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Synthesis of 1,3,4-trisubstituted pyrrolidines as meropenem adjuvants targeting New Delhi metallo-β-lactamase†

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The effective strategies to neutralize the New Delhi metallo-β-lactamase (NDM-1) activity offer unique opportunities to combination therapy because NDM-1 inactivates all classes of carbapenem antibiotics, which are widely regarded as the last resort of drugs for treating serious bacterial infections. Here we describe the efficient construction of a series of *trans*-1,3,4-trisubstituted pyrrolidines *via* boric acid-catalyzed 1,3-dipolar cycloaddition of *N*-benzylazomethine ylide with methyl ferulate for the biological evaluation of their cytotoxicity and synergistic activity in combination with meropenem towards NDM-1 positive carbapenem-resistant *Enterobacteriaceae* (CRE). The cell-based screens generated one promising hit, namely compound **10e**, which exhibited low cytotoxicity (IC_{50} > 128 μM), moderate NDM-1 enzyme inhibition (IC_{50} = 51 μM), and potent synergistic activity against a panel of clinically isolated NDM-1 positive CRE with fractional inhibitory concentration indexes ranging from 0.01 to 0.25. Structure–activity relationship studies revealed that the zinc-chelating moiety of 2-(bis(pyridin-2-ylmethyl)-amino)acetyl group of compound **10e** plays a pivotal role for potent activity. Regarding the inhibition mechanism, a series of biochemical assays revealed that compound **10e** may inactivate NDM-1 activity by displacing both zinc ions from the active site of the enzyme. Altogether, our studies indicate that compound **10e** represents an important pyrrolidine-type scaffold targeting NDM-1, providing a promising starting point to be further developed as carbapenem antibiotic adjuvants.

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Introduction

Carbapenem antibiotics are highly effective "last-resort" β-lactam antibiotics commonly used for treating severe bacterial infections such as bloodstream infections and pneumonia.[1] Unfortunately, the worldwide dissemination of carbapenem-resistant *Enterobacteriaceae* (CRE) over the past decade severely limits their therapeutic options, rendering severe clinical infections difficult to treat.[2] The World Health Organization has recently prioritized CRE as one of the Priority 1 ("CRITICAL") microorganisms for which new drugs are urgently needed.[3] CRE are a large family of Gram-negative bacteria that acquire carbapenem resistance by overexpressing carbapenemases, which are powerful β-lactamases capable of inactivating all carbapenem antibiotics by breaking down the β-lactam ring of the molecules. Worse still, these β-lactamases are not only encoded by genes that are horizontally transferable by plasmids but also associated commonly with genes encoding for other resistant determinants, rendering CRE a veritable "superbug" which exhibits drug resistance to all current antibiotics.

Metallo-β-lactamases (MBLs) constitute one of the major groups of clinically important carbapenemases, particularly New Delhi metallo-β-lactamase (NDM-1). NDM-1 is one of the zinc-dependent β-lactamases that can inactivate β-lactam antibiotics without proceeding *via* a covalent intermediate. Therefore, NDM-1 is generally regarded as the MBLs of greatest

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† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra of compounds **4–10**; MIC screening of compound alone and MRM in the presence of the compound at 100 μM against *E. coli* BL21 (NDM-1), calculated *c* log *P*, topological polar surface area (tPSA) and reduction fold (RF); X-ray crystal structure of **9b**; HPLC chromatogram of **6a**; crystal data and structure refinement for compound **9b**. CCDC 2046394. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/d0nj06090a

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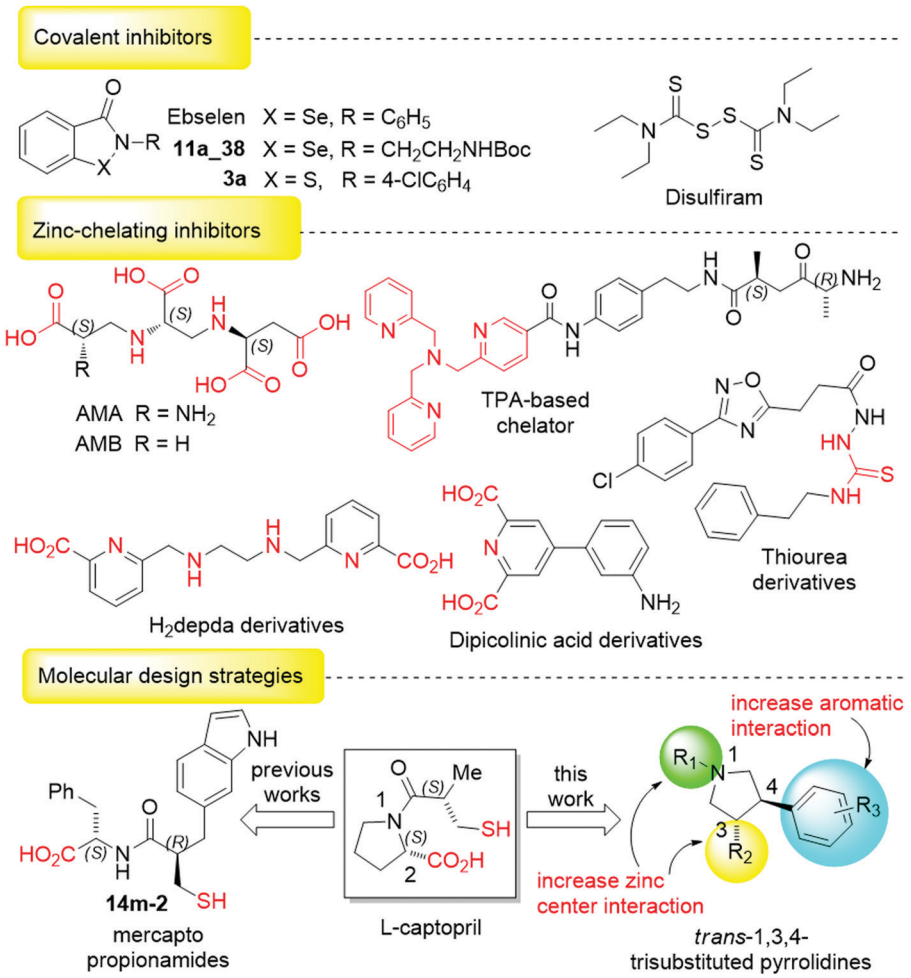


Fig. 1 The chemical structures of reported NDM-1 inhibitors and molecular design strategies from captopril.

concerns as it is not only the most easily communicable but also capable of compromising the clinical efficacy of almost all β-lactam antibiotics except aztreonam. There are no inhibitors of MBLs in current clinical use. Therefore, effective strategies to neutralize the activity of NDM-1 are highly desirable. Over the past decade of research efforts, two major classes of promising NDM-1 inhibitors including covalent inhibitors and zinc chelating inhibitors have been reported in the literature throwing light on the importance of metal chelation and anchor residue binding features.[4–6] Meanwhile, docking platform also shed light on the key interaction of molecules with the amino acids of NDM-1 active site.[7] As depicted in Fig. 1, disulfiram,[8] ebselen (Eb)[9] as well as its derivatives **11a_38**[10] and ebsulfur derivative **3a**[11,12] are examples of potent covalent NDM-1 inhibitors targeting important amino acid residue Cys221 of NDM-1 enzyme. On the other hand, zinc chelating inhibitors, such as AMA and its amino carboxylic acid analog AMB,[13,14] tris-picolylamine (TPA)-based chelators,[15] H_2dedpa derivatives,[16,17] thiourea derivatives,[18,19] and dipicolinic acid derivatives[20] are also reported to potently inhibit NDM-1 with the half-maximal inhibitory concentration (IC_{50}) at single-digit micromolar concentrations *via* a zinc-depletion mechanism.

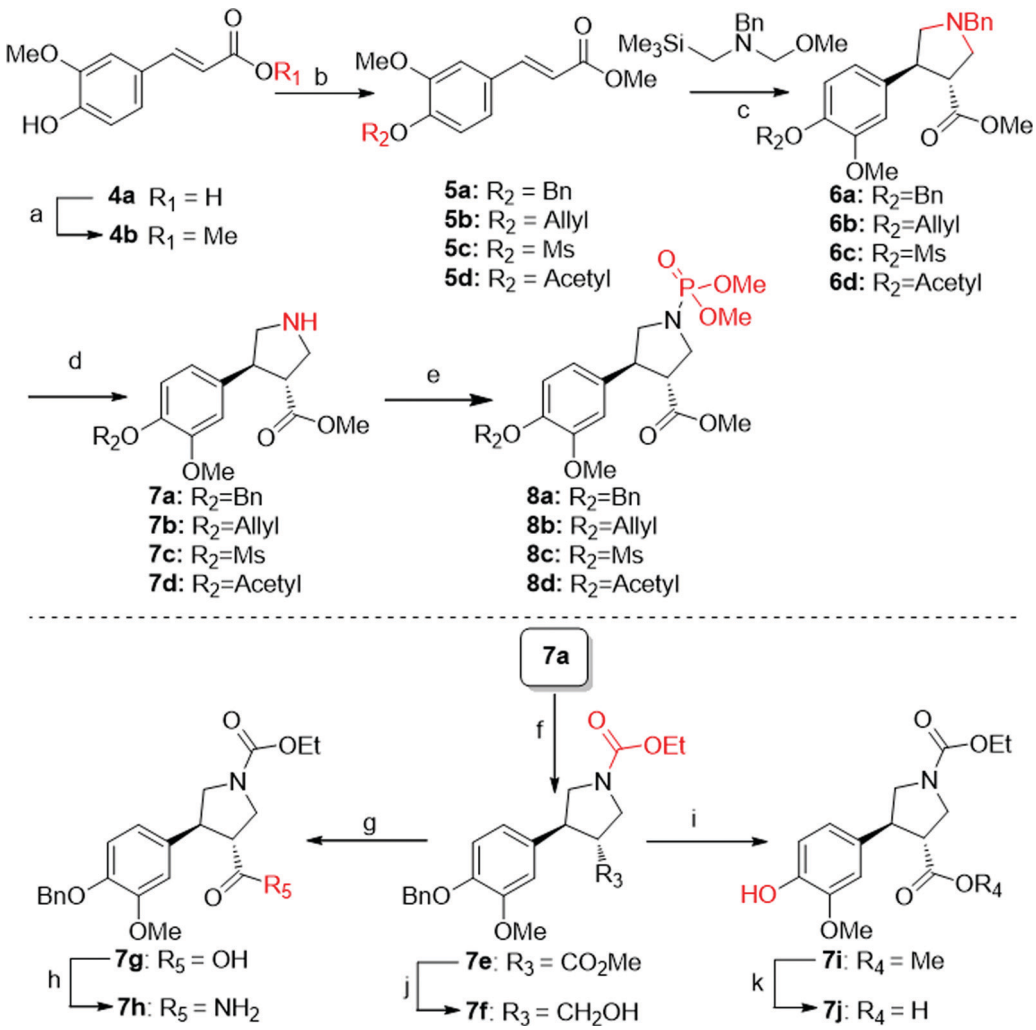
Among the reported sulfur-containing NDM-1 inhibitors, both D- and L-captopril (Fig. 1) have been documented as the most promising candidates for inhibition of NDM-1 upon imipenem hydrolysis with IC_{50} values of 7.9 and 202.0 μM, respectively.[21,22] The thiol and carboxylic acid moieties of captopril are likely to interact with the dinuclear zinc center of NDM-1 by displacing the catalytic hydroxyl ions.[23] Pioneering studies of captopril modifications by other research groups have identified a series of chiral mercapto propionamides **14m-2** and other derivatives with potent NDM-1 inhibition (Fig. 1).[24–26] However, adverse effects associated with thiol-containing compounds, such as rashes and loss of taste, may present a substantial barrier for further development.[27,28] In the present study, we describe our strategy to further modify captopril by constructing a small library of 1,3,4-trisubstituted pyrrolidine derivatives without bearing thiol groups *via* boric acid-catalyzed 1,3-dipolar cycloaddition of *N*-benzylazomethine ylide with methyl ferulate. Subsequent biological evaluation of their cytotoxicity and synergistic activity in combination with meropenem (MRM) towards NDM-1 positive CRE allows us to identify a promising lead targeting NDM-1.

Results and discussion

1. Chemical synthesis

Our molecular design strategy is shown in Fig. 1. The core pyrrolidine ring is retained for maintaining the hydrophobic interaction with Trp64 of NDM-1.[29] Functional group R_1 and carboxylate R_2 are selectively installed at positions 1 and 3 of the pyrrolidine ring respectively. Oxygen-rich R_1 groups that may provide a stronger interaction with the dinuclear zinc center in the active site of the NDM-1 enzyme will be selected. The substituted phenyl ring R_3 is installed at position 4 as this aromatic group may enhance π–π interaction with the amino acid residues such as histidine and tryptophan in the active site of the NDM-1 enzyme. Moreover, the substitutes at positions 3 and 4 of the 1-pyrroline ring of hydrolyzed MRM are in *trans* configuration (please refer to the graphical abstract).[30] We envisioned that the R_2 and R_3 groups should also be arranged similarly for better fitting into the NDM-1 substrate-binding site.

Inspired by previous works,[31,32] we initiated our study by constructing a 1,3,4-trisubstituted pyrrolidine library *via* a key intermediate pyrrolidine **7a**, which was obtained from a concise synthetic method with four steps in good chemical yield. As shown in Scheme 1, esterification of ferulic acid (**4a**) with methanol in the presence of a catalytic amount of sulphuric acid provided methyl ferulate (**4b**). The phenol group of ester **4b** was protected with benzyl bromide under the basic medium to afford benzyl ether **5a** in high yield.[33] Subsequent intermolecular 1,3-dipolar cycloaddition of **5a** as a dipolarophile with nonstabilized *N*-benzylazomethine ylide, which was generated *in situ* from *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzyl-amine in the presence of a catalytic amount of boric acid, furnished desired pyrrolidine **6a** in good yield.[34,35] The chemical structure of the



Scheme 1 *Reagents and reaction conditions*: (a) MeOH, conc. H_2SO_4 (cat.), reflux, 6 h, 62%; (b) for **5a**, BnBr, K_2CO_3, acetone, reflux, 3 h, 80%; for **5b**, allyl bromide, K_2CO_3, acetone, reflux, 3 h, 67%; for **5c**, mesyl chloride, Et_3N, DCM, 0 °C, 4 h, 66%; for **5d**, acetic anhydride, pyridine, rt, 3 h, 54%; (c) $B(OH)_3$ (cat.), DCM, rt, 48 h, for **6a**, 53%, for **6b**, 50%, for **6c**, 57%, for **6d**, 40%; (d) (i) α-chloroethyl chloroformate, DCM, reflux, 3 h; (ii) MeOH, reflux, 3 h, for **7a**, 73%, for **7b**, 68%, for **7c**, 49%, for **7d**, 65%; (e) dimethyl chlorophosphate, NEt_3, DMAP, DCM, 0 °C to rt, 14 h, for **8a**, 76%, for **8b**, 73%, for **8c**, 75%, for **8d**, 53%; (f) ethyl chloroformate, NEt_3, DCM, 0 °C, 4 h, 73%; (g) **7e**, KOH, H_2O, rt to 60 °C, 16 h, 35%; (h) (1) ethyl chloroformate, NEt_3, THF, 0 °C to rt, 16 h; (2) NH_4OH, THF, 0 °C to rt, 16 h, 43%; (i) **7e**, 10% Pd/C, H_2, THF, rt, 16 h, 49%; (j) LiCl, $NaBH_4$, EtOH, THF, 0 °C to rt, 2 h, 44%. (k) KOH, H_2O, rt to 60 °C, 16 h, 43%.

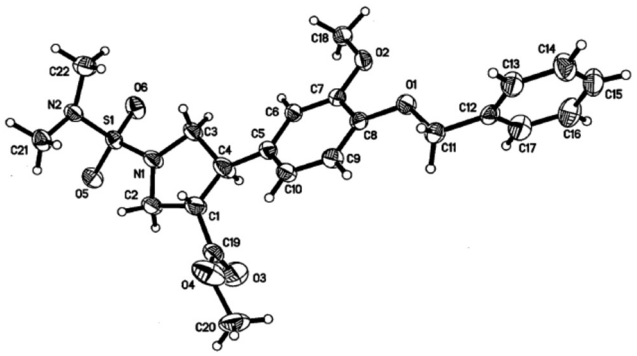


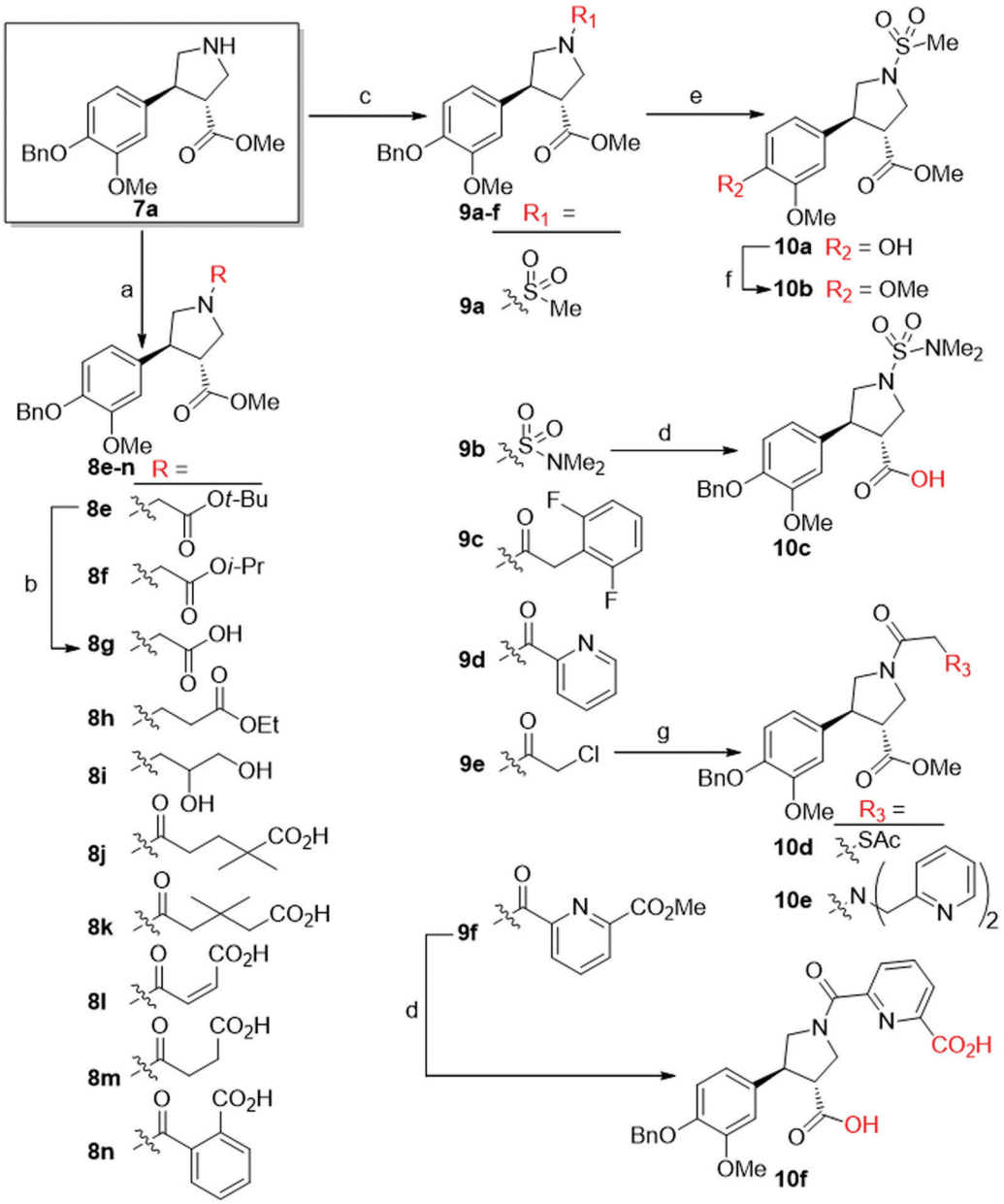
Fig. 2 X-Ray crystal structure of **9b**.

1,3,4-trisubstituted pyrrolidine **6a** was fully supported by the proton and carbon NMR. Moreover, the stereochemistry of pyrrolidine **6a** was initially assigned as a *trans* configuration based on two reasons: (1) a weaker vicinal coupling constant between protons at positions 3 and 4 of the pyrrolidine ring; (2) the inherent *trans* configuration of alkene **5a**. This result was subsequently confirmed by a crystal structure of compound **9b**, indicating that the protons at positions 3 and 4 of pyrrolidine ring are *trans* to each other (Fig. 2). It should be noted that pyrrolidine **6a** was obtained as a racemic mixture (Fig. S44, ESI†). Alternative reaction conditions to produce nonstabilized *N*-benzylazomethine ylide *in situ* by using trifluoroacetic acid (TFA), acetic acid, *p*-toluenesulfonic acid sulphuric acid or potassium fluoride resulted in a low yield of the desired product. Under a mild reaction condition, selective *N*-debenzylation of tertiary amine of pyrrolidine **6a** by treating with α-chloroethyl chloroformate followed by refluxing in methanol delivered multigram quantities of the key intermediate pyrrolidine **7a** for the ensuing modification.[36] Similarly, by replacing the phenol protecting group in the second step with other groups, such as allyl, mesyl, or acetyl groups, pyrrolidines **7b–d** were furnished smoothly without further purification. Having constructed the skeletal of pyrrolidines **7**, we next targeted modifying the secondary amine moiety of pyrrolidines **7a–d**. Treatment of pyrrolidines **7a–d** with dimethyl chlorophosphate in the presence of 4-(dimethylamino)pyridine (DMAP) as a catalyst furnished pyrrolidines **8a–d** in good yield. Moreover, pyrrolidines **7a** was selected to react with ethyl chloroformate using DCM as a solvent at an ice bath temperature, providing carbamate **7e** in good yield. The methyl ester group at position 3 of carbamate **7e** was sequentially converted into carboxylic acid **7g** and amide **7h** under standard reaction conditions. Additionally, selective reduction of the methyl ester group of carbamate **7e** by using alcoholic solutions of lithium borohydride afforded alcohol **7f** without destroying the carbamate group.[37] Under the hydrogen atmosphere at balloon pressure, the benzyl group of carbamate **7e** was removed by Pd/C-catalyzed hydrogenation providing pyrrolidine **7i**, which was further converted to the carboxylic acid **7j** by treating with potassium hydroxide in moderate yield.

As depicted in Scheme 2, the same route could be repurposed to allow diversified functionalization of the secondary amine moiety of pyrrolidine **7a**. Amine alkylation of pyrrolidine **7a** with various bromoesters, such as *t*-butyl 2-bromoacetate, i-propyl 2-bromoacetate, and ethyl 3-bromopropanoate, or 3-chloropropane-1,2-diol under the basic medium provided pyrrolidine **8e–f** and **8h–i** respectively. Subsequent acidic treatment for removing the *tert*-butyl group of **8e** with TFA using DCM as solvent delivered carboxylic acid **8g** in moderate yield. To obtain more pyrrolidine derivatives bearing a carboxylic acid group at position 1 for the biological study, treatment of pyrrolidine **7a** with a series of cyclic anhydrides, including 2,2-dimethylglutaric anhydride, 3,3-dimethylglutaric anhydride, maleic anhydride, succinic anhydride, and phthalic anhydride in the presence of trimethylamine afforded acids **8j–n** in an operationally simple and scalable way. Similarly, treatment of pyrrolidine **7a** with mesyl chloride or *N*,*N*-dimethylsulfamoyl chloride using DCM as a solvent in the presence of trimethylamine afforded pyrrolidines **9a–b** in good yield respectively. The X-ray crystallographic structure of compound **9b**, crystallized in ethyl acetate and hexane, confirmed the existence of the *trans* configuration of these pyrrolidine derivatives (Fig. 2). Besides, direct amidation of a series of acid chlorides, including 2-(2,6-difluorophenyl)acetyl chloride, picolinoyl chloride, 2-chloroacetyl chloride, and methyl 6-(chlorocarbonyl)picolinate with pyrrolidine **7a** under mild condition delivered desired pyrrolidines **9c–f** respectively in moderate yield. Deprotection of the benzyl group of **9a** by using Pd/C-catalyzed hydrogenation under hydrogen atmosphere followed by methylation with iodomethane under basic medium furnished smoothly pyrrolidine **10a** and **10b**. Pyrrolidines **9b** and **9f** were further converted to carboxylic acid **10c** and diacid **10f** respectively by using the same reaction condition (potassium hydroxide) in moderate yield. Finally, the substitution of the chloride moiety of **9e** with thioacetic acid or bis(pyridin-2-ylmethyl)amine provided pyrrolidines **10d** and **10e** in moderate yield respectively. Collectively, all compounds were easily accessible from commercially available building blocks within three to four synthetic steps, allowing the rapid construction of a compound library for biological screening. The important parameters of "drug-likeness", such as *c* log *P* and topological polar surface area (tPSA) of all synthesized compounds were calculated as a guide to prioritize the chemical syntheses (Table 1).[38]

2. Biological evaluation

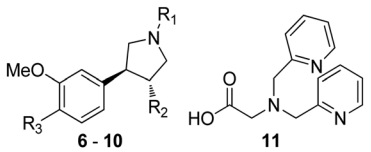
2.1 Cell-based screen using *E. coli* BL21 (NDM-1) strain identified compound 10e as a promising MRM adjuvant. With a sufficient amount of compounds in hand, the next stage was set for a cell-based screen for inhibitors of the NDM-1. To increase the screen sensitivity, a test strain with a clean background of MRM-resistant *E. coli* BL21 (NDM-1) carrying only an isopropyl β-D-1-thiogalacto-pyranoside (IPTG)-inducible plasmid pET28b-bla_{NDM-1} was produced from a parental *E. coli* BL21 strain without producing NDM-1.[9] All antimicrobial tests were performed according to the Clinical and Laboratory Standards Institute (CLSI) approved standard guidelines.[39] The minimum inhibition concentration (MIC) of MRM towards this test strain of *E. coli* BL21 (NDM-1) was found to be 128 μg mL^{-1} (Table 1, entry 1), which was 1024-fold higher than the parental *E. coli* BL21 (MIC of Mem = 0.125 μg mL^{-1}). This result indicated that



Scheme 2 *Reagents and reaction conditions*: (a) for **8e**, *t*-butyl 2-bromoacetate, K_2CO_3, KI (cat.), DCM, 0 °C to rt, 14 h, 80%; for **8f**, i-propyl 2-bromoacetate, K_2CO_3, KI (cat.), DCM, 0 °C to rt, 14 h, 67%; for **8h**, ethyl 3-bromopropanoate, K_2CO_3, KI (cat.), DCM, 0 °C to rt, 14 h, 53%; for **8i**, (±)-3-chloropropane-1,2-diol, NEt_3, DCM, 0 °C to reflux, 16 h, 55%; for **8j**, 2,2-dimethylglutaric anhydride, NEt_3, DCM, 0 °C to rt, 16 h, 71%; for **8k**, 3,3-dimethylglutaric anhydride, NEt_3, DCM, 0 °C to rt, 16 h, 63%; for **8l**, maleic anhydride, NEt_3, DCM, 0 °C to rt, 16 h, 59%; for **8m**, succinic anhydride, NEt_3, DCM, 0 °C to rt, 16 h, 59%; for **8n**, phthalic anhydride, NEt_3, DCM, 0 °C to rt, 16 h, 51%; (b) TFA, DCM, 0 °C to rt, 16 h, 46%. (c) For **9a**, mesyl chloride, NEt_3, DCM, 0 °C, 4 h, 63%; for **9b**, *N*,*N*-dimethylsulfamoyl chloride, NEt_3, DCM, 0 °C, 4 h, 54%; for **9c**, 2-(2,6-difluorophenyl)acetyl chloride, NEt_3, DCM, rt, 4 h, 34%; for **9d**, picolinoyl chloride, NEt_3, DCM, 0 °C to rt, 12 h, 18%; for **9e**, 2-chloroacetyl chloride, NEt_3, DCM, 0 °C to rt, 12 h, 50%; for **9f**, methyl 6-(chlorocarbonyl)picolinate, NEt_3, DCM, 0 °C to rt, 12 h, 41%; (d) KOH, H_2O, MeOH, reflux, 6 h, for **10c**, 39%; For **10f**, 63%; (e) **9a**, 10% Pd/C, H_2, THF, rt, 16 h, 51%; (f) MeI, K_2CO_3, acetone, reflux, 16 h, 71%; (g) for **10d**, thioacetic acid, NEt_3, DCM, 0 °C to rt, 16 h, 71%; for **10e**, bis(pyridin-2-ylmethyl)amine, K_2CO_3, acetone, 0 °C to reflux, 16 h, 33%.

the test strain was capable of overexpressing the NDM-1 enzyme and suitable for the cell-based screen of NDM-1 inhibitors. To assess the off-target effect of these compounds, the antimicrobial activities of all compounds were tested individually by evaluating their MICs towards this test strain. The summarized results are presented in Table 1 (see Table S1 for all compounds, ESI†). Generally, all compounds exhibited very weak inhibitory activities against the growth of the test strain even at a concentration of 128 μg mL^{-1}, implying that these compounds are relatively non-toxic and well-tolerated to bacterial cells. Moreover, at a concentration below 128 μg mL^{-1}, this class of compounds is very unlikely to undergo non-specific interaction with drug targets that may induce cellular toxicity, which is the prerequisite of being a safe antibiotic adjuvant.[40]

Table 1 MIC screening of compound alone and MRM in the presence of the compound at 100 μM towards *E. coli* BL21 (NDM-1), calculated *c* log *P*, topological polar surface area (tPSA), and reduction fold (RF)



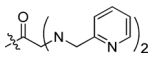
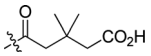
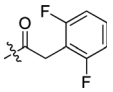
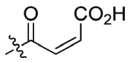
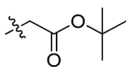
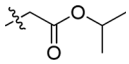
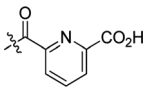
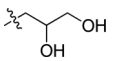
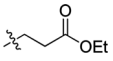
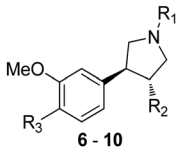
Entry	Cpd no.	R_1	R_2	R_3	*c* log *P*[a]	tPSA[a]	MIC (μg mL^{-1}) Cpd	MRM[b]	RF[c]
1	MRM	N.A.[d]	N.A.	N.A.	N.A.	N.A.	128	N.A.	N.A.
2	**10e**		CO_2Me	OBn	3.68	93.03	>128	0.5	256
3	**11**	N.A.	N.A.	N.A.	0.53	65.26	>128	4	32
4	**8k**		CO_2Me	OBn	3.80	102.37	>128	8	16
5	**9d**		CO_2Me	OBn	2.97	77.43	>128	8	16
6	**9c**		CO_2Me	OBn	4.82	65.07	>128	8	16
7	**8l**		CO_2Me	OBn	3.15	102.37	>128	16	8
8	**9e**		CO_2Me	OBn	3.04	65.07	>128	16	8
9	**8e**		CO_2Me	OBn	4.79	74.30	>128	32	4
10	**8f**		CO_2Me	OBn	4.39	74.30	>128	32	4
11	**9b**		CO_2H	OBn	2.37	96.38	>128	32	4
12	**7j**		CO_2H	OH	1.52	96.30	>128	32	4
13	**8d**		CO_2Me	OAc	0.68	100.60	>128	32	4
14	**10f**		CO_2H	OBn	2.76	125.73	>128	32	4
15	**8i**		CO_2Me	OBn	2.61	88.46	>128	32	4
16	**7a**		CO_2Me	OBn	3.31	56.79	>128	64	2
17	**9a**		CO_2Me	OBn	2.89	82.14	>128	64	2
18	**10a**		CO_2Me	OH	0.64	93.14	>128	64	2
19	**10b**		CO_2Me	OMe	1.12	82.14	>128	64	2
20	**7e**		CO_2Me	OBn	4.23	74.30	>128	64	2
21	**8g**		CO_2Me	OBn	1.24	85.30	>128	64	2
22	**7g**		CO_2H	OBn	3.76	85.30	>128	64	2
23	**8h**		CO_2Me	OBn	4.36	74.30	>128	64	2
24	**7f**		CH_2OH	OBn	3.66	68.23	>128	64	2

Table 1 (*continued*)



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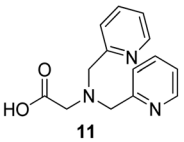
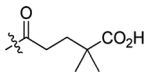
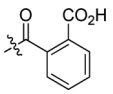


11

Entry	Cpd no.	R_1	R_2	R_3	$c\log P^a$	tPSA[a]	MIC (μg mL^{-1}) Cpd	MRM[b]	RF[c]
25	**7h**		$CONH_2$	OBn	3.05	91.09	>128	64	2
26	**8j**		CO_2Me	OBn	3.71	102.37	>128	64	2
27	**8a**		CO_2Me	OBn	3.01	83.53	>128	64	2
28	**6c**		CO_2Me	OMs	2.83	82.14	>128	64	2
29	**6b**		CO_2Me	OAllyl	4.25	48.00	>128	64	2
30	**8b**		CO_2Me	OAllyl	2.02	83.53	>128	64	2
31	**8n**		CO_2Me	OBn	4.07	102.37	>128	64	2

[a] Compound's $c\log P$ and tPSA values were calculated using the ChemDraw Ultra (version 12.0). [b] MIC value of MRM in the presence of a compound at 100 μM. [c] Reduction fold (RF) was calculated by MIC of MRM alone divided by MIC of MRM in the presence of 100 μM of the test compound. [d] N.A.: not applicable; N = 1–3 independent experiments.

To examine the synergistic activities of these compounds in combination with MRM, the MICs of MRM were systematically evaluated in the presence of compounds at a fixed concentration of 100 μM. The reduction folds (RF), which were defined as the ratio of MIC of MRM along to MIC of MRM in the presence of compounds at 100 μM, were employed to compare the compound's synergistic activity directly. The compound showing a larger RF value has a more prominent effect on reversing the antimicrobial activity of MRM. The screen results are summarized in Table 1, in which only MICs of MRM $\leq$64 μg mL^{-1} are shown. MICs of MRM $\geq$64 μg mL^{-1} are shown in the ESI† (Table S1). In general, all compounds exhibited relatively weak synergistic activity with RF ranged from 2 to 16 (Table 1, entries 4–31), except compound **10e**. We reasoned that may due to several reasons: (1) the weak interactions between the dinuclear zinc center of NDM-1 and the R_1 groups; (2) the intrinsic low permeability of compound itself to pass through the cell membrane of *E. coli*; (3) the active membrane efflux pumps presented in the *E. coli*. More experiments are required to test these hypotheses. Encouragingly, compound **10e** was found to display the most promising synergistic activity with an RF value of 256 (Table 1, entry 2). In the presence of 100 μM **10e**, the MIC of MRM dramatically reduced from 128 μg mL^{-1} to 0.5 μg mL^{-1}. Structurally, this compound possesses not only optimum drug-likeness properties ($c\log P$ = 3.68, tPSA = 93.03) but also important warheads of a 2-(bis(pyridin-2-ylmethyl)-amino)acetyl group (R_1), a methyl ester group, and a phenyl benzyl ether group at positions 1, 3 and 4 of the pyrrolidine respectively. Removal of the R_1 group of compound **10e** (compound **7a**) dramatically reduced the RF from 256 to 2 (Table 1, entry 16), suggesting that the 2-(bis(pyridin-2-ylmethyl)-amino)acetyl group plays a pivotal role for the potent activity. Interestingly, 2-(bis(pyridin-2-ylmethyl)-amino)acetic acid (**11**) (Table 1) has been reported to be a zinc chelator, forming a stable zinc–ligand complex *via* the carboxylic acid group and three nitrogen atoms.[41] Therefore, it was synthesized and tested, demonstrating a potent synergistic activity with an RF value of 32. However, replacements of the R_1 group of compound **10e** with other zinc-chelating moieties, such as picolinoyl group (Table 1, entry 5) and 6-(hydroxycarbonyl)-picolinoyl group (Table 1, entry 14) weakened the synergistic activity. Modification of the R_2 and R_3 groups also resulted in no improvement in the synergistic activity. Taken together, compound **10e** demonstrated the most promising RF value. We reasoned that compound **10e** may inhibit NDM-1 activity by acting as a zinc chelator just like compound **11**. Compound **10e** was, therefore, selected for detailed mechanistic characterization.

2.2 Compound 10e exhibited low cytotoxicity and moderate inhibition of NDM-1 enzyme *via* zinc depletion mechanism. The next question we need to answer is whether the observed synergism of **10e** and MRM combination is due to the inhibition of NDM-1. To address this question, we sought to conduct a series of standard biochemical assays using the purified NDM-1 enzyme and colorimetric β-lactamase substrate nitrocefin as previously described.[9] Enzyme inhibition assay revealed that

compound **10e** inhibited moderately the NDM-1 activity with a half-maximal inhibitory concentration (IC_{50}) of 51 ± 7 μM with a calculated K_i of 4.6 ± 0.8 μM (Fig. 3a, blue line). As mentioned before, compound **10e** may inhibit NDM-1 activity by acting as a zinc chelator. Next, we sought to test whether the compound could inhibit NDM-1 activity at a higher concentration of zinc content. The enzyme inhibition assay was then conducted in the presence of 50 μM zinc sulfate. Surprisingly, compared with the results without adding zinc sulfate, the calculated IC_{50} and K_i values of compound **10e** were increased by two-fold to 103 ± 12 μM and 9.4 ± 1.1 μM, respectively (Fig. 3a, red line), resulting in stronger enzyme activity and weaker NDM-1 inhibition of compound **10e**. These results suggested that compound **10e** may form a stable zinc-compound **10e** complex probably *via* the 2-(bis(pyridin-2-ylmethyl)-amino)acetyl group at high zinc ion concentration, resulting in a weaker NDM-1 inhibition. To further investigate the mechanism of the interaction between compound **10e** and NDM-1 enzyme, nano ESI-MS analysis was conducted (Fig. 3c–e). The molecular weight of the native NDM-1 enzyme was found to be approximately 26 052 Da (Fig. 3c). Incubating the NDM-1 enzyme with excessive compound **10e** at a ratio of 1 : 10 led to an obvious mass shift to a peak showing the molecular weight of 25 920 Da (Fig. 3d). The delta mass (132 Da) was approximate to the loss of two zinc ions, suggesting that compound **10e** inhibited NDM-1 activity by complete removal of both zinc ion in the active site. On the other hand, a well-known zinc chelator, ethylenediaminetetracetic acid (EDTA), has been reported to inhibit NDM-1 activity with an IC_{50} of 0.4 μM *via* zinc depletion mechanism.[42,43] Incubating the NDM-1 enzyme with EDTA also led to a similar mass shift to a peak with the molecular weight of 25 919 Da (Fig. 3e). Collectively, these data confirmed that compound **10e** could extract two zinc ions from the NDM-1 enzyme and therefore inhibit its activity. One of the potential problems usually associated with zinc chelators is their non-specific interactions with other metalloproteins, causing relatively high toxicity towards eukaryotic cells. To confirm the *in vitro* cytotoxicity of compound **10e** to normal cells, MTT (thiazolyl blue tetrazolium bromide) assay was employed next.[44] HEK293 (human embryonic kidney) cells viability was assessed in the presence of various concentrations of compound **10e**. As shown in Fig. 3b, compound **10e** exhibited relatively low cytotoxicity with 80% of HEK293 cells survived in the presence of

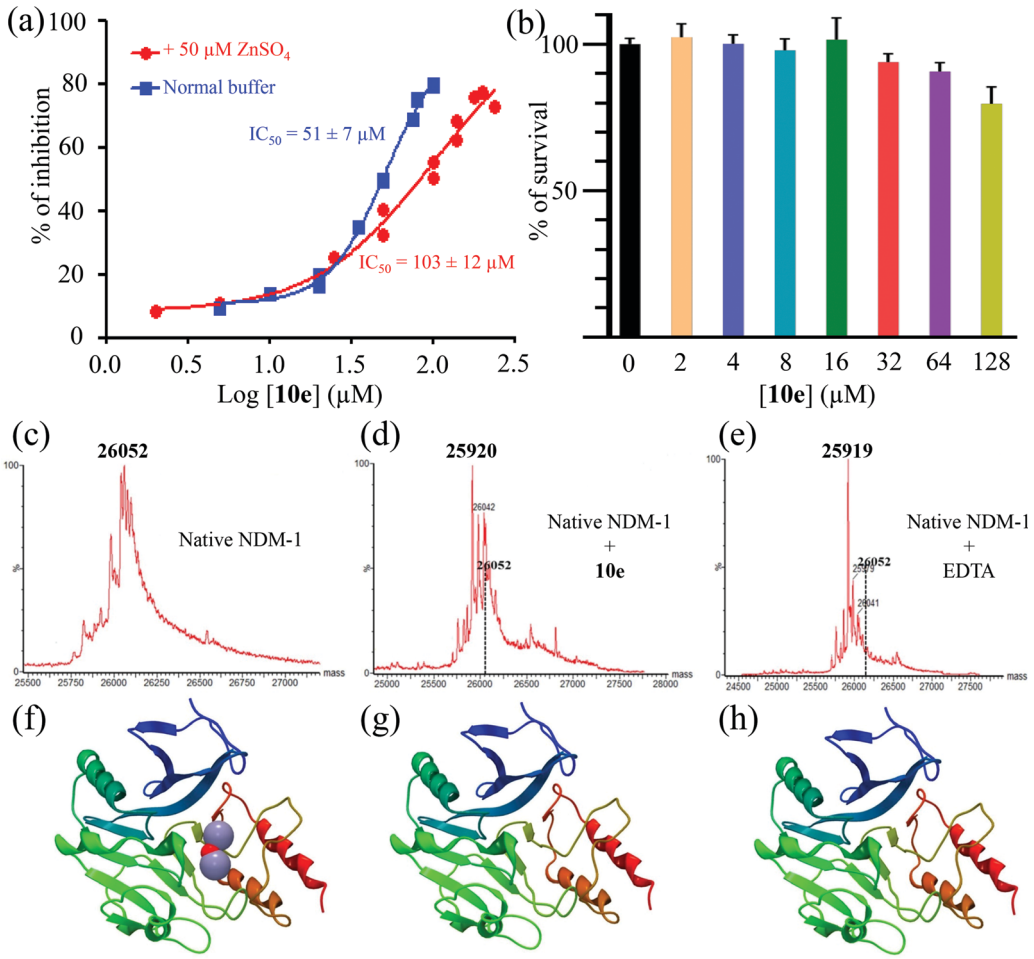


Fig. 3 (a) Inhibition of NDM-1 enzyme by **10e** in the normal buffer (blue line) or with the addition of 50 μM $ZnSO_4$ solution (red line); (b) cytotoxicity of **10e** towards HEK cell lines; nano-ESI-MS analysis of native NDM-1 (c), native NDM-1 treated with **10e** (d), native NDM-1 treated with ETDA (e) and their cartoon representations (f–h). Grey balls represent the zinc ions.

compound **10e** at 128 μM after 24 hours treatment, indicating that compound **10e** is relatively non-toxic and well-tolerated to eukaryotic cells. Moreover, this concentration is much higher than the effective concentration in the combination study.

2.3 Docking study of both enantiomers of compound 10e. To gain more insights into the molecular interaction of compound **10e** with NDM-1 enzyme, computational docking studies of both enantiomers of compound **10e** using previously reported crystal structure NDM-1 (PDB ID: 4EXS) was conducted (Fig. 4).[45] The results of docking studies revealed that the highest docking score positioned both enantiomers of compound **10e** into the substrate-binding site of NDM-1. The warheads of 2-(bis(pyridin-2-ylmethyl)-amino)acetyl group of both enantiomers were well-situated in the active site of NDM-1, interacting with the dinuclear zinc center. For 3*R*,4*S*-**10e** enantiomer, a conventional hydrogen-bonding interaction was predicted to occur between the amide group of His122 and the ester group of **10e** (Fig. 4a). For 3*S*,4*R*-**10e** enantiomer, two hydrogen-bonding interactions were predicted to occur between (1) the amine group of Lys211 and the ester group of **10e**; (2) the amide group of His122 and pyridinyl group of **10e** (Fig. 4b). Several important amino acid residues of NDM-1 enzyme were predicted to be closely adjacent to both enantiomers of **10e**, such as Glu152, Met194, Asp223, Gly222, Ala224, Leu221, His189, Asn220, Gly219, Leu218, Lys211, His250, Met67, Pro68, Leu65, Trp93, Asp124, Gln123, His122, His120, implying that these amino acids may also be involved in the van der Waals interaction with compound **10e**. Importantly, the top-ranked scores of both enantiomers of compound **10e** are −79, suggesting that both enantiomers may bind to the NDM-1 enzyme with similar binding affinity.

2.4 Combination of compound 10e and MRM against clinically isolated NDM-1 positive CRE. Based on the promising results of **10e** and MRM combination towards *E. coli* BL21 (NDM-1) strain, we sought to examine whether the NDM-1-mediated resistance to MRM in the screening strain could also be reproduced in our in-house collection of clinically isolated NDM-1-producing CRE strains, including three *E. coli*, one *K. oxytoca*, one *C. freundii*, one *E. cloacae*, and two *M. morganii* strains. These CRE strains were highly MRM-resistant owing to the overexpression of the NDM-1 enzyme with half of the CRE strains exhibiting the MICs of MRM $\geq$128 μg mL^{-1} (Table 2, entries 3–6). In addition to producing NDM-1, four of these strains also co-expressed other β-lactamases such as CTX-M-3, CTX-M-14, TEM-1, and SHV-12. As illustrated in Table 2, compound **10e** exhibited no antibacterial activity itself (MICs $\geq$ 128 μg mL^{-1}) but a strong synergy with the combination of MRM across this panel of clinical isolates with fractional inhibitory concentration index (FICI) ranging from 0.01 to 0.25. Compared with the positive control Eb, compound **10e** exhibited much stronger synergy, meriting further development as carbapenem antibiotic adjuvant.

The worldwide dissemination of NDM-1 positive CRE has become a major threat to human health. However, there are currently no FDA-approved NDM-1 inhibitors available in clinical use. Therefore, it is very important to keep on the development of new compounds to meet clinical needs. In this study, we have modified the structure of well-known NDM-1 inhibitor captopril by synthesizing a compound library of trisubstituted pyrrolidines

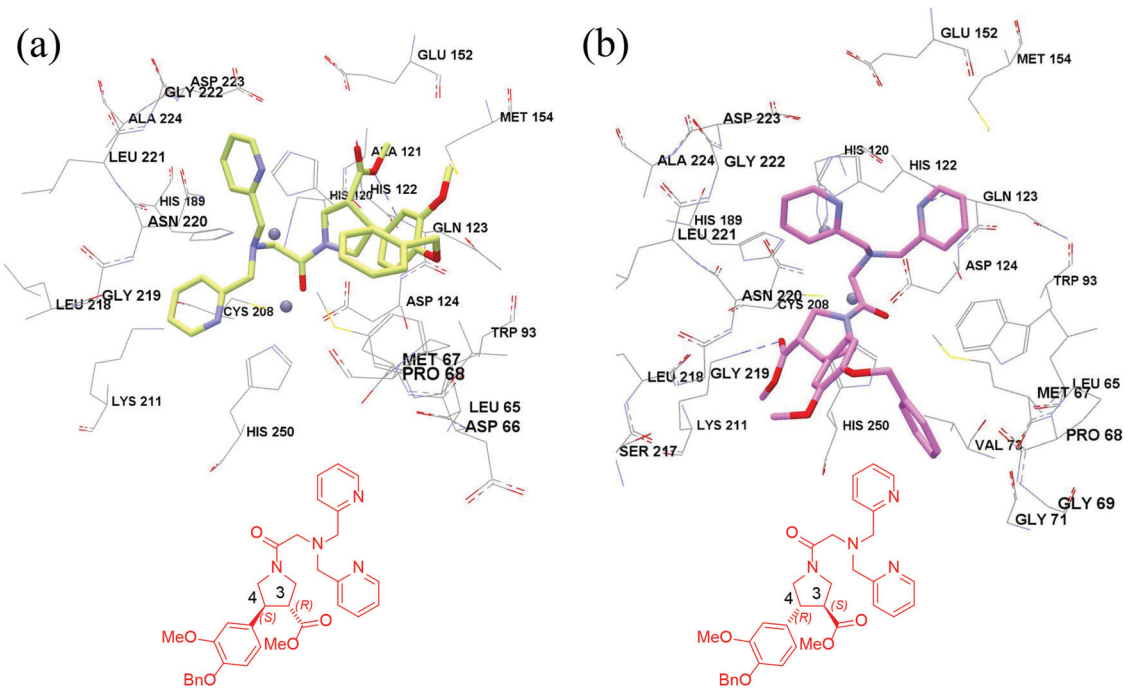
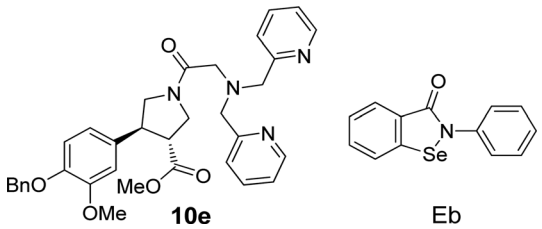


Fig. 4 Models of compound **10e** ((a) 3*R*,4*S*-**10e**, (b) 3*S*,4*R*-**10e**) docked into the substrate-binding site of NDM-1 using the X-ray crystal structure of NDM-1 (PDB ID: 4EXS) with labeled important amino acid residues. Grey balls and blue dotted lines represent zinc ion and hydrogen-bonding interaction respectively.

Table 2 MIC (μg mL^{-1}) screening of compound **10e** and Eb in combination with MRM against clinically isolated NDM-1 positive CRE strains[a]



Entry	CRE strains	Additional β-lactamase determinants	MIC (μg mL^{-1}) MRM	**10e**	**10e** + MRM	FICI	MIC (μg mL^{-1}) Eb	Eb + MRM	FICI
1	EC4-1	None	64	≥128	1	0.02	—	—	—
2	EC36-2	None	32	≥128	0.25	0.01	—	—	—
3	EC06	b1, b2, b4	≥128	≥128	16	0.25	≥128	≥128	2.00
4	KO03	b4	≥128	≥128	4	0.06	≥128	32	0.50
5	CF05	b4	≥128	≥128	8	0.13	≥128	16	0.25
6	EL22	b2, b3, b4	≥128	≥128	2	0.03	≥128	≥128	2.00
7	MM23	None	64	≥128	4	0.09	≥128	64	1.50
8	MM26	None	64	≥128	8	0.19	≥128	64	1.50

[a] EC, *Escherichia coli*; KO, *Klebsiella oxytoca*; CF, *Citrobacter freundii*; EL, *Enterobacter cloacae*; MM, *Morganella morganii*. Additional β-lactamase determinants: b1: CTX-M-3, b2: CTX-M-14, b3: TEM-1, and b4: SHV-12. The synergistic effect is depicted by the FICI, which is calculated as FIC (cpd) + FIC (MRM), where FIC (cpd) is the (MIC of cpd in combination with MRM)/(MIC of cpd alone) while FIC (MRM) is (MIC of cpd in combination with MRM)/(MIC of MRM alone). If the MIC value is ≥128, then 128 was used for calculating the FICI. The drug combination is considered synergistic if the FICI ≤ 0.5; —: not tested; N = 1–3 independent experiments.

without bearing thiol groups. These newly developed compounds are potential inhibitors neutralizing the activity of NDM-1 metallo-β-lactamase, particularly compound **10e** which demonstrates the ability to restore the activity of meropenem against clinically isolated NDM-1 positive CRE. The advantage of these compounds avoids the adverse effects associated with thiol-containing compounds. Moreover, the scaffold of compound **10e** provides a valuable template for deeper exploration in structural diversity and could be a potential source of new, more drug-like NDM-1 inhibitors. It is likely that the structural exploration of other similar classes of pyrrolidine derivatives without the hydrophobic phenyl rings, especially those capable of metal coordination, may lead to more active NDM-1 inhibitors with the improved physiochemical property.

Conclusion

In this study, based on the rational design on the captopril scaffold, a total of 36 *trans*-1,3,4-trisubstituted pyrrolidine derivatives without bearing thiol group have been synthesized *via* boric acid-catalyzed 1,3-dipolar cycloaddition of *N*-benzylazomethine ylide with methyl ferulate for biological evaluation as MRM adjuvant targeting NDM-1. Through a cell-based screen, compound **10e** bearing a zinc-chelating moiety of 2-(bis(pyridin-2-ylmethyl)-amino)acetyl group at R_1 was found to exhibit low cytotoxicity (IC_{50} > 128 μM), moderate NDM-1 enzyme inhibition (IC_{50} = 51 μM), and potent synergistic activity against a panel of clinically isolated NDM-1 positive CRE with FICI ranged from 0.01 to 0.25. A series of biochemical assays further confirmed that compound **10e** inhibits NDM-1 activity *via* displacing two zinc ions from NDM-1, which was similar to EDTA. Altogether, our studies indicate that **10e** represents an important pyrrolidine-type scaffold targeting NDM-1, providing a promising starting point to be further developed as carbapenem antibiotic adjuvants.

Experimental section

General

All commercially available starting materials, reagents, and solvents, unless otherwise stated, were used as received without further purification. All reactions were monitored by using the thin-layer chromatography (TLC) (silica gel 60-F_{254}, E. Merck) and visualized under short and long UV light. Flash column chromatography was carried out on the silica-gel 60 (200–300 mesh). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured at room temperature on a Bruker Advance-III spectrometer (400 MHz FT-NMR System) with tetramethylsilane as an internal standard. Chemical shifts are expressed in δ (ppm) and coupling constants (J) in Hz. Low-resolution (LRMS) and high-resolution mass spectra (HRMS) were obtained on a Micromass Q-TOF-2 by electron spray ionization (ESI) mode.

Synthesis of methyl ferulate (4b). Ferulic acid (**4a**) (5.0 g, 26 mmol) was dissolved in MeOH (100 mL) in the presence of two drops of concentrated sulfuric acid as a catalyst. The solution was heated under reflux for 6 hours. The reaction mixture was cooled to room temperature, concentrated under reduced pressure, and redissolved in ethyl acetate (EA) (80 mL). The reaction mixture was washed with water (200 mL × 3). The organic portion was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford the desired product as a pale yellow oil (3.3 g, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, J = 16.6 Hz, 1H), 7.07 (d, J = 7.8 Hz, 1H), 7.02 (s, 1H), 6.92 (d, J = 7.8 Hz, 1H), 6.30 (d, J = 15.7 Hz, 1H), 3.91 (s, 3H), 3.80 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.9, 148.2,

147.0, 145.1, 126.8, 123.0, 115.0, 114.9, 109.7, 55.9, 51.6; LRMS (ESI) *m*/*z* $C_{11}H_{13}O_4$ (M + H^+) 209.2.

Synthesis of (*E*)-methyl 3-(4-(benzyloxy)-3-methoxyphenyl)acrylate (5a). Compound **4b** (3.3 g, 16 mmol) was dissolved in acetone and subsequently mixed with benzyl bromide (4.1 g, 24 mmol) and potassium carbonate (2.2 g, 16 mmol). The resulting mixture was heated under reflux for 3 hours. Excess triethylamine (2.4 g, 24 mmol) was added to the reaction mixture and heated under reflux for 12 hours. A white solid was precipitated. The crude reaction mixture was cooled to room temperature and subsequently filtered, concentrated under reduced pressure and redissolved in EA (80 mL). The crude reaction mixture was washed with water (5 mL HCl blended, 200 mL × 3). The organic portion was dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to afford the desired product as a white powder (3.8 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 15.65 Hz, 1H), 7.43–7.49 (m, 2H), 7.40 (t, J = 7.34 Hz, 2H), 7.34 (d, J = 6.85 Hz, 1H), 7.02–7.13 (m, 2H), 6.89 (d, J = 8.80 Hz, 1H), 6.33 (d, J = 15.65 Hz, 1H), 5.21 (s, 2H), 3.94 (s, 3H), 3.82 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7, 150.3, 149.8, 144.8, 136.6, 128.7, 128.0, 127.7, 127.2, 122.4, 115.6, 113.4, 110.2, 70.9, 56.0, 51.6; LRMS (ESI) *m*/*z* $C_{18}H_{19}O_4$ (M + H^+) 299.2.

Synthesis of (*E*)-methyl 3-(4-(allyloxy)-3-methoxyphenyl)acrylate (5b). This compound (0.8 g, 67%) was prepared from compound **4b** (1.0 g, 4.8 mmol), allyl bromide (0.7 g, 5.8 mmol), acetone (20 mL) and potassium carbonate (1.0 g, 7.2 mmol) according to the same preparation procedure of compound **5a** described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 16.63 Hz, 1H), 6.99–7.10 (m, 2H), 6.84 (d, J = 7.82 Hz, 1H), 6.30 (d, J = 15.65 Hz, 1H), 5.96–6.14 (m, 1H), 5.33–5.46 (m, 1H), 5.29 (d, J = 8.80 Hz, 1H), 4.62 (d, J = 4.89 Hz, 2H), 3.88 (s, 3H), 3.78 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.6, 150.1, 149.5, 144.7, 132.8, 127.5, 122.4, 118.3, 115.5, 112.8, 110.0, 69.7, 55.9, 51.6; LRMS (ESI) *m*/*z* $C_{14}H_{17}O_4$ (M + H^+) 249.1.

Synthesis of (*E*)-methyl 3-(3-methoxy-4-((methylsulfonyl)oxy)-phenyl)acrylate (5c). Compound **4b** (1.0 g, 4.8 mmol) was dissolved in DCM (20 mL) and subsequently mixed with mesyl chloride (0.7 g, 5.8 mmol) and triethylamine (5 mL) in an ice bath for 4 hours. The crude reaction mixture was warmed to room temperature and subsequently washed with water (50 mL × 2). The organic portion was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to furnish a crude mixture, which was subjected to purification by using the flash column chromatography on silica gel with isocratic elution (EA/Hex, 10%) to afford the desired product (0.9 g, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 15.65 Hz, 1H), 7.26 (d, J = 7.83 Hz, 1H), 7.06–7.14 (m, 2H), 6.38 (d, J = 15.65 Hz, 1H), 3.88 (s, 3H), 3.77 (s, 3H), 3.18 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.0, 151.7, 143.4, 139.5, 134.6, 124.8, 121.1, 119.0, 111.9, 56.1, 51.8, 38.5, 31.6; LRMS (ESI) *m*/*z* $C_{12}H_{15}O_6S$ (M + H^+) 287.1.

Synthesis of (*E*)-methyl 3-(4-acetoxy-3-methoxyphenyl)acrylate (5d). To a well-stirred solution of compound **4b** (1.0 g, 4.8 mmol) in pyridine (10 mL) was added acetic anhydride (0.6 g, 5.8 mmol) and stirred at room temperature for 3 hours. The crude reaction mixture was purified by using the flash column chromatography on silica gel with isocratic elution (EA/Hex, 10%) to afford the desired product (0.65 g, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 15.65 Hz, 1H), 7.04–7.11 (m, 2H), 6.97–7.04 (m, 1H), 6.36 (d, J = 15.65 Hz, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.7, 167.2, 151.4, 144.1, 141.4, 133.3, 123.2, 121.1, 118.0, 111.2, 55.8, 51.7, 20.6; LRMS (ESI) *m*/*z* $C_{13}H_{15}O_5$ (M + H^+) 251.1.

Synthesis of (±)-*trans*-methyl 1-benzyl-4-(4-(benzyloxy)-3-methoxyphenyl)-pyrrolidine-3-carboxylate (6a). Compound **5a** (3.8 g, 13 mmol) was mixed with *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzylamine (4.5 g, 19 mmol) in the presence of boric acid (50 mg) as a catalyst in DCM (60 mL). The solution mixture was stirred at room temperature for 48 hours. The crude reaction mixture was purified by using the flash column chromatography on silica gel with isocratic elution (EA/Hex, 7%) to afford desired product as a pale yellow oil (2.9 g, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.54 (m, 2H), 7.32–7.43 (m, 7H), 7.27–7.32 (m, 1H), 6.94–7.03 (m, 1H), 6.78–6.89 (m, 2H), 5.16 (s, 2H), 3.93 (s, 3H), 3.61–3.80 (m, 6H), 3.08–3.18 (m, 2H), 2.98 (t, J = 8.80 Hz, 1H), 2.84–2.90 (m, 1H), 2.80 (dd, J = 5.87, 9.29 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.7, 149.7, 146.9, 138.9, 137.8, 137.4, 128.7, 128.6, 128.3, 127.8, 127.3, 127.1, 119.3, 114.0, 111.2, 71.2, 61.7, 59.9, 57.5, 56.0, 52.0, 51.8, 46.6; LRMS (ESI) *m*/*z* $C_{27}H_{30}NO_4$ (M + H^+) 433.1.

Synthesis of (±)-*trans*-methyl 4-(4-(allyloxy)-3-methoxyphenyl)-1-benzylpyrrolidine-3-carboxylate (6b). This compound (2.9 g, 50%) was prepared from compound **5b** (3.8 g, 15 mmol), *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzylamine (4.5 g, 19 mmol), and boric acid (50 mg) in DCM (25 mL) according to the same procedure of compound **6a** described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.47 (m, 2H), 7.34 (t, J = 7.34 Hz, 2H), 7.25–7.31 (m, 1H), 6.96 (d, J = 1.96 Hz, 1H), 6.78–6.88 (m, 2H), 6.03–6.16 (m, 1H), 5.35–5.48 (m, 1H), 5.24–5.33 (m, 1H), 4.60 (d, J = 4.89 Hz, 2H), 3.90 (s, 3H), 3.73–3.81 (m, 1H), 3.67–3.73 (m, 4H), 3.61–3.67 (m, 1H), 3.08–3.20 (m, 2H), 2.99 (t, J = 8.80 Hz, 1H), 2.76–2.92 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.7, 149.5, 146.7, 138.9, 137.6, 133.6, 128.7, 128.3, 127.1, 119.2, 117.8, 113.4, 111.0, 70.0, 61.7, 59.9, 57.5, 55.9, 52.0, 51.9, 46.6; HRMS *m*/*z* calcd for $C_{23}H_{28}NO_4$ (M + H)$^+$ 382.1940, found 382.1945.

Synthesis of (±)-*trans*-methyl 1-benzyl-4-(3-methoxy-4-((methylsulfonyl)oxy)phenyl)pyrrolidine-3-carboxylate (6c). This compound (3.1 g, 57%) was prepared from compound **5c** (3.7 g, 13 mmol), *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzylamine (4.5 g, 19 mmol), and boric acid (50 mg) in DCM (25 mL) according to the same procedure of compound **6a** described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25–7.45 (m, 5H), 7.18–7.24 (m, 1H), 7.09 (s, 1H), 6.93 (dd, J = 1.96, 8.80 Hz, 1H), 3.90 (s, 3H), 3.75–3.83 (m, 1H), 3.63–3.73 (m, 5H), 3.09–3.29 (m, 5H), 2.92–3.03 (m, 1H), 2.78–2.92 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.0, 151.4, 136.9, 128.8, 128.4, 127.4, 124.3, 119.9, 112.1, 61.1, 59.7, 57.3, 56.0, 52.1, 51.7, 46.5, 38.2; HRMS *m*/*z* calcd for $C_{21}H_{26}NO_6S$ (M + H)$^+$ 420.1481, found 420.1473.

Synthesis of (±)-*trans*-methyl 4-(4-acetoxy-3-methoxyphenyl)-1-benzylpyrrolidine-3-carboxylate (6d). This compound (0.40 g, 40%) was prepared from compound **5d** (0.65 g, 2.6 mmol), *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzylamine (0.81 g, 3.4 mmol), and boric acid (50 mg) in DCM (25 mL) according to the same procedure of compound **6a** described above. ^{1}H NMR

(400 MHz, $CDCl_3$) δ 7.31–7.44 (m, 4H), 7.25–7.31 (m, 1H), 7.04 (s, 1H), 6.89–7.00 (m, 2H), 3.86 (s, 3H), 3.59–3.81 (m, 6H), 3.08–3.23 (m, 2H), 2.91–3.01 (m, 1H), 2.78–2.89 (m, 2H), 2.33 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.6, 169.2, 151.0, 143.7, 138.8, 138.2, 128.7, 128.4, 127.1, 122.6, 119.5, 111.6, 61.6, 59.8, 57.5, 55.8, 52.0, 51.8, 46.6, 20.7; HRMS *m*/*z* calcd for $C_{22}H_{26}NO_5$ $(M + H)^+$ 384.1733, found 384.1742.

Synthesis of (±)-*trans*-methyl 4-(4-(benzyloxy)-3-methoxyphenyl)-pyrrolidine-3-carboxylate (7a). Compound **6a** (1.0 g, 2.3 mmol) was dissolved in DCM and mixed with 1-chloroethyl chloroformate (0.5 g, 3.5 mmol). The solution was heated under reflux for 3 hours. After that, the solution was dried under high vacuum. The crude product was redissolved in MeOH and refluxed for 3 hours. The mixture was purified by using the flash column chromatography on silica gel with gradient elution (MeOH/DCM, 3% to 10%) to afford desired product as a brown oil (0.58 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.45 (m, 2H) 7.34 (s, 2H) 7.28 (s, 1H) 6.78–6.85 (m, 2H) 6.72 (d, J = 6.6 Hz, 1H) 5.10 (s, 2H) 3.87 (s, 3H) 3.64 (s, 3H) 3.48 (br. s, 2H) 3.39 (d, J = 11.5 Hz, 1H) 3.32 (br. s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.9, 149.8, 147.2, 137.2, 134.5, 128.5, 127.8, 127.3, 119.2, 114.3, 111.4, 71.1, 56.1, 55.3, 52.3, 52.0, 51.4, 49.9. HRMS (ESI) calcd for $C_{20}H_{24}NO_4$ $(M + H^+)$ 342.1700, found 342.1715.

Synthesis of (±)-*trans*-methyl 4-(4-(benzyloxy)-3-methoxyphenyl)-1-(dimethoxyphosphoryl)pyrrolidine-3-carboxylate (8a). A mixture of compound **7a** (0.33 g, 1.0 mmol), dimethyl chlorophosphate (0.22 g, 1.5 mmol) and excess triethylamine (0.30 g, 3.0 mmol) in dry DCM (25 mL) was stirred at 0 °C for 30 min with a little amount of DMAP as the catalyst. The solution was allowed to warm to room temperature and stir for 14 h. The organic solvent was evaporated to dryness to give a crude residue. Subsequent purification of the residue was performed by using flash column chromatography on silica gel with gradient elution (EA/Hex, 10% to 30%) to afford the desired compound as a reddish oil (0.33 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.44 (m, 2H), 7.31 (t, J = 7.34 Hz, 2H), 7.21–7.27 (m, 1H), 6.73–6.85 (m, 2H), 6.65–6.73 (m, 1H), 5.08 (s, 2H), 3.83 (s, 3H), 3.54–3.75 (m, 12H), 3.42–3.52 (m, 1H), 3.25 (dt, J = 2.93, 8.80 Hz, 1H), 3.02–3.16 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.1, 150.0, 147.8, 137.0, 130.1, 128.5, 127.9, 127.2, 119.6, 114.2, 111.2, 71.0, 56.1, 53.2, 53.2, 52.3, 51.0, 50.0, 47.5, 47.3; HRMS *m*/*z* calcd for $C_{22}H_{29}NO_7P$ $(M + H)^+$ 450.0124, found 450.0112.

Synthesis of (±)-*trans*-methyl 4-(4-(allyloxy)-3-methoxyphenyl)-1-(dimethoxyphosphoryl)pyrrolidine-3-carboxylate (8b). Compound **7b** (1.5 g, 68%) was prepared from **6b** (2.9 g, 7.6 mmol) and α-chloroethyl chloroformate (1.7 g, 12 mmol) according to the same preparation procedure of compound **7a** described above. A mixture of compound **7b** (0.33 g, 1.0 mmol), dimethyl chlorophosphate (0.22 g, 1.5 mmol) and excess triethylamine (0.30 g, 3.0 mmol) in dry DCM (25 mL) was stirred at 0 °C for 30 minutes in the presence of catalytic amount of DMAP. The solution was allowed to warm to room temperature and stir for 14 h. The organic solvent was evaporated to dryness to give a crude mixture. Subsequent purification of the mixture was performed by using the flash column chromatography on silica gel with gradient elution (EA/Hex, 10% to 30%) to afford the desired compound as a reddish oil (0.33 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.59–6.75 (m, 3H), 5.85–6.02 (m, 1H), 5.19–5.32 (m, 1H), 5.09–5.18 (m, 1H), 4.45 (d, J = 4.89 Hz, 2H), 3.69–3.78 (m, 3H), 3.62 (s, 3H), 3.58–3.61 (m, 3H), 3.54–3.58 (m, 2H), 3.52 (s, 3H), 3.46–3.51 (m, 1H), 3.32–3.43 (m, 1H), 3.12–3.21 (m, 1H), 2.97–3.08 (m, 1H); HRMS *m*/*z* calcd for $C_{18}H_{27}NO_7P$ $(M + H)^+$ 400.1525, found 400.1524.

Synthesis of (±)-*trans*-methyl 1-(dimethoxyphosphoryl)-4-(3-methoxy-4-((methylsulfonyl)oxy)phenyl)pyrrolidine-3-carboxylate (8c). Compound **7c** (1.2 g, 49%) was prepared from **6c** (3.1 g, 7.4 mmol) and α-chloroethyl chloroformate (1.6 g, 11 mmol) according to the same preparation procedure of compound **7a** described above. A mixture of compound **7c** (0.31 g, 1.0 mmol), dimethyl chlorophosphate (0.22 g, 1.5 mmol) and excess triethylamine (0.30 g, 3 mmol) in dry DCM (25 mL) was stirred at 0 °C for 30 minutes in the presence of catalytic amount of DMAP. The solution was allowed to warm to room temperature and stir for 14 h. The organic solvent was evaporated to dryness to give a crude mixture. Subsequent purification of the residue was performed by using the flash column chromatography on silica gel with gradient elution (EA/Hex, 10% to 30%) to afford the desired compound as a reddish oil (0.31 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (d, J = 8.80 Hz, 1H), 6.82 (d, J = 1.96 Hz, 1H), 6.71–6.79 (m, 1H), 3.72–3.84 (m, 3H), 3.64 (s, 3H), 3.61 (s, 3H), 3.58 (d, J = 7.83 Hz, 2H), 3.55 (s, 3H), 3.35–3.44 (m, 1H), 3.15–3.23 (m, 1H), 3.03–3.10 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.0, 151.4, 136.9, 128.8, 128.4, 127.4, 124.3, 119.9, 112.1, 77.5, 77.1, 76.8, 61.1, 59.7, 57.3, 56.0, 52.1, 51.7, 46.5, 38.2; HRMS *m*/*z* calcd for $C_{16}H_{25}NO_9PS$ $(M + H)^+$ 438.0988, found 438.0982.

Synthesis of (±)-*trans*-methyl 4-(4-acetoxy-3-methoxyphenyl)-1-(dimethoxyphosphoryl)pyrrolidine-3-carboxylate (8d). Compound **7d** (0.40 g, 65%) was prepared from **6d** (0.80 g, 2.1 mmol) and α-chloroethyl chloroformate (0.45 g, 3.1 mmol) according to the same preparation procedure of compound **7a** described above. A mixture of compound **7d** (0.29 g, 1.0 mmol), dimethyl chlorophosphate (0.22 g, 1.5 mmol) and excess triethylamine (0.30 g, 3.0 mmol) in dry DCM (25 mL) was stirred at 0 °C for 30 minutes in the presence of catalytic amount of DMAP. The solution was allowed to warm to room temperature and stir for 14 h. The organic solvent was evaporated to dryness to give a crude mixture. Subsequent purification of the mixture was performed by using the flash column chromatography on silica gel with gradient elution (EA/Hex, 10% to 30%) to afford the desired compound as a reddish oil (0.21 g, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (d, J = 7.82 Hz, 1H), 6.77–6.87 (m, 2H), 3.78 (s, 3H), 3.62–3.74 (m, 12H), 3.43–3.53 (m, 1H), 3.21–3.34 (m, 1H), 3.13 (q, J = 7.83 Hz, 1H), 2.27 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 168.9, 151.2, 138.9, 138.8, 122.9, 119.1, 111.6, 55.8, 53.3, 53.3, 53.2, 53.2, 53.2, 53.1, 52.1, 51.2, 51.1, 49.7, 49.7, 48.2, 48.1, 20.6; HRMS *m*/*z* calcd for $C_{17}H_{25}NO_8P$ $(M + H)^+$ 402.1240, found 402.1236.

Synthesis of (±)-*trans*-1-ethyl 3-methyl 4-((4-benzyloxy)-3-methoxyphenyl)pyrrolidine-1,3-dicarboxylate (7e). To a mixture of compound **7a** (1 g, 3 mmol) and trimethylamine (0.87 g, 8.6 mmol) in dry DCM (25 mL) was added ethyl chloroformate (0.49 g, 4.5 mmol) at 0 °C with stirring for 4 h. Removal of the

organic layer under reduced pressure gave a brownish residue. Subsequent purification of the residue was performed by using the flash column chromatography on silica gel with gradient elution (EA/Hex, 20% to 50%) to afford the desired compound as a brown oil (0.90 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.46 (m, 2H), 7.34 (t, J = 7.34 Hz, 2H), 7.23–7.30 (m, 1H), 6.82 (d, J = 8.80 Hz, 1H), 6.77 (s, 1H), 6.67–6.75 (m, 1H), 5.10 (s, 2H), 4.08–4.23 (m, 2H), 3.88–3.96 (m, 1H), 3.82–3.88 (m, 4H), 3.55–3.67 (m, 5H), 3.35–3.52 (m, 1H), 3.05–3.23 (m, 1H), 1.26 (q, J = 6.85 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 154.7, 149.8, 147.5, 137.1, 128.5, 127.8, 127.2, 119.2, 114.2, 111.2, 71.0, 61.2, 56.0, 52.3, 52.1, 50.5, 49.0, 48.6, 47.4, 46.6, 14.8; HRMS m/z calcd for $C_{23}H_{28}NO_6$ $(M + H)^+$ 414.1917, found 414.1912.

Synthesis of (±)-*trans*-4-(4-(benzyloxy)-3-methoxyphenyl)-1-(ethoxycarbonyl)-pyrrolidine-3-carboxylic acid (7g). A well-stirred mixture of compound **7e** (1.0 g, 3.0 mmol) and KOH (0.34 g, 8.6 mmol) in water (25 mL) was heated at 60 °C for 16 h until the TLC showed the disappearance of starting material. The resulting solution was poured into 6 M HCl ice water (80 mL) and extracted with EA (100 mL × 3). The organic layers were dried over anhydrous $MgSO_4$ and filtered. Removal of the organic layers under reduced pressure gave a brownish residue and subsequent purification was performed by using the flash column chromatography on silica gel with gradient elution (EA/Hex, 30% to 50%) to afford the desired compound as a brown oil (0.42 g, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.22 (br. s, 1H), 7.23–7.53 (m, 5H), 6.78–6.94 (m, 2H), 6.74 (d, J = 7.82 Hz, 1H), 5.12 (br. s, 2H), 4.17 (d, J = 5.87 Hz, 2H), 3.87 (br. s, 5H), 3.54–3.72 (m, 2H), 3.34–3.54 (m, 1H), 3.07–3.28 (m, 1H), 1.28 (d, J = 6.85 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.1, 155.1, 149.8, 147.5, 137.1, 132.1, 128.5, 127.9, 127.3, 119.3, 114.2, 111.3, 71.1, 61.6, 56.1, 52.4, 50.3, 49.4, 48.9, 48.5, 47.2, 46.4, 14.7; HRMS m/z calcd for $C_{22}H_{26}NO_6$ $(M + H)^+$ 400.1760, found 400.1769.

Synthesis of (±)-*trans*-ethyl 3-(4-(benzyloxy)-3-methoxyphenyl)-4-carbamoylpyrrolidine-1-carboxylate (7h). To a 50 mL round-bottomed flask was added a mixture of compound **7g** (0.36 g, 0.9 mmol) and excess triethylamine (0.25 g, 2.5 mmol) in THF (25 mL) at 0 °C followed by the addition of ethyl chloroformate (0.13 g, 1.2 mmol). The solution was allowed to warm to room temperature and stir overnight. The organic solvent was removed under reduced pressure to yield the desired intermediate with sufficient purity to be used directly for the next step. To this intermediate in THF (25 mL) was slowly added ammonium hydroxide solution (20 mL) at 0 °C with stirring. The reaction mixture was allowed to warm to room temperature slowly and stirred for 16 h. Finally, removal of the organic solvent under reduced pressure furnished a brownish residue and subsequent purification of the residue was performed by using the flash column chromatography on silica gel with gradient elution (MeOH/DCM, 5% to 10%) to afford the desired compound as a yellowish oil (0.15 g, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23–7.52 (m, 5H), 6.70–6.90 (m, 3H), 5.98 (br. s, 1H), 5.73 (br. s, 1H), 5.11 (s, 2H), 4.14 (d, J = 6.85 Hz, 2H), 3.74–3.99 (m, 5H), 3.66 (br. s, 1H), 3.50 (br. s, 1H), 3.42 (br. s, 1H), 2.99 (br. s, 1H), 1.26 (t, J = 6.36 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.5, 154.9, 150.0, 147.6, 137.0, 131.9, 128.6, 127.9, 127.4, 127.3, 119.4, 114.5, 111.4, 71.1, 61.3, 56.2, 52.9, 14.8; HRMS m/z calcd for $C_{22}H_{27}N_2O_5$ $(M + H)^+$ 399.4654, found 399.4635.

Synthesis of (±)-*trans*-ethyl 3-(4-(benzyloxy)-3-methoxyphenyl)-4-(hydroxymethyl)pyrrolidine-1-carboxylate (7f). To a 50 mL round-bottomed flask was added a mixture of LiCl (0.025 g, 0.6 mmol) and $NaBH_4$ (0.023 g, 0.6 mmol) in EtOH and THF at a ratio of 5 : 3 (25 mL) at 0 °C followed by the addition of compound **7e** (0.12 g, 0.3 mmol). The solution was allowed to warm to room temperature and stir for 2 h. The organic solvent was removed under reduced pressure and the crude mixture was purified by the flash column chromatography on silica gel with gradient elution (MeOH/DCM, 5% to 10%) to afford the desired compound as a colorless oil (0.058 g, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24–7.54 (m, 5H), 6.62–6.92 (m, 3H), 5.13 (s, 2H), 4.07–4.21 (m, 2H), 3.74–3.97 (m, 5H), 3.62–3.72 (m, 1H), 3.47–3.59 (m, 1H), 3.27–3.46 (m, 2H), 3.09 (d, J = 7.83 Hz, 1H), 2.47 (br. s, 1H), 2.31 (m, 2H), 1.17–1.43 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.2, 149.9, 147.3, 137.2, 133.1, 128.5, 127.9, 127.3, 119.6, 114.3, 111.3, 71.2, 62.5, 61.1, 56.1, 53.2, 49.1, 14.8; HRMS m/z calcd for $C_{22}H_{28}NO_5$ $(M + H)^+$ 386.1975, found 385.1991.

Synthesis of (±)-*trans*-1-ethyl 3-methyl 4-(4-hydroxy-3-methoxyphenyl)-pyrrolidine-1,3-dicarboxylate (7i). To a solution of compound **7e** (0.70 g, 1.7 mmol) in dry THF (35 mL) was added 10% palladium on carbon (0.22 g, 2.1 mmol) at room temperature with stirring for 16 h under hydrogen atmosphere at balloon pressure. The catalyst was filtered and washed with THF (30 mL) and the filtrate was removed under reduced pressure. The residue was purified by using the flash column chromatography on silica gel with gradient elution (EA/Hex, 20% to 50%) to afford the desired compound as a colourless oil (0.27 g, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (d, J = 8.80 Hz, 1H), 6.66–6.76 (m, 2H), 5.82 (s, 1H), 4.15 (q, J = 6.85 Hz, 2H), 3.81–3.97 (m, 5H), 3.52–3.67 (m, 5H), 3.33–3.52 (m, 1H), 3.13 (t, J = 8.31 Hz, 1H), 1.17–1.33 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 154.9, 146.8, 145.0, 130.7, 130.7, 119.8, 114.7, 110.0, 61.3, 55.9, 52.4, 52.4, 52.1, 50.6, 49.7, 49.0, 48.7, 47.5, 46.8, 14.7; HRMS m/z calcd for $C_{16}H_{22}NO_6$ $(M + H)^+$ 324.1447, found 324.1440.

Synthesis of (±)-*trans*-1-(ethoxycarbonyl)-4-(4-hydroxy-3-methoxyphenyl)pyrrolidine-3-carboxylic acid (7j). A well-stirred mixture of compound **7i** (0.26 g, 0.8 mmol) and KOH (0.08 g, 2 mmol) in a mixture of water and MeOH (25 mL) was heated for 16 h until the TLC showed the disappearance of starting material. The resulting solution was poured into 6 M HCl ice water (80 mL), extracted with EA (100 mL × 3), dried over anhydrous $MgSO_4$ and filtered. Removal of the organic layers under reduced pressure gave a brownish residue and subsequent purification of the residue was performed by using the flash column chromatography on silica gel with gradient elution (EA/Hex, 30% to 50%) to afford the desired compound as a brown oil (0.11 g, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83–6.95 (m, 1H), 6.68–6.82 (m, 2H), 4.18 (q, J = 6.85 Hz, 2H), 3.78–4.00 (m, 5H), 3.55–3.75 (m, 2H), 3.34–3.54 (m, 1H), 3.18 (t, J = 8.31 Hz, 1H), 1.28 (d, J = 5.87 Hz, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.7, 155.0, 146.7, 145.0, 130.6, 119.9, 114.7, 109.9, 61.5, 55.9, 52.5, 48.9, 48.5, 47.4, 46.6, 14.7; HRMS m/z calcd for $C_{15}H_{20}NO_6$ $(M + H)^+$ 310.3320, found 310.3302.

Synthesis of (±)-*trans*-methyl 4-(4-(benzyloxy)-3-methoxyphenyl)-1-(2-(*tert*-butoxy)-2-oxoethyl)pyrrolidine-3-carboxylate (8e). To a mixture of compound **7a** (1.0 g, 3 mmol) and K_2CO_3 (1.2 g, 8.6 mmol) in dry DCM (25 mL) with a small amount of KI as catalyst was added *t*-butyl 2-bromoacetate (0.88 g, 4.5 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 14 hours. The crude reaction mixture was washed with water (200 mL × 3). The organic portion was dried with anhydrous $MgSO_4$ and filtered. Removal of the organic layer under reduced pressure gave a brownish residue and subsequent purification of the residue was performed by using the flash column chromatography on silica gel with gradient elution (EA/Hex, 20% to 50%) to afford the desired compound as a colourless oil (1.1 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, J = 7.82 Hz, 2H), 7.33 (t, J = 7.34 Hz, 2H), 7.27 (d, J = 7.82 Hz, 1H), 6.92 (s, 1H), 6.80 (s, 2H), 5.10 (s, 2H), 3.88 (s, 3H), 3.60–3.69 (m, 4H), 3.31–3.42 (m, 1H), 3.15–3.24 (m, 2H), 3.00–3.14 (m, 3H), 2.96 (dd, J = 7.34, 9.29 Hz, 1H), 1.46 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.3, 169.7, 149.7, 147.0, 137.4, 136.6, 128.5, 127.7, 119.3, 114.2, 111.4, 81.1, 71.1, 61.1, 56.9, 56.8, 56.1, 51.9, 51.6, 46.9, 28.2; HRMS *m*/*z* calcd for $C_{26}H_{34}NO_6$ $(M + H)^+$ 456.2386, found 456.2377.

Synthesis of 2-((±)-*trans*-3-(4-benzyloxy)-3-methoxyphenyl)-4-((methoxycarbonyl)pyrrolidin-1-yl)acetic acid (8g). To a well-stirred solution of compound **8e** (0.9 g, 2.0 mmol) in DCM (20 mL), TFA (0.25 g, 2.2 mmol) was added dropwise at 0 °C with stirring for 16 h. Removal of the solvent *in vacuo* followed by the flash column chromatographic purification on silica gel using a mixture of 10% MeOH in DCM as eluent afforded the desired compound **8g** (0.37 g, 46%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.81 (br. s, 1H), 7.22–7.48 (m, 5H), 6.92 (s, 1H), 6.79 (s, 2H), 5.08 (s, 2H), 3.85 (m, 4H), 3.51–3.81 (m, 9H), 3.37–3.51 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 170.0, 149.9, 147.7, 137.1, 131.4, 128.5, 127.8, 127.3, 119.5, 114.2, 111.5, 71.0, 59.9, 57.7, 56.2, 56.2, 52.3, 49.9, 46.7; HRMS *m*/*z* calcd for $C_{22}H_{26}NO_6$ $(M + H)^+$ 400.1760, found 400.1778.

Synthesis of (±)-*trans*-methyl 4-(4-(benzyloxy)-3-methoxyphenyl)-1-(2-isopropoxy-2-oxoethyl)pyrrolidine-3-carboxylate (8f). A mixture of compound **7a** (1.0 g, 3.0 mmol), i-propyl 2-bromoacetate (0.81 g, 4.5 mmol) and K_2CO_3 (0.62 g, 6.0 mmol) in dry DCM (25 mL) was stirred in the presence of small amount of KI as catalyst at 0 °C for 30 minutes. The solution was allowed to warm to room temperature and stirred for 14 h. The resulting solution was diluted with DCM (40 mL), washed with water (50 mL), dried over anhydrous $MgSO_4$ and filtered. The organic layer was evaporated to dryness to give a crude residue. Subsequent purification of the residue was performed by using flash column chromatography on silica gel with gradient elution (EA/Hex, 10% to 30%) to afford the desired compound as brown oil (0.89 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24–7.49 (m, 5H), 6.95 (s, 1H), 6.75–6.88 (m, 2H), 4.97–5.23 (m, 3H), 3.84–3.98 (m, 3H), 3.61–3.75 (m, 4H), 3.40–3.54 (m, 1H), 3.19–3.37 (m, 2H), 3.04–3.18 (m, 3H), 3.00 (dd, J = 6.85, 8.80 Hz, 1H), 1.28 (d, J = 5.87 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.3, 169.9, 149.7, 147.0, 137.3, 136.5, 128.5, 127.8, 127.3, 119.3, 114.2, 111.4, 71.1, 68.2, 61.1, 57.0, 56.3, 56.1, 52.0, 51.6, 46.9, 21.9. HRMS *m*/*z* calcd for $C_{25}H_{32}NO_6$ $(M + H)^+$ 442.2230, found 442.2244.

Synthesis of (±)-*trans*-methyl 4-(4-(benzyloxy)-3-methoxyphenyl)-1-(3-ethoxy-3-oxopropyl)pyrrolidine-3-carboxylate (8h). A mixture of compound **7a** (1 g, 3 mmol), ethyl 3-bromopropionate (0.81 g, 4.5 mmol) and K_2CO_3 (0.62 g, 6.0 mmol) in dry DCM (25 mL) was stirred in the presence of catalytic amount of KI at 0 °C for 30 minutes. The solution was allowed to warm to room temperature and stir for 14 h. The resulting solution was then diluted with DCM (40 mL), washed with water (50 mL), dried over anhydrous $MgSO_4$ and filtered. The organic layers were evaporated to dryness giving a brownish crude residue. Subsequent purification was performed by the flash column chromatography on silica gel with gradient elution (EA/Hex, 10% to 30%) to afford the desired compound as a brown oil (0.70 g, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.50 (m, 2H), 7.35 (t, J = 7.34 Hz, 2H), 7.22–7.31 (m, 1H), 6.90 (d, J = 1.96 Hz, 1H), 6.73–6.84 (m, 2H), 5.12 (s, 2H), 4.07–4.22 (m, 2H), 3.89 (s, 3H), 3.67 (s, 3H), 3.53–3.63 (m, 1H), 3.00–3.17 (m, 2H), 2.92–3.00 (m, 1H), 2.81–2.92 (m, 2H), 2.70–2.81 (m, 2H), 2.53 (t, J = 7.34 Hz, 2H), 1.26 (t, J = 7.34 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.5, 172.2, 149.8, 147.0, 137.4, 137.3, 128.5, 127.7, 127.2, 119.3, 114.2, 111.4, 71.1, 61.6, 60.3, 57.4, 56.0, 51.9, 51.6, 50.9, 46.6, 34.1, 14.2; HRMS *m*/*z* calcd for $C_{25}H_{32}NO_6$ $(M + H)^+$ 442.2230, found 442.2221.

Synthesis of (±)-*trans*-methyl 4-(4-(benzyloxy)-3-methoxyphenyl)-1-((*R*)-2,3-dihydroxypropyl)pyrrolidine-3-carboxylate (8i). Compound **7a** (0.3 g, 0.9 mmol) was dissolved in MeOH (30 mL). (±)-3-Chloropropane-1,2-diol (0.06 g, 0.6 mmol) and triethylamine (0.2 g, 1.7 mmol) were added to the compound **7a** solution under an ice bath. The reaction mixture was heated under reflux for 16 h. The reaction mixture was concentrated under reduced pressure and subsequently purified by using the flash column chromatography on silica gel with isocratic elution (MeOH/DCM, 4%) to afford the desired product as a yellow oil (0.2 g, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.49 (m, 2H), 7.38 (t, J = 7.34 Hz, 2H), 7.32 (d, J = 7.82 Hz, 1H), 6.84 (d, J = 8.80 Hz, 2H), 6.74–6.80 (m, 1H), 5.15 (s, 2H), 3.91 (s, 3H), 3.74–3.87 (m, 2H), 3.70 (s, 3H), 3.54–3.66 (m, 2H), 3.14–3.26 (m, 1H), 3.05–3.14 (m, 2H), 2.96–3.05 (m, 1H), 2.76–2.90 (m, 2H), 2.71 (dd, J = 7.34, 9.29 Hz, 1H), 2.43–2.56 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.5, 149.8, 147.1, 137.3, 136.2, 128.5, 127.8, 127.3, 119.1, 114.3, 111.4, 71.2, 68.7, 65.0, 62.1, 58.0, 57.7, 57.6, 56.1, 52.1, 51.2, 46.8, 46.8; HRMS *m*/*z* calcd for $C_{23}H_{30}NO_6$ $(M + H)^+$ 416.2068, found 416.2069.

Synthesis of 5-((±)-*trans*-3-(4-(benzyloxy)-3-methoxyphenyl)-4-(methoxycarbonyl)pyrrolidin-1-yl)-2,2-dimethyl-5-oxopentanoic acid (8j). A mixture of compound **7a** (1.0 g, 3.0 mmol), 2,2-dimethylglutaric anhydride (0.64 g, 4.5 mmol) and excess triethylamine (0.61 g, 6.0 mmol) in dry DCM (25 mL) was stirred at 0 °C for 30 mintues. The solution was allowed to warm to room temperature and stir for 16 h. The organic solvent was evaporated to dryness to give a crude residue. Subsequent purification of was performed by using the flash column chromatography on silica gel with gradient elution (MeOH/DCM, 5% to 10%) to afford the desired compound as a brown oil (1.0 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (br. s, 1H), 7.27–7.56 (m, 5H), 6.62–6.90 (m, 3H), 5.01–5.20 (m, 2H), 3.82–4.11 (m, 5H), 3.39–3.82 (m, 5H), 3.23 (m, 1H), 2.22–2.46 (m, 2H), 1.94 (d, J = 5.87 Hz, 2H), 1.24 (s., 3H), 1.21 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.4, 171.9,

149.9, 137.0, 128.5, 127.9, 127.3, 119.2, 114.2, 111.2, 71.1, 56.2, 52.2, 48.8, 47.6, 41.5, 35.0, 30.3, 25.1, 25.0, 24.9; HRMS *m*/*z* calcd for $C_{27}H_{34}NO_7$ $(M + H)^+$ 484.2335, found 484.2332.

Synthesis of (±)-*trans*-5-(3-(4-(benzyloxy)-3-methoxyphenyl)-4-(methoxycarbonyl)pyrrolidin-1-yl)-3,3-dimethyl-5-oxopentanoic acid (8k). A mixture of compound **7a** (0.33 g, 1 mmol), 3,3-dimethylglutaric anhydride (0.32 g, 2.3 mmol) and excess triethylamine (0.31 g, 3 mmol) in dry DCM (25 mL) was stirred at 0 °C for 30 minutes. The solution was allowed to warm to room temperature and stir for 16 h. The organic solvent was evaporated to dryness to give a crude residue. Subsequent purification of the residue was performed by using the flash column chromatography on silica gel with gradient elution (MeOH/DCM, 5% to 10%) to afford the desired compound as a brown oil (0.31 g, 63%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.50 (d, J = 7.82 Hz, 2H), 7.40 (t, J = 7.34 Hz, 2H), 7.30–7.37 (m, 1H), 7.08 (dd, J = 1.96, 10.76 Hz, 1H), 7.00 (dd, J = 3.42, 8.31 Hz, 1H), 6.90 (dt, J = 1.96, 8.31 Hz, 1H), 5.11 (d, J = 3.91 Hz, 2H), 4.12–4.21 (m, 1H), 4.05 (dd, J = 8.31, 12.23 Hz, 1H), 3.85 (s, 3H), 3.58–3.76 (m, 2H), 3.31–3.57 (m, 2H), 2.49–2.56 (m, 2H), 2.40–2.48 (m, 2H), 1.10–1.20 (m, 6H); ^{13}C NMR (101 MHz, acetone-d_6) δ 171.7, 171.1, 150.1, 147.8, 147.7, 137.7, 132.2, 128.3, 127.7, 127.6, 127.5, 119.7, 119.5, 114.3, 114.2, 111.9, 111.7, 70.6, 55.4, 55.4, 54.1, 52.3, 50.1, 50.0, 48.9, 48.3, 47.8, 46.1, 45.9, 45.9, 42.9, 42.7, 33.5; HRMS *m*/*z* calcd for $C_{27}H_{34}NO_7$ $(M + H)^+$ 484.1326, found 484.1315.

Synthesis of (*E*)-4-((±)-*trans*-3-(4-(benzyloxy)-3-methoxyphenyl)-4-(methoxycarbonyl)pyrrolidin-1-yl)-4-oxobut-2-enoic acid (8l). A mixture of compound **7a** (0.33 g, 1 mmol), maleic anhydride (0.23 g, 2.3 mmol) and excess triethylamine (0.31 g, 3 mmol) in dry DCM (25 mL) was stirred at 0 °C for 30 minutes. The solution was allowed to warm to room temperature and stir for 16 h. The organic solvent was evaporated to dryness to afford a crude mixture. Subsequent purification of the residue was performed by using the flash column chromatography on silica gel with gradient elution (MeOH/DCM, 5% to 10%) to afford the desired compound as a brown oil (0.26 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30–7.51 (m, 5H), 6.88 (d, J = 8.80 Hz, 1H), 6.79 (d, J = 1.96 Hz, 1H), 6.69–6.76 (m, 1H), 6.37–6.60 (m, 2H), 5.17 (s, 2H), 4.05–4.24 (m, 2H), 4.00 (t, J = 9.29 Hz, 1H), 3.65–3.94 (m, 8H), 3.21–3.40 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 172.2, 171.6, 149.8, 147.7, 137.1, 128.5, 127.9, 127.3, 119.2, 114.2, 111.1, 77.4, 77.1, 76.8, 71.1, 56.1, 53.1, 52.2, 50.7, 48.8, 47.6, 46.1, 41.5, 35.0; HRMS *m*/*z* calcd for $C_{24}H_{26}NO_7$ $(M + H)^+$ 440.3564, found 440.3579.

Synthesis of 4-((±)-*trans*-3-(4-(benzyloxy)-3-methoxyphenyl)-4-(methoxycarbonyl)pyrrolidine-1-yl)-4-oxobutanoic acid (8m). A mixture of compound **7a** (0.33 g, 1 mmol), succinic anhydride (0.23 g, 2.3 mmol) and excess triethylamine (0.31 g, 3 mmol) in dry DCM (25 mL) was stirred at 0 °C for 30 minutes. The solution was allowed to warm to room temperature with stirring for 16 h. The organic solvent was evaporated under reduced pressure to give a crude mixture. Subsequent purification of the mixture was performed by using the flash column chromatography on silica gel with gradient elution (MeOH/DCM, 5% to 10%) to afford the desired compound as a brown oil (0.26 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (br. s, 1H), 7.33–7.43 (m, 2H), 7.15–7.33 (m, 3H), 6.63–6.84 (m, 3H), 4.95–5.11 (m, 2H), 3.99 (dd, J = 8.80, 11.74 Hz, 1H), 3.84–3.94 (m, 1H), 3.80 (d, J = 6.85 Hz, 3H), 3.65–3.72 (m, 1H), 3.56 (d, J = 4.89 Hz, 3H), 3.40–3.52 (m, 2H), 3.05–3.26 (m, 1H), 2.59–2.72 (m, 2H), 2.47–2.59 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 172.2, 170.6, 149.8, 147.5, 137.0, 128.5, 128.5, 127.8, 127.8, 127.3, 119.2, 114.2, 111.2, 71.0, 56.0, 56.0, 52.2, 52.2, 48.8, 31.5, 29.1, 29.0; HRMS *m*/*z* calcd for $C_{24}H_{28}NO_7$ $(M + H)^+$ 442.1866, found 442.1863.

Synthesis of 2-(3-(4-(benzyloxy)-3-methoxyphenyl)-4-(methoxycarbonyl)pyrrolidine-1-carbonyl)benzoic acid (8n). Phthalic anhydride (0.20 g, 1.4 mmol) and triethylamine (0.30 g, 3.0 mmol) were added to compound **7a** (0.30 g, 0.9 mmol) in DCM (30 mL) under an ice bath. The mixture was stirred at room temperature for 16 h. The reaction mixture was then concentrated under reduced pressure and purified by using the flash column chromatography on silica gel with isocratic elution (MeOH/DCM, 5%) to afford the desired product as a yellow oil (0.22 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, J = 7.82, 14.67 Hz, 1H), 7.58 (dd, J = 8.31, 16.14 Hz, 1H), 7.30–7.51 (m, 7H), 6.83–6.90 (m, 1H), 6.68–6.82 (m, 2H), 5.11 (d, J = 12.72 Hz, 2H), 4.19–4.30 (m, 1H), 3.82–3.96 (m, 4H), 3.69–3.80 (m, 1H), 3.61–3.66 (m, 2H), 3.56–3.61 (m, 2H), 3.45–3.55 (m, 1H), 3.14–3.37 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 172.2, 170.4, 168.6, 149.8, 147.6, 138.6, 137.1, 132.9, 131.1, 129.1, 128.5, 127.8, 127.3, 126.6, 119.4, 114.2, 111.3, 71.0, 56.2, 54.9, 52.1, 51.9, 50.6, 49.2, 48.7, 47.6, 46.5. HRMS *m*/*z* calcd for $C_{28}H_{28}NO_7$ $(M + H)^+$ 490.1860, found 490.1864.

Synthesis of (±)-*trans*-methyl 4-(4-(benzyloxy)-3-methoxyphenyl)-1-(methylsulfonyl)pyrrolidine-3-carboxylate (9a). To a solution of mesyl chloride (0.52 g, 4.5 mmol) in dry DCM (25 mL) and excess triethylamine (0.87 g, 8.6 mmol) was added dropwise a solution of compound **7a** (1.0 g, 3.0 mmol) at 0 °C with stirring for 4 h. Removal of the organic layer under reduced pressure furnished a brownish residue and subsequent purification of the residue was performed by using the flash column chromatography on silica gel with gradient elution (EA/Hex, 30% to 50%) to afford the desired compound as a brown oil (0.79 g, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.52 (m, 5H), 6.74–6.89 (m, 3H), 5.14 (s, 2H), 3.90 (s, 3H), 3.73–3.85 (m, 2H), 3.57–3.72 (m, 5H), 3.50 (dd, J = 7.83, 9.78 Hz, 1H), 3.17–3.29 (m, 1H), 2.86–2.96 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 149.9, 147.7, 137.0, 132.0, 128.6, 127.9, 127.3, 119.1, 114.2, 111.0, 71.0, 56.1, 53.6, 52.4, 50.6, 50.0, 47.5, 35.4; LRMS (ESI) *m*/*z* $C_{21}H_{26}NO_6S$ $(M + H^+)$ 420.2.

Synthesis of (±)-*trans*-methyl 4-(4-hydroxy-3-methoxyphenyl)-1-(methylsulfonyl)pyrrolidine-3-carboxylate (10a). To a solution of compound **9a** (0.6 g, 1.4 mmol) in dry tetrahydrofuran (THF) (25 mL) was added 10% palladium on carbon (0.22 g, 2.1 mmol) at room temperature with stirring for 16 h under hydrogen atmosphere at balloon pressure. The catalyst was filtered and washed with THF (20 mL) and the filtrate was concentrated under reduced pressure. The crude reaction mixture was purified by using flash column chromatography on silica gel with gradient elution (EA/Hex, 30% to 50%) to afford the desired compound as a colourless oil (0.23 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83–6.91 (m, 1H), 6.72–6.82 (m, 2H), 5.76 (br. s, 1H),

3.88 (s, 3H), 3.74–3.86 (m, 2H), 3.57–3.71 (m, 5H), 3.49 (dd, J = 7.83, 9.78 Hz, 1H), 3.16–3.26 (m, 1H), 2.93 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 146.9, 145.1, 130.7, 119.8, 114.7, 109.9, 109.7, 56.0, 53.7, 52.4, 50.7, 50.0, 47.7, 35.4; LRMS (ESI) m/z $C_{14}H_{20}NO_6S$ ($M + H^+$) 330.1.

Synthesis of (±)-*trans*-methyl 4-(3,4-dimethoxyphenyl)-1-(methylsulfonyl)-pyrrolidine-3-carboxylate (10b). A well-stirred mixture of compound **10a** (0.20 g, 0.67 mmol), anhydrous potassium carbonate (0.28 g, 2.01 mmol) and methyl iodide (0.29 g, 2.01 mmol) in acetone (20 mL) was refluxed for 16 h. After cooling, the reaction mixture was filtered. Removal of the solvent under reduced pressure followed by flash column chromatographic purification on silica gel using a mixture of 30% EA in Hex as eluent afforded the desired compound **10b** (0.16 g, 71%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68–6.91 (m, 3H), 3.85–3.92 (m, 6H), 3.73–3.85 (m, 2H), 3.58–3.72 (m, 5H), 3.47–3.57 (m, 1H), 3.21 (q, J = 7.82 Hz, 1H), 2.94 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 149.3, 148.5, 131.4, 119.1, 111.5, 110.4, 56.0, 55.9, 53.6, 52.4, 50.7, 50.0, 47.6, 35.5; LRMS (ESI) m/z $C_{15}H_{22}NO_6S$ ($M + H^+$) 344.2.

Synthesis of (±)-*trans*-methyl 4-(4-(benzyloxy)-3-methoxyphenyl)-1-(*N*,*N*-dimethylsulfamoyl)pyrrolidine-3-carboxylate (9b). To a 50 mL round-bottomed flask was added a mixture of compound **7a** (1 g, 3 mmol) and excess triethylamine (0.87 g, 8.6 mmol) in DCM (25 mL) at 0 °C followed by the addition of *N*,*N*-dimethylsulfamoyl chloride (0.65 g, 4.5 mmol). After 4 h, the organic solvent was removed under reduced pressure and the crude mixture was purified by the flash column chromatography on silica gel with gradient elution (EA/Hex, 10% to 30%) to afford the desired compound as pale brown solid (0.73 g, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25–7.49 (m, 5H), 6.80–6.91 (m, 2H), 6.76 (d, J = 7.83 Hz, 1H), 5.14 (s, 2H), 3.90 (s, 3H), 3.73–3.85 (m, 2H), 3.63–3.70 (m, 4H), 3.56–3.63 (m, 1H), 3.44 (t, J = 9.29 Hz, 1H), 3.20 (d, J = 7.83 Hz, 1H), 2.87 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 149.9, 147.6, 137.1, 132.2, 128.5, 127.8, 127.3, 119.2, 114.2, 111.0, 71.1, 56.1, 54.5, 52.2, 50.9, 50.6, 47.4, 38.0; HRMS m/z calcd for $C_{22}H_{29}N_2O_6S$ ($M + H$)$^+$ 449.1746, found 449.1754. CCDC 2046394.†

Synthesis of (±)-*trans*-4-(4-(benzyloxy)-3-methoxyphenyl)-1-(*N*,*N*-dimethylsulfamonyl)pyrrolidine-3-carboxylic acid (10c). A well-stirred mixture of compound **9b** (0.45 g, 1.0 mmol) and KOH (0.08 g, 2 mmol) in a mixture of water and MeOH (25 mL) was heated for 16 h until the TLC showed the disappearance of starting material. The resulting solution was poured into 6 M HCl ice water (80 mL), extracted with EA (100 mL × 3), dried over anhydrous $MgSO_4$ and filtered. Removal of the organic layers under reduced pressure afforded a brownish residue and subsequent purification of the residue was performed by using the flash column chromatography on silica gel with gradient elution (EA/Hex, 30% to 50%) to afford the desired compound as a brown oil (0.17 g, 39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.54 (m, 5H), 6.70–6.89 (m, 3H), 5.15 (s, 2H), 3.90 (s, 3H), 3.81 (td, J = 9.17, 17.85 Hz, 2H), 3.58–3.73 (m, 2H), 3.43 (t, J = 8.80 Hz, 1H), 3.24 (d, J = 8.80 Hz, 1H), 2.88 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.0, 149.9, 147.7, 137.0, 132.0, 128.5, 127.9, 127.3, 119.3, 114.3, 111.1, 71.1, 56.2, 54.7, 50.6, 50.3, 47.3, 38.0; HRMS m/z calcd for $C_{21}H_{27}N_2O_6S$ ($M + H$)$^+$ 435.1516, found 435.1521.

Synthesis of (±)-*trans*-methyl 4-(4-(benzyloxy)-3-methoxyphenyl)-1-(2-(2,6-difluorophenyl)acetyl)pyrrolidine-3-carboxylate (9c). 2-(2,6-Difluorophenyl)acetic acid (0.8 g, 4.6 mmol) was dissolved in DCM (20 mL) and mixed with oxalyl chloride (1.7 g, 13.4 mmol) and DMF (3 drops) as catalyst under an ice bath. The reaction mixture was stirred at room temperature for 6 h. The reaction mixture was concentrated under reduced pressure to remove excess oxalyl chloride and a crude product of 2-(2,6-difluorophenyl)acetyl chloride was obtained. To a well-stirred solution of compound **7a** (0.4 g, 1.17 mmol) in DCM (20 mL) under an ice-bath was added dropwise the crude product of 2-(2,6-difluorophenyl)acetyl chloride and triethylamine (0.4 g, 3.5 mmol). The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure and subsequently purified by using the flash column chromatography on silica gel with isocratic elution (EA/Hex, 50%) to afford the desired product as a yellow oil (0.19 g, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (br. s, 2H), 7.34–7.42 (m, 2H), 7.27–7.34 (m, 1H), 7.18–7.26 (m, 1H), 6.86–6.95 (m, 3H), 6.73–6.86 (m, 2H), 5.15 (d, J = 5.87 Hz, 2H), 4.01–4.13 (m, 2H), 3.90 (d, J = 10.76 Hz, 3H), 3.70–3.81 (m, 2H), 3.64–3.70 (m, 5H), 3.55–3.63 (m, 1H), 3.20 (d, J = 9.78 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 172.2, 167.1, 162.8, 160.4, 149.9, 147.7, 137.1, 131.8, 131.7, 128.9, 128.8, 128.7, 128.6, 127.9, 127.9, 127.3, 119.2, 114.3, 111.2, 111.1, 111.0, 110.9, 71.1, 56.1, 53.2, 52.3, 52.2, 50.9, 49.2, 48.9, 47.7, 46.2; HRMS m/z calcd for $C_{28}H_{28}F_2NO_5$ ($M + H$)$^+$ 496.1936, found 496.1930.

Synthesis of (±)-methyl 4-(4-(benzyloxy)-3-methoxyphenyl)-1-picolinoylpyrrolidine-3-carboxylate (9d). Picolinic acid (1.0 g, 8.1 mmol) was dissolved in DCM (20 mL) and mixed with oxalyl chloride (1.45 g, 12.2 mmol) and DMF (2 drops) as the catalyst. The reaction mixture was heated under reflux for 3 h. The reaction mixture was concentrated under reduced pressure to afford a crude product of picolinoyl chloride. To a well-stirred solution of compound **7a** (1.0 g, 3.0 mmol) in DCM (20 mL) in an ice-bath was added dropwise the crude product of picolinoyl chloride and triethylamine (0.9 g, 9.0 mmol). The reaction mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure and subsequently purified by using the flash column chromatography on silica gel with gradient elution (MeOH/DCM, 1% to 5%) to afford the desired product as a yellow oil (0.24 g, 18%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54–8.64 (m, 1H), 7.90–7.99 (m, 1H), 7.81–7.90 (m, 1H), 7.26–7.47 (m, 6H), 6.74–6.89 (m, 3H), 5.14 (d, J = 6.85 Hz, 2H), 4.23 (ddd, J = 5.38, 7.58, 12.47 Hz, 2H), 3.92–4.15 (m, 2H), 3.89 (d, J = 3.91 Hz, 3H), 3.61–3.73 (m, 4H), 3.19–3.33 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 172.3, 165.6, 153.1, 149.8, 147.5, 137.5, 131.9, 131.5, 128.5, 127.8, 127.2, 125.2, 124.4, 119.4, 114.2, 111.2, 71.1, 56.1, 55.5, 53.2, 52.2, 51.8, 51.4, 49.9, 48.7, 48.4, 45.7; HRMS m/z calcd for $C_{26}H_{27}N_2O_5$ ($M + H$)$^+$ 447.1914, found 447.1913.

Synthesis of (±)-*trans*-methyl 4-(4-(benzyloxy)-3-methoxyphenyl)-1-(2-chloroacetyl)pyrrolidine-3-carboxylate (9e). Compound **7a** (0.6 g, 1.8 mmol) was dissolved in DCM (20 mL) and mixed with 2-chloroacetyl chloride (0.4 g, 3.5 mmol) and trimethylamine

(0.53 g, 5.2 mmol) under an ice-bath. The reaction mixture was then stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure and subsequently purified by using the flash column chromatography on silica gel with isocratic elution (EA/Hex, 50%) to afford the desired product as a yellow oil (0.38 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.48 (m, 2H), 7.39 (t, J = 7.34 Hz, 2H), 7.33 (d, J = 6.85 Hz, 1H), 6.86 (br. s, 1H), 6.70–6.81 (m, 2H), 5.16 (s, 2H), 4.05 (d, J = 11.74 Hz, 4H), 3.82–3.95 (m, 4H), 3.75 (d, J = 9.78 Hz, 1H), 3.60–3.71 (m, 5H), 3.19 (d, J = 7.83 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.3, 171.9, 164.7, 149.9, 147.7, 137.0, 131.6, 131.4, 128.5, 127.9, 127.3, 119.1, 114.3, 111.2, 77.6, 71.0, 56.1, 52.8, 52.3, 50.8, 49.1, 49.0, 48.7, 47.7, 45.9, 41.7; HRMS m/z calcd for $C_{22}H_{25}ClNO_5$ $(M + H)^+$ 418.1416, found 418.1417.

Synthesis of (±)-*trans*-methyl 1-(2-(acetylthio)acetyl)-4-(4-(benzyloxy)-3-methoxyphenyl)pyrrolidine-3-carboxylate (10d). To a well-stirred mixture of compound **9e** (0.8 g, 1.91 mmol) and excess triethylamine (5 mL) in DCM (20 mL) was added thioacetic acid (0.22 g, 2.87 mmol) dropwise under an ice bath. After the addition, the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was washed with water (100 mL × 3). The organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford a crude mixture, which was subsequently purified by using the flash column chromatography on silica gel with isocratic elution (EA/Hex, 25%) to afford the desired product as a yellow oil (0.62 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.47 (m, 2H), 7.31 (t, J = 7.34 Hz, 2H), 7.20–7.27 (m, 1H), 6.62–6.88 (m, 3H), 5.07 (d, J = 4.89 Hz, 2H), 3.94–4.03 (m, 2H), 3.83 (d, J = 7.83 Hz, 3H), 3.63–3.71 (m, 3H), 3.57–3.62 (m, 4H), 3.45–3.56 (m, 1H), 3.06–3.28 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.5, 172.3, 172.0, 165.8, 165.8, 149.9, 147.6, 137.1, 137.1, 128.5, 128.5, 127.8, 127.3, 119.2, 119.2, 114.2, 111.2, 71.0, 56.1, 53.1, 50.8, 47.7, 46.0, 32.5, 32.3, 30.1; HRMS m/z calcd for $C_{24}H_{28}NO_6S$ $(M + H)^+$ 458.1632, found 458.1639.

Synthesis of (±)-*trans*-methyl 4-(4-(benzyloxy)-3-methoxyphenyl)-1-(2-(bis(pyridin-2-ylmethyl)amino)acetyl)pyrrolidine-3-carboxylate (10e). Compound **9e** (0.26 g, 0.62 mmol) was dissolved in acetone (30 mL). Bis(pyridin-2-ylmethyl)amine (0.25 g, 1.3 mmol) and potassium carbonate (0.26 g, 1.9 mmol) were added to the solution of **9e** under an ice bath. The reaction mixture was subsequently heated under reflux for 16 h. The reaction mixture was concentrated under reduced pressure and re-dissolved in EA (50 mL). The mixture was washed with water (100 mL × 3). The organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude mixture was subsequently purified by using the flash column chromatography on silica gel with gradient elution (MeOH/DCM, 5% to 7%) to afford the desired product as a yellow oil (0.12 g, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, J = 4.89 Hz, 1H), 8.55 (d, J = 3.91 Hz, 1H), 7.59–7.71 (m, 2H), 7.50–7.59 (m, 2H), 7.41–7.48 (m, 2H), 7.34–7.41 (m, 2H), 7.31 (dd, J = 2.93, 6.85 Hz, 1H), 7.10–7.22 (m, 2H), 6.78–6.88 (m, 1H), 6.72–6.77 (m, 1H), 6.69 (t, J = 7.34 Hz, 1H), 5.13 (d, J = 7.82 Hz, 2H), 3.98 (d, J = 7.83 Hz, 5H), 3.87 (d, J = 7.82 Hz, 3H), 3.78 (t, J = 9.29 Hz, 1H), 3.54–3.71 (m, 5H), 3.44–3.52 (m, 1H), 3.31–3.44 (m, 2H), 3.01–3.20 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 172.2, 168.9, 158.8, 149.8, 148.9, 147.6, 137.1, 136.6, 131.7, 128.6, 127.9, 127.9, 127.3, 123.8, 122.2, 119.2, 114.3, 111.2, 71.1, 60.5, 60.4, 56.2, 56.1, 52.4, 52.2, 51.9, 50.9, 48.8, 48.6, 48.5, 47.7, 45.8; HRMS m/z calcd for $C_{34}H_{37}N_4O_5$ $(M + H)^+$ 581.2758, found 581.2761.

Synthesis of (±)-*trans*-methyl 6-(-3-(4-(benzyloxy)-3-methoxyphenyl)-4-(methoxycarbonyl)pyrrolidine-1-carbonyl)picolinate (9f). 6-(Methoxycarbonyl)picolinic acid (0.5 g, 2.9 mmol) was dissolved in DCM (20 mL) and mixed with oxalyl chloride (1.1 g, 8.7 mmol) and DMF (2 drops) as catalyst under an ice-bath. The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure to remove excess oxalyl chloride. A crude product of methyl 6-(chlorocarbonyl)-picolinate was obtained. To a well-stirred solution of compound **7a** (0.5 g, 1.5 mmol) in DCM (20 mL) under an ice-bath was added the crude methyl 6-(chlorocarbonyl)picolinate dropwise. The reaction mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure and subsequently purified by using the flash column chromatography on silica gel with gradient elution (EA/Hex, 30% to 50%) to afford the desired product as a yellow oil (0.3 g, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (dd, J = 7.83, 11.74 Hz, 1H), 8.10–8.15 (m, 1H), 7.96–8.03 (m, 1H), 7.42–7.48 (m, 2H), 7.35–7.41 (m, 2H), 7.32 (dd, J = 3.91, 6.85 Hz, 1H), 6.82–6.89 (m, 2H), 6.75–6.81 (m, 1H), 5.15 (d, J = 7.83 Hz, 2H), 4.25–4.33 (m, 1H), 4.07–4.18 (m, 1H), 3.87–4.07 (m, 7H), 3.62–3.82 (m, 5H), 3.27 (d, J = 8.80 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 172.2, 165.1, 153.5, 153.5, 149.8, 147.6, 146.3, 138.2, 137.1, 128.5, 127.9, 127.4, 127.2, 126.3, 119.4, 119.3, 114.2, 111.2, 71.1, 71.1, 56.1, 55.4, 53.3, 52.9, 52.2, 51.8, 51.4, 50.1, 48.7, 48.4, 45.7; HRMS m/z calcd for $C_{28}H_{29}N_2O_7$ $(M + H)^+$ 505.1969, found 505.1973.

Synthesis of (±)-*trans*-6-(3-(4-(benzyloxy)-3-methoxyphenyl)-4-carboxypyrrolidine-1-carbonyl)picolinic acid (10f). Compound **9f** (0.10 g, 0.2 mmol) was dissolved in MeOH (25 mL). Potassium hydroxide (0.022 g, 0.39 mmol) was dissolved in a minimum amount of water and added to the MeOH solution of **9f** under an ice bath. The reaction mixture was stirred at reflux for 6 h. The reaction mixture was concentrated under reduced pressure and redissolved in EA. A yellow solid was deposited on the surface of the round bottom flask, which was rinsed with 1 M HCl solution. The solution was then extracted with EA (10 mL × 2) and the organic portions were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to afford the desired product as a yellow powder (0.06 g, 63%). ^{1}H NMR (400 MHz, MeOH-d_4) δ 3.65 (d, J = 18.6 Hz, 2H) 3.84 (d, J = 12.7 Hz, 3H) 4.11 (d, J = 6.8 Hz, 2H) 4.18 (d, J = 8.8 Hz, 2H) 5.05 (d, J = 11.7 Hz, 2H) 6.84 (br. s, 1H) 6.87–7.04 (m, 2H) 7.24–7.38 (m, 3H) 7.39–7.48 (m, 2H) 8.01 (br. s, 1H) 8.10 (d, J = 10.8 Hz, 1H) 8.23 (d, J = 7.8 Hz, 1H); ^{13}C NMR (101 MHz, MeOH-d_4) δ 174.1, 173.8, 165.9, 159.6, 153.1, 149.9, 147.5, 145.3, 138.6, 137.3, 132.6, 128.1, 127.5, 127.3, 126.6, 126.1, 119.5, 119.4, 114.5, 111.6, 70.9, 55.2, 53.2, 51.6, 50.8, 49.8, 45.8. HRMS m/z calcd for $C_{26}H_{25}N_2O_7$ $(M + H)^+$ 477.1656, found 477.1658.

Biological studies

Bacterial strains. *E. coli* strain BL21 (NDM-1) carrying only NDM-1 marker was constructed for the screening of test

compounds by determining MIC as previously described.[9] Briefly, the bla_{NDM-1} gene was synthesized, PCR amplified and constructed on the IPTG-inducible pET28b vector. Then the constructed plasmid was transformed with *E. coli* BL21 to form *E. coli* BL21 (NDM-1) for MIC determination. *E. coli* BL21 (NDM-1) carrying the recombinant plasmid pET28b-bla_{H6}-mNDM-1, which encoded G36 to R270 and carried an N-terminal His6 tag for the purification of NDM-1 enzyme. Clinically isolated strains shown in Table 2 were isolated from the patients' urine, feces, and sputum in the Second People's Hospital of Jiaxing in Zhejiang Province, China.[46]

MIC determination

Bacterial cells of *E. coli* BL21 (NDM-1) were incubated on Mueller–Hinton agar (MHA) plate at 37 °C overnight under aerobic conditions. The bacteria were then transferred to normal saline where the OD_{600} value of the solution was measured to 0.08–0.1. The *E. coli* BL21 (NDM-1) saline solution was transferred to a 96-well plate and incubated with Mueller–Hinton broth (MHB), 1 mM of IPTG, and a serial concentration of MRM alone, the freshly prepared compound alone in DMSO, or a combination of various concentrations of MRM and test compound at 100 μM. After being incubated at 37 °C overnight, the MIC values were determined following the CLSI guidelines.[39]

NDM-1 enzyme inhibition assay

Expression and purification of NDM-1 protein were prepared following our previous report.[47]

Kinetic assay of NDM-1 was exhibited to determine the inactivation constants of inhibitors as previously published.[9] Briefly, followed by the addition of 7 fold of K_m of the reporter substrate called nitrocefin, 1 nM of pure NDM-1 was mixed with different concentrations of compound **10e** in 500 μL of 50 mM phosphate buffer with or without 50 μM $ZnSO_4$. Bovine serum albumin (BSA) was then added to stabilize the activity of diluted mNDM-1. The readout of the velocity can be recorded by the wavelength change at 482 nm. Three independent assays for **10e** were performed in triplicate.

Cytotoxicity assay

This assay was based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to dark blue formazan by succinate dehydrogenase in living cells.[48] HEK cells were seeded into three 96-well plates at a density of 1×10^4 cells per well in DMEM (10% FBS) and incubated for 12 h at 37 °C. Then, the cells were exposed to various concentrations of compound **10e** in DMSO for 24 h. Medium containing 0.5% DMSO was used as a negative control. Medium without cells was used as blank control. After treatment, MTT at a concentration of 0.5 mg mL^{-1} in PBS was added to each well and the cells were further incubated for 3 h at 37 °C. The medium was removed to afford the formazan crystals followed by dissolving in DMSO. The optical density was measured at 490 nm using a Microplate Reader (Clariostar, BMG). The percentage of surviving cells was calculated using the following formula: (corrected reading from the test well − corrected reading from the blank well)/(corrected reading from negative well − corrected reading from the blank well) × 100%. IC_{50} was calculated by the software IC_{50} calculator.

ESI-MS analysis

Waters synapt g2-si electrospray ionization/quadrupole-ion mobility-time-of flight mass spectrometer was employed to perform Electrospray ionization mass spectrometry (ESI-MS) experiments. For qualitative detection of the binding between NDM-1 and compound under non-denaturing conditions, after incubating 20 μM of mNDM-1 in 20 mM ammonia acetate with an equal molar of compound **10e** in the same buffer system for 20 minutes, the reaction mixture was infused directly into a nanospray emitter (Econo12, New Objectives, Woburn, USA), which was mounted onto a nano-ESI source for analysis. The spray voltage was carefully raised to start the spray process and the spray was maintained for around 20 minutes at the voltage of 150 V.[49] During data acquisition, positive ion mode was exhibited in the operation of the mass spectrometer in the *m*/*z* range of 200–5000 for the detection of multiply charged ions. Transform program (MassLynx 4.1, Waters) was used to analyze the obtained raw multiply charged mass spectra.

Computational docking studies

CLC Drug Discovery Workbench (Version 2.5, QIAGEN) software was used for docking study. The 2D structures of both enantiomers of the compound **10e** were generated from SMILES and imported into the software. The X-ray crystal structure of the NDM-1 enzyme (PDB ID: 4EXS) obtained from the Protein Data Bank (https://www.rcsb.org/) was used directly for docking without any changes. Using the software function of "Find Binding Pockets", the software was able to identify the substrate-binding site of NDM-1 as potential binding pockets. The identification of ligand binding modes was done iteratively by evaluating 30 000 ligand conformations and estimating the binding energy of their interactions with these binding pockets. The binding pose with the top 5% highest scores were returned for further visual inspection. The highest scores positioned both enantiomers of the compound **10e** into the substrate-binding site of NDM-1 with the potential binding poses, important amino acids, and hydrogen bonding interactions shown in Fig. 4.

Abbreviations

CRE	Carbapenem-resistant *Enterobacteriaceae*
MBLs	Metallo-β-lactamases
NDM-1	New Delhi metallo-β-lactamase-1
Eb	Ebselen
IC_{50}	Half-maximal inhibitory concentration
TPA	Tris-picolylamine
MRM	Meropenem
TFA	Trifluoroacetic acid
DMAP	4-(Dimethylamino)pyridine
tPSA	Topological polar surface area
IPTG	Isopropyl β-D-1-thiogalacto-pyranoside

CLSI	Clinical and Laboratory Standards Institute
MIC	Minimum inhibition concentration
RF	Reduction fold
FICI	Fractional inhibitory concentration index
EDTA	Ethylenediaminetetracetic acid

Author contributions

The manuscript was written through the contributions of all authors. All authors have approved the final version of the manuscript.

Conflicts of interest

The authors declare no competing financial interest.

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References

1 K. M. Papp-Wallace, A. Endimiani, M. A. Taracila and R. A. Bonomo, *Antimicrob. Agents Chemother.*, 2011, **55**, 4943–4960.
2 L. K. Logan and R. A. Weinstein, *J. Infect. Dis.*, 2017, **215**, S28–S36.
3 https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.
4 A. Y. Chen, R. N. Adamek, B. L. Dick, C. V. Credille, C. N. Morrison and S. M. Cohen, *Chem. Rev.*, 2019, **119**, 1323–1455.
5 P. Linciano, L. Cendron, E. Gianquinto, F. Spyrakis and D. Tondi, *ACS Infect. Dis.*, 2019, **5**, 9–34.
6 Y. H. Yan, G. Li and G. B. Li, *Med. Res. Rev.*, 2020, **40**, 1558–1592.
7 M. Danishuddin, A. Kumar, F. Mobeen and A. U. Khan, *J. Biomol. Struct. Dyn.*, 2018, **36**, 2966–2975.
8 C. Chen, K.-W. Yang, L.-Y. Wu, J.-Q. Li and L.-Y. Sun, *Chem. Commun.*, 2020, **56**, 2755–2758.
9 J. Chiou, S. Wan, K.-F. Chan, P.-K. So, D. He, E. W.-C. Chan, T.-H. Chan, K.-Y. Wong, J. Tao and S. Chen, *Chem. Commun.*, 2015, **51**, 9543–9546.
10 W. B. Jin, C. Xu, Q. Cheng, X. L. Qi, W. Gao, Z. Zheng, E. W. C. Chan, Y.-C. Leung, T. H. Chan, K.-Y. Wong, S. Chen and K.-F. Chan, *Eur. J. Med. Chem.*, 2018, **155**, 285–302.
11 J. Su, J. Liu, C. Chen, Y. Zhang and K. Yang, *Bioorg. Chem.*, 2019, **84**, 192–201.
12 W. B. Jin, C. Xu, Q. Cheung, W. Gao, P. Zeng, J. Liu, E. W. C. Chan, Y.-C. Leung, T. H. Chan, K.-Y. Wong, S. Chen and K.-F. Chan, *Bioorg. Chem.*, 2020, **100**, 103873.
13 A. M. King, S. A. Reid-Yu, W. Wang, D. T. King, G. De Pascale, N. C. Strynadka, T. R. Walsh, B. K. Coombes and G. D. Wright, *Nature*, 2014, **510**, 503–506.
14 K. H. M. E. Tehrani, H. Fu, N. C. Brüchle, V. Mashayekhi, A. Prats Luján, M. J. van Haren, G. J. Poelarends and N. I. Martin, *Chem. Commun.*, 2020, **56**, 3047–3049.
15 C. Schnaars, G. Kildahl-Andersen, A. Prandina, R. Popal, S. Radix, M. Le Borgne, T. Gjøen, A. M. S. Andresen, A. Heikal, O. A. Økstad, C. Fröhlich, Ø. Samuelsen, S. Lauksund, L. P. Jordheim, P. Rongved and O. A. H. Åstrand, *ACS Infect. Dis.*, 2018, **4**, 1407–1422.
16 X.-F. Shi, M.-M. Wang, S.-C. Huang, J.-X. Han, W.-C. Chu, C. Xiao, E. Zhang and S. Qin, *Eur. J. Med. Chem.*, 2019, **167**, 367–376.
17 D.-Y. Cui, Y. Yang, M.-M. Bai, J.-X. Han, C.-C. Wang, H.-T. Kong, B.-Y. Shen, D.-C. Yan, C.-L. Xiao, Y.-S. Liu and E. Zhang, *Bioorg. Chem.*, 2020, **101**, 103965.
18 A. U. Khan, A. Ali, M. Danishuddin, G. Srivastava and A. Sharma, *Sci. Rep.*, 2017, **7**, 9207.
19 B. Zhao, X. Zhang, T. Yu, Y. Liu, X. Zhang, Y. Yao, X. Feng, H. Liu, D. Yu, L. Ma and S. Qin, *Acta Pharm. Sin. B*, 2021, **11**, 203–221.
20 A. Y. Chen, P. W. Thomas, A. C. Stewart, A. Bergstrom, Z. Cheng, C. Miller, C. R. Bethel, S. H. Marshall, C. V. Credille, C. L. Riley, R. C. Page, R. A. Bonomo, M. W. Crowder, D. L. Tierney, W. Fast and S. M. Cohen, *J. Med. Chem.*, 2017, **60**, 7267–7283.
21 D. T. King, L. J. Worrall, R. Gruninger and N. C. J. Strynadka, *J. Am. Chem. Soc.*, 2012, **134**, 11362–11365.
22 Y. Guo, J. Wang, G. J. Niu, W. Q. Shui, Y. N. Sun, H. G. Zhou, Y. Z. Zhang, C. Yang, Z. Y. Lou and Z. H. Rao, *Protein Cell*, 2011, **2**, 384–394.
23 I. García-Sáez, J. Hopkins, C. Papamicael, N. Franceschini, G. Amicosante, G. M. Rossolini, M. Galleni, J.-M. Frère and O. Dideberg, *J. Biol. Chem.*, 2003, **278**, 23868–23873.
24 N. N. Li, Y. T. Xu, Q. Xia, C. G. Bai, T. Y. Wang, L. Wang, D. D. He, N. N. Xie, L. X. Li, J. Wang, H. G. Zhou, F. Xu, C. Yang, Q. Zhang, Z. Yin, Y. Guo and Y. Chen, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 386–389.
25 Z. Meng, M.-L. Tang, L. Yu, Y. Liang, J. Han, C. Zhang, F. Hu, J.-M. Yu and X. Sun, *ACS Infect. Dis.*, 2019, **5**, 903–916.
26 Y. Yusof, D. T. C. Tan, O. K. Arjomandi, G. Schenk and R. P. McGeary, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 1589–1593.
27 I. A. Jaffe, *Am. J. Med.*, 1986, **80**, 471–476.
28 A. A. Patchett, E. Harris, E. W. Tristram, M. J. Wyvratt, M. T. Wu, D. Taub, E. R. Peterson, T. J. Ikeler, J. ten Broeke, L. G. Payne, D. L. Ondeyka, E. D. Thorsett, W. J. Greenlee, N. S. Lohr, R. D. Hoffsommer, H. Joshua, W. V. Ruyle, J. W. Rothrock, S. D. Aster, A. L. Maycock, F. M. Robinson, R. Hirschmann, C. S. Sweet, E. H. Ulm, D. M. Gross, T. C. Vassil and C. A. Stone, *Nature*, 1980, **288**, 280–283.
29 J. Brem, S. S. van Berkel, D. Zollman, S. Y. Lee, O. Gileadi, P. J. McHugh, T. R. Walsh, M. A. McDonough and C. J. Schofield, *Antimicrob. Agents Chemother.*, 2016, **60**, 142–150.

30 H. Feng, X. Liu, S. Wang, J. Fleming, D.-C. Wang and W. Liu, *Nat. Commun.*, 2017, **8**, 2242.
31 K. M. Belyk, C. D. Beguin, M. Palucki, N. Grinberg, J. DaSilva, D. Askin and N. Yasuda, *Tetrahedron Lett.*, 2004, **45**, 3265–3268.
32 A. Ohigashi, T. Kikuchi and S. Goto, *Org. Process Res. Dev.*, 2010, **14**, 127–132.
33 J. W. Bode, M. P. Doyle, M. N. Protopopova and Q.-L. Zhou, *J. Org. Chem.*, 1996, **61**, 9146–9155.
34 I. Coldham and R. Hufton, *Chem. Rev.*, 2005, **105**, 2765–2810.
35 T. Hashimoto and K. Maruoka, *Chem. Rev.*, 2015, **115**, 5366–5412.
36 B. V. Yang, D. O'Rourke and J. Li, *Synlett*, 1993, 195–196.
37 J. Kollonitsch, O. Fuchs and V. GÁBor, *Nature*, 1954, **173**, 125–126.
38 M. D. Shultz, *J. Med. Chem.*, 2019, **62**, 1701–1714.
39 Clinical and Laboratory Standards Institute, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard CLSI document M07-A7, Clinical and Laboratory Standards Institute, Wayne, PA, 7th edn, 2006.
40 M. Tyers and G. D. Wright, *Nat. Rev. Microbiol.*, 2019, **17**, 141–155.
41 Y. H. Chiu and J. W. Canary, *Inorg. Chem.*, 2003, **42**, 5107–5116.
42 T. Li, Q. Wang, F. Chen, X. Li, S. Luo, H. Fang, D. Wang, Z. Li, X. Hou and H. Wang, *PLoS One*, 2013, **8**, e61914.
43 P. W. Thomas, M. Zheng, S. Wu, H. Guo, D. Liu, D. Xu and W. Fast, *Biochemistry*, 2011, **50**, 10102–10113.
44 P. Zeng, C. Xu, C. Liu, J. Liu, Q. Cheng, W. Gao, X. Yang, S. Chen, K.-F. Chan and K.-Y. Wong, *ACS Appl. Bio Mater.*, 2020, **3**, 1738–1752.
45 D. T. King, L. J. Worrall, R. Gruninger and N. C. J. Strynadka, *J. Am. Chem. Soc.*, 2012, **134**, 11362–11365.
46 X. Wang, G. Chen, X. Wu, L. Wang, J. Cai, E. W. Chan, S. Chen and R. Zhang, *Front. Microbiol.*, 2015, 6.
47 J. Chiou, T. Y.-C. Leung and S. Chen, *Antimicrob. Agents Chemother.*, 2014, **58**, 5372–5378.
48 F. Denizot and R. Lang, *J. Immunol. Methods*, 1986, **89**, 271–277.
49 A. Schmidt, M. Karas and T. Dülcks, *J. Am. Soc. Mass Spectrom.*, 2003, **14**, 492–500.

