

EUROPEAN QUALIFYING EXAMINATION 1999

PAPER B CHEMISTRY

THIS PAPER COMPRISES:

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DESCRIPTION OF THE APPLICATION

The present application deals with certain polyamines and their use in the treatment of diseases.

Natural polyamines are well known. These include, e.g.

- 5 $\text{H}_2\text{N}-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_4-\text{NH}-(\text{CH}_2)_3-\text{NH}_2$ (spermine),
 $\text{H}_2\text{N}-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_4-\text{NH}_2$ (spermidine) and
 $\text{H}_2\text{N}-(\text{CH}_2)_5-\text{NH}_2$ (cadaverine).

Our recent research concentrated on trialkylene tetramines and N-substituted trialkylene tetramines, such
10 as spermine and N-substituted spermines.

These compounds are easily available by simple synthetic methods. Spermine can be obtained by the
process described by O. Bayer (Angew. Ch. 61, 235 (1949)), i.e. by reacting

- 15 (1) $\text{H}_2\text{N}-(\text{CH}_2)_4-\text{NH}_2$ (1,4-diamino butane) with two equivalents of
 $\text{H}_2\text{C}=\text{CH}-\text{CN}$ (acrylonitrile) to yield
 $\text{NC}-(\text{CH}_2)_2-\text{NH}-(\text{CH}_2)_4-\text{NH}-(\text{CH}_2)_2-\text{CN}$, and

 (2) catalytically hydrogenating the product of step (1).

20

Whereas the unsubstituted trialkylene tetramines (i.e. the compounds of the
formula $\text{H}_2\text{N}-(\text{CH}_2)_x-\text{NH}-(\text{CH}_2)_y-\text{NH}-(\text{CH}_2)_z-\text{NH}_2$) are well known in the art, N-substituted trialkylene
tetramines have not been described up to now.

25 In a first embodiment, the present invention relates to new compounds which may be described by the
following general formula:



30 wherein

m is an integer from 3 to 6,

n is an integer from 3 to 6, and

the groups **R**, **R'** and **R''** are each directly bonded to a nitrogen atom, may be the same or different and are, independently of one another, hydrogen atoms or optionally substituted hydrocarbon groups, with the proviso that at least one of the **R**, **R'** and **R''** groups is not hydrogen.

5 Preferred **R**, **R'** and **R''** groups are hydrogen atoms or hydrocarbon groups having from 1 to 16 carbon atoms, e.g. a C₅-, C₆- or C₇-hydrocarbon group.

Examples of such groups are alkyl groups, such as methyl, ethyl, propyl, butyl, n-pentyl, n-hexyl, n-octyl, n-decyl and n-hexadecyl; aryl groups, such as phenyl; alkaryl groups, such as methylphenyl
10 (-C₆H₄-CH₃) or dimethylphenyl ((-C₆H₃(CH₃)₂), and aralkyl groups, such as benzyl (-CH₂-C₆H₅).

The compounds of formula (I) may be prepared by reacting a polyamine of the formula



wherein **n** and **m** have the same meaning as above, with compounds which replace at least one of the hydrogen atoms of compound (IV) by at least one hydrocarbon group in accordance with the above definitions for **R**, **R'** and **R''** in the presence of a strong base, such as NaH. Examples of such compounds
20 are hydrocarbyl halides, in particular iodides.

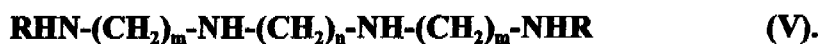
If not all the hydrogens in the amino groups are to be substituted by the above hydrocarbon groups, the reaction is carried out in three steps:

25 All of the **R**, **R'** and **R''** groups which are to be hydrogen atoms in the desired product (i.e. which shall not be substituted by a hydrocarbon group), have to be replaced in a first step by protective groups which can in a last step easily be replaced again by hydrogen atoms. Thus, the polyamine of formula (IV) is reacted with the appropriate amount of p-toluene sulphonyl chloride to yield the respective sulfonamide.

30 The remaining **N-H** groups are then reacted with the said reactants to replace the hydrogens bonded to nitrogen atoms by hydrocarbon groups.

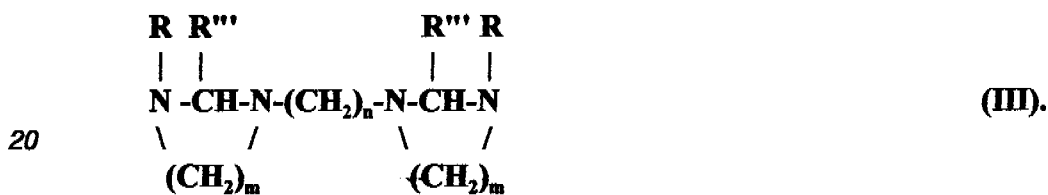
Subsequently, all the said sulfonamide groups, introduced in the first step, are replaced by hydrogen atoms under reducing conditions to yield the product of the invention.

Preferably, both R'' groups are hydrogen atoms. Most preferably, all the R' and R'' groups are hydrogen atoms. These preferred compounds are described by formula (V):



Epecially preferred are those compounds of formulae (I) and (V), where m has a value of 3 and n has a value of 4. These compounds are most easily accessible due to the fact that spermine can be used as a starting compound in the process for making them.

Compounds of the formula (V) can be modified in a further step by condensation reaction with aldehydes of the formula R'''-CHO (II), wherein R''' is a hydrogen atom, a lower alkyl, an aryl or an alkaryl group (preferably an alkyl group having from 1 to 12 carbon atoms, an aryl or alkaryl group having up to 12 carbon atoms), to form compounds of the formula (III)



The compounds of formulae (III) and (V) are particularly useful in the treatment of cancer, such as lung cancer and leukaemia.

Example 1

Tests were conducted with murine L1210 leukaemia cell cultures. The cells were treated with filtered aqueous solutions of the polyamine derivatives of the present invention. The cells were then reseeded at 0.3·10⁵ cells/ml and then incubated. Cell samples were removed for counting at the indicated time periods (48 hours, 96 hours). The IC₅₀ was determined (i.e. the concentration of the compound necessary to reduce cell growth to 50% of control growth). The lower the IC₅₀-value, the more effective against leukaemia is the respective compound. The results are shown in Tables I and II, below.

Example 2

A murine Lewis lung carcinoma was maintained as s.c. (subcutaneous) tumour in mice. A 2-4 mm

fragment of this donor tumour was implanted s.c. in the axillary region on day 0. DPS spermine, i.e. the compound of formula (V) where both R groups are $-C_5H_{11}$ was administered (intraperitoneal) injection every 8 h for 5 days beginning with day 5. Equal numbers of mice treated with saline injections served as controls. The parameter measured was mean survival time. The results are shown in Table III.

Table I

Compound of formula (V) with $m=3$; $n=4$; the R groups being identical	IC ₅₀ -value	
	after 48 h	after 96 h
R = $-CH_3$ (methyl)	16 micrograms	0.17 micrograms
R = $-C_2H_5$ (ethyl)	15 micrograms	0.09 micrograms
R = $-C_5H_{11}$ (n-pentyl)	8 micrograms	0.07 micrograms
R = $-C_6H_{13}$ (n-hexyl)	20 micrograms	0.21 micrograms
R = $-C_8H_{17}$ (n-octyl)	27 micrograms	0.28 micrograms
R = $-C_{16}H_{33}$ (n-hexadecyl)	32 micrograms	0.32 micrograms
R = $-C_6H_5$ (phenyl)	35 micrograms	0.40 micrograms
R = $-CH_2-C_6H_5$ (benzyl)	4 micrograms	0.02 micrograms
R = $-C_6H_4-CH_3$ (toluyl)	34 micrograms	0.30 micrograms

Table II

Compound of formula (III) with $m=3$; $n=4$; R'''=H; R groups being identical	IC ₅₀ -value	
	after 48 h	after 96 h
R = $-CH_3$ (methyl)	12 micrograms	0.10 micrograms
R = $-C_2H_5$ (ethyl)	8 micrograms	0.07 micrograms
R = $-C_6H_5$ (phenyl)	35 micrograms	0.42 micrograms
R = $-CH_2-C_6H_5$ (benzyl)	3 micrograms	0.01 micrograms

Table III

Dose	Mean survival time in days
0 (Control)	9.6 ± 0.5
15 mg/kg/8 h	14.9 ± 1.3
20 mg/kg/8 h	16.7 ± 2.6

Claims

1. Compounds of the formula



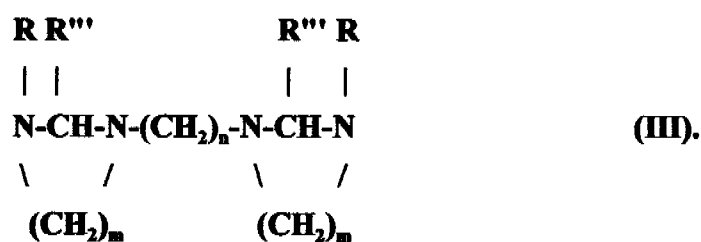
wherein

m is an integer from 3 to 6,

n is an integer from 3 to 6, and

the **R**, **R'** and **R''** groups are each directly bonded to a nitrogen atom, may be the same or different and are, independently of one another, hydrogen atoms or optionally substituted hydrocarbon groups, with the proviso that at least one of the **R**, **R'** and **R''** groups is not hydrogen.

2. Compounds of claim 1, wherein both **R''** groups are hydrogen atoms.
3. Compounds of claim 1, wherein all the **R'** and **R''** groups are hydrogen atoms.
4. Process for the condensation reaction of compounds of claim 3 with aldehydes of the formula **R'''-CHO (II)**, wherein **R'''** is a hydrogen atom, a lower alkyl, an aryl or an alkaryl group, to form a compound of the formula **(III)**



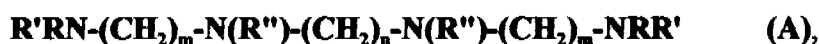
5. Compounds of formula **(III)** as defined in claim 4.
6. Compositions containing the compounds as defined in claims 1 to 3 and 5.
7. Use of the compounds of any of the claims 1 to 3 and 5 or of the compositions according to claim 6 for the treatment of diseases.

COMMUNICATION

1. The subject-matter of claims 1 to 7 is not novel. Your attention is drawn to **Document I** (2) which describes the compounds, the process and the compositions of present claims 1 to 6. Your attention is also drawn to **Document II (DII)** which additionally discloses the use as claimed in present claim 7.
2. The subject-matter of claim 7 is not susceptible of industrial application (Art. 52 (4) EPC).
3. Claim 4 is unclear due to the vague expression "a lower alkyl, an aryl or an alkaryl group". Reference is made to the decision T337/95 (see OJ EPO 1996, 628); lack of clarity due to the relative term "lower alkyl").
4. If you wish to continue with the application, you are invited to submit claims which take the aforementioned objections into account and comply with the requirements of the EPC, especially with regard to novelty, inventive step, clarity, disclosure in the application documents as originally filed and, if necessary, unity (Art. 54 (1) and (2), 56, 84, 123(2) and 82 EPC).
5. In your reply, you should also identify the difference between the new claims and the state of the art and its significance, and present the invention in such a way that both the technical problem to be solved vis-à-vis the state of the art and the solution found (see Rule 27(1)(c) EPC and Guidelines C-IV, 9.5), as well as your position on the question of inventive step, can be understood.
6. According to the Guidelines (C-III, 4.4) an independent claim should specify clearly all the essential features needed to define the invention, i.e. each independent claim must indicate all the features necessary to solve the problem on which the invention is based.
7. Your attention is drawn to the fact that the application may not be amended in such a way that its subject-matter extends beyond the content of the application as filed (Art. 123 (2) EPC). Therefore, and also in view of the Guidelines E-II, 1 and C-VI, 5.4, you should explain from where in the original application documents the new features in any newly formulated claims have been directly and unambiguously derived.
8. It is suggested not to file an adapted description until the Examining Division has indicated that the amended claims are allowable.

DOCUMENT I (State of the Art)

The present invention deals with insecticidal compositions for mosquitos containing certain polyan... compounds. These compounds are selected from those of formula (A):



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wherein

m is an integer from 3 to 6,

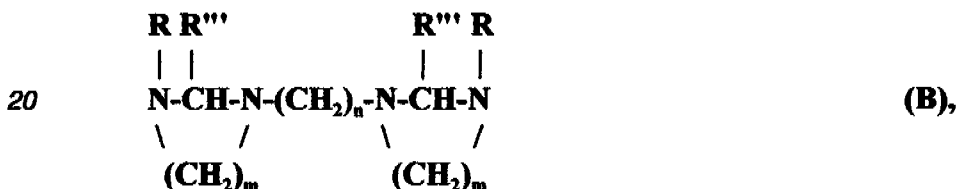
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n is an integer from 3 to 6, and

the **R**, **R'** and **R''** groups are each directly bonded to a nitrogen atom, may be the same or different and are, independently of one another, hydrogen atoms or optionally substituted hydrocarbon groups, with the proviso that at least one of the radicals **R**, **R'** and **R''** is not hydrogen;

15

and those of the formula (B):



wherein **R'''** is a hydrogen atom, a lower alkyl, an aryl or an alkaryl group, and wherein **R**, **n** and **m** in the formula have the meanings indicated above.

The groups **R**, **R'** and **R''** may, e.g., be hydrogen atoms or hydrocarbon groups having from 1 to 16 carbon atoms, especially from 4 to 16, most preferably from 5 to 10 carbon atoms. Examples of such groups are alkyl groups, such as methyl, ethyl, propyl, n-pentyl, n-hexyl, n-octyl and n-hexadecyl; aryl groups, such as phenyl; alkaryl groups, such as methylphenyl (toluyl, -C₆H₄-CH₃) or dimethylphenyl (-C₆H₃(CH₃)₂), and aralkyl groups.

Preferably, **R'''** is a hydrogen atom, an alkyl group having from 1 to 12 carbon atoms, an aryl or alkaryl

group having up to 12 carbon atoms. Examples of R''' groups are methyl, ethyl, n-propyl, isopropyl, phenyl groups.

Preferably, the R'' groups in formula (A) are both hydrogen atoms. Most preferably, all the R' and R'' groups in formula (A) are hydrogen atoms.

These compounds are described by formula (C):



10

Especially preferred are those compounds of formulae (A), (B) and (C), where m is 3 and n is 4 and both R groups are identical.

Most preferred are those compounds of formula (C) where the R groups are alkyl groups having from 1 to 16 carbon atoms or the phenyl group.

Preparation examples (N, N', N'', N''' refer to the individual nitrogen atoms in the compounds, see the formula (D) below)

20 1. Preparation of N,N'''-diethyl spermine

1.1 Preparation of N,N',N'',N'''-tetratosyl spermine

To a mixture of spermine tetrahydrochloride (4.53 g, 13.0 mmol) and 10% aqueous NaOH (200 ml; 132 mmol) was added dropwise a solution of p-toluene sulphonyl chloride (tosyl chloride; 9.98 g; 52.3 mmol) in CH₂Cl₂ at 0°C. After stirring for two days at room temperature, the organic phase was separated, washed with water and dried and purified on silica gel to give 9.69 g (91% of the theoretical yield) of the tetratosylspermine (each nitrogen in the compound being substituted by one tosyl group).

30 1.2 Preparation of N,N'''-diethyl- N,N',N'',N'''-tetratosyl spermine

To the tetratosyl spermine prepared in step 1.1 (1.75 g; 2.14 mmol) in dry THF (12 ml) was added

80% NaH (sodium hydride; 0.25 g; 8.33 mmol) and then ethyl iodide (1.0 ml; 12.5 mmol). After heating under nitrogen (10 h; 55°C), the mixture was poured on ice and extracted with CH₂Cl₂. The organic phase was then washed with water, dried, and the solvents were distilled off to yield 1.6 g (87% of the theoretical yield) of the product.

5

1.3 Preparation of N,N'''-diethyl spermine

10

Into a solution of the product of step 1.2 (2.78 g; 3.18 mmol) in THF (200 ml) at -78°C was condensed 300 ml of NH₃ (liquid ammonia). Spheres of sodium metal (3.0 g; 0.13 mol) were added and the reaction mixture was stirred at -78°C for 4 h. The reaction mixture was allowed to warm up to room temperature to distil off the ammonia. Diethyl ether was added and then, cautiously, ethanol and finally, after evolution of gas had ceased, water. The ether phase was separated from the basic aqueous phase. The aqueous phase was further extracted with diethyl ether. The combined ether phases were dried, filtered and the ether was distilled off. The liquid product was distilled, the diethyl spermine was dissolved in a mixture of ether and ethanol and precipitated with hydrochloric acid and recrystallised to yield 790 mg (63% of the theoretical yield) of the respective hydrochloride salt (N,N'''-diethyl spermine·4 HCl). The pure base (N,N'''-diethyl spermine according to formula C, each R being -C₂H₅) may be recovered when the hydrochloride salt is treated with a base, e.g. with an aqueous solution of NaOH.

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2. Preparation of the compound of formula (B) wherein both R groups are -C₂H₅; m=3; n=4; R'''=H.

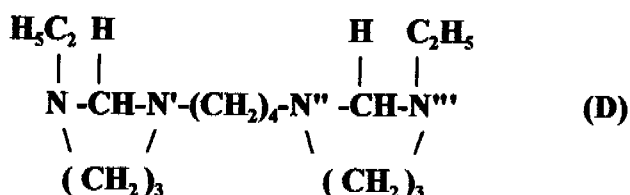
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To the N,N'''-diethyl spermine·4 HCl of step 1.3 (36.1 mg; 0.0893 mmol) in 0.17 M NaOH (2.0 ml; 0.34 mmol) was added formaldehyde solution (0.20 mmol). The mixture was stirred for 3 h at room temperature, NaOH and aqueous NaCl-solution were added, the product was extracted with CH₂Cl₂, the extracts were dried and purified to yield 22 mg (88% of the theoretical yield) of the product.

Said product may be described by the formula depicted below:

30

5



Application example

A thousand mosquito eggs were hatched at 25°C in a culture medium. The larvae were transferred to test tubes, each test tube containing 10 mosquito larvae and 3 ml of culture medium. The polyamines according to the present invention were then introduced into said test tubes.

15

The resulting data are reported as LD₅₀-values for each compound, i.e. the polyamine concentration necessary to kill 50% of the larvae. Said concentrations are given in ppm, i.e. in mg/kg of the culture medium. The lower the LD₅₀-value, the more effective the respective compound.

20

Compound of formula (C) with m=3; n=4; the R groups being identical	LD ₅₀ -value	
	after 48 h	after 96 h
R = -CH ₃ (methyl)	10 ppm	8 ppm
R = -C ₂ H ₅ (ethyl)	2 ppm	---
R = -C ₃ H ₇ (n-propyl)	8 ppm	6 ppm
R = -C ₅ H ₁₁ (n-pentyl)	7 ppm	5 ppm
R = -C ₆ H ₁₃ (n-hexyl)	8 ppm	6 ppm
R = -C ₁₀ H ₂₁ (n-decyl)	10 ppm	9 ppm
R = -C ₁₆ H ₃₃ (n-hexadecyl)	12 ppm	10 ppm
R = -C ₆ H ₅ (phenyl)	20 ppm	14 ppm
R = -C ₆ H ₄ -CH ₃ (toluyl)	22 ppm	17 ppm
R = -C ₆ H ₃ (CH ₃) ₂ (dimethylphenyl)	25 ppm	18 ppm

25

30

Compound of formula (B) with m=3; n=4; R''' = H; the R groups being identical	LD ₅₀ -value	
	after 48 h	after 96 h
R = -CH ₃ (methyl)	30 ppm	20 ppm
R = -C ₂ H ₅ (ethyl)	35 ppm	25 ppm
R = -C ₆ H ₅ (phenyl)	50 ppm	30 ppm

DOCUMENT II (State of the Art)

We have developed new derivatives of spermine which are useful as disinfectants and for the treatment of intestinal infections of humans and animals.

These new derivatives of spermine are those of the formula (1):

5



wherein

10 \mathbf{m} is an integer from 3 to 6,

\mathbf{n} is an integer from 3 to 6, and

15 the \mathbf{R} , \mathbf{R}' and \mathbf{R}'' groups are each directly bonded to a nitrogen atom, may be the same or different and are, independently of one another, hydrogen atoms or optionally substituted hydrocarbon groups, with the proviso that at least one of the \mathbf{R} , \mathbf{R}' and \mathbf{R}'' groups is not hydrogen.

Preferred \mathbf{R} , \mathbf{R}' and \mathbf{R}'' groups are hydrogen atoms or hydrocarbon groups having not more than 16 carbon atoms, preferably from 6 to 16 carbon atoms.

20

Especially preferred are compounds of formula (1) where all the \mathbf{R}'' groups are hydrogen atoms and the \mathbf{R} groups are the same or different and are $\text{C}_6\text{-C}_{16}$ -alkyl groups. Examples of such alkyl radicals are n-hexyl, n-heptyl, n-octyl, iso-octyl, n-decyl, n-dodecyl and n-hexadecyl groups. Such compounds are particularly effective in the treatment of intestinal infections.

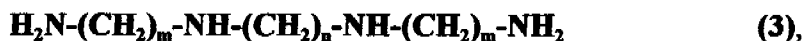
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The preferred compounds of this kind are described by formula (2):



Especially preferred are those compounds of formulae (1) and (2), where m is 3 and n is 4.

5 The compounds of the present invention are prepared starting from a compound of the formula (3):



wherein n and m have the same meaning as above.

10

In a first step, those of the hydrogen atoms which are not to be replaced by an alkyl group are reacted with *p*-toluene sulphonyl chloride to yield the respective sulfonamides.

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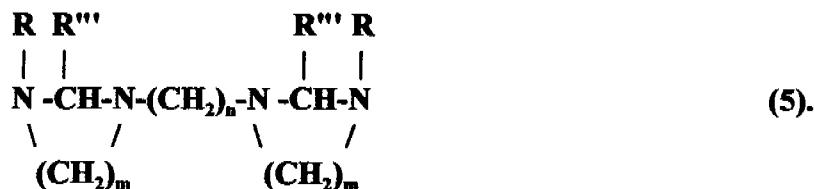
In a second step the remaining hydrogen atoms are replaced by alkyl groups in a reaction with alkylating agents in the presence of a strong base, e.g. NaH.

20

In a third step, the sulfonamide groups are again replaced by hydrogen atoms under conditions known in the art. This is most easily achieved under reducing conditions, e.g. by reaction with sodium metal in liquid ammonia (Na/NH_3).

Compounds of the formula (2) can be condensed with aldehydes of the formula $\text{R}'''\text{-CHO}$ (4), wherein R''' is a hydrogen atom, alkyl, aryl or alkaryl group (preferably an alkyl group having from 1 to 12 carbon atoms, an aryl or alkaryl group having up to 12 carbon atoms), to form compounds of the formula (5)

25



30

The present compounds of the formulae (1), (2) and (5) are useful as disinfectants and as active agents

in compositions for the treatment of intestinal infections of humans and animals.

They may be admixed with suitable pharmaceutical carriers or excipients to form pharmaceutical compositions, e.g. in the form of solutions and dispersions, tablets, capsules, suppositories, and the like.

5

They are preferably administered to humans and animals at a dose of from 0,05 to 5 mg/kg.

Example 1

The following compounds of the present invention have been prepared under the reaction conditions mentioned above:

10

Compound of formula (2) with m=3; n=4; the R groups being identical	Compound No.	Melting Point
R = -C ₇ H ₁₅ (n-heptyl)	1	122 - 123°C
R = -C ₈ H ₁₇ (n-octyl)	2	118 - 119°C
R = -C ₁₂ H ₂₅ (n-dodecyl)	3	116 - 117°C
R = -C ₁₆ H ₃₃ (n-hexadecyl)	4	113 - 114°C
R = -C ₆ H ₅ (phenyl)	5	123 - 124°C
R = -C ₆ H ₄ -CH ₃ (toluyl)	6	128°C

20

Compound of formula (5) with m=3; n=4; the R''' = H; the R groups being identical	Compound No.	Melting Point
R = -C ₇ H ₁₅ (n-heptyl)	7	151 - 152°C
R = -C ₈ H ₁₇ (n-octyl)	8	150°C
R = -C ₁₂ H ₂₅ (n-dodecyl)	9	148 - 149°C
R = -C ₁₆ H ₃₃ (n-hexadecyl)	10	130 - 131°C
R = -C ₆ H ₅ (phenyl)	11	148°C
R = -C ₆ H ₄ -CH ₃ (toluyl)	12	156 - 157°C

25

30

Example 2

The new compounds exhibit a good to excellent antimicrobial activity. The following table lists the results

obtained from tests in vitro on a culture medium in successive dilution of the compounds according to the present invention. Each of the culture media contained one of the pathogenic microorganisms mentioned below.

5 The minimum concentrations inhibiting the growth of the microorganisms are given in micrograms/ml.

Compound No.	Staph. aur.*	E. coli*	Pseudomonas * aeruginosa
1	10	125	375
2	3	33	53
3	2	10	15
4	5	531	500
5	12.5	100	100
6	2.5	31.2	31.2
7	0.3	6.2	6.2
8	1.2	50	3.1
9	6.3	62.5	10
10	1.2	15.6	125
11	1.2	5.0	5.0
12	0.6	5.0	2.5

* pathenogenic microorganism