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Scaffold hopping in the oxadiazole antibiotic structure leads to more active compounds

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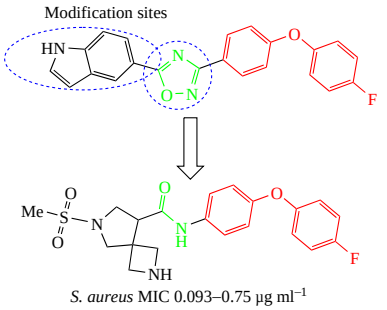
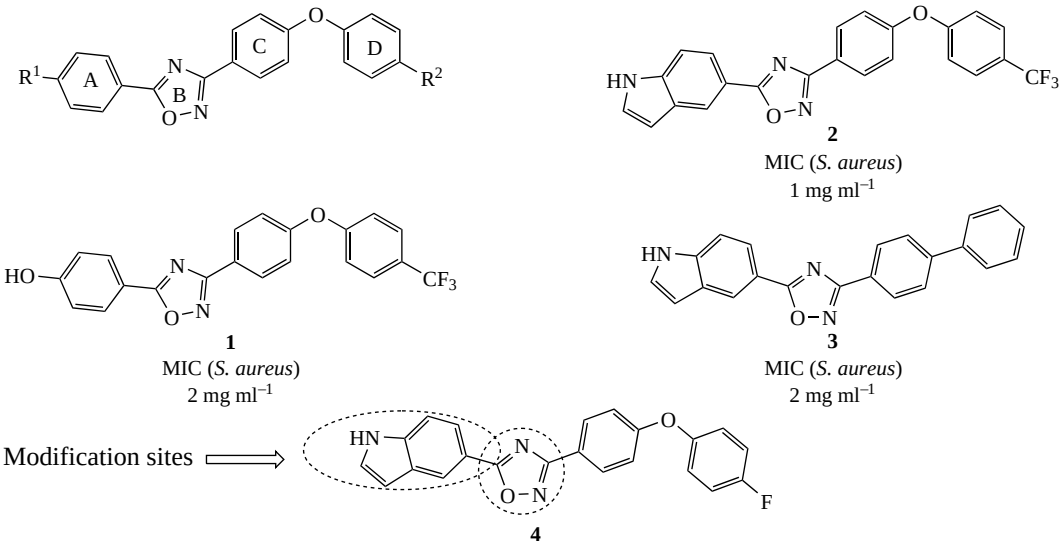
Isosteric replacement of the oxadiazole ring by amide bond in the structure of new non-β-lactam antibiotics led to compounds with higher activity against Gram-positive pathogens of ESKAPE panel. A series of 17 compounds were synthesized by acylation of 4-(4-fluorophenoxy)aniline with various amino acids. The spirocyclic derivative with 6-methylsulfonyl-2,6-diazaspiro[3.4]octane moiety showed excellent minimum inhibitory concentrations of 0.093–0.75 μg ml^{-1} against a number of methicillin-resistant *Staphylococcus aureus* strains.



Keywords: non-β-lactam antibiotics, ESKAPE pathogens, methicillin-resistant bacteria, isosteric replacement, antibiotic resistance, organofluorine compounds, carboxamides, 2,6-diazaspiro[3.4]octane.

Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a threat to human health due to its ease of transmission and difficult treatment conditions.[1] Bacteria MRSA are capable of becoming resistant to new drugs introduced very quickly.[2] The effective drug development to combat MRSA strains has great potential. A new class of non-β-lactam bactericidal antibiotics active against Gram-positive bacteria was recently proposed[3] after *in silico* screening 1.2 million structures. These compounds inhibit PBP2a (penicillin-binding protein 2a), an important enzyme in cell-wall building of *S. aureus*.[4] Protein PBP2a is insensitive to inhibition by all commercially available β-lactams and is responsible for at least one mechanism of MRSA drug resistance. Following *in vitro* and *in vivo* evaluation, several optimization steps[5–7] produced a few lead candidates **1**–**3** using an oxadiazole-based scaffold (Figure 1). This type of antibacterials began to be called 'oxadiazole antibiotics'. These substances exhibit synergism with oxacillin against MRSA[8] and can be used against other Gram-positive pathogens such as *Clostridioides difficile*.[9] The review by Verma[10] summarizes the oxadiazole derivatives development as agents against multidrug-resistant MRSA strains and discusses structure–activity relationships (SAR).

Previous SAR studies[5,7] have examined variations in cores A and D of the base scaffold. However, the oxadiazole core B



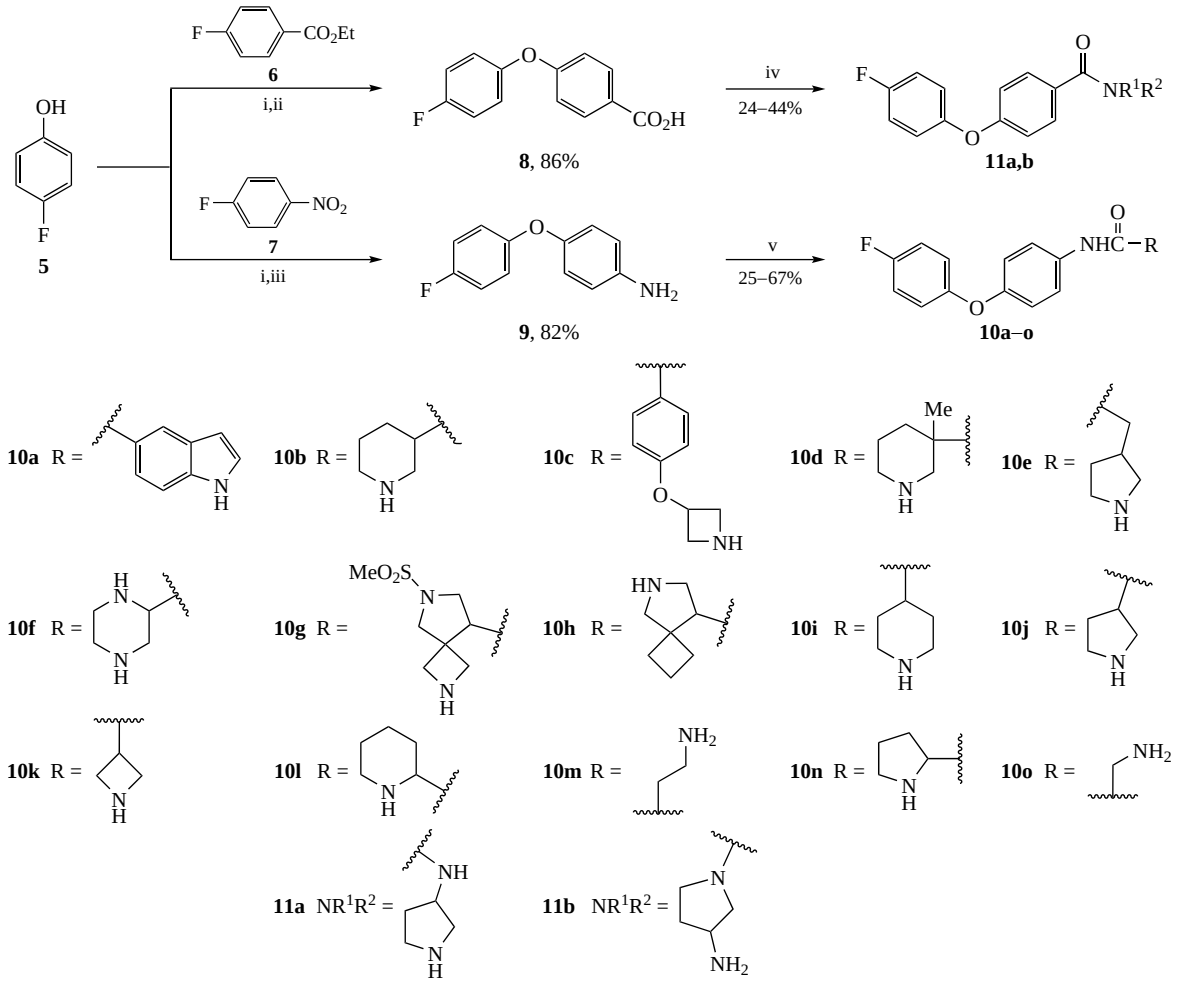
1
MIC (*S. aureus*)
2 mg ml^{-1}

2
MIC (*S. aureus*)
1 mg ml^{-1}

3
MIC (*S. aureus*)
2 mg ml^{-1}

4

Figure 1 Basic scaffold, its derivative lead compounds **1**–**3** and modification sites in structure **4**.



Scheme 1 *Reagents and conditions*: i, K_2CO_3, DMF, 130 °C, 12 h; ii, NaOH, H_2O, MeOH, 50 °C, 4 h, then HCl (aq.); iii, H_2 (1 atm.), Pd/C, MeOH, room temperature, 4 h; iv, R^1R^2NH, HBTU, Et_3N, DMF, room temperature, 12 h; v, RCO_2H, HBTU, Et_3N, DMF, room temperature, 12 h.
For LK codes of compounds **10a**–**o** and **11a**,**b**, see Online Supplementary Materials.

remained an unchanged component of the scaffold. The oxadiazole moiety is often used for bioisosteric amide bond replacement in the design of peptidomimetics and inhibitors. However, the reverse replacement is not impossible as well. In this work, we decided to test the potential effects of this scaffold modification on antibacterial activity.

Based on our previous investigations[11–13] on changing pharmacophore structures, we proposed the following synthesis scheme (Scheme 1). As the starting point of modification, we chose 6-{3-[4-(4-fluorophenoxy)phenyl]-1,2,4-oxadiazol-5-yl}-1*H*-indole **4** possessing MIC value of 2 μg ml^{-1} measured for *S. aureus* ATCC 29213.[5] Alkylation of 4-fluorophenol **5** with ethyl 4-fluorobenzoate **6** or 4-fluoronitrobenzene **7** and subsequent saponification/reduction afforded the starting derivatives 4-(4-fluorophenoxy)benzoic acid **8** and 4-(4-fluorophenoxy)aniline **9**, respectively. The ultimate HBTU-mediated condensation of **8** or **9** with protected amides or amino acids leads after final work up to the target derivatives **10a**–**o** and **11a**,**b** as hydrochlorides at side amino moieties, respectively (for synthetic details, see Online Supplementary Materials; Scheme 1 depicts structures **10** and **11** as free bases).

All synthesized compounds were tested against Gram-positive (*S. aureus* and *E. faecalis*) or Gram-negative (*P. aeruginosa*, *A. baumannii*, *K. pneumoniae*, *E. cloacae*) pathogens of the so-called ESKAPE panel,[14] which includes the five bacterial families with high ability to obtain multi-drug resistance. Ciprofloxacin was used as the positive control and comparator. The compounds were initially screened at a single concentration to determine the presence and the diameter of the bacterial growth inhibition zone (IZ) around the drug-treated disk. Those compounds that displayed growth inhibition were tested in serial dilution mode to determine the MIC (methods for antimicrobial activity measuring can be found in Online Supplementary Materials). Active compounds (MICs ⩽ 6 μg ml^{-1}) and their testing results are shown in Table 1.

The most active compound **10g** (LK1819) was screened against a panel of MRSA multiresistant clinical isolates. Its MIC values were in the range of 0.09–0.75 μg ml^{-1} and, in most cases, were 10–100 times lower than the control MICs (a full antimicrobial activity data set can be found in Online Supplementary Materials). They were significantly lower than those for compound **4** (1–4 μg ml^{-1}) against multiresistant MPSA strains.[5]

The initial set of compounds consisted of compound **4** isosteric analogue, *N*-[4-(4-fluorophenoxy)phenyl]-1*H*-indole-5-carboxamide **10a** (inactive), and some derivatives with simple amino acids. From this series, compound **10n** turned out to be active. Its close analogues **10b** and **10j** also showed good results. However, the placement of the amide group in substances **11a**,**b** in the inverted position leads to decreased activity. The expansion of the substituent set made it possible to identify the lead compound **10g** with maximum activity against MRSA. Interestingly, the resulting antibacterials have significant activity not only against Gram-positive but also against Gram-negative microorganisms such as *A. baumannii* and *E. cloacae*. This is not

Table 1 Antibacterial activity [disk diffusion method inhibition zone (IZ) and minimal inhibitory concentration (MIC)] of compounds **10b**,**c**,**g**,**h**,**j**,**n** and ciprofloxacin (positive control) against the ESKAPE panel of pathogens.

Compound	IZ (mm)/MIC (μg ml^{-1}) values					
	E. faecalis	*S. aureus*	*K. pneumoniae*	*A. baumannii*	*P. aeruginosa*	*E. cloacae*
10b	9/0.75	7/ > 6	15/0.75	0/ > 6	13/3.00	19/1.50
10c	0/n.t.[a]	12/0.37	0/ n.t.[a]	10/0.74	10/0.74	0/1.48
10g[b]	9/0.33	9/0.33	0/1.30	0/1.30	0/n.t.[a]	0/2.6
10h	9/3.10	11/0.39	0/ n.t.[a]	0/ n.t.[a]	0/n.t.[a]	0/1.55
10j	9/0.33	13/0.65	0/2.60	0/2.60	0/n.t.[a]	11/2.60
10n	9/6.20	7/0.39	0/1.55	0/3.10	0/n.t.[a]	0/3.10
Control	17/1.25	17/1.25	13/0.60	14/2.50	9/0.60	21/3.00

[a] 'n.t.' stands for not tested. [b] The most active compound **10g** (LK1819) is highlighted.

characteristic of the parent oxadiazole antibiotics and may indicate another mechanism of action. Indeed, substances with similar chemical structures have recently been proposed for use against MRSA[15] and as general antibiotics.[16] These molecules are built as a linear chain of aromatic cores with a 1,3,4-oxadiazole moiety in the middle.[17] Other mechanisms of action have been postulated for them.

In summary, the results obtained demonstrate the high potential of the new class of antibiotics and require further work to optimize the lead compound and to establish the precise mechanism of action of these substances.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.04.016.

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