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Synthesis and antibacterial evaluation of oxazolidin-2-ones structurally related to linezolid

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Abstract

Compounds structurally related to the known antimicrobial drug linezolid were selected in order to evaluate the influence of electron-withdrawing properties and altered geometric features as a result of the *N*-substituent modification. After a preliminary study of molecular modeling, cinnamoyl-, pyridin- and pyrimidinoxazolidin-2-ones were synthesized. None of the new compounds showed antibacterial activity.

Keywords: Oxazolidin-2-ones; Antibacterial activity; Linezolid

1. Introduction

Linezolid (ZYVOX®) is the first member of a new class of totally synthetic antimicrobial agents, the oxazolidinones [1–3]; it has been recently marketed in the USA and it is available in oral and i.v. formulations. The oxazolidinones are particularly efficacious in treating skin and soft tissues infections, nosocomial and community-acquired pneumonia and bacteremia caused by methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VREF) and penicillin-resistant *Streptococcus pneumoniae* (PRSP).

Oxazolidinones act by inhibiting the protein synthesis at very early stage; they bind the 50S subunit, so preventing the bacterial translation [4,5]. This represents a unique mechanism of action, and cross-resistance with other protein synthesis inhibitors has not been reported. Some rare cases of oxazolidinone resistance have been described, but only after a long treatment with linezolid [6].

Several SAR studies have been carried out on antibacterial oxazolidinones, focused primarly on substituents at carbon position 5 and at phenyl bound to the nitrogen in position 3 on the heterocycle [7–9]. Very little is known about the replacement of the above phenyl with other electron-rich groups. In literature are described only some attempts to space out the aromatic system to the oxazolidinone ring by small groups, like carbonyl or methylene; the result is a total loss of activity [10].

In this article we describe the synthesis of novel oxazolidin-2-ones structurally related to linezolid, obtained by replacement of phenyl with a cinnamoyl group and some heteroaromatic rings (Fig. 1).

2. Experimental

2.1. Chemistry

Melting points were determined on an Electrothermal IA 9100 apparatus and are uncorrected. Optical rotations were measured on a Perkin Elmer model 241 polarimeter. The infrared spectra were recorded on an FT-IR 1600 Perkin Elmer spectrometer. The NMR spectra were run at 300 MHz on a Varian spectrometer; chemical shifts (δ) are reported in ppm. Microanalyses were carried out with an Eurovector Euro EA 3000 model analyzer and the analytical results are within 0.4% of the theoretical values. GC analyses were run on an autosystem GC Perkin Elmer apparatus using a fused

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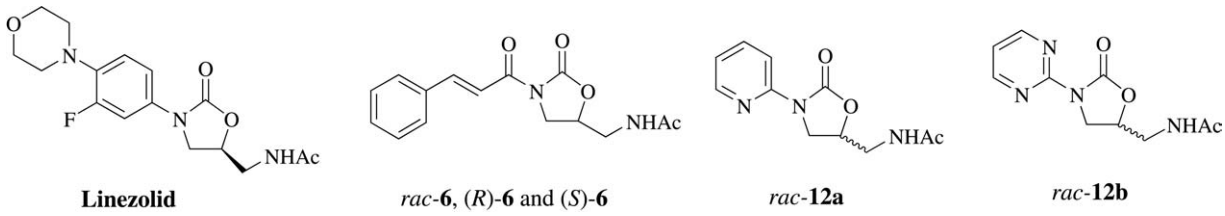


Fig. 1. Chemical structures of linezolid and new oxazolidin-2-ones.

silica capillary column (30 m, 0.53 mm ID), SPB-5 Supelco. Commercial reagents were used as received from Aldrich. THF was distilled from sodium/benzophenone.

2.2. Synthesis of compound rac-*6*

2.2.1. Racemic 5-(hydroxymethyl)-1,3-oxazolidin-2-one (rac-*1*)

A solution of 3-amino-1,2-propandiole (5.08 g, 55.8 mmol) and sodium carbonate (20.70 g, 195.3 mmol) in water (60 ml) was stirred at room temperature whilst triphosgene (4.70 g, 16.74 mmol) was carefully added. After 4 h, the mixture was extracted with CH_2Cl_2. The aqueous phase was neutralized with HCl and then evaporated under reduced pressure. The solid residue was suspended in absolute ethanol and the inorganic salts were filtered off. The filtrate was evaporated to give an oil, purified by chromatography on silica gel (eluent $CH_2Cl_2/CH_3OH/NH_4OH$, 10:4:0.5) to give the oxazolidinone *rac*-**1** (colourless oil, 50% yield). IR (KBr) 3324, 1736 cm^{-1}; ^1H NMR (DMSO) δ 3.17–3.54 (m, 4H, NHCH_2 and CH_2OH), 4.46–4.54 (m, 1H, OC*H*CH_2), 5.05 (t, 1H, O*H*), 7.38 (s, 1H, N*H*); ^{13}C NMR (DMSO) δ 41.9 (*C*H_2 ox), 62.5 (*C*H_2OH), 76.6 (*C*H), 159.6 (*C*=O).

2.2.2. Racemic (2-oxo-1,3-oxazolidin-5-yl)methyl 4-methylbenzenesulfonate (rac-*2*)

A mixture of alcohol *rac*-**1** (3.15 g, 26.9 mmol), toluene-*p*-sulfonyl chloride (6.15 g, 32.28 mmol) and triethylamine (4.88 ml, 34.97 mmol) in CH_2Cl_2 (30 ml) was stirred at room temperature for 21 h. The solution was diluted with CH_2Cl_2, washed with H_2O and a saturated aqueous solution of $NaHCO_3$, dried on Na_2SO_4 and evaporated under reduced pressure. The crude material was purified by chromatography on silica gel (eluent CH_2Cl_2/CH_3OH 95:5) to give the tosylate *rac*-**2** (white solid, 69% yield, m.p. 99 °C). IR (KBr) 3289, 1754, 1703 $cm^{-1;}$ ^1H NMR ($CDCl_3$) δ 2.45 (s, 3H, CH_3), 3.60 (dd, 1H, NHC*H*H), 3.69 (t, 1H, NHCH*H*), 4.15 (d, 2H, CH_2OSO_2), 4.76–4.84 (m, 1H, OC*H*CH_2), 5.27 (s, 1H, N*H*), 7.37 (d, 2H, aromatic), 7.79 (d, 2H, aromatic); ^{13}C NMR ($CDCl_3$) δ 21.9 (*C*H_3), 42.2 (*C*H_2 ox), 68.8 (*C*H_2OS), 73.1 (*C*H), 128.2 (*C*H aromatic), 130.3 (*C*H aromatic), 132.2 (*C*–S aromatic), 145.8 (*C*–C aromatic), 159.0 (*C*=O).

2.2.3. Racemic (3-cinnamoyl-2-oxo-1,3-oxazolidin-5-yl) methyl 4-methylbenzenesulfonate (rac-*3*)

Buthyllithium (11.3 ml, 18.1 mmol, 1.6 M solution in hexane) in dry THF (20 ml) and after 15 min, cinnamoyl chloride (4.22 g, 25.34 mmol) in THF (20 ml), were added to a stirred solution of *rac*-**2** (4.92 g, 18.1 mmol) in dry THF (60 ml), at –78 °C under nitrogen atmosphere. After 5 h at –78 °C, the mixture was allowed to warm to room temperature and stirred for further 20 h. The solvent was evaporated under reduced pressure to lead a crude product, which was dissolved in CH_2Cl_2 (60 ml). The organic phase was washed with H_2O, dried over Na_2SO_4 and concentrated at reduced pressure. The resulting oily product was purified by column chromatography on silica gel (eluent CH_2Cl_2/acetone, 98:2) to give the *rac*-**3** (white solid, 65% yield, m.p. 169–171 °C). IR (KBr) 1767, 1680, cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.43 (s, 3H, ArCH_3), 4.13–4.29 (m, 4H, NCH_2 e CH_2OSO_2), 4.78–4.81 (m, 1H, OC*H*CH_2), 7.35–7.89 (m, 11H, aromatic and C*H*=C*H*); ^{13}C NMR ($CDCl_3$) δ 21.9 (*C*H_3), 44.5 (*C*H_2N ox), 68.2 (*C*H_2OS), 70.4 (*C*H), 116.5 (*C*HCO), 128.3 (*C*H aromatic), 128.9 (*C*H aromatic), 129.2 (*C*H aromatic), 130.4 (*C*H aromatic), 131.1 (*C* aromatic), 132.1 (*C* aromatic), 134.6 (*C* aromatic), 145.9 (*C* aromatic), 147.0 (*C*HPh), 152.4 (*C*O ox), 165.2 (*C*OCHCH).

2.2.4. Racemic 5-(azidomethyl)-3-cinnamoyl-1,3-oxazolidin-2-one (rac-*4*)

Sodium azide (0.81 g, 12.41 mmol) was added to a solution of *rac*-**3** (4.53 g, 11.28 mmol) in dry DMSO (40 ml), at 60 °C under nitrogen atmosphere. After 2 h the mixture was diluted with H_2O and extracted with ethyl acetate. The organic phase was washed with a saturated aqueous solution of NaCl, dried over Na_2SO_4 and concentrated at reduced pressure. The crude product *rac*-**4** (white solid, 90% yield) was used in the following reaction without purification.

2.2.5. Racemic 5-(aminomethyl)-3-cinnamoyl-1,3-oxazolidin-2-one (rac-*5*)

A mixture of *rac*-**4** (2.61 g, 9.59 mmol), triphenylphosphine (2.77 g, 10.55 mmol) and H_2O (1.72 ml, 95.5 mmol) in THF (40 ml) was heated at 40 °C for 23 h. After cooling, the reaction mixture was diluted with HCl 2 N and extracted with ethyl acetate. Aqueous NaOH was added to the aqueous phase and the resulting basic solution extracted with ethyl acetate. The organic phase was washed with H_2O, dried and concentrated under reduced pressure to give the compound *rac*-**5** (white solid, 72% yield). ^1H NMR (DMSO) δ 2.19 (broad s, 2H, NH_2), 3.06–3.11 (m, 2H,CH_2NH_2), 4.25–4.33 (m, 2H, CH_2 ox), 4.44–4.49 (m, 1H, C*H*), 7.14 (d, 1H, CH=C*H*–CO), 7.44–7.80 (m, 6H, C*H* aromatic and CH=C*H*Ph); ^{13}C NMR (DMSO) δ 43.4 (*C*H_2NH_2), 47.4

(CH_2 ox), 76.8 (*C*H), 119.2 (*C*HCO), 128.1 (*C*H aromatic), 128.9 (*C*H aromatic), 136.2 (*C* aromatic), 145.0 (*C*HPh), 150.4 (*C*O ox), 166.2 (*C*OCHCH).

2.2.6. Racemic N-*[(3-cinnamoyl-2-oxo-1,3-oxazolidin-5-yl) methyl]acetamide (rac-**6**)*

Pyridine (1.01 ml, 12.50 mmol) and acetic anhydride (0.83 ml, 8.75 mmol) were added to *rac*-**5** (1.54 g, 6.25 mmol) under ice cooling, and the mixture was stirred at room temperature for 2 h. Diluted HCl was added and the solution extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting oily product was purified on silica gel (eluent ethyl acetate) to give the acetamide *rac*-**7** (white solid, 40% yield). IR (KBr) 1784, 1691 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.42 (s, 3H, CH_3), 3.42 (dd, 1H,C*H*HNH), 3.66 (t, 1H, CH*H*NH), 4.12 (d, 2H, CH_2CH), 4.72–4.81 (m, 1H, C*H*), 6.42 (s, 1H, N*H*), 6.47 (d, 1H, C*H*CHCO), 7.34 (d, 2H, aromatic), 7.57 (d, 1H, CHC*H*CO), 7.76 (d, 2H aromatic); ^{13}C NMR ($CDCl_3$) δ 21.9 (*C*H_3), 42.3 (*C*H_2NH), 69.0 (*C*H_2 ox), 73.2 (*C*H), 120.1 (CH*C*HCO), 128.2 (*C*H aromatic), 129.1 (1 *C*H aromatic), 130.2 (*C*H aromatic), 141.7 (CH*C*HCO), 146.0 (*C*O ox), 159.8 (CHCH*C*O), 168.2 (*C*ONH).

*2.3. Synthesis of compounds rac-**12a–b***

The compounds **9–12b** were obtained following the same synthetic procedures described for the corresponding **9–12a**. The starting material is the commercial 2-aminopyrimidine.

*2.3.1. Isobutyl pyridin-2-yl carbamate (**7a**)*

To a solution of 2-aminopyridine (10 g, 106.2 mmol) in dry THF (80 ml), triethylamine (14.80 ml, 106.2 mmol) and isobutyl chloroformate (13.89 ml, 106.2 mmol) were added under nitrogen atmosphere. After stirring at room temperature for 22 h, the THF was evaporated under reduced pressure; the resulting crude material was dissolved in CH_2Cl_2, washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. After purification by chromatography on silica gel (eluent ciclohexane/ethyl acetate, 8:2) was obtained the carbamate **7a** (white solid, 46% yield, m.p. 72–75 °C). IR (KBr) 1731, 1536, cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.97 (d, 6H, CH_3), 2.00–2.09 (m, 1H, C*H*), 3.98 (d, 2H, CH_2), 6.95–6.99 (dd, 1H, C*H* pyr), 7.7 (dt, 1H, C*H* pyr), 8.00 (d, 1H, C*H* pyr), 8.30 (dd, 1H, C*H* pyr), 8.92 (s broad, 1H, N*H*); ^{13}C NMR ($CDCl_3$) δ 19.3 (*C*H_3), 28.2 (*C*H), 71.6 (*C*H_2), 112.8 (*C*H pyr), 118.6 (*C*H pyr), 138.7 (*C*H pyr), 147.8 (*C*H pyr), 152.7 (*C* pyr), 154.1 (*C*=O).

*2.3.2. Isobutyl pyrimidin-2-yl carbamate (**7b**)*

White solid, 40% yield, m.p. 94–95 °C. IR (KBr) 3429, 1749 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.96 (d, 6H, CH_3), 1.96–2.05 (m, 1H, C*H*), 4.02 (d, 2H, CH_2), 6.99 (t, 1H, C*H* pyr), 8.63 (d, 2H, C*H* pyr), 9.09 (s broad, 1H, N*H*); ^{13}C NMR ($CDCl_3$) δ 19.3 (*C*H_3), 28.1 (*C*H), 71.9 (*C*H_2), 116.1 (*C*H pyr), 152.1 (*C* pyr), 158.0 (*C*=O), 158.7 (*C*H pyr).

*2.3.3. Oxiran-2-ylmethyl-4-methylbenzensulfonate (rac-**8**)*

Triethylamine (13.66 ml, 98.02 mmol) and toluene-*p*-sulfonyl chloride (17.25 g, 90.48 mmol) were added to a solution of glicidol (5.0 ml, 75.4 mmol) in CH_2Cl_2 (40 ml), at 0 °C. The mixture was allowed to warm to room temperature and after 2 h was washed with brine and a saturated aqueous solution of $NaHCO_3$. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified on silica gel (eluent ciclohexane/ethyl acetate, 8:2) to give the oxirane *rac*-**8** (colourless oil, 67% yield, b.p. 285 °C). IR (KBr) 1596 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.43 (s, 3H, CH_3), 2.56–2.58 (dd, 1H, C*H*H oxirane), 2.79 (t, 1H, CH*H* oxirane), 3.16–3.21 (m, 1H, C*H*), 3.91–3.96 (dd, 1H, C*H*HOS), 4.22–4.28 (dd, 1H, CH*H*OS), 7.35 (d, 2H, aromatic), 7.80 (d, 2H, aromatic); ^{13}C NMR ($CDCl_3$) δ 21.9 (*C*H_3), 44.8 (*C*H_2 oxirane), 49.1 (*C*H), 70.7 (*C*H_2OS), 128.2 (*C*H aromatic), 130.2 (*C*H aromatic), 132.8 (*C*–S aromatic), 145.4 (*C*–C aromatic).

*2.3.4. (2-Oxo-3-pyridin-2-yl-1,3-oxazolidin-5-yl)methyl 4-methylbenzensulfonate (rac-**9a**)*

A mixture of **7a** (9.20 g, 47.4 mmol), *rac*-**8** (10.81 g, 47.4 mmol) and triethylamine (0.06 ml, 0.47 mmol) was heated to 150 °C for 3 h. After cooling, the resulting oil was dissolved in CH_2Cl_2, washed with H_2O and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (eluent ciclohexane/ethyl acetate, 8:2) to give *rac*-**9a** (white solid, 16% yield, m.p. 149–151 °C). IR (KBr) 1748, 1363, 1195 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.43 (s, 3H, CH_3), 4.02–4.35 (m, 4H, CH_2 ox e CH_2OS), 4.79–4.85 (m, 1H, C*H*), 7.04 (dt, 1H, C*H* pyr), 7.34 (d, 2H, aromatic), 7.66 (dt, 1H, C*H* pyr), 7.78 (d, 2H, aromatic), 8.14 (d, 1H, C*H* pyr), 8.29 (dd, 1H, C*H* pyr); ^{13}C NMR ($CDCl_3$) δ 21.9 (*C*H_3), 45.7 (*C*H_2 ox.), 68.7 (*C*H_2OS), 70.2 (*C*H), 113.1 (*C*H pyr), 119.6 (*C*H pyr), 128.2 (*C*H aromatic), 130.3 (*C*H aromatic), 132.3 (*C*–S aromatic), 138.1 (*C*H pyr), 145.7 (*C*–C aromatic), 147.8 (*C*H pyr), 150.5 (*C* pyr), 153.7 (*C*=O).

*2.3.5. (2-Oxo-3-pyrimidin-2-yl-1,3-oxazolidin-5-yl)methyl 4-methylbenzensulfonate (rac-**9b**)*

White solid, 13% yield, m.p. 131–134 °C. IR (KBr) 1758 $cm^{-1;}$ ^1H NMR ($CDCl_3$) δ 2.43 (s, 3H, CH_3), 4.08–4.34 (m, 4H, CH_2 ox e CH_2OS), 4.78–4.86 (m, 1H, C*H*), 7.06 (t, 1H, C*H* pyr), 7.34 (d, 2H, C*H* aromatic), 7.76 (d, 2H, C*H* aromatic), 8.65 (d, 2H, C*H* pyr); ^{13}C NMR ($CDCl_3$) δ 21.9 (*C*H_3), 46.3 (*C*H_2 ox), 68.6 (*C*H_2OS), 69.6 (*C*H), 116.9 (*C*H pyr), 128.2 (*C*H aromatic), 130.4 (*C*H aromatic), 132.1 (*C*–S aromatic), 145.8 (*C*–C aromatic), 151.8 (*C* pyr), 156.7 (*C*=O), 158.5 (*C*H pyr).

*2.3.6. 5-(Azidomethyl)-3-pyridin-2-yl-1,3-oxazolidin-2-one (rac-**10a**)*

Sodium azide (0.51 g, 7.89 mmol) was added to a solution of *rac*-**9a** (2.50 g, 7.18 mmol) in dry DMSO (70 ml), at 60 °C under nitrogen atmosphere. After 8 h the mixture was diluted

with H_2O and extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product *rac*-**10a** (yellow oil, 99% yield) was used in the following reaction without purification.

*2.3.7. 5-(Azidomethyl)-3-pyrimidin-2-yl-1,3-oxazolidin-2-one (rac-**10b**)*

Yellow oil, 69% yield. IR (KBr) 2104, 1766 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.57–3.69 (dq, 2H, CH_2N_3), 4.01–4.35 (m, 2H, CH_2 ox), 4.75–4.81 (m, 1H, C*H*), 7.07 (t, 1H, C*H* pyr), 8.68 (d, 2H, 2 C*H* pyr); ^{13}C NMR ($CDCl_3$) δ 47.2 (*C*H_2 ox), 53.3 (*C*H_2N_3), 71.0 (*C*H), 116.8 (*C*H pyr), 151.8 (*C* pyr), 156.7 (*C*=O), 158.5 (*C*H pyr).

*2.3.8. 5-(Aminomethyl)-3-pyridin-2-yl-1,3-oxazolidin-2-one (rac-**11a**)*

A solution of *rac*-**10a** (1.42 g, 6.48 mmol), triphenylphosphine (1.87 g, 7.13 mmol) and H_2O (1.17 ml, 64.8 mmol) in THF (20 ml) was heated at 40 °C for 24 h. After cooling, the mixture was diluted with HCl 2 N and extracted with ethyl acetate. Aqueous NaOH was added and the resulting basic solution was extracted with ethyl acetate. The organic phase was washed with H_2O, dried over Na_2SO_4 and concentrated under reduced pressure to give the desired *rac*-**11a** (yellow oil, 46% yield).

IR (KBr) 3378, 1749 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.44 (s broad, 2H, N*H*$_2$), 2.93–3.00 (dq, 2H, CH_2NH_2), 3.98–4.04 (dd, 1H, C*H*H ox), 4.25–4.31 (dd, 1H, CH*H* ox), 4.62–4.69 (m, 1H, C*H*), 6.99–7.03 (dt, 1H, C*H* pyr), 7.65–7.71 (dt, 1H, C*H* pyr), 8.18–8.22 (d, 1H, C*H* pyr), 8.29–8.31 (dd, 1H, C*H* pyr); ^{13}C NMR ($CDCl_3$) δ 45.5 (*C*H_2NH_2), 46.8 (*C*H_2 ox.), 75.0 (*C*H), 113.2 (*C*H pyr), 119.3 (*C*H pyr), 138.0 (*C*H pyr), 147.7 (*C*H pyr), 151.1(*C*=O), 154.7 (*C* pyr).

*2.3.9. 5-(Aminomethyl)-3-pyrimidin-2-yl-1,3-oxazolidin-2-one (rac-**11b**)*

Yellow oil, 45% yield. IR (KBr) 3421, 2104, 1754 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.00 (s broad, 2H, N*H*$_2$), 2.93–2.99 (dq, 2H, CH_2NH_2), 3.94–4.00 (dd, 1H, C*H*H ox), 4.27–4.31 (dd, 1H, CH*H* ox), 4.62–4.71 (m, 1H, C*H*), 7.07 (t, 1H, C*H* pyr), 8.66 (d, 2H, C*H* pyr); ^{13}C NMR ($CDCl_3$) δ 45.3 (*C*H_2 ox), 45.8 (*C*H_2NH_2), 72.5 (*C*H), 111.1 (*C*H pyr), 157.4 (*C* pyr), 158.4 (*C*H pyr), 158.5 (*C*=O).

*2.3.10. N-[(2-oxo-3-pyridin-2-yl-1,3-oxazolidin-5-yl)methyl]acetamide (rac-**12a**)*

Pyridine (0.39 ml, 4.86 mmol) and acetic anhydride (0.32 ml, 3.40 mmol) were added to *rac*-**11a** (0.47 g, 2.43 mmol) under ice cooling. After stirring at room temperature for 1 h, the mixture was treated with HCl 2 N and extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified on silica gel (eluent ethyl acetate) to give the acetamide *rac*-**12a** (white solid, 35% yield, m.p. 101.5–102.5 °C). IR (KBr) 3311, 1775, 1648 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.02 (s, 3H, CH_3), 3.40–3.49 (m, 1H, C*H*HNH), 3.79–3.87 (m, 1H, CH*H*NH), 3.91–3.97 (dd, 1H, C*H*H ox), 4.32–4.35 (dd, 1H, CH*H* ox), 4.74–4.83 (m, 1H, C*H*), 6.04 (s broad, 1H, N*H*), 7.02–7.06 (dd, 1H, C*H* pyr), 7.67–7.73 (dt, 1H, C*H* pyr), 8.16 (d, 1H C*H* pyr), 8.30–8.32 (dd, 1H C*H* pyr); ^{13}C NMR ($CDCl_3$) δ 23.4 (*C*H_3), 42.6 (*C*H_2NH), 46.7 (*C*H_2 ox.), 72.7 (*C*H), 113.1 (*C*H pyr), 119.6 (*C*H pyr), 138.1 (*C*H pyr), 147.8 (*C*H pyr), 150.7 (*C*=O ox), 153.3 (*C* pyr), 170.8 (*C*ONH).

*2.3.11. N-[(2-oxo-3-pyrimidin-2-yl-1,3-oxazolidin-5-yl)methyl]acetamide (rac-**12b**)*

White solid, 35% yield. ^1H NMR ($CDCl_3$) δ 2.05 (s, 3H, CH_3), 3.41–3.98 (m, 4H, CH_2NH e CH_2 ox), 4.98–5.00 (m, 1H, C*H*), 6.02 (t, 1H, N*H*), 6.55 (t, 1H, C*H* pyr), 8.65 (d, 2H, C*H* pyr); ^{13}C NMR ($CDCl_3$) δ 23.5 (*C*H_3), 40.0 (*C*H_2NH), 41.7 (*C*H_2 ox), 71.9 (*C*H), 111.2 (*C*H pyr), 157.4 (*C* pyr), 158.2 (*C*H pyr), 158.5 (*C*=O ox), 170.9 (*C*ONH).

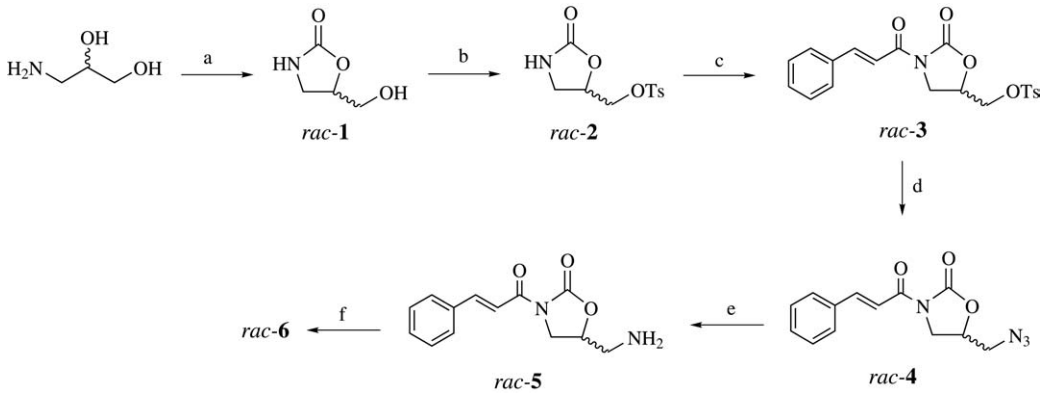
2.4. Biological evaluation

The minimum inhibitory concentration (MIC) values of the synthesized compounds were determined by agar dilution or microtiter broth dilution methods, corresponding to the National Committee for Clinical Laboratory Standards. The bacterial strains used were clinically isolated and from the American Type Culture Collection. Cultures were maintained on Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) or grown at 37 °C in Mueller-Hinton broth (GIBCO Laboratories, Grand Island, NY).

The compounds were dissolved in DMSO and the solutions were diluted with distilled water. To ensure that DMSO had no effect on bacterial growth, a control test was performed with test medium supplemented with solvent at the same dilutions as used in the experiments. Vancomycin was used as reference drug. All tested compounds exhibited MIC values >128 μg/ml against a number of Gram-positive microorganisms selected for this study (*Staphylococcus aureus, Staphylococcus haemolyticus, Streptococcus pyogenes, Streptococcus agalactiae, Enterococcus faecalis, Enterococcus faecium, Staphylococcus epidermidis*).

3. Results and discussion

Compounds *rac*-**6**, (*R*)-**6**, (*S*)-**6**, *rac*-**12a** and *rac*-**12b** were selected after a preliminary study of molecular modeling. The molecular electrostatic potentials (MEPs) were calculated by using the Grid 17 software [11]; we chose an hydrophobic probe (DRY) and water to explore MEPs distribution in a number of designed compounds and some active oxazolidinones. This approach allowed us to select compounds with enhanced electron-withdrawing properties and altered geometric features, as a result of the *N*-substituent modifications. We performed these structural changes by keeping the acetylaminomethylic group at carbon in position 5, resulted one of the best substituents.



Scheme 1. Reagents and conditions: (a) $POCl_3$, Na_2CO_3, H_2O, r.t.; (b) TsCl, NEt_3, CH_2Cl_2, r.t.; (c) cinnamoyl chloride, BuLi, THF, –78 °C; (d) NaN_3, DMSO, 60 °C; (e) PPh_3, THF, 40 °C; (f) Ac_2O, Pyr, r.t.

The synthesis of cinnamoyl oxazolidinone *rac*-**6** is shown in Scheme 1. The hydroxymethyloxazolidinone *rac*-**1** was prepared by reaction of commercially available 3-amino-1,2-propandiole with triphosgene, in the presence of Na_2CO_3; afterwards it was treated with tosyl chloride (TsCl) and NEt_3 to give the tosylate *rac*-**2**. The reaction of *rac*-**2** with BuLi and cinnamoyl chloride, in freshly distilled THF at –78 °C, afforded the intermediate *rac*-**3**, that was reacted with sodium azide to obtain the azido-derivative *rac*-**4**. Reduction of *rac*-**4** with triphenylphosphine, followed by acylation with acetic anhydride and pyridine, led to the desired compound *rac*-**6**.

The same synthetic route was followed in order to obtain the chiral derivatives (*R*)-**6** and (*S*)-**6**, starting from commercially available (*R*)- and (*S*)-3-amino-1,2-propandiole.

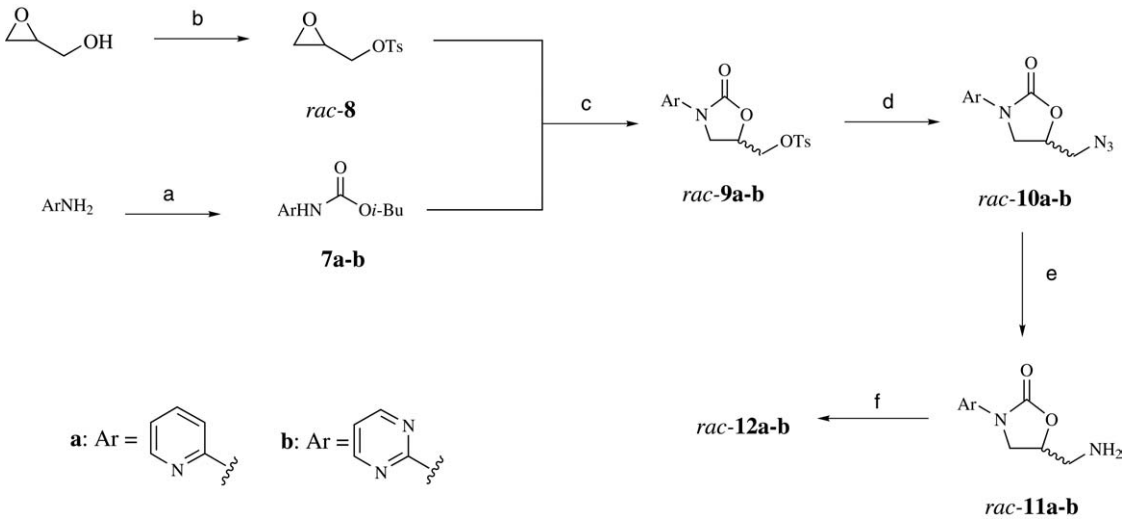
The pyridin- and pyrimidinoxazolidinones *rac*-**12a** and *rac*-**12b** were synthesized as depicted in Scheme 2, following the same synthetic route described for linezolid [12]. Reaction of 2-aminopyridine with isobutyl chloroformate, in the presence of triethylamine in dry THF, afforded the carbamate **7a**. Treatment of **7a** with glicidyl tosylate *rac*-**8**, previously obtained from glicidol and TsCl, gave *rac*-**9a**. This reaction was carried out by mixing the carbamate and the oxirane without solvent, using triethylamine as catalyst and heating the mixture until 150 °C [13]. The mechanism of this reaction proceeds via the initial formation of an aminoalcohol intermediate, which undergoes an intramolecular exchange of alcohols, to give the desired oxazolidinone.

Nucleophilic substitution with sodium azide gave *rac*-**10a**, which was reduced with triphenylphosphine; the resulting amine *rac*-**11a** was acylated with acetic anhydride to obtain the oxazolidinone *rac*-**12a**.

The compound *rac*-**12b** was synthesized in a similar manner, starting from commercial 2-aminopyrimidine.

All the intermediates and the final compounds were analytically characterized by means of TLC, GC, NMR, MS and IR techniques.

The synthesized oxazolidinones were tested for antibacterial activity against both standard and clinically isolated strains of important Gram-positive pathogens. Compounds were dissolved in DMSO and vancomycin was used as control. The antibacterial activity was completely lost (MIC > 128) for all derivatives tested.



Scheme 2. Reagents and conditions: (a) CICOO*i*-Bu, NEt_3, THF, r.t.; (b) TsCl, NEt_3, CH_2Cl_2, 0 °C; (c) NEt_3, 150 °C; (d) NaN_3, DMSO, 60 °C; (e) PPh_3, THF, 40 °C; (f) Ac_2O, Pyr, r.t.

Referring to linezolid structure and SAR studies we have designed and synthesized novel compounds of oxazolidin-2-one class, in order to investigate the relationships between antimicrobial activity and increased electron-withdrawing features. The total loss of activity of compound **6** is not so surprising: the literature describes other oxazolidinones, in which the aromatic ring is not directly linked to heterocyclic nitrogen, resulted totally inactive. Regarding pyridine- and pyrimidine-derivatives **12a–b**, we have synthesized, at present, only the racemic forms: this could have halved the biological activity, because only (*S*)-oxazolidinones show antibacterial properties, while (*R*)-forms are completely inactive. Nevertheless, only this consideration does not explain the total loss of biological activity.

Further work is in progress in our laboratory in order to evaluate the effect of substitution on heteroaromatic ring with electron-withdrawing groups, in the attempt for the discovery and development of new active agents.

Acknowledgements

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