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Published
With international search report.

(54) Title: BAFILOMYCINE MACROLIDES FOR TREATMENT OF BONE AFFECTING DISEASES

(57) Abstract

A method for the treatment of diseases related to loss of bone mass such as osteoporosis, Paget's disease of bone, hyperparathyroidism, malignant neoplasms causing hypercalcemia, parodontal diseases and implant-related bone loss comprising administration to a patient suffering therefrom an amount of a compound of formula (I), or a pharmaceutically acceptable salt thereof, effective to mitigate the symptoms of the bone disease.
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Bafilomycine macrolides for treatment of bone affecting diseases.

Field of the Invention

The present invention is related to a novel method for the treatment of several bone affecting diseases, especially osteoporosis, which are characterized by loss of bone mass.

Background of the Invention

The balance in normal subjects between on the one hand bone formation, which is associated with the number and activity of osteoblasts, that is cells associated with the production of bone in the organism, and on the other hand bone loss, which is associated with the number and activity of osteoclasts, that is cells associated with the absorption and removal of bone, is disturbed in several bone affecting diseases. At the present time there is no good treatment for any of these diseases, among which can be mentioned osteoporosis, Paget’s disease of bone, hyperparathyroidism and related disorders, and several malignant neoplasms where tumor cells are producing osteoclast-activating factors and cause hypercalcemia.

Worldwide the most urgent need is for the treatment of osteoporosis and tumor associated hypercalcemia. In some areas, e.g. in England and in some other parts of Europe there is also high incidence of Paget’s disease of bone.

In osteoporosis bone formation as well as bone resorption are disturbed, resulting in decreased bone mass. Osteoporosis
predominantly affects the elderly, but also other groups such as postmenopausal women, where an estrogen deficit is believed to be a significant etiological factor, and immobilized patients. At this point it is not possible to clear up the whole picture of the disease mechanism and estimate which is the primary cause of osteoporosis. However, about 25% of osteoporotic females belong to what is called "rapid bone losers" and at least in those patients the bone resorption rate is probably increased. Landry and Fleisch showed in immobilization induced osteoporosis that bone resorption rate was accelerated, (Landry, M. and Fleisch, H,: The influence of immobilization on bone formation as evaluated the incorporation of tetracyclines. J. Bone Joint Surg. 46B:764, 1964).

The clinical manifestations of osteoporosis comprise fractures, especially hip fractures, but also vertebral fractures and fractures of the proximal radius, and complication of such fractures.

In Finland it has been estimated that about 10% of all surgical hospital beds are used for the treatment of osteoporosis related fractures (Lüthje, P.: Reisiluunkaulan ja trokantterin murtumapotilaiden hoito ja ennuste sekä hiodon kustannukset. Thesis. Helsinki 1983).

The present methods for the treatment of osteoporosis include exercise; administration of estrogen, especially for postmenopausal women; and consumption of calcium or calcium containing material such as milk. Calcitonin, a hormone associated with calcium metabolism, has also been used in the treatment of osteoporosis. None of these methods of treating osteoporosis results in increase of the bone mass.
Several malignant tumors are known to be associated by hypercalcemia which is due to increased osteoclastic activity. This is a common complication for instance in the case of breast cancer and prostate cancer which are both one of the most common malignant tumors. Hypercalcemia is due to both systemic and local factors. Some malignant cells are known to secrete agents which stimulate bone resorption (Sato, K., Fujii, Y. Kachivehi, T., Kasono, K., Shizume, K.: Production of interleukin 1 alpha (IL-1α)-like activity and colony stimulating activity by clonal squamous cell carcinomas derived from patients with hypercalcemia and leucocytosis. In: Calcium Regulation and Bone Metabolism Vol. 9 (eds. D.V. Cohu, T.J. Martin, P.J. Meunier), 1986).

In malignant hypercalcemia calcitonin and diphosphonate treatment has been used.

Paget’s disease (or osteitis deformans) of bone is a disease of unknown etiology where bone resorption and remodelling are increased leading sometimes even to the fractures of affected bone. Bone pain is the main indication of treatment in these patients. In these patients there is highly elevated local osteoclastic bone destruction. The incidence of osteitis deformans is very low in Scandinavian countries. In England it has been estimated to be present in 3-4 % of population on the basis of autopsy studies (Anderson’s Textbook of Pathology 1986). It is very rare in patients under 40 years. Calcitonin and diphosphonates are also used in the treatment of Paget’s disease.
Other disease states for the treatment of which antagonists to osteoclastic activity might be useful, are parodontal diseases and prosthetic and implant bone losses.

It is an object of the present invention to provide compounds which by affecting the balance between osteoblast and osteoclast activity can be useful for the prophylactic and therapeutic treatment of diseases as indicated above which are associated with bone loss. It is believed that the use of these compounds will also ultimately result in an increase of the bone mass.

Prior art


Outline of the present invention

According to the present invention it has been found that compounds of the general formula I, which are known as Bafilomycin compounds, Hygrolidin compounds, and related compounds, as well as pharmaceutically acceptable salts thereof are effective as inhibitors of basal and stimulated bone resorption and are useful as medicals for the treatment of diseases related to bone loss
and increased bone resorption, such as osteoporosis, Paget's disease of bone, hyperparathyroidism, both primary and secondary, malignant neoplasms where tumor cells are producing osteoclast-activating factors and cause hypercalcemia, immobilization-induced ostoporosis, parodontal diseases and prosthetic and implant-related bone losses.

The compounds of the invention are of the following formula I, and pharmaceutically acceptable salts thereof:

\[
\text{I}
\]

wherein

1. \( R^1 = \text{OME}, \ R^2 = \text{H}, \ R^4 = \text{H}, \ R^3 = \)

\[
\text{R}^5 = \text{H, MeCO, HOOCCH} \text{CHO, NHCOCHCHCO,}
\]

and \( R^6 = \text{H, Me}, \)

or \( R^5 = \text{Me}, \)

\[
\text{and } R^6 = \text{H};
\]
2. $R^1 = \text{OMe}$  $R^2 = \text{MeCO}$  $R^4 = \text{H}$

$R^3 = \begin{array}{c}
\text{OR}^5 \\
\text{OR}^6 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{H} \\
\text{Me}
\end{array}$

$R^5 = \text{MeCO}$  $R^6 = \text{H, Me}$

3. $R^1 = \text{Me}$  $R^2 = \text{H}$  $R^4 = \text{H}$

$R^3 = \begin{array}{c}
\text{OR}^8 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{HCOCHCHCO} \\
\text{HO}
\end{array}$

$R^8 = \text{H, HOOCCHCHCO, COCHCHCO or H}_2\text{NCOCHCHCO}$

4. $R^1 = \text{OMe}$  $R^2 = \text{MeCO}$  $R^4 = \text{MeCO}$  and

$R^3 = \begin{array}{c}
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{OH}
\end{array}$
"Me" in the formulas designates methyl. The formula I specifically indicates those stereoisomers which are described in the literature referred to above. Thus, the formula I includes the following compounds: Bafilomycin $A_1$, $A_2$, $B_1$, $B_2$, $C_1$, $C_2$, and D (according to the nomenclature used by Werner et al (1984); see above) as well as Hygrolidin, Hygrolidin amide, Defumaryhygrolidin and Oxohygroolidin (according to the nomenclature used by Seto et al. (1984) and Kretschmer et al. (1985); see above).

Preferred compounds of the invention are those of formula I, wherein $R^1$ is OMe, $R^2$ and $R^4$ are H, $R^3$ is

![Chemical structure](image)

and $R^5$ and $R^6$ are as described above. Further preferred of these compounds are those wherein $R^5$ is H, HOOCCHCHCO or

![Chemical structure](image)

and $R^6$ is as described above. Still further preferred of these compounds are those wherein $R^5$ is H or HOOCCHCHCO and $R^6$ is as described above. Particularly preferred of these compounds are the compound wherein $R^5$ and $R^6$ are both H (Bafilomycin $A_1$) and the compound wherein $R^5$ is HOOCCHCHCO and $R^6$ is H (Bafilomycin $C_1$).

Accordingly, the invention relates to

- a method for the prophylactic and therapeutic treatment of each of the ailments above by administering to a host in need thereof of an effective amount of a compound of the formula I optionally together with a pharmaceutically acceptable carrier

- a pharmaceutical preparation for use in the prophylactic and therapeutic treatment of each of the ailments above comprising a compound of the formula I as active ingredient
- the use of a compound of the formula I in the manufacture of a medicament for the prophylactic and therapeutic treatment of each of the ailments above

- a method for improving the healing rate of bone fractures by administering to a host in need thereof of an effective amount of a compound of the formula I

**Pharmacological tests**

In order to evaluate the inhibitory effect on bone resorption, an *in vitro* model, the mouse calvaria explant model (described in Reynolds, J.J. Organ cultures of bone: Studies on the physiology and pathology of bone resorption. In: Organ culture in biomedical research (Bullen M., and Monnichendam M.A. eds) Cambridge University Press, p.p. 355-366, 1976) was used. In this model the effects of the compounds on the basal and the parathyroid hormone (PTH)-induced bone resorption are measured.

**Results**

The compound of structure I where \( R^1 = \text{OMe}, R^2 = \text{H}, R^4 = \text{H}, R^3 = \)

\[
\begin{align*}
\text{CH}_3 & \text{C} \\
\text{C} & \text{CH}_3
\end{align*}
\]

\( R^5 = \text{H} \) and \( R^6 = \text{H} \), bafilomycin \( A_1 \), was tested. As can be seen from Table 1 the compound significantly inhibits PTH-induced bone resorption.
Table 1. Effect of Bafilomycin A₁ on parathyroid hormone induced bone resorption in vitro

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<tr>
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<th>% Release of $^{45}$Ca$^{2+}$</th>
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<tbody>
<tr>
<td>Control (=basal)</td>
<td>5.4±0.35</td>
</tr>
<tr>
<td>PTH</td>
<td>30±4.3</td>
</tr>
<tr>
<td>PTH + 10$^{-10}$ mol/l Bafilomycin A₁</td>
<td>27±5.0</td>
</tr>
<tr>
<td>PTH + 10$^{-9}$ mol/l Bafilomycin A₁</td>
<td>18±2.2</td>
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<tr>
<td>PTH + 10$^{-8}$ mol/l Bafilomycin A₁</td>
<td>6.3±0.41</td>
</tr>
<tr>
<td>PTH + 10$^{-7}$ mol/l Bafilomycin A₁</td>
<td>3.5±0.52</td>
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</table>

PTH=Parathyroid hormone. PTH was used at a concentration of 10$^{-8}$ mol/l. The results are mean±SEM of 5 half-calvaria in each group. The calvarial bones were cultured with PTH and Bafilomycin A₁ for 72 hours. The estimated IC$_{50}$ value (IC$_{50}$=the concentration of drug that gives 50 % inhibition of the response) was estimated to 10$^{-9}$ mol/l.
For clinical use the compounds of the formula I are formulated into pharmaceutical formulations for oral, rectal, parenteral or other mode of administration. The pharmaceutical formulation contains a compound of the formula I in combination with a pharmaceutically acceptable carrier. The carrier may be in the form of a solid, semi-solid or liquid diluent, or a capsule. These pharmaceutical preparations are a further object of the invention. Usually the amount of active compounds is between 0.1-95 % by weight of the preparation, between 0.2-20 % by weight in preparations for parenteral use and between 1 and 50 % by weight in preparations for oral administration.

The typical daily dose of the active substance varies within a wide range and will depend on various factors such as for example the individual requirement of each patient, the route of administration and the disease. In general, oral and parenteral dosages will be in the range of 0.1 to 500 mg per day of active substance.
What we claim is:

1. A method for the treatment of osteoporosis by administering to a host in need of such treatment of a therapeutically effective amount of a compound of the formula I below, or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable carrier:

![Chemical Structure](image)

wherein

1. \( R^1 = \text{OMe} \quad R^2 = \text{H} \quad R^4 = \text{H} \),

\[ R^3 = \]

\[ R^5 = \text{H}, \text{MeCO}, \text{HOOCHCHCO}, \]

and \( R^6 = \text{H}, \text{Me} \),

or \( R^5 = \text{Me} \),

\[ \text{and } R^6 = \text{H}; \]
2. \( R^1 = \text{OMe} \quad R^2 = \text{MeCO} \quad R^4 = \text{H} \)
   \( R^3 = \)
   \( R^5 = \text{MeCO} \quad R^6 = \text{H, Me} \)

3. \( R^1 = \text{Me} \quad R^2 = \text{H} \quad R^4 = \text{H} \)
   \( R^3 = \)
   \( R^8 = \text{H, HOOCCHCHCO, COCHCHCO} \quad \text{or H}_2\text{NCOCHCHCO} \)

4. \( R^1 = \text{OMe} \quad R^2 = \text{MeCO} \quad R^4 = \text{MeCO and} \)
   \( R^3 = \)

2. A method for the treatment of Paget's disease of bone by administering to a host in need of such treatment of a therapeutically effective amount of a compound of the formula I as defined in claim 1, optionally together with a pharmaceutically acceptable carrier.
3. A method for the treatment of primary and secondary hyperparathyroidism by administering to a host in need of such treatment of a therapeutically effective amount of a compound of the formula I as defined in claim 1, optionally together with a pharmaceutically acceptable carrier.

4. A method for the treatment of such malignant neoplasms where tumor cells are producing osteoclast-activating factors, by administering to a host in need of such treatment of a therapeutically effective amount of a compound of the formula I as defined in claim 1, optionally together with a pharmaceutically acceptable carrier.

5. A method for the treatment of parodontal diseases, by administering to a host in need of such treatment of a therapeutically effective amount of a compound of the formula I as defined in claim 1, optionally together with a pharmaceutically acceptable carrier.

6. A method for the treatment of prosthetic and implant-related bone loss, by administering to a host in need of such treatment of a therapeutically effective amount of a compound of the formula I as defined in claim 1, optionally together with a pharmaceutically acceptable carrier.
7. A method for the treatment of disease connected with increased bone resorption by administering to a host in need of such treatment of a therapeutically effective amount of a compound of the formula I as defined in claim 1, optionally together with a pharmaceutically acceptable carrier.

8. A method according to claims 1-7 wherein Bafilomycin A₁ is used.

9. A method according to claims 1-7 wherein Bafilomycin C₁ is used.

10. The use of a compound of the formula I as defined in claim 1 in the manufacture of a medicament for the treatment of osteoporosis.

11. The use of a compound of the formula I as defined in claim 1 in the manufacture of a medicament for the treatment of Paget's disease of bone.

12. The use of a compound of the formula I as defined in claim 1 in the manufacture of a medicament for the treatment of primary and secondary hyperparathyroidism.

13. The use of a compound of the formula I as defined in claim 1 in the manufacture of a medicament for the treatment of such malignant neoplasms where tumor cells are producing osteoclast activating factors.
14. The use of a compound of the formula I as defined in claim 1 in the manufacture of a medicament for the treatment of parodontal diseases.

15. The use of a compound of the formula I as defined in claim 1 in the manufacture of a medicament for the treatment of prosthetic and implant-related bone loss.

16. The use of a compound of the formula I as defined in claim 1 in the manufacture of a medicament for the treatment of diseases connected with increased bone resorption.

17. The use according to any of claims 10-16 wherein the compound I is Baflomycin A₁.

18. The use according to any of claims 10-16 wherein the compound I is Baflomycin C₁.

19. A pharmaceutical preparation for use in the treatment of diseases connected with increased bone resorption; osteoporosis; Paget's disease of bone; primary and secondary hyperparathyroidism; such malignant neoplasms where tumor cells are producing osteoclast-activating factors; such parodontal diseases which are associated with bone loss; or prosthetic and implant-related bone loss; and comprising a compound of the formula I as defined in claim 1 as active ingredient.
20. A pharmaceutical preparation according to claim 19 comprising Bafilomycin A₁ as active ingredient.

21. A pharmaceutical preparation according to claim 19 comprising Bafilomycin C₁ as active ingredient.

22. A method for improving the healing rate of bone fractures by administering to a host in need thereof an effective amount of a compound as defined in claim 1.

23. A method according to claim 22 wherein the compound of the formula I is Bafilomycin A₁.

24. A method according to claim 22 wherein the compound of the formula I is Bafilomycin C₁.

25. A compound of the formula I as defined in claim 1 for use in the manufacture of a medicament for improving the healing rate of bone fractures.

26. Bafilomycin A₁ for use according to claim 25.

27. Bafilomycin C₁ for use according to claim 25.

28. A pharmaceutical preparation for use in improving the healing rate of bone fractures, comprising a compound of the formula I as defined in claim 1 as active ingredient.

29. A pharmaceutical preparation according to claim 28 comprising Bafilomycin A₁ as active ingredient.
30. A pharmaceutical preparation according to claim 28 comprising Bafilomycin C₁ as active ingredient.
**INTERNATIONAL SEARCH REPORT**

**International Application No.** PCT/SE 90/00684

**I. CLASSIFICATION OF SUBJECT MATTER**

According to International Patent Classification (IPC) or to both National Classification and IPC

**IPC5:** A 61 K 31/365, C 07 D 313/00, 407/06

**II. FIELDS SEARCHED**

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Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched

SE, DK, FI, NO classes as above

**III. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>The Journal of Antibiotics, Vol. 37, No. 9, 1984 L. Huang et al.: &quot;Discovery, production and purification of the Na+, K+ activated ATPase inhibitor, L-681,110 from the fermentation broth of Streptomyces sp. MA-5038&quot;, page 970 - page 975 see the whole article</td>
<td>10-21, 28-30</td>
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* 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 cited to establish the publication date of another citation or other special reason (as specified)
  - "O" document referring to an oral disclosure, use, exhibition or other means
  - "P" document published prior to the international filing date but later than the priority date claimed

**IV. CERTIFICATION**

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International Searching Authority: SWEDISH PATENT OFFICE

Signature of Authorized Officer: Göran Karlsson

Form PCT/ISA/210 (second sheet) (January 1995)
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<td>Y</td>
<td>Endocrinology, Vol. 113, No. 1, 1983 Nancy S. Krieger et al.: &quot;Inhibition of stimulated bone resorption by vanadate&quot;, page 324 - page 328 see especially page 328</td>
<td>10-21, 28-30</td>
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V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSCALABLE

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. Claim numbers 1-9, because they relate to subject matter not required to be searched by this Authority, namely:
   A method for treatment of the human or animal body, see rule 39.

2. Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claim numbers ....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This International Searching Authority found multiple inventions in this international application as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest
- The additional search fees were accompanied by applicant’s protest.
- No protest accompanied the payment of additional search fees.
This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 90-11-28. The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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