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(54) Title: METHOD OF TREATING CANCER BY CONJUNCTIVE THERAPY WITH 2'-HALOMETHYLIDENE DE- RIVATIVES AND AN S-PHASE OR M-PHASE SPECIFIC ANTINEOPLASTIC AGENT (57) Abstract The present invention relates to a method of treating a patient suffering from a neoplastic disease state comprising admin- istering to said patient an effective antineoplastic amount of a 2'-halomethylidene derivative in conjunctive therapy with an effec- tive antineoplastic amount of an S-phase or M-phase specific agent.		

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METHOD OF TREATING CANCER BY CONJUNCTIVE THERAPY WITH 2'-
HALOMETHYLIDENE DERIVATIVES AND A S-PHASE OR M-PHASE
SPECIFIC ANTINEOPLASTIC AGENT

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BACKGROUND OF THE INVENTION

Neoplastic disease states in humans are recognized throughout the world as being serious and oftentimes life-threatening conditions. These neoplastic diseases, which
10 are characterized by rapidly-proliferating cell growth, have been and continue to be the subject of worldwide research efforts directed toward the identification of therapeutic agents which are effective in the treatment of patients suffering therefrom. Effective therapeutic agents can be
15 characterized as those which prolong the survivability of the patient, which inhibit the rapidly-proliferating cell growth associated with the neoplasm, or which effect a regression of the neoplasm. Research in this area is primarily focused toward identifying agents which would be
20 therapeutically effective in humans. Typically, compounds are tested for antineoplastic activity in small mammals, such as mice, in experiments designed to be predictive of antineoplastic activity not only in those animals but also in humans against specific neoplastic disease states.

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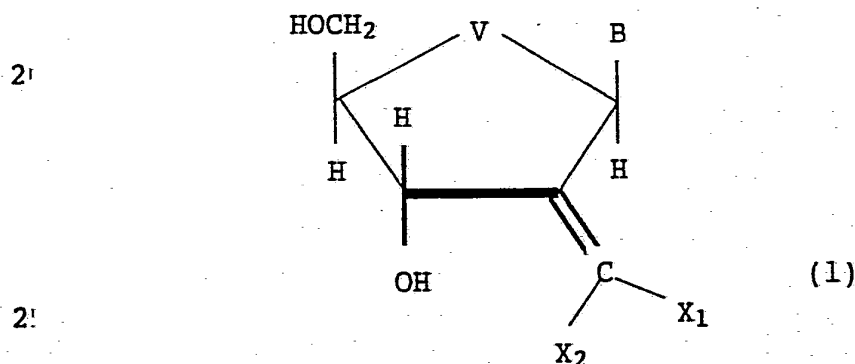
Certain S-phase specific antimetabolites and M-phase specific vinca alkaloids are well known as effective

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antineoplastic agents [See Corr, R. T., and Fritz, W. L., "CANCER CHEMOTHERAPY HANDBOOK", 1980, Elsevier North Holland, Inc., New York, New York and Calabresi, P., and Chabner, B. A., "CHEMOTHERAPY OF NEOPLASTIC DISEASES",
 5 Section XII, GOODMAN AND GILLMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, 8th ed., 1990, Pergamon Press Inc., Elmsford, New York]. Cytarabine (ARA-C), fluorouracil (5-FU), mercaptopurine (6-MP), methotrexate (MTX), thioguanine (6-TG), hydroxyurea, prednisone, procarbazine and
 10 diglycoaldehyde are examples of antimetabolites with antineoplastic properties. Vincristine and vinblastine are examples of vinca alkaloids with antineoplastic properties. These agents are proven to be useful in the treatment of patients suffering from a variety of neoplastic disease
 15 states.

Certain 2'-halomethylidene derivatives of the formula

(1)



wherein

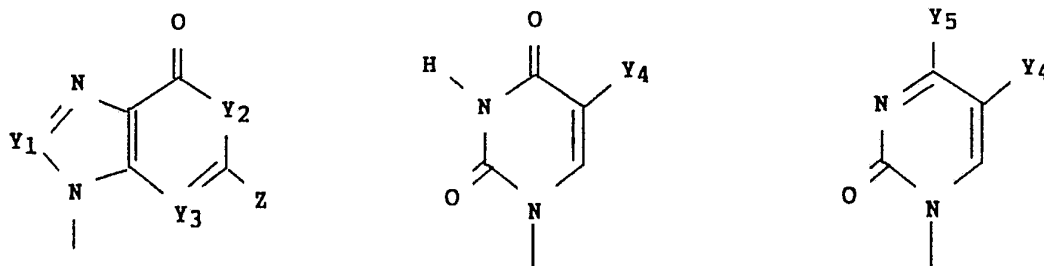
V is oxy, methylene, or thio,

X₁ and X₂ are each independently hydrogen or halogen,
 30 with the proviso that at least one of X₁ and X₂ is halogen,

B is a radical of the formula

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wherein Y_1 is nitrogen, a CH group, a CCl group, a CBr group or a CNH_2 group; Y_2 and Y_3 are each independently
 10 nitrogen or a CH group; Y_4 is hydrogen, C_1 - C_4 alkyl, C_1 - C_4 alkoxy or halogen; Y_5 is amino or C_1 - C_4 alkoxy; and Z is hydrogen, halogen, or NH_2 ;

or a pharmaceutically acceptable salt thereof, such as (E)-
 2'-deoxy-2'-fluoromethylidenecytidine, are also well known
 15 as effective antineoplastic agents [European Patent Application Publication No. 0 372 268, published June 13, 1990]. These 2'-halomethylidene derivatives of formula (1) are ribonucleotide reductase inhibitors with potent
 20 antiproliferative and antitumor activity which are also useful in the treatment of patients suffering from a variety of neoplastic disease states.

It has now been found that in treating a patient
 25 afflicted with certain neoplastic disease states, conjunctive therapy with a 2'-halomethylidene derivatives of formula (1), such as (E)-2'-deoxy-2'-fluoromethylidenecytidine, and a S-phase specific antineoplastic antimetabolite or M-phase specific vinca
 30 alkaloid will provide a synergistic antineoplastic effect.

SUMMARY OF THE INVENTION

The present invention provides a method of treating a patient suffering from a neoplastic disease state comprising administering to said patient an effective antineoplastic amount of a 2'-halomethylidene derivative of formula (1) in conjunctive therapy with an effective antineoplastic amount of a S-phase or M-phase specific antineoplastic antimetabolite or vinca alkaloid.

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More specifically, the present invention provides a method of treating a patient suffering from a neoplastic disease state comprising administering an effective antineoplastic amount of 2'-halomethylidene derivative of formula (1) in conjunctive therapy with an effective antineoplastic amount of cytarabine (ARA-C), fluorouracil (5-FU), mercaptopurine (6-MP), methotrexate (MTX), thioguanine (6-TG), hydroxyurea, prednisone, procarbazine, diglycoaldehyde, vincristine or vinblastine.

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DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "halogen" or "halo-" refers to a fluorine, chlorine, bromine, or iodine atom and the term "nitrogen" refers to a trivalent nitrogen atom attached to two radicals. The term "C₁-C₄ alkyl" refers to a saturated straight or branched chain hydrocarbyl radical of one to four carbon atoms and includes methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl, tertiary butyl and the like. The term "C₁-C₄ alkoxy" refers to a C₁-C₄ alkyl bearing an oxy group and includes methoxy, ethoxy, propoxy, butoxy and the like.

As used herein, the term "S phase specific agent" refers to those agents which exert their cytotoxic activity in the

S phase or DNA synthetic phase of the cell cycle. Included within the meaning of the term are agents which exert their cytotoxic effect during the S phase of the cell cycle and subsequently result in cell death. Examples of cytotoxic agents in this class are the antimetabolites cytarabine (ARA-C), fluorouracil (5-FU), mercaptopurine (6-MP), methotrexate (MTX), thioguanine (6-TG), hydroxyurea, prednisone, procarbazine and diglycoaldehyde. Also included within the meaning of the term "S phase specific agent" are agents which exert their cytotoxic effect during the S phase but result in cell death while the cell is in the M phase or mitosis phase of the cell cycle. Examples of cytotoxic agents in this class are the nitrosoureas and bleomycin.

As used herein, the term "M phase specific agent" refers to those agents which exert their cytotoxic activity in the M-phase or mytic phase of the cell cycle. Included within the meaning of the term are agents which exert their cytotoxic effect during the M phase of the cell cycle and subsequently result in cell death. Examples of cytotoxic agents in this class are the etoposid, vincristine and vinblastine.

2'-Halomethylidene derivatives of formula (1) can be prepared as described in European Patent Application Publication No. 0 372 268, published June 13, 1990. In order to illustrate the preparation of the 2'-halomethylidene derivatives of formula (1), the following example illustrating the preparation of (E)-2'-deoxy-2'-fluoromethylidenecytidine is provided. The example is illustrative only and is not intended to limit the invention in any way. All temperatures are in degrees Celsius and the following abbreviations are used: (g) is grams, (mol) is moles, (ml) is milliliters, (l) is liters, (lb/in²) is pounds per square inch, (TLC) is thin layer chromatography,

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(THF) is tetrahydrofuran, (DMF) is dimethylformamide, (mp) is melting point, (mm/Hg) is pressure expressed as millimeters of mercury, and (bp) is boiling point.

5 EXAMPLE 1

(Z)- and (E)-2'-DEOXY-2'-FLUOROMETHYLIDENECYTIDINE

Step a: 4-Ethoxy-1-[(3,5-O-tetraisopropylidisiloxan-1,3-diyl)- β -D-erythro-pentofuran-2-(2-fluoro-2-phenylsulfonyl methylidene)osyl]-2(1H)-pyrimidone

- 10 Prepare diethylfluoromethylphenylsulfonylphosphonate as follows: To a solution of fluoromethylphenyl sulfone (500 mg, 2.87 mmol) in dry THF (30 ml) which has been cooled to about -60°C in a dry 3-necked 100ml flask with stirring bar, argon inlet valve, thermometer and rubber septum, add
- 15 diethyl chlorophosphate (500 mg, 0.42 ml, 2.87 mmol) via syringe. To this mixture, add a solution of 1.65 M lithium diisopropylamide in cyclohexane (3.48 ml, 5.74 mmol) via syringe and follow the formation of diethylfluoromethylphenylsulfonylphosphonate by gas-liquid chromatography
- 20 (GLC).

To the diethylfluoromethylphenylsulfonylphosphonate solution above add a solution of 4-ethoxy-1-[(3,5-O-tetraisopropylidisiloxan-1,3-diyl)-2-keto- β -D-erythro-

25 pentofuranosyl]-2(1H)-pyrimidone (732 mg, 2 mmol) in dry THF (about 5 ml) and allow the reaction mixture to warm to room temperature overnight under an argon atmosphere. Pour the mixture into a saturated, ice-cold solution of ammonium chloride and extract the mixture with ethyl acetate (3

30 times, 75 ml each time). Combine the organic layers, dry with anhydrous magnesium sulfate, and evaporate to dryness. Chromatograph the residue on a silica gel flash column eluting with ethyl acetate/hexane (1/1, v/v) to provide the title compound.

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Step b: 4-Ethoxy-1-[β -D-erythro-pentofuran-2-(2-fluoro-2-phenylsulfonylmethylidene)osyl]-2(1H)-pyrimidone

To a solution of 1.0 M tetrabutylammonium fluoride in THF (2.2 ml, 2.2 mmol) add 4-ethoxy-1-[(3,5-O-
5 tetraisopropylidisiloxan-1,3-diyl)- β -D-erythro-pentofuran-2-(2-fluoro-2-phenylsulfonylmethylidene)osyl]-2(1H)-pyrimidone (668 mg, 1 mmol) and stir the mixture at room temperature for 2 hours. Neutralize the mixture with acetic acid, add flash silica gel to the mixture and evaporate to dryness *in*
10 *vacuo*. Apply the residue to a flash silica gel column and elute with chloroform/ethanol (20/1, v/v) to provide the title compound.

Step c: (Z)- and (E)-2'-Deoxy-2'-fluoromethylidenecytidine

15 To a solution of 4-ethoxy-1-[β -D-erythro-pentofuran-2-(2-fluoro-2-phenylsulfonylmethylidene)osyl]-2(1H)-pyrimidone (854 mg, 2 mmol) in 10% aqueous THF (100 ml) under a nitrogen atmosphere, add aluminum amalgam (made from 0.04 gm aluminum in 2% aqueous HgCl₂). Stir and vent the mixture
20 while refluxing for 2 hours. Filter the mixture and evaporate most of the THF *in vacuo*. Extract the residue with ethyl acetate (3 times, 25 ml each time), combine the organic layers and dry with anhydrous Na₂SO₄. Evaporate to dryness *in vacuo* and apply the residue to a flash silica gel
25 column and elute with chloroform/ethanol (9/1, v/v) to provide (Z)- and (E)-4-ethoxy-1-[β -D-erythro-pentofuran-2-(2-fluoromethylidene)osyl]-2(1H)-pyrimidone as a mixture of geometric isomers.

30 Heat a solution of (Z)- and (E)-4-ethoxy-1-[β -D-erythro-pentofuran-2-(2-fluoromethylidene)osyl]-2(1H)-pyrimidone (858 mg, 3 mmol) in methanolic ammonia (10 ml, saturated at 0°C) in a sealed tube at 100°C for 2 days. Evaporate the solution to dryness and separate the (Z) and (E) isomers of
35 the title compound by chromatography by applying the residue

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to a column packed with Dowex 1-X2 (OH⁻ form) and eluting with methanol.

The antineoplastic antimetabolites, such as cytarabine
5 (ARA-C), fluorouracil (5-FU), mercaptopurine (6-MP),
methotrexate (MTX), thioguanine (6-TG), hydroxyurea,
prednisone, procarbazine and diglycoaldehyde and the
antineoplastic vinca alkaloids, such as vincristine and
vinblastine, are readily available and their use as
10 antineoplastic agents is well known and appreciated in the
art [For example, See Corr, R. T., and Fritz, W. L., "CANCER
CHEMOTHERAPY HANDBOOK", 1980, Elsevier North Holland, Inc.,
New York, New York and Calabresi, P., and Chabner, B. A.,
"CHEMOTHERAPY OF NEOPLASTIC DISEASES", Section XII, GOODMAN
15 AND GILLMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, 8th
ed., 1990, Pergamon Press Inc., Elmsford, New York].

The present invention provides a method of treating a
patient suffering from a neoplastic disease state comprising
20 conjunctive therapy with an effective antineoplastic amount
of a 2'-halomethylidene of formula (1) and an effective
antineoplastic amount of a S-phase specific antineoplastic
antimetabolite or M-phase specific vinca alkaloid. This
conjunctive therapy unexpectedly provides a synergistic
25 antineoplastic effect.

As used herein, the term "patient" refers to a warm-
blooded animal such as a mammal which is afflicted with a
neoplastic disease state. It is understood that dogs, cats,
30 rats, mice, horses, bovine cattle, sheep, and humans are
examples of animals within the scope of the meaning of the
term.

The term "neoplastic disease state" as used herein
35 refers to an abnormal state or condition characterized by

rapidly proliferating cell growth or neoplasm. Neoplastic disease states for which conjunctive therapy according to the present invention will be particularly useful include: Leukemias such as, but not limited to, acute lymphoblastic, 5 chronic lymphocytic, acute myeloblastic and chronic myelocytic; Carcinomas, such as, but not limited to, those of the cervix, oesophagus, stomach, small intestines, brain, colon and lungs; Sarcomas, such as, but not limited to, osteosarcoma, osteosarcoma, lipoma, liposarcoma, hemangioma and 10 hemangiosarcoma; Melanomas, including amelanotic and melanotic; and mixed types of neoplasias such as, but not limited to carcinosarcoma, lymphoid tissue type, follicular reticulum, cell sarcoma and Hodgkin's disease. Of course, one skilled in the art will recognize that not every 15 combination of conjunctive therapy according to the present invention will be equally effective against each of the neoplastic disease states. Selection of the most appropriate combination is within the ability of one of ordinary skill in the art and will depend on a variety of 20 factors including assessment of results obtained in standard animal cancer models and the effectiveness of the individual agents as monotherapy in treating particular neoplastic disease states.

25 For example, conjunctive therapy with a 2'-halomethylidene derivatives of formula (1) and cytarabine will be particularly effective in the treatment of a patient afflicted with acute granulocytic and acute lymphocytic leukemias. Conjunctive therapy with a 2'-halomethylidene 30 derivative of formula (1) and hydroxyurea will be particularly effective in the treatment of a patient afflicted with chronic granulocytic leukemia, polycythemia vera, essential thrombocytosis and malignant melanoma. Conjunctive therapy with a 2'-halomethylidene derivative of 35 formula (1) and prednisone will be particularly effective in

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the treatment of a patient afflicted with acute and chronic lymphocytic leukemias, non-Hodgkin's lymphomas, Hodgkin's disease and breast carcinoma. Conjunctive therapy with a 2'-halomethylidene derivative of formula (1) and

5 methotrexate will be particularly effective in the treatment of a patient afflicted with acute lymphocytic leukemia, choriocarcinoma, mycosis fungoides, breast, head and neck carcinoma, and lung osteogenic sarcoma. Conjunctive therapy with a 2'-halomethylidene derivative of formula (1) and

10 fluorouracil will be particularly effective in the treatment of a patient afflicted with breast, colon, stomach, pancreas, ovary, head and neck carcinoma, urinary bladder carcinoma and premalignant skin lesions. Conjunctive therapy with a 2'-halomethylidene derivative of formula (1)

15 and mercaptopurine will be particularly effective in the treatment of a patient afflicted with acute lymphocytic, acute granulocytic and chronic granulocytic leukemias. Conjunctive therapy with a 2'-halomethylidene derivative of formula (1) and thioguanine will be particularly effective

20 in the treatment of a patient afflicted with acute granulocytic, acute lymphocytic, and chronic granulocytic leukemias. Conjunctive therapy with a 2'-halomethylidene derivative of formula (1) and procarbazine will be particularly effective in the treatment of a patient

25 afflicted with Hodgkin's disease. Conjunctive therapy with a 2'-halomethylidene derivative of formula (1) and vincristine will be particularly effective in the treatment of a patient afflicted with acute lymphocytic leukemia, neuroblastoma, Wilms' tumor, rhabdomyosarcoma, Hodgkin's

30 disease and small-cell lung carcinoma. Conjunctive therapy with a 2'-halomethylidene derivative of formula (1) and vinblastine will be particularly effective in the treatment of a patient afflicted with Hodgkin's disease, non-Hodgkin's lymphomas, breast and testis carcinoma.

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In effecting treatment of a patient afflicted with a neoplastic disease state as described above, a 2'-halomethylidene derivative of formula (1) is administered in conjunctive therapy with a S-phase specific antineoplastic antimetabolite or M-phase specific vinca alkaloid. As used herein, the term "conjunctive therapy" contemplates co-administration of a 2'-halomethylidene derivative of formula (1) along with the S-phase specific antineoplastic antimetabolite or M-phase specific vinca alkaloid. This co-administration may take place at essentially the same time, it may take place sequentially, or it may take place alternately.

In providing co-administration at essentially the same time, the courses of treatment with a 2'-halomethylidene derivative of formula (1) and the selected S-phase specific antineoplastic antimetabolite or M-phase specific vinca alkaloid run essentially concomitantly. In providing sequential co-administration, a full course of treatment of one of the agents is terminated and then followed by a full course of treatment of the other. In providing alternate co-administration, a partial course of treatment of one of the agents is terminated and then followed by a partial course of treatment of the other in an alternating manner until a full treatment of each agent is administered. When the 2'-halomethylidene derivative of formula (1) and the selected S-phase specific antineoplastic antimetabolite or M-phase specific vinca alkaloid are co-administered in a sequential or an alternate manner, it is generally preferred to administer the 2'-halomethylidene derivative of formula (1) first and the S-phase specific antineoplastic antimetabolite or M-phase specific vinca alkaloid last.

In effecting the conjunctive therapy according to the present invention, it is preferred to co-administer the 2'-

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halomethylidene derivative of formula (1) and the selected S-phase specific antineoplastic antimetabolite or M-phase specific vinca alkaloid in a sequential or an alternate manner. It is most preferred to co-administer the 2'-
5 halomethylidene derivative of formula (1) and the selected S-phase specific antineoplastic antimetabolite or M-phase specific vinca alkaloid in a sequential manner.

As used herein, the term "effective antineoplastic
10 amount" refers to an amount which is effective, upon single or multiple dose administration to the patient, in controlling the growth of the neoplasm or in prolonging the survivability of the patient beyond that expected in the absence of such treatment. As used herein, "controlling the
15 growth" of the neoplasm refers to slowing, interrupting, arresting or stopping its growth and does not necessarily indicate a total elimination of the neoplasm.

An effective antineoplastic amount of a 2'-
20 halomethylidene derivative of formula (1) is expected to vary from about 10 milligram per kilogram of body weight per day (mg/kg/day) to about 100 mg/kg/day and preferably will be about 5 mg/kg/day to about 50 mg/kg/day.

25 The effective antineoplastic amounts of the various S-phase specific antineoplastic antimetabolites and M-phase specific vinca alkaloids are well known and appreciated in the art. For example, an effective antineoplastic amount of cytarabine (ARA-C) is expected to vary from about 1
30 mg/m²/day to about 200 mg/m²/day. An effective antineoplastic amount of fluorouracil (5-FU) is expected to vary from about 6 mg/m²/day to about 800 mg/m²/day. An effective antineoplastic amount of mercaptopurine (6-MP) is expected to vary from about 2.5 mg/m²/day to about 700
35 mg/m²/day. An effective antineoplastic amount of

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methotrexate (MTX) is expected to vary from about 2.5 mg/m²/day to about 30 mg/m²/day. An effective antineoplastic amount of thioguanine (6-TG) is expected to vary from about 2 mg/kg/day to about 3 mg/kg/day. An effective
5 antineoplastic amount of hydroxyurea is expected to vary from about 20 mg/kg/day to about 80 mg/kg/day. An effective antineoplastic amount of prednisone is expected to vary from about 15 mg/m²/day to about 100 mg/m²/day. An effective antineoplastic amount of procarbazine is expected to vary
10 from about 1 mg/kg/day to about 4 mg/kg/day. An effective antineoplastic amount of diglycoaldehyde is expected to vary from about 0.25 mg/kg/day to about 5 mg/kg/day. An effective antineoplastic amount of vincristine is expected to vary from about 0.4 mg/M²/week to about 2 mg/M²/week. An
15 effective antineoplastic amount of vinblastine is expected to vary from about 4.0 mg/m²/week to about 2 mg/m²/day.

In effecting treatment of a patient afflicted with a disease state described above, the 2'-halomethylidene
20 derivatives of formula (1) can be administered in any form or mode which makes the compound bioavailable in effective amounts, including oral and parenteral routes. For example, it can be administered orally, subcutaneously, intramuscularly, intravenously, transdermally, intranasally,
25 rectally, and the like. Oral administration is generally preferred. One skilled in the art of preparing formulations can readily select the proper form and mode of administration depending upon the particular circumstances, including the disease state to be treated, the stage of the
30 disease, the form of administration of the selected S-phase specific antineoplastic antimetabolite or vinca alkaloid, the manner of co-administration selected, and the like.

In effecting treatment of a patient afflicted with a
35 disease state described above, the 2'-halomethylidene

derivatives of formula (1) can be administered in combination with the various S-phase specific antineoplastic antimetabolites or M-phase specific vinca alkaloids in the proportions of antineoplastic amount of a 2'-halomethylidene derivative of formula (1) to antineoplastic amount of a S-phase or M-phase specific agent in the range of about 1:0.1 to about 1:50, more preferably in the range of about 1:1 to about 1:20 and most preferably in the range of about 1:1 to about 1:10.

10

The 2'-halomethylidene derivatives of formula (1) can be administered alone or in the form of a pharmaceutical composition in combination with pharmaceutically acceptable carriers or excipients, the proportion and nature of which are determined by the solubility and chemical properties of the 2'-halomethylidene derivatives of formula (1), the chosen route of administration, and standard pharmaceutical practice. The 2'-halomethylidene derivative of formula (1), while effective itself, may be formulated and administered in the form of its pharmaceutically acceptable acid addition salt for purposes of stability, convenience of crystallization, increased solubility and the like.

The selected S-phase specific antineoplastic antimetabolite or M-phase specific vinca alkaloid can be administered in a manner as is well known and accepted for the particular agent. For example, cytarabine, fluorouracil, methotrexate, thioguanine, hydroxyurea, procarbazine, mercaptopurine (as its sodium salt), vinblastine and vincristine may be administered intravenously. Mercaptopurine, methotrexate, thioguanine, hydroxyurea, prednisone, procarbazine and diglycoaldehyde may be administered orally. Methotrexate may also be administered via intramuscular, intraarterial and intrathecal routes.

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The following examples are provided in order to illustrate the method of use of the present invention. These examples are intended to be illustrative only and are not to be construed to limit the scope of the invention in any way.

Example 2Synergistic Antitumor Activity of A Combination of MDL10 101,731 and Ara-C Against B16 Melanoma in Mice

Inject B16 melanoma cells (1×10^5 cells/mouse) subcutaneously on day 0. Administer compounds i.p. once daily as indicated. Sacrifice the animals on day 15, dissect the 15 tumors and weigh.

Table 1
Antitumor Activity against B16 Melanoma

20	Treatment	Tumor Weight (g) (Mean $\pm$ S.D. ^a)	% Inhibition
	Control	2.6 ± 0.5	-
	101,731 ^b (5 mg/kg, day 1-7)	2.4 ± 0.9	10
25	Ara-C (10 mg/kg, day 7-14)	3.6 ± 0.8	0
	101,731 + Ara-C	1.0 ± 0.3	59

^a S.D. = Standard Deviation

30 ^b 101,731 = (E)-2'-deoxy-2'-fluoromethylenecytidine

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Example 3Synergistic Antitumor Activity of A Combination of MDL 101,731 and Ara-C Against Lewis Lung Carcinoma in Mice

5 Inject 3LL cells (1×10^5 /mouse) s.c. on day 0. Administer compounds i.p., once daily as indicated. Sacrifice animals on day 15, dissect the tumors and weigh. Also count pulmonary metastatic foci.

Table 2
Antitumor Activity against Lewis Lung Carcinoma

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Treatment	Tumor Weight (g) (Mean $\pm$ S.D. ^a)	% Inhibition	No. of Foci (Mean $\pm$ S.D. ^a)	% Inhibition
Control	3.5 $\pm$ 0.4	-	11.3 $\pm$ 4	-
101,731 ^b (5 mg/kg, day 1-7)	2.8 $\pm$ 0.6	20	15.5 $\pm$ 6	0
Ara-C (10 mg/kg, day 7-14)	3.83 $\pm$ 0.8	0	14.6 $\pm$ 5	0
101,731 + Ara-C	1.12 $\pm$ 0.3	69	0	100

a S.D. = Standard Deviation

b 101,731 = (E)-2'-deoxy-2'-fluoromethylidenecytidine

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Example 4Synergistic Antiproliferative Activity of MDL 101,731 in Combination with S Phase Specific Antitumor Agents Against HeLa Cells

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Plate HeLa cells (2×10^3 cells/well) and allow to grow for 18 hours. Treat with MDL 101,731 (15ng/mL) for 24 hours. Wash the compound and expose cells to the indicated drugs for another 72 hours. Determine the cell viability by a colorimetric assay, essentially as described by Carmichael et al. [*Cancer Res.* 47, 936 (1987)], whereby the cellular reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] is measured. Treatment with MDL 101,731 alone as described did not show any effect on the growth of the tumor cells.

Calculate IC_{50} values for the individual treatments as well as for the combined treatments of MDL 101,731 with the various other agents. The IC_{50} values at the various concentrations of test agents are presented in Table 3.

Table 3
Antiproliferative Activity of 101,731 in Combination with S Phase Specific Anti-tumor Agents Against HeLa Cells

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Drug	Drug Alone IC_{50} (μ g/mL)	Drug + 101,731 ^a IC_{50} (μ g/mL)
Cytosine Arabinoside (Ara-C)	13.7	3.2
5-Fluorouracil (5Fu)	41.1	18.9

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^a101,731 = (E)-2'-deoxy-2'-fluoromethylidenecytidine

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Table 3
Antiproliferative Activity of 101,731 in Combination with S
Phase Specific Anti-tumor Agents Against HeLa Cells

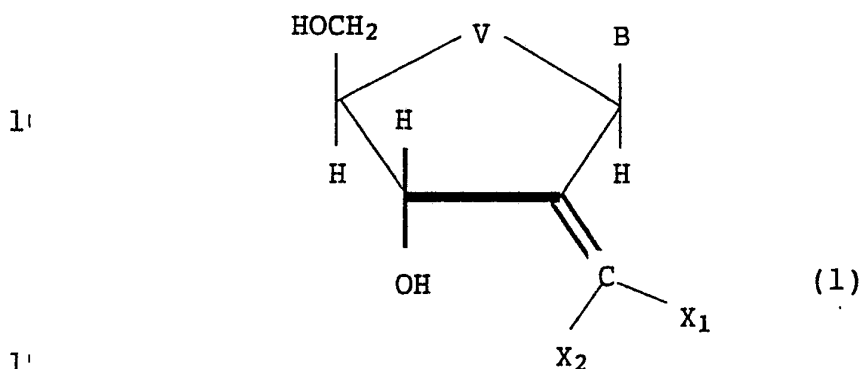
Drug	Drug Alone IC ₅₀ (μg/mL)	Drug + 101,731 ^a IC ₅₀ (μg/mL)
Vinblastine (VLB)	86.7	13.6

^a101,731 = (E)-2'-deoxy-2'-fluoromethylenecytidine

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WHAT IS CLAIMED IS:

1. A method of treating a patient suffering from a
 5 neoplastic disease state comprising administering to said
 patient an effective antineoplastic amount of a 2'-
 halomethylidene derivative of the formula (1)

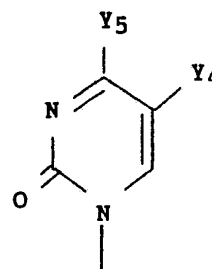
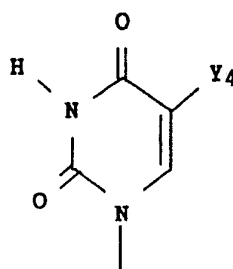
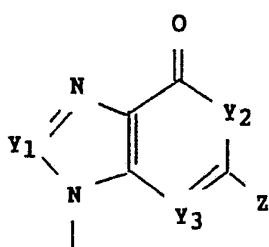


wherein

V is oxy, methylene, or thio,

X₁ and X₂ are each independently hydrogen or halogen,
 with the proviso that at least one of X₁ and X₂ is
 20 halogen,

B is a radical of the formula



- 30 wherein Y₁ is nitrogen, a CH group, a CCl group, a CBr
 group or a CNH₂ group; Y₂ and Y₃ are each independently
 nitrogen or a CH group; Y₄ is hydrogen, C₁-C₄ alkyl, C₁-
 C₄ alkoxy or halogen; Y₅ is amino or C₁-C₄ alkoxy; and Z
 is hydrogen, halogen, or NH₂;

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or a pharmaceutically acceptable salt thereof in conjunctive therapy with an effective antineoplastic amount of a S-phase or M-phase specific agent.

5 2. A method according to Claim 1 wherein the 2'-halomethylidene derivative of the formula (1) is (E)-2'-deoxy-2'-fluoromethylidenecytidine.

3. A method according to Claim 1 wherein the S-phase
10 specific agent is cytarabine.

4. A method according to Claim 1 wherein the S-phase specific agent is fluorouracil.

15 5. A method according to Claim 1 wherein the M-phase specific agent is vinblastine.

6. A method according to Claim 1 wherein the neoplastic disease state is a leukemia.

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7. A method according to Claim 1 wherein the neoplastic disease state is a carcinoma.

8. A method according to Claim 2 wherein the S-phase
25 specific agent is cytarabine.

9. A method according to Claim 2 wherein the S-phase specific agent is fluorouracil.

30 10. A method according to Claim 2 wherein the M-phase specific agent is vinblastine.

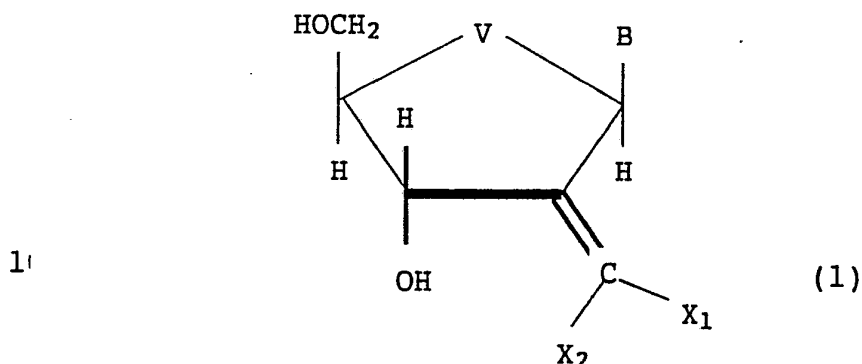
11. A method according to Claim 2 wherein the neoplastic disease state is a leukemia.

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12. A method according to Claim 2 wherein the neoplastic disease state is a carcinoma.

13. A compound of the formula (1)

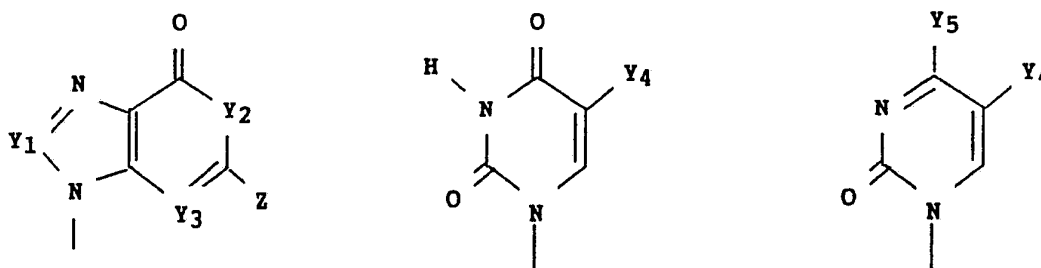


wherein

V is oxy, methylene, or thio,

15 X₁ and X₂ are each independently hydrogen or halogen, with the proviso that at least one of X₁ and X₂ is halogen,

B is a radical of the formula



25

wherein Y₁ is nitrogen, a CH group, a CCl group, a CBr group or a CNH₂ group; Y₂ and Y₃ are each independently nitrogen or a CH group; Y₄ is hydrogen, C₁-C₄ alkyl, C₁-C₄ alkoxy or halogen; Y₅ is amino or C₁-C₄ alkoxy; and Z is hydrogen, halogen, or NH₂;

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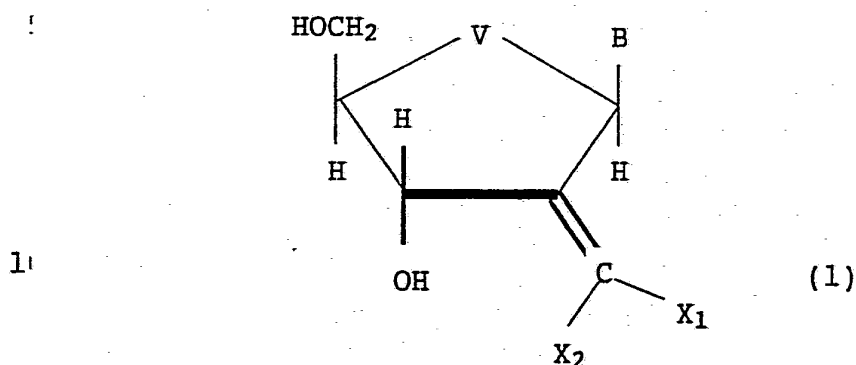
or a pharmaceutically acceptable salt thereof in conjunction with an effective antineoplastic amount of a S-phase or M-phase specific agent, optionally in combination with a

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pharmaceutically acceptable carrier, for use as a pharmaceutically active compound.

14. A compound of the formula (1)

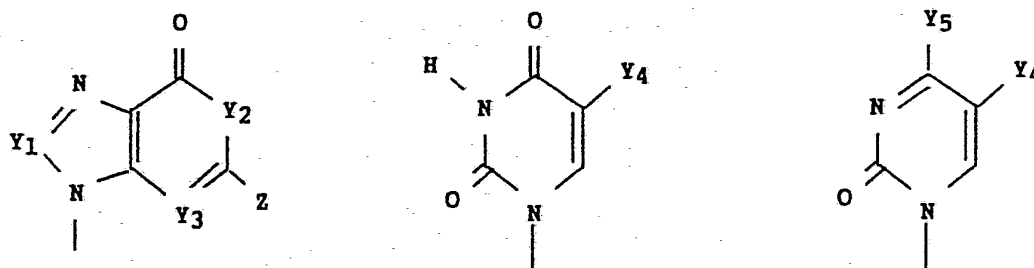


wherein

V is oxy, methylene, or thio,

15 X₁ and X₂ are each independently hydrogen or halogen, with the proviso that at least one of X₁ and X₂ is halogen,

B is a radical of the formula



25

wherein Y₁ is nitrogen, a CH group, a CCl group, a CBr group or a CNH₂ group; Y₂ and Y₃ are each independently nitrogen or a CH group; Y₄ is hydrogen, C₁-C₄ alkyl, C₁-C₄ alkoxy or halogen; Y₅ is amino or C₁-C₄ alkoxy; and Z is hydrogen, halogen, or NH₂;

30

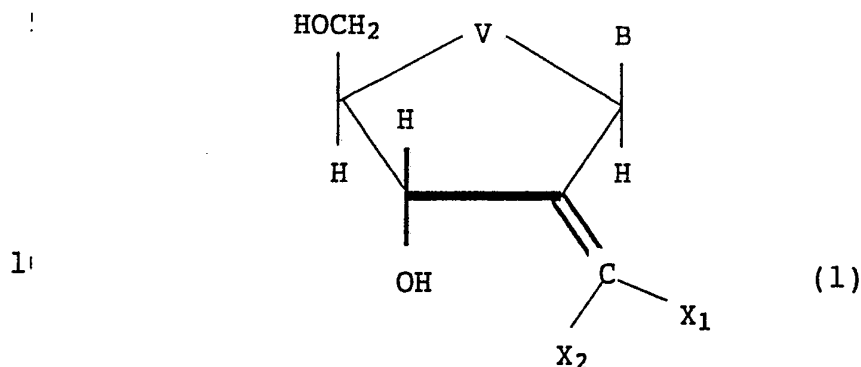
or a pharmaceutically acceptable salt thereof in conjunction with an effective antineoplastic amount of a S-phase or M-phase specific agent, optionally in combination with a

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pharmaceutically acceptable carrier, for use in the treatment of a neoplastic disease state.

15. A compound of the formula (1)

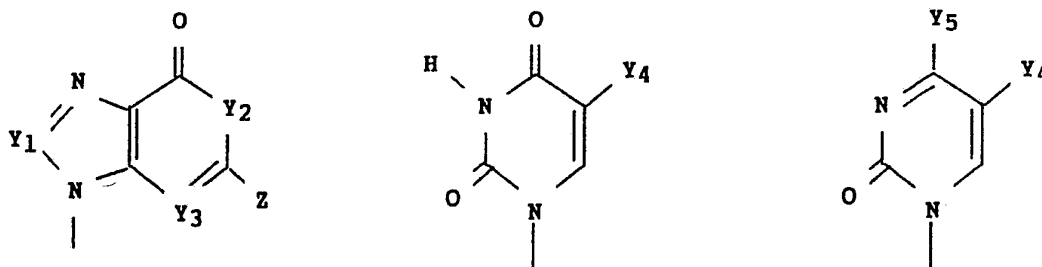


wherein

V is oxy, methylene, or thio,

15 X₁ and X₂ are each independently hydrogen or halogen, with the proviso that at least one of X₁ and X₂ is halogen,

B is a radical of the formula



25

wherein Y₁ is nitrogen, a CH group, a CCl group, a CBr group or a CNH₂ group; Y₂ and Y₃ are each independently nitrogen or a CH group; Y₄ is hydrogen, C₁-C₄ alkyl, C₁-C₄ alkoxy or halogen; Y₅ is amino or C₁-C₄ alkoxy; and Z is hydrogen, halogen, or NH₂;

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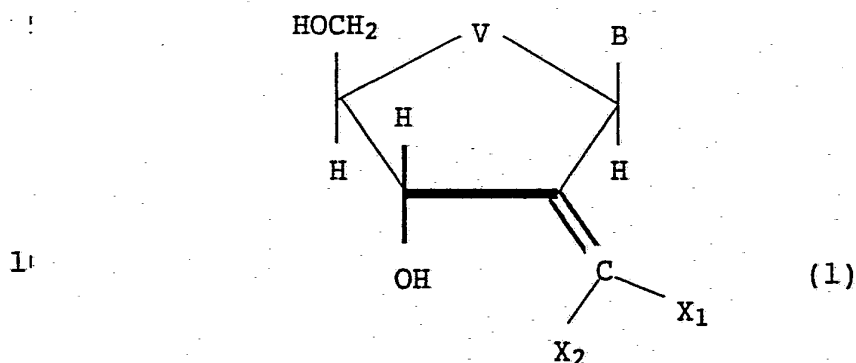
or a pharmaceutically acceptable salt thereof in conjunction with an effective antineoplastic amount of a S-phase or M-phase specific agent, optionally in combination with a

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pharmaceutically acceptable carrier, for use in the treatment of a leukemia.

16. A compound of the formula (1)

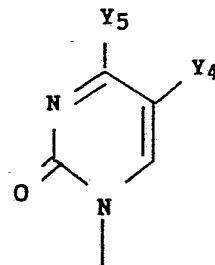
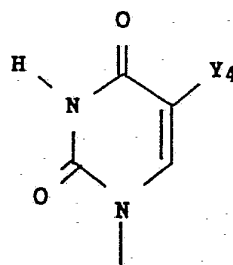
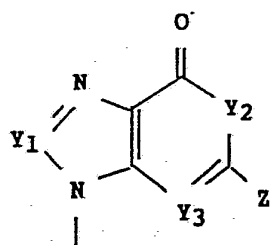


wherein

V is oxy, methylene, or thio,

15 X₁ and X₂ are each independently hydrogen or halogen, with the proviso that at least one of X₁ and X₂ is halogen,

B is a radical of the formula



25

wherein Y₁ is nitrogen, a CH group, a CCl group, a CBr group or a CNH₂ group; Y₂ and Y₃ are each independently nitrogen or a CH group; Y₄ is hydrogen, C₁-C₄ alkyl, C₁-C₄ alkoxy or halogen; Y₅ is amino or C₁-C₄ alkoxy; and Z is hydrogen, halogen, or NH₂;

30

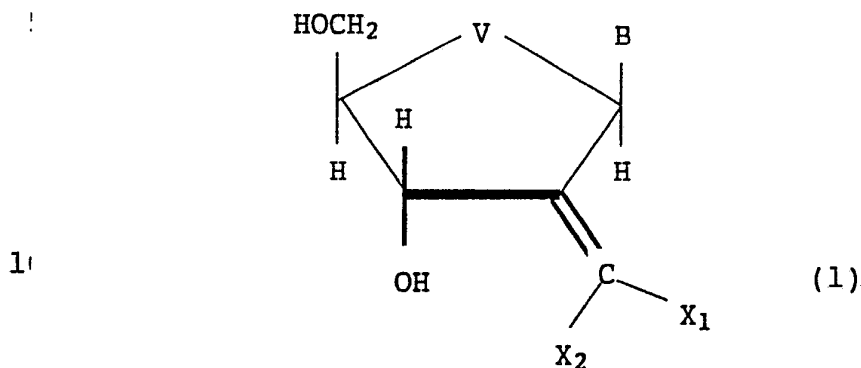
or a pharmaceutically acceptable salt thereof in conjunction with an effective antineoplastic amount of a S-phase or M-phase specific agent, optionally in combination with a

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pharmaceutically acceptable carrier, for use in the treatment of a carcinoma.

17. Use of a compound of the formula (1)

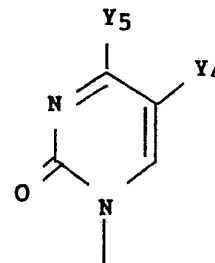
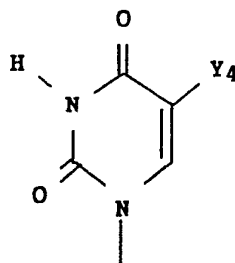
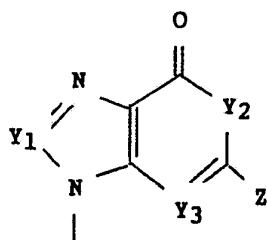


wherein

V is oxy, methylene, or thio,

15 X₁ and X₂ are each independently hydrogen or halogen, with the proviso that at least one of X₁ and X₂ is halogen,

B is a radical of the formula



25

wherein Y₁ is nitrogen, a CH group, a CCl group, a CBr group or a CNH₂ group; Y₂ and Y₃ are each independently nitrogen or a CH group; Y₄ is hydrogen, C₁-C₄ alkyl, C₁-C₄ alkoxy or halogen; Y₅ is amino or C₁-C₄ alkoxy; and Z is hydrogen, halogen, or NH₂;

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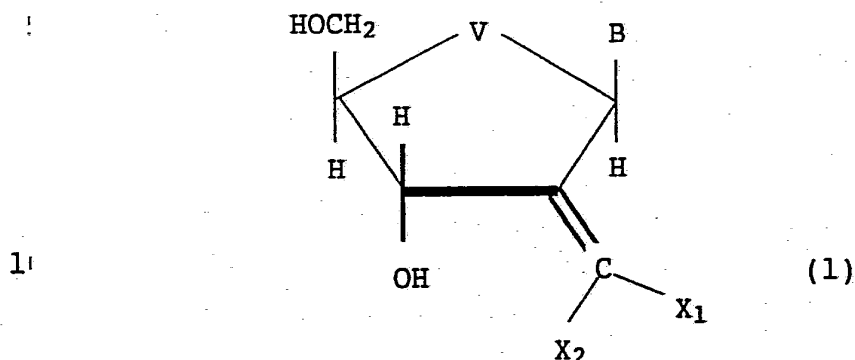
or a pharmaceutically acceptable salt thereof in conjunctive therapy with an effective antineoplastic amount of a S-phase or M-phase specific agent, optionally in combination with a pharmaceutically acceptable carrier, for the preparation of

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a pharmaceutical composition for the treatment of a neoplastic disease state.

18. Use of a compound of the formula (1)

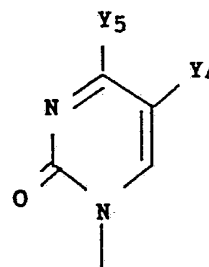
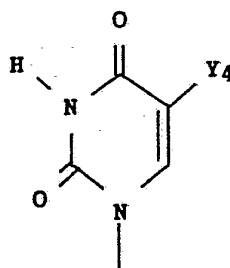
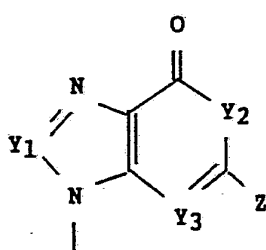


wherein

V is oxy, methylene, or thio,

15 X₁ and X₂ are each independently hydrogen or halogen, with the proviso that at least one of X₁ and X₂ is halogen,

B is a radical of the formula



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wherein Y₁ is nitrogen, a CH group, a CCl group, a CBr group or a CNH₂ group; Y₂ and Y₃ are each independently nitrogen or a CH group; Y₄ is hydrogen, C₁-C₄ alkyl, C₁-C₄ alkoxy or halogen; Y₅ is amino or C₁-C₄ alkoxy; and Z is hydrogen, halogen, or NH₂;

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or a pharmaceutically acceptable salt thereof in conjunctive therapy with an effective antineoplastic amount of a S-phase or M-phase specific agent, optionally in combination with a pharmaceutically acceptable carrier, for the preparation of

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a pharmaceutical composition for the treatment of a leukemia
or a carcinoma.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/02490

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A61K 31/70, 31/505, 31/52, 31/44

US CL : 514/45, 49, 256, 261, 274, 283, 299, 303

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)

U.S. : 514/45, 49, 256, 261, 274, 283, 299, 303

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. Am. Chem. Soc., 1991, McCarthy et al., p. 7439-7440, Stereospecific Method to E and Z Terminal Fluoro Olefins and its Application to the Synthesis of 2'-deoxy-2'-fluoromethylene Nucleosides as potential inhibitors of Ribonucleoside Diphosphate Reductase - See entire reference Chemotherapy of Cancer, 2nd ED, 13 August 1981, pages 76-83.	1-18
Y	Chemotherapy of Cancer, 2nd ED, 13 August 1981, pages 76-83	1-18

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be part of particular relevance	"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
"E" earlier document published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

Date of the actual completion of the international search

29 JULY 1993

Date of mailing of the international search report

23 SEP 1993

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

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