Compounds of general formula (I) wherein the nature of the group R is not particularly critical and typically takes various forms, include the natural compound halitulin of formula (I) have antitumor activity.
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CYTOTOXIC ALKALOIDS (HALITULIN)

The present invention relates to a new class of anti-tumor agents based on a new active compound isolated from a sponge.

BACKGROUND OF THE INVENTION

In connection with our long-standing interest in the chemistry and bioactivity of marine sponges, we found that extracts of the Indo-Pacific sponge *Haliclona tulearensis* (class Demospongiae, order Haplosclerida, family Chalinidae, genus Haliclona), collected in Sodwana Bay, Durban, South Africa, were quite cytotoxic. Many interesting N-containing metabolites came out from the genus *Haliclona*\(^1\text{-}\text{6}^\)\). Recently, we reported the isolation of haliclorensin (1), a new N-(3'-aminopropyl)-3-methylazacyclocdecane, from *H. tulearensis*\(^7\) of the following formula (A):

\[
\text{N} \quad \text{NH}_2
\]

We have now discovered a new N-containing metabolite named halitulin with cytotoxic activity, which in turn has led us to a new class of active compounds.
SUMMARY OF THE INVENTION

According to the present invention, there is provided a compound of the general formula (I):

![Chemical Structure]

and related derivatives.

PREFERRED EMBODIMENTS

In particular, the present invention provides compounds of the given formula (I) wherein R is selected from:

a) a cycloamino-N-alkylene group of formula (II):

\[ (\text{CH}_2)_m \text{N} \circlearrowleft (\text{CH}_2)_n \text{H} \]

where \( m \) is typically 1 to 6, especially 3, and \( n \) is typically 3 to 20, especially 9, and which may be substituted on the ring, for example with one or more C\(_1\)-C\(_6\) alkyl groups, especially with one methyl group \( \beta \) to the ring nitrogen;

b) a similar cycloaliphatic-alkylene group lacking the ring nitrogen;

c) an optionally substituted alkyl group, cycloalkyl group, aryl group, aralkyl group or any other substituent group which does not undermine the activity of the compound, for example an aminoalkylene group of formula \(-(\text{CH}_2)_p \text{NR}^1 \text{R}^2\),

SUBSTITUTE SHEET (RULE 26)
where p is typically 1 to 6, especially 3, and R\textsuperscript{1} and R\textsuperscript{2} are hydrogen, aryl or aralkyl;

d) hydrogen.

The nature of the group R is not critical.

Moreover, there can be one or more substituents on the quinoline rings, for example alkyl groups at one or more of the 2-, 3- and 4-positions of one or both quinoline rings.

The present invention further extends to related derivatives of the compounds of formula (I), which include:

a) derivatives of the phenolic hydroxy groups, such as ethers or esters;
b) oxidised forms such as N-oxides and o-quinolinoquinones;
c) pharmaceutical acceptable acid addition salts

In particular, we provide the compound halitinulin of formula:
Halitulin, a substituted pyrrole, can be isolated from the sponge *Haliclona tulearensis*, and can be characterised by the following data:

\[ \alpha_0 = +7.5^\circ; \quad (c=2.8, \text{MeOH}) \]

IR \( \delta_{\text{max}} \): 3000-3400, 1623, 1597 cm\(^{-1}\)

\( \lambda_{\text{max}}(\text{MeOH}): 212 (29200), 252 (31600), 364 (4400); \)

and other data given later.

Over 250 pyrrole-containing compounds are known from marine organisms. A few that resemble the structure of halitulin are polycitone A, polycitins A & B\(^8\), the lamellarins\(^9\) from ascidians and the storniamide\(^10\) and arcyriarubins\(^11\) from sponges. Halitulin, to the best of our knowledge, is the first natural compound to be discovered that embodies a 7,8-dihydroxyquinoline system. A very few other dihydroxyquinoline-containing compounds are known e.g. luzopeptin, a terrestrial Actinomadura antimicrobial metabolite\(^20\) and the marine sponge *Verongia aerophoba* metabolite 3,4-dihydroxyquinoline-2-carboxylic acid \(^21\).

Halitulin (2) was found to have cytotoxic activity. The activity, IC\(_{50}\) values, against cell cultures of P-388 murine leukemia, A-549 human lung carcinoma, HT-29 human colon carcinoma and MEL-28 human melanoma is 0.025, 0.012, 0.012 and 0.025 µg/ml respectively.

We know that the compound haliclorens is inactive. From this knowledge, we can predict the activity in the compounds of the present invention.

The present invention also relates to pharmaceutical preparations which contain as active ingredient a compound of this invention, as well as processes for preparation. Methods of administration to patients are also envisaged.

Examples of pharmaceutical compositions include any solid (tablets, pills, capsules, granules, etc.) or liquid (solutions, suspensions or emulsions) with suitable
composition of oral, topical or parental administration, and they may contain the pure
compound or in combination with any carrier or other pharmacologically active
compounds. These compositions may need to be sterile when administered
parenterally.

The correct dosage of a pharmaceutical composition comprising a compound of
this invention will vary according to the pharmaceutical formulation, the mode of
application, and the particular situs, host and tumor being treated. Other factors like
age, body weight, sex, diet, time of administration, rate of excretion, condition of the
host, drug combinations, reaction sensitivities and severity of the disease shall be taken
into account. Administration can be carried out continuously or periodically within the
maximum tolerated dose.

The compounds may be provided in the pharmaceutical compositions of this
invention in the form of a prodrug or precursor, which upon administration converts or
is metabolised to the active compound.

The compositions of this invention may be used with other drugs to provide a
combination therapy. The other drugs may form part of the same composition, or be
provided as a separate composition for administration at the same time or a different
time. The identity of the other drug is not particularly limited, and suitable candidates
include:
- drugs with antimitotic effects, especially those which target cytoskeletal elements,
  including microtubule modulators such as taxane drugs (such as taxol, paclitaxel,
  taxotere, docetaxel), podophyllotoxins or vinca alkaloids (vincristine, vinblastine);
- antimetabolite drugs such as 5-fluorouracil, cytarabine, gemcitabine, purine analogues
  such as pentostatin, methotrexate);
- alkylating agents such as nitrogen mustards (such as cyclophosphamide or ifosfamide);
- drugs which target DNA such as the antracycline drugs adriamycin, doxorubicin,
  phmarorubicin or epirubicin;
drugs which target topoisomerases such as etoposide;
hormones and hormone agonists or antagonists such as estrogens, antiestrogens
(tamoxifen and related compounds) and androgens, flutamide, leuprolelin, goserelin,
cyproterone or octreotide;
drugs which target signal transduction in tumour cells including antibody derivatives
such as herceptin;
alkylating drugs such as platinum drugs (cis-platin, carboplatin, oxaliplatin, paraplatin)
or nitrosoureas;
drugs potentially affecting metastasis of tumours such as matrix metalloproteinase
inhibitors;
gene therapy and antisense agents;
antibody therapeutics; and
other bioactive compounds of marine origin, notably the ecteinascidins such as ET-743,
or the didemmins such as aplidine.

EXAMPLES OF THE INVENTION

The present invention will be further illustrated with reference to the following
examples which aid in the understanding of the present invention, but which are not to
be construed as limitations thereof. All percentages reported herein, unless otherwise
specified, are present by weight. All temperatures are expressed in degrees Celsius.
All incubations are carried out at 28°C and flasks are shaken in an orbital shaker at 250
rpm. All media and recipients are sterile and all culture processes aseptic.

IR spectra were recorded on a Nicolet 205 Ft-ir spectrophotometer. High
resolution mass spectra ((HRMS) were obtained on a VG Fison autospec Q instrument.
$^1$H and $^{13}$C-nmr were recorded on Bruker AMX-360 and ARX-500 spectrometers. All
chemical shifts are reported with respect to TMS ($\delta_{H}=0$). Optical rotations were
measured on a Perkin-Elmer Model 141 polarimeter using a 1-cm microcell.

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Haliclona tulearensis was collected in Sodwana Bay, Durban, South Africa. A voucher sample of the organism (the sponge Haliclona tulearensis) from which halitulin (2) has been isolated, has been deposited at the Oceanographic Research Institute in Durban, South Africa, having the Deposit Number TASA-121 and having been deposited in July 1992.

Freshly collected H. tulearensis was frozen on site and kept frozen until needed. Freeze-dried sponge tissue (32g, dry wt) was extracted with methanol-EtOAc (1:1) to give a brown gum (2.9g) after evaporation. The two latter fractions were individually fractionated by repeated chromatography on Sephadex LH-20 (eluting with CHCl₃:MeOH, 1:1) to afford halitulin (2, 180 mg, 0.56% dry wt)¹².

Halitulin (2) was analyzed for C₃₃H₄₇N₄O₄ by HREIMS (m/z 580.3054, 100% Δmμu - 0.5) confirmed by positive and negative FABMS (m/z 581 and 579, respectively). The ¹³C NMR spectrum (Table 1) showed, however, only 24 resonances - thirteen sp-3 carbons (one methyl, eleven methylenes and one methine) and eleven sp² carbons (five methine and six quaternary carbons) implying, therefore, the duplication of eleven carbon atoms. The duplicated part, according to the integration of the proton signals, was determined to be the aromatic portion of the molecule. Comparing the NMR data of the aliphatic part of 2 (Table 1) with haliclorensin (1)⁷, together with the COSY, TOCSY, and HMBC data, established their identity. The incorporation of 1 in halitulin was further supported by the two MS fragments at m/z 168 (C₁₁H₂₂N⁺, 48%) and m/z/ 399 (MH⁺-C₁₂H₂₄N, 90%), resulting from the preferable αβ to the aliphatic nitrogen-atoms' fragmentation at C-13,14 and C-12,13 respectively. Determination of the haliclorensin moiety in 2 left C₂₂H₁₄N₃O₄ (including the adjoining N-atom) with 17 degrees of unsaturation to be accounted for. Strong absorptions centred at 3200 cm⁻¹ in the IR spectrum, the presence of four oxygen atoms in 2 and the absence of carbonyls and ethereal C-atoms in the ¹³C NMR spectrum suggested four OH groups. Indeed, acetylation of 2, with a 1:1 mixture of Ac₂O/pyridine, overnight at room temperature,
afforded a very unstable tetra phenol acetate (3), on the basis of the HREIMS which
gave the suitable molecular ion and also four subsequential losses of 42 mass units and
no loss of 60 mass units\textsuperscript{13}. The four phenol acetate groups were in full agreement with
the 1773 cm\textsuperscript{-1} IR absorption and the new methyls in the proton NMR. Furthermore, the
$^1$H-NMR spectrum, showing only two new signals at $\delta$ 2.37 and 2.50 ppm integrating
for 6H each (in comparison with H$_3$-25), pointed clearly to symmetry in the aromatic
part of 2. The eleven sp\textsuperscript{2} C-atoms, suggested six different double bonds, of which at
least one has to be a C=N bond. A priori more than one structure is possible, however,
accounting for the above data, especially the 1D and 2D NMR spectra (Table 1), only
one structure (discussed below) is possible, namely a diquinolinylpyrrole. Two of the
double bonds, carrying H-2 ($\delta$ 8.56) and H-10 ($\delta$ 7.04), with $^1$J\textsuperscript{CH} values of 179 and 184
Hz, respectively, have to be adjacent to N-atoms, and moreover to be part of quinoline
and pyrrole\textsuperscript{14}. The $^1$H and $^{13}$C chemical shifts of the aromatic pat (Table 1) implied a
substituted quinoline system. Furthermore, the three proton spin system, confirmed by
a COSY experiment ($\delta$ 8.56 d(J$_{2,3}$=4.9 Hz), 7.20 dd (J= 8.3 Hz) and 8.51 d(J$_{3,4}$ = 8.3
Hz) indicated, according to the 4.9 Hz coupling characteristic for a coupling constant
next to a nitrogen atom, that the pyridine ring of the system is free of substitution. On
the other hand, the adjacent benzene ring, carrying a single proton (\$ 7.28 brs) has to be
three substituted. That is, one position being the linkage to the rest of the molecule and
two others bear OH groups. Empirical calculations of the carbon chemical shifts\textsuperscript{15}
agreed best with a 5-substituted-7,8-dihydroxyquinoline, a suggestion that was
confirmed in two ways: a reaction of halitulin with NaIO\textsubscript{4} (known to oxidize catechols
to o-quinones)\textsuperscript{16} in a 1:1 mixture of EtOH:H\textsubscript{2}O, afforded, on the basis of the change in
the UV spectrum\textsuperscript{17} and change in color from orange to red, an o-quinone and b. from the
measured NOE's vide infra. All the above data suggested a 3,4-diquinolinylpyrrole,
which is in full agreement with the results from the HMBC experiment. To distinguish
between very close shifts of C-4a, 6 and 10, a 1D INAPT experiment was undertaken\textsuperscript{18}.
Two key NOE's that supported unequivocally the suggested structure, were between H-6
and H-10 and between H-4 and H--10. Both NOE's being possible due to rotation
around the C-5,9 bond, and only possible for the suggested isomer.
Compound 2 is sensitive to light and air, conditions under which, most likely, the azacyclodecane-nitrogen oxidizes. This is seen from the appearance of a weak peak at $m/z$ M+16 in the mass spectrum. As a result, two N-oxide isomers are obtained causing the appearance of two new double methyl resonances (Me-25) in the NMR spectrum. Subsequently, the dihydroxy quinoline also oxidizes to the o-quinolinoquinone.

The structure of halitulin, as far as the aromatic part is concerned, resembles the polycitone$^1$ and the lamellarins$^2$. However, the two phenyl-C$_3$ units of the latter's biogenthnic precursors, are suggested in this case, to be replaced by two 5-substituted-quinoline-C$_3$ compounds, the biogenesis of which is not simple. The halicloresin (1) biogenesis (replacing the phenethyl amine, as suggested earlier, for the above compounds$^7$) is similar to the one suggested for manzamine C$^{22}$.

Table 1. $^1$H(500 MHz) and $^{13}$C(125 MHz) NMR data of halitulin (2) in CDCl$_3$.

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<thead>
<tr>
<th>No.</th>
<th>$\delta_C$ (m)</th>
<th>$\delta_H$ (m, J in Hz)</th>
<th>HMBC (H to C)</th>
<th>No.</th>
<th>$\delta_C$ (m)</th>
<th>$\delta_H$ (m, J in Hz)</th>
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<td>2</td>
<td>145.1d</td>
<td>8.56 (d,4,9)</td>
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<td>26.1t</td>
<td>2.56(m)</td>
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<td>3</td>
<td>117.1 d</td>
<td>7.20 (dd, 8.3, 4.9)</td>
<td>2,4a</td>
<td>14</td>
<td>54.4 t</td>
<td>3.23 (m)</td>
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<td>141.6 d</td>
<td>8.51 (d, 8.3)</td>
<td>2,5, 8a</td>
<td>16</td>
<td>57.7 t</td>
<td>3.27 (m), 2.97 (m)</td>
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<td>4a</td>
<td>122.9</td>
<td></td>
<td></td>
<td>17</td>
<td>28.3</td>
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<td>5</td>
<td>126.7s</td>
<td></td>
<td></td>
<td>18</td>
<td>32.7 t</td>
<td>1.60 (m)</td>
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<tr>
<td>6</td>
<td>123.2 d</td>
<td>7.28 brs</td>
<td>4a,7,8,9</td>
<td>19</td>
<td>24.4 t</td>
<td>1.36-1.62 (m)</td>
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<tr>
<td>7</td>
<td>148.1 s</td>
<td></td>
<td></td>
<td>20</td>
<td>24.2 t</td>
<td>1.36-1.62 (m)</td>
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<tr>
<td>8</td>
<td>131.2 s</td>
<td></td>
<td></td>
<td>21</td>
<td>23.9 t</td>
<td>1.36-1.62</td>
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<tr>
<td>8a</td>
<td>130.1 s</td>
<td></td>
<td></td>
<td>22</td>
<td>23.6</td>
<td>1.36-1.62 (m)</td>
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<tr>
<td>9</td>
<td>118.8 s</td>
<td></td>
<td></td>
<td>23</td>
<td>22.0 t</td>
<td>1.96 (m)</td>
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<tr>
<td>10</td>
<td>122.6 d</td>
<td>7.04 brs</td>
<td>5,9,12</td>
<td>24</td>
<td>50.9 t</td>
<td>3.40 (m), 3.75 (m)</td>
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<td>12</td>
<td>46.8 t</td>
<td>4.23 (t, 6.3)</td>
<td>10,13,14</td>
<td>25</td>
<td>20.07 q</td>
<td>1.09 (brs)</td>
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</table>
exchangeable. The chemical shifts are strongly influenced by concentration and pH e.g. C-4a, 6 and 10 resonated in another experiment at 122.5, 122.5 and 122.1 respectively. The following $^{1}J_{CH}$ values have been measured for C-2,3,4,6 and 10: 179, 166, 161, 160 and 184 Hz respectively. Besides the COSY correlations of H-2-4, the aliphatic protons gave the expected COSY, TOCSY and HMBC correlations as detailed for haliclorens earlier. INAPT correlations were found between (H to C): 2/8a, 3/4a, 4/5,8a 6/4a,8,9 and 10/9.

References and Notes


12. 2, orange foaming oil; [α]D +7.5 (c 2.8, MeOH), νmax 3000-3400, 1623, 1597 cm⁻¹, λmax (MeOH) 212(29200), 252(31600), 364(4400), λmax(MeOH+OH) 214(24700), 264(14800), 350(4650).

13. 3: EIMS m/z (748(5%), 706(50), 664(100), 622(100), 580(48), 525(10), 483(25), 441(42), 399(98), 168(37), HREI 664.3261(Δmmu 1.1), 399,1220(Δmmu - 0.1). δH (J in Hz) 0.90 (d, 3H, J=6), 1.51 (bres, SH), 1.61-1.69 (m, 10H), 1.86 (m, 1H), 1.97 (m, 1H) 2.22 (m, 1H), 2.24 (m, 1H), 2.37 (bres, 6H), 2.50 (bres, 6H), 2.65 (m, 1H), 2.84 (bbrt, 1H), 4.16 (m, 2H), 7.03 (bres, 2H), 7.15 (dd, 2H, J=8.4), 7.28 (bres, 2H), 8.23 (d, 2H, J=8.6), 8.76 (d, 2H, J=2); δC 19.59, 20.6 qx2, 20.7 qx2, 22.3t, 24.5t, 24.7t, 26.0t, 26.5t, 29.0t, 30.2 t, 31.9 t, 48.5t, 52.3t, 53.5t, 60.8t, 120.65, 120.7d, 121.7d, 123.4d, 125.9s, 132.4s, 134.6d, 14.7s, 142.1s, 150.5d, 168.1s, 168.5.


15. Spec Tool, version 1.0, Chemical Concepts Gub H.


17. 2-o-quinone; λmax (MeOH) 219 (12700), 248 (7400), 348 (3300), 457 (800) - see reference 14.


19. Because of the symmetry of 2, the discussion regarding the one half is, of course, also valid for the identical second half.

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Biological Activity

The compound of the present invention, Halitulin (2) exhibits antitumor activity against cell line derived from human tumors, such as P-388 mouse lymphoma, A-549 human lung carcinoma, HT-29 human colon carcinoma and MEL-28 human melanoma. Also provides a method of treating any mammal affected by a malignant tumor sensitive to Halitulin (2) which comprises administering to the affected individual a therapeutically effective amount of Halitulin (2) or a pharmaceutical composition thereof.

Cell Cultures. Cells were maintained in logarithmic phase of growth in eagle’s Minimum Essential Medium, with Earle’s Balanced Salts, with 2.0 mm L-glutamine, with non-essential amino acids, without sodium bicarbonate (EMEM/neaa); supplemented with 10% Fetal Calf Serum (FCS), 10^{-2} M sodium bicarbonate and 0.1 g/l penicillin-G+ streptomycin sulfate.

A simple screening procedure has been carried out to determine and compare the antitumor activity of these compounds, using an adapted form of the method described by Bergeron et al. (1984)(1). The antitumor cells employed have been P-388
(suspension culture of a lymphoid neoplasm from DBA/2 mouse), A0549 (monolayer culture of a human lung carcinoma), HT-29 (monolayer culture of a human colon carcinoma) and MEL-28 (monolayer culture of a human melanoma).

P-388 cells were seeded into 16 mm wells at $1 \times 10^4$ cells per well in 1 ml aliquots of MEM 5FCS containing the indicated concentration of drug. A separate set of cultures without drug was seeded as control growth to ensure that cells remained in exponential phase of growth. All a 98% humid atmosphere, an approximately IC50 was determined by comparing the growth in wells with drug to the growth in wells control.

A-549, HT-29 and MEL-28 cells were seeded into 16 mm wells at $2 \times 10^4$ cells per well in 1 ml aliquots of MEM 10FCS containing the indicated concentration of drug. A separate set of cultures without drug was seeded as control growth to ensure that cells remained in exponential phase of growth. All determinations were carried out in duplicate. After three days of incubation at 37°C, 10% CO2 in a 98% humid atmosphere, the wells were stained with 0.1% Crystal Violet. An approximately IC50 was determined by comparing the growth in wells with drug to the growth in wells control.


<table>
<thead>
<tr>
<th>Compound</th>
<th>P-388</th>
<th>A-549</th>
<th>HT-29</th>
<th>MEL-28</th>
</tr>
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<tbody>
<tr>
<td>Halitulin</td>
<td>0.025</td>
<td>0.0125</td>
<td>0.0125</td>
<td>0.025</td>
</tr>
</tbody>
</table>
1. A compound of the general formula (I):

wherein R is selected from:

a) a cycloamino-N-alkylene group of formula (II):

\[ \text{-(CH}_2\text{)}_m\text-N\text-(CH}_2\text{)}_n\text-H \]

where m is 1 to 6 and n is 3 to 20, and which may be substituted on the ring with one or more C\textsubscript{1}-C\textsubscript{6} alkyl groups;

b) a cycloaliphatic-alkylene group

\[ \text{-(CH}_2\text{)}_m\text-CH\text-(CH}_2\text{)}_n\text-H \]

where m is 1 to 6 and n is 3 to 20, and which may be substituted on the ring with one or more C\textsubscript{1}-C\textsubscript{6} alkyl groups;

c) a C\textsubscript{1}-C\textsubscript{6} alkyl group, cyclo-(C\textsubscript{1}-C\textsubscript{6})-alkyl group, aryl group, C\textsubscript{7}-C\textsubscript{12}-aralkyl group, an aminoalkylene group of formula \text{-(CH}_2\text{)}_p\text-NR}^1\text{R}^2, where p is 1 to 6, and R\textsuperscript{1} and R\textsuperscript{2} are hydrogen, aryl or C\textsubscript{7}-C\textsubscript{12}-aralkyl;

d) hydrogen;

and wherein there is one or more C\textsubscript{1}-C\textsubscript{6} alkyl groups at one or more of the 2-, 3- and 4-positions of one or both quinoline rings;

the compounds including derivatives:
a) ethers or ester derivatives of the phenolic hydroxy group;
b) oxidised forms which are N-oxides and o-quinolinoquinones;
c) pharmaceutical acceptable acid addition salts

2. A compound according to claim 1 where m is 3, and n is 9.

3. A compound according to claim 1(a), with one methyl group β to the ring nitrogen

4. A compound according to claim 1 which is halitulin of formula:

5. A pharmaceutical preparation which contain a compound according to any preceding claim, together with a pharmaceutically acceptable carrier.

7. The use of claim 6, wherein the medicament is for use in the treatment of a tumor.

8. A method of preparation of halitulin, which comprises isolation from the sponge *Haliclona tulearensis.*
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7: C07D401/14 A61K31/40

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)
IPC 7: C07D

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
<th>Category</th>
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</tr>
</thead>
</table>

X Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier document but published on or after the international filing date
  "L" document which may throw doubts on priority claim(s) or which is citable to establish the publication date of another citation or other special reason (as specified)
  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed
  "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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  "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
  "&" document member of the same patent family

Date of the actual completion of the international search
27 January 2000

Date of mailing of the international search report
15/02/2000

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 551 epc nl, Fax (+31-70) 340-3016

Authorized officer
Stellmach, J
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<tr>
<td>Y</td>
<td>PALERMO J A ET AL: &quot;Storniamides A-D: Alkaloids from a Patagonian Sponge Cliona sp&quot;&lt;br&gt;<strong>TETRAHEDRON.NL,ELSEVIER SCIENCE</strong>&lt;br&gt;PUBLISHERS, AMSTERDAM,&lt;br&gt;vol. 52, no. 8, 1996, page 2727-2734&lt;br&gt;X0004104363&lt;br&gt;ISSN: 0040-4020&lt;br&gt;cited in the application&lt;br&gt;the whole document</td>
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<td>Y</td>
<td>REDDY M V R ET AL: &quot;New Lamellarin Alkaloids from an Unidentified Ascidian from the Arabian Sea&quot;&lt;br&gt;<strong>TETRAHEDRON.NL,ELSEVIER SCIENCE</strong>&lt;br&gt;PUBLISHERS, AMSTERDAM,&lt;br&gt;vol. 53, no. 10, 1997, page 3457-3466&lt;br&gt;X0004105426&lt;br&gt;ISSN: 0040-4020&lt;br&gt;the whole document</td>
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<td>KOREN-GOLDSHLAGER G. ET AL.: &quot;Haliclorensin, a Novel Alkaloid from the Marine Sponge Haliclona tulearensis&quot;&lt;br&gt;<strong>J.NAT.PROD.</strong>&lt;br&gt;vol. 61, February 1998 (1998-02), pages 282-284, X0002128243&lt;br&gt;COLUMBUS&lt;br&gt;cited in the application&lt;br&gt;the whole document</td>
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<td>CHARAN R D ET AL: &quot;Haliconacyclamines A and B, Cytotoxic Alkaloids from the Tropical Marine Sponge Haliclona sp&quot;&lt;br&gt;<strong>TETRAHEDRON.NL,ELSEVIER SCIENCE</strong>&lt;br&gt;PUBLISHERS, AMSTERDAM,&lt;br&gt;vol. 52, no. 27, July 1996 (1996-07), page 9111-9120 X0004103999&lt;br&gt;ISSN: 0040-4020&lt;br&gt;the whole document</td>
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<td>CLARK R J ET AL: &quot;The Haliconacyclamines, Cytotoxic Tertiary Alkaloids from the Tropical Marine Sponge Haliclona sp&quot;&lt;br&gt;<strong>TETRAHEDRON.NL,ELSEVIER SCIENCE</strong>&lt;br&gt;PUBLISHERS, AMSTERDAM,&lt;br&gt;vol. 54, no. 30, page 8811-8826&lt;br&gt;X0004124047&lt;br&gt;ISSN: 0040-4020&lt;br&gt;the whole document</td>
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