innate insensitivity towards ampicillin and *Pseudomonas aeruginosa* is naturally insensitive to sulphonamides, tetracycline, chloramphenicol and trimethoprim. Apart from innate resistance, Gram-negative bacteria can also become resistant to antibiotics through genomic mutations.

According to the World Health Organization the most critical bacteria for which new antibiotics are urgently needed are gram-negative bacteria, namely carbapenem-resistant ***Acinetobacter baumannil***, carbapenem-resistant ***Pseudomonas aeruginosa***, and 3rd generation cephalosporin-resistant ***Enterobacteriaceae*** *(Klebsiella pneumonia, Escherichia coli, Enterobacter spp, Serratia spp, Proteus spp, Providentia spp,* and *Morganella spp).*

Clinical isolates where only two, or fewer, unrelated antibiotics are active against the bacterium are commonly referred to as multidrug-resistant (MDR) bacteria. Several multiresistant isolates have been observed in hospitals. They include *Acinetobacter baumannii* susceptible to two or fewer of meropenem or imipenem, third-generation cephalosporins, piperacillin/tazobactam, (tigecycline), aminoglycosides, quinolones, trimethoprim, colistin, ; *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp. and *Citrobacter* spp. that are susceptible to two or fewer of carbapenems, third-generation cephalosporins, including with β-lactamase inhibitors, piperacillin/tazobactam, temocillin, tigecycline, aminoglycosides, quinolones, trimethoprim or colistin; *Proteus* spp., *Morganella* spp. and *Providencia* spp. that are resistant to third-generation cephalosporin, piperacillin/tazobactam, and aminoglycosides and susceptible only to carbapenems, and the new BL/BLI combinations (ceftolozane/tazobactam or ceftazidime/avibactam). These *Proteeae* are inherently resistant to tigecycline and colistin.

There is therefore an urgent need in the art for a new Gram-negative antibacterial therapy.

SUMMARY ~~OF THE INVENTION~~

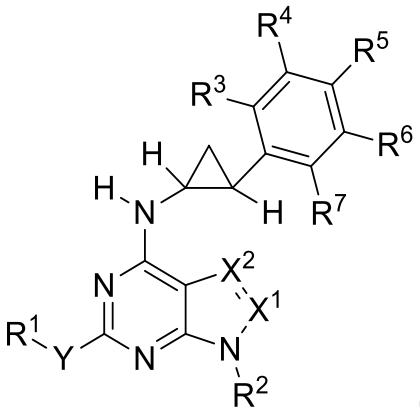
We have surprisingly found pyrimidine derivatives that possess antibacterial activity against Gram-negative bacteria when administered together with a membrane penetrating agent and can be used in the treatment or prevention of bacterial infection in a host mammal and in the treatment and/or prevention of bacterial contamination and fouling.

The pyrimidine derivatives, optionally with a detectable isotope, can also be used as radiotracer in diagnosis or prognosis of bacterial infection.

It is also found that such pyrimidine derivatives can be used in a method for controlling Gram-negative bacterial growth in biofilm formation at early stage such as step 1 or 2 or for killing

bacteria at all steps of biofilm formation including the last step 3 wherein the biofilm has reached its maturation stage of matrix formation and the bacteria start detachment from the surface with a consequent spreading of the bacteria into other locations. In preferred ~~embodiments~~ examples of this use, the pyrimidine derivatives of the disclosure ~~invention~~ are used in combination with a membrane penetrating agent.

~~In one aspect, t~~The ~~invention~~ disclosure provides pyrimidine derivatives represented by formula (I)



(I)

or isomers, racemic mixtures thereof, pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, or alkylated ammonium salts or prodrug thereof;

for use together with a membrane penetrating agent in a treatment or prevention of Gram-negative bacterial infection in a host mammal in need of such treatment or prevention,

wherein:

X^1 and X^2 are independently N, CH, CR^8 wherein R^8 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl; with the exception that if one of X^1 or X^2 is equal to N, then the remaining of X^1 or X^2 are selected from CH, CR^8;

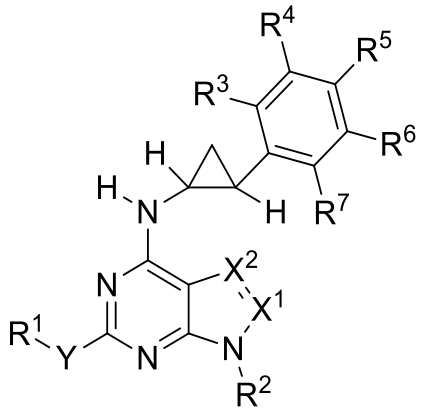
-Y- is -O- or –S-;

R^1 and R^2 are independently C_{1-6} -alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, C_{3-6}-cycloalkyl, aryl, aryl-C_{1-6}-alkyl wherein the alkyl or cycloalkyl moiety is optionally mono or polysubstituted with OH or an halogen and the aryl moiety is optionally mono or polysubstituted with an halogen, -C_{1-6} alkyl, -C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or -$SO_2N(R^8)_2$;

R^3, R^4, R^5, R^6 and R^7 are independently H, an halogen, a C_{1-6} alkyl, C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or –

$SO_2N(R^8)_2$.

In one aspect, the disclosure relates to use of a pyrimidine derivative represented by formula (I)



(I)

or isomers, racemic mixtures thereof, pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, or alkylated ammonium salts thereof;

together with a membrane penetrating agent in the manufacture of a medicament for the treatment or prevention of Gram-negative bacterial infection in a host mammal in need of such treatment or prevention,

wherein:

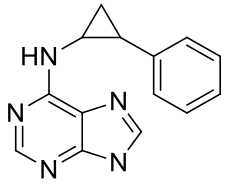
X^1 and X^2 are independently N, CH, CR^8 wherein R^8 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl; with the exception that if one of X^1 or X^2 is equal to N, then the remaining of X^1 or X^2 are selected from CH, CR^8;

-Y- is -O- or –S-;

R^1 and R^2 are independently C_{1-6} -alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, C_{3-6}-cycloalkyl, aryl, aryl-C_{1-6}-alkyl wherein the alkyl or cycloalkyl moiety is optionally mono or polysubstituted with OH or an halogen and the aryl moiety is optionally mono or polysubstituted with an halogen, -C_{1-6} alkyl, -C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or -$SO_2N(R^8)_2$;

R^3, R^4, R^5, R^6 and R^7 are independently H, an halogen, a C_{1-6} alkyl, C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or –$SO_2N(R^8)_2$.

In particular ~~embodiments~~examples, R^3 and R^7 are hydrogen and R^4 and R^5 are independently an halogen. In particular examples~~embodiments~~, X^1 is CH or CR^8 and X^2 is N. In particular examples~~embodiments~~, the pyrimidine derivative comprises a *N*-(2-phenylcyclopropyl)-9*H*-purin-6-amine scaffold represented by formula (V)



(V).

In particular examples~~embodiments~~, the pyrimidine derivative is selected from:

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-2-(propylthio)-9*H*-purin-6-amine (1c);

9-methyl-*N*-((1*R*,2*S*)-2-phenylcyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (2c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-ethyl-2-(propylthio)-9*H*-purin-6-amine (3c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-propyl-2-(propylthio)-9*H*-purin-6-amine (4c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-isopropyl-2-(propylthio)-9*H*-purin-6-amine (5c);

9-cyclopropyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (6c);

9-butyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (7c);

9-(*sec*-butyl)-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (8c);

9-(*tert*-butyl)-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (9c);

9-cyclobutyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (10c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-pentyl-2-(propylthio)-9*H*-purin-6-amine (11c);

9-cyclopentyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (12c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-hexyl-2-(propylthio)-9*H*-purin-6-amine (13c);

9-cyclohexyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (14c);

9-allyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (15c);

2-(6-(((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)amino)-2-(propylthio)-9*H*-purin-9-yl)ethanol (16c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-(prop-2-yn-1-yl)-2-(propylthio)-9*H*-purin-6-amine (17c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9-(2,2,2-trifluoroethyl)-9*H*-purin-6-amine (18c);

(1*S*,2*R*,3*S*,4*R*)-4-(6-(((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)amino)-2-(propylthio)-9*H*-purin-9-yl)cyclopentane-1,2,3-triol (19d);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(ethylthio)-9-methyl-9*H*-purin-6-amine (20c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-ethyl-2-(ethylthio)-9*H*-purin-6-amine (21c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-2-(methylthio)-9*H*-purin-6-amine (22c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-2-propoxy-9*H*-purin-6-amine hydrochloride (23t.HCl);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-ethyl-2-(methylthio)-9*H*-purin-6-amine (24c);

2-(butylthio)-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-9*H*-purin-6-amine (25c);

2-(butylthio)-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-ethyl-9*H*-purin-6-amine (26c);

or isomers, racemic mixtures thereof, pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, or alkylated ammonium salts or prodrug thereof.

In particular examples~~embodiments~~, the pyrimidine derivatives are selected from:

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-2-(propylthio)-9*H*-purin-6-amine (1c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-ethyl-2-(propylthio)-9*H*-purin-6-amine (3c);

9-allyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (15c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-(prop-2-yn-1-yl)-2-(propylthio)-9*H*-purin-6-amine (17c);

(1*S*,2*R*,3*S*,4*R*)-4-(6-(((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)amino)-2-(propylthio)-9*H*-purin-9-yl)cyclopentane-1,2,3-triol (19d);

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-2-(methylthio)-9*H*-purin-6-amine (22c);

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-ethyl-2-(methylthio)-9*H*-purin-6-amine (24c);

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(ethylthio)-9-methyl-9*H*-purin-6-amine (20c);

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-ethyl-2-(ethylthio)-9*H*-purin-6-amine (21c);

2-(butylthio)-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-9*H*-purin-6-amine (25c);

or isomers, racemic mixtures thereof, pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, or alkylated ammonium salts or prodrug thereof.

In particular examples~~embodiments~~, the pyrimidine derivative is

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-2-(propylthio)-9*H*-purin-6-amine (1c); or an isomer, a racemic mixture thereof, a pharmaceutically acceptable acid addition salt, pharmaceutically acceptable metal salt, or alkylated ammonium salt or prodrug thereof.

In particular examples~~embodiments~~, the pyrimidine derivatives are selected from

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-2-(propylthio)-9*H*-purin-6-amine (1c);

9-allyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (15c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-(prop-2-yn-1-yl)-2-(propylthio)-9*H*-purin-6-amine (17c); or

(1*S*,2*R*,3*S*,4*R*)-4-(6-(((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)amino)-2-(propylthio)-9*H*-purin-9-yl)cyclopentane-1,2,3-triol (19d).

In particular examples~~embodiments~~, X^1 is N and X^2 is CH or CR^8. In particular embodiments, the pyrimidine derivative is selected from:

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-methyl-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (27k);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-6-(ethylthio)-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine hydrochloride (28x.HCl);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-6-(propylthio)-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine hydrochloride (29x.HCl);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-ethyl-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (30k);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-ethyl-6-(ethylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine hydrochloride (31x.HCl);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-ethyl-6-(propylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine hydrochloride (32x.HCl);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-isopropyl-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine hydrochloride (33k.HCl);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-6-(methylthio)-1-propyl-1*H*-pyrazolo[3,4-

d]pyrimidin-4-amine hydrochloride (34k.HCl);

or isomers, racemic mixtures thereof, pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, or alkylated ammonium salts or prodrug thereof.

In particular examples~~embodiments~~, the pyrimidine derivative is selected from:

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-methyl-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (27k);

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-ethyl-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine(30k);

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-6-(ethylthio)-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine hydrochloride (28x.HCl);

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-isopropyl-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine hydrochloride(33k.HCl);

or isomers, racemic mixtures thereof, pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, or alkylated ammonium salts or prodrug thereof.

In particular examples~~embodiments~~, X^1 and X^2 are CH or CR^8.

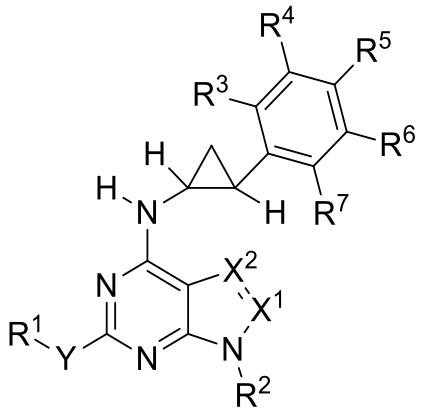
In particular examples~~embodiments~~, the pyrimidine derivative is:

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-7-ethyl-2-(methylthio)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine hydrochloride (35p.HCl);

or an isomer, racemic mixture thereof, pharmaceutically acceptable acid addition salt, pharmaceutically acceptable metal salt, or alkylated ammonium salt or prodrug thereof.

In particular ~~embodiments~~examples, R^3 and R^7 are H and R^4, R^5 is a fluorine.

In another aspect, the ~~The invention~~ disclosure ~~further~~ relates to the use of pyrimidine derivative represented by formula (I)



(I)

wherein:

X^1 and X^2 are independently N, CH, CR^8 wherein R^8 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl; with the exception that if one of X^1 or X^2 is equal to N, then the remaining of X^1 or X^2 are selected from CH, CR^8;

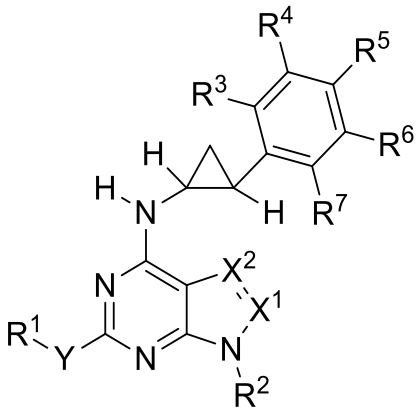
-Y- is -O- or –S-;

R^1 and R^2 are independently C_{1-6} -alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, C_{3-6}-cycloalkyl, aryl, aryl-C_{1-6}-alkyl wherein the alkyl or cycloalkyl moiety is optionally mono or polysubstituted with OH or an halogen and the aryl moiety is optionally mono or polysubstituted with an halogen, -C_{1-6} alkyl, -C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or -$SO_2N(R^8)_2$;

R^3, R^4, R^5, R^6 and R^7 are independently H, an halogen, a C_{1-6} alkyl, C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or –$SO_2N(R^8)_2$;

together with a membrane penetrating agent~~-~~ ex vivo as inhibitor of Gram-negative bacterial growth in biofilm formation on a surface of a medical device~~.~~

The ~~invention~~ disclosure further relates to a method for killing or preventing Gram-negative bacterial growth in biofilm formation comprising applying on a surface of a medical device, an effective amount of pyrimidine derivatives represented by formula (I) together with a membrane penetrating agent



(I)

wherein:

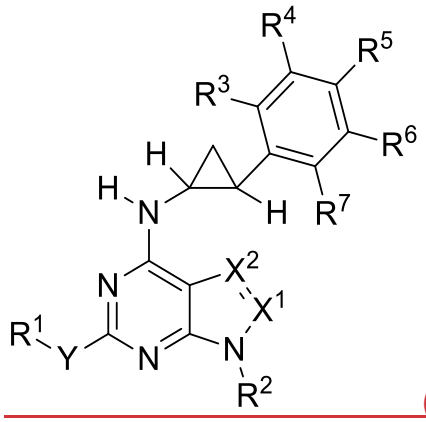
X^1 and X^2 are independently N, CH, CR^8 wherein R^8 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl; with the exception that if one of X^1 or X^2 is equal to N, then the remaining of X^1 or X^2 are selected from CH, CR^8;

-Y- is -O- or –S-;

R^1 and R^2 are independently C_{1-6} -alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, C_{3-6}-cycloalkyl, aryl, aryl-C_{1-6}-alkyl wherein the alkyl or cycloalkyl moiety is optionally mono or polysubstituted with OH or an halogen and the aryl moiety is optionally mono or polysubstituted with an halogen, -C_{1-6} alkyl, -C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or -$SO_2N(R^8)_2$;

R^3, R^4, R^5, R^6 and R^7 are independently H, an halogen, a C_{1-6} alkyl, C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or –$SO_2N(R^8)_2$, wherein said method is not a method of treatment of the human or animal body.

In another aspect, the disclosure relates to a method for killing or preventing Gram-negative bacterial growth in biofilm formation comprising applying on a surface of a medical device, preferably a cardiovascular device, an effective amount of pyrimidine derivatives represented by formula (I) together with a membrane penetrating agent



(I)

wherein:

X^1 and X^2 are independently N, CH, CR^8 wherein R^8 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl; with the exception that if one of X^1 or X^2 is equal to N, then the remaining of X^1 or X^2 are selected from CH, CR^8;

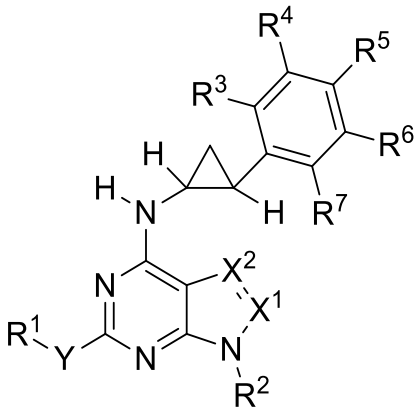
-Y- is -O- or –S-;

R^1 and R^2 are independently C_{1-6} -alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, C_{3-6}-cycloalkyl, aryl, aryl-C_{1-6}-alkyl wherein the alkyl or cycloalkyl moiety is optionally mono or polysubstituted with OH or an halogen and the aryl moiety is optionally mono or polysubstituted with an halogen, -C_{1-6} alkyl, -C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or -$SO_2N(R^8)_2$;

R^3, R^4, R^5, R^6 and R^7 are independently H, an halogen, a C_{1-6} alkyl, C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or –$SO_2N(R^8)_2$, wherein said method is not a method of treatment of the human or animal body.

In particular examples~~embodiments~~, the medical device is a cardiovascular device.

The ~~invention~~ disclosure further relates to the pyrimidine derivative represented by formula (I)



(I)

wherein:

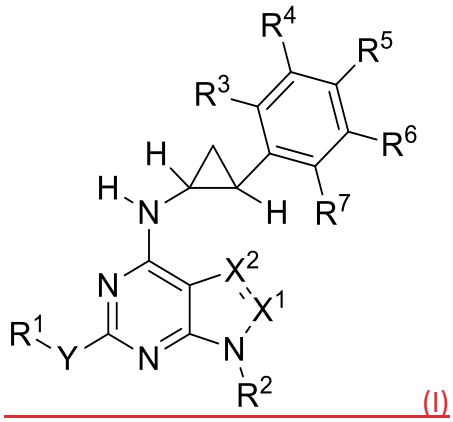
X^1 and X^2 are independently N, CH, CR^8 wherein R^8 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl; with the exception that if one of X^1 or X^2 is equal to N, then the remaining of X^1 or X^2 are selected from CH, CR^8;

-Y- is -O- or –S-;

R^1 and R^2 are independently C_{1-6} -alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, C_{3-6}-cycloalkyl, aryl, aryl-C_{1-6}-alkyl wherein the alkyl or cycloalkyl moiety is optionally mono or polysubstituted with OH or an halogen and the aryl moiety is optionally mono or polysubstituted with an halogen, -C_{1-6} alkyl, -C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or -$SO_2N(R^8)_2$;

R^3, R^4, R^5, R^6 and R^7 are independently H, an halogen, a C_{1-6} alkyl, C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or –$SO_2N(R^8)_2$. comprising a marker for use together with a membrane penetrating agent– in diagnosing or prognosing Gram-negative bacterial infection.

In another aspect, the disclosure relates to use of a pyrimidine derivative represented by formula (I) together with a membrane penetrating agent in the manufacture of a medicament for the diagnosis or prognosis of Gram-negative bacterial infection



(I)

wherein:

X^1 and X^2 are independently N, CH, CR^8 wherein R^8 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl; with the exception that if one of X^1 or X^2 is equal to N, then the remaining of X^1 or X^2 are selected from CH, CR^8;

-Y- is -O- or –S-;

R^1 and R^2 are independently C_{1-6} -alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, C_{3-6}-cycloalkyl, aryl, aryl-C_{1-6}-alkyl wherein the alkyl or cycloalkyl moiety is optionally mono or polysubstituted with OH or an halogen and the aryl moiety is optionally mono or polysubstituted with an halogen, -C_{1-6} alkyl, -C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or -$SO_2N(R^8)_2$;

R^3, R^4, R^5, R^6 and R^7 are independently H, an halogen, a C_{1-6} alkyl, C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or –$SO_2N(R^8)_2$; and

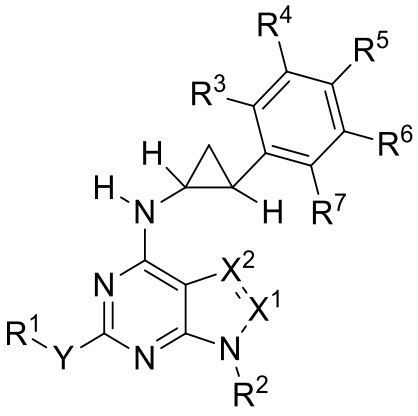
wherein the pyrimidine derivative comprises a detectable marker.

In particular ~~emodiments~~ examples of the compositions, uses and methods described herein, the membrane penetrating agent is polymyxin B nonapeptide.

The ~~invention~~ disclosure further relates to pharmaceutical compositions comprising a pyrimidine derivative of formula (I) or isomars, racemic mixtures thereof, pharmaceutically acceptable acid addition salts thereof as provided herein for use in the prevention or treatment of gram-negative bacterial infections. In particular examples~~embodiments~~, the pharmaceutical composition further comprises a membrane-penetrating agent.

CLAIMS:

1. Use of a ~~A~~ pyrimidine derivative represented by formula (I)



(I)

or isomers, racemic mixtures thereof, pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, or alkylated ammonium salts ~~or prodrug~~ thereof;

~~for use~~ together with a membrane penetrating agent in the manufacture of a medicament for the ~~in a~~ treatment or prevention of Gram-negative bacterial infection in a host mammal in need of such treatment or prevention,

wherein:

X^1 and X^2 are independently N, CH, CR^8 wherein R^8 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl; with the exception that if one of X^1 or X^2 is equal to N, then the remaining of X^1 or X^2 are selected from CH, CR^8;

-Y- is -O- or –S-;

R^1 and R^2 are independently C_{1-6} -alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, C_{3-6}-cycloalkyl, aryl, aryl-C_{1-6}-alkyl wherein the alkyl or cycloalkyl moiety is optionally mono or polysubstituted with OH or an halogen and the aryl moiety is optionally mono or polysubstituted with an halogen, -C_{1-6} alkyl, -C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or -$SO_2N(R^8)_2$;

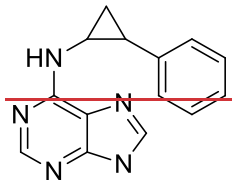
R^3, R^4, R^5, R^6 and R^7 are independently H, an halogen, a C_{1-6} alkyl, C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or –$SO_2N(R^8)_2$.

2. The ~~pyrimidine derivative for~~ use according to claim 1 wherein R^3 and R^7 are hydrogen and R^4

and R^5 are independently a halogen.

3. The ~~pyrimidine derivative for~~ use according to claim 1 or 2 wherein X^1 is CH or CR^8 and X^2 is N.

~~4. The pyrimidine derivative for use according to claim 3 comprising a *N*-(2-phenylcyclopropyl)-9*H*-purin-6-amine scaffold represented by formula (V)~~



~~(V).~~

~~5~~4. The ~~pyrimidine derivative for~~ use according to claim 3 ~~or 4~~ selected from:

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-2-(propylthio)-9*H*-purin-6-amine (1c);

9-methyl-*N*-((1*R*,2*S*)-2-phenylcyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (2c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-ethyl-2-(propylthio)-9*H*-purin-6-amine (3c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-propyl-2-(propylthio)-9*H*-purin-6-amine (4c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-isopropyl-2-(propylthio)-9*H*-purin-6-amine (5c);

9-cyclopropyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (6c);

9-butyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (7c);

9-(*sec*-butyl)-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (8c);

9-(*tert*-butyl)-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (9c);

9-cyclobutyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (10c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-pentyl-2-(propylthio)-9*H*-purin-6-amine (11c);

9-cyclopentyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (12c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-hexyl-2-(propylthio)-9*H*-purin-6-amine (13c);

9-cyclohexyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (14c);

9-allyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (15c);

2-(6-(((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)amino)-2-(propylthio)-9*H*-purin-9-yl)ethanol (16c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-(prop-2-yn-1-yl)-2-(propylthio)-9*H*-purin-6-amine (17c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9-(2,2,2-trifluoroethyl)-9*H*-purin-6-amine (18c);

(1*S*,2*R*,3*S*,4*R*)-4-(6-(((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)amino)-2-(propylthio)-9*H*-purin-9-yl)cyclopentane-1,2,3-triol (19d);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(ethylthio)-9-methyl-9*H*-purin-6-amine (20c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-ethyl-2-(ethylthio)-9*H*-purin-6-amine (21c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-2-(methylthio)-9*H*-purin-6-amine (22c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-2-propoxy-9*H*-purin-6-amine hydrochloride (23t.HCl);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-ethyl-2-(methylthio)-9*H*-purin-6-amine (24c);

2-(butylthio)-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-9*H*-purin-6-amine (25c);

2-(butylthio)-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-ethyl-9*H*-purin-6-amine (26c);

or isomers, racemic mixtures thereof, pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, or alkylated ammonium salts ~~or prodrug~~ thereof.

~~6~~5. The ~~pyrimidine derivative for~~ use according to ~~anyone of~~ claim~~s~~ 3 ~~to 5~~ or 4 selected from:

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-2-(propylthio)-9*H*-purin-6-amine (1c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-ethyl-2-(propylthio)-9*H*-purin-6-amine (3c);

9-allyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (15c);

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-(prop-2-yn-1-yl)-2-(propylthio)-9*H*-purin-6-amine (17c);

(1*S*,2*R*,3*S*,4*R*)-4-(6-(((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)amino)-2-(propylthio)-9*H*-purin-9-yl)cyclopentane-1,2,3-triol (19d);

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-2-(methylthio)-9*H*-purin-6-amine (22c);

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-ethyl-2-(methylthio)-9*H*-purin-6-amine (24c);

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(ethylthio)-9-methyl-9*H*-purin-6-amine (20c);

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-ethyl-2-(ethylthio)-9*H*-purin-6-amine (21c);

2-(butylthio)-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-9*H*-purin-6-amine (25c);

or isomers, racemic mixtures thereof, pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, or alkylated ammonium salts ~~or prodrug~~ thereof.

~~7~~6. The ~~pyrimidine derivative for~~ use according to anyone of claims 3 to ~~6~~ 5 which is selected from

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-methyl-2-(propylthio)-9*H*-purin-6-amine (1c);

9-allyl-*N*-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-2-(propylthio)-9*H*-purin-6-amine (15c);
N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-9-(prop-2-yn-1-yl)-2-(propylthio)-9*H*-purin-6-amine (17c); or

(1*S*,2*R*,3*S*,4*R*)-4-(6-(((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)amino)-2-(propylthio)-9*H*-purin-9-yl)cyclopentane-1,2,3-triol (19d).

~~8~~7. The ~~pyrimidine derivative~~ use according to claim 1 or 2 wherein X^1 is N and X^2 is CH or CR^8, preferably wherein the pyrimidine derivative is selected from:

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-methyl-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (27k);
N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-6-(ethylthio)-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine hydrochloride (28x.HCl);
N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-6-(propylthio)-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine hydrochloride (29x.HCl);
N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-ethyl-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (30k);
N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-ethyl-6-(ethylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine hydrochloride (31x.HCl);
N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-ethyl-6-(propylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine hydrochloride (32x.HCl);
N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-isopropyl-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine hydrochloride (33k.HCl);
N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-6-(methylthio)-1-propyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine hydrochloride (34k.HCl);

or isomers, racemic mixtures thereof, pharmaceutically acceptable acid addition salts,

pharmaceutically acceptable metal salts, or alkylated ammonium salts ~~or prodrug~~ thereof, most preferably, wherein the pyrimidine derivative is selected from:

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-methyl-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (27k);

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-ethyl-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine(30k);

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-6-(ethylthio)-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine hydrochloride (28x.HCl);

N-((*1R,2S*)-2-(3,4-difluorophenyl)cyclopropyl)-1-isopropyl-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine hydrochloride(33k.HCl);

or isomers, racemic mixtures thereof, pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, or alkylated ammonium salts ~~or prodrug~~ thereof.

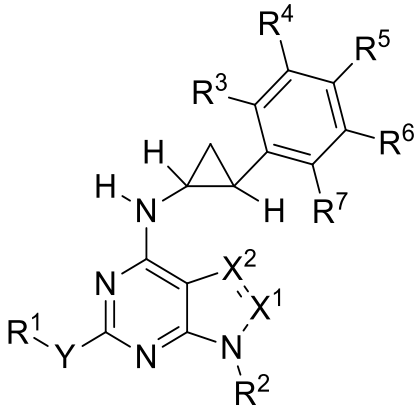
~~98~~. The ~~pyrimidine derivative for~~ use according to claim 1 or 2, wherein X^1 and X^2 are CH or CR^8, preferably wherein the pyrimidine derivative is selected from

N-((1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl)-7-ethyl-2-(methylthio)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine hydrochloride (35p.HCl);

or isomers, racemic mixtures thereof, pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, or alkylated ammonium salts ~~or prodrug~~ thereof.

~~109~~. The ~~pyrimidine derivative for~~ use according to anyone of claim 1 to ~~9~~ 8 wherein R^3 and R^7 are H and R^4, R^5 is a fluorine.

~~11~~10. Use of a pyrimidine derivative represented by formula (I)



(I)

wherein:

X^1 and X^2 are independently N, CH, CR^8 wherein R^8 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl; with the exception that if one of X^1 or X^2 is equal to N, then the remaining of X^1 or X^2 are selected from CH, CR^8;

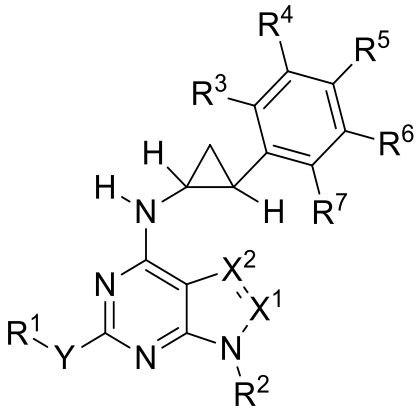
-Y- is -O- or –S-;

R^1 and R^2 are independently C_{1-6} -alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, C_{3-6}-cycloalkyl, aryl, aryl-C_{1-6}-alkyl wherein the alkyl or cycloalkyl moiety is optionally mono or polysubstituted with OH or an halogen and the aryl moiety is optionally mono or polysubstituted with an halogen, -C_{1-6} alkyl, -C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or -$SO_2N(R^8)_2$;

R^3, R^4, R^5, R^6 and R^7 are independently H, an halogen, a C_{1-6} alkyl, C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or –$SO_2N(R^8)_2$;

together with a membrane penetrating agent -ex vivo as inhibitor of Gram-negative bacterial growth in biofilm formation on a surface of a medical device.

~~12~~11. A method for killing or preventing Gram-negative bacterial growth in biofilm formation comprising applying on a surface of a medical device, preferably a cardiovascular device, an effective amount of pyrimidine derivatives represented by formula (I) together with a membrane penetrating agent



(I)

wherein:

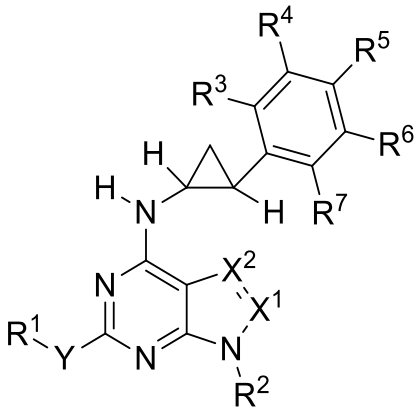
X^1 and X^2 are independently N, CH, CR^8 wherein R^8 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl; with the exception that if one of X^1 or X^2 is equal to N, then the remaining of X^1 or X^2 are selected from CH, CR^8;

-Y- is -O- or –S-;

R^1 and R^2 are independently C_{1-6} -alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, C_{3-6}-cycloalkyl, aryl, aryl-C_{1-6}-alkyl wherein the alkyl or cycloalkyl moiety is optionally mono or polysubstituted with OH or an halogen and the aryl moiety is optionally mono or polysubstituted with an halogen, -C_{1-6} alkyl, -C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or -$SO_2N(R^8)_2$;

R^3, R^4, R^5, R^6 and R^7 are independently H, an halogen, a C_{1-6} alkyl, C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or –$SO_2N(R^8)_2$, wherein said method is not a method of treatment of the human or animal body.

~~13~~12. Use of a ~~The~~ pyrimidine derivative represented by formula (I) ~~for use~~ together with a membrane penetrating agent in the manufacture of a medicament for the diagnosis or prognosis of ~~in diagnosing or prognosing~~ Gram-negative bacterial infection



(I)

wherein:

X^1 and X^2 are independently N, CH, CR^8 wherein R^8 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl; with the exception that if one of X^1 or X^2 is equal to N, then the remaining of X^1 or X^2 are selected from CH, CR^8;

-Y- is -O- or –S-;

R^1 and R^2 are independently C_{1-6} -alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, C_{3-6}-cycloalkyl, aryl, aryl-C_{1-6}-alkyl wherein the alkyl or cycloalkyl moiety is optionally mono or polysubstituted with OH or an halogen and the aryl moiety is optionally mono or polysubstituted with an halogen, -C_{1-6} alkyl, -C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or -$SO_2N(R^8)_2$;

R^3, R^4, R^5, R^6 and R^7 are independently H, an halogen, a C_{1-6} alkyl, C_{1-6} alkoxy, -OH, -NO_2, -CN, -NH_2, -NHR^8, -$N(R^8)_2$ -COOH, -$COOR^8$, -$CONH_2$, -$CONHR^8$, -$CON(R^8)_2$, -SO_2NH_2, -SO_2NHR^8, or –$SO_2N(R^8)_2$; and

wherein the pyrimidine derivative comprises a detectable marker.

~~14~~13. The ~~pyrimidine derivative for~~ use according to anyone of claims 1 to ~~10~~ 9 or ~~13~~ 12 wherein the membrane penetrating agent is polymyxin B nonapeptide.

~~15~~14. The method according to claim ~~12~~11, wherein the membrane penetrating agent is polymixyn B nonapeptide.

15. The method according according to claim 11 or 14 wherein the medical device is a prosthetic heart valve or a catheter.

16. The use according to anyone of claims 1 to 9, 12 or 13 for topical administration