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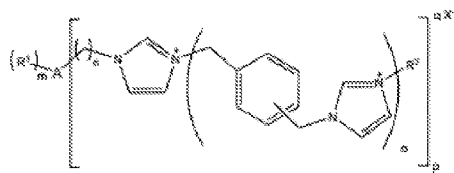
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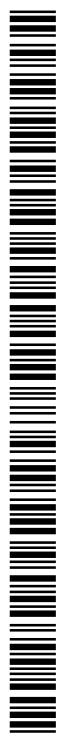
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(54) Title: IMIDAZOLIUM COMPOUNDS, METHODS OF SYNTHESIS AND USES THEREOF



(I)

(57) Abstract: The present invention generally relates to a non-linear imidazolium compound of Formula (I), comprising a planar or stereo core structure. In addition, the present invention relates to the method of preparing the imidazolium compound as disclosed herein. Advantageously, the imidazolium compound of the present disclosure may demonstrate antimicrobial properties. The present invention also relates to the therapeutic and non-therapeutic use of the imidazolium compound as disclosed herein.



Description

Title of Invention: Imidazolium Compounds, Methods of Synthesis and Uses thereof

5 Technical Field

The present invention generally relates to imidazolium compounds, their methods of synthesis and uses thereof. The disclosed compounds may be useful in therapeutic and non-therapeutic applications for killing microbes.

Background Art

10 Infectious diseases and the increasing threat of worldwide pandemics have underscored the importance of antibiotics and hygiene. Small molecular antimicrobial agents, such as triclosan and guanidine derivatives, are commonly used in consumer care products to inhibit microbial growth for preventing infections. However, these antimicrobial agents may exert adverse effects on the

15 human health and environment. For instance, triclosan may lead to abnormal endocrine function in a human being and may be harmful to the human immune system. Moreover, triclosan is found in many consumer products (e.g. soap and detergent) and gets washed down as wastewater into water treatment systems. However, triclosan is not readily eliminated during wastewater treatment. Hence,

20 the disposal of treated water into rivers, lakes and seas may cause disruption to the ecosystem. It is expected that these conventional antimicrobial agents may be banned from use in the consumer products and alternatives would need to be discovered as viable substitutes. Furthermore, many strains of microbes have intrinsic resistance to these small molecular antimicrobial agents. The overuse of

25 these antimicrobial agents has also led to the proliferation of drug-resistant microbes.

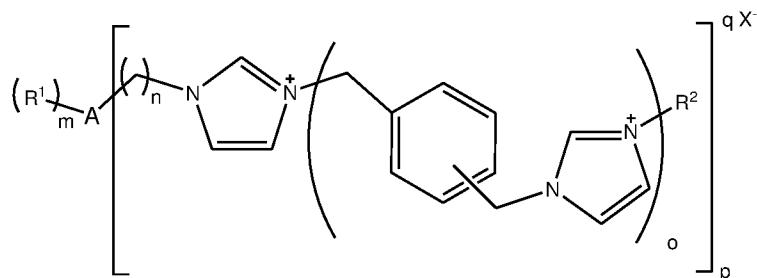
Antimicrobial peptides (AMPs) have attracted attention as an alternative to traditional antibiotics. AMPs have been shown to exhibit a selective membrane-disruptive activity, demonstrating a fast killing mechanism and the potential to address the issue of microbes developing resistance to conventional antibiotics.

5 However, the high cost of manufacture and poor half-lives of AMPs *in vivo* has also limited their applications in healthcare and hygiene.

Therefore, there is a need to provide alternative compounds or compositions that overcome, or at least ameliorate, one or more of the disadvantages described above. There is also a need to provide methods of killing
10 or inhibiting the growth of microorganisms using such compounds or compositions.

Summary of Invention

In one aspect, there is provided a compound of Formula (I),



[Formula (I)]

15 wherein

A is a phenyl ring or an anionic boron;

R¹ is independently selected from hydrogen and an optionally substituted aliphatic group;

R² is independently selected from an optionally substituted aliphatic group that
20 is linear, cyclic, saturated, unsaturated or any combination thereof;

m is an integer of 0-3;

n is an integer of 0-1;

o is an integer of at least 1;

p is an integer of 3-6;

q is an integer representing the number of counterions required for electron neutrality; and

5 X⁻ is an anionic counterion.

The disclosed compounds may be non-linear e.g., planar or may possess a stereochemical structure such as trigonal planar, trigonal pyramidal, T-shaped, tetrahedral, seesaw, square planar, etc. Advantageously, the disclosed compound may be capable of killing bacteria by a membrane-lytic antimicrobial mechanism.

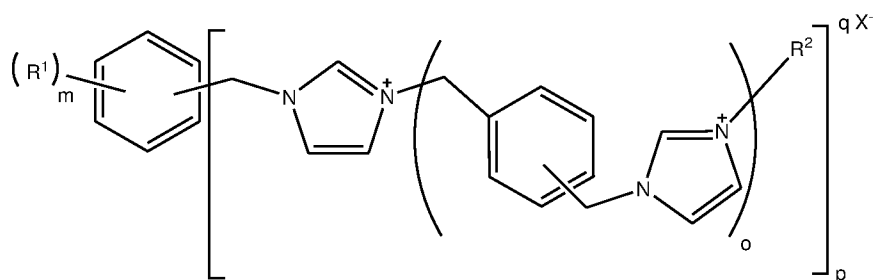
10 Also, advantageously, the disclosed compound may have a broad spectrum of antimicrobial activities against multidrug-resistant bacteria, fungi and biofilms, and be capable of preventing microbes from developing resistance to the drug. In addition, the disclosed compound may be an AMP-mimicking imidazolium linear polymer having antibacterial and/or anti-fungal properties. Further advantageously,

15 the disclosed compound may possess a broad spectrum of antimicrobial effects against Gram-positive, Gram-negative or fungal microorganisms with high selectivity. Additionally, the disclosed compounds may also advantageously exhibit high antimicrobial activity, e.g. having a minimum inhibitory concentration (MIC) of not more than 2000 µg/ml, not more than 1000 µg/ml, not more than 500 µg/ml, not

20 more than 250 µg/ml, not more than 125 µg/ml, not more than 63 µg/ml, not more than 32 µg/ml, not more than 16 µg/ml, not more than 8 µg/ml, or not more than 4 µg/ml against microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. Further advantageously, the disclosed compound may be substantially non-toxic to mammalian cells. Further

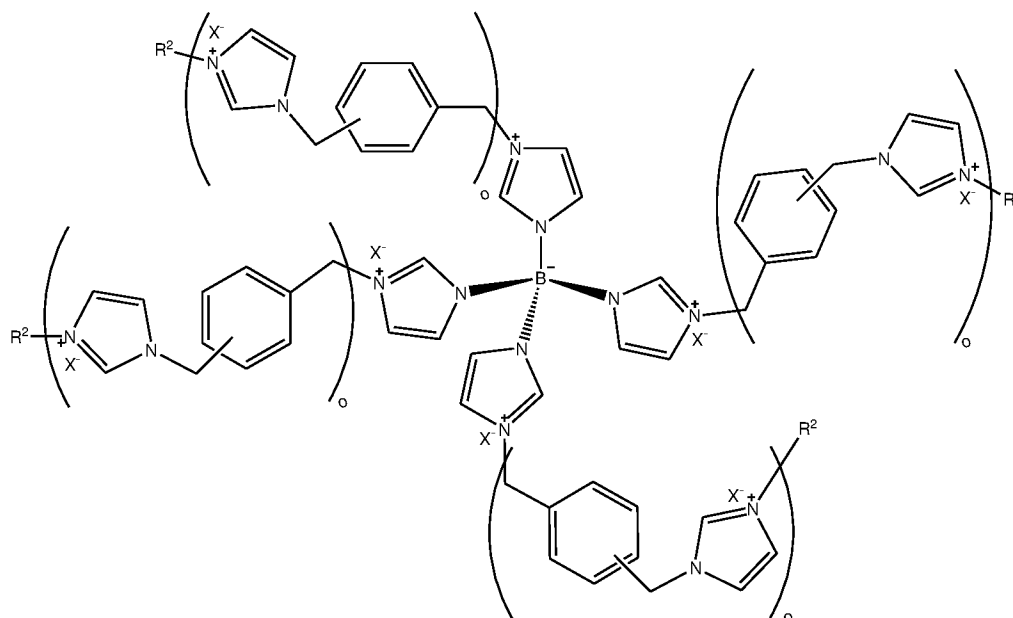
25 advantageously, the disclosed compound may be suitable for *in vivo* application or may be used in an ex-vivo environment for non-therapeutic applications e.g., disinfection or sterilization of surfaces/equipment.

In embodiments, the present disclosure relates to a compound of Formula (IIa)



Formula (IIa).

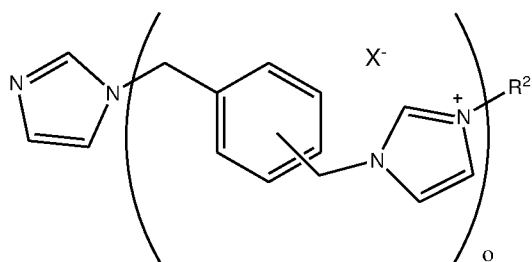
5 In other embodiments, the present disclosure relates to a compound of Formula (IIb)



Formula (IIb).

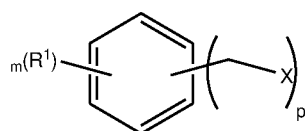
In another aspect, there is provided a method of preparing an imidazolium compound of Formula (IIa) comprising reacting a compound of Formula (2)

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Formula (2)

with a compound of Formula (3)

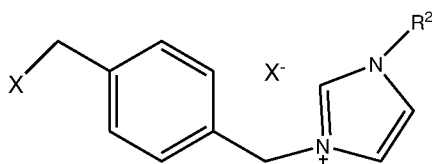


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Formula (3),

wherein R¹, R², m, o, p and X⁻ are as defined herein and X is the neutral form of X⁻.

In another aspect, there is provided a method of preparing an imidazolium compound of Formula (10a) comprising reacting a compound of Formula (10a)



10

Formula (10a),

wherein R² and X⁻ are as defined herein and X is the neutral form of X⁻,
with a sodium tetrakis(1-imidazolyl)borate.

Advantageously, the compound of the present disclosure can be prepared
by the disclosed method. Further, the disclosed method advantageously requires
only simple mixing or stirring at mild conditions, i.e. at temperature lower than or
equal to about 90 °C.

In a further aspect, there is provided a method of killing or inhibiting the growth of a microorganism, comprising contacting said microorganism with an imidazolium compound as defined herein.

In another aspect, there is provided an imidazolium compound as defined
5 herein for use as a medicament.

In yet another aspect, there is provided a use of an imidazolium compound as defined herein in the manufacture of a medicament, for preventing infection.

In another aspect, there is provided a non-therapeutic use of an imidazolium compound as defined herein, for killing a microorganism.

10

Definitions

The following words and terms used herein shall have the meaning indicated:

In the definitions of a number of substituents below it is stated that “the
15 group may be a terminal group or a bridging group”. This is intended to signify that the use of the term is intended to encompass the situation where the group is a linker between two other portions of the molecule as well as where it is a terminal moiety. Using the term alkyl as an example, some publications would use the term “alkylene” for a bridging group and hence in these other publications there is a
20 distinction between the terms “alkyl” (terminal group) and “alkylene” (bridging group). In the present application no such distinction is made and most groups may be either a bridging group or a terminal group.

“Alkyl” as a group or part of a group refers to a straight, linear or branched aliphatic hydrocarbon group, preferably a C₁–C₂₀ alkyl. Examples of suitable

straight and branched C₁ - C₂₀ alkyl substituents include methyl, ethyl, n-propyl, 2-propyl, n-butyl, sec-butyl, t-butyl, n-hexyl, n-octyl and the like. The group may be a terminal group or a bridging group.

A "bond" is a linkage between atoms in a compound or molecule. The bond
5 may be a single bond, a double bond, or a triple bond.

"Halogen" represents chlorine, fluorine, bromine or iodine. The term
"halogenide" when use herein refers to a halogen bearing a negative charge.

"Stereo" compound, as used herein, refers to a molecule wherein the atoms
that make up the molecule are in a three-dimensional, non-linear, spatial
10 arrangement. An example of a suitable stereo compound is one having a trigonal
or a tetrahedral structure.

It is understood that included in the family of compounds of Formula (I) are
isomeric forms including diastereoisomers, enantiomers, tautomers, and
geometrical isomers in "E" or "Z" configurational isomer or a mixture of E and Z
15 isomers. It is also understood that some isomeric forms such as diastereomers,
enantiomers, and geometrical isomers can be separated by physical and/or
chemical methods and by those skilled in the art.

Some compounds of the disclosed embodiments may exist as single
stereoisomers, racemates, and/or mixtures of enantiomers and/or diastereomers.
20 All such single stereoisomers, racemates and mixtures thereof, are intended to be
within the scope of the subject matter described and claimed.

The compounds of the present disclosure when referred to herein are to be
interpreted broadly to include their solvates, hydrates and pharmaceutically
acceptable salts.

The term "pharmaceutically acceptable salts" when used herein, refers to salts that retain the desired biological activity of the above-identified compounds, and include pharmaceutically acceptable acid addition salts and base addition salts. Suitable pharmaceutically acceptable acid addition salts of compounds of Formula (I) may be prepared from an inorganic acid or from an organic acid. Examples of such inorganic acids are hydrochloric, sulfuric, and phosphoric acid. Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, heterocyclic carboxylic and sulfonic classes of organic acids, examples of which are formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, fumaric, maleic, alkyl sulfonic, arylsulfonic. Additional information on pharmaceutically acceptable salts can be found in Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing Co., Easton, PA 1995. In the case of agents that are solids, it is understood by those skilled in the art that the inventive compounds, agents and salts may exist in different crystalline or polymorphic forms, all of which are intended to be within the scope of the present disclosure and specified formulae.

The term "optionally substituted" as used herein means the group to which this term refers may be unsubstituted, or may be substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, thioalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, halo, carboxyl, haloalkyl, haloalkynyl, hydroxyl, alkoxy, thioalkoxy, alkenyloxy, haloalkoxy, haloalkenyloxy, nitro, amino, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroheterocyclyl, alkylamino, dialkylamino, alkenylamine, alkynylamino, acyl, alkenoyl, alkynoyl, acylamino, diacylamino, acyloxy, alkylsulfonyloxy, heterocycloxy, heterocycloamino, haloheterocycloalkyl, alkylsulfenyl, alkylcarbonyloxy, alkylthio, acylthio, phosphorus-containing groups such as phosphono and phosphinyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cyano, cyanate, isocyanate, $-C(O)NH(alkyl)$, and $-C(O)N(alkyl)_2$.

The word "substantially" does not exclude "completely" e.g. a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

5 Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of
10 components of the formulations, typically means +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the stated value, more typically, +/- 2% of the stated value, even more typically +/- 1% of the stated value, and even more typically +/- 0.5% of the stated value.

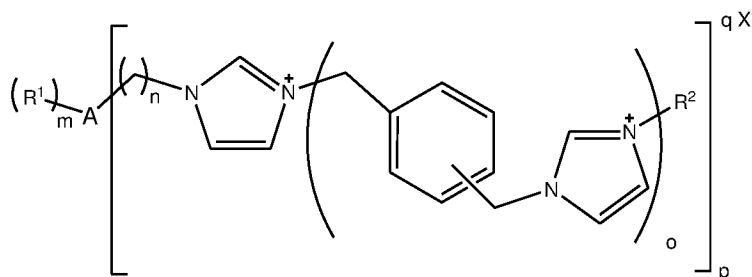
Throughout this disclosure, certain embodiments may be disclosed in a
15 range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a
20 range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges 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.

Certain embodiments may also be described broadly and generically herein.
25 Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the disclosure. This includes the generic description of

the embodiments with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Detailed Disclosure of Embodiments

5 The present disclosure relates to a compound having the following Formula (I):



Formula (I)

wherein A may be selected from a phenyl ring or an anionic boron; R¹ may be independently selected from hydrogen and an optionally substituted aliphatic group, R² may be independently selected from an optionally substituted aliphatic group that is linear, cyclic, saturated, unsaturated or any combination thereof; m may be an integer of 0-3; n may be an integer of 0-1; o may be an integer of at least 1; p may be an integer of 3-6; q may be an integer signifying the number of counterions required for electron neutrality and X⁻ may be an anionic counterion.

R¹ may be independently selected from hydrogen and an optionally substituted C₁-C₁₀alkyl, or an optionally substituted C₁-C₈alkyl, or an optionally substituted C₁-C₆alkyl, or an optionally substituted C₁-C₄alkyl. In one embodiment, R¹ may be linear. Also, R¹ may be selected from methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl. R¹ may be a methyl group or an ethyl group. In one embodiment, R¹ may be hydrogen.

In one embodiment, where A is phenyl, the disclosed compounds may have an R¹ group selected from hydrogen, or a short chain alkyl having 1 to 6 carbon atoms, 1 to 4 carbon atoms, or 1 to 3 carbon atoms. Advantageously, it has been observed that such compounds may possess superior microbial killing properties (e.g., a reduction of bacteria count by up to a factor of 10,000 over 24 hours).

R² may be a hydrophobic moiety independently selected from an optionally substituted aliphatic group that is linear. R² may be an optionally substituted C₁-C₁₆alkyl, or an optionally substituted C₃-C₁₆alkyl, or an optionally substituted C₅-C₁₆alkyl, or an optionally substituted C₅-C₁₄alkyl, or an optionally substituted C₅-C₁₂alkyl, or an optionally substituted C₅-C₁₀alkyl, or an optionally substituted C₅-C₈alkyl, or an optionally substituted C₆-C₈alkyl. R² may be a hexyl group or an octyl group.

Each occurrence of R² may be the same or different. In one embodiment, each occurrence of R² is independently selected from an aliphatic C₆₋₁₂ alkyl. In another embodiment, each occurrence of R² is independently selected from an aliphatic C₆₋₈ alkyl. In another embodiment, when p is 3-4, each occurrence of R² is an octyl group. Advantageously, compounds of the present invention may express a degree of hydrophobicity sufficient to cause perturbation or disruption of a lipid membrane, while expressing little to no hemolytic effects. This allows the compounds to act as effective antimicrobials while remain non-toxic to mammalian cells.

In one embodiment of the compounds disclosed herein, wherein A is phenyl, each occurrence of the phenyl ring that is not A is independently ortho-substituted or para-substituted.

In another embodiment, wherein A is phenyl, each occurrence of the phenyl ring that is not A is independently para-substituted. In another embodiment, wherein A is phenyl, each occurrence of the phenyl ring that is not A is independently ortho-substituted. Advantageously, the compounds of the present invention may possess a MIC of not more than 250 $\mu\text{g/ml}$, not more than 125 $\mu\text{g/ml}$, not more than 63 $\mu\text{g/ml}$, not more than 32 $\mu\text{g/ml}$, not more than 16 $\mu\text{g/ml}$, not more than 8 $\mu\text{g/ml}$ or not more than 4 $\mu\text{g/ml}$ against microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*

The substituent A, wherein A is a phenyl ring, each occurrence of the phenyl ring that is not A may be independently substituted at the meta-position, ortho-position, para-position or any combination thereof. In some embodiments, wherein when p is 3, the phenyl ring may be substituted in an alternating substitution pattern, i.e. every substituent containing the imidazolium moiety may be placed in meta position to each other on the phenyl ring. In another embodiment, wherein A is phenyl and p is 3, each occurrence of the phenyl ring that is not A is independently para-substituted. Advantageously, the compounds of the present invention may be non-hemolytic even when used at a concentration of about 2000 $\mu\text{g/ml}$.

In embodiments, wherein A is an anionic boron, m may be an integer of 0. In other embodiments, wherein A is an anionic boron, n may be an integer of 0. In further embodiments, wherein A is an anionic boron, p may be an integer of 4. Advantageously, the compounds of the present disclosure may demonstrate rapid microbe killing wherein total loss of bacterial cell viability (e.g. *E. coli*) was observed within 6 hours after exposure to the compounds.

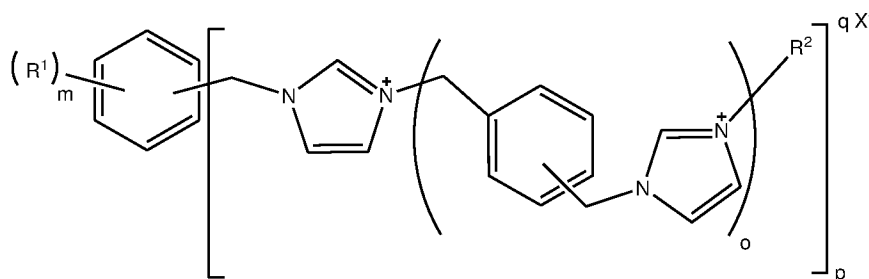
The integers m and p may be selected as valency permits, i.e. the sum of m and p in embodiments, wherein A is an anionic boron may not exceed 4 and in embodiments, wherein A is phenyl, the sum of m and p may not exceed 6.

q may be an integer signifying the number of counterions required for electron neutrality. In embodiments, wherein A is an anionic boron, the number of counterions required may therefore be calculated as follows: $q = [(\text{the integer value of } o + 1) \times \text{the integer value of } p] - 1$. In embodiments, wherein A is a phenyl, the number of counterions required may therefore be calculated as follows: $q = (\text{the integer value of } o + 1) \times \text{the integer value of } p$. In embodiments, the integer p may be 3 or 4 where A is phenyl.

X^- may be an anionic counterion, wherein X^- may be halogenide, carbonate, phosphate, nitrate, sulfate, carboxylate or any combination thereof. X^- may be a halogenide, selected from the group consisting of fluoride, chloride, bromide and iodide. In an embodiment, X^- may be bromide or chloride.

In some embodiments, A may be phenyl and n may be 1.

Accordingly, in some embodiments, there is provided a compound having the following Formula (IIa):

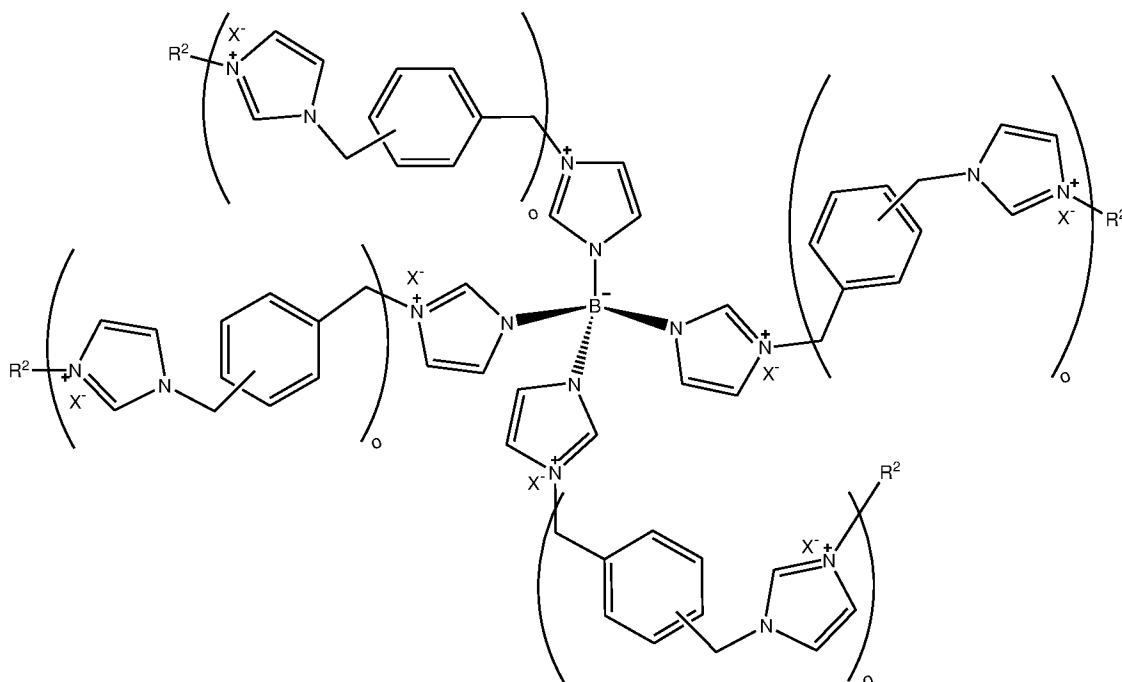


Formula (IIa),

wherein R^1 , R^2 , m, o, p, q and X^- may be as defined above.

In a preferred embodiment of compounds described with Formula (IIa), p is selected from an integer value 3 and 6. When p is 3, m may be 3. When p is 6, m may be 0.

In other embodiments, A may be an anionic boron, m and n may be 0 and p may be 4. Accordingly, in other embodiments, there is provided a compound having the following Formula (IIb):



Formula (IIb),

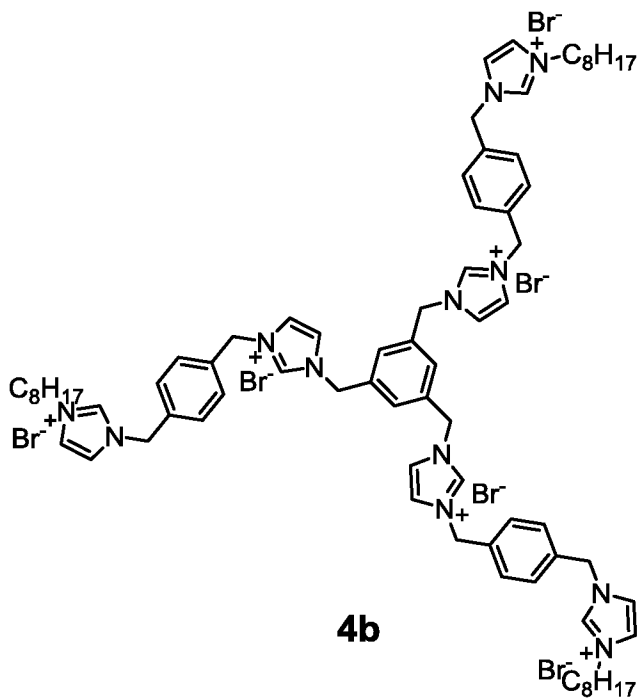
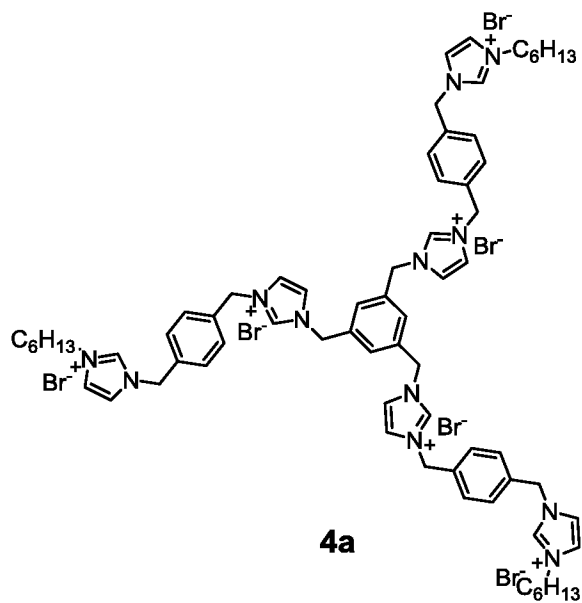
wherein R^2 and o may be as defined above.

Each occurrence of the phenyl group may be independently meta-, ortho-, or para-substituted. In an embodiment, the phenyl group may be para-substituted.

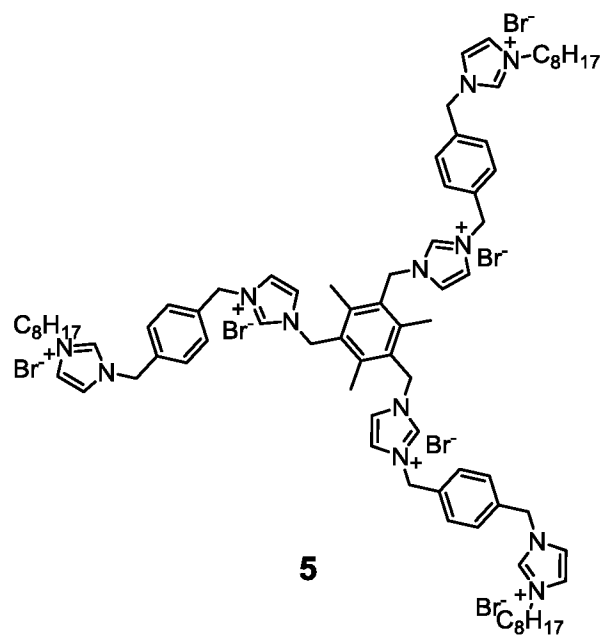
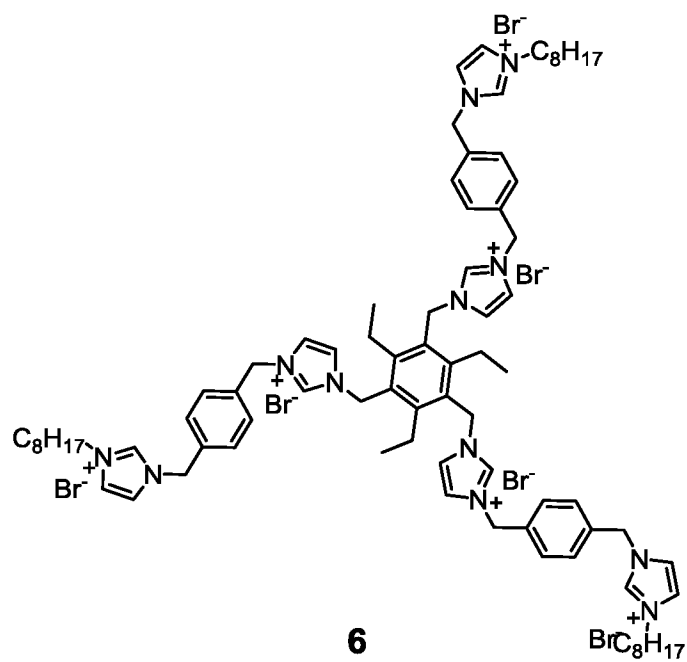
In embodiments of Formula (IIb), wherein the four substituents of the anionic boron are different, the compounds may exist as single stereoisomers, racemates, and/or mixtures of enantiomers. All such single stereoisomers,

racemates and mixtures thereof, are intended to be within the scope of the subject matter described and claimed.

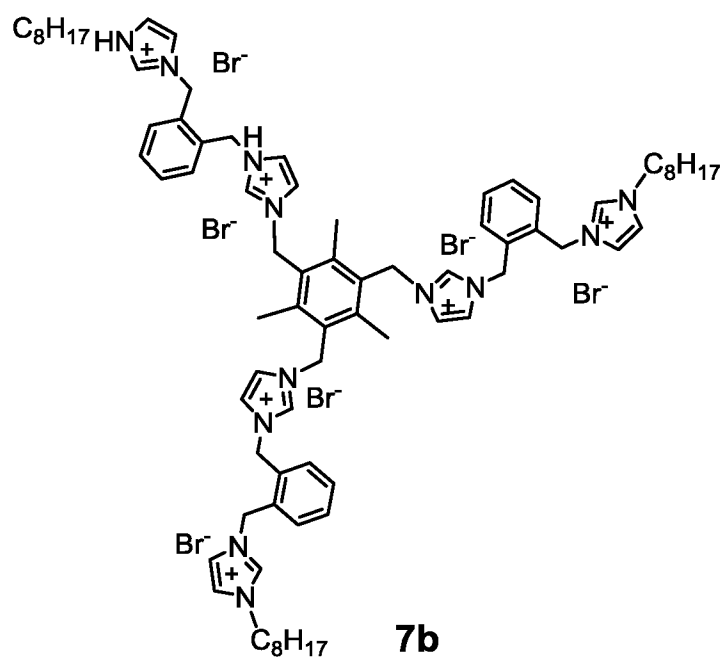
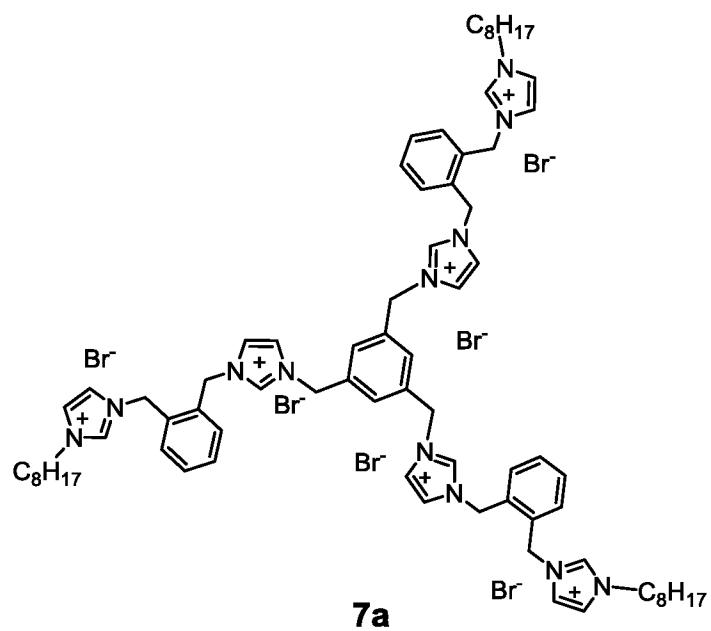
The compounds as disclosed above may be selected from the following compounds:



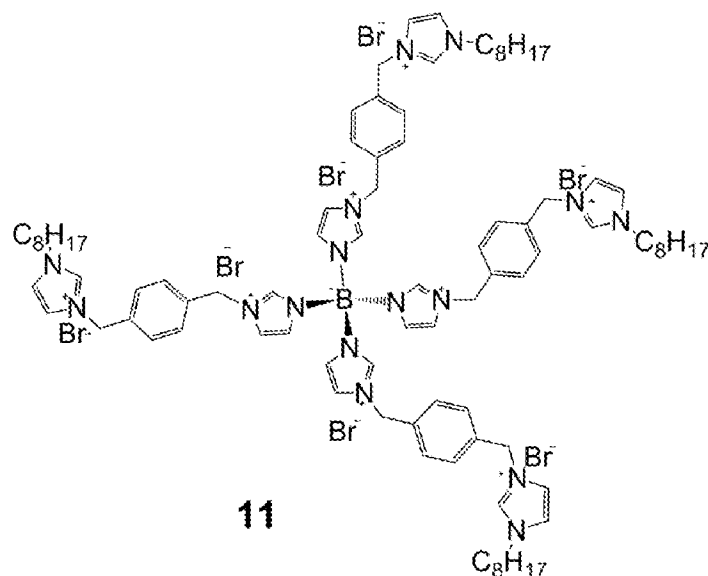
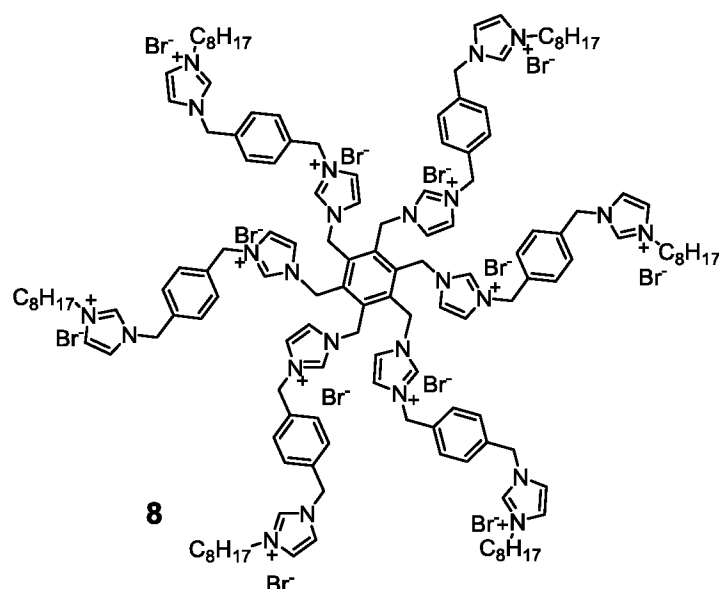
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**5****6**

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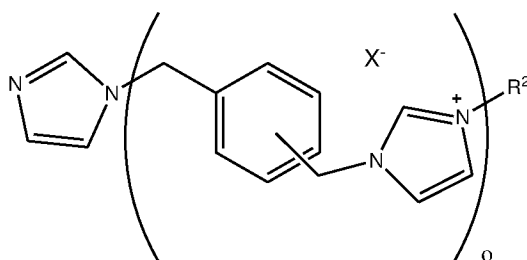
The compounds **(4)** to **(8)** may possess a common planar benzene core
 5 with three or six arms. Each arm may contain two imidazolium units that may be
 linked by *o*-xylene or *p*-xylene and ends with a linear alkyl chain ($-\text{C}_6\text{H}_{13}$ or $-\text{C}_8\text{H}_{17}$).
 In addition, a stereo imidazolium compound **(11)** may have a pyramid $\text{B}(\text{Im})_4$ core
 and four imidazolium arms. Each arm may also end with a linear alkyl chain ($-\text{C}_8\text{H}_{17}$).

In an embodiment, the compound may be amphiphilic. The amphiphilicity may be due to the presence of both a hydrophilic group (quaternary ammonium salts) and lipophilic group (amphiphilic group). Advantageously, the compound as defined above may be amphiphilic because the hydrophilic groups are in the core and the hydrophobic groups are in the shell of the core-shell type structure of the molecule.

In an embodiment, the disclosed compounds may comprise a planar or a stereo core structure. In one embodiment, the disclosed compounds do not exhibit a linear structure.

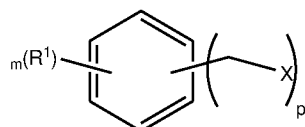
Surprisingly, the planar and stereo compounds of the present disclosure may be particularly effective in killing or inhibiting the growth of specific bacterial species, such as *Staphylococcus aureus*, compared to other types of bacteria. The disclosed compounds may also demonstrate superior activity against *Staphylococcus aureus* relative to linear, amphiphilic imidazolium compounds. In addition, the disclosed planar and stereo imidazolium compounds are easier to prepare than their linear counterparts. For instance, the disclosed planar and stereo imidazolium compounds may be prepared under mild temperature conditions of less than 100 °C, and may not require the use of complex reagents and/or catalysts.

The present disclosure also relates to a method for synthesizing a compound having the structure of Formula (IIa) as disclosed herein, comprising reacting a compound of Formula (2),



Formula (2)

with a compound of Formula (3),

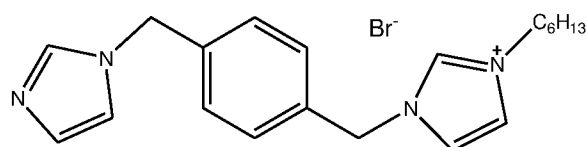


Formula (3)

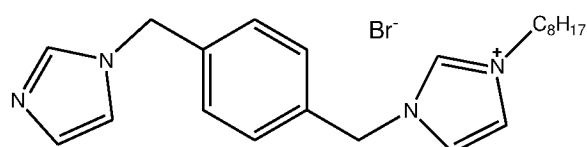
- 5 wherein R^1 , R^2 , m , p , o and X^- may be as defined above and X is the neutral form of X^- . The phenyl ring between the two imidazolium moieties of Formula (2) may be substituted at the meta-position, ortho-position or para-position. Preferably, the phenyl ring between the two imidazolium moieties of Formula (2) may be substituted at the ortho- or para- position. In an embodiment, m is 0 or 3. In
- 10 another embodiment, p is 3 or 6. When p is 3, the arm with the X substituent and the arm with the R^1 substituent may be substituted in alternating substitution pattern. R^1 may be a methyl group or an ethyl group. R^2 may be a hexyl group or an octyl group.

Formula (2) may be selected from the group consisting of Formula (2a), (2b)

- 15 and (2c).

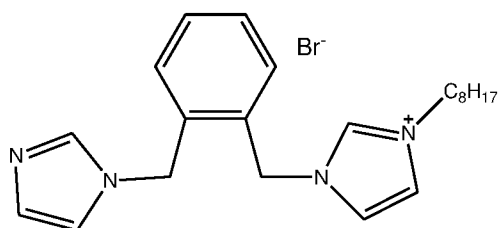


Formula (2a)



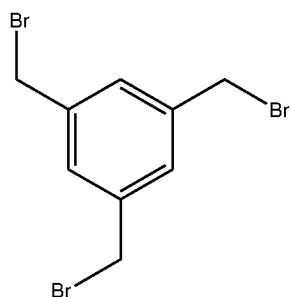
21

Formula (2b)

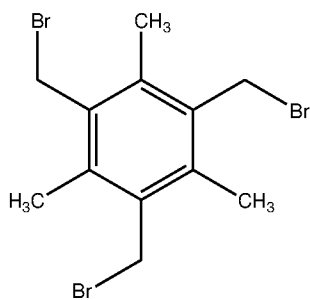


Formula (2c)

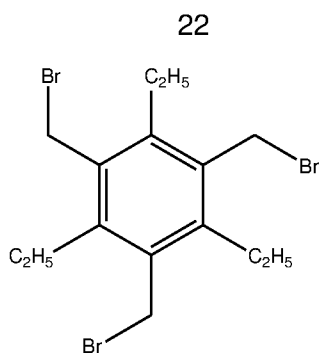
Formula (3) may be selected from the group consisting of Formula (3a),
 5 (3b), (3c) and (3d).



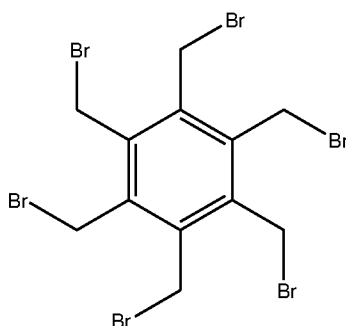
Formula (3a)



Formula (3b)

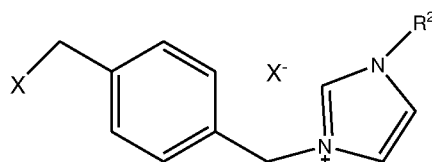


Formula (3c)



Formula (3d)

- 5 The present disclosure also relates to a method for synthesizing a compound having the structure of Formula (IIb) as disclosed above, comprising reacting a compound of Formula (10a) with a sodium tetrakis(1-imidazolyl)borate.

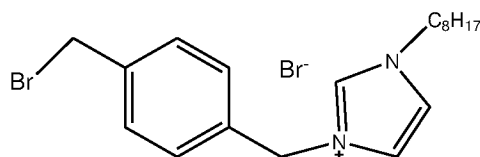


Formula (10a)

- 10 wherein R^2 and X may be as defined above.

In a preferred embodiment, Formula (10a) may be Formula (10).

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Formula (10)

The method of synthesis of Formula (IIa) or (IIb) may take place via an organic solvent. The organic solvent may be selected from the group comprising tetrahydrofuran (THF), dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). In a preferred embodiment, the organic solvent may be DMF. Advantageously, it has been found that the provision of DMF solvent provides improved yield on the compounds of Formula (4), (4a), (5), (6), (7a), (7b), (8) and (11).

The reaction may take place at a temperature of about 150 °C, 120 °C, 110 °C, 100 °C, 90 °C, 80 °C, 70 °C, 60 °C, 50 °C, 40°C, 30°C or 20°C. The reaction may be at a temperature range comprising an upper and lower limit selected from any two of the above values. Preferably, the reaction mixture may be heated up to 90 °C. Also preferably, the reaction mixture may be heated up to 40 °C.

The reaction may take place at the temperature defined above for at least 12 hour, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 24 hours (1 day), 48 hours (2 days) or 72 hours (3 days). Preferably, the reaction mixture may be heated to the desired temperature for at least 15 hours. Also preferably, the reaction mixture may be heated to the desired temperature for at least 2 days.

The reaction mixture may then be allowed to cooled. The cooled reaction mixture may be centrifuged and the solution decanted. The precipitate may then be washed with an organic solvent and further purified by re-precipitation from another organic solvent.

Advantageously, the compounds as described above may be an antimicrobial peptide (AMP)-mimicking imidazolium main-chain polymer that has antibiotic characteristics. More advantageously, the AMP-mimicking imidazolium polymer may exhibit selective membrane-disruptive activity, demonstrating a fast
5 killing mechanism and the potential to deal with drug-resistance issues usually associated with conventional antibiotics.

More advantageously, the hydrophilic groups in the core and the hydrophobic groups in the shell of the core-shell structure of the compound as defined above may be modified to possess antimicrobial activities while preventing
10 hemolysis.

The present disclosure also relates to a method of killing or inhibiting the growth of a microorganism, comprising contacting said microorganism with an imidazolium compound as disclosed herein. In embodiments, the contacting step may be performed on an inanimate surface. Further, the present disclosure relates
15 to non-therapeutic use of an imidazolium compound as defined herein, for killing a microorganism.

The present disclosure also relates to an imidazolium compound as defined herein for use as a medicament. The present disclosure also relates to the use of an imidazolium compound as defined herein in the manufacture of a medicament,
20 for preventing or treating infection, particularly bacterial infection.

The microorganism may be selected the group consisting of Gram-positive, Gram-negative and fungal microorganisms. The microorganism may be selected from the group consisting of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*.

Advantageously, the compounds as described above exhibit antimicrobial activity against the microorganism as defined above. The compounds defined above may possess antibacterial effect against any one of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*.

5 More advantageously, the longer the hydrophobic alkyl chain in the lipophilic shell, the higher the antimicrobial activity. In one embodiment, there is provided the use of any one of compounds of Formula (4b), (5), (6), (7a), (7b), (8) and (11) as an antibacterial against *Staphylococcus aureus*. In a preferred embodiment, there is provided the use of any one of compounds of Formula (4b),
10 (5), (6), (7a) and (7b) as an antibacterial against *Staphylococcus aureus*.

Advantageously, the presence of *ortho*-xylylene linker resulted in higher antimicrobial activity than *para*-xylylene linker. Accordingly, in another embodiment, there is provided the use of any one of compounds of Formula (4b), (5), (6), (7a), (7b), (8) and (11) as an antibacterial against *Escherichia coli*. In a
15 preferred embodiment, there is provided the use of any one of compounds of Formula (4b), (5), (6), (7a), (7b) and (11) as an antibacterial against *Escherichia coli*. In a more preferred embodiment, there is provided the use of a compound of Formula (7a) as an antibacterial against *Escherichia coli*.

The compounds of Formula (4b), (5), (6), (7a), (7b), (8) and (11) may be an
20 effective antibacterial agent against *Pseudomonas aeruginosa*. In a preferred embodiment, the compounds of Formula (6), (7a), (7b) and (11) may be an effective antibacterial agent against *Pseudomonas aeruginosa*. In a more preferred embodiment, the compound of Formula (8) may be an effective antibacterial agent against *Pseudomonas aeruginosa*.

Advantageously, stereo compounds may be faster than planar compounds in killing bacteria. In particular, compound (11) exhibits the fastest speed in killing the *Escherichia coli* compared to compounds (4b), (5) and (6).

Advantageously, no significant hemolysis may be observed for compounds (4a), (4b), (5) and (6) even at concentration of 200 µg/ml.

Brief Description of Drawings

The accompanying drawings illustrate a disclosed embodiment and serves to explain the principles of the disclosed embodiment. It is to be understood, however, that the drawings are designed for purposes of illustration only, and not as a definition of the limits of the invention.

Fig. 1

[Fig. 1] shows the SEM images of *E. coli* after being treated with 62.5 µg/ml of each of the planar imidazolium compounds 4b, 5 and 6 and stereo compound 11 at 37 °C for 3 h. The cell wall was disrupted after exposure to the imidazolium compounds of the present disclosure. Bacteria treated with Tryptic Soy broth (TSB) without the imidazolium compounds were used as control. Scale bars represent 1 µm.

Fig. 2

[Fig. 2] shows the graph of colony formation units (CFU) of *E. coli* after being treated with 62.5 µg/ml of each of the planar imidazolium compounds 4b, 5, 6 and 8 and stereo compound 11 at 37 °C for 3 h. The cell wall was disrupted after exposure. Bacteria treated with TSB without the imidazolium compounds were used as control. Scale bars represent 1 µm.

Fig. 3

[Fig. 3] shows the graph of the percentage hemolysis caused by the planar imidazolium compounds **4a**, **4b**, **5(Br)**, **5(Cl)**, **6**, **7a**, **7b** and **8** and stereo compound **11**.

5 Examples

Non-limiting examples of the invention will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

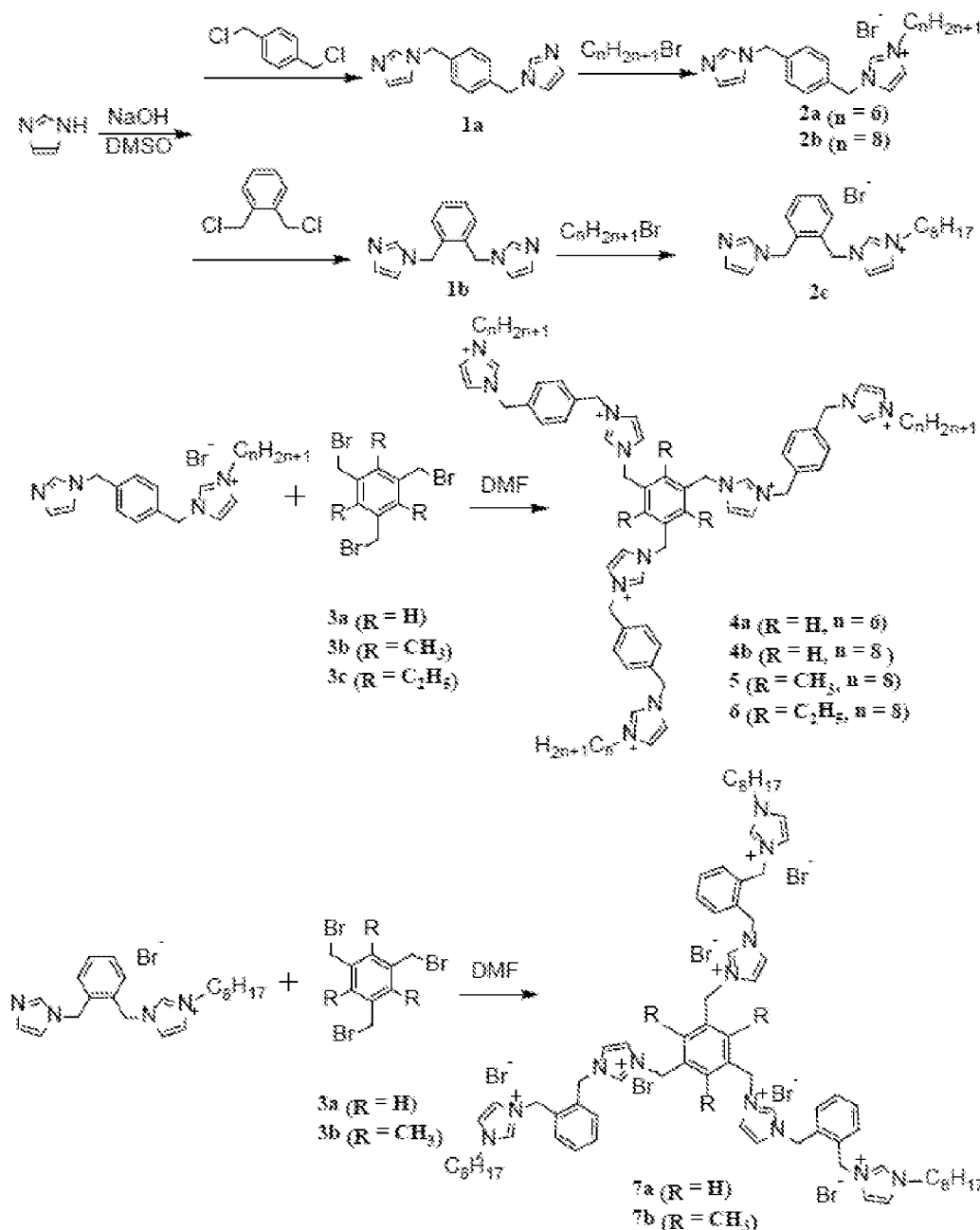
Example 1 – Synthesis of imidazolium compounds**10 Example 1.1 – Materials and Characterization**

All solvents and chemicals were used as obtained from commercial suppliers, unless otherwise indicated. Triton-X was obtained from Aldrich. PBS buffer was used in all experiments.

Nuclear magnetic resonance (NMR) spectra were obtained using a Bruker
15 AV-400 (400 MHz) spectrometer. Chemical shifts were reported in ppm from tetramethylsilane with the solvent resonance as the internal standard. ESI-TOF-MS spectra were obtained from a Bruker MicroTOF-Q system. The samples were directly injected into the chamber at 20 $\mu\text{L}\cdot\text{min}^{-1}$. Typical instrument parameters: capillary voltage, 4 kV; nebulizer, 0.4 bars; dry gas, 2 $\text{L}\cdot\text{min}^{-1}$ at 120 $^{\circ}\text{C}$; m/z range,
20 40 – 3000.

Example 1.2 – Synthesis of 1a and 1b intermediates

The overview of the synthesis of procedure of planar imidazolium compounds (**4a**), (**4b**), (**5**), (**6**), (**7a**) and (**7b**) is as follows.



Synthesis of **1,4-bis(N-imidazole-1-ylmethyl)benzene (1a)**: A mixture of imidazole (0.9 g, 13.0 mmol) and sodium hydroxide (0.5 g, 12.5 mmol) in DMSO (5 mL) was heated to 90 °C for 2 hours and then cooled to room temperature. A solution of α, α' -dichloro-p-xylene (0.99 g, 5.7 mmol) in DMSO (10 mL) was added to the mixture and heated slowly to 40 °C for 1 hour with constant stirring. The solution obtained was poured into ice-cold water (40 mL). The precipitate was collected, washed with water, and recrystallized from methanol/water to give 1,4-

bis(N-imidazole-1-ylmethyl)benzene (**1a**) as a white solid (0.95 g, 79 %). ¹H NMR (CDCl₃): δ 7.55 (s, 2H), 7.13 (s, 4H), 7.10 (s, 2H), 6.89 (s, 2H), 5.12 (s, 4H). MS (GC-MS) m/z 238 (M⁺).

A similar procedure was used for the synthesis of **1,2-bis(N-imidazole-1-ylmethyl)benzene (1b)**, with a solution of α,α'-dichloro-o-xylene instead of α,α'-dichloro-p-xylene. ¹H NMR (CDCl₃): δ 7.45 (s, 2H), 7.38 (d, 2H), 7.12 (s, 2H), 7.08 (d, 2H), 6.80 (s, 2H), 5.03 (s, 4H). MS (GC-MS) m/z 238 (M⁺).

Example 1.3 – Synthesis of 2a, 2b and 2c intermediates

Synthesis of **2a**: A mixture of **1,4-bis(N-imidazole-1-ylmethyl)benzene (1a)** (10.0 mmol, 2.4 g) and 1-bromohexane (9.8 mmol, 1.4 mL) was stirred in THF (15 mL) at 70 °C. After overnight stirring, the precipitate was washed 2 times with THF and 3 times with diethyl ether (1.98 g, 50 %). ¹H NMR (DMSO-d₆): δ 9.43 (s, 1H), 7.85 (m, 3H), 7.42 (d, 2H), 7.30 (d, 2H), 7.19 (s, 1H), 6.87 (s, 1H), 5.45 (s, 2H), 5.22 (s, 2H), 4.18 (m, 2H), 1.77 (m, 2H), 1.23 (m, 6H), 0.83 (t, 3H).

A similar procedure was used to synthesize compounds **(2b)** and **(2c)**.

2b: ¹H NMR (MeOD): δ 9.21 (s, 1H), 7.76 (s, 1H), 7.65 (d, 2H), 7.45 (d, 2H), 7.34 (d, 2H), 7.12 (s, 1H), 6.98 (s, 1H), 5.43 (s, 2H), 5.27 (s, 2H), 4.23 (m, 2H), 1.90 (m, 2H), 1.32 (b, 10H), 0.89 (t, 3H).

2c: ¹H NMR (DMSO-d₆): δ 9.32 (s, 1H), 7.90 (m, 1H), 7.85 (s, 1H), 7.75 (t, 1H), 7.42 (m, 2H), 7.33 (m, 1H), 7.17 (m, 1H), 7.09 (m, 1H), 6.99 (m, 1H), 5.65 (s, 2H), 5.47 (s, 2H), 4.21 (m, 2H), 1.80 (m, 2H), 1.25 (m, 10H), 0.86 (t, 3H).

Example 1.4 – Synthesis of planar imidazolium compounds 4a, 4b, 5, 6, 7a and 7b

Synthesis of **4a**: A mixture of (**2a**) (2.35 g, 3.3 mmol) and (**3a**) (357 mg, 1.0 mmol) in DMF (30 mL) was stirred at 90 °C for 15 h. After cooling down, the reaction mixture was centrifuged and the solution decanted. The precipitate was washed with DMF thoroughly and further purified by re-precipitation from methanol.

5 (**4a**) was obtained as white solid (1.46 g, 93%). ¹H NMR (DMSO-d₆): δ 9.82 (s, 3H), 9.53 (s, 3H), 7.88-7.98 (m, 12H), 7.69 (s, 3H), 7.53-7.61 (m, 12H), 5.51-5.58 (m, 18H), 4.20 (t, 6H), 1.80 (m, 6H), 1.26 (m, 18H), 0.85 (t, 9H).

Synthesis of **4b**: A mixture of (**2b**) (4.10 g, 8.3 mmol) and (**3a**) (892 mg, 2.5 mmol) in DMF (50 mL) was stirred at 90 °C for 15 h. After cooling down, the reaction mixture was centrifuged and the solution decanted. The precipitate was washed with DMF thoroughly and further purified by re-precipitation from methanol.

10 (**4b**) was obtained as white solid (3.74 g, 91%). ¹H NMR (DMSO-d₆): δ 9.80 (s, 3H), 9.54 (s, 3H), 7.87-7.98 (m, 12H), 7.70 (s, 3H), 7.53-7.61 (m, 12H), 5.52-5.58 (m, 18H), 4.21 (t, 6H), 1.80 (m, 6H), 1.25 (m, 30H), 0.86 (t, 9H).

15 The similar procedure was used to synthesize compounds (**5**), (**6**), (**7a**) and (**7b**).

5: ¹H NMR (DMSO-d₆): δ 9.66 (s, 3H), 9.55 (s, 3H), 7.86-7.91 (m, 12H), 7.50-7.57 (m, 12H), 5.51-5.62 (M, 18H), 4.20 (t, 6H), 2.31 (s, 9H), 1.80 (m, 6H), 1.25 (m, 30H), 0.85 (t, 9H).

20 **6**: ¹H NMR (DMSO-d₆): δ 9.78 (s, 3H), 9.54 (s, 3H), 7.86-8.00 (m, 12H), 7.49-7.58 (m, 12H), 5.50-5.63 (M, 18H), 4.19 (t, 6H), 2.62 (m, 6H), 1.80 (m, 6H), 1.25 (m, 30H), 0.86 (t, 18H).

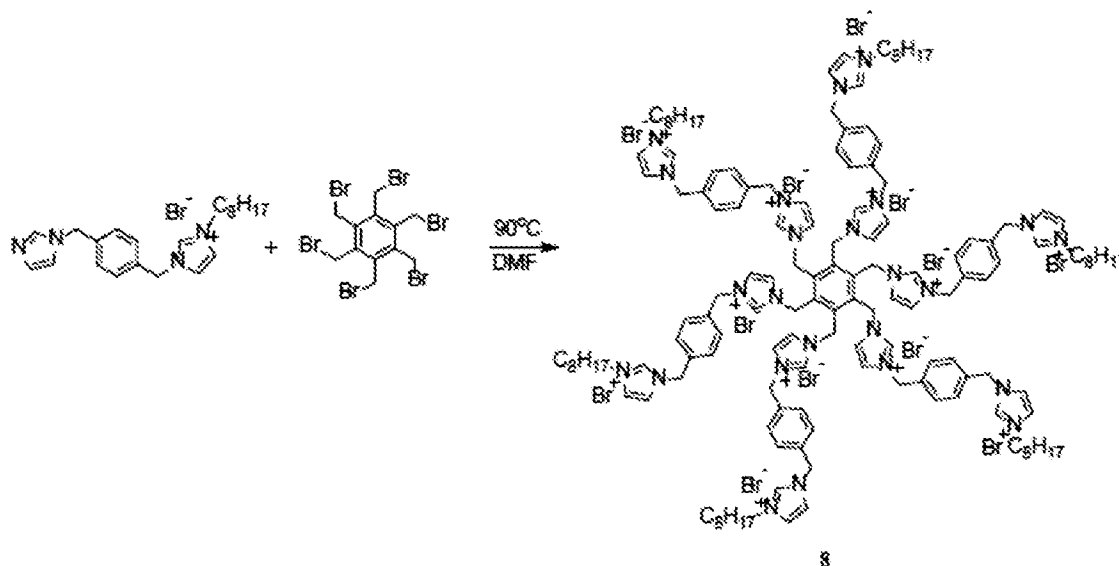
7a: ¹H NMR (DMSO-d₆): δ 9.74 (s, 3H), 9.55 (s, 3H), 7.88-7.92 (m, 9H), 7.75 (s, 3H), 7.32-7.49 (m, 12H), 5.82 (s, 6H), 5.79 (s, 6H), 5.59 (s, 6H), 4.23 (t, 6H), 1.82 (m, 6H), 1.26 (m, 30H), 0.86 (t, 9H).

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7b: ¹H NMR (DMSO-d₆): δ 9.66 (s, 3H), 9.48 (s, 3H), 7.95 (s, 3H), 7.90 (s, 3H), 7.87 (s, 3H), 7.80 (s, 3H), 7.74 (m, 6H), 7.24 (m, 6H), 5.79 (s, 6H), 5.73 (s, 6H), 5.58 (s, 6H), 4.20 (t, 6H), 1.80 (m, 6H), 1.24 (m, 30H), 0.85 (t, 18H).

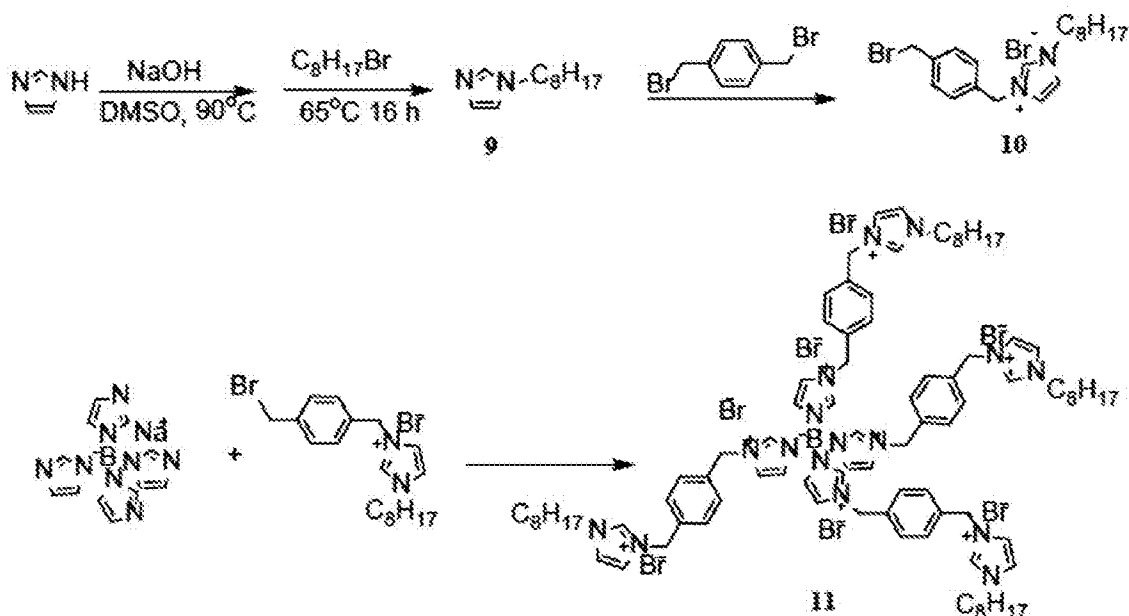
Example 1.5 – Synthesis of planar imidazolium compound 8



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Synthesis of **8**: A mixture of (**2b**) (3.82 g, 6.6 mmol) and 1,2,3,4,5,6-hexakis(bromomethyl)benzene (636 mg, 1.0 mmol) in DMF (50 mL) was stirred at 90 °C for 15 h. After cooling down, the reaction mixture was centrifuged and the solution decanted. The precipitate was washed with DMF thoroughly and further purified by re-precipitation from methanol. **8** was obtained as white solid (2.97 g, 92%). ¹H NMR (DMSO-d₆): δ 9.74 (s, 6H), 9.61 (s, 6H), 7.94 (s, 6H), 7.88 (s, 6H), 7.79 (s, 6H), 7.63 (d, 12H), 7.52 (d, 12H), 7.35 (s, 6H), 5.89 (s, 12H), 5.52 (d, 12H), 4.21 (t, 12H), 1.81 (m, 12H), 1.25 (m, 60H), 0.86 (t, 18H).

Example 1.6 – Synthesis of stereo imidazolium compound 11



Synthesis of **9**: A mixture of imidazole (1.36 g, 20.0 mmol) and sodium hydroxide (0.80 g, 20.0 mmol) in DMSO was heated to 90 °C for 2 h, and then cooled to room temperature. A solution of 1-bromooctane (3.46 g, 19.0 mmol) in DMSO was added dropwise to the mixture. After stirring at room temperature for 3 h, the mixture was heated up slowly to 65 °C for 16 h with constant stirring. The solution obtained was mixed with water and the product was extracted 4 times with diethyl ether. The diethyl ether phases were combined and dried with sodium sulfate. Diethyl ether was removed under vacuum and the intermediate (**9**) was obtained as yellow liquid (2.89 g, 89 %). ¹H NMR (DMSO-d₆): δ 7.61 (s, 2H), 7.15 (s, 1H), 6.87 (s, 1H), 3.93 (t, 2H), 1.68 (m, 2H), 1.25 (m, 10H), 0.85 (t, 3H).

Synthesis of **10**: A mixture of (**9**) (1.80 g 10.0 mmol,) and α,α'-dibromo-p-xylene (13.20 g, 50.0 mmol) was stirred in THF (150 mL) at 70 °C. After overnight stirring, the precipitate was washed with diethyl ether 3 times and then dried under vacuum (2.5 g, 56 %). ¹H NMR (DMSO-d₆): δ 9.43 (s, 1H), 7.87 (m, 2H), 7.42 (d, 2H), 7.30 (d, 2H), 7.19 (s, 1H), 6.87 (s, 1H), 5.45 (s, 2H), 5.22 (s, 2H), 4.18 (m, 2H), 1.77 (m, 2H), 1.23 (m, 6H), 0.83 (t, 3H).

Synthesis of **11**: A mixture of (**10**) (2.67 g, 6.0 mmol) and sodium tetrakis(1-imidazolyl) borate (0.30 g, 1.0 mmol) in DMF (50 mL) was stirred at 40 °C for 2 days. After cooling down, DMF was removed under vacuum and the solid was washed with acetone 3 times. Stereo imidazolium compound (**11**) was obtained as white solid (1.27 g, yield 64%). ¹H NMR (DMSO-d₆): δ 9.56 (s, 4H), 9.36 (s, 4H), 7.84-7.95 (m, 16H), 7.52-7.63 (m, 16H), 5.51 (d, 16H), 4.20 (t, 8H), 1.80 (m, 8H), 1.24 (m, 40H), 0.85 (t, 12H). MS (ESI) m/z: 908.34 (calcd. 908.31 for [M-2Br]²⁺).

Example 2 – Antimicrobial Studies

Example 2.1 – Minimum Inhibitory Concentration

Staphylococcus aureus (ATCC 6538, Gram-positive), *Escherichia coli* (ATCC 8739, Gram-negative), *Pseudomonas aeruginosa* (Gram-negative), and *Candida albicans* (ATCC 10231, fungus) were used as representative microorganisms to evaluate the antimicrobial activity of the imidazolium compounds synthesized according to Example 1. All bacteria and fungus were stored frozen at -80 °C. Bacteria were grown overnight at 37 °C in Tryptic Soy broth (TSB) prior to experiments. Fungus was grown overnight at 22 °C in Yeast Mold (YM) broth. Subsamples of these cultures were grown for a further 3 h and diluted to give an optical density value of 0.07 at 600 nm, corresponding to 3 × 10⁸ CFU mL⁻¹ (McFarland' Standard 1).

The imidazolium compounds of the present disclosure were dissolved in TSB at a concentration of 4 mg mL⁻¹ and the minimal inhibitory concentrations (MICs) were determined by microdilution assay. Typically, 100 µL of microbial solution (containing 3 × 10⁸ cells mL⁻¹) was added to 100 µL of TSB or YM broth containing the test imidazolium compounds (normally ranging from 2 µg mL⁻¹ to 2 mg mL⁻¹ in serial two-fold dilutions) in each well of the 96-well microtiter plate. The plates were incubated at 37 °C for 24 h with shaking at 300 rpm. For *C. albicans*,

the incubation was at room temperature for 48 h. The MICs were taken as the concentration of the antimicrobial imidazolium compound at which no microbial growth was observed with the microplate reader. Broth solution containing microbial cells alone was used as negative control. Experiments were run in quadruplicates.

Table 1. Antimicrobial activities (MIC) of the imidazolium compounds of the present disclosure. Values are shown in $\mu\text{g mL}^{-1}$.

	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
4a	32	250	2000	63
4b	4	16	500	63
5	4	16	1000	63
6	4	16	250	63
7a	4	8	250	63
7b	4	16	250	63
8	16	62.5	62.5	125
11	8	16	125	63

The MIC values against four different and clinically relevant microbes of all the eight imidazolium compounds are presented in Table 1. All the synthesized planar and stereo compounds exhibit antimicrobial activity against the tested microbes. Compound (**4b**) has shown a higher antimicrobial activity than compound (**4a**), which may be due to the more hydrophobic *n*-octyl chain at the terminal end compared to the *n*-hexyl chain. The long aliphatic ending group triggers a self-assembly process, facilitating the interaction between the compound and the cell membrane, therefore enhancing antimicrobial activity.

The MICs of compounds (**7a**) and (**7b**) against *P. aeruginosa* are lower than that of compounds **4b** and **5**, respectively. It is postulated that the *ortho*-xylylene

linker may be more suitable for the formation of amphiphilic topology, thus enhancing the ability of polymers to bind to the lipid membrane, leading to membrane perturbation and eventually cell death. Compound **8** showed a relatively weak activity against, which may be due to its low solubility in water and rigid structure.

Example 2.2 – Morphology of Microbes

The morphology of the microbes was observed using a field emission SEM (JEOL JSM-7400F) operated at an accelerating voltage of 5 keV. Samples containing *E. coli* cells (3×10^8 CFU/ml), with and without imidazolium compounds (62.5 ppm), were grown in TSB for 3 h. The mixtures were collected and centrifuged at 5000 rpm for 6 min. The precipitate was washed with PBS buffer and then fixed with paraformaldehyde (2.5% in PBS) for 3 h. Subsequently, the precipitate was washed with DI water twice. Dehydration of the samples was performed using a series of ethanol/water solution (35%, 50%, 75%, 90%, 95% and 100%). The dehydrated samples were mounted on copper tape. After drying for 2 days, the samples were coated with platinum for imaging with JEOL JSM-7400F (Japan) field emission SEM.

The morphological changes of bacteria after being treated with imidazolium compounds (**4b**), (**5**), (**6**), and (**11**) were as shown in Figure 1. The cell wall of *E. coli* was disrupted and subsequently dissolved after 3 h exposure the imidazolium compounds.

Example 2.3 – Time-kill Studies

B. subtilis and *E. coli* were grown overnight in TSB at 37 °C. The cells were diluted to $2 \sim 5 \times 10^8$ CFU/mL and a 100 µL of this suspension was then added to TSB broth or broth containing 125 ppm of imidazolium compounds, respectively.

Sampling aliquots were withdrawn from cultures at 1, 3, 6 and 24 h after the addition of imidazolium compounds. The aliquots were plated on solid Lysogeny broth (LB) plates and were incubated at 37 °C overnight. Subsequently, the colony number was counted. Data from duplicate plate was collected and the mean of the colony counts were taken. The resulting values were plotted on a log scale against time.

Table 2. Log reduction of *E. coli* after being treated with 62.5 µg/ml of the planar and stereo compounds for 1h, 3h, 6h and 24h compared to the control sample. TSB media without tested compounds was used as control.

Compounds	1 h	3 h	6 h	24 h
4b	>1	>4	>8	>8
5	<1	>2	>6	8
6	<1	2	>6	8
8	<1	>1	>5	3
11	>1	>5	>8	>8

The killing kinetics of the planar and stereo compounds against *E. coli* were studied. Table 2 above displays the reduction in the colony count after being subjected to treatment with the planar and stereo imidazolium compounds of the present disclosure. The colony formation units of *E. coli* after being treated with the compounds at 62.5 µg/ml for various periods were as shown in Figure 2. Although compounds **(4b)**, **(5)**, **(6)** and **(11)** have the same MIC against *E. coli*, the speed at which bacteria were killed are different. The stereo compound **(11)** showed the fastest killing speed compared to the other planar compounds. Total loss of cell viability was observed within 6 h after exposure. Among the planar compounds **(4b)**, **(5)**, **(6)** and **(8)**, **(4b)** has the fastest killing speed. The substituents on the benzene core affect the flexibility of the molecule, resulting in the different killing efficiencies.

Example 2.4 – Hemolysis Studies

For biomedical applications, the biocompatibility of the compounds with blood is important. Hemolysis assay, which examines the hemoglobin release due to rupturing of red blood cells, is a commonly used method to evaluate the toxicity of antimicrobial compounds. Fresh rat red blood cells (RBCs) were diluted with PBS buffer to give a RBC stock suspension (4 vol% blood cells). 100 μ L aliquot of RBC stock was added to a 96-well plate containing 100 μ L of the imidazolium compounds stock solutions of various concentrations (obtained from serial 2-fold dilution in PBS). After incubating for 1 h at 37°C, The content of each well was pipetted into a microcentrifuge tube and then centrifuged at 4000 rpm for 5 min. Hemolytic activity was determined as a function of hemoglobin release by measuring the OD₅₇₆ of 100 μ L of the supernatant. A control solution which contains only PBS was used as a reference for 0% hemolysis. 100% hemolysis was measured by adding 0.5% Triton-X to the RBCs.

$$\text{Hemolysis}(\%) = \frac{OD_{576 \text{ polymer}} - OD_{576 \text{ blank}}}{OD_{576 \text{ Triton-X } 100} - OD_{576 \text{ blank}}} \times 100$$

Figure 3 shows the percentage hemolysis caused by planar and stereo compounds. The best non-hemolytic properties were found for compounds **(4a)**, **(4b)**, **(5)** and **(6)**. No significant hemolysis was observed even at 2000 μ g/ml, the highest concentration tested. These compounds are primarily qualified as active and nontoxic compounds. In contrast, compound **(8)** has shown to cause serious hemoglobin leakage from RBCs at 125 μ g/ml. This may be because compound **(8)** has six arms and six *octyl* ending groups, resulting in the highest hydrophobicity as compared to the other planar compounds with three arms and three terminal alkyl groups. It has been reported that biomolecules of higher hydrophobicity are

generally more potent as a biocide, but are concurrently more toxic. However, compound **(8)** exhibited less potency against various microbes which may due to its highly crowded core structure.

In relation to compounds **(7a)** and **(7b)**, with *ortho*-xylylene linkers, these
5 compounds have shown remarkably high hemolytic activity while compounds **(4b)**, **(5)** and **(6)**, with *para*-xylylene linkers, are non-hemolytic. In the structures of **(7a)** and **(7b)** with *ortho*-xylylene linkers, all six imidazolium units may tend to localize into the core part. In comparison, the three exterior imidazolium units of compounds **(4b)**, **(5)** and **(6)** (with *para*-xylylene linkers) are stretched out and the
10 positive charges on these molecules are more delocalized from the core.

In conclusion, a series of imidazolium compounds with planar and stereo core structure has been designed and synthesized. These compounds have symmetric structures with different cores, tails and linkers. In vitro assays have demonstrated a desirable set of bioactivities against four types of clinically relevant
15 microbes.

Industrial Applicability

The imidazolium compounds of the present disclosure may be useful in both therapeutic and non-therapeutic applications. For instance, the imidazolium compounds may be used in healthcare, hygiene and consumer care products to
20 provide antimicrobial or disinfecting properties.

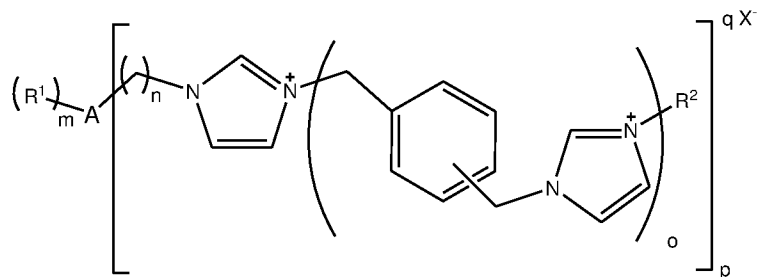
Furthermore, the imidazolium compounds may also be used in the medical field. The compounds of the present disclosure may be used in the preparation of a medicament for preventing infection. For instance, the compounds may be used as an alternative to conventional antibiotics. Advantageously, the disclosed compound
25 may not result in the microbes developing resistance to the compound.

It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the

5 appended claims.

Claims

1. A compound of Formula (I),



[Formula (I)]

wherein

A is a phenyl ring or an anionic boron;

R^1 is independently selected from hydrogen and an optionally substituted aliphatic group;

R^2 is independently selected from an optionally substituted aliphatic group that is linear, cyclic, saturated, unsaturated or any combination thereof;

m is an integer of 0-3;

n is an integer of 0-1;

o is an integer of at least 1;

p is an integer of 3-6;

q is an integer representing the number of counterions required for electron neutrality; and

X^- is an anionic counterion.

2. The compound according to claim 1, wherein each occurrence of R^1 is independently selected from the group consisting of hydrogen, and an optionally substituted C_1 - C_{10} alkyl.

3. The compound according to claim 2, wherein each occurrence of R^1 is independently selected from the group consisting of hydrogen, methyl, ethyl, propyl and butyl.

4. The compound according to any one of the preceding claims, wherein each occurrence of R^2 is independently selected from an optionally substituted aliphatic C_{6-12} group.

5. The compound of claim 4, wherein each occurrence of R^2 is independently selected from an optionally substituted aliphatic C_{6-8} group.

6. The compound according to any one of the preceding claims, wherein A is phenyl, and wherein each occurrence of the phenyl ring that is not A is independently ortho-substituted or para-substituted.

7. The compound according to any one of the preceding claims, wherein A is phenyl, and each occurrence of the phenyl ring that is not A is independently para-substituted.

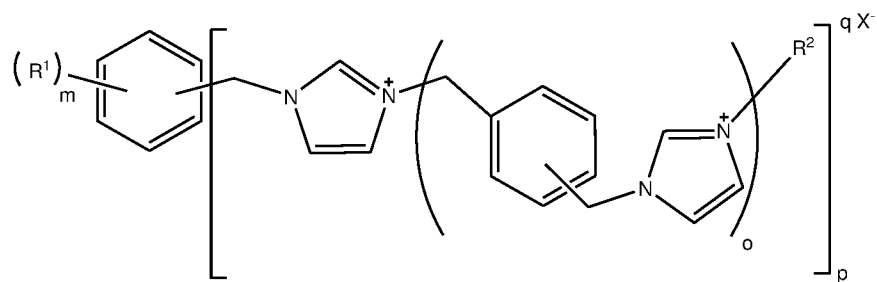
8. The compound according to any one of the preceding claims, wherein A is phenyl, and p is 3, said A is substituted in an alternating substitution pattern.

9. The compound according to any one of the preceding claims, wherein X^- is selected from the group consisting of halogenide, carbonate, phosphate, nitrate, sulfate, carboxylate and any combination thereof.

10. The compound according to any one of the preceding claims, wherein X^- is bromide or chloride.

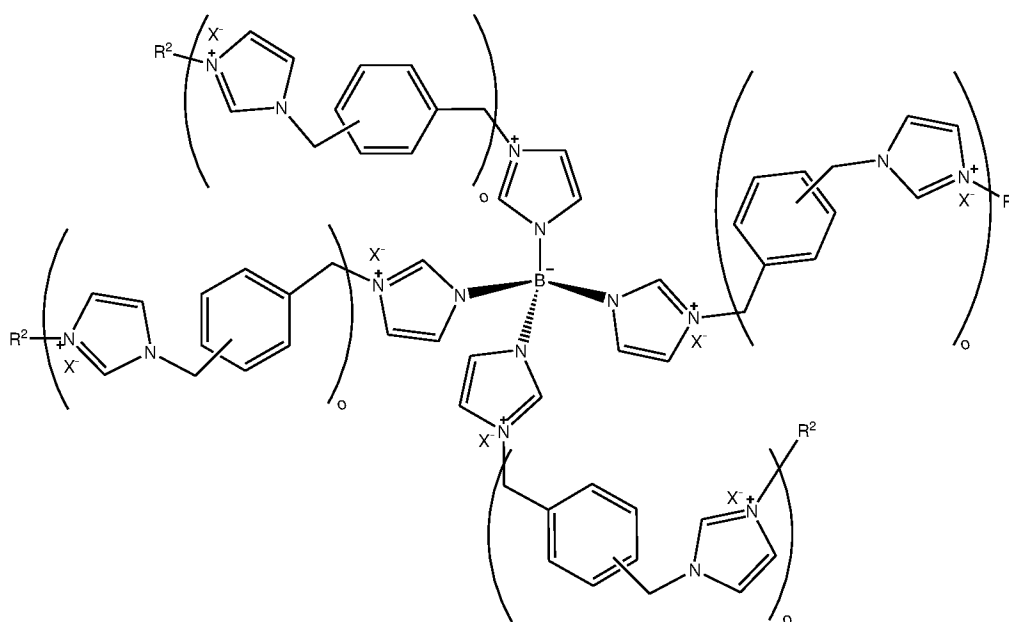
11. The compound according to any one of the preceding claims, wherein said compound is a compound of Formula (IIa),

42



[Formula (IIa)].

12. The compound of claim 11, wherein p is 3 or 6.
13. The compound of claim 12, wherein p is 3, and m is 3.
14. The compound of claim 12, wherein p is 6 and m is 0.
15. The compound according to any one of claims 1 to 5, wherein said compound is a compound of Formula (IIb),



[Formula (Iib)].

16. The compound of claim 15, wherein each occurrence of R² is an optionally substituted aliphatic C₆₋₁₂ group.
17. The compound of claim 16, wherein each occurrence of R² is an aliphatic C₆₋₈ group.

18. The compound according to any one of claims 15 to 17, each occurrence of the phenyl group is independently ortho- or para-substituted.

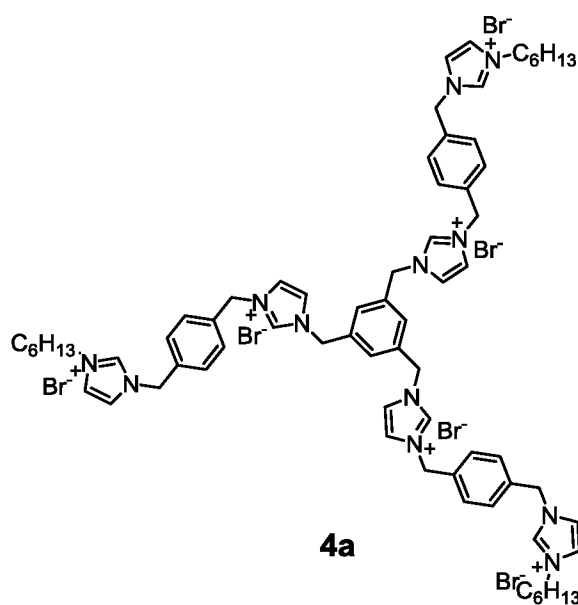
19. The compound of claim 18, wherein each occurrence of the phenyl group is para-substituted.

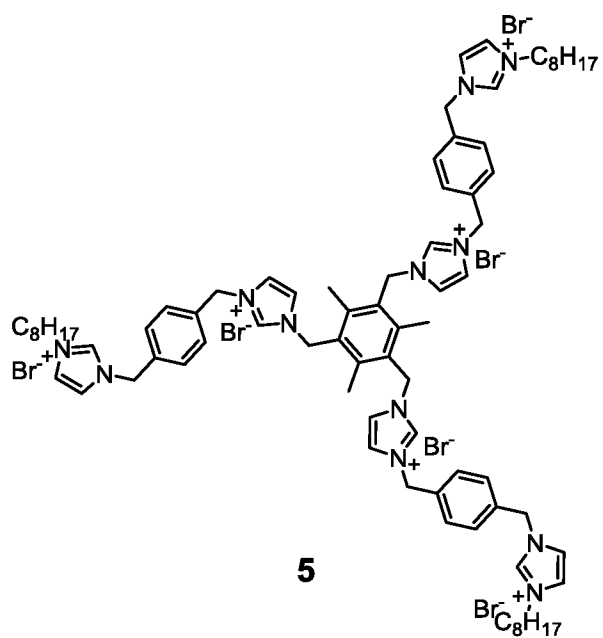
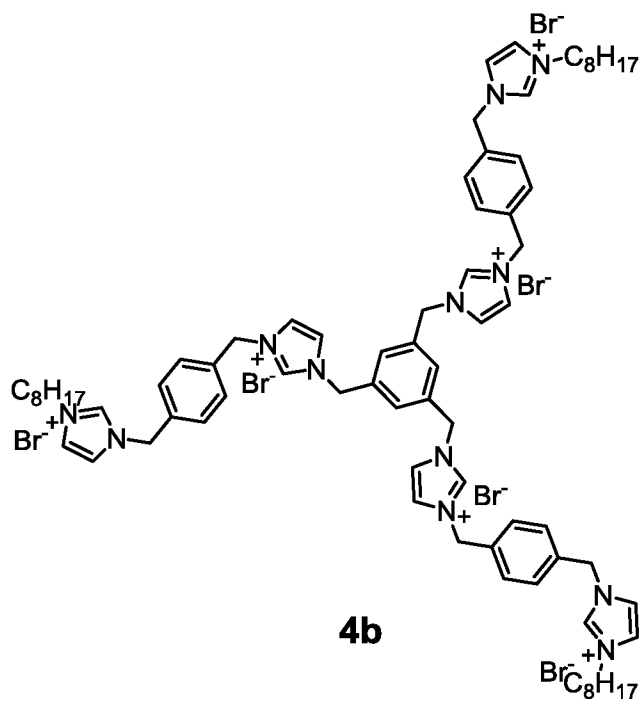
20. The compound according to any one of the preceding claims, wherein said compound is a non-linear structure.

21. The compound according to any one of claims 1 to 14, wherein said compound comprises a planar core structure.

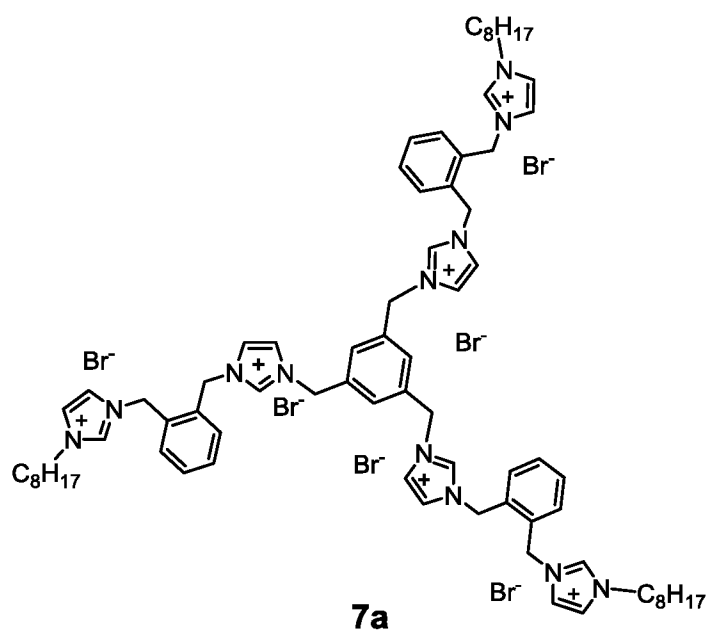
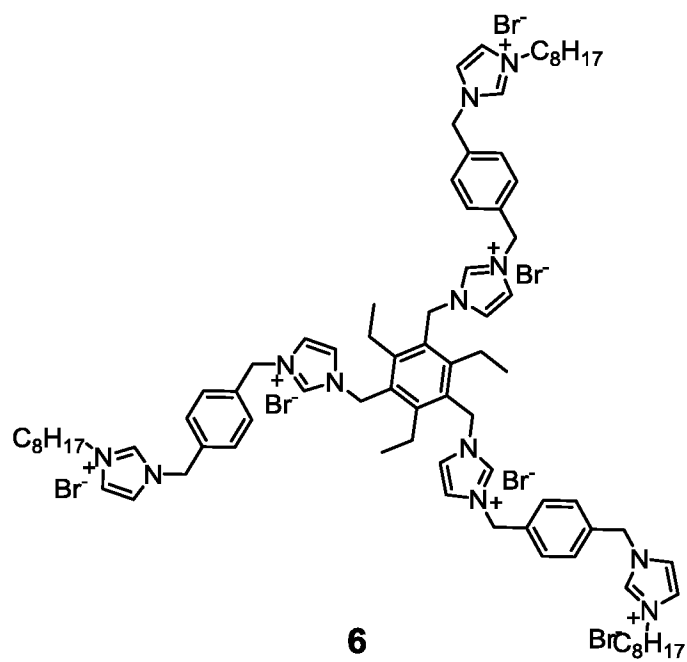
22. The compound of according to any one of claims 1 to 5 and 15 to 19, wherein said compound comprises a stereo core structure.

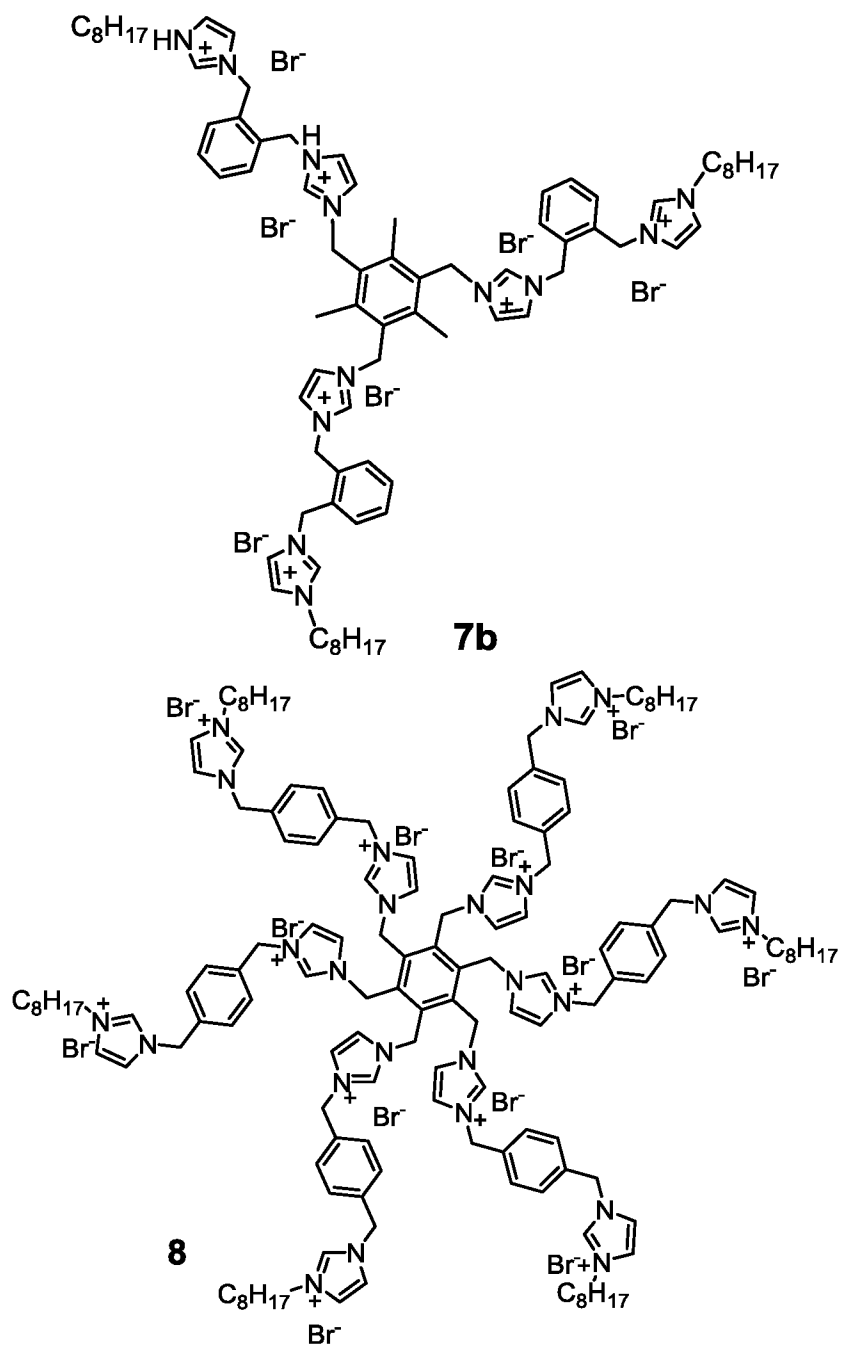
23. A compound selected from the group consisting of:



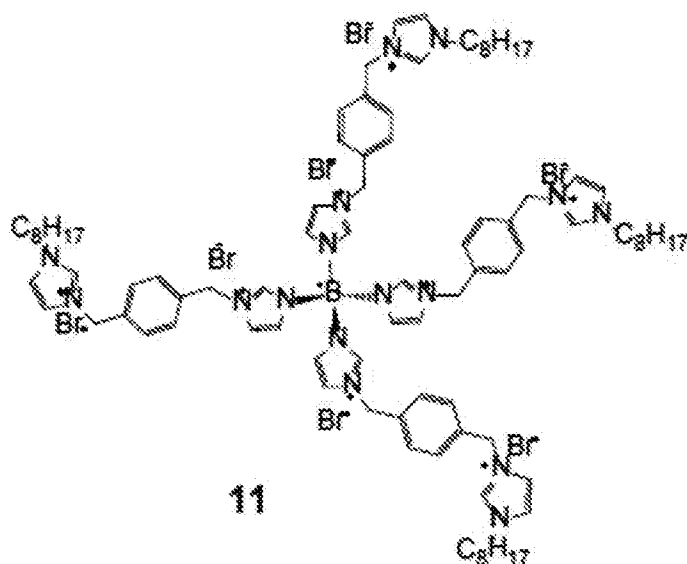


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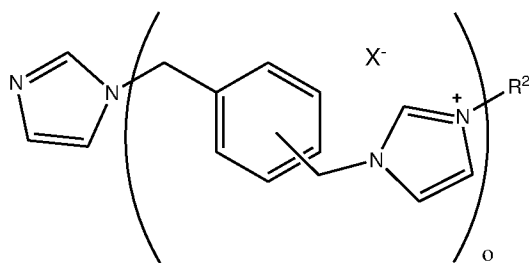


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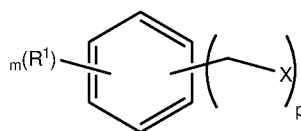
and

24. A method of preparing an imidazolium compound of Formula (IIa), the method comprising: reacting a compound of Formula (2)



Formula (2)

with a compound of Formula (3)

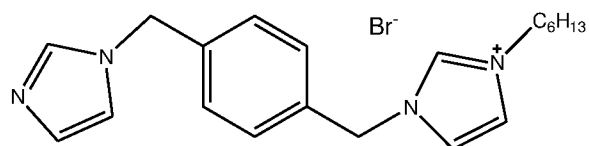


Formula (3),

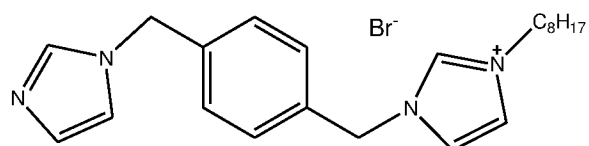
wherein R^1 , R^2 , m , o , p and X^- are as defined in any one of the preceding claims and X is the neutral form of X^- .

25. The method of claim 24, wherein the compound of Formula (2) is a compound selected from the group consisting of

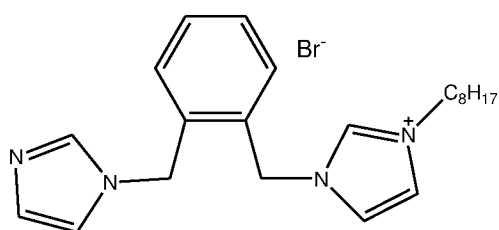
48



Formula (2a),

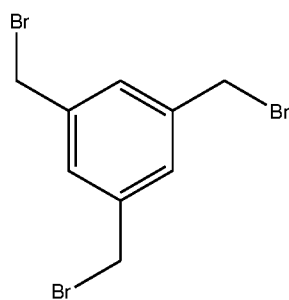


Formula (2b) and

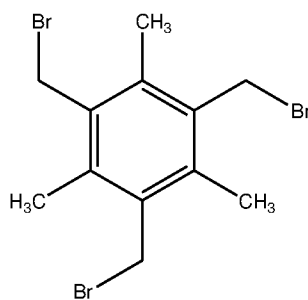


Formula (2c).

26. The method of claim 24 or 25, wherein the compound of Formula (3) is a compound selected from the group consisting of

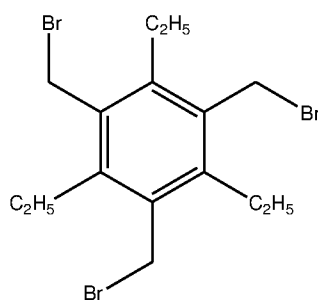


Formula (3a),

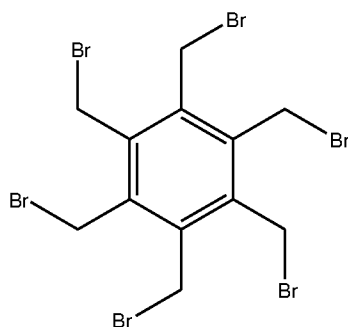


Formula (3b),

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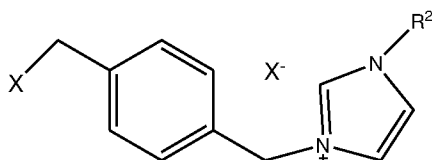


Formula (3c) and



Formula (3d).

27. A method of preparing an imidazolium compound of Formula (IIb) comprising reacting a compound of Formula (10a)

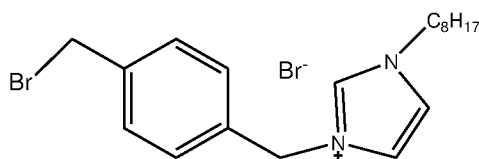


Formula (10a),

wherein R^2 and X^- are as defined in any one of the preceding claims and X is the neutral form of X^- ,

with a sodium tetrakis(1-imidazolyl)borate.

28. The method of claim 27, wherein the compound of Formula (10a) is a compound of Formula (10)



Formula (10).

29. The method according to any one of claims 24 to 28, wherein the reaction takes place in an organic solvent.

30. The method of claim 29, wherein the organic solvent is selected from the group comprising tetrahydrofuran (THF), dimethylformamide (DMF) and dimethyl sulfoxide (DMSO).

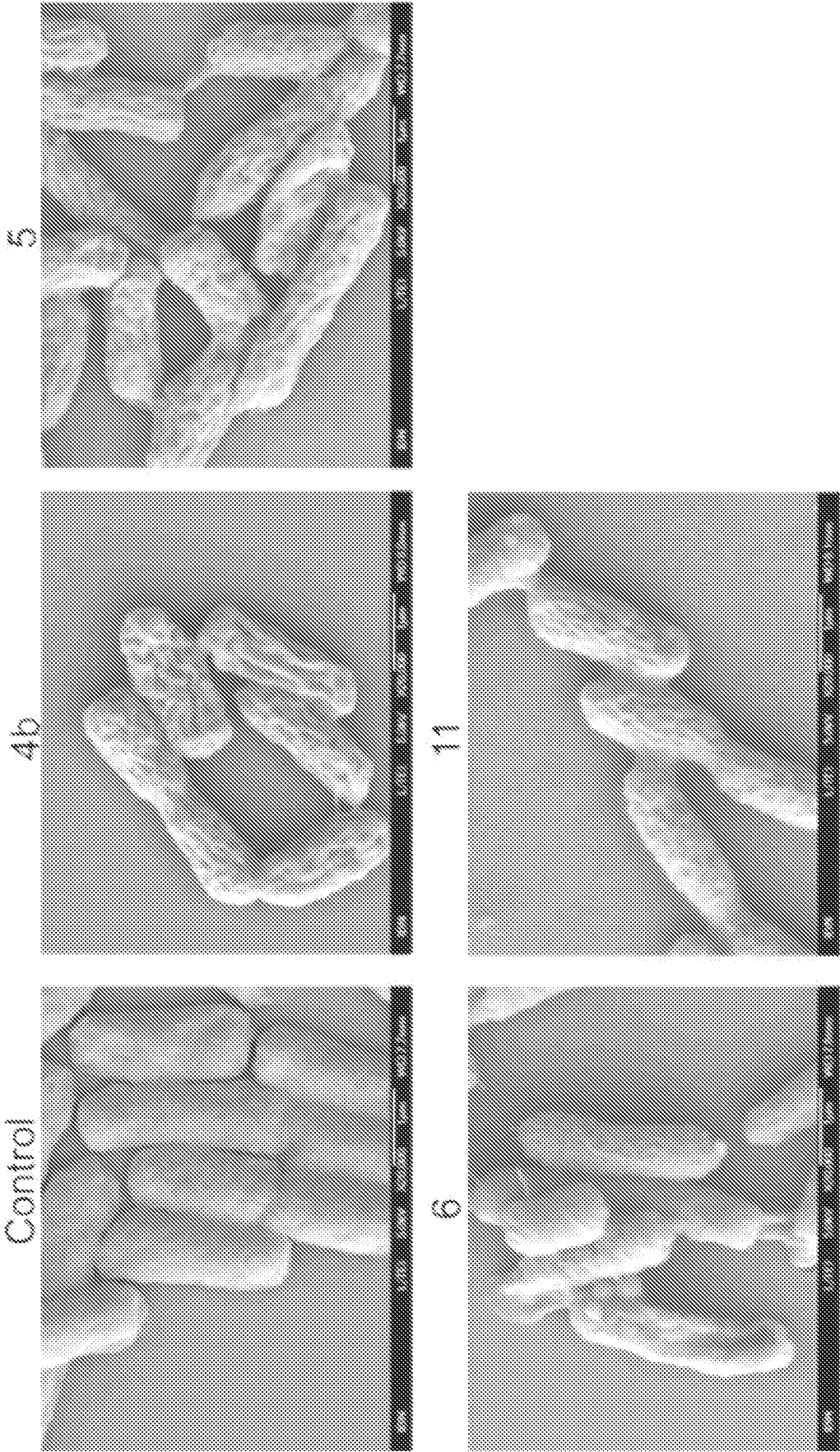
31. A method of killing or inhibiting the growth of a microorganism, comprising contacting said microorganism with an imidazolium compound as defined in any one of claims 1 to 23.

32. A compound as defined in any one of claims 1 to 23 for use as a medicament.

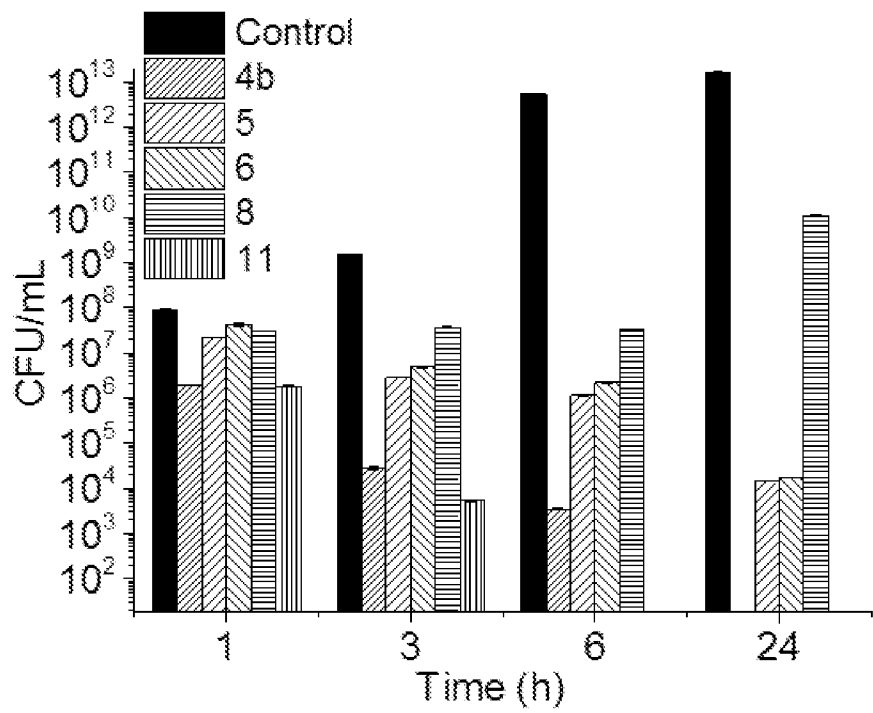
33. Use of a compound as defined in any one of claims 1 to 23 in the manufacture of a medicament, for treating or preventing infection caused by a microbe.

34. An *ex-vivo* or non-therapeutic use of a compound as defined in any one of claims 1 to 23, for killing or inhibiting the growth of a microorganism.

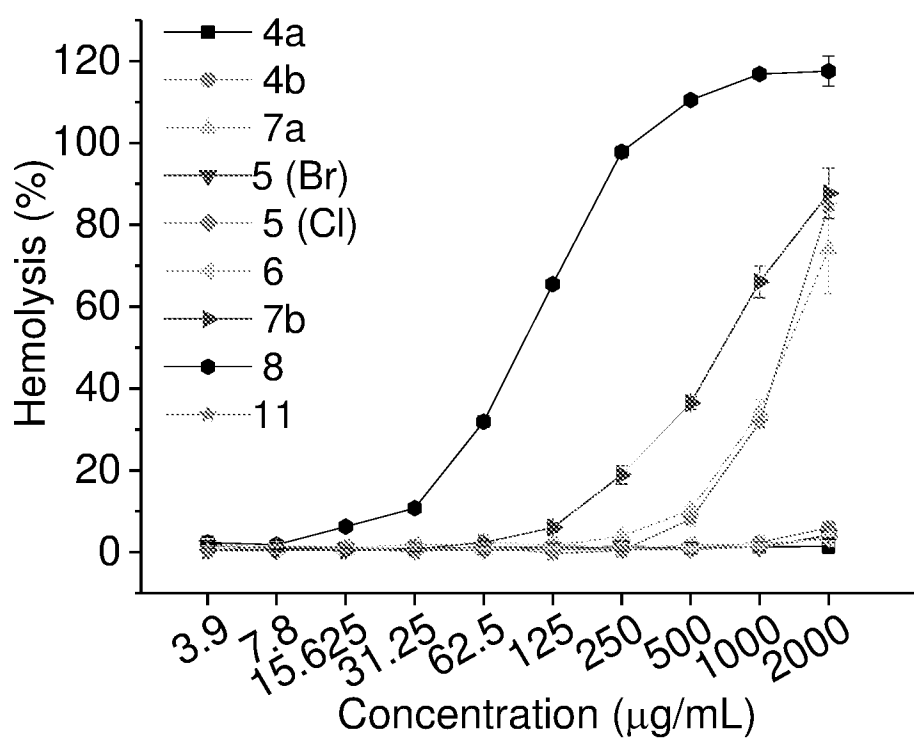
[Fig. 1]



[Fig. 2]



[Fig. 3]



INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2017/050467

A. CLASSIFICATION OF SUBJECT MATTER

See Supplemental Box

According to International Patent Classification (IPC)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D, A61K, A01N, A61P, A01P

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)

EPODOC/WPI/REGISTRY/CAPLUS: structure search of Formula I, IIa, IIb, 4a, 4b, 5, 11, 2, 3 and 10a, imidazolium, diazole, microbial, microbes, bacteria, fungi, fungus, bactericide, biocide, anti-infection, disinfection, sterilization, branched, non-linear, dendrimeric and other search terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y A	WO 2016/043660 A1 (AGENCY FOR SCIENCE, TECHNOLOGY AND RESEARCH) 24 March 2016 Examples 1-7; Figure 1	1-14, 20, 21, 23-26, 29-34 15-19, 22, 27, 28
Y A	WO 2012/149523 A1 (THE UNIVERSITY OF AKRON) 1 November 2012 Paragraphs [0109] - [0116]	1-14, 20, 21, 23-26, 29-34 15-19, 22, 27, 28
P,X	YUAN, Y. & ZHANG Y., Synthesis of Imidazolium Oligomers with Planar and Stereo Cores and Their Antimicrobial Applications. <i>ChemMedChem</i> , 16 May 2017, Vol. 12, No. 11, pages 835 - 840 [Retrieved on 23/10/2017] <DOI: 10.1002/CMDC.201700167> Abstract	1-34

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

*Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23/10/2017

(day/month/year)

Date of mailing of the international search report

31/10/2017

(day/month/year)

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG2017/050467

Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2016/043660 A1	24/03/2016	CN 107108518 A EP 3194371 A1 SG 11201702009 W	29/08/2017 26/07/2017 27/04/2017
WO 2012/149523 A1	01/11/2012	AU 2012249262 A1 CA2833886 A1 CN 103547154 A EP 2701511 A1 US 2014142307 A1	07/11/2013 01/11/2012 29/01/2014 05/03/2014 22/05/2014

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2017/050467

Supplemental Box

(Classification of Subject Matter)

Int. Cl.

C07D 233/56 (2006.01)

A61K 31/4178 (2006.01)

A01N 43/50 (2006.01)

A61P 31/00 (2006.01)

A01P 1/00 (2006.01)