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(21) International Application Number: PCT/JP99/04338 (22) International Filing Date: 11 August 1999 (11.08.99) (30) Priority Data: 10/228300 12 August 1998 (12.08.98) JP (71) Applicant (for all designated States except US): TAKEDA CHEMICAL INDUSTRIES, LTD. [JP/JP]; 1-1, Doshomachi 4-chome, Chuo-ku, Osaka-shi, Osaka 541-0045 (JP). (72) Inventors; and (75) Inventors/Applicants (for US only): MAKINO, Haruhiko [JP/JP]; 17-8, Wakaba 1-chome, Inagawa-cho, Kawabe-gun, Hyogo 666-0251 (JP). FUJISAWA, Yukio [JP/JP]; 12-5-605, Takezono 2-chome, Tsukuba-shi, Ibaraki 305-0032 (JP). HAZAMA, Masatoshi [JP/JP]; 7-28-102, Satsukigaoka 2-chome, Ikeda-shi, Osaka 563-0029 (JP). SOHDA, Takashi [JP/JP]; 27-20, Higashikanmaki 2-chome, Takatsuki-shi, Osaka 569-0002 (JP). (74) Agents: ASAHINA, Tadao et al.; Osaka Plant of Takeda Chemical Industries, Ltd., 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka-shi, Osaka 532-0024 (JP). (81) Designated States: AE, AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CR, CU, CZ, DM, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>		
(54) Title: ENHANCER OF CELL DIFFERENTIATION INDUCTION FACTOR		
$\begin{array}{c} \text{B} \\ \\ (\text{CH}_2)_k \\ \\ \text{C} - \text{S}(=\text{O})_{k'} \\ \quad \\ \text{X} \quad \text{R} \end{array}$ (I)		
(57) Abstract This invention provides an enhancer of cell differentiation induction factor comprising a compound of formula (I), wherein ring A is an optionally substituted benzene ring; R is a hydrogen atom or an optionally substituted hydrocarbon group; B is an optionally esterified or amidated carboxyl group; X is -CH(OH)- or -CO-; k is 0 or 1; and k' is 0, 1 or 2, or its salt, which can be advantageously used as an enhancer of cell differentiation induction factor.		

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DESCRIPTION

Enhancer of Cell Differentiation Induction Factor

Technical Field

5 The present invention relates to an enhancer of cell differentiation induction factor effective in the treatment and prevention of bone diseases such as osteoporosis, bone fractures, and bone re-fractures, in the reconstruction of bone, and in the treatment and prevention
10 of nervous diseases such as Alzheimer's disease, cerebrovascular dementia, amyotrophic lateral sclerosis (Lou Gehrig's disease), and diabetic peripheral neuropathy.

15 Background Art

 Bone morphogenetic protein (BMP), isolated from decalcified bone, is the only group of protein factors known to be capable of ectopic bone induction [A.E. Wang, Trends in Biotechnology, Vol. 11, pp. 379-383 (1993)].

20 Because BMP directly promotes osteoblast differentiation, it is assumed to play a role as a coupling factor in bone remodeling, and is closely involved in bone metabolism. Also, it has been reported that the BMP content in bone substrate in aged animals has been considerably
25 decreased [M.L. Urist, Bone and Mineral Research, Vol. 6 (edited by W.A. Peck), pp. 57-112, Elsevier, 1989], suggesting that BMP is profoundly involved in the maintenance of bone mass.

 In view of the above aspects, it may be common
30 knowledge for a skilled artisan that any compound enhancing the action of BMP, for example, could be useful as a therapeutic drug for bone diseases as described above by being administered in vivo because of its own activity to enhance the action of present BMP. To date, a number of
35 such substances with BMP action-enhancing activity have

been reported, i.e., retinoic acid, vitamin D3, estrogen, and glucocorticoid [V. Rosen & R.S. Thies, Trends in Genetics, Vol. 8, pp. 97-102 (1992); Y. Takuwa et al., Biochemical and Biophysical Research Communications, Vol. 174, pp. 96-101 (1991)]. However, these substances, when administered in vivo, are known to promote bone resorption and have side effects such as hypercalcemia and ovarian cancer onset, and are not always appropriate for use as therapeutic drugs for bone diseases. Therefore it has been a long time desire for the skilled person to find a substance which is useful for prevention and treatment of such bone diseases.

Neurotrophic factors, a group of proteinous factors playing an important role in the survival and functional expression of neurons, include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3). NGF promotes the differentiation and maturation of the sympathetic ganglia and dorsal root ganglia of the neural tube in the peripheral nervous system [A.M. Davies & R.M. Lindsay, Developmental Biology, Vol. 111, pp. 62-72 (1985); R. Levi-Montalcini, EMBO Journal, Vol. 6, 1145-1154 (1987)], and acts on the cholinergic neurons of septa (procephalic basal ganglia) in the central nervous system [H. Gnahn et al., Developmental Brain Research, Vol. 9, pp. 45-52 (1983); H. Hatanaka & H. Tsukui, Developmental Brain Research, Vol. 30, pp. 47-56 (1986); F. Hefti, Journal of Neuroscience, Vol. 6, pp. 2155-2162 (1986)]. NGF is essential for the maintenance of nervous function even after completion of neuron differentiation. BDNF acts on the dorsal spinal root ganglia and nodal ganglia in the peripheral nervous system but does not act on sympathetic ganglia [R.M. Lindsay & H. Rohrer, Developmental Biology, Vol. 112, pp. 30-48 (1985); R.M. Lindsay et al., Developmental Biology, Vol. 112, pp. 319-328 (1985); A.M. Davies et al., Journal of Neuroscience,

Vol. 6, pp. 1897-1904 (1986)]. On the other hand, in the central nervous system, BDNF acts on the cholinergic neurons and GABA (γ -aminobutyric acid)-acting neurons of septa, and the dopaminergic neurons of the midbrain [R.F. Alderson et al., *Neuron*, Vol. 5, pp. 297-306 (1990); C. Hyman et al., *Nature*, Vol. 350, pp. 230-232 (1991); B. Knusel et al., *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 88, pp. 961-965 (1991)]. NT-3 is characterized by potent action on the sensory neurons of neural plate origin, although its action overlaps those of NGF and BDNF in the peripheral nervous system [P. Ernfors et al., *Proc. Natl. Acad. Sci. USA*, Vol. 87, pp. 5454-5458 (1990); A. Rosenthal et al., *Neuron*, Vol. 4, pp. 767-773 (1990)].

Therefore, any compound enhancing the action of NGF, for example, would be useful as anti-dementia drug or anti-peripheral neuropathy drug as described above by being administered in vivo due to its own activity to enhance the action of present NGF. As a substance exhibiting such action, sabeluzole [4-(2-benzothiazolylmethylamino)- α [(p-fluorophenoxy)methyl]-1-(piperidineethanol)] has been reported [*New Current*, Vol. 4, No. 26, p. 14 (1993)]; however, its action mechanism remains to be clarified; because clinical studies have demonstrated side effects such as headache, dizziness, and fatigue, it is not always appropriate for use as a therapeutic drug for nervous diseases. Also, as compounds exhibiting NGF secretion-inducing action, steroids, catechols, and cytokines have been reported [*Experimental Neurology*, Vol. 124, pp. 36-42 (1993)]. Japanese Patent Unexamined Publication No. 81218/1991 discloses idebenone as such a compound. However, some of these compounds exhibit undesirable actions such as neurotoxicity, immunity reduction, hypercalcemia, and bone resorption promotion; in fact, their NGF secretion-inducing action is not always

distinguishable from their adverse effects on non-nervous tissues, so they are unsatisfactory for practical application. It has been also desired to find a substance useful for treatment or prevention for these disease.

5 Alzheimer dementia has been characterized by extensive disorder and fall of cerebral cortical neurons, as well as denaturation and fall of cholinergic neurons of the procephalic basal ganglia, including the septa; NGF and new neurotrophic factors are considered as candidates for
10 therapeutic drugs therefor [F. Hefti & W.J. Weiner, Annual Neurology, Vol. 20, pp. 275-281 (1986)]. Also, in Parkinson's disease, characterized by denaturation and fall of dopaminergic neurons of the midbrain, BDNF, a neurotrophic factor for said neurons, is expected to serve
15 as a therapeutic drug. Because these neurotrophic factors are proteins, however, their application is subject to limitation.

BMP has been reported to possess an activity like that of neurotrophic factors [V.M. Paralkar et al., Journal of
20 Cell Biology, Vol. 119, pp. 1721-1728 (1992)]. Also, it is known that the BMP gene is strongly expressed in brain tissue [E. Ozkaynak et al., Biochemical and Biophysical Research Communications, Vol. 179, pp. 116-123 (1991)]. BMP has also been suggested as playing an important role
25 in neural tube formation in embryogenesis [K. Basler et al., Cell, Vol. 73, pp. 687-702 (1993)]. BMP is therefore profoundly involved in the differentiation or maintenance of function of neurons.

In addition, because cell differentiation induction
30 factors, typically exemplified by BMP, are proteins, their administration to the living body is subject to limitation. It is therefore preferable that the compound that enhances the action of a cell differentiation induction factor present or administered in vivo be of low molecular weight.

35 In view of the above-described situation, the present

invention is directed to discover a low-molecular compound that enhances the action of cell differentiation induction factors, typically exemplified by BMP, and to provide a cell differentiation induction factor action enhancer useful in the treatment and prevention of various bone diseases or nervous diseases.

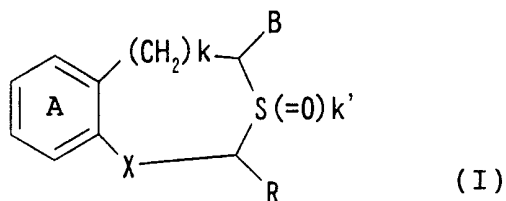
Disclosure of Invention

Against this technical background, the present inventors conducted extensive investigation in search for a low-molecular compound that enhances the action of cell differentiation induction factors, with the aim of developing a drug that specifically enhances the induction of osteoblast or neuron differentiation by BMP, and found that an optically active benzothiepine derivative represented by formula (I) below possesses excellent activity to enhance the action of BMP. The inventors conducted further investigation based on this finding, and developed the present invention.

Accordingly, the present invention relates to:

(1) An enhancer of cell differentiation induction factor action, which comprises benzothiepine or benzothiopilane derivatives or its salts;

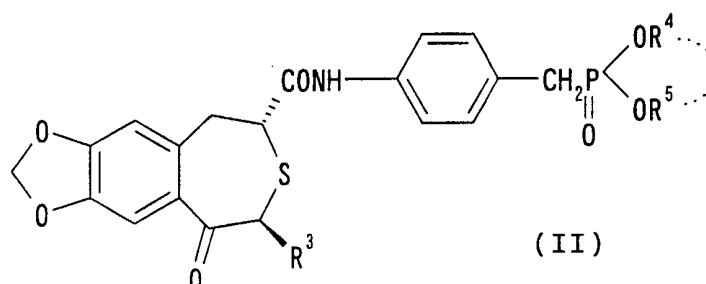
(2) An enhancer of cell differentiation induction factor, which comprises a compound (I) of the formula:



wherein ring A is an optionally substituted benzene ring; R is a hydrogen atom or an optionally substituted hydrocarbon group; B is an optionally esterified or amidated carboxyl group; X is -CH(OH)- or -CO-; k is 0 or 1; and k' is 0, 1 or 2, or its salt;

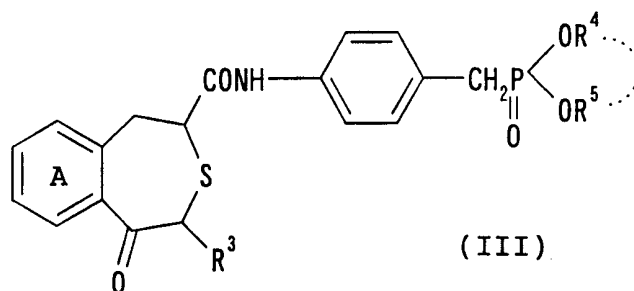
(3) An enhancer according to (2), which comprises the compound (I) wherein the ring A is a benzene ring which may be substituted by 1 or 2 substituents selected from the group consisting of a halogen, a C₁₋₁₀ alkyl, a C₁₋₁₀ alkoxy, an alkylenedioxy group of the formula: -O-(CH₂)_n-O- wherein n is an integer from 1 to 3 and a C₁₋₁₀ alkylthio group; R is a hydrogen atom, a C₁₋₆ alkyl or a phenyl group; B is -CON(R¹)(R²) wherein R¹ is a hydrogen atom or a C₁₋₁₀ alkyl group and R² is a phenyl or a phenyl-C₁₋₃ alkyl group which those groups may be substituted by a halogen, a C₁₋₆ alkoxy, a mono- or di-C₁₋₆ alkoxyphosphoryl, a mono- or di-C₁₋₆ alkoxyphosphoryl-C₁₋₃ alkyl wherein two alkyl groups of these di-C₁₋₆ alkoxy group optionally may bind together to form a C₁₋₆ alkylene group and C₁₋₆ alkoxycarbonyl group;

(4) An enhancer according to (2), wherein the compound is an optically active compound (II) of the formula:



wherein R³ is a C₁₋₆ alkyl group; and R⁴ and R⁵ are independently a C₁₋₆ alkyl group or bind together to form a C₁₋₆ alkylene group;

(5) An enhancer according to (2), wherein the compound is a compound (III) of the formula:



wherein the ring A is a benzene ring substituted by two methoxy groups; R^3 is a hydrogen atom; and R^4 and R^5 are independently a C_{1-6} alkyl group or bind together to form a C_{1-6} alkylene group;

(6) An enhancer according to (4) or (5), wherein R_4 and R_5 are independently a C_{1-4} alkyl group;

(7) An enhancer according to (4), wherein the optically active compound (II) is (2R,4S)-(-)-N-[4-(diethoxyphosphorylmethyl)phenyl]-1,2,4,5-tetrahydro-4-methyl-7,8-methylenedioxy-5-oxo-3-benzothiepine-2-carboxamide;

(8) An enhancer according to (5), wherein the compound (III) is N-[4-(diethoxyphosphorylmethyl)phenyl]-1,2,4,5-tetrahydro-7,8-dimethoxy-5-oxo-3-benzothiepine-2-carboxamide;

(9) A pharmaceutical composition comprising the enhancer defined in (2);

(10) A pharmaceutical composition according to (9), which is in the form of a sustained-release preparation comprising a biodegradable polymer;

(11) A pharmaceutical composition according to (9), which is for local administration;

(12) A pharmaceutical composition according to (9), which is for oral administration;

(13) A pharmaceutical composition according to (9), which is for injection;

(14) A pharmaceutical composition according to (9), which is for prevention or treatment of bone diseases;

(15) A pharmaceutical composition according to (9), which is for prevention or treatment of nervous diseases;

(16) An enhancer defined in (2), wherein the cell differentiation induction factor is a bone morphogenetic protein;

(17) An enhancer defined in (2), wherein the cell differentiation induction factor is a neurotrophic factor;

(18) An enhancer defined in (17), wherein the neurotrophic factor is one selected from a family of NGF;

(19) A method for enhancing an action of cell differentiation induction factor, which comprises administering compound (I) or its salts defined in (2) to mammal in need; and

(20) Use of the compound (I) or its salts defined in (2) for manufacturing a medicament for enhancing an action of cell differentiation induction factor.

The chemical structures of benzothiepine or benzothiopilane derivatives of the present invention are different from them of the above mentioned known-substance having enhancing effect of the action of BMP or NGF completely.

With respect to the compound (I) or its salt, the substituents of the substituted benzene represented by the ring A is exemplified by a halogen atom, a nitro group, an optionally substituted alkyl group, an optionally substituted hydroxy group, an optionally substituted mercapto group, an optionally substituted amino group, an optionally substituted acyl group, a mono- or di-alkoxyphosphoryl group, a phosphono group, an optionally substituted aryl group, an optionally substituted aralkyl group and an optionally substituted aromatic heterocyclic group. Of these substituents, 1 to 4, preferably 1 or 2, whether identical or different, may be present on the benzene ring.

The halogen atom includes fluorine, chlorine,

bromine and iodine.

The alkyl group of the optionally substituted alkyl group includes an alkyl group having 1 to 10 carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, neo-pentyl, hexyl, heptyl, octyl, nonyl and decyl, and a cycloalkyl group having 3 to 7 carbon atoms such as cyclopropyl, cyclobutyl, cyclohexyl and cycloheptyl. These alkyl groups may be substituted by 1 to 3 substituents selected from a halogen atom (e.g., fluorine, chlorine, bromine, iodine), a hydroxy group, an alkoxy group having 1 to 6 carbon atoms (e.g., methoxy, ethoxy, propoxy, butoxy, hexyloxy), a mono- or di-C₁₋₆ alkoxyphosphoryl group (e.g. methoxyphosphoryl, ethoxyphosphoryl, dimethoxyphosphoryl, diethoxyphosphoryl) and a phosphono group.

The substituted alkyl group includes trifluoromethyl, trifluoroethyl, trichloromethyl, hydroxymethyl, 2-hydroxyethyl, methoxyethyl, 1-methoxyethyl, 2-methoxyethyl, 2,2-diethoxyethyl, 2-diethoxyphosphorylethyl, phosphonomethyl and so on.

The substituted hydroxy group includes alkoxy group, an alkenyloxy group, an aralkyloxy group, an acyloxy group, an aryloxy group and so on. Preferable alkoxy groups is an alkoxy group having 1 to 10 carbon atoms (e.g., methoxy, ethoxy, propoxy, butoxy, tert-butoxy, pentyloxy, hexyloxy, heptyloxy, nonyloxy) and a cycloalkoxy group having 4 to 6 carbon atoms (e.g., cyclobutoxy, cyclopentoxy, cyclohexyloxy). Preferable alkenyloxy group is an alkenyloxy group having 2 to 10 carbon atoms such as allyloxy, crotyloxy, 2-pentenyl, 3-hexenyl, 2-cyclopentenylmethoxy and 2-cyclohexenylmethoxy. Preferable aralkyloxy group is an aralkyloxy group having 7 to 19 carbon atoms, with more preference given to a C₆₋₁₄ aryl-C₁₋₄ alkyloxy group (e.g., benzyloxy, phenethyloxy). Preferable acyloxy group is an alkanoyloxy group such as

one having 2 to 10 carbon atoms (e.g., acetyloxy, propionyloxy, n-butyryloxy, hexanoyloxy). Preferable aryloxy group is aryloxy group having 6 to 14 carbon atoms (e.g., phenoxy, biphenyloxy). Further, these substituted hydroxy groups may be substituted by 1 to 3 substituents selected from the above-mentioned halogen atom, a hydroxy group, an alkoxy group having 1 to 6 carbon atoms, a mono- or di-C₁₋₆ alkoxyphosphoryl group, a phosphono group, etc. Specifically, the substituted hydroxy group includes trifluoromethoxy, 2,2,2-trifluoroethoxy, difluoromethoxy, 2-methoxyethoxy, 4-chlorobenzoyloxy and 2-(3,4-dimethoxyphenyl)ethoxy, and so on.

The substituted mercapto group includes an alkylthio group, an aralkylthio group and an acylthio group. Preferable alkylthio group is an alkylthio group having 1 to 10 carbon atoms (e.g., methylthio, ethylthio, propylthio, butylthio, pentylthio, hexylthio, heptylthio, nonylthio) and a cycloalkylthio group having 4 to 6 carbon atoms (e.g., cyclobutylthio, cyclopentylthio, cyclohexylthio). Preferable aralkylthio group is an aralkylthio group having 7 to 19 carbon atoms, more preferably a C₆₋₁₄ aryl-C₁₋₄ alkylthio group such as benzylthio and phenethylthio. Preferable acylthio group is alkanoylthio group such as one having 2 to 10 carbon atoms (e.g., acetylthio, propionylthio, n-butyrylthio, hexanoylthio). Further, these substituted mercapto groups may be substituted by 1 to 3 substituents selected from the above-mentioned halogen atom, a hydroxy group, an alkoxy group having 1 to 6 carbon atoms, a mono- or di-C₁₋₆ alkoxyphosphoryl group, a phosphono group, etc. Specifically, the substituted mercapto group includes tri-fluoromethylthio, 2,2,2-trifluoroethylthio, 2-methoxyethylthio, 4-chlorobenzylthio, 3,4-dichlorobenzylthio, 4-fluorobenzylthio, 2-(3,4-dimethoxyphenyl)ethylthio, and so on.

As substituents of the substituted amino group, there may be used 1 or 2 of identical or different substituents selected from the above-mentioned groups such as the alkyl group having 1 to 10 carbon atoms, the alkenyl group having 2 to 10 carbon atoms (e.g., allyl, vinyl, 2-penten-1-yl, 3-penten-1-yl, 2-hexen-1-yl, 3-hexen-1-yl, 2-cyclohexenyl, 2-cyclopentenyl, 2-methyl-2-propen-1-yl, 3-methyl-2-buten-1-yl), the aryl group having 6 to 14 carbon atoms (e.g. phenyl, naphthyl) and the aralkyl group having 7 to 19 carbon atoms (e.g. benzyl, phenetyl). These substituents may be substituted by the above-mentioned group such as the halogen atom, the alkoxy group having 1 to 6 carbon atoms, the mono- or di-C₁₋₆ alkoxyphosphoryl group, the phosphono group, etc. Specifically, the substituted amino group includes methylamino, dimethylamino, ethylamino, diethylamino, dibutylamino, diallylamino, cyclohexylamino, phenylamino, N-methyl-N-phenylamino, N-methyl-N-(4-chlorobenzyl)amino and N,N-di(2-methoxyethyl)amino, and so on.

The acyl group of the optionally substituted acyl group includes an organic carboxylic acid acyl group and a sulfonic acid acyl group with a hydrocarbon group having 1 to 6 carbon atoms (e.g., C₁₋₆ alkyl group such as methyl, ethyl, n-propyl, hexyl) or phenyl. Useful organic carboxylic acyl group is formyl, a C₁₋₁₀ alkyl-carbonyl group (e.g., acetyl, propionyl, butyryl, valeryl, pivaloyl, hexanoyl, octanoyl, cyclobutanecarbonyl, cyclohexanecarbonyl, cycloheptanecarbonyl), a C₂₋₁₀ alkenyl-carbonyl group (e.g., crotonyl, 2-cyclohexenecarbonyl), a C₆₋₁₄ aryl-carbonyl group (e.g., benzoyl), a C₇₋₁₉ aralkyl-carbonyl group (e.g., benzylcarbonyl, benzhydrylcarbonyl), a 5- or 6-membered aromatic heterocyclic carbonyl group (e.g, nicotinoyl, 4-thiazolylcarbonyl) or a 5- or 6-membered aromatic heterocyclic acetyl group (e.g., 3-pyridylacetyl, 4-

thiazolylacetyl). Useful sulfonic acyl group having 1 to 6 carbon atoms are methanesulfonyl and ethanesulfonyl, and so on. These acyl groups may be substituted by 1 to 3 substituents selected from the above-mentioned halogen atom, a hydroxy group, an alkoxy group having 1 to 6 carbon atoms, an amino group, etc. Specifically, the substituted acyl group includes trifluoroacetyl, trichloroacetyl, 4-methoxybutyryl, 3-cyclohexyloxypropionyl, 4-chlorobenzoyl and 3,4-dimethoxybenzoyl, and so on.

The mono- or di-alkoxyphosphoryl group includes a mono-C₁₋₆ alkoxyphosphoryl group such as methoxyphosphoryl, ethoxyphosphoryl, propoxyphosphoryl, isopropoxyphosphoryl, butoxyphosphoryl, pentyloxyphosphoryl and hexyloxyphosphoryl, and a di-C₁₋₆ alkoxyphosphoryl group such as dimethoxyphosphoryl, diethoxyphosphoryl, dipropoxyphosphoryl, diisopropoxyphosphoryl, dibutoxyphosphoryl, dipentyloxyphosphoryl and dihexyloxyphosphoryl, with preference given to a di-C₁₋₆ alkoxyphosphoryl group such as dimethoxyphosphoryl, diethoxyphosphoryl, dipropoxyphosphoryl, diisopropoxyphosphoryl, ethylenedioxyphosphoryl, dibutoxyphosphoryl, etc..

The aryl group of the optionally substituted aryl group includes an aryl group having 6 to 14 carbon atoms such as phenyl, naphthyl and anthryl. These aryl groups may be substituted by 1 to 3 substituents selected from the above-mentioned alkyl group having 1 to 10 carbon atoms, a halogen atom, a hydroxy group, an alkoxy group having 1 to 6 carbon atoms, etc. Specifically, the substituted aryl group includes 4-chlorophenyl, 3,4-dimethoxyphenyl, 4-cyclohexylphenyl and 5,6,7,8-tetrahydro-2-naphthyl.

The aralkyl group of the optionally substituted aralkyl group includes aralkyl group having 7 to 19 carbon atoms such as benzyl, naphthylethyl and trityl. These aralkyl groups may be substituted by 1 to 3 substituents

selected from the above-mentioned alkyl group having 1 to 10 carbon atoms, a halogen atom, a hydroxy group, an alkoxy group having 1 to 6 carbon atoms, etc. on the aromatic ring. Specifically, the substituted aralkyl group includes 4-chlorobenzyl, 3,4-dimethoxybenzyl, 4-cyclohexylbenzyl and 5,6,7,8-tetrahydro-2-naphthylethyl.

The aromatic heterocyclic group of the optionally substituted aromatic heterocyclic group includes a 5- or 6-membered aromatic heterocyclic group having 1 to 4 atoms of nitrogen, oxygen and/or sulfur, such as furyl, thienyl, imidazolyl, thiazolyl, oxazolyl and thiadiazolyl. These aromatic heterocyclic groups may be substituted by 1 to 3 substituents selected from the above-mentioned alkyl group having 1 to 10 carbon atoms, a halogen atom, a hydroxy group, an alkoxy group having 1 to 6 carbon atoms, etc.

Provided that two alkyl groups are present as mutually adjoining substituents on the benzene ring A, they may bind together to form an alkylene group represented by the formula: $-(CH_2)_m-$ wherein m is an integer from 3 to 5 (e.g., trimethylene, tetramethylene, pentamethylene). Provided that two alkoxy groups are present as mutually adjoining substituents on the benzene ring A, they may bind together to form an alkylenedioxy group represented by the formula: $-O-(CH_2)_n-O-$ wherein n is an integer from 1 to 3 (e.g., methylenedioxy, ethylenedioxy, trimethylenedioxy). In these cases, a 5- to 7-membered ring is formed in cooperation with carbon atoms of the benzene ring.

With respect to the compound (I), R is a hydrogen atom or an optionally substituted hydrocarbon group.

The hydrocarbon group of the optionally substituted hydrocarbon group represented by R is exemplified by the above-mentioned alkyl group (preferably an alkyl group having 1 to 10 carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, iso-pentyl, neo-pentyl and hexyl), the alkenyl group

(preferably an alkenyl group having 2 to 10 carbon atoms), the aryl group (preferably an aryl group having 6 to 14 carbon atoms) and the aralkyl group (preferably an aralkyl group having 7 to 19 carbon atoms). Useful substituents on the hydrocarbon group include the above-mentioned 5- or 6-membered aromatic heterocyclic group such as furyl, thienyl, imidazolyl, thiazolyl, oxazolyl and thiadiazolyl, the halogen atom, the di-C₁₋₆ alkoxyphosphoryl group and the phosphono group.

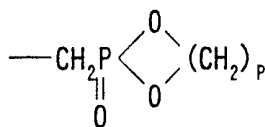
With respect to the compound (I), B is an optionally esterified or amidated carboxyl group.

The esterified carboxyl group represented by B is exemplified by an alkoxycarbonyl group, preferably a C₁₋₁₀ alkoxy-carbonyl group (e.g., methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, butoxycarbonyl), an aryloxycarbonyl group, preferably C₆₋₁₄ aryloxycarbonyl group (e.g., phenoxycarbonyl), and an aralkyloxycarbonyl group, preferably a C₇₋₁₉ aralkyloxy-carbonyl group (e.g., benzyloxycarbonyl).

The amidated carboxyl group represented by B is exemplified by an optionally substituted carbamoyl group represented by the formula: -CON(R¹)(R²) wherein R¹ and R² independently are a hydrogen atom, an optionally substituted hydrocarbon group or an optionally substituted 5- to 7-membered heterocyclic group.

The hydrocarbon group of the optionally substituted hydrocarbon group represented by R¹ or R² is exemplified by the above-mentioned alkyl group, preferably an alkyl group having 1 to 10 carbon atoms (e.g., methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, neopentyl, hexyl, heptyl, octyl, nonyl, decyl), the alkenyl group, preferably an alkenyl group having 2 to 10 carbon atoms (e.g., allyl, vinyl, 2-penten-1-yl, 3-penten-1-yl, 2-hexen-1-yl, 3-hexen-1-yl, 2-cyclohexenyl, 2-cyclopentenyl, 2-methyl-2-propen-1-yl,

3-methyl-2-buten-1-yl), the aryl group, preferably aryl group having 6 to 14 carbon atoms (e.g., phenyl, naphthyl, anthryl), and the aralkyl group, preferably aralkyl groups having 7 to 19 carbon atoms (e.g., benzyl, naphthylethyl, trityl). These hydrocarbon groups may be substituted by 1 to 3 substituents selected from (i) a halogen atom (e.g., fluorine, chlorine, bromine, iodine), (ii) a hydroxy group, (iii) an alkoxy group having 1 to 6 carbon atoms (e.g., methoxy, ethoxy, propoxy, butoxy, tert-butoxy, pentyloxy, hexyloxy), (iv) an amino group which may be substituted by an alkyl group having 1 to 6 carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, iso-pentyl, neo-pentyl and hexyl (e.g., amino, methylamino, ethylamino, dimethylamino, diethylamino, dipropylamino), (v) an amino group which may be substituted by an acyl group such as a C₁₋₁₀ alkanoyl group (e.g., acetylamino, propionylamino, benzoylamino), (vi) a carbamoyl group which may be substituted by an alkyl group having 1 to 6 carbon atoms (e.g., carbamoyl, methylcarbamoyl, dimethylcarbamoyl, diethylcarbamoyl), (vii) a C₁₋₆ alkoxy-carbonyl group (e.g., methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl), (viii) a mono- or di-alkoxyphosphoryl group (e.g. a mono- or di-C₁₋₆ alkoxyphosphoryl group such as dimethoxyphosphoryl, diethoxyphosphoryl, ethylenedioxyphosphoryl), (ix) a mono- or di-alkoxyphosphorylalkyl group (e.g. a mono- or di-C₁₋₆ alkoxyphosphoryl-C₁₋₃ alkyl group such as methoxyphosphorylmethyl, ethoxyphosphorylmethyl, methoxyphosphorylethyl, ethoxyphosphorylethyl, dimethoxyphosphorylmethyl, diethoxyphosphorylmethyl, dimethoxyphosphorylethyl, diethoxyphosphorylethyl), (x) a moiety:



wherein p is an integer from 2 to 4, (xi) a phosphono group, (xii) the above-mentioned aromatic heterocyclic group, etc.

5 The 5- to 7-membered heterocyclic group of the optionally substituted 5- to 7-membered heterocyclic group represented by R^1 or R^2 is exemplified by a 5- to 7-membered heterocyclic group containing a sulfur, nitrogen or oxygen atom, 5- or 6-membered heterocyclic groups containing 2 to 4 nitrogen atoms, and 5- or 6-membered heterocyclic groups containing 1 or 2 nitrogen atom(s) and a sulfur or oxygen atom. These heterocyclic groups may be condensed with a 6-membered ring containing 2 or less nitrogen atoms, a benzene ring or a 5-membered ring containing a sulfur atom.

10 As a substituent of the substituted 5- to 7-membered heterocyclic group represented by R^1 and R^2 , there may be used 1 to 4 of the same substituents as those for the substituted hydrocarbon group represented by R^1 and R^2 above.

20 Preferable examples of the 5- to 7-membered heterocyclic group represented by R^1 and R^2 include 2-pyridyl, pyrimidyl, pyrazinyl, pyridazinyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, tetrazolyl, thiadiazolyl, oxadiazolyl, triazinyl, triazolyl, thienyl, pyrrolyl, pyrrolinyl, furyl, pyrrolidinyl, benzothienyl, indolyl, 25 imidazolidinyl, piperidyl, piperidino, piperazinyl, morpholinyl, morpholino, pyrido[2,3-d]pyrimidyl, benzopyranyl, 1,8-naphthyridyl, quinolyl, thieno[2,3-b]pyridyl.

30 The moiety: $-N(R^1)(R^2)$ may form a 5- to 7-membered ring by binding together with R^1 and R^2 . Such rings include morpholine, piperidine, thiomorpholine, homopiperidine, piperidine, pyrrolidine, thiazolidine and azepine.

The substituted alkyl group as preferable examples of the optionally substituted hydrocarbon group represented by R^1 and R^2 include trifluoromethyl,

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trifluoroethyl, difluoromethyl, trichloromethyl, 2-hydroxyethyl, 2-methoxyethyl, 2-ethoxyethyl, 2,2-dimethoxyethyl, 2,2-diethoxyethyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-(2-thienyl)ethyl, 3-(3-furyl)propyl, 2-morpholinoethyl, 3-pyrrolylbutyl, 2-piperidinoethyl, 2-(N,N-dimethylamino)ethyl, 2-(N-methyl-N-ethylamino)ethyl, 2-(N,N-diisopropylamino)ethyl, 5-(N,N-dimethylamino)pentyl, N,N-dimethylcarbamoylethyl, N,N-dimethylcarbamoypentyl, ethoxycarbonylmethyl, isopropoxycarbonylethyl, tert-butoxy-carbonylpropyl, 2-diethoxyphosphorylethyl, 3-dipropoxyphosphorylpropyl, 4-dibutoxyphosphorylbutyl, ethylenedioxyphosphorylmethyl, 2-phosphonoethyl and 3-phosphonopropyl. The preferable substituted aralkyl groups include 4-chlorobenzyl, 3-(2-fluorophenyl)propyl, 3-methoxybenzyl, 3,4-dimethoxyphenethyl, 4-ethylbenzyl, 4-(3-trifluoromethylphenyl)butyl, 4-acetylamino benzyl, 4-dimethylaminophenethyl, 4-diethoxy-phosphorylbenzyl and 2-(4-dipropoxyphosphorylmethylphenyl)ethyl. The preferable substituted aryl groups include 4-chlorophenyl, 4-cyclohexylphenyl, 5,6,7,8-tetrahydro-2-naphthyl, 3-trifluoromethylphenyl, 4-hydroxyphenyl, 3,4,5-trimethoxyphenyl, 6-methoxy-2-naphthyl, 4-(4-chlorobenzoyloxy)phenyl, 3,4-methylenedioxyphenyl, 4-(2,2,2-trifluoroethoxy)phenyl, 4-propionylphenyl, 4-cyclohexanecarbonylphenyl, 4-dimethyl-aminophenyl, 4-benzoylamino phenyl, 4-diethoxycarbamoypenyl, 4-tert-butoxycarbonylphenyl, 4-diethoxyphosphorylphenyl, 4-diethoxyphosphorylmethylphenyl, 4-(2-diethoxyphosphorylethyl)phenyl, 2-diethoxyphosphorylmethylphenyl, 3-diethoxyphosphorylmethylphenyl, 4-dipropoxyphosphorylphenyl, 4-(2-phosphonoethyl)phenyl, 4-phosphonomethylphenyl and 4-phosphonophenyl. The preferable substituted 5- to 7-membered heterocyclic

groups include 5-chloro-2-pyridyl, 3-methoxy-2-pyridyl, 5-methyl-2-benzothiazolyl, 5-methyl-4-phenyl-2-thiazolyl, 3-phenyl-5-isoxazolyl, 4-(4-chlorophenyl)-5-methyl-2-oxazolyl, 3-phenyl-1,2,4-thiadiazol-5-yl, 5-methyl-1,3,4-thiadiazol-2-yl, 5-acetylamino-2-pyrimidyl, 3-methyl-2-thienyl, 4,5-dimethyl-2-furanyl and 4-methyl-2-morpholinyl.

With respect to compound (I), the ring A is preferably a benzene ring which may be substituted by 1 or more, more preferably 1 or 2 substituents selected from halogen atoms, an optionally substituted alkyl group, an optionally substituted hydroxy group, an optionally substituted mercapto group and/or an optionally substituted amino group.

More preferably, the ring A is a benzene ring which may be substituted by 1 or 2 substituents selected from the above-mentioned halogen atom, an alkyl group having 1 to 10 carbon atoms (furthermore preferably 1 to 5 carbon atoms), an alkoxy group having 1 to 10 carbon atoms (furthermore preferably 1 to 5 carbon atoms), an alkylenedioxy group represented by the formula: $-O-(CH_2)_n-O-$ wherein n is an integer from 1 to 3, and/or an alkylthio group having 1 to 10 carbon atoms (furthermore preferably 1 to 5 carbon atoms).

Most preferably, the ring A is a benzene ring which may be substituted by an alkylenedioxy group represented by the formula: $-O-(CH_2)_n-O-$ wherein n is an integer from 1 to 3.

R is preferably a hydrogen atom, an alkyl group having 1 to 6 carbon atoms (e.g. methyl, ethyl) or a phenyl group.

B is preferably an alkoxycarbonyl group or a group represented by the formula: $-CON(R^1)(R^2)$ wherein R^1 and R^2 independently are a hydrogen atom, an optionally substituted hydrocarbon group or an optionally substituted

5- to 7-membered heterocyclic group.

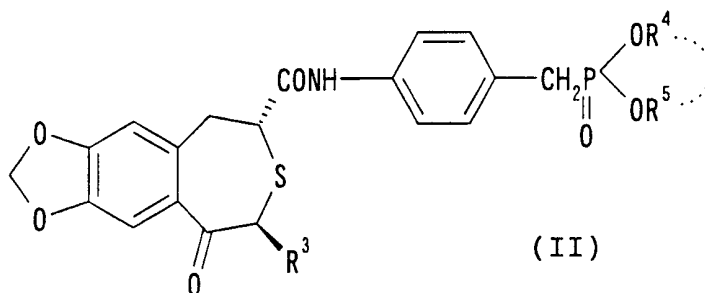
With respect to R^1 and R^2 above, R^1 is preferably a hydrogen atom or an alkyl group having 1 to 10 carbon atoms (e.g. methyl, ethyl, propyl), and R^2 is preferably a phenyl or phenyl- C_{1-3} alkyl group which may be substituted by a halogen atom (e.g. fluorine, chlorine, bromine), a C_{1-6} alkoxy (e.g. methoxy, ethoxy), a mono- or di-alkoxyphosphoryl (preferably a mono- or di- C_{1-6} alkoxyphosphoryl such as dimethoxyphosphoryl, diethoxyphosphoryl), a mono- or di-alkoxyphosphorylalkyl (preferably a mono- or di- C_{1-6} alkoxyphosphoryl- C_{1-3} alkyl such as dimethoxyphosphorylmethyl, diethoxyphosphorylmethyl) wherein dialkyl groups of these di- C_{1-6} alkoxy group may bind together to form a C_{1-6} alkylene group or a C_{1-6} alkoxycarbonyl (e.g. methoxycarbonyl, ethoxycarbonyl), or a 5- or 6-membered heterocyclic group (e.g. pyridyl) which may be substituted by a phenyl and that contains 1 or 2 nitrogen atom(s) or a nitrogen atom and a sulfur atom.

More preferable example of R^1 and R^2 is a hydrogen atom, and a phenyl group substituted by a mono- or di- C_{1-6} alkoxyphosphoryl- C_{1-3} alkyl, respectively (e.g. 4-diethoxyphosphorylmethylphenyl).

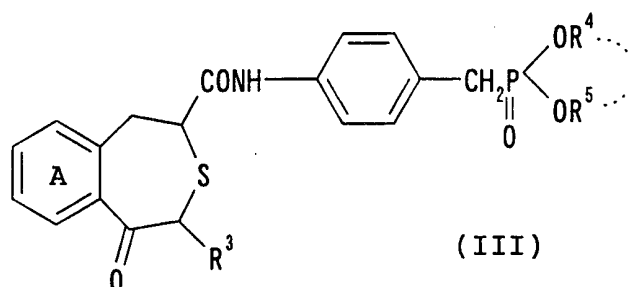
With respect to compound (I), X is $-CH(OH)-$ or $-CO-$, preferably $-CO-$.

With respect to compound (I), k is 0 or 1, and k' is 0, 1 or 2, preferably k is 1, and k' is 0.

Compound (I) is preferably an optically active benzothiepine derivative represented by the formula (II):

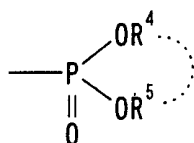


wherein R^3 is a C_{1-6} alkyl group; and R^4 and R^5 are independently a C_{1-6} alkyl group or bind together to form a C_{1-6} alkylene group, or a benzothiepine derivative represented by the formula (III):

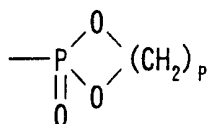


wherein the ring A is a benzene ring substituted by two methoxy groups; R^3 is a hydrogen atom; and R^4 and R^5 are independently a C_{1-6} alkyl group or bind together to form a C_{1-6} alkylene group.

The C_{1-6} alkyl group represented by R^4 or R^5 in the compound (II) and the compound (III) is exemplified by an alkyl group such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, neo-pentyl and hexyl, and preferably a C_{1-4} alkyl group. R^4 and R^5 may bind together to form a C_{1-6} alkylene group. In this case, a moiety:



may represent a moiety:



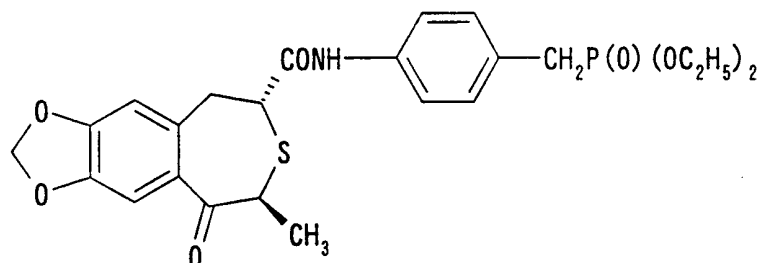
wherein p is an integer from 2 to 4.

Preferable groups for R^4 and R^5 include alkyl groups

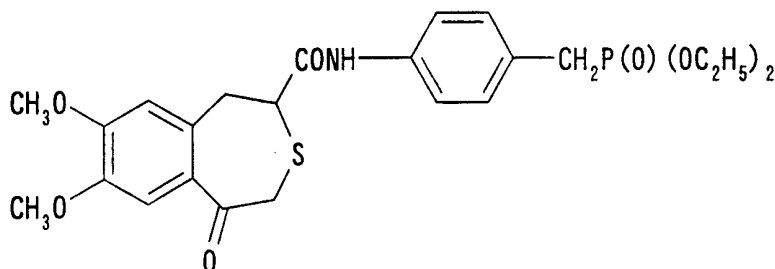
having 1 to 4 carbon atoms such as methyl and ethyl.

The compound (II) is an optically active compound of the (2R,4S) configuration, and contains substantially no compound of the (2S,4R) configuration. The compound (II) of which optical purity is nearly 100% is preferable.

Most preferably, the compound (II) is, for example, (2R,4S)-(-)-N-[4-(diethoxyphosphorylmethyl)phenyl]-1,2,4,5-tetrahydro-4-methyl-7,8-methylenedioxy-5-oxo-3-benzothiepine-2-carboxamide (hereinafter also referred to as Compound A) or its salt. The Compound A is represented as below.



And most preferably, the compound (III) is, for example, N-[4-(diethoxyphosphorylmethyl)phenyl]-1,2,4,5-tetrahydro-7,8-dimethoxy-5-oxo-3-benzothiepine-2-carboxamide (hereinafter also referred to as Compound B) or its salt. The Compound B is represented as below.



The salt of compound (I) is preferably a pharmaceutically acceptable salt. Pharmaceutically acceptable salts include salts with inorganic or organic bases, salts with inorganic or organic acids, and basic or

acidic amino acids. Inorganic basic salts include alkali metal salts (e.g., sodium salts, potassium salts) and alkaline earth metal salts (e.g., calcium salts, magnesium salts). Such organic basic salts include the salts with
5 trimethylamine, triethylamine, pyridine, picoline, N,N-dibenzyl-ethylenediamine or diethanolamine. Such inorganic acidic salts include the salts with hydrochloric acid, hydrobromic acid, hydroiodic acid, phosphoric acid, nitric acid and sulfuric acid. Such organic acidic salts
10 include the salts with formic acid, acetic acid, trifluoroacetic acid, oxalic acid, tartaric acid, fumaric acid, maleic acid, methanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid and citric acid. Such the salts with basic or acidic amino acids include the salts
15 with arginine, lysine, aspartic acid and glutamic acid.

Compound (I) or its salt included in the pharmaceutical composition of the present invention can be produced by the method described in Japanese laid-open patent applications 232880/1991 (corresponding to EP-A-
20 0376197), 364179/1992 (corresponding to EP-A-0460488), and 231569/1996 (corresponding to EP-A-0719782) or a modification thereof.

The cell differentiation induction factors serving as subjects of the present invention include factors that
25 induce characteristic of the process of differentiation of undifferentiated precursors of cells that maintain living body function in a particular tissue, such as osteoblasts or neurons, e.g., factors belonging to the TGF- β superfamily such as bone morphogenetic factor,
30 neurotrophic factors, tumor growth factor (TGF)- β , and activin; factors belonging to the FGF superfamily such as basic fibroblast growth factor (bFGF) and acidic fibroblast growth factor (aFGF); factors belonging to the neuropoietic cytokine family such as leukemia inhibitory factor (LIF,
35 or also called cholinergic differentiation factor (CDF))

and ciliary neurotrophic factor (CNTF); interleukin 1 (IL-1, hereinafter similarly abbreviated), IL-2, IL-3, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, tumor necrosis factor- α (TNF- α), and interferon- γ (IFN- γ), with preference given to BMP
5 as bone morphogenetic factor or neurotrophic factors.

Bone morphogenetic factors include members of the BMP family of proteins that promote osteogenesis and chondrogenesis, such as BMP-2, -4, -5, -6, -7, -8, -9, -10, -11, and -12, with preference given to BMP-2, -4, -6, and
10 -7. BMP may be a homo-dimer of each of the above-mentioned factors or a hetero-dimer consisting of any possible combination thereof.

Neurotrophic factors include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and
15 neurotrophin-3 (NT-3), with preference given to the NGF family.

The enhancer of cell differentiation induction factor comprising compound (I) can be used singly or in combination with a substance showing cell differentiation induction
20 factor action, such as BMP or a neurotrophic factor, for the promotion of healing from bone fractures, promotion of bone reconstruction, treatment and prevention of various bone diseases such as osteoporosis, and treatment and prevention of various cerebral dysfunctions or nervous
25 diseases such as nerve-degenerating diseases in cerebrovascular dementia, senile dementia, Alzheimer's disease, or the like, amyotrophic lateral sclerosis (Lou Gehrig's disease), and diabetic peripheral neuropathy. The enhancer of cell differentiation induction factor of
30 the present invention is also used for prevention or/and treatment of diseases wherein the pathologic condition is improved as a result of enhancement of these actions by BMP, neurotrophic factors etc., as well as their roles in vivo described above.

35 When the enhancer comprising the compound (I) is

administered in combination with a substance showing cell differentiation induction factor action (e.g. BMP, neurotrophic factors etc.), they may be administered at the same time or at time intervals orally or non-orally. The substance showing cell differentiation induction factor action may be prepared individually or mixed together with the enhancer of the present invention, and also may be formulated with pharmaceutically acceptable carriers, excipients, binders, and diluents.

The pharmaceutical composition comprising compound (I) of the present invention can be administered orally or non-orally, as formulated with a pharmaceutically acceptable carrier, in the form of solid preparations such as tablets, capsules, granules and powders, or liquid preparations such as syrups and injectable preparations.

Pharmaceutically acceptable carriers are various organic or inorganic carrier substances in common use as pharmaceutical materials. They include excipients, lubricants, binders and disintegrants for solid preparations; and solvents, dissolution aids, suspending agents, isotonizing agents, buffers and soothing agents for liquid preparations. Other pharmaceutical additives such as preservatives, antioxidants, stabilizing agents, coloring agents and sweetening agents may be used as necessary.

Preferable excipients include lactose, sucrose, D-mannitol, starch, crystalline cellulose and light silicic anhydride.

Preferable lubricants include magnesium stearate, calcium stearate, talc and colloidal silica.

Preferable binders include binding cellulose, pregelatinized starch, sucrose, D-mannitol, dextrin, hydroxypropyl cellulose, hydroxypropylmethyl cellulose and polyvinylpyrrolidone.

Preferable disintegrants include starch,

carboxymethyl cellulose, carboxymethyl cellulose calcium, croscarmellose sodium and carboxymethyl starch sodium, low-substituted hydroxypropyl cellulose.

5 Preferable solvents include water for injection, alcohol, propylene glycol, macrogol, sesame oil and corn oil.

10 Preferable dissolution aids include polyethylene glycol, propylene glycol, D-mannitol, benzyl benzoate, ethanol, tris-aminomethane, cholesterol, triethanolamine, sodium carbonate and sodium citrate.

15 Preferable suspending agents include surfactants such as stearyltriethanolamine, sodium lauryl sulfate, lauryl-aminopropionic acid, lecithin, benzalkonium chloride, benzethonium chloride and monostearic glycerol; and hydrophilic polymers such as polyvinyl alcohol, polyvinylpyrrolidone, carboxymethyl cellulose sodium, methyl cellulose, hydroxymethyl cellulose, hydroxyethyl cellulose and hydroxypropyl cellulose.

20 Preferable isotonizing agents include sodium chloride, glycerol and D-mannitol.

 Preferable buffers include buffer solutions of phosphates, acetates, carbonates and citrates.

 Preferable soothing agents include benzyl alcohol.

25 Preferable preservatives include p-oxybenzoic acid esters, chlorobutanol, benzyl alcohol, phenethyl alcohol, dehydroacetic acid and sorbic acid.

 Preferable antioxidants include sulfites and ascorbic acid.

30 The pharmaceutical composition of the present invention can be produced by dissolving or dispersing compound (I) into an appropriate solvent and forming into microcapsules, spheres, rods, needles, pellets, films or the like, by an appropriate method.

35 The pharmaceutical composition of the present invention can be mixed in a carrier for bone reconstruction

as an osteogenesis promoter in bone repair and bone transplantation because it possesses potent osteogenesis-promoting activity. For example, the compound (I) can be used as adhered to, or contained in, artificial bones etc. prepared from metals, ceramics or high-molecular substances. The artificial bone is preferably made porous on the surface thereof to allow the effective release of the pharmaceutical composition of the present invention in the living tissue upon its transplantation to a bone defect. The compound (I) can be adhered to, or contained in, an artificial bone by dispersing it in an appropriate dispersant, binder, diluent or the like (e.g., collagen, physiological saline, citric acid solution, acetic acid solution, hydroxyapatite, fibrin, mixture thereof) and applying it to, or impregnating it in, the artificial bone. Such artificial bone is transplanted to a bone defect and firmly fixed to the defect. An artificial bone fixative can be prepared by mixing the active ingredient (i.e., compound (I)) with pharmaceutically acceptable dispersants, binders, diluents, other components effective on bone (e.g., calcium), etc. The artificial bone fixative can also be used as filled in the gap between the artificial bone transplanted to the bone defect in the host and the bone defect, without adhering it to, or containing it in, artificial bone. It should also be noted that the non-oral composition described here can also be used with an osteogenesis-promoting protein such as the BMP family adhered thereto or contained therein.

In addition, the pharmaceutical composition of the present invention can also be a sustained-release preparation comprising a biodegradable polymer dispersed compound (I) by a method such as that described in Japanese laid-open patent application 231569/1996 (corresponding to EP-A-0719782).

The biodegradable polymer of the present invention

is a polymer that is poorly soluble or insoluble in water and degradable in vivo in an appropriate period for treatment. Examples of such polymers include fatty acid polyesters such as polymers, copolymers and their mixture
5 of one or more kinds of α -hydroxycarboxylic acids (e.g., lactic acid, glycolic acid, 2-hydroxybutyric acid, 2-hydroxyvaleric acid, 2-hydroxy-3-methylbutyric acid, 2-hydroxycaproic acid, 2-hydroxyisocaproic acid, 2-hydroxycaprylic acid), hydroxydicarboxylic acids (e.g.,
10 malic acid) and hydroxytricarboxylic acids (e.g., malic acid), lactic acid caprolactones, valerolactones, etc., and derivatives thereof (e.g., block polymers of polylactic acid, polyglycolic acid and polyethylene glycol), poly- α -cyanoacrylates, polyalkylene oxalates (e.g.,
15 polytrimethylene oxalate, polytetramethylene oxalate), polyortho-esters, polyortho-carbonates, polycarbonates (e.g., polyethylene carbonate, polyethylene-propylene carbonate), polyamino acids (e.g., poly- γ -benzyl-L-glutamic acid, poly-L-alanine, poly- γ -methyl-L-glutamic
20 acid), hyarulonates, polystyrene, polymethacrylic acid, acrylic acid-methacrylic acid copolymers, polyamino acids, dakin stearate, ethyl cellulose, acetyl cellulose, nitro-cellulose, maleic anhydride copolymers, collagen, gelatin, fibrin and hydroxyapatite.

25 These biodegradable polymers may be in the form of homopolymers or copolymers of two or more kinds, or these mixtures.

 Polymerization may be of the random, block or graft type.

30 Preferable biodegradable polymers include aliphatic polyesters.

 From the viewpoint of biodegradability and biocompatibility, polymers and copolymers synthesized from one or more kinds of α -hydroxycarboxylic acids are
35 preferred. Specifically, copolymers synthesized from one

or more kinds of lactic acid, glycolic acid, 2-hydroxybutyric acid, 2-hydroxyvaleric acid etc., or mixtures thereof are used.

5 The biodegradable copolymer for the present invention can be produced by commonly known methods such as that described in Japanese laid-open patent application 28521/1986 (EP172636), or a modification thereof.

10 Although the above-mentioned α -hydroxycarboxylic acids may be of the D-, L- or D,L-configuration, the D,L-configuration is preferred.

Homopolymers of the above-mentioned α -hydroxycarboxylic acids include homopolymers of lactic acid, glycolic acid and 2-hydroxybutyric acid. The preferable α -hydroxycarboxylic acid is lactic acid.
15 Copolymers of the above-mentioned α -hydroxycarboxylic acids include copolymers of glycolic acid and the other α -hydroxycarboxylic acids. Preferable α -hydroxycarboxylic acids are lactic acid and 2-hydroxybutyric acid. Specifically, useful copolymers
20 include lactic acid-glycolic acid copolymers and 2-hydroxybutyric acid-glycolic acid copolymers, with preference given to lactic acid-glycolic acid copolymers, etc.

25 The average molecular weight of these biodegradable polymers for the present invention is preferably chosen from the range of about 2,000 to 800,000, more preferably about 5,000 to 200,000.

30 The weight-average molecular weight of a lactic acid homopolymer (hereinafter also referred to as polylactic acid) is preferably about 5,000 to 100,000, more preferably about 6,000 to 50,000. A polylactic acid can, for example, be synthesized by commonly known production methods such as that described in Japanese laid-open patent application 28521/1986 (EP172636).

35 The content ratio of lactic acid and glycolic acid

in a lactic acid-glycolic acid copolymer is preferably about 100/0 to 50/50 (w/w), and more preferably about 90/10 to 50/50 (w/w). The weight-average molecular weight of the lactic acid-glycolic acid copolymer is preferably about 5,000 to 100,000, more preferably about 8,000 to 50,000. The lactic acid-glycolic acid copolymer can be synthesized by a commonly known production method such as that described in Japanese laid-open patent application 28521/1986 (EP172636). The copolymer is preferably synthesized by catalyst-free dehydration polymerization condensation.

With respect to the 2-hydroxybutyric acid-glycolic acid copolymer, the content ratio is preferably such that glycolic acid accounts for about 40 to 70 mol%, and 2-hydroxybutyric acid accounts for the remaining portion. The weight-average molecular weight of the 2-hydroxybutyric acid-glycolic acid copolymer is preferably about 5,000 to 100,000, more preferably about 8,000 to 50,000. The 2-hydroxybutyric acid-glycolic acid copolymer can be synthesized by a commonly known production method such as that described in Japanese laid-open patent application 28521/1986 (EP172636). The copolymer is preferably synthesized by catalyst-free dehydration polymerization condensation.

The above-described 2-hydroxybutyric acid-glycolic acid copolymer may be used in mixture with polylactic acid. When the 2-hydroxybutyric acid-glycolic acid copolymer is used in mixture with polylactic acid, the mixing ratio of 2-hydroxybutyric acid/glycolic acid is about 10/90 to 90/10 (% by weight), preferably about 25/75 to 75/25 (% by weight).

In the present specification, weight-average molecular weight is defined as that based on polystyrene measured by gel permeation chromatography (GPC). Measurements were taken using a GPC column KF804L×2 (produced by Showa Denko) and an RI monitor L-3300 (produced

by Hitachi Ltd.) with chloroform as a mobile phase.

The amount of biodegradable polymer is variable according to the strength of the pharmacological activity of compound (I), the speed and duration of drug release from the biodegradable polymer and so on, as long as the desired purpose is accomplished. For example, the biodegradable polymer is used in amounts about 0.2 to 10,000 times (ratio by weight), preferably about 1 to 1,000 times, more preferably about 1 to 100 times, for the amount of the active substance.

The pharmaceutical composition of the present invention can be produced by ordinary methods of producing a pharmaceutical composition, for example, it can be produced by dispersing a non-peptide osteogenetic promoting substance in a bio-degradable polymer, or by filling a non-peptide osteogenetic promoting substance in a previously shaped hollow biodegradable polymer. Specifically, useful methods include the in-water drying method, the phase separation method, the spray drying method, and modifications thereof.

Example methods of producing microcapsules of the present invention are described below.

(1) In-water drying method (o/w method)

In this method, an organic solvent solution comprising a biodegradable polymer is first prepared. The organic solvent used to produce the pharmaceutical composition of the present invention preferably has a boiling point of not higher than 120°C. Such organic solvents include halogenated hydrocarbons (e.g., dichloromethane, chloroform, chloroethane, dichloroethane, trichloroethane, carbon tetrachloride), aliphatic esters (e.g., ethyl asetate, butyl asetate), ethers (e.g., ethyl ether, isopropyl ether) and aromatic hydrocarbons (e.g., benzene, toluene, xylene). These solvents may be used in combination of two or more kinds

in appropriate ratios. The organic solvent is preferably dichloromethane or acetonitrile. The organic solvent is more preferably dichloromethane. The concentration of biodegradable polymer in the organic solvent solution is normally chosen over the range of about 0.01 to 80% (w/w), preferably about 0.1 to 70% (w/w), and more preferably about 1 to 60% (w/w), although varying depending on molecular weight of biodegradable polymer and organic solvent type, etc.

Compound (I) is added and dissolved into the organic solvent solution comprising the biodegradable polymer thus obtained, if necessary after lyophilized or vacuum dried. The amount of compound (I) is about 0.001 to 90% (w/w), preferably about 0.01 to 80% (w/w), and more preferably about 0.1 to 50% (w/w), based on the concentration of biodegradable polymer in the organic solvent solution, although varying depending on drug type, mechanism of action on cartilage destruction suppressing effect or chondrogenesis promoting effect, effect duration, etc..

The organic solvent solution thus prepared is then added to an aqueous phase to form an o/w emulsion using a turbine type mechanical stirrer or the like. The volume of the aqueous phase is normally chosen from the range of about 1 to 10,000 times, preferably about 2 to 5,000 times, and more preferably about 5 to 2,000 times, for the volume of the oil phase.

An emulsifier may be added to the aqueous phase. The emulsifier may be any one as long as it is capable of forming a stable o/w emulsion. Examples of such emulsifiers include anionic surfactants, nonionic surfactants, polyoxyethylene castor oil derivatives, polyvinyl pyrrolidone, polyvinyl alcohol, carboxymethyl cellulose, lecithin, gelatin and hyaluronic acid. These may be used in combination as appropriate. The concentration of emulsifier in the aqueous phase is preferably about 0.001

to 20% (w/w), more preferably about 0.01 to 10% (w/w), and further more preferably about 0.05 to 5% (w/w).

Solvent evaporation from the oil phase can be achieved by commonly used methods, including the method in which the solvent is evaporated under normal or gradually reduced pressure during stirring using a propeller stirrer or magnetic stirrer, etc., and the method in which the solvent is evaporated while the degree of vacuum is adjusted using a rotary evaporator, etc.. The obtained microcapsules are separated by centrifugal method or filtration, after which they are washed with, for example, water or heptane, several times to remove free compound (I), emulsifier, etc. adhering to the microcapsule surface. The microcapsules are then again dispersed in distilled water, etc. and lyophilized. To prevent particle flocculation during washing, antiflocculants: water-soluble sugars such as mannitol, lactol, glucose and starches (e.g., corn starch), amino acids such as glycine and alanine, and proteins such as gelatin, fibrin and collagen may be added.

In the above-described o/w method, microcapsules may be produced by the s/o/w method, in which the compound (I) is dispersed in an organic solvent solution comprising a biodegradable polymer.

(2) In-water drying method (w/o/w method)

In this method, the compound (I) is first dissolved or dispersed in water to obtain a concentration specified above to yield an internal aqueous phase, if necessary with dissolving or suspending by adding a drug-retaining substance such as a protein (e.g., gelatin), seaweed (e.g., agar), polysaccharide (e.g., alginic acid), synthetic high-molecular substance (e.g., polyvinyl alcohol), basic amino acid (e.g., arginine, lysine) or the like. The internal aqueous phase may be supplemented with an organic acid such as acetic acid, oxalic acid or citric acid, an

inorganic acid such as carbonic acid or phosphoric acid, an alkali metal hydroxide such as sodium hydroxide, a basic amino acid such as arginine or lysine or a salt thereof (e.g., salts with organic acids such as acetic acid, oxalic acid, citric acid or salts with inorganic acids such as carbonic acid, phosphoric acid and hydrochloric acid) as a pH regulator for keeping the stability and solubility of the compound (I) or its salt thereof. As a stabilizer for the compound (I), there may be added a protein (e.g., albumin, gelatin), starch derivative (e.g. dextrin, pullulan), organic acid (e.g., citric acid), ethylenediaminetetraacetic acid alkali metal salt (e.g., sodium ethylenediamine-tetraacetate), sulfurous acid hydrogen alkali metal salt (e.g., sodium hydrogen sulfite), synthetic high-molecular substance (e.g., polyethylene glycol) or the like. Commonly preservatives may also be added p-oxybenzoates (e.g., methyl paraben, propyl paraben), benzyl alcohol, chlorobutanol and thimerosal. The additional amount of the compound (I) is about 0.001 to 90% (w/w), preferably about 0.01 to 80% (w/w), and more preferably about 0.1 to 50% (w/w), although varying depending on drug type, mechanism of action on cartilage destruction suppressing effect or chondrogenesis promoting effect or effect duration, etc.

The obtained internal aqueous phase is added to a solution (oil phase) containing the biodegradable polymer, followed by emulsifying treatment, to yield a w/o emulsion. This emulsification is achieved by a known dispersing methods which include the intermittent shaking method, the method using a mixer such as a propeller shaker or a turbine shaker, the colloidal mill method, the homogenizer method and the ultra-sonication method. The above-described solution (oil phase) containing the biodegradable polymer is a solution prepared by dissolving the biodegradable polymer in an organic solvent. This solvent may be any

solvent as long as its boiling point is not higher than about 120°C and it is immiscible with water. Such solvents include halogenated hydrocarbons (e.g., dichloromethane, chloroform, chloroethane, dichloroethane, trichloroethane, carbon tetrachloride), aliphatic esters (e.g., ethyl acetate, butyl acetate), ethers (e.g., ethyl ether, isopropyl ether) and aromatic hydrocarbons (e.g., benzene, toluene, xylene). These solvents may be used in combination of two or more kinds in appropriate ratios. To prevent particle flocculation during washing, antiflocculants: water-soluble sugars such as mannitol, lactol, glucose and starches (e.g., corn starch), amino acids such as glycine and alanine, and proteins such as gelatin, fibrin and collagen may be added.

The produced w/o emulsion is then added to an aqueous phase to yield a w/o/w emulsion, from which the oil phase solvent is evaporated off, to yield microcapsules. The specific procedure for this production is the same as that described in (1) above.

(3) Phase separation method

In this method, a coacervating agent is gradually added to the above-described w/o emulsion under the stirring to precipitate and solidify the biodegradable polymer. The coacervating agent can be used silicon oil, vegetable oils and fats (e.g., sesame oil, soybean oil, corn oil, cotton seed oil, coconut oil, linseed oil), mineral oils, hydrocarbons (e.g., n-hexane, n-heptane) as long as it is a polymeric, mineral oil or vegetable oil compound which can be mixed with the solvent of the biodegradable polymer and which does not dissolve the polymer for encapsulation. These may be used in combination of two or more kinds.

The obtained microcapsules are, after filtration and separation of them, repeatedly washed with heptane, etc. to remove the coacervating agent. The free drug and solvent

are then removed by using the same manner as in-water drying method. To prevent particle flocculation during washing, antiflocculants: water-soluble sugars such as mannitol, lactol, glucose and starches (e.g., corn starch), amino acids such as glycine and alanine, and proteins such as gelatin, fibrin and collagen may be added.

(4) Spray drying method

For producing microcapsules by this method, the above-described w/o emulsion is sprayed via a nozzle into the drying chamber of a spray drier to volatilize the organic solvent and water in the fine droplets in a very short time, and microcapsules are obtained. The nozzle is exemplified by the double-fluid nozzle, pressure nozzle and rotary disc nozzle. To prevent microcapsule flocculation, an aqueous solution of the above-described antiflocculant may be sprayed via another nozzle, while the w/o emulsion is sprayed. The microcapsules thus obtained may be warmed under reduced pressure to facilitate the removal of the water and solvent contained them.

When microcapsules are used as an injectable suspension, for instance, their particle size is chosen over the range from about 0.1 to 300 μm of average particle diameter, as long as the requirements concerning the degree of dispersion and needle passage are met. Preferably, the particle size is about 1 to 150 μm , more preferably about 2 to 100 μm .

Methods of preparing microcapsules as a sterile preparation include, but are not limited to, the method in which the entire production process is sterile, the method in which gamma rays are used as sterilant, and the method in which an antiseptic is added.

In addition to the above-described microcapsules, the sustained-release preparation of the present invention can be produced by dissolving a biodegradable polymer dispersed the compound (I) therein and forming the solution

into spheres, rods, needles, pellets, films or the like, by an appropriate method.

In addition, the sustained-release preparation of the present invention can also be produced by pulverizing to appropriate particle size a biodegradable polymer dispersed the compound (I) therein by a method such as that described in Japanese laid-open patent applications 234656/1994, which employs a turbo counter jet mill pulverizer or an ultrasonic jet pulverizer. Specifically, the compound (I) is added to an organic solvent containing the biodegradable polymer, and dissolved therein. The solid solution obtained by vacuum drying is then coarsely pulverized and sieved, followed by solvent removal, after which the coarse particles are pulverized to controlled particle size using an ultrasonic jet pulverizer to yield the sustained-release preparation of the present invention.

On the above mentioned preparation of the pharmaceutical composition, the content ratio of the compound (I) based on the pharmaceutical composition is about 0.01 to 95% (w/w), and preferably about 0.1 to 20% (w/w).

However, the pharmaceutical composition of the present invention can be administered as an oral agent, a non-oral agent for local administration (e.g., injectable preparations of intramuscular, subcutaneous, organs or joints, etc., solid preparations such as, indwellable preparations, granules and powders, liquid preparations such as suspensions, and ointments) is more preferable.

The practical injectable preparation can be prepared as aqueous suspension by suspending the compound (I) in water, along with a dispersing agent (e.g., surfactants such as Tween 80 and HCO-60, polysaccharides such as carboxymethyl cellulose, sodium alginate and hyarulonic acid, and polysorbate), a preservative (e.g.,

methy1 paraben, propyl paraben), an isotonizing agent (e.g., sodium chloride, mannitol, sorbitol, glucose), buffer (e.g. calcium carbonate), pH adjusting agent (e.g. sodium phosphate, potassium phosphate), etc., and may be also prepared as an oily suspension by dispersing the compound (I) in a vegetable oil such as sesame oil or corn oil with or without a phospholipid such as lecithin, or a moderatelength fatty acid triglyceride (e.g., MIGLYOL 812).

When the pharmaceutical composition of the present invention is administered locally to a joint of a patient of osteoarthritis, the pharmaceutical composition may be the preparation in which the compound (I) is dispersed in the injectable hyaluronic acid pharmaceutical composition (e.g., KAKENSEIYAKU, trade name: *ALTZ). The hyaluronic acids can be used as its pharmaceutically acceptable salts. The salts include alkali metal salts (e.g., sodium salts, potassium salts) and alkaline earth metal salts (e.g., calcium salts, magnesium salts), preferably sodium salts. The weight-average molecular weight of the hyaluronic acid or a salt thereof about 200,000 to 5,000,000, preferably about 500,000 to 3,000,000, more preferably about 700,000 to 2,500,000.

The concentration of Hyaluronic acid or the sodium salts in the dispersion medium dispersing the compound (I) therein is less than 1%(W/v), preferably about 0.02 to 1%(W/v), more preferably about 0.1 to 1%(W/v), because its viscosity is proper to administrate by injection.

The dispersion medium can include pH regulators, local anesthetics, antiiotics, dissolution aids, isotonizing agents, adsorption preventing agents, glycosaminoglycans, polysaccharides and the like, which are conventionally used in this field.

Preferable examples include mannitol, sorbitol, sodium chloride, glycine, ammonium acetate or water-

soluble protein which is substantially inactive in the body.

Preferable glycosaminoglycan include hyaluronic acid, chondroitin, chondroitin sulfate A, chondroitin sulfate C, dermatan sulfate, heparin, heparan sulfate, and the like.

Preferable water-soluble protein include, which is dissolved in water or physiological salt solution, include human serum albumin, human serum globulin, collagen, gelatin, etc.

Preferable pH regulators include glycine, ammonium acetate, citric acid, hydrochloric acid, sodium hydroxide, etc.

Preferable local anesthetics include chlorobutanol, lidocaine hydrochloride, etc.

Preferable antibiotics include gentamycin, etc.

Preferable dissolution aids include glycerin, polyethyleneglycol-400, etc.

Preferable isotonizing agents include mannitol, sorbitol, sodium chloride, etc.

Preferable adsorption preventing agents include polyoxyethylene sorbitan monooleate, etc.

The dose of the water-soluble protein may be about 0.05 to 50 mg, preferably about 0.5 to 20 mg, more preferably about 0.75 to 10 mg per a injectable preparation.

The phosphate or its salts (e.g., sodium phosphate, potassium phosphate) can enhance the activity of the pharmaceutical composition of the present invention. The concentration of sodium phosphate or potassium phosphate in the injectable preparation is about 0.1 mM to 500 mM, preferably about 1 mM to 100 mM.

The preferable preparation of the present invention is as follows.

(A) a lactic acid-glycolic acid copolymer:

wherein the ratio of lactic acid/glycolic acid is about 90/10 to 50/50 (w/w) and the weight-average

molecular weight is about 8000 to 50000,

(B) Compound A or Compound B, and

(C) phosphoric acid or its salt: sodium phosphate.

5 The content ratio of (B) based on (A) is about 5 to 30% (w/w). The content ratio of (C) based on (A) and (B) is about 0.1 to 20% (w/w).

The pharmaceutical composition of the present invention is preferably a suspension as described above.

10 The pharmaceutical composition of the present invention is preferably in the form of fine particles. This is because said pharmaceutical composition is less likely to cause excess pain to the patient when administered through an injection needle for ordinary subcutaneous or
15 intramuscular injection.

The pharmaceutical composition of the present invention is preferably an injectable preparation.

Methods of preparing the pharmaceutical composition of the present invention as a sterile preparation include,
20 but are not limited to, the method in which the entire production process is sterile, the method in which gamma rays are used as sterilant, and the method in which an antiseptic is added.

25 The sustained-release composition of the present invention can release the compound (I) usually for a period from a week to three months, although depending on type of biodegradable polymer and the dose of the compound (I). The sustained-release composition of the present invention can safely be used in mammals mentioned above because of its
30 low toxicity. The sustained-release composition of the present invention is expected to serve as a safe preparation of high efficacy proving a constant drug effect with low toxicity and meeting the requirements of the prevention and treatment of bone diseases.

35 The dose of the sustained-release composition of the

present invention may be an effective amount of the compound (I), although depending on type and content of the compound (I), type of formulation, release time of the drug and subject animals, etc. For example, when the
5 pharamaceutical composition of the present invention is used in the form of microcapsules to treat a bone fracture portion, it may be administered at about 0.01 to 500 mg, preferably about 5 to 50 mg, based on the active ingredient content (e.g., compound (I)), per adult (weighing 50 kg)
10 per dosing, once every week to every 3 months.

Best Mode for Carrying out the Invention

The present invention is hereinafter described in more detail by means of the following working examples,
15 which are not to be construed as limitative.

Compound A: (2R,4S)-(-)-N-[4-(diethoxyphosphorylmethyl)phenyl]-1,2,4,5-tetrahydro-4-methyl-7,8-methylenedioxy-5-oxo-3-benzothiepine-2-carboxamide

20 Compound B: N-[4-(diethoxyphosphorylmethyl)phenyl]-1,2,4,5-tetrahydro-7,8-dimethoxy-5-oxo-3-benzothiepine-2-carboxamide

These compounds are prepared according to the method described in Example 1 and Reference Examples of Japanese Patent Unexamined Publication 231569/1996 (corresponding
25 to EP-A-0719782).

Examples

Experimental Example

30 Induction of alkaline phosphatase (ALP) production in a line of mouse osteoblast

The mouse-derived osteoblast line MC3T3-E1 was sown over an α -minimum essential medium (MEM) containing 10% fetal calf serum (FCS) in a 96-well plate (4,000 cells/well).
35 Three days later, the sample, previously diluted with a

medium containing or not containing 3 ng/ml of the BMP-4/7 hetero dimer (described in Japanese Patent Unexamined Publication No. 265083/1995) to each concentration shown in Table 1, was added to a layer of uniformly grown (confluent) cells; cultivation was continued for 72 hours. After being once washed with physiological saline, the plate was incubated at room temperature in the presence of a substrate solution for 15 minutes. The reaction was stopped by the addition of 0.05 N sodium hydroxide; the absorbance at 405 nm was determined. As a result, as shown in Table 1, the compound A and compound B were proven to enhance BMP activity, i.e., induction by BMP of APL production, and to possess excellent ALP production-inducing activity irrespective of the presence or absence of BMP.

Table 1

Samples	Concentration (μ M)	Alkaline Phosphatase Activity nmol/min/well(1000XA ₄₀₅ ±SE)	
		BMP 0	BMP 3ng/ml
Compound A	0	118±1	134±2
	0.1	124±2	141±3
	1	154±3##	191±3**
	10	195±8##	284±10**
Compound B	0	118±1	134±2
	0.1	118±1	133±2
	1	126±2#	144±3
	10	164±4##	199±6**

** : P<0.01 vs. well without Samples

: P<0.01, ## : P<0.05 vs. well without BMP and Samples

Example 1

(1) Compound A	5g
(2) Lactose	82.5g
(3) Hydroxypropylcellulose	2.8g
(4) Magnesium Stearate	0.4g

(5) Hydroxypropylmethylcellulose 2910	2.994g
(6) Corn Starch	19.3g
(7) Macrogol 6000	0.6g
(8) Titanium Oxide	0.4g
(9) Yellow Ferric Oxide	0.006g

Ingredients (1) to (6) are mixed and compressed with a tablet machine to provide 1,000 tablets included 5mg of the compound A having 6.5mm in diameter. The tablets are coated with ingredients (7) to (9) to provide film-coated tablets having 6.6mm in diameter.

5

Example 2

(1) Compound A	5g
(2) Lactose-Crystalline Cellulose(granule)	330g
(3) D-Mannitol	29g
(4) Low Substituted Hydroxypropylcellulose	0.4g
(5) Talc	25g
(6) Hydroxypropylcellulose	50g
(7) Aspartame	3g
(8) Dipotassium Glycyrrhizinate	3g
(9) Hydroxypropylmethylcellulose 2910	30g
(10) Titanium Oxide	3.5g
(11) Yellow Ferric Oxide	0.5g
(12) Light Anhydrous Silicic Acid	1g

The granule of (2) is coated with aqueous solution or suspension of ingredients (1), (3), (4), (5), (6), (7) and (8) to provide crude granule. The crude granule is coated with ingredients (9) to (11), and mixed with ingredient (12) to provide about 500g of granule included 1% of the compound A. The granule is divided into each 500mg of granule.

10

Example 3

A dichloromethane solution of a lactic acid-glycolic acid copolymer (hereinafter also referred to as PLGA; the lactic acid-glycolic acid composition ratio (mol%) and

15

weight-average molecular weight as determined by GPC measurement given in Table 2) or a lactic acid homopolymer (hereinafter also referred to as PLA), was prepared with each formulation shown in Table 2 (hereinafter also referred to as solution A). Similarly, a solution was prepared from 0.1 g of Compound A and 1.0 ml of dichloromethane (hereinafter also referred to as solution B). Solutions A and B were uniformly mixed, poured over a 0.1% aqueous solution of polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry Co., Ltd.) (PVA solution) in a volume shown in Table 2, previously adjusted to 15°C, and stirred using a turbine type homo-mixer at 7,000 rpm to yield an O/W emulsion. This O/W emulsion was stirred at room temperature for 3 hours to volatilize the dichloromethane and solidify the oil phase, which phase was collected via centrifugation using a centrifuge (05PR-22, Hitachi Ltd.) at 2,000 rpm. This oil phase was again dispersed in distilled water, after which it was further centrifuged and washed to remove the free drug etc. The microcapsules collected were re-dispersed in a small amount of distilled water and freeze-dried.

Table 2

Micro-capsules	Solution A				PVA Liquid Volume (ml)	Particle Size (μ m)
	Polymer	L/G Content Ratio * (mol%)	Weight-average Molecular Weight	Polymer Weight (g)	Di-chloro-methane (ml)	
No. 1	PLGA	75/25	10500	2.4	2.5	24
No. 2	PLGA	75/25	6500	2.4	1.0	50
No. 3	PLGA	75/25	17100	2.4	1.5	38
No. 4	PLA	100/0	12000	2.4	2.0	37
No. 5	PLGA	90/10	20000	2.4	2.0	65
No. 6	PLGA	85/15	12100	2.4	2.0	51

* Lactic acid/Glycolic acid Content Ratio

Example 4

About 8 g of a lactic acid-valerolactone copolymer (PLV 2500ML, produced by Taki Chemical, hereinafter also referred to as PLV) or a glycolic acid-caprolactone copolymer (PGC 2500MG, produced by Taki Chemical, hereinafter also referred to as PGC) was placed in a centrifugal tube and heated to about 50 °C in a water bath. About 80 mg of Compound A was mixed in each tube, followed by uniform dispersion, to yield an ointment preparation, which was stored at a cold place.

Example 5

500 mg of microcapsule prepared at Example 3 was uniformly dispersed in two test tubes of fibrinogen solution for Tisseel (produced by Nippon Zoki Pharmaceutical). Thrombin solution of two test tubes for Tisseel were gradually added. Subsequently, the mixture was immediately aspirated into a plastic syringe. The syringe was kept standing at 37 °C for 30 minutes to solidify the content. After solidification, the content was extruded from the syringe tip and cut using a razor into pellets about 200 μ l in volume.

Industrial Applicability

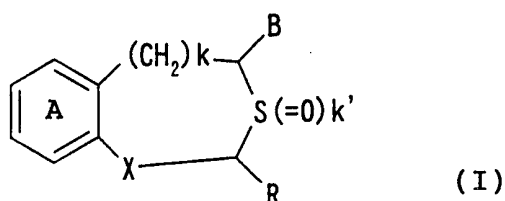
The cell differentiation induction factor action enhancer of the present invention, which comprises an optically active benzothiepine derivative represented by formula (I), possesses potent BMP action-enhancing activity and osteogenesis promotion-enhancing activity, for example, and acts on bone tissue to increase bone mass and strength. This agent is therefore useful in the treatment and prevention of various bone diseases such as osteoporosis, promotion of healing from bone fractures, and promotion of bone reconstruction. The present enhancer also possesses neurotrophic factor action-enhancing activity, and is useful in the treatment and prevention of

various nervous diseases such as Alzheimer dementia, ordinary senile dementia, motor neuron disorders (amyotrophic lateral sclerosis etc.), and diabetic peripheral neuropathy.

CLAIMS

1. An enhancer of cell differentiation induction factor, which comprises a benzothiepine or a benzothiopilane derivative or its salt.

2. An enhancer of cell differentiation induction factor, which comprises a compound (I) of the formula:

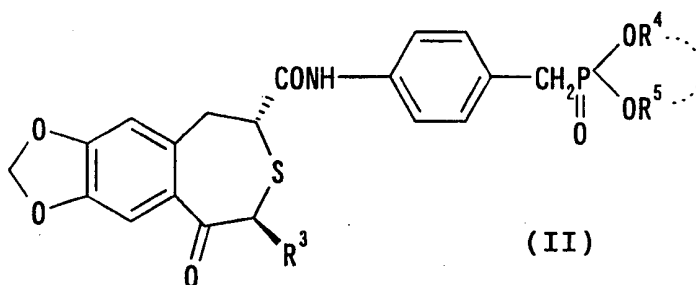


wherein ring A is an optionally substituted benzene ring; R is a hydrogen atom or an optionally substituted hydrocarbon group; B is an optionally esterified or amidated carboxyl group; X is -CH(OH)- or -CO-; k is 0 or 1; and k' is 0, 1 or 2, or its salt.

3. An enhancer according to claim 2, wherein the ring A is a benzene ring which may be substituted by 1 or 2 substituents selected from the group consisting of a halogen, a C₁₋₁₀ alkyl, a C₁₋₁₀ alkoxy, an alkylenedioxy group of the formula: -O-(CH₂)_n-O- wherein n is an integer from 1 to 3 and a C₁₋₁₀ alkylthio group; R is a hydrogen atom, a C₁₋₆ alkyl or a phenyl group; B is -CON(R¹)(R²) wherein R¹ is a hydrogen atom or a C₁₋₁₀ alkyl group and R² is a phenyl or a phenyl-C₁₋₃ alkyl group which those groups may be substituted by a halogen, a C₁₋₆ alkoxy, a mono- or di-C₁₋₆ alkoxyphosphoryl, a mono- or di-C₁₋₆ alkoxyphosphoryl-C₁₋₃ alkyl wherein two alkyl groups of these di-C₁₋₆ alkoxy group optionally may bind together to form a C₁₋₆ alkylene group and C₁₋₆ alkoxycarbonyl group.

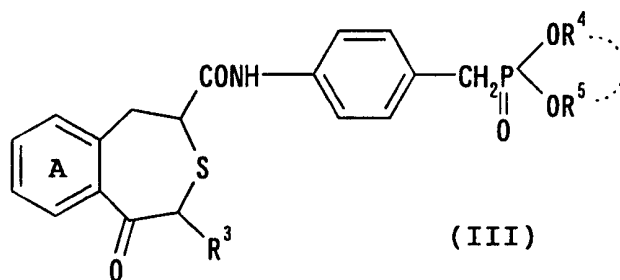
4. An enhancer according to claim 2, wherein the compound is an optically active compound (II) of the formula;

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wherein R^3 is a C_{1-6} alkyl group; and R^4 and R^5 are independently a C_{1-6} alkyl group or bind together to form a C_{1-6} alkylene group.

5. An enhancer according to claim 2, wherein the compound is a compound (III) of the formula;



wherein the ring A is a benzene ring substituted by two methoxy groups; R^3 is a hydrogen atom; and R^4 and R^5 are independently a C_{1-6} alkyl group or bind together to form a C_{1-6} alkylene group.

6. An enhancer according to claim 4 or 5, wherein R_4 and R_5 are independently a C_{1-4} alkyl group.

7. An enhancer according to claim 4, wherein the optically active compound (II) is (2R,4S)-(-)-N-[4-(diethoxyphosphorylmethyl)phenyl]-1,2,4,5-tetrahydro-4-methyl-7,8-methylenedioxy-5-oxo-3-benzothiepine-2-carboxamide.

8. An enhancer according to claim 5, wherein the compound (III) is N-[4-(diethoxyphosphorylmethyl)phenyl]-1,2,4,5-tetrahydro-7,8-dimethoxy-5-oxo-3-benzothiepine-2-carboxamide.

9. A pharmaceutical composition comprising the enhancer defined in claim 2.

10. A pharmaceutical composition according to claim 9, which is in the form of a sustained-release preparation comprising a biodegradable polymer.

11. A pharmaceutical composition according to claim 9, which is for local administration.

12. A pharmaceutical composition according to claim 9, which is for oral administration.

13. A pharmaceutical composition according to claim 9, which is for injection.

14. A pharmaceutical composition according to claim 9, which is for treatment or prevention of bone diseases.

15. A pharmaceutical composition according to claim 9, which is for treatment or prevention of nervous diseases.

16. An enhancer defined in claim 2, wherein the cell differentiation induction factor is a bone morphogenetic protein.

17. An enhancer defined in claim 2, wherein the cell differentiation induction factor is a neurotrophic factor.

18. An enhancer defined in claim 17, wherein the neurotrophic factor is one selected from a family of NGF.

19. A method for enhancing an action of cell differentiation induction factor, which comprises administering the compound (I) or its salt defined in claim 2 to mammal in need.

20. Use of the compound (I) or its salt defined in claim 2 for manufacturing a medicament for enhancing an action of cell differentiation induction factor.