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Synthesis, SAR, and antibacterial activity of novel oxazolidinone analogues possessing urea functionality☆

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Abstract—The syntheses of a number of novel oxazolidinone analogues possessing an urea functionality are reported. While the urea derivatives possessing aliphatic and aromatic groups were prepared by the more conventional isocyanate method, the derivatives possessing heterocyclic rings were synthesized by a relatively uncommon but otherwise efficient carbamate chemistry. Though the SAR resulted in novel compounds possessing in vitro activity equivalent to Linezolid, the compounds possess a range of substituents that are amenable for altering physicochemical properties of the resultant drug. The antibacterial activity was found to be not sensitive to the functional groups attached to the urea site regardless of the size and electronic characteristics. Based on in vivo results, one molecule has been identified as a candidate and additional work such as salt selection, scale-up, etc., are currently underway to take the molecule further through development.
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The emergence of bacterial resistance to the antibiotics poses a serious concern for medical professionals during the last decade.[1] In particular, multi-drug-resistant Gram-positive bacteria[2] including methicillin-resistant *Staphylococcus aureus* (MRSA)[3] and *Staphylococcus epidermidis* (MRSE), and vancomycin resistant enterococci (VRE) are of major concern.[4] Oxazolidinones are a new class of synthetic antibacterials with activity against Gram-positive bacteria and anaerobic bacteria.[5,6] They have shown to selectively bind to the 50S ribosomal subunit and inhibit bacterial translation at the initiation phase of the protein synthesis.[7] This class of compounds is particularly active against Gram-positive organisms such as MRSA, MRSE, and VRE. The novel mechanism of action combined with the biological activity against resistant organisms aroused widespread attention and stimulated others to explore chemistry in the oxazolidinone class.[4]

Linezolid **1** and Eperozolid **2** were jointly taken for development by the erstwhile Pharmacia and Upjohn (Fig. 1). Owing to certain toxicity concerns, development of Eperozolid **2** was terminated and Linezolid **1** became the first compound commercialized worldwide from oxazolidinone class of antibacterials.[8] Further chemistry in this class of compounds involved modifications on the right-hand side acetamide group and changes on the left-hand side piperazine ring. After having thoroughly studied the SAR on the acetamide functional group, we turned our attention to modify the piperazine ring.[4] As Eperozolid **2** was said to be toxic and as Linezolid **1** has not been approved for long-term

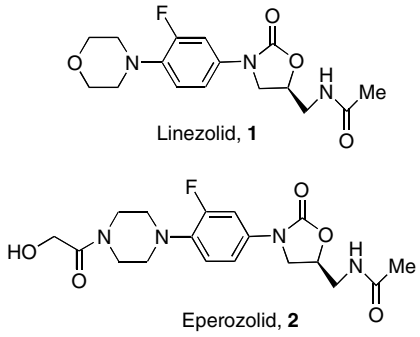

Figure 1.

Keywords: Oxazolidinones; Antibacterial; Linezolid; Urea.
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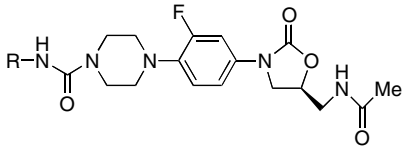
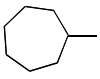
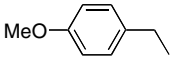
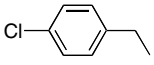
therapy owing to myelosuppression issues, we envisioned that altering the piperazine ring with a more drug-like urea functionality would provide an answer for the continous search for safer second generation oxazolidinone drug. Toward such a goal, we have designed urea functionality on the piperazine nitrogen with a range of substituents on the urea function that have the potential to lead to analogues having varied physicochemical properties. In this letter, we report our results on the antibacterial activity of the novel oxazolidinone molecules possessing the said urea functionality.

A number of new molecules (compounds **4**–**22**) having substituted urea functionality have been synthesized and summarized in Tables 1–3 along with their in vitro antibacterial activity. The syntheses of the

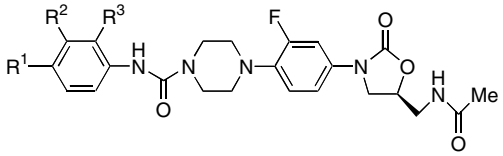
Table 1. In vitro (MIC, μg/mL)[a] and in vivo (ED_{50}, mg/kg) antibacterial activity of novel urea oxazolidinone compounds[b,c]



Entry	Compound	R	S.a. 035	S.a. 019	S.a. 446	E.f. 034	E.f. 153	E.fm 154	ED_{50}
1	**4**	H	4	2	2	2	2	2	13.8
2	**5**	OH	4	4	2	4	2	2	
3	**6**	Me	4	4	4	4	2	4	
4	**7**	$COCH_3$	16	8	8	8	4	4	
5	**8**	$CNHCH_3$	4	4	4	2	2	4	
6	**9**		16	8	8	8	4	4	
7	**10**		8	8	8	4	4	4	
8	**11**		4	4	4	4	2	4	
9	**12**		4	2	2	2	2	2	
10	**13**		4	4	4	4	2	4	
11	Linezolid		2	2	2	2	2	2	3.91
12	Vancomycin		1	2	1	2	>32	>32	3.93[d]

[a] S.a. 035 = *Staphylococcus aureus* ATCC 29213; S.a. 019 = *Staphylococcus aureus* ATCC 33591 (methicillin resistant); S.a. 446 = *Staphylococcus aureus* (clinical isolate); E.f. 034 = *Enterococcus faecalis* ATCC 29212 (vancomycin sensitive); E.f. 153 = *Enterococcus faecalis* NCTC 12201 (vancomycin resistant) and E.fm 154 = *Enterococcus faecium* ATCC 12202 (vancomycin resistant).
[b] The MIC and ED_{50} values were determined as described previously.[4]
[c] All the compounds were prepared by Method A (Scheme 1).
[d] The ED_{50} values are for oral route of administration but for vancomycin (SC route).

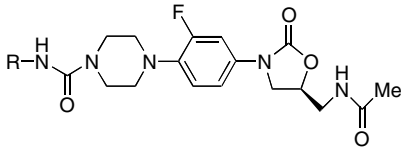
Table 2. In vitro (MIC, μg/mL)[a] and in vivo (ED_{50}, mg/kg) antibacterial activity of novel aromatic urea oxazolidinones[b]



Entry	Compound	R^1	R^2	R^3	S.a. 035	S.a. 019	S.a. 446	E.f. 034	E.f. 153	E.fm 154	ED_{50}
1	**14**	H	H	H	4	4	2	2	2	2	>10
2	**15**	H	F	H	4	2	2	2	1	2	18.4
3	**16**	H	H	OCH_3	2	2	2	2	2	2	9.1
4	**17**	OCH_3	H	OCH_3	4	4	4	2	2	4	
5	**18**	H	H	Cl	4	2	2	2	2	2	
6	**19**	Cl	H	Cl	2	1	2	2	1	2	>10
7	Linezolid				2	2	2	2	2	2	3.91
8	Vancomycin				1	2	1	2	>32	>32	3.93[c]

[a] See the footnote given in Table 1 for the details about the organisms.
[b] Compound **15** was prepared as per Method A and the rest of the compounds as per Method B (Scheme 1).
[c] The ED_{50} values are for oral route of administration but for vancomycin (SC route).

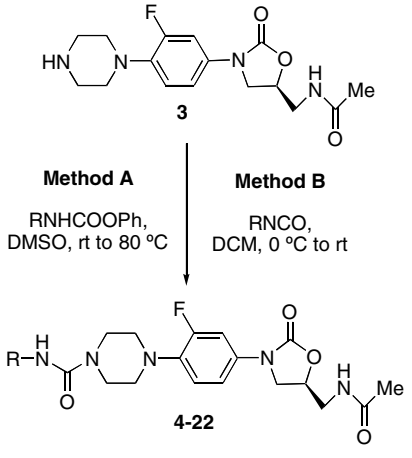
Table 3. In vitro (MIC, μg/mL)[a] (ED_{50}, mg/kg) antibacterial activity of novel heterocyclic urea oxazolidinones[b]



Entry	Compound[b]	R	S.a. 035	S.a. 019	S.a. 446	E.f. 034	E.f. 153	E.fm 154	E.fm 154
1	**20**		4	4	4	2	2	4	
2	**21**		2	2	2	1	1	1	18.4
3	**22**		2	2	2	1	1	1	
4	Linezolid		2	2	2	2	2	2	3.91
5	Vancomycin		1	2	1	2	>32	>32	3.93[c]

[a] See the footnote given in Table 1 for the details about the organisms.
[b] All the compounds were prepared by Method A (Scheme 1).
[c] The ED_{50} values are for oral route of administration but for vancomycin (SC route).

compounds **4**–**22** were accomplished, starting from the known intermediate **3**,[8] by following two methods (Scheme 1).[9,10] The first method involved the treatment of the intermediate **3** with phenylcarbamates of appropriate amines in DMSO. This method is versatile that the syntheses of carbamate reagents and the subsequent treatment of these reagents with the intermediate **3** have been performed in parallel. It is important to note that this method of preparing urea is relatively uncommon, which renders access to important heterocyclic derivatives such as **20**, **21**, and **22** that are otherwise difficult to prepare by conventional methods. While all the new compounds were synthesized using this carbamate method, the aromatic urea compounds disclosed in the Table 2 were synthesized by a rather conventional method which involved the treatment of the intermediate **3** with the corresponding aryl isocyanates. In addition to the above two methods, it is interesting to note that the synthesis of compound **7** warranted the hitherto unknown acetyl carbamate which was prepared by treating acetyl isocyanate[11] with phenol.



Scheme 1.

The analogues of Eperozolid **2** prepared above possessing the substituted urea functionality were screened for in vitro activity against a panel of Gram-positive organisms and the results are summarized in Tables 1–3. The unsubstituted urea compound **4** exhibited activity equivalent to Linezolid having MIC values ranging 2–4 μg/mL. The hydroxyl and methyl substituted compounds **5** and **6** were found to have activity slightly inferior to that of Linezolid. However, the acetyl urea compound **7** was found to possess poor activity (4–16 μg/mL). But, it is interesting to note that the corresponding imidate compound **8** exhibited activity equivalent to Linezolid. The in vitro activity of the cycloalkyl substituted compounds **9**–**11** suggests the larger cycloalkyl ring (compound **11**) to be better than the smaller ones. The substituted benzyl urea compounds **12** and **13** were also found to be good having same activity as that of Linezolid.

Having accomplished preparing many analogues possessing activity equal to Linezolid, we turned our attention toward molecules having aryl substituted urea functionality (Table 2) and heterocycles substituted urea functionality (Table 3). The substituted aryl urea compounds **14**–**19** exhibited activity equivalent to Linezolid irrespective of aryl ring substituents such as fluorine, methoxy, and chlorine. However, the heterocycle substituted urea compounds **20**–**22** showed improved activity. In particular, the MIC values of vancomycin sensitive and resistant *Enterococcus faecalis* and *Enterococcus faecium* of the compounds **21** and **22** were one dilution better (1 μg/mL) compared to Linezolid. This observa-

tion indicates the possibility of getting even superior compounds if a SAR is carried out on the thiazole and pyridine ring of the compounds **21** and **22**.

It is important to note that most of the new analogues possessing substituted urea functionality were exhibiting activity equal to Linezolid. Based on the in vitro activity, the compounds **4**, **14**, **15**, **16**, **19**, and **21** were scaled up and subjected for in vivo studies in mice by systemic infection model.[4] The ED_{50} values of the in vivo experiments following the oral route of administration for all these compounds were >10 mg/kg except for compound **16** for which the value was 9.1 mg/kg.

In conclusion, a number of new oxazolidinone molecules having substituted urea functionality have been synthesized following an uncommon method of treating the compound **3** with various carbamates and evaluated for their antibacterial activity. It was found that most of the new analogues were exhibiting activity equal to Linezolid suggesting that the antibacterial activity is not sensitive to the functional groups attached to the urea site regardless of the size and electronic characteristics. Selected compounds were subjected to in vivo studies that revealed the compound **16** to possess acceptable ED_{50} value. Further work, to develop this compound, such as salt selection, PK, scale-up, and then toxicity studies, is currently underway.

Acknowledgments

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Supplementary data

The spectral data of all the final compounds are provided as supplementary data and can be found in the online version. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.09.024.

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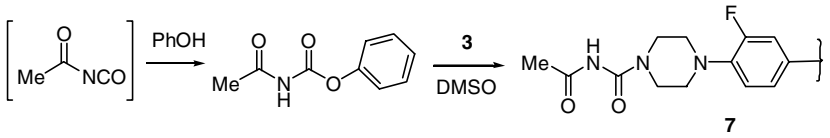
9. Representative experimental procedure: a solution of the intermediate **3** (100 mg, 0.3 mmol) and phenyl carbamate (50 mg, 0.36 mmol) in DMSO (2 mL) was stirred at 60 °C over 3 h. After ascertaining the completion of the reaction by TLC, the reaction mixture was allowed to attain room temperature and diluted with half saturated brine (4 mL). The resultant mixture was extracted with DCM and the organic extract was washed with water, brine, and dried. The residue obtained upon evaporation of the solvents was purified by silica gel chromatography to afford the urea compound **4** (85 mg, 75%) as a colorless solid. Mp 201 °C. IR (KBr) 1738, 1518, 1234, 990 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 8.22 (br t, J = 5.6 Hz, 1H), 7.48 (dd, J = 15.0, 2.7 Hz, 1H), 7.17 (dd, J = 8.7, 2.4 Hz, 1H), 7.16–7.07 (m, 1H), 6.02 (s, 2H), 4.72–4.68 (m, 1H), 4.10–4.05 (m, 1H), 3.70 (dd, J = 9.1, 6.4 Hz, 1H), 3.45–3.38 (m, 6H), 2.91–2.90 (m, 4H), 1.83 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.0, 158.0, 154.7 (d, J = 242.4 Hz, 1C), 154.0, 135.5 (d, J = 8.7 Hz, 1C), 133.5 (d, J = 10.6 Hz, 1C), 119.7 (d, J = 4.2 Hz, 1C), 114.0 (d, J = 2.7 Hz, 1C), 106.6 (d, J = 26.2 Hz, 1C), 71.5, 50.4 (2C), 47.3, 43.5 (2C), 41.4, 22.4. MS (Electrospray) 380 (M^++1), 337. HRMS: (Electrospray method for Na adduct) Calcd for $C_{17}H_{22}N_5O_4FNa$ 402.1554, found 402.1553. HPLC (System 1)[4] 99.41% purity.
10. Spectral data for selected compounds: compound **14**: Mp 245 °C. IR (KBr) 1722, 1660, 1537, 1242 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 8.59 (s, 1H), 8.22 (br t, J = 5.9 Hz, 1H), 7.52–7.46 (m, 3H), 7.25–6.91 (m, 5H), 4.72–4.68 (m, 1H), 4.10–4.06 (m, 1H), 3.71 (dd, J = 9.0, 6.6 Hz, 1H), 3.62–3.59 (m, 4H), 3.41–3.39 (m, 2H), 3.00–2.90 (m, 4H), 1.83 (s, 3H). ^{13}C NMR (50 MHz, DMSO-d_6) δ 170.0, 154.9, 154.6 (d, J = 242.4 Hz, 1C), 154.0, 140.5, 135.4 (d, J = 8.8 Hz, 1C), 133.6 (d, J = 10.7 Hz, 1C), 128.3 (2C), 121.8, 119.8, 119.7 (2C), 114.0 (d, J = 2.7 Hz, 1C), 106.6 (d, J = 25.8 Hz, 1C), 71.6, 50.4 (2C), 47.3, 43.9 (2C), 41.4, 22.4. MS (Electrospray) 456 (M^++1), 337. HRMS: (Electrospray method for Na adduct) Calcd for $C_{23}H_{26}N_5O_4FNa$ 478.1867, found 478.1870. HPLC (System 1)[4] 98.39% purity. Compound **21**: Mp 217 °C. IR (KBr) 1730, 1656, 1425 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.97 (br s, 1H), 8.21 (br t, J = 5.6 Hz, 1H), 7.49 (dd, J = 14.8, 2.4 Hz, 1H), 7.34 (br s, 1H), 7.19–7.00 (m, 3H), 4.72–4.68 (m, 1H), 4.10–4.06 (m, 1H), 3.72–3.60 (m, 5H), 3.41–3.38 (m, 2H), 2.98–2.95 (m, 4H), 1.83 (s, 3H). ^{13}C NMR (50 MHz, DMSO-d_6) δ 170.0, 162.2, 154.8, 154.7 (d, J = 242.4 Hz, 1C), 154.0, 135.4 (d, J = 9.1 Hz, 1C), 133.7 (d, J = 4.5 Hz, 1C), 119.9, 119.8, 114.0 (d, J = 2.7 Hz, 1C), 111.8, 106.6 (d, J = 26.2 Hz, 1C), 71.6, 50.3 (2C), 47.3, 43.6 (2C), 41.4, 22.4. MS (Electrospray) 463 (M^++1), 337. HRMS: (Electrospray method for M^++1 peak) Calcd for $C_{20}H_{24}N_6O_4FS$ 463.1564, found 463.1569. HPLC (System 1)[4] 98.61% purity.
11. The acetyl isocyanate was prepared as reported (Deng, M.-Z.; Caubere, P.; Senet, J. P.; Lecolier, S. *Tetrahedron* **1988**, *44*, 6079) and was treated in situ with phenol to generate the carbamate (colorless solid) as shown below, which on treatment with compound **3** following Method A led to the analogue **7**. It is pertinent to note that the



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treatment of acetyl isocyanate in situ with compound **3** as per Method B did not yield even trace of compound **7**, which neccessitated the preparation of the carbamate as above for the route A.