C), 128.78 (2C), 128.73, 128.60 (2C), 128.39 (2C), 127.69, 127.29, 127.05 (2C), 126.71, 65.34, 54.38, 50.18, 37.47, 37.15.

*4.5.15. (R)-(S)-2-Benzamido-3-phenylpropyl 2-(thiophene-2-carboxamido)-3-phenyl propanoate (**4c**)*

Yield: 90.4%; M.p.: 153–154 °C; $[\alpha]_D^{20}$ -22.7 (*c* 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 535.1668 $[M+Na]^+$ (Calcd. for $C_{30}H_{28}N_2O_4SNa$, 535.1667); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.06 (m, 18H, ArH), 6.54 (d, 1H, *J* = 8.4 Hz, NH), 6.46 (d, 1H, *J* = 6.6 Hz, NH), 4.93 (m, 1H, 2-H), 4.61 (m, 1H, 5-H), 4.46 (dd, 1H, *J* = 11.4, 3.6 Hz, 4H-a), 4.05 (dd, 1H, *J* = 11.4, 4.2 Hz, 4H-b), 3.22 (m, 2H, 1-H), 2.87 (dd, 1H, *J* = 13.8, 6.6 Hz, 6H-a), 2.71 (dd, 1H, *J* = 13.8, 7.8 Hz, 6H-b); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.11, 167.12, 161.93, 137.76, 136.91, 135.58, 134.02, 131.51, 130.75, 129.13 (2C), 129.02 (2C), 128.83 (2C), 128.68, 128.60 (2C), 128.46 (2C), 127.80, 127.36, 127.07 (2C), 126.73, 65.60, 54.04, 49.96, 37.50, 37.24.

*4.5.16. (S)-(R)-2-Benzamido-3-phenylpropyl 2-(thiophene-2-carboxamido)-3-phenyl propanoate (**4d**)*

Yield: 91.1%; M.p.: 158–160 °C; $[\alpha]_D^{20}$ 23.5 (*c* 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 535.1668 $[M+Na]^+$ (Calcd. for $C_{30}H_{28}N_2O_4SNa$, 535.1667); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.06 (m, 18H, ArH), 6.54 (d, 1H, *J* = 8.4 Hz, NH), 6.46 (d, 1H, *J* = 6.6 Hz, NH), 4.93 (m, 1H, 2-H), 4.61 (m, 1H, 5-H), 4.46 (dd, 1H, *J* = 11.4, 3.6 Hz, 4H-a), 4.05 (dd, 1H, *J* = 11.4, 4.2 Hz, 4H-b), 3.22 (m, 2H, 1-H), 2.87 (dd, 1H, *J* = 13.8, 6.6 Hz, 6H-a), 2.71 (dd, 1H, *J* = 13.8, 7.8 Hz, 6H-b); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.11, 167.12, 161.93, 137.76, 136.91, 135.58, 134.02, 131.51, 130.75, 129.13 (2C), 129.02 (2C), 128.83 (2C), 128.68, 128.60 (2C), 128.46 (2C), 127.80, 127.36, 127.07 (2C), 126.73, 65.60, 54.04, 49.96, 37.50, 37.24.

*4.5.17. (S)-(S)-2-Benzamido-3-phenylpropyl 2-methylsulfonamido-3-phenyl propanoate (**7a**)*

Yield: 78.6%; M.p.: 186–187 °C; $[\alpha]_D^{20}$ -26.2 (*c* 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 503.1616 $[M+Na]^+$ (Calcd. for $C_{26}H_{28}N_2O_5SNa$, 503.1617); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.17 (m, 15H, ArH), 6.40 (d, 1H, *J* = 8.4 Hz, NH), 4.81 (d, 1H, *J* = 9.0 Hz, NH), 4.66 (m, 1H, 2-H), 4.36 (m, 1H, 5-H), 4.40 (dd, 1H, *J* = 11.4, 3.6 Hz, 4H-a), 4.18 (dd, 1H, *J* = 11.4, 4.8 Hz, 4H-b), 3.15 (dd, 1H, *J* = 13.8, 5.4 Hz, 1H-a), 2.97 (dd, 1H, *J* = 13.8, 7.2 Hz, 1H-b), 2.95 (dd, 1H, *J* = 14.4, 6.0 Hz, 6H-a), 2.71 (dd, 1H, *J* = 14.4, 7.8 Hz, 6H-b), 2.64 (s, 3H, -CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.56, 167.16, 136.71, 135.33, 133.92, 131.59, 129.31 (2C), 129.15 (2C), 128.79 (2C), 128.70 (2C), 128.50 (2C), 127.46, 126.98 (2C), 126.88, 66.12, 57.44, 49.90, 41.37, 38.86, 37.14.

*4.5.18. (R)-(R)-2-Benzamido-3-phenylpropyl 2-methylsulfonamido-3-phenyl propanoate (**7b**)*

Yield: 74.6%; M.p.: 185–187 °C; $[\alpha]_D^{20}$ 25.9 (*c* 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 503.1616 $[M+Na]^+$ (Calcd. for $C_{26}H_{28}N_2O_5SNa$, 503.1617); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.17 (m, 15H, ArH), 6.40 (d, 1H, *J* = 8.4 Hz, NH), 4.81 (d, 1H, *J* = 9.0 Hz, NH), 4.66 (m, 1H, 2-H), 4.36 (m, 1H, 5-H), 4.40 (dd, 1H, *J* = 11.4, 3.6 Hz, 4H-a), 4.18 (dd, 1H, *J* = 11.4, 4.8 Hz, 4H-b), 3.15 (dd, 1H, *J* = 13.8, 5.4 Hz, 1H-a), 2.97 (dd, 1H, *J* = 13.8, 7.2 Hz, 1H-b), 2.95 (dd, 1H, *J* = 14.4, 6.0 Hz, 6H-a), 2.71 (dd, 1H, *J* = 14.4, 7.8 Hz, 6H-b), 2.64 (s, 3H, -CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.56, 167.16, 136.71, 135.33, 133.92, 131.59, 129.31 (2C), 129.15 (2C), 128.79 (2C), 128.70 (2C), 128.50 (2C), 127.46, 126.98 (2C), 126.88, 66.12, 57.44, 49.90, 41.37, 38.86, 37.14.

*4.5.19. (R)-(S)-2-Benzamido-3-phenylpropyl 2-methylsulfonamido-3-phenyl propanoate (**7c**)*

Yield: 75.7%; M.p.: 192–195 °C; $[\alpha]_D^{20}$ -13.1 (*c* 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 503.1616 $[M+Na]^+$ (Calcd. for $C_{26}H_{28}N_2O_5SNa$, 503.1617); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.16 (m, 15H, ArH), 6.42 (d, 1H, *J* = 8.4 Hz, NH), 4.77 (d, 1H, *J* = 9.6 Hz, NH), 4.39 (m, 1H, 2-H), 4.33 (m, 1H, 5-H), 4.35 (dd, 1H, *J* = 11.4, 3.6 Hz, 4H-a), 4.17 (dd, 1H, *J* = 11.4, 4.8 Hz, 4H-b), 3.00 (dd, 1H, *J* = 12.0, 6.6 Hz, 1H-a), 2.96 (dd, 1H, *J* = 12.0, 6.0 Hz, 1H-b), 3.14 (dd, 1H, *J* = 13.8, 5.4 Hz, 6H-a), 2.89 (dd, 1H, *J* = 13.8, 7.8 Hz, 6H-b), 2.64 (s, 3H, -CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.15, 167.12, 136.86, 135.44, 133.95, 131.56, 129.27 (2C), 129.18 (2C), 128.80 (2C), 128.68 (2C), 128.46 (2C), 127.47, 127.01 (2C), 126.82, 66.20, 57.59, 49.83, 41.23, 38.88, 37.28.

*4.5.20. (S)-(R)-2-Benzamido-3-phenylpropyl 2-methylsulfonamido-3-phenyl propanoate (**7d**)*

Yield: 75.5%; M.p.: 195–197 °C; $[\alpha]_D^{20}$ 12.8 (*c* 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 503.1616 $[M+Na]^+$ (Calcd. for $C_{26}H_{28}N_2O_5SNa$, 503.1617); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.16 (m, 15H, ArH), 6.42 (d, 1H, *J* = 8.4 Hz, NH), 4.77 (d, 1H, *J* = 9.6 Hz, NH),

4.39 (m, 1H, 2-H), 4.33 (m, 1H, 5-H), 4.35 (dd, 1H, J = 11.4, 3.6 Hz, 4H-a), 4.17 (dd, 1H, J = 11.4, 4.8 Hz, 4H-b), 3.00 (dd, 1H, J = 12.0, 6.6 Hz, 1H-a), 2.96 (dd, 1H, J = 12.0, 6.0 Hz, 1H-b), 3.14 (dd, 1H, J = 13.8, 5.4 Hz, 6H-a), 2.89 (dd, 1H, J = 13.8, 7.8 Hz, 6H-b), 2.64 (s, 3H, -CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.15, 167.12, 136.86, 135.44, 133.95, 131.56, 129.27 (2C), 129.18 (2C), 128.80 (2C), 128.68 (2C), 128.46 (2C), 127.47, 127.01 (2C), 126.82, 66.20, 57.59, 49.83, 41.23, 38.88, 37.28.

4.5.21. (S)-(S)-2-Benzamido-3-phenylpropyl 2-(4-methylphenylsulfonamido)-3-phenyl propanoate (**8a**)

Yield: 85.8%; M.p.: 143–145 °C; $[\alpha]_D^{20}$ -45.8 (c 0.4 $CHCl_3$:MeOH = 1:1); HR-MS: 579.1930 $[M+Na]^+$ (Calcd. for $C_{32}H_{32}N_2O_5SNa$, 579.1930); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.04 (m, 19H, ArH), 6.50 (d, 1H, J = 8.4 Hz, NH), 5.06 (d, 1H, J = 9.6 Hz, NH), 4.51 (m, 1H, 2-H), 4.20 (m, 1H, 5-H), 4.17 (dd, 1H, J = 11.4, 3.6 Hz, 4H-a), 4.05 (dd, 1H, J = 11.4, 4.2 Hz, 4H-b), 3.04 (dd, 1H, J = 13.8, 6.0 Hz, 1H-a), 2.95 (dd, 1H, J = 13.8, 7.2 Hz, 1H-b), 2.90 (dd, 1H, J = 13.8, 6.6 Hz, 6H-a), 2.75 (dd, 1H, J = 13.8, 7.8 Hz, 6H-b), 2.35 (s, 3H, -CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.51, 166.97, 143.78, 136.95, 136.26, 134.88, 133.92, 131.56, 129.59 (2C), 129.15 (4C), 128.64 (2C), 128.62 (2C), 128.47 (2C), 127.25, 127.02 (2C), 126.99 (2C), 126.77, 65.89, 56.88, 50.19, 38.83, 36.99, 21.42.

4.5.22. (R)-(R)-2-Benzamido-3-phenylpropyl 2-(4-methylphenylsulfonamido)-3-phenyl propanoate (**8b**)

Yield: 85.9%; M.p.: 145–146 °C; $[\alpha]_D^{20}$ 45.5 (c 0.4 $CHCl_3$:MeOH = 1:1); HR-MS: 579.1930 $[M+Na]^+$ (Calcd. for $C_{32}H_{32}N_2O_5SNa$, 579.1930); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.04 (m, 19H, ArH), 6.50 (d, 1H, J = 8.4 Hz, NH), 5.06 (d, 1H, J = 9.6 Hz, NH), 4.51 (m, 1H, 2-H), 4.20 (m, 1H, 5-H), 4.17 (dd, 1H, J = 11.4, 3.6 Hz, 4H-a), 4.05 (dd, 1H, J = 11.4, 4.2 Hz, 4H-b), 3.04 (dd, 1H, J = 13.8, 6.0 Hz, 1H-a), 2.95 (dd, 1H, J = 13.8, 7.2 Hz, 1H-b), 2.90 (dd, 1H, J = 13.8, 6.6 Hz, 6H-a), 2.75 (dd, 1H, J = 13.8, 7.8 Hz, 6H-b), 2.35 (s, 3H, -CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.51, 166.97, 143.78, 136.95, 136.26, 134.88, 133.92, 131.56, 129.59 (2C), 129.15 (4C), 128.64 (2C), 128.62 (2C), 128.47 (2C), 127.25, 127.02 (2C), 126.99 (2C), 126.77, 65.89, 56.88, 50.19, 38.83, 36.99, 21.42.

4.5.23. (R)-(S)-2-Benzamido-3-phenylpropyl 2-(4-methylphenylsulfonamido)-3-phenyl propanoate (**8c**)

Yield: 83.6%; M.p.: 150–152 °C; $[\alpha]_D^{20}$ -23.3 (c 0.4 $CHCl_3$:MeOH = 1:1); HR-MS: 579.1930 $[M+Na]^+$ (Calcd. for $C_{32}H_{32}N_2O_5SNa$, 579.1930); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.02 (m, 19H, ArH), 6.57 (d, 1H, J = 8.4 Hz, NH), 4.99 (d, 1H, J = 9.0 Hz, NH), 4.56 (m, 1H, 2-H), 4.10 (m, 1H, 5-H), 4.32 (dd, 1H, J = 11.4, 3.6 Hz, 4H-a), 3.95 (dd, 1H, J = 11.4, 4.2 Hz, 4H-b), 3.03 (dd, 1H, J = 13.8, 6.0 Hz, 1H-a), 2.96 (dd, 1H, J = 13.8, 7.8 Hz, 1H-b), 2.94 (dd, 1H, J = 13.8, 6.6 Hz, 6H-a), 2.82 (dd, 1H, J = 13.8, 7.8 Hz, 6H-b), 2.38 (s, 3H, -CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.89, 167.08, 143.80, 137.05, 136.02, 134.87, 134.05, 131.47, 129.61 (2C), 129.20 (2C), 129.07 (2C), 128.70 (2C), 128.62 (2C), 128.40 (2C), 127.30, 127.09 (2C), 127.03 (2C), 126.73, 65.84, 57.05, 49.92, 38.83, 37.14, 21.45.

4.5.24. (S)-(R)-2-Benzamido-3-phenylpropyl 2-(4-methylphenylsulfonamido)-3-phenyl propanoate (**8d**)

Yield: 84.3%; M.p.: 150–151 °C; $[\alpha]_D^{20}$ 23.8 (c 0.4 $CHCl_3$:MeOH = 1:1); HR-MS: 579.1930 $[M+Na]^+$ (Calcd. for $C_{32}H_{32}N_2O_5SNa$, 579.1930); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.02 (m, 19H, ArH), 6.57 (d, 1H, J = 8.4 Hz, NH), 4.99 (d, 1H, J = 9.0 Hz, NH), 4.56 (m, 1H, 2-H), 4.10 (m, 1H, 5-H), 4.32 (dd, 1H, J = 11.4, 3.6 Hz, 4H-a), 3.95 (dd, 1H, J = 11.4, 4.2 Hz, 4H-b), 3.03 (dd, 1H, J = 13.8, 6.0 Hz, 1H-a), 2.96 (dd, 1H, J = 13.8, 7.8 Hz, 1H-b), 2.94 (dd, 1H, J = 13.8, 6.6 Hz, 6H-a), 2.82 (dd, 1H, J = 13.8, 7.8 Hz, 6H-b), 2.38 (s, 3H, -CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.89, 167.08, 143.80, 137.05, 136.02, 134.87, 134.05, 131.47, 129.61 (2C), 129.20 (2C), 129.07 (2C), 128.70 (2C), 128.62 (2C), 128.40 (2C), 127.30, 127.09 (2C), 127.03 (2C), 126.73, 65.84, 57.05, 49.92, 38.83, 37.14, 21.45.

4.6. General procedure for the synthesis of compounds **5**–**6**

IM 3 (0.3 g, 0.6 mmol) was treated with 1.5 mL 1.35 mol/L dry hydrochloride gas in ethyl acetate for 4 h at room temperature. The solvent was removed under reduced pressure to give white solid and suspended in anhydrous CH_2Cl_2 (10 mL). Triethylamine (0.4 mL) and 0.4 equivalents of triphosgene were added at 0 °C. After stirred for 15 min, various heterocycles were added. The reaction mixture was stirred at room temperature for 24 h. Then it was concentrated and the residue upon column chromatographic purification using 200–300 mesh silica gel and 10% acetone-chloroform (v/v) as eluent, yielding target compound as a white solid.

4.7. Spectroscopy data of compounds **5**–**6**

4.7.1. (S)-(S)-2-Benzamido-3-phenylpropyl 2-(1H-imidazole-1-carboxamido)-3-phenylpropanoate (5a)

Yield: 45.2%; M.p.: 173–174 °C; $[\alpha]_D^{20}$ -24.5 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 519.2007 $[M+Na]^+$ (Calcd. for $C_{29}H_{28}N_4O_4Na$, 519.2008); ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.32 (d, 2H, J = 9.0 Hz, ArH), 7.74 (d, 2H, J = 7.2 Hz, ArH), 7.40 (m, 3H, ArH), 7.15 (m, 9H, ArH), 7.08 (d, 2H, J = 7.2 Hz, ArH), 6.51 (d, 2H, J = 7.8 Hz, ArH), 4.37 (m, 2H, 2-H, 5-H), 4.10 (m, 2H, 4-H), 2.93 (dd, 1H, J = 13.8, 5.4 Hz, 1H-a), 2.77 (m, 3H, 1H-b, 6-H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.54, 166.58, 157.25, 138.73, 137.29, 134.83, 131.54, 129.48 (3C), 129.46 (3C), 129.04 (3C), 128.59 (3C), 128.57 (3C), 127.56 (3C), 126.87, 126.56, 65.81, 54.39, 50.16, 37.55, 36.65.

4.7.2. (R)-(R)-2-Benzamido-3-phenylpropyl 2-(1H-imidazole-1-carboxamido)-3-phenylpropanoate (5b)

Yield: 45.7%; M.p.: 177–179 °C; $[\alpha]_D^{20}$ 24.5 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 519.2007 $[M+Na]^+$ (Calcd. for $C_{29}H_{28}N_4O_4Na$, 519.2008); ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.32 (d, 2H, J = 9.0 Hz, ArH), 7.74 (d, 2H, J = 7.2 Hz, ArH), 7.40 (m, 3H, ArH), 7.15 (m, 9H, ArH), 7.08 (d, 2H, J = 7.2 Hz, ArH), 6.51 (d, 2H, J = 7.8 Hz, ArH), 4.37 (m, 2H, 2-H, 5-H), 4.10 (m, 2H, 4-H), 2.93 (dd, 1H, J = 13.8, 5.4 Hz, 1H-a), 2.77 (m, 3H, 1H-b, 6-H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.54, 166.58, 157.25, 138.73, 137.29, 134.83, 131.54, 129.48 (3C), 129.46 (3C), 129.04 (3C), 128.59 (3C), 128.57 (3C), 127.56 (3C), 126.87, 126.56, 65.81, 54.39, 50.16, 37.55, 36.65.

4.7.3. (R)-(S)-2-Benzamido-3-phenylpropyl 2-(1H-imidazole-1-carboxamido)-3-phenylpropanoate (5c)

Yield: 44.3%; M.p.: 167–168 °C; $[\alpha]_D^{20}$ 11.7 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 519.2007 $[M+Na]^+$ (Calcd. for $C_{29}H_{28}N_4O_4Na$, 519.2008); ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.33 (d, 2H, J = 8.4 Hz, ArH), 7.77 (d, 2H, J = 8.4 Hz, ArH), 7.41 (m, 3H, ArH), 7.09 (m, 9H, ArH), 6.56 (d, 2H, J = 7.8 Hz, ArH), 4.36 (m, 2H, 2-H, 5-H), 4.12 (dd, 1H, J = 10.8, 5.4 Hz, 4H-a), 4.01 (dd, 1H, J = 10.8, 6.0 Hz, 4H-a), 2.78 (m, 4H, 1H, 6-H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.91, 169.90, 166.58, 138.70, 137.76, 134.81, 131.57, 129.44 (3C), 129.31 (3C), 128.60 (6C), 127.57 (3C), 126.87, 126.58, 65.80, 54.12, 50.06, 36.85, 36.68.

4.7.4. (S)-(R)-2-Benzamido-3-phenylpropyl 2-(1H-imidazole-1-carboxamido)-3-phenylpropanoate (5d)

Yield: 41.3%; M.p.: 165–167 °C; $[\alpha]_D^{20}$ -12.7 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 519.2007 $[M+Na]^+$ (Calcd. for $C_{29}H_{28}N_4O_4Na$, 519.2008); ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.33 (d, 2H, J = 8.4 Hz, ArH), 7.77 (d, 2H, J = 8.4 Hz, ArH), 7.41 (m, 3H, ArH),

7.09 (m, 9H, ArH), 6.56 (d, 2H, J = 7.8 Hz, ArH), 4.36 (m, 2H, 2-H, 5-H), 4.12 (dd, 1H, J = 10.8, 5.4 Hz, 4H-a), 4.01 (dd, 1H, J = 10.8, 6.0 Hz, 4H-a), 2.78 (m, 4H, 1H, 6-H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.91, 169.90, 166.58, 138.70, 137.76, 134.81, 131.57, 129.44 (3C), 129.31 (3C), 128.60 (6C), 127.57 (3C), 126.87, 126.58, 65.80, 54.12, 50.06, 36.85, 36.68.

4.7.5. *(S)-(S)-2-Benzamido-3-phenylpropyl 3-phenyl-2-(1H-1,2,4-triazole-1-carboxamido)propanoate (6a)*

Yield: 56.2%; M.p.: 154–155 °C; $[\alpha]_D^{20}$ -21.7 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 520.1961 $[M+Na]^+$ (Calcd. for $C_{29}H_{28}N_4O_4Na$, 520.1961); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.71 (s, 1H, ArH), 7.91 (s, 1H, ArH), 7.66 (d, 2H, J = 7.2 Hz, ArH), 7.19 (m, 13H, ArH), 6.31 (d, 1H, J = 8.4 Hz, NH), 4.85 (m, 1H, 2-H), 4.63 (m, 1H, 5-H), 4.40 (dd, 1H, J = 10.2, 4.8 Hz, 4H-a), 4.11 (dd, 1H, J = 11.4, 5.4 Hz, 4H-b), 3.28 (dd, 1H, J = 14.4, 6.0 Hz, 1H-a), 3.19 (dd, 1H, J = 13.8, 7.2 Hz, 1H-b), 2.94 (dd, 1H, J = 13.8, 6.0 Hz, 6H-a), 2.82 (dd, 1H, J = 13.8, 7.8 Hz, 6H-b); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.52, 167.12, 152.49, 147.25, 143.40, 136.60, 134.84, 134.11, 131.60, 129.14 (2C), 128.99 (2C), 128.90 (2C), 128.71 (2C), 128.47 (2C), 127.54, 126.90 (3C), 65.99, 54.60, 49.85, 37.68, 37.23.

4.7.6. *(R)-(R)-2-Benzamido-3-phenylpropyl 3-phenyl-2-(1H-1,2,4-triazole-1-carboxamido)propanoate (6b)*

Yield: 55.6%; M.p.: 156–157 °C; $[\alpha]_D^{20}$ 21.9 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 520.1961 $[M+Na]^+$ (Calcd. for $C_{29}H_{28}N_4O_4Na$, 520.1961); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.71 (s, 1H, ArH), 7.91 (s, 1H, ArH), 7.66 (d, 2H, J = 7.2 Hz, ArH), 7.19 (m, 13H, ArH), 6.31 (d, 1H, J = 8.4 Hz, NH), 4.85 (m, 1H, 2-H), 4.63 (m, 1H, 5-H), 4.40 (dd, 1H, J = 10.2, 4.8 Hz, 4H-a), 4.11 (dd, 1H, J = 11.4, 5.4 Hz, 4H-b), 3.28 (dd, 1H, J = 14.4, 6.0 Hz, 1H-a), 3.19 (dd, 1H, J = 13.8, 7.2 Hz, 1H-b), 2.94 (dd, 1H, J = 13.8, 6.0 Hz, 6H-a), 2.82 (dd, 1H, J = 13.8, 7.8 Hz, 6H-b); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.52, 167.12, 152.49, 147.25, 143.40, 136.60, 134.84, 134.11, 131.60, 129.14 (2C), 128.99 (2C), 128.90 (2C), 128.71 (2C), 128.47 (2C), 127.54, 126.90 (3C), 65.99, 54.60, 49.85, 37.68, 37.23.

4.7.7. *(R)-(S)-2-Benzamido-3-phenylpropyl 3-phenyl-2-(1H-1,2,4-triazole-1-carboxamido)propanoate (6c)*

Yield: 55.6%; M.p.: 147–149 °C; $[\alpha]_D^{20}$ -11.3 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 520.1961 $[M+Na]^+$ (Calcd. for $C_{29}H_{28}N_4O_4Na$, 520.1961); ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.07 (s, 1H, ArH), 9.03 (d, 1H, J = 8.4 Hz, NH), 8.35 (d, 1H, J = 8.4 Hz, NH), 8.25 (s, 1H, ArH), 7.72 (d, 2H, J = 7.8 Hz, ArH), 7.16 (m, 13H, ArH), 4.68 (m, 1H, 2-H), 4.40 (m, 1H, 5-H), 4.22 (dd, 1H, J = 10.8, 5.4 Hz, 4H-a), 4.11 (dd, 1H, J = 10.8, 5.4 Hz, 4H-b), 3.18 (m, 2H, 1-H), 2.82 (m, 2H, 6-H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.64, 166.67, 153.06, 148.06, 144.85, 138.65, 137.73, 134.84, 131.54, 129.41 (2C), 129.40 (2C), 128.68 (2C), 128.60 (2C), 128.57 (2C), 127.56 (2C), 126.96, 126.60, 66.26, 55.10, 50.13, 36.63, 35.83.

4.7.8. *(S)-(R)-2-Benzamido-3-phenylpropyl 3-phenyl-2-(1H-1,2,4-triazole-1-carboxamido)propanoate (6d)*

Yield: 55.6%; M.p.: 147–149 °C; $[\alpha]_D^{20}$ 11.8 (c 0.5 $CHCl_3$:MeOH = 1:1); HR-MS: 520.1961 $[M+Na]^+$ (Calcd. for $C_{29}H_{28}N_4O_4Na$, 520.1961); ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.07 (s, 1H, ArH), 9.03 (d, 1H, J = 8.4 Hz, NH), 8.35 (d, 1H, J = 8.4 Hz, NH), 8.25 (s, 1H, ArH), 7.72 (d, 2H, J = 7.8 Hz, ArH), 7.16 (m, 13H, ArH), 4.68 (m, 1H, 2-H), 4.40 (m, 1H, 5-H), 4.22 (dd, 1H, J = 10.8, 5.4 Hz, 4H-a), 4.11 (dd, 1H, J = 10.8, 5.4 Hz, 4H-b), 3.18 (m, 2H, 1-H), 2.82 (m, 2H, 6-H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.64, 166.67, 153.06, 148.06, 144.85, 138.65, 137.73, 134.84, 131.54, 129.41 (2C), 129.40 (2C), 128.68 (2C), 128.60 (2C), 128.57 (2C), 127.56 (2C), 126.96, 126.60, 66.26, 55.10, 50.13, 36.63, 35.83.

4.8. Cell culture

MCF-7 human breast cancer cells, HeLa human cervical cancer cells and BEL-7402 human hepatic cancer cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 1 mM-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in 100% humidified and 5% CO_2.

4.9. Cell growth inhibition assay

The growth inhibitory effects of asperphenamate analogs on various tumor cells *in vitro* were measured by MTT assay. The cells seeded in 96-well plates were treated with different concentrations. DMSO (end-concentration of 0.1%) was used as a control group. After 48 and 72 h incubation, 5.0 mg/mL MTT solution was added, and the plates were incubated for another 4 h at 37 °C. The purple formazan crystals were dissolved in 100 μl DMSO and the plates were read on an ELISA reader at 570 nm. The cell viability was calculated as the ratio of the absorbance of the treated cells to the absorbance of the control groups. The IC_{50} values were calculated using the software of Statistical Product and Service Solutions. All experiments were performed in triplicate in three independent experiments.

4.10. AO and MDC staining assay for autophagy detection

As a marker of autophagy, the volume of the cellular acidic compartment can be visualized after AO staining. Three cell lines seeded in 24-well plate were exposed to various concentrations of **8c** or 0.1% DMSO for indicated times. Cells were then incubated with medium containing 1 mg/mL AO for 15 min. After washing with PBS, the fluorescent micrographs were taken using an inverted fluorescent microscope. MDC staining was used to confirm the abundance of autophagic vacuoles in cells. Following **8c** treatment, cells were stained with 50 μM MDC for 1 h at 37 °C, and then examined by fluorescence microscopy or analyzed on a flow cytometry using CellQuest software.

4.11. Western blot analysis

Three cell lines (1×10^6 cells) were incubated with various concentrations of **8c** or 0.1% DMSO for indicated times. After harvesting by trypsinisation, the cells were washed with PBS, and harvested by centrifugation. The pellets were resuspended in RIPA lysis buffer [50 mM Tris-HCl, 150 mM NaCl, 0.1% sodium dodecyl sulfate (SDS), 1% nonidet P-40, 0.5% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride (PMSF), 100 μM leupeptin, and 2 μg/mL aprotinin, pH 8.0] on ice for 30 min and centrifuged. After mixed with 2 × SDS-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer [20% glycerol, 4% SDS, 0.04% bromphenol blue, 200 nM L-mercaptoethanol, and 100 mM Tris-HCl, (pH 6.8)]. Equal amounts of protein extracts (10 μg) were separated by 8–15% SDS-PAGE, and transferred onto polyvinylidene difluoride membranes. The membranes were treated with specified antibodies, and then incubated with horseradish peroxidaseconjugated secondary antibody. Blots were detected using enhanced chemiluminescence western blotting detection reagents. All experiments were repeated at least three times.

Acknowledgements

The work was supported by the National Nature Science Foundation of China (81302638) and Specialized Research Fund for the Doctoral Program of Higher Education (20112134120007).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.01.020.

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