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(54) **TREATMENT OF CANCER USING THE
SODIUM SALT OF A BENZOIC ACID
DERIVATIVE**

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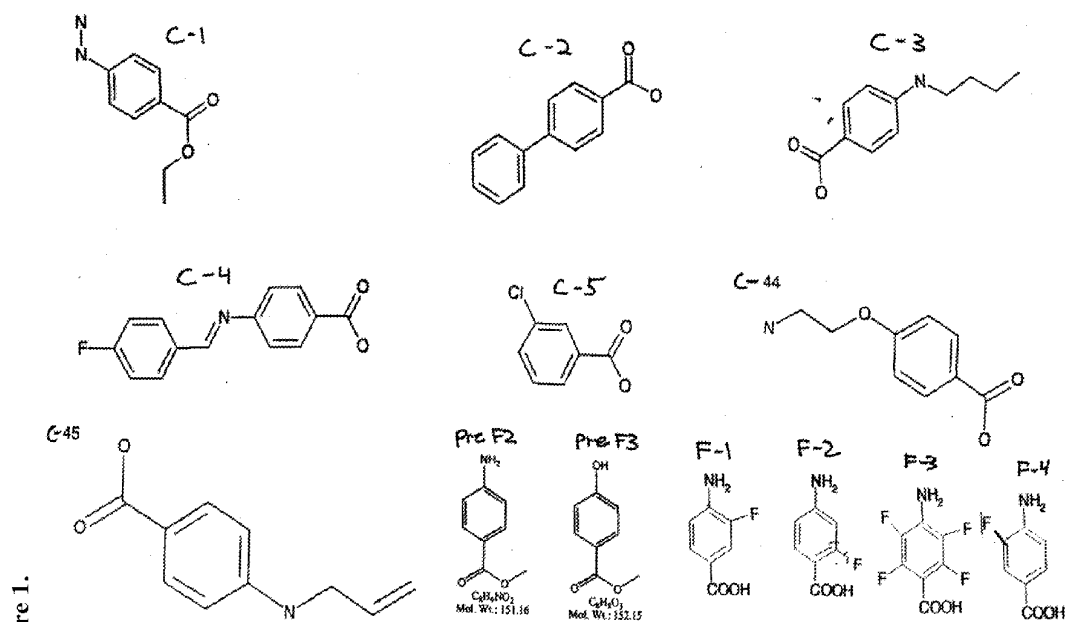
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(57) **ABSTRACT**

The present invention provides a method of treating cancer
using the sodium salt of a benzoic acid derivative, alone or in
combination with standard treatments such as chemotherapy
and radiotherapy.



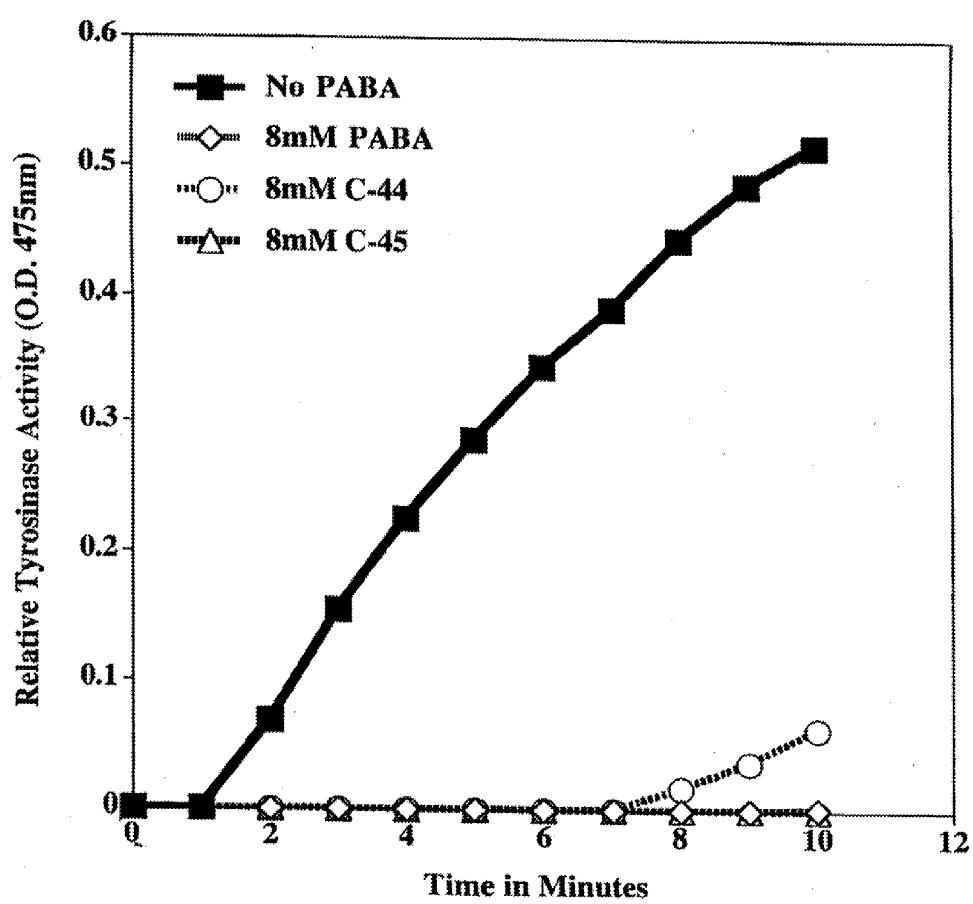


Figure 2.

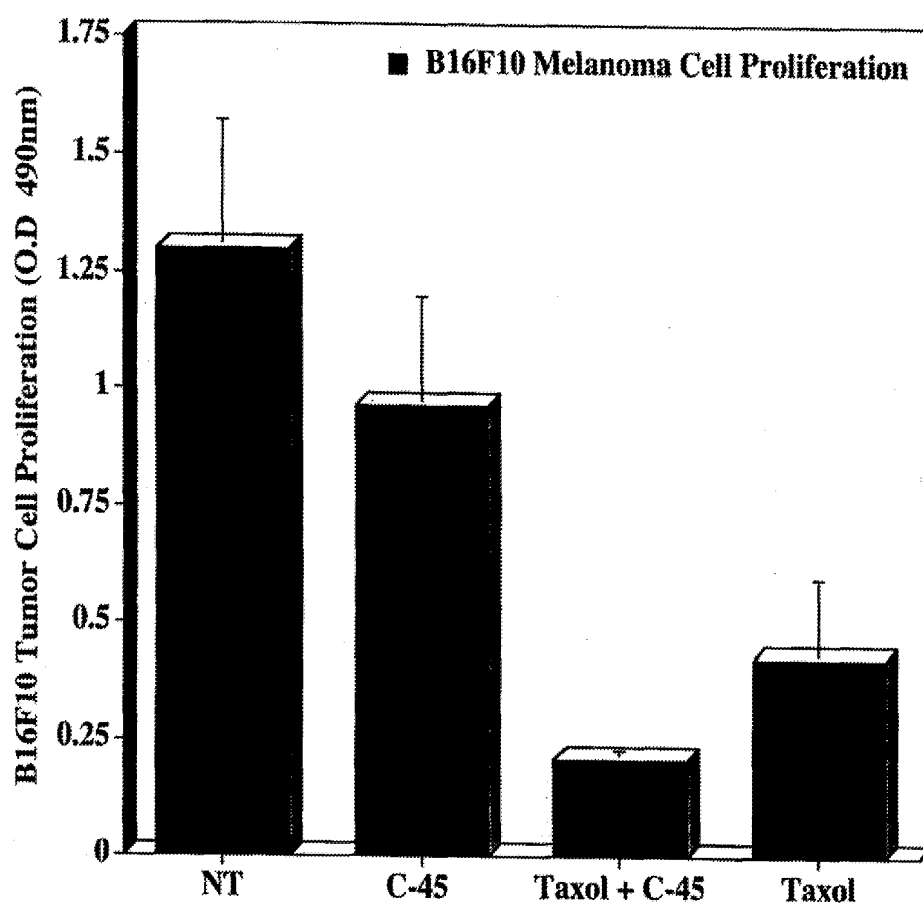
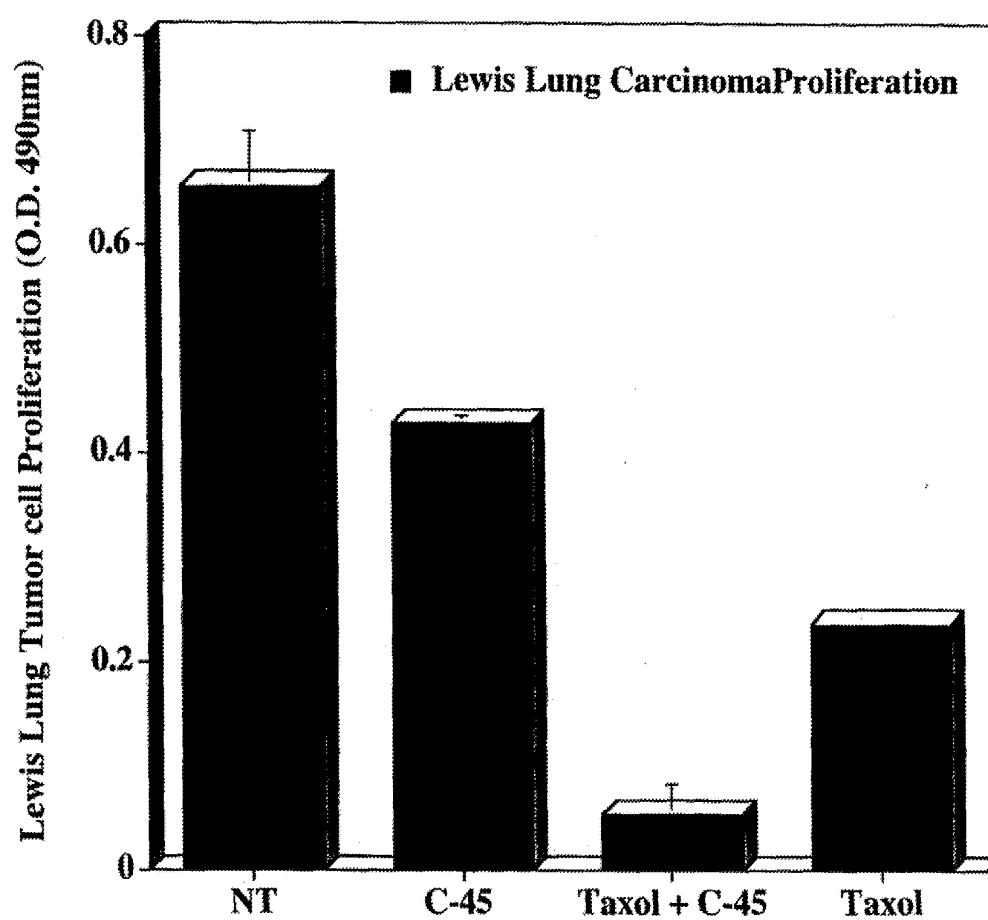


Figure 3A.

Figure 3B.



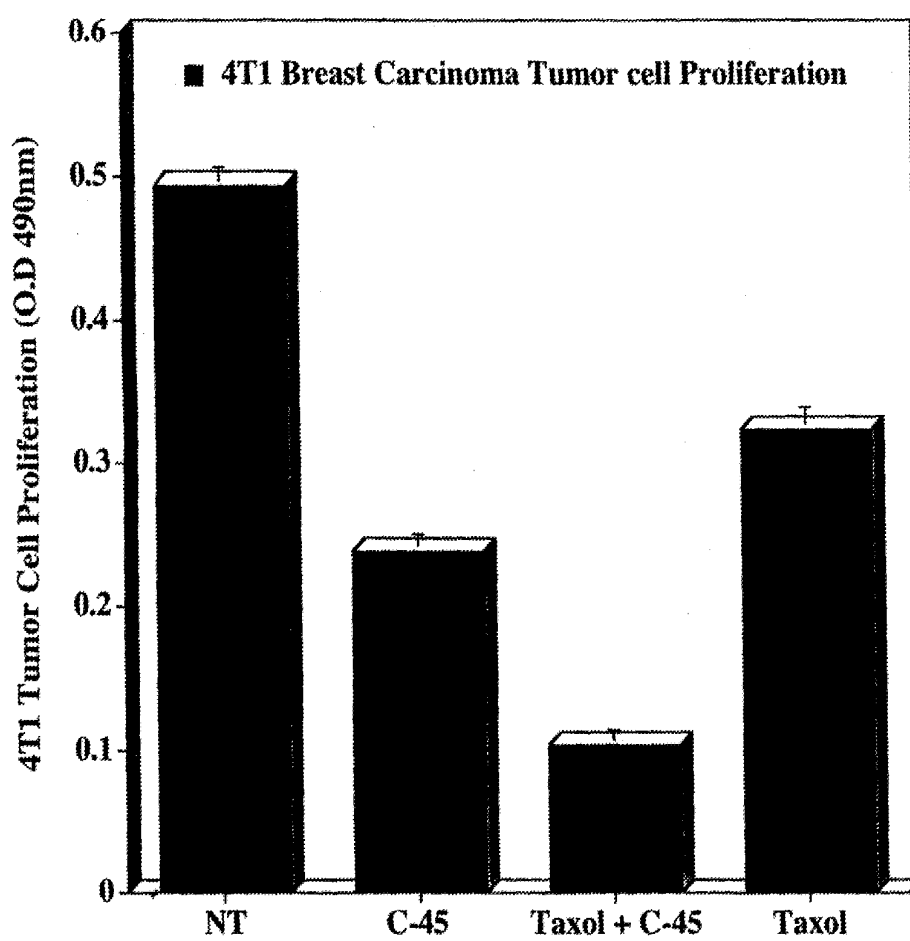


Figure 3C.

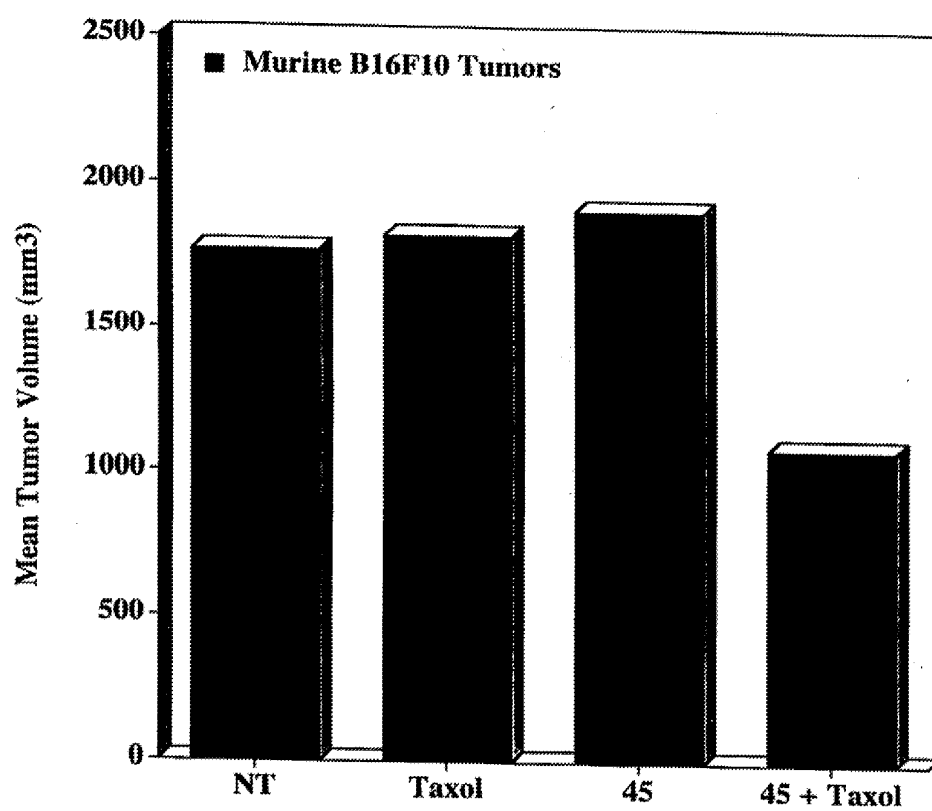


Figure 4.

Effects of C-45 on the Anti-Tumor Activity of Radiation

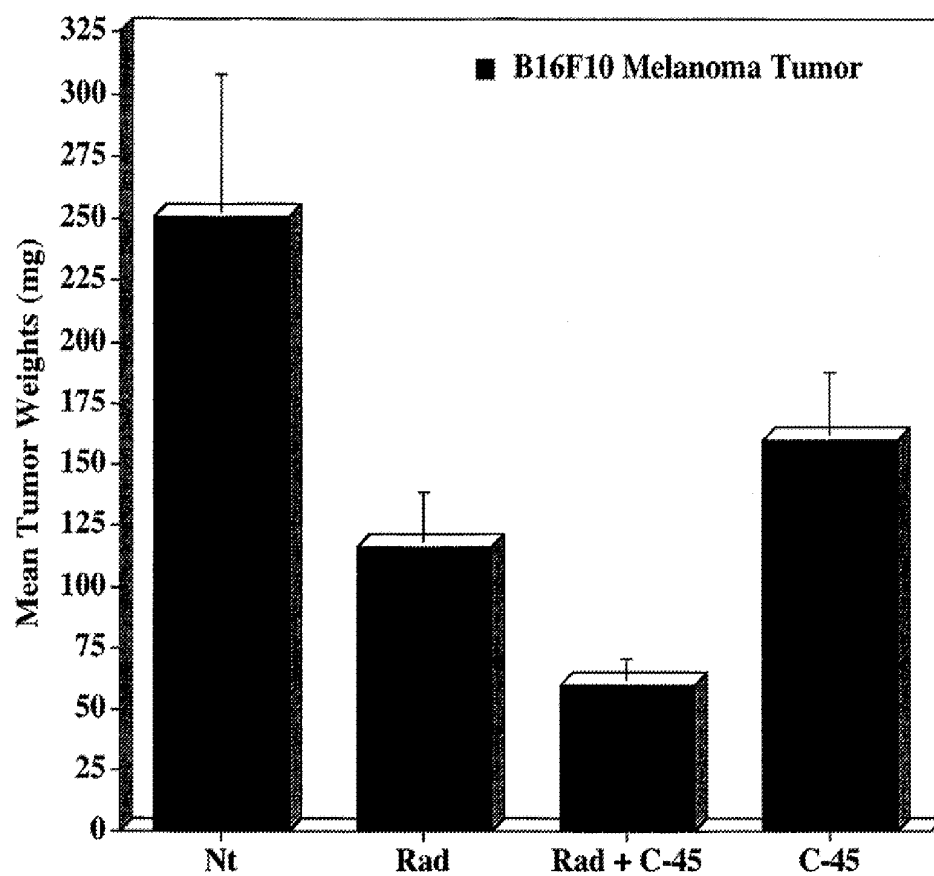


Figure 5A.

Effects of Compound C-45 on the Anti-tumor Activity of Taxol

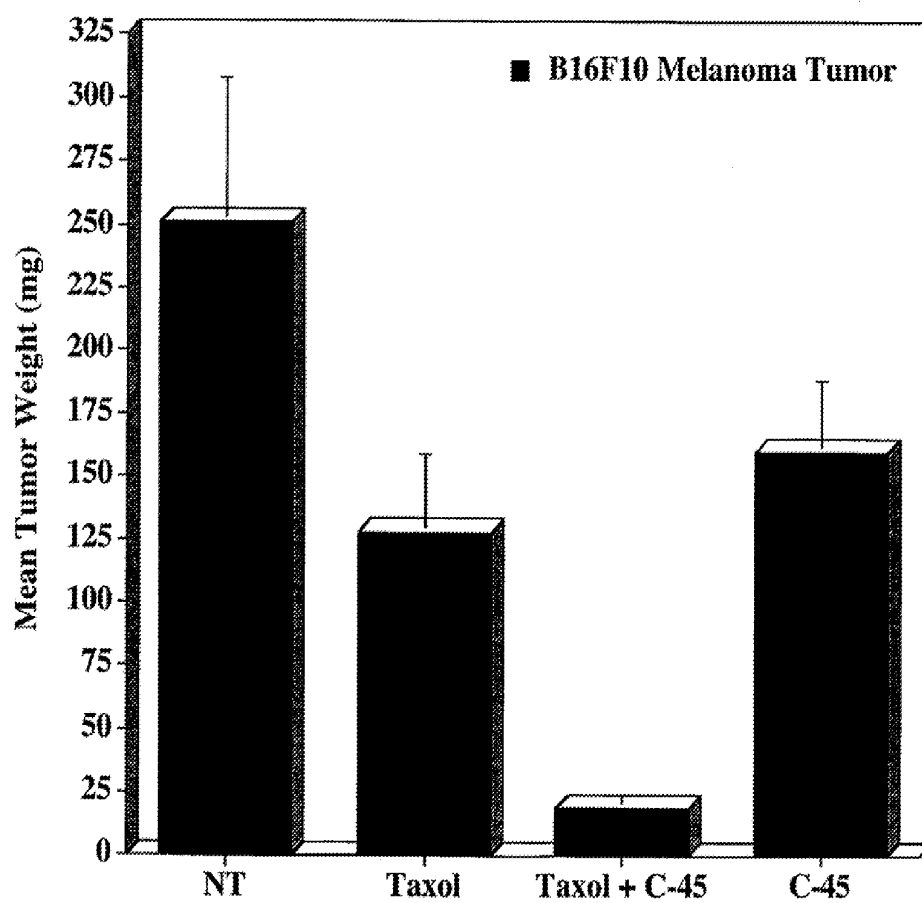


Figure 5B.

Figure 6.

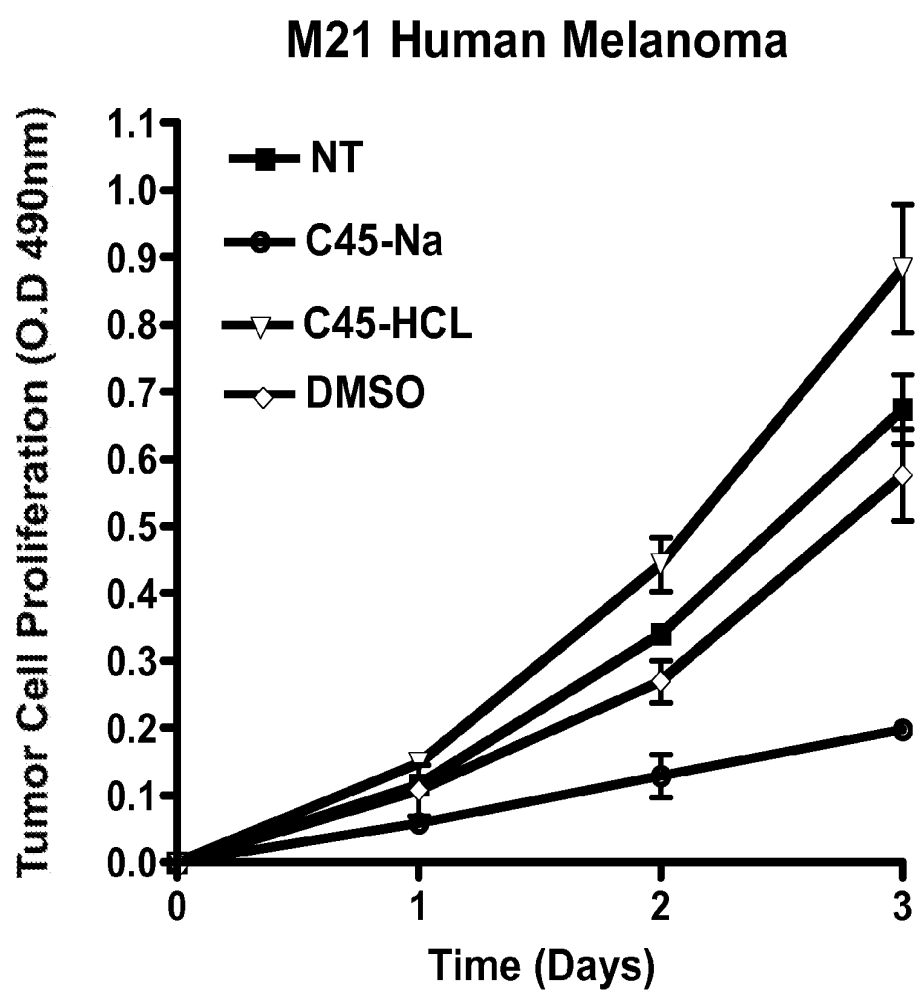


Figure 7.

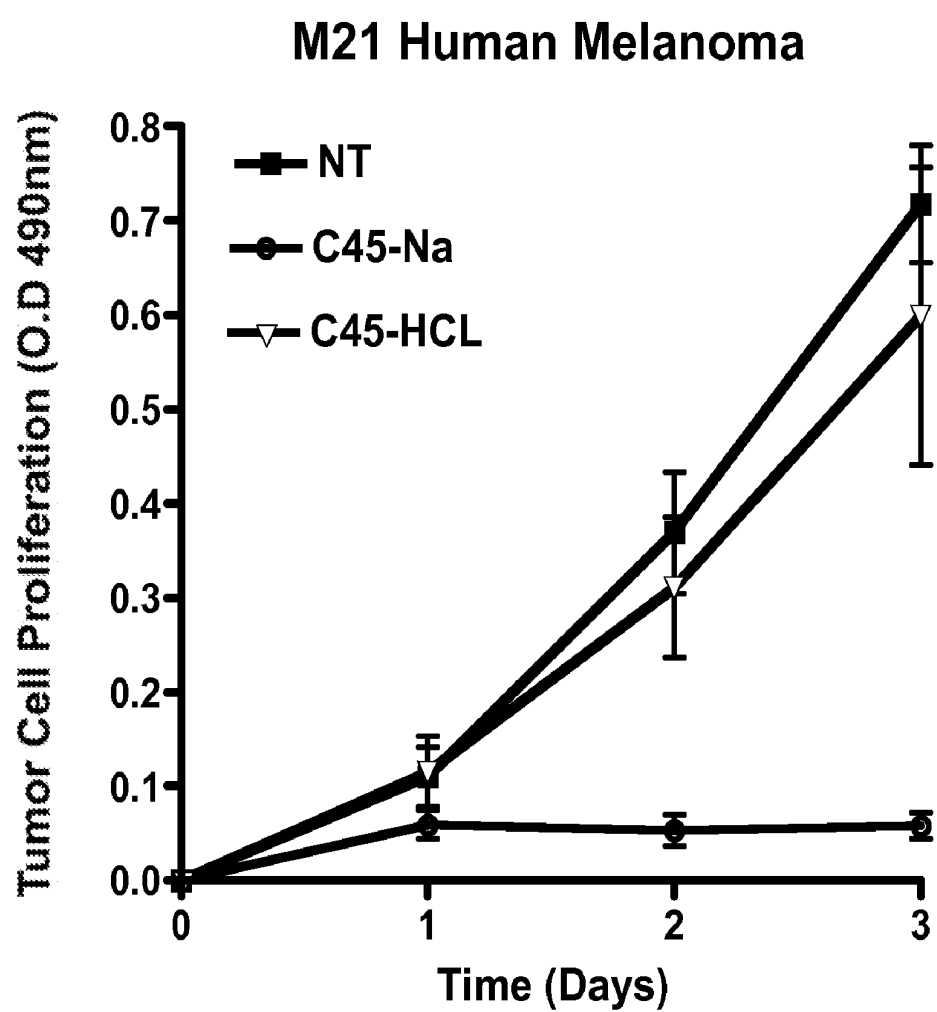


Figure 8.

Effects of C45 (1ug/day) on M21 Tumor Growth in Mice

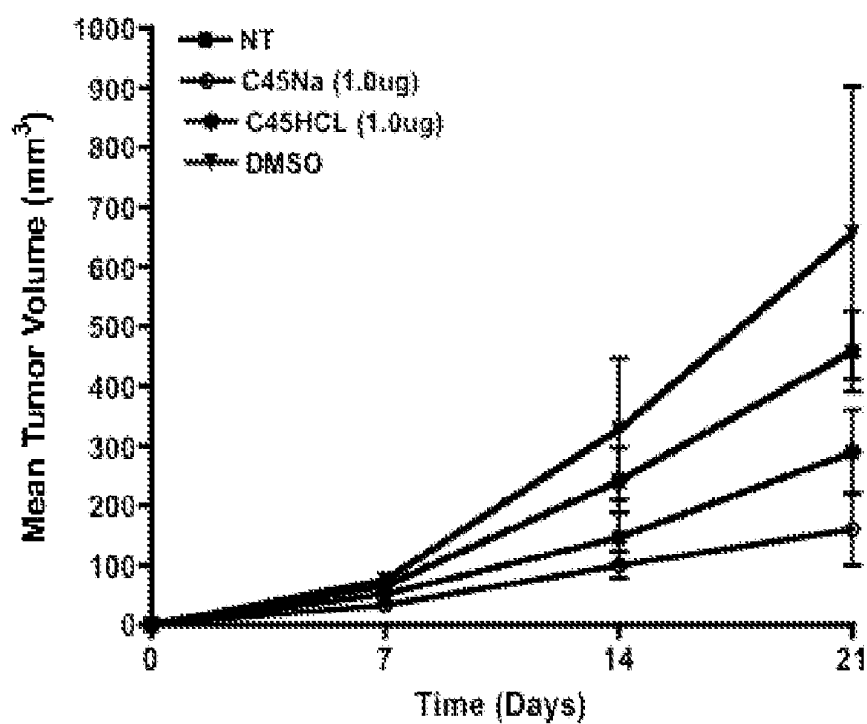


Figure 9.

Effects of C45 (10ug/day) on M21 Tumor Growth in Mice

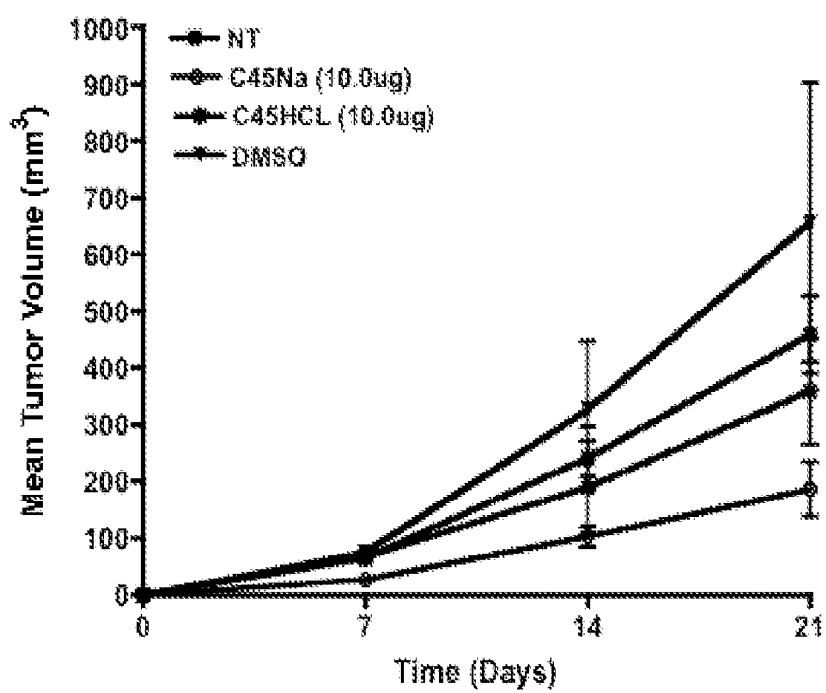


Figure 10.

Effects of C45 (100ug/day) on M21 Tumor Growth in Mice

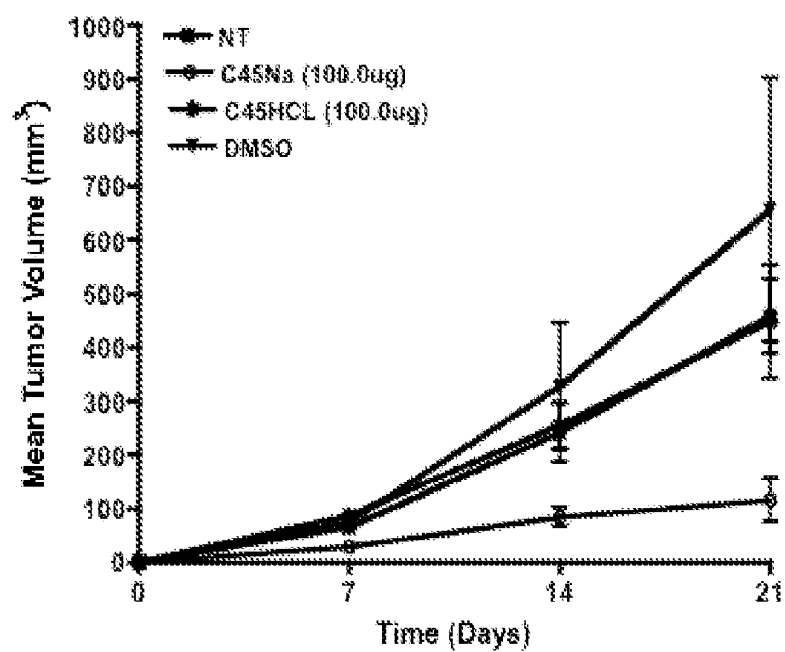


Figure 11.

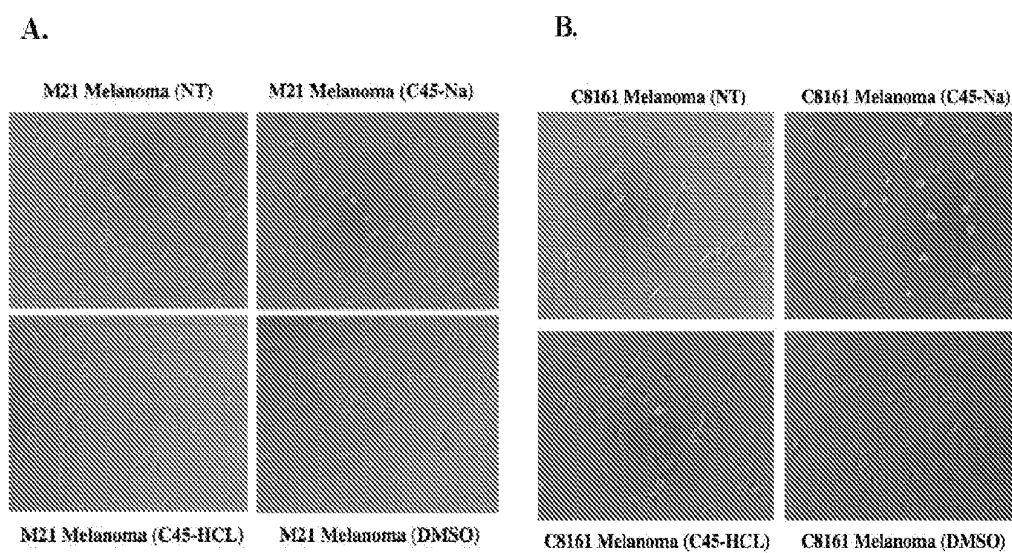


Figure 12.

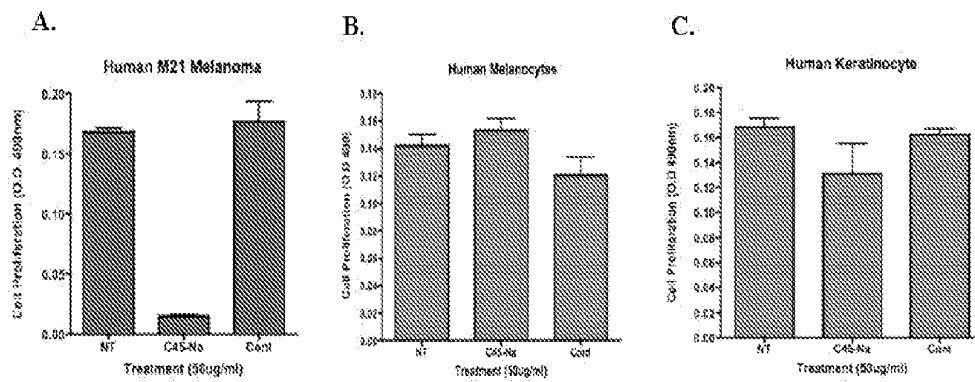


Figure 13.

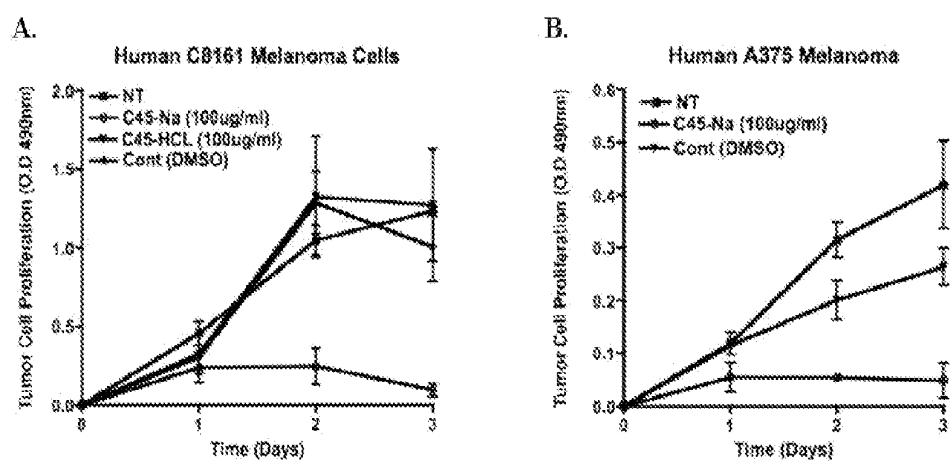
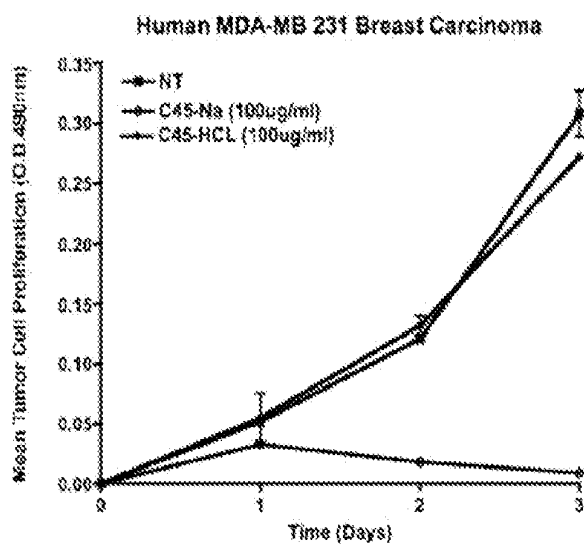


Figure 14.

A.



B.

Human Panc-1 Pancreatic Carcinoma

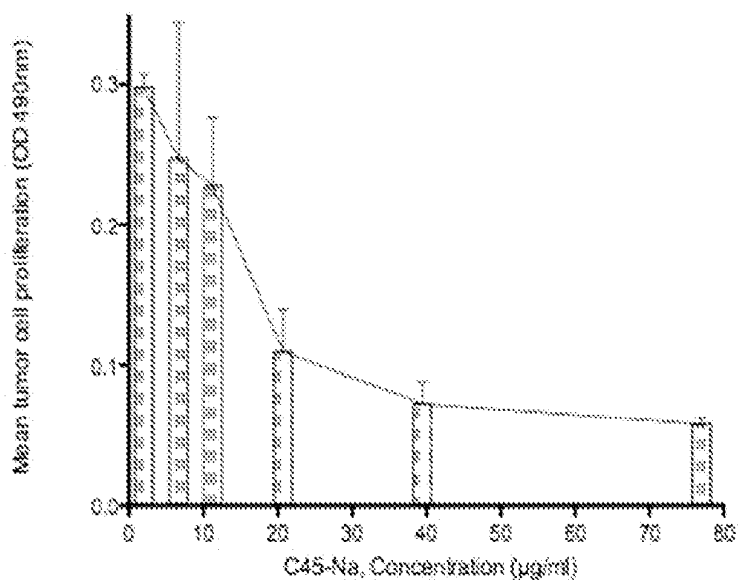


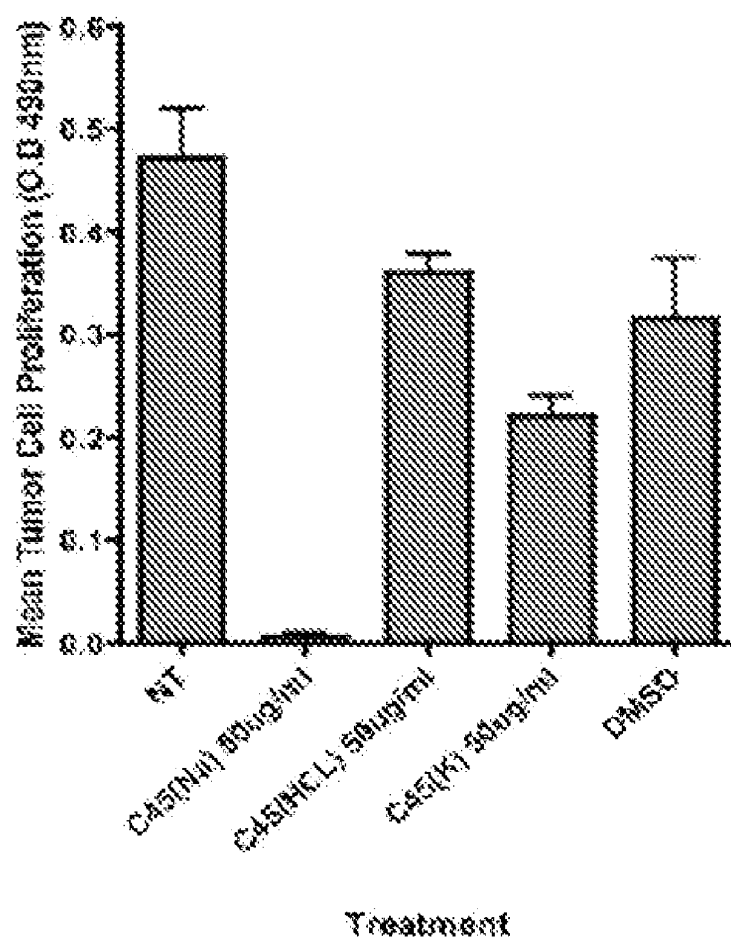
Figure 15.**Human M21 Melanoma Cell Proliferation Day 3**

Figure 16.

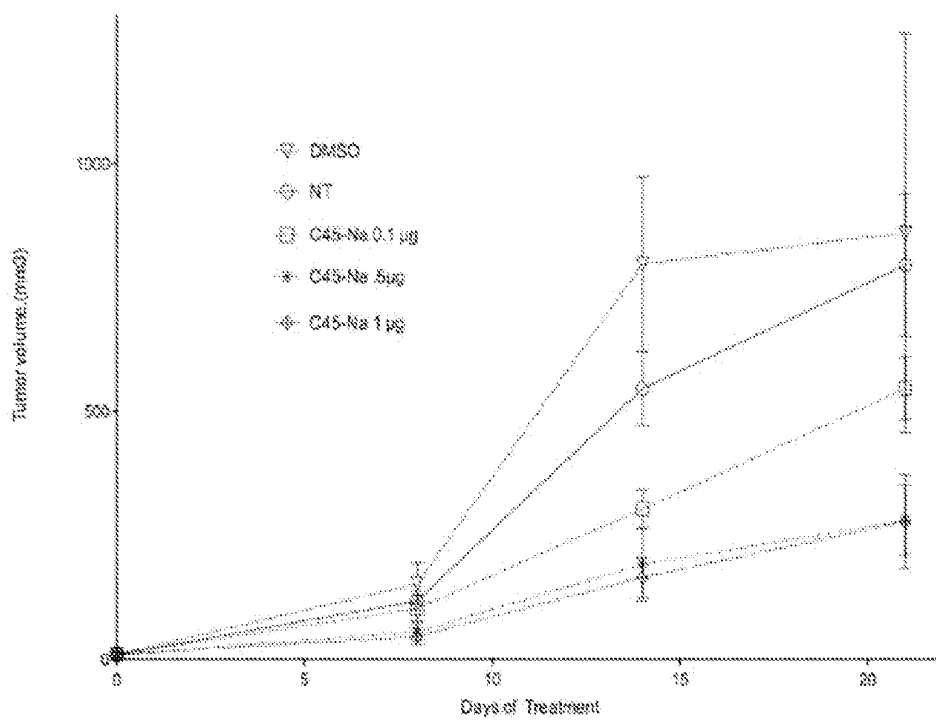
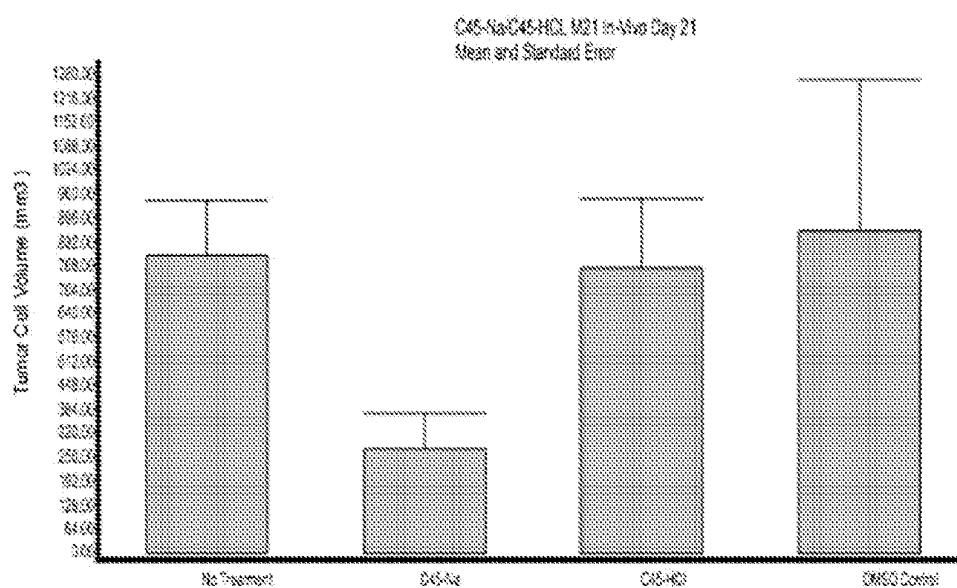


Figure 17.



TREATMENT OF CANCER USING THE SODIUM SALT OF A BENZOIC ACID DERIVATIVE

REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional Application No. 61/276,421, filed Sep. 10, 2009, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to the treatment of cancer using the sodium salt of a derivative of p-amino-benzoic acid (PABA). The invention also relates to the potentiation of radiotherapy, chemotherapy, or a combination thereof, with the sodium salt of the PABA derivative.

BACKGROUND OF THE INVENTION

[0003] Cancer

[0004] Cancer accounts for nearly one-quarter of deaths in the United States, exceeded only by heart disease. In the year 2000, there were 553,091 cancer deaths in the US. In 2003, the American Cancer Society estimates that this number will increase to approximately 556,500, due to aging and growth of the population. Lung cancer is the most common fatal cancer in men (31%), followed by prostate (10%), and colon & rectum (10%). In women, lung (25%), breast (15%), and colon & rectum (11%) are the leading sites of cancer death. Among children, leukemia is the most common cancer among children ages 0-14 years and it comprises approximately 30% of all childhood cancers and accounts for the most childhood deaths. Acute lymphocytic cancer is the most common form of leukemia in children. It is estimated that 1.33 million new cases of cancer were diagnosed in 2003 (American Cancer Society, 2003 Cancer Statistics Slide Set 2003).

[0005] Melanoma

[0006] Studies have indicated that nearly 45,000 new cases of melanoma are diagnosed each year in the U.S., and approximately 20% of patients will die of metastatic disease. Melanomas arise from the malignant conversion of melanocytes, which in turn are derived from mesenchymal neural crest cells. Melanomas undergo melanogenesis, a complex process that results in the production of melanin. Melanogenesis is initiated by the hydroxylation of L-tyrosine, to form L-dihydroxyphenylalanine (L-DOPA), which is then converted to DOPAchrome by specific melanocyte-associated enzymes, including tyrosinase. A further series of oxidation and reduction reactions ultimately convert DOPAchrome to melanin. It has been suggested that melanogenesis may account for the resistance of melanomas to treatment with ionizing radiation and chemotherapy. It has also been suggested that byproducts of melanogenesis are responsible for other adverse effects including immunosuppression, fibrosis and mutagenesis.

[0007] Current Treatments

[0008] Chemotherapy (CT) and radiation therapy (RT), and combinations thereof, remain the leading defenses against cancer, although recent advances in the field have led to widespread uses of specialized treatments such as angiogenesis inhibitors, biological therapies, including adjuvant therapy to boost the patient's immune system, antibody therapy, vaccine therapy, and photodynamic therapy.

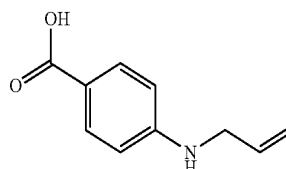
[0009] In addition to numerous adverse effects of RT and CT, a major limiting factor is the development of drug resistance by the tumors, and induction of tumor cell growth arrest and senescence. While senescent tumors do not increase in size per se, they still retain the capacity to produce and secrete tumor stimulating mitogens and pro-angiogenic factors that can lead to tumor progression.

[0010] The present invention shows that the sodium salt of the 4-(allylamino)benzoic acid derivative C-45 has unexpected benefits for the treatment of melanotic tumors and non-melanotic tumors. Further, the sodium salt of C-45 can surprisingly can potentiate treatment of melanotic and non-melanotic carcinomas with RT and/or CT.

SUMMARY OF THE INVENTION

[0011] The present invention provides a method of treating cancer, comprising administering an effective amount of the sodium salt of the benzoic acid derivative C-45, wherein C-45 has the following Formula I:

(Formula I)



[0012] In one embodiment, the sodium salt of C-45 is administered as monotherapy.

[0013] In another embodiment, the sodium salt of C-45 is administered in combination with chemotherapy and/or radiation therapy.

[0014] In one aspect of the invention, the cancer is melanoma. In another aspect of the invention, the cancer is breast carcinoma. In a further aspect of the invention, the cancer is lung carcinoma.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIG. 1 FIG. 1 depicts benzoic acid derivatives which can be used to treat cancer according to the method of the present invention.

[0016] FIG. 2 FIG. 2 demonstrates the inhibition of tyrosinase over time with PABA and two derivative compounds.

[0017] FIG. 3A-C FIG. 3 shows in vitro effects on the proliferation of B16F10 (3A), Lewis Lung carcinoma (3B), and 4T1 (3C) breast carcinoma cells treated with compound C-45, TAXOL®, and a combination of C-45 and TAXOL®.

[0018] FIG. 4 FIG. 4 shows the in vivo effects of compound C-45, TAXOL®, and a combination thereof, on tumor growth in nude mice.

[0019] FIG. 5A-B FIG. 5 demonstrates the effects of C-45, alone, and in combination with radiation (5A) and chemotherapy (5B), on growth of the B16F10 melanoma tumors in chick embryos.

[0020] FIG. 6 FIG. 6 is a graph illustrating the effects of no treatment (NT), C45-Na treatment, C-45-HCl treatment, and DMSO treatment on M21 human melanoma tumor cell proliferation.

[0021] FIG. 7 FIG. 7 is a graph illustrating the effects of no treatment (NT), C-45-Na treatment, and C-45-HCl treatment on M21 human melanoma tumor cell proliferation.

[0022] FIG. 8 FIG. 8 is a graph illustrating the effects of no treatment (NT), C-45-Na (1 µg/day), C-45-HCl (1 µg/day), and DMSO treatment on M21 tumor growth in mice.

[0023] FIG. 9 FIG. 9 is a graph illustrating the effects of no treatment (NT), C-45-Na (10 µg/day), C-45-HCl (10 µg/day), and DMSO treatment on M21 tumor growth in mice.

[0024] FIG. 10 FIG. 10 is a graph illustrating the effects of no treatment (NT), C-45-Na (100 µg/day), C-45-HCl (100 µg/day), and DMSO treatment on M21 tumor growth in mice.

[0025] FIG. 11 FIG. 11 is a microscope image illustrating the effects of no treatment (NT), C-45-Na (100 mg/mL), C-45-HCl (100 mg/mL), and DMSO treatment of human M21 cells (A) and C8161 melanoma cells.

[0026] FIG. 12 FIG. 12 is a graph illustrating the effects of no treatment (NT), C-45-Na (50 mg/mL), and DMSO treatment of human MDA-231 breast carcinoma cells (A), human melanocytes (B), and human keratinocytes (C).

[0027] FIG. 13 FIG. 13 illustrates a plot of tumor cell proliferation over time with no treatment (NT), C-45-Na (100 mg/mL), C-45-HCl (100 mg/mL), and DMSO treatment of human C8161 melanoma cells (A) and human A375 melanoma cells (B).

[0028] FIG. 14 FIG. 14(A) illustrates a plot of tumor cell proliferation over time with no treatment (NT), C-45-Na (100 mg/mL), and C-45-HCl (100 mg/mL) treatment of human MDA-231 breast carcinoma cells. FIG. 14 (B) illustrates a plot of tumor cell proliferation with treatment with various concentrations of C-45-Na of human panc-1 pancreatic carcinoma cells.

[0029] FIG. 15 FIG. 15 illustrates a graph of tumor cell proliferation with no treatment (NT), C-45-Na (50 mg/mL), C-45-K (50 mg/mL), C-45-HCl (50 mg/mL) and DMSO treatment of human M treatment of human M21 melanoma cells.

[0030] FIG. 16 FIG. 16 illustrates a plot of tumor cell growth over time with no treatment (NT), C-45-Na (0.1 mg, 0.5 mg, and 1 mg) and DMSO treatment of M21 tumors.

[0031] FIG. 17 FIG. 17 illustrates a plot of in vivo tumor cell growth over 21 days with no treatment (NT), C-45-Na (1 mg), C-45-HCl (1 mg) and DMSO treatment of M21 tumors.

DETAILED DESCRIPTION

[0032] It has now been surprisingly discovered that the sodium salt of C-45 can specifically target tumor cells (including non-melanotic tumors) and potentiate tumoricidal activities of CT and/or RT in such tumor cells. Unexpectedly, the sodium salt of C-45 has activity against tumor cells that markedly exceeds that of C-45 and other salts (i.e., potassium salt) of C-45.

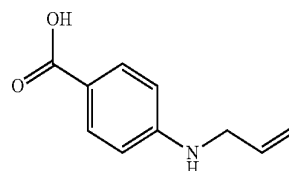
[0033] The present invention is, in part, based on the findings described in the Examples presented herein.

DEFINITIONS

[0034] “para-amino-benzoic-acid” (PABA) is commercially available from, e.g., Sigma-Aldrich Chemical Co., St. Louis, Mo.

[0035] “PABA derivative” as used herein refer to compounds chemically related to benzoic acid, including alkyl and acyl analogues, chlorobenzoic acids, aminobenzoic acids and nitrobenzoic acids, and salts and esters thereof.

[0036] The compound designated C-45 has the following Formula I:



(Formula I)

[0037] The compound of the present invention is the sodium salt of C-45.

[0038] “Cancer” refers to abnormal, malignant proliferations of cells originating from epithelial cell tissue (carcinomas), blood cells (leukemias, lymphomas, myelomas), connective tissue (sarcomas), or glial or supportive cells (gliomas). In one embodiment, the invention relates to the treatment of carcinomas and blood cell tumors. In a preferred embodiment, the invention relates to the treatment of lung tumors, breast tumors, ovarian tumors, pancreatic tumors, glioblastoma tumors, and sarcomas.

[0039] The term cancer includes but is not limited to the following: acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, carcinoma, aids-related cancers, aids-related lymphoma, anal cancer, astrocytoma (cerebellar), bile duct cancer (extrahepatic), bladder cancer, bone cancer (osteosarcoma/malignant fibrous histiocytoma), brain stem glioma, ependymoma, childhood visual pathway and hypothalamic glioma, breast cancer (including male), bronchial adenomas/carcinoids, carcinoid tumor (gastrointestinal), islet cell carcinoma, carcinoma of unknown primary origin, central nervous system lymphoma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, clear cell sarcoma of tendon sheaths, colon cancer, colorectal cancer, cutaneous T-cell lymphoma, endometrial cancer, ependymoma, ovarian epithelial cancer, esophageal cancer, Ewing’s family of tumors, extracranial germ cell tumor, extragonadal germ cell tumor, intraocular melanoma, retinoblastoma, gallbladder cancer, gastric (stomach) cancer, germ cell tumor, ovarian germ cell tumor, gestational trophoblastic tumor, hairy cell leukemia, head and neck cancer, hepatocellular (liver) cancer, Hodgkin’s lymphoma, hypopharyngeal cancer, Kaposi’s sarcoma, kidney cancer, laryngeal cancer, lip and oral cavity cancer, liver cancer, lung cancer (non-small cell and small cell), lymphoma, macroglobulinemia, Waldenström’s, malignant mesothelioma, medulloblastoma, melanoma, Merkel cell carcinoma, metastatic squamous neck cancer with occult primary, multiple endocrine neoplasia syndrome, multiple myeloma/plasma cell neoplasm, mycosis fungoides, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, multiple myeloma, chronic myeloproliferative disorders, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, oral cancer, oral cavity and lip cancer, oropharyngeal cancer, ovarian low malignant potential tumor, pancreatic cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pheochromocytoma, pineal and supratentorial primitive neuroectodermal tumors, pituitary tumor, pleuropulmonary blastoma, prostate cancer, rectal cancer, renal cell (kidney) cancer, renal pelvis and ureter, transitional cell cancer, rhabdomyosarcoma, sali-

vary gland cancer, soft tissue sarcoma, Sezary syndrome, skin cancer (non-melanotic), small intestine cancer, supratentorial primitive neuroectodermal tumors, T-cell lymphoma, cutaneous testicular cancer, thymoma, thymic carcinoma, thyroid cancer, urethral cancer, endometrial uterine cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilm's tumor.

[0040] The term also includes childhood cancers of all or any of the above-identified cancers.

[0041] In a specific embodiment, the cancer is melanoma. The term "radiation therapy" or "radiotherapy" refers to use of high-energy radiation to treat cancer. Radiation therapy includes externally administered radiation, e.g., external beam radiation therapy from a linear accelerator, and brachytherapy, in which the source of irradiation is placed close to the surface of the body or within a body cavity. Common radioisotopes used include but are not limited to cesium (^{137}Cs), cobalt (^{60}Co), iodine (^{131}I), phosphorus-32 (^{32}P), gold-198 (^{198}Au), iridium-192 (^{192}Ir), yttrium-90 (^{90}Y), and palladium-109 (^{109}Pd). Radiation is generally measured in Gray units (Gy), where 1 Gy=100 rads.

[0042] "Chemotherapy" (CT) refers to treatment with anti-cancer drugs. The term encompasses numerous classes of agents including platinum-based drugs, alkylating agents, anti-metabolites, anti-mitotic agents, anti-microtubule agents, plant alkaloids, and anti-tumor antibiotics, kinase inhibitors, proteasome inhibitors, EGFR inhibitors, HER dimerization inhibitors, VEGF inhibitors, and antisense molecules, and includes antibodies. Such drugs include but are not limited to adriamycin, melphalan, ara-C, BiCNU, busulfan, CCNU, pentostatin, the platinum-based drugs carboplatin, cisplatin and oxaliplatin, cyclophosphamide, daunorubicin, epirubicin, dacarbazine, 5-fluorouracil (5-FU), fludarabine, hydroxyurea, idarubicin, ifosfamide, methotrexate, altretamine, mithramycin, mitomycin, bleomycin, chlorambucil, mitoxantrone, nitrogen mustard, mercaptopurine, mitozantrone, paclitaxel (TAXOL®), vinblastine, vincristine, vindesine, etoposide, gemcitabine, monoclonal antibodies such as Herceptin®, Rituxan®, Campath®, Zevelin® and Bexxar®, irinotecan, leustatin, vinorelbine, STI-571 (Gleevec®), tamoxifen, docetaxel, topotecan, capecetabine (Xeloda®), raltitrexed, streptozocin, tegafur with uracil, temozolomide, thioguanine, thiotepa, podophyllotoxin, filgristim, proflimer sodium, letrozole, amifostine, anastrozole, temozolomide, arsenic trioxide, epthalones A and B tretinoin, interleukins (e.g., 2 and 12) and interferons, e.g., alpha and gamma, bortezomib, huBr-E3, Genasense, Ganite, FIT-3 ligand, MLN491RL, MLN2704, MLN576, and MLN518. Antiangiogenic agents include but are not limited to BMS-275291, Dalteparin (Fragmin®) 2-methoxyestradiol (2-ME), thalidomide, CC-5013 (thalidomide analog), maspin, combretastatin A4 phosphate, LY317615, soy isoflavone (genistein; soy protein isolate), AE-941 (Neovastat™; GW786034), anti-VEGF antibody (Bevacizumab; Avastin™), PTK787/ZK 222584, VEGF-trap, ZD6474, EMD 121974, anti- $\alpha\text{v}\beta_3$ integrin antibody (Medi-522; Vitaxin™) carboxyamidotriazole (CAI), celecoxib (Celebrex®), halofuginone hydrobromide (Tempostat™), and Rofecoxib (VIOXX®).

[0043] The term "chemotherapy" also includes gene therapy with agents such as interferon and the interleukins, i.e., administration of a vector encoding genes for the interferons or interleukins. See e.g., Heller et al., *Technol Cancer Res Treat.* 2002; 1(3):205-9.

[0044] The term "PXR/SXR" refers to a mammalian steroid and xenobiotic-sensing nuclear receptor. For example, the nucleotide and amino acid sequences for the human PXR/SXR can be found in GenBank Accession No. AY091855, or in Blumberg et al., *Genes Dev.* 1988; 12(20): 3195-3205.

[0045] "Cell cycle regulatory proteins" are those proteins, including enzymes, which are required for progression through the cell cycle, i.e., mitosis, or arrest of the cell cycle, i.e., senescence. Cell cycle progression proteins include but are not limited to CDC25A, CDC2, Wee-1, Myt-1, cyclin A, cyclin B, and LATS1, and associated cyclin dependent kinases. Proteins involved in cell cycle arrest, which include DNA repair proteins, include but are not limited to Id-1, Id-2, Id-3, ATM, ATR, p53, BRCA-1, BRCA-2, chk-1, Rad-53, and the cyclin dependent kinase inhibitors.

[0046] As used herein, the terms "treatment" or "treat" mean the lessening or ameliorating of at least one abnormal or undesirable condition associated with cancer. Treatment may, for example, cause a reduction in the rate or amount of growth of a tumor. Treatment also includes reducing or ameliorating the undesirable symptoms of cancer. The foregoing are merely non-limiting examples of the treatment of cancer. In a specific embodiment, the term "treatment" refers to enhancing tumor cell death by RT and/or CT by administering the sodium salt of C-45.

[0047] As used herein, a "therapeutically effective amount" of an agent is an amount sufficient to ameliorate at least one symptom associated with a pathological, abnormal or otherwise undesirable condition, e.g., cancer, an amount sufficient to prevent or lessen the probability that such a condition will occur or re-occur, or an amount sufficient to delay worsening of such a condition. In one embodiment, the term "therapeutically effective amount" is used to refer to an amount having antiproliferative effect.

[0048] Preferably, the therapeutically effective amount has apoptotic activity, or is capable of inducing cell death, and preferably death of benign or malignant tumor cells, in particular cancer cells. Efficacy can be measured in conventional ways, depending on the condition to be treated. For cancer therapy, efficacy can, for example, be measured by assessing the time for disease progression, or determining the response rates. In a preferred embodiment, an effective amount of the sodium salt of C-45 is an amount that reduces or inhibits the growth and/or proliferation of tumor cells in an individual in need of treatment alone, or in combination with RT and/or CT.

[0049] As used herein, the phrase "individual or mammal in need of such treatment" refers to a mammal suffering from at least one type of hyperproliferative disorder or who has been diagnosed with cancer.

[0050] The phrase "in combination with" refers to a method of treatment in which two or more treatments are administered collectively or according to a specific sequence, such that they produce a desirable effect.

[0051] The term "potentiate" means to increase the effect of, or act synergistically with, a drug or a biologic. In one embodiment of the present invention, PABA derivatives potentiate the tumoricidal activity or inhibition of tumor growth effected by RT or CT.

[0052] The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar toxicity (for example, gastric upset, dizziness and the like) when administered to an individual. Preferably, and particu-

larly where a formulation is used in humans, the term “pharmaceutically acceptable” may mean approved by a regulatory agency (for example, the U.S. Food and Drug Agency) or listed in a generally recognized pharmacopeia for use in animals (e.g., the U.S. Pharmacopeia). The term “salt” denotes any form of an active ingredient in which the active ingredient assumes an ionic form and is coupled to a counter ion (a cation or anion) or is in solution. This also includes complexes of the active ingredient with other molecules and ions, in particular complexes which are complexed by ion interaction.

[0053] The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in “Remington’s Pharmaceutical Sciences” by E. W. Martin, 18th Edition.

[0054] The terms “about” and “approximately” shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Typical, exemplary degrees of error are within 20 percent (%), preferably within 10%, and more preferably within 5% of a given value or range of values. Alternatively, and particularly in biological systems, the terms “about” and “approximately” may mean values that are within an order of magnitude, preferably within 10- or 5-fold, and more preferably within 2-fold of a given value. Numerical quantities given herein are approximate unless stated otherwise, meaning that the term “about” or “approximately” can be inferred when not expressly stated.

[0055] Molecular Biology

[0056] The term “purified” as used herein refers to material that has been isolated under conditions that reduce or eliminate the presence of unrelated materials, i.e., contaminants, including native materials from which the material is obtained. For example, a purified protein is preferably substantially free of other proteins or nucleic acids with which it is associated in a cell; a purified nucleic acid molecule is preferably substantially free of proteins or other unrelated nucleic acid molecules with which it can be found within a cell. As used herein, the term “substantially free” is used operationally, in the context of analytical testing of the material. Preferably, purified material substantially free of contaminants is at least 95% pure; more preferably, at least 97% pure, and more preferably still at least 99% pure. Purity can be evaluated by chromatography, gel electrophoresis, immunoassay, composition analysis, biological assay, and other methods known in the art. In a specific embodiment, purified means that the level of contaminants is below a level acceptable to regulatory authorities for administration to a human or non-human animal.

[0057] A “gene” is a sequence of nucleotides which code for a functional “gene product”. Generally, a gene product is a functional protein. However, a gene product can also be another type of molecule in a cell, such as an RNA (e.g., a tRNA or a rRNA). For the purposes of the present invention, a gene product also refers to an mRNA sequence which may be found in a cell.

[0058] The term “express” and “expression” means allowing or causing the information in a gene or DNA sequence to become manifest, for example producing RNA (such as

rRNA or mRNA) or a protein by activating the cellular functions involved in transcription and translation of a corresponding gene or DNA sequence. A DNA sequence is expressed by a cell to form an “expression product” such as an RNA (e.g., a mRNA or a rRNA) or a protein. The expression product itself, e.g., the resulting RNA or protein, may also said to be “expressed” by the cell.

[0059] “Expression level” correspond to levels of a detectable cellular product e.g., mRNA or a corresponding gene product, or an activity of such a gene product. For example, according to the screening method of present invention, changes in the expression level of a reporter gene operatively associated with the PXR/SXR response element can be used to determine whether a compound binds to and activates the PXR/SXR receptor.

[0060] The term “transfection” means the introduction of a foreign nucleic acid into a cell. The term “transformation” means the introduction of a “foreign” (i.e., extrinsic or extracellular) gene, DNA or RNA sequence into a host cell so that the host cell will express the introduced gene or sequence to produce a desired substance, in this invention typically an RNA coded by the introduced gene or sequence, but also a protein or an enzyme coded by the introduced gene or sequence. The introduced gene or sequence may also be called a “cloned” or “foreign” gene or sequence, may include regulatory or control sequences (e.g., start, stop, promoter, signal, secretion or other sequences used by a cell’s genetic machinery). The gene or sequence may include nonfunctional sequences or sequences with no known function. A host cell that receives and expresses introduced DNA or RNA has been “transformed” and is a “transformant” or a “clone”. The DNA or RNA introduced to a host cell can come from any source, including cells of the same genus or species as the host cell or cells of a different genus or species.

[0061] The terms “vector”, “cloning vector” and “expression vector” mean the vehicle by which a DNA or RNA sequence (e.g., a foreign gene) can be introduced into a host cell so as to transform the host and promote expression (e.g., transcription and translation) of the introduced sequence.

[0062] The term “expression system” means a host cell and compatible vector under suitable conditions, e.g., for the expression of a protein coded for by foreign DNA carried by the vector and introduced to the host cell. Common expression systems include *E. coli* host cells and plasmid vectors, insect host cells such as Sf9, Hi5 or S2 cells and Baculovirus vectors, and expression systems, and mammalian host cells, including tumor cells and cell lines, and vectors.

[0063] By “vector” is meant any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc., which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

[0064] Formulations and Administration

[0065] Formulations

[0066] For use in the present invention, the sodium salt of C-45 may be formulated into a pharmaceutical composition. The pharmaceutical composition may include additives, such as a pharmaceutically acceptable carrier or diluent, a flavorant, a sweetener, a preservative, a dye, a binder, a suspending agent, a dispersing agent, a colorant, a disintegrant, an excipient, a film forming agent, a lubricant, a plasticizer, an edible oil or any combination of two or more of the foregoing.

[0067] Suitable pharmaceutically acceptable carriers or diluents include, but are not limited to, ethanol, water, glycerol, propylene glycol, aloe vera gel, allantoin, glycerin, vitamin A and E oils, mineral oil, PPG2 myristyl propionate, magnesium carbonate, potassium phosphate, vegetable oil, animal oil, and solketal. Preferred carriers are vegetable and mineral oils.

[0068] Suitable binders include, but are not limited to, starch, gelatin, natural sugars, such as glucose, sucrose and lactose; corn sweeteners, natural and synthetic gums, such as acacia, tragacanth, vegetable gum, and sodium alginate, carboxymethylcellulose, hydroxypropylmethylcellulose, polyethylene glycol, povidone, waxes; and the like.

[0069] Suitable disintegrants include, but are not limited to, starch, e.g., corn starch, methyl cellulose, agar, bentonite, xanthan gum, sodium starch glycolate, croscopovidone and the like.

[0070] Suitable lubricants include, but are not limited to, sodium oleate, sodium stearate, sodium stearyl fumarate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like.

[0071] A suitable suspending agent is, but is not limited to, bentonite, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, agar-agar and tragacanth, or mixtures of two or more of these substances, and the like.

[0072] Suitable dispersing and suspending agents include, but are not limited to, synthetic and natural gums, such as vegetable gum, tragacanth, acacia, alginate, dextran, sodium carboxymethylcellulose, methylcellulose, polyvinyl-pyrrolidone and gelatin.

[0073] Suitable film forming agents include, but are not limited to, hydroxypropylmethylcellulose, ethylcellulose and polymethacrylates. Suitable plasticizers include, but are not limited to, polyethylene glycols of different molecular weights (e.g., 200-8000 Da) and propylene glycol.

[0074] Suitable colorants include, but are not limited to, ferric oxide(s), titanium dioxide and natural and synthetic lakes.

[0075] Suitable edible oils include, but are not limited to, cottonseed oil, sesame oil, coconut oil and peanut oil.

[0076] Examples of additional additives include, but are not limited to, sorbitol, talc, stearic acid, dicalcium phosphate and polydextrose.

[0077] Dosages and Dosage Forms

[0078] The pharmaceutical composition or unit dosage form of the present invention may be administered according to a dosage and administration regimen defined by routine testing in order to obtain optimal activity while minimizing toxicity or side-effects for a particular patient. Typically, dosages will be determined by those skilled in the art on a case-by-case basis, depending upon the tumor type, stage, location, and prognosis of the individual, and other factors such as weight, sex and age of the individual, the particular dosage form employed, and the route of administration utilized. Pharmacokinetics and pharmacodynamics such as half-life ($t_{1/2}$), peak plasma concentration (c_{max}), time to peak plasma concentration (t_{max}), and exposure as measured by area under the curve (AUC) can be obtained using ordinary methods known in the art.

[0079] Data obtained from cell culture assay or animal studies may be used to formulate a therapeutic dosage range for use in humans and non-human animals. The dosage of compounds used in therapeutic methods of the present inven-

tion preferably lie within a range of circulating concentrations that includes the ED_{50} concentration (effective for 50% of the tested population) but with little or no toxicity.

[0080] A therapeutically effective dose may be initially estimated from cell culture assays and formulated in animal models to achieve a circulating concentration range that includes the IC_{50} . The IC_{50} concentration of a compound is the concentration that achieves a half-maximal inhibition of symptoms (e.g., as determined from the cell culture assays). Appropriate dosages for use in a particular individual, for example in human patients, may then be more accurately determined using such information.

[0081] In one embodiment, the compound may be administered, alone or in combination with CT or RT in the range of about 10 g/day, preferably in a range from about 10 mg/day to about 6 g/day, more preferably in a range from about 250 mg/day to about 5 g/day.

[0082] For combination therapy with radiation, the radiation is typically administered in doses of 1 cGy to 100 Gy. More preferably, radiation is administered in doses of 2 cGy to 20 Gy. Factors such as dose rate delivered, tumor size, and radiosensitivity play a major role in determining therapeutic response, while target-to-nontarget ratios and, particularly, circulating radioactivity to the bone marrow determine the major dose-limiting toxicities.

[0083] Dosages of chemotherapy are not only drug-type specific, but also depend to a large extent on the individual patient, the tumor-type, and the stage of the disease, and accordingly, are determined by one of ordinary skill in the art. By way of example, standard doses of paclitaxel (TAXOL®) in combination with carboplatin for ovarian and lung cancer are 175 mg/m² of the former and AUC 5 mg/ml·min of the latter.

[0084] Unit dosage forms. The above compositions of the sodium salt of C-45 may be formulated as unit dosage forms such as tablets, pills, capsules, caplets, boluses, powders, granules, sterile parenteral solutions, sterile parenteral suspensions, sterile parenteral emulsions, elixirs, tinctures, metered aerosol or liquid sprays, drops, ampoules, autoinjector devices or suppositories. Unit dosage forms may be used for oral, parenteral, intranasal, sublingual or rectal administration, or for administration by inhalation or insufflation, transdermal patches, and a lyophilized composition. In general, any delivery of active ingredients that results in systemic availability of them can be used.

[0085] Preferably the unit dosage form of the sodium salt of C-45 is an oral dosage form, most preferably a solid oral dosage form, therefore the preferred dosage forms are tablets, pills, caplets and capsules. Sodium salt of C-45-containing solutions and suspensions for oral administration are also preferred. However, the compound can also be formulated for parenteral administration. Parenteral preparations (e.g., injectable preparations in saline and preparations for powder jet systems) are preferred for CT and RIT.

[0086] Solid unit dosage forms may be prepared by mixing an active agent of the present invention with a pharmaceutically acceptable carrier and any other desired additives as described above. The mixture is typically mixed until a homogeneous mixture of the active agents of the present invention and the carrier and any other desired additives is formed, i.e., until the active agent is dispersed evenly throughout the composition. In this case, the compositions can be formed as dry or moist granules.

[0087] Dosage forms with predetermined amounts of the benzoic acid derivatives may be formulated starting with compositions with known quantities of the compounds using methods well known in the art. In a preferred embodiment a dosage form is obtained by mixing compositions comprising known quantities of the derivatives.

[0088] Dosage forms can be formulated as, for example, "immediate release" dosage forms. "Immediate release" dosage forms are typically formulated as tablets that release at least 70%-90% of the active ingredient within 30-60 min when tested in a drug dissolution test, e.g., U.S. Pharmacopeia standard <711>. In a preferred embodiment, immediate dosage forms release 75% of active ingredients in 45 min.

[0089] Dosage forms can also be formulated as, for example, "controlled release" dosage forms. "Controlled," "sustained," "extended" or "time release" dosage forms are equivalent terms that describe the type of active agent delivery that occurs when the active agent is released from a delivery vehicle at an ascertainable and modifiable rate over a period of time, which is generally on the order of minutes, hours or days, typically ranging from about sixty minutes to about 3 days, rather than being dispersed immediately upon entry into the digestive tract or upon contact with gastric fluid. A controlled release rate can vary as a function of a multiplicity of factors. Factors influencing the rate of delivery in controlled release include the particle size, composition, porosity, charge structure, and degree of hydration of the delivery vehicle and the active ingredient(s), the acidity of the environment (either internal or external to the delivery vehicle), and the solubility of the active agent in the physiological environment, i.e., the particular location along the digestive tract. Typical parameters for dissolution test of controlled release forms are found in U.S. Pharmacopeia standard chapter <724>.

[0090] Dosage forms can also be formulated to deliver active agent in multiphasic stages whereby a first fraction of an active ingredient is released at a first rate and at least a second fraction of active ingredient is released at a second rate. In a preferred embodiment, a dosage form can be formulated to deliver active agent in a biphasic manner, comprising a first "immediate release phase", wherein a fraction of active ingredient is delivered at a rate set forth above for immediate release dosage forms, and a second "controlled release phase," wherein the remainder of the active ingredient is released in a controlled release manner, as set forth above for controlled release dosage forms.

[0091] Tablets or pills can be coated or otherwise compounded to form a unit dosage form which has delayed and/or prolonged action, such as time release and controlled release unit dosage forms. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of a layer or envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release.

[0092] Biodegradable polymers for controlling the release of the active agents, include, but are not limited to, polylactic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydro-pyrans, polycyanoacrylates and cross-linked or amphipathic block copolymers of hydrogels. For liquid dosage forms, the active substances or their physiologically acceptable salts are brought into solution, suspension or emulsion, optionally with the

usually employed substances such as solubilizers, emulsifiers or other auxiliaries. Solvents for the active combinations and the corresponding physiologically acceptable salts, can include water, physiological salt solutions or alcohols, e.g. ethanol, propane-diol or glycerol. Additionally, sugar solutions such as glucose or mannitol solutions may be used. A mixture of the various solvents mentioned may further be used in the present invention.

[0093] A transdermal dosage form also is contemplated by the present invention. Transdermal forms may be a diffusion-driven transdermal system (transdermal patch) using either a fluid reservoir or a drug-in-adhesive matrix system. Other transdermal dosage forms include, but are not limited to, topical gels, lotions, ointments, transmucosal systems and devices, and iontophoretic (electrical diffusion) delivery system. Transdermal dosage forms may be used for timed release and controlled release of the active agents of the present invention.

[0094] Pharmaceutical compositions and unit dosage forms of the present invention for administration parenterally, and in particular by injection, typically include a pharmaceutically acceptable carrier, as described above. A preferred liquid carrier is vegetable oil. Injection may be, for example, intravenous, intrathecal, intramuscular, intratracheal, or subcutaneous.

[0095] The active agent also can be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

[0096] The sodium salt of C-45 of the present invention also may be coupled with soluble polymers as targetable drug carriers. Such polymers include, but are not limited to, polyvinyl-pyrrolidone, pyran copolymer, polyhydroxypropyl-methacryl-amidephenol, polyhydroxy-ethylaspartamidephenol, and polyethyl-eneoxidepolylysine substituted with palmitoyl residues.

[0097] Administration

[0098] The pharmaceutical composition or unit dosage forms of the present invention may be administered by a variety of routes such as intravenous, intratracheal, subcutaneous, oral, intratumoral, mucosal parenteral, buccal, sublingual, rectal, ophthalmic, pulmonary, transmucosal, transdermal, and intramuscular. Unit dosage forms also can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches known to those of ordinary skill in the art. Oral administration of the derivatives is preferred. Also preferred is administration by local intratumoral injection.

[0099] The pharmaceutical composition or unit dosage forms of the present invention may be administered to a mammal, preferably a human being, in need of cancer treatment.

[0100] The pharmaceutical composition or unit dosage form may be administered in a single daily dose, or the total daily dosage may be administered in divided doses. In addition, co-administration or sequential administration of other active agents may be desirable. The derivatives and mixtures thereof of the invention may be combined with any known drug therapy, preferably RT and/or CT, for the treatment of cancer.

[0101] The pharmaceutical composition or unit dosage form may be administered in a single daily dose, or the total daily dosage may be administered in divided doses. The combination of the benzoic acid derivative and RT or CT may be co-administered simultaneously or sequentially administered. The compounds preferably will be provided as separate dosage forms.

[0102] The exact dosage and administration regimen utilizing the combination therapy of the present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the route of administration; the renal and hepatic function of the patient; the treatment history of the patient; and the responsiveness of the patient. Optimal precision in achieving concentrations of compounds within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the absorption, distribution, metabolism, excretion of a drug, and responsiveness of the patient to the dosage regimen. However, such fine tuning of the therapeutic regimen is routine in light of the guidelines given herein.

EXAMPLES

[0103] The present invention is further described by means of the examples presented below. The use of such examples is illustrative only and in no way limits the scope and meaning of the invention or of any exemplified term. Likewise, the invention is not limited to any particular preferred embodiments described herein. Indeed, many modifications and variations of the invention will be apparent to those skilled in the art upon reading this specification and can be made without departing from its spirit and scope. The invention is therefore to be limited only by the terms of the appended claims along with the full scope of equivalents to which the claims are entitled.

Example 1

PABA and Derivatives Inhibit Tyrosinase Activity In Vitro

[0104] Methods

[0105] Activity of purified tyrosinase was measured by monitoring the formation of DOPACHrome. Briefly, a fixed concentration of L-DOPA (8.0 mM) was incubated with 800 U/ml tyrosinase (from mushrooms-Sigma, St. Louis, Mo.) in the absence or presence of increasing concentrations of PABA, compound 44 (C-44) and compound 45 (C-45) (see FIG. 1 for structures) for indicated time points (see FIG. 2 for concentrations and time points). At each time point, 100 μ l of the reaction mixture was measured. Data points represent the mean optical density at 475 nm \pm SD from triplicate wells.

[0106] Results

[0107] As shown in FIG. 2, PABA, C-44, and C-45 all inhibited tyrosinase activity at 8.0 mM up to 10 minutes, the last time point measured. By contrast, untreated samples demonstrated linearly increasing tyrosinase activity up to 10 minutes. This approach can be applied to evaluate other benzoic acid derivatives or other compounds for inhibitory activity on tyrosinase.

Example 2

Effects of PABA Derivative C-45 on Carcinoma Cells Treated with Chemotherapy

[0108] Methods

[0109] Tumor cell proliferation was assessed by monitoring mitochondrial dehydrogenase activity as detected by tetrazolium salt cleavage using a commercially available kit (WST-1; Roche, Indianapolis, Ind.). Briefly, B16F10 melanoma cells, Lewis Lung carcinoma cells, and 4T1 breast carcinoma cells (1×10^6) were resuspended in PABA-free growth medium supplemented with 2.0% fetal bovine serum and 50 μ g/ml of test compound (as shown in FIG. 1) Following incubation for 3 days, the cells were either treated or not treated with 0.01 μ M TAXOL[®] and incubated for an additional 48 hours. Proliferation was measured; data points represent the mean optical density at 490 nm \pm SD from triplicate wells.

[0110] In addition, tumor growth in vivo of xenografts of B16F10 melanoma cells in nude mice was determined. Briefly, subconfluent cultures of B16F10 cells were harvested, washed, and resuspended in sterile PBS. Female nude mice were injected subcutaneously with 1×10^6 cells per mouse and tumors were allowed to grow for 3 days. After 3 days, mice were either untreated or treated daily for 15 days by intraperitoneal injection of TAXOL[®] (0.5 μ M), C-45 (100 μ g), or a combination of TAXOL[®] and C-45. Tumor growth was determined weekly by caliper measurements. After 15 days, the mice were sacrificed and tumor volume was determined by standard methods using the formula $V=L \times W^2/2$, where V=volume, L=length, and W=width.

[0111] Results

[0112] FIG. 3A-C demonstrate that both TAXOL[®] and compound C-45 individually inhibited in vitro proliferation of B16F10, Lewis Lung carcinoma, and 4T1 breast carcinoma cells compared to untreated control, and the inhibition was even more pronounced with a combination of C-45 and TAXOL[®].

[0113] As shown in FIG. 4, neither TAXOL[®] nor C-45 inhibited in vivo tumor growth compared to untreated controls. By contrast, a combination of TAXOL[®] and C-45 inhibited in vivo growth by more than about 33%.

Example 3

The Effect of C-45 on Radio- and Chemosensitivity In Vivo

[0114] Methods

[0115] The chick embryo tumor growth model was used to determine whether C-45 enhances the ability of TAXOL[®] or ionizing radiation to inhibit tumor growth. Briefly, B16F10 melanoma cells were cultured in growth medium in the presence or absence of 50 μ g/ml C-45 for at least 7 days. Following harvesting and washing, about 2×10^5 tumor cells were implanted onto the chorioallantoic membranes of 10 day-old chick embryos and incubated for at least 24 hours. Following incubation, the embryos were treated with either a single fractionated dose of radiation (5.0 Gy) or TAXOL[®] (paclitaxel) (0.01 μ M) and incubated for an additional 7 days. The embryos were sacrificed and tumors were resected and weighed (5-10 embryos per condition).

[0116] Results

[0117] FIG. 5A-B demonstrates the effect of C-45 on radiation- and chemotherapy-treated tumor growth in chick embryos. As shown in FIG. 5A, while treatment with radiation and C-45 alone reduced tumor weight significantly, the combination of radiation and C-45 was even more effective at inhibiting tumor growth in vivo. Similarly, while TAXOL® and C-45 individually inhibited the growth of the B16F10 tumors significantly, the combination was far more effective (FIG. 5B).

Example 4

**Screening for Compounds that Bind to and Activate
the PXR/SXR Receptor**

[0118] Rationale

[0119] It has been previously demonstrated that PABA and other benzoic acid derivatives bind to the PXR/SXR receptor (Moore et al., *Mol. Endocrinol.* 2002; 16: 977-86). The present inventors have shown that when treated with PABA, G361 human melanoma cells exhibit decreased protein expression of DNA repair protein BRCA-2 and increase expression of cell-cycle progression checkpoint protein CDC25A (data not shown). G361 melanoma cells also express the PXR/SXR receptor whereas non-transformed melanocytes do not (data not shown). Accordingly, it is expected that PABA-like benzoic acid derivatives may have similar agonist activity at this receptor may be useful in the treatment of PXR/SXR over-expressing tumors.

[0120] Methods

[0121] To assess PXR/SXR binding and activation by test compounds and other benzoic acid derivatives, standard cell-based reporter assays can be employed. For example, target cells can be transfected with a reporter construct carrying the xenobiotic response elements (e.g., DR3 motif 5' AGTTCA 3') upstream of e.g., a luciferase reporter gene. Following treatment of cells with e.g., PABA or any other test compound, activation of the PXR/SXR receptors will be quantified by measuring the increase in luciferase activity as a result of activation of the receptors and their binding to the xenobiotic response elements (Kliwer et al., *Cell* 1998; 92: 73-82; and Takeshita et al., *J. Biol. Chem.* 2002; 277: 32453-8).

Example 5

**Determining Expression of PXR/SXR and Cell
Cycle Regulatory Proteins in Tumor Cells in
Response to Drug Treatment**

[0122] Methods

[0123] To determine whether tumor cells abnormally express and signal through the PXR/SXR receptor compared to their non-transformed counterparts, and thus, candidates for treatment with PXR/SXR agonists, immortalized, non-transformed cells and sub-confluent transformed, tumorigenic cells will be cultured in the presence or absence of various concentrations of a known PXR/SXR activator, e.g., benzoic acid derivatives, and a negative control, e.g., streptomycin, for a pre-determined time e.g., 5 days. Cell lysates will be achieved as described above. Following lyses, about 15 µg/lane of protein will be loaded and separated by 10% SDS-PAGE, followed by transfer to nitrocellulose membranes. Membranes will then incubated in the presence of e.g., mono-

clonal antibody 39048, specific for the PXR/SXR nuclear receptor, followed by washing and labeling with a peroxidase-labeled secondary antibody.

[0124] In addition, RT-PCR and/or Northern hybridization can be used to determine nucleic acid expression, e.g., mRNA expression of the PXR/SXR receptor. For example, for RT-PCR primers were designed from the human PXR/SXR cDNA sequence (GenBank Accession No. AY091855) are as follows:

5' Forward	AGCAATTCGCCATTACT (SEQ ID NO: 2)
3' Reverse	GCTACCTGTGATACCGAAC (SEQ ID NO: 3)

[0125] Using a standard amplification protocol consisting of e.g., 40 cycles of 94° denaturation for 30 sec, 55° annealing for 30 sec and 72° amplification for 1 min will yield a product of about 203 bp. Detection can be achieved by, e.g., electrophoresis on a 2% agarose gel stained with ethidium bromide.

[0126] Similarly, to evaluate expression of cell cycle proteins that may be regulated by PXR/SXR activation in response to drug treatment, membranes as prepared above will be incubated with commercially available monoclonal antibodies such as PC410 (Chemicon, Temecula, Calif.), directed against β-integrin, as a positive control, monoclonal antibody 3303, specific for BRCA-2 (QED Biosciences, San Diego, Calif.), and with monoclonal antibody AB-3, directed against CDC25A (NeoMakers Inc). Additional antibodies may include anti-Id-1 monoclonal antibody B30-1, anti-Id-2 monoclonal antibody B31-1 (BD PharMingen, San Jose, Calif.) and Id-3 monoclonal antibody 56209 (QED Biosciences, San Diego, Calif.). Peroxidase-labeled goat anti-mouse secondary antibodies will then be incubated after washing. Additional controls consisted of membranes probed with only either the primary or secondary antibodies.

[0127] Detection will be performed using laser scanning densitometry using a Nuclear Vision 760 system to compare relative expression levels of the proteins.

[0128] Results

[0129] As stated above, it has been previously demonstrated that G361 melanomas, but not immortalized but untransformed melanocytes, express high levels of PXR/SXR (data not shown), and increased levels of CDC25A and decreased levels of DNA repair protein BRCA-2 when treated with PXR/SXR agonist PABA. This suggests that therapeutic agents like PABA or related derivatives which are agonists for this receptor will be useful in the treatment of PXR/SXR over-expressing tumors.

[0130] This finding led to the hypothesis that that PABA derivatives may also enhance the effects of chemotherapy and ionizing radiation in a manner similar to previously shown by PABA, by preventing tumor cells senescence upon treatment. A compound that simultaneously inhibits cell cycle repair proteins and increasing the concentration of proteins that induce cell cycle progression will be a preferred drug candidate. Accordingly, it is expected that similar effects on DNA repair and cell cycle regulatory protein expression will be observed with other PXR/SXR agonists depicted in FIG. 1 or identified by the screening methods of the present invention.

Example 6

Effects of Candidate Compounds on the Cell Cycle and Anti-Proliferative Effects of Ionizing Radiation or Chemotherapy In Vitro

[0131] Methods

[0132] To test the hypothesis stated above for benzoic acid derivatives that bind to the PXR/SXR receptor, cell cycle analysis can be performed as follows using Fluorescent Activated Cell Sorting (FACS) in cells treated with drug candidates and CT or RT. Immortalized cells and PXR/SXR-expressing transformed tumorigenic cells will be cultured in the presence or absence of various concentrations of a test compound, and positive and negative control compounds (e.g., PABA and streptomycin) for a designated time period. Next, cells will be synchronized by serum starvation for at least 24 hours, then cultured in serum-containing medium and treated with a test compound and control compounds, with or without various single fraction doses of radiation (e.g., 1, 2, 5, 10 and 20 Gy) or chemotherapy. At various time points (e.g., 1, 3, 6, 12 and 24 h) following serum stimulation, cells will be removed and washed with PBS and fixed for 1 hr with 70% ethanol at 4° C. Cells will then be washed several times with PBS and resuspended in RNase staining buffer, commercially available from BD PharMingen, for 30 minutes on ice. Cell cycle analysis will be determined using a FACS Scan flow cytometer (Becton-Dickenson, San Jose, Calif.), and data collected using e.g., a Hewlett Packard HP9000 equipped with FAC-Scan software and analyzed using PC-Lysis software (also from Becton-Dickenson). At least 10,000 events will be collected with identical gating parameters on single cell populations and percentages of cells within G0/G1, S, G2 and M determined along with the apoptotic population.

[0133] In addition to cell cycle analysis, cell proliferation can be measured using methods such as e.g., tetrazolium salt cleavage or incorporation of tritiated thymidine.

[0134] For example, cells can be treated with test and control compounds, plated and incubated for 2 hours to permit cell adhesion. Plates will then treated or not with various concentrations of ionizing radiation, e.g., 1, 2 10 and 20 Gy, or chemotherapy e.g., 0-0.2 μ M TAXOL®. Proliferation of the cells will then measured using e.g., the WST-1 kit (tetrazolium salt cleavage). Data points representing the mean optical density at 490 nm from triplicate wells \pm SD will be obtained.

[0135] Results

[0136] It is expected that treatment of non-transformed cells, or cells not expressing the PXR/SXR receptor, will not have altered proliferation when contacted with test compounds, radiation or chemotherapy, or a combination of the test compound with either agent. To the contrary, tumor cells which express PXR/SXR will respond to agonist compounds, as demonstrated by increased cell death, which will be more pronounced in combination with radiation or chemotherapy. Such compounds will be designated candidate or lead compounds.

[0137] Conclusion

[0138] The results presented herein are the first demonstration that benzoic acid derivatives can potentiate radio- and chemosensitivity of tumor cells which overexpress the PXR/SXR nuclear receptor. Given the potential of PABA to induce radiosensitivity via a PXR/SXR dependent mechanism, taken together with the relatively limited expression of PXR/SXR

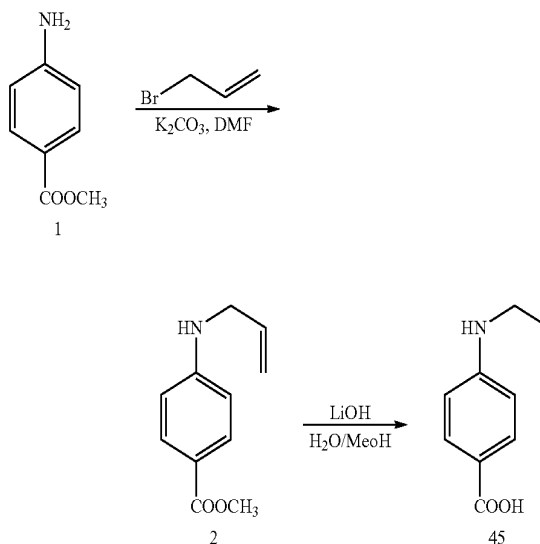
receptor in normal cell types, it is likely that specific agonists will provide a novel, effective approach to enhance the anti-tumor activity of RT and CT in malignant cells but not normal cells.

[0139] In addition, the present invention discloses the surprising discovery that benzoic acid derivatives that inhibit tyrosinase, and hence, melanogenesis, can potentiate RT and CT activities in melanotic as well as non-melanin expressing carcinoma cells.

Example 7

Synthesis of sodium 4-(allylamino)benzoic acid (C45 sodium salt)

[0140]



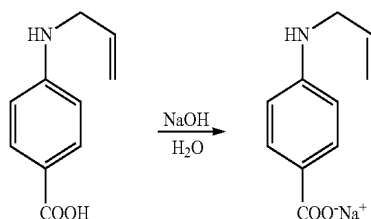
[0141] Methyl 4-aminobenzoate(1), allyl bromide, and LiOH.H₂O were purchased from AcrosOrganics.

[0142] Methyl 4-allylaminobenzoate (2) A 250 mL round-bottom flask, equipped with a magnetic stir bar, was charged with methyl 4-aminobenzoate (1) (6.04 g, 40 mmol), K_2CO_3 (11.06 g, 80 mmol) and 85 mL anhydrous DMF. The mixture was cooled to 0° C. and allyl bromide (3.13 mL, 36 mmol) was added dropwise with stirring. After the reaction mixture was heated at 60° C. for 12 hours, DMF was removed under high vacuum. The residual was extracted with CH_2Cl_2 /H₂O=150 mL/200 mL, the organic phase was removed and the aqueous phase was then extracted again with CH_2Cl_2 (150 mL). The combine organic phases were dried over $MgSO_4$. After removal of solvent, the crude product was purified by silica gel chromatography (eluent: Hexane/Ethyl acetate from 100/0 to 100/7) to give compound 2 as a white solid (5.38 g, 78.2% yield). 1H NMR (400 MHz, $CDCl_3$): δ 3.72 (d, 2H), 3.76 (s, 3H), 5.16 (dd, 1H), 5.21 (dd 1H), 5.80-5.85 (m, 1H), 6.50 (d, 2H), 7.80 (d, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 45.89, 51.53, 111.72, 116.80, 118.66, 131.51, 134.29, 144.91, 151.67. MP: 71-73° C.

[0143] 4-allylaminobenzoic acid (45)

[0144] A 500 mL round-bottom flask, equipped with a magnetic stir bar and condenser was charged with compound 2 (2 g, 10.4 mmol), LiOH.H₂O (1.18 g, 15.7 mmol), 100 mL

MeOH and 60 mL H₂O. The mixture was heated at 55° C. for 24 hours. The solution was concentrated in vacuo to remove methanol and most of H₂O. Ether/H₂O=100 mL/100 mL was used to remove most of the unreacted starting material. The aqueous layer was acidified to pH 4.0 with 1M HCl and some white precipitation was formed. The product was extracted with ethyl acetate (3×70 mL). The combined organic layer was washed with brine and dried over NaSO₄. After removal of the solvent, the crude product was purified by silica gel chromatography (eluent: Hexane/Ethyl acetate from 100:5 to 100:25). Recrystallization from ethyl acetate/hexane twice yielded white crystal (0.85 g, 46.1% yield). ¹H NMR (400 MHz, CDCl₃): δ 3.82 (d, 2H), 5.10 (dd, 1H), 5.23 (dd, 1H), 5.80-5.96 (m, 1H), 6.62 (d, 2H), 7.78 (d, 2H); ¹³C NMR (400 MHz, CDCl₃): δ 46.07, 112.19, 116.06, 118.44, 132.30, 136.14, 153.63, 168.01; ESI-MS: Calcd for C₁₀H₁₂NO₂ [M+1] 178.1. found 178.0. Anal. Calcd for C₁₀H₁₁NO₂: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.81; H, 6.28; N, 7.86. MP: 144-146° C.



[0145] Sodium 4-allylaminobenzoate (45 sodium salt)

[0146] A 100 mL round-bottom flask, equipped with a magnetic stir bar, was charged with compound 45 (296 mg, 1.67 mmol) and H₂O (10 mL). NaOH water solution (2.35 M, 730 μL) was added before the mixture was heated at 80° C. After 1 h, the solid was dissolved and the solution was cooled to room temperature. The solvent was removed by lyophilization and the residual was further dried under vacuum to afford the Na-salt 45 in white powder. ¹H NMR (400 MHz, D₂O): δ 7.69 (d, J=8.7 Hz, 2H), 6.71 (d, J=8.7 Hz, 2H), 5.83-5.94 (m, 1H), 5.19 (d, J=17.5 Hz, 1H), 5.11 (d, J=10.5 Hz, 1H), 3.72 (d, J=5.4 Hz, 2H); ¹³C NMR (400 MHz, D₂O): δ 175.7, 151.0, 135.1, 130.9, 125.2, 116.1, 113.1, 45.6; ESI-MS: Calcd for C₁₀H₁₀NNaO₂ [M+1] 200.1. found 177.9, 199.9.

Experiment 1

Effects of Candidate Compounds on M21 Human Melanoma Tumor Cell Proliferation

[0147] Methods

[0148] Human M21 melanoma cells were washed and re-suspended in proliferation buffer containing 1.0% serum in the presence or absence of 100 ng/mL of C-45-Na, or C-45-HCl. DMSO control samples lacking C-45-Na or C-45-HCl were also prepared. Treated cells (1,500/well) were added to individual wells of a cell culture microtiter plate and allowed to grow over a time course of 3 days. Proliferation was monitored using a commercially available MTT assay (Millipore). Data points represent the mean±standard deviations from triplicate wells.

[0149] Results

[0150] As shown in FIG. 6, C-45-Na (100 ng/mL) has a very potent anti-proliferative activity against M21 human tumor cells. Notably, no significant inhibitory activity was observed in M21 melanoma cells treated with C-45-HCl or DMSO.

Experiment 2

Effects of Candidate Compounds on M21 Human Melanoma Tumor Cell Proliferation

[0151] Methods

[0152] Human M21 melanoma cells were washed and re-suspended in proliferation buffer containing 1.0% serum in the presence or absence of 100 ng/mL of C-45-Na, or C-45-HCl. Treated cells (1,500/well) were added to individual wells of a cell culture microtiter plate and allowed to grow over a time course of 3 days. Proliferation was monitored using a commercially available MTT assay (Millipore). Data points represent the mean±standard deviations from triplicate wells.

[0153] Results

[0154] As shown in FIG. 7, C-45-Na (100 ng/mL) has a very potent anti-proliferative activity against M21 human tumor cells. Notably, no significant inhibitory activity was observed in M21 melanoma cells treated with C-45-HCl or DMSO.

Experiment 3

Effects of Candidate Compounds on M21 Human Melanoma Tumor Cell Proliferation

[0155] Methods

[0156] Human M21 melanoma cells were washed and re-suspended in proliferation buffer containing 0.5% serum in the presence of 50 μg/mL of C-45-Na, C-45-K, or C-45-HCl. DMSO control samples lacking C-45-Na, C-45-K, and C-45-HCl were also prepared. Treated cells (1,500/well) were added to individual wells of a cell culture microtiter plate and allowed to grow over a time course of 3 days. Proliferation was monitored using a commercially available MTT assay (Millipore). Data points represent the mean±standard deviations from triplicate wells.

[0157] Results

[0158] As shown in FIG. 15, C-45-Na (50 μg/mL) has a very potent anti-proliferative activity against M21 human tumor cells. Surprisingly, no significant inhibitory activity was observed in M21 melanoma cells treated with C-45-HCl, C-45-K, or DMSO. These results establish that C-45-Na exhibits significantly higher activity than the HCl or K salt versions of C-45.

Experiment 4

Effects of Candidate Compounds on C8161 and A375 Cell Proliferation

[0159] Methods

[0160] C8161 and A375 human melanoma cells were washed and re-suspended in proliferation buffer containing 0.5% serum in the presence of 100 μg/mL of C-45-Na or C-45-HCl. DMSO control samples lacking C-45-Na and

C-45-HCl were also prepared. Treated cells (1,500/well) were added to individual wells of a cell culture microtiter plate and allowed to grow over a time course of 3 days. Proliferation was monitored using a commercially available MTT assay (Millipore). Data points represent the mean \pm standard deviations from triplicate wells.

[0161] Results

[0162] As shown in FIG. 13A, C-45-Na (100 μ g/mL) has a very potent anti-proliferative activity against C8161 human tumor cells. Further, FIG. 13B illustrates that C-45-Na (100 μ g/mL) also has a very potent anti-proliferative activity against A375 human tumor cells. Surprisingly, no significant inhibitory activity was observed in C8161 and A375 human melanoma cells treated with C-45-HCl or DMSO. These results establish that C-45-Na exhibits significantly higher activity than the HCl salt versions of C-45.

Experiment 5

Effects of Candidate Compounds on MDA-MB 231 Breast Cell Carcinoma Proliferation

[0163] Methods

[0164] MDA-MB 231 human breast carcinoma cells were washed and re-suspended in proliferation buffer containing 0.5% serum in the presence of 100 μ g/mL of C-45-Na or C-45-HCl. DMSO control samples lacking C-45-Na and C-45-HCl were also prepared. Treated cells (1,500/well) were added to individual wells of a cell culture microtiter plate and allowed to grow over a time course of 3 days. Proliferation was monitored using a commercially available MTT assay (Millipore). Data points represent the mean \pm standard deviations from triplicate wells.

[0165] Results

[0166] As shown in FIG. 14A, C-45-Na (100 μ g/mL) has a very potent anti-proliferative activity MDA-MB 231 human breast carcinoma cells. Surprisingly, no significant inhibitory activity was observed in MDA-MB 231 human breast carcinoma cells treated with C-45-HCl or DMSO. These results establish that C-45-Na exhibits significantly higher activity than the HCl salt version of C-45.

Experiment 6

Effects of Candidate Compounds on Panc-1 human Pancreatic Carcinoma Proliferation

[0167] Methods

[0168] Panc-1 human pancreatic carcinoma cells were allowed to proliferate over a 3-day time course in the presence or absence of a series of concentrations of C-45-Na (4.7, 9.3, 17.3, 37.5, and 75 μ g/mL) of or C-45-HCl. DMSO control samples lacking C-45-Na and were also prepared. Treated cells (1,500/well) were added to individual wells of a cell culture microtiter plate and allowed to grow over a time course of 3 days. Proliferation was monitored using a commercially available MTT assay (Millipore). Data points represent the mean \pm standard deviations from triplicate wells.

[0169] Results

[0170] As shown in FIG. 14B, C-45-Na has a very potent anti-proliferative activity panc-1 human pancreatic carcinoma cells

Experiment 7

Effects of Candidate Compounds on M21 Human Melanoma Tumor Cell Morphology

[0171] Methods

[0172] Human M21 melanoma cells or C8161 melanoma cells were incubated in the presence of 100 μ g/mL of C-45-Na or C-45-HCl for 24 hours. DMSO control samples lacking C-45-Na and C-45-HCl were also prepared.

[0173] Results

[0174] As shown in FIGS. 11A and 11B, incubation of these human melanoma cells with C-45-Na (100 μ g/mL) resulted in significant cell rounding as compared to the non-treated or C45-HCl samples. These findings suggest that C-45-Na may alter cytoskeletal organization at the concentration indicated.

Experiment 8

Effects of Candidate Compounds on Malignant Human M21 Melanoma Cell Proliferation

[0175] Methods

[0176] In Example 6, the differential effects of C-45-Na on normal human melanocytes, normal human keratinocytes and human melanoma cells were examined. Normal human melanocytes, keratinocytes and M21 melanoma cells were allowed to proliferate in the presence or absence of C-45-Na (50 μ g/mL) for 24 hours. Proliferation was monitored using a commercially available MTT assay (Millipore).

[0177] Results

[0178] As shown in FIG. 12A, C-45-Na (50 μ g/mL) has a very potent anti-proliferative activity against M21 human tumor cells. In contrast, C-45-Na exhibited very little effect on normal human melanocytes (FIG. 12B) or keratinocytes (FIG. 12C). These studies establish that C-45-Na is selective for tumor cell proliferation rather than normal human cells.

Experiment 9

Effects of Candidate Compounds on Tumor Growth in Mice

[0179] Methods

[0180] Given the highly potent anti-proliferative activity of C-45-Na on human melanoma cells in vitro, the effects of this compound on M21 melanoma tumor growth in vivo were studied. Briefly, M21 melanoma cells (4.0×10^6) were injected subcutaneously in nude mice. Following establishment of palpable tumors (4 days), mice were either left untreated or treated daily by i.p injection with DMSO (50 μ l/day), or with C-45-Na (50 μ l/day) or C-45-HCl (50 μ l/day) at a concentration range of 1-100 μ g/day. Tumor size was monitored by caliper measurements and tumor volumes were estimated using the formula ($L^2 \times W/2$) where "L" represents length, and "W" represents width.

[0181] Results

[0182] As shown in FIGS. 8-10, C-45-Na potently inhibited M21 melanoma tumor growth by approximately 75% to 80% in 21 days. Significant inhibition was observed at low doses (1.0 μ g/day) of C-45-Na. At doses of 10 and 100 μ g/day, no significant inhibition ($P > 0.05$) was observed with C-45-

HCl as compared with the control. These findings suggest that C-45-Na alone may represent a highly potent new anti-tumor compound in vivo.

Experiment 10

Effects of Varying C45-Na Concentration on Tumor Growth in Mice

[0183] Methods

[0184] The effects of C45-Na on M21 melanoma tumor growth in vivo were studied. Briefly, M21 melanoma cells (4.0×10^6) were injected subcutaneously in nude mice. Following establishment of palpable tumors (4 days), mice were either left untreated or treated daily by i.p injection with DMSO (50 μ l/day), or with C-45-Na concentrations of 0.1, 0.5, or 1.0 μ g/day. Tumor size was monitored by caliper measurements and tumor volumes were estimated using the formula ($L^2 \times W/2$) where "L" represents length, and "W" represents width.

[0185] Results

[0186] As shown in FIG. 16, significant inhibition of tumor growth ($P < 0.05$) was observed at days 8, 14, and 21, at all dosing levels tested.

Experiment 11

Effects of Candidate Compounds on Tumor Growth in Mice

[0187] Methods

[0188] In experiment 11, an activity comparison between C-45-Na (1.0 μ g) and C45-HCl (1.0 μ g) on the in vivo growth of M21 tumors was examined following daily treatment after 21 days. Tumor size was monitored by caliper measurements and tumor volumes were estimated using the formula ($L^2 \times W/2$) where "L" represents length, and "W" represents width.

[0189] Results

[0190] As shown in FIG. 17, significant inhibition of tumor growth ($P < 0.05$) was observed at day 21 at the dosing levels of C45-Na salt but not for the HCl salt.

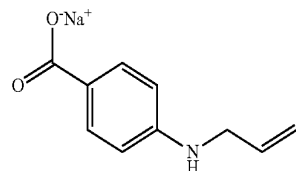
REFERENCES CITED

[0191] Numerous references, including patents, patent applications and various publications, are cited and discussed in the description of this invention. The citation and/or discussion of such references is provided merely to clarify the description of the present invention and is not an admission that any such reference is "prior art" to the invention described herein. All references cited and discussed in this specification are incorporated herein by reference in their entirety and to the same extent as if each reference was individually incorporated by reference.

What is claimed:

1. A method of treating cancer in a mammal, comprising administering to a mammal in need of such treatment a ther-

apeutically effective amount of a sodium salt of the benzoic acid (PABA) benzoic acid derivative C-45 having the following structure:



2. The method of claim 1, wherein the treatment comprises administering chemotherapy to the mammal.

3. The method of claim 1, wherein the treatment comprises administering radiation therapy to the mammal.

4. The method of claim 1, wherein the cancer is selected from the group consisting of melanoma, breast carcinoma and lung carcinoma.

5. The method of claim 1, wherein the mammal is a human.

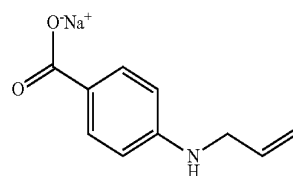
6. The method of claim 5, wherein the cancer is melanoma.

7. The method of claim 5, wherein the cancer is breast carcinoma.

8. The method of claim 5, wherein the cancer is lung carcinoma.

9. The method of claim 1, wherein the sodium salt of C-45 is administered in combination with an effective amount of paclitaxel.

10. A pharmaceutical composition comprising a therapeutically effective amount of a sodium salt of the benzoic acid (PABA) benzoic acid derivative C-45 having the following structure:



and a pharmaceutically acceptable carrier.

11. The pharmaceutical composition of claim 13, wherein the composition is suitable for oral administration to a mammal.

12. The pharmaceutical composition of claim 13, wherein the composition is suitable for parenteral administration to a mammal.

13. The pharmaceutical composition of claim 15, wherein the composition is suitable for intravenous administration to a mammal.

* * * * *