

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 2014210359 B2**

(54) Title
New bioactive polymers

(51) International Patent Classification(s)
C08G 73/02 (2006.01)

(21) Application No: **2014210359**

(22) Date of Filing: **2014.01.22**

(87) WIPO No: **WO14/113835**

(30) Priority Data

(31) Number
A 53/2013

(32) Date
2013.01.25

(33) Country
AT

(43) Publication Date: **2014.07.31**

(44) Accepted Journal Date: **2017.08.24**

(71) Applicant(s)
Sealife Pharma GmbH

(72) Inventor(s)
Pretsch, Alexander; Nagl, Michael; Wiesner, Christoph; Burgmann, Heinz

(74) Agent / Attorney
Wrays Pty Ltd formerly EKM patent & trade marks, L 15 330 Collins St, Melbourne, VIC, 3000, AU

(56) Related Art
US 2011/0269936 A1

(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES
PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

(19) Weltorganisation für geistiges
Eigentum

Internationales Büro

(43) Internationales
Veröffentlichungsdatum
31. Juli 2014 (31.07.2014)



(10) Internationale Veröffentlichungsnummer
WO 2014/113835 A1

(51) Internationale Patentklassifikation:
C08G 73/02 (2006.01)

(21) Internationales Aktenzeichen: PCT/AT2014/050026

(22) Internationales Anmeldedatum:
22. Januar 2014 (22.01.2014)

(25) Einreichungssprache: Deutsch

(26) Veröffentlichungssprache: Deutsch

(30) Angaben zur Priorität:
A 53/2013 25. Januar 2013 (25.01.2013) AT

(71) Anmelder: SEALIFE PHARMA GMBH [AT/AT];
Technopark 1, A-3430 Tulln (AT).

(72) Erfinder: PRETSCH, Alexander; Grossreinprechts 60,
A-3524 Gainbrunn (AT). NAGL, Michael; Novaragasse
51/19, A-1020 Wien (AT). WIESNER, Christoph;
Brunnengasse 69/18, A-1160 Wien (AT). BURGMANN,
Heinz; Karl-Bekehrty-Strasse 19, A-1140 Wien (AT).

(74) Anwalt: ELLMEYER, Wolfgang; Häupl & Ellmeyer KG,
Mariahilfer Straße 50, A-1070 Wien (AT).

(81) Bestimmungsstaaten (soweit nicht anders angegeben, für
jede verfügbare nationale Schutzrechtsart): AE, AG, AL,
AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, 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, IR, IS, JP, KE, KG, 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, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU,
RW, SA, 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) Bestimmungsstaaten (soweit nicht anders angegeben, für
jede verfügbare regionale Schutzrechtsart): ARIPO (BW,
GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ,
TZ, UG, ZM, ZW), eurasisches (AM, AZ, BY, KG, KZ,
RU, TJ, TM), europäisches (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, KM, ML, MR, NE, SN, TD, TG).

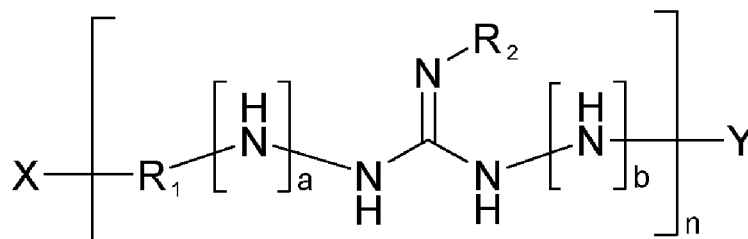
Veröffentlicht:

— mit internationalem Recherchenbericht (Artikel 21 Absatz
3)

— vor Ablauf der für Änderungen der Ansprüche geltenden
Frist; Veröffentlichung wird wiederholt, falls Änderungen
eingehen (Regel 48 Absatz 2 Buchstabe h)

(54) Title: NEW BIOACTIVE POLYMERS

(54) Bezeichnung : NEUE BIOAKTIVE POLYMERE



(I)

(57) Abstract: The invention relates to new polycondensation products of aminoguanidine and/or 1,3-diaminoguanidine with one or more diamines, i.e. polyguanidine derivatives of formula (I) below: wherein X is selected from -NH₂, aminoguanidino, and 1,3-diaminoguanidino; Y is selected from -H and -R₁-NH₂; or X and Y together stand for a chemical bond in order to produce a cyclic structure; R₁ is selected from divalent organic groups having 2 to 20 carbon atoms, in which one or more carbon atoms are optionally replaced by O or N; a and b are 0 or 1, wherein a+b ≠ 2 if no 1,3-diaminoguanidine units are present; R₂ is selected from -H and -NH₂, wherein R₂ is -NH₂ if a+b = 0, R₂ is -H or -NH₂ if a+b = 1, and R₂ is -H if a+b = 2; and n ≥ 2; or salts thereof. The invention further relates to a production method and various applications therefor.

(57) Zusammenfassung: Die Erfindung betrifft neue Polykondensationsprodukte von Aminoguanidin und/oder 1,3-Diaminoguanidin mit einem oder mehreren Diaminen, d.h. Polyguanidin-Derivate der nachstehenden Formel (I); worin X aus -NH₂, Aminoguanidino und 1,3-Diaminoguanidino ausgewählt ist; Y aus -H und -R₁-NH₂ ausgewählt ist; oder X und Y zusammen für eine chemische Bindung stehen, um eine zyklische Struktur zu ergeben; R₁ aus zweiwertigen organischen Resten mit 2 bis 20 Kohlenstoffatomen ausgewählt ist, in denen gegebenenfalls ein oder mehrere Kohlenstoffatome durch O oder N ersetzt sind; a und b jeweils 0 oder 1 sind, wobei a+b ≠ 2 ist,

[Fortsetzung auf der nächsten Seite]



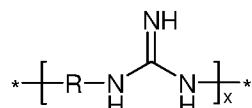
New bioactive polymers

The present invention relates to new bioactive polymers as well as their use as biocides.

5

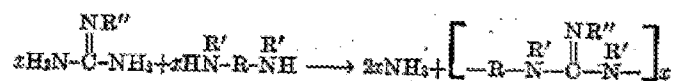
STATE OF THE ART

Polyguanidines of the following general formula as well as various derivatives thereof have been known for a long time.

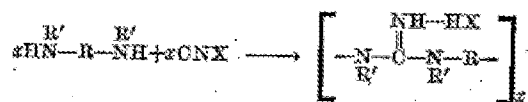


10 Already in 1943, patent literature described in US Patent 2,325,586 several methods for producing various polyguanidines by polycondensation of i) guanidine or salts thereof, ii) cyanohalides, iii) dicyanoamides, or iv) isocyanide dihalides with diamines, or of v) two dicyanodiamides together (which results in cyano-substituted polyguanidines), as well as the use of polyguanidines thus produced as dyeing aids:

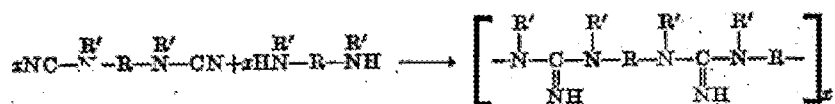
15 i)



ii)

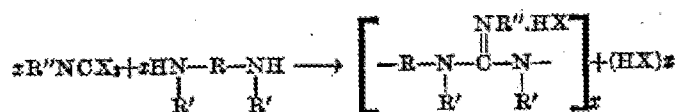


iii)

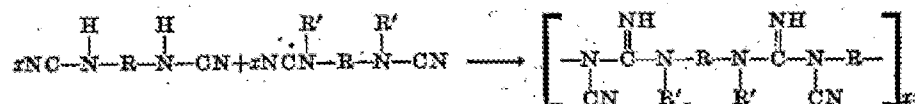


20

iv)



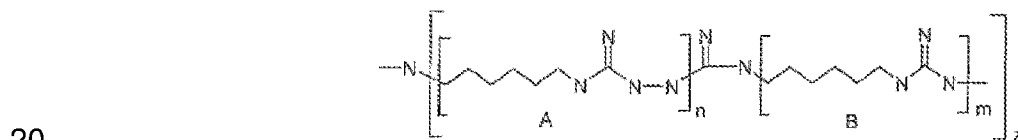
v)



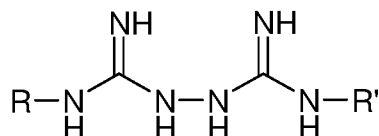
Already at that time, the diamines in the reactions i) to iv) disclosed were alkylene and phenylene diamines as well as oxyalkylene or other polyether diamines, which later also became known as Jeffamines[®].

- 5 Decades later, such polyguanidines have proven to be excellent biocides. A group around Oskar Schmidt discloses in WO 99/54291 A1 the production of microbiocidal poly(hexamethylene guanidines), in WO 01/85676 A1 biocidal polyguanidines that are produced by condensation of guanidine with polyoxyalkylenes, and in WO 2006/047800 A1 polyguanidine derivatives acting as biocides, in particular as fungicides,
 10 which are produced by polycondensation of guanidine with a mixture of alkylene diamine and oxyalkylene diamine and are said to possess lower toxicity than polymers containing only one of the two types of the divalent radical R₁.

WO 02/30877 A1 describes similar polyguanidines used as disinfectants, which additionally contain phenylene moieties in the chains. A Russian group of researchers (Tets, Tets und Krasnov) discloses in WO 2011/043690 A1, from which US 2011/0269936 A1 and EP 2,520,605 A1 were derived, biocidal polyguanidines of the following formula, which are produced by polycondensation of guanidine and hexamethylene diamine in the presence of hydrazine hydrate:

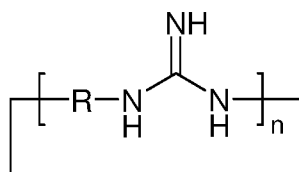


Thus, during polycondensation, the hydrazine replaces – at least formally – an amino group of only one guanidine moiety or also of two guanidine moieties, which is said to result in block copolymers with alternating poly(hexamethylene guanidine) blocks and
 25 poly(hexamethylene aminoguanidine) blocks, wherein the two types of blocks are linked via guanidine dimers, as shown below:



These polymers and acid addition salts thereof are also said to act as biocides against bacteria, viruses, and fungi. However, the examples given in this application, in which 7 different polymers were produced, do not contain any physical data on the products obtained except for the statement that the polymer of Example 1 is a “solid,
5 almost colorless, transparent substance.”

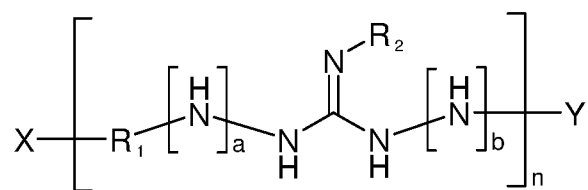
Regarding the possible structures that may form during polycondensation of guanidines with diamines, there are several articles by a group of researchers from Graz University of Technology, e.g. Albert et al., *Biomacromolecules* 4(6), 1811-1817
10 (2003), and Feiertag et al., *Macromol. Rap. Comm.* 24(9), 567-570 (2003). In addition to the different possibilities of terminating the linear polymer chains with one of the starting monomers, usually cyclic molecules of the following general formula are also formed at a portion not to be neglected, which, among other things, depends on the chain length of the diamine:



The main disadvantages of practically all polyguanidine derivatives described above is, on the one hand, the toxicity of these products that is not to be neglected as well as – in case highly reactive components are used – their comparatively laborious
20 production methods, in addition to the use of, as is known from the toxicological field, problematic components such as hydrazine, which is why the object of the invention was the production of new, less toxic, but still biocidally effective polyguanidines in a fashion as simple and economic as possible and avoiding the above disadvantages.

25 DISCLOSURE OF THE INVENTION

The present invention achieves this object by providing new polycondensation products of aminoguanidine and/or 1,3-diaminoguanidine with one or more diamines, i.e. of polyguanidine derivatives of the following formula (I):



(I)

wherein

X is selected from $-NH_2$, aminoguanidino, and 1,3-diaminoguanidino;

5

Y is selected from $-H$ and $-R_1-NH_2$;

or X and Y together represent a chemical bond to give a cyclic structure;

10 R_1 is selected from divalent organic radicals having 2 to 20 carbon atoms, in which optionally one or more carbon atoms are replaced by O or N;

a and b are each 0 or 1,

wherein $a+b \neq 2$ if no 1,3-diaminoguanidine units are contained;

15

R_2 is selected from $-H$ and $-NH_2$,

wherein R_2 is $-NH_2$ if $a+b = 0$,

R_2 is $-H$ or $-NH_2$ if $a+b = 1$, and

R_2 is $-H$ if $a+b = 2$; and

20

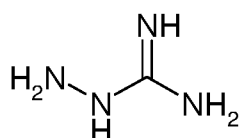
$n \geq 2$;

or of salts thereof.

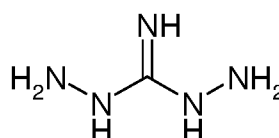
25 In activity assays, the new polyguanidine derivatives of formula (I) have proven to be effective antimicrobial substances, which, however, surprisingly show much lower toxicity than the structurally similar polymers of the above documents WO 2011/043690 A1, US 2011/0269936 A1 and EP 2,520,605 A1, as will be substantiated by embodiments of the invention and comparative examples below. Without wishing to

be bound by any theory, the inventors assume that amino- and diaminoguanidino moieties are better tolerated by human eukaryotic cells than guanidino moieties and in particular than the polymers containing the hydrazo-bridged guanidine dimers shown above. In addition, the method disclosed avoids the use of the toxic component, hydrazine hydrate, in the polymerization process, which may be contained as a residual monomer in some polymers according to the state of the art.

The above formula (I) refers to polycondensation products of (mono)aminoguanidine, in the following referred to as MAG, as well as of 1,3-diaminoguanidine, in the following referred to as DAG.



MAG



DAG

Formula (I) may be explained by the fact that during polycondensation proceeding with concomitant separation of ammonia, MAG and DAG radicals can take part in this polycondensation via their amino or tautomeric imino groups as well as their hydrazo (hydrazinyl) moieties. Consequently, there are three different possibilities for MAG and DAG as starting monomers to be integrated into the chains of the inventive polymers. In the case of MAG, the only hydrazo moiety in formula (I), in the case of DAG the only imino/amino moiety, can point to the left, to the right or upwards.

For MAG, this means the following possible parameter in formula (I):

$a = 1, b = 0, R_2$ is $-H$: hydrazo moiety points to the left;

$a = 0, b = 1, R_2$ is $-H$: hydrazo moiety points to the right; or

$a = 0, b = 0, R_2$ is $-NH_2$: hydrazo moiety points upwards.

For DAG, there are the following parameter combinations:

$a = 0, b = 1, R_2$ is $-NH_2$: amino/imino moiety points to the left;

$a = 1, b = 0, R_2$ is $-NH_2$: amino/imino moiety points to the right; or

$a = 1, b = 1, R_2$ is $-H$: amino/imino moiety points upwards.

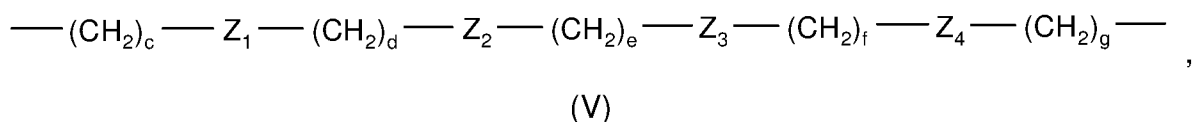
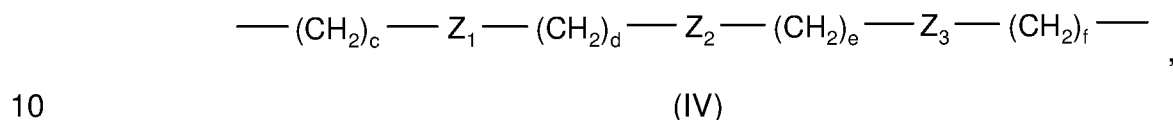
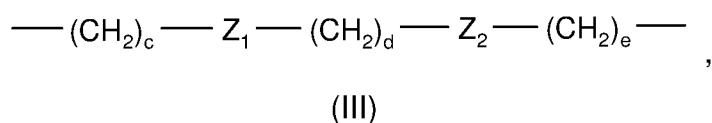
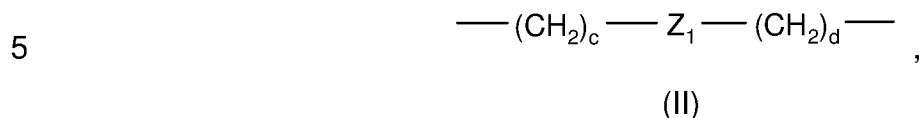
Without being limited thereto, NMR spectra of the polycondensates obtained seem, as will be described in later examples of the invention, to prove that the polycondensation reactions consistently result in mixtures of several of the three possible orientations, which leads to the presumption that several orientations of one and the same monomer are present within a chain (which has not been clarified 100% yet).

In this connection, it should be explicitly mentioned that the position of C=N double bonds of guanidino moieties – as well as the spatial position of substituent R₂ at the double bond – is subject to the usual effects of tautomerism. This means that the double bond of guanidine may be within or outside the chain and that R₂ may point to the left or to the right. Such tautomers of the above polycondensation products of formula (I) are thus also within the scope of the present invention.

The above options for X and Y result from the different possibilities of terminating chains – depending on whether MAG, DAG, or a mixture of both was used as starting monomer(s) – including the possibility of a cyclization to obtain a cyclic polycondensate. See also the articles mentioned above by Albert et al. and by Feiertag et al. Of course, the same options are available for terminal aminoguanidino (MAG) and 1,3-diaminoguanidino (DAG) moieties as for moieties within a chain, i.e. the attachment to the chain may be via any nitrogen atom.

According to the present invention, the radical R₁ may be a linear, branched or cyclic, saturated or unsaturated, divalent hydrocarbon radical having 2 to 20 carbon atoms, preferably 4 to 18 carbon atoms, more preferably 6 to 12 carbon atoms, in which some C atoms may be replaced by O and/or N. The above preferences are the result of the following considerations. In the case of very short radicals R₁, the active MAG or DAG moieties are very close to each other, which may reduce the activity of the polymers; with longer radicals, however, they are quite far apart. Radicals having more than 20 atoms are thus basically possible, however, they are not preferred from the economic point of view because they result in polymers of formula (I) in which relatively few antiinfectively effective guanidino moieties are contained per weight unit.

Preferably, the radical R_1 is selected from alkylene radicals in which optionally one or more carbon atoms are replaced by O or N to increase the hydrophilicity of the chain, more preferably R_1 is selected from radicals of the following general formulas (II) to (V):



wherein Z_1 to Z_4 are each independently a heteroatom selected from O and N, and the indexes c to g are each independently integers in the range of 1 to 12, so that the total number of atoms of radical R_1 does not exceed 20. Especially preferred is that all heteroatoms Z within one radical R_1 are either O or N.

The best results in assays on biocidal effect or toxicity were achieved with compounds in which R_1 represents the divalent radical of a polyether diamine such as 4,9-dioxadodecane-1,12-diamine, a polyoxyethylene and/or propylene diamine, wherein n is preferably 2 to 15, more preferably 2 to 10, most preferably 2 to 6.

Useful salts of the new polyguanidines of formula (I) are any acid addition salts with one or more inorganic or organic acids, such as hydrohalic acids, oxygen acids of nitrogen, sulphur or phosphor, boric acid, carbonic acid, carboxylic, thiocarboxylic, carbamic, sulfonic, phosphonic or phosphinic acids, as well as partial esters or amides of multivalent forms of these acids. According to the invention, pharmaceutically acceptable salts are preferably used, more preferably acid addition salts in the form of a hydrogen chloride, hydrogen bromide, hydrogen iodide, sulfate, methylsulfate, carbonate, borate, cyanate, thiocyanate, phosphate, mesylate, nitrate, acetate,

benzoate, lactate, tartrate, citrate, maleate, fumarate or partial esters of these acids in case they are difunctional or higher. A preferred alcoholic component of such partial esters is a pharmaceutically acceptable alcohol, in particular ethanol.

- 5 If the radical R_1 contains one or more OH or COOH groups, salts with inorganic or organic bases are also within the scope of the present invention, preferably with pharmaceutically acceptable bases, more preferably with a guanidine derivative, in particular with amino or diamino guanidine, i.e. with the guanidine derivative which is the basis for the production of the inventive new polyguanidines. Usually, an inner
10 salt of acidic and basic moieties will form within the respective molecules in any case.

In a second aspect of the invention, a method for producing inventive polyguanidine derivatives according to the first aspect by polycondensation of a guanidine derivative or a salt thereof with a diamine is provided, which method is characterized in that
15 MAG and/or DAG, or an acid addition salt thereof, is polycondensated with at least one diamine $H_2N-R_1-NH_2$ by heating.

Contrary to the state of the art, the method of the invention comprises reacting MAG or DAG with one or more diamines, preferably only one single diamine. This allows
20 for the production of more clearly defined products than in the works mentioned above conducted by the Russian researchers, because in the course of the reaction no free hydrazine was detectable in the reaction mixtures produced according to the invention – neither chromatographically nor by wet chemistry. (Side) reactions with hydrazine, which were desirable in the state of the art mentioned, but are completely
25 undesirable herein, could thus be effectively avoided.

Preferably, the method of the invention is conducted by heating a salt of MAG or DAG, in particular the hydrogen chloride thereof, together with the diamine, which is preferably used at a small molar excess, e.g. of 3 to 5 molar % or, for economic
30 reasons, of 10 molar % at a maximum, in relation to (di-)aminoguanidine, in order to guarantee the complete conversion of the guanidine derivative, wherein heating is initially carried out to a first, lower temperature, preferably approximately 80-150°C,

more preferably 110-130°C, and then to a second, higher temperature, preferably 150-250°C, more preferably 160-180°C, in order to control the reaction rate and thus also the formation of gas. The reaction mixture is held at the first temperature for preferably 1 to 3 h, more preferably 2 h, and then at the second temperature for preferably 1 to 8 h, more preferably 3 to 5 h, in order to guarantee a complete reaction.

The reaction is preferably conducted at normal pressure and with the exclusion of water, which can, for example, be achieved by initially purging the reaction vessel with inert gas and equipping the reaction vessel with a drying tube. However, applying a vacuum is also possible, in particular at the end of the reaction in the course of a purification step in order to evaporate free ammonia as well as residual monomer, i.e. mainly excess diamines, as completely as possible.

After completion of the reaction, the polyguanidine derivative obtained is preferably dissolved in water, e.g. in the 3- to 10-fold amount of water. This serves, on the one hand, to separate any water-insoluble components and, on the other hand, an aqueous solution is a preferred formulation for the use of the new polymers, which means that it might – if applicable, after the addition of optional adjuvants – be usable directly as such.

Further purification options, which are less preferred at the moment, include for example evaporating the water from the aqueous solution and drying the polymers in a vacuum or salting out from the aqueous solution by the addition of acid and subsequent drying, where the pharmaceutically acceptable acids described as preferred are useful. One embodiment of salting out includes the introduction of CO₂ and salting out the polyguanidines as carbonates or hydrogen carbonates. If the desired polyguanidine is not to be used as a salt, but as a free base, salting out might be followed by treatment with a base, which may be provided in an aqueous or non-aqueous solution or suspension.

In a third aspect, the invention provides a polyguanidine derivative according to the first aspect of the invention or produced by a method according to the second aspect

of the invention for the use in the human and veterinary medical fields for antagonizing bacterial, fungal and viral infections and their aftereffects, as a pesticide and disinfectant in the agricultural and environmental fields, generally as a disinfectant (biocide) for reducing and eliminating germs, as an antiparasitic, as a supplement for stabilizing (sterilizing) products, or as a nebulization substance in a dissolved form for cold/wet nebulization, micronization and vapor sterilization.

Below, the present invention will be described in more detail by means of non-limiting exemplary embodiments together with comparative examples. The only figure, Fig 1, summarizes the results of toxicity assays.

EXAMPLES

Examples 1 to 6 & Comparative Examples 1 and 2 – Production of the polymers

Example 1

23 mmol of 1,3-diaminoguanidinium hydrochloride and 24 mmol of 4,9-dioxadodecane-1,12-diamine were heated in a reaction vessel closed with a drying tube at 120°C for 90 min with stirring, then the temperature was increased to 180°C for 100 min, at the end of this reaction time under reduced pressure (50 mbar) for 45 min. After the reaction mixture had cooled off to below 80°C, 25 ml of water were added to the gelatinous reaction product. After several hours, a clear solution was obtained.

The water was evaporated from a sample of the aqueous solution obtained, and the residue obtained was dried in vacuum, which resulted in a reddish, viscous liquid. It was dissolved in 2 ml of D₂O (with a deuterization degree > 99,5%), and a ¹H nuclear resonance (¹H-NMR-) spectrum was obtained. The positions of methylene proton groups of the radical R₁ in the product distinguishable in this way are as follows:

¹H-NMR (D₂O), δ (ppm): 1.54-1.67 (m, OCH₂CH₂CH₂CH₂O), 1.80-1.95 (m, NCH₂CH₂), 3.23-3.38 ppm (m, NCH₂), 3.42-3.65 ppm (m, CH₂CH₂OCH₂CH₂).

This confirms the structure of the diamine component used, 4,9-dioxadodecane-1,12-diamine.

Example 2

4.6 mmol of 1,3-diaminoguanidinium hydrochloride and 4.8 mmol of 4,9-dioxadodecane-1,12-diamine were heated in a reaction vessel closed with a drying tube at 120°C for 90 min with stirring, then the temperature was increased to 180°C for 8 h, at the end of this reaction time under reduced pressure (50 mbar) for 45 min. After the reaction mixture had cooled off to below 80°C, 16 ml of water were added to the gelatinous reaction product. After several hours, a clear solution was obtained.

Example 3

4.6 mmol of N-aminoguanidinium hydrochloride and 4.8 mmol of 4,9-dioxadodecane-1,12-diamine were heated in a reaction vessel closed with a drying tube at 120°C for 90 min with stirring, then the temperature was increased to 180°C for 3.5 h, at the end of this reaction time under reduced pressure (50 mbar) for 60 min. After the reaction mixture had cooled off to below 80°C, 16 ml of water were added to the gelatinous reaction product. After several hours, a clear solution was obtained.

Example 4

1.16 mmol of 1,3-diaminoguanidinium hydrochloride and 1.21 mmol of tris(2-aminoethyl)amine were heated in a reaction vessel closed with a drying tube at 120°C for 150 min with stirring, then the temperature was increased to 160°C for 2.5 h, at the end of this reaction time under reduced pressure (50 mbar) for 45 min. After the reaction mixture had cooled off to below 80°C, 4 ml of water were added to the gelatinous reaction product. After several hours, a clear solution was obtained.

Example 5

8.12 mmol of 1,3-diaminoguanidinium hydrochloride and 8.47 mmol of tris(2-aminoethyl)amine were heated in a reaction vessel closed with a drying tube at 130°C for 120 min with stirring, then the temperature was increased to 180°C for 8 h, at the end of this reaction time under reduced pressure (50 mbar) for 90 min. After the reaction mixture had cooled off to below 80°C, 28 ml of water were added to the gelatinous reaction product. After several hours, a clear solution was obtained.

Example 6

2.32 mmol of 1,3-diaminoguanidinium hydrochloride and 2.43 mmol of 3,6-dioxaoctane-1,8-diamine were heated in a reaction vessel closed with a drying tube at 120°C for 60 min with stirring, then the temperature was increased to 170°C for 4 h, at the end of this reaction time under reduced pressure (50 mbar) for 60 min. After the reaction mixture had cooled off to below 80°C, 7 ml of water were added to the gelatinous reaction product. After several hours, a clear solution was obtained.

Comparative Example 1

23.2 mmol of guanidinium hydrochloride, 5.4 mmol of 3,6-dioxaoctane-1,8-diamine and 18.1 mmol of 1,6-diaminohexane were heated in a reaction vessel closed with a drying tube at 120°C for 90 min with stirring, then the temperature was increased to 170°C for 8 h, at the end of this reaction time under reduced pressure (50 mbar) for 90 min. After the reaction mixture had cooled off to below 80°C, 60 ml of water were added to the gelatinous reaction product. After several hours, a clear solution was obtained.

The structure of the polymer obtained corresponds to that disclosed in WO 2006/047800 A1.

Comparative Example 2

2.00 mmol of guanidinium hydrochloride, 1.70 mmol of 1,6-hexamethylene diamine and 0.3 mmol of hydrazine hydrate were heated in a reaction vessel closed with a drying tube at 160°C for 90 min with stirring, then the temperature was increased to 180°C for 3.5 h, at the end of this reaction time under reduced pressure (50 mbar) for 60 min. After the reaction mixture had cooled off to below 80°C, 4 ml of water were added to the gelatinous reaction product. After several hours, a clear solution was obtained.

The structure of the polymer obtained corresponds to that disclosed in WO 2011/043690 A1.

Example 7 – Determination of activity: antimicrobial/antifungal/antiviral effects

The activities of the new compounds were tested in screening systems in multiple. The antibacterial and antifungal activities were tested in a MIC assay. MIC refers to “minimal inhibitory concentration” and is the lowest concentration of a substance that will inhibit the growth of microorganisms discernible with the naked eye. The MIC is determined using a so-called titer method, where the substance is diluted and then the pathogen is added.

Usually this allows for the determination of the concentration of an antibiotic that is just high enough to inhibit growth of a bacterial strain. The MIC is specified in micrograms per milliliter ($\mu\text{g/ml}$) or in % per volume, and the dilutions are generally conducted in log₂ steps. Herein, an initial concentration of 1 % each was 2-fold diluted, which consequently resulted in test concentrations of 0.5 %, 0.25 %, 0.125 %, etc. Lower values thus reflect better activity as anti-infective.

The assays were conducted according to the standards required by EUCAST (European Committee for Antimicrobial Susceptibility Testing) and according to the AFST (“Antifungal Susceptibility Testing”) regulations of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID).

The screening system for viruses is an infection system in which host cells are infected *in vitro*, and the test substance is added before or after the infection and its activity determined. All these assays were conducted according to internal standard regulations of SeaLife Pharma for drug screening, wherein analogous serial dilutions were used like in the antibacterial/antifungal assay.

The following tables 1 to 3 summarize the test results regarding the anti-infective effect of the inventive new compounds of Examples 1, 3, 4 and 5 against multiresistant bacteria and fungi as well as viruses. The data are mean values of multiple determinations.

It is obvious that the new compounds of the invention show excellent activity against Gram-positive as well as Gram-negative pathogens:

Table 1

MIC assay results	MRSA	Staphylococcus epidermis	Streptococcus pneumoniae	Enterococcus faecalis	Propionibacter acne	E. coli	Klebsiella pneumoniae	Pseudomonas aeruginosa	Acinetobacter baumannii	Enterobacter cloacae	Salmonella enterica
Example 1	0.001 %	0.001 %	0.004 %	0.008 %	0.001 %	0.016 %	0.02 %	0.02 %	0.06 %	0.03 %	0.03 %
Example 3	0.001 %	0.001 %	0.001 %	0.008 %	0.001 %	0.02 %	0.02 %	0.02 %	0.06 %	0.2 %	0.03 %
Example 4	0.001 %	0.001 %	0.001 %	0.008 %	0.001 %	0.016 %	0.016 %	0.030 %	0.02 %	0.016 %	0.030 %
Example 5	0.001 %	0.001 %	0.002 %	0.002 %	0.001 %	0.020 %	0.02 %	0.04 %	0.04 %	0.13 %	0.03 %

5

Also against fungi and yeasts:

Table 2

MIC assay results	Candida albicans	Candida papilliosis	Candida glabrata	Candida kruzei	Aspergillus terreus	Aspergillus fumigatus	Fusarium rosei	Trichophyton sp.	Alternaria alternaria	Microsporum canis	Dematiacea sp.
Example 1	0.008 %	0.03 %	0.02 %	0.02 %	0.02 %	0.03 %	0.03 %	0.02 %	0.02 %	0.03 %	0.02 %
Example 3	0.02 %	0.02 %	0.02 %	0.02 %	0.03 %	0.03 %	0.03 %	0.02 %	0.02 %	0.02 %	0.02 %
Example 4	0.008 %	0.016 %	0.016 %	0.008 %	0.125 %	0.125 %	n.t.	n.t.	n.t.	n.t.	n.t.
Example 5	0.02 %	0.02 %	0.02 %	0.020 %	0.016 %	0.016 %	n.t.	n.t.	n.t.	n.t.	n.t.

10

As well as against viruses:

Table 3

Virological assay results	Influenza A and B	Human rhinovirus	Parainfluenza virus	Herpes simplex virus
Example 1	0,008 %	0,008 %	0,008 %	0,02 %
Example 3	0,02 %	0,02 %	0,02 %	0,02 %
Example 4	0,04 %	0,02 %	0,04 %	0,02 %
Example 5	0,04 %	0,04 %	0,04 %	0,02 %

5

Thus, all new compounds tested show very good to excellent activity against various pathogens – with significantly lower toxicity than the polyguanidine derivatives known from prior art, as is shown by the following toxicity assays.

10 **Example 8 – Toxicity assays**

AlamarBlue[®] Assays as described below were used to study 4 polymers with regard to their toxicological potential (including proliferation, cell death, cell metabolism), and the IC₅₀ value and the non-toxic concentration were determined with primary keratinocytes (HKER) and primary endothelial cells (HUVEC). Fig. 1 shows the toxic effect of the various polymers depending on their concentration.

AlamarBlue[®] Assay: 20,000 human keratinocytes (HKER) or endothelial cells (HUVEC) were plated in 96 well plates and incubated for 24 h, before different concentrations (5 % to 0.005 %) of the new polymers of Examples 1 and 3 as well as of the comparative substances of Comparative Examples 1 and 2 were added. After 24 hours, 10 µl AlamarBlue[®] were added to each well (100 µl medium), and after 3 hours of incubation, the color reaction was detected using a multiplate reader (ex: 530 nm; em: 590 nm). HKER: “human primary keratinocytes”; HUVEC: “human umbilical vein endothelial cells”.

25

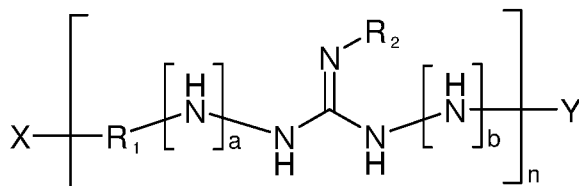
The polymers of Comparative Examples 1 and 2 show significant toxic effects against HKER as well as HUVEC at already very low concentrations, i.e. an IC₅₀ of

approximately 0.01 % or below. In comparison, the new polymers produced by the inventors of Examples 1 and 3 show toxic effects at significantly higher concentrations: for Example 1, the IC_{50} for both cell types is approximately 1 %, and for Example 3, it ranges between 0.05 % and 0.1 %. The toxicity produced by the comparative examples is reached by the polymer of Example 3 only at the 5-fold concentration, and by that of Example 1 only at the at least 100-fold concentration. The DAG derivative thus showed much better results in this assay than the MAG polymer.

Consequently, the new compounds show very good to excellent activity against various pathogens – with significantly lower toxicity than polyguanidine derivatives known from prior art.

CLAIMS

1. A polycondensation product of aminoguanidine and/or 1,3-diaminoguanidine with one or more diamines, i.e. a polyguanidine derivative of the following formula (I):



(I)

wherein

X is selected from $-\text{NH}_2$, aminoguanidino, and 1,3-diaminoguanidino;

Y is selected from $-\text{H}$ und $-\text{R}_1-\text{NH}_2$;

or X and Y together represent a chemical bond to give a cyclic structure;

R_1 is selected from divalent organic radicals having 2 to 20 carbon atoms, in which optionally one or more carbon atoms are replaced by O or N;

a and b are each 0 or 1,

wherein $a+b \neq 2$ if no 1,3-diaminoguanidine units are contained;

R_2 is selected from $-\text{H}$ und $-\text{NH}_2$,

wherein R_2 is $-\text{NH}_2$ if $a+b = 0$,

R_2 is $-\text{H}$ or $-\text{NH}_2$ if $a+b = 1$, and

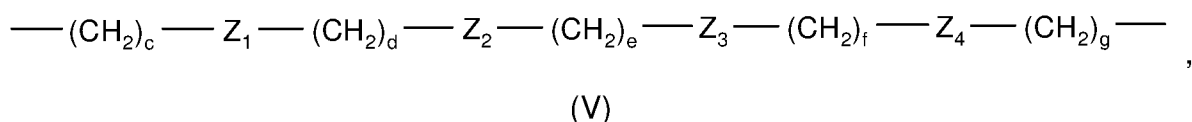
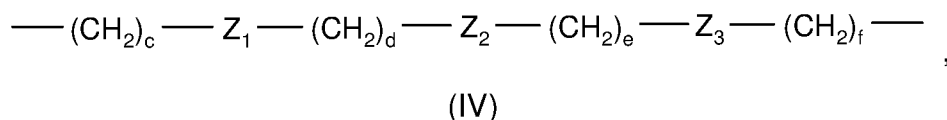
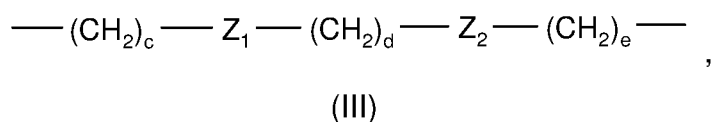
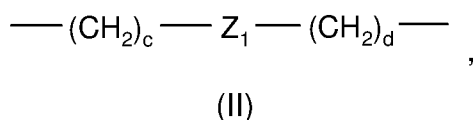
R_2 is $-\text{H}$ if $a+b = 2$; and

$n \geq 2$;

or a salt thereof.

2. The polyguanidine derivative according to claim 1, characterized in that R₁ is selected from alkylene radicals, in which optionally one or more carbon atoms are replaced by O or N.

5 3. The polyguanidine derivative according to claim 2, characterized in that R₁ is selected from radicals of the following general formulas (II) to (V):



15 wherein Z₁ to Z₄ are each independently a heteroatom selected from O and N, and the indexes c to g are each independently integers in the range of 1 to 12, so that the total number of atoms of radical R₁ does not exceed 20.

4. The polyguanidine derivative according to claim 3, characterized in that all heteroatoms Z within one radical R₁ are either O or N.

5. The polyguanidine derivative according to claim 4, characterized in that R₁ represents the divalent radical of a polyether diamine.

25 6. The polyguanidine derivative according to any one of the claims 1 to 5, characterized in that n = 2 to 6.

7. The polyguanidine derivative according to any one of the claims 1 to 6, characterized in that it is present as an acid addition salt in the form of a hydrogen chloride, hydrogen bromide, hydrogen iodide, sulfate, carbonate, borate, cyanate, thiocyanate,

30

phosphate, mesylate, nitrate, acetate, benzoate, lactate, tartrate, citrate, maleate, fumarate, a partial ester of one of these acids, in case they are difunctional or higher, or as a mixture of two or more of these salts and/or partial esters.

- 5 8. A method for producing polyguanidine derivatives according to any one of the claims 1 to 7 by polycondensation of a guanidine derivative, or a salt thereof, with a diamine, characterized in that aminoguanidine or 1,3-diaminoguanidine, or an acid addition salt thereof, is polycondensed with at least one diamine $H_2N-R-NH_2$ by heating.
- 10 9. The method according to claim 8, characterized in that the at least one diamine is used at an excess of 3 to 5 molar % in relation to the guanidine derivative.
- 15 10. The method according to claim 8 or 9, characterized in that a salt of aminoguanidine or 1,3-diaminoguanidine is heated together with the at least one diamine, initially to a first, lower temperature and then to a second, higher temperature.
- 20 11. The method according to claim 10, characterized in that the salt of aminoguanidine or 1,3-diaminoguanidine is heated together with the at least one diamine, initially to 110-130°C and then to 160-180°C.
- 25 12. The method according to claim 10 or 11, characterized in that the reaction mixture is kept at the first temperature for 1 to 3 h and at the second temperature for 1 to 8 h.
- 30 13. The method according to any one of the claims 8 to 12, characterized in that the polyguanidine derivative obtained is purified by dissolution in approximately the 3- to 10-fold amount of water.
14. The polyguanidine derivative according to any one of the claims 1 to 7 or produced by the method according to any one of the claims 8 to 13 for use in the human and veterinary medical fields for antagonizing bacterial, fungal and viral infections

and their aftereffects, as a pesticide and disinfectant in the agricultural and environmental fields, generally as a disinfectant (biocide) for reducing and eliminating germs, as an antiparasitic, as a supplement for stabilizing (sterilizing) products, or as a nebulization substance in a dissolved form for cold/wet nebulization, micronization and vapor sterilization.

15. The polyguanidine derivative according to any one of the claims 1 to 7 or produced by the method according to any one of the claims 8 to 13 for use according to claim 14, characterized in that an effective amount of the polyguanidine derivative is present as a solution in the 3- to 10-fold amount of water.

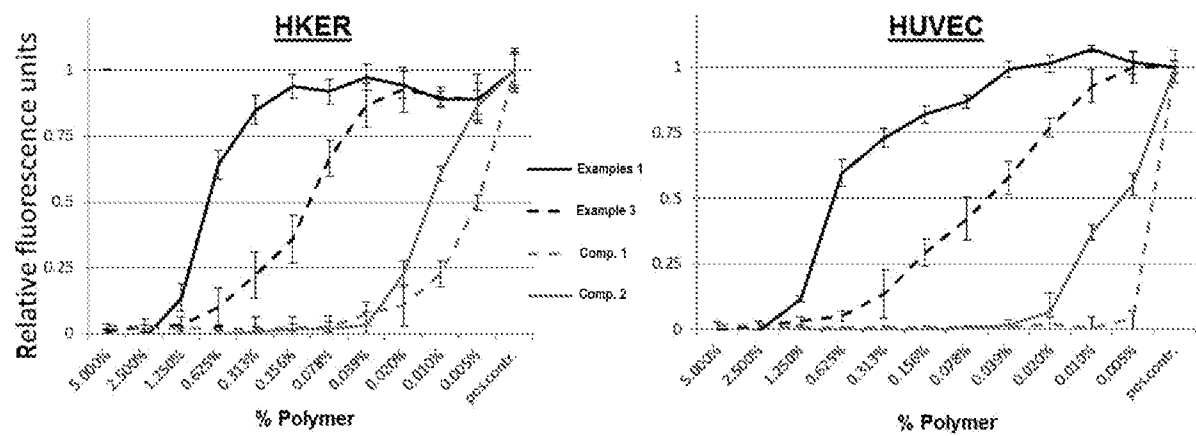


Figure 1