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Side chain SAR of bicyclic β-lactamase inhibitors (BLIs). 2. N-Alkylated and open chain analogs of MK-8712

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ABSTRACT

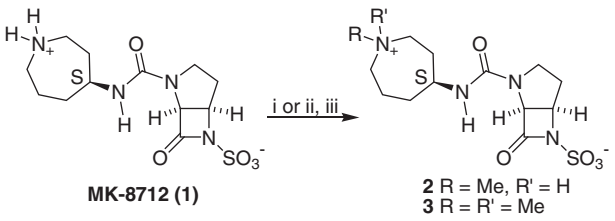
The bridged monobactam β-lactamase inhibitor MK-8712 (**1**) effectively inhibits class C β-lactamases. Side chain N-alkylated and ring-opened analogs of **1** were prepared and evaluated for combination with imipenem to overcome class C β-lactamase mediated resistance. Although some analogs were more potent inhibitors of AmpC, none exhibited better synergy with imipenem than **1**.

Carbapenem antibiotics (e.g., imipenem) are an important therapeutic option for the treatment of hospital-acquired bacterial infections.[1] Recently, some *Pseudomonas* strains have developed resistance to carbapenems through either porin loss or expression of β-lactamase enzymes (BLs) that can hydrolyze and inactivate carbapenems.[2] The hundreds of known BLs are divided into four classes (A–D) based on their structures.[3] While Class A BLs are a major cause of penicillin resistance in bacteria, carbapenem resistance in *Pseudomonas* is primarily mediated by class C BLs such as AmpC.[4] Over the years, several β-lactamase inhibitors (BLIs) have been developed that effectively restore antibacterial activity against class A BL-expressing strains when combined with a penicillin (e.g., clavulanate with amoxicillin).[5] Likewise, addition of an effective class C BLI to a carbapenem antibiotic should restore antibacterial efficacy against class C BL-producing strains. To date, no class C BLI has been brought to market although several have been reported.[6]

We recently reported the discovery of MK-8712 (**1**),[6a] a class C BLI for combination with imipenem/cilastatin (IPM/CIL). MK-8712 is an analog of Ro48-1256.[6b,6c]

In the search for a backup for **1**, we synthesized and report herein numerous N-substituted and open chain analogs. The *N*-methyl (**2**) and *N*,*N*-dimethyl (**3**) analogs were prepared from MK-8712 by reductive amination (for **2**) or alkylation with methyl iodide (for **3**), albeit in low yield (Scheme 1). The hydroxyl-alkyl analogs **4** and **5** were readily synthesized from the chiral side chain amine **16**[6a] as outlined in Scheme 2. BOC protection of the free amine followed by CBZ removal afforded the protected diamine **17** in 82% overall yield. Alkylation with unprotected bromoethanol was messy but reaction with the TIPS-protected alcohol proceeded smoothly with moderate yields. Simultaneous TFA removal of the TIPS and BOC protecting groups afforded the requisite hydroxyalkyl amine intermediates **18** and **19**. Activation of the amine as the hydroxysuccinimide mixed carbonate followed by coupling with the core amine **8**[6b,7] provided **4** and **5**.

The amino-alkyl analogs **6** and **7** were similarly prepared as outlined in Scheme 3. Alkylation of **17** with dimethylamino ethyl tosylate or *N*-Cbz-iodoethyl amine followed by deprotection affor-



Scheme 1. Reagents and conditions: (i) CH_2O, $NaCNBH_3$, AcOH, ACCN, 23%; (ii) MeI, Et_3N, DMAP, DMF, 29%; (iii) HPLC on Phenominex Synergi Polar RP80 column eluted with MeOH/H_2O.

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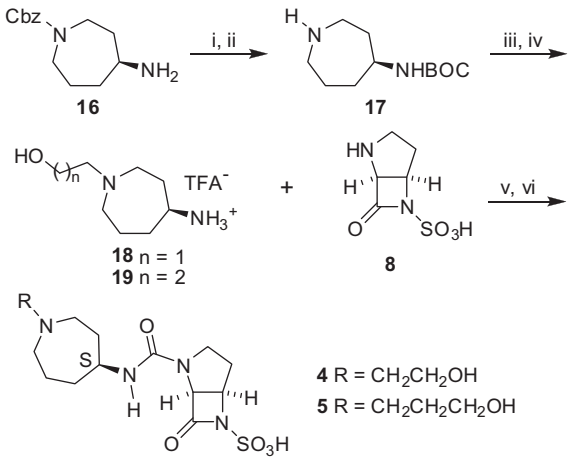
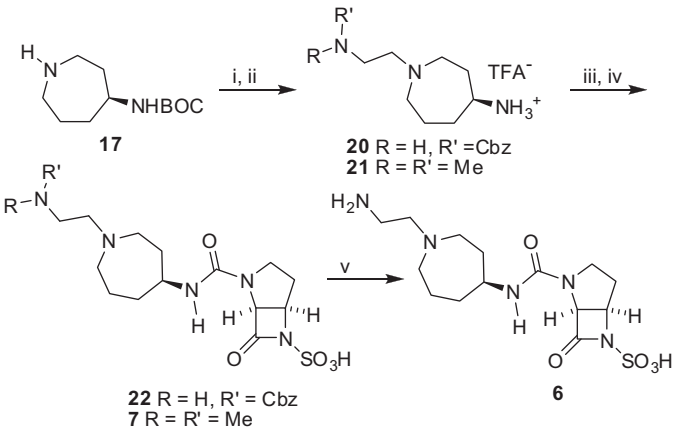


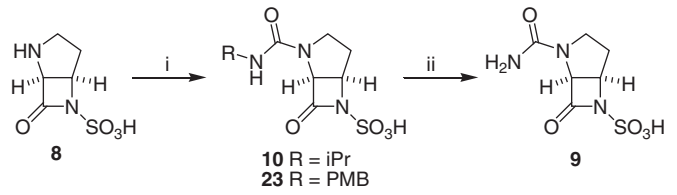
Scheme 2. Reagents and conditions: (i) Boc_2O, Hunig's base, DMAP, THF, 82%; (ii) 20% $Pd(OH)_2/C$, 40 psi H_2, MeOH; (iii) $Br(CH_2)_nCH_2OTIPS$, Hunig's base, DMF, rt, 48 h, 53% ($n = 1$), 48% ($n = 2$); (iv) TFA, CH_2Cl_2; (v) **18** or **19**, N,N′-disuccinimidyl carbonate, MeCN; Et_3N; (vi) **8**, $NaHCO_3$, MeCN, H_2O, 31%; (vii) HPLC on Phenominex Synergi Polar RP80 column eluted with MeOH/H_2O.



Scheme 3. Reagents and conditions: (i) $ICH_2CH_2NHCO_2Bn$, Hunig's base, DMF, rt, 24 h, 70–96%, or $TsOCH_2CH_2NMe_2$, Hunig's base, NaI, DMF, rt, 24 h, 73%; (ii) TFA, CH_2Cl_2; (iii) N,N′-disuccinimidyl carbonate, MeCN, Et_3N, 10% (R = R′ = Me), 49–54% (R = H, R′ = Cbz); (iv) **8**, $NaHCO_3$, MeCN, H_2O, 43–49%; (v) **22**, 20% Pd black, H_2, MeOH, AcOH, 68%.

ded intermediates **20** or **21** in good to excellent yield. Coupling to the core **8** provided **7** directly and the advanced intermediate **22**, which was further deprotected to afford amino-ethyl analog **6**.

Reaction of the bridged monobactam core **8** and isopropyl isocyanate or *p*-methoxybenzyl isocyanate afforded the isopropyl amide **10** directly and the protected amide **23** (Scheme 4). Deprotection of PMB amide **23** afforded unsubstituted amide **9** in good yield.



Scheme 4. Reagents and conditions: (i) RNCO, H_2O, THF, 19% (R = *i*Pr) or 54% (R = PMB); (ii) **23**, CAN, H_2O, MeCN, 66%.

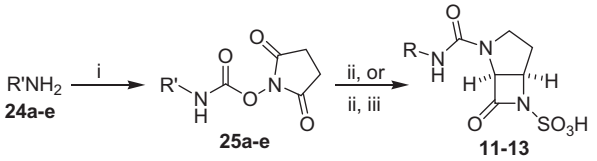
Open chain analogs **11**–**15** were prepared by coupling of the BLI core with the appropriate hydroxysuccinimide carbamate (Table 2).

In general, N-substitution on **1** was discovered to be detrimental to in vitro synergy with IPM (Table 1). As seen in the simple *N*-methyl analog **2**, both enzymatic inhibition against pseudomonal class C β-lactamase AmpC, and synergy were decreased about 3-fold. Although the *N*,*N*-dimethyl analog **3** restored enzymatic activity, a continued decrease in synergy with IPM was observed. By tethering a longer hydrophilic side chain to the azepine ring, compounds (**4**, **5,** and **6)** with comparable or improved enzymatic inhibition over **1** could be obtained; however, all of these analogs suffered substantial loss in synergy. This loss in synergy was even more pronounced in the amino-alkyl analog **7**.

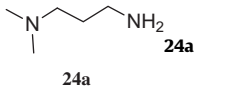
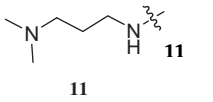
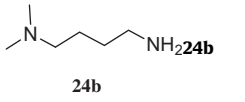
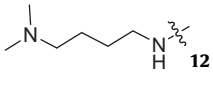
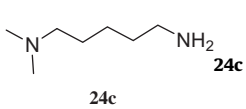
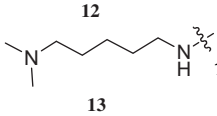
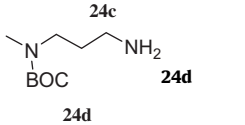
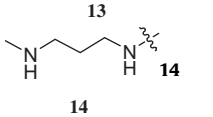
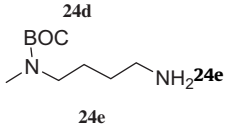
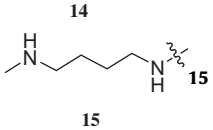
Investigation into replacing the azepine side chain was also pursued. Truncating the side chain to the unsubstituted urea **9** partially restored enzymatic inhibition relative to the inactive bridged monobactam core **8**. By incorporating an isopropyl group, a compound (**10**) with comparable enzymatic inhibition to **1** was obtained, albeit with a complete loss of synergy. Experiments with **10** in efflux-deleted mutants strongly suggest that the loss of in vitro synergy with IPM is due primarily to increased efflux.[9] Open chain analogs **11–13** demonstrated that extending a tertiary amine, up to 5-carbons, was also well tolerated by the enzyme, but remained less effective in the synergy assay with IPM. Surprisingly, the corresponding secondary amines (**14** and **15**) offered no improvement in synergy as hoped, and proved to be inferior in the enzymatic inhibition against class C BLs.

The structures of **6** and **15** bound to AmpC were solved to 1.4 and 1.6 Ang resolution, respectively.[10] Figure 1 shows a comparison of the binding of MK-8712 (**1**) (panel A), compound **6** (panel

Table 2
Synthesis of Analogs **11**–**15**

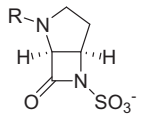


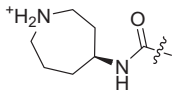
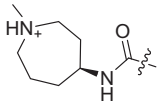
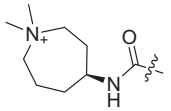
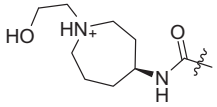
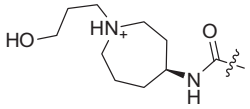
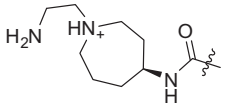
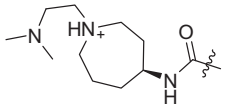
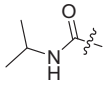
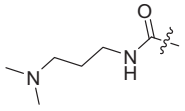
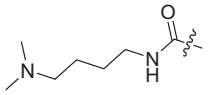
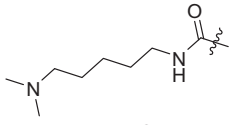
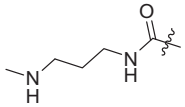
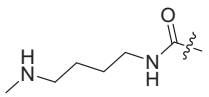
Conditions: (i) N,N′-Disuccinimidyl carbonate, MeCN; (ii) 8, $NaHCO_3$, MeCN, H_2O; (iii) TsOH, AcOH, H_3PO_4

R′NH_2	R	Yields (i, ii, iii)[a]
		(35, 11, n/a)
		(95, 18, n/a)
		(NI[b], 41, n/a)
		(NI[b], 23, 20)
		(NI[b], 35, 29)

[a] Yields (%) for steps (i), (ii), and (iii) (if applicable).
[b] NI = not isolated, crude used in next step without further purification.

Table 1
Enzyme inhibition and *in vitro* synergy



#	R	P.a. AmpC IC_{50}[a] (μM)	conc. (μM) to restore IPM susc. CL5701 [b]
1		1.0	9.4
2		2.6	25
3		1.5	50
4		0.9	25
5		1.1	50
6		0.6	25
7		0.9	100
8	H	>250	>100
9		16	>100
10		1.8	>100
11		2.1	25
12		1.6	25
13		1.9	100
14		5.3	50
15		3.5	25

[a] IC_{50} for inhibition of the hydrolysis of nitrocefin; see Ref. 8 for details.
[b] Concentration of BLI (μM) required to reduce the imipenem (IPM) MIC for *Pseudomonas aeruginosa* strain CL5701 from 32 μg/mL to the susceptibility breakpoint of 4 μg/mL; see Ref. 8 for details.

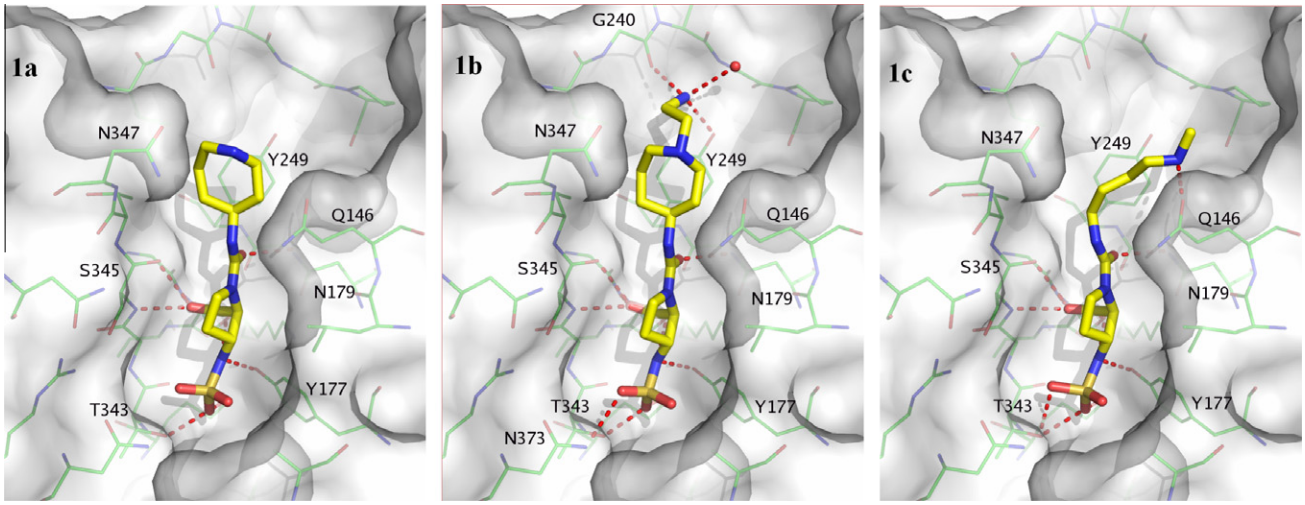


Figure 1. X-ray crystal structures of (a) MK-8712 (**1**), (b) compound **6**, and (c) compound **15** bound to AmpC.

B) and compound **15** (panel C). The three compounds are covalently bound to Ser 90, and extend along a narrow groove on the surface of the protein. All three compounds are anchored at the bottom of the groove by several hydrogen bonds to protein atoms. The *s*-azepine of MK-8712 and **6** are stacked against the side chain of Tyr249. In addition, the amino alkyl chain of **6** hydrogen bonds to the main chain oxygen of Gly 240, the side chain oxygen of Tyr 249 and a water molecule. These interactions are not present in MK-8712, and may account for the slightly higher enzymatic activity observed for **6**. Conversely, the slight loss in potency observed for **15** can be explained by the fact that the open alkyl chain does not optimally interact with Tyr249; the reduced hydrophobic interactions may be partially compensated by the hydrogen bond made by the amino group.

In conclusion, our efforts to identify a back-up to **1**, demonstrated that the azepine side chain is optimal both for enzymatic inhibition against pseudomonal class C β-lactamase AmpC, and synergy with IPM. Although several N-alkylated azepine and open chain analogs have been discovered with slightly improved or similar enzymatic inhibition against class C BLs, none showed improved in vitro synergy with IPM. Further reports from this laboratory will describe our efforts to clarify the factors contributing to this loss in synergy and to identify an optimal β-lactamase inhibitor for combination with imipenem.

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7. An improved synthesis of **8** was developed by Merck Process Research and will be published separately.
8. (a) Blizzard, T.A.; Chen, H.Y.; Wu, J.Y.; Kim, S.; Ha, S.; Mortko, C.; Variankaval, N.; Chiu, A. WO 2008039420. Detailed experimental procedures for the enzyme inhibition assay and the in vitro synergy assay are provided in Example 32 of this patent. For convenience, brief summaries of the protocols for these assays are provided below. (b) Enzyme inhibition assay: Hydrolysis of the commercially available substrate nitrocefin by AmpC in the presence of the BLI was measured in a spectrophotometric assay. The enzyme AmpC (from *Pseudomonas aeruginosa*) and the substrate were dissolved in 100 mM KH_2PO_4 buffer (pH 7) containing 0.005% BSA. The BLI was dissolved in DMSO and serially diluted in a 96-well microplate. The BLI and AmpC were incubated for 40 min at room temperature then the substrate solution was added and the incubation continued for another 40 min. The spectrophotometric reaction was quenched by the addition of 2.5 N acetic acid and the absorbance at 492 nm was measured. The IC_{50} was determined from semi-logarithmic plots of enzyme inhibition versus inhibitor concentration. (c) *In vitro synergy Assay*: The assay determines the concentration of BLI required to reduce the MIC of imipenem by one-half, one-quarter, one-eighth, one-sixteenth and one-thirty-second against resistant bacteria. The BLI was titrated in a serial dilution across a microtiter plate while at the same time imipenem was titrated in a serial dilution down the microtiter plate. The plate was inoculated with the bacterial strain in question then incubated overnight and evaluated for bacterial growth. Each well in the microplate checkerboard contains a different combination of concentrations of the inhibitor and the antibiotic thus allowing determination of synergy between the two.
9. Young, K. et al. manuscript in preparation.
10. Coordinates for the two complexes described in the paper have been deposited with the Protein Data Bank (PDB), accession codes 3S1Y for compound **6**, and 3S22 for compound **15**.