Published in final edited form as:
Bioorg Med Chem Lett. 2010 February 1; 20(3): 1302. doi:10.1016/j.bmcl.2009.10.018.

Syntheses and antibacterial activity studies of new oxazolidinones from nitroso Diels–Alder chemistry

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Abstract

A series of novel oxazolidinone antibiotics having [2.2.1] and [2.2.2] bicyclic oxazine moieties at the C-5 side chain of the A ring was synthesized by nitroso Diels–Alder reactions, from three linezolid analogs containing morpholine, piperazine and thiomorpholine, respectively, as the C-ring components. Subsequent N–O bond cleavage generated oxazolidinones with 4-amino cyclo-2- en-1-ol substituents. The *in vitro* antibacterial activities of these oxazolidinone analogs were evaluated.

Oxazolidinones are a new class of synthetic antimicrobial agents which are now clinically useful. They inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit at the translation step.[1] The most promising feature of these compounds is their oral activity against multidrug-resistant Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) as well as select anaerobic organisms.[2] Linezolid (Figure 1) is the first oxazolidinone drug approved for the treatment of Gram-positive bacterial infections in humans.[2b,3] Unfortunately, resistance to linezolid has already been observed in Gram-positive bacteria such as *S. aureus* and *Enterococcus faecium*.[4] More recently, resistant strains of MRSA, *E. coli* and other bacteria have also been identified.[5] Thus, there is significant need for the rapid development of novel antibacterial agents. Four Types of chemical modifications of linezolid and oxazolidinone-type antibiotics have been reported, including modifications on each of the A-, B- and C-rings as well as the C-5 side chain of the A ring substructure.[2b,6] The structure-activity- relationship studies of oxazolidinone antibiotics show that the stereochemistry (*S*) at the C-5 position of the A ring is critical in terms of biological activity. While an acetamide substituent on the 5-methyl group is found to produce optimal antibacterial activity, modification of the C-5 side chain would still benefit the development of new oxazolidinone antibiotics. In this regard, a frequently employed strategy involves the addition of heterocycles, such as triazoles,[7] and pyridones.[8] However, to the best of our knowledge, the incorporation of bicyclic oxazines into this class of antibiotics has not been disclosed.

Syntheses of interesting biologically relevant molecules using nitroso Diels–Alder chemistry (NDA) is of growing interest. As a synthetic tool for the formation of 4-amino cyclopent-2-en-1-ol derivatives in one step, acylnitroso Diels–Alder reactions are useful for the creation of unique structural and functional diversity.[9] We and others have demonstrated that the nitroso

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bicyclic oxazine **3** derived from NDA reaction between *in situ* generated acylnitroso **2** and 1,3-cyclopentadiene can undergo various chemical transformations (Scheme 1).[9b] We envisioned that modification at the A-ring C-5 side chain of linezolid-type oxazolidinone antibiotics using nitroso Diels–Alder chemistry would generate novel analogs with incorporation of bicyclic oxazine functionalities. These new analogs were anticipated to not only enhance the available set of oxazolidinone antibiotics, but also provide new scaffolds for further transformations. Herein we report the syntheses and biological evaluation of a series of oxazolidinone antibiotics in which the C-5 side chain has been modified by nitroso Diels–Alder reactions and subsequent chemical transformations.

To begin our investigation, three types of oxazolidinone precursors **14**–**16** were synthesized from commercial piperazine (**4**), morpholine (**6**) and thiomorpholine (**7**), respectively, as shown in Scheme 2. The syntheses began by separate S_NAr reactions of 3,4-difluoronitrobenzene with mono Boc-protected piperazine, **5**, morpholine, **6**, and thiomorpholine, **7**, under basic conditions to afford para-substituted nitrobenzene derivatives **8**–**10** in moderate to good yields. Palladium catalyzed hydrogenolyses of **8** and **9**, followed by reactions with CbzCl gave protected anilines **11**–**12**. The synthesis of thiomorpholine analog **13** required modification to avoid poisoning of the catalyst by the sulfur atom during the hydrogenation step. Thus, use of indium powder and ammonium chloride[10] successfully reduced the nitro group of **10** and gave compound **13** in 62% yield after Cbz protection. Then, as reported,[11] compounds **11**–**13** were treated with *n*BuLi and *R*-glycidyl butyrate sequentially at −78 °C to form oxazolidinone analogs **14**–**16** in 70–80% yields with defined stereochemistry at the A-ring C-5 position.

With the desired aza-, oxa-, and thia-analogs, **14**–**16**, in hand, introduction of the *N*-hydroxy carbamate moiety at the C-5 side chain and subsequent nitroso Diels–Alder reactions were explored (Scheme 3). Reactions of**14** and **15** with *O*-benzylhydroxylamine hydrochloride (OBHA·HCl) and carbonyldiimidazole (CDI) generated protected *N*-hydroxy carbamates **17** and **18** in 96% and 94% yields, respectively. Hydrogenolyses afforded the desired free *N*-hydroxy carbamates, **20** and **21**, which were directly used in nitroso Diels–Alder reactions without further purification. Toward this end, *N*-hydroxy carbamate **20** was chosen as the model substrate. The cycloaddition reaction was carried out by *in situ* oxidation of **20** in the presence of 1,3-cyclohexadiene. Among several oxidants attempted, including sodium periodate ($NaIO_4$), Dess-Martin and $FeCl_3/H_2O_2$,[12] we found that use of sodium periodate gave cycloadduct **23b** in the best yield (56%) as one regioisomer after chromatography purification. For the thiomorpholine series, in order to again avoid catalyst poisoning during the subsequent deprotection step, *O*-p-methoxybenzyl hydroxylamine hydrochloride ($PMBONH_2$·HCl)[13] instead of OBHA·HCl was used in the coupling reaction with **16**. The PMB group was removed under acidic conditions to give *N*-hydroxy carbamate **22** in good yield. Using the optimized NDA reaction conditions, a series of new oxazolidinones **23a**–**25b** with bicyclic oxazines at the C-5 side chain were synthesized. Among them, cycloadducts **25a** and **25b** were obtained with the sulfur atom in the thiomorpholinyl ring oxidized. N–O bond cleavage reactions of these cycloadducts were also carried out, with intent to generate the corresponding oxazolidinones with additional 1,4-amino alcohol substituents. Thus, $Mo(CO)_6$[14] successfully reduced the N–O bond of compounds **23a**–**25b** to generate the corresponding oxazolidinone analogs, **26a**–**28b**, that contain aminocycloalkenol functional groups, in moderate yields. In addition, under $Mo(CO)_6$ conditions, the sulfoxide group of **25a** and **25b** was also reduced. For comparison during the biological testing, an oxazolidinone analog **29** with a C-5 acetate substituent was synthesized from compound **15** (Scheme 4).

With the biological profile of parent linezolid, **1**, known for comparison, all the oxazolidinone analogs **23a**–**28b**, **29**, as well as precursors **14**–**16**, and ciprofloxacin, as a positive control, were subjected to broad antibacterial studies against various strains of Gram-positive and

Gram-negative bacteria as well as *Mycobacterium vaccae*, using agar diffusion assays (Table 1).[15] Compound **29** was found to be comparable to linezolid (**1**) itself for all tested organisms, as expected. Interestingly, all the oxazolidinone precursors **14**–**16** were roughly equipotent *in vitro* with linezolid (**1**) against several Gram-positive organisms, including *Bacillus subtilis*, *S. aureus*, *E. faecalis* and *M. luteus*. They also exhibited antimycobacterial activity and could potentially be useful for treatment of *M. tuberculosis* as they induced large inhibition zones against *Mycobacterium vaccae*, a common model for *M. tuberculosis*. Most oxazolidinone analogs with bicyclic oxazine or aminocycloalkenol moieties at the C-5 side chain, generated from NDA chemistry, had antimicrobial profiles similar to linezolid (**1**), but at a generally diminished level of activity. In general, oxazolidinone analogs substituted with [2.2.1] bicyclic oxazines (**23a**–**25a**) were more active than those with [2.2.2] bicyclic oxazines (**23b**–**25b**). Among them, compound **25a** derived from thiomorpholine exhibited the best activity. Analogs **26a**–**28b**, derived from reduction of the N–O bonds were relatively inactive compared to their parent cycloadducts.

In summary, we have synthesized a series of novel oxazolidinone antibiotics with [2.2.1] and [2.2.2] bicyclic oxazine as well as aminocycloalkenol moieties at the C-5 side chain through nitroso Diels–Alder chemistry. These oxazolidinone analogs exhibited *in vitro* antibacterial profiles similar to that of linezolid.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge the NIH (GM 075885) for support of this research. We thank Uta Wohlfeld (HKI) for performing antibacterial assays. We also thank the Lizzadro Magnetic Resonance Research Center at Notre Dame for NMR facility and Nonka Sevova for mass spectroscopic analyses. TAW acknowledges the University of Notre Dame Chemistry-Biochemistry-Biology Interface (CBBI) Program and NIH Training Grant T32GM075762 for a fellowship.

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15. The *in vitro* biological testing of *N*-hydroxy carbamates **20–22** (>90% pure based on LC/MS) showed these compounds also retained antimicrobial profiles similar to linezolid (**1**), but at a generally diminished level of activity. For detailed data, see Table 1 in Supplementary Content.

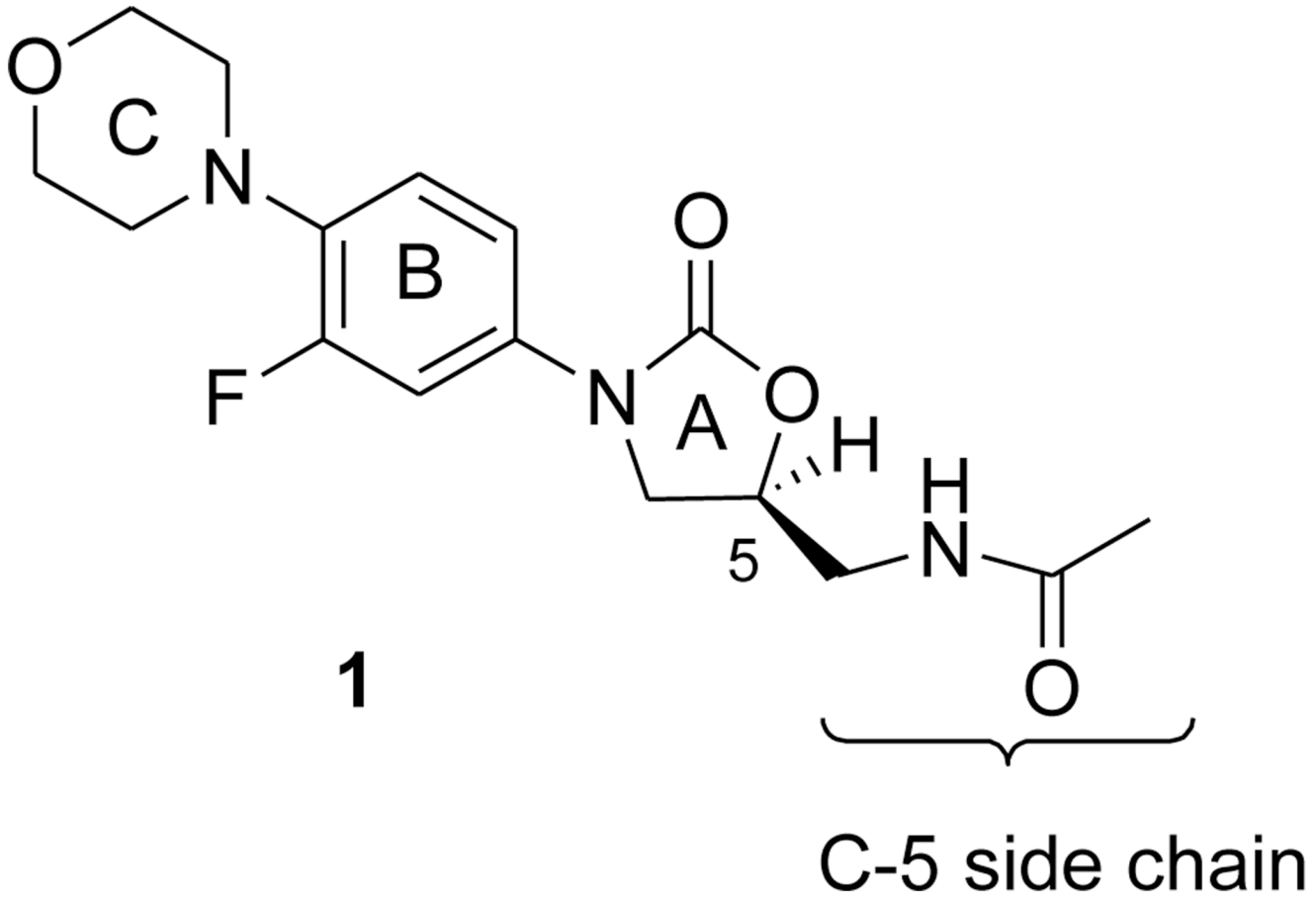
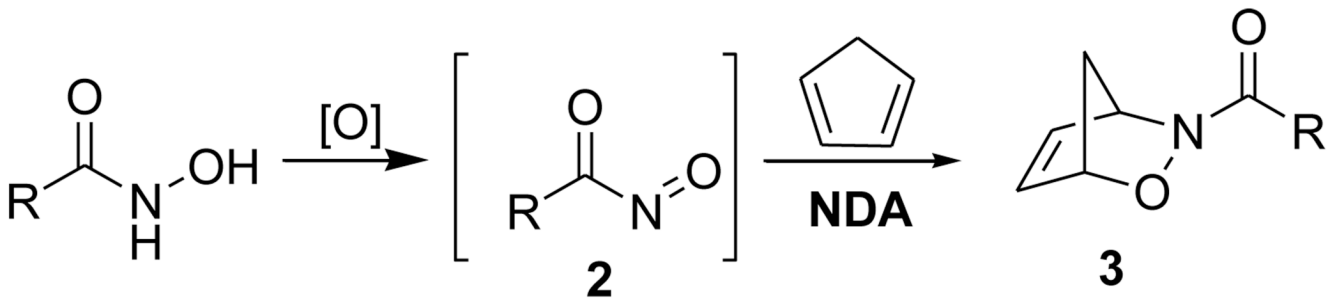
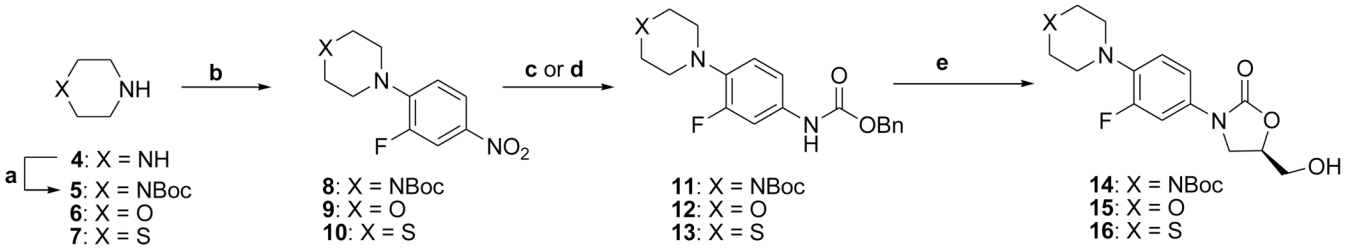


Figure 1.
Linezolid antibiotic (**1**).

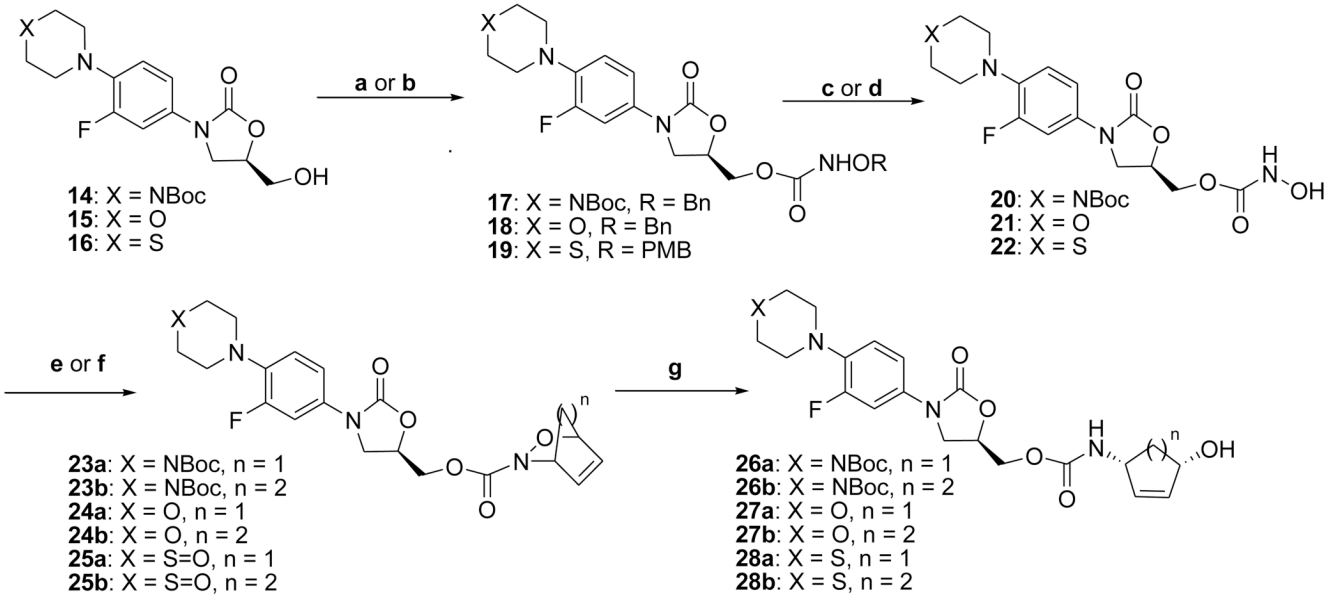


Scheme 1.
Acylnitroso Diels–Alder (NDA) reaction



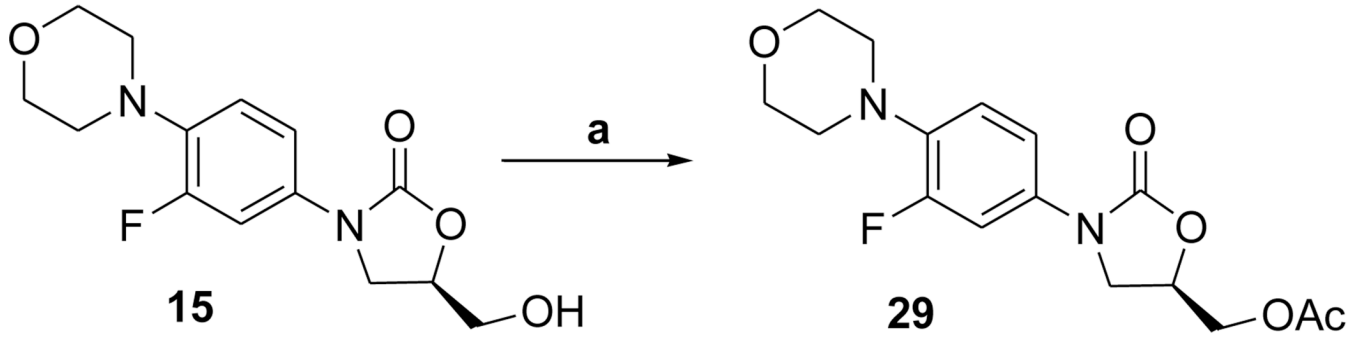
Scheme 2.

Reagents and conditions: (a) Boc_2O, DCM, 50%; (b) 3,4-difluoronitrobenzene, Hunig's base, CH_3CN, 49% (for **8**), 84% (for **9**), 70% (for **10**); (c) (i) Pd/C, H_2, MeOH, (ii) CbzCl, $NaHCO_3$, THF/H_2O, 89% (for **11**), 92% (for **12**); (d) (i) In, NH_4Cl, EtOH, reflux, (ii) CbzCl, $NaHCO_3$, THF/H_2O, 62% (for **13**); (e) (i) *n*BuLi, THF, −78 °C, (ii) *R*-glycidyl butyrate, 71% (for **14**), 70% (for **15**), 80% (for **16**).



Scheme 3.

Reagents and conditions: (a) (i) CDI, CH_3CN (ii) OBHA·HCl, imidazole, 96% (for **17**), 94% (for **18**); (b) CDI, CH_3CN, (ii) $PMBONH_2$·HCl, imidazole, 92% (for **19**); (c) Pd/C, H_2, MeOH; (d) (i) 10% TFA, DCM, (ii) sat. Na_2CO_3; (e) $NaIO_4$, 1,3-cyclopentadiene, MeOH/H_2O, 0°C, 50% (for **23a**, from **17**), 49% (for **24a**, from **18**), 40% (for **25a**, from **19**); (e) $NaIO_4$, 1,3-cyclopentadiene, MeOH/H_2O, 0°C, 50% (for **23a**, from **17**), 49% (for **24a**, from **18**), 40% (**25a**, from **19**); (f) $NaIO_4$, 1,3-cyclohexadiene, MeOH/H_2O, 0°C, 56% (for **23b**, from **17**), 47% (for **24b**, from **18**), 42% (**25b**, from **19**); (g) $Mo(CO)_6$, CH_3CN/H_2O, 80°C, 50% (for **26a**), 43% (for **26b**), 42% (for **27a**), 62% (for **27b**), 54% (for **28a**), 55% (for **28b**).



Scheme 4.
Reagents and conditions: (a) Ac_2O, Pyridine, DMAP, DCM, 75%.

Table 1

Antimicrobial activity in the agar diffusion assay (Diameter of inhibition zone, measured in mm).

Compds	Gram-positive bacteria						Gram-negative bacteria			
	Bacillus subtilis	*Micrococcus luteus*	*Staphylococcus aureus*		*Enterococcus faecalis*		*Escherichia coli*	*Pseudomonas aeruginosa*		*Mycobacterium vaccae*
	ATCC 6633	ATCC 10240	SG 511[a]	134/93 (MRSA)	ATCC 49532	1528 (VRE)	ATCC 25922	K799/WT[a]	K799/61[b]	IMET 10670
1	31/35P	38	27/36P	NT	25	NT	0	0	10P	42
29	26.5	38/46p	26/35P	NT	24	NT	0	0	17h	43
14	23/26P	34.5	24/33P	NT	21	NT	0	0	10P	NT
15	26/29P	38	23/32P	NT	21	NT	0	0	11P	43
16	26/29P	41	24/35P	NT	21.5	NT	0	0	11P	43
23a	12/18P	16	13/19p	19	19	16	0	14/20p	0	19p
23b	18P	NT	16P	14p	NT	12P	NT	NT	0	13
24a	17/30P	NT	15/21p	25	NT	19	NT	NT	0	30
24b	19P	NT	16P	18p	NT	16	NT	NT	0	15
25a	21/28P	22/32P	18/22p	NT	19	NT	0	0	11P	35
25b	17P	15p	15p	NT	17	NT	0	0	10P	18
26a	13h	12P	14p	NT	12.5	NT	0	0	10P	12h
26b	0	0	0	NT	12p	NT	0	0	12P	0
27a	14P	NT	15P	14p	NT	12P	NT	NT	0	18
27b	13P	NT	14P	14P	NT	12P	NT	NT	0	0
28a	14P	15h	12.5p	NT	12.5	NT	0	0	10P	18
28b	12h	14	0	NT	12	NT	0	0	10P	0
cipro	29.5	0	24	0	13	15	31/40P	35	39	19p

[a] wild type.

[b] permeability mutant.

p, partially clear inhibition zone/colonies in the inhibition zone.

P, unclear inhibition zone/many colonies in the inhibition zone.

h, faint indication of inhibition zone.

Exactly 50 μl of a 2.0 mM solution of each compound dissolved in 1:9 DMSO:MeOH was filled in 9 mm wells in agar media (Standard I Nutrient Agar, Serva or Mueller Hinton II Agar, Becton, Dickinson and Company). Inhibition zones read after incubation at 37 °C for 24 h. Cipro (ciprofloxacin) was dissolved in H_2O to give 5 μg/mL solution.