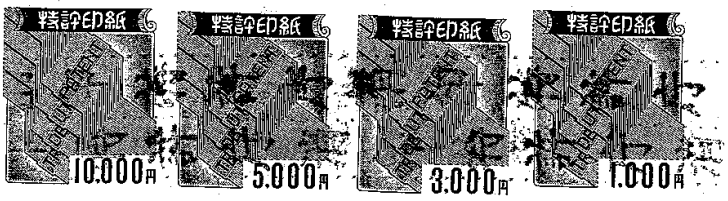
【公報種別】特許法第１７条の２の規定による補正の掲載
【部門区分】第３部門第２区分
【発行日】平成17年2月10日(2005.2.10)

【公表番号】特表2000-514405(P2000-514405A)
【公表日】平成12年10月31日(2000.10.31)
【出願番号】特願平9-542811
【国際特許分類第７版】
　Ａ６１Ｋ　47/48
　Ａ６１Ｋ　31/337
　Ａ６１Ｐ　35/00
【ＦＩ】
　Ａ６１Ｋ　47/48
　Ａ６１Ｋ　31/337
　Ａ６１Ｐ　35/00

【誤訳訂正書】
【提出日】平成16年5月28日(2004.5.28)
【誤訳訂正１】
【訂正対象書類名】明細書
【訂正対象項目名】補正の内容のとおり
【訂正方法】変更
【訂正の内容】



適

誤訳訂正書

平成１６年５月２８日

適

（１９，０００円）

特許庁長官　殿

１．　事件の表示

平成９年特許願第５４２８１１号

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４．　訂正の対象　　明細書

５．　訂正の内容

１）明細書、１頁１１～１２行の「タキサス　ブレビホリア（Taxus brevifoolia）」の記載を「タキサス　ブレビホリア（Taxus brevifolia）」と訂正する。

２）同、４頁７～８行の「[ドクタキセル（docctaxel）]」の記載を「[ドセタキセル（docetaxel）]」と訂正する。

３）同、１１頁下から７行の「ポリエチレン」の記載を「プロピレン」と訂正する。

４）同、１１頁下から６行の「アルファートコフェノール」の記載を「アルファートコフェロール」と訂正する。

５）同、１１頁末行の「１－βカロテンおよびα－トコフェノール。」の記載を「ｔ－βカロテンおよびα－トコフェロール。」と訂正する。

6）同、１２頁２～３行の「タキサス　ブレビホリア（Taxus brevifolia)」の記載を「タキサス　ブレビホリア（Taxus brevifolia)」と訂正する。

7）同、１４頁８行の「４０℃」の記載を「４℃」と訂正する。

8）同、１５頁６行の「＋５０．０および－５０．０」の記載を「＋５０、０および－５０」と訂正する。

9）同、１５頁１４行の「ｌｏｇ１０」の記載を「log_{10}」と訂正する。

１０）同、１５頁１５～１６行の「＋５０．０～－５０.」の記載を「＋５０、０および－５０」と訂正する。

１１）同、１５頁下から４行の「有効であった。」の記載を「有効であった（約３ｌｏｇまで)。」と訂正する。

１２）同、１２２頁２行の「モノオルジ」の記載を「モノまたはジ」と訂正する。

６．　訂正の理由等

訂正の理由１）

この箇所の国際出願の明細書の表記は、ＷＯ９７／４４０６３公報１頁（訂正の理由の説明に必要な資料１）１０行には「Taxus brevifolia」と記載されていたところ、誤訳訂正前は錯誤して「タキサス　ブレビホリア（Taxus brevifoolia)」と翻訳していた。該資料１の１０行からもこれは明らかな誤訳であるから、「タキサス　ブレビホリア（Taxus brevifolia)」と訂正する。

訂正の理由２）

この箇所の国際出願の明細書の表記は、ＷＯ９７／４４０６３公報３頁（訂正の理由の説明に必要な資料２）１８行には「(docetaxel)」と記載されていたところ、誤訳訂正前は錯誤して「[ドクタキセル（docctaxel)]」と翻訳していた。該資料２の１８行からもこれは明らかな誤訳であるから、「[ドセタキセル(docetaxel)]」と訂正する。

訂正の理由３）

この箇所の国際出願の明細書の表記は、ＷＯ９７／４４０６３公報９頁（訂正の

理由の説明に必要な資料３）２６行には「propylene」と記載されていたところ、誤訳訂正前は錯誤して「ポリエチレン」と翻訳していた。該資料３の２６行からもこれは明らかな誤記であるから、「プロピレン」と訂正する。

訂正の理由４）

この箇所の国際出願の明細書の表記は、該資料３の２６行には「alph-tocopherol」と記載されていたところ、誤訳訂正前は錯誤して「アルファートコフェノール」と翻訳していた。該資料３の２６行からもこれは明らかな誤記であるから、「アルファートコフェロール」と訂正する。

訂正の理由５）

この箇所の国際出願の明細書の表記は、該資料３の３０行には「t-β carotene and α-tocopherol.」と記載されていたところ、誤訳訂正前は錯誤して「１－βカロテンおよびα－トコフェノール。」と翻訳していた。該資料３の３０行からもこれは明らかな誤記であるから「ｔ－βカロテンおよびα－トコフェロール。」と訂正する。

訂正の理由６）

この箇所の国際出願の明細書の表記は、該資料３の３２行には「(Taxus brevifolia)」と記載されていたところ、誤訳訂正前は錯誤して「タキサス　ブレビホリア (Taxus brevifoolia)」と翻訳していた。訂正の理由の説明に必要な資料３の３２行からもこれは明らかな誤記であるから「タキサス　ブレビホリア (Taxus brevifolia)」と訂正する。

訂正の理由７）

この箇所の国際出願の明細書の表記は、ＷＯ９７／４４０６３公報１１頁（訂正の理由の説明に必要な資料４）２２行には「４℃」と記載されていたところ、誤訳訂正前は錯誤して「４０℃」と翻訳していた。該資料４の２２行からもこれは明らかな誤記であるから「４℃」と訂正する。

訂正の理由８）

この箇所の国際出願の明細書の表記は、ＷＯ９７／４４０６３公報１２頁（訂正

の理由の説明に必要な資料５）２５行には「+50, 0, and -50」と記載されていたところ、誤訳訂正前は錯誤して「＋５０．０および－５０．０」と翻訳していた。該資料５の２５行からもこれは明らかな誤記であるから「＋５０、０および－５０」と訂正する。

訂正の理由９）

この箇所の国際出願の明細書の表記は、ＷＯ９７／４４０６３公報１３頁（訂正の理由の説明に必要な資料６）３行には「log_{10}」と記載されていたところ、誤訳訂正前は錯誤して「ｌｏｇ１０」と翻訳していた。該資料６の３行からもこれは明らかな誤記であるから「ｌｏｇ$_{10}$」と訂正する。

訂正の理由１０）

この箇所の国際出願の明細書の表記は、該資料６の５行には「+50, 0, and -50」と記載されていたところ、誤訳訂正前は錯誤して「＋５０．０～－５０.」と翻訳していた。訂正の理由の説明に必要な資料６の５行からもこれは明らかな誤記であるから「＋５０、０および－５０」と訂正する。

訂正の理由１１）

この箇所の国際出願の明細書の表記は、該資料６の１５～１６行には「was more effective against ..... (by about 3 logs),」と記載されていたところ、誤訳訂正前は錯誤して「有効であった。」と翻訳していた。該資料６の１５～１６行からもこれは明らかな誤記であるから「有効であった（約３１ｏｇまで）。」と訂正する。

訂正の理由１２）

この箇所の国際出願の明細書の表記は、ＷＯ９７／４４０６３公報６１頁（訂正の理由の説明に必要な資料７）３０行には「mono ordi」と記載されていたところ、誤訳訂正前は錯誤して「モノオルジ」と翻訳していた。該資料７の３０行からもこれは明らかな誤記であるから「モノまたはジ」と訂正する。

７．　添付書類の目録

（１）訂正の理由の説明に必要な資料　　７

以上

訂正の理由の説明に必要な資料1

DHA-PHARMACEUTICAL AGENT CONJUGATES

Background of the Invention

Improving drug selectivity for target tissue is an established goal in the medical arts. In general, it is desirable to deliver a drug selectively to its target, so that dosage and, consequently, side effects can be reduced. This is particularly the case for toxic agents such as anti-cancer agents because achieving therapeutic doses effective for treating the cancer is often limited by the toxic side effects of the anti-cancer agent on normal, healthy tissue. The problems relating to lack of drug selectivity can be exemplified by Taxol®.

Taxol® (paclitaxel) was first isolated in 1971 from the bark of Taxus brevifolia and was approved in 1992 by the US Food and Drug Administration for treatment of metastatic ovarian cancer and later for breast cancer. Its mechanism of action is believed to involve promoting formation and hyperstabilization of microtubules, thereby preventing the disassembly of microtubules necessary for completion of cell division. It also has been reported that Taxol induces expression of cytokines, affects the activity of kinases and blocks processes essential for metastasis, in as yet uncharacterized mechanisms of action.

Taxol has attracted unusually strong scientific attention, not only because of its unique antiproliferative mechanism of action, but also because it is active against nearly all cancers against which it has been tested and because it has been discovered to be an analog of numerous closely related compounds occurring naturally. These compounds, taxanes, are now recognized as a new class of anticancer compounds.

Taxol's strength against cancers of diverse tissue origin also represents a significant drawback. An ideal anticancer agent has tissue specificity, thereby reducing side-effects on normal (dividing) cells. Taxol analogs with tissue specificity therefore are desired. Another drawback of Taxol is its extreme insolubility. Taxol can be administered effectively in a solvent including cremophor, which combination can provoke severe hypersensitive immune responses. As a result of these drawbacks, and also as a result of the potential for modifying Taxol at numerous sites as demonstrated by other naturally-occurring taxanes with anticancer activity, a search for more selective taxanes was launched.

To date, more than 200 taxanes have been synthesized (or isolated) and tested *in vitro* or *in vivo* for anticancer activity. The results, however, have been so disappointing that the National Cancer Institute (NCI) generally no longer is interested in testing Taxol analogs. In general with Taxol analogs, the solubility problems remain, and/or potency is sharply reduced, and/or selectivity

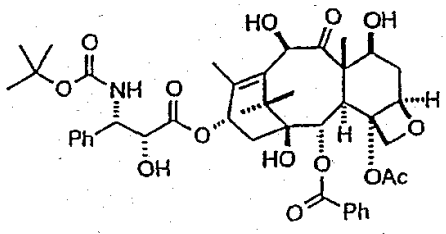
anhydride), sulfonic acids, amino acids and phosphates. Generally, activity was reduced although some success was obtained with certain derivatives. Again, no particular pattern emerged permitting one to predict reliably which groups could be substituted on Taxol to yield a therapeutically useful product, although it was suggested that the 2' OH derivatives may cleave more easily than the C7 OH derivatives.

Several other factors add to the problem of predicting which Taxol analogs will be effective. Multiple mechanisms of action have been proposed in the literature, and a change in one position may have no effect on activity on one such mechanism but may eliminate activity on another mechanism. In addition, changes that favorably influence activity may unfavorably influence bioavailability. For example, Taxol affects microtubule formation inside a cell, but a change in structure that increases intracellular activity may adversely affect the ability of Taxol to gain entry into a cell. Taxol also is known to bind to proteins, and the effect on activity that results from a change in Taxol's binding to protein (in terms of conformation, cellular absorption and solubility) is unknown.

It has been reported that Taxol does not get into the brain, apparently excluded by the blood brain barrier. It is not known why this is so, as Taxol is lipophilic, gets into cells and might be expected to cross the blood brain barrier.

Among the most promising of the two hundred analogs tested is Taxotere (docetaxel), because of its slightly increased activity and solubility. Oddly, however, Taxotere differs from Taxol at sites which typically do not have a strong influence on activity, and one would not predict the improvements in Taxotere from these differences, even in hindsight.

Taxotere has the following formula:



DHA (docosahexaenoic acid) is a 22 carbon naturally-occurring, unbranched fatty acid that previously has been attached to drugs to help deliver them across the blood brain barrier. DHA is attached via the acid group to hydrophilic drugs and renders these drugs more hydrophobic (lipophilic). DHA is an important constituent of the brain and recently has been approved in Europe

Figure 25 is a graph plotting concentration of Taxol versus percent growth of renal cancer cells.

Figure 26 is a graph plotting concentration of Taxol versus percent growth of prostate cancer cells.

Figure 27 is a graph plotting concentration of Taxol versus percent growth of breast cancer cells.

Detailed Description of the Invention

Cis-docosahexaenoic acid (DHA) is a naturally occurring fatty acid. It is an unbranched chain fatty acid with six double bonds, all *cis*. Its structure is as follows:

DHA can be isolated, for example, from fish oil or can be chemically synthesized. These methods, however, can generate *trans* isomers, which are difficult and expensive to separate and which may present safety problems in humans. The preferred method of production is biological synthesis to produce the all *cis* isomer. The preferred source of DHA is from Martek Biosciences Corporation of Columbia, Maryland. Martek has a patented system for manufacturing DHA using microalgae which synthesize only a single isomer of DHA, the all *cis* isomer. Martek's patents include U.S. Pat. Nos. 5,374,657, 5,492;938, 5,407,957 and 5,397,591.

DHA also is present in the milk of lactating women, and Martek's licensee has obtained approval in Europe of DHA as a nutritional supplement for infant formula.

It is known that DHA can be unstable in the presence of oxygen. To stablizie DHA and its conjugates it is important to add anti-oxidants to the material after it is synthesized. One method of stablization is to make-up the newly synthesized material in the following solution:
100 g neat DHA-taxol plus 100 g of vehicle (100ml propylene glycol, 70 mg alph-tocopherol, 5 mg dialaurylthiodipropionic acid, 50 mg ascorbic acid) prepared and held under argon in amber, sealed vials and stored at four degrees centigrade. The following anti-oxidants may also be employed: ascorbic acid, ascorbyl palmitate, dilauryl ascorbate, hydroquinone, butyated hydroxyanisole, sodium meta bisulfite, *t*-β carotene and α-tocopherol. A heavy metal chelator such as ethylenediamine tetra-acetic acid (EDTA) may also be used.

Paclitaxel was first isolated from the bark of Taxus brevifolia (Wani et al., J. Am. Chem.

The production of analog 2 involves several steps including a number of protection-acylation-deprotection steps. A solution of Taxol (59μmol) in methylene chloride (2.5mL) was mixed at ambient temperature under argon with imidazole (147μmol) and triethylsilyl chloride (147μmol). The reaction mixture was stirred for thirty minutes, diluted with additional methylene chloride, washed with water, saturated aqueous sodium chloride, dried, and concentrated. Chromatography of the residue produced 50mg (88%) of intermediate A plus 5mg of the 2', 7-di(triethylsilyl) ether derivative. A solution of intermediate A (52μmol) in methylene chloride (3mL) was mixed at ambient temperature under argon with 4-dimethylaminopyridine (52μmol), dicyclohexylcarbodiimide (104μmol), and DHA (52μmol). The reaction mixture was stirred for ten hours, diluted with ether, passed through celite, and concentrated. Chromatography of the residue produced 65.9mg of intermediate B. A solution of intermediate B (51μmol) in acetonitrile (2mL) at 0°C under argon was mixed with 49% aqueous HF (0.2mL) and the reaction mixture was stirred for one hour. After dilution with ether, the reaction mixture was washed with water, saturated aqueous sodium chloride, dried, and concentrated. Radial chromatography of the residue produced 44.6mg (75%) of Taxol-DHA conjugate 2.

Example 3

Conjugates 1 and 2 were sent to the United States National Cancer Institute (NCI) for screening in the NCI's anticancer screening program. The conjugates were provided in ethanol (approximately 40mg analog/2ml ethanol). The conjugates were sealed in vials under argon to avoid exposure of the conjugates to oxygen because the conjugates were believed to be sensitive to oxygen. Instructions were provided to store at 4°C and to open the vials only when ready for immediate experimental use. Instructions also were provided to use the ethanol solutions containing the conjugates directly or to dissolve the analogs further in DMSO (dimethylsulfoxide) at appropriate concentrations, with vortexing if necessary for adequate dispersal.

The activities of conjugates 1 and 2 were tested against 57 cancer cell lines. The results are presented in Figs. 1-9 for conjugate 1, Figs. 10-18 for conjugate 2 and Figs 19-27 for Taxol. To understand the data, reference is made to the guides provided by the NCI, excerpted as follows:

訂正の理由の説明に必要な資料５

The Calculated Measurement of Effect: Percentage Growth (PG)

The measured effect of the compound on a cell line is currently calculated according to one or the other of the following two expressions:

If (Mean OD_{test} -Mean OD_{tzero}) ≥ 0, then

$$PG = 100 \times (\text{Mean } OD_{test} - \text{Mean } OD_{tzero})/(\text{Mean } OD_{ctrl} - \text{Mean } Od_{tzero})$$

If (Mean OD_{test} -Mean OD_{tzero}) < 0, then

$$PG = 100 \times (\text{Mean } OD_{test} - \text{Mean } Od_{tzero})/\text{Mean } Od_{tzero}$$

Where:

Mean OD_{tzero} = The average of optical density measurements of SRB-derived color just before exposure of cells to the test compound.

Mean OD_{test} = The average of optical density measurements of SRB-derived color after 48 hours exposure of cells to the test compound.

Mean OD_{ctrl} = The average of optical density measurements of SRB-derived color after 48 hours with no exposure of cells to the test compound.

Experimental data was collected against each cell line. ... Each concentration is expressed as the $\log_{10}$ (molar or µg/ml). ... The response parameters GI50, TGI, and LC50 are interpolated values representing the concentrations at which the PG is +50, 0, and -50, respectively. Sometimes these response parameters cannot be obtained by interpolation. If, for instance, all of the PGs in a given row exceed +50, then none of the three parameters can be obtained by interpolation. In such a case, the value given for each response parameter is the highest concentration tested. ... This practice is extended similarly to the other possible situations where a response parameter cannot be obtained by interpolation.

訂正の理由の説明に必要な資料6

Dose-Response Curves:

The dose-response curve page of the data package is created by plotting the PGs against the $\log_{10}$ of the corresponding concentration for every cell line. The cell line curves are grouped by subpanel. Horizontal lines are provided at the PG values of +50, 0, and -50. The concentrations corresponding to points where the curves cross these lines are the GI50, TGI and LC50, respectively.

Several important distinctions are apparent from the data. Most important, the patterns of anticancer actively for conjugates 1 and 2 differ from that of Taxol. In one sense, conjugates 1 and 2 are effective anticancer agents against a more restricted set of cancer cell lines. For example, conjugates 1 and 2 were not very effective against any of the six leukemia cancer cell lines tested, whereas Taxol was somewhat effective against all four leukemia cell lines against which Taxol was tested. (See Figs. 1, 10 and 19.)

The relative activity against members within a class of cancers also was altered. For example, at TGI (horizontal line at zero in the graphs), Taxol was more effective against non-small cell lung cancer line H522 than against H460 (by about 3 logs), whereas conjugates 1 and 2 were slightly more effective against H460 than H522. As another example, Taxol was least effective at TGI against CNSU251, whereas conjugate 1 was most effective against CNSU251 and conjugates 2 was also very effective against CNSU251(relative to other CNS cell lines). As a further example, Taxol was equivalent in activity toward MDA-N and MDA-MB-435 breast cancer cell lines at all concentrations tested, whereas conjugates 1 and 2 were more effective against MDA-N than MDA-MB-435 at all concentrations tested.

To further illustrate the differences in the activity of conjugates 1 and 2 versus that of Taxol, the NCI subjected the data to a statistical analysis designed by the NCI to reflect differences in the pattern of activity of anticancer agents. Conjugate 1 and conjugate 2 were determined to be statistically different in their pattern of activity versus Taxol in this unique measurement by the NCI.

It also is to be noted that, in general, conjugates 1 and 2 were one thousand to ten thousand times less potent than Taxol for many cell lines tested. This reduction in activity is important, especially since conjugates 1 and 2 maintained strong activity against some cell lines. Conjugates 1 and 2 will be sufficiently active against certain cell lines, but will have, on average, a substantially and disproportionately lower activity against other cell lines, reducing potential side effects. For

pharmacologically and pharmaceutically acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulfonic, tartaric, citric, methane sulfonic, formic, malonic, succinic, naphthalene-2-sulfonic, and benzene sulfonic. Also, pharmaceutically acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

Suitable buffering agents include: acetic acid and a salt (1-2% W/V); citric acid and a salt (1-3% W/V); boric acid and a salt (0.5-2.5% W/V); and phosphoric acid and a salt (0.8-2% W/V).

Suitable preservatives include benzalkonium chloride (0.003-0.03% W/V); chlorobutanol (0.3-0.9% W/V); parabens (0.01-0.25% W/V) and thimerosal (0.004-0.02% W/V).

The active compounds of the present invention may be a pharmaceutical composition having a therapeutically effective amount of a conjugate of the invention optionally included in a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid filler, dilutants or encapsulating substances which are suitable for administration to a human or other animal. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions are capable of being commingled with the molecules of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

Compositions suitable for parenteral administration conveniently comprise a sterile preparation of the conjugates of the invention. This preparation may be formulated according to known methods. Formulations for taxanes can be found in Chapter 9 of Taxol: Science and Applications, CRC Press, Inc., 2000 Corporate Boulevard, N.W., Boca Raton, FL 33431. In general, Taxol has been formulated as a 6 mg/ml cremophor EL (polyoxyethylated castor oil)/ethanol mixture, which is diluted to final volume with normal saline or 5% dextrose. A 15mg/ml solution of taxotere has been formulated in polysorbate 80 (polyoxyethylene sorbitanmonooleate)/ethanol mixture, diluted with 5% dextrose.

The sterile preparation thus may be a sterile solution or suspension in a non-toxic parenterally-acceptable diluent or solvent. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono ordi-glycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables. Carrier formulations suitable for oral, subcutaneous, intravenous, intramuscular, etc. can be found in Remington's Pharmaceutical Sciences, Mack