[00104] Figure 1 is a graph that compares the pharmacokinetics of BID dosing versus QD dosing in patients, where the patients were dosed at 500 mg during each dose. Medication was administered with a four-hour interval in between the two doses during the same day for the 500 mg BID regimen.

[00105] Figure 2 is a graph that compares the pharmacokinetics of two different formulations of the Compound of the Invention. The two formulations result in different sizes of the capsule.

[00106] Figure 3A consists of photographic images of tumor tissue samples from CRC patients visualized through immunohistochemistry using antibodies against phosphorylated STAT3 and DAPI (lower row).

[00107] Figure 3B is a chart showing a trend towards improvement in survival for patients with high p-STAT3 (compared with patients having low or negative p-STAT3).

[00108] Figure 4A consists of photographic images of tumor tissue samples from CRC patients visualized through immunohistochemistry using antibodies against β-catenin and DAPI (lower row).

[00109]

[00110] Figure 5 shows $CD44^{high}$ cells growth being blocked by a Compound of the Invention. $CD44^{high}$ cells were isolated by FACS (FaDu) and were cultured in the absence of attachment and serum for 5 days to form primary spheres. Primary spheres were then dissociated in Accumax (eBioscience, San Diego, CA) to single cells, and were cultured as above for 72 hours before the addition of the indicated concentrations of therapeutic agents. After five days of treatment, representative sphere images were captured.

[00111] Figure 6 shows an *in vivo* study of nude mice with xenografted human colon cancer tumor tissues where a Compound of the Invention was shown to be effective in reducing or clearing p-STAT3 and β-catenin levels. Formaldehyde-fixed tumors from mice treated daily for 15 days with oral gavage of the Co pound of the Invention or Vehicle (Control) were sectioned and analyzed by immunofluorescence staining using antibodies specific for human –STAT2 and β-catenin.

[00112] Figure 7 shows that in a mice study, a Compound of the Invention targeted cancer stem cells. Xenograft bearing mice were administered with either vehicle, gemcitabine (120mg/kg (MIA PaCa-2)), carboplatin (30mg/kg (FaDu)), or 20mg/kg of a

Patient Screening using Putative Biomarker

[00211] Based on the discovery that phosphorylated STAT3 (p-STAT3) positivity and β-catenin expression in cellular nucleus can both, individually or in combination, serve as predictive biomarkers for higher likelihood of treatment efficacy using the Compound of the Invention (see Examples 9 and 10), the present invention provides ways to screen patients for recommendation of cancer treatments that involve the Compound of the Invention. Our data indicates a direct correlation between the level of p-STAT3 in tumor tissues before treatment and the chance of survival or treatment success with the Compound of the Invention. In other words, the higher the level of p-STAT3 found in a cancer patient before treatment, at least in colorectal cancer (CRC) patients, the higher overall survival (OS) is once treatment using the Compound of the Invention and related compositions (Figure 3B). Accordingly, the present invention provides a method of treating cancer in a selected patient population or screening potential cancer patients for treatment, the method comprising the steps of: measuring a level of phosphorylated STAT3 (p-STAT3) in a biological sample (e.g., tumor tissue before treatment) obtained from a patient candidate diagnosed of a cancer (e.g., colorectal adenocarcinoma); confirming that the patient candidate's p-STAT3 level is above a benchmark level; and administering to the patient candidate a therapeutically effective amount of the Compound of the Invention or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof. The benchmark level may differ for different demographic sectors, and can be determined by one skilled artisan through routine experimentation.

[00212] Similarly, because our data indicates a direct correlation between the level of expression or localization of β-catenin, an oncogene closely linked to STAT3, in the cell nucleus and the chance of survival or treatment success with the Compound of the Invention. In other words, the higher the expression level of β-catenin in cancer cell nucleus as opposed to the cell membrane in a cancer patient, at least in CRC patients, the higher overall survival (OS) is. Accordingly, the present invention provides a method of treating cancer in a selected patient population or screening potential cancer patients for treatment, the method comprising the steps of: detecting a locus of β-catenin expression in a biological sample (e.g., tumor tissue before treatment) obtained from a patient candidate diagnosed of a cancer; confirming that significant β-catenin expression is detected in cell nucleus in the sample from the patient candidate; and administering to the patient candidate a therapeutically effective amount of the Compound of the Invention or a pharmaceutically

inhibiting p-STAT3 expression. Even with dosage as low as 100 QD (single daily dosage), there was almost no longer any detectable p-STAT3 in the patient tissue after treatment. And as the chart in Figure 3B shows, for patients receiving the Compound of the Invention treatment (N=13), the overall survival (OS) is much more optimistic in those who had previously exhibited relatively high levels of p-STAT3. For instance, 40% of the patients with high p-STAT3 levels before treatment survived longer than 100 weeks whereas only 10% of those with low or no p-STAT3 levels before treatment survived beyond 100 weeks. This further confirms that the Compound of the Invention downregulates that STAT3 pathway, and that the STAT3 pathway is implicated in colorectal cancers.

[00307] The direct correlation between the p-STAT3 level and OS of CRC patient receiving treatment with the Compound of the Invention makes p-STAT3 a promising diagnostic biomarker that can be used to predict treatment effectiveness. Accordingly, p-STAT3 level can be used to screen patient pools for treatment with the Compound of the Invention.

EXAMPLE 10: Nuclear β-catenin as predicative biomarker

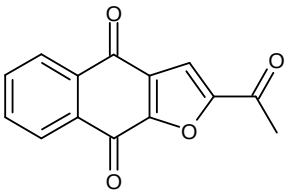
[00308] CRC patient's archival tumor tissue samples were analyzed through immunohistochemistry (IHC) using labeled antibodies against β-catenin. As shown in Figure 4A, the Compound of the Invention was effective in removing or preventing the accumulation of β-catenin in cell nucleus in tumor tissues.

For patients receiving treatment with the Compound of the Invention (N=13), the overall survival (OS) is much more optimistic in those found to have previously shown high levels of nuclear β-catenin prior to treatment. For instance, close to 40% of the patients with high nuclear β-catenin levels before treatment survived longer than 100 weeks whereas none of those with high levels of membranous β-catenin survived beyond 25 weeks. This further confirms that the Compound of the Invention disrupts or somehow modulates the β-catenin function, and that the β-catenin pathway is implicated in colorectal cancers.

[00309] The direct correlation between the nuclear β-catenin level and OS of CRC patient receiving treatment with the Compound of the Invention makes nuclear β-catenin level a promising diagnostic biomarker that can be used to predict treatment effectiveness. Accordingly, nuclear β-catenin level can be used to screen patient pools for treatment with the Compound of the Invention.

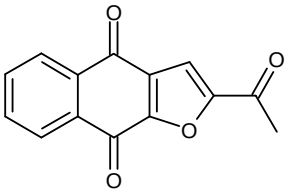
Claims

1. Use of a compound having the structure of



or a pharmaceutically acceptable salt, solvate, hydrate, thereof, in the manufacture of a medicament for use in combination therapy of cancer in a subject, wherein the medicament is to be administered in combination with an antimitotic agent or a pharmaceutically acceptable salt, solvate or hydrate thereof, and wherein the compound is to be administered at a total daily dose in a range from about 80 mg to about 2000 mg,wherein the antimitotic agent is selected from the group consisting of docetaxel (taxotere), BMS-275183, xyotax, tocosal, vinorlebine, vincristine, vinblastine, vindesine, vinzolidine, etoposide (VP-16), teniposide (VM-26), ixabepilone, larotaxel, ortataxel, tesetaxel, and ispinesib.

2. Use of a compound having the structure of



or a pharmaceutically acceptable salt, solvate, or hydrate thereof, in the manufacture of a medicament for use in a combination therapy of a cancer in a subject, wherein the medicament is to be administered in combination of paclitaxel, the cancer is gastric cancer, gastroesophageal junction cancer, or esophageal cancer, and the compound is to be administered at a total daily dose in a range from about 80 mg to about 2000 mg.

3. The use according to claim 2, wherein the paclitaxel is to be administered at a total weekly dose in a range from about 40 mg/ m^2 to about 100 mg/m^2.

4. The use according to claim 3, wherein the paclitaxel is to be administered at a total weekly dose of

about 80 mg/ m^2.

5. The use according to claim 4, wherein the paclitaxel is to be administered to the subject through IV.

6. The use according to claim 2, wherein the cancer is refractory, recurrent, or/and metastatic.

7. The use according to claim 2, wherein the medicament comprises a therapeutically effective amount of the compound, polyoxylglycerides with a hydrophilic lipophilic balance (HLB) value of more than 10, and polyoxylglycerides with a hydrophilic lipophilic balance (HLB) value of less than 10.

8. The use according to claim 7, wherein the medicament further comprises a surfactant.

9. The use according to claim 8, wherein the surfactant is sodium lauryl sulfate (SLS) or sodium dodecyl sulfate (SDS).

10. The use according to any one of claims 7 to 9, wherein the polyoxylglycerides with a hydrophilic lipophilic balance (HLB) value of more than 10 comprise lauroyl polyoxylglycerides or stearoyl polyoxylglycerides.

11. The use according to any one of claims 7 to 9, wherein the polyoxylglycerides with an HLB value of less than 10 comprise linoleoyl polyoxylglycerides.

12. The use according to claim 2, wherein the medicament is in a capsule.

13. The use according to claim 12, wherein the capsule comprises about 80 mg of the compound.