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Novel Mercapto Propionamide Derivatives with Potent New Delhi Metallo-β-Lactamase-1 (NDM-1) Inhibitory Activity and Low Toxicity

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The emergence and worldwide prevalence of New Delhi metallo-β-lactamase 1 (NDM-1) expressing Gram-negative bacteria with resistance against most β-lactam antibiotics pose a serious threat to human health. However, no NDM-1 inhibitors are clinically approved at present. Herein, based on the lead compound captopril, a series of compounds were designed, synthesized and evaluated for NDM-1 inhibitory activities. All designed compounds showed single digit

micromolar or submicromolar NDM-1 inhibitory activities, which were much more potent than that of captopril. Among them, compounds **14a** and **14m** exhibited excellent NDM-1 inhibitory activities, with IC_{50} values of 0.10 and 0.12 μM, respectively. Further studies demonstrated that compound **14m** displayed low cytotoxicity, good water solubility, high metabolic stability and low acute toxicity in mice. Importantly, compound **14m** exhibited potent synergistic antimicrobial activities with meropenem (MEM) for the treatment of clinically isolated NDM-1-expressing strains.

Keywords: NDM-1, Gram-negative bacteria, resistance, inhibitors, captopril, inhibitory activities, meropenem

Antibiotics play a crucial role in the treatment of infectious disease.[1] However, the emergence of antimicrobial resistance (AMR) presents a threat to public health worldwide: even worse, it has been exacerbated by the abuse of antibiotics.[2,3] Carbapenem-resistant Enterobacteriaceae (CRE) consists of multiple Gram-negative bacteria that exhibit AMR to many antibiotics.[4,5] CRE infections have become a serious threat to human health and have caused the eventual death of half of the patients who receive CRE-associated bloodstream infections.[6,7] A list of AMR "priority pathogens" recently published by the World Health Organization (WHO) demonstrated that CRE are among the most critical infection-causing bacteria that urgently necessitate the development of new antibiotics.[8]

One of the main resistance mechanisms of CRE is the generation of β-lactamases, which can be divided into two types on the basis of their catalytic mechanisms.[9] One type is the serine-β-lactamases (SBLs; classes A, C and D); these SBLs hydrolyze β-lactam rings by a serine nucleophilic mechanism. The other type is the metallo-β-lactamases (MBLs; class B); MBLs

utilize active-site zinc to coordinate a nucleophilic hydroxide to breakdown the β-lactam ring.[10] Combination therapy has been identified as a more economical and effective option compared to monotherapy, and it has been widely adopted in clinical practice.[11,12] Several SBL inhibitors, such as clavulanic acid, sulbactam, tazobactam and avibactam, have been used in combination with appropriate β-lactam antibiotics to treat infections caused by SBL-expressing bacteria.[13-15] In contrast, Me1071 (CP-3242) was the only MBL inhibitor in phase I/II clinical trials for the treatment of bacterial infection. However, no recent development has been reported for this compound.[16]

According to amino acid sequence and metal ion features, MBLs could be further grouped into three subclasses (*i.e.*, B1, B2 and B3).[17] Among the B1 subfamily, Imipenemase-1 (IMP-1), Verona integron-encoded MBL (VIM) and New Delhi metallo-β-lactamase-1 (NDM-1) are the most prevalent MBLs observed in the clinic.[18] In particular, NDM-1 represents one of the most troublesome MBLs. NDM-1 can inactivate nearly all β-lactam antibiotics, including carbapenems, which are known as the "last resort" for antibiotic treatment.[19] Additionally, NDM-1 has been widely expressed by a variety of clinically relevant Gram-negative species and spreads worldwide. Furthermore, the NDM-1 gene is carried on easily transmissible plasmids that contribute to its wide spread, and plasmids with the NDM-1 gene are often relevant to other MBL genes.[18,20,21] To overcome the prevalence of AMR caused by NDM-1, a number of inhibitors have been discovered over the last decade, such as the natural product aspergillomarasmine A (AMA),[22] metal chelator tris-picolylamine (TPA) derivative,[3] 2,6-dipicolinic acid (DPA),[18] cyclic boronate,[23] covalent inhibitor cefaclor,[24] triazolylthioacetamide,[21] rhodanine derivative ML302[25] and L-captopril[26] (**Figure 1**).

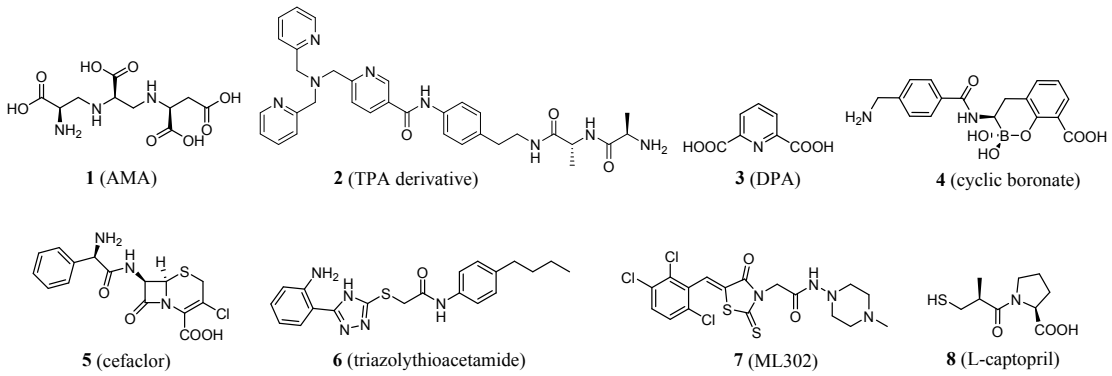


Figure 1. Representative NDM-1 inhibitors

Among the reported NDM-1 inhibitors, sulfur-containing compounds were found to be one of the most promising categories, exemplified by the anti-hypertension drug captopril.[27-29] Both L-captopril and D-captopril demonstrated only moderate NDM-1 inhibitory activity, with IC_{50} values of 202.0 μM and 7.9 μM, respectively.[30] However, very limited studies designing and testing captopril analogues as NDM-1 inhibitors were reported in the literature.[31-37] Importantly, L-captopril was successfully cocrystallized with NDM-1 (PDB: 4EXS), which laid the foundation for the rational design of more potent NDM-1 inhibitors.[38] The crystal structure of L-captopril with NDM-1 reveals that the thiol group could coordinate to zinc ions and that the carboxyl group could form hydrogen bond with Asn220, suggesting that both thiol and carboxyl groups were pivotal for NDM-1 inhibitory activities and should be maintained in the new designed analogues. Additionally, the active site of NDM-1 contains a large hydrophobic domain and no obvious interactions of the pyrrolidine ring in captopril with NDM-1 were observed, implying that the pyrrolidine ring may not be essential for NDM-1 inhibitory activities (**Figure 2A, Figure 2B**).[38] This hydrophobic channel of the active domain is a long corridor which might be suitable for chain-like large substituents. Thus, different bulky amino acids could be introduced to maintain hydrogen bonds by carboxylic acids with protein residues (such as Asn220, Ile210) and increase the hydrophobic interactions. Meanwhile, due to the relatively large hydrophobic domain near the

methyl group (**Figure 2B**), diversified aromatic rings were simultaneously imported to enhance the hydrophobic interactions with protein residues in the active site of NDM-1 (**Figure 2C**). Taken together, twenty-nine novel NDM-1 inhibitors were designed and synthesized, and their biological activities were evaluated.

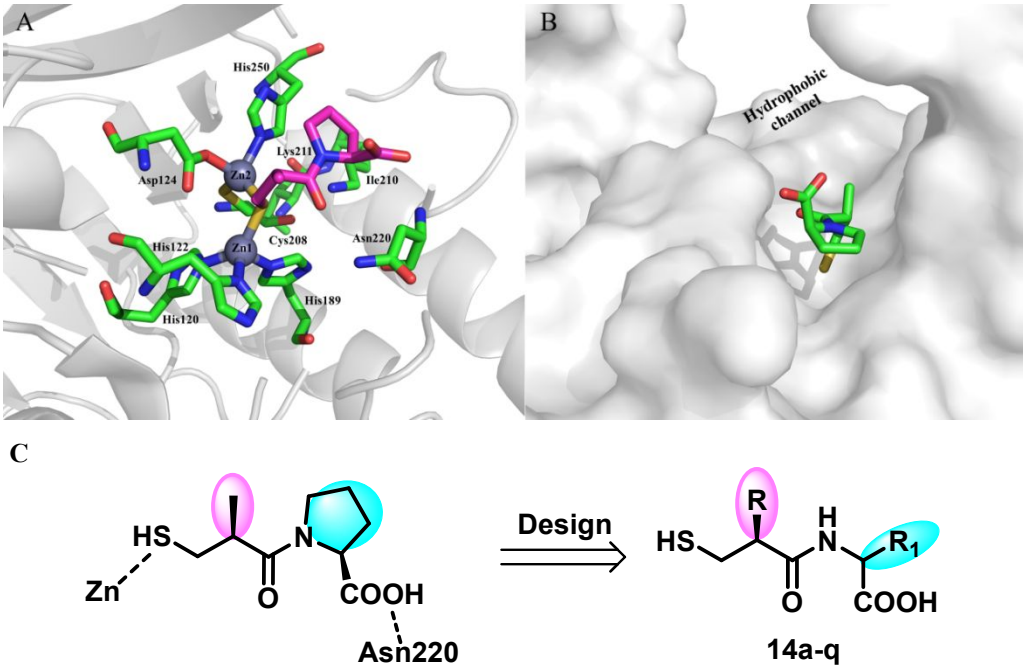
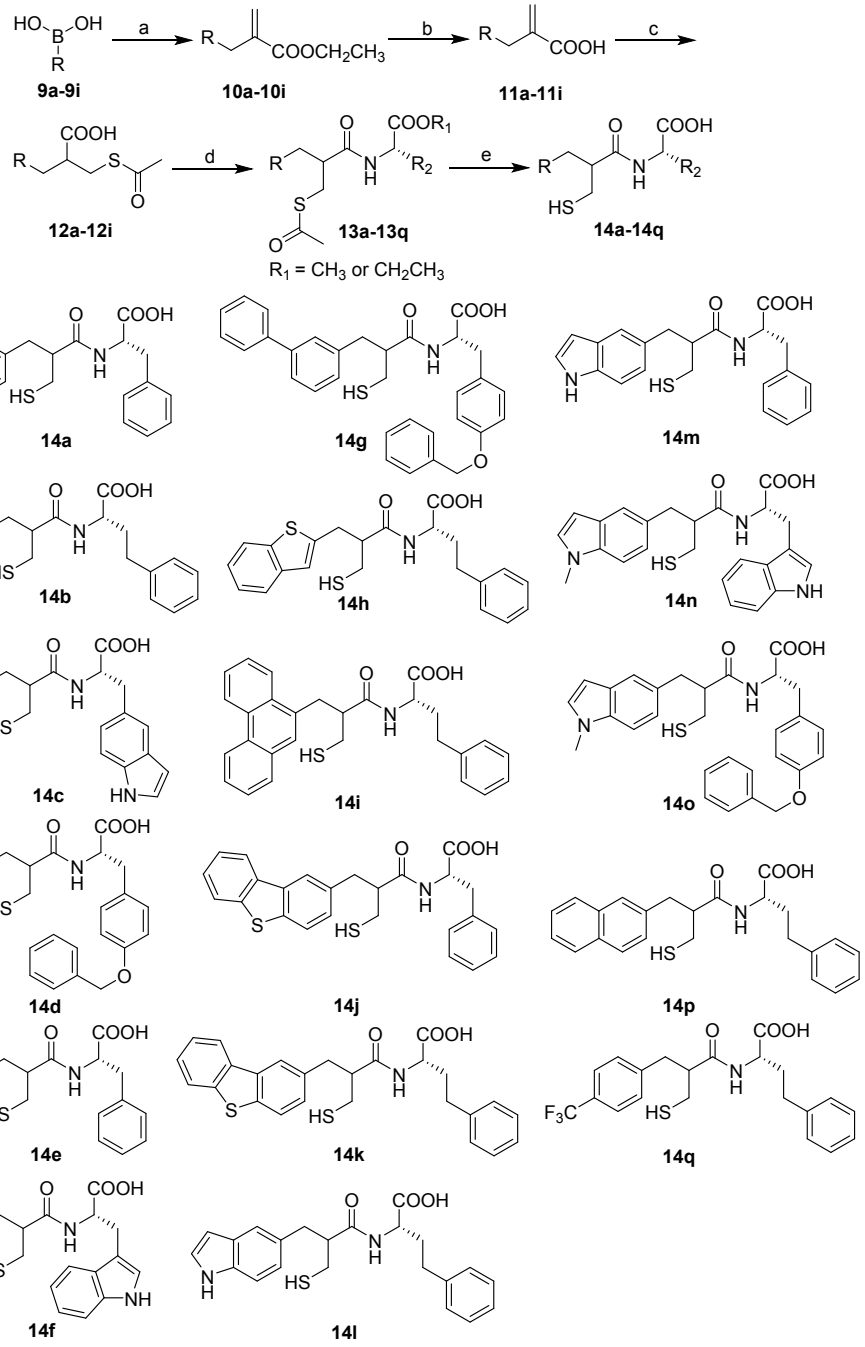


Figure 2. (A and B) Binding modes of L-captopril in the active site of NDM-1(PDB code: 4EXS). (C) Design of novel NDM-1 inhibitors **14a-q**.

RESULTS AND DISCUSSION

Chemistry. Starting from the commercially available boronic acids and ethyl 2-(bromomethyl)-acrylate, intermediates **10a-i** were formed via a Suzuki coupling reaction based on the reported methods[39] prior to hydrolysis with aqueous NaOH to afford the acrylic derivatives **11a-i.** Next**,** compounds **11a-i** were reacted with thioacetic acid via Michael addition reaction to obtain intermediates **12a-i,** which were coupled with various methyl or ethyl amino acid esters to yield their corresponding mercapto propionamide derivatives **13a-q**. Finally, these esters **13a-q** were subsequently hydrolyzed to obtain target compounds **14a-q** (**Scheme 1**).

Scheme 1. Synthesis of compounds 14a-q[α]

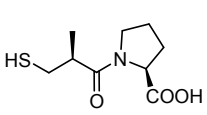
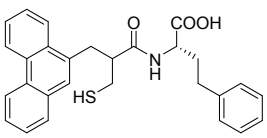


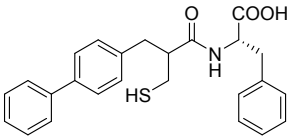
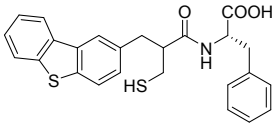
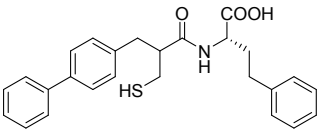
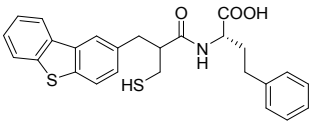
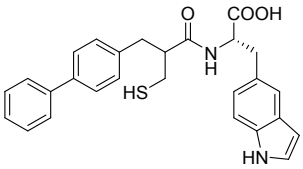
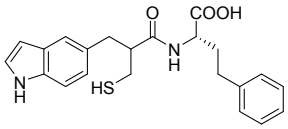
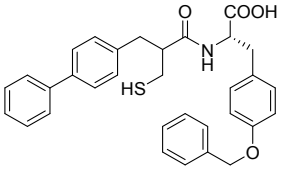
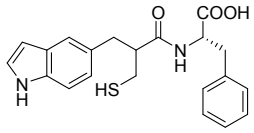
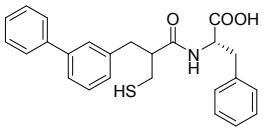
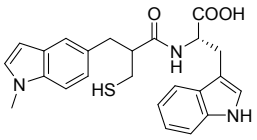
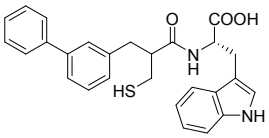
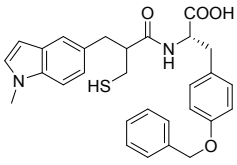
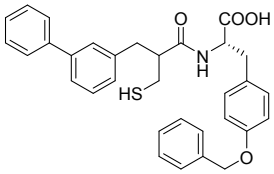
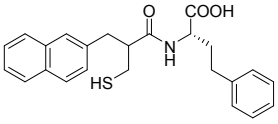
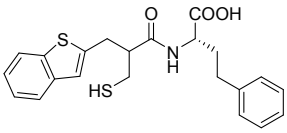
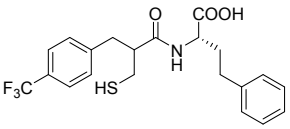
[α]Reagents and conditions: (a) ethyl 2-(bromomethyl)acrylate, $Pd(TFA)_2$, KOH, H_2O, 90 °C, 3 h, 47-72% yields; (b) THF-3 M aqueous NaOH (1:6), 90 °C, 5 h, 95-100% yields; (c) thioacetic acid, Et_3N, CH_2Cl_2, N_2, rt, 30 h, 38-57% yields; (d) amino acid methyl/ethyl ester, EDCI, HOBt, *N*-methylmorpholine, CH_2Cl_2, rt, overnight; (e) THF-3 M aqueous NaOH (1:6), N_2, rt, 10 h,

21-63% yields (two steps).

NDM-1 Inhibitory Activities. Initially, diastereomers **14a-q** were tested against NDM-1 by the fluorescence-based assay using L-captopril as a positive control.[18,26,40] As shown in **Table 1**, all compounds showed single digit micromolar or submicromolar activity against NDM-1 and much more potent activity than L-captopril, indicating that most of the substitution patterns (*i.e.*, L-phenylalanine, L-homophenylalanine or L-tryptophan at the R_1 position and biphenyl, indole, or 2-benzo[b]thiophene at the R position) were well tolerated. L-Phenylalanine analogue **14a** was 19-fold more potent than its corresponding O-benzyl-L-tyrosine analog **14d**. In general, all compounds bearing O-benzyl-L-tyrosine at the R_1 position (**14d**, **14 g** and **14o**) were shown to exhibit relatively weak activity in this series. One possible reason for this is that the hydrophobic pocket at the R_1 position was smaller and deeper. When the R_1 group was substituted with tryptophan, all compounds (**14c**, **14n** and **14f**) exhibited good NDM-1 inhibitory activity, with IC_{50} values of 0.98, 0.22 and 0.45 μM, respectively. If the R group was substituted by a bulkier group (*i.e.*, phenanthrene or dibenzo[b,d]thiophene), compounds **14i-k** demonstrated some degree of decreased activity. Overall, compounds **14a** and **14m** exhibited the most potent NDM-1 inhibitory activity in this series, with IC_{50} values of 0.10 μM and 0.12 μM, respectively.

Table 1. NDM-1 Inhibitory Activities of Target Compounds **14a-q.**

ID	Chemical structure	IC_{50} (μM)	ID	Chemical structure	IC_{50} (μM)
8		208.60 ± 12.01	**14i**		1.78 ± 0.59

14a		0.10 ± 0.05	**14j**		2.66 ± 0.79
14b		0.18 ± 0.02	**14k**		1.33 ± 0.35
14c		0.98 ± 0.10	**14l**		1.30 ± 0.24
14d		1.90 ± 0.49	**14m**		0.12 ± 0.02
14e		0.39 ± 0.07	**14n**		0.45 ± 0.06
14f		0.22 ± 0.09	**14o**		6.28 ± 0.18
14g		2.41 ± 0.47	**14p**		0.78 ± 0.13
14h		0.55 ± 0.27	**14q**		3.90 ± 0.23

It was interesting to further separate the diastereomers of these analogues. Compounds **14a**, **14b**, **14e**, **14g**, **14j** and **14m** were chosen to be purified again by preparative TLC to obtain their counterparts. Their optical rotations were measured by a polarimeter. To determine their absolute

configurations, a single crystal of compound **14j-2** was cultivated, and X-ray crystallographic analysis showed that the absolute configuration of **14j-2** was ***R***, ***S*** (**Figure 3**). Meanwhile, the stereochemistry of compound **14j-1** was identified as ***S***, ***S***. Furthermore, the absolute configurations of compounds **14m-1** and **14m-2** were confirmed by electrostatic circular dichroism (ECD) analyses.[41] The theoretical calculated ECD curves of (***S***,***S***)-**14m** and (***R***,***S***)-**14m** were consistent with the experimental ECD curves of **14m-1** and **14m-2**, respectively (**Figure 4**). Therefore, the stereochemical configurations of **14m-1** and **14m-2** were identified as **(*S*,*S*)-14m-1** and **(*R*,*S*)-14m-2**, respectively. Finally, by the comparison of specific rotations or experimental ECD curves of compounds **14a**, **14b**, **14e** and **14g** to those of compounds **14j-1**, **14j-2**, **14m-1** and **14m-2**, the absolute configurations of diastereomers were also determined (**Table S1** and **Figure S2**).

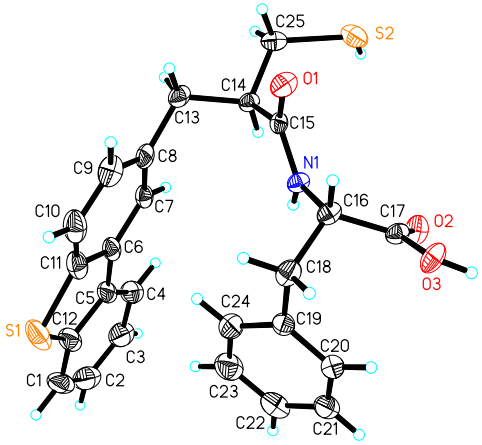


Figure 3. X-ray crystallographic analysis of **14j-2**

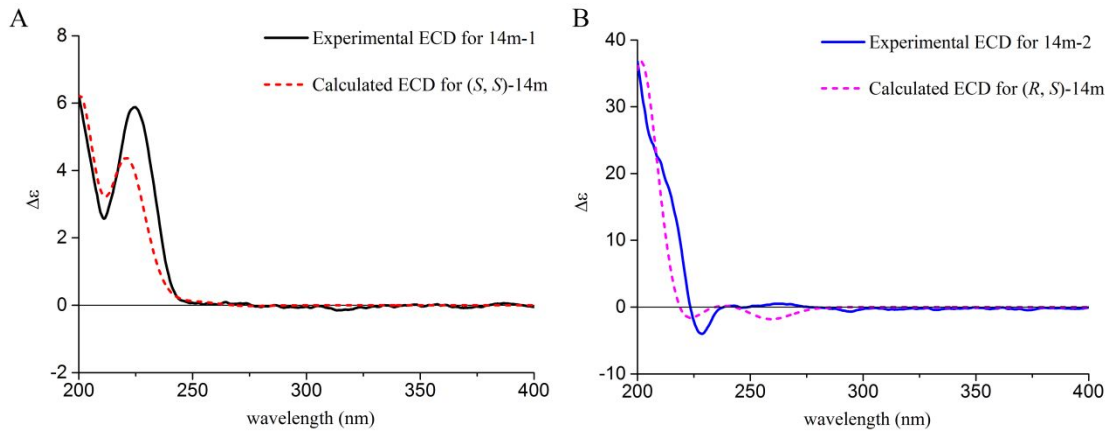
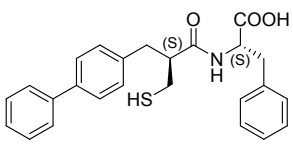
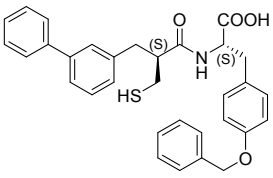
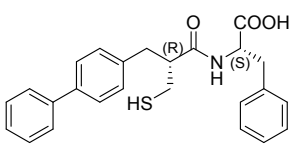
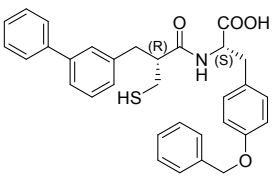
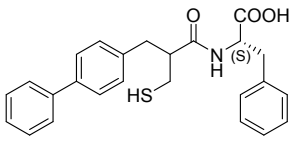
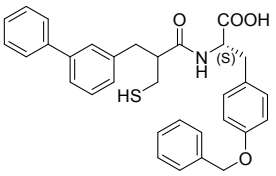
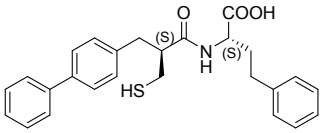
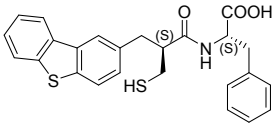
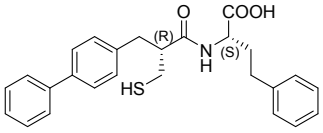
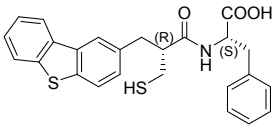
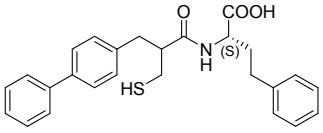
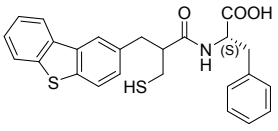
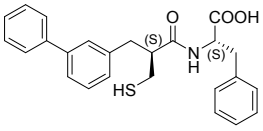
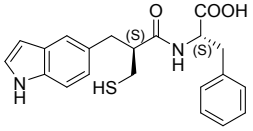
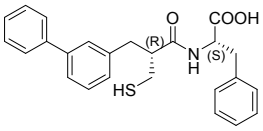
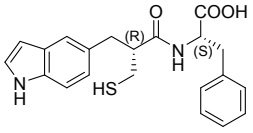
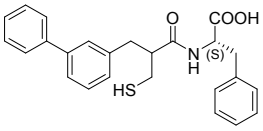
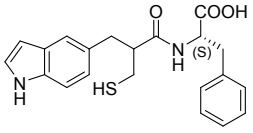


Figure 4. (A) Experimental ECD spectra of **14m-1** and calculated ECD spectra of (***S***,***S***)-**14m**. (B) Experimental ECD spectra of **14m-2** and calculated ECD spectra of (***R***,***S***)-**14m**.

As shown in **Table 2**, the two most potent compounds, **14a** and **14m**, displayed similar NDM-1 inhibitory activities to their diastereomeric counterparts, (***R***,***S***)-**14a-2** and (***R***,***S***)-**14m-2**, respectively, and were more potent than their corresponding (***S***,***S***)-**14a-1** and (***S***,***S***)-**14m-1** diastereomer. A similar trend could be found within compounds **14b**, **14e**, **14g** and **14j**. Therefore, diastereomers were chosen for follow-up studies.

Table 2. Comparison of NDM-1 Inhibitory Activities of Compounds **14a, 14b, 14e, 14g, 14j** and **14m** with Their Corresponding Diastereomers

ID	Chemical structure	IC_{50} (μM)	ID	Chemical structure	IC_{50} (μM)
14a-1		0.47 ± 0.12	**14g-1**		3.11 ± 0.54
14a-2		0.16 ± 0.04	**14g-2**		1.67 ± 0.43

14a		0.10 ± 0.05	**14g**		2.41 ± 0.47
14b-1		0.33 ± 0.11	**14j-1**		5.78 ± 0.61
14b-2		0.16 ± 0.07	**14j-2**		2.07 ± 0.19
14b		0.18 ± 0.02	**14j**		2.66 ± 0.79
14e-1		1.31 ± 0.15	**14m-1**		0.48 ± 0.06
14e-2		0. 46 ± 0.09	**14m-2**		0.17 ± 0.05
14e		0.39 ± 0.07	**14m**		0.12 ± 0.02

Cytotoxicity and Water Solubility. Cytotoxicity is an important aspect in defining reliable inhibitors.[27,36] Taking account of the inhibition of NDM-1 and structural diversity, the toxicities of compounds **14a**, **14b**, **14h**, **14i**, **14l**, **14m** and **14p** to HUVEC were measured using the CCK-8 assay.[42] As shown in **Figure 5**, the IC_{50} values of all tested compounds showed inhibitory activity above 200 μM, which preliminarily suggested that the toxicities of these compounds to HUVEC were very weak. To further examine the cytotoxicity of compound **14m**, cell viability and the

release of lactate dehydrogenase (LDH) from HUVEC, L02 and HEK293 cells were measured at concentrations of 100 and 200 μM, respectively. As shown in **Figure 6A**, the growth of three cultured human cell lines was barely influenced by compound **14m** at the concentration of 100 μM, and the compound only slightly impacted cell proliferation even at 200 μM. In the LDH release assay, compound **14m** did not increase the amount of LDH release at all but could decrease the LDH level (**Figure 6B**). These results implied that the cytotoxicity of compound **14m** was quite low. In addition, the aqueous solubility values of compounds **14a** and **14m** were measured by reported methods,[42] and the results showed that the water solubility of compound **14m** (800.3 μM) was far superior to compound **14a** (15.6 μM).

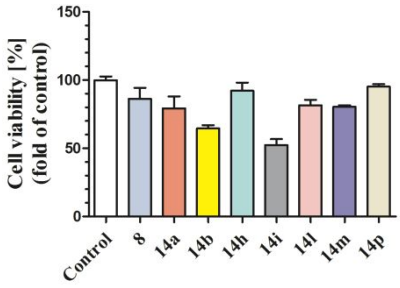


Figure 5. HUVEC viability when treated by different compounds at the concentration of 200 μM.

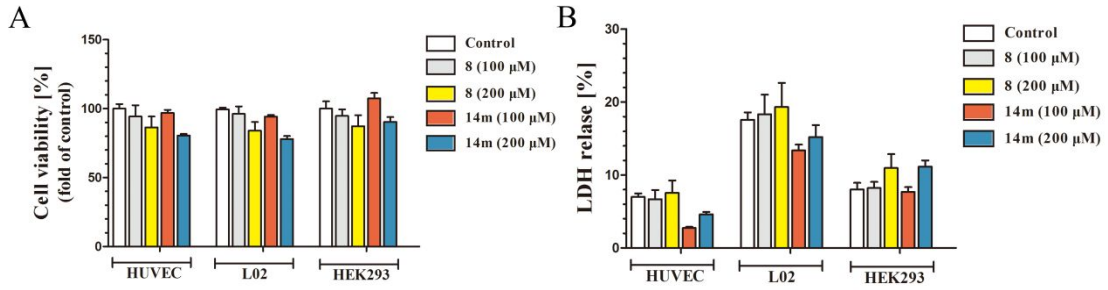


Figure 6. Cell viability (**A**) and LDH release (**B**) of HUVEC, L02 and HEK293 cells treated by **8** and **14m** at concentrations of 100 and 200 μM.

Microdilution Broth Minimum Inhibitory Concentrations (MICs). To determine the synergistic activities of compound **14m** in combination with carbapenem class antibiotics to

resistant clinical strains expressing bla_{NDM-1}, the microdilution broth MICs of compound **14m** and meropenem (MEM) were measured on NDM-1 positive clinical isolates of K. *pneumoniae* and E. *coli* (**Table 3**). The fractional inhibitory concentration index (FICI), which evaluated the degree of interaction between antibiotics and adjuvant, was calculated by adding the following two fractional values: (MIC compound A in combination/MIC of compound A alone) + (MIC compound B in combination/MIC of compound B alone), and the synergistic interaction was determined by FICI ≤0.5.[43] The NDM-1-harboring isolates were identified to be resistant to MEM, with MICs ≥64 μg/mL, which was far larger than the breakpoint value (8 μg/mL) for MEM against *Enterobacteriaceae* as defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[44] Inhibitor **14m** itself displayed little to no influence on the growth of the tested *K. pneumoniae* (MICs >128 μg/mL) and *E. coli* (MIC >64 μg/mL) isolates. Except *K. pneumoniae*-13443, the MIC data of MEM for most of the tested strains were decreased when compound **14m** was used in combination, suggesting that **14m** could enhance the susceptibility of clinically resistant strains expressing bla_{NDM-1} to MEM. Of particular note, the MIC values of MEM against NDM-1-producing *E. coli* and *K. pneumoniae*-2470 isolate were reduced from 64 to 8 μg/mL and from >128 to 32 μg/mL, respectively. Meanwhile, corresponding FICIs were calculated to be <0.375 and <0.5, indicating the potent synergistic interaction between compound **14m** and MEM. Furthermore, the inhibitory concentrations of compound **14m** to all three cultured human cell lines (HUVEC, L02 and HEK293) were higher than 200 μM (76.4 μg/mL). This means that the cell viabilities of these cells were not influenced by compound **14m** at 100 μM (38.2 μg/mL), which was higher than the concentrations (16 μg/mL, 32 μg/mL) relevant to the MIC assay. Thus, compound **14m** exhibited an obvious synergism effect with

MEM toward some of the clinically isolated NDM-1-producing strains when applied within the safe concentration range, and the concentrations used in MIC studies were lower than those of most of the previously reported NDM-1 inhibitors.[18,25,31,32]

Table 3. Microdilution Broth MICs (μg/mL) of MEM Tested Alone or in Combination with Compound **14m** Against Clinical *K. pneumoniae* and *E. coli* Strains Expressing bla_{NDM-1}.

Isolates	MIC [a] (fold change [b])	
	MEM	MEM + **14m**
K. pneumoniae-2470	>128	32 (>4)
K. pneumoniae-2472	>128	64 (>2)
K. pneumoniae-13443	>128	>128
E. coli	64	8 (8)

[a]Compound **14m,** in combination with MEM, was tested at 32 and 16 μg/mL for K. *pneumoniae* and E. *coli*, respectively. [b]The fold change was defined as the MIC of MEM used alone divided by the MIC of MEM used in combination with compound **14m**.

Metabolic Stability in the Presence of Human Liver Microsomes. The in vitro metabolic stability of compound **14m** was measured using the human liver microsomes (HLM) assay. As shown in **Table 4**, compound **14m** displayed high metabolic stability with the half-life of 135.5 min, which was far superior to the reference substances. After 60 min, 70.4% of compound **14m** remained in this HLM assay. The intrinsic clearance (CL) of **14m** was 9.2 mL/min/kg.

Table 4. In Vitro Metabolic Stability of Compound **14m** in the Presence of Human Liver Microsomes

Compound	HLM (final concentration: 0.5 mg protein/mL)				
	R^2	$T_{1/2}$[a] (min)	$CL_{int(liver)}$[b] (mL/min/kg)	Remaining (T = 60 min)	Remaining[c] (NCF = 60 min)
14m	0.9346	135.5	9.2	70.4%	88.3%
Testosterone	0.9687	13.4	93.4	4.4%	88.1%

Diclofenac	0.9796	10.5	118.2	1.9%	91.7%
Propafenone	0.9828	7.7	162.1	0.6%	99.8%

[b]Half-time. [b]Intrinsic clearance. [c]No co-factor.

In Vivo Acute Toxicity. Acute toxicity of compound **14m** in vivo was determined by a single-dose intravenous administration and intraperitoneal injection in ICR mice. Compound **14m** was injected into mice at doses of 50, 100, 200, 300 and 400 mg/kg, respectively, and survival data were recorded until two weeks after administration. As shown in **Table 5**, almost all of the mice survived in the intraperitoneal injection group, except for one mouse that was injected with compound **14m** at the highest concentration (400 mg/kg). Furthermore, the median lethal dose (LD_{50}) value of the intravenous injection group was calculated to be 350.1 mg/kg. Thus, these results suggested that **14m** possessed a low acute toxicity and was well tolerated in mice.

Table 5. Acute Toxicity of Compound **14m** in Mice

Dose (mg/kg)	No. of mice	Intraperitoneal injection		Intravenous injection	
		No. of dead mice	Survival	No. of dead mice	Survival
50	8	0	100%	0	100%
100	8	0	100%	0	100%
200	8	0	100%	0	100%
300	8	0	100%	1	87.5%
400	8	1	87.5%	7	12.5%

CONCLUSION

In summary, the present study provided the design and synthesis of twenty-nine novel compounds based on the lead compound captopril. All designed compounds showed single digit micromolar or submicromolar NDM-1 inhibitory activities, which were much more potent than that of captopril. Among them, compounds **14a** and **14m** exhibited excellent NDM-1 inhibitory activities, with IC_{50} values of 0.10 and 0.12 μM, respectively. Further studies demonstrated that

compound **14m** displayed low cytotoxicity, good water solubility, high metabolic stability and low acute toxicity in mice. Importantly, compound **14m** exhibited strong synergistic antimicrobial activities with MEM for the treatment of clinically isolated NDM-1-expressing strains. The SAR studies of captopril demonstrated that the methyl and proline fragments were not essential and could be replaced by large hydrophobic substituents that can improve NDM-1 inhibitory activities. According to the results, compound **14m** could represent a promising lead compound for developing clinically useful NDM-1 inhibitors. Further structural optimization, mechanistic studies and biological evaluations are in progress.

EXPERIMENTAL SECTION

Chemistry

Unless otherwise noted, all reagents and solvents were purchased from commercial sources and used without further purification. Thin-layer chromatography (TLC) analysis was carried out on GF254 silica gel plates (QindaoHaiyang Chemical, China). Silica gel column chromatography was performed with 60G silica gel (QindaoHaiyang Chemical, China). ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Mercury Plus400 or Bruker AVANCE600 spectrometers, with TMS as an internal standard and $CDCl_3$, CD_3COCD_3 or DMSO-*d*6 as solvents. Chemical shifts and coupling constants (*J*) were expressed in parts per million (ppm, δ) and Hz, respectively. Abbreviations applied for proton coupling patterns are described as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quadruplet (q) and multiplet (m). Low-resolution mass spectra (ESI) were recorded on an Agilent 1100 LC/MSD, and high resolution mass spectra (HRMS) were collected on an AB Sciex Triple TOF® 5600+. All of the target compounds exhibited purity greater than 95% by HPLC.

General procedure for the synthesis of **10a-i** exemplified by **10a.** To a suspension of ethyl 2-(bromomethyl)acrylate (1.0 mL, 7.25 mmol, 1 eq) in distilled H_2O (50 mL), 4-biphenylboronic acid (1.7 g, 8.70 mmol, 1.2 eq), $Pd(TFA)_2$ (2.4 mg, 7.25 μM, 0.001 eq) and KOH (610.2 mg, 10.88 mmol, 1.5 eq) were added. The reaction mixture was heated to 90 °C for 3 h before being extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layer was washed with saturated NaCl, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography (PE:EA = 10:1) to afford compound **10a** (1.0 g, 53% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.58 (dd, *J* = 8.0, 8.0 Hz, 4H), 7.41 (dd, *J* = 4.0, 4.0 Hz, 2H), 7.31 (dd, *J* = 8.0, 8.0 Hz, 3H), 6.31 (s, 1H), 5.51 (s, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.71 (s, 2H), 1.20-1.33 (m, 3H).

Ethyl 2-(dibenzo[b,d]thiophen-2-ylmethyl)acrylate (**10b**). Yield 72%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.03-8.13 (m, 1H), 7.95 (s, 1H), 7.76 (dd, *J* = 8.0, 8.0 Hz, 2H), 7.22-7.44 (m, 3H), 6.26 (s, 1H), 5.48 (s, 1H), 4.08-4.21 (m, 2H), 3.77 (s, 2H), 1.23 (t, *J* = 6.6 Hz, 3H).

Ethyl 2-(phenanthren-9-ylmethyl)acrylate (**10c**). Yield 66%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.60 (dd, *J* = 8.0, 8.0 Hz, 2H), 7.90 (dd, *J* = 4.0, 4.0 Hz, 1H), 7.73-7.81 (m, 1H), 7.46-7.63 (m, 5H), 6.23 (s, 1H), 5.19 (s, 1H), 4.19-4.32 (m, 2H), 4.06 (s, 2H), 1.21-1.35 (m, 3H).

Ethyl 2-(4-(trifluoromethyl)benzyl)acrylate (**10d**). Yield 47%, colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 7.8 Hz, 2H), 6.43 (s, 1H), 5.65 (s, 1H), 3.68 (s, 2H).

Ethyl 2-([1,1'-biphenyl]-3-ylmethyl)acrylate (**10e**). Yield 63%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.56 (d, *J* = 7.6 Hz, 2H), 7.40 (dd, *J* = 7.0, 7.0 Hz, 4H), 7.28-7.35 (m, 2H), 7.17 (d, *J* = 7.5 Hz, 1H), 6.25 (s, 1H), 5.48 (s, 1H), 4.14-4.19 (m, 2H), 3.68 (s, 2H), 1.25 (t, *J* = 6.0,

3H).

Ethyl 2-(benzo[b]thiophen-2-ylmethyl)acrylate (**10f**). Yield 51%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.68 (t, *J* = 7.5 Hz, 1H), 7.60 (t, *J* = 7.4 Hz, 1H), 7.17-7.27 (m, 2H), 6.99 (d, *J* = 7.1 Hz, 1H), 6.27 (d, *J* = 7.2 Hz, 1H), 5.59 (d, *J* = 7.0 Hz, 1H), 4.13-4.19 (m, 2H), 3.84 (s, 2H), 1.13-1.26 (m, 3H).

Ethyl 2-((1H-indol-5-yl)methyl)acrylate (**10g**). Yield 68%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.26 (s, 1H), 7.43 (s, 1H), 7.13 (d, *J* = 8.3 Hz, 1H), 6.94-6.99 (m, 2H), 6.40 (s, 1H), 6.20 (s, 1H), 5.42 (s, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 3.71 (s, 2H), 1.18(t, *J* = 6.0 Hz, 3H).

Ethyl 2-((1-methyl-1H-indol-5-yl)methyl)acrylate (**10h**). Yield 49%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42 (s, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 7.03 (d, *J* = 8.0 Hz, 1H), 6.94 (t, *J* = 4.0 Hz, 1H), 6.38 (s, 1H), 6.19 (s, 1H), 5.40 (s, 1H), 4.12-4.17 (m, 2H), 3.71 (s, 2H), 3.64 (s, 3H), 1.23 (t, *J* = 6.0 Hz, 3H).

Ethyl 2-(naphthalen-2-ylmethyl)acrylate (**10i**). Yield 71%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.78 (dd, *J* = 10.6, 8.8 Hz, 3H), 7.64 (s, 1H), 7.41-7.45 (m, 2H), 7.32 (d, *J* = 8.4 Hz, 1H), 6.26 (s, 1H), 5.48 (s, 1H), 4.14-4.20 (m, 2H), 3.78 (s, 2H), 1.23 (t, *J* = 6.0 Hz, 3H).

General procedure for the synthesis of **11a-i** exemplified by **11a**. To a solution of **10a** (980.0 mg， 3.68 mmol) in THF (10 mL), 3 M NaOH (60 mL) was added. The resulting mixture was stirred for 5 h at 90 °C. Upon completion, the solvent was removed, and the residual aqueous solution was adjusted to pH 3-4 with 3 N HCl. After extraction with ethyl acetate (3 × 100 mL), the combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The obtained residue was purified by silica gel column chromatography (DCM:CH_3OH = 50:1) to afford target compound **11a** (849.7 mg, 97% yield) as a white solid. ^{1}H NMR (400 MHz,

DMSO-d_6) δ: 12.57 (s, 1H), 7.62 (dd, *J* = 8.0, 8.0 Hz, 4H), 7.45 (t, *J* = 7.4 Hz, 2H), 7.25-7.39 (m, 3H), 6.14 (s, 1H), 5.64 (s, 1H), 3.60 (s, 2H).

2-(Dibenzo[b,d]thiophen-2-ylmethyl)acrylic acid (**11b**). Yield 97%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.60 (s, 1H), 7.90-8.42 (m, 4H), 7.35-7.52 (m, 3H), 6.17 (s, 1H), 5.63 (s, 1H), 3.76 (s, 2H).

2-(Phenanthren-9-ylmethyl)acrylic acid (**11c**). Yield 100%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.68 (s, 1H), 8.84 (dd, *J* = 8.0, 8.0 Hz, 2H), 7.96 (dd, *J* = 3.6, 3.6 Hz, 2H), 7.62-7.67 (m, 5H), 6.17 (s, 1H), 5.32 (s, 1H), 4.07 (s, 2H).

2-(4-(Trifluoromethyl)benzyl)acrylic acid (**11d**). Yield 95%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.56 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 7.8 Hz, 2H), 6.43 (s, 1H), 5.65 (s, 1H), 3.68 (s, 2H).

2-([1,1'-Biphenyl]-3-ylmethyl)acrylic acid (**11e**). Yield 98%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.58 (d, *J* = 7.3 Hz, 2H), 7.43 (t, *J* = 7.2 Hz, 4H), 7.32-7.37 (m, 2H), 7.19-7.27 (m, 1H), 6.41 (s, 1H), 5.64 (s, 1H), 3.69 (s, 2H).

2-(Benzo[b]thiophen-2-ylmethyl)acrylic acid (**11f**). Yield 95%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.73 (s, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.74 (d, *J* = 8.0 Hz, 1H), 7.25-7.34 (m, 2H), 7.16 (s, 1H), 6.19 (s, 1H), 5.79 (s, 1H), 3.86 (s, 2H).

2-[(1H-indol-5-yl)methyl]acrylic acid (**11g**). Yield 96%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.75 (s, 1H), 11.31 (s, 1H), 7.70 (s, 1H), 7.67 (s, 1H), 7.61 (s, 1H), 7.29 (d, *J* = 8.1 Hz, 1H), 6.71 (s, 1H), 6.46 (s, 1H), 5.83 (s, 1H), 3.98 (s, 2H).

2-[(1-Methyl-1H-indol-5-yl)methyl]acrylic acid (**11h**). Yield 98%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.45 (s, 1H), 7.26-7.27 (m, 1H), 7.03-7.08 (m, 2H), 6.41 (s, 1H), 6.35 (s, 1H),

5.55 (s, 1H), 3.78-3.80 (m, 3H), 3.72 (s, 2H).

2-(Naphthalen-2-ylmethyl)acrylic acid (**11i**). Yield 98%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.77 (d, J = 7.8, 3H), 7.64 (s, 1H), 7.42-7.46 (m, 2H), 7.25-7.35 (m, 1H), 6.41 (s, 1H), 5.61 (d, J = 4.0 Hz, 1H), 3.78 (s, 2H).

General procedure for the synthesis of **12a-i** exemplified by **12a**. To a stirred solution of compound **11a** (830 mg, 3.49 mmol, 1.0 eq) and Et_3N (0.3 mL, 0.22 mmol, 0.06 eq) in CH_2Cl_2 (30 mL) a solution of thioacetic acid (320 μL, 4.5 mmol, 1.3 eq) in CH_2Cl_2 (10 mL) was added in a dropwise manner, and the reaction mixture was continuously stirred at room temperature for 30 h under a nitrogen atmosphere. The mixture was evaporated to obtain compound **12a** (857.6 mg, 49% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.15 (s, 1H), 7.30-7.48 (m, 6H), 7.23 (d, J = 8.0 Hz, 3H), 2.80-3.16 (m, 5H), 2.26 (s, 3H).

3-(Acetylthio)-2-(dibenzo[b,d]thiophen-2-ylmethyl)propanoic acid (**12b**). Yield 42%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.83 (s, 1H), 8.05 (s, 1H), 7.90 (s, 1H), 7.75 (s, 1H), 7.66 (s, 1H), 7.35 (s, 2H), 7.18 (s, 1H), 3.02-3.12 (m, 5H), 2.21 (s, 3H).

3-(Acetylthio)-2-(phenanthren-9-ylmethyl)propanoic acid (**12c**). Yield 57%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.74 (d, J = 8.0 Hz, 1H), 8.66 (d, J = 8.0 Hz, 1H), 8.10 (d, J = 5.3 Hz, 1H), 7.83 (d, J = 7.4 Hz, 1H), 7.56-7.70 (m, 5H), 3.58 (d, J = 8.0 Hz, 1H), 3.15-3.36 (m, 4H), 2.32 (s, 3H).

3-(Acetylthio)-2-(4-(trifluoromethyl)benzyl)propanoic acid (**12d**). Yield 38%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.46 (s, 1H), 7.46 (d, J = 7.7 Hz, 2H), 7.22 (d, J = 7.7 Hz, 2H), 2.84-3.06 (m, 5H), 2.23 (s, 3H).

3-([1,1'-Biphenyl]-3-yl)-2-((acetylthio)methyl)propanoic acid (**12e**). Yield 54%, white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.58 (d, *J* = 7.5 Hz, 2H), 7.47 (d, *J* = 7.5 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.35 (d, *J* = 7.7 Hz, 2H), 7.18 (d, *J* = 7.5 Hz, 1H), 2.96-3.17 (m, 5H), 2.33 (s, 3H).

3-(Acetylthio)-2-(benzo[b]thiophen-2-ylmethyl)propanoic acid (**12f**). Yield 40%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.73 (s, 1H), 7.89 (s, 2H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.43 (s, 1H), 7.32 (s, 1H), 7.20 (s, 1H), 3.10-3.20 (m, 3H), 2.89 (s, 1H), 2.34 (s, 3H).

2-[(1H-Indol-5-yl)methyl]-3-(acetylthio)propanoic acid (**12g**). Yield 39%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.44 (s, 1H), 11.01 (s, 1H), 7.31 (d, *J* = 6.6 Hz, 3H), 6.90 (d, *J* = 8.0 Hz, 1H), 6.34 (s, 1H), 2.73-3.04 (m, 5H), 2.31 (s, 3H).

3-(Acetylthio)-2-((1-methyl-1H-indol-5-yl)methyl)propanoic acid (**12h**). Yield 44%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.05 (s, 1H), 7.40 (s, 1H), 7.22 (s, 1H), 7.01 (s, 2H), 6.39 (s, 1H), 3.73 (s, 3H), 2.98-3.12 (m, 5H), 2.29 (s, 3H).

3-(Acetylthio)-2-(naphthalen-2-ylmethyl)propanoic acid (**12i**). Yield 51%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (t, *J* = 9.0 Hz, 3H), 7.65 (s, 1H), 7.45 (t, *J* = 4.0 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 1H), 3.03-3.24 (m, 5H), 2.33 (s, 3H).

General procedure for the synthesis of **14a-q** exemplified by **14a**. To a solution of **12a** (200.0 mg, 0.64 mmol, 1.0 eq) in anhydrous CH_2Cl_2 (50 mL), EDCI (159.5 mg, 0.83 mmol, 1.3 eq), HOBt (112.4 mg, 0.83 mmol, 1.3 eq) and *N*-methylmorpholine (100 μL, 0.91 mmol, 1.4 eq) were added. The resulting mixture was stirred for 30 min under nitrogen in an ice-salt bath. A solution of ethyl L-phenylalaninate hydrochloride (190.7 mg, 0.83 mmol, 1.3 eq) and *N*-methylmorpholine (90 μL, 0.85 mmol, 1.3 equiv) in CH_2CH_2 (20 mL) was then added to the mixture. The reaction was warmed to room temperature and stirred overnight under nitrogen. After completion, the reaction mixture was diluted with CH_2CH_2 (100 mL) and washed with 5% aqueous citric acid and

brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to yield crude compound **13a**, which was used directly for the next step without further purification. Then, compound **13a** was dissolved in degassed THF (5 mL), and 3 M NaOH (30 mL) was added. The mixture was stirred for 10 h at room temperature under nitrogen. After removal of most of the THF, the residual aqueous solution was adjusted to pH 3-4 with 3 N HCl and extracted with ethyl acetate (3 × 30 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The obtained residue was purified by silica gel column chromatography (DCM: CH_3OH = 50: 1) to afford target compound **14a** (85.8 mg, 32% yield) as a white solid. The racemic **14a** was further separated by preparative TLC, developing with CH_2Cl_2/MeOH/HCOOH (20:1:0.1) to obtain (*S*,*S*)-**14a-1** and (*R*,*S*)-**14a-2**.

(3-([1,1'-Biphenyl]-4-yl)-2-(mercaptomethyl)propanoyl)-L-phenylalanine (**14a**). Yield 62%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.53 (d, *J* = 7.6 Hz, 2H), 7.46 (d, *J* = 7.9 Hz, 2H), 7.41 (t, *J* = 7.6 Hz, 2H), 7.32-7.37 (m, 1H), 7.28 (d, *J* = 5.4 Hz, 3H), 7.16 (s, 2H), 7.14 (s, 2H), 5.73 (d, *J* = 7.4 Hz, 1H), 4.76- 4.88 (m, 1H), 3.17-3.21 (m, 1H), 2.88-3.02 (m, 2H), 2.78-2.87 (m, 2H), 2.47-2.57 (m, 2H), 1.33 (t, *J* = 8.7 Hz, 1H).

((*S*)-3-([1,1'-Biphenyl]-4-yl)-2-(mercaptomethyl)propanoyl)-L-phenylalanine ((*S*,*S*)-**14a-1**). Yield 19%, white solid. [α]25 D +26.4 (c = 0.1, CH_3OH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.77 (s, 1H), 8.39 (d, *J* = 8.0 Hz, 1H), 7.64 (d, *J* = 4.0 Hz, 2H), 7.54 (d, *J* = 8.0 Hz, 2H), 7.44 (t, *J* = 6.0 Hz, 2H), 7.33 (t, *J* = 6.0 Hz, 1H), 7.24 (s, 8H), 4.50 (q, *J* = 8.0 Hz, 1H), 2.88 (t, *J* = 8.0 Hz, 2H), 2.68-2.70 (m, 2H), 2.53-2.57 (m, 1H), 2.33 (t, *J* = 8.0 Hz, 1H), 1.81 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 172.73, 172.30, 139.91, 138.32, 137.83, 137.50, 129.31, 129.00, 128.72, 127.99, 127.02, 126.33, 126.19, 53.09, 50.34, 36.61, 25.39. HRMS m/z calcd for

$C_{25}H_{25}NO_3S$ $[M + H]^+$ 420.1628, found 420.1641.

((*R*)-3-([1,1'-Biphenyl]-4-yl)-2-(mercaptomethyl)propanoyl)-L-phenylalanine ((*R*,*S*)-**14a-2**). Yield 32%, white solid. [α]25 D -15.1 (c = 0.1, CH_3OH). ^1H NMR (400 MHz, DMSO-d_6) δ: 12.78 (s, 1H), 8.37 (d, *J* = 8.0 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.44 (t, *J* = 8.0 Hz, 2H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.20 (s, 1H), 7.17 (d, *J* = 8.0 Hz, 2H), 7.12 (t, *J* = 8.0 Hz, 4H), 4.46 (q, *J* = 8.0 Hz, 1H), 2.99 (dd, *J* = 4.0, 4.0 Hz, 1H), 2.68-2.84 (m, 3H), 2.59-2.63 (m, 2H), 2.35-2.41 (m, 1H), 2.20 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 172.84, 172.38, 139.86, 138.26, 137.78, 137.33, 129.27, 128.88, 128.72, 127.93, 127.03, 126.30, 126.17, 53.05, 50.15, 36.59, 25.41. MS m/z 420.0 $[M + H]^+$.

(2S)-2-(3-([1,1'-Biphenyl]-4-yl)-2-(mercaptomethyl)propanamido)-4-phenylbutanoic acid (**14b**). Yield 63%, white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.9 Hz, 4H), 7.41 (s, 2H), 7.34 (s, 1H), 7.26 (s, 2H), 7.21 (s, 3H), 7.15 (s, 2H), 5.85 (s, 1H), 4.55 (s, 1H), 2.82-2.99 (m, 3H), 2.56-2.69 (m, 4H), 2.18 (s, 1H), 1.92 (s, 1H), 1.26 (s, 1H).

(*S*)-2-((*S*)-3-([1,1'-Biphenyl]-4-yl)-2-(mercaptomethyl)propanamido)-4-phenylbutanoic acid ((*S*,*S*)-**14b-1**). Yield 22%, white solid. [α]25 D +21.6 (c = 0.1, CH_3OH). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.54 (d, *J* = 8.0 Hz, 2H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.41 (t, *J* = 8.0 Hz, 2H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.28 (s, 1H), 7.25 (s, 2H), 7.21 (d, *J* = 8.0 Hz, 2H), 7.14 (d, *J* = 8.0 Hz, 2H), 5.83 (d, *J* = 8.0 Hz, 1H), 4.55 (q, *J* = 5.0 Hz, 1H), 2.93-2.98 (m, 1H), 2.86 (d, *J* = 8.0 Hz, 2H), 2.64 (t, *J* = 6.0 Hz, 2H), 2.55-2.59 (m, 2H), 2.15-2.23 (m, 1H), 1.89-1.94 (m, 1H), 1.58 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 177.96, 177.38, 145.48, 144.81, 142.89, 133.82, 133.05, 132.74, 132.57, 131.40, 130.95, 130.16, 55.85, 41.89, 37.71, 36.03, 33.66, 30.33. HRMS m/z calcd for $C_{26}H_{27}NO_3S$ $[M + H]^+$ 434.1784, found 434.1788.

(*S*)-2-((*R*)-3-([1,1'-Biphenyl]-4-yl)-2-(mercaptomethyl)propanamido)-4-phenylbutanoic acid ((*R*,*S*)-**14b-2**). Yield 36%, white solid. [α]25 D -10.7 (c = 0.1, CH_3OH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.61 (s, 1H), 8.34 (d, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.38 (t, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 3H), 7.12 (t, *J* = 6.0 Hz, 3H), 6.94 (d, *J* = 8.0 Hz, 2H), 4.05 (q, *J* = 8.0 Hz, 1H), 2.81-2.87 (m, 3H), 2.71-2.78 (m, 1H), 2.53-2.55 (m, 1H), 2.22-2.6 (m, 3H), 1.80-1.90 (m, 1H), 1.58 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 173.47, 172.47, 140.73, 139.81, 138.35, 137.87, 129.31, 128.61, 128.09, 128.00, 126.95, 126.28, 125.59, 51.01, 50.49, 36.93, 32.43, 31.07, 26.20. HRMS m/z calcd for $C_{26}H_{27}NO_3S$ $[M + H]^+$ 434.1784, found 434.1787.

(2*S*)-2-(3-([1,1'-Biphenyl]-4-yl)-2-(mercaptomethyl)propanamido)-3-(1H-indol-5-yl)-propanoic acid (**14c**). Yield 41%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.69 (s, 1H), 10.89 (s, 1H), 8.38 (d, *J* = 8.0 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 7.21 (d, *J* = 8.0 Hz, 3H), 7.08 (t, *J* = 6.0 Hz, 1H), 6.99 (t, *J* = 6.0 Hz, 1H), 4.58 (q, *J* = 6.0 Hz, 1H), 3.20 (dd, *J* = 4.0, 4.0 Hz, 1H), 3.03-3.08 (m, 1H), 2.88 (m, 1H), 2.56-2.77 (m, 3H), 2.34-2.40 (m, 1H), 1.95 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 173.19, 172.35, 139.91, 138.31, 137.80, 135.96, 129.32, 128.70, 127.02, 126.30, 123.52, 120.75, 118.17, 118.08, 111.18, 109.76, 52.56, 50.39, 36.72, 27.07, 25.33. HRMS m/z calcd for $C_{27}H_{26}N_2O_3S$ $[M + H]^+$ 459.1737, found 459.1740.

(2*S*)-2-(3-([1,1'-Biphenyl]-4-yl)-2-(mercaptomethyl)propanamido)-3-(4-(benzyloxy)phenyl)propanoic acid (**14d**). Yield 38%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.76 (s, 1H), 8.34 (d, *J* = 8.0 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.34 (s, 1H), 7.33 (s, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 8.0 Hz,

2H), 7.02 (d, *J* = 8.0 Hz, 2H), 6.83 (d, *J* = 6.0 Hz, 2H), 5.02 (s, 1H), 4.95 (s, 1H), 4.41 (q, *J* = 8.0 Hz, 1H), 2.95 (dd, *J* = 4.0, 4.0 Hz, 1H), 2.74-2.85 (m, 2H), 2.54-2.69 (m, 3H), 2.32-2.45 (m, 1H), 2.24 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 172.88, 172.38, 156.81, 139.84, 138.81, 137.77, 136.93, 130.04, 129.90, 129.47, 129.30, 128.71, 128.22, 128.17, 127.54, 127.35, 127.02, 126.30, 126.27, 114.34, 68.90, 53.25, 49.99, 36.53, 35.73, 25.54. HRMS m/z calcd for $C_{32}H_{31}NO_4S$ $[M + H]^+$ 526.2047, found 526.2051.

(3-([1,1'-Biphenyl]-3-yl)-2-(mercaptomethyl)propanoyl)-*L*-phenylalanine (**14e**). Yield 47%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.77 (s, 1H), 8.36 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 7.7 Hz, 2H), 7.43-7.53 (m, 4H), 7.30-7.38 (m, 2H), 7.03-7.15 (m, 6H), 4.42-4.47 (m, 1H), 2.90-2.99 (m, 1H), 2.71-2.85 (m, 3H), 2.65-2.68 (m, 2H), 2.37-2.45 (m, 1H), 2.19 (t, *J* = 7.8 Hz, 1H).

((*S*)-3-([1,1'-Biphenyl]-3-yl)-2-(mercaptomethyl)propanoyl)-L-phenylalanine ((*S*,*S*)-**14e-1**). Yield 15%, white solid. [α]25 D +17.1 (c = 0.2, CH_3OH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.78 (s, 1H), 8.36 (d, *J* = 8.0 Hz, 1H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 4.0 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 4.0 Hz, 1H), 7.32 (t, *J* = 6.0 Hz, 1H), 7.15 (s, 1H), 7.14 (s, 2H), 7.07 (d, *J* = 8.0 Hz, 3H), 4.44 (q, *J* = 6.7 Hz, 1H), 2.93 (dd, *J* = 4.0, 4.0 Hz, 1H), 2.72-2.81 (m, 3H), 2.61-2.68 (m, 2H), 2.37-2.43 (m, 1H), 2.20 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 172.78, 172.38, 140.14, 140.09, 139.92, 139.64, 137.21, 128.94, 128.85, 128.69, 128.60, 127.89, 127.18, 126.55, 126.15, 124.38, 53.04, 50.32, 37.26, 36.70, 25.31. MS m/z 420.0 $[M + H]^+$.

((*R*)-3-([1,1'-Biphenyl]-3-yl)-2-(mercaptomethyl)propanoyl)-L-phenylalanine ((*R*,*S*)-**14e-2**). Yield 26%, white solid. [α]25 D -23.7 (c = 0.2, CH_3OH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.76 (s, 1H), 8.38 (d, *J* = 8.0 Hz, 1H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.46 (s, 3H), 7.44 (s, 1H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.23 (s, 4H), 7.15 (t, *J* = 6.0 Hz, 2H), 4.50 (q, *J* = 6.7 Hz, 1H), 3.10 (dd, *J* = 4.0, 4.0

Hz, 1H), 2.85-2.92 (m, 2H), 2.68-2.77 (m, 2H), 2.51-2.55 (m, 1H), 2.27-2.34 (m, 1H), 1.84 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 172.71, 172.32, 140.13, 140.02, 139.68, 137.44, 129.00, 128.69, 128.63, 127.98, 127.91, 127.18, 127.14, 126.59, 126.19, 124.40, 53.06, 50.23, 37.06, 36.70, 25.21. HRMS m/z calcd for $C_{25}H_{25}NO_3S$ $[M + H]^+$ 420.1628, found 420.1631.

(3-([1,1'-Biphenyl]-3-yl)-2-(mercaptomethyl)propanoyl)-L-tryptophan (**14f**). Yield 36%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.64 (s, 1H), 10.87 (s, 1H), 8.38 (d, *J* = 4.0 Hz, 1H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.50 (s, 1H), 7.46 (t, *J* = 6.0 Hz, 3H), 7.33 (d, *J* = 8.0 Hz, 3H), 7.16 (t, *J* = 8.0 Hz, 2H), 7.07 (t, *J* = 8.0 Hz, 1H), 6.99 (t, *J* = 8.0 Hz, 1H), 4.59 (q, *J* = 6.7 Hz, 1H), 3.21 (dd, *J* = 4.0, 4.0 Hz, 1H), 3.03-3.10 (m, 1H), 2.90-2.97 (m, 1H), 2.68-2.83 (m, 3H), 2.56-2.63 (m, 1H), 1.94 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 173.17, 172.43, 140.13, 140.02, 139.69, 135.93, 128.69, 128.62, 127.17, 127.05, 126.59, 124.40, 123.48, 123.31, 120.75, 118.17, 111.17, 109.69, 52.56, 50.20, 37.21, 27.18, 25.14. HRMS m/z calcd for $C_{27}H_{26}N_2O_3S$ $[M + H]^+$ 459.1737, found 459.1737.

(2S)-2-(3-([1,1'-Biphenyl]-3-yl)-2-(mercaptomethyl)propanamido)-3-(4-(benzyloxy)phenyl)-propanoic acid (**14g**). Yield 54%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.72 (s, 1H), 8.30 (d, *J* = 7.9 Hz, 1H), 7.65 (d, *J* = 7.4 Hz, 2H), 7.29-7.54 (m, 11H), 7.08 (d, *J* = 7.4 Hz, 1H), 6.94 (d, *J* = 8.3 Hz, 2H), 6.76 (d, *J* = 8.4 Hz, 2H), 4.95 (s, 2H), 4.40 (d, *J* = 5.0 Hz, 1H), 2.62-2.90 (m, 6H), 2.43 (s, 1H), 2.21 (t, *J* = 8.1 Hz, 1H).

(*S*)-2-((*S*)-3-([1,1'-Biphenyl]-3-yl)-2-(mercaptomethyl)propanamido)-3-(4-(benzyloxy)phenyl propanoic acid ((*S*,*S*)-**14g-1**). Yield 17%, white solid. [α]25 D +25.5 (c = 0.1, CH_3OH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.71 (s, 1H), 8.29 (d, J = 8.0 Hz, 1H), 7.63 (d, J = 8.0 Hz, 2H), 7.45 (t, J = 8.0 Hz, 4H), 7.36 (s, 4H), 7.31 (t, J = 8.0 Hz, 3H), 7.07 (d, J = 8.0 Hz, 1H), 6.93 (d, J = 8.0 Hz,

2H), 6.73 (d, J = 8.0 Hz, 2H), 4.93 (s, 2H), 4.39 (q, J = 6.7 Hz, 1H), 2.78-2.86 (m, 2H), 2.60-2.75 (m, 4H), 2.38-2.45 (m, 1H), 2.20 (t, J = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 172.81, 172.35, 156.73, 140.11, 139.88, 139.64, 136.97, 129.96, 129.87, 129.27, 128.69, 128.57, 128.21, 127.89, 127.56, 127.35, 127.20, 126.56, 124.36, 114.20, 68.86, 53.24, 50.27, 37.28, 35.84, 25.44. MS m/z 526.2 $[M + H]^+$.

(S)-2-((R)-3-([1,1'-Biphenyl]-3-yl)-2-(mercaptomethyl)propanamido)-3-(4-(benzyloxy)phenyl) propanoic acid ((R,S)-14g-2). Yield 29%, white solid. [α]25 D -3.4 (c = 0.1, CH_3OH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.70 (s, 1H), 8.32 (d, *J* = 8.0 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.47 (s, 2H), 7.45 (s, 2H), 7.40 (t, *J* = 6.0 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.32 (t, *J* = 4.0 Hz, 2H), 7.31 (s, 1H), 7.13 (t, *J* = 6.0 Hz, 3H), 6.87 (t, *J* = 8.0 Hz, 2H), 5.02 (s, 2H), 4.43 (q, *J* = 6.7 Hz, 1H), 3.00 (dd, *J* = 4.0, 4.0 Hz, 1H), 2.88-2.93 (m, 1H), 2.63-2.81 (m, 3H), 2.55-2.60 (m, 1H), 2.29-2.35 (m, 1H), 1.89 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 172.79, 172.36, 156.89, 140.17, 140.06, 139.75, 137.08, 130.09, 129.56, 128.74, 128.67, 128.28, 127.98, 127.62, 127.44, 127.23, 127.18, 126.64, 124.44, 114.37, 69.01, 53.35, 50.24, 37.10, 35.93, 25.31. HRMS m/z calcd for $C_{32}H_{31}NO_4S$ $[M + H]^+$ 526.2047, found 526.2049.

(2*S*)-2-(3-(Benzo[b]thiophen-2-yl)-2-(mercaptomethyl)propanamido)-4-phenylbutanoic acid (**14h**). Yield 21%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.66 (s, 1H), 8.48 (d, *J* = 8.0 Hz, 1H), 7.85 (t, *J* = 6.0 Hz, 1H), 7.01 (t, *J* = 6.0 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 1H), 7.20 (d, *J* = 8.0 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 3H), 6.86 (d, *J* = 8.0 Hz, 2H), 4.07 (q, *J* = 5.3 Hz, 1H), 3.11-3.20 (m, 2H), 2.92-2.97 (m, 1H), 2.75-2.79 (m, 1H), 2.58-2.63 (m, 1H), 2.29-2.43 (m, 3H), 1.74-1.93 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 173.45, 172.06, 142.59, 140.61, 139.52, 138.65, 128.00, 125.59, 124.03, 123.53, 122.69, 121.97, 51.12, 50.08, 32.34,

31.76, 31.05, 26.23. HRMS m/z calcd for $C_{22}H_{23}NO_3S_2$ $[M + H]^+$ 414.1192, found 414.1205.

(2*S*)-2-(3-Mercapto-2-(phenanthren-9-ylmethyl)propanamido)-4-phenylbutanoic acid (**14i**). Yield 38%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.61 (s, 1H), 8.90 (d, *J* = 4.0 Hz, 1H), 8.80 (d, *J* = 8.0 Hz, 1H), 8.51 (d, *J* = 8.0 Hz, 1H), 8.27 (s, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.73 (s, 3H), 7.63 (t, *J* = 8.0 Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 2H), 7.18 (s, 3H), 4.20 (s, 1H), 3.39-3.44 (m, 1H), 3.25-3.29 (m, 1H), 3.01-3.10 (m, 1H), 2.85 (q, *J* = 8.0 Hz, 1H), 2.58-2.72 (m, 3H), 2.40 (q, *J* = 8.0 Hz, 1H), 1.86-2.04 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 173.15, 172.76, 140.73, 132.83, 131.01, 130.50, 129.97, 129.00, 127.96, 127.87, 127.81, 126.93, 126.35, 126.23, 125.91, 125.82, 125.39, 124.11, 122.75, 121.86, 51.17, 49.22, 34.47, 32.83, 31.30, 26.36. HRMS m/z calcd for $C_{28}H_{27}NO_3S$ $[M + H]^+$ 458.1784, found 458.1788.

(3-(Dibenzo[b,d]thiophen-2-yl)-2-(mercaptomethyl)propanoyl)-*L*-phenylalanine (**14j**). Yield 46%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.86 (s, 1H), 8.45 (d, *J* = 8.0 Hz, 1H), 8.34 (s, 1H), 8.20 (s, 1H), 8.05 (s, 1H), 7.92 (d, *J* = 8.1 Hz, 1H), 7.55 (d, *J* = 2.9 Hz, 2H), 7.35 (d, *J* = 7.9 Hz, 1H), 7.17-7.27 (m, 5H), 4.54 (d, *J* = 3.8 Hz, 1H), 3.04-3.20 (m, 2H), 2.85-2.95 (m, 3H), 2.63 (s, 1H), 2.40 (s, 1H), 1.90 (s, 1H).

((*S*)-3-(Dibenzo[b,d]thiophen-2-yl)-2-(mercaptomethyl)propanoyl)-L-phenylalanine ((*S*,*S*)-**14j-1**). Yield 14%, white solid. [α]25 D +23.3 (c = 0.2, CH_3OH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.82 (s, 1H), 8.41 (d, *J* = 8.0 Hz, 1H), 8.30 (s, 1H), 8.16 (s, 1H), 8.01 (s, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.51 (t, *J* = 6.0 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 1H), 7.22 (s, 3H), 7.18 (d, *J* = 8.0 Hz, 2H), 4.50 (q, *J* = 8.0 Hz, 1H), 3.03-3.13 (m, 2H), 2.81-2.91 (m, 3H), 2.56-2.63 (m, 1H), 2.33-2.40 (m, 1H), 1.86 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 172.76, 172.29, 138.68, 137.52, 136.16, 135.77, 134.95, 134.78, 128.99, 128.11, 127.96, 126.77, 126.17, 124.46, 122.85, 122.49,

121.93, 121.77, 53.17, 50.43, 36.91, 36.63, 25.34. HRMS m/z calcd for $C_{25}H_{23}NO_3S_2$ $[M + H]^+$ 450.1192, found 450.1199.

((*R*)-3-(Dibenzo[b,d]thiophen-2-yl)-2-(mercaptomethyl)propanoyl)-L-phenylalanine ((*R*,*S*)-**14j-2**). Yield 26%, white solid. [α]25 D -5.7 (c = 0.2, CH_3OH). ^1H NMR (400 MHz, DMSO-d_6) δ: 12.83 (s, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 8.28 (s, 1H), 8.10 (s, 1H), 8.01 (s, 1H), 7.86 (d, *J* = 8.0 Hz, 1H), 7.51 (s, 2H), 7.23 (d, *J* = 4.0 Hz, 1H), 7.01 (s, 5H), 4.42 (q, *J* = 8.0 Hz, 1H), 2.67-2.94 (m, 5H), 2.63-2.65 (m, 1H), 2.42-2.47 (m, 1H), 2.22 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 172.79, 172.34, 138.71, 137.21, 136.18, 135.72, 134.90, 134.78, 128.74, 128.03, 127.74, 126.77, 126.02, 124.44, 122.87, 122.48, 121.88, 121.70, 53.10, 50.61, 37.12, 36.61, 25.55.

(2*S*)-2-(3-(Dibenzo[b,d]thiophen-2-yl)-2-(mercaptomethyl)propanamido)-4-phenylbutanoic acid (**14k**). Yield 21%, white solid. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.61 (s, 1H), 8.45 (d, *J* = 8.0 Hz, 1H), 8.32 (d, *J* = 8.0 Hz, 1H), 8.21 (s, 1H), 8.00 (d, *J* = 6.0 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.50 (d, *J* = 6.0 Hz, 2H), 7.40 (d, *J* = 8.0 Hz, 1H), 7.25 (t, *J* = 6.0 Hz, 2H), 7.16 (t, *J* = 8.0 Hz, 3H), 4.18 (q, *J* = 8.0 Hz, 1H), 3.09-3.15 (m, 1H), 2.87-2.92 (m, 2H), 2.70-2.76 (m, 2H), 2.55-2.68 (m, 2H), 2.28 (t, *J* = 8.0 Hz, 1H), 1.98-2.04 (m, 1H), 1.84-1.90 (m, 1H). ^{13}C NMR (150 MHz, DMSO-d6) δ: 173.35, 172.57, 140.90, 138.70, 136.20, 135.74, 134.98, 134.80, 128.22, 128.14, 126.77, 125.73, 124.46, 122.85, 122.51, 121.99, 121.78, 51.11, 50.39, 37.21, 32.74, 31.25, 25.34. HRMS m/z calcd for $C_{26}H_{25}NO_3S_2$ $[M + H]^+$ 464.1349, found 464.1353.

(2*S*)-2-(2-((1H-Indol-5-yl)methyl)-3-mercaptopropanamido)-4-phenylbutanoic acid (**14l**). Yield 26%, white solid. ^1H NMR (400MHz, DMSO-d_6) δ: 10.96 (s, 1H), 8.28 (d, *J* = 8.0 Hz, 1H), 7.36 (s, 1H), 7.28 (s, 1H), 7.26 (s, 1H), 7.19 (t, *J* = 8.0 Hz, 2H), 7.11 (d, *J* = 8.0 Hz, 1H), 6.95 (d, *J* = 8.0 Hz, 1H), 6.89 (d, *J* = 8.0 Hz, 2H), 6.32 (s, 1H), 4.02 (q, *J* = 8.0 Hz, 1H), 2.78-2.90 (m, 4H),

2.70-2.73 (m, 1H), 2.44-2.47 (m, 1H), 2.37-2.42 (m, 2H), 1.73-1.91(m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 173.63, 172.96, 140.78, 134.49, 129.10, 128.26, 128.10, 128.02, 127.57, 125.57, 125.07, 122.11, 119.73, 110.83, 100.50, 51.33, 51.08, 37.68, 32.43, 31.05, 25.93. HRMS m/z calcd for $C_{22}H_{24}N_2O_3S$ $[M + H]^+$ 397.1580, found 397.1588.

(2-((1H-Indol-5-yl)methyl)-3-mercaptopropanoyl)-*L*-phenylalanine (**14m**). Yield 43%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 8.39-8.49 (m, 1H), 7.24-7.43 (m, 4H), 6.80-7.10 (m, 4H), 6.32-6.54 (m, 2H), 6.02-6.06 (m, 1H), 4.77-4.81 (m, 1H), 2.75-3.10 (m, 5H), 2.49 (s, 2H), 1.47 (t, *J* = 7.8 Hz, 1H).

((*S*)-2-((1H-Indol-5-yl)methyl)-3-mercaptopropanoyl)-L-phenylalanine ((*S*,*S*)-**14m-1**). Yield 11%, white solid. [α]25 D +9.7 (c = 0.1, CH_3OH). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.96 (s, 1H), 8.26 (d, *J* = 7.9 Hz, 1H), 7.28 (t, *J* = 2.7 Hz, 1H), 7.26 (s, 1H), 7.24 (s, 1H), 7.14 (d, *J* = 3.9 Hz, 1H), 7.13 (d, *J* = 2.0 Hz, 2H), 7.08 (d, *J* = 3.5 Hz, 1H), 7.07 (d, *J* = 2.0 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 1H), 6.33 (s, 1H), 4.43-4.47 (m, 1H), 2.97 (dd, *J* = 4.92, 4.86 Hz, 1H), 2.81-2.85 (m, 1H), 2.72-2.77 (m, 2H), 2.59-2.63 (m, 2H), 2.34-2.36 (m, 1H), 2.09 (s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 172.93, 172.76, 137.37, 134.48, 129.00, 128.89, 127.85, 127.54, 126.10, 125.10, 122.14, 119.77, 110.87, 100.54, 53.12, 51.22, 37.57, 36.57, 25.10. MS m/z 383.0 $[M + H]^+$.

((*R*)-2-((1H-Indol-5-yl)methyl)-3-mercaptopropanoyl)-L-phenylalanine ((*R*,*S*)-**14m-2**). Yield 26%, white solid. [α]25 D -6.1 (c = 0.1, CH_3OH). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.96 (s, 1H), 8.30 (d, *J* = 7.8 Hz, 1H), 7.31 (s, 1H), 7.28 (d, *J* = 3.3 Hz, 1H), 7.26 (s, 1H), 7.25 (s, 2H), 7.23 (s, 2H), 7.19 (d, *J* = 4.1 Hz, 1H), 7.17 (d, *J* = 4.3 Hz, 1H), 6.90 (d, *J* = 8.3 Hz, 1H), 6.33 (s, 1H), 4.46-4.50 (m, 1H), 3.11 (dd, *J* = 4.68, 4.62 Hz, 1H), 2.88-2.94 (m, 2H), 2.64-2.71 (m, 1H), 2.63 (d, *J* = 8.22 Hz, 1H), 2.53-2.56 (m, 1H), 2.25-2.27 (m, 1H), 1.73 (s, 1H). ^{13}C NMR (150

MHz, DMSO-d_6) δ: 172.95, 172.69, 137.67, 134.46, 129.02, 127.95, 127.58, 126.12, 125.11, 122.13, 119.74, 110.91, 100.54, 53.27, 51.18, 37.40, 36.65, 24.94. HRMS m/z calcd for $C_{21}H_{22}N_2O_3S$ $[M + H]^+$ 383.1424, found 383.1437.

(3-Mercapto-2-((1-methyl-1H-indol-5-yl)methyl)propanoyl)-*L*-tryptophan (**14n**). Yield 43%, white solid. ^{1}H NMR (400 MHz, CD_3COCD_3) δ: 11.18 (s, 1H), 10.06 (s, 1H), 8.11 (s, 1H), 7.49 (d, *J* = 8.0 Hz, 1H), 7.37 (s, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.23 (s, 1H), 7.16 (d, *J* = 8.0 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 1H), 7.01 (d, *J* = 8.0 Hz, 2H), 6.34 (d, *J* = 8.0 Hz, 1H), 4.86 (q, *J* = 6.7 Hz, 1H), 3.78 (s, 3H), 3.33 (dd, *J* = 4.0, 4.0 Hz, 1H), 3.07-3.24 (m, 2H), 2.94-3.05 (m, 1H), 2.53-2.74 (m, 2H), 2.36-2.47 (m, 1H), 1.83 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, CD_3COCD_3) δ: 174.13, 173.54, 162.34, 137.48, 136.76, 130.70, 130.20, 129.82, 128.70, 124.44, 123.60, 122.19, 121.63, 119.64, 112.51, 110.87, 101.60, 53.88, 53.52, 39.15, 32.96, 28.43, 26.21. HRMS m/z calcd for $C_{24}H_{25}N_3O_3S$ $[M + H]^+$ 436.1689, found 436.1696.

(2*S*)-3-(4-(Benzyloxy)phenyl)-2-(3-mercapto-2-((1-methyl-1H-indol-5-yl)methyl)-propanamidpropanoic acid (**14o**). Yield 38%, white solid. ^{1}H NMR (400 MHz, CD_3COCD_3) δ: 11.24 (s, 1H), 7.45 (t, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 3H), 7.27 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 3H), 7.03 (t, *J* = 8.0 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 6.79 (d, *J* = 8.0 Hz, 1H), 6.67 (d, *J* = 8.0 Hz, 1H), 6.36 (d, *J* = 8.0 Hz, 1H), 5.06 (s, 1H), 5.01 (s, 1H), 4.73 (q, *J* = 6.7 Hz, 1H), 3.78 (s, 3H), 3.15 (dd, *J* = 4.0, 4.0 Hz, 1H), 2.89-2.99 (m, 2H), 2.79-2.84 (m, 1H), 2.68-2.78 (m, 2H), 2.37-2.78 (m, 1H), 1.83 (t, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, CD_3COCD_3) δ: 174.01, 172.95, 158.73, 138.59, 136.78, 131.40, 131.28, 130.53, 130.54, 129.33, 128.63, 128.42, 123.70, 123.53, 121.57, 115.54, 110.10, 101.18, 70.48, 54.43, 53.55, 39.06, 37.38, 32.93, 23.40. HRMS m/z calcd for $C_{29}H_{30}N_2O_4S$ $[M + H]^+$ 503.1999, found 503.2002.

(2S)-2-(3-Mercapto-2-(naphthalen-2-ylmethyl)propanamido)-4-phenylbutanoic acid (**14p**). Yield 34%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.68 (s, 1H), 8.49 (d, *J* = 8.0 Hz, 1H), 7.87 (t, *J* = 8.8 Hz, 3H), 7.76 (s, 1H), 7.48 (dd, *J* = 5.6,9.2 Hz, 3H), 7.27 (t, *J* = 7.2 Hz, 2H), 7.18 (d, *J* = 7.2 Hz, 3H), 4.16-4.26 (m, 1H), 3.11-3.16 (m, 1H), 2.86-2.98 (m, 2H), 2.55-2.77 (m, 4H), 2.29 (t, *J* = 8.0 Hz, 1H), 1.98-2.09 (m, 1H), 1.85-1.93 (m, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 173.38, 172.63, 140.91, 136.72, 132.93, 131.58, 128.24, 128.15, 127.50, 127.27, 127.25, 126.84, 125.78, 125.74, 125.17, 51.11, 50.16, 37.39, 32.77, 31.27, 25.42. HRMS m/z calcd for $C_{24}H_{25}NO_3S$ $[M + H]^+$ 408.1628, found 408.1632.

(2*S*)-2-(3-Mercapto-2-(4-(trifluoromethyl)benzyl)propanamido)-4-phenylbutanoic acid (**14q**). Yield 23%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.46 (s, 1H), 8.22 (d, *J* = 7.6 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.25 (d, *J* = 7.6 Hz, 2H), 7.07 (t, *J* = 7.2 Hz, 3H), 6.95 (d, *J* = 6.4 Hz, 1H), 6.76 (d, *J* = 7.2 Hz, 1H), 3.95-4.00 (m, 1H), 2.76-2.83 (m, 1H), 2.58-2.70 (m, 4H), 2.31-2.40 (m, 2H), 2.11-2.17 (m, 1H), 2.29 (t, *J* = 8.0 Hz, 1H), 1.78-1.86 (m, 1H). ^{19}F NMR (376 MHz, DMSO-d_6) δ: -62.31. HRMS m/z calcd for $C_{21}H_{22}F_3NO_3S$ $[M + H]^+$ 426.1345, found 426.1349.

Fluorescence-based assay. The NDM-1 enzyme was purchased from Biorbyt (orb197650). NDM-1 enzymatic activity was evaluated at room temperature in black polypropylene 96-well plates (Corning) using dicefalotinodifluorescescein, known as Fluorocillin (Thermo Fisher), as the substrate to transfer the action of the NDM-1.[25] The test compounds were dissolved in DMSO (100 mM) and diluted into different concentrations in assay buffer (50 mM HEPES, pH 7.5 containing 0.01% Triton X-100). Then, 10 μL of the dilution was incubated with 80 μL of protein (final concentration 0.1 nM) in the assay buffer at room temperature for 30 min. In addition, negative controls (no enzyme) were included with 90 μL of assay buffer, and the positive controls

(no added inhibitor) were included with 10 μL of assay buffer, which replaced the test compound. Next, 10 μL of the Fluorocillin substrate was added (final concentration 0.1 μg) to produce a final volume of 100 μL, and each well contained the same concentration of 1% DMSO. The fluorescence emitted by the substrate product difluorofluorescein was determined every 45 s for 30 cycles using an Infinite 200 TECAN fluorescence plate reader. The rate of the enzymatic reaction was acquired by dividing the quantity of the fluorescent product (RFU) by time (minutes).[18] The IC_{50} values were calculated using data obtained from measurements, applying a sigmoidal dose response (variable slope with four parameters) equation using GraphPad Prism 5.0 software. All experiments were independently measured in triplicate.

CCK-8 assay. HUVEC and L02 and HEK293 cells were cultured in DMEM (HyClone, Logan, Utah, USA) supplemented with 1 mM PSP, 10% fetal bovine serum (FBS), 2 mg/mL sodium bicarbonate, 4.5 mg/mL glucose, 100 μg/mL streptomycin and 100 U/mL penicillin in a 5% CO_2 atmosphere in a humidified incubator at 37 °C. The cells were plated at a density of 1×10^4 per well in 96-well transparent plates and were incubated for 24 h. Test compounds were added at different concentrations, and 0.1% DMSO was used for control. After 24 h, the above medium was removed, and 100 μL of new medium supplemented with 10% Cell Counting Kit-8 (CCK-8) was added to each well and incubated for 1-1.5 h. The absorbance (OD) was measured using a Multiskan GO (Thermo Scientific) at 450 nm. The IC_{50} values were calculated using GraphPad Prism 5.0 software. All experiments were independently measured in triplicate.

LDH Release. The assay was measured following the manufacturer's protocol.[45] Cells were seeded at a constant density of 1×10^4 per well in 96-well plates and incubated for 24 h at 37 °C. The medium was substituted by DMEM with 10% FBS and test compounds at different

concentrations and cultured for 24 h. LDH release solutions (10%) were added 1 h before measurement to the medium as positive controls. After the incubation time, an amount of 120 μL of the supernatant was transferred to new 96-well plates. Then, 60 μL of LDH substrate solution was added to each well and incubated at room temperature for 30 min. The absorbance was monitored on a spectrophotometer (Multiskan GO, Thermo Scientific) at 490 nm. All experiments were independently performed three times.

Microdilution Broth Minimum Inhibitory Concentration (MIC) Assay. MIC values were determined according to the standard broth micro-dilution method of the Clinical and Laboratory Standards Institute (CLSI) guidelines.[46] Briefly, K. *pneumoniae* and E. *coli* clinical isolates expressing $bla_{\text{NDM-1}}$ were cultured in Mueller-Hinton broth (MHB) overnight at 37 °C, and the OD values were measured at 600 nm (OD_{600}). The bacterial density was then diluted to 1×10^6 colony forming units (CFU) per mL in MHB. Then, MEM (final concentration between 0.06-128 μg/mL in 2-fold dilution series), the compound (final concentration between 0.5-32 μg/mL in 2-fold dilution series) and the prepared bacterial suspension were added to 96-well microtiter plates. Wells with no bacteria and no MER served as the negative and positive controls, respectively. The plates were incubated for 24 h at 37 °C. MIC values were determined as the lowest concentration of the drugs that could visually inhibit the growth of bacteria. Each MIC experiment was performed in duplicate. The FICI was defined according to the following equation: FICI = FICA + FICB = CA/MICA + CB/MICB, where MICA and MICB were the MIC values of compounds A and B, respectively, when functioning alone, and CA and CB were the concentrations of compounds A and B at the effective combinations. Synergism was defined by FICI $\leq$ 0.5, indifference was defined by FICI > 0.5 but $\leq$ 4, and antagonism was defined by FICI $\geq$ 4.

In Vivo Acute Toxicity of Compound 14m in Mice. Female ICR mice aged 4-5 weeks old and weighing 20-24 g were purchased from Shanghai Bikai Laboratory Animal Co. Ltd, and they were acclimatized for a week before the experiment. Then, the mice were divided into 12 groups of 8 mice each, and compound **14m** was intravenously or intraperitoneally injected (200 μL per mouse) at doses of 50, 100, 200, 300 and 400 mg/kg, respectively. The other two groups were left untreated and served as control groups. Survival, behavior and changes in the weight of mice were recorded within 14 days. The LD_{50} values were evaluated using SPSS 19.0 software. All experiments in mice were approved by the Laboratory Animal Ethics Committee of Fudan University.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: XXXXXX/acs.XXXXXXX.

^{1}H and ^{13}C NMR spectra, high resolution mass spectra, HPLC purity spectra, optical rotations, experimental ECD spectra, ECD computational section. (PDF)

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Notes

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ABBREVIATIONS LIST

NDM-1, New Delhi Metallo-β-Lactamase-1; MEM, meropenem; AMR, antimicrobial resistance; CRE, Carbapenem-resistant Enterobacteriaceae; WHO, World Health Organization; SBLs, serine-β-lactamases; MBLs, metallo-β-lactamases; IMP-1, Imipenemase-1; VIM, Verona integrin-encoded MBL; AMA, aspergillomarasmine A; DPA, 2, 6-dipicolinic acid; IC_{50}, half maximal inhibitory concentration; ECD, electrostatic circular dichroism; LDH, lactate dehydrogenase; MICs, minimum inhibitory concentrations; FICI, fractional inhibitory concentration index; HLM, human liver microsomes; CL, intrinsic clearance; LD_{50}, median lethal dose;

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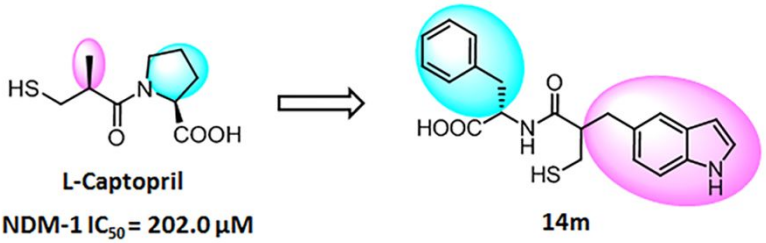


L-Captopril

NDM-1 IC_{50} = 202.0 µM

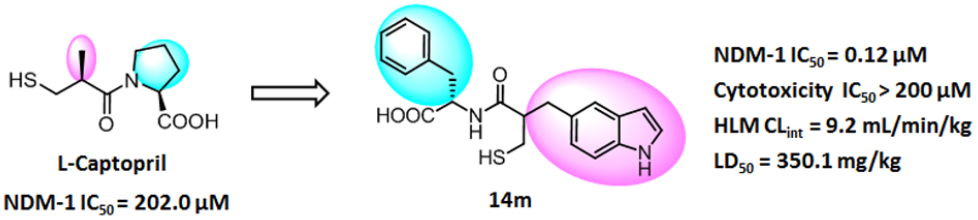
14m

NDM-1 IC_{50} = 0.12 µM

Cytotoxicity IC_{50} > 200 µM

HLM CL_{int} = 9.2 mL/min/kg

LD_{50} = 350.1 mg/kg



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