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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2391–2395

Synthesis and antibacterial activity of novel oxazolidinones bearing *N*-hydroxyacetamidine substituent☆

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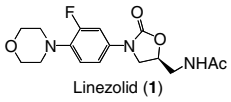
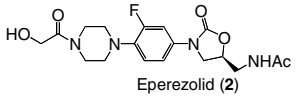
Received 2 December 2005; revised 21 January 2006; accepted 27 January 2006
Available online 20 February 2006

Abstract—Novel oxazolidinone antibacterials containing *N*-hydroxyacetamidine moiety are synthesized with the diversity at C-5 terminus. These compounds have been evaluated against a panel of clinically relevant Gram-positive and Gram-negative pathogens. Most of the analogs in this series displayed activity superior to Linezolid and in vivo efficacies of selected oxazolidinones are also disclosed herein.

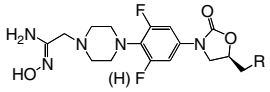
The growing incidence of bacterial resistance to antibiotics represents a serious medical and socio-economical problem. Of particular concern are infections caused by multidrug-resistant Gram-positive pathogens. Principal players among these problematic organisms are isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermis* (MRSE),[1] vancomycin-resistant *Enterococcus faecalis*, and *Enterococcus faecium* (VREF)[2] and cephalosporin-resistant *Streptococcus pneumoniae*.[3] These pathogens are responsible for significant morbidity and mortality in both the hospital[4] and community settings.[5]



Linezolid (**1**)



Eperezolid (**2**)



N-Hydroxyacetamidine series (**3**)
R = N or O linkage

Oxazolidinones, typified by Linezolid (**1**) (marketed as Zyvox™) and Eperezolid (**2**), represent a new class of synthetic antibacterial agents with potent activity against clinically important susceptible and resistant Gram-positive pathogens.[6] The many attractive traits of oxazolidinone series have encouraged further work in this area, and also the literature reveals extensive chemical programs exist.[7] In addition, this class of compounds has a novel mechanism of action that shows selective and unique binding to 50S ribosomal subunit, inhibiting bacterial translation at initiation phase of protein synthesis.[8] It is suggested that oxazolidinones act by disrupting the processing of *N*-formylmethionyl t-RNA by the ribosome.[9] However, some Linezolid-resistant clinical isolates of VREF and *S. aureus* have recently been reported.[10] This unexpected early resistance development emphasizes the need for further exploration of features of oxazolidinone series to overcome these issues.

We have implemented a research program to develop second-generation oxazolidinones with a primary goal of identifying compounds with increased potency against Gram-positive as well as fastidious Gram-negative pathogens compared to Linezolid.[11] To this end, we have explored replacing hydroxyacetamido group of Eperezolid (**2**) with *N*-hydroxyacetamidine moiety (as shown in **3**). We anticipated that such modification would improve the solubility as *N*-hydroxyacetamidines are polar in nature.[12] A series of novel oxazolidinones with various N- or O-linked groups at C-5 terminus has been made. The synthesis and antibacterial activity of these derivatives are disclosed herein.

Keywords: Oxazolidinone; Antibacterial; Linezolid; Acetamidine.
☆ DRL Publication No. 520.
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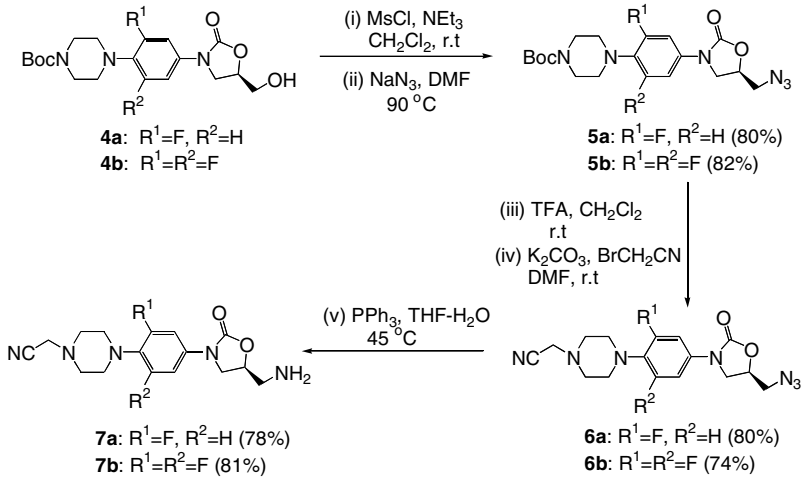
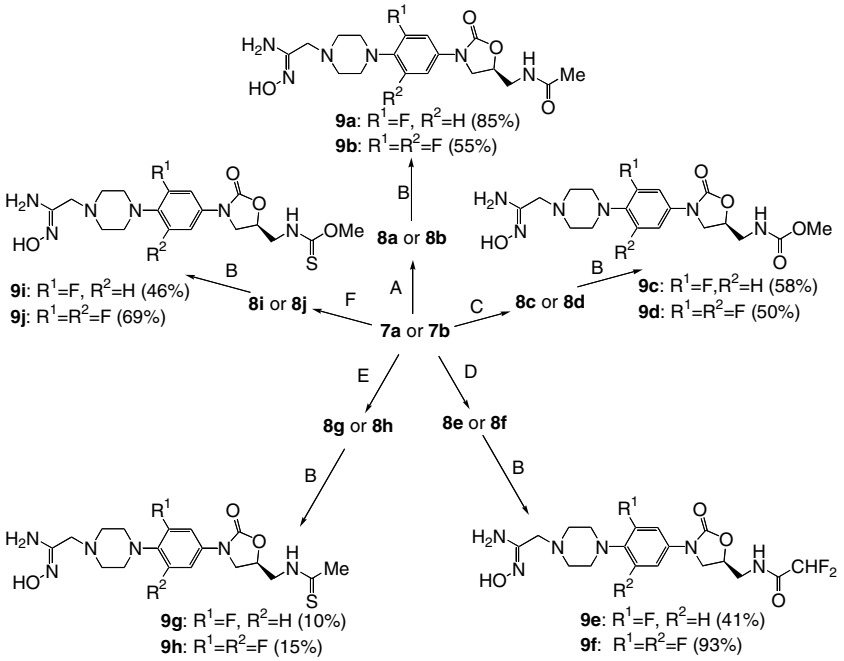
doi:10.1016/j.bmcl.2006.01.109

The key intermediates **4a**, **4b**, **5a**, and **5b** were conveniently prepared according to the reported procedure.[13] As depicted in Scheme 1, Boc group of **5a** or **5b** was cleaved using TFA and the resulting amine was subjected to base-promoted alkylation with bromoacetonitrile in the presence of potassium carbonate to yield **6a** or **6b**. The transformation of azide to amine **7a** or **7b** was readily achieved with PPh_3 in THF–H_2O mixture.

As outlined in Scheme 2, the conversion of amine **7a** or **7b** to acetamide **8a** or **8b** was carried out using Ac_2O and Et_3N and, the latter was transformed into **9a** or **9b** by reacting with *N*-hydroxylamine. The carbamate derivative **8c** or **8d** was accessed by the treatment of **7a** or **7b** with triethylamine and methylchloroformate. Finally, the reaction of **8c** or **8d** with *N*-hydroxylamine afforded **9c** or **9d**. Acylation of amine **7a** or **7b** with difluoroacetic acid in the presence of EDCI furnished difluoroacetamide **8e** or **8f**, which was further reacted with *N*-hydroxylamine to yield acetamidine derivative **9e** or **9f**. Sequential treatment of amine **7a** or **7b** with ethyl dithioacetate and then *N*-hydroxylamine gave rise to thioacetamide **9g** or **9h**. The thiocarbamate **8i** or **8j** was smoothly obtained by the reaction of amine **7a** or **7b** with triethylamine, carbon disulfide, and ethyl chloroformate to produce the corresponding isothiocyanate, which in turn reacted with excess methanol under reflux conditions. Final transformation of **8i** or **8j** to the corresponding **9i** or **9j** was routine.



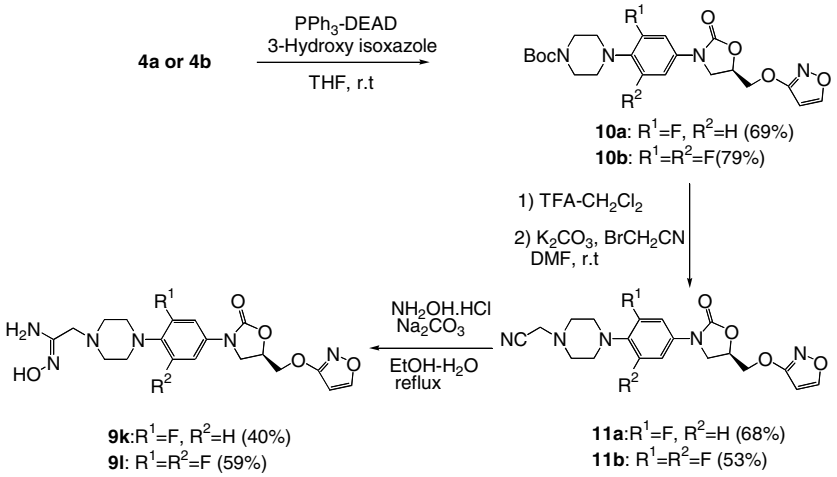
Scheme 1.



Scheme 2. Reagents and conditions: (A) Ac_2O, Et_3N, CH_2Cl_2, rt, yield **8a** (78%) and **8b** (91%); (B) $NH_2OH{\cdot}HCl$, Na_2CO_3, EtOH–H_2O, reflux; (C) ClCOOMe, DIPA, CH_2Cl_2, rt, yield **8c** (61%) and **8d** (87%); (D) CHF_2COOH, EDCI·HCl, NMM, CH_2Cl_2, rt, yield **8e** (55%) and **8f** (54%); (E) $CH_3C(S)SEt$, Et_3N, THF, rt, yield **8g** (81%) and **8h** (86%); (F) Et_3N, CS_2, ClCOOEt then MeOH, reflux, yield **8i** (62%) and **8j** (66%).

As described in Scheme 3, compound **4a** or **4b** was exposed to Mitsunobu conditions using 3-hydroxy isoxazole as a nucleophile to yield **10a** or **10b**. The *t*-Boc group was cleaved by trifluoroacetic acid treatment and the resulting amine was subjected to alkylation with bromoacetonitrile to produce **11a** or **11b**. The compound **11a** or **11b** was advanced to the corresponding *N*-hydroxyacetamidine **9k** or **9l** uneventfully by reacting with hydroxylamine hydrochloride.[14]

The synthesized oxazolidinones **9a–l** were screened against a panel of Gram-positive and Gram-negative organisms such as *Haemophilus influenzae* and *Moraxella catarrhalis* with Linezolid as a standard.[15] In a gratifying result, many of these compounds exhibited good to excellent in vitro antibacterial activity including against Gram-negative organisms. The results are summarized in Table 1. Among the C-5 aminomethyl derivatives, the compounds **9b**, **9d**, **9f**, **9g**, **9h**, **9i**, and **9j** exhibited in vitro antibacterial activity comparable or superior to that of Linezolid, whereas analogs **9a**, **9c**, and **9e** were inferior. In particular, thioacetamide **9h** and thiocarbamate **9j** were 3- to 4-fold more active against most of the targeted Gram-positive stains and Gram-negative *M. catarrhalis*, and 1-fold better against *H. influenzae* compared to standard molecule. Difluoroacetamide **9f** and thiocarbamate **9i** were found to be 1- to 2-fold more active than standard compound. Addition of two fluorines in C-5 acetamides **9a** and **9b** to produce corresponding difluoroacetamides **9e** and **9f** showed not much variation in their MIC values. It is also evident from Table 1 that thiocarbonyl compounds **9g**, **9h**, **9i**, and **9j** are more potent than the



Scheme 3.

Table 1. In vitro antibacterial activity (MIC (μg/mL)) of oxazolidinone derivatives **9a–l**

Compd	Sa[a]	Sa[b]	Sa[c]	Ef[d]	Ef[e]	Ef[f]	Mc[g]	Hi[h]
9a	16	4	4	8	2	8	8	8
9b	2	1	1	1	1	1	4	4
9c	8	16	16	4	4	16	>16	32
9d	2	1	1	1	1	2	2	8
9e	4	4	4	4	2	4	8	4
9f	2	1	1	1	0.5	1	2	4
9g	2	1	1	1	1	1	N.D.	4
9h	1	0.25	0.25	0.5	0.5	0.25	0.5	4
9i	1	0.5	0.5	0.5	0.5	0.5	2	8
9j	0.5	0.25	0.25	0.25	0.25	0.25	0.5	4
9k	16	4	4	16	8	16	8	256
9l	4	2	2	4	2	4	16	>32
LNZ	2	1	2	2	2	2	8	8

N.D., not determined.
LNZ, Linezolid.
MIC, minimum inhibitory concentration.
[a] Sa, *Staphylococcus aureus* DRCC 035 (methicillin-susceptible *S. aureus*).
[b] Sa, *Staphylococcus aureus* DRCC 019 (methicillin-resistant *S. aureus*).
[c] Sa, *Staphylococcus aureus* DRCC 446 (methicillin-resistant *S. aureus*, Ciprofloxacin-resistant).
[d] Ef, *Enterococcus faecalis* DRCC 034 (vancomycin-susceptible *E. faecalis*).
[e] Ef, *Enterococcus faecalis* DRCC 153 (vancomycin-resistant *E. faecalis*).
[f] Ef, *Enterococcus faecium* DRCC154 (vancomycin-resistant *E. faecium*).
[g] Mc, *Moraxella catarrhalis* DRCC 300 (β-lactamase −ve).
[h] Hi, *Haemophilus influenzae* DRCC 433 (β-lactamase −ve).

Table 2. In vivo efficacy in a systemic mouse infection model by oral route

Compd	ED_{50} (mg/kg/day)
9d	32.2 (18.6–56.8)[a]
9e	12.1 (8.0–18.4)
9f	18.3 (12.5–26.8)
9h	22.1 (14.2–36.3)
9i	21.2 (15.1–29.6)
9j	32.2 (18.6–56.8)
Linezolid	5.3 (2.6–9.0)

[a] Numbers in parentheses are 95% confidence ranges.

corresponding oxocarbonyl derivatives **9a**, **9b**, **9c**, and **9d**. This can be attributed to the change in electronic character and lipophilicity of the molecules.[16] Among the two O-linked analogs **9k** and **9l**, the activity of **9l** was comparable to that of Linezolid against Gram-positive pathogens. In all the cases, compounds having difluoro substitution on phenyl ring (**9b**, **9d**, **9f**, **9h**, **9j**, and **9l**) showed enhanced activity compared to their monofluoro congeners (**9a**, **9c**, **9e**, **9g**, **9i** and **9k**).

Selected oxazolidinones **9d**, **9e**, **9f**, **9h**, **9i**, and **9j** were evaluated for in vivo efficacy in a lethal systemic mouse infection model by oral route, employing *S. aureus* ATCC 29213 as the infectious organism (Table 2). These compounds protected the mice from infection following oral administration, however, at higher doses compared to Linezolid despite their potent in vitro antibacterial profile. This difference in in vivo efficacy may be due to the suboptimal pharmacokinetics.

In summary, introduction of *N*-hydroxyacetamidine group to the oxazolidinones afforded a potent series with in vitro antibacterial activity comparable or superior to Linezolid against Gram-positive and clinically significant Gram-negative bacteria. Some of the analogs from this series also exhibited in vivo activity in a lethal mouse infection model when administered orally, but were less efficacious than Linezolid.

Acknowledgments

We thank Dr. R. Rajagopalan, President, Discovery Research, Dr. Reddy's Laboratories Ltd, for his constant encouragement and valuable suggestions. We would also like to thank Analytical Department for spectral data and Formulation Department for solubility data.

References and notes

1. Ayliffe, G. A. *Clin. Infect. Dis.* **1997**, *24*(Suppl. 1), S74.
2. Leclercq, R. *Clin. Infect. Dis.* **1997**, *24*(Suppl. 1), S80.
3. (a) Thornsberry, C.; Ogilvie, P. T.; Holley, H. P., Jr.; Sahm, D. F. *Antimicrob. Agents Chemother.* **1999**, *43*, 2612; (b) Pfaller, M. A.; Kugler, K.; Freeman, J.; Jones, R. N. *Clin. Infect. Dis.* **1998**, *27*(Suppl. 1), 764.
4. Pfaller, M. A.; Jones, R. N.; Doern, G. V.; Sader, H. S.; Kugler, K. C.; Beach, M. L. *Diagn. Microbiol. Infect. Dis.* **1999**, *33*, 283.
5. (a) Abi-Hanna, P.; Frank, A. L.; Quinn, J. P.; Kelkar, S.; Schreck-enberger, P. C.; Hayden, M. K.; Marcinak, J. F. *Clin. Infect. Dis.* **2000**, *30*, 630; (b) Collignon, P. *J. Infect. Dis.* **1999**, *179*, 1592; (c) Merlino, J.; Leroi, M.; Bradbury, R.; Veal, D.; Harbour, C. *J. Clin. Microbiol.* **2000**, *38*, 2378; (d) Cookson, B. D. *Infect. Control Hosp. Epidemiol.* **2000**, *21*, 398.
6. Clemett, D.; Markham, A. *Drug* **2000**, *59*, 815.
7. (a) Gravestock, M. B.; David, G. A.; Betts, M. J.; Dennis, M.; Hatter, G.; McGregor, A.; Swain, M. L.; Wilson, R. G.; Woods, L.; Wookey, A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4179; (b) Philips, O. A.; Udo, E. E.; Ali, A. A. M.; Samuel, S. M. *Bioorg. Med. Chem.* **2005**, *13*, 4113; (c) Jang, S. Y.; Ha, Y. H.; Ko, S. W.; Lee, W.; Lee, J.; Kim, S.; Kim, Y. W.; Lee, W. K.; Ha, H. J. *Bioorg. Med. Chem. Lett.* **2004**, *12*, 3881; (d) Lee, C. S.; Allwine, D. A.; Barbachyn, M. R.; Grega, K. C.; Dolak, L. A.; Ford, C. W.; Jensen, R. M.; Seest, E. P.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H.; Zurenko, G. E.; Genin, M. J. *Bioorg. Med. Chem.* **2001**, *9*, 3243; (e) Griera, R.; Llopart, C. C.; Amat, M.; Bosch, J.; Castilo, J. C. D.; Huguet, J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2515.
8. Shinabarger, D. L.; Marotti, K. R.; Murray, R. W.; Lin, A. H.; Melchoir, E. P.; Swaney, S. M.; Dunyak, D. S.; Demyan, W. F.; Buysse, J. M. *Antimicrob. Agents Chemother.* **1997**, *41*, 2132.
9. Kloss, P.; Xiong, L.; Mankin, A. S.; Shinabarger, D. L.; Mankin, A. S. *J. Mol. Biol.* **1999**, *294*, 93.
10. (a) Tsirodas, S.; Gold, H. S.; Sakoulas, G.; Eliopoulas, G. M.; Wennerstein, C.; Venkataraman, L.; Moellering, R. C., Jr.; Ferro, M. J. *Lancet* **2001**, *358*, 207; (b) Johnson, A. P.; Tysall, L.; Stockdale, M. W.; Woodford, N.; Kaufmann, M. E.; Warner, M.; Livermoore, D. M.; Asboth, F.; Alleberger, F. J. *Eur. J. Clin. Microbial. Infect. Dis.* **2002**, *21*, 751.
11. (a) Selvakumar, N.; Srinivas, D.; Khera, M. K.; Kumar, M. S.; Mamidi, R. N. V. S.; Sarnaik, H.; Charavaryamath, C.; Rao, B. S.; Raheem, M. A.; Das, J.; Iqbal, J.; Rajagopalan, R. *J. Med. Chem.* **2002**, *45*, 3953; (b) Das, J.; Rao, C. V. L.; Sastry, T. V. R. S.; Roshiah, M.; Sankar, P. G.; Khadeer, A.; Kumar, M. S.; Mallik, A.; Selvakumar, N.; Iqbal, J.; Trehan, S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 337; (c) Selvakumar, N.; Raheem, M. A.; Khera, M. K.; Rajale, T. V.; Kumar, M. S.; Kandepu, S.; Das, J.; Rajagopalan, R.; Iqbal, J.; Trehan, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4169.
12. Solubility of compound **9a** in MQ water was determined by UV-spectrophotometry method at 25 °C after 4 h shaking. The water solubility of **9a** was found to be 5.07 mg/mL, whereas the solubility of Linezolid is 3.06 mg/mL under the same conditions.
13. Hutchinson, D. K.; Brickner, S. J.; Barbachyn, M. R.; Gammill, R. B. PCT Int. Appl. WO 93/23384.
14. Typical procedure (**9l**): To a solution of compound **11b** (330 mg, 0.79 mmol) in ethanol (30 mL) were added hydroxylamine hydrochloride (219 mg, 3.19 mmol) and saturated aqueous solution of sodium carbonate (250 mg). The mixture was refluxed for overnight and the solvent was removed under vacuum. The residue was diluted with ethylacetate and washed with water and brine successively. The organic layers were dried over sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography over silica gel using 3% MeOH/$CHCl_3$ as eluent. Compound **9l** was obtained as a white solid (210 mg, 59%). ^1H NMR (DMSO-d_6): δ 8.97 (s, 1H, D_2O exchangeable), 8.69 (d, J = 1.9 Hz, 1H), 7.28 (d, J = 11.5 Hz, 2H), 6.37 (d, J = 1.9 Hz, 1H), 5.28 (s, 2H, D_2O exchangeable), 5.10–5.05 (m, 1H), 4.50–4.42 (m, 2H), 4.16 (t, J = 9.1 Hz, 1H), 3.88

(dd, J_1 = 6.5 Hz, J_2 = 9.1 Hz, 1H), 3.17–3.05 (m, 4H), 2.89 (s, 2H), 2.55–2.49 (m, 4H); IR (KBr pellet) 3453, 2923, 1753, 1664, 1582, 1513, 1451, 1245, 1120, 1023 cm^{-1}; MS: (*m*/*z*) 453 (M+1).

15. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial-susceptibility tests for bacteria that grow aerobically. Approved Standard, 5th ed.; NCCLS: USA, 2000; M7-A5, Vol. 20, No. 2.
16. (a) Tokuyama, R.; Takahashi, Y.; Tomita, Y.; Tsubochi, M.; Yoshida, T.; Iwasaki, N.; Kado, N.; Okezaki, E.; Nagata, O. *Chem. Pharm. Bull.* **2001**, *49*, 353; (b) Tokuyama, R.; Takahashi, Y.; Tomita, Y.; Tsubochi, M.; Iwasaki, N.; Kado, N.; Okezaki, E.; Nagata, O. *Chem. Pharm. Bull.* **2001**, *49*, 361; (c) Tokuyama, R.; Takahashi, Y.; Tomita, Y.; Suzuki, T.; Yoshida, T.; Iwasaki, N.; Kado, N.; Okezaki, E.; Nagata, O. *Chem. Pharm. Bull.* **2001**, *49*, 347; (d) Ciske, F. L.; Barbachyn, M.; Genin, M. J.; Grega, K. C.; Lee, C. S.; Dolak, L. A.; Seest, E. P.; Watt, W.; Adams, W. J.; Friis, J. M.; Ford, C. W.; Zurenko, G. E. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4235; (e) Thomasco, L. M.; Gadwood, R. C.; Weaver, E. A.; Ochoada, J. M.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Stapert, D.; Moerman, J. K.; Schaadt, R. D.; Yagi, B. H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4193.