Pure Appl. Chem., Vol. 77, No. 1, pp. 195–200, 2005.
DOI: 10.1351/pac200577010195
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Novel anti-infective compounds*

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Abstract: A set of substituted piperazinyloxazolidinone derivatives has been studied for their antibacterial activity in a few gram-positive bacteria. The structural modifications have provided a superior compound than linezolid, the only drug of this class in the market at present.

Nature has a wide spectrum of biodiversity. On one hand, a large number of microbes coexist with each other, while a number of natural products inhibit their growth. The discovery of antibacterial activity of natural products has led to the invention and introduction of antibiotics such as penicillins, cephalosporins, aminoglycosides, tetracyclines, erythromycin, and other macrolides, vancomycin, teicoplanin, etc. [1,2]. Although these natural gifted antibiotics reduce mortality due to bacterial infections, their biodiversity has also given microorganisms the opportunity to learn, mutate, and survive the onslaught, and develop multidrug-resistance properties. This has posed a great challenge for health care practitioners [3–5]. Reports of vancomycin-resistant enterococcus (VRE) [6,7] are alarming since VRE strains also carry resistance to many known natural antibiotics [8]. Owing to growing resistance to natural antibiotics, scientists started to look for antibiotics that do not have a similar structural motif in nature, so that organisms that are resistant to multidrugs have not been exposed to such antibiotics. Extensive research in this area led to the oxazolidinone class of antibacterials, which is considered a breakthrough invention in this direction.

A large number of publications, reviews, and patents testify to the interest of the various research groups in the oxazolidinone class of synthetic compounds [9,10]. Furazolidone **1** (Fig. 1), the first member of the oxazolidinone class discovered in 1950, appears to be the initial candidate responsible for the genesis of further work on oxazolidinones [11]. The early developed candidate Dup. 721 **2** (Fig. 1) [12], was discontinued following lethal toxicity shown in rats [13]. Extensive research work by Upjohn and

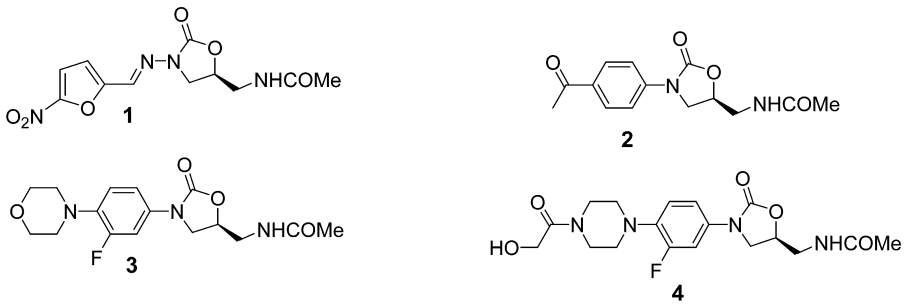

Fig. 1

*Paper based on a presentation at the 24th International Symposium on the Chemistry of Natural Products and the 4th International Congress on Biodiversity, held jointly in Delhi, India, 26–31 January 2004. Other presentations are published in this issue, pp. 1–344; ZRC Communication number 119.

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Pharmacia [13] led to the discovery of linezolid **3** and eperezolid **4** (Fig. 1). Linezolid, the first member of the oxazolidinone class of antibacterial agents, was launched in 2000 as zyvox, whereas eperezolid remained as a lead for further modification owing to its inferior antibacterial efficacy and more toxicity than linezolid [10a].

The reports of resistance to linezolid and its toxicity at higher doses, as well as other side effects, have prompted the pharmaceutical industries and academic institutions [10,14] to further fine tune the phenyloxazolidinone pharmacophore. Earlier, we have reported the synthesis of **5**, wherein the five-membered oxazolidinone ring in linezolid is replaced with a six-membered ring [15].

The present article will describe some of our exploratory approaches toward studying the effect of further modification on ring "A" and ring "C" in **6** (Fig. 2). It has been reported that replacement of 5-acetamide group by a thioacetamide or a thiourea group has a tremendous impact on the in vitro antibacterial activity [16]. The results in our laboratory have indicated that substituting the acetamide group at 5-position of linezolid by a thiocarbonyl or thiourea group (**7**,**8**) improves the antibacterial activity by two to four times (Table 2). This encouraged us to explore the effect of replacing the carbonyl group of ring "A" by a thiocarbonyl group. Thus, we synthesized compounds **9** and **10** (Fig. 2) and studied their antibacterial activity in gram-positive bacteria. The compounds **9** and **10**, interestingly, showed no antibacterial activity up to a concentration of 64 μg/ml in the bacteria used (Table 2). This was rather surprising to us since oxygen atom vs. sulfur atom normally does not bring drastic change in the activity profile of molecules. Therefore, we tried to look at the binding of these compounds at the RNA level. It has been reported [17] that there is a cavity of sufficient size in the P-site of RNA, which can accommodate the inhibitor oxazolidinone. This cavity contains a potassium ion, which may be required for the catalytic activity. Our preliminary modeling studies based on this model indicated that replacement of oxygen atom by a sulfur atom hinders the binding of thio compounds **9** and **10** into this cavity, resulting in a very high binding energy. Although there may be differences between archael and eubacterial ribosome, the wet lab data is explained by the unfavorable binding obtained in our modeling studies characterized by reverse orientation and a fewer number of hydrogen bonds [18]. The minimum inhibitory concentration (MIC) for 90 % bacterial growth inhibition was determined by the microbroth dilution technique using the National Committee for Clinical and Laboratory Standards (NCCLS) method [19].

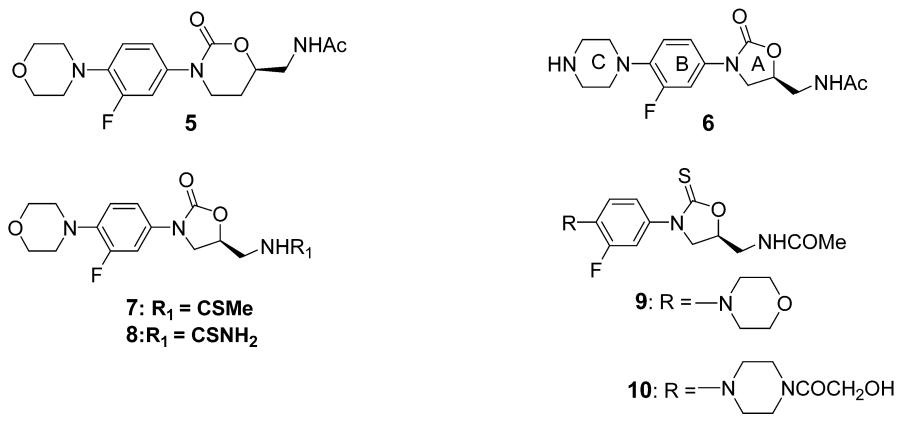
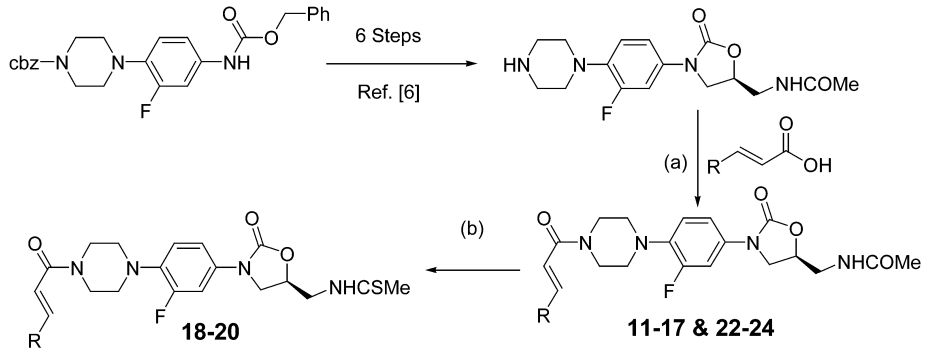
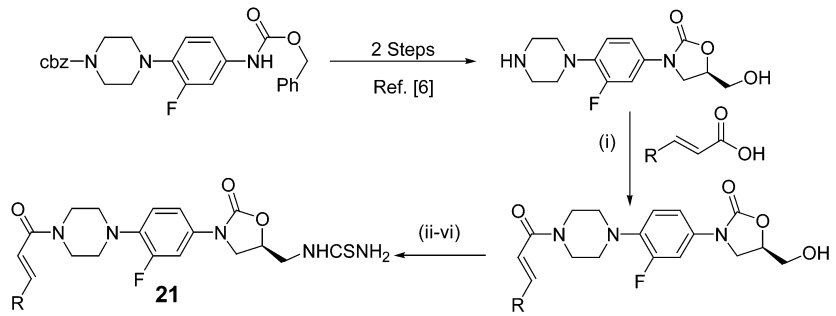


Fig. 2

Further, we focused on the modification of ring "C" in **6**. We first selected cinnamoyl group as the simplest novel substituent on **6**, which was incorporated by a simple amidation reaction. We synthesized several novel 3-(4-piperazinophenyl) substituted oxazolidinones (Schemes 1, 2 and Table 1) and evaluated them in a panel of gram-positive bacteria. These compounds inhibited the growth of *staphylococcus aureus*, *staphylococcus epidermidis*, and *enterococcus faecalis* with MIC values superior to linezolid and eperezolid (Table 3).



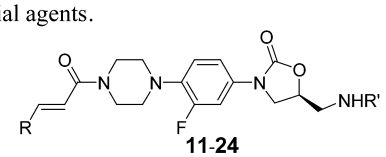
Scheme 1 (a) EDC-HCl, HOBt·H_2O, TEA, CH_2Cl_2, 26–28 °C, 15–20 min. (b) Lawesson's reagent, THF, 65–70 °C, 1 h.



Scheme 2 (i) EDC-HCl, HOBt·H_2O, TEA, CH_2Cl_2, 27–28 °C, 30 min. (ii) $MeSO_2Cl$, TEA, CH_2Cl_2, 0–5 °C, 1 h. (iii) NaN_3, DMF, 70–80 °C, 2–3 h. (iv) $P(Ph)_3$, 1,4-dioxane, MOH, NH_3(aq), 27–28 °C, 30 min. (v) CS_2 solution, ethyl chloroformate, TEA, 20–30 min. (vi) Methanolic ammonia, 0–5 °C, 5–10 min.

The parent compound **11** containing the unsubstituted cinnamoyl group is nearly as active as linezolid in in vitro MIC assay and is superior to eperezolid in all of the strains of bacteria studied. Substitution of phenyl ring of the cinnamoyl group showed an interesting structure–activity relationship. When the 4-position of phenyl ring (in cinnamoyl moiety) was substituted with the –OMe group, the resulting compound **12** showed a moderate decrease in the antibacterial activity. This suggests that the electron-donating group on the cinnamoyl moiety is not favorable. Further, we substituted the 4^{th} position of the phenyl ring with electron-withdrawing groups such as $–NO_2$ (**13**) and –F (**14**). The antibacterial activity of these compounds indicated that electron-withdrawing groups are not preferred on the phenyl ring of the cinnamoyl moiety. Thus, we synthesized a compound having a 4-OH (**15**) group on the phenyl group of cinnamoyl moiety, which showed improved antibacterial activity. Replacement of 4-OH group in **15** by a 3-OH group (**16**) retained the antibacterial activity. However, introduction of a 3,4-dihydroxy group (**17**) led to complete loss of antibacterial activity.

Table 1 New oxazolidinones as antibacterial agents.



11-**24**

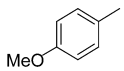
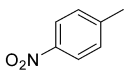
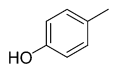
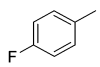
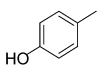
Compd.	R	R'	% Yield	Compd.	R	R'	% Yield
11		$COCH_3$	80	**18**		$CSCH_3$	78
12		$COCH_3$	47	**19**		$CSCH_3$	31
13		$COCH_3$	55	**20**		$CSCH_3$	49
14		$COCH_3$	73	**21**		$CSNH_2$	67
15		$COCH_3$	52	**22**		$COCH_3$	57
16		$COCH_3$	70	**23**		$COCH_3$	78
17		$COCH_3$	25	**24**		$COCH_3$	47

Table 2 Antibacterial activity (MIC values in μg/ml) of compounds containing thiocarbonyl moieties.

Compd.	S.a	S.a	S.e	B.s	E.f
7	0.5	1	0.5	0.25	1
8	0.5	1	0.5	0.25	0.5
9	>64	>64	>64	>64	>64
10	>64	>64	>64	>64	>64
Linezolid	1	4	0.5	1	4
Eperezolid	2	4	4	2	2

S.a = *Staphylococcus aureus* **ZYABL 006**.
S.a = *S. aureus* **ATCC 33591**.
S.e = *Staphylococcus epidermidis* **ATCC 12228**.
B.s = *Bacillus subtilis* **ATCC 6633, MTCC 441**.
E.f = *Enterococcus faecalis* **ATCC 29212**.

Compounds **11**, **14**, and **15** were taken for further modification in order to get better antibacterial compounds. In these compounds, the acetamide group (R' = $–COCH_3$) was replaced by a thioacetamide group (R' = $–CSCH_3$) to yield **18**–**20**, respectively. Compounds **18**–**20** showed very good antibacterial activities even in MRSA, and compound **20** also showed activity against linezolid partially resistant gram-negative *Klebsiella pneumoniae* (Table 3).

Compound **11** was further modified to furnish **21** (R' = $-CSNH_2$), which showed superior antibacterial activity than its acetamide analog **11** and thioacetamide analog **18**.

A few five/six-membered heteroaryl-substituted compounds **22**–**24** were also synthesized and screened against the same panel of gram-positive bacteria, which showed improved MIC values against a variety of strains (Table 3).

Table 3 MIC (minimum inhibitory concentration in μg/ml) values of compounds in several gram-positive and gram-negative bacteria.

Compd.	B.p	B.c	S.p	S.e	E.f 1	E.f 2	S.a 1	S.a 2	S.a 3	S.a 4	K.p
11	1	1	0.5	2	2	1	4	4	2	4	>16
12	2	1	1	4	4	2	4	8	4	4	>16
13	2	1	1	4	4	4	4	16	4	4	>16
14	4	4	2	8	4	4	16	>16	16	>16	>16
15	1	0.5	2	0.25	1	2	2	2	1	2	>16
16	0.5	0.5	0.25	2	1	1	2	4	4	1	>16
17	8	16	4	16	8	16	8	16	16	8	>16
18	0.5	0.5	0.25	0.5	0.5	0.5	2	2	1	1	>16
19	0.5	0.5	0.5	2	0.5	2	1	1	4	4	>16
20	0.25	0.25	≤0.12	0.5	1	0.25	0.25	0.5	0.25	0.5	4
21	1	0.25	0.25	1	0.5	0.5	1	2	2	1	>16
22	2	2	0.5	4	2	4	4	16	8	4	>16
23	1	0.5	4	0.5	2	2	2	4	2	4	>16
24	1	1	ND	2	1	1	2	4	1	1	>16
Linezolid	2	2	0.5	4	2	4	4	8	4	4	>16
Eperezolid	2	2	1	4	4	2	4	4	4	4	>16

B.p = *Bacillus pumilus* **MTCC 1607**.
B.c = *Bacillus cereus* **MTCC 430**.
S.p = *Streptococcus pyogenes* **MTCC 442**.
S.e = *Staphylococcus epidermidis* **ATCC 155**.
E.f 1 = *Enterococcus faecalis* **MTCC 439**.
E.f 2 = *E. faecalis* **ATCC 14506**.
S.a 1 = *Staphylococcus aureus* **MTCC 96**.
S.a 2 = *S. aureus* **ATCC 14154**.
S.a 3= *S. aureus* **ATCC 25923**.
S.a 4 = *S. aureus* **ATCC 29213**.
K.p = *Klebsiella pneumoniae* **ATCC 10031**.
ND = not done.

In summary, hydroxy substituent on the cinnamoyl phenyl group imparts good antibacterial activity. Thiourea and thioacetamide derivatives possess better antibacterial activity compared to their corresponding acetamide derivatives. Further study toward selecting a right candidate that could be a true antibacterial agent meeting all the physicochemical, in vitro, in vivo, and pharmacokinetic properties is in progress.

ACKNOWLEDGMENTS

We thank Mr. Pankaj R. Patel, CMD and the management of Zydus Group for encouragement. We also thank Mr. Binu Philip and Ms. Rina Soni for their help in preparing the manuscript and the analytical department for support.

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