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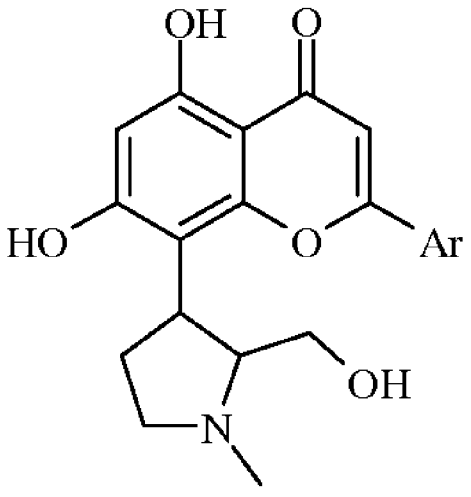
(54) Title: A SYNERGISTIC PHARMACEUTICAL COMBINATION FOR THE TREATMENT OF PANCREATIC CANCER



(I)

(57) Abstract: In accordance with the present invention there is provided a pharmaceutical combination useful for the treatment of pancreatic cancer comprising a CDK inhibitor selected from the compounds of Formula (I); and a compound capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity. The present invention also provides a combination wherein said pharmaceutical combination useful for the treatment of pancreatic cancer further comprises gemcitabine. The present invention also provides a method for the treatment of pancreatic cancer in a subject comprising administering said pharmaceutical combination(s) to said subject.

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A SYNERGISTIC PHARMACEUTICAL COMBINATION FOR THE TREATMENT OF PANCREATIC CANCER

FIELD OF INVENTION:

The present invention relates to a pharmaceutical combination for use in the treatment of pancreatic cancer. The invention particularly relates to a pharmaceutical combination comprising a cyclin dependent kinase inhibitor selected from the compounds of formula I (as described herein) or a pharmaceutically acceptable salt or a solvate thereof and a compound capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity for use in the treatment of pancreatic cancer. The present invention further relates to including gemcitabine or a salt thereof to said combination for use in the treatment of pancreatic cancer. The invention also relates to pharmaceutical composition comprising said combinations; a method for the treatment of pancreatic cancer in a subject comprising administering said pharmaceutical combinations to said subject.

BACKGROUND OF THE INVENTION:

Cancer is a group of diseases characterized by aberrant control of cell growth. Cancer cells can invade nearby tissues and can spread through the bloodstream and lymphatic system to other parts of the body. There are different types of cancers such as bladder cancer, breast cancer, colon cancer, rectal cancer, head and neck cancer, endometrial cancer, kidney (renal cell) cancer, leukemia, small cell lung cancer, non-small cell lung cancer, pancreatic cancer, prostate cancer, thyroid cancer, skin cancer, Non-Hodgkin's lymphoma and melanoma. Although all type of cancers are fatal, pancreatic cancer remains the fourth and fifth most common cause of cancer-related mortality in North America and Europe, respectively, with an estimated 38,000 new cases in the United States and approximately 58,000 new cases in Europe occurring annually (J. Clin. Oncol.; Volume 27 No. 13, 2231 – 2237 (2009)). In fact, pancreatic cancer has an extremely poor prognosis, with an overall 5 year survival rate of less than 5% (Pharmacol Ther 1999; 82: 241–250).

Further, patients with locally advanced and metastatic pancreatic cancer have poor prognoses, and diagnosis generally occurs too late for surgery or radiotherapy to be curative. Chemotherapy can provide symptom relief for some patients with advanced pancreatic cancer, but its impact on survival has been modest to date. Historically, 5-fluorouracil (5-FU) was the drug of choice for the systemic treatment of advanced pancreatic cancer, but in

single-agent studies conducted in the era of computed tomographic (CT) assessment of tumor response, response rates rarely exceed 20%, with median survival times of 4.2–5.5 months (J. Clin. Oncol.; 15:2403–2413 (1997)). Compared with 5-FU, first-line treatment with gemcitabine (Gemzar®) produced a modest median survival advantage (5.7 months versus 4.4 months) and was shown to be more effective for palliation of patients with symptomatic, advanced disease in a randomized clinical trial.

Although the systemic administration of gemcitabine has been associated with both clinical benefit and prolongation of survival in patients with advanced disease, objective tumor responses occur in fewer than 10% of patients and survival times are generally less than 6 months (J. Clin. Oncol.; 15:2403–2413 (1997)). However, over the period it has been observed that treatment of pancreatic cancer with gemcitabine failed to significantly improve the condition of pancreatic cancer patients because of the pre-existing or acquired chemo resistance of most of the tumor cells to the drug (*Oncogene*; 22(21): 3243-51 (2003)). In a review of clinical trials with gemcitabine as a single-agent in the treatment of pancreatic cancer, it has been reported that pulmonary and allergic toxicities were assessed using World Health Organization recommended toxicity grading in 2,704 patients. As per this study, 7.4% of patients had mild symptoms (grade 1), 7.6% patients experienced dyspnea on exertion (grade 2), dyspnea at rest (grade 3) occurred in 3.1% of treated patients, and complete bed rest (grade 4) was required in 0.8% of patients (J. Pancreas (Online) 2008; 9(6):708-714). Thus, treatment with gemcitabine raises concerns due to the toxic effects reported in patients treated with gemcitabine as a monotherapy. There have been attempts to combine gemcitabine with several other cytotoxic drugs or agents and the results from phase II studies of gemcitabine combined with cisplatin, 5-FU, irinotecan, and oxaliplatin, in advanced and metastatic pancreatic cancer have suggested that efficacy could be improved.

However, phase III trials of these combinations have not confirmed their superiority to the single agent, gemcitabine. Further, survival times associated with combinations of gemcitabine/5-FU, gemcitabine/irinotecan, gemcitabine/cisplatin, and gemcitabine/oxaliplatin appear to be either equivalent or only marginally superior to those associated with the single-agent, gemcitabine (J. Clin. Oncol.: Vol. 24 No. 3 327 – 329 (2006)). Combination of gemcitabine with erlotinib (Tarceva®), an orally bioavailable EGFR tyrosine inhibitor, when tested in animal models showed only additive effect (J Clin Invest 1992; 90: 1352–1360). However, a randomized phase III trial involving treatment of 569 patients having untreated inoperable pancreatic cancer with the combination of gemcitabine

and erlotinib demonstrated a small but significant survival benefit for the combination of gemcitabine and erlotinib versus gemcitabine alone (Oncology (Williston Park) 2003; 17: 11–16). This was the first trial to show a survival benefit for any combination therapy in pancreatic cancer and led to Food and Drug Administration (FDA) approval of this combination in the front-line therapy of pancreatic cancer in 2005. Most recently, phase III clinical trial comparing patients with advanced pancreatic cancer randomized to receive gemcitabine plus the EGFR inhibitor, erlotinib, or gemcitabine alone showed a significant improvement in median survival of 6.2 versus 5.9 months as well as 1 year survival of 23% versus 17%, respectively (Semin. Oncol. 2005;32:5-6). There have been a report of a clinical trial involving addition of a second targeted agent namely bevacizumab to the combination of gemcitabine and erlotininb to further improve outcomes in patients with metastatic pancreatic cancer. However, it was observed that the addition of bevacizumab to gemcitabine-erlotinib combination did not lead to a statistically significant improvement in overall survival in patients with metastatic pancreatic cancer (J. Clin. Oncol.; 27: 2231 – 2237 (2009)).

Further, a CDK inhibitor particularly a flavone derivative has been found to potentiate synergistically the cytotoxic response of gemcitabine in human pancreatic cell lines (PCT Appln. Publication No. WO 2008/139271). A combination comprising a VEGF receptor kinase inhibitor and erlotinib or gemcitabine showing good anti-tumor activity is reported in US Published Patent Appln. No. US 2010-0048503.

Based on the discussion of the current treatment options available for pancreatic cancer, particularly for the locally advanced or metastatic pancreatic cancer, it is evident that in spite of the efforts made to date, there is still the need to find new therapeutic approaches which not only provide improvement in efficacy but also provide added survival benefits to the patients suffering from pancreatic cancer. Further, from the above discussion it is also clear that although the protocol involving combination of anticancer agents having different mechanism of action may work in case of some combinations, it may not work in the same manner for other combination of anticancer agents and such combination may not always result in a combination having advantageous therapeutic effects. However, the invention described herein provides a drug combination having improved efficacy, and methods for using the drug combination in the treatment of pancreatic cancer. The present inventors have found that a combination of known anticancer agents comprising a compound capable of inhibiting EGFR kinase activity and a cyclin dependant kinase inhibitor selected from compounds represented by formula I (as described herein); when used in the treatment of

pancreatic cancer provides unexpectedly greater efficacy. Addition of the known cytotoxic agent, gemcitabine to afore said combination further improves efficacy of the combination when used in the treatment of pancreatic cancer.

SUMMARY OF THE INVENTION:

In one aspect, the present invention relates to a pharmaceutical combination comprising a cyclin dependent kinase (CDK) inhibitor selected from the compounds represented by formula I and a compound capable of inhibiting EGFR kinase activity; for use in the treatment of pancreatic cancer.

In another aspect, the present invention relates to a method for the treatment of pancreatic cancer in a subject, comprising administering to the subject a therapeutically effective amount of a cyclin dependent kinase (CDK) inhibitor selected from the compounds represented by formula I in combination with a therapeutically effective amount of a compound capable of inhibiting EGFR kinase activity.

In yet another aspect, the present invention relates to a pharmaceutical composition comprising a therapeutically effective amount of a cyclin dependent kinase (CDK) inhibitor selected from the compounds represented by formula I and a therapeutically effective amount of a compound capable of inhibiting the EGFR kinase activity; for use in the treatment of pancreatic cancer.

In another further aspect, the present invention relates to a pharmaceutical combination comprising a cyclin dependent kinase (CDK) inhibitor selected from the compounds represented by formula I; and a compound capable of inhibiting the EGFR kinase activity; wherein said combination exhibits synergistic effect in the treatment of pancreatic cancer.

In yet another further aspect, the present invention relates to the pharmaceutical combination comprising a cyclin dependent kinase (CDK) inhibitor selected from the compounds represented by formula I; and a compound capable of inhibiting the EGFR kinase activity; further comprising gemcitabine; for use in the treatment of pancreatic cancer.

In yet another aspect, the present invention relates to the pharmaceutical composition comprising a therapeutically effective amount of a cyclin dependent kinase (CDK) inhibitor selected from the compounds represented by formula I and a therapeutically effective amount of a compound capable of inhibiting the EGFR kinase activity; further comprising therapeutically effective amount of gemcitabine; for use in the treatment of pancreatic cancer.

In yet another aspect, the present invention relates to a method for the treatment of pancreatic cancer in a subject, comprising administering to the subject a therapeutically effective amount of a cyclin dependent kinase (CDK) inhibitor in combination with a therapeutically effective amount of a compound capable of inhibiting EGFR kinase activity; further in combination with gemcitabine.

In yet another further aspect, the present invention relates to a kit comprising a cyclin dependent kinase (CDK) inhibitor selected from compounds represented by formula I; a compound capable of inhibiting the EGFR kinase activity and optionally gemcitabine; wherein said kit may further include a package insert comprising printed instructions directing the use of the combined treatment as a method for treating pancreatic cancer.

Other aspects and further scope of applicability of the present invention will become apparent from the detailed description to follow.

BRIEF DESCRIPTION OF THE DRAWINGS:

Figure 1a provides graphical representation of the percentage inhibition results of single and combination dosing of compound A and erlotinib in Panc-1 cells at the end of 72 hrs.

Figure 1b provides graphical representation of the percentage inhibition results of single and combination dosing of compound A and erlotinib in Panc-1 cells at the end of 96 hrs.

Figure 2a provides graphical representation of the percentage inhibition results of single and combination dosing of compound A and erlotinib in AsPc-1 cells at the end of 72 hrs.

Figure 2b provides graphical representation of the percentage inhibition results of single and combination dosing of compound A and erlotinib in AsPc-1 cells at the end of 96 hrs.

Figure 3a provides graphical representation of the determination of IC_{50} of compound A, erlotinib, lapatinib and gemcitabine in Panc-1 cells.

Figure 3b provides graphical representation of the determination of IC_{50} of compound A, erlotinib, lapatinib and gemcitabine in MiaPaca-2 cells.

Figure 4a provides graphical representation of the percentage cytotoxicity results of single and combination dosing of gemcitabine (IC_{30}), compound A (IC_{30}) and erlotinib(IC_{30})/lapatinib(IC_{30}) in Panc-1 cells.

Figure 4b provides graphical representation of the percentage cytotoxicity results of single and combination dosing of gemcitabine (IC_{50}), compound A(IC_{30}) and erlotinib(IC_{30})/lapatinib(IC_{30}) in Panc-1 cells.

Figure 5a provides graphical representation of the percentage cytotoxicity results of single and combination dosing of gemcitabine (IC_{30}), compound A (IC_{30}) and erlotinib(IC_{30})/lapatinib(IC_{30}) in MiaPaca-2 cells.

Figure 5b provides graphical representation of the percentage cytotoxicity results of single and combination dosing of gemcitabine (IC_{50}), compound A (IC_{30}) and erlotinib(IC_{30})/lapatinib(IC_{30}) in MiaPaca-2 cells.

Figure 6 provides graphical representation of the percentage cytotoxicity results of single and combination dosing of gemcitabine (IC_{30} or IC_{50}), compound A (IC_{30} or IC_{50}) and erlotinib (IC_{30} or IC_{50}) in HPAC cells.

Figure 7 provides graphical representation of the percentage cytotoxicity results of single and combination dosing of gemcitabine (IC_{30} or IC_{50}), compound A (IC_{30} or IC_{50}) and erlotinib (IC_{30} or IC_{50}) in CAPAN cells.

Figure 8a provides graphical representation of the inhibition of pEGFR – Y1173 in Panc-1 cells with gemcitabine(IC_{30}) in combination with compound A + erlotinib or with compound A + lapatinib at 8 hours.

Figure 8b provides graphical representation of the inhibition of pEGFR – Y845 in Panc-1 cells with gemcitabine(IC_{30} or IC_{50})in combination with compound A + erlotinib or with compound A + lapatinib at 8 hours.

Figure 8c provides graphical representation of the inhibition of pAKT – S473 in Panc-1 cells with gemcitabine(IC_{30} or IC_{50}) in combination with compound A + erlotinib or with compound A + lapatinib at 12 hours.

Figure 8d provides graphical representation of the inhibition of pRB – S780 in Panc-1 cells with gemcitabine(IC_{30} or IC_{50}) in combination with compound A + erlotinib or with compound A + lapatinib at 12 hours.

Figure 8e provides graphical representation of the inhibition of Cyclin D in Panc-1 cells with gemcitabine(IC_{30} or IC_{50}) in combination with compound A + erlotinib or with compound A + lapatinib at 12 hours.

Figure 9 provides graphical representation of activation of Caspase 3 in Panc-1 cells and MiaPaca-2 cells with gemcitabine(IC_{30} or IC_{50}) in combination with compound A + erlotinib or with compound A + lapatinib at 48 hours.

DETAILED DESCRIPTION OF THE INVENTION:

The present invention is based, in part, on the recognition that CDK inhibitors selected from the compounds of formula I potentiate and synergize with, enhance the effectiveness of, improve the tolerance of, and/or reduce side effects caused by, the compounds capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity for use in the treatment of pancreatic cancer.

Encompassed by the present invention are pharmaceutical combinations that have synergistic effects where the therapeutic efficacy of the combination is greater than additive when used in the treatment of pancreatic cancer. Preferably, such combinations also reduce or avoid unwanted or adverse effects. In certain embodiments, the combinations encompassed by the invention provide an improved overall therapy relative to administration of the CDK inhibitor or the compound capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity or gemcitabine.

Accordingly, the present invention relates to pharmaceutical combinations designed to treat, or manage pancreatic cancer in a subject, wherein the combination comprises a CDK inhibitor selected from the compounds of formula I in combination with the compound capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity and said combination further comprises gemcitabine added to it. In particular, the present invention provides methods of preventing, or managing pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula I in combination with a therapeutically effective amount of the compound capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity.

The general terms used hereinbefore and hereinafter preferably have within the context of this disclosure the following meanings, unless otherwise indicated. Thus, the definitions of the general terms as used in the context of the present invention are provided herein below:

The singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.

The phrase “a cyclin dependent kinase (CDK) inhibitor” or “CDK inhibitor” as used herein means a compound that exhibits activity against one or more known cyclin dependent kinases. In the context of the present invention the CDK inhibitor is a pyrrolidine substituted flavone compound disclosed in US Application Publication No. No. 20070015802A1 and PCT Patent Publication No. WO2007148158 which applications are incorporated herein by

reference in its entirety. The CDK inhibitor according to the present invention is specifically selected from a compound of formula I as described herein below or a pharmaceutically acceptable salt or solvate thereof. Further, the term "CDK inhibitor" as used herein may refer to the compound of formula I and/or the (+)-trans isomer of the compound of formula I, as indicated in Formula IA (as described herein below) and/or a pharmaceutically acceptable salt or solvate of the compound of formula I or the (+)-trans isomer of the compound of formula I.

The phrase "a compound capable of inhibiting EGFR kinase activity" as used herein refers to a compound that acts by inhibiting, blocking, antagonizing or otherwise blocking EGFR kinase activity in cells and tissues. The "epidermal growth factor receptor (EGFR)" is a member of the ErbB family of transmembrane tyrosine kinase receptors, which includes ErbB1 (or HER1, or EGFR), ErbB2 (or HER2/neu), ErbB3 (or HER3), and ErbB4 (or HER4). The expression of EGFR is common in a number of normal epithelial tissues and expression of EGFR is elevated in several solid tumors including pancreatic cancer.

As used herein, the term "combination" or "pharmaceutical combination", means the combined administration of the anti-cancer agents namely the CDK inhibitor and the compound capable of inhibiting EGFR kinase activity or the combined administration of the anti-cancer agents namely the CDK inhibitor and the compound capable of inhibiting EGFR kinase activity and gemcitabine; which anti-cancer agents may be administered independently at the same time or separately within time intervals that especially allow that the combination partners show a synergistic effect.

The phrase "double combination" generally refers to a combination of two known anticancer agents comprising a cyclin dependant kinase inhibitor selected from compounds represented by formula I and a compound capable of inhibiting EGFR kinase activity. The term may also refer to combined administration of the anti-cancer agents namely the CDK inhibitor and the compound capable of inhibiting EGFR kinase activity.

The phrase "triple combination" refers to a combination of known anticancer agents comprising, gemcitabine, a compound capable of inhibiting EGFR kinase activity and a cyclin dependant kinase inhibitor selected from the compounds represented by formula I. The term may also refer to combined administration of the anti-cancer agents namely gemcitabine, the CDK inhibitor and the compound capable of inhibiting EGFR kinase activity.

As used herein, the term "synergistic" means that the effect achieved with the

methods and combinations of this invention is greater than the sum of the effects that result from using the anti-cancer agents namely the CDK inhibitor; the compound capable of inhibiting EGFR kinase activity and gemcitabine as a monotherapy. Advantageously, such synergy provides greater efficacy at the same doses, and/or prevents or delays the build-up of multi-drug resistance.

A “therapeutically effective amount”, in reference to the treatment of pancreatic cancer, refers to an amount capable of invoking one or more of the following effects in a subject receiving the combination of the present invention: (i) inhibition, to some extent, of tumor growth, including, slowing down and complete growth arrest; (ii) reduction in the number of tumor cells; (iii) reduction in tumor size; (iv) inhibition (i.e., reduction, slowing down or complete stopping) of tumor cell infiltration into peripheral organs; (v) inhibition (i.e., reduction, slowing down or complete stopping) of metastasis; (vi) enhancement of anti-tumor immune response, which may, but does not have to, result in the regression or rejection of the tumor; and/or (vii) relief, to some extent, of one or more symptoms associated with pancreatic cancer.

The term "subject" as used herein, refers to an animal, preferably a mammal, most preferably a human, who is in the need of treatment of pancreatic cancer. The term subject may be interchangeably used with the term patient in the context of the present invention.

The phrase "non-responsive/refractory" as used herein, is used to describe subjects or patients having pancreatic cancer having been treated with currently available cancer therapies for the treatment of pancreatic cancer such as chemotherapy, radiation therapy, surgery, hormonal therapy and/or biological therapy/immunotherapy wherein the therapy is not clinically adequate to treat the patients such that these patients need additional effective therapy, e.g., remain unsusceptible to therapy. The phrase can also describe subjects or patients who respond to therapy yet suffer from side effects, relapse, develop resistance, etc. In various embodiments, "non-responsive/refractory" means that at least some significant portions of the cancer cells are not killed or their cell division arrested. The determination of whether the cancer cells are "non-responsive/refractory" can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of treatment on cancer cells, using the art-accepted meanings of "refractory" in such a context. In various embodiments, a cancer is "non-responsive/refractory" where the number of cancer cells has not been significantly reduced, or has increased.

As used herein, the term "potentiate" refers to an improvement in the efficacy of a

therapeutic agent at its common or approved dose. In the context of the present invention the therapeutic agent is an anticancer agent such as the compound capable of inhibiting EGFR kinase activity or gemcitabine.

As used herein, the terms "manage", "managing" and "management" refer to the beneficial effects that a subject or a patient derives from the pharmaceutical combination of the present invention when administered to said patient or subject so as to prevent the progression or worsening of pancreatic cancer.

The term “overexpression,” as used herein, refers to overexpression of a gene and/or its encoded protein in a cell, such as a cancer cell. A cancer cell that “overexpresses” a protein is one that has significantly higher levels of that protein compared to a noncancerous cell of the same tissue type.

The term "dosage form" as used herein shall mean the physical form in which a drug is produced and dispensed, such as a tablet, capsule, or an injectable.

The term “active ingredient” as used herein shall mean a CDK inhibitor selected from the compounds of formula I or a compound capable of inhibiting EGFR kinase activity or gemcitabine. The term “active ingredient” when used in plural form i.e. active ingredients shall mean a CDK inhibitor selected from the compounds of formula I in combination with a compound capable of inhibiting EGFR kinase activity and gemcitabine. The term “active ingredient” may be used interchangeably with the term “therapeutic agent.”

The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which a compound of the invention is administered.

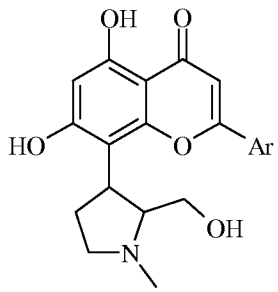
It has now been found that a pharmaceutical combination which comprises a CDK inhibitor selected from the compounds of formula I (as described herein) and a compound capable of inhibiting EGFR kinase activity; exhibits synergistic effect when used in the treatment of cancer, particularly pancreatic cancer. In particular, a compound capable of inhibiting EGFR kinase activity such as erlotinib when used in combination with the CDK inhibitor of formula I produces significantly better effects than any one of erlotinib and the CDK inhibitor of formula I when used alone for treating pancreatic cancer.

It has also been observed by the present inventors that the synergistic effect of the pharmaceutical combination comprising a CDK inhibitor selected from the compounds of

formula I (as described herein) and a compound capable of inhibiting EGFR kinase activity is further enhanced by adding a known cytotoxic agent, gemcitabine or a pharmaceutically acceptable salt thereof to said combination. Accordingly, the present invention also encompasses within its scope a pharmaceutical combination which comprises a CDK inhibitor selected from the compounds of formula I (as described herein); a compound capable of inhibiting EGFR kinase activity and gemcitabine or a pharmaceutically acceptable salt thereof; for use in the treatment of pancreatic cancer.

The CDK inhibitor used in the pharmaceutical combination of the present invention is selected from the compounds of formula I as described herein below. The compounds of formula I are promising CDK inhibitors, which can inhibit proliferation of many cancer cells.

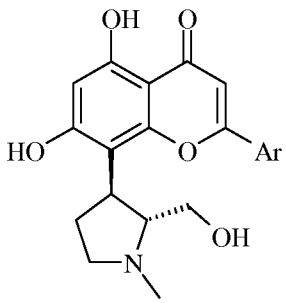
In one embodiment, the CDK inhibitor used in the pharmaceutical combination of present invention is selected from the compounds represented by the following formula I,



Formula I

wherein Ar is a phenyl group, which is unsubstituted or substituted by 1, 2, or 3 identical or different substituents selected from: halogen; nitro, cyano, C_1-C_4-alkyl, trifluoromethyl, hydroxyl or C_1-C_4-alkoxy; or a pharmaceutically acceptable salt or solvate thereof.

In an embodiment of the invention, the CDK inhibitor is the (+)-trans isomer of the compound of formula I, as indicated in Formula IA below,



Formula IA

wherein Ar is a phenyl group, which is unsubstituted or substituted by 1, 2, or 3 identical or different substituents selected from :halogen, nitro, cyano, C_1-C_4-alkyl, trifluoromethyl, hydroxyl or C_1-C_4-alkoxy; or a pharmaceutically acceptable salt or solvate thereof.

In another embodiment, the CDK inhibitor used in the pharmaceutical combination of present invention is a compound of formula I wherein the phenyl group is substituted by 1, 2, or 3 identical or different substituents selected from: chlorine, bromine, fluorine or iodine, C_1-C_4-alkyl or trifluoromethyl; or a pharmaceutically acceptable salt or solvate thereof.

In another embodiment, the CDK inhibitor used in the pharmaceutical combination of present invention is a compound of formula I wherein the phenyl group is substituted by 1, 2, or 3 halogens selected from chlorine, bromine, fluorine or iodine; or a pharmaceutically acceptable salt or solvate thereof.

In another embodiment, the CDK inhibitor used in the pharmaceutical combination of present invention is a compound of formula I wherein the phenyl group is substituted by chlorine; or a pharmaceutically acceptable salt or solvate thereof.

In another embodiment, the CDK inhibitor used in the pharmaceutical combination of present invention is a compound of formula I wherein the phenyl group is substituted by 2 different substituents selected from chlorine and trifluoromethyl; or a pharmaceutically acceptable salt or solvate thereof.

It will be appreciated by those skilled in the art that the CDK inhibitors represented by the compounds of formula I contain at least two chiral centers and hence, exist in the form of two different optical isomers (i.e. (+) or (-) enantiomers). All such enantiomers and mixtures thereof including racemic mixtures are included within the scope of the invention. The enantiomers of the compound of formula I can be obtained by methods disclosed in PCT Application Publication Nos. WO2004004632, WO2008007169 and WO2007148158 incorporated herein by reference or the enantiomers of the compound of formula I can also be obtained by methods well known in the art, such as chiral HPLC and enzymatic resolution. Alternatively, the enantiomers of the compounds of formula I can be synthesized by using optically active starting materials.

The manufacture of the compounds of formula I, which may be in the form of pharmaceutically acceptable salts and solvates, and the manufacture of oral and/or parenteral pharmaceutical composition containing the above compounds are generally disclosed in PCT Application Publication No. WO2004004632. This patent, which is incorporated herein by reference, discloses that the CDK inhibitors represented by formula I exhibit significant

anticancer efficacy.

As indicated herein above the CDK inhibitors of formula I may be used in the form of their salts. Preferred salt of compounds of formula I include hydrochloride, methanesulfonic acid and trifluoroacetic acid salt.

Accordingly, in another embodiment, the CDK inhibitor used in the pharmaceutical combination of present invention is selected from (+)-*trans*-2-(2-Chloro-phenyl)-5,7-dihydroxy-8-(2-hydroxy-methyl-1-methyl-pyrrolidin-3-yl)-chromen-4-one hydrochloride (referred to herein as compound A) or (+)-trans-3-[2[(2-Chloro-4-trifluoromethyl-phenyl)-5,7-dihydroxy-8-(2-hydroxymethyl-1-methyl-pyrrolidin-3-yl)-chromen-4-one hydrochloride (referred to herein as compound B).

In another further embodiment, the CDK inhibitor used in the pharmaceutical combination of the present invention is (+)-*trans*-2-(2-Chloro-phenyl)-5,7-dihydroxy-8-(2-hydroxy-methyl-1-methyl-pyrrolidin-3-yl)-chromen-4-one hydrochloride (compound A).

In another embodiment, the CDK inhibitor used in the pharmaceutical combination of the present invention is (+)-trans-3-[2[(2-Chloro-4-trifluoromethyl-phenyl)-5,7-dihydroxy-8-(2-hydroxymethyl-1-methyl-pyrrolidin-3-yl)-chromen-4-one hydrochloride (compound B).

According to the present invention, the compound capable of inhibiting EGFR kinase activity included in the pharmaceutical combination of the present invention can be selected from a group consisting of: gefitinib (also known as IRESSA® or ZD1839; AstraZeneca), erlotinib (also known as TARCEVA® or OSI-774; OSI Pharmaceuticals, Inc.), lapatinib or lapatinib ditosylate (also known as GW572016 or TYKERB®; GlaxoSmithKline), AG 1478 (4-(3-chloroanillino)-6,7-dimethoxyquinazoline A.G. Scientific, Inc.), EKB-569 (Pelitinib; Wyeth Pharmaceuticals), EKI-785 (Wyeth Pharmaceuticals), PKI-166 (Novartis Pharmaceuticals), canertinib dihydrochloride (also known as CI-1033; Pfizer), D-69491 (also known as SU11464; Baxter Oncology), vandetanib (also known as ZD6474 or ZACTIMA®; AstraZeneca) , XL-147 (also known as SAR-245408; Exelixis, Inc,) afatinib (Boehringer Ingelheim Corp.), CUDC-101 (Curis Inc.), S-222611 (Shionogi & Co. Ltd.), AZD-8931 (AstraZeneca) or neratinib (Wyeth Pharmaceuticals).

According to an embodiment of the invention, the compound capable of inhibiting EGFR kinase activity that may be used in the pharmaceutical combination of the present invention is selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof.

Erlotinib is an EGFR inhibitor. This drug follows gefitinib (Iressa®), which was the first drug of this type. Erlotinib specifically targets the epidermal growth factor receptor

(EGFR) tyrosine kinase, which is highly expressed and occasionally mutated in various forms of cancer. It binds in a reversible fashion to the adenosine triphosphate (ATP) binding site of the receptor (J Clin Oncol 2007;25: 1960-1966). Erlotinib is commercially available.

Lapatinib is a 4-anilinoquinoline derivative that is able to reversibly inhibit the tyrosine kinase activity of EGFR and HER2. Like other small molecule tyrosine kinase inhibitors, lapatinib competes with ATP for its binding site on the tyrosine kinase domain. In cell-free biochemical kinase assays, lapatinib inhibits the recombinant EGFR and HER2 tyrosine kinases by 50% (IC50) at concentrations of 10.8 and 9.3 nmol/l, respectively. It acts as a reversible inhibitor, with an estimated dissociation constant (Ki) value of 3 and 13 nmol/l for EGFR and HER2, respectively [*Drugs* 60 Suppl 1: 15–23]. Lapatinib is commercially available.

Gemcitabine is the generic name assigned to 2'-deoxy-2',2'-difluorocytidine. It is commercially available as the monohydrochloride salt, and as the β-isomer. Gemcitabine is also known as Gemzar® and it is a cytotoxic agent. It is an anti-metabolite which causes inhibition of DNA synthesis. Gemcitabine is disclosed in U.S. Pat. Nos. 4,808,614 and 5,464,826, which are incorporated herein by reference for their teaching of how to synthesize and use gemcitabine for treating susceptible cancers. The commercial formulation of gemcitabine hydrochloride as a single agent is indicated as first-line treatment for patients with locally advanced or metastatic adenocarcinoma of the pancreas. Gemcitabine is commercially available.

In one embodiment, the present invention relates to a pharmaceutical combination for the treatment of pancreatic cancer wherein the combination comprises a cyclin dependent kinase (CDK) inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt or a solvate thereof; a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention is directed to a pharmaceutical combination for the treatment of pancreatic cancer wherein the combination comprises a cyclin dependent kinase (CDK) inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt or a solvate thereof and erlotinib or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention is directed to a pharmaceutical combination for the treatment of pancreatic cancer wherein the combination comprises a cyclin dependent kinase (CDK) inhibitor selected from the compounds of formula I or a

pharmaceutically acceptable salt or a solvate thereof and lapatinib or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention is directed to a pharmaceutical combination for the treatment of pancreatic cancer wherein the combination comprises a CDK inhibitor selected from the compound A or compound B and a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention is directed to a pharmaceutical combination for the treatment of pancreatic cancer wherein the combination comprises the compound A and erlotinib or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention is directed to a pharmaceutical combination for the treatment of pancreatic cancer wherein the combination comprises the compound A and lapatinib or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention encompasses within its scope inclusion of gemcitabine to the pharmaceutical combination comprising a CDK inhibitor selected from the compounds of formula I and a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof. Accordingly, in this embodiment, the present invention relates to a pharmaceutical combination comprising a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt or a solvate thereof; a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof; and gemcitabine.

In another embodiment, the present invention is directed to a pharmaceutical combination for the treatment of pancreatic cancer wherein the combination comprises a cyclin dependent kinase (CDK) inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt or a solvate thereof; erlotinib or a pharmaceutically acceptable salt thereof; and gemcitabine or a pharmaceutically acceptable salt.

In another embodiment, the present invention is directed to a pharmaceutical combination for the treatment of pancreatic cancer wherein the combination comprises a cyclin dependent kinase (CDK) inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt or a solvate thereof; lapatinib or a pharmaceutically acceptable salt thereof and gemcitabine or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention is directed to a pharmaceutical

combination for the treatment of pancreatic cancer wherein the combination comprises a cyclin dependent kinase (CDK) inhibitor selected from the compound A or compound B; a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof; and gemcitabine or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention is directed to a pharmaceutical combination for the treatment of pancreatic cancer wherein the combination comprises the compound A; erlotinib or a pharmaceutically acceptable salt thereof and gemcitabine or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention is directed to a pharmaceutical combination for the treatment of pancreatic cancer wherein the combination comprises the compound A; lapatinib or a pharmaceutically acceptable salt thereof and gemcitabine or a pharmaceutically acceptable salt thereof.

In one embodiment, the pharmaceutical combination comprising the CDK inhibitor selected from the compounds of formula I and erlotinib or lapatinib, or the pharmaceutical combination comprising the CDK inhibitor; erlotinib or lapatinib and gemcitabine; is not exclusively limited to those combinations which are obtained by physical association of said ingredients, but also encompass those which permit a separate administration, which can be simultaneous, sequential or spaced out over a period of time so as to obtain maximum efficacy of the combination. Thus, the pharmaceutical combination may be administered simultaneously or spaced out over a period of time for an effective treatment of pancreatic cancer.

In one embodiment, the present invention relates to a pharmaceutical composition which comprises a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt or solvate thereof and a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof; in association with a pharmaceutically acceptable excipient or carrier.

In another embodiment, the present invention relates to a pharmaceutical composition which comprises a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt or solvate thereof and erlotinib or a pharmaceutically acceptable salt thereof; in association with a pharmaceutically acceptable excipient or carrier.

In another further embodiment, the present invention relates to a pharmaceutical composition which comprises a CDK inhibitor selected from the compounds of formula I or a

pharmaceutically acceptable salt or solvate thereof and lapatinib or a pharmaceutically acceptable salt thereof; in association with a pharmaceutically acceptable excipient or carrier.

In another further embodiment, the present invention relates to a pharmaceutical composition which comprises a CDK inhibitor selected from the compound A or compound B and a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof; in association with a pharmaceutically acceptable excipient or carrier.

In another further embodiment, the present invention relates to a pharmaceutical composition which comprises the compound A and erlotinib or a pharmaceutically acceptable salt thereof; in association with a pharmaceutically acceptable excipient or carrier.

In another further embodiment, the present invention relates to a pharmaceutical composition which comprises the compound A and lapatinib or a pharmaceutically acceptable salt thereof; in association with a pharmaceutically acceptable excipient or carrier.

According to one embodiment, the present invention relates to a pharmaceutical composition comprising a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt or solvate thereof and a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof; in association with a pharmaceutically acceptable excipient or carrier; which composition further comprises gemcitabine or a pharmaceutically acceptable salt thereof. Accordingly, in this embodiment, the present invention relates to pharmaceutical composition comprising a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt or solvate thereof; a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof; and gemcitabine; in association with a pharmaceutically acceptable excipient or carrier.

In another embodiment, the present invention relates to a pharmaceutical composition which comprises a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt or solvate thereof; erlotinib or a pharmaceutically acceptable salt thereof and gemcitabine or a pharmaceutically acceptable salt thereof; in association with a pharmaceutically acceptable excipient or carrier.

In another further embodiment, the present invention relates to a pharmaceutical composition which comprises a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt or solvate thereof; lapatinib or a pharmaceutically acceptable salt thereof; and gemcitabine or a pharmaceutically acceptable salt thereof; in

association with a pharmaceutically acceptable excipient or carrier.

In another embodiment, the present invention relates to a pharmaceutical composition comprising a CDK inhibitor selected from the compound A or compound B; a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof; and gemcitabine or a pharmaceutically acceptable salt thereof; in association with a pharmaceutically acceptable excipient or carrier.

In another further embodiment, the present invention relates to a pharmaceutical composition comprising the compound A; erlotinib or a pharmaceutically acceptable salt thereof; and gemcitabine or a pharmaceutically acceptable salt thereof; in association with a pharmaceutically acceptable excipient or carrier.

In another further embodiment, the present invention relates to a pharmaceutical composition comprising the compound A; lapatinib or a pharmaceutically acceptable salt thereof; and gemcitabine or a pharmaceutically acceptable salt thereof; in association with a pharmaceutically acceptable excipient or carrier.

According to one embodiment, the present invention is directed to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt thereof; and a therapeutically effective amount of a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof; wherein said CDK inhibitor and said compound capable of inhibiting EGFR kinase activity are administered simultaneously or sequentially.

In another embodiment, the present invention is directed to a method for the treatment of pancreatic cancer in a subject, which comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of erlotinib or a pharmaceutically acceptable salt thereof; wherein said CDK inhibitor and erlotinib are administered simultaneously or sequentially .

In another further embodiment, the present invention is directed to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of lapatinib or a pharmaceutically acceptable salt thereof, wherein said CDK inhibitor and lapatinib are administered simultaneously or sequentially.

In another further embodiment, the present invention is directed to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compound A or compound B and a therapeutically effective amount of a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof; wherein said compound A or compound B and erlotinib are administered simultaneously or sequentially.

In another further embodiment, the present invention is directed to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of the compound A and a therapeutically effective amount of erlotinib or a pharmaceutically acceptable salt thereof; wherein said compound A and erlotinib are administered simultaneously or sequentially.

In another further embodiment, the present invention is directed to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of the compound A and a therapeutically effective amount of lapatinib or a pharmaceutically acceptable salt thereof; wherein said compound A and lapatinib are administered simultaneously or sequentially.

In another embodiment, the present invention is directed to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of the compound A, and a therapeutically effective amount of erlotinib or a pharmaceutically acceptable salt thereof; wherein said compound A and erlotinib are administered sequentially such that the compound A is administered before or after erlotinib.

In another further embodiment, the present invention is directed to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of compound A, and a therapeutically effective amount of lapatinib or a pharmaceutically acceptable salt thereof; wherein compound A and lapatinib are administered sequentially such that the compound A is administered before or after lapatinib.

According to one embodiment of the present invention, said method for the treatment of pancreatic cancer in a subject; further comprising administering to the subject a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; in addition to administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt

thereof and a therapeutically effective amount of a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof. Accordingly, in this embodiment the present invention also relates to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt thereof; a therapeutically effective amount of a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof; and a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; wherein said CDK inhibitor, said compound capable of inhibiting EGFR kinase activity and gemcitabine are administered simultaneously or sequentially.

In another embodiment , the present invention relates to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt thereof; a therapeutically effective amount of a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof; and a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; wherein said CDK inhibitor, said compound capable of inhibiting EGFR kinase activity and gemcitabine are administered simultaneously.

In another embodiment , the present invention relates to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt thereof; a therapeutically effective amount of a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof; and a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; wherein said CDK inhibitor; said compound capable of inhibiting EGFR kinase activity and gemcitabine are administered sequentially such that gemcitabine is administered before or after said CDK inhibitor and said compound capable of inhibiting EGFR kinase activity are administered.

According to another embodiment, the present invention is directed to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula

I or a pharmaceutically acceptable salt thereof; a therapeutically effective amount of erlotinib or a pharmaceutically acceptable salt thereof; and a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; wherein the CDK inhibitor, erlotinib and gemcitabine are administered simultaneously or sequentially.

According to another embodiment, the present invention is directed to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt thereof; a therapeutically effective amount of lapatinib or a pharmaceutically acceptable salt of erlotinib; and a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; wherein the CDK inhibitor, lapatinib and gemcitabine are administered simultaneously or sequentially.

According to another embodiment, the present invention is directed to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt thereof; a therapeutically effective amount of erlotinib or a pharmaceutically acceptable salt thereof; and a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; wherein the CDK inhibitor, erlotinib and gemcitabine are administered simultaneously.

According to another embodiment, the present invention is directed to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt thereof; a therapeutically effective amount of erlotinib or a pharmaceutically acceptable salt thereof; and a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; wherein the CDK inhibitor, erlotinib and gemcitabine are administered sequentially such that gemcitabine is administered before or after the CDK inhibitor and erlotinib are administered.

According to another embodiment, the present invention is directed to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt thereof; a therapeutically effective amount of lapatinib or a pharmaceutically acceptable salt thereof; and a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; wherein the CDK inhibitor, lapatinib and gemcitabine are administered sequentially such that gemcitabine is

administered before or after the CDK inhibitor and lapatinib are administered.

According to another embodiment, the present invention relates to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from compound A or compound B; a therapeutically effective amount of a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib or a pharmaceutically acceptable salt thereof; and a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; wherein said CDK inhibitor, said compound capable of inhibiting EGFR kinase activity and gemcitabine are administered simultaneously or sequentially.

According to another embodiment, the present invention relates to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of compound A; a therapeutically effective amount of erlotinib or a pharmaceutically acceptable salt thereof; and a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; wherein said compound A, erlotinib and gemcitabine are administered simultaneously or sequentially.

According to another embodiment, the present invention relates to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of compound A; a therapeutically effective amount of erlotinib or a pharmaceutically acceptable salt thereof; and a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; wherein said compound A, erlotinib and gemcitabine are administered simultaneously.

According to another embodiment, the present invention relates to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of compound A; a therapeutically effective amount of erlotinib or a pharmaceutically acceptable salt thereof; and a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; wherein said compound A, erlotinib and gemcitabine are administered sequentially such that gemcitabine is administered before or after the administration of compound A and erlotinib.

According to another embodiment, the present invention relates to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of compound A; a therapeutically effective amount of lapatinib or a pharmaceutically acceptable salt thereof; and a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; wherein said compound A,

lapatinib and gemcitabine are administered simultaneously or sequentially.

According to another embodiment, the present invention relates to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of compound A; a therapeutically effective amount of lapatinib or a pharmaceutically acceptable salt thereof; and a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; wherein said compound A, lapatinib and gemcitabine are administered simultaneously.

According to another embodiment, the present invention relates to a method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of compound A; a therapeutically effective amount of lapatinib or a pharmaceutically acceptable salt thereof; and a therapeutically effective amount of gemcitabine or a pharmaceutically acceptable salt thereof; wherein said compound A, lapatinib and gemcitabine are administered sequentially such that gemcitabine is administered before or after the administration of compound A and lapatinib.

The administration of the triple combination consisting of the CDK inhibitor of formula I , a compound capable of inhibiting EGFR kinase activity selected from erlotinib or lapatinib and gemcitabine may produce effects, such as anti-cancer effects, greater than those achieved with any of the CDK inhibitor of formula I or erlotinib/lapatinib and gemcitabine used alone, greater than those achieved with the combination of the CDK inhibitor of formula I and erlotinib or the CDK inhibitor of formula I and lapatinib.

In one embodiment, the constituents comprised in the combination may have to be administered by different routes, because of their different physical and chemical characteristics. For example, the CDK inhibitors of formula I may be administered either orally or parenterally to generate and maintain good blood levels thereof, while gemcitabine and the compound capable of inhibiting EGFR kinase may be administered orally or parenterally, by intravenous, subcutaneous or intramuscular route.

Combinations and compositions intended for pharmaceutical use may be prepared according to any method known in the art for the manufacture of pharmaceutical combinations and compositions, e.g. *Remington – The Science and Practice of Pharmacy (21st Edition)* (2005), *Goodman & Gilman's The Pharmacological Basis of Therapeutics (11th Edition)* (2006) and *Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems (9th Edition)*, edited by Allen et al., Lippincott Williams & Wilkins, (2011), *Solid-State Chemistry of Drugs (2nd Edition)*(1999), each of which is hereby incorporated by reference."

The compositions described herein may be in a form suitable for oral administration, for example as a tablet or capsule, for nasal administration or administration by inhalation, for example as a powder or solution, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) for example as a sterile solution, suspension or emulsion, for topical administration for example as an ointment or cream, for rectal administration for example as a suppository or the route of administration may be by direct injection into the tumor or by regional delivery or by local delivery

For oral use, the CDK inhibitors of formula I may be administered, for example, in the form of tablets or capsules, powders, dispersible granules, or cachets, or as aqueous solutions or suspensions. In the case of tablets for oral use, carriers which are commonly used include lactose, corn starch, magnesium carbonate, talc, and sugar, and lubricating agents such as magnesium stearate are commonly added. For oral administration in capsule form, useful carriers include lactose, corn starch, magnesium carbonate, talc and sugar.

For intramuscular, intraperitoneal, subcutaneous and intravenous use, sterile solutions of the active ingredient (the CDK inhibitor as described herein or the compound capable of inhibiting EGFR kinase activity or gemcitabine) are usually employed, and the pH of the solutions should be suitably adjusted and buffered.

In a preferred embodiment, the therapeutic agents contained in the combination of the invention are formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to humans. Typically, the therapeutic agents contained in the combination of the present invention for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the compositions may also include a solubilizing agent. Compositions for intravenous administration may optionally include a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachet indicating the quantity of active agent. Where one or more of the therapeutic agents contained in the combination of the present invention is/are to be administered by infusion, it can be dispensed, for example, with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the therapeutic agents contained in the combination of the present invention is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

Compositions for oral delivery may be in the form of tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixirs. Orally administered compositions may contain one or more optional agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation. Moreover, wherein tablet or pill form, the compositions may be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving compound are also suitable for orally administered compounds or anticancer agents contained in the combination of the invention. In these later platforms, fluid from the environment surrounding the capsule is imbibed by the driving compound (the anticancer agent(s)), which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time delay material such as glycerol monostearate or glycerol stearate may also be used. Oral compositions can include standard vehicles such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Such vehicles are preferably of pharmaceutical grade.

Further, the effect of the compounds (anticancer agents) contained in the pharmaceutical combination of this invention may be delayed or prolonged by proper formulation. For example, a slowly soluble pellet of the compound may be prepared and incorporated in a tablet or capsule. The technique may be improved by making pellets of several different dissolution rates and filling capsules with a mixture of the pellets. Tablets or capsules may be coated with a film which resists dissolution for a predictable period of time. Even the parenteral preparations may be made long-acting, by dissolving or suspending the compound (anticancer agent) in oily or emulsified vehicles which allow it to disperse only slowly in the serum.

The compound capable of inhibiting EGFR kinase activity can be administered either separately or together with the CDK inhibitor of formula I when used in double combination or together with the CDK inhibitor of formula I and gemcitabine when used in triple combination by the same or different routes, and in a wide variety of different dosage forms. For example, the compound capable of inhibiting EGFR kinase activity is preferably administered orally or parenterally where the compound capable of inhibiting the EGFR

kinase activity is erlotinib HCl (TARCEVA®), oral administration is preferable.

The compound capable of inhibiting the EGFR kinase activity can be administered with various pharmaceutically acceptable inert carriers in the form of tablets, capsules, lozenges, troches, hard candies, powders, sprays, creams, salves, suppositories, jellies, gels, pastes, lotions, ointments, elixirs, syrups, and the like. Administration of such dosage forms can be carried out in single or multiple doses. Carriers include solid diluents or fillers, sterile aqueous media and various non-toxic organic solvents, etc. Oral pharmaceutical compositions can be suitably sweetened and/or flavored.

In another embodiment, the present invention relates to a method for the treatment of pancreatic cancer, which method comprises administering to a subject in need of such a treatment a therapeutically effective amount of said combination. Accordingly, in the method of the present invention, pancreatic cancer is treated in a subject by administering to the subject a therapeutic amount of gemcitabine to treat the cancer, in combination with a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula I or a pharmaceutically acceptable salt or a solvate thereof and a compound capable of inhibiting EGFR kinase activity, wherein a synergistic effect results.

Synergism between the therapeutic agents (anticancer agents) namely the CDK inhibitors selected from the compounds of formula I , a compound capable of inhibiting EGFR kinase activity such as erlotinib or lapatinib and gemcitabine when used in combination are assayed in chosen pancreatic cell lines. Standard cytotoxicity assays are performed in the cell line, essentially as described in the experimental section, using fixed or variable ratios of the therapeutic agents when used in combination compared to either therapeutic agent alone indicating synergism. The results are analyzed using the Combination Index (CI) method by which a CI value less than 1 indicates synergy, equal to 1 indicates additive effect, and greater than 1 indicates antagonism.

The present invention encompasses methods for treating or managing pancreatic cancer in a patient comprising administering to the patient a CDK inhibitor selected from the compounds of formula I in combination with a compound capable of inhibiting EGFR kinase and further in combination with gemcitabine. The term "combination" is not limited to the administration of the therapeutic agents (anticancer agents) at exactly the same time, but rather it is meant that CDK inhibitor selected from the compounds of formula I and the other therapeutic agents are administered to a subject in a sequence and within a time interval such that the CDK inhibitor can act together with the other therapeutic agents to provide an

increased benefit than if they were administered otherwise. For example, each therapeutic agent may be administered at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic effect. Each therapeutic agent can be administered separately, in any appropriate form and by any suitable route of administration.

In an embodiment of the invention, the anticancer agents contained in the combination can be administered in a staggered regimen, i.e., with the CDK inhibitor being given at a different time during the course of the cycle than the compound capable of inhibiting EGFR kinase activity and of gemcitabine. This time differential may range from several minutes, hours, days, weeks, or longer between administration of the at least two anticancer agents. Therefore, the term combination (or combined) does not necessarily mean administered at the same time or as a unitary dose or single composition, but that each of the components are administered during a desired treatment period. The anticancer agents may also be administered by different routes. In one embodiment, 1 “cycle” includes 21 days. These regimens or cycles may be repeated, or alternated, as desired. Other dosage regimens and variations are foreseeable, and are determined through physician guidance."

In specific embodiments, the therapeutic agents are administered in a time frame where both agents are still active. One skilled in the art would be able to determine such a time frame by determining the half life of the administered therapeutic agents.

As indicated herein before, the active ingredients contained in the pharmaceutical composition can be administered simultaneously or sequentially.

For effective administration of the therapeutic agents contained in the pharmaceutical combination of the present invention, particularly when the double combination is used, include administering erlotinib in a general dose range of 25 mg to 300 mg; and the CDK inhibitor of formula I e.g. the compound A in a general dose range of 100 mg/m2/day to 185 mg/m2/day.

For effective administration of the therapeutic agents contained in the pharmaceutical combination of the present invention, particularly when the triple combination is used, include administering erlotinib in a general dose range of 25 mg to 300 mg; and the CDK inhibitor of formula I e.g. the compound A in a general dose range of 100 mg/m2/day to 200 mg/m2/day and gemcitabine in a general dose range of 200mg/m2 Dose to 1000mg/m2 Dose.

Those skilled in the art will recognize that several variations are possible within the

scope and spirit of this invention. The invention will now be described in greater detail by reference to the following non-limiting examples. The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

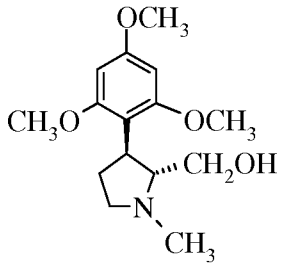
Example 1:

A) General procedure for the preparation of the CDK inhibitors compounds of Formula I:

The compounds of formula I may be prepared according to the methods disclosed in PCT Patent Publication No. WO2004004632 and PCT Patent Publication No. WO2007148158 which are incorporated herein by reference.

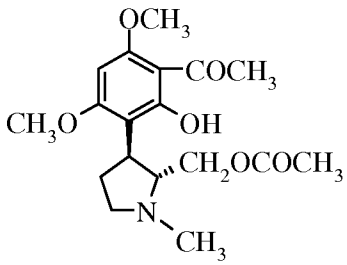
The general process for the preparation of the compound of formula I, or a pharmaceutically acceptable salt thereof, comprises the following steps:

(a) treating the resolved enantiomerically pure (-)-trans enantiomer of the intermediate compound of formula VIA,



VIA

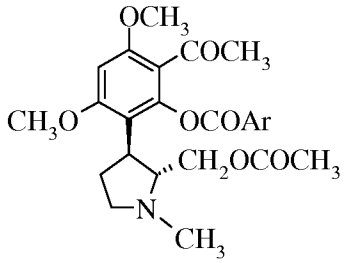
with acetic anhydride in the presence of a Lewis acid catalyst to obtain a resolved acetylated compound of formula VIIA,



VIIA

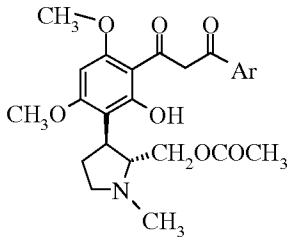
(b) reacting the resolved acetylated compound of formula VIIA with an acid of formula ArCOOH or an acid chloride of formula ArCOCl or an acid anhydride of formula $(ArCO)_2O$ or an ester of formula $ArCOOCH_3$, wherein Ar is as defined hereinabove in reference to the compound of formula I, in the presence of a base and a solvent to obtain a resolved

compound of formula VIIIA;



VIIIA

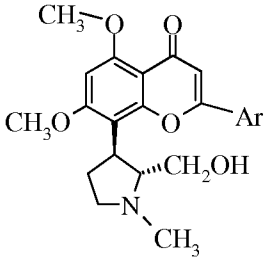
(c) treating the resolved compound of formula VIIIA with a base in a suitable solvent to obtain the corresponding resolved β-diketone compound of formula IXA;



IXA

where Ar is as defined above;

(d) treating the resolved β-diketone compound of formula IXA with an acid such as hydrochloric acid to obtain the corresponding cyclized compound of formula XA,



XA

(e) subjecting the compound of formula XA to dealkylation by heating it with a dealkylating agent at a temperature ranging from 120-180 °C to obtain the (+)-*trans* enantiomer of the compound of formula I and, optionally, converting the subject compound into its pharmaceutically acceptable salt.

The Lewis acid catalyst utilized in the step (a) above may be selected from: BF_3, Et_2O, zinc chloride, aluminium chloride and titanium chloride.

The base utilized in the process step (b) may be selected from triethylamine, pyridine and a DCC-DMAP combination (combination of N, N'-dicyclohexyl carbodiimide and 4-dimethylaminopyridine).

It will be apparent to those skilled in the art that the rearrangement of the compound of formula VIIIA to the corresponding β-diketone compound of formula IXA is known as a *Baker-Venkataraman* rearrangement (J. Chem. Soc., 1381 (1933) and Curr. Sci., 4, 214 (1933)).

The base used in the process step (c) may be selected from: lithium hexamethyl disilazide, sodium hexamethyldisilazide, potassium hexamethyldisilazide, sodium hydride and potassium hydride. A preferred base is lithium hexamethyl disilazide.

The dealkylating agent used in process step (e) for the dealkylation of the compound of formula IXA may be selected from: pyridine hydrochloride, boron tribromide, boron trifluoride etherate and aluminium trichloride. A preferred dealkylating agent is pyridine hydrochloride.

Preparation of the starting compound of formula VIA involves reacting 1-methyl-4-piperidone with a solution of 1,3,5-trimethoxybenzene in glacial acetic acid, to yield 1-methyl-4-(2,4,6-trimethoxyphenyl)-1,2,3,6-tetrahydropyridine, which is reacted with boron trifluoride diethyl etherate, sodium borohydride and tetrahydrofuran to yield 1-methyl-4-(2,4,6-trimethoxyphenyl)piperidin-3-ol. Conversion of 1-methyl-4-(2,4,6-trimethoxyphenyl)piperidin-3-ol to the compound of formula VlA involves converting the hydroxyl group present on the piperidine ring of the compound, 1-methyl-4-(2,4,6-trimethoxyphenyl)piperidin-3-ol to a leaving group such as tosyl, mesyl, triflate or halide by treatment with an appropriate reagent such as p-toluenesulfonylchloride, methanesulfonylchloride, triflic anhydride or phosphorous pentachloride in the presence of oxygen nucleophiles such as triethylamine, pyridine, potassium carbonate or sodium carbonate, followed by ring contraction in the presence of oxygen nucleophiles such as sodium acetate or potassium acetate in an alcoholic solvent such as isopropanol, ethanol or propanol.

B) Preparation of (+)-trans-2-(2-Chlorophenyl)-5, 7-dihydroxy-8-(2-hydroxymethyl-1-methyl-pyrrolidin-3-yl)-chromen-4-one hydrochloride (Compound A)

Molten pyridine hydrochloride (4.1 g, 35.6 mmol) was added to (+)-trans-2-(2-

chloro-phenyl)-8-(2-hydroxymethyl-1-methyl-pyrrolidin-3-yl)-5,7-dimethoxy-chromen-4-one (0.4 g, 0.9 mmol) and heated at 180 °C for 1.5 h. The reaction mixture was cooled to 25 °C, diluted with MeOH (10 mL) and basified using Na_2CO_3 to pH 10. The mixture was filtered and the organic layer was concentrated. The residue was suspended in water (5 mL), stirred for 30 min., filtered and dried to obtain the compound, (+)-trans-2-(2-chloro-phenyl)-5,7-dihydroxy-8-(2-hydroxymethyl-1-methyl-pyrrolidin-3-yl)-chromen-4-one .

Yield: 0.25 g (70 %);

IR (KBr): 3422, 3135, 1664, 1623, 1559 cm-1;

1H NMR (CDCl3, 300MHz): δ 7.56 (d, 1H), 7.36 (m, 3H), 6.36 (s, 1H), 6.20 (s, 1H), 4.02 (m, 1H), 3.70 (m, 2H), 3.15 (m, 2H), 2.88 (m, 1H), 2.58 (s, 3H), 2.35 (m, 1H), 1.88 (m, 1H);

MS (ES+): m/z 402 (M+1);

Analysis: C21H20ClNO5 C, 62.24 (62.71); H, 5.07 (4.97); N, 3.60 (3.48); Cl, 9.01 (8.83).

The compound (0.2 g, 0.48 mmol) as obtained above was suspended in IPA (5 mL) and 3.5 % HCl (25 mL) was added. The suspension was heated to get a clear solution. The solution was cooled and solid filtered to obtain the compound, (+)-trans-2-(2-Chlorophenyl)-5,7-dihydroxy-8-(2-hydroxymethyl-1-methyl-pyrrolidin-3-yl)-chromen-4-one hydrochloride.

Yield: 0.21 g (97 %); mp: 188 – 192 °C ; [α]D25 = +21.3° (c = 0. 2, methanol);

1H NMR (CD3OD, 300MHz): δ 7.80 (d, 1H), 7.60 (m, 3H), 6.53 (s, 1H), 6.37 (s, 1H), 4.23 (m, 1H), 3.89 (m, 2H), 3.63 (m, 1H), 3.59 (dd, 1H), 3.38 (m, 1H), 2.90 (s, 3H), 2.45 (m, 1H), 2.35 (m, 1H); MS (ES+): m/z 402 (M +1)(free base).

This compound was subjected to chiral HPLC. Chiral HPLC was done using column Chiralcel OD-H (250 x 4.6 mm) and solvent system haxane:ethanol (92:08) with TFA (0.4%). The results are recorded at 264nm with solvent flow rate of 1mL/min. As depicted in Figure 3, the chiral HPLC showed 100% e.e of the compound, (+)-trans-2-(2-chloro-phenyl)-5,7-dihydroxy-8-(2-hydroxy-methyl-1-methyl-pyrrolidin-3-yl)-chromen-4-one hydrochloride

C) Preparation of (+)-trans-2-(2-chloro-4-trifluoromethyl-phenyl)-5,7-dihydroxy-8-(2-hydroxymethyl-1-methyl-pyrrolidin-3-yl)-chromen-4-one hydrochloride (Compound B)

A mixture of the compound, (+)-trans-2-(2-Chloro-4-trifluoromethylphenyl)-8-(2-hydroxymethyl-1-methyl pyrrolidin-3-yl)-5,7-dimethoxy-chromen-4-one (0.25 g, 0.5 mmol), pyridine hydrochloride (0.25 g, 2.16 mmol) and a catalytic amount of quinoline was heated at 180 °C for a period of 2.5 hrs. The reaction mixture was diluted with methanol (25 mL)

and basified with solid Na_2CO_3 to pH 10. The reaction mixture was filtered, and washed with methanol. The organic layer was concentrated and the residue purified by column chromatography using 0.1 % ammonia and 4.5 % methanol in chloroform as eluent to yield the compound, (+)-trans-2-(2-chloro-4-trifluoromethylphenyl)-5,7-dihydroxy-8-(2-hydroxy-methyl-1-methylpyrrolidin-3-yl)-chromen-4-one, as a yellow solid.

Yield: 0.15 g (63.7 %);

1H NMR (CDCl3, 300MHz): δ 7.99 (m, 2H), 7.83 (d, 1H), 6.65 (s, 1H), 6.41 (s, 1H), 4.24 (m, 1H), 3.90 (m, 2H), 3.70 (m, 1H), 3.60 (m, 1H), 3.41 (m, 1H), 2.99 (s, 3H), 2.54 (m, 1H), 2.28 (m, 1H); MS (ES+): m/z 470 (M+1).

The compound (0.1 g, 0.2 mmol) as obtained above was suspended in methanol (2 mL) and treated with ethereal HCl and the organic solvent evaporated to yield the compound, (+)-trans-2-(2-chloro-4-trifluoromethyl-phenyl)-5,7-dihydroxy-8-(2-hydroxymethyl-1-methyl-pyrrolidin-3-yl)-chromen-4-one hydrochloride.

Yield: 0.1g (92.8 %);

1H NMR (CDCl3, 300MHz): δ 8.02 (d, 2H), 7.83 (d, 1H), 6.64 (s, 1H), 6.41 (s, 1H), 4.23 (m, 1H), 3.73 (m, 2H), 3.68 (m, 1H), 3.51 (m, 1H), 3.39 (m, 1H), 2.99 (s, 3H), 2.54 (m, 1H), 2.31 (m, 1H).

IN VITRO STUDIES INVOLVING USE OF THE DOUBLE COMBINATION CONSISTING OF THE COMPOUND A AND ERLOTINIB

Example 2:

Combination studies of compound A with erlotinib in cytotoxicity assay using Propidium Iodide (PI)

The propidium iodide fluorescence assay (PI) was carried out according to the procedure mentioned in Anticancer Drugs, 1995, 6, 522–32 which is incorporated herein by reference.

The assay was developed to characterize the *in vitro* growth of human tumor cell lines as well as to test the cytotoxic activity of the anticancer agents ("the test compounds"). Propidium iodide (PI) was used as a dye, which penetrates the damaged cellular membranes only. Intercalation complexes are formed by PI with double-stranded DNA, which effect an amplification of the fluorescence. After freezing the cells at –70°C for 24 h, PI had access to total DNA leading to total cell population counts. Background readings were obtained

from cell-free wells containing only the media and propidium iodide.

The human pancreatic cancer cell lines, Panc-1 and AsPc-1 (Source: ATCC (American Type Culture collection)) were seeded at a density of 1500-3000 cells/well in 180 µL of MEM (Minimum Essential medium, SAFC, USA) and RPMI 1460 (Sigma, USA), along with 10 % FCS (fetal calf serum) in a 96-well plate and incubated for about 16 h to allow the cells to adhere. The cells were then treated with the compound A at different concentrations corresponding to IC_{30}, IC_{50} or IC_{70} together with erlotinib (LC Laboratories, USA) at different concentrations corresponding to IC_{30}, IC_{50} or IC_{70} or the cells were treated with different concentrations (IC_{30}, IC_{50} or IC_{70}) of compound A alone or with different concentrations (IC_{30}, IC_{50} or IC_{70}) of erlotinib alone as shown in the Figures 1a, 1b, 2a and 2b. The IC_{30}, IC_{50} and IC_{70} concentrations of compound A and of erlotinib for Panc-1 and AsPc-1 cells are specified in Table 3 as presented herein below. The period of treatment was either 72 hours or 96 hours. The plates were incubated in humidified 5 % CO_2 incubator at 37 °C ± 1°C. Control wells were treated with vehicle (DMSO). At the end of the incubation periods, the medium was removed from the wells and washed with phosphate buffered saline (PBS). 100 µL of PI working solution (7 µg/mL) per well was added and the plates were stored at -80°C for about 16 hours. The plates were thawed and the fluorescence was measured using the POLARstar optima plate reader (USA) at excitation 536 nm and emission 590 nm.

The percentage (%) inhibition was calculated and the combination index was determined using the Compusyn software (ComboSyn, Inc. USA). See *Therapeutical Basis, Experimental Design, and Computerized Simulation of Synergism and Antagonism in Drug Combination Studies,* CHOU, Ting-Chao. Pharmacol. Rev., vol. 58, no. 3, pgs. 621-681 (2006), which is hereby incorporated by reference. Combination index (C.I) is used to evaluate the synergism between 2 or more compounds. C.I <1 indicates that the combination is synergistic, C.I = 1 indicates that the combination is additive and C.I > 1 indicates that the combination is antagonistic. In one embodiment of the invention, the C.I is < 1.

(PI stock solution of 1mg/mL was prepared by dissolving 1 mg PI in 1 mL of distilled water. PI working solution was prepared by adding 140 µL of stock solution to PBS to make up the volume to 220 mL (7 µg/mL))

The results are presented in the following Tables 1a, 1b, 2a and 2b. Further, a graphical representation in Figures 1a and 1b provides the percentage inhibition results of single and combination dosing of compound A and erlotinib in Panc-1 cells at the end of

72 hrs and 96 hrs respectively. The Figures 2a and 2b provides graphical representation of the percentage inhibition results of single and combination dosing of compound A and erlotinib in AsPc-1 cells at the end of 72 hrs and 96 hrs respectively.

In the following tables 1a, 1b, 2a and 2b, the symbol "+" indicates that the anticancer agents (the test compounds) are used simultaneously.

Table 1a - Combination index (C.I) calculated for the combination of compound A (at different concentration) with erlotinib (at different concentration) in Panc-1 cells after 72 h of treatment

Combination of compound A and Erlotinib	Combination index (C.I) Values
IC_{30} Compound A+ IC_{50} Erlotinib	0.5
IC_{30} Compound A+ IC_{70} Erlotinib	0.2
IC_{50} Compound A+ IC_{30} Erlotinib	0.8
IC_{50} Compound A+ IC_{50} Erlotinib	0.4
IC_{50} Compound A+ IC_{70} Erlotinib	0.1
IC_{70} Compound A+ IC_{30} Erlotinib	0.7
IC_{70} Compound A+ IC_{50} Erlotinib	0.5
IC_{70} Compound A+ IC_{70} Erlotinib	0.2

Table 1b - Combination index (C.I) calculated for the combination of compound A (at different concentration) with erlotinib (at different concentration) in Panc-1 cells after 96 h of treatment

Combination of compound A and Erlotinib	Combination index (C.I) Values
IC_{30} Compound A+ IC_{50} Erlotinib	0.5
IC_{30} Compound A+ IC_{70} Erlotinib	0.1
IC_{50} Compound A+ IC_{30} Erlotinib	0.8
IC_{50} Compound A+ IC_{50} Erlotinib	0.4
IC_{50} Compound A+ IC_{70} Erlotinib	0.1
IC_{70} Compound A+ IC_{30} Erlotinib	0.7
IC_{70} Compound A+ IC_{50} Erlotinib	0.4
IC_{70} Compound A+ IC_{70} Erlotinib	0.1

Table 2a - Combination index (C.I) calculated for the combination of compound A (at different concentration) with erlotinib (at different concentration) in AsPc-1 cells after 72 h of treatment

Combination of compound A and Erlotinib	Combination index (C.I) Values
IC_{30} Compound A+ IC_{30} Erlotinib	0.6
IC_{30} Compound A+ IC_{50} Erlotinib	0.3
IC_{50} Compound A+ IC_{30} Erlotinib	0.3
IC_{50} Compound A+ IC_{50} Erlotinib	0.3
IC_{70} Compound A+ IC_{30} Erlotinib	0.5
IC_{70} Compound A+ IC_{50} Erlotinib	0.6

Table 2b - Combination index (C.I) calculated for the combination of compound A (at different concentration) with erlotinib (at different concentration) in AsPc-1 cells after 96 h of treatment

Combination of compound A and Erlotinib	Combination index (C.I) Values
IC_{30} Compound A+ IC_{30} Erlotinib	0.4
IC_{30} Compound A+ IC_{50} Erlotinib	0.1
IC_{50} Compound A+ IC_{30} Erlotinib	0.3
IC_{50} Compound A+ IC_{50} Erlotinib	0.2
IC_{70} Compound A+ IC_{30} Erlotinib	0.4
IC_{70} Compound A+ IC_{50} Erlotinib	0.4

Table 3 - The 30, 50 and 70 percent inhibitory concentrations (IC_{30}, IC_{50} and IC_{70}) of the compound A and erlotinib in Panc-1 and AsPc-1 cell lines

Cell lines	Compounds	Concentration (μM)		
		IC_{30}	IC_{50}	IC_{70}
Panc-1	Compound A	0.4	0.6	0.7
	Erlotinib	5	12	22
AsPc-1	Compound A	0.7	1.5	3.0
	Erlotinib	10	30	-

IN VITRO STUDIES INVOLVING USE OF THE TRIPLE COMBINATION CONSISTING OF THE COMPOUND A, ERLOTINIB/LAPATINIB AND GEMCITABINE

Example 3:

Materials: Gemcitabine, lapatinib and erlotinib were obtained from LC Labs, USA. CCK-8 cytotoxicity kit was procured from Dojindo Molecular Technologies, Japan Culture media and fetal bovine serum (FBS) was obtained from Sigma (St. Louis, MO) and Gibco (Paisley, Scotland) respectively. Panc-1 and Mia Paca-2 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% FBS, Penicillin-Streptomycin Solution Stabilized, sterile-filtered, with 5,000 units penicillin and 5 mg streptomycin/mL (P-4458, Sigma-Aldrich, USA). The cells were grown in 75-cm^2 culture flasks and kept in a humidified (37°C, 5% CO_2) incubator. Cells were passaged on reaching 80% confluence.

Cell proliferation assay: Logarithmically growing cells were plated at a density of 3 x 10^3 cells/well and allowed to recover overnight. The cells were challenged with varying concentration in the range of 10 nM to 30 μM of different anticancer agents (gemcitabine, compound A, lapatinib and erlotinib) and control cells received Dulbecco's modified Eagle's medium (DMEM) with 10% serum containing dimethyl sulfoxide (DMSO) vehicle at a concentration of 0.2%. After 72 hrs, cell toxicity was determined by CCK-8 reagent

(Dojindo Molecular Technologies, Japan); (WST-1 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfophenyl)]-2H-tetrazolium, monosodium salt assay). In accordance with the manufacturer's instructions, 5µl/well CCK-8 reagent was added and plates were incubated for 2 hours. The toxicity was determined by measuring the absorbance on Tecan Sapphire multi-fluorescence micro-plate reader (Tecan, Germany, GmbH) at a wavelength of 450 nm corrected to 650 nm and normalized to controls.

A CCK-8 (Cell Counting Kit-8) non-radioactive colorimetric assay was carried out to characterize the *in vitro* growth of Panc1 and Mia Paca2 cells as well as to test the cytotoxic activity of the anticancer agents , gemcitabine, compound A, lapatinib and erlotinib when used in combination. CCK-8 allows convenient assays using Dojindo's tetrazolium salt, WST 8-(2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H- tetrazolium, monosodium salt), which produces a water-soluble formazan dye upon bioreduction in the presence of an electron carrier, 1-Methoxy PMS. CCK-8 solution is added directly to the cells; no pre-mixing of components is required. CCK-8 is a sensitive nonradioactive colorimetric assay for determining the number of viable cells in cell proliferation and cytotoxicity assays. WST-8 is bio-reduced by cellular dehydrogenases to an orange formazan product that is soluble in tissue culture medium. The amount of formazan produced is directly proportional to the number of living cells. The detection sensitivity of cell proliferation assays using WST-8 is higher than assays using the other tetrazolium salts such as MTT, XTT, MTS or WST-1 (PCT Published Appln. WO97/38985). Optical Density was determined at measurement wavelength of 450nm and reference wave length of 630nm.

Determination of 50 percent inhibitory concentrations (IC_{50}) of the compound A , erlotinib/lapatinib and gemcitabine in Panc-1 cells, Mia Paca-2 cells, HPAC cells and Capan1 cells

In order to determine the IC_{50} for gemcitabine, compound A, erlotinib and lapatinib in Panc-1 cells, Mia Paca-2 cells, HPAC cells and Capan1 cells, the cells were treated with the specified anticancer agents ("the test compounds") at the below mentioned concentrations. All the anticancer agents in the following doses of final concentration 0.03 µM, 0.1 µM, 0.3 µM, 1 µM, 3 µM, 10 µM, 30 µM and 100 µM were analyzed for their capacity to exhibit cytotoxicity particularly to exhibit 50 % cytotoxicity. The cells were seeded at a density of 3000 cells/well, in a 200 µL in tissue culture grade 96 well plate and were allowed to recover for 24 hrs in a humidified 5% ± 0.2 CO_2 incubator at 37 ^{0}C ± 0.5 ^{0}C. After 24 hrs, 1 µL

of 200 X test compound (gemcitabine, compound A, erlotinib and lapatinib (200 times higher than required concentration is denoted as 200 X)) dissolved in neat dimethyl sulfoxide (DMSO) was added to the cells. The final DMSO concentration was 0.5% in wells. Plates were incubated for 48 hrs in humidified 5% ± 0.2 CO_2 incubator at 37 ± 0.5 ^{0}C. After 48 hrs the plates were removed from CO_2 incubator and 5 µL of Cell counting Kit (CCK-8) per well was added. The same plate was kept at 37 ^{0}C for 3 hrs, and allowed to come to room temperature. The absorbance at a wavelength of 450nm was read on Tecan Multimode safire. The percent cytotoxicity was calculated using the following formula.

$$\text{Percent Cytotoxicity} = \left(\frac{\text{OD of DMSO control} - \text{OD Treated cells}}{\text{OD DMSO control}}\right] \times 100)$$

Dose-response studies at 72 hr in panc-1 cells showed that the gemcitabine, compound A, erlotinib and lapatinib inhibited 50% growth (IC_{50}) at 0.06 µM, 0.51 µM, 7.0 µM and 4.8 µM respectively. The results are presented in Table 4a and are graphically presented in Figure 3a.

Dose-response studies at 72 hr in MiaPaca-2 cells showed that the gemcitabine, compound A, erlotinib and lapatinib inhibited 50% growth (IC_{50}) at 0.22 µM, 0.72 µM, 9.0 µM and 7.6 µM respectively. The results are presented in Table 4b and are graphically presented in Figure 3b.

Dose-response studies at 72 hr in HPAC cells showed that the gemcitabine, compound A and erlotinib inhibited 50% growth (IC_{50}) at 1.6 µM, 1.1 µM and 2.3 µM respectively. The results are presented in Table 4c.

Dose-response studies at 72 hr in Capan1 cells showed that the gemcitabine, compound A and erlotinib inhibited 50% growth (IC_{50}) at 2.9 µM, 3.6 µM and 3.9 µM respectively. The results are presented in Table 4d.

Similarly the IC_{30}, IC_{70} and IC_{90} concentrations for the all the tested compounds (anticancer compounds) were established from dose in which particular compound shows 30%, 70% and 90% activity respectively in the cytotoxicity assay.

Table 4a - 30 %, 50 %, 70 % and 90 % inhibitory concentrations (IC_{30}, IC_{50}, IC_{70} and IC_{90}) of gemcitabine, compound A, erlotinib and lapatinib in Panc-1 cells

Anti-cancer agents	Panc-1 cells (Inhibitory conc. in µM)			
	IC_{30}	IC_{50}	IC_{70}	IC_{90}
Compound A	0.1	0.5	3.1	9.4
Gemcitabine	0.02	0.06	3	7.3
Erlotinib	1.6	4.8	12	26
Lapatinib	2.8	7	10	35

Table 4b - 30 %, 50 %, 70 % and 90 % inhibitory concentrations (IC_{30}, IC_{50}, IC_{70} and IC_{90}) of gemcitabine, compound A, erlotinib and lapatinib in MiaPaca-2 cells

Anti-cancer agents	MiaPaca-2 cells (Inhibitory conc. in µM)			
	IC_{30}	IC_{50}	IC_{70}	IC_{90}
Compound A	0.3	0.72	5.3	9.5
Gemcitabine	0.05	0.22	3.1	7.1
Erlotinib	2.6	7.6	15	34
Lapatinib	3	9	20	45

Table 4c - 30 %, 50 %, 70 % and 90 % inhibitory concentrations (IC_{30}, IC_{50}, IC_{70} and IC_{90}) of gemcitabine, compound A and erlotinib in HPAC cells

Anti-cancer agents	HPAC cells (Inhibitory conc. in µM)			
	IC_{30}	IC_{50}	IC_{70}	IC_{90}
Compound A	0.4	1.1	4.6	21.4
Gemcitabine	0.9	1.6	8.7	28.9
Erlotinib	1.4	2.3	6.3	10.7

Table 4d - 30 %, 50 %, 70 % and 90 % inhibitory concentrations (IC_{30}, IC_{50}, IC_{70} and IC_{90}) of gemcitabine, compound A and erlotinib in Capan1 cells

Anti-cancer agents	Capan1 cells (Inhibitory conc. in µM)			
	IC_{30}	IC_{50}	IC_{70}	IC_{90}
Compound A	1.6	3.6	7.8	22.3
Gemcitabine	1.1	2.9	11.7	23.7
Erlotinib	1.1	3.9	12.6	20.6

Example 4:

Combination studies of gemcitabine with compound A and erlotinib or with compound A and lapatinib in Panc-1 cells

Gemcitabine in a dose of final concentration of 0.02 µM (IC_{30}) or 0.06 µM (IC_{50}), the compound A in the dose of final concentration 0.1 µM (IC_{30}), lapatinib in the dose of final concentration of 2.8 µM (IC_{30}) and erlotinib in the dose of final concentration of 1.6 µM (IC_{30}) were analyzed for sequential combinations of the three specified anticancer agents). The following sequence of treatment was followed: the Panc-1 cells were treated with gemcitabine with either IC_{30} or IC_{50} for 0 to 24 hrs. At the end of 24 hrs the cells were washed two times with plain minimum essential medium (MEM). Fresh minimum essential medium with 10% serum (200 µL/well) was added, followed by treatment with both, the compound A and erlotinib or the compound A and lapatinib from 24 hrs to 96 hrs. At the 96 hrs cytotoxicity was measured using the CCK-8 method. The combination index was determined using the Combosyn software. Combination index (C.I) is used to evaluate the synergism between 2 or more compounds. CI <1 indicates that the combination is synergistic, CI = 1 indicates that the combination is additive and CI > 1 indicates that the combination is antagonistic.

The combination of gemcitabine at IC_{30} concentration with compound A and lapatinib was found to be synergistic at the IC_{30} concentration of each compound respectively. Gemcitabine at IC_{30} showed cytotoxicity of 12% and compound A at IC_{30} in combination with lapatinib IC_{30} showed cytotoxicity of 26%. However, when used as a combination of gemcitabine IC_{30} for 24hrs, followed by compound A at IC_{30} in combination with lapatinib at IC_{30} for 72 hrs showed an increase in cytotoxicity to the extent of 73% was noted, which

is 35% more cytotoxicity than the additive effect suggesting a synergistic effect between the three anticancer agents in Panc-1 cells. The results are presented in Table 5a and are graphically presented in Figure 4a.

The combination of gemcitabine with compound A and erlotinib was found to be synergistic at the IC_{30} concentration of each anticancer agent respectively. Gemcitabine at IC_{30} showed cytotoxicity of 12% and compound A IC_{30} in combination with erlotinib IC_{30} showed cytotoxicity of 28%. However, when used as a combination of gemcitabine at IC_{30} concentration for 24hrs, followed by compound A at IC_{30} concentration in combination with erlotinib at IC_{30} concentration for 72hrs showed an increase in cytotoxicity to the extent of 72%, which is 32% more cytotoxicity than the additive effect suggesting a synergistic effect between the three anticancer agents in Panc-1 cells . The results are presented in Table 5a and are graphically presented in Figure 4a.

In the following tables, the symbol "+" indicates that the anticancer agents (the test compounds) are used simultaneously.

Table 5a - Combination studies of gemcitabine at IC_{30} concentration with compound A and erlotinib or with compound A and lapatinib in Panc-1 cells

S.No	Anti-cancer agent	% Cytotoxicity	Combination index (C.I) Values
1	Gemcitabine IC_{30}	12	-
2	Compound A IC_{30}	13	-
3	Lapatinib IC_{30}	14	-
4	Compound A IC_{30} + Lapatinib IC_{30}	26	-
5	Erlotinib IC_{30}	15	-
6	Compound A IC_{30} + Erlotinib IC_{30}	28	-
7	Gemcitabine IC_{30} + (Compound A IC_{30} + Lapatinib IC_{30})	73	0.44
8	Gemcitabine IC_{30} + (Compound A IC_{30} + Erlotinib IC_{30})	72	0.17

The combination of gemcitabine at IC_{50} concentration with compound A at IC_{30} concentration and lapatinib at IC_{30} concentration was found to be synergistic in Panc-1 cells. Gemcitabine at IC_{50} concentration showed cytotoxicity of 21% and compound A at IC_{30}

concentration in combination with lapatinib IC_{30} concentration showed cytotoxicity of 26%. However, when used as a combination of gemcitabine at IC_{50} concentration for 24hrs, followed by compound A at IC_{30} concentration in combination with lapatinib at IC_{30} concentration for 72 hrs showed an increase in cytotoxicity to the extent of 89%, which is 42% more cytotoxicity than the additive effect suggesting a synergistic effect between the three anticancer agents in Panc-1 cells. The results are presented in Table 5b and are graphically presented in Figure 4b.

The combination of gemcitabine at IC_{50} concentration with compound A at IC_{30} concentration and erlotinib at IC_{30} concentration was found to be more synergistic in Panc-1 cells. Gemcitabine at IC_{50} concentration showed cytotoxicity of 21% and compound A at IC_{30} concentration in combination with erlotinib at IC_{30} concentration showed cytotoxicity of 28%. However, when used as a combination of gemcitabine at IC_{50} concentration for 24hrs, followed by compound A at IC_{30} concentration in combination with erlotinib at IC_{30} concentration for 72hrs showed an increase in cytotoxicity to the extent of 86%, which is 37% more cytotoxicity than the additive effect suggesting a synergistic effect between the three anticancer agents in Panc-1 cells. The results are presented in Table 5b and are graphically presented in Figure 4b.

Table 5b - Combination studies of gemcitabine at IC_{50} concentration with compound A and erlotinib or with compound A and lapatinib in Panc-1 cells

S.No	Anti-cancer agent	% Cytotoxicity	Combination index (C.I) Values
1	Gemcitabine IC_{50}	21	-
2	Compound A IC_{30}	13	-
3	Lapatinib IC_{30}	14	-
4	Compound A IC_{30} + Lapatinib IC_{30}	26	-
5	Erlotinib IC_{30}	15	-
6	Compound A IC_{30} + Erlotinib IC_{30}	28	-
7	Gemcitabine IC_{50} + (Compound A IC_{30} + Lapatinib IC_{30})	89	0.19
8	Gemcitabine IC_{50} + (Compound A IC_{30} + Erlotinib IC_{30})	86	0.11

Example 5:

Combination studies of gemcitabine with compound A and erlotinib or with compound A and lapatinib in MiaPaca-2 cells

Gemcitabine in a dose of final concentration of 0.05 μM (IC_{30}) and 0.22 μM (IC_{50}), Compound A in a dose of final concentration 0.3 μM (IC_{30}), lapatinib in a dose of final concentration of 3.0 μM (IC_{30}), erlotinib in a dose of final concentration of 2.6 μM (IC_{30}) were analyzed for sequential combinations of the three anticancer agents mentioned above. The following sequence of treatment was followed: the MiaPaca2 cells were treated with gemcitabine with either IC_{30} concentration or IC_{50} concentration for 0 to 24 hrs. At the end of 24 hrs the cells were washed two times with plain minimum essential medium (MEM). Fresh minimum essential medium with 10% serum (200 μL/well) was added, followed by treatment with both, the compound A and erlotinib or the compound A and lapatinib from 24 hrs to 96 hrs. At the 96 hrs cytotoxicity was measured using CCK-8 method. The combination index was determined using the Combosyn software. Combination index (C.I) is used to evaluate the synergism between 2 or more compounds. CI <1 indicates that the combination is synergistic, CI = 1 indicates that the combination is additive and CI > 1 indicates that the combination is antagonistic.

The combination of gemcitabine at IC_{30} concentration with compound A and lapatinib was found to be synergistic at the IC_{30} concentration of each compound respectively. Gemcitabine at IC_{30} concentration showed cytotoxicity of 16% and Compound A at IC_{30} concentration in combination with lapatinib at IC_{30} concentration showed cytotoxicity of 26%. However, when used as a combination of gemcitabine at IC_{30} for 24hrs, followed by compound A at IC_{30} in combination with lapatinib at IC_{30} for 72hrs showed an increase in cytotoxicity to the extent of 64%, which is 22% more cytotoxicity than the additive effect suggesting a synergistic effect between the three anticancer agents in MiaPaca-2 cells. The results are presented in Table 6a and are graphically presented in Figure 5a.

The combination of gemcitabine at IC_{30} concentration with compound A and erlotinib was found to be synergistic at the IC_{30} concentration of each compound respectively. Gemcitabine at IC_{30} concentration showed cytotoxicity of 16% and compound A at IC_{30} concentration in combination with erlotinib at IC_{30} concentration showed cytotoxicity of 27%. However, when used as a combination of gemcitabine at IC_{30} concentration for 24hrs, followed by compound A at IC_{30} concentration in combination with erlotinib at IC_{30} concentration for 72hrs showed an increase in cytotoxicity to the extent of 69%, which is

26% more cytotoxicity than the additive effect suggesting a synergistic effect between the three anticancer agents in MiaPaca-2 cells. The results are presented in Table 6a and are graphically presented in Figure 5a. In the following tables , the symbol "+" indicates that the anticancer agents (the test compounds) are used simultaneously.

Table 6a - Combination studies of gemcitabine at IC_{30} concentration with compound A and erlotinib or with compound A and lapatinib in MiaPaca-2 cells

S.No	Anti-cancer agent	% Cytotoxicity	Combination index (C.I) Values
1	Gemcitabine IC_{30}	16	-
2	Compound A IC_{30}	14	-
3	Lapatinib IC_{30}	17	-
4	Compound A IC_{30} + Lapatinib IC_{30}	26	-
5	Erlotinib IC_{30}	19	-
6	Compound A IC_{30} + Erlotinib IC_{30}	27	-
7	Gemcitabine IC_{30} + (Compound A IC_{30} + Lapatinib IC_{30})	64	0.29
8	Gemcitabine IC_{30} + (Compound A IC_{30} + Erlotinib IC_{30})	69	0.32

The combination of gemcitabine at IC_{50} concentration with compound A at IC_{30} concentration and lapatinib at IC_{30} concentration was found to be more synergistic in MiaPaca2 cells. Gemcitabine at IC_{50} concentration showed cytotoxicity of 21% and compound A at IC_{30} concentration in combination with lapatinib at IC_{30} concentration showed cytotoxicity of 26%. However, when used as a combination of gemcitabine at IC_{50} concentration for 24hrs, followed by compound A at IC_{30} concentration in combination with lapatinib at IC_{30} concentration for 72hrs showed an increase in cytotoxicity to the extent of 91%, which is 44% more cytotoxicity than the additive effect suggesting a synergistic effect between the three anticancer agents in MiaPaca-2 cells. The results are presented in Table 6b and are graphically presented in Figure 5b.

The combination of gemcitabine (IC_{50}) with compound A (IC_{30}) and erlotinib(IC_{30}) was found to be more synergistic in MiaPaca2 cells. Gemcitabine at IC_{50} concentration showed cytotoxicity of 21% and compound A at IC_{30} concentration in combination with erlotinib at IC_{30} concentration showed cytotoxicity of 27%. However, when used as a

combination of gemcitabine at IC_{50} concentration for 24hrs, followed by compound A at IC_{30} concentration in combination with erlotinib at IC_{30} concentration for 72hrs showed an increase in cytotoxicity to the extent of 88%, which is 40% more cytotoxicity than the additive effect suggesting a synergistic effect between the three anticancer agents in MiaPaca-2 cells. The results are presented in Table 6b and are graphically presented in Figure 5b. In the following tables , the symbol "+" indicates that the anticancer agents (the test compounds) are used simultaneously.

Table 6b - Combination studies of gemcitabine at IC_{50} concentration with compound A and erlotinib or with compound A and lapatinib in MiaPaca-2 cells

S.No	Anti-cancer agent	% Cytotoxicity	Combination index (C.I) Values
1	Gemcitabine IC_{50}	21	-
2	Compound A IC_{30}	14	-
3	Lapatinib IC_{30}	17	-
4	Compound A IC_{30} + Lapatinib IC_{30}	26	-
5	Erlotinib IC_{30}	19	-
6	Compound A IC_{30} + Erlotinib IC_{30}	27	-
7	Gem IC_{50} + (Compound A IC_{30} + Lapatinib IC_{30})	91	0.14
8	Gem IC_{50} + (Compound A IC_{30} + Erlotinib IC_{30})	88	0.15

Example 6:

Combination studies of gemcitabine with compound A and erlotinib in HPAC cells

The combination of gemcitabine with compound A and erlotinib was found to be synergistic at the IC_{30} concentration of each anticancer agent respectively. Gemcitabine at IC_{30} concentration showed cytotoxicity of 17% and compound A at IC_{30} concentration in combination with erlotinib at IC_{30} concentration showed cytotoxicity of 33%. However, when used as a combination of gemcitabine at IC_{30} concentration for 24hrs, followed by compound A at IC_{30} concentration in combination with erlotinib at IC_{30} concentration for 72hrs showed an increase in cytotoxicity to the extent of 88%, which is 38% more cytotoxicity than the additive effect suggesting a synergistic effect between the three anticancer agents in HPAC cells with combination index (C.I) of 0.21. The results are presented in Table 7 and are graphically presented in Figure 6

Table 7 - Combination studies of gemcitabine at IC_{30} or IC_{50} concentration with compound A (at IC_{30} or IC_{50} concentration) and erlotinib (at IC_{30} or IC_{50} concentration) in HPAC cells

Sr. No	Anti-cancer agents		% Cytotoxicity at 96 hr	Combination index (C.I) Values
	Day 1	Day 2		
1	Gemcitabine IC_{30}	-	17.29	-
2	Gemcitabine IC_{50}	-	21.82	-
3	Compound A IC_{30}	-	16.95	-
4	Compound A IC_{50}	-	23.48	-
5	Erlotinib IC_{30}	-	14.21	-
6	Erlotinib IC_{50}	-	24.84	-
7	Gemcitabine IC_{30}	Compound A IC_{30}	51.83	0.92
8	Gemcitabine IC_{30}	Compound A IC_{50}	62.37	0.57
9	Gemcitabine IC_{50}	Compound A IC_{30}	63.47	0.66
10	Gemcitabine IC_{50}	Compound A IC_{50}	71.47	0.42
11	Erlotinib IC_{30}	Compound A IC_{30}	47.44	0.96
12	Erlotinib IC_{30}	Compound A IC_{50}	54.99	0.65
13	Erlotinib IC_{50}	Compound A IC_{30}	64.10	0.88
14	Erlotinib IC_{50}	Compound A IC_{50}	73.37	0.59
15	No Gemcitabine	Compound A IC_{30} + Erlotinib IC_{30}	33.29	-
16	No Gemcitabine	Compound A IC_{30} + Erlotinib IC_{50}	39.94	-
17	Gemcitabine IC_{30}	Compound A IC_{30} + Erlotinib IC_{30}	88.42	0.21
18	Gemcitabine IC_{30}	Compound A IC_{30} + Erlotinib IC_{50}	89.53	0.27
19	Gemcitabine IC_{50}	Compound A IC_{30} + Erlotinib IC_{30}	87.34	0.57
20	Gemcitabine IC_{50}	Compound A IC_{30} + Erlotinib IC_{50}	96.51	0.63

Example 7

Combination studies of gemcitabine with compound A and erlotinib in Capan1 cells

The combination of gemcitabine with compound A and erlotinib was found to be synergistic at the IC_{30} concentration of each anticancer agent respectively. Gemcitabine at IC_{30} concentration showed cytotoxicity of 11% and compound A at IC_{30} concentration in combination with erlotinib at IC_{30} concentration showed cytotoxicity of 28%. However, when used as a combination of gemcitabine at IC_{30} concentration for 24hrs, followed by compound A at IC_{30} concentration in combination with erlotinib at IC_{30} concentration for 72hrs showed an increase in cytotoxicity to the extent of 78% was noted, which is 40% more cytotoxicity than the additive effect suggesting a synergistic effect between the three anticancer agents in Capan1 cells with combination index (C.I) of 0.34. The results are presented in Table 8 and graphically presented in Figure 7.

Table 8 - **Combination studies of gemcitabine at IC_{30} or IC_{50} concentration with compound A (at IC_{30} or IC_{50} concentration) and erlotinib (at IC_{30} or IC_{50} concentration) in Capan1 cells**

Sr. No	Anti-cancer agents		% Cytotoxicity at 96 hr	Combination index (C.I) Values
	Day 1	Day 2		
1	Gemcitabine IC_{30}	-	11.78	-
2	Gemcitabine IC_{50}	-	19.67	-
3	Compound A IC_{30}	-	17.94	-
4	Compound A IC_{50}	-	28.50	-
5	Erlotinib IC_{30}	-	17.00	-
6	Erlotinib IC_{50}	-	27.42	-
7	Gemcitabine IC_{30}	Compound A IC_{30}	48.00	0.81
8	Gemcitabine IC_{30}	Compound A IC_{50}	63.81	0.9
9	Gemcitabine IC_{50}	Compound A IC_{30}	66.01	0.47
10	Gemcitabine IC_{50}	Compound A IC_{50}	74.07	0.71
11	Erlotinib IC_{30}	Compound A IC_{30}	41.20	0.95
12	Erlotinib IC_{30}	Compound A IC_{50}	47.79	1.75
13	Erlotinib IC_{50}	Compound A IC_{30}	53.68	0.96
14	Erlotinib IC_{50}	Compound A IC_{50}	67.31	1.02
15	No Gemcitabine	Compound A IC_{30} + Erlotinib IC_{30}	28.14	-
16	No Gemcitabine	Compound A IC_{30} + Erlotinib IC_{50}	36.12	-
17	Gemcitabine IC_{30}	Compound A IC_{30} + Erlotinib IC_{30}	78.01	0.34
18	Gemcitabine IC_{30}	Compound A IC_{30} + Erlotinib IC_{50}	84.81	0.39
19	Gemcitabine IC_{50}	Compound A IC_{30} + Erlotinib IC_{30}	81.66	0.45
20	Gemcitabine IC_{50}	Compound A IC_{30} + Erlotinib IC_{50}	91.06	0.46

Example 8

Determination of protein expression

A) High content screening (*In vitro* protein estimation by Cellomics VTI array scan)

The cells were seeded in 96-well plates at a density of 7.5 X 10^3 cells/well. 24 h post seeding, the minimum essential medium (MEM) was replaced with a fresh minimum essential medium with 10% serum. The anticancer agents were treated at certain specific concentration and the cells were incubated for 4 hrs and 12 hrs respectively. At end of every time points, to determine the protein expression, the cells were fixed with 3.7% formaldehyde (Sigma St. Louis, MO) in PBS for 10 minutes at room temperature, followed by permeabilization with 0.15% Triton X-100 (Sigma St. Louis, MO) for 10 minutes. After permeabilization, the cells were blocked with 5% Bovine Serum Albumin (BSA) for 2 hours. After blocking step specific primary antibody were added for 1h. Following primary antibody incubation, the nucleus was stained with Hoechst 3342 (blue), and primary antibodies of different protein (pEGFR^{Y1171} pEGFR^{Y875} pAKT, pRB and CyclinD) were localized by secondary antibody labeled with Dylight548 (red). Immunofluorescence of pEGFR^{Y1171} pEGFR^{Y875} pAKT, pRB and CyclinD was determined by scanning the plates on Cellomics Array Scan® VTI HCS Reader (Thermo Fisher Scientific Inc. Waltham, MA). All the data points were analyzed using the Target Activation and Molecular translocation bio-algorithm of Cellomics and the quantitative data was expressed as percentage (%) activation in comparison to the DMSO control cells. 1000 cells or twenty fields were counted for each replicate well and the results were presented as an average ± SE.

B) Analysis of different protein expression in Panc-1 cells using array scan

Gemcitabine in a dose of final concentration of 0.02 µM (IC_{30}) and 0.06 µM (IC_{50}), Compound A in a dose of final concentration 0.1 µM (IC_{30}), lapatinib in a dose of final concentration of 2.8 µM (IC_{30}), erlotinib in a dose of final concentration of 1.6 µM (IC_{30}) were analyzed for sequential combinations of the three anticancer agents mentioned above. It has been termed as three anticancer agents because in the triple combination of anticancer agents either erlotinib or lapatinib is used. The sequence of treatment is as follows; the Panc-1 cells were treated with gemcitabine with either IC_{30} or IC_{50} for 0 to 24 hrs. At the end of 24 hrs the cells were washed two times with plain minimum essential medium. Fresh minimum essential medium with 10% serum (200 µL/well) was added, followed by treatment with

both, the compound A and erlotinib or the compound A and lapatinib for 4 hrs and 12 hrs respectively. After 4 hrs and 12 hrs each plate was fixed and stained for specific proteins using the same method as described above.

C) Protein expression analysis in Panc-1 cells for pEGFR-Y1173

The combination of gemcitabine with compound A and lapatinib or compound A with erlotinib was found to be synergistic at the IC_{30} concentration of each compound (the anticancer agent) in Panc-1 cells. Gemcitabine at IC_{30} or IC_{50} concentration and Compound A at IC_{30} concentration showed no inhibition of pEGFR-Y1173. The single anticancer agent, erlotinib and lapatinib at IC_{30} concentration showed only 40% and 47% inhibition respectively; while combination of gemcitabine IC_{30} with either (Compound A and erlotinib) or (compound A and lapatinib) showed 59.2 & 72.5 % inhibition of pEGFR-Y1173 levels respectively in Panc-1 cells at 8 hours.

The results are graphically presented in Figure 8a.

D) Protein expression analysis in Panc-1 cells for pEGFR-Y845

The combination of gemcitabine with compound A and lapatinib or Compound A with erlotinib was found to be synergistic at the IC_{30} concentration of each anticancer agent in Panc-1 cells. Gemcitabine at IC_{30} or IC_{50} concentration and Compound A at IC_{30} concentration showed no inhibition of pEGFR-Y845. The single drug erlotinib and lapatinib at IC_{30} concentration showed only 33% and 31% inhibition, while combination of gemcitabine IC_{30} with either (Compound A or erlotinib) or (compound A and lapatinib) showed 65.2 & 78.2 % inhibition of pEGFR-Y1845 levels respectively in Panc-1 cells at 8 hours. The results are graphically presented in Figure 8b.

E) Protein expression analysis in Panc-1 cells for pAKT-S473

The combination of gemcitabine with compound A and lapatinib or Compound A with erlotinib was found to be synergistic at the IC_{30} concentration of each anticancer agent in Panc-1 cells. The compound A, erlotinib and lapatinib at IC_{30} concentration showed no significant inhibition at 12 hrs, while gemcitabine at IC_{30} and IC_{50} concentration showed 19.5% and 25.7% inhibition of pAKT-S473. The combination of compound A with erlotinib and that with lapatinib at IC_{30} concentration showed only 32.9% and 29.1% inhibition, while combination of gemcitabine IC_{50} with either (Compound A or erlotinib) or [compound A and

lapatinib] showed 62.5 & 72.2 % inhibition of pAKT-S473 levels respectively in Panc-1 cells at 12 hours. The results are graphically presented in Figure 8c.

F) Protein expression analysis in Panc-1 cells for pRB-S780

The combination of gemcitabine with compound A and lapatinib or compound A with erlotinib was found to be synergistic at the IC_{30} concentration of each anticancer agent in Panc-1 cells. Gemcitabine at IC_{30}, and IC_{50} concentration, erlotinib and lapatinib showed no significant inhibition at 12 hrs, while Compound A at IC_{30} concentration showed 25% inhibition of pRB-S780. The combination of compound A with erlotinib and that with lapatinib at IC_{30} concentration showed only 17% and 23.5% inhibition, while combination of gemcitabine IC_{50} with either (Compound A or erlotinib) or (compound A and lapatinib) showed 53.4 & 72.1 % inhibition of pRB-S480 levels respectively in Panc-1 cells at 12 hours. The results are graphically presented in Figure 8d

G) Protein expression analysis in Panc-1 cells for CyclinD

The combination of gemcitabine with compound A and lapatinib or erlotinib was found to be synergistic at the IC_{30} concentration of each anticancer agent in Panc-1 cells. Gemcitabine at IC_{30} or IC_{50} concentration and compound A at IC_{30} concentration showed marginal inhibition of 27.7%, 20.3 and 27.8% respectively when compared to control. The anticancer agents, erlotinib and lapatinib either alone or with compound A did not show any notable inhibition at 12 hrs, while combination of gemcitabine IC_{50} with either (Compound A or erlotinib) or (compound A and lapatinib) showed 69.8 & 63.6 % inhibition of CyclinD levels respectively in Panc-1 cells at 12 hours.

The results are graphically presented in Figure 8e.

Example 9

Analysis of cleaved caspase-3 expression levels

This study was conducted to evaluate the mechanisms by which the triple combination consisting of gemcitabine, (erlotinib or lapatinib) with compound A blocks proliferation and whether it can induce apoptosis in pancreatic cancer cells.

The cells were seeded in 96-well plates at a density of 7.5 X 10^3 cells/well. 24 h post seeding, the minimum essential medium was replaced with a fresh minimum essential medium with 10% serum. The anticancer agents (gemcitabine, erlotinib or lapatinib and

compound A) were treated with specific concentration as mentioned below in Panc-1 and Miapaca-2 cells and incubated for 48 hrs. At the end of 48 hrs, to determine the protein expression, the cells were in 96 well plate spin down at 800g for 5 minutes. Culture supernatant was removed and 200 µL of caspase-3 assay buffer was added and plates were again spin down at 800g for 5 minutes. Supernatant were removed and cells were lysed with 100 µL caspase-3 lysis buffer and incubated for 30 min in orbital shaker at 300 rpm in room temperature. Further plates were spin down at 800g for 10 minutes and 90 µL of the supernatant was transferred into new black well plate. To 90 µL of lysis solution 100 µL of caspase-3 substrate was added and incubated for 30 minutes at 37'C. At the end of incubation plates were read in Tecan Safire multimode reader with an excitation wavelength of 485 nm and emission wavelength of 535 nm.

A) Treatment pattern in Panc-1 cells for assessing caspase-3 activity

Gemcitabine a dose of final concentration 0.02 µM (IC_{30}) and 0.06 µM (IC_{50}), compound A in a dose of final concentration 0.1 µM (IC_{30}), lapatinib a dose of final concentration of 2.8 µM (IC_{30}), erlotinib in a dose of final concentration of 1.6 µM (IC_{30}) were analyzed for sequential combinations of the three anticancer agents mentioned above. The sequence of treatment is as follows; the Panc-1 cells were treated with gemcitabine with either IC_{30} or IC_{50} for 0 to 24 hrs. At the end of 24 hrs the cells were washed two times with plain MEM medium. Fresh MEM with 10% serum (200 µL/well) was added, followed by treatment with both compound A and erlotinib or compound A and lapatinib for 48 hrs. After 48 hrs, the plates were processed for caspase-3 activity assay as described above.

B) Treatment pattern in MiaPaca-2 cells for assessing caspase-3 activity

Gemcitabine a dose of final concentration 0.05 µM (IC_{30}) and 0.22 µM (IC_{50}), compound A in a dose of final concentration 0.3 µM (IC_{30}), lapatinib in a dose of final concentration of 3.0 µM (IC_{30}), erlotinib in a dose of final concentration of 2.6 µM (IC_{30}) were analyzed for sequential combinations of the three anticancer agents mentioned above. The sequence of treatment is as follows; the MiaPaca2 cells were treated with gemcitabine with either IC_{30} or IC_{50} for 0 to 24 hrs. At the end of 24 hrs the cells were washed two times with plain MEM medium. Fresh MEM with 10% serum (200 µL/well) was added, followed by treatment with both compound A and erlotinib or compound A and lapatinib for 48 hrs. After 48 hrs, the plates were processed for caspase-3 activity assay as described above.

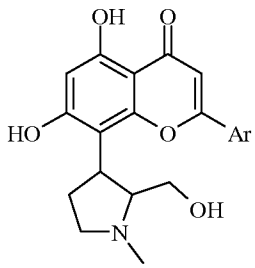
C) Protein expression analysis in Panc-1 and MiaPaca-2 cells for Caspase3

Gemcitabine with compound A in combination with lapatinib or compound A in combination with erlotinib in pancreatic cancer cells enhances gemcitabine chemoresistance by apoptosis through significant induction of apoptosis.

Single treatment with gemcitabine at IC_{30} or IC_{50} concentration, erlotinib, lapatinib and compound A did not show significant induction of caspase-3 activity. The combination of compound A with erlotinib and that with lapatinib at IC_{30} concentration showed only 30% to 35% activation of caspase3 when compared to the control in both Panc-1 and MiaPaca-2 cells. While gemcitabine and compound A with erlotinib or lapatinib showed significant induction of caspase3 levels up to 75% to 80% in both the cells at 48 hours. The results are graphically presented in Figure 9.

We claim:

1. A pharmaceutical combination comprising a CDK inhibitor selected from the compounds of formula I ;



Formula I

wherein Ar is a phenyl group, which is unsubstituted or substituted by 1, 2, or 3 identical or different substituents selected from :halogen; nitro, cyano, C_1-C_4-alkyl, trifluoromethyl, hydroxyl or C_1-C_4-alkoxy; or a pharmaceutically acceptable salt or solvate thereof; and a compound capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity.

2. The pharmaceutical combination according to claim 1, wherein in the compound of formula I the phenyl group is substituted by 1, 2, or 3 identical or different substituents selected from: chlorine, bromine, fluorine or iodine, C_1-C_4-alkyl or trifluoromethyl; or a pharmaceutically acceptable salt or solvate thereof.

3. The pharmaceutical combination according to claim 2; wherein in the compound of formula I the phenyl group is substituted by chlorine.

4. The pharmaceutical combination according to claim 3; wherein the compound of formula I is (+)-*trans*-2-(2-Chloro-phenyl)-5,7-dihydroxy-8-(2-hydroxy-methyl-1-methyl-pyrrolidin-3-yl)-chromen-4-one hydrochloride (compound A).

5. The pharmaceutical combination according to claim 2; wherein in the compound of formula I the phenyl group is a substituted group substituted by 2 different substituents selected from chlorine and trifluoromethyl.

6. The pharmaceutical combination according to claim 5; wherein the compound of formula I is (+)-trans-3-[2[(2-Chloro-4-trifluoromethyl-phenyl)-5,7-dihydroxy-8-(2-hydroxymethyl-1-methyl-pyrrolidin-3-yl)-chromen-4-one hydrochloride (compound B).

7. The pharmaceutical combination according to claim 1, wherein the compound capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity is selected from erlotinib or lapatinib.

8. The pharmaceutical combination according to claim 7, wherein the compound capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity is erlotinib.

9. The pharmaceutical combination according to claim 7, wherein the compound capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity is lapatinib.

10. The pharmaceutical combination according to claim 1; wherein said combination further comprises gemcitabine.

11. The pharmaceutical combination according to claim 2; wherein said combination further comprises gemcitabine.

12. The pharmaceutical combination according to claim 4; wherein said combination further comprises gemcitabine.

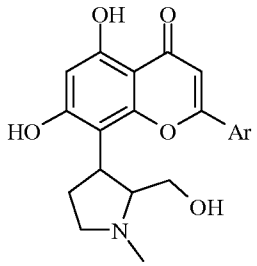
13. The pharmaceutical combination according to claim 6; wherein said combination further comprises gemcitabine.

14. The pharmaceutical combination according to claim 7; wherein said combination further comprises gemcitabine.

15. The pharmaceutical combination according to claim 1; wherein said combination is adapted for the treatment of pancreatic cancer.

16. The pharmaceutical combination according to claim 10; wherein said combination is adapted fro the treatment of pancreatic cancer.

17. A method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula I ;



Formula I

wherein Ar is a phenyl group, which is unsubstituted or substituted by 1, 2, or 3 identical or different substituents selected from :halogen; nitro, cyano, C_1-C_4-alkyl, trifluoromethyl, hydroxyl or C_1-C_4-alkoxy; or a pharmaceutically acceptable salt or solvate thereof; in combination with a therapeutically effective amount of a compound capable of inhibiting EGFR kinase activity.

18. The method according to claim 17; wherein in the compound of formula I the phenyl group is substituted by chlorine.

19. The method according to claim 18; wherein the compound of formula I is (+)-*trans*-2-(2-Chloro-phenyl)-5,7-dihydroxy-8-(2-hydroxy-methyl-1-methyl-pyrrolidin-3-yl)-chromen-4-one hydrochloride (compound A).

20. The method according to claim 17; wherein in the compound of formula I the phenyl group is a substituted group substituted by 2 different substituents selected from chlorine and trifluoromethyl.

21. The method according to claim 20; wherein the compound of formula I is (+)-trans-3-[2[(2-Chloro-4-trifluoromethyl-phenyl)-5,7-dihydroxy-8-(2-hydroxymethyl-1-methyl-

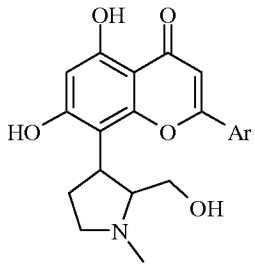
pyrrolidin-3-yl)-chromen-4-one hydrochloride (compound B).

22. The method according to claim 17, wherein the compound capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity is selected from erlotinib or lapatinib.

23. The method according to claim 22, wherein the compound capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity is erlotinib.

24. The method according to claim 22, wherein the compound capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity is lapatinib.

25. A method for the treatment of pancreatic cancer in a subject comprising administering to said subject a therapeutically effective amount of a CDK inhibitor selected from the compounds of formula I ;



Formula I

wherein Ar is a phenyl group, which is unsubstituted or substituted by 1, 2, or 3 identical or different substituents selected from :halogen; nitro, cyano, C_1-C_4-alkyl, trifluoromethyl, hydroxyl or C_1-C_4-alkoxy; or a pharmaceutically acceptable salt or solvate thereof; in combination with a therapeutically effective amount of a compound capable of inhibiting EGFR kinase activity and a therapeutically effective amount of gemcitabine.

26. The method according to claim 25; wherein in the compound of formula I the phenyl group is substituted by chlorine.

27. The method according to claim 26; wherein the compound of formula I is (+)-*trans*-2-(2-Chloro-phenyl)-5,7-dihydroxy-8-(2-hydroxy-methyl-1-methyl-pyrrolidin-3-yl)-chromen-4-one hydrochloride (compound A).

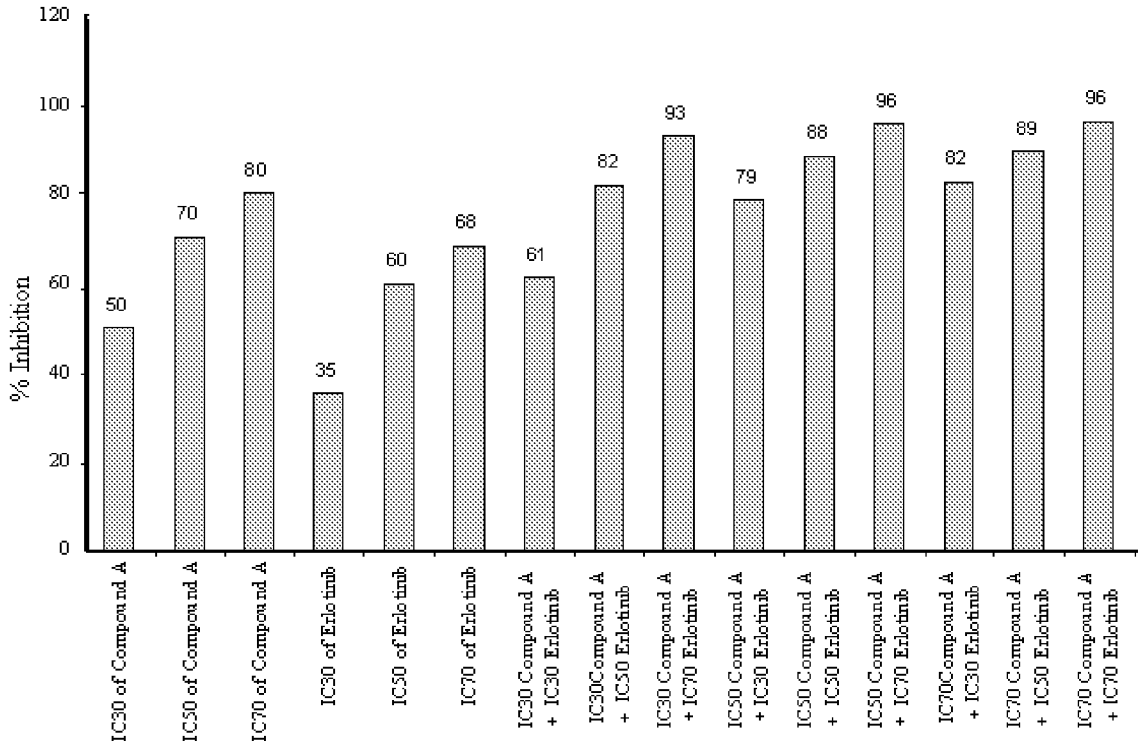
28. The method according to claim 25; wherein in the compound of formula I the phenyl group is a substituted group substituted by 2 different substituents selected from chlorine and trifluoromethyl.

29. The method according to claim 28; wherein the compound of formula I is (+)-trans-3-[2[(2-Chloro-4-trifluoromethyl-phenyl)-5,7-dihydroxy-8-(2-hydroxymethyl-1-methyl-pyrrolidin-3-yl)-chromen-4-one hydrochloride (compound B).

30. The method according to claim 25, wherein the compound capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity is selected from erlotinib or lapatinib.

31. The method according to claim 30, wherein the compound capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity is erlotinib.

32. The method according to claim 30, wherein the compound capable of inhibiting epidermal growth factor receptor (EGFR) kinase activity is lapatinib.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IC30 of Erlotinib
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IC70 of Erlotinib
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IC50 Compound A + IC50 Erlotinib
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IC70Compound A + IC30 Erlotinib
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Figure 1a

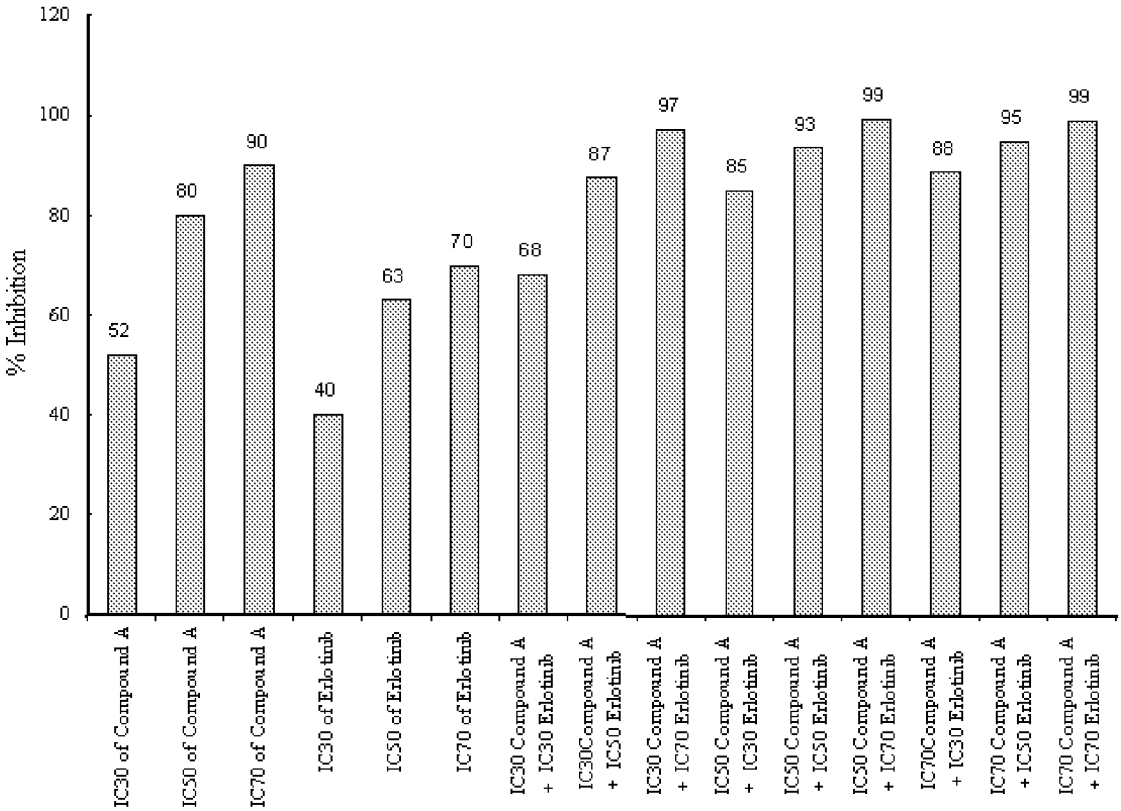
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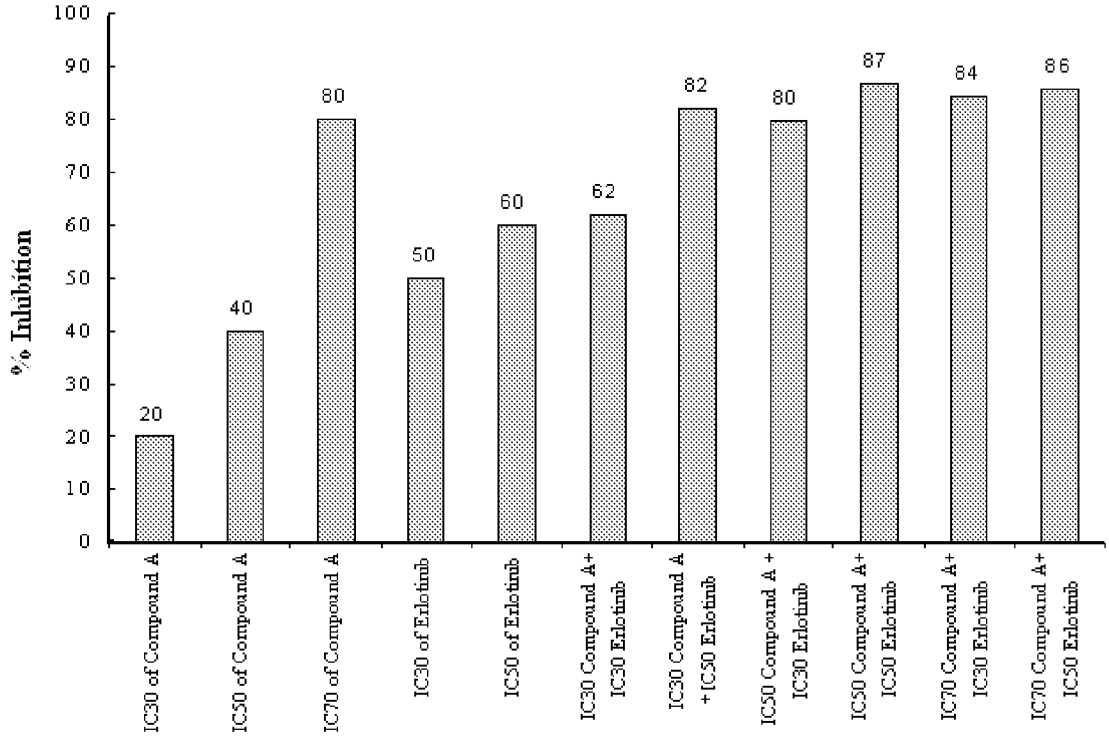
Figure 1b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IC30 of Compound A
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IC30 Compound A +IC50 Erlotinib
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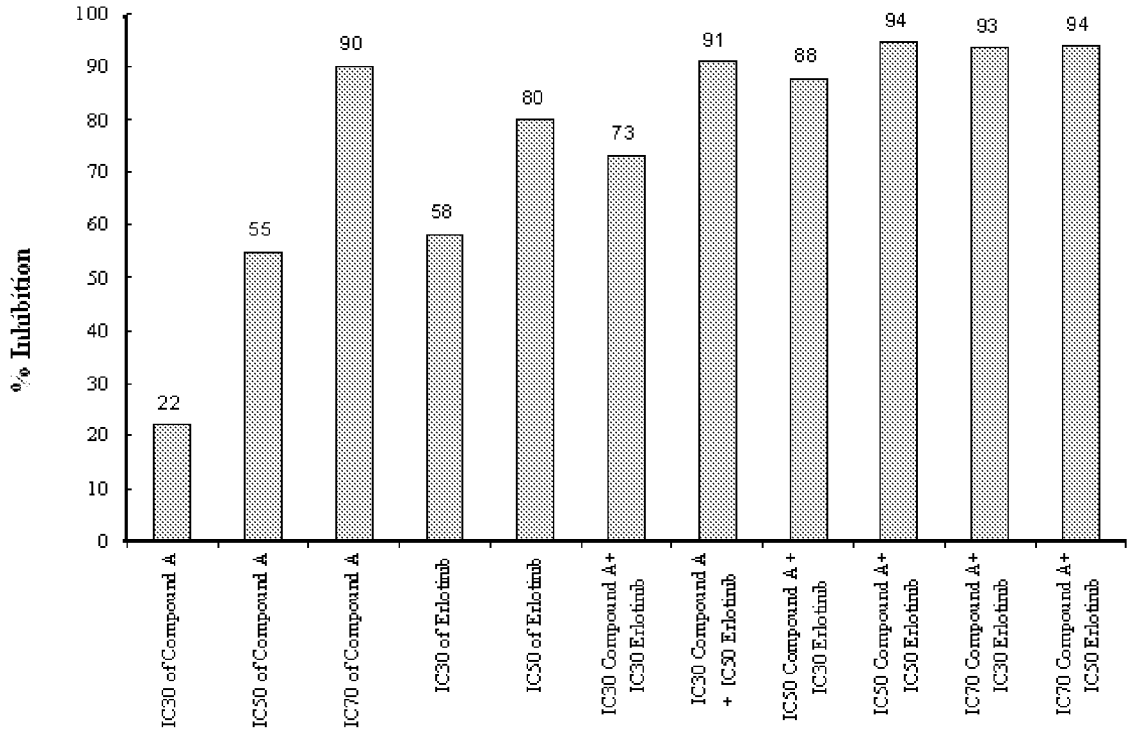
IC50 Compound A+ IC50 Erlotinib
IC70 Compound A+ IC30 Erlotinib
IC70 Compound A+ IC50 Erlotinib

Figure 2a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IC30 of Compound A
IC50 of Compound A
IC70 of Compound A
IC30 of Erlotinib
IC50 of Erlotinib
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IC30 Compound A + IC30 Erlotinib
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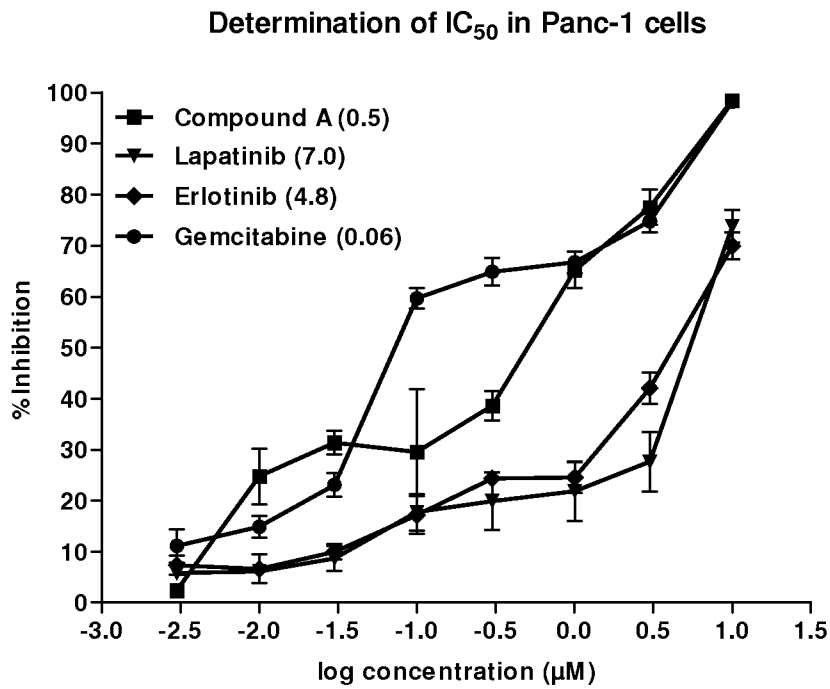
IC70 Compound A+ IC30 Erlotinib
IC70 Compound A+ IC50 Erlotinib

Figure 2b



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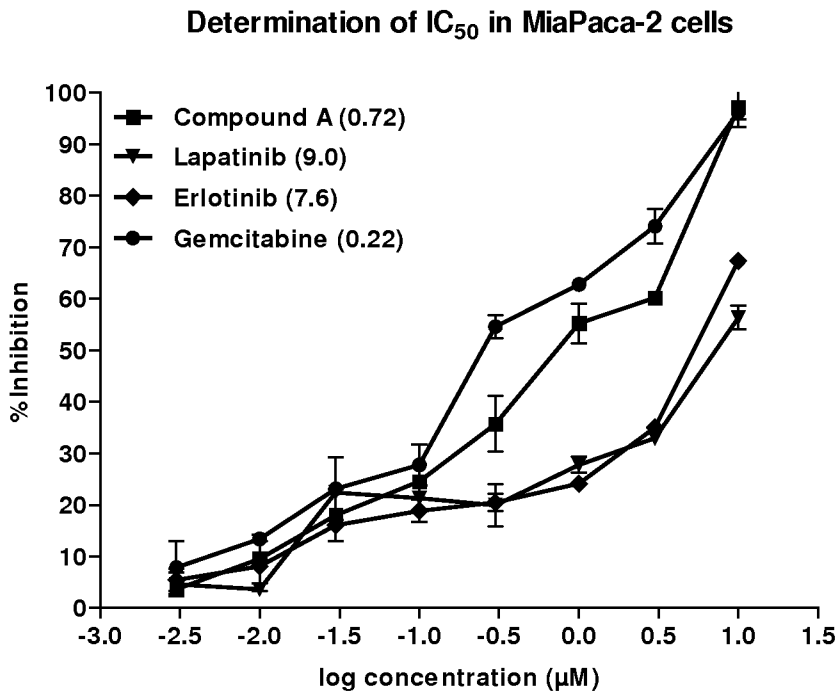
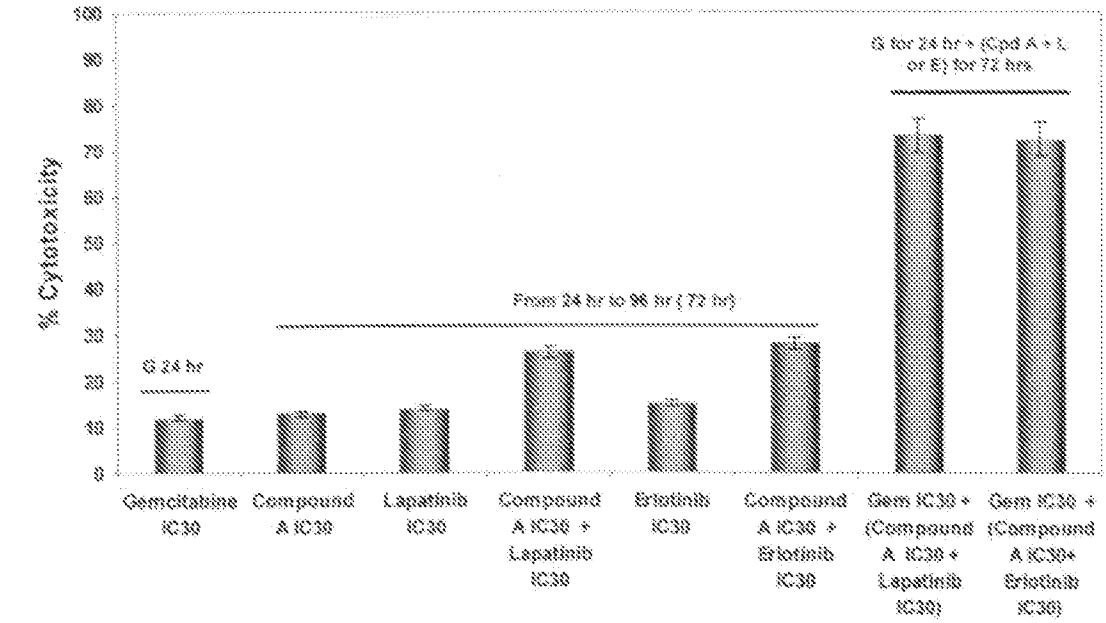
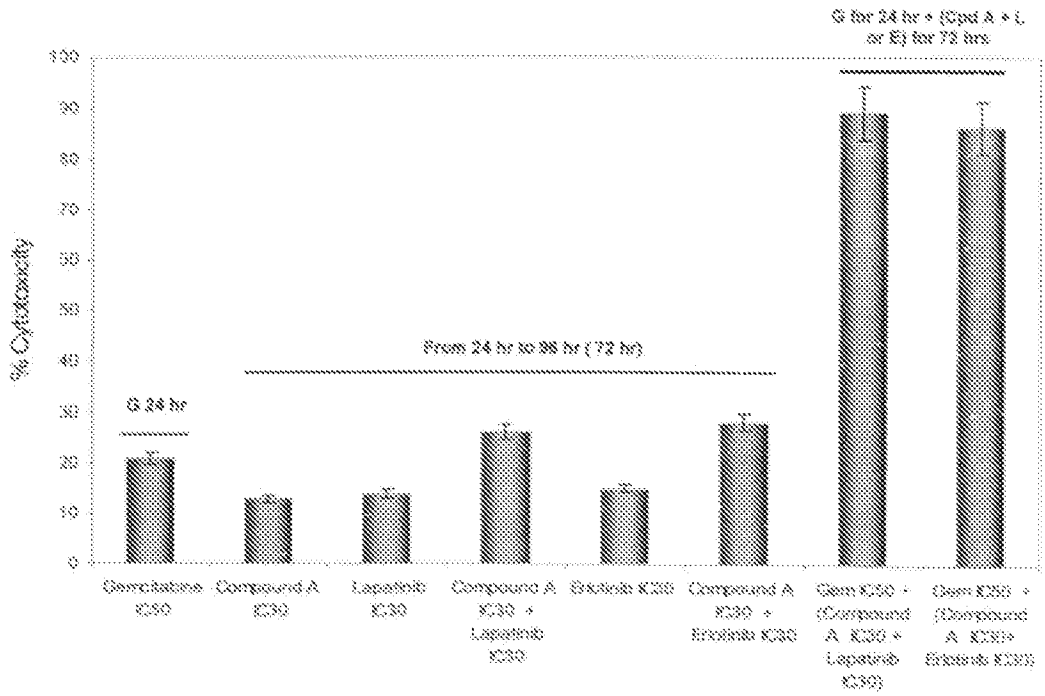


Figure 3b



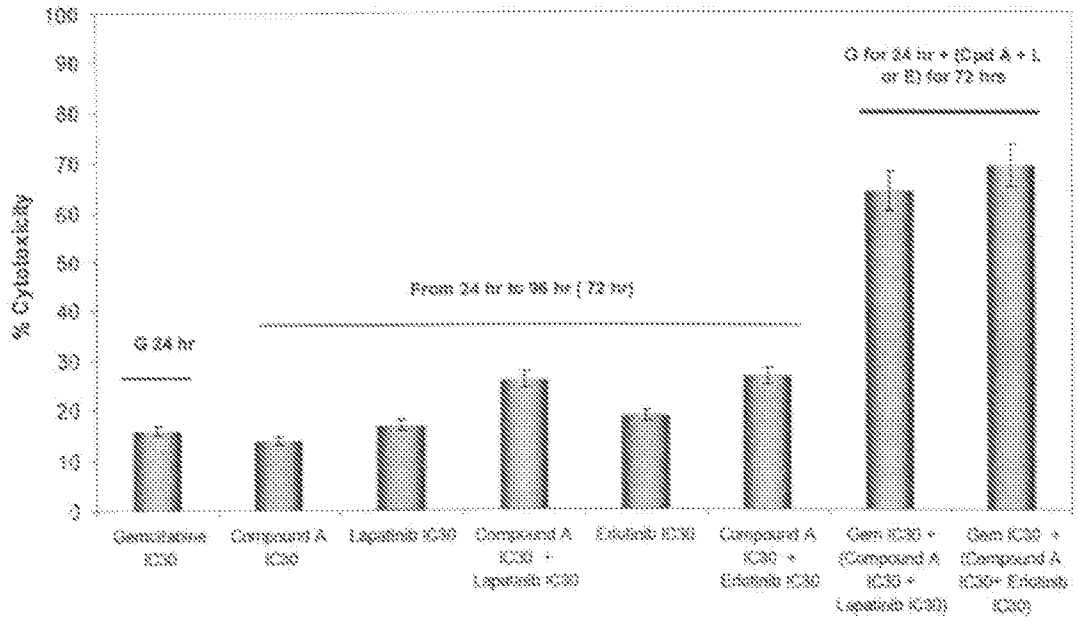
Gem - Gemcitabine

Figure 4a



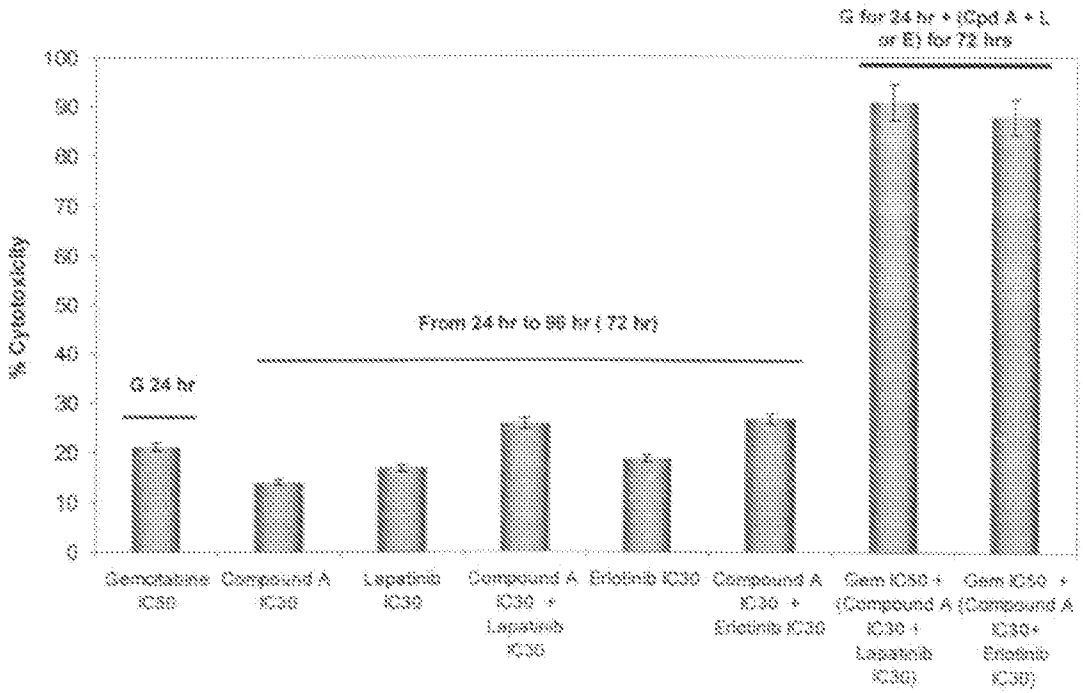
Gem - Gemcitabine

Figure 4b



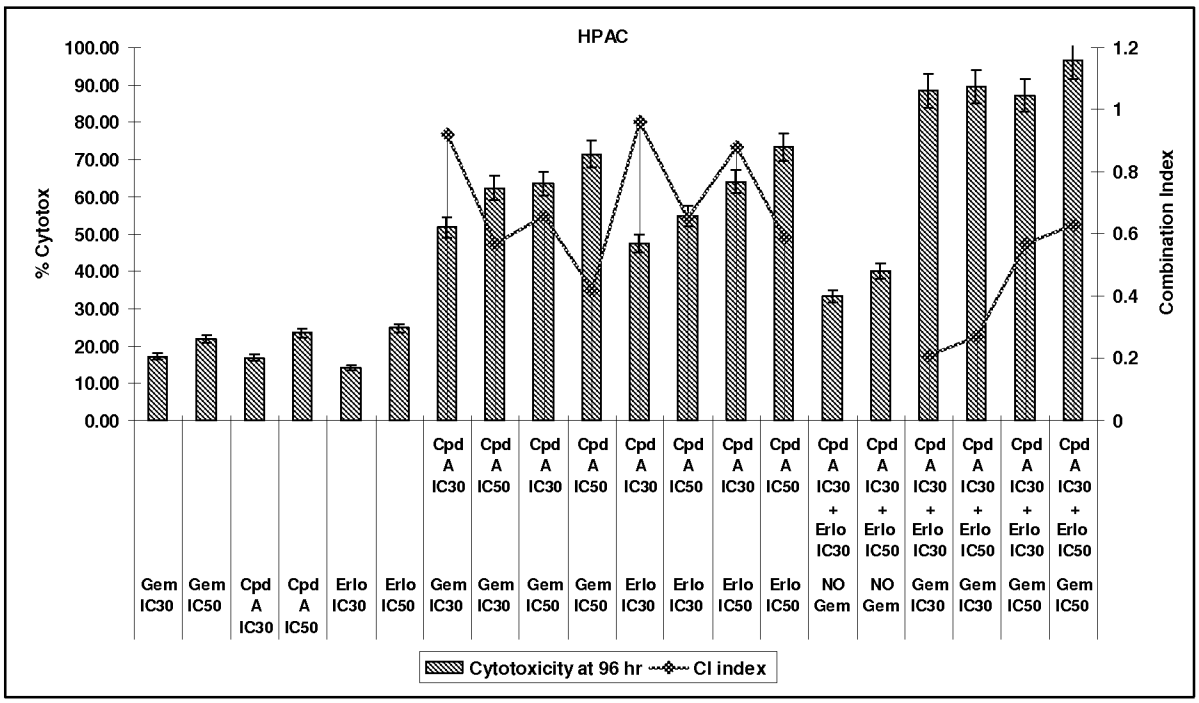
Gem - Gemcitabine

Figure 5a



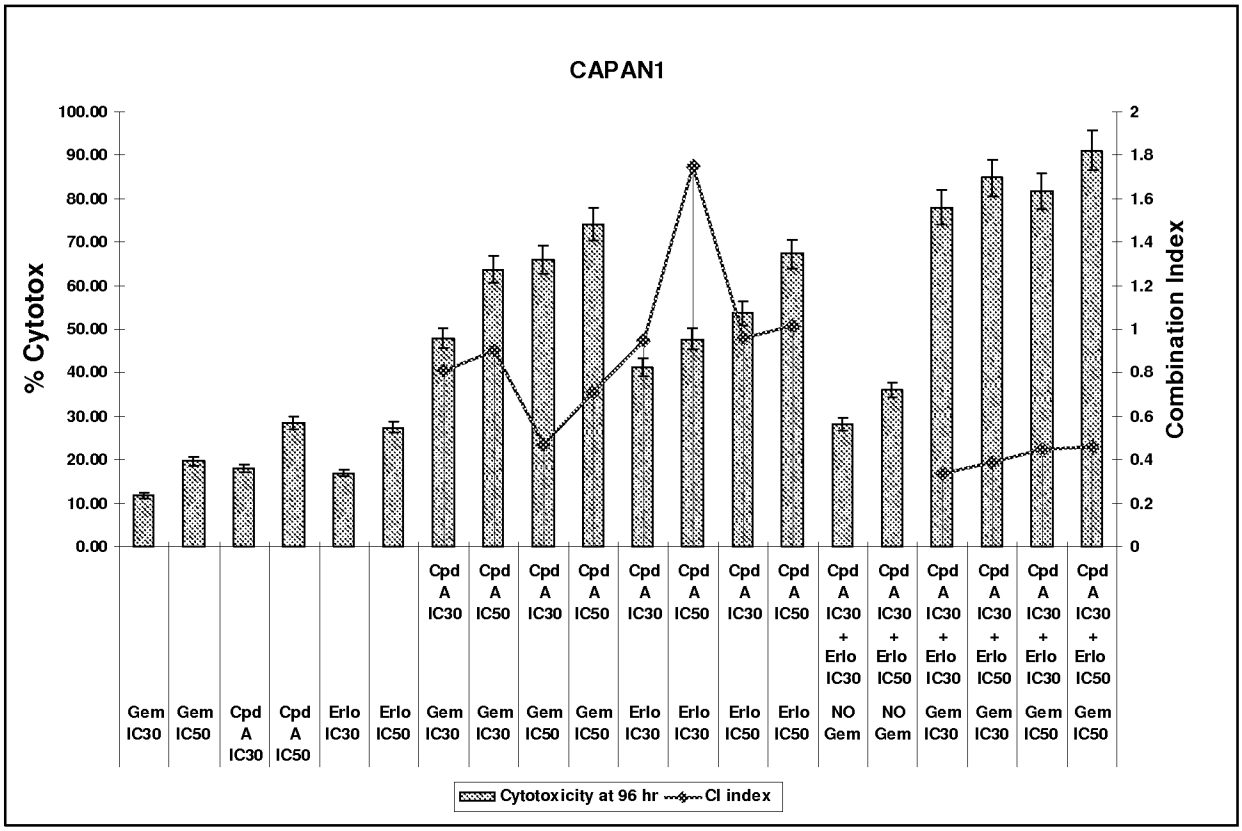
Gem - Gemcitabine

Figure 5b



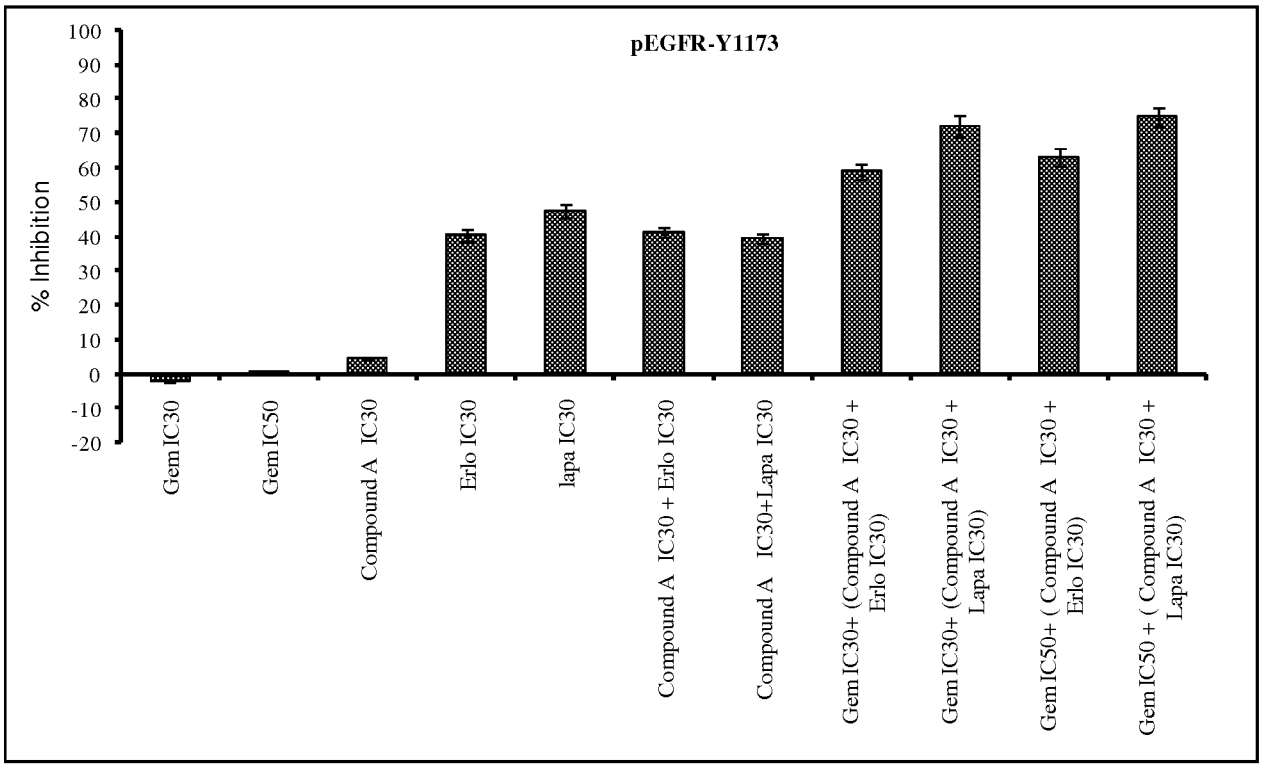
Gem - Gemcitabine
Cpd A – Compound A
Erlo - Erlotinib

Figure 6



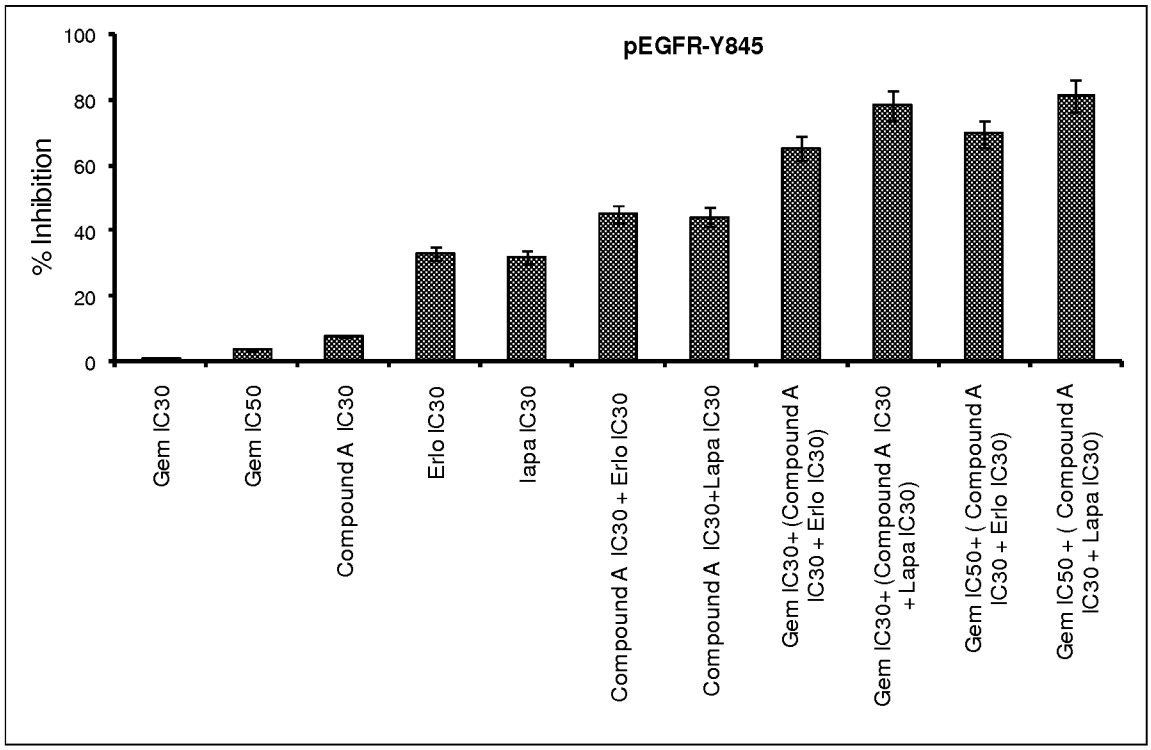
Gem - Gemcitabine
Cpd A – Compound A
Erlo - Erlotinib

Figure 7



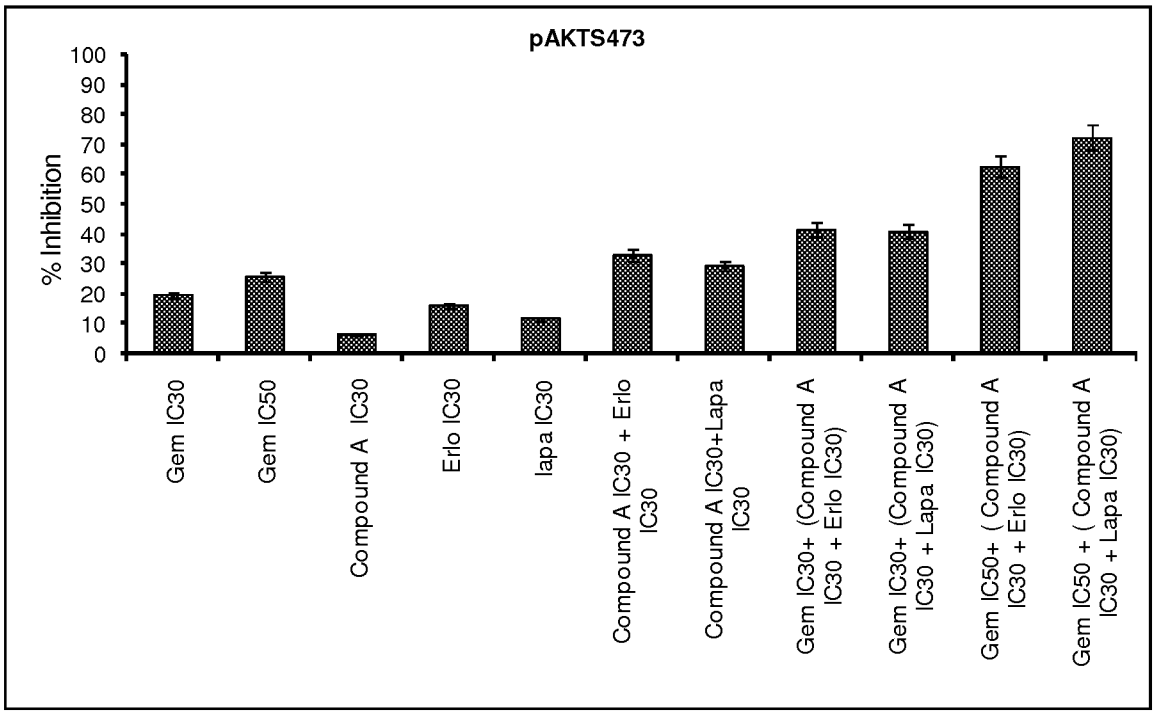
Gem – Gemcitabine
Erlo – Erlotinib
Lapa - Lapatinib

Figure 8a



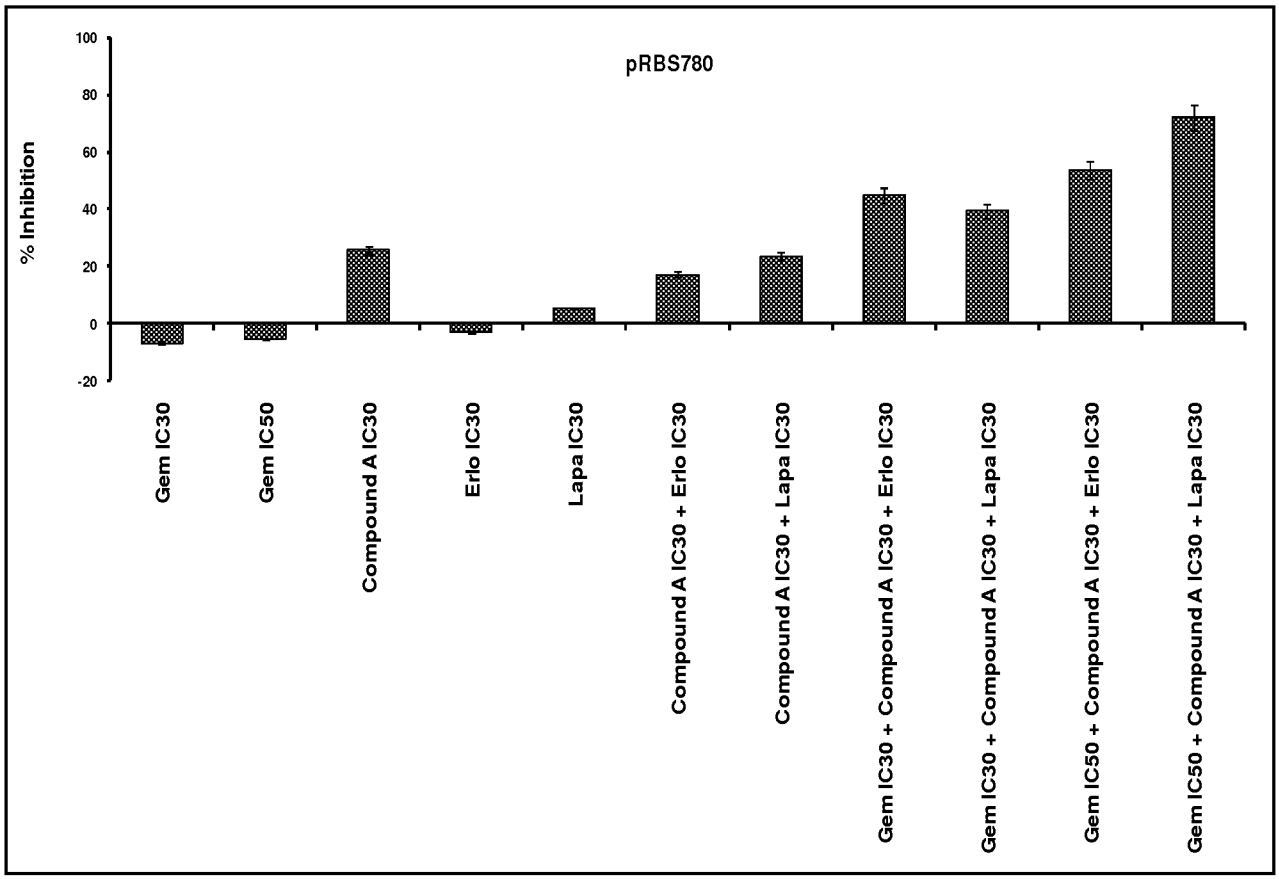
Gem – Gemcitabine
Erlo – Erlotinib
Lapa - Lapatinib

Figure 8b



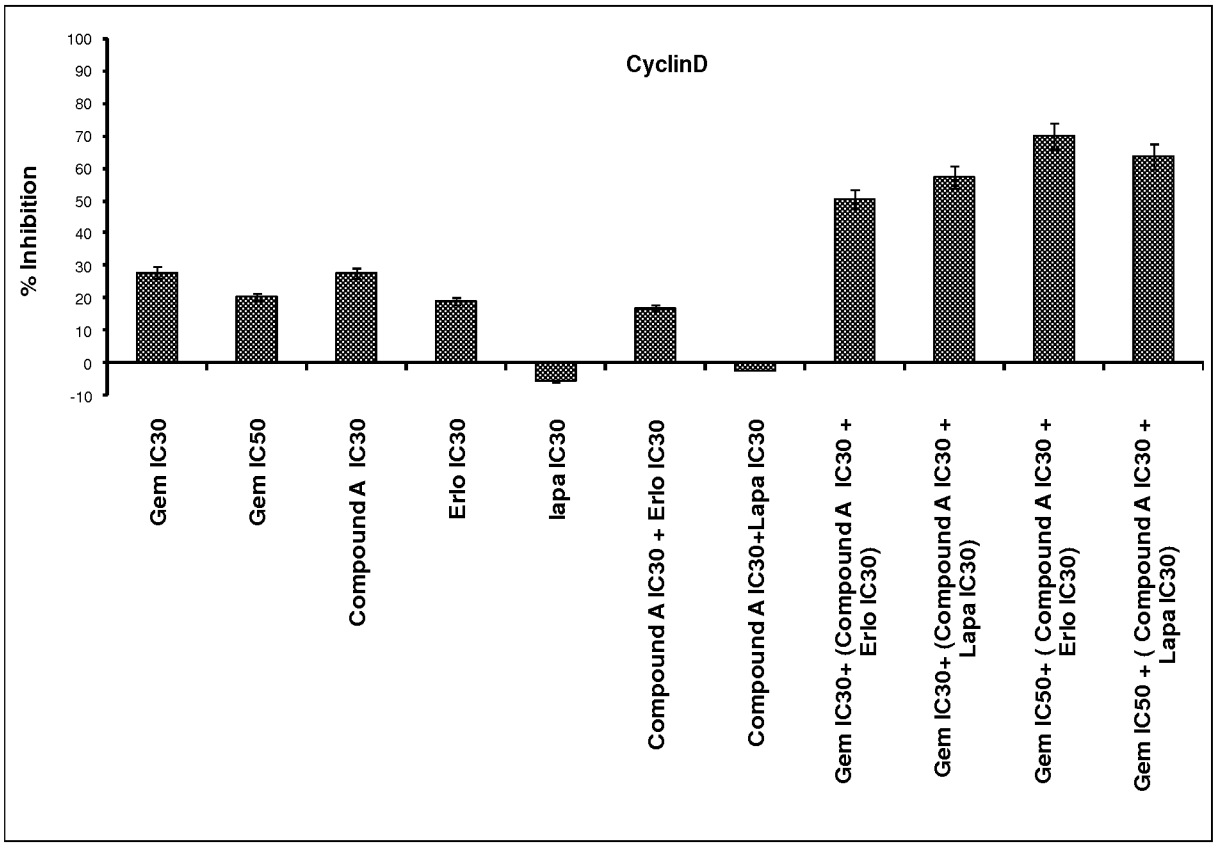
Gem – Gemcitabine
Erlo – Erlotinib
Lapa - Lapatinib

Figure 8c



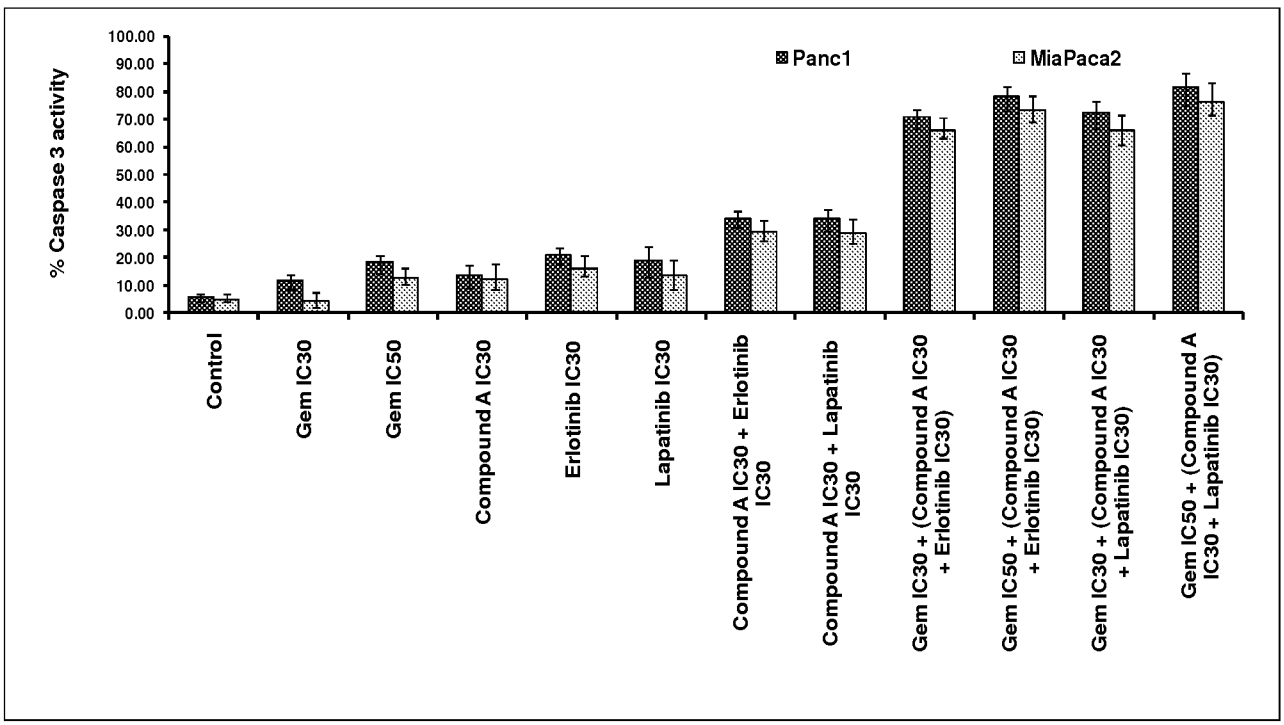
Gem – Gemcitabine
Erlo – Erlotinib
Lapa - Lapatinib

Figure 8d



Gem – Gemcitabine
Erlo – Erlotinib
Lapa - Lapatinib

Figure 8e



Gem – Gemcitabine

Figure 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/051168

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/4025 A61K31/517 A61K31/7068 A61P35/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2007/129062 A1 (ASTEX THERAPEUTICS LTD [GB]; SQUIRES MATTHEW SIMON [GB]) 15 November 2007 (2007-11-15) page 1, line 3 - line 8 page 57, line 27 page 116, line 19 page 117, line 6 ----- -/--	1-32

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 May 2012	31/05/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Terenzi, Carla

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/051168

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2008/139271 A2 (PIRAMAL LIFE SCIENCES LTD [IN]; RATHOS MAGGIE [IN]; JOSHI KALPANA [IN]) 20 November 2008 (2008-11-20) cited in the application page 4, line 31 - line 34 page 5, line 1 - line 3 page 8, line 4 - line 14 page 15, line 9 - line 11 page 16, line 13 - line 15 claim 6 -----	1-32
Y	WO 2010/128443 A1 (PIRAMAL LIFE SCIENCES LTD [IN]; RATHOS MAGGIE JOYCE [IN]; JOSHI KALPAN) 11 November 2010 (2010-11-11) page 4, line 5 - line 8 page 6, line 7 - line 15 page 11, line 30 - line 33 page 12, line 27 -----	1-32
Y	FURUGAKI K ET AL: "Antitumor activity of erlotinib in combination with gemcitabine in in vitro and in vivo models of KRAS-mutated pancreatic cancers", ONCOLOGY LETTERS 2010 SPANDIDOS PUBLICATIONS GRC LNKD-DOI:10.3892/OL_00000041, vol. 1, no. 2, March 2010 (2010-03), pages 231-235, XP002676219, ISSN: 1792-1074 abstract page 235, left-hand column, line 10 - line 15 -----	1-32

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2012/051168

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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