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# New potent antibacterials against Gram-positive multiresistant pathogens: Effects of side chain modification and chirality in linezolid-like 1,2,4-oxadiazoles

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ABSTRACT

The effects of side chain modification and chirality in linezolid-like 1,2,4-oxadiazoles have been studied to design new potent antibacterials against Gram-positive multidrug-resistant pathogens. The adopted strategy involved a molecular modelling approach, the synthesis and biological evaluation of new designed compounds, enantiomers separation and absolute configuration assignment. Experimental determination of the antibacterial activity of the designed (*S*)-1-((3-(4-(3-methyl-1,2,4-oxadiazol-5-yl)phenyl)-oxazolidin-2-one-5-yl)methyl)-3-methylthiourea and (*S*)-1-((3-(3-fluoro-4-(3-methyl-1,2,4-oxadiazol-5-yl)phenyl)-oxazolidin-2-one-5-yl)methyl)-3-methylthiourea against multidrug resistant linezolid bacterial strains was higher than that of linezolid.



## 1. Introduction

The urgent need for new antibiotics due to the increase in the frequency of bacterial infections with Gram-positive multidrug-resistant (MDR) strains—such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and penicillin-resistant *Streptococcus pneumoniae* (PNSSP)—in both the hospital and the community settings, has not been met by the development of effective and broad spectrum antibiotics.[1] Among them linezolid, a synthetic oxazolidinone which entered the market in 2000, appeared to have a unique mechanism of action and was not cross-resistant with existing resistant mechanisms in bacteria. Moreover, linezolid is characterized by excellent pharmacokinetic properties with 100% oral bioavailability and effective penetration at therapeutic concentrations to almost every organ in the body is making a valuable option in the treatment of both community and hospital acquired infections.[2] However, since 2001 linezolid resistance began to appear[3–5] and an intense search for novel oxazolidinones with promising antibacterial activity started and structure–activity relationships (SAR) have been extensively reported.[6–12] In order to rationalize the site of modifications, the structure of linezolid is usually divided into four portions, as elucidated in Scheme 1.

It was suggested that fluorination of phenyl B-ring flanking a morpholine or piperazine C-ring improved the bacterial activity and that a C-5 acylaminomethyl group in the side chain was essential for activity.[13] Subsequently, substitution of the C-5 side-chain

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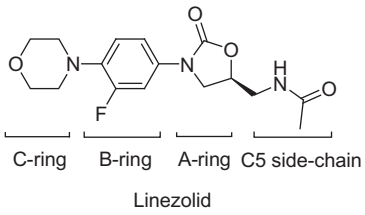



**Scheme 1.** Structure and portions nomenclature of Linezolid.

of linezolid with thiocarbonyl groups (i.e. thioamide, dithiocarbamate, thiourea, and thiocarbamate) demonstrated that these compounds are in vivo more active than linezolid.[14] The introduction of heteroaromatic moieties into the C5 side-chain was able to maintain or even enhance the antibacterial activity.[15]

Considering the known properties of 1,2,4-oxadiazoles in bioactive compounds[16–18] and as biocompatible moiety in functional biomaterials[19–21], we have recently approached the synthesis of linezolid-like compounds by introduction of a 1,2,4-oxadiazolyl moiety. By following this approach, we have recently shown that the replacement of the oxazolidinone (A-ring) with 1,2,4-oxadiazole resulted in the loss of activity,[22] while replacement of the morpholine or piperazine C-ring with 1,2,4-oxadiazole allowed to maintain a satisfactory antibacterial activity against Gram-positive multiresistant pathogens.[23] Moreover, the presence of fluorine in the benzene B-ring of these new oxadiazoles exhibited no significant effect on activity and produced a slight decrease in cytotoxicity, while C-5 acetamidomethyl or thioacetamidomethyl moieties appeared essential structural requirements for activity.[23] However, in previous work[22,23] the newly synthesized compounds were tested as a racemic mixture. An homogeneous comparison with the activity of Linezolid *S* enantiomer can only be achieved by the measurement of the antibacterial activity of the *S* enantiomers of new linezolid-like 1,2,4-oxadiazoles. In this context we here report the design, synthesis, enantiomer separation, and in vitro antibacterial evaluation for both S and R enantiomers to gain a deeper insight into the effects of C-5 side chain modification and chirality.

## 2. Results and discussion

### 2.1. Synthesis and biological evaluation of new linezolid-like 1,2,4-oxadiazoles

In order to investigate the effects of side chain modification, compounds **1**–**5a**,**b** (see Chart 1) were synthesized and tested against multidrug-resistant clinical isolates. In Table 1 we report the calculated p$K_a$, Log*P*, LogD75, and the logarithm of the predicted intrinsic solubility for compounds **1**–**5 a**,**b**.

**Table 1**
Chemical-physical calculated values for compounds **1**–**5 a**,**b**

| Compound | p$K_a$[a] | Log*P*[a] | Log*D* 7.5[a] | Log (intrinsic solubility)[b] |
|---|---|---|---|---|
| **1a** | – | 0.2 | 0.2 | −2.3 |
| **1b** | – | 0.4 | 0.4 | −2.3 |
| **2a** | – | 3.2 | 3.2 | −3.3 |
| **2b** | – | 3.3 | 3.3 | −3.3 |
| **3a** | 10.37 | 1.7 | 1.7 | −3.5 |
| **3b** | 10.37 | 1.8 | 1.8 | −3.5 |
| **4a** | 10.93 | 1.7 | 1.7 | −3.6 |
| **4b** | 10.93 | 1.9 | 1.9 | −3.6 |
| **5a** | – | 1.2 | 1.2 | −3.4 |
| **5b** | – | 1.4 | 1.4 | −3.3 |

[a] Calculated using the MoKa 2.6 software.[24]
[b] The logarithm of experimental intrinsic solubility (mol/Litre at 25 °C) Log (intrinsic solubility) was predicted using the VolSurf + software.[25]

The synthesis was achieved by initially following the *amidoxime route* scheme for the construction of the 1,2,4-oxadiazole's moiety[22,26] (Scheme 2).

For the subsequent step, the typical reactivity of 5-fluoroaryl-1,2,4-oxadiazoles towards the nucleophilic aromatic substitution,[27–30] was a key feature to access allylamine derivatives **9a**,**b** precursors for the construction of the oxazolidinone ring (Scheme 3) through a iodo-cyclization reaction leading to oxazolidinones **10a**,**b**.[23]

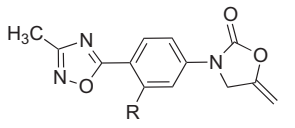
The synthetic pathway towards final derivatives **1**–**5** was planned by taking advantage of the iodomethylene moiety of key derivatives **10** as illustrated in Scheme 4.

In particular, methylidene derivatives **1** were obtained in good yield and under mild conditions by a base promoted elimination reaction. Isothiocyanates **2**, azides **11**, and triazoles **5**, were instead obtained through a nucleophilic substitution with either potassium thiocyanate, sodium azide, or 1,2,3-triazole, respectively. Further functionalization of derivatives **2** has been achieved by addition of ammonia leading to the corresponding thioureas **3a**,**b**. Reduction of azides **11** into amines **12**, followed by reaction with methylisothiocyanate, yielded the corresponding methylthioureas **4**.

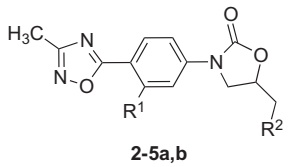
### 2.2. Microbiological evaluation of compounds 1–5a,b

The antibacterial tests against 3 MDR clinical isolates of MRSA, compared with those against 2 standard ATCC *S. aureus* strains reported in Table 2 show that **4a** and **4b** exhibit an excellent activity expressed in low Minimal Inhibitory Concentration (MIC) values against MRSA strains with potency comparable to that of linezolid, while **5a** and **5b** are also active; other compounds are inactive.

The presence of thioacetamidomethyl or 1,2,3-triazole moieties in the side chain appear to be essential structural requirements for activity, while the aromatic F substituent has no effect.




**a:** R = H
**b:** R = F




**a:** $R^1$ = H
**b:** $R^1$ = F

| Compound | $R^2$ |
|---|---|
| **2** | NCS |
| **3** | $NHC(=S)NH_2$ |
| **4** | $NHC(=S)NHCH_3$ |
| **5** | 1,2,3-triazol-1-yl |

**Chart 1.**