PHARMACEUTICAL COMPOSITION FOR PREVENTING OR TREATING CHRONIC MYELOID LEUKEMIA AND METHOD USING THE SAME

Title: PHARMACEUTICAL COMPOSITION FOR PREVENTING OR TREATING CHRONIC MYELOID LEUKEMIA AND METHOD USING THE SAME

Abstract: Provided is a pharmaceutical composition for preventing or treating chronic myeloid leukemia and a method of preventing or treating chronic myeloid leukemia using the same, thereby being effectively applied to the prevention or treatment of chronic myeloid leukemia.

(51) International Patent Classification:
A61K 31/4439 (2006.01)
A61K 31/437 (2006.01)
A61K 31/478 (2006.01)
A61K 31/4196 (2006.01)


(60) Classification:
A61K 31/4439; C07D 401/14


Published:
— with international search report (Art. 21(3))
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(10) International Publication Number
WO 2016/126028 A1

(12) International Application Published Under the Patent Cooperation Treaty (PCT)
(19) World Intellectual Property Organization
International Bureau
(43) International Publication Date
11 August 2016 (11.08.2016)
Description

Title of Invention: PHARMACEUTICAL COMPOSITION FOR PREVENTING OR TREATING CHRONIC MYELOID LEUKEMIA AND METHOD USING THE SAME

Technical Field

[1] The present disclosure relates to a pharmaceutical composition for preventing or treating chronic myeloid leukemia and a method of preventing or treating chronic myeloid leukemia using the same.

Background Art

[2] Chronic myeloid leukemia (CML) is an abnormal myeloproliferative disease derived from abnormal expansion of the clone of hematopoietic stem cells. CML is caused by a Philadelphia chromosome, which results from the translocation between chromosomes 9 and 22 (t(9;22)(q34;q11)). This chromosomal translocation results in the fusion between ABL gene on chromosome 9 and BCR gene on chromosome 22, and this BCR-ABL fusion gene produces a BCR-ABL fusion protein with abnormal tyrosine kinase activity. BCR-ABL tyrosine kinase induces abnormal cell division. Recently, tyrosine kinase inhibitor (TKI)-insensitive CML problematically occurs in CML patients after TKI therapy. Since TKI targets only actively dividing CML cells, it does not eliminate quiescent CML stem cells. For radical treatment of CML, therefore, there is a need to kill CML stem cells as well as CML cells.

[3] Transforming growth factor (TGF)-β is a cytokine that modulates cell proliferation and differentiation, wound healing, extracellular matrix production, etc. TGF-β family belongs to TGF-β superfamily, and this TGF-β superfamily includes activins, inhibins, bone morphogenetic proteins, and anti-Mullerian hormone. The tumor cells and the stromal cells within the tumors in late stages of various cancers generally overexpress TGF-β. TGF-β would lead to stimulation of angiogenesis and cell motility, suppression of the immune system, and increased interaction of tumor cells with the extracellular matrix. TGF-β receptor is a serine/threonine kinase receptor, and divided into TGF-β receptor 1, TGF-β receptor 2, and TGF-β receptor 3. Of them, TGF-β receptor 1 is also called activin A receptor type II-like kinase (ALK5).

[4] Accordingly, for effective prevention or treatment of CML, there is a demand for a pharmaceutical composition capable of effectively inhibiting tyrosine kinase and TGF-β signaling pathway.

Disclosure of Invention

Solution to Problem
Provided is a pharmaceutical composition for preventing or treating chronic myeloid leukemia, the composition including a compound having a TGF-β signaling pathway-inhibiting activity and a tyrosine kinase inhibitor.

Provided is a method of preventing or treating chronic myeloid leukemia, the method including administering a compound having a TGF-β signaling pathway-inhibiting activity and a tyrosine kinase inhibitor to a subject.

**Advantageous Effects of Invention**

A pharmaceutical composition for preventing or treating chronic myeloid leukemia according to an aspect and a method of preventing or treating chronic myeloid leukemia using the same may be used to effectively prevent or treat chronic myeloid leukemia.

**Brief Description of Drawings**

These and/or other aspects will become apparent and more readily appreciated from the following description of the embodiments, taken in conjunction with the accompanying drawings in which:

FIG. 1 is a graph showing colony formation of LT-CML stem cells cultured in the presence of imatinib, nilotinib, dasatinib, ponatinib, or TEW-7197 alone, or a combination thereof;

FIG. 2A is a graph showing survival rate (%) of TT-CML affected mouse according to drug administration, FIG. 2B is a graph showing survival rate (%) of tg-CML affected mouse over time after the end of doxycycline treatment, and FIG. 2C is a graph showing survival rate (%) of TKI-resistant T315I TT-CML affected mouse according to drug administration;

FIGS. 3A through 3C are a graph showing the number of leukocyte in the peripheral blood of the drug-administered TT-CML affected mouse, a photograph of the spleen thereof, and a graph showing the weight of the spleen thereof, respectively and FIG. 3D is a graph showing percentages (%) of T cell, B cell, and bone marrow cell among the total GFP/BCR-ABL + cells in the peripheral blood of TEW-7197-administered TT-CML affected mouse;

FIG. 4A is the flow cytometry result of GFP/BCR-ABL + KLS+ cells (bold box) and KLS cells (dotted box) in drug-administered TT-CML affected mouse, FIG. 4B is a graph showing the percentage (%) of CML KLS cells among GFP+/CML cells, and FIG. 4C is a graph showing the percentage (%) of CML KLS+ cells among GFP+/CML cells;

FIG. 5A is the flow cytometry result of T315I BCR-ABL-GFP + KLS+ cells (bold box) and KLS cells (dotted box) in drug-administered TT-CML affected mouse, FIG. 5B is a graph showing the percentage (%) of T315I KLS cells among T315I BCR-
ABL-GFP+ cells, and FIG. 5C is a graph showing the percentage (%) of T315I KLS+ cells among T315I BCR-ABL-GFP+ cells; and

FIGS. 6A through 6C are graphs showing colony formation of human CML-initiating cells derived from three patients.

Mode for the Invention

An aspect provides a pharmaceutical composition for preventing or treating chronic myeloid leukemia (CML), the composition including a compound of the following Chemical Formula I, a pharmaceutically acceptable salt, solvate, or stereoisomer thereof, or a combination thereof; and a tyrosine kinase inhibitor:

[Chemical Formula I]

[Diagram]

In the chemical formula I, R handfuls may be independently H, halo, C handfuls, haloalkyl, cycloalkyl, OH, -0-C handfuls alkyl, -O-Q handfuls haloalkyl, -0-C handfuls cycloalkyl, NH q, -NH-C handfuls alkyl, -NH-C handfuls haloalkyl, -NH-C handfuls cycloalkyl, -S-C handfuls alkyl, -S-C handfuls haloalkyl, -S-C handfuls cycloalkyl, CN, or NO q.

m may be 0, 1, 2, 3 or 4.

Any one of A handfuls and A handfuls may be N and the other may be NR handfuls, in which R handfuls is H, OH, C handfuls alkyl, Ci handfuls haloalkyl, or C handfuls cycloalkyl.

X may be -NR handfuls, -O- or -S-, in which R handfuls is H or C handfuls alkyl.

R handfuls may be each independently H, halo, C handfuls alkyl, Ci handfuls haloalkyl, C handfuls cycloalkyl, C handfuls alkenyl, (CH handfuls q-OR handfuls 3, -(CH handfuls q)-NR handfuls R handfuls 3, -(CH handfuls q)-SR, -(CH handfuls q)-N0 handfuls 2, -(CH handfuls q)-CONHOH, -(CH handfuls q)-CN, -(CH handfuls q)-COR, -(CH handfuls q)-C0 handfuls R handfuls 3, -(CH handfuls q)-CONR handfuls R handfuls 4, -(CH handfuls q)-tetrazole, -(CH handfuls q)-CH=CH-CN, -(CH handfuls q)-CH=CH-C0 handfuls R handfuls 3, -(CH handfuls q)-CH=CH-CONR handfuls R handfuls 4, -(CH handfuls q)-CH=CH-tetrazole, -(CH handfuls q)-NHCOR, -(CH handfuls q)-NHC0 handfuls R handfuls 3, -(CH handfuls q)-CONHS0 handfuls R handfuls 3, -(CH handfuls q)-NHS0 handfuls R handfuls 3, -(CH handfuls q)-C=C-CN, -(CH handfuls q)-C=C-C0 handfuls R handfuls 3, -(CH handfuls q)-C=C-CONR handfuls R handfuls 4, -(CH handfuls q)-C=C-tetrazole, -(CH handfuls q)-SOR, -(CH handfuls q)-S0 handfuls R handfuls 3, or -(CH handfuls q)-(OR handfuls) 2, in which R handfuls and R handfuls are each independently H, Ci handfuls alkyl, Ci handfuls haloalkyl, or C handfuls cycloalkyl, or taken together with the nitrogen atom bound thereto to form a mono-cyclic ring, for example, imidazole, pyrrolidine, piperidine, morpholine, piperazine and homopiperazine; R handfuls is C handfuls alkyl, Ci handfuls haloalkyl, or C handfuls cycloalkyl; q is 0, 1, 2, 3, or 4; and r is 1, 2, 3, or 4.

n may be 0, 1, 2, 3, 4 or 5.
The alkyl group may be straight or branched. Examples of the alkyl group include methyl, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, and n-hexyl. The alkyl group may be substituted with one or more of alkoxy, cycloalkoxy, amino, nitro, carboxy, cyano, halo, hydroxyl, sulfo, or mercapto.

The cycloalkyl group is, for example, cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

The halo is, for example, fluorine, chlorine, bromine, or iodine.

The alkenyl group may be straight or branched. The alkenyl group is, for example, vinyl, allyl, isoprenyl, 2-butenyl, and 2-hexenyl. The alkenyl group may be substituted with, for example, alkoxy, cycloalkoxy, amino, nitro, carboxy, cyano, halo, hydroxyl, sulfo, or mercapto.

The alkynyl group may be straight or branched. The alkynyl group is, for example, ethynyl, propargyl, and 2-butynyl. The alkynyl group may be substituted with, for example, alkoxy, cycloalkoxy, amino, nitro, carboxy, cyano, halo, hydroxyl, sulfo, or mercapto.

The compound may be a compound of the following Chemical Formula II:

The compound of Chemical Formula II is N-((4-((I,2,4]triazolo[l,5-a]
)pyridin-6-yl)-5-(6-methylpyridin-2-yl)-IH-imidazol-2-yl)methyl)-2-fluoroaniline(called "TEW-7197").

The compound of Chemical Formula II may selectively inhibit TGF-β receptor 1(ALK5) and/or activin receptor type-IB (ACVR1B, or ALK-4).

The pharmaceutically acceptable salt may be a salt that does not cause significant irritation to an organism to which the compound is administered and does not abrogate the biological activity and properties of the compound. The salt may be, for example, an inorganic acid salt, organic acid salt, or metal salt. The inorganic acid salt may be a salt of hydrochloric acid, bromic acid, phosphoric acid, sulfuric acid, or disulfuric acid. The organic acid salt may be a salt of mesylic acid, formic acid, acetic acid, propionic acid, lactic acid, oxalic acid, tartaric acid, malic acid, maleic acid, citric acid, fumaric acid, besylic acid, camsylic acid, edisylic acid, trichloroacetic acid, trifluoroacetic acid,
benzoic acid, gluconic acid, methanesulfonic acid, glycolic acid, succinic acid, 4-toluenesulfonic acid, galacturonic acid, embonic acid, glutamic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, or aspartic acid. The metal salt may be a calcium salt, sodium salt, magnesium salt, strontium salt, or potassium salt.

The solvate may be a compound formed by attractive forces between solute and solvent molecules. The solvate may be, for example, a hydrate.

The stereoisomers refer to molecules that have the same molecular formula and connectivity of their atoms, but differ in spatial arrangement of atoms. The stereoisomers may be diastereomers or enantiomers of the compound of Chemical Formula I.

The term "tyrosine kinase (TK)" refers to a protein capable of transferring phosphate groups of ATP to tyrosine residues of proteins. Tyrosine kinase plays an important role in cell activity, for example, signal transduction regulating cell division. The tyrosine kinase may be Bcr-Abl tyrosine kinase. Bcr-Abl tyrosine kinase may be a protein that is produced from a BCR-ABL fusion gene between ABL gene on chromosome 9 and BCR gene on chromosome 22, resulting from the translocation between human chromosomes 9 and 22 (t(9;22)(q34;q11)).

The term "tyrosine kinase inhibitor (TKI)" refers to a drug inhibiting tyrosine kinase. The tyrosine kinase inhibitor may be a Bcr-Abl tyrosine kinase inhibitor. The tyrosine kinase inhibitor may be, for example, imatinib, dasatinib (brand name: sprycel), nilotinib (brand name: Tasigna), bosutinib, ponatinib, or a combination thereof. Imatinib may be imatinib mesylate (brand name: Gleevec or Glivec).

The CML may be tyrosine kinase inhibitor-resistant. The tyrosine kinase inhibitor resistance includes tyrosine kinase inhibitor resistance acquired during CML treatment as well as initial resistance to tyrosine kinase inhibitors. The tyrosine kinase inhibitor resistance may be caused by Bcr-Abl dependent and independent mechanisms. Bcr-Abl dependent mechanism may include amplification of Bcr-Abl gene, mutation of Bcr-Abl gene, mutation of tyrosine kinase binding site, or a combination thereof. The mutation of Bcr-Abl gene may be phosphate binding loop (p-loop) mutation (e.g., G250E, Q252H, Y253F, Y253H, E255K, and E255V). The mutation of tyrosine kinase binding site may be, for example, T315I, T315A, F317L, and F317V. For example, tyrosine kinase inhibitor-resistance may be caused by a BCR-ABL fusion protein (T315I) in which a tyrosine residue is mutated by an isoleucine residue at position 315 from the N-terminus. Bcr-Abl independent mechanism may include drug efflux caused by P-glycoproteins, drug import by organic cation transporter 1 (OCT 1), and activation of alternative signaling pathway, for example, Src family kinase. Since TEW-7197 inhibits CML stem cells, it may have a prophylactic or therapeutic effect on any tyrosine kinase inhibitor-resistant CML having different mechanisms.

As used herein, the term "prevention" means all of the actions by which the oc-
currence of chronic myeloid leukemia is restrained or retarded by administration of the pharmaceutical composition, and the term "treatment" means all of the actions by which the symptoms of chronic myeloid leukemia have taken a turn for the better or been modified favorably by administration of the pharmaceutical composition.

The pharmaceutical composition may include a pharmaceutically acceptable carrier. The carrier includes an excipient, a diluent or an auxiliary agent. The carrier may be selected from the group consisting of lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starch, acacia rubber, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methylcellulose, polyvinylpyrrolidone, water, physiological saline, a buffer such as PBS, methylhydroxybenzoate, propylhydroxybenzoate, talc, magnesium stearate, and mineral oils. The composition may include a filler, an anti-coagulating agent, a lubricant, a humectant, a flavor, an emulsifier, an antiseptic, etc.

The pharmaceutical composition may be prepared into any formulation by a general method. The composition may be prepared into, for example, an oral formulation (e.g., powder, tablet, capsule, syrup, pill, granule) or a parenteral formulation (e.g., injectable formulation). Further, the composition may be prepared into a topical or systemic formulation.

The pharmaceutical composition may be prepared as a single composition or individual compositions.

The pharmaceutical composition may include the compound of Chemical Formula I, a pharmaceutically acceptable salt, solvate, or stereoisomer thereof, or a combination thereof; and a tyrosine kinase inhibitor in an effective amount. The term "effective amount" refers to an amount sufficient to exhibit a prophylactic or therapeutic effect when administered to a subject in need of prevention or treatment. The effective amount may be properly selected by those skilled in the art according to a cell or a subject to be selected. The effective amount may be determined depending on the severity of disease, a patient's age, body weight, health conditions, gender, and drug sensitivity, administration time, administration route, excretion rate, treatment period, a drug blended with or co-administered with the composition, and other factors well known in the medical field. The effective amount of the compound of Chemical Formula I, a pharmaceutically acceptable salt, solvate, or stereoisomer thereof, or a combination thereof may be about 0.5 µg to about 2 g, about 1 µg to about 1 g, about 10 µg to about 500 mg, about 100 µg to about 100 mg, about 1 mg to about 90 mg, about 5 mg to about 80 mg, about 10 mg to about 70 mg, about 15 mg to about 60 mg, or about 20 mg to about 50 mg, based on the pharmaceutical composition. The effective amount of the tyrosine kinase inhibitor may be about 0.5 µg to about 2 g, about 1 µg to about 1 g, about 10 µg to about 500 mg, about 100 µg to about 100 mg, about 1 mg to about 50 mg, about 5 mg to about 40 mg, or about 10 mg to about 30
mg, based on the pharmaceutical composition.

The administration dose of the pharmaceutical composition may be, for example, in the range from about 0.001 mg/kg to about 100 mg/kg, about 0.01 mg/kg to about 10 mg/kg, or about 0.1 mg/kg to about 1 mg/kg per adult. The administration may be performed once a day, several times a day, twice or three times a week, once to four times a month, or once or twelve times a year.

Another aspect provides a method of preventing or treating chronic myeloid leukemia of a subject, the method including administering the compound of Chemical Formula I according to an aspect, a pharmaceutically acceptable salt, solvate, or stereoisomer thereof, or a combination thereof; and a tyrosine kinase inhibitor to the subject.

The compound of Chemical Formula I, the pharmaceutically acceptable salt, solvate, or stereoisomer thereof, the tyrosine kinase, the tyrosine kinase inhibitor, the chronic myeloid leukemia, the prevention and treatment are the same as described above.

The subject may be a mammal, for example, human, cow, horse, pig, dog, sheep, goat or cat. The subject may be a subject having chronic myeloid leukemia or at risk of having chronic myeloid leukemia.

The compound, a pharmaceutically acceptable salt, solvate, or stereoisomer thereof, or a combination thereof, and the tyrosine kinase inhibitor may be directly administered into a subject by any means such as oral, intravenous, intramuscular, buccal, transdermal, mucosal, intranasal, intratracheal, or subcutaneous administration. The compound, a pharmaceutically acceptable salt, solvate, or stereoisomer thereof, or a combination thereof, and the tyrosine kinase inhibitor may be systemically or locally administered singly or together with other pharmaceutically active compound.

The compound, a pharmaceutically acceptable salt, solvate, or stereoisomer thereof, or a combination thereof, and the tyrosine kinase inhibitor may be administered simultaneously, individually, or sequentially. For example, after administering dasatinib to a subject, the compound of Chemical Formula I, a pharmaceutically acceptable salt, solvate, or stereoisomer thereof, or a combination thereof may be administered to the subject.

A preferred administration dose of the compound, a pharmaceutically acceptable salt, solvate, or stereoisomer thereof, or a combination thereof, and tyrosine kinase inhibitor may differ depending on a patient's conditions and body weight, severity of the disease, drug formulation, administration route and period, but it may be properly selected by those skilled in the art. The administration dose may be, for example, within the range of about 0.001 mg/kg to about 100 mg/kg, about 0.01 mg/kg to about 10 mg/kg, or about 0.1 mg/kg to about 1 mg/kg per adult. The administration may be performed once a day, several times a day, twice or three times a week, once to four
times a month, or once or twelve times a year.

Reference will now be made in detail to embodiments, examples of which are illustrated in the accompanying drawings, wherein like reference numerals refer to like elements throughout. In this regard, the present embodiments may have different forms and should not be construed as being limited to the descriptions set forth herein. Accordingly, the exemplary embodiments are merely described below, by referring to the figures, to explain aspects of the present description. As used herein, the term "and/or" includes any and all combinations of one or more of the associated listed items.

Hereinafter, the present invention will be described in more detail with reference to Examples. However, these Examples are for illustrative purposes only, and the invention is not intended to be limited by these Examples.

**Example 1.** Test of CML therapeutic effect of combination of tyrosine kinase inhibitor and TEW-7197

1. Preparation of mouse model and CML stem cell

Scl/Tall-tTA x TRE-BCR-ABL1 double transgenic (tg) mice was prepared by mating Scl/Tall-tTA tg mouse (JAX® mice, Stock No. 006209, Jackson Laboratory) and TRE-BCR-ABL1 transgenic mouse (JAX® mice, Stock No. 006202, Jackson Laboratory). These mice was maintained while supplying water containing 20 mg/L of doxycycline (Sigma-Aldrich). 5 weeks after birth, water containing doxycycline was replaced by water containing no doxycycline (doxycycline-end) to induce expression of BCR-ABL1 oncogene. About 2 weeks to 5 weeks after the doxycycline end date, CML-like disease occurred in the transgenic mice. These mice are Scl/Tall/BCR-ABL-tetO double positive mice, and designated as tetracycline(tet)-inducible "tg-CML affected mouse".

The primitive, long term (LT)-CML stem cells (CD150+CD135 CD48 c-Kit+Lineage- Sca-1+ cell) was separated from the tet-inducible tg-CML affected mice.

Meanwhile, hematopoietic stem cells of human BCR-ABL1-GFP gene-introduced mouse was transplanted to C57BL/6 (Sankyo-Lab Service, Japan) mouse to prepare a transduction/transplantation (TT)-CML affected mouse model (Naka et. al., Nature 2010; 463: 676-680). A CML-MPP (multipotent progenitor) fraction containing GFP/BCR-ABL1 -positive and GFP/BCR-ABL1 T315I-positive c-Kit+Lineage Sea-1+(KLS+) cells was separated from the TT-CML affected mouse (Naka et. al., Nature 2010; 463: 676-680). Further, human BCR-ABL1 T315I mutant-GFP gene-introduced mouse hematopoietic stem cells was transplanted to mice by the above described method to prepare TKI-resistant T315I TT-CML affected mice (Naka et. al., Nature 2010; 463: 676-680).

2. Preparation of drug

As a vehicle, 7 ml of 37%(v/v) gastric acid, 2.0 g of NaCl, 3.2 g of pepsin
(Sigma-Aldrich), and distilled water was mixed to prepare 1000 ml of artificial gastric fluid.

As an administration drug, N-((4-((1,2,4)triazolo[1,5-a]pyridin-6-yl)-5-(6-methylpyridin-2-yl)-IH-imidazol-2-yl)methyl)-2-fluoroaniline (hereinbelow, referred to as "TEW-7197") (Syngene, India) was dissolved in the vehicle to prepare 2 mg/ml of TEW-7197 solution. As a comparative control to TEW-7197, Ly2157299 (Selleck Chemicals) was dissolved in the vehicle to prepare a Ly2157299 solution.

Further, as a tyrosine kinase inhibitor (TKI), each of imatinib mesylate (Gleevec®, Novartis), nilotinib (Tasigna®, Novartis), dasatinib (Sprycel®, Bristo-Myers Squibb), and ponatinib (Selleck Chemicals, AP24534) was dissolved in the vehicle to prepare drugs.

3. In vitro test of CML therapeutic effect of combination of tyrosine kinase inhibitor and TEW-7197

About 100,000 of mouse mesenchymal stem cell, OP-9 cell (ATCC® CRL-2749) were cultured as a monolayer in a 24-well plate for about 1 day. 100,000 of LT-CML stem cells prepared in 1. were added to the cultured OP-9 cells to prepare a cell culture broth.

5 µM of TEW-7197 was added to the cell culture broth, and the cells were cultured under conditions of 3% oxygen and 37°C for about 1 day. Next day, 1 µM of imatinib mesylate, 1 µM of nilotinib, 1 µM of dasatinib, or 1 µM ponatinib was added to the cultured cells, respectively and the cells were cultured under conditions of 3% oxygen and 37°C for about 2 day. A co-culture period of LT-CML stem cells and OP-9 cells was total 3 days. As a control group, dimethyl sulfoxide (DMSO) was used instead of the drugs. Thereafter, the cell culture broths were washed with a phosphate buffer and cells were collected. The collected cells was cultured in methyl cellulose (Stem cell technologies, GFM3434) under conditions of 3% oxygen and 37°C for about 1 week, and colony formation of CML stem cells was measured in the cultured cells.

Colony formation of LT-CML stem cells cultured in the presence of TEW-7197, imatinib, nilotinib, dasatinib, or ponatinib alone, or a combination thereof is shown in FIG. 1. As shown in FIG. 1, a significant reduction in colony formation was observed in co-treatment of TEW-7197 and imatinib, nilotinib, dasatinib, or ponatinib, compared to single treatment of TEW-7197, imatinib, nilotinib, or dasatinib. In particular, a combination of TEW-7197 and dasatinib and a combination of TEW-7197 and ponatinib significantly inhibited colony formation. Therefore, it was confirmed that a combination of TEW-7197 and a tyrosine kinase inhibitor has an inhibitory effect on CML stem cells.

4. In vivo test of mouse survival rate by combination of tyrosine kinase inhibitor and
TEW-7197

(1) Effect of combination of imatinib and TEW-7197 in TT-CML-affected mouse
Cells were transplanted to the prepared TT-CML affected mouse as described in 1. and on day 8 post-transplantation, the vehicle or imatinib (200 mg/kg/ day) was orally administered. From day 15 to day 90 post-transplantation, vehicle; vehicle and TEW-7197 (2.5 mg/kg, every three days); vehicle and imatinib (200 mg/kg/day); imatinib (200 mg/kg/ day) and Ly2157299 (150 mg/kg, every three days), or imatinib (200 mg/kg/ day) and TEW-7197 (2.5 mg/kg, every three days) were administered.

After drug administration, mouse survival was monitored until day 125 post-transplantation. From this result, survival rate (%) of TT-CML affected mouse over time was calculated and the result is shown in FIG. 2A (- - - - : vehicle (n=23), — - - - - - : vehicle and TEW-7197 (n=24), bold line: vehicle and imatinib (n=24), — - - - : imatinib and Ly2157299 (n=24), solid line: imatinib and TEW-7197 (n=24)).

As shown in FIG. 2A, the concurrent administration of imatinib and TEW-7197 increased survival rate of TT-CML-affected mouse, compared to single administration of imatinib or TEW-7197, and concurrent administration of imatinib and Ly2157299. Therefore, the combination of TEW-7197 and imatinib exhibits a prophylactic or therapeutic effect on CML in vivo.

(2) Effect of combination of dasatinib and TEW-7197 in tg-CML affected mouse
To examine in vivo therapeutic effects of combination of TEW-7197 and dasatinib in tg-CML affected mouse, tg-CML affected mice were prepared as described in 1.
Water for feeding the tg-CML affected mice was replaced by water containing no doxycycline. The tg-CML affected mice were orally administered with dasatinib at a dose of 5 mg/kg/day once a day for 1 day to 36 days after the end of doxycycline treatment. Additionally, dasatinib alone, or dasatinib and TEW-7197 (2.5 mg/kg/day) was/were orally administered every two days for 8 days to 36 days after the end of doxycycline treatment. As a control group, an vehicle containing no drug was used.

After the end of doxycycline treatment, survival rate(%) of tg-CML affected mouse over time was calculated and the result is shown in FIG. 2B (dotted line: vehicle (n=20), bold line: dasatinib alone (n=12), solid line: dasatinib and TEW-7197(n=11)).

As shown in FIG. 2B, the concurrent administration of dasatinib and TEW-7197 increased survival rate of tg-CML affected mouse, compared to the control group and single administration of dasatinib. Therefore, it was confirmed that the combination of TEW-7197 and dasatinib exhibits a prophylactic or therapeutic effect on CML in vivo.

(3) Effect of combination of ponatinib and TEW-7197 in TKI-resistant T315I TT-CML affected mouse
Cells were transplanted to the prepared TKI-resistant T315I TT-CML affected mouse as described in 1., and on day 8 post-transplantation, the vehicle or ponatinib (15 mg/
kg/day) was orally administered. From day 15 to day 60 post-transplantation, vehicle; vehicle and TEW-7197 (2.5 mg/kg, every three days); vehicle and ponatinib (15 mg/kg/day); or ponatinib (15 mg/kg/day) and TEW-7197 (2.5 mg/kg, every three days) were administered.

After drug administration, mouse survival was monitored until day 100 post-transplantation. From this result, survival rate (%) of TKI-resistant T315I TT-CML affected mouse over time was calculated and the result is shown in FIG. 2C (---: vehicle (n=23), solid line: vehicle and TEW-7197 (n=37), bold line: vehicle and ponatinib (n=32), —-—-: ponatinib and TEW-7197 (n=32)).

As shown in FIG. 2C, the concurrent administration of ponatinib and TEW-7197 increased survival rate of TKI-resistant T315I TT-CML affected mouse, compared to single administration of ponatinib or TEW-7197. Therefore, it was confirmed that the combination of TEW-7197 and ponatinib exhibits a prophylactic or therapeutic effect on tyrosine kinase-resistant CML in vivo.

**5. In vivo effect of combination of tyrosine kinase inhibitor and TEW-7197**

(1) CML therapeutic effect of combination of dasatinib and TEW-7197 in TT-CML-affected mouse

Dasatinib (5 mg/kg/day), TEW-7197 (2.5 mg/kg/day, every three days), or dasatinib (5 mg/kg/day) and TEW-7197 (2.5 mg/kg/day, every three days) were orally administered for about 30 days to the prepared TT-CML affected mouse as described in 1.

The number of leukocyte in the peripheral blood of the drug-administered TT-CML affected mouse is shown in FIG. 3A (n=5, NS: not significant), a photograph of the spleen thereof is shown in FIG. 3B (bar: 10 mm), and the weight of the spleen thereof is shown in FIG. 3C (n=3). Further, percentages (%) of T cell (detected using anti-CD4 antibody and anti-CD8 antibody), B cell (detected using anti-B220 antibody), and bone marrow cell (detected using anti-Mac 1 antibody and anti-Gr-1 antibody) among the total BCR-ABL1-GFP+ cells in the peripheral blood of the TEW-7197-administered TT-CML affected mouse are shown in FIG. 3D (n=3, NS: not significant).

As shown in FIGS. 3A though 3C, the number of leukocyte in the peripheral blood was increased and splenomegaly was promoted in the TT-CML affected mouse administered with TEW-7197, compared to the control group administered with the vehicle. However, the increase in the number of leukocyte in the peripheral blood and splenomegaly were inhibited in the TT-CML affected mouse administered with dasatinib and TEW-7197. As shown in FIG. 3D, administration of TEW-7197 did not affect differentiation of bone marrow cells among the BCR-ABL1-GFP+ cells. Therefore, it was confirmed that dasatinib inhibits proliferation of CML cells of which differentiation is induced by TEW-7197 in vivo.
After cell transplantation, dasatinib (5 mg/kg/day), TEW-7197 (2.5 mg/kg/day, every three days), or dasatinib (5 mg/kg/day) and TEW-7197 (2.5 mg/kg/day, every three days) were orally administered for 30 days to the TT-CML affected mouse. GFP/BCR-ABL + c-Kit+Lineage Sea-1+(KLS+) cells (dotted box) and c-Kit+Lineage Sea-1- (KLS-) cells (dotted box) in the peripheral blood were analyzed by flow cytometry, and the results are shown in FIG. 4A. Cells were gated on GFP+ and Lineage-, and the percentages (%) of GFP/BCR-ABL+KLS+ cells and KLS+ cells among the total GFP/BCR-ABL+ cells of TT-CML affected mouse are shown in FIGS. 4B and 4C, respectively (n=3, NS: not significant).

As shown in FIGS. 4A through 4C, and the percentages (%) of KLS cells and KLS+ cells among the BCR-ABL1-GFP+CML cells were remarkably decreased in the TT-CML affected mouse administered with TEW-7197. Single administration of dasatinib decreased the percentage of CML KLS cells, but concurrent administration of dasatinib and TEW-7197 further decreased the percentage of CML KLS cells, indicating that TEW-7197 inhibits self-renewal ability of primitive CML-MPP, but did not inhibit proliferation of differentiated CML cells. Therefore, concurrent administration of dasatinib and TEW-7197 eliminates TKI-insensitive CML-MPP to provide a therapeutic effect for CML patients.

(2) TKI-resistant CML therapeutic effect of combination of ponatinib and TEW-7197 in TKI-resistant T315I TT-CML affected mouse

Ponatinib (15 mg/kg/day), TEW-7197 (2.5 mg/kg/day, every three days), or ponatinib (15 mg/kg/day) and TEW-7197 (2.5 mg/kg/day, every three days) were orally administered for 30 days to the prepared TKI-resistant T315I TT-CML affected mouse as described in 1. T315I BCR-ABL-GFP+KLS+ cells (bold box) and KLS cells (dotted box) in the peripheral blood were analyzed by flow cytometry, and the results are shown in FIG. 5A. Cells were gated on GFP+ and Lineage-, and the percentages (%) of T315I BCR-ABL-GFP+KLS+ cells and KLS+ cells among the total T315I BCR-ABL-GFP+ cells of TKI-resistant T315I TT-CML affected mouse are shown in FIGS. 5B and 5C, respectively (n=3, NS: not significant).

As shown in FIGS. 5A through 5C, the percentage (%) of T315I BCR-ABL-GFP+KLS+ cells was remarkably decreased in the TKI-resistant T315I TT-CML affected mouse administered with TEW-7197. Concurrent administration of ponatinib and TEW-7197 further decreased the percentage of T315I BCR-ABL-GFP+KLS+ cells. Therefore, concurrent administration of ponatinib and TEW-7197 as well as single administration of TEW-7197 effectively blocks self-renewal ability of T315I-CML KLS+ cells, thereby providing a therapeutic effect for CML patients.

6. Inhibitory effect of combination of dasatinib and TEW-7197 on colony formation of human CML-initiating cells in vitro
Bone marrow mononuclear cells (Allcells, Cat. No. 06-255, 06-620, and 147742, CA) of three patients with chronic CML were prepared.

Bone marrow mononuclear cells were stained with anti-CD34(8G12) antibody (BD Biosciences), anti-CD38(HIT2) antibody (BD Biosciences), anti-CD3(SK7) antibody (BD Biosciences), anti-CD 16(3G8) antibody (BD Biosciences), anti-CD 19(SJ25C1) antibody (BD Biosciences), anti-CD20(L27) antibody (BD Biosciences), anti-CD14(MfP9) antibody (BD Biosciences), and anti-CD56(NCAM16.2) antibody (BD Biosciences). A mouse antibody cocktail recognizing CD3, CD16, CD19, CD20, CD14 and CD56 was used to identify Lineage- (Lin-) cells, and CD34+CD38 Lin- cells were separated. CD34+CD38 Lin- cells were co-cultured with OP-9 stromal cells (ATCC® CRL-2749) in the presence of TEW-7197 alone, dasatinib alone, or TEW-7197 and dasatinib under conditions of 3% oxygen and 37°C. A control group was prepared in the same manner, except that DMSO was used. Cells were harvested and washed with PBS, and then cultured in methyl cellulose (Stem cell technologies, GFM3434) to measure colony formation of human CML-initiating cells. Colony formations of human CML-initiating cells of the three patients thus measured are shown in FIGS. 6A through 6C.

As shown in FIGS. 6A through 6C, TEW-7197 significantly inhibited colony formation of human CML-initiating cells in vitro. Further, co-treatment of TEW-7197 and dasatinib significantly inhibited colony formation of human CML-initiating cells, compared to single treatment of dasatinib. Therefore, it was confirmed that combination of TKI and TEW-7197 eliminates primitive CML-initiating cells in human CML patients.

It should be understood that exemplary embodiments described herein should be considered in a descriptive sense only and not for purposes of limitation. Descriptions of features or aspects within each exemplary embodiment should typically be considered as available for other similar features or aspects in other exemplary embodiments.

While one or more exemplary embodiments have been described with reference to the figures, it will be understood by those of ordinary skill in the art that various changes in form and details may be made therein without departing from the spirit and scope of the inventive concept as defined by the following claims.
Claims

[Claim 1] A pharmaceutical composition for preventing or treating chronic myeloid leukemia, the composition comprising a compound of the following Chemical Formula I, a pharmaceutically acceptable salt, solvate, or stereoisomer thereof, or a combination thereof:

[Chemical Formula I]
[Claim 2] The pharmaceutical composition of claim 1, wherein the compound of Chemical Formula I is a compound of the following Chemical Formula II:

[Chemical Formula II]

[Claim 3] The pharmaceutical composition of claim 1, wherein the tyrosine kinase inhibitor is imatinib, dasatinib, nilotinib, bosutinib, ponatinib, or a combination thereof.

[Claim 4] The pharmaceutical composition of claim 1, wherein the chronic myeloid leukemia is tyrosine kinase inhibitor-resistant.

[Claim 5] The pharmaceutical composition of claim 4, wherein the tyrosine kinase inhibitor-resistance is caused by a BCR-ABL fusion protein having a mutation of replacing a tyrosine residue by an isoleucine residue at position 315.

[Claim 6] The pharmaceutical composition of claim 1, wherein the pharmaceutical composition is a single composition or individual compositions.

[Claim 7] A method of preventing or treating chronic myeloid leukemia of a subject, the method comprising administering the compound of Chemical Formula I of claim 1, a pharmaceutically acceptable salt, solvate, or stereoisomer thereof, or a combination thereof; and a tyrosine kinase inhibitor to the subject.

[Claim 8] The method of claim 7, wherein the administering is performed simultaneously, individually or sequentially.

[Claim 9] The method of claim 7, wherein the administering is administering the compound of Chemical Formula I of claim 1, a pharmaceutically acceptable salt, solvate, or stereoisomer thereof, or a combination thereof to the subject, after administering the tyrosine kinase inhibitor to the subject.

[Claim 10] The method of claim 7, wherein the subject is a subject having chronic myeloid leukemia or at risk of having chronic myeloid leukemia.
[Fig. 2A]

**Graph:**
- **Y-axis:** Survival rate (%)
- **X-axis:** Time post-transplantation (day)
- **Legend:**
  - IM plus TEW-7197 (n=24)
  - IM plus Ly2157299 (n=24)
  - IM plus Vehicle (n=24)
  - Vehicle plus TEW-7197 (n=24)
  - Vehicle (n=23)

**Key Points:**
- The graph compares the survival rates of different treatment groups over time.
- The administration period is indicated.
- The survival rate is shown to decrease over time for all groups.
- A statistical significance mark (P=0.028) indicates a significant difference between some groups.

**Note:**
- The specific values and statistical results are not provided in the image.
[Fig. 2B]

- Dasatinib plus TEW-7197 (n=11)
- Dasatinib plus Vehicle (n=12)
- Vehicle (n=20)

Survival rate (%) vs. time post-DOX withdrawal (day)

Administration Period
[Fig. 2C]

- Ponatinib plus TEW-7197 (n=32)
- Ponatinib plus Vehicle (n=32)
- Vehicle plus TEW-7197 (n=37)
- Vehicle (n=34)

Administration Period

Survival Rate (%) vs. Time Post-Transplantation (day)

\[ P = 0.001 \]
[Fig. 3A]

![Graph showing number of leukocytes in peripheral blood (μL)].

**NS**

$P=0.0031$

[Fig. 3B]

![Images of tissues or samples under different conditions: Vehicle and TEW-7197 for VEHICLE and DASATINIB groups].

**Vehicle**

**TEW-7197**

**Vehicle**

**TEW-7197**

**Vehicle**

**DASATINIB**
[Fig. 4C]

PERCENTAGE OF CMKL+ CELLS AMONG GFP+ CML CELLS (%)

VEHICLE  TEW-7197  VEHICLE  TEW-7197

VEHICLE  DASATINIB

P = 0.016

P = 0.0081

P = 0.0096
[Fig. 5A]

**VEHICLE**

**TEW-7197**

**Ponatinib plus Vehicle**

**Ponatinib plus TEW-7197**
## A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/4439(2006.01)i, A61K 31/437(2006.01)i, A61K 31/4178(2006.01)i, A61K 31/4196(2006.01)i, C07D 401/14(2006.01)i, C07D 403/14(2006.01)i, C07D 471/04(2006.01)i, A61P 35/02(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 31/4439; C07D 471/04; A61K 31/437; C07D 401/14; A61P 35/04; A61K 31/4178; A61K 31/4196; C07D 403/14; A61P 35/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal), STN(Registry, Caplus), Google & Keywords: chronic myeloid leukemia, tyrosine kinase, inhibit, triazolo, pyridine

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

<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>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>Y0 2012-002680 A2 (Ewha UNIVERSITY-INDUSTRY COLLABORATION FOUNDATION) 5 January 2012 See abstract; claims 1-3, 8; and Table 1.</td>
<td>1–6</td>
</tr>
<tr>
<td>Y</td>
<td>Y0 2009-115572 A2 (NOVARTIS AG) 24 Sept ember 2009 See abstract; paragraphs [0167]-[0169], [0178]; and Table 1.</td>
<td>1–6</td>
</tr>
<tr>
<td>A</td>
<td>A0 2011-0319408 Al (KIM, DAE-KEE et al.) 29 December 2011 See abstract; claims 1, 6; and Table 1.</td>
<td>1–6</td>
</tr>
<tr>
<td>A</td>
<td>A0 IN, C. H. et al., “Discovery of N-[4-(1,2,4)Triazole-1,5-a]pyrimidine-6-yl] -5-(6-methylpyridin-2-yl)-2-[(imidazol-2-yl)methyl]2-f luoromalene (EW-7197): A Highly Potent Ent, Sel ective, and Oral Ly Bisovale labile Inhibitor of TGF-β Type I Receptor or Kinase as Cancer Immunotherapeutic ic/Ant ifibrotic Agent ”, Journal of Medizing Chemi stry, 2 May 2014, Vol. 57, No. 10, pp. 4213-4238 See abstract; and Figure 1.</td>
<td>1–6</td>
</tr>
<tr>
<td>A</td>
<td>A0 SON, J I YEON et al., “EW-7197, a Novel ALK-5 Kinase Inhibitor, Pot ent ent y Inhibits Breast to Lung Metastasis”, Mol ecular Cancer Therap eutics, 9 May 2014, Vol. 13, No. 7, pp. 1704-1716 See abstract; and Figure 1.</td>
<td>1–6</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C. See patent family annex.

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

Date of the actual completion of the international search
21 June 2016 (21.06.2016)

Date of mailing of the international search report
22 June 2016 (22.06.2016)

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea
Facsimile No. +82-42-481-8578

Authorized officer
LEE, Jeong A
Telephone No. +82-42-481-8740

Form PCT/ISA/210 (second sheet) (January 2015)
INTERNATIONAL SEARCH REPORT

Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☒ Claims Nos.: 7-10
   because they relate to subject matter not required to be searched by this Authority, namely:
   Claims 7-10 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.

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

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

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

2. ☑ As all searchable claims could be searched without effort justifying any additional fees, this Authority did not invite payment of any additional fees.

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

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

Remark on Protest ☑ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☒ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>WO 2012-002680 A2</td>
<td>05/01/2012</td>
<td>AU 2011-272149 A</td>
<td>17/01/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 2011-272149 B2</td>
<td>22/05/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2803577 A</td>
<td>05/01/2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2803577 C</td>
<td>20/10/2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 103025731 A</td>
<td>03/04/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 103025731 B</td>
<td>13/01/2016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CO 6620028 A2</td>
<td>15/02/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 2588479 A2</td>
<td>08/05/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 2588479 A4</td>
<td>20/11/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 2588479 Bl</td>
<td>04/03/2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 2947081 A</td>
<td>25/11/2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ES 2535876 T3</td>
<td>18/05/2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 05732131 B2</td>
<td>10/06/2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2013-533252 A</td>
<td>22/08/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KR 10-1500665 Bl</td>
<td>09/03/2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KR 10-2013-0028749 A</td>
<td>19/03/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MX 2012015260 A</td>
<td>03/04/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT 2588479 E</td>
<td>26/03/2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RU 2518069 CI</td>
<td>10/06/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SG 186773 A</td>
<td>28/02/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 8080568 Bl</td>
<td>20/12/2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2012-002680 A3</td>
<td>26/04/2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 2009-227013 A</td>
<td>24/09/2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 227013 B2</td>
<td>10/01/2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2718936 A</td>
<td>24/09/2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 102015686 A</td>
<td>13/04/2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 102015686 B</td>
<td>02/07/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CO 6311078 A2</td>
<td>22/08/2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CR 11681 A</td>
<td>29/10/2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DO P2010000280 A</td>
<td>15/09/2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EA 201001456 A</td>
<td>30/06/2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EC SP10010559 A</td>
<td>30/11/2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 2274300 A2</td>
<td>19/01/2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL 208063 D0</td>
<td>30/12/2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2011-515371 A</td>
<td>19/05/2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2014-040452 A</td>
<td>06/03/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 5384611 B2</td>
<td>08/01/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KR 10-1408517 Bl</td>
<td>17/06/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KR 10-2010-0136516 A</td>
<td>28/12/2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MA 32232 Bl</td>
<td>01/04/2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MX 2010010317 A</td>
<td>04/10/2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE 16562009 A</td>
<td>20/11/2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SM AP201000115 A</td>
<td>19/01/2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SV 2010003673 A</td>
<td>05/07/2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TW 201000457 A</td>
<td>01/01/2010</td>
</tr>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
<td>Publication date</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------</td>
<td>--------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>US 2010-0003246 Al</td>
<td>07/01/2010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>US 8129394 B2</td>
<td>06/03/2012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UY 31724 A</td>
<td>10/11/2009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WO 2009-115572 A3</td>
<td>03/12/2009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>US 2011-0319408 Al</td>
<td>29/12/2011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>US 8513222 B2</td>
<td>20/08/2013</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Form PCT/ISA/2 10 (patent family annex) (January 2015)