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Preliminary communication

Synthesis and preliminary antibacterial evaluation of Linezolid-like 1,2,4-oxadiazole derivatives

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ABSTRACT

In the present study the synthesis of new Linezolid-like molecules has been achieved by substitution of the oxazolidinone central heterocyclic moiety with a 1,2,4-oxadiazole ring. Two series of 1,2,4-oxadiazoles, bearing different side-chains and containing a varying number of fluorine atoms, were synthesized and preliminarily tested for biological activity against some Gram-positive and Gram-negative bacteria using Linezolid and Ceftriaxone as reference drugs.

1. Introduction

The alarming rate of increase of the multidrug resistance phenomenon among bacterial pathogens in hospitals represents one of the major challenges for researchers [1–7]. For approximately four decades, from the 40's until the mid 70's, the pharmaceutical industry provided a steady flow of new antibiotics, including several with new mechanisms of action that circumvented the problems caused by bacterial resistance to earlier agents.

Since then only three antibiotics, Quinupristin/Dalfopristin, Linezolid, and Daptomycin, have been marketed in Europe to treat infections caused by multidrug-resistant Gram-positive bacteria [4]. In particular, Linezolid (Fig. 1) is the lead compound of oxazolidinones [8,9] and was approved in 2000 by the FDA for the treatment of community-acquired and nosocomial pneumonia, complicated and uncomplicated skin and soft-tissue infections, and infections caused by MRSA and VRE [10–12]. It is the only marketed oxazolidinone, although others are in development. It binds the 50S ribosomal subunit preventing the formation of a functional initiation complex 70S for the protein synthesis [13,14]. This unique mechanism, among protein synthesis inhibitors, explains why Linezolid retained antibacterial activity against Gram-positive organisms which were resistant to others antibacterials as well as why cross-resistance does not occur with other classes of antibiotics [8,15]. Nevertheless, resistance towards Linezolid has been recently triggered by mutations within regions of 50S large-subunit ribosomal proteins, which closely interact with the oxazolidinone binding site in the peptidyl transferase center, as well as by enhanced efflux pumps or inactivation of the endogenous ribosomal methyltransferase [16,17].

In order to rationalize the type of modifications, the structure of Linezolid can formally be divided into four portions according to oxazolidinone antibacterials nomenclature [8]: i) the A-Ring, consisting of the oxazolidinone central heterocycle; ii) the B-ring, consisting of a *N*-aryl moiety linked to the oxazolidinone nitrogen; iii) the C-ring, consisting of either a carbo- or heterocyclic functional group, not necessarily aromatic; iv) the side-chain, consisting of any functional group linked to the oxazolidinone C(5) or in an isosteric position with respect to an A-Ring of general type (Fig. 1).

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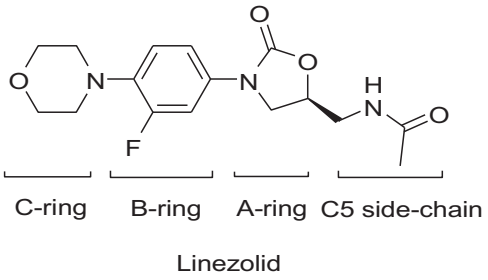


Fig. 1. Structure and nomenclature of Linezolid.

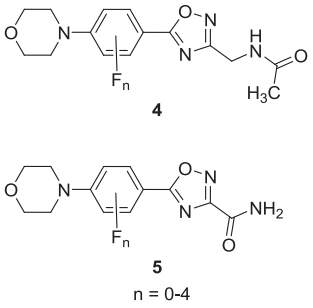


Chart 2.

To date, numerous structural modifications of Linezolid have been reported, many of them involving the introduction of heterocyclic moieties replacing the A-, B- or C-ring [8,10,11,15,18–21]. For instance, 1,2,4-oxadiazoles have been introduced in compound **1** (Chart 1) as terminal heterocyclic rings, far from the oxazolidinonic core [21]. Additionally, it has been shown that the antibacterial activity of Linezolid analogues could be maintained by replacing the oxazolidinone ring with five-membered heteroaromatic rings, such as isoxazole in compound **2** (Chart 1). Contrary to initial claims [15], this result reveals that the presence of the oxazolidinone C5 asymmetric center is not necessary for antibacterial activity [18,22].

Surprisingly, despite the well-known biological activity of 1,2,4-oxadiazoles [23–25] and the non-fluorinated series of 3-morpholinophenyl-1,2,4-oxadiazoles [26], there are no reports of Linezolid analogues containing a 1,2,4-oxadiazole as the A-ring portion.

We, therefore, decided to exploit the synthesis of a series of Linezolid-like derivatives bearing a 1,2,4-oxadiazole moiety in place of the oxazolidinone ring. As for the choice of the side-chain linked to the C(3) of the 1,2,4-oxadiazole ring, in addition to the typical methylene–acetamide moiety of Linezolid, we decided to introduce a carboxamide moiety. In fact, it has been reported that oxazolidinones **3** (Chart 1), endowed with a *reversed amide* side-chain, showed a reduction of toxic effects and an extension of the spectrum of activity associated with monoamine oxidase (MAO) inhibition [27].

Additionally, since the content of fluorine atoms on the B-ring can affect the biological activity [28,29], we planned the synthesis of derivatives with a varying number of fluorine atoms on the phenyl ring (B-ring). The general structure of our target molecules is illustrated in Chart 2.

2. Results and discussion

2.1. Chemistry

5-Morpholinoaryl-substituted 1,2,4-oxadiazoles **4a**–**e**, containing a different number of fluorine atoms in the phenyl ring, were obtained by initially building the oxadiazole heterocycle by following the amidoxime synthetic route (Scheme 1) [23]. When taking advantage of this approach, one should consider that the amidoxime should have been previously functionalized either with the final side-chain or with one of its precursors. Therefore, 2-aminoacetonitrile **6** was previously acetylated to obtain the *N*-(cyanomethyl)acetamide **7** which was subsequently treated with hydroxylamine to produce amidoxime **8**.

The latter was acylated by reaction with benzoyl chloride and subsequent thermal cyclization of the obtained crude *O*-aroylamidoxime under solvent-free conditions produced the corresponding oxadiazole **9**. The same approach was also applied to the synthesis of fluorinated oxadiazoles **11a**–**e** by using the corresponding fluorinated benzoyl chlorides **10a**–**e**. In the last step, compounds **4a**–**e** were obtained by reaction of the corresponding oxadiazoles **11a**–**e** with morpholine through a SN_{Ar} which involved the displacement of the fluorine atom occupying the *para* position with respect to the oxadiazole ring [30–34].

Similarly, 1,2,4-oxadiazole-3-carboxamides **5a**–**e,** bearing a *reversed amide* moiety with respect to the side-chain of Linezolid, were obtained by initially constructing the oxadiazole ring through acylation of amidoxime **12** with aroyl chlorides **10a**–**e** and subsequent solvent-free thermal cyclization of the corresponding *O*-acylamidoxime into 1,2,4-oxadiazoles **13a**–**e** (Scheme 2).

3-Carboxyethyl-5-fluoroaryl-1,2,4-oxadiazoles **13a**–**d** were then converted into the corresponding 3-carboxamides **14a**–**d** by treatment with methanolic ammonia solution at room temperature. In the final step, compounds **14a**–**d** were functionalized by a SN_{Ar} introducing morpholine on the 4′ position of the aryl moiety and producing target compounds **5a**–**d** (Scheme 2).

This approach could not be applied to the more reactive pentafluorophenyl-oxadiazole **13e**, which upon treatment with the ammonia solution underwent a displacement of the 4′-fluorine atom by the methanolic solvent. In turn, the pronounced reactivity of the pentafluorophenyl moiety of compound **13e** allowed the introduction of the morpholine moiety and this gave rise to

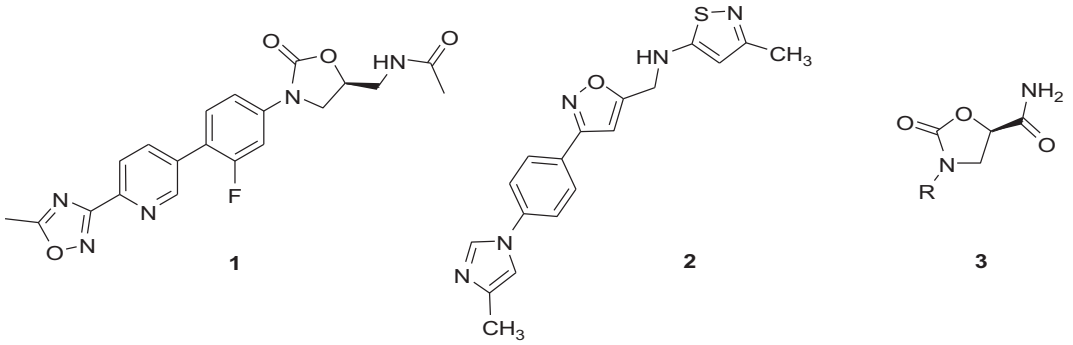
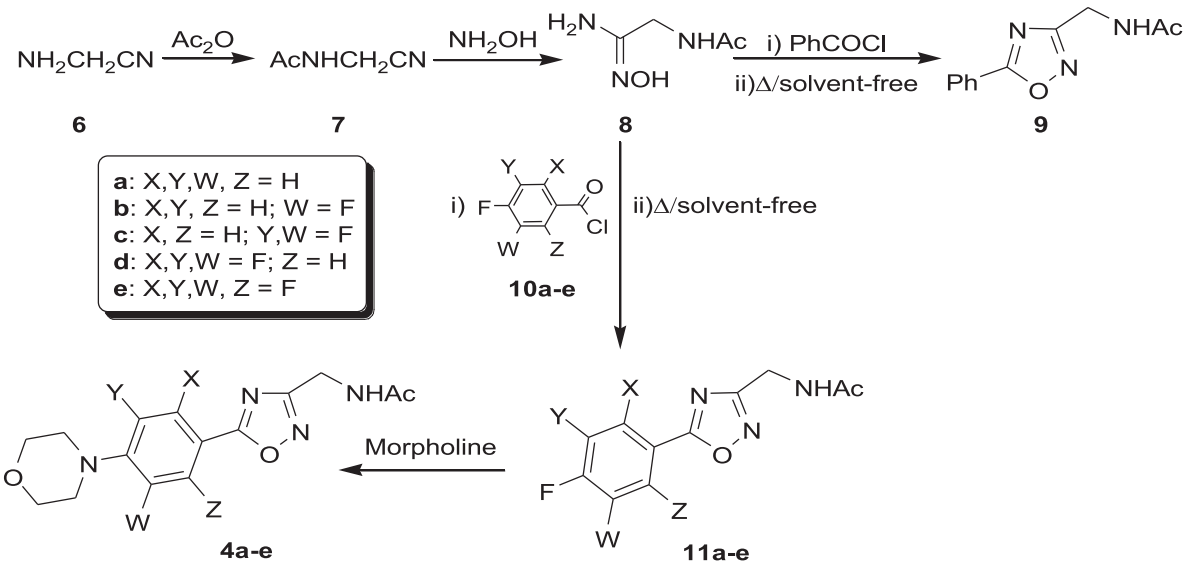
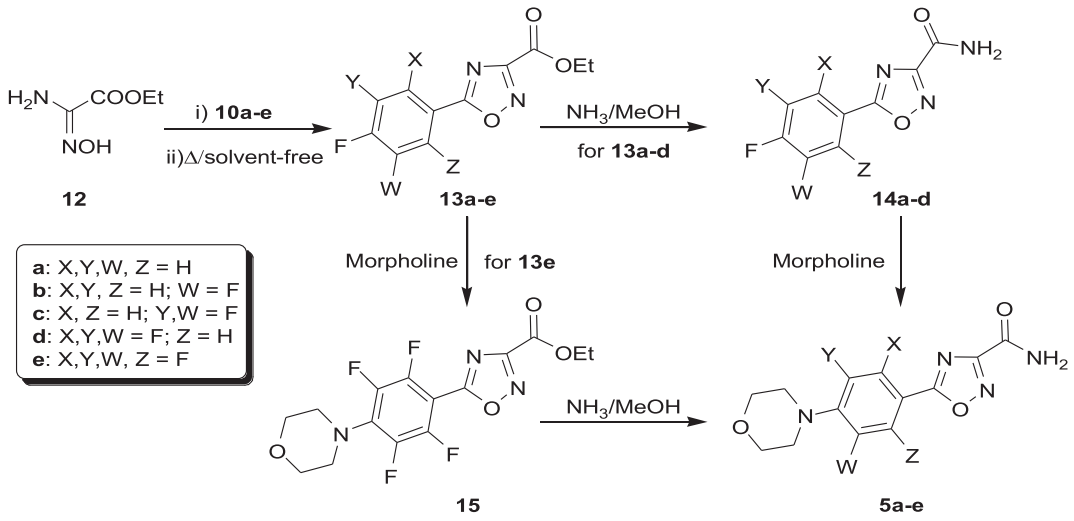


Chart 1.



Scheme 1.



Scheme 2.

Table 1
Antimicrobial activities, expressed in MIC values (mg/L) of synthetized compounds against Gram-positive and Gram-negative pathogens.

	4a	4b	4c	4d	4e	5a	5b	5c	5d	5e	Ceftriaxone	Linezolid
S. pyogenes ATCC 19615	128	128	128	128	>256	128	128	128	128	>256	<0.12	1
S. pyogenes EryR 6	128	128	128	128	>256	128	128	128	128	>256	<0.12	0,12
S. pyogenes EryR 24	64	128	>256	>256	>256	>256	128	>256	>256	>256	<0.12	0,12
S. pyogenes EryR 47	64	>256	128	128	>256	>256	>256	128	128	>256	<0.12	1
S. pyogenes EryS 3	128	>256	>256	>256	>256	128	128	>256	>256	>256	<0.12	1
S. pyogenes EryS 9	128	>256	>256	>256	>256	128	>256	>256	>256	>256	<0.12	1
S. pyogenes EryS 12	128	128	>256	>256	>256	128	128	>256	>256	>256	<0.12	0,25
S aureus ATCC 29213	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	4	4
S aureus ATCC 25923	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	1	1
S aureus ATCC 43300	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	8	2
S aureus GISA MU 50	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	4	2
MRSA C530	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	2
S. pneumoniae PEN I	128	>256	>256	>256	>256	128	>256	>256	>256	>256	0.12	1
E. coli ATCC 25922	>256	128	128	128	128	>256	128	128	128	128	0.12	4
E. coli 3350	128	128	128	128	>256	128	128	128	128	>256	4	>256
S. marcescens Mel 522	128	>256	>256	>256	>256	128	128	>256	>256	>256	<0.12	128

compound **15**. Finally, compound **15** was converted into the target carboxamide **5e** by reaction with ammonia.

It is worth noting that the synthetic strategy used to obtain compounds **5a**–**e**, the reverse approach (i.e. an initial reaction of **13a**–**e** with morpholine followed by reaction in methanolic ammonia), could not be applied to all compounds **13a**–**e** since reaction of morpholine with the ester moiety was observed for the less fluorinated substrates.

2.2 Biological activity

In this preliminary study, a screening of the antibacterial activity was carried out on compounds **4a**–**e** and **5a**–**e** against a series of clinical isolates. Tested Gram-positive and Gram-negative bacterial pathogens included: *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *and Serratia marcescens*. Antimicrobial activities are summarized in Table 1 and were determined by the microbroth dilution method using the Clinical Laboratory Standards Institute (CLSI) recommendations (See Experimental). MIC (Minimal Inhibition Concentrations) values were expressed in mg/L.

The synthesized compounds showed weak or no antibacterial activity against tested strains. Considering that the 1,2,4-oxadiazole ring is isosteric with the oxazolidinone ring and possesses very similar hydrogen bond acceptors sites, one may ascribe the observed weak activity to the introduction of an heteroaromatic core which could affect the cellular uptake. Although weak, the activity was observed more frequently for the non-fluorinated or less fluorinated compounds. In particular, only compound **4a** demonstrated a better activity against *S. pyogenes* (64 mg/L). Interestingly, this activity was shown for two clinical isolates (EryR 24 and EryR 47) both expressing ribosomal constitutive resistance for macrolide class compounds (MIC of Erythromycin = >256 mg/L).

3. Conclusion

Two series of 1,2,4-oxadiazoles analogues of Linezolid were synthesized by using the *amidoxime route* approach [23]. The two series differ for the type of side-chain, while, within a given series, the number of fluorine atoms on the phenyl moiety varies. The sequence of the synthetic steps was crucial for the synthesis of compounds **5a**–**e** since the fluorine content was strongly affecting the competition between the ester and the fluoroaryl moiety for the nucleophilic reagent. Compounds **4a**–**e** and **5a**–**e** were tested to evaluate their antibacterial activity against a series of standard and clinical isolates. Tested Gram-positive and Gram-negative bacterial pathogens included: *S. pyogenes*, *S. pneumoniae*, *S. aureus*, *E. coli* and *S. marcescens*. The antibacterial assay against other Gram-positive strains is in progress, because none of the presented 1,2,4-oxadiazoles was effective against the tested microorganisms in comparison with used drugs. In order to optimize the pharmacological properties of this class of compounds, the obtained biological activity data will be used as inactive compounds references in the construction of a QSAR model based on a chemoinformatic approach recently used by some of us [35].

4 Experimental

4.1. Materials and methods

Melting points were determined on a Reichart-Thermovar hot-stage apparatus and are uncorrected. IR spectra (Nujol) were determined with a Shimadzu FTIR-8300 instrument; ^{1}H NMR spectra were recorded on a Bruker 300 Avance spectrometer using TMS as an internal standard. GC–MS determinations were carried out on a Shimadzu GCMS-QP2010 system. Flash chromatography was performed by using silica gel (0.040–0.063 mm) and mixtures of ethyl acetate and petroleum ether (fraction boiling in the range of 40–60 °C) in various ratios. Compound **12** was prepared according to reported procedures [36]. Biological assays were performed by using a broth microdilution method as described by CLSI [37].

4.2. N-(Cyanomethyl)-acetamide (**7**)

Acetic anhydride (2.03 g, 19.9 mmol) was added at 0 °C to a solution of 2-aminoacetonitrile sulfate (2.04 g, 13.2 mmol) in Pyridine. The reaction mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and the residue treated with water and rendered alkaline (pH 8) by adding NaOH(aq) 1 M. The aqueous phase was extracted with EtOAc (3 × 75 mL). The organic layers were collected, dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure to give the required product **7** as a white solid (0.62 g, 48%); mp 73–74 °C (lit. 77 °C [38]); IR (Nujol) 3301, 3060, 2258, 1634 cm^{-1}; GC–MS *m*/*z*: 98 (M^+, 100%); ^{1}H NMR (300 MHz-$CDCl_3$) δ 7.80 (bs, 1H, exch. with D_2O), 4.10 (d, 2H, J = 5.7 Hz), 2.03 (s, 3H); Anal. Found (calc) for $C_4H_6N_2O$ (%): C, 48.97 (48.90); H, 6.16 (6.10); N, 28.56 (28.50).

4.3. 2-(N-Acetylamino)-acetamidoxime (***8***)

A solution of hydroxylamine hydrochloride (0.65 g, 9.5 mmol) and NaOH (0.3 g, 9.5 mmol) in water (8 mL) was added at 0 °C to a solution of **7** (0.62 g, 6.3 mmol) in MeOH (10 mL). The reaction mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and the residue treated with water (50 mL) and extracted with EtOAc (3 × 75 mL). The organic layers were collected, dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure to give the required product **8** as a white solid (0.74 g, 90%); mp 123–125 °C; IR (Nujol) 3366, 3228, 1670, 1625 cm^{-1}; GC–MS *m*/*z*: 131 (M^+, 100%); ^{1}H NMR (300 MHz-DMSO-d_6) δ 9.07 (bs, 1H, exch. with D_2O), 8.16 (bs, 1H, exch. with D_2O), 5.35 (bs, 2H, exch. with D_2O), 3.66 (d, 2H, J = 5.7 Hz), 1.93 (s, 3H); Anal. Found (calc) for $C_4H_9N_3O_2$ (%): C, 36.64 (36.60); H, 6.92 (6.90); N, 32.04 (32.00).

4.4. General procedure for the preparation of compounds ***9*** and ***11a–e***

Either benzoyl chloride or fluorinated aroyl chlorides **10a**–**e** (2.2 mmol) were added to a solution of **8** (0.26 g; 2 mmol) in Acetone (20 mL) containing also K_2CO_3 (0.30 g, 2.2 mmol). The mixture was stirred at room temperature for 1 h after which the solvent was removed under reduced pressure. The residue was treated with water and the solid precipitate was collected by filtration. The *O*-acylamidoxime so formed was heated, without any further purification, at 170 °C for 5 min in a sealed tube. The crude material obtained was purified by column chromatography to give the desired products 1,2,4-oxadiazoles **9** and **11a**–**e**.

4.4.1. N-((5-Phenyl-1,2,4-oxadiazol-3-yl)-methyl)-acetamide (***9***)

White solid (0.39 g, 90%); mp 128–130 °C; IR (Nujol) 3261, 1646 cm^{-1}; GC–MS *m*/*z*: 217 (M^+, 100%); ^{1}H NMR (300 MHz; DMSO-d_6) δ 8.67 (t, 1H, J = 5.4 Hz; exch. with D_2O), 8.17–8.14 (m, 2H), 7.80–7.67 (m, 3H), 4.50 (d, 2H, J = 5.4 Hz), 1.94 (s, 3H). Anal. Found (calc) for $C_{11}H_{11}N_3O_2$ (%): C, 60.82 (60.80); H, 5.10 (5.15); N, 19.34 (19.30).

*4.4.2. N-((5-(4′-Fluorophenyl)-1,2,4-oxadiazol-3-yl)-methyl)-acetamide (**11a**)*

White solid (0.30 g, 64%); mp 146–148 °C; IR (Nujol) 3300, 1651 cm^{-1}; GC–MS *m/z*: 235 (M^+, 100%); ^1H NMR (300 MHz-DMSO-d_6) δ 8.64 (t, 1H, J = 5.6 Hz; exch. with D_2O), 8.26–8.19 (m, 2H), 7.58–7.51 (m, 2H), 4.49 (d, 2H, J = 5.6 Hz), 1.94 (s, 3H). Anal. Found (calc) for $C_{11}H_{10}FN_3O_2$ (%): C, 56.17 (56.10); H, 4.29 (4.20); N, 17.86 (17.80).

*4.4.3. N-((5-(3′,4′-Difluorophenyl)-1,2,4-oxadiazol-3-yl)-methyl)-acetamide (**11b**)*

White solid (0.30 g, 59%); mp 145–146 °C; IR (Nujol) 3302, 1622 cm^{-1}; GC–MS *m/z*: 253 (M^+, 100%); ^1H NMR (300 MHz; DMSO-d_6) δ 8.65 (t, 1H, J = 5.8 Hz; exch. with D_2O), 8.25–8.19 (m, 1H), 8.06–8.03 (m, 1H), 7.83–7.74 (m, 1H), 4.50 (d, 2H, J = 5.8 Hz), 1.94 (s, 3H). Anal. Found (calc) for $C_{11}H_9F_2N_3O_2$ (%): C, 52.18 (52.10); H, 3.58 (3.50); N, 16.60 (16.65).

*4.4.4. N-((5-(3′,4′,5′-Trifluorophenyl)-1,2,4-oxadiazol-3-yl)-methyl)-acetamide (**11c**)*

White solid (0.35 g, 65%); mp 161–163 °C; IR (Nujol) 3292, 1659, cm^{-1}; GC–MS *m/z*: 271 (M^+, 100%); ^1H NMR (300 MHz; DMSO-d_6) δ 8.64 (t, 1H, J = 6.0 Hz; exch. with D_2O), 8.14 (t, 2H, J = 7.2 Hz), 4.50 (d, 2H, J = 6.0 Hz), 1.94 (s, 3H). Anal. Found (calc) for $C_{11}H_8F_3N_3O_2$ (%): C, 48.72 (48.70); H, 2.97 (2.90); N, 15.49 (15.40).

*4.4.5. N-((5-(2′,3′,4′,5′-Tetrafluorophenyl)-1,2,4-oxadiazol-3-yl)-methyl)-acetamide (**11d**)*

White solid (0.50 g, 87%); mp 117–119 °C; IR (Nujol) 3300, 1651 cm^{-1}; GC–MS *m/z*: 289 (M^+, 100%); ^1H NMR (300 MHz; DMSO-d_6) δ 8.67 (t, 1H, J = 5.7 Hz; exch. with D_2O), 8.22–8.13 (m, 1H), 4.50 (d, 2H, J = 5.7 Hz), 1.94 (s, 3H). Anal. Found (calc) for $C_{11}H_7F_4N_3O_2$ (%): C, 45.69 (45.70); H, 2.44 (2.40); N, 14.53 (14.50).

*4.4.6. N-((5-Pentafluorophenyl-1,2,4-oxadiazol-3-yl)-methyl)-acetamide (**11e**)*

White solid (0.46 g, 75%); mp 130–132 °C; IR (Nujol) 3296, 1653 cm^{-1}; GC–MS *m/z*: 307 (M^+, 100%); ^1H NMR (300 MHz; DMSO-d_6) δ 8.70 (t, 1H, J = 6.0 Hz; exch. with D_2O), 4.56 (d, 2H, J = 6.0 Hz), 1.94 (s, 3H). Anal. Found (calc) for $C_{11}H_6F_5N_3O_2$ (%): C, 43.01 (43.00); H, 1.97 (1.90); N, 13.68 (13.60).

*4.5. Synthesis of compounds **4a–e***

*4.5.1. N-((5-(4′-(Morpholin-N′-yl)-phenyl)-1,2,4-oxadiazol-3-yl)-methyl)-acetamide (**4a**)*

Compound **11a** (0.23 g; 1 mmol) was dissolved in morpholine (3 mL) and the mixture refluxed for 5 h. The reaction mixture was concentrated under reduced pressure and treated with water (25 mL). The formed precipitate of compound **4a** was collected by filtration as a white solid (0.21 g, 70%); mp 197–199 °C; IR (Nujol) 3283, 1653 cm^{-1}; GC–MS *m/z*: 302 (M^+, 100%); ^1H NMR (300 MHz; DMSO-d_6) δ 8.59 (t, 1H, J = 5.7 Hz; exch. with D_2O), 7.96 (d, 2H, J = 9.0 Hz), 7.16 (d, 2H, J = 9.0 Hz), 4.44 (d, 2H, J = 5.7 Hz), 3.80 (t, 4H, J = 4.8 Hz), 3.37 (t, 4H, J = 4.8 Hz), 1.94 (s, 3H); ^{13}C NMR (62.5 MHz; DMSO-d_6) δ 175.5, 169.7, 168.8, 154.1, 129.3, 114.1, 112. 5, 66.0, 46.9, 34.6, 22.6. Anal. Found (calc) for $C_{15}H_{18}N_4O_3$ (%): C, 59.59 (59.50); H, 6.00 (6.05); N, 18.53 (18.50).

*4.5.2. N-(5-(3′-Fluoro-(4′-(morpholin-N′-yl)-phenyl)-1,2,4-oxadiazol-3-yl)-methyl)-acetamide (**4b**)*

Morpholine (0.18 g; 2 mmol) was added to a solution of oxadiazole **11b** (0.25 g; 1 mmol) in DMF (3 mL). The mixture was refluxed for 1 h, concentrated under reduced pressure and treated with water. The formed precipitate of compound **4b** was collected by filtration as a white solid (0.23 g, 72%); mp 202–204 °C; IR (Nujol) 3281, 1659 cm^{-1}; GC–MS *m/z*: 320 (M^+, 100%); ^1H NMR (300 MHz; DMSO-d_6) δ 8.63 (t, 1H, J = 6.0 Hz; exch. with D_2O), 7.91 (d, 1H, J = 9.0 Hz), 7.84 (d, 2H, J = 14.7 Hz), 7.27 (t, 1H, J = 9.0 Hz), 4.46 (d, 2H, J = 5.7 Hz), 3.81 (t, 4H, J = 4.2 Hz), 3.24 (t, 4H, J = 4.2 Hz), 1.94 (s, 3H). ^{13}C NMR (62.5 MHz; DMSO-d_6) δ 174.4, 169.7, 169.1, 154.0 (d, J_{C-F} = 244.6 Hz), 143.8, 125.3, 119.5, 115.9, 115.5 (d, J_{C-F} = 23.7 Hz), 66.1, 49.9, 34.6, 22.6. Anal. Found (calc) for $C_{15}H_{17}FN_4O_3$ (%): C, 56.24 (56.20); H, 5.35 (5.30); N, 17.49 (17.40).

*4.5.3. N-((5-(3′,5′-Difluoro-4′-(morpholin-N′-yl)-phenyl)-1,2,4-oxadiazol-3-yl)-methyl)-acetamide (**4c**)*

Morpholine (0.18 g; 2 mmol) was added to a solution of oxadiazole **11c** (0.27 g; 1 mmol) in DMF (3 mL). The mixture was refluxed for 1 h, concentrated under reduced pressure and treated with water. The formed precipitate of compound **4c** was collected by filtration as a white solid (0.23 g, 68%); mp 196–198 °C; IR (Nujol) 3292, 1659 cm^{-1}; GC–MS *m/z*: 338 (M^+, 100%); ^1H NMR (300 MHz; DMSO-d_6) δ 8.62 (t, 1H, J = 5.7 Hz; exch. with D_2O), 7.78 (d, 2H, J = 9.0 Hz), 4.48 (d, 2H, J = 5.7 Hz), 3.77 (t, 4H, J = 4.5 Hz), 3.33 (t, 4H, J = 4.5 Hz), 1.94 (s, 3H). Anal. Found (calc) for $C_{15}H_{16}F_2N_4O_3$ (%): C, 53.25 (53.20); H, 4.77 (4.70); N, 16.56 (16.50).

*4.5.4. N-((5-(2′,3′,5′,-Trifluoro-4′-(morpholin-N′-yl)-phenyl)-1,2,4-oxadiazol-3-yl)-methyl)-acetamide (**4d**)*

Morpholine (0.18 g; 2 mmol) was added to a solution of oxadiazole **11d** (0.29 g; 1 mmol) in DMF (3 mL). The mixture was stirred for 24h at r.t, concentrated under reduced pressure and treated with water. The formed precipitate of compound **4d** was collected by filtration as a white solid (0.30 g, 84%); mp 199–201 °C; IR (Nujol) 3292, 1659 cm^{-1}; GC–MS *m/z*: 356 (M^+, 100%); ^1H NMR (300 MHz; DMSO-d_6) δ 8.64 (t, 1H, J = 5.7 Hz; exch. with D_2O), 7.72 (ddd, 1H, J_1 = 8.4 Hz, J_2 = 6.3 Hz, J_3 = 2.1 Hz), 4.50 (d, 2H, J = 5.7 Hz), 3.77 (t, 4H, J = 4.5 Hz), 3.41 (t, 4H, J = 4.5 Hz), 1.94 (s, 3H). Anal. Found (calc) for $C_{15}H_{15}F_3N_4O_3$ (%): C, 50.56 (50.55); H, 4.24 (4.20); N, 15.72 (15.70).

*4.5.5. N-((5-(2′,3′,5′,6′-Tetrafluoro-4′-(morpholin-N′-yl)-phenyl)-1,2,4-oxadiazol-3-yl)-methyl)-acetamide (**4e**)*

Morpholine (0.18 g; 2 mmol) was added to a solution of oxadiazole **11e** (0.31 g; 1 mmol) in DMF (3 mL). The mixture was stirred for 24 h at r.t, concentrated under reduced pressure and treated with water. The formed precipitate of compound **4e** was collected by filtration as a white solid (0.30 g, 80%); mp 189–191 °C; IR (Nujol) 3288, 1653 cm^{-1}; GC–MS *m/z*: 374 (M^+, 100%); ^1H NMR (300 MHz; DMSO-d_6) δ 8.69 (t, 1H, J = 6.0 Hz, exch. with D_2O), 4.52 (d, 2H, J = 6.0 Hz), 3.78 (t, 4H, J = 4.5 Hz), 3.45 (t, 4H, J = 4.5 Hz), 1.94 (s, 3H). Anal. Found (calc) for $C_{15}H_{14}F_4N_4O_3$ (%): C, 48.13 (48.10); H, 3.77 (3.70); N, 14.97 (14.90).

*4.6. General procedure for the preparation of compounds **13a–e***

A 10% excess of the appropriate fluorinated aroyl chlorides **10a–e** (4.4 mmol) was added to a solution of compound **12** (0.52 g, 4.0 mmol) in acetone (20 mL) together with K_2CO_3 (0.60 g, 4.4 mmol). The mixture was stirred at room temperature for 1 h after which the solvent was removed under reduced pressure. The residue was treated with water and the solid collected by filtration and heated, without any previous purification, at 180 °C for 30 min in a sealed tube. Chromatography of the residue yielded the corresponding oxadiazole compounds **13a–e**.

*4.6.1. 3-Carboxyethyl-5-(4′-fluorophenyl)-1,2,4,oxadiazole (**13a**)*

White solid (0.81 g, 86%); mp 75–76 °C (lit. 65 °C [39]); IR (Nujol) 1743, 1608 cm^{-1}; GC–MS *m/z*: 236 (M^+, 100%); ^1H NMR

(300 MHz; $CDCl_3$) δ 8.26–8.22 (m, 2H), 7.30–7.22 (m, 2H), 4.56 (q, 2H, J = 7.2 Hz), 1.48 (t, 3H, J = 7.2 Hz). Anal. Found (calc) for $C_{11}H_9FN_2O_3$ (%): C, 55.93 (55.90); H, 3.84 (3.80); N, 11.86 (11.80).

4.6.2. 3-Carboxyethyl-5-(3′,4′-difluorophenyl)-1,2,4,oxadiazole (**13b**)

White solid (0.75 g, 74%); mp 86–87 °C; IR (Nujol) 1741 cm^{-1}; GC–MS m/z: 254 (M^+, 100%); ^1H NMR (300 MHz-$CDCl_3$) δ 8.07–8.00 (m, 2H), 7.42–7.33 (m, 1H), 4.55 (q, 2H, J = 6.9 Hz), 1.48 (t, 3H, J = 6.9 Hz). Anal. Found (calc) for $C_{11}H_8F_2N_2O_3$ (%): C, 51.98 (51.90); H, 3.17 (3.10); N, 11.02 (11.0).

4.6.3. 3-Carboxyethyl-5-(3′,4′,5′-trifluorophenyl)-1,2,4,oxadiazole (**13c**)

White solid (0.80 g, 74%); mp 103–105 °C; IR (Nujol) 1747 cm^{-1}; GC–MS m/z: 272 (M^+, 100%); ^1H NMR (300 MHz; $CDCl_3$) δ 7.91 (dd, 2H, J_1 = 7.2 Hz, J_2 = 6.3 Hz), 4.57 (q, 2H, J = 7.2 Hz), 1.48 (t, 3H, J = 7.2 Hz). Anal. Found (calc) for $C_{11}H_7F_3N_2O_3$ (%): C, 48.54 (48.50); H, 2.59 (2.50); N, 10.29 (10.20).

4.6.4. 3-Carboxyethyl-5-(2′,3′,4′,5′-tetrafluorophenyl)-1,2,4,oxadiazole (**13d**)

White solid (0.86 g, 74%); mp 77–78 °C; IR (Nujol) 1749 cm^{-1}; GC–MS m/z: 290 (M^+, 100%); ^1H NMR (300 MHz; $CDCl_3$) δ 7.92–7.88 (m, 1H), 4.56 (q, 2H, J = 7.2 Hz), 1.48 (t, 3H, J = 7.2 Hz). Anal. Found (calc) for $C_{11}H_6F_4N_2O_3$ (%): C, 45.53 (45.50); H, 2.08 (2.00); N, 9.65 (9.60).

4.6.5. 3-Carboxyethyl-5-(pentafluorophenyl)-1,2,4,oxadiazole (**13e**)

White solid (0.92 g, 75%); mp 69–71 °C; IR (Nujol) cm^{-1} 1749; GC–MS m/z: 308 (M^+, 100%); ^1H NMR (300 MHz-$CDCl_3$) δ 4.56 (q, 2H, J = 7.0 Hz), 1.48 (t, 3H, J = 7.0 Hz); Anal. Found (calc) for $C_{11}H_5F_5N_2O_3$ (%): C, 48.87 (48.80); H, 1.64 (1.60); N, 9.09 (9.0).

4.7. General procedure for the preparation of carboxamides **14a–d**

An ammonia saturated methanolic solution (2 mL) was added to a solution of the appropriate esters **13a–d** (2.5 mmol) in MeOH (10 mL). The mixture was stirred for 30 min at room temperature, after which the solvent was removed under reduced pressure and the residue re-crystallized from MeOH.

4.7.1. 5-(4′-Fluorophenyl)-1,2,4-oxadiazole-3-carboxamide (**14a**)

White solid (0.50 g, 97%); mp 215–216 °C; IR (Nujol) 3477, 3217, 1689 cm^{-1}; GC–MS m/z: 207 (M^+, 100%); ^1H NMR (300 MHz-DMSO-d_6) δ 8.46 (bs, 1H, exch. with D_2O), 8.30–8.24 (m, 3H, overlapped signals), 7.60–7.54 (m, 2H). Anal. Found (calc) for $C_9H_6FN_3O_2$ (%): C, 52.18 (52.10); H, 2.92 (2.90); N, 20.28 (20.25).

4.7.2. 5-(3′,4′-Difluorophenyl)-1,2,4-oxadiazole-3-carboxamide (**14b**)

White solid (0.52 g, 92%); mp 201–203 °C; IR (Nujol) 3419, 3283, 1686 cm^{-1}; GC–MS m/z: 225 (M^+, 100%); ^1H NMR (300 MHz-DMSO-d_6) δ 8.48 (bs, 1H, exch. with D_2O), 8.30–8.24 (m, 2H), 8.08 (bs, 1H, exch. with D_2O), 7.86–7.77 (m, 1H). Anal. Found (calc) for $C_9H_5F_2N_3O_2$ (%): C, 48.01 (48.00); H, 2.24 (2.20); N, 18.66 (18.60).

4.7.3. 5-(3′,4′,5′-Trifluorophenyl)-1,2,4-oxadiazole-3-carboxamide (**14c**)

White solid (0.59 g, 97%); mp 200–202 °C; IR (Nujol) 3367, 3180, 1689, cm^{-1}; GC–MS m/z: 243 (M^+, 100%); ^1H NMR (300 MHz-DMSO-d_6) δ 8.48 (bs, 1H exch. with D_2O), 8.27 (bs, 1H exch. with D_2O), 8.19 (dd, 2H, J_1 = 7.8 Hz, J_2 = 6.6 Hz). Anal. Found (calc) for $C_9H_4F_3N_3O_2$ (%): C, 44.46 (44.40); H, 1.66 (1.60); N, 17.28 (17.20).

4.7.4. 5-(2′,3′,4′,5′-Tetrafluorophenyl)-1,2,4-oxadiazole-3-carboxamide (**14d**)

White solid (0.64 g, 98%); mp 173–176 °C; IR (Nujol) 3481, 3331, 1686 cm^{-1}; GC–MS m/z: 261 (M^+, 100%); ^1H NMR (300 MHz-DMSO-d_6) δ 8.49 (bs, 1H, exch. with D_2O), 8.29 (bs, 1H exch. with D_2O), 8.28–8.19 (m, 1H). Anal. Found (calc) for $C_9H_3F_4N_3O_2$ (%): C, 41.40 (41.40); H, 1.16 (1.10); N, 16.09 (16.00).

4.8. 3-Carboxyethyl-5-(2′,3′,5′,6′-tetrafluoro-4′-(morpholin-N-yl)-phenyl)-1,2,4-oxadiazole **15**

A solution of**13e** (0.31 g, 1 mmol) in DMF (3 mL) containing morpholine (0.09 g, 1 mmol) was stirred at room temperature for 3 days. The reaction mixture was concentrated under reduced pressure, treated with water (50 mL) and extracted with EtOAc (3 × 75 mL). The organic layers were collected, dried over anhydrous Na_2SO_4, filtered and the solvent removed. The residue was chromatographed to give the required product **15** as a white solid (0.30 g, 80%); mp 160–162 °C; IR (Nujol) 1748 cm^{-1}; GC–MS m/z: 375 (M^+, 100%); ^1H NMR (300 MHz-$CDCl_3$) δ 4.56 (q, 2H, J = 7.2 Hz), 3.84 (t, 4H, J = 4.5 Hz), 3.46 (t, 4H, J = 4.5 Hz), 1.48 (t, 3H, J = 7.2 Hz). Anal. Found (calc) for $C_{15}H_{13}F_4N_3O_4$ (%): C, 48.01 (48.00); H, 3.49 (3.50); N, 11.20 (11.25).

4.9. General procedure for the preparation of compounds **5a–d**

Morpholine (1.74 g; 20 mmol) was added to a solution of **14a–d** (2.5 mmol) in DMF (5 mL). The resulting solution was refluxed for 1 h after which the reaction mixture was concentrated under reduced pressure, treated with water (50 mL) and extracted with EtOAc (3 × 75 mL). The organic layers were collected and dried over anhydrous Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The residue was chromatographed yielding the corresponding compounds **5a–d**.

4.9.1. 5-(4′-(Morpholin-N-yl)-phenyl)-1,2,4-oxadiazole-3-carboxamide (**5a**)

White solid (0.51 g, 74%); mp 247–249 °C; IR (Nujol) 3487, 3240, 1686 cm^{-1}; GC–MS m/z: 274 (M^+, 100%); ^1H NMR (300 MHz-DMSO-d_6) δ 8.37 (s, 1H, exch. with D_2O), 8.16 (s, 1H, exch. with D_2O), 8.01 (d, 2H, J = 9.0 Hz), 7.18 (d, 2H, J = 9.0 Hz), 3.80 (t, 4H, J = 4.5 Hz), 3.40 (t, 4H, J = 4.5 Hz); ^{13}C NMR (62.5 MHz; DMSO-d_6) δ 176.2, 164.4, 158.3, 154.3, 129.6, 114.1, 111.9, 66.0, 46.8. Anal. Found (calc) for $C_{13}H_{14}N_4O_3$ (%): C, 56.93 (56.90); H, 5.14 (5.10); N, 20.43 (20.40).

4.9.2. 5-(3′-Fluoro-4′-(morpholin-N-yl)-phenyl)-1,2,4-oxadiazole-3-carboxamide (**5b**)

White solid (0.61 g, 84%); mp 217–218 °C; IR (Nujol) 3469, 1721, 1687 cm^{-1}; GC–MS m/z: 292 (M^+, 100%); ^1H NMR (300 MHz-DMSO-d_6) δ 8.41 (s, 1H, exch. with D_2O), 8.20 (s, 1H, exch. with D_2O), 7.94 (dd, 1H, J_1 = 13.8 Hz, J_2 = 2.1 Hz), 7.90 (dd, 1H, J_1 = 19.2 Hz, J_2 = 2.1 Hz), 7.33–7.27 (m, 1H), 3.82 (t, 4H, J = 4.5 Hz), 3.27 (t, 4H, J = 4.5 Hz); ^{13}C NMR (62.5 MHz; DMSO-d_6) δ 175.2, 164.5, 158.0, 153.9 (d, J_{C-F} = 244.2 Hz), 144.0 (d, J_{C-F} = 6.7 Hz), 125.6, 119.5, 115.8 (d, J_{C-F} = 24.2 Hz), 115.5, 66.1, 49.8. Anal. Found (calc) for $C_{13}H_{13}FN_4O_3$ (%): C, 53.42 (53.40); H, 4.48 (4.40); N, 19.17 (19.10).

4.9.3. 5-(3′,5′-Difluoro-4′-(morpholin-N-yl)-phenyl)-1,2,4-oxadiazole-3-carboxamide (**5c**)

White solid (0.70 g, 90%); mp 208–210 °C; IR (Nujol) 3371, 3193, 1684 cm^{-1}; GC–MS m/z: 310 (M^+, 100%); ^1H NMR (300 MHz-DMSO-d_6) δ 8.44 (s, 1H, exch. with D_2O), 8.23 (s, 1H, exch. with D_2O), 7.86–7.80 (m, 2H), 3.77 (t, 4H, J = 4.5 Hz), 3.35 (t, 4H, J = 4.5 Hz). Anal. Found (calc) for $C_{13}H_{12}F_2N_4O_3$ (%): C, 50.33 (50.30); H, 3.90 (3.95); N, 18.06 (18.00).

4.9.4. 5-(2′,3′,5′,-Trifluoro-4′-(morpholin-N-yl)-phenyl)-1,2,4-oxadiazole-3-carboxamide (**5d**)

White solid (0.70 g, 85%); mp 204–206 °C; IR (Nujol) 3441, 3256, 1697 cm^{-1}; GC–MS *m*/*z*: 328 (M^+, 100%); ^1H NMR (300 MHz-DMSO-d_6) δ 8.44 (s, 1H, exch. with D_2O), 8.25 (s, 1H, exch. with D_2O), 7.82 (ddd, 1H, $J_1 = 12.6$ Hz, $J_2 = 6.3$ Hz, $J_3 = 2.1$ Hz), 3.78 (t, 4H, $J = 4.5$ Hz), 3.42 (t, 4H, $J = 4.5$ Hz). Anal. Found (calc) for $C_{13}H_{11}F_3N_4O_3$ (%): C, 47.57 (47.50); H, 3.38 (3.40); N, 17.07 (17.00).

4.10. 5-(2,3,5,6-Tetrafluoro-4-morpholinphenyl)-1,2,4-oxadiazole-3-carboxamide (**5e**)

An ammonia saturated methanolic solution (2 mL) was added to a solution of compound **15** (0.94 g, 2.5 mmol) in MeOH (20 mL). The mixture was stirred for 30 min at room temperature, after which the solvent was removed under reduced pressure and the residue re-crystallized from MeOH to give oxadiazole **5e** as a white solid (0.67 g, 77%); mp 252–253 °C; IR (Nujol) 3367, 3201, 1683 cm^{-1}; GC–MS *m*/*z*: 346 (M^+, 100%); ^1H NMR (300 MHz-DMSO-d_6) δ 8.47 (s, 1H, exch. with D_2O), 8.29 (s, 1H, exch. with D_2O), 3.78 (t, 4H, $J = 4.5$ Hz), 3.47 (t, 4H, $J = 4.5$ Hz). Anal. Found (calc) for $C_{13}H_{10}F_4N_4O_3$ (%): C, 45.10 (45.15); H, 2.91 (2.90); N, 16.18 (16.10).

4.11. Determination of minimum inhibitory concentrations (MICs)

The *in vitro* antibacterial activity of compounds **4a**–**e** and **5a**–**e** was studied by determining the Minimum Inhibitory Concentrations (MICs) by means of the broth microdilution method, according to the protocols approved by the Clinical and Laboratory Standards Institute (CLSI) [37].

Serial two-fold dilutions of each antibiotic stock solution were obtained using Mueller–Hinton broth in 96 wells microtitre plates. Compound stocks were prepared in 100% DMSO at 1000 g/L. Serial dilutions were made for compound concentrations of 0.12–>256 mg/L. An equal volume of 1×10^6 CFU/mL bacterial inoculum Colony Forming Unit/mL was added to each well of the microtitre plate containing 0.05 mL of serial antibiotic concentrations. The microtitre plate was then incubated at 35 °C for 16–20 h; subsequently each well was analyzed for the presence of visible bacterial growth. MIC was defined as the lowest concentration of the tested compound able to inhibit visible growth of the microorganism after overnight incubation. Controls with DMSO and uninoculated media were run parallel to the tested compounds under the same conditions. The *in vitro* antibacterial activity of compounds **4a**–**e** and **5a**–**e** was tested and compared to that of Linezolid used as oxazolidinones reference. *S. pyogenes* ATCC 19615, *S. aureus* ATCC 29213(MSSA), *S. aureus* ATCC 25923(MSSA), *S. aureus* ATCC 700699, also known as MU50 (MRSA) and *E. coli* 25922 were used as standard controls for MIC determinations.

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