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Title: ANTIMICROBIAL COMPOSITIONS, ANTIBIOFILM COMPOSITIONS AND USES THEREOF

Abstract: Methods, articles and compositions utilizing 4-fluorophenylboronic acid or esters thereof, having Formulae A or A’ as described herein, in combination with an additional active agent, for inhibiting the growth of a pathogenic microorganism and/or for preventing and/or reducing the formation of microbial biofilms and/or for disrupting microbial biofilms in living tissues or inanimate objects, are disclosed. Further disclosed are compounds represented by general Formula A as described herein, which exhibit antimicrobial and anti-biofilm formation activity, as well as methods, articles and compositions using the same.
ANTIMICROBIAL COMPOSITIONS, ANTIBIOFILM COMPOSITIONS AND USES THEREOF

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application No. 61/472,679, filed April 7, 2011, and U.S. Provisional Patent Application No. 61/528,347, filed August 29, 2011, which are incorporated herein in their entirety.

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to pharmacology, and, more particularly, but not exclusively, to treatments which are useful in inhibiting bacterial growth and/or in preventing, reducing and/or interfering with biofilm formation. Antibiotics, which are also referred to herein and in the art as antibacterial or antimicrobial agents, are natural substances of relatively small size in molecular terms, which are typically released by bacteria or fungi. These natural substances, as well as derivatives and/or modifications thereof, are used for many years as medications for treating infections caused by bacteria. However, over the decades, almost all the prominent infection-causing bacterial strains have developed resistance to antibiotics.

The rapid spread of antibiotic resistance in pathogenic bacteria has prompted a continuing search for new agents capable of antibacterial activity. Indeed, microbiologists today warn of a "medical disaster" which could lead back to the era before penicillin, when even seemingly small infections were potentially lethal. Thus, research into the design of new antibiotics is of high priority.

The mechanism of drug resistance varies with respect to the pathogen and the therapy employed. For example, strains of Staphylococcus aureus exhibiting resistance to penicillin G appeared shortly after this antibiotic was introduced. The frequency of drug resistance has increased such that over 80% of both hospital and community-acquired strains of S. aureus are now resistant. Other strains of S. aureus have emerged that are highly resistant to all beta-lactam antibiotics. National prospective surveillance of over 24,000 invasive bacterial isolates show disease-associated S. aureus strains with methicillin resistance (MRSA) have increased dramatically and are now frequently...
identified in community-acquired infections as well as in hospital settings. There is also
a dramatic increase in incidences of nosocomial infection and colonization with
vancomycin-resistant Enterococci (VRE) throughout the world. In addition, pathogenic
Gram-negative bacteria are often multi-resistant.

One way to delay the emergence of antibiotic-resistance is to develop new
synthetic materials that can selectively inhibit bacterial enzymes, via novel mechanisms
of action. An additional strategy involves circumventing existing bacterial resistance
mechanisms and thereby restoring usefulness to anti-bacterial agents that have become
compromised by resistance. The remarkable advances in recent years in elucidating the
mechanisms of resistance to various clinical antibiotics on the molecular level provide
complementary tools to this approach via structure-based and mechanism-based design.

Many bacteria are planktonic, namely they move freely around in water and
other liquid media, however, many pathogenic and harmful microorganisms are or
become sessile, namely attached to a surface where they form biofilms. Biofilms are a
multicellular high density ecological environment of mostly bacteria and/or fungi and
their secretions, but it is not considered a multicellular organism per se. Once a
microorganism attach to a surface it undergoes a series of changes, the most obvious of
which is the excretion of a slimy material consisting mostly of extra-cellular
polysaccharides (EPS). EPS, soluble microbiological products (SMP), dispersed
bacterial cells, and a well characterized natural organic matter (NOM) have all been
identified as part of a "conditioning layer" that may help retain other fouling materials,
as well as directly cause membrane biofouling.

A dramatic phenotypic change occurs as the bacteria turn or switch from a
planktonic to a biofilm state and attach to a surface; a whole different suite of genes is
activated, making sessile bacteria significantly different to planktonic bacteria
suspended in the water. Biofilm bacteria have been found to remain viable at MICs
(minimal inhibitory concentration) up to 1,000 times higher than those of their
planktonic counterparts. Biofilms have been shown to alter the local environment to
enhance their survival, changing such properties as pH and the dissolved oxygen
concentration. These changes can reduce the effectiveness of some treatments. For
example, biofilms are known to vary the local pH, and some oral biofilms have regions
of pH less than 4.9.
In addition to their complex, heterogeneous composition, biofilms are also dynamic hydrogels which capriciously move, detach and reform on a wide variety of environmental or engineered surfaces. Thus, when water-borne bacteria congregate in sufficient numbers they may form a film on the surface of pipes, tanks, and indeed any piece of equipment, and biofouling usually results. Biofouling can be defined as the unspecific adsorption of biological material onto surfaces upon their immersion in a fluid. EPS secreted by bacteria and other colonizing microorganisms envelope and anchor them to the substrate thereby altering the local surface chemistry which can stimulate further growth such as the recruitment and settlement of microorganisms. Biofouling via biofilm formation causes the deterioration in the microbiological quality of water by inducing biocorrosion termed microbiologically influenced corrosion and biofouling of piping, membranes, containers and reservoirs.

Biofilms also interrupt the flow of ions and water to and from the substrate surface by acting as a diffusion barrier. The reduction of localized oxygen by cathodic reactions within the electrolyte can accelerate the corrosion of a metallic substrate by creating a differential aeration concentration cell. The corrosion and weathering caused by biofilm can lead to considerable damage to heat exchangers, unexpected corrosion of stainless steel, and premature destruction of membranes, and many other technological, industrial and homestead aliments.

Both bacteria and fungi share the same habitat in the oral cavity, although they belong to different kingdoms in the evolution hierarchy. They both harbor mixed biofilms which cover oral tissues. *Streptococcus mutans* is a cardinal member of the oral biofilm associated with dental caries while *Candida albicans* is associated with oral candidiasis.

Bacteria can communicate therebetween by what is known as quorum sensing (QS) which is effected by secreting small molecules termed auto inducers (AFs) into their environment. Quorum sensing takes place especially in biofilms were the microbes are at close proximity to one another. This phenomenon affects many physiological and metabolic pathways of bacteria, including the formation of biofilms and antibacterial resistance.

Inter species QS may affect microbes' physiology and virulence properties resulting in enhanced virulent properties of biofilms. Small peptides, AI-2 (furanosyl furanose (furanosyl-ß-D-ribopyranosyl) dimeric pyrophosphate) ...
borate diester), AI-1 (N-acylhomoserine lactones) and C-AI (Cholera AI) have been shown to act as signal molecules in QS in many types of bacteria, including oral bacteria.

The study of bacterial QS has suggested several ideal targets for manipulation of QS, mainly the AI-2 signal molecule or its sensor-2, due to the wide distribution of the AI-2 cascade in many types of bacteria.

Eukaryotic cells such as fungi have also been shown to communicate with each other by producing signal molecules (AIs), however, the AIs of bacteria and fungi differ chemically; production of farnesol by C. albicans at high cell densities is the first QS system which has been discovered in eukaryotes. Farnesol has been identified as QS agent that blocks the morphological transition from yeast to the filament form and affects biofilm formation in C. albicans. The mechanism by which farnesol is sensed by C. albicans is not yet known. Farnesolic acid and tyrosol were also shown to posses AI properties in C. albicans.

Eukaryotes seem to have evolved efficient mechanisms to manipulate bacterial QS and thereby protect themselves from pathogenic bacterial attack and competition [Hogan, D.A., 2006, Eukaryot Cell, 5, 613-9]. Thus, by producing quorum sensing inhibitors (QSIs), the eukaryotic host may be simultaneously conversing with a variety of different bacterial strains that it encounters in its natural habitat, potentially encouraging the beneficial ones and antagonizing the harmful strains. Parallel to this, certain bacteria have evolved mechanisms to fine-tune gene regulation of eukaryotic with their QS signals [Hogan D.A. et al, 2004, Mol. Microbiol, 54, 1212-23]. This eukaryote-bacterial cross talk could be exploited to model manipulative techniques that interfere with bacterial QS.

It has been established that bacteria and fungi immobilized in the form of biofilms are inherently more robust and resistant to antibiotics and antifungal agents than the planktonic forms. Those mixed biofilms constitute a basis of numerous infections and diseases and are responsible in part to the growing emergence of resistance to antimicrobial agents. Thus, small compounds that can interfere with QS can offer a new approach to the development of novel antimicrobial and anti-biofilm agents. It is now evident that influencing QS in microbes harboring biofilms bears great
potential as a novel non-antimicrobial, alternative means of affecting pathogenic microbes.

Boron containing compounds have received increasing attention as therapeutic agents over the past few years as technology in organic synthesis has expanded to include this atom. Boron containing compounds have been shown to have various biological activities, including an antibacterial activity [Bailey et al., Antimicrobial Agents and Chemotherapy, (1980), 17, 549-553]. For example, U.S. Patent Nos. 6,075,014 and 6,184,363 disclose that a number of phenyl boronic acids are effective against bacteria resistant to beta-lactam antibiotics. These compounds, or pharmaceutically acceptable salts thereof, are antibacterial by themselves, although at higher concentrations than beta-lactam antibiotics.

WO 2005/021559 discloses a novel family of oxazaborolidines which can act as antibacterial agents, particularly in preventing or reducing biofilm formation.

Additional background art includes WO 2009/003090; WO 2003/009689; and U.S. Patent Nos. 6,448,238 and 7,666,813.

In U.S. Provisional Patent Application No. 61/472,679, it is disclosed that 4-phenylboronic acid can be used, in combination with antibiotics, and particularly non beta-lactam antibiotics, in inhibiting bacterial growth and in prevention or reduction of biofilm formation.

**SUMMARY OF THE INVENTION**

The present inventors have uncovered that 4-phenylboronic acid and/or an ester thereof can be efficiently used, either alone or in combination with an anti-microbial agent (e.g., an antibiotic), in inhibiting a growth of various bacterial strains and importantly, in preventing or reducing biofilm formation.
According to an aspect of some embodiments of the present invention there is provided a composition comprising a compound having general Formula A':

![Chemical Structure Image]

Wherein R₃ and R₄ are each independently selected from the group consisting of hydrogen, alkyl, aryl and cycloalkyl, or, alternatively, Rᵢ and R₂ are joined together so as to form a ring, an antimicrobial agent and a pharmaceutically acceptable carrier.

According to some embodiments of the invention, R₃ and R₄ are joined together so as to form a ring.

According to some embodiments of the invention, the ring is selected from the group consisting of 5-, 6-, 7-, 8-, 9- and 10-membered ring.

According to some embodiments of the invention, the ring comprises a nitrogen atom.

According to some embodiments of the invention, the ring comprises a N-methyldiethylamine moiety.

According to some embodiments of the invention, R₃ and R₄ are each hydrogen.

According to some embodiments of the invention, the composition is packaged in a packaging material and identified in print, in or on the packaging material, for use in inhibiting a growth of a pathogenic microorganism in or on a substrate.

According to some embodiments of the invention, the substrate is a living tissue, the composition being identified for use in the treatment of a medical condition associated with the pathogenic microorganism in a subject comprising the living tissue.

According to some embodiments of the invention, the composition is packaged in a packaging material and identified in print, in or on the packaging material, for use
in reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate.

According to some embodiments of the invention, the substrate is a living tissue, the composition being identified for use in the treatment of a medical condition in which reducing or preventing the formation of the biofilm and/or disrupting the biofilm in the living tissue is beneficial.

According to some embodiments of the invention, the compound and the antimicrobial agent act in synergy.

According to an aspect of some embodiments of the present invention there is provided a method of inhibiting a growth of a pathogenic microorganism in or on a substrate, the method comprising co-contacting the substrate with an antimicrobial agent and with antimicrobial effective amount of a compound having Formula A', as described herein, an antibacterial agent and a pharmaceutically acceptable carrier.

According to some embodiments of the invention, the substrate is a living tissue, the method being for treating a medical condition associated with the pathogenic microorganism in a subject comprising the living tissue.

According to an aspect of some embodiments of the present invention there is provided a method of reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate, the method comprising co-contacting the substrate with an antimicrobial agent and an antimicrobial effective amount of a compound having Formula A', as described herein.

According to some embodiments of the invention, the substrate is a living tissue, the method being for treating a medical condition in which reducing or preventing the formation of the biofilm and/or disrupting the biofilm in the living tissue is beneficial.

According to some embodiments of the invention, the compound and the antimicrobial agent act in synergy.

According to an aspect of some embodiments of the present invention there is provided a use of a compound having general Formula A', as described herein, in the manufacture of a product for inhibiting growth of a pathogenic microorganism in or on a substrate, the product further comprising an antimicrobial agent.
According to some embodiments of the invention, the substrate is a living tissue and the product is a medicament for treating a medical condition associated with the pathogenic microorganism in a subject comprising the living tissue.

According to an aspect of some embodiments of the present invention there is provided a use of a compound having general Formula A’, as described herein, in the manufacture of a product for reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate, the product further comprising an antimicrobial agent.

According to some embodiments of the invention, the substrate is a living tissue and the product is a medicament for treating a medical condition in which reducing or preventing the formation of the biofilm and/or disrupting the biofilm in the living tissue is beneficial.

According to some embodiments of the invention, the compound and the antimicrobial agent act in synergy.

According to an aspect of some embodiments of the present invention there is provided an article comprising a substrate, a compound having general Formula A’, as described herein, and an antimicrobial agent, each being incorporated in or on the substrate.

According to some embodiments of the invention, the antimicrobial agent is an antibacterial agent.

According to some embodiments of the invention, the antibacterial agent is a non beta-lactam antibacterial agent.

According to some embodiments of the invention, the antibacterial agent is selected from the group consisting of gentamicin and tobramycin.
According to an aspect of some embodiments of the present invention there is provided an antimicrobial composition comprising a compound having a general Formula A:

![Formula A](image)

wherein \( R_1 \) and \( R_2 \) are each independently selected from the group consisting of alkyl, aryl and cycloalkyl, or, alternatively, \( R_1 \) and \( R_2 \) are joined together so as to form a ring.

According to some embodiments of the invention, \( R_1 \) and \( R_2 \) are joined together so as to form a ring.

According to some embodiments of the invention, the ring is selected from the group consisting of 5-, 6-, 7-, 8-, 9- and 10-membered ring.

According to some embodiments of the invention, the ring comprises a nitrogen atom.

According to some embodiments of the invention, the ring comprises a N-methyl diethylamine moiety.

According to some embodiments of the invention, the composition is packaged in a packaging material and identified in print, in or on the packaging material, for use in inhibiting a growth of a pathogenic microorganism in or on a substrate.

According to some embodiments of the invention, the substrate is selected from the group consisting of a living tissue and an inanimate object.

According to some embodiments of the invention, the substrate is a living tissue, the composition being a pharmaceutical composition which further comprises a pharmaceutically acceptable carrier.

According to some embodiments of the invention, the composition is packaged in a packaging material and identified in print, in or on the packaging material, for use
in the treatment of a medical condition associated with a pathogenic microorganism in a subject comprising the living tissue.

According to some embodiments of the invention, composition is an antibiofilm composition.

According to some embodiments of the invention, the composition is packaged in a packaging material and identified in print, in or on the packaging material, for use in reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate.

According to some embodiments of the invention, the substrate is selected from the group consisting of a living tissue and an inanimate object.

According to some embodiments of the invention, the composition being identified for use in the treatment of a medical condition in which reducing or preventing the formation of the biofilm and/or disrupting the biofilm in the living tissue is beneficial.

According to some embodiments of the invention, the composition is for use in combination with an additional active agent.

According to some embodiments of the invention, the composition further comprises an additional active agent.

According to some embodiments of the invention, the additional active agent is an antimicrobial agent.

According to some embodiments of the invention, the compound and the antimicrobial agent act in synergy.

According to an aspect of some embodiments of the present invention there is provided a method of inhibiting a growth of a pathogenic microorganism in or on a substrate, the method comprising contacting the substrate with an antimicrobial effective amount of a compound having a general Formula A, as described herein.

According to some embodiments of the invention, the substrate is selected from the group consisting of a living tissue and an inanimate object.

According to some embodiments of the invention, the substrate is a living tissue, the method being for use in the treatment of a medical condition associated with the pathogenic microorganism in a subject comprising the living tissue.
According to an aspect of some embodiments of the present invention there is provided a method of reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate, the method comprising contacting the substrate with an antimicrobial effective amount of a compound having general Formula A, as described herein.

According to some embodiments of the invention, the substrate is selected from the group consisting of a living tissue and an inanimate object.

According to some embodiments of the invention, the substrate is a living tissue, the method being for treating a medical condition in which reducing or preventing the formation of the biofilm and/or disrupting the biofilm in the living tissue is beneficial.

According to some embodiments of the invention, the method further comprises contacting the substrate with an additional active agent.

According to some embodiments of the invention, the additional active agent is an antimicrobial agent.

According to some embodiments of the invention, the compound and the antimicrobial agent act in synergy.

According to an aspect of some embodiments of the present invention there is provided a compound having general Formula A, as described herein, for use in a method of inhibiting a growth of a pathogenic microorganism in or on a substrate.

According to an aspect of some embodiments of the present invention there is provided a use of a compound having general Formula A, as described herein, in the manufacture of a product for inhibiting a growth of a pathogenic microorganism in or on a substrate.

According to some embodiments of the invention, the substrate is selected from the group consisting of a living tissue and an inanimate object.

According to some embodiments of the invention, the substrate is a living tissue, and the compound is being for use in the treatment of a medical condition associated with the pathogenic microorganism in a subject comprising the living tissue.

According to some embodiments of the invention, the substrate is a living tissue and the product is a medicament for treating a medical condition associated with the pathogenic microorganism in a subject comprising the living tissue.
According to an aspect of some embodiments of the present invention there is provided a compound having general Formula A, as described herein, for use in a method of reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate.

According to an aspect of some embodiments of the present invention there is provided a use of the compound having general Formula A, as described herein, in the manufacture of a product for reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate.

According to some embodiments of the invention, the substrate is selected from the group consisting of a living tissue and an inanimate object.

According to some embodiments of the invention, the substrate is a living tissue, the compound being for use in a method of treating a medical condition in which reducing or preventing the formation of the biofilm and/or disrupting the biofilm in the living tissue is beneficial.

According to some embodiments of the invention, the substrate is a living tissue and the product is a medicament for treating a medical condition in which reducing or preventing the formation of the biofilm and/or disrupting the biofilm in the living tissue is beneficial.

According to some embodiments of the invention, the compound as described herein is for use in combination with an additional active agent.

According to some embodiments of the invention, the product is for use in combination with an additional active agent.

According to some embodiments of the invention, the product further comprises an additional active agent.

According to some embodiments of the invention, the additional active agent is an antimicrobial agent.

According to some embodiments of the invention, the compound and the antimicrobial agent act in synergy.

According to an aspect of some embodiments of the present invention there is provided an article comprising a substrate and a compound having general Formula A, as described herein, incorporated in or on the substrate.
According to some embodiments of the invention, the article further comprises an antimicrobial agent being incorporated in or on the substrate.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

As used herein the term "about" refers to ± 10%.

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of means "including and limited to".

The term "consisting essentially of means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

The word "exemplary" is used herein to mean "serving as an example, instance or illustration". Any embodiment described as "exemplary" is not necessarily to be construed as preferred or advantageous over other embodiments and/or to exclude the incorporation of features from other embodiments.

The word "optionally" is used herein to mean "is provided in some embodiments and not provided in other embodiments". Any particular embodiment of the invention may include a plurality of "optional" features unless such features conflict.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should
be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

As used herein, the terms "treating" and "treatment" includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, abrogating, substantially inhibiting or slowing the propagation of at least one cause of a (medical) condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

The phrase "a subject in need thereof as used herein describes a human, or mammal, who has bacterial infection or is at the risk of developing microbial infection (i.e., predisposed).

As used herein, the phrase "microbial infection" encompasses diseases and conditions resulting from or associated with presence of pathogenic microorganism in a subject.
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BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIG. 1 is a comparative bar plot presenting the results of the optical density measurements (blue) and bacterial count (light blue) of methicillin resistant Staphylococcus aureus USA300 strain with different concentrations of Compound K;

FIG. 2 is a comparative bar plot presenting the results of the optical density measurements (orange) and bacterial count (yellow) of Pseudomonas aeruginosa ATCC27312 strain with different concentrations of Compound K;

FIG. 3 is a bar graph presenting methicillin resistant Staphylococcus aureus USA300 strain inhibition zones by different concentrations of Compound K (denoted EDP-5), measured in cm² using planimetry method;

FIG. 4 is a bar graph presenting the Pseudomonas aeruginosa ATCC27312 strain inhibition zones by different concentrations of Compound K, measured in cm² using planimetry method;

FIGs. 5A-B are comparative bar plots presenting the results of the optical density measurements of methicillin resistant Staphylococcus aureus USA300 strain in the presence of different concentrations of Compound K (yellow bars) and Compound F (light blue bars) (FIG. 5A) and of different concentrations of Gentamicin (FIG. 5B);

FIGs. 6A-B are comparative bar plots presenting the results of the optical density measurements of Pseudomonas aeruginosa ATCC27312 strain with different concentrations of Compound K (orange bars) and Compound F (blue bars) (FIG. 6A) and of different concentrations of Tobramycin (FIG. 6B);

FIGs. 7A-D present representative photographs of wounds induced in tested pigs during, before and after treatments (following inoculation with bacterial strains), as well as throughout the assessment days, wherein gauze in all treatment groups were in place and moist on day 1 showing a slight adherence to the wound bed (FIG. 7A), while re-
injury was not observed (FIG. 7B), and wherein wound fluid was observed from all treatment groups after 24 hours biofilm formation in wounds assigned to biofilm elimination assessment (FIG. 7C and Figure 7D);

FIGs. 8A-B present photographs of a wound induced in a tested pig and infected with methicillin resistant *Staphylococcus aureus* (FIG. 8B), and treated within 20 minutes after inoculation with vehicle (FIG. 8A);

FIG. 9 is a bar graph showing methicillin resistant *Staphylococcus aureus* USA300 biofilm inhibition in the presence of 100 mg/ml (light blue bars), 40 mg/ml (yellow bars), and 10 mg/ml (green bars) of Compound K (denoted EDP-5), of vehicle only (purple bars) and of mupirocin (blue bars) control treatments, compared to control untreated wounds (orange bars);

FIG. 10 is a bar graph showing inhibition of biofilm of methicillin resistant *S. aureus* USA300 in the presence of various concentrations of Compound K (denoted EDP-5) and control treatments following 24 hours (Day 1) of a first treatment (1 Rx) (light blue bars) and following additional 24 hours (Day 2) of a second treatment (2 Rx);

FIGs. 11A-B are bar graphs showing bacterial counts of methicillin resistant *Staphylococcus aureus* USA300 biofilm in wounds (light blue bars) compared with bacterial count in gauze (red bars), 24 hours (Day 1) after one treatment application (1 Rx) of various concentrations of Compound K (denoted EDP-5) and control treatments (FIG. 11A), and bacterial counts of *S. aureus* USA300 in wounds (yellow bars) compared with bacterial count in gauze (orange bars) 24 hours (Day 2) after a second treatment application (2 Rx) of various concentrations of Compound K (denoted EDP-5) and control treatments (FIG. 11B);

FIG. 12 is a bar graph showing elimination of methicillin resistant *Staphylococcus aureus* USA300 biofilm after 24 hours of biofilm formation, following treatment of various concentrations of Compound K (denoted EDP-5) and control treatments;

FIG. 13 is a bar graph showing bacterial count of methicillin resistant *Staphylococcus aureus* USA300 after 24 hours of biofilm formation in wounds (different color bars), compared with bacterial count in gauze (green bars), following
one treatment with various concentrations of Compound K (denoted EDP-5) and control
treatments;

FIG. 14 is a bar graph showing inhibition of *Pseudomonas aeruginosa* ATCC 27312 biofilm formation after one application (Day 1, 1 Rx) and two applications (Day 2, 2 Rx) of treatment with 40 mg/ml (light blue bars), 10 mg/ml (yellow bars), 4 mg/ml (green bars) of Compound K (denoted EDP-5) and vehicle (purple bars), silver sulfadiazine (blue bars) and control (brown bars) treatments;

FIG. 15 is a bar graph comparing inhibition of *Pseudomonas aeruginosa* ATCC 27312 biofilm after one application (Day 1, 1 Rx; light blue bars) and two applications (Day 2, 2 Rx; yellow bars) of treatment with various concentrations of Compound K (denoted EDP-5) and control treatments;

FIGs. 16A-B are bar graphs showing bacterial count of *Pseudomonas aeruginosa* ATCC 27312 in wounds (light blue bars), compared with bacterial count in gauze (red bars), 24 hours (Day 1) after one treatment application (1 Rx) (FIG. 16A) and bacterial count of *Pseudomonas aeruginosa* ATCC 27312 in wounds (yellow bars), compared with bacterial count in gauze (orange bars), additional 24 hours (Day 2) after second treatment application (2 Rx) (FIG. 16B) of treatment with various concentrations of Compound K (denote EDP-5) and control treatments;

FIG. 17 is a bar graph showing elimination of *Pseudomonas aeruginosa* ATCC 27312 biofilm after 24 hours of biofilm formation, following treatment of various concentrations of Compound K (denoted EDP-5) and control treatments;

FIG. 18 is a bar graph showing bacterial count of *Pseudomonas aeruginosa* ATCC 27312 after 24 hours of biofilm formation in wounds (different color bars), compared with bacterial count in gauze (green bars), following one treatment with various concentrations of Compound K (denote EDP-5) and control treatments;

FIGs. 19A-B present a bar graph showing inhibition of biofilm formation *Pseudomonas aeruginosa* ATCC27312 after one treatment with BL 6031 (Compound F) 10 mg/ml (light blue bars) and 30 mg/ml (yellow bars), with BL 6031 (Compound K) in 30 % PEG vehicle, 20 mg/ml (grey blue bars) and 60 mg/ml (pink bars), of BL 6030 (Compound K) in 30 % PG vehicle, 20 mg/ml (dark yellow bars) and 60 mg/ml (lavender bars), with Tobramycin 100 µg/ml (purple bars) and 50 µg/ml (blue bars) and in untreated wound (brown bars) (FIG. 19A) and a bar graph showing inhibition of
**Pseudomonas aeruginosa** ATCC27312 (PA biofilm formation after one application (Day 1, 1 Rx) (light blue bars) and two applications (Day 2, 2 Rx) (yellow bars) of treatment with the various concentrations of BL 6030, BL 6031 and control treatments (FIG. 19B) (see, Design 1);

FIGs. 20A-B present bar graphs showing inhibition of biofilm formation of **Pseudomonas aeruginosa** ATCC27312 in wounds (light blue bars), compared with bacterial count in gauze (red bars), following one treatment with various concentrations of BL 6030 (Compound K) and BL 6031 (Compound F) and control treatments, 24 hours (Day 1) following one treatment application (1 Rx) (FIG. 20A) and additional 24 hours (Day 2) following a second treatment application (FIG. 20B) (see, Design 1);

FIGs. 21A-B present bar graphs showing inhibition of methicillin resistant **Staphylococcus aureus** (MRSA) biofilm formation 24 hours after application of treatment with 75 mg/ml (pink bars) and 37.5 mg/ml (blue bars) of BL 6031 (Compound F), with Gentamicin 200 µg/ml (green bars) and 100 µg/ml (yellow bars), with 150 mg/ml (red bars) and 75 mg/ml (light blue bars) of BL 6030 (Compound K) in 30 % PG vehicle, with 150 mg/ml (bright green bars) and 75 mg/ml (brown bars) of BL 6030 (Compound K) in 30 % PEG vehicle, with Mupirocin (purple bars) and in untreated wound (sea green bars) (FIG. 21A), and showing inhibition of biofilm formation of MRSA in wounds (light blue bars), compared with bacterial count in gauze (red bars), following one treatment with the various preparations of BL 6030 (Compound K) and BL 6031 (Compound F) and control treatments (FIG. 21B), 24 hours (Day 1) following one treatment application (1 Rx) (see, Design 2);

FIGs. 22A-B present bar graphs showing elimination of methicillin resistant **Staphylococcus aureus** (MRSA) USA300 biofilm 24 hours after application of treatment with 75 mg/ml (plum bars) and 37.5 mg/ml (blue bars) of BL 6031 (Compound F), with Gentamicin 200 µg/ml (green bars) and 100 µg/ml (yellow bars), with 150 mg/ml (red bars) and 75 mg/ml (light blue bars) of BL 6030 (Compound K) in 30 % PG vehicle, with 150 mg/ml (bright green bars) and 75 mg/ml (brown bars) of BL 6030 (Compound K) in 30 % PEG vehicle, with Mupirocin (purple bars) and in untreated wound (sea green bars) (FIG. 22A), and showing elimination of MRSA biofilm in wounds (yellow bars), compared with bacterial count in gauze (orange bars), following treatment with various preparations of BL 6030 (Compound K) and BL 6031
(Compound F) and control treatments (FIG. 22B), after 24 hours of biofilm formation, and additional 24 hours (Day 2) following one treatment application (1 Rx) (see, Design 2);

FIGs. 23A-B present bar graphs showing inhibition of *Pseudomonas aeruginosa* ATCC27312 biofilm formation 24 hours after application of treatment with 10 mg/ml (plum bars) and 30 mg/ml (blue bars) of BL 6031 (Compound F), with 20 mg/ml (green bars) and 60 mg/ml (yellow bars) of BL 6030 (Compound K), with Tobramycin 200 µg/ml (red bars), 100 µg/ml (light blue bars) and 50 µg/ml (bright green bars), with 30 % PEG vehicle (brown bars), with Silver Sulfadiazine (purple bars) and of untreated wound (sea green bars) (FIG. 23A), and showing inhibition of biofilm formation of *Pseudomonas aeruginosa* ATCC27312 in wounds (light green bars), compared with bacterial count in gauze (blue bars), following one treatment with the various preparations of BL 6030 (Compound K) and BL 6031 (Compound F) and control treatments (FIG. 23B), 24 hours (Day 1) following one treatment application (1 Rx) (see, Design 3);

FIGs. 24A-B present bar graphs showing elimination of *Pseudomonas aeruginosa* ATCC27312 biofilm 24 hours after application of treatment with 10 mg/ml (plum bars) and 30 mg/ml (blue bars) of BL 6031 (Compound F), with 20 mg/ml (green bars) and 60 mg/ml (yellow bars) of BL 6030 (Compound K), with Tobramycin 200 µg/ml (red bars), 100 µg/ml (light blue bars) and 50 µg/ml (bright green bars), with 30 % PEG vehicle (brown bars), with Silver Sulfadiazine (purple bars) and of untreated wound (sea green bars) (FIG. 24A), and showing elimination of *Pseudomonas aeruginosa* ATCC27312 biofilm in wounds (pink bars), compared with bacterial count in gauze (purple bars), following treatment with the various preparations of BL 6030 (Compound K) and BL 6031 (Compound F) and control treatments (FIG. 24B), after 24 hours of biofilm formation, and additional 24 hours (Day 2) following one treatment application (1 Rx) (see, Design 3);

FIGs. 25A-B present bar graphs showing inhibition of methicillin resistant *Staphylococcus aureus* (MRSA) biofilm formation 24 hours after application of treatment with Gentamicin 25 µg/ml (plum bars), 37.5 mg/ml (blue bars) BL 6031 (Compound F), with Gentamicin 50 µg/ml (green bars), with 75 mg/ml (yellow bars) BL 6031 (Compound F), with a combined therapy of Gentamicin 25 µg/ml and 37.5
mg/ml BL 6031 (Compound F) (rose bars), with Gentamicin 100 µg/ml (bright green bars), with 30% PEG vehicle (brown bars), with Mupirocin (purple bars) and in untreated wound (sea green bars) (FIG. 25A), and showing inhibition of biofilm formation of MRSA in wounds (light blue bars), compared with bacterial count in gauze (green bars), following one treatment with the various preparations of BL 6031 and/or Gentamicin and control treatments (FIG. 25B), 24 hours (Day 1) following one treatment application (1 Rx) (see, Design 4);

FIGs. 26A-B present bar graphs showing elimination of methicillin resistant Staphylococcus aureus (MRSA) USA300 biofilm 24 hours after application of treatment with Gentamicin 25 µg/ml (plum bars), 37.5 mg/ml (blue bars) BL 6031 (Compound F), with Gentamicin 50 µg/ml (green bars), with 75 mg/ml (yellow bars) BL 6031 (Compound F), with a combined therapy of Gentamicin 25 µg/ml and 37.5 mg/ml BL 6031 (Compound F) (rose bars), with Gentamicin 100 µg/ml (bright green bars), with 30% PEG vehicle (brown bars), with Mupirocin (purple bars) and in untreated wound (sea green bars) (FIG. 26A), and showing elimination of MRSA biofilm in wounds (yellow bars), compared with bacterial count in gauze (orange bars), following treatment with various preparations of BL 6031 (Compound F) and/or Gentamicin and with control treatments (FIG. 26B), after 24 hours of biofilm formation, and additional 24 hours (Day 2) following one treatment application (1 Rx) (see, Design 4);

FIGs. 27A-B present bar graphs showing inhibition of Pseudomonas aeruginosa ATCC27312 biofilm formation 24 hours after application of treatment with Tobramycin 25 µg/ml (plum bars), 10 mg/ml (blue bars) BL 6031 (Compound F), with Tobramycin 50 µg/ml (green bars), with 30 mg/ml (yellow bars) BL 6031 (Compound F), with a combined therapy of Tobramycin 25 µg/ml and 10 mg/ml BL 6031 (Compound F) (rose bars), with a combined therapy of Tobramycin 50 µg/ml and 30 mg/ml BL 6031 (Compound F) (light blue bars), with Tobramycin 100 µg/ml (bright green bars), with 30% PEG vehicle (brown bars), with Silver Sulfadiazine (purple bars) and in untreated wound (sea green bars) (FIG. 27A), and showing inhibition of biofilm formation of Pseudomonas aeruginosa ATCC27312 in wounds (light blue bars), compared with bacterial count in gauze (light yellow bars), following one treatment with the various...
preparations of BL 6031 and/or Tobramycin and control treatments (FIG. 27B), 24 hours (Day 1) following one treatment application (1 Rx) (see, Design 5); and

FIGs. 28A-B present bar graphs showing elimination of *Pseudomonas aeruginosa* ATCC27312 biofilm 24 hours after application of treatment with Tobramycin 25 µg/ml (plum bars), 10 mg/ml (blue bars) BL 6031 (Compound F), with Tobramycin 50 µg/ml (green bars), with 30 mg/ml (yellow bars) BL 6031 (Compound F), with a combined therapy of Tobramycin 25 µg/ml and 10 mg/ml BL 6031 (Compound F) (rose bars), with a combined therapy of Tobramycin 50 µg/ml and 30 mg/ml BL 6031 (Compound F) (light blue bars), with Tobramycin 100 µg/ml (bright green bars), with 30 % PEG vehicle (brown bars), with Silver Sulfadiazine (purple bars) and in untreated wound (sea green bars) (FIG. 28A), and showing elimination of *Pseudomonas aeruginosa* ATCC27312 biofilm in wounds (yellow bars), compared with bacterial count in gauze (orange bars), following treatment with the various preparations of BL 6031 (Compound F) and/or Tobramycin and with control treatments (FIG. 28B), after 24 hours of biofilm formation, and additional 24 hours (Day 2) following one treatment application (1 Rx) (see, Design 5).

**DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION**

The present invention, in some embodiments thereof, relates to pharmacology, and, more particularly, but not exclusively, to treatments which are useful in inhibiting bacterial growth and/or in preventing, reducing and/or interfering with biofilm formation.

The principles and operation of the present invention may be better understood with reference to the accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

As discussed hereinabove, resistance to antimicrobial agents, as well as formation of biofilm of bacteria, fungi, algae and other microorganisms, present a formidable challenge in the battle against infections and other medical conditions associated with pathogenic microorganisms, biofouling of medical devices, particularly
in internal medicinal and dentistry fields, as well as in fields such as water treatment, containment and transportation.

U.S. Patent No. 6,448,238 teaches 4-fluorophenylboronic acid and other phenylboronic acids and their use as beta-lactamase inhibitors. The compounds described in this patent are taught to be used in combination with beta-lactam antibiotics for treatment of infections of bacteria which are resistant to beta-lactam antibiotics.

The present inventors have uncovered that exposing pathogenic bacteria to 4-fluorophenylboronic acid (designated herein as Compound F or BL 6031), combined with anti-bacterial agents which are not beta-lactam antibiotics ("non beta-lactam antibiotics") resulted in surprisingly strong inhibition of bacterial growth.

The present inventors have postulated that esters of 4-fluorophenylboronic acid can perform similarly to 4-fluorophenylboronic acid.

While reducing the present invention to practice, the present inventors have prepared an exemplary such ester, designated Compound K (and also referred to herein as BL 6030 and EDP-5), which has a 6-methyl-2-phenyl-1,3,6,2-dioxazaborocane motif, and have uncovered that such an esterified boronic acid derivative exerts antimicrobial activity per se as well as quorum sensing inhibitory activity, and hence can be used as antimicrobial and/or anti-biofilm agent, as defined herein.

The present inventors have further uncovered that 4-phenylboronic acid and/or esterified derivatives thereof are effective as antimicrobial and/or anti-biofilm agents when used in combination with other antimicrobial agents, showing mostly synergistic activity effect.

The 4-fluorophenylboronic acid and esters thereof:

4-Fluorophenylboronic acid is also referred to herein as Compound F or BL 6031 and has the following structure:

![Compound F](image-url)
The herein disclosed esterified derivatives of 4-fluorophenylboronic acid are also referred to herein as 4-fluorophenylboronic esters or esters of 4-fluorophenylboronic acid, or simply as ester compounds or esters, and can be collectively represented by general Formula A, as follows:

\[
\begin{align*}
\text{Formula A} \\
R_1\text{O} & \quad \text{B} & \quad \text{OR}_2 \\
\text{F} & \\
\end{align*}
\]

wherein \( R_1 \) and \( R_2 \) are each independently selected from the group consisting of alkyl, aryl and cycloalkyl, or, alternatively, \( R_1 \) and \( R_2 \) are joined together so as to form a ring.

The term "alkyl", as used herein, describes a saturated aliphatic hydrocarbon including straight chain and branched chain groups. In some embodiments, the alkyl group has 1 to 20 carbon atoms. Whenever a numerical range; *e.g.*, "1-20", is stated herein, it implies that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 20 carbon atoms. In some embodiments, the alkyl is a medium size alkyl having 1 to 10 carbon atoms. In some embodiments, the alkyl is a lower alkyl having 1 to 4 carbon atoms. The alkyl group may be substituted or unsubstituted. Substituted alkyl may have one or more substituents, whereby each substituent group can independently be, for example, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, amine, halide, sulfonate, sulfoxide, phosphonate, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, azo, sulfonamide, C-carboxylate, O-carboxylate, N-thiocarbamate, O-thiocarbamate, urea, thiourea, N-carbamate, O-carbamate, C-amide, N-amide, guanyl, guanidine and hydrazine, as defined herein.

The alkyl group can be an end group, wherein it is attached only to the respective oxygen atom in Formula A, or a linking group, which is connected to the
respective oxygen moiety and further to one or more other moieties (e.g., in cases where 
R_i and R_2 form together a ring).

The term "cycloalkyl" describes an all-carbon monocyclic or fused ring (i.e., rings which share an adjacent pair of carbon atoms) group where one or more of the rings does not have a completely conjugated pi-electron system. The cycloalkyl group may be substituted or unsubsstituted. Substituted cycloalkyl may have one or more substituents, whereby each substituent group can independently be, for example, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, amine, halide, sulfonate, sulfoxide, phosphonate, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, azo, sulfonamide, C-carboxylate, O-carboxylate, N-thiocarbamate, O-thiocarbamate, urea, thiourea, N-carbamate, O-carbamate, C-amide, N-amide, guanyl, guanidine and hydrazine, as defined herein.

The cycloalkyl group can be an end group, wherein it is attached only to the respective oxygen atom in Formula A, or a linking group, which is connected to the respective oxygen moiety and further to one or more other moieties (e.g., in cases where 
R_i and R_2 form together a ring) at one or more positions thereof.

The term "aryl" describes an all-carbon monocyclic or fused-ring polycyclic (i.e., rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. The aryl group may be substituted or unsubsstituted. Substituted aryl may have one or more substituents, whereby each substituent group can independently be, for example, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, amine, halide, sulfonate, sulfoxide, phosphonate, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, azo, sulfonamide, C-carboxylate, O-carboxylate, N-thiocarbamate, O-thiocarbamate, urea, thiourea, N-carbamate, O-carbamate, C-amide, N-amide, guanyl, guanidine and hydrazine, as defined herein. The aryl group can be an end group, wherein it is attached only to the respective oxygen atom in Formula A, or a linking group, which is connected to the respective oxygen moiety and further to one or more other moieties (e.g., in cases where 
R_i and R_2 form together a ring) at one or more positions thereof.

In some embodiments, 
R_i and R_2 are joined together so as to form a ring with the -O-B-O- skeleton of the boronic acid. The ring can comprise from 4 to 12 atoms, or from 5 to 10 atoms, and thus can be a 5-, 6-, 7-, 8-, 9- or 10-membered ring.
In some embodiments, the ring is a monocylic ring.

A ring formed by \( R_1 \) and \( R_2 \) is naturally a heterocyclic ring since it includes at least one boron atom and 2 oxygen atoms derived from the boronic acid skeleton.

In some embodiments, the ring formed by \( R_1 \) and \( R_2 \) includes a moiety that bridges the two oxygen atoms of the boronic acid of which the compounds disclosed herein are esters.

Thus, \( R_1 \) and \( R_2 \) can be joined so as form a bridging moiety which bridges the two oxygen atoms of the boronic acid of which the compounds disclosed herein are esters.

This bridging moiety can be, for example, a hydrocarbon chain, optionally substituted, and optionally interrupted by one or more heteroatoms such as, but not limited to, nitrogen (NR'), -O- or -S-.

As used herein, a "hydrocarbon chain" describes a moiety having a backbone chain that is comprised of carbon atoms, which can be unsubstituted (namely, have hydrogens attached to the carbon atoms) or substituted by various substituents, as described hereinabove for an alkyl.

A hydrocarbon moiety can therefore be comprised of one or more alkyls, one or more cycloalkyls and/or one or more aryls, as these are defined herein, wherein each can be substituted or unsubstituted.

In some embodiments, the hydrocarbon chain is 2 to 8 carbon atoms in length, and can thus be 2, 3, 4, 5, 6, 7 or 8 carbon atom in length.

In some embodiments, the ring comprises an alkylene chain, namely, a hydrocarbon chain as described herein, comprised of one or more alkyls. The alkylene chain can be linked via one carbon atom to one oxygen of the boronic acid skeleton and via another carbon to the other oxygen of the boronic acid skeleton.

In some embodiments, the alkylene chain is 2 to 8 carbon atoms in length, and can thus be 2, 3, 4, 5, 6, 7 or 8 carbon atom in length.

In some embodiments, the hydrocarbon chain comprises one or more heteroatoms, as described herein.

Such hydrocarbon chains can be comprised of an alkylene chain, as described herein, which is interrupted by one or more heteroatoms. Alternatively, such hydrocarbon chains can comprise one or more of a heteroalicyclic moiety or a
heteroaryl moiety, whereby a heteroatom of such moieties forms a part of the hydrocarbon chain.

In some embodiments, the ring comprises a hydrocarbon as described herein, which is interrupted by one or more nitrogen atoms, such that the ring formed by Ri and R2 in Formula A comprises one or more nitrogen atoms.

In some embodiments, the ring comprises an alkylene chain, as described herein, interrupted by one or more nitrogen atoms.

In an exemplary embodiment, the ring comprises a N-methyldiethylamine moiety, bridging the two oxygen atoms in Formula A.

Exemplary compounds according to some embodiments of the present invention have Formula A as described herein, wherein:

Ri is methyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is ethyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is propyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is butyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is pentyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is hexyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is heptyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is octyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is nonyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is decyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is cyclopropyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is cyclobutyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is cyclopentyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is cyclohexyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is cycloheptyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is any other cycloalkyl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is cyclopropyl and R2 is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl or any other cycloalkyl;
Ri is cyclobutyl and R2 is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl or any other cycloalkyl;
Ri is cyclopentyl and R2 is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl or any other cycloalkyl;
Ri is cyclohexyl and R2 is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl or any other cycloalkyl;
Ri is cycloheptyl and R2 is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl or any other cycloalkyl;
Ri is cyclooctyl and R2 is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl or any other cycloalkyl;
Ri is phenyl, naphthalenyl or any other aryl and R2 is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl or any other cycloalkyl;
Ri is phenyl, naphthalenyl or any other aryl and R2 is ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl;
Ri is phenyl, naphthalenyl or any other aryl and R2 is phenyl, naphthalenyl or any other aryl, including any combination thereof.

Additional exemplary compounds have Formula A as described herein, wherein Ri, O, B, O and R2 form a ring such that Ri and R2 form together any of the following
bridging moieties (in a biradical form thereof, which is also referred to herein as a linking moiety):

- ethyl, propyl, butyl, pentyl, hexyl, pentyl, octyl, cyclopropyl, cyclobutyl,
cyclopentyl, cyclohexyl, phenyl, naphthalenyl, each being substituted or unsubstituted;

- a moiety having the formula: \( \text{R}^a \), wherein \( \text{R}^1 \) and \( \text{R}^2 \) are each independently an alkyl linking moiety, a cycloalkyl linking moiety or an aryl linking moiety, as defined herein, and \( \text{R}^a \) is hydrogen, alkyl, cycloalkyl or aryl, as defined herein, such as, but not limited to, the following moieties:
  - N-methylethaneamine, N-dimethylamine, N-methylpropaneamine, N-methylbutaneamine, N-methylpentaneamine, N-methylhexanamine, N-diethylamine, N-ethylpropaneamine, N-ethylbutaneamine, N-ethylpentaneamine, N-ethylhexanamine, N-dipropylamine, N-propylbutaneamine, N-propylpentaneamine, N-propylhexanamine, N-dibutylamine, N-butylpentaneamine, N-butylhexanamine, N-methylpropaneamine, N-methylbutaneamine, N-methylpentaneamine, N-methylhexanamine, whereby in each of the above the nitrogen can be substituted by an alkyl, cycloalkyl or aryl, as defined herein; and

- a moiety having the formula: \( \text{R}^1 \), wherein \( \text{R}^1 \) and

In some embodiments, the bridging moiety comprises an N-methyldiethylamine moiety.

In some embodiments, \( \text{R}^1 \), O, B, O and \( \text{R}^2 \) form a 1,3,6,2-dioxazaborocane ring, such as 6-methyl-1,3,6,2-dioxazaborocane ring.

Thus, an exemplary ester compound according to some embodiments of the present invention is Compound K, as described in the Examples section that follows.

The term "heteroaryl" describes a monocyclic or fused ring (i.e., rings which share an adjacent pair of atoms) group having in the ring(s) one or more atoms, such as, for example, nitrogen, oxygen and sulfur and, in addition, having a completely conjugated pi-electron system. Examples, without limitation, of heteroaryl groups include pyrrole, furane, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine,
pyrimidine, quinoline, isoquinoline and purine. The heteroaryl group may be substituted or unsubstituted. Substituted heteroaryl may have one or more substituents, whereby each substituent group can independently be, for example, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, amine, halide, sulfonate, sulfoxide, phosphonate, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, azo, sulfonamide, C-carboxylate, O-carboxylate, N-thiocarbamate, O-thiocarbamate, urea, thiourea, O-carbamate, N-carbamate, C-amide, N-amide, guanyl, guanidine and hydrazine. The heteroaryl group can be an end group, as this phrase is defined hereinabove, where it is attached to a single adjacent atom, or a linking group, as this phrase is defined hereinabove, connecting two or more moieties at two or more positions thereof. Representative examples are pyridine, pyrrole, oxazole, indole, purine and the like.

The term "heteroalicyclic" describes a monocyclic or fused ring group having in the ring(s) one or more atoms such as nitrogen, oxygen and sulfur. The rings may also have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. The heteroalicyclic may be substituted or unsubstituted. Substituted heteroalicyclic may have one or more substituents, whereby each substituent group can independently be, for example, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, amine, halide, sulfonate, sulfoxide, phosphonate, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, azo, sulfonamide, C-carboxylate, O-carboxylate, N-thiocarbamate, O-thiocarbamate, urea, thiourea, O-carbamate, N-carbamate, C-amide, N-amide, guanyl, guanidine and hydrazine. The heteroalicyclic group can be an end group, as this phrase is defined hereinabove, where it is attached to a single adjacent atom, or a linking group, as this phrase is defined hereinabove, connecting two or more moieties at two or more positions thereof. Representative examples are piperidine, piperazine, tetrahydrofurane, tetrahydropyrane, morpholino and the like.

As used herein, the term "amine" describes both a -NR'R" group and a -NR- group, wherein R' and R" are each independently hydrogen, alkyl, cycloalkyl, aryl, as these terms are defined hereinbelow.
The amine group can therefore be a primary amine, where both $R'$ and $R''$ are hydrogen, a secondary amine, where $R'$ is hydrogen and $R''$ is alkyl, cycloalkyl or aryl, or a tertiary amine, where each of $R'$ and $R''$ is independently alkyl, cycloalkyl or aryl.

Alternatively, $R'$ and $R''$ can each independently be hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, amine, halide, sulfonate, sulfoxide, phosphonate, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, azo, sulfonamide, carbonyl, C-carboxylate, O-carboxylate, N-thiocarbamate, O-thiocarbamate, urea, thiourea, N-carbamate, O-carbamate, C-amide, N-amide, guanyl, guanidine and hydrazine.

The term "amine" is used herein to describe a -$NR'R''$ group in cases where the amine is an end group, as defined hereinunder, and is used herein to describe a -$NR'$-group in cases where the amine is a linking group.

The term "hydroxyl" describes a -OH group.

The term "alkoxy" describes both an -O-alkyl and an -O-cycloalkyl group, as defined herein.

The term "aryloxy" describes both an -O-aryl and an -O-heteroaryl group, as defined herein.

The term "thiohydroxy" describes a -SH group.

The term "thioalkoxy" describes both a -S-alkyl group, and a -S-cycloalkyl group, as defined herein.

The term "thioaryloxy" describes both a -S-aryl and a -S-heteroaryl group, as defined herein.

The term "cyano" describes a -C≡N group.

The term "isocyanate" describes an -N=C=O group.

The term "nitro" describes an -NO$_2$ group.

The term "halide" and "halo" describes fluorine, chlorine, bromine or iodine.

The term "haloalkyl" describes an alkyl group as defined above, further substituted by one or more halide.

The term "sulfate" describes a -$0-S(=0)\_2^{-}$OR'$ end group, as this term is defined hereinabove, or an -$0-S(=0)\_2^{-}$-OR linking group, as these phrases are defined hereinabove, where $R'$ is as defined hereinabove.
The term "thiosulfate" describes a -O-S(=S)(=0)OR' end group or a -O-S(=S)(=0)-O- linking group, as these phrases are defined hereinabove, where R' is as defined hereinabove.

The term "sulfite" describes an -O-S(=0)-O-R' end group or a -O-S(=0)-O- linking group, as these phrases are defined hereinabove, where R' is as defined hereinabove.

The term "thiosulfite" describes a -O-S(=S)-O-R' end group or an -O-S(=S)-O- linking group, as these phrases are defined hereinabove, where R' is as defined hereinabove.

The term "sulfinate" describes a -P(=0)(OR')(OR") end group or an -P(=0)(OR')(0)- linking group, as these phrases are defined hereinabove, with R' and R" as defined herein.

The term "thiophosphonate" describes a -P(=S)(OR')(OR") end group or an -P(=S)(OR')(0)- linking group, as these phrases are defined hereinabove, with R' and R" as defined herein.
The term "phosphinyl" describes a -PR'R" end group or a -PR'-linking group, as these phrases are defined hereinabove, with R' and R" as defined hereinabove.

The term "phosphine oxide" describes a -P(=0)(R')(R") end group or a -P(=0)(R')-linking group, as these phrases are defined hereinabove, with R' and R" as defined herein.

The term "phosphine sulfide" describes a -P(=S)(R')(R") end group or a -P(=S)(R')-linking group, as these phrases are defined hereinabove, with R' and R" as defined herein.

The term "phosphite" describes an -0-PR'(=0)(OR") end group or an -O-PH(=0)(0)-linking group, as these phrases are defined hereinabove, with R' as defined herein.

The term "carbonyl" or "carbonate" as used herein, describes a -C(=0)-R' end group or a -C(=0)-linking group, as these phrases are defined hereinabove, with R' as defined herein.

The term "thiocarbonyl" as used herein, describes a -C(=S)-R' end group or a -C(=S)-linking group, as these phrases are defined hereinabove, with R' as defined herein.

The term "oxime" describes a =N-OH end group or a =N-O- linking group, as these phrases are defined hereinabove.

The term "acyl halide" describes a -(C=0)R" group wherein R" is halide, as defined hereinabove.

The term "azo" or "diazo" describes an -N=NR' end group or an -N=N- linking group, as these phrases are defined hereinabove, with R' as defined herein.

The term "peroxo" describes an -O-OR' end group or an -O-O- linking group, as these phrases are defined hereinabove, with R' as defined hereinabove.

The term "C-carboxylate" describes a -C(=0)-OR' end group or a -C(=0)-0-linking group, as these phrases are defined hereinabove, where R' is as defined herein.

The term "O-carboxylate" describes a -OC(=0)R' end group or a -OC(=0)-linking group, as these phrases are defined hereinabove, where R' is as defined herein.

The term "C-thiocarboxylate" describes a -C(=S)-OR' end group or a -C(=S)-0-linking group, as these phrases are defined hereinabove, where R' is as defined herein.
The term "O-thiocarboxylate" describes a -OC(=S)R' end group or a -OC(=S)-linking group, as these phrases are defined hereinabove, where R' is as defined herein.

The term "N-carbamate" describes an R"OC(=0)-NR' end group or a -OC(=0)-NR'- linking group, as these phrases are defined hereinabove, with R' and R" as defined herein.

The term "O-carbamate" describes a -OC(=0)-NR'R" end group or a -OC(=0)-NR'- linking group, as these phrases are defined hereinabove, with R' and R" as defined herein.

The term "O-thiocarbamate" describes a -OC(=S)-NR'R" end group or a -OC(=S)-NR'- linking group, as these phrases are defined hereinabove, with R' and R" as defined herein.

The term "N-thiocarbamate" describes an R"OC(=S)NR' end group or a -OC(=S)NR'- linking group, as these phrases are defined hereinabove, with R' and R" as defined herein.

The term "S-dithiocarbamate" describes a -SC(=S)-NR'R" end group or a -SC(=S)-NR'- linking group, as these phrases are defined hereinabove, with R' and R" as defined herein.

The term "N-dithiocarbamate" describes an R"SC(=S)NR' end group or a -SC(=S)NR'- linking group, as these phrases are defined hereinabove, with R' and R" as defined herein.

The term "urea", which is also referred to herein as "ureido", describes a -NR'C(=0)-NR"R' end group or a -NR'C(=0)-NR"- linking group, as these phrases are defined hereinabove, where R' and R" are as defined herein and R" is as defined herein for R and R'.

The term "thiourea", which is also referred to herein as "thioureido", describes a -NR'-C(=S)-NR"R' end group or a -NR'-C(=S)-NR"- linking group, with R', R" and R"' as defined herein.

The term "C-amide" describes a -C(=0)-NR'R" end group or a -C(=0)-NR'-linking group, as these phrases are defined hereinabove, where R' and R" are as defined herein.
The term "N-amide" describes a R'C(=0)-NR" end group or a R'C(=0)-N-linking group, as these phrases are defined hereinabove, where R' and R" are as defined herein.

The term "guanyl" describes a R'R"NC(=N)- end group or a -R'NC(=N)-linking group, as these phrases are defined hereinabove, where R' and R" are as defined herein.

The term "guanidine" describes a -R'NC(=N)-NR"R' end group or a -R'NC(=N)-NR"-linking group, as these phrases are defined hereinabove, where R', R", and R'" are as defined herein.

The term "hydrargyrene" describes a -NR'-NR"R" end group or a -NR'-NR"-linking group, as these phrases are defined hereinabove, with R', R", and R'" as defined herein.

4-Fluorophenylboronic acid and the ester derivatives thereof, represented by general Formula A as described herein are represented herein collectively as compounds having general Formula A' as follows:

![Formula A']

wherein R3 and R4 are each independently selected from the group consisting of hydrogen, alkyl, aryl and cycloalkyl, or, alternatively, R3 and R4 are joined together so as to form a ring.

In some embodiments, R3 and R4 are each independently selected from the group consisting alkyl, aryl and cycloalkyl, or, alternatively, R3 and R4 are joined together so as to form a ring, and are as described herein for R1 and R2, in Formula A.

In some embodiments, at least one of R3 and R4 is hydrogen and the other is as described herein for R1 and R2 (when R1 and R2 do not form a ring).
In some embodiments, each of R₃ and R₄ is hydrogen, such that the compound having Formula A’ is 4-fluorophenylboronic acid.

Embodiments of the present invention in which a compound having Formula A’ is Compound F, relate to a combined use of Compound F and an additional active agent (e.g., an antibacterial agent), as described herein.

**Antimicrobial effect:**

The present inventors have demonstrated that an exemplary ester of 4-fluorophenylboronic acid, Compound K, exhibits an antimicrobial activity, and was further found to prevent or reduce biofilms both *ex-vivo* and *in-vivo*, and that these activities can be enhanced synergistically in combination with antibacterial agents.

Thus, it was shown that 4-fluorophenylboronic esters as described herein can be used effectively both in antimicrobial compositions, and in compositions for preventing, reducing and eliminating biofilms both *ex-vivo* and *in-vivo*, and that these activities can be enhanced synergistically in combination with antibacterial agents.

The present inventors have further demonstrated that 4-fluorophenylboronic acid (Compound F) can be used effectively both in antimicrobial compositions, and in compositions for preventing, reducing and eliminating biofilms both *ex-vivo* and *in-vivo*, and that these activities can be enhanced, additively and even synergistically, in combination with antibacterial agents.

The phrase “antimicrobial” as used herein, refers to a property of a substance (e.g., a compound or a composition) that can effect a parameter of microorganism, as defined herein, including death, eradication, elimination, reduction in number, reduction of growth rate, inhibition of growth, change in population distribution of one or more species of microbial life forms. This term encompasses antibacterial agents, which are also referred to herein as antibiotics.

The effect of an antimicrobial substance (e.g., a compound or a composition) can be manifested, for example, by reducing or increasing adhesion of the microorganism to the substrate; by effecting enzymatic activity; and/or by effecting the viability of the microorganism (e.g., bacteria), as further discussed herein.
According to an aspect of embodiments of the present invention, there is provided a composition which includes, as an active ingredient, a compound having general Formula A as described herein and optionally a carrier.

According to another aspect of embodiments of the present invention, there is provided a composition which includes, as an active ingredient, a compound having general Formula A’ as described herein (e.g., Compound F), an additional active agent (e.g., an antimicrobial or antibacterial agent), as described herein, and optionally a carrier.

Each of these compositions is referred to herein as an antimicrobial or an antibacterial composition.

According to some embodiments of the present invention, the composition is packaged in a packaging material and is identified in print, in or on the packaging material, for use in inhibiting a growth of a pathogenic microorganism in or on a substrate.

Microorganisms can exist on living tissues or on non-living, organic or inorganic substrates, referred to herein an inanimate objects or substrates, and constitute a prevalent mode of microbial life in natural, industrial and medical settings.

Thus, a substrate as defined herein encompasses living tissues and inanimate objects.

In the context of embodiments of the present invention, the phrase "living tissue" is meant to encompass any part of a living organism, a bodily site or a living organ.

As used herein, the phrase "bodily site" includes any organ, tissue, membrane, cavity, blood vessel, tract, biological surface or muscle, which contacting therewith (e.g., delivering thereto or applying thereon) the compound disclosed herein is beneficial. Exemplary bodily sites include, but are not limited to, the skin, a dermal layer, the scalp, an eye, an ear, a mouth, a throat, a stomach, a small intestines tissue, a large intestines tissue, a kidney, a pancreas, a liver, the digestive system, the respiratory tract, a bone marrow tissue, a mucosal membrane, the blood system, a blood vessel, a muscle, a pulmonary cavity, an artery, a vein, a capillary, a heart, a heart cavity, a male or female reproductive organ and any visceral organ or cavity. Any organ or tissue onto which microorganism can exist in contemplated.
The phrase "living tissue" encompasses also samples of a living organism or subject, namely a human or an animal, which have been removed from the organism and maintained viable for any purpose, and encompasses the living subject itself as a whole, e.g., a plant, a human or an animal (e.g., a mammal).

In the context of embodiments of the present invention, the phrase "inanimate object" is meant to encompass any surface of an object which may harbor a microorganism, such as, but not limited to, an implantable medical device such as a gastric or duodenal sleeve, a topical medical device such as a wound dressing, a subcutaneous medical device such as a subcutaneous injection port, a percutaneous medical device such as a catheter, a syringe needle or an endoscopic device, a vessel, a tube, a lid, a wrap, a package, a work surface or area, a warehouse, a package and the like, as is further described hereinafter in the context of "substrate".

As used herein, the phrase "inhibiting the growth of a microorganism" refers to an effect of a compound, a composition or a combination of a compound with another active agent, which stops and/or reverses the propagation of a microorganism, such that at least one cell or a culture thereof is no longer multiplying or growing and/or is killed as a result of coming in contact with the compound, the composition or the combination of compounds. In embodiments of the present invention where the microorganism is a pathogenic microorganism, the effect of inhibiting the growth thereof is oftentimes beneficial.

According to some embodiments of the present invention, the antimicrobial composition is packaged in a packaging material and is identified in print, in or on the packaging material for use in inhibiting a growth of a microorganism in or on inanimate objects, as discussed herein. Such a composition may be in a form of, for example, solution, paste, liquid, spray or powder.

According to some embodiments of this aspect of the present invention, when the substrate is a living tissue, the composition is a pharmaceutical composition.

Hence, according to an aspect of some embodiments of the present invention, there is provided a pharmaceutical composition which comprises a compound having general Formula A as described herein and a pharmaceutically acceptable carrier.

According to another aspect of some embodiments of the present invention, there is provided a pharmaceutical composition which comprises a compound having
general Formula A’ as described herein (e.g., Compound F), an additional active agent (e.g., an antimicrobial or antibacterial agent) and a pharmaceutically acceptable carrier.

As used herein the phrase "pharmaceutical composition" refers to a preparation of any boronic acid or ester compound as described herein, with other chemical components such as pharmaceutically acceptable and suitable carriers and excipients, and optionally with additional active agents, such as another antimicrobial agent. The purpose of a pharmaceutical composition is to facilitate administration of the compound (or combination of compounds) to a subject, or optionally to facilitate its application (contacting) in or on an inanimate object.

Hereinafter, the term "pharmaceutically acceptable carrier" refers to a carrier or a diluent that does not cause significant irritation to an organism and does not inhibit the distribution, therapeutic properties or otherwise does not abrogate the biological activity and properties of the administered or applied compound.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration or application of a drug.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences" Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more pharmaceutically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredient(s) into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient’s condition (see e.g., Fingl et al, 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.l).

The pharmaceutical composition may be formulated for administration in either one or more of routes depending on whether local or systemic treatment or administration is of choice, and on the area to be treated. Administration may be done orally, by inhalation, or parenterally, for example by intravenous drip or intraperitoneal,
subcutaneous, intramuscular or intravenous injection, or topically (including transdermally, ophtalmically, vaginally, rectally, intranasally).

In some embodiments, the pharmaceutical composition is formulated as a wound dressing, using methods known in the art.

The amount of a composition to be administered or otherwise applied will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as an FDA (the U.S. Food and Drug Administration) approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as, but not limited to a blister pack or a pressurized container (for inhalation). The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accompanied by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions for human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising active ingredient(s) according to embodiments of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of a particular medical condition, disease or disorder, as is detailed herein.

According to some embodiments, the pharmaceutical composition presented herein is packaged in a packaging material and identified in print, in or on the packaging material, for use in inhibiting a growth of a pathogenic microorganism in a subject in need thereof.

Further according to an aspect of some embodiments of the present invention, there is provided a use of a compound having general Formula A as described herein in the manufacture of a product for inhibiting a growth of a pathogenic microorganism in or on a substrate, as defined herein.

When the substrate is a living tissue as defined herein, the product is a medicament.
In the context of some embodiments of the present invention, the term "medicament" is used interchangeably with the phrase "pharmaceutical composition".

When the substrate is an inanimate object as defined herein, the product is also referred to herein as an article or article-of-manufacture.

Further according to an aspect of some embodiments of the present invention there is provided a compound having general Formula A as described herein, for use in a method of inhibiting a growth of a pathogenic microorganism in or on a substrate, as defined herein.

In some embodiments, when the substrate is a living tissue, the compound having general Formula A as described herein, is for use in a method of inhibiting a growth of a pathogenic microorganism in a subject which comprises the living tissue.

Further according to an aspect of some embodiments of the present invention there is provided a compound having general Formula A' as described herein (e.g., Compound F), for use in a method of inhibiting a growth of a pathogenic microorganism in or on a substrate, as defined herein, wherein the compound having general Formula A' is used in combination with an additional active agent (e.g., antimicrobial agent or antibacterial agent).

In some embodiments, when the substrate is a living tissue, the compound having general Formula A' as described herein (e.g., Compound F), is for use in a method of inhibiting a growth of a pathogenic microorganism in a subject which comprises the living tissue, wherein the compound having general Formula A' is used in combination with an additional active agent (e.g., antimicrobial agent or antibacterial agent).

According to an aspect of the invention, there is provided a method of inhibiting a growth of a pathogenic microorganism in or on a substrate, which is effected by contacting the substrate with an antimicrobial effective amount of a compound having general Formula A as described herein.

According to another aspect of the invention, there is provided a method of inhibiting a growth of a pathogenic microorganism in or on a substrate, which is effected by co-contacting, as described herein, the substrate with an antimicrobial effective amount of a compound having general Formula A' as described herein (e.g.,
Compound F) and with an additional active agent (e.g., an antimicrobial or antibacterial agent).

As used in the context of embodiments of this aspect of the present invention, the phrase "antimicrobial effective amount" describes an amount of an antimicrobial agent which will effect one or more parameters of a microorganism, including death, eradication, elimination, reduction in number, reduction of growth rate, inhibition of growth, change in population distribution of one or more species of microbial life forms, as described herein. In some embodiments, an antimicrobial effective amount is an amount that reduces to some extent the population of a microorganism in or on a substrate.

In some embodiments, an antimicrobial effective amount is an amount that reduces by at least 10%, 20%, 30%, 40%, 50% or to a higher extent, the population of a microorganism in or on a substrate.

In some embodiments, reduction in the population of a microorganism in or on a substrate is determined by measuring the number of colony forming units of the microorganism grown upon contacting an antimicrobial agent and comparing it to the number of colony forming units of the microorganism when grown without an antimicrobial agent.

As described hereinabove, a substrate can be a living tissue or an inanimate object, as these terms are defined hereinabove.

According to some embodiments of the present invention, when the substrate is a living tissue, such as a bodily site of a subject, the method is effected by administering to the subject an antimicrobial effective amount of a compound having general Formula A as described herein.

According to some embodiments, the administration can be effected orally, rectally, intravenously, topically, intranasally, intradermally, transdermally, subcutaneously, intramuscularly, intraperitoneally or by intrathecal catheter.

A compound having general Formula A or A’ as described herein can be administered either per se or as a part of a pharmaceutical composition as described herein.

The mode of administration is selected to suite the medical condition which is being treated. For example, in treating a systemic infection where rapid distribution of
the therapeutic agent(s) is needed, drug(s) are typically administered orally or intravenously. When treating a local infection, the drug is administered locally, topically, transdermally, subcutaneously or intramuscularly.

According to some embodiments, an exemplary method of inhibiting a growth of a pathogenic microorganism in a subject is effected topically by applying a composition containing the active compound (a compound having general Formula A as described herein) onto a wound.

When the substrate is an inanimate object, as described hereinabove, the mode of inhibiting a growth of a pathogenic microorganism in or on the substrate is effected typically by dipping, spraying, coating, or otherwise applying the active compound(s) or a composition comprising the same, as described herein, in or on the substrate. For example, a catheter for prolonged percutaneous use can be coated with a composition containing a compound having general Formula A as described herein so as to inhibit the growth of a pathogenic microorganism therein or thereon. A catheter for prolonged percutaneous use can also be coated with a composition containing a compound having general Formula A' as described herein (e.g., Compound F) and an additional active agent (e.g., antimicrobial or antibacterial agent) so as to inhibit the growth of a pathogenic microorganism therein or thereon. Other medical devices can be similarly coated, as is detailed hereinbelow.

Herein throughout, the phrase "pathogenic microorganism" is used to describe any microorganism which can cause a disease or infection in a higher organism, such as humans or any animals grown for commercial or recreational purposes, fish, poultry, insects (e.g., bees) and mammals. In some embodiments, a pathogenic microorganism is one that causes diseases and adverse effects in humans, hence, a pathogenic microorganism in the context of embodiments of the present invention is regarded as a cause of a medical condition associated therewith.

The pathogenic microorganism may belong to any family of organisms such as, but not limited to, prokaryotic organisms, eubacterium, archaeabacterium, eukaryotic organisms, yeast, fungi, algae, protozoa, and other microscopic parasites.

As is demonstrated in the Examples section that follows, Compound K was found to be a highly efficient agent against a wide spectrum of bacteria, including...
Gram-negative bacteria and Gram-positive bacteria, when used either alone or in combination with an antibiotic.

As is further demonstrated in the Examples section that follows, Compound F was found to be a highly efficient agent against a wide spectrum of bacteria, including Gram-negative bacteria and Gram-positive bacteria, when used either alone or in combination with an antibiotic.

In some embodiments, the phrase "pathogenic microorganism" refers to a bacterium (or a bacterial strain).

The terms "bacterium" or "bacteria", as used herein, refers to all prokaryotic organisms, including those within all of the phyla in the Kingdom Procaryotae. It is intended that these terms encompass all microorganisms considered to be bacteria including *Mycoplasma, Chlamydia, Actinomyces, Streptomyces, and Rickettsia.* All forms of bacteria are included within this definition including *cocci, bacilli, spirochetes, spheroplasts, protoplasts,* etc. Also included within these terms are prokaryotic organisms that are Gram-negative or Gram-positive. "Gram-negative" and "Gram-positive" refer to staining patterns with the Gram-staining process, which is well known in the art. (See e.g., Finegold and Martin, Diagnostic Microbiology, 6th Ed., CV Mosby St. Louis, pp. 13-15 (1982)). "Gram-positive bacteria" are bacteria that retain the primary dye used in the Gram stain, causing the stained cells to generally appear dark blue to purple under the microscope. "Gram-negative bacteria" do not retain the primary dye used in the Gram stain, but are stained by the counterstain. Thus, Gram-negative bacteria generally appear red. In some embodiments, bacteria are continuously cultured. In some embodiments, bacteria are uncultured and existing in their natural environment (e.g., at the site of a wound or infection) or obtained from patient tissues (e.g., via a biopsy). Bacteria may exhibit pathological growth or proliferation.

Non-limiting examples of bacteria include bacteria of a genus selected from the group including *Salmonella, Shigella, Escherichia, Enterobacter, Serratia, Proteus, Yersinia, Citrobacter, Edwardsiella, Providencia, Klebsiella, Hafnia, Ewingella, Kluyvera, Morganella, Planococcus, Stomatococcus, Micrococcus, Staphylococcus, Vibrio, Aeromonas, Plesiomonas, Haemophilus, Actinobacillus, Pasteurella, Mycoplasma, Ureaplasma, Rickettsia, Coxiella, Rochalimaea, Ehrlichia, Streptococcus, Enterococcus, Aerococcus, Gemella, Lactococcus, Leuconostoc, Pedicoccus, Bacillus,*

In some embodiments of the present invention the pathogenic bacteria are one or more of the following species: Acinetobacter baumanii, Belicobacter pylori, Burkholderia multivorans, Campylobacter jejuni, Deinococcus radiodurans, E. coli, Enterobacter cloaca, Enterococcus faecalis, Haemophilus influenzae, Klebsiella pneumoniae, Klebsiella oxytoca, Mycobacterium tuberculosis, Neisseria gonorrhoeae, Neisseria meningitidis, Pseudomonas aeruginosa, Pseudomonas phosphoreui, Escherichia coli, Bacillus Subtis, Borrelia burgfrferi, Nisseria meningitidis, Nisseria gonorrhoeae, Yersinia pestis, Campylobacter jejuni, Deinococcus radiodurans, Mycobacterium tuberculosis, Enterococcus faecalis, Streptococcus pneumoniae, Streptococcus pyogenes and Staphyllococcus aureus, Salmonella typhimurium, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus pneumoniae, Staphylococcus sanguis, Staphylococcus viridans, Vibrio harveyi, Vibrio cholerae, Vibrio paraahienoltyicus, Vibrio alginolyticus, Yersinia enterocolitica or Yersinia pestis, including any strain or mutant thereof.

In some embodiments the bacteria are of the species Staphylococcus aureus.
In some embodiments the bacteria are of the species Pseudomonas aeruginosa.
In some embodiments the bacteria are of the species Staphylococcus epidermidis.
A wide-spectrum activity range is oftentimes indicative of activity in inhibiting the growth of eukaryotic microorganisms such as fungi, as well as prokaryotic microorganisms such as bacteria.

Trichosporon asahii, Trichosporon cutaneum, Trichosporon inkin, Trichosporon mucoides.

Representative examples of pathogenic parasites and protozoa, against which a compound having general Formula A as described herein may be used according to the present embodiments include, without limitation, various types of amoeba, Leishmania spp, Plasmodium falciparum Trypanosoma cruzi (causing Chagas' disease), Trypanosoma brucei (causing "sleeping sickness"), Plasmodium vivax (causing malaria), Cryptosporidium parvum (causing cryptosporidiosis), Cyclospora cayetanensis, Giardia lamblia (causing giardiasis) and many others.

Resistance of microorganism to antimicrobial agents is the ability of a microorganism to withstand the antimicrobial effects of any given agents (antibiotics). The antimicrobial action of any given agent is putting an environmental pressure on the target (and also non-targeted) microorganisms. The microorganisms which have a mutation that will allow it to survive will live on to reproduce. These newly evolved strain(s) will then pass this trait to their offspring, which will constitute a fully resistant generation.

As demonstrated in the Example section that follows, an exemplary compound having general Formula A as described herein, designated herein as Compound K, was shown to be effective against methicillin resistant Staphylococcus aureus (MRSA). As further demonstrated in the Example section that follows, Compound F as described herein, was shown to be effective against methicillin resistant Staphylococcus aureus (MRSA). Both exemplary Compounds K and F were shown to be effective when used either alone or in combination with an antibiotic. Since this strain is associated with severe medical conditions, the use of a compound having general Formula A or A' as described herein may be one of the means to combat this and other antibiotic resistant pathogens and ameliorate or cure the medical conditions associated therewith.

Hence, according to some embodiments of the present invention, the pathogenic microorganism is a pathogenic, drug-resistant microorganism. The pathogenic, drug resistant microorganism can be any of the microorganisms selected from the group consisting of prokaryotic organisms, eubacteria, archaebacteria, eukaryotic organisms, yeast, fungi, algae, protozoa and other parasites.
The drug-resistant microorganism can be resistant to one or more conventionally used drug or other antimicrobial agent. The phrase "conventional antimicrobial agent" as used herein refers to antimicrobial agents commonly used in clinical therapies, and encompasses, for example, antibacterial agents commonly used in clinical therapies, which are also referred to herein as "clinical antibiotics".

Non-limiting examples of conventional antibacterial agents (antibiotics) include, but are not limited to, gentamicin, ampicillin, amikacin (AK), cefazolin, ceftriaxone, clindamycin, cephalothin, ciprofloxacin, chloramphenicol, ceftazidime (CAZ), cefepime (CPE), erythromycin, trimethoprim/sulfamethoxazole (T/S), gatifloxacin, piperacillin/tazobactam (P/T), aztreonam (AZT), imipenem, levofloxacin, penicillin, oxacillin, nitrofurantoin, linezolid, moxifloxacin, meropenem (MER), tobramycin (TO), ciprofloxacin (CP), tetracycline, vancomycin, rifampin synercid, streptomycin Synergy, colistin (CT) and chloramphenicol (C).

Additional non-limiting examples of conventional antimicrobial agent include polyene-based antifungal agents such as amphotericin, amphotericin B, nystatin and pimaricin, amphotericin B liposomal formulations (AmBisome, Abelcet, Amphocil),azole-based antifungal agents such as fluconazole, itraconazole and ketoconazole,allylamine- or morpholine-based antifungal agents such as allylamines (naftifine, terbinafme), and antimetabolite-based antifungal agents such as 5-fluorocytosine, andfungal cell wall inhibitor such as echinocandins like caspofungin, micafungin and anidulafungin.

According to some embodiments, the pharmaceutical composition described herein is packaged in a packaging material and identified in print, in or on the packaging material, for use in the treatment of a medical condition associated with the pathogenic microorganism in a subject in need thereof.

Further according to some embodiments of the present invention, the medicament as described herein for inhibiting a growth of a pathogenic microorganism in a subject presented herein above is being for use in the treatment of a medical condition associated with the pathogenic microorganism in a subject in need thereof.

Further according to some embodiments of the present invention, a compound having general Formula A as described herein is for use in a method of treating a
medical condition associated with the pathogenic microorganism in a subject in need thereof.

Further according to some embodiments of the present invention, the method as described herein is being for treating a medical condition associated with the pathogenic microorganism in a subject in need thereof.

Further according to some embodiments of the present invention, a compound having general Formula A' as described herein (e.g., Compound F), in combination with an additional active agent (e.g., an antimicrobial or antibacterial agent) is for use in a method of treating a medical condition associated with the pathogenic microorganism in a subject in need thereof.

Further according to some embodiments of the present invention, the method as described herein is being for treating a medical condition associated with the pathogenic microorganism in a subject in need thereof.

"A subject in need thereof" describes a subject that has one or more tissues, organs or any bodily site, infected by the pathogenic microorganism.

In some embodiments the subject is an animal, preferably a mammal, more preferably a human being.

As used herein the term "associated" in the context embodiments of the present invention means that at least one adverse manifestation of the medical condition is caused by a pathogenic microorganism. The phrase "medical condition associated with a pathogenic microorganism" therefore encompasses medical conditions of which the microorganism may be the primary cause of the medical condition or a secondary effect of the main medical condition(s).

Medical conditions associated with a pathogenic microorganism include infections, infestation, contaminations and transmissions by or of pathogenic microorganisms such as those described herein. In general, a disease causing infection is the invasion into the tissues of a plant or an animal by pathogenic microorganisms. The invasion of body tissues by parasitic worms and other higher pathogenic organisms such as lice is oftentimes referred to as infestation.

Invading organisms such as bacteria typically produce toxins that damage host tissues and interfere with normal metabolism; some toxins are actually enzymes that break down host tissues. Other bacterial substances may inflict their damage by
destroying the host's phagocytes, rendering the body more susceptible to infections by other pathogenic microorganisms. Substances produced by many invading organisms cause allergic sensitivity in the host. Infections may be spread via respiratory droplets, direct contact, contaminated food, or vectors, such as insects. They can also be transmitted sexually and from mother to fetus.

Examples of medical conditions and diseases caused by bacterial infections, which are treatable by a compound having general Formula A as described herein according to the present embodiments, include, without limitation, actinomycosis, anthrax, aspergillosis, bacteremia, bacterial skin diseases, bartonella infections, botulism, brucellosis, burkholderia infections, Campylobacter infections, candidiasis, cat-scratch disease, chlamydia infections, cholera, Clostridium infections, coccidioidomycosis, cryptococcosis, dermatomycoses, diphtheria, ehrlichiosis, epidemic louse borne typhus, Escherichia coli infections, fusobacterium infections, gangrene, general infections, general mycoses, gonorrhea, gram-negative bacterial infections, gram-positive bacterial infections, histoplasmosis, impetigo, klebsiella infections, legionellosis, leprosy, leptospirosis, listeria infections, lyme disease, malaria, maduromycosis, melioidosis, mycobacterium infections, mycoplasma infections, necrotizing fasciitis, nocardia infections, onychomycosis, ornithosis, pneumococcal infections, pneumonia, pseudomonas infections, Q fever, rat-bite fever, relapsing fever, rheumatic fever, rickettsia infections, Rocky-mountain spotted fever, salmonella infections, scarlet fever, scrub typhus, sepsis, sexually transmitted bacterial diseases, staphylococcal infections, streptococcal infections, surgical site infection, tetanus, tick-borne diseases, tuberculosis, tularemia, typhoid fever, urinary tract infection, vibrio infections, yaws, yersinia infections, Yersinia pestis plague, zoonoses and zygomycosis.

Medical conditions that are associated with fungi and which may be treatable by a compound having general Formula A as described herein, according to some embodiments, mainly include fungal infections or mycoses. Fungal infections or mycoses are classified depending on the degree of tissue involvement and mode of entry into the host. Main classes are superficial, subcutaneous, systemic and opportunistic infections. Non-limiting examples of medical conditions associated with fungi or other eukaryotes include superficial mycoses infections, "ringworm" or "tinea", candidiasis or "thrush", subcutaneous mycoses, sporotrichosis, systemic mycoses, histoplasmosis,
blastomycosis, coccidiomycosis, paracoccidiodomycosis, aspergillosis, systemic candidosis and cryptococcosis and Pneumocystis.

Medical conditions associated with pathogenic parasites and protozoa which may be treatable by a compound having general Formula A as described herein, according to some embodiments, include, without limitation, acanthamoeba infection, African trypanosomiasis (sleeping sickness), alveolar echinococcosis, amebiasis (entamoeba histolytica infection), American trypanosomiasis (Chaga's disease), ancylostoma infection (hookworm infection, cutaneous larva migrans, CLM), angiostrongylus infection (angiostrongyliasis), angiostrongyliasis (angiostrongylus infection), anisakis infection (anisakiasis), anisakiasis (anisakis infection), ascariasis (intestinal roundworms), babesia infection (babesiosis), babesiosis (babesia infection), balantidiasis (balantidium infection), balantidium infection (balantidiasis), baylisascaris infection (racon roundworm), bilharzia (schistosomiasis), blastocystis hominis infection, body and public lice infestation ("the crabs"), capillaria infection (capillariosis), capillariosis (capillaria infection), cercarial dermatitis (swimmer's itch), chilomastix mesnili infection, clonorchis infection (clonorchiasis), clonorchiasis (clonorchis infection), cryptosporidiosis (Cryptosporidium infection), cutaneous larva migrans (CKM, hookworm infection, ancylostoma infection), cyclospora infection (cyclosporiasis), cysticercosis (neurocysticercosis), delusional parasitosis, dientamoeba fragilis infection, diphyllobothrium infection (diphyllobothriasis), dipylidium infection (dog or cat tapeworm infection), dracunculiasis (guinea worm disease), dog tapeworm (dipylidium), E. histolytica infection (amebiasis), echinococcosis (alveolar hydatid disease), elephantiasis (filariasis, lymphatic filariasis), endolimax nana infection, Entamoeba coli infection, Entamoeba dispar infection, Entamoeba hartmanni infection, Entamoeba histolytica infection (amebiasis), Entamoeba polecki infection, Enterobiasis (pinworm infection), fasciola infection (fascioliasis), fascioliasis (fasciola infection), fasciolopsisis (fasciolopsis infection), fasciolopsis infection (fasciolopsisis), filariasis (lymphatic filariasis, elephantiasis), foodborne diseases, giardiasis (giardia infection, "beaver fever"), gnathostoma infection (gnathostomiasis), gnathostomiasis (gnathostoma infection), guinea worm disease (dracunculiasis), heterophyes infection (heterophyiasis), hymenolepis infection (hymenolepiasis), hookworm infection (ancylostoma infection, cutaneous larva migrans), intestinal roundworms (ascariasis),
iodamoeba buetschlii infection, isospora infection (isosporiasis), leishmaniasis (kala-azar, leishmania infection), loa-loa infection (loaiasis), lymphatic filariasis (filariasis, elephantiasis), malaria, microsporidia infection (microsporidiosis), naegleria infection, neurocysticercosis (cysticercosis), nonpathogenic intestinal amebae infection, onchocerciasis (river blindness), opisthorchis infection (opisthorchiasis), paragonimus infection (paragonimiasis), pediculosis (head lice infestation), pinworm infection (enterobiasis), Pneumocystis carinii pneumonia (PCP), raccoon roundworm infection (baylisascaris infection), river blindness (onchocerciasis), scabies (mite infestation), schistosomiasis (bilharzia), strongyloides infection (strongyloidiasis), trichinellosis (trichinosis), toxocara infection (toxocariasis, ocular larva migrans, visceral larva migrans), toxocariasis (toxocara infection, ocular larva migrans, visceral larva migrans), toxoplasmosis (toxoplasma infection), trichinellosis (trichinosis), trichinosis (trichinellosis), trichomonas infection (trichomoniasis), trichomoniasis (trichomonas infection), trichuriasis (whipworm infection, trichuris infection), travelers' diarrhea, waterborne diseases and zoonotic diseases (diseases spread from animals to people).

**Anti-Biofilm effect:**

As discussed hereinabove, the formation of biofilms of microorganisms presents a formidable challenge in medicine and other practices. As demonstrated in the Examples section that follows, an exemplary ester, Compound K, was found to exert both a biofilm elimination activity and prevention of biofilm formation, and overall to exert an anti-biofilm formation (ABF) activity. As further demonstrated in the Examples section that follows, Compound F was found to exert both a biofilm elimination activity and prevention of biofilm formation, and overall to exert an anti-biofilm formation (ABF) activity. Both exemplary Compounds K and F were found to extent ABF activity when used in combination with an antibiotic.

The term "biofilm", as used herein, refers to an aggregate of living cells which are stuck to each other and/or immobilized onto a surface as colonies. The cells are frequently embedded within a self-secreted matrix of extracellular polymeric substance (EPS), also referred to as "slime", which is a polymeric sticky mixture of nucleic acids, proteins and polysaccharides.
In the context of the present embodiments, the living cells forming a biofilm can be cells of a unicellular microorganism (prokaryotes, archaea, bacteria, eukaryotes, protists, fungi, algae, euglena, protozoan, dinoflagellates, apicomplexa, trypanosomes, amoebae and the likes), or cells of multicellular organisms in which case the biofilm can be regarded as a colony of cells (like in the case of the unicellular organisms) or as a lower form of a tissue.

According to some embodiments of the present invention, the cells are of microorganism origins, and the biofilm is a biofilm of microorganisms, such as bacteria and fungi. The cells of a microorganism growing in a biofilm are physiologically distinct from cells in the "planktonic form" of the same organism, which by contrast, are single-cells that may float or swim in a liquid medium. Biofilms can go through several life-cycle steps which include initial attachment, irreversible attachment, one or more maturation stages, and dispersion. Major differences in protein expression of biofilm forming microorganisms in their sessile phase, compared to that of stationary-phase planktonic cells, make biofilms a completely different mode of microorganism, and one manifestation of this difference is the cells become resistant to a range of antibiotics and disinfectants.

The phrases "anti-biofilm formation activity" or "anti-quorum sensing activity", as these equivalent terms are used herein interchangeably, refer to the capacity of a substance to effect the prevention of formation of a biofilm of bacterial, fungal and/or other cells; and/or to effect a disruption and/or the eradication of an established and/or matured biofilm of bacterial, fungal and/or other cells; and/or to effect a reduction in the rate of buildup of a biofilm of bacterial, fungal and/or other cells on a surface of a substrate.

The phrases "anti-biofilm formation compound/composition/agent", "ABF compound/composition/agent", "anti-quorum sensing compound/composition/agent", "AQS compound/composition/agent", "quorum sensing inhibitor", "QSI" and "anti-biofouling compound/composition/agent", as these equivalent terms are used herein interchangeably, refer to a substance having an anti-biofilm formation activity, as defined herein.

As demonstrated hereinbelow, a compound having general Formula A or A' as described herein was shown to exert ABF activity and can thus prevent or reduce the
formation or the mass of a biofilm, and/or disrupt an existing (established) biofilm. Therefore, the ester compounds described herein can be used in methods and compositions which are directed at anti-biofilm formation purposes, as detailed hereinbelow. The acid compound as described herein can also be used in methods and compositions which are directed at anti-biofilm formation purposes, as detailed hereinbelow.

According to embodiments of the present invention, the activity of preventing or reducing the formation of a biofilm, and the activity of disrupting a biofilm which has been established before treatment, may be achieved by identical or different ABF agents. The prevention or reducing of forming a biofilm assumes that the biofilm has not yet been formed, and hence the presence of the ABF agent is required also in cases where no biofilm is present or detected. In other applications and embodiments, the biofilm has already been formed and the disruption thereof is desirable; thus in these cases the ABF agent according to embodiments of the present invention, may be introduced before, during or after the detection of presence of the biofilm.

As used herein, the term "preventing" in the context of the formation of a biofilm, indicates that the formation of a biofilm is essentially nullified or is reduced by at least 20 % of the appearance of the biofilm in a comparable situation lacking the presence of the ABF agent. Alternatively, preventing means a reduction to at least 15 %, 10 % or 5 % of the appearance of the biofilm in a comparable situation lacking the presence of the ABF agent. Methods for determining a level of appearance of a biofilm are known in the art.

As used herein, the term "disrupting" in the context of the formation of a biofilm, indicates that the mass of a biofilm is reduced to at least 20 % of its mass prior to the introduction of the ABF agent presented herein. Alternatively, disrupting means a reduction in the mass of the biofilm to at least 30 %, 40 % or 50 % of its original mass prior to the introduction of the ABF agent.

In some embodiments, disrupting a biofilm, or reducing a biofilm mass, results in converting at least a portion of the biofilm (e.g., at least 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 % and even 100 %) into planktonic cells that formed the biofilm.
According to an aspect of some embodiments of the present invention there is provided an anti-biofilm composition which comprises a compound having general Formula A as described herein, and can optionally further comprise a carrier.

According to another aspect of some embodiments of the present invention there is provided an anti-biofilm composition which comprises a compound having general Formula A’ as described herein (e.g., Compound F), and an additional active agent (e.g., an antimicrobial or antibacterial agent), and can optionally further comprise a carrier.

According to some embodiments of the present invention, a composition as presented herein is packaged in a packaging material and identified in print, in or on the packaging material, for use in reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate, as described herein.

Biofilms may form on living tissues or form on non-living, organic or inorganic substrates, referred to herein an inanimate objects or substrates, and constitute a prevalent mode of microbial life in natural, industrial and medical settings. Hence, in the context of these embodiments the substrate can be a living tissue or an inanimate object, as described herein.

According to some embodiments of the present invention, when the substrate is a living tissue, a composition comprising a compound having general Formula A or A’ as described herein is referred to as a pharmaceutical composition, as described herein.

According to some embodiments of the present invention, the pharmaceutical composition presented herein is packaged in a packaging material and identified in print, in or on the packaging material, for use in reducing or preventing the formation of a biofilm and/or disrupting a biofilm in the living tissue or in a subject in need thereof, which comprises said living tissue, as described herein.

According to some embodiments, the pharmaceutical composition is identified for use in the treatment of a medical condition in which reducing or preventing the formation of the biofilm and/or disrupting the biofilm in the living tissue is beneficial.

Further according to an aspect of some embodiments of the present invention there is provided a use of a compound having general Formula A as described herein in the manufacture of a product for reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate.
Further according to an aspect of some embodiments of the present invention there is provided a use of a compound having general Formula A' as described herein (e.g., Compound F) in the manufacture of a product for reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate, wherein the medicament further comprises an additional active agent or is for use in combination with an additional active agent (e.g., an antimicrobial or antibacterial agent).

When the substrate is a living tissue, the product is a medicament for reducing or preventing the formation of a biofilm and/or disrupting a biofilm in the living tissue or in a subject in need thereof, which comprises said living tissue, as described hereinabove.

According to some embodiments, the medicament is for the treatment of a medical condition in which reducing or preventing the formation of the biofilm and/or disrupting the biofilm in the living tissue is beneficial.

Further according to an aspect of some embodiments of the present invention there is provided a compound having general Formula A as described herein for use in a method of reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate.

Further according to an aspect of some embodiments of the present invention there is provided a compound having general Formula A' as described herein (e.g., Compound F) for use, in combination with an additional active agent (e.g., an antimicrobial or antibacterial agent) in a method of reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate.

When the substrate is a living tissue, a compound having general Formula A or A' as described herein is for use in a method of reducing or preventing the formation of a biofilm and/or disrupting a biofilm the living tissue or in a subject in need thereof, which comprises said living tissue, as described hereinabove.

In some embodiments, a compound having general Formula A or A' as described herein is identified for use in the treatment of a medical condition in which reducing or preventing the formation of the biofilm and/or disrupting the biofilm in the living tissue is beneficial.

Further according to an aspect of some embodiments of the present invention there is provided a method of reducing or preventing the formation of a biofilm and/or
disrupting a biofilm in or on a substrate, which is effected by contacting the substrate
with an antimicrobial effective amount of a compound having general Formula A as
described herein.

Further according to an aspect of some embodiments of the present invention
there is provided a method of reducing or preventing the formation of a biofilm and/or
disrupting a biofilm in or on a substrate, which is effected by co-contacting the substrate
with an antimicrobial effective amount of a compound having general Formula A' as
described herein (e.g., Compound F) and with an additional active agent (e.g., an
antimicrobial or antibacterial agent).

As used in the context of embodiments of this aspect of the present invention,
the phrase "antimicrobial effective amount" describes an amount of an anti-biofilm
ingredient which will reduce, prevent and/or disrupt, at least to some extent, the
formation of a biofilm of a microorganism, as described herein, in or on a substrate
harboring the microorganism.

According to some embodiments of the present invention, when the substrate is
a living tissue, such as a bodily site of a subject, the method is effected by administering
to the subject an antimicrobial effective amount of a compound having general Formula
A or A' as described herein.

According to some embodiments, the administration can be effected orally,
rectally, intravenously, topically, intranasally, intradermally, transdermally,
subcutaneously, intramuscularly, intraperitoneally or by intrathecal catheter.

A compound having general Formula A or A' as described herein can
administered either per se or as a part of a pharmaceutical composition as described
herein.

According to some embodiments of the present invention, the method is being
for treating a medical condition in which reducing or preventing the formation of the
biofilm and/or disrupting the biofilm in the living tissue is beneficial.

When the substrate is a subject, the mode of administration in the context of
reducing, preventing and/or disrupting a biofilm formation is similar to the mode of
administration in the context of inhibiting the growth of a microorganism.

According to some embodiments, and as demonstrated in the Examples section
that follows, an exemplary method of reducing, preventing and/or disrupting a biofilm
in a subject is effected topically by applying a composition containing the active ingredient(s) on a wound. This method is also effective in reducing, preventing and/or disrupting a biofilm formation on the wound dressing used to treat the wound.

A well known case of a pathologic case of biofilm formation in a living tissue, are streptococcal infections, which is the basis for the intense virulence of many streptococcal species.

When the substrate is an inanimate object, as described hereinabove, the mode of reducing, preventing and/or disrupting a biofilm formation in or on the substrate is effected typically by dipping, spraying, coating, or otherwise applying a formulation containing the active ingredient(s) in or on the substrate. For example, a catheter for prolonged percutaneous use can be coated with a formulation containing a compound having general Formula A or A’ as described herein so as to reduce, prevent and/or disrupt, at least to some extent, the formation of a biofilm therein or thereon.

In some embodiments, the anti-biofilm composition as described herein is used to reduce, prevent and/or disrupt, at least to some extent, the formation of a biofilm in a medical device by coating or impregnating the device with an amount of the composition capable of reducing, preventing and/or disrupting formation of the biofilm.

As used herein, the phrase "medical device" includes any material or device that is used on, in, or through a subject's body, for example, in the course of medical treatment (e.g., for a disease or injury). Medical devices include, but are not limited to, such items as medical implants, wound care devices, drug delivery devices, and body cavity and personal protection devices. The medical implants include, but are not limited to, urinary catheters, intravascular catheters, dialysis shunts, wound drain tubes, skin sutures, vascular grafts, implantable meshes, intraocular devices, heart valves, and the like. Wound care devices include, but are not limited to, general wound dressings, biologic graft materials, tape closures and dressings, and surgical incise drapes. Drug delivery devices include, but are not limited to, needles, drug delivery skin patches, drug delivery mucosal patches and medical sponges. Body cavity and personal protection devices, include, but are not limited to, tampons, sponges, surgical and examination gloves, and toothbrushes. Birth control devices include, but are not limited to, intrauterine devices (IUDs), diaphragms and condoms.
Coating or impregnating the medical device with the anti-biofilm composition as described herein may be effected via various techniques known in the art (see, for example, in US Pat. Nos. 4,107,121; 4,442,133; 4,895,566; 4,917,686; 5,013,306; 5,624,704; 5,688,516; 5,756,145; 5,853,745; 5,902,283; 6,719,991).

**Combined antimicrobial effect:**

As demonstrated in the Examples section hereinbelow, the antimicrobial and ABF activity of 4-fluorophenylboronic acid (Compound F) and of an exemplary ester compound having general Formula A as described herein (Compound K) has been shown to be enhanced when acting in the presence of another active agent or bioactive agent, such as another antimicrobial agent (e.g., an antibiotic). Similarly, the antimicrobial and ABF activity of various antimicrobial agents (e.g., antibiotics) was shown to be enhanced when acting in the presence of 4-fluorophenylboronic acid and of an exemplary ester compound having general Formula A as described herein.

4-Fluorophenylboronic acid and the compounds having general Formula A as described herein are represented herein collectively as compounds having general Formula A' as described herein.

According to embodiments of the present invention, any of the compounds, compositions or medicaments presented herein may be used in combination with an additional active agent, or alternatively, the compositions or medicaments as presented herein may include an additional active agent.

According to some embodiments of the present invention, there are provided antimicrobial compositions, pharmaceutical compositions and ABF compositions, as described herein for compounds having general Formula A, which comprise a compound having general Formula A' as described herein and an additional active agent, as described herein.

Further according to some embodiments of the present invention there are provided methods and uses, as described herein for compounds having general Formula A, which utilize a compound having general Formula A' as described herein in combination with an additional active agent, as described herein.

In embodiments wherein the additional active agent (e.g., an additional antimicrobial agent) is not an ingredient of the composition or medicament containing a compound having general Formula A' as described herein, it can be held as a separate
composition in a separate container and packaged together with the container holding the composition containing a compound having general Formula A' as described herein, or it can be packaged separately.

Further according to some embodiments of the present invention, the methods as described herein, are effected by co-contacting the substrate with a compound having general Formula A' as described herein and an additional active agent as described herein.

When using an additional active agent in any of the methods described herein, the additional agent can be administered to or otherwise contacted with the substrate, concomitantly, concurrently, simultaneously, consecutively or sequentially with a compound having general Formula A' as described herein.

According to an aspect of the present invention, there is provided a composition which comprises both a compound having general Formula A' as described herein and an additional active agent, and optionally further comprises a carrier. The composition can be an antimicrobial composition, an anti-biofilm composition or a pharmaceutical composition, as described herein.

Further according to an aspect of some embodiments of the present invention there is provided a use of a compound having general Formula A' as described herein in the manufacture of a product (e.g., a medicament), as described herein, wherein the product (e.g., medicament) comprises an additional active agent or is identified for use in combination with an additional active agent.

Further according to an aspect of some embodiments of the present invention there is provided a compound having general Formula A' as described herein for use in any of the methods described herein, in combination with an additional active agent.

Further according to an aspect of some embodiments of the present invention there is provided a method as described herein, which is effected by co-contacting the substrate with a compound having general Formula A' as described herein and with an additional active agent.

In some embodiments, a method as described herein is effected by co-administering to a subject in need thereof a compound having general Formula A' as described herein and an additional active agent.
Co-contacting and co-administering can be effected concomitantly, concurrently, sequentially or consecutively.

According to an aspect of the present invention, there is provided a method of inhibiting a growth of a pathogenic microorganism in a subject which is effected by co-administering to a subject in need thereof an synergistically effecting amount of a compound having general Formula A’ as described herein and an antimicrobial agent.

Accordingly, there is provided a method of reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate, which is effected co-contacting the substrate with an antimicrobial effective amount of a compound having general Formula A’ as described herein and an antimicrobial agent.

According to some embodiments of the invention, the additional active agent is an antimicrobial agent, and according to some embodiments, the additional antimicrobial agent acts in synergy with a compound having general Formula A’ as described herein in inhibiting a growth of a microorganism and/or within the ABF activity exhibited thereby.

The terms "synergy", "synergism", and any grammatical diversion thereof, as used herein, describe a cooperative action encountered in combinations of two or more active compounds in which the combined effect exhibited by the two compounds when used together exceeds the sum of the effect of each of the compounds when used alone.

"Synergy" is therefore often determined when a value representing an effect of a combination of two active agents is greater than the sum of the same values obtained for each of these agents when acting alone.

A synergy between two antimicrobial agents may be determined by methods well known in the art. An exemplary method is demonstrated in Example 2 in the Examples section that follows.

A "synergistically effective amount" describes an amount of a compound having general Formula A’ as described herein that when combined with an additional agent results in synergy, as defined herein.

Any of the antimicrobial agents described herein are contemplated.

In some embodiments, the antimicrobial agent is an antibacterial agent (an antibiotic).
Non-limiting examples of antibacterial agents which are suitable for use in the context of these embodiments of the present invention include, without limitations, amikacin, amoxicillin, ampicillin, azithromycin, Aztreonam, cefepime, cefonicid, cefotetan, ceftazidine, cephalosporin, cefamycin, Chloramphenicol, chlorotetracycline, ciprofloxacin, clarithromycin, clindamycin, colistin, cycloserine, dalfopristin, doxycycline, ephalothin, erythromycin, gentamicin, kanamycin, levofloxacin, lincosamide, linezolid, meropenem, moxifloxacin, neomycin, oxytetracycline, piperacillin, penicillin, quinupristin, rifampicin, spectinomycin, streptomycin, sulfanilamide, sulfamethoxazole, tazobactam, Tobramycin, trimethoprim and vancomycin, as well as any of combinations and any derivatives thereof.

According to some embodiments of the present invention, the additional antimicrobial agent used together with a compound having general Formula A' as described herein is other than a beta-lactam antibiotic agent, or is a non-beta-lactam antimicrobial agent.

Beta-lactam antimicrobial/antibacterial agents are those agents having a "azetidin-2-one" motif, and include, without limitation, amoxicillin, ampicillin, azlocillin, aztreonam (Azactam), benzathine penicillin, benzylpenicillin (penicillin G), carbenicillin, cefaclor, cefamandole, cefazolin, cefepime, cefixime, cefotaxime, cefotetan, cefoxitin, cefpirome, cepodoxime (ATDOX-200), ceftazidime, ceftriaxone, cefuroxime, cephalexin, cephalothin, clavulanic acid, cloxacillin, dicloxacillin, doripenem, ertapenem, faropenem, flucloxacillin, imipenem, meropenem, methicillin, mezlocillin, nafcillin, nocardicin A, oxacillin, penicillin, phenoxymethylpenicillin (penicillin V), piperacillin, procaine penicillin, sulbactam, tabtoxinine -P-lactam, tazobactam, temocillin, ticarcillin and tigemonam.

The phrase "non beta-lactam antimicrobial agent", as used herein, refers to a bacteriostatic or a bactericidal (antibiotic) agent which is not of the beta-lactam class of antibiotics or one not having a "azetidin-2-one" motif. Exemplary non beta-lactam antibacterial agent include, without limitation, mafenide, cephalothin, vancomycin, cefazolin, imipenemen, clindamycin, synercid, erythromycin, tetracycline, ciprofloxacin, tigecycline and ertapenem, as well as aminoglycoside antibiotics such as, but not limited to, amikacin, apramycin, arbekacin, butirosin, dibekacin, fortimycin, G-418, gentamicin, hygromycin, habekacin, dibekacin, netlimicin, istamycin, isepamycin,
kanamycin, lividomycin, neamine, neomycin, paromomycin, ribostamycin, sisomycin, spectinomycin, streptomycin and tobramycin.

According to some embodiments of the present invention, in any of the compositions, methods and uses as described herein, which utilize a compound having Formula A' as described herein and an additional active agent, the compound having Formula A' is 4-fluorophenylboronic acid (Compound F, BL 6031) and the additional active agent is gentamicin.

According to some embodiments of the present invention, in any of the compositions, methods and uses as described herein, which utilize a compound having Formula A' as described herein and an additional active agent, the compound having Formula A' is 4-fluorophenylboronic acid (Compound F, BL 6031) and the additional active agent is Tobramycin.

According to some embodiments of the present invention, in any of the compositions, methods and uses as described herein, which utilize a compound having Formula A' as described herein and an additional active agent, the compound having Formula A' is Compound K (BL 6030) and the additional active agent is gentamicin.

According to some embodiments of the present invention, in any of the compositions, methods and uses as described herein, which utilize a compound having Formula A' as described herein and an additional active agent, the compound having Formula A' is Compound K (BL 6030) and the additional active agent is Tobramycin.

**Articles:**

Compounds having Formula A or A' as described herein, either alone or in combination with an additional active agent (e.g., an antimicrobial agent) can be applied on or in various substrates, for exhibiting antimicrobial or anti-biofouling effect, as described herein.

The term "substrate" as used herein, is as described hereinabove, and further refers to any surface, structure, product or material which can support, harbor or promote the growth of a microorganism. Non-limiting examples include the inner walls of a storage container that is routinely treated with anti-microbial preferably anti-fungal agents, a soil and/or soil enrichment supplements, any agricultural product or crop such as wood, fiber, fruit, vegetable, flower, extract, horticultural crop and any other processed or unprocessed agricultural product or crop which are produced from organic
 origins such living plants or animals, a cosmetic product, a building, warehouse, compartment, container or transport vehicle, a dye or a paint and any other materials and industrial compounds used for which require protection of their surfaces against microbes, moulds and fungi attacks, such as, for example, construction materials.

Such products include, for example, food products, agricultural products, cosmetic products and many more. Due to their effect in reducing the load of microorganisms, the compounds described herein can be utilized as a preservative in such products.

In some embodiments, the substrate is a medical device or any other device which is intended for contacting a living tissue, as defined herein.

In some embodiments, there is provided a wound dressing having a compound represented by Formula A or A’ as described herein, either alone or in combination with an additional active agent (e.g., an antimicrobial agent).

Thus, according to a further aspect of some embodiments of the present invention there is provided a method of reducing the load of a pathogenic microorganism in or on a substrate, the method being effected by contacting the substrate an antimicrobial effective amount of a compound having Formula A or A’ as described herein, optionally in combination with an additional antimicrobial agent.

The term "reducing the load" refers to a decrease in the number of the microorganism(s), or to a decrease in the rate of their growth or both in the substrate as compared to a non-treated substrate.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.
Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non-limiting fashion.

EXAMPLE 1

**Antibacterial Activity of 4-fluorophenylboronic acid in Combination with Non Beta-Lactam Antibiotics**

The anti-bacterial and/or anti biofilm activity of 4-phenylboronic acid (designated herein as Compound F) in combination with commonly used clinical antibiotics was tested, as follows.

**Materials and Methods**

**Test Compound:**

4-phenylboronic acid (designated 'Compound P):

![Compound F]

**Bacteria:**

*Staphylococcus aureus* (MRSA; strain USA 400), *Staphylococcus epidermidis* (strain ATCC 35984) and *Pseudomonas aeruginosa* (strain ATCC 27853).
Clinical Antibiotics:
Cephalothin, Cefazolin, Ciprofloxacin, Chloramphenicol, Clindamycin, Erythromycin, Etrapenem, Gentamicin, Imipenem, Nitrofurantoin, Piperacillin, Tazobactam, Synercid, Tetracycline, Tigecycline and Vancomycin.

Determination of Minimum inhibitory concentration (MIC) and minimum biofilm eliminating concentration (MBEC):
MIC and MBEC values for each antibacterial agent (antibiotic), the tested compound and combination thereof are determined according to the procedure described by Ceri et al. (Methods Enzymol 337:377-85, 2001) using SENSITITRE® Gram Positive and Gram Negative MIC Plates (TREC Diagnostics Systems, Cleveland, OH) and Biofilm™ test panels (Inovotech Inc., Edmonton, AB Canada).

Determination of synergy between Compound F and antibacterial agents:
The synergy between Compound F and clinical antibiotics is expressed in terms of the fractional inhibitory concentrations (FIC) (Elion et al., Journal of Biological Chemistry 208: 477-88, 1954). The FIC is calculated by the following formula:

\[
FIC = \frac{\text{Lowest MIC Agent} \times_{\text{combination}}}{\text{Lowest MIC Agent} \times_{\text{single agent}}} + \frac{\text{Lowest MIC Agent} \times_{\text{combination}}}{\text{Lowest MIC Agent} \times_{\text{single agent}}}
\]

The combined treatment with Compound F and the tested antibiotic is considered synergic when the resulting FIC value is equal to or lower than 0.5.

Results
Tables 1 and 2 below show the effect of Compound F in combination with clinical non beta-lactam antibiotics on the growth of methicillin-resistant *Staphylococcus aureus*. The combinations of Compound F with Cephalothin, Vancomycin, Cefazolin, Imipenemen, Clindamycin, Synercid, Erythromycin, or Tetracycline resulted in synergic inhibition of bacterial growth.
Minimum Inhibitory Concentration (MIC) of antibacterial agents (compound F and clinical antibiotics) provided to *Staphylococcus aureus* (methicillin resistance; MRSA USA 400)

<table>
<thead>
<tr>
<th>Antibacterial Agent</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound F</td>
<td>2800</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>8</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>8</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.25</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.5</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>32.5</td>
</tr>
<tr>
<td>Synercid</td>
<td>1</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>2</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>4</td>
</tr>
</tbody>
</table>

Antibacterial-agent combinations (compound F combined with clinical antibiotics) at minimal concentrations for inhibiting methicillin-resistant *Staphylococcus aureus* USA 400 (sorted by FIC)

<table>
<thead>
<tr>
<th>Compound F Concentration fag/ml</th>
<th>Antibiotic</th>
<th>Antibiotic Concentration fag/ml</th>
<th>FIC*</th>
<th>FIC Code**</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0028</td>
<td>Cephalothin</td>
<td>1</td>
<td>0.13</td>
<td>SY</td>
</tr>
<tr>
<td>0.028</td>
<td>Cephalothin</td>
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<td>SY</td>
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<td>Vancomycin</td>
<td>1</td>
<td>0.25</td>
<td>SY</td>
</tr>
<tr>
<td>0.000028</td>
<td>Vancomycin</td>
<td>1</td>
<td>0.25</td>
<td>SY</td>
</tr>
<tr>
<td>0.00028</td>
<td>Cephalothin</td>
<td>2</td>
<td>0.25</td>
<td>SY</td>
</tr>
<tr>
<td>0.00028</td>
<td>Vancomycin</td>
<td>1</td>
<td>0.25</td>
<td>SY</td>
</tr>
<tr>
<td>Compound F Concentration (W/mL)</td>
<td>Antibiotic</td>
<td>Antibiotic Concentration (W/mL)</td>
<td>FIC*</td>
<td>FIC Code**</td>
</tr>
<tr>
<td>-------------------------------</td>
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<td>---------------------------------</td>
<td>------</td>
<td>------------</td>
</tr>
<tr>
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<td>Cefazolin</td>
<td>2</td>
<td>0.25</td>
<td>SY</td>
</tr>
<tr>
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<td>Imipenem</td>
<td>0.125</td>
<td>0.25</td>
<td>SY</td>
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<tr>
<td>0.0028</td>
<td>Vancomycin</td>
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<td>0.25</td>
<td>SY</td>
</tr>
<tr>
<td>0.028</td>
<td>Cefazolin</td>
<td>2</td>
<td>0.26</td>
<td>SY</td>
</tr>
<tr>
<td>0.028</td>
<td>Imipenem</td>
<td>0.125</td>
<td>0.26</td>
<td>SY</td>
</tr>
<tr>
<td>0.028</td>
<td>Vancomycin</td>
<td>1</td>
<td>0.26</td>
<td>SY</td>
</tr>
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<td>Cefazolin</td>
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<td>0.35</td>
<td>SY</td>
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<tr>
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<td>Clindamycin</td>
<td>0.0625</td>
<td>0.35</td>
<td>SY</td>
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<tr>
<td>0.28</td>
<td>Imipenem</td>
<td>0.125</td>
<td>0.35</td>
<td>SY</td>
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<td>0.28</td>
<td>Synercid</td>
<td>0.25</td>
<td>0.35</td>
<td>SY</td>
</tr>
<tr>
<td>0.28</td>
<td>Vancomycin</td>
<td>1</td>
<td>0.35</td>
<td>SY</td>
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<td>Erythromycin</td>
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<td>0.5</td>
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</tr>
<tr>
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<td>Synercid</td>
<td>0.5</td>
<td>0.5</td>
<td>SY</td>
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<td>Tetracycline</td>
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<td>0.5</td>
<td>SY</td>
</tr>
<tr>
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<td>Vancomycin</td>
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<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Cephalothin</td>
<td>4</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Clindamycin</td>
<td>0.125</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Erythromycin</td>
<td>0.5</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Synercid</td>
<td>0.5</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
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<td>Tetracycline</td>
<td>1</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Clindamycin</td>
<td>0.125</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Imipenem</td>
<td>0.25</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Synercid</td>
<td>0.5</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Tetracycline</td>
<td>1</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Cefazolin</td>
<td>4</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Clindamycin</td>
<td>0.125</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Erythromycin</td>
<td>0.5</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Gentamicin</td>
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<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Synercid</td>
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<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Tetracycline</td>
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<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Clindamycin</td>
<td>0.125</td>
<td>0.51</td>
<td>AD</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Erythromycin</td>
<td>0.5</td>
<td>0.51</td>
<td>AD</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Synercid</td>
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<td>0.51</td>
<td>AD</td>
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<td>0.0000028</td>
<td>Tetracycline</td>
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<td>0.51</td>
<td>AD</td>
</tr>
<tr>
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<td>Erythromycin</td>
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<td>AD</td>
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<td>Gentamicin</td>
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<td>0.6</td>
<td>AD</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Tetracycline</td>
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<td>0.6</td>
<td>AD</td>
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<tr>
<td>0.0000028</td>
<td>Nitrofurantoin</td>
<td>32</td>
<td>0.98</td>
<td>AD</td>
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</tbody>
</table>

* FIC = [(lowest MIC of compound F in combination) / (lowest MIC of compound F as single agent)] + [(lowest MIC of antibiotic in combination) / [(lowest MIC of antibiotic as single agent)].
** SY = synergy; the interaction between Compound F and the clinical antibiotic is considered synergic when the resulting FIC value is equal to or lower than 0.5.

** AD = additive, the interaction between Compound F and the clinical antibiotic is considered additive when the resulting FIC value is equal to or lower than 1.0 but higher than 0.5.

Tables 3 and 4 below show the effect of Compound F in combination with clinical non beta-lactam antibiotics on the growth of *Staphylococcus epidermitis*. The combinations of Compound F with Imipenem, Cephalothin, Tetracycline, Cefazoline, or Ciprofloxacin resulted in synergic inhibition of bacterial growth.

**Table 3**

Minimum Inhibitory Concentration (MIC) of antibacterial agents (compound F and clinical antibiotics) provided to *Staphylococcus epidermitis* ATCC 35984

<table>
<thead>
<tr>
<th>Antibacterial Agent</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound F</td>
<td>2800</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>4</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>4</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>8</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.25</td>
</tr>
<tr>
<td>Imipenem</td>
<td>4</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>16</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>4</td>
</tr>
<tr>
<td>Synercid</td>
<td>0.5</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Table 4**

Antibacterial-agent combinations (compound F combined with clinical antibiotics) at minimal concentrations for inhibiting *Staphylococcus epidermitis* ATCC 35984 (sorted by FIC)

<table>
<thead>
<tr>
<th>Compound F Concentration (µg/ml)</th>
<th>Antibiotic</th>
<th>Antibiotic Concentration (µg/ml)</th>
<th>FIC*</th>
<th>FIC Code**</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.028</td>
<td>Imipenem</td>
<td>0.07</td>
<td>SY</td>
<td></td>
</tr>
<tr>
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<td>Imipenem</td>
<td>0.13</td>
<td>SY</td>
<td></td>
</tr>
<tr>
<td>0.28</td>
<td>Imipenem</td>
<td>&lt;0.125</td>
<td>0.13</td>
<td>SY</td>
</tr>
<tr>
<td>MIC</td>
<td>Compound</td>
<td>FIC</td>
<td>MIC</td>
<td>Compound</td>
</tr>
<tr>
<td>----------</td>
<td>-------------------</td>
<td>-------</td>
<td>----------</td>
<td>-------------------</td>
</tr>
<tr>
<td>0.0028</td>
<td>Impenem</td>
<td>1</td>
<td>0.25</td>
<td>SY</td>
</tr>
<tr>
<td>0.028</td>
<td>Cephalophin</td>
<td>2</td>
<td>0.26</td>
<td>SY</td>
</tr>
<tr>
<td>0.28</td>
<td>Tetracycline</td>
<td>0.125</td>
<td>0.35</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Cefazolin</td>
<td>2</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Cephalothin</td>
<td>2</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Ciprofloxacin</td>
<td>0.125</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Tetracycline</td>
<td>0.25</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Cefazolin</td>
<td>2</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Cephalophin</td>
<td>2</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0000028</td>
<td>Tetracycline</td>
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<td>0.5</td>
<td>SY</td>
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<tr>
<td>0.0028</td>
<td>Cefazolin</td>
<td>2</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0028</td>
<td>Cephalophin</td>
<td>2</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0028</td>
<td>Impenem</td>
<td>2</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0028</td>
<td>Tetracycline</td>
<td>0.25</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.028</td>
<td>Cefazolin</td>
<td>2</td>
<td>0.51</td>
<td>AD</td>
</tr>
<tr>
<td>0.028</td>
<td>Piperacillin/Tazobactam</td>
<td>2</td>
<td>0.51</td>
<td>AD</td>
</tr>
<tr>
<td>0.028</td>
<td>Tetracycline</td>
<td>0.25</td>
<td>0.51</td>
<td>AD</td>
</tr>
<tr>
<td>0.28</td>
<td>Cefazolin</td>
<td>2</td>
<td>0.6</td>
<td>AD</td>
</tr>
<tr>
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<td>Cephalophin</td>
<td>2</td>
<td>0.6</td>
<td>AD</td>
</tr>
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<td>0.28</td>
<td>Chloramphenical</td>
<td>4</td>
<td>0.6</td>
<td>AD</td>
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<tr>
<td>0.28</td>
<td>Nitrofurantoin</td>
<td>8</td>
<td>0.6</td>
<td>AD</td>
</tr>
<tr>
<td>0.28</td>
<td>Piperacillin/Tazobactam</td>
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<td>0.6</td>
<td>AD</td>
</tr>
<tr>
<td>0.28</td>
<td>Synercid</td>
<td>0.25</td>
<td>0.6</td>
<td>AD</td>
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</tbody>
</table>

* FIC = [(lowest MIC of compound F in combination) / (lowest MIC of compound F as single agent)] + [(lowest MIC of antibiotic in combination) / [(lowest MIC of antibiotic as single agent)].

** SY = synergy; the interaction between Compound F and the clinical antibiotic is considered synergic when the resulting FIC value is equal to or lower than 0.5.

** AD = additive, the interaction between Compound F and the clinical antibiotic is considered additive when the resulting FIC value is equal to or lower than 1.0 but higher than 0.5.

Tables 5 and 6 below show the effect of Compound F in combination with clinical non beta-lactam antibiotics on the growth of *Psuedomonas aeruginosa*. The combinations of Compound F with Tigecycline, Tetracycline, or Ertapenem resulted in synergic inhibition of bacterial growth.
Table 5
Minimum Inhibitory Concentration (MIC) of antibacterial agents (compound F and clinical antibiotics) provided to *Psuedomonas aeruginosa* ATCC 27853

<table>
<thead>
<tr>
<th>Antibacterial Agent</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound F</td>
<td>2800</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>8</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>16</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 6
Antibacterial-agent combinations (compound F combined with clinical antibiotics) at minimal concentrations for inhibiting *Psuedomonas aeruginosa* ATCC 27853 (sorted by FIC)

<table>
<thead>
<tr>
<th>Compound F Concentration (µg/ml)</th>
<th>Antibiotic</th>
<th>Antibiotic Concentration (µg/ml)</th>
<th>FIC*</th>
<th>FIC Code**</th>
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<tr>
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<td>Tigecycline</td>
<td>4</td>
<td>0.5</td>
<td>SY</td>
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<td>Tigecycline</td>
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<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.0028</td>
<td>Tetracycline</td>
<td>8</td>
<td>0.5</td>
<td>SY</td>
</tr>
<tr>
<td>0.28</td>
<td>Ertapenem</td>
<td>4</td>
<td>0.6</td>
<td>AD</td>
</tr>
</tbody>
</table>

*FIC = [(lowest MIC of compound F in combination) / (lowest MIC of compound F as single agent)] + [(lowest MIC of antibiotic in combination) / (lowest MIC of antibiotic as single agent)].

** SY = synergy; the interaction between Compound F and the clinical antibiotic is considered synergic when the resulting FIC value is equal to or lower than 0.5.

** AD = additive, the interaction between Compound F and the clinical antibiotic is considered additive when the resulting FIC value is equal to or lower than 1.0 but higher than 0.5.

The results obtained indicate that Compound F is uniquely and unexpectedly capable of potentiating the activity of non beta-lactam antibiotics commonly used in therapy.
EXAMPLE 2

Chemical Syntheses of exemplary esters of phenylboronic acid and derivatives thereof

General Procedure:

Phenyloxaboronidines are synthesized by reacting a respective phenylboronic acid with an equimolar amount of N-methyldiethanolamine in an organic solvent (e.g., toluene), optionally in the presence of a dehydrating agent (e.g., molecular sieves).

Synthesis of Compound K (2-(4-fluorophenyl)-6-methyl-1,3,6,2-dioxazaborocane):

Compound K is also referred to as BL-6030 or EDP-5.

An exemplary synthesis of Compound K is depicted in Scheme 1 below.

Scheme 1

All glassware equipment (500 ml round bottom flask, nitrogen tap, a magnetic egg stirrer bar) and molecular sieves 4 Å were dried in oven prior to use.

4-Fluorophenylboric acid (10 grams, 71.4 mmol), dry toluene (300 ml) and 50 grams of molecular sieves 4 Å were added to a 500 ml round bottom flask under dry nitrogen. The solution was stirred and N-methyldiethanolamine (8.5 ml, 71.4 mmol) was added. The solution turned from opaque to clear and the resulting mixture was vigorously stirred under dry nitrogen at room temperature for five hours.

After five hours the molecular sieves were filtered off in vacuum with a sintered funnel and washed with toluene (2 x 20 ml). The clear filtrate was evaporated under vacuum to obtain oil.
Azeotropic distillation of the obtained oil was performed with two portions of 25 ml of toluene in order to remove water from the reaction until viscous oil was obtained. The product was dried under high vacuum for 24 hours, followed by drying under diaphragm vacuum pump for the weekend to afford colorless viscous oil.

The obtained viscous oil was solidified to a white solid upon standing on the bench at 20 °C for several days. This solid was filtered under vacuum and washed with petroleum ether several times. The solid was dried on a sintered funnel, then transferred to a vial and dried for 24 hours in a desiccator connected to a diaphragm pump, so as to afford Compound K as a white solid powder (14.9 grams, 94 % yield).

**Synthesis of Compound B (6-methyl-2-(4-vinylphenyl)-1,3,6,2-dioxazaborocane):**

Compound B was prepared as described hereinabove for Compound K, using 4-vinylphenylboric acid as the starting material, as depicted in Scheme 2 below.

![Scheme 2](image)

**Synthesis of CompoundA (6-methyl-2-phenyl-1,3,6,2-dioxazaborocane):**

Compound A was prepared as described hereinabove for Compound K, using phenylboronic acid as the starting material, as depicted in Scheme 3 below.
EXAMPLE 3

In vitro Studies

Studies were conducted in order to determine the effects of quorum sensing inhibitors (QSI) on minimum inhibitory concentration (MIC) values of Gram-negative and Gram-positive organisms using antibiotic test panels, on minimum bactericidal concentration (MBC) values of Gram-negative and Gram-positive organisms using antibiotic test panels, and on minimum biofilm eradication concentration (MBEC) values of Gram-negative and Grand-positive organisms using antibiotic test panels.

The bioFILM PA™, developed by Innovotech™ Inc., Canada, is a broth dilution antimicrobial susceptibility panel test was designed for use in determining antimicrobial agent susceptibility of both planktonic and biofilm Pseudomonas aeruginosa. This test has a panel of various antimicrobial agents which are diluted in recovery buffer at categorical breakpoint concentrations defined by Clinical and Laboratory Standard Institute™ (CLSI).

Panel wells were inoculated with planktonic and biofilm Pseudomonas aeruginosa using a 95 peg inoculation lid. Panels and pegged lids were then incubated at 35 °C for a minimum of 16 hours. Planktonic susceptibility and resistance was determined by measuring inhibition and growth in the presence of antimicrobial agents after 16-24 hours incubation at 35 °C. The pegged lid containing the biofilm bacteria that have been exposed to the antimicrobial agents was placed in a recovery media.

Biofilm susceptibility and resistance was determined by measuring inhibition and growth after incubation for additional 16-24 hours at 35 °C.
The Gram-positive test panel was designed for use in determining antimicrobial agent susceptibility of both planktonic and biofilms of Gram-positive organisms. This broth dilution antimicrobial susceptibility test had various antimicrobial agents which are diluted in recovery buffer at categorical breakpoint concentrations defined by Clinical and Laboratory Standard Institute™ (CLSI).

Panel wells were inoculated with planktonic and biofilm of clinically significant Gram-positive organisms of choice using a 96 peg inoculation lid. Panels and pegged lids were then incubated at 35 °C for a minimum of 16 hours. Planktonic susceptibility and resistance was determined by measuring inhibition and growth in the presence of antimicrobial agents after 16-24 hours incubation at 35 °C. The pegged lid containing the biofilm bacteria that have been exposed to the antimicrobial agents was placed in a recovery media. Biofilm susceptibility and resistance was determined by measuring inhibition and growth after incubation for additional 16-24 hours at 35 °C.

**Sample Preparation:**

The following test compounds were used:

Compound A, Compound B and Compound K, described in Example 2 hereinabove.

Stock solutions were prepared by either weighing each test compound or by weighing the vial containing the test compound; dissolving the entire amount of the compound in a known volume of 100 % DMSO and weighting the empty vial to get the amount dissolved in the DMSO. The working concentrations of the compounds were made to be higher than the test concentration because the compounds were diluted when the inoculum was added.

A working concentration of 6.154 gram/L of each compound was made.

The compounds for the Gram-negative panels (A) were setup as follows:

Compound A: 0.1524 gram was dissolved in 24.764 ml of cation-adjusted Mueller-Hinton broth (CAMHB) with 2 % DMSO Compound B: 0.1517 gram was dissolved in 24.651 ml of cation-adjusted Mueller-Hinton broth (CAMHB) with 2 % DMSO.

Compound K was pre-dissolved in 100 % DMSO making a 3 ml solution of 0.3181 mg/µl of Compound K in DMSO. To make 25 ml of the working solution 0.484 µl of the Compound K solution was added to 24.516 ml CAMHB.
The compounds for the Gram-positive panels (C) were setup as follows:

Compound A: 0.1535 gram was dissolved in 24.944 ml of CAMHB with 2% DMSO.

Compound B was pre-dissolved in 100% DMSO making a 5.5 ml solution of 0.1022 mg/µl of Compound B in DMSO. To make 25 ml of the working solution 1.505 µl of the Compound B solution was added to 23.495 ml CAMHB.

Compound K was pre-dissolved in 100% DMSO making a 3 ml solution of 0.3181 mg/µl of Compound K in DMSO. To make 25 ml of the working solution 484 µl of the Compound K solution was added to 24.516 ml CAMHB.

For plates B/D a working concentration of 4.776 grams/L of each compound was made.

The compounds for the Gram-negative panels (B) were setup as follows:

Compound A: 0.1195 gram was dissolved in 25.021 ml of CAMHB with 2% DMSO.

Compound B: 0.1084 gram was dissolved in 22.697 ml of CAMHB with 2% DMSO.

Compound K was pre-dissolved in 100% DMSO making a 3 ml solution of 0.3181 mg/µl of Compound K in DMSO. To make 25 ml of the working solution 375 µl of the Compound K solution was added to 24.625 ml CAMHB.

The compounds for the gram positive panels (D) were setup as follows:

Compound A: 0.1108 gram was dissolved in 23.199 ml of CAMHB with 2% DMSO.

Compound B was pre-dissolved in 100% DMSO making a 5.5 ml solution of 0.1022 mg/µl of Compound B in DMSO. To make 25 ml of the working solution 1.168 µl of the Compound B solution was added to 23.832 ml CAMHB.

Compound K was pre-dissolved in 100% DMSO making a 3 ml solution of 0.3181 mg/µl of Compound K in DMSO. To make 25 ml of the working solution 375 µl of the Compound K solution was added to 24.625 ml CAMHB.

Each working solution of compound was used to make the 4 dilutions by serially diluting in 10 fold steps. From the working solution 2.5 ml was taken and mixed with 22.5 ml CAMHB, 2.5 ml was then removed from the second tube and mixed with 22.5 ml in the third tube. This was repeated until the fourth dilution, giving the five 10-fold
These dilutions end up being 4, 0.4, 0.04, 0.004 and 0.0004 gram/L after the antibiotics and organism are added.

**Antibiotics:**

The following antibiotics were tested either alone or in combination with the tested compounds, as described hereinafore: Gentamicin (GM), Amikacin (AK), Ceftazidime (CAZ), Cefepime (CPE), trimethoprim/sulfamethoxazole (T/S), piperacillin/tazobactam (P/T), Aztreonam (AZT), Meropenem (MER), Tobramycin (TO), Ciprofloxacin (CP), Colistin (CT) and Chloramphenicol (ChA).

Samples containing an antibiotic and a tested compound were prepared by mixing a sample of the tested compound with the antibiotic so as to afford the indicated concentration.

**Microorganisms:**

Two exemplary microorganisms were tested: *Staphylococcus epidermidis* (ATCC 35984) and *Pseudomonas aeruginosa* (ATCC 27853).

**Inoculum Preparation:**

Using a cryogenic stocks (at -70 °C), the first sub-cultures of the bacterial organisms listed above were streaked out on TSA. The organisms were incubated at 35 °C for 24 hours. Following growth the plate(s) were wrapped in parafilm at 4 °C. From the first sub-cultures, a second sub-culture was streaked out on TSA and incubated at 35 °C for 24 hours. The second sub-cultures were used within 24 hours starting from the time it was first removed from incubation.

Using the second sub-cultures, an inoculum in 3 ml sterile water that matches a 0.5 McFarland Standard (1.5 x 108 cells per ml) in a glass test tube using a sterile cotton swab, was created. This solution was diluted 250 x 100 ml CAMHB. The diluted organism was gently stirred by swirling the flask to achieve uniform mixing of the organism. One sample (100 µL) of the diluted organism was used for an inoculum check by serially diluting and spot plating on Tyrptic Soy Agar (TSA).

**Challenge:**

To each well of the test panels 20 µL of the diluted organism was added.

Gram-negative panels: 20 µL of sterile CAMHB was added to well H12.

Gram-positive panels: 20 µL of sterile CAMHB was added to well Al.
The lid of the 95 peg MBEC device (Gram-Ve) or 96 peg MBEC device (G+Ve) was placed on the bottom plate containing organism, test compound and antibiotics.

The device was placed on an orbital in a humidified incubator at 35 °C for 24 hours set at 110 rpm. Following incubation the peg lid was rinsed by dipping it in a 96 well microtitre plate where each well contained 200 μl of sterile saline for 1 minute. The peg lid was inserted into a recovery plate where each well contained 200 μl of CAMHB. The device was incubated at 35 °C for 24 hours.

**Determination of Planktonic MIC:**

The challenge plate was incubated at 35 °C for 24 hours and read visually to determine MIC values (minimum inhibitory concentration) for each of the tested compounds for each organism shed from the biofilm during the challenge incubation. The MIC is defined as the minimum concentration of a compound that inhibits growth of the organism.

**Determination of Planktonic MBC:**

After the specified contact time, 20 μl from each well of the challenge plate was removed, and placed into the corresponding wells of a fresh 96 well Nunc plate containing 180 μl CAMHB. The wells were incubated at 35 °C for 24 hours. MBC results were determined following the 24 hour incubation by +/- growth.

**Determination of Biofilm MBEC:**

The recovery plate was incubated at 35 °C for 24 hours and read visually to determine MBEC values. A microtiter plate reader was used to obtain optical density measurements at 630 nm (OD630). Clear wells (OD630 < 0.1) were evidence of biofilm eradication.

The MBEC is defined as the minimum concentration of a compound that inhibits growth of the biofilm.

**Data Collection:**

MIC results were determined following the 24 hour incubation from the bioFILM PA and Gram+VE panels using the plate reader. To determine the minimum inhibitory concentration (MIC) values, turbidity (visually) in the wells of the challenge plate were looked for. Alternatively, a microtiter plate reader was used to obtain optical density measurements at 630 nm (OD$_{630}$).
The MIC is defined as the minimum concentration of the tested antibacterial agent that inhibits growth of the organism. Clear wells (OD_{630} < 0.1) were evidence of inhibition following a suitable period of incubation.

MBC value represents the lowest concentration which kills 99.9% of the microorganism population. Results were determined following the 24 hour incubation from the Test panels using the plate reader. To determine the minimum bactericidal concentration (MBC) values, turbidity (visually) in the wells of the challenge plate was looked for. Alternatively, a microtiter plate reader was used to obtain optical density measurements at 630 nm (OD_{630}).

MBEC results were determined following the 24 hour incubation from the MBEC recovery panels using the plate reader to determine the minimum biofilm eradication concentration (MBEC) values. Turbidity (visually) in the wells of the recovery plate was looked for. Alternatively, a microtiter plate reader was used to obtain optical density measurements at 630 nm (OD_{630}). Clear wells (OD_{630} < 0.1) were evidence of biofilm eradication.

The MBEC is defined as the minimum concentration of the antibacterial agent that inhibits growth of the biofilm.

**FIC Analysis:**

It is noted herein that although the fractional inhibitory concentration (FIC) index is most frequently used to define or to describe drug interactions, it has some limitations when used for drugs against filamentous fungi (molds) and other biofilm-forming microbial life forms. These limitations include observer's bias in the determination of the MIC and lack of agreement on the endpoints (MIC-0, MIC-1 and MIC-2 or >95%, >75% and >50% growth inhibition, respectively) when studying drug combinations. Furthermore, statistical analysis and comparisons are oftentimes difficult to make and standardize.

To determine the fractional inhibitory concentrations (FICs), values were determined according to $\text{FIC} = (\text{MIC}_{X_t}/\text{MIC}_{X_s}) + (\text{MIC}_{Y_t}/\text{MIC}_{Y_s})$, wherein MIC_{X_t} is the lowest MIC measured for test compound X in the combined formulation, MIC_{X_s} is the lowest MIC measured for test compound X as a single agent, MIC_{Y_t} is the lowest MIC measured for antibiotic agent Y in the combined formulation and MIC_{Y_s} is the lowest MIC measured for antibiotic agent Y as a single agent.
In order to calculate the FICs:

(a) MICs were determined for the test compound alone (to be used as the values for \( \text{MIC}_{X} \));

(b) MICs were determined for the antibiotic agent alone (to be used as the values for \( \text{MIC}_{Y} \)). Only single antibiotic agents were analyzed for FIC calculation.

(c) The MIC was determined for each antibiotic at each test compound concentration (i.e., at a particular test compound concentration, used as Lowest MIC Agent X(combination), the corresponding concentration of antibiotic which was the minimum antibiotic concentration to result in bacterial growth inhibition at that test compound concentration was determined, and used as \( \text{MIC}_{X} \)).

(d) The MIC was determined for each test compound at each antibiotic concentration (i.e., at a particular antibiotic concentration, used as \( \text{MIC}_{X} \text{c} \), the corresponding concentration of test compound which was the minimum compound concentration to result in bacterial growth inhibition at that antibiotic concentration was determined, and used as \( \text{MIC}_{Xc} \)).

(e) The FIC index was calculated using the MICs determined in both (c) and (d). MICs less than or equal to the value selected in (c) or (d) indicate that the MIC value selected was the lowest concentration tested. MICs greater than the value selected in (c) or (d) indicate that there was growth at the highest concentration tested.

**Results:**

Codes were assigned to all FIC indexes calculated as follows:

If \( \text{FIC} \leq 0.5 \); the combination is synergistic (designated SY in Tables 1 and 2 below);

If \( 0.5 < \text{FIC} \leq 1.0 \), the combination is additive (designated AD in Tables 1 and 2 below);

If \( 1.0 < \text{FIC} \leq 4.0 \), the combination is indifferent (designated IN in Tables 1 and 2 below);

If \( \text{FIC} > 4.0 \), the combination is antagonistic or indeterminate (not clinically achievable).

The lowest FIC index calculated for each compound-antibiotic combination was selected as the final FIC value to be tabulated.

The obtained data is presented in Tables 7-10 below.
Table 7 below presents the Minimum Inhibitory Concentration (MIC) of antibacterial agents (tested compounds and clinical antibiotics) obtained for \textit{Pseudomonas aeruginosa} ATCC 27853.

<table>
<thead>
<tr>
<th>Antibacterial Agent</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound A</td>
<td>4000</td>
</tr>
<tr>
<td>Compound B</td>
<td>4000</td>
</tr>
<tr>
<td>Compound K</td>
<td>4000</td>
</tr>
<tr>
<td>AK</td>
<td>4</td>
</tr>
<tr>
<td>AZT</td>
<td>4</td>
</tr>
<tr>
<td>CT</td>
<td>1</td>
</tr>
<tr>
<td>GM</td>
<td>2</td>
</tr>
<tr>
<td>TO</td>
<td>2</td>
</tr>
<tr>
<td>ChA</td>
<td>4</td>
</tr>
<tr>
<td>CP</td>
<td>0.25</td>
</tr>
<tr>
<td>CAZ</td>
<td>2</td>
</tr>
<tr>
<td>CPE</td>
<td>4</td>
</tr>
<tr>
<td>MER</td>
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</tr>
</tbody>
</table>

Table 8 presents the Antibacterial-agent combinations (test compounds combined with clinical antibiotics) at minimal concentrations for inhibiting \textit{Pseudomonas aeruginosa} ATCC 27853 (sorted by FIC).

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Test Compound Concentration (µg/ml)</th>
<th>Antibiotic</th>
<th>Antibiotic Concentration (µg/ml)</th>
<th>FIC</th>
<th>FIC Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.004</td>
<td>AK</td>
<td>2</td>
<td>0.50</td>
<td>AD</td>
</tr>
<tr>
<td>A</td>
<td>0.0004</td>
<td>AZT</td>
<td>4</td>
<td>1.00</td>
<td>IN</td>
</tr>
<tr>
<td>A</td>
<td>0.0004</td>
<td>CT</td>
<td>1</td>
<td>1.00</td>
<td>IN</td>
</tr>
<tr>
<td>A</td>
<td>0.004</td>
<td>GM</td>
<td>1</td>
<td>0.50</td>
<td>AD</td>
</tr>
<tr>
<td>A</td>
<td>0.4</td>
<td>TO</td>
<td>0.5</td>
<td>0.35</td>
<td>SY</td>
</tr>
<tr>
<td>A</td>
<td>0.0004</td>
<td>ChA</td>
<td>4</td>
<td>1.00</td>
<td>IN</td>
</tr>
<tr>
<td>A</td>
<td>0.04</td>
<td>CP</td>
<td>0.25</td>
<td>1.01</td>
<td>IN</td>
</tr>
<tr>
<td>A</td>
<td>0.004</td>
<td>CAZ</td>
<td>1</td>
<td>0.50</td>
<td>AD</td>
</tr>
<tr>
<td>A</td>
<td>0.04</td>
<td>CPE</td>
<td>1</td>
<td>0.26</td>
<td>SY</td>
</tr>
</tbody>
</table>
As can be seen in Table 8, Compound K, in most of the tested combinations, was shown capable of substantially potentiating the activity of most of the tested antibiotics.

Table 9 below presents the Minimum Inhibitory Concentration (MIC) of antibacterial agents (tested compounds and clinical antibiotics) obtained for to Staphylococcus epidermidis ATCC 35984.

### Table 9

<table>
<thead>
<tr>
<th>Antibacterial Agent</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound K</td>
<td>4000</td>
</tr>
<tr>
<td>Compound A</td>
<td>4000</td>
</tr>
<tr>
<td>Compound B</td>
<td>4000</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>4</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>8</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>16</td>
</tr>
</tbody>
</table>
Table 10 presents the Antibacterial-agent combinations (test compounds combined with clinical antibiotics) at minimal concentrations for inhibiting *Staphylococcus epidermidis* ATCC 35984 (sorted by FIC)

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Test Compound Concentration (µg/ml)</th>
<th>Antibiotic</th>
<th>Antibiotic Concentration (µg/ml)</th>
<th>FIC*</th>
<th>FIC Code**</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0004</td>
<td>Ampicillin</td>
<td>4</td>
<td>1.00</td>
<td>IN</td>
</tr>
<tr>
<td>A</td>
<td>0.0004</td>
<td>Cefazolin</td>
<td>4</td>
<td>0.50</td>
<td>AD</td>
</tr>
<tr>
<td>A</td>
<td>0.0004</td>
<td>Ceftriaxone</td>
<td>16</td>
<td>1.00</td>
<td>IN</td>
</tr>
<tr>
<td>A</td>
<td>0.04</td>
<td>Cephalothin</td>
<td>2</td>
<td>0.26</td>
<td>SY</td>
</tr>
<tr>
<td>A</td>
<td>0.0004</td>
<td>Chloramphenicol</td>
<td>8</td>
<td>1.00</td>
<td>IN</td>
</tr>
<tr>
<td>A</td>
<td>0.0004</td>
<td>Ciprofloxacin</td>
<td>0.25</td>
<td>1.00</td>
<td>IN</td>
</tr>
<tr>
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<td>0.04</td>
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<td>0.51</td>
<td>AD</td>
</tr>
<tr>
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<td>Erythromycin</td>
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<td>1.00</td>
<td>IN</td>
</tr>
<tr>
<td>A</td>
<td>0.0004</td>
<td>Gatifloxacin</td>
<td>0.125</td>
<td>1.00</td>
<td>IN</td>
</tr>
<tr>
<td>A</td>
<td>0.0004</td>
<td>Gentamicin</td>
<td>4</td>
<td>1.00</td>
<td>IN</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>-----</td>
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</tr>
<tr>
<td>A</td>
<td>0.04</td>
<td>Gentamicin Synergy</td>
<td>62.5</td>
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<td>Imipenem</td>
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<td>0.07</td>
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<td>Levofloxacin</td>
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<td>0.50</td>
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<td>Linezolid</td>
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<td>Nitrofurantoin</td>
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<tr>
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<td>1.00</td>
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</tr>
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<td>Levofloxacin</td>
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</tr>
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<td>Moxifloxacin</td>
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<td>1.00</td>
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<td>Nitrofurantoin</td>
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<tr>
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<td>SY</td>
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<tr>
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<td>0.13</td>
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</tbody>
</table>

As can be seen in Table 10, compound K, in almost all of the tested combinations, was shown capable of substantially potentiating the activity of all of the tested antibiotics.

**EXAMPLE 4**

**Antibacterial Activity of Compound F and Compound K Combined with Non Beta-Lactam Antibiotics**

The anti-bacterial activity of Compound F and Compound K in combination with commonly used non beta-lactam antibiotics was tested as follows.

**MATERIALS AND METHODS**

**Bacterial strains:**

*Staphylococcus aureus* (MRSA; strain USA 400), *Staphylococcus epidermidis* (strain ATCC 35984) and *Pseudomonas aeruginosa* (strain ATCC 27853).

**Clinical antibiotics:**

Ciprofloxacin, Gentamicin, Tetracycline, Tobramycin and Vancomycin.
**Determination of Minimum Inhibitory Concentration (MIC):**

MIC values for each antibacterial agent (antibiotic), the tested compound and combination thereof were determined according to the procedure described by Ceri et al. (Methods Enzymol 337:377-85, 2001) using SENSITITRE® Gram Positive and Gram Negative MIC Plates (TREC Diagnostics Systems, Cleveland, OH) and Biofilm™ test panels (Inovotech Inc., Edmonton, AB Canada).

**Determination of synergy between test compound and antibacterial agents:**

The synergy between Compound F or Compound K and clinical antibiotics is expressed in terms of the fractional inhibitory concentrations (FIC) (Elion et al., Journal of Biological Chemistry 208: 477-88, 1954). The FIC is calculated by the following formula:

\[
\text{FIC} = \frac{\text{Lowest MIC Agent}_{(\text{combination})}}{\text{Lowest MIC Agent}_{(\text{single})}} + \frac{\text{Lowest MIC Agent}_{(\text{combination})}}{\text{Lowest MIC Agent}_{(\text{single})}}
\]

The combined treatment with Compound F or Compound K and the tested antibiotic was considered synergic when the resulting FIC value is equal to or lower than 0.5.

**RESULTS**

Table 11 below presents the Minimum Inhibitory Concentration (MIC) of the tested compounds (Compound F, Compound K) and the known non-beta lactam antibacterial agents (clinical antibiotics) provided to bacterial strains *Staphylococcus aureus* strain USA 400 (MRSA), *Staphilococcus epidermitis* ATCC 35984 (S. epidermitis) and *Psuedomonas aeruginosa* ATCC 27853 (P. aeruginosa)

<table>
<thead>
<tr>
<th>Antibacterial Agent</th>
<th>Bacterial Strain</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound F</td>
<td>MRSA</td>
<td>2.8 mg/ml</td>
</tr>
<tr>
<td></td>
<td><em>S. epidermidis</em></td>
<td>0.28 mg/ml</td>
</tr>
<tr>
<td></td>
<td><em>P. aeruginosa</em></td>
<td>2.8 mg/ml</td>
</tr>
<tr>
<td>Compound K</td>
<td>MRSA</td>
<td>4.5 mg/ml</td>
</tr>
</tbody>
</table>
Table 12 below shows the effect of Compound F combined with clinical non-beta-lactam antibiotics on the clinical bacterial strains tested (sorted by FIC). As shown therein, the combination of Compound F with Gentamicin, Ciprofloxacin, Tetracycline, Tobramycin or Vancomycin resulted in synergic inhibition of bacterial growth.

### Table 12

<table>
<thead>
<tr>
<th><strong>Bacterial Strain</strong></th>
<th><strong>Compound F Concentration (µg/ml)</strong></th>
<th><strong>Antibiotic</strong></th>
<th><strong>Antibiotic Concentration (µg/ml)</strong></th>
<th><strong>FIC</strong></th>
<th><strong>FIC Code</strong></th>
</tr>
</thead>
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<tr>
<td>MRSA</td>
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<td>Ciprofloxacin</td>
<td>0.125</td>
<td>0.22</td>
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</tr>
<tr>
<td>MRSA</td>
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<td>0.25</td>
<td>SY</td>
</tr>
<tr>
<td>MRSA</td>
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<td>Ciprofloxacin</td>
<td>0.5</td>
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<td>SY</td>
</tr>
<tr>
<td>MRSA</td>
<td>0.28</td>
<td>Gentamicin</td>
<td>0.125</td>
<td>0.23</td>
<td>SY</td>
</tr>
<tr>
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<td>SY</td>
</tr>
<tr>
<td>MRSA</td>
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<td>SY</td>
</tr>
<tr>
<td><strong>S. epidermidis</strong></td>
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<td>Tetracycline</td>
<td>0.125</td>
<td>0.26</td>
<td>SY</td>
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<tr>
<td><strong>S. epidermidis</strong></td>
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<td>0.0625</td>
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<td><strong>S. epidermidis</strong></td>
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<tr>
<td><strong>S. epidermidis</strong></td>
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<td>Tetracycline</td>
<td>0.25</td>
<td>0.50</td>
<td>SY</td>
</tr>
</tbody>
</table>
Table 13 below shows the effect of Compound K combined with clinical non-beta-lactam antibiotics on the clinical bacterial strains tested (sorted by FIC). As shown therein, the combination of Compound K with Gentamicin, Ciprofloxacin, Tetracycline, Tobramycin or Vancomycin resulted in synergic inhibition of bacterial growth.

* FIC = [(lowest MIC of compound F in combination) / (lowest MIC of compound F as single agent)] + [(lowest MIC of antibiotic in combination) / [(lowest MIC of antibiotic as single agent)].

** SY = synergy; the interaction between Compound F and the clinical antibiotic is considered synergic when the resulting FIC value is equal to or lower than 0.5.

** AD = additive, the interaction between Compound F and the clinical antibiotic is considered additive when the resulting FIC value is equal to or lower than 1.0 but higher than 0.5.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Compound K Concentration W/ml</th>
<th>Antibiotic</th>
<th>Antibiotic Concentration (μg/ml)</th>
<th>FIC *</th>
<th>FIC Code **</th>
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<tbody>
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<td>P. aeruginosa</td>
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<td>P. aeruginosa</td>
<td>0.000045</td>
<td>Tobramycin</td>
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<td>0.50</td>
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</table>
The results obtained indicate that Compound F and Compound K are uniquely and unexpectedly capable of potentiating the activity of non beta-lactam antibiotics commonly used in therapy.

**EXAMPLE 5**

*Antibacterial Activity of Compound K*

The antimicrobial effect of Compound K described hereinabove, referred to herein also as EDP-5 or BL-6030, and of Compound F, referred to herein also as BL-6031, on methicillin resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* was further tested *in vitro*.

**MATERIALS AND METHODS**

**Inoculum Preparation:**

A fresh culture of pathogenic isolate obtained directly from American Type Culture Collection (ATCC), Rockville, Maryland, was used in these studies. The bacterial strains were methicillin resistant *Staphylococcus aureus* (MRSA: USA300) and *Pseudomonas aeruginosa* (PA: ATCC27312).
The freeze-dried bacteria culture was recovered per ATCC standard recovering protocol. All challenge inoculum suspensions were prepared by swabbing a 3-cm diameter area of the growth from a culture plate into 4.5 ml of sterile water, providing a suspension consisting of approximately $10^{10}$ colony forming units/ml (CFU/ml). Two (2) ml of this suspension were diluted into 150 ml of sterile tryptic soy broth (TSB), making the inoculum suspension approximately $10^6$ CFU/ml. The concentration was confirmed using historical optical density measurements. In addition, serial dilutions of the suspension were plated onto specific media for this microorganism using the Spiral Plater System that deposits a small amount (50 μl) of suspension over the surface of a rotating culture media agar plate to quantitate the exact concentration of viable organisms prior to the experiment.

**Minimum Inhibitory Concentration (MIC) Broth Tube Dilution Assay:**

The following treatment groups were assayed:

A. Compound K (between 10 mg/ml to 0.4 mg/ml)

B. Vehicle (10% 1,2-propanediol in water for injection)

C. Negative Control TSB (Turbidity Control)

The tube dilution test is the standard method for determining levels of resistance to a compound (antibiotic). Dilutions of the antibiotic were made in a liquid inoculum with a known number of organisms after overnight incubation. The lowest concentration (highest dilution) of antibiotic preventing appearance of turbidity was considered to be the minimum inhibitory concentration (MIC). At this dilution the antibiotic is bacteriostatic.

**MIC Assay:**

Fourteen (14) sterile, capped test tubes were labeled 1 through 14. All steps were carried out using aseptic technique.

Twelve different concentrations (10, 5.0, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.75, 0.5 and 0.4 mg/ml) were evaluated to determine the minimum inhibitory concentration (MIC).

1.0 ml of TSB with bacterial inoculum was added to each tube.

1.0 ml of each concentration of the tested compound was added to first 12 tubes.

1.0 ml of vehicle was added to the tube number thirteen.
1.0 ml of sterile TSB was added to the tube number fourteen. This tube was served as a negative control and received none of the tested compound.

All of the test tubes were incubated for 24 hours at 37 °C. After the incubation period, the tubes were examined for visible signs of bacterial growth, seen as turbidity in the suspension and were mixed by pipetting, and 200 µl were deposited into 3 wells on a 96 wells plate. The optical density (OD) of the solution was measured in a spectrophotometer (BioRad Laboratories Inc.). As compared to the control diluted culture suspension, the without growth (no turbidity) was determined to be the minimum inhibitory concentration (MIC) for each strain of bacteria.

**Minimum Bactericidal Concentration (MBC):**

The contents of the tubes were also subcultured onto antibiotic free medium (TSA) to examine bacterial growth (MBC).

**Inhibition Zone (Agar Diffusion Assay):**

Four concentrations of the Compound K and vehicle control were examined using the zone of inhibition test (measurement using the planimetry method).

The following treatment groups were assayed:

A. Compound K (Concentrations 10, 4.0, 2.5, and 1.0 mg/ml).
B. Vehicle
C. Mupirocin 2% (Positive Control to MRSA)
D. Silver Sulfadiazine 1% and Sulfamylon 8.5% (Positive Control to P. aeruginosa).
E. Negative Control (Sterile PBS)

A total of six plates for each concentration were evaluated. The inoculum was evenly spread on the total surface of the culture media plate in order to grow a continuous lawn of bacteria. After 2 hours of plate inoculation, one punch (10 mm) was made in the central area of the plates (agar was removed) and approximately 200 µl of the testing compounds were placed in the well of the Tryptic Soy agar with 5% sheep's blood culture media plates. The initial diameter of each test compound and the diameter to which it spreads were noted. All the media plates were incubated for 24 hours at 37 °C. After the incubation period, the zones of bacteria growth inhibition were measured using planimetry method.
RESULTS

Data were analyzed by comparing quantification of bacteria (per bacterial strain) as a function of the effect of each treatment versus controls, measured by zone of inhibition and estimated by mean optical densities (at 655 nm) per each concentration. The arithmetic mean and standard deviation of the OD, bacterial counts Log (CFU/ml), and zones of bacteria growth inhibition were calculated for each treatment.

Figure 1 presents a bar graph presenting the results of the optical density measurements and bacterial count of methicillin resistant *Staphylococcus aureus* USA300 strain with different concentrations of Compound K (denoted as EDP-5).

As can be seen in Figure 1, all treatment groups with Compound K at 24 hours assessment reduced bacterial count compared to Vehicle and Negative Control. Negative control group resulted in a greater MRSA count and higher OD compared with all different Compound K concentrations. Similar results were observed in the vehicle group. After 24 hours, bacterial counts and OD measurements of methicillin resistant *Staphylococcus aureus* recovered from the plates treated with Compound K 10 mg/ml were $3.10 \pm 0.06$ log CFU/ml and -0.005 OD average, respectively. Compound K 10 mg/ml showed the largest reduction of associated bacteria compared to all treatments. Bacterial counts were increased from Compound K 5 mg/ml ($5.18 \pm 0.04$ log CFU/ml) until Compound K 0.4 mg/ml ($8.92 \pm 0.04$ log CFU/ml).

As can further be seen in Figure 1, OD values increased with the increasing of bacterial counts in all treatments groups. Compound K 0.4 mg/ml had highest bacterial counts compared with the other Compound K concentrations.

Figure 2 is a bar graph presenting the results of the optical density measurements and bacterial count of *Pseudomonas aeruginosa* ATCC27312 strain (PA) with different concentrations of Compound K.

As can be seen in Figure 2, all treatment groups with Compound K at 24 hours assessment reduced bacterial count compared to vehicle and negative control. Negative control group resulted in a greater total bacterial count and higher OD compared with all different Compound K concentrations. Similar results were observed in the vehicle group. After 24 hours bacterial counts and OD measurements of *P. aeruginosa* recovered from the plates treated with Compound K 10 mg/ml were $1.30 \pm 0.0$ (equivalent to limit of quantification) log CFU/ml and -0.004 OD average respectively.
Treatment with Compound K 10 mg/ml showed the higher reduction of associated PA compared to all treatments. Log CFU/ml of associate *P. aeruginosa* in the other treatments were between $5.16 \pm 0.03\ log\ CFU/ml$ and $9.80 \pm 0.05\ log\ CFU/ml$.

As can further be seen in Figure 2, an increase of the OD value with the increasing of bacterial counts was observed in all treatment groups. Compound K 0.4 mg/ml showed largest bacterial counts compared with the others concentrations of Compound K.

Figure 3 is bar graph presenting methicillin resistant *Staphylococcus aureus* USA300 strain inhibition zones by various concentrations of Compound K measured in cm² using planimetry method.

As can be seen in Figure 3, all treatment groups using Compound K for 24 hours showed some zone of inhibition. Treatment with 10 mg/ml Compound K showed the largest zone of bacteria growth inhibition (5.33 cm²) compared to all treatments. This was followed by the results observed for 4.0 mg/ml Compound K (3.23 cm²), 2.5 mg/ml Compound K (2.51 cm²) and 1.0 mg/ml Compound K (1.88 cm²). Mupirocin positive control resulted in an inhibition zone of 51.06 cm². Negative control group and vehicle control did not show any zone of inhibition.

Figure 4 is a bar graph presenting the *Pseudomonas aeruginosa* ATCC27312 bacteria growth inhibition zones by various concentrations of Compound K, measured in cm² using planimetry method.

As can be seen in Figure 4, all treatment groups using Compound K for 24 hours showed some zone of inhibition. Treatment with 10 mg/ml Compound K showed the largest zone of inhibition (21.08 cm²) compared to all treatments. This was followed by the results observed for 4.0 mg/ml Compound K (16.25 cm²), 2.5 mg/ml Compound K (5.96 cm²) and 1.0 mg/ml Compound K (1.84 cm²). Negative control group and vehicle did not show zone of inhibition. All samples using Compound K showed a greater inhibition zone than silver sulfadiazine 1% (2.59 cm²), except the sample using 1.0 mg/ml Compound K. The inhibition zone with sulfamylon 8.5% was 31.29 cm².

The data obtained in these studies clearly demonstrate that all treatments at different concentration of Compound K showed reduction of bacteria count in the *in vitro* study. Compound K at a concentration of 10 mg/ml challenge with both strains
showed the largest reduction of bacteria count in the \textit{in vitro} study compared to all treatments.

Inhibition zone assay showed that 10 mg/ml Compound K had the largest zone of bacteria growth inhibition of methicillin resistant \textit{Staphylococcus aureus} USA300 and \textit{Pseudomonas aeruginosa} ATCC27312 (5.33 and 21.08 cm$^2$, respectively) compared to any treatment. This concentration resulted in better inhibition zone than silver sulfadiazine when was tested against \textit{P. aeruginosa}.

\textbf{EXAMPLE 6}\n
\textbf{Antibacterial Activity of Compound K or Compound F in combination with non-beta lactam antibiotics}\n
The antimicrobial effect of Compound K described herein above, referred to herein also as EDP-5 or BL-6030, and of Compound F, referred to herein also as BL-6031, on methicillin resistant \textit{Staphylococcus aureus} (MRSA) and \textit{Pseudomonas aeruginosa} was further tested \textit{in vitro}.

\textbf{MATERIALS AND METHODS}\n
\textbf{Inoculum Preparation:}\n
A fresh culture of pathogenic isolate obtained directly from American Type Culture Collection (ATCC), Rockville, Maryland, was used in these studies. The bacterial strains were methicillin resistant \textit{Staphylococcus aureus} (MRSA: USA300) and \textit{Pseudomonas aeruginosa} (PA: ATCC27312).

The freeze-dried bacteria culture was recovered per ATCC standard recovering protocol. All challenge inoculum suspensions were prepared by swabbing a 3-cm diameter area of the growth from a culture plate into 4.5 ml of sterile water, providing a suspension consisting of approximately $10^{10}$ colony forming units/ml (CFU/ml). Two (2) ml of this suspension were diluted into 150 ml of sterile tryptic soy broth (TSB), making the inoculum suspension approximately $10^6$ CFU/ml. The concentration was confirmed using historical optical density measurements. In addition, serial dilutions of the suspension were plated onto specific media for this microorganism using the Spiral Plater System that deposits a small amount (50 µl) of suspension over the surface of a rotating culture media agar plate to quantitate the exact concentration of viable organisms prior to the experiment.
Minimum Inhibitory Concentration (MIC) Broth Tube Dilution Assay:

The following treatment groups were assayed for the MRSA strain:

A. Compound K (between 5 mg/ml and 0.2 mg/ml)
B. Compound F (between 5 mg/ml and 0.2 mg/ml)
C. Gentamicin (between 4 µg/ml and 0.0625 µg/ml)
D. Vehicle (2% DMSO)
E. Negative Control TSB (Turbidity Control)

The following treatment groups were assayed for the PA strain:

A. Compound K (between 5 mg/ml and 0.2 mg/ml)
B. Compound F (between 5 mg/ml and 0.2 mg/ml)
C. Tobramycin (between 4 µg/ml and 0.0625 µg/ml)
D. Vehicle (2% DMSO)
E. Negative Control TSB (Turbidity Control)

The tube dilution test is the standard method for determining levels of resistance to a compound (antibiotic). Dilutions of the antibiotic were made in a liquid inoculum with a known number of organisms after overnight incubation. The lowest concentration (highest dilution) of antibiotic preventing appearance of turbidity was considered to be the minimum inhibitory concentration (MIC). At this dilution the antibiotic is bacteriostatic.

MIC Assay:

Fourteen (14) sterile, capped test tubes were labeled 1 through 14. All steps were carried out using aseptic technique.

Twelve different concentrations (5.0, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.75, 0.5, 0.4 and 0.2 mg/ml) were evaluated for Compound K and Compound F; and seven different concentrations (4.0, 2.0, 1.0, 0.5, 0.25, 0.125 and 0.0625 µg/ml) were used in antibiotics (Gentamicin or Tobramycin) to determine the minimum inhibitory concentration (MIC).

1.0 ml of TSB with bacterial inoculum was added to each tube.

1.0 ml of each concentration of the tested compound was added to first 12 tubes of Compound K or Compound F or first 7 tubes of antibiotics.

1.0 ml of vehicle was added to the tube number thirteen of Compound F or Compound K, or the tube number eight of the antibiotics.
1.0 ml of sterile TSB was added to the tube number fourteen of Compound F or Compound K, or the tube number nine of the antibiotics. These tubes served as a negative control and received none of the tested compound.

All of the test tubes were incubated for 24 hours at 37 °C. After the incubation period, the tubes were examined for visible signs of bacterial growth, seen as turbidity in the suspension and were mixed by pipetting, and 200 μl were deposited into 3 wells on a 96 wells plate. The optical density (OD) of the solution was measured in a spectrophotometer (BioRad Laboratories Inc.). As compared to the control diluted culture suspension, the without growth (no turbidity) was determined to be the minimum inhibitory concentration (MIC) for each strain of bacteria.

RESULTS

Data were analyzed by comparing quantification of bacteria (per bacterial strain) as a function of the effect of each treatment versus controls, measured by zone of inhibition and estimated by mean optical densities (at 655 nm) per each concentration. The arithmetic mean and standard deviation of the OD, bacterial counts Log (CFU/ml), and zones of bacteria growth inhibition were calculated for each treatment.

Figure 5A is a bar graph presenting the results of the optical density measurements and bacterial count of methicillin resistant Staphylococcus aureus USA300 strain with different concentrations of Compound K and Compound F.

As can be seen in Figure 5A, after 24 hours bacterial OD measurements of Methicillin Resistant Staphylococcus aureus treated with Compound F remain undetectable (values between 0.004 - 0.007 OD) for concentrations from 5 to 2.5 mg/ml. The OD values increased with the decreasing of concentrations of both Compound F and Compound K, starting from 5 mg/ml. Negative control and vehicle showed the largest OD measurement compared with all different concentrations tested for both compounds.

Figure 5B is a bar graph presenting the results of the optical density measurements and bacterial count of methicillin resistant Staphylococcus aureus USA300 strain with different concentrations of Gentamicin.

After 24 hours bacterial OD measurements of Methicillin Resistant Staphylococcus aureus treated with Gentamicin showed the more lower OD with 4
µg/ml (0.025 OD) followed by 2 µg/ml (0.176 OD). Concentrations tested from 1.0 - 0.0625 µg/ml resulted in a higher OD measurement (between 0.387 - 0.440). Negative control and vehicle showed the largest OD measurement compared with all different concentrations tested for gentamicin.

Figure 6A is a bar graph presenting the results of the optical density measurements and bacterial count of *Pseudomonas aeruginosa* ATCC27312 strain (PA) with different concentrations of Compound K and Compound F.

As can be seen in Figure 6A, bacterial OD measurements of *Pseudomonas aeruginosa* ATCC27312 after 24 hours challenged with Compound F remained undetectable for concentrations from 5 to 1.5 mg/ml (values between 0.001 - 0.008 OD). Concentrations examined for Compound F between 2.0 to 0 µg/ml had an inversely proportional increase with the OD measurement.

The OD with Compound K remained undetectable for 5.0 and 4.0 µg/ml. A similar trend to Compound F was observed from concentration between 3.5 - 0.2 µg/ml, with the OD value increasing with the decreased concentrations. Optical density for negative control and vehicle showed the largest measurement compared with all different concentrations tested for both compounds. As can further be seen in Figure 6A, an increase of the OD value with the increasing of bacterial counts was observed in all treatment groups. Compound K 0.4 mg/ml showed largest bacterial counts compared with the others concentrations of Compound K.

Figure 6B is a bar graph presenting the results of the optical density measurements and bacterial count of *Pseudomonas aeruginosa* ATCC27312 strain (PA) with different concentrations of Tobramycin. As can be seen in Figure 6B, after 24 hours bacterial OD measurements of *Pseudomonas aeruginosa* ATCC 27312 treated with Tobramycin showed the more lower OD with 4 µg/ml (0.019 OD) followed by 2 µg/ml (0.037 OD). Concentrations tested from 1.0 - 0.0625 µg/ml resulted in a higher OD measurement (between 0.108 - 0.448). Negative control and vehicle showed the largest OD measurement compared with all different concentrations tested for Tobramycin.
EXAMPLE 7

In Vivo Studies for Compound K

The antimicrobial effect of Compound K (also denoted EDP-5) described hereinabove was tested on meticillin resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa in vivo.

MATERIALS AND METHODS

Experimental Animals:

Swine were used as experimental research animal since their skin is morphologically and biochemically similar to human skin. Two animals were studied: one (1) animal with S. aureus and another animal with P. aeruginosa. The young female specific pathogen free (SPF: Looper Farms, North Carolina) pigs weighing 35-40 kg were kept in-house for at least one week prior to initiating the experiment. The animals were fed a basal diet ad libitum and housed in animal facilities (American Association for Accreditation of Laboratory Animal accredited) with controlled temperature (19-21 °C) and lights (12 hour/12 hour LD).

Animal Preparation:

Animals were anesthetized with Telazol HC1 (5 mg/kg), Xylazine (0.2 mg/kg), Atropine (0.05 mg/kg) intramuscularly, and inhalation of an isoflurane and oxygen combination. Hair on the back of the pigs was clipped with standard animal clippers. Skin on the back and sides of the animals, surrounding and including the wound area, were prepared by washing with a non-antibiotic soap (Neutrogena®) and sterile water. The animals were blotted dry with sterile gauze.

Deep Partial Thickness Wound:

Wounding:

Fifty-four (54) rectangular wounds measuring 10 mm x 7 mm x 0.5 mm deep was made in the paravertebral and thoracic area with a specialized electrokeratome fitted with a 7 mm blade. Punctate bleeding was controlled by pressure with sterile gauze pads. The wounds were separated from one another by 15 mm of unwounded skin. The wounds were inoculated and treated as described in the experimental design hereinbelow.
Experimental Design:

One animal was inoculated with Methicillin Resistant *Staphylococcus aureus* (MRSA) and the other animal with *Pseudomonas aeruginosa* (PA). On each animal nine wounds were randomly assigned to one of six treatment groups (see below). All nine wounds were inoculated (as described hereinbelow). Six of the wounds were treated within 20 minutes to assess bacteria counts prior to biofilm development, for "biofilm inhibition"; and the other three wounds were covered with a polyurethane film to observe the effect of the treatments on established biofilms, for "biofilm elimination".

For methicillin resistant *Staphylococcus aureus*, the following treatment groups were assayed:

A.  S1 (Compound K; 100 mg/ml)
B.  S2 (Compound K; 40 mg/ml)
C.  S2 (Compound K; 10 mg/ml)
D.  S4 (Vehicle; 10% propylene glycol/H₂O)
E.  Mupirocin (as positive control to methicillin resistant *S. aureus*; cream was applied by adding approximately between 150 - 200 µl to the top of the wound and covering with sterile gauze (2 x 2 inch 4 ply thick)
F.  Untreated

24 hour-biofilms were established prior to the first treatment on Day 1.

For *Pseudomonas aeruginosa* ATCC27312 strain, the following treatment groups were assayed:

A.  P1 (Compound K; 40 mg/ml)
B.  P2 Compound K; 10 mg/ml)
C.  P3 (Compound K; 5 mg/ml)
D.  P4 (Vehicle; 10% propylene glycol/H₂O)
E.  Silver Sulfadiazine (as Positive Control to *P. aeruginosa*; Cream was applied by adding approximately between 150 - 200 µl to the top of the wound and covering with sterile gauze (2 x 2 inch 4 ply thick)
F.  Untreated
Treatment Regimen:

Immediately after wounding, the wounds were inoculated with the appropriate bacterial strain as described in hereinbelow. The wounds were divided into six groups of nine wounds each. Six wounds were treated on day 0 within 20 minutes of inoculation to determine the biofilm inhibition.

Treatments A, B, C and D were effected by adding 1.5 ml of the tested formulation to completely saturate a sterile gauze pad 2 x 2 inch in size and 4 ply thick (Curity™ Tyco Healthcare Group, Mansfield, MA). Gauze saturation with the formulation helped maintaining the formulation in direct contact with the wounds. Each saturated gauze pad was put on each wound, and covered with a polyurethane film dressing.

Wounds that were treated with positive controls (Mupirocin or Silver Sulfadiazine) received about 150 - 200 µl of material which was sufficient to completely cover the wound area and surrounding skin. Positive controls were spread out gently with a sterile Teflon spatula and covered with a sterile gauze pad and redressed with a polyurethane film dressing.

The remaining three wounds were left untreated and remained in place for 24 hours to allow formation of a bacterial biofilm in the wounds. All dressings were covered and secured by wrapping the animal with self-adherent elastic bandages (Coban; 3M, St. Paul, MN). After 24 hours (day 1), the dressings were removed. Three of the wounds were recovered as described hereinbelow. Three wounds were treated again (see above design "Biofilm inhibition") and the remaining three wounds were treated to determine the "Biofilm elimination".

Microbiological Techniques:

Wound Inoculation:

Fresh cultures of methicillin resistant Staphylococcus aureus USA300 (for the first pig) and Pseudomonas aeruginosa ATCC27312 (for the second pig) were used in this study. The challenge inoculum suspension was prepared by scraping the overnight growth from a culture plate into 5 ml of normal saline, so as to provide a suspension concentration of approximately $10^{10}$ colony forming units/ml (CFU/ml) for each bacteria. Serial dilutions were made until a concentration of $10^6$ CFU/ml was achieved. The inoculum was vortexed and 25 ml of the suspension were inoculated into each
wound. In addition, serial dilutions of the suspension were plated onto culture media to quantify the exact concentration of viable organisms used for this experiment.

**Recovery Methods:**

Prior to wound recovery, the gauze materials from all three wounds were placed in a sterile 50 ml polyurethane tube. Five (5) ml of all purpose neutralizer solution was added, vortexed and serially diluted for quantitation using spiral plater method described above. After removal of gauze, three wounds for each treatment group were recovered on day 1 after first treatment application (within 20 minutes after inoculation). The remaining six wounds for each treatment group were recovered on day 2 after second treatment application and after 24 hours biofilm formation and first treatment application.

To recover bacteria from the wounds, a sterile surgical steel cylinder (22 mm inside diameter) was placed around the wound area. One (1) ml of all purpose neutralizer solution was drawn into the cylinder and the site was scrubbed with a sterile Teflon spatula for 30 seconds.

Serial dilutions were made from all culture samples and the extent of microbiological contamination assessed using the Spiral Plater System as described hereinabove. Oxicillin Resistance Screening Agar (ORSAB) was used to isolate MRSA USA 300 from the wounds and a selective media for *Pseudomonas aeruginosa* (Pseudomonas Agar with CN supplement) was used to quantify *Pseudomonas aeruginosa* present in the suspension. All plates were incubated aerobically overnight (16-24 hours) at 37 °C, after which the number of viable colonies were counted.

**Observations:**

The terms used to describe swelling and erythema from light to heavy are absent & slight < mild < moderate < marked < exuberant.

Figures 7A-D present photographs of wounds during, before and after treatments, as well as throughout the assessment days, wherein gauze in all treatment groups were in place and moist on day 1 showing a slight adherence to the wound bed (Figure 7A), while re-injury was not observed (Figure 7B), and wherein wound fluid was observed from all treatment groups after 24 hours biofilm formation in wounds assigned to biofilm elimination assessment (Figure 7C and Figure 7D).
Figures 8A-B present photographs of wounds infected with methicillin resistant *Staphylococcus aureus* and treated within 20 minutes after inoculation with vehicle (Figure 8A) and untreated wounds (Figure 8B).

As can be seen in Figures 8A-B, on day 1 after one treatment application, treated wounds (Figure 8A) and untreated wounds (Figure 8B) showed slight swelling and erythema. On day 6, gauze continued to show slight adherence to the wound bed without re-injury. No wounds from any of the six treatment groups showed signs of erythema or swelling.

**RESULTS**

After the incubation period, colonies were counted, the data was tabulated and the Log of colony forming units/ml (log CFU/ml) for methicillin resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (PA) determined. The arithmetic mean of the log (CFU/ml) and standard deviation were calculated for each treatment.

Figure 9 is a bar graph showing methicillin resistant *Staphylococcus aureus* USA300 biofilm inhibition after treatment application.

As can be seen in Figure 9, all treatment groups on day 1 reduced bacterial count compared to the untreated group. Wounds treated with 40 mg/ml Compound K showed the lowest bacterial count (6.52 ± 0.36 log CFU/ml) of *S. aureus* in wounds. Treatment with 40 mg/ml Compound K showed a bacterial reduction of 1.84 ± 0.09 log CFU/ml compared with untreated wounds. This value corresponds to a 98.54 % reduction of *S. aureus* on day 1 when compared to untreated wounds. Use of 100 and 10 mg/ml Compound K resulted in 6.98 ± 0.87 and 7.42 ± 0.34 log CFU/ml of *S. aureus* in wounds, respectively. Untreated wounds had the higher log CFU/ml (8.36 ± 0.26).

As can further be seen in Figure 9, after second treatment application all treatments groups showed a reduction in *S. aureus* counts compared with untreated wounds. Compound K at concentration of 100 mg/ml, showed the lowest *S. aureus* counts (6.46 ± 0.16 log CFU/ml). Wounds treated with 100 mg/ml Compound K resulted in a reduction of 1.60 ± 0.04 log CFU/ml compared with untreated wounds, representing a 97.47 % reduction of *S. aureus* on day 2 when compared to untreated wounds. The lower bacterial count in wounds treated with 100 mg/ml Compound K
was followed by 40 mg/ml Compound K (7.41 ± 0.43 log CFU/ml), 10 mg/ml Compound K (7.59 ± 0.25 log CFU/ml) and vehicle (7.76 ± 0.09 log CFU/ml). Untreated wounds resulted in highest *S. aureus* count log CFU/ml (10.77 ± 0.13).

Figure 10 is a bar graph presenting data obtained for inhibition of biofilm of methicillin resistant *S. aureus* USA300 after treatment application.

As can be seen in Figure 10, wounds treated with mupirocin (positive control) had *S. aureus* log CFU/ml counts at day 1 and 2 recovery times of 5.12 ± 0.79 and 4.42 ± 0.18, respectively. On day 2 after second treatment with 100 mg/ml Compound K reduced *S. aureus* counts (0.65 log CFU/ml) compared with day 1 after first treatment. Similar bacterial reduction was found with mupirocin (positive control) when this treatment was applied for 2 days (0.71 log CFU/ml). Treatment with either 40 mg/ml or 5 mg/ml Compound K and vehicle increased MRSA count as compared to same treatments on day 1.

Figures 11A-B are bar graphs presenting the data obtained for biofilm inhibition of methicillin resistant *Staphylococcus aureus* USA300 strain, compared with bacterial count in gauze, after 1 treatment application (Figure 11A), and biofilm inhibition of *S. aureus* USA300, compared with bacterial count in gauze, after 2 treatment applications (Figure 11B).

As can be seen in Figures 11A-B, on days 1 and 2 after first and second treatment applications for biofilm elimination, the *S. aureus* counts in gauze observed in the tests using Compound K and mupirocin (red bars) were lower than bacterial count in untreated wounds (light blue bars), except vehicle on day 1. Similar *S. aureus* counts in gauze were observed with the treatment using 10 mg/ml Compound K and vehicle on day 2 after second treatment. Gauze associated *S. aureus* counts (orange bars) resulted in a lesser *S. aureus* count than wounds.

Figure 12 is a bar graph presenting the data obtained for elimination of methicillin resistant *Staphylococcus aureus* USA300 biofilm after 24 hours of biofilm formation, followed by treatment application.

As can be seen in Figure 12, after 24 hours biofilm formation and treatment application, wounds treated with 100 mg/ml Compound K (6.91 ± 0.25 log CFU/ml) and mupirocin (6.35 ± 0.11 log CFU/ml) showed the lowest *S. aureus* count compared with untreated wounds. In wounds treated with 40 mg/ml Compound K, 10 mg/ml
Compound K and vehicle, counts of $7.46 \pm 0.49 \log \text{CFU/ml}$, $7.58 \pm 0.41 \log \text{CFU/ml}$, and $7.66 \pm 0.60 \log \text{CFU/ml}$, were observed, respectively. The untreated group had an MRSA count of $8.37 \pm 0.47 \log \text{CFU/ml}$. All treatments resulted in fewer bacterial counts than the untreated wounds.

As can further be seen in Figure 12, wounds treated with 100 mg/ml Compound K and mupirocin after 24 hours of biofilm formation showed the largest reduction of wound-associated bacteria compared to untreated wounds. The results indicate that wounds treated with 100 mg/ml Compound K had $(1.46 \pm 0.22 \log \text{CFU/ml})$ reduction of *S. aureus* compared to untreated wounds. These values corresponded to a 96.56% reduction of *S. aureus* associated bacteria count.

Figure 13 is a bar graph presenting the data obtained for elimination of biofilm of methicillin resistant *Staphylococcus aureus* USA300, compared with bacterial count in gauze, after 24 hours of biofilm formation followed by treatment application.

As can be seen in Figure 13, after 24 hours of biofilm formation and treatment application for biofilm elimination, the *S. aureus* count in gauze (green bars) with Compound K and mupirocin (positive control) were lower than bacterial count in the wounds. Bacterial counts from gauze associate with untreated wounds and gauze recovered from wounds treated with vehicle control had a similar higher bacterial count (8.03 and 7.82 log CFU/gauze, respectively) compared to sample treated with Compound K.

Figure 14 is a bar graph presenting the data obtained for inhibition of *Pseudomonas aeruginosa* ATCC 27312 biofilm formation after treatment application.

As can be seen in Figure 14, on day 1 after the first treatment, all treatment groups exhibited reduced bacterial counts compared to untreated wounds. On day 1 after first treatment application, the lowest bacterial count ($4.73 \pm 0.51 \log \text{CFU/ml}$) and ($4.33 \pm 0.54 \log \text{CFU/ml}$) of *P. aeruginosa* was observed in wounds treated with 40 mg/ml Compound K and silver sulfadiazine, respectively, compared with the bacterial counts observed in untreated wounds. Wounds treated with 40 mg/ml Compound K exhibited a reduction of $3.76 \pm 0.15 \log \text{CFU/ml}$ compared with untreated wounds, representing a 99.98% reduction of biofilm-associated *P. aeruginosa* on day 1 when compared to wounds untreated. Bacterial counts observed in wounds treated Compound K followed a dose-response mode going from 40 mg/ml Compound K, to 10
mg/ml Compound K (5.60 ± 0.40 log CFU/ml) when compared with untreated wounds. The P. aeruginosa count was reduced with this treatment in 4.04 ± 0.36 log CFU/ml in treated wounds (99.99 % of reduction). Tests using 10 mg/ml Compound K and 4 mg/ml Compound K resulted in 7.51 ± 0.79 and 7.89 ± 0.12 log CFU/ml of P. aeruginosa in wounds respectively, indicating a dose-response mode. Untreated wounds had the greatest amount of P. aeruginosa log CFU/ml (9.64 ± 0.04).

As can be further seen in Figure 14, wounds treated with silver sulfadiazine (positive control) exhibited the lowest bacterial count at day 1 and 2 recovery times (4.33 ± 0.54 and 5.20 ± 0.62 log CFU/ml, respectively). Similar P. aeruginosa count reduction with silver sulfadiazine was observed on both day 1 and day 2 (4.16 ± 0.19 and 4.44 ± 0.58 log CFU/ml, respectively). These values represent a 99.99 % reduction P. aeruginosa on day 1 and 2 when compared to untreated wounds.

Figure 15 is a bar graph presenting the data obtained for inhibition of Pseudomonas aeruginosa ATCC 27312 biofilm formation after treatment application.

As can be seen in Figure 15, on day 2 after second treatment with Compound K at different concentration, an increase in at least (0.65 log CFU/ml) of bacterial count was observed. Wounds treated with positive control silver sulfadiazine showed an increase of 0.88 log CFU/ml of P. aeruginosa, and P. aeruginosa counts and untreated wounds exhibited an increase of 1.55 log CFU/ml compared day 1 with day 2.

Figures 16A-B are bar graphs presenting the data obtained for Pseudomonas aeruginosa ATCC 27312 biofilm inhibition, compared with bacterial count in gauze, after 1 treatment application (Figure 16A), and biofilm inhibition of Pseudomonas aeruginosa ATCC 27312, compared with bacterial count in gauze, after 2 treatment applications (Figure 16B).
As can be seen in Figures 16A-B, on day 1 and 2 after first and second treatment application for biofilm elimination the *P. aeruginosa* counts in gauze (red bars in Figure 16A and orange bars in Figure 16B) with 40 mg/ml Compound K and silver sulfadiazine were lower than bacterial count in wounds. Similar counts in gauze were observed with the treatment 10 mg/ml and 4 mg/ml Compound K and vehicle on both day 1 and day 2.

Figure 17 is a bar graph presenting the data obtained for elimination of *Pseudomonas aeruginosa* ATCC 27312 biofilm after 24 hours of biofilm formation and treatment application.

As can be seen in Figure 17, wounds colonized with *P. aeruginosa* after 24 hours of biofilm formation, treated once with Compound K at different concentrations and recovered after 24 hours, showed a reduction in *P. aeruginosa* counts compared with untreated wounds. Treatment with 40 mg/ml Compound K resulted in the lowest *P. aeruginosa* count (5.28 ± 0.94 log CFU/ml) compared with untreated wounds (9.10 ± 0.06 log CFU/ml). This was followed by treatment with 4 mg and 10 mg Compound K and silver sulfadiazine (7.12 ± 0.27, 7.62 ± 0.64 and 7.80 ± 0.19 log CFU/ml, respectively). Vehicle and untreated wounds had similar *P. aeruginosa* counts (8.89 ± 0.32 and 9.10 ± 0.06 log CFU/ml, respectively).

As can further be seen in Figure 17, treatment with 40 mg/ml Compound K resulted in a considerable reduction (2.52 ± 0.75 log CFU/ml) of *P. aeruginosa* bacterial biofilm compared to positive control (silver sulfadiazine). *P. aeruginosa* biofilm treated with 40 mg/ml Compound K had a 99.70% of reduction compared with silver sulfadiazine.

Figure 18 is a bar graph presenting the data obtained for elimination of *Pseudomonas aeruginosa* ATCC 27312 biofilm, compared with bacterial count in gauze, after 24 hours of biofilm formation and treatment application.

As can be seen in Figure 18, treatment with 40 mg/ml Compound K after 24 hours biofilm formation showed similar largest reduction of wound-associated bacteria compared to untreated wounds and vehicle control (3.82 ± 0.88 and 3.61 ± 0.62 log CFU/ml). These values corresponded to a 99.98% reduction of *P. aeruginosa*. All treatments had an increase in the bacterial count of *P. aeruginosa* in gauze after 24 hours of biofilm formation and treatment application. Silver sulfadiazine (positive
control) in gauze showed the lowest bacterial count compared with \textit{P. aeruginosa} counts in wounds.

The results in both methicillin resistant \textit{Staphylococcus aureus} USA300 and \textit{Pseudomonas aeruginosa} ATCC27312 with Compound K at higher concentration indicated dose-response characteristics in the \textit{in vivo} study. This study demonstrated that Compound K at different concentrations was able to reduce bacterial counts in the partial thickness wound infection porcine model.

On day 1, wounds treated with 40 mg/ml Compound K showed the lowest bacterial biofilm inhibition count (6.52 ± 0.36 log CFU/ml) of \textit{S. aureus} in wounds. Bacterial reduction was by 1.84 ± 0.09 log CFU/ml compared with untreated wounds. This value corresponded to a 98.54% reduction of biofilm-associated \textit{S. aureus} on day 1.

Wounds assessed on day 2 resulted in a biofilm elimination count of \textit{S. aureus} with 100 mg/ml Compound K of 6.46 ± 0.16 log CFU/ml with a reduction of 1.60 ± 0.04 log CFU/ml compared with untreated wounds. This value represents a 97.47% reduction of \textit{S. aureus} on day 2 when compared to wounds untreated.

On day 2 after second treatment with 100 mg/ml Compound K reduced bacterial count of MRSA USA300 (0.65 log CFU/ml) compared with day 1 after first treatment. Similar bacterial reduction were found with mupirocin (positive control) when this treatment was applied per 2 days (0.71 log CFU/ml).

Wounds treated with 100 mg/ml Compound K and mupirocin after 24 hours biofilm formation showed the largest reduction of \textit{S. aureus} compared to untreated wounds. Results indicate that wounds treated with 100 mg/ml Compound K had (1.46 ± 0.22 log CFU/ml) reduction of \textit{S. aureus} compared to untreated wounds. These values corresponded to a 96.56% reduction of \textit{S. aureus} counts.

Treatment with 40 mg/ml Compound K showed a lowest bacterial count (4.73 ± 0.51 log CFU/ml) of \textit{P. aeruginosa} in wounds compared with untreated wounds. Percentage of reduction with this concentration was 99.98%. \textit{P. aeruginosa} counts after second treatment application with the same 40 mg/ml concentration of Compound K was lowest (5.60 ± 0.40 log CFU/ml) compared with 1 treatment application.

\textit{P. aeruginosa} biofilm elimination in wounds treated with 40 mg/ml Compound K after 24 hours of biofilm formation was 5.28 ± 0.94 log CFU/ml. 40 mg/ml
Compound K showed a considerable reduction in *P. aeruginosa* counts compared with the results obtained in the untreated wounds and wounds treated with silver sulfadiazine (3.82 ± 0.88 and 2.52 ± 0.75 log CFU/ml, respectively). These values corresponded to a 99.98 % and 99.70 % reduction of *P. aeruginosa* after a 24 hours treatment application when compared to untreated wounds or wounds treated with silver sulfadiazine, respectively.

Overall various formulations of Compound K at different concentrations appeared to be effective against both pathogenic bacteria methicillin resistant *Staphylococcus aureus* USA300 and *Pseudomonas aeruginosa* ATCC 27312 colonization. Reducing *P. aeruginosa* and *S. aureus* counts carries important clinical implications for wound treatment and infection prevention.

Reduction and elimination of pathogen biofilms can be even more efficient at other concentrations and/or different treatment regimens of Compound K.

**EXAMPLE 8**

*In Vivo Studies for Compound K and Compound F in combination with non beta-lactam antibiotics*

The antimicrobial effect of Compound K (also referred to as BL 6030) and Compound F (also referred to as BL 6031) described hereinabove was tested on meticillin resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* *in vivo* in swine, similarly to the procedure described in Example 7 hereinabove.

**MATERIALS AND METHODS**

*Experimental Animals:*

Five animals were studied: two (2) animals were inoculated with *meticillin resistant* Staphylococcus aureus (MRSA; *S. aureus*) and three (3) animals with *P. aeruginosa*. The young female specific pathogen free (SPF: Looper Farms, North Carolina) pigs weighing 35-40 kg were kept in-house for at least one week prior to initiating the experiment. The animals were fed a basal diet *ad libitum* and housed in animal facilities (American Association for Accreditation of Laboratory Animal accredited) with controlled temperature (19-21 °C) and lights (12 hour/12 hour LD).
**Animal Preparation:**

Animals were anesthetized with Telazol HC1 (5 mg/kg), Xylazine (0.2 mg/kg), Atropine (0.05 mg/kg) intramuscularly, and inhalation of an isoflurane and oxygen combination. Hair on the back of the pigs was clipped with standard animal clippers. Skin on the back and sides of the animals, surrounding and including the wound area, were prepared by washing with a non-antibiotic soap (Neutrogena®) and sterile water. The animals were blotted dry with sterile gauze.

**Deep Partial Thickness Wound:**

**Wounding:**

Sixty (60) rectangular wounds measuring 10 mm x 7 mm x 0.5 mm deep was made in the paravertebral and thoracic area with a specialized electrokeratome fitted with a 7 mm blade. Punctate bleeding was controlled by pressure with sterile gauze pads. The wounds were separated from one another by 15 mm of unwounded skin. The wounds were inoculated and treated as described in the experimental design hereinbelow.

Wounds were divided into ten (10) groups to animal treated with the tested compound alone or combined with antibiotic, respectively. Immediately after wounding, the wounds were inoculated with the appropriate bacterial strain as described hereinbelow. Wounds were then inoculated and treated as described hereinbelow.

**Wound Inoculation:**

Fresh cultures of methicillin resistant *Staphylococcus aureus* USA300 (for the first pig) and *Pseudomonas aeruginosa* ATCC27312 (for the second pig) were used in this study. The challenge inoculum suspension was prepared by scraping the overnight growth from a culture plate into 5 ml of normal saline, so as to provide a suspension concentration of approximately $10^{10}$ colony forming units/ml (CFU/ml) for each bacteria. Serial dilutions were made until a concentration of $10^6$ CFU/ml was achieved. The inoculum was vortexed and 25 ml of the suspension were inoculated into each wound. In addition, serial dilutions of the suspension were plated onto culture media to quantify the exact concentration of viable organisms used for this experiment.
**Experimental Design:**

Treatments were randomly assigned as follows:

**Design 1** - animal inoculated with *Pseudomonas aeruginosa* (PA) and the 10 wounds were assigned to the following treatment groups:

- A. BL 6031 30% PEG 10 mg/ml
- B. BL 6031 30% PEG 30 mg/ml
- C. BL 6030 30% PEG 20 mg/ml
- D. BL 6030 30% PEG 60 mg/ml
- E. BL 6030 30% PG 20 mg/ml
- F. BL 6030 30% PG 60 mg/ml
- G. Tobramycin 100 µg/ml
- H. Tobramycin 50 µg/ml
- I. Untreated
- J. Positive control (silver sulfadiazine)

Treatment was applied within 30 minutes after inoculation. Assessment of inhibition of biofilm formation (biofilm inhibition) was made 24 hours after treatment.

**Design 2** - animal inoculated with methicillin Resistant *Staphylococcus aureus* (MRSA) and the 10 wounds were assigned to the following treatment groups:

- A. BL 6031 75 mg/ml
- B. BL 6031 37.5 mg/ml
- C. Gentamicin 200 µg/ml
- D. Gentamicin 100 µg/ml
- E. BL 6030 PG 150 mg/ml
- F. BL 6030 PG 75 mg/ml
- G. BL 6030 PEG 150 mg/ml
- H. BL 6030 PEG 75 mg/ml
- I. Positive control (Mupirocin)
- J. Untreated

Treatment was applied within 30 minutes after inoculation for 30 wounds, for assessment of Biofilm inhibition, and the remaining 30 wounds were inoculated and were covered with polyurethane film for 24 hours so as to allow biofilm formation.
Thereafter, treatment was applied and Biofilm elimination was assessed 24 hours following treatment.

**Design 3** - animal inoculated with *Pseudomonas aeruginosa* (PA) and the 10 wounds were assigned to the following treatment groups:

A. BL 6031 PEG 400 30 % 10 mg/ml
B. BL 6031 PEG 400 30 % 30 mg/ml
C. BL 6030 PEG 400 30 % 20 mg/ml
D. BL 6030 PEG 400 30 % 60 mg/ml
E. Tobramycin 200 μg/ml
F. Tobramycin 100 μg/ml
G. Tobramycin 50 μg/ml
H. Vehicle PEG 400 30 %
I. Positive control (silver sulfadiazine)
J. Untreated

Treatment was applied within 30 minutes after inoculation for 30 wounds, for assessment of Biofilm inhibition. The remaining 30 wounds were inoculated and were covered with polyurethane film for 24 hours so as to allow biofilm formation. Treatment was thereafter applied and after 24 hours Biofilm elimination was assessed.

**Design 4** - animal inoculated with methicillin Resistant *Staphylococcus aureus* (MRSA) and the 10 wounds were assigned to the following treatment groups:

A. Gentamicin 25 μg/ml
B. BL 6031 37.5 mg/ml
C. Gentamicin 50μg/ml
D. BL 6031 75 mg/ml
E. Gentamicin 25 μg/ml + BL 6031 37.5 mg/ml
F. Gentamicin 50 μg/ml + BL 6031 75 mg/ml
G. Gentamicin 100 μg/ml
H. Vehicle (30 % PEG)
I. Positive control (Mupirocin)
J. Untreated

Treatment was applied within 30 minutes after inoculation for 30 wounds, for assessment of biofilm inhibition following 24 hours treatment. The remaining 30
wounds were inoculated and were covered with polyurethane film for 24 hours, so as to allow biofilm formation. Treatment was thereafter applied, and biofilm elimination was assessed after 24 hours.

**Design 5** - animal inoculated with *Pseudomonas aeruginosa* (PA) and the 10 wounds were assigned to the following treatment groups:

A. Tobramycin 25 µg/ml  
B. BL 6031 10 mg/ml  
C. Tobramycin 50 µg/ml  
D. BL 6031 30 mg/ml  
E. Tobramycin 25 µg/ml + BL 6031 10 mg/ml  
F. Tobramycin 50 µg/ml + BL 6031 30 mg/ml  
G. Tobramycin 100 µg/ml  
H. Vehicle (30% PEG)  
I. Positive control (Silver Sulfadiazine)  
J. Untreated

Treatment was applied within 30 minutes after inoculation for 30 wounds, for assessment of biofilm inhibition following 24 hours. The remaining 30 wounds were inoculated and were covered with polyurethane film for 24 hours, so as to allow biofilm formation. Treatment was thereafter applied and biofilm elimination was assessed after 24 hours.

**Treatment Regimen:**

Immediately after wounding, the wounds were inoculated with the appropriate bacterial strain as described in hereinbelow. The wounds were divided into 10 groups of six wounds each.

For biofilm formation, all six wounds were treated on day 0 within 30 minutes of inoculation. Three of wounds were recovered as described hereinbelow. The three remaining wounds were treated again with appropriate treatment (see above design) and were recovered on day 2 (after second treatment application).

For biofilm inhibition, three wounds were treated on day 0 within 30 minutes of inoculation. The remaining 3 wounds were left untreated and remained in place for 24 hours to allow formation of a bacterial biofilm in the wounds.
Treatments were applied by adding 1.5 ml up to complete saturation of sterile 2"x2" gauze sponge 4ply (Curity™ Tyco Healthcare Group, Mansfield, MA). Gauze saturation with the treatment helped maintaining the treatment in direct contact with the wounds. Each of saturated gauzes was put on each wound, and was covered with a polyurethane film dressing.

Wounds that were treated with positive controls (Mupirocin or Silver Sulfadiazine) received about 150 - 200 mg preparation, so as to completely cover the wounded area and surrounding normal skin. Positive controls were spread out gently with a sterile Teflon spatula and covered with a sterile 2"x2" gauze sponge 4ply (Curity™ Tyco Healthcare Group, Mansfield, MA) and redressed with a polyurethane film dressing.

Unless otherwise indicated, all tested compounds were formulated using 30 % PEG-400 as a vehicle.

All dressings were covered and secured by wrapping the animal with self-adherent elastic bandages (Coban; 3M, St. Paul, MN). After 24 hours (day 1), the dressings were removed. Three of the wounds were recovered as described hereinbelow. Three wounds were treated again with appropriate treatment and the remaining three wounds were treated to determine Biofilm elimination.

**Recovery Methods:**

Prior to wound recovery, the gauze materials from all three wounds were placed in a sterile 50 ml polyurethane tube. Five (5) ml of all purpose neutralizer solution was added, vortexed and serially diluted for quantitation using spiral plater method described above. After removal of gauze, three wounds for each treatment group were recovered on day 1 after first treatment application (within 30 minutes after inoculation). The remaining three wounds for each treatment group were recovered on day 2 after second treatment application (see, Design 1) or after 24 hours biofilm formation and 24 hours treatment application (see, Designs 2, 3, 4 and 5).

To recover bacteria from the wounds, a sterile surgical steel cylinder (22 mm inside diameter) was placed around the wound area. One (1) ml of all purpose neutralizer solution was drawn into the cylinder and the site was scrubbed with a sterile Teflon spatula for 30 seconds.
Serial dilutions were made from all culture samples and the extent of microbiological contamination assessed using the Spiral Plater System as described hereinabove. Oxacillin Resistance Screening Agar (ORSAB) was used to isolate MRSA USA 300 from the wounds and a selective media for *Pseudomonas aeruginosa* (Pseudomonas Agar with CN supplement) was used to quantify *Pseudomonas aeruginosa* present in the suspension. All plates were incubated aerobically overnight (16-24 hours) at 37 °C, after which the number of viable colonies were counted.

**Observations:**

The terms used to describe swelling and erythema from light to heavy are absent < slight < mild < moderate < marked < exuberant.

Representative photos of wounds were taken during the study. Observations were made on day 1 after 24 hours of first treatment application (wounds assigned to biofilm inhibition) and on day 2 after 24 hours of second treatment application (wounds assigned to biofilm elimination).

During the entire study (in all 5 animals) the gauze materials remained in place except for one incidence where gauze was found partially removed. During removal of the gauze materials slight rewounding was noted.

In the three animals where wounds were inoculated with *Pseudomonas aeruginosa*, erythema or swelling were not observed. In wounds which were inoculated with MRSA USA 300 a slight erythema was observed.

**RESULTS**

After the incubation period, colonies were counted, the data was tabulated and the Log of colony forming units/ml (log CFU/ml) for methicillin resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (PA) determined. The arithmetic mean of the log (CFU/ml) and standard deviation were calculated for each treatment.

**Animal 1 (see, Designed 1) - Biofilm inhibition after treatment application (PA27312):**

The obtained data is presented in Figure 19A, and show that all treatment groups on day 1 reduced bacterial count compared to Untreated (see also Figure 19B). Wounds treated with BL 6030 (in 30 % PG) at a concentration of 20 mg/ml, showed the lowest
bacterial count (5.55 ± 0.62 Log CFU/ml) of PA27312 in wounds. Treatment with BL
6030 30 % PG at a concentration of 20 mg/ml showed a bacterial reduction of 2.67 ±
0.20 Log CFU/ml compared with untreated wounds. This value corresponded to a
99.79 % reduction of PA27312 on day 1 when compared to untreated wounds. Positive
control silver sulfadiazine showed a 6.11 Log CFU/ml of PA27312 in wounds after 24
hours of biofilm inhibition. Tobramycin 100 and 50 µg/ml resulted in 4.40 ± 0.68 and
4.90 ± 0.19 Log CFU/ml of PA27312 in wounds, respectively. Untreated wounds had
the higher Log CFU/ml (8.22 ± 0.43).

As can further be seen in Figure 19A, after second treatment application all
treatment groups showed a reduction in PA27312 counts compared with untreated
wounds. BL 6030 in 30 % PEG at a concentration of 20 mg/ml showed the lowest
PA27312 counts (6.29 ± 0.33 Log CFU/ml). Wounds treated with BL 6030 in 30 %
PEG at a concentration of 20 mg/ml resulted in a reduction of 2.70 ± 0.16 Log CFU/ml
compared with untreated wounds, representing a 99.80 % reduction of PA27312 on day
2 when compared to untreated wounds. The following lower bacterial count in wounds
was exhibited by BL 6030 in 30 % PG at a concentration of 20 mg/ml (6.53 ± 0.30 Log
CFU/ml) and BL 6031 in 30 %PEG at a concentration of 20 mg/ml (6.57 ± 0.18 Log
CFU/ml). All other tested preparations (BL 6031 in 30 % PEG at a concentration of 30
mg/ml, BL 6030 in 30 %PEG at a concentration of 60 mg/ml and BL 6030 in 30 % PG
at a concentration of 60 mg/ml) resulted in similar PA27312 bacterial count (7.06 ±
0.36, 6.89 ± 0.56 and 6.94 ± 0.12 Log CFU/ml, respectively). Untreated wounds
resulted in highest PA27312 count Log CFU/ml (8.99±0.17).

As shown in Figure 19B, wounds treated with Silver sulfadiazine (positive
control) had similar bacterial inhibition counts at day 1 and 2 recovery times (6.11 ±
0.12 and 6.01 ± 0.55 Log CFU/ml, respectively). Tobramycin at 100 and 50 µg/ml
resulted in 5.16 ± 0.49 and 5.79 ± 0.17 Log CFU/ml of PA27312, respectively.

As further shown in Figure 19B, BL 6031 in 30 % PEG at a concentration of 10
mg/ml and BL 6030 in 30 % PEG at a concentration of 20 mg/ml resulted in similar
PA27312 bacteria counts after 2 treatment applications for biofilm inhibition. With the
other tested formulations, bacterial count was increased after the second treatment
application in at least 0.23 Log CFU/ml compared with day 1 after first treatment.
Similar bacterial count increase was observed in wounds treated with Tobramycin at 100 and 50 µg/ml when treatment was applied for 2 days (0.75 and 0.89 Log CFU/ml).

Figures 20A and 20B present the bacterial counts in the treated wounds and in the gauzes after 24 hours (Day 1) from the first treatment (1Rx) (Figure 20A) and after 24 hours (Day 2) from the second treatment (2Rx) (Figure 20B).

As shown in Figure 20A, on day 1 after first treatment application for biofilm inhibition the PA27312 counts in gauze (red bars) were lower than bacterial count in wounds (light blue bars) for all tested compounds, except for BL 6031 in 30 % PEG at a concentration of 10 mg/ml and BL 6030 in 30 % PEG at a concentration of 20 mg/ml.

As shown in Figure 20B, after the second treatment application bacterial recovered from the gauze (orange bars) was lower or similar to bacterial counts in wounds (yellow bar) except for BL 6031 in 30 % PEG at a concentration of 10mg/ml, BL 6030 in 30 %PG at a concentration of 60 mg/ml and Tobramycin 50 µg/ml. Gauze associated PA27312 counts resulted in a lesser PA27312 count with Tobramycin 100 µg/ml.

Animal 2 (see, Design 2) - Biofilm inhibition and elimination after treatment application (MRSA):

Animal 2 Biofilm inhibition after treatment application (MRSA):

As shown in Figure 21A, with all treatment groups on day 1 after first treatment bacterial count was reduced, compared to Untreated. On day 1 after treatment application with BL 6031 75 mg/ml, the lowest bacterial count was observed (5.12 ± 0.19 Log CFU/ml), compared with Untreated wounds. Wounds treated with Gentamicin 200 and 100 µg/ml resulted in lower bacterial counts (1.46 ± 0.28 and 2.94 ± 0.30 Log CFU/ml, respectively). Both concentrations tested for Gentamicin (200 and 100 µg/ml) showed a considerable greater bacterial reduction compared with untreated wounds (6.56 ± 0.26 and 5.08 ± 0.24 Log CFU/ml, respectively), corresponding to a 99.99 % of bacterial reduction. BL 6031 75mg/ml resulted in a reduction of 2.89 ± 0.35 Log CFU/ml compared with untreated wounds, corresponding to 99.87 % reduction of biofilm-associated MRSA on day 1, compared to untreated wounds. Wounds treated with Mupirocin (positive control) had a large reduction in bacterial counts (6.20 ± 0.30 Log CFU/ml) at day 1 compared with untreated wounds (99.99 % of bacterial reduction) 1.
FIG. 2B presents the MRSA counts in gauze (red bars) and in wounds (light blue bars) 24 hours after first treatment, and show that bacterial counts in gauze were lower than bacterial count in wounds, for all tested compounds. The bacterial count in gauze associated for Gentamicin 200 and 100 µg/ml was higher than bacterial counts in wounds. Similar lowers count was observed in wounds or gauze with Mupirocin.

Animal 2, Biofilm elimination after treatment application (MRSA):

As shown in Figure 22A, wounds inoculated with MRSA for 24 hours, and then treated once and recovered after 24 hours showed a reduction in MRSA counts compared with untreated wounds, for all tested compounds. BL 603 1 75 mg/ml resulted in the lowest MRSA count (5.50 ± 0.25 Log CFU/ml) compared with Untreated wounds (9.37 ± 0.51 Log CFU/ml), and the largest reduction of wound-associated bacteria compared to untreated wounds (3.87 ± 0.26 Log CFU/ml), corresponding to a 99.99 % reduction of MRSA. This treatment also resulted in considerable reduction (0.44 Log CFU/ml) of MRSA bacterial biofilm (biofilm elimination) compared to Positive control (Mupirocin).

Figure 22B presents the bacterial count of MRSA in gauze (orange bars) and in wounds (yellow bars) after 24 hours of biofilm formation, following by treatment application. Mupirocin (positive control) in gauze showed the lowest bacterial count compared with MRSA counts in wounds.

Animal 3 (see, Design 3) - Biofilm inhibition and elimination after treatment application (PA27312):

Animal 3, Biofilm inhibition after treatment application (PA27312):

The obtained data is presented in Figures 23A and 23B. As shown in Figure 23A, on day 1 after treatment application within 30 minutes after bacterial inoculation all treatment groups reduced bacterial counts as compared to Untreated. On day 1, wounds treated with BL6031 in PEG-400 30 % at a concentration of 10 mg/ml showed the lowest bacterial count (5.27 ± 0.18 Log CFU/ml) of PA27312, and a reduction of 1.50 ± 0.04 and 2.59 ± 0.04 Log CFU/ml compared with vehicle and untreated wounds, respectively, corresponding to 96.81 % and 99.74 % reduction of biofilm-associated PA27312 on day 1 when compared to wounds treated with vehicle and untreated, respectively. The lower bacterial count in wounds treated with the antibiotic Tobramycin was observed at 200 µg/ml (2.06 ± 0.41 Log CFU/ml). All concentrations
tested for Tobramycin had a 99.99% reduction of PA27312 bacteria counts compared with vehicle and untreated control. Untreated and vehicle wounds had the higher Log CFU/ml (7.86 ± 0.14 and 6.76 ± 0.14).

Figure 23B presents PA27312 counts in gauze (blue bars) and in wounds (green bars) on day 1 after treatment application for biofilm inhibition, showing bacterial counts in gauze were higher than bacterial count in wounds, for all tested compounds except for BL 6031 in PEG-400 30% at a concentration of 10 mg/ml and 20 mg/ml, and vehicle control.

**Animal 3, Biofilm elimination after treatment application (PA27312):**

The obtained data is presented in Figures 24A and 24B. As shown in Figure 24A, after 24 hours biofilm formation and treatment application on day 2, all tested compounds reduced PA count compared with Untreated and vehicle control. Wounds treated with BL 6031 in PEG-400 30% at a concentration of 30 mg/ml and BL 6030 in PEG-400 30% at a concentration of 60 mg/ml and Silver sulfadiazine (positive control).

Considerable PA reductions with Silver sulfadiazine (positive control) were found compared with the vehicle and untreated wounds (1.25 ± 0.23 and 1.95 ± 0.26 Log CFU/ml, respectively, corresponding to 94.38% and 98.89% reduction for PA27312 biofilm associated wounds compared with vehicle and untreated wounds, respectively.

Figure 24B presents PA27312 counts in gauze (violet bars) and in wounds (pink bars) on day 1 after treatment application for biofilm inhibition, showing bacterial counts in gauze were lower than bacterial count in wounds, for all tested compounds except for BL 6031 in PEG-400 30% at a concentration of 10 mg/ml and 20 mg/ml, and vehicle control.
Animal 4 (see, Design 4) - Biofilm inhibition and elimination after treatment application (MRSA):

Animal 4, Biofilm inhibition after treatment application (MRSA):

All treatment groups on day 1 after first treatment reduced bacterial count compared to Untreated and Vehicle. As shown in Figure 25A, on day 1 after treatment application with BL 6031 37.5mg/ml combined with gentamicin 25 µg/ml, the lowest bacterial count (3.93 ± 1.00 Log CFU/ml) of MRSA was observed, compared with Untreated wounds. Gentamicin 50µg/ml + BL6031 75mg/ml and BL 6031 75 mg/ml also showed low bacterial count (5.02 ± 0.49 and 5.24 ± 0.32 Log CFU/ml). Wounds treated with Gentamicin alone at different concentrations (100, 50 and 25µg/ml) resulted in lesser bacterial counts. All these concentrations reduced the bacterial count at 99.99 % compared with untreated wounds. Wounds treated with Mupirocin (positive control) reduced bacterial count (3.22±0.53 Log CFU/ml) at day 1 compared with untreated wounds (99.94 % of bacterial reduction).

Figure 25B presents MRSA counts in gauze (green bars) and in wounds (light blue bars) on day 1 after treatment application for biofilm inhibition, showing bacterial counts in gauze were higher than bacterial count in wounds, for all tested compounds except for BL 6031 at a concentration of 37.5 mg/ml and Gentamicin 50 µg/ml + BL 6031 75 mg/ml.

Animal 4, Biofilm elimination after treatment application (MRSA):

As shown in Figure 26A, after 24 hours biofilm formation and treatment application, all treatments resulted in fewer bacterial counts than the Untreated wounds. Wounds treated with Gentamicin 50 µg/ml + BL 6031 75 mg/ml showed the lowest MRSA count compared with Untreated wounds (5.07 ± 0.76 Log CFU/ml). Similar counts were observed in wounds treated with BL6031 37.5 mg/ml, BL 6031 75 mg/ml, Gentamicin 25µg/ml + BL 6031 37.5mg/ml and Gentamicin 100µg/ml (6.06 ± 0.35 Log CFU/ml), (5.84 ± 0.73 Log CFU/ml), (5.74 ± 0.44 Log CFU/ml) and (5.93 ± 0.11 Log CFU/ml), respectively. These treatments reduced the bacterial counts compared to untreated group between 3.49 - 3.81 Log CFU/ml, corresponding to approximately 99.98 % of bacterial reduction of MRSA associated bacteria biofilm. Untreated wounds had the highest Log CFU/ml (9.55 ± 0.41).
Figure 26B presents MRSA counts in gauze (orange bars) and in wounds (yellow bars) after 24 hours of biofilm formation and treatment application, showed a decrease in bacterial counts in gauze, for all tested compounds. Mupirocin (positive control), Gentamicin 50 µg/ml + BL 6031 75 mg/ml and Gentamicin 100 µg/ml in gauze showed the lowest bacterial count in gauze compared with MRSA counts in wounds (1.73 Log CFU/gauze).

Animal 5 (see, Design 5) - Biofilm inhibition and elimination after treatment application (PA27312):

Animal 5, Biofilm inhibition after treatment application (PA27312):

Wounds treated within 30 minutes after bacterial inoculation were recovered 24 hours after treatment application. Biofilm elimination was observed for all tested compounds. As shown in Figure 27A, bacterial counts demonstrated that all treatment groups assessed on day 1 reduced bacterial population compared to Untreated. On day 1 wounds treated with Tobramycin 50 µg/ml + BL 6031 30 mg/ml showed the lowest bacterial count (4.23 ± 0.33 Log CFU/ml) of PA27312 in wounds, and in a reduction of 2.00 ± 0.12 and 3.60 ± 0.19 Log CFU/ml compared with vehicle and untreated wounds, respectively, corresponding to a 99.00% and 99.97% reduction of biofilm-associated PA27312 on day 1 when compared to wounds treated with treatment vehicle and untreated, respectively. The lower bacterial count in wounds treated Tobramycin was observed at 100 µg/ml (3.17 ± 0.47 Log CFU/ml), followed by 50 and 25 µg/ml (3.70 ± 0.06 and 4.50 ± 0.46 Log CFU/ml, respectively). All concentrations tested for Tobramycin had more than 99.95% of reduction of PA27312 bacteria counts compared with untreated control. Untreated and vehicle wounds had the higher Log CFU/ml recovered (7.83 ± 0.14 and 6.23 ± 0.21).

Wounds treated with Silver sulfadiazine (positive control) were found to be the treatment with the third lowest Log CFU/ml (4.19 ± 0.51).

Figure 27B presents bacterial counts in gauze (light yellow bars) and in wounds (light green bars) on day 1 after treatment application for biofilm inhibition, showing bacterial counts in gauze were higher than bacterial count in wounds, for all tested compounds except for BL6031 37.5 mg/ml and Gentamicin 50 µg/ml + BL 6031 75 mg/ml.
**Animal 5, Biofilm elimination after treatment application (PA27312):**

As shown in Figure 28A, all treatment groups reduced PA27312 counts when compared to untreated group wounds. Wounds treated with Tobramycin 50 µg/ml + BL 6031 30 mg/ml showed the lowest biofilm bacterial count (4.31 ± 0.62 Log CFU/ml) of PA27312 in wounds, and in a biofilm bacterial reduction of 1.37 ± 0.27 and 2.29 ± 0.63 Log CFU/ml compared with vehicle and untreated wounds, respectively, corresponding to 95.73 % and 99.49 % reduction of biofilm-associated PA27312 when compared to wounds treated with vehicle and untreated. Increased concentrations of Tobramycin (from 25 to 50 and from 50 to 100 µg/ml), resulted in a decrease in bacterial biofilm count of PA27312 (0.48 ± 0.14 and 0.62 ± 0.36 Log CFU/ml). Increased concentrations of Tobramycin (from 25 µg/ml to 50 µg/ml) combined with BL 6031 resulted in increase in of bacterial reduction.

Wounds treated with Silver sulfadiazine (positive control) were found to exhibit considerably lower reduction of CFU/ml (5.89 ± 0.33).

Figure 28B presents bacterial counts in gauze (orange bars) and in wounds (yellow bars) after 24 hours of biofilm formation and treatment application for biofilm elimination, showing bacterial counts in gauze were higher than bacterial count in wounds, for all tested compounds.

These studies clearly show the efficacy Compound K and of Compound F, particularly when combined with antibiotics, against pathogenic bacteria. The following can be noted:

Wounds treated with BL 6030 (Compound K) in 30 % PG at 20 mg/ml eliminated biofilm on day 1 and resulted in a low bacterial count (5.55 ± 0.62 Log CFU/ml) of PA27312 in wounds (99.79 % reduction) compared with untreated wounds. Tobramycin 100 µg/ml resulted on day 1 and 2 in a lowest bacterial count (4.40 ± 0.68 and 5.16 ± 0.49 Log CFU/ml, respectively) of PA27312 in wounds.

Wounds inoculated with MRSA and treated with BL 6031 75 mg/ml resulted in low bacterial count for biofilm inhibition and elimination (5.12 ± 0.19 and 5.50 ± 0.25 Log CFU/ml, respectively) of MRSA in wounds when compared with Untreated wounds (99.87 % and 99.99 % of reduction, respectively). Both concentrations tested for Gentamicin (200 and 100 µg/ml) showed a greater bacterial reduction compared
with untreated wounds (6.56 ± 0.26 and 5.08 ± 0.24 Log CFU/ml, respectively; 99.99 % of bacterial reduction).

Wounds inoculated with PA27312 and treated with BL 6030 in PEG-400 30 % at 60 mg/ml resulted in low bacterial count for biofilm inhibition and elimination (5.47 ± 0.42 5.97 ± 0.55 Log CFU/ml, respectively) of PA27312 when compared with Untreated wounds (99.59 % and 98.65 % of reduction, respectively).

On day 1 after treatment application with a combination of BL 6031 at 37.5 mg/ml and Gentamicin at 50 µg/ml, the lowest bacterial count (3.93 ± 1.00 Log CFU/ml) of MRSA in wounds was observed. Wounds treated with Gentamicin alone at different concentrations (100, 50 and 25 µg/ml) resulted in lesser bacterial counts.

Wounds treated with Gentamicin 50µg/ml + BL 6031 75 mg/ml showed the lowest MRSA count compared with Untreated wounds (5.07 ± 0.76 Log CFU/ml).

Wounds treated with a combination of Tobramycin 50 µg/ml + BL 6031 30 mg/ml showed the lowest bacterial count (4.23 ± 0.33 Log CFU/ml) of PA27312 in wounds. Wounds treated with Tobramycin 50 µg/ml + BL 6031 30 mg/ml resulted in a reduction of 2.00 ± 0.12 and 3.60 ± 0.19 Log CFU/ml compared with vehicle and untreated wounds, respectively.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.
WHAT IS CLAIMED IS:

1. A composition comprising a compound having general Formula A':

   \[
   \begin{array}{c}
   \text{R}_30 \text{B} \text{CR}_4 \\
   \text{F} \\
   \text{F}
   \end{array}
   \]

   Formula A'

   wherein \( R_3 \) and \( R_4 \) are each independently selected from the group consisting of hydrogen, alkyl, aryl and cycloalkyl, or, alternatively, \( R_1 \) and \( R_2 \) are joined together so as to form a ring, an antimicrobial agent and a pharmaceutically acceptable carrier.

2. The composition of claim 1, packaged in a packaging material and identified in print, in or on said packaging material, for use in inhibiting a growth of a pathogenic microorganism in or on a substrate.

3. The composition of claim 2, wherein said substrate is a living tissue, the composition being identified for use in the treatment of a medical condition associated with said pathogenic microorganism in a subject comprising said living tissue.

4. The composition of claim 1, packaged in a packaging material and identified in print, in or on said packaging material, for use in reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate.

5. The composition of claim 4, wherein said substrate is a living tissue, the composition being identified for use in the treatment of a medical condition in which reducing or preventing the formation of said biofilm and/or disrupting said biofilm in said living tissue is beneficial.
6. The composition of any of claims 1-5, wherein said compound and said antimicrobial agent act in synergy.

7. A method of inhibiting a growth of a pathogenic microorganism in or on a substrate, the method comprising co-contacting the substrate with an antimicrobial agent and with antimicrobial effective amount of a compound having Formula A':

\[
\begin{array}{c}
\text{R}_3\text{O} \quad \text{B} \quad \text{OR}_4 \\
\text{F} \\
\end{array}
\]

Formula A'

wherein \( \text{R}_3 \) and \( \text{R}_4 \) are each independently selected from the group consisting of hydrogen, alkyl, aryl and cycloalkyl, or, alternatively, \( \text{R}_1 \) and \( \text{R}_2 \) are joined together so as to form a ring, an antibacterial agent and a pharmaceutically acceptable carrier.

8. The method of claim 7, wherein said substrate is a living tissue, the method being for treating a medical condition associated with said pathogenic microorganism in a subject comprising said living tissue.
9. A method of reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate, the method comprising co-contacting the substrate with an antimicrobial agent and an antimicrobial effective amount of a compound having Formula A':

\[
\begin{align*}
\text{Formula A'} & \\
\end{align*}
\]

wherein \( R_3 \) and \( R_4 \) are each independently selected from the group consisting of hydrogen, alkyl, aryl and cycloalkyl, or, alternatively, \( R_i \) and \( R_2 \) are joined together so as to form a ring, an antimicrobial agent and a pharmaceutically acceptable carrier.

10. The method of claim 9, wherein the substrate is a living tissue, the method being for treating a medical condition in which reducing or preventing the formation of said biofilm and/or disrupting said biofilm in said living tissue is beneficial.

11. The method of any of claims 7-10, wherein said compound and said antimicrobial agent act in synergy.
12. Use of a compound having general Formula A':

\[
\text{Formula A'}
\]

wherein \( R_3 \) and \( R_4 \) are each independently selected from the group consisting of hydrogen, alkyl, aryl and cycloalkyl, or, alternatively, \( R_i \) and \( R_j \) are joined together so as to form a ring,

in the manufacture of a product for inhibiting growth of a pathogenic microorganism in or on a substrate, said product further comprising an antimicrobial agent.

13. The use of claim 12, wherein said substrate is a living tissue and said product is a medicament for treating a medical condition associated with said pathogenic microorganism in a subject comprising said living tissue.

14. Use of a compound having general Formula A':

\[
\text{Formula A'}
\]
wherein \( R_3 \) and \( R_4 \) are each independently selected from the group consisting of hydrogen, alkyl, aryl and cycloalkyl, or, alternatively, \( R_1 \) and \( R_2 \) are joined together so as to form a ring,

in the manufacture of a product for reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate, said product further comprising an antimicrobial agent.

15. The use of claim 14, wherein said substrate is a living tissue and said product is a medicament for treating a medical condition in which reducing or preventing the formation of said biofilm and/or disrupting said biofilm in said living tissue is beneficial.

16. The use of any of claims 12-15, wherein said compound and said antimicrobial agent act in synergy.

17. An article comprising a substrate, a compound having general Formula A':

\[
\begin{align*}
\text{Formula A'}
\end{align*}
\]

wherein \( R_3 \) and \( R_4 \) are each independently selected from the group consisting of hydrogen, alkyl, aryl and cycloalkyl, or, alternatively, \( R_1 \) and \( R_2 \) are joined together so as to form a ring,

and an antimicrobial agent, each being incorporated in or on said substrate.
18. The composition, method, use or article of any of claims 1-17, wherein R₃ and R₄ are joined together so as to form a ring.

19. The composition, method, use or article of any of claims 1-18, wherein said ring is selected from the group consisting of 5-, 6-, 7-, 8-, 9- and 10-membered ring.

20. The composition, method, use or article of any of claims 18 and 19, wherein said ring comprises a nitrogen atom.

21. The composition, method, use or article of claim 20, wherein said ring comprises a N-methyldiethylamine moiety.

22. The composition, method, use or article of any of claims 1-17, wherein R₃ and R₄ are each hydrogen.

23. The composition, method, use or article of any of claims 1-22, wherein said antimicrobial agent is an antibacterial agent.

24. The composition, method, use or article of claim 23, wherein said antibacterial agent is a non beta-lactam antibacterial agent.

25. The composition, method, use or article of claim 24, wherein said antibacterial agent is selected from the group consisting of gentamicin and tobramycin.

26. The composition, method, use or article of claim 22, wherein said antimicrobial agent is gentamicin.

27. The composition, method, use or article of claim 22, wherein said antimicrobial agent is tobramycin.
28. An antimicrobial composition comprising a compound having a general Formula A:

\[
\text{Formula A}
\]

wherein \( R_1 \) and \( R_2 \) are each independently selected from the group consisting of alkyl, aryl and cycloalkyl, or, alternatively, \( R_1 \) and \( R_2 \) are joined together so as to form a ring.

29. The composition of claim 28, packaged in a packaging material and identified in print, in or on said packaging material, for use in inhibiting a growth of a pathogenic microorganism in or on a substrate.

30. The composition of claim 29, wherein said substrate is selected from the group consisting of a living tissue and an inanimate object.

31. The composition of claim 29, wherein said substrate is a living tissue, the composition being a pharmaceutical composition which further comprises a pharmaceutically acceptable carrier.

32. The composition of claim 31, packaged in a packaging material and identified in print, in or on said packaging material, for use in the treatment of a medical condition associated with a pathogenic microorganism in a subject comprising said living tissue.

33. The composition of claim 28, being an antibiofilm composition.
34. The composition of claim 33, packaged in a packaging material and identified in print, in or on said packaging material, for use in reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate.

35. The composition of claim 34, wherein said substrate is selected from the group consisting of a living tissue and an inanimate object.

36. The composition of claim 35, wherein said substrate is a living tissue, the composition being identified for use in the treatment of a medical condition in which reducing or preventing the formation of said biofilm and/or disrupting said biofilm in said living tissue is beneficial.

37. The composition of any of claims 29-36, being for use in combination with an additional active agent.

38. The composition of any of claims 28-36, further comprising an additional active agent.

39. A method of inhibiting a growth of a pathogenic microorganism in or on a substrate, the method comprising contacting the substrate with an antimicrobial effective amount of a compound having a general Formula A:

```
R_1O
\underline{\text{B}}\underline{\text{O}}R_2
```

Formula A
wherein \( R_i \) and \( R_2 \) are each independently selected from the group consisting of alkyl, aryl and cycloalkyl, or, alternatively, \( R_i \) and \( R_2 \) are joined together so as to form a ring.

40. The method of claim 39, wherein said substrate is selected from the group consisting of a living tissue and an inanimate object.

41. The method of claim 40, wherein said substrate is a living tissue, the method being for use in the treatment of a medical condition associated with said pathogenic microorganism in a subject comprising said living tissue.

42. A method of reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate, the method comprising contacting the substrate with an antimicrobial effective amount of a compound having general Formula A:

\[
\text{Formula A}
\]

\[
\begin{array}{c}
\text{R}_1 \text{O} \\
\text{B} \\
\text{F} \\
\text{R}_2
\end{array}
\]

wherein \( R_i \) and \( R_2 \) are each independently selected from the group consisting of alkyl, aryl and cycloalkyl, or, alternatively, \( R_i \) and \( R_2 \) are joined together so as to form a ring.

43. The method of claim 42, wherein the substrate is selected from the group consisting of a living tissue and an inanimate object.

44. The method of claim 43, wherein the substrate is a living tissue, the method being for treating a medical condition in which reducing or preventing the
formation of said biofilm and/or disrupting said biofilm in said living tissue is beneficial.

45. The method of any of claims 39-44, further comprising contacting said substrate with an additional active agent.

46. A compound having general Formula A:

\[
\begin{align*}
& \text{R}_1 \text{O} - \text{B} - \text{OR}_2 \\
& \text{F} \\
\end{align*}
\]

Formula A

wherein Ri and R₂ are each independently selected from the group consisting of alkyl, aryl and cycloalkyl, or, alternatively, Ri and R₂ are joined together so as to form a ring, for use in a method of inhibiting a growth of a pathogenic microorganism in or on a substrate.

47. Use of a compound having general Formula A:

\[
\begin{align*}
& \text{R}_1 \text{O} - \text{B} - \text{OR}_2 \\
& \text{F} \\
\end{align*}
\]

Formula A
wherein \( R_i \) and \( R_2 \) are each independently selected from the group consisting of alkyl, aryl and cycloalkyl, or, alternatively, \( R_i \) and \( R_2 \) are joined together so as to form a ring, in the manufacture of a product for inhibiting a growth of a pathogenic microorganism in or on a substrate.

48. The compound of claim 46 or the use of claim 47, wherein said substrate is selected from the group consisting of a living tissue and an inanimate object.

49. The compound of claim 48, wherein said substrate is a living tissue, the compound being for use in the treatment of a medical condition associated with said pathogenic microorganism in a subject comprising said living tissue.

50. The use of claim 48, wherein said substrate is a living tissue and said product is a medicament for treating a medical condition associated with said pathogenic microorganism in a subject comprising said living tissue.

51. A compound having general Formula A:

\[
\begin{align*}
\text{R}_1 \text{O} & \quad \text{B} \quad \text{OR}_2 \\
\end{align*}
\]

wherein \( R_i \) and \( R_2 \) are each independently selected from the group consisting of alkyl, aryl and cycloalkyl, or, alternatively, \( R_i \) and \( R_2 \) are joined together so as to form a ring, for use in a method of reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate.
52. Use of the compound having general Formula A:

\[
\begin{align*}
R_1O & \quad B \quad OR_2 \\
\end{align*}
\]

wherein \( R_1 \) and \( R_2 \) are each independently selected from the group consisting of alkyl, aryl and cycloalkyl, or, alternatively, \( R_1 \) and \( R_2 \) are joined together so as to form a ring,

in the manufacture of a product for reducing or preventing the formation of a biofilm and/or disrupting a biofilm in or on a substrate.

53. The compound or claim 51 or the use of claim 52, wherein said substrate is selected from the group consisting of a living tissue and an inanimate object.

54. The compound of claim 53, wherein said substrate is a living tissue, the compound being for use in a method of treating a medical condition in which reducing or preventing the formation of said biofilm and/or disrupting said biofilm in said living tissue is beneficial.

55. The use of claim 53, wherein said substrate is a living tissue and said product is a medicament for treating a medical condition in which reducing or preventing the formation of said biofilm and/or disrupting said biofilm in said living tissue is beneficial.

56. The compound of any of claims 46, 48, 49, 51, 53 and 54, being for use in combination with an additional active agent.
57. The use of any of claims 47, 48, 50, 52, 53 and 55, wherein said product is for use in combination with an additional active agent.

58. The use of any of claims 47, 48, 50, 52, 53 and 55, wherein said product further comprises an additional active agent.

59. The compound of claim 56 or the use of any of claims 57 and 58, wherein said additional active agent is an antimicrobial agent.

60. An article comprising a substrate and a compound having general Formula A:

\[
\begin{align*}
R_1O & \\
\text{B} & \\
R_2 &
\end{align*}
\]

\[
\text{F}
\]

wherein \( R_1 \) and \( R_2 \) are each independently selected from the group consisting of alkyl, aryl and cycloalkyl, or, alternatively, \( R_1 \) and \( R_2 \) are joined together so as to form a ring, incorporated in or on said substrate.

61. The article of claim 60, further comprising an antimicrobial agent being incorporated in or on said substrate.

62. The compound, method, use or article of any of claims 28-61, wherein \( R_1 \) and \( R_2 \) are joined together so as to form a ring.

63. The compound, method, use or article of claim 62, wherein said ring is selected from the group consisting of 5-, 6-, 7-, 8-, 9- and 10-membered ring.
64. The compound, method, use or article of any of claims 62 and 63, wherein said ring comprises a nitrogen atom.

65. The compound, use, method or article of claim 64, wherein said ring comprises a N-methyldiethyamine moiety.
FIG. 3

S. aureus MRSA USA300 area of Inhibition zone (cm²) with different concentrations of EDP-5.

FIG. 4

Pseudomonas aeruginosa ATCC 273132 area of Inhibition zone (cm²) with different concentrations of EDP-5.
FIG. 5A
Optical density of MRSA USA300 with different concentrations of compound F and K.

FIG. 5B
Optical density of MRSA USA300 with different concentrations of Gentamicin.
FIG. 6A
Optical density of PA27312 with different concentrations of compound F and K.

FIG. 6B
Optical density of PA27312 with different concentrations of Tobramycin.

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FIG. 9

Biofilm inhibition of Staphylococcus aureus (MRSA) USA 300 after treatment application

![Graph showing biofilm inhibition results]

FIG. 10

Biofilm inhibition of Staphylococcus aureus (MRSA) USA 300 after treatment application

![Graph showing biofilm inhibition results]
FIG. 11A

Biofilm inhibition of Staphylococcus aureus (MRSA) USA 300 after 1 treatment application compare with bacterial count in gauze

Log CFU/mL

Day 1 after 1Rx  LogCFU/gauze after 1 Rx application

FIG. 11B

Biofilm inhibition of Staphylococcus aureus (MRSA) USA 300 after 2 treatment application compare with bacterial count in gauze

Log CFU/mL

Day 2 after 2Rx  LogCFU/gauze after 2 Rx application
**FIG. 12**

Biofilm elimination of *Staphylococcus aureus* (MRSA) USA 300 after 24 hours biofilm formation and treatment application.

**FIG. 13**

Biofilm elimination of *Staphylococcus aureus* (MRSA) USA 300 after 24 hours biofilm formation and treatment application compared with bacterial count in gauze.
FIG. 14

Biofilm inhibition of *Pseudomonas aeruginosa* ATCC27312 after treatment application

Day 1 after 1Rx
Day 2 after 2Rx

- EDP-5 40mg/ml
- EDP-5 10mg/ml
- EDP-5 4mg/ml
- Vehicle
- Silver Sulfadiazine
- Untreated

FIG. 15

Biofilm inhibition of *Pseudomonas aeruginosa* ATCC27312 after treatment application

Day 1 after 1Rx
Day 2 after 2Rx

- EDP-5 40mg/ml
- EDP-5 10mg/ml
- EDP-5 4mg/ml
- Vehicle
- Silver Sulfadiazine
- Untreated
FIG. 16A

Biofilm inhibition of *Pseudomonas aeruginosa* ATCC27312 after 1 treatment application compare with bacterial count in gauze

- **Day 1 after 1Rx**
- **LogCFU/gauze after 1 Rx application**

FIG. 16B

Biofilm inhibition of *Pseudomonas aeruginosa* ATCC27312 after 2 treatment application compare with bacterial count in gauze

- **Day 2 after 2Rx**
- **LogCFU/gauze after 2 Rx application**
FIG. 17

Biofilm elimination of *Pseudomonas aeruginosa* ATCC27312 after 24 hours biofilm formation and treatment application.

![Graph showing biofilm elimination comparison](image17.png)

FIG. 18

Biofilm elimination of *Pseudomonas aeruginosa* ATCC27312 after 24 hours biofilm formation and treatment application compared with bacterial count in gauze

![Graph showing biofilm inhibition comparison](image18.png)

LogCFU/gauze Biofilm Inhibition after 1 Rx application
FIG. 19A
Biofilm inhibition of Pseudomonas aeruginosa ATCC27312 after treatment application

Log CFU/ml

Day 1 after 1Rx  Day 2 after 2Rx

BL 6031 30%PEG 10mg/ml  BL 6031 30%PEG 30mg/ml  BL 6030 30%PEG 20mg/ml  BL 6030 30%PEG 60mg/ml
BL 6030 30%PG 20mg/ml  BL 6030 30%PG 60mg/ml  Tobramycin 100μg/ml  Tobramycin 50μg/ml

FIG. 19B
Biofilm inhibition of Pseudomonas aeruginosa ATCC27312 after treatment application

Log CFU/ml

Day 1 after 1Rx  Day 2 after 2Rx

BL 6031 30%PEG 10mg/ml  BL 6031 30%PEG 30mg/ml  BL 6030 30%PEG 20mg/ml  BL 6030 30%PEG 60mg/ml
BL 6030 30%PG 20mg/ml  BL 6030 30%PG 60mg/ml  Tobramycin 100μg/ml  Tobramycin 50μg/ml  Silver  Sulfadiazine

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**FIG. 21A**
Biofilm inhibition of *Staphylococcus aureus* (MRSA) USA 300 after treatment application

**FIG. 21B**

- **Day 1 after 1Rx Biofilm inhibition**
- **LogCFU/gauze after 1 Rx application Biofilm inhibition**

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FIG. 22A
Biofilm elimination of *Staphylococcus aureus* (MRSA) USA 300 after treatment application

FIG. 22B
Biofilm inhibition of *Staphylococcus aureus* (MRSA) USA 300 after 1 treatment application. Biofilm elimination compared with bacterial count in gauze.

- Day 2 after 1Rx Biofilm elimination
- LogCFU/gauze after 1 Rx application Biofilm elimination

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**FIG. 23A**

Biofilm inhibition of *Pseudomonas aeruginosa* ATCC27312 after treatment application

**FIG. 23B**

- Day 1 after 1Rx Biofilm inhibition
- LogCFU/gauze after 1 Rx application Biofilm inhibition
**FIG. 24A**

Biofilm elimination of *Pseudomonas aeruginosa* ATCC27312 after treatment application

**FIG. 24B**

Day 2 after 1Rx Biofilm elimination

LogCFU/gauze after 1 Rx application Biofilm elimination

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**FIG. 25A**

Biofilm inhibition of *Staphylococcus aureus* (MRSA) USA 300 after treatment application.

**FIG. 25B**

Biofilm inhibition of *Staphylococcus aureus* (MRSA) USA 300 after 1 treatment application. Biofilm inhibition compared with bacterial count in gauze.

LOQ = Limit of Quantification

Day 1 after 1Rx Biofilm inhibition.
FIG. 26A
Biofilm elimination of Staphylococcus aureus (MRSA) USA 300 after treatment application

FIG. 26B
Biofilm inhibition of Staphylococcus aureus (MRSA) USA 300 after 1 treatment application. Biofilm elimination compared with bacterial count in gauze.

- Day 2 after 1 Rx Biofilm elimination
- LogCFU/gauze after 1 Rx application Biofilm elimination
FIG. 27A
Biofilm inhibition of Pseudomonas aeruginosa ATCC27312 after treatment application

FIG. 27B

Day 1 after 1Rx Biofilm inhibition
LogCFU/gauze after 1 Rx application Biofilm inhibition
FIG. 28A
Biofilm elimination of *Pseudomonas aeruginosa* ATCC27312 after treatment application

![Bar graph showing biofilm elimination of *Pseudomonas aeruginosa* ATCC27312](image)

FIG. 28B
Biofilm elimination of *Pseudomonas aeruginosa* ATCC27312 after 1 treatment application. Biofilm elimination compared with bacterial count in gauze.

![Bar graph showing biofilm elimination compared with bacterial count in gauze](image)

**Legend:**
- □ Day 2 after 1Rx Biofilm elimination
- □ LogCFU/gauze after 1 Rx application Biofilm elimination

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**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

**INV.** A61K31/69 A61P31/00

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, CHEMABS Data, EMBASE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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abstract
page 57; example 15.8
claims
page 37, paragraph 198

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**X** Further documents are listed in the continuation of Box C.

**X** See patent family annex.

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Date of the actual completion of the international search:

12 June 2012

Date of mailing of the international search report:

19/06/2012

Name and mailing address of the ISA:

European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HJ Rijswijk
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