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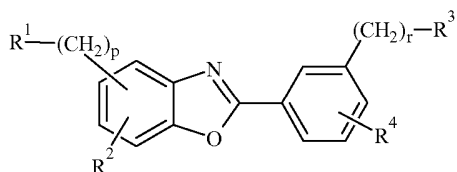
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(VI)

(57) Abstract: The application concerns compounds for use as antibacterial agents, processes for their preparation, and compositions comprising said agents, wherein said compounds have formula (VI) and r, p, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> have defined meanings.

## ANTIBACTERIAL AGENTS

### FIELD OF THE INVENTION

The present application concerns antibacterial agents, processes for the preparation of the disclosed agents, compositions comprising said agents, and uses of said agents including  
5 *inter alia* therapeutic uses.

### BACKGROUND OF THE INVENTION

The therapeutic significance of antibiotics in the prevention and treatment of infectious diseases is widely recognised. Moreover, emergence of bacterial resistance towards existing antibiotics is of great concern and necessitates active discovery and development of further  
10 substances useful as antibacterial agents.

Useful targets of antibacterial therapy include constituents involved in the synthesis of bacterial cell wall, *inter alia* because said synthesis process and its products are fairly specific to the bacterial world. An important player in bacterial cell wall synthesis is a family of enzymes known as DD-ligases. DD-ligases contribute to the synthesis of peptidoglycan, a  
15 major component of the bacterial cell wall that is required for bacterial survival. In particular, DD-ligases catalyse the synthesis of a dimer of D-amino acids in an early step of peptidoglycan synthesis.

In most bacteria, DD-ligases catalyse the formation of a dimer of D-alanine (*i.e.*, D-alanine-D-alanine ligases). However, some DD-ligases can use D-serine or D-lactate instead of one of  
20 said two D-alanines to form D-alanine-D-serine dipeptide or D-alanine-D-lactate depsipeptide, respectively. For example, DD-ligases with so-altered specificity can be found in vancomycin-resistant *Enterococci* (VRE), vancomycin-resistant *Staphylococcus aureus*, or in some species of lactic bacteria, *etc.* Vancomycin is an antibiotic commonly used as a last resort treatment for infections caused by multiresistant *Enterococci* or *S. aureus*. Because vancomycin acts by  
25 binding to the D-Ala-D-Ala motif, bacteria containing DD-ligases capable of employing other substrates such as D-serine or D-lactate can display resistance to vancomycin.

Only a handful of inhibitors of DD-ligase have been previously described: D-cycloserine, a competitive inhibitor of DD-ligases and of alanine racemase has been used as an antibiotic in the early 1950's and 1960's, but has been largely abandoned due to toxic effects on the  
30 central nervous system. Moreover, the antibiotic activity of D-cycloserine appears to be

mainly mediated by inhibition of alanine racemase which converts L-Ala to D-Ala. Further, methylphosphinate inhibitors display high affinity for DD-ligases, but their use as antibiotics is hampered because their high polarity prevents them from crossing the bacterial membrane and reaching their target. More recently described DD-ligase inhibitors include 2-(2-Amino-2-carboxy-ethyl)-1-phenyl-cyclopropanecarboxylic acid, 3-chloro-2,2-dimethyl-N-[4(trifluoromethyl)phenyl]propanamide, and diazenedicarboxamides.

Consequently, there exists a pressing need in the art to develop substances applicable as further and/or improved inhibitors of DD-ligases suitable as antibacterial agents.

Further related prior art: WO 03/074516 discloses 2-(3-nitrophenyl)-1,3-benzoxazole-6-carboxylic acid and 2-(3-aminophenyl)-1,3-benzoxazole-6-carboxylic acid as intermediates in a synthesis process, but does not attribute any function to said molecules. 3-(1,3-benzoxazol-2-yl)aniline has been described as antihelmintic but not as an antibacterial agent.

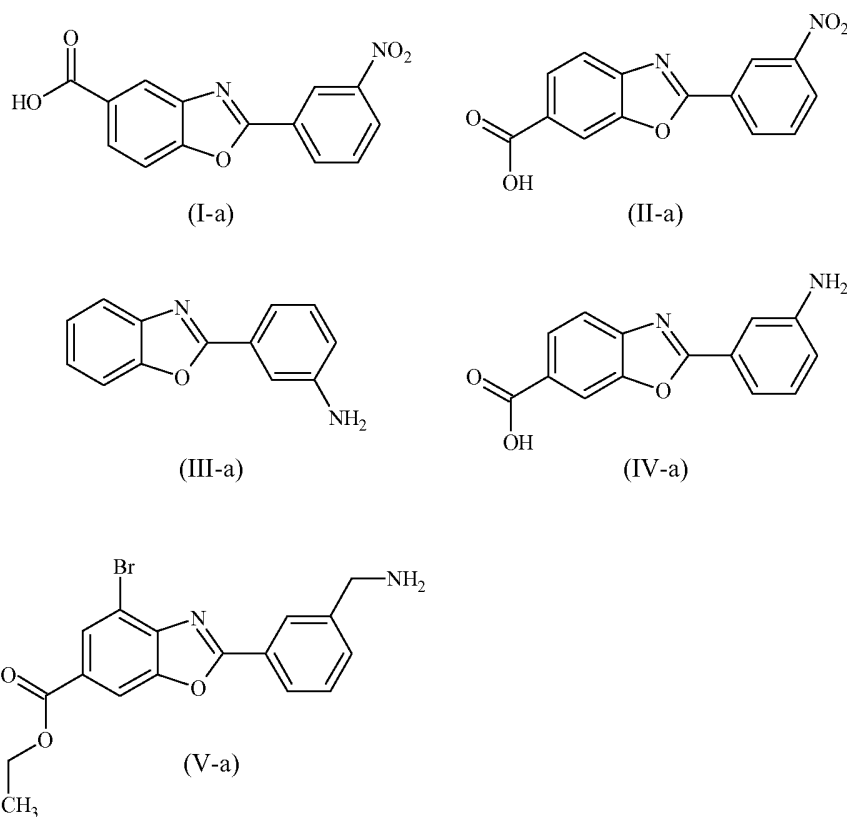
## SUMMARY OF THE INVENTION

The invention addresses the above discussed needs in the art. More specifically, the invention teaches further compounds useful as inhibitors of DD-ligases and as antibacterial agents.

The present substances may also entail one or more advantages over previously known DD-ligase inhibitors and/or over existing antibiotics. For example, the compounds can show improved penetration of bacterial cell membrane, for example as compared to methylphosphinate inhibitors, whereby they can more powerfully act on intact, living bacteria. Moreover, the present molecules are structurally distinct from D-cycloserine and can therefore avoid undesired side effects such as CNS toxicity. Also, the molecules have been designed to act selectively on DD-ligases – since D-amino acids are not naturally encountered in eukaryotic cells, this ensures high degree of specificity of the present compounds to bacteria, thereby further reducing the possibility of unwanted side effects. In addition, because DD-ligases represent a so far largely unexploited target, it is expected that bacterial resistance to the present compounds in clinics would be minimal. Also, given that the chemical structure and mode of action of the present molecules is considerably different from other antibiotic classes in common use, said molecules would not display cross-resistance with other existing antibiotics, and therefore could target bacteria that have acquired resistance to such existing antibiotics. For example, because the present molecules

target DD-ligase and not the D-Ala-D-Ala dimer, they can be effective against both vancomycin-sensitive and vancomycin-resistant bacteria. Also, since DD-ligases are essential enzymes for all bacteria relying on the existence of a cell wall – which encompass most known pathogenic bacteria – the present compounds are expected to show broad spectrum of activity.

Accordingly, in an aspect the invention provides the use of a compound of any of formulas (I-a), (II-a), (III-a), (IV-a) or (V-a):



or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug or solvate thereof, for the manufacture of a medicament for the treatment of a bacterial infection.

In a related aspect, the invention provides a compound of any of formulas (I-a), (II-a), (III-a), (IV-a) or (V-a) as shown above, or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug or solvate thereof, for use in the treatment of a bacterial infection. Also provided is a pharmaceutical composition comprising a compound of any of formulas (I-a), (II-a), (III-a), (IV-a) or (V-a) as shown above, or a pharmaceutically acceptable *N*-oxide form, addition salt,

prodrug or solvate thereof, and one or more pharmaceutically acceptable carriers, for use in the treatment of a bacterial infection.

Another related aspect provides a method for treating a bacterial infection in a subject in need of such treatment, comprising administering to said subject a therapeutically or prophylactically effective amount of a compound of any of formulas (I-a), (II-a), (III-a), (IV-a) or (V-a) as shown above, or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug or solvate thereof.

Also, the invention provides a compound of any of formulas (I-a), (II-a), (IV-a) or (V-a) as shown above, or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug or solvate thereof, for use as a medicament. Accordingly, the invention also provides a pharmaceutical composition comprising a compound of any of formulas (I-a), (II-a), (IV-a) or (V-a) as shown above, or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug or solvate thereof, and one or more pharmaceutically acceptable carriers. The invention also provides a compound of any of formulas (I-a) or (V-a) as shown above, an *N*-oxide form, addition salt, prodrug or solvate thereof.

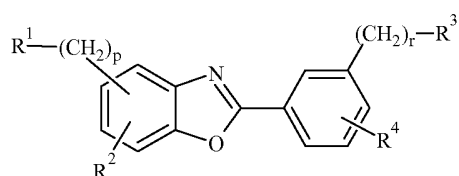
Also provided is a method for inhibition of DD-ligase comprising contacting a DD-ligase, or a composition or a bacterium comprising such, with a compound of any of formulas (I-a), (II-a), (III-a), (IV-a) or (V-a) as shown above, or an *N*-oxide form, addition salt, prodrug or solvate thereof, in an amount sufficient to obtain said inhibition. In an embodiment, said DD-ligase inhibition may occur *in vivo*, *i.e.*, within the body of a subject in particular of a human or animal subject. In other embodiments, said inhibition of DD-ligase may take place *in vitro*, more particularly encompassing inhibition of isolated DD-ligase, inhibition of isolated DD-ligase when being a part of a composition, as well as inhibition of DD-ligase in bacteria when said bacteria are outside of a human or animal body. The term "isolated" when referring to a protein, means that said protein has been identified and separated and/or recovered from a component of its natural environment. For instance, an isolated protein can be substantially separated from cellular material or other proteins of a cell source from which it is derived. For example, an isolated protein may be isolated from its natural source or produced recombinantly.

Further aspect concerns a method for inhibiting the growth, proliferation and/or survival of bacteria comprising contacting said bacteria with a compound of any of formulas (I-a), (II-a),

(III-a), (IV-a) or (V-a) as shown above, or an *N*-oxide form, addition salt, prodrug or solvate thereof, in an amount sufficient to obtain said inhibition.

In an embodiment, said inhibition of bacterial growth, proliferation and/or survival may occur *in vivo*, *i.e.*, within the body of a subject in particular of a human or animal subject. In other  
 5 embodiments, said inhibition of bacterial growth, proliferation and/or survival may take place *ex vivo*, which refers to treatments of bacteria outside of a human or animal body. A non-limiting example of such *ex vivo* inhibition may pertain to a method for preserving, disinfecting or sterilising a composition such as for example a liquid solution, device or apparatus contaminated or suspected to be contaminated with bacteria, comprising contacting said  
 10 composition, device or apparatus with a herein disclosed compound. Exemplary devices or apparatuses to be so disinfected or sterilised may include, without limitation, medical or surgical instruments or appliances, such as for example catheters, prosthetic implants, stents, *etc.* Advantageously, surfaces of such devices or apparatuses may be disinfected or sterilised by applying a present compound thereto, *e.g.*, in a solution, in a spray aerosol, *etc.*  
 15 Exemplary compositions to be so preserved, disinfected or sterilised may include, without limitation, cell culture media for animal cells including *inter alia* insect or mammalian cells, *etc.* In another non-limiting example, the growth, proliferation and/or survival of bacteria may be inhibited in an *in vitro* bacterial cell culture, such as for example to assess the sensitivity of a particular bacterial species, subspecies or strain to present compounds, *e.g.*, in clinical or  
 20 research settings.

In an aspect the invention provides the use of a compound of formula (VI):



(VI)

or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, for the manufacture of a medicament for the  
 25 treatment of a bacterial infection, wherein:

*p* is 0, 1 or 2 and when *p* is 0 a direct bond is intended;

*r* is 0, 1 or 2 and when *r* is 0 a direct bond is intended;

R<sup>1</sup> is selected from hydrogen, -C(=O)R<sup>5</sup>, -S(=O)OR<sup>8</sup>, -S(=O)<sub>2</sub>OR<sup>8</sup>, -PH(=O)OR<sup>8</sup> and -P(=O)(OR<sup>8</sup>)(OR<sup>9</sup>),

R<sup>5</sup> is selected from hydrogen, C<sub>1-6</sub>alkyl, -OR<sup>6</sup>, -SR<sup>6</sup>, -NR<sup>6</sup>R<sup>7</sup>, and C<sub>1-6</sub>alkyl substituted with one or more substituents selected from halo, hydroxy and oxo,

5 R<sup>6</sup> and R<sup>7</sup> are each independently selected from hydrogen, C<sub>1-6</sub>alkyl, and C<sub>1-6</sub>alkyl substituted with one or more substituents selected from halo, hydroxy, C<sub>1-6</sub>alkyloxy, carboxyl, C<sub>1-6</sub>alkyloxycarbonyl and C<sub>1-6</sub>alkylcarbonyloxy,

R<sup>8</sup> and R<sup>9</sup> are each independently selected from hydrogen, C<sub>1-6</sub>alkyl, and C<sub>1-6</sub>alkyl substituted with one or more substituents selected from halo, hydroxy and  
10 C<sub>1-6</sub>alkyloxy;

R<sup>2</sup> is selected from hydrogen, halo, hydroxy, cyano, nitro, amino, mono- or di(C<sub>1-6</sub>alkyl)amino, C<sub>1-6</sub>alkylcarbonylamino, C<sub>1-6</sub>alkyl, polyhaloC<sub>1-6</sub>alkyl, C<sub>1-6</sub>alkyloxy, carboxyl, C<sub>1-6</sub>alkylcarbonyloxy, C<sub>1-6</sub>alkyloxycarbonyl, each of said groups optionally substituted with one or more substituents selected from halo, hydroxy and C<sub>1-6</sub>alkyloxy;

15 R<sup>3</sup> is selected from -NHR<sup>10</sup> and nitro,

R<sup>10</sup> is selected from hydrogen, C<sub>1-6</sub>alkyl, C<sub>1-6</sub>alkylcarbonyl and C<sub>1-6</sub>alkyloxycarbonyl, each of said groups optionally substituted with one or more substituents selected from halo, hydroxy, amino, nitro and C<sub>1-6</sub>alkyloxy;

20 R<sup>4</sup> is selected from hydrogen, halo, hydroxy, cyano, nitro, amino, mono- or di(C<sub>1-6</sub>alkyl)amino, C<sub>1-6</sub>alkylcarbonylamino, C<sub>1-6</sub>alkyl, polyhaloC<sub>1-6</sub>alkyl and C<sub>1-6</sub>alkyloxy, each of said groups being optionally substituted with one or more substituents selected from halo, hydroxy, amino, nitro and C<sub>1-6</sub>alkyloxy.

In a related aspect, the invention provides a compound of formula (VI) wherein p, r, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are as defined above, or a pharmaceutically acceptable *N*-oxide form, addition salt, 25 prodrug, solvate or a stereochemically isomeric form thereof, for use in the treatment of a bacterial infection. Also provided is a pharmaceutical composition comprising a compound of formula (VI) wherein p, r, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are as defined above, or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, and one or more pharmaceutically acceptable carriers, for use in the treatment of a  
30 bacterial infection. Another related aspect provides a method for treating a bacterial infection in a subject in need of such treatment, comprising administering to said subject a therapeutically or prophylactically effective amount of a compound of formula (VI) wherein p,

r, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are as defined above, or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof.

Also provided is a method for inhibition of DD-ligase comprising contacting a DD-ligase, or a composition or a bacterium comprising such, with a compound of formula (VI) wherein p, r, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are as defined above, or an *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, in an amount sufficient to obtain said inhibition. In an embodiment, said DD-ligase inhibition may occur *in vivo*, *i.e.*, within the body of a subject in particular of a human or animal subject. In other embodiments, said inhibition of DD-ligase may take place *in vitro*, more particularly encompassing inhibition of isolated DD-ligase, inhibition of isolated DD-ligase when being a part of a composition, as well as inhibition of DD-ligase in bacteria when said bacteria are outside of a human or animal body.

Further aspect concerns a method for inhibiting the growth, proliferation and/or survival of bacteria comprising contacting said bacteria with a compound of formula (VI) wherein p, r, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are as defined above, or an *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, in an amount sufficient to obtain said inhibition. In an embodiment, said inhibition of bacterial growth, proliferation and/or survival may occur *in vivo*, *i.e.*, within the body of a subject in particular of a human or animal subject. In other embodiments, said inhibition of bacterial growth, proliferation and/or survival may take place *ex vivo*, which refers to treatments of bacteria outside of a human or animal body. Exemplary *ex vivo* applications are described elsewhere in this application.

A first group of preferred compounds of formula (VI) in the foregoing aspects consists of those compounds of formula (VI) or an *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, wherein any one or more or all of the following restrictions apply:

- a) p is 0 or 1;
- b) r is 0 or 1;
- c) R<sup>1</sup> is selected from hydrogen and -C(=O)R<sup>5</sup>;
- d) R<sup>5</sup> is selected from hydrogen and -OR<sup>6</sup>,
- e) R<sup>10</sup> is selected from hydrogen and C<sub>1-6</sub>alkyl optionally substituted with one or more substituents selected from halo, hydroxy, amino, nitro and C<sub>1-6</sub>alkyloxy.

A second group of preferred compounds of formula (VI) in the foregoing aspects consists of those compounds of formula (VI) or an *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, wherein any one or more or all of the following restrictions apply:

- 5 a)  $p$  is 0;
- b)  $r$  is 0 or 1;
- c)  $R^1$  is selected from hydrogen and  $-C(=O)R^5$ ;
- d)  $R^5$  is selected from hydrogen and  $-OR^6$ ;
- e)  $R^6$  is selected from hydrogen and  $C_{1-6}$ alkyl;
- 10 f)  $R^2$  is selected from hydrogen, halo, hydroxy,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl and  $C_{1-6}$ alkyloxy;
- g)  $R^{10}$  is selected from hydrogen and  $C_{1-6}$ alkyl;
- h)  $R^4$  is selected from hydrogen, halo, hydroxy,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl and  $C_{1-6}$ alkyloxy.

A third group of preferred compounds of formula (VI) in the foregoing aspects consists of those compounds of formula (VI) or an *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, wherein any one or more or all of the following restrictions apply:

- a)  $p$  is 0;
- b)  $r$  is 0 or 1;
- c)  $R^1$  is selected from hydrogen,  $-C(=O)OH$  and or  $-C(=O)OC_{1-6}$ alkyl;
- 20 d)  $R^2$  is selected from hydrogen and halo;
- e)  $R^3$  is selected from  $-NH_2$  and nitro;
- f)  $R^4$  is hydrogen.

A fourth group of preferred compounds of formula (VI) in the foregoing aspects consists of those compounds of formula (VI) or an *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, wherein any one or more or all of the following restrictions apply:

- a)  $p$  is 0;
- b)  $r$  is 0;
- c)  $R^1$  is selected from hydrogen,  $-C(=O)OH$  and or  $-C(=O)OC_{1-6}$ alkyl;
- 30 d)  $R^2$  is selected from hydrogen and halo;
- e)  $R^3$  is nitro;

f)  $R^4$  is hydrogen.

A fifth group of preferred compounds of formula (VI) in the foregoing aspects consists of those compounds of formula (VI) or an *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, wherein any one or more or all of the following  
5 restrictions apply:

a)  $p$  is 0;

b)  $r$  is 0 or 1;

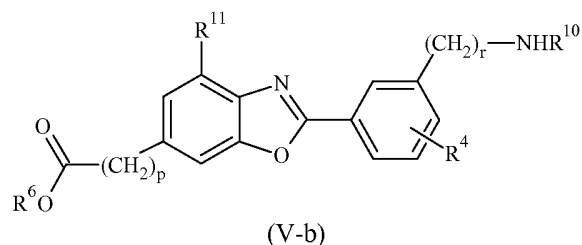
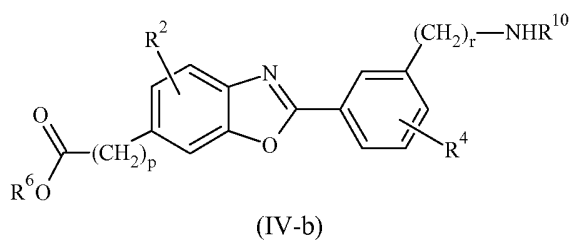
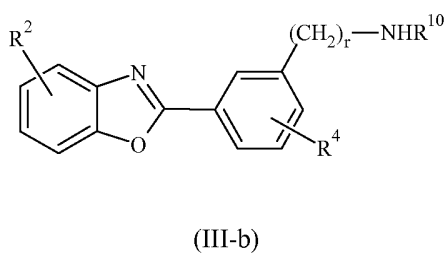
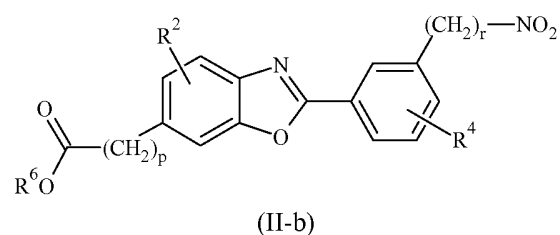
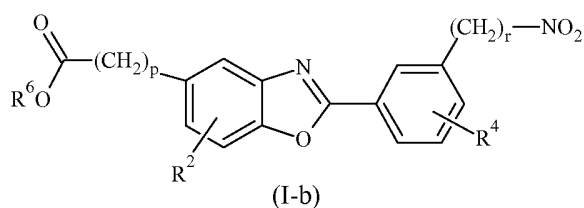
c)  $R^1$  is selected from hydrogen,  $-C(=O)OH$  and or  $-C(=O)OC_{1-6}alkyl$ ;

d)  $R^2$  is selected from hydrogen and halo;

10 e)  $R^3$  is  $-NH_2$ ;

f)  $R^4$  is hydrogen.

A sixth group of preferred compounds of formula (VI) in the foregoing aspects consists of compounds of any of formulas (I-b), (II-b), (III-b), (IV-b) or (V-b):



or an *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, wherein:

*p* is 0, 1 or 2, preferably *p* is 0 or 1, more preferably *p* is 0;

*r* is 0, 1 or 2, preferably *r* is 0 or 1, very preferably *r* is 0 or very preferably *r* is 1;

- 5  $R^6$  is selected from hydrogen,  $C_{1-6}$ alkyl, and  $C_{1-6}$ alkyl substituted with one or more substituents selected from halo, hydroxy,  $C_{1-6}$ alkyloxy, carboxyl,  $C_{1-6}$ alkyloxycarbonyl and  $C_{1-6}$ alkylcarbonyloxy, preferably  $R^6$  is hydrogen or  $C_{1-6}$ alkyl, very preferably  $R^6$  is hydrogen or very preferably  $R^6$  is  $C_{1-6}$ alkyl;

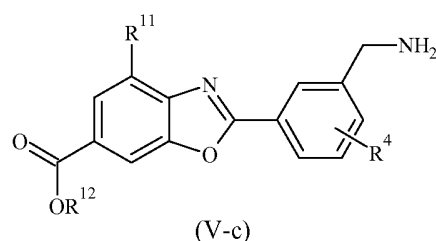
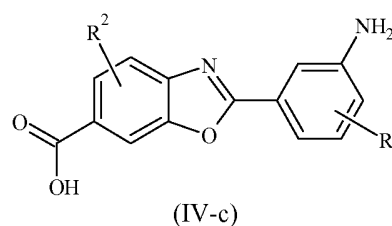
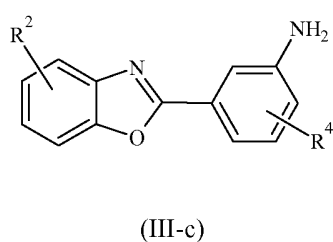
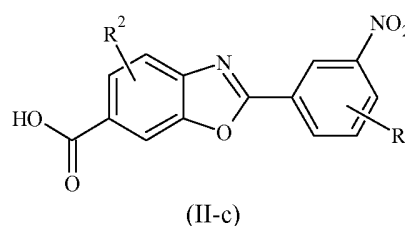
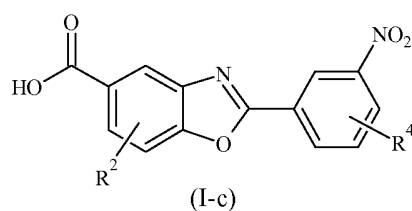
- $R^2$  is selected from hydrogen, halo, hydroxy, cyano, nitro, amino, mono- or di( $C_{1-6}$ alkyl)amino,   
10  $C_{1-6}$ alkylcarbonylamino,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl,  $C_{1-6}$ alkyloxy, carboxyl,  $C_{1-6}$ alkylcarbonyloxy,  $C_{1-6}$ alkyloxycarbonyl, each of said groups optionally substituted with one or more substituents selected from halo, hydroxy and  $C_{1-6}$ alkyloxy, preferably  $R^2$  is selected from hydrogen, halo, hydroxy,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl and  $C_{1-6}$ alkyloxy, more preferably  $R^2$  is hydrogen or halo;

- 15  $R^{10}$  is selected from hydrogen,  $C_{1-6}$ alkyl,  $C_{1-6}$ alkylcarbonyl and  $C_{1-6}$ alkyloxycarbonyl, each of said groups optionally substituted with one or more substituents selected from halo, hydroxy, amino, nitro and  $C_{1-6}$ alkyloxy, preferably  $R^{10}$  is selected from hydrogen and  $C_{1-6}$ alkyl optionally substituted with one or more substituents selected from halo, hydroxy, amino, nitro and  $C_{1-6}$ alkyloxy, more preferably  $R^{10}$  is hydrogen or  $C_{1-6}$ alkyl, even more preferably  $R^{10}$  is hydrogen;

- 20  $R^4$  is selected from hydrogen, halo, hydroxy, cyano, nitro, amino, mono- or di( $C_{1-6}$ alkyl)amino,  $C_{1-6}$ alkylcarbonylamino,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl and  $C_{1-6}$ alkyloxy, each of said groups being optionally substituted with one or more substituents selected from halo, hydroxy, amino, nitro and  $C_{1-6}$ alkyloxy, preferably  $R^4$  is selected from hydrogen, halo, hydroxy,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl and  $C_{1-6}$ alkyloxy; more preferably  $R^4$  is hydrogen;

- 25  $R^{11}$  is halo, preferably  $R^{11}$  is fluoro, chloro, bromo or iodo, more preferably  $R^{11}$  is bromo.

A seventh group of preferred compounds of formula (VI) in the foregoing aspects consists of compounds of any of formulas (I-c), (II-c), (III-c), (IV-c) or (V-c):



or an *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, wherein:

- 5  $R^2$  is selected from hydrogen, halo, hydroxy, cyano, nitro, amino, mono- or di( $C_{1-6}$ alkyl)amino,  $C_{1-6}$ alkylcarbonylamino,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl,  $C_{1-6}$ alkyloxy, carboxyl,  $C_{1-6}$ alkylcarbonyloxy,  $C_{1-6}$ alkyloxycarbonyl, each of said groups optionally substituted with one or more substituents selected from halo, hydroxy and  $C_{1-6}$ alkyloxy, preferably  $R^2$  is selected from hydrogen, halo, hydroxy,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl and  $C_{1-6}$ alkyloxy, more preferably  $R^2$  is hydrogen or halo;
- 10  $R^4$  is selected from hydrogen, halo, hydroxy, cyano, nitro, amino, mono- or di( $C_{1-6}$ alkyl)amino,  $C_{1-6}$ alkylcarbonylamino,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl and  $C_{1-6}$ alkyloxy, each of said groups being optionally substituted with one or more substituents selected from halo, hydroxy, amino, nitro and  $C_{1-6}$ alkyloxy, preferably  $R^4$  is selected from hydrogen, halo, hydroxy,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl and  $C_{1-6}$ alkyloxy; more preferably  $R^4$  is hydrogen;
- 15  $R^{11}$  is halo, preferably  $R^{11}$  is fluoro, chloro, bromo or iodo, more preferably  $R^{11}$  is bromo;  $R^{12}$  is  $C_{1-6}$ alkyl, preferably  $R^{12}$  is methyl or ethyl, more preferably  $R^{12}$  is ethyl.

An eighth group of preferred compounds of formula (VI) in the foregoing aspects consists of compounds of any of formulas (I-a), (II-a), (III-a), (IV-a) or (V-a), or an *N*-oxide form, addition salt, prodrug or solvate thereof, as defined above wherein.

Also, the invention provides a compound of any of formulas (I-b), (II-b), (IV-b) or (V-b) as defined above, or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, for use as a medicament. Accordingly, the invention also provides a pharmaceutical composition comprising a compound of any of formulas (I-b), (II-b), (IV-b) or (V-b) as defined above, or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, and one or more pharmaceutically acceptable carriers.

As well, the invention provides a compound of any of formulas (I-c), (II-c), (IV-c) or (V-c) as defined above, or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, for use as a medicament. Hence, the invention also provides a pharmaceutical composition comprising a compound of any of formulas (I-c), (II-c), (IV-c) or (V-c) as defined above, or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, and one or more pharmaceutically acceptable carriers.

The invention also provides a compound of any of formulas (I-b) or (V-b) as defined above, an *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof.

As well provided is a compound of any of formulas (I-c) or (V-c) as defined above, an *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof.

In addition, when the inventors docked the present molecules *in silico* into the binding site of D-alanine-D-alanine ligase (using the published 2.3-resolution structure of the ddIB gene product of *Escherichia coli* as published by Fan *et al.* 1994. Science 266: 439-443) using the Glide software package (Friesner *et al.* 2004. J Med Chem 47: 1739-49), the molecules were predicted to establish bonds with specific amino acids in the enzyme cavity, comprising Glu68, Tyr210 and Arg255. Accordingly, an aspect relates to further compounds capable of establishing bonds with amino acids of DD-ligase enzyme cavity comprising bonds with Glu68, Tyr210 and Arg255 or amino acids equivalent thereto, as well as to uses of such compounds as DD-ligase inhibitors and antibacterial agents, analogously as described herein.

The invention also relates to processes for the preparation of the herein disclosed compounds, and particularly compounds of formula (VI), (I-a), (II-a), (III-a), (IV-a), (V-a), (I-b), (II-b), (III-b), (IV-b), (V-b), (I-c), (II-c), (III-c), (IV-c) or (V-c) as defined herein, *N*-oxide forms, addition salts, prodrugs, solvates or stereochemically isomeric forms thereof, as taught  
5 herein.

These and further aspects and preferred embodiments of the invention are described in the following sections and in the appended claims.

### BRIEF DESCRIPTION OF FIGURES

**Figure 1** illustrates inhibition of isolated D-alanine-D-alanine ligase (Ddl) by the present  
10 compounds compared to D-cycloserine. X-axis: tested compound. Y-axis: residual activity (% of control).

**Figure 2** illustrates Ddl concentration-inhibition relationships for the present compounds II-a (B), I-a (C) and IV-a (D) compared to D-cycloserine (A). X-axis: log[inhibitor] (mM). Y-axis: activity of the ligase (%).

### DETAILED DESCRIPTION OF THE INVENTION

  
15

#### Definitions

As used herein, the singular forms "a", "an", and "the" include both singular and plural referents unless the context clearly dictates otherwise.

The terms "comprising", "comprises" and "comprised of" as used herein are synonymous with  
20 "including", "includes" or "containing", "contains", and are inclusive or open-ended and do not exclude additional, non-recited members, elements or method steps.

The recitation of numerical ranges by endpoints includes all numbers and fractions subsumed within the respective ranges, as well as the recited endpoints.

The term "about" as used herein when referring to a measurable value such as a parameter,  
25 an amount, a temporal duration, and the like, is meant to encompass variations of +/-10% or less, preferably +/-5% or less, more preferably +/-1% or less, and still more preferably +/-0.1% or less of and from the specified value, insofar such variations are appropriate to perform in the disclosed invention. It is to be understood that the value to which the modifier "about" refers is itself also specifically, and preferably, disclosed.

All documents cited in the present specification are hereby incorporated by reference in their entirety.

Unless otherwise defined, all terms used in disclosing the invention, including technical and scientific terms, have the meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. By means of further guidance, term definitions are included to better appreciate the teaching of the present invention.

### Compounds

The Summary section discloses aspects and embodiments of the invention that involve compounds, more particularly compounds of any of formulas (VI), (I-a), (II-a), (III-a), (IV-a), (V-a), (I-b), (II-b), (III-b), (IV-b), (V-b), (I-c), (II-c), (III-c), (IV-c) or (V-c) as defined above, or pharmaceutically acceptable *N*-oxide forms, addition salts, prodrugs, solvates or stereochemically isomeric forms thereof, as well as preferred subgroups of such substances.

As used herein, halo is generic to fluoro, chloro, bromo and iodo. C<sub>1-6</sub>alkyl defines straight- and branched-chain saturated hydrocarbon radicals having from 1 to 6 carbon atoms such as, e.g., methyl, ethyl, propyl, butyl, pentyl, hexyl, 1-methylethyl, 2-methylpropyl, 2-methyl-butyl, 2-methylpentyl and the like. PolyhaloC<sub>1-6</sub>alkyl defines a C<sub>1-6</sub>alkyl as defined herein, wherein two or more hydrogens of said C<sub>1-6</sub>alkyl are replaced with identical or different halogen substituents; this also encompasses perhaloC<sub>1-6</sub>alkyl, *i.e.*, C<sub>1-6</sub>alkyl as defined herein wherein all hydrogens of said C<sub>1-6</sub>alkyl are replaced with identical or different halogen substituents – for example, trihalomethyl defines methyl containing three identical or different halo substituents, such as, e.g., trifluoromethyl. Further, oxo defines =O; nitro defines -NO<sub>2</sub>.

When any variable, e.g., halogen or C<sub>1-6</sub>alkyl, occurs more than one time in any constituent, each definition is independent.

The term "substituted" denotes that one or more hydrogens on one or more atoms (typically C, N, O or S atoms, usually C atoms) of a group indicated by the modifier "substituted" is replaced with a selection from the recited group, provided that the normal valency of the atoms of the indicated group is not exceeded, and that the substitution results in a chemically stable compound, *i.e.*, a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an useful agent. The term "one or more" covers the possibility of one, more or all available atoms of an indicated group to be

substituted where appropriate; preferably of one, two or three, more preferably of one or two, and even more preferably of one available atoms of an indicated group to be substituted.

The term "addition salt" comprises salts which the present compounds are able to form with organic or inorganic bases such as amines, alkali metal bases and earth alkaline metal bases, or quaternary ammonium bases, or with organic or inorganic acids, such as mineral  
5 acids, sulfonic acids, carboxylic acids or phosphorus containing acids.

The term "addition salt" further comprises pharmaceutically acceptable salts, metal complexes and the salts thereof, that the present compounds are able to form.

The term "pharmaceutically acceptable salts" means pharmaceutically acceptable acid or  
10 base addition salts. The pharmaceutically acceptable acid or base addition salts are meant to comprise therapeutically active non-toxic acid and non-toxic base addition salt forms which the present compounds are able to form. The present compounds which have basic properties can be converted in their pharmaceutically acceptable acid addition salts by treating said base form with an appropriate acid. Appropriate acids comprise, for example,  
15 inorganic acids such as hydrohalic acids, *e.g.*, hydrochloric or hydrobromic acid, sulphuric, nitric, phosphoric and the like acids; or organic acids such as, for example, acetic, propanoic, hydroxyacetic, lactic, pyruvic, oxalic, malonic, succinic (*i.e.*, butanedioic acid), maleic, fumaric, malic, tartaric, citric, methanesulfonic, ethanesulfonic, benzenesulfonic, *p*-toluenesulfonic, cyclamic, salicylic, *p*-aminosalicylic, pamoic and the like acids.

20 The present compounds which have acidic properties may be converted in their pharmaceutically acceptable base addition salts by treating said acid form with a suitable organic or inorganic base. Appropriate base salt forms comprise, for example, the ammonium salts, the alkali and earth alkaline metal salts, *e.g.*, the lithium, sodium, potassium, magnesium, calcium salts and the like, salts with organic bases, *e.g.*, the benzathine, *N*-methyl-D-glucamine, hydrabamine salts, and salts with amino acids such as, for example,  
25 arginine, lysine and the like.

The terms acid or base addition salt also comprise the hydrates and the solvent addition forms thereof which the present compounds are able to form. Examples of such forms are, *e.g.*, hydrates, alcoholates and the like.

30 The term "metal complexes" means a complex formed between a present compound and one or more organic or inorganic metal salt or salts. Examples of said organic or inorganic salts

comprise the halogenides, nitrates, sulfates, phosphates, acetates, trifluoroacetates, trichloroacetates, propionates, tartrates, sulfonates, *e.g.*, methylsulfonates, 4-methylphenylsulfonates, salicylates, benzoates and the like of the metals of the second main group of the periodical system, *e.g.*, the magnesium or calcium salts, of the third or  
5 fourth main group, *e.g.*, aluminium, tin, lead, as well as the first to the eighth transition groups of the periodical system such as, for example, chromium, manganese, iron, cobalt, nickel, copper, zinc and the like.

The term "stereochemically isomeric forms" of present compounds as used herein, defines all possible compounds made up of the same atoms bonded by the same sequence of bonds  
10 but having different three-dimensional structures which are not interchangeable, which the present compounds may possess. Unless otherwise mentioned or indicated, the chemical designation of a compound encompasses the mixture of all possible stereochemically isomeric forms which said compound may possess. Said mixture may contain all diastereomers and/or enantiomers of the basic molecular structure of said compound. All  
15 stereochemically isomeric forms of the present compounds both in pure form or in admixture with each other are intended to be embraced.

Of special interest are those present compounds which are stereochemically pure.

Pure stereoisomeric forms of the compounds and intermediates as mentioned herein are defined as isomers substantially free of other enantiomeric or diastereomeric forms of the  
20 same basic molecular structure of said compounds or intermediates. In particular, the term "stereoisomerically pure" concerns compounds or intermediates having a stereoisomeric excess of at least 80% (*i.e.*, minimum 90% of one isomer and maximum 10% of the other possible isomers) up to a stereoisomeric excess of 100% (*i.e.*, 100% of one isomer and none of the other), more preferably, compounds or intermediates having a stereoisomeric excess of  
25 90% up to 100%, even more preferably having a stereoisomeric excess of 94% up to 100% and most preferably having a stereoisomeric excess of 97% up to 100%. The terms "enantiomerically pure" and "diastereomerically pure" should be understood in a similar way, but then having regard to the enantiomeric excess and the diastereomeric excess of the mixture in question, respectively.

30 The *N*-oxide forms of the present compounds are meant to comprise those compounds wherein one or several nitrogen atoms are oxidized to the so-called *N*-oxide.

The present compounds may form solvates, for example, with water (*i.e.*, hydrates) or common organic solvents. As used herein, the term "solvate" means a physical association of the present compounds with one or more solvent molecules. This physical association involves varying degrees of ionic and other bonding, including hydrogen bonding. In certain instances the solvate will be capable of isolation, for example, when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. The term "solvate" is intended to encompass both solution-phase and isolatable solvates. Non-limiting examples of suitable solvates include ethanlates, methanlates, and the like.

Furthermore, the present compounds may have one or more crystalline or amorphous polymorph forms, as such intended to be embraced.

Also contemplated are any isotopes of atoms present in the present compounds. For example, isotopes of hydrogen include tritium and deuterium and isotopes of carbon include  $^{13}\text{C}$  and  $^{14}\text{C}$ .

Prodrugs of the present compounds are substances that readily undergo chemical changes under physiological conditions (*in vivo*) to provide the present compounds. Additionally, prodrugs can be converted to the present compounds by chemical or biochemical methods in an *ex vivo* environment. For example, prodrugs can be slowly converted to the present compounds when placed in a trans-dermal patch reservoir with a suitable enzyme or chemical reagent.

Hence, the term "prodrug" means pharmacologically acceptable derivatives such as, *e.g.*, esters, amides and phosphates, such that the resulting *in vivo* biotransformation product of the derivative are the active compounds as defined above. See, *e.g.*, Goodman and Gilman (The Pharmacological Basis of Therapeutics, 8th ed, McGraw-Hill, Int. Ed. 1992, "Biotransformation of Drugs", p 13–15) generally describing prodrugs. Prodrugs of the present compounds are prepared by modifying functional groups present in a compound in such a way that the modifications are cleaved, either in routine manipulation or *in vivo* or *ex vivo*, to the parent compound. Prodrugs may include substances wherein a hydroxy group or an amino group is bonded to any group that, when the prodrug is administered to a patient, cleaves to form a free hydroxyl or free amino, respectively. Typical examples of prodrugs are described for instance in WO 99/33795, WO 99/33815, WO 99/33793 and WO 99/33792 all incorporated herein by reference. Prodrugs are usually characterised by excellent aqueous

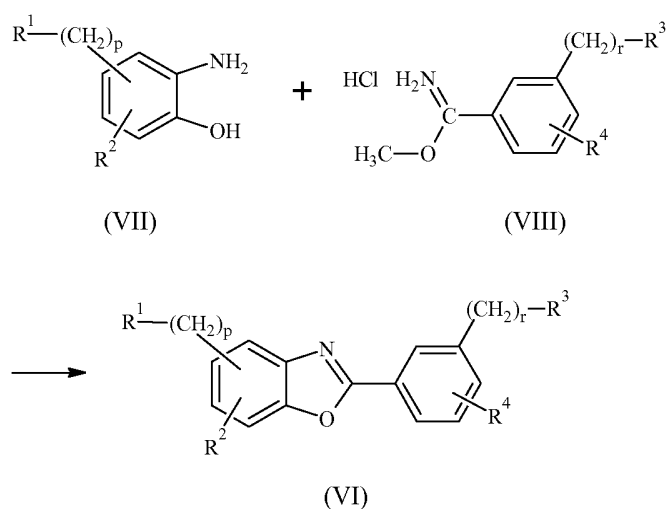
solubility, increased bioavailability and are readily metabolised into the active inhibitors *in vivo*.

Whenever used hereinafter, the term "present compounds" is meant to include also the N-oxide forms, the acid or base addition salts particularly the pharmaceutically acceptable acid or base addition salts, the prodrugs, the solvates and all stereoisomeric forms of the compounds as defined in the Summary section, more particularly of compounds of any of formulas (VI), (I-a), (II-a), (III-a), (IV-a), (V-a), (I-b), (II-b), (III-b), (IV-b), (V-b), (I-c), (II-c), (III-c), (IV-c) or (V-c) as defined above, or compounds of any preferred subgroups thereof.

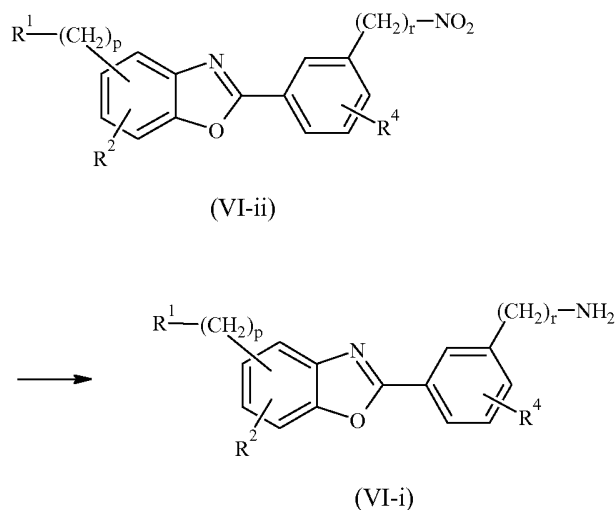
#### Synthesis of the present compounds

- 10 The present compounds, their N-oxides, pharmaceutically acceptable salts, prodrugs, solvates, and stereochemically isomeric forms thereof may be prepared in conventional manner. The starting materials and some of the intermediates are known compounds and are commercially available or may be prepared according to conventional reaction procedures as generally known in the art.
- 15 A number of such preparation methods for compounds of formula (VI), which are directly applicable to synthesis of the more particular compounds (I-a), (II-a), (III-a), (IV-a), (V-a), (I-b), (II-b), (III-b), (IV-b), (V-b), (I-c), (II-c), (III-c), (IV-c) or (V-c), will be described hereinafter in more detail. Other methods for obtaining preferred final compounds are described in the examples.
- 20 The compounds of formula (VI) can be prepared by reacting an intermediate of formula (VII) with an intermediate of formula (VIII) in an appropriate protic solvent such as methanol:

19

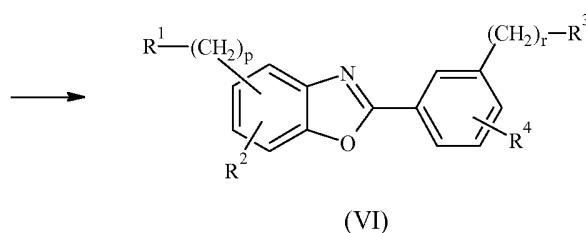
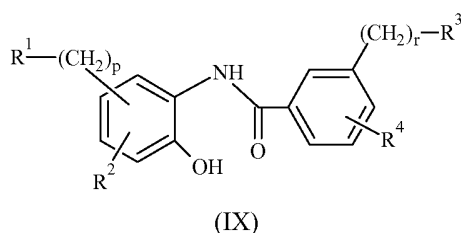


The compounds of formula (VI) wherein R<sup>3</sup> is -NH<sub>2</sub>, herein termed compounds of formula (VI-i) can be prepared from compounds of formula (VI) wherein R<sup>3</sup> is -NO<sub>2</sub>, herein termed compounds of formula (VI-ii), by a nitro to amine reduction reaction, for example in the presence of a metal catalyst such as Raney Nickel and an appropriate reductant such as hydrogen, or using sodium dithionite or another suitable reducing agent, in a suitable solvent such as methanol or ethanol:



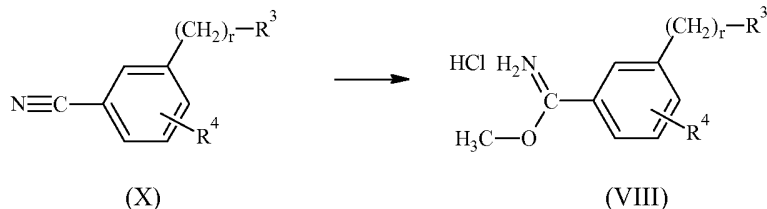
The compounds of formula (VI) can also be prepared from intermediates of formula (IX) by cyclocondensation at elevated temperatures in the presence of a suitable acid catalyst such as HCl or *p*-toluenesulfonic acid in an appropriate solvent such ethanol:

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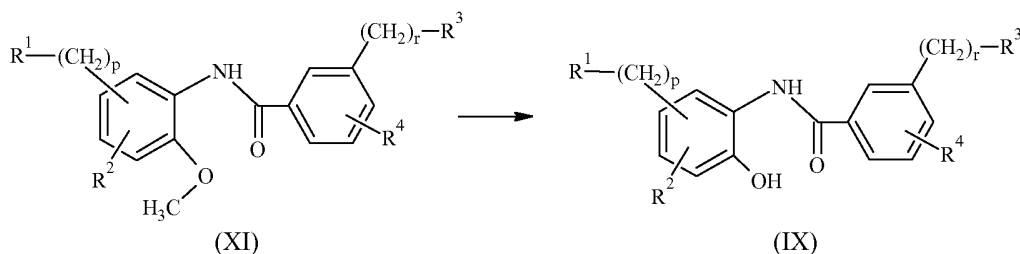


The compounds of formula (VI) wherein R<sup>1</sup> is -C(=O)OH or wherein R<sup>1</sup> is -C(=O)OC<sub>1-6</sub>alkyl can be interconverted by esterification and de-esterification processes known *per se*.

Intermediates of formula (VIII) can be prepared from respective intermediates of formula (X) by reacting the latter with gaseous HCl in a suitable solvent such as methanol:



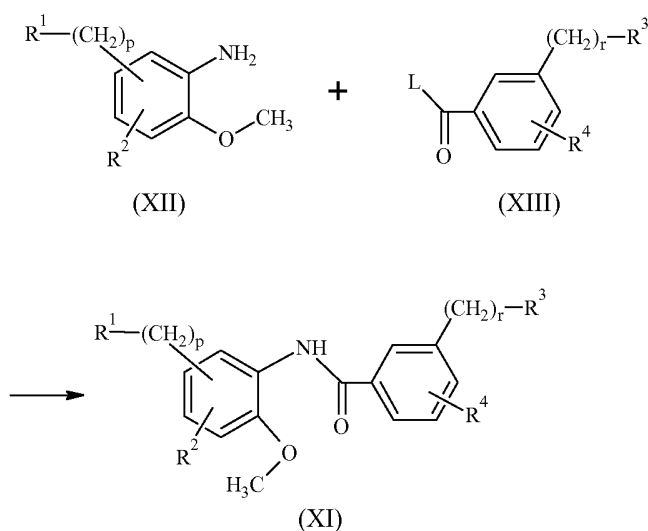
Intermediates of formula (IX) can be prepared from intermediates of formula (XI) by demethylation such as using boron tribromide (BBr<sub>3</sub>) in a suitable solvent such as dichloromethane:



10

Intermediates of formula (XI) can be prepared by reacting intermediates of formula (XII) and intermediates of formula (XIII), wherein -C(=O)L represents carboxyl or optionally a reactive functional derivative thereof, such as, e.g., carbonyl imidazole derivative (imidazolide), acyl

halide (such as, e.g., acyl chloride or bromide), mixed anhydride, 2-chloropyridinium or 3-chloroisoxazolium derivative, thioester or the like:



During any of the processes for preparation of the present compounds and intermediates, it may be necessary and/or desirable to protect sensitive or reactive groups on any of the molecules concerned. This may be achieved by means of conventional protecting groups, such as those described in *Protecting Groups*, P. Kocienski, Thieme Medical Publishers, 2000; and T.W. Greene & P.G.M. Wuts, *Protective Groups in Organic Synthesis*, 3rd ed. Wiley Interscience, 1999. The protecting groups may be removed at a convenient subsequent stage using methods known in the art.

The reactions of the synthetic methods described herein are carried out in suitable solvents which may be readily selected by one of skill in the art of organic synthesis, said suitable solvents generally being any solvent which is substantially non-reactive with the starting materials (reactants), the intermediates, or products at the temperatures at which the reactions are carried out, i.e., temperatures which may range from the solvent's freezing temperature to the solvent's boiling temperature. A given reaction may be carried out in one solvent or a mixture of more than one solvent. Depending on the particular reaction step, suitable solvents for a particular reaction step may be selected.

After any of the reactions is carried out or after removing protecting group(s), intermediates and/or final compounds may, if necessary for the following steps and/or for monitoring the reaction, be worked-up and/or purified by methods known in the field of organic synthetic

chemistry, such as filtering, solvent extraction, solvent evaporation by heat or under vacuum, re-crystallisation, trituration, chromatography or a method using an ion exchange resin.

#### Applications of the present compounds

The present compounds represent useful antibacterial agents, *i.e.*, substances which are  
5 destructive to or prevent the growth of bacteria, particularly suitable for the treatment of bacterial infections in subjects in need thereof. The present compounds are also suitable for inhibiting the growth, proliferation and/or survival of bacteria *in vivo* (*i.e.*, within a subject's body), as well as in *ex vivo* (*i.e.*, outside of a subject's body) situations.

The terms "subject" or "patient" refer to animals, preferably warm-blooded animals, more  
10 preferably vertebrates, and even more preferably mammals specifically including humans and non-human mammals, that have been the object of treatment, observation or experiment. The term "mammal" includes any animal classified as such, including, but not limited to, humans, domestic and farm animals, zoo animals, sport animals, pet animals, companion animals and experimental animals, such as, for example, mice, rats, hamsters, rabbits, dogs, cats, guinea  
15 pigs, cattle, cows, sheep, horses, pigs and primates, *e.g.*, monkeys and apes. Preferred patients are human subjects, including both genders and all age categories thereof.

As used herein, a phrase such as "a subject in need of treatment" includes subjects that would benefit from treatment of a given condition, particularly of a bacterial infection. Such subjects may include, without limitation, those that have been diagnosed with said condition,  
20 those prone to contract or develop said condition and/or those in whom said condition is to be prevented.

The terms "treat" or "treatment" encompass both the therapeutic treatment of an already developed disorder, such as the therapy of an already developed bacterial infection, as well as prophylactic or preventative measures, wherein the aim is to prevent or lessen the  
25 chances of incidence of an undesired affliction, such as to prevent the chances of contraction and progression of a bacterial infection. Beneficial or desired clinical results may include, without limitation, alleviation of one or more symptoms or one or more biological markers (*e.g.*, fever, bacterial load, *etc.*), diminishment of extent of disease, stabilised (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or  
30 palliation of the disease state, and the like. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

The term "prophylactically effective amount" refers to an amount of an active compound or pharmaceutical agent that inhibits or delays in a subject the onset of a disorder as being sought by a researcher, veterinarian, medical doctor or other clinician. The term "therapeutically effective amount" as used herein, refers to an amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a subject that is being sought by a researcher, veterinarian, medical doctor or other clinician, which may include *inter alia* alleviation of the symptoms of the disease or disorder being treated. Methods are known in the art for determining therapeutically and prophylactically effective doses for the present compounds.

The term "bacterial infection" refers to the entry and proliferation of a bacterium that is pathogenic to a subject in a bodily tissue of said subject, producing subsequent tissue injury and disease.

The term also encompasses a "bacterial superinfection" which refers to a secondary infection that occurs after a previous infection; this secondary infection is generally more destructive than the first and is often attributed to bacteria which have become resistant to the antibiotics used to treat the first infection. The terms also encompass nosocomial, *i.e.*, hospital-acquired bacterial infections and/or superinfections. The present compounds can reduce bacterial infections and/or superinfections and prevent further infection from developing.

In an embodiment, the present compounds may be employed for the treatment of infections caused by bacteria, or for inhibiting the growth, proliferation and/or survival of bacteria, wherein said bacteria comprise DD-ligase activity. The term "DD-ligase" refers to a class of enzymes which catalyse the formation of a dimer of D-amino acids from the corresponding monomers at the expense of ATP. Particularly, the term encompasses D-alanine-D-alanine ligases (EC 6.3.2.4) which catalyse the reaction  $\text{ATP} + 2 \text{ D-alanine} \rightarrow \text{ADP} + \text{phosphate} + \text{D-alanyl-D-alanine}$ . Also, the term encompasses DD-ligases which can employ other D-amino acid substrates, such as for example D-alanine-D-serine ligases and D-alanine-D-lactate ligases. The presence of DD-ligase activity in bacteria or lysates thereof may be tested in known enzymatic assays, such as ones analogous to that shown in the examples.

In a further embodiment, the present compounds may be employed for the treatment of infections caused by bacteria, or for inhibiting the growth, proliferation and/or survival of bacteria, wherein said bacteria comprise peptidoglycan. The term "peptidoglycan" as used herein refers to a glycopeptide polymer that is a component of bacterial cell walls, and is

generally characterised as comprising  $\beta$ -(1,4) linked N-acetylglucosamine (NAG) and N-acetylmuramic acid chains cross-linked by oligopeptide units comprising D-amino acids. The present compounds can advantageously interfere with the synthesis of peptidoglycan and hence combat bacteria comprising such.

- 5 In a further embodiment, the present compounds may be employed for the treatment of infections caused by bacteria, or for inhibiting the growth, proliferation and/or survival of bacteria, wherein said bacteria are Gram-positive bacteria, Gram-negative bacteria, aerobic bacteria, anaerobic bacteria or mycobacteria.

- 10 In a preferred embodiment, the present compounds may be employed for the treatment of infections caused by bacteria, or for inhibiting the growth, proliferation and/or survival of bacteria, wherein said bacteria are Gram-positive bacteria. Due to high peptidoglycan content in Gram-positive bacteria, the present compounds which inhibit peptidoglycan synthesis may be particularly effective in said bacteria.

- 15 In an embodiment, the present compounds may be employed for the treatment of infections caused by bacteria, or for inhibiting the growth, proliferation and/or survival of bacteria, wherein said bacteria are chosen, by preference but without limitation, from the genera *Acinetobacter*, *Bacillus*, *Bacteroides*, *Campylobacter*, *Clostridium*, *Corynebacterium*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Fusobacterium*, *Helicobacter*, *Haemophilus*, *Klebsiella*, *Legionella*, *Listeria*, *Moraxella*, *Mycobacterium*, *Mycoplasma*, *Neisseria*,  
20 *Porphyromonas*, *Prevotella*, *Proteus*, *Pseudomonas*, *Salmonella*, *Serratia*, *Staphylococcus*, *Streptococcus*, and *Vibrio*, particularly wherein said bacteria represent pathogenic species of said genera.

- 25 In an embodiment, the present compounds may be employed for the treatment of infections caused by bacteria, or for inhibiting the growth, proliferation and/or survival of bacteria, wherein said bacteria are chosen, by preference but without limitation, from *Staphylococcus aureus*; *Staphylococcus epidermidis* and other coagulase-negative staphylococci; *Streptococcus pyogenes*; *Streptococcus pneumoniae*; *Streptococcus agalactiae*; *Enterococcus* species; *Corynebacterium diphtheriae*; *Listeria monocytogenes*; *Bacillus anthracis*; *Neisseria meningitidis*; *Neisseria gonorrhoeae*; *Moraxella catarrhalis*; *Vibrio cholerae*;  
30 *Campylobacter jejuni*; Enterobacteriaceae (including *Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter*); *Pseudomonas aeruginosa*; *Acinetobacter* species; *Haemophilus influenzae*; *Clostridium tetani*; *Clostridium botulinum*; *Bacteroides* species; *Prevotella* species;

*Porphyromonas* species; *Fusobacterium* species; *Mycobacterium tuberculosis* and *Mycobacterium leprae*. Examples of infections caused by these microorganisms are provided in Table 1.

Table 1

Organism	Disease
GRAM-POSITIVE COCCI	
<i>Staphylococcus aureus</i>	Major human pathogen, bacteremia, pneumonia
<i>Staphylococcus epidermidis</i> and other coagulase-negative staphylococci	Urinary tract infections, osteomyelitis, bacteremia
<i>Streptococcus pyogenes</i>	Bacteremia, lymphangitis, pneumonia
<i>Streptococcus pneumoniae</i>	Pneumonia, otitis media, sinusitis
<i>Streptococcus agalactiae</i>	Primary bacteremia, pneumonia, endocarditis, osteomyelitis
<i>Enterococcus</i> sp.	Urinary tract infections, bacteremia, endocarditis, intra-abdominal and pelvic infections, neonatal sepsis
GRAM-POSITIVE BACILLI	
<i>Corynebacterium diphtheriae</i>	Respiratory tract diphtheria, anterior nasal, faucial
<i>Listeria monocytogenes</i>	Bacteremia, meningoenzephalitis
<i>Bacillus anthracis</i>	Acute infection
GRAM-NEGATIVE COCCI	
<i>Neisseria meningitidis</i>	Endemic and epidemic disease
<i>Neisseria gonorrhoeae</i>	Genital infection, perihepatitis
<i>Moraxella catarrhalis</i>	Otitis media, lower respiratory tract infections, pneumonia, bacteremia
GRAM-NEGATIVE BACILLI	
<i>Vibrio cholerae</i>	Epidemic diarrheal disease
<i>Campylobacter jejuni</i>	Acute enteritis, acute colitis, bacteremia
<i>Enterobacteriaceae</i> (incl. <i>Escherichia</i> , <i>Salmonella</i> , <i>Klebsiella</i> , <i>Enterobacter</i> )	Enteric infections, urinary tract infections, respiratory infections, bacteremia, bacillary dysentery
<i>Pseudomonas aeruginosa</i>	Endocarditis, respiratory infections, bacteremia, CNS infections
<i>Acinetobacter</i> sp.	Respiratory tract infections, bacteremia, genitourinary infections
<i>Haemophilus influenzae</i>	Pneumonia, meningitis, epiglottitis, bacteremia
ANAEROBIC BACTERIA	
<i>Clostridium tetani</i>	Tetanus
<i>Clostridium botulinum</i>	Botulism
<i>Bacteroides</i> sp.; <i>Prevotella</i> sp.; <i>Porphyromonas</i> sp.; <i>Fusobacterium</i> species	Postaspiration pleuropulmonary infection, genital tract infection, intra-abdominal abscesses
MYCOBACTERIAL DISEASE	
<i>Mycobacterium tuberculosis</i>	Affects virtually every organ, most importantly the lungs
<i>Mycobacterium leprae</i>	Leprosy, Hansen's disease

In an embodiment, the present compounds may be employed for the treatment of infections caused by bacteria, or for inhibiting the growth, proliferation and/or survival of bacteria, wherein said bacteria are resistant to other inhibitors of cell wall synthesis, such as particularly to  $\beta$ -lactam antibiotics (e.g., penicillins, cephalosporins) or to vancomycin. Indeed, due to a different mode of action, the present compounds may be particularly useful for so-resistant microorganisms.

In a particular embodiment, the present compounds may be employed for the treatment of infections caused by bacteria, or for inhibiting the growth, proliferation and/or survival of bacteria, wherein said bacteria are vancomycin-sensitive or vancomycin-resistant. Specific vancomycin-resistant bacteria that may be inhibited include, without limitation, vancomycin-resistant *Staphylococcus aureus*, *Staphylococcus epidermis*, vancomycin-resistant *Enterococci* such as for example vancomycin-resistant *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus casseliflavus* and *Enterococcus gallinarum*, and vancomycin-resistant *Clostridium innocuum*.

As noted, the present compounds may also represent useful inhibitors of DD-ligases as defined above. The term "inhibition" encompasses any extent of inhibition of DD-ligase activity, in particular of its enzymatic activity, such as, e.g., inhibition by at least about 10%, by at least about 20%, by at least about 30%, by at least about 40%, by at least about 50%, by at least about 60%, by at least about 70%, by at least about 80%, by at least about 90%, or even by about 100%, compared to DD-ligase activity in the absence of the inhibitor. The extent of DD-ligase inhibition may be measured by assays known in the art, such as described in the examples.

#### Pharmaceutical formulations comprising the present compounds

The present compounds may be advantageously formulated as pharmaceutical formulations for treating bacterial infections.

Such pharmaceutical compositions comprise one or more present compounds or pharmaceutically acceptable N-oxide forms, acid or base addition salts, prodrugs, solvates or stereoisomeric forms thereof (i.e., one or more "active substances"), and one or more pharmaceutically acceptable carrier/excipient.

As used herein, "carrier" or "excipient" includes any and all solvents, diluents, buffers (such as, e.g., neutral buffered saline or phosphate buffered saline), solubilisers, colloids,

dispersion media, vehicles, fillers, chelating agents (such as, *e.g.*, EDTA or glutathione), amino acids (such as, *e.g.*, glycine), proteins, disintegrants, binders, lubricants, wetting agents, emulsifiers, sweeteners, colorants, flavourings, aromatisers, thickeners, agents for achieving a depot effect, coatings, antifungal agents, preservatives, antioxidants, tonicity  
5 controlling agents, absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active substance, its use in the therapeutic compositions may be contemplated.

Illustrative, non-limiting carriers for use in formulating the pharmaceutical compositions  
10 include, for example, oil-in-water or water-in-oil emulsions, aqueous compositions with or without inclusion of organic co-solvents suitable for intravenous (IV) use, liposomes or surfactant-containing vesicles, microspheres, microbeads and microsomes, powders, tablets, capsules, suppositories, aqueous suspensions, aerosols, and other carriers apparent to one of ordinary skill in the art.

Pharmaceutical compositions of the invention may be formulated for essentially any route of  
15 administration, such as without limitation, oral administration (such as, *e.g.*, oral ingestion or inhalation), intranasal administration (such as, *e.g.*, intranasal inhalation or intranasal mucosal application), parenteral administration (such as, *e.g.*, subcutaneous, intravenous, intramuscular, intraperitoneal or intrasternal injection or infusion), transdermal or  
20 transmucosal (such as, *e.g.*, oral, sublingual, intranasal) administration, topical administration, rectal, vaginal or intra-tracheal instillation, and the like. In this way, the therapeutic effects attainable by the methods and compositions of the invention can be, for example, systemic, local, tissue-specific, *etc.*, depending of the specific needs of a given application of the invention.

For example, for oral administration, pharmaceutical compositions may be formulated in the  
25 form of pills, tablets, lacquered tablets, coated (*e.g.*, sugar-coated) tablets, granules, hard and soft gelatin capsules, aqueous, alcoholic or oily solutions, syrups, emulsions or suspensions. In an example, without limitation, preparation of oral dosage forms may be suitably accomplished by uniformly and intimately blending together a suitable amount of the active  
30 compound in the form of a powder, optionally also including finely divided one or more solid carrier, and formulating the blend in a pill, tablet or a capsule. Exemplary but non-limiting solid carriers include calcium phosphate, magnesium stearate, talc, sugars (such as, *e.g.*, glucose,

mannose, lactose or sucrose), sugar alcohols (such as, *e.g.*, mannitol), dextrin, starch, gelatin, cellulose, polyvinylpyrrolidone, low melting waxes and ion exchange resins. Compressed tablets containing the pharmaceutical composition can be prepared by uniformly and intimately mixing the active ingredient with a solid carrier such as described above to  
5 provide a mixture having the necessary compression properties, and then compacting the mixture in a suitable machine to the shape and size desired. Moulded tablets maybe made by moulding in a suitable machine, a mixture of powdered compound moistened with an inert liquid diluent. Suitable carriers for soft gelatin capsules and suppositories are, for example, fats, waxes, semisolid and liquid polyols, natural or hardened oils, *etc.*

10 For example, for oral or nasal aerosol or inhalation administration, pharmaceutical compositions may be formulated with illustrative carriers, such as, *e.g.*, as in solution with saline, polyethylene glycol or glycols, DPPC, methylcellulose, or in mixture with powdered dispersing agents, further employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilising or  
15 dispersing agents known in the art. Suitable pharmaceutical formulations for administration in the form of aerosols or sprays are, for example, solutions, suspensions or emulsions of the compounds of the invention or their physiologically tolerable salts in a pharmaceutically acceptable solvent, such as ethanol or water, or a mixture of such solvents. If required, the formulation can also additionally contain other pharmaceutical auxiliaries such as surfactants,  
20 emulsifiers and stabilizers as well as a propellant. Illustratively, delivery may be by use of a single-use delivery device, a mist nebuliser, a breath-activated powder inhaler, an aerosol metered-dose inhaler (MDI) or any other of the numerous nebuliser delivery devices available in the art. Additionally, mist tents or direct administration through endotracheal tubes may also be used.

25 Examples of carriers for administration via mucosal surfaces depend upon the particular route, *e.g.*, oral, sublingual, intranasal, *etc.* When administered orally, illustrative examples include pharmaceutical grades of mannitol, starch, lactose, magnesium stearate, sodium saccharide, cellulose, magnesium carbonate and the like, with mannitol being preferred. When administered intranasally, illustrative examples include polyethylene glycol,  
30 phospholipids, glycols and glycolipids, sucrose, and/or methylcellulose, powder suspensions with or without bulking agents such as lactose and preservatives such as benzalkonium chloride, EDTA. In a particularly illustrative embodiment, the phospholipid 1,2 dipalmitoyl-sn-

glycero-3-phosphocholine (DPPC) is used as an isotonic aqueous carrier at about 0.01-0.2% for intranasal administration of the compound of the subject invention at a concentration of about 0.1 to 3.0 mg/ml.

For example, for parenteral administration, pharmaceutical compositions may be advantageously formulated as solutions, suspensions or emulsions with suitable solvents, diluents, solubilisers or emulsifiers, *etc.* Suitable solvents are, without limitation, water, physiological saline solution or alcohols, *e.g.* ethanol, propanol, glycerol, in addition also sugar solutions such as glucose, invert sugar, sucrose or mannitol solutions, or alternatively mixtures of the various solvents mentioned. The injectable solutions or suspensions may be formulated according to known art, using suitable non-toxic, parenterally-acceptable diluents or solvents, such as mannitol, 1,3-butanediol, water, Ringer's solution or isotonic sodium chloride solution, or suitable dispersing or wetting and suspending agents, such as sterile, bland, fixed oils, including synthetic mono- or diglycerides, and fatty acids, including oleic acid. The compounds and pharmaceutically acceptable salts thereof of the invention can also be lyophilised and the lyophilisates obtained used, for example, for the production of injection or infusion preparations. For example, one illustrative example of a carrier for intravenous use includes a mixture of 10% USP ethanol, 40% USP propylene glycol or polyethylene glycol 600 and the balance USP Water for Injection (WFI). Other illustrative carriers for intravenous use include 10% USP ethanol and USP WFI; 0.01-0.1% triethanolamine in USP WFI; or 0.01-0.2% dipalmitoyl diphosphatidylcholine in USP WFI; and 1-10% squalene or parenteral vegetable oil-in-water emulsion. Illustrative examples of carriers for subcutaneous or intramuscular use include phosphate buffered saline (PBS) solution, 5% dextrose in WFI and 0.01-0.1% triethanolamine in 5% dextrose or 0.9% sodium chloride in USP WFI, or a 1 to 2 or 1 to 4 mixture of 10% USP ethanol, 40% propylene glycol and the balance an acceptable isotonic solution such as 5% dextrose or 0.9% sodium chloride; or 0.01-0.2% dipalmitoyl diphosphatidylcholine in USP WFI and 1 to 10% squalene or parenteral vegetable oil-in-water emulsions.

Where aqueous formulations are preferred, such may comprise one or more surfactants. For example, the composition can be in the form of a micellar dispersion comprising at least one suitable surfactant, *e.g.*, a phospholipid surfactant. Illustrative examples of phospholipids include diacyl phosphatidyl glycerols, such as dimyristoyl phosphatidyl glycerol (DPMG), dipalmitoyl phosphatidyl glycerol (DPPG), and distearoyl phosphatidyl glycerol (DSPG), diacyl

phosphatidyl cholines, such as dimyristoyl phosphatidylcholine (DPMC), dipalmitoyl phosphatidylcholine (DPPC), and distearoyl phosphatidylcholine (DSPC); diacyl phosphatidic acids, such as dimyristoyl phosphatidic acid (DPMA), dipalmitoyl phosphatidic acid (DPPA), and distearoyl phosphatidic acid (DSPA); and diacyl phosphatidyl ethanolamines such as  
5 dimyristoyl phosphatidyl ethanolamine (DPME), dipalmitoyl phosphatidyl ethanolamine (DPPE) and distearoyl phosphatidyl ethanolamine (DSPE). Typically, a surfactant:active substance molar ratio in an aqueous formulation will be from about 10:1 to about 1:10, more typically from about 5:1 to about 1:5, however any effective amount of surfactant may be used in an aqueous formulation to best suit the specific objectives of interest.

10 When rectally administered in the form of suppositories, these formulations may be prepared by mixing the compounds according to the invention with a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at ordinary temperatures, but liquidify and/or dissolve in the rectal cavity to release the drug.

Suitable carriers for microcapsules, implants or rods are, for example, copolymers of glycolic  
15 acid and lactic acid.

One skilled in this art will recognize that the above description is illustrative rather than exhaustive. Indeed, many additional formulations techniques and pharmaceutically-acceptable excipients and carrier solutions are well-known to those skilled in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions  
20 described herein in a variety of treatment regimens.

The present active substances may be used alone or in combination with any antimicrobial therapies and antibiotics known in the art ("combination therapy"). Combination therapies as contemplated herein may comprise the administration of at least one active substance of the present invention and at least one other pharmaceutically or biologically active ingredient.  
25 Said present active substance(s) and said pharmaceutically or biologically active ingredient(s) may be administered in either the same or different pharmaceutical formulation(s), simultaneously or sequentially in any order.

Exemplary antibiotics in combination therapy with which the present active substances may be employed include, without limitation,  $\beta$ -lactam antibiotics (such as, e.g., penicillins and  
30 cephalosporins), sulfonamide antibiotics, aminoglycoside antibiotics, carbapenems,

trimethoprim, chloramphenicol, glycopeptide antibiotics such as vancomycin, macrolides, quinoline antibiotics, tetracyclines, etc.

The dosage or amount of the present active substances used, optionally in combination with one or more other active compound to be administered, depends on the individual case and is, as is customary, to be adapted to the individual circumstances to achieve an optimum effect. Thus, it depends on the nature and the severity of the disorder to be treated, and also on the sex, age, body weight, general health, diet, mode and time of administration, and individual responsiveness of the human or animal to be treated, on the route of administration, efficacy, metabolic stability and duration of action of the compounds used, on whether the therapy is acute or chronic or prophylactic, or on whether other active compounds are administered in addition to the agent(s) of the invention.

Without limitation, depending on the type and severity of the disease, a typical daily dosage might range from about 1 µg/kg to 100 mg/kg of body weight or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. A preferred dosage of the active substance of the invention may be in the range from about 0.05 mg/kg to about 10 mg/kg of body weight. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g., every week or every three weeks.

#### Applications of the present compounds in plants

This invention also provides a method for treating or protecting plants from bacterial infection by applying an effective amount of the present compounds to the foliage, roots or the soil surrounding the plants or roots. In particular, said plants may be treated with compounds of any of formulas (VI), (I-a), (II-a), (III-a), (IV-a), (V-a), (I-b), (II-b), (III-b), (IV-b), (V-b), (I-c), (II-c), (III-c), (IV-c) or (V-c) as defined above, or *N*-oxide forms, addition salts, prodrugs, solvates or stereochemically isomeric forms thereof, as well as preferred subgroups of such substances. The present compounds can be combined with known pesticides or insecticides.

In order to achieve good dispersion and adhesion of the compounds as used to treat plants, it may be advantageous to formulate the compounds with components that aid dispersion and adhesion. Suitable formulations will be known to those skilled in the art. For example,

compounds within the present invention when used to treat or protect plants from antibiotic resistant bacterial infections can be formulated as wettable powders, granules and the like, or can be microencapsulated in a suitable medium and the like. Examples of other formulations include, but are not limited to soluble powders, wettable granules, dry flowables, aqueous flowables, wettable dispersible granules, emulsifiable concentrates and aqueous suspensions. Other suitable formulations will be known to those skilled in the art.

The above aspects and embodiments are further supported by the following non-limiting examples.

## EXAMPLES

### 10 Example 1: Purification of D-Ala-D-Ala ligase (Ddl ligase)

#### Construction of expression vector for Ddl ligase of *Enterococcus faecalis*:

Plasmids pGEM-T easy (Promega) and pBAD/Myc-His-A (Invitrogen) was used for the cloning of DNA fragments. *Escherichia coli* LMG194 (Invitrogen) was the host strain for recombinant plasmids. Total DNA from *Enterococcus faecalis* JH2-2 (Jacob & Hobbs 1974. J Bacteriol 117: 360-372) was prepared as described (Le Bouguenec *et al.* 1990. J Bacteriol 172: 727-734) and D-Ala-D-Ala gene was amplified by PCR using the *pfu* polymerase (Promega) and the primers 1 and 2

(1 : 5'-CGGGATCCATGGCTAAGATTATTTTGTGTATGGCGGCAGAAG-3' (SEQ ID NO 1));

2 : 5'-CGAATTCTGCAGTTTAAAACGATTCAAAGCTAAC-3' (SEQ ID NO 2).

20 Primer 1 containing an *NcoI* restriction site and the beginning of the Ddl gene and primer 2 containing the end of the gene with the Stop codon and an *EcoRI* restriction site. The purified PCR-amplified fragment was controlled by sequencing in the plasmid pGEM-T easy and was then subcloned in pBAD/Myc-His A at *NcoI* and *EcoRI* restriction sites. The resulting plasmid encodes for Ddl-His6 protein under the control of a L-arabinose inducible promoter (Guzman *et al.* 1995. J Bacteriol 177: 4121-4130). Constructed plasmid was transformed into compete strain of LMG194 *E. coli* for expression.

#### Overproduction and purification of the Ddl-His6 enzyme:

Bacterial cultures were inoculated in a minimal medium (RM media, Invitrogen, Gambetta & Lagarias 2001. PNAS 98: 10566-10571) containing 0.2% of glucose for the repression of His-

tagged Ddl and 100µg/ml of ampicillin. The inoculum was grown at 37°C to a DO600 of 0.4. At this point, L-arabinose was added to a final concentration of 2% for induction of His-tagged Ddl expression, and induced bacterial culture grown overnight at 25°C. The cultures were centrifuged 15 minutes at 4000 rpm and cell pellets were resuspended in cold buffer A (Buffer A : Hepes 50 mM pH 8.0, MgCl<sub>2</sub> 5mM, imidazole 10 mM, glycerol 10%) by concentrating 20x. All following steps of purification were performed in 4°C. Bacteria were disrupted twice by passage in a French Press (operated at 1000 psi) and centrifuged at 18000 g during 30 min.

The supernatant was incubated during 1h30min with HisLink resin (Promega) (5 ml of HisLink slurry for 10 ml of lysate) with a soft shaking. Lysate was removed and the resin was placed on a column and washed with buffer A (30 ml of buffer A for 5 ml of HisLink slurry). His-tagged proteins were eluted by adding buffer B (10 ml for 5 ml of HisLink slurry) ( Buffer B : Hepes 50 mM pH 8.0, MgCl<sub>2</sub> 5mM, NaCl 300 mM, imidazole 500 mM, glycerol 10%). Elution fraction was dialysed twice (for 2h and overnight) against buffer C (Buffer C : Hepes 50 mM pH 7.2, KCl 150 mM, MgCl<sub>2</sub> 5 mM, GSH 5 mM and glycerol 20% ; 2 L of buffer C for 150ml of elution buffer). After dialysis, proteins fractions were analyzed for purity by SDS-polyacrylamide gel electrophoresis (Novex tris-Glycine Gels 14%, Invitrogen); one unique band was obtained at ~42 kDa. Protein concentrations were determined by the method of Bradford using bovine serum albumin as standard (Quick Start Bradford Protein Assay, BioRad). Pure fractions are stored in buffer C at -80°C. 555 ml of pure proteins concentrated at 2.24 mg/ml were obtained from 12 L of induced bacterial culture.

## **Example 2: Ddl enzyme assay by TLC and inhibition studies**

### Protocol

Activity of the His-tagged Ddl ligase was determined by measuring the quantity of D- Ala-D- Ala produced from D-Ala using D-[<sup>14</sup>C]Ala. Assay mixtures (10 µl) contained Tris-HCl 20 mM pH 7.4, MgCl<sub>2</sub> 10 mM, KCl 10 mM, ATP 5 mM, enzyme ( 0.1 to 3 µg) and mixture of D-[1-<sup>14</sup>C]Ala 10 mM (Moravek: 51 mCi/mmol, 0.1mCi/ml) and unlabeled D-Ala to a final concentration of 10 mM at 0.02 µCi.

For testing the effect of inhibitors, molecules (at suitable concentration(s)) were preincubated for 5 min with the enzyme in the above assay mixture before addition of ATP and D-[<sup>14</sup>C]Ala (in a volume of 20 µl). Depending of the water-solubility of each inhibitor, up to 10% of DMSO was added in reaction buffer.

The mixture was incubated 30 min at 30°C. The reaction was stopped by boiling the samples for 5 min (immersion in a water bath set at 100°C), and centrifuged 10 min at 13,000 rpm. 2 µl of the reaction mixture was spotted on a 0.1-mm microcrystalline cellulose thin-layer chromatography sheet (Polygram Cel 400; Macherey-Nagel) and developed in butanol-glacial acetic acid-water (12:3:5 vol/vol/vol). After migration, plates were dried for 10 min at room temperature, and cutted in bands covering the position of D-Ala and D-Ala-D-Ala, respectively, based on the migration pattern of standard of D-Ala and D-Ala-D-Ala (revealed by staining with 0.25% ninhydrin in acetone following by drying 3 min at 120°C). The corresponding fragments were used for determination of their radioactivity by placing them in vials containing 10 ml of scintillation mixture (Ultima Gold from PerkinElmer) and by counting samples in a Packard TriCarb Liquid scintillation analyser (1900TR) with an efficiency of 95%. The percentage of conversion of D-Ala into the dimmer was calculated, and enzyme activity expressed as moles of product released per minute and per mg of enzyme.

### Results

D-Ala-D-Ala ligase has been purified from *Enterococcus faecalis* JH2-2. More than 1 g has been obtained from 12 L of induced bacterial culture with a purification factor of about 2 and a yield of about 65%. The activity of the pure enzyme was about 1.5 nmol/min/µg at 30°C.

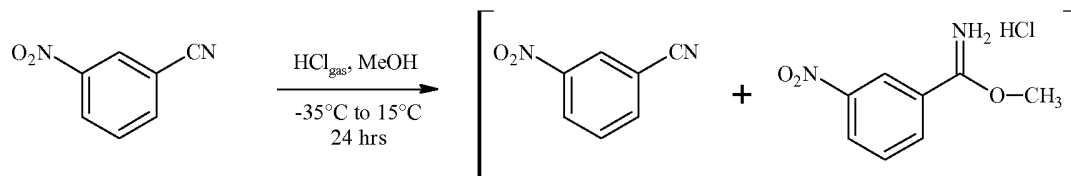
Initially, the present compounds of formulas (I-a), (II-a), (III-a), (IV-a) and (V-a) were tested at a fixed concentration corresponding to the IC<sub>50</sub> of D-cycloserine (0.6 mM), in comparison with cycloserine, with a 5 min preincubation of the enzyme with each inhibitor. Results shown in **Figure 1** demonstrate that said compounds cause significant Ddl inhibition.

Subsequently, concentration-inhibition relationships were examined for present compounds of formulas (I-a), (II-a) and (IV-a) as compared to D-cycloserine. Results are shown in **Figure 2**. Figure 2A also shows that DMSO used to solubilise some of the present compounds did not affect the Ddl ligase activity.

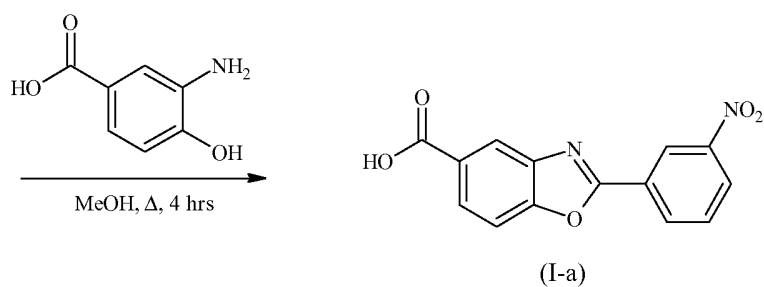
### **Example 3: Preparation of compounds of formula (I-a), (II-a), (III-a), (IV-a) and (V-a)**

The compound of formula (I-a) was prepared by the following reaction scheme 1:

35

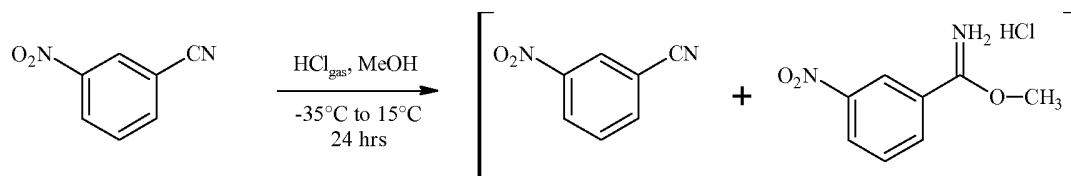
Scheme 1

Conversion: 30-60%

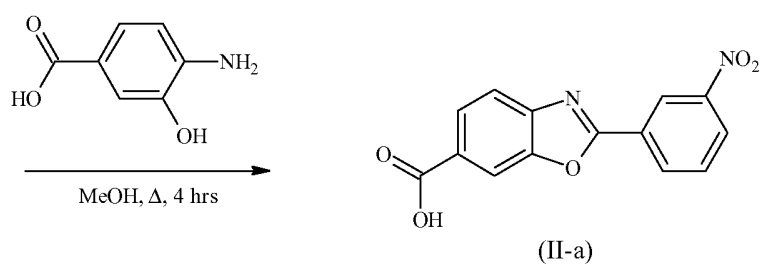


Yield: 25-32%

The compound of formula (II-a) was prepared using the following reaction scheme 2:

Scheme 2

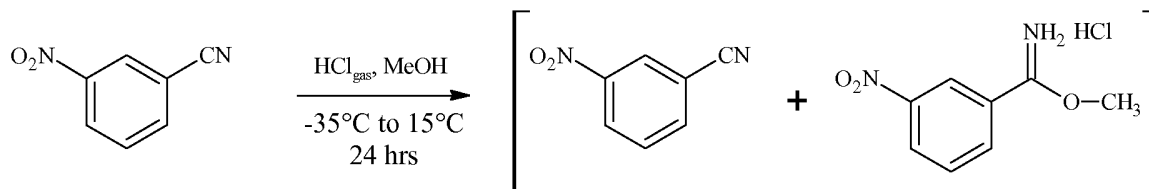
Conversion: 30-60%



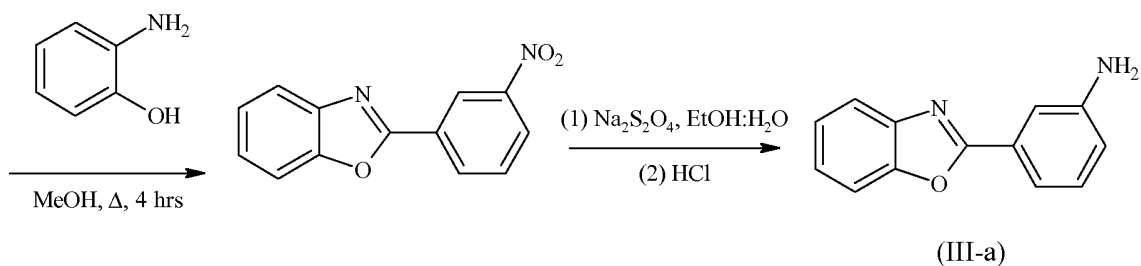
Yield: 25-32%

The compound of formula (III-a) was prepared following reaction scheme 3:

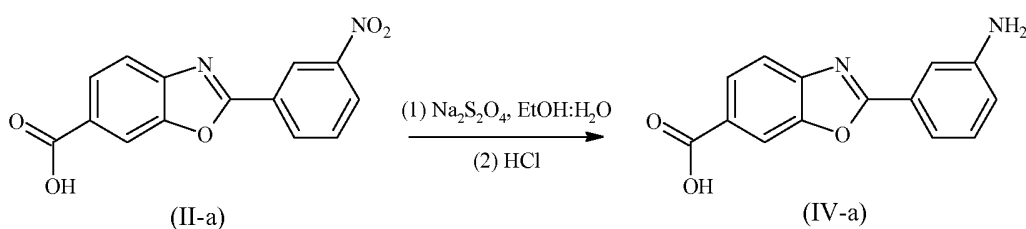
36

Scheme 3

Conversion: 30-60%



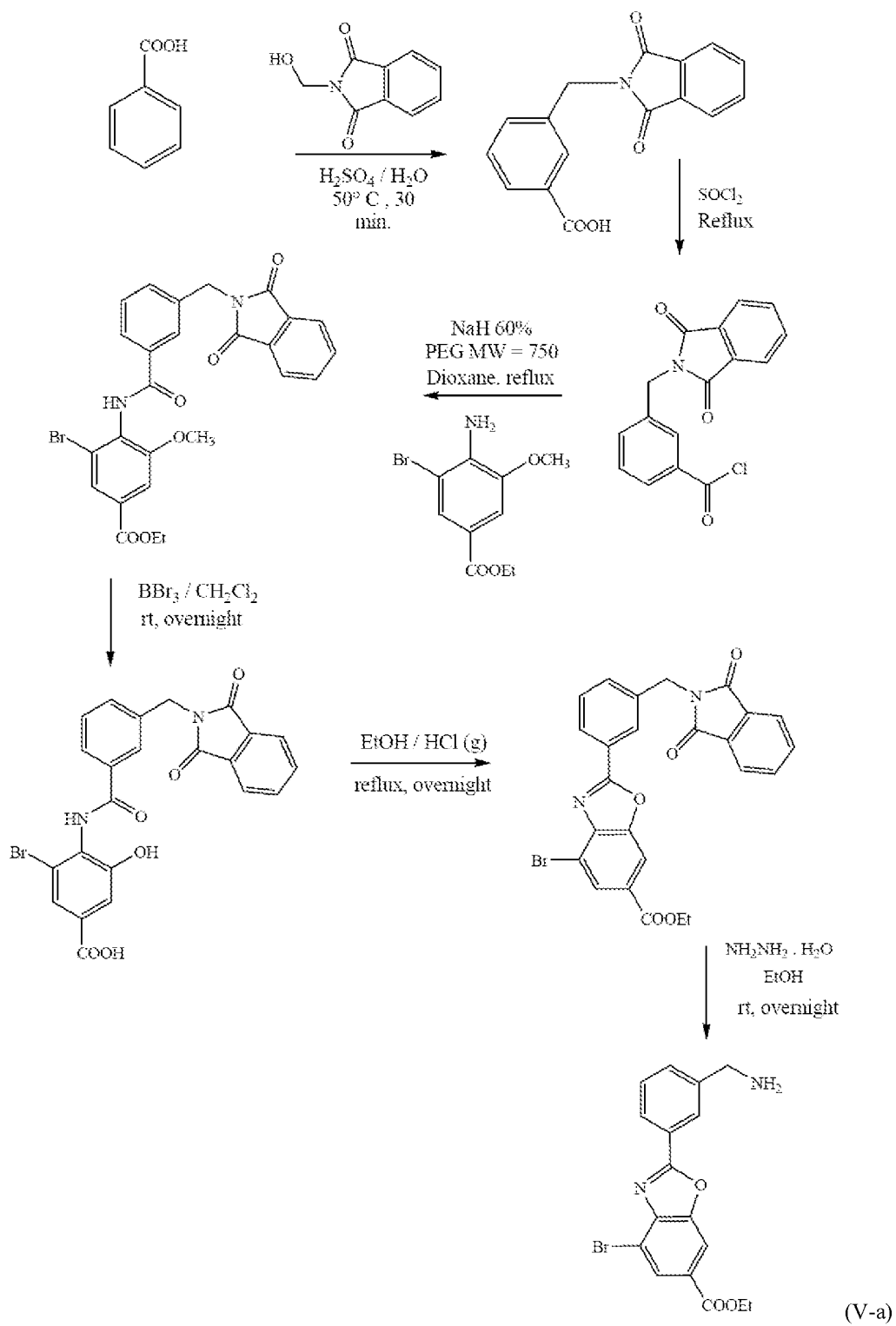
The compound (IV-a) was prepared from compound (II-a) following the reaction scheme 4:

Scheme 4

5

The compound (V-a) was prepared following the reaction scheme 5:

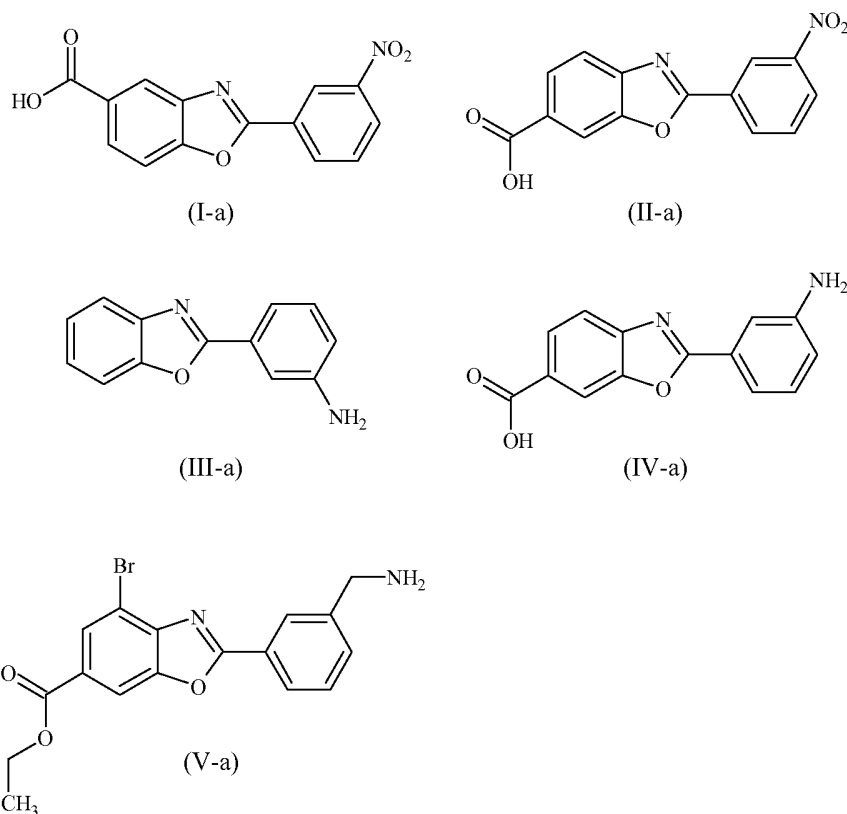
## Scheme 5



Accordingly, the invention also relates to a process for preparation of a compound of any of formula (I-a), (II-a), (III-a) (IV-a) and (V-a) as taught in above schemes 1, 2, 3, 4 and 5, respectively.

## CLAIMS

1. A compound of any of formulas (I-a), (II-a), (III-a), (IV-a) or (V-a):



5 or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug or solvate thereof, for use in the treatment of a bacterial infection.

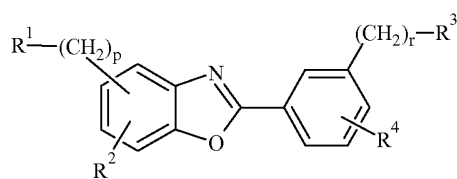
2. A compound of any of formulas (I-a), (II-a), (IV-a) or (V-a), or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug or solvate thereof, for use as a medicament.

3. A compound of any of formulas (I-a) or (V-a), an *N*-oxide form, addition salt, prodrug or solvate thereof.

10 4. A method for inhibition of DD-ligase *in vitro* comprising contacting a DD-ligase, or a composition or a bacterium comprising such, with a compound of any of formulas (I-a), (II-a), (III-a), (IV-a) or (V-a), or an *N*-oxide form, addition salt, prodrug or solvate thereof, in an amount sufficient to obtain said inhibition.

5. A method for preserving, disinfecting or sterilising a composition, device or apparatus contaminated or suspected to be contaminated with bacteria, comprising contacting said composition, device or apparatus with a compound of any of formulas (I-a), (II-a), (III-a), (IV-a) or (V-a), or an *N*-oxide form, addition salt, prodrug or solvate thereof.

5 6. A compound of formula (VI):



(VI)

or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, for use in the treatment of a bacterial infection, wherein:

10 p is 0, 1 or 2 and when p is 0 a direct bond is intended;

r is 0, 1 or 2 and when r is 0 a direct bond is intended;

$R^1$  is selected from hydrogen,  $-C(=O)R^5$ ,  $-S(=O)OR^8$ ,  $-S(=O)_2OR^8$ ,  $-PH(=O)OR^8$  and  $-P(=O)(OR^8)(OR^9)$ ,

15  $R^5$  is selected from hydrogen,  $C_{1-6}$ alkyl,  $-OR^6$ ,  $-SR^6$ ,  $-NR^6R^7$ , and  $C_{1-6}$ alkyl substituted with one or more substituents selected from halo, hydroxy and oxo,

$R^6$  and  $R^7$  are each independently selected from hydrogen,  $C_{1-6}$ alkyl, and  $C_{1-6}$ alkyl substituted with one or more substituents selected from halo, hydroxy,  $C_{1-6}$ alkyloxy, carboxyl,  $C_{1-6}$ alkyloxycarbonyl and  $C_{1-6}$ alkylcarbonyloxy,

20  $R^8$  and  $R^9$  are each independently selected from hydrogen,  $C_{1-6}$ alkyl, and  $C_{1-6}$ alkyl substituted with one or more substituents selected from halo, hydroxy and  $C_{1-6}$ alkyloxy;

$R^2$  is selected from hydrogen, halo, hydroxy, cyano, nitro, amino, mono- or di( $C_{1-6}$ alkyl)amino,  $C_{1-6}$ alkylcarbonylamino,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl,  $C_{1-6}$ alkyloxy, carboxyl,  $C_{1-6}$ alkylcarbonyloxy,  $C_{1-6}$ alkyloxycarbonyl, each of said groups optionally substituted with one or more substituents selected from halo, hydroxy and  $C_{1-6}$ alkyloxy;

25  $R^3$  is selected from  $-NHR^{10}$  and nitro,

R<sup>10</sup> is selected from hydrogen, C<sub>1-6</sub>alkyl, C<sub>1-6</sub>alkylcarbonyl and C<sub>1-6</sub>alkyloxycarbonyl, each of said groups optionally substituted with one or more substituents selected from halo, hydroxy, amino, nitro and C<sub>1-6</sub>alkyloxy;

R<sup>4</sup> is selected from hydrogen, halo, hydroxy, cyano, nitro, amino, mono- or di(C<sub>1-6</sub>alkyl)amino, C<sub>1-6</sub>alkylcarbonylamino, C<sub>1-6</sub>alkyl, polyhaloC<sub>1-6</sub>alkyl and C<sub>1-6</sub>alkyloxy, each of said groups being optionally substituted with one or more substituents selected from halo, hydroxy, amino, nitro and C<sub>1-6</sub>alkyloxy.

7. A method for inhibition of DD-ligase *in vitro* comprising contacting a DD-ligase, or a composition or a bacterium comprising such, with a compound of formula (VI) as defined in claim 6, or an *N*-oxide form, addition salt, prodrug or solvate thereof, in an amount sufficient to obtain said inhibition.

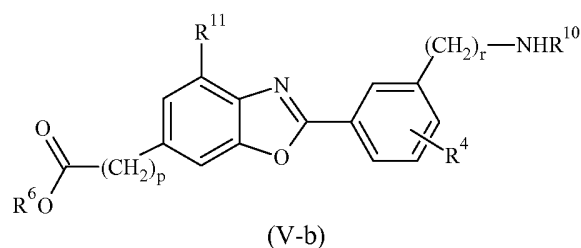
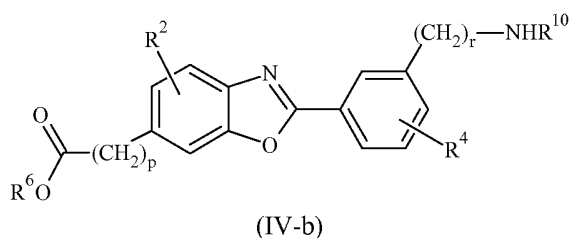
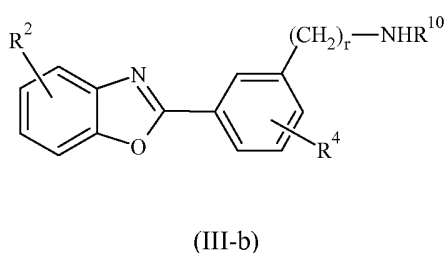
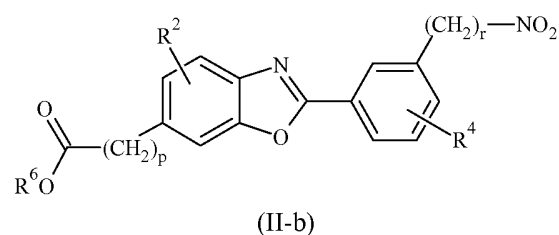
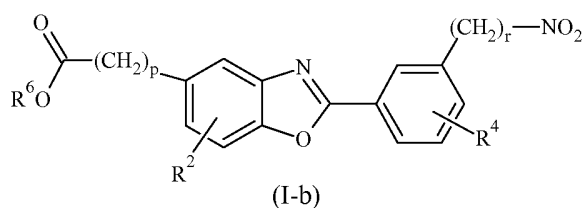
8. A method for preserving, disinfecting or sterilising a composition, device or apparatus contaminated or suspected to be contaminated with bacteria, comprising contacting said composition, device or apparatus with a compound of formula (VI) as defined in claim 6, or an *N*-oxide form, addition salt, prodrug or solvate thereof.

9. A method for treating or protecting plants from bacterial infection by applying an effective amount of a compound of formula (VI) as defined in claim 6, or an *N*-oxide form, addition salt, prodrug or solvate thereof, to the foliage, roots or the soil surrounding the plants or roots.

10. The compound for use in the treatment of a bacterial infection according to claim 6, or the methods of any of claims 7, 8 or 9, wherein any one or more or all of the following restrictions apply:

- a) p is 0;
- b) r is 0 or 1;
- c) R<sup>1</sup> is selected from hydrogen, -C(=O)OH and or -C(=O)OC<sub>1-6</sub>alkyl;
- d) R<sup>2</sup> is selected from hydrogen and halo;
- e) R<sup>3</sup> is -NH<sub>2</sub>;
- f) R<sup>4</sup> is hydrogen.

11. The compound for use in the treatment of a bacterial infection according to claim 6, or the methods of any of claims 7, 8 or 9, wherein compound of formula (VI) is chosen from compounds of any of formulas (I-b), (II-b), (III-b), (IV-b) or (V-b):



or an *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, wherein:

*p* is 0, 1 or 2, preferably *p* is 0 or 1, more preferably *p* is 0;

5 *r* is 0, 1 or 2, preferably *r* is 0 or 1, very preferably *r* is 0 or very preferably *r* is 1;

*R*<sup>6</sup> is selected from hydrogen, C<sub>1-6</sub>alkyl, and C<sub>1-6</sub>alkyl substituted with one or more substituents selected from halo, hydroxy, C<sub>1-6</sub>alkyloxy, carboxyl, C<sub>1-6</sub>alkyloxycarbonyl and C<sub>1-6</sub>alkylcarbonyloxy, preferably *R*<sup>6</sup> is hydrogen or C<sub>1-6</sub>alkyl, very preferably *R*<sup>6</sup> is hydrogen or very preferably *R*<sup>6</sup> is C<sub>1-6</sub>alkyl;

10 *R*<sup>2</sup> is selected from hydrogen, halo, hydroxy, cyano, nitro, amino, mono- or di(C<sub>1-6</sub>alkyl)amino, C<sub>1-6</sub>alkylcarbonylamino, C<sub>1-6</sub>alkyl, polyhaloC<sub>1-6</sub>alkyl, C<sub>1-6</sub>alkyloxy, carboxyl, C<sub>1-6</sub>alkylcarbonyloxy, C<sub>1-6</sub>alkyloxycarbonyl, each of said groups optionally substituted with one or more substituents selected from halo, hydroxy and C<sub>1-6</sub>alkyloxy, preferably *R*<sup>2</sup> is selected from hydrogen, halo, hydroxy, C<sub>1-6</sub>alkyl, polyhaloC<sub>1-6</sub>alkyl and C<sub>1-6</sub>alkyloxy, more preferably *R*<sup>2</sup> is hydrogen or halo;

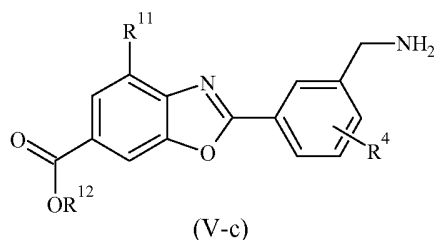
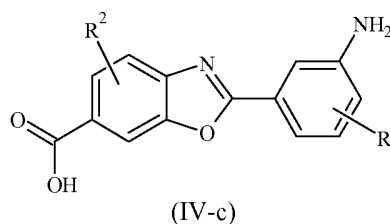
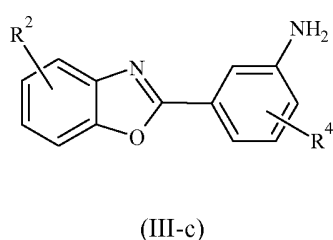
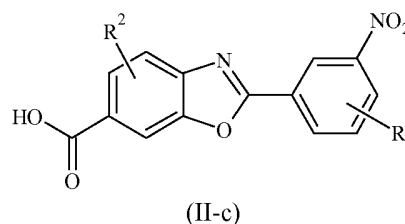
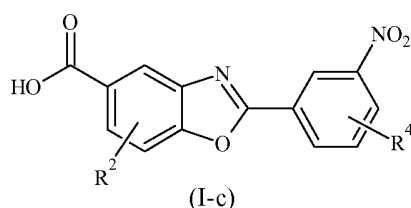
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$R^{10}$  is selected from hydrogen,  $C_{1-6}$ alkyl,  $C_{1-6}$ alkylcarbonyl and  $C_{1-6}$ alkyloxycarbonyl, each of said groups optionally substituted with one or more substituents selected from halo, hydroxy, amino, nitro and  $C_{1-6}$ alkyloxy, preferably  $R^{10}$  is selected from hydrogen and  $C_{1-6}$ alkyl optionally substituted with one or more substituents selected from halo, hydroxy, amino, nitro and  $C_{1-6}$ alkyloxy, more preferably  $R^{10}$  is hydrogen or  $C_{1-6}$ alkyl, even more preferably  $R^{10}$  is hydrogen;

$R^4$  is selected from hydrogen, halo, hydroxy, cyano, nitro, amino, mono- or di( $C_{1-6}$ alkyl)amino,  $C_{1-6}$ alkylcarbonylamino,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl and  $C_{1-6}$ alkyloxy, each of said groups being optionally substituted with one or more substituents selected from halo, hydroxy, amino, nitro and  $C_{1-6}$ alkyloxy, preferably  $R^4$  is selected from hydrogen, halo, hydroxy,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl and  $C_{1-6}$ alkyloxy; more preferably  $R^4$  is hydrogen;

$R^{11}$  is halo, preferably  $R^{11}$  is fluoro, chloro, bromo or iodo, more preferably  $R^{11}$  is bromo.

12. The compound for use in the treatment of a bacterial infection according to claim 6, or the methods of any of claims 7, 8 or 9, wherein compound of formula (VI) is chosen from compounds of any of formulas (I-c), (II-c), (III-c), (IV-c) or (V-c):



or an *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, wherein:

$R^2$  is selected from hydrogen, halo, hydroxy, cyano, nitro, amino, mono- or di( $C_{1-6}$ alkyl)amino,  $C_{1-6}$ alkylcarbonylamino,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl,  $C_{1-6}$ alkyloxy, carboxyl,  $C_{1-6}$ alkylcarbonyloxy,  $C_{1-6}$ alkyloxycarbonyl, each of said groups optionally substituted with one or more substituents selected from halo, hydroxy and  $C_{1-6}$ alkyloxy, preferably  $R^2$  is selected from hydrogen, halo, hydroxy,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl and  $C_{1-6}$ alkyloxy, more preferably  $R^2$  is hydrogen or halo;

$R^4$  is selected from hydrogen, halo, hydroxy, cyano, nitro, amino, mono- or di( $C_{1-6}$ alkyl)amino,  $C_{1-6}$ alkylcarbonylamino,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl and  $C_{1-6}$ alkyloxy, each of said groups being optionally substituted with one or more substituents selected from halo, hydroxy, amino, nitro and  $C_{1-6}$ alkyloxy, preferably  $R^4$  is selected from hydrogen, halo, hydroxy,  $C_{1-6}$ alkyl, polyhalo $C_{1-6}$ alkyl and  $C_{1-6}$ alkyloxy; more preferably  $R^4$  is hydrogen;

$R^{11}$  is halo, preferably  $R^{11}$  is fluoro, chloro, bromo or iodo, more preferably  $R^{11}$  is bromo;

$R^{12}$  is  $C_{1-6}$ alkyl, preferably  $R^{12}$  is methyl or ethyl, more preferably  $R^{12}$  is ethyl.

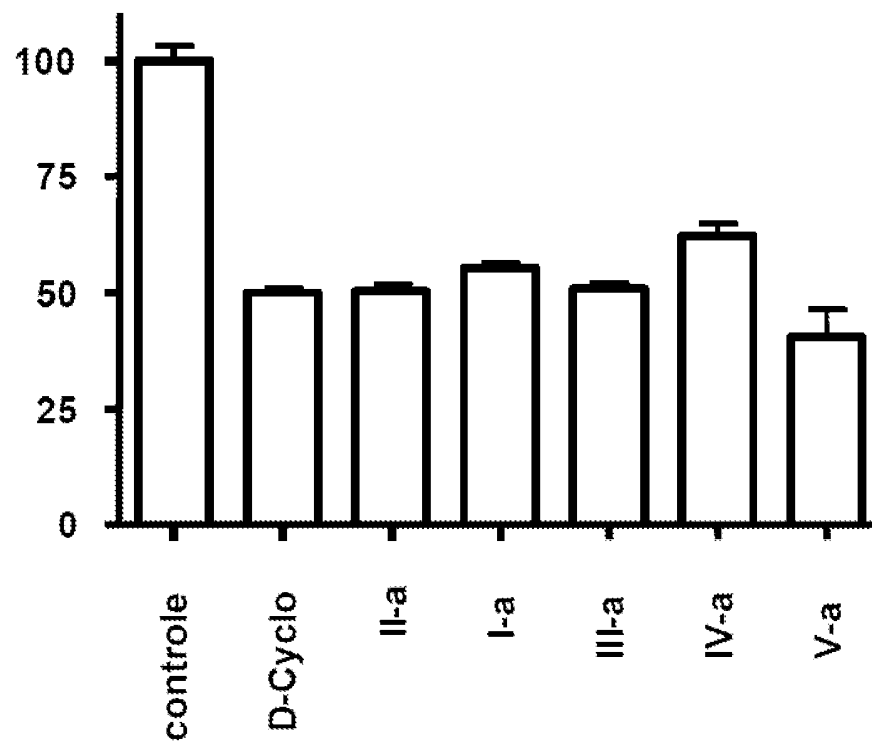
13. A compound of any of formulas (I-b), (II-b), (IV-b) or (V-b) wherein each  $p$ ,  $r$ ,  $R^6$ ,  $R^2$ ,  $R^{10}$ ,  $R^4$  and  $R^{11}$  is as defined in claim 11, or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, for use as a medicament.

14. A compound of any of formulas (I-c), (II-c), (IV-c) or (V-c), wherein each  $R^2$ ,  $R^4$ ,  $R^{11}$  and  $R^{12}$  is as defined in claim 12, or a pharmaceutically acceptable *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof, for use as a medicament.

15. A compound of any of formulas (I-b) or (V-b) wherein each  $p$ ,  $r$ ,  $R^6$ ,  $R^2$ ,  $R^{10}$ ,  $R^4$  and  $R^{11}$  is as defined in claim 11, an *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof.

16. A compound of any of formulas (I-c) or (V-c) wherein each  $R^2$ ,  $R^4$ ,  $R^{11}$  and  $R^{12}$  is as defined in claim 12, an *N*-oxide form, addition salt, prodrug, solvate or a stereochemically isomeric form thereof.

1 / 2  
FIGURE 1



2 / 2  
FIGURE 2