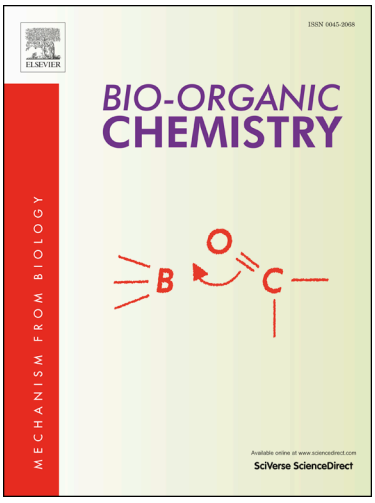

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Discovery of a novel cathepsin inhibitor with dual autophagy-inducing and metastasis-inhibiting effects on breast cancer cells

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Abstract: Drug resistance and cancer cells metastasis have been the leading causes of chemotherapy failure and cancer-associated death in breast cancer patients. In present, various active molecules either exhibiting novel mechanism of action such as inducing autophagy or inhibiting metastasis have been developed to address these problems. However, the compounds exhibiting such dual functions have rarely been described. Previous work in our group showed that **TSA**, as a synthetic analog of asperphenamate, induced autophagic cell death in breast cancer cells instead of apoptosis. Furthermore, the target enzyme of **TSA** was predicted to be cathepin L (Cat L) by natural product consensus pharmacophore strategy. Accumulated evidences have shown that cathepsins are closely associated with migration and invasion of breast cancer cells. It seemed likely that **TSA**-like molecules may possess the dual functions of inducing autophagy and inhibiting metastasis. Therefore, sixty optically active derivatives were firstly designed and synthesized by replacing the A-ring moiety of **TSA** with other substituted-phenyl sulfonyl groups. Further cathepsin inhibitory activity assay showed that (*S*, *S*) and (*S*, *R*) isomers displayed no activity against four kinds of cathepsins (L, S, K, B), while all derivatives tested were inactive toward K and B subtypes. Compound **6a** with *meta*-bromo substituent displayed the greatest inhibitory activity, and its inhibitory capability against Cat L and S was 3.9 and 11.5-fold more potent than that of **TSA**, respectively. Molecular docking also exhibited that **6a** formed more hydrogen bonds or π-π contacts with Cat L or S than **TSA**. In order to determine whether **6a** could play dual roles, its anti-cancer mechanism was further investigated. On the one hand, MDC staining experiment and western blotting analysis validated that **6a** can induce autophagy in MDA-MB-231 cells. On the other hand, its metastatic inhibitory ability was also confirmed by wound healing and transwell chamber experiment.

Key words: cathepsin inhibitor; anti-metastatic effect; inducing autophagy; docking calculations; anti-breast cancer effects.

1. Introduction

The cancer epidemiology data coming from the Cancer Statistics 2017 provided a reasonably accurate portrayal of current situation of breast cancer. The statistics showed that 252,710 new cases of breast carcinoma and 40,610 cancer deaths in 2017 are projected to occur in the United States. Breast cancer still ranks as the number one cause of new cancer cases in women and the second most common cause of cancer-related death after lung cancer [1].

Chemotherapy is one of the most important strategies used in breast cancer therapy which

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displays multiple actions, such as inhibiting tumor cell division, angiogenesis or inducing cell death [2, 3]. Since most anti-breast cancer drugs induce apoptosis of tumor cells, breast cancer cells have the tendency to become remarkably resistant to proapoptotic stimuli [4-6]. Meanwhile, metastasis is also a major obstacle to effective cancer treatment with chemotherapeutic drugs and has been the leading cause of breast cancer associated mortality [7, 8]. Obviously, drug resistance and cancer cells metastasis have become the critical impediment to breast cancer treatment. Therefore, double functional chemotherapeutic agents with both novel mechanisms of action against apoptosis-resistant cancer cells and inhibiting cancer metastasis ability are urgently needed.

Natural products are always visualized as an arsenal of battling against various diseases. They exhibit a wide scope of significant biological effects and are always being used in the design and discovery of the target-based drugs in the pharmaceutical field [9-12]. Certainly, they show more important and tremendous contribution to novel anti-cancer drug development. Asperphenamate is always paid attention by us because asperphenamate and its analogues displayed a novel anticancer mechanism of action, fully inducing autophagic death in different kinds of cancer cell lines [13-15] (**Figure 1**). In further work, we did our best to determine cysteine cathepsin was the target enzyme of asperphenamate by a natural product consensus pharmacophore strategy. The inhibitory effects of some previous synthetic analogs with more potent activity than asperphenamate on Cat L, S, K and B were further studied. The results showed that an A-ring tosyl substituted derivative, **TSA (Figure 1)**, displayed the greatest potency against Cat L [16].

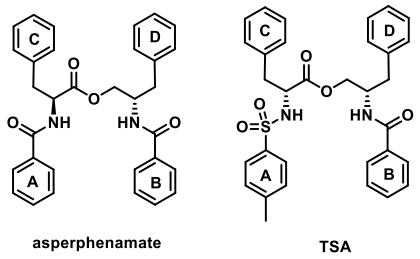


Figure 1. The structues of asperphenamate and **TSA**.

Human cysteine cathepsins includes eleven members which can be secreted from lysosomes into the extracellular milieu to degrade various extracellular matrix (ECM) proteins which facilitate the invasion, angiogenesis and metastasis of tumor cells [17-20]. Accumulated evidences

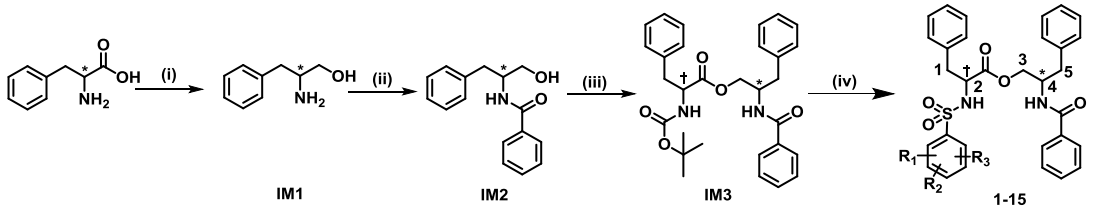
have shown that cathepsins in human breast cancer are responsible for metastasis [21-25]. In view that **TSA** targeted cathepsin, we hypothesized that it could also possess anti-metastatic ability. At the same time, our previous study had revealed that **TSA** exhibited novel anti-tumor effect by inducing autophagy instead of apoptosis. It indicated that A-ring sulfonyl-substituted derivatives of asperphenamate may present both autophagy-inducing and metastasis-inhibiting effects on tumor cells.

In this paper, we report the synthesis of A-ring phenylsulfonyl substituted analogues of **TSA.** Furthermore, the inhibitory activity of all derivatives against Cat L, S, K and B were tested. From this screen results, compound **6a** was shown to possess the most potent activity and was further used to explore its ability to both induce autophagy and inhibit metastasis in breast cancer cells.

2. Results and discussion

2.1 Chemistry.

Considering that toluenesulfonyl substituent in A-ring moiety of **TSA** contributed to the inhibitory ability against cathepsin, other substitution-type phenylsulfonyl groups would be chosen to replace the toluenesulfonyl fragment. Compounds **1-15** were synthesized by similar approach with the previously published procedures illustrated in **Scheme 1** [15]. Briefly, phenylalaninol **IM1** was prepared by directly reduction of optically pure phenylalanine with $NaBH_4/I_2$ [26]. **IM1** reacted with benzoyl chloride to give *N*-benzoyl-phenylalaninol **IM2** in presence of K_2CO_3 [27]. The key intermediate **IM3** was synthesized by esterification of **IM2** with optically pure *N*-(*tert*-butoxycarbonyl)-phenylalanine (*N*-Boc-phenylalanine) using 1, 1'-carbonyldiimidazole (CDI) as condensation reagent. Treatment of **IM3** with a 1.5 M solution of hydrogen chloride gas in ethyl acetate yielded the deprotected product, which was then sulfonylated to furnish the target derivatives **1-15**.



1: 4-Br	**8:** 2-F, 4-F
2: 4-OMe	**9:** 2-F, 6-F
3: 4-CF_3	**10:** 2-OMe, 5-Br
4: 4-F	**11:** 2-OMe, 5-OMe
5: -H	**12:** 2-OMe, 4-OMe
6: 3-Br	**13:** 2-NO_2, 4-CF_3
7: 2-Br	**14:** 4-NO_2, 3-CF_3
	15: 2-F, 4-F, 5-Cl

a	*=d	†=l
b	*=d	†=d
c	*=l	†=d
d	*=l	†=l

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, 70-87%; (iv) (a) 1.5 M HCl/ethyl acetate, rt, 77-90%; (b) Different substituted phenylsulfonyl chlorides, triethylamine, CH_2Cl_2, rt, 91-96%.

2.2 Cathepsin inhibitory activity assay.

All derivatives were screened for their inhibitory activities toward Cat L, S, B and K. **TSA** was chosen as control. The results were summarized in **Table 1**. All synthetic compounds were inactive (IC_{50}>100 μM) against Cat B and K. The (*S*, *S*) and (*S*, *R*) isomers **1-15c** and **d** showed no inhibitory effect on four cathepsins. Among all derivatives, the *meta*-bromo substituted derivative **6a** showed the greatest potency against Cat L and S with an average IC_{50} value of 7.66±0.83 and 6.94±0.67 μM, respectively. Unsubstituted derivatives **5** totally lost its inhibition ability against all cathepsins.

As far as mono-substituted derivatives are concerned, *ortho*-bromo substituted analog **7** was non-effective. With the exception of the 4-methoxyl substituted derivative **2**, almost other *para*-substituted (*R*, *S*) stereoisomers with electron-withdrawing group (F **4**, Br **1**, CF_3 **3**) showed inhibitory potency. Although, corresponding (*R*, *R*) isomers were inactive. Only the 4-fluoro derivative **4a** showed moderate inhibitory activity against Cat S and 3.6-fold decreased activity compared with **6a**.

Among di-substituted derivatives, the 2, 6-difluoro derivative **9** displayed no inhibitory ability against four cathepsins. 5-Bromo-2-methoxyl derivative **10a** was more potent than other compounds and displayed a 1.6-fold and 4.6-fold decreased activity against Cat L and S compared with **6a**, respectively. And the 2, 5-dimethoxyl analog **11** also showed inhibitory effect but was significantly less potent than **10a**. Despite the fluoro and trifluoromethyl groups at 4-position still remain, the inhibitory potency of compound **8** and **13** decreased evidently while the other electron-withdrawing group was introduced into 2-position, such as fluoro and nitro groups. Interestingly, although 4-position was substituted by methoxyl group with electron-donating effect, the (*R*, *S*) and (*R*, *R*) isomers of the 2, 4-dimethoxyl analog **12** showed comparable activity with **10a** against Cat L. The derivative **14b** remained the inhibitory activity even after electron-withdrawing groups were introduced at the 3- and 4-positions. Although the 2, 4-difluoro derivative **8** showed weak inhibitory effect, tri-substituted derivative **15** showed moderate inhibitory ability after chloro group was further introduced in 5-position of **8**. In addition, only **10a** and **15a** displayed moderate inhibitory potency toward Cat S in all di- or tri-substituted

derivatives.

In general, both the electronic nature and the position of the substituents played important roles in cathepsin inhibitory potency. It indicated that the substituents at the 2-position displayed a significant impact on activity, which electron-donating groups being more beneficial to enzyme inhibition effect than electron-withdrawing groups. Meanwhile, electron-withdrawing groups at the 5-position are also contributive to activity.

(**Table 1** should be listed here)

2.3 Docking study.

To discover potential cathepsin inhibitors, molecular docking was carried out to reveal the interaction modes of A-ring phenylsulfonyl substituted derivatives in the active sites of Cat L and S. Compound **6a**, which displayed the most pronounced activity against Cat L and S, was selected for the molecular docking studies. And as a control, the molecular docking of lead compound, **TSA**, was also performed along with **6a**. The crystal structures of Cat L (PDB ID: 2XU4) [28] and S (PDB ID: 3OVX) [29] were used for the docking calculations [30].

For Cat L, the key residue Gly68 formed dual hydrogen-bond interactions with two compounds. And the carbonyl oxygen in the B-ring region of **TSA** only made a hydrogen-bond interaction with His163. Although, comparing with **TSA**, both amide oxygen and NH in the B-ring moiety of **6a** formed two hydrogen bonds with the backbone NH of Gln19 and carbonyl oxygen of Asp162, respectively. And the *meta*-bromo group in **6a** interacted with a hydrophobic pocket being composed by Leu69, Met70 and Ala214 (**Figure 2**). For Cat S, hydrogen-bond interactions appeared between residue Gly69 and two compounds. In the same time, their B-ring moieties formed the other hydrogen-bond interactions which amide carbonyl oxygen of **TSA** made a contact with residue His164, and amide NH of **6a** formed an interaction with Asn163. Both A and D-phenyl rings of **TSA** and **6a** participated in the π-π contacts with Phe70. The additional π-π interaction between B-phenyl ring of **6a** and imidazolyl ring of His164 residue was also observed (**Figure 3**).

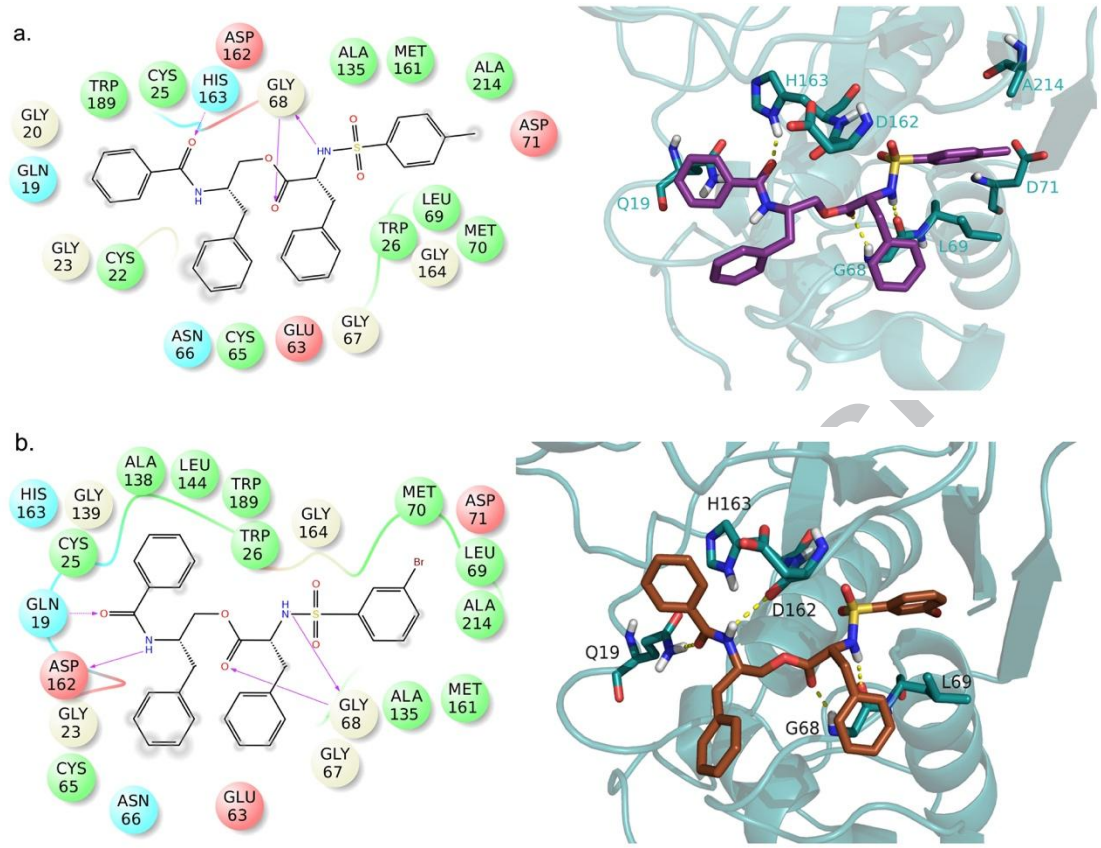


Figure 2. 2D and 3D binding modes of **TSA** (**a**, purple stick), compounds **6a** (**b**, brown stick) with Cat L. The Cat L protein was displayed in ribbon and amino acid residues were displayed as blue thin stick.

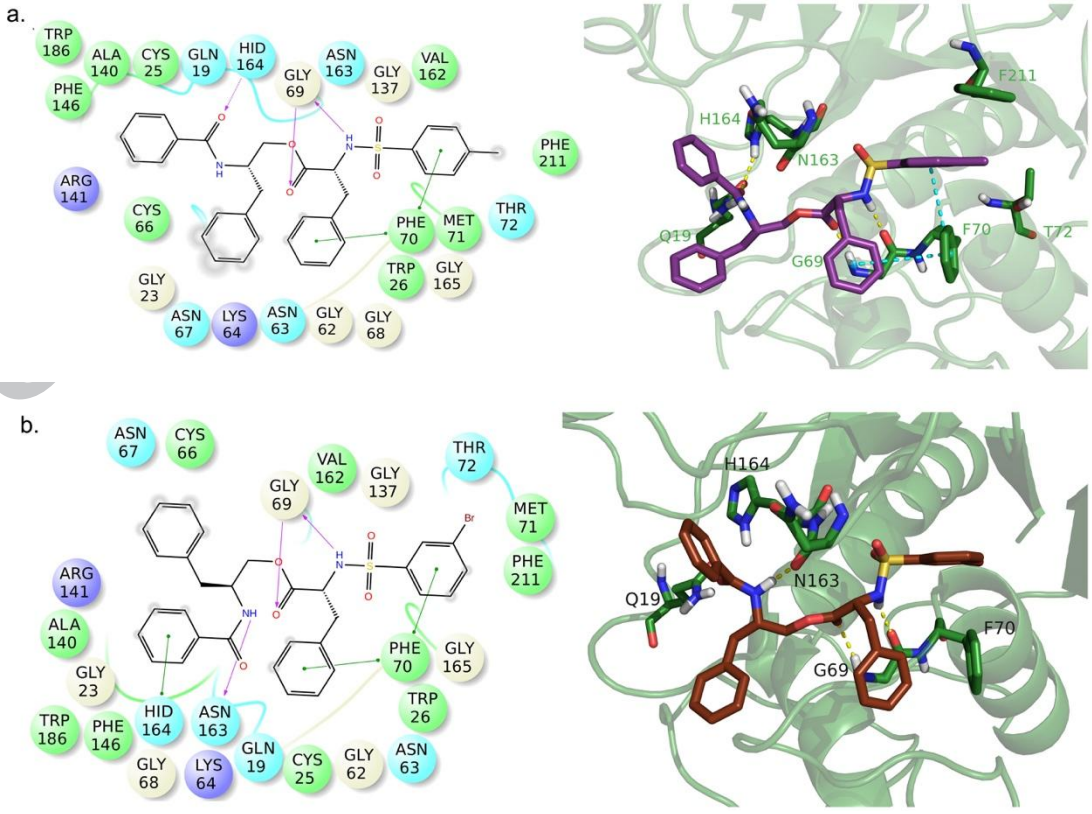


Figure 3. 2D and 3D binding modes of **TSA** (**a**), compounds **6a** (**b**) with Cat S. The Cat S protein was displayed in

ribbon and amino acid residues were displayed as blue thin stick.

The shape of binding pockets of Cat L and S were displayed as molecular surfaces (**Figure 4** and **5**). In Cat L, **TSA** and **6a** exhibited key interactions with S1, S2 and S3 pockets [31] utilized by their A- and C-phenyl rings. In addition, the B-rings of **TSA** and **6a** interacted with S2' pocket being composed of Cys22, Gly23 and Cys25 residues and the S1' pocket including Ala138, Leu144 and Trp189 residues, respectively. Hydrophobic interactions of *meta*-bromo group seem to alter the orientation of B-ring in **6a** to make it interact with S1' pocket. In Cat S, **TSA** and **6a** showed similar interactions. In addition to accommodation into S1, S2 and S3 pockets well [32], they provided hydrophobic interactions with the side chains of Ala140, Phe146 and Trp186 by their B-phenyl rings.

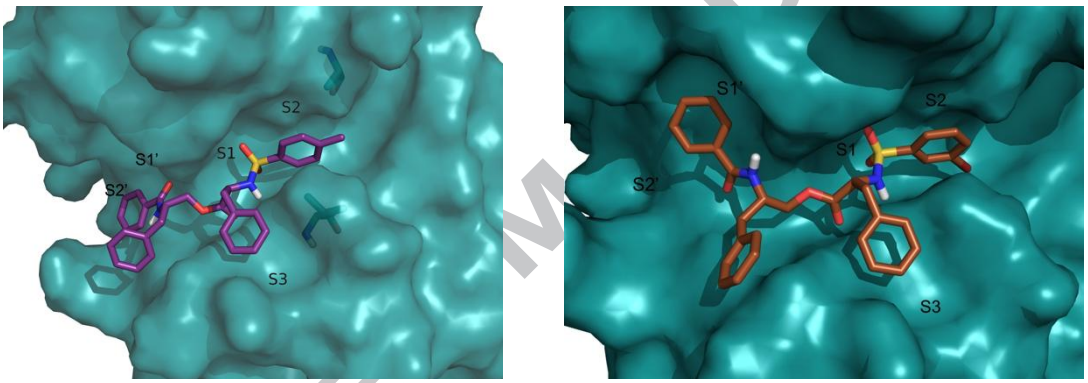


Figure 4. Binding modes of **TSA** (purple stick) and compound **6a** (brown stick) with Cat L (blue surface).

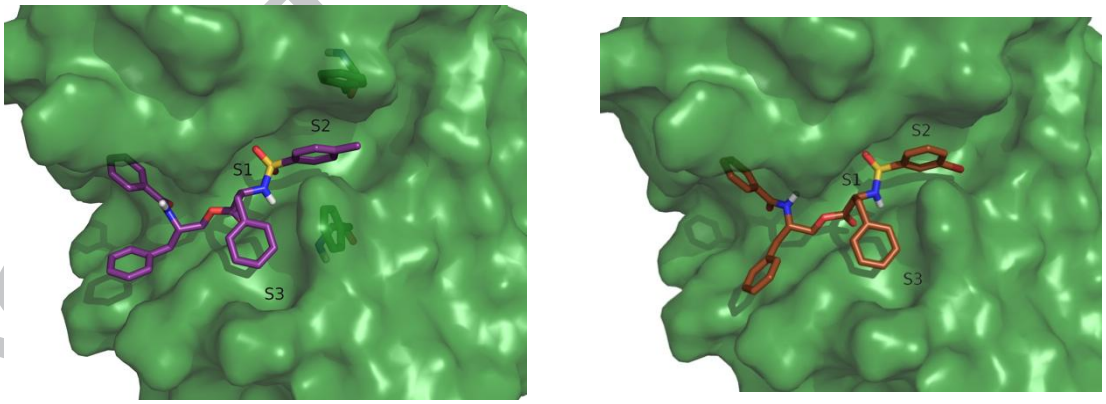


Figure 5. Binding modes of **TSA** (purple stick) and compound **6a** (brown stick) with Cat S (green surface).

In general, the docking results showed that **6a** formed more hydrogen bonds than **TSA** with Cat L. And for Cat S, **6a** also displayed more π-π contacts than **TSA**. It was obvious that molecular simulation results were consistent with the cathepsin inhibitory assay results. The *N*-phenylsulfonyl-phenylalanine moiety was the key fragment whose A-and C-phenyl ring occupied S2 and S3 pockets well, and the sulfonyl linker filled in the S1 pocket in Cat L and S.

The hydrophobic bromo atom at the 3-position in the A-ring of **6a** made the B-ring more tightly interact with S1' rather than S2' pocket, leading to an enhancement of inhibitory ability against Cat L. It meant that S1' hydrophobic pocket was the essential binding domain for this kind of compound. However, the D-ring did not show any fixed interaction with Cat L and S. Due to the D-ring being surrounded by the region composed of Cys65 and Asn66, we hypothesized that some hydrogen-bond acceptors would provide more benefit for their interactions. Therefore, follow-up work will focus on modification of the 3-position of the A-ring and introduction carbonyl and cyano substituents into D-ring to further increase the interactions with cathepsin based on keeping *N*-phenylsulfonyl-phenylalanine moiety.

*2.4 Compound **6a** suppressed breast cancer cells migration.*

An increasing literature described the important role of cathepsin in tumor progression, especially in the process of migration and invasion. Compared with primary tumor, the relative activity of cathepsins in metastatic tumor significantly increased. Therefore, it indicates that inhibition of cathepsins may be favorable in reducing the occurrence of tumor migration and invasion. Our cathepsin inhibitory activity results displayed that compound **6a** showed the greatest potency against Cat L and S. And hence, its inhibitory activity against tumor migration and invasion was further studied in two kinds of breast cancer cells, MCF-7 and MDA-MB-231.

*2.4.1 **6a** and cathepsin inhibitor, **E-64d** had no effect on the proliferation of MDA-MB-231 and MCF-7 cells.*

Firstly, we determined the non-cytotoxic concentration of **6a** by MTT assay for the purpose of eliminating the impact of **6a** on cell proliferation in the subsequent anti-migration and invasion experiments. MDA-MB-231 and MCF-7 cells were treated with **6a** at different concentrations of 3.125, 6.25 and 12.5 μM for 48 h, after which MTT assay were performed to evaluate the cell viability.

Figure 6 displayed that 3.125-12.5 μM of **6a** showed no anti-proliferative effect on two types of breast cancer cell lines. Along with **6a**, cathepsin inhibitor **E-64d** as control at the concentration of 12.5 μM also exhibited no cytotoxity against two cells. Therefore, we defined upper limit of concentration of **6a** at 12.5 μM in following studies.

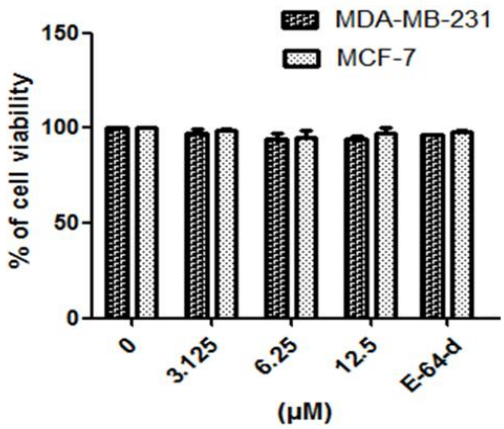


Figure 6. Cell viability of MDA-MB-231 and MCF-7 cells after treatment with **6a** and **E-64d**.

*2.4.2 Compound **6a** inhibited breast cancer cells migration and invasion.*

In order to investigate the effect of **6a** on migration and invasion of the two breast cancer cell lines, wound healing and transwell chamber assays were carried out. Previous research indicated that MDA-MB-231 cells displayed stronger metastatic ability than MCF-7 cells [16]. Therefore, MDA-MB-231 cells were chosen to study further.

MDA-MB-231 cells were incubated with DMSO, **6a** (at 3.125, 6.25 and 12.5 μM, respectively) and **E64d** at 12.5 μM for 48 h. Then, the anti-metastatic potential was examined by the wound healing assay. As expected, breast cancer cells in DMSO control displayed the strongest migratory ability. A dose-dependent inhibitory effect of **6a** was observed in MDA-MB-231 cells. The width of scratch directly reflected the decreasing migration capability of MDA-MB-231 cells by 25%, 38% and 46% in correspondence to the concentrations of **6a**. Its migration inhibitory potency at 12.5 μM was close to **E64d** (**Figure 7a**).

On the other hand, transwell chamber assay results showed that compound **6a** significantly attenuated the ability of cells invasion by treating the cells using **6a** at concentrations of 3.125, 6.25 and 12.5 μM. Under these conditions, the number of cells that permeated the membrane was reduced by 18%, 36% and 47%, respectively. In conclusion, all results illustrated that **6a** displayed a significant anti-metastatic ability against MDA-MB-231 cells (**Figure 7b**).

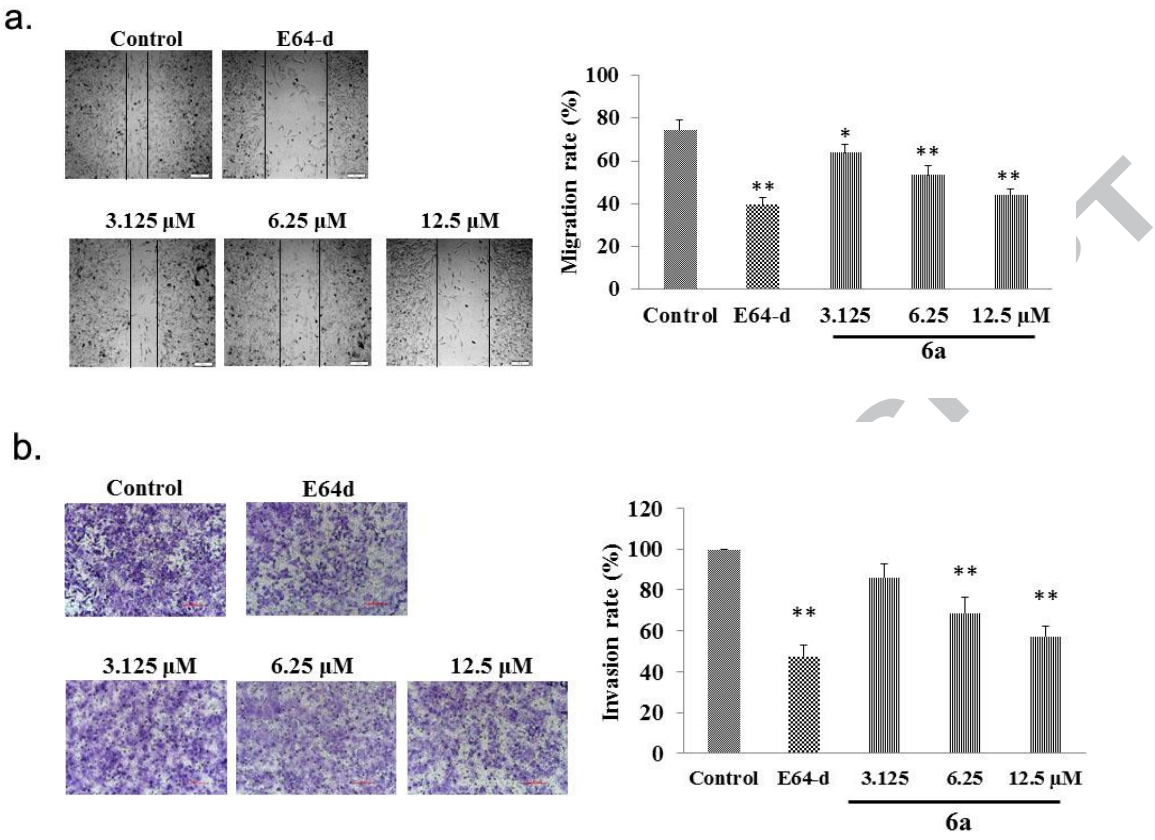


Figure 7. Compound **6a** inhibited migration of MDA-MB-231 cells. **a.** Wound healing assay; **b.** Transwell chamber assay were used to investigate the effect of compound **6a** on migration of MDA-MB-231 cells.

2.5 ***6a*** *induced autophagy in MDA-MB-231*.

Because our previous work showed that **TSA** was an autophagy inducer, we guessed that **6a** as an analog of **TSA**, could also possess autophagy-inducing potency. For the sake of determining the suitable concentration of **6a** in subsequent assay, antitumor activity of **6a** against MDA-MB-231 cells was screened *in vitro* through the standard MTT method using cisplatin as control (see **Table 1s**). The results showed that the IC_{50} value for **6a** was 26.24±1.43μM. Accordingly, two concentrations (12.5 and 25 μM) were selected for following study.

To investigate autophagy-inducing effect of **6a** on MDA-MB-231 cells, we first observed the morphological changes using fluorescence microscopy after MDC staining [33]. As shown in Figure **8a**, 12.5 and 25 μM of **6a** induced the formation of acidic vesicle organelles (a characteristic of autophagy), suggesting that autophagy occurred in MDA-MB-231 cells **6a** treated [34]. The conversion of LC3-I to LC3-II was also examined so as to further assess the development of autophagy in **6a**-treated MDA-MB-231 cells [35]. **Figure 8b** showed that autophagy marker LC3-II was detected by western blot analysis. These results proved that **6a** can

induce autophagy in MDA-MB-231 cells.

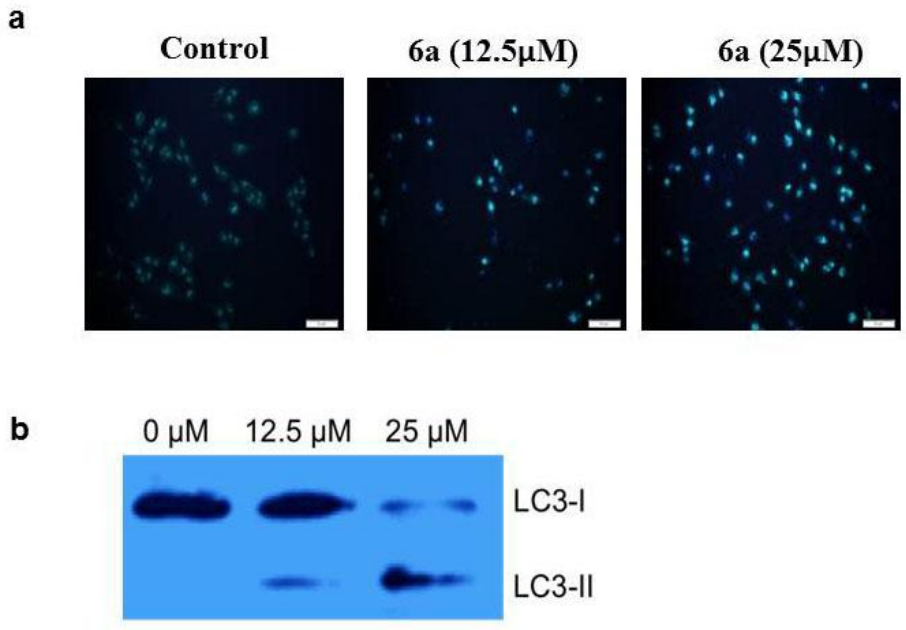


Figure 8. 6a induced autophagy in MDA-MB-231 cells. **a.** Autophagosomes observation. MDA-MB-231 cells treated with **6a** (12.5 and 25 μM) for 72h, and then the cells were stained with MDC and observed by fluorescence microscope; **b.** Western blotting analysis for autophagy. MDA-MB-231 cells were treated with **6a** (12.5 and 25 μM) for 72h, and then the total proteins were extracted and detected by western blot analysis.

3. Conclusion

In summary, sixty optically active derivatives of **TSA** were designed and synthesized. The structures of all derivatives were characterized by NMR and HR-MS. Subsequent cathepsin inhibitory assay showed compound **6a** displayed the greatest inhibitory activity against both Cat L and S. The enzyme inhibitory ability of **6a** against Cat L and S was 3.9 and 11.5-fold more potent than that of **TSA**, respectively. These results were also supported by molecular simulation study that **6a** formed more hydrogen bonds and π-π interactions with Cat L and S than **TSA**. In order to determine whether **6a** could possess the dual functions of inducing autophagy and inhibiting metastasis, MDC staining experiment and western blotting analysis were firstly undertaken. The overall results showed that **6a** induced the autophagy in MDA-MB-231 cells. On the other hand, evidence for the inhibitory effect of **6a** on migration and invasion of breast cancer cells was furthermore provided by wound healing and transwell chamber experiments.

4. Experimental

4.1. Reagents and apparatus

Melting points were measured by use of a Büchi Melting Point B-540 apparatus (Büchi

Labortechnik, Flawil, Switzerland) without correction. High resolution mass spectra were carried out on a Bruker Micromass Time of Flight mass spectrometer using electrospray ionisation (ESI). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker ARX-600 (600 MHz). Unless otherwise stated, reagents came from commercial sources and used without further purification. All crude target compounds and intermediates were purified by chromatographic column using silica gel (200-300 mesh) from Qingdao Ocean Chemicals with chloroform/acetone mixture as eluent. The intermediates **IM1**, **IM2 and IM3** were prepared followed by the methods in references [26, 27, 15], respectively.

4.2 General procedure for the preparation of derivatives 1-15

To a solution of hydrogen chloride gas in dry ethyl acetate (1.5 M, 3.0 mL) was added intermediate **IM 3** (0.3 g, 0.6 mmol). The reaction mixture was stirred for 4 h at room temperature. The solvent was removed under reduced pressure to give solid deprotected product. Various substituted-phenyl sulfonyl chlorides were added dropwise in a mixture of deprotected product and triethylamine (0.5 mL) in anhydrous CH_2Cl_2 (10 mL). After stirring at room temperature overnight, the reaction mixture was concentrated using a rotary evaporator. The crude product was purified by chromatography on silica gel utilized a mixture of chloroform and acetone (35:1, v/v) as eluent to furnish the desired product.

4.2.1 (*R*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(4-bromophenylsulfonamido)-3-phenyl propanoate (1a)

mp 164-167 ºC; yield 63.9 %; $[\alpha]_D^{20}$ -23.3 (*c* 0.4 $CHCl_3$/MeOH=1:1 , v/v); HR-MS: $C_{31}H_{29}BrN_2O_5SNa$, calcd. 643.0878 $[M+Na]^+$, found: 643.0878; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.73-7.75 (d, *J* = 6.6 Hz, 2H, ArH), 7.42-7.50 (m, 7H, ArH), 7.18-7.32 (m, 8H, ArH), 7.00 (m, 2H, ArH), 6.53 (d, 1H, *J* = 7.8 Hz, CONH), 5.18 (d, *J* = 9.0 Hz, 1H, CONH), 4.61 (m, 1H, 4-H), 4.34-4.36 (d, *J* = 9.6 Hz, 1H, 3a-H), 4.11 (d, *J* = 5.4 Hz, 1H, 2-H), 4.03 (dd, *J* = 7.2 Hz, 1H, 3b-H), 3.06-3.10 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.97-3.00 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.87-2.93 (dd, *J* = 13.8, 6.6 Hz, 2H, 1b-H, 5b-H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.6, 167.1, 138.2, 136.9, 134.8, 134.0, 132.2×2, 131.6, 129.2×2, 129.0×2, 128.7×2, 128.7×2, 128.4×2, 128.4×2, 127.8, 127.3, 127.0×2, 126.8, 66.2, 57.3, 49.8, 38.7, 37.2.

4.3.2 (*R*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(4-bromophenylsulfonamido)-3-phenyl propanoate (1b)

mp 162-164 ºC; yield: 60.2 % ; $[\alpha]_D^{20}$ 47.1 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); ^1H NMR (600 MHz, $CDCl_3$): δ 7.72-7.73 (d, *J* = 7.8 Hz, 2H, ArH), 7.41-7.52 (m, 7H, ArH), 7.19-7.34 (m, 8H, ArH), 7.02 (m, 2H, ArH), 6.45 (d, 1H, *J* = 8.4 Hz, CONH), 5.17 (d, *J* = 9.6 Hz, 1H, CONH), 4.59 (m, 1H, 4-H), 4.25-4.28 (dd, *J* = 11.4, 3.0 Hz, 1H, 3a-H), 4.17-4.21 (dd, *J* = 14.4, 8.4 Hz, 1H, 2-H), 4.12 -4.15 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.08-3.11 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.98-2.95 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.92-2.88 (dd, *J* = 13.8, 6.6 Hz, 1H, 1b-H), 2.83-2.87 (dd, *J* = 13.8, 6.6 Hz, 1H, 5b-H).

4.3.3 (*S*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(4-bromophenylsulfonamido)-3-phenyl propanoate (1c)

mp: 164-168 ºC; yield: 65.4 %; $[\alpha]_D^{20}$ 22.8 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); ^1H NMR (600 MHz, $CDCl_3$): δ 7.73-7.75 (d, *J* = 6.6 Hz, 2H, ArH), 7.42-7.50 (m, 7H, ArH), 7.18-7.32 (m, 8H, ArH), 7.00 (m, 2H, ArH), 6.53 (d, 1H, *J* = 7.8 Hz, CONH), 5.18 (d, *J* = 9.0 Hz, 1H, CONH), 4.61 (m, 1H, 4-H), 4.34-4.36 (d, *J* = 9.6 Hz, 1H, 3a-H), 4.11 (d, *J* = 5.4 Hz, 1H, 2-H), 4.03 (dd, *J* = 7.2 Hz, 1H, 3b-H), 3.06-3.10 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.97-3.00 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.87-2.93 (dd, *J* = 13.8, 6.6 Hz, 2H, 1b-H, 5b-H).

4.3.4 (*S*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(4-bromophenylsulfonamido)-3-phenyl propanoate (1d)

mp: 162-163 ºC; yield: 64.9 %; $[\alpha]_D^{20}$ -46.6 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); ^1H NMR (600 MHz, $CDCl_3$): δ 7.72-7.73 (d, *J* = 7.8 Hz, 2H, ArH), 7.41-7.52 (m, 7H, ArH), 7.19-7.34 (m, 8H, ArH), 7.02 (m, 2H, ArH), 6.45 (d, 1H, *J* = 8.4 Hz, CONH), 5.17 (d, *J* = 9.6 Hz, 1H, CONH), 4.59 (m, 1H, 4-H), 4.25-4.28 (dd, *J* = 11.4, 3.0 Hz, 1H, 3a-H), 4.17-4.21 (dd, *J* = 14.4, 8.4 Hz, 1H, 2-H), 4.12 -4.15 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.08-3.11 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.98-2.95 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.92-2.88 (dd, *J* = 13.8, 6.6 Hz, 1H, 1b-H), 2.83-2.87 (dd, *J* = 13.8, 6.6 Hz, 1H, 5b-H).

4.3.5 (*R*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(4-methoxyphenylsulfonamido)-3-phenyl propanoate (2a)

mp: 158-160 ºC; yield: 61.1 %; $[\alpha]_D^{20}$ -17.5 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); HR-MS: $C_{32}H_{32}N_2O_6SNa$, calcd. 595.1879 $[M+Na]^+$, found: 595.1880; ^1H NMR (600 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.8, 2H, ArH), 7.58 (d, *J* = 9.0, 2H, ArH), 7.40-7.50 (m, 3H, ArH), 7.18-7.32 (m, 8H, ArH), 7.04 (m, 2H, ArH), 6.82 (d, *J* = 9.0, 2H, ArH), 6.60 (d, 1H, *J* = 8.4 Hz, CONH), 5.10 (d, *J* = 9.0

Hz, 1H, CONH), 4.57 (m, 1H, 4-H), 4.32-4.34 (dd, *J* = 10.8, 3.0 Hz, 1H, 3a-H), 4.09-4.12 (dd, *J* = 14.4, 7.8 Hz, 1H, 2-H), 3.97-4.00 (dd, *J* = 11.4, 4.2 Hz, 1H, 3b-H), 3.83 (s, 3H, 8-H), 3.03-3.06 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 2.94-2.98 (m, 2H, 5a-H, 1b-H), 2.82-2.86 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.6, 166.9, 163.0, 136.9, 134.9, 133.9, 131.6, 130.6, 129.2×2, 129.2×4, 128.7×2, 128.6×2, 128.5×2, 127.3, 127.0×2, 126.8, 114.1×2, 65.9, 56.8, 55.5, 50.2, 38.8, 37.0.

4.3.6 (*R*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(4-methoxyphenylsulfonamido)-3-phenyl propanoate (2b)

mp: 153-154 ºC; yield: 57.7 %; $[\alpha]_D^{20}$ 34.8 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.2, 2H, ArH), 7.59 (d, *J* = 9.0, 2H, ArH), 7.42-7.52 (m, 3H, ArH), 7.20-7.32 (m, 8H, ArH), 7.05 (m, 2H, ArH), 6.82 (d, *J* = 9.0, 2H, ArH), 6.54 (d, 1H, *J* = 7.8 Hz, CONH), 5.05 (d, *J* = 9.0 Hz, 1H, CONH), 4.54 (m, 1H, 4-H), 4.17-4.22 (m, 2H, 3a-H, 2-H), 4.07-4.09 (dd, *J* = 10.8, 3.6 Hz, 1H, 3b-H), 3.81 (s, 3H, 8-H), 3.04-3.07 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 2.90-2.97 (m, 2H, 5a-H, 1b-H), 2.77-2.80 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.7 (*S*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(4-methoxyphenylsulfonamido)-3-phenyl propanoate (2c)

mp: 157-158 ºC; yield: 60.4 %; $[\alpha]_D^{20}$ 17.3 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.8, 2H, ArH), 7.58 (d, *J* = 9.0, 2H, ArH), 7.40-7.50 (m, 3H, ArH), 7.18-7.32 (m, 8H, ArH), 7.04 (m, 2H, ArH), 6.82 (d, *J* = 9.0, 2H, ArH), 6.60 (d, 1H, *J* = 8.4 Hz, CONH), 5.10 (d, *J* = 9.0 Hz, 1H, CONH), 4.57 (m, 1H, 4-H), 4.32-4.34 (dd, *J* = 10.8, 3.0 Hz, 1H, 3a-H), 4.09-4.12 (dd, *J* = 14.4, 7.8 Hz, 1H, 2-H), 3.97-4.00 (dd, *J* = 11.4, 4.2 Hz, 1H, 3b-H), 3.83 (s, 3H, 8-H), 3.03-3.06 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 2.94-2.98 (m, 2H, 5a-H, 1b-H), 2.82-2.86 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H);

4.3.8 (*S*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(4-methoxyphenylsulfonamido)-3-phenyl propanoate (2d)

mp: 153-154ºC; yield: 58.7 %; $[\alpha]_D^{20}$ -34.6 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.2, 2H, ArH), 7.59 (d, *J* = 9.0, 2H, ArH), 7.42-7.52 (m, 3H, ArH), 7.20-7.32 (m, 8H, ArH), 7.05 (m, 2H, ArH), 6.82 (d, *J* = 9.0, 2H, ArH), 6.54 (d, 1H, *J* = 7.8 Hz, CONH), 5.05 (d, *J* = 9.0 Hz, 1H, CONH), 4.54 (m, 1H, 4-H), 4.17-4.22 (m, 2H, 3a-H, 2-H), 4.07-4.09 (dd, *J* = 10.8, 3.6 Hz, 1H, 3b-H), 3.81 (s, 3H, 8-H), 3.04-3.07 (dd, *J* = 13.8, 6.0 Hz, 1H,

1a-H), 2.90-2.97 (m, 2H, 5a-H, 1b-H), 2.77-2.80 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.9 (*R*)-(*S*)-2-Benzamido-3-phenylpropyl

2-(4-trifluoromethylphenylsulfonamido)-3-phenyl propanoate (3a)

mp: 168-169 ºC; yield: 48.9 %; $[\alpha]_D^{20}$ -26.1 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); HR-MS: $C_{32}H_{29}F_3N_2O_5SNa$, Calcd. 633.1647 $[M+Na]^+$, found: 633.1647; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.8 Hz, 2H, ArH), 7.69 (d, *J* = 7.8 Hz, 2H, ArH), 7.59 (d, *J* = 7.8 Hz, 2H, ArH), 7.41-7.52 (m, 3H, ArH), 7.22-7.34 (m, 5H, ArH), 7.13-7.16 (m, 3H, ArH), 6.99 (d, *J* = 6.6 Hz, 2H, ArH), 6.52 (d, 1H, *J* = 8.4 Hz, CONH), 5.30 (d, *J* = 8.4 Hz, 1H, CONH), 4.64 (m, 1H, 4-H), 4.35-4.38 (dd, *J* = 11.4, 3.0 Hz, 1H, 3a-H), 4.11-4.15 (m, 1H, 2-H), 4.05-4.07 (dd, *J* = 10.8, 4.2 Hz, 1H, 3b-H), 3.09-3.12 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.99-3.02 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.86-2.93 (m, 2H, 1b-H, 5b-H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 171.1, 167.1, 143.0, 136.7, 134.9, 134.3, 133.9, 131.6, 129.1×2, 129.1×2, 128.7×2, 128.7×2, 128.5×2, 127.4, 127.3×2, 127.0×2, 126.9, 126.0, 123,.9, 122.1, 66.3, 57.3, 50.0, 38.6, 37.1.

4.3.10 (*R*)-(*R*)-2-Benzamido-3-phenylpropyl

2-(4-trifluoromethylphenylsulfonamido)-3-phenyl propanoate (3b)

mp: 165-168 ºC; yield: 51.3 %; $[\alpha]_D^{20}$ 52.4 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74 (d, *J* = 7.2 Hz, 2H, ArH), 7.67 (d, *J* = 7.8 Hz, 2H, ArH), 7.58 (d, *J* = 7.8 Hz, 2H, ArH), 7.41-7.52 (m, 3H, ArH), 7.22-7.34 (m, 5H, ArH), 7.14-7.18 (m, 3H, ArH), 7.01 (d, *J* = 6.6 Hz, 2H, ArH), 6.46 (d, 1H, *J* = 8.4 Hz, CONH), 5.31 (d, *J* = 7.8 Hz, 1H, CONH), 4.62 (m, 1H, 4-H), 4.29-4.30 (m, 1H, 3a-H), 4.19-4.23 (m, 1H, 2-H), 4.14 -4.17 (dd, *J* = 10.8, 4.8 Hz, 1H, 3b-H), 3.11-3.14 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.96-3.00 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.85-2.90 (m, 2H, 1b-H, 5b-H).

4.3.11 (*S*)-(*R*)-2-Benzamido-3-phenylpropyl

2-(4-trifluoromethylphenylsulfonamido)-3-phenyl propanoate (3c)

mp: 168-170 ºC; yield: 50.6 %; $[\alpha]_D^{20}$ 26.7 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.8 Hz, 2H, ArH), 7.69 (d, *J* = 7.8 Hz, 2H, ArH), 7.59 (d, *J* = 7.8 Hz, 2H, ArH), 7.41-7.52 (m, 3H, ArH), 7.22-7.34 (m, 5H, ArH), 7.13-7.16 (m, 3H, ArH), 6.99 (d, *J* = 6.6 Hz, 2H, ArH), 6.52 (d, 1H, *J* = 8.4 Hz, CONH), 5.30 (d, *J* = 8.4 Hz, 1H, CONH), 4.64 (m, 1H, 4-H), 4.35-4.38 (dd, *J* = 11.4, 3.0 Hz, 1H, 3a-H), 4.11-4.15 (m, 1H, 2-H), 4.05-4.07 (dd, *J* = 10.8, 4.2 Hz, 1H, 3b-H), 3.09-3.12 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.99-3.02 (dd, *J* = 13.8, 6.6 Hz, 1H,

5a-H), 2.86-2.93 (m, 2H, 1b-H, 5b-H)

4.3.12 (*S*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(4-trifluoromethylphenylsulfonamido)-3-phenyl propanoate (3d)

mp: 163-166 ºC; yield: 59.9 %; $[\alpha]_D^{20}$ -51.9 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74 (d, *J* = 7.2 Hz, 2H, ArH), 7.67 (d, *J* = 7.8 Hz, 2H, ArH), 7.58 (d, *J* = 7.8 Hz, 2H, ArH), 7.41-7.52 (m, 3H, ArH), 7.22-7.34 (m, 5H, ArH), 7.14-7.18 (m, 3H, ArH), 7.01 (d, *J* = 6.6 Hz, 2H, ArH), 6.46 (d, 1H, *J* = 8.4 Hz, CONH), 5.31 (d, *J* = 7.8 Hz, 1H, CONH), 4.62 (m, 1H, 4-H), 4.29-4.30 (m, 1H, 3a-H), 4.19-4.23 (m, 1H, 2-H), 4.14 -4.17 (dd, *J* = 10.8, 4.8 Hz, 1H, 3b-H), 3.11-3.14 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.96-3.00 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.85-2.90 (m, 2H, 1b-H, 5b-H).

4.3.13 (*R*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(4-fluorophenylsulfonamido)-3-phenyl propanoate (4a)

mp: 171-172 ºC; yield: 45.7%; $[\alpha]_D^{20}$ -32.9 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); HR-MS: $C_{31}H_{29}FN_2O_5SNa$, Calcd. 583.1679 $[M+Na]^+$, found: 583.1680; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.2 Hz, 2H, Ar), 7.60-7.62 (m, 2H, ArH), 7.41-7.51 (m, 3H, ArH), 7.18-7.33 (m, 8H, ArH), 7.00-7.03 (m, 4H, ArH), 6.52 (d, 1H, *J* 8.4 Hz, CONH), 5.14 (d, *J* = 9.0 Hz, 1H, CONH), 4.61 (m, 1H, 4-H), 4.34-4.36 (dd, *J* = 11.4, 3.6 Hz, 1H, 3a-H), 4.10-4.13 (m, 1H, 2-H), 4.02-4.05 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.06-3.09 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.97-3.01 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.91-2.95 (dd, *J* = 13.8, 8.4 Hz, 1H, 1b-H), 2.87-2.90 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.3, 167.1, 165.8, 164.1, 136.8, 135.4, 134.9, 133.9, 131.6, 129.7, 129.6, 129.1×3, 128.7×2, 128.7×2, 128.5×2, 127.3, 127.0×2, 126.9, 116.2, 116.1, 66.1, 57.1, 50.1, 38.7, 37.1.

4.3.14 (*R*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(4-fluorophenylsulfonamido)-3-phenyl propanoate (4b)

mp: 167-169 ºC; yield: 45.9% ; $[\alpha]_D^{20}$ 65.8 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74 (d, *J* = 7.8 Hz, 2H, ArH), 7.59-7.61 (m, 2H, ArH), 7.41-7.52 (m, 3H, ArH), 7.19-7.33 (m, 8H, ArH), 6.99-7.04 (m, 4H, ArH), 6.49 (d, 1H, *J* 7.8 Hz, CONH), 5.19 (d, *J* = 9.0 Hz, 1H, CONH), 4.58 (m, 1H, 4-H), 4.24-4.26 (dd, *J* = 10.8, 3.0 Hz, 1H, 3a-H), 4.17-4.20 (m, 1H, 2-H), 4.11-4.14 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.08-3.11 (dd, *J* = 14.4, 6.0 Hz, 1H, 1a-H), 2.94-2.97 (dd, *J* = 13.8, 7.2 Hz, 1H, 5a-H), 2.89-2.93 (dd, *J* = 13.8, 7.8 Hz, 1H, 1b-H), 2.82-2.86

(dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.15 (*S*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(4-fluorophenylsulfonamido)-3-phenyl propanoate (4c)

mp: 171-172 ºC; yield: 54.5% ; $[\alpha]_D^{20}$ 33.2 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.2 Hz, 2H, Ar), 7.60-7.62 (m, 2H, ArH), 7.41-7.51 (m, 3H, ArH), 7.18-7.33 (m, 8H, ArH), 7.00-7.03 (m, 4H, ArH), 6.52 (d, 1H, *J* = 8.4 Hz, CONH), 5.14 (d, *J* = 9.0 Hz, 1H, CONH), 4.61 (m, 1H, 4-H), 4.34-4.36 (dd, *J* = 11.4, 3.6 Hz, 1H, 3a-H), 4.10-4.13 (m, 1H, 2-H), 4.02-4.05 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.06-3.09 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.97-3.01 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.91-2.95 (dd, *J* = 13.8, 8.4 Hz, 1H, 1b-H), 2.87-2.90 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.16 (*S*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(4-fluorophenylsulfonamido)-3-phenyl propanoate (4d)

mp: 166-169 ºC; yield: 60.1 % ; $[\alpha]_D^{20}$ -66.3 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74 (d, *J* = 7.8 Hz, 2H, ArH), 7.59-7.61 (m, 2H, ArH), 7.41-7.52 (m, 3H, ArH), 7.19-7.33 (m, 8H, ArH), 6.99-7.04 (m, 4H, ArH), 6.49 (d, 1H, *J* = 7.8 Hz, CONH), 5.19 (d, *J* = 9.0 Hz, 1H, CONH), 4.58 (m, 1H, 4-H), 4.24-4.26 (dd, *J* = 10.8, 3.0 Hz, 1H, 3a-H), 4.17-4.20 (m, 1H, 2-H), 4.11-4.14 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.08-3.11 (dd, *J* = 14.4, 6.0 Hz, 1H, 1a-H), 2.94-2.97 (dd, *J* = 13.8, 7.2 Hz, 1H, 5a-H), 2.89-2.93 (dd, *J* = 13.8, 7.8 Hz, 1H, 1b-H), 2.82-2.86 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.17 (*R*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(phenylsulfonamido)-3-phenyl propanoate (5a)

mp: 173-175 ºC; yield: 66.3 % ; $[\alpha]_D^{20}$ -31.3 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); HR-MS: $C_{31}H_{30}N_2O_5SNa$, Calcd. 565.1773 $[M+Na]^+$, found: 565.1774; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.8 Hz, 2H, ArH), 7.65 (d, *J* = 7.8 Hz, 2H, ArH), 7.48-7.52 (m, 2H, ArH), 7.17-7.43 (m, 12H, ArH), 7.02-7.03 (m, 2H, ArH), 6.54 (d, 1H, *J* = 8.4 Hz, CONH), 5.15 (d, *J* = 9.0 Hz, 1H, CONH), 4.56 (m, 1H, 4-H), 4.28-4.29 (dd, *J* = 11.4, 4.8 Hz, 1H, 3a-H), 4.13-4.17 (m, 1H, 2-H), 3.97-3.99 (dd, *J* = 11.4, 4.2 Hz, 1H, 3b-H), 3.04-3.07 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 2.94-2.99 (m, 2H, 5a-H, 1b-H), 2.82-2.86 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.8, 167.1, 139.1, 137.0, 134.8, 134.0, 132.9, 131.5, 129.2×2, 129.0×2, 129.0×2, 128.7×2, 128.6×2, 128.4×2, 127.4, 127.1×2, 126.9×2, 126.8, 65.9, 57.1, 49.9, 38.8, 37.2.

4.3.18 (*R*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(phenylsulfonamido)-3-phenyl propanoate

(5b)

mp: 168-169 ºC; yield: 69.2 %; $[\alpha]_D^{20}$ 62.4 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74 (d, *J* = 7.2 Hz, 2H, ArH), 7.66 (d, *J* = 8.4 Hz, 2H, ArH), 7.41-7.51 (m, 4H, ArH), 7.18-7.36 (m, 10H, ArH), 7.03-7.05 (m, 2H, ArH), 6.46 (d, 1H, *J* = 8.4 Hz, CONH), 5.16 (d, *J* = 9.0 Hz, 1H, CONH), 4.53 (m, 1H, 4-H), 4.21-4.24 (m, 1H, 2-H), 4.16-4.18 (dd, *J* = 11.4, 3.6 Hz, 1H, 3a-H), 4.05-4.08 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.05-3.08 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 2.91-2.98 (m, 2H, 5a-H, 1b-H), 2.76-2.80 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.19 (*S*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(phenylsulfonamido)-3-phenyl propanoate (5c)

mp: 170-171 ºC; yield: 70.1 %; $[\alpha]_D^{20}$ 31.0 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.8 Hz, 2H, ArH), 7.65 (d, *J* = 7.8 Hz, 2H, ArH), 7.48-7.52 (m, 2H, ArH), 7.17-7.43 (m, 12H, ArH), 7.02-7.03 (m, 2H, ArH), 6.54 (d, 1H, *J* = 8.4 Hz, CONH), 5.15 (d, *J* = 9.0 Hz, 1H, CONH), 4.56 (m, 1H, 4-H), 4.28-4.29 (dd, *J* = 11.4, 4.8 Hz, 1H, 3a-H), 4.13-4.17 (m, 1H, 2-H), 3.97-3.99 (dd, *J* = 11.4, 4.2 Hz, 1H, 3b-H), 3.04-3.07 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 2.94-2.99 (m, 2H, 5a-H, 1b-H), 2.82-2.86 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.20 (*S*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(phenylsulfonamido)-3-phenyl propanoate (5d)

mp: 168-170 ºC; yield: 68.7 %; $[\alpha]_D^{20}$ -61.8 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74 (d, *J* = 7.2 Hz, 2H, ArH), 7.66 (d, *J* = 8.4 Hz, 2H, ArH), 7.41-7.51 (m, 4H, ArH), 7.18-7.36 (m, 10H, ArH), 7.03-7.05 (m, 2H, ArH), 6.46 (d, 1H, *J* = 8.4 Hz, CONH), 5.16 (d, *J* = 9.0 Hz, 1H, CONH), 4.53 (m, 1H, 4-H), 4.21-4.24 (m, 1H, 2-H), 4.16-4.18 (dd, *J* = 11.4, 3.6 Hz, 1H, 3a-H), 4.05-4.08 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.05-3.08 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 2.91-2.98 (m, 2H, 5a-H, 1b-H), 2.76-2.80 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.21 (*R*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(3-bromophenylsulfonamido)-3-phenyl propanoate (6a)

mp: 160-161 ºC; yield: 50.7 %; $[\alpha]_D^{20}$ -15.2 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); HR-MS: $C_{31}H_{29}BrN_2O_5SNa$, Calcd. 643.0878 $[M+Na]^+$, found: 643.0878; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.78-7.79 (m, 1H, ArH), 7.73 (d, *J* = 7.2 Hz, 2H, ArH), 7.49-7.60 (m, 3H, ArH), 7.41-7.44 (m, 2H, ArH), 7.31-7.34 (m, 2H, ArH), 7.19-7.26 (m, 7H, ArH), 7.03-7.04 (m, 2H, ArH), 6.43 (d, 1H, *J* = 8.4 Hz, CONH), 5.20 (d, *J* = 9.0 Hz, 1H, CONH), 4.58 (m, 1H, 4-H), 4.21-4.26 (m, 2H, 3a-H, 2-H), 4.12-4.14 (dd, *J* = 11.4, 5.4 Hz, 1H, 3b-H), 3.09-3.12 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 2.91-2.98 (m, 2H, 5a-H, 1b-H), 2.83-2.86 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H); ^{13}C NMR (150 MHz,

$CDCl_3$) δ 170.6, 167.2, 141.1, 136.9, 135.8, 134.7, 134.0, 131.6, 130.4, 129.8, 129.2×2, 129.0×2, 128.7×2, 128.7×2, 128.5×2, 127.5, 127.0×2, 126.8, 125.4, 122.9, 66.2, 57.3, 49.8, 38.7, 37.2.

4.3.22 (*R*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(3-bromophenylsulfonamido)-3-phenyl propanoate (6b)

mp: 157-158 ºC; yield: 52.7% ; $[\alpha]_D^{20}$ 31.2 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.81-7.79 (m, 1H, ArH), 7.74 (d, *J* = 7.2 Hz, 2H, ArH), 7.49-7.64 (m, 3H, ArH), 7.41-7.44 (m, 2H, ArH), 7.31-7.33 (m, 2H, ArH), 7.17-7.26 (m, 7H, ArH), 7.01-7.02 (m, 2H, ArH), 6.48 (d, 1H, *J* = 8.4 Hz, CONH), 5.19 (d, *J* = 9.0 Hz, 1H, CONH), 4.61 (m, 1H, 4-H), 4.33-4.35 (dd, *J* = 11.4, 3.6 Hz, 1H, 3a-H), 4.14-4.18 (m, 1H, 2-H), 4.03 -4.06 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.06-3.10 (dd, *J* = 14.4, 6.0 Hz, 1H, 1a-H), 2.96-3.00 (dd, *J* = 13.8, 7.2 Hz, 1H, 5a-H), 2.91-2.95 (dd, *J* = 14.4, 8.4 Hz, 1H, 1b-H), 2.87-2.90 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.23 (*S*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(3-bromophenylsulfonamido)-3-phenyl propanoate (6c)

mp: 160-162 ºC; yield: 53.3 %; $[\alpha]_D^{20}$ 15.8 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.78-7.79 (m, 1H, ArH), 7.73 (d, *J* = 7.2 Hz, 2H, ArH), 7.49-7.60 (m, 3H, ArH), 7.41-7.44 (m, 2H, ArH), 7.31-7.34 (m, 2H, ArH), 7.19-7.26 (m, 7H, ArH), 7.03-7.04 (m, 2H, ArH), 6.43 (d, 1H, *J* = 8.4 Hz, CONH), 5.20 (d, *J* = 9.0 Hz, 1H, CONH), 4.58 (m, 1H, 4-H), 4.21-4.26 (m, 2H, 3a-H, 2-H), 4.12-4.14 (dd, *J* = 11.4, 5.4 Hz, 1H, 3b-H), 3.09-3.12 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 2.91-2.98 (m, 2H, 5a-H, 1b-H), 2.83-2.86 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.24 (*S*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(3-bromophenylsulfonamido)-3-phenyl propanoate (6d)

mp: 157-158 ºC; yield: 56.1 %; $[\alpha]_D^{20}$ -32.1 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.81-7.79 (m, 1H, ArH), 7.74 (d, *J* = 7.2 Hz, 2H, ArH), 7.49-7.64 (m, 3H, ArH), 7.41-7.44 (m, 2H, ArH), 7.31-7.33 (m, 2H, ArH), 7.17-7.26 (m, 7H, ArH), 7.01-7.02 (m, 2H, ArH), 6.48 (d, 1H, *J* = 8.4 Hz, CONH), 5.19 (d, *J* = 9.0 Hz, 1H, CONH), 4.61 (m, 1H, 4-H), 4.33-4.35 (dd, *J* = 11.4, 3.6 Hz, 1H, 3a-H), 4.14-4.18 (m, 1H, 2-H), 4.03 -4.06 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.06-3.10 (dd, *J* = 14.4, 6.0 Hz, 1H, 1a-H), 2.96-3.00 (dd, *J* = 13.8, 7.2 Hz, 1H, 5a-H), 2.91-2.95 (dd, *J* = 14.4, 8.4 Hz, 1H, 1b-H), 2.87-2.90 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.25 (*R*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(2-bromophenylsulfonamido)-3-phenyl propanoate (7a)

mp: 157-158 ºC; yield: 46.8 %; $[\alpha]_D^{20}$ -15.9 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); HR-MS: $C_{31}H_{29}BrN_2O_5SNa$, Calcd. 643.0878 $[M+Na]^+$, found: 643.0878; ^1H NMR (600 MHz, $CDCl_3$): δ 7.95-7.97 (m, 1H, ArH), 7.74 (d, *J* = 8.4 Hz, 2H, ArH), 7.61-7.62 (m, 1H, ArH), 7.49-7.52 (m, 1H, ArH), 7.23-7.44 (m, 7H, ArH), 7.16-7.20 (m, 5H, ArH), 7.06-7.08 (m, 2H, ArH), 6.50 (d, 1H, *J* = 8.4 Hz, CONH), 5.55 (d, *J* = 8.4 Hz, 1H, CONH), 4.57 (m, 1H, 4-H), 4.28-4.31 (dd, *J* = 11.4, 3.0 Hz, 1H, 3a-H), 4.20-4.23 (m, 1H, 2-H), 3.98-4.00 (dd, *J* = 11.4, 4.2 Hz, 1H, 3b-H), 3.04-3.11 (m, 2H, 1-H), 2.94-2.97 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.80-2.84 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.6, 167.0, 138.4, 137.0, 135.2, 134.6, 134.1, 133.8, 131.5, 130.8, 129.2×2, 129.0×2, 128.8×2, 128.6×2, 128.4×2, 127.5, 127.5, 127.1×2, 126.7, 120.1, 65.8, 57.5, 49.9, 38.6, 37.2.

4.3.26 (*R*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(2-bromophenylsulfonamido)-3-phenyl propanoate (7b)

mp: 151-153 ºC; yield: 45.6 %; $[\alpha]_D^{20}$ 31.9 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); ^1H NMR (600 MHz, $CDCl_3$): δ 7.97-7.98 (m, 1H, ArH), 7.72 (d, *J* = 7.2 Hz, 2H, ArH), 7.60-7.62 (m, 1H, ArH), 7.50-7.52 (m, 1H, ArH), 7.42-7.45 (m, 2H, ArH), 7.23-7.34 (m, 5H, ArH), 7.14-7.19 (m, 5H, ArH), 7.07-7.08 (m, 2H, ArH), 6.41 (d, 1H, *J* = 7.8 Hz, CONH), 5.59 (d, *J* = 8.4 Hz, 1H, CONH), 4.52 (m, 1H, 4-H), 4.30-4.33 (m, 1H, 2-H), 4.10-4.11 (d, *J* = 4.0 Hz, 1H, 3-H), 3.08-3.12 (dd, *J* = 13.8, 6.6 Hz, 1H, 1a-H), 3.03-3.06 (dd, *J* = 13.8, 6.6 Hz, 1H, 1b-H), 2.88-2.92 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.72-2.76 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.27 (*S*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(2-bromophenylsulfonamido)-3-phenyl propanoate (7c)

mp: 156-158 ºC; yield: 47.8 %; $[\alpha]_D^{20}$ 16.2 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); ^1H NMR (600 MHz, $CDCl_3$): δ 7.95-7.97 (m, 1H, ArH), 7.74 (d, *J* = 8.4 Hz, 2H, ArH), 7.61-7.62 (m, 1H, ArH), 7.49-7.52 (m, 1H, ArH), 7.23-7.44 (m, 7H, ArH), 7.16-7.20 (m, 5H, ArH), 7.06-7.08 (m, 2H, ArH), 6.50 (d, 1H, *J* = 8.4 Hz, CONH), 5.55 (d, *J* = 8.4 Hz, 1H, CONH), 4.57 (m, 1H, 4-H), 4.28-4.31 (dd, *J* = 11.4, 3.0 Hz, 1H, 3a-H), 4.20-4.23 (m, 1H, 2-H), 3.98-4.00 (dd, *J* = 11.4, 4.2 Hz, 1H, 3b-H), 3.04-3.11 (m, 2H, 1-H), 2.94-2.97 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.80-2.84 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.28 (*S*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(2-bromophenylsulfonamido)-3-phenyl propanoate (7d)

mp: 151-152 ºC; yield: 45.5 %; $[\alpha]_D^{20}$ -32.3 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.97-7.98 (m, 1H, ArH), 7.72 (d, *J* = 7.2 Hz, 2H, ArH), 7.60-7.62 (m, 1H, ArH), 7.50-7.52 (m, 1H, ArH), 7.42-7.45 (m, 2H, ArH), 7.23-7.34 (m, 5H, ArH), 7.14-7.19 (m, 5H, ArH), 7.07-7.08 (m, 2H, ArH), 6.41 (d, 1H, *J* = 7.8 Hz, CONH), 5.59 (d, *J* = 8.4 Hz, 1H, CONH), 4.52 (m, 1H, 4-H), 4.30-4.33 (m, 1H, 2-H), 4.10-4.11 (d, *J* = 4.0 Hz, 1H, 3-H), 3.08-3.12 (dd, *J* = 13.8, 6.6 Hz, 1H, 1a-H), 3.03-3.06 (dd, *J* = 13.8, 6.6 Hz, 1H, 1b-H), 2.88-2.92 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.72-2.76 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.29 (*R*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(2, 4-difluorophenylsulfonamido)-3-phenyl propanoate (8a)

mp: 165-166 ºC; yield: 50.9 %; $[\alpha]_D^{20}$ -21.3 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); HR-MS: $C_{31}H_{28}F_2N_2O_5SNa$, Calcd. 601.1585 $[M+Na]^+$, found: 601.1585; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.71-7.75 (m, 3H, ArH), 7.50-7.52 (m, 1H, ArH), 7.42-7.44 (m, 2H, ArH), 7.25-7.34 (m, 3H, ArH), 7.18-7.22 (m, 5H, ArH), 7.06-7.07 (m, 2H, ArH), 6.87-6.90 (m, 1H, ArH), 6.72-6.76 (m, 1H, ArH), 6.45 (d, 1H, *J* = 8.4 Hz, CONH), 5.34 (d, *J* = 8.4 Hz, 1H, CONH), 4.62 (m, 1H, 4-H), 4.31-4.34 (dd, *J* = 11.4, 3.6 Hz, 1H, 3a-H), 4.27-4.31 (m, 1H, 2-H), 4.07-4.10 (dd, *J* = 11.4, 4.2 Hz, 1H, 3b-H), 3.10-3.13 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.97-3.03 (m, 2H, 5a-H, 1b-H), 2.86-2.89 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.7, 167.1, 165.8, 159.4, 136.9, 134.8, 134.0, 131.5, 131.3, 129.2×2, 129.0×2, 128.7×2, 128.7×2, 128.4×2, 127.4, 127.0×2, 126.8, 124.2, 111.5, 106.6, 66.0, 57.4, 49.8, 38.7, 37.2.

4.3.30 (*R*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(2, 4-difluorophenylsulfonamido)-3-phenyl propanoate (8b)

mp: 160-162 ºC; yield: 54.6% ; $[\alpha]_D^{20}$ 42.3 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ7.71-7.76 (m, 3H, ArH), 7.50-7.52 (m, 1H, ArH), 7.42-7.44 (m, 2H, ArH), 7.16-7.33 (m, 8H, ArH), 7.07-7.08 (m, 2H, ArH), 6.84-6.87 (m, 1H, ArH), 6.73-6.76 (m, 1H, ArH), 6.41 (d, 1H, *J* = 8.4 Hz, CONH), 5.42 (d, *J* = 9.0 Hz, 1H, CONH), 4.58 (m, 1H, 4-H), 4.35-4.38 (m, 1H, 2-H), 4.21-4.24 (dd, *J* = 11.4, 4.2 Hz, 1H, 3a-H), 4.13-4.16 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.11-3.14 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 2.99-3.02 (dd, *J* = 13.8, 7.2 Hz, 1H, 1b-H), 2.93-2.97 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.82-2.85 (dd, *J* = 14.4, 7.8 Hz, 1H, 5b-H).

4.3.31 (*S*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(2, 4-difluorophenylsulfonamido)-3-phenyl propanoate (8c)

mp: 166-168 ºC; yield: 56.7 %; $[\alpha]_D^{20}$ 20.9 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.71-7.75 (m, 3H, ArH), 7.50-7.52 (m, 1H, ArH), 7.42-7.44 (m, 2H, ArH), 7.25-7.34 (m, 3H, ArH), 7.18-7.22 (m, 5H, ArH), 7.06-7.07 (m, 2H, ArH), 6.87-6.90 (m, 1H, ArH), 6.72-6.76 (m, 1H, ArH), 6.45 (d, 1H, *J* = 8.4 Hz, CONH), 5.34 (d, *J* = 8.4 Hz, 1H, CONH), 4.62 (m, 1H, 4-H), 4.31-4.34 (dd, *J* = 11.4, 3.6 Hz, 1H, 3a-H), 4.27-4.31 (m, 1H, 2-H), 4.07-4.10 (dd, *J* = 11.4, 4.2 Hz, 1H, 3b-H), 3.10-3.13 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.97-3.03 (m, 2H, 5a-H, 1b-H), 2.86-2.89 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.32 (*S*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(2, 4-difluorophenylsulfonamido)-3-phenyl propanoate (8d)

mp: 161-162 ºC; yield: 54.4% ; $[\alpha]_D^{20}$ -41.9 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.71-7.76 (m, 3H, ArH), 7.50-7.52 (m, 1H, ArH), 7.42-7.44 (m, 2H, ArH), 7.16-7.33 (m, 8H, ArH), 7.07-7.08 (m, 2H, ArH), 6.84-6.87 (m, 1H, ArH), 6.73-6.76 (m, 1H, ArH), 6.41 (d, 1H, *J* = 8.4 Hz, CONH), 5.42 (d, *J* = 9.0 Hz, 1H, CONH), 4.58 (m, 1H, 4-H), 4.35-4.38 (m, 1H, 2-H), 4.21-4.24 (dd, *J* = 11.4, 4.2 Hz, 1H, 3a-H), 4.13-4.16 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.11-3.14 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 2.99-3.02 (dd, *J* = 13.8, 7.2 Hz, 1H, 1b-H), 2.93-2.97 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.82-2.85 (dd, *J* = 14.4, 7.8 Hz, 1H, 5b-H).

4.3.33 (*R*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(2, 6-difluorophenylsulfonamido)-3-phenyl propanoate (9a)

mp: 154-155 ºC; yield: 45.1 %; $[\alpha]_D^{20}$ -24.1 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); HR-MS: $C_{31}H_{28}F_2N_2O_5SNa$, Calcd. 601.1585 $[M+Na]^+$, found: 601.1586; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.2 Hz, 2H, ArH), 7.48-7.51 (m, 1H, ArH), 7.41-7.45 (m, 3H, ArH), 7.23-7.32 (m, 3H, ArH), 7.14-7.20 (m, 5H, ArH), 7.08-7.10 (m, 2H, ArH), 6.87-6.90 (m, 2H, ArH), 6.48 (d, 1H, *J* = 8.4 Hz, CONH), 5.60 (d, *J* = 8.4 Hz, 1H, CONH), 4.59 (m, 1H, 4-H), 4.44-4.47 (m, 1H, 2-H), 4.29-4.32 (dd, *J* = 11.4, 3.6 Hz, 1H, 3a-H), 4.04-4.06 (dd, *J* = 11.4, 4.2 Hz, 1H, 3b-H), 3.13-3.16 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 3.05-3.09 (dd, *J* = 13.8, 7.2 Hz, 1H, 1b-H), 2.94-2.97 (dd, *J* = 13.8, 5.4 Hz, 1H, 5a-H), 2.83-2.87 (dd, *J* = 14.4, 8.4 Hz, 1H, 5b-H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.5, 167.0, 160.1, 158.3, 137.0, 134.7, 134.5, 133.9, 131.5, 129.2×2, 129.0×2, 128.8×2, 128.6×2, 128.4×2, 127.4, 127.0×2, 126.8, 117.8, 113.0, 112.9, 66.0, 57.4, 49.9, 38.7, 37.1.

4.3.34 (*R*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(2, 6-difluorophenylsulfonamido)-3-phenyl propanoate (9b)

mp: 150-152 ºC; yield: 43.8 %; $[\alpha]_D^{20}$ 47.6 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.72 (d, *J* = 7.2 Hz, 2H, ArH), 7.49-7.52 (m, 1H, ArH), 7.37-7.44 (m, 3H, ArH), 7.15-7.32 (m, 8H, ArH), 7.10-7.11 (m, 2H, ArH), 6.84-6.87 (m, 2H, ArH), 6.39 (d, 1H, *J* = 8.4 Hz, CONH), 5.56 (d, *J* = 9.0 Hz, 1H, CONH), 4.50-4.57 (m, 2H, 4-H, 2-H), 4.15-4.18 (dd, *J* = 11.4, 4.2 Hz, 1H, 3a-H), 4.08-4.11 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.13 -3.16 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 3.06-3.09 (dd, *J* = 13.8, 7.2 Hz, 1H, 1b-H), 2.89-2.92 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.76-2.79 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.35 (*S*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(2, 6-difluorophenylsulfonamido)-3-phenyl propanoate (9c)

mp: 154-155 ºC; yield: 46.2 %; $[\alpha]_D^{20}$ 22.3 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.2 Hz, 2H, ArH), 7.48-7.51 (m, 1H, ArH), 7.41-7.45 (m, 3H, ArH), 7.23-7.32 (m, 3H, ArH), 7.14-7.20 (m, 5H, ArH), 7.08-7.10 (m, 2H, ArH), 6.87-6.90 (m, 2H, ArH), 6.48 (d, 1H, *J* = 8.4 Hz, CONH), 5.60 (d, *J* = 8.4 Hz, 1H, CONH), 4.59 (m, 1H, 4-H), 4.44-4.47 (m, 1H, 2-H), 4.29-4.32 (dd, *J* = 11.4, 3.6 Hz, 1H, 3a-H), 4.04-4.06 (dd, *J* = 11.4, 4.2 Hz, 1H, 3b-H), 3.13-3.16 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 3.05-3.09 (dd, *J* = 13.8, 7.2 Hz, 1H, 1b-H), 2.94-2.97 (dd, *J* = 13.8, 5.4 Hz, 1H, 5a-H), 2.83-2.87 (dd, *J* = 14.4, 8.4 Hz, 1H, 5b-H).

4.3.36 (*S*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(2, 6-difluorophenylsulfonamido)-3-phenyl propanoate (9d)

mp: 149-152 ºC; yield: 45.5 %; $[\alpha]_D^{20}$ -45.7 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.72 (d, *J* = 7.2 Hz, 2H, ArH), 7.49-7.52 (m, 1H, ArH), 7.37-7.44 (m, 3H, ArH), 7.15-7.32 (m, 8H, ArH), 7.10-7.11 (m, 2H, ArH), 6.84-6.87 (m, 2H, ArH), 6.39 (d, 1H, *J* = 8.4 Hz, CONH), 5.56 (d, *J* = 9.0 Hz, 1H, CONH), 4.50-4.57 (m, 2H, 4-H, 2-H), 4.15-4.18 (dd, *J* = 11.4, 4.2 Hz, 1H, 3a-H), 4.08-4.11 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.13 -3.16 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 3.06-3.09 (dd, *J* = 13.8, 7.2 Hz, 1H, 1b-H), 2.89-2.92 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.76-2.79 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.37 (*R*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(5-bromo-2-methoxy phenylsulfonamido)-3-phenyl propanoate (10a)

mp: 148-150 ºC; yield: 47.8 %; $[\alpha]_D^{20}$ -34.9 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); HR-MS: $C_{32}H_{31}BrN_2O_6SNa$, Calcd. 673.0984 $[M+Na]^+$, found: 673.0985; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.92 (d, *J* = 2.4 Hz, 1H, ArH), 7.72 (d, *J* = 8.4 Hz, 2H, ArH), 7.57-7.59 (dd, J = 2.4 Hz, 9.0 Hz, 1H,

ArH), 7.50-7.53 (m, 1H, ArH), 7.43-7.45 (m, 2H, ArH), 7.16-7.33 (m, 8H, ArH), 7.08-7.09 (m, 2H, ArH), 6.77 (d, *J* = 8.4 Hz, 1H, ArH), 6.44 (d, 1H, *J* = 8.4 Hz, CONH), 5.46 (d, *J* = 6.6 Hz, 1H, CONH), 4.52 (m, 1H, 4-H), 4.29-4.32 (dd, *J* = 11.4, 3.6 Hz, 1H, 3a-H), 4.21-4.25 (dd, *J* = 13.8, 7.2 Hz, 1H, 2-H), 3.94-3.96 (dd, *J* = 10.8, 3.6 Hz, 1H, 3b-H), 3.73 (s, 3H, 8-H), 3.09-3.12 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 3.03-3.07 (dd, *J* = 13.8, 7.2 Hz, 1H, 1b-H), 2.91-2.95 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.81-2.84 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.9, 167.1, 155.2, 137.3, 137.0, 134.9, 134.1, 132.1, 131.5, 129.2×2, 129.0×2, 128.7×2, 128.6×2, 128.4×2, 128.4, 127.4, 127.0×2, 126.7, 113.8, 112.3, 65.7, 57.4, 56.4, 49.9, 38.9, 37.1.

4.3.38 (*R*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(5-bromo-2-methoxy phenylsulfonamido)-3-phenyl propanoate (10b)

mp: 145-146 ºC; yield: 42.2 %; $[\alpha]_D^{20}$ 69.1 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.94 (d, *J* = 2.4 Hz, 1H, ArH), 7.69 (d, *J* = 7.8 Hz, 2H, ArH), 7.50-7.52 (m, 2H, ArH), 7.42-7.45 (m, 2H, ArH), 7.17-7.33 (m, 8H, ArH), 7.09-7.10 (m, 2H, ArH), 6.75 (d, *J* = 9.0 Hz, 1H, ArH), 6.33 (d, 1H, *J* = 8.4 Hz, CONH), 5.50 (d, *J* = 7.8 Hz, 1H, CONH), 4.50 (m, 1H, 4-H), 4.28-4.31 (dd, *J* = 13.8, 6.6 Hz, 1H, 2-H), 4.08-4.15 (m, 2H, 3-H), 3.76 (s, 3H, 8-H), 3.09-3.12 (dd, *J* = 13.8, 6.6 Hz, 1H, 1a-H), 3.03-3.07 (dd, *J* = 13.8, 6.6 Hz, 1H, 1b-H), 2.89-2.92 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.75-2.78 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.39 (*S*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(5-bromo-2-methoxy phenylsulfonamido)-3-phenyl propanoate (10c)

mp: 148-151 ºC; yield: 47.6 %; $[\alpha]_D^{20}$ 34.6 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.92 (d, *J* = 2.4 Hz, 1H, ArH), 7.72 (d, *J* = 8.4 Hz, 2H, ArH), 7.57-7.59 (dd, J = 2.4 Hz, 9.0 Hz, 1H, ArH), 7.50-7.53 (m, 1H, ArH), 7.43-7.45 (m, 2H, ArH), 7.16-7.33 (m, 8H, ArH), 7.08-7.09 (m, 2H, ArH), 6.77 (d, *J* = 8.4 Hz, 1H, ArH), 6.44 (d, 1H, *J* = 8.4 Hz, CONH), 5.46 (d, *J* = 6.6 Hz, 1H, CONH), 4.52 (m, 1H, 4-H), 4.29-4.32 (dd, *J* = 11.4, 3.6 Hz, 1H, 3a-H), 4.21-4.25 (dd, *J* = 13.8, 7.2 Hz, 1H, 2-H), 3.94-3.96 (dd, *J* = 10.8, 3.6 Hz, 1H, 3b-H), 3.73 (s, 3H, 8-H), 3.09-3.12 (dd, *J* = 13.8, 6.0 Hz, 1H, 1a-H), 3.03-3.07 (dd, *J* = 13.8, 7.2 Hz, 1H, 1b-H), 2.91-2.95 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.81-2.84 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.40 (*S*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(5-bromo-2-methoxy phenylsulfonamido)-3-phenyl propanoate (10d)

mp: 144-145 ºC; yield: 43.4 %; $[\alpha]_D^{20}$ -70.5 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz,

$CDCl_3$): δ 7.94 (d, *J* = 2.4 Hz, 1H, ArH), 7.69 (d, *J* = 7.8 Hz, 2H, ArH), 7.50-7.52 (m, 2H, ArH), 7.42-7.45 (m, 2H, ArH), 7.17-7.33 (m, 8H, ArH), 7.09-7.10 (m, 2H, ArH), 6.75 (d, *J* = 9.0 Hz, 1H, ArH), 6.33 (d, 1H, *J* = 8.4 Hz, CONH), 5.50 (d, *J* = 7.8 Hz, 1H, CONH), 4.50 (m, 1H, 4-H), 4.28-4.31 (dd, *J* = 13.8, 6.6 Hz, 1H, 2-H), 4.08-4.15 (m, 2H, 3-H), 3.76 (s, 3H, 8-H), 3.09-3.12 (dd, *J* = 13.8, 6.6 Hz, 1H, 1a-H), 3.03-3.07 (dd, *J* = 13.8, 6.6 Hz, 1H, 1b-H), 2.89-2.92 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.75-2.78 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.41 (*R*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(2, 5-dimethoxyphenylsulfonamido)-3-phenyl propanoate (11a)

mp: 151-152 ºC; yield: 67.7 %; $[\alpha]_D^{20}$ -36.5 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); HR-MS: $C_{33}H_{34}N_2O_7SNa$, Calcd. 625.1984 $[M+Na]^+$, found: 625.1985; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.72 (d, *J* = 7.2 Hz, 2H, ArH), 7.47-7.49 (m, 1H, ArH), 7.40-7.42 (m, 2H, ArH), 7.14-7.34 (m, 9H, ArH), 7.07-7.08 (m, 2H, ArH), 7.00-7.02 (dd, J = 3.6 Hz, 9.0 Hz, 1H, ArH), 6.81 (d, *J* = 9.0 Hz, 1H, ArH), 6.49 (d, 1H, *J* = 8.4 Hz, CONH), 5.49 (d, *J* = 6.6 Hz, 1H, CONH), 4.50 (m, 1H, 4-H), 4.25-4.28 (dd, *J* = 11.4, 3.0 Hz, 1H, 3a-H), 4.18-4.21 (dd, *J* = 13.8, 6.6 Hz, 1H, 2-H), 3.89-3.91 (dd, *J* = 11.4, 3.6 Hz, 1H, 3b-H), 3.74 (s, 3H, -OCH_3), 3.68 (s, 3H, -OCH_3), 3.06-3.08 (m, 2H, 1-H), 2.89-2.92 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.76-2.79 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.3, 166.9, 152.9, 150.1, 136.9, 134.9, 134.0, 131.5, 129.2×2, 129.2×2, 128.6×2, 128.6×2, 128.5×2, 127.5, 127.3, 127.0×2, 126.7, 120.3, 114.0, 113.5, 65.7, 57.2, 56.6, 55.9, 50.2, 39.0, 37.0.

4.3.42 (*R*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(2, 5-dimethoxyphenylsulfonamido)-3-phenyl propanoate (11b)

mp: 143-144 ºC; yield: 61.2 %; $[\alpha]_D^{20}$ 71.5 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.69 (d, *J* = 7.2 Hz, 2H, ArH), 7.48-7.51 (m, 1H, ArH), 7.40-7.43 (m, 2H, ArH), 7.15-7.36 (m, 9H, ArH), 7.08-7.09 (m, 2H, ArH), 6.95-6.97 (dd, J = 3.0 Hz, 9.0 Hz, 1H, ArH), 6.80 (d, *J* = 9.0 Hz, 1H, ArH), 6.37 (d, 1H, *J* = 8.4 Hz, CONH), 5.53 (d, *J* = 7.2 Hz, 1H, CONH), 4.44 (m, 1H, 4-H), 4.25-4.29 (dd, *J* = 14.4, 7.2 Hz, 1H, 3a-H), 4.06-4.09 (dd, *J* = 11.4, 4.2 Hz, 1H, 2-H), 4.03-4.05 (dd, *J* = 11.4, 4.2 Hz, 1H, 3b-H), 3.72 (s, 3H, -OCH_3), 3.70 (s, 3H, -OCH_3), 3.06-3.09 (dd, *J* = 10.8, 6.6 Hz, 1H, 1a-H), 3.03-3.06 (dd, *J* = 10.8, 6.6 Hz, 1H, 1b-H), 2.84-2.88 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.68-2.72 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.43 (*S*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(2, 5-dimethoxyphenylsulfonamido)-3-phenyl

propanoate (11c)

mp: 150-153 ºC; yield: 64.5 %; $[\alpha]_D^{20}$ 35.1 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.72 (d, *J* = 7.2 Hz, 2H, ArH), 7.47-7.49 (m, 1H, ArH), 7.40-7.42 (m, 2H, ArH), 7.14-7.34 (m, 9H, ArH), 7.07-7.08 (m, 2H, ArH), 7.00-7.02 (dd, J = 3.6 Hz, 9.0 Hz, 1H, ArH), 6.81 (d, *J* = 9.0 Hz, 1H, ArH), 6.49 (d, 1H, *J* = 8.4 Hz, CONH), 5.49 (d, *J* = 6.6 Hz, 1H, CONH), 4.50 (m, 1H, 4-H), 4.25-4.28 (dd, *J* = 11.4, 3.0 Hz, 1H, 3a-H), 4.18-4.21 (dd, *J* = 13.8, 6.6 Hz, 1H, 2-H), 3.89-3.91 (dd, *J* = 11.4, 3.6 Hz, 1H, 3b-H), 3.74 (s, 3H, -OCH_3), 3.68 (s, 3H, -OCH_3), 3.06-3.08 (m, 2H, 1-H), 2.89-2.92 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.76-2.79 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.44 (*S*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(2，5-dimethoxyphenylsulfonamido)-3-phenyl propanoate (11d)

mp: 144-145 ºC; yield: 67.1 %; $[\alpha]_D^{20}$ -71.2 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.69 (d, *J* = 7.2 Hz, 2H, ArH), 7.48-7.51 (m, 1H, ArH), 7.40-7.43 (m, 2H, ArH), 7.15-7.36 (m, 9H, ArH), 7.08-7.09 (m, 2H, ArH), 6.95-6.97 (dd, J = 3.0 Hz, 9.0 Hz, 1H, ArH), 6.80 (d, *J* = 9.0 Hz, 1H, ArH), 6.37 (d, 1H, *J* = 8.4 Hz, CONH), 5.53 (d, *J* = 7.2 Hz, 1H, CONH), 4.44 (m, 1H, 4-H), 4.25-4.29 (dd, *J* = 14.4, 7.2 Hz, 1H, 3a-H), 4.06-4.09 (dd, *J* = 11.4, 4.2 Hz, 1H, 2-H), 4.03-4.05 (dd, *J* = 11.4, 4.2 Hz, 1H, 3b-H), 3.72 (s, 3H, -OCH_3), 3.70 (s, 3H, -OCH_3), 3.06-3.09 (dd, *J* = 10.8, 6.6 Hz, 1H, 1a-H), 3.03-3.06 (dd, *J* = 10.8, 6.6 Hz, 1H, 1b-H), 2.84-2.88 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.68-2.72 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.45 (*R*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(2，4-dimethoxyphenylsulfonamido)-3-phenyl propanoate (12a)

mp: 153-154 ºC; yield: 69.1 %; $[\alpha]_D^{20}$ -28.3 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); HR-MS: $C_{33}H_{34}N_2O_7SNa$, Calcd. 625.1984 $[M+Na]^+$, found: 625.1984; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.69-7.73 (m, 3H, ArH), 7.40-7.49 (m, 3H, ArH), 7.07-7.29 (m, 10H, ArH), 6.35-6.54 (m, 2H, ArH), 6.43 (d, 1H, *J* = 7.8 Hz, CONH), 5.35 (d, *J* = 6.0 Hz, 1H, CONH), 4.49 (m, 1H, 4-H), 4.29-4.31 (dd, *J* = 10.8, 2.4 Hz, 1H, 3a-H), 4.09-4.12 (dd, *J* = 13.2, 6.6 Hz, 1H, 2-H), 3.85-3.88 (dd, *J* = 11.4, 3.0 Hz, 1H, 3b-H), 3.80 (s, 3H, -OCH_3), 3.67 (s, 3H, -OCH_3), 3.05 (m, 2H, 1-H), 2.88-2.91 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.76-2.79 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.2, 167.0, 165.0, 157.7, 137.2, 135.1, 134.2, 131.6, 131.4, 129.2×2, 129.1×2, 128.7×2, 128.6×2, 128.4×2, 127.4, 127.1×2, 126.7, 118.6, 104.0, 99.3, 65.4, 57.5, 56.0,

55.6, 50.0, 38.9, 37.0.

4.3.46 (*R*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(2, 4-dimethoxyphenylsulfonamido)-3-phenyl propanoate (12b)

mp: 148-149ºC; yield: 60.8 %; $[\alpha]_D^{20}$ 57.2 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.62-7.65 (m, 3H, ArH), 7.34-7.44 (m, 3H, ArH), 7.02-7.22 (m, 10H, ArH), 6.28-6.29 (m, 2H, ArH), 6.33 (d, 1H, *J* = 7.8 Hz, CONH), 5.34 (d, *J* = 7.2 Hz, 1H, CONH), 4.38 (m, 1H, 4-H), 4.12-4.15 (dd, *J* = 13.8, 7.2 Hz, 1H, 3a-H), 3.94-3.97 (dd, *J* = 11.4, 4.2 Hz, 1H, 2-H), 3.88-3.90 (dd, *J* = 11.4, 3.0 Hz, 1H, 3b-H), 3.71 (s, 3H, -OCH_3), 3.64 (s, 3H, -OCH_3), 2.97 (d, *J* = 6.6 Hz, 2H, 1-H), 2.71-2.75 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.57-2.61 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.47 (*S*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(2, 4-dimethoxyphenylsulfonamido)-3-phenyl propanoate (12c)

mp: 150-152 ºC; yield: 59.8 %; $[\alpha]_D^{20}$ 27.2 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.69-7.73 (m, 3H, ArH), 7.40-7.49 (m, 3H, ArH), 7.07-7.29 (m, 10H, ArH), 6.35-6.54 (m, 2H, ArH), 6.43 (d, 1H, *J* = 7.8 Hz, CONH), 5.35 (d, *J* = 6.0 Hz, 1H, CONH), 4.49 (m, 1H, 4-H), 4.29-4.31 (dd, *J* = 10.8, 2.4 Hz, 1H, 3a-H), 4.09-4.12 (dd, *J* = 13.2, 6.6 Hz, 1H, 2-H), 3.85-3.88 (dd, *J* = 11.4, 3.0 Hz, 1H, 3b-H), 3.80 (s, 3H, -OCH_3), 3.67 (s, 3H, -OCH_3), 3.05 (m, 2H, 1-H), 2.88-2.91 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.76-2.79 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.48 (*S*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(2, 4-dimethoxyphenylsulfonamido)-3-phenyl propanoate (12d)

mp: 146-147 ºC; yield: 63.4 %; $[\alpha]_D^{20}$ -56.3 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.62-7.65 (m, 3H, ArH), 7.34-7.44 (m, 3H, ArH), 7.02-7.22 (m, 10H, ArH), 6.28-6.29 (m, 2H, ArH), 6.33 (d, 1H, *J* = 7.8 Hz, CONH), 5.34 (d, *J* = 7.2 Hz, 1H, CONH), 4.38 (m, 1H, 4-H), 4.12-4.15 (dd, *J* = 13.8, 7.2 Hz, 1H, 3a-H), 3.94-3.97 (dd, *J* = 11.4, 4.2 Hz, 1H, 2-H), 3.88-3.90 (dd, *J* = 11.4, 3.0 Hz, 1H, 3b-H), 3.71 (s, 3H, -OCH_3), 3.64 (s, 3H, -OCH_3), 2.97 (d, *J* = 6.6 Hz, 2H, 1-H), 2.71-2.75 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.57-2.61 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.49 (*R*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(2-nitro-4-(trifuoromethyl)phenylsulfonamido)-3-phenyl propanoate (13a)

mp: 141-142 ºC; yield: 54.3 %; $[\alpha]_D^{20}$ -25.3 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); HR-MS:

$C_{32}H_{28}F_3N_3O_7SNa$, Calcd. 678.1498 $[M+Na]^+$, found: 678.1498; ^1H NMR (600 MHz, $CDCl_3$): δ 7.93 (s, 1H, ArH), 7.93 (d, *J* = 7.8 Hz, 1H, ArH), 7.79 (d, *J* = 7.8 Hz, 1H, ArH), 7.70 (d, *J* = 7.2 Hz, 2H, ArH), 7.47-7.50 (m, 1H, ArH), 7.39-7.41 (m, 2H, ArH), 7.21-7.33 (m, 5H, ArH), 7.03-7.06 (m, 5H, ArH), 6.37 (d, 1H, *J* = 7.8 Hz, CONH), 6.03 (d, *J* = 4.2 Hz, 1H, CONH), 4.64 (m, 1H, 4-H), 4.40-4.43 (dd, *J* = 13.2, 7.8 Hz, 1H, 3a-H), 4.26-4.29 (dd, *J* = 10.8, 2.4 Hz, 1H, 2-H), 4.14-4.17 (dd, *J* = 10.8, 4.2 Hz, 1H, 3b-H), 3.19-3.22 (dd, *J* = 13.8, 4.8 Hz, 1H, 1a-H), 2.94-2.99 (m, 2H, 1b-H, 5a-H), 2.87-2.91 (dd, *J* = 13.8, 7.2 Hz, 1H, 1b-H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.4, 167.1, 147.1, 137.4, 136.6, 134.9, 133.8, 131.7, 130.8, 129.7, 129.7, 129.1×2, 129.0×2, 128.7×2, 128.6×4, 127.4, 126.9×2, 122.8, 122.6, 121.1, 66.3, 58.4, 49.7, 38.3, 37.2.

4.3.50 (*R*)-(*R*)-2-Benzamido-3-phenylpropyl

2-(2-nitro-4-(trifuoromethyl)phenylsulfonamido)-3-phenyl propanoate (13b)

mp: 136-138 ºC; yield: 50.3 %; $[\alpha]_D^{20}$ 49.4 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^1H NMR (600 MHz, $CDCl_3$): δ 7.97 (s, 1H, ArH), 7.91 (d, *J* = 7.8 Hz, 1H, ArH), 7.78 (d, *J* = 8.4 Hz, 1H, ArH), 7.71 (d, *J* = 8.4 Hz, 2H, ArH), 7.48-7.51 (m, 1H, ArH), 7.41-7.43 (m, 2H, ArH), 7.21-7.32 (m, 5H, ArH), 7.02-7.06 (m, 5H, ArH), 6.39 (d, 1H, *J* = 8.4 Hz, CONH), 6.02 (d, *J* = 9.0 Hz, 1H, CONH), 4.62 (m, 1H, 4-H), 4.49-4.53 (dd, *J* = 13.8, 9.0 Hz, 1H, 3a-H), 4.26-4.28 (dd, *J* = 10.8, 3.6 Hz, 1H, 2-H), 4.14-4.17 (dd, *J* = 10.8, 4.8 Hz, 1H, 3b-H), 3.20-3.23 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.87-2.99 (m, 3H, 1b-H, 5-H).

4.3.51 (*S*)-(*R*)-2-Benzamido-3-phenylpropyl

2-(2-nitro-4-(trifuoromethyl)phenylsulfonamido)-3-phenyl propanoate (13c)

mp: 143-144 ºC; yield: 56.8 %; $[\alpha]_D^{20}$ 25.1 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^1H NMR (600 MHz, $CDCl_3$): δ 7.93 (s, 1H, ArH), 7.93 (d, *J* = 7.8 Hz, 1H, ArH), 7.79 (d, *J* = 7.8 Hz, 1H, ArH), 7.70 (d, *J* = 7.2 Hz, 2H, ArH), 7.47-7.50 (m, 1H, ArH), 7.39-7.41 (m, 2H, ArH), 7.21-7.33 (m, 5H, ArH), 7.03-7.06 (m, 5H, ArH), 6.37 (d, 1H, *J* = 7.8 Hz, CONH), 6.03 (d, *J* = 4.2 Hz, 1H, CONH), 4.64 (m, 1H, 4-H), 4.40-4.43 (dd, *J* = 13.2, 7.8 Hz, 1H, 3a-H), 4.26-4.29 (dd, *J* = 10.8, 2.4 Hz, 1H, 2-H), 4.14-4.17 (dd, *J* = 10.8, 4.2 Hz, 1H, 3b-H), 3.19-3.22 (dd, *J* = 13.8, 4.8 Hz, 1H, 1a-H), 2.94-2.99 (m, 2H, 1b-H, 5a-H), 2.87-2.91 (dd, *J* = 13.8, 7.2 Hz, 1H, 1b-H).

4.3.52 (*S*)-(*S*)-2-Benzamido-3-phenylpropyl

2-(2-nitro-4-(trifuoromethyl)phenylsulfonamido)-3-phenyl propanoate (13d)

mp: 136-137 ºC; yield: 51.8 %; $[\alpha]_D^{20}$ -52.2 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^1H NMR (600 MHz,

$CDCl_3$): δ 7.97 (s, 1H, ArH), 7.91 (d, *J* = 7.8 Hz, 1H, ArH), 7.78 (d, *J* = 8.4 Hz, 1H, ArH), 7.71 (d, *J* = 8.4 Hz, 2H, ArH), 7.48-7.51 (m, 1H, ArH), 7.41-7.43 (m, 2H, ArH), 7.21-7.32 (m, 5H, ArH), 7.02-7.06 (m, 5H, ArH), 6.39 (d, 1H, *J* = 8.4 Hz, CONH), 6.02 (d, *J* = 9.0 Hz, 1H, CONH), 4.62 (m, 1H, 4-H), 4.49-4.53 (dd, *J* = 13.8, 9.0 Hz, 1H, 3a-H), 4.26-4.28 (dd, *J* = 10.8, 3.6 Hz, 1H, 2-H), 4.14-4.17 (dd, *J* = 10.8, 4.8 Hz, 1H, 3b-H), 3.20-3.23 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.87-2.99 (m, 3H, 1b-H, 5-H).

4.3.53 (*R*)-(*S*)-2-Benzamido-3-phenylpropyl

2-(4-nitro-3-(trifuoromethyl)phenylsulfonamido)-3-phenyl propanoate (14a)

mp: 160-162 ºC; yield: 54.7 %; $[\alpha]_D^{20}$ -27.4 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); HR-MS: $C_{32}H_{28}F_3N_3O_7SNa$, Calcd. 678.1498 $[M+Na]^+$, found: 678.1499; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.67-7.75 (m, 3H, ArH), 7.41-7.51 (m, 4H, ArH), 7.07-7.33 (m, 11H, ArH), 6.47 (d, 1H, *J* = 7.2 Hz, CONH), 4.98 (d, *J* = 6.6 Hz, 1H, CONH), 4.57 (m, 1H, 4-H), 4.19-4.22 (dd, *J* = 11.4, 6.0 Hz, 1H, 3a-H), 4.14-4.17 (dd, *J* = 10.8, 4.2 Hz, 1H, 3b-H), 4.09-4.11 (dd, *J* = 11.4, 3.0 Hz, 1H, 2-H), 3.07 (d, *J* = 6.0 Hz, 2H, 1-H), 2.92-2.96 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.81-2.85 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7, 167.45, 149.62, 144.3, 136.3, 135.1, 133.8, 131.8, 131.4, 129.1×2, 129.0×2, 128.8×2, 128.7×2, 128.6×2, 127.4, 127.1, 126.9×2, 126.5, 126.5, 125.5, 124.2, 66.8, 57.9, 49.7, 38.4, 37.2.

4.3.54 (*R*)-(*R*)-2-Benzamido-3-phenylpropyl

2-(4-nitro-3-(trifuoromethyl)phenylsulfonamido)-3-phenyl propanoate (14b)

mp: 158-160 ºC; yield: 56.6 %; $[\alpha]_D^{20}$ 55.2 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74-7.75 (m, 3H, ArH), 7.41-7.51 (m, 4H, ArH), 7.14-7.31 (m, 11H, ArH), 6.46 (d, 1H, *J* = 7.8 Hz, CONH), 4.98 (d, *J* = 6.6 Hz, 1H, CONH), 4.57 (m, 2H, 4-H, 3a-H), 4.31 (m, 1H, 3b-H), 4.09-4.11 (dd, *J* = 11.4, 3.0 Hz, 1H, 2-H), 3.07 (d, *J* = 6.6 Hz, 2H, 1-H), 2.93-2.96 (dd, *J* = 13.8, 6.0 Hz, 1H, 5a-H), 2.77-2.81 (dd, *J* = 13.8, 6.4 Hz, 1H, 5b-H).

4.3.55 (*S*)-(*R*)-2-Benzamido-3-phenylpropyl

2-(4-nitro-3-(trifuoromethyl)phenylsulfonamido)-3-phenyl propanoate (14c)

mp: 163-167 ºC; yield: 50.0 %; $[\alpha]_D^{20}$ 27.8 (*c* 0.4 $CHCl_3$/MeOH=1:1， v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.67-7.75 (m, 3H, ArH), 7.41-7.51 (m, 4H, ArH), 7.07-7.33 (m, 11H, ArH), 6.47 (d, 1H, *J* = 7.2 Hz, CONH), 4.98 (d, *J* = 6.6 Hz, 1H, CONH), 4.57 (m, 1H, 4-H), 4.19-4.22 (dd, *J* = 11.4, 6.0 Hz, 1H, 3a-H), 4.14-4.17 (dd, *J* = 10.8, 4.2 Hz, 1H, 3b-H), 4.09-4.11 (dd, *J* = 11.4, 3.0 Hz, 1H,

2-H), 3.07 (d, *J* = 6.0 Hz, 2H, 1-H), 2.92-2.96 (dd, *J* = 13.8, 6.6 Hz, 1H, 5a-H), 2.81-2.85 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.56 (*S*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(4-nitro-3-(trifuoromethyl)phenylsulfonamido)-3-phenyl propanoate (14d)

mp: 155-156 ºC; yield: 59.0 %; $[\alpha]_D^{20}$ -56.4 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74-7.75 (m, 3H, ArH), 7.41-7.51 (m, 4H, ArH), 7.14-7.31 (m, 11H, ArH), 6.46 (d, 1H, *J* = 7.8 Hz, CONH), 4.98 (d, *J* = 6.6 Hz, 1H, CONH), 4.57 (m, 2H, 4-H, 3a-H), 4.31 (m, 1H, 3b-H), 4.09-4.11 (dd, *J* = 11.4, 3.0 Hz, 1H, 2-H), 3.07 (d, *J* = 6.6 Hz, 2H, 1-H), 2.93-2.96 (dd, *J* = 13.8, 6.0 Hz, 1H, 5a-H), 2.77-2.81 (dd, *J* = 13.8, 6.4 Hz, 1H, 5b-H).

4.3.57 (*R*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(5-chloro-2, 4-difluorophenylsulfonamido)-3-phenyl propanoate (15a)

mp: 163-165 ºC; yield: 58.3 %; $[\alpha]_D^{20}$ -16.8 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); HR-MS: $C_{31}H_{27}ClF_2N_2O_5SNa$, Calcd. 635.1195 $[M+Na]^+$, found: 635.1195; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.76-7.79 (m, 1H, ArH), 7.73 (d, *J* = 7.2 Hz, 2H, ArH), 7.50-7.52 (m, 1H, ArH), 7.41-7.44 (m, 2H, ArH), 7.17-7.34 (m, 8H, ArH), 7.04-7.06 (m, 2H, ArH), 6.80-6.83 (m, 1H, ArH), 6.40 (d, 1H, *J* = 8.4 Hz, CONH), 5.40 (d, *J* = 9.0 Hz, 1H, CONH), 4.64 (m, 1H, 4-H), 4.30-4.35 (m, 2H, 3a-H, 2-H), 4.13-4.16 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.11-3.14 (dd, *J* = 14.4, 5.4 Hz, 1H, 1a-H), 2.95-3.01 (m, 2H, 1b-H, 5a-H), 2.88-2.91 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.5, 167.1, 160.7, 157.4, 136.8, 134.8, 133.9, 131.6, 131.0, 129.2×2, 129.0×2, 128.7×2, 128.7×2, 128.5×2, 127.4, 127.0×2, 126.9, 125.2, 117.1, 106.7, 66.2, 57.5, 49.8, 38.7, 37.3.

4.3.58 (*R*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(5-chloro-2, 4-difluorophenylsulfonamido)-3-phenyl propanoate (15b)

mp: 155-157 ºC; yield: 60.9 %; $[\alpha]_D^{20}$ 33.5 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.76-7.79 (m, 1H, ArH), 7.70 (d, *J* = 7.2 Hz, 2H, ArH), 7.49-7.52 (m, 1H, ArH), 7.41-7.43 (m, 2H, ArH), 7.17-7.34 (m, 8H, ArH), 7.06-7.07 (m, 2H, ArH), 6.81-6.84 (m, 1H, ArH), 6.37 (d, 1H, *J* = 8.4 Hz, CONH), 5.49 (d, *J* = 9.0 Hz, 1H, CONH), 4.62 (m, 1H, 4-H), 4.38-4.42 (m, 1H, 2-H), 4.27-4.29 (dd, *J* = 14.4, 4.8 Hz, 1H, 3a-H), 4.16-4.19 (dd, *J* = 14.4, 5.4 Hz, 1H, 3b-H), 3.13-3.17 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.96-2.99 (m, 2H, 1b-H, 5a-H), 2.86-2.89 (dd, *J* = 13.8, 7.2 Hz, 1H, 5b-H).

4.3.59 (*S*)-(*R*)-2-Benzamido-3-phenylpropyl 2-(5-chloro-2, 4-difluorophenylsulfonamido)-3-phenyl propanoate (15c)

mp: 161-164 ºC; yield: 56.9 %; $[\alpha]_D^{20}$ 17.2 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.76-7.79 (m, 1H, ArH), 7.73 (d, *J* = 7.2 Hz, 2H, ArH), 7.50-7.52 (m, 1H, ArH), 7.41-7.44 (m, 2H, ArH), 7.17-7.34 (m, 8H, ArH), 7.04-7.06 (m, 2H, ArH), 6.80-6.83 (m, 1H, ArH), 6.40 (d, 1H, *J* = 8.4 Hz, CONH), 5.40 (d, *J* = 9.0 Hz, 1H, CONH), 4.64 (m, 1H, 4-H), 4.30-4.35 (m, 2H, 3a-H, 2-H), 4.13-4.16 (dd, *J* = 11.4, 4.8 Hz, 1H, 3b-H), 3.11-3.14 (dd, *J* = 14.4, 5.4 Hz, 1H, 1a-H), 2.95-3.01 (m, 2H, 1b-H, 5a-H), 2.88-2.91 (dd, *J* = 13.8, 7.8 Hz, 1H, 5b-H).

4.3.60 (*S*)-(*S*)-2-Benzamido-3-phenylpropyl 2-(5-chloro-2, 4-difluorophenylsulfonamido)-3-phenyl propanoate (15d)

mp: 153-157 ºC; yield: 61.1 %; $[\alpha]_D^{20}$ 34.4 (*c* 0.4 $CHCl_3$/MeOH=1:1，v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.76-7.79 (m, 1H, ArH), 7.70 (d, *J* = 7.2 Hz, 2H, ArH), 7.49-7.52 (m, 1H, ArH), 7.41-7.43 (m, 2H, ArH), 7.17-7.34 (m, 8H, ArH), 7.06-7.07 (m, 2H, ArH), 6.81-6.84 (m, 1H, ArH), 6.37 (d, 1H, *J* = 8.4 Hz, CONH), 5.49 (d, *J* = 9.0 Hz, 1H, CONH), 4.62 (m, 1H, 4-H), 4.38-4.42 (m, 1H, 2-H), 4.27-4.29 (dd, *J* = 14.4, 4.8 Hz, 1H, 3a-H), 4.16-4.19 (dd, *J* = 14.4, 5.4 Hz, 1H, 3b-H), 3.13-3.17 (dd, *J* = 13.8, 5.4 Hz, 1H, 1a-H), 2.96-2.99 (m, 2H, 1b-H, 5a-H), 2.86-2.89 (dd, *J* = 13.8, 7.2 Hz, 1H, 5b-H).

4.4 Molecular Docking

AutoDock 4 software was applied to predict the binding patterns of investigated molecules. The crystal structures of cysteine protease Cat L (PDB code: 2XU4) and S (PDB code: 3OVX) were chosen in docking calculations. Structures of **TSA** and **6a** were built and minimized with Accelrys Discovery Studio 3.0 software package, with flexible torsions assigned to allow all dihedral angles to freely rotate. We used Lamarckian genetic algorithm to define the optimized parameters, as follows: the maximum number of energy evaluations per run was increased to 25 000 000, the iterations of Solis &Wets local search were set as 3000, the number of individuals in population was set as 300, and the number of generations was set as 100. The generated poses differing by < 2 Å in a positional root mean square deviation (RMSD) were divided into one cluster, which the conformer with lowest binding energy and highest percentage frequency was selected as the result representative.

4.5 Cell culture

Human breast cancer MDA-MB-231 cells were cultured in L15 medium supplemented with 10% FBS, 1 mM-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin under the normal culture conditions. Human breast cancer MCF-7 cells were cultured in DMEM medium supplemented with 10% FBS, 1 mM-glutamine, 100 U/ml penicillin and 100 μg/mL streptomycin at routine culture conditions.

4.6 MTT assay

MDA-MB-231 or MCF-7 cells cultured in 96-well plates were stimulated by different concentrations of **6a** or **E64d** for 72 h, and MTT solution was then added and incubated for 4 h. After discarding the supernatant and adding 100 μL DMSO, the plates were read by a ELISA reader at 570 nm. IC_{50} values were obtained by the Statistical Product and Service Solutions software.

4.7 Enzyme binding inhibition assay

Cathepsin L, S, B and K were diluted to 4 nM, 10 nM, 2 nM and 2 nM with the buffer solution, respectively. The activity of cathepsin K and L was detected in pH 5.5 Mes-NaOH buffer solution (including 2.5 mM DTT, 2.5 mM EDTA and 10% DMSO), and 20μM Z-Phe-Arg-AMC was used as substrate. Cathepsin B activity assay was carried out in pH 6.0 Mes-NaOH buffer solution (including 2.5 mM DTT, 2.5 mM EDTA, 0.001% Tween 20 and 10% DMSO), and 20 μM Z-Phe-Arg-AMC was used as substrate. Cathepsin S activity assay was carried out in pH 6.5 Mes-NaOH buffer solution (including 100 mM NaCl, 2.5 mM DTT, 2.5 mM EDTA, 0.001% BSA and 10% DMSO), and 20 μM Z-VVR-AMC was used as substrate. The enzyme was mixed with or without 1μL of different concentrations of the samples or **E64-d** in 96-well plate. After incubating at 37 °C for 30 minutes, 50 μL of fluorescent substrate was added and the fluorescence intensity of each well was measured by automatic microplate reader in kinetic mode for 6 min (excitation wavelength 380 nm, emission wavelength 460 nm). A curve was generated using the fluorescence intensity of cleaved substrate versus unit time (minute), and two time points in the liner range of the sample curve were used for the calculation of the slope of curve. Enzyme activity and enzyme binding inhibition can be observed by comparing the slope of curve.

4.8 Cell migration and invasion determination by wound healing assay and transwell chamber assay

4.8.1 Wound healing assay.

The cells were grown in 6-well plates for the wound-healing assay. After confluency was obtained, the cells were scratched with a pipette tip, rinsed to remove debris, and incubated in a fresh medium in the presence or absence of compound **6a** or **E64d** for 48 h. Cell migration images were captured at 0 and 48 h. The wound healing index was determined as a percentage and quantitatively analyzed as follows: (1- [current wound size/initial wound size]) *100%.

4.8.2 Transwell chamber assay.

Transwell chamber assay was performed using 24-well Matrigel invasion chambers (BD Biosciences). The cells were trypsinized and re-seeded in the upper chamber at a concentration of $1*10^5$/ mL in 200 μL of the medium in the presence or absence of **6a** or **E64d**. The lower chamber contained 500 μL of the medium supplemented with 10% FBS. After 24 h, the cells on the upper surface of the filters were removed, and the cells on the lower surface were fixed with methanol and stained with crystal violet.

4.9 MDC staining assay for autophagy detection.

MDA-MB-231 cells cultured in 24-well plate were treated with various concentrations of **6a** or 0.1% DMSO for 72 h, and the cells were then stained with 50 μM MDC for 1 h at 37 °C. After washing with PBS, the pictures were taken using an inverted fluorescent microscope to detect the acidic vesicular organelle formation.

4.10 Western blot analysis.

Western blot was performed as decribed in our published paper [14]. In brief, 20 μg of protein extracts from **6a**-treated MDA-MB-231 cells were separated by 10% SDS-PAGE and transferred onto PVDF membranes. The PVDF membranes were incubated with primary antibodies, and then treated with HRP-conjugated secondary antibody. Blots were finally detected using electrochemiluminescence.

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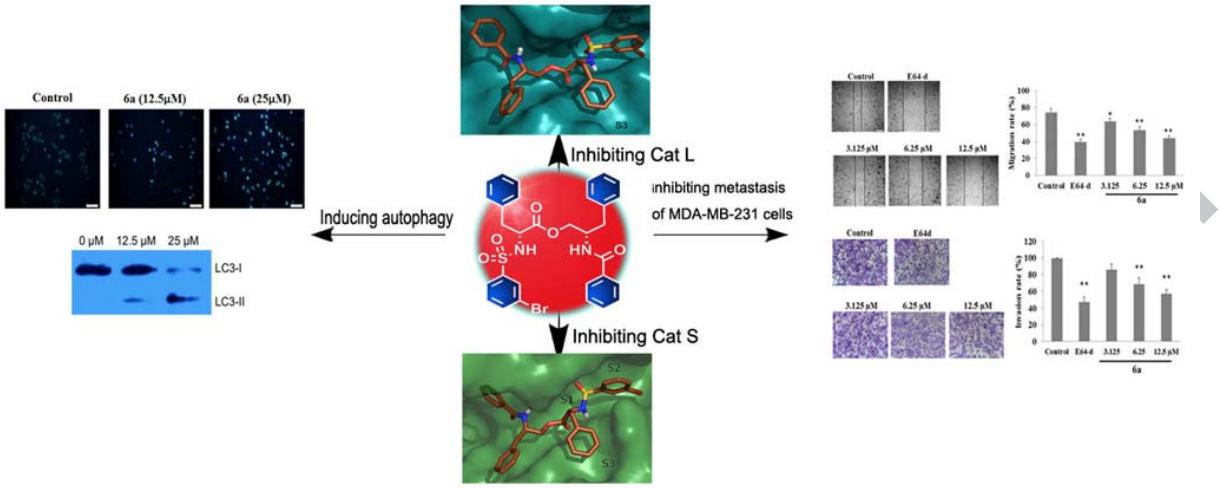
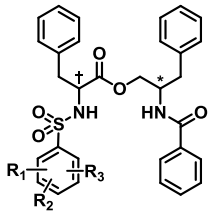


- **TSA** was chosen as lead compound whose A-ring tolyl moiety was replaced with other substituted-phenyl sulfonyl groups. And sixty optically active derivatives were obtained.
- Cathepsin inhibitory activity assay showed that compound **6a** with *meta*-bromo substituent displayed the greatest inhibitory activity whose inhibitory capability against Cat L and S was 3.9 and 11.5-fold more potent than that of **TSA**, respectively.
- MDC staining experiment and western blot analysis validated that **6a** induced the autophagy in MDA-MB-231 cells.
- The metastatic inhibitory ability of **6a** were also confirmed by wound healing and transwell chamber experiment.
- Compound **6a** displayed dual anti-tumor effects by induction of autophagy and inhibition of metastasis in breast cancer cells.

Table 1. Inhibitory activities of **TSA** and all synthetic derivatives against cathepsins L, S, K and B.



Compound	Substituent			IC_{50} (μM)[a]				Configuration
	R1	R2	R3	Cat L	Cat S	Cat B	Cat K	
1a	4-Br	H	H	14.37±1.27	>100	>100	>100	*(R, S)*
1b				>100	>100	>100	>100	*(R, R)*
1c				>100	>100	>100	>100	*(S, R)*
1d				>100	>100	>100	>100	*(S, S)*
2a	4-OMe	H	H	>100	>100	>100	>100	*(R, S)*
2b				>100	>100	>100	>100	*(R, R)*
2c				>100	>100	>100	>100	*(S, R)*
2d				>100	>100	>100	>100	*(S, S)*
3a	4-CF_3	H	H	20.06±2.11	>100	>100	>100	*(R, S)*
3b				>100	>100	>100	>100	*(R, R)*
3c				>100	>100	>100	>100	*(S, R)*
3d				>100	>100	>100	>100	*(S, S)*
4a	4-F	H	H	10.24±2.08	25.27±2.33	>100	>100	*(R, S)*
4b				>100	>100	>100	>100	*(R, R)*
4c				>100	>100	>100	>100	*(S, R)*
4d				>100	>100	>100	>100	*(S, S)*
5a	H	H	H	>100	>100	>100	>100	*(R, S)*
5b				>100	>100	>100	>100	*(R, R)*
5c				>100	>100	>100	>100	*(S, R)*
5d				>100	>100	>100	>100	*(S, S)*
6a	3-Br	H	H	7.66±0.83	6.94±0.67	>100	>100	*(R, S)*
6b				>100	>100	>100	>100	*(R, R)*
6c				>100	>100	>100	>100	*(S, R)*
6d				>100	>100	>100	>100	*(S, S)*
7a	2-Br	H	H	>100	>100	>100	>100	*(R, S)*
7b				>100	>100	>100	>100	*(R, R)*
7c				>100	>100	>100	>100	*(S, R)*
7d				>100	>100	>100	>100	*(S, S)*
8a	2-F	4-F	H	61.42±6.99	>100	>100	>100	*(R, S)*
8b				>100	>100	>100	>100	*(R, R)*
8c				>100	>100	>100	>100	*(S, R)*
8d				>100	>100	>100	>100	*(S, S)*

9a	2-F	6-F	H	>100	>100	>100	>100	*(R, S)*
9b				>100	>100	>100	>100	*(R, R)*
9c				>100	>100	>100	>100	*(S, R)*
9d				>100	>100	>100	>100	*(S, S)*
10a	2-OMe	5-Br	H	12.34±2.57	32.15±3.18	>100	>100	*(R, S)*
10b				>100	>100	>100	>100	*(R, R)*
10c				>100	>100	>100	>100	*(S, R)*
10d				>100	>100	>100	>100	*(S, S)*
11a	2-OMe	5-OMe	H	53.12	>100	>100	>100	*(R, S)*
11b				40.64	>100	>100	>100	*(R, R)*
11c				>100	>100	>100	>100	*(S, R)*
11d				>100	>100	>100	>100	*(S, S)*
12a	2-OMe	4-OMe	H	18.49	>100	>100	>100	*(R, S)*
12b				16.68	>100	>100	>100	*(R, R)*
12c				>100	>100	>100	>100	*(S, R)*
12d				>100	>100	>100	>100	*(S, S)*
13a	$2\text{-}NO_2$	$4\text{-}CF_3$	H	>100	>100	>100	>100	*(R, S)*
13b				66.39	>100	>100	>100	*(R, R)*
13c				>100	>100	>100	>100	*(S, R)*
13d				>100	>100	>100	>100	*(S, S)*
14a	$4\text{-}NO_2$	$3\text{-}CF_3$	H	>100	>100	>100	>100	*(R, S)*
14b				23.18	>100	>100	>100	*(R, R)*
14c				>100	>100	>100	>100	*(S, R)*
14d				>100	>100	>100	>100	*(S, S)*
15a	2-F	4-F	5-Cl	25.06±1.80	48.98±5.49	>100	>100	*(R, S)*
15b				>100	>100	>100	>100	*(R, R)*
15c				>100	>100	>100	>100	*(S, R)*
15d				>100	>100	>100	>100	*(S, S)*
TSA[b]	$4\text{-}CH_3$	H	H	30.2±1.12	79.68±1.13	>100	>100	*(R, S)*

[a] Data were shown as mean ± SD from three independent experiments.

[b] Used as a positive control.