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3-Arylpiperidines as Potentiators of Existing Antibacterial Agents

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Abstract—Important resistance patterns in Gram-negative pathogens include active efflux of antibiotics out of the cell via a cellular pump and decreased membrane permeability. A 3-arylpiperidine derivative (**1**) has been identified by high-throughput assay as a potentiator with an IC_{50} ~90 μM. This report details the evaluation of the tether length, aryl substitution and the importance of the fluorine on antibiotic accumulation. Evaluation of various tether lengths demonstrated that the two-carbon tethered analogues are optimal. Removal of the fluorine has a modest effect on antibiotic accumulation and the defluorinated analogue **17** is equally potent to the original lead **1**.

The emergence of mutant bacterial pathogens resistant against a wide range of available antibiotics has resulted in what some researchers call the 'post-antimicrobial era' when a simple bacterial infection can result in a life-threatening infection.[1] Resistance patterns in bacteria can be classified into a few separate groups:[2] (1) Bacterial modification of the antimicrobial target in such a way that the drug no longer has affinity for binding. (2) Bacterial production of enzymes that deactivate the drug. (3) Decrease in the permeability of the bacterial membrane or active efflux of the drug, resulting in less drug availability inside the cell.

These patterns of resistance have resulted in increased research activity in both academic and industrial settings for the discovery of antibiotics that overcome this challenge of multidrug resistance.[3–5] The identification of efflux pumps in microorganisms as an important pathway for generating resistance against antibiotics is a relatively new discovery.[6] A diverse set of pumps have been discovered in a wide variety of both Gram-positive and Gram-negative organisms. The various transport proteins in microorganisms have been characterized based on their origins.[7] These pumps show remarkably broad substrate specificity. A large number of antibiotics are extruded by these pumps including β-lactams and tetracycline.[8] AcrAB/TolC in *Escherichia coli,* which is probably the efflux pump mainly responsible for resistance in *E. coli*, is composed of three proteins that in combination form the functional pump.

Efflux pumps are attractive targets for antibacterial research since inhibition of them would render many currently available antibiotics considerably more effective. There has not been an intense effort in that regard, and only a handful of reports have appeared in the literature. The screening of inhibitors of tetracycline-specific efflux pumps has been described by Yamaguchi et al.[9] They discovered several indane inhibitors of the pump as monitored by an accumulation of radiolabeled tetracycline. Another approach to discover novel antibiotics has been described by Lewis, which demonstrated that compounds considered inactive as antibacterial agents, such as Berberine, have activity in mutants lacking the efflux pump. This approach allows for the potential identification of alternative classes of antibiotics if the compound could be subsequently altered to minimize efflux.[10] Researchers from Microcide have described arginine derivatives such as **MC-207110** as effective efflux pump inhibitors, thus increasing the sensitivity of numerous existing antibiotics.[11] An alternative approach to decrease the resistance to

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pumps is increasing the permeability of the bacterial membrane. The interplay between membrane permeability and efflux pumps has recently been demonstrated in *Pseudomonas aeruginosa*.[12] This sensitizing approach should improve the activity of existing antibiotics, and such an approach has recently been described utilizing cholic acid derivatives.[13]

Lead Identification

In an effort aimed at discovering novel efflux pump inhibitors, our compound collection was screened in a high-throughput assay with a sub-lethal concentration of novobiocin. Any compound that exhibited antibacterial activity against *E. coli* at the same concentration (100 μM) in the absence of novobiocin and compounds that had been reported in the literature as antibacterial agents were disregarded as hits. Any remaining compound that inhibited growth against *E. coli* in the presence of novobiocin was considered a putative inhibitor of the pump. In this assay, **1** was identified as a putative inhibitor (Fig. 1). An accumulation assay of ^{14}C labeled linezolid has been utilized as a secondary assay for a direct measurement of increased intracellular concentration of antibiotics in the presence of putative pump inhibitors.[14] In this accumulation assay it has been determined that (±)-**1** has an IC_{50} ~90 μM. In order to simplify the preparation of analogues of **1**, we elected to evaluate the importance of the individual functional groups contained in (±)-**1**. In this report, we describe the synthesis of analogues specifically prepared to evaluate the importance of the tether length and the 4-substituted fluorine.

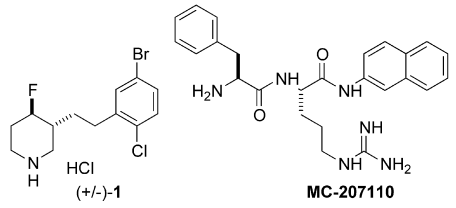
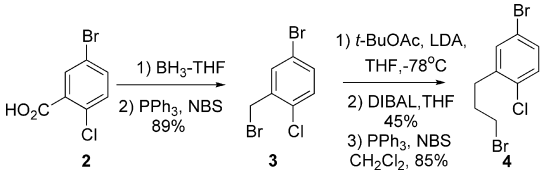
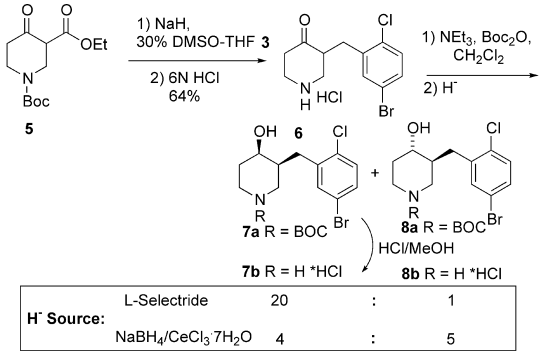


Figure 1. The piperidine derivative **1** and the Microcide compound **MC-207110**.



Scheme 1.

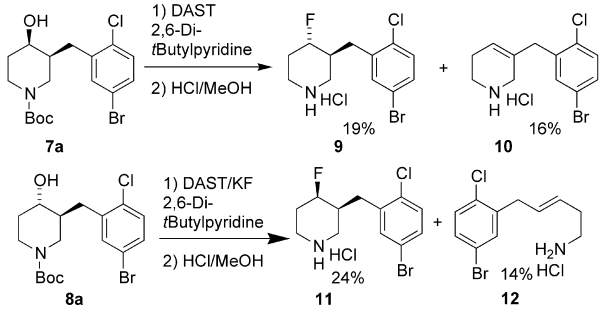


Scheme 2.

Alkylation of 4-ketopiperidine is an attractive approach to access the different tethered aryl analogues of **1** along with the corresponding desfluoro analogue.[15] The desired alkylating side chains were prepared by a multistep synthesis similar to reported literature procedures (Scheme 1).[16] Deprotonation of the ketoester **5** with various metal hydrides in a range of solvents afforded the desired alkylated product along with recovered starting material. The optimal reaction conditions which were deprotonation of **5** with NaH in 30% DMSO–THF resulted in a clear reaction mixture; addition of **3** gave the alkylated product in quantitative yield (Scheme 2). Decarboxylation of **5** in refluxing 6 N HCl afforded the decarboxylated product **6** in 64% yield. Reprotection of **6**, followed by hydride reduction, provided the corresponding alcohols. These diastereomers were easily separated by silica gel chromatography. Boc removal provided the *cis* alcohol **7b** and the *trans* alcohol **8b**.

The alcohols (±)-**7a/8a** proved to be prone to elimination during fluoride displacement via the activated intermediate (Scheme 3). Utilizing different bases or absence of base had no effect upon this result. The surprising discovery was made that the product isolated from elimination of the *trans* alcohol was not the expected cyclic alkene, rather the primary amine **12** resulting from fragmentation. The fragmentation can be explained in that the activated hydroxyl has the correct stereoelectronic overlap with the nitrogen, allowing the nitrogen to assist in the fragmentation, resulting after workup in the primary amine **12**, which was extensively characterized by NMR and MS.

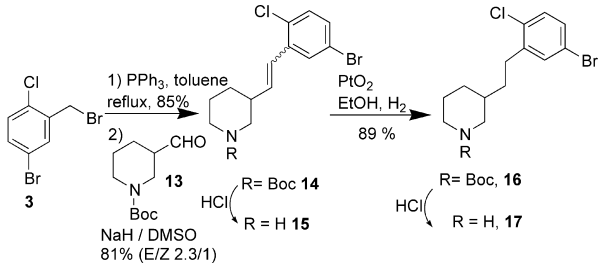
Numerous attempts to alkylate keto-ester **5** with a two-carbon electrophile were unsuccessful. Since it was imperative for us to gain access to the desfluoro analogue of **1**, attention was directed to an alternative bond disconnection utilizing a Wittig olefination as the key event. The challenge of this route is that hydrogenation of an alkene in the presence of an aryl bromide is very difficult. Exploration of the literature revealed only a



Scheme 3.

few examples of a styryl olefin being saturated in the presence of an aryl bromide.[17] The Wittig reagent was easily prepared by refluxing in toluene triphenylphosphine and the arylbromide **3**, affording the pure ylide (Scheme 4).[18] The ylide was deprotonated with NaH in DMSO at 60 °C followed by the addition of the aldehyde **13** to afford the desired alkene **14** as a ~2/1 (*E*/*Z*) mixture. The alkene **14** was then saturated under an atmosphere of hydrogen in EtOH utilizing Adams catalyst affording the alkane **16** in excellent yield with minimal dehalogenation. This approach afforded access to a range of different aryl substituted two-carbon analogues (Table 2).

The three-carbon analogue was prepared in a similar fashion as previously described for the preparation of **9** (Scheme 5). In this instance, the displacement of alcohol **19** with fluoride proved to be very problematic due to the predomination of alkene formation (alkene/fluoride, 7/3) affording desired analogue, **20** in poor yield.



Scheme 4.

Table 1. Accumulation data and representative minimum inhibitory concentration (MIC) data for selected compounds

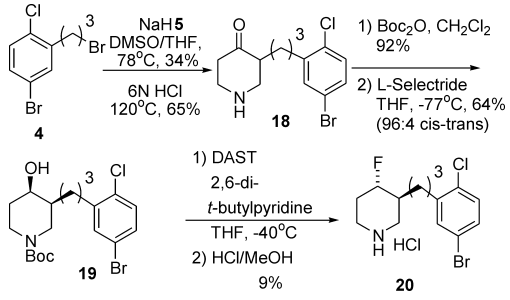
Compd	Accumulation IC_{50} (μM)	MIC	
		S. aureus[a] (μg/mL)	*E. coli*[b] (μg/mL)
MC207110	730	—	256
1	90	64	64
6	>900	>128	>256
7b	>900	>128	>256
8b	>900	>128	>256
9	>900	>125	256
10	600	128	256
11	480	>128	128
15	140	32	64
17	185	64	128
20	400	—	—

[a]*S. aureus* strain ATCC 29213.
[b]*E. coli* strain ATCC 25922.

Each of the analogues were evaluated in the accumulation assay, which measures the accumulation of ^{14}C-labeled linezolid within wild-type *E. coli* K12 cells in the presence of the potentiator.[19]

Using these assays it was determined that the lead compound **1** has an IC_{50} ~90 μM. As seen in Table 1, one-carbon tether analogues displayed marginal activity. The desfluoro analogues **17** and **15** have comparable activity to the lead compound, while the three-carbon tether analogue **20** had a lower inhibitory effect than the lead compound. The importance of aryl substitution was evaluated in a desfluoro series (Table 2). The halogens are essential for activity since 4-EtO, 4-CN, 3,5-Me and 2-Cl-4-(2MeOPh)Ph substitution afforded inactive compounds. The Br in **17** could be replaced with Cl without affecting activity. In general, the 3,4 and 2,5 disubstitution affords the most active compounds, with activity comparable to the initial lead. Several of the analogues display very weak antibacterial activity of their own, but there is no correlation between their antibacterial activity and their potency as potentiators (compare compounds **24**, **27**, and **29**, Table 2). The MIC of linezolid against *E. coli* in the presence of three potentiators is displayed in Figure 2. In the absence of the potentiator, the MIC of linezolid is >128 μg/mL.

In this report, we described the discovery of a novel piperidine derivative with the ability to increase



Scheme 5.

Table 2. Accumulation data and representative minimum inhibitory concentration (MIC) data for selected two-carbon analogues

	Compd	Accumulation IC_{50} (μM)	MIC	
			S. aureus[a] (μg/mL)	*E. coli*[b] (μg/mL)
Ph	**21**	>900	>128	>128
4-EtOPh	**22**	>900	>128	>128
4-CNPh	**23**	>900	>128	>128
2,5-ClPh	**24**	195	64	128
2,5-MePh	**25**	>900	>128	>128
3,5-CF_3Ph	**26**	300	64	64
2,6-ClPh	**27**	290	>128	>128
3,4-ClPh	**28**	200	128	128
2-Cl-4-(2-MeOPh)Ph	**29**	>900	32	64

[a]*S. aureus* strain ATCC 29213.
[b]*E. coli* strain ATCC 25922.

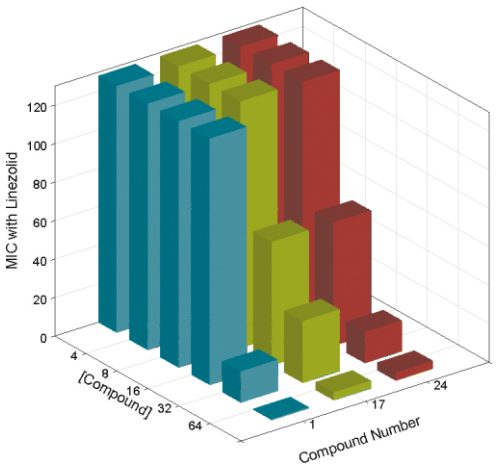


Figure 2. MIC of linezolid against *E. coli* K12 in the presence of three potentiators. In the absence of the potentiator, the MIC of linezolid is >128 μg/mL. The MIC of all of the potentiators is equal or greater than 128 μg/mL for this strain of *E. coli*. The concentration of the compounds and the MICs of linezolid are reported in μg/mL.

intracellular concentration of existing antibiotics. Several analogues have been prepared aimed at addressing two questions: first, the importance of the tether length connecting the aryl ring and the piperidine ring, and second, the importance of the 4-fluoro substituent in **1**. The results demonstrate that shortening the tether by one carbon eliminates accumulation of linezolid, while the three-carbon tether is significantly less potent than the two-atom tether. Removal of the fluorine substituent had little effect on activity. These results have allowed us to explore the importance of aryl substitution in a simpler system lacking the fluorine substituent where we have found 3,4- and 2,5-dihalogen substitution to be optimal.

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19. An accumulation assay only measures the direct increase of intracellular concentration of radiolabel. The increased concentration can be the result of pump inhibition or improved import into the cell. At this stage, we are unable to differentiate the two phenomena, but the described compounds are not lytic (Stephen E. Buxser, unpublished results).