Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 35–41

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Antibacterial Activity of 5-Substituted Oxazolidinones

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Received 10 June 2002; accepted 20 August 2002

Abstract—A series of 5-substituted oxazolidinones with varying substitution at the 5-position of the oxazolidinone ring were synthesized and their in vitro antibacterial activity was evaluated. The compounds demonstrated potent to weak antibacterial activity. A novel compound (**PH-027**) demonstrated potent antibacterial activity, which is comparable to or better than those of linezolid and vancomycin against antibiotic-susceptible standard and clinically isolated resistant strains of gram-positive bacteria. Although the presence of the C-5-acetamidomethyl functionality at the C-5 position of the oxazolidinones has been widely claimed and reported as a structural requirement for optimal antimicrobial activity in the oxazolidinone class of compounds, our results from this work identified the C-5 triazole substitution as a new structural alternative for potent antibacterial activity in the oxazolidinone class.

Introduction

The rising prevalence of multi-drug resistant gram-positive bacteria continues to provide impetus for the search and discovery of novel antimicrobial agents active against these pathogens. Oxazolidinones are a novel class of totally synthetic antimicrobial agents active against gram-positive pathogenic bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP) and vancomycin-resistant *Enterococcus faecium* (VREF).[1,2] Linezolid (ZYVOX®, **1**; Chart 1) is the first member of this class recently licensed in the USA and Europe, and available in both iv and oral formulations. Linezolid is efficacious in treating skin and soft tissue infections, pneumonia and bacteremia; and is particularly effective against infections caused by MRSA, PRSP and VREF.[3]

Oxazolidinones are antibacterial agents that act primarily against a wide spectrum of gram-positive and anaerobic bacteria by inhibiting protein synthesis.[1] Recent studies have indicated that the oxazolidinones bind to 50S subunits, of the 70S ribosome, but not to 30S subunits; thus inhibiting bacterial translation at the initiation phase of protein synthesis.[4,5] Linezolid has been shown to inhibit the binding of *N*-formylmethionine-transfer-RNA (fMet-tRNA) to 70S ribosomes.[6] Since the oxazolidinones have a unique mode of action,[5] by inhibiting protein synthesis early in translation, cross-resistance with other antimicrobial agents is believed to be unlikely. However, the potential for the development of resistance cannot be ignored. Already resistance to linezolid has been reported in clinical isolates of VREF[7] and *S. aureus*[8] in the USA. The potential for resistance developments, warrants investigation of newer and more effective antimicrobial agents.

Concerning the C-5 substituent on the oxazolidinone ring, Dupont[9] and Pharmacia[10] groups concluded that the acetylaminomethyl moiety was the best substituent at this position of the oxazolidinone for optimal antibacterial activity. However, recent studies have reported the introduction of substituents such as the 5-thiourea **2a** and 5-thiocarbamate[11] **2b**, 5-dithiocarbamate[12] **2c** and nitrogen substituted heterocyclic aryl moieties[13,14] in **3** as potential replacements for the acetamidomethyl side chain at the C-5 position (Chart 1).

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PII: S0968-0896(02)00423-6

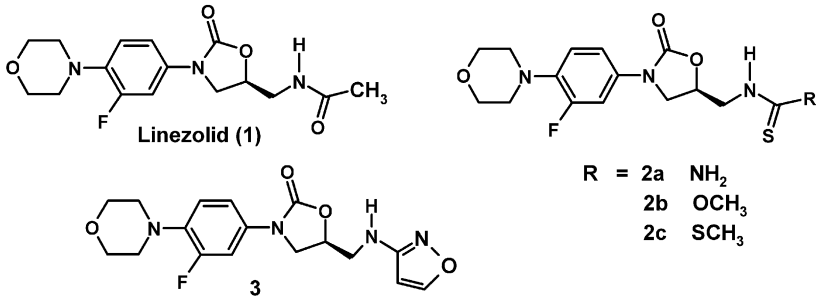


Chart 1. Structures of oxazolidinone antibacterial agents.

In this paper, we describe the synthesis and antibacterial activity of a series of oxazolidinone derivatives bearing the five-membered ring nitrogen heterocycles (triazolyl and imidazolyl moieties) and the hydroxamic acid functionality at the C-5 position.

Chemistry

The key intermediates, 5-methanesulfonate oxazolidinone **6** and 5-azide oxazolidinone **7** were prepared from **4** and **5** according to the usual method.[10,15] Further chemical transformation of the azide intermediate **7** by condensation with 3-butyn-2-one in dimethoxyethane (DME) gave 4-acetyltriazolyl and 5-acetyltriazolyl oxazolidinones **8** and **9**, respectively. Reaction of **8** with methylhydroxylamine hydrochloride gave **10** as mixture of the *E* and *Z* isomers. Treatment of **7** with acetylene afforded the 5-triazole oxazolidinone **PH-027** (Scheme 1). The disubstituted triazole derivative **11** was prepared by treatment of **7** with dimethyl acetylenedicarboxylate, while basic hydrolysis of the ester functionalities gave the disodium salt **12**. The reaction of intermediate **6** with sodium hydride treated imidazole in DMF afforded the imidazole derivative **13**. Treatment of **6** with *tert*-butyl *N*-(*tert*-butoxycarbonyloxy)carbamate and sodium hydride in DMF gave **14**, which was deprotected with trifluoroacetic acid in DCM to give the *N*-hydroxylamine **15**. Diacetylation of the hydroxylamine **15** with acetic anhydride in the presence of triethylamine to give **16**, which was followed by basic hydrolysis of the ester to give the hydroxamic acid derivative **17** (Scheme 2).

Results and Discussion

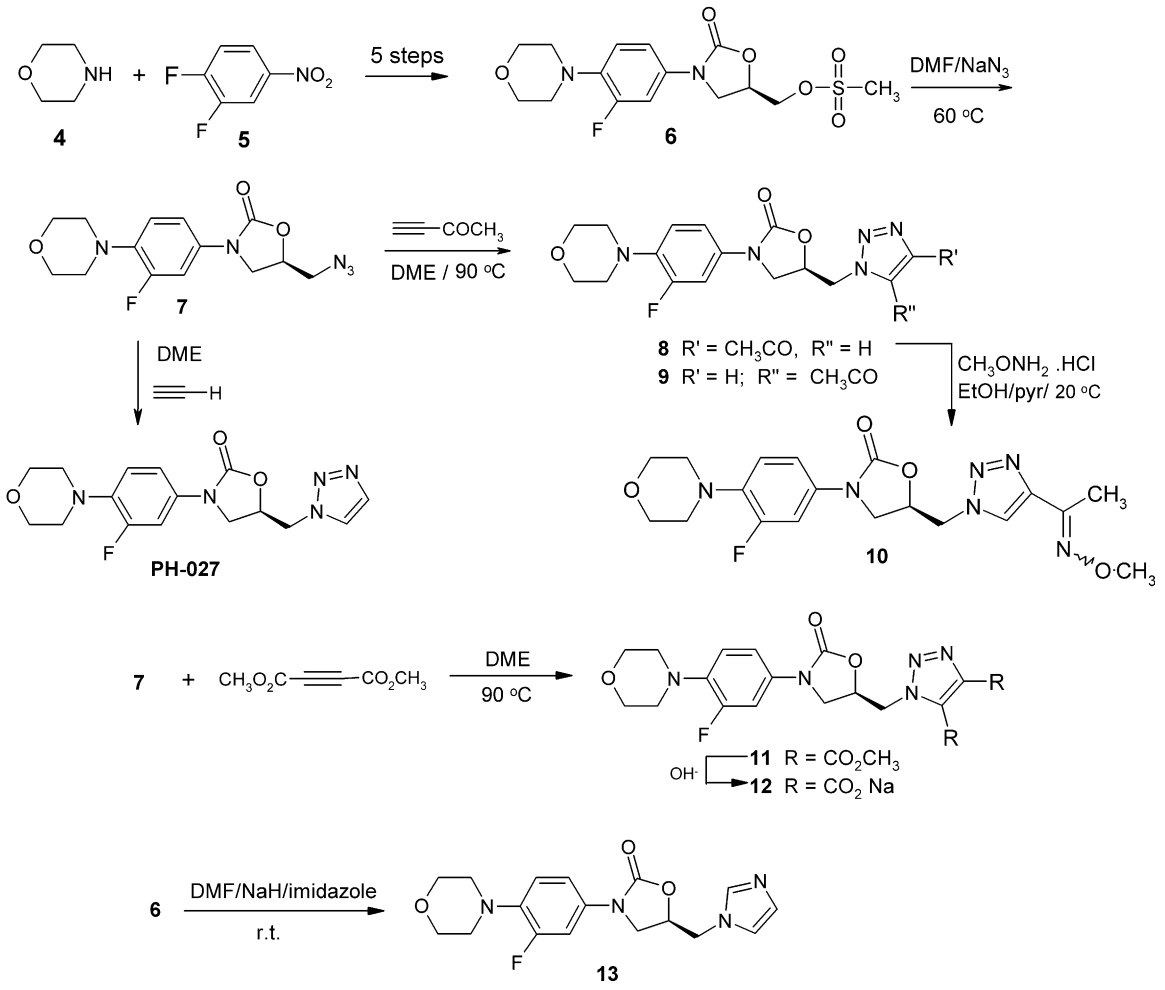
Chemistry

The reaction of the 5-azide oxazolidinone **7** with 3-butyn-2-one yielded the 4-acetyltriazolyl oxazolidinone **8** ($R' = -CH_3CO$, $R'' = H$) and 5-acetyltriazolyl oxazolidinone **9** ($R' = H$, $R'' = -CH_3CO$; Scheme 1) respectively in approximately 10:1 ratio as observed in the ^1H NMR spectrum. The structural assignment to these two positional isomers was accomplished by nuclear Overhauser enhancement (NOE) studies. Irradiation of the triazole C-5′ proton signal (8.80 ppm) of **8** resulted in about 12% enhancement of the methylene protons signal ($-CH_2-$, 4.88 ppm) bonded to the C-5 position of the oxazolidinone ring as observed in the NMR spectra and vice versa. This observed NOE indicated the closer proximity of the triazole C-5′ proton to the methylene protons (4.88 ppm) in **8**, compared to the lack of NOE observed for **9** (ca. 5.10 ppm).

Antimicrobial activity

All newly synthesized oxazolidinone derivatives were tested for antibacterial activity against both standard and clinically isolated strains of gram-negative and gram-positive pathogenic bacteria. Initially, susceptibility testing was carried out by measuring the inhibitory zone diameters on Mueller-Hinton (MH) agar, with conventional paper disk containing 30 μg of each compound; and the inhibitory zone diameters were read and rounded up to the nearest whole numbers (mm) for analysis. From the inhibition zone diameter data analysis, the 5-triazole oxazolidinone compound **PH-027** was identified as the most active compound with an inhibitory zone diameter of 39 mm for *S. aureus* ATCC 25923, MRSA, and methicillin-sensitive *Staphylococci epidermidis* ATCC 12228 (coagulase-negative staphylococci) strain. The 4-acetyltriazole derivative **8** and the hydroxamic acid derivative **17** demonstrated moderate activity with inhibition zone diameter of 19 mm for antibiotic-susceptible strains of *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228; and clinical isolates of MRSA, methicillin-sensitive *S. aureus* (MSSA), methicillin-resistance coagulase-negative staphylococci (MR-CNS) and methicillin-sensitive coagulase-negative staphylococci (MS-CNS). The other related compounds in this study demonstrated only weak antibacterial activity. Furthermore, none of the compounds evaluated in this class were active against gram-negative bacteria strains including *Escherichia coli* (*E. coli* ATCC 25922), *Haemophilus influenzae* (*H. influenzae* ATCC 49247) and *Pseudomonas aeruginosa* (*P. aeruginosa* ATCC 27853), with inhibitory zone diameter of <17 mm. The reported inhibitory zone diameters for linezolid **1** (Chart 1) of ≥ 21 mm for susceptible and ≤ 17 mm for resistance[16] were referred to as standard reference values in this study.

The minimum inhibitory concentrations (MIC's, μg/mL) of compounds **8**, **10**, **15**, **17** and **PH-027** in comparison to those of linezolid and vancomycin against antibiotic-susceptible standard and clinically isolated resistant strains of gram-positive bacteria were determined. The results are presented in Tables 1 and 2. Amongst all the compounds tested, the triazole oxazo-

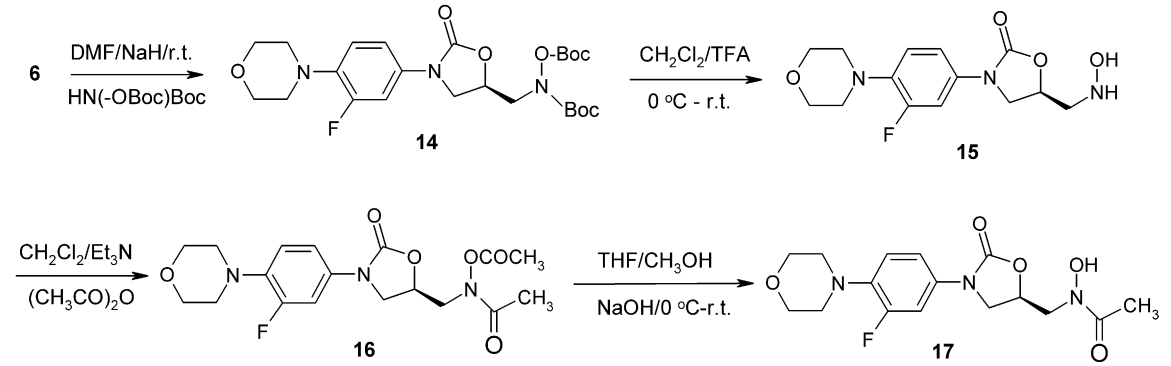


Scheme 1. Synthesis of 5-triazolyl and 5-imidazolyl oxazolidinone derivatives.

lidinone **PH-027**, demonstrated the most potent antibacterial activity against both sensitive and resistant gram-positive bacteria strains, including MRSA, MSSA, MS-CNS, MR-CNS, PRSP and enterococci (*Enterococcus faecium* and *Enterococcus faecalis*). None of the compounds showed activity against gram-negative bacteria including *E. coli*, *H. influenzae* and *P. aeruginosa* with MIC's of $>32\,\mu g/mL$.

Against some of the gram-positive bacteria strains tested, the triazole oxazolidinone **PH-027** demonstrated MIC values comparable to or 1- to 2-fold lower than those of linezolid and vancomycin (Table 1). In particular, the MIC values of **PH-027** against vancomycin-susceptible *E. faecium* (VSE), vancomycin-intermediate resistant *E. faecalis* (VIRE) and vancomycin-resistant *E. faecalis* (VRE) were 0.5, 0.5 and $2\,\mu g/mL$, respectively, compared to an MIC of $2\,\mu g/mL$ demonstrated by linezolid against these strains. However, the MIC's of vancomycin against the same strains were 4, 8 and $>32\,\mu g/mL$, respectively.

Attempts were made to correlate the antibacterial activities of these compounds to the calculated log of



Scheme 2. Synthesis of hydroxamic acid oxazolidinone derivatives.

Table 1. Spectrum of activity against susceptible and resistant gram-positive clinical isolates

Organism	Strain no.	MIC (μg/mL) for						
		8	10	15	17	PH-027	Lin	Van
S. aureus	ATCC25923	>32	>32	>32	>32	1	2	2
S. aureus	ATCC25913	32	>32	>32	>32	1	1	1
MRSA	1669	32	>32	>32	16	2	1	2
MRSA	3014	32	16	32	32	0.5	0.5	1
MSSA	249189	32	>32	>32	16	0.5	2	2
MSSA	1191918	16	>32	>32	32	1	2	2
S. epidermidis	ATCC12228	8	32	>32	16	0.5	2	2
S. epidermidis[a]	1777365	32	>32	>32	16	1	1	2
S. haemolyticus	8186366	32	>32	>32	>32	0.5	0.5	2
S. pneumoniae	ATCC49619	>32	>32	>32	>32	2	1	1
S. pneumoniae[b]	99.388	>32	>32	>32	>32	0.5	1	1
E. faecalis	ATCC29212	16	32	>32	>32	1	2	2
E. faecium (VSE)	1	>32	>32	>32	>32	0.5	2	4
E. faecalis (VIRE)	7	>32	>32	>32	>32	0.5	2	8
E. faecalis (VRE)	ENT 90	>32	>32	>32	>32	2	2	>32
E. faecalis (VRE)	ENT 11U	>32	>32	>32	>32	2	2	>32

[a]Methicillin resistant strain (MR-CNS).
[b]Penicillin resistant strain (PRSP).

partition coefficient (Clog P).[17] However, no direct correlation could be established between the Clog P and antibacterial activity (Table 2). The Clog P values of compounds **8** (0.78) and **9** (0.78) were identical and were comparable to that of linezolid (Clog P = 0.76). However, both of these compounds **8** and **9** demonstrated only weak antibacterial activity with their MIC values in the range 8–>32 μg/mL against both the sensitive control and clinically resistant bacteria strains. Although, studies from other laboratories have reported that compounds in the 5-thiocarbonyl and 5-thiocarbamate oxazolidinone series with Clog P values within a favorable range of −1 to +2 show strong in vitro antibacterial activity against gram-positive bacteria including MRSA and VRE;[11,12] a similar correlation could not be established in this series of 5-heterocycle and hydroxamic acid substituted oxazolidinones. It becomes apparent that the criteria relating to favorable Clog P value range may not be the sole predicting factor for antibacterial activity; since most of the compounds reported in the present study showed Clog P values (0.5-1.31) within the reported optimal range.[12]

From our study, mono- and di-substitution on the triazole C-4 and C-5 positions as found in **8**, **9**, **10**, **11** and **12** resulted in complete loss of antibacterial activity against both gram-negative and gram-positive bacteria irrespective of their Clog P values. This observation may suggest the negating effects of the bulky group(s) on antibacterial activity, which may result from increased steric effects at these positions, due to substitutions on the triazole moiety. Previous studies[9,18] have suggested that the oxazolidinone-binding site is very sensitive to steric environment about the 5-position of the oxazolidinone. Furthermore, the replacement of the triazole moiety in **PH-027** with Clog P = 0.89, for imidazole as in **13** (Clog P = 1.26) also resulted in complete loss of antibacterial activity. The imidazole oxazolidinone, compound **13** demonstrated an inhibitory zone diameter of <17 mm and MIC of >32 μg/mL against all the bacteria strains tested. The superior antibacterial activity of **PH-027** may be interpreted as an indication of a stronger intrinsic 'binding attraction' for the triazole-oxazolidinone at the site of action.

The hydroxamic acid oxazolidinone derivative **17** with Clog P value of 1.31 demonstrated only minimal antibacterial activity with inhibition zone diameter of 18 mm against *S. aureus*, and coagulase-negative staphylococci strains. Compound **17** demonstrated an MIC range of 16–>32 μg/mL against all sensitive and resistant clinically isolated bacteria strains tested in this study. This further strengthened previously indicated significance of the amide-hydrogen (N–H) present in linezolid for antibacterial activity,[18] compared to the hydroxamic acid-hydrogen (N–OH) present in **17**. The replacement of the amide hydrogen (NH) by methyl group (NCH_3)[19] and substitution of the amide functionality for other groups, namely NH_2, OH, and morpholinyl[18] resulted in compounds with weaker antibacterial activity. However, other structural requirements including the presence of the 3-fluoro and 4-morpholinyl groups on the phenyl ring, and the oxazolidinone functionality are highly essential for antibacterial activity of this class of compounds among other factors.[18] In addition, all the intermediates (**14**, **15**, and **16**) leading to **17** were void of antibacterial activity; this probably is expected for **16**, with very high Clog P value of 4.38.

Table 2. Summary of activity against susceptible and resistant gram-positive and gram-negative clinical isolates

Compd	Clog P[a]	MIC's (μg/mL) ranges against							
		MSSA ($n=8$)	MRSA ($n=20$)	MS-CNS ($n=5$)	MR-CNS ($n=5$)	H. influ. ($n=7$)	S. pn. ($n=8$)	VSE ($n=11$)	VRE ($n=6$)
8	0.78	>32	16–32	8–32	32	>32	>32	16–>32	>32
9	0.78	>32	32	>32	>32	>32	>32	>32	>32
10	1.92	>32	8–>32	>32	>32	>32	>32	16–>32	>32
15	0.57	>32	>32	16–>32	16–>32	>32	>32	>32	>32
17	1.31	16–32	16–>32	16–>32	16–>32	>32	>32	>32	>32
PH-027	0.89	0.5–1	0.5–2	1	0.5–1	32	0.5–1	0.5–2	0.5—2
Lin	0.76	2	0.5–2	0.5—1	0.5–1	8	0.5–1	1–2	1—2
Van	n.d.	1–2	0.5–2	1—2	2	>32	0.25–1	1–4	8–>32

[a]ref 17.

Conclusion

In conclusion, 5-triazole, 5-imidazole and 5-hydroxamate oxazolidinone derivatives were synthesized and their antibacterial activities against the antibiotic susceptible standard and clinically isolated resistant gram-positive bacteria including MRSA, MR-CNS, PRSP, and VRE were evaluated. The 5-triazole oxazolidinone **PH-027** demonstrated strong in vitro activity, comparable to or better than those of linezolid and vancomycin. No correlation between activity and lipophilicity (Clog P values) could be established. The 5-imidazole and 5-hydroxamate oxazolidinones were void of antibacterial activity. We have identified the C-5 triazole substitution as a new structural alternative for strong antibacterial activity in the oxazolidinone class. Further work in this area is on going in our laboratories.

Experimental

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The Science Analytical Facilities (SAF), Faculty of Science, Kuwait University, performed all the analyses. Elemental analyses were determined on LECO elemental analyzer CHNS 932 apparatus, and were within ±0.4% of the calculated values. ^{1}H NMR spectra were recorded on Bruker DPX 400 NMR (400 MHz) spectrometer using tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra were measured on a Finnigan MAT INCOS XL mass spectrometer. Infrared (IR) spectra were recorded on Perkin–Elmer System 2000 FT-IR spectrometer. Column chromatography was carried out with silica gel [Kieselgel 60, 70–230 mesh (Aldrich)]. TLC was conducted on 0.25 mm precoated silica gel plates ($60F_{254}$, Merck). All extracted solvents were dried over Na_2SO_4, followed by evaporation in vacuo. The calculated partition coefficient (Clog P) values were determined by using the CS ChemDraw Ultra version 6.01, computer software by CambridgeSoft.Com.[17]

Syntheses

(*R*)-5-{(4-Acetyl-[1,2,3]triazol-1-ylmethyl)-3-(3-fluoro-4-morpholin-4-yl-phenyl)} oxazolidin-2-one (8). A solution of the 5-azidomethyl[10] **7** (2.0 g, 6.2 mmol) in dimethoxyethane (40 mL) was treated with 3-butyn-2-one (0.64 g, 9.3 mmol) and heated at 90 °C overnight for 24 h. The reaction mixture was cooled and concentrated to give a foam 2.50 g. Recrystallization from a dimethoxyethane-ethyl acetate mixture gave the more polar component **8** (1.5 g, 62%) as a light brown solid, mp 169–170 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.80 (s, 1H), 7.43 (dd, 1H, J = 2.4 Hz, J = 14.9 Hz), 7.15 (dd, 1H, J = 2.3 Hz, J = 9.1 Hz), 7.06 (t, 1H, J = 9.1), 5.17 (m, 1H), 4.88 (d, 2H, J = 6.80 Hz), 4.22 (t, 1H, J = 9.2 Hz), 3.91 (dd, 1H, J = 4.8 Hz, J = 9.2 Hz), 3.73 (t, 4H, J = 4.5 Hz), 2.96 (t, 4H, J = 4.5 Hz), 2.55 (s, 3H). IR (KBr pellet, cm^{-1}): ν 3350, 1735, 1693, 1448, 1247. MS: m/z 389 (100, M$^+$). Anal. CHN: calcd 55.52, 5.18, 17.99, found 55.42, 5.06, 17.88.

(*R*)-5-{(5-Acetyl-[1,2,3]triazol-1-ylmethyl)-3-(3-fluoro-4-morpholin-4-yl-phenyl)} oxazolidin-2-one (9). The concentrated filtrate (800 mg) from above, containing approximately 1:1 ratio of two components, was subjected to silica gel column chromatography eluting with ethyl acetate–benzene 5:1, to give a less polar compound as a foam 200 mg. The foam was digested with ether to give a yellow solid, which was recrystallized from ethyl acetate–hexane to afford **9** (127 mg, 5%) as a light yellow amorphous solid, mp 152–153 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.62 (s, 1H), 7.45 (dd, 1H, J = 2.4 Hz, J = 14.9 Hz), 7.17 (dd, 1H, J = 2.3 Hz, J = 9.1 Hz), 7.07 (t, 1H, J = 9.1), 5.10 (m, 3H), 4.22 (t, 1H, J = 9.0 Hz), 3.91 (dd, 1H, J = 4.8 Hz, J = 9.2 Hz), 3.74 (t, 4H, J = 4.5 Hz), 2.97 (t, 4H, J = 4.5), 2.59 (s, 3H). IR (KBr pellet, cm^{-1}): ν 3351, 1744, 1679, 1445, 1252. MS: m/z 389 (100, M$^+$). Anal. CHN: calcd 55.52, 5.18, 17.99, found 55.78, 5.20, 17.79.

(*R*)-3-{3-Fluoro-4-morpholin-4-yl-phenyl)-5-[4-(*E*/*Z*)(1-methoxyiminoethyl)-[1,2,3]triazol-1-ylmethyl]}oxazolidin-2-one (10). A mixture of **8** (300 mg, 0.78 mmol), methoxylamine hydrochloride (85 mg, 1.0 mmol) and pyridine (86 μL, 1.0 mmol) in dimethoxyethane-ethanol (20:10 mL) was stirred at room temperature for 6 h. The reaction mixture was concentrated to dryness, and the residue treated with ethyl acetate and water, the organic layer was separated, washed with water, brine, dried (Na_2SO_4) and concentrated to afford a beige foam (280 mg). Recrystallized from ethyl acetate–ether afforded **10** as an off-white amorphous solid (215 mg, 67%), mp 142–143 °C. This compound was obtained as mixture of inseparable *E*/*Z*-isomer. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.72 and 8.42 (two sets of s, 1H), 7.43 (overlapping dd, 1H, J = 2.4 Hz, J = 14.9 Hz), 7.15 (overlapping dd, 1H, J = 2.4 Hz, J = 8.8 Hz), 7.06 (t, 1H, J = 9.4), 5.14 (m, 1H), 4.88 and 4.83 (two sets of d, 2H, J = 5.3 Hz), 4.21 (t, 1H, J = 9.0 Hz), 3.92 and 3.90 (two sets of s, 3H), 3.89 (m, 1H), 3.74 (t, 4H, J = 4.5 Hz), 2.96 (t, 4H, J = 4.5 Hz) 2.27 and 2.22 (two sets of s, 3H). IR (KBr pellet, cm^{-1}): ν 1751, 1625, 1517, 1478, 1451, 1256. Anal. CHN: calcd 54.54, 5.54, 20.08, found 54.87, 5.50, 20.10.

(*R*)-3-(3-Fluoro-4-morpholin-4-yl-phenyl)-5-[1,2,3]triazol-1-ylmethyl oxazolidin-2-one (PH-027). A solution of the 5-azidomethyl[10] **7** (1.01 g, 3.14 mmol) in dimethoxyethane (30 mL) was placed in a steel bomb and cooled to −78 °C in a dry ice-acetone bath. A stream of excess acetylene gas was passed into the bomb to condense over a period of 4 min, the steel bomb was tightly closed and the mixture was heated at 90 °C for 12 h. The bomb was cooled to 0 °C and excess acetylene was released carefully and the mixture was concentrated to give a yellow solid 1.1 gm, which was recrystallized from tetrahydrofuran–diethyl ether mixture to afford **PH-027**; 809 mg, 74% as a crystalline solid, mp 169–170 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.17 (s, 1H), 7.77 (s, 1H), 7.41 (dd, 1H, J = 2.4 Hz, J = 14.9 Hz), 7.13 (dd, 1H, J = 2.4, J = 9.4 Hz), 7.05 (t, 1H, J = 9.4 Hz), 5.13 (m, 1H), 4.83 (d, 2H, J = 5.1 Hz), 4.20 (t, 1H, J = 9.2 Hz), 3.86 (dd, 1H, J = 5.7 Hz, J = 9.3 Hz), 3.73 (t, 4H, J = 4.6 Hz), 2.96 (t, 4H, J = 4.6 Hz). IR (KBr pellet,

cm^{-1}): ν 1749, 1625, 1518, 1472, 1447, 1288. MS: *m/z* 347 (100, M^+). Anal. CHN: calcd 55.33, 5.22, 20.16, found 55.26, 5.20, 20.03.

(*R*)-1-[3-(3-Fluoro-4-morpholin-4-yl-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-1*H*-[1,2,3]triazole-4,5-dicarboxylic acid methyl ester (11). A mixture of 5-azidomethyl **7**[10] (1.0 g, 3.1 mmol) and dimethyl acetylenedicarboxylate (671 mg, 4.7 mmol) in dimethoxyethane (30 mL) was heated at 90 °C for 24 h. The mixture was concentrated to give a yellow solid, which was recrystallized from ethyl acetate to afford **11** (1.07 g, 73%) as a crystalline solid, mp 144–145 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.46 (dd, 1H, J = 2.4 Hz, J = 14.9 Hz), 7.17 (dd, 1H, J = 2.4 Hz, J = 8.8 Hz), 7.07 (t, 1H, J = 9.5), 5.14 (m, 1H), 5.03 (d, 2H, J = 5.0 Hz), 4.23 (t, 1H, J = 9.2 Hz), 3.94 (s, 3H), 3.90 (dd, 1H, J = 5.6 Hz, J = 9.5 Hz), 3.89 (s, 3H), 3.74 (t, 4H, J = 4.5 Hz), 2.97 (t, 4H, J = 4.5 Hz). IR (KBr pellet, cm^{-1}): ν 1766, 1727, 1561, 1519, 1483, 1465, 1272. MS: *m/z* 463 (95%, M^+). Anal. CHN: calcd 51.84, 4.79, 15.11, found 51.97, 4.66, 15.12.

(*R*)-1-[3-(3-Fluoro-4-morpholin-4-yl-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-1*H*-[1,2,3]triazole-4,5-dicarboxylic acid, sodium salt (12). A solution of dimethyl ester **11** (700 mg, 1.5 mmol) in methanol–tetrahydrofuran (15 mL:20 mL) was cooled to 0 °C and treated with a solution of sodium hydroxide (123 mg, 3.01 mmol) in 10 mL water, and stirred for 2 h. The mixture was evaporated to remove methanol and tetrahydrofuran, and the aqueous solution was freeze dried to give a solid, which was recrystallized from methanol–ether to afford **12** (610 mg, 84%) as a white fluffy solid mp 270–272 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.48 (dd, 1H, J = 2.5 Hz, J = 14.6 Hz), 7.17 (dd, 1H, J = 2.5 Hz, J = 8.8 Hz), 7.06 (t, 1H, J = 9.3), 5.15 (s, 2H), 4.78 (m, 1H), 4.20 (t, 1H, J = 9.2 Hz), 3.97 (dd, 1H, J = 5.6 Hz, J = 9.5 Hz), 3.72 (t, 4H, J = 4.5 Hz), 2.96 (t, 4H, J = 4.5 Hz). IR (KBr pellet, cm^{-1}): ν 3437, 1747, 1627, 1518, 1449, 1239. Anal. CHN: calcd 45.10, 3.36, 14.61 found 45.33, 3.46, 14.90

(*S*)-3-(3-Fluoro-4-morpholin-4-yl-phenyl)-5-imidazol-1-ylmethyl-oxazolidin-2-one (13). A solution of imidazole (183 mg, 2.68 mmol) in anhydrous DMF (25 mL) under nitrogen, treated with 60% NaH in mineral oil (120 mg) was stirred at room temp for 20 min, a solution of **6** (1.0 g, 2.68 mmol) in anhydrous DMF (10 mL) was added in rapid drops and the mixture stirred at room temp under nitrogen for 48 h. The reaction mixture was diluted with water and ethyl acetate; and the ethyl acetate layer separated. The aqueous layer was further extracted with ethyl acetate and the combined ethyl acetate extracts were washed with water, brine, dried (Na_2SO_4), and concentrated to give a white solid 500 mg. Purification by silica gel column chromatography eluting with ethyl acetate–methanol gave a white solid. Recrystallization from methanol/ethyl acetate afforded **13** (200 mg, 22%) as a white amorphous solid, mp 153–154 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.68 (s, 1H), 7.43 (dd, 1H, J = 2.4 Hz, J = 14.9 Hz), 7.23 (s, 1H), 7.16 (dd, 1H, J = 2.4, J = 8.9 Hz), 7.05 (t, 1H, J = 9.42 Hz), 6.91 (s, 1H), 4.98 (m, 1H), 4.37 (m, 2H), 4.14 (t, 1H, J = 9.0 Hz) 3.77 (dd, 1H, J = 5.7 Hz, J = 9.3 Hz), 3.74 (t, 4H, J = 4.6 Hz), 2.96 (t, 4H, J = 4.6 Hz). IR (KBr pellet, cm^{-1}): ν 1729, 1625, 1517, 1480, 1271. MS: *m/z* 346 (100, M^+). Anal. CHN: calcd 58.95, 5.53, 16.18, found 58.55, 5.53, 15.99.

(*R*)-[*N*-3-(3-Fluoro-4-morpholin-4-ylphenyl)-2-oxo-5-oxazolidinyl] methyl *N*,*O*-bis-(*tert*-butoxycarbonyl)hydroxylamine (14). A solution of *tert*-butyl *N*-(*tert*-butoxycarbonyloxy)carbamate (3.14 g, 13.5 mmol) in anhydrous DMF (50 mL) under an atmosphere of N_2 gas, was treated with sodium hydride (584 mg of 60% NaH in mineral oil, 14.59 mmol). The reaction was stirred for 30 min at room temp and treated with a solution of the methanesulfonate **6** (4.2 g, 11.2 mmol) in DMF (50 mL). The mixture was stirred at room temp for 48 h, diluted with water and extracted with methylene chloride (3 × 80 mL). The methylene chloride extracts were pooled, washed with water, brine, dried (Na_2SO_4), and concentrated to give a brown oil 6.50 g. Purification by silica gel column chromatography eluting with ethyl acetate–hexane 1:1.5 afforded **14** (4.01 g, 70%) as a brown viscous oil. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.49 (dd, 1H, J = 2.45 Hz, J = 14.9 Hz), 7.18 (dd, 1H, J = 2.6 Hz, J = 8.8 Hz), 7.07 (t, 1H, J = 9.4 Hz), 4.86 (m, 1H), 4.13 (t, 1H, J = 6.9 Hz), 3.85 (dd, 1H, J = 7.5 Hz, J = 14.9 Hz), 3.73 (m, 5H), 3.35 (m, 1H, overlaps with D_2O signal), 2.96 (t, 4H, J = 4.6 Hz), 1.45 (s, 9H), 1.40 (s, 9H). MS: *m/z* 511.55 (M^+).

(*R*) 3-(3-Fluoro-4-morpholin-4-yl-phenyl)-5-*N*-hydroxyaminomethyl-oxazolidin-2-one (15). A solution of **14** (3.41 g, 6.67 mmol) in anhydrous dichloromethane (40 mL) at 0 °C was treated with trifluoroacetic acid (21 mL; 274 mmol), then stirred at 0 °C for 2.5 h, and concentrated to dryness. The crude was diluted with dichloromethane 100 mL and 10% potassium carbonate solution. The DCM layer was separated and the aqueous layer further extracted with additional DCM (3 × 60 mL). The organic layers were combined, dried (Na_2SO_4), filtered and concentrated to give a white solid. Recrystallization from dichloromethane-ether afforded **15** (1.2 g, 58%) as white crystalline solid, mp 148–149 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.50 (dd, 1H, J = 2.5 Hz, J = 15.1 Hz), 7.44 (s, 1H, NOH exchangeable with D_2O), 7.19 (t, 1H, J = 2.1 Hz, J = 8.8 Hz), 7.06 (t, 1H, J = 9.5 Hz), 6.03 (br. s, 1H, NH exchangeable with D_2O), 4.80 (m, 1H), 4.10 (t, 1H, J = 8.9 Hz), 3.82 (dd, 1H, J = 6.8 Hz, J = 8.9 Hz), 3.74 (t, 4H, J = 4.5 Hz), 3.03 (ABq, 2H, J = 6.19 Hz, J = 13.6 Hz) 2.96 (t, 4H, J = 4.5 Hz). IR (KBr pellet, cm^{-1}): ν 3275, 3180, 1719, 1631, 1523, 1478, 1272. Anal. CHN: calcd 54.01, 5.83, 13.50, found 54.05, 5.71, 13.50.

(*R*)-*N*-[3-(3-Fluoro-4-morpholin-4-yl-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-*N*-hydroxyacetamide (17). A solution of **15** (1.1 g, 3.5 mmol) in anhydrous CH_2Cl_2 (30 mL) with stirring at room temp was treated with triethylamine (3.0 mL; 21.1 mmol); then acetic anhydride (833 μL; 8.83 mmol) was added and the mixture stirred at room temp for 3 h. The reaction mixture was

diluted with a solution of 10% potassium carbonate and the dichloromethane layer was separated, washed with brine, dried (Na_2SO_4), and concentrated to give *N*-acetoxy-*N*-[3-(3-fluoro-4-morpholin-4-yl-phenyl)-2-oxo-oxazolidin-5-ylmethyl]acetamide **16** (1.50 g, quantitative yield) as a viscous oil. A solution of **16** (1.30 g, 3.3 mmol) in methanol–THF (36 mL:9 mL) at 0 °C was treated with NaOH solution (132 mg in 30 mL water) and stirred for 1 h 10 min at this temp. The mixture was acidified with dilute HCl solution adjusting the pH of the solution to ~7.0, and concentrated to remove THF and methanol. The aqueous layer was extracted with dichloromethane (2 × 30 mL), the organic layer was separated, dried (Na_2SO_4), and concentrated to give a crude. Purification by silica gel column chromatography eluting with ethyl acetate-hexane afforded **17** (920 mg, 79%) as a brown foam. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.08 (s, 1H, –NO<u>H</u> exchangeable with D_2O), 7.49 (dd, 1H, J = 2.4 Hz, J = 15.0 Hz), 7.19 (dd, 1H, J = 2.32 Hz, J = 8.8 Hz), 7.07 (t, 1H, J = 9.5 Hz), 4.84 (m, 1H), 4.13 (t, 1H, J = 8.9 Hz), 4.03 (dd, 1H, J = 7.0 Hz, J = 14.7 Hz), 3.73 (t, 4H, J = 4.5 Hz), 3.70 (ABq, 2H, J = 4.4 Hz, J = 14.6 Hz) 2.96 (t, 4H, J = 4.5 Hz), 2.02 (s, 3H). IR (KBr pellet, cm^{-1}): ν 3435, 1751, 1630, 1518, 1419, 1228. MS: m/z 353 (M^+). Anal. CHN: calcd 54.39, 5.71, 11.89, found 54.58, 5.96, 10.95.

Microbiology

In vitro antibacterial tests. Antibacterial susceptibility testing was performed by disk diffusion and the agar dilution methods of the National Committee for Clinical Laboratory Standards.[20] Inhibitory zone diameters were measured on Mueller-Hinton (MH) agar, with conventional paper disk (Whatman, Grade AA Discs, 6.00 mm in diameter) containing 30 μg of each compound. The inhibitory zone diameters were read with a caliper, and all results were rounded up to the nearest whole numbers (mm) for analysis. The Minimum Inhibitory Concentrations (MIC's, μg/mL) were determined on MH agar with medium containing dilutions of antibacterial agents ranging from 0.25 to 32 mg/L. The new compounds were dissolved in 20% water in DMSO, while linezolid and vancomycin were dissolved in 40% water in ethanol and water, respectively.

The test compounds were diluted in MH broth for all staphylococci and enterococci, and in MH broth supplemented with 5% sheep blood to facilitate the growth of *S. pneumoniae* and *H. influenzae*. The Gram-positive organisms utilized in this study consisted of MRSA, MSSA, MR-CNS, MS-CNS, *S. pneumoniae*, PRSP, VSE, VIRE and VRE. The Gram-negative species include *E. coli*, *P. aeruginosa* and *H. influenzae*. The final bacterial concentration for inocula was of 10^7 CFU/mL, and was incubated at 35 °C for 18 h. The MIC was defined as the lowest drug concentration that completely inhibited growth of the bacteria.

Acknowledgements

This project was supported by Kuwait University Research Grant PC01/00, and the Science Analytical Facilities (SAF) Project no. GS01/01.

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