Synthesis of novel oxazolidinone antimicrobial agents

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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.
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. This result supports the idea that triazoles can act as amide surrogates.

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. 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.

Replacement of amide group in compound 5 with a triazole was easily accomplished using Sharpless click chemistry conditions on intermediate 10. 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. 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. 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...
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 \( \mu \text{g/mL} \) 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 \( \mu \text{g/mL} \). 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. \(^1\)H NMR and \(^{13}\)C
NMR data were collected at 300 MHz on a Bruker NMR spectrometer.

4.1.1. 2-Nitrodibenzo[e][1,4]dioxine (6). Catechol (20.0 g, 0.182 mol) and K$_2$CO$_3$ (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): $\delta$ 6.93 (m, 5H), 7.74 (d, $J = 2.44$ Hz, 1H), 8.73 (dd, $J = 8.85$ Hz, $J' = 2.75$ Hz, 1H). $^{13}$C NMR (CDCl$_3$, 300 MHz): $\delta$ 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$_{14}$H$_{13}$NO$_4$ + Na, exact 252.0274; found 252.0274.

4.1.2. Benzyl dibenzo[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 $-20^\circ$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 24 h. The reaction mixture was warmed to ambient temperature while stirring for 24 h. The reaction mixture was diluted with CH$_2$Cl$_2$, washed with water (3x) and brine, and dried over Na$_2$SO$_4$. Concentration in vacuo yielded 2.75 g (99%) of 7 as a white solid. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 3.11 (s, 3H), 3.91 (dd, $J = 9.16$ Hz, $J' = 6.11$ 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 (m, $J = 8.85$ Hz, $J' = 2.74$ Hz, 1H), 7.18 (d, $J = 2.75$ Hz, 1H). $^{13}$C NMR (CDCl$_3$, 300 MHz): $\delta$ 153.5, 142.3, 142.1, 141.5, 138.9, 133.3, 124.0, 123.9, 116.4, 116.3, 113.3, 107.3, 69.3, 67.9, 46.6, 37.8. HRMS(ESI) calcd for C$_{22}$H$_{15}$N$_2$O$_4$ + Na, exact 400.0461; found 400.0447.

4.1.5. (R)-5-(azidomethyl)-3-(dibenzo[e][1,4]dioxin-7-yl)oxazolidin-2-one (10). 9 (0.39 g, 2.68 mmol) was dissolved in THF (250 mL) with EtOAc. The solution was washed with water (3x) and brine, and dried over Na$_2$SO$_4$. Concentration in vacuo yielded 2.77 g (99%) of 10 as a white solid. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 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, $J' = 6.11$ Hz, 1H), 4.04 (t, $J = 8.5$ Hz, 1H), 4.77 (m, 1H), 6.79 (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): $\delta$ 154.1, 141.6, 141.6, 141.3, 137.6, 134.8, 124.8, 124.7, 116.8, 113.7, 113.0, 71.8, 53.0, 47.2. HRMS(ESI) calcd for C$_{17}$H$_{15}$NO$_4$ + Na, exact 347.0751, found 347.0742.

4.1.6. (S)-5-(aminomethyl)-3-(dibenzo[e][1,4]dioxin-7-yl)oxazolidin-2-one (11). Azide 10 (0.39 g, 2.68 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): $\delta$ 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): $\delta$ 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$_{25}$H$_{14}$N$_2$O$_4$ + H, exact 299.0124, found 299.0126.

4.1.7. N-((S)-(3-(dibenzo[e][1,4]dioxin-7-yl)-5-oxooxazolidin-5-yl)methyl)acetamide (5). Amine 11 (0.247 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. 1H NMR (CDCl3, 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 (dd, J = 2.44 Hz, 1H). 13C NMR (CDCl3, 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, 42.0, 23.2. HRMS(ESI) calcd for C18H16N2O5 + Na, exact mass 373.0907, found 373.0907.

5. General procedure for preparation of antimicrobial drugs

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/CH3Cl2 solution was filtered and the CH3Cl2 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 ATTC 25922 strains were maintained in Muller Hinton broth (MB; Oxoid, Ogdenburg, 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 12c 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 16,000g, 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**

1H 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**


