PHARMACEUTICAL COMPOSITION CONTAINING BLOCK COPOLYMER COMPRISING BORIC ACID COMPOUND

The present invention provides a pharmaceutical composition. The pharmaceutical composition includes a block copolymer including: a hydrophilic segment; a hydrophobic segment; and a boronic acid compound bonded to a side chain of the hydrophobic segment via a linker moiety including a heterocyclic structure, wherein: a cyclic skeleton of the heterocyclic structure has a boron atom derived from the boronic acid compound, atoms X’s bonded to the boron atom and each selected from an oxygen atom and a nitrogen atom, and carbon atoms bonded to the atoms X’s; and the block copolymer further comprises an organic group bonded to the carbon atoms, the organic group containing an aromatic group or cyclic alkyl group as a structure for protecting a boronic acid ester bond and/or a boron amide bond resulting from bonding between the boron atom and the atoms X’s.
Description

Technical Field

[0001] The present invention relates to a pharmaceutical composition including a block copolymer containing a boronic acid compound typified by Velcade (trademark: bortezomib as generic name).

Background Art

[0002] A boronic acid compound is expected to find use in various medical applications. For example, bortezomib is known as a potent anti-cancer agent that suppresses growth of myeloma cells by inhibiting an action of an enzyme (proteasome) for degrading an unnecessary protein in cells and inhibiting activation of NF-\(\kappa\)B. The proteasome is a biological mechanism for degrading a structurally abnormal protein or an extra protein. A cell growth rate is extremely high in cancer cells as compared to normal cells, and protein synthesis is carried out actively in cancer cells as compared to normal cells, resulting in an increased synthesis amount of the structurally abnormal protein as well. Therefore, a function of the proteasome is inhibited by bortezomib to increase an intracellular concentration of an abnormal protein. Further, the activation of NF-\(\kappa\)B plays important roles in survival, growth, and invasion of tumor cells. NF-\(\kappa\)B is generally present as an inactive form in a state of being bound to its inhibitory protein I\(\kappa\)B\(\alpha\). NF-\(\kappa\)B is activated by degradation of I\(\kappa\)B\(\alpha\) with the proteasome. The activation of NF-\(\kappa\)B is inhibited by inhibition of the function of the proteasome by bortezomib. Those phenomena caused by bortezomib can lead to dysfunction and cell death of cancer cells. However, the proteasome is present in normal cells as well, and hence bortezomib has strong side effects. As the side effects, there have been reported, for example, myelosuppression, lung disorder, tumor lysis syndrome, gastrointestinal disorders, peripheral neuropathy, pneumonia, and cardiovascular disorders.

[0003] The inventors of the present invention have advanced development of a drug delivery system (DDS) with a polymer micelle from the viewpoint of enhancing efficacy of a drug while reducing side effects thereof. One of the goals of the DDS resides in achieving sustained release of a drug through micelle formation, in other words, stable retention of a drug in a micelle under a physiological condition, to thereby prevent an abrupt rise in concentration of the drug in blood to avoid occurrence of side effects.

[0004] However, a polymer micelle capable of sufficiently stably retaining a boronic acid compound such as bortezomib under a physiological condition or a block copolymer suitable for forming the polymer micelle has not yet been obtained. It should be noted that the following literatures are given as related art literatures about the boronic acid compound.

Citation List

Patent Literature

[0005]

[PTL 1] WO 96/13266 A1
[PTL 2] US 5780454 B
[PTL 3] WO 2010/019718 A1

Summary of Invention

Technical Problem

[0006] A main object of the present invention is to provide a polymer micelle capable of stably retaining a boronic acid compound such as bortezomib under a physiological condition, which may be used as a pharmaceutical composition, and a block copolymer composition suitable for forming the polymer micelle.

Solution to Problem

[0007] The inventors of the present invention have found that, when a boronic acid compound is bonded to a hydrophobic segment of a block copolymer via a specified chemical structure, stable retention property of the boronic acid compound under a physiological condition through micelle formation is improved to a great extent. Thus, the present invention has been completed. That is, the present invention provides a pharmaceutical composition, including a block copolymer including: a hydrophilic segment; a hydrophobic segment; and a boronic acid compound bonded to a side
chain of the hydrophobic segment via a linker moiety including a heterocyclic structure, in which: a cyclic skeleton of the heterocyclic structure has a boron atom derived from the boronic acid compound, atoms X’s bonded to the boron atom and each selected from an oxygen atom and a nitrogen atom, and carbon atoms bonded to the atoms X’s; and the block copolymer further includes an organic group bonded to the carbon atoms, the organic group containing an aromatic group or cyclic alkyl group as a structure for protecting a boronic acid ester bond and/or a boron amide bond resulting from bonding between the boron atom and the atoms X’s.

Advantageous Effects of Invention

[0008] According to one embodiment of the present invention, the stable retention property of the boronic acid compound under a physiological condition in a polymer micelle type DDS can be improved.

Brief Description of Drawings

[0009] [FIG. 1] A graph showing drug release test results of polymer micelle compositions formed of block copolymer compositions of Example 1, Example 2, and Comparative Example 1.

[FIG. 2] A graph showing concentrations of bortezomib in plasma of rats to which the polymer micelle composition formed of the block copolymer composition of Example 2 and a bortezomib aqueous solution were administered, respectively.

[FIG. 3A] A bar graph showing relative values of tumor volumes 7 days after administration to tumor volumes on the administration date in mice to which the polymer micelle composition formed of the block copolymer composition of Example 1, the bortezomib aqueous solution, and a control solution were administered, respectively.

[FIG. 3B] A bar graph showing relative values of body weights 7 days after administration to body weights on the administration date in mice to which the polymer micelle composition formed of the block copolymer composition of Example 1, the bortezomib aqueous solution, and the control solution were administered, respectively.

Description of Embodiments

A. Pharmaceutical composition

[0010] A pharmaceutical composition of the present invention includes a block copolymer including: a hydrophilic segment; a hydrophobic segment; and a boronic acid compound bonded to a side chain of the hydrophobic segment via a linker moiety including a heterocyclic structure. The block copolymer has, in its linker moiety, a specified chemical structure for protecting the bond to the boronic acid compound, and thus is capable of stably retaining the boronic acid compound under a physiological condition in a form of, for example, a polymer micelle.

[0011] Specifically, a cyclic skeleton of the heterocyclic structure has a boron atom derived from the boronic acid compound, atoms X1 and X2 bonded to the boron atom and each independently selected from an oxygen atom and a nitrogen atom, and carbon atoms bonded to the atoms X’s. The carbon atoms are bonded to an organic group containing an aromatic group or cyclic alkyl group as a structure for protecting a boronic acid ester bond and/or a boron amide bond resulting from bonding between the boron atom and the atoms X’s.

[0012] More specifically, the cyclic skeleton of the heterocyclic structure has a boron atom derived from the boronic acid compound, atoms X1 and X2 bonded to the boron atom and each independently selected from an oxygen atom and a nitrogen atom, a carbon atom bonded to the atom X1, and a carbon atom bonded to the atom X2. At least one of the carbon atom bonded to the atom X1 and the carbon atom bonded to the atom X2 is bonded to an organic group containing an aromatic group or cyclic alkyl group as a structure for protecting a boronic acid ester bond and/or a boron amide bond resulting from bonding between the boron atom and the atoms X1 and X2.

[0013] In a preferred embodiment of the present invention, the heterocyclic structure may be represented by the following chemical structure (I) or (II):
where:

B represents the boron atom derived from the boronic acid compound;
at least one of R¹, R², R³, and R⁴ represents the organic group;
X¹ and X² each independently represent an oxygen atom or a nitrogen atom;
R⁵ and R⁶ each independently represent hydrogen or a substituted or unsubstituted alkyl group having 1 to 6 carbon
atoms, provided that R⁵ and R⁶ are absent when X¹ and X² each represent an oxygen atom;
L¹ represents - (CH₂)ₚ₁ - (where p₁ represents an integer of 0 to 5); and
L² represents - (CH₂)ₚ₂-M-(CH₂)ₚ₃ - (where M represents CH or N and p₂ and p₃ each independently represent
an integer of 0 to 2).

[0014] As described above, at least one of R¹, R², R³, and R⁴ represents an organic group containing an aromatic
group or cyclic alkyl group as a structure for protecting a boronic acid ester bond and/or a boron amide bond (hereinafter sometimes simply referred to as "protective structure"). The aromatic group or cyclic alkyl group as the protective structure is desirably disposed in proximity to carbon atoms for forming the cyclic skeleton of the heterocyclic structure in at least one of R1, R2, R3, and R4. Specifically, the protective structure is bonded to carbon atoms for forming the cyclic skeleton of the heterocyclic structure preferably via 1 to 4 atoms or directly, more preferably via 1 or 2 atoms or directly, still more preferably via 1 atom or directly. More specifically, an aromatic ring (aromatic ring derived from the aromatic group) or a cycloalkyl ring (cycloalkyl ring derived from the cyclic alkyl group) in the protective structure is bonded to carbon atoms for forming the cyclic skeleton of the heterocyclic structure preferably via 1 to 4 atoms or directly, more preferably via 1 or 2 atoms or directly, still more preferably via 1 atom or directly. The stable retention property of the boronic acid compound can be suitably improved by disposing a bulky ring structure in proximity to the boronic acid ester bond and/or the boron amide bond.

Specific examples of the aromatic group include a phenyl group, a benzyl group, a naphthyl group, an anthracenyl group, a biphenyl group, and a triphenyl group. Further, specific examples of the cyclic alkyl group include a cycloalkyl group having 3 to 10 carbon atoms. A plurality of (e.g., two or three) cycloalkyl groups may be linked together. Those aromatic groups and cyclic alkyl groups may be substituted by any appropriate substituent. Specific examples of the substituent include an alkyl group (more specifically, a linear or branched alkyl group having 1 to 4 carbon atoms), a halogen, a cyano group, a formyl group, a carboxyl group, an amino group, an alkoxy carbonyl group, an acylamide group, a siloxy group, a tri(alkyl)siloxyl group, and a silylamino group. The aromatic group is preferably a phenyl group or a benzyl group. The cyclic alkyl group is preferably a cyclopentyl group or a cyclohexyl group.

The protective structure is preferably contained in two or more of R1, R2, R3, and R4. In one embodiment, both of any one of R1 and R2 and any one of R3 and R4 represent an aromatic group (e.g., a phenyl group or a benzyl group). Groups containing no protective structure out of R1, R2, R3, and R4 may each independently be a linear or branched alkyl group having 1 to 16 carbon atoms. A plurality of (e.g., two or three) cycloalkyl groups may be linked together. Those aromatic groups and cyclic alkyl groups may be substituted by any appropriate substituent. Specific examples of the substituent include an alkyl group (more specifically, a linear or branched alkyl group having 1 to 4 carbon atoms), a halogen, a cyano group, a formyl group, a carboxyl group, an amino group, an alkoxy carbonyl group, an acylamide group, a siloxy group, a tri(alkyl)siloxyl group, and a silylamino group.

The atoms X1 and X2 are each independently selected from an oxygen atom or a nitrogen atom, and are bonded to a boron atom to form a boronic acid ester bond or a boron amide bond. Valence electrons of nitrogen more easily enter the empty p-orbital of the boron atom than valence electrons of oxygen. Hence, the boron amide bond would have higher stability than the boronic acid ester bond, and thus is considered to be advantageous from the viewpoint of the stable retention property of the boronic acid compound.

R5 and R6 each independently represent hydrogen or a substituted or unsubstituted alkyl group having 1 to 6 carbon atoms when X1 and/or X2 represents a nitrogen atom. Examples of the substituent include a halogen, a cyano group, a formyl group, a carboxyl group, an amino group, an alkoxy carbonyl group, an acylamide group, a siloxy group, a tri(alkyl)siloxyl group, and a silylamino group.

L1 and L2 each independently represent hydrogen or an alkyl group having 1 to 6 carbon atoms when X1 and/or X2 represents a nitrogen atom. Examples of the alkyl group include a linear or branched alkyl group having 1 to 6 carbon atoms.

In a more preferred embodiment of the present invention, the heterocyclic structure is at least one selected from the group consisting of the following chemical structures (III) to (V):

[0015]

[0016]

[0017]

[0018]

[0019]

[0020]

[0021]
[Chem. 3]

[Chem. 4]

(III)

(IV)
where B, R₁ to R₆, X₁, and X² in each of the chemical structures (III) to (V) are as defined in the chemical structure (I) or (II).

[0022] The hydrophilic segment in the block copolymer is formed of a hydrophilic polymer chain. Any appropriate hydrophilic polymer may be adopted as the hydrophilic polymer. Specific examples of the hydrophilic polymer include polyethylene glycol, a polysaccharide, polyvinylpyrrolidone, polyvinyl alcohol, polyacrylamide, polyacrylic acid, polymethacrylamide, polymethacrylic acid, polyethylene glycol, polyethylene glycol, and polyethylene glycol, and derivatives thereof. Specific examples of the polysaccharide include starch, dextran, fructan, and galactan. Of those, polyethylene glycol is preferred. This is because end-reactive polyethylene glycols having various functional groups at their ends are commercially available, ones having various molecular weights are also commercially available, and hence ones having characteristics appropriate for purposes are easily available.

[0023] The hydrophobic segment in the block copolymer is formed of a hydrophobic polymer chain. Any appropriate hydrophobic polymer may be adopted as the hydrophobic polymer. Specific examples of the hydrophobic polymer include polyamino acid chains of polyglutamic acid, polyaspartic acid, and an ester or amide derivative thereof. Such ester or amide derivative can be formed by subjecting a corresponding hydroxy compound or amino compound having a hydrophobic organic group to a reaction with a reactive derivative (e.g., an ester) of polyglutamic acid or polyaspartic acid. Specific examples of the hydrophobic organic group include an alkyl phenyl group whose alkyl group has 1 to 6 carbon atoms, cholesterol, and an alkyl group having 8 to 18 carbon atoms. Specific examples of the derivative include a poly(β-alkyl aspartate-co-aspartic acid), poly(β-alkyl aspartate-co-aspartic acid), apoly(β-alkyl aspartate-co-aspartic acid), a poly(γ-aralkyl glutamate-co-glutamic acid), a poly(γ-aralkyl glutamate-co-glutamic acid), poly(β-aralkyl glutamate-co-glutamic acid), poly(γ-aralkyl glutamate-co-glutamic acid), and poly(γ-benzyl-L-glutamate).

[0024] An introduction ratio of the protective structure into the side chain of the hydrophobic segment may be, for example, 50% or more, or for example, 60% or more, or for example, 70% or more, or for example, 90% or more when one of the groups R¹, R², R³, and R⁴ contains the protective structure. When two or more of the groups R¹, R², R³, and R⁴ contain the protective structure, the introduction ratio may be adjusted to less than 50%, for example, less than 40%, or for example, less than 30% depending on an increase in steric hindrance associated with the incorporation of such bulky structure. It is considered that the increase in steric hindrance in the vicinity of the bond to the boronic acid compound through the introduction of the protective structure inhibits the access of water molecules to the bond, which results in the cleavage of the bond, to thereby improve the stable retention property of the boronic acid compound under a physiological condition. It should be noted that, even when a linear structure is introduced in place of the protective structure, the stable retention property of the boronic acid compound cannot be significantly improved.

[0025] In one embodiment, the block copolymer is represented by the following formula (1) or (2). It should be noted that the "block copolymer" as used herein also encompasses a pharmaceutically acceptable salt of the block copolymer.
In the formulae (1) and (2), $R^7$'s each independently represent a hydrogen atom, a methyl group, or a linear, branched, or cyclic C$_1$ to C$_{12}$ alkyl group that may have a substituent. Examples of the substituent include an acetalated formyl group, a cyano group, a formyl group, a carboxyl group, an amino group, a C$_1$ to C$_8$ alkoxy carbonyl group, a C$_2$ to C$_7$ acylamide group, a siloxy group having three C$_1$ to C$_6$ alkyl groups identical to or different from each other, a siloxy group, a silylamino group, a maleimide group, a thiol group, a hydroxyl group, and an active ester group. Such substituent may be protected by any appropriate protective group. $R^8$ represents a hydrogen atom, a saturated or unsaturated C$_1$ to C$_{30}$ aliphatic carbonyl group, or a C$_6$ to C$_{30}$ aryl carbonyl group. $R^9$'s each independently represent a hydroxyl group, an amino group, an acylamino group, a carboxyl group, or a carboxylic acid ester (e.g., a benzyl ester or a C$_1$ to C$_6$ alkyl ester) for the respective repeating units. $R^{10}$ represents a hydroxyl group, a saturated or unsaturated C$_1$ to C$_{30}$ aliphatic oxy group, or a C$_6$ to C$_{30}$ aryl-lower alkyl oxy group. $L^3$ and $L^4$ each independently represent a linking group. $m$ represents an integer of 5 to 20,000, $n$ represents an integer of 2 to 5,000, and $x$ represents an integer of 0 to 5,000 (provided that the integer $x$ is smaller than the integer $n$). $y_1$ represents an integer of 0 to 5, and $y_2$ represents an integer of 1 to 5. W represents a heterocyclic structure represented by the chemical structure (I) or (II). Q represents a residue of a boronic acid compound. The mark * represents a single bond or a divalent linking group. It should be noted that details of the boronic acid compound and a method of introducing the boronic acid compound into the block copolymer are described later.

[0026] $m$ represents preferably an integer of 5 to 20,000, more preferably an integer of 10 to 5,000, particularly preferably an integer of 40 to 500. $n$ represents preferably an integer of 2 to 5,000, more preferably an integer of 10 to 100, still more preferably an integer of 20 to 80, particularly preferably an integer of 30 to 50, most preferably an integer of about 40. Thus, the term “poly (polymer)” as used herein encompasses a so-called “oligo (oligomer).” Further, those
numerical values mean average values (peak values) of molecular weight distributions.

[0028] x defines an introduction ratio of the boronic acid compound into the side chain of the hydrophobic segment in the block copolymer. As described above, x represents preferably an integer of 0 to 5,000 (provided that the integer x is smaller than the integer n). When x does not represent 0, the respective repeating units may be arranged in a random, alternating, or block configuration, or a combination thereof. A ratio of n-x to n (i.e., an introduction ratio of the boronic acid compound into the side chain of the hydrophobic segment) may be, for example, 50% or more, or for example, 60% or more, or for example, 70% or more, or for example, 90% or more when one of R¹, R², R³, and R⁴ contains the protective structure, and may be less than 50%, for example, less than 40%, or for example, less than 30% when two or more of R¹ R², R³, and R⁴ each contain the protective structure.

[0029] Any appropriate linking group may be adopted as each of the linking groups L³ and L⁴ as long as it can link together a hydrophilic segment (e.g., a polyethylene glycol chain) and a hydrophobic segment (e.g., a polyamino acid chain). Specific examples of the linking group L³ include - (CH₂)b-NH-. In the formula, b represents an integer of 1 to 5. Specific examples of the linking group L⁴ include - (CH₂)c-CO-. In the formula, c represents an integer of 1 to 5.

[0030] The structure W is preferably selected from heterocyclic structures represented by the chemical structures (III) to (V). The structures W's may each independently be selected for the respective repeating units.

[0031] For example, when W represents the chemical structure (I), the mark * may represent a single bond. Further, for example, when W represents the chemical structure (II), the mark * may represent a divalent linking group. Examples of the divalent linking group include a divalent linking group having 0 to 5 carbon atoms that may include an amide bond, an ester bond, an ether bond, and the like. More specific examples thereof include - (CH₂)d-, -NHCO-, -CONH-, -COO-, -O-, -CO-, and a combination thereof. In the formula, d represents an integer of 1 to 5, preferably 1 or 2.

[0032] The boronic acid compound maybe any appropriate compound having a boronic acid group. The boronic acid compound is typically represented by the following formula (3):

![Chem. 8]

where R¹¹ represents a hydrogen atom, a linear or branched alkyl group having 1 to 16 carbon atoms, or an aromatic group, preferably a hydrogen atom, a benzyl group, a phenyl group, more preferably a benzyl group, and a ring A is a heterocycle. Specific examples of the heterocycle include a pyridyl group, a pyrimidyl group, a furanyl group, a thiophenyl group, a pyrrolyl group, a pyrazolyl group, an imidazolyl group, a tetrazolyl group, a benzofuranyl group, a benzothiophenyl group, an indolyl group, an indolenyl group, a quinolinyl group, an isoquinolinyl group, a benzimidazolyl group, a piperidinyl group, a pyrrolidinyl group, a 2-pyrolidonyl group, a pyrrolinyl group, a tetrahydrofuranyl group, a tetrahydroquinolinyl group, a tetrahydroisoquinolinyl group, a decahydroquinolinyl group, an octahydroisoquinolinyl group, an azocinyl group, a triazinyl group, a 6H-1,2,5-thiazinyl group, a 2H,6H-1,5,2-dithiazinyl group, a thiophene (yl) group, an thianthrenyl group, a furanyl group, a pyranyl group, an isobenzofuranyl group, a chromenyl group, a xanthynyl group, a phenoxathiinyl group, a 2H-pyrrolyl group, a pyrrole group, an imidazolyl group, a pyrazolyl group, an isothiazolyl group, an isoazolyl group, a pyridinyl group, a pyrazinyl group, a pyrimidinyl group, a pyridazinyl group, an indolizinyl group, an isoindolyl group, a 3H-indolyl group, an indolyl group, a 1H-indazolyl group, a purinyl group, a 4H-quinolinyl group, an isoquinolinyl group, a quinolinyl group, a phthalazinyl group, a naphthynyl group, a quinoxalinyl group, a quinazolinyl group, a cinnolinyl group, a pteridinyl group, a 4aH-carbazolyl group, a carbazolyl group, a β-carbolinyl group, a phenanthridinyl group.
group, an acridinyl group, a phenanthrolinyl group, a phenazinyl group, a phenoxyazinyl group, an isochromanyl group, a chromanyl group, a pyrrolidinyl group, an imidazolidinyl group, an imidazolinyl group, a pyrazolidinyl group, a pyrazolinyl group, a piperazinyl group, an indolinyl group, an isoindolinyl group, a quinuclidinyl group, a morpholiny group, and an oxazolidinyl group. Of those, a pyrazinyl group is preferred.

The boronic acid compound is particularly preferably bortezomib (the formula (3) where R1 represents a benzyl group and the ring A is a pyrazinyl group). The reason for this is as described below. According to one embodiment of the present invention, when the boronic acid compound is bonded to the block copolymer via a linker moiety having the protective structure, the retention property of the boronic acid compound under a physiological condition is remarkably improved. As a result, for bortezomib, which is known to have serious side effects among the boronic acid compounds, an effect of reducing its side effects is large, and thus the advantage of the present invention becomes remarkable.

A method of introducing a boronic acid compound into a side chain of a hydrophobic segment of a block copolymer having a hydrophilic segment and a hydrophobic segment via a linker moiety including a heterocyclic structure represented by the chemical structure (III) is described below. For simplicity, a method of introducing bortezomib into a block copolymer having polyethylene glycol as the hydrophilic segment and polyglutamic acid as the hydrophobic segment is described as an example. The introduction of bortezomib is carried out according to the following reaction scheme:

[Chem. 9]
subjected to a reaction with a block copolymer of polyethylene glycol and polyglutamic acid. Specifically, water is eliminated through a reaction between a carboxyl group of a side chain of a polyglutamic acid block and an amino group of the compound 2. Then, deprotection is carried out by alkali treatment to yield a copolymer 3. Finally, the copolymer 3 is subjected to a dehydration reaction with bortezomib to yield a block copolymer 4 having a protective structure for a boronic acid ester bond (block copolymer contained in the pharmaceutical composition of the present invention). The introduction ratio of the boronic acid compound (e.g., bortezomib) in the block copolymer is, for example, 50% or more, or for example, 60% or more, or for example, 70% or more, or for example, 90% or more. It should be noted that, in this description, "/" shown between the repeating units of the structural formula of the copolymer means that these repeating units may be arranged in any appropriate configuration such as a random, alternating, or block configuration, or a combination thereof.

B. Polymer micelle composition

According to another aspect of the present invention, a polymer micelle composition can be provided. The polymer micelle composition of the present invention includes the block copolymer described in the above-mentioned section A, and may be suitably used as an anti-tumor composition. The block copolymer can undergo association in an aqueous solution to suitably form micelle particles. The micelle particles each have an average particle diameter of, for example, 5 nm to 5 μm, preferably 5 to 500 nm, more preferably 10 to 300 nm.

Examples

Hereinafter, the present invention is more specifically described by way of examples. However, the present invention is not limited by these examples.

(Example 1)

According to the above-mentioned reaction scheme, bortezomib was introduced into a block copolymer of polyethylene glycol and polyglutamic acid via a linker moiety including a heterocyclic structure represented by the chemical structure (III). The introduction is specifically described below.

<1. Synthesis of compound 2>

A compound 1 (430 mg, 1.25 mmol) was dissolved in THF (10 mL) and subjected to a reaction with phenethylboronic acid (376 mg, 0.25 mmol) at room temperature in the presence of molecular sieves 4A and p-toluenesulfonic acid monohydrate (50 mg, 0.26 mmol). After 2 hours, the completion of the reaction was confirmed by TLC, the molecular sieves were separated by filtration, and then the filtrate was concentrated under reduced pressure. The resultant residue was dissolved in ethyl acetate (50 mL), washed with a saturated sodium bicarbonate aqueous solution (50 mLx3) and brine (50 mLx3), dried over anhydrous magnesium sulfate, and concentrated.

The residue was purified by silica gel chromatography (eluent: hexane/ethyl acetate=5/2 (v/v)) to yield a compound 2 (515 mg, yield: 89.9%) as a colorless oily product.

$^1$H NMR (CDCl$_3$) δ: 0.70 (3H, s), 1.28 (2H, t, J=8.0 Hz), 1.46 (3H, s), 1.48 (3H, s), 2.84 (2H, t, J=8.0 Hz), 4.37 (2H, d, J=5.9 Hz), 5.06 (1H, br s), 5.14 (2H, s), 7.13-7.35 (14H, m)

<2. Synthesis of block copolymer 3>

The compound 2 (515 mg, 1.13 mmol) was dissolved in ethanol (20 mL), and a hydrogen gas was blown into the solution in the presence of 10% palladium carbon (50 mg). After the completion of the reaction, palladium carbon was separated by filtration, and the filtrate was concentrated under reduced pressure. The residue was subjected to a reaction with a separately synthesized block copolymer of polyethylene glycol and polyglutamic acid (355 mg, 0.023 mmol, average molecular weight of polyethylene glycol: 10 KDa, average polymerization degree of polyglutamic acid: 40, average molecular weight of block copolymer: 15,200) through the use of N,N-diisopropylcarbodiimide (146 mL, 0.94 mmol) and 4-dimethylaminopyridine (114 mg, 0.93 mmol) in dry DMF (5 mL) under an argon atmosphere for 3 days. After the reaction, the reaction liquid was crystallized with a mixed solvent (100 mL) of hexane and ethyl acetate (hexane/ethyl acetate=1/1 (v/v)), and the precipitated polymer was filtered by suction. The polymer powder collected by the filtration was dispersed in the same solvent as described above, washed, and filtered by suction. The same operation was carried out once more, and the resultant polymer powder was dried under reduced pressure at room temperature overnight.

The resultant polymer was treated with a 0.5 N sodium hydroxide aqueous solution (3 mL), and the alkali was
removed by dialysis (molecular weight cut-off: 1,000) treatment. After that, a 0.5 N hydrochloric acid aqueous solution (3 mL) was added, and the dialysis treatment was continued. After the removal of the acid, the polymer aqueous solution was collected and lyophilized to yield a block copolymer 3 (520 mg) to which a linker was bonded. The number of linker molecules introduced was 29 molecules per molecule of the block copolymer based on 1H NMR spectrum analysis.

<3. Synthesis of block copolymer composition 4>

[0043] The resultant block copolymer 3 (100 mg, 4.83×10⁻³ mmol) was dissolved in dry DMF (2 mL) under an argon atmosphere and subjected to a reaction with bortezomib (58.9 mg, 0.153 mmol) in the presence of molecular sieves 4A and p-toluenesulfonic acid monohydrate (5.5 mg, 0.029 mmol) at room temperature the whole day and night. After the reaction, the reaction liquid was crystallized with a mixed solvent (50 mL) of hexane and ethyl acetate (hexane/ethyl acetate=1/1 (v/v)), and the precipitated polymer was filtered by suction. The polymer powder collected by the filtration was dispersed in the same solvent (50 mL) as described above, washed, and filtered by suction. This operation was carried out once more, and the resultant polymer powder was dried under reduced pressure at room temperature overnight to yield a block copolymer composition 4 (95 mg) in which bortezomib was bonded via a protective structure for a boronic acid ester bond, as the pharmaceutical composition of the present invention. The number of bortezomib molecules introduced was 29 molecules per molecule of the block copolymer based on 1H NMR spectrum analysis.

(Comparative Example 1)

[0044] An aspartic acid side chain of a block copolymer of polyethylene glycol and polyaspartic acid-β benzyl ester (1.72 g, 0.095 mmol, average molecular weight of polyethylene glycol: 10 kDa, average polymerization degree of polyaspartic acid: 40, average molecular weight of block copolymer: 18,200) was bonded to bortezomib via a predetermined linker moiety to yield a block copolymer composition 5 (90 mg) shown below. The number of bortezomib molecules introduced was 15 molecules per molecule of the block copolymer based on 1H NMR spectrum analysis.

[Chem. 10]

(Example 2)

[0045] A glutamic acid side chain of a block copolymer of polyethylene glycol and polyglutamic acid (680 mg, 0.045 mmol, average molecular weight of polyethylene glycol: 10 kDa, average polymerization degree of polyglutamic acid:
40, average molecular weight of block copolymer: 15,200) was bonded to bortezomib via a predetermined linker moiety to yield a block copolymer composition 6 (320 mg) shown below as the pharmaceutical composition of the present invention. The number of bortezomib molecules introduced was 9.5 molecules per molecule of the block copolymer based on 1H NMR spectrum analysis.

[Chem. 11]

![Chemical Structure]

<Drug release test>

1. Preparation of sample solution

[0046] Ultrapure water was added to the block copolymer composition in which bortezomib was bonded obtained in each of Examples and Comparative Example so as to achieve a block copolymer composition concentration of 1.0 mg/mL. The mixture was stirred at room temperature for 10 minutes and then ultrasonicated under cooling with ice water for 10 minutes to prepare a sample solution.

2. Measurement of bortezomib content

[0047] 500 μL of 2.0 N NaOH were added to 500 μL of the sample solution (as a result, block copolymer composition concentration: 0.5 mg/mL, alkali concentration: 1.0 N), and the mixture was incubated under light shielding at 37°C and then 200 μL thereof were fractionated. 200 μL of 1.0 N HCl were added to neutralize and dilute the fraction (as a result, block copolymer composition concentration: 0.25 mg/mL), and a bortezomib content was measured under the following HPLC conditions.

[0048] HPLC conditions:

- System: HITACHI Inter-face D-7000 (L-7100, L-7200, L-7300, L-7405)
- Column: Waters XTerra™ MSC18 (4.6x100 mm, 3.5 μm)
- Mobile phase: A (water:acetonitrile:formic acid=7:3:0.1), B (water:acetonitrile:formic acid=2:8:0.1)
- Gradient: 0 (100), 15 (100), 20 (0), 32 (0), 34 (100), 40 (100) min (A%)
- Column temperature: 35°C
To 1.0 mL of the sample solution was added 1.0 mL of a 200 mM sodium phosphate buffer (pH 7.4), and the mixture was stirred. After that, 350 μL of the mixture were fractionated for each of measurements, and incubated under light shielding at 37°C for each measurement time (0, 1, 3, 6, or 24 hours). After that, 200 μL thereof were fractionated, 200 μL of ultrapure water were added to the fraction, and an amount of bortezomib released was measured under the above-mentioned HPLC conditions. A drug release ratio was calculated with the following calculation equation.

Drug release ratio (%) = \frac{(Amount of bortezomib released)}{(Total amount of bortezomib)} \times 100

FIG. 1 shows a relationship between an elapsed time after the preparation of the sample solution and a drug release ratio. As apparent from FIG. 1, 90% or more of bortezomib bonded in the block copolymer composition 5 of Comparative Example 1 were released in 1 hour after the sample preparation. On the other hand, the block copolymer composition 4 of Example 1 and the block copolymer composition 6 of Example 2 (these block copolymer compositions are substantially polymer micelle compositions), in each of which bortezomib was bonded via a linker moiety having a protective structure, showed drug release ratios after a lapse of 6 hours from the sample preparation of less than 30% and less than 10%, respectively, and showed drug release ratios after a lapse of 24 hours from the sample preparation of 50% and 24% (not shown), respectively, showing that both the compositions remarkably suppressed the release of bortezomib. This reveals that, according to one embodiment of the present invention, the boronic acid compound such as bortezomib can be stably retained in water (e.g., under a physiological condition).

<Evaluation of retention property of drug (in vivo)>

1. Preparation of standard solution of bortezomib

Bortezomib was dissolved with DMSO so as to achieve a concentration of 10 mg/mL. 100 μL of the resultant solution were added to a mixed solution of 1 mL of DMSO, 2 mL of a 50% sucrose solution, and 6.9 mL of water for injection. Thus, a solution (10% DMSO and 10% sucrose solution) containing bortezomib at 100 μg/mL was prepared. The solution was diluted with 0.1% formic acid to prepare solutions having bortezomib concentrations of 2.5, 9.8, 39.1, 78.1, 156.3, 625, and 1,250 ng/mL. 40 μL of normal rat plasma were sampled in a microtube and then supplemented with 0.1% formic acid. To the resultant solution were added 150 μL of acetonitrile, and the mixture was stirred at room temperature for about 10 seconds to precipitate a plasma protein. The resultant was centrifuged at about 4°C at 10,000 rpm for about 10 minutes, and 100 μL of the supernatant were transferred to a plastic vial for HPLC as a standard solution.

2. Preparation of measurement sample

A drug was administered into the tail vein of Crlj:WI rats (male, 6-week-old, CHARLES RIVER LABORATORIES JAPAN, INC.). The rats were divided into two groups consisting of: a control group (administration of a 100 μg/mL bortezomib aqueous solution, n=3) ; and a group to which the block copolymer composition 6 (polymer micelle composition) of Example 2 was administered (n=3). The dosage was 100 μg/kg in terms of bortezomib for each of the groups. The administration of the block copolymer composition 6 of Example 2 was carried out with a solution (10% DMSO and 10% sucrose solution, bortezomib concentration: 100 μg/mL) of the copolymer composition. Blood was collected 5 minutes, 1 hour, 3 hours, and 6 hours after the administration to obtain plasma samples. The resultant plasma samples were cryopreserved at -80°C for a period of time before the measurement of a bortezomib concentration.
a plasma protein. The resultant was centrifuged at about 4°C at 10,000 rpm for about 10 minutes, and 100 μL of the supernatant were transferred to a plastic vial for HPLC as a measurement sample. It should be noted that the plasma sample estimated to have a high free bortezomib concentration was diluted with normal rat plasma in advance to prepare a measurement sample according to the above-mentioned method.

3. Measurement of bortezomib concentration

[0054] 10 μL of the measurement sample were injected into an LC/MS/MS, and a concentration of free bortezomib in plasma was measured under the conditions shown in Table 1. FIG. 2 shows the results.

[0055] As shown in FIG. 2, the group to which the block copolymer composition of Example 2 was administered maintained a concentration of free bortezomib in plasma about 16 times as high as that of the control group 1 hour after the administration, and maintained concentrations of free bortezomib in plasma about 11 times and about 7 times as high as those of the control group even 3 hours and 6 hours after the administration, respectively. The results reveal that the block copolymer composition of Example 2 can stably retain bortezomib in blood and may be used as a pharmaceutical composition excellent in sustained-release property.

<Drug efficacy test>

[0057] Human prostatic cancer PC-3 cells were inoculated under the dorsal skin of male nude mice (Balb nu/nu, 5-
week-old, CHARLES RIVER LABORATORIES JAPAN, INC.) at $3 \times 10^6$ cells per mouse. At the time when the tumor volume reached $90.7 \pm 4.5 \text{ mm}^3$ (mean $\pm$ standard error (SE)) (on day 10 after the inoculation), a drug was administered into the tail vein. The mice were divided into the following three groups (n=6, provided that n=10 for the control group): (1) a group to which a control solution (10% sucrose/1% DMSO) was administered; (2) a group to which a bortezomib aqueous solution was administered (1 mg/kg) (Comparative Example 2); and (3) a group to which the block copolymer composition (polymer micelle composition) of Example 1 was administered (0.3 mg/kg). It should be noted that the dosage is an amount in terms of a drug for each of the groups. FIG. 3A shows relative values of tumor volumes after 7 days from the administration date to tumor volumes on the administration date, and FIG. 3B shows relative values of body weights after 7 days from the administration date to body weights on the administration date. As apparent from FIG. 3A, according to the block copolymer composition (polymer micelle composition) of Example 1, an increase in tumor volume was remarkably suppressed as compared to Comparative Example 2 and the control group. FIG. 3B revealed that there was no significant difference in mouse body weight. It should be noted that, when the dosage of the bortezomib aqueous solution was set to more than 1 mg/kg, toxicity due to the active pharmaceutical ingredient became remarkable and an amount of body weight loss reached a serious level (e.g., loss by 20% or more).

[0058] As described above, according to one embodiment of the present invention, the boronic acid compound such as bortezomib can be stably retained in water (e.g., under a physiological condition) for a long period of time. Hence, an excellent anti-tumor effect of the boronic acid compound is provided while side effects thereof are reduced.

Claims

1. A pharmaceutical composition, comprising a block copolymer comprising:
   - a hydrophilic segment;
   - a hydrophobic segment; and
   - a boronic acid compound bonded to a side chain of the hydrophobic segment via a linker moiety including a heterocyclic structure, wherein:
     - a cyclic skeleton of the heterocyclic structure has a boron atom derived from the boronic acid compound, atoms X's bonded to the boron atom and each selected from an oxygen atom and a nitrogen atom, and carbon atoms bonded to the atoms X's; and
     - the block copolymer further comprises an organic group bonded to the carbon atoms, the organic group containing an aromatic group or cyclic alkyl group as a structure for protecting a boronic acid ester bond and/or a boron amide bond resulting from bonding between the boron atom and the atoms X's.

2. The pharmaceutical composition according to claim 1, wherein the cyclic skeleton has two atoms X$^1$ and X$^2$ bonded to the boron atom as the atoms X's.

3. The pharmaceutical composition according to claim 1 or 2, wherein the heterocyclic structure is represented by the following chemical structure (I) or (II):
where:

B represents the boron atom derived from the boronic acid compound;

at least one of R1, R2, R3, and R4 represents the organic group;

X1 and X2 each independently represent O or N;

R5 and R6 each independently represent hydrogen or a substituted or unsubstituted alkyl group having 1 to 6 carbon atoms, provided that R5 and R6 are absent when X1 and X2 each represent O;

L1 represents -(CH2)p1- where p1 represents an integer of 0 to 5; and

L2 represents -(CH2)p2-M-(CH2)p3- where M represents CH or N and p2 and p3 each independently represent an integer of 0 to 2.

4. A pharmaceutical composition according to claim 3, wherein the heterocyclic structure comprises at least one selected from the group consisting of the following chemical structures (III) to (V):
[Chem. 3]

[Chem. 4]
where B, R¹ to R⁶, X¹, and X² in each of the chemical structures are as defined in the chemical structure (I) or (II).

5. The pharmaceutical composition according to any one of claims 1 to 4, wherein an aromatic ring derived from the aromatic group or a cycloalkyl ring derived from the cyclic alkyl group is bonded to the carbon atoms for forming the cyclic skeleton directly or via 1 or 2 atoms.

6. The pharmaceutical composition according to any one of claims 1 to 5, wherein the boronic acid compound comprises bortezomib.

7. The pharmaceutical composition according to any one of claims 1 to 6, wherein the hydrophilic segment is formed of a polyethylene glycol chain and the hydrophobic segment is formed of a polyamino acid chain.
[Fig. 1]

The graph shows the release ratio (%) over time (h) for different examples.

- **Example 1**: Data points are scattered across different time intervals, indicating a varying release ratio.
- **Example 2**: Data points are clustered at lower release ratios, consistently over time.
- **Comparative Example 1**: Data points are shown for a comparative analysis, highlighting differences or similarities with Example 1.

The x-axis represents time in hours (0 to 8), while the y-axis represents the release ratio in percentage (0 to 120).
[Fig. 2]

- CONTROL GROUP
- GROUP TO WHICH BLOCK COPOLYMER COMPOSITION OF EXAMPLE 2 WAS ADMINISTERED

CONCENTRATION IN PLASMA (ng/mL)

TIME AFTER ADMINISTRATION (h)
# INTERNATIONAL SEARCH REPORT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
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</table>

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Date of the actual completion of the international search 17 May, 2012 (17.05.12)

Date of mailing of the international search report 29 May, 2012 (29.05.12)

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