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Effect of Aryl Ring Fluorination on the Antibacterial Properties of C_4 Aryl-Substituted *N*-Methylthio β-Lactams

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Abstract—4-Aryl-substituted *N*-thiolated β-lactams are a new family of antibacterial agents possessing unique structure–activity profiles and a mode of action. Unlike traditional β-lactam antibiotics, which require highly polar enzyme-binding groups, these lactams bear hydrophobic groups on their side chains. In this study, we examine the effect that increasing hydrophobicity, through fluorine substitution in the C_4 aryl ring, has on the antibacterial properties.

Introduction

N-Methylthio-substituted β-lactams represent a new class of antibacterial agents effective against drug-resistant strains of *Staphylococcus aureus* (*MRSA*).[1] In a previous paper we described a series of C_4 aryl substituted analogues **1** whose ring substituent X included several different types of functionalities such as nitro, cyano, chloro, methoxy, methyl, and ester moieties.[2] Although the mechanism of action of these *N*-thiolated lactams has not yet been identified, the compounds do not appear to interact with the penicillin binding proteins (PBPs) or to affect cell wall crosslinking like other β-lactam antibiotics. We attribute this to the absence of polar functionality needed for binding to the PBPs.[3] The antibacterial activity instead seems to be related to blockage of intracellular events, wherein it may be possible to further enhance bioactivity by making the side chains more lipophilic. One way to possibly do this is by incorporating fluorine atoms into these peripheral groups.[4] In this paper, we address this by examining the effects that fluorination in the C_4 aryl ring of *N*-methylthio β-lactams (**1**, $X = F_n$ where $n = 1$–5) has on antibacterial activity.

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Results and Discussion

The first series of analogues examined in our study were monofluorinated compounds **2**–**4**, which were prepared using a previously reported procedure as represented in Scheme 1. Staudinger coupling of methoxyacetyl chloride to the corresponding fluorobenzaldehyde *N*-(4-methoxyphenyl)imine afforded *exclusively* the *cis*-3,4-disubstituted β-lactam adduct, which was converted to the *N*-methylthio derivative in two steps.[5] The *cis*-stereochemistry of the lactam intermediates and final products was assigned based on the vicinal coupling constants ($J = 4$–5 Hz) for the C_3 and C_4 protons on the ring.[6]

Our initial screening of the lactams was performed by a standard Kirby–Bauer disk diffusion assay against a panel of common bacteria including penicillin-susceptible *Staphylococcus* species and methicillin-resistant strains. Prior to this testing, a series of trial experiments were first conducted using *ortho*-fluorophenyl lactam **2** to determine the optimal amount of compound for disk diffusion measurements. Figure 1a and b compares the relative effectiveness of lactam **2** and penicillin, respectively, against a methicillin-susceptible strain of *S. aureus* (*MSSA*) and a methicillin-resistant isolate (*MRSA*). The plot in Figure 1a reveals a linear relationship between the amount of lactam **2** used for testing and the size of the zones that are produced. Against the *MSSA*,

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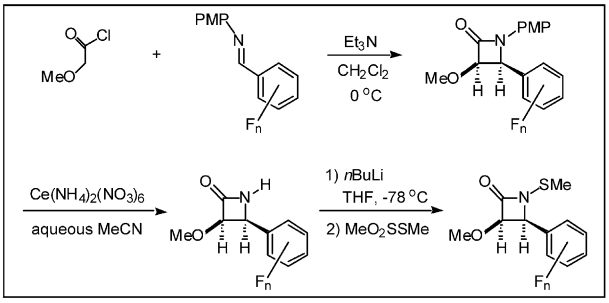
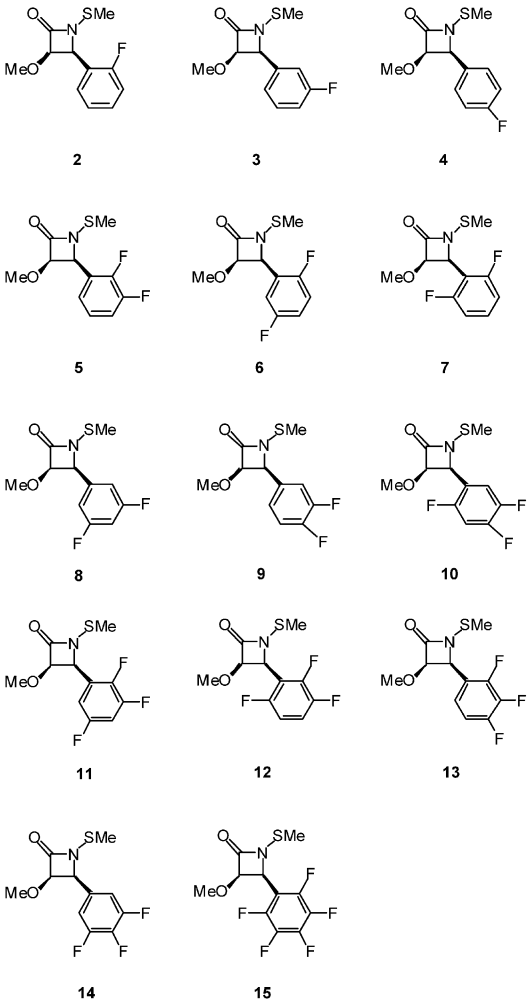


Fluorinated *N*-Methylthio β-Lactams 2-15



Scheme 1.

the inhibitory activity of **2** rises steadily as the amount of antibiotic is increased from 2 to 48 μg/disk. The same trend is observed against the *MRSA*, indicating that lactam **2** retains its full antibacterial activity against the β-lactamase-producing strain at all concentrations. In direct contrast, Figure 1b shows that the effectiveness of penicillin G against the *MRSA* strain is precipitously lower relative to the *MSSA*. It is also apparent that against *MSSA*, penicillin is effective at all concentrations, but against *MRSA*, more than 28 μg of penicillin/disk is required to bring on an inhibitory effect. The marked decrease in activity of penicillin towards *MRSA* is a consequence of the detrimental effect that bacterial

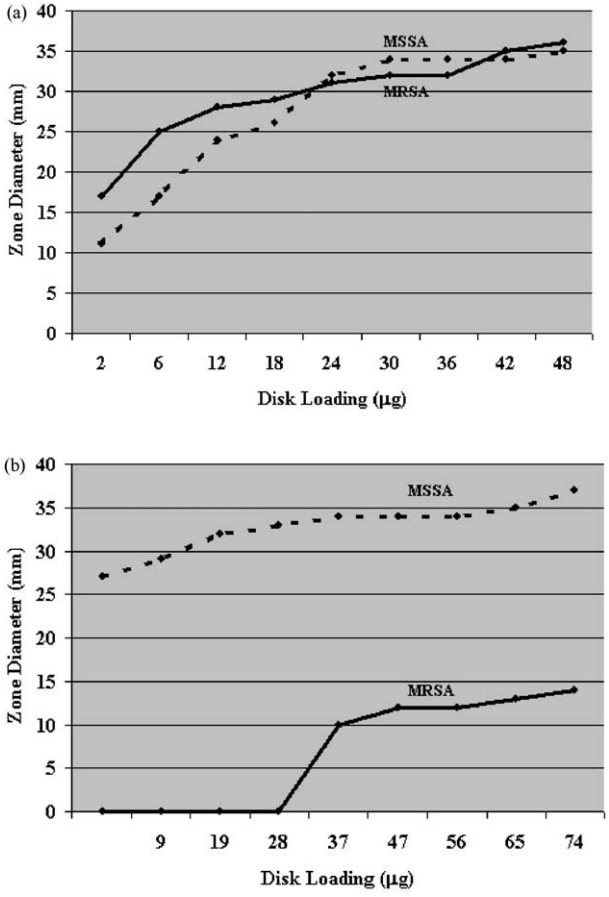


Figure 1. Disk loading versus zone of growth inhibition of (a) lactam **2**, (b) penicillin G against *MSSA* and *MRSA*.

β-lactamases have on penicillin at lower concentrations, which is not observed for lactam **2**. This is in agreement with our previous finding that *N*-methylthio β-lactams are stable to penicillinases.[1]

Figure 2a and b compares the performance of lactam **2** to *MSSA* and *MRSA* versus that of two reference antibiotics, penicillin G and vancomycin. As shown in Figure 2a, lactam **2** yields smaller zones against the *MSSA* than penicillin up to 175 nmol/disk (equivalent to 46 μg of **2**, or 64 μg of penicillin G) before the two compounds begin to show equal effectiveness. The figure also indicates lactam **2** and vancomycin produce equivalent zone sizes at 25 nmol (equivalent to 6 μg of **2**, or 14 μg of vancomycin), and at higher disk loadings, lactam **2** is consistently more active than vancomycin. In Figure 2b, a similar comparison of activities is made for the three antibiotics against the *MRSA*. In this case, lactam **2** is much stronger than penicillin over the entire 10–200 nmol range, and more potent than vancomycin when more than 75 nmol of compound is used. In addition to the disk diffusion measurements, minimum inhibitory concentration (MIC) values were determined for lactams **2**–**12** by agar dilution. Table 2 shows that the MIC's of the lactams for *S. aureus* and the *MRSA* strains are similar and in the range of 10–15 μg/mL. For comparison, MIC_{90}'s determined for penicillin G using this method are >64 μg/mL for the eight *MRSA* variants.

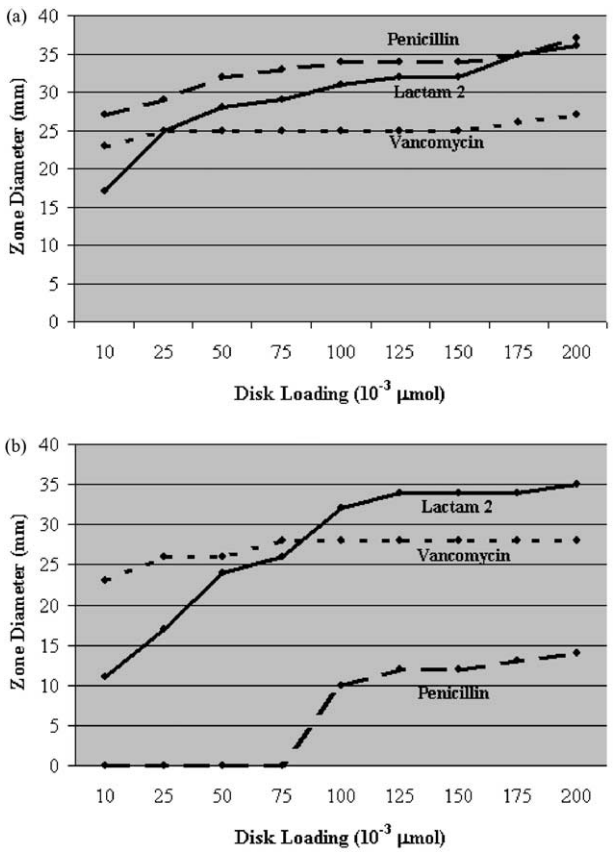


Figure 2. Disk Loadings versus zones of growth inhibition against (a) Methicillin-Susceptible *Staphylococcus aureus (MSSA)*, (b) Methicillin-Resistant *Staphylococcus aureus (MRSA)*.

Since 20 µg/disk of lactam **2** appears to be optimal for Kirby–Bauer testing, we began to measure susceptibility data for a selection of other fluorinated analogues (**3**–**15**, Scheme 1) that differ in the location or number of fluorine atoms on the aryl ring. Table 1 summarizes these data for all the compounds, along with a non-fluorinated derivative (**1**, X = H) and penicillin G as controls. The three monofluorophenyl-substituted lactam isomers **2**–**4** have similar activity, with the *ortho*-fluoro derivative generating slightly larger zones than the *meta*- and *para*-analogues. All three isomers retain their effectiveness against the *MRSA* strains as well. It is also apparent that the activities of the five difluorophenyl analogues **5**–**9** are similar to monofluorinated lactams **2**–**4**, and that the *location* of the halogen on the aryl ring in these lactams has little effect on the antibacterial properties.

The trifluorophenyl derivatives **10**–**14** and pentafluorophenyl analogue **15** were also examined. These were found to exhibit similar antimicrobial activities as the mono or difluoro compounds, with the strongest activity being against the *Staphylococcus* and *Micrococcus* species. Weak activity was also observed against the Gram-negative microbe *Neiserria gonorrhoeae*; however, lactams **2**–**15** had no inhibitory effect on other Gram-negative bacteria such as *Serratia marcessens*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobactor cloacae*, or *Bacteroides fragalis*. Thus, like the parent non-fluorinated lactam **1**, these fluorinated systems display a very narrow spectrum of activity selective for *Staphylococcus* and *MRSA*. The fluorine atoms on the C_4 aryl ring of *N*-methylthio β-lactam antibacterials do not alter in vitro activity.[7] Experiments are now directed to examining the in vivo properties of these fluorinated β-lactam systems.

Experimental

All reagents needed for the synthesis of the β-lactams were purchased from Sigma-Aldrich Chemical Company and used without further purification. Solvents were obtained from Fisher Scientific Company. Thin-layer chromatography (TLC) was carried out using EM Reagent plates with a fluorescence indicator (SiO_2-60, F-254). Products were purified by flash chromatography using J.T. Baker flash chromatography silica gel (40 µm). NMR spectra were recorded in $CDCl_3$. ^{13}C NMR

Table 1. Growth inhibition zones obtained from agar disk diffusion experiments using 6-mm air-dried disks impregnated with 20 µg of the test compound. The values correspond to the diameters in mm (average of three runs) for the zone of growth inhibition observed after 24 h. Penicillin G (Pen G) is used as a reference antibiotic

Microorganism	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	Pen G
Staphylococcus aureus	25	22	20	23	20	18	26	16	17	20	20	26	23	19	18	33
MRSA USF652	30	27	21	25	24	19	27	19	18	23	23	27	25	18	22	8
MRSA USF653	30	28	24	27	27	21	28	18	18	22	22	28	26	14	20	15
MRSA USF654	26	25	18	22	20	14	26	13	12	19	20	24	22	17	16	10
MRSA USF655	25	23	18	21	20	15	25	13	11	19	19	24	21	17	16	14
MRSA USF656	28	26	19	25	22	19	27	14	12	20	22	26	23	19	18	12
MRSA USF657	27	26	21	25	23	19	26	16	11	20	20	24	22	19	16	12
MRSA USF658	26	25	20	23	20	16	25	18	13	19	19	23	20	17	17	19
MRSA USF659	24	11	18	10	18	17	21	15	9	16	15	21	20	17	15	16
Staphylococcus epidermidis	31	25	25	28	25	21	33	17	20	24	25	31	28	20	20	50
Staphylococcus saprophyticus	22	20	15	21	19	14	23	13	15	19	19	21	20	18	15	30
Staphylococcus simulans	14	25	16	13	15	15	18	14	10	17	17	18	19	18	15	13
Micrococcus luteus	21	25	24	24	25	25	24	25	25	26	25	22	27	25	25	40
Neiserria gonorrhoeae	14	14	13	14	12	12	12	11	12	12	14	12	12	11	12	0

Staphylococcus aureus (ATCC 25923), *MRSA* USF652-659 are β-lactamase-producing strains of methicillin-resistant *Staphylococcus aureus* obtained from Lakeland Regional Medical Center (Lakeland, FL), *Staphylococcus epidermidis* (environmental isolate), *Staphylococcus saprophyticus* (ATCC 3552), *Staphylococcus simulans* (ATCC 11631), *Micrococcus luteus* (environmental isolate), *Neiserria gonorrhoeae* (β-lactamase-producing strain from Tampa Branch State Laboratory). 'nt' indicates 'not tested'.

Table 2. In vitro activities of lactams **2**–**12** against *MSSA* (ATCC 25923) and eight *MRSA* strains

	MSSA (MIC, μg/mL)	*MRSA* (MIC_{90}, μg/mL)
2	10	10
3	10	10
4	10	10
5	10	10
6	15	15
7	5	10
8	10	10
9	5	10
10	15	15
11	15	20
12	5	5

Minimum inhibitory concentration (MIC) values were determined by agar dilution following NCCLS protocols.

spectra were proton broad-band decoupled, but not fluorine broad-band decoupled, and therefore some signals in the spectra are split by J_{C-F}-coupling. β-Lactams **2**–**15** were prepared from the commercially available aldehydes as described in ref 1, and their *cis*-relative stereochemistry confirmed by NMR.[6]

(±)-(3*S*,4*R*)-4-(2-Fluorophenyl)-3-methoxy-1-(methylthio)azetidin-2-one (2)

White crystal; mp 62–65 °C. IR (neat) 1772 cm^{-1} (C=O). ^1H NMR (250 MHz) δ 7.28–6.99 (m, 4H), 5.15 (d, 1H, $J=4.7$ Hz), 4.77 (d, 1H, $J=4.7$ Hz), 3.16 (s, 3H), 2.36 (s, 3H); ^{13}C NMR (63 MHz) δ 170.8, 161.6 (d, app $J_{C-F}=220$ Hz), 130.7, 130.3, 128.2, 124.5, 115.4 (d, app $J_{C-F}=22$ Hz), 86.9, 59.7, 59.0, 22.3.

(±)-(3*S*,4*R*)-4-(3-Fluorophenyl)-3-methoxy-1-(methylthio)azetidin-2-one (3)

White crystal; mp 43–44 °C. IR (neat) 1741 cm^{-1} (C=O). ^1H NMR (250 MHz) δ 7.36 (m, 1H), 7.17–7.07 (m, 3H), 4.85–4.80 (m, 2H), 3.18 (s, 3H), 2.37 (s, 3H); ^{13}C NMR (63 MHz) δ 170.1, 162.6 (d, app $J_{C-F}=248$ Hz), 136.3, 129.9, 124.5, 115.8 (d, app $J_{C-F}=21$ Hz), 115.6 (d, app $J_{C-F}=23$ Hz), 86.5, 65.5, 58.4, 22.0.

(±)-(3*S*,4*R*)-4-(4-Fluorophenyl)-3-methoxy-1-(methylthio)azetidin-2-one (4)

White crystal; mp 46–48 °C. IR (neat) 1767 cm^{-1} (C=O). ^1H NMR (250 MHz) δ 7.54 (dd, 1H, $J=8.1$, 5.7 Hz), 7.23 (t, 2H, $J=8.5$ Hz), 5.00 (app dd, 2H, $J=10.0$, 4.8 Hz), 3.37 (s, 3H), 2.55 (s, 3H); ^{13}C NMR (63 MHz) δ 170.3, 163.0 (d, app $J_{C-F}=249$ Hz), 130.6, 129.1, 115.4 (d, app $J_{C-F}=22$ Hz), 86.4, 65.4, 58.3, 22.1.

(±)-(3*S*,4*R*)-4-(2,3-Difluorophenyl)-3-methoxy-1-(methylthio)azetidin-2-one (5)

Colorless crystal; mp 42–45 °C. IR (neat) 1772 cm^{-1} (C=O). ^1H NMR (250 MHz) δ 7.23–7.09 (m, 3H), 5.24 (d, 1H, $J=4.9$ Hz), 4.88 (d, 1H, $J=4.9$ Hz), 3.29 (s, 3H), 2.46 (s, 3H); ^{13}C NMR (63 MHz) δ 170.0, 124.2–123.4 (overlapping m), 117.3 (d, app $J_{C-F}=17$ Hz), 86.5, 59.0, 58.7, 21.9.

(±)-(3*S*,4*R*)-4-(2,5-Difluorophenyl)-3-methoxy-1-(methylthio)azetidin-2-one (6)

Colorless crystal; mp 57–60 °C. IR (neat) 1757 cm^{-1} (C=O). ^1H NMR (250 MHz) δ 7.09–7.00 (m, 3H), 5.19 (d, 1H, $J=4.9$ Hz), 4.85 (d, 1H, $J=5.0$ Hz), 3.27 (s, 3H), 2.45 (s, 3H); ^{13}C NMR (63 MHz) δ 169.9, 158.5 (d, app $J_{C-F}=243$ Hz), 157.3, (d, app $J_{C-F}=241$ Hz), 122.9 (overlapping m), 117.0–115.9 (overlapping m), 86.5, 59.0, 58.7, 21.8.

(±)-(3*S*,4*R*)-4-(2,6-Difluorophenyl)-3-methoxy-1-(methylthio)azetidin-2-one (7)

Pale-yellow solid; mp 51–53 °C. IR (neat) 1762 cm^{-1} (C=O). ^1H NMR (250 MHz) δ 7.25 (m, 1H), 6.93 (t, 1H, $J=8.8$ Hz), 5.26 (d, 1H, $J=5.0$ Hz), 4.87 (d, 1H, $J=5.0$ Hz), 3.32 (s, 3H), 2.39 (s, 3H); ^{13}C NMR (63 MHz) δ 170.5, 162.7 (dd, app $J_{C-F}=251$, 7 Hz), 131.2 (t, app $J_{C-F}=11$ Hz), 112.5 (d, app $J_{C-F}=22$ Hz), 110.1 (d, app $J_{C-F}=14$ Hz), 86.3, 59.3, 57.6, 22.1.

(±)-(3*S*,4*R*)-4-(3,5-Difluorophenyl)-3-methoxy-1-(methylthio)azetidin-2-one (8)

Colorless crystal; mp 82–83 °C. IR (neat) 1757 cm^{-1} (C=O). ^1H NMR (250 MHz) δ 6.87–6.85 (m, 3H), 4.75 (s, 2H), 3.16 (s, 3H), 2.35 (s, 3H); ^{13}C NMR (63 MHz) δ 170.3, 163.2 (dd, app $J_{C-F}=250$, 13 Hz), 138.3 (t, app $J_{C-F}=9$ Hz), 112.1 (d, app $J_{C-F}=25$ Hz), 104.8 (t, app $J_{C-F}=25$ Hz), 87.0, 65.6, 59.0, 22.5.

(±)-(3*S*,4*R*)-4-(3,4-Difluorophenyl)-3-methoxy-1-(methylthio)azetidin-2-one (9)

Colorless crystal; mp 49–51 °C. IR (neat) 1757 cm^{-1} (C=O). ^1H NMR (250 MHz) δ 7.25–7.14 (m, 3H), 4.80 (app s, 2H), 3.24 (s, 3H), 2.40 (s, 3H); ^{13}C NMR (63 MHz) δ 170.0, 150.9 (m), 130.6, 125.0, 117.5 (m), 86.4, 65.0, 58.5, 22.1.

(±)-(3*S*,4*R*)-3-Methoxy-1-(methylthio)-4-(2,4,5-trifluorophenyl)azetidin-2-one (10)

Yellow solid; mp 56–59 °C. IR (neat) 1767 cm^{-1} (C=O). ^1H NMR (250 MHz) δ 7.17 (m, 1H), 7.02 (td, 1H, $J=9.6$, 6.5 Hz), 5.16 (d, 1H, $J=4.8$ Hz), 4.84 (d, 1H, $J=4.9$ Hz), 3.30 (s, 3H), 2.46 (s, 3H).

(±)-(3*S*,4*R*)-3-Methoxy-1-(methylthio)-4-(2,3,5-trifluorophenyl)azetidin-2-one (11)

Pale-yellow crystal; mp 69–72 °C. IR (neat) 1777 cm^{-1} (C=O). ^1H NMR (250 MHz) δ 6.96 (m, 1H), 6.83 (m, 1H), 5.22 (d, 1H, $J=5.0$ Hz), 4.88 (d, 1H, $J=5.0$ Hz), 3.32 (s, 3H), 2.47 (s, 3H); ^{13}C NMR (63 MHz) δ 169.7, 161.8, 157.7, 151.7, 124.7, 110.8, 105.7, 86.5, 58.8, 58.8, 21.8.

(±)-(3*S*,4*R*)-3-Methoxy-1-(methylthio)-4-(2,3,6-trifluorophenyl)azetidin-2-one (12)

Brown solid; mp 65–66 °C. IR (neat) 1770 cm^{-1} (C=O). ^1H NMR (250 MHz) δ 7.12 (tdd, 1H, $J=9.2$, 3.7, 2.2

Hz), 6.82 (td, 1H, $J=9.2$, 5.0 Hz), 5.17 (d, 1H, $J=5.1$ Hz), 4.81 (d, 1H, $J=5.1$ Hz), 3.29 (s, 3H), 2.34 (s, 3H).

(±)-(3*S*,4*R*)-3-Methoxy-1-(methylthio)-4-(2,3,4-trifluorophenyl)azetidin-2-one (13)

Oil. IR (neat) 1767 cm^{-1} (C=O). ^1H NMR (250 MHz) δ 7.09–7.04 (m, 2H), 5.16 (d, 1H, $J=4.9$ Hz), 4.84 (d, 1H, $J=4.9$ Hz), 3.32 (s, 3H), 2.46 (s, 3H).

(±)-(3*S*,4*R*)-3-Methoxy-1-(methylthio)-4-(3,4,5-trifluorophenyl)azetidin-2-one (14)

White solid; mp 93–96 °C. IR (neat) 1762 cm^{-1} (C=O). ^1H NMR (250 MHz) δ 7.05 (dd, 2H, $J=7.4$, 6.9 Hz), 4.82 (m, 2H), 3.28 (s, 3H), 2.43 (s, 3H).

(±)-(3*S*,4*R*)-3-Methoxy-1-(methylthio)-4-(pentafluorophenyl)azetidin-2-one (15)

White solid; mp 72–74 °C. IR (neat) 1762 cm^{-1} (C=O). ^1H NMR (250 MHz) δ 5.15 (d, 1H, $J=5.1$ Hz), 4.81 (d, 1H, $J=5.1$ Hz), 3.35 (s, 3H), 2.35 (s, 3H).

Testing of antimicrobial susceptibilities (Kirby–Bauer disk diffusion)

Staphylococcus aureus (ATCC 25923), *Escherichia coli* (ATCC 23590), *Serratia marcescens* (ATCC 29634), *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus saprophyticus* (ATCC 3552), *Staphylococcus simulans* (ATCC 11631) were purchased from ATCC sources. *Vibrio cholerae* (biotype E1 Tor Ogawa, cholera toxin positive, CDC E5906) was obtained from the Centers for Disease Control in Atlanta, GA. *Staphylococcus aureus* (methicillin-resistant, β-lactamase-producing, labeled as *MRSA* USF652-659 in Table 1), *Staphylococcus epidermidis*, *Micrococcus luteus*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Salmonella typhimurium* were obtained from Lakeland Regional Medical Center (Lakeland, FL). *Bacteroides fragalis* was obtained from Smith-Kline Laboratories. *Neisseria gonorrhoeae* (β-lactamase positive) was obtained from the Tampa Branch State Laboratory.

Culture preparation. From a freezer stock in tryptic soy broth (Difco Laboratories, Detroit, MI) and 20% glycerol, a culture of each microorganism was transferred with a sterile Dacron swab to Trypticase® Soy Agar (TSA) plates (Becton-Dickinson Laboratories, Cockeysville, MD), streaked for isolation and incubated at 37 °C for 24 h. A 10^8 standardized cell count suspension was then made in sterile phosphate buffered saline (pH 7.2) and swabbed across fresh TSA plates.

Disc preparation. Sterile 6-mm susceptibility discs (Becton-Dickinson Laboratories, Cockeysville, MD) were impregnated with 20 μL of a 1 mg/mL stock solution of the test lactam compound in dimethylsulfoxide (DMSO) and placed onto the inoculated TSA plates. The plates were incubated for 24 h at 37 °C and the antimicrobial susceptibilities were determined by measuring the zones of growth inhibition around each disc.

Determination of minimum inhibitory concentrations

Media preparation. The minimum inhibitory concentration (MIC) values of the lactams were determined for *Staphylococcus aureus* and *MRSA* by agar dilution according to NCCLS protocols.[8] The test medium was prepared in 24-well plates (Costar 3524, Cambridge, MA) by adding a known quantity of the test drug in DMSO to Mueller-Hinton II agar (Becton-Dickinson Laboratories, Cockeysville, MD) to bring the total volume in each well to 1.0 mL. The medium was allowed to solidify at room temperature for 24 h before inoculation with the bacteria. Using a sterilized inoculating loop, a small amount of each standardized *Staphylococcus* strain cultured on TSA plates for 24 h was transferred into sterile test tubes containing 5 mL of TSA broth, and incubated at 37 °C for 24 h. One microliter of each culture was then applied to the appropriate well of Mueller–Hinton agar and incubated at 37 °C overnight. After 24 h, the MIC's were determined as being the lowest concentration of drug where bacterial growth was inhibited. MIC_{90}'s correspond to the MIC for 90% of the *MRSA* strains tested.

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References and Notes

1. Turos, E.; Konaklieva, M. I.; Ren, R. X. F.; Shi, H.; Gonzalez, J.; Dickey, S.; Lim, D. *Tetrahedron* **2000**, *56*, 5571.
2. Turos, E.; Long, T. E.; Konaklieva, M. I.; Coates, C.; Shim, J.-Y.; Dickey, S.; Lim, D. V.; Cannons, A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2229.
3. *Chemistry and Biology of β-Lactam Antibiotics*; Morin, R. B., Gorman, M., Eds.; Academic: New York, 1982; Vols. 1–3.
4. (a) Barbachyn, M. R.; Toops, D. S.; Grega, K. C.; Hendges, S. K.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H.; Buysse, J. M.; Demyan, W. F.; Kilburn, J. O.; Glickman, S. E. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1009. (b) Park, C.-H.; Brittelli, D. R.; Wang, C. L.-J.; March, F. D.; Gregory, W. A.; Wuonola, M. A.; McRipley, R. J.; Eberly, V. S.; Slee, A. M.; Forbes, M. *J. Med. Chem.* **1992**, *35*, 1156.
5. Ren, X.-F.; Konaklieva, M. I.; Shi, H.; Dickey, S.; Lim, D. V.; Gonzalez, J.; Turos, E. *J. Org. Chem.* **1998**, *63*, 8898.
6. *cis*-Disubstituted β-lactams typically display a coupling constant of 4.8–6. 0 Hz for the two vicinal protons on the ring, while for the trans isomer, the value is normally in the range of 1–3 Hz.
7. The suggestion that antibacterial activity of these fluorinated compounds is independent of the degree of C_4 lipophilicity is in line with our recent studies on C_4 saturated side-chain analogues. See Coates, C.; Long, T.E.; Turos, E.; Dickey, S.; Lim, D.V. *Bioorg. Med. Chem.* **2003**, *11*, 193.
8. NCCLS (National Committee for Clinical Laboratory Standards) *Methods for Dilution of Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. NCCLS Document M7-A4, Vol. 17, No. 2, 1997.