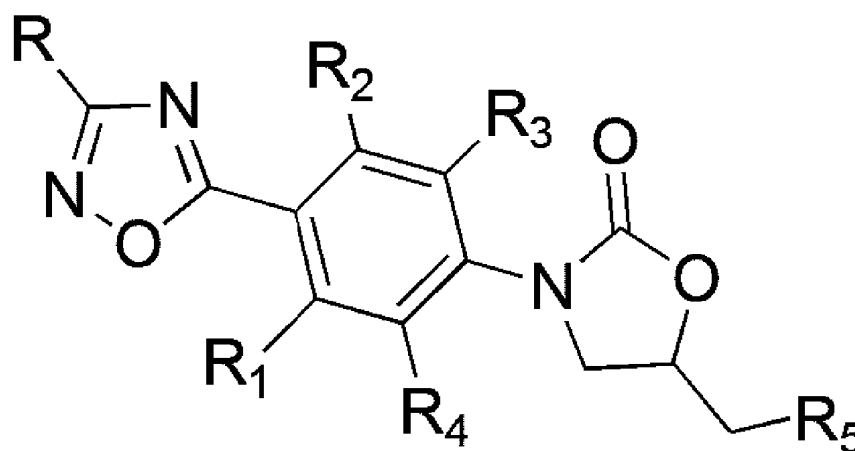




(86) Date de dépôt PCT/PCT Filing Date: 2014/03/17
(87) Date publication PCT/PCT Publication Date: 2014/09/18
(45) Date de délivrance/Issue Date: 2021/04/20
(85) Entrée phase nationale/National Entry: 2015/09/15
(86) N° demande PCT/PCT Application No.: IB 2014/059896
(87) N° publication PCT/PCT Publication No.: 2014/141218
(30) Priorités/Priorities: 2013/03/15 (ITRM2013A000155);
2013/03/15 (US13/839,485)

(51) Cl.Int./Int.Cl. *C07D 413/14* (2006.01),
A61K 31/422 (2006.01), *A61K 31/4245* (2006.01),
A61P 31/04 (2006.01), *C07D 413/10* (2006.01)
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(54) Titre : COMPOSES 1,2,4-OXADIAZOLE ACTIFS CONTRE LES PATHOGENES A GRAM POSITIF
(54) Title: 1, 2, 4-OXADIAZOL COMPOUNDS ACTIVE AGAINST GRAM-POSITIVE PATHOGENS



Formula (I)

(57) Abrégé/Abstract:

The present invention relates to new oxazolidinone compounds of general formula (I) having antibiotic activity even against multiresistant bacterial strains (I).

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1, 2, 4-OXADIAZOL COMPOUNDS ACTIVE AGAINST GRAM-POSITIVE PATHOGENS

Description

STATE OF THE PRIOR ART

5 Use and misuse of antibacterial agents have resulted in the development of
bacterial resistance to all antibiotics in clinical use, irrespective of the chemical class
or molecular target of the drug. Infections caused by multiresistant Gram-positive
cocci, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-
resistant enterococci (VRE) and penicillin-resistant *Streptococcus pneumoniae*
10 (PNSSP), have emerged as major public health concern, both in hospital and
community settings worldwide. The need for new antibiotics urged the Infectious
Disease Society of America (IDSA) to issue the challenge to develop ten new
antibiotics by 2020.

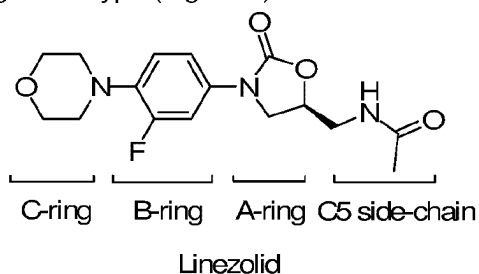
 Oxazolidinones are a class of antibacterial agents which displayed activity
15 against a variety of Gram-positive pathogens and are highly potent against
multidrug-resistant bacteria. In particular, oxazolidinones are used to treat skin and
respiratory tract infections caused by *Staphylococcus aureus* and streptococci
strains, as well as being active against vancomycin-resistant *Enterococcus faecium*.
Linezolid (Figure 1), the first oxazolidinone antibiotic approved for clinical use, has
20 been shown to inhibit translation at the initiation phase of protein synthesis in
bacteria by binding to the 50S ribosomal subunit. Since 2001, however, linezolid
resistance began to appear in *Staphylococcus aureus* and *Enterococcus faecium*
clinical isolates and the rate of resistance raised especially among enterococci and
Staphylococcus epidermidis strains with its usage.[1-4] In addition, linezolid therapy
25 is not without side effects such as reversible myelosuppression and inhibition of
monoamine oxidases (MAO).

 A number of solutions to the problem of bacterial resistance are possible.
Successful strategies include combination of existing antibacterial agents with other
drugs as well as the development of improved diagnostic procedures that may lead
30 to rapid identification of the causative pathogen and permit the use of antibacterial
agents with a narrow spectrum of activity. Another strategy is the discovery of novel
classes of antibacterial agents acting through new mechanisms of action. However,
the most common approach, and still the most promising one, is the modification of
existing classes of antibacterial agents to provide new analogues with improved
35 activities, although activity and toxicity of the new analogues are not easily
predictable.

 In this context, many researchers have attempted to modify, without even

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obtaining results such as to lead to approval for use of new molecules, the structure of linezolid to improve the antibacterial activity. In order to rationalize the site of modifications, the structure of linezolid can formally be divided into four portions according to oxazolidinone antibacterials nomenclature[5]: 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 a carbo-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 (Figure 1).



10

Figure 1

Different types of modifications are reported in literature; the most common one regards the C-ring, while only few modifications were reported for the A-ring, and in some cases good activity was retained.[6-7]

Our group previously reported that the replacement of the oxazolidinone (A-ring) with an isosteric 1,2,4-oxadiazole heteroaromatic ring resulted in a lack of activity.[8] Therefore, these compounds have been chosen as references for inactive linezolid-like compounds in a virtual screening approach.

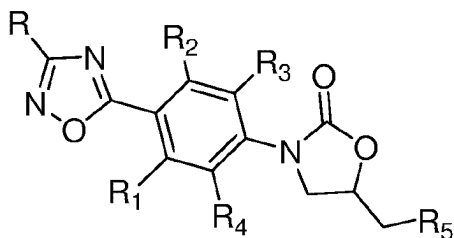
The purpose of the present invention is to find new molecules suitable as medicaments which exceed the limits and disadvantages of the prior art molecules, in terms of antibacterial activity, especially against resistant strains, and harmlessness.

SUMMARY OF THE INVENTION

The present invention is based on the discovery that the substitution of the C-ring of linezolid-like molecules, with a five-membered heterocyclic ring, also substituted, containing 2 or 3 heteroatoms, is effective for the obtainment of new oxazolidinone antibiotics with a tunable activity by the presence of further modifications at the B-ring and at the C(5) side-chain of the oxazolidinone nucleus.

Therefore, objects of the present invention are new compounds with a general formula (I), and their use for the treatment of infections preferably caused by Gram-positive bacteria,

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Formula (I)

as racemic mixtures or pure enantiomers or mixtures enriched with one of
 5 the S or R enantiomers

where:

R=, F, Cl, Br, I, (C1-C3) alkyl (methyl, ethyl, n-propyl, iso-propyl), (C3-C6)
 cyclo-alkyl, phenyl, aryl, heteroaryl, NH₂, OH, SH, NHR₆, N(R₆)₂, OR₆ with R₆= (C1-
 C3) alkyl, (C3-C6) cyclo-alkyl, aryl, heteroaryl, (C1-C4) acyl;

10 R₁₋₄= independently H, F, Cl, Br, CH₃, OH, OCH₃;

R₅= -NH₂; -I; -N₃; -OH; -NCS, -NHC(X)CH₃ with X= O or S; -NHC(X)CH₂Z
 with X= O, S, Z= F, Cl; -NHC(X)CHZ₂ with X= O, S, Z= F, Cl; -NHC(X)CZ₃ with X=
 O, S, Z= F, Cl; -NHC(X)NHR₇ with X= O, S, R₇= H, (C1-C3) alkyl, (C3-C6) cyclo-
 alkyl, aryl, heteroaryl, (C1-C3) acyl.

15 Specific embodiment of the invention consists on compounds with general
 formula (I) where R is methyl, ethyl, n-propyl, iso-propyl;

or compounds with general formula (I) where at least one between R₁, R₂, R₃
 or R₄ is a fluorine atom, while the other are H;

20 or compounds with general formula (I) where R₅ is selected between: -
 NHC(=O)CH₃, -NHC(=S)CH₃, -NHC(=O)CH₂F, -NHC(=S)CH₂F, -NHC(=O)CH₂Cl, -
 NHC(=S)CH₂Cl, -NHC(=S)NH₂, NHC(=O)NH₂, -NHC(=O)NHCH₃, -NHC(=S)NHCH₃,
 -NHC(=O)NHC₂H₅, -NHC(=S)NHC₂H₅, -NCS; 1,2,3-triazol-1-yl;

25 or compounds with general formula (I) where R is a methyl and R₅ is
 selected between: -NHC(=O)CH₃, -NHC(=S)CH₃, -NHC(=O)CH₂F, -NHC(=S)CH₂F, -
 NHC(=O)CH₂Cl, -NHC(=S)CH₂Cl, -NHC(=S)NH₂, NHC(=O)NH₂, -NHC(=O)NHCH₃, -
 NHC(=S)NHCH₃, -NHC(=O)NHC₂H₅, -NHC(=S)NHC₂H₅, -NCS; 1,2,3-triazol-1-yl;

30 or compounds with general formula (I) where R₁ is F, R₂, R₃ and R₄ are H
 and R is a methyl and R₅ is selected between: -NHC(=O)CH₃, -NHC(=S)CH₃, -
 NHC(=O)CH₂F, -NHC(=S)CH₂F, -NHC(=O)CH₂Cl, -NHC(=S)CH₂Cl, -NHC(=S)NH₂,
 NHC(=O)NH₂, -NHC(=O)NHCH₃, -NHC(=S)NHCH₃, -NHC(=O)NHC₂H₅, -
 NHC(=S)NHC₂H₅, -NCS; 1,2,3 triazol-1-yl.

In a preferred embodiment of the invention, all compounds indicated above are pure S enantiomer or in a mixture enriched with the S enantiomer.

5 In a further embodiment of the invention the disclosed compounds are intended for use in the treatment of infections caused by Gram-positive bacteria, preferably multi-antibiotic resistant (also called multi-resistant), for example in the treatment of infections caused by *Staphylococcus* spp, *Enterococcus* spp, *Streptococcus* spp, in particular of infection caused by *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Streptococcus pneumoniae*. Especially if resistant to one or
10 more of the antibiotics methicillin, vancomycin, penicillin, macrolides, fluoroquinolones and linezolid.

A second object of the invention are pharmaceutical compositions comprising the compounds of the invention as active ingredients and a pharmaceutically acceptable excipient.

15 Such compositions are intended for use in the treatment of infections by both Gram-positive and Gram-negative bacteria including multi-resistant strains.

A third object of the invention are processes for preparing the compounds of the invention which comprises the steps shown in diagrams 1, 2 and 3.

20 In one embodiment of the invention the methods comprise one or more steps of separation of the enantiomers S and R or enrichment of the racemic mixture in one of the enantiomers, preferably the S enantiomer.

A fourth object of the invention are processes for the preparation of pharmaceutical compositions comprising the step of mixing the active ingredients with a pharmacologically acceptable excipient.

25 A further object of the invention is the use of the compounds of the invention for the preparation of a medicament for the treatment of infections by multi-resistant Gram-positive strains.

Advantages offered by the present invention reside in obtaining new antibiotic compounds with activity equivalent to or comparable to that of linezolid
30 against linezolid-susceptible bacterial strains but with greater effectiveness than linezolid against bacterial strains resistant to linezolid and / or to other antibiotics. In addition some of these substances possess cytotoxicity levels comparable to or less than that of linezolid. Finally, replacing the morpholine ring of linezolid with the oxadiazole ring, as described herein, prevents the opening of the ring and the
35 formation of inactive metabolites such as PNU-142586 and PNU-142300.

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Description of the Figures

Figure 1. Formula of linezolid with structural elements that compose it and nomenclature.

5 Figure 2. Results of cell viability assays on PK15 cells treated with the **A4b** compound (compound 23 of table 1) and linezolid. Limits of significance: * = P <0.05, ** = P <0.01.

Figure 3. Results of cell viability assays on HaCaT cells treated with the **A4b** compound (compound 23 of table 1) and linezolid. Limits of significance: * = P <0.05, ** = P <0.01.

10 Figure 4. Results of cell viability on HepG2 cells treated with the **A4b** compound (compound 23 of table 1) and linezolid. Limits of significance: * = P <0.05, ** = P <0.01.

15 Figure 5. Results of cell viability on HepG2 cells treated with **B4a** and **B4b** compounds (compounds 106 and 107 of Table 1) in the form of their respective enantiomers.

Figure 6: Results of OXPPOS assay on HepG2 cells treated with **A4aS** and **A4bS** compounds (compounds 22 and 23 of Table 1) in the form of their respective S enantiomers.

Figure 7: Scheme 1 of chemical synthesis of compounds 1-5 and A1.

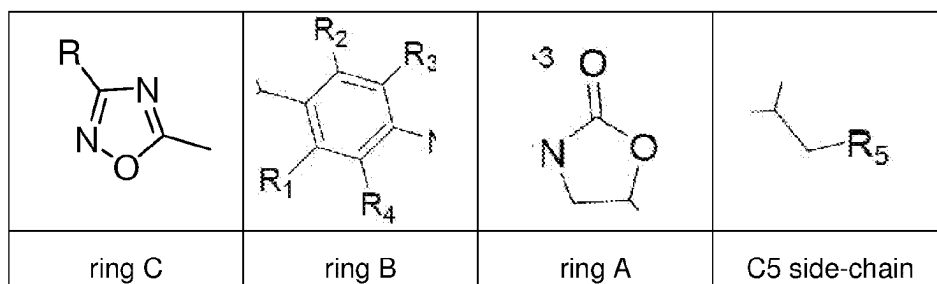
20 Figure 8: Scheme 2 of chemical synthesis of A and B compounds.

Figure 9: Scheme 3 of chemical synthesis of the compounds of interest A and B.

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DETAILED DESCRIPTION OF THE INVENTIONCompounds:

The chemical structure of the compounds of the present invention [formulas (I)] consists of an oxazolidinone ring (ring A), a phenyl ring (ring B), an oxadiazole ring (ring C) and a side-chain linked to the C5 position of the oxazolidinone (C5-linked side-chain).

Ring C

The ring C is an 1,2,4-oxadiazole heterocycle linked via the C (5) to the ring B. The R substituent on the ring C can be a substituent chosen among: F, Cl, Br, I, methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *sec*-butyl, *ter*-butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, phenyl, aryl, heteroaryl, -NH₂, NHCH₃, NHC₂H₅, -N(CH₃)₂, N(CH₃)(C₂H₅), -NC(=O)CH₃, -NC(=O)C₂H₅, -NH(cyclopropyl), NH(cyclobutyl), NH(cyclopentyl), NH(cyclohexyl), -OH, -OCH₃, -OC₂H₅, -*On*-Propyl, *Oi*-Propyl, -SH, SCH₃.

Ring B

Groups R₁, R₂, R₃, R₄ are, independently from each other, H, F, Cl, Br, CH₃, OH, OCH₃. At least one of them is an halogen atom, for example R₁ is F, Cl, or Br, or R₁ and R₂ are F, Cl, or Br, or R₁, R₂, and R₃ are F or Cl. In a specific embodiment the halogen atom is F and the remaining R groups are hydrogen atoms. In a preferred formula, either R₁ or R₂ are F and R₃ and R₄ are H.

C5 side-chain

The R₅ substituent in the C5 side-chain linked at the position 5 of the oxazolidinone nucleus is chosen within a group comprising the following radicals: I, -N₃, -NHC(=O)CH₃, -NHC(=S)CH₃, -NHC(=O)CH₂F, -NHC(=S)CH₂F, -NHC(=O)CH₂Cl, -NHC(=S)CH₂Cl, -NHC(=O)CH₂Br, -NHC(=S)CH₂Br, -NHC(=O)CHF₂, -NHC(=S)CHF₂, -NHC(=O)CHCl₂, -NHC(=S)CHCl₂, -NHC(=O)CHBr₂, -NHC(=S)CHBr₂, -NHC(=O)CF₃, -NHC(=S)CF₃, -NHC(=O)CCl₃, -NHC(=S)CCl₃, -NHC(=O)CBr₃, -NHC(=S)CBr₃, -NHC(=S)NH₂, -NHC(=O)NH₂, -NHC(=O)NHCH₃, -NHC(=S)NHCH₃, -NHC(=O)NHC₂H₅, -NHC(=S)NHC₂H₅, -NHC(=O)NH-*n*C₃H₇, -NHC(=S)NH-*n*C₃H₇, -NHC(=O)NH-*i*C₃H₇, -NHC(=S)NH-*i*C₃H₇,

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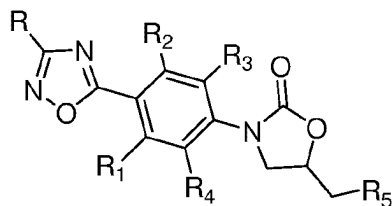
NHC(=S)NH-cyclopropyl, -NHC(=O)NH-cyclopropyl, NHC(=S)NH-cyclobutyl, -
 NHC(=O)NH-cyclobutyl, NHC(=S)NH-cyclopentyl, -NHC(=O)NH-cyclopentyl,
 NHC(=S)NH-cyclohexyl, -NHC(=O)NH-cyclohexyl, NHC(=O)NHC(=O)CH₃,
 NHC(=S)NHC(=O)CH₃, NHC(=O)NHC(=O)C₂H₅, NHC(=O)NH-heteroaryl, -NCS,
 5 pyrrolyl, pyrazolyl, imidazolyl, 1,2,3-triazol-1-yl, 1,2,4-triazol-1-yl.

It was observed that compounds comprising a thio group as indicated above seem to present a better solubility and a greater ability to cross biological membranes.

10 Considering the asymmetric configuration of the carbon atom in position 5 of the ring A, all above identified compounds are optically active. Therefore, the present invention concerns: racemic mixtures of these compounds, mixtures enriched in either one of the enantiomers, and either one of the isolated enantiomers. For the scopes of the present invention it is understood as racemic mixture a 50%:50% mixture of the two R and S enantiomers. It is understood as
 15 mixture enriched in one of the enantiomers a mixture containing more than 50% of one enantiomer (either S or R), for example 55%, 60%, 65%, 70%, 75%, or more. As isolated enantiomer it is understood a pure enantiomer, i.e. 100% or a mixture highly enriched of that enantiomer, for example 98%, 95%, 93%, 90%, 88%, 85%,
 20 80%.

A specific form of embodiment of the invention implies compounds consisting of the S enantiomer or compositions comprising the S enantiomer as either enriched mixture or pure enantiomer. A second specific form of embodiment of the invention comprises compounds consisting of the R/S racemic mixtures or compositions comprising the R/S racemic mixtures. A further form of specific embodiment, less
 25 preferred, implies mixture enriched in the R enantiomer.

Preferred compounds having general formula (I) are listed in Table 1 below.



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Table 1

	R	R1	R2	R3	R4	R5
1	Ph	H	H	H	H	NHC(=O)CH ₃
2	Ph	F	H	H	H	NHC(=O)CH ₃
3	Ph	F	F	H	H	NHC(=O)CH ₃
4	Ph	F	F	F	H	NHC(=O)CH ₃
5	Ph	F	F	F	H	NHC(=O)CH ₃
6	Ph	Cl	H	H	H	NHC(=O)CH ₃
7	Ph	Cl	Cl	H	H	NHC(=O)CH ₃
8	Ph	H	H	H	H	NHC(=S)CH ₃
9	Ph	F	H	H	H	NHC(=S)CH ₃
10	Ph	F	F	H	H	NHC(=S)CH ₃
11	Ph	Cl	H	H	H	NHC(=S)CH ₃
12	Ph	Cl	Cl	H	H	NHC(=S)CH ₃
13	Ph	F	F	F	H	NHC(=S)CH ₃
14	Ph	Br	H	H	H	NHC(=S)CH ₃
15 (A3a)	CH ₃	H	H	H	H	NHC(=O)CH ₃
16 (A3b)	CH ₃	F	H	H	H	NHC(=O)CH ₃
17	CH ₃	F	F	H	H	NHC(=O)CH ₃
18	CH ₃	F	F	F	H	NHC(=O)CH ₃
19	CH ₃	Cl	H	H	H	NHC(=O)CH ₃
20	CH ₃	Cl	Cl	H	H	NHC(=O)CH ₃
21	CH ₃	Br	H	H	H	NHC(=O)CH ₃
22 (A4a)	CH ₃	H	H	H	H	NHC(=S)CH ₃

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23 (A4b)	CH ₃	F	H	H	H	NHC(=S)CH ₃
24	CH ₃	F	F	H	H	NHC(=S)CH ₃
25	CH ₃	Cl	H	H	H	NHC(=S)CH ₃
26	CH ₃	Cl	Cl	H	H	NHC(=S)CH ₃
27	CH ₃	F	F	F	H	NHC(=S)CH ₃
28	CH ₃	Br	H	H	H	NHC(=S)CH ₃
29	C ₂ H ₅	H	H	H	H	NHC(=O)CH ₃
30	C ₂ H ₅	F	H	H	H	NHC(=O)CH ₃
31	C ₂ H ₅	F	F	H	H	NHC(=O)CH ₃
32	C ₂ H ₅	F	F	F	H	NHC(=O)CH ₃
33	C ₂ H ₅	Cl	H	H	H	NHC(=O)CH ₃
34	C ₂ H ₅	Cl	Cl	H	H	NHC(=O)CH ₃
35	C ₂ H ₅	Br	H	H	H	NHC(=O)CH ₃
36	C ₂ H ₅	H	H	H	H	NHC(=S)CH ₃
37	C ₂ H ₅	F	H	H	H	NHC(=S)CH ₃
38	C ₂ H ₅	F	F	H	H	NHC(=S)CH ₃
39	C ₂ H ₅	Cl	H	H	H	NHC(=S)CH ₃
40	C ₂ H ₅	Cl	Cl	H	H	NHC(=S)CH ₃
41	C ₂ H ₅	F	F	F	H	NHC(=S)CH ₃
42	C ₂ H ₅	Br	H	H	H	NHC(=S)CH ₃
43	Ph	H	H	H	H	NHC(=O)NH ₂
44	Ph	F	H	H	H	NHC(=O)NH ₂
45	Ph	F	F	H	H	NHC(=O)NH ₂
46	Ph	F	F	F	H	NHC(=O)NH ₂
47	Ph	Br	H	H	H	NHC(=O)NH ₂
48	Ph	Cl	H	H	H	NHC(=O)NH ₂

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49	Ph	Cl	Cl	H	H	NHC(=O)NH ₂
50	Ph	H	H	H	H	NHC(=S)NH ₂
51	Ph	F	H	H	H	NHC(=S)NH ₂
52	Ph	F	F	H	H	NHC(=S)NH ₂
53	Ph	Cl	H	H	H	NHC(=S)NH ₂
54	Ph	Cl	Cl	H	H	NHC(=S)NH ₂
55	Ph	F	F	F	H	NHC(=S)NH ₂
56	Ph	Br	H	H	H	NHC(=S)NH ₂
57	CH ₃	H	H	H	H	NHC(=O)NH ₂
58	CH ₃	F	H	H	H	NHC(=O)NH ₂
59	CH ₃	F	F	H	H	NHC(=O)NH ₂
60	CH ₃	F	F	F	H	NHC(=O)NH ₂
61	CH ₃	Cl	H	H	H	NHC(=O)NH ₂
62	CH ₃	Cl	Cl	H	H	NHC(=O)NH ₂
63	CH ₃	Br	H	H	H	NHC(=O)NH ₂
64 (B3a)	CH ₃	H	H	H	H	NHC(=S)NH ₂
65 (B3b)	CH ₃	F	H	H	H	NHC(=S)NH ₂
66	CH ₃	F	F	H	H	NHC(=S)NH ₂
67	CH ₃	Cl	H	H	H	NHC(=S)NH ₂
68	CH ₃	Cl	Cl	H	H	NHC(=S)NH ₂
69	CH ₃	F	F	F	H	NHC(=S)NH ₂
70	CH ₃	Br	H	H	H	NHC(=S)NH ₂
71	C ₂ H ₅	H	H	H	H	NHC(=O)NH ₂
72	C ₂ H ₅	F	H	H	H	NHC(=O)NH ₂
73	C ₂ H ₅	F	F	H	H	NHC(=O)NH ₂
74	C ₂ H ₅	F	F	F	H	NHC(=O)NH ₂

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75	C ₂ H ₅	Cl	H	H	H	NHC(=O)NH ₂
76	C ₂ H ₅	Cl	Cl	H	H	NHC(=O)NH ₂
77	C ₂ H ₅	Br	H	H	H	NHC(=O)NH ₂
78	C ₂ H ₅	H	H	H	H	NHC(=S)NH ₂
79	C ₂ H ₅	F	H	H	H	NHC(=S)NH ₂
80	C ₂ H ₅	F	F	H	H	NHC(=S)NH ₂
81	C ₂ H ₅	Cl	H	H	H	NHC(=S)NH ₂
82	C ₂ H ₅	Cl	Cl	H	H	NHC(=S)NH ₂
83	C ₂ H ₅	F	F	F	H	NHC(=S)NH ₂
84	C ₂ H ₅	Br	H	H	H	NHC(=S)NH ₂
85	Ph	H	H	H	H	NHC(=O)NHCH ₃
86	Ph	F	H	H	H	NHC(=O)NHCH ₃
87	Ph	F	F	H	H	NHC(=O)NHCH ₃
88	Ph	F	F	F	H	NHC(=O)NHCH ₃
89	Ph	Br	H	H	H	NHC(=O)NHCH ₃
90	Ph	Cl	H	H	H	NHC(=O)NHCH ₃
91	Ph	Cl	Cl	H	H	NHC(=O)NHCH ₃
92	Ph	H	H	H	H	NHC(=S)NHCH ₃
93	Ph	F	H	H	H	NHC(=S)NHCH ₃
94	Ph	F	F	H	H	NHC(=S)NHCH ₃
95	Ph	Cl	H	H	H	NHC(=S)NHCH ₃
96	Ph	Cl	Cl	H	H	NHC(=S)NHCH ₃
97	Ph	F	F	F	H	NHC(=S)NHCH ₃
98	Ph	Br	H	H	H	NHC(=S)NHCH ₃
99	CH ₃	H	H	H	H	NHC(=O)NHCH ₃
100	CH ₃	F	H	H	H	NHC(=O)NHCH ₃
101	CH ₃	F	F	H	H	NHC(=O)NHCH ₃
102	CH ₃	F	F	F	H	NHC(=O)NHCH ₃

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103	CH ₃	Cl	H	H	H	NHC(=O)NHCH ₃
104	CH ₃	Cl	Cl	H	H	NHC(=O)NHCH ₃
105	CH ₃	Br	H	H	H	NHC(=O)NHCH ₃
106 (B4a)	CH ₃	H	H	H	H	NHC(=S)NHCH ₃
107 (B4b)	CH ₃	F	H	H	H	NHC(=S)NHCH ₃
108	CH ₃	F	F	H	H	NHC(=S)NHCH ₃
109	CH ₃	Cl	H	H	H	NHC(=S)NHCH ₃
110	CH ₃	Cl	Cl	H	H	NHC(=S)NHCH ₃
111	CH ₃	F	F	F	H	NHC(=S)NHCH ₃
112	CH ₃	Br	H	H	H	NHC(=S)NHCH ₃
113	C ₂ H ₅	H	H	H	H	NHC(=O)NHCH ₃
114	C ₂ H ₅	F	H	H	H	NHC(=O)NHCH ₃
115	C ₂ H ₅	F	F	H	H	NHC(=O)NHCH ₃
116	C ₂ H ₅	F	F	F	H	NHC(=O)NHCH ₃
117	C ₂ H ₅	Cl	H	H	H	NHC(=O)NHCH ₃
118	C ₂ H ₅	Cl	Cl	H	H	NHC(=O)NHCH ₃
119	C ₂ H ₅	Br	H	H	H	NHC(=O)NHCH ₃
120	C ₂ H ₅	H	H	H	H	NHC(=S)NHCH ₃
121	C ₂ H ₅	F	H	H	H	NHC(=S)NHCH ₃
122	C ₂ H ₅	F	F	H	H	NHC(=S)NHCH ₃
123	C ₂ H ₅	Cl	H	H	H	NHC(=S)NHCH ₃
124	C ₂ H ₅	Cl	Cl	H	H	NHC(=S)NHCH ₃
125	C ₂ H ₅	F	F	F	H	NHC(=S)NHCH ₃
126	C ₂ H ₅	Br	H	H	H	NHC(=S)NHCH ₃
127 (B2a)	CH ₃	H	H	H	H	NCS

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128 (B2b)	CH ₃	F	H	H	H	NCS
129	CH ₃	F	F	H	H	NCS
130	CH ₃	Cl	H	H	H	NCS
131	CH ₃	Cl	Cl	H	H	NCS
132	CH ₃	F	F	F	H	NCS
133	CH ₃	Br	H	H	H	NCS
134	CH ₃	H	H	H	H	NHC(=O)NHC(=O)CH ₃
135	CH ₃	F	H	H	H	NHC(=O)NHC(=O)CH ₃
136	CH ₃	F	F	H	H	NHC(=O)NHC(=O)CH ₃
137	CH ₃	Cl	H	H	H	NHC(=O)NHC(=O)CH ₃
138	CH ₃	Cl	Cl	H	H	NHC(=O)NHC(=O)CH ₃
139	CH ₃	F	F	F	H	NHC(=O)NHC(=O)CH ₃
140	CH ₃	Br	H	H	H	NHC(=O)NHC(=O)CH ₃
141	CH ₃	H	H	H	H	NHC(=S)NHC(=O)CH ₃
142	CH ₃	F	H	H	H	NHC(=S)NHC(=O)CH ₃
143	CH ₃	F	F	H	H	NHC(=S)NHC(=O)CH ₃
144	CH ₃	Cl	H	H	H	NHC(=S)NHC(=O)CH ₃
145	CH ₃	Cl	Cl	H	H	NHC(=S)NHC(=O)CH ₃
146	CH ₃	F	F	F	H	NHC(=S)NHC(=O)CH ₃
147	CH ₃	Br	H	H	H	NHC(=S)NHC(=O)CH ₃
148 (A1a)	CH ₃	H	H	H	H	I
149 (A1b)	CH ₃	F	H	H	H	I
150	CH ₃	F	F	H	H	I
151	CH ₃	Cl	H	H	H	I

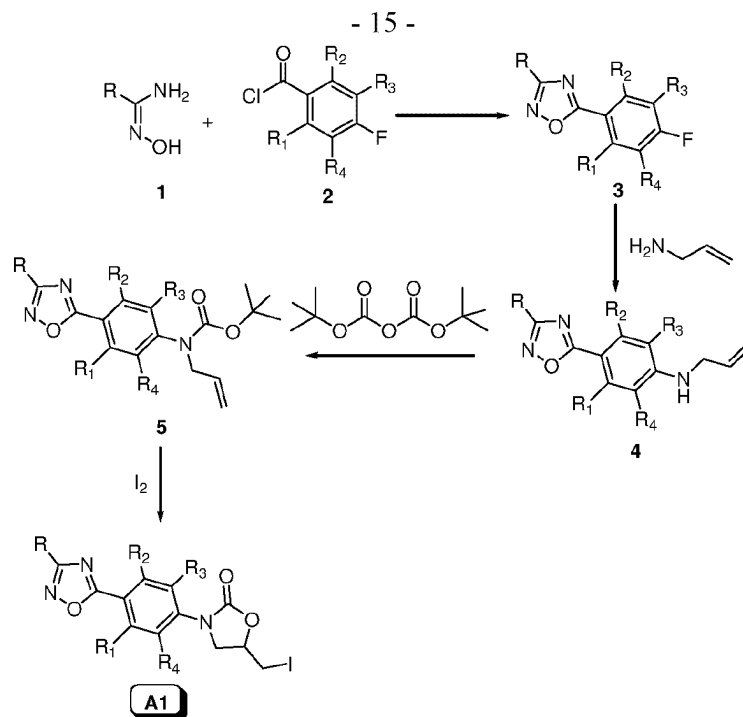
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152	CH ₃	Cl	Cl	H	H	I
153	CH ₃	F	F	F	H	I
154	CH ₃	Br	H	H	H	I
155 (B1a)	CH ₃	H	H	H	H	1,2,3-triazol-1-yl
156 (B1b)	CH ₃	F	H	H	H	1,2,3-triazol-1-yl
157	CH ₃	F	F	H	H	1,2,3-triazol-1-yl
158	CH ₃	Cl	H	H	H	1,2,3-triazol-1-yl
159	CH ₃	Cl	Cl	H	H	1,2,3-triazol-1-yl
160	CH ₃	F	F	F	H	1,2,3-triazol-1-yl
161	CH ₃	Br	H	H	H	1,2,3-triazol-1-yl

Each compound identified above is intended as the S enantiomer as well as a mixture enriched with the S enantiomer or a racemic mixture., For compounds 127-133 and 148-161 is understood as the R-enantiomer is preferred as pure as a mixture enriched in R-enantiomer.

5 Preparation of invented compounds

The synthesis of compounds of interest A and B and of the corresponding intermediates, is described below. The invented compounds were synthesized starting from the construction of the 1,2,4-oxadiazole ring by following the classic *amidoxime route* (Scheme 1) as reported in [9]. Thus, amidoxime **1** was reacted with the corresponding benzoyl chloride **2**, producing 1,2,4-oxadiazoles **3**. The latter compounds, where the *para* position is activated to undergo an Aromatic Nucleophilic Substitution, [10-13] were with allylamine, yielding compounds **4**. Reaction with di-(*t*-butyl)-dicarbonate and subsequent cyclization [14] of the resulting derivatives **5**, yielded oxazolidinones of interest **A1** as ideal precursors for further side-chain modifications.



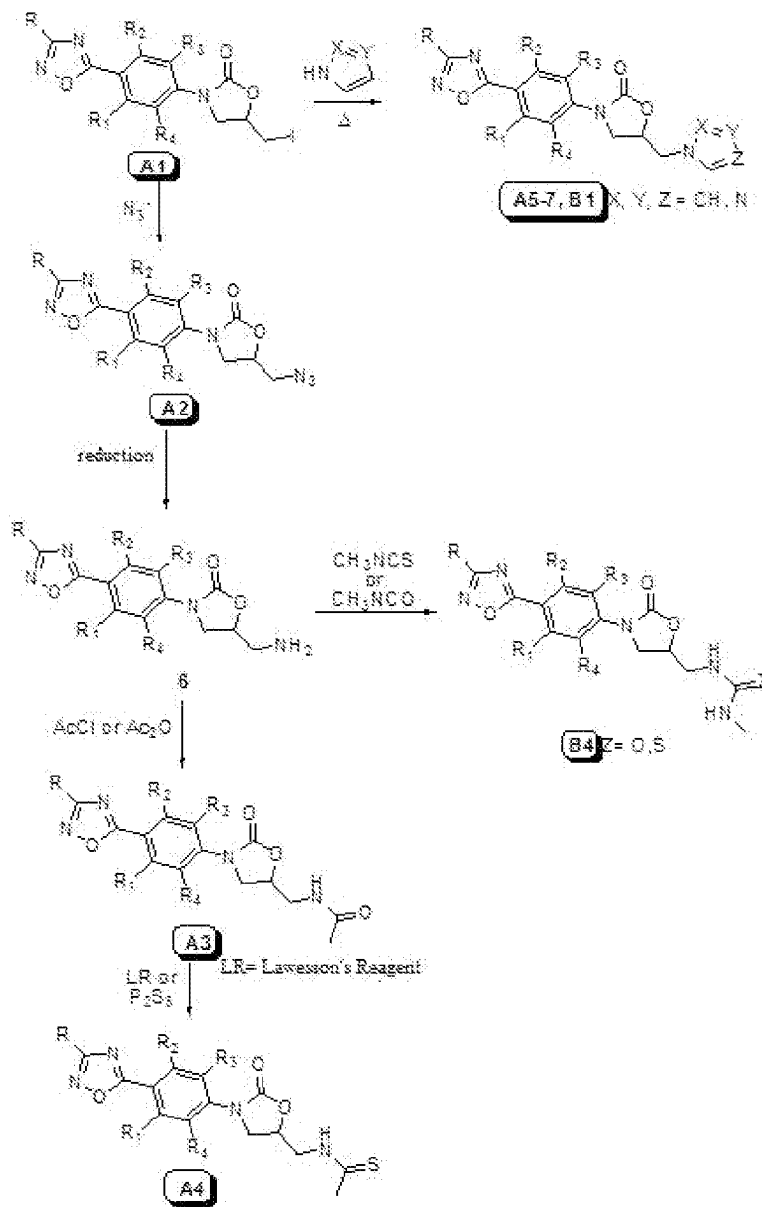
Scheme 1

The subsequent functionalization of the side-chain (Scheme 2) included the acetamidomethyl moiety **A3**, as well as the corresponding thioamides **A4**, thioureas **B4** and azolic derivatives **A5-7**, **B1**.

The azide precursors **A2** were obtained by reaction of compounds **A1** with an azide source. Their subsequent reduction yielded the corresponding amino derivatives **6** [15]. The amino derivatives **6** were readily reacted with acetyl chloride or acetic anhydride, giving compounds **A3**. The acetamidomethyl derivatives **A3**, were reacted with sulfating reagents (i.e. Lawesson's Reagent or P_2S_5) yielding thioamide derivatives **A4** (Scheme 2).

The azole derivatives **A5-7**, **B1**, were obtained by means of nucleophilic substitution starting from iodo-derivatives **A1**, while (thio)ureas **B4** were obtained through reactions of amines **6** with iso(thio)cyanates (Scheme 2).

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Scheme 2

The so obtained compounds, synthesized as racemic mixtures, were resolved into the corresponding enantiomers (S or R) through HPLC separations by using a chiral stationary phase.

The pharmaceutical compositions

Pharmaceutical compositions suitable for administration of the compounds of the invention are compositions designed for oral, parenteral or topical usage.

Oral compositions may be, for example, in the form of tablet, coated tablet,

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hard capsule, soft capsule, syrup, solution, suspension, emulsion. Parenteral compositions may be, for example, in the form of aqueous or oily solution or emulsion. Topical compositions may be for example in the form of ointment, cream, gel, solution, O/W or W/O emulsion, or suspension.

5 In a specific embodiment the compositions are administered via inhalation.

In the preparation of pharmaceutical compositions one or more compounds of the invention are mixed with various therapeutically acceptable excipients suitable for solid, liquid or pasty compositions.

10 The suspensions/emulsions, whatever were their route of administration, may comprise nanoparticles and/or liposomes as a vehicle or carrier of the medicament.

15 Since some persistent lung infections often show a low rate of response to conventional therapy, in part due to the lack of selectivity of the drugs, in part to their low bioavailability, especially when administered systemically, particular attention has been given, within of the present invention, to the route of administration by endotracheal inhalation as an alternative to non-invasive systemic delivery of the compounds described herein.

Therefore, a specific embodiment of the invention involves the administration by endotracheal inhalation of the drug, preferably encapsulated in nanoparticles.

20 In fact, the nano-encapsulation of drugs and their pulmonary release promote a higher accumulation and retention of the drug within the lungs. The main advantage of this formulation and the route of administration is to be able to treat topically compartmentalized diseases such as those within the lung or bronchus, allowing the administration of high doses of the drug involved in the district and with reduced systemic toxicity, and therefore a reduced probability of giving rise to systemic side effects;

25 Formulations based on nanoparticle-carrier offer the additional advantage of improving the crossing of biological membranes such as the outer membrane of the bacteria, expanding the spectrum of action of drugs active also against Gram-negative bacteria;

30 In particular, we have developed solid lipid nanoparticles nebulizer - compatible (Nebulizer -Compatible Solid Lipid Nanoparticle (SLN)s) for the release of antimicrobial agents of the invention. SLN can be used as a vehicle for the pulmonary or bronchial release of antimicrobial drugs, improving stability as well as the in vivo retention time in the lungs, thus obtaining an increased bioavailability.

35 The studies on the pharmacokinetics and biodistribution of SLN have shown that interstitial lung macrophages, in more close contact with the circulation

compared to alveolar macrophages, significantly contribute to the SLN uptake. Moreover, it was seen that factors such as prolonged circulation time, lower exposure of drugs at the renal level and markedly increased deposit in lung tissues are important characteristics for antimicrobial compounds also effective in the treatment of pneumonia caused by beta-lactams resistant bacteria (eg. methicillin-resistant *Staphylococcus aureus* (MRSA)).

Therapeutic Applications

The disclosed compounds are new antibiotics intended for use in the treatment of infections caused by bacteria, essentially extremely resistant by Gram-positive bacteria. For example, but not limited to, *Staphylococcus* spp, *Enterococcus* spp, *Streptococcus* spp, in particular in the treatment of infections caused by *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Moraxella catarrhalis*. The compounds of the invention have proved to be active also on bacteria resistant to other antibiotics or resistant to the reference compound, linezolid. Advantageously, the compounds of the invention are effective even against bacteria resistant to more than one antibiotic, against multi-resistant bacteria, for example to two or more antibiotics selected from methicillin, vancomycin, penicillin, macrolides, fluoroquinolones or linezolid.

Furthermore, the novel compounds of the invention combine the inhibitory or bactericidal activity against both susceptible or (multi)resistant bacteria to known antibiotics to an entirely acceptable toxicity or even less than that of the reference compound, linezolid, thus offering an entirely beneficial clinical/therapeutic profile.

Without linking the invention to some particular scientific theories, the effectiveness of the compounds of the invention in the treatment of bacterial infections, especially those caused by bacteria also resistant to other antibiotics, seems to be based on mechanisms of action involving modulation and/or inhibition of bacterial protein synthesis and/or activity. The effectiveness of the molecules of the invention seems to be linked, in theory, not only to an interaction with proteins responsible for the resistance mechanisms developed by bacteria, such as for example, the protein expressed by PBP2a MRSA strains (methicillin-resistant *Staphylococcus aureus*), but also to an interaction of the compounds of the invention with mechanisms of ribosomal protein synthesis.

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EXPERIMENTAL SECTION**Evaluation of the pharmacological activity****Microbiological assays**

(i) Bacterial strains

5 Several well characterized for their antibiotic-susceptibility phenotype *Staphylococcus aureus* isolates were used for the determination of the *in vitro* antibacterial activity of the studied compounds. In particular, *S. aureus* ATCC 29213 reference standard strain and *S. aureus* M923 (collection strain) were used as MSSA strains. Among MRSA, *S. aureus* MU50 (ATCC 700699) reference standard
10 strain and two collection strains (433 and F511) were used for susceptibility assays.

In particular, eleven linezolid-resistant coagulase-negative staphylococci (CoNS) (ten *S. epidermidis* and one *S. hominis*) were investigated. The eleven linezolid-resistant strains were isolated in several hospital settings between 2010 and 2011 from positive blood cultures. For the comparison of the antimicrobial
15 activities of the different compounds a collection of forty linezolid-susceptible MRSA, recently isolated from patients with cystic fibrosis, showing different profiles of multi-resistance to different classes of antimicrobials, was used (Table 4 and 5).

(ii) Determination of Minimum Inhibitory Concentrations (MICs)

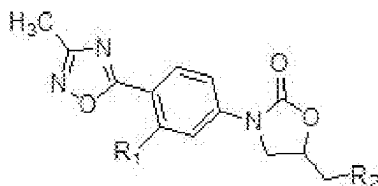
The *in vitro* antibacterial activity of the new agents was studied by
20 determining their minimum inhibitory concentrations (MICs) by means of the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. [16] Briefly, serial 2-fold dilutions of each compound were made using the Cation adjusted Mueller-Hinton broth (CAMHB) in microtitre plates with 96 wells. Dimethyl sulfoxide (DMSO) was used as solvent for all the synthesized
25 compounds. An equal volume of the bacterial inoculum (1×10^6 CFU/mL) was added to each well on the microtitre plate containing 0.05 mL of the serial antibiotic dilutions. The microtitre plate was then incubated at 37°C for 18-24 h after which each well was analysed for the presence of bacterial growth. The MIC was defined as the lowest concentration of antimicrobial agent able to cause inhibition of
30 bacterial growth as shown by the lack of turbidity of the culture medium. The *in vitro* antibacterial activities of new linezolid-like 1,2,4-oxadiazoles were tested and compared to that of reference oxazolidinone in clinical use: Linezolid (Sigma-Aldrich). Final DMSO concentrations were also taken into account in all the biological assays.

35 **Minimum inhibitory concentration test**

Fourteen new compounds in racemic mixture (group A), as following shown, were analyzed for their antibacterial activity against strains of *Staphylococcus*

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aureus in terms of reference standard strains and clinical strains, both methicillin-susceptible (MSSA) or methicillin-resistant (MRSA).



A1-7a,b

	R ₁	R ₂
A1a	H	H
A1b	H	H
A2a	H	N ₃
A2b	H	N ₃
A3a	H	NH(C=O)CH ₃
A3b	H	NH(C=O)CH ₃
A4a	H	NH(C-S)CH ₃
A4b	H	NH(C-S)CH ₃
A5a	H	pyrazol-1-yl
A5b	H	pyrazol-1-yl
A6a	H	imidazol-1-yl
A6b	H	imidazol-1-yl
A7a	H	1,2,4-triazol-1-yl
A7b	H	1,2,4-triazol-1-yl

- 5 The antimicrobial activities, summarized in Table 2, were determined by the "gold standard" method of broth microdilution, as recommended by the Clinical Laboratory Standards Institute (CLSI) (See the Experimental Section). The minimum inhibitory concentrations (MIC) values were expressed in $\mu\text{g/mL}$, and cell viability tests were performed to evaluate the antibacterial selective toxicity of most active compounds.
- 10 Linezolid has been used as a reference antibiotic. In detail, the bacterial strains were tested: *Staphylococcus aureus* ATCC 29213, a clinical strain of methicillin-susceptible *S. aureus* (M923), *S. aureus* MU50 (methicillin-resistant - MRSA), and two methicillin-resistant clinical strains, 433 and F511. All strains tested were found to be linezolid-susceptible. Among these molecules the most active, in racemic form,
- 15 have proved to be **A4a** and **A4b** compounds.

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Table 2
MIC ($\mu\text{g/mL}$)

Comp. A	ATCC	MSSA	MRSA	MRSA	MRSA
	29213	M923	MU50	433	F511
A1a	>50	>50	50	25	50
A1b	>50	>50	50	50	>50
A2a	>50	>50	>50	>50	>50
A2b	>50	>50	>50	>50	>50
A3a	12.5	6.25	6.25	1.6	12.5
A3b	12.5	6.25	6.25	1.6	12.5
A4a	3.13	1.6	≤ 0.4	1.6	1.6
A4b	1.6	1.6	≤ 0.4	0.8	1.6
A5a	>50	>50	>50	>50	>50
A5b	>50	>50	>50	>50	>50
A6a	>50	>50	>50	>50	>50
A6b	>50	>50	>50	>50	>50
A7a	>50	>50	>50	>50	>50
A7b	>50	>50	>50	>50	>50
Linezolid	≤ 0.4	3.13	0.8	1.6	3.13

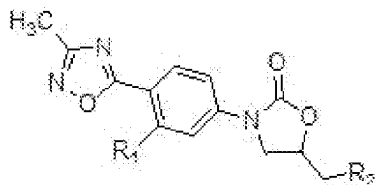
Compounds **A3a**, **A3b**, **A4a**, **A4b**, **A1a**, **A1b** correspond to the compounds 15, 16, 22, 23, 148 and 149 of Table 1

Four of the fourteen tested compounds (see Table 2) showed MIC values, both against MSSA and MRSA strains, with potency comparable or superior to that of linezolid. Furthermore, a better activity against MSSA and MRSA strains compared to linezolid has been displayed by derivatives containing sulfur **A4a** and **A4b**, while compounds **A3a**, and **A3b** were shown to be less active than linezolid, except for the MRSA strain 433. The comparison with the linezolid should take account of the fact that the tested compounds were used as a racemic mixture, then the antibacterial activity of **A3a**, **A3b**, **A4a** and **A4b** is presumed to be

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underestimated compared to the pure more active enantiomer.

Of other compounds (group B), shown below, were assessed the activities of both the racemic mixture and S and R enantiomers.



B14a,b

	R ₁	R ₂
B1a	II	1,2,3-triazol-1-yl
B1b	III	1,2,3-triazol-1-yl
B2a	II	NCS
B2b	III	NCS
B3a	II	NH(C=S)NH ₂
B3b	III	NH(C=S)NH ₂
B4a	II	NH(C=S)NHCH ₃
B4b	III	NH(C=S)NHCH ₃

5 The antimicrobial activities, summarized in Table 3, were determined by the "gold standard" method of broth microdilution, as recommended by the Clinical Laboratory Standards Institute (CLSI) (See the Experimental Section). The minimum inhibitory concentration (MIC) values were expressed in µg/mL. Linezolid has been used as a reference antibiotic. In detail, the tested bacterial strains were:

10 *Staphylococcus aureus* ATCC 29213, a clinical strain of methicillin-susceptible *S. aureus* (M923), *S. aureus* MU50 (methicillin-resistant - MRSA) strain, and two methicillin-resistant clinical strains, 433 and F511. All strains tested were found to be linezolid-susceptible. Among tested new molecules the most active, in racemic form, have proved to be **B4a** and **B4b** compounds, followed by **B1a** and **B1b** possessing

15 a fair amount of activity (Table 3).

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Table 3

Comp. B	MIC ($\mu\text{g/mL}$)				
	ATCC	MSSA	MRSA	MRSA	MRSA
	29213	M923	MU50	433	F511
B1a	25	25	3,125	12,5	12,5
B1b	25	25	1,6	6,25	12,5
B2a	>50	>50	>50	>50	50
B2b	>50	>50	>50	>50	>50
B3a	>50	>50	>50	>50	>50
B3b	>50	>50	>50	>50	>50
B4a	6,25	6,25	1,6	3,125	6,25
B4b	6,25	6,25	1,6	3,125	6,25
Linezolid	≤ 0.4	3.125	0.8	1.6	3.125

Among these, **B4a** and **B4b** compounds (corresponding to compounds 106 and 107 of Table 1) showed an antibacterial activity very similar to that of linezolid against linezolid-susceptible *S. aureus* strains.

5 In a completely surprising manner, the same compounds, resolved into their enantiomers, have demonstrated efficacy from 8 to 32 times higher than linezolid against linezolid-resistant *Staphylococcus* spp. strains. The results are reported in Tables 4 and 5. In one case (A4bS) it is quite completely reversed resistance to linezolid into susceptibility. Of these molecules enantiomeric separations have
10 allowed to assign the power to the S enantiomer, while the R proved to be inactive (see Table 4).

The compounds **B4a** and **B4b** correspond to racemic mixtures of the two compounds **B4a** and **B4b**, the compounds **B4bS** and **B4bR** and **B4aS** and **B4aR** are resolved S and R enantiomers, respectively.

Table 4

MIC values

MIC-range ≤0,06 a >128 µg/ml								
tested strains: 6 ATCC (4MSSA, 2 MRSA), plus 45 MRSA all LZD sensitive								
Compound	B4b	B4bS	B4bR	B4a	B4aS	B4aR	LZD	DA
MIC - range	0,5-16	0,5-8	64->128	1-16	0,5-8	128->128	0,25-16	<0,06- >128
MIC ₅₀	4	2	>128	8	2	>128	2	<0,06
MIC ₉₀	16	4	>128	16	4	>128	4	>128
tested strains: 12 MRSE all LZD sensitive								
MIC range	32->128	8-16	>128	32->128	8-32	>128	32-64	0,12-1
MIC ₅₀	64	8	>128	64	16	>128	32	0,5
MIC ₉₀	128	8	>128	>128	32	>128	64	1

Table 5

Strains	MIC-range 0.06 – 128 µg/mL				
	A4aS	A4aR	A4bS	A4bR	LZD ₅
ATCC <i>S. aureus</i> 29213	8	128	4	64	4
ATCC <i>E. faecalis</i> 29212	4	> 128	2	32	1
11 Linezolid-resistant CoNS					
<i>S. epidermidis</i> Strain 1	8	> 128	8	128	64
<i>S. epidermidis</i> Strain 2	32	> 128	8	> 128	64
<i>S. epidermidis</i> Strain 3	4	> 128	4	> 128	64
<i>S. epidermidis</i> Strain 4	32	> 128	4	128	104
<i>S. epidermidis</i> Strain 5	4	> 128	2	128	64
<i>S. epidermidis</i> Strain 6	4	> 128	4	64	32
<i>S. epidermidis</i> Strain 7	32	> 128	8	> 128	32
<i>S. epidermidis</i> Strain 8	32	> 128	2	128	32
<i>S. epidermidis</i> Strain 9	1	> 128	1	> 128	32
<i>S. epidermidis</i> Strain 10	8	> 128	4	128	32
<i>S. hominis</i> Strain 11	8	> 128	4	128	32
					15
MIC-range	1-32	>128	1-8	64->128	32 – 64
MIC₅₀	8	>128	4	128	32
MIC₉₀	32	>128	8	128	64
45 Linezolid-susceptible MRSA					
MIC₅₀	2	>128	0.5	128	2

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Cell viability (citotoxicity assay)

To assess if the effect shown against bacterial cells could be related to a selected toxicity or to a more general toxic effect, we performed a first level assay in different types of eukaryotic cell lines to screen the new compounds for their general cytotoxic activity.

Cell viability

The effects of A4b, (compound 23 of Table 1) and linezolid on cells viability were *in vitro* studied on PK15 (porcine kidney epithelial), HaCaT (human keratinocytes), and HepG2 (human hepatocellular carcinoma) cell lines. [17-19] HepG2 and HaCat cells were grown in Dulbecco's modified eagles medium (DMEM) whereas PK15 in DMEM/M199 (1:1). All media were supplemented with 10% heat inactivated foetal bovine serum (FBS), 2mM L-glutamine, 100 units/mL penicillin and 100 µg/mL streptomycin. Cells were maintained at 37°C in a 5% CO₂ atmosphere. All reagents for cell culture were from Euroclone (Pero, Italy).

Cell viability was measured by the MTT assay.[20] Briefly, MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] stock solution (5 mg/mL) was added to each well to a final concentration of 1.2 mM, and cells were incubated for 1 hour and 30 minutes at 37°C. After removing MTT solution, the reaction was stopped by adding 90% ethanol. Resuspended cells were centrifuged 10 min at 800 x g. The absorbance was measured with the multilabel Victor³ spectrophotometer (Perkin Elmer, Turku, Finland) at wavelength of 570 nm. Data are means ± S.E. of 3 separate experiments performed in triplicate.

Statistical analysis

Statistical significance was obtained with Student's t test in comparison with controls * = $P < 0.05$, ** = $P < 0.001$. Data are means ± S.E. of 3 separate experiments performed in triplicate.

All tested cell lines were treated with increasing concentrations (5-400 µg/mL) of **A4a**, and linezolid as reference compound. Another control was DMSO used as solvent.

The **A4b** molecule induced a moderate reduction of viability (less than 10%) in the PK15 cell line, with statistical significance at the concentrations of 25 (P < 0.01), 50 (P < 0.05) and 200 µg/mL (P < 0.05), respectively (Figure 2). This trend is comparable to that obtained with linezolid at the same concentrations.

The reduction of cell viability caused by the **A4b** molecule was slightly more evident in the HaCaT cell line, reaching levels of statistically significant mortality compared to the values obtained with linezolid only at a concentration of 400 µg/mL (P < 0.01; Figure 3).

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HepG2 cells showed a reduction in viability from 50 μ g/mL of the A4b compound (Figure 4).

They were then *in vitro* evaluated the effects of **B4a** and **B4b** molecules on cell viability on human hepatoma cell line, HepG2 and comparison with cytotoxicity induced by linezolid (negative control).

The cells are cultured in Dulbecco's modified eagles medium (DMEM) supplemented by 10% heat inactivated fetal bovine serum (FBS), L-glutamine at a final concentration of 2 mM, 100 units/mL of penicillin and 100 micrograms/mL of streptomycin. The cells were maintained at 37°C in a 5% of CO₂ atmosphere.

Cytotoxic treatment: cells, plated at a density of 40,000 cell/cm² and maintained in culture for two days, were treated for 48 hours with increasing concentrations (25-100 μ g/ml) of both enantiomers of **B4a** and **B4b** substances.

Cell viability was evaluated by an PrestoBlue® Cell Viability Reagent assay, a solution containing resazurin that permeates into cells and exploits the reducing power when they are alive and metabolically active. Briefly, the PrestoBlue® solution is administered directly to the medium of the cells in culture following the instructions of the manufacturer that has supplied the product. The cells are incubated for 1 hour at 37°C, at which time the PrestoBlue® solution, metabolized by living cells changes the staining from blue to red. The absorbance is measured using a Victor3 multifunction spectrophotometer (Perkin Elmer, Turku, Finland) at a wavelength of 570 nm. The obtained results and represented in the graph correspond to the mean \pm SE of independent experiments performed in triplicate.

The HepG2 cell line was subjected to treatment with increasing concentrations (25-100 μ g/mL) of both enantiomers of the **B4a** and **B4b** molecules. Linezolid is used as a reference molecule only to a final concentration of 100 micrograms/mL. Moreover, as an additional control, cells are also treated with 0.9% DMSO, used as a solvent of the substances.

Both enantiomers of the **B4b** molecule have induced a moderate reduction of viability (\leq of 12%) in the HepG2 cell line at all the tested concentrations (Figure 5).

The S enantiomer of the **B4a** molecule has a slight concentration-independent cytotoxic effect in HepG2 cells (evident only at 25 micrograms/mL), while the R-enantiomer does not determine an apparent reduction in cell viability. HepG2 cells, as expected, is subject to a mortality of 20% after treatment with 100 micrograms/mL of linezolid.

35 **Oxidative phosphorylation (OXPHOS) assay**

This assay (Nadaciva S. et al., 2010) is used to monitor the level of mitochondrial protein synthesis of some key proteins in the process of oxidative

phosphorylation of eukaryotic cells, comparing it with the level of synthesis of mitochondrial proteins encoded by nuclear DNA. This study allows us to analyze the effects of A4bS on the proteins encoded by mitochondrial DNA (mtDNA).

5 The results, shown in figure 6, confirm that linezolid (100 µg/mL) acts negatively on mitochondrial protein synthesis. In fact, the proteins of complex I, III (core 2) and IV (synthesized by mtDNA) undergo a significant decrease after the treatment of linezolid. In parallel, one can compare the A4bS molecule (10-100 µg/mL), which as linezolid, causing a reduction of the synthesis of proteins of complex I and IV with respect to the control (non-treated cells). However, it is to be
10 noted that the decrease in the protein synthesis induced by the compound A4bS is of a lower entity than that induced by linezolid, such an effect highlights a decrease of the side effect linked to the reversible myelosuppression. The results reported in Figure 6 were obtained as followed: the levels of the proteins encoded by the mitochondrial DNA (mtDNA) synthesized on mitochondrial ribosome (Complex IV, Complex I) and of the proteins encoded by the nuclear DNA synthesized on the
15 ribosome in the cytosol (Complex II subunit α V complex), and imported in mitochondria, were analyzed by MitoProfile® Total OXPHOS human WB antibody after treatment of HepG2 cells (human hepatocellular carcinoma cells) with the compound A4bS. The data represent the mean \pm SEM of three separate
20 experiments performed in triplicate. Statistical significance is obtained by the Student's test compared to the compounds.

* = $p < 0.05$; ** = $p < 0.01$.

Chemical Synthesis

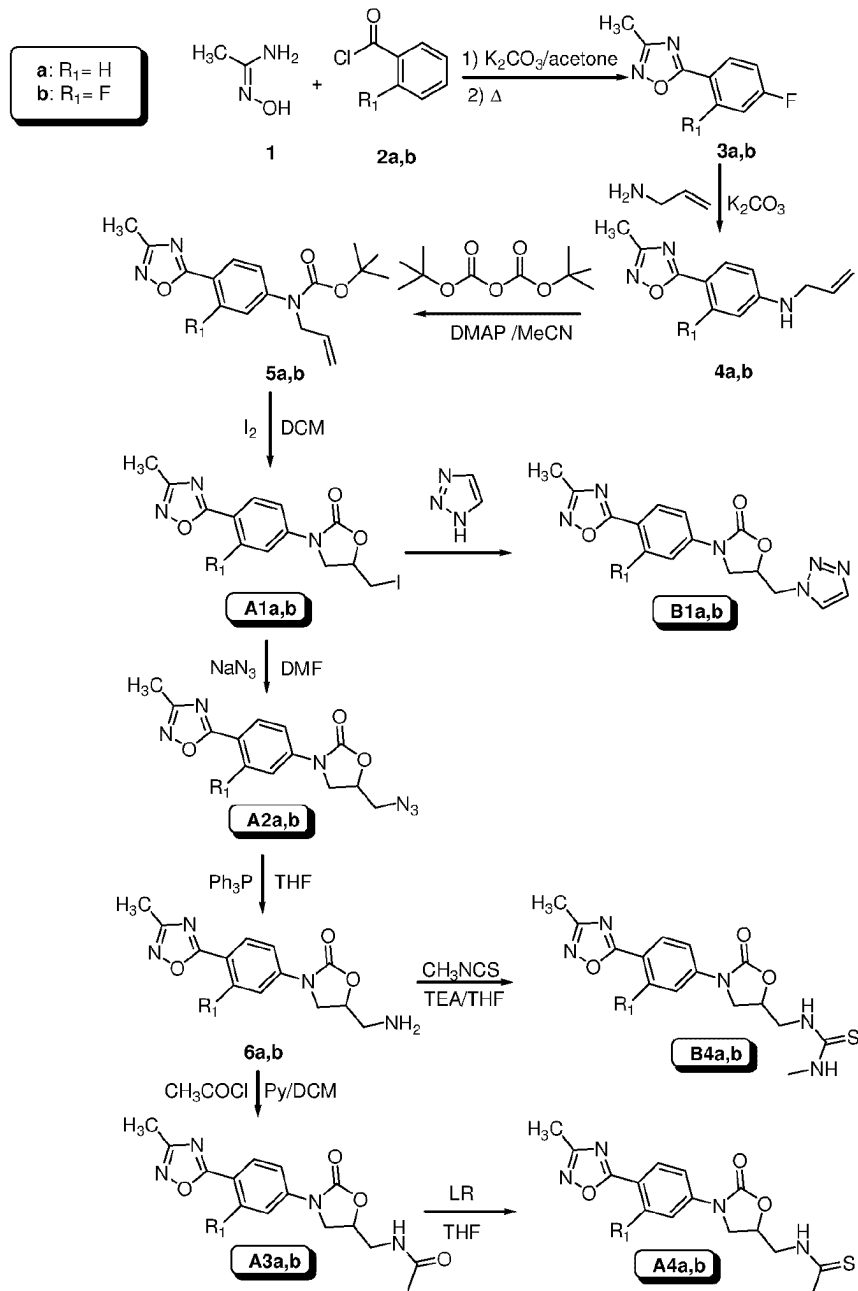
25 Melting points were determined on a Reichart-Thermovar hotstage apparatus and are uncorrected. IR spectra (Nujol) were determined with a Shimadzu FTIR-8300 instrument; H NMR spectra were recorded on a Bruker 300 Avance spectrometer using TMS as an internal standard. Flash chromatography was performed 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. The
30 purity of compounds, in all cases higher than 95 %, has been checked by both NMR and HPLC analyses. Separation of racemates was performed by means of HPLC with chiral stationary phase (Daicel, Chiralpak™-IA), by using hexane-iPrOH (70:30) as mobile phase, and 1 mL/min flux. In every case an ee>99% was obtained.

The most interesting compounds:

35 **A1a** (compound 148 table 1), **A1b** (compound 149 table 1), **A3a** (compound 15 table 1), **A3b** (compound 16 table 1), **A4a** (compound 22 table 1), **A4b** (compound 23 table 1), **B1a** (compound 155 table 1), **B1b** (compound 156 table 1),

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B4a (compound 106 table 1), **B4b** (compound 107 table 1); reported in table 2 (group A) and 3 (group B) and corresponding intermediates **1-6**, were obtained accordingly to general methodologies reported on schemes 1 and 2, following specifications indicated below and on scheme 3.



5

Scheme 3

General procedure for the preparation of compounds **3a,b**

A solution of hydroxylamine hydrochloride (1.00 g, 14.4 mmol) and NaOH (0.57 g, 14.4 mmol) in water (5 mL) was added (in about 15 minutes) to 15 mL of

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CH₃CN. The reaction mixture was stirred at room temperature for 24 hours. The solvent was removed under reduced pressure and the residue treated with ethanol; the resulting suspension was filtered and the solvent was removed under reduced pressure producing 1.659 g of acetamidoxime **1** (77%). Then, either 4-fluorobenzoyl
5 (**2a**) chloride or 2,4-difluorobenzoyl chloride (**2b**) (14.8 mmol) were added to a solution of **1** (1.00 g; 13.5 mmol) in Acetone (35 mL) containing also K₂CO₃ (2.05 g, 14.8 mmol). The mixture was stirred at room temperature for about 90 minutes after which the solvent was removed under reduced pressure. The residue was treated with water and the solid precipitate was collected by filtration. The obtained O-acylamidoxime was heated, without any further purification, at about 130°C for 90
10 minutes in a sealed tube. The obtained residue was chromatographed yielding the corresponding 1,2,4-oxadiazoles **3a** and **3b**.

3-methyl-5-(4'-fluorophenyl)-1,2,4-oxadiazole (**3a**): Yield (72 %); mp 80.0-81.0°C; ¹H NMR (300 MHz; CDCl₃) 2.45 (s, 3H, Me); 7.16-7.23 (m, 2H, Ar); 8.08-8.14 (m, 2H, Ar). Anal. Found (calc) for C₉H₇FN₂O (%): C, 60.65 (60.67); H, 3.90 (3.96); N, 15.70 (15.72).
15

3-methyl-5-(2',4'-difluorophenyl)-1,2,4-oxadiazole (**3b**): Yield (72 %); mp 57.0-60.0°C; ¹H-NMR (300 MHz; CDCl₃) 2.46 (s, 3H, Me); 6.95-7.07 (m, 2H, Ar); 8.04-8.14 (m, 1H, Ar). Anal. Found (calc) for C₉H₆F₂N₂O (%): C, 55.15 (55.11); H, 3.10 (3.08); N, 14.25 (14.28).
20

Preparation of N-allyl-4-(3'-methyl-1,2,4-oxadiazol-5'-yl)-aniline (**4a**)

Compound **3a** (0.61g; 3.43 mmol) was heated, with allylamine (3.0 mL; 2.28 g; 40.0 mmol) and K₂CO₃ (2.00 g; 14.5 mmol), at about 60°C for 8 days. The reaction mixture was treated with water and extracted with EtOAc. The organic
25 layers were collected, dried over anhydrous Na₂SO₄, filtered and the solvent removed. The residue was chromatographed yielding compound **3a**: Yield (54%); mp 63.9-65.5°C; IR (Nujol) 3335 (NH), 1607 (C=N) cm⁻¹; ¹H-NMR (300 MHz; DMSO-d₆) 2.31 (s, 3H, Me); 3.76-3.79 (m, 2H, CH₂); 5.12 (dd, 1H, J₁ = 10.5 Hz, J₂ = 1.8 Hz, -CH=CH₂); 5.22 (dd, 1H, J₁ = 17.1 Hz, J₂ = 1.8 Hz, -CH=CH₂); 5.82-5.93 (m, 1H, -CH=CH₂); 6.68 (d, 2H, J = 9.0 Hz, Ar); 6.87 (t, 1H, J = 5.7 Hz, NH, exch. with D₂O); 7.76 (d, 2H, J = 9.0 Hz, Ar). Anal. Found (calc) for C₁₂H₁₃N₃O (%): C, 66.95 (66.96); H, 6.10 (6.09); N, 19.45 (19.52).
30

Preparation of N-allyl-3-fluoro-4-(3'-methyl-1,2,4-oxadiazol-5'-yl)-aniline (**4b**)

To a solution of **3b** (0,86g; 4.38 mmol) in DMF (2.0 mL) was added
35 allylamine (1.64 mL; 1.25 g; 22.0 mmol). The reaction mixture was stirred for 2 days, after which the solution was treated with water and extracted with EtOAc. The organic layers were collected, dried over anhydrous Na₂SO₄, filtered and the solvent

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removed. The residue was chromatographed yielding compound **4b**: Yield (49%); mp 57.9-59.9°C; IR (Nujol) 3335 (NH), 1626 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (300 MHz; DMSO- d_6) 2.34 (s, 3H, Me); 3.77-3.81 (m, 2H, CH_2); 5.13 (dd, 1H, $J_1 = 13.2$ Hz, $J_2 = 1.2$ Hz, $-\text{CH}=\text{CH}_2$); 5.23 (dd, 1H, $J_1 = 17.4$ Hz, $J_2 = 1.2$ Hz, $-\text{CH}=\text{CH}_2$); 5.81-5.93 (m, 1H, $-\text{CH}=\text{CH}_2$); 6.46 (dd, 1H, $J_1 = 14.4$ Hz, $J_2 = 1.8$ Hz, Ar); 6.56 (dd, 1H, $J_1 = 8.7$ Hz, $J_2 = 1.8$ Hz, Ar); 7.17-7.21 (bs, 1H, NH, exch. with D_2O); 7.72-7.77 (m, 1H, Ar). Anal. Found (calc) for $\text{C}_{12}\text{H}_{12}\text{FN}_3\text{O}$ (%): C, 61.80 (61.79); H, 5.10 (5.19); N, 18.15 (18.02).

General procedure for the preparation of compounds **5a,b**

Either compound **4a** or **4b** (2.15 mmol) were dissolved in CH_3CN (25 mL); di-(*t*-butyl)-dicarbonate (0.51 g; 2.36 mmol) and 4-dimethylaminopyridine (0.29 g; 2.36 mmol) were added and the mixture was stirred for 2 days or 2.5 hours, respectively. The solvent was removed under reduced pressure and the obtained residue was chromatographed yielding the corresponding compounds **5a** and **5b**.

tert-butyl N-allyl-(4-(3'-methyl-1,2,4-oxadiazol-5'-yl)-phenyl)-carbamate (**5a**): oil; Yield (73%); IR (Nujol) 1711 (NCO_2), 1614 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (300 MHz; CDCl_3) 1.27 (s, 9H, *t*-Bu); 2.25 (s, 3H, Me); 4.10 (d, 2H, $J = 5.1$ Hz, CH_2); 4.95-4.97 (m, 1H, $-\text{CH}=\text{CH}_2$); 4.99-5.01 (m, 1H, $-\text{CH}=\text{CH}_2$); 5.67-5.78 (m, 1H, $-\text{CH}=\text{CH}_2$); 7.23 (d, 2H, $J = 9.0$ Hz, Ar); 7.84 (d, 2H, $J = 9.0$ Hz, Ar). Anal. Found (calc) for $\text{C}_{17}\text{H}_{21}\text{N}_3\text{O}_3$ (%): C, 64.70 (64.74); H, 6.80 (6.71); N, 13.35 (13.32).

tert-butyl N-allyl-(3-fluoro-4-(3'-methyl-1,2,4-oxadiazol-5'-yl)-phenyl)-carbamate (**5b**): oil; Yield (72%); IR (Nujol) 1713 (NCO_2), 1615 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (300 MHz; CDCl_3) 1.53 (s, 9H, *t*-Bu); 2.53 (s, 3H, Me); 4.36 (d, 2H, $J = 5.1$ Hz, CH_2); 5.21-5.28 (m, 2H, $-\text{CH}=\text{CH}_2$); 5.91-6.02 (m, 1H, $-\text{CH}=\text{CH}_2$); 7.28-7.36 (m, 2H, Ar); 8.02-8.08 (m, 1H, Ar). Anal. Found (calc) for $\text{C}_{17}\text{H}_{20}\text{FN}_3\text{O}_3$ (%): C, 61.25 (61.25); H, 6.10 (6.05); N, 12.65 (12.61).

General procedure for the preparation of compounds **A1a,b**

To a solution of 1.70 mmol of either compound **5a** or **5b** in CH_2Cl_2 (10 mL) was added I_2 sublimate (1.29 g; 5.10 mmol). The solution was stirred for 24 hours, after which the reaction was treated with a solution of Na_2SO_3 ; the organic layer was dried over anhydrous Na_2SO_4 , filtered and the solvent removed. The residue was chromatographed yielding the corresponding compounds **A1a** and **A1b**.

3-(4'-(3''-methyl-1,2,4-oxadiazol-5''-yl)-phenyl)-5-(iodomethyl)-oxazolidin-2-one (**A1a**): Yield (89%); mp 145.0-147.0 °C; IR (Nujol) 1763 (NCO_2), 1618 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (300 MHz; DMSO- d_6) 2.47 (s, 3H, Me); 3.62-3.73 (m, 2H, $\text{CH}_2\text{-I}$); 3.80 (dd, 1H, $J_1 = 9.3$ Hz, $J_2 = 6.0$ Hz, $\text{C}_4\text{-H}$); 4.34 (dd, 1H, $J_1 = 9.3$ Hz, $J_2 = 9.0$ Hz, $\text{C}_4\text{-H}$); 4.81-4.90 (m, 1H, $\text{C}_5\text{-H}$); 7.88 (d, 2H, $J = 9.0$ Hz, Ar); 8.17 (d, 2H, $J = 9.0$ Hz, Ar). Anal. Found (calc) for $\text{C}_{13}\text{H}_{12}\text{IN}_3\text{O}_3$ (%): C, 40.55 (40.54); H, 3.15 (3.14); N,

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10.85 (10.91).

3-(3'-fluoro-4'-(3''-methyl-1,2,4-oxadiazol-5''-yl)-phenyl)-5-(iodomethyl)-oxazolidin-2-one (**A1b**): Yield (76%); mp 148.0-149.0 °C; IR (Nujol) 1743 (NCO₂), 1637 (C=N) cm⁻¹; ¹H-NMR (300 MHz; DMSO-d₆) 2.48 (s, 3H, Me); 3.61-3.72 (m, 2H, CH₂-I); 3.81 (dd, 1H, J₁ = 9.6 Hz, J₂ = 6.0 Hz, C₄-H); 4.33 (dd, 1H, J₁ = 9.6 Hz, J₂ = 9.0 Hz, C₄-H); 4.83-4.93 (m, 1H, C₅-H); 7.68 (dd, 1H, J₁ = 8.7 Hz, J₂ = 2.1 Hz, Ar); 7.80 (dd, 1H, J₁ = 13.8 Hz, J₂ = 2.1 Hz, Ar); 8.16 (dd, 1H, J₁ = 8.7 Hz, J₂ = 8.5 Hz, Ar). Anal. Found (calc) for C₁₃H₁₁FIN₃O₃ (%): C, 38.75 (38.73); H, 2.55 (2.75); N, 10.35 (10.42).

10 General procedure for the preparation of compounds **A2a,b**

To a solution of 0.75 mmol of compound **A1a** or **A1b** in DMF (6 mL) was added NaN₃ (0.39 g; 6.00 mmol). The solution was stirred for 24 hours, after which the reaction was treated with water and extracted with EtOAc; the organic layers were dried over anhydrous Na₂SO₄, filtered and the solvent removed. The residue

15 was chromatographed yielding the corresponding compounds **A2a** and **A2b**.

3-(4'-(3''-methyl-1,2,4-oxadiazol-5-yl)-phenyl)-5-(azidometil)-oxazolidin-2-one (**A2a**): Yield (94%); mp 133.9-135.0 °C; IR (Nujol) 2095 (N₃), 1765 (NCO₂), 1727 (NCO₂), 1618 (C=N) cm⁻¹; ¹H-NMR (300 MHz; DMSO-d₆) 2.46 (s, 3H, Me); 3.75-3.88 (m, 2H, CH₂-N₃); 3.92 (dd, 1H, J₁ = 9.3 Hz, J₂ = 6.0 Hz, C₄-H); 4.28 (t, 1H, J = 9.3 Hz, C₄-H); 4.96-5.03 (m, 1H, C₅-H); 7.86 (d, 2H, J = 9.0 Hz, Ar); 8.16 (d, 2H, J = 9.0 Hz, Ar). Anal. Found (calc) for C₁₃H₁₂N₆O₃ (%): C, 52.05 (52.00); H, 4.10 (4.03); N, 27.85 (27.99).

3-(3'-fluoro-4'-(3''-methyl-1,2,4-oxadiazol-5-yl)-phenyl)-5-(azidometil)-oxazolidin-2-one (**A2b**): Yield (99%); mp 126.2-127.7 °C; IR (Nujol) 2107 (N₃), 1758 (NCO₂), 1743 (NCO₂), 1630 (C=N) cm⁻¹; ¹H-NMR (300 MHz; DMSO-d₆) 2.41 (s, 3H, Me); 3.69-3.82 (m, 2H, CH₂-N₃); 3.86 (dd, 1H, J₁ = 9.3 Hz, J₂ = 6.0 Hz, C₄-H); 4.21 (t, 1H, J = 9.3 Hz, C₄-H); 4.91-4.99 (m, 1H, C₅-H); 7.60 (dd, 1H, J₁ = 9.0 Hz, J₂ = 1.8 Hz, Ar); 7.72 (dd, 1H, J₁ = 13.5 Hz, J₂ = 1.8 Hz, Ar); 8.08-8.14 (m, 1H, Ar). Anal. Found (calc) for C₁₃H₁₁FN₆O₃ (%): C, 49.10 (49.06); H, 3.50 (3.48); N, 26.45 (26.41).

30 General procedure for the preparation of compounds **6a,b**

To a solution of 0.45 mmol of compound **A2a** or **A2b** in THF (15 mL) was added PPh₃ (0.16 g; 0.60 mmol). The solution was stirred for about 90 minutes, after which 100 mL of distilled water was added and the resulting mixture was refluxed for

35 4 hours. The THF was removed under reduced pressure, the resulting residue was neutralized with hydrochloric acid and extracted with EtOAc. A solution of NaOH (pH~9) was added to the aqueous phase, which was extracted with EtOAc; the

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organic layers were dried over anhydrous Na₂SO₄, filtered and the solvent removed, yielding the corresponding compounds **6a** and **6b**.

3-(4'-(3''-methyl-1,2,4-oxadiazol-5-yl)-phenyl)-5-(aminomethyl)-oxazolidin-2-one (**6a**): Yield (66%); mp 139.3-141.3 °C; IR (Nujol) 3390 (NH), 3361 (NH), 1748 (NCO₂), 1616 (C=N) cm⁻¹; ¹H-NMR (300 MHz; DMSO-d₆) 2.22 (bs, 2H, NH₂, exch. with D₂O); 2.39 (s, 3H, Me); 2.77-2.91 (m, 2H, CH₂-NH₂); 3.94 (dd, 1H, J₁ = 9.0 Hz, J₂ = 6.3 Hz, C₄-H); 4.13 (t, 1H, J = 9.0 Hz, C₄-H); 4.61-4.70 (m, 1H, C₅-H); 7.80 (d, 2H, J = 9.0 Hz, Ar); 8.09 (d, 2H, J = 9.0 Hz, Ar). Anal. Found (calc) for C₁₃H₁₄N₄O₃ (%): C, 56.90 (56.93); H, 5.15 (5.14); N, 20.45 (20.43).

3-(3'-fluoro-4'-(3''-methyl-1,2,4-oxadiazol-5-yl)-phenyl)-5-(aminomethyl)-oxazolidin-2-one (**6b**): Yield (88%); mp 137.0-140.0 °C; IR (Nujol) 3372 (NH), 1743 (NCO₂), 1630 (C=N) cm⁻¹; ¹H-NMR (300 MHz; DMSO-d₆) 2.21 (bs, 2H, NH₂, exch. with D₂O); 2.41 (s, 3H, Me); 2.77-2.91 (m, 2H, CH₂-NH₂); 3.93 (dd, 1H, J₁ = 9.3 Hz, J₂ = 6.3 Hz, C₄-H); 4.13 (t, 1H, J = 9.0 Hz, C₄-H); 4.63-4.71 (m, 1H, C₅-H); 7.60 (dd, 1H, J₁ = 9.0 Hz, J₂ = 2.1 Hz, Ar); 7.73 (dd, 1H, J₁ = 10.8 Hz, J₂ = 2.1 Hz, Ar); 8.08-8.14 (m, 1H, Ar). Anal. Found (calc) for C₁₃H₁₃FN₄O₃ (%): C, 53.40 (53.42); H, 4.45 (4.48); N, 19.25 (19.17).

General procedure for the preparation of compounds **A3a,b**.

Acetyl chloride (40 µl; 44 mg; 0.56 mmol) was added to a solution of either compound **A3a** or **A3b** (0.28 mmol) in CH₂Cl₂ (3 mL) containing also pyridine (1 mL; 0.97 g; 12.3 mmol). The solution was stirred for 30 minutes after which the solvent was removed and the residue treated with HCl 1M (20 mL) and extracted with EtOAc; the organic layers were dried over anhydrous Na₂SO₄, filtered and the solvent removed. The residue was chromatographed yielding the corresponding compounds **A3a** and **A3b**.

3-(4'-(3''-methyl-1,2,4-oxadiazol-5-yl)-phenyl)-5-(N-acetylaminoethyl)-oxazolidin-2-one (**A3a**): Yield (58%); mp 214.0-216.0 °C; IR (Nujol) 3257 (NH), 1751 (NCO₂), 1646 (amide), 1616 (C=N) cm⁻¹; ¹H-NMR (300 MHz; DMSO-d₆) 1.89 (s, 3H, COMe); 2.46 (s, 3H, Me); 3.50 (t, 2H, J = 5.7 Hz, CH₂-NHCOMe); 3.88 (dd, 1H, J₁ = 9.0 Hz, J₂ = 6.6 Hz, C₄-H); 4.25 (t, 1H, J = 9.0 Hz, C₄-H); 4.79-4.87 (m, 1H, C₅-H); 7.84 (d, 2H, J = 8.7 Hz, Ar); 8.16 (d, 2H, J = 8.7 Hz, Ar); 8.32 (t, 1H, J = 5.7 Hz, NH, exch. with D₂O); ¹³C-NMR (75 MHz; DMSO-d₆) 11.4, 22.6, 41.5, 47.2, 72.0, 118.1 (overlapped signals), 128.9, 142.6, 154.1, 167.7, 170.2, 174.5. Anal. Found (calc) for C₁₅H₁₆N₄O₄ (%): C, 56.95 (56.96); H, 5.05 (5.10); N, 17.85 (17.71).

3-(3'-fluoro-4'-(3''-methyl-1,2,4-oxadiazol-5-yl)-phenyl)-5-(N-acetylaminoethyl)-oxazolidin-2-one (**A3b**): Yield (62%); mp 184.0-186.0 °C; IR (Nujol) 3343 (NH), 1751 (NCO₂), 1666 (amide), 1628 (C=N) cm⁻¹; ¹H-NMR (300

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MHz; DMSO-d₆) 1.89 (s, 3H, COMe); 2.48 (s, 3H, Me); 3.50 (t, 2H, J = 5.4 Hz, CH₂-NHCOMe); 3.88 (dd, 1H, J₁ = 9.3 Hz, J₂ = 6.3 Hz, C₄-H); 4.25 (t, 1H, J = 9.0 Hz, C₄-H); 4.81-4.88 (m, 1H, C₅-H); 7.64 (dd, 1H, J₁ = 9.0 Hz, J₂ = 1.8 Hz, Ar); 7.77 (dd, 1H, J₁ = 13.8 Hz, J₂ = 1.8 Hz, Ar); 8.15-8.21 (m, 1H, Ar), 8.31 (m, 1H, NH, exch. with D₂O); ¹³C-NMR (75 MHz; DMSO-d₆) 11.32, 22.6, 41.5, 47.3, 72.2, 105.7 (d, J_{C-F} = 32 Hz), 106.2 (d, J_{C-F} = 14 Hz), 114.1, 131.4, 144.3 (d, J_{C-F} = 14 Hz), 153.9, 160.4 (d, J_{C-F} = 305 Hz), 167.5, 170.2, 171.6. Anal. Found (calc) for C₁₅H₁₅FN₄O₄ (%): C, 53.90 (53.89); H, 4.65 (4.52); N, 16.65 (16.76).

General procedure for the preparation of compounds **A4a,b**

The Lawesson's reagent (0.2 g; 0.49 mmol) was added to a solution of either **A3a** or **A3b** (0.49 mmol) in THF (14 mL). The reaction mixture was refluxed for 2 hours, after which the solvent was removed under reduced pressure. The residue was chromatographed yielding the corresponding compounds **A4a** and **A4b**.

3-(4'-(3''-methyl-1,2,4-oxadiazol-5-yl)-phenyl)-5-(N-thioacetylaminoethyl)-oxazolidin-2-one (**A4a**): Yield (77%); mp 199.4-201.0°C; IR (Nujol) 3217 (NH), 1721 (NCO₂), 1618 (thioamide) cm⁻¹; ¹H-NMR (300MHz; DMSO-d₆) 2.47 (s, 3H, Me); 2.51 (s, 3H, CSMe); 3.95-4.03 (m, 3H, overlapped signals); 4.28-4.34 (m, 1H, C₄-H); 5.01-5.11 (m, 1H, C₅-H); 7.85 (d, 2H, J = 9.0 Hz, Ar); 8.18 (d, 2H, J = 9.0 Hz, Ar); 10.45 (bs, 1H, NH, exch. with D₂O). Anal. Found (calc) for C₁₅H₁₆N₄O₃S (%): C, 54.15 (54.20); H, 4.85 (4.85); N, 16.90 (16.86).

3-(3'-fluoro-4'-(3''-methyl-1,2,4-oxadiazol-5-yl)-phenyl)-5-(N-thioacetylaminoethyl)-oxazolidin-2-one (**A4b**): Yield (93%); mp 166.5-167.7°C; IR (Nujol) 3262 (NH), 1746 (NCO₂), 1633 (thioamide) cm⁻¹; ¹H-NMR (300MHz; DMSO-d₆) 2.48 (s, 3H, Me); 2.51 (s, 3H, CSMe); 3.94-4.00 (m, 3H, overlapped signals); 4.28-4.34 (m, 1H, C₄-H); 5.04-5.12 (m, 1H, C₅-H); 7.65 (dd, 1H, J₁ = 9 Hz, J₂ = 1.8 Hz, Ar); 7.78 (dd, 1H, J₁ = 13.5 Hz, J₂ = 1.8 Hz, Ar); 8.16-8.22 (m, 1H, Ar); 10.45 (bs, 1H, NH exch. with D₂O). Anal. Found (calc) for C₁₅H₁₄FN₄O₃S (%): C, 51.35 (51.42); H, 4.30 (4.32); N, 16.05 (15.99).

General procedure for the preparation of compounds **B1a,b**

In a glass tube, to 0.45 mmol of compound **A1a** or **A1b** was added 1,2,3-triazole (0.124 g; 1.8 mmol). The mixture was heated until complete consumption of the starting material monitored by TLC. The residue was chromatographed yielding the corresponding compounds **B1a** and **B1b**.

((3-(4-(3-methyl-1,2,4-oxadiazol-5-yl)phenyl)-oxazolidin-2-on-5-yl)methyl)-4,5-dihydro-1H-1,2,3-triazole (**B1a**): Yield (73%); mp 208-210 °C; IR (Nujol) 1751 cm⁻¹; ¹H-NMR (300MHz; CDCl₃) 2.46 (s, 3H), 4.03 (dd, J₁ = 6.3 Hz, J₂ = 9.3 Hz, 1H), 4.25 (dd, J₁ = 9.3 Hz, J₂ = 9.0 Hz, 1H), 4.82-4.83 (m, 2H), 5.08-5.14 (m, 1H),

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7.59 (d, J = 9.0 Hz, 1H), 7.75 (s, 1H), 7.80 (s, 1H), 8.08 (d, J = 9.0 Hz, 1H); Anal. Found (calc) for C₁₅H₁₄N₆O₃ (%): C, 55.30 (55.21); H, 4.39 (4.32); N, 25.69 (25.75).

((3-(3-fluoro-4-(3-methyl-1,2,4-oxadiazol-5-yl)phenyl)-oxazolidin-2-on-5-yl)methyl)-4,5-dihydro-1H-1,2,3-triazole (**B1b**): Yield (64%); mp 176.2-177.8 °C; IR (Nujol) 1751 cm⁻¹; ¹H-NMR (300MHz; CDCl₃) 2.48 (s, 3H), 4.03 (dd, J₁ = 9.3 Hz, J₂ = 6.0 Hz, 1H), 4.25 (dd, J₁ = 9.6 Hz, J₂ = 9.0 Hz, 1H), 4.82-4.83 (m, 2H), 5.15-5.30 (m, 1H), 7.27 (dd, J₁ = 8.3 Hz, J₂ = 1.8 Hz, 1H), 7.56 (dd, J₁ = 12.6 Hz, J₂ = 1.8 Hz, 1H), 7.75 (s, 1H), 7.79 (s, 1H), 8.02 (t, J = 8.3 Hz, 1H); Anal. Found (calc) for C₁₅H₁₃FN₆O₃ (%): C, 52.37 (52.33); H, 3.85 (3.81); N, 24.47 (24.41).

10 General procedure for the preparation of compounds **B4a,b**

To a solution of 0.55 mmol of compound **6a** or **6b** in THF (5 mL) was added CH₃NCS (0.041 mL; 0.60 mmol) and triethylamine (0.084 mL; 0.60 mmol). The solution was stirred for 3 hours at room temperature. The solvent was then removed under vacuum. The residue was chromatographed yielding the corresponding compounds **B4a** and **B4b**.

15 1-((3-(4-(3-methyl-1,2,4-oxadiazol-5-yl)phenyl)-oxazolidin-2-one-5-yl)methyl)-3-methylthiourea (**B4a**): Yield (80%); mp 189.4-191.8 °C; IR (Nujol) 3364, 1732 cm⁻¹; ¹H-NMR (300MHz; CDCl₃) 2.39 (s, 3H), 2.82 (bs, 3H), 3.82-4.00 (m, 3H), 4.20 (dd, J₁ = 8.7 Hz, J₂ = 6.0 Hz, 1H), 4.91 (bs, 1H), 7.77-7.80 (m, 3H), 8.09 (d, J = 6.9 Hz, 2H); Anal. Found (calc) for C₁₅H₁₇N₅O₃S (%): C, 51.91 (51.86); H, 5.00 (4.93); N, 20.20 (20.16).

25 1-((3-(3-fluoro-4-(3-methyl-1,2,4-oxadiazol-5-yl)phenyl)-oxazolidin-2-one-5-yl)methyl)-3-methylthiourea (**B4b**): Yield (88%); mp 170.7-172.4 °C; IR (Nujol) 3370, 1739 cm⁻¹; ¹H-NMR (300MHz, DMSO) 2.48 (s, 3H), 2.89 (bs, 3H), 3.89-4.07 (m, 3H), 4.24-4.30 (m, 1H), 4.89 (bs, 1H), 7.74 (s, 1H), 7.79 (dd, J₁ = 13.5 Hz, J₂ = 2.1 Hz, 2H), 8.20 (t, J = 9.0 Hz, 2H); Anal. Found (calc) for C₁₅H₁₆FN₅O₃S (%): C, 49.21 (49.31); H, 4.35 (4.41); N, 19.10 (19.17).

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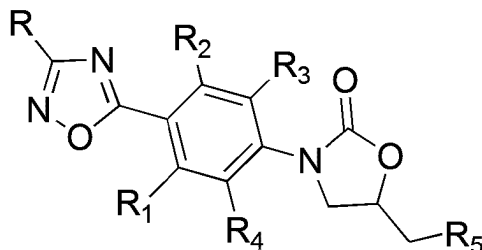
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CLAIMS

1. Compounds of general formula (I):



Formula (I)

as racemic mixtures or pure enantiomers or mixtures enriched with one of the S or R enantiomer,

where:

R= F, Cl, Br, I, C1-C3 alkyl, C3-C6 cyclo-alkyl, aryl, heteroaryl, NH₂, OH, SH, NHR₆, N(R₆)₂, or OR₆, with R₆= C1-C3 alkyl, C3-C6 cyclo-alkyl, aryl, heteroaryl, or C1-C4 acyl;

R₁₋₄= independently H, F, Cl, Br, CH₃, OH, or OCH₃;

R₅= -NH₂, -OH, -NCS, -NHC(X)CH₃ with X= O or S; -NHC(X)CH₂Z with X= O or S and Z= F or Cl; -NHC(X)CHZ₂ with X= O or S and Z= F or Cl; -NHC(X)CZ₃ with X= O or S and Z= F or Cl; or -NHC(X)NHR₇ with X= O or S, and R₇= H, C1-C3 alkyl, C3-C6-cyclo-alkyl, aryl, heteroaryl, or C1-C3-acyl

for the use in the treatment of infections caused by Gram-positive bacteria.

2. The compound for use according to claim 1 where R is a methyl, ethyl or phenyl group.

3. The compound for use according to claim 1 or 2 where at least one of the R₁, R₂, R₃ or R₄ substituents is a fluorine atom, while the others are H.

4. The compound for use according to any one of claims from 1 to 3 where R₅ is selected among: -NHC(=O)CH₃, -NHC(=S)CH₃, -NHC(=O)CH₂F, -NHC(=S)CH₂F, -NHC(=O)CH₂Cl, -NHC(=S)CH₂Cl, -NHC(=S)NH₂, NHC(=O)NH₂, -NHC(=O)NHCH₃, -NHC(=S)NHCH₃, -NHC(=O)NHC₂H₅, -NHC(=S)NHC₂H₅, -NCS; and 1,2,3 triazol-1-yl.

5. The compounds for use according to any one of claims from 1 to 4 selected among compounds where:

R₅ is -NHC(=S)CH₃ and R is CH₃;

R₅ is -NHC(=S)NHCH₃ and R is CH₃;

R₅ is -NHC(=O)CH₃ and R is CH₃; and

R₅ is -NHC(=S)NH₂ and R is CH₃.

6. The compound for use according to any one of claims from 1 to 5 selected among compounds:

	R	R1	R2	R3	R4	R5
1	Ph	H	H	H	H	NHC(=O)CH ₃
2	Ph	F	H	H	H	NHC(=O)CH ₃
3	Ph	F	F	H	H	NHC(=O)CH ₃
4	Ph	F	F	F	H	NHC(=O)CH ₃
5	Ph	F	F	F	H	NHC(=O)CH ₃
6	Ph	Cl	H	H	H	NHC(=O)CH ₃
7	Ph	Cl	Cl	H	H	NHC(=O)CH ₃
8	Ph	H	H	H	H	NHC(=S)CH ₃
9	Ph	F	H	H	H	NHC(=S)CH ₃
10	Ph	F	F	H	H	NHC(=S)CH ₃
11	Ph	Cl	H	H	H	NHC(=S)CH ₃
12	Ph	Cl	Cl	H	H	NHC(=S)CH ₃
13	Ph	F	F	F	H	NHC(=S)CH ₃
14	Ph	Br	H	H	H	NHC(=S)CH ₃
15 (A3a)	CH ₃	H	H	H	H	NHC(=O)CH ₃
16 (A3b)	CH ₃	F	H	H	H	NHC(=O)CH ₃
17	CH ₃	F	F	H	H	NHC(=O)CH ₃

18	CH ₃	F	F	F	H	NHC(=O)CH ₃
19	CH ₃	Cl	H	H	H	NHC(=O)CH ₃
20	CH ₃	Cl	Cl	H	H	NHC(=O)CH ₃
21	CH ₃	Br	H	H	H	NHC(=O)CH ₃
22 (A4a)	CH ₃	H	H	H	H	NHC(=S)CH ₃
23 (A4b)	CH ₃	F	H	H	H	NHC(=S)CH ₃
24	CH ₃	F	F	H	H	NHC(=S)CH ₃
25	CH ₃	Cl	H	H	H	NHC(=S)CH ₃
26	CH ₃	Cl	Cl	H	H	NHC(=S)CH ₃
27	CH ₃	F	F	F	H	NHC(=S)CH ₃
28	CH ₃	Br	H	H	H	NHC(=S)CH ₃
29	C ₂ H ₅	H	H	H	H	NHC(=O)CH ₃
30	C ₂ H ₅	F	H	H	H	NHC(=O)CH ₃
31	C ₂ H ₅	F	F	H	H	NHC(=O)CH ₃
32	C ₂ H ₅	F	F	F	H	NHC(=O)CH ₃
33	C ₂ H ₅	Cl	H	H	H	NHC(=O)CH ₃
34	C ₂ H ₅	Cl	Cl	H	H	NHC(=O)CH ₃
35	C ₂ H ₅	Br	H	H	H	NHC(=O)CH ₃
36	C ₂ H ₅	H	H	H	H	NHC(=S)CH ₃
37	C ₂ H ₅	F	H	H	H	NHC(=S)CH ₃
38	C ₂ H ₅	F	F	H	H	NHC(=S)CH ₃
39	C ₂ H ₅	Cl	H	H	H	NHC(=S)CH ₃
40	C ₂ H ₅	Cl	Cl	H	H	NHC(=S)CH ₃
41	C ₂ H ₅	F	F	F	H	NHC(=S)CH ₃
42	C ₂ H ₅	Br	H	H	H	NHC(=S)CH ₃

43	Ph	H	H	H	H	NHC(=O)NH ₂
44	Ph	F	H	H	H	NHC(=O)NH ₂
45	Ph	F	F	H	H	NHC(=O)NH ₂
46	Ph	F	F	F	H	NHC(=O)NH ₂
47	Ph	Br	H	H	H	NHC(=O)NH ₂
48	Ph	Cl	H	H	H	NHC(=O)NH ₂
49	Ph	Cl	Cl	H	H	NHC(=O)NH ₂
50	Ph	H	H	H	H	NHC(=S)NH ₂
51	Ph	F	H	H	H	NHC(=S)NH ₂
52	Ph	F	F	H	H	NHC(=S)NH ₂
53	Ph	Cl	H	H	H	NHC(=S)NH ₂
54	Ph	Cl	Cl	H	H	NHC(=S)NH ₂
55	Ph	F	F	F	H	NHC(=S)NH ₂
56	Ph	Br	H	H	H	NHC(=S)NH ₂
57	CH ₃	H	H	H	H	NHC(=O)NH ₂
58	CH ₃	F	H	H	H	NHC(=O)NH ₂
59	CH ₃	F	F	H	H	NHC(=O)NH ₂
60	CH ₃	F	F	F	H	NHC(=O)NH ₂
61	CH ₃	Cl	H	H	H	NHC(=O)NH ₂
62	CH ₃	Cl	Cl	H	H	NHC(=O)NH ₂
63	CH ₃	Br	H	H	H	NHC(=O)NH ₂
64 (B3a)	CH ₃	H	H	H	H	NHC(=S)NH ₂
65 (B3b)	CH ₃	F	H	H	H	NHC(=S)NH ₂
66	CH ₃	F	F	H	H	NHC(=S)NH ₂
67	CH ₃	Cl	H	H	H	NHC(=S)NH ₂

68	CH ₃	Cl	Cl	H	H	NHC(=S)NH ₂
69	CH ₃	F	F	F	H	NHC(=S)NH ₂
70	CH ₃	Br	H	H	H	NHC(=S)NH ₂
71	C ₂ H ₅	H	H	H	H	NHC(=O)NH ₂
72	C ₂ H ₅	F	H	H	H	NHC(=O)NH ₂
73	C ₂ H ₅	F	F	H	H	NHC(=O)NH ₂
74	C ₂ H ₅	F	F	F	H	NHC(=O)NH ₂
75	C ₂ H ₅	Cl	H	H	H	NHC(=O)NH ₂
76	C ₂ H ₅	Cl	Cl	H	H	NHC(=O)NH ₂
77	C ₂ H ₅	Br	H	H	H	NHC(=O)NH ₂
78	C ₂ H ₅	H	H	H	H	NHC(=S)NH ₂
79	C ₂ H ₅	F	H	H	H	NHC(=S)NH ₂
80	C ₂ H ₅	F	F	H	H	NHC(=S)NH ₂
81	C ₂ H ₅	Cl	H	H	H	NHC(=S)NH ₂
82	C ₂ H ₅	Cl	Cl	H	H	NHC(=S)NH ₂
83	C ₂ H ₅	F	F	F	H	NHC(=S)NH ₂
84	C ₂ H ₅	Br	H	H	H	NHC(=S)NH ₂
85	Ph	H	H	H	H	NHC(=O)NHCH ₃
86	Ph	F	H	H	H	NHC(=O)NHCH ₃
87	Ph	F	F	H	H	NHC(=O)NHCH ₃
88	Ph	F	F	F	H	NHC(=O)NHCH ₃
89	Ph	Br	H	H	H	NHC(=O)NHCH ₃
90	Ph	Cl	H	H	H	NHC(=O)NHCH ₃
91	Ph	Cl	Cl	H	H	NHC(=O)NHCH ₃
92	Ph	H	H	H	H	NHC(=S)NHCH ₃
93	Ph	F	H	H	H	NHC(=S)NHCH ₃
94	Ph	F	F	H	H	NHC(=S)NHCH ₃

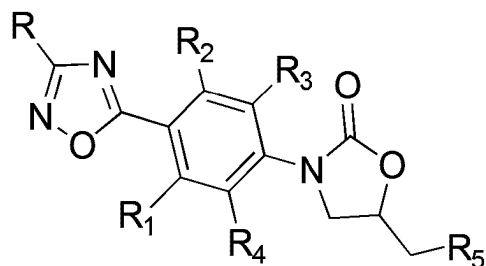
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97	Ph	F	F	F	H	NHC(=S)NHCH ₃
98	Ph	Br	H	H	H	NHC(=S)NHCH ₃
99	CH ₃	H	H	H	H	NHC(=O)NHCH ₃
100	CH ₃	F	H	H	H	NHC(=O)NHCH ₃
101	CH ₃	F	F	H	H	NHC(=O)NHCH ₃
102	CH ₃	F	F	F	H	NHC(=O)NHCH ₃
103	CH ₃	Cl	H	H	H	NHC(=O)NHCH ₃
104	CH ₃	Cl	Cl	H	H	NHC(=O)NHCH ₃
105	CH ₃	Br	H	H	H	NHC(=O)NHCH ₃
106 (B4a)	CH ₃	H	H	H	H	NHC(=S)NHCH ₃
107 (B4b)	CH ₃	F	H	H	H	NHC(=S)NHCH ₃
108	CH ₃	F	F	H	H	NHC(=S)NHCH ₃
109	CH ₃	Cl	H	H	H	NHC(=S)NHCH ₃
110	CH ₃	Cl	Cl	H	H	NHC(=S)NHCH ₃
111	CH ₃	F	F	F	H	NHC(=S)NHCH ₃
112	CH ₃	Br	H	H	H	NHC(=S)NHCH ₃
113	C ₂ H ₅	H	H	H	H	NHC(=O)NHCH ₃
114	C ₂ H ₅	F	H	H	H	NHC(=O)NHCH ₃
115	C ₂ H ₅	F	F	H	H	NHC(=O)NHCH ₃
116	C ₂ H ₅	F	F	F	H	NHC(=O)NHCH ₃
117	C ₂ H ₅	Cl	H	H	H	NHC(=O)NHCH ₃
118	C ₂ H ₅	Cl	Cl	H	H	NHC(=O)NHCH ₃
119	C ₂ H ₅	Br	H	H	H	NHC(=O)NHCH ₃

120	C ₂ H ₅	H	H	H	H	NHC(=S)NHCH ₃
121	C ₂ H ₅	F	H	H	H	NHC(=S)NHCH ₃
122	C ₂ H ₅	F	F	H	H	NHC(=S)NHCH ₃
123	C ₂ H ₅	Cl	H	H	H	NHC(=S)NHCH ₃
124	C ₂ H ₅	Cl	Cl	H	H	NHC(=S)NHCH ₃
125	C ₂ H ₅	F	F	F	H	NHC(=S)NHCH ₃
126	C ₂ H ₅	Br	H	H	H	NHC(=S)NHCH ₃
127 (B2a)	CH ₃	H	H	H	H	NCS
128 (B2b)	CH ₃	F	H	H	H	NCS
129	CH ₃	F	F	H	H	NCS
130	CH ₃	Cl	H	H	H	NCS
131	CH ₃	Cl	Cl	H	H	NCS
132	CH ₃	F	F	F	H	NCS
133	CH ₃	Br	H	H	H	NCS
134	CH ₃	H	H	H	H	NHC(=O)NHC(=O)CH ₃
135	CH ₃	F	H	H	H	NHC(=O)NHC(=O)CH ₃
136	CH ₃	F	F	H	H	NHC(=O)NHC(=O)CH ₃
137	CH ₃	Cl	H	H	H	NHC(=O)NHC(=O)CH ₃
138	CH ₃	Cl	Cl	H	H	NHC(=O)NHC(=O)CH ₃
139	CH ₃	F	F	F	H	NHC(=O)NHC(=O)CH ₃
140	CH ₃	Br	H	H	H	NHC(=O)NHC(=O)CH ₃
141	CH ₃	H	H	H	H	NHC(=S)NHC(=O)CH ₃
142	CH ₃	F	H	H	H	NHC(=S)NHC(=O)CH ₃
143	CH ₃	F	F	H	H	NHC(=S)NHC(=O)CH ₃
144	CH ₃	Cl	H	H	H	NHC(=S)NHC(=O)CH ₃

145	CH ₃	Cl	Cl	H	H	NHC(=S)NHC(=O)CH ₃
146	CH ₃	F	F	F	H	NHC(=S)NHC(=O)CH ₃
147	CH ₃	Br	H	H	H	NHC(=S)NHC(=O)CH ₃
148 (A1a)	CH ₃	H	H	H	H	I
149 (A1b)	CH ₃	F	H	H	H	I
150	CH ₃	F	F	H	H	I
151	CH ₃	Cl	H	H	H	I
152	CH ₃	Cl	Cl	H	H	I
153	CH ₃	F	F	F	H	I
154	CH ₃	Br	H	H	H	I
155 (B1a)	CH ₃	H	H	H	H	1,2,3-triazol-1-yl
156 (B1b)	CH ₃	F	H	H	H	1,2,3-triazol-1-yl
157	CH ₃	F	F	H	H	1,2,3-triazol-1-yl
158	CH ₃	Cl	H	H	H	1,2,3-triazol-1-yl
159	CH ₃	Cl	Cl	H	H	1,2,3-triazol-1-yl
160	CH ₃	F	F	F	H	1,2,3-triazol-1-yl
161	CH ₃	Br	H	H	H	1,2,3-triazol-1-yl

7. The compound for use according to any one of claims from 1 to 6 in the form of pure S enantiomer or mixture enriched with the S enantiomer.

8. A pharmaceutical composition containing a compound having the general formula (I):



Formula (I)

as racemic mixtures or pure enantiomers or mixtures enriched with one of the S or R enantiomer,

where:

R= F, Cl, Br, I, C1-C3 alkyl, C3-C6 cyclo-alkyl, aryl, heteroaryl, NH₂, OH, SH, NHR₆, N(R₆)₂, or OR₆, with R₆= C1-C3 alkyl, C3-C6 cyclo-alkyl, aryl, heteroaryl, or C1-C4 acyl;

R₁₋₄= independently H, F, Cl, Br, CH₃, OH, or OCH₃;

R₅= -NH₂, -OH, -NCS, -NHC(X)CH₃ with X= O or S; -NHC(X)CH₂Z with X= O or S and Z= F or Cl; -NHC(X)CHZ₂ with X= O or S and Z= F or Cl; -NHC(X)CZ₃ with X= O or S and Z= F or Cl; -NHC(X)NHR₇ with X= O or S, and R₇= H, C1-C3 alkyl, C3-C6-cyclo-alkyl, aryl, heteroaryl, or C1-C3-acyl

and at least one pharmaceutically acceptable excipient,

for the use in the treatment of infections caused by Gram-positive bacteria.

9. The pharmaceutical composition for use according to claim 8 wherein R is a methyl, ethyl or phenyl group.

10. The pharmaceutical composition for use according to claim 8 or 9 wherein at least one of the substituents R₁, R₂, R₃ or R₄ is a fluorine atom, while the others are H.

11. The pharmaceutical composition for use according to any one of claims from 8 to 10 wherein R₅ is selected from: NHC(=O)CH₃, NHC(=S)CH₃, -NHC(=O)CH₂F, -NHC(=S)CH₂F, -NHC(=O)CH₂Cl, -NHC(=S)CH₂Cl, -NHC(=S)NH₂, NHC(=O)NH₂, -NHC(=O)NHCH₃, -NHC(=S)NHCH₃, -NHC(=O)NHC₂H₅, -NHC(=S)NHC₂H₅, -NCS; and 1,2,3 triazol-1-yl.

12. The pharmaceutical composition for use according to any one of claims from 8 to 11 containing a compound wherein:

R₅ is NHC(=S)CH₃ and R is CH₃;

R₅ is NHC(=S)NHCH₃ and R is CH₃;

R₅ is NHC(=O)CH₃ and R is CH₃; and

R₅ is NHC(=S)NH₂ and R is CH₃.

13. The pharmaceutical composition for use according to any one of claims from 8 to 12 wherein the compound is selected from compounds:

	R	R1	R2	R3	R4	R5
1	Ph	H	H	H	H	NHC(=O)CH ₃
2	Ph	F	H	H	H	NHC(=O)CH ₃
3	Ph	F	F	H	H	NHC(=O)CH ₃
4	Ph	F	F	F	H	NHC(=O)CH ₃
5	Ph	F	F	F	H	NHC(=O)CH ₃
6	Ph	Cl	H	H	H	NHC(=O)CH ₃
7	Ph	Cl	Cl	H	H	NHC(=O)CH ₃
8	Ph	H	H	H	H	NHC(=S)CH ₃
9	Ph	F	H	H	H	NHC(=S)CH ₃
10	Ph	F	F	H	H	NHC(=S)CH ₃
11	Ph	Cl	H	H	H	NHC(=S)CH ₃
12	Ph	Cl	Cl	H	H	NHC(=S)CH ₃
13	Ph	F	F	F	H	NHC(=S)CH ₃
14	Ph	Br	H	H	H	NHC(=S)CH ₃
15 (A3a)	CH ₃	H	H	H	H	NHC(=O)CH ₃
16 (A3b)	CH ₃	F	H	H	H	NHC(=O)CH ₃
17	CH ₃	F	F	H	H	NHC(=O)CH ₃
18	CH ₃	F	F	F	H	NHC(=O)CH ₃
19	CH ₃	Cl	H	H	H	NHC(=O)CH ₃

20	CH ₃	Cl	Cl	H	H	NHC(=O)CH ₃
21	CH ₃	Br	H	H	H	NHC(=O)CH ₃
22 (A4a)	CH ₃	H	H	H	H	NHC(=S)CH ₃
23 (A4b)	CH ₃	F	H	H	H	NHC(=S)CH ₃
24	CH ₃	F	F	H	H	NHC(=S)CH ₃
25	CH ₃	Cl	H	H	H	NHC(=S)CH ₃
26	CH ₃	Cl	Cl	H	H	NHC(=S)CH ₃
27	CH ₃	F	F	F	H	NHC(=S)CH ₃
28	CH ₃	Br	H	H	H	NHC(=S)CH ₃
29	C ₂ H ₅	H	H	H	H	NHC(=O)CH ₃
30	C ₂ H ₅	F	H	H	H	NHC(=O)CH ₃
31	C ₂ H ₅	F	F	H	H	NHC(=O)CH ₃
32	C ₂ H ₅	F	F	F	H	NHC(=O)CH ₃
33	C ₂ H ₅	Cl	H	H	H	NHC(=O)CH ₃
34	C ₂ H ₅	Cl	Cl	H	H	NHC(=O)CH ₃
35	C ₂ H ₅	Br	H	H	H	NHC(=O)CH ₃
36	C ₂ H ₅	H	H	H	H	NHC(=S)CH ₃
37	C ₂ H ₅	F	H	H	H	NHC(=S)CH ₃
38	C ₂ H ₅	F	F	H	H	NHC(=S)CH ₃
39	C ₂ H ₅	Cl	H	H	H	NHC(=S)CH ₃
40	C ₂ H ₅	Cl	Cl	H	H	NHC(=S)CH ₃
41	C ₂ H ₅	F	F	F	H	NHC(=S)CH ₃
42	C ₂ H ₅	Br	H	H	H	NHC(=S)CH ₃
43	Ph	H	H	H	H	NHC(=O)NH ₂
44	Ph	F	H	H	H	NHC(=O)NH ₂

45	Ph	F	F	H	H	NHC(=O)NH ₂
46	Ph	F	F	F	H	NHC(=O)NH ₂
47	Ph	Br	H	H	H	NHC(=O)NH ₂
48	Ph	Cl	H	H	H	NHC(=O)NH ₂
49	Ph	Cl	Cl	H	H	NHC(=O)NH ₂
50	Ph	H	H	H	H	NHC(=S)NH ₂
51	Ph	F	H	H	H	NHC(=S)NH ₂
52	Ph	F	F	H	H	NHC(=S)NH ₂
53	Ph	Cl	H	H	H	NHC(=S)NH ₂
54	Ph	Cl	Cl	H	H	NHC(=S)NH ₂
55	Ph	F	F	F	H	NHC(=S)NH ₂
56	Ph	Br	H	H	H	NHC(=S)NH ₂
57	CH ₃	H	H	H	H	NHC(=O)NH ₂
58	CH ₃	F	H	H	H	NHC(=O)NH ₂
59	CH ₃	F	F	H	H	NHC(=O)NH ₂
60	CH ₃	F	F	F	H	NHC(=O)NH ₂
61	CH ₃	Cl	H	H	H	NHC(=O)NH ₂
62	CH ₃	Cl	Cl	H	H	NHC(=O)NH ₂
63	CH ₃	Br	H	H	H	NHC(=O)NH ₂
64	CH ₃	H	H	H	H	NHC(=S)NH ₂
(B3a)						
65	CH ₃	F	H	H	H	NHC(=S)NH ₂
(B3b)						
66	CH ₃	F	F	H	H	NHC(=S)NH ₂
67	CH ₃	Cl	H	H	H	NHC(=S)NH ₂
68	CH ₃	Cl	Cl	H	H	NHC(=S)NH ₂
69	CH ₃	F	F	F	H	NHC(=S)NH ₂

70	CH ₃	Br	H	H	H	NHC(=S)NH ₂
71	C ₂ H ₅	H	H	H	H	NHC(=O)NH ₂
72	C ₂ H ₅	F	H	H	H	NHC(=O)NH ₂
73	C ₂ H ₅	F	F	H	H	NHC(=O)NH ₂
74	C ₂ H ₅	F	F	F	H	NHC(=O)NH ₂
75	C ₂ H ₅	Cl	H	H	H	NHC(=O)NH ₂
76	C ₂ H ₅	Cl	Cl	H	H	NHC(=O)NH ₂
77	C ₂ H ₅	Br	H	H	H	NHC(=O)NH ₂
78	C ₂ H ₅	H	H	H	H	NHC(=S)NH ₂
79	C ₂ H ₅	F	H	H	H	NHC(=S)NH ₂
80	C ₂ H ₅	F	F	H	H	NHC(=S)NH ₂
81	C ₂ H ₅	Cl	H	H	H	NHC(=S)NH ₂
82	C ₂ H ₅	Cl	Cl	H	H	NHC(=S)NH ₂
83	C ₂ H ₅	F	F	F	H	NHC(=S)NH ₂
84	C ₂ H ₅	Br	H	H	H	NHC(=S)NH ₂
85	Ph	H	H	H	H	NHC(=O)NHCH ₃
86	Ph	F	H	H	H	NHC(=O)NHCH ₃
87	Ph	F	F	H	H	NHC(=O)NHCH ₃
88	Ph	F	F	F	H	NHC(=O)NHCH ₃
89	Ph	Br	H	H	H	NHC(=O)NHCH ₃
90	Ph	Cl	H	H	H	NHC(=O)NHCH ₃
91	Ph	Cl	Cl	H	H	NHC(=O)NHCH ₃
92	Ph	H	H	H	H	NHC(=S)NHCH ₃
93	Ph	F	H	H	H	NHC(=S)NHCH ₃
94	Ph	F	F	H	H	NHC(=S)NHCH ₃
95	Ph	Cl	H	H	H	NHC(=S)NHCH ₃
96	Ph	Cl	Cl	H	H	NHC(=S)NHCH ₃

97	Ph	F	F	F	H	NHC(=S)NHCH ₃
98	Ph	Br	H	H	H	NHC(=S)NHCH ₃
99	CH ₃	H	H	H	H	NHC(=O)NHCH ₃
100	CH ₃	F	H	H	H	NHC(=O)NHCH ₃
101	CH ₃	F	F	H	H	NHC(=O)NHCH ₃
102	CH ₃	F	F	F	H	NHC(=O)NHCH ₃
103	CH ₃	Cl	H	H	H	NHC(=O)NHCH ₃
104	CH ₃	Cl	Cl	H	H	NHC(=O)NHCH ₃
105	CH ₃	Br	H	H	H	NHC(=O)NHCH ₃
106 (B4a)	CH ₃	H	H	H	H	NHC(=S)NHCH ₃
107 (B4b)	CH ₃	F	H	H	H	NHC(=S)NHCH ₃
108	CH ₃	F	F	H	H	NHC(=S)NHCH ₃
109	CH ₃	Cl	H	H	H	NHC(=S)NHCH ₃
110	CH ₃	Cl	Cl	H	H	NHC(=S)NHCH ₃
111	CH ₃	F	F	F	H	NHC(=S)NHCH ₃
112	CH ₃	Br	H	H	H	NHC(=S)NHCH ₃
113	C ₂ H ₅	H	H	H	H	NHC(=O)NHCH ₃
114	C ₂ H ₅	F	H	H	H	NHC(=O)NHCH ₃
115	C ₂ H ₅	F	F	H	H	NHC(=O)NHCH ₃
116	C ₂ H ₅	F	F	F	H	NHC(=O)NHCH ₃
117	C ₂ H ₅	Cl	H	H	H	NHC(=O)NHCH ₃
118	C ₂ H ₅	Cl	Cl	H	H	NHC(=O)NHCH ₃
119	C ₂ H ₅	Br	H	H	H	NHC(=O)NHCH ₃
120	C ₂ H ₅	H	H	H	H	NHC(=S)NHCH ₃
121	C ₂ H ₅	F	H	H	H	NHC(=S)NHCH ₃

122	C ₂ H ₅	F	F	H	H	NHC(=S)NHCH ₃
123	C ₂ H ₅	Cl	H	H	H	NHC(=S)NHCH ₃
124	C ₂ H ₅	Cl	Cl	H	H	NHC(=S)NHCH ₃
125	C ₂ H ₅	F	F	F	H	NHC(=S)NHCH ₃
126	C ₂ H ₅	Br	H	H	H	NHC(=S)NHCH ₃
127 (B2a)	CH ₃	H	H	H	H	NCS
128 (B2b)	CH ₃	F	H	H	H	NCS
129	CH ₃	F	F	H	H	NCS
130	CH ₃	Cl	H	H	H	NCS
131	CH ₃	Cl	Cl	H	H	NCS
132	CH ₃	F	F	F	H	NCS
133	CH ₃	Br	H	H	H	NCS
134	CH ₃	H	H	H	H	NHC(=O)NHC(=O)CH ₃
135	CH ₃	F	H	H	H	NHC(=O)NHC(=O)CH ₃
136	CH ₃	F	F	H	H	NHC(=O)NHC(=O)CH ₃
137	CH ₃	Cl	H	H	H	NHC(=O)NHC(=O)CH ₃
138	CH ₃	Cl	Cl	H	H	NHC(=O)NHC(=O)CH ₃
139	CH ₃	F	F	F	H	NHC(=O)NHC(=O)CH ₃
140	CH ₃	Br	H	H	H	NHC(=O)NHC(=O)CH ₃
141	CH ₃	H	H	H	H	NHC(=S)NHC(=O)CH ₃
142	CH ₃	F	H	H	H	NHC(=S)NHC(=O)CH ₃
143	CH ₃	F	F	H	H	NHC(=S)NHC(=O)CH ₃
144	CH ₃	Cl	H	H	H	NHC(=S)NHC(=O)CH ₃
145	CH ₃	Cl	Cl	H	H	NHC(=S)NHC(=O)CH ₃
146	CH ₃	F	F	F	H	NHC(=S)NHC(=O)CH ₃

147	CH ₃	Br	H	H	H	NHC(=S)NHC(=O)CH ₃
148 (A1a)	CH ₃	H	H	H	H	I
149 (A1b)	CH ₃	F	H	H	H	I
150	CH ₃	F	F	H	H	I
151	CH ₃	Cl	H	H	H	I
152	CH ₃	Cl	Cl	H	H	I
153	CH ₃	F	F	F	H	I
154	CH ₃	Br	H	H	H	I
155 (B1a)	CH ₃	H	H	H	H	1,2,3-triazol-1-yl
156 (B1b)	CH ₃	F	H	H	H	1,2,3-triazol-1-yl
157	CH ₃	F	F	H	H	1,2,3-triazol-1-yl
158	CH ₃	Cl	H	H	H	1,2,3-triazol-1-yl
159	CH ₃	Cl	Cl	H	H	1,2,3-triazol-1-yl
160	CH ₃	F	F	F	H	1,2,3-triazol-1-yl
161	CH ₃	Br	H	H	H	1,2,3-triazol-1-yl

14. The pharmaceutical composition for use according to claim 13 wherein compounds 1-126 and 134-147 are in the form of S-enantiomer or a mixture enriched in S-enantiomer.

15. The pharmaceutical composition for use according to claim 13 wherein compounds 127-133 and 148-161 are in the form of R-enantiomer or a mixture enriched in R-enantiomer.

16. The pharmaceutical composition for use according to any one of claims from 8 to 15 for oral use in the form of tablet, capsule, syrup, or solution, for parenteral use in the form of aqueous or oily solution or emulsion, for topical use in the form of ointment, cream, gel, solution, O/W or W/O emulsion, suspension emulsion, or suspension or by inhalation in the form of solution, emulsion or dispersion.

17. The pharmaceutical composition according to claim 16 wherein the suspension comprises nanoparticles, nanocapsule and/or liposomes.

18. The pharmaceutical composition according to claim 17, wherein nanoparticles are solid lipid nanoparticles, compatible with the administration through the nebulizer.

19. A pharmaceutical composition for use in the treatment of infections by Gram-positive bacteria being multi antibiotic-resistant bacteria, the use comprising administration to a patient of a pharmaceutically active amount of a composition as defined in any one of claims from 8 to 18.

20. The pharmaceutical composition for use according to claim 19 in the treatment of infections caused by *Staphylococcus* spp, *Enterococcus* spp, *Streptococcus* spp, being resistant to antibiotics.

21. Use of a pharmaceutically active amount of a compound as defined in any one of claims 1 to 7 or a composition as defined in any one of claims 8 to 18 for the treatment of infections by Gram-positive bacteria being multi antibiotic-resistant bacteria.

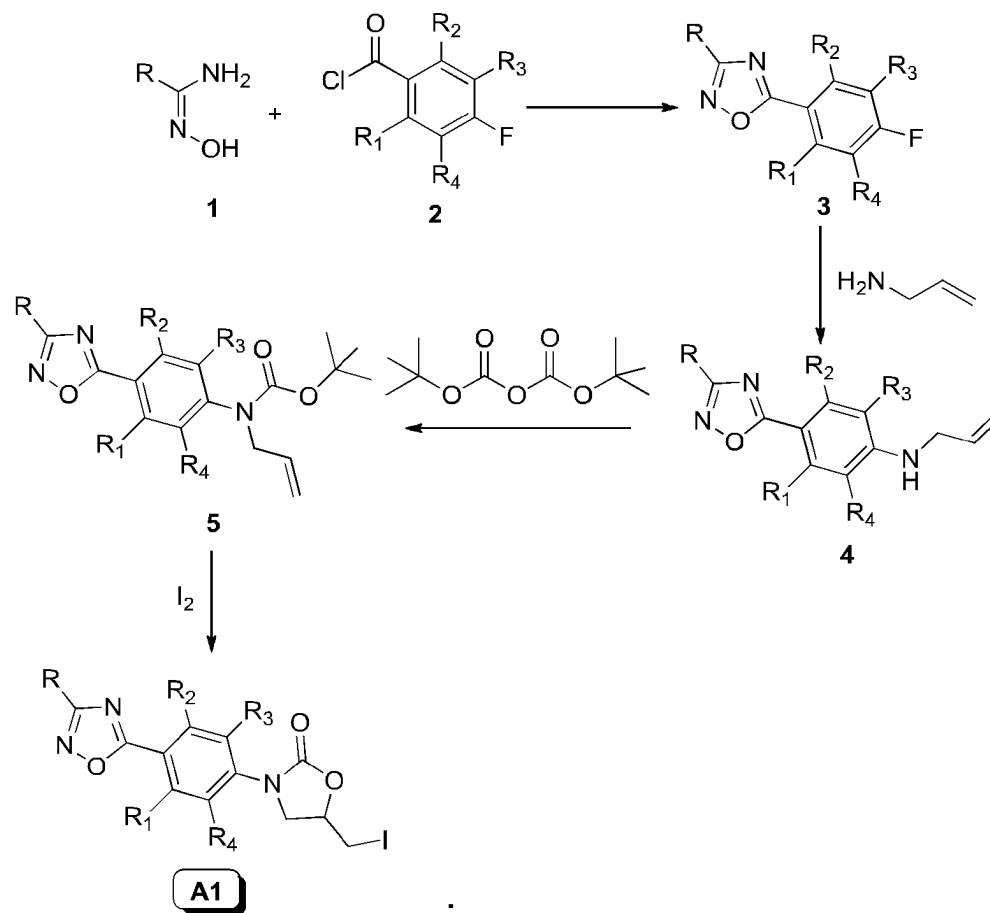
22. The use according to claim 21 for the treatment of infections caused by *Staphylococcus* spp, *Enterococcus* spp, *Streptococcus* spp, being resistant to antibiotics.

23. Use of a compound as defined in any one of claims 1 to 7 or a composition as defined in any one of claims 8 to 18 in the manufacture of a medicament for the treatment of infections by Gram-positive bacteria being multi antibiotic-resistant bacteria.

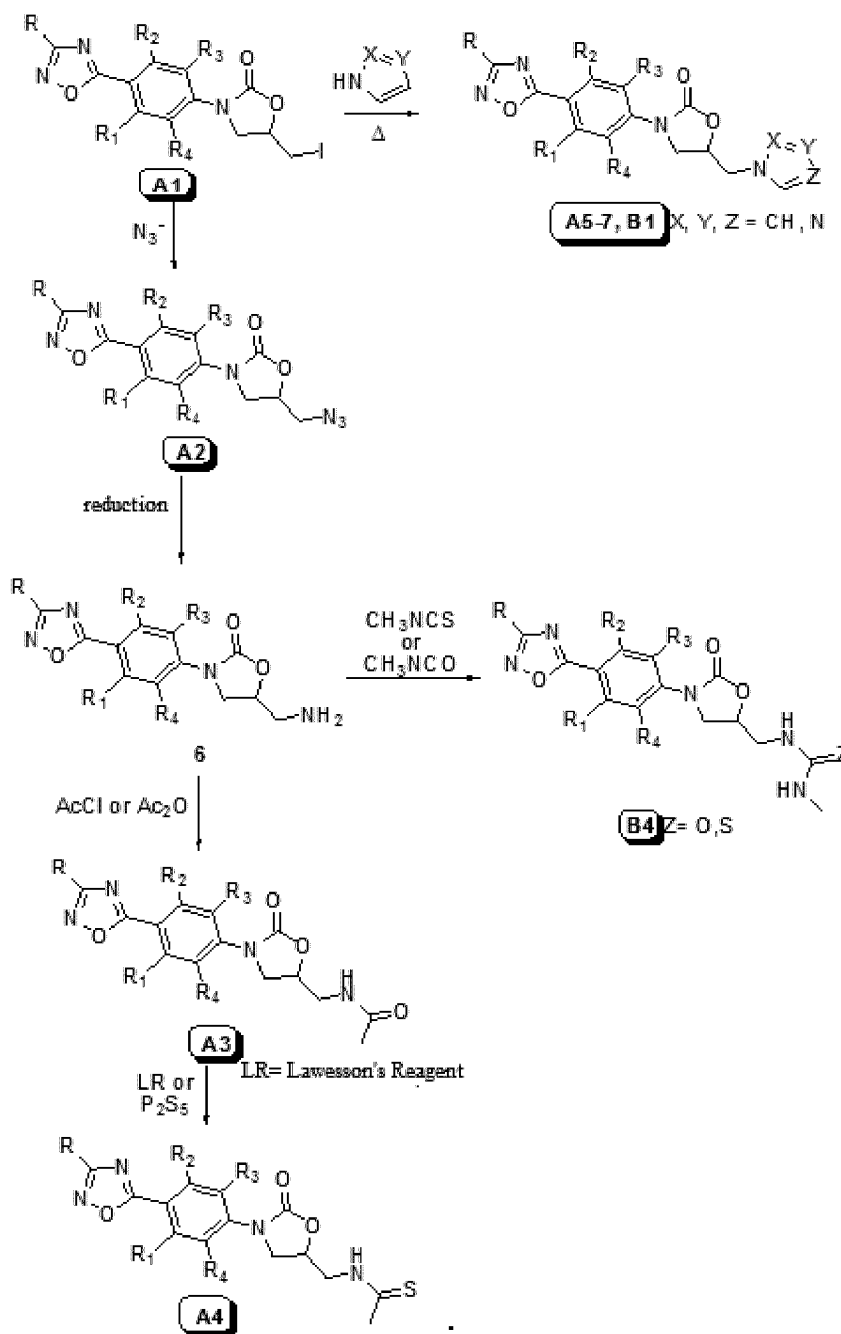
24. The use according to claim 23 in the manufacture of a medicament for the treatment of infections caused by *Staphylococcus* spp, *Enterococcus* spp, *Streptococcus* spp,

being resistant to antibiotics.

25. A process for the preparation of the compounds as defined in any one of claims from 1 to 7 comprising the steps shown in Scheme 1:

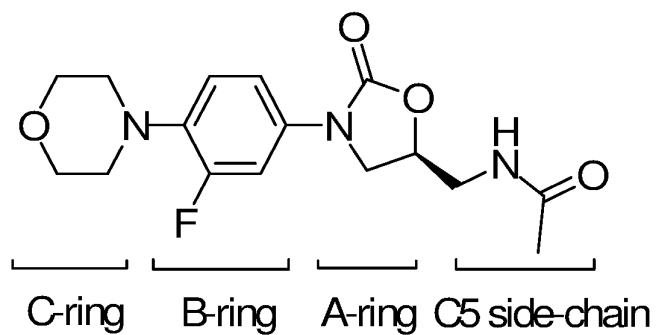


26. The process according to claim 25 comprising the steps shown in Scheme 2:



27. The process according to any one of claims 25 or 26 comprising a step of separation of the enantiomers S and R or enrichment of the racemic mixture in one of the enantiomers.

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Linezolid

Figure 1

PK15

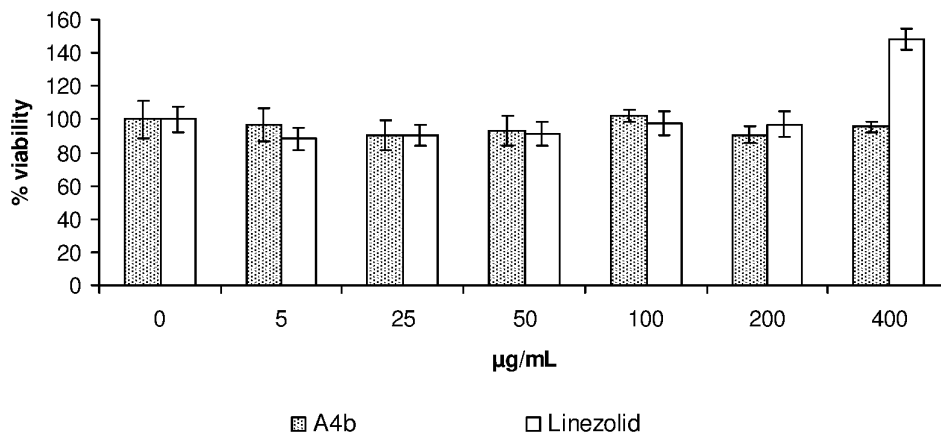


Figure 2

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HaCat

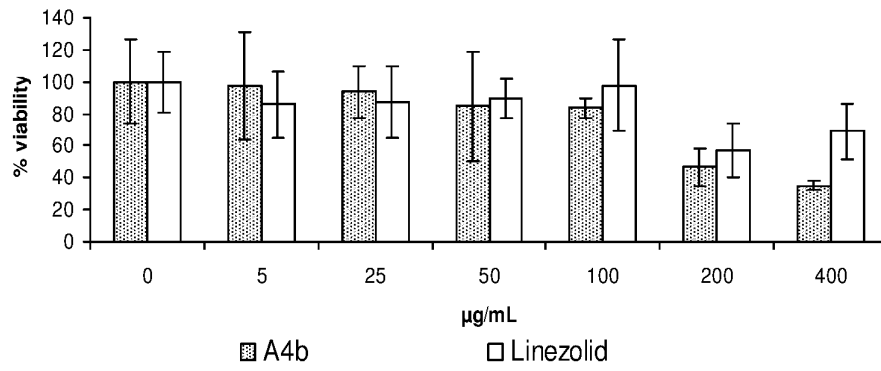


Figure 3

HepG2

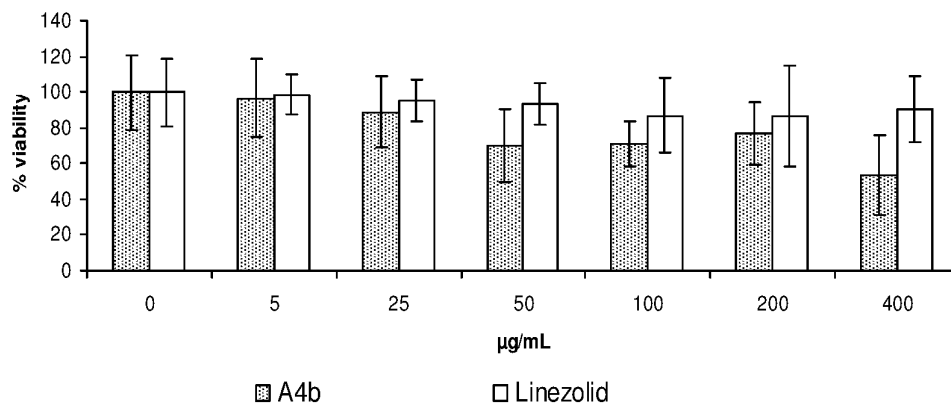


Figure 4

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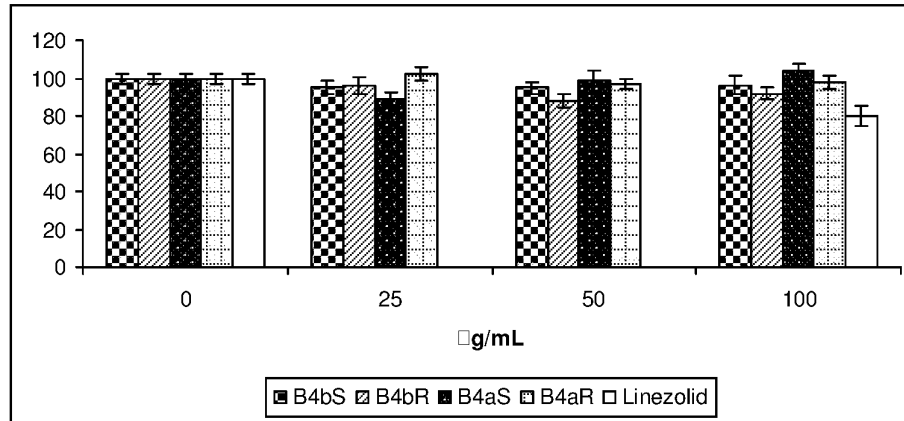


Figure 5

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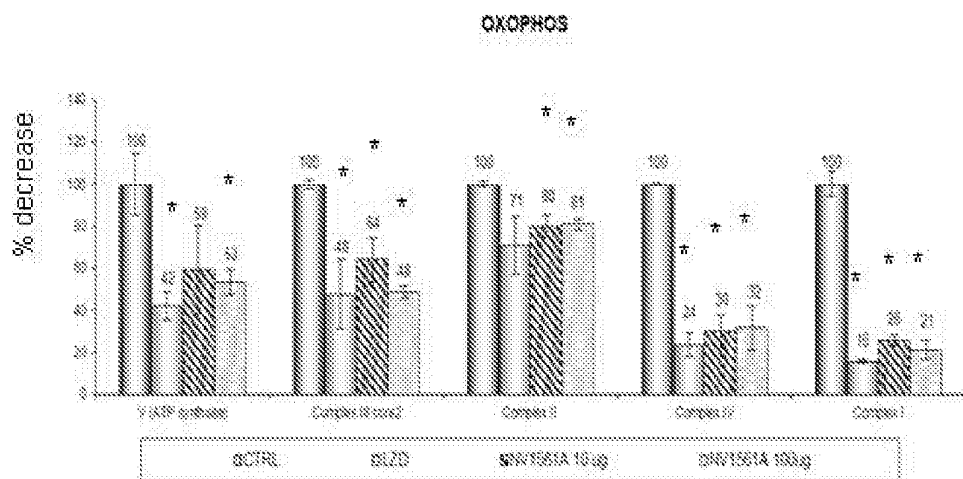
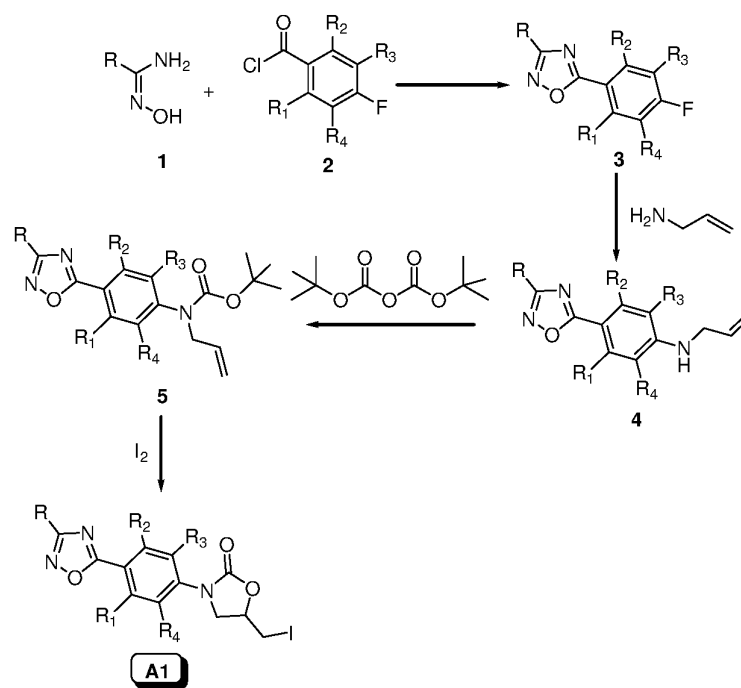


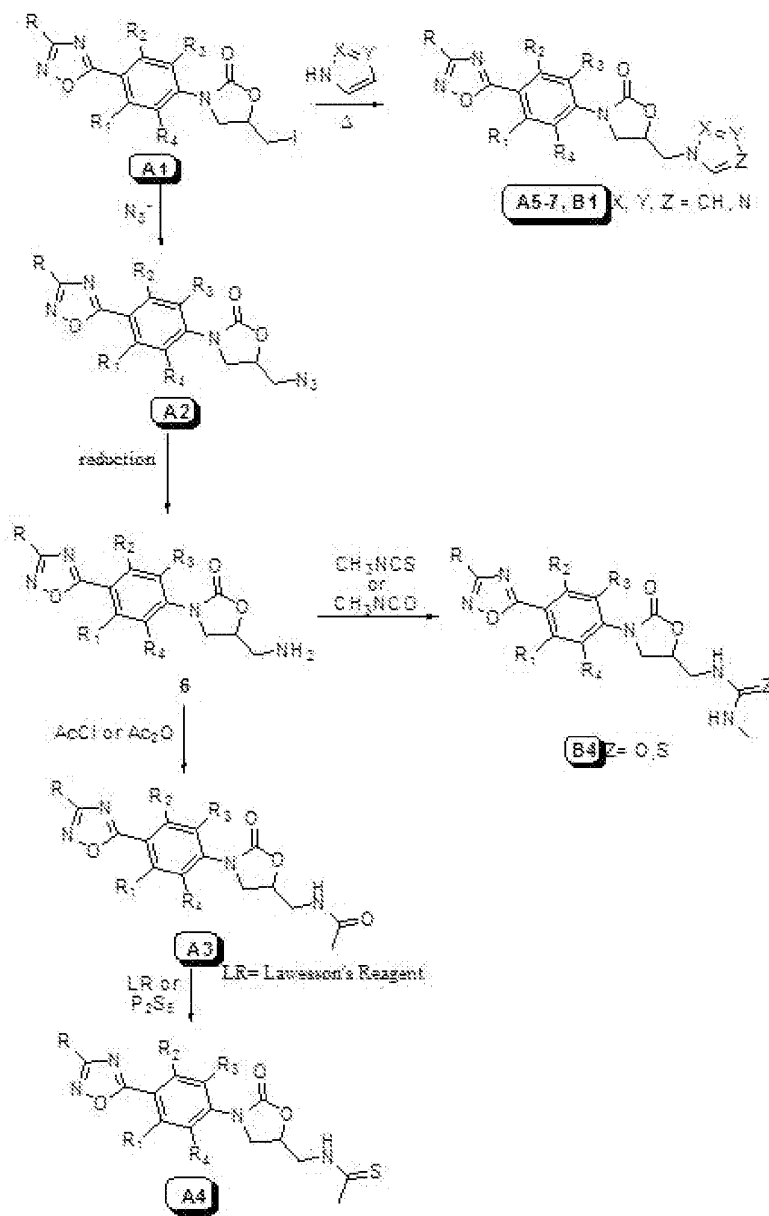
Figure 6



SCHEME 1

Figure 7

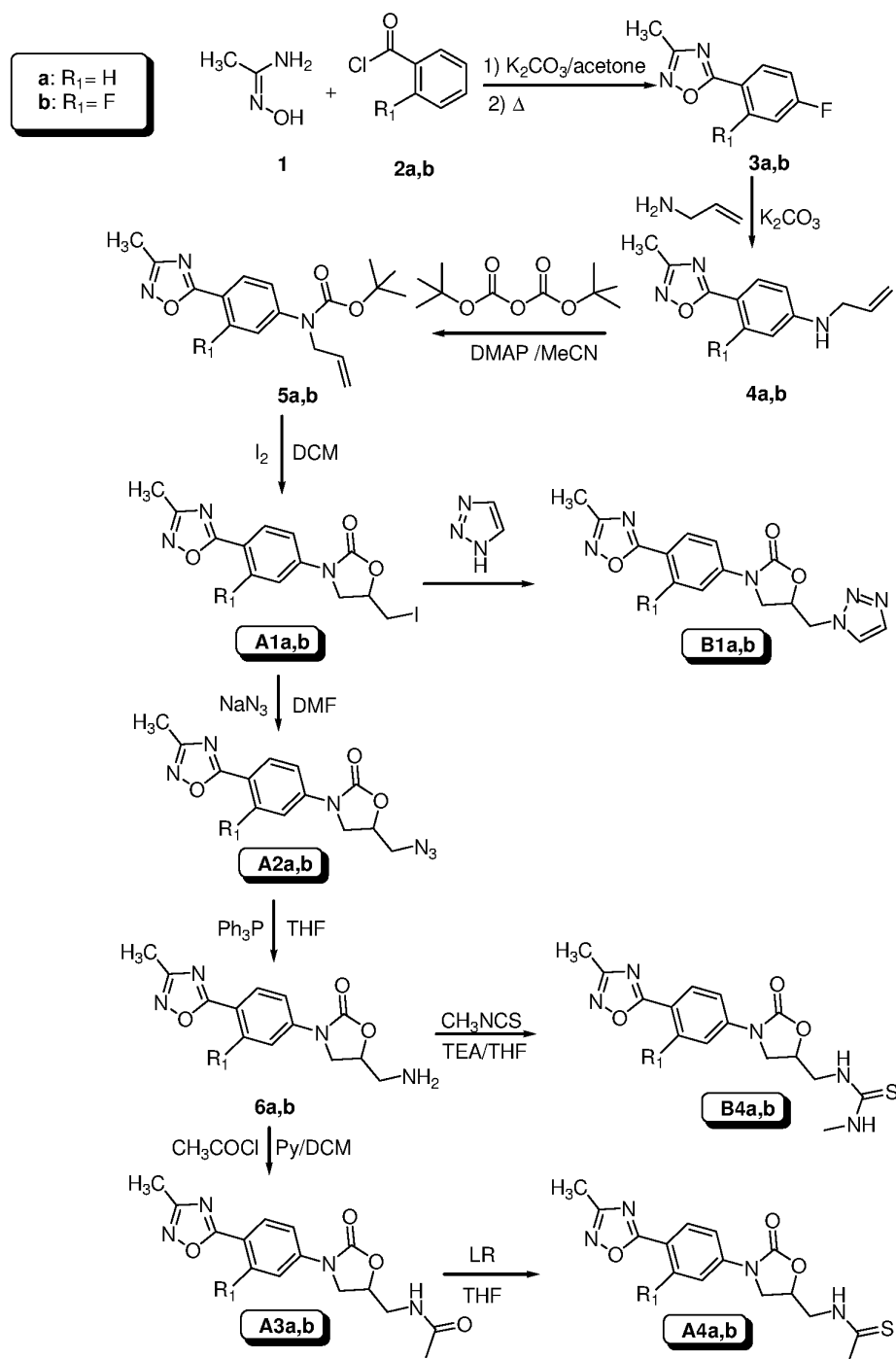
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SCHEME 2

Figure 8

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SCHEME 3

Figure 9