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Synthesis and evaluation of PSSRI-based inhibitors of *Staphylococcus aureus* multidrug efflux pumps

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Abstract—Phenylpiperidine selective serotonin reuptake inhibitors (PSSRIs) block the function of selected multidrug efflux pumps of *Staphylococcus aureus*. In this study PSSRI-based piperidine derivatives were prepared, evaluated for inhibition of two multidrug resistance (MDR)-conferring efflux pump systems, and tested for potentiation of antimicrobial activity of antibacterial efflux pump substrates. It is demonstrated that the 4-phenyl moiety of PSSRI-based efflux pump inhibitors (EPIs) is not an absolute structural requirement for inhibiting the NorA and MepA MDR efflux pumps. Potency of efflux inhibition is maintained or enhanced by replacing the aryloxymethyl substituent at position-3 of PSSRIs with arylalkene and arylthioether moieties. Novel 3-aryl piperidine EPIs that significantly increase substrate antibiotic activity against strains of *S. aureus* expressing NorA and MepA are described.

Membrane-based bacterial efflux pump systems have been implicated in bacterial pathogenicity,[1] and contribute to antimicrobial resistance in bacteria.[2–4] Efflux pumps diminish intracellular drug concentrations as a direct resistance mechanism, and indirectly predispose organisms to the emergence of high-level, target-based resistance mechanisms.[5] Efflux pumps have also been implicated in reduced postantibiotic effect.[6,7] Constitutive expression of drug efflux pumps that are capable of extruding multiple structurally unrelated compounds contributes to the innate multidrug resistance (MDR) phenotype of some organisms.[4,8,9] Several families of efflux systems capable of multiple drug extrusion have been described. Some efflux systems require ATP hydrolysis for drug transport (ATP binding cassette, or ABC pumps [primary transporters]), while others require a sodium or proton gradient for drug efflux (major facilitator superfamily [MFS], small multidrug resistance [SMR], resistance-nodulation-division [RND], and multiple drug and toxin extrusion [MATE] pumps [secondary transporters]).[2,4,10–12] Structures of various components of some efflux pump systems have begun to be elucidated thus affording clues to begin understanding the mechanisms of drug recognition and transport.[13,14]

Bacterial efflux pump inhibitors (EPIs) are being pursued by academic and commercial laboratories.[15] A number of structurally diverse EPIs have been identified; examples include those from screening of compound libraries,[16] from natural sources,[17] and from the evaluation of other drug classes.[18] Inhibiting efflux pumps potentiates activity of antibiotics that are pump substrates.[15] EPIs are also known to suppress the emergence of adaptive resistance mechanisms, such as target mutations.[19] Co-administration of EPIs that inhibit different pump types in the same organism has shown remarkable synergy when used in combination with certain substrates in Gram-negative organisms.[20] Most EPIs discovered to date inhibit a limited number of efflux systems, thus presenting one obstacle to EPIs finding broad clinical utility as an adjuvant in antimicrobial therapy. Indeed, EPIs honed to block the major efflux pump systems of select MDR pathogens are the focus of early translational studies.[21]

MDR *Staphylococcus aureus* has emerged as a significant problem in both community and hospital acquired infections.[22,23] Efflux-mediated resistance to fluoroquinolones (FQ) in *S. aureus* is primarily mediated by the

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norA-encoded (MFS class) protein, although other secondary transporters such as MepA (MATE class) also contribute.[23,24] The discovery of potent inhibitors of multiple pump types in *S. aureus* is a critical first step toward beginning to evaluate the potential of *S. aureus*-targeted EPIs as potential adjuvants to antimicrobial therapy to maintain antimicrobial potency and potentially attenuate the development of target-mediated resistance.

Previous work by Kaatz and co-investigators demonstrated that phenylpiperidine selective serotonin reuptake inhibitors (PSSRIs) interfere with multidrug efflux pumps in *S. aureus*.[25] In subsequent studies we demonstrated that substituents on the phenylether moiety of paroxetine-like PSSRIs play an important role in modulating potency of efflux inhibition against NorA and non-NorA efflux pumps in *S. aureus* (Fig. 1, **1**).[18] Substitution of the piperidine amine as *N*-methyl or *N*-acetyl significantly diminished inhibition of the NorA pump, but had less of an effect on non-NorA efflux. Thorarensen et al. described 3-aryl piperidines that promote antibiotic accumulation and potentiate antibiotic activity with *E. coli* that predominately expressed the AcrAB pump system (Fig. 1, **2**).[26] These agents lack the 4-phenyl ring of PSSRIs, but otherwise have a putative pharmacophore similar to the PSSRIs.

Previous studies have shown that different substituents on the aromatic rings of the PSSRIs (Fig. 2, **3**) and 3-(2-arylethyl)piperidines (Fig. 2, **4**) afford variable effects on EPI activity.[18,25,26] No significant effect on activity has been observed for the different 3,4-(±)-*trans*-isomers. Whether the 4-phenyl substituent of PSSRIs is required for EPI activity has not been studied, and no SAR for the two-atom aryloxymethyl linker at position-3 of the PSSRI piperidine ring has been determined.

In this study we set out to determine if the 4-phenyl ring of PSSRIs is required for EPI activity, and to investigate the effect of structural changes in the two-atom aryloxymethyl linker of PSSRIs on EPI potency (Fig. 2, **5**). To this end, a series of PSSRI-like derivatives were prepared where the aryloxymethyl two-atom linker was replaced with thioether, amine and alkene linker moieties (Fig. 2, **5**). Moreover, because of the overlapping structural similarities between PSSRIs that inhibit

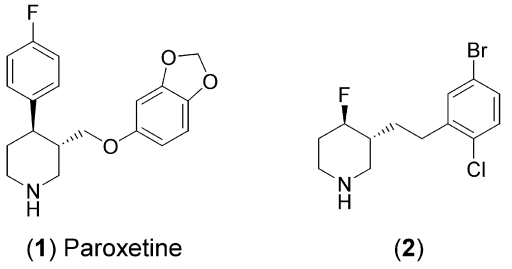


Figure 1. Parent PSSRI paroxetine (**1**) that inhibits NorA (MFS-type pump) and MepA (MATE-type pump) and 3-(2-arylethyl)piperidine (**2**) that increases antibiotic accumulation and potentiates antibiotic activity against *E. coli* expressing the AcrAB efflux pump (RND-type pump).

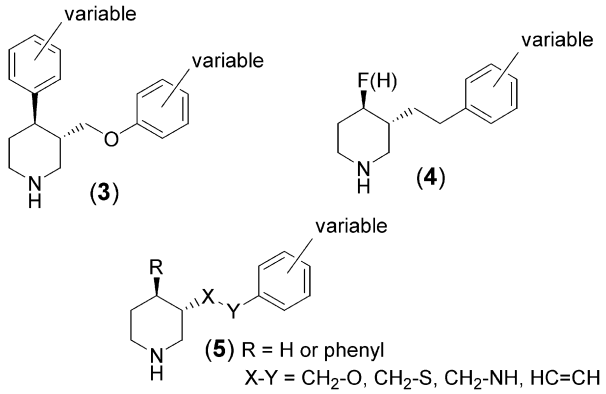


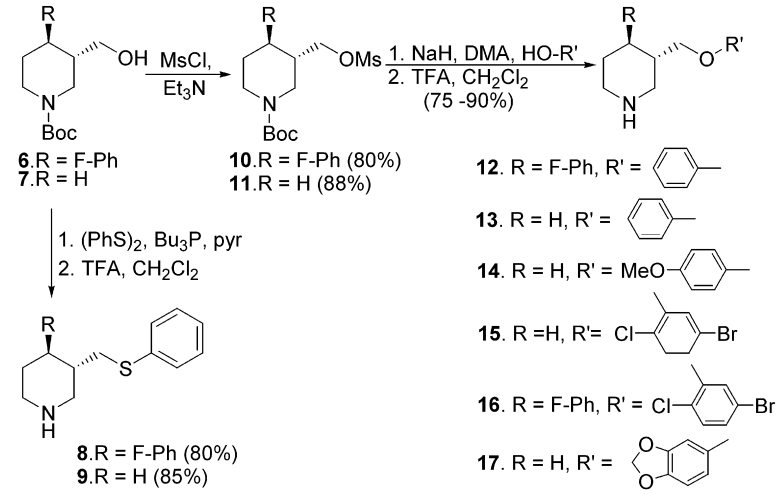
Figure 2. General structures of previously described PSSRI-based NorA and MepA inhibitors of efflux in *S. aureus* (±**3**) and 3-aryl piperidine inhibitors of transport in *E. coli* (±**4**) indicated the possibility of a common pharmacophore (±**5**) to inhibit diverse types of efflux pumps.

NorA (MFS-type) and MepA (MATE-type) efflux pumps in *S. aureus*, and the 3-(2-arylethyl)piperidines that increase antibiotic accumulation and potentiate activity of antibiotics against *E. coli* expressing the AcrAB (RND-type) pump system, we anticipated select EPIs of structure **5** might potentiate antibacterial activity of substrate antibiotics against organisms expressing each of these three different types of efflux pump systems.

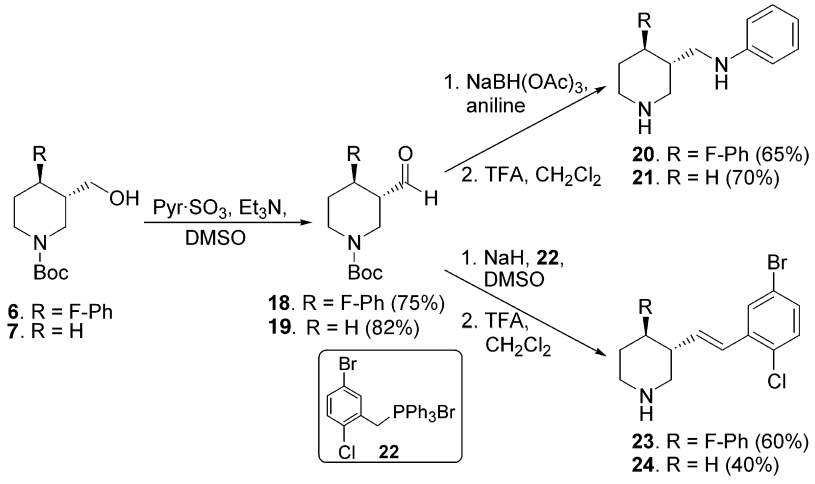
To account for effects of different 3-aryl moieties on EPI activity a number of 3-aryl substituents consistent with those found in paroxetine (**1**, methylenedioxy), femoxetine (4-methoxy), the most potent 3-arylethyl piperidines (**2**, 5-bromo-2-chloro), and unsubstituted phenyl were employed in the synthesis of target PSSRI-based EPIs. Derivatives of 3-aryloxymethy-piperidine with or without a phenyl group at position 4 of the piperidine ring were prepared by first converting 3-hydroxymethyl piperidine derivatives **6** and **7** to mesylates **10** and **11**, respectively (Scheme 1).[27] Displacement of mesylate with phenols followed by removal of Boc groups under acidic conditions afforded 3-aryloxymethyl piperidines **12**–**17** (Scheme 1).[28] Thioethers **8** and **9**, for comparison to phenoxy derivatives **12** and **13**, were prepared by treating 3-hydroxymethyl piperidines **6** and **7** with diphenylsulfide and tributylphosphine in pyridine, followed by TFA-mediated removal of Boc groups (Scheme 1).[29]

Synthesis of *N*-phenyl-3-piperidinemethanamine derivatives (**20**, **21**) and 3-[2-(5-bromo-2-chlorophenyl)-ethenyl]piperidines (**23**, **24**) was achieved by first oxidizing 3-hydroxymethyl piperidines **6** and **7** to aldehydes **18** and **19** (Scheme 2).[30] Reductive amination with aniline followed by Boc removal afforded amine-linked analogs **20** and **21**,[31] having a secondary amine in place of the phenoxy oxygen of PSSRIs. Wittig olefination with **22**[32] followed by amine deprotection afforded the 3-ethenyl-linked analogs **23** and **24**.[33]

Inhibition of NorA-mediated efflux in *S. aureus* was evaluated by comparing percent inhibition of ethidium



Scheme 1. Synthesis of PSSRI derivatives having ether-linked and sulfide-linked 3-aryl moieties (for R = F–Ph, ± trans; for R = H, ±).



Scheme 2. Synthesis of amine-linked and alkene-linked 3-aryl PSSRI derivatives (for R = F–Ph, ± trans; for R = H, ±).

bromide (EtBr) efflux against SA-K2361 (Fig. 3).[34] Derivatives lacking a 4-F-phenyl substituent are generally less active than the corresponding 4-fluorophenyl analogs. However, ether-linked and alkenyl-linked 5-bromo-2-chloro derivatives **15** and **24** do achieve 50% inhibition of NorA at 40–50 μM. This more potent inhibition of NorA by 4-unsubstituted piperidine derivatives bearing the 5-bromo-2-chloro aryl ring was similarly observed in the 4-fluorophenyl series of compounds, where aryloxy-linked **16** and alkene-linked **23** are the most potent NorA inhibitors (Fig. 3 and Table 1).

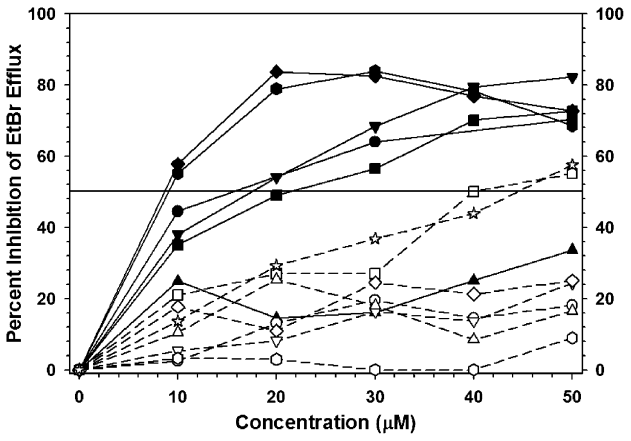


Figure 3. Inhibition of NorA-mediated ethidum efflux from SA-K2361. PSSRI-based EPIs that inhibit 50% efflux below 50 μM (♦, 16; ●, 23; ⬢, 1 (Paroxetine); ▼, 8; ■, 12; □, 15; ☆, 24). EPIs where 50% inhibition was not achieved (○, 14; ▽, 17; ◊, 9; △, 13; ⬡, 21; ▲, 20).

Direct comparison of **16**–**23** and **8**–**12** suggests the two-atom ether, thioether, and alkene linking groups afford equipotent NorA inhibitors in the absence of other structural differences. Amine-linked **20** and **21** are significantly less active.

Having demonstrated 4-fluorophenyl and 4-unsubstituted piperidines bearing the 5-bromo-2-chloro group at position-3 were the most potent NorA inhibitors within each series, ether-linked and alkene-linked derivatives **16**, **13**, **15**, and **24** were evaluated for inhibition of MepA-mediated efflux of EtBr by *S. aureus* strain SA-K2886 (Fig. 4 and Table 1).[34] In contrast to inhibition

Table 1. Comparison of IC_{50} (μM) values for EPIs against NorA-mediated efflux (SA-K2361) and MepA-mediated efflux (SA-K2886)

EPI	NorA (K2361)	MepA (K2886)
1 (Paroxetine)	15	<10
15	40	<10
16	<10	13
23	<10	20
24	45	<10
8	18	nd
12	22	nd
9, **13**, **14**, **17**, **20**, **21**	>50	nd

Concentrations at which 50% inhibition of efflux was achieved were determined from Figures 3 and 4.

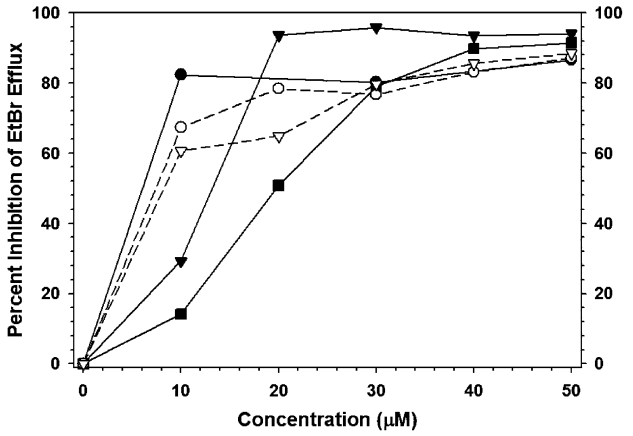


Figure 4. Inhibition of ethidium efflux from SA-K2886 (MepA overexpressor) by select PSSRI-based derivatives shown to inhibit NorA. ●, 1 (Paroxetine); ▼, 16; ■, 23 ○, 15; ▽, 24.

of NorA, potent inhibition of MepA does not require the presence of a fluorophenyl ring at position 4 of these PSSRI-based derivatives. Moreover, 4-unsubstituted analogs **15** and **24** are modestly more potent inhibitors of MepA than 4-F-phenyl substituted **16** and **23**. Inhibition of MepA by the other ether-linked and thioether-linked derivatives similarly showed 50% inhibition of efflux in the 10–20 μM range. This trend of an apparently more defined, narrow, SAR for PSSRI-like compounds to inhibit NorA than MepA is consistent with previous work where only some PSSRI derivatives that inhibited non-NorA efflux in *S. aureus* were also potent inhibitors of NorA.[18]

The identification of PSSRI-based inhibitors of NorA (MFS-type pump) and MepA (MATE-type pump) bearing the 5-bromo-2-chloro group at position 3 of the piperidine ring suggested the possibility that such compounds might display even broader-spectrum efflux pump inhibition because of structural similarity to phenylpiperidines that promote antibiotic accumulation and potentiate antibiotic activity with *E. coli* expressing the AcrAB efflux pump (RND-type) (see Fig. 2). Evaluating the EPIs here for direct inhibition of the AcrAB and MexB efflux pumps of *E. coli* and *P. aeruginosa* at one-half MIC did not reveal direct pump inhibition (data not shown, see Table 2 for MICs). However, test concentrations of one-half MIC are well below IC_{50} values reported for enhanced antibiotic accumulation by 3-arylpiperidines.[26]

Table 2. MIC (μg/mL) values for EPIs against parent and efflux pump overexpressing strains of *S. aureus*, *P. aeruginosa*, and *E. coli*

Strain	**24**	**23**	**15**	**16**
SA-K1902	100	25	100	12.5
SA-K2361 (*norA*+)	100	25	>100	12.5
SA-K2885	100	25	100	6.25
SA-K2886 (*mepA*+)	50	25	>100	6.25
PA-K3082	100	100	100	100
PA-K2184 (*mexB*+)	100	100	100	100
EC-K2201	50	100	100	25
EC-K2203 (*acrB*+)	100	100	100	50

MIC value for paroxetine against each test strain was >50 μg/mL.

MIC values were determined for **15**, **16**, **23**, and **24** against parent and efflux pump overexpressing strains of *S. aureus*, *P. aeruginosa*, and *E. coli* (Table 2). The fact that MICs are not different between the parent and pump overexpressing strains demonstrates these EPIs are not efflux pump substrates. Derivatives **16** and **23** have surprisingly low MICs against *S. aureus*. EPI-substrate antibiotic combination studies against parent and pump overexpressing strains of *S. aureus* were pursued to further confirm EPI activity and to evaluate the ability of these Novel EPIs to potentiate antibiotic activity of antibiotic pump substrate.[26,35] At concentrations of 1/4 and 1/2 MIC EPIs **15**, **16**, **23**, and **24** were found to significantly lower MICs of ethidium bromide (potentiate antibiotic activity) in strains of *S. aureus* expressing either the NorA or MepA efflux pump system (Table 3). In comparison, at 1/4 MIC paroxetine was shown to lower ethidium bromide MIC 2- to 8-fold against NorA and non-NorA efflux pump expressing strains of *S. aureus*.[25]

Because potentiation data are determined relative to MIC of each EPI (at 1/4 and 1/2 MIC), differences in the inherent MIC of each EPI against each test strain have a significant effect on molar concentration of the EPI in these studies. For example, the equivalent 4-fold reduction in EtBr MIC against SA-K2866 by **16** and **24**

Table 3. Antibiotic potentiation of PSSRI-based EPIs against *S. aureus* strains expressing the NorA and MepA efflux pumps

EPI	Fold reduction in EtBr MIC			
	SA-K2361 (*norA*+)		SA-K2886 (*mepA*+)	
	1/4 MIC[a]	1/2 MIC	1/4 MIC[a]	1/2 MIC
24	64	>128	4	16
23	16	64	4	>32
15	16	64	2	8
16	4	32	4	8

[a] MIC values ranged from 6.25 to 100 μg/mL against test strains. Molar concentrations for each derivative at 1/4 MIC against each test strain were: (SA-K2361) **24**, 8.32 μM; **23**, 1.58 μM; **15**, 8.21 μM; **16**, 0.79 μM. (SA-K2886) **24**, 4.16 μM; **23**, 1.58 μM; **15**, 4.1 μM; **16**, 0.39 μM.

at 1/4 MIC translates to **16** being over 10-fold more potent than **24**, where molar concentrations of **16** and **24** at 1/4 MIC are 0.39 μM and 4.16 μM, respectively. None of the EPIs lowered ethidium MIC against parent strains SA-K1902 and SA-K2885 at a concentration of 1/2 MIC, further supporting that lowering of EtBr MIC against strains of *S. aureus* expressing the NorA and MepA pumps is due to pump inhibition.

Derivatives **15**, **16**, **23**, and **24** were also evaluated in combination studies with ciprofloxacin using wild-type *E. coli* and *P. aeruginosa* strains and derivatives overexpressing the AcrAB and MexAB pumps, respectively, where ciprofloxacin is a substrate for these pump systems (see Table 2 for strains used).[35] No significant potentiation of ciprofloxacin activity (greater than 4-fold lowering of ciprofloxacin MIC by 1/2 MIC of EPI) was observed, indicating these EPIs are not potent inhibitors of these RND-type efflux pumps. This result is consistent with parent PSSRIs showing no significant changes in antibiotic susceptibility against efflux pump overproducing strains of *P. aeruginosa* and *E. coli*.[25]

In summary, we have demonstrated that a 4-phenyl moiety on the piperidine ring of PSSRI-based EPIs is not required for inhibition of the NorA and MepA efflux pumps of *S. aureus*. A number of two-atom linker groups for the 3-aryl piperidine moiety of PSSRI-based EPIs have been shown to maintain EPI activity. This work reveals, in general, a new structural scaffold for inhibitors of *S. aureus* efflux pumps that although originally derived from PSSRIs is no longer restricted to the core structure of PSSRIs. We have identified novel 3-aryl piperidine inhibitors of the NorA and MepA efflux pumps in *S. aureus* that are potent potentiators of substrate antibiotic activity against strains of *S. aureus* expressing these efflux systems.

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References and notes

1. Piddock, L. J. *Nat. Rev. Microbiol.* **2006**, *4*, 629.
2. Li, X.-Z.; Nikaido, H. *Drugs* **2004**, *64*, 159.
3. Piddock, L. J. *Clin. Microbiol. Rev.* **2006**, *19*, 382.
4. Poole, K. *J. Antimicrob. Chemother.* **2005**, *56*, 20.
5. (a) DeMarco, C. E.; Cushing, L. A.; Frempont-Manso, E.; Seo, S. M.; Jaravaza, T. A. A.; Kaatz, G. W. . *Antimicrob. Agents Chemother.* **2007**, *51*, 3235; (b) Markham, P. N. *Antimicrob. Agents Chemother.* **1999**, *43*, 988; (c) Markham, P.; Neyfakh, A. *Antimicrob. Agents Chemother.* **1996**, *40*, 2673; (d) Lomovskaya, O.; Lee, A.; Hoshino, K.; Ishida, H.; Mistry, A.; Warren, M. S.; Boyer, E.; Chamberland, S.; Lee, V. J. *Antimicrob. Agents Chemother.* **1999**, *43*, 1340; (e) Oethinger, M.; Kern, W. V.; Jellen-Ritter, A. S.; McMurry, L. M.; Levy, S. B. *Antimicrob. Agents Chemother.* **2000**, *44*, 10; (f) Nakajima, A.; Sugimoto, Y.; Yoneyama, H.; Nakae, T. *Microbiol. Immunol.* **2002**, *46*, 391; (g) Hocquet, D.; Vogne, C.; Garch, F.; Vejux, A.; Gotoh, N.; Lee, A.; Lomovskaya, O.; Plesiat, P. *Antimicrob. Agents Chemother.* **2003**, *47*, 1371.
6. Stubbings, W.; Bostock, J.; Ingham, E.; Chopra, I. *Antimicrob. Agents Chemother* **2005**, *49*, 1206.
7. Aeschlimann, J. R.; Dresser, L. D.; Kaatz, G. W.; Rybak, M. J. *Antimicrob. Agents Chemother.* **1999**, *43*, 335.
8. Vila, J.; Martí, S.; Sánchez-Céspedes, J. *J. Antimicrob. Chemother.* **2007**, *59*, 1210.
9. Yang, S.; Clayton, S. R.; Zechiedrich, E. L. *J. Antimicrob. Chemother.* **2003**, *51*, 545.
10. Langton, K. P.; Henderson, P. J. F.; Herbert, R. B. *Nat. Prod. Rep.* **2005**, *22*, 439.
11. Higgins, C. F. *Nature* **2007**, *446*, 749.
12. Kumar, A.; Schweizer, H. P. *Adv. Drug Deliv. Rev.* **2005**, *57*, 1486.
13. For example, see (a) Dawson, R. J. P.; Locker, K. P. *Nature* **2006**, *443*, 180; (b) Murakami, S.; Nakashima, R.; Yamashita, E.; Yamaguchi, A. *Nature* **2002**, *419*, 587.
14. (a) Yu, E.; McDermott, G.; Zgurskaya, H.; Nikaido, H.; Koshlander, D., Jr. *Science* **2003**, *300*, 976; (b) Lomovskaya, O.; Zgurskaya, H. I.; Totrov, M.; Watkins, W. J. *Nat. Rev. Drug Discov.* **2007**, *6*, 56; (c) McKeegan, K. S.; Borges-Walmsley, M. I.; Walmsley, A. R. *Curr. Opin. Pharmacol.* **2004**, *4*, 479; (d) Yu, E. W.; Aires, J. R.; Nikaido, H. *J. Bacteriol.* **2003**, *185*, 5657.
15. (a) Kaatz, G. W. *Curr. Opin. Investig. Drugs* **2005**, *6*, 191; (b) Renau, T. E.; Lemoine, R. C. *Drugs of the Future* **2001**, *26*, 1171; (c) Barrett, J. *Curr. Opin. Invest. Drugs* **2001**, *2*, 212; (d) Lynch, A. S. *Biochem. Pharmacol.* **2006**, *71*, 949; (e) Lomovskaya, O.; Bostian, K. A. *Biochem. Pharmacol.* **2006**, *71*, 910; (f) Lomovskaya, O.; Watkins, W. J. *Curr. Med. Chem.* **2001**, *8*, 1699; (g) Kristiansen, J. E.; Hendricks, O.; Delvin, T.; Butterworth, T. S.; Aagaard, L.; Christensen, J. B.; Flores, V. C.; Keyzer, H. *J. Antimicrob. Chemother.* **2007**, *59*, 1271.
16. Markham, P. N.; Westhaus, E.; Klyachko, K.; Johnson, M. E.; Neyfakh, A. A. *Antimicrob. Agents Chemother.* **1999**, *43*, 2404.
17. Stavri, M.; Piddock, L. J. V.; Gibbons, S. *J. Antimicrob. Chemother* **2007**, *59*, 1247.
18. Wei, P.; Kaatz, G. W.; Kerns, R. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3093.
19. Louie, A.; Brown, D. L.; Liu, W.; Kulawy, R. W.; Deziel, M. R.; Drusano, G. L. *Antimicrob. Agents Chemother.* **2007**, *51*, 3988, See also, Ref. 5c.
20. Tegos, G.; Stermitz, F. R.; Lomovskaya, O.; Lewis, K. *Antimicrob. Agents Chemother.* **2002**, *46*, 3133.
21. For reviews that discuss many of the issues surrounding potential clinical application of EPIs in antimicrobial therapy see Ref. 15d,e.
22. Carleton, H. A.; Diep, B. A.; Charlebois, E. D.; Sensabaugh, G. F.; Perdreau-Remington, F. *J. Infect. Dis.* **2004**, *190*, 1730.
23. The expression of different efflux pumps has been used to estimate the frequency of efflux-related resistance in 232 bloodstream isolates of *S. aureus*, see Ref. 5a.
24. Kaatz, G. W.; Mcaleese, F.; Seo, S. M. *Antimicrob. Agents Chemother.* **2005**, *49*, 1857.
25. Kaatz, G. W.; Moudgal, V. V.; Seo, S. M.; Bondo Hansen, J.; Kristiansen, J. E. *Int. J. Antimicrob. Agents* **2003**, *22*, 254.
26. Thorarensen, A.; Presley-Bodnar, A. L.; Marotti, K. R.; Boyle, T. P.; Heckaman, C. L.; Bohanon, M. J.; Tomich, P. K.; Zurenko, G. E.; Sweeney, M. T.; Yagi, B. H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1903.
27. Known mesylates **10** and **11** were synthesized from commercially available alcohols +/−**7** and +/−*trans*-**6**. using methodology previously reported for preparing **10**

Johnson, T. A.; Curtis, M. D.; Beak, P. *J. Am. Chem. Soc.* **2001**, *123*, 1004.

28. 3-Aryloxymethyl piperidines **12**–**17** were synthesized from mesylates **10** and **11** using conditions for mesylate displacement and Boc removal as previously described (Johnson et al., see Ref. 27). Characterization of previously described **12**, **13**, and **14** by NMR and Mass. Spec. confirmed structure. For unreported derivatives **15**–**17**: (**15**) ^{1}H NMR (300 MHz, ($CDCl_3$) δ = 7.21 (d, J = 8.4 Hz), 7.06 (dd, J = 2.0, J = 4 Hz, 1H), 7.00 (d, J = 2.0 Hz, 1H), 3.99 (ddd, J = 4.0, J = 6.0, J = 9 Hz, 2H), 3.51 (m, 2H), 2.91 (m, 2H), 2.5 (m, 1H), 1.99 (m,3H), 1,62 (m, 2H), 1.20 (m, 2H). ^{13}C NMR (75 MHz, ($CDCl_3$) δ = 21.70, 25.43, 33.69, 44.07, 46.26, 70.60, 116.79, 120.63, 122.23, 124.96, 131.16, 154.38. HRMS (ESI): calcd for ($M+H^+$); 307.0005, found; 306.9976. (**16**) ^{1}H NMR (300 MHz, ($CDCl_3$) δ = 7.20 (m, 4H), 7.0 (m, 3 H), 6.74 (d, J = 1.5 Hz, 1H), 4.83 (bs, 1H), 3.68 (m, 4H), 3.23 (m, 1H), 3.00 (m, 2H), 2.52 (m, 1H), 2.13 (m, 2H). ^{13}C NMR (75 MHz, (dmso-d6) δ = 30.57, 38.87, 44.15, 46.30, 68.87, 115.96, 116.25, 117.29, 120.93, 121.41, 125.12, 129.42, 129.49, 129.52, 131.72, 138.73, 143.81, 154.61, 159.96, 163.20. HRMS (ESI): calcd for ($M+H^+$) 401.0224, found; 401.0194. (**17**) ^{1}H NMR (300 MHz, ($CDCl_3$) δ = 6.68 (d, J = 8.1 Hz, 1H), 6.44 (s, 1H), 6.26 (d, J = 8 Hz, 1H), 5.91 (s, 2H), 3.79 (m, 2H), 3.52(m, 2H), 2.87 (m, 2H), 2.36 (m, 1H), 1.89 (m, 3H). ^{13}C NMR (75 MHz, ($CDCl_3$) δ = 21.78, 25.32, 33.86, 44.74, 47.12, 60.45, 70.31, 98.05, 101.22, 105.54, 107.93, 142.06, 148.31, 153.88. HRMS (ESI): calcd for ($M+H^+$); 236.1208, found; 236.1248.
29. For previously unreported thioethers **8** and **9**: Bu_3P (96 μL, 0.38 mmol) was added to a stirred solution of **6** or **7** (0.12/0.14 mmol) in pyridine (3 mL) containing $(PhS)_2$ (91 mg, 0.42 mmol). After 24 hr at rt the mixture was poured onto stirred crushed ice. Extraction with ethyl acetate was followed by washing with 10% HCL, 25% NaOH, then brine. After separation of product by flash chromatography (1:4 Ethyl Acetate/Hexanes), product was dissolved in dichloromethane (5 mL) and treated with *p*-toluenethiol (1 equiv)/trifluoroacetic acid (0.5 mL). After 5 h the mixture was concentrated by evaporation, washed with hexanes (5 × 20 mL) and solids dried to give **8** (80%) and **9** (85%). (**8**) ^{1}H NMR (300 MHz, ($CDCl_3$) δ = 7.2 (m, 5H), 7.03 (m, 4H), 3.86 (d, J = 12 Hz, 1H), 3.53 (d, J = 12 Hz, 1H), 3.02 (q, J = 9.6, J = 13.2 Hz, 1H), 2.87 (m, 2H), 2.64 (dt, J = 4.2 Hz, J = 11 Hz, 1H), 2.43 (m, 2H), 2.03 (m, 2H).)^{13}C NMR (75 MHz, ($CDCl_3$) δ = 30.75, 35.88, 38.78, 44.52, 44.95, 47.91, 115.92, 127.08, 128.88, 130.62, 134.73, 136.708. (**9**) ^{1}H NMR (300 MHz, ($CDCl_3$) δ = 7.31 (m, 5H), 3.62 (d, J = 11.4 Hz, 1H), 3.43 (d, J = 12 Hz, 1H), 2.88 (m, 3H), 2.70 (t, J = 12 Hz, 1H), 1.93 (m, 4H), 1.31 (m, 1H)^{13}C NMR (75 MHz, ($CDCl_3$) δ = 21.81, 28.48, 33.43, 37.94, 44.23, 48.11, 126.88, 129.17, 130.21, 135.27.
30. Oxidation of primary alcohols **6** and **7** to previously unreported aldehyde **18** and known **19** employed Parikh–Doering conditions as previously described for Boc-protected hydroxymethyl pyrrolidines, see; Wallén, E. A. A.; Christiaans, J. A. M.; Saario, S. M.; Forsberg, M. M.; Venäläinen, J. I.; Paso, H. M.; Männistö, P. T.; Gynther, J. *Bioorg. Med. Chem.* **2002**, *10*, 2199, For **18**:^{1}H NMR (300 MHz, ($CDCl_3$) δ = 9.47 (s, 1H), 7.18 (m, 2H), 6.97 (m, 2H), 4.39 (m, 1H), 2.21 (m,1H), 2.82 (m, 4H), 1.84 (m, 1H), 1.70 (dt, J = 12 Hz, J = 4 Hz, 1H), 1.48 (s, 9H). ^{13}C NMR (75 MHz, ($CDCl_3$) δ = 28.49, 33.72, 41.02, 43.88, 53.81, 80.21, 115.92, 128.90, 154.55, 160.18, 163.43, 202.35.
31. Reductive amination employed standard conditions using sodium triacetoxyborohydride. After purification of Boc-protected intermediates by flash chromatography the Boc groups were removed as described in Ref. 29 to provide previously unreported **20** and **21**. (**20**) ^{1}H NMR (300 MHz, (MeOD) δ = 7.32 (m, 2H), 7.13 (m, 4H), 6.68 (dt, J = 1.3, J = 7.2 Hz, 1H), 6.48 (dd, J = 4.2, J = 0.9 Hz, 2H), 3.69 (ddd, J = 1.2 Hz, J = 3.1 Hz, J = 6.6 Hz, 1H), 3.48 (dt, J = 1.5 Hz, J = 13 Hz, 1H), 3.32 (m, 1H), 3.11 (dt, J = 4.2 Hz, J = 12.7 Hz, 1H), 3.02–2.84 (m, 3H), 2.74 (dt, J = 4.6 Hz, J = 11.0 Hz, 1H), 2.37 (m, 1H), 2.01 (m, 2H).^{13}C NMR (75 MHz, (MeOD) δ = 30.64, 38.49, 43, 43.10, 44.49, 112.64, 115.25, 117.14, 128.92, 138.16, 147.57, 160.42, 163.66. HRMS (ESI): calcd for ($M+H^+$); 285.1722, found; 285.1723. (**21**) ^{1}H NMR (300 MHz, (acetone-d^6) δ = 7.10 (m, 2H), 6.65 (m, 3H), 3.67 (d, J = 12 Hz, 1H), 3.54 (d, J = 12.7 Hz, 1H), 3.18 (dd, J = 2.3, J = 6.6 Hz, 2H), 3.09 (m, 1H), 2.94 (t, J = 12 Hz, 1H), 2.31 (m, 1H), 1.77–2.12 (m, 7H), 1.43(m, 1H).^{13}C NMR (75 MHz, ($CDCl_3$) δ = 30.64, 38.49, 43, 43.10, 44.49, 112.64, 115.25, 117.14, 128.92, 138.16, 147.57, 160.42, 163.66. HRMS (ESI): calcd for ($M+H^+$); 191.1504, found; 191.1514.
32. Wittig salt **22** was synthesized in four steps from 5-bromo-2-chlorobenzoic acid and has been previously reported, see; Plater, M. J. *J. Chem. Soc. Perkin Trans. I* **1997**, *19*, 2903.
33. Standard Witting olefination procedures using NaH in DMSO at 60°C followed by work-up and flash chromatography (1:6, AcOEt:Hexane) afforded the Boc-protected intermediates, which were subjected to Boc removal as described in Ref.29 to provide previously unreported **23** and **24**. (**23**)^{1}H NMR (300 MHz, ($CDCl_3$) δ = 7.45 (d, J = 14.4 Hz, 1H), 7.23 (m, 3H), 7.10 (d, J = 8.5 H), 6.94 (t, J = 8.5, J = 10 Hz, 2H), 6.39 (d, J = 18.6 Hz, 1H), 6.00 (dd, J = 9 Hz, J = 16 Hz, 1H), ^{13}C NMR (75 MHz, (DMSO-d_6) δ = 30.57, 44.14, 46.30, 68.87, 115.96, 116.25, 117.29, 120.93, 121.40, 125.12, 129.52, 131.72, 138.66, 138.73, 1143.81, 154.61, 159.60 (J_{CF} = 210 Hz). HRMS (ESI): calcd for ($M+H^+$); 396.0275, found; 396.0772. **24**: ^{1}H NMR (300 MHz, (DMSO-d_6) δ = 9.06 (br s, 1H), 7.87 (s, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 6.65 (d, J = 15.9 Hz, 1H), 6.42 (dd, J = 6, J = 15.9 Hz, 1H), 3.24 (m, 2H), 2.75 (m, 3H), 1.83 (m, 2H), 1.44 (q, J = 10 Hz, 1H). ^{13}C NMR (75 MHz, (DMSO-d_6) δ = 21.74, 28.34, 36.76, 43.38, 47.38, 120.94, 124.82, 129.80, 131.30, 131.93, 132.10, 136.29, 137.06. HRMS (ESI): calcd for ($M+H^+$); 303.0056, found; 303.0026.
34. Inhibition of NorA-mediated efflux of EtBr in *S. aureus* strain K2361, which overexpresses the NorA efflux pump system, and inhibition of MepA-mediated efflux of EtBr in *S. aureus* strain K2886, which overexpresses the MepA efflux pump system, was performed as previously described; see Kaatz, G. W.; Seo, S. M.; OBrien, L.; Wahiduzzaman, M.; Foster, T. J. *Antimicrob. Agents Chemother.* **2000**, *44*, 1404, Experiments were performed in duplicate, and the results were expressed as mean total efflux over a 5 min time course.
35. MIC and combination studies to evaluate lowering of antibiotic MIC by EPIs against the various strains were performed as previously reported in Ref. 26 and as described in (a) Smith, E. C.; Kaatz, G. W.; Seo, S. M.; Wareham, N.; Williamson, E. M.; Gibbons, S. *Antimicrob. Agents Chemother.* **2007**, *51*, 4480; (b) Eliopoulos, G. M.; Moellering, Jr. R. C. In *Antibiotics in laboratory medicine*, Lorian, V. Ed.; Williams and Wilkins, Baltimore, Md, 1991; Antimicrobial combinations, pp. 432–492.