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

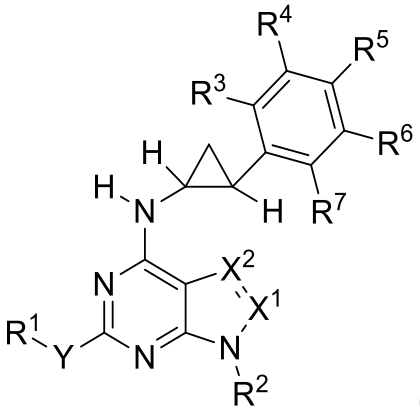
We have surprisingly found pyrimidine derivatives that possess antibacterial activity against Gram-negative bacteria when administered together with a membrane penetrating agent and can be used in the treatment or prevention of bacterial infection in a host mammal and in the treatment and/or prevention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

The 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;

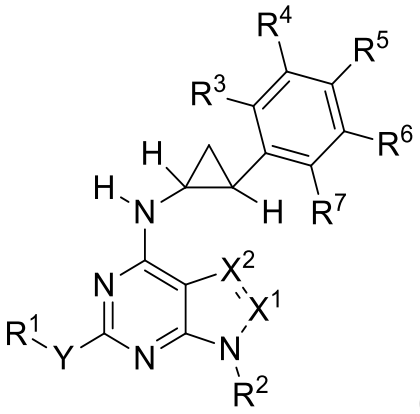
-Y- is -O- or –S-;

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:

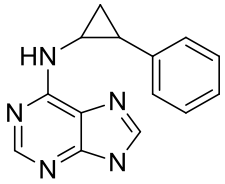
X^1 and X^2 are independently N, CH, CR^8 wherein R^8 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl; with the exception that if one of X^1 or X^2 is equal to N, then the remaining of X^1 or X^2 are selected from CH, CR^8;

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



(V).

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

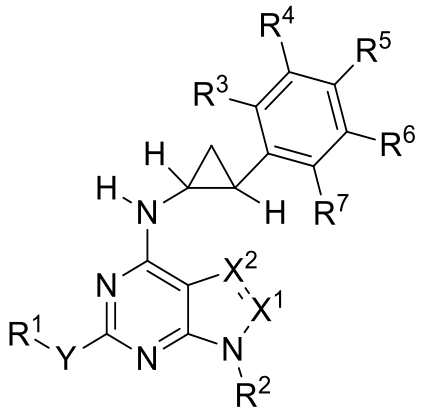
In particular examples, the pyrimidine derivative is:

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 examples, R^3 and R^7 are H and R^4, R^5 is a fluorine.

In another aspect, the disclosure 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;

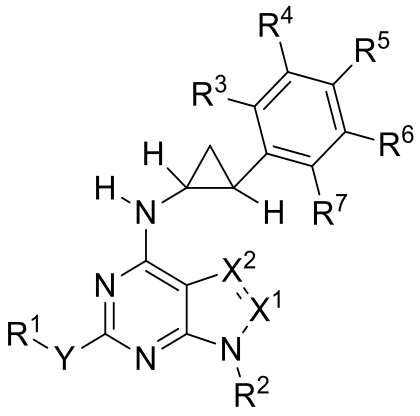
-Y- is -O- or –S-;

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 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:

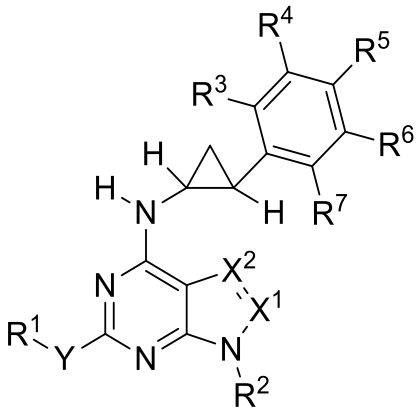
X^1 and X^2 are independently N, CH, CR^8 wherein R^8 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl; with the exception that if one of X^1 or X^2 is equal to N, then the remaining of X^1 or X^2 are selected from CH, CR^8;

-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;

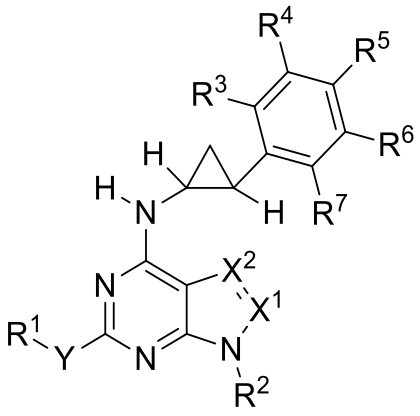
-Y- is -O- or –S-;

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, the medical device is a cardiovascular device.

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



(I)

wherein:

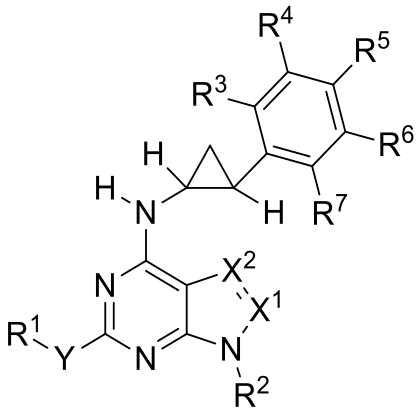
X^1 and X^2 are independently N, CH, CR^8 wherein R^8 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl; with the exception that if one of X^1 or X^2 is equal to N, then the remaining of X^1 or X^2 are selected from CH, CR^8;

-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

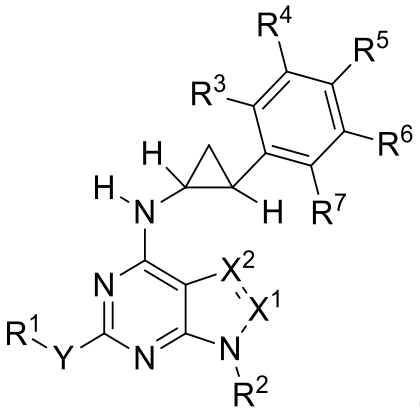
wherein the pyrimidine derivative comprises a detectable marker.

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

The 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, the pharmaceutical composition further comprises a membrane-penetrating agent.

CLAIMS:

1. 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 –$SO_2N(R^8)_2$.

2. The 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 use according to claim 1 or 2 wherein X^1 is CH or CR^8 and X^2 is N.

4. The use according to claim 3 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 thereof.

5. The 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);

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 thereof.

6. The use according to anyone of claims 3 to 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).

7. The 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 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 thereof.

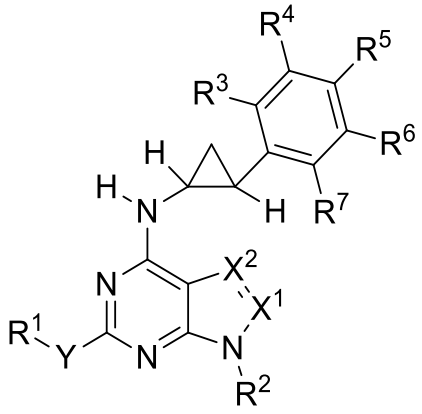
8. The use according to claim 1 or 2, wherein X^1 and X^2 are CH or CR^8, preferably wherein the pyrimidine derivative is selected from

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 thereof.

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

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-;

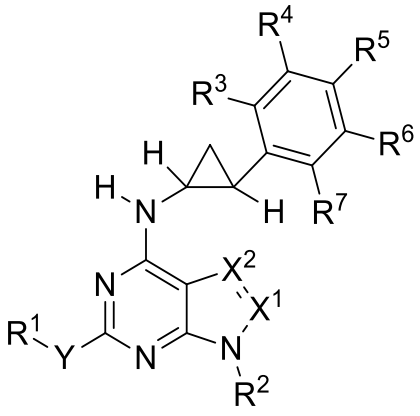
R^1 and R^2 are independently C_{1-6} -alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, C_{3-6}-cycloalkyl, aryl, aryl-C_{1-6}-alkyl wherein the alkyl or cycloalkyl moiety is optionally mono or polysubstituted with OH or an halogen and the aryl moiety is optionally mono or polysubstituted with an halogen, -C_{1-6} 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.

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;

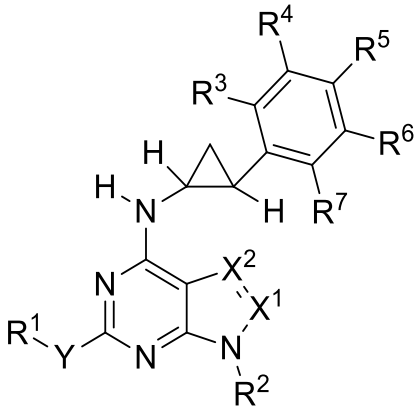
-Y- is -O- or –S-;

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.

12. 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.

13. The use according to anyone of claims 1 to 9 or 12 wherein the membrane penetrating agent is polymyxin B nonapeptide.

14. The method according to claim 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

5