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Synthesis and antibacterial activity of novel oxazolidinones with methylene oxygen- and methylene sulfur-linked substituents at C5-position

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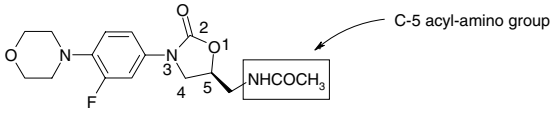
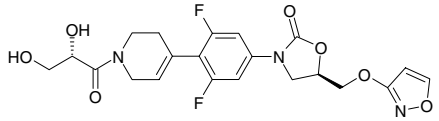
Abstract—Novel oxazolidinone derivatives of the lead compound RBx 8700, containing methylene oxygen- and methylene sulfur-linked substituents at the C5-position, were synthesized. Antibacterial screening of these compounds against a panel of resistant and susceptible Gram-positive and fastidious Gram-negative bacteria gave compounds **2** and **4** as new antibacterial agents.

Oxazolidinone antibacterial agents represented by linezolid[1] (Zyvox™), approved for use in humans in April 2000, emerged as the first totally synthetic, structurally distinct, and mechanistically novel class of antibacterial agents since the discovery of trimethoprim in 1968. Linezolid is active against Gram-positive pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), vancomycin-resistant *Enterococcus faecium* (VRE), *Streptococcus pneumoniae* (S.pn), and *Streptococcus pyogenes* (S.py), which cause various nosocomial and community-acquired infections, including bacteremia, pneumonia, and skin infections.[2,3] Linezolid exhibits its antibacterial activity by inhibiting bacterial protein synthesis via binding to the 50S ribosomal subunit and interfering with the fMet-tRNA binding to the P-site of the ribosomal peptidyltransferase center.[4] The therapy profile of linezolid is less than ideal[5] because it is inactive against Gram-negative bacilli, requires a twice daily dosing regimen, and may cause bone marrow toxicity on usage of more than two weeks. Thus, an oxazolidinone antibiotic with a broader antibacterial spectrum, and a better pharmacokinetic and safety profile than linezolid, may be a useful addition in the armamentarium of antibiotics for treating infections.



Linezolid (Zyvox™, Pharmacia/Pfizer)

Early SAR emerging from work of Barbachyn et al. on oxazolidinones indicated that the C5-acylamino group was essential for good activity.[6] However, it has now been shown that compounds bearing thio-amides, thio-ureas, halogen-substituted methyl-amides, ureas, *N*-carbamates, etc. have superior or similar activity to linezolid,[5,7–9] and indeed a radical modification involving the introduction of O-linked and N-linked heterocycles at the C5-position led to the discovery of a clinical compound **AZD-2563**.[10–12] The replacement of the C5 *N*-acetamido group with thiocarbonyl functional groups has provided compounds with enhanced spectra of antibacterial activity such as activity against fastidious Gram-negative bacteria *Haemophilus influenzae* and *Moraxella catarrhalis*.[5]



AZD 2563

From our in-house oxazolidinone discovery program, we previously obtained RBx 8700 (**1**) as a potent

Keywords: Antibacterial; Oxazolidinone; RBx 8700; Nitro-thiophene.
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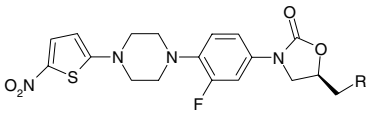
Table 1. Synthesized compounds **2**–**21**



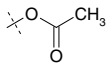
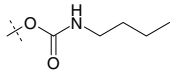
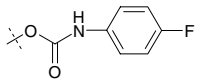
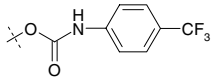
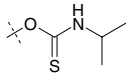
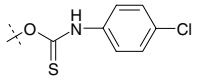
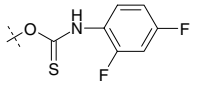
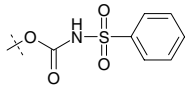
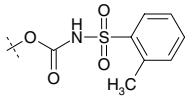
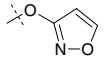
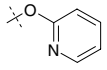
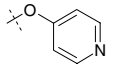
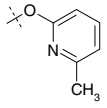
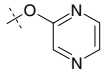
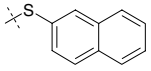
Compound[a]	R	%Yield[b]
2	–OH	55
3	$-OCH_3$	32
4		40
5		40
6		84
7		59
8		42
9		40
10		37
11		65
12		62
13		13
14		27
15		41
16		47
17		48
18		22
19		57

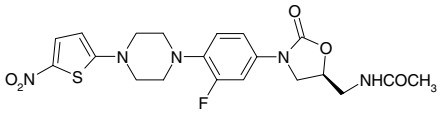
Table 1 (*continued*)

Compound[a]	R	%Yield[b]
20		22
21		45

[a] The compounds were characterized by 400 MHz NMR (Bruker) spectroscopy and Mass spectroscopy (ESI, Micro mass ZQ2000). The qualitative purity of the compounds was $\geqslant$85% as observed by NMR spectroscopy.

[b] %Yield is the chemical yield in the final step of the synthetic sequence.

antibacterial compound active against Gram-positive bacteria and mycobacteria, and modestly active against fastidious Gram-negative bacteria such as *H. influenzae* and *M. catarrhalis*.[13–16] With the opportunities offered by C5-substitution in mind, we wished to explore the effect of various methylene oxygen- and methylene sulfur-linked substituents at the C5-position of RBx 8700. As shown in Table 1, various O-linked carbamates and thiocarbamates (**5**–**12**), O-linked heterocycles (**13** and **14**, **16**–**18**), and S-linked alkyl and aryl derivatives (**19**–**21**) were synthesized.[17]

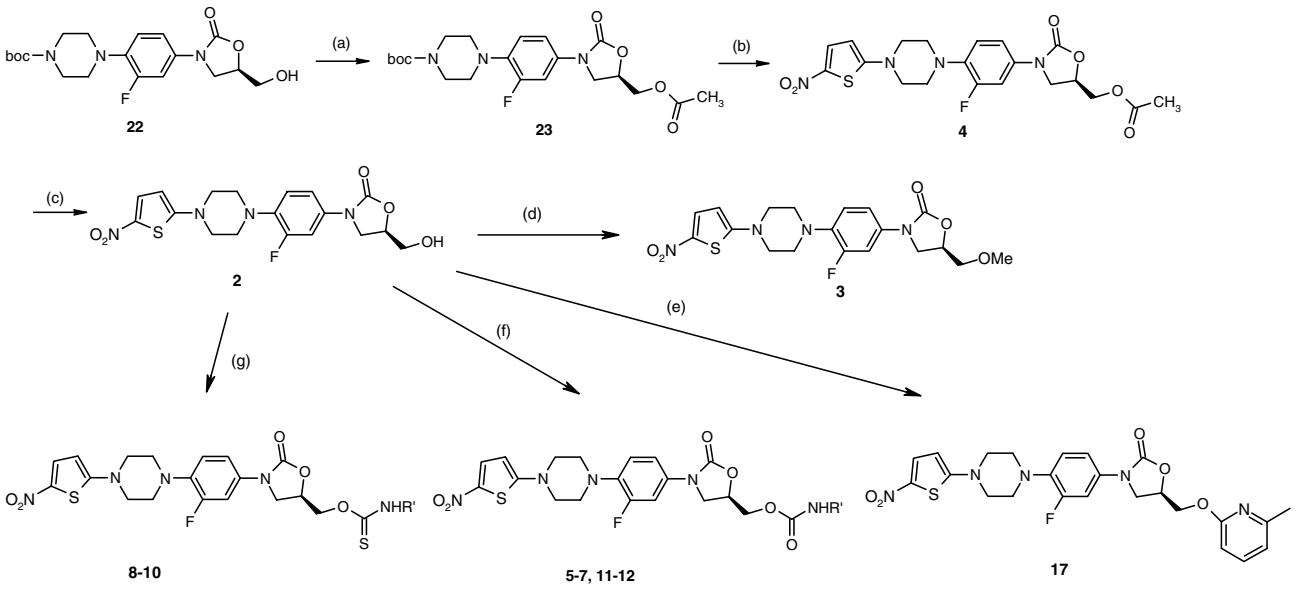


1 (RBx 8700)

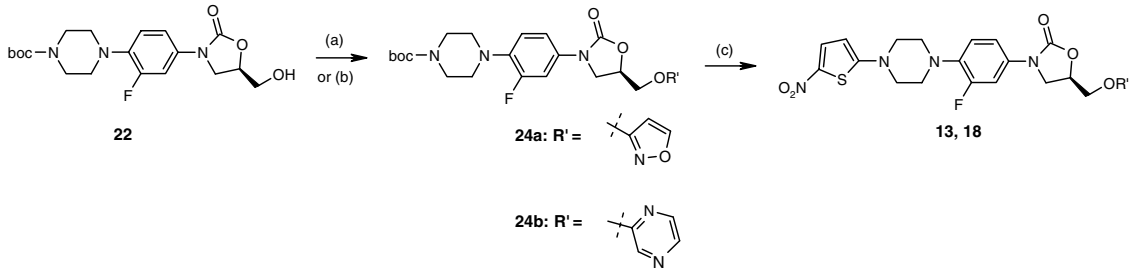
For the synthesis of the target compounds, the oxazolidinone-methanol derivative **22**[18] was acylated with acetic anhydride in pyridine to give compound **23** (Scheme 1). Boc-deprotection, followed by aromatic substitution of 2-bromo-5-nitro-thiophene, led to compound **4**. Hydrolysis of the acetate group of **4** by heating in 3 N HCl produced compound **2**. The alcohol **2** was alkylated with methyliodide using sodium hydride as base to give **3**. Mitsunobu reaction of **2** with 2-hydroxy-6-methylpyridine led to the ether-linked derivative **17**. Treatment of **2** with sodium hydride in THF at 0 °C followed by substituted isocyanates produced the carbamate derivatives **5**–**7** and **11** and **12**. Similarly, the thiocarbamate derivatives **8**–**10** were obtained by treatment of compound **2** with the corresponding isothiocyanates.

The synthesis of compounds **13** and **18** is given in Scheme 2. Mitsunobu reaction of the alcohol **22** with 3-hydroxyisoxazole produced the ether-linked heterocyclic intermediate **24a**. Heating **22** with sodium hydride and 2-chloropyrazine led to the ether-linked intermediate **24b**. Boc-deprotection of **24a**,**b** followed by aromatic substitution as described earlier led to compounds **13** and **18**.[19]

For the synthesis of compounds **14**–**16** (Scheme 3), the alcohol **22** was converted to the methanesulfonate derivative **25**, which on heating with sodium hydride and 2-pyridone gave the ether-inked intermediate **26a**



Scheme 1. Reagents and conditions: (a) acetic anhydride, pyridine, rt, 17 h; (b) 0.3 N HCl in ethanol, 0 °C → rt, 2 h; 2-bromo-5-nitro-thiophene, *N*-ethyldiisopropyl amine, acetonitrile, 60 °C, 30 h; (c) 3 N HCl, 100 °C, 2 h; (d) MeI, NaH, THF, 0 °C → rt, 55 h; (e) 2-hydroxy-6-methylpyridine, DEAD, PPh_3, THF, rt 17 h; (f) NaH, THF, 0 °C, 10 min, then suitably substituted isocyanates, rt, 25 h (for **5**), 9 h (for **6** and **7**), 1 h (for **11** and **12**); (g) NaH, THF, 0 °C, 10 min, then suitably substituted isothiocyanates, rt, 1 h (for **8**), 17 h (for **10**), 12 h (for **9**).



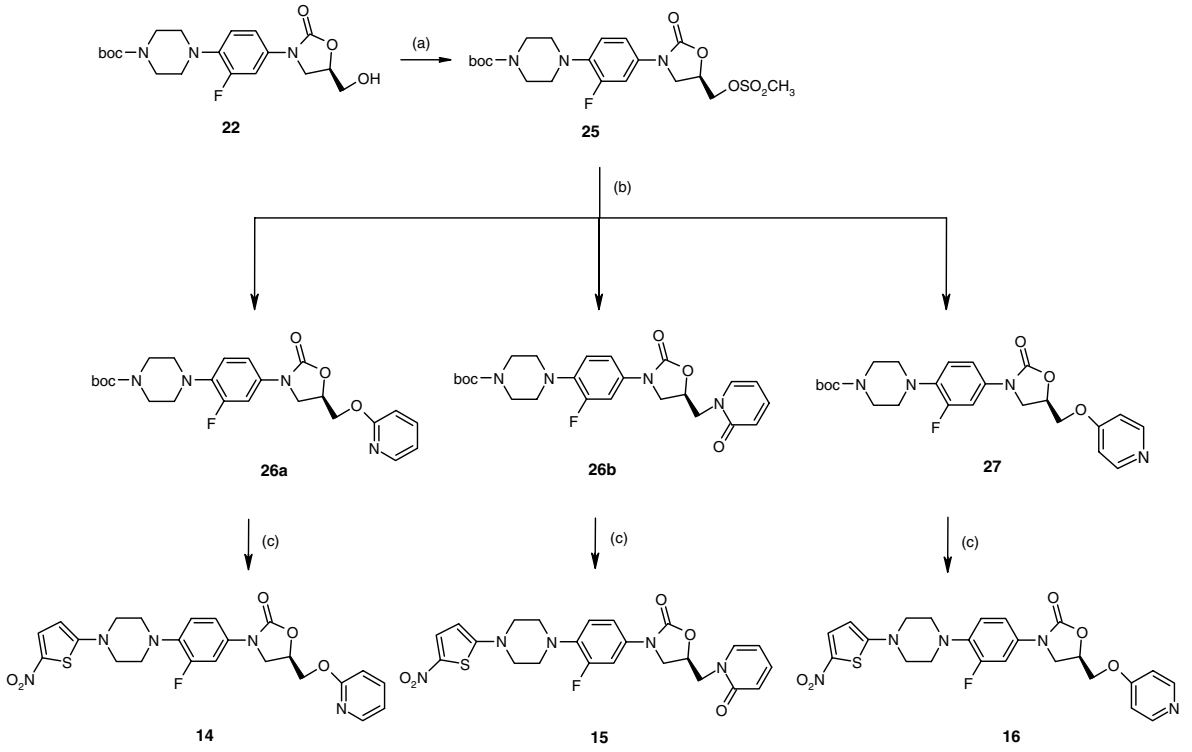
Scheme 2. Reagents and conditions: (a) compound **24a**: 3-hydroxyisoxazole, DIAD, PPh_3, THF, rt, 18 h; (b) compound **24b**: NaH, DMF, 2-chloropyrazine, 80 °C, 4 h; (c) TFA, dichloromethane, 0 °C → rt, 2 h; 2-bromo-5-nitro-thiophene, *N*-ethyldiisopropylamine, acetonitrile, 60 °C, 17–24 h.

along with the *N*-alkylated product **26b**. Similarly, on using 4-hydroxypyridine the intermediate **27** was obtained. Compounds **26a**,**b** were differentiated by IR: compound **26b** showed a strong conjugated amide peak at 1661 cm^{-1}. The intermediates **26a**,**b** and **27** were converted to the compounds **14**–**16** by methods described earlier.

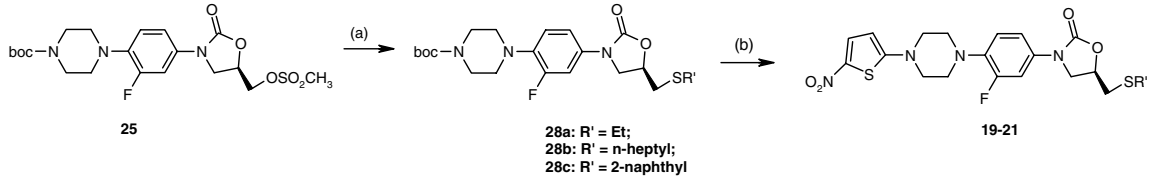
The sulfur-linked compounds **19**–**21** were prepared by the methods shown in Scheme 4. The methanesulfonate derivative **25** was converted to the thio-ether compounds **28a**–**c** by heating with substituted thiols. Transformation of the intermediates **28a**–**c** to compounds **19**–**21** was carried out by the methods described earlier.

Compounds **1**–**21** were screened for their antibacterial activity against *S. pneumoniae*, *S. aureus*, *Enterococcus faecalis*, *E. facium*, and *H. influenzae* using an agar diffusion assay.[20] Compounds **1**, **2**, **4**, and **12** showed comparable or better zone of inhibition sizes than the standards linezolid and vancomycin, and so their minimum inhibitory concentrations (MIC)[21] were determined as per NCCLS guidelines (Table 2). The potencies of the hydroxyl compound **2** and its acetate derivative **4** were comparable to RBx 8700 against Staphylococci and Enterococci, and superior to linezolid. The *o*-tosyl derivative **12** was weakly active. The in vivo activity of compound **2** against MRSA 33 was weaker than RBx 8700. The in vivo activity of compound **4** was not evaluated because its in vitro activity against fastidious Gram-negative organisms was weaker than RBx 8700.

In general, with the exception of compounds **2** and **4**, the compounds in Table 1 containing methylene oxygen-linked substituents and isosteric methylene sulfur-linked substituent, were weaker in antibacterial activity than the C5-acetamido analog RBx 8700. The calculated log *P* value[22] of RBx 8700 was 2.82 whilst that of linezolid was 0.43. The calculated log *P* values of AZD 2563 and its direct congener compound **13** were 1.60 and 4.36, respectively. Indeed, the calculated log *P* values of compounds **2**–**21** were found to be in the range 3.16–7.84, with many being toward the higher side (>5). Tokuyama et al. have



Scheme 3. Reagents and conditions: (a) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C, 30 min; (b) 2-pyridone (for **26a**,**b**), 4-hydroxypyridine (for **27**), NaH (60% w/w), DMF, 80 °C, 2 h; (c) 20% TFA/CH_2Cl_2; 2-bromo-5-nitro-thiophene, *N*-ethyldiisopropylamine, acetonitrile, 60 °C, 17–24 h.



Scheme 4. Reagents and conditions: (a) NaH (60% w/w), R′SH, DMF, 55 °C, 5 h (for **28a**), 6 h (for **28b**); 80 °C, 15 min (for **28c**); (b) 2-bromo-5-nitro-thiophene, *N*-ethyldiisopropylamine, acetonitrile, 60 °C, 17 h (for **19**, **20**), 2 h (for **21**).

reported[9] that a log *P* of −1 to +2 is favored for strong in vitro MRSA and VRE activity in a series of thiocarbonyl oxazolidinones. However, other authors have reported no direct correlation between log *P* and MIC values[23] and that activities were more dependent on the appendages at the *para* position of the phenyl ring.[23,24] In our case,

Table 2. In vitro and In vivo activity

Microorganisms[a]	Minimum inhibitory concentrations (MIC) μg/mL					
	Compound **2**	Compound **4**	Compound **12**	Compound **1** (RBx 8700)	Linezolid	Vancomycin
S.au 25923	0.5	0.5	16	0.25	2	1
S.au 15187	0.5	0.5	16	0.25	2	0.5
MRSA 562	0.5	0.5	16	0.25	2	0.5
MRSA 33	0.5	0.5	16	0.25	2	0.5
E.fa 29212	0.5	0.5	8	0.25	2	4
VRE 6A	0.5	1	1	0.25	2	>16
S.py 19615	0.5	0.25	0.5	0.125	2	0.5
S.pn 6303	1	1	1	0.125	2	0.5
M.cat M2	2	>16	>8	0.25	>4	>16
H.flu 49247	8	>16	>16	8	4	>64
ED_{50} (MRSA 33) mg/kg po	>25	ND	ND	11.15	5.6	ND

[a] Microorganisms: S.au 25923, *Staphylococcus aureus* ATCC 25923; S.au 15187, *Staphylococcus aureus* 15187; MRSA 562, methicillin-resistant *Staphylococcus aureus* 562; MRSA 33, methicillin-resistant *Staphylococcus aureus* 33; E.fa 29212, *Enterococcus faecalis* ATCC 29212; VRE 6A, vancomycin-resistant *Enterococcus faecium* 6A; S.py 19615, *Streptococcus pyogenes* ATCC 19615; S.pn 6303, *Streptococcus pneumoniae* ATCC 6303; M.cat M2, *Moraxella catarrhalis* M2; H.flu 49247, *Haemophilus influenzae* ATCC 49247.

compounds **2** and **4** with calculated log *P* values of 3.16 and 4.05, respectively, were active in vitro, whereas compounds **3**, **13**, and **15** with log *P* values 3.79, 4.36, and 3.68, respectively, were less active. Hence, a direct correlation between MIC values and physicochemical properties could not be made.

C5 substituted *N*-carbamates in the tetrahydroquinoline series of oxazolidinones are reported to have good antibacterial activities against *S. aureus* and *S. pneumoniae* strains.[5] In a tricyclic imidazolyl-oxazolidinone series *N*-thiocarbamates have 4- to 8-fold more antibacterial activities than *N*-carbamates.[5] The *N*-thiocarbamate analogs of thiomorpholine-phenyl oxazolidinones also have potent antibacterial activities against Staphylococci and Enterococccci.[9] In our series of compounds, reversing the *N*-carbamates and *N*-thiocarbamates to *O*-carbamates and *O*-thiocarbamates (compounds **5**–**12**) led to weaker antibacterial compounds. These compounds have bulky hydrophobic groups on the *O*-carbamates and *O*-thiocarbamates and so steric constraints may be a limiting factor to their biological activities. RBx 8700 and compound **2** are the most potent compounds in this series, both containing hydrogen bond donor substituents at C5-position, which too may be an important feature in this series of compounds.

In conclusion, in the RBx 8700 series of compounds, which contain a thienyl-piperazine group, only small O-linked groups can be tolerated at the C5-position, and SAR is more closely aligned with that of linezolid than that of other O-linked compounds.[10]

Acknowledgments

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19. Analytical data of compound **18**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.28 (s, 1H, pyrazinyl-*H*), 8.21 (d, 1H, J = 2.4 Hz, pyrazinyl-*H*), 8.09 (d, 1H, pyrazinyl-*H*), 7.81 (d, 1H, J = 4.8 Hz, thienyl-*H*), 7.51 (dd, 1H, J = 14.1 Hz, 1.8 Hz, phenyl-*H*), 7.18 (d, 1H, J = 7.8 Hz, phenyl-*H*), 6.97 (t, 1H, J = 9 Hz, phenyl-*H*), 6.02 (d, 1H, J = 4.8 Hz thienyl-*H*), 5.06 (m, 1H, oxazolidinyl C_5-*H*), 4.62 (m, 2H, –CH_2–O), 4.17 (t, 1H, oxazolidinyl C_4-*H*), 3.55 (m, 4H, piperazinyl-*H*), 3.22 (m, 4H, piperazinyl-*H*); Mass *m*/*z* (rel. int.): 501.3 (100%, M^++H), 523 (45%, M^++Na), 455.1 (25%, M^+-NO_2).
20. Agar diffusion assay was performed against fastidious and facultative bacteria. For fastidious bacteria, *S. pneumoniae*—Mueller Hinton agar with 5% sheep blood was used and for *Haemophilus*—*Haemophilus* test medium with supplements was used. For facultative bacteria, *S. aureus* and Enterococci—Mueller Hinton agar was used. Standard antibiotics used were linezolid and vancomycin. Bacterial cultures (500 μL of 0.5–0.8 Mc Farland per 50 mL of molten agar) were added into agar plates. Plates were allowed to settle. Wells of 6 mm size were punched into the agar. Compounds and standard antibiotics were subjected to serial 2-fold dilutions in DMSO and sterile distilled water, respectively. Fifty microliters of each dilution was added into the wells. The plates were incubated at 37 °C for 18–24 h. For fastidious organisms CO_2 incubator was used for incubation. Zone of inhibition sizes were measured using vernier caliper.
21. MIC's were determined by agar dilution method (NCCLS) using doubling dilutions in Mueller Hinton agar. Stock solutions of the compounds and standard antibiotics were prepared (1 mg/mL) in DMSO and respective solvents, respectively. Serial 2-fold dilutions were prepared in respective diluents to get a concentration range of 16–0.015 μg/mL. Two hundred and ninety microliters of respective drug dilution was added in 20 mL of molten agar to get the required concentration range. Direct colony suspensions of bacterial cultures were made in saline and were adjusted to 0.5 McFarland turbidity standard. The inoculum was diluted 10-fold in normal saline. Thirty cultures were replicated

onto the drug containing agar plates using a replicator. Plates were allowed to dry and incubated in ambient air at 35 °C for 18–24 h. For fastidious organisms the plates were incubated in 5% CO_2 atmosphere at 35 °C for 18–24 h.

22. log *P* values were calculated using Advanced Chemistry Development Software V 9.14.
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