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(54) Benævnelse: **Kombinationsterapi med et antitumor-alkaloid**

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DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention relates to the combination of PM01183 with antimetabolites and to the use of these combinations in the treatment of cancer.

BACKGROUND OF THE INVENTION

[0002] Cancer develops when cells in a part of the body begin to grow out of control. Although there are many kinds of cancer, they all arise from out-of-control growth of abnormal cells. Cancer cells can invade nearby tissues and can spread through the bloodstream and lymphatic system to other parts of the body. There are several main types of cancer. Carcinoma is a malignant neoplasm, which is an uncontrolled and progressive abnormal growth, arising from epithelial cells. Epithelial cells cover internal and external surfaces of the body, including organs, lining of vessels, and other small cavities. Sarcoma is cancer arising from cells in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue. Leukemia is cancer that arises in blood-forming tissue such as the bone marrow, and causes large numbers of abnormal blood cells to be produced and enter the bloodstream. Lymphoma and multiple myeloma are cancers that arise from cells of the immune system.

[0003] In addition, cancer is invasive and tends to infiltrate the surrounding tissues and give rise to metastases. It can spread directly into surrounding tissues and also may be spread through the lymphatic and circulatory systems to other parts of the body.

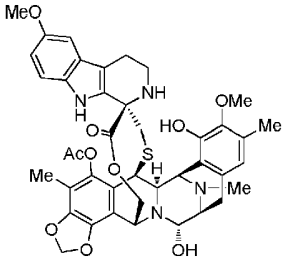
[0004] Many treatments are available for cancer, including surgery and radiation for localised disease, and chemotherapy. However, the efficacy of available treatments for many cancer types is limited, and new, improved forms of treatment showing clinical benefits are needed. This is especially true for those patients presenting with advanced and/or metastatic disease and for patients relapsing with progressive disease after having been previously treated with established therapies which become ineffective or intolerable due to acquisition of resistance or to limitations in administration of the therapies due to associated toxicities.

[0005] Since the 1950s, significant advances have been made in the chemotherapeutic management of cancer. Unfortunately, more than 50% of all cancer patients either do not respond to initial therapy or experience relapse after an initial response to treatment and ultimately die from progressive metastatic disease. Thus, the ongoing commitment to the design and discovery of new anticancer agents is critically important.

[0006] Chemotherapy, in its classic form, has been focused primarily on killing rapidly proliferating cancer cells by targeting general cellular metabolic processes, including DNA, RNA, and protein biosynthesis. Chemotherapy drugs are divided into several groups based on how they affect specific chemical substances within cancer cells, which cellular activities or processes the drug interferes with, and which specific phases of the cell cycle the drug affects. The most commonly used types of chemotherapy drugs include: DNA-alkylating drugs (such as cyclophosphamide, ifosfamide, cisplatin, carboplatin, dacarbazine), antimetabolites (5-fluorouracil, capecitabine, 6-mercaptopurine, methotrexate, gemcitabine, cytarabine, fludarabine), mitotic inhibitors (such as paclitaxel, docetaxel, vinblastine, vincristine), anticancer antibiotics (such as daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone), topoisomerase I and/or II inhibitors (such as topotecan, irinotecan, etoposide, teniposide), and hormone therapy (such as tamoxifen, flutamide). Scaife et al.: "Antimetabolites in Cancer Therapy" in "Anticancer Therapeutics" (2008, John Wiley & Sons) provides a review on antimetabolites commonly employed to treat cancer.

[0007] The ideal antitumor drug would kill cancer cells selectively, with a wide index relative to its toxicity towards non-cancer cells and it would also retain its efficacy against cancer cells, even after prolonged exposure to the drug. Unfortunately, none of the current chemotherapies with these agents posses an ideal profile. Most posses very narrow therapeutic indexes and, in addition, cancerous cells exposed to slightly sublethal concentrations of a chemotherapeutic agent may develop resistance to such an agent, and quite often cross-resistance to several other antitumor agents.

[0008] PM01183, also known as tryptamicidin, is a synthetic alkaloid which is currently in clinical trials for the treatment of cancer, and has the following chemical structure:



[0009] PM01183 has demonstrated a highly potent in vitro activity against solid and non-solid tumour cell lines as well as a significant in vivo activity in several xenografted human tumor cell lines in mice, such as those for breast, kidney and ovarian cancer. PM01183 exerts its anticancer effects through the covalent modification of guanines in the DNA minor groove that eventually give rise to DNA double-strand break, S-phase arrest and apoptosis in cancer cells. Further information regarding this compound can be found in WO 03/01427; 100th AACR Annual Meeting, April 18-22, 2009, Denver, CO, Abstract Nr. 2679 and Abstract Nr. 4525; and Leal JFM et al. Br. J. Pharmacol. 2010, 161, 1099-1110.

[0010] Since cancer is a leading cause of death in animals and humans, several efforts have been and are still being undertaken in order to obtain a therapy active and safe to be administered to patients suffering from a cancer. The problem to be solved by the present invention is to provide anticancer therapies that are useful in the treatment of cancer.

SUMMARY OF THE INVENTION

[0011] Subject-matter which is not encompassed by the scope of the claims does not form part of the presently claimed invention.

[0012] The present invention establishes that PM01183 potentiates the antitumor activity of antimetabolites 5-fluorouracil, gemcitabine, cytarabine, capecitabine, decitabine, floxuridine, aminopterin, methotrexate, pemetrexed and raltitrexed. Therefore PM01183 and said other antimetabolite can be successfully used in combination therapy for the treatment of cancer.

[0013] Thus, pharmaceutical compositions, kits using these combination therapies and uses of both drugs in the treatment of cancer and in the manufacture of medicaments for synergistic combination therapies are herein disclosed.

[0014] In accordance with one aspect of this invention, the invention is directed to PM01183, or a pharmaceutically acceptable salt thereof, for use in the treatment of cancer comprising administering a therapeutically effective amount of PM01183, or a pharmaceutical acceptable salt thereof, in synergistic combination with a therapeutically effective amount of an antimetabolite selected from 5-fluorouracil, gemcitabine, cytarabine, capecitabine, decitabine, floxuridine, aminopterin, methotrexate, pemetrexed and raltitrexed.

[0015] In another aspect, the invention is directed to PM01183, or a pharmaceutically acceptable salt thereof, for use in increasing or potentiating the therapeutic efficacy of an antimetabolite in the treatment of cancer, which comprises administering to a patient in need thereof a therapeutically effective amount of PM01183, or a pharmaceutically acceptable salt thereof, in synergistic combination with this antimetabolite, wherein the antimetabolite is selected from 5-fluorouracil, gemcitabine, cytarabine, capecitabine, decitabine, floxuridine, aminopterin, methotrexate, pemetrexed and raltitrexed.

[0016] The use of PM01183, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for the treatment of cancer by synergistic combination therapy employing PM01183, or a pharmaceutically acceptable salt thereof, with an antimetabolite as defined above is also herein disclosed.

[0017] Also herein disclosed is a pharmaceutical composition comprising PM01183, or a pharmaceutically acceptable salt thereof, and/or an antimetabolite as defined above, and a pharmaceutically acceptable carrier, to be used in synergistic combination therapy for the treatment of cancer.

[0018] The invention also encompasses a kit for use in the treatment of cancer which comprises a dosage form of PM01183, or a pharmaceutically acceptable salt thereof, and a dosage form of an antimetabolite as defined above, and instructions for the use of both drugs in synergistic combination.

BRIEF DESCRIPTION OF THE FIGURES

[0019]

Fig 1-2. In vitro activity data of PM01183 in combination with 5-fluorouracil and gemcitabine respectively against A549 cells.

Fig 3-4. In vitro activity data of PM01183 in combination with cytarabine and gemcitabine respectively against A673 cells.

Fig 5-7. In vitro activity data of PM01183 in combination with 5-fluorouracil, cytarabine and methotrexate respectively against SK-MEL-2 cells.

Fig 8-11. In vitro activity data of PM01183 in combination with 5-fluorouracil, cytarabine, gemcitabine and methotrexate respectively against PC-3 cells.

Fig 12-14. In vitro activity data of PM01183 in combination with cytarabine, gemcitabine and methotrexate respectively against PANC-1 cells.

Fig 15-18. In vitro activity data of PM01183 in combination with 5-fluorouracil, cytarabine, gemcitabine and methotrexate respectively against HGC-27 cells.

Fig 19-22. In vitro activity data of PM01183 in combination with 5-fluorouracil, cytarabine, gemcitabine and methotrexate respectively against IGROV-1 cells.

Fig 23-26. In vitro activity data of PM01183 in combination with 5-fluorouracil, cytarabine, gemcitabine and methotrexate respectively against HEP-G2 cells.

Fig 27-30. In vitro activity data of PM01183 in combination with 5-fluorouracil, cytarabine, gemcitabine and methotrexate respectively against MDA-MB-231 cells.

Fig 31-33. In vitro activity data of PM01183 in combination with 5-fluorouracil, cytarabine and gemcitabine respectively against HT-29 cells.

Fig 34-37. In vitro activity data of PM01183 in combination with 5-fluorouracil, cytarabine, gemcitabine and methotrexate respectively against RXF-393 cells.

Fig 38-40. In vitro activity data of PM01183 in combination with 5-fluorouracil, gemcitabine and methotrexate respectively against U87-MG cells.

Fig 41. Tumor volume evaluation of HGC-27 tumors in mice treated with placebo, PM01183, 5-fluorouracil and PM01183 plus 5-fluorouracil.

Fig 42. Tumor volume evaluation of SW1990 tumors in mice treated with placebo, PM01183, gemcitabine and PM01183 plus gemcitabine.

Fig 43. Effects of the combination of PM01183 with methotrexate in JURKAT cell line.

Fig 44. Effects of the combination of PM01183 with methotrexate in MOLT-4 cell line.

Fig 45. Effects of the combination of PM01183 with cytarabine in RAMOS cell line.

Fig 46. Effects of the combination of PM01183 with methotrexate in RAMOS cell line.

Fig 47. Effects of the combination of PM01183 with methotrexate in U-937 cell line.

Fig 48. Effects of the combination of PM01183 with gemcitabine in RAMOS cell line.

Fig 49. Effects of the combination of PM01183 with gemcitabine in U-937 cell line.

DETAILED DESCRIPTION OF THE INVENTION

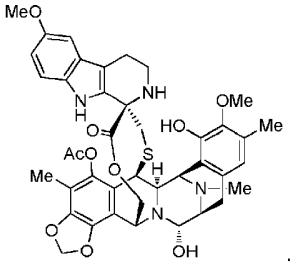
[0020] We surprisingly found that PM01183 greatly enhances the anticancer activity of antimetabolites 5-fluorouracil, gemcitabine, cytarabine, capecitabine, decitabine, floxuridine, aminopterin, methotrexate, pemetrexed and raltitrexed when these anticancer drugs are combined with PM01183. Thus, the present invention is directed to provide an efficacious treatment of cancer based on the synergistic combination of PM01183, or a pharmaceutically acceptable salt thereof, with an antimetabolite selected from 5-fluorouracil, gemcitabine, cytarabine, capecitabine, decitabine, floxuridine, aminopterin, methotrexate, pemetrexed and raltitrexed.

[0021] In the present application, by "cancer" it is meant to include tumors, neoplasias, and any other malignant disease having as cause malignant tissue or cells.

[0022] The term "treating", as used herein, unless otherwise indicated, means reversing, alleviating, or inhibiting the progress of the disease or condition to which such term applies, or one or more symptoms of such disorder or condition. The term "treatment", as used herein, unless otherwise indicated, refers to the act of treating as "treating" is defined immediately above.

[0023] The term "combination" as used throughout the specification, is meant to encompass the administration to a patient suffering from cancer of the referred therapeutic agents in the same or separate pharmaceutical formulations, and at the same time or at different times. If the therapeutic agents are administered at different times they should be administered sufficiently close in time to provide for the potentiating or synergistic response to occur.

[0024] As mentioned above, PM01183 is a synthetic alkaloid, having the following structure:



[0025] The term "PM01183" is intended here to cover any pharmaceutically acceptable salt, solvate, hydrate, prodrug, or any other compound which, upon administration to the patient is capable of providing (directly or indirectly) the compound as described herein. The preparation of salts, solvates, hydrates, and prodrugs can be carried out by methods known in the art.

[0026] Pharmaceutically acceptable salts can be synthesized from the parent compound, which contains a basic or acidic moiety, by conventional chemical methods. Generally, such salts are, for example, prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent or in a mixture of the two. Generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol or acetonitrile are preferred. Examples of the acid addition salts include mineral acid addition salts such as, for example, hydrochloride, hydrobromide, hydroiodide, sulphate, nitrate, phosphate, and organic acid addition salts such as, for example, acetate, trifluoroacetate, maleate, fumarate, citrate, oxalate, succinate, tartrate, malate, mandelate, methanesulphonate and p-toluenesulphonate. Examples of the alkali addition salts include inorganic salts such as, for example, sodium, potassium, calcium and ammonium salts, and organic alkali salts such as, for example, ethylenediamine, ethanolamine, N,N-dialkylenethanolamine, triethanolamine and basic aminoacids salts.

[0027] The term "prodrug" is used in its broadest sense and encompasses those derivatives that are converted in vivo to PM01183. The prodrug can hydrolyze, oxidize, or otherwise react under biological conditions to provide PM01183. Examples of prodrugs include derivatives and metabolites of PM01183 that include biohydrolyzable moeities such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Prodrugs can typically be prepared using well-known methods, such as those described by Burger in "Medicinal Chemistry and Drug Discovery" 6th ed. (Donald J. Abraham ed., 2001, Wiley) and "Design and Applications of Prodrugs" (H. Bundgaard ed., 1985, Harwood Academic Publishers).

[0028] In addition, any drug referred to herein may be in amorphous form or crystalline form either as free compound or as solvates (e.g. hydrates) and it is intended that both forms are within the scope of the present invention. Methods of solvation are generally known within the art.

[0029] Moreover, PM01183 for use in accordance with the present invention may be prepared following the synthetic process such as the one disclosed in WO 03/014127.

[0030] Pharmaceutical compositions of PM01183, or of a pharmaceutically acceptable salt thereof, that can be used include solutions, suspensions, emulsions, lyophilised compositions, etc., with suitable excipients for intravenous administration. Preferably, PM01183 may be supplied and stored as a sterile lyophilized product, comprising PM01183 and excipients in a formulation adequate for therapeutic use. For further guidance on pharmaceutical compositions of PM01183, or a pharmaceutically acceptable salt thereof, see for example the formulations described in WO 2006/046079.

[0031] Administration of PM01183, or a pharmaceutically acceptable salt thereof, or pharmaceutical compositions comprising the compound is preferably by intravenous infusion. Infusion times of up to 72 hours can be used, more preferably between 1 and 24 hours, with either about 1 hour or about 3 hours most preferred. Short infusion times which allow treatment to be carried out without an overnight stay in hospital are especially desirable. However, infusion may be around 24 hours or even longer if required.

[0032] Preferably the administration of PM01183 is performed in cycles. In a preferred administration schedule an intravenous infusion of PM01183 is given to the patients the first week of each cycle and the patients are allowed to recover for the remainder of the cycle. The preferred duration of each cycle is of either 3 or 4 weeks. Multiple cycles can be given as needed. Administration of PM01183, or a pharmaceutically acceptable salt thereof, by intravenous infusion during about 1 hour once every 3 weeks is the most preferred administration schedule, although other protocols can be devised as variations.

[0033] The present invention relates to the synergistic combination of PM01183, or a pharmaceutically acceptable salt thereof, with an antimetabolite selected from 5-fluorouracil, gemcitabine, cytarabine, capecitabine, decitabine, floxuridine, aminopterin, methotrexate, pemetrexed and raltitrexed in the treatment of cancer.

[0034] Particularly preferred cancer types are those selected from lung cancer, sarcoma, malignant melanoma, bladder carcinoma, prostate cancer, pancreas carcinoma, thyroid cancer, gastric carcinoma, ovarian cancer, hepatoma (also known as liver cancer), breast cancer, colorectal cancer, kidney cancer, esophageal cancer, neuroblastoma, brain cancer, cervical cancer, anal cancer, testicular cancer, leukemia, multiple myeloma and lymphoma.

[0035] In apreferred embodiment, the invention is directed to the synergistic combination of PM01183, or a pharmaceutically acceptable salt thereof, with an antimetabolite selected from 5-fluorouracil, gemcitabine, cytarabine, capecitabine, decitabine, floxuridine, aminopterin, methotrexate, pemetrexed and raltitrexed in the treatment of cancer, and more particularly in the treatment of a cancer selected from lung cancer, sarcoma, malignant melanoma, bladder carcinoma, prostate cancer, pancreas carcinoma, gastric carcinoma, ovarian cancer, hepatoma, breast cancer, colorectal cancer, kidney cancer, esophageal cancer, brain cancer, anal cancer, leukaemia and lymphoma. Particularly preferred is the synergistic combination of PM01183, or a pharmaceutically acceptable salt thereof, with 5-fluorouracil, gemcitabine, cytarabine and methotrexate in the treatment of cancer, and more particularly in the treatment of a cancer selected from lung cancer, sarcoma, malignant melanoma, prostate cancer, pancreas carcinoma, gastric carcinoma, ovarian cancer, hepatoma, breast cancer, colorectal cancer, kidney cancer, brain cancer, leukemia and lymphoma.

[0036] The invention includes any pharmaceutically acceptable salt of any drug referred to herein, which can be synthesized from the parent compound by conventional chemical methods as disclosed before.

[0037] The invention relates to synergistic combinations employing PM01183, or a pharmaceutically acceptable salt thereof, and another anticancer drug selected from the list of drugs given above. An indication of synergism can be obtained by testing the combinations and analyzing the results, for example by the Chou-Talalay method or by any other suitable method, such as those provided in the Examples section.

[0038] The possible favorable outcomes for synergism include 1) increasing the efficacy of the therapeutic effect, 2) decreasing the dosage but increasing or maintaining the same efficacy to avoid toxicity, 3) minimizing or slowing down the development of drug resistance, and 4) providing selective synergism against target (or efficacy synergism) versus host (or toxicity antagonism). Accordingly, in a combination of two chemotherapeutic agents having synergism, the treatment regimen will be different of those in which the combination of the two drugs shows only an additive effect. In this regard, if there is synergism less dosage of one or both of the agents (compared with the amounts used in single therapy) may be required to obtain the same or even a greater efficacy, and the possible toxic side effects may be reduced or even avoided. Alternatively, if the dosage of both drugs in the combination is the same as those when given alone (as single agents), an increase in efficacy of the combination can be expected. Therefore, the existence of synergism in a given drug combination will modify the length of the treatment and/or the

treatment regimen.

[0039] In another embodiment, the invention relates to PM01183, or a pharmaceutically acceptable salt thereof, for use in increasing or potentiating the therapeutic efficacy of an antimetabolite selected from 5-fluorouracil, gemcitabine, cytarabine, capecitabine, decitabine, floxuridine, aminopterin, methotrexate, pemetrexed and raltitrexed in the treatment of cancer, which comprises administering to a patient in need thereof a therapeutically effective amount of PM01183, or a pharmaceutically acceptable salt thereof, in synergistic combination with this antimetabolite. An indication of increase or potentiation of the therapeutic efficacy can be obtained by testing the combinations and analyzing the results, for example the tumor growth inhibition. This tumor growth inhibition can be assessed by comparing the mean tumor volume of the treatment combining the two drugs (PM01183 and the other drug) with those of the other drug monotherapy treatment. In this regard, increase or potentiation of the therapeutic efficacy is determined when the response of the combination therapy is greater than the best response of the most active drug administered as single agent (monotherapy) on the same schedule and dose as used in the combination therapy. This aspect of the invention is further illustrated in the Examples section, specifically in Examples 13-14.

[0040] Also disclosed herein is the use of PM01183, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for the treatment of cancer by synergistic combination therapy employing PM01183, or a pharmaceutically acceptable salt thereof, with another anticancer drug selected from the list of drugs given above..

[0041] In another aspect, the invention is directed to PM01183, or a pharmaceutically acceptable salt thereof, for use in the treatment of cancer comprising administering a therapeutically effective amount of PM01183, or a pharmaceutical acceptable salt thereof, in synergistic combination with a therapeutically effective amount of an antimetabolite selected from 5-fluorouracil, gemcitabine, cytarabine, capecitabine, decitabine, floxuridine, aminopterin, methotrexate, pemetrexed and raltitrexed.

[0042] According to the present invention, PM01183, or a pharmaceutically acceptable salt thereof, and the other anticancer drug may be provided in the same medicament or as separate medicaments for administration at the same time or at different times. Preferably, PM01183, or a pharmaceutically acceptable salt thereof, and the other anticancer drug are provided as separate medicaments for administration at different times. When administered separately and at different times, either PM01183, or a pharmaceutically acceptable salt thereof, or the other anticancer drug, may be administered first. In addition, both drugs can be administered in the same day or at different days, and they can be administered using the same schedule or at different schedules during the treatment cycle. Additionally, the administration of both drugs can be done by using the same route of administration or different routes. For instance, both drugs can be administered by intravenous administration or, alternatively, one drug can be administered orally and the other one by intravenous administration.

[0043] Thus, the pharmaceutical compositions of the present invention may comprise all the components (drugs) in a single pharmaceutically acceptable formulation or, alternatively, the components may be formulated separately and administered in combination with one another. Various pharmaceutically acceptable formulations well known to those of skill in the art can be used in the present invention. Moreover, selection of an appropriate formulation for use in the present invention can be performed by those skilled in the art by taking into account the route of administration and the solubility characteristics of the components of the composition.

[0044] The correct dosage of both drugs in combination will vary according to the particular formulation, the mode of application, and the particular site, patient and tumour being treated. Other factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the patient, other drug combinations, reaction sensitivities and severity of the disease shall be taken into account. Administration can be carried out continuously or periodically within the maximum tolerated dose.

[0045] The combination of the invention may be used alone or in combination with one or more of a variety of anticancer agents or supportive care agents.

[0046] In addition, depending on the type of tumor and the development stage of the disease, anticancer effects of the treatments of the present invention include inhibition of tumor growth, tumor growth delay, regression of tumor, shrinkage of tumor, increased time to regrowth of tumor on cessation of treatment, slowing of disease progression, and prevention of metastasis. It is expected that when a treatment of the present invention is administered to a patient, such as a human patient, in need of such treatment, said treatment will produce an effect, as measured by, for example, the extent of the anticancer effect, the response rate, the time to disease progression, or the survival rate. In particular, the treatments of the invention are suited for human patients, especially those who are relapsing or refractory to previous chemotherapy. First line therapy is also envisaged.

[0047] In another aspect, the present invention is directed to a kit for use in the treatment of cancer, comprising a supply of

PM01183, or a pharmaceutically acceptable salt thereof, in dosage units for at least one cycle, and printed instructions for the use of PM01183, or a pharmaceutically acceptable salt thereof, with an antimetabolite selected from 5-fluorouracil, gemcitabine, cytarabine, capecitabine, decitabine, floxuridine, aminopterin, methotrexate, pemetrexed and raltitrexed in synergistic combination.

[0048] In a related aspect, the present invention is directed to a kit for use in the treatment of cancer, comprising a supply of PM01183, or a pharmaceutically acceptable salt thereof, in dosage units for at least one cycle, a supply of an antimetabolite selected from 5-fluorouracil, gemcitabine, cytarabine, capecitabine, decitabine, floxuridine, aminopterin, methotrexate, pemetrexed and raltitrexed in dosage units for at least one cycle, and printed instructions for the use of both drugs in synergistic combination.

[0049] Also disclosed herein is a pharmaceutical composition comprising PM01183, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or excipient, for use in synergistic combination with another anticancer drug selected from the list of drugs given above in the treatment of cancer.

[0050] Further disclosed herein is a pharmaceutical composition comprising PM01183, or a pharmaceutically acceptable salt thereof, another anticancer drug selected from the list of drugs given above, and a pharmaceutically acceptable carrier. This pharmaceutical composition is preferable for use in the treatment of cancer.

[0051] Also disclosed herein is the use of PM01183, or a pharmaceutically acceptable salt thereof, in the preparation of a composition for use in synergistic combination with another anticancer drug selected from the list of drugs given above in the treatment of cancer.

[0052] In one embodiment, cancer cells are contacted, or otherwise treated, with a combination of PM01183, or a pharmaceutically acceptable salt thereof, and another anticancer drug selected from the list of drugs given above. The cancer cells are preferably human and include carcinoma cells, sarcoma cells, leukemia cells, lymphoma cells, and myeloma cells. More preferably, the cancer cells are cells of lung cancer, sarcoma, malignant melanoma, bladder carcinoma, prostate cancer, pancreas carcinoma, thyroid cancer, gastric carcinoma, ovarian cancer, hepatoma, breast cancer, colorectal cancer, kidney cancer, esophageal cancer, neuroblastoma, brain cancer, cervical cancer, anal cancer, testicular cancer, leukemia, multiple myeloma and lymphoma. In addition, the combination provides a synergistic inhibitory effect against the cancer cells, particularly against the human cancer cells mentioned above.

[0053] For example, the combination inhibits proliferation or survival of contacted cancer cells. A lower level of proliferation or survival of the contacted cancer cells compared to the non-contacted cancer cells supports the combination of PM01183, or a pharmaceutically acceptable salt thereof, and another anticancer drug selected from the list of drugs given above as being effective for treating a patient with cancer.

[0054] Also disclosed herein is a method for inhibiting the growth of cancer cells comprising contacting said cancer cells with an effective amount of PM01183, or a pharmaceutically acceptable salt thereof, in synergistic combination with another anticancer drug selected from the list of drugs given above.

[0055] Also disclosed herein is a method for inhibiting the growth of cancer cells comprising contacting said cancer cells with a synergistic combination of PM01183, or a pharmaceutically acceptable salt thereof, and another anticancer drug selected from the list of drugs given above, wherein said combination provides improved inhibition against cancer cell growth as compared to (i) PM01183, or a pharmaceutically acceptable salt thereof, in the absence of the other anticancer drug, or (ii) the other anticancer drug in the absence of PM01183.

[0056] Also disclosed herein is a pharmaceutical composition comprising a synergistic combination of PM01183, or a pharmaceutically acceptable salt thereof, and another anticancer drug selected from the list of drugs given above for inhibiting the growth of cancer cells, wherein said combination provides improved inhibition against cancer cell growth as compared to (i) PM01183, or a pharmaceutically acceptable salt thereof, in the absence of the other anticancer drug, or (ii) the other anticancer drug in the absence of PM01183.

[0057] Also disclosed herein is a synergistic combination of PM01183, or a pharmaceutically acceptable salt thereof, and another anticancer drug selected from the list of drugs given above for use in inhibiting tumor growth or reducing the size of a tumor *in vivo*. In particular, the combination inhibits *in vivo* growth and/or reduces the size of carcinoma, sarcoma, leukemia, lymphoma, and myeloma. Preferably, the combination inhibits *in vivo* tumor growth of lung, sarcoma, malignant melanoma, bladder, prostate, pancreas, thyroid, gastric, ovarian, hepatoma, breast, colorectal, kidney, esophageal, neuroblastoma, brain,

cervical, anal, testicular, leukemia, multiple myeloma and lymphoma tumours.

[0058] For example, these combinations inhibit tumor growth or reduce the size of human cancer xenografts, particularly human gastric, pancreas, sarcoma, lung, colorectal and ovary tumors xenografts, in animal models. A reduced growth or reduced size of human cancer xenografts in animal models administered with these combinations further supports the combination of PM01183, or a pharmaceutically acceptable salt thereof, and another anticancer drug selected from the list of drugs given above as being effective for treating a patient with cancer.

[0059] Therefore, also disclosed herein is PM01183, or a pharmaceutically acceptable salt thereof for use in reducing the size of a tumor, comprising administering an effective amount of PM01183, or a pharmaceutically acceptable salt thereof, in synergistic combination with another anticancer drug selected from the list of drugs given above.

[0060] Also disclosed herein is PM01183, or a pharmaceutically acceptable salt thereof for use in inhibiting tumor growth, comprising administering an effective amount of PM01183, or a pharmaceutically acceptable salt thereof, in combination with another anticancer drug selected from the list of drugs given above.

[0061] The following examples further illustrate the invention. These examples should not be interpreted as a limitation of the scope of the invention.

[0062] To provide a more concise description, some of the quantitative expressions given herein are not qualified with the term "about". It is understood that, whether the term "about" is used explicitly or not, every quantity given herein is meant to refer to the actual given value, and it is also meant to refer to the approximation to such given value that would reasonably be inferred based on the ordinary skill in the art, including equivalents and approximations due to the experimental and/or measurement conditions for such given value.

EXAMPLES

EXAMPLE 1. In vitro studies to determine the effect of PM01183 in combination with chemotherapeutic agents on human lung carcinoma cell lines.

[0063] The objective of this study was to determine the ability of PM01183 to potentiate the antitumor activity of chemotherapeutic agents used in the treatment of lung carcinoma.

[0064] The following agents were evaluated in combination with PM01183: 5-fluorouracil (5-FU) and gemcitabine (stock solutions of these compounds prepared in pure DMSO and stored at -20°C). Additional serial dilutions were prepared in serum-free culture medium to achieve a final 4X concentration. Aliquots of 50 µL of each diluted compound were added per well.
A549 was the human lung carcinoma cell line selected for this assay. A549 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine and 100 units/mL of Penicillin-Streptomycin, at 37 °C, 5% CO2 and 95% humidity.
The screening was performed in two parts:
a. In the first set of assays, IC_{50} values were determined for each drug in A549 cells after 72 hours of drug exposure. Briefly, cells were harvested and seeded in 96 well microtiter plates at a density of 5,000 cells in 150 µL of culture medium and incubated for 24 hours in drug-free medium before treatment with vehicle alone or test compounds for 72 h.
The cytotoxic effect was measured by the MTT reduction assay, in which 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole, which is reduced to purple formazan in the mitochondria of living cells, was used. MTT (50µL of 1mg/mL stock solution) was added to the wells and incubated for 8 hours at 37°C until formazan crystals were formed. After gently removing the culture medium, DMSO was added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of the wells was quantified by measuring the optical density at 540 nm. Results were expressed as percentage of control cell growth. The IC50 values (concentration of drug that produces a 50% inhibition of cell growth) used for the combination studies were calculated using Prism v5.02 software (GraphPad). The results were expressed as molar concentration and represented the average of 2-4 independent assays.
The IC_{50} values (72 hours drug exposure) of each individual agent for the A549 tumor cell line are shown in table 1.
Table 1: IC_{50} values in molar concentration (M) for each of the agent

Compound	IC_{50} (M)
PM01183	3.60E-09
Gemcitabine	2.80E-10
5-FU	9.23E-05

b. In a second set of assays, A549 human tumor cells were incubated with PM01183 in combination with each of the agents mentioned above. The previously obtained IC_{50} values were used as starting concentrations for each compound (100% concentration). Arbitrary dilutions, as percentage of the initial IC_{50} value (100%, 75%, 70%, 60%, 50%, 40%, 30%, 25%, and 0%), were performed for each pair of compounds and tested in combined complementary (opposite concentrations) dose-response curves as follows:

IC_{50} of PM01183	IC_{50} of Agent
100%	0%
75%	25%
70%	30%
60%	40%
50%	50%
40%	60%
30%	70%
25%	75%
0%	100%

[0065] As a visual aid, response values were plotted on a scatter plot with dose ratios given on the x-axis and % response values on the y-axis. A horizontal line was drawn between the two endpoint response values (E.g. between the response values for 100% IC_{50} PM01183 and 100% IC_{50} standard chemotherapeutic agent). In cases where response values at the two endpoints were approximately equivalent, points lying above or below this predicted line of additivity could be interpreted as representing antagonistic or synergistic drug interaction, respectively.

[0066] The *in vitro* combinations of each drug with PM01183 have the potential to be synergistic, additive or antagonistic. Synergistic cytotoxicity to tumor cells is an optimal effect and implies that the combination of PM01183 with another drug is more effective than either drug alone.

[0067] According to this assay, it was found that in A549 human lung carcinoma cell line the combination of PM0183 with 5-fluorouracil (Figure 1) and PM01183 with gemcitabine (Figure 2) showed synergism at almost all dose ratios.

EXAMPLE 2. In vitro studies to determine the effect of PM01183 in combination with chemotherapeutic agents on human sarcoma cell lines.

[0068] The objective of this study was to determine the ability of PM01183 to potentiate the antitumor activity of chemotherapeutic agents used in the treatment of sarcoma.

[0069] The following agents were evaluated in combination with PM01183: gemcitabine and cytarabine (stock solutions of these compounds prepared in pure DMSO and stored at -20°C). Additional serial dilutions were prepared in serum-free culture medium to achieve a final 4X concentration. Aliquots of 50 µL of each diluted compound were added per well.
A673 was the human rhabdomyosarcoma cell line selected for this assay. A673 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine and 100 units/mL of Penicillin-Streptomycin, at 37 °C, 5% CO2 and 95% humidity.

[0070] The screening was performed in two parts as disclosed in example 1:
a. In the first set of assays, IC_{50} values were determined for each drug after 72 hours of drug exposure in the A673 tumor cell line.

The IC_{50} values (72 hours drug exposure) of each individual agent for the A673 tumor cell line were calculated by using the same methodology disclosed in example 1 and are shown in table 2.

Table 2: IC_{50} values in molar concentration (M) for each of the agent

Compound	IC_{50} (M)
PM01183	2.20E-09
Cytarabine	1.97E-07
Gemcitabine	4.34E-10

b. In a second set of assays, A673 human tumor cells were incubated with PM01183 in combination with each of the agents mentioned above in the same combination of unique IC_{50} concentrations as those described in example 1. Cell culture and cell plating were performed as described before and the cytotoxic effect was measured by the MTT Assay as disclosed in example 1.

[0071] According to this assay, it was found that in A673 human sarcoma cell line the combination of PM01183 with cytarabine exhibited strong synergism (Figure 3), while the combination of PM01183 with gemcitabine showed synergism (Figure 4) at the 75/25-70/30 dose ratios.

EXAMPLE 3. In vitro studies to determine the effect of PM01183 in combination with chemotherapeutic agents on human malignant melanoma cell lines.

[0072] The objective of this study was to determine the ability of PM01183 to potentiate the antitumor activity of chemotherapeutic agents used in the treatment of malignant melanoma.

[0073] The following agents were evaluated in combination with PM01183: 5-fluorouracil, cytarabine and methotrexate (stock solutions of these compounds prepared in pure DMSO and stored at -20°C). Additional serial dilutions were prepared in serum-free culture medium to achieve a final 4X concentration. Aliquots of 50 µL of each diluted compound were added per well.
SK-MEL-2 was the human melanoma cell line selected for this assay. SK-MEL-2 cells were maintained in Minimum Essential Medium Eagle (MEME) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine and 100 units/mL of Penicillin-Streptomycin, at 37 °C, 5% CO2 and 95% humidity.

[0074] The screening was performed in two parts as disclosed in example 1:
a. In the first set of assays, IC_{50} values were determined for each drug after 72 hours of drug exposure in the SK-MEL-2 tumor cell line.
The IC_{50} values (72 hours drug exposure) of each individual agent for the SK-MEL-2 tumor cell line were calculated by using the same methodology disclosed in example 1 and are shown in table 3.

Table 3: IC_{50} values in molar concentration (M) for each of the agent

Compound	IC_{50} (M)
PM01183	2.00E-09
Cytarabine	3.89E-06
Methotrexate	1.00E-04
5-FU	7.00E-04

b. In a second set of assays, SK-MEL-2 tumor cells were incubated with PM01183 in combination with each of the agents mentioned above in the same combination of unique IC_{50} concentrations as those described in example 1. Cell culture and cell plating were performed as described before and the cytotoxic effect was measured by the MTT Assay as disclosed in example 1.

[0075] According to this assay, it was found that in SK-MEL-2 human melanoma cell line the combination of PM01183 with 5-fluorouracil (Figure 5), PM01183 with cytarabine (Figure 6), and PM01183 with methotrexate (Figure 7) exhibited strong synergism.

EXAMPLE 4. In vitro studies to determine the effect of PM01183 in combination with chemotherapeutic agents on human prostate carcinoma cell lines.

[0076] The objective of this study was to determine the ability of PM01183 to potentiate the antitumor activity of chemotherapeutic agents used in the treatment of prostate cancer.

[0077] The following agents were evaluated in combination with PM01183: 5-fluorouracil, gemcitabine, cytarabine and methotrexate (stock solutions of these compounds prepared in pure DMSO and stored at -20°C). Additional serial dilutions were prepared in serum-free culture medium to achieve a final 4X concentration. Aliquots of 50 µL of each diluted compound were added per well.
PC-3 was the human prostate adenocarcinome cell line selected for this assay. PC-3 cells were maintained in Roswell Park Memorial Institute medium (RPMI) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine and 100 units/mL of Penicillin-Streptomycin, at 37 °C, 5% CO2 and 95% humidity.

[0078] The screening was performed in two parts as disclosed in example 1:
a. In the first set of assays, IC_{50} values were determined for each drug after 72 hours of drug exposure in the PC-3 tumor cell line.
The IC_{50} values (72 hours drug exposure) of each individual agent for the PC-3 tumor cell line were calculated by using the same methodology disclosed in example 1 and are shown in table 4.
Table 4: IC_{50} values in molar concentration (M) for each of the agent

Compound	IC_{50} (M)
PM01183	2.60E-09
5-FU	1.00E-03
Methotrexate	1.20E-04
Cytarabine	4.00E-05
Gemcitabine	4.00E-07

b. In a second set of assays, PC-3 human tumor cells were incubated with PM01183 in combination with each of the agents mentioned above in the same combination of unique IC_{50} concentrations as those described in example 1. Cell culture and cell plating were performed as described before and the cytotoxic effect was measured by the MTT Assay as disclosed in examples 1.

[0079] According to this assay it was found that in PC-3 human prostate cancer cell line the combination of PM01183 with 5-fluorouracil (Figure 8) and PM01183 with cytarabine (Figure 9) exhibited synergism at almost all dose ratios, and the combination of PM01183 with gemcitabine exhibited strong synergism (Figure 10). Finally, the combination of PM01183 with methotrexate showed synergism (Figure 11) at the 30/70-25/75 dose ratios.

EXAMPLE 5. In vitro studies to determine the effect of PM01183 in combination with chemotherapeutic agents on human pancreas carcinoma cell lines.

[0080] The objective of this study was to determine the ability of PM01183 to potentiate the antitumor activity of chemotherapeutic agents used in the treatment of pancreatic carcinoma.

[0081] The following agents were evaluated in combination with PM01183: gemcitabine, cytarabine and methotrexate (stock solutions of these compounds prepared in pure DMSO and stored at -20°C). Additional serial dilutions were prepared in serum-free culture medium to achieve a final 4X concentration. Aliquots of 50 µL of each diluted compound were added per well.
PANC-1 was the human pancreatic carcinoma cell line selected for this assay. PANC-1 cells were maintained in Roswell Park Memorial Institute medium (RPMI) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine and 100 units/mL of Penicillin-Streptomycin, at 37 °C, 5% CO2 and 95% humidity.

[0082] The screening was performed in two parts as disclosed in example 1:
a. In the first set of assays, IC_{50} values were determined for each drug after 72 hours of drug exposure in the PANC-1 tumor cell line.
The IC_{50} values (72 hours drug exposure) of each individual agent for the PANC-1 tumor cell line were calculated by using the same methodology disclosed in example 1 and are shown in table 5.
Table 5: IC_{50} values in molar concentration (M) for each of the agent

Compound	IC_{50} (M)
PM01183	2.80E-09
Cytarabine	9.00E-05
Gemcitabine	1.00E-06
Methotrexate	1.00E-05

b. In a second set of assays, PANC-1 human tumor cells were incubated with PM01183 in combination with each of the agents mentioned above in the same combination of unique IC_{50} concentrations as those described in example 1. Cell culture and cell plating were performed as described before and the cytotoxic effect was measured by the MTT Assay as disclosed example 1.

[0083] According to this assay it was found that in PANC-1 human pancreas carcinoma cell line the combination of PM01183 with cytarabine (Figure 12) showed synergism at almost all dose ratios, while the combination of PM01183 with gemcitabine (Figure 13) and PM01183 with methotrexate (Figure 14) exhibited strong synergism.

EXAMPLE 6. In vitro studies to determine the effect of PM01183 in combination with chemotherapeutic agents on human gastric carcinoma cell lines.

[0084] The objective of this study was to determine the ability of PM01183 to potentiate the antitumor activity of chemotherapeutic agents used in the treatment of gastric cancer.

[0085] The following agents were evaluated in combination with PM01183: 5-fluorouracil, gemcitabine, cytarabine and methotrexate (stock solutions of these compounds prepared in pure DMSO and stored at -20°C). Additional serial dilutions were prepared in serum-free culture medium to achieve a final 4X concentration. Aliquots of 50 µL of each diluted compound were added per well.
HGC-27 was the human gastric carcinoma cell line selected for this assay. HGC-27 cells were maintained in Iscove's modified Dulbeco's medium (IDMD) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine and 100 units/mL of Penicillin-Streptomycin, at 37 °C, 5% CO2 and 95% humidity.

[0086] The screening was performed in two parts as disclosed in example 1:
a. In the first set of assays, IC_{50} values were determined for each drug after 72 hours of drug exposure in the HGC-27 tumor cell line.
The IC_{50} values (72 hours drug exposure) of each individual agent for the HGC-27 tumor cell line were calculated by using the same methodology disclosed in example 1 and are shown in table 6.
Table 6: IC_{50} values in molar concentration (M) for each of the agent

Compound	IC_{50} (M)
PM01183	8.50E-10
5-FU	1.00E-05
Methotrexate	3.30E-08
Cytarabine	5.00E-05
Gemcitabine	5.34E-10

b. In a second set of assays, HGC-27 human tumor cells were incubated with PM01183 in combination with each of the agents mentioned above in the same combination of unique IC_{50} concentrations as those described in example 1. Cell culture and cell plating were performed, as described before and the cytotoxic effect was measured by the MTT Assay, as disclosed in example 1.

[0087] According to this assay it was found that in HGC-27 human gastric carcinoma cell line the combination of PM01183 with 5-fluorouracil (Figure 15) and PM01183 with cytarabine (Figure 16) exhibited synergism, even being strong in some dose ratios. The combination of PM01183 with gemcitabine (Figure 17) and PM01183 with methotrexate (Figure 18) showed synergism at almost all dose ratios.

EXAMPLE 7. In vitro studies to determine the effect of PM01183 in combination with chemotherapeutic agents on human ovarian carcinoma cell lines.

[0088] The objective of this study was to determine the ability of PM01183 to potentiate the antitumor activity of chemotherapeutic agents used in the treatment of ovarian cancer.

[0089] The following agents were evaluated in combination with PM01183: 5-fluorouracil, gemcitabine, cytarabine and methotrexate (stock solutions of these compounds prepared in pure DMSO and stored at -20°C). Additional serial dilutions were prepared in serum-free culture medium to achieve a final 4X concentration. Aliquots of 50 µL of each diluted compound were added per well.
IGROV-1 was the human ovarian adenocarcinoma cell line selected for this assay. IGROV-1 cells were maintained in Roswell Park Memorial Institute medium (RPMI) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine and 100 units/mL of Penicillin-Streptomycin, at 37 °C, 5% CO2 and 95% humidity.

[0090] The screening was performed in two parts as disclosed in example 1:
a. In the first set of assays, IC_{50} values were determined for each drug after 72 hours of drug exposure in the IGROV-1 tumor cell line.
The IC_{50} values (72 hours drug exposure) of each individual agent for the IGROV-1 tumor cell line were calculated by using the same methodology disclosed in example 1 and are shown in table 7.
Table 7: IC_{50} values in molar concentration (M) for each of the agent

Compound	IC_{50} (M)
PM01183	3.20E-09
5-FU	9.00E-05
Methotrexate	1.00E-04
Cytarabine	1.17E-05
Gemcitabine	6.34E-09

b. In a second set of assays, IGROV-1 human tumor cells were incubated with PM01183 in combination with each of the agents mentioned above in the same combination of unique IC_{50} concentrations as those described in example 1. Cell culture and cell plating were performed as described before and the cytotoxic effect was measured by the MTT Assay as disclosed in example 1.

[0091] According to this assay it was found that in IGROV-1 human ovarian carcinoma cell line the combination of PM01183 with 5-fluorouracil (Figure 19) and PM01183 with cytarabine (Figure 20) showed synergism at almost all dose ratios. The combination of PM01183 with gemcitabine (Figure 21) and PM01183 with methotrexate (Figure 22) exhibited synergism.

EXAMPLE 8. In vitro studies to determine the effect of PM01183 in combination with chemotherapeutic agents on human hepatocellular carcinoma cell lines.

[0092] The objective of this study was to determine the ability of PM01183 to potentiate the antitumor activity of chemotherapeutic agents used in the treatment of hepatocellular cancer.

[0093] The following agents were evaluated in combination with PM01183: 5-fluorouracil, gemcitabine, cytarabine and methotrexate (stock solutions of these compounds prepared in pure DMSO and stored at -20°C). Additional serial dilutions were prepared in serum-free culture medium to achieve a final 4X concentration. Aliquots of 50 µL of each diluted compound were added per well.
HepG2 was the human hepatocellular liver carcinoma cell line selected for this assay. HepG2 cells were maintained in Minimum Essential Medium Eagle (MEME) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine and 100 units/mL of Penicillin-Streptomycin, at 37 °C, 5% CO2 and 95% humidity.

[0094] The screening was performed in two parts as disclosed in example 1:
a. In the first set of assays, IC_{50} values were determined for each drug after 72 hours of drug exposure in the HepG2 tumor cell line.
The IC_{50} values (72 hours drug exposure) of each individual agent for the HepG2 tumor cell line were calculated by using the same methodology disclosed in example 1 and are shown in table 8.

Table 8: IC_{50} values in molar concentration (M) for each of the agent

Compound	IC_{50} (M)
PM01183	2.50E-09
5-FU	4.50E-06
Methotrexate	3.96E-08
Cytarabine	2.06E-05
Gemcitabine	5.34E-09

b. In a second set of assays, HepG2 human tumor cells were incubated with PM01183 in combination with each of the agents mentioned above in the same combination of unique IC_{50} concentrations as those described in example 1. Cell culture and cell plating were performed as described before and the cytotoxic effect was measured by the MTT Assay as disclosed in example 1.

[0095] According to this assay it was found that in HepG2 human hepatocellular cell line the combination of PM01183 with 5-fluorouracil (Figure 23) showed synergism at the 75/25, 50/50 and 30/70 dose ratios. The combination of PM01183 with cytarabine (Figure 24), PM01183 with gemcitabine (Figure 25) and PM01183 with methotrexate (Figure 26) exhibited strong synergism.

EXAMPLE 9. In vitro studies to determine the effect of PM01183 in combination with chemotherapeutic agents on human breast carcinoma cell lines.

[0096] The objective of this study was to determine the ability of PM01183 to potentiate the antitumor activity of chemotherapeutic agents used in the treatment of breast cancer.

[0097] The following agents were evaluated in combination with PM01183: 5-fluorouracil, gemcitabine, cytarabine and methotrexate (stock solutions of these compounds prepared in pure DMSO and stored at -20°C). Additional serial dilutions were prepared in serum-free culture medium to achieve a final 4X concentration. Aliquots of 50 µL of each diluted compound were added per well.
MDA-MB-231 was the human breast adenocarcinoma cell line selected for this assay. MDA-MB-231 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine and 100 units/mL of Penicillin-Streptomycin, at 37 °C, 5% CO2 and 95% humidity.

[0098] The screening was performed in two parts as disclosed in example 1:
a. In the first set of assays, IC_{50} values were determined for each drug after 72 hours of drug exposure in the MDA-MB-231 tumor cell line.
The IC_{50} values (72 hours drug exposure) of each individual agent for the MDA-MB-231 tumor cell line were calculated by using the same methodology disclosed in example 1 and are shown in table 9.
Table 9: IC_{50} values in molar concentration (M) for each of the agent

Compound	IC_{50} (M)
PM01183	3.50E-09
5-FU	9.00E-05
Methotrexate	5.94E-06
Cytarabine	9.57E-06
Gemcitabine	8.50E-09

b. In a second set of assays, MDA-MB-231 human tumor cells were incubated with PM01183 in combination with each of the agents mentioned above in the same combination of unique IC_{50} concentrations as those described in example 1. Cell culture and cell plating were performed as described before and the cytotoxic effect was measured by the MTT Assay as disclosed in example 1.

[0099] According to this assay it was found that in MDA-MB-231 human breast carcinoma cell line the combination of PM01183 with 5-fluorouracil (Figure 27) showed synergism at almost all dose ratios. The combination of PM01183 with cytarabine (Figure

28) and PM01183 with gemcitabine (Figure 29) exhibited strong synergism, while the combination of PM01183 with methotrexate (Figure 30) showed synergism at the 75/25-70/30 and 50/50 dose ratios.

EXAMPLE 10. In vitro studies to determine the effect of PM01183 in combination with chemotherapeutic agents on human colorectal carcinoma cell lines.

[0100] The objective of this study was to determine the ability of PM01183 to potentiate the antitumor activity of chemotherapeutic agents used in the treatment of colorectal cancer.

[0101] The following agents were evaluated in combination with PM01183: 5-fluorouracil, gemcitabine and cytarabine (stock solutions of these compounds prepared in pure DMSO and stored at -20°C). Additional serial dilutions were prepared in serum-free culture medium to achieve a final 4X concentration. Aliquots of 50 µL of each diluted compound were added per well.
HT-29 was the human colon adenocarcinoma cell line selected for this assay. HT-29 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine and 100 units/mL of Penicillin-Streptomycin, at 37 °C, 5% CO2 and 95% humidity.

[0102] The screening was performed in two parts as disclosed in example 1:
a. In the first set of assays, IC_{50} values were determined for each drug after 72 hours of drug exposure in the HT-29 tumor cell line.
The IC_{50} values (72 hours drug exposure) of each individual agent for the HT-29 tumor cell line were calculated by using the same methodology disclosed in example 1 and are shown in table 10.
Table 10: IC_{50} values in molar concentration (M) for each of the agent

Compound	IC_{50} (M)
PM01183	3.70E-09
5-FU	9.00E-06
Cytarabine	7.80E-06
Gemcitabine	4.00E-07

b. In a second set of assays, HT-29 human tumor cells were incubated with PM01183 in combination with each of the agents mentioned above in the same combination of unique IC_{50} concentrations as those described in example 1. Cell culture and cell plating were performed as described before and the cytotoxic effect was measured by the MTT Assay as disclosed in example 1.

[0103] According to this assay it was found that in HT-29 human colorectal carcinoma cell line the combination of PM01183 with 5-fluorouracil (Figure 31) and PM01183 with gemcitabine (Figure 33) showed synergism at almost all dose ratios, and the combination of PM01183 with cytarabine (Figure 32) exhibited strong synergism.

EXAMPLE 11. In vitro studies to determine the effect of PM01183 in combination with chemotherapeutic agents on human kidney carcinoma cell lines.

[0104] The objective of this study was to determine the ability of PM01183 to potentiate the antitumor activity of chemotherapeutic agents used in the treatment of kidney cancer.

[0105] The following agents were evaluated in combination with PM01183: 5-fluorouracil, gemcitabine, methotrexate and cytarabine (stock solutions of these compounds prepared in pure DMSO and stored at -20°C). Additional serial dilutions were prepared in serum-free culture medium to achieve a final 4X concentration. Aliquots of 50 µL of each diluted compound were added per well.
RXF-393 was the human kidney carcinoma cell line selected for this assay. RXF-393 cells were maintained in Roswell Park Memorial Institute medium (RPMI) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine and 100 units/mL of Penicillin-Streptomycin, at 37 °C, 5% CO2 and 95% humidity.

[0106] The screening was performed in two parts as disclosed in example 1:
a. In the first set of assays, IC_{50} values were determined for each drug after 72 hours of drug exposure in the RXF-393 tumor cell line.

The IC_{50} values (72 hours drug exposure) of each individual agent for the RXF-393 tumor cell line were calculated by using the same methodology disclosed in example 1 and are shown in table 11.

Table 11: IC_{50} values in molar concentration (M) for each of the agent

Compound	IC_{50} (M)
PM01183	5.00E-09
Cytarabine	5.00E-05
Gemcitabine	5.00E-07
5-FU	3.00E-04
Methotrexate	1.75E-04

b. In a second set of assays, RXF-393 human tumor cells were incubated with PM01183 in combination with each of the agents mentioned above in the same combination of unique IC_{50} concentrations as those described in example 1. Cell culture and cell plating were performed as described before and the cytotoxic effect was measured by the MTT Assay as disclosed in example 1.

[0107] According to this assay it was found that in RXF-393 human kidney carcinoma cell line the combination of PM01183 with 5-fluorouracil (Figure 34), PM01183 with cytarabine (Figure 35), PM01183 with gemcitabine (Figure 36), and PM01183 with methotrexate (Figure 37) showed synergism at almost all dose ratios.

EXAMPLE 12. In vitro studies to determine the effect of PM01183 in combination with chemotherapeutic agents on human glioblastoma cell lines.

[0108] The objective of this study was to determine the ability of PM01183 to potentiate the antitumor activity of chemotherapeutic agents used in the treatment of glioblastoma.

[0109] The following agents were evaluated in combination with PM01183: 5-fluorouracil, gemcitabine and methotrexate (stock solutions of these compounds prepared in pure DMSO and stored at -20°C). Additional serial dilutions were prepared in serum-free culture medium to achieve a final 4X concentration. Aliquots of 50 µL of each diluted compound were added per well.
U87-MG was the human glioblastoma cell line selected for this assay. U87-MG cells were maintained in Minimum Essential Medium Eagle (MEME) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine and 100 units/mL of Penicillin-Streptomycin, at 37 °C, 5% CO2 and 95% humidity.

[0110] The screening was performed in two parts as disclosed in example 1:
a. In the first set of assays, IC_{50} values were determined for each drug after 72 hours of drug exposure in the U87-MG tumor cell line.
The IC_{50} values (72 hours drug exposure) of each individual agent for the U87-MG tumor cell line were calculated by using the same methodology disclosed in example 1 and are shown in table 12.

Table 12: IC_{50} values in molar concentration (M) for each of the agent

Compound	IC_{50} (M)
PM01183	4.50E-09
5-FU	1.00E-03
Gemcitabine	4.50E-07
Methotrexate	5.00E-05

b. In a second set of assays, U87-MG human tumor cells were incubated with PM01183 in combination with each of the agents mentioned above in the same combination of unique IC_{50} concentrations as those described in example 1. Cell culture and cell plating were performed as described before and the cytotoxic effect was also measured by the MTT Assay as disclosed in example 1.

[0111] According to this assay it was found that in U87-MG human glioblastoma cell line the combination of PM01183 with 5-fluorouracil (Figure 38) and PM01183 with methotrexate (Figure 40) exhibited synergism. The combination of PM01183 with gemcitabine (Figure 39) showed synergism at almost all dose ratios.

EXAMPLE 13. In vivo studies to determine the effect of PM01183 in combination with 5-fluorouracil in human gastric tumor xenografts.

[0112] The aim of these studies was to evaluate the ability of PM01183 to potentiate the antitumor activity of 5-fluorouracil by using a xenograft model of human gastric carcinoma.

[0113] Female athymic nude mice (Harlan Laboratories Models, S.L. (Barcelona, Spain) were utilized for all experiments. Animals were housed in individually ventilated cages, up to ten per cage in a 12-hour light-dark cycle at 21-23 °C and 40-60 % humidity. The mice were allowed free access to irradiated standard rodent diet and sterilized water. Animals were acclimated for at least 5 days prior to tumor implantation with a tumor cell suspension.

[0114] The tumor model used in these studies was HGC-27 cell line, which was obtained from the European Collection of Cell Cultures (ECACC n° 94042256).

[0115] HGC-27 cells were grown at 37 °C with 5 % CO_2 in Iscove's modified Dulbeco's medium (IDMD). Each animal was subcutaneously implanted on the right flank, using 26G needle and a 1 cc syringe, with $5x10^6$ HGC-27 cells (from *in vitro* passage 4 in PM01183 and cisplatin study, and passage 6 in PM01183 and 5-fluorouracil study), in 0.05 mL suspension of 50% Matrigel and 50% serum free medium, without antibiotics.

[0116] Tumor measurements were determined by using digital caliper (Fowler Sylvac, S235PAT). The formula to calculate volume for a prolate ellipsoid was used to estimate tumor volume (mm^3) from 2-dimensional tumor measurements: Tumor volume (mm^3) = [L x W^2] ÷ 2, where L is the length and it is the longest diameter in mm, and W is the width and it is the shortest diameter in mm of a tumor. Assuming unit density, volume was converted to weight (i.e., 1 mm^3 = 1 mg). Tumor volume and animal body weights were measured 2-3 times per week starting form the first day of treatment (Day 0).

[0117] Treatment tolerability was assesed by monitoring body weight evolution, clinical signs as well as evidences of local damage in the injection site.

[0118] When tumors reached a volume of about 165.5 mm^3 in the study of PM01183 with cisplatin and a volume of about 170 mm^3 in the study of PM01183 with 5-fluorouracil, mice were randomly allocated into the treatments and control groups (N = 5-7/group) based on body weight and tumor volumen measurements by using NewLab Oncology Software (version 2.25.06.00).

[0119] PM01183 was provided in the form of vials of lyophilized PM01183 cake which was reconstituted with water for infusion to a concentration of 0.2 mg/mL. The PM01183 stock solution was further diluted in 5% glucose solution for injection to the dosing formulation concentrations. 5-fluorouracil was provided as a solution prepared by diluting the product with 0.9 % saline solution for injection to the target final concentration.

[0120] In these experiments, PM01183 and 5-fluorouracil treatment, as well as placebo, were intravenously administered once per week up to 2 consecutive weeks on Days 0 and 7. Dose level groups were administered either as single agents or in combination.

[0121] Comparison of the median tumor volume in the treatment groups (T) to the median tumor volume in the control group (T/C x 100%) was used for evaluation of the antitumor efficacy.

[0122] In addition, potentiation was determined when the response of the combination group was greater than the best response of the most active agent administered as single agent (monotherapy) on the same schedule and dose as those used in the combination therapy.

[0123] Finally, the combination index (CI), that quantitatively measures the degree of drug interactions, was obtained from the fractions affected by the treatment, Fa (defined as 1 -T/C) for each experimental group at the last measurement day using the median-effect principle (Chou T.C. Pharmacol. Rev. 2006, 58, 621-681).

[0124] Table 13 reports the % T/C values obtained with PM01183 and 5-fluorouracil both administered as single agents and in combination for each dose level, and Figure 41 shows the tumor volume evaluation of HGC-27 tumors in mice treated with

placebo, PM01183, 5-fluorouracil, and the corresponding combinations combinations for the groups dosed at the two highest ratios.

Table 13

			% T/C on day						
Group	**Dose**	**Test materials**	**0**	**2**	**5**	**7**	**9**	**12**	**14**
G01 (Control group)	10 ml/kg	Placebo	-	-	-	-	-	-	-
G02	0.18 mg/kg	PM01183	99.6	78.6	50.9	43.3	41.0	33.0	29.2
G03	0.135 mg/kg	PM01183	100.2	81.5	58.7	61.4	60.2	54.6	55.1
G04	0.09 mg/kg	PM01183	100.6	90.5	87.6	83.4	82.6	76.7	67.7
G05	0.045 mg/kg	PM01183	99.9	84.3	103.2	104.6	103.5	101.6	85.0
G06	50.0 mg/kg	5-Fluorouracil	100.3	81.2	82.3	81.1	75.6	69.6	60.7
G07	37.5 mg/kg	5-Fluorouracil	99.4	86.9	86.9	78.6	73.2	76.7	83.1
G08	25.0 mg/kg	5-Fluorouracil	100.6	89.8	97.0	111.4	102.6	93.9	82.8
G09	12.5 mg/kg	5-Fluorouracil	100.7	81.7	101.3	102.8	98.6	90.5	83.8
G10	0.18 mg/kg 50.0 mg/kg	PM01183 5-Fluorouracil	99.6	73.0	44.2	35.9	31.5	25.3	22.0
G11	0.135 mg/kg 37.5 mg/kg	PM01183 5-Fluorouracil	100.8	73.4	63.5	53.1	50.6	42.8	51.1
G12	0.09 mg/kg 25.0 mg/kg	PM01183 5-Fluorouracil	99.6	95.8	97.7	98.9	90.0	74.7	69.9
G13	0.045 mg/kg 12.5 mg/kg	PM01183 5-Fluorouracil	99.5	80.6	87.3	88.5	99.3	87.1	84.2
Placebo: lyophilised cake containing 100 mg Sucrose + Potassium dihydrogen phosphate 6.8 mg + Phosphoric acid q.s. pH 3.8-4.5, which was reconstituted with 1 mL of water for infusion..									

[0125] According to these assays it was found that the combination treatment of PM01183 and 5-fluorouracil was effective in the inhibition of the growth of the HGC-27 gastric cells, resulting in a statistically significant ($P<0.01$) tumor reduction compared to the control group with T/C values of 22.0% and 51.1 % (Day 14) in the two highly-dosed groups. Moreover, the combination of PM01183 and 5-fluorouracil produced lower T/C values than the more active single agent in this experiment (PM01183 at a dose of 0.18 mg/kg). Specifically, the TC (%) values of the combination (50 mg/kg 5-fluorouracil + 0.18 mg/kg PM01183) vs PM01183 alone (0.18 mg/kg PM01183) were 35.9 vs 43.3 (day 7), 31.5 vs 41.0 (day 9), 25.3 vs 33.0 (day 12), and 22.0 vs 29.2 (day 14). Therefore, when PM01183 is combined with 5-fluorouracil a potentiation of the antitumor activity is clearly observed.
Additionally, based on the median-effect principle, the combination of PM01183 and 5-fluorouracil resulted in CI values of 0.78 (at Fa equal to 0.97), indicating moderate synergism in mice bearing gastric HGC-27 xenografted tumors.

EXAMPLE 14. In vivo studies to determine the effect of PM01183 in combination with gemcitabine in human pancreatic tumor xenografts.

[0126] The aim of these studies was to evaluate the ability of PM01183 to potentiate the antitumor activity of gemcitabine by using a xenograft model of human pancreatic cancer.

[0127] Female athymic nude mice (Harlan Laboratories Models, S.L. (Barcelona, Spain) were utilized for all experiments. Animals were housed in individually ventilated cages, up to ten per cage in a 12-hour light-dark cycle at 21-23 °C and 40-60 % humidity. The mice were allowed free access to irradiated standard rodent diet and sterilized water. Animals were acclimated for at least 5 days prior to tumor implantation with a tumor cell suspension.

[0128] The tumor model used in these studies was SW1990 cell line, which was obtained from the American Type Culture Collection (ATCC: CRL-2172™).

[0129] SW1990 cells were grown at 37 °C with 5 % CO_2 in RPMI-1640 medium. Each animal was subcutaneously implanted on the right flank, using 26G needle and a 1 cc syringe, with $5x10^6$ SW1990 cells, from *in vitro* passage 12, in 0.05 mL suspension of 50% Matrigel and 50% serum free medium, without antibiotics.

[0130] Tumor measurements and treatment tolerability were performed and determined as disclosed in Example 13.

[0131] When tumors reached a volume of about 210 mm^3 mice were randomly allocated into the treatments and control groups (*N* = 5-7/group) based on body weight and tumor volumen measurements by using NewLab Oncology Software (version 2.25.06.00).

[0132] PM01183 was provided in the form of vials of lyophilized PM01183 cake which was reconstituted with water for infusion to a concentration of 0.2 mg/mL. The PM01183 stock solution was further diluted in 5% glucose solution for injection to the dosing formulation concentrations. Gemcitabine was provided as a solution prepared by reconstituting the product with 0.9 % saline solution for injection to a concentration of 40 mg/ml stock solution. The gemcitabine stock solution was further diluted with 0.9 % saline solution for injection to the target final concentration.

[0133] In these experiments, PM01183 and gemcitabine treatment, as well as placebo, were intravenously administered once per week up to 3 consecutive weeks on Days 0, 7 and 14. Dose level groups were administered either as single agents or in combination.

[0134] Comparison of the median tumor volume in the treatment groups (T) to the median tumor volume in the control group (T/C x 100%) was used for evaluation of the antitumor efficacy. In addition, potentiation and combination index were determined as disclosed in Example 13.

[0135] Table 14 reports the % T/C values obtained with PM01183 and gemcitabine both administered as single agents and in combination for each dose level, and Figure 42 shows the tumor volume evaluation of SW1990 tumors in mice treated with placebo, PM01183, gemcitabine, and the corresponding combinations for the groups dosed at the two highest ratios.

Table 14

			% T/C on day					
Group	**Dose**	**Test materials**	**0**	**3**	**6**	**8**	**10**	**13**
G01 (Control group)	10 ml/kg	Placebo	-	-	-	-	-	-
G02	0.18 mg/kg	PM01183	100.0	74.3	61.3	59.4	56.7	56.1
G03	0.135 mg/kg	PM01183	99.6	81.3	71.0	73.1	65.6	63.1
G04	0.09 mg/kg	PM01183	101.1	81.5	72.8	68.7	68.4	74.4
G05	0.045 mg/kg	PM01183	100.2	83.6	82.8	93.3	82.9	88.1
G06	180.0 mg/kg	Gemcitabine	102.2	84.1	73.9	66.1	60.9	59.4
G07	135.0mg/kg	Gemcitabine	102.3	78.3	71.9	63.7	55.4	52.7
G08	90.0 mg/kg	Gemcitabine	103.8	70.0	73.8	63.3	55.6	54.8
G09	45.0 mg/kg	Gemcitabine	102.3	85.5	70.3	70.5	63.3	64.8
G10	0.18 mg/kg 180.0 mg/kg	PM01183 Gemcitabine	102.1	69.7	51.2	46.2	36.0	34.1
G11	0.135 mg/kg 135.0 mg/kg	PM01183 Gemcitabine	100.4	64.6	52.8	51.5	48.9	46.0
G12	0.09 mg/kg 90.0 mg/kg	PM01183 Gemcitabine	98.2	83.2	64.4	59.7	50.6	49.6
G13	0.045 mg/kg 45.0 mg/kg	PM01183 Gemcitabine	97.7	81.6	70.9	68.8	65.9	65.7

			% T/C on day					
Group	**Dose**	**Test materials**	**15**	**17**	**20**	**22**	**24**	**28**
G01 (Control group)	10 ml/kg	Placebo	-	-	-	-	-	-
G02	0.18 mg/kg	PM01183	53.2	47.8	44.2	45.3	44.8	38.9
G03	0.135 mg/kg	PM01183	56.3	56.7	56.9	56.5	53.0	51.7
G04	0.09 mg/kg	PM01183	74.7	80.7	71.9	75.4	77.3	63.9
G05	0.045 mg/kg	PM01183	92.6	86.5	85.1	84.5	85.8	85.4
G06	180.0 mg/kg	Gemcitabine	58.5	52.1	49.1	48.6	46.9	39.3
G07	135.0mg/kg	Gemcitabine	54.8	51.2	49.5	48.7	49.8	49.5
G08	90.0 mg/kg	Gemcitabine	49.9	47.4	47.6	47.0	45.9	49.2
G09	45.0 mg/kg	Gemcitabine	63.1	58.5	58.7	57.3	65.2	59.3
G10	0.18 mg/kg 180.0 mg/kg	PM01183 Gemcitabine	34.7	31.6	31.7	28.0	26.0	22.7
G11	0.135 mg/kg 135.0 mg/kg	PM01183 Gemcitabine	42.4	38.2	36.6	34.6	31.5	25.8
G12	0.09 mg/kg 90.0 mg/kg	PM01183 Gemcitabine	47.4	46.0	43.8	49.1	46.0	42.9
G13	0.045 mg/kg 45.0 mg/kg	PM01183 Gemcitabine	57.9	59.9	55.9	54.9	52.1	50.5

Placebo: as disclosed in table 13.

[0136] According to this assay it was found that:

1. a. The combination treatment of PM01183 and gemcitabine was effective in the inhibition of the growth of the SW 1990 pancreatic cells, resulting in a statistically significant (P<0.01) tumor reduction compared to the control group with T/C values of 22.7% and 25.8% (Day 28) in the two highly-dosed groups. Moreover, the combination of PM01183 and gemcitabine produced lower T/C values than the more active single agent in this experiment (PM01183 at a dose of 0.18 mg/kg). Specifically, the TC (%) values of the combination (180 mg/kg gemcitabine + 0.18 mg/kg PM01183) vs PM01183 alone (0.18 mg/kg PM01183) were 31.7 vs 44.2 (day 20), 28.0 vs 45.3 (day 22), 26.0 vs 44.8 (day 24), and 22.7 vs 38.9 (day 28).Therefore, when PM01183 is combined with gemcitabine a potentiation of the antitumor activity is clearly observed.

Additionally, based on the median-effect principle, the combination of PM01183 and gemcitabine resulted in CI values less than 1 (at Fa higher than 0.8), indicating synergism in mice bearing pancreatic SW 1990 xenografted tumors.

EXAMPLE 15. In vitro studies to determine the effect of PM01183 in combination with methotrexate on human leukemia cell lines.

[0137] The following agent was evaluated in combination with PM01183: methotrexate (stock solution of this compound prepared in pure DMSO and stored at -20°C). Additional serial dilutions were prepared in serum-free culture medium to achieve a final 4X concentration. Aliquots of 50 µL of the diluted compound were added per well.
JURKAT and MOLT-4 were the human leukemia cell lines selected for this assay, which were obtained from the American Type Culture Collection (ATCC). JURKAT and MOLT-4 cells were grown in phenol red-free RPMI medium supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine and 100 units/mL of Penicillin-Streptomycin, at 37 °C, 5% CO2 and 95% humidity.
The screening was performed in two parts:
a. In the first set of assays, the relative potency of each compound against the different cell lines was determined using a 72 hours exposure *in vitro* cytotoxicity assay.
Briefky, cells were seeded in 96 well microtiter plates at a density of 50000 cells per well in 150 µL of culture medium and incubated for 4-6 hours in drug-free medium before treatment with vehicle alone or test compounds for 72 hours.
After incubation, the cytotoxic effect was evaluated using a MTT reduction assay. 50 µL of MTT solution (1 mg/mL) were added to the wells and incubated for 15-17 hours at 37°C until formazan crystals were formed. After gently removing the culture medium, DMSO was added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of the wells was quantified by measuring the optical density at 540 nm. Results were expressed as percentage of control cell growth. The EC50 values (half-maximal effective concentration) used for the combination studies were calculated using Prism v5.02 software (GraphPad). EC50 was expressed as molar concentration and represented the mean of at least three independent assays.
The individual EC50 values obtained for each drug are shown in tables 15 and 16.
Table 15: EC50 values in molar concentration (M) for each of the agents for the JURKAT tumor cell line.

Compound	EC50 (M)
Methotrexate	1.45E-07
PM01183	1.55E-09

Table 16: EC50 values in molar concentration (M) for each of the agents for the MoLT-4 tumor cell line.

Compound	EC50 (M)
Methotrexate	4.39E-08
PM01183	8.57E-10

b. In a second set of experiments, concentration-response curves for the agents tested, both alone and in two-drug combination, were performed, using the same methodology described in the previous paragraph.

[0138] Given the significant differences between the respective EC50 values for PM01183 and the other standard drugs in this satudy, different ratios of fixed concentrations for the two drugs were used. Normally, the selection of the fixed ratios of concentrations were the equipotent ratio (1:1) at the EC50 value for each drug, and some other ratios representing different percentages of the corresponding EC50 values for each drug above or below it. Using these starting concentrations, constant serial dilutions were performed to generate the concentration-response curves for each set of drugs, alone and in combination.

[0139] The effect of the two-drug combination, as compared with the effect of each drug alone, on the viability of tumor cells, was evaluated using the Chou and Talalay method which is based on the median-effect principle (Chou and Talalay, Adv. Enzyme Regul. 1984, 22, 27-55). The median-effect equation: $f_a / f_u = (C / C_m)^m$ (where C is the drug concentration, C_m the median-effect concentration (i.e., IC50, ED50, or LD50, that inhibits the system under study by 50%), f_a the cell fraction affected by the drug concentration C, f_u the unaffected fraction, and m the sigmoidicity coefficient of the concentration-response curve), describes the relationship between the concentration and the effect of a drug on a given biological system.

[0140] Based on this equation, the term "combination index" (CI) is used as a quantitative measure of the degree of drug interactions. The combination index (CI) is determined by the equation:

$CI = (C)_1/(C_x)_1 + (C)_2/(C_x)_2$

where $(C_x)_1$ is the concentration of drug 1 alone that inhibits an x percentage of a system, $(C_x)_2$ the concentration of drug 2 alone that inhibits the same x percentage of the system, and $(C_1) + (C)_2$ the concentrations of drug 1 and drug 2 that in combination also inhibitis an X percentage of the system. CI values were calculated by solving the equation for different values of f_a (i.e., for different degrees of cell groth inhibition). CI values of <1 indicate synergy, the value of 1 indicates additive effects, and values >1 indicate antagonism.

Data were analyzed using CalcuSyn software (Biosoft, Cambridge, UK). For statistical analysis and graphs Prism software (GraphPad, San Diego, USA) was used. All the results represent the mean of at least three independent experiments.

[0141] The effect of the tested drug combinations on cell proliferation is shown in Figures 43-44:

- Combination of PM01183 with methotrexate. The combination of PM01183 with methotrexate in JURKAT (Figure 43) cell line resulted in some synergistic effects (CI<1) at determined concentrations of both drugs. The effects of PM01183 in combination with methotrexate in MOLT-4 (Figure 44) cell line were mostly additive.

EXAMPLE 16. In vitro studies to determine the effect of PM01183 in combination with chemotherapeutic agents on human lymphoma cell lines.

[0142] The following agents were evaluated in combination with PM01183: gemcitabine, cytarabine and methotrexate, (stock solutions of these compounds prepared in pure DMSO and stored at -20°C). Additional serial dilutions were prepared in serum-free culture medium to achieve a final 4X concentration. Aliquots of 50 µL of each diluted compound were added per well.

[0143] RAMOS and U-937 were the human lymphoma cell lines selected for this assay, which were obtained from the American Type Culture Collection (ATCC). RAMOS and U-937 cells were grown in phenol red-free RPMI medium supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine and 100 units/mL of Penicillin-Streptomycin, at 37 °C, 5% CO2 and 95% humidity.

[0144] The screening was performed in two parts, as previously described in example 15.

[0145] In the first set of assays, the individual EC50 values were determined for each drug as shown in tables 17 and 18.
Table 17: EC50 values in molar concentration (M) for each of the agents for the RAMOS tumor cell line.

Compound	EC50 (M)
Gemcitabine	2.51 E-08
Cytarabine	3.64E-08
Methotrexate	5.02E-06
PM01183	1.39E-09

Table 18: EC50 values in molar concentration (M) for each of the agents for the U-937 tumor cell line.

Compound	EC50 (M)
Gemcitabine	3.27E-08
Methotrexate	2.63E-08
PM01183	1.03E-09

[0146] In the second set of assays, concentration-response curves for the agents tested, both alone and in two-drug combination, were performed. The effects of the drug combinations were evaluated using the Chou and Talalay method as described in the example 15
The effect of the tested drug combinations on cell proliferation is shown in Figures 45-49:

- Combination of PM01183 with cytarabine. The combination of PM01183 with cytarabine in RAMOS (Figure 45) cell line resulted in some synergistic effects (CI<1).
- Combination of PM01183 with methotrexate. The combination of PM01183 with methotrexate in RAMOS (Figure 46) cell line resulted in some synergistic effects (CI<1) at determined concentrations of both drugs. The effects of PM01183 in combination with methotrexate in U-937 (Figure 47) cell line resulted in some synergistic effects at determined concentrations.
- Combination of PM01183 with gemcitabine. The combination of PM01183 with gemcitabine in RAMOS (Figure 48) cell line was additive or synergistic (CI<1) at determined concentrations of both drugs. The combination of PM01183 with gemcitabine in U-937 (Figure 49) cell line resulted in synergistic effects (CI<l).

REFERENCES CITED IN THE DESCRIPTION

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PATENTKRAV

1. PM01183 eller et farmaceutisk acceptabelt salt deraf til anvendelse til behandling af cancer, omfattende administration af en terapeutisk effektiv mængde PM01183 eller et farmaceutisk acceptabelt salt deraf i synergistisk kombination med en terapeutisk effektiv mængde af en antimetabolit, hvor antimetabolitten er valgt blandt 5-fluoruracil, gemcitabin, cytarabin, capecitabin, decitabin, floxuridin, aminopterin, methotrexat, pemetrexed og raltitrexed.

2. PM01183 eller et farmaceutisk acceptabelt salt deraf til anvendelse til forøgelse af den terapeutiske effektivitet af en antimetabolit ved behandling af cancer, som omfatter administration til en patient, som har behov derfor, af en terapeutisk effektiv mængde PM01183 eller et farmaceutisk acceptabelt salt deraf i synergistisk kombination med antimetabolitten, hvor antimetabolitten er valgt blandt 5-fluoruracil, gemcitabin, cytarabin, capecitabin, decitabin, floxuridin, aminopterin, methotrexat, pemetrexed og raltitrexed.

3. PM01183 eller et farmaceutisk acceptabelt salt deraf til anvendelse ifølge krav 1 eller 2, hvor PM01183 eller et farmaceutisk acceptabelt salt deraf og antimetabolitten udgør en del af det samme lægemiddel.

4. PM01183 eller et farmaceutisk acceptabelt salt deraf til anvendelse ifølge krav 1 eller 2, hvor PM01183 eller et farmaceutisk acceptabelt salt deraf og antimetabolitten tilvejebringes som separate lægemidler til administration på samme tid eller på forskellige tidspunkter.

5. PM01183 eller et farmaceutisk acceptabelt salt deraf til anvendelse ifølge krav 4, hvor PM01183 eller et farmaceutisk acceptabelt salt deraf og antimetabolitten tilvejebringes som separate lægemidler til administration på forskellige tidspunkter.

6. PM01183 eller et farmaceutisk acceptabelt salt deraf til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antimetabolitten er valgt blandt 5-fluoruracil, capecitabin, gemcitabin, cytarabin og methotrexat.

7. PM01183 eller et farmaceutisk acceptabelt salt deraf til anvendelse ifølge krav 6, hvor antimetabolitten er valgt blandt 5-fluoruracil, gemcitabin, cytarabin og methotrexat.

8. PM01183 eller et farmaceutisk acceptabelt salt deraf til anvendelse ifølge et hvilket som helst af de foregående krav, hvor den cancer, der skal behandles, er valgt blandt lungecancer, sarkom, malignt melanom, blærecarcinom, prostatacancer, carcinom i bugspytkirtlen, cancer i skjoldbruskkirtlen, gastrisk carcinom, ovariecancer, hepatom, brystcancer, colorektal cancer, nyrecancer, cancer i spiserøret, neuroblastom, hjernecancer, livmoderhalscancer, anal cancer, testikelcancer, leukæmi, multipelt myelom og lymfom.

9. PM01183 eller et farmaceutisk acceptabelt salt deraf til anvendelse ifølge krav 8, hvor den cancer, der skal behandles, er valgt blandt lungecancer, sarkom, malignt melanom, prostatacancer, carcinom i bugspytkirtlen, gastrisk carcinom, ovariecancer, hepatom, brystcancer, colorektal cancer, nyrecancer, hjernecancer, leukæmi og lymfom.

10. Kit til anvendelse til behandling af cancer, som omfatter

en doseringsform af PM01183 eller et farmaceutisk acceptabelt salt deraf; og

en doseringsform af en antimetabolit, hvor antimetabolitten er valgt blandt 5-fluoruracil, gemcitabin, cytarabin, capecitabin, decitabin, floxuridin, aminopterin, methotrexat, pemetrexed og raltitrexed; og

instruktioner til anvendelse af begge lægemidler i synergistisk kombination som beskrevet i et hvilket som helst af de foregående krav.

DRAWINGS

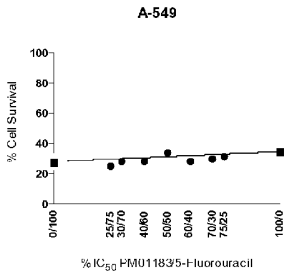

Figure 1

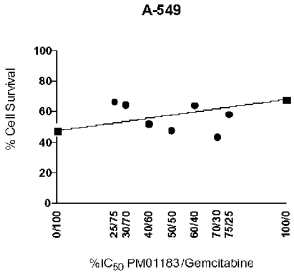

Figure 2

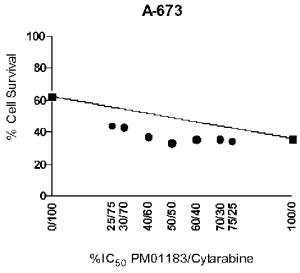

Figure 3

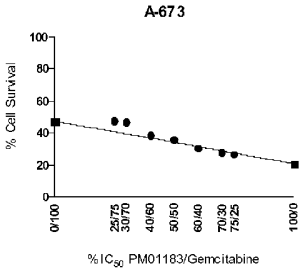

Figure 4

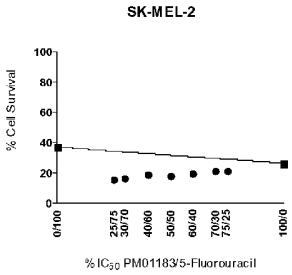

Figure 5

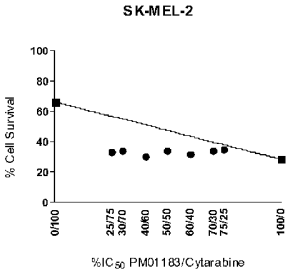

Figure 6

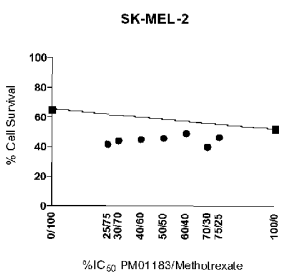

Figure 7

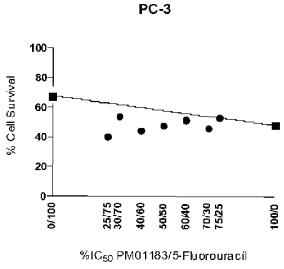

Figure 8

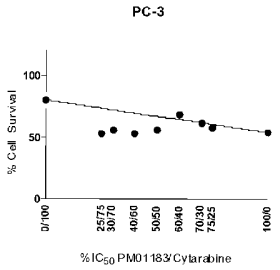

Figure 9

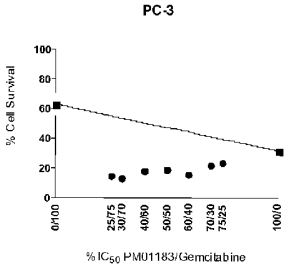

Figure 10

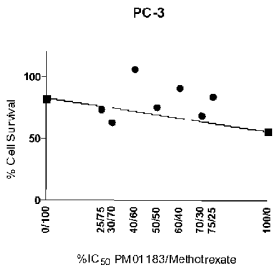

Figure 11

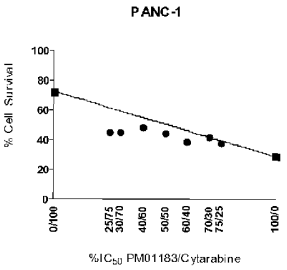

Figure 12

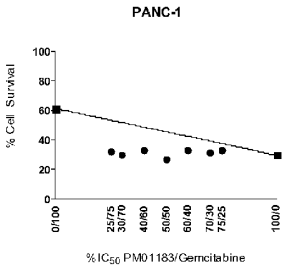

Figure 13

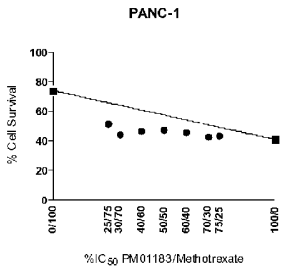

Figure 14

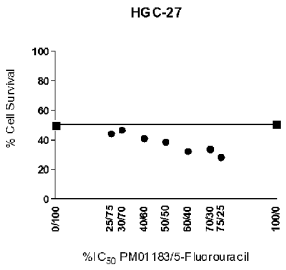

Figure 15

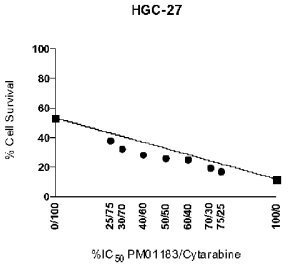

Figure 16

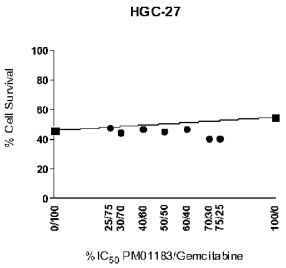

Figure 17

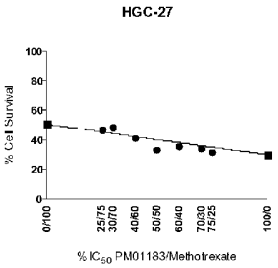

Figure 18

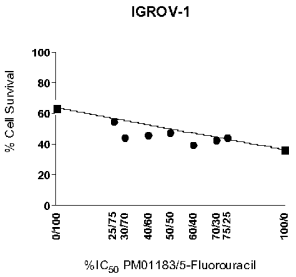

Figure 19

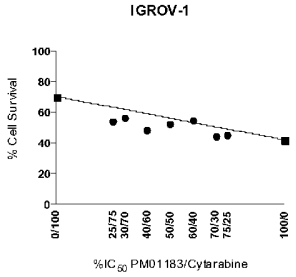

Figure 20

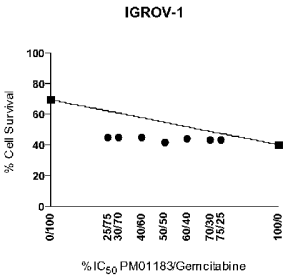

Figure 21

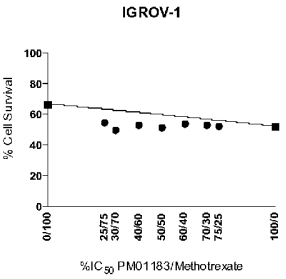

Figure 22

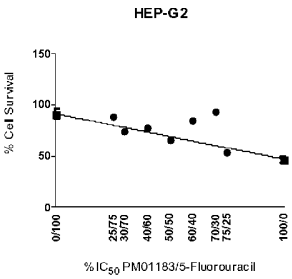

Figure 23

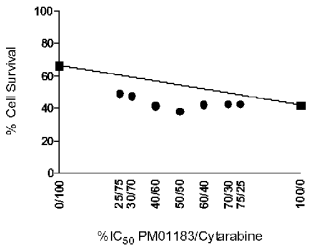

Figure 24

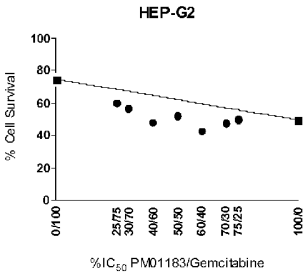

Figure 25

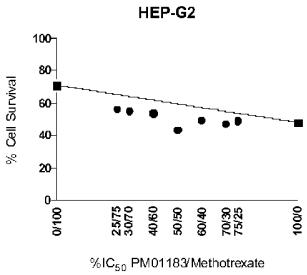

Figure 26

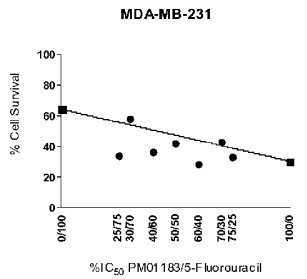

Figure 27

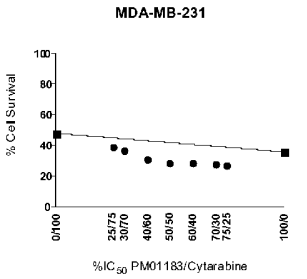

Figure 28

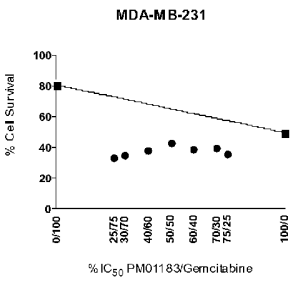

Figure 29

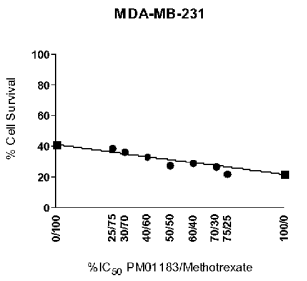

Figure 30

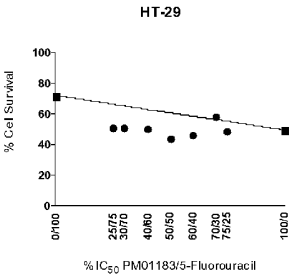

Figure 31

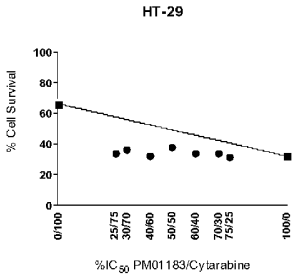

Figure 32

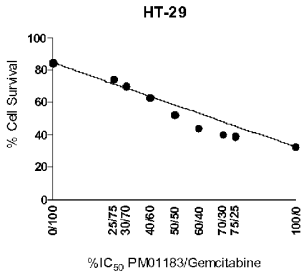

Figure 33

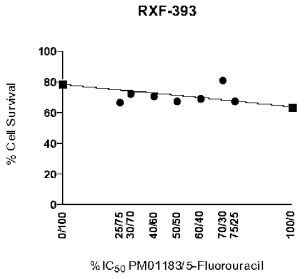

Figure 34

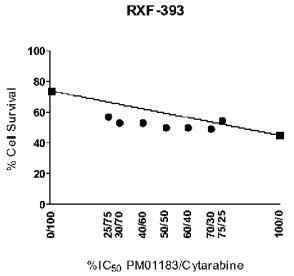

Figure 35

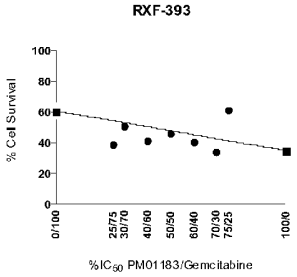

Figure 36

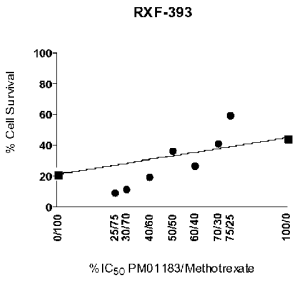

Figure 37

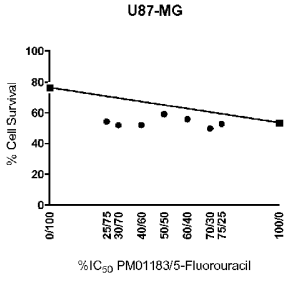

Figure 38

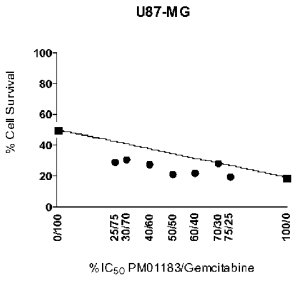

Figure 39

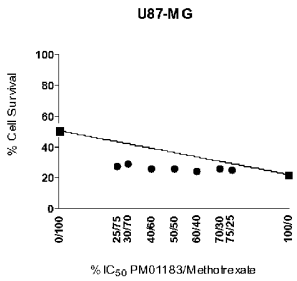

Figure 40

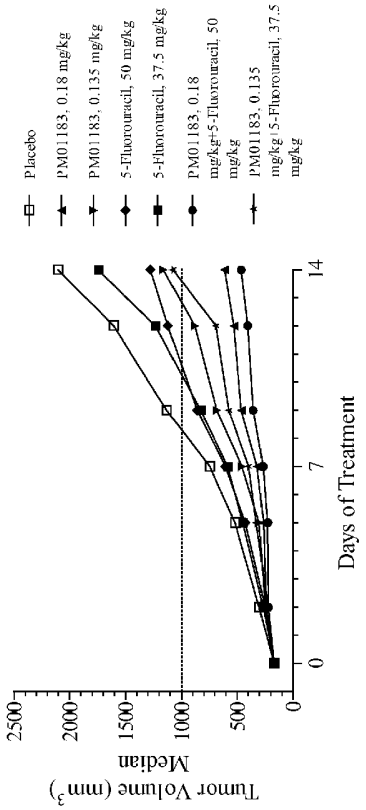
Placebo
PM01183, 0.18 mg/kg
PM01183, 0.135 mg/kg
5-Fluorouracil, 50 mg/kg
5-Fluorouracil, 37.5 mg/kg
PM01183, 0.18 mg/kg+5-Fluorouracil, 50 mg/kg
PM01183, 0.135 mg/kg+5-Fluorouracil, 37.5 mg/kg
Tumor Volume (mm3) Median
2500
2000
1500
1000
500
0
0
7
14
Days of Treatment

Figure 41

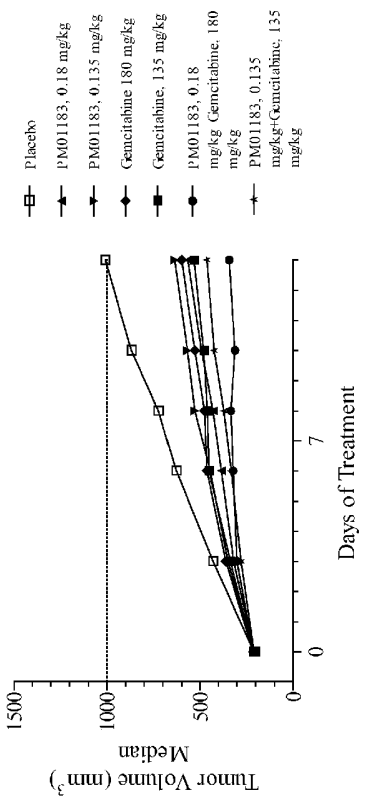
Placebo
PM01183, 0.18 mg/kg
PM01183, 0.135 mg/kg
Gemcitabine 180 mg/kg
Gemcitabine, 135 mg/kg
PM01183, 0.18 mg/kg Gemcitabine, 180 mg/kg
PM01183, 0.135 mg/kg+Gemcitabine, 135 mg/kg
Tumor Volume (mm3) Median
1500
1000
500
0
0
7
Days of Treatment

Figure 42

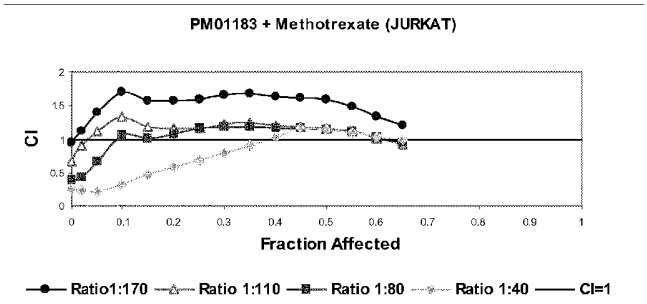

Figure 43

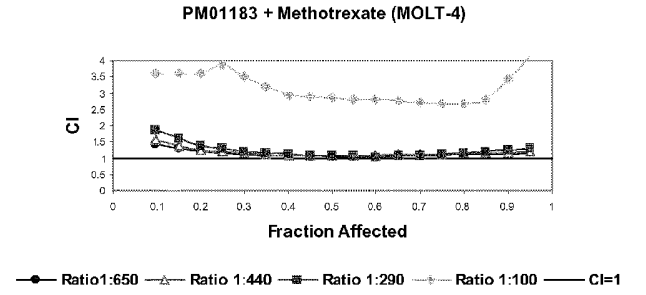

Figure 44

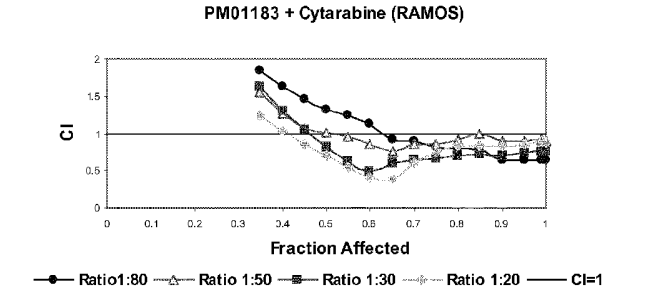

Figure 45

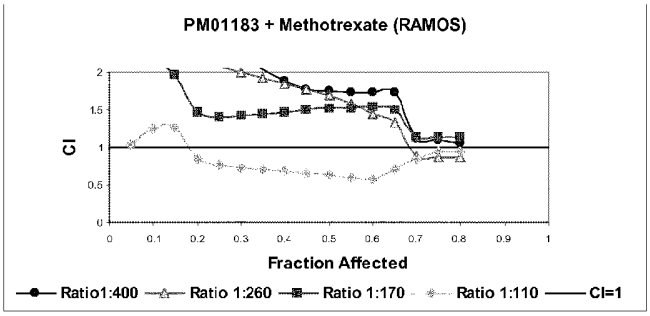

Figure 46

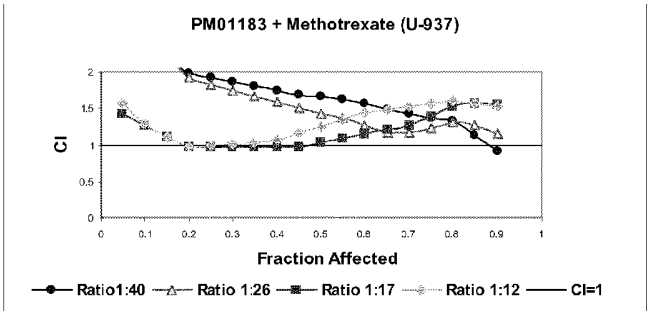

Figure 47

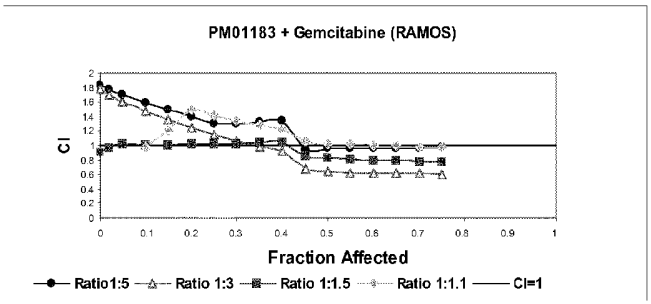

Figure 48

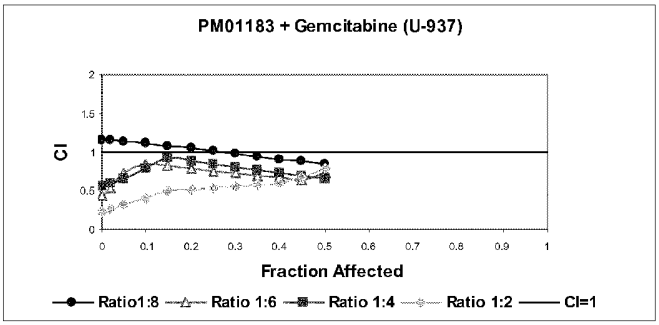
PM01183 + Gemcitabine (U-937)
CI
2
1.5
1
0.5
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Fraction Affected
Ratio1:8
Ratio 1:6
Ratio 1:4
Ratio 1:2
CI=1

Figure 49