(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 April 2012 (05.04.2012)

PCT

(10) International Publication Number
WO 2012/041934 A1

(51) International Patent Classification:
C07D 233/88 (2006.01) *A61P 31/00* (2006.01)
A61K 31/4168 (2006.01)

(21) International Application Number:
PCT/EP2011/066933

(22) International Filing Date:
28 September 2011 (28.09.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
1016261.8 28 September 2010 (28.09.2010) GB

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

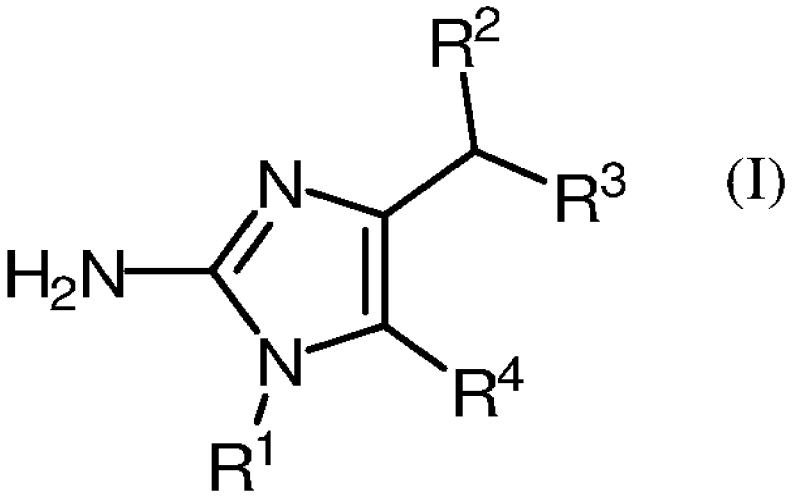
Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

(54) **Title**: POLYSUBSTITUTED 2-AMINOIMIDAZOLES FOR CONTROLLING BIOFILMS AND PROCESS FOR THEIR PRODUCTION



(I)

(57) **Abstract**: The present invention relates to compounds, compositions and methods for controlling and/or preventing biofilms and bacterial infections, being polysubstituted 2-amino-imidazoles with the structural formula (I). wherein R^1 is H, an aliphatic group or a cycloaliphatic group; R^2 is H, an aliphatic group or a cycloaliphatic group; R^3 is an aliphatic group, a cycloaliphatic group, an aromatic group or a heterocyclic group; and R^4 is an aliphatic group, a cycloaliphatic group, an aromatic group or a heterocyclic group; and pharmaceutically acceptable salts, hydrates, solvates, prodrugs, stereoisomeric forms or polymorphic substances thereof.

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POLYSUBSTITUTED 2-AMINOIMIDAZOLES FOR CONTROLLING BIOFILMS AND PROCESS FOR THEIR PRODUCTION

The present invention relates to new polysubstituted 2-aminoimidazoles being active against biofilm formation. The present invention also relates to antimicrobial, antifungal and insecticidal compositions comprising a microbial biofilm formation inhibiting amount of such polysubstituted 2-aminoimidazoles optionally in combination with excipients and/or with other antimicrobial, antifungal or insecticidal agents. The present invention also relates to the use of such polysubstituted 2-aminoimidazoles in methods for inhibiting or controlling microbial biofilm formation in a plant, a body part of a human or an animal, or a surface with which a human or an animal may come into contact. The present invention also relates to new synthetic methods for producing the relevant polysubstituted 2-aminoimidazoles

FIELD OF THE INVENTION

The present invention relates to polysubstituted 2-aminoimidazole compounds, compositions and methods for controlling biofilms and microbial, in particular bacterial, growth and for reducing bacterial colonization. The present invention relates to the treatment and prevention of infectious diseases caused by microbial biofilm formation, in particular to antimicrobial prophylactic and therapeutic compositions containing an effective amount of a biofilm formation inhibitor to reduce or eliminate colonization with potentially pathogenic microorganisms, more particularly bacteria (including bacterial strains resistant to many or most common antimicrobial agents), thereby reducing the risk of subsequent disease occurrence. Furthermore the present invention relates to polysubstituted 2-aminoimidazole compounds, and to compositions and methods involving these compounds, for inhibiting, reducing or preventing the formation of a biofilm on a surface of a medical device such as a catheter, or on a tissue such as teeth, urethra or lungs of a human (e.g. a cystic fibrosis patient). These polysubstituted 2-

aminoimidazole compounds, compositions and methods of present invention are in particular useful for preventing biofilm formation in a tissue to prevent or control a chronic bacterial infection or sepsis, and also useful for sanitation when applied to a substrate with which a human or an animal may come into contact. Furthermore the present invention relates to antifungal and insecticidal compositions containing such polysubstituted 2-aminoimidazole compounds.

BACKGROUND OF THE INVENTION

Biofilms are defined in the art as structured communities or aggregates of microorganisms (e.g. bacterial cells) in which cells adhere to each other and/or to a living or inert (non-living) surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance. Biofilms represent a prevalent mode of microbial life in natural, industrial and hospital settings. The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism. Biofilm cells exhibit profound changes in gene expression and cell physiology compared with planctonic cells, and multiple genetic pathways mediate the regulation of biofilm formation. Microorganisms in biofilms form microbial colonies or condominiums that make it easy to carry out chemical reactions that are impossible for individual microbial cells. Biofilms can contain many different types of microorganism, e.g. bacteria, archaea, protozoa, fungi and algae.

The use of effective antimicrobial compositions to avoid biofilm formation is recommended for any surface in contact with water, such as swimming pool liners, water cooling surfaces, hoses, water dispensers, water storage and distribution systems for drinking water or aquaculture, and for surfaces of medical devices such as catheters, medical implants, wound dressings and the like, especially when intended for patients with metabolic disorders.

Biofilms are known to provide cells with an array of advantages as compared with planktonic cells including the ability to resist challenges from predators, antibiotics, disinfectants and host immune systems. Biofilms offer a selective advantage to a microorganism to ensure its survival, or allow it a certain amount of time to exist in a dormant state until suitable growth conditions arise which for instance provide bacteria protection from antibiotics and from a host's immune system. This causes serious problems and costs in medical, hospital and industrial settings.

Biofilms are believed to be involved in 80% of human bacterial infections such as periodontitis, gingivitis, urethritis, endocarditis, benign prostatic hyperplasia, chronic prostatitis, biliary tract infections, urinary tract infections, cystitis, lung infections, sinus infections, ear infections (e.g. otitis media, otitis externa), acne and other skin infections, rosacea, dental caries, dental plaque formation, nosocomial infections, open wounds, chronic wounds and device-associated infections.

There is thus a need in the art to control biofilm formation in many situations of daily life. A biofilm inhibitor may be useful to clear the way for a bactericide (antibiotics) to penetrate the affected cells and eradicate the infection while using the antibiotics in a smaller amount. A biofilm inhibitor can also provide an alternative treatment approach for certain types of infections where certain antibiotics fail or where drug resistance has developed.

Biofilms can contribute to contaminating food (food industry), decreasing flow through pipelines by colonization of the pipe interior or mild steel corrosion (oil industry), initiating biofouling on vessel hulls (shipping industry), and infesting hospitals, in particular surgery rooms and medical devices used therein, as well as other living areas (private and public places). Biofilm formation in combination with mineral deposition may reduce heat transfer in water based cooling plants.

There is thus also a need in the art for new compounds and methods for preventing biofilm formation and improving sanitization in industrial as well as agricultural environment.. For instance valuable plants such as those producing fruits and

vegetables, forestry crops, corn, cotton, rice, soybeans and wheat are affected in a deleterious manner by insects and by biofilm formation from various microorganisms (including fungi) and hence need protection against biofilm formation.

For instance *Pseudomonas aeruginosa* is an opportunistic pathogen implicated in respiratory infections, urinary tract infections, gastrointestinal infections, keratitis, otitis media, and bacteremia. *P. aeruginosa* is found in an estimated 10 to 20% of all hospital-acquired infections. Patients with compromised host defenses, such as those infected with human immunodeficiency virus (HIV), burn patients, or those with cystic fibrosis (CF) (80% colonization rate of *P. aeruginosa*), are particularly susceptible to *P. aeruginosa* infections. There is evidence that biofims are involved in chronic *P. aeruginosa* infections such as recurrent ear infections (otitis media), chronical bacterial prostatitis and lung infections in CF patients. The latter being extremely harmful as *P. aeruginosa* colonization and chronic lung infection is the major causative agent of morbidity and mortality in CF patients. Moreover, *P. aeruginosa* biofilms can colonize a variety of medical devices such as intravascular catheters, urinary catheters, orthopedic devices, and dialysis machines. Obviously there is an urgent need in the art for agents that can prevent or eradicate *P. aeruginosa* biofilms on infected tissues and on medical devices.

Salmonella is worldwide one of the most important foodborne pathogens. In the European Union, more than 165.000 cases of salmonellosis were reported in 2006 (incidence 34.6 per 100.000 persons). Most patients infected with *Salmonella* develop a self-limiting gastro-enteritis, however in severe cases, the *Salmonella* infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics. Infants, elderly persons, and those with impaired immune systems are more likely than others to develop severe illness. *Salmonella Typhimurium* (mostly associates with contaminated pigs) and *Salmonella Enteritidis* (associated with poultry and eggs) are the most prevalent causing human infections. *Salmonella* is able to form microcolonies or even mature biofilms on a lot of different surfaces, ranging from abiotic surfaces (e.g. concrete, plastics, glass,

polystyrene) to biotic surfaces (gallstones, plant surfaces and epithelial cell layers). *Salmonella* biofilm formation is an important survival strategy in non-host environments, which are fundamentally different from typical host environments. In this context *Salmonella* biofilms on installations in farms, slaughterhouses and food processing industry form a serious risk given the fact that they are difficult to remove by classical disinfectants. Also biofilms on vegetables, fruits and seeds are problematic as they are not removed by simply washing. *Salmonella* also forms biofilms as a strategy to induce chronic infections and even colonize host organisms. In the biofilm mode of growth the *Salmonella* cells are more resistant to antibiotics. Additional to this biofilm related resistance, *Salmonella* has also reached a high level of genetic resistance, acquired by mutations or transfer of resistance genes. 40% of *S.* Typhimurium isolates in Europe are resistant to 4 or more antibiotics and resistance to fluoroquinolones is also emerging, which are critically important for use in humans. There is therefore a strong need in the art for alternative treatments that can prevent or eradicate *Salmonella* biofilm formation both inside and outside the host.

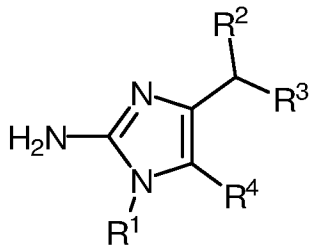
2-Aminoimidazole is emerging as an important pharmacophore, and is widely found in numerous biologically active marine sponge alkaloids. In particular, polysubstituted 2-aminoimidazoles have recently been reported as human β-secretase (BACE1) inhibitors, and tubulin-binding agents. Although a variety of synthetic methods to prepare a highly functionalized 2-aminoimidazole core has greatly advanced due to the rising demand in natural product synthesis and medicinal chemistry, most of them involve long experimental procedures with many protection-deprotection steps and the use of unstable precursors, such as α-aminoketones, α-bromoaldehydes or organomagnesium and organolithium compounds.

Synthesis of polysubstituted 2-aminoimidazoles via lanthanide-mediated hydroamination of propargylic cyanamides is rather limited by the diversity and availability of the starting electron-rich secondary benzylamines as well as by drastic reaction conditions. Accordingly, there is a need in the art for straightforward and more

generally applicable procedures for making diverse polysubstituted 2-aminoimidazoles from readily available precursors.

SUMMARY OF THE INVENTION

The above-mentioned objectives have been achieved by developing a rapid and highly efficient synthesis of disubstituted and trisubstituted 2-aminoimidazoles from propargylamines. The present invention relates, in a first aspect, to a class of disubstituted or trisubstituted 2-aminoimidazoles compounds as defined in claim 1, i.e. represented by the structural formula (I)



(I)

and pharmaceutically acceptable salts, hydrates, solvates, prodrugs, stereiisomeric forms or polymorphic substances thereof, wherein:

$\mathbf{R^1}$ is H, an aliphatic group or a cycloaliphatic group;

$\mathbf{R^2}$ is H, an aliphatic group or a cycloaliphatic group;

$\mathbf{R^3}$ is an aliphatic group, a cycloaliphatic group, an aromatic group or a heterocyclic group

$\mathbf{R^4}$ is an aliphatic group, a cycloaliphatic group, an aromatic group or a heterocyclic group.

The present invention relates, in a second aspect, to a unique method for producing such disubstituted or trisubstituted 2-aminoimidazoles compounds in a limited number of steps starting from a propargylamine and successively passing through a propargylguanidine intermediate and a protected 2-iminoimidazolidine intermediate.

The present invention also relates, in a third aspect, to pharmaceutical, anti-bacterial, anti-fungal and insecticidal compositions comprising an effective amount of a

compound represented by the structural formula (I), or any specific embodiment thereof, optionally together with one or more excipients, carriers or diluents, and further optionally in combination with one or more anti-bacterial, anti-fungal or insecticidal active ingredient.

The present invention also relates to medicinal and non-medicinal uses, in particular disinfecting and insecticidal uses, of a compound represented by the structural formula (I). The present invention also relates to methods of treatment of individuals or plants infected with biofilms, and to methods of disinfection of surfaces infected with biofilms.

DEFINITIONS

Unless otherwise stated herein, the term '' disubstituted '' in relation to the imidazole structure means that only the carbon atoms being in positions 4 and 5 of the imidazole moiety (according to standard atom numbering for this moiety) are substituted with an atom or group of atoms other than hydrogen. Unless otherwise stated herein, the term '' trisubstituted '' in relation to the imidazole structure means that all three carbon atoms being in positions 1, 4 and 5 of the imidazole moiety (according to standard atom numbering for this moiety) are substituted with an atom or group of atoms other than hydrogen.

The term "optionally substituted" indicates that the specified group is either unsubstituted, or substituted by one or more suitable substituents. A "substituent" as defined herein is a monovalent atom or group of atoms replacing a hydrogen atom on a hydrocarbon chain or cycle (ring) of an organic molecule, for example halogen, hydroxy, acyl, alkyl, haloalkyl, alkenyl, alkynyl, cycloaliphatic, heterocyclo, aryl (in particular phenyl), heteroaryl, alkoxy, aryloxy, amino, amido, sulfhydryl, alkylthio, arylthio, alkylsulfonyl, nitro, carbonyl, carboxy, amino-acid (both natural and synthetic, and whether attached either through the carboxyl moiety thereof or through the amino moiety

thereof) and peptido, or a divalent atom replacing two hydrogen atoms on the same carbon atom of a hydrocarbon chain, for instance oxo or thioxo. Each of the above-listed substituents may, where applicable, be defined as set forth in any of the specific meanings below. The number of admissible substituents depends upon the number of hydrogen atoms that can be replaced, thus the chain length, the type of substituent and parameters such as steric hindrance which are all well known to the skilled person.

The term "aliphatic" as used herein refers to "Alkyl", "Alkenyl" or "Alkynyl".

The term "alkyl" or "saturated aliphatic" as used herein, and unless otherwise specified, refers to a straight or branched hydrocarbon chain containing from 1 to 20 (e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, etc.) carbon atoms, hence the notation C_{1-20} alkyl. Suitable representative examples of C_{1-20} alkyl thus include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, *tert*-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, 3-methylhexyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-dodecyl and the like. Such alkyl groups may optionally be substituted with one or more (e.g. 2, 3, 4 or more) substituents as defined hereinabove.

"Alkenyl," as used herein, and unless otherwise specified, refers to a straight or branched hydrocarbon chain containing from 2 to 20 (e.g. 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, etc.) carbon atoms, hence the notation C_{2-20} alkenyl, and containing at least one carbon-carbon double bond. The carbon-carbon double bond needs not be terminal, but may be located at any place within the hydrocarbon chain. Suitable representative examples of "alkenyl" thus include, but are not limited to, ethenyl, 2-propenyl, 2-methyl-2-propenyl, 3-butenyl, 4-pentenyl, 5-hexenyl, 2-heptenyl, 2-methyl-l-heptenyl, 3-decenyl, butadienyl, hexadienyl and the like. Such alkenyl groups may optionally be substituted with one or more (e.g. 2, 3, 4 or more) substituents as defined hereinabove.

"Alkynyl" as used herein, and unless otherwise specified, refers to a straight or branched hydrocarbon chain containing from 2 to 20 (e.g. 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, etc.) carbon atoms, hence the notation C_{2-20} alkynyl, and containing at least

one carbon-carbon triple bond. The carbon-carbon triple bond needs not be terminal, but may be located at any place within the hydrocarbon chain. Suitable representative examples of C_{2-20} alkynyl thus include, but are not limited, to acetylenyl, 1-propynyl, 2-propynyl, 3-butynyl, 2-pentynyl, 1-butynyl and the like. Such alkynyl groups may optionally be substituted with one or more (e.g. 2, 3, 4 or more) substituents as defined hereinabove.

The term "cycloaliphatic", as used herein and unless otherwise specified, refers to a saturated or ethylenically unsaturated monocyclic or polycyclic hydrocarbon group containing from 3 to 12 (e.g. 4, 5, 6, 7, 8, 9 or 10) carbon atoms, which is not aromatic (as defined below), hence the notations C_{3-12} cycloalkyl (saturated) and C_{3-12} cycloalkenyl (unsaturated). Such cycloaliphatic groups may optionally be substituted with one or more (e.g. 2, 3, 4 or more) substituents as defined hereinabove. Suitable representative examples of cycloalkyl thus include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, tricyclodecyl, cyclododecyl, adamantyl, nornornyl, 5,6-trimethylenenorborn-2-yl and cyclooctyl. Suitable representative examples of cycloalkenyl thus include, but are not limited to, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl, cyclononenyl, cyclodecenyl, 1,3-cyclohexadienyl, 1,4-cyclohexadienyl, 1,3-cycloheptadienyl, 1,5-cyclooctadienyl.

The term “heterocyclic”, as used herein, and unless otherwise specified, refers to a saturated or ethylenically unsaturated but not aromatic (as defined below) monocyclic or polycyclic (e.g. bicyclic) ring system comprising at least one heteroatom preferably selected from the group consisting of nitrogen, oxygen and sulfur in at least one ring.

Monocyclic heterocyclic ring systems are exemplified by any ring having from 3 to 8 (e.g. 4, 5 or 6 or 7) members and containing 1, 2, 3, or 4 heteroatoms independently selected from the group consisting of O, N, and S. For instance a 5 member ring system may have from 0 to 2 (e.g. 1) double bond(s), and a 6 member ring system may have from 0 to 3 (e.g. 1 or 2) double bond(s). Depending upon the number of double bonds, the heterocyclic ring system may be heteroaromatic (see specific definition below) or not.

Suitable representative examples of monocyclic non-aromatic heterocyclic ring systems thus include, but are not limited to, azetidine, azepine, aziridine, diazepine, 1,3-dioxolane, 1,2-dioxane, 1,3-dioxane, 1,4-dioxane, 1,2-dithiane, 1,3-dithiane, 1,4-dithiane, imidazoline, imidazolidine, isothiazoline, isothiazolidine, isoxazoline, isoxazolidine, morpholine, oxadiazoline, oxadiazolidine, oxazoline, oxazolidine, piperazine, piperidine, pyrazine, pyrazoline, pyrazolidine, pyrroline, pyrrolidine, tetrahydrofuran, tetrahydrothiophene, thiadiazoline, thiadiazolidine, thiazoline, thiazolidine, thiomorpholine, thiomorpholine sulfone, thiomorpholine sulfoxide, and the like. Bicyclic ring systems are exemplified by any of the above monocyclic ring systems fused to one or more (e.g. two) aryl or cycloalkyl or heterocyclic groups as defined herein. Suitable representative examples of bicyclic ring systems thus include but are not limited to, for example, benzimidazole, benzothiazole, benzothiadiazole, benzothiophene, benzoxadiazole, benzoxazole, benzofuran, benzopyran, benzothiopyran, benzodioxine, 1,3- benzodioxole, cinnoline, indazole, indole, indoline, indolizine, naphthyridine, isobenzofuran, isobenzothiophene, isoindole, isoindoline, isoquinoline, phthalazine, pyranopyridine, quinoline, quinolizine, quinoxaline, quinazoline, tetrahydroisoquinoline, tetrahydroquinoline, thiopyranopyridine, and the like.

"Aromatic" as used herein, and unless otherwise specified, refers to a ring system having one or more aromatic rings, which may be homoaromatic or heteroaromatic.

The term "Homoaromatic" or ''aryl'' as used herein, and unless otherwise specified, refers to an aromatic ring system in which no carbon atoms have been replaced with heteroatoms. The homoaromatic group can be unsubstituted or substituted with from 1 to 5 (e.g. 2, 3 or 4) suitable substituents as defined hereinabove in each aromatic ring, and wherein two adjacent substituents may be linked to form a cycle such as methylenedioxy. Suitable representative examples of aryl thus include, but are not limited to, azulenyl, indanyl, indenyl, naphthyl, phenyl, tetrahydronaphthyl, anthracenyl and mono- and polysubstituted versions thereof.

The term "Heteroaromatic" or ''heteroaryl'' as used herein, and unless otherwise specified, refers to an aromatic ring system in which one or more carbon atoms have been replaced with heteroatoms independently selected from the group consisting of nitrogen, oxygen and sulfur in at least one ring. Suitable representative examples of heteroaryl thus include, but are not limited to, pyridyl, pyrimidinyl, imidazolyl, thienyl, furyl, pyrazinyl, pyrrolyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, indolyl, isoindolyl, indolizinyl, triazolyl, pyridazinyl, indazolyl, purinyl, quinolizinyl, isoquinolyl, quinolyl, phthalazinyl, naphthyridinyl, quinoxalinyl, isothiazolyl, isoxazolyl, oxazolyl, dioxazolyl, pyrazolyl, tetrazinyl, tetrazolyl, thiadiazolyl, thiazolyl, triazinyl and benzo[b]thienyl. The heteroaryl group may be optionally substituted with one or more, e.g. 2, 3 or 4, suitable substituents (as defined hereinabove) in each ring.

The term " C_{1-20} alkoxy " as used herein, and unless otherwise specified, refers to substituents wherein a carbon atom of a C_{1-20} alkyl group (such as defined herein), is attached to an oxygen atom through a single bond such as, but not limited to, methoxy, ethoxy, propoxy, isopropoxy, butoxy, iso-butoxy, sec-butoxy, *tert*-butoxy, pentoxy, 3-pentoxy, or *n*-hexyloxy.

The term " C_{1-20} alkylthio " as used herein, and unless otherwise specified, refers to substituents wherein a carbon atom of a C_{1-20} alkyl group (such as defined herein), is attached to an sulfur atom through a single bond such as, but not limited to, methylthio, ethylthio, etc.

The term " aryloxy " as used herein, and unless otherwise specified, refers to substituents wherein a carbon atom of an aryl group (such as defined herein), is attached to an oxygen atom through a single bond such as, but not limited to, phenoxy, naphthoxy, etc.

The term " arylthio " as used herein, and unless otherwise specified, refers to substituents wherein a carbon atom of an aryl group (such as defined herein), is attached to an sulfur atom through a single bond such as, but not limited to, phenylthio.

As used herein and unless otherwise stated, the term halogen means any atom selected from the group consisting of fluorine, chlorine, bromine and iodine.

As used herein and unless otherwise stated, the term haloalkyl refers to an alkyl group (such as defined hereinabove) wherein one or more, optionally all, hydrogen toms are replaced with halogen atoms. Suitable representative examples of haloalkyl thus include, but are not limited to, fluoromethyl, difluoromethyl, trifluoromethyl, chloromethyl, dichloromethyl, trichloromethyl, pentafluoroethyl and the like.

The term " amino acid " as used herein, and unless otherwise specified, refers to a group derived (through abstraction of a hydrogen atom, either from the carboxyl moiety thereof or from the amino moiety thereof) from any " natural amino acid " (i.e. Alanine (ala), Arginine (Arg), Asparagine (asn), Aspartic acid (Asp), Cysteine (cys), Glutamine (gln), Glutamic acid (glu), Glycine (gly), Histidine (his), Hydroxylysine (Hyl), Hydroxyproline (Hyp), Isoleucine (ile), Leucine (leu), Lysine (lys), Methionine (met), Phenylalanine (phe), Proline (pro), Serine (ser), Threonine (thr), Tryptophan (trp), Tyrosine (tyr), or Valine (val)) in D or L conformation, as well as from any " non-natural (or synthetic) amino acid " known in the art (e.g., but not limited to, phosphoserine, phosphothreonine, phosphotyrosine, hydroxyproline, gamma-carboxyglutamate, hippuric acid, octahydro-indole-2-carboxylic acid, statine, 1,2,3,4,-tetrahydroisoquinoline-3-carboxylic acid, penicillarnine, ornithine, citruline, α-methyl-alanine, para-benzoylphenylalanine, phenyl-glycine, propargylglycine, sarcosine, *tert*-butylglycine), and amino-acids bearing one or more sulfonic and/or phosphonic groups. This term also comprises natural and non-natural amino acids being protected at their carboxylic terminus, e.g. as a C_{1-20} alkyl, phenyl or benzyl ester or as an amide, such as for example, a mono-C_{1-20} alkyl or di-(C_{1-}

$_{20}$ alkyl) amide, or another other suitable carboxy-protecting group well known to those skilled in the art e.g. from T.W. Greene, Protecting Groups In Organic Synthesis, Wiley, New York, (1981) and references cited therein, the content of which is incorporated herein by reference).

The term " peptide " as used herein, and unless otherwise specified, refers to a sequence of 2 to 100 amino-acids (e.g. 3, 4, 5, 6, 8, 10, 20 or 50) as defined hereinabove.

The terms " amino-protecting group '' and '' imino-protecting group '' as used herein, and unless otherwise specified, refer to groups which are able to replace a hydrogen atom of an amino or imino group respectively to avoid reaction of this hydrogen atom in a certain reaction type, and which are capable of being simply cleaved off after said reaction has been performed, thus avoiding intereference of said amino or imino group in said reaction. Suitable amino-protecting groups and imino-protecting groups are well known in the art and are preferably selected from the group consisting of arylcarbonyl, alkyloxycarbonyl and arylalkyloxycarbonyl. A few non-limiting examples of suitable amino-protecting groups include benzyloxycarbonyl (which may be introduced by reaction with benzylchloroformate under alkaline conditions, e.g. making use of sodium hydroxide or hydrogenocarbonate) and 9-fluorenylmethoxycarbonyl (which may be introduced by reaction with 9-fluorenylmethyl chloroformate). Another example of an amino-protecting group is a *tert*-butoxycarbonyl group which may be introduced by reaction with di-tert-butyl dicarbonate under alcaline conditions. Other suitable amino-protecting groups include, but are not limited to aralkyl type protecting groups comprising benzyl, p-methoxybenzyl, p-nitrobenzyl, p-bromobenzyl and triphenylmethyl (trityl); alternative acyl type protecting groups comprising formyl, acetyl, chloroacetyl, dichloroacetyl, trichloroacetyl, trifluoroacetyl, phenylacetyl, o-nitrophenoxyacetyl, sec-butyryl, pivaloyl- (also known as tert-butyryl), cyclopropanoyl, benzoyl, o-nitrobenzoyl, and alpha-chlorobutyryl; or other protecting groups comprising benzyloxycarbonyl, *p*-chlorobenzyloxycarbonyl, *p*-nitrobenzyloxycarbonyl, *p*-bromo-

benzyloxycarbonyl, *p*-methoxybenzyloxycarbonyl, *o*-nitrobenzyloxycarbonyl, tert-butyloxycarbonyl, *tert*-amyloxycarbonyl, diisopropylmethoxycarbonyl, isopropyloxy-carbonyl, allyloxycarbonyl, cyclopentyloxycarbonyl, adamantyloxycarbonyl and cyclo-hexyloxycarbonyl.

As used herein and unless otherwise stated, the term '' stereoisomeric form '' refers to all possible different isomeric as well as conformational forms which the compounds of this invention may possess, in particular all possible stereochemically and conformationally isomeric forms, all diastereomers, enantiomers and/or conformers of the basic molecular structure.

As used herein and unless otherwise stated, the term '' enantiomer '' means each individual optically active form of a compound of this invention, having an optical purity or enantiomeric excess (as may be determined by methods standard in the art) of at least 80% (i.e. at least 90% of one enantiomer and at most 10% of the other enantiomer), preferably at least 90% and more preferably at least 98%.

As used herein and unless otherwise stated, the term '' solvate '' includes any combination which may be formed by combining, in a molar ratio readily determinable by a person skilled in the art, a compound of this invention with a suitable inorganic solvent (e.g. hydrates) or with an organic solvent such as but not limited to alcohols (e.g. methanol, etanol or isopropanol), ketones (e.g. acetone), esters (e.g. ethyl acetate), nitriles (e.g. acetonitrile) and the like.

The term " microorganism " as used herein, and unless otherwise specified, refers to unicellular or cell-cluster microscopic organisms including eukaryotes such as fungi and protists, and prokaryotes, especially microorganisms that are susceptible to cause a disease in humans, such as bacteria, but excluding a virus or a prion. As is well known to those skilled in the art, these microorganisms can be organized in the form of a biofilm, thus the term ''microbial biofilm ''.

The term '' sepsis '' as used herein, and unless otherwise specified, refers to a systemic inflammatory response syndrome associated to an infection. Septic shock is

characterized namely by (a) hypotension persisting despite adequate fluid resuscitation, and (b) abnormalities related to hypoperfusion or organ dysfunction.

The terms " decreasing bacterial growth " as used herein, and unless otherwise specified, refers to one of the following effects ; decrease in the number of viable bacteria of at least one species ; decrease in the rate of growth of the number of viable bacteria (although the number itself may increase); elimination of substantially all viable bacteria; prevention of the formation and accumulation of bacteria on a specific target.

The terms " increasing susceptibility to cytotoxic effects of antibacterial agents" as used herein, and unless otherwise specified, refers to the fact that in the presence of the compound of the invention other anti-bacterial agents (antibiotics) can be administered in smaller amounts to obtain the same level of cytotoxic effect as compared to their effective amount in the absence of the compound of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The first aspect (new compounds) of the present invention will now be described with respect to a number of specific valuable embodiments.

One specific embodiment of this aspect of the present invention relates to a disubstituted 2-aminoimidazole compound as defined in claim 2.

Another specific embodiment of this aspect of the present invention relates to a trisubstituted 2-aminoimidazole compound as defined in claim 3.

Another specific embodiment of this aspect of the present invention relates to a trisubstituted 2-aminoimidazole compound as defined in claim 4.

Another specific embodiment of this aspect of the present invention relates to a trisubstituted 2-aminoimidazole compound as defined in claim 5.

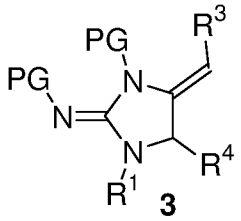
Another specific embodiment of this aspect of the present invention relates to a trisubstituted 2-aminoimidazole compound as defined in claim 6.

Another specific embodiment of this aspect of the present invention relates to a trisubstituted 2-aminoimidazole compound as defined in claim 7.

Another specific embodiment of this aspect of the present invention relates to compounds selected of the group consisting of 4-benzyl-1-methyl-1H-imidazol-2-amine, 4-(4-methoxybenzyl)-1-methyl-1H-imidazol-2-amine, 1,4-dibenzyl-1H-imidazol-2-amine, 1,4-bis(4-methoxybenzyl)-1H-imidazol-2-amine, 1,4-bis(4-hydroxybenzyl)-1H-imidazol-2-amine, 4-((2-amino-4-(4-methoxybenzyl)-1-methyl-1H-imidazol-5-yl)methyl)phenol, 4,5-bis(benzo[*d*][1,3]dioxol-5-ylmethyl)-1-methyl-1H-imidazol-2-amine, 4-benzyl-1-*tert*-butyl-5-isobutyl-1H-imidazol-2-amine, 4-benzyl-1-cycloheptyl-5-isobutyl-1H-imidazol-2-amine, 4-benzyl-1-cyclododecyl-5-isobutyl-1H-imidazol-2-amine, 4-benzyl-5-isobutyl-1-(3-methoxyphenethyl)-1*H*-imidazol-2-amine, 1-benzyl-4-phenethyl-5-phenyl-1H-imidazol-2-amine, 1-benzyl-4-(4-(pentyloxy)benzyl)-5-*p*-tolyl-1H-imidazol-2-amine, 4-benzyl-1-cyclopropyl-5-isobutyl-1H-imidazol-2-amine, 4-benzyl-1-cyclobutyl-5-isobutyl-1H-imidazol-2-amine, 4-benzyl-1-cyclopentyl-5-isobutyl-1H-imidazol-2-amine, 4-benzyl-1-cyclohexyl-5-isobutyl-1H-imidazol-2-amine, 4-benzyl-1-cyclooctyl-5-isobutyl-1H-imidazol-2-amine, 4-(4-butoxybenzyl)-1-benzyl-5-isobutyl-1H-imidazol-2-amine, 4-(4-(pentyloxy)benzyl)-1-benzyl-5-isobutyl-1H-imidazol-2-amine, 4-(4-propylbenzyl)-1-benzyl-5-isobutyl-1H-imidazol-2-amine, 4-(4-bytylbenzyl)-1-benzyl-5-isobutyl-1H-imidazol-2-amine, 4-(4-pentylbenzyl)-1-benzyl-5-isobutyl-1H-imidazol-2-amine, 1-cyclobutyl-4-(2-cyclopentylethyl)-5-cyclopropyl-1H-imidazol-2-amine, 4-(4-methoxybenzyl)-5-cyclohexyl-1-((S)-1-phenylethyl)-1H-imidazol-2-amine, 1-butyl-4-(4-methylphenyl)-5-isobutyl-1H-imidazol-2-amine, 1-benzyl-4-(4-methylphenyl)-5-isopropyl-1H-imidazol-2-amine, 1-benzyl-4-phenyl-5-cyclopropyl-1H-imidazol-2-amine.

In another aspect the present invention relates to a process for producing a disubstituted or trisubstituted 2-aminoimidazole represented by the structural formula (I) as defined in any one of claims 1 to 7, wherein $\mathbf{R^2}$ is H, said process comprises the step of

deprotecting and isomerizing a bis-protected 2-iminoimidazolidine represented by the structural formula **3**:



3

wherein $\mathbf{R^1}$, $\mathbf{R^3}$ and $\mathbf{R^4}$ are as defined in formula (I) and wherein PG is an amino-protecting group or an imino-protecting group such as defined hereinabove.

The second aspect (new process) of the present invention will now be described with respect to a number of specific valuable embodiments.

The step of deprotecting and isomerizing a bis-protected 2-iminoimidazolidine intermediate may be carried out by methods well known in the art for cleaving an amino- or imino-protecting group. These methods obviously depend upon the type of relevant amino- or imino-protecting group. For instance a tert-butoxycarbonyl group can be cleaved off by means of trifluoracetic acid (TFA) at room temperature during about 1 to 6 hours in a suitable solvent such as but not limited to dichloromethane.

Another specific embodiment of this aspect of the present invention relates to a process as defined in claim 14.

Another specific embodiment of this aspect of the present invention relates to a process as defined in claim 15.

According to the present invention, the bis-protected 2-iminoimidazolidine intermediate may thus be obtained from a suitable propargylamine in a metal-catalyzed coupling and cyclization reaction or guanylation reaction with a protected isothiourea as shown in scheme 3 below. For instance the reaction may be performed at room temperature during about 3 minutes to about 3 hours in a suitable solvent such as but not limited to dichloromethane. Some of the bis-protected 2-iminoimidazolidine intermediates described below are new compounds.

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Another specific embodiment of this aspect of the present invention relates to a process as defined in claim 16, i.e. the catalytic metal is a silver compound such as but not limited to silver nitrate, silver triflate, or silver hexafluoroantimonate.

Another specific embodiment of this aspect of the present invention relates to a process as defined in claim 14, and wherein the catalytic metal is a compound of a metal selected from the group consisting of copper, palladium and mercury such as but not limited to copper chloride, copper triflate, mercury triflate, or palladium bis-acetate.

In the two latter embodiments of the present invention, the bis-protected 2-iminoimidazolidine intermediate is readily obtained in very high yield (from 75% to 100%) within a short time period.

Another specific embodiment of this aspect of the present invention relates to a process as defined in claim 17, i.e. combining all of the above recited process steps.

Suitably substituted starting secondary propargylamines for performing the above methods can be readily obtained by reacting a R^1–substituted primary amine with a R^4–substituted aldehyde and a R^3–substituted alkyne, for instance at about 100°C in toluene during about 25 minutes in the presence of copper bromide. Some of the secondary propargylamines intermediates described below are new compounds.

The third aspect (new compositions) and further aspects (medical and non-medical uses) of the present invention will now be described with respect to a number of specific valuable embodiments.

The present invention further concerns a composition for increasing the susceptibility of bacteria to the cytotoxic effects of other antibacterial agents comprising the compound of formula (I) as defined in the present invention.

The present invention encompass the above described compounds of formula (I) for use in a veterinarian or human treatment of curing, ameliorating or preventing a microbial infection or a sepsis.

The present invention relates to compounds for interrupting one or more of the microbial intra- and interspecies signaling pathways that are in association with or are controlling biofilm formation. It is the discovery of the present invention that some classes of compounds can effectively decrease biofilm formation and increase biofilm's sensitivity to anti-microbial treatment. The present invention provides furthermore methods and compositions useful for preventing or treating biofilm formation or microbial infections associated with biofilm formation. The compounds of present invention which inhibit biofilm formation are suitable for such methods or for the manufacture of such compositions.

One feature of the present invention provides a method for preventing or treating biofilm formation on surfaces. According to an embodiment of the present invention, the formation of a biofilm can be prevented or treated by 1) contacting a surface, e.g., a biological surface or an inanimate or dynamic solid industrial surface such as a heat exchanger or "clean room" surfaces with an agent of present invention or by administering this agent to a subject, e.g., a human or an animal in need of such treatment. The compounds of present invention which inhibit biofilm formation are suitable for preventing or treating biofilm formation on surfaces.

Moreover, such agent of present invention can be administered to a population of bacteria, e.g., more than one bacterium to prevent or decrease the biofilm formation of the bacteria.

According to another feature of the present invention, bacteria, biofilms, or surfaces containing thereof can be sensitized for anti-microbial, e.g. anti-bacterial treatment using the agents of the present invention. For example, the agents of the present invention can be administered to a population of bacteria or bacteria within a biofilm so that these bacteria are primed or sensitized to be less resistant or more susceptible to anti-microbial treatments, e.g., antibiotics, target specific therapeutic agents, detergents, and biocides.

Bacteria or biofilms can be sensitized either prior to or simultaneously with any anti-microbial treatment. For example, the agents of the present invention can be used prior to or in combination with an anti-microbial agent to treat a surface, e.g., industrial or biological surface or subject, e.g., human for biofilm formation or microbial infections such as bacterial infection associated with biofilm formation. The subject in need of such treatment can be any suitable subject, e.g., a human or an animal including a domestic animal such as a horse, dog, or cat. Some of the compounds of present invention combine anti-microbial properties with biofilm formation properties and have an exceptional biofilm penetrating and microantimicrobial activity in a treatment by a single drug structure.

In particular embodiments of the present invention agents have been demonstrated to be suitable for inhibiting biofilm formations and thus interact with microbial intra- or interspecies signaling pathways that are association with or are controlling biofilm formation. These compounds of such embodiment of present invention can be acting via any mechanism known or later discovered that is suitable for inhibiting biofilm formations.

The methods provided by the present invention can be used to prevent or treat any biofilm. Biofilms in general are coatings formed via bacteria adhering to a surface, e.g., solid surface. Usually a population of bacteria interact or signal with each other directly or indirectly to form a highly hydrated matrix of exopolymers, typically polysaccharide, and other biopolymers on a surface. The formation of biofilm can take several steps and usually includes initial attachment and full maturity into a stable community.

According to the present invention, biofilms prevented or treated by the present invention can contain single species or multiple species bacteria. In one embodiment, the biofilms are associated with microbial infection or a disease condition including, without limitation, dental caries, periodontal disease, prostatitis, osteomyelitis, septic arthritis, and cystic fibrosis. In another embodiment, the biofilms contain gram positive bacteria including without limitation Streptococcus, e.g. *S. mutans* or gram negative bacteria

without limitation including *Salmonella*, e.g. *S.* Typhimurium, *S. Enteritidis, S. arizonae, S. bongori, S. cholerae-suis, S. choleraesuis, S. enterica, S. paratyphi, S. pullorum, S. subterranea, and S. typhi* or *Pseudomonas*, e.g; a bacterium of the *Pseudomonas aeruginosa* group such as *P. aeruginosa group P. aeruginosa, P. alcaligenes, P. anguilliseptica, P. argentinensis, P. borbori, P. citronellolis, P. flavescens, P. mendocina, P. nitroreducens, P. oleovorans, P. pseudoalcaligenes, P. resinovorans or P. straminea.*

Without wishing to be bound by theory, it is believed that the active compounds represented by the structural formula (I) are able to perform biofilm inhibition in the method of treatment of this invention through one or more of the following mechanisms of action:

- Activation of the PhoPQ regulon,
- Downregulation of csgD, a master regulator for biofilm formation, both in a PhoPQ dependent manner, and in a PhoPQ independent manner,
- Decreasing motility of microorganisms,
- Interfering with nucleotide biosynthesis, in particular purine nucleotide biosynthesis and pyrimidine nucleotide biosynthesis, thus potentially decreasing bioavailability of the messenger molecule c-di-GMP which is crucial for biofilm formation.

The biofilm inhibitor of this invention may be capable of complexing, or reversibly binding to, the organic matrix material, thereby rendering the organic matrix water insoluble. The biofilm inhibitor may exhibit greater binding affinity for functional groups in cellular proteins of microorganisms. When a microorganism contacts the anti-biofilm material of this invention, the organic material engages or disrupts at least the outer portion of the lipid bilayer of the microorganism's cell membrane sufficiently to permit insinuation of the biofilm inhibitor into the microorganism, where cell proteins or proteins in the lipid bilayer compete effectively for the biofilm inhibitor due to favourable binding constants. Stated another way, the biofilm inhibitor binds to or forms a complex with the organic material in which the association between the organic material and

biofilm inhibitor is sufficiently strong that the layer or film does not elute anti-biofilm amounts of the biofilm inhibitor into a contacting solution. However, the biofilm inhibitor preferentially binds to certain proteins in the microorganism and thus is transferred from the matrix to the microorganism. The result is a contact-biofilm preventing delivery system that selectively transfers the biofilm inhibitor or into the microorganism's cell membrane upon contact, without elution or dissolution of the biofilm inhibitor into solution, thereby maintaining the long term anti-biofilm efficacy of the composition.

In still another embodiment, the biofilms are associated with a surface, e.g., a solid surface. Such surface can be the surface of any industrial structure, e.g., pipeline or the surface of any structure in animals or humans. For example, such surface can be any epithelial surface, mucosal surface, or any host surface associated with bacterial infection, e.g., persistent and chronic bacterial infections. The surface can also include any surface of a bio-device in animals or humans, including without limitation, bio-implants such as bone prostheses, heart valves, and pacemakers.

In addition to surfaces associated with biofilm formation in a biological environment, the surfaces treated by the present invention can also be any surface associated with industrial biofilm formation. For example, the surfaces being treated can be any surface associated with biofouling of pipelines, heat exchangers, air filtering devices, or contamination of computer chips or water-lines in surgical units like those associated with dental hand-pieces.

The surface for present invention can include a plastic. The formation of biofilm on plastics for instance on silicons have been considered to be the key negative effect of microbes as it influences the hygiene and aesthetics of the concerned goods. There is a necessity to keep the surface of the plastic clean and hygienic. Moreover microorganisms have the capability to use any component of the material formulation as a nutrient. When this occurs, a reduction in the degree of mechanical properties of the plastic or silicon is observed. This will lead to brittleness, shrinkage, and loss of tensile strength. There is thus a need to prevent biofilm formation on certain plastics, which need can be answered

by the solution of present invention. As plastic materials are expected to ensure long-term performance and reliability, the introduction of an antimicrobial system into the plastic is essential to nullify the effect of microbial activity. Fungi and bacteria have been mainly responsible for undesired effects such as breakdown of polymer chain and degradation of ingredients. In yet another particular embodiment of present invention the biofilm inhibiting compounds of present invention are incorporated into the plastic in order to prevent biofilm formation. Such plastics which comprise the compounds effective in preventing biofilm formation or having antibacterial properties of present invention can be used for healthcare such as medical devices, packaging of medical devices, pharmaceutical packaging, hospital environment and utilities. Moreover the plastics that comprise the compounds of present invention can be used for industrial applications such as HVAC applications, food processing equipment, building and construction applications (for instance clean rooms), automotive, hydroponics installations, aquaculture systems and general packaging. There are also several consumer applications that benefit of the plastics which comprise the compounds of present invention such as electronic products, home appliances, sport wear and equipment, personal care products, infant care, toys and kitchenware. Silicone formulation that comprise the antimicrobial and/or biofilm inhibiting compounds of present inventions are useful for urinary tract stents, catheters, nephrostomy tubes and prosthetic bladder material for partial or eventually even total replacement of the bladder.

According to yet another feature the present invention provides drug compositions useful for treating biofilm formation and infections associated with biofilm formation. These compositions may contain an anti-microbial or anti-fungal entity and an active agent of the present invention. The anti-microbial entity can be any that is suitable for the present invention, e.g. detergents, biocides, antibiotics or target specific therapeutic entities. The antibiotics include, without limitation, penicillin, quinoline, vancomycin, sulfonamide, ampicillin, ciprofloxacin, and sulfisoxazole. The target specific therapeutic entities can include a targeting moiety coupled to an anti-microbial peptide moiety. U.S.

application Ser. No. 10/077,624 discloses various target specific anti-microbial entities and is incorporated herein by reference. The anti-fungal entity include, without limitation, ketoconazole, itraconazole, miconazole, or other compounds inhibiting P450-enzymes, and mixtures thereof in any proportions.

Such drug combinations are able to provide synergistic effects against biofilm formation or biofilm growth. As is conventional in the art, the evaluation of a synergistic effect in a drug combination may be made by analyzing the quantification of the interactions between individual drugs, using the median effect principle described by Chou et al. in *Adv. Enzyme Reg.* (1984) 22:27. Briefly, this principle states that interactions (synergism, additivity, antagonism) between two drugs can be quantified using the combination index (hereinafter referred as CI) defined by the following equation:

$$CI_x = \frac{ED_x^{1c}}{ED_x^{1a}} + \frac{ED_x^{2c}}{ED_x^{2a}}$$

wherein ED_x is the dose of the first or respectively second drug used alone (1a, 2a), or in combination with the second or respectively first drug (1c, 2c), which is needed to produce a given effect. The said first and second drug have synergistic or additive or antagonistic effects depending upon CI < 1, CI = 1, or CI > 1, respectively.

This principle may be applied to a combination of different drugs of the invention or to a combination of the drugs of the invention with other drugs that exhibit similar therapeutic effects on biofilm formation or biofilm growth.

In a particular embodiment the present invention provides methods for prophylactically treating a patient, and methods for disinfecting or sterilizing a surface ex-vivo to remove a biofilm or prevent biofilm growth are also disclosed, as well as implantable articles susceptible to biofilm growth to which a prophylactic coating of the surface with the compounds of present invention has been applied.

The composition or agent of the present invention can also include one or more other non-active ingredients, e.g., ingredients that do not interfere with the function of the active ingredients. For example, the composition or agent of the present invention can include a suitable carrier or be combined with other therapeutic agents.

A suitable carrier can be a powder, encapsulated solid, or an aqueous carrier including any safe and effective materials for use in the compositions of the present invention. In one embodiment, an aqueous carrier is used for the compositions of the present invention in oral formations and includes, without limitation, thickening materials, humectants, water, buffering agents, abrasive polishing materials, surfactants, titanium dioxide, flavor system, sweetening agents, coloring agents, and mixtures thereof.

The pure biofilm forming inhibiting compounds of present invention can be of value in managing a large variety of bacterial infections because particular compounds are highly specific and very effective in preventing the biofilm formation and are absolutely safe to humans and do not affect the growth planktonic microbials.

The present invention envisions using the biofilm formation inhibitors found (in combination with antibiotics or alone) to prophylactically or therapeutically eliminate various bacteria capable of causing diseases of the gastrointestinal, genitourinary, and respiratory tracts, and skin, oral cavity, and bloodstream or for preventing them colonizing on or in surfaces. In accordance with this invention, the selected biofilm formation inhibitors can be administered in a number of ways, in various formulations, including: (i) orally, in tablets or liquids, (ii) locally, in tampons, rinses or creams, (iii) aerosols, and (iv) intravenously.

The compounds of present invention can also be formulated into gels and foams. Thus, devices that dispense gels or foams can be used for their administration or application.

One benefit of biofilm formulation inhibiting therapy when compared to antibiotic / antimicrobial therapy relates to the relative specificity of the two therapeutic modalities. Selected biofilm formation inhibitors can be specific for biofilm, while antibiotics /

antifungals / antimicrobials typically are affect the growth of bacterial species, yeasts and molds and their genera.

According to this invention, for human or animal treatment the biofilm formation inhibiting compounds are preferably formulated in pharmaceutical compositions containing the biofilm formation inhibitor and a pharmaceutically acceptable carrier, and can be stored as a concentrated aqueous solution or lyophilized powder preparation. The biofilm formation inhibitor may be formulated for oral administration by suspending or diluting compound preparation in aqueous medium that are non-toxic to humans. The pharmaceutical composition may contain other components so long as the other components do not reduce the effectiveness (ineffectively) of the biofilm formation inhibitor so much that the therapy is negated. Pharmaceutically acceptable carriers are well known, and one skilled in the pharmaceutical art can easily select carriers suitable for particular routes of administration (Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985).

Those of ordinary skill in the art are familiar with formulation and administration techniques, e.g., as discussed in Goodman and Gilman's The Pharmacological Basis of Therapeutics, current edition; Pergamon Press; and Remington's Pharmaceutical Sciences (current edition.) Mack Publishing Co., Easton, Pa. These techniques can be employed in appropriate aspects and embodiments of the invention.

The compounds utilized in the methods of the instant invention can be administered either alone or in combination with pharmaceutically acceptable carriers, excipients or diluents, in a pharmaceutical composition, according to standard pharmaceutical practice. The compounds can be administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration.

For example, the therapeutic or pharmaceutical compositions of the invention can be administered locally to the area in need of treatment. This can be achieved by, for example, but not limited to, local infusion during surgery, topical application, e.g., cream,

ointment, injection, catheter, or implant, said implant made, e.g., out of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. The administration can also be by direct injection at the site (or former site) of a tumor or neoplastic or pre-neoplastic tissue.

Still further, the therapeutic or pharmaceutical composition can be delivered in a vesicle, e.g., a liposome (see, for example, Langer, 1990, Science, 249:1527-1533; Treat et al., 1989, Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Bernstein and Fidler (eds.), Liss, N.Y., pp. 353-365).

The pharmaceutical compositions used in the methods of the present invention can be delivered in a controlled release system. In one embodiment, a pump can be used (see, Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald et al., 1980, Surgery, 88:507; Saudek et al., 1989, N. Engl. J. Med., 321:574). Additionally, a controlled release system can be placed in proximity of the therapeutic target (see, Goodson, 1984, Medical Applications of Controlled Release, Vol. 2, pp. 115-138).

The pharmaceutical compositions used in the methods of the instant invention can contain the active ingredient in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients, which are suitable for the manufacture of tablets. These excipients can be, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, such as microcrystalline cellulose, sodium crosscarmellose, corn starch, or alginic acid; binding agents, for example starch, gelatin, polyvinyl-pyrrolidone

or acacia, and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques to mask the taste of the drug or delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a water soluble taste masking material such as hydroxypropylmethyl cellulose or hydroxypropyl cellulose, or a time delay material such as ethyl cellulose, or cellulose acetate butyrate can be employed.

Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluents, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatine capsules wherein the active ingredient is mixed with water soluble carrier such as polyethyleneglycol or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions can contain the active material in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients can act as suspending agents and include, e.g., sodium carboxymethyl cellulose, methyl cellulose, hydroxypropylmethyl cellulose, sodium alginate, polyvinyl pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylene-oxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin or aspartame.

Oily suspensions can be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents can be added to provide a palatable oral preparation. These compositions can be preserved by the addition of an anti-oxidant, e.g., butylated hydroxyanisol, alpha-tocopherol, or ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present. These compositions can be preserved by the addition of antioxidant(s).

The pharmaceutical compositions used in the methods of the instant invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents can be naturally-occurring phosphatides, for example soy bean lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening, flavoring agents, preservatives and antioxidants.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations can also contain a demulcent, a preservative, flavoring and coloring agents and antioxidant.

The pharmaceutical compositions can be in the form of composition suitable for use as an inhalant.

The pharmaceutical compositions can be in the form of a sterile injectable aqueous solution. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution.

The sterile injectable preparation can also be a sterile injectable oil-in-water microemulsion where the active ingredient is dissolved in the oily phase. For example, the active ingredient can be first dissolved in a mixture of soybean oil and lecithin. The oil solution then introduced into a water and glycerol mixture and processed to form a microemulsion.

The injectable solutions or microemulsions can be introduced into a patient's blood stream by local bolus injection. Alternatively, it can be advantageous to administer the solution or microemulsion in such a way as to maintain a constant circulating concentration of the instant compound. In order to maintain such a constant concentration, a continuous intravenous delivery device can be utilized. An example of such a device is the Deltec CADD-PLUS™ model 5400 intravenous pump.

The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleagenous suspension for intramuscular and subcutaneous administration. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butane-diol. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The compounds used in the methods of the present invention can also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the inhibitors with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and

will therefore melt in the rectum to release the drug. Such materials include cocoa butter, glycerinated gelatine, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing a compound of this invention can be used. As used herein, topical application can include mouthwashes and gargles.

The compounds used in the methods of the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles and delivery devices, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

The methods and compounds of the instant invention can also be used in conjunction with other well known therapeutic agents that are selected for their particular usefulness against the condition that is being treated.

Preferably the pharmaceutical preparation is in unit dosage form. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component, e.g., an amount that is effective to achieve the desired purpose.

The actual dosage employed can be varied depending upon the requirements of the patient and the severity of the condition being treated. Determination of the proper dosage for a particular situation is within the skill of the art. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small amounts until the optimum effect under the circumstances is reached. For convenience, the total daily dosage can be divided and administered in portions during the day if desired.

The amount and frequency of administration of the compounds used in the methods of the present invention and, if applicable, other chemotherapeutic agents will be regulated according to the judgment of the attending clinician (physician) considering

such factors as age, condition and size of the patient as well as severity of the disease being treated.

In general, compounds of the invention and, in embodiments where combinational therapy is employed, other agents do not have to be administered in the same pharmaceutical composition, and may, because of different physical and chemical characteristics, have to be administered by different routes. The determination of the mode of administration and the advisability of administration, where possible, in the same pharmaceutical composition, is well within the knowledge of the skilled clinician. The initial administration can be made according to established protocols known in the art, and then, based upon the observed effects, the dosage, modes of administration and times of administration can be modified by the skilled clinician. The particular choice of compounds used will depend upon the diagnosis of the attending physicians and their judgment of the condition of the patient and the appropriate treatment protocol. The compounds can be administered concurrently (e.g., simultaneously, essentially simultaneously or within the same treatment protocol) or sequentially, depending upon the nature of the proliferative disease, the condition of the patient, and the actual choice of compounds used.

The determination of the order of administration, and the number of repetitions of administration of each therapeutic agent during a treatment protocol, is well within the knowledge of the skilled physician after evaluation of the disease being treated and the condition of the patient. The pharmaceutical compositions containing a biofilm formation inhibitor may be administered by parenteral (subcutaneously, intramuscularly, intravenously, intraperitoneally, intrapleurally, intravesicularly or intrathecally), topical, oral, rectal, inhalation, ocular, otic, or nasal route, as necessitated by choice of drug and disease.

Injection of specific biofilm formation inhibiting compounds directly into the bloodstream can target the compounds to tissues where biofilm formation has to be inhibited or prevented. If, after either oral or local administration, compounds get into the

bloodstream in sufficient numbers to eliminate bacteria from the bloodstream, septicemia may be treated by administering compound s orally (or locally). If the compounds do not get into the bloodstream in sufficient numbers to eliminate bacteria from the bloodstream, the utility of direct i.v. injection of compounds for treating septic infections can be used to treat bloodstream infections caused by pathogenic bacteria, and can provide an urgently needed means for dealing with currently untreatable septicemic infections.

One of the major concerns about the use of antibacterial compounds in clinical settings is the possible development of bacterial resistance against them. However, as with antimicrobial resistance, the development of resistance to compounds takes time. The successful use of compounds in clinical settings will require continual monitoring for the development of resistance, and, when resistance appears, the substitution of other compounds to which the bacterial mutants are not resistant. In general, compound preparations may be constructed by mixing several separately grown and well-characterized biofilm formation inhibiting monocompounds, in order to (i) achieve the desired, broad target activity of the compound preparation, (ii) ensure that the preparation has stable biofilm formation inhibiting properties, and (iii) minimize the development of resistance against the preparation.

A suitable carrier can also be a pharmaceutically acceptable carrier that is well known to those in the art. Such carriers include, without limitation, large, slowly metabolized macromolecules, e.g., proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles.

Pharmaceutically acceptable salts can also be used in the composition, for example, mineral salts such as sodium or stannous fluorides, or sulfates, as well as the salts of organic acids such as acetates, proprionates, carbonates, malonates, or benzoates. The composition can also contain liquids, e.g., water, saline, glycerol, and ethanol, as well as substances, e.g., wetting agents, emulsifying agents, or pH buffering agents.

According to another feature of the present invention, the compositions or agents of the present invention can be used to treat or prevent disease conditions associated with biofilm formation. For example, an effective amount of the composition or agent of the present invention can be administered to a subject, e.g., a human or an animal to treat or prevent disease conditions associated with biofilm formation. Alternatively, the agent of the present invention and an anti-microbial agent can be administered as separate compositions, either sequential or simultaneously to a subject to treat or prevent disease conditions associated with biofilm formation. Various disease conditions are associated with biofilm formation including, without limitation, dental caries, periodontal disease, prostatitis, osteomyelitis, septic arthritis, cystic fibrosis, and heart valve vegetations. In one embodiment, the disease conditions are on an epithelial surface or a mucosal surface, e.g., mouth, vagina, gastrointestinal tract, and esophageal tract.

In general, an effective amount of the agent or composition of the present invention to be administered to a subject can be determined on a case-by-case basis. Factors to be considered usually include the total surface area to be treated, age, body weight, stage of the condition, other disease conditions, duration of the treatment, and the response to the initial treatment.

Typically, the agents or compositions used in the present invention are prepared as a topical or an injectable, either as a liquid solution or suspension. However, solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The composition can also be formulated into an enteric-coated tablet or gel capsule according to known methods in the art. In one embodiment, the composition of the present invention can be formulated onto the surfaces of industrial or biological structures or as coatings thereto, e.g., coatings to prosthetic heart valves, prosthetic hearts, vascular stents, or prosthetic joints.

The agents or compositions of the present invention may be administered in any way which is industrially or medically acceptable which may depend on the condition or injury being treated. Possible administration routes include injections, by parenteral

routes such as intravascular, intravenous, intraepidural or others, as well as oral, nasal, ophthalmic, rectal, vaginal, topical, or pulmonary, e.g., by inhalation. The compositions may also be directly applied to industrial or tissue surfaces. Sustained release, pH dependent release, or other specific chemical or environmental condition mediated release administration is also specifically included in the invention, by such means as depot injections or erodible implants.

According to the present invention there is provided a biofilm inhibiting composition comprising: a liquid carrier, a surfactant, an emulsifier, an antioxidant, a stabilizer, or a detergent; a biofilm inhibitor and, bound to or complexed with the biofilm inhibitor, a film-forming organic polymeric material that interacts with the biofilm inhibitor such that the biofilm inhibitor is not eluted into contacting water at levels capable of imparting disinfecting action to said water, wherein said composition, when applied to a surface forms a non-permanent, adherent, water-insoluble film, and wherein the film is removable by treatment with an alcoholic solution containing a surfactant.

Furthermore biofilm inhibiting compositions that when applied on a surface, prevent biofilm formation on of or kill microorganisms contacting the films, but which do not leach or elute significant amounts of biofilm inhibitorsinto the contacting liquids at levels sufficient to provide disinfection in the contacting solution.

More particularly, biofilm inhibiting compositions of the invention comprise a liquid comprising a solution, dispersion, emulsion or suspension of a polymeric, film-forming material and a biofilm inhibitorin a liquid carrier, which, when applied to a surface, forms a water-insoluble polymeric film on the surface in which the biofilm inhibitor is non-leachably bound to, complexed with, associated with or dispersed. The polymeric film forming material preferably comprises a polymer, copolymer or adduct which contains segments that, when the polymer forms a film on a surface, are capable of engaging microorganisms that come in contact with it. The biofilm inhibitorpreferably is non-leachably attached to, complexed or associated with or dispersed within said film, but

is capable of being preferentially transferred directly from the polymeric film to the contacting microorganism due to a higher affinity for proteins within the microorganisms.

In one aspect, the composition comprises a combination of (i) an organic polymeric material which is capable of forming a layer, film or matrix, and (ii) a biofilm inhibitor which, upon application, becomes intercalated in the layer, film or matrix and that interacts sufficiently strongly with the organic material so that the biofilm inhibitor does not dissolve into or elute from the matrix into the surrounding environment. The organic material should possess two important properties: it should be capable of reversibly binding or complexing with the biofilm inhibitor, and should be capable of insinuating the biofilm inhibitor into the cell membrane of a microorganism in contact with it. The organic material preferably is capable of dissolving into or adhering to the cell membrane surrounding the microorganism. Preferred organic materials are those which can be applied on a surface as water-insoluble films and which bind the biofilm inhibitorin such a manner as to permit transfer of the biofilm inhibitorinto the microorganism, but will not release the biofilm inhibitorinto the surrounding environment, e.g., into the air or into any liquid in contact with the coated surface.

The biofilm inhibitor preferably is a low molecular weight material that prevents biofilm fomation of the microorganisms and is capable of complexing with or reversibly binding to the organic matrix material, thereby rendering the organic matrix water insoluble. The biofilm inhibitor exhibits greater binding affinity for functional groups in cellular proteins of microorganisms. When a microorganism contacts the antibiofilm material, the organic material engages or disrupts at least the outer portion of the lipid bilayer of the microorganism's cell membrane sufficiently to permit insinuation of the biofilm inhibitor into the microorganism, where cell proteins or proteins in the lipid bilayer compete effectively for the biofilm inhibitordue to favorable binding constants. Stated another way, the biofilm inhibitor binds to or forms a complex with the organic material in which the association between the organic material and biofilm inhibitoris sufficiently strong that the layer or film does not elute antibiofilmamounts of the biofilm

inhibitorinto a contacting solution. However, the biofilm inhibitor preferentially binds to certain proteins in the microorganism and thus is transferred from the matrix to the microorganism. The result is a contact-biofilm preventing delivery system that selectively transfers the biofilm inhibitor or into the microorganism's cell membrane upon contact, without elution or dissolution of the biofilm inhibitorinto solution, thereby maintaining the long term antibiofilmefficacy of the composition.

The compounds of present invention would be particularly useful in treating equipment or environments inhabited by bacterial genera such as *Pseudomonas* which can become resistant to commonly used disinfectants. The compounds are thus particularly useful for hospital environments, surgery rooms or environments where wounded human or animal subjects are housed since treatment of Pseudomonas infections are difficult to treat by the methods of the art.

Another environmental application is in food processing or preparation environments. The compounds of present invention would be particularly useful in treating equipment or environments inhabited by bacterial genera such as *Salmonella*. Compound compositions used to treat objects or the environment can be sprayed, painted, or poured, onto such objects or surfaces in solutions or the objects can be immersed in a solution containing a compound of present invention. The optimal numbers and timing of applications of compound compositions remains to be determined and would be predicated by the exact usage of such products.

This invention also contemplates compound cocktails which can be custom tailored to the pathogens that are prevalent in a certain situation. Typically, pathogenic bacteria would be initially isolated from a particular source and susceptibility testing of the pathogens to various biofilm formation inhibitor compounds and/or antibacterial compounds of present invention would be performed, analogous to antimicrobial susceptibility testing. Once each pathogen's compound susceptibility profile is determined, the appropriate compound cocktail can be formulated from compound strains

to which the pathogens are susceptible and administered to the patient or contacted with the surface or the environment.

Since the compound of present invention will often be used in institutional settings where pathogens are resistant to many of the currently used antimicrobial agents, compound cocktails will often consist of the biofilm formation inhibiting compounds of present invention for the most prevalent institutional pathogens of human and life stock. Also since enterococci are often involved in polymicrobial infections along with other gastrointestinal commensals, such as in pelvic wound infections, the approach of therapeutically using cocktails of biofilm formation inhibiting compounds of present invention against different bacterial species would be most appropriate.

Biofilm formation inhibitor cocktails can be applied contemporaneously, i.e. they can be applied at the same time (e.g., in the same application), or can be applied in separate applications spaced in time such that they are effective at the same time. The biofilm formation inhibitor can be applied as a single application, periodic applications, or as a continuous application.

The compounds of present invention can be used as sanitation agents in a variety of fields. Although the terms "compound " or "biofilm formation inhibitor " can be used below, it should be noted that, where appropriate, this term should be broadly construed to include a single biofilm formation inhibitor, multiple biofilm formation inhibitors, such as a biofilm formation inhibitor cocktail, and mixtures of a biofilm formation inhibitor with an agent, such as a disinfectant, a detergent, a surfactant, water, etc.

The efficacy of compound treatment to reduce bacterial load can be determined by quantification of the bacteria periodically in samples taken from the treated environment. In one embodiment, this can be performed daily. If administration of compound reduced bacterial load by at least 1 log as compared to the control (e.g., before treatment) within 48-98 hours after compound administration, then this dose of the particular compound is deemed efficacious. More preferably, colonization will be reduced by at least 3 logs.

According to some embodiments of the present invention, biofilm formation inhibitors can be used for food and agriculture sanitation (including meats, fruits and vegetable sanitation), hospital sanitation, home sanitation, military sanitation (including anti-bioterrorism applications and military vehicle and equipment sanitation), industrial sanitation, etc. Other applications not specifically mentioned are within the contemplation of the present invention.

The broad concept of biofilm formation inhibitor sanitation can be applied to other agricultural applications and organisms. Produce, including fruits and vegetables, dairy products, and other agricultural products consumed by humans can become contaminated with many pathogenic organisms, including *Listeria* and highly virulent organisms such as *E. coli* O157:H7.

The application of biofilm formation inhibiting preparations of this invention to agricultural produce can substantially reduce or eliminate the possibility of food-borne illness through application of a single compound or compound cocktails with specificity toward species of bacteria associated with food-borne illness. Biofilm formation inhibitor can be applied at various stages of production and processing to reduce bacterial contamination at that point or to protect against contamination at subsequent points. Specific biofilm formation inhibitor s can be applied to produce in restaurants, grocery stores, produce distribution centres, etc. For example, compound can be periodically or continuously applied to the fruit and vegetable contents of a salad bar. This can be through a misting or spraying process, washing process, etc., and can be provided as a supplement or a substitute or supplement to chemical sanitizers, such as hypochlorite, sulphur dioxide, etc.

In another embodiment, compound can be periodically or continuously applied to produce in a grocery store. In still another embodiment, compound can be applied to produce in produce distribution centres, in shipment vehicles, etc. Other applications are within the contemplation of the present invention.

Another embodiment of this application contemplates inclusion of a biofilm formation inhibitor of present invention or matrices or support media containing biofilm formation inhibitors with packaging containing meat, produce, cut fruits and vegetables, and other foodstuffs. Biofilm formation inhibiting preparations containing single biofilm formation inhibitor s or cocktails of biofilm formation inhibitors specific for the desired pathogen(s) can be sprayed, coated, etc. onto the foodstuff or packaging material prior to packaging. The biofilm formation inhibitor preparation can also be introduced into the package as part of a matrix that can release adsorbed or otherwise incorporated compound at a desirable rate by passive means, or can comprise part of a biodegradable matrix designed to release compound at a desirable rate as it degrades. Examples of passive release devices can include absorbent pads made of paper or other fibrous material, sponge, or plastic materials.

In another embodiment, a polymer that is suitable for packaging can be impregnated with a biofilm formation inhibiting preparation of this invention. Suitable methods for impregnating a polymer with a biofilm formation inhibiting preparation are well known in the art. Suitable polymers can include those polymers approved by the U. S. Food and Drug Administration for food packaging.

Biofilm formation inhibitors can be used to sanitize hospital facilities, including operating rooms, patient rooms, waiting rooms, lab rooms, or other miscellaneous hospital equipment. This equipment can include electrocardiographs, respirators, cardiovascular assist devices, intraaortic balloon pumps, infusion devices, other patient care devices, televisions, monitors, remote controls, telephones, beds, etc. The present invention provides a fast and easy way to sanitize certain sensitive equipment and devices.

In some situations, it can be desirable to apply the compound through an aerosol canister; in other situations, it can be desirable to wipe the compound on the object with a transfer vehicle; in still other situations, it can be desirable to immerse the object in a container containing compound s; and in others, a combination of methods, devices, or

techniques can be used. Any other suitable technique or method can be used to apply the compound to the area, object, or equipment.

The compounds of present invention can be used in conjunction with patient care devices. In one embodiment, compound can be used in conjunction with a conventional ventilator or respiratory therapy device to clean the internal and external surfaces between patients. Examples of ventilators include devices to support ventilation during surgery, devices to support ventilation of incapacitated patients, and similar equipment. This can include automatic or motorized devices or manual bag-type devices such as are commonly found in emergency rooms and ambulances. Respiratory therapy devices can include inhalers to introduce medications such as bronchodilators as commonly used with chronic obstructive pulmonary disease or asthma, or devices to maintain airway patency such as continuous positive airway pressure devices.

In another embodiment, biofilm formation inhibitors can be used to sanitize a living area, such as a house, apartment, condominium, dormitory, barracks, etc. The compound can also be used to sanitize public areas, such as theatres, concert halls, museums, train stations, airports, etc. The compounds can be applied to various rooms of a house, including the kitchen, bedrooms, bathrooms, garage, basement, etc. In embodiment, the compound can be used in the same manner as conventional cleaners (e.g., Lysol® cleaner, 409® cleaner, etc.).

The present invention with its antimicrobial and biofilm formation inhibiting compounds can be used in many industrial applications, including the animal husbandry industry. This includes, but is not limited to, the breeding, raising, storing, and slaughter of livestock or other animals.

The present invention provides a method for reducing the risk of bacterial infection or sepsis in a susceptible patient by treating the susceptible patient with a pharmaceutical composition containing a biofilm formation inhibitor of present invention of one or more strains which produce biofilm formation inhibiting infections in pathogenic bacteria. Preferably, treatment of the patient reduces the level of colonization

with pathogenic bacteria susceptible to the biofilm formation inhibitor of present invention by at least one log. In a typical embodiment, the susceptible patient is an immunocompromised patient selected from the group consisting of leukemia patients, lymphoma patients, carcinoma patients, sarcoma patients, allogeneic transplant patients, congenital or acquired immunodeficiency patients, cystic fibrosis patients, and AIDS patients. In a preferred mode, the patients treated by this method are colonized with the pathogenic bacteria subject to infection by said biofilm formation inhibitor.

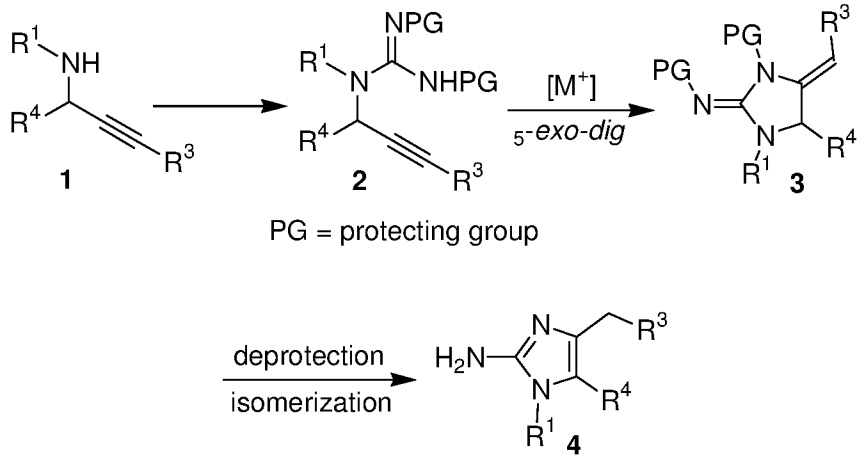
The following examples are purely illustrative of a few specific embodiments of the invention but should not be construed as limiting the scope thereof.

Example 1 - production methods

Herein, we report a rapid and highly efficient Ag^{I}-mediated synthesis of polysubstituted 2-aminoimidazoles from secondary propargylamines.

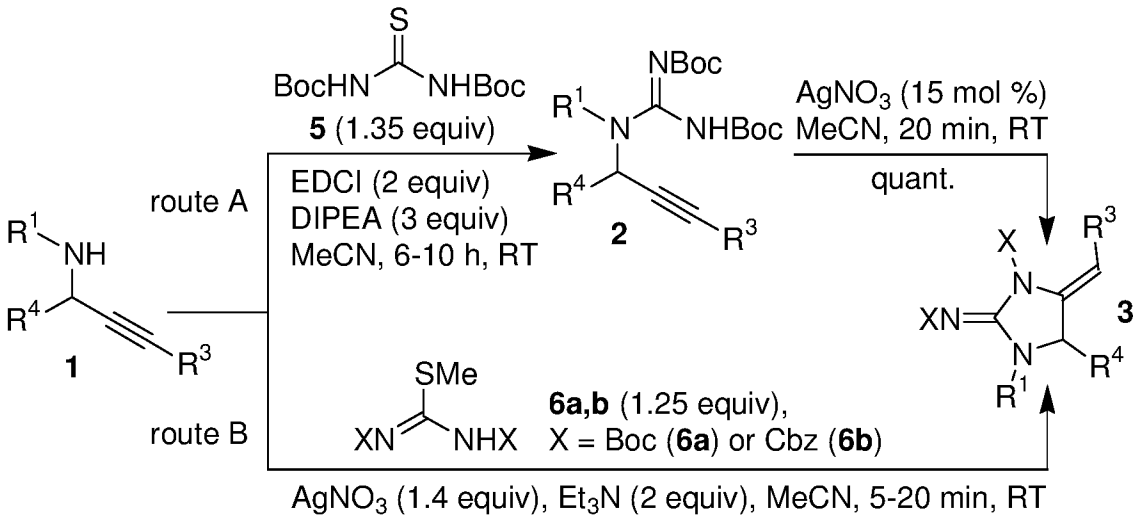
Propargylguanidine **2** was assembled from propargylamine **1** using a known guanylation procedure (Scheme 1 below). The subsequent intramolecular π-philic metal-catalyzed 5-*exo*-dig heterocyclization of propargylguanidine **2** into protected 2-iminoimidazoline **3** followed by deprotection and isomerization would provide the target 1,4,5-trisubstituted 2-aminoimidazole **4**.

Scheme 1



As recently reported, the microwave-assisted Cu^{I}-catalyzed three-component coupling of an aldehyde, an alkyne and a primary amine (A^3-coupling) provides a direct access to diverse secondary propargylamines **1**.

Initially, we focused on the guanylation of the sterically hindered propargylamines **1a-h** (Table 2, entries 1-8), using 1.35 equivalent of *N,N'*-bis-Boc-protected thiourea (**5**) in the presence of 2 equiv of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) and 3 equiv of Hünig's base (Scheme 2 below, route A). The resulting propargylguanidine **2a-h** were cyclized in the presence of various catalysts. Gratifyingly, the desired Boc-protected 2-iminoimidazolines **3a-h** were formed in quantitative yield within 20 minutes using 15 mol % of $AgNO_3$ in MeCN. Comparable results were obtained using 5 - 10 mol % of AgOTf or $Hg(OTf)_2$, while other catalysts such as CuCl, CuBr and $Cu(OTf)_2$ were less efficient, and other catalysts from gold, scandium, ytterbium and platinum were not efficient.



Scheme 2

Table 1 below provides yields obtained for a few exemplary compounds in this series.

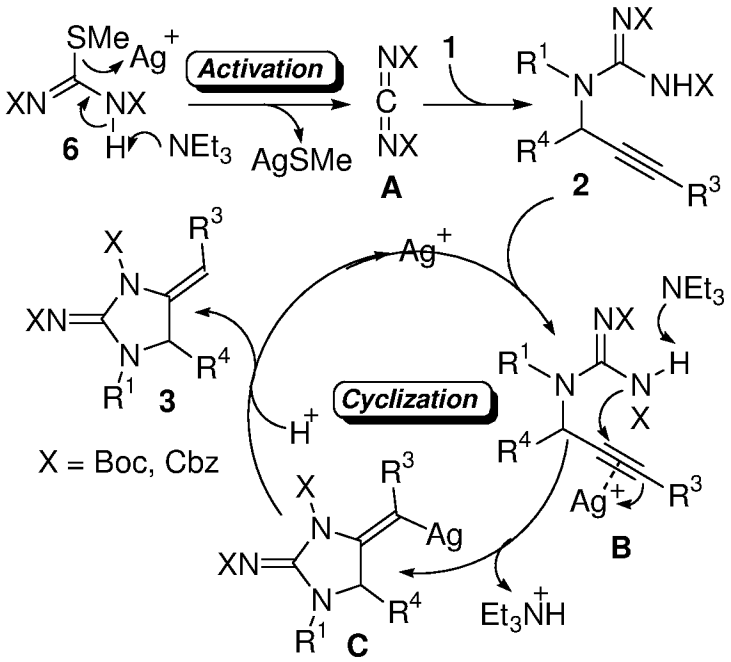
Table 1

Entry	**3**	X	R^1	R^4	R^3	Yield [%][a]
1	**3a**	Boc	Me	H	H	100, (98)[b]
2	**3b**	Boc	Bn	*c*Pr	Ph	98, (94)[b]
3	**3c**	Boc	Bn	*n*Pr	Ph	86, (87)[b]
4	**3d**	Boc	Bn	*i*Pr	*p*Tolyl	97, (91)[b]
5	**3e**	Boc	PMB	*p*FPh	*t*BuPh	84, (81)[b]
6	**3f**	Boc	*c*Oct	*n*Am	Ph	88, (87)[b]
7	**3g**	Boc	*c*Doc	*i*Bu	Ph	77, (68)[b]
8	**3h**	Boc	*n*Bu	*i*Bu	*p*Tolyl	98, (79)[b]
9	**3i**	Cbz	3,4-DMB-CH_2	H	Me	84
10	**3j**	Cbz	Bn	*c*Hex	Ph	99
11	**3k**	Boc	Bn	Ph	*c*Am	82
12	**3l**	Boc	Bn	*p*Tolyl	*p*AmOPh	80
13	**3m**	Boc	Bn	Ph	Bn	85
14	**3n**	Boc	(*S*)-1-MeBn	*c*Hex	PMP	79[c]
15	**3o**	Boc	*c*Bu	*c*Pr	*c*AmCH_2	83
16	**3p**	Cbz	Me	*p*BnOBn	PMP	87
17	**3q**	Boc	*t*Bu	*i*Bu	Ph	86
18	**3r**	Boc	*c*Hep	*i*Bu	Ph	78
19	**3s**	Cbz	Bn	*n*Pr	Ph	95
20	**3t**	Boc	3-MeOBn-CH_2	*i*Bu	Ph	99
21	**3u**	Cbz	Bn	*i*Pr	*p*Tolyl	98

22	**3v**	Boc	*p*Tolyl	*p*FPh	Ph	84

[a] Yield of isolated product via route B. [b] Yield of isolated product after two steps via route A. [c] Determined by ^{1}H NMR d.r. = 65:35.

Surprisingly, when we attempted the guanylation[14] of propargylamines **1** using 1.25 equiv of protected *S*-methylisothioureas **6** in the presence of 1.4 equiv of $AgNO_3$ and 2 equiv of Et_3N in MeCN, the corresponding protected 2-iminoimidazolines **3** were obtained quantitatively in a single step within 5 min at RT (Scheme 2, route B). All the Boc- and Cbz-protected 2-iminoimidazolines **3a-v** were obtained in a one-pot manner in high yields within 5-20 min. Remarkably, sterically hindered propargylamines efficiently underwent the cyclization (entries 6, 7, 14, 17 and 18) as well as *N*-arylpropargylamine (entry 22).



We presume that the reaction proceeds through a carbodiimide mechanism (Scheme 3 above). In the first step protected *S*-methylisothiourea **6** undergoes Ag^{I}-promoted methylsulfide elimination in the presence of base to form reactive carbodiimide intermediate **A**. Then the addition of propargylamine **1** to carbodiimide **A** gives the protected propargylguanidine intermediate **2**. The Ag^{I}-catalyzed cyclization and subsequent proton transfer to intermediate **B** finally provides the protected 2-iminoimidazoline **3**. The resulting insoluble AgSMe can be easily recovered into $AgNO_3$ and reused again. Remarkably, the presence of Boc or Cbz protecting groups facilitates the key steps of the process: 1) The activation of thiourea towards methylsulfide elimination; 2) the activation of carbodiimide towards addition of propargylamine; 3) the activation of guanidine function towards intramolecular hydroamination of alkyne.

The Boc-deprotection step was achieved using TFA-DCM (1:2) at RT (Scheme 4), and desired 2-aminoimidazoles **4a-f** were isolated as free bases in high yields (Table 2, entries 1-6). 2-Aminoimidazoles **4a-f** were found to effectively inhibit biofilm formation by pathogenic *Salmonella* Typhimurium and *Pseudomonas aeruginosa* bacteria without a significant influence on the planktonic growth (Table 2, entries 3, 5 and 6). Thus, it is the first evidence of anti-biofilm activity of polysubstituted 2-aminoimidazoles against Gram-negative bacteria.

Next we applied our protocol to the total synthesis of 1,4,5-trisubstituted derivatives of the so-called naamine family (Scheme 5 below). The starting *N*-methylpropargylamines **11a-f** were easily accessed via microwave-assisted Cu^{I}-catalyzed A^3-coupling reaction, followed by Pd^{0}-catalyzed deallylation in the presence of 1,3-dimethylbarbituric acid (DMBA). The Ag^{I}-promoted cycloguanylation of propargylamines and the subsequent removal of Boc and Bn protective groups provided the target naamines A, C, E-G (**13a-e**) and leucettamine A (**13f**) in high overall yields (Scheme 5). Characterizing data (in particular nuclear magnetic resonance and infrared spectra) of such compounds are given below.

Scheme 4 - deprotection (Reaction conditions: 2-iminoimidazoline **3** (0.2 mmol), TFA (2.0 mL), dichloromethane (4.0 mL), 1-3 h, RT.) of bis-Boc 2-iminoimidazolines **3**

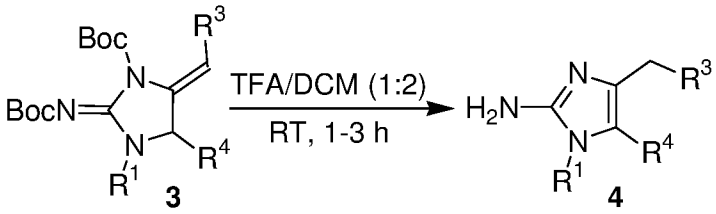
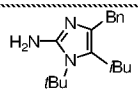
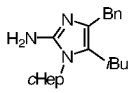
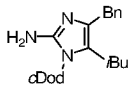
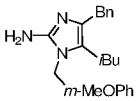
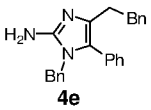
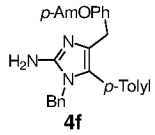
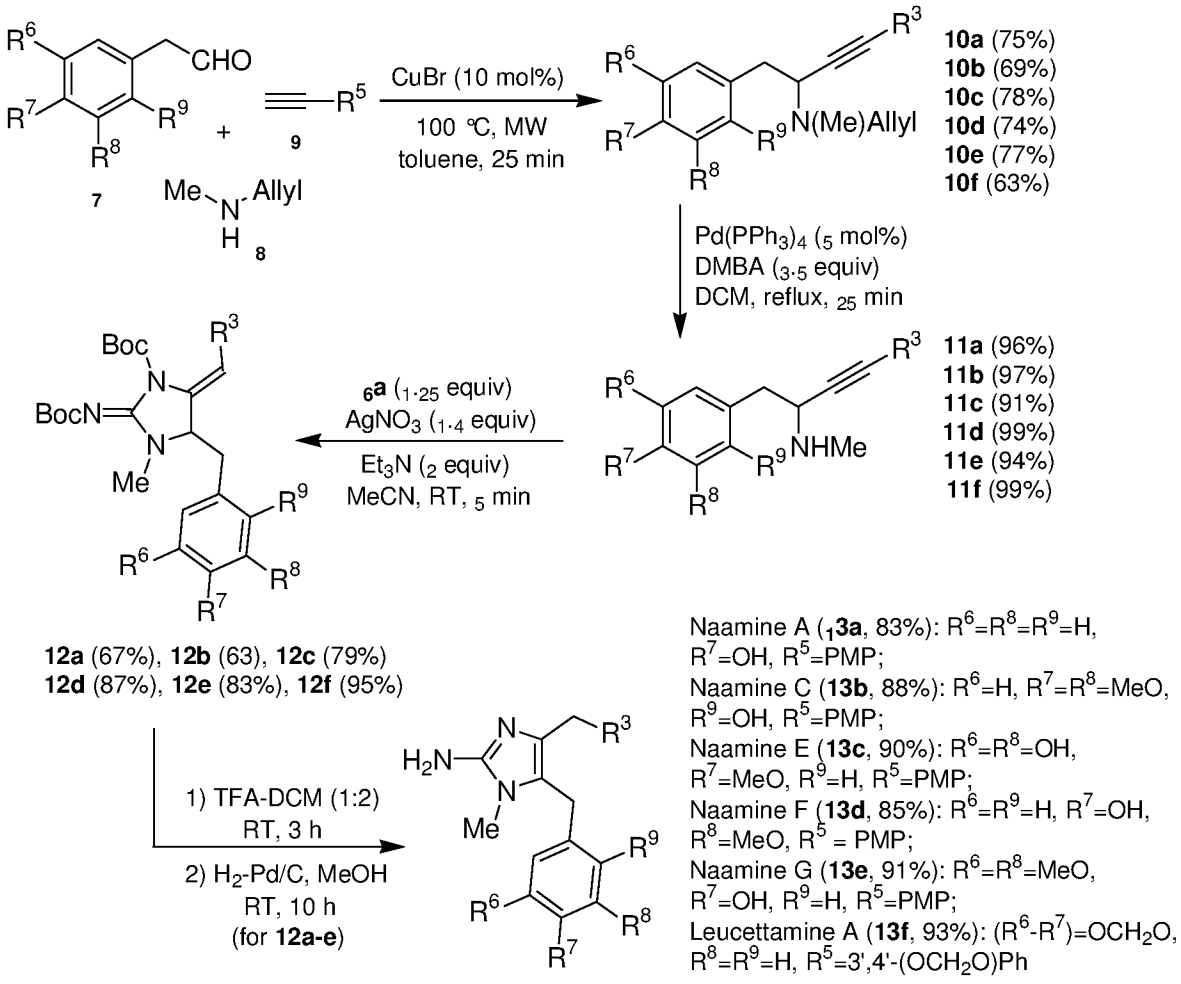


Table 2 - ***c***ompounds made through deprotection of bis-Boc 2-iminoimidazolines **3** and inhibitory activity against bacterial biofilm formation.

Entry	**4**	Yield[b] [%]	*S.* Typhimurium ATCC14028[c]	PA14[c]
1	 **4a**	85	42.4	377.1
2	 **4b**	95	74.4	109.0
3	 **4c**	78	18.3	17.4
4	 **4d**	80	100.3	85.6
5	 **4e**	75	20.1	19.8

6	 **4f**	92	10.3	27.4

[b] Yield of isolated product. [c] IC_{50} values are given in μM.



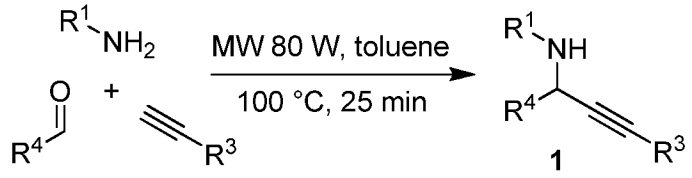
Scheme 5

Moreover, as for the above amino- or imino-protective groups, a prodrug of the compound of the invention can be produced by introducing a pro-drug forming group

well known in the art (e.g. phosphate, phosphonate, carboxylic acid) at the starting material level or at any intermediate stage of the above synthetic methods.

The following is a detailed description of exemplary precursors, intermediates and final compounds that have been made according to the above stated synthetic principles, including their NMR and infrared characterizing data.

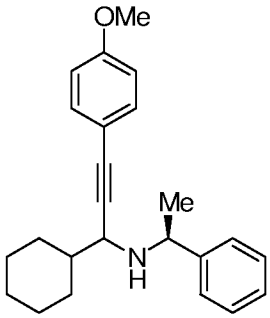
Synthesis of Secondary Propargylamines **1**



To a microwave vial equipped with a magnetic stir bar were added amine (1.5 mmol), aldehyde (1.0 mmol), acetylene (3.0 mmol), copper bromide (0.2 mmol) and toluene (1.0 mL). The mixture was degassed and backfilled with argon. The reaction vessel was sealed and irradiated in the cavity of CEM-Discover microwave reactor at a ceiling temperature of 100 °C and a maximum power of 80 W for 25 min. The resulting reaction mixture was cooled to ambient temperature and diluted with dichloromethane (2 mL), loaded on a silica gel column and flashed with 10-20% EtOAc in heptane to afford the product as light yellow oil.

Illustrative but non-limiting examples are given below, together with characterizing data:

1-Cyclohexyl-3-(4-methoxyphenyl)-*N*-((*S*)-1-phenylethyl)prop-2-yn-1-amine **(1n)**



Minor diastereoisomer:

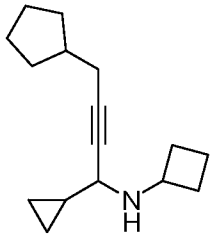
- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.45-7.22 (m, 7H), 6.83 (d, *J* = 8.7 Hz, 2H), 4.14 (q, *J* = 6.4 Hz, 1H), 3.81 (s, 3H), 3.50 (d, *J* = 5.7 Hz, 1H), 1.84-1.07 (m, 13H).
- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 160.0, 146.3, 133.6 (×2), 133.5, 128.5 (×2), 126.9 (×2), 115.8, 114.2, 113.9 (×2), 88.9, 84.1, 83.7, 55.8, 55.5, 53.8, 42.5, 30.5, 28.4, 26.6, 26.4, 26.2, 22.3.
- DEPT-135 NMR (75.5 Hz, $CDCl_3$): δ = 133.6, 133.1, 128.5, 127.0, 126.9, 114.0, 113.9, 55.5, 55.3, 53.8, 42.5, - 30.5, - 28.4, - 26.6, - 26.4, - 26.2, 22.3.
- HRMS (EI) *m/z* Calculated for $C_{24}H_{29}NO$: 347.2249, Observed: 347.2251.

Major diastereoisomer:

- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.45-7.22 (m, 7H), 6.86 (d, *J* = 8.7 Hz, 2H), 4.23 (q, *J* = 6.4 Hz, 1H), 3.82 (s, 3H), 3.05 (d, *J* = 5.7 Hz, 1H), 1.84-1.07 (m, 13H).
- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 159.2, 145.3, 133.1 (×2), 128.4 (×2), 127.1 (×2), 127.0, 115.9, 114.0, 113.9 (×2), 89.1, 84.1, 75.8, 55.4, 55.3, 53.7, 43.1, 30.2, 29.0, 26.6, 26.3, 26.2, 25.5.
- DEPT-135 NMR (75.5 Hz, $CDCl_3$): δ = 133.1, 128.4, 127.1, 126.9, 113.9, 113.8, 55.8, 55.3, 53.7, 43.1, - 30.2, - 29.0, - 26.6, - 26.3, - 26.2, 25.5.

- HRMS (EI) *m/z* Calculated for $C_{24}H_{29}NO$: 347.2249, Observed: 347.2251.

N-(4-Cyclopentyl-1-cyclopropylbut-2-ynyl)cyclobutanamine **(1o)**



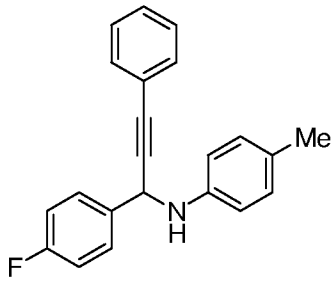
- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.51 (m, 1H), 3.22 (d, *J* = 6.4 Hz, 1H), 2.24 (m, 2H), 2.17 (d, *J* = 6.7 Hz, 2H), 2.01 (m, 1H), 1.88-1.53 (m, 10H), 1.26 (m, 2H), 1.04 (m, 1H), 0.48-0.33 (m, 4H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 81.6, 77.8, 50.9, 50.2, 37.8, 30.5 (×2), 30.4, 30.0, 23.9 (×2), 23.1, 14.4, 13.7, 1.8, 0.02.

- DEPT-135 NMR (75.5 Hz, $CDCl_3$): δ = - 50.9, - 50.2, -37.8, 30.5 (×2), 30.4, 30.0, 23.9 (×2), 23.1, - 14.4, 13.7, 1.8, 0.02.

- HRMS (EI) *m/z* Calculated for $C_{16}H_{25}N$: 231.1987, Observed: 231.1998.

N-(1-(4-Fluorophenyl)-3-phenylprop-2-ynyl)-4-methylbenzenamine **(1v)**

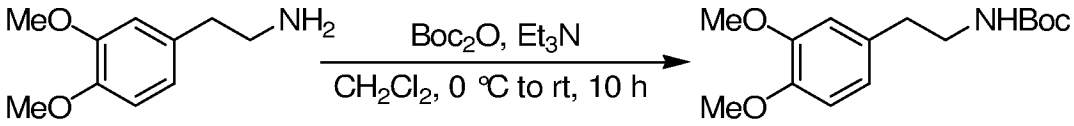


- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.61 (m, 2H), 7.40 (m, 2H), 7.28 (m, 3H), 7.10-7.00 (m, 4H), 6.67 (d, *J* = 8.3 Hz, 2H), 5,44 (s, 1H), 2.25 (s, 3H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 164.1, 160.1, 144.1, 135.7 (d, *J* = 3.3 Hz), 132.1, 131.8 (×2), 129.7 (×2), 128.9 (d, *J* = 8.2 Hz), 128.4, 128.3, 128.2 (×2), 128.1, 122.7, 115.7, 115.4, 144.4 (×2), 88.4, 85.2, 50.3, 20.5.

- HRMS (EI) *m/z* Calculated for $C_{22}H_{18}FN$: 315.1423, Observed: 315.1428.

Preparation of *tert*-butyl 3,4-dimethoxyphenethylcarbamate



In a 100 mL two-necked RBF containing a magnetic stir were added 2-(3,4-dimethoxyphenyl)ethanamine (2.4 g, 13.3 mmol), triethylamine (2.8 mL, 20 mmol, 1.5 eq), 4'-dimethylaminopyridine (32 mg, 2 mol.%) and CH_2Cl_2 (25 mL). The reaction mixture was cooled on an ice bath, and a solution of Boc-anhydride (3.5 g, 16 mmol, 1.2 eq) in CH_2Cl_2 (15 mL) was added dropwise upon vigorous stirring. The reaction was allowed to proceed for 10 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (150 mL) and washed with 1N HCl (100 mL), saturated $NaHCO_3$ (50 mL) and brine (100 mL). The organic layer was then collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification of the material was accomplished by flash column chromatography eluting with 40% EtOAc in heptane (R_f 0.35). The fractions containing product were combined and then concentrated under reduced pressure to give Boc-protected amine (2.91 g, 78% yield) as colorless oil characterized as follows:

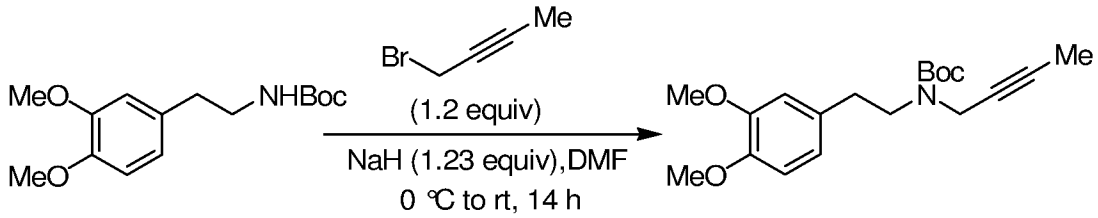
- ^1H NMR (300 MHz, $CDCl_3$): δ = 6.83-6.71 (m, 3H), 4.55 (br, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.35 (m, 2H), 2.74 (t, *J* = 7.3 Hz,), 1.44 (s, 9H);

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 156.3, 149.4, 148.0, 131.9, 121.1, 112.4, 111.7, 79.6, 56.3, 42.3, 36.2, 28.8.

- IR 3372, 2980, 2940, 1682, 1512, 1463, 1448, 1364, 1294, 1277, 1260, 1229, 1141, 1065, 1025, 990, 868, 779 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{15}H_{23}NO_4$: 281.1627, Observed: 281.1631.

Preparation of *tert*-butyl 3,4-dimethoxyphenethylbut-2-ynylcarbamate



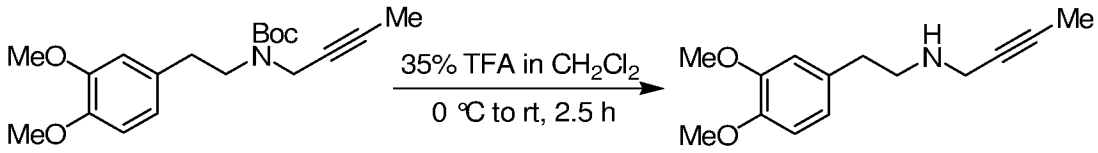
In a 100 mL two-necked RBF containing a magnetic stir under inert atmosphere the Boc-protected 2-(3,4-dimethoxyphenyl)ethanamine (2.0 g, 7.1 mmol) was dissolved in DMF (30 mL). The reaction mixture was cooled on an ice bath, and sodium hydride (60% in mineral oil, 0.35 g, 8.7 mmol) was added y small portions upon vigorous stirring. The reaction was allowed to proceed for 14 h at room temperature. Then the reaction mixture was quenched by water (20 mL) and extracted by diethyl ether (2 × 100 mL). The organic layer was then collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification of the material was accomplished by flash column chromatography eluting with 20% EtOAc in heptane (R_f 0.27). The fractions containing product were combined and then concentrated under reduced pressure to give Boc-protected propargylamine (2.02 g, 85% yield) as viscous yellow oil characterized as follows:

- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.79 (m, 3H), 3.97 (br, 2H), 3.88 (s, 3H), 3.86 (s, 3H), 3.50 (t, *J* = 7.3 Hz, 2H), 2.80 (t, *J* = 7.3 Hz, 2H), 1.82 (s, 3H), 1.45 (s, 9H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 155.0, 148.9, 147.5, 120.8, 112.1, 111.3, 79.9, 74.9, 56.0, 48.3, 36.5, 34.4, 28.4, 14.2, 3.6.

- HRMS *m/z* (EI) Calculated for $C_{19}H_{27}NO_4$: 333.1940, Observed: 333.1939.

Preparation of *N*-(3,4-dimethoxyphenethyl)but-2-yn-1-amine



To a 50 mL two-necked RBF containing a magnetic stir and the Boc-protected propargylamine (1.0 g, 3 mmol) 35% solution of TFA in CH_2Cl_2 (15 mL) was added upon cooling on an ice bath. After 0.5 h the ice bath was removed and the reaction was allowed to proceed for another 2.5 h at room temperature. Then the solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (150 mL), washed with saturated $NaHCO_3$ (50 mL) and brine (100 mL). The organic layer was then collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure to give free propargylamine (0.64 g, 91% yield) as yellowish oil characterized as follows:

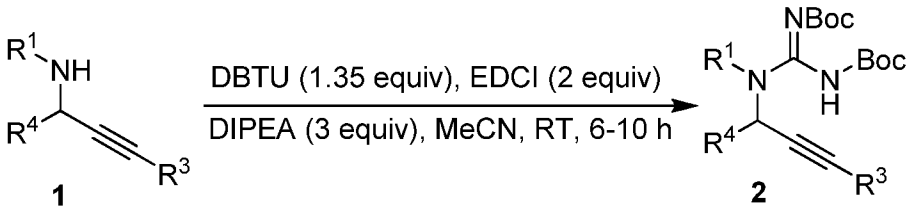
- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.78 (m, 3H), 3.88 (s, 3H), 3.87 (s, 3H), 3.38 (m, 2H), 2.92 (t, *J* = 7.3 Hz, 2H), 2.77 (t, *J* = 7.3 Hz, 2H), 1.81 (s, 3H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 148.9, 147.5, 132.5, 120.6, 112.0, 111.3, 79.0, 56.0, 50.1, 38.6, 35.8, 3.5.

- IR 2917, 2834, 1667, 1605, 1590, 1562, 1513, 1451, 1417, 1359, 1332, 1259, 1233, 1190, 1154.

- HRMS *m/z* (EI) Calculated for $C_{14}H_{19}NO_2$: 233.1416, Observed: 233.1420.

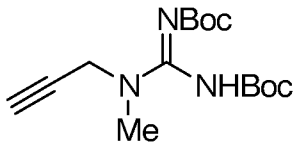
Guanylation of Propargylamines 1 with di-Boc-isothiourea



A stirred solution of propargylamine (0.3 mmol), 1,3-bis(*tert*-butoxycarbonyl)thiourea (DBTU, 115 mg, 0.41 mmol, 1.35 equiv), and diisopropylethylamine (112 mg, 0.9 mmol, 3 equiv) in anhydrous MeCN (2 mL) was cooled to 0 °C. EDCI (115 mg, 0.6 mmol, 2 equiv) was added, and the solution was stirred at RT overnight. The reaction mixture was diluted with EtOAc (50 mL), and washed with water (3×100 mL), followed by brine and dried over anhydrous Na_2SO_4. The residue remaining after removal of the solvent was subjected to column chromatography on silica gel using 20% EtOAc in heptane as eluent (R_f 0.25-0.3) .

Illustrative but non-limiting examples are given below, together with characterizing data:

1-Methyl-1-propargyl-2,3-di-(*tert*-butoxycarbonyl)guanidine **(2a)**

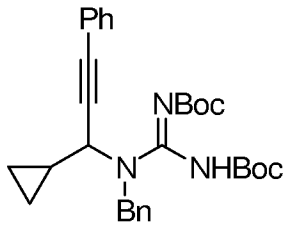


- ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.65 (br, 1H), 4.31 (d, *J* = 2.5 Hz, 2H), 3.11 (s, 3H), 2.32 (t, *J* = 2.5 Hz, 1H), 1.51 (br, 18H).

- ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.6, 151.9, 118.2, 85.9, 77.8, 73.2, 36.5, 28.1, 27.9.

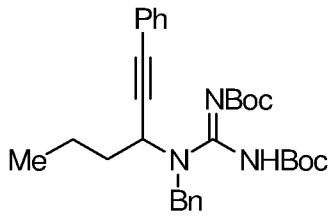
- HRMS *m/z* (EI) Calculated for $C_{15}H_{25}N_3O_4$: 311.1845, Observed: 311.1850.

1-Benzyl-1-(1'-cyclopropyl-3'-phenylprop-2'-ynyl)-2,3-di-(*tert*-butoxycarbonyl)guanidine **(2b)**



- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.81 (br, 1H), 7.38-7.21 (m, 10H), 5.58 (br, 1H), 4.87 (dd, *J* = 45.6, 16.6 Hz, 2H), 1.49 (m, 18H), 0.65 (m, 1H), 0.51 (m, 3H).
- ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 162.8, 156. 8, 155.1, 139.0, 134.8, 131.2, 128.9, 128.8, 128.7, 128.5, 128.1, 128.0, 121.1, 87.5, 85.7, 79.5, 79.1, 65.1, 49.8, 48.2, 8.5, 4.3.
- HRMS *m/z* (EI) Calculated for $C_{30}H_{37}N_3O_4$: 503.2784, Observed: 503.2787.

1-Benzyl-1-(1'-propyl-3'-phenylprop-2'-ynyl)-2,3-di-(*tert*-butoxycarbonyl)guanidine **(2c)**



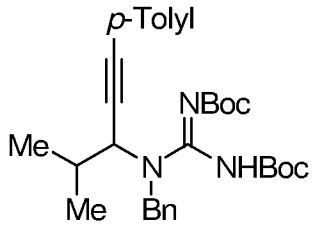
- ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.06 (br, 1H), 7.40-7.21 (m, 10H), 4.95 (d, *J* = 16.1 Hz, 1H), 4.78 (d, *J* = 16.1 Hz, 1H), 4.40 (m, 1H), 1.95-1.66 (m, 3H), 1.60-1.16 (br, 18H), 1.00-0.87 (m, 5H).

- ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.4, 154.0, 137.9, 134.1, 131.7, 128.9, 128.7, 128.5, 128.3, 128.2, 128.1, 128.0, 127.3, 127.2, 122.6, 121.5, 87.3, 85.6, 79.9, 79.4, 64.7, 49.5, 48.5, 36.8, 28.3, 28.1, 19.5, 14.0.

- IR 2962, 2930, 2872, 1737, 1638, 1391, 1364, 1285, 1250, 1142, 1076, 1050, 755, 727 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{30}H_{39}N_3O_4$: 505.2941, Observed: 505.2957.

1-Benzyl-1-(1'-isopropyl-3'-(*p*-tolyl)-2'-ynyl)-2,3-di-(*tert*-butoxycarbonyl)guanidine **(2d)**



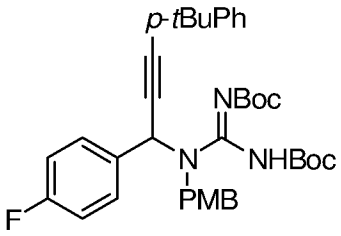
- ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.42 (br, 1H), 7.33-7.09 (m, 9H), 5.27 (m, 1H), 4.85 (d, *J* = 19.1 Hz, 1H), 4.55 (d, *J* = 19.0 Hz, 1H), 2.28 (s, 3H), 2.05 (m, 1H), 1.42-1.22 (m, 18H), 1.10 (br, 3H), 0.94 (br, 3H).

- ^{13}C NMR (100 MHz, DMSO-d_6): δ = 161.9, 159.9, 138.9, 138.8, 137.4, 131.5, 129.7, 129.6, 129.3, 128.4, 127.9, 127.0, 119.4, 80.0, 79.1, 77.6, 70.1, 47.9, 28.5, 28.4, 21.4, 20.0, 19.3.

- IR: 3209, 2980, 2930, 1737, 1472, 1300, 1251, 1231, 1105, 1048, 903, 852 cm^{-1}.

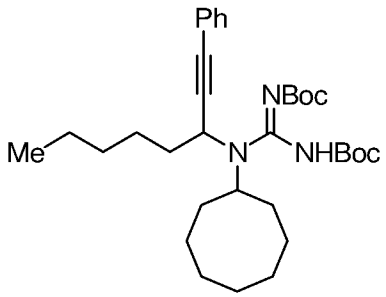
- HRMS *m/z* (EI) Calculated for $C_{31}H_{41}N_3O_4$: 519.3097, Observed: 519.3116.

1-(*p*-Methoxybenzyl)-1-(1'-(*p*-fluorophenyl)-3'-(*p*-tert-butylphenyl)-2'-ynyl)-2,3-di-(*tert*-butoxycarbonyl)guanidine **(2e)**



- ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.59 (br, 1H), 7.62 (m, 2H), 7.43-7.26 (m, 7H), 7.05 (d, *J* = 8.3 Hz, 2H), 6.77 (d, *J* = 8.7 Hz, 2H), 4.55 (d, *J* = 16.2 Hz, 1H), 4.33 (d, *J* = 16.2 Hz, 1H), 3.67 (s, 3H), 3.34 (s, 1H), 1.37 (s, 9H), 1.27 (br, 18H) .
- ^{13}C NMR (100 MHz, DMSO-d_6): δ = 159.2, 158.0, 133.3, 131.2, 129.5, 129.4, 128.0, 125.4, 118.5, 115.7, 115.3, 113.4, 85.0, 77.4, 54.9, 47.1, 34.8, 34.5, 30.4, 27.9, 27.7, 25.8.
- DEPT-135 (100 MHz, DMSO-d_6): δ = 131.3, 129.5, 129.4, 125.5, 115.7, 115.3, 113.4, - 54.9, - 30.4, - 27.9, - 27.7.
- IR: 2975, 2928, 2852, 1743, 1681, 1607, 1365, 1292, 1210, 1177, 1146, 1124, 1092, 991 cm^{-1}.

1-Cyclooctyl-1-(1'-pentyl-3'-phenylprop-2'-ynyl)-2,3-di-(*tert*-butoxycarbonyl)guanidine **(2f)**



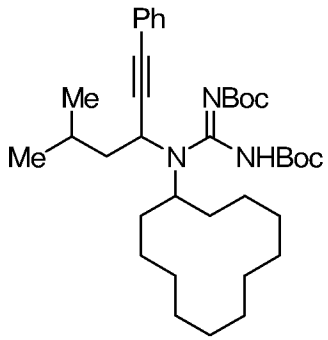
- ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41 (m, 2H), 7.32 (m, 3H), 4.08 (br, 1H), 2.09-1.28 (m, 40H), 0.91 (s, 3H).

- ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.1, 150.4, 136.0, 131.7, 128.8, 128.3, 128.0, 115.6, 87.9, 81.6, 78.7, 58.4, 50.2, 43.1, 31.3, 28.3, 28.2, 27.3, 26.5, 26.4, 26.2, 25.5, 22.5, 14.0.

- IR 2979, 2974, 1732, 1680, 1621, 1447, 1391, 1365, 1286, 1244, 1146, 752, 732, 696 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{33}H_{51}N_3O_4$: 553.3880, Observed: 553.3869.

1-Cyclododecyl-1-(1'-isobutyl-3'-phenylprop-2'-ynyl)-2,3-di-(*tert*-butoxycarbonyl)-guanidine **(2g)**

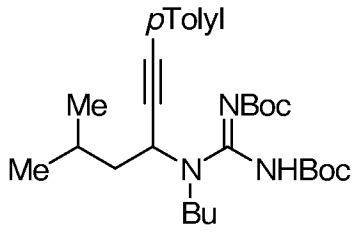


- ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.14 (br, 1H), 7.52-7.17 (m, 5H), 4.41 (br, 1H), 3.92 (m, 1H), 1.94-0.87 (m, 49H).

- ^{13}C NMR (100 MHz, $CDCl_3$): δ = 159.6, 148.5, 135.9, 134.0, 131.8, 128.8, 128.3, 128.1, 128.0, 127.2, 116.1, 82.6, 81.4, 78.6, 57.8, 51.2, 43.5, 31.3, 28.3, 28.1, 27.5, 27.3, 25.7, 25.1, 24.6, 24.0, 23.4, 22.9, 22.3, 21.9, 21.4.

- HRMS *m/z* (EI) Calculated for $C_{36}H_{57}N_3O_4$: 595.4349, Observed: 595.4362.

1-Butyl-1-(1'-isobutyl-3'-(*p*-tolyl)-2'-ynyl)-2,3-di-(*tert*-butoxycarbonyl)guanidine **(2h)**



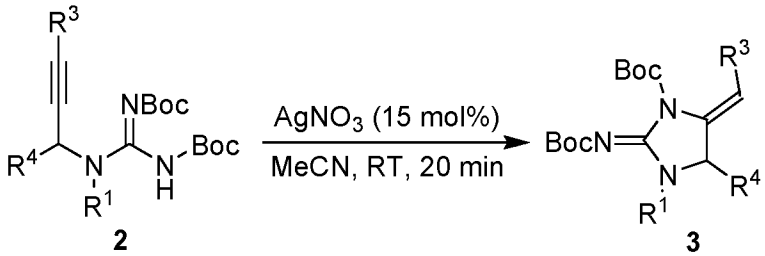
- ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.24 (br, 1H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.11 (d, *J* = 8.1 Hz, 2H), 3.54 (t, *J* = 7.6 Hz, 1H), 2.34 (s, 3H), 1.87-1.65 (m, 6H), 1.50 (br, 18H), 1.36 (m, 2H), 0.96 (m, 10H).

- ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.2, 153.5, 150.9, 138.4, 131.5, 129.0, 128.3, 119.7, 86.6, 85.0, 79.6, 46.0, 43.4, 35.4, 31.0, 28.3, 28.2, 28.1, 25.1, 23.1, 22.4, 21.8, 21.4, 20.4, 13.8.

- IR: 2970, 2931, 1735, 1638, 1591, 1509, 1455, 1389, 1365, 1300, 1249, 1149, 815 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{22}H_{38}N_3O_4$: 408.2862, Observed : 408.2864.

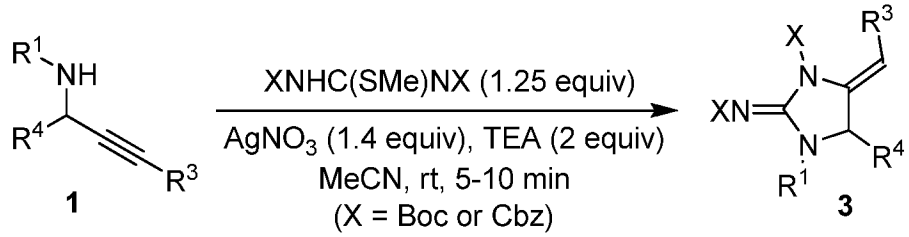
Cyclization of *N*-propargylguanidines 2 into 2-iminoimidazolines **3**



To a solution of *N*-propargylguanidine **2** (0.5 mmol) in MeCN (2 mL) was added silver nitrate (12.8 mg, 15 mol%) and the reaction was stirred until the starting material has disappeared (20 min). Then the reaction was diluted with EtOAc, followed by

filtration through short Celite pad. The resulting organic layer was evaporated under reduced pressure, and the residue was subjected to silica gel column chromatography (eluted with 20-30% EtOAc in heptane) providing the target compound **3** as amorphous white foam.

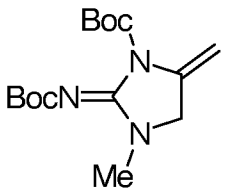
Coupling and Cyclization of Propargylamines 1 with Protected Isothioureas



To a solution of propargylamine (0.5 mmol) and *N,N'*-bis-protected *S*-methylisothiourea (0.63 mmol, 1.25 equiv) in MeCN (2.5 mL) was added triethylamine (0.14 mL, 1 mmol, 2 equiv) under an argon atmosphere. After dissolution, silver nitrate (119 mg, 0.7 mmol, 1.4 equiv) was added and the heterogeneous reaction was vigorously stirred until the starting material has disappeared. Then the reaction was diluted with EtOAc, followed by filtration through Celite pad. The resulting organic layer was washed with brine (2 × 100 mL) and dried over anhydrous Na_2SO_4. Filtration, followed by removal of the solvent under reduced pressure resulted in a yellow oil which was subjected to silica gel column chromatography (eluted with 5-10% diethyl ether in CH_2Cl_2) providing the target compound as amorphous white foam.

Illustrative but non-limiting examples are given below, together with characterizing data:

tert-Butyl-2-(*tert*-butoxycarbonyl)-3-methyl-5-methyleneimidazolidine-1-carboxylate **(3a)**

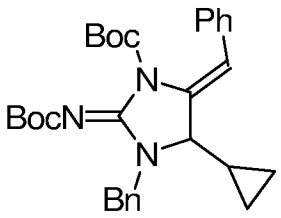


- ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.21 (m, 1H), 4.48 (m, 1H), 4.09 (m, 2H), 2.96 (s, 3H), 1.58 (s, 9H), 1.53 (s, 9H).

- ^{13}C NMR (100 MHz, $CDCl_3$): δ = 159.3, 153.0, 149.4, 137.0, 92.0, 84.2, 78.9, 51.4, 31.8, 28.4, 28.1, 28.0. IR (neat) 2978, 2933, 1782, 1706, 1601, 1478, 1455, 1391, 1366, 1245, 1135, 1048, 869, 775 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{15}H_{25}N_3O_4$: 311.1845, Observed: 311.1851.

(5*Z*)-*tert*-Butyl-3-benzyl-5-benzylidene-2-(*tert*-butoxycarbonylimino)-4-cyclopropyl-imidazolidine-1-carboxylate **(3b)**



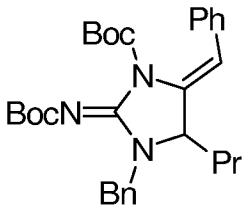
^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.45 (d, *J* = 7.53 Hz, 2H), 7.35-7.18 (m, 8H), 6.01 (s, 1H), 5.15 (d, *J* = 15.45 Hz, 1H), 4.45 (d, *J* = 15.6 Hz, 1H), 3.45 (d, *J* = 6.78 Hz, 1H), 1.59 (s, 9H), 1.03 (s, 9H), 0.93-0.82 (m, 1H), 0.59-0.54 (m, 2H), 0.27-0.26 (m, 2H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 158.2, 150.4, 146.8, 134.8, 134.4, 131.0, 127.4, 127.1, 126.6, 126.2, 126.0, 125.7, 115.2, 81.8, 77.7, 61.8, 44.6, 26.7, 25.8, 11.1, 1.8, -1.4.

IR 2972, 2929, 1742, 1489, 1451, 1389, 1361, 1280, 1240, 1218, 1188, 1150, 1133, 1111, 1087, 1048, 1025, 1001, 725 cm^{-1}.

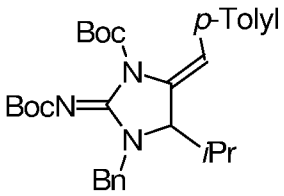
HRMS *m/z* (EI) Calculated for $C_{30}H_{37}N_3O_4$: 503.2784, Observed : 503.2757.

(*Z*)-*tert*-Butyl 3-benzyl-5-benzylidene-2-(*tert*-butoxycarbonyl)-4-propylimidazoli-dine-1-carboxylate **(3c)**



- ^1H NMR (300 MHz, $CDCl_3$): δ = 7.45 (d, *J* = 7.3 Hz, 2H), 7.34–7.18 (m, 8H), 5.84 (s, 1H), 5.05 (d, *J* = 14.6 Hz, 1H), 4.25 (d, *J* = 14.6 Hz, 1H), 3.87 (m, 1H), 1.59 (s, 9H), 1.48 (m, 2H), 1.25 (m, 2H), 1.03 (s, 9H), 0.82 (t, *J* = 7.3 Hz, 3H).
- ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 159.8, 152.5, 148.4, 136.1, 135.9, 133.3, 128.8 (×4), 128.2 (×2), 128.1 (×2), 127.8, 127.2, 116.2, 83.2, 79.3, 59.8, 46.6, 34.4, 28.3, 27.3, 16.6, 13.9.
- IR: 2973, 2935, 1747, 1681, 1630, 1446, 1389, 1360, 1290, 1246, 1215, 1126, 1103, 765, 717 cm^{-1}.
- HRMS *m/z* (EI) Calculated for $C_{30}H_{39}N_3O_4$: 505.2941, Observed : 505.2944.

(*Z*)-Benzyl 3-benzyl-5-*p*-tolylidene-2-(benzyloxycarbonyl)-4-*i*-propylimidazolidine-1-carboxylate **(3d)**



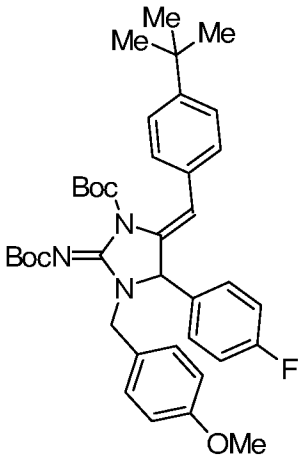
- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35 (m, 7H), 7.14 (d, *J* = 8.2 Hz, 2H), 5.78 (s, 1H), 5.15 (d, *J* = 15.5 Hz, 1H), 4.23 (d, *J* = 15.5 Hz, 1H), 3.68 (m, 1H), 2.34 (s, 3H), 1.59 (s, 9H), 1.48 (m, 2H), 1.05 (s, 9H), 0.99 (d, *J* = 7.3 Hz, 3H), 0.79 (d, *J* = 7.3 Hz, 3H).

- ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 159.8, 153.0, 148.1, 137.0, 136.2, 133.1, 130.6, 128.8 (×4), 128.7, 128.0 (×2), 127.7, 118.3, 83.0, 79.2, 64.9, 46.9, 30.2, 28.3, 27.3, 21.3, 18.5, 15.4.

- IR: 2975, 2929, 1742, 1677, 1628, 1514, 1499, 1451, 1389, 1367, 1281, 1242, 1218, 1176, 1149, 1129, 1113, 1080, 1041, 1028, 1003, 721 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{31}H_{41}N_3O_4$: 519.3097, Observed.: 519.3103.

<u>*tert*-Butyl (*Z*)-1-(*tert*-butoxycarbonyl)-5-(4-*tert*-butylbenzylidene)-3-(4-methoxybenzyl)-4-(4-fluorophenyl)imidazolidin-2-ylidenecarbamate</u> **(3e)**



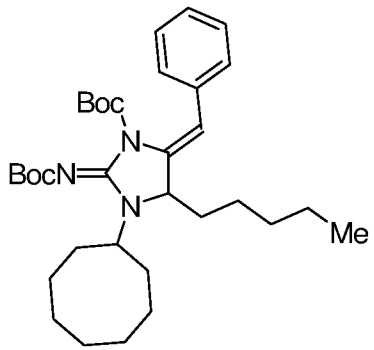
- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.31 (s, 4H), 7.14 (m, 2H), 7.05 (m, 4H), 6.80 (d, *J* = 8.7 Hz, 2H), 5.67 (d, *J* = 1.5 Hz, 1H), 5.17 (d, *J* = 15.1 Hz, 1H), 4.78 (d, *J* = 1.5 Hz, 1H), 3.78 (s, 3H), 3.70 (d, *J* = 15.1 Hz, 1H), 1.63 (s, 9H), 1.29 (s, 9H), 0.96 (s, 9H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 164.6, 161.3, 159.7, 159.2, 151.8, 150.5, 148.5, 133.4, 133.2, 130.0, 129.2 (d, *J* = 8.2 Hz), 128.5, 127.3, 124.9, 117.4, 116.0 (d, *J* = 22.0 Hz), 114.0, 83.5, 79.5, 62.6, 55.3, 45.4, 34.6, 31.2, 28.3, 27.1.

- DEPT-135 NMR (75.5 Hz, $CDCl_3$): δ = 130.0, 129.2 (d, *J* = 8.2 Hz), 128.5, 124.9, 117.4, 116.0 (d, *J* = 22.0 Hz), 114.0, 83.5, 79.5, 62.6, 55.3, - 45.4, 31.2, 28.3, 27.1. IR (neat) 2967, 1744, 1685, 1633, 1417, 1240, 1170, 1144, 1119, 1102, 1033, 1013, 844, 747 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{38}H_{46}FN_3O_5$: 643.3421, Observed : 643.3371.

tert-Butyl (*Z*)-1-(*tert*-butoxycarbonyl)-5-benzylidene-3-cyclooctyl-4-pentylimidazolidin-2-ylidenecarbamate **(3f)**



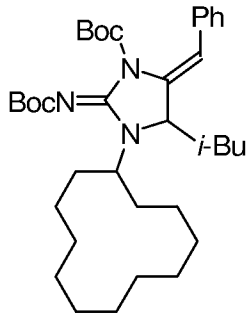
- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.45 (d, *J* = 7.5 Hz, 2H), 7.31 (m, 2H), 7.19 (t, *J* = 7.0 Hz, 1H), 5.87 (s, 1H), 4.15-4.05 (m, 2H), 1.96 (m, 1H), 1.79-1.27 (m, 31H), 1.01 (s, 9H), 0.88 (t, *J* = 5.7 Hz, 3H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 160.0, 151.4, 148.3, 136.0, 134.0, 128.8, 128.0, 127.0, 115.5, 82.7, 78.9, 59.0, 55.2, 34.9, 32.2, 31.8, 31.3, 31.2, 28.3, 27.3, 26.9, 25.4, 25.0, 24.4, 23.0, 22.5, 14.1.

- DEPT-135 NMR (75.5 Hz, $CDCl_3$): δ = 128.8, 128.0, 127.0, 115.5, 59.0, 55.2, - 34.9, - 32.2, - 31.8, - 31.2, 28.3, 27.3, - 26.9, - 25.4, - 25.0, - 24.4, - 23.0, - 22.5, 14.1.

- IR: 2925, 2858, 1742, 1681, 1619, 1448, 1284, 1242, 1144, 1104, 696 cm^{-1}.

(5*Z*)-*tert*-Butyl-5-benzylidene-2-(*tert*-butoxycarbonylimino)-3-cyclododecyl-4-isobutyl-imidazolidine-1-carboxylate **(3g)**



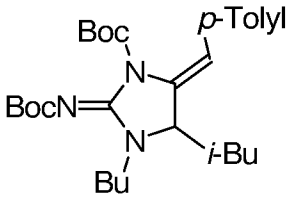
- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.47 (d, *J* = 7.3 Hz, 2H), 7.34-7.17 (m, 3H), 5.89 (s, 1H), 4.17 (bs, 1H), 3.91 (t, *J* = 6.7 Hz, 1H), 1.88-1.58 (m, 3H), 1.55 (s, 9H), 1.45-1.32 (m, 22H), 1.03-1.02 (m, 12H), 0.92 (d, *J* = 6.7 Hz, 3H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 159.6, 151.4, 148.4, 135.8, 133.9, 128.7,128.0, 127.1, 116.0, 82.5, 78.5, 57.7, 51.1, 43.3, 29.8, 28.8, 27.7, 27.2, 24.6, 24.5, 24.4, 23.9, 23.4, 23.3, 23.0, 22.8, 22.6, 22.6, 22.3, 21.8.

- IR: 2980, 2930, 1740, 1710, 1615, 1364, 1287, 1250, 1149, 1096, 1071, 696 cm^{-1}.

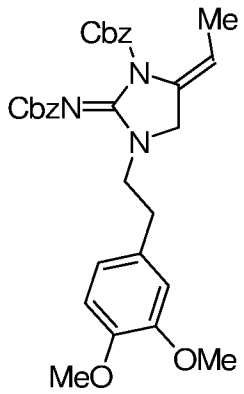
- HRMS *m/z* (EI) Calculated for $C_{36}H_{57}N_3O_4$: 595.4349, Observed : 595.4345.

tert-Butyl (*Z*)-1-(*tert*-butoxycarbonyl)-5-(4-methylbenzylidene)-3-butyl-4-isobutylimidazolidin-2-ylidenecarbamate **(3h)**



- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35 (d, *J*= 8.3 Hz, 2H), 7.12 (d, *J*= 8.3 Hz, 2H), 5.89 (s, 1H), 3.93 (m, 1H), 2.99 (m, 1H), 2.34 (s, 3H), 1.80 (m, 1H), 1.63-1.25 (m, 17H), 1.03-0.90 (m, 18H).
- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 159.9, 151.6, 148.7, 137.0, 132.9, 132.6, 128.7, 116.6, 82.7, 79.0, 59.2, 42.3, 41.1, 29.8, 28.3, 27.3, 24.2, 23.7, 22.3, 21.3, 20.1, 13.9.
- DEPT-135 NMR (75.5 Hz, $CDCl_3$): δ = 128.7, 116.6, 59.2, - 42.3, - 41.1, - 29.8, 28.3, 27.3, 24.2, 23.7, 22.3, 21.3, - 20.1, 13.9.
- IR: 2979, 2930, 2872, 1744, 1715, 1683, 1628, 1514, 1456, 1389, 1365, 1291, 1239, 1141, 1085, 990 cm^{-1}.
- HRMS *m/z* (EI) Calculated for $C_{29}H_{45}N_3O_4$: 499.341, Observed.: 499.344.

Benzyl (*Z*)-3-((benzyloxy)carbonyl)-1-(3,4-dimethoxyphenethyl)-4-ethylidene-imidazolidin-2-ylidenecarbamate **(3i)**



- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.34 (m, 10H), 6.71 (m, 3H), 5.13 (s, 2H), 5.05 (m, 1H), 5.04 (s, 2H), 3.84 (s, 3H), 3.77 (s, 3H), 3.61 (t, *J* = 7.3 Hz, 2H), 2.85 (t, *J* = 7.3 Hz, 2H), 1.55 (d, *J* = 6.4 Hz, 3H).

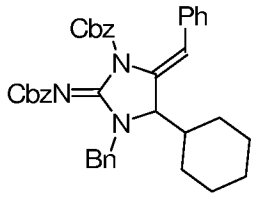
- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 160.4, 152.6, 150.9, 149.5, 148.1, 137.2, 135.3, 131.3, 129.4, 129.1, 128.9, 128.7, 128.2, 121.0, 114.1, 112.3, 111.7, 69.4, 67.9, 56.3, 56.2, 51.1, 46.9, 33.2, 14.7.

- DEPT-135 NMR (75.5 Hz, $CDCl_3$): δ = 129.4, 129.0, 128.9, 128.2, 120.9, 114.1, 112.2, 111.7, - 69.4, - 67.9, 56.3, 56.2, 51.1, 46.9, - 33.2, 14.7.

- IR: 2955, 2835, 1741, 1714, 1688, 1619, 1514, 1497, 1376, 1334, 1281, 1259, 1234, 1202, 1155, 1139, 1115, 1024 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{31}H_{33}N_3O_6$: 543.2369, Observed.: 543.2377.

(5*Z*)-Benzyl-3-benzyl-5-benzylidene-2-(benzyloxycarbonylimino)-4-cyclohexyl-imidazolidine-1-carboxylate **(3j)**



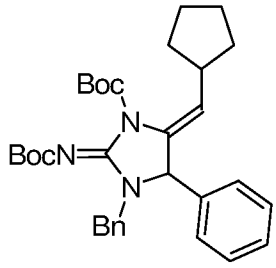
- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.45 (d, *J* = 7.71 Hz, 2H), 7.37-7.17 (m, 16H), 7.00 (d, *J* = 7.35 Hz, 2H), 5.84 (s, 1H), 5.22-5.10 (m, 3H), 5.01 (d, *J* = 11.9 Hz, 1H), 4.53 (d, *J* = 11.9 Hz, 1H), 4.19 (d, *J* = 15.4 Hz, 1H), 3.71 (s, 1H), 1.57-1.45 (m, 5H), 1.25-1.08 (m, 2H), 0.96-0.90 (m, 1H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 160.2, 152.7, 149.2, 136.9, 135.6, 135.1, 134.7, 130.9, 128.8, 128.6, 128.3, 128.3, 128.2, 128.2, 128.2, 127.9, 127.9, 127.7, 127.3, 127.3, 118.5, 68.4, 67.5, 64.4, 46.8, 40.2, 28.6, 26.1, 25.9, 25.8.

- IR: 2929, 1748, 1675, 1629, 1494, 1466, 1449, 1383, 1368, 1272, 1232, 1219, 1123, 1077, 1025, 1001, 761 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{39}H_{39}N_3O_4$: 613.2941, Observed.: 613.2944.

(5*Z*)-*tert*-Butyl-3-benzyl-2-(*tert*-butoxycarbonylimino)-5-(cyclopentylmethylene)-4-phenylimidazolidine-1-carboxylate **(3k)**



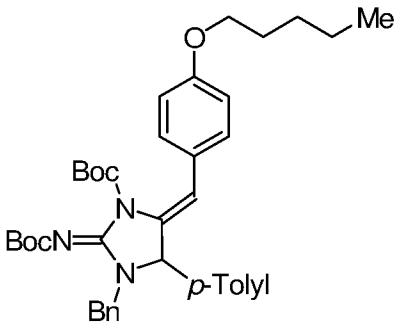
- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.32-7.27 (m, 6H), 7.13-7.08 (m, 4H), 5.24 (d, *J* = 15.06 Hz, 1H), 4.85 (d, *J* = 9.03 Hz, 1H), 4.58 (s, 1H), 3.72 (d, *J* = 15.27 Hz, 1H), 2.93-2.80 (m, 1H), 1.86-1.77 (m, 2H), 1.56 (s, 9H), 1.52-1.51 (m, 4H), 1.46 (s, 9H), 1.25-1.13 (m, 2H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 159.4, 153.3, 149.8, 137.5, 135.5, 132.4, 128.9,128.6, 128.4, 127.6, 126.8, 125.3, 83.6, 79.0, 62.8, 46.05, 38.3, 32.9, 28.3, 28.0, 25.1, 25.1.

- IR: 2971, 1742, 1689, 1626, 1495, 1454, 1391, 1366, 1285, 1251, 1137, 1105, 1075, 1028, 1000, 945, 845, 744 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{32}H_{41}N_3O_4$: 531.3097, Observed.: 531.2142.

(5*Z*)-*tert*-Butyl-3-benzyl-2-(*tert*-butoxycarbonylimino)-5-(4-(pentyloxy)benzylidene)-4-*p*-tolylimidazolidine-1-carboxylate **(3l)**



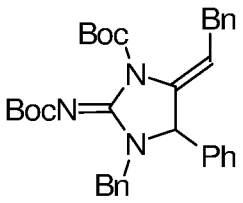
- ^1H NMR (300 MHz, $CDCl_3$): δ = 7.31-7.26 (m, 6H), 7.17-7.13 (m, 4H), 7.04 (d, *J* = 7.44 Hz, 2H), 6.81 (d, *J* = 8.1 Hz, 2H), 5.67 (s, 1H), 5.26 (d, *J* = 15.4 Hz, 2H), 4.73 (s, 1H), 3.93 (t, *J* = 6.6 Hz, 2H), 2.35 (s, 3H), 1.78-1.74 (m, 2H), 1.61 (s, 9H), 1.40 (br, 4H), 1.03 (s, 9H), 0.92 (t, *J* = 6.96 Hz, 3H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 159.7, 158.2, 152.2, 148.7, 138.7, 135.5,134.2, 132.2,130.1, 129.6, 128.6, 128.6, 128.3, 127.6, 127.2, 117.0, 114.0, 83.2, 79.3, 68.0, 63.0, 53.4, 45.6, 28.8, 28.2, 28.1, 27.2, 22.4, 21.2, 14.0.

- IR: 2928, 2844, 1740, 1699, 1623, 1441, 1284, 1242, 1144, 1044, 678 cm^{-1}.

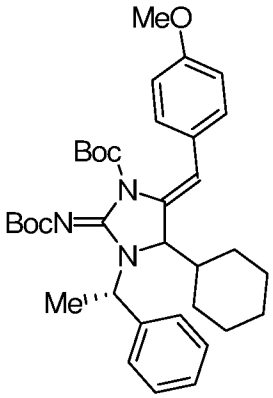
- HRMS *m/z* (EI) Calculated for $C_{39}H_{49}N_3O_5$: 639.3672, Observed: 639.3687.

(5*Z*)-*tert*-Butyl-3-benzyl-2-(*tert*-butoxycarbonylimino)-4-phenyl-5-(2-phenylethylidene)-imidazolidine-1-carboxylate **(3m)**



- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.32-7.11 (m, 15H), 5.30-5.25 (m, 1H), 5.11-5.07 (m, 1H), 4.62 (s, 1H), 3.76-3.69 (m, 1H), 3.62-3.33 (m, 2H), 1.58 (s, 9H), 1.47 (s, 9H).
- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 159.5, 152.9, 149.8, 140.0, 137.3, 135.4, 134.1, 129.3, 129.0, 128.7, 128.6, 128.5, 128.4, 127.7, 126.6, 126.2, 118.4, 83.9, 79.2, 62.6, 60.4, 46.0, 35.0, 28.3, 28.0, 21.0, 14.2.
- IR: 2928, 1745, 1684, 1657, 1357, 1235, 1222, 1141, 1100, 690 cm^{-1}.
- HRMS *m/z* (EI) Calculated for $C_{34}H_{39}N_3O_4$: 553.2941, Observed.: 553.2911.

tert-Butyl (*Z*)-1-(*tert*-butoxycarbonyl)-5-(4-methoxybenzylidene)-4-cyclohexyl-3-((*S*)-1-phenylethyl)imidazolidin-2-ylidenecarbamate **(3n)**



Minor diastereoisomer:

- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.52 (d, *J* = 6.4 Hz, 2H), 7.43-7.23 (m, 5H), 6.85 (d, *J* = 8.7 Hz, 2H), 5.75 (m, 2H), 3.86 (d, *J* = 2.3 Hz, 2H), 3.82 (s, 3H), 1.83 (m, 1H), 1.62 (d, *J* = 7.2 Hz, 3H), 1.57 (s, 9H), 1.55-1.10 (m, 10H), 1.05 (s, 9H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 160.4, 158.6, 153.3, 148.3, 140.4, 130.2 (×2), 128.7 (×2), 128.6 (×2), 117.4, 113.4 (×2), 62.6, 55.4, 51.0, 40.7, 29.3, 28.3 (×2), 27.4 (×2), 26.4, 26.3, 25.4, 25.3, 15.8.

- DEPT-135 NMR (75.5 Hz, $CDCl_3$): δ = 130.2 (×2), 128.7 (×2), 128.6 (×2), 117.4, 113.4 (×2), 82.7, 79.2, 62.6, 55.4, 51.0, 40.7, - 29.4, 28.3 (×2), 27.4 (×2), - 26.4, - 26.3, - 25.4, - 25.3, 15.8.

- IR: 2975, 2928, 2852, 1743, 1681, 1624, 1607, 1511, 1292, 1244, 1210, 1177, 1146, 1124, 1092, 1027, 991 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{35}H_{47}N_3O_5$: 589.3516, Observed.: 589.3526.

Major diastereoisomer:

- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.38 (d, *J* = 8.7 Hz, 2H), 7.35-7.23 (m, 5H), 6.84 (d, *J* = 8.7 Hz, 2H), 5.62 (m, 2H), 3.81 (s, 3H), 3.36 (d, *J* = 2.6 Hz, 1H), 1.79 (m, 1H), 1.68 (d, *J* = 7.2 Hz, 3H), 1.60 (s, 9H), 1.55-1.10 (m, 10H), 1.07 (s, 9H).

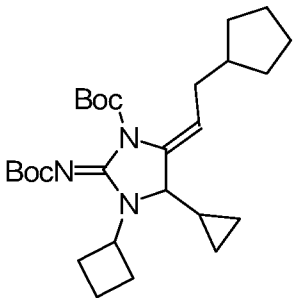
- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 160.2, 158.6, 153.1, 148.3, 139.0, 130.2 (×2), 128.7 (×2), 128.4, 127.5 (×2), 117.7, 113.4 (×2), 82.7, 79.2, 63.7, 55.4, 52.5, 42.7, 29.4, 28.3 (×2), 27.4 (×2), 26.6, 26.5, 26.0, 26.7, 18.1.

- DEPT-135 NMR (75.5 Hz, $CDCl_3$): δ = 130.2 (×2), 128.7 (×2), 128.4, 127.5 (×2), 117.7, 113.4 (×2), 63.8, 55.4, 52.5, 42.7, - 29.4, 28.3 (×2), 27.4 (×2), - 26.6, - 26.5, - 26.0, - 26.7, 18.1.

- IR: 2975, 2928, 2852, 1743, 1681, 1624, 1607, 1511, 1292, 1244, 1210, 1177, 1146, 1124, 1092, 1027, 991 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{35}H_{47}N_3O_5$: 589.3516, Observed.: 589.3526.

tert-Butyl (*Z*)-1-(*tert*-butoxycarbonyl)-3-cyclobutyl-5-(2-cyclopentylethylidene)-4-cyclopropylimidazolidin-2-ylidenecarbamate **(3o)**



- ^1H NMR (300 MHz, $CDCl_3$): δ = 4.96 (m, 1H), 4.60 (m, 1H), 3.87 (d, *J* = 5.3 Hz, 1H), 2.33-2.06 (m, 6H), 1.88-1.46 (m, 30H), 1.28-0.91 (m, 3H), 0,57 (m, 1H), 0.45-0.34 (m, 2H), 0.27 (m, 1H).

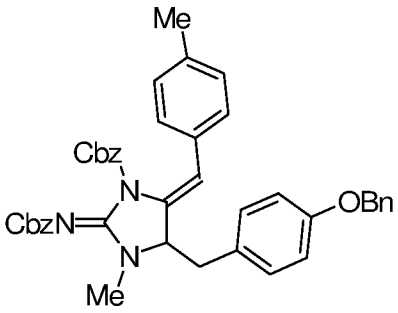
- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 157.9, 149.8, 147.6, 129.5, 117.6, 81.4, 77.0, 58.9, 46.5, 37.8, 32.9, 30.8, 30.6, 28.1, 26.7, 26.5, 26.4, 26.2, 23.5, 13.5, 12.6, 1.58, 0.02, - 1.74.

- DEPT-135 NMR (75.5 Hz, $CDCl_3$): δ = - 117.6, - 58.9, - 46.5, - 37.8, 32.9, 30.8, 30.6, 28.1, 26.7, - 26.5, 23.5, 13.5, 0.02, - 12.6, 1.58, - 1.74.

- IR: 2979, 1742, 1619, 1365, 1291, 1248, 1132, 1092, 1059, 733 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{27}H_{45}N_3O_4$: 473.3254, Obsd.: 473.3269.

Benzyl (*Z*)-1-((benzyloxy)carbonyl)-4-(4-(benzyloxy)benzyl)-5-(4-methylbenzylidene)-3-methylimidazolidin-2-ylidenecarbamate **(3p)**



- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.39-7.18 (m, 13H), 7.03-6.82 (m, 4H), 6.75 (d, *J* = 8.7 Hz, 2H), 5.50 (s, 1H), 5.03 (d, *J* = 2.6 Hz, 2H), 4.97 (s, 2H), 4.90 (d, *J* = 11.7 Hz, 1H), 4.50 (d, *J* = 11.7 Hz, 1H), 4.03 (m, 1H), 3.07 (s, 3H), 2.95 (m, 1H), 2.71 (m, 1H), 2.29 (s, 3H).

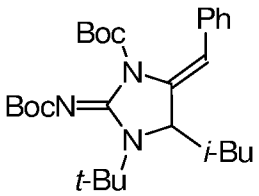
- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 160.1, 157.8, 152.1, 149.3, 137.1, 136.9, 136.8, 134.4, 132.2, 130.9, 130.1, 128.9, 128.8, 128.7 (×2), 128.6 (×2), 128.4 (×2), 128.3 (×2), 128.2, 128.2, 128.1, 128.0, 127.9, 127.7, 127.4, 126.8, 117.7, 114.8, 69.9, 68.7, 67.6, 64.4, 37.7, 30.7, 21.4.

- DEPT-135 NMR (75.5 Hz, $CDCl_3$): δ = 130.9, 128.9, 128.7 (×2), 128.6 (×2), 128.5 (×2), 128.3 (×2), 128.2, 128.1, 128.0, 127.8, 127.7, 127.4, 117.7, 114.8, - 69.9, - 68.7, - 67.6, 64.4, - 37.7, 30.7, 21.4.

- IR: 3032, 2980, 2359, 1744, 1682, 1625, 1509, 1454, 1377, 1272, 1176, 1133, 1056, 1024, 992, 908, 733, 695 cm^{-1}.

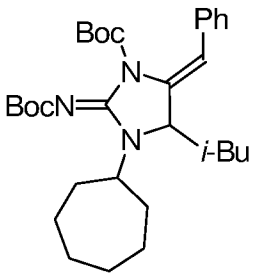
- HRMS *m/z* (EI) Calculated for $C_{42}H_{39}N_3O_5$: 665.289, Observed.: 665.2903.

(5*Z*)-*tert*-Butyl-5-benzylidene-2-(*tert*-butoxycarbonylimino)-3-*tert*-butyl-4-isobutyl-imidazolidine-1-carboxylate **(3q)**



- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.48 (d, *J* = 7.3 Hz, 2H), 7.34-7.26 (m, 2H), 7.22-7.17 (m. 1H), 5.88 (s, 1H), 4.12 (t, *J* = 6.7 Hz, 1H), 1.83-1.74 (m, 2H), 1.71-1.64 (m, 2H), 1.56 (s, 9H), 1.53 (s, 9H), 1.04-0.90 (m, 15H).
- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 159.3, 149.8, 148.7, 135.7, 133.3, 128.8, 128.0, 127.1, 116.1, 82.3, 78.6, 59.9, 55.7, 43.0, 28.4, 28.2, 27.3, 24.6, 23.9, 21.3.
- IR: 2970, 2917, 1743, 1682, 1628, 1416, 1364, 1286, 1251, 1231, 1212, 1151, 1129, 1095, 1060, 1028, 1003, 933, 919, 874, 771 cm^{-1}.
- HRMS *m/z* (EI) Calculated for $C_{28}H_{43}N_3O_4$: 485.3254, Observed.: 485.3254.

(5*Z*)-*tert*-Butyl-5-benzylidene-2-(*tert*-butoxycarbonylimino)-3-cycloheptyl-4-isobutyl-imidazolidine-1-carboxylate **(3r)**



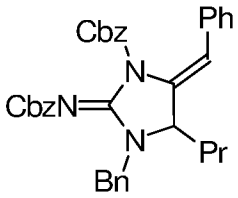
- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.47 (d, *J* = 7.3 Hz, 2H), 7.34-7.17 (m, 3H), 5.88 (s, 1H), 4.13-4.0 (m, 2H), 1.90-1.79 (m, 3H), 1.65-1.48 (m, 21H), 1.05-1.01 (m, 12H), 0.92 (d, *J* = 6.6 Hz, 3H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 160.1, 151.1, 148.6, 135.7, 133.6, 128.8, 128.0, 127.1, 116.3, 82.5, 78.9, 57.5, 56.0, 53.4, 43.3, 34.1, 33.3, 28.2, 27.6, 27.4, 27.2, 24.6, 24.6, 24.5, 24.0, 21.7.

- IR: 2980, 2928, 1743, 1683, 1625, 1388, 1365, 1284, 1240, 1145, 1108, 1093, 696 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{31}H_{47}N_3O_4$: 525.3567, Observed.: 525.3597.

(*Z*)-Benzyl 3-benzyl-5-benzylidene-2-(benzyloxycarbonyl)-4-propylimidazolidine-1-carboxylate **(3s)**



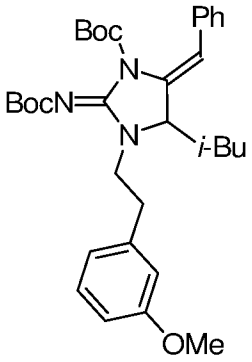
- ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.46 (d, *J* = 7.3 Hz, 2H), 7.38–7.18 (m, 16H), 6.95 (m, 2H), 5.88 (s, 1H), 5.20–5.10 (m, 4H), 4.98 (d, *J* = 11.9 Hz, 1H), 4.59 (d, *J* = 11.9 Hz, 1H), 4.17 (d, *J* = 15.5 Hz, 1H), 3.94 (m, 1H), 1.54 (m, 2H), 1.17 (m, 2H), 0.75 (t, *J* = 7.3 Hz, 3H).

- ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 160.1, 152.3, 149.6, 136.9, 135.5, 135.2, 134.6, 132.3, 128.9 (×2), 128.7, 128.5 (×2), 128.4 (×2), 128.2 (×2), 128.0, 127.8, 127.4, 116.4, 69.7, 67.9, 67.6, 59.5, 53.5, 46.6, 34.2, 16.3, 13.8.

- IR: 3062, 3032, 2967, 2932, 2875, 1751, 1676, 1634, 1263, 1225, 1202, 1075, 799, 780, 733 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{36}H_{35}N_3O_4$: 573.2628, Observed.: 573.2637.

(5*Z*)-*tert*-Butyl-5-benzylidene-2-(*tert*-butoxycarbonylimino)-4-isobutyl-3-(3-methoxy-phenethyl)imidazolidine-1-carboxylate **(3t)**



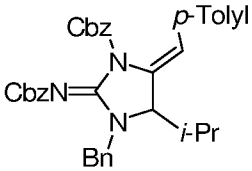
- ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.44 (d, *J* = 7.53Hz, 2H), 7.34-7.13 (m, 4H), 6.82-6.70 (m, 3H), 5.68 (s, 1H), 4.17-4.08(m, 1H), 3.67 (s, 3H), 3.40-3.35 (m, 1H), 3.19-3.10 (m, 1H), 3.04-2.94 (m, 1H), 2.86-2.78 (m, 1H) 1.58 (s, 9H), 1.38-1.18 (m, 3H), 1.00 (s, 9H), 0.89-0.84 (m, 6H).

- ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 159.8, 159.6, 151.0, 148.5, 140.5, 135.8, 133.3, 129.6, 128.7,128.0, 127.1, 121.1, 116.4, 114.2, 112.2, 82.8, 79.1, 59.9, 55.0, 53.4, 44.3, 41.2, 34.0, 28.2, 27.9, 29.2, 24.0, 23.4, 22.1.

- IR: 2970, 2930, 1745, 1684, 1628, 1490, 1454, 1390, 1366, 1287, 1256, 1147, 1128, 1111, 1056, 1041, 1020, 951 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{33}H_{45}N_3O_5$: 563.3359, Observed.: 563.3343.

(*Z*)-Benzyl 3-benzyl-5-*p*-tolylidene-2-(benzyloxycarbonyl)-4-*i*-propylimidazolidine-1-carboxylate **(3u)**



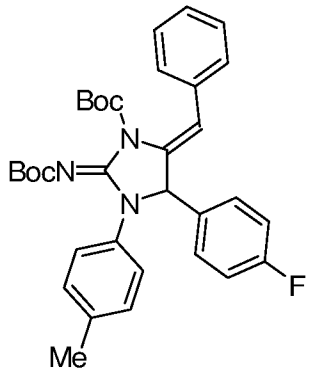
- ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.45–7.15 (m, 15 H), 6.97 (m, 4H), 5.83 (s, 1H), 5.30–5.09 (4H), 4.96 (d, J = 11.9 Hz, 1H), 6.71 (d, J = 11.9 Hz, 1H), 4.15 (d, J = 15.5 Hz, 1H), 3.75 (s, 1H), 2.30 (s, 3H), 1.96 (m, 2H), 0.95 (d, J = 7.3 Hz, 3H), 0.67 (d, J = 7.3 Hz, 3H).

- ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 160.2, 152.9, 149.4, 137.1, 137.0, 135.6, 134.7, 132.2, 129.6, 128.9 (×2), 128.7 (×2), 128.3 (×2), 128.2 (×2), 128.0 (×2), 127.8, 119.0, 68.7, 67.6, 64.5, 53.5, 46.9, 30.0, 21.4, 18.1, 15.1.

- IR : 3031, 2961, 2875, 1744, 1682, 1624, 1513, 1496, 1452, 1376, 1266, 1226, 1205, 1172, 1128, 1110, 1076, 1040, 1026, 1000 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{37}H_{37}N_3O_4$: 587.2784, Observed.: 587.2796.

tert-Butyl (*Z*)-1-(*tert*-butoxycarbonyl)-5-benzylidene-4-(4-fluorophenyl)-3-*p*-tolylimidazolidin-2-ylidenecarbamate **(3v)**



- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.45 (d, J = 7.2 Hz, 2H), 7.35-7.19 (m, 7H), 7.07-6.97 (m, 4H), 5.92 (s, 1H), 5.44 (s, 1H), 2.26 (s, 3H), 1.55 (s, 9H), 0.99 (s, 9H).

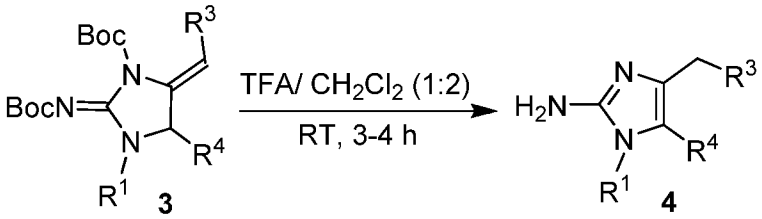
- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 149.4, 148.5, 135.9, 135.6, 134.6, 134.0, 133.9, 133.4, 129.6 (×2), 128.8 (×2), 128.4, 128.3, 128.1 (×2), 127.5, 124.4 (×2), 117.1, 116.2, 115.9, 83.6, 79.7, 66.5, 28.2, 27.2, 21.0.

- DEPT-135 NMR (75.5 Hz, $CDCl_3$): δ = 129.6 (×2), 128.8 (×2), 128.4, 128.3, 128.1 (×2), 127.5, 124.4 (×2), 117.1, 116.2, 115.9, 66.5, 28.2, 27.2, 21.0.

- IR : 2979, 2929, 1744, 1687, 1636, 1602, 1509, 1476, 1451, 1425, 1391, 1366, 1282, 1251, 1229, 1128, 1069, 1014, 922 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{33}H_{36}FN_3O_4$: 557.2690, Observed.: 557.2694.

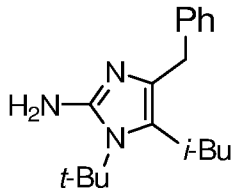
Boc-deprotection of Protected 2-Iminoimidazolines 3 into Polysubstituted 2-Aminoimidazoles **4**



In a 50 mL RBF fitted with a magnetic stirring bar bis-protected 2-iminoimidazoline (0.3 mmol) was dissolved in CH_2Cl_2 (4 mL). The reaction mixture was cooled on an ice bath and then added TFA (2 mL). After 0.5 h the reaction mixture was warmed to the room temperature and allowed to stir for 4-5 h. After completion the reaction mixture was neutralized with saturated solution of Na_2CO_3 (50 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure to dryness. The residue was subjected to flash chromatography on silica gel (CH_2Cl_2 : MeOH : NH_3 (6N in MeOH) = 90: 8: 2) to get the polysubstituted 2-aminoimidazole as a free base.

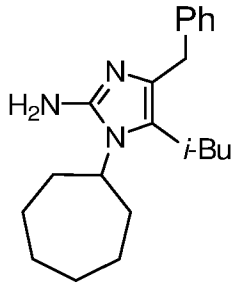
Illustrative but non-limiting examples are given below, together with characterizing data:

4-Benzyl-1-*tert*-butyl-5-isobutyl-1*H*-imidazol-2-amine **(4a)**



- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.27-7.14 (m, 5H), 4.47 (bs, 2H), 3.74 (s, 2H), 2.49 (d, *J* = 7.17, 2H), 1.83-1.74 (m, 1H), 1.65 (s, 9H), 0.88 (d, *J* = 6.57 Hz, 6H).
- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 147.7, 140.3, 132.0, 131.3, 128.5, 128.2, 125.8, 123.6, 57.8, 35.1, 33.1, 30.5, 30.4, 22.8, 22.1.
- IR : 2971, 2917, 1743, 1682, 1628, 1416, 1388, 1364, 1286, 1251, 1212, 1151, 1095, 1060, 933, 919, 874 cm^{-1}.
- HRMS *m/z* (EI) Calculated for $C_{18}H_{27}N_3$: 285.2205, Observed.: 285.2210.

4-Benzyl-1-cycloheptyl-5-isobutyl-1*H*-imidazol-2-amine **(4b)**



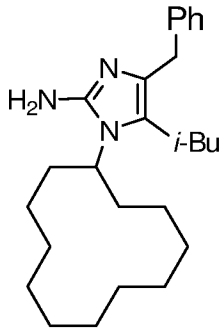
- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.26-7.16 (m, 5H), 5.48 (bs, 2H), 3.87-3.80 (m, 1H), 3.74 (s, 2H), 2.30 (d, *J* = 7.35, 2H), 2.10-2.03 (m, 2H), 1.87-1.79 (m, 4H), 1.62-1.52 (m, 8H), 0.90 (d, *J* = 6.57 Hz, 6H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 145.7, 139.2, 128.5, 128.4, 126.8, 126.2, 121.6, 56.6, 32.7, 32.3, 31.9, 29.3, 27.2, 26.0, 22.2.

- IR : 2927, 2861, 1657, 1602, 1521, 1495, 1454, 1426, 1384, 1368, 1198, 1129, 1074, 1028, 827, 798, 719 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{21}H_{31}N_3$: 325.2518, Observed.: 325.2514.

4-Benzyl-1-cyclododecyl-5-isobutyl-1*H*-imidazol-2-amine **(4c)**



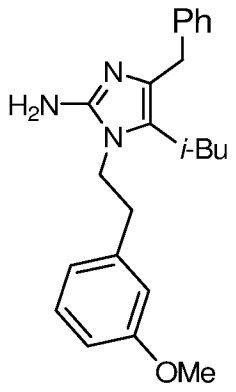
- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.31-7.21 (m, 5H), 6.83 (bs, 2H), 4.06-4.01 (m, 1H), 3.78 (s, 2H), 2.21(d, *J* = 7.14 Hz, 2H), 2.04-1.97 (m, 2H), 1.73-1.62 (m, 3H), 1.38 (bs, 18H), 0.92 (d, *J* = 6.54 Hz, 6H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 145.9, 137.3, 128.7, 128.4, 126.8, 122.7, 51.1, 32.1, 30.5, 28.8, 27.8, 24.0, 23.7, 22.8, 22.2, 22.2, 21.9.

- IR : 2927, 2854, 1655, 1521, 1496, 1454, 1197, 1130, 1065, 913, 829, 764, 719, 701, 520 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{26}H_{41}N_3$: 395.3300, Observed.: 395.3322.

4-Benzyl-5-isobutyl-1-(3-methoxyphenethyl)-1*H*-imidazol-2-amine **(4d)**



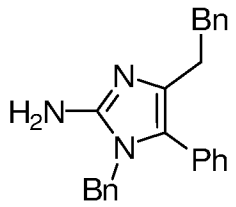
- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.27-7.15 (m, 6H), 6.78 (dd, *J* = 2.07, 8.28 Hz, 1H), 6.66 (d, *J* = 7.53, 1H), 6.53 (s, 1H), 3.97 (bs, 2H), 3.86 (t, *J* = 6.78 Hz, 2H), 3.68 (s, 3H), 2.88 (t, *J* = 6.78 Hz, 2H), 2.20 (d, *J* = 7.35 Hz, 2H), 1.71-1.67 (m, 1H), 0.88 (d, *J* = 6.6 Hz, 6H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 159.8, 146.5, 140.5, 139.4, 130.4, 129.9, 128.5, 128.2, 125.8, 122.1, 121.0, 114.4, 112.6, 55.1, 44.3, 36.1, 32.8, 32.3, 29.3, 22.3.

- IR : 2980, 2956, 1669, 1599, 1584, 1489, 1435, 1357, 1294, 1258, 1200, 1166, 1152, 1041, 778 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{23}H_{29}N_3O$: 363.2311, Observed.: 363.2309.

1-Benzyl-4-phenethyl-5-phenyl-1*H*-imidazol-2-amine **(4e)**



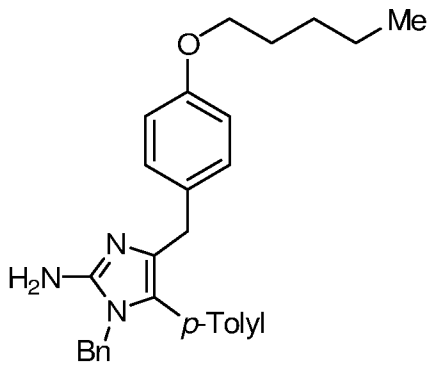
- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.31-7.16 (m, 9H), 7.09 (d, *J* = 6.6 Hz, 2H), 6.98 (d, *J* = 6.39 Hz, 2H), 6.90-6.86 (m, 2H), 5.04 (bs, 2H), 4.81 (s, 2H), 2.96 (t, *J* = 7.14 Hz, 2H), 2.74 (t, *J* = 7.92 Hz, 2H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 147.5, 141.5, 135.7, 130.9, 130.0, 129.0, 128.6, 128.5, 128.1, 127.9, 127.8, 126.1, 125.8, 125.1, 46.2, 35.6, 28.4.

- IR : 3059, 3027, 1668, 1597, 1494, 1451, 1380, 1355, 1199, 1175, 1129, 1073, 750, 719, 694, 553 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{24}H_{23}N_3$: 353.1892, Observed.: 353.1880.

1-Benzyl-4-(4-(pentyloxy)benzyl)-5-*p*-tolyl-1*H*-imidazol-2-amine **(4f)**



- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.34-7.26 (m, 4H), 7.13-7.04 (m, 7H), 6.76 (d, *J* = 8.49 Hz, 2H), 4.81(s, 2H), 4.00 (br, 2H), 3.88 (t, *J* = 6.57 Hz, 2H), 3.73 (s, 2H), 2.33 (s, 3H), 1.79-1.70 (m, 2H), 1.40-1.37 (m, 4H), 0.91 (t, *J* = 6.96 Hz, 3H).

- ^{13}C NMR (75.5 Hz, $CDCl_3$): δ = 157.2, 148.0, 137.2, 136.6, 133.6, 133.1, 129.8, 129.3, 129.3, 129.0, 128.5, 127.6, 127.4, 126.0, 125.3, 114.3, 67.9, 46.3, 32.5, 29.0, 28.2, 22.4, 21.2, 14.0.

- IR : 2957, 2930, 1607, 1552, 1507, 1466, 1452, 1436, 1378, 1357, 1242, 1173, 819, 720, 694 cm^{-1}.

- HRMS *m/z* (EI) Calculated for $C_{29}H_{33}N_3O$: 439.2624, Observed.: 439.2632.

Example 2 - Test organisms/test system

(1) Test organisms/ Test system

Relevant model microbials *Pseudomonas aeruginosa* and *Salmonella* Typhimurium have been used to test inhibiting activity on biofilm formation.

(2) Biofilm formation inhibiting Action

As described in more detail in the Material and Methods section below, the evaluation of the biofilm inhibitory activity of the compounds of the invention was carried out by growing up biofilms of *S.* Typhimurium ATCC14028 or SL1344 or *Pseudomonas aeruginosa* PA14 in the presence of different concentrations (1/2 dilution series) of biofilm inhibitors, by using the Calgary biofilm device. After an incubation period of 24 hours at 25°C the biofilms were visualized by crystal violet staining. In the next step the stain was extracted from the biofilm with 30% acetic acid. The absorbance of the resolubilized stain at 570 nm is a measure of the amount of biofilm formed. From these measurements the concentration of the test compound at which 50% biofilm inhibition was observed (IC_{50} value) was calculated. From the above results, it was revealed that the compounds of the invention had a biofilm formation inhibiting activity .

Some of the compounds show a concentration range in which biofilm formation is inhibited but no effect on the planktonic growth is observed. These compounds are particularly interesting for prevention of biofilm formation in different applications, as the development of resistance against these compounds is less likely.

(3) Material and methods

Stock solutions

Stock solutions of all compounds assayed for biological activity were prepared in DMSO or EtOH and stored at -20 °C. The amount of solvent used in biofilm inhibition screens and growth inhibition screens did not exceed 2% (by volume).

Static peg assay for prevention of Salmonella Typhimurium *biofilm formation*

The device used for biofilm formation is a platform carrying 96 polystyrene pegs (Nunc no. 445497) that fits as a microtiter plate lid with a peg hanging into each microtiter plate well (Nunc no. 269789). Biofilm formation assays were carried out at 25°C.

Two-fold serial dilutions of the compounds in 100 µl liquid TSB 1/20 broth per well were prepared in the microtiter plate. Subsequently, an overnight culture of *S.* Typhimurium ATCC14028 was diluted 1:100 into TSB 1/20 broth and 100 µl (ca. 1. 10^6 cells) was added to each well of the microtiter plates, resulting in a total amount of 200 µl medium per well. The pegged lid was placed on the microtiter plate and the plate was incubated for 24 h at 25°C without shaking. During this incubation period biofilms were formed on the surface of the pegs. The optical density at 600 nm (OD600) was measured for the planktonic cells in the first plate using a VERSAmax microtiter plate reader (Molecular Devices) and the growth retarding concentration IC50 for growth inhibition was determined as the concentration that decreases the OD600 of the planktonic cells 50%. For quantification of biofilm formation, the pegs were washed once in 200 µl phosphate buffered saline (PBS). The remaining attached bacteria were stained for 30 min with 200 µl 0.1% (w/v) crystal violet in an isopropanol/methanol/PBS solution (v/v 1:1:18). Excess stain was rinsed off by placing the pegs in a 96-well plate filled with 200 µl distilled water per well. After the pegs were air dried (30 min), the dye bound to the adherent cells was extracted with 30% glacial acetic acid (200 µl). The OD570 of each well was measured using the VERSAmax microtiter plate reader. The IC50 value for each

compound was determined from concentration gradients in two independent experiments (with 2 or 3 repeats per experiment), by using the GraphPad software of Prism.

Static peg assay for prevention of Pseudomonas aeruginosa biofilm formation

The device used for *Pseudomonas aeruginosa* biofilm formation is the same platform carrying polystyrene pegs as described for *Salmonella* Typhimurium.
Two-fold serial dilutions of the compounds in 100 µl liquid broth per well were prepared in the microtiter plate. Subsequently, an overnight culture of *P. aeruginosa* PA14 (grown up in TSB medium) was diluted 1:100 into liquid broth and 100 µl was added to each well of the microtiter plates, resulting in a total amount of 200 µl medium per well. The pegged lid was placed on the microtiter plate and the plate was incubated for 24 hours at 25 °C without shaking. During this incubation period biofilms were formed on the surface of the pegs. The liquid broth used for the tests at 25°C was TSB 1/20. Quantification of biofilm formation was performed similarly as described previously for the *S.* Typhimurium biofilm assays.

Bioscreen assay for measuring growth inhibition

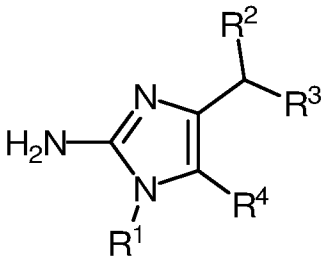
The Bioscreen device (Oy Growth Curves Ab Ltd) was used for measuring the influence of the chemical compounds on the planktonic growth of *Salmonella* Typhimurium and *Pseudomonas aeruginosa*. The Bioscreen is a computer controlled incubator/reader/shaker that uses 10x10 well microtiter plates and measures light absorbance of each well at a specified wave length in function of time.

An overnight culture of S. Typhimurium ATCC14028 or *Pseudomonas aeruginosa* PA14 (grown up in TSB medium) was diluted 1:200 in liquid broth. The broth used was TSB 1/20. 300 µl of the diluted overnight culture was added to each well of the 10x10 well microtiter plate. Subsequently serial dilutions of the chemical

compounds were prepared in DMSO or EtOH. 3 µl of each diluted stock solution was added to the wells (containing the 300 µl bacterial culture) in 3-fold. As a control 3 µl of the appropriate solvent (DMSO) was also added to the plate in 3- or 4-fold. The microtiter plate was incubated in the Bioscreen device at 25°C for at least 36 hours, with continious medium shaking. The absorbance of each well was measured at 600 nm each 15 minutes. Excel was used to generate the growth curves for treated the treated wells and the untreated control wells.

CLAIMS

1. A polysubstituted 2-aminoimidazole compound represented with the structural formula (I):



(I)

wherein:

$\mathbf{R^1}$ is H, an aliphatic group or a cycloaliphatic group;

$\mathbf{R^2}$ is H, an aliphatic group or a cycloaliphatic group;

$\mathbf{R^3}$ is an aliphatic group, a cycloaliphatic group, an aromatic group or a heterocyclic group;

$\mathbf{R^4}$ is an aliphatic group, a cycloaliphatic group, an aromatic group or a heterocyclic group;

and pharmaceutically acceptable salts, hydrates, solvates, prodrugs, stereoisomeric forms or polymorphic substances thereof.

2. A compound as defined in claim 1, wherein $\mathbf{R^2}$ is H.

3. A compound as defined in claim 1, wherein $\mathbf{R^4}$ is selected from the group consisting of propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, tert-butyl, cyclobutyl, n-pentyl, isoamyl, cyclopentyl, n-hexyl, cyclohexyl, heptyl, octyl, nonyl, decyl, dodecyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cyclododecyl, benzyl, phenethyl, piperonyl, 4-hydroxybenzyl, 4-methoxybenzyl, phenyl, 4-methylphenyl, 3,4-dimethoxybenzyl, 3-methoxyphenethyl, 4-chlorophenyl, 4-fluorophenyl, 4-trifluoromethylphenyl, 4-methylsulfonylphenyl and 4-biphenylyl.

4. A compound as defined in claim 2 or claim 3, wherein $\mathbf{R^1}$ is H.

5. A compound as defined in claim 2 or claim 3, wherein $\mathbf{R^1}$ is selected from the group consisting of methyl, ethyl, propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, tert-butyl, cyclobutyl, n-pentyl, isoamyl, cyclopentyl, n-hexyl, cyclohexyl, heptyl, octyl, nonyl, decyl, dodecyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cyclododecyl, benzyl, phenethyl, piperonyl, 4-hydroxybenzyl, 4-methoxybenzyl, phenyl, 4-methylphenyl, 4-methoxyphenyl, 3,4-methoxyphenyl, 4-propylphenyl, 4-butylphenyl, 4-pentylphenyl, 4-butoxyphenyl, 4-pentoxyphenyl, 3,4-dimethoxybenzyl, 3-methoxyphenethyl, 4-chlorophenyl, 4-fluorophenyl, 4-trifluoromethylphenyl, 4-methylsulfonylphenyl, 4-biphenylyl, and (*S*)-1-CHMePh.

6. A compound as defined in any one of claims 1 to 5, wherein $\mathbf{R^3}$ is selected from the group consisting of benzyl, phenethyl, piperonyl, 4-hydroxybenzyl, 4-methoxybenzyl, phenyl, 4-methylphenyl, 4-methoxyphenyl, 3,4-methoxyphenyl, 4-propylphenyl, 4-butylphenyl, 4-pentylphenyl, 4-butoxyphenyl, 4-pentoxyphenyl, 3,4-dimethoxybenzyl, 3-methoxyphenethyl, 4-chlorophenyl, 4-fluorophenyl, 4-trifluoromethylphenyl, 4-methylsulfonylphenyl, 4-biphenylyl, and cyclopentylmethyl,.

7. A compound as defined in any one of claims 1 to 6, being selected from the group consisting of 4-benzyl-1-methyl-1H-imidazol-2-amine, 4-(4-methoxybenzyl)-1-methyl-1H-imidazol-2-amine, 1,4-dibenzyl-1H-imidazol-2-amine, 1,4-bis(4-methoxybenzyl)-1H-imidazol-2-amine, 1,4-bis(4-hydroxybenzyl)-1H-imidazol-2-amine, 4-((2-amino-4-(4-methoxybenzyl)-1-methyl-1H-imidazol-5-yl)methyl)phenol, 4,5-bis(benzo[*d*][1,3]dioxol-5-ylmethyl)-1-methyl-1H-imidazol-2-amine, 4-benzyl-1-*tert*-butyl-5-isobutyl-1H-imidazol-2-amine, 4-benzyl-1-cycloheptyl-5-isobutyl-1H-imidazol-2-amine, 4-benzyl-1-cyclododecyl-5-isobutyl-1H-imidazol-2-amine, 4-benzyl-5-isobutyl-1-(3-methoxy-

phenethyl)-1*H*-imidazol-2-amine, 1-benzyl-4-phenethyl-5-phenyl-1H-imidazol-2-amine, 1-benzyl-4-(4-(pentyloxy)benzyl)-5-*p*-tolyl-1H-imidazol-2-amine, 4-benzyl-1-cyclopropyl-5-isobutyl-1H-imidazol-2-amine, 4-benzyl-1-cyclobutyl-5-isobutyl-1H-imidazol-2-amine, 4-benzyl-1-cyclopentyl-5-isobutyl-1H-imidazol-2-amine, 4-benzyl-1-cyclohexyl-5-isobutyl-1H-imidazol-2-amine, 4-benzyl-1-cyclooctyl-5-isobutyl-1H-imidazol-2-amine, 4-(4-butoxybenzyl)-1-benzyl-5-isobutyl-1H-imidazol-2-amine, 4-(4-(pentyloxy)-benzyl)-1-benzyl-5-isobutyl-1H-imidazol-2-amine, 4-(4-propylbenzyl)-1-benzyl-5-isobutyl-1H-imidazol-2-amine, 4-(4-bytylbenzyl)-1-benzyl-5-isobutyl-1H-imidazol-2-amine, 4-(4-pentylbenzyl)-1-benzyl-5-isobutyl-1H-imidazol-2-amine, 1-cyclobutyl-4-(2-cyclopentylethyl)-5-cyclopropyl-1H-imidazol-2-amine, 4-(4-methoxybenzyl)-5-cyclohexyl-1-((S)-1-phenylethyl)-1H-imidazol-2-amine, 1-butyl-4-(4-methylphenyl)-5-isobutyl-1H-imidazol-2-amine, 1-benzyl-4-(4-methylphenyl)-5-isopropyl-1H-imidazol-2-amine, and 1-benzyl-4-phenyl-5-cyclopropyl-1H-imidazol-2-amine.

8. A composition comprising a compound, as defined in any one of claims 1 to 7 and one or more carriers, excipients or diluents.

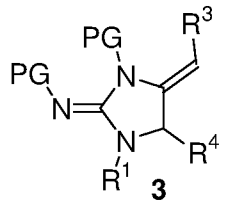
9. A composition according to claim 8, being a pharmaceutical composition wherein the one or more carriers, excipients or diluents are pharmaceutically acceptable.

10. A composition according to claim 8 or claim 9, further comprising another anti-bacterial or anti-fungal agent.

11. A compound as defined in any one of claims 1 to 7 for use in treating or preventing an infection from a biofilm in a patient.

12. A compound for use according to claim 11, wherein said infection originates from one or more species of bacteria.

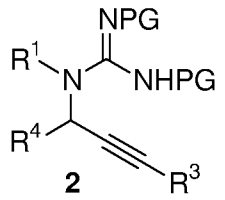
13. A process for producing a disubstituted or trisubstituted 2-aminoimidazole as defined in any one of claims 1 to 7, wherein $\mathbf{R^2}$ is H, said process comprises the step of deprotecting and isomerizing a bis-protected 2-iminoimidazolidine represented by the structural formula **3**:



3

wherein $\mathbf{R^1}$, $\mathbf{R^3}$ and $\mathbf{R^4}$ are as defined in formula (I) and wherein PG is an amino-protecting group or an imino-protecting group.

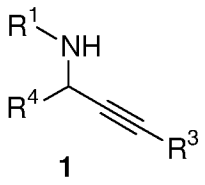
14. A process according to claim 13, wherein said protected 2-iminoimidazolidine represented by the structural formula **3** results from intramolecular π-philic metal-catalyzed 5-*exo*-dig heterocyclization of a propargylguanidine represented by the structural formula **2** :



2

wherein $\mathbf{R^1}$, $\mathbf{R^3}$ and $\mathbf{R^4}$ are as defined in formula (I) and wherein PG is an amino-protecting group or an imino-protecting group.

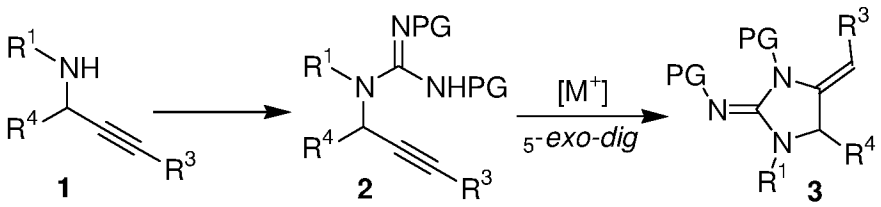
15. A process according to claim 14, wherein said propargylguanidine represented by the structural formula **2** results from guanylation of a propargylamine represented by the structural formula **1** :



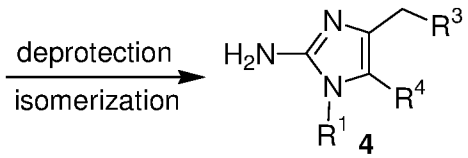
1

16. A process according to claim 14 or claim 15, wherein the metal catalyst for said metal-catalyzed 5-*exo*-dig heterocyclization of a propargylguanidine comprises a silver compound.

17. A process according to any one of claims 13 to 16, being performed according to the following synthetic scheme:



PG = protecting group



4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/066933

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D233/88 A61K31/4168 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)
C07D A61K A61P

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 practical, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SU, ZHAOMING. ET AL: "A nitroenolate approach to the synthesis of 4,5-disubstituted-2-aminoimidazoles. Pilot library assembly and screening for antibiotic and antibiofilm activity", ORGANIC & BIOMOLECULAR CHEMISTRY, vol. 8, no. 12, 29 April 2010 (2010-04-29) , pages 2814-2822, XP55011751, ISSN: 1477-0520, DOI: 10.1039/c001479f table 7; compounds 1-20 ----- -/--	1-4,6, 8-12

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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

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Date of the actual completion of the international search	Date of mailing of the international search report
11 November 2011	30/11/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Johnson, Claire

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	GILES ET AL: "Addition-Hydroamination Reactions of Propargyl Cyanamides: Rapid Access to Highly Substituted 2-Aminoimidazoles", ANGEWANDTE CHEMIE INTERNATIONAL EDITION, vol. 48, no. 17, 14 April 2009 (2009-04-14), pages 3116-3120, XP55011715, ISSN: 1433-7851, DOI: 10.1002/anie.200900160 Scheme 3;	1-3,5-7
X	WO 2009/108550 A1 (MERCK & CO INC [US]; HILLS IVORY D [US]; COBURN CRAIG A [US]; GRAHAM S) 3 September 2009 (2009-09-03) claims 1,15; examples 9,20,22	1,4,8,9
X,P	DENIS S. ERMOLAT'EV ET AL: "Concise and Diversity-Oriented Route toward Polysubstituted 2-Aminoimidazole Alkaloids and Their Analogues", ANGEWANDTE CHEMIE INTERNATIONAL EDITION, vol. 49, no. 49, 20 October 2010 (2010-10-20), pages 9465-9468, XP55011731, ISSN: 1433-7851, DOI: 10.1002/anie.201004256 the whole document	1-17
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/066933

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