Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 2651–2656
Bioorganic & Medicinal Chemistry

Synthesis of novel oxazolidinone antimicrobial agents

David C. Ebner,[a] Jeffrey C. Culhane,[a] Tyler N. Winkelman,[a] Mitchell D. Haustein,[b] Jayna L. Ditty[b] and J. Thomas Ippoliti[a,*]

[a]*Department of Chemistry, University of St. Thomas, St. Paul, MN 55105, USA*
[b]*Department of Biology, University of St. Thomas, St. Paul, MN 55105, USA*

Received 16 October 2007; revised 9 November 2007; accepted 13 November 2007
Available online 19 November 2007

Abstract—The oxazolidinone class of antimicrobials represents a promising advance in the fight against resistant Gram-positive bacterial infections. Four novel oxazolidinone antimicrobial compounds, each containing a benzodioxin ring system, have been prepared. The general synthesis of each compound begins with the construction of a benzodioxin ring system containing a nitro substituent that ultimately becomes the nitrogen of the oxazolidinone ring. Three of the compounds utilize high yielding 'click chemistry' in their final step. The antimicrobial activities of the new oxazolidinones have been measured and the MIC against *Staphylococcus aureus* for one of the antimicrobials was determined to be 2–3 μg/mL, which is comparable to the well-known oxazolidinone, linezolid.

1. Introduction

Multi-drug resistance among Gram-positive bacterial pathogens represents a serious challenge for health practitioners in treating both nosocomial and community-acquired infections.[1] Several reports have shown that antibacterial resistance is increasing at a rapid rate in both community and hospital settings.[2,3] This continued rise in antibacterial resistance has called for antimicrobial agents to be developed that are effective against resistant strains of bacteria. In April 2000, the Food and Drug Administration approved the release of linezolid, **1**, trademarked Zyvox (Fig. 1).[4]

Zyvox, an antimicrobial oxazolidinone first marketed by the Pharmacia Corporation, became the first approved drug that was effective against antibacterial resistant bacteria.[5] Zyvox prevents initiation of protein synthesis via a novel mechanism in Gram-positive bacteria including *Enterococcus* strains, *Staphylococcus* strains, and *Streptococcus pneumoniae*.[6,7] Specifically, the fMet-tRNA necessary for bacterial protein synthesis initiation is blocked by Zyvox's interaction with the 23S rRNA of the 50S subunit.

Brickner et al. described the syntheses of Zyvox, **1**, and an analog, **2**, in 1996.[8] The common structural elements in these analogs are the ortho electron donating groups, a fluorine and an amino nitrogen on the aryl ring connected to the nitrogen of the oxazolidinone ring, as well as the stereochemistry at carbon 5 of the oxazolidinone ring. It has also been reported that an acetamide group connected to carbon 5 of the oxazolidinone ring is required for high activity.[9]

Since the introduction of Zyvox there has been much research directed toward the synthesis of oxazolidinone antimicrobials.[10,11] Wright has recently reviewed the discovery of Zyvox and syntheses of new members of this class.[12]

To explore the ability of conjugated oxygens as electron donors to affect the antibacterial activity versus the nitrogen and fluorine atoms of linezolid, we have designed an oxazolidinone containing a benzodioxin moiety. Others have been successful in attaching fused rings to the phenyl group to yield molecules with desirable activity. For example, oxazolidinones containing fused tricyclic ring systems such as **3** and **4** have been synthesized (Fig. 2).[13] These compounds have MIC values less than 0.125–1 μg/mL.

Keywords: Oxazolidinone; Benzodioxin; Triazole; Antimicrobial agents.
* Corresponding author. Tel.: +1 651 962 5582.; e-mail: jtippoliti@stthomas.edu

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.11.040

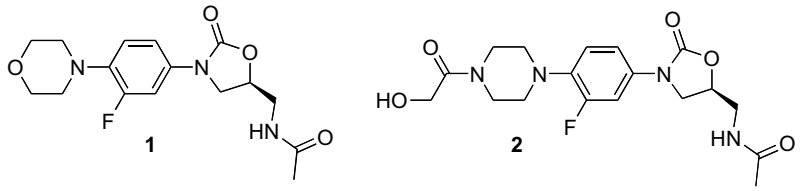


Figure 1. Zyvox (**1**) and analog (**2**).

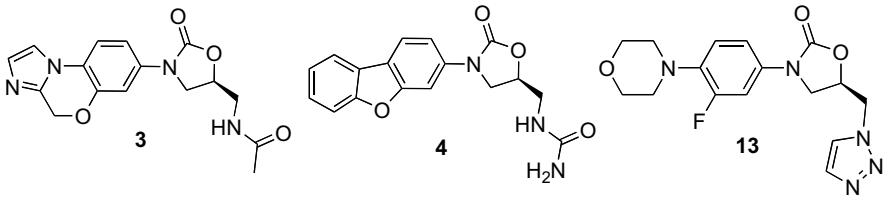


Figure 2. Oxazolidinones that demonstrate high antibacterial activity.

In addition, Phillips et al. have replaced the amide group of linezolid with a triazole ring to give compound **13** and found comparable antibacterial activity to linezolid.[14] This result supports the idea that triazoles can act as amide surrogates.[15]

Herein we describe the synthesis and antibacterial activity of four novel oxazolidinones. The first compound (**5**) contains a benzodioxin ring system with the benzodioxin oxygens in the same positions as the fluorine and amino nitrogen of linezolid (Fig. 3). We have also explored the effect of replacing the amide functional group on compound **5** with a substituted triazole ring by synthesizing compounds **12a**–**12c**.

2. Results and discussion

The synthesis of the new antimicrobial agent **5** was accomplished in seven steps as shown in Scheme 1.

The benzodioxin ring was readily constructed by a double nucleophilic aromatic substitution reaction using catechol and 3,4-difluoronitrobenzene in 85% yield. Reduction of the nitro group and formation of the benzyl carbamate was carried out in the same reaction vessel without isolation of the amine in an overall 74% yield. The formation of the oxazolidinone ring was accomplished in good yield (83%) starting with enantiomerically pure (*R*)-(−)-glycidyl butyrate. Although the enantiomeric excess for this reaction was not measured, the identical reaction conditions used by Brickner et al. were followed and the authors reported greater than 99.7% ee.[8] Mesylation followed by substitution with azide and then reduction of the azide gave amine **11** in high overall yield. Acylation of the amine with acetic anhydride proceeded smoothly to give the final product **5** in 83% yield. Purification of the product did not require chromatography and was accomplished readily by washing with cold ethanol.

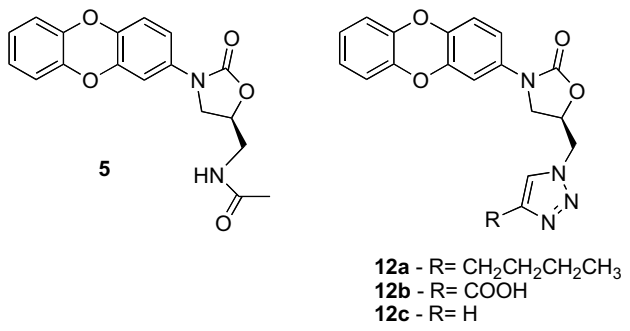
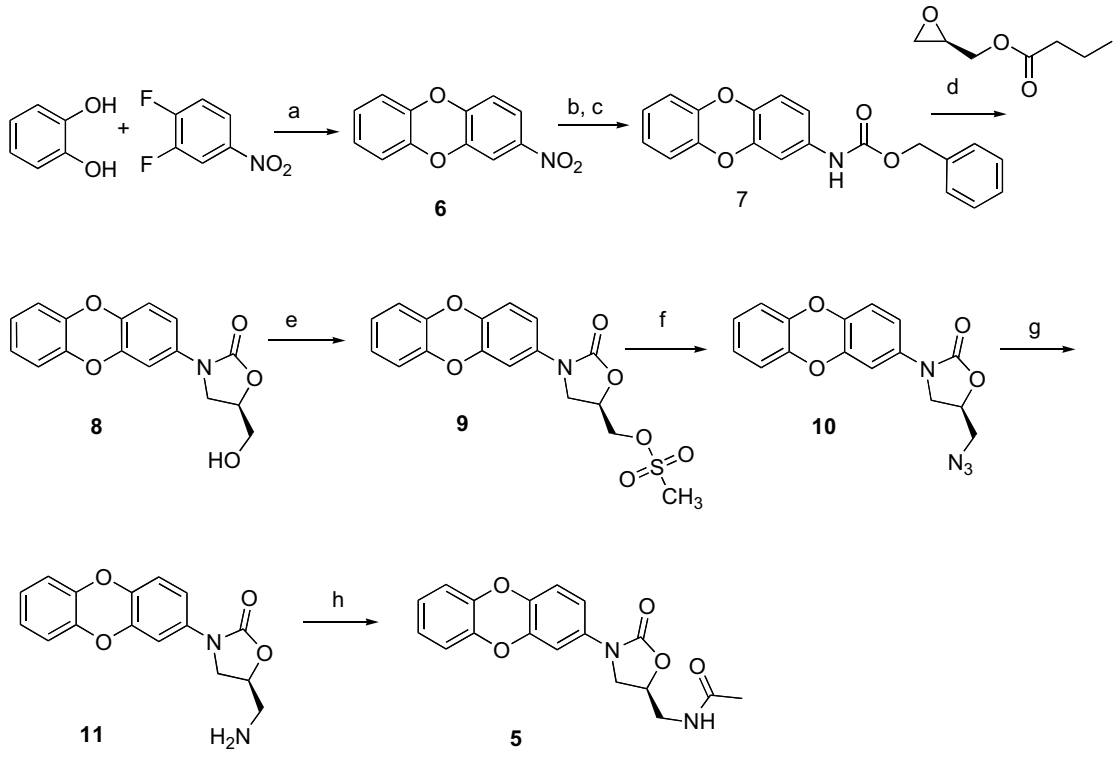


Figure 3. Novel oxazolidinones.

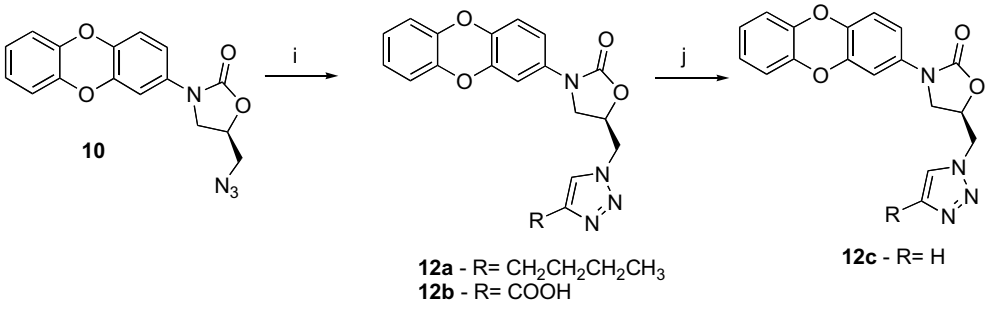
Replacement of amide group in compound **5** with a triazole was easily accomplished using Sharpless click chemistry conditions on intermediate **10**.[16] Three novel oxazolidinones containing triazole rings were synthesized according to Scheme 2. Substituted alkynes reacted readily with the azide **10** to give **12a** with a nonpolar butyl group and **12b** with a polar carboxylic acid group. Synthesis of the unsubstituted triazole was not straightforward. Phillips et al. reacted azides with acetylene gas under high pressure in a steel bomb. To avoid these potentially hazardous conditions we used methodology reported by Biagi et al. to form the unsubstituted triazole ring.[17] We first synthesized a triazole substituted with a carboxylic acid group (**12b**). Decarboxylation proceeded smoothly by refluxing in DMF to give compound **12c** in excellent yield.

2.1. In vitro antimicrobial testing

The MIC of the oxazolidinone derivative **5** for Gram-positive *Staphylococcus aureus* ATCC 29213 was 2–3 μg/mL. The determined MIC concentration is similar in efficacy range compared to other published oxazolidinone derivative MIC values.[7] The MICs of the oxazolidinone derivatives **12a**, **12b**, and **12c** for *S. aureus* were greater than 50 μg/mL. Concentrations above 50 μg/mL were not tested of derivatives **12a**, **12b**, and **12c**. None of the oxazolidinone derivatives were effective against the Gram-negative *Escherichia coli* ATCC 25922 up to a concentration of 100 μg/mL. The 100 μg/mL



Scheme 1. Reagents: (a) K_2CO_3, DMF/toluene (80:20); (b) H_2, 10% Pd/C, THF; (c) CbzCl; (d) $LiN(Si(CH_3)_3)_2$ followed by addition of (*R*)-(−)-glycidyl butyrate; (e) $MeSO_2Cl/NEt_3/CH_2Cl_2$; (f) NaN_3/DMF; (g) H_2, 10% Pd/C, THF; (h) acetic anhydride, pyridine.



Scheme 2. Reagents and condition: (i) sodium ascorbate, $CuSO_4{\cdot}5H_2O$, *t*-BuOH/H_2O, for **12a**: 1-hexyne, for **12b**: propiolic acid; (j) from **12b**, DMF, reflux.

concentration was the highest concentration of the oxazolidinone derivative that could be administered without limiting the growth of *E. coli* due to the presence of greater than 5% EtOH in the growth medium. The oxazolidinone derivative was determined to have a bacteriostatic mode of action, as cells that were harvested from post MIC incubations were viable after removal of the antimicrobial.

3. Conclusions

Four novel oxazolidinones containing a benzodioxin moiety have been synthesized. The antimicrobial activity of each of these compounds has been measured. The MIC against *S. aureus* was determined to be 2–3 μg/mL for compound **5**. This compound has similar antimicrobial activity against *S. aureus* compared to linezolid, which has been reported to have an MIC of 4 μg/mL. It appears that the benzodioxin ring system does not alter the activity of the oxazolidinone with an amide attached. However, in contrast to the results of Phillips et al., our compounds with triazole rings show no antimicrobial activity, which suggests there is a link between the benzodioxin moiety and the amide functionality for activity of these oxazolidinones.

4. Experimental

4.1. Chemistry

All reagents and solvents were purchased from Aldrich Chemical Co. and used as received. ^1H NMR and ^{13}C

NMR data were collected at 300 MHz on a Bruker NMR spectrometer.

4.1.1. 2-Nitrodibenzo[b,e][1,4]dioxine (6). Catechol (20.0 g, 0.182 mol) and K_2CO_3 (50.2 g, 0.363 mol) were stirred in a solution of 80:20 DMF:toluene (1000 mL). The solution was treated by dropwise addition of 3,4-difluoronitrobenzene (20.1 mL, 0.182 mol) and brought to reflux for 2 h. The reaction mixture was cooled and then poured over ice water (1500 mL). The resulting yellow precipitate was vacuum filtered and recrystallized from MeOH. The procedures yielded 28.0 g (68%) of **6** as a yellow solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.93 (m, 5H), 7.74 (d, J = 2.44 Hz, 1H), 7.83 (dd, J = 8.85 Hz, J' = 2.75 Hz, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 147.4, 143.5, 141.9, 140.7, 140.7, 124.9, 124.6, 119.9, 116.5, 116.5, 116.3, 112.2. HRMS(ESI) calcd for $C_{12}H_7NO_4$ + Na, exact 252.0267; found 252.0274.

4.1.2. Benzyl dibenzo[b,e][1,4]dioxin-7-ylcarbamate (7). The nitro compound **6** (15.0 g, 65.4 mmol) was dissolved in THF (300 mL) followed by the addition of 10% Pd on carbon (1.0 g). Hydrogenation in a Parr Shaker was carried out for 20 h. The reaction mixture was filtered through Celite, treated with saturated $NaHCO_3$ solution, and cooled to −20 °C. After addition of benzyl chloroformate (14.0 mL, 98.1 mmol) the reaction mixture was allowed to warm to ambient temperature while stirring for 20 h. The solution was concentrated to half the volume on a rotary evaporator followed by dilution with EtOAc. The solution was washed with water (4×) and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was washed with hot hexanes to yield 19.6 g (78%) of **7** as a beige solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.19 (s, 2 H), 6.53 (br s, 0.5H), 6.84 (m, 7H), 7.38 (m, 5H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 154.2, 142.2, 142.1, 141.7, 138.1, 135.9, 133.6, 128.6, 128.4, 128.3, 123.7, 116.4, 116.3, 116.3, 67. HRMS(ESI) calcd for $C_{20}H_{15}NO_4$ + Na, exact 356.0893; found 356.0898.

4.1.3. (*R*)-3-(Dibenzo[b,e][1,4]dioxin-7-yl)-5-(hydroxymethyl)oxazolidin-2-one (8). A solution of compound **7** (3.85 g, 11.6 mmol) in THF (200 mL) was cooled to −78 °C. 1.0 M lithium bis(trimethylsilyl)amide (12.8 mL, 12.8 mmol) was added dropwise to the solution and allowed to stir for 30 min. (*R*)-(−)-glycidyl butyrate (1.64 mL, 11.6 mmol) was added dropwise to the solution. The reaction mixture was warmed to ambient temperature while stirring for 24 h. The reaction mixture was diluted with EtOAc, washed with water and saturated NaCl solution (2×), and dried over Na_2SO_4. Precipitation of product during in vacuo concentration yielded 2.75 g (79%) of **8** as a white solid. ^{1}H NMR (DMF, 300 MHz): δ 3.56 (ddd, J = 12.21 Hz, J' = 9.15 Hz, J'' = 3.66 Hz, 1H), 3.67 (ddd, J = 12.21 Hz, J' = 9.15 Hz, J'' = 3.66 Hz, 1H), 3.81 (dd, J = 8.85 Hz, J' = 6.91 Hz, 1H), 4.02 (t, J = 8.85 Hz,1H), 4.63 (m, 1H), 5.23 (t, J = 5.8 Hz, 1H), 6.85 (m, 5H), 6.99 (dd, J = 8.85 Hz, J' = 2.44 Hz, 1H), 7.27 (d, J = 2.44 Hz, 1H). ^{13}C NMR (DMSO, 300 MHz): δ 154.434, 141.4, 141.235, 141.0, 137.1, 134.9, 124.6, 124.3, 116.5, 116.5, 116.3, 113.2, 106.2, 73.2, 61.7, 46.1. HRMS(ESI) calcd for $C_{16}H_{13}NO_5$ + Na, exact 322.0686, found 322.0708.

4.1.4. ((2*R*)-4-(dibenzo[b,e][1,4]dioxin-7-yl)-tetrahydro-5-oxofuran-2-yl)methyl methanesulfonate (9). 8 (2.68 g, 8.95 mmol) and NEt_3 (2.19 mL, 15.7 mmol) in CH_2Cl_2 (250 mL) were cooled to 0 °C and allowed to stir for 30 min. Methanesulfonyl chloride (0.867 mL, 11.2 mmol) was added dropwise to the solution. The reaction mixture was warmed to ambient temperature while stirring for 24 h. The reaction mixture was diluted over CH_2Cl_2, washed with water (3×) and brine, and dried over Na_2SO_4. Concentration in vacuo yielded 3.29 g (97%) of **9** as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.11 (s, 3H), 3.91 (dd, J = 9.16 Hz, J' = 6.11 Hz, 1H), 4.11 (t, J = 9.16 Hz, 1H), 4.43 (dd, J = 11.59 Hz, J' = 3.96 Hz, 1H), 4.50 (dd, J = 11.59 Hz, J' = 3.96 Hz, 1H), 4.91 (m, 1H), 6.87 (m, 5H), 6.98 (dd, J = 8.85 Hz, J' = 2.74 Hz, 1H), 7.18 (d, J = 2.75 Hz, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 153.5, 142.3, 141.8, 141.5, 138.9, 133.3, 124.0, 123.9, 116.4, 116.3, 116.3, 113.3, 107.3, 69.3, 67.9, 46.6, 37.8. HRMS(ESI) calcd for $C_{17}H_{15}NO_7S$ + Na, exact 400.0461; found 400.0447.

4.1.5. (*R*)-5-(azidomethyl)-3-(dibenzo[b,e][1,4]dioxin-7-yl)oxazolidin-2-one (10). 9 (3.25 g, 8.61 mmol) in DMF (250 mL) was treated with sodium azide (5.60 g, 86.1 mmol) and heated to 60 °C for 18 h. The reaction mixture was diluted with EtOAc, washed with water (5×) and brine, and dried with Na_2SO_4. Concentration in vacuo yielded 2.77 g (99%) of **10** as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.59 (dd, J = 13.12 Hz, J' = 4.27 Hz, 1H), 3.70 (dd, J = 13.42 Hz, J' = 4.88 Hz, 1H), 3.81 (dd, J = 8.85 Hz, J' = 6.1 Hz, 1H), 4.04 (t, 8.85 Hz, 1H), 4.77 (m, 1H), 6.87 (m, 5H), 6.99 (dd, J = 8.85 Hz, J' = 2.75 Hz, 1H), 7.18 (d, J = 2.75 Hz, 1H). ^{13}C NMR (DMSO, 300 MHz): δ 154.1, 141.6, 141.6, 141.3, 137.6, 134.8, 124.8, 124.7, 116.8, 113.7, 106.8, 71.5, 53.0, 47.2. HRMS(ESI) calcd for $C_{16}H_{12}N_4O_4$ + Na, exact 347.0751, found 347.0742.

4.1.6. (*S*)-5-(aminomethyl)-3-(dibenzo[b,e][1,4]dioxin-7-yl)oxazolidin-2-one (11). Azide **10** (2.75 g, 8.48 mmol) was dissolved in THF (250 mL) followed by the addition of 10% Pd on carbon (0.90 g). Reaction vessel was charged with H_2 and hydrogenation for 20 h ensued in a Parr Shaker. The reaction mixture was filtered through Celite and concentrated in vacuo to yield 2.50 g (99%) of **11** as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.97 (dd, J = 14.19 Hz, J' = 5.8 Hz, 1H), 3.11 (dd, J = 13.73 Hz, J' = 5.27 Hz, 1H), 3.80 (dd, J = 8.54 Hz, J' = 6.71 Hz, 1H), 4.00 (t, J = 8.44 Hz, 1H), 4.66 (m, 1H), 6.86 (m, 5H), 7.00 (dd, J = 8.85 Hz, J' = 2.44 Hz, 1H), 7.20 (d, J = 2.75 Hz, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 154.6, 142.3, 142.0, 141.7, 138.5, 134.2, 124.0, 123.8, 116.4, 116.4, 113.1, 107.1, 73.8, 47.7, 45.0. HRMS(ESI) calcd for $C_{16}H_{14}N_2O_4$ + H, exact 299.1024, found 299.1026.

4.1.7. *N*-(((*S*)-3-(dibenzo[b,e][1,4]dioxin-7-yl)-2-oxooxazolidin-5-yl)methyl)acetamide (5). Amine **11** (2.47 g,

8.28 mmol) in pyridine (300 mL) was treated with acetic anhydride (0.94 mL, 9.94 mmol) and stirred for 21 h. Concentration in vacuo yielded 2.35 g (83%) of crude product **5** as a beige solid. Crude product **5** (2.00 g) was washed with cold absolute EtOH while stirring for 20 min to yield 1.15 g (58%) of **5** as a white solid. mp 233.0–233.3 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.03 (s, 3H), 3.66 (m, 3H), 4.01 (t, J = 9.15 Hz, 1H), 4.76 (m, 1H), 6.01 (br s, 1H), 6.89 (m, 6H), 7.17 (d, J = 2.44 Hz, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 171.0, 154.3, 142.4, 142.0, 141.7, 138.8, 133.8, 124.1, 123.9, 116.4, 116.4, 113.2, 107.2, 71.8, 47.7, 42.0, 23.2. HRMS(ESI) calcd for $C_{18}H_{16}N_2O_5$ + Na, exact 363.0951, found 363.0951.

4.1.8. (*R*)-5-((4-butyl-1H-1,2,3-triazol-1-yl)methyl)-3-(dibenzo[b,e][1,4]dioxin-8-yl)oxazolidin-2-one (12a). To azide **10** (252.0 mg) in 20 mL H_2O were added (+)-sodium ascorbate (82.3 mg) and copper(II) sulfate pentahydrate (7.4 mg). The solution was stirred and hexyne (67.0 mg) was added. The reaction mixture was stirred for 5 days under ambient conditions and then filtered. After washing with water and 1.0 M HCl, 152.0 mg (60.3%) of **12a** was obtained. mp 215.8–216.2 °C. ^{1}H NMR (DMSO, 300 MHz): δ 0.85 (t, J = 7.34 Hz, 3H), 1.26 (m, 2H), 1.513 (m, 2H), 2.59 (t, J = 7.34 Hz, 2H), 3.82 (dd, J = 9.41 Hz, J' = 5.38 Hz, 1H), 4.19 (t, J = 9.17 Hz, 1H), 4.73 (d, J = 4.89 Hz, 2H), 5.09 (m, 1H), 6.99 (m, 6H), 7.19 (d, J = 1.47 Hz, 1H), 7.87 (s, J = Hz, 1H). ^{13}C NMR (DMSO, 300 MHz): δ 153.4, 146.9, 141.2, 141.1, 140.8, 137.2, 134.2, 124.5, 124.3, 122.7, 116.4, 113.5, 106.5, 70.7, 51.8, 47.0, 31.1, 24.5, 21.5, 13.7. HRMS(ESI) calcd for $C_{22}H_{22}N_4O_4$ + H, exact 407.1714, found 407.1721.

4.1.9. 1-(((*R*)-3-(dibenzo[b,e][1,4]dioxin-8-yl)-2-oxooxazolidin-5-yl)methyl)-1H-1, 2, 3-triazole-4-carboxylic acid (12b). To azide **10** (209.0 mg) in 10 mL *t*-BuOH were added (+)-sodium ascorbate (76.4 mg) and copper(II) sulfate pentahydrate (45.5 mg). The solution was stirred and propiolic acid (80% in THF) (3 mL) was added. The reaction was refluxed overnight under N_2 pressure and constant stirring. Reaction mixture was filtered resulting in a purple solid. The purple solid was stirred in 0.5 M HCl overnight, filtered, and washed with 50 mL H_2O and 2× 25 mL MeOH to yield 220.0 mg (87%) of **12b** as a light gray solid. ^{1}H NMR (DMSO, 300 MHz): δ 3.90 (m, 1H), 4.22 (t, J = 8.19 Hz, 1H), 4.84 (d, J = 4.40 Hz, 2H), 5.16 (m, 1H), 7.00 (m, 6H), 7.23 (s, 1H), 8.72 (s, 1H). ^{13}C NMR (DMSO, 300 MHz): δ 161.4, 153.3, 141.2, 141.1, 140.8, 139.6, 137.3, 134.2, 129.8, 124.5, 124.3, 116.4, 113.6, 106.6, 70.5, 52.1, 47.1. HRMS(ESI) calcd for $C_{19}H_{14}N_4O_6$ + Na, exact 417.0806, found 417.0803.

4.1.10. (*R*)-5-((1H-1,2,3-triazol-1-yl)methyl)-3-(dibenzo[b,e][1,4]dioxin-8-yl)oxazolidin-2-one (12c). 12b (250.0 mg) was added to 30 mL DMF. The solution was refluxed for 3.25 h under N_2 pressure and constant stirring. The reaction mixture was cooled to room temperature and a brown solid was precipitated into 150 mL H_2O. The solid was filtered and washed with 3× 25 mL EtOAc yielding 200.0 mg **12c** (90.1%) as a light tan solid. Decomposes at 243 °C. ^{1}H NMR (DMSO, 300 MHz): δ 3.86 (dd, J = 9.17 Hz, J' = 5.75 Hz, 1H), 4.20 (t, J = 8.80 Hz, 1H), 4.83 (d, J = 5.01 Hz, 2H), 5.12 (m, 1H), 7.00 (m, 6H), 7.21 (s, 1H), 7.77 (s, 1H), 8.18 (s, 1H). ^{13}C NMR (DMSO, 300 MHz): δ 153.4, 141.2, 141.1, 140.8, 137.3, 134.2, 125.8, 124.5, 124.3, 116.4, 116.4, 113.6, 106.6, 70.7, 51.7, 47.1. HRMS(ESI) calcd for $C_{18}H_{14}N_4O_4$ + -Na, exact 373.0907, found 373.0907.

5. General procedure for preparation of antimicrobial drugs[18]

A 2.0 mg/mL stock solution of linezolid (Pharmacia Corporation, New York) and the oxazolidinone derivative was prepared using 100% ethanol (EtOH) or 100% dimethylsulfoxide (DMSO, for derivative **12c** only because of insolubility in EtOH) as the solvent. Linezolid was prepared by grinding Zyvox pills with a mortar and pestle and placing in 100 mL of methylene chloride. The Zyvox/CH_2Cl_2 solution was filtered and the CH_2Cl_2 was evaporated. The resultant white crystalline powder was analyzed by TLC and NMR and shown to be pure linezolid. This material was used to make the linezolid stock solution.

5.1. Strains, growth conditions, and minimum inhibitory concentration (MIC)

Staphylococcus aureus ATCC 29213 and *Escherichia coli* ATCC 25922 strains were maintained in Muller Hinton broth (MHB; Oxoid, Ogdensburg, New York). Overnight cultures incubated at 37 °C for 24 h were used to test antimicrobial efficacy. The MIC of Zyvox and the oxazolidinone derivatives were determined as previously described.[18] The gradient of both Zyvox and oxazolidinone derivative **5** tested against *S. aureus* ranged from final concentrations of 0.5 to 10.0 μg/mL. The gradient of oxazolidinone derivatives **12a**, **12b**, and **12 c** tested against *S. aureus* ranged from final concentrations of 0.5 to 50.0 μg/mL. The range of antimicrobial concentration used against *E. coli* was from 10 to 100 μg/mL. Cultures exposed to the antimicrobials and either EtOH or DMSO controls were statically incubated at 37 °C for 24 h and subsequently visualized for microbial growth.

5.2. Determination of cell viability

To determine if oxazolidinone derivative **5** had a bacteriostatic or bacteriocidal mode of action, cell viability was determined after exposure to the oxazolidinone derivative. Bacterial cultures exposed to oxazolidinone derivative **5** concentrations around the determined MIC and the EtOH control were selected. The 1.0 mL MIC culture was placed into a 1.5 mL microfuge tube and centrifuged for 2 min at 16,000*g*. The supernatant was removed and the pellet was resuspended in 1 mL of MHB medium to wash the cell pellet. The cell suspension was centrifuged again for 2 min at 16,000*g*, the supernatant was removed, and a final 1 mL of MHB medium was used to resuspend the cell pellet. Dilutions were plated in duplicate on MHB agar (purchased from

Oxoid) and incubated at 37 °C for 24 h to determine cell number and viability.

Supplementary data

^{1}H NMR spectra of all compounds are given. This material is available free of charge via the internet. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.11.040.

References and notes

1. Klevens, R. M.; Morrison, M. A.; Nadle, J.; Petit, S.; Gershman, K.; Ray, S.; Harrison, L. H.; Lynfield, R.; Dumyati, G.; Townes, J. M.; Craig, A. S.; Zell, E. R.; Fosheim, G. E.; McDougal, L. K.; Carey, R. B.; Fridkin, S. K. *J. Am. Med. Assn.* **2007**, *298*, 1763–1771.
2. Ament, P. W.; Jamshd, N.; Horne, J. P. *Clin. Pharmacol.* **2002**, *65*, 663–670.
3. Livermore, D. M. *Antimicrob. Chemother.* **2003**, *51*(Suppl. S2), ii9–ii16.
4. Moellering, R. C., Jr. *Ann. Intern. Med.* **2003**, *138*, 135–142.
5. Brickner, S. J. *Curr. Pharm. Des.* **1996**, *2*, 175–194.
6. Lin, A. H.; Murray, R. W.; Vidmar, T. J.; Marotti, K. R. *Antimicrob. Agents Chemother.* **1997**, *41*, 2127–2131.
7. Shinabarger, D. L.; Marotti, K. R.; Murray, R. W.; Lina, A. H.; Melchior, E. P.; Swaney, S. M.; Dunyak, D. S.; Demyan, W. F.; Buysse, J. M. *Antimicrob. Agents Chemother.* **1997**, *41*, 2132–2136.
8. Brickner, S. J.; Hutchinson, D. K.; Barbachy, M. R.; Manninen, P. R.; Ulanowicz, D. A.; Garmon, S. A.; Gregra, K. C.; Hendges, S. K.; Toops, D. S.; Ford, C. W.; Zurenko, G. E. *J. Med. Chem.* **1996**, *39*, 673–679.
9. Gregory, W. A.; Brittelli, D. R.; Want, C. J.; Wuonola, M. A.; McRipley, R. J.; Eustice, D. C.; Eberly, V. S.; Bartholomew, P. T.; Slee, A. M.; Forbes, M. *J. Med. Chem.* **1989**, *32*, 1673–1681.
10. Sbardella, G.; Mai, A.; Artico, M.; Loddo, R.; Setzu, M. G.; LaColla, P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1537–1541.
11. Singh, U.; Raju, B.; Lam, S.; Zhou, J.; Gadwood, R. C.; Ford, C. W.; Zurenko, G. E.; Schaadt, R. D.; Morin, S. E.; Adam, W. J.; Friis, J. M.; Courtney, M.; Palandra, J.; Hackbarth, C. J.; Lopez, S.; Wu, C.; Mortell, K. H.; Trias, J.; Yuan, Z.; Patel, D. V.; Gordeev, M. F. . *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4209–4212.
12. Mukhtar, T. A.; Wright, G. D. *Chem. Rev.* **2005**, *105*, 529–542.
13. Bayer AG:WO9903846 (1999), Bayer AG:WO9940094 (1999).
14. (a) Phillips, O. A.; Udo, E. E.; Ali, A. A. M.; Al-Hassawi, N. *Bioorg. Med. Chem.* **2003**, *11*, 35–41; (b) Phillips, O. A.; Udo, E. E.; Ali, A. A. M.; Samuel, S. M. *Bioorg. Med. Chem.* **2005**, *13*, 4113–4123.
15. Brik, A.; Alexandratos, J.; Lin, Y. C.; Elder, J. H.; Olson, A. J.; Wlodawer, A.; Goodsell, D. S.; Wong, C. H. *Chem. Biol. Chem.* **2006**, *6*, 1167–1169.
16. Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128–1137.
17. Biagi, G.; Calderone, V.; Giorgi, I.; Livi, O.; Martinotti, E.; Martelli, A.; Nardi, A. *Il Farmaco* **2004**, *59*, 397–404.
18. National Committee for Clinical Laboratory Standards. 1993. Methods for Dilution of Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically-Third Edition; approved Standard; NCCLS Document M7-A3 (ISBN 1-56238-209-8); NCCLS: 771 Lancaster Ave. Villanova, PA 19085.