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Optimization of LpxC inhibitor lead compounds focusing on efficacy and formulation for high dose intravenous administration

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KEYWORDS: Antibacterial, formulability, Gram-negative pathogens, hydroxamic acid, iv administration, LpxC inhibitors, solubility.

ABSTRACT: LpxC inhibitors were optimized starting from lead compounds with limited efficacy and solubility, and with the goal to provide new options for the treatment of

serious infections caused by Gram-negative pathogens in hospital settings. In order to enable the development of an aqueous formulation for intravenous administration of the drug at high dose, improvements in both solubility and antibacterial activity in vivo were prioritized early on. This lead optimization program resulted in the discovery of compounds such as **13** and **30**, which exhibited high solubility and potent efficacy against Gram-negative pathogens in animal infection models.

INTRODUCTION

Infections caused by multidrug-resistant (MDR) Gram-negative bacteria are nowadays considered as one of the greatest threats to global health.[1, 2] In particular, the incidence of infections caused by resistant pathogens such as *Acinetobacter*, *Pseudomonas aeruginosa* (Pae) and Enterobacteriaceae including *Klebsiella pneumoniae* (Kpn) and *Escherichia coli* (Eco), is increasing at a steady and alarming pace.[3, 4] Development of novel antibiotics active against these MDR Gram-negative bacteria (GNB) is urgently needed to provide new therapeutic options for difficult-to-treat infections, and to avoid

antimicrobial resistance (AMR) jeopardizing modern medical practice such as general intensive care, joint replacement, transplant surgery, and cancer therapy.

LpxC has drawn attention as a promising target for new antibiotic discovery ever since specific inhibitors exhibiting potent growth inhibition of Pae and Enterobacteriaceae were discovered.[5-7] In addition, such antibiotics with novel mechanisms of action may overcome cross-resistance with marketed antibiotics since no LpxC inhibitors are currently used in clinical settings. LpxC (UDP-3-O-((*R*)-3-hydroxymyristoyl)-*N*-acetylglucosamine deacetylase; EC 3.5.1.108) is a zinc-dependent enzyme localized in the cytosol of GNB.[8] It catalyzes the first committed step in the biosynthesis of lipid A, a major component of lipopolysaccharides (LPS), and is essential for Gram-negative outer membrane integrity and cell envelope biogenesis.[9] The gene encoding LpxC is broadly distributed across the genomes of GNB while no homologues have been found in genomes of Gram-positive bacteria and mammals. This offers the possibility of developing a selective treatment, which would avoid promoting resistance development

in Gram-positive organisms and be devoid of side effects linked to the inhibition of a homologous human target.

In the accompanying manuscript,[10] we reported the discovery of new LpxC inhibitors **1**-**3** (Figure 1), which exhibited dose-dependent efficacy in a mouse thigh infection model. Even though the results of these proof-of-concept studies were encouraging, daily doses of 150 mg/kg did not lead to a reduction of bacterial load below that at treatment start. Similarly, methylsulfone hydroxamate derivatives reported by Pfizer, such as **4**, suffered from limited free exposures in rodents, mainly due to their high plasma clearance.[11] The optimization strategy reported in this latter case focused on lowering the human predicted dose by reducing plasma protein binding, which successfully yielded compounds such as **5** with improved free exposures and efficacy whereas clearance remained moderate to high.[12, 13]

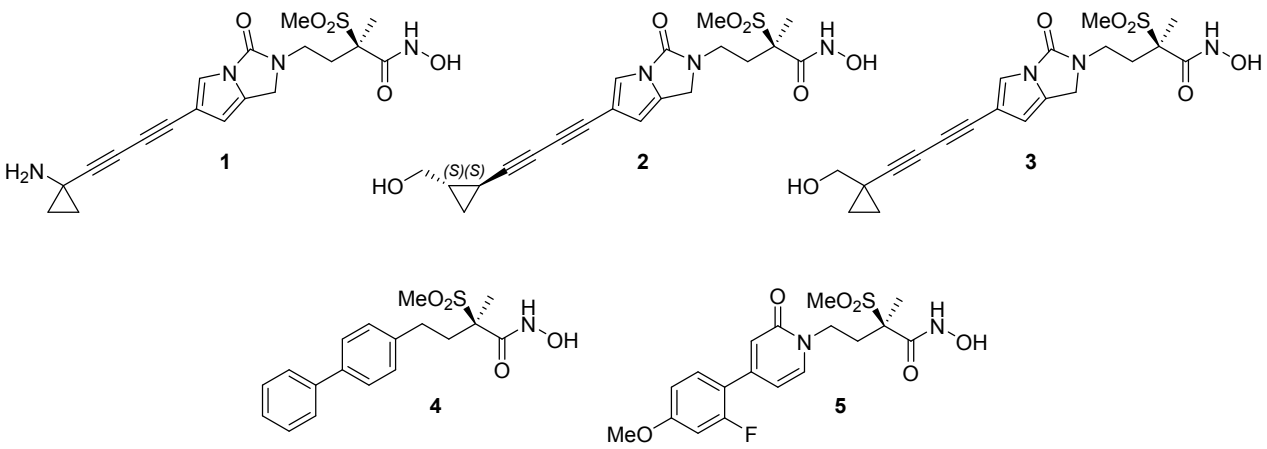


Figure 1. Methylsulfone hydroxamate LpxC inhibitors reported by Idorsia (**1**-**3**) and Pfizer (**4**, **5**).

Compounds with low clearance in humans are usually preferred since they minimize the therapeutic doses needed, apart from the case described above that represents an exception. In addition, low clearance in animals is also advantageous for preclinical development since toxicology studies are conducted at exposure levels that are multiples of those estimated for human efficacy. The identification of compounds that can be formulated as concentrated solutions for conducting such toxicology studies represents an additional challenge in the context of development of antibiotics for Gram-

negative pathogens, where patients are typically treated in hospitals with high daily doses intravenously.[14]

Starting from the reported lead compounds **1**-**3**, we aimed at improving antibacterial activity in vivo while concomitantly ensuring high solubility in a vehicle adequate for future safety studies. We took advantage of the various leads' structural features to follow a two-pronged approach. Starting from lead compound **1**, we improved the intrinsic solubility and formulability by using the potential of its amino moiety for ionization. In parallel, we targeted improving efficacy and metabolic stability for lead compounds **2** and **3**, while their hydroxy group represented a potential handle to attach a solubilizing moiety that would enable the development of a prodrug approach later on if needed.

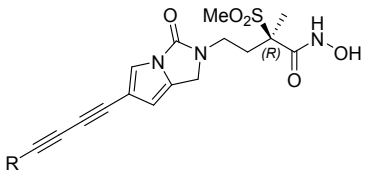
RESULTS AND DISCUSSION

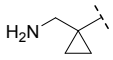
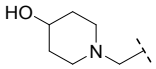
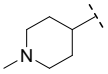
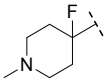
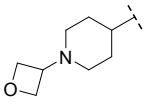
Antibacterial activity, solubility and pKa. Compound **1** was regarded as a particularly appealing starting point for investigating the solubility and formulability of this series of LpxC compounds. As previously seen in X-ray structures of compounds from this

chemical series, the terminal substituent on the distal alkyne lies on the outside of the LpxC enzyme, which allowed for structural modification without detrimental effects on the enzyme binding affinity.[10] The compound showed moderate solubility at pH 4, 7 and 9 and potent antibacterial activity with MICs of < 1 μg/mL against the three strains tested (Table 1). With a measured logD of 0.7 at pH 7.4, **1** was relatively polar among the compounds evaluated. The various pKa's of the compound were determined to investigate the impact of ionization on solubility. The primary amine turned out to be weakly basic (pKa 6.5) and the hydroxamic acid weakly acidic (pKa 7.5). Therefore, several ionized species exist under equilibrium at physiological pH, which may limit the potential to formulate the compound at high concentrations. The target concentration for formulation was set at 25 mg/mL at a pH ranging from 4.5 to 8.5 to avoid local tolerance issues at the injection site.[15] Given the pKa values of **1**, the pH of a suitable formulation would have to be <4.5 in order to allow for sufficient solubility with >99% of the compound in the protonated state. Therefore, we decided to look for compounds which possess a more basic amine since a higher pKa would allow complete protonation of

the amine at a pH closer to physiological conditions, thus facilitating the preparation of concentrated formulations.

Table 1. Exploration of side chains containing an amine function.



Cmpd	R	MIC (µg/mL)[a]			ΔTm (K)[b]		logD7.4	pKa[c] R	Solubility (µg/mL)		
		Eco	Kpn	Pae	Eco	Pae			pH 4	pH 7	pH 9
1		0.25	0.25	0.5	10.0	11.6	0.7	6.5	571	418	536
6		0.12	0.25	0.5	10.6	12.2	1.4	6.5	551	182	486
7		0.25	0.5	0.5	11.6	13.4	2.1	5.5	412	15	95
8		8	16	16	9.6	10.7	-0.4	9.2	>730	552	>730
9		1	2	1	9.5	11.5	0.4	6.5	>810	>810	>810
10		4	4	1	10.6	11.3	0.2	9.0	>780	>780	574
11		0.5	1	1	9.6	12.0	1.0	8.0	>810	384	203
12		0.5	1	2	11.1	13.2	1.1	6.2	>850	329	>850
13		0.5	0.5	0.5	8.9	9.9	0.3	8.4	>730	>730	>730

[a]*E. coli* ATCC 25922; *K. pneumoniae* T 6474, QR and ESBL; *P. aeruginosa* ATCC 27853. [b]DSF on isolated enzyme, determined at 25 µM compound concentration. [c]pKa of the hydroxamic acid varied between 7.3 and 7.7.

Addition of methyl groups to **1** resulted in derivatives **6** and **7**, which displayed comparable antibacterial activity despite having slightly increased binding affinity for the enzymes (ΔTm values, determined by DSF). However, their solubility decreased in line with the logD increase.

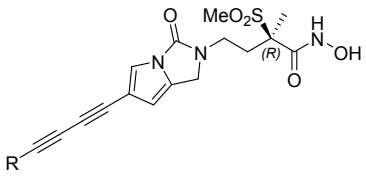
The introduction of an extra carbon atom in **8** resulted in a significant pKa shift with a more basic nitrogen (pKa 9.2), which would be almost fully protonated under physiological conditions. This was reflected in the solubility and logD measurements. Unfortunately, compound **8** had almost completely lost its antibacterial activity even though it had retained significant binding to the enzymes (ΔTm). It was hypothesized that the basic primary amine may lead to a reduced uptake into the GNBs under investigation since the MIC shifts between the WT Pae strain and the isogenic efflux mutant were comparable for all compounds shown in Table 1 (4- to 8-fold shift, Table S-1).

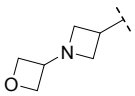
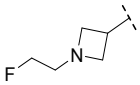
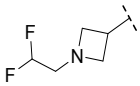
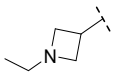
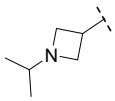
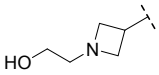
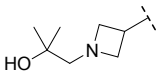
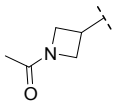
Various nitrogen-containing rings were also considered as side chains. Hydroxypiperidine derivative **9** displayed moderate antibacterial activity against the

three strains as well as very good solubility at all pHs but its pKa was identical to that of **1**, thus preventing us from fully exploiting the nitrogen atom's potential for ionization. An inversion of the piperidine ring as in **10** led to a pKa increase but still resulted in a weaker antibacterial activity. Although critical for achieving potent activity against these three bacteria, neither the basicity nor the location of the nitrogen influenced the binding to the enzyme. Modification with a fluorine[16] or oxetane[17] substituent in **11** and **12** resulted in a step-wise decrease in the pKa along with variable effects on lipophilicity and solubility, but this did not result in recovering the targeted level of antibacterial activity. Only **13**, with its smaller azetidine ring, displayed antibacterial activity below 1 μg/mL against the three strains, was very soluble at all pHs tested and possessed a basic pKa of 8.4, which was deemed ideal to allow sufficient solubility for developing a concentrated formulation suitable for iv dosing. Surprisingly, it had an MIC of 0.5 μg/mL against Pae despite having the lowest ΔTm among the compounds tested, suggesting again that the antibacterial potency is driven not only by target potency but also by drug accumulation in the cytoplasm.

The potential of the azetidine side chains was further explored (Table 2). Since amides are usual metabolites resulting from aldehyde oxidase-mediated reduction of hydroxamic acids, we first checked whether the presence of the hydroxamic acid moiety was indeed required for activity. As expected, the corresponding amide derivative **14** did not show any inhibitory activity and did not bind to the enzymes. Then, the effect of the azetidine substitution on antibacterial activity and solubility was investigated.

Table 2. Exploration of the azetidine side chain substitution.



Cmpd	R	MIC (μg/mL)[a]			ΔTm (K)[b]		$logD_{7.4}$	pKa[c] R	Solubility (μg/mL)		
		Eco	Kpn	Pae	Eco	Pae			pH 4	pH 7	pH 9
13		0.5	0.5	0.5	8.9	9.9	0.3	8.4	>730	>730	>730
14[d]		>32	>32	>32	0	0.3	0.5	8.4	>700	706	92
15		8	4	1	8.0	9.3	-1.0	9.4	>710	>710	>710
16		0.25	0.5	1	10.5	12.6	0.5	5.5	>800	128	681
17		0.12	0.5	2	8.1	11.6	1.1	6.7	>760	219	>760
18		1	2	2	7.1	10.0	0.3	8.0	>740	175	279
19		0.06	0.25	0.5	10.6	12.9	1.0	6.7	>780	247	>780
20		0.06	0.25	1	10.6	13.2	1.4	5.2	>820	209	>820
21		0.25	0.5	0.5	9.3	10.4	0.6	8.5	>750	>750	>750
22		0.25	0.25	0.5	9.6	11.2	1.0	8.3	>780	398	420
23		0.5	1	0.5	9.7	11.2	0.1	7.9	>780	>780	>780
24		0.5	1	0.5	10.3	12.1	0.7	7.8	>830	>830	>830
25		1	1	1	9.7	12.4	0.3	-	>780	>780	>780

[a]*E. coli* ATCC 25922; *K. pneumoniae* T 6474, QR and ESBL; *P. aeruginosa* ATCC 27853. [b]DSF on isolated enzyme, determined at 25 μM compound concentration. [c]pKa of the hydroxamic acid varied between 7.2 and 7.7. [d]Amide instead of hydroxamic acid.

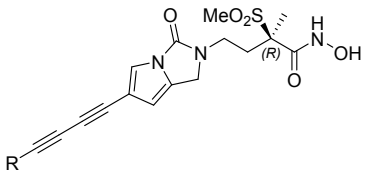
Unsubstituted azetidine **15** retained some level of antibacterial activity, notably against Pae, despite the basic pKa and the high polarity as indicated by the negative logD. Oxetane derivative **16** displayed MICs comparable to those of **13** but was significantly less soluble at pH 7 likely due to the reduced nitrogen basicity. In comparison, the pKa increased with the two fluorinated azetidines **17** and **18**. While the former had overall properties comparable to **16** despite its lower affinity to the enzymes, the latter had antibacterial activity and solubility that were not satisfactory. When the fluorine was present on the substituent of the azetidine nitrogen, as in **19**, MICs improved but the solubility at pH 7 remained limited, in line with the pKa and logD. An attempt to further modify the antibacterial activity by modulating logD and pKa with an additional fluorine atom[16] in **20** proved unsuccessful. As expected, it led to increased logD and concomitantly decreased pKa, but the antibacterial activity did not improve. Ethyl-substituted analogue **21** displayed overall properties comparable to those of **13**, while isopropyl derivative **22** exhibited higher logD and lower MICs against Enterobacteriaceae. Surprisingly, the MIC against Pae was not negatively affected by the increased lipophilicity. Even though **22** had a pKa in the desired range, it suffered

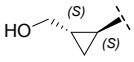
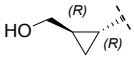
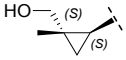
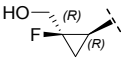
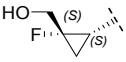
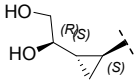
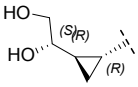
from limited solubility at pH 7 and 9. Hydroxy-containing side chains were also tolerated, as highlighted by **23** and **24**. These derivatives displayed overall properties comparable to those of **13**, though a somewhat slightly lower pKa. One advantage of such derivatives may be the possible use of the hydroxy function as a handle to attach a promoiety in using a prodrug approach, which could be an option to reach higher concentrations in formulations if needed. Interestingly, the slightly less active amide **25** was highly soluble over the entire pH range tested even though it did not contain a protonable nitrogen. In summary, the azetidine ring has been shown to be a privileged motif that allows combining potent antibacterial activity and high solubility. Several compounds of interest were therefore progressed into in vivo evaluation (vide infra).

Metabolic stability. The other chemical series reported in the accompanying manuscript featured a hydroxymethyl substituent on the cyclopropyl moiety in place of the amino group found in **1**. These early leads **2** and **3** also exhibited dose-dependent efficacy in the murine thigh infection model, but net static efficacy over 24 h was not achieved even at relatively high doses. To improve the efficacy in this model, one could

focus on improving the intrinsic antibacterial activity (MIC) and/or the exposure (PK). It had already been reported that most of the metabolism of methylsulfone derivatives such as **4** occur on the hydroxamic acid moiety via glucuronide formation and aldehyde oxidase-mediated reduction to the corresponding amide.[11] In order to gain a more detailed understanding of our compounds' metabolism, and the impact of the solvent-exposed groups in particular, a metabolite identification study was performed on a compound related to **2** and possessing the same cyclopropyl substitution. Upon incubation in suspended fresh rat hepatocytes, a glucuronide and the corresponding amide were indeed observed as expected. The hydroxymethyl side chain also appeared to be a metabolically labile spot, which, upon oxidation, gave the corresponding carboxylic acid in both rat microsomes and hepatocytes. We therefore decided to increase the sterics around the cyclopropyl moiety to block this metabolically labile site. The effects of these modifications on antibacterial activity and metabolic stability in vitro are reported in Table 3.

Table 3. Exploration of side chains containing a hydroxy group.



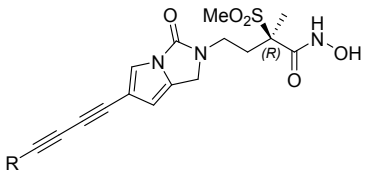
Cmpd	R	MIC (μg/mL)[a]			$Cl_{int,tot}$		$logD_{7.4}$	Solubility (μg/mL)		
		Eco	Kpn	Pae	RHep [b]	RLM [c]		pH 4	pH 7	pH 9
2		0.12	0.25	0.25	19	<10	1.3	600	588	628
26		0.12	0.25	0.5	13.4	<10	1.1	600	602	552
3		0.25	1	0.5	8.5	24	0.9	149	384	553
27		0.25	0.25	0.25	55	<10	1.6	344	401	506
28		0.25	0.5	0.5	13	16	0.9	354	613	>760
29		0.25	0.5	0.5	13	12	0.9	220	248	>760
30		0.5	1	1	10.2	<10	0.5	>780	>780	>780
31		0.25	1	0.5	10	<10	0.5	618	>780	>780

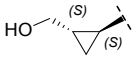
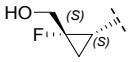
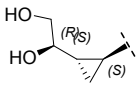
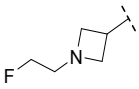
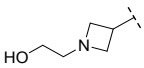
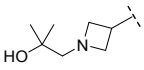
[a]*E. coli* ATCC 25922; *K. pneumoniae* T 6474, QR and ESBL; *P. aeruginosa* ATCC 27853. [b]Intrinsic clearance in μL/(min*10^6 cells) measured in fresh rat hepatocytes in suspension. The fraction unbound of compounds in media used for all Cl_{int} investigations were all in a similar range. [c]Intrinsic clearance in μL/(min*mg) measured in rat liver microsomes.

As reported in the accompanying manuscript, the difference in the stereochemistry of the cyclopropyl ring in **2** and **26** impacted neither the MIC nor the Cl_{int} measured in vitro in rat hepatocytes and microsomes. However, when the hydroxymethyl group was appended to a different position on the cyclopropane ring as in **3**, the antibacterial activity was slightly reduced and hepatocytes Cl_{int} slightly decreased together with a concomitant increase in metabolic liability in microsomes. The presence of a methyl group in **27**, which displayed MICs comparable to those of **2**, resulted in an increase of about 3-fold in Cl_{int} in hepatocytes but not in microsomes, potentially indicating an enhanced glucuronidation. The opposite trend was observed with an additional fluorine atom at the same position, and both diastereomers **28** and **29** displayed a slightly lower Cl_{int}. This additional atom had no impact on the antibacterial activity when compared to **2** and **26**. Incorporation of an additional hydroxymethyl unit on this substituent led to **30** and **31**, which, in addition to improved metabolic stability, also displayed excellent solubility.

Pharmacology. Hydroxy-containing derivatives with acceptable Cl_{int} values as well as azetidine analogs showing the targeted antibacterial activity in vitro were assessed for efficacy against *E. coli* in the neutropenic murine thigh infection model after subcutaneous administration (Table 4). The fluorinated derivative **29** reduced CFU below net stasis, i.e. below the level measured at treatment start 2 h after mice were infected, and exhibited significantly higher efficacy in this model as compared to the previous lead compounds **2** and **3**. While the 3-fold increase in free AUC could explain the higher efficacy compared to **2**, **29** also displayed a stronger CFU reduction than **3** despite a 2-fold lower free AUC and comparable MIC. Diol **30** also displayed an improved efficacy and a larger free AUC. With its roughly 5-fold higher free AUC, the soluble azetidine **13** proved more efficacious than its previous amine lead compound **1**. Fluoroethyl derivative **19** displayed efficacy below net stasis, comparable to that of **13**, with the 8-fold improved MIC of **19** over **13** compensating for its 6-fold reduced free AUC. Finally, the two hydroxy derivatives **23** and **24** revealed different results. While the efficacy and MIC of **23** was comparable to that of **13**, the free exposure of **24** was low and did only result in marginal reduction of the bacterial burden in vivo.

Table 4. Efficacy and exposure of selected derivatives in mice.



Cmpd	R	MIC[a] (μg/mL)	Efficacy[b] (Δlog_{10}CFU/thigh)	mPPB[c] (%)	AUC_{24h}[d] (μg*h/mL)	free AUC_{24h} (μg*h/mL)
2		0.12	+0.8	99.1	124	1.11
3		0.25	+0.8	91.5	89.5	7.64
29		0.25	-1.2	98.6	236	3.30
30		0.5	-0.6	97.3	347	9.54
1		0.25	+1.8	92.2	44.7	3.49
13		0.5	-0.8	63.3	52.9	19.4
19		0.06	-0.6	93.5	49.1	3.19
23		0.5	-0.9	66.1	63.2	21.4
24		0.5	+2.2	90.0	47.3	4.73

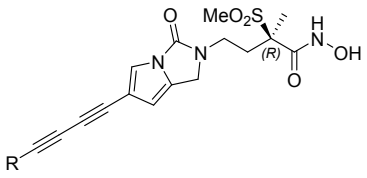
[a]*E. coli* ATCC 25922. [b]Mice were treated sc at 150 mg/kg starting at 2 h post infection and sacrificed at 24 h post infection. Median Δlog_{10}CFU/thigh of animal treated with compounds compared to CFU at treatment start is reported. [c]Determined at 1 μM compound concentration. [d]AUC values originated from PK studies performed in mouse dosed sc at 150 mg/kg, except for **2** for which the AUC was extrapolated from a sc dosing at 50 mg/kg.

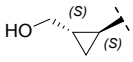
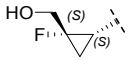
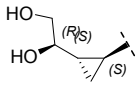
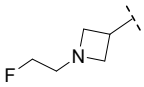
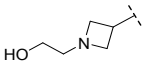
ADME properties. The compounds that exhibited interesting efficacy in the murine infection model were further characterized in rats for their PK properties after intravenous dosing, with the goal to investigate the correlation between in vitro and in vivo clearances as a first step towards human PK modeling. Fluorinated cyclopropyl **29** displayed a 7-fold lower unbound clearance compared to **2** (Table 5). It showed a proportionally higher free exposure, highlighting the positive effect of the fluorine. Diol **30** displayed the lowest clearance among all hydroxy derivatives. Among the compounds with amine side chains, **13** had lower unbound clearance than **1** in rats. This was reflected in the in vitro Cl_{int} data from hepatocytes but not liver microsomes. Data from hepatocytes are therefore better suited to predict in vivo clearance, consistent with the important role played by phase II metabolism for this series of compounds. The more lipophilic fluoroethyl derivative **19** displayed higher clearance while the hydroxy-containing azetidine derivative **23** was comparable to **13** overall. In general, the more metabolically stable the compound was in rat hepatocytes in vitro, the

lower its unbound clearance and the higher its free AUC in vivo. Given the intrinsic clearance shown in human in vitro systems, a lower metabolic clearance is expected in human as compared to that measured in animals.

Additional in vitro investigations on reactive metabolite formation and drug drug interactions were performed. Upon incubation of the compounds at 10 μM with human liver microsomes, the rates of trapping of reactive entities by dansyl glutathione were in all instances lower than 100 pmol/mg·h. The compounds did not inhibit any of the major cytochromes P450 enzymes CYP3A4/2C9/2D6 (IC_{50} > 50 μM), and neither did **29**, **30**, **1**, **13**, and **23** activate the human pregnane X receptor (PXR) at concentration up to 30 μM (**2** and **19** not tested in this latter assay).

Table 5. In vitro and rat PK parameters of selected derivatives.



Cmpd	R	logD$_{7.4}$	Cl$_{int,tot}$				rPPB [e] (%)	Cl$_u$ (Cl)[f] (mL/min*kg)	free AUC (ng*h/mL)
			RHep [a]	RLM[b]	HHep[c]	HLM[d]			
2		1.3	19	<10	3.2	<10	99.2	2125 (17)	80.8
29		0.9	13	12	<2	<10	98.3	306 (5.2)	550
30		0.5	10.2	<10	<2	<10	98.2	172 (3.1)	968
1		0.7	17	14	<2	<10	88.9	261 (29)	637
13		0.3	8.6	665	<2	48	81.0	95 (18)	1777
19		1.0	15.4	>1250	<2	<10	93.3	567 (38)	291
23		0.1	7.3	198	<2	14	69.9	93 (28)	1774[g]

[a]Intrinsic clearance in µL/(min*10^6 cells) measured in fresh rat hepatocytes in suspension. All Cl$_{int}$ were determined at 1 µM compound concentration, and the fraction unbound of compounds in media were all in a similar range. [b]Intrinsic clearance in µL/(min*mg) measured in rat liver microsomes. [c]Intrinsic clearance in µL/(min*10^6 cells) measured in cryopreserved human hepatocytes in suspension. [d]Intrinsic clearance in µL/(min*mg) measured in human liver microsomes. [e]Determined at 1 µM compound concentration. [f]Rats were dosed iv at 10 mg/kg; Cl$_u$: unbound clearance calculated by correcting total Cl (in parenthesis) with the free fraction in plasma. [g]Calculated from iv PK at 50 mg/kg.

Antibacterial activity on an extended panel of strains. Five compounds were further evaluated against a panel of recent clinical isolates, including drug-resistant pathogens, such as fluoroquinolone- or carbapenem-resistant strains (Table 6). All compounds showed potent activity against Enterobacteriaceae with MIC_{90} values of ≤4 μg/mL and against Pae isolates with MIC_{90} of ≤ 2μg/mL. While excellent activity was observed against the respiratory-tract pathogen *Haemophilus influenzae*, there was no useful activity against *Acinetobacter baumannii* and *Moraxella catarrhalis*. This is in agreement with the published findings that in these GNB the activity of LpxC is dispensable for growth.[18] In addition, all compounds exhibited only weak binding affinity in DSF against an isolated *A. baumannii* enzyme (data not shown). Similarly, it is reasonable to assume that it is also the case for *Stenotrophomonas maltophila*. In summary, these compounds have an antibacterial spectrum that would be well suited for the treatment of serious infections caused by GNB, especially Enterobacteriaceae and *P. aeruginosa*.

Table 6. MIC_{90} (μg/mL) of selected derivatives and reference compounds.

Strains (n)	13	19	23	29	30	Cipro[a]	Tige[b]	Imi[c]
C. freundii (10)	1	0.5	1	1	1	0.125	1	1
E. aerogenes (11)	1	1	1	2	2	0.03	1	2
E. cloacae (10)	4	1	4	4	4	0.06	4	1
E. coli (21)	1	0.25	1	2	2	>8	1	0.25
K. oxytoca (9)	4	0.125	4	4	4	≤0.016	0.5	0.5
K. pneumoniae (21)	4	2	2	4	4	>8	4	>16
M. morganii (10)	0.5	0.25	1	0.5	1	≤0.016	8	16
P. mirabilis (10)	1	0.5	1	2	1	0.125	4	4
S. marcescens (11)	0.5	0.25	1	1	0.5	0.25	2	1
A. baumanii (15)	>16	>16	>16	>16	>16	>8	16	>16
B. cepacia (11)	16	4	16	2	8	8	>16	>16
H. influenzae (9)	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.016	0.25	0.5
M. catarrhalis (10)	>16	>16	>16	>16	>16	1	0.5	0.06
P. aeruginosa (16)	2	1	2	2	2	>8	>16	>16
S. maltophilia (10)	>16	>16	>16	>16	>16	8	4	>16

[a]Ciprofloxacin. [b]Tigecycline. [c]Imipenem.

Formulation investigations. The identification of a formulation suitable for toxicity studies often represents a challenge in the field of antibacterial compounds targeting GNB since single doses of ≥1 g are required for human use, and multiples of the predicted human exposure are needed in animals. Highly concentrated formulations are

therefore required since the volume that can be injected is limited and only vehicles that do not lead to any tolerability or toxicity issues on their own can be used.[14]

We decided to test our compounds at various concentrations in an aqueous solution containing 5% mannitol as the only excipient for osmolarity adjustment and modify the pH to maximize solubility. The lowest acceptable pH was set at pH 4.5 to avoid local tolerance issues at the injection site. As the ionization of compounds comprising a hydroxy-containing side chain devoid of any basic amine takes place at basic pH by deprotonation of the hydroxamic acid, the upper pH threshold was set at pH 8.5. Formulations trials were conducted at concentrations of 1, 5, 10 and 25-30 mg/mL, and the highest concentration for each derivative where a clear solution was obtained is reported in Table 7. Azetidines **13**, **19**, and **25** could be successfully formulated at 25-30 mg/mL under acidic conditions. For the latter two, slightly turbid formulations were obtained at the highest concentrations. Filtration through a 0.22 µm PVDF filter yielded clear solutions, which showed the expected concentration after HPLC analysis. Diol **30** also turned out to be soluble at high concentration in a slightly basic formulation,

whereas the limited solubility of **29** prevented clear solutions at concentration >1 mg/mL.

Table 7. Formulations resulting in a clear solution in 5% mannitol/water.

Cmpd	Concentration [mg/mL]	pH
13	30	4.5
19	25	4.5
23	30	4.5
29	1	8.4
30	25	8.2

CONCLUSION

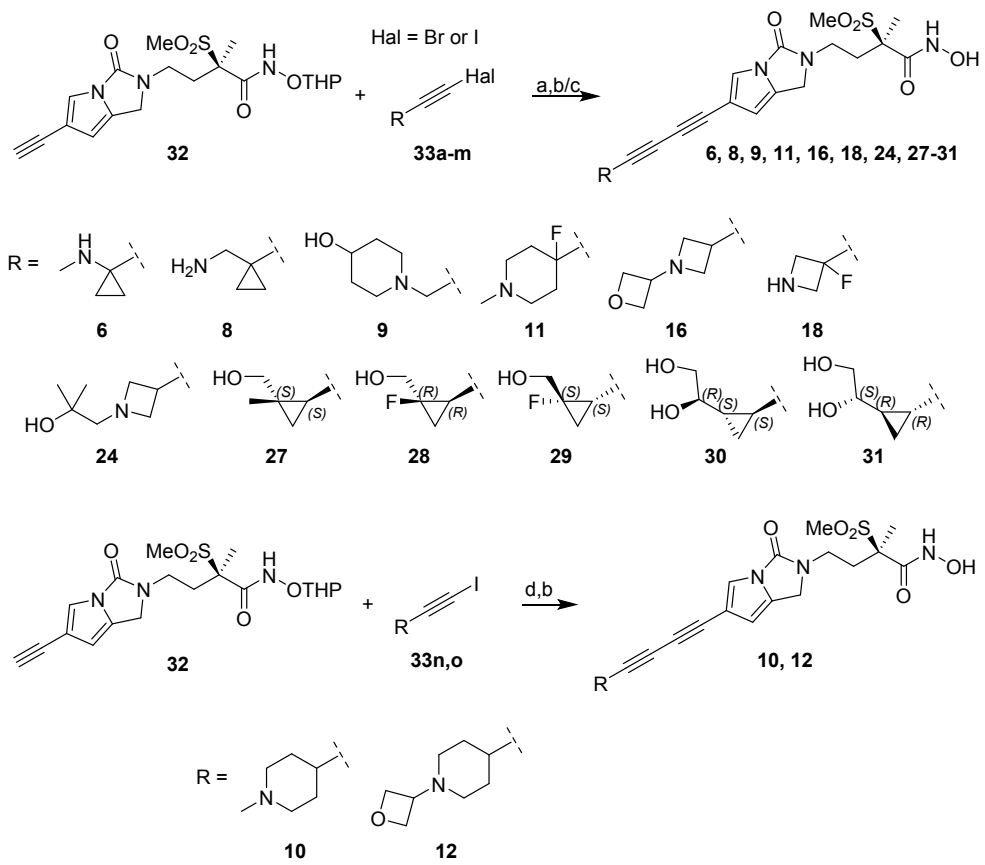
We successfully completed a lead optimization program focused on improving solubility and formulability parameters as well as antibacterial efficacy rather than simply in vitro potency. Focusing on solubility in addition to activity early in the lead optimization phase led to the discovery of a set of compounds fulfilling the targeted attributes, i.e., improved antibacterial activity against GNB causing complicated urinary tract, intraabdominal space, or lower respiratory tract infections, and improved properties for intravenous

administration. In addition to displaying suitable physicochemical properties enabling the development of a formulation for intravenous administration of high doses, the compounds exhibited improved metabolic stability resulting in lower clearance leading to promising efficacy in an animal infection model. A few of these compounds will be assessed further and results will be reported in due course.

CHEMISTRY

Compounds **6**, **8**, **9**, **11**, **16**, **18**, **24** and **27-31** were prepared by reacting alkyne **32** and halo-alkynes **33a-m** in a Cadiot-Chodkiewicz cross-coupling reaction [19] followed by cleavage of the THP protecting group under suitable acidic conditions, using either hydrochloric acid in a 9:1 mixture of methanol and water, or PPTS in ethanol (Scheme 1).[20, 21] Alternatively, coupling alkyne **32** with iodoalkynes **33n-o** in a Sonogashira reaction gave **10**, respectively **12**, after deprotection.

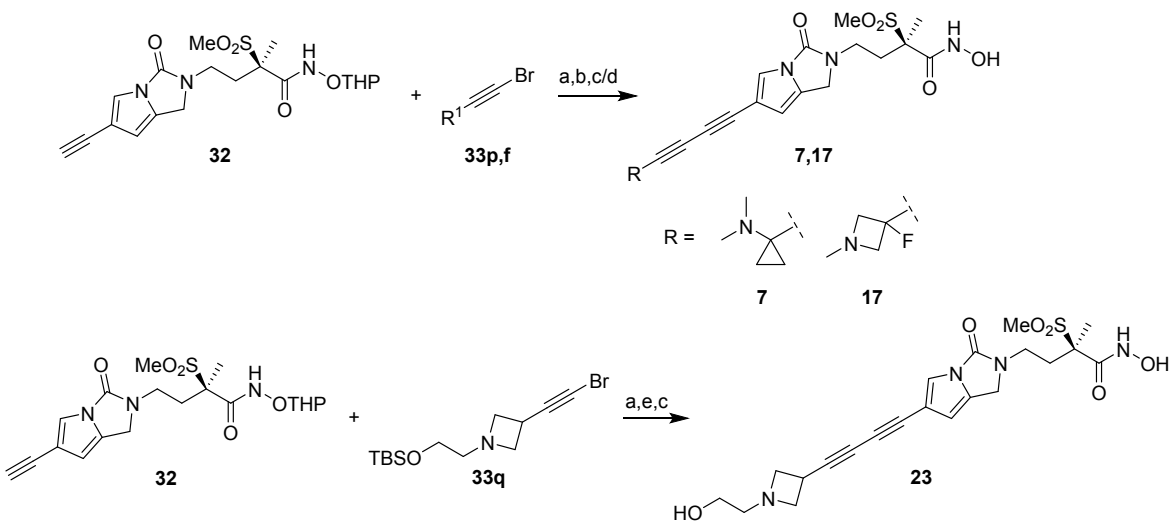
Scheme 1. Synthesis of derivatives using Cadiot-Chodkiewicz and Sonogashira coupling [a]



[a]Reagents and conditions: (a) CuCl, $NH_2OH{\cdot}HCl$, n-$BuNH_2$, 0 °C-rt, 2 h. (b) HCl, 9:1 MeOH/H_2O, rt, 1 h. (c) PPTS, EtOH, 80 °C, 2 h. (d) CuI, $PdCl_2(PPh_3)_2$, Et_3N, THF, 40 °C, 1 h.

The side chain of **7**, **17** and **23** was functionalized by undergoing a reductive amination or a deprotection reaction under standard conditions before the final hydroxamic acid deprotection (Scheme 2).

Scheme 2. Synthesis of derivatives **7**, **17** and **23** [a]

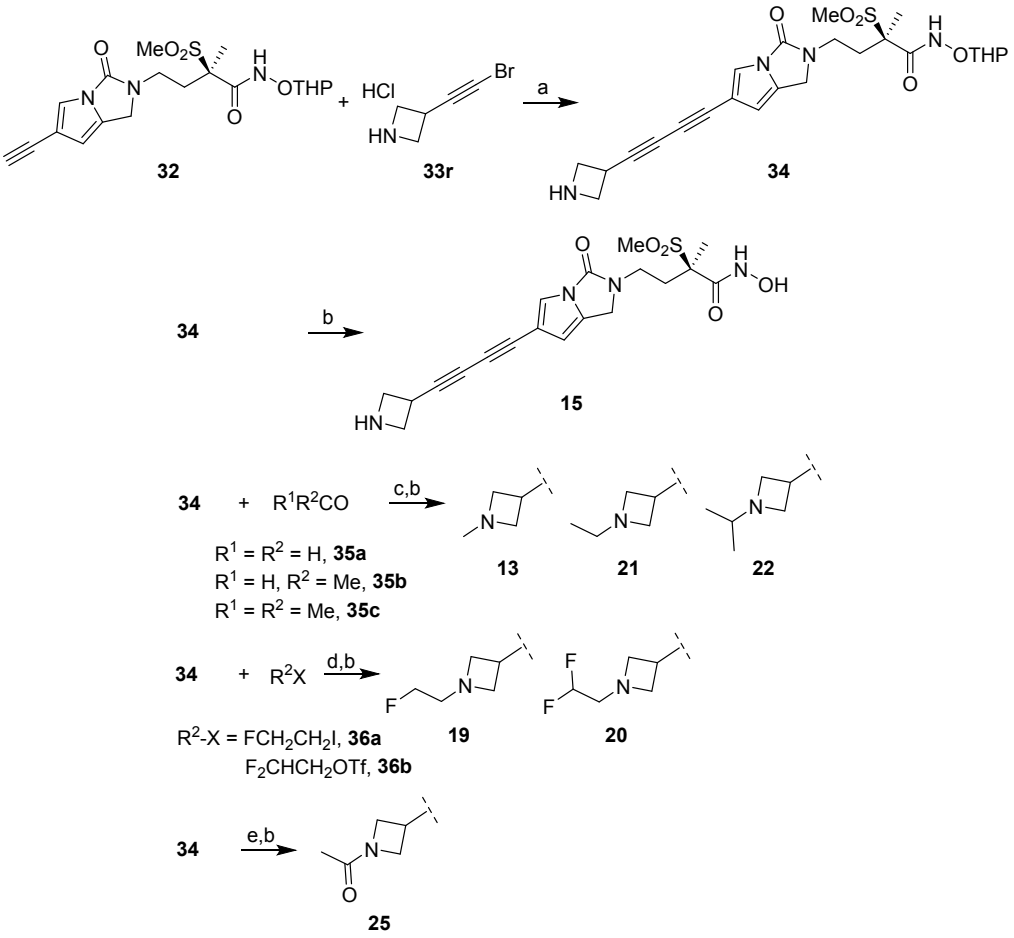


[a]Reagents and conditions: (a) CuCl, $NH_2OH{\cdot}HCl$, n-$BuNH_2$, 0 °C-rt, 2 h. (b) HCHO, $NaBH(OAc)_3$, DCM, rt, 1-16 h. (c) HCl, 9:1 MeOH/H_2O, rt, 1 h. (d) PPTS, EtOH, 80 °C, 2 h. (e) TBAF, THF, rt, 3 h.

The azetidine-containing derivatives **13**, **15**, **19-22** and **25** were prepared from the common intermediate **34** resulting from Cadiot-Chodkiewicz cross-coupling reaction between **32** and **33r**[22] (Scheme 3). An acidic treatment delivered **15**, whereas performing first a reductive alkylation with formaldehyde, acetaldehyde or acetone (**35a-c**), or employing a nucleophilic substitution with iodo- or trifluoromethanesulfonate electrophiles (**36a** or **36b**), gave **13**, **21-22**, respectively **19-20**. Finally, amide **25** was prepared under standard conditions upon reaction with acetic acid. The detailed

synthesis of all bromo- and iodo-alkynes as well as amide **14** are described in the Supporting Information.

Scheme 3. Synthesis of azetidine derivatives from intermediate **34** [a]



[a]Reagents and conditions: (a) CuCl, $NH_2OH{\cdot}HCl$, n-$BuNH_2$, 0 °C-rt, 2 h. (b) HCl, 9:1 MeOH/H_2O, rt, 1 h. (c) $NaBH(OAc)_3$, DCM, rt, 1-2 h. (d) DIPEA, THF, rt - reflux, 2-4 h. (e) AcOH, HATU, DIPEA, THF, rt, 18 h.

EXPERIMENTAL SECTION

Chemistry. **1**, **2**, **3**, **26** and **32** was prepared as described.[10] All solvents and chemicals were used as purchased without further purification. Analytical TLC characterizations were performed with 0.2 mm plates: Merck, Silica gel 60 F254. Column chromatography (CC) were performed using Brunschwig 60A silica gel (0.032-0.63 mm), or using an ISCO CombiFlash system and prepacked SiO_2 cartridges, and the elution was performed with an appropriate gradient of the indicated solvents. Unless indicated otherwise, the gradient started from 100% of apolar solvent and stopped at 100% of the polar solvent. When the polar solvent was MeOH, 100% corresponded to a 90:10 mixture of DCM/MeOH. In cases of compounds containing a basic function (e.g. amine), 1% of NH_4OH (25% aq) was added to the polar eluent(s). Prep-HPLCs were performed on XBridge Prep C18 columns from Waters. Eluents: A, H_2O + 0.5% acidic or basic additive; B, MeCN; Gradient, 5% B to 95% B over 5 min. Detection: UV/vis and/or MS and/or ELSD. Prep-HPLC (acidic): additive in A was HCO_2H. Prep-HPLC (basic): additive in A was NH_4OH (25% aq). Chiral prep-HPLC were performed on a ChiralPak AD-H column from Daicel. Column, 5 µm 30 x 250 mm. Eluent, heptane/EtOH 3:7; flow: 16 mL/min; detection, UV 224 nm. NMR spectra were recorded on a Bruker

Ascend 500 (500 MHz for ^{1}H; 125 MHz for ^{13}C) spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to deuterated solvent as the internal standard (δH: $CDCl_3$ 7.26 ppm, d_6-DMSO 2.50 ppm); multiplicities, s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, br = broad signal; coupling constants are given in Hz. LC-HRMS were performed on Waters Acquity UPLC-MS; pump, Waters Acquity Binary, Solvent Manager; MS, SYNAPT G2 MS; DAD, Acquity UPLC PDA detector; column, Acquity UPLC CSH C18 1.7 µm 2.1 x 50 mm from Waters, thermostated in the Acquity UPLC Column Manager at 60 °C. Eluents: A, H_2O + 0.05% HCO_2H; B, MeCN + 0.05% HCO_2H; gradient, 2% B to 98% B over 2.0 min; flow, 0.6 mL/min; detection, UV 214 nm and MS; the retention time ^{t}R is given in min. The purity of all final compounds was ≥ 95%. Chiral LC (Method 1) were performed on a ChiralPak AY-H column from Daicel. Column, 5 µm 4.6 x 250 mm. Eluent, heptane/EtOH 3:7; flow: 0.8 mL/min; detection, UV 210 nm. Chiral LC (Method 2) were performed on a ChiralPak IF column from Daicel. Column, 5 µm 4.6 x 250 mm. Eluent, MeCN/EtOH + 0.2% TFA 1:4; flow: 1.2 mL/min; detection, UV 210 nm. Other LC-MS were obtained under the following conditions: pump, Agilent G4220A binary gradient pump; MS detector, Thermo

Finnigan MSQPlus or equivalent; DAD detector, Agilent G4212A; ELS detector, Sedere SEDEX 90; column, Zorbax SB-aq 3.5 µm 4.6 x 50 mm from Agilent, thermostated at 40 °C. Eluents: A, H_2O + 0.04% TFA; B, MeCN; gradient, 25% B to 95% B (0.0 min – 1.0 min), 95% B (1.0 min – 1.45 min); flow, 4.5 mL/min; detection, UV 214 nm and MS; the retention time ^tR is given in min. The number of decimals given for the corresponding [M+H$^+$] peak(s) of each tested compound depends upon the accuracy of the LC-MS device used.

General Procedure 1 (Cadiot-Chiodkiewicz Coupling). CuCl (0.2 equiv) was added to a solution of *n*-BuNH_2 (0.5 mL; 5.0 equiv) in water (1.5 mL) at rt. NH_2OH hydrochloride (2.0 equiv) was added and the solution was stirred at rt for 15 min. The terminal alkyne (1.0 mmol; 1.0 equiv) was added and the solution was cooled to 0 °C. A solution of the bromoalkyne (1.2 equiv) in *n*-BuNH_2 (0.5 mL) was added and the reaction mixture was stirred for 2 h and allowed to warm to rt during that time. In case the reaction was not complete at that stage, as many rounds of cooling the reaction mixture to 0 °C, adding bromoalkyne (0.05-0.2 equiv) followed by stirring at rt for 1 h as necessary were

undertaken. Upon completion, the reaction mixture was diluted with water (20 mL) and extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. Purification of the residue gave the desired product.

General Procedure 2 (OTHP Deprotection using HCl in MeOH). To a solution of the THP-protected hydroxamic acid derivative (0.1 mmol) in MeOH (1.1 mL) and water (0.1 mL) was added a 2 M aq HCl solution (0.5 mL). The reaction proceeded at rt for 1 h. A 25% aq NH_4OH solution was added until pH 8 was reached and the resulting mixture was purified by prep-HPLC.

General Procedure 3 (**Reductive Amination**). To a solution of the amine (0.1 mmol; 1.0 equiv) in DCM (1.0 mL) were added the aldehyde (1.1-3.0 equiv) and $NaBH(OAc)_3$ (2.0-6.0 equiv). The reaction mixture was stirred at rt and monitored by LC-MS. Upon reaction completion, a saturated aq $NaHCO_3$ solution (3 mL) was added. The two layers were separated, and the aqueous layer was extracted with 9:1 DCM/MeOH (3 x 3 mL).

The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue gave the desired product.

General Procedure 4 (OTHP Deprotection using PPTS). To a solution of the THP-protected hydroxamic acid derivative (0.1 mmol) in EtOH (5 mL) was added PPTS (1.5 equiv). The reaction mixture was stirred at 80 °C for 2 h and concentrated under reduced pressure. Purification of the residue gave the desired product.

General Procedure 5 (Bisalkyne formation via Pd/Cu catalysis). To the alkyne (1.0 mmol; 1.0 equiv), the iodoalkyne (1.1 equiv), CuI (0.2 equiv) and $PdCl_2(PPh_3)_2$ (0.1 equiv) was added dry THF (0.8 mL) and Et_3N (3.5 equiv) at rt under inert atmosphere (N_2). The resulting reaction mixture was purged with N_2 for 5 min, stirred at 40 °C for 1 h and concentrated under reduced pressure. Purification of the residue gave the desired product.

General Procedure 6 (Azetidine Alkylation). To a suspension of the azetidine derivative (1.0 mmol; 1.0 equiv) in THF (10 mL) at 0 °C were added DIPEA (2.0 equiv) and the alkylating agent (1.5 equiv). The reaction mixture was stirred and monitored by

LC-MS. Upon reaction completion, DCM (10 mL) and saturated aq $NaHCO_3$ solution (10 mL) were added. The two layers were separated, and the aqueous layer was extracted with DCM (2 x 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue gave the desired product.

General Procedure 7 (**Silyl Ether Deprotection**). To a solution of the silyl ether (1.0 mmol; 1.0 equiv) in THF (10 mL) at rt was added a 1 M TBAF solution in THF (2.0 equiv). The reaction mixture was stirred for 3 h and most of the THF was removed under reduced pressure. EtOAc (20 mL) and water (20 mL) were added, and the two layers were separated. The aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. Purification of the residue gave the desired product.

(*2R*)-*N*-hydroxy-2-methyl-4-(6-((1-(methylamino)cyclopropyl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-2-(methylsulfonyl)butanamide (6). Prepared according to General Procedure 1 (62% yield) from **32** (0.100 g; 0.23 mmol) and **33a**

(0.070 g; 0.33 mmol), followed by General Procedure 2 (62% yield). Purification by prep-HPLC (basic) gave **6** as a yellowish solid (0.039 g). ^{1}H NMR (d_6-DMSO) δ: 10.91 (br. s, 1H); 9.16 (br. s, 1H); 7.54 (d, J = 0.9 Hz, 1H); 6.24 (d, J = 1.2 Hz, 1H); 4.42 (s, 2H); 3.48 (m, 1H); 3.38 (m, 1H); 3.05 (s, 3H); 2.72 (br. s, 1H); 2.57 (m, 1H); 2.31 (d, J = 1.8 Hz, 3H); 1.96 (m, 1H); 1.51 (s, 3H); 0.95-0.90 (m, 2H), 0.87-0.81 (m, 2H). ^{13}C NMR (d_6-DMSO) δ: 163.8; 150.4; 130.9; 117.7; 108.9; 105.9; 87.4; 74.4; 72.4; 68.3; 65.1; 45.2; 39.5 (overlaid); 37.0; 33.5; 31.9; 31.0; 17.7 (2C); 13.4. MS (ESI, m/z): 433.0 [M+H$^+$] for $C_{20}H_{24}N_4O_5S$; ^{t}R = 0.52 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{20}H_{24}N_4O_5S$ 433.1546, found 433.1552; ^{t}R = 0.87 min.

(*2R*)-4-(6-((1-(dimethylamino)cyclopropyl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)butanamide (7). Prepared according to General Procedure 1 (83% yield) from **32** (2.50 g; 5.90 mmol) and **33p**[10] (1.43 g; 7.31 mmol), followed by General Procedure 3 (66% yield) starting from the isolated amine (0.150 g; 0.30 mmol) using a 37% aq formaldehyde solution (0.06 mL; 0.77 mmol) and stirring at rt overnight, followed by General

Procedure 4 (47% yield). Purification by CC (DCM-MeOH) gave **7** as a yellowish solid (0.039 g). ^{1}H NMR (*d*$_6$-DMSO) δ: 10.95 (s, 1H); 9.19 (s, 1H); 7.58 (m, 1H); 6.27 (m, 1H); 4.44 (s, 2H); 3.49 (m, 1H); 3.39 (m, 1H); 3.06 (s, 3H); 2.60 (m, 1H); 2.26 (s, 6H); 1.97 (m, 1H); 1.53 (s, 3H); 1.00-0.97 (m, 2H); 0.92-0.88 (m, 2H). ^{13}C NMR (*d*$_6$-DMSO): 163.9; 150.4; 130.9; 117.8; 108.7; 105.9; 83.4; 74.2; 72.3; 68.3; 67.7; 45.2; 42.4 (2C); 39.5 (overlaid); 38.3; 37.0; 31.0; 18.4 (2C); 13.3. MS (ESI, m/z): 447.1 [M+H$^+$] for $C_{21}H_{26}N_4O_5S$; ^tR = 0.54 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{21}H_{26}N_4O_5S$ 447.1702, found 447.1712; ^tR = 0.95 min.

(*R*)-4-(6-((1-(aminomethyl)cyclopropyl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)butanamide (8). Prepared according to General Procedure 1 (80% yield) from **32** (0.100 g; 0.24 mmol) and **33b** (0.073 g; 0.35 mmol), followed by General Procedure 2 (49% yield). Purification by prep-HPLC (basic) gave **8** as a white solid (0.040 g). ^{1}H NMR (*d*$_6$-DMSO) δ: 7.53 (s, 1H); 6.23 (d, *J* = 1.2 Hz, 1H); 4.42 (s, 2H); 3.53-3.44 (m, 1H); 3.42-3.34 (m, 1H); 3.05 (s, 3H); 2.62-2.54 (m, 3H); 2.00-1.91 (m, 1H); 1.51 (s, 3H); 0.91-0.83 (m, 4H); hydroxamic

acid OH and NH as well as primary amine NH_2 missing. ^{13}C NMR (d_6-DMSO) δ: 163.7; 150.4; 130.9; 117.6; 108.9; 105.9; 89.3; 74.9; 70.6; 68.3; 62.5; 47.9; 45.2; 39.5 (overlaid); 37.0; 31.0; 16.1; 14.0 (2C); 13.4. MS (ESI, m/z): 474.0 [M+MeCN+H^+] for $C_{20}H_{24}N_4O_5S$; ^tR = 0.53 min. LC-HRMS (ESI, m/z): [M+H^+] calcd for $C_{20}H_{24}N_4O_5S$ 433.1546, found 433.1551; ^tR = 0.88 min.

(*2R*)-*N*-hydroxy-4-(6-(5-(4-hydroxypiperidin-1-yl)penta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-2-methyl-2-(methylsulfonyl)butanamide (9).

Prepared according to General Procedure 1 (37% yield) from **32** (0.150 g; 0.35 mmol) and **33c** (0.108 g; 0.40 mmol), followed by General Procedure 4 (54% yield). Purification by prep-HPLC (basic) gave **9** as a white solid (0.033 g). ^1H NMR (d_6-DMSO) δ: 10.95 (s, 1H); 9.19 (s, 1H); 7.59 (s, 1H); 6.28 (d, J = 1.1 Hz, 1H); 4.46-4.42 (br s, 2H); 3.49-3.44 (m, 4H); 3.07 (s, 3H); 2.72-2.66 (m, 2H); 2.61-2.57 (m, 1H); 2.23 (t, J = 9.5 Hz, 2H); 2.01-1.93 (m, 1H); 1.74-1.71 (m, 2H); 1.53 (s, 3H); 1.45-1.36 (m, 2H); 1.10 (t, J = 7.0 Hz, 2H). ^{13}C NMR (d_6-DMSO) δ: 163.8; 150.4; 130.9; 117.9; 108.5; 105.9; 80.5; 74.0; 72.3; 69.8; 68.3; 65.4; 49.9 (2C); 47.3; 45.2; 39.5 (overlaid); 37.0; 34.7 (2C); 31.0;

13.3. MS (ESI, m/z): 477.0 [M+H$^+$] for $C_{22}H_{28}N_4O_6S$; ^tR = 0.49 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{22}H_{28}N_4O_6S$ 477.1808, found 477.1804; ^tR = 0.79 min.

(*2R*)-*N*-hydroxy-2-methyl-4-(6-((1-methylpiperidin-4-yl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-2-(methylsulfonyl)butanamide (10). Prepared according to General Procedure 5 (30% yield) from **32** (0.100 g; 0.23 mmol) and **33n** (0.065 g; 0.26 mmol), followed by General Procedure 2 (51% yield). Purification by prep-HPLC (basic) gave **10** as a white solid (0.016 g). ^{1}H NMR (d_6-DMSO) δ: 10.91 (br s, 1H); 9.21 (br s, 1H); 7.57 (s, 1H); 6.27 (s, 1H); 4.44 (s, 2H); 3.59-3.33 (overlaid m, 4H); 3.07 (s, 3H); 2.66-2.50 (overlaid m, 3H); 2.15 (s, 3H); 2.12-2.02 (m, 1H); 2.01-1.91 (m, 1H); 1.86-1.75 (m, 2H); 1.64-1.53 (m, 2H); 1.53 (s, 3H). ^{13}C NMR (d_6-DMSO) δ: 163.8; 150.4; 130.9; 117.7; 108.8; 105.9; 87.6; 74.4; 71.6; 68.3; 66.2; 54.0 (2C); 46.6; 45.2; 39.5 (overlaid); 37.0; 31.4 (2C); 31.0; 27.0; 13.3. MS (ESI, m/z): 461.1 [M+H$^+$] for $C_{22}H_{28}N_4O_5S$; ^tR = 0.53 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{22}H_{28}N_4O_5S$ 461.1859, found 461.1862; ^tR = 0.88 min.

(*2R*)-4-(6-((4-fluoro-1-methylpiperidin-4-yl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)butanamide (11). Prepared according to General Procedure 1 (68% yield) from **32** (0.130 g; 0.307 mmol) and **33d** (0.074g; 0.338 mmol), followed by General Procedure 2 (72% yield). Purification by prep-HPLC (basic) gave **11** as a yellowish solid (0.071 g). ^{1}H NMR (d_6-DMSO) δ: 10.90 (br. s, 1H); 9.19 (br. s, 1H); 7.68 (s, 1H); 6.32 (d, J = 1.2 Hz, 1H); 4.45 (s, 2H); 3.55-3.46 (m, 1H); 3.45-3.36 (overlaid m, 1H); 3.06 (s, 3H); 2.66-2.55 (overlaid m, 1H); 2.48-2.29 (overlaid m, 4H); 2.19 (s, 3H); 2.04-1.93 (m, 5H); 1.53 (s, 3H). ^{13}C NMR (d_6-DMSO) δ: 163.8; 150.3; 131.1; 118.7; 107.8; 106.0; 88.7 (d, J = 167 Hz, 1C); 81.0 (d, J = 30 Hz, 1C); 77.0 (d, J = 4 Hz, 1C); 72.7 (d, J = 6 Hz, 1C); 72.1; 68.3; 51.5 (2C); 45.9; 45.2; 39.5 (overlaid); 37.0; 36.8 (2C); 31.0; 13.3. MS (ESI, m/z): 479.1 [M+H$^+$] for $C_{22}H_{27}N_4O_5FS$; ^{t}R = 0.56 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{22}H_{27}N_4O_5FS$ 479.1764, found 479.1763; ^{t}R = 0.91 min.

(*2R*)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)-4-(6-((1-(oxetan-3-yl)piperidin-4-yl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)butanamide (12). Prepared

according to General Procedure 5 (78% yield) from **32** (0.100 g; 0.23 mmol) and the **33o** (0.075 g; 0.26 mmol), followed by General Procedure 2 (12% yield). Purification by prep-HPLC (basic) gave **12** as a white solid (0.011 g). ^{1}H NMR (d_6-DMSO) δ: 10.96 (br. s, 1H); 9.21 (br. s, 1H); 7.57 (s, 1H); 6.27 (s, 1H); 4.52 (t, J = 6.5 Hz, 2H); 4.44 (s, 2H); 4.41 (t, J = 6.1 Hz, 2H); 3.55-3.45 (m, 1H); 3.45-3.34 (overlaid m, 2H); 3.08 (s, 3H); 2.70-2.54 (overlaid m, 2H); 2.49-2.43 (overlaid m, 1H); 2.09-1.90 (m, 4H); 1.89-1.77 (m, 2H); 1.65-1.53 (m, 2H); 1.54 (s, 3H). ^{13}C NMR (d_6-DMSO): 163.8; 150.4; 130.9; 117.7; 108.7; 105.9; 87.5; 75.1 (2C); 74.3; 71.7; 68.3; 66.3; 59.1; 48.3 (2C); 45.2; 39.5 (overlaid); 37.0; 31.1 (2C); 31.0; 27.3; 13.3. MS (ESI, m/z): 503.1 [M+H$^+$] for $C_{24}H_{30}N_4O_6S$; ^tR = 0.53 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{24}H_{30}N_4O_6S$ 503.1964, found 503.1968; ^tR = 0.88 min.

(*2R*)-N-hydroxy-2-methyl-4-(6-((1-methylazetidin-3-yl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-2-(methylsulfonyl)butanamide (13). Prepared according to General Procedure 3 (64% yield) from **34** (2.37 g; 4.72 mmol) and a 37% aq formaldehyde solution **35a** (1.10 mL; 14.1 mmol) at rt for 30 min, followed by

General Procedure 2 (58% yield). Purification by prep-HPLC (basic) gave **13** as a white solid (1.30 g). ^{1}H NMR (d_6-DMSO) δ: 10.94 (br. s, 1H); 9.19 (br. s, 1H); 7.56 (d, J = 1.0 Hz, 1H); 6.25 (d, J = 1.2 Hz, 1H); 4.43 (s, 2H); 3.53-3.45 (m, 3H); 3.43-3.36 (m, 1H); 3.35-3.30 (overlaid m, 1H); 3.06 (s, 3H); 2.99-2.94 (m, 2H); 2.63-2.55 (m, 1H); 2.18 (s, 3H); 2.00-1.93 (m, 1H); 1.52 (s, 3H). ^{13}C NMR (d_6-DMSO) δ: 163.8; 150.4; 130.9; 117.8; 108.6; 105.9; 85.1; 74.2; 72.6; 68.3; 67.0; 62.0 (2C); 46.0; 45.2; 39.5 (overlaid); 37.0; 31.0; 20.9; 13.3. MS (ESI, m/z): 433.0 [M+H$^+$] for $C_{20}H_{24}N_4O_5S$; ^tR = 0.50 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{20}H_{24}N_4O_5S$ 433.1546, found 433.1532; ^tR = 0.80 min.

(2*R*)-4-(6-(azetidin-3-ylbuta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)butanamide (15). Prepared according to General Procedure 2 from **34** (0.15 g; 0.30 mmol). Purification by prep-HPLC (basic) gave **15** as a white solid (0.074 g; 59% yield). ^{1}H NMR (d_6-DMSO) δ: 8.99 (br. s, 1H); 7.56 (s, 1H); 6.25 (d, J = 1.2 Hz, 1H); 4.43 (s, 2H); 3.63-3.45 (m, 6H); 3.42-3.35 (overlaid m, 1H); 3.05 (s, 3H); 2.63-2.54 (overlaid m, 1H); 2.00-1.92 (m, 1H); 1.49 (s, 3H); hydroxamic

acid NH and OH missing. ^{13}C NMR (d_6-DMSO) δ: 163.7; 150.4; 131.0; 117.8; 108.7; 105.9; 85.4; 74.2; 72.9; 68.3; 67.8; 52.9 (2C); 45.2; 39.5 (overlaid); 37.0; 31.0; 24.9; 13.4. MS (ESI, m/z): 419.0 [M+H$^+$] for $C_{19}H_{22}N_4O_5S$; ^tR = 0.49 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{19}H_{22}N_4O_5S$ 419.1389, found 419.1388; ^tR = 0.79 min.

(*R*)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)-4-(6-((1-(oxetan-3-yl)azetidin-3-yl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)butanamide (16). Prepared according to General Procedure 1 (84% yield) from **32** (0.400 g; 0.95 mmol) and **33e** (0.265 g; 1.23 mmol), followed by General Procedure 2 (44% yield). Purification by prep-HPLC (basic) gave **16** as a yellowish solid (0.159 g). ^{1}H NMR (d_6-DMSO) δ: 10.95 (br. s, 1H); 9.19 (br. s, 1H); 7.56 (d, J = 1.2 Hz, 1H); 6.26 (d, J = 1.2 Hz, 1H); 4.53 (t, J = 6.6 Hz, 2H); 4.43 (s, 2H); 4.31 (dd, J = 5.3, 6.4 Hz, 2H); 3.67 (m, 1H); 3.52 (overlaid t, J = 7.6 Hz, 2H); 3.49-3.30 (overlaid m, 3H); 3.14-3.10 (m, 2H); 3.05 (s, 3H); 2.63-2.57 (m, 1H); 2.01-1.93 (m, 1H); 1.51 (s, 3H). ^{13}C NMR (d_6-DMSO) δ: 163.9; 150.4; 131.0; 117.8; 108.6; 105.9; 84.9; 74.2; 74.1 (2C); 72.8; 68.4; 67.3; 58.6; 56.4 (2C); 45.2; 39.5 (overlaid); 37.0; 31.0; 21.1; 13.3. MS (ESI, m/z): 475.1 [M+H$^+$] for

$C_{22}H_{26}N_4O_6S$; ^tR = 0.51 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{22}H_{26}N_4O_6S$ 475.1651, found 475.1648; ^tR = 0.82 min.

(*2R*)-4-(6-((3-fluoro-1-methylazetidin-3-yl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)butanamide (17). Prepared according to General Procedure 1 (41% yield) from **32** (0.195 g; 0.46 mmol) and **33f** (0.111 g; 0.51 mmol), followed by General Procedure 3 (49% yield) using a 37% aq formaldehyde solution (0.06 mL; 0.769 mmol) at rt for 2 h and General Procedure 4 (38% yield). Purification by CC (DCM-MeOH) gave **17** as a yellowish solid (0.015 g). ^{1}H NMR (d_6-DMSO) δ: 10.94 (s, 1H); 9.18 (s, 1H); 7.71-7.68 (m, 1H); 6.35-6.32 (m, 1H); 4.44 (s, 2H); 3.61-3.56 (m, 2H); 3.52-3.36 (m, 4H); 3.06 (s, 3H); 2.63-2.58 (m, 1H); 2.30 (s, 3H); 2.01-1.94 (m, 1H); 1.52 (s, 3H). ^{13}C NMR (d_6-DMSO) δ: 163.8; 150.3; 131.1; 118.9; 107.6; 106.0; 83.6 (d, J = 201 Hz, 1C); 78.3 (d, J = 33 Hz, 1C); 77.9 (d, J = 3 Hz, 1C); 72.8 (d, J = 10 Hz, 1C); 72.5 (d, J = 6 Hz, 1C); 68.3; 67.6 (d, J = 23 Hz, 2C); 45.7; 45.2; 39.5 (overlaid); 37.0; 31.0; 13.4. MS (ESI, m/z): 451.0

[M+H$^+$] for $C_{20}H_{23}N_4O_5FS$; ^tR = 0.51 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{20}H_{23}N_4O_5FS$ 451.1452, found 451.1452; ^tR = 0.85 min.

(*2R*)-4-(6-((3-fluoroazetidin-3-yl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)butanamide (18). Prepared according to General Procedure 1 (24% yield) from **32** (0.126 g; 0.297 mmol) and **33f** (0.070 g; 0.326 mmol), followed by General Procedure 2 (27% yield). Purification by prep-HPLC (basic) gave **18** as a yellowish solid (0.08 g). ^{1}H NMR (d_6-DMSO) δ: 9.20 (br s, 1H); 7.69 (s, 1H); 6.33 (d, J = 1.3 Hz, 1H); 4.44 (s, 2H); 3.84-3.73 (m, 4H); 3.52-3.45 (m, 1H); 3.42-3.35 (overlaid m, 1H); 3.06 (s, 3H); 2.63-2.56 (m, 1H); 2.00-1.92 (m, 1H); 1.52 (s, 3H); hydroxamic acid NH and OH missing. ^{13}C NMR (d_6-DMSO) δ: 163.7; 150.3; 131.1; 118.8; 107.6; 106.0; 87.8 (d, J = 204 Hz, 1C); 78.6 (d, J = 33 Hz, 1C); 77.9 (d, J = 3 Hz, 1C); 73.1 (d, J = 10 Hz, 1C); 72.6 (d, J = 6 Hz, 1C); 68.3; 58.9 (d, J = 25 Hz, 2C); 45.2; 39.5 (overlaid); 37.0; 30.9; 13.3. MS (ESI, m/z): 478.0 [M+MeCN+H$^+$] for $C_{19}H_{21}N_4O_5FS$; ^tR = 0.52 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{19}H_{21}N_4O_5FS$ 437.1295, found 437.1294; ^tR = 0.84 min.

(*2R*)-4-(6-((1-(2-fluoroethyl)azetidin-3-yl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)butanamide (19). Prepared according to General Procedure 6 (45% yield) from **34** (0.600 g; 1.19 mmol) and 1-iodo-2-fluoroethane **36a** (0.120 mL; 1.44 mmol) at reflux for 4 h and adding an additional portion of 1-iodo-2-fluoroethane after 2 h, followed by General Procedure 2 (72% yield). Purification by prep-HPLC (basic) gave **19** as a white solid (0.163 g). ^{1}H NMR (d_6-DMSO) δ: 10.94 (br. s, 1H); 9.18 (br. s, 1H); 7.57 (s, 1H); 6.27 (d, J = 1.2 Hz, 1H); 4.49-4.39 (m, 3H); 4.33 (t, J = 4.8 Hz, 1H); 3.55 (t, J = 7.2 Hz, 2H); 3.52-3.45 (m, 1H); 3.44-3.36 (overlaid m, 2H); 3.08 (t, J = 6.3 Hz, 2H); 3.06 (s, 3H); 2.69 (t, J = 4.8 Hz, 1H); 2.63 (overlaid t, J = 4.4 Hz, 1H); 2.62-2.58 (overlaid m, 1H); 2.01-1.93 (m, 1H); 1.53 (s, 3H). ^{13}C NMR (d_6-DMSO) δ: 163.8; 150.4; 130.9; 117.8; 108.6; 105.9; 84.9; 83.1 (d, J = 164 Hz, 1C); 74.2; 72.7; 68.3; 67.2; 60.9 (2C); 58.4 (d, J = 4 Hz, 1C); 45.2; 39.5 (overlaid); 37.0; 31.0; 21.8; 13.4. MS (ESI, m/z): 465.1 [M+H$^+$] for $C_{21}H_{25}N_4O_5FS$; ^{t}R = 0.52 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{21}H_{25}N_4O_5FS$ 465.1608, found 465.1604; ^{t}R = 0.83 min.

(*2R*)-4-(6-((1-(2,2-difluoroethyl)azetidin-3-yl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)butanamide (20). Prepared according to General Procedure 6 (23% yield) from **34** (0.500 g; 0.995 mmol) and 2,2-difluoroethyl trifluoromethanesulfonate **36b** (0.203 mL; 1.49 mmol) at rt for 2 h, followed by General Procedure 2 (73% yield). Purification by prep-HPLC (basic) gave **20** as a white solid (0.073 g). ^{1}H NMR (d_6-DMSO) δ: 10.85 (br. s, 1H); 9.17 (br. s, 1H); 7.57 (d, J = 0.9 Hz, 1H); 6.26 (d, J = 1.2 Hz, 1H); 5.95 (tt, J = 55.2, 4.2 Hz, 1H); 4.43 (s, 2H); 3.59 (t, J = 7.4 Hz, 2H); 3.53-3.37 (m, 3H); 3.19 (t, J = 6.9 Hz, 2H); 3.06 (s, 3H); 2.81 (td, J = 16.2, 4.1 Hz, 2H); 2.62-2.55 (m, 1H); 2.01-1.91 (m, 1H); 1.52 (s, 3H). ^{13}C NMR (d_6-DMSO) δ: 163.8; 150.4; 131.0; 117.8; 116.2 (t, J = 239 Hz, 1C); 108.6; 105.9; 84.7; 74.1; 72.8; 68.3; 67.3; 61.3 (2C); 59.7 (t, J = 23 Hz, 1C); 45.2; 39.5 (overlaid); 37.0; 31.0; 22.0; 13.4. MS (ESI, m/z): 483.0 [M+H$^+$] for $C_{21}H_{24}N_4O_5F_2S$; ^tR = 0.54 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{21}H_{24}N_4O_5F_2S$ 483.1514, found 483.1510; ^tR = 0.96 min.

(*2R*)-4-(6-((1-ethylazetidin-3-yl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)butanamide (21). Prepared according to General Procedure 3 (45% yield) from **34** (0.511 g; 0.10 mmol) and a 5 M acetaldehyde solution in THF **35b** (0.62 mL; 3.1 mmol) at rt for 1 h, followed by General Procedure 2 (58% yield). Purification by prep-HPLC (basic) gave **21** as a white solid (0.160 g). ^{1}H NMR (*d*$_6$-DMSO) δ: 10.72 (br. s, 1H); 9.20 (br. s, 1H); 7.57 (s, 1H); 6.26 (s, 1H); 4.43 (s, 2H); 3.54-3.42 (m, 3H); 3.41-3.28 (overlaid m, 2H); 3.06 (s, 3H); 2.93 (t, *J* = 6.5 Hz, 2H); 2.65-2.55 (m, 1H); 2.35 (q, *J* = 7.1 Hz, 2H); 2.02-1.90 (m, 1H); 1.52 (s, 3H); 0.84 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (*d*$_6$-DMSO) δ: 163.8; 150.4; 130.9; 117.8; 108.7; 105.9; 85.2; 74.2; 72.6; 68.3; 67.0; 59.9 (2C); 53.2; 45.2; 39.5 (overlaid); 37.0; 31.0; 21.0; 13.3; 12.6. MS (ESI, m/z): 447.0 [M+H$^+$] for $C_{21}H_{26}N_4O_5S$; ^tR = 0.52 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{21}H_{26}N_4O_5S$ 447.1702, found 447.1710; ^tR = 0.84 min.

(*2R*)-*N*-hydroxy-4-(6-((1-isopropylazetidin-3-yl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-c]imidazol-2(3*H*)-yl)-2-methyl-2-(methylsulfonyl)butanamide (22). Prepared according to General Procedure 3 (60% yield) from **34** (0.100 g; 0.20 mmol) and

acetone **35c** (0.045 mL; 0.60 mmol) at rt for 2 h, followed by General Procedure 2 (63% yield). Purification by prep-HPLC (basic) gave **22** as a white solid (0.030 g). ^{1}H NMR (*d*$_6$-DMSO) δ: 10.94 (br. s, 1H); 9.20 (br. s, 1H); 7.57 (s, 1H); 6.26 (d, *J* = 1.2 Hz, 1H); 4.43 (s, 2H); 3.54-3.23 (overlaid m, 5H); 3.06 (s, 3H); 2.99-2.90 (m, 2H); 2.63-57 (m, 1H); 2.29-2.21 (m, 1H); 2.01-1.93 (m, 1H); 1.53 (s, 3H); 0.83 (d, *J* = 6.2 Hz, 6H). ^{13}C NMR (*d*$_6$-DMSO) δ: 163.8; 150.4; 130.9; 117.8; 108.7; 105.9; 85.3; 74.2; 72.6; 68.3; 66.9; 58.8 (2C); 58.1; 45.2; 39.5 (overlaid); 37.0; 31.0; 19.9; 19.8 (2C); 13.4. MS (ESI, m/z): 461.0 [M+H$^+$] for $C_{22}H_{28}N_4O_5S$; ^tR = 0.54 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{22}H_{28}N_4O_5S$ 461.1859, found 461.1863; ^tR = 0.86 min.

(*2R*)-*N*-hydroxy-4-(6-((1-(2-hydroxyethyl)azetidin-3-yl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-2-methyl-2-(methylsulfonyl)butanamide (23). Prepared according to General Procedure 1 (80% yield) from **32** (3.50 g; 8.26 mmol) and **33q** (3.16 g; 9.92 mmol), followed by General Procedure 7 (52% yield) and General Procedure 2 (80% yield). Purification by prep-HPLC (basic) gave **23** as a white solid (1.19 g). ^{1}H NMR (*d*$_6$-DMSO) δ: 10.94 (br. s, 1H); 9.20 (br. s, 1H); 7.57 (d, *J* = 1.0 Hz,

1H); 6.26 (d, *J* = 1.3 Hz, 1H); 4.43 (s, 2H); 4.42 (t, *J* = 5.5 Hz, 1H); 3.51 (t, *J* = 6.9 Hz, 2H); 3.54-3.45 (overlaid m, 1H); 3.42-3.31 (overlaid m, 4H); 3.06 (s, 3H); 3.03 (t, *J* = 6.9 Hz, 2H); 2.63-55 (m, 1H); 2.43 (t, *J* = 6.0 Hz, 2H); 2.00-1.93 (m, 1H); 1.53 (s, 3H). ^{13}C NMR (*d*$_6$-DMSO) δ: 163.8; 150.4; 130.9; 117.8; 108.7; 105.9; 85.2; 74.2; 72.7; 68.3; 67.1; 61.5; 60.9 (2C); 59.7; 45.2; 39.5 (overlaid); 37.0; 31.0; 21.8; 13.3. MS (ESI, m/z): 463.0 [M+H$^+$] for $C_{21}H_{26}N_4O_6S$; ^tR = 0.49 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{21}H_{26}N_4O_6S$ 463.1651, found 463.1649; ^tR = 0.79 min.

(*R*)-*N*-hydroxy-4-(6-((1-(2-hydroxy-2-methylpropyl)azetidin-3-yl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-2-methyl-2-(methylsulfonyl)butanamide (24). Prepared according to General Procedure 1 (93% yield) from **32** (0.300 g; 0.71 mmol) and **33g** (0.197 g; 0.85 mmol), followed by General Procedure 2 (78% yield). Purification by prep-HPLC (basic) gave **24** as a yellowish solid (0.250 g). ^{1}H NMR (*d*$_6$-DMSO) δ: 10.93 (br s, 1H); 9.18 (s, 1H); 7.57 (d, *J* = 0.8 Hz, 1H); 6.26 (d, *J* = 1.2 Hz, 1H); 4.44 (s, 2H); 4.06 (s, 1H); 3.57 (t, *J* = 7.4 Hz, 2H); 3.49 (m, 1H); 3.42-3.35 (overlaid m, 2H); 3.08-3.05 (m, 2H); 3.06 (overlaid s, 3H); 2.59 (m, 1H); 2.28 (s, 2H); 1.97 (m,

1H); 1.53 (s, 3H); 1.03 (s, 6H). ^{13}C NMR (d_6-DMSO) δ: 163.8; 150.4; 130.9; 117.8; 108.7; 105.9; 85.2; 74.2; 72.7; 71.2; 70.0; 68.3; 67.1; 62.2 (2C); 45.2; 39.5 (overlaid); 37.0; 31.0; 28.4 (2C); 21.9; 13.3. MS (ESI, m/z): 491.1 [M+H$^+$] for $C_{23}H_{30}N_4O_6S$; ^tR = 0.53 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{23}H_{30}N_4O_6S$ 491.1964, found 491.1963; ^tR = 0.85 min.

(*2R*)-4-(6-((1-acetylazetidin-3-yl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)butanamide (25). To a solution of **34** (0.150 g; 0.298 mmol) in THF (10 mL) at rt were added DIPEA (0.319 mL; 1.87 mmol), HATU (0.170 mg; 0.448 mmol) and AcOH (0.025 mL, 0.448 mmol). The reaction mixture was stirred at rt for 18 h, was poured into water (20 mL) and extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with brine, filtered, and concentrated. Purification by CC (DCM-MeOH) gave a beige solid (0.131 g; 81% yield). It was subsequently reacted according to General Procedure 2. Purification by prep-HPLC (basic) gave **25** as a white solid (0.063 g; 57% yield). ^{1}H NMR (d_6-DMSO) δ: 10.95 (br. s, 1H); 8.90 (br. s, 1H); 7.59 (s, 1H); 6.26 (s, 1H); 4.43 (s, 2H); 4.37 (t,

J = 8.5 Hz, 1H); 4.13-4.07 (m, 2H); 3.76 (m, 1H); 3.66 (m, 1H); 3.48 (m, 1H); 3.38 (m, 1H); 3.05 (s, 3H); 2.56 (m, 1H); 1.92 (m, 1H); 1.75 (s, 3H); 1.47 (s, 3H). ^{13}C NMR (d_6-DMSO) δ: 170.4; 163.8; 150.4; 131.0; 118.0; 108.4; 105.9; 84.2; 73.9; 73.3; 68.3; 68.1; 56.0; 53.9; 45.2; 39.5 (overlaid); 37.0; 31.0; 19.2; 19.1; 13.4. MS (ESI, m/z): 461.0 [M+H$^+$] for $C_{21}H_{24}N_4O_6S$; ^tR = 0.67 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{21}H_{24}N_4O_6S$ 461.1495, found 461.1497; ^tR = 1.05 min.

(*R*)-*N*-hydroxy-4-(6-(((*1R,2S*)-2-(hydroxymethyl)-2-methylcyclopropyl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-2-methyl-2-(methylsulfonyl)butanamide (27). Prepared according to General Procedure 1 (70% yield) from **32** (0.100 g; 0.236 mmol) and **33h** (0.071 g; 0.307 mmol), however further stirred for 3 days at rt upon completion of coupling reaction in order for the acetate cleavage to be complete, followed by General Procedure 4 (36% yield). Purification by prep-HPLC (acidic) followed by trituration in water (0.2 mL) gave **27** as a white solid (0.025 g). ^{1}H NMR (d_6-DMSO) δ: 10.9 (br. s, 1H); 9.18 (br. s, 1H); 7.53 (s, 1H); 6.25 (d, *J* = 1.1 Hz, 1H); 4.74 (t, *J* = 5.8 Hz, 1H); 4.43 (s, 2H); 3.48 (m, 1H); 3.39 (m, 1H); 3.29 (dd, *J* = 5.9, 11.2 Hz, 1H);

3.21 (dd, *J* = 5.6, 11.2 Hz, 1H); 3.06 (s, 3H); 2.59 (m, 1H); 1.96 (m, 1H); 1.54 (overlaid m, 1H); 1.53 (br. s, 3H); 1.17 (s, 3H); 1.03 (m, 1H); 0.61 (m, 1H). ^{13}C NMR (*d*$_6$-DMSO) δ: 163.9; 150.4; 130.9; 117.6; 109.0; 106.0; 86.2; 75.0; 70.0; 68.4; 66.6; 63.0; 45.2; 39.5 (overlaid); 37.0; 31.0; 27.2; 20.3; 17.6; 13.3; 10.8. MS (ESI, m/z): 448.0 [M+H$^+$] for $C_{21}H_{25}N_3O_6S$; ^tR = 0.68 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{21}H_{25}N_3O_6S$ 448.1542, found 448.1534; ^tR = 1.13 min.

(*R*)-4-(6-(((*1R*,*2R*)-2-fluoro-2-(hydroxymethyl)cyclopropyl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)butanamide (28). Prepared according to General Procedure 1 (84% yield) from **32** (0.151 g; 0.356 mmol) and **33i** (0.116 g; 0.390 mmol), followed by General Procedure 4 (86% yield). Purification by CC (heptane-EtOAc) gave **28** as a white solid (0.115 g). ^{1}H NMR (*d*$_6$-DMSO) δ: 10.94 (s, 1 H); 9.18 (s, 1H); 7.57 (d, *J* = 0.9 Hz, 1H); 6.26 (d, *J* = 1.2 Hz, 1H); 5.23 (t, *J* = 6.1 Hz, 1H); 4.43 (s, 2H); 3.72-3.58 (m, 2H); 3.52-3.45 (m, 1H); 3.43-3.35 (m, 1H); 3.06 (s, 3H); 2.63-2.55 (m, 1H); 1.99-1.90 (m, 2H); 1.53 (s, 3H); 1.38-1.32 (m, 2H). ^{13}C NMR (*d*$_6$-DMSO) δ: 163.9; 150.4; 130.9; 117.9; 108.7; 106.0;

82.8 (d, *J* = 230 Hz); 82.1 (d, *J* = 5 Hz); 74.7; 70.8; 68.3; 63.8; 62.9 (d, *J* = 22 Hz); 45.2; 39.5 (overlaid); 37.0; 31.0; 18.4 (d, *J* = 11 Hz); 13.3; 10.8 (d, *J* = 11 Hz). MS (ESI, m/z): 452.0 [M+H$^+$] for $C_{20}H_{22}N_3O_6FS$; ^tR = 0.65 min. Chiral LC (Method 2): ^tR = 3.33 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{20}H_{22}N_3O_6FS$ 452.1291, found 452.1290; ^tR = 1.08 min.

(*R*)-4-(6-(((*1S*,*2S*)-2-fluoro-2-(hydroxymethyl)cyclopropyl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)butanamide (29). Prepared according to General Procedure 1 (76% yield) from **32** (0.150 g; 0.354 mmol) and **33j** (0.116 g; 0.390 mmol), followed by General Procedure 4 (85% yield). Purification by CC (heptane-EtOAc) gave **29** as a white solid (0.103 g). Alternatively, prepared according to General Procedure 1 (96% yield) from **32** (0.90 g; 2.13 mmol) and **33k** (0.513 g; 2.66 mmol), followed by General Procedure 4 (80% yield). Purification by CC (heptane-9:1 EtOAc/MeOH) gave **29** as a yellowish solid (0.729 g). ^{1}H NMR (*d*$_6$-DMSO) δ: 10.94 (s, 1H); 9.18 (s, 1H); 7.56 (d, *J* = 0.9 Hz, 1H); 6.25 (d, *J* = 1.2 Hz, 1H); 5.23 (t, *J* = 6.1 Hz, 1H); 4.42 (s, 2H); 3.71-3.59 (m, 2H); 3.53-

3.45 (m, 1H); 3.42-3.35 (m, 1H); 3.06 (s, 3H); 2.63-2.55 (m, 1H); 1.97-1.90 (m, 2H); 1.52 (s, 3H); 1.36-1.31 (m, 2H). ^{13}C NMR (d_6-DMSO) δ: 163.9; 150.4; 130.9; 117.9; 108.7; 106.0; 83.6; 82.8 (d, J = 230 Hz); 82.1 (d, J = 5 Hz); 74.6; 70.8; 68.3; 63.8; 62.9 (d, J = 22 Hz); 45.2; 39.5 (overlaid); 37.0; 31.0; 18.4 (d, J = 11 Hz); 13.3; 10.8 (d, J = 11 Hz). MS (ESI, m/z): 452.0 [M+H$^+$] for $C_{20}H_{22}N_3O_6FS$; t_R = 0.67 min. Chiral LC (Method 2): ^tR = 4.71 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{20}H_{22}N_3O_6FS$ 452.1292, found 452.1296; ^{t}R = 1.08 min.

(*2R*)-4-(6-(((*1S*,*2S*)-2-((*R*)-1,2-dihydroxyethyl)cyclopropyl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)butanamide (30). Prepared according to General Procedure 1 (79% yield) from **32** (2.25 g; 5.31 mmol) and **33l** (1.61 g; 7.83 mmol), followed by General Procedure 4 (53% yield). Purification by prep-HPLC (acidic) followed by recrystallization (CH_3CN/hexane) gave **30** as a white solid (1.03 g). ^{1}H NMR (d_6-DMSO) δ: 10.94 (br. s, 1H); 9.18 (br. s, 1H); 7.52 (s, 1H); 6.23 (d, J = 1.1 Hz, 1H); 4.70 (d, J = 4.8 Hz, 1H); 4.61 (t, J = 5.6 Hz, 1H); 4.42 (s, 2H); 3.53-3.45 (m, 1H); 3.42-3.31 (overlaid m, 3H); 3.31-3.25 (m, 1H); 3.06 (s,

3H); 2.63-2.56 (m, 1H); 2.00-1.92 (m, 1H); 1.52 (s, 3H); 1.48-1.43 (m, 1H); 1.37-1.33 (m, 1H); 0.96-0.90 (m, 1H); 0.84-0.78 (m, 1H). ^{13}C NMR (d_6-DMSO) δ: 163.8; 150.4; 130.9; 117.6; 108.9; 106.0; 87.9; 74.9; 71.0; 70.1; 68.3; 65.9; 61.2; 45.2; 39.5 (overlaid); 37.0; 31.0; 26.0; 13.3; 12.1; 3.7. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{21}H_{25}N_3O_7S$ 464.1491, found 464.1488; ^tR = 1.01 min.

(*2R*)-4-(6-(((*1R*,*2R*)-2-((*S*)-1,2-dihydroxyethyl)cyclopropyl)buta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-*N*-hydroxy-2-methyl-2-(methylsulfonyl)butanamide (31). Prepared according to General Procedure 1 (54% yield) from **32** (0.250 g; 0.59 mmol) and **33m** (0.157 g; 0.766 mmol), followed by General Procedure 4 (74% yield). Purification by prep-HPLC (acidic) gave **31** as a white solid (0.108 g). ^{1}H NMR (d_6-DMSO) δ: 10.93 (br. s, 1H); 9.18 (br. s, 1H); 7.52 (s, 1H); 6.23 (d, J = 1.2 Hz, 1H); 4.69 (d, J = 5.1 Hz, 1H); 4.61 (t, J = 5.7 Hz, 1H); 4.42 (s, 2H); 3.53-3.44 (m, 1H); 3.41-3.36 (overlaid m, 3H); 3.31-3.25 (m, 1H); 3.06 (s, 3H); 2.63-2.55 (m, 1H); 1.99-1.92 (m, 1H); 1.52 (s, 3H); 1.49-1.43 (m, 1H); 1.37-1.31 (m, 1H); 0.96-0.90 (m, 1H); 0.84-0.78 (m, 1H). ^{13}C NMR (d_6-DMSO) δ: 163.9; 150.4; 130.9; 117.6; 109.0; 106.0; 87.9; 74.9;

71.0; 70.2; 68.4; 65.9; 61.2; 45.2; 39.5 (overlaid); 37.0; 31.0; 26.0; 13.3; 12.2; 3.7. MS (ESI, m/z): 464.0 [M+H$^+$] for $C_{21}H_{25}N_3O_7S$; ^tR = 0.48 min. LC-HRMS (ESI, m/z): [M+H$^+$] calcd for $C_{21}H_{25}N_3O_7S$ 464.1491, found 464.1488; ^tR = 1.01 min.

(*2R*)-4-(6-(azetidin-3-ylbuta-1,3-diyn-1-yl)-3-oxo-1*H*-pyrrolo[1,2-*c*]imidazol-2(3*H*)-yl)-2-methyl-2-(methylsulfonyl)-*N*-((*2RS*)-(tetrahydro-2*H*-pyran-2-yl)oxy)butanamide (34). Prepared according to General Procedure 1 from **32** (4.00 g; 9.45 mmol) and **33r** (3.24 g; 16.5 mmol). Purification by CC (DCM-MeOH) gave **34** as a yellow solid (3.06 g; 65% yield). ^{1}H NMR (d_6-DMSO) δ (1:1 mixture of diastereoisomers): 11.34 (m, 2 x 1H); 7.56 (s, 1H); 7.55 (s, 1H); 6.26 (m, 2 x 1H); 4.85 (s, 1H); 4.49 (m, 1H); 4.47-4.37 (m, 2 x 2H); 4.01 (m, 1H); 3.95 (m, 1H); 3.64-3.57 (m, 2 x 3H); 3.56-3.46 (m, 2 x 4H); 3.45-3.38 (m, 2 x 2H); 3.07 (s, 3H); 3.04 (s, 3H); 2.62 (m, 2 x 1H); 1.97 (m, 2 x 1H); 1.73-1.60 (m, 2 x 2H); 1.59-1.43 (overlaid m, 2 x 4H); 1.56 (s, 3H); 1.54 (s, 3H). MS (ESI, m/z): 503.2 [M+H$^+$] for $C_{24}H_{30}N_4O_6S$; ^tR = 0.59 min.

ASSOCIATED CONTENT

Supporting Information. The following files are available free of charge.

Experimental details on preparation of recombinant Pae and Eco LpxC proteins, DSF measurement, MIC testing, logD, pKa and solubility determination, formulation trials, neutropenic murine thigh infection model, pharmacokinetics in the mouse and in the rat, Table S-1: MICs against PAO1 and PA2616 strains, Figure and Table S-2 to S-11: graphical representation of in vivo efficacy and descriptive statistics, and preparation of compounds **33a-r** and **14** (PDF)

Molecular formula strings (CSV)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest. All authors were full-time employees of Idorsia Pharmaceuticals Limited at the time the manuscript was written.

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ABBREVIATIONS

AMR, antimicrobial resistance; AUC, area under the plasma concentration-time curve; CDI, carbonyl-diimidazole; CFU, colony forming unit; Cipro, ciprofloxacin; Cl, total clearance; Cl_{int}, intrinsic clearance; Cl_u, unbound clearance; DIPEA, *N*,*N*-diisopropylethylamine; DSF, Differential scanning fluorimetry; Eco, *Escherichia coli*; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; ESBL, extended-spectrum beta-lactamase; Et_2O, diethyl ether; EtOAc, ethyl acetate; EtOH, ethanol; GNB, Gram-negative bacteria; HOBt, 1-hydroxybenzotriazole; Imi, imipenem; Kpn, *Klebsiella pneumoniae*; MeCN, acetonitrile; MeOH, methanol; MIC, minimal inhibitory concentration; $nBuNH_2$, n-butyl amine; NH_2OTHP, O-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine; LpxC, UDP-3-O-((*R*)-3-hydroxymyristoyl)-*N*-acetylglucosamine deacetylase; Pae, *Pseudomonas aeruginosa*; QR, quinolone-resistant; T3P,

propylphosphonic anhydride; TEA, triethylamine; Tige, tigecycline; THF, tetrahydrofuran.

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