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Discovery of MK-7655, a β-lactamase inhibitor for combination with Primaxin®

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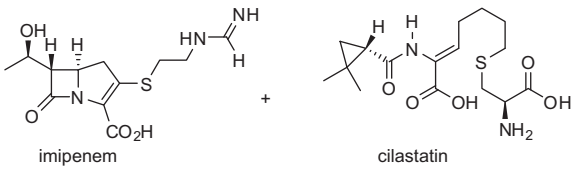
ABSTRACT

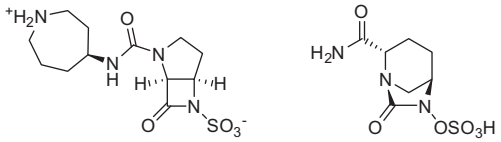
β-Lactamase inhibitors with a bicyclic urea core and a variety of heterocyclic side chains were prepared and evaluated as potential partners for combination with imipenem to overcome class A and C β-lactamase mediated antibiotic resistance. The piperidine analog **3** (MK-7655) inhibited both class A and C β-lactamases in vitro. It effectively restored imipenem's activity against imipenem-resistant *Pseudomonas* and *Klebsiella* strains at clinically achievable concentrations. A combination of MK-7655 and Primaxin® is currently in phase II clinical trials for the treatment of Gram-negative bacterial infections.

Beta-lactam antibiotics have played a key role in the treatment of bacterial infections for over half a century.[1] Carbapenems, a subset of beta-lactam antibiotics, have been especially important for treatment of serious nosocomial infections.[2] One of the most important carbapenems is Merck's Primaxin®, a combination of the carbapenem antibiotic imipenem and the renal DHP inhibitor cilastatin.



Beta-lactams kill bacteria by inhibiting the cell wall construction enzymes known as PBPs. However, some bacteria have developed enzymes that can destroy beta-lactams before they can inactivate the PBPs. These enzymes, known as β-lactamases, thus enable the bacteria to survive even in the presence of high concentrations of beta-lactams.[3] There are hundreds of known β-lactamases, divided into four broad classes (A, B, C, and D) based on their structures.[4] A proven strategy to restore antibacterial activity is to add a β-lactamase inhibitor (BLI) to the antibiotic.[5,6] For example, the class A β-lactamases responsible for resistance of Gram-positive bacteria to amoxicillin can be inhibited by clavulanic acid. Addition of clavulanic acid to amoxicillin successfully restores the original spectrum of amoxicillin. In recent years some strains of Gram-negative bacteria, especially *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, have developed imipenem resistance mediated by class C (e.g. ampC)[7] and class A (e.g. KPC-2)[8] β-lactamases, respectively.[9] In order to overcome this resistance, we began a program aimed at discovering a class A/C BLI for combination with Primaxin®. We report herein the discovery of MK-7655, the clinical candidate that resulted from this program.

We have previously reported the discovery of MK-8712, a bridged monobactam BLI that is effective against class C β-lactamases (e.g. ampC) but not the class A KPC enzymes.[10,11]



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The development of MK-8712 was discontinued due, in part, to its lack of class A β-lactamase coverage and we turned our attention to the discovery of class A/C dual inhibitors. We pursued several leads including de novo design and modification of existing BLIs. One very promising lead series was the bridged bicyclic urea BLIs discovered at Sanofi-Aventis.[12] This series included a clinical candidate, NXL 104, which is currently in development by Astra Zeneca in combination with ceftazidime.[13] Although this series is much more synthetically challenging than the bridged monobactams, it has the advantage of being active against both class A and class C β-lactamases. Based on our experience in the monobactam series, we decided to examine a series of bridged bicyclic ureas with basic heterocyclic side chains.

The (*S*)-azepine side chain of MK-8712 was attached to the bicyclic urea core, as outlined in Scheme 1. Starting with the known acid **19**,[14] amide formation with amine **20** afforded the protected amide **21**. Deprotection of the benzyl ether followed by sulfation and BOC removal afforded the (*S*)-azepine analog **1**.

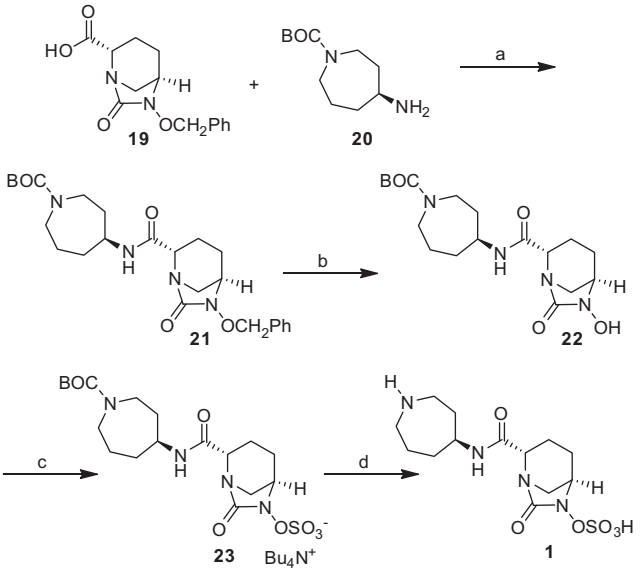
The (*R*) azepine analog **2**, the piperidine analog **3**, and the isomeric pyrrolidine analogs **4** and **5** were prepared by analogous routes using the appropriate protected amines as starting materials.

The *N*-methyl piperidine analog **6** was synthesized from acid **19** as outlined in Scheme 2. Acylation of **19** with amine **24** followed by debenzylation and sulfonylation afforded the desired *N*-methyl analog **6** in good overall yield.

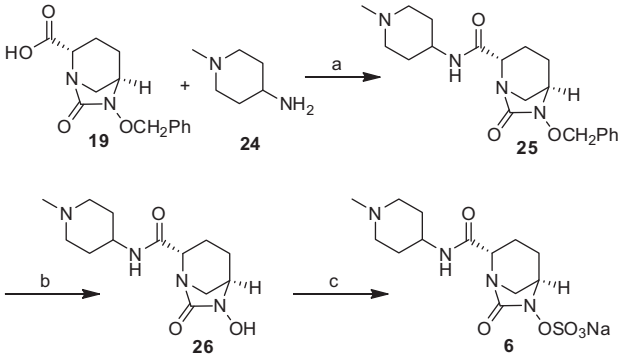
The corresponding sulfone analog **7** which has a neutral side chain, in contrast to analogs **1**–**6** which have side chains that would be positively charged at physiological pH, was similarly prepared as summarized in Scheme 3. BOP[15] mediated acylation of aminosulfone **27** with acid **19** afforded the intermediate amide **28**. Debenzylation of **28** followed by sulfation of the free hydroxyl group afforded the desired sulfone **7**.

The *para*-substituted pyridine **8** was prepared via the synthetic route outlined in Scheme 4. Coupling of acid **19** and 4-amino-pyridine **30** afforded the protected amide **31**. Protecting group removal followed by sulfation with sulfur trioxide pyridine complex afforded the *para*-pyridyl analog **8**.

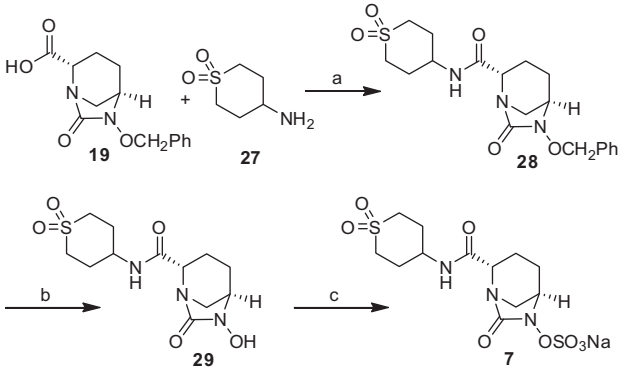
The *meta*-pyridyl analog **9** and the *ortho*-pyridyl analog **10** were synthesized via analogous routes from the appropriate aminopyridines. Although analogs **8**–**10** have weakly basic side chains, the side chains would be neutral at physiological pH.



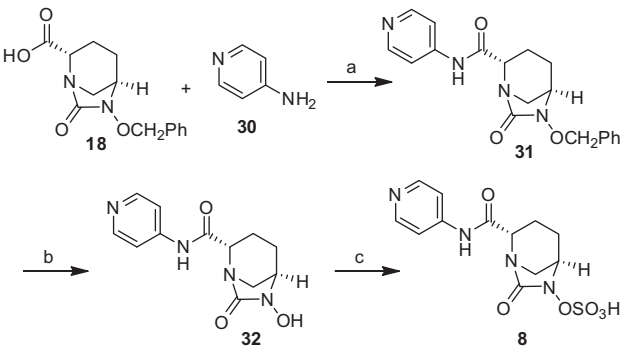
Scheme 1. Reagents and conditions: (a) EDC, HOBT, Et_3N, CH_2Cl_2, 25 °C, 56%; (b) Pd/C, H_2, CH_3OH, 25 °C; (c) SO_3·pyridine complex, pyridine, Bu_4NSO_3H, 25 °C; (d) TFA, CH_2Cl_2, 33% over three steps.



Scheme 2. Reagents and conditions: (a) 2-chloro-*N*-methylpyridinium iodide, Et_3N, CH_2Cl_2, 50 °C, 1 h, 84%; (b) 10% Pd/C, H_2, CH_3OH, 25 °C, 99% crude; (c) SO_3·pyridine complex, pyridine, 25 °C, 31%.



Scheme 3. Reagents and conditions: (a) BOP, Hunig's base, DMF, 25 °C, 1 h 77%; (b) Pd/C, H_2, CH_3OH, 25 °C, 99% crude; (c) SO_3·pyridine complex, pyridine, 25 °C, 15%.



Scheme 4. Reagents and conditions: (a) 2-chloro-*N*-methylpyridinium iodide, Et_3N, CH_2Cl_2, 50 °C, 1.5 h, 68%; (b) 10% Pd/C, H_2, CH_3OH, CH_2Cl_2, 25 °C, 47%; (c) SO_3·pyridine complex, pyridine, 25 °C, 47%.

The 6-aminopyridine analog **11** was targeted since the side chain would be more basic (due to electron donation by the second *ortho* nitrogen) than the unsubstituted pyridines in analogs **8**–**10**, although it would likely not be protonated at physiological pH (calculated p*K*a 4.7 for 11 vs 3.6 for 9). Analog **11** was prepared as outlined in Scheme 5. Acylation of the core acid **19** with protected aminopyridine **33** afforded amide **34** in modest yield. Debenzylation followed by sulfation and BOC removal afforded the desired aminopyridyl analog **11** in low overall yield. In order to probe the effect of a positively charged aromatic side chain, the aminomethylpyridine analog **12** was prepared in a similar manner by substituting 4-BOC-aminomethylpyridine for **33**.

A 'hard quat' analog, **13**, with an *N*-methylpyridinium side chain that would be positively charged at all pHs, was also targeted. It was prepared in a straightforward manner but very poor overall yield from acid **19** and aminopyridine **37** as outlined in Scheme 6.

To determine the effect of a negatively charged (at physiological pH) side chain, the carboxylic acid analog **14** was targeted. Amidation of acid **19** with protected aminobenzoic acid **40** proceeded in very good yield to afford amide **41**. Debenzylation proceeded smoothly but sulfation and cleavage of the *t*-butyl ester formed **14** in disappointingly low overall yield (Scheme 7).

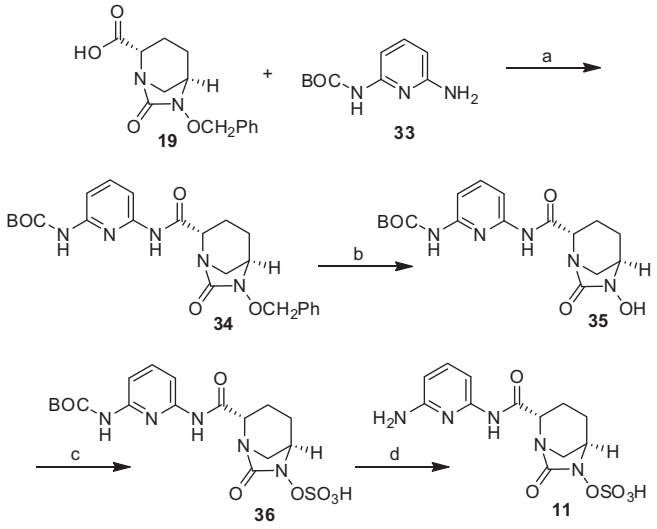
To complement the positively and negatively charged side chain analogs, one analog with a zwitterionic side chain was also targeted. Amino acid **15** was synthesized from acid **19** and the bis-protected aminophenylalanine **44** as outlined in Scheme 8.

The effect of a slightly longer spacer between the bicyclic core and the side chain was probed by the extended analog **16**, in which a methylene spacer has been inserted between the core amide and the piperidine ring of **3**. The synthesis of **16** was relatively straightforward, as outlined in Scheme 9, and proceeded in good overall yield.

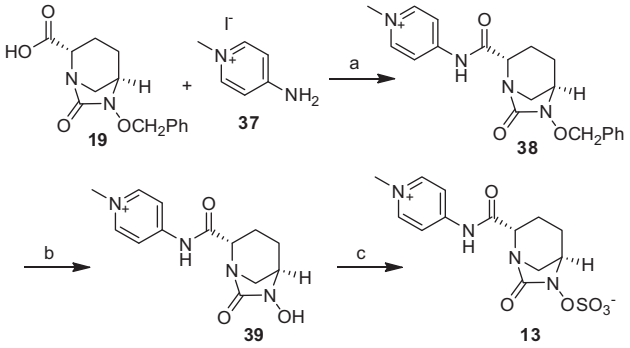
To further probe the effect of modification of the amide linker of **3**, the *N*-methyl amide **17** was prepared (Scheme 10). Since the desired BOC-protected starting material was not available commercially, it was necessary to use the corresponding CBZ-protected analog **52** instead and switch protecting groups during the debenzylation step. Thus, amide coupling of core acid **19** and amine **52** afforded amide **53** in good yield. Debenzylation of **53** in the presence of BOC-anhydride resulted in in situ protection of the intermediate free piperidine nitrogen to provide the desired BOC-protected intermediate **54** which was then converted to **17** by our usual protocol.

Finally, the ester-linked analog **18** was prepared by substitution of 4-hydroxy-BOC-piperidine for amine 48 in the synthetic route outlined in Scheme 9.

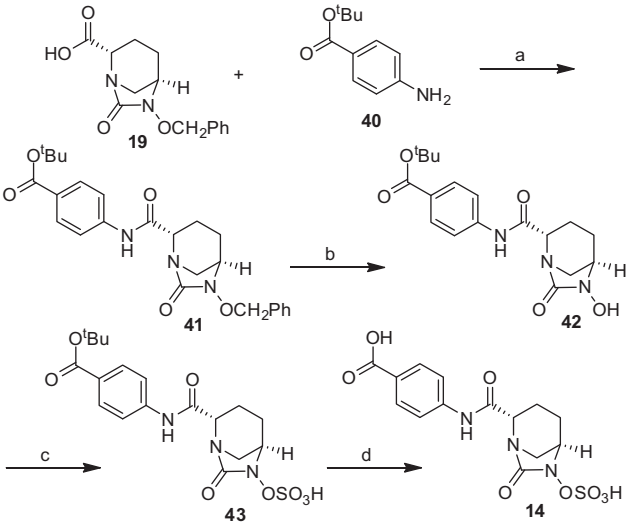
All compounds were evaluated in an enzyme inhibition assay and in an in vitro synergy assay (Table 1). The enzyme assay measured the compound's ability to inhibit the hydrolysis of nitrocefin by four β-lactamases (one class A BL, two class C BLs, and one class D BL), reported as an IC_{50} (nM).[16] The synergy assay measured the compound's ability to reduce the MIC of imipenem against clinically relevant β-lactamase producing strains of *Pseudomonas*, *Klebsiella*, and *Acinetobacter*. The synergy data in Table 1 is reported as the concentration (μM) of BLI required to reduce the imipenem MIC for each strain to 4 μg/mL.[16]



Scheme 5. Reagents and conditions: (a) 2-chloro-*N*-methylpyridinium iodide, Et_3N, CH_2Cl_2, 50 °C, 2.5 h, 50%; (b) 10% Pd/C, H_2, CH_3OH, 25 °C, 100% crude; (c) SO_3·pyridine complex, pyridine, 25 °C; (d) TFA, CH_2Cl_2, 10% over two steps.



Scheme 6. Reagents and conditions: (a) 2-chloro-*N*-methylpyridinium iodide, Et_3N, CH_3CN, 60 °C, 1 h, 29%; (b) 10% Pd/C, H_2, CH_3OH, 25 °C, 100% crude; (c) SO_3·pyridine complex, pyridine, 25 °C, 5%.

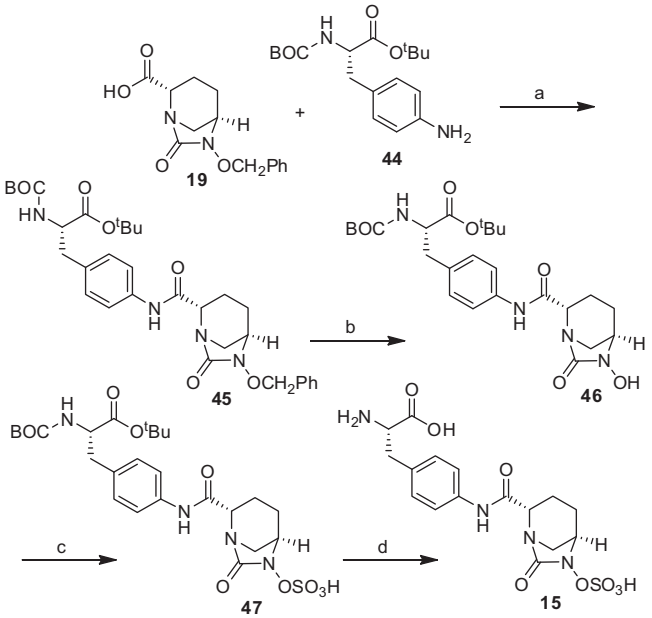


Scheme 7. Reagents and conditions: (a) 2-chloro-*N*-methylpyridinium iodide, Et_3N, CH_2Cl_2, 50 °C, 1 h, 75%; (b) 10% Pd/C, H_2, CH_3OH, 25 °C, 96% crude; (c) SO_3·pyridine complex, pyridine, 25 °C; (d) TFA, CH_2Cl_2, 4% over two steps.

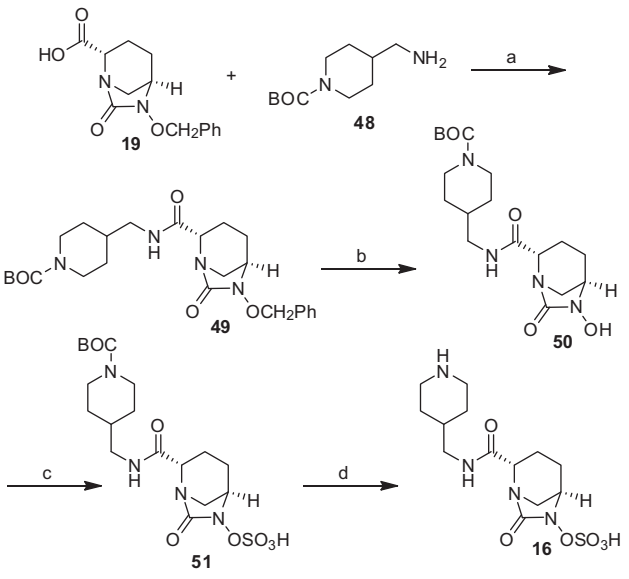
We were pleased to find that a wide variety of heterocyclic side chains were tolerated and that many of the new compounds had excellent activity against class A and class C β-lactamases (Table 1). For example, the piperidine analog 3 inhibits both KPC-2, a key class A enzyme responsible for carbapenem resistance in *Klebsiella pneumoniae*, and AmpC, the enzyme responsible for resistance in *Pseudomonas*. It is less effective against AmpC from *Acinetobacter* species and is ineffective against the class D enzyme Oxa-40, also from acinetobacter. However, resistant *Acinetobacter* accounts for only a very small percentage of clinical cases and is much less important than *Pseudomonas* and *Klebsiella*. Piperidine 3 also effectively synergized imipenem against imipenem-resistant *Klebsiella* and *Pseudomonas*, with concentrations of 12.5 and 4.7 micromolar, respectively, reducing the imipenem MIC to 4 μg/mL.

An X-ray crystal structure of **3** bound in the active site of AmpC (*Pseudomonas*) confirmed the assigned structure of **3** and provided insights into its key interactions with the enzyme (Fig. 1; coordinates for the structure have been deposited with the Protein Data Bank, entry 4NK3). As expected, **3** binds to AmpC in a very similar manner to our previous BLI MK-8712.[10]

The seven and five membered ring analogs (**1**–**2** and **4**–**5**, respectively) demonstrated comparable activity in both the enzyme and synergy assays.



Scheme 8. Reagents and conditions: (a) 2-chloro-*N*-methylpyridinium iodide, Et_3N, CH_2Cl_2, 50 °C, 1 h, 81%; (b) 10% Pd/C, H_2, CH_3OH, 25 °C; (c) SO_3·pyridine complex, pyridine, 25 °C; (d) TFA, CH_2Cl_2, 6% over three steps.

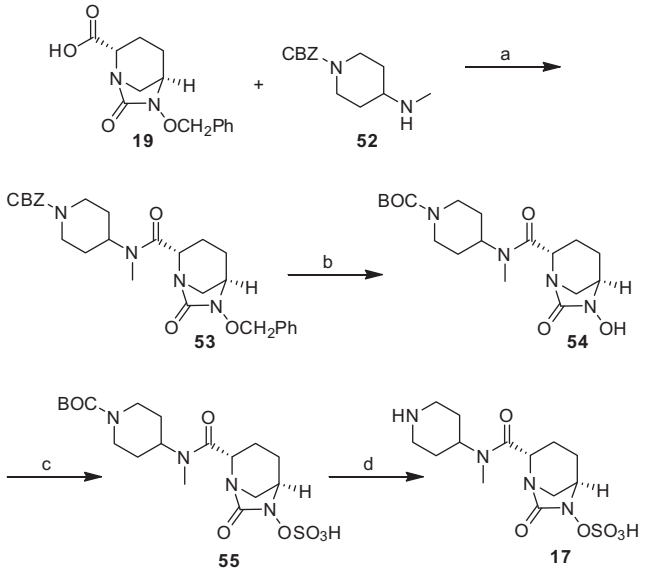


Scheme 9. Reagents and conditions: (a) EDC, 4-DMAP, CH_2Cl_2, 25 °C, 67%; (b) 10% Pd/C, H_2, CH_3OH, 25 °C; (c) SO_3·pyridine complex, pyridine, 25 °C; (d) TFA, CH_2Cl_2, 36% over three steps.

Alkylation of the piperidine nitrogen analog (e.g. *N*-methyl analog **6**), had little, if any, effect on enzyme activity and synergy.

Replacement of the piperidine nitrogen with a sulfone group (analog **7**) resulted in a significant improvement in β-lactamase enzyme inhibition but a substantial loss of synergistic activity with imipenem. This is consistent with our previous observation, during the course of our work on MK-8712, that a positive charge in the side chain was essential for prevention of efflux from the bacterial cell.

Interestingly, replacement of the piperidine side chain with aromatic side chains also resulted in a significant increase in enzyme inhibition but a decrease in synergy with imipenem. For example, the pyridine analog **8** had a nearly 80-fold improvement in its AmpC IC50 but was about five-fold less effective in restoring imipenem's activity against pseudomonas. As noted above for



Scheme 10. Reagents and conditions: (a) 2-chloro-*N*-methylpyridinium iodide, Et_3N, CH_2Cl_2, 50 °C, 1 h, 68%; (b) 10% Pd/C, H_2, BOC-anhydride, CH_3OH:THF (2:1), 25 °C; (c) SO_3·pyridine complex, pyridine, 25 °C; (d) TFA, CH_2Cl_2, 2 h, 0 °C, 5% over two steps.

sulfone **7**, we believe the observed lack of synergy for pyridine analog **8** may also be due to increased efflux since the side chain nitrogen would not be protonated at physiological pH. Indeed, addition of a basic nitrogen to to the aromatic side chain, as in the methylamino analog **12**, does improve synergy, but not to an extent commensurate with the increased enzyme inhibitory activity. In fact, even the most potent β-lactamase inhibitors required a concentration of ~3 μM or greater to synergize imipenem to 4 μg/mL. This observed activity 'floor' is most likely due to a combination of not only the inherent potency of the compound, but also the concentration of enzyme in the bacterial cell and the turnover number for effective inhibition (both MK-7655 and avibactam are reversible covalent inhibitors). Attempts to improve synergy by lowering the p*K*a of the pyridine side chain by incorporating an electron donating nitrogen (as in analog **11**) were unsuccessful. Analog **11** (and similar analogs which are not shown in Table 1) retained excellent enzyme inhibitory activity but were not active in the synergy assay. Interestingly, the 'hard quat' analog **13** was much less active than the other aromatic analogs in the enzyme inhibition assay.

The carboxylic acid analog **14**, with a side chain that would be negatively charged at physiological pH, exhibited excellent enzyme inhibitory activity but was essentially inactive in the synergy assay. Similarly, the zwitterionic amino acid analog **15** was a very effective β-lactamase inhibitor but did not effectively synergize imipenem against resistant bacteria. It is unclear if the reduced cellular activity of **14** and **15** is due to poor penetration into the bacterial cell or due to efflux.

Insertion of a methylene unit between the heterocycle and the core (analog **16**) improved enzyme activity but not synergy with imipenem, possibly due to increased lipophilicity which we believe would increase susceptibility to efflux. Alkylation of the amide nitrogen linker, as in the *N*-methyl analog **17**, however, resulted in a substantial loss of enzyme inhibition. We also found that replacement of the amide linker with an ester (analog **18**) resulted in improved enzyme activity without significant loss of activity in the synergy assay. However, due to the presumably better in vivo stability of the amide, we focused our effort on the amide series.

The piperidine analog **3** in combination with imipenem had excellent activity against an extended panel of resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* clinical isolates.[17] In

Table 1
SAR of bridged bicyclic urea β-lactamase inhibitors

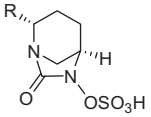
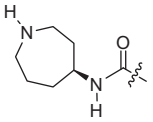
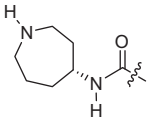
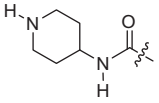
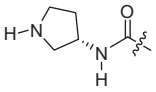
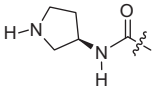
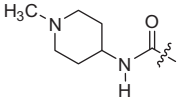
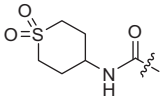
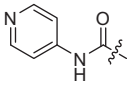
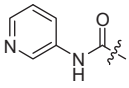
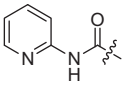
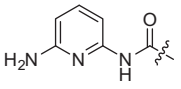
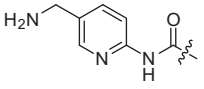
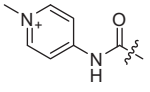
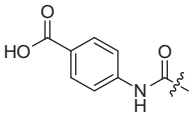
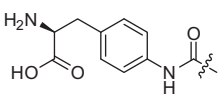
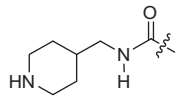
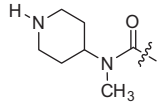
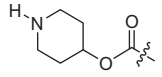
#		Enzyme inhibition (IC_{50}, nM)				In vitro synergy BLI (μM) to reduce IPM MIC to 4 μg/mL		
		KPC-2	AmpC	AmpC	Oxa40	CL 6569	CL 5701	CL 6188
	R	*K. pneum.*	*P. aerug.*	*A. baum.*	*A. baum*	*K. pneum.*	*P. aerug.*	*A. baum.*
1		245	69	1700	>50,000	12.5	6.25	>100
2		225	520	1200	>50,000	12.5	6.25	>100
3		210	465	4100	>50,000	12.5	4.7	>100
4		410	140	5600	>50,000	6.25	3.12	>100
5		260	1900	27,000	>50,000	6.25	6.25	>100
6		240	500	2700	>50,000	12.5	12.5	>100
7		150	9.5	470	>50,000	50	50	>100
8		54	6	1050	20,000	50	25	>100
9		42	37	1,200	20,000	50	100	>100
10		36	1.2	23	530	100	12.5	>100
11		11	8	100	360	25	50	>100
12		42	3	26	4400	12.5	6.25	>100
13		260	210	12,000	>50,000	25	25	>100
14		10	11	460	4800	>100	50	>100
15		3	11	1100	28,000	50	25	>100

Table 1 (*continued*)

#	R	Enzyme inhibition (IC_{50}, nM)				In vitro synergy BLI (μM) to reduce IPM MIC to 4 μg/mL		
		KPC-2	AmpC	AmpC	Oxa40	CL 6569	CL 5701	CL 6188
		K. pneum.	*P. aerug.*	*A. baum.*	*A. baum*	*K. pneum.*	*P. aerug.*	*A. baum.*
16		77	30	2200	>50,000	6.25	6.25	>100
17		2500	290	2100	>50,000	50	25	>100
18		9	170	1100	13,000	6.25	6.25	>100

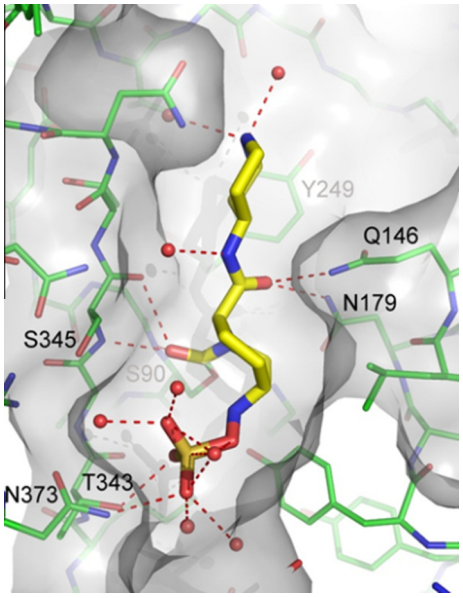


Figure 1. X-ray crystal structure of 3 in AmpC from *Pseudomonas*.

addition, it also had pharmacokinetics that were well matched with those of imipenem, an essential property for a combination product.[18] Finally, **3** was very effective in three mouse models of infection with clinically relevant imipenem-resistant *Pseudomonas* and *Klebsiella* strains.[19]

Based on its superior in vitro and in vivo activity, acceptable PK and physical properties, and lack of significant off-target activities or toxicity in preliminary safety testing, **3** was selected for development and assigned the development number MK-7655. An improved large scale synthesis of MK-7655 was recently reported by a Merck Process Chemistry group.[20] As of this writing, MK-7655 has completed phase I clinical trials[21] and is currently in phase II.[22] Further developments in this area will be reported in future communications from this laboratory.

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