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FULL PAPER

Exploration of the structure–activity relationship and druggability of novel oxazolidinone-based compounds as Gram-negative antibacterial agents

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Abstract

To gain further knowledge of the structure–activity relationship and druggability of novel oxazolidinone-based UDP-3-*O*-acyl-*N*-acetylglucosamine deacetylase (LpxC) inhibitors as Gram-negative antibacterial agents, compounds containing the hydrophobic tails with different lengths and terminal substitutions were synthesized and their antibacterial activities against standard and clinically isolated Gram-negative strains were evaluated. We summarized their structure–activity relationships and found that oxazolidinone-based compounds exhibited a narrower antibacterial spectrum compared with threonine-based compounds. Furthermore, we parallelly compared the metabolic stabilities of the compounds with the classic threonine scaffold and the novel oxazolidinone scaffold in liver microsomes. The results indicated that the druggability of the oxazolidinone scaffold may be inferior to the classic threonine scaffold in the design of LpxC inhibitors.

KEYWORDS
antibacterial activity, oxazolidine, QSAR

1 | INTRODUCTION

Multi-resistant Gram-negative bacteria infections, especially caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* have evolved into a public health crisis recently.[1] Unfortunately, compared with the increasing quantities of drugs against Gram-positive bacteria infections, only a few novel Gram-negative antibiotics are in the clinical development pipeline.[2] Therefore, there is an urgent need for novel and broad-spectrum Gram-negative antibiotics with new mechanisms of action and chemical classes.

UDP-3-*O*-acyl-*N*-acetylglucosamine deacetylase (LpxC) inhibitors have been developed for more than 20 years as new-type antibacterial agents against Gram-negative bacteria infections since the discovery of L-161,240 (**1**) in 1996.[3] The X-ray crystal structure of LpxC from *Aquifex aeolicus* demonstrates the existence of a catalytic zinc ion and a hydrophobic tunnel accommodating a myristate fatty acid chain.[4,5] Thus, the classic LpxC inhibitors are usually composed of a zinc-binding group to disable the catalytic zinc ion, a hydrophobic tail to mimic the myristate in the natural substrate, and a scaffold to connect them. The most common zinc-binding group is hydroxamic acid, which could exhibit the strongest binding activity to the zinc ion.[6,7] The hydrophobic tail is usually composed of linear hydrophobic chains with different lengths and substitutions, which is quite similar except slight differences.[8,9] Therefore, scientists preferred to spend more efforts to study new scaffolds to obtain a revolutionary breakthrough. The oldest scaffold of LpxC inhibitors was

the threonine moiety, represented by CHIR-090 (**2**) and LPC-011 (**3**), which displayed remarkable antibacterial activity against a wide range of Gram-negative bacteria.[10,11] Besides, the LpxC inhibitors consisting of other scaffolds (**4**, **5**) also exhibit excellent antibacterial activities, although none of them have reached the market to our knowledge (Figure 1).[12,13]

In 2016, Kurasaki et al.[14] reported a series of oxazolidinone-based LpxC inhibitors, aimed to replace the threonine moiety. These compounds, especially **6**, exhibited remarkable antibacterial activities against *E. coli* and *K. pneumoniae*, and aroused our interest. To have a further exploration of the structure–activity relationship and druggability of this novel scaffold, a series of oxazolidinone-based LpxC inhibitors were designed, synthesized, and biologically evaluated.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

2.1.1 | Method A: Synthesis of *N*-methyl imidazolidone-based LpxC inhibitors (DD-1)

The target compound **DD-1** was afforded according to the procedure illustrated in Scheme 1. (*S*)-Glycidol was protected by PMBCl with the existence of NaH to give **A2**. Ring-open reaction was proceeded using NaN_3 in ethanol/H_2O to afford **A3**, then converted to aziridine **A4** through a one-pot process. Intermediate **A5** was obtained by another ring-open reaction between 4-iodoaniline and **A4**, followed by self-cyclization and methylation to yield imidazolidone **A6**, then deprotected by trifluoroacetic acid to give **A7**. Subsequently, oxidization and methylation reactions were proceeded successively to get intermediate **A9**. The Sonogashira cross-coupling reaction catalyzed by $Pd(PPh_3)_2Cl_2$ was carried out between intermediate **A9** and phenylacetylene to afford the corresponding intermediate **A10** and then converted to corresponding hydroxamic acid **DD-1** using the aqueous solution of NH_2OH.

2.1.2 | Method B: Synthesis of oxazolidinone-based LpxC inhibitors (DD-2–DD-5, DD-9–DD-10, DD-12–DD-16)

The target compounds **DD-2**–**DD-5**, **DD-9**–**DD-10**, **DD-12**–**DD-16** were afforded according to the procedure illustrated in Scheme 2. The ring-open reaction was adopted between commercially available materials (*R*)-glycidol and 4-iodoaniline to yield dihydroxyl intermediate **B-02**, which was further cyclized using diethyl carbonate under alkaline condition to give oxazolidinone **B-03**. The oxidization reaction was carried to transfer hydroxyl oxazolidinone **B-03** to the corresponding carboxylic acid **B-04**, and then methylated to afford corresponding carboxylic ester **B-05**. Intermediate **B-06** was obtained by Sonogashira cross-coupling reaction catalyzed by $Pd(PPh_3)_2Cl_2$ from ester **B-05** and trimethylsilylacetylene, then deprotected by TBAF to get corresponding terminal alkyne **B-07**. The Suzuki coupling reaction catalyzed by $Pd(PPh_3)_4$ was adopted to afford intermediate **B-08**. The Sonogashira cross-coupling reactions catalyzed by $Pd(PPh_3)_2Cl_2$ were carried out between **B-05** and alkynes with different substitutions to afford the corresponding intermediates **B-09**–**B-11**.The Eglinton cross-coupling reactions catalyzed by $Cu(OAc)_2$ were carried out separately between alkyne **B-07** and other alkynes to get corresponding intermediates **B-12**–**B-19**. At last, intermediates **B-08**–**B-19** were converted to corresponding hydroxamic acids (**DD-2**–**DD-5**, **DD-9**–**DD-10**, **DD-12**–**DD-16**, **23e**) using the aqueous solution of NH_2OH.

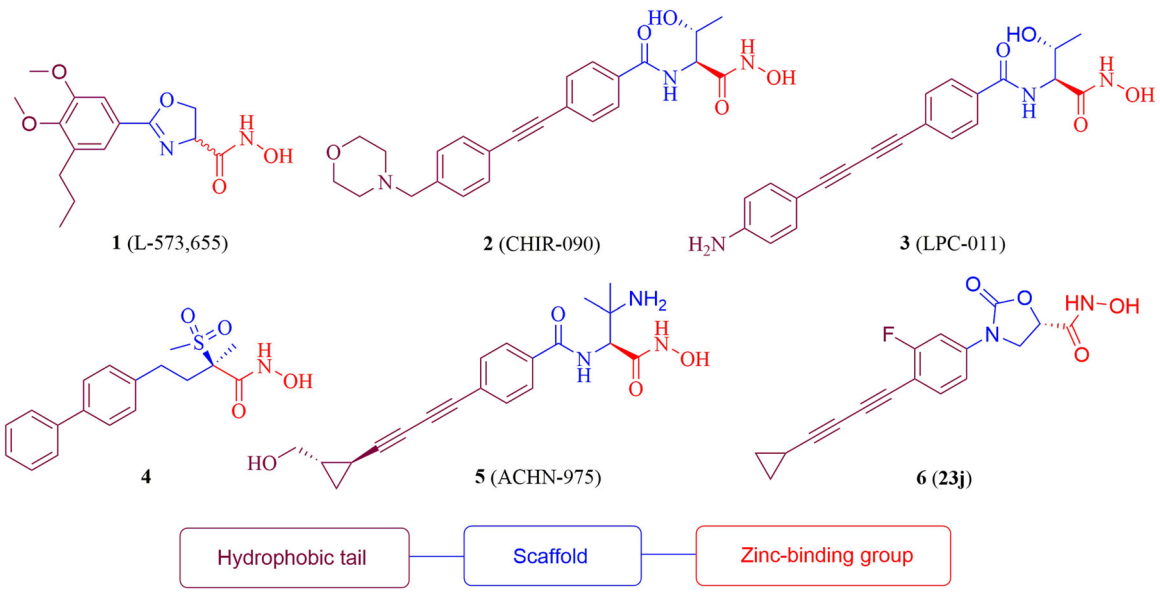
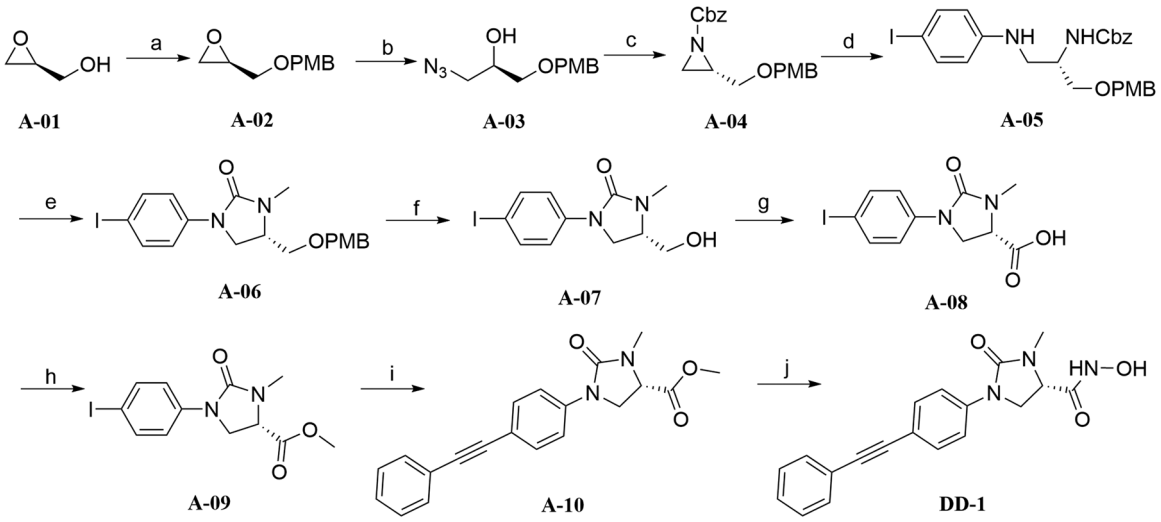
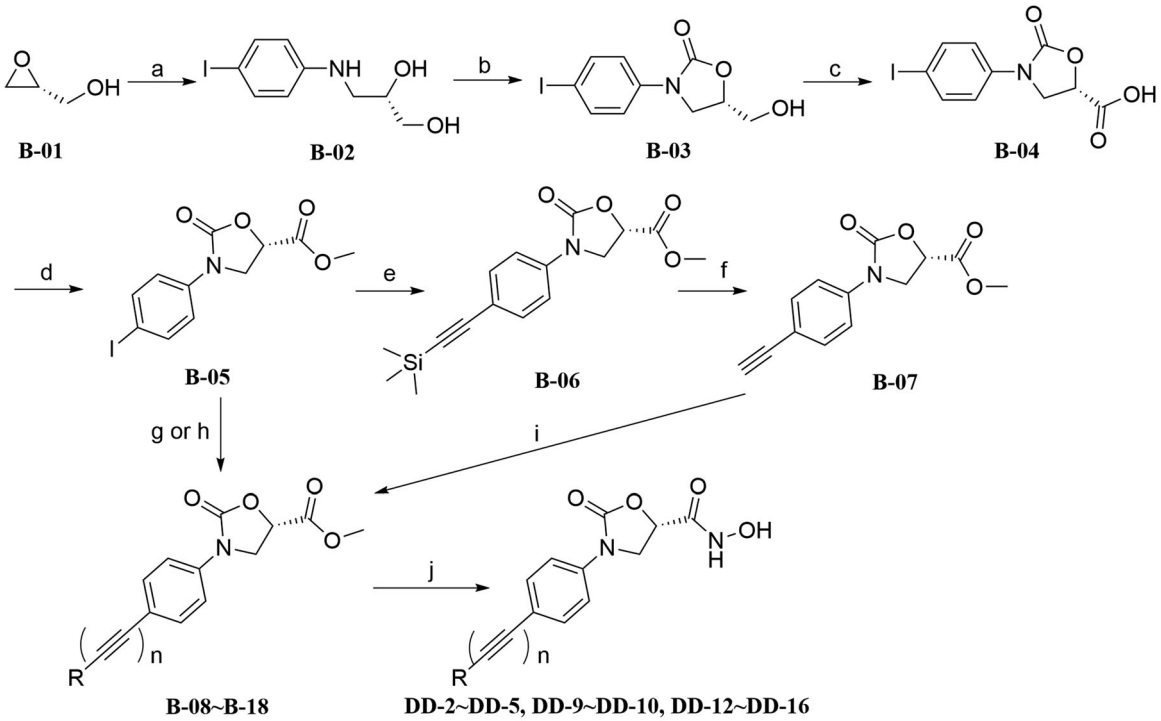


FIGURE 1 Structures of reported UDP-3-*O*-acyl-*N*-acetylglucosamine deacetylase inhibitors



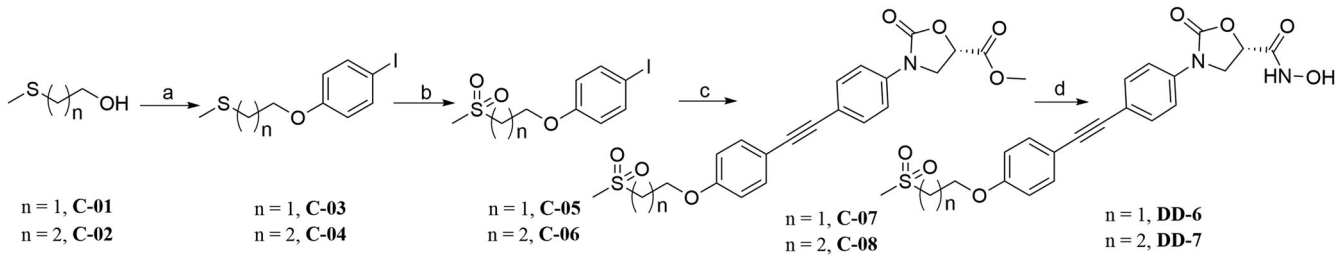
SCHEME 1 Reagents and conditions: (a) PMBCl, NaH, TBAI, THF; (b) NaN_3, NH_4Cl, EtOH/H_2O; (c) PPh_3, Et_3N, CbzCl, acetonitrile; (d) 4-iodoaniline, $LiClO_4$, acetonitrile/H_2O; (e) NaH, CH_3I, TBAI; (f) TFA, DCM; (g) TEMPO, NaClO, $NaClO_2$, CH_3CN/H_2O; (h) CH_3I, Cs_2CO_3, DMF; (i) phenylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, TEA, THF; (j) NH_2OH (50% in water), DCM/MeOH



	n	R
B-08, DD-2	0	benzene
B-09, DD-3	1	cyclopropane
B-10, DD-4	1	benzene
B-11, DD-5	1	aniline
B-12, DD-9	2	benzene
B-13, DD-10	2	aniline

	n	R
B-14, DD-12	2	cyclohexane
B-15, DD-13	2	cyclopentane
B-16, DD-14	2	1-cyclopentanol
B-17, DD-15	2	isopropane
B-18, DD-16	2	isopropanol
B-19, 23e	2	cyclopropyl

SCHEME 2 Reagents and conditions: (a) 4-Iodoaniline, EtOH; (b) diethyl carbonate, MeOH/MeONa, toluene; (c) TEMPO, NaClO, $NaClO_2$, CH_3CN/H_2O; (d) CH_3I, Cs_2CO_3, DMF; (e) ethynyltrimethylsilane, $Pd(PPh_3)_2Cl_2$, CuI, TEA, THF; (f) TBAF, THF; (g) alkynes, $Pd(Ph_3)_2Cl_2$, CuI, TEA, THF; (h) phenylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, H_2O/isopropanol; (i) alkynes, $Cu(OAc)_2$, MeOH/pyridine; (j) NH_2OH (50% in water), DCM/MeOH



SCHEME 3 Reagents and conditions: (a) 4-Iodophenol, DIAD, PPh_3, THF; (b) mCPBA, DCM; (c) **B-07**, $Pd(PPh_3)_2Cl_2$, CuI, TEA, THF; (d) NH_2OH (50% in water), DCM/MeOH

2.1.3 | Method C: Synthesis of oxazolidinone-based LpxC inhibitors (DD-6–DD-7)

The target compounds **DD-6**–**DD-7** were afforded according to the procedure illustrated in Scheme 3. The Mitsunobu reaction was adopted between commercially available thiols **C-01**–**C-02** and 4-iodoaniline to yield ethers **C-03**–**C-04**, and then further oxidized using mCPBA to give corresponding sulfones **C-05**–**C-06**. Intermediates **C-07**–**C-08** were obtained by Sonogashira cross-coupling reactions catalyzed by $Pd(PPh_3)_2Cl_2$ from sulfones **C-05**–**C-06** and intermediate **B-07**, and then converted to the corresponding hydroxamic acids **DD-6**–**DD-7** using the aqueous solution of NH_2OH.

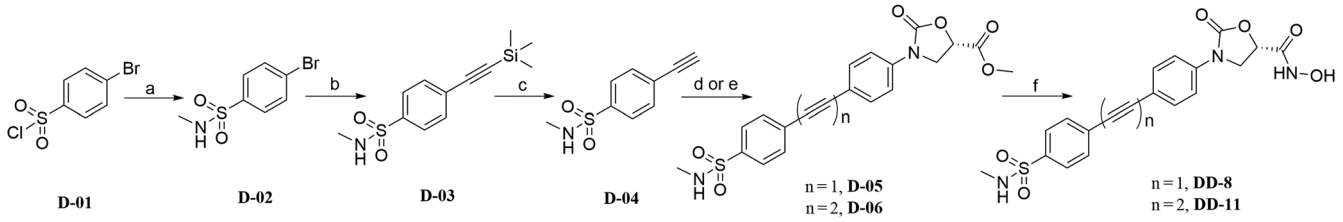
2.1.4 | Method D: Synthesis of oxazolidinone-based LpxC inhibitors (DD-8, DD-11)

The target compounds **DD-8** and **DD-11** were afforded according to the procedure illustrated in Scheme 4. The nucleophilic substitution was adopted between methylamine and intermediate **D-01** to yield sulfamide **D-02**, then coupled with ethynyltrimethylsilane by Sonogashira cross-coupling reaction catalyzed by $Pd(PPh_3)_2Cl_2$ to afford **D-03**. Subsequently, the deprotection reaction was proceeded under alkaline condition to give terminal alkyne **D-04**. The Sonogashira cross-coupling reaction catalyzed by $Pd(PPh_3)_2Cl_2$ and the Eglinton cross-coupling reaction catalyzed by $Cu(OAc)_2$ were carried out separately between intermediate **D-04** and oxazolidinone-based intermediates (**B-05**, **B-07**) to give intermediates **D-05**–**D-06**, which were converted to corresponding hydroxamic acids **DD-8** and **DD-11** using the aqueous solution of NH_2OH.

2.2 | Biology

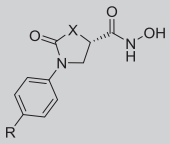
2.2.1 | Minimum inhibitory concentration values

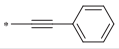
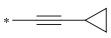
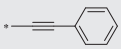
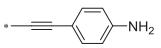
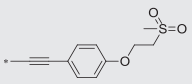
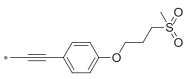
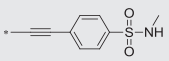
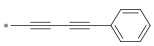
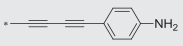
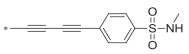
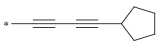
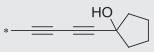
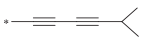
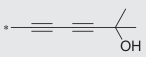
The minimum inhibitory concentration (MIC) values of all tested compounds are listed in Table 1. All of them exhibited more potent antibacterial activities against *E. coli* species than *P. aeruginosa* species. At first, we compared the antibacterial activity between **DD-1** and **DD-4**, because they had the same structures except the scaffold parts. Unfortunately, **DD-1** with the *N*-methyl imidazolidone scaffold showed lower antibacterial activities against both *E. coli* and *P. aeruginosa*, compared with the oxazolidinone-based compound **DD-4**. This result confirms that oxazolidinone scaffold is a more appropriate structure than imidazolidone scaffold in the design of LpxC inhibitors. Then, we refocused our research to the oxazolidinone-based series. The antibacterial activities against *E. coli* species increased with the length of the hydrophobic tails (**DD-2**–**DD-4**, **DD-9**). At the same time, compounds containing linear tails (**DD-9**) showed stronger antibacterial activities than those containing nonlinear tails (**DD-6**–**DD-7**). For the compounds containing the structure of "benzene–alkyne–benzene," the introduction of small terminal substitutions only caused marginal effects to their antibacterial activities (**DD-4**–**DD-5**, **DD-8**). For the compounds containing the structure of "benzene–alkyne–alkyne–benzene", the introduction of small terminal substitutions brought a boost to their antibacterial activities (**DD-9**–**DD-11**). Then we replaced the benzene ring of **DD-9** with aliphatic ring of different sizes and get compounds **DD-12**–**DD-13** and known compound **23e**. Compound **DD-13** exhibited strongest antibacterial activity against *E. coli* species, but barely inhibited the *P. aeruginosa* species. The introduction of hydroxyl to the compounds containing terminal aliphatic substitutions decreased their antibacterial activity dramatically (**DD-13** vs. **DD-14**, **DD-15** vs. **DD-16**). In conclusion, compared with classic LpxC inhibitor CHIR-090,



SCHEME 4 Reagents and conditions: (a) Methylamine, DCM; (b) ethynyltrimethylsilane, $Pd(PPh_3)_2Cl_2$, CuI, DIEA, THF; (c) K_2CO_3, MeOH; (d) **B-05**, $Pd(PPh_3)_2Cl_2$, CuI, TEA, THF; (d) **B-07**, $Cu(OAc)_2$, MeOH/pyridine; (f) NH_2OH (50% in water), DCM/MeOH

TABLE 1 In vitro antibacterial activities of compounds against the *Escherichia coli* species and the *Pseudomonas aeruginosa* species



Compound	X	R	MIC (μg/ml)			
			E. coli[a]	*E. coli*[b]	*P. aeruginosa*[c]	*P. aeruginosa*[d]
DD-1	N–CH_3		20	20	>256	>256
DD-2	O		50	50	200	200
DD-3	O		50	50	>256	>256
DD-4	O		1.25	0.625	10	10
DD-5	O		1.25	0.625	10	10
DD-6	O		50	50	125	125
DD-7	O		6.25	6.25	>256	>256
DD-8	O		3.12	6.25	>256	>256
DD-9	O		0.12	0.31	100	100
DD-10	O		0.020	0.025	100	100
DD-11	O		0.078	0.078	>256	>256
DD-12	O		0.62	0.78	>256	>256
DD-13	O		0.015	0.020	>256	>256
DD-14	O		1.56	1.56	>256	>256
DD-15	O		0.156	0.156	25	25
DD-16	O		3.12	3.12	>256	>256
23e	O		0.03	0.04	100	100
CHIR-090	–	–	0.156	0.156	2.5	2.5

Abbreviation: MIC, minimum inhibitory concentration.

[a]*E. coli*, DH5α.

[b]*E. coli*, AB1157.

[c]*P. aeruginosa*, PAO1.

[d]*P. aeruginosa*, PA14.

oxazolidinone compounds **DD-9**–**DD-10**, **DD-13** and known compound **23e** exhibit improved antibacterial activity against *E. coli* species and declined antibacterial activity against *P. aeruginosa* species.

To further explore the antibacterial activities and spectrum of this novel series, compounds **DD-10** and **23e** were selected to experiment with clinical isolated multidrug-resistant strains. Meanwhile, CHIR-090 and aztreonam were also selected as control drugs. Their MIC values are listed in Table 2. For *E. coli* and multidrug-resistant *K. pneumoniae*, compounds containing oxazolidinone scaffold (**DD-10**, **23e**) exhibited similar antibacterial activities to CHIR-090 and much stronger antibacterial activities than aztreonam. For multidrug-resistant *E. cloacae*, the oxazolidinone-based compounds (**DD-10**, **23e**) exhibited similar antibacterial activities to CHIR-090, and weaker antibacterial activities than aztreonam. For multidrug-resistant *A. baumannii*, all of these compounds exhibited weak inhibitory activities. For multidrug-resistant and standard *P. aeruginosa*, the antibacterial activities of oxazolidinones (**DD-10**, **23e**) were slightly weaker than aztreonam, but much weaker than CHIR-090.

TABLE 2 In vitro antibacterial activities of compounds against clinical isolated strains

Compound	MIC (μg/ml)					
	E. coli[a]	*K. pneumoniae*[b]	*E. cloacae*[c]	*A. baumannii*[d]	*P. aeruginosa*[e]	*P. aeruginosa*[f]
DD-10	≤0.125–1	0.25–8	0.5–2	≥16	16	16
23e	≤0.125–2	≤0.125–1	0.25–2	8–16	8–16	16
CHIR-090	≤0.125–0.5	0.25–1	0.25–1	4–16	0.25–1	2
Aztreonam	1 to >16	≥16	≤0.25–0.5	≥16	4–16	4

Abbreviation: MIC, minimum inhibitory concentration.
[a]*E. coli*, 5 strains.
[b]Multidrug-resistant *K. pneumoniae*, 5 strains.
[c]Multidrug-resistant *E. cloacae*, 5 strains.
[d]Multidrug-resistant *A. baumannii*, 5 strains.
[e]Multidrug-resistant *P. aeruginosa*, 5 strains.
[f]*P. aeruginosa*, ATCC 27853.

2.2.2 | Stabilities in the liver microsomes

Moreover, to study the metabolism characters of the novel oxazolidinone-based series, compounds **23e** and **YDL-2** (synthesized according to published procedures[7]) were selected to parallelly test their metabolism stabilities in liver microsomes of different species.[8] The results are listed in Table 3. Compound **23e**, containing the oxazolidinone scaffold, exhibited much weaker metabolism stability than **YDL-2** containing the threonine scaffold in all species.

3 | CONCLUSION

In summary, the compounds containing novel oxazolidinone scaffold exhibit a narrower antibacterial spectrum and worse biological metabolism stability in liver microsomes compared with the compounds containing the threonine scaffold, which indicated that the oxazolidinone scaffold may be inferior to the threonine scaffold in the design of LpxC inhibitors.

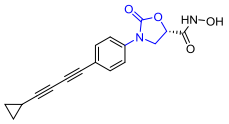
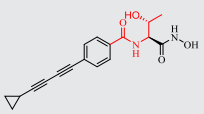
4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Proton nuclear magnetic resonance spectra were obtained on a Bruker Biospin Avance III 400 spectrometer or Bruker Biospin Avance III 500. Spectra were given in ppm (δ) and coupling constants, J values, were reported in hertz. Splitting patterns were designated as follows: s, singlet; brs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Tetramethylsilane was used as an internal reference standard. Mass spectra were obtained on a Finnigan MAT95 spectrometer (EI-MS) or Finnigan LCQ-DECA spectrometer (ESI-MS). High resolution mass spectrum was obtained on a Micromass Ultra Q-TOF spectrometer (ESI-MS). HPLC techniques (Agilent 1100 series) were used to determine the purity of compounds. All reagents and solvents were obtained from commercial suppliers and used without further purifcation. Room temperature refers to 20–25°C. All reaction mixtures were monitored using thin-layer chromatography (TLC) on silica gel F-254 TLC plates. All products, unless otherwise noted, were purified by column chromatography using silica gel (200–300 mesh). All final compounds were purified to >95% purity as determined by an Agilent 1100 series LC system (PLATISIL ODS 5 μm 250 × 4.6 mm) with two solvent systems (acetonitrile/water or acetonitrile/buffer [0.1% CF_3COOH in water]). The following abbreviations are used: deuterated chloroform; petroleum ether; ethyl acetate (EtOAc); dichloromethane (DCM); *N*,*N*-dimethylformamide (DMF); magnesium sulfate; tetrahydrofuran (THF); ammonium chloride (NH_4Cl); methanol (MeOH); sodium bicarbonate ($NaHCO_3$); trifluoroacetic acid (TFA); sodium azide (NaN_3); tetrabutylammonium iodide (TBAI); lithium perchlorate ($LiClO_4$); 2,2,6,6-tetramethylpiperidinooxy (TEMPO); sodium chlorite ($NaClO_2$) solution; sodium hypochlorite (NaClO); bis(triphenylphosphine)palladium(II) chloride

TABLE 3 Half-lives and intrinsic clearances of oxazolidinone-based compound and threonine-based compound

Compound	Structure	Species	Microsomal $t_{1/2}$, min	Cl_{int}, (ml·min^{-1}·g^{-1}·protein)
23e		Human	46	46
		Rat	21	99
		Mouse	41	51
YDL-2		Human	632.6	3
		Rat	189.5	11
		Mouse	904.1	2

($Pd(PPh_3)_2Cl_2$); tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$); cupric acetate ($Cu(OAc)_2$); diisopropyl azodiformate (DIAD); triphenylphosphine (PPh_3); tetrabutylammonium fluoride (TBAF); 3-chloroperoxybenzoic acid (mCPBA).

The original NMR spectra of the investigated compounds are provided as Supporting Information, as are their InChI codes together with some biological activity data.

4.1.2 | Compounds synthesized by Method A (Scheme 1)

*(R)-2-{[(4-Methoxybenzyl)oxy]methyl}oxirane (**A-02**)*

Step (a). NaH (60% dispersion in mineral oil, 0.65 g) was dispersed in the dry DMF (40 ml) under argon protection at 0℃; then **A-01** (1.7 g, 11 mmol) and TBAI (0.5 g, 1.4 mmol) were added. The resulting solution was stirred for 8 hr at room temperature and monitored by TLC. Water was added, and it was extracted with EtOAc. The organic phase was washed with saturated NH_4Cl solution and brine, dried over anhydrous Na_2SO_4, and filtered, and then the filtrate was concentrated in vacuo. The residue was purified by chromatography to give 1.4 g (83%) of **A-02** as a colorless liquid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.30 (d, J = 8.6 Hz, 2H), 6.91 (d, J = 8.6 Hz, 2H), 4.54 (q, J = 11.5 Hz, 2H), 3.83 (s, 3H), 3.76 (dd, J = 11.4, 3.1 Hz, 1H), 3.44 (dd, J = 11.4, 5.8 Hz, 1H), 3.20 (ddt, J = 5.7, 3.8, 2.9 Hz, 1H), 2.82 (dd, J = 5.0, 4.1 Hz, 1H), 2.63 (dd, J = 5.0, 2.7 Hz, 1H). MS (EI) *m/z*: (M^+, 194).

*(R)-1-Azido-3-[(4-methoxybenzyl)oxy]propan-2-ol (**A-03**)*

Step (b). Intermediate **A-02** (0.90 g, 6.0 mmol) was dissolved in the mixed solvent of ethanol (30 ml) and water (80 ml). Then NaN_3 (0.84 g, 13 mmol) and NH_4Cl (0.75 g, 14 mmol) were added. The resulting solution was stirred for 10 hr at 100℃ and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 1.9 g (74%) of **A-03** as a colorless liquid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.33–7.10 (m, 2H), 6.97–6.82 (m, 2H), 4.51 (s, 2H), 4.05–3.92 (m, 1H), 3.83 (s, 3H), 3.59–3.46 (m, 2H), 3.41–3.30 (m, 2H), 2.48 (d, J = 5.3 Hz, 1H). MS (EI) *m/z*: (M^+, 237).

*Benzyl (S)-2-{[(4-methoxybenzyl)oxy]methyl}aziridine-1-carboxylate (**A-04**)*

Step (c). Intermediate **A-03** (1.9 g, 8.0 mmol) was dissolved in the dry acetonitrile (80 ml) under argon protection at room temperature; then triphenylphosphine (2.1 g, 8.0 mmol) was added. The resulting solution was stirred for 6 hr at 70℃ and then warmed to room temperature. After that, triethylamine (1.2 g, 12 mmol) and benzyl chloroformate (1.6 g, 9.6 mmol) were added, and the mixed solution was stirred for 2 hr and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 2.2 g (84%) of **A-04** as a colorless liquid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.37 (s, 5H), 7.24 (d, J = 8.5 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 5.15 (s, 2H), 4.60–4.38 (m, 2H), 3.82 (s, 3H), 3.61 (d, J = 4.7 Hz, 2H), 2.76–2.70 (m, 1H), 2.37 (d, J = 6.1 Hz, 1H), 2.22 (d, J = 3.7 Hz, 1H). MS (EI) *m/z*: (M^+, 327).

*Benzyl (S)-{1-[(4-iodophenyl)amino]-3-[(4-methoxybenzyl)oxy]propan-2-yl}carbamate (**A-05**)*

Step (d). Intermediate **A-04** (2.2 g, 6.7 mmol) was dissolved in the mixed solvent of acetonitrile (80 ml) and water (20 ml), then $LiClO_4$ (1.1 g, 10 mmol) and 4-iodoaniline (2.2 g, 10 mmol) were added. The resulting solution was stirred for 10 hr at 95℃ and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 2.6 g (71%) of **A-04** as a yellow liquid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.52–7.26 (m, 8H), 7.23 (d, J = 8.7 Hz, 2H), 6.89 (d, J = 8.8 Hz, 2H), 6.37 (d, J = 8.5 Hz, 2H), 5.09 (s, 2H), 4.54–4.35 (m, 2H), 3.82 (s, 3H), 3.65–3.41 (m, 4H), 3.27 (d, J = 6.4 Hz, 2H). MS (EI) *m/z*: (M^+, 546).

*(S)-1-(4-Iodophenyl)-4-{[(4-methoxybenzyl)oxy]methyl}-3-methylimidazolidin-2-one (**A-06**, Scheme 1)*

Step (e). NaH (60% dispersion in mineral oil, 66 mg) was dispersed in 40 ml of dry DMF under argon protection at 0℃, then **A-05** (0.15 g, 0.28 mmol) and TBAI (10 mg, 0.03 mmol) were added. The resulting solution was stirred for 8 hr at room temperature. After that, CH_3I (78 mg, 0.55 mmol) was added, and the mixed solution was stirred for 8 hr and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 0.11 g (85%) of **A-06** as a white solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.59 (d, J = 9.1 Hz, 2H), 7.33 (d, J = 9.2 Hz, 2H), 7.24 (d, J = 8.8 Hz, 2H), 6.89 (d, J = 8.7 Hz, 2H), 4.49 (s, 2H), 3.86–3.79 (m, 4H), 3.79–3.69 (m, 1H), 3.61 (dd, J = 9.6, 4.6 Hz, 1H), 3.51 (dt, J = 8.7, 5.6 Hz, 2H), 2.88 (s, 3H). MS (EI) *m/z*: (M^+, 452).

*(S)-4-(Hydroxymethyl)-1-(4-iodophenyl)-3-methylimidazolidin-2-one (**A-07**)*

Step (f). Intermediate **A-06** (0.35 g, 0.77 mmol) was dissolved in the mixed solvent of DCM (10 ml) and TFA (3.0 ml), and the resulting solution was stirred for 15 hr at room temperature and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 0.22 g (86%) of **A-07** as a white solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.62 (d, J = 9.1 Hz, 2H), 7.38 (d, J = 9.1 Hz, 2H), 3.94–3.83 (m, 2H), 3.80–3.68 (m, 3H), 2.94 (s, 3H), 2.27–2.22 (m, 1H). MS (EI) *m/z*: (M^+, 332).

*(S)-1-(4-Iodophenyl)-3-methyl-2-oxoimidazolidine-4-carboxylic acid (**A-08**)*

Step (g). Intermediate **A-07** (0.22 g, 0.66 mmol) was dissolved in the mixed solvent of acetonitrile (16 ml) and phosphate buffer (pH 6.7, 3.0 mL), then TEMPO (26 mg, 0.16 mmol), $NaClO_2$ solution (0.60 g in 6.0 ml H_2O), NaClO solution (0.10 g in 2.0 ml H_2O) were added. The resulting solution was stirred for 15 hr at 80℃ and monitored by TLC. Then diluted hydrochloric acid (1 N) was added to adjust the pH value to 1–2. The post-treatment process was the same as Step (a) in method A. The concentrated product was used to the next step without any further purification. MS (EI) *m/z*: (M^+, 346).

*Methyl (S)-1-(4-iodophenyl)-3-methyl-2-oxoimidazolidine-4-carboxylate (**A-09**)*

Step (h). Intermediate **A-08** (obtained by the last step) was dissolved in the dry DMF (10 ml); then Cs_2CO_3 (0.22 g, 0.66 mmol) and CH_3I (0.24 g, 1.7 mmol) were added. The resulting solution was stirred for 12 hr at room temperature and monitored by TLC. The post-treatment process was the same as Step (a) in method A to give 0.12 g (50%, for two steps) of **A-09** as a white solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.63 (d, J = 8.8 Hz, 2H), 7.34 (d, J = 8.8 Hz, 2H), 4.25 (dd, J = 10.1, 5.2 Hz, 1H), 4.06 (t, J = 9.8 Hz, 1H), 3.85 (m, 4H), 2.99 (s, 3H). MS (EI) *m/z*: (M^+, 360).

*Methyl (S)-3-methyl-2-oxo-1-[4-(phenylethynyl)phenyl]imidazolidine-4-carboxylate (**A-10**)*

Step (i). Intermediate **A-09** (88 mg, 0.22 mmol) was dissolved in the dry THF (10 ml), then TEA (74 mg, 0.73 mmol), $Pd(PPh_3)_2Cl_2$ (17 mg, 0.024 mmol), CuI (3.0 mg, 0.012 mmol), phenylacetylene (50 mg, 0.49 mmol) were added under argon protection at room temperature. The resulting solution was stirred for 12 hr at 80℃ and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 73 mg (89%) of **A-10** as a yellow solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.67–7.45 (m, 6H), 7.43–7.30 (m, 3H), 4.27 (dd, J = 10.0, 5.0 Hz, 1H), 4.12 (t, J = 9.7 Hz, 1H), 3.91 (dd, J = 9.5, 5.2 Hz, 1H), 3.86 (s, 3H), 3.01 (s, 3H). MS (EI) *m/z*: (M^+, 334).

*(S)-N-Hydroxy-3-methyl-2-oxo-1-[4-(phenylethynyl)phenyl]imidazolidine-4-carboxamide (**DD-1**)*

Step (j). Intermediate **A-10** (70 mg, 0.21 mmol) was dissolved in the mixed solvent of DCM (6.0 ml) and MeOH (2.0 ml), then hydroxylamine (50% in water) was added. The resulting solution was stirred for 6 hr at room temperature and monitored by TLC, then concentrated in vacuo. The residue was washed by DCM three times and dried to give 50 mg (71%) of **DD-1** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.64 (d, J = 8.7 Hz, 2H), 7.59–7.47 (m, 4H), 7.45–7.29 (m, 3H), 4.09–3.94 (m, 2H), 3.68 (dd, J = 6.4, 2.3 Hz, 1H), 2.70 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.14, 156.92, 141.13, 132.45 (overlap), 131.65 (overlap), 129.18 (overlap), 128.91, 123.11, 116.80 (overlap), 115.28, 90.13, 88.83, 54.82, 45.75, 29.51. MS(ESI) *m/z*: [$(M+H)^+$, 336.0]. HRMS (ESI): Anal. calc. for $C_{19}H_{18}O_3N_3$: 336.1348, found: 336.1339.

4.1.3 | Compounds synthesized by Method B (Scheme 2)

*(S)-3-[(4-Iodophenyl)amino]propane-1,2-diol (**B-02**)*

Step (a). 4-Iodoaniline (2.5 g, 12 mmol) was dissolved in the anhydrous EtOH (30 ml), then intermediate **B-01** was added under argon protection. The resulting solution was stirred for 12 hr at 75℃ and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 2.5 g (81%) of **B-02** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.31 (d, J = 8.5 Hz, 2H), 6.45 (d, J = 8.6 Hz, 2H), 5.72 (t, J = 5.8 Hz, 1H), 4.77 (d, J = 4.9 Hz, 1H), 4.60 (t, J = 5.6 Hz, 1H), 3.60 (h, J = 5.6 Hz, 1H), 3.43–3.32 (m, 2H), 3.12 (dt, J = 13.0, 5.6 Hz, 1H), 2.86 (dt, J = 12.2, 5.8 Hz, 1H). MS (ESI) *m/z*: [$(M-H)^-$, 291.8].

*(S)-5-(Hydroxymethyl)-3-(4-iodophenyl)oxazolidin-2-one (**B-03**)*

Step (b). Intermediate **B-02** (2.4 g, 8.1 mmol) was dissolved in the anhydrous methylbenzene (80 ml), then diethyl carbonate (2.9 g, 24 mmol) and sodium methanolate (30% in methanol, 1.5 ml) were added under argon protection. The resulting solution was stirred for 8 hr at 105℃ and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 1.8 g (68%) of **B-03** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 (d, J = 9.2 Hz, 2H), 7.42 (d, J = 8.8 Hz, 2H), 5.23 (t, J = 5.6 Hz, 1H), 4.70 (ddt, J = 9.5, 6.4, 3.6 Hz, 1H), 4.06 (t, J = 9.0 Hz, 1H), 3.80 (dd, J = 8.8, 6.2 Hz, 1H), 3.71–3.64 (m, 1H), 3.55 (dt, J = 12.3, 4.9 Hz, 1H). MS (ESI) *m/z*: [$(M-H)^-$, 317.5].

*(S)-3-(4-Iodophenyl)-2-oxooxazolidine-5-carboxylic acid (**B-04**)*

Step (c). Intermediate **B-03** (0.37 g, 1.1 mmol) was dissolved in the mixed solvent of acetonitrile (16 ml) and phosphate buffer (pH 6.7, 3.0 ml), then TEMPO (26 mg, 0.16 mmol), $NaClO_2$ solution (0.60 g in 6.0 ml H_2O), NaClO solution (0.10 g in 2.0 ml H_2O) were added. The resulting solution was stirred for 15 hr at 80℃ and monitored by TLC. Then diluted hydrochloric acid (1 N) was added to adjust the pH value to 1–2. The posttreatment process was the same as Step (a) in method A. The concentrated product was used to the next step without any further purification. MS (EI) *m/z*: (M^+, 333).

*Methyl (S)-3-(4-iodophenyl)-2-oxooxazolidine-5-carboxylate (**B-05**)*

Step (d). Intermediate **B-04** (obtained by the last step) was dissolved in the dry DMF (10 ml), then Cs_2CO_3 (0.41 g, 1.3 mmol) and CH_3I (0.24 g, 1.7 mmol) were added. The resulting solution was stirred for 12 hr at room temperature and monitored by TLC. The post-treatment process was the same as Step (a) in method A to give 0.30 g (75%, for two steps) of **B-05** as a white solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.70 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.5 Hz, 2H), 5.09 (dd, J = 9.5, 5.3 Hz, 1H), 4.29 (t, J = 9.4 Hz, 1H), 4.13 (dd, J = 9.1, 5.3 Hz, 1H), 3.89 (s, 3H). MS (ESI) *m/z*: [$(M+H)^+$, 348.0].

*Methyl (S)-2-oxo-3-{4-[(trimethylsilyl)ethynyl]phenyl}oxazolidine-5-carboxylate (**B-06**)*

Step (e). Intermediate **B-05** (1.3 g, 3.7 mmol) was dissolved in the dry THF (10 ml), then TEA (1.5 g, 15 mmol), $Pd(PPh_3)_2Cl_2$ (0.22 g, 0.31 mmol), CuI (60 mg, 0.31 mmol), trimethylsilylacetylene (0.31 g, 3.1 mmol) were added under argon protection at room temperature. The resulting solution was stirred for 12 hr at 80℃ and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 1.1 g (93%) of **B-06** as a yellow solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.47 (s, 4H), 5.06 (dd, J = 9.7, 5.3 Hz, 1H), 4.28 (dd, J = 9.7, 9.3 Hz, 1H), 4.13 (dd, J = 9.3, 5.3 Hz, 1H), 3.86 (s, 3H), 0.24 (s, 9H). MS (ESI) *m/z*: [$(M+H)^+$, 318.3].

*Methyl (S)-3-(4-ethynylphenyl)-2-oxooxazolidine-5-carboxylate (**B-07**)*

Step (f). Intermediate **B-06** (0.30 g, 0.95 mmol) was dissolved in the dry THF (10 ml), then TBAF (1.0 M in THF, 1.0 ml) was added at 0°C. The resulting solution was stirred for 10 min at room temperature and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 80 mg (34%) of **B-07** as a white solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.50 (s, 4H), 5.08 (dd, J = 9.6, 5.3 Hz, 1H), 4.29 (t, J = 9.5 Hz, 1H), 4.15 (dd, J = 9.3, 5.3 Hz, 1H), 3.87 (s, 3H), 3.07 (s, 1H). MS (ESI) *m/z*: [(M+K)$^+$, 284.1].

*Methyl (S)-3-([1,1′-biphenyl]-4-yl)-2-oxooxazolidine-5-carboxylate (**B-08**)*

Step (h). Intermediate **B-07** (0.20 g, 0.58 mmol) was dissolved in the mixed solvent of isopropanol (10 ml) and H_2O (3.0 ml), then Na_2CO_3 (0.12 g, 1.2 mmol), $Pd(PPh_3)_4$ (33 mg, 0.029 mmol), phenylboronic acid (0.31 g, 3.1 mmol) were added under argon protection at room temperature. The resulting solution was stirred for 12 hr at 70°C and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 90 mg (53%) of **B-08** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (d, J = 8.6 Hz, 2H), 7.69–7.64 (m, 4H), 7.46 (t, J = 7.4 Hz, 2H), 7.36 (t, J = 7.1 Hz, 1H), 5.36 (dd, J = 9.8, 5.1 Hz, 1H), 4.43 (t, J = 9.6 Hz, 1H), 4.22 (dd, J = 9.3, 5.1 Hz, 1H), 3.78 (s, 3H). MS (ESI) *m/z*: [(M+Na)$^+$, 320.0].

*(S)-3-([1,1′-Biphenyl]-4-yl)-N-hydroxy-2-oxooxazolidine-5-carboxamide (**DD-2**)*

Step (j). Intermediate **B-08** (60 mg, 0.20 mmol) was dissolved in the mixed solvent of DCM (3.0 ml) and MeOH (1.0 ml), then hydroxylamine (50% in water, 1.0 ml) was added. The resulting solution was stirred for 6 hr at room temperature and monitored by TLC, then concentrated in vacuo. The residue was washed by DCM three times and dried to give 40 mg (66%) of **DD-2** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78–7.58 (m, 6H), 7.44 (t, J = 7.7 Hz, 2H), 7.33 (t, J = 7.3 Hz, 1H), 4.95 (dd, J = 9.3, 5.6 Hz, 1H), 4.28 (t, J = 9.1 Hz, 1H), 4.05 (dd, J = 9.0, 5.6 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.83, 154.16, 139.80, 137.94, 135.73, 129.41 (overlap), 127.70, 127.56 (overlap), 126.82 (overlap), 118.74 (overlap), 69.85, 47.77. MS (ESI) *m/z*: [(M–H)$^-$, 296.9]. HRMS (ESI): Anal. calc. for $C_{16}H_{15}O_4N_2$: 299.1032, found: 299.1027.

*Methyl (S)-3-[4-(cyclopropylethynyl)phenyl]-2-oxooxazolidine-5-carboxylate (**B-09**)*

Step (g). Intermediate **B-05** (78 mg, 0.22 mmol) was dissolved in the dry THF (10 ml), then TEA (74 mg, 0.73 mmol), $Pd(PPh_3)_2Cl_2$ (17 mg, 0.024 mmol), CuI (3.0 mg, 0.012 mmol), cyclopropylacetylene (32 mg, 0.49 mmol) were added under argon protection at room temperature. The resulting solution was stirred for 12 hr at 80°C and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 50 mg (78%) of **B-09** as a yellow solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.47–7.35 (m, 4H), 5.07 (dd, J = 9.6, 5.3 Hz, 1H), 4.28 (t, J = 9.5 Hz, 1H), 4.13 (dd, J = 9.3, 5.4 Hz, 1H), 3.87 (s, 3H), 1.48–1.38 (m, 1H), 0.90–0.84 (m, 2H), 0.83–0.76 (m, 2H). MS (ESI) *m/z*: [(M+H)$^+$, 286.1].

*(S)-3-[4-(Cyclopropylethynyl)phenyl]-N-hydroxy-2-oxooxazolidine-5-carboxamide (**DD-3**)*

Compound **DD-3** was prepared from intermediate **B-09** in the same manner as described for compound **DD-2**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.21 (s, 1H), 9.27 (s, 1H), 7.54 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 4.97 (dd, J = 9.2, 5.0 Hz, 1H), 4.26 (t, J = 9.2 Hz, 1H), 4.05–3.96 (m, 1H), 1.53 (p, J = 6.8 Hz, 1H), 1.02–0.79 (m, 2H), 0.75–0.64 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.92, 153.87, 137.86, 132.45 (overlap), 118.66, 118.15 (overlap), 93.84, 75.65, 69.43, 47.60, 8.81, 0.24. MS (ESI) *m/z*: [(M–H)$^-$, 284.9]. HRMS (ESI): Anal. calc. for $C_{15}H_{13}O_4N_2$: 285.0881, found: 285.0881.

*Methyl (S)-2-oxo-3-[4-(phenylethynyl)phenyl]oxazolidine-5-carboxylate (**B-10**)*

Intermediate **B-10** was prepared from intermediate **B-05** and phenylacetylene in the same manner as described for intermediate **B-09**. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.47–7.35 (m, 4H), 5.07 (dd, J = 9.6, 5.3 Hz, 1H), 4.28 (t, J = 9.5 Hz, 1H), 4.13 (dd, J = 9.3, 5.4 Hz, 1H), 3.87 (s, 3H), 1.48–1.38 (m, 1H), 0.90–0.84 (m, 2H), 0.83–0.76 (m, 2H). MS (ESI) *m/z*: [(M+H)$^+$, 286.1].

*(S)-N-Hydroxy-2-oxo-3-[4-(phenylethynyl)phenyl]oxazolidine-5-carboxamide (**DD-4**)*

Compound **DD-4** was prepared from intermediate **B-10** in the same manner as described for compound **DD-2**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.78 (s, 1H), 7.65–7.56 (m, 4H), 7.55–7.52 (m, 2H), 7.44–7.35 (m, 3H), 4.97 (dd, J = 9.3, 5.4 Hz, 1H), 4.28 (t, J = 9.2 Hz, 1H), 4.03 (dd, J = 9.1, 5.5 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.82, 153.91, 138.73, 132.63 (overlap), 131.75 (overlap), 129.23 (overlap), 129.17, 122.81, 118.23 (overlap), 117.53, 89.54, 89.47, 69.53, 47.61. MS(ESI) *m/z*: [(M–H)$^-$, 321.0]. HRMS (ESI): Anal. calc. for $C_{18}H_{13}O_4N_2$: 321.0875, found: 321.0882.

*Methyl (S)-3-{4-[(4-aminophenyl)ethynyl]phenyl}-2-oxooxazolidine-5-carboxylate (**B-11**)*

Intermediate **B-11** was prepared from intermediate **B-05** and 4-ethynylaniline in the same manner as described for intermediate **B-09**. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.53 (s, 4H), 7.35 (d, J = 8.4 Hz, 2H), 6.66 (d, J = 8.4 Hz, 2H), 5.10 (dd, J = 9.6, 5.3 Hz, 1H), 4.33 (t, J = 9.4 Hz, 1H), 4.18 (dd, J = 9.2, 5.3 Hz, 1H), 3.90 (s, 3H). MS (ESI) *m/z*: [(M+H)$^+$, 337.0].

*(S)-3-{4-[(4-Aminophenyl)ethynyl]phenyl}-N-hydroxy-2-oxooxazolidine-5-carboxamide (**DD-5**)*

Compound **DD-5** was prepared from intermediate **B-11** in the same manner as described for compound **DD-2**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 1H), 9.28 (s, 1H), 7.60 (d, J = 8.8 Hz, 2H), 7.48 (d, J = 8.7 Hz, 2H), 7.19 (d, J = 8.5 Hz, 2H), 6.55 (d, J = 8.5 Hz, 2H), 5.57 (s, 2H), 4.99 (dd, J = 9.3, 5.5 Hz, 1H), 4.29 (t, J = 9.2 Hz, 1H), 4.04 (dd, J = 9.2, 5.5 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.91, 153.89,

149.89, 137.81, 132.94 (overlap), 132.01 (overlap), 118.88, 118.24 (overlap), 114.08 (overlap), 108.61, 91.40, 86.59, 69.45, 47.63. MS (ESI) *m/z*: [(M−H)$^-$, 335.9]. HRMS (ESI): Anal. calc. for $C_{18}H_{14}O_4N_3$: 336.0984, found: 336.0979.

*Methyl (S)-2-oxo-3-[4-(phenylbuta-1,3-diyn-1-yl)phenyl]oxazolidine-5-carboxylate (**B-12**)*

Intermediate **B-07** (90 mg, 0.36 mmol) was dissolved in the mixed solvent of MeOH (3.0 ml) and pyridine (3.0 ml), then phenylacetylene (74 mg, 0.73 mmol), $Cu(OAc)_2$ (0.53 g, 2.9 mmol) were added under argon protection at room temperature. The resulting solution was stirred for 18 hr at room temperature and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 40 mg (32%) of **B-12** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.71–7.55 (m, 6H), 7.54–7.39 (m, 3H), 5.36 (dd, J = 9.8, 5.1 Hz, 1H), 4.41 (t, J = 9.7 Hz, 1H), 4.21 (dd, J = 9.4, 5.1 Hz, 1H), 3.76 (s, 3H). MS (ESI) *m/z*: [(M+H)$^+$, 345.1].

*(S)-N-Hydroxy-2-oxo-3-[4-(phenylbuta-1,3-diyn-1-yl)phenyl]oxazolidine-5-carboxamide (**DD-9**)*

Compound **DD-9** was prepared from intermediate **B-12** in the same manner as described for compound **DD-2**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.23 (s, 1H), 9.29 (s, 1H), 7.80–7.53 (m, 6H), 7.48–7.33 (m, 3H), 5.00 (dd, J = 9.1, 5.3 Hz, 1H), 4.29 (t, J = 9.2 Hz, 1H), 4.05 (dd, J = 9.1, 5.3 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.82, 153.84, 139.71, 133.81 (overlap), 132.83 (overlap), 130.42, 129.39 (overlap), 120.95, 118.29 (overlap), 115.49, 82.22, 74.11, 73.74, 69.53, 47.59. MS (ESI) *m/z*: [(M−H)$^-$, 344.8]. HRMS (ESI): Anal. calc. for $C_{20}H_{15}O_4N_2$: 347.1032, found: 347.1022.

*Methyl (S)-3-{4-[(4-aminophenyl)buta-1,3-diyn-1-yl]phenyl}-2-oxooxazolidine-5-carboxylate (**B-13**)*

Intermediate **B-13** was prepared from intermediate **B-07** and 4-ethynylaniline in the same manner as described for intermediate **B-12**. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.54 (s, 4H), 7.35 (d, J = 8.3 Hz, 2H), 6.62 (d, J = 8.2 Hz, 2H), 5.11 (dd, J = 9.7, 5.2 Hz, 1H), 4.32 (t, J = 9.5 Hz, 1H), 4.17 (dd, J = 9.5, 5.8 Hz, 1H), 3.90 (s, 3H). MS (ESI) *m/z*: [(M+H)$^+$, 361.2].

*(S)-3-{4-[(4-Aminophenyl)buta-1,3-diyn-1-yl]phenyl}-N-hydroxy-2-oxooxazolidine-5-carboxamide (**DD-10**)*

Compound **DD-10** was prepared from intermediate **B-13** in the same manner as described for compound **DD-2**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 1H), 9.28 (s, 1H), 7.75–7.52 (m, 4H), 7.24 (d, J = 8.2 Hz, 2H), 6.54 (d, J = 8.1 Hz, 2H), 5.81 (s, 2H), 4.99 (dd, J = 9.0, 5.2 Hz, 1H), 4.29 (t, J = 9.4 Hz, 1H), 4.04 (dd, J = 8.9, 5.3 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.84, 153.84, 151.10, 139.18, 134.31 (overlap), 133.43 (overlap), 118.28 (overlap), 116.37, 114.04 (overlap), 105.99, 84.84, 81.01, 74.88, 71.78, 69.50, 47.60. MS (ESI) *m/z*: [(M−H)$^-$, 359.9]. HRMS (ESI): Anal. calc. for $C_{20}H_{14}O_4N_3$: 360.0990, found: 360.0991.

*Methyl (S)-3-[4-(cyclohexylbuta-1,3-diyn-1-yl)phenyl]-2-oxooxazolidine-5-carboxylate (**B-14**)*

Intermediate **B-14** was prepared from intermediate **B-07** and cyclohexylacetylene in the same manner as described for intermediate **B-12**. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.51 (s, 4H), 5.10 (dd, J = 9.6, 5.2 Hz, 1H), 4.31 (t, J = 9.5 Hz, 1H), 4.16 (dd, J = 9.3, 5.3 Hz, 1H), 3.90 (s, 3H), 2.57 (tt, J = 8.9, 3.8 Hz, 1H), 1.92–1.79 (m, 2H), 1.80–1.68 (m, 2H), 1.57–1.47 (m, 3H), 1.38–1.32 (m, 3H). MS (ESI) *m/z*: [(M+H)$^+$, 352.1].

*(S)-3-[4-(Cyclohexylbuta-1,3-diyn-1-yl)phenyl]-N-hydroxy-2-oxooxazolidine-5-carboxamide (**DD-12**)*

Compound **DD-12** was prepared from intermediate **B-14** in the same manner as described for compound **DD-2**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 1H), 9.27 (s, 1H), 7.60 (q, J = 8.9 Hz, 4H), 5.33 (t, J = 4.8 Hz, 1H), 4.99 (dd, J = 9.3, 5.5 Hz, 1H), 4.28 (t, J = 9.3 Hz, 1H), 4.04 (dd, J = 9.3, 5.5 Hz, 1H), 1.83–1.75 (m, 2H), 1.68–1.59 (m, 2H), 1.53–1.40 (m, 4H), 1.36–1.28 (m, 4H). MS (ESI) *m/z*: [(M−H)$^-$, 350.9]. HRMS (ESI): Anal. calc. for $C_{20}H_{21}O_4N_2$: 353.1496, found: 353.1487.

*Methyl (S)-3-[4-(cyclopentylbuta-1,3-diyn-1-yl)phenyl]-2-oxooxazolidine-5-carboxylate (**B-15**)*

Intermediate **B-15** was prepared from intermediate **B-07** and cyclopentylacetylene in the same manner as described for intermediate **B-12**. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.50 (s, 4H), 5.10 (dd, J = 9.6, 5.2 Hz, 1H), 4.31 (t, J = 9.5 Hz, 1H), 4.16 (dd, J = 9.3, 5.3 Hz, 1H), 3.89 (s, 3H), 2.85–2.74 (m, 1H), 2.07–1.87 (m, 2H), 1.83–1.53 (m, 6H). MS (EI) *m/z*: (M$^+$, 337).

*(S)-3-[4-(Cyclopentylbuta-1,3-diyn-1-yl)phenyl]-N-hydroxy-2-oxooxazolidine-5-carboxamide (**DD-13**)*

Compound **DD-13** was prepared from intermediate **B-15** in the same manner as described for compound **DD-2**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 1H), 9.27 (s, 1H), 7.91–7.50 (m, 4H), 5.20–4.81 (m, 1H), 4.35–4.22 (m, 1H), 4.11–3.97 (m, 1H), 2.86 (q, J = 7.3, 6.7 Hz, 1H), 2.02–1.89 (m, 2H), 1.73–1.57 (m, 6H). MS (ESI) *m/z*: [(M−H)$^-$, 337.0]. HRMS (ESI): Anal. calc. for $C_{19}H_{19}O_4N_2$: 339.1339, found: 339.1343.

*Methyl (S)-3-{4-[(1-hydroxycyclopentyl)buta-1,3-diyn-1-yl]phenyl}-2-oxooxazolidine-5-carboxylate (**B-16**)*

Intermediate **B-16** was prepared from intermediate **B-07** and 1-ethynylcyclopentanol in the same manner as described for intermediate **B-12**. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.49 (s, 4H), 5.08 (dd, J = 9.6, 5.2 Hz, 1H), 4.29 (t, J = 9.5 Hz, 1H), 4.13 (dd, J = 9.3, 5.3 Hz, 1H), 3.87 (s, 3H), 2.07–1.93 (m, 5H), 1.89–1.81 (m, 2H), 1.80–1.73 (m, 2H). MS (ESI) *m/z*: [(M+H)$^+$, 353.1].

*(S)-N-Hydroxy-3-{4-[(1-hydroxycyclopentyl)buta-1,3-diyn-1-yl]phenyl}-2-oxooxazolidine-5-carboxamide (**DD-14**)*

Compound **DD-14** was prepared from intermediate **B-16** in the same manner as described for compound **DD-2**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.23 (s, 1H), 9.29 (s, 1H), 7.61 (q, J = 8.5 Hz, 4H), 5.52 (s,

1H), 5.00 (dd, *J* = 9.2, 5.4 Hz, 1H), 4.29 (t, *J* = 9.3 Hz, 1H), 4.05 (dd, *J* = 9.1, 5.4 Hz, 1H), 1.98–1.82 (m, 4H), 1.76–1.54 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.82, 153.83, 139.46, 133.69 (overlap), 118.24 (overlap), 115.77, 89.13, 78.39, 73.72, 73.37, 69.50, 66.70, 47.58, 42.26, 23.50. MS (ESI) *m/z*: [(M–H)$^-$, 352.9]. HRMS (ESI): Anal. calc. for $C_{19}H_{19}O_5N_2$: 355.1288, found: 355.1279.

*Methyl (S)-3-[4-(5-methylhexa-1,3-diyn-1-yl)phenyl]-2-oxooxazolidine-5-carboxylate (**B-17**)*

Intermediate **B-17** was prepared from intermediate **B-07** and 3-methyl-1-butyne in the same manner as described for intermediate **B-12**. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.51 (s, 4 H), 5.10 (dd, *J* = 9.7, 5.3 Hz, 1H), 4.32 (t, *J* = 9.5 Hz, 1H), 4.21–4.12 (m, 1H), 3.90 (s, 3H), 2.74 (p, *J* = 6.9 Hz, 1H), 1.26 (d, *J* = 6.9 Hz, 6H). MS (ESI) *m/z*: (M$^+$, 311).

*(S)-N-Hydroxy-3-[4-(5-methylhexa-1,3-diyn-1-yl)phenyl]-2-oxooxazolidine-5-carboxamide (**DD-15**)*

Compound **DD-15** was prepared from intermediate **B-17** in the same manner as described for compound **DD-2**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.18 (s, 1H), 9.54–9.18 (m, 1H), 7.80–7.14 (m, 4H), 4.97 (dd, *J* = 9.2, 5.4 Hz, 1H), 4.26 (t, *J* = 9.3 Hz, 1H), 4.01 (dd, *J* = 9.2, 5.4 Hz, 1H), 2.77 (p, *J* = 6.8 Hz, 1H), 1.16 (d, *J* = 6.9 Hz, 6H). MS (ESI) *m/z*: (M$^+$, 312). HRMS (ESI): Anal. calc. for $C_{17}H_{17}O_4N_2$: 313.1183, found: 313.1189.

*Methyl (S)-3-[4-(5-hydroxy-5-methylhexa-1,3-diyn-1-yl)phenyl]-2-oxooxazolidine-5-carboxylate (**B-18**)*

Intermediate **B-18** was prepared from intermediate **B-07** and 3-methyl butynol in the same manner as described for intermediate **B-12**. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.50 (s, 4H), 5.09 (dd, *J* = 9.6, 5.2 Hz, 1H), 4.30 (t, *J* = 9.5 Hz, 1H), 4.14 (dd, *J* = 9.3, 5.3 Hz, 1H), 3.87 (s, 3H), 2.08 (s, 1H), 1.58 (s, 6H). MS (ESI) *m/z*: [(M+H)$^+$, 327.1].

*(S)-N-Hydroxy-3-[4-(5-hydroxy-5-methylhexa-1,3-diyn-1-yl)phenyl]-2-oxooxazolidine-5-carboxamide (**DD-16**)*

Compound **DD-16** was prepared from intermediate **B-18** in the same manner as described for compound **DD-2**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.23 (s, 1H), 9.29 (s, 1H), 7.61 (q, *J* = 8.5 Hz, 4H), 5.67 (s, 1H), 5.00 (dd, *J* = 9.3, 5.5 Hz, 1H), 4.29 (t, *J* = 9.3 Hz, 1H), 4.04 (dd, *J* = 9.2, 5.5 Hz, 1H), 1.43 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.82, 153.84, 139.50, 133.71 (overlap), 118.25 (overlap), 115.70, 90.01, 78.30, 73.57, 69.50, 65.75, 64.31, 47.58, 31.52 (overlap). MS (ESI) *m/z*: [(M–H)$^-$, 328.1]. HRMS (ESI): Anal. calc. for $C_{17}H_{15}O_5N_2$: 327.0981, found: 327.0987.

4.1.4 | Compounds synthesized by Method C (Scheme 3)

*[2-(4-Iodophenoxy)ethyl](methyl)sulfane (**C-03**)*

Step (a). Intermediate **C-01** (0.31 g, 3.4 mmol) was dissolved in the dry THF (6.0 ml) under argon protection at room temperature, then 4-iodophenol (0.82 g, 3.7 mmol), DIAD (1.0 g, 5.1 mmol), PPh_3 (1.8 g, 6.8 mmol) were added. The resulting solution was stirred for 1 hr at room temperature and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 0.86 g (86%) of **C-03** as a white solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.55 (d, *J* = 9.0 Hz, 2H), 6.68 (d, *J* = 9.0 Hz, 2H), 4.12 (t, *J* = 6.8 Hz, 2H), 2.87 (t, *J* = 6.8 Hz, 2H), 2.21 (s, 3H). MS (ESI) *m/z*: [(M+H)$^+$, 295.0].

*1-Iodo-4-[2-(methylsulfonyl)ethoxy]benzene (**C-05**)*

Step (b). Intermediate **C-03** (0.50 g, 1.7 mmol) was dissolved in the dry DCM (15 ml) at room temperature, then mCPBA (0.62 g, 3.6 mmol) was added. The resulting solution was stirred for 2 hr at room temperature and monitored by TLC. Saturated sodium bisulfite solution was added, and it was extracted with EtOAc. The organic phase was washed with saturated NH_4Cl solution and brine, dried over anhydrous Na_2SO_4, and filtered, and then the filtrate was concentrated in vacuo. The residue was purified by chromatography to give 0.50 g (90%) of **C-05** as a white solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.60 (d, *J* = 9.0 Hz, 2H), 6.69 (d, *J* = 9.0 Hz, 2H), 4.41 (t, 2H), 3.44 (t, *J* = 5.3 Hz, 2H), 3.05 (s, 3H). MS (ESI) *m/z*: [(M+H)$^+$, 327.0].

*(S)-3-[4-({4-[2-(Methylsulfonyl)ethoxy]phenyl}ethynyl)phenyl]-2-oxooxazolidine-5-carboxylate (**C-07**)*

Step (c). Intermediate **C-07** was prepared from intermediate **C-05** and **B-05** in the same manner as described for intermediate **B-09** in method A. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.53 (s, 4H), 7.49 (d, *J* = 9.0 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 5.09 (dd, *J* = 9.6, 5.3 Hz, 1H), 4.49–4.45 (m, 2H), 4.32 (t, *J* = 9.5 Hz, 1H), 4.17 (dd, *J* = 9.3, 5.3 Hz, 1H), 3.89 (s, 3H), 3.51–3.39 (m, 2H), 3.08 (s, 3H). MS (ESI) *m/z*: [(M+H)$^+$, 445.0].

*(S)-N-Hydroxy-3-[4-({4-[2-(methylsulfonyl)ethoxy]phenyl}ethynyl)-phenyl]-2-oxooxazolidine-5-carboxamide (**DD-6**)*

Step (d). Compound **DD-6** was prepared from intermediate **C-07** in the same manner as described for compound **DD-2**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.88 (s, 1H), 7.64 (d, *J* = 8.9 Hz, 2H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.51 (d, *J* = 8.6 Hz, 2H), 7.05 (d, *J* = 8.7 Hz, 2H), 5.01 (dd, *J* = 9.3, 5.5 Hz, 1H), 4.39 (t, *J* = 5.6 Hz, 2H), 4.30 (t, *J* = 9.1 Hz, 1H), 4.04 (dd, *J* = 9.2, 5.5 Hz, 1H), 3.65 (t, *J* = 5.7 Hz, 2H), 3.09 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.84, 158.31, 153.91, 138.46, 133.41 (overlap), 132.43 (overlap), 118.25 (overlap), 117.94, 115.52, 115.43 (overlap), 89.41, 88.39, 69.45, 62.52, 53.74, 47.63, 42.77. MS (ESI) *m/z*: [(M–H)$^-$, 442.8]. HRMS (ESI): Anal. calc. for $C_{21}H_{21}O_7N_2S$: 445.1064, found: 445.1075.

*[3-(4-Iodophenoxy)propyl](methyl)sulfane (**C-04**)*

Intermediate **C-04** was prepared from intermediate **C-02** in the same manner as described for intermediate **C-03**. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.60–7.52 (m, 2H), 6.72–6.67 (m, 2H), 4.05 (t, *J* = 6.1 Hz, 2H), 2.70 (t, *J* = 7.1 Hz, 2H), 2.14 (s, 3 H), 2.12–2.04 (m, 2H). MS (ESI) *m/z*: [(M+H)$^+$, 309.0].

*1-Iodo-4-[3-(methylsulfonyl)propoxy]benzene (**C-06**)*

Intermediate **C-06** was prepared from intermediate **C-04** in the same manner as described for intermediate **C-05**. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.56 (d, J = 9.1 Hz, 2H), 6.66 (d, J = 9.0 Hz, 2H), 4.08 (t, J = 5.8 Hz, 2H), 3.28–3.18 (m, 2H), 2.95 (s, 3H), 2.40–2.28 (m, 2H). MS (ESI) *m/z*: [(M+H)$^+$, 441.0].

*Methyl (S)-3-[4-({4-[3-(methylsulfonyl)propoxy]phenyl}ethynyl)phenyl]-2-oxooxazolidine-5-carboxylate (**C-08**)*

Intermediate **C-08** was prepared from intermediate **C-06** and **B-05** in the same manner as described for intermediate **B-09** in method A. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96–7.78 (m, 4H), 7.72–7.59 (m, 5H), 5.37 (dd, J = 9.8, 5.1 Hz, 1H), 4.42 (t, J = 9.6 Hz, 1H), 4.22 (dd, J = 9.3, 5.1 Hz, 1H), 3.77 (s, 3H), 2.44 (d, J = 5.0 Hz, 3H). MS (ESI) *m/z*: [(M–H)$^-$, 437.0].

*(S)-N-Hydroxy-3-[4-({4-[3-(methylsulfonyl)propoxy]phenyl}ethynyl)-phenyl]-2-oxooxazolidine-5-carboxamide (**DD-7**)*

Compound **DD-7** was prepared from intermediate **C-08** in the same manner as described for compound **DD-2**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.24 (s, 1H), 9.30 (s, 1H), 7.82 (s, 4H), 7.68 (s, 4H), 7.63 (d, J = 5.1 Hz, 1H), 5.05–4.96 (m, 1H), 4.31 (t, J = 9.4 Hz, 1H), 4.11–4.00 (m, 1H), 2.44 (d, J = 5.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.80, 153.84, 140.43, 139.98, 133.98 (overlap), 133.58 (overlap), 127.52 (overlap), 125.00, 118.29 (overlap), 115.05, 83.67, 80.67, 76.59, 73.35, 69.53, 47.59, 29.09. MS (ESI) *m/z*: [(M–H)$^-$, 437.8]. HRMS (ESI): Anal. calc. for $C_{21}H_{16}O_6N_3S$: 438.0760, found: 438.0756.

4.1.5 | Compounds synthesized by Method D (Scheme 4)

*4-Bromo-N-methylbenzenesulfonamide (**D-02**)*

Step (a). 4-Bromobenzenesulfonyl chloride (0.51 g, 2.0 mmol) was dissolved in the dry THF (15 ml) at 0℃, then methylamine (dissolved in ethanol, 5.0 ml) was added. The resulting solution was stirred for 4 hr at 0℃ and monitored by TLC. The posttreatment process was the same as Step (a) in method A. The concentrated product was used in the next step without any further purification. MS (EI) *m/z*: (M$^+$, 249).

*N-Methyl-4-[(trimethylsilyl)ethynyl]benzenesulfonamide (**D-03**)*

Step (b). Intermediate **D-02** (1.1 g, 4.5 mmol) was dissolved in the dry THF (10 ml), then DIEA (2.3 g, 18 mmol), $Pd(PPh_3)_2Cl_2$ (95 mg, 0.14 mmol), CuI (26 mg, 0.14 mmol), trimethylsilylacetylene (0.53 g, 5.4 mmol) were added under argon protection at room temperature. The resulting solution was stirred for 12 hr at 80℃ and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 0.90 g (75.0%) of **D-03** as a yellow solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.85–7.73 (m, 2H), 7.63–7.55 (m, 2H), 4.37 (t, J = 5.4 Hz, 1H), 2.66 (d, J = 5.4 Hz, 3H), 0.26 (s, 9H). MS (ESI) *m/z*: [(M–H)$^-$, 266.0].

*4-Ethynyl-N-methylbenzenesulfonamide (**D-04**)*

Step (c). Intermediate **D-03** (0.90 g, 3.4 mmol) was dissolved in the dry MeOH (10 ml), then K_2CO_3 (0.70 g, 5.1 mmol) was added under argon protection at room temperature. The resulting solution was stirred for 2 hr and monitored by TLC. The posttreatment process was the same as Step (a) in method A to give 0.59 g (90%) of **D-04** as a yellow solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.87–7.79 (m, 2H), 7.67–7.56 (m, 2H), 4.47 (d, J = 5.4 Hz, 1H), 3.26 (d, J = 0.8 Hz, 1H), 2.67 (dd, J = 5.4, 0.8 Hz, 3H). MS (ESI) *m/z*: [(M–H)$^-$, 194.1].

*Methyl (S)-3-(4-{[4-(N-methylsulfamoyl)phenyl]ethynyl}phenyl)-2-oxooxazolidine-5-carboxylate (**D-05**)*

Step (d). Intermediate **D-05** was prepared from intermediate **D-04** and **B-05** in the same manner as described for intermediate **B-09** in method A. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.83 (d, J = 8.7 Hz, 2H), 7.65 (d, J = 8.7 Hz, 2H), 7.57 (s, 4H), 5.11 (dd, J = 9.6, 5.2 Hz, 1H), 4.39–4.29 (m, 2H), 4.18 (dd, J = 9.3, 5.3 Hz, 1H), 3.89 (s, 3H), 2.69 (d, J = 5.4 Hz, 3H). MS (ESI) *m/z*: [(M–H)$^-$, 413.0].

*(S)-N-Hydroxy-3-(4-{[4-(N-methylsulfamoyl)phenyl]ethynyl}phenyl)-2-oxooxazolidine-5-carboxamide (**DD-8**)*

Step (f). Compound **DD-8** was prepared from intermediate **D-05** in the same manner as described for compound **DD-2**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (s, 1H), 7.85–7.75 (m, 4H), 7.72–7.62 (m, 4H), 7.58 (q, J = 5.1 Hz, 1H), 5.01 (dd, J = 9.3, 5.4 Hz, 1H), 4.31 (t, J = 9.2 Hz, 1H), 4.06 (dd, J = 9.2, 5.4 Hz, 1H), 2.44 (d, J = 4.3 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.81, 153.89, 139.29, 139.21, 132.91 (overlap), 132.41 (overlap), 127.50 (overlap), 126.85, 118.27 (overlap), 116.82, 92.43, 88.25, 69.51, 47.61, 29.12. MS (ESI) *m/z*: [(M–H)$^-$, 413.8]. HRMS (ESI): Anal. calc. for $C_{19}H_{18}O_6N_3S$: 416.0911, found: 416.0904.

*(S)-3-(4-{[4-(N-Methylsulfamoyl)phenyl]buta-1,3-diyn-1-yl}phenyl)-2-oxooxazolidine-5-carboxylate (**D-06**)*

Step (e). Intermediate **D-06** was prepared from intermediate **B-04** and **B-07** in the same manner as described for intermediate **B-12**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96–7.78 (m, 4H), 7.72–7.59 (m, 5H), 5.37 (dd, J = 9.8, 5.1 Hz, 1H), 4.42 (t, J = 9.6 Hz, 1H), 4.22 (dd, J = 9.3, 5.1 Hz, 1H), 3.77 (s, 3H), 2.44 (d, J = 5.0 Hz, 3H). MS (ESI) *m/z*: [(M–H)$^-$, 437.0].

*(S)-N-Hydroxy-3-(4-{[4-(N-methylsulfamoyl)phenyl]buta-1,3-diyn-1-yl}phenyl)-2-oxooxazolidine-5-carboxamide (**DD-11**)*

Compound **DD-11** was prepared from intermediate **D-06** in the same manner as described for compound **DD-2**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.24 (s, 1H), 9.30 (s, 1H), 7.82 (s, 4H), 7.68 (s, 4H), 7.63 (d, J = 5.1 Hz, 1H), 5.05–4.96 (m, 1H), 4.31 (t, J = 9.4 Hz, 1H), 4.11–4.00 (m, 1H), 2.44 (d, J = 5.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.80, 153.84, 140.43, 139.98, 133.98 (overlap), 133.58 (overlap), 127.52 (overlap), 125.00, 118.29 (overlap), 115.05, 83.67, 80.67, 76.59, 73.35, 69.53, 47.59, 29.09. MS(ESI)

m/z: [(M–H)$^-$, 437.8]. HRMS (ESI): Anal. calc. for $C_{21}H_{16}O_6N_3S$: 438.0760, found: 438.0756.

4.2 | Biochemical pharmacology

4.2.1 | MIC tests

MIC of each compound against standard Gram-negative bacteria (DH5α, AB1157, PAO1, PA14) was tested by the modified National Committee for Clinical Laboratory Standards, which is adapted to 96-well plates and LB media in the presence of 5% DMSO.[15]

The MICs of the target compounds against clinical isolated Gram-negative bacteria were determined using the broth micro-dilution protocol according to the methods of the Clinical and Laboratory Standards Institute, in the Mueller–Hinton broth.[16] These clinical isolated strains were obtained from hospitals in Sichuan and Beijing. All of the tested compounds were dissolved in DMSO, and then serially diluted in growth medium. The strains were incubated at 35–37°C, and the MIC values were determined at 18 hr.

4.2.2 | Liver microsomal stability assay

The assay was performed with liver microsomes from male human, rat, and mouse. The incubation was performed as follows: Microsomes in 0.10 M trishydroxymethyl aminomethane/hydrochloric acid buffer (pH 7.4, 0.33 mg/ml microsomal protein), cofactor $MgCl_2$ (5.0 mM), tested compound (final concentration 0.10 μM, 0.01% DMSO, 0.005% bovine serum albumin). Then NADPH (1.0 mM) was added at 37°C and incubated for 60 min. The reaction can be started by the addition of liver microsomes or the tested compounds or NADPH. Aliquots were sampled at 0, 7, 17, 30, and 60 min incubation, and enzymatic reaction was stopped by protein precipitation in methanol. After centrifugation, the samples were then analyzed by LC/MS/MS. The assay evaluated the metabolic stability of compounds by measuring the in vitro half-life ($t_{1/2}$) and liver microsomal clearance (Cl_{int}).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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