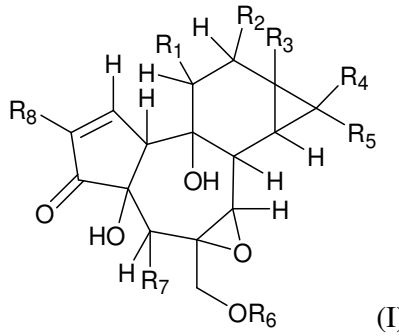
Of these 'non-antibiotic' strategies, disrupting the integrity of the EPS matrix of established biofilms is a particularly attractive. By making the EPS matrix more permeable, disaggregating the bacteria and interfering with the pathogenic signalling network, this strategy theoretically makes the individual bacteria more accessible to the immune system and/or to the potentiating the action of antibiotics (Gunn *et al.* 2016; Fleming & Rumbaugh 2017). Using this approach, a range of therapies involving enzymes (proteases, DNases, glycoside hydrolases), peptides, monoclonal antibodies and polymers that target specific components of the matrix (e.g. exopolysaccharides and eDNA) are under investigation (Koo *et al.* 2017).

Epoxytiglienones are small molecules with a range of bioactive properties. They are potent anti-tumour compounds, have antiparasitic properties, and stimulate immune and other cellular responses that promote healing of acute and chronic wounds. Previously, epoxytiglienones have been reported to have direct antibiotic activity against a range of Gram-positive bacteria (WO2007/070985 and WO2014/169356). However, in recent studies to more specifically evaluate the antibiotic properties of epoxytiglienone compounds against pathogenic strains of three Gram-negative pathogens (*E. coli, P. aeruginosa* and *A. baumannii*), no antibiotic effects have been found and it was not possible to establish a minimal inhibitory concentration (MIC) in conventional planktonic culture systems used for routine antibiotic screening.

The present invention is predicated, at least in part, on the subsequent, unexpected discovery that despite the absence of any direct antibiotic activity against planktonic Gram-negative bacteria, epoxytiglienones have potent, 'non-antibiotic' effects on established biofilms of a range of Gram-negative bacteria.

Summary

In an example of the disclosure there is provided a method of dispersing a biofilm comprising Gram-negative bacteria comprising exposing the biofilm to an epoxytiglienone compound for formula (I):



(I)

wherein

R_1 is selected from hydrogen and $C_{1\text{-}6}$alkyl;

R_2 is selected from –O$C_{1\text{-}8}$alkyl, -O$C_{2\text{-}8}$alkenyl, -O$C_{2\text{-}8}$alkynyl, -OC(O)$C_{1\text{-}7}$alkyl, -OC(O)$C_{2\text{-}7}$alkenyl and -OC(O)$C_{2\text{-}7}$alkynyl;

R_3 is selected from -OH, –O$C_{1\text{-}8}$alkyl, -O$C_{2\text{-}8}$alkenyl, -O$C_{2\text{-}8}$alkynyl, -OC(O)$C_{1\text{-}7}$alkyl, -OC(O)$C_{2\text{-}7}$alkenyl and -OC(O)$C_{2\text{-}7}$alkynyl;

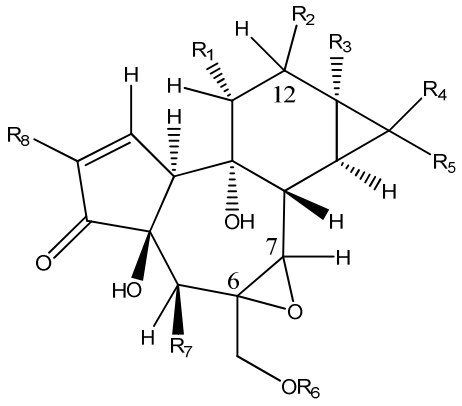
R_4 and R_5 are independently selected from hydrogen and $C_{1\text{-}6}$alkyl;

R_6 is selected from hydrogen, -$C_{1\text{-}6}$alkyl, -$C_{2\text{-}6}$alkenyl, -$C_{2\text{-}6}$alkynyl, -C(O)$C_{1\text{-}6}$alkyl, -C(O)$C_{2\text{-}6}$alkenyl, –C(O)$C_{2\text{-}6}$alkynyl, -C(O)$C_{3\text{-}8}$cycloalkyl, -C(O)$C_{1\text{-}6}$alkyl$C_{3\text{-}8}$cycloalkyl, -C(O)$C_{2\text{-}6}$alkenyl$C_{3\text{-}8}$cycloalkyl, -C(O)$C_{2\text{-}6}$alkynyl$C_{3\text{-}8}$cycloalkyl, -C(O)aryl, -C(O)$C_{1\text{-}6}$alkylaryl, -C(O)$C_{2\text{-}6}$alkenylaryl and -OC(O)$C_{2\text{-}6}$alkynylaryl;

R_7 is selected from hydroxy, -O$C_{1\text{-}6}$alkyl, -O$C_{2\text{-}6}$alkenyl, -O$C_{2\text{-}6}$alkynyl, -OC(O)$C_{1\text{-}6}$alkyl, -OC(O)$C_{2\text{-}6}$alkenyl, –OC(O)$C_{2\text{-}6}$alkynyl, -C(O)$C_{3\text{-}8}$cycloalkyl, -C(O)$C_{1\text{-}6}$alkyl$C_{3\text{-}8}$cycloalkyl, -C(O)$C_{2\text{-}6}$alkenyl$C_{3\text{-}8}$cycloalkyl, -C(O)$C_{2\text{-}6}$alkynyl$C_{3\text{-}8}$cycloalkyl, -OC(O)aryl, -OC(O)$C_{1\text{-}6}$alkylaryl, -C(O)$C_{2\text{-}6}$alkenylaryl and -C(O)$C_{2\text{-}6}$alkynylaryl;

R_8 is selected from hydrogen and $C_{1\text{-}6}$alkyl; or a salt thereof.

In an aspect, there is provided an *ex vivo* method of dispersing a biofilm comprising Gram-negative bacteria comprising exposing the biofilm to an epoxytiglienone compound for formula (II):



(II)

wherein

R_1 is selected from hydrogen and C_{1-6}alkyl;

R_2 is selected from -OC(O)C_{1-7}alkyl, -OC(O)C_{2-7}alkenyl and -OC(O)C_{2-7}alkynyl;

R_3 is selected from-OC(O)C_{1-7}alkyl, -OC(O)C_{2-7}alkenyl and -OC(O)C_{2-7}alkynyl;

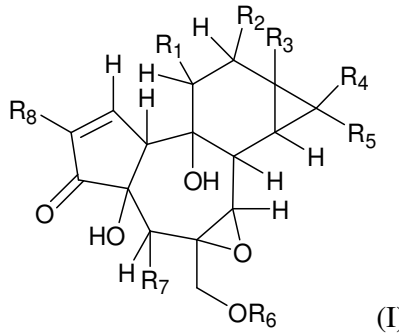
R_4 and R_5 are independently selected from hydrogen and C_{1-6}alkyl;

R_6 is selected from hydrogen and -C(O)C_{1-6}alkyl;

R_7 is hydroxy; and

R_8 is selected from hydrogen or C_{1-6}alkyl; or a pharmaceutically acceptable salt thereof.

In an example of the disclosure there is provided a method of treating a bacterial infection comprising a biofilm comprising Gram-negative bacteria, said method comprising locally administering to the bacterial infection an epoxytiglienone compound of formula (I):



(I)

wherein

R_1 is selected from hydrogen and $C_{1\text{-}6}$alkyl;

R_2 is selected from –O$C_{1\text{-}8}$alkyl, -O$C_{2\text{-}8}$alkenyl, -O$C_{2\text{-}8}$alkynyl, -OC(O)$C_{1\text{-}7}$alkyl, -OC(O)$C_{2\text{-}7}$alkenyl and -OC(O)$C_{2\text{-}7}$alkynyl;

R_3 is selected from -OH, –O$C_{1\text{-}8}$alkyl, -O$C_{2\text{-}8}$alkenyl, -O$C_{2\text{-}8}$alkynyl, -OC(O)$C_{1\text{-}7}$alkyl, -OC(O)$C_{2\text{-}7}$alkenyl and -OC(O)$C_{2\text{-}7}$alkynyl;

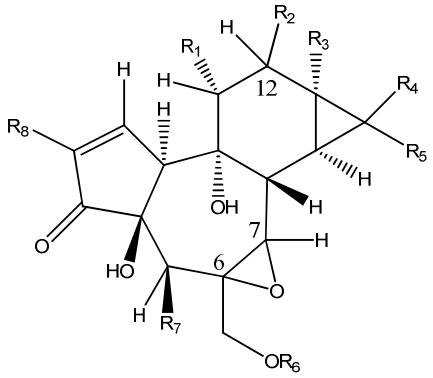
R_4 and R_5 are independently selected from hydrogen and $C_{1\text{-}6}$alkyl;

R_6 is selected from hydrogen, -$C_{1\text{-}6}$alkyl, -$C_{2\text{-}6}$alkenyl, -$C_{2\text{-}6}$alkynyl, -C(O)$C_{1\text{-}6}$alkyl, -C(O)$C_{2\text{-}6}$alkenyl, –C(O)$C_{2\text{-}6}$alkynyl, -C(O)$C_{3\text{-}8}$cycloalkyl, -C(O)$C_{1\text{-}6}$alkyl$C_{3\text{-}8}$cycloalkyl, -C(O)$C_{2\text{-}6}$alkenyl$C_{3\text{-}8}$cycloalkyl, -C(O)$C_{2\text{-}6}$alkynyl$C_{3\text{-}8}$cycloalkyl, -C(O)aryl, -C(O)$C_{1\text{-}6}$alkylaryl, -C(O)$C_{2\text{-}6}$alkenylaryl and -OC(O)$C_{2\text{-}6}$alkynylaryl;

R_7 is selected from hydroxy, -O$C_{1\text{-}6}$alkyl, -O$C_{2\text{-}6}$alkenyl, -O$C_{2\text{-}6}$alkynyl, -OC(O)$C_{1\text{-}6}$alkyl, -OC(O)$C_{2\text{-}6}$alkenyl, –OC(O)$C_{2\text{-}6}$alkynyl, -C(O)$C_{3\text{-}8}$cycloalkyl, -C(O)$C_{1\text{-}6}$alkyl$C_{3\text{-}8}$cycloalkyl, -C(O)$C_{2\text{-}6}$alkenyl$C_{3\text{-}8}$cycloalkyl, -C(O)$C_{2\text{-}6}$alkynyl$C_{3\text{-}8}$cycloalkyl, -OC(O)aryl, -OC(O)$C_{1\text{-}6}$alkylaryl, -C(O)$C_{2\text{-}6}$alkenylaryl and -C(O)$C_{2\text{-}6}$alkynylaryl;

R_8 is selected from hydrogen and $C_{1\text{-}6}$alkyl; or a pharmaceutically acceptable salt thereof.

In a further aspect of the disclosure there is provided a use of an epoxytiglienone compound of formula (II):



(II)

wherein

R_1 is selected from hydrogen and C_{1-6}alkyl;

R_2 is selected from -OC(O)C_{1-7}alkyl, -OC(O)C_{2-7}alkenyl and -OC(O)C_{2-7}alkynyl;

R_3 is selected from -OC(O)C_{1-7}alkyl, -OC(O)C_{2-7}alkenyl and -OC(O)C_{2-7}alkynyl;

R_4 and R_5 are independently selected from hydrogen and C_{1-6}alkyl;

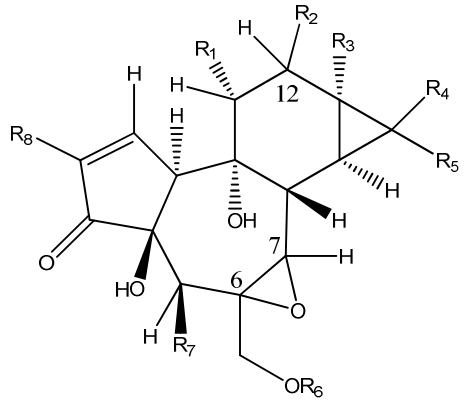
R_6 is selected from hydrogen and -C(O)C_{1-6}alkyl;

R_7 is hydroxy;

R_8 is selected from hydrogen and C_{1-6}alkyl; or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a bacterial infection comprising a biofilm comprising Gram-negative bacteria wherein the compound of formula (II) is to be administered locally to the bacterial infection.

In a further aspect of the disclosure there is provided an *ex vivo* method of preventing a biofilm comprising Gram-negative bacteria forming on a medical device or dispersing a biofilm comprising Gram-negative bacteria on a medical device, said method comprising

applying an epoxytiglienone compound of formula (I) to the medical device: the compound of formula (I) comprising



wherein

R_1 is selected from hydrogen and $C_{1\text{-}6}$alkyl;

R_2 is selected from -OC(O)$C_{1\text{-}7}$alkyl, -OC(O)$C_{2\text{-}7}$alkenyl and -OC(O)$C_{2\text{-}7}$alkynyl;

R_3 is selected from -OC(O)$C_{1\text{-}7}$alkyl, -OC(O)$C_{2\text{-}7}$alkenyl and -OC(O)$C_{2\text{-}7}$alkynyl;

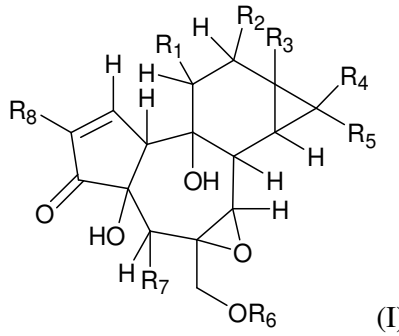
R_4 and R_5 are independently selected from hydrogen and $C_{1\text{-}6}$alkyl;

R_6 is selected from hydrogen and -C(O)$C_{1\text{-}6}$alkyl;

R_7 is hydroxy; and

R_8 is selected from hydrogen and $C_{1\text{-}6}$alkyl; or a pharmaceutically acceptable salt thereof

In a further example of the disclosure there is provided a method of preventing a biofilm comprising Gram-negative bacteria forming or dispersing a biofilm comprising Gram-negative bacteria on a medical device, said method comprising applying an epoxytiglienone compound of formula (I) to the medical device, the compound of formula (I) being:



(I)

wherein

R_1 is selected from hydrogen and C_{1-6}alkyl;

R_2 is selected from –OC_{1-8}alkyl, -OC_{2-8}alkenyl, -OC_{2-8}alkynyl, -OC(O)C_{1-7}alkyl, -OC(O)C_{2-7}alkenyl and -OC(O)C_{2-7}alkynyl;

R_3 is selected from -OH, –OC_{1-8}alkyl, -OC_{2-8}alkenyl, -OC_{2-8}alkynyl, -OC(O)C_{1-7}alkyl, -OC(O)C_{2-7}alkenyl and -OC(O)C_{2-7}alkynyl;

R_4 and R_5 are independently selected from hydrogen and C_{1-6}alkyl;

R_6 is selected from hydrogen, -C_{1-6}alkyl, -C_{2-6}alkenyl, -C_{2-6}alkynyl, -C(O)C_{1-6}alkyl, -C(O)C_{2-6}alkenyl, –C(O)C_{2-6}alkynyl, -C(O)C_{3-8}cycloalkyl, -C(O)C_{1-6}alkylC_{3-8}cycloalkyl, -C(O)C_{2-6}alkenylC_{3-8}cycloalkyl, -C(O)C_{2-6}alkynylC_{3-8}cycloalkyl, -C(O)aryl, -C(O)C_{1-6}alkylaryl, -C(O)C_{2-6}alkenylaryl and -OC(O)C_{2-6}alkynylaryl;

R_7 is selected from hydroxy, -OC_{1-6}alkyl, -OC_{2-6}alkenyl, -OC_{2-6}alkynyl, -OC(O)C_{1-6}alkyl, -OC(O)C_{2-6}alkenyl, –OC(O)C_{2-6}alkynyl, -C(O)C_{3-8}cycloalkyl, -C(O)C_{1-6}alkylC_{3-8}cycloalkyl, -C(O)C_{2-6}alkenylC_{3-8}cycloalkyl, -C(O)C_{2-6}alkynylC_{3-8}cycloalkyl, -OC(O)aryl, -OC(O)C_{1-6}alkylaryl, -C(O)C_{2-6}alkenylaryl and -C(O)C_{2-6}alkynylaryl;

R_8 is selected from hydrogen and C_{1-6}alkyl; or a pharmaceutically acceptable salt thereof.

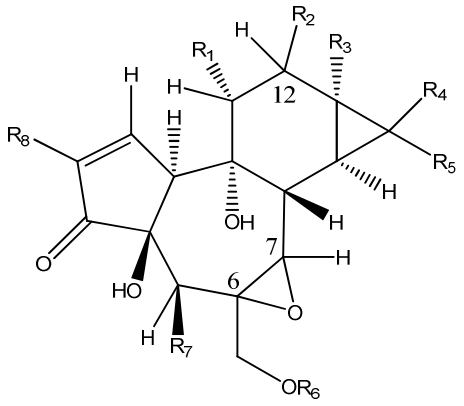
Detailed Description

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

Claims

1. An *ex vivo* method of dispersing a biofilm comprising Gram-negative bacteria comprising exposing the biofilm to an epoxytiglienone compound for formula (II):



(II)

wherein

R_1 is selected from hydrogen and C_{1-6}alkyl;

R_2 is selected from -OC(O)C_{1-7}alkyl, -OC(O)C_{2-7}alkenyl and -OC(O)C_{2-7}alkynyl;

R_3 is selected from-OC(O)C_{1-7}alkyl, -OC(O)C_{2-7}alkenyl and -OC(O)C_{2-7}alkynyl;

R_4 and R_5 are independently selected from hydrogen and C_{1-6}alkyl;

R_6 is selected from hydrogen and -C(O)C_{1-6}alkyl;

R_7 is hydroxy; and

R_8 is selected from hydrogen or C_{1-6}alkyl; or a pharmaceutically acceptable salt thereof.

2. The method according to claim 1 wherein R_1 is C_{1-3} alkyl.

3. The method according to claim 1 or claim 2, wherein R_2 is selected from -OC(O)C_{3-6}alkyl and -OC(O)C_{3-6}alkenyl.

4. The method according to any one of claims 1 to 3, wherein R_3 is selected from -OC(O)C_{3-6}alkyl, -OC(O)C_{3-6}alkenyl and -OC(O)C_{3-6}alkynyl.

5. The method according to any one of claims 1 to 4, wherein R_4 and R_5 are each methyl.

6. The method according to any one of claims 1 to 5, wherein R_6 is selected from hydrogen, -C(O)CH_3, -C(O)CH_2CH_3, -C(O)$CH(CH_3)_2$ or -C(O)$CH_2CH_2CH_3$.

7. The method according to any one of claims 1 to 6, wherein R_8 is C_{1-3}alkyl.

8. The method according to any one of claims 1 to 7, wherein the alkyl or alkenyl group of R_2 and/or R_3 are branched alkyl or branched alkenyl groups.

9. The method according to any one of claims 1 to 7, wherein the alkyl or alkenyl group of R_2 and/or R_3 are linear alkyl or linear alkenyl groups.

10. The method according to claim 1, wherein the compound of formula (II) is selected from:

12-tigloyl-13-(2-methylbutanoyl)-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tiglien-3-one (Compound 1);

12,13-di-(2-methylbutanoyl)-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tiglien-3-one (Compound 2);

12-hexanoyl-13-(2-methylbutanoyl)-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tiglien-3-one (Compound 3);

12,13-dihexanoyl-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tiglien-3-one (Compound 4);

12-tigloyl-13-(2-methylbutanoyl)-6,7-epoxy-4,5,9,12,13-pentahydroxy-20-acetyloxy-1-tiglien-3-one (Compound 5);

12-propanoyl-13-(2-methylbutanoyl)-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tiglien-3-one (Compound 6);

12,13-ditigloyl-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tiglien-3-one (Compound 7);

12-(2-methylbutanoyl)-13-tigloyl-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tiglien-3-one (Compound 8);

12-butanoyl-13-(2-methylbutanoyl)-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tiglien-3-one (Compound 9);

12-(3,3-dimethylbut-2-enoyl)-13-(2-methylbutanoyl)-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tiglien-3-one (Compound 10);

12-hex-2,4-dienoyl-13-(2-methylbutanoyl)-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tiglien-3-one (Compound 11);

12-tigloyl-13-(2-methylpropanoyl)-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tiglien-3-one (Compound 12);

12-but-2-enoyl-13-(2-methylbutanoyl)-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-

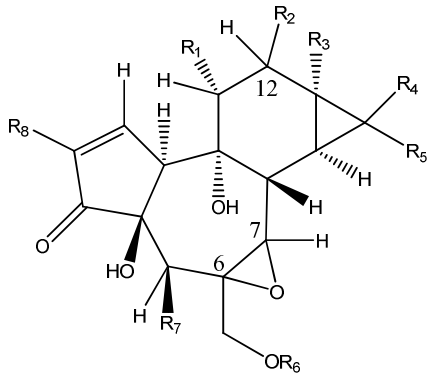
tiglien-3-one (Compound 13);
12-tigloyl-13-butanoyl-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tiglien-3-one (Compound 14);
12,13-dibutanoyl-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tiglien-3-one (Compound 15);
12,13-dipentanoyl-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tiglien-3-one (Compound 16);
12,13-di-(2*E*,4*E*)-hexa-2,4-dienoyl-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tiglien-3-one (Compound 17);
12-(2-methylprop-2-enoyl)-13-(2-methylbutanoyl)-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tigliaen-3-one (Compound 19);
12,13-di-heptanoyl-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tigliaen-3-one (Compound 20); and
12,13-di-(3-methylbutanoyl)-6,7-epoxy-4,5,9,12,13,20-hexahydroxy-1-tigliaen-3-one (Compound 21);
or a pharmaceutically acceptable salt thereof.

11. The method according to any one of claims 1 to 10, wherein the biofilm comprising Gram-negative bacteria comprises at least one Gram-negative bacteria selected from *Pseudomonas* species, *Acinetobacter* species, *Aeromonas* species, *Bacteroides* species, *Bordetella* species, *Borrelia* species, *Burkholderia* species, *Citrobacter* species, *Compylobacter* species, *Escherichia* species, *Enterobacter* species, *Flavobacterium* species, *Fusobacterium* species, *Klebsiella* species, *Leptospira* species, *Neisseria* species, *Helicobacter* species, *Hemophilus* species, *Legionella* species, *Moraxella* species, *Yersinia* species, *Oligella* species, *Pantoea* Species, *Porphyromonas* species, *Prevotella* species, *Proteus* species, *Raoutella* species, *Salmonella* species, *Serratia* species, *Shigella* species, *Sphingomonas* species, *Stenotophomonas* species, *Treponema* species, *Veillonella* species and *Vibrio* species.

12. The method according to claim 11, wherein the Gram-negative bacteria is selected from *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Hemophilus influenzae*, *Legionella pneumophila*, *Yersinia pestis*, *Yersinia enterocolitica*, *Salmonella*

enterica, *Salmonella bongori*, *Proteus mirabilis*, *Enterobacter cloacae*, *Serratia marcescens, Bacteroides fragilis*, *Fusobacterium necrophorum*, *Burkholderia cepacian* and *Prevotella intermedia*.

13. Use of an epoxytiglienone compound of formula (II):



(II)

wherein

R_1 is selected from hydrogen and $C_{1\text{-}6}$alkyl;

R_2 is selected from -OC(O)$C_{1\text{-}7}$alkyl, -OC(O)$C_{2\text{-}7}$alkenyl and -OC(O)$C_{2\text{-}7}$alkynyl;

R_3 is selected from -OC(O)$C_{1\text{-}7}$alkyl, -OC(O)$C_{2\text{-}7}$alkenyl and -OC(O)$C_{2\text{-}7}$alkynyl;

R_4 and R_5 are independently selected from hydrogen and $C_{1\text{-}6}$alkyl;

R_6 is selected from hydrogen and-C(O)$C_{1\text{-}6}$alkyl;

R_7 is hydroxy; and

R_8 is selected from hydrogen and $C_{1\text{-}6}$alkyl; or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a bacterial infection comprising a biofilm comprising Gram-negative bacteria wherein the compound of formula (II) is to be administered locally to the bacterial infection.

14. The use according to claim 13, wherein the compound of formula (I) is to be administered topically.

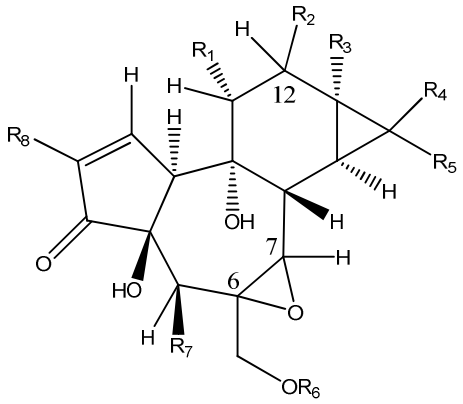
15. The use according to claim 13 or claim 14, wherein the infection is a post-surgical infection or an infection at the site of insertion of a medical device or implantation of an implant.

16. The use according to any one of claims 13 to 15, wherein the bacterial infection

is a chronic infection.

17. The use according to any one of claims 13 to 16, wherein the compound of formula (I) is to be administered in combination with an antibiotic to which the Gram-negative bacteria is susceptible when in planktonic state.

18. An *ex vivo* method of preventing a biofilm comprising Gram-negative bacteria forming on a medical device or dispersing a biofilm comprising Gram-negative bacteria on a medical device, said method comprising applying an epoxytiglienone compound of formula (II) to the medical device: the compound of formula (I) comprising



(II)

wherein

R_1 is selected from hydrogen and C_{1-6}alkyl;

R_2 is selected from -OC(O)C_{1-7}alkyl, -OC(O)C_{2-7}alkenyl and -OC(O)C_{2-7}alkynyl;

R_3 is selected from - -OC(O)C_{1-7}alkyl, -OC(O)C_{2-7}alkenyl and -OC(O)C_{2-7}alkynyl;

R_4 and R_5 are independently selected from hydrogen and C_{1-6}alkyl;

R_6 is selected from hydrogen and -C(O)C_{1-6}alkyl;

R_7 is hydroxy; and

R_8 is selected from hydrogen and C_{1-6}alkyl; or a pharmaceutically acceptable salt thereof.

19. A method according to claim 18, wherein the medical device is a surgical instrument, catheter or medical implant.

20. A method according to claim 18 or claim 19, wherein the medical device is a catheter.

21. A method according to claim 19, wherein the medical device is a dental implant.