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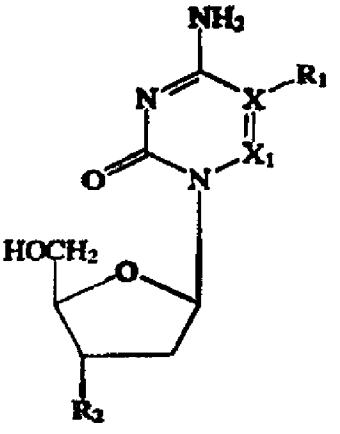
(54) **PROCEDE DE TRAITEMENT DES TROUBLES CARACTERISES PAR UNE SUREXPRESSION DE LA CYTIDINE-DESAMINASE OU DE LA DESOXYCYTIDINE-DESAMINASE**

(54) **METHOD OF TREATING DISORDERS CHARACTERIZED BY OVEREXPRESSION OF CYTIDINE DEAMINASE OR DEOXYCYTIDINE DEAMINASE**



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(57) L'invention concerne un procédé de traitement des troubles caractérisés par une surexpression de la cytidine-désaminase ou de la désoxycytidine-désaminase chez les patients affectés. Le procédé consiste à administrer aux patients un composé de la formule (I). Dans ladite formule, X et X_1 sont indépendamment C ou N; R_1 est alkyle inférieur, alcényle inférieur, alcynyl inférieur, halogène ou haloalkyle; R_2 est H, $-N_3$, -OH, amino, ou halogène; ou bien un sel pharmaceutiquement acceptable de ce type de composé. On administre les composés dans une proportion efficace pour traiter les troubles. Il est également fait référence à des formulations pharmaceutiques susceptibles d'être utilisées dans le cadre du traitement.

(57) A method of treating a disorder characterized by the overexpression of deaminase or deoxycytidine deaminase in a subject in need of such treatment is disclosed. The method comprises administering a compound of Formula (I) wherein X and X_1 are each independently C or N; R_1 is lower alkyl, lower alkenyl, lower alkynyl, halogen, or haloalkyl; and R_2 is H, $-N_3$, -OH, amino, or halogen; or a pharmaceutically acceptable salt thereof, to the subject in an amount effective to treat the disorder. Pharmaceutical formulations useful in the method of the present invention are also disclosed.

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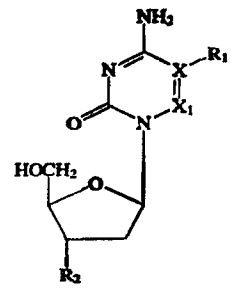
(54) Title: METHOD OF TREATING DISORDERS CHARACTERIZED BY OVEREXPRESSION OF CYTIDINE DEAMINASE OR DEOXYCYTIDINE DEAMINASE

(57) Abstract

A method of treating a disorder characterized by the overexpression of deaminase or deoxycytidine deaminase in a subject in need of such treatment is disclosed. The method comprises administering a compound of Formula (I) wherein X and X_1 are each independently C or N; R_1 is lower alkyl, lower alkenyl, lower alkynyl, halogen, or haloalkyl; and R_2 is H, $-N_3$, -OH, amino, or halogen; or a pharmaceutically acceptable salt thereof, to the subject in an amount effective to treat the disorder. Pharmaceutical formulations useful in the method of the present invention are also disclosed.



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PCT/US 96/20649
IPEA/US 13 FEB 1998

AGENT & METHOD FOR TREATING DISORDERS ASSOCIATED WITH CYTIDINE DEAMINASE OR DEOXYCITIDINE DEAMINASE OVER EXPRESSION

Related Application

This application is a continuation-in-part application of U.S. Patent Application Serial No. 08/577,185, filed 22 December 1995.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to an active agent of the formula I and to treating a disorder associated in part with the overexpression of cytidine deaminase or deoxycytidine deaminase, comprising administering 5-methyl-2', 3'-dideoxy-3'-azidocytidine (5mAZC), analog thereof or a pharmaceutically effective salt thereof to a subject in need of such treatment in an amount effective to treat the disorder and ameliorate its symptoms.

The overexpression of cytidine deaminase (CD) is associated with a number of human disorders. For example, certain kinds of leukemias are refractory to the widely used anti-cancer agent cytosine arabinoside (araC). The lack of response to araC has been found to be due primarily either to inactivation of the deoxycytidine kinase gene locus (whose product is required for activation of araC to its cancer-killing form), or to the overexpression of cytidine deaminase or deoxycytidine deaminase (which deactivates araC by deaminating it to an inactive form, araUracil). There is currently no adequate treatment to overcome this resistance to araC due to cytidine deaminase or deoxycytidine deaminase overexpression.

The overexpression of cytidine deaminase is also implicated in the infective cycle of the human

immunodeficiency virus (HIV), the virus implicated in AIDS. The initial states of HIV infection are characterized by the overexpression of cytidine deaminase by the CD-4+T lymphocytes targeted by the virus. The elimination of this original set of infected cells could be critically important in controlling the level of subsequent infection.

Currently, one treatment for HIV infection and AIDS is the administration of 3'-azido-3'deoxythymidine associated with this treatment. Problems with toxicity, however, have been associated with this treatment. Such toxicity occurs in part due to the fact that AZT is administered systemically, and damages host cells in all replicating tissue compartments.

There is currently a need for a treatment for HIV infection that has the same or improved efficacy of AZT, without the side effects associated with toxicity. One solution to this problem would be a prodrug for AZT which exhibited reduced toxicity to replicating cells, and was preferentially activated to its virus-killing form (AZT) in HIV-infected cells.

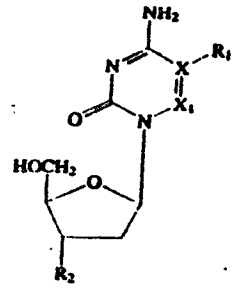
In addition, inflammatory cells associated with the symptoms of arthritis have also been shown to overexpress cytidine deaminase.

It would therefore be desirable to provide a relatively non-toxic prodrug that is preferentially activated to a metabolite that is toxic to arthritis mediating inflammatory cells.

SUMMARY OF THE INVENTION

A method of treating a disorder associated with the overexpression of cytidine deaminase or deoxycytidine deaminase comprises administering to a subject in need of such treatment, of 5-methyl-2',3'dideoxy-3'azidocytidine (5mAZC), analog thereof or pharmaceutically acceptable salts thereof, hereinafter referred to as the "active compound", an amount effective to treat the disorder.

A method of treating a disorder associated with the overexpression of cytidine deaminase or deoxycytidine deaminase comprises administering to a subject in need of the treatment, an amount of a compound of the chemical formula I



wherein

X and X_1 are each independently C or N;

R^1 is lower alkyl, lower alkenyl, lower alkynyl, halogen, or haloalkyl; and

R^2 is H, $-N_3$, -OH, amino, or halogen;

or a pharmaceutically acceptable salt thereof, effective to treat the disorder.

A method of combating leukemia resistant to cytosine arabinoside (araC) comprises administering to a subject in need of the treatment an anti-araC resistant leukemia effective amount of a compound of the chemical formula I.

A method of combating HIV-infection comprises administering to a subject in need of the treatment an anti-HIV effective amount of a compound of the chemical formula I.

A method of combating arthritis comprises administering an anti-arthritis effective amount of a compound of the chemical formula I to an arthritic subject.

A method of combating a cancer associated with the overexpression of cytidine deaminase, comprising administering to a subject in need of the treatment an

anti-cancer effective amount of a compound of the chemical formula I.

The agent of this invention is suitable for the preparation of medicament for application in the treatments given above. The medicaments are provided in the form of oral, parenteral, topical, and transdermal formulations, as well as an implant and in the form of a kit.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 is a graph of a dose response curve comparing the efficacy of 5mAZC in HT-29SF^{CD} cells, which overexpress cytidine deaminase, and HT-29SF cells, which do not. HT-29SF cells were plated in the bottom compartment, and HT-29SF^{CD} cells in the top compartment of a "Transwell" culture dish (1,000 cells each). A semipermeable membrane separates the two compartments of the Transwell, such that any drug administered in the tissue culture media bathe both compartments equally. The numbers in the x-axis indicate the concentration (in μM) at which 5mAZC was administered continuously for 72 hours. The numbers on the y-axis indicate the number of surviving cells. The circular (●) data points represent the dose response for the HT-29SF^{CD} cells. The square (■) data points represent the dose response for the HT-29SF cells.

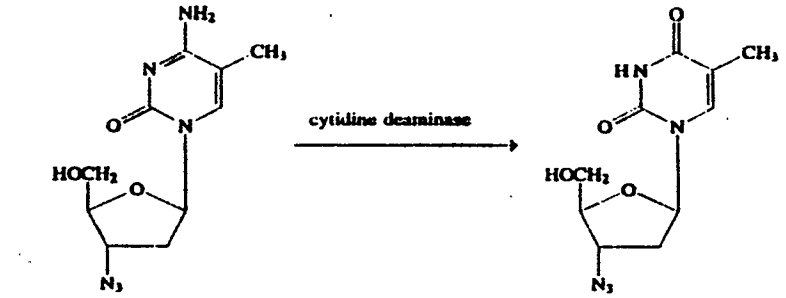
DETAILED DESCRIPTION OF THE INVENTION

The method of the present invention may be used to treat a disorder associated with the overexpression of cytidine deaminase and/or deoxycytidine deaminase by administering an amount of the active compound of this invention to a subject in need of the treatment, effective to treat the disorder. That is 5-methyl 2',3'-deoxy-cytidine deaminase(5mAZC) an analog thereof, or pharmaceutically acceptable salts thereof (i.e., the active compounds). Examples of the disorders that may be treated include, but are not limited to, cancer, leukemia

PCT/US 96/20649
IPEA/US 13 FEB 1998

that is resistant to cytosine arabinoside (araC), HIV infection, and arthritis. In a preferred embodiment, the method of the present invention is used to treat a subject suffering from a cancer that is characterized by the overexpression of cytidine deaminase. Examples of these cancers include adenocarcinoma of the colon, adenocarcinoma of the lung, adenocarciona of the stomach, adenocarcinoma of the breast, Wilm's tumor, chondrocarcinoma, chondrosarcoma, leukemia, prostate tumors, brain tumors (e.g., glioma, astrocytoma), kidney tumors, pancreatic tumors, cervical tumors, liver tumors (e.g., hepatoblastoma, hepatocarcinoma), neuroblastoma, retinoblastoma, melanoma, basal cell carcinoma, sarcoma, and cancers metastatic to the liver (e.g., colon cancer).

While applicants do not wish to be bound to any particular theory of the instant invention, 5mAZC appears to be metabolized to 3'-azido-3'deoxythymidine (AZT) by cytidine deaminase and deoxycytidine deaminase, as illustrated in **Scheme 1**, below.



5-methyl-2',3'-dideoxy-3'-azidocytidine (5mAZC)

2',3'-dideoxy-3'-azidothymidine (also 3'-azido-3'-deoxythymidine) (AZT)

While not adversely affecting cells that do not overexpress cytidine deaminase, 5mAZC appears to be preferentially deaminated to AZT in tumor cells that overexpress cytidine deaminase.

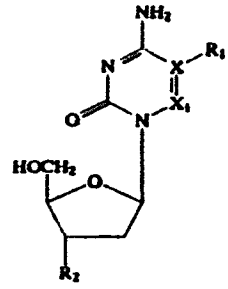
The present invention is concerned primarily with the treatment of human subjects but may also be employed in

PCT/US 96/20649
IPEA/US 13 FEB 1998

the treatment of other mammalian subjects, such as dogs and cats, for veterinary purposes.

As used herein, the term "lower alkyl", refers to C^1 to C^4 linear or branched alkyl, such as methyl, ethyl, propyl, butyl, isopropyl, sec-butyl, and tert-butyl. Methyl is currently preferred. As used herein the term "lower alkenyl", refers to C^2 to C^5 linear or branched alkenyl, such as ethenyl, propenyl, and butendyl. As sued herein, the term "lower alkynyl", refers to C^2 to C^5 linear or branched alykynl, such as propynyl and butynl. AS used herein, the term "haloalkyl" refers to a lower alkyl as defined above wherein one or more hydrogens is substituted with a halo-group, e.g., a chloro-, fluor-, bromo- or iodo-group, with $-CF_3$ being currently preferred.

Active compounds useful in the practice of the present invention include compounds of the chemical formula I



wherein

X and X_1 are each independently C or N;

R^1 is lower alkyl, lower alkenyl, lower alkynyl, halogen, or haloalkyl; and

R^2 is H, $-N_3$, -OH, amino, or halogen.

In a preferred embodiment of the present invention, R^1 is methyl or $-CF_3$, and R^2 is $-N_3$ or -OH. In a particularly preferred embodiment of the invention, R^1 is methyl and R^2

is $-N_3$. Also encompassed herein are the pharmaceutically acceptable salts of these compounds.

Analogs of 5mAZC useful in the practice of the present invention include, but are not limited to, 5-methyl-2', 3'-dideoxycytidine, 5-ethyl-2',3'-dideoxycytidine, 5-ethyl-2', 3'-dideoxy-3'-azidocytidine, 5-propyl-2', 3'-dideoxycytidine, 5-propyl-2', 3'-dideoxy-3'-azidocytidine, 5-propene-2',3'dideoxycytidine, 5 propene-2', 3'-dideoxy-3'-azidocytidine, 5-propyne-2',3'-dideoxycytidine, and 5-propyne-2',3'-dideoxy-3'-azidocytidine.

The structure of 5mAZC is known. See, e.g., T.S. Lin et al., J. Med. Chem 26: 1691-1696 (1983). 5mAZC is available from ChemSyn Laboratories (Lenexa, Kansas, USA). Compounds useful for carrying out the present invention may be synthesized in accordance with known procedures which will be apparent to those skilled in the art. See, e.g., T.S. Lin et al., supra; T. Kulikowski et al., Acta Biochim. Polonica 16, 201-217 (1969); J.J. Fox et al., J. Amer. Chem. Soc. 81, 178-187 (1959).

The active compounds disclosed herein may, as noted above, be prepared in the form of their pharmaceutically acceptable salts. "Pharmaceutically acceptable salts" are salts that retain the desired biological activity of the parent compound utilized and do not impart undesired toxicological effects. Examples of such salts are (a) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; and salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalene sulfonic acid, methane sulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; (b) salts formed from elemental anions such

as chlorine, bromine, and iodine, and (c) salts derived from bases, such as ammonium salts, alkali metal salts such as those of sodium and potassium, alkaline earth metal salts such as those of calcium and magnesium, and salts with organic bases such as dicyclohexylamine and N-methyl-D-glucamine.

The dosage of active compound for treatment will vary depending on the condition and the state of the subject being treated. Generally, the dosage may be as low as 0.1 μmol/kg, but more preferably is at least 1.0 μmol/kg and most preferably is at least 25 μmol/kg. The dosage of the active compound may generally be as high as 1000 μmol/kg, more preferably is less than 500 μmol/kg and still most preferably is less than 100 μmol/kg. Depending on the solubility of the particular formulation of active compound administered, the daily dose may be divided among one or several unit dose administrations. The administration of the active compound of the invention may be carried out therapeutically, i.e., as a rescue treatment, or prophylactically.

Pharmaceutical compositions for use in the present method of treating disorders associated with the overexpression of cytidine deaminase and/or deoxycytidine deaminase include those suitable for inhalation, oral, rectal, parenteral (including subcutaneous, intradermal, intramuscular, intravenous) and transdermal administration. The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods which are well known in the art. The most suitable route of administration in any given case may depend upon the anatomic location of the disorder in the subject, the nature and severity of the condition being treated, and the particular formulation that is being used. The formulations may conveniently be presented in unit dosage form and may be prepared by any method well known in the art.

In the present method of treating leukemia or other disorders associated with the overexpression of cytidine deaminase or deoxycytidine deaminase, the active compound is administered in a dose range as given above. The dose of active agent may vary according to the condition being treated and the dose at which adverse pharmacological effects occur. One skilled in the art will take such factors into account when determining dosage.

In one embodiment of the present invention, the active compound is administered to a subject with araC-resistant leukemia in an amount effective so that it is metabolized by cytidine deaminase or deoxycytidine deaminase to produce an anti-araC-resistant cancer effective amount of AZT.

The active compound may be used alone or in combination with one or more anti-leukemic agents for the prophylaxis or treatment of araC-resistant leukemia.

In the manufacture of a pharmaceutical composition according to the invention into a "formulation", the active agent(s) or physiologically acceptable salts thereof (the "active compound") are typically admixed with, inter alia, an acceptable carrier. The carrier must be acceptable so that it is compatible with any other ingredients in the formulation and not deleterious to the subject's health in general. The carrier may be solid or liquid, or a mixture of both, and is preferably formulated with the active compound as a unit-dose formulation, for example, a tablet, which may contain from 0.5% to 99% by weight of the active compound. One or more active compounds may be incorporated in the formulation of the invention, e.g., the formulation may contain one or more additional agents as noted above. The formulations may be prepared by any of the well known techniques of pharmacy comprising admixing the components with one or more carriers, and optionally including one or more accessory therapeutic ingredients.

Formulations suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges or tablets, each containing a predetermined amount of the active compound; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such formulations may be prepared by any suitable method of pharmacy which includes bringing into association the active compound and a suitable carrier (which may contain one or more accessory ingredients as noted above). In general, the formulations of the invention are prepared by uniformly and intimately admixing the active compound with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture. For example, a tablet may be prepared by compressing or molding a powder or granules containing the active compound, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the compound in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s). Molded tablets may be made by molding in a suitable machine, the powdered compound moistened with an inert liquid binder. Liquid formulations for oral administration may optionally include enteric coatings known in the art to prevent degradation of the formulation in the stomach and provide release of the drug in the small intestine.

Formulations suitable for buccal (sub-lingual) administration include lozenges comprising the active compound in a flavored base, usually sucrose and acacia or tragacanth; and pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

Formulations of the present invention suitable for parenteral administration comprise sterile aqueous and non-aqueous injection solutions of the active compound,

PCT/US 96/20649
IPEA/US 13 FEB 1998

which preparations are preferably isotonic with the blood of the intended recipient. These preparations may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions may include suspending agents and thickening agents. The formulations may be presented in unit/dose or multi-dose containers, for example sealed capsules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use. Injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations suitable for rectal administration are preferably presented as unit dose suppositories. These may be prepared by admixing the active compound with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Formulations suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Formulations suitable for transdermal administration may also be delivered by iontophoresis, and typically take the form of an optionally buffered aqueous solution of the active compound. See e.g., Pharmaceutical Research **3**, 318 (1986).

The following examples are provided to more fully illustrate the present invention and should not be construed as restrictive thereof. In the following examples, g means grams, h means hours, kg means kilogram, ml means milliliter, M means molar, μg means microgram, and μmol means micromoles, nmol means nanomoles, pmol means picomoles, μCi means microCuries.

PCT/US 96/20649
IPEA/US 13 FEB 1998

EXAMPLES

Example 1: Effect of 5mAZC in Overexpressing Cytidine Deaminase Cells

HT-29SF human adenocarcinoma cells naturally express a high level of cytidine deaminase (CD). HT-29SF^{CD} is a subline of the HT-29SF line that express even higher CD levels than normal (about 5.4 times the expression level of HT-29SF cells). In order to see whether cells that overexpress cytidine deaminase are more sensitive to 5mAZC than other cells, HT-29SF^{CD} cells were plated in the top compartment of a Transwell culture dish, while HT-29SF cells were placed in the bottom compartment (1000 cells each). A semipermeable membrane separated the two compartments of the Transwell, such that drugs administered in the tissue culture media bathe both compartments equally. 5mAZC was administered continuously for 72 hrs at concentrations ranging from 0 μM to 50 μM. The results of this experiment are shown in **Figure 1**. The results of this experiment confirm the idea that cells that highly overexpress CD are sensitive to 5mAZC.

Example 2: In Vitro Activity of 5mAZC

Based upon the results obtained in example 1 above, a dose of 50 μM 5mAZC was selected for more in-depth analysis. Two experiments were performed, in quintuplicate, in the same Transwell dishes, containing the HT-29SF cells and the HT-29SF^{CD} cells in compartments separated only by a membrane permeable to 5mAZC. The results are given in Table 1 below.

PCT/US 96/20649
IPEA/US 13 FEB 1998

Table 1. Effects of exposure of HT-29SF and HF-29SF^{CD} human colonic adenocarcinoma cells to 50μM 5mAZC (72h) in Transwell culture system dishes.

Experiment 1 Number of viable cells		Example 2 Number of viable cells	
HT-29SF^{CD}	HT-29SF	HT-29SF^{CD}	HT-29SF
33.8 ± 17.1	797± 68	45.4 ± 15.6	867.2 ± 29.4

Example 3: In vivo Activity of 5mAZC: Human Tumor Xenograft Model

To demonstrate that 5mAZC exhibits in vivo activity, two sixteen week old female Balb/C nu/nu mice were subcutaneously inoculated with 2.0 x 10^6 HT-29SF (non cytidine deaminase producing)cells in the left scapular region, and 2.0 x 10^6 HT-29SF^{CD} (cytidine deaminase producing)cells in the right scapular region. One of these mice received twice daily intraperitoneal doses of 150 mg/kg of 5mAZC. The other mouse received saline only. This treatment continued for 5 weeks, at which time the animals were euthanized and the solid tumors were dissected free and weighed. The examined tumors had the following weights:

1- HT-29SF^{CD} + 5mAZC: 0.22 g ± 0.31 g (N=2)

2- HT-29SF + 5mAZC: 1.02 ± 0.13 g (N=2)

3- HT-29SF^{CD} + saline: 0.96 g (N=1)

4- HT-29SF + saline: 1.13 g (N=1)

In another experiment, thirty BALB/c nude mice received subcutaneous injections of 5 x 10^6 HT-29SF cells. Twelve of the animals received 600 mg/kg/day 5mAZC in the flank for 28 days. Eighteen animals received saline injections on the same schedule. On Day 29, the animals were sacrificed and the tumor volume was quantified by standard methods. The average tumor volume for the control group receiving saline was 509 ± 292 mm^3. In contrast, the animals treated with 5mAZC had an average tumor volume of 273 ± 97 mm^3, an average 46% reduction in

size. The results of Examples 2 and 3 show clearly that 5mAZC is activated *in vitro* and *in vivo* into molecular species exclusively toxic to tumor cells that overexpress cytidine deaminase.

Example 4: CD Overexpression in Colon Tumors: Ex vivo Patient Data

The hypothesis that cytidine deaminase (CD) is overexpressed in certain tumors as compared with normal tissue was demonstrated in experiments conducted with surgical specimens obtained from patient undergoing bowel resection for colon cancer. Specimens of tumor and adjacent normal tissue obtained from pathological evaluation ere assayed for CD activity using [^{3}H]-cytidine as a substrate, according to the method of R.L. Momparler and J. Laliberte, *J. Leukemia Res.*, 14(9), 751-54 (1990). Briefly, colon tumors and adjacent mucosa from surgical specimens were homogenized in 5mM Tris-C1, pH 7.4, sonicated on ice in three 5-second pulses, and then adjusted to 50mM Tris-HC1,pH 7.4. The homogenates were centrifuged at 12,000 x g for 15 minutes at 4°C, and the homogenates stored at -70°C until analysis. Protein concentration was determined using a BioRad protein assay kit and bovine gamma globulin was used as a standard.

For determination of cytidine deaminase activity, the reaction mixture (100μL) contained 25 mM Tris-C1, pH 7.4, 0.5 μCi of [^{3}H]-cytidine and 0.02-0.05 mg of homogenate tissue. Reactions were carried out at 37°C for 30 minutes, then stopped with 3 mL of cold 0.001 N HC1. The reaction mixture was placed on Whatman P-81 phosphocellulose discs that were washed with 3 mL of H_2O, 1 mL of 0.1 N HC1, and 3 mL of H2O twice before use. The mixture was allowed to flow gently by gravity for 1 hr and then assayed for radioactivity. The results were normalized for protein content and expressed as nmol/min/mg protein. The results of this experiment are presented in Table 2 below.

Example 5
Preferential Deamination of 5mAZC to AZT in Colonic Tumors:

The hypothesis that 5mAZC is preferentially deaminated to AZT in human colonic tumors versus in normal adjacent mucosa was demonstrated in the same *ex vivo* samples described above in Example 4. Using [^{3}H]-5mAZC as a substrate, the [^{3}H]-AZT product of the enzymatic reaction was isolated by HPLC and the radiolabeled product quantitated as described in J.W. Nyce et al., *Proc. Natl. Acad. Sci USA* **90**, 2960-2964 (1993). Briefly, procedures described in Example 4 were repeated with the following exceptions: Each reaction mixture contained 0.26 μCi of [^{3}H]-5mAZC. The reactions were terminated with 50 μL of 2M perchloric acid and the mixture separated by HPLC using a 4.6 x 25 cm Beckman Ultrasphere ODS with a mobile phase of 12.5% acetonitrile in 40 mM sodium acetate pH 7.0. Unlabeled 5mAZC and AZT were used to authenticate the resulting peaks. The fractions corresponding to radiolabeled 5mAZC and AZT were collected and assayed for radioactivity by scintillation counting. The results were normalized for protein content and expressed as pmol/min/mg protein, and are provided below in **Table 3**.

To confirm that the AZT found in the colonic tumor tissue was being produced by cytidine deaminase activity (see **Scheme 1**, above), the cytidine deaminase inhibitor tetrahydrouridine (THU) was added to the reaction mixtures containing tumor tissue homogenate and radiolabeled 5mAZC; the amount of radiolabeled AZT produced was assayed as above, then compared to the amount of radiolabeled AZT produced in reaction mixtures not containing THU. The results of this experiment are also provided below in **Table 3**.

The foregoing Examples are illustrative of the present invention, and are not to be construed as limiting thereof. The invention is defined by the

following claims, with equivalents of the claims to be included therein.

TABLE 2

Ex Vivo Patient Data:

Cytidine Deaminase Activity in Tumor vs. Normal Adjacent Mucosa

Patient	Activity In Normal Mucosa Adjacent to Tumor nmol/min/mg protein	Activity in Adenocarcimoma · Tumor nmol/min/mg protein	Percentage Tumor CD Activity of Normal	p value
1	13.26 ± 0.67	17.36 ± 0.24	130.92	<.01
2	12.00 ± 0.50	15.49 ± 0.33	129.08	<.01
3	8.81 ± 0.79	29.20 ± 0.54	331.44	<.01
4	10.82 ± 0.63	12.98 ± 0.58 18.38 ± 0.34	119.96 169.87	<.01
5	7.78 ± 0.22	9.25 ± 0.48	118.89	<.05
6	17.32 ± 0.93	22.35 ± 0.39	129.04	<.01
7	6.37 ± 0.89	18.69 ± 1.27	294.98	<.01
8	9.63 ± 0.77	14.55 ± 1.17	151.09	<.05
9	14.01 ± 0.89	20.09 ± 0.20	143.40	<.01
10	6.06 ± 0.83	18.51 ± 0.89	305.22	<.01
Average	10.61 ±3.6	17.91 ± 5.2	184	
HT-29SF	11.96 ± 0.16			

TABLE 3

Ex vivo patient data:
Preferential 5mAZC deamination to AZT in tumor vs. normal adjacent mucosa

Patient #	Radiolabeled AZT in Normal Adjacent Tissue pmol/min/mg protein	Radiolabeled AZT in Colon Tumor Tissue pmol/min/mg protein	Percentage AZT in Tumor Tissue of Normal	p value
1	117.14 ± 7.61	134.51 ± 14.92 After THU: 30.56 ± 4.22	114.83	
2	128.76 ± 2.17	153.34 ± 8.70 After THU: 50.48 ± 7.30	119.09	< .05
3	40.42 ± 1.18	304.04 ± 17.63 After THU: 0.31 ± 0.31	752.17	< .01
4	77.52 ± 8.83	80.01 ± 20.23 After THU: 6.47 ± 6.47 138.58 ±1.44 After THU: 34.83 ± 2.17	103.33 178.88	polyp < .01
5	144.28 ± 20.39	225.21 ± 44.91 After THU: 90.90 ± 26.41	156.09	
6	126.75 ± 9.75	235.07 ± 11.74 After THU: 74.84 ± 6.51	185.46	< .01
7	70.58 ± 16.31	164.62 ± 20.63 After THU: 26.81 ± 1.46	233.23	< .05
8	51.91 ± 14.01	88.59 ± 13.80 After THU: 61.31 ± 4.57	170.64	
9	89.05 ± 13.05	132.10 ± 4.83 After THU: 39.88 ± 24.18	148.34	< .05
10	64.28 ± 6.00	147.80 ± 10.95 After THU: 80.34 ± 16.86	229.93	< .01
Average	91.1 ±36.0	172.5 ±63	229	

PCT/US 96/20649

IPEA/US 13 FEB 1998

WHAT IS CLAIMED IS:

1. An agent having the chemical formula

$C_7N_3H_8O_2R^1R^2XX^1$,

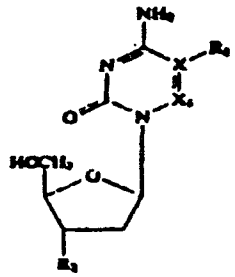
wherein X and X^1 are each independently C or N but both may not be C; R^1 is selected from the group consisting of lower alkyl, alkenyl and alkynyl, halogen and haloalkyl; and R^2 is selected from the group consisting of H, $-N_3$, -OH, amino and halogen; or pharmaceutically acceptable salts thereof.

2. The agent of claim 1, wherein R^1 is methyl and R^2 is $-N_3$.

3. The agent of claims 1 or 2, wherein R^1 is CF_3.

4. The agent of claims 1 to 3, which is selected from the group consisting of 5-methyl-2', 3-dideoxycytidine, 5-ethyl-2',3' dideoxy-3' - azidocytidine, 5-propyl-2',3-dideoxycytidine, 5-propyl-2',3'-dideoxy-3-azidocytidine, 5-propene-2', 3'-dideoxy-3'-azidocytidine, 5-propyne-2',3'-dideoxy-3'-azidocytidine, 5-propyne-2',3'-dideoxycytidine and 5-propyne-2',3'-dideoxy-3'-azidocytidine.

5. The agent of claims 1 to 4, or pharmaceutically acceptable salts thereof, having the chemical formula



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6. The agent of claims 1 to 5, wherein X comprises N and X^1 comprises C.

7. The agent of claims 1 to 5, wherein X comprises C and X^1 comprises N.

8. The agent of claims 1 to 5, wherein X comprises N and X^1 comprises N.

9. The agent of claims 1 to 8, further comprising a radiolabel.

10. The agent of claims 1 to 9, which is freeze-dried or lyophilized.

11. The agent of claims 1 to 10, wherein the R^1 haloalkyl is selected from the group consisting of alkyl substituted by one or more chloro, fluoro, bromo and iodo.

12. The agent of claims 1 to 11, wherein the R^1 fluoroalkyl comprises $-CF_3$.

13. A composition, comprising
the agent of claims 1 to 12; and
a pharmaceutically acceptable carrier.

14. The composition of claim 13, comprising about 0.5 to about 99 % of the agent.

15. The composition of claims 13 to 14, in unit dosage form.

16. The composition of claims 13 to 15, in multi-dosage form.

17. The composition of claims 13 to 16, in a form selected from the group consisting of capsules, cachets, pastilles, lozanges, powder, granules, solution, suspension, emulsion and tablets.

18. The composition of claims 13 to 17, wherein the carrier is selected from the group consisting of solid and liquid carriers.

19. The composition of claim 13 to 18, further comprising an agent selected from the group consisting of other therapeutic agents, flavorings, lubricants, suspending and thickening agents, binders, inert diluents, surface active agents, dispersants, antioxidants, buffers, bacteriostats and solutes to attain isotonicity.

20. The composition of claims 13 to 19, wherein the therapeutic agent comprises an anti-leukemia agent.

21. The composition of claims 13 to 20, wherein the anti-leukemia agent comprises cytosine arabinoside.

22. An oral formulation comprising the composition of claims 13 to 21, and an enteric coating.

PCT/US 96/20649
IPEA/US 13 FEB 1998

23. A sub-lingual formulation comprising the composition of claims 13 to 22, wherein the flavoring and inert diluent are selected from the group consisting of sucrose, acacia, tragacanth, gelatin and glycerin.

24. A parenteral formulation comprising the composition of claims 13 to 21, comprising a solution, suspension or emulsion.

25. An ampule or vial comprising the formulation of claims 13 to 21 and 24.

26. A rectal formulation comprising the composition of claims 13 to 21, in unit dosage form.

27. A transdermal formulation comprising the composition of claims 13 to 21, and an iontophoretic medium.

28. A transdermal device, comprising a patch which comprises the formulation of claims 13 to 21 and 27.

29. An iontophoretic device comprising the transdermal device of claim 28, and means for iontophoretic delivery.

30. A .therapeutic kit, comprising in a sealed container the composition of claims 13 to 29, and instructions for its administration.

31. A method of preventing or treating a disorder associated with cytidine deaminase or deoxycytidine deaminase over-expression, comprising administering to a subject in need of such treatment an anti-disorder effective amount of an agent selected from the group consisting of the agent of the chemical formula $C_7N_3H_8O_2R^1R^2XX^1$, wherein X and X_1 are each independently C or N, R^1 is selected from the group consisting of lower alkyl, alkenyl and alkynyl, halogen and haloalkyl, and R^2 is selected from the group consisting of H, $-N_3$, -OH, amino and halogen; or pharmaceutically acceptable salts thereof.

32. The method of claim 31, wherein R^1 is methyl and R^2 is $-N_3$.

33. The method of claim 31 or 32, wherein the agent is selected from the group consisting of 5- methyl- 2', 3- dideoxycytidine, 5- ethyl- 2', 3' dideoxy- 3' -

azidocytidine, 5- propyl- 2', 3- dideoxycytidine, 5-propyl-2',3'-dideoxy-3-azidocytidine, 5-propene-2', 3'-dideoxy-3'-azidocytidine, 5-propyne-2',3'-dideoxy-3'-azidocytidine, 5-propyne-2',3'-dideoxycytidine and 5-propyne-2',3'-dideoxy-3'-azidocytidine.

34. The method of claims 31 to 33, wherein the disorder is cytosine arabinoside (araC) -resistant leukemia.

35. The method of claims 31 to 34, further comprising administering to the subject an anti-leukemia effective amount of an anti-leukemia agent.

36. The method of claims 31 to 35, wherein the anti-leukemia agent is administered concurrently with the agent.

37. The method of claims 31 to 36, wherein the anti-leukemia agent comprises cytosine arabinoside (araC).

38. The method of claims 31 to 37 , wherein the disorder comprises arthritis.

39. The method of claims 31 to 38, which is a prophylactic method.

40. The method of claims 31 to 38, which is a therapeutic method.

41. The method of claims 31 to 40, wherein the agent is administered parenterally.

42. The method of claims 31 to 40 , wherein the agent is administered orally.

43. The method of claims 31 to 40, wherein the agent is administered transdermally.

44. The method of claims 31 to 43, wherein the agent is administered in an amount of about 0.1 to about 1000 μmol/kg body weight.

45. The method of claims 31 to 44, wherein the subject is human.

46. The method of claims 31 to 45, wherein the subject is a non-human animal.

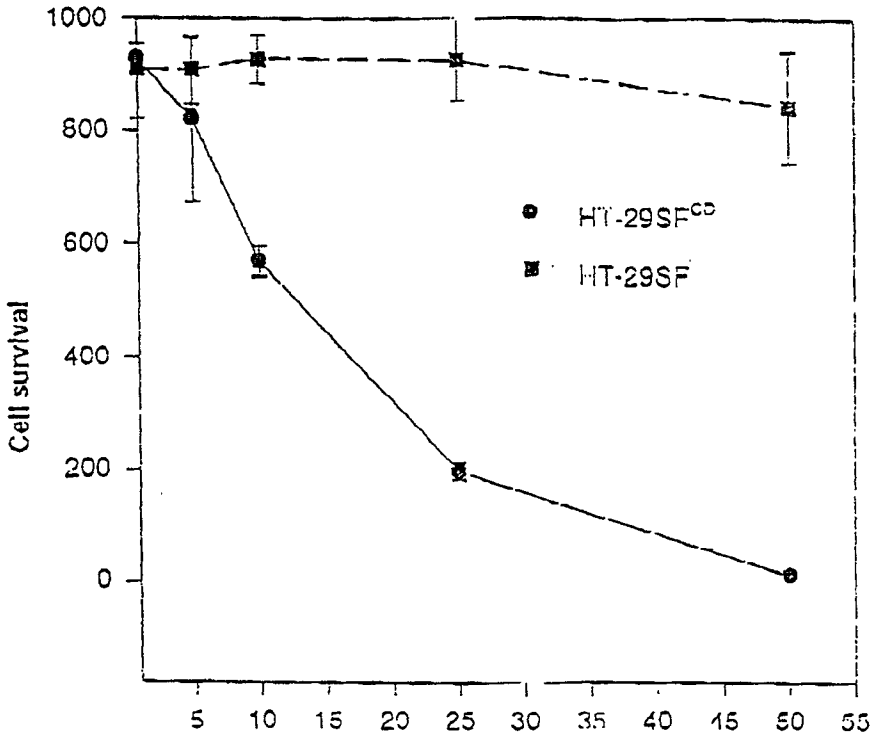
47. The method of claims 31 to 46, wherein the non-human animal is selected from the group consisting of animals in the care of a veterinarian.

48. The method of claims 31 to 47, for treating a cancer associated with cytidine deaminase or deoxycytidine deaminase over-expression, wherein the agent is administered in an anti-cancer effective amount.

49. The method of claims 31 to 47, for preventing or treating an HIV infection, wherein the agent is comprising administered in an anti-HIV effective amount.

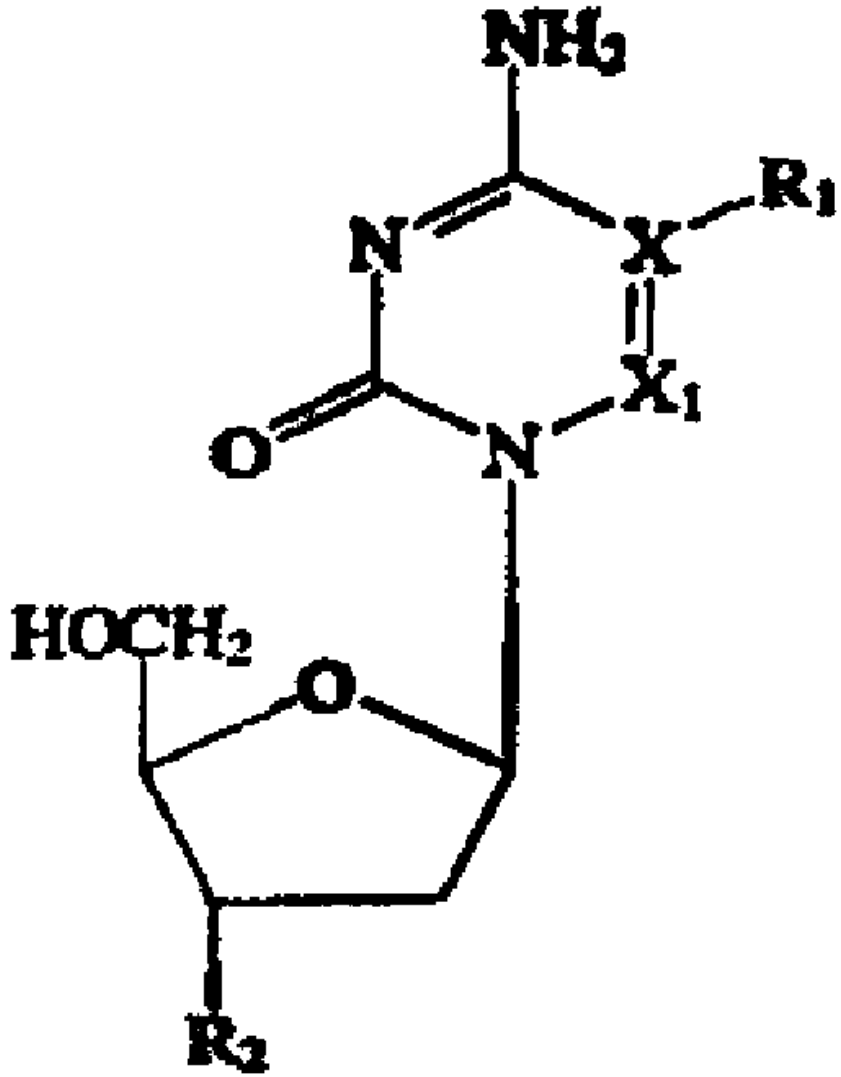
50. The method of claims 51 to 49, for reducing the number or toxicity of proinflammatory cells which over-express cytidine deaminase or deoxycytidine deaminase, wherein the agent is administered in an anti-inflammation effective amount.

Dose response curve for 5-mAZC in HT-29SF^{CD} and HT-29SF cells



HT-29SF^{CD} cells are a subline of HT-29SF human colonic adenocarcinoma cells which were selected in this laboratory for overexpression of cytidine deaminase. HT-29SF were plated in the bottom compartment, and HT-29SF^{CD} in the top compartment of a "Transwell" culture dish (1,000 cells each). A semipermeable membrane separates the two compartments of the Transwell, such that drugs administered in the tissue culture media bathe both compartments equally. 5-mAZC was administered continuously for 72 h at the indicated concentrations.

FIGURE 1



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