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Bioorganic & Medicinal Chemistry Letters 15 (2005) 2834–2839
Bioorganic & Medicinal Chemistry Letters

Synthesis and antibacterial activity of dihydro-1,2-oxazine and 2-pyrazoline oxazolidinones: novel analogs of linezolid

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Received 18 June 2004; revised 22 March 2005; accepted 25 March 2005
Available online 28 April 2005

Abstract—The synthesis and antibacterial activity of oxazolidinones containing dihydro-1,2-oxazine and 2-pyrazoline ring systems are described. Linezolid analogs utilizing dihydro-1,2-oxazines as morpholine mimics were prepared utilizing a nitrosoamine/diene 4+2 cycloaddition strategy. Pyrazolidine, hexahydro-pyridazine, and 2-pyrazoline analogs more closely related to eperezolid were also prepared. The most active of these new oxazolidinones were the dihydro-1,2-oxazine **6** and the 2-pyrazoline **20** both of which had potency similar to linezolid against a panel of Gram-positive bacteria.

The oxazolidinone antibacterial linezolid (**1**) discovered and developed by Upjohn/Pharmacia (now marketed by Pfizer as Zyvox™) has been described as the first new class of antibacterial agents to be marketed in the last 30 years. The related eperezolid (**2**) (Fig. 1) was not advanced to Phase 3 studies presumably due to its shorter human $T_{1/2}$.[1]

The urgency of discovering new classes of antibacterial agents was highlighted when the first clinical case of vancomycin-resistant *Staphylococcus aureus* (VRSA) infection was reported in 2002.[2] Clinically, one of linezolid's most valuable characteristics is that it is one of the few orally active agents available to treat MRSA[1] and VRSA[2] infections. Another distinguishing feature of this class of antibacterial agents is its synthetic origin.

Efforts to enhance the spectrum and potency of this class of antibiotics have been ongoing throughout the pharmaceutical industry.[3] It has been argued that the most urgent improvement needed for the oxazolidinone class is an enhanced safety profile.[4] Hematological toxicity (possibly due to myelo-suppression) is a side effect especially during prolonged treatment (>14 days). Based on our analysis of the limited published oxazolidinone toxicity data available at the time, we concluded that the most prudent approach would be to maintain the amine functionality at the 4-position of the phenyl ring.

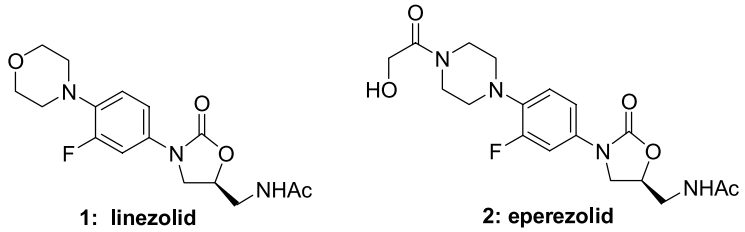

Figure 1. Linezolid and eperezolid.

Keywords: Antibacterial; Oxazolidinones; Linezolid.
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doi:10.1016/j.bmcl.2005.03.099

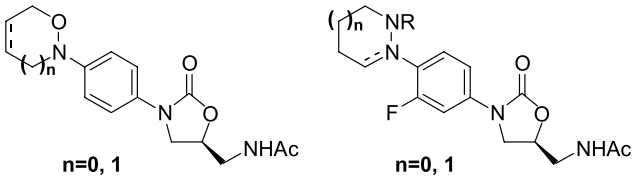
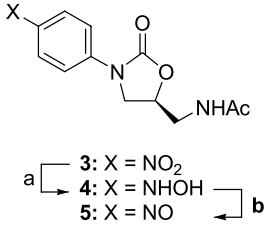


Figure 2. Pseudo-isomers of linezolid and eperezolid.

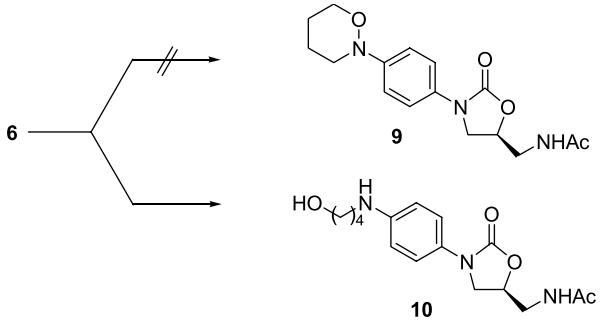


Scheme 1. Synthesis of hydroxylamine and nitroso derivative. Reagents and conditions: (a) Zn, NH_4Cl, 60 °C, 10 min; (b) PCC, rt, 10 min, THF/CH_3CN (5:2).

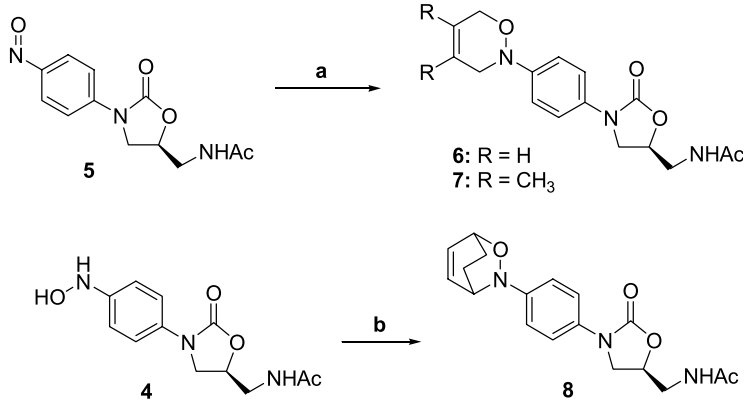
Further, we felt that modulating (either reducing or increasing) the electron-donating ability of the nitrogen in the 4-position of the phenyl ring could have a beneficial effect on the safety profile.[5] It should be emphasized that these presumed structure/toxicity relationships remain unproven to date. The SAR of amines in the 4-position was studied in detail by Upjohn/Pharmacia, but 4-amino groups imparting useful properties continue to emerge.[6–8] In this paper, we will detail our efforts to replace the linezolid morpholine by the pseudo-isomeric dihydro-1,2-oxazine and isoxazolidine ring systems. In addition, we will describe the replacement of the eperezolid piperazine by pyrazolidine, hexahydropyridazine, and 2-pyrazoline rings (Fig. 2).

These morpholine and piperazine replacements were chosen based on their modest structural resemblance, their modified electronic properties,[9] and the fact that they remained unexplored by previous researchers. In general, these cyclic amines containing an N–O or N–N linkage are less basic and less electron donating than the corresponding morpholine, piperazine, and pyrrolidine ring systems.[10] Inspired by a synthetic strategy used to functionalize the 7-position of quinolone antibacterials,[11] the known nitro oxazolidinone **3**[12] was reduced with Zn/NH_4Cl to give the hydroxylamine **4** in 94% yield. Oxidation of **4** with PCC provided the nitroso derivative **5** in 56% isolated yield (Scheme 1). The [4+2] cycloaddition of **5** with butadiene and 2,3-dimethylbuta-1,3-diene gave rise to dihydro-1,2-oxazines **6** and **7** (89% and 57% yield, respectively) (Scheme 2). In some cases, it was possible to carry out this two step sequence in one pot by generating the nitroso derivative **5** in situ in the presence of the appropriate diene. For instance, this in situ oxidation procedure carried out in the presence of cyclohexadiene provided the dihydro-1,2-oxazine **8** (31%) (Scheme 2). Attempted catalytic hydrogenation of the olefinic double bond in **6** did not result in the formation of **9**, instead, the N–O bond was severed and the ring-opened product **10** was formed (Scheme 3). This result was somewhat surprising based on the successful catalytic hydrogenation of similar dihydro-1,2-oxazines.[13] Attempted diimide reduction of **8** resulted in no reaction.

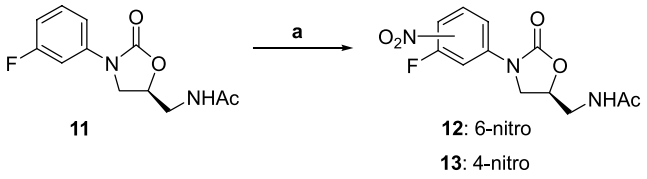
The fluoro analog of **5** was sought as a key intermediate to prepare a fluorinated series of dihydro-1,2-oxazines. Nitration of 3-fluoro-oxazolidinone **11**[14] provided a 1:1 ratio of the 6-nitro oxazolidinone **12** and the desired 4-nitro regioisomer **13** (Scheme 4). Separation by preparative HPLC gave a 20% yield of each pure



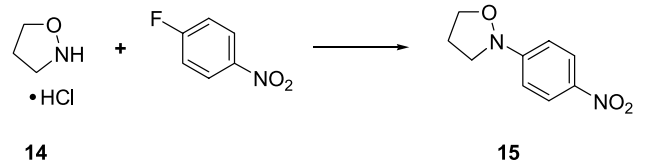
Scheme 3. Reduction of dihydro-1,2-oxazine **6** (H_2, 5% Pd/C, EtOAc, Parr shaker, 2 h).



Scheme 2. Nitroso amine/diene 4+2 cycloadditions. Reagents and conditions: (a) butadiene or 2,3-dimethylbuta-1,3-diene, 0 °C to rt, 3 h, CH_2Cl_2; (b) 4.3 equiv Bu_4NIO_4, cyclohexadiene, DMF, rt, 3 h.



Scheme 4. Nitration of **11**. Reagents and conditions: (a) KNO_3, H_2SO_4, 0 °C to rt, 4 h.



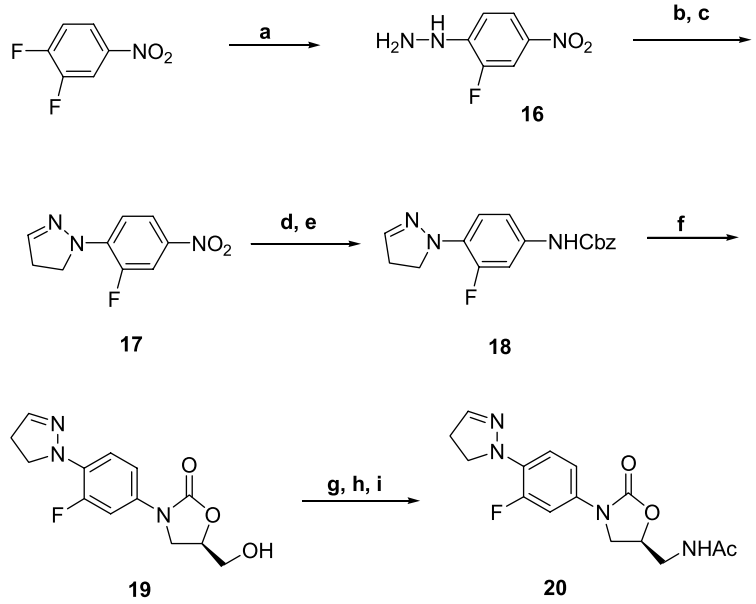
Scheme 5. Isoxazolidine analog.

regioisomer. Unfortunately, we were unsuccessful in our attempt to reduce **13** to the hydroxylamine. This prevented our synthesis of the fluorinated dihydro-1,2-oxazine series. Alternate, more laborious routes to this series were considered but regrettably never undertaken.

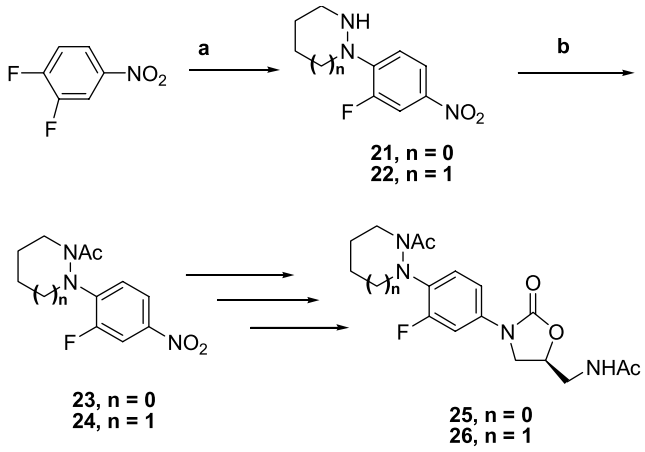
An approach to the five-membered ring analog of **9** is shown in Scheme 5. Isoxazolidine **14** reacted smoothly with 4-fluoronitrobenzene to give isoxazolidine **15** (86%). While selective reduction of the nitro group seemed to be a risky proposition, we gained some confidence after finding precedence for the selective reduction of a Cbz group,[15] a urea,[16] and an oxime moiety[17] all in the presence of a N–O bond. Unfortunately, all attempts to selectively reduce the nitro group in **15** failed (Pd/NH_4OCHO, $SnCl_2$, or $Na_2S_2O_4$).

The 2-pyrazoline **20** was prepared as shown in Scheme 6. Hydrazine was added to 3,4-difluoronitrobenzene to give a 98% yield of arylhydrazine **16**. Dialkylation of the hydrazine moiety with 1,3-dibromopropane followed by air oxidation led to a 39% yield of 2-pyrazoline **17**. Optimization of these reaction conditions was not undertaken, so it remains uncertain whether other oxidants would be superior to air for this conversion. Under the air oxidation conditions employed, no sign of over oxidation to the pyrazole was observed although minor impurities were not investigated. Phase transfer catalytic hydrogenation provided the aniline (84% yield), which was treated with benzylchloroformate to give the carbamate **18** (80% yield). Note that these are the same catalytic phase transfer reduction conditions which led to over-reduction (N–O bond cleavage) in the above case (see Scheme 5). At this point, general methodology documented by Upjohn/Pharmacia[1] was employed. The anion of carbamate **18**, formed using *n*-BuLi, was quenched by the addition of (*R*)-glycidyl butyrate. The hydroxymethyl oxazolidinone **19** is obtained upon workup (75% yield). Mesylate formation followed by aminolysis and acetylation ultimately gave the final product **20** (50% yield over three steps).

Analogs **25** and **26**, which are related to eperezolid, were prepared similarly as shown in Scheme 7. The bis TFA salts of pyrazolidine and hexahydro-pyridazine[18] were reacted with 3,4-difluoronitrobenzene in the presence of excess Hünig's base to give nitro derivatives **21** and **22** (30% and 83% yield, respectively). Acylation provided *N*-acetate derivatives **23** and **24**, which were



Scheme 6. Synthesis of 2-pyrazoline analog **20**. Reagents and conditions: (a) hydrazine hydrate, pyridine, rt, 50 min; (b) NaH, 1,3-dibromopropane, DMF, 0 °C to rt, 3 h; (c) air; (d) 10% Pd/C, HCO_2NH_4, THF/MeOH (1:2); (e) CbzCl, $NaHCO_3$, H_2O/acetone (1:2), 0 °C to rt, 2 h; (f) *n*-BuLi, (*R*)-glycidyl butyrate, THF, −78 °C to rt, 18 h; (g) MsCl, NEt_3, CH_2Cl_2, rt, 1 h; (h) NH_4OH, THF, *i*PrOH, 100 °C, 5 h, sealed tube; (i) Ac_2O, triethylamine, CH_2Cl_2, rt, 2 h.

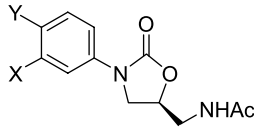


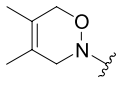
Scheme 7. Synthesis of **25** and **26**. Reagents and conditions: (a) iPr$_2$NEt, and pyrazolidine or hexahydro-pyridazine, CH_3CN, 90 °C, 10 h; (b) Ac_2O, pyridine, DMAP, THF, rt, 1 h.

converted to oxazolidinones **25** and **26** via standard methods.[1]

The minimum inhibitory concentrations[19] (MICs) are shown in Table 1. This panel is composed of some representative Gram-positive bacteria, including streptococci, staphylococci, and enterococci. It should be noted that the *staph aureus* strain is methicillin-susceptible and penicillinase positive, and the enterococci are not vancomycin-resistant strains. The dihydro-1,2-oxazine series appeared to be sensitive to the overall size of the oxazine ring. The smallest, least substituted member **6** showed the best potency against this panel of Gram-positive bacteria, while the most bulky dihydro-1,2-oxazine **8** was the least potent. This is consistent with established oxazolidinone SAR indicating that excessive steric bulk adjacent to the aryl ring is detrimental to activity.[20] Oxazolidinone **6** is roughly equivalent in potency versus linezolid against this panel. It is

Table 1. Minimum inhibitory concentrations (μg/mL)



Compd	X	Y	Minimum inhibitory concentration (μg/mL)[a]					
			S. aureus[b]	*S. pneumo*[c]	MRSA[d]	MRSA[e]	*E. faecalis*[f]	*E. faecium*[g]
1 Linezolid	F	Morpholine	1	1	2	2	4	2
2 Eperezolid	F	$HOCH_2C(O)$piperazine	1	0.5	1	1	2	2
Ref.	H	NH_2	16	16	128	64	128	128
3	H	NO_2	0.5	2	2	1	4	4
13	F	NO_2	>128	64	128	128	>128	>128
4	H	NHOH	0.5	4	4	2	8	4
5	H	NO	2	1	2	1	4	2
6	H		1(1)	2	2(2)	1(1)	4	2
7	H		4	8	8	4	16	8
8	H		16	16	8	4	32	16
25	F		32	0.5	32	16	32	32
26	F		32	4	32	16	32	32
20	F		1(1)	1	1(1)	1(1)	2	4

[a] Values in parentheses are MIC's in the presence of 50% calf serum.
[b] *Staphylococcus aureus*/Pen$^+$ A15090.
[c] *Streptococcus pneumoniae* A9585.
[d] *Staphylococcus aureus*/heterogeneously methicillin-resistant A27218.
[e] *Staphylococcus aureus*/homogeneously methicillin-resistant A27223.
[f] *Enterococcus faecalis* A20688.
[g] *Enterococcus faecium* A24885.

quite possible that the fluoro analog of **6** would have been more active than linezolid against this panel of Gram-positive bacteria. This 'fluoro' effect is evident in most of the oxazolidinone series (e.g., piperazines) in which sufficient MIC data have been published.[1]

Turning to the eperezolid analogs, the pyrazolidine derivative **25** and the hexahydro-pyridazine **26** had moderate to poor antibacterial activity.[21] It is possible that the potency of these derivatives could be enhanced by more closely mimicking the eperezolid SAR and derivatizing the pyrazolidine and hexahydropyridazine rings with the 2-hydroxyacetyl group in place of the acetate.[1] One can speculate further on why compounds **25** and **26** did not maintain antibacterial potency on par with eperezolid. Perhaps acylation of the pyrazolidine and hexahydropyridazine rings diminishes the electron-donating ability of these heteroatom ring systems relative to an acylated piperazine. Since the chemical space occupied by the acetyl group in **25** and **26** is quite distinct from eperezolid, it's possible that steric interaction of the acetate forces the aryl-nitrogen bond to rotate out of the plane. The 2-pyrazoline analog **20** had potency similar to linezolid and eperezolid. In fact, **20** appears to have slightly lower MICs than linezolid against MRSA and *Enterococcus faecalis*. Some of the oxazolidinone intermediates were also assayed for in vitro antibacterial activity. Interestingly, hydroxylamine **4** was significantly more potent than the corresponding aniline.[22] The two nitro derivatives **3** and **13** represent a notable exception to the 'fluoro effect' mentioned above. An alternate explanation is that the highly electrophilic *ortho*-fluoronitrophenyl moiety is insoluble, or unstable under the assay conditions.

Oxazolidinones **6** and **20** were dosed orally in an experimental murine in vivo infection model.[23] Based on the in vitro potency of these compounds in the presence of 50% calf serum, protein binding does not appear to explain the lack of in vivo activity. Since these oxazolidinones had modest to poor activity (PD_{50} >50 mg/kg/day) in this in vivo model, no further characterization was undertaken.

In summary, oxazolidinones related to linezolid and eperezolid were synthesized[24] and found to exhibit potent in vitro activity against a panel of Gram-positive bacteria. The dihydro-1,2-oxazine present in **6** and the 2-pyrazoline present in **20** showed the most promise as morpholine and piperazine replacements, respectively.

Acknowledgements

The authors would like to thank the analytical chemistry group at Bristol-Myers Squibb for support in characterizing the compounds reported herein.

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19. The minimum inhibitory concentrations (MICs) were determined using a broth microdilution assay in accordance with that recommended by the National Committee for Clinical Laboratory Standards (NCCLS).

Mueller-Hinton medium was used except for streptococci, which was tested in Todd Hewitt broth. The final bacterial inoculate contained approximately 5×10^5 cfu/mL and the plates were incubated at 35 °C for 18 h in ambient air (streptococci in 5% CO_2). The MIC was defined as the lowest drug concentration that prevented physical growth.

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21. The MIC of **25** against *Streptococcus pneumoniae* A9585 is actually quite impressive, but this determination was not repeated. Also, compound **25** had MIC's of 8 μg/mL against two other strains (*S. pneumoniae* A27881 and *S. pneumoniae* A28272) not included in Table 1.
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24. All new compounds were characterized by analytical methods (^{1}H NMR and LC/MS).