Commonwealth of Australia
The Patents Act 1952

DECLARATION IN SUPPORT

In support of the (Convention) Application made by:
SRI INTERNATIONAL
333 Ravenswood Avenue, Menlo Park, California 94025, United States of America

for a patent for an invention entitled:
1,2,4-BENZOTRIAZINE OXIDES AS RADIOSENSITIZERS AND SELECTIVE CYTOTOXIC AGENTS

I (~~We~~) Richard P. Lange

of and care of the applicant company do solemnly and sincerely declare as follows:

---

a) ~~I am (We are) the applicant(s) for the patent.~~
or
b) I am (~~We are~~) authorised by the applicant(~~s~~) for the patent to make this declaration on its behalf.

Delete the following if not a Convention Application.

The basic application(~~s~~) as defined by section 141 (~~142)~~ of the Act was (~~were~~) made

on 18 March 1988 in United States of America

on ~~15 March 1989~~ in ~~Patent Cooperation Treaty~~

on in

by William W. Lee, J. Martin Brown, Edward W. Grange, Abelardo P. Martinez & Michael Tracy

The basic application(~~s~~) referred to in this paragraph is (~~are~~) the first application(s) made in a Convention country in respect of the invention the subject of the application.

---

a) ~~I am (We are) the actual inventor(s) of the invention~~.

or
b) William W. Lee 991 N. California Avenue, Palo Alto, California 94303;
J. Martin Brown 33 Peter Coutts Circle, Stanford, California 94305;
Edward W. Grange 3480 Waverley Street, Palo Alto, Califonria 94306;
Abelardo P. Martinez 1111 Beaumont Drive, San Jose, California 94306;
Michael Tracy 2495 Chabot Terrace, Palo Alto, California 94303, all in the

~~is~~ (are) the actual inventor(s) of the invention and the facts upon which United States of America the applicant is (~~are~~) entitled to make the application are as follows:

by virtue of assignment dated 17 March 1988 from said Inventors, the applicant is the assignee of the invention from the said actual inventors.

Declared at Menlo Park, California United States of America this 20 day of December 19 90

Signed Richard P. Lange Status Patent Counsel

Declarant's Name Richard P. Lange

F. B. RICE & CO PATENT ATTORNEYS

This form is suitable for any type of Patent Application. No legalisation required.

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(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 637572

(54) Title
1,2,4-BENZOTRIAZINE OXIDES AS RADIOSENSITIZERS AND SELECTIVE CYTOTOXIC AGENTS

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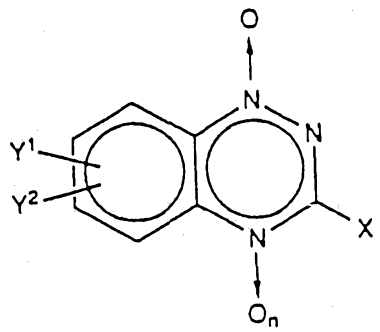
(56) Prior Art Documents
WO 88/02366
EP 0001090
FR 2322140

(57) Claim

5. A compound having the structural formula:



wherein X is H or a hydocarbyl (1-4C);

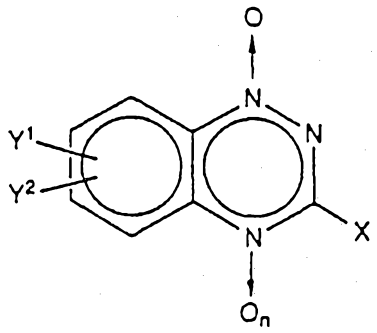
n is 1; and

Y^1 and Y^2 are independently either H; halogen; hydrocarbyl (1-14C) including cyclic and unsaturated

.../2

hydrocarbyl, if hydrocarbyl, either unsubstituted or substituted with 1 or 2 substituents selected from the group consisting of halogen, hydroxy, epoxy, alkoxy (1-4C), alkylthio (1-4C), primary amino (NH_2), morpholino, acyloxy (1-4C), acylamido (1-4C) and thio analogs thereof, carboxy, alkoxycarbonyl (1-4C), carbamyl, alkylcarbamyl (1-4C), and wherein the hydrocarbyl may be interrupted by a single ether (-O-) linkage; or wherein Y^1 and Y^2 are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR')R' in which R' is a hydrocarbyl as defined above, or a pharmacologically acceptable salt of said compound.

1. A method of selectively killing hypoxic tumor cells comprising administering to said cells a pharmaceutical composition comprising a compound of the formula



wherein X is H or a hydrocarbyl (1-4C);

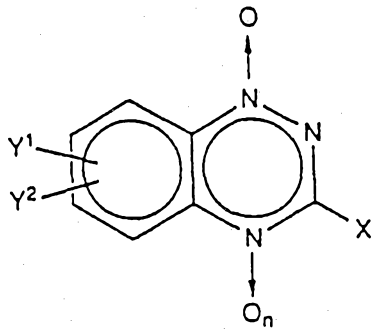
n is 1; and

Y^1 and Y^2 are independently either H; halogen; hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, if hydrocarbyl, either unsubstituted or substituted with 1 or 2 substituents selected from the group consisting of halogen, hydroxy, epoxy, alkoxy

(1-4C), alkylthio (1-4C), primary amino (NH_2), morpholino, acyloxy (1-4C), acylamido (1-4C) and thio analogs thereof, carboxy, alkoxycarbonyl (1-4C), carbamyl, alkylcarbamyl (1-4C), and wherein the hydrocarbyl may be interrupted by a single ether (-O-) linkage; or wherein Y^1 and Y^2 are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR')R' in which R' is a hydrocarbyl as defined above,

or a pharmacologically acceptable salt of said compound.

2. A method of selectively killing hypoxic tumor cells comprising administering to said cells a pharmaceutical composition comprising a compound of the formula



wherein X is H or hydrocarbyl (1-4C), and, if hydrocarbyl, may be substituted with OH, NH_2, alkoxy (1-4C), or halogen substituents;

n is 1; and

Y^1 and Y^2 are independently either H; halogen; hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, if hydrocarbyl, either unsubstituted or substituted with 1 or 2 substituents selected from the group consisting of halogen, hydroxy, epoxy, alkoxy (1-4C), alkylthio (1-4C), primary amino (NH_2), morpholino, acyloxy (1-4C), acylamido (1-4C) and thio analogs thereof, carboxy, alkoxycarbonyl (1-4C),

carbamyl, alkylcarbamyl (1-4C), and wherein the hydrocarbyl may be interrupted by a single ether (-O-) linkage; or wherein Y^1 and Y^2 are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR')R' in which R' is a hydrocarbyl as defined above, or a pharmacologically acceptable salt of said compound.

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(54) Title: 1,2,4-BENZOTRIAZINE OXIDES AS RADIOSENSITIZERS AND SELECTIVE CYTOTOXIC AGENTS

(57) Abstract

A method of using 1,2,4-benzotriazine oxides as radiosensitizers and selective cytotoxic agents is disclosed. The compounds are shown to specifically radiosensitive hypoxic tumor cells and are additionnally disclosed to be useful as specific cytotoxic agents for these cells.

1,2,4-BENZOTRIAZINE OXIDES AS RADIOSENSITIZERS AND SELECTIVE CYTOTOXIC AGENTS

Reference to Government Grant or Contract

The invention described herein was made in the course of work under grant or contract form the Department of health and Human Services. The Government has certain rights in this invention.

Technical Field

The invention relates to cytotoxic agents and radiotherapy effective against hypoxic cells. Specifically, the invention relates to selectively killing tumor cells and to sensitizing tumor cells to radiation using 1,2,4-benzotriazine oxides.

Background Art

Hypoxic cell radiosensitizers are compounds that selectively increase the sensitivity of hypoxic cells to destructive radiation. Cytotoxins which have enhanced activity under hypoxic conditions also provide a means for selective destruction of cells under low oxygen pressure. This specificity for hypoxic cells is important because it is tumors that are typically characterized by such cells. Virtually all tumors which

~~1,2,4-BENZOTRIAZINE OXIDES AS~~ RADIOSENSITIZERS AND SELECTIVE CYTOTOXIC AGENTS

The herein application is a continuation-in-part of U.S. Application Serial No. 911,906, filed 25 September 1986.

Reference to Government Grant or Contract

The invention described herein was made in the course of work under grant or contract from the Department of Health and Human Services. The Government has certain rights in this invention.

Technical Field

The invention relates to cytotoxic agents and radiotherapy effective against hypoxic cells. Specifically, the invention relates to selectively killing tumor cells and to sensitizing tumor cells to radiation using 1,2,4-benzotriazine oxides.

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Hypoxic cell radiosensitizers are compounds that selectively increase the sensitivity of hypoxic cells to destructive radiation. Cytotoxins which have enhanced activity under hypoxic conditions also provide a means for selective destruction of cells under low oxygen pressure. This specificity for hypoxic cells is important because it is tumors that are typically ~~characterized by such cells. Virtually all tumors which~~

are present as solid masses contain these cells, while normal cells generally have an adequate supply of oxygen. Accordingly, anti-tumor agents can be made selective for tumors by virtue of high activity under hypoxic conditions, and radiation can be employed more effectively in the presence of these sensitizers.

Of course, the use of radiation treatment to destroy tumor cells is only practical if damage to the surrounding normal tissue can be minimized or avoided. The effects of radiation are enhanced by the presence of oxygen, and it is established that as the dose of radiation is increased, the effectiveness of the radiation in destroying target cells is enhanced most dramatically when oxygen is present. Therefore, selectivity for tumor cells toward radiation is difficult to achieve -- normal cells, in view of their oxygen supply, are generally more susceptible to radiation than the target tumor cells. It is therefore desirable to provide a means of sensitizing tumor cells, but not the surrounding tissue, to radiation treatment. One solution would be to increase the supply of oxygen to these tumor cells. This, however, has proved difficult to do.

Various heterocyclic compounds and in particular those with oxidized nitrogen moieties, have been used to radiosensitize hypoxic tumor cells. Indeed, it has been postulated that the oxidized nitrogen functionality is responsible for this activity. Nitroimidazoles, particularly misonidazole (MIS) and metronidazole have been studied extensively, and MIS is commonly used as a standard in in vitro and in vivo tests for radiosensitizing activity. (See, e.g., Asquith, et al, Radiation Res (1974) 60:108-118; Hall,

et al, Brit J Cancer (1978) 37: 567-569; Brown, et al, Radiation Res (1980) 82:171-190; and U.S. patent 4,371,540. The radiosensitizing activities of certain 1-substituted 3(5)-nitro-s-triazoles and of various quinoxaline-1,4-dioxide derivatives have also been disclosed.

In addition, US Serial Nos. 730,761, filed 3 May 1985, and 788,762, filed 18 October 1985 assigned to the same assignee and incorporated by reference disclose a group of radiosensitizers that do not contain oxidized nitrogen -- the substituted benzamides and nicotinamides and their thio analogs. These compounds, nevertheless, are radiosensitizers. It is important to distinguish the ability to sensitize hypoxic cells selectively, for instance, by enhancing their oxygen supply, from another mechanism commonly encountered for "sensitizing" cells: inhibition of the enzyme poly(ADP-ribose)polymerase, which is believed to be essential in the repair of irradiated cells after radiation. This repair mechanism is operative in both hypoxic tumor cells and in normal cells. Hence, administration of "radiosensitizers" which operate according to this latter mechanism does not accomplish the desired purpose of selectively sensitizing the target tumor cells.

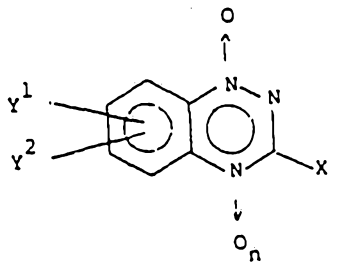
A group of compounds which has not previously been suggested for use in either selectively killing hypoxic cells or in radiosensitizing such cells is 3-amino-1,2,4-benzotriazine 1,4-di-N-oxide and related compounds. Related US patents 3,980,779; 3,868,371; and 4,001,410 disclose the preparation of a group of these compounds and their use as anti-microbial agents, particularly by addition of these materials to livestock fodder. US patents 3,991,189 and 3,957,799 disclose

derivatives of these compounds bearing substituents on the nitrogen of the 3-amino group. These compounds also have anti-microbial activity.

The present invention provides additional compounds which specifically radiosensitize hypoxic cells in vitro and which, furthermore, are directly cytotoxic to hypoxic cells both in vitro and in vivo. Therefore, administration of these compounds prior to or following radiation treatment of tumors selectively kills the hypoxic (tumor) cells which survive the radiation dose. Both the ability of these compounds to radiosensitize hypoxic cells in vitro and especially their ability to selectively kill hypoxic cells directly are unexpected properties of these compounds.

Disclosure of the Invention

The invention provides a valuable addition to the group of compounds currently available as selective radiosensitizers and selective cytotoxic agents for hypoxic tumor cells. Some of the compounds useful in this regard are known compounds, others are novel. One aspect of the invention, therefore, is a method of radiosensitizing or selectively killing hypoxic tumor cells with a compound of the formula:



wherein X is H, hydrocarbyl (1-4C), OH, OR, NH_2, NHR or NR_2 where each R is independently an alkyl of 1-4 carbon atoms, an amide, or a morpholino moiety

and may further be substituted with hydroxy, alkoxy, amino, or halogeno substituents;

wherein n is 0 or 1; and

Y^1 and Y^2 are independently either H, halogeno, hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, optionally substituted with 1 or 2 substituents selected from the group consisting of halogeno, hydroxy, epoxy, alkoxy, alkylthio, amino (including morpholino), acyloxy, acylamido and their thio analogs, alkylsulfonyl, alkylphosphonyl, carboxy, alkoxycarbonyl, carbamyl or alkylcarbamyl, and wherein the hydrocarbyl can optionally be interrupted by a single ether (-O-) linkage, or wherein Y^1 and Y^2 are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR)R' in which R' is a hydrocarbyl optionally substituted as defined above.

The compounds of the invention, therefore, are the mono- or dioxides of optionally substituted 1,2,4-benzotriazine which may contain a hydrocarbyl (1-4C), hydroxyl or amino group, either substituted or unsubstituted, in the 3 position. While all of the compounds defined by Formula 1 are generally effective as radiosensitizers, only compounds unsubstituted at the 3-position or having a 3-amino or 3-hydrocarbyl (1-4C) substituent (i.e., X=H, hydrocarbyl (1-4C), NH_2, NHR or NR_2 with R as defined above) and which are di-N-oxides (n=1) are effective cytotoxic agents.

Certain of the compounds encompassed by Formula 1 are already known in the art as being useful for other purposes; other compounds are novel. The novel compounds encompassed by the present invention and which may be prepared by methods disclosed herein include compounds represented by the formula above, in

the following three classes: I. X is OH, OR, or NR_2 with R as defined above, n is 0 or 1, and Y^1 and Y^2 are as defined above; II. X is NH_2 or NHR with R as defined above, n is 0, and Y^1 and Y^2 are as defined above; III. X is NH_2, n is 1, and Y^1 and Y^2 are as defined above but not halogeno, saturated alkyl (1-6C) unsubstituted or halogen-substituted, alkoxy (1-6C), carbamyl, carboxy or carboalkoxy (1-6C); IV. X is H or hydrocarbyl (1-4C), n is 1, and Y^1 and Y^2 are as defined above, with the proviso that when Y^1 and Y^2 are H, X is other than methyl.

Brief Description of the Drawings

Figures 1A, 1B and 1C show the selective cytotoxicity of 3-amino-1,2,4-benzotriazine 1,4-dioxide for hypoxic cells derived from hamster, mouse and human tissues.

Figure 2 shows the in vivo efficacy of 3-amino-1,2,4-benzotriazine 1,4-dioxide in enhancing the killing of tumor cells when combined with radiation.

Figure 3 shows the killing of tumor cells in vivo by 3-amino-1,2,4-benzotriazine 1,4-dioxide when the tumor has been made hypoxic by the intraperitoneal administration of the antihypertensive drug hydralazine.

Modes of Carrying Out the Invention

A. The Compounds Useful in the Invention

The compounds useful in radiosensitizing hypoxic tumor cells as described herein are derivatives of 1,2,4-benzotriazine oxide.

The hydrocarbyl group represented by Y^1 or Y^2 may contain 1-14 carbon atoms, may be saturated or

unsaturated, cyclic or acyclic, and may optionally be interrupted by a single ether linkage. Thus, the unsubstituted form of Y^1 or Y^2 can be, for example, methyl, ethyl, n-propyl, s-butyl, n-hexyl, 2-methyl-n-pentyl, 2-ethoxyethyl, 3-(n-propoxy)-n-propyl, 4-methoxybutyl, cyclohexyl, tetrahydrofurfuryl, furfuryl, cyclohexenyl, 3-(n-decyloxy)-n-propyl, 4-methyloctyl, 4.7-dimethyloctyl, and the like.

The hydrocarbyl may be substituted with one or two substituents as follows: The halogeno substituents are fluoro, chloro, bromo, or iodo. The alkoxy substituents represented by OR' may contain 1 to 4 carbon atoms, and include, for example, methoxy, n-propoxy, and t-butoxy. The amino substituent may be NH_2, NHR or NR_2, where each R is independently an alkyl of 1-4 carbons or a morpholino moiety. R may optionally be substituted with 1-2 hydroxy, alkoxy, amino, or halogeno substituents.

The acyloxy and acylamido groups are represented by R'COO- and R'CONH-, respectively, where R' contains 1-4 carbons, and their thio analogs are represented by R'CSO- and R'CSNH-. Alkyl sulfonyl and alkyl phosphonyl are, respectively, $R'SO_2$ and R'P(OR')O- wherein each R' is independently as above defined. Carboxy is the group -C(O)OH; alkoxycarbonyl is -C(O)OR'; carbamyl is $-C(O)NH_2$; and alkylcarbamyl is -C(O)NHR'.

Where X is OH, of course, the compounds may also be prepared and used as the pharmaceutically acceptable salts formed from inorganic bases, such as sodium, potassium, or calcium hydroxide, or from organic bases, such as caffeine, ethylamine, and lysine.

When X is NH_2, pharmaceutically acceptable acid addition salts may be used. These salts are those with inorganic acids such as hydrochloric, hydrobromic or phosphoric acids or organic acids such as acetic acid, pyruvic acid, succinic acid, mandelic acid, p-toluene sulfonic acid, and so forth. (Amino substituents on the hydrocarbyl side chain can also, of course, be converted to salts.)

The 1,2,4-benzotriazine may be used as the mono- or dioxide. Either the 1-nitrogen of the triazino ring may be oxidized, or both the 1-and 4-nitrogens may be oxidized.

Specific particularly preferred compounds which are useful in the radiosensitization and cytotoxic procedures of the invention include

3-hydroxy-1,2,4-benzotriazine 1-oxide;
3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;
3-amino-1,2,4-be zotriazine 1-oxide;
3-amino-1,2,4-benzotriazine 1,4-di-oxide;
6(7)-methoxy-3-hydroxy-1,2,4-benzotriazine 1-oxide;
6(7)-methoxy-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;
6(7)-methoxy-3-amino-1,2,4-benzotriazine 1-oxide;
6(7)-methoxy-3-amino-1,2,4-benzotriazine 1,4-dioxide;
6(7)-ethoxy-3-hydroxy-1,2,4-benzotriazine 1-oxide;
6(7)-ethoxy-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;
6(7)-ethoxy-3-amino-1,2,4-benzotriazine 1-oxide;
6(7)-ethoxy-3-amino-1,2,4-benzotriazine 1,4-dioxide;
6(7)-[4-acetamido-n-butanoxy]-3-hydroxy-1,2,4-benzotriazine 1-oxide;
6(7)-[4-acetamido-n-butanoxy]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;
6(7)-[4-acetamido-n-butanoxy]-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-[4-acetamido-n-butanoxy]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[1-(2,3-dihydroxy)propoxy]-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-[1-(2,3-dihydroxy)propoxy]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[1-(2,3-dihydroxy)propoxy]-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-[1-(2,3-dihydroxy)propoxy]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[(2-furyl)methylamino]-3-hydroxy-1,2,4-benzotriazinel-oxide;

6(7)-[(2-furyl)methylamino]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[(2-furyl)methylamino]-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-[(2-furyl)methylamino]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-(2-methoxyethylamino)-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-(2-methoxyethylamino)-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-(2-methoxyethylamino)-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-(2-methoxyethylamino)-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-carbethoxymethoxy-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-carbethoxymethoxy-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-carbethoxymethoxy-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-carbethoxymethoxy-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[(2-methoxyethyl)carbamylmethoxy]-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-[(2-methoxyethyl)carbamylmethoxy]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[(2-methoxyethyl)carbamylmethoxy]-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-[(2-methoxyethyl)carbamylmethoxy]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[(2-hydroxyethyl)carbamylmethoxy]-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-[(2-hydroxyethyl)carbamylmethoxy]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[(2-hydroxyethyl)carbamylmethoxy]-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-[(2-hydroxyethyl)carbamylmethoxy]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[1-(2-hydroxy-3-morpholino)propoxy]-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-[1-(2-hydroxy-3-morpholino)propoxy]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[1-(2-hydroxy-3-morpholino)propoxy]-3-amino-1,2,4 benzotriazine 1-oxide;

6(7)-[1-(2-hydroxy-3-morpholino)propoxy]-3-amino-1,2,4 benzotriazine 1,4-dioxide;

6(7)-[3-amino-n-propoxy]-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-[3-amino-n-propoxy]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[3-amino-n-propoxy]-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-[3-amino-n-propoxy]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[2,3-epoxypropoxy]-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-[2,3-epoxypropoxy]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[2,3-epoxypropoxy]-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-[2,3-epoxypropoxy]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[3-methoxy-2-hydroxy-n-propoxy]-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-[3-methoxy-2-hydroxy-n-propoxy]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[3-methoxy-2-hydroxy-n-propoxy]-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-[3-methoxy-2-hydroxy-n-propoxy]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[4-ethoxy-3-hydroxy-n-butoxy]-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-[4-ethoxy-3-hydroxy-n-butoxy]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[4-ethoxy-3-hydroxy-n-butoxy]-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-[4-ethoxy-3-hydroxy-n-butoxy]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[3,4-dihydroxy-n-butoxy]-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-[3,4-dihydroxy-n-butoxy]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[3,4-dihydroxy-n-butoxy]-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-[3,4-dihydroxy-n-butoxy]-3-amino-1,2,4-benzotriazine 1,4-dioxide;
6(7)-methyl-3-hydroxy-1,2,4-benzotriazine 1-oxide;
6(7)-methyl-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;
6(7)-methyl-3-amino-1,2,4-benzotriazine 1-oxide;
6(7)-methyl-3-amino-1,2,4-benzotriazine 1,4-dioxide;
6(7)-ethyl-3-hydroxy-1,2,4-benzotriazine 1-oxide;
6(7)-ethyl-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;
6(7)-ethyl-3-amino-1,2,4-benzotriazine 1-oxide;
6(7)-ethyl-3-amino-1,2,4-benzotriazine 1,4-dioxide;
6(7)-chloroacetamido-3-hydroxy-1,2,4-benzotriazine 1-oxide;
6(7)-chloroacetamido-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;
6(7)-chloroacetamido-3-amino-1,2,4-benzotriazine 1-oxide;
6(7)-chloroacetamido-3-amino-1,2,4-benzotriazine 1,4-dioxide;
6(7)-[(2-hydroxyethyloxy)acetamido]-3-hydroxy-1,2,4-benzotriazine 1-oxide;
6(7)-[(2-hydroxyethyloxy)acetamido]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;
6(7)-[(2-hydroxyethyloxy)acetamido]-3-amino-1,2,4-benzotriazine 1-oxide;
6(7)-[(2-hydroxyethyloxy)acetamido]-3-amino-1,2,4-benzotriazine 1,4-dioxide;
6,7-dimethoxy-3-hydroxy-1,2,4-benzotriazine 1-oxide;
6,7-dimethoxy-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;
6,7-dimethoxy-3-amino-1,2,4-benzotriazine 1-oxide;
6,7-dimethoxy-3-amino-1,2,4-benzotriazine 1,4-dioxide;
6,7-diethoxy-3-hydroxy-1,2,4-benzotriazine 1-oxide;
6,7-diethoxy-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;
6,7-diethoxy-3-amino-1,2,4-benzotriazine 1-oxide;

6,7-diethoxy-3-amino-1,2,4-benzotriazine 1,4-dioxide;
6(7)-propionyl-3-hydroxy-1,2,4-benzotriazine 1-oxide;
6(7)-propionyl-3-hydroxy-1,2,4-benzotriazine
1,4-dioxide;
6(7)-propionyl-3-amino-1,2,4-benzotriazine 1-oxide;
6(7)-propionyl-3-amino-1,2,4-benzotriazine 1,4-dioxide;
6(7)-(2-acetoxyethoxy)-3-hydroxy-1,2,4-benzotriazine
1-oxide;
6(7)-(2-acetoxyethoxy)-3-hydroxy-1,2,4-benzotriazine
1,4-dioxide;
6(7)-(2-acetoxyethoxy)-3-amino-1,2,4-benzotriazine
1-oxide;
6(7)-(2-acetoxyethoxy)-3-amino-1,2,4-benzotriazine
1,4-dioxide;
6(7)-n-hexyloxy-3-hydroxy-1,2,4-benzotriazine 1-oxide;
6(7)-n-hexyloxy-3-hydroxy-1,2,4-benzotriazine
1,4-dioxide;
6(7)-n-hexyloxy-3-amino-1,2,4-benzotriazine 1-oxide;
6(7)-n-hexyloxy-3-amino-1,2,4-benzotriazine 1,4-dioxide;
6(7)-ethylamino-3-hydroxy-1,2,4-benzotriazine 1-oxide;
6(7)-ethylamino-3-hydroxy-1,2,4-benzotriazine
1,4-dioxide;
6(7)-ethylamino-3-amino-1,2,4-benzotriazine 1-oxide;
6(7)-ethylamino-3-amino-1,2,4-benzotriazine 1,4-dioxide;
6(7)-(2-methoxyethoxy)-3-hydroxy-1,2,4-benzotriazine
1-oxide;
6(7)-(2-methoxyethoxy)-3-hydroxy-1,2,4-benzotriazine
1,4-dioxide;
6(7)-(2-methoxyethoxy)-3-amino-1,2,4-benzotriazine
1-oxide;
6(7)-(2-methoxyethoxy)-3-amino-1,2,4-benzotriazine
1,4-dioxide;

6(7)-(aminoacetamido)-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-(aminoacetamido)-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-(aminoacetamido)-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-(aminoacetamido)-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-(carbamylmethoxy)-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-(carbamylmethoxy)-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-(carbamylmethoxy)-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-(carbamylmethoxy)-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-(carboxymethoxy)-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-(carboxymethoxy)-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-(carboxymethoxy)-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-(carboxymethoxy)-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[1,2-dihydroxyethyl]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[1-(3-ethylamino-2-hydroxypropoxy)]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[2-ethylamino-1-hydroxyethyl]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[2-hydroxyethyl]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[1-hydroxyethyl]-3-amino-1,2,4-benzotriazine 1,4-dioxide;
3-(2-hydroxyethylamino)-1,2,4-benzotriazine 1-oxide;
3-(2-hydroxyethylamino)-1,2,4-benzotriazine 1,4-dioxide;
6(7)-chloro-3-(2-hydroxyethylamino)-1,2,4-benzotriazine 1-oxide;
6(7)-chloro-3-(2-hydroxyethylamino)-1,2,4-benzotriazine 1,4-dioxide;
3-(1-hydroxyethylamino)-1,2,4-benzotriazine 1-oxide;
3-(1-hydroxyethylamino)-1,2,4-benzotriazine 1,4-dioxide;
1,2,4-benzotriazine 1-oxide;
1,2,4-benzotriazine 1,4-dioxide;
3-methyl-1,2,4-benzotriazine 1,4-dioxide;
3-ethyl-1,2,4-benzotriazine 1,4-dioxide;
3-propyl-1,2,4-benzotriazine 1,4-dioxide;
6(7)-amino-1,2,4-benzotriazine 1,4-dioxide;
6(7)-amino-3-methyl-1,2,4-benzotriazine 1,4-dioxide;
6(7)-amino-3-ethyl-1,2,4-benzotriazine 1,4-dioxide;
6(7)-methoxy-1,2,4-benzotriazine 1,4-dioxide;
6(7)-methoxy-3-methyl-1,2,4-benzotriazine 1,4-dioxide;
6(7)-[1-(2,3-dihydroxypropoxy]-1,2,4-benzotriazine 1,4-dioxide;
6(7)-[1,2-dihydroxyethyl]-1,2,4-benzotriazine 1,4-dioxide;
6(7)-[1-(3-ethylamino-2-hydroxypropoxy)]-1,2,4-benzotriazine 1,4-dioxide;
6(7)-[2-ethylamino-1-hydroxyethyl]-1,2,4-benzotriazine 1-4 dioxide;
6(7)-chloro-1,2,4-benzotriazine 1,4-dioxide;
6(7)-[2-hydroxyethyl]-1,2,4-benzotriazine 1,4-dioxide;
6(7)-[1-hydroxyethyl]-1,2,4-benzotriazine 1,4-dioxide;

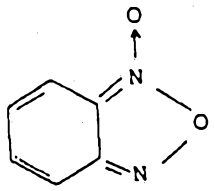
and their pharmaceutically acceptable salts and the thioamide analogs of the foregoing list of compounds. It should be noted that the "Y^1 or Y^2" substituents set forth in most of the above compounds as present in either the 6 or 7 positions (designated "6(7)") or in both the 6 and 7 positions (designated "6,7") may also be present at the 5 and/or 8 ring positions.

Of the above compounds useful in the method of the present invention as selective cytotoxic agents or radiosensitizers, the following compounds are novel: compounds given by the formula above wherein I. X is OH, OR, or NR_2, where each R is independently an alkyl of 1-4 carbon atoms, an amide, or a morpholino moiety and may further be substituted with hydroxy, alkoxy, amino, or halogeno substituents, n is 0 or 1, and Y^1 and Y^2 are independently either H, halogeno, hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, optionally substituted with 1 or 2 substituents selected from the group consisting of halogeno, hydroxy, epoxy, alkoxy, alkylthio, amino (including morpholino), acyloxy, acylamido and their thio analogs, alkylsulfonyl, alkylphosphonyl, carboxy, alkoxycarbonyl, carbamyl or alkylcarbamyl, and wherein the hydrocarbyl can optionally be interrupted by a single ether (-O-) linkage, or wherein Y^1 and Y^2 are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR)R' in which R' is a hydrocarbyl optionally substituted as defined above; II. X is NH_2 or NHR with R as defined above, n is 0, and Y^1 and Y^2 are as defined in I; III. X is NH_2, n is 1, and Y^1 and Y^2 are independently either H, hydrocarbyl (7-14C; saturated or unsaturated), unsaturated hydrocarbyl (1-6C), either hydrocarbyl substituent being either unsubstituted or substituted

with halogen, hydroxy, epoxy, alkoxy, alkylthio, amino (including morpholino), acyloxy, acylamido and their thio analogs, alkylsulfonyl or alkylphosphonyl, and wherein the hydrocarbyl can optionally be interrupted by a single ether (-O-) linkage, or wherein Y^1 and Y^2 are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR)R' in which R' is a hydrocarbyl optionally substituted as defined above; IV. X is H or hydrocarbyl (1-4C), n is 1, and Y^1 and Y^2 are as defined above, with the proviso that when Y^1 and Y^2 are H, X is other than methyl.

B. Preparation of the Compounds of the Invention

General methods for preparing some 3-amino derivatives are found in the above reference patents to Ley et al., for example US 3,980,779. The compounds are prepared from benzofuroxan of the formula:



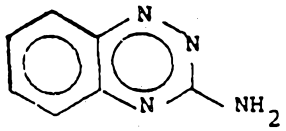
by reaction with a salt of cyanamide, followed by acidification of the reaction mixture. The benzofuroxan starting material is not symmetric with respect to its own 5 and 6 positions (which are the 6 and 7 positions of the resulting 3-amino benzotriazine oxide). Therefore, a mixture of the 6- and 7-substituted materials may result. If desired, this mixture can be separated using conventional means into individual components having a substituent in either the 6 or 7 position.

The dioxide may also be prepared from the parent monoxide or 1,2,4-benzotriazine by peracid oxidation (see Robbins et al, J Chem Soc 3186 (1957) and Mason et al, J Chem Soc B 911 (1970)).

In addition, the monoxide may be prepared by:

(1) cyclization of a 1-nitro-2-aminobenzene compound using H_2NCN;

(2) oxidation of the parent compound given by the structure

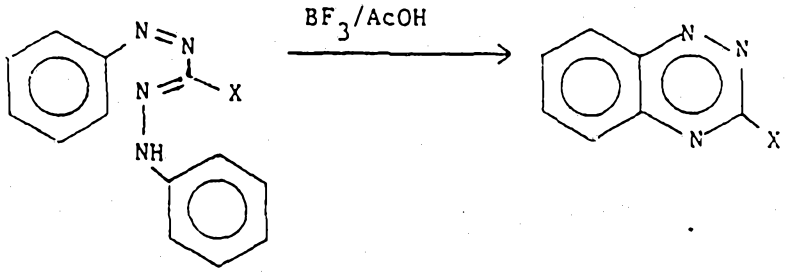


or by controlled reduction of the corresponding dioxide (see Mason, supra, and Wolf et al, J Am Chem Soc 76:355 (1954)).

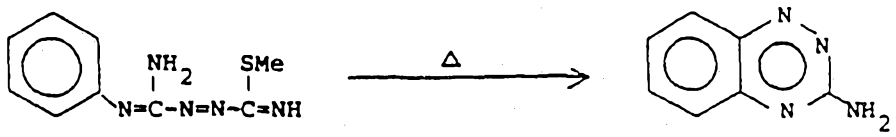
The 1,2,4-benzotriazines may be prepared by cyclization of formazan precursors using BF_3/AcOH (see Scheme I and Atallah and Nazer, Tetrahedron 38:1793 (1982)).

3-amino-1,2,4-benzotriazines may be prepared either by cyclization of a parent compound (see Scheme II and Arndt, Chem. Ber. 3522 (1913)) or by reduction of the monoxide or dioxide as above.

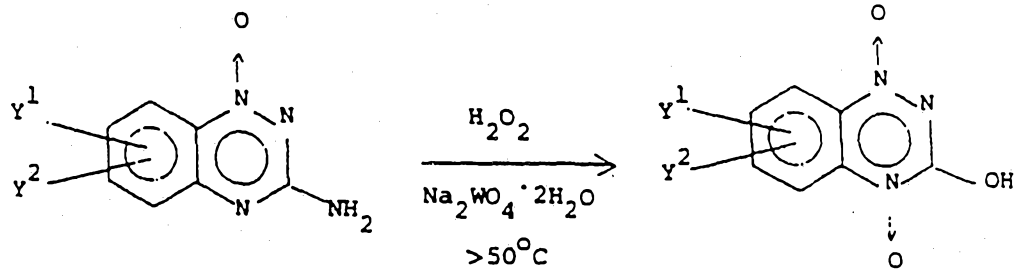
The 3-hydroxy-1,2,4-benzotriazine oxides may be prepared using peroxide and and tungsten oxide (Scheme III), a novel synthetic procedure for making the 3-hydroxy-1,4-dioxide compound, or concentrated sulfuric acid and sodium nitrate (Scheme IV).



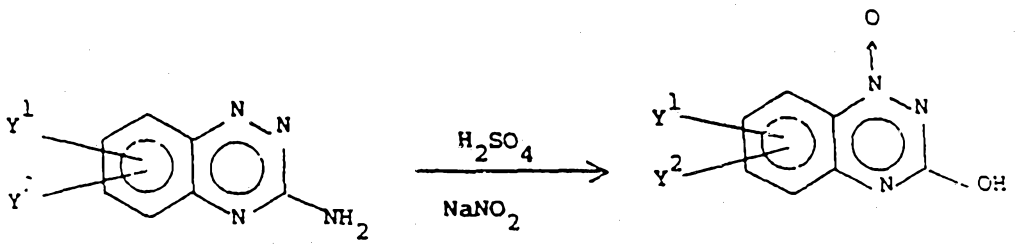
Scheme I



Scheme II



Scheme III



Scheme IV

C. Formulation and Administration

As demonstrated below, the oxidized benzotriazines of the invention may be used to radiosensitize or selectively kill hypoxic tumor cells in warm-blooded animal hosts. A way in which they may be used is in conjunction with agents known to selectively create hypoxia in tumors. Such methods include the use of antihypertensive drugs such as hydralazine, or agents which affect the amount of oxygen carried by the blood. While these compounds will typically be used in cancer therapy of human patients, they may be used to kill hypoxic tumor cells in other warm blooded animal species such as other primates, farm animals such as cattle, and sports animals and pets such as horses, dogs, and cats.

Hypoxia is believed to be associated with all types of solid malignant neoplasms. The compounds of the invention may, therefore, be used to radiosensitize or to kill neoplastic epithelial cells, endothelial cells, connective tissue cells, bone cells, muscle cells, nerve cells, and brain cells. Examples of carcinomas and sarcomas include carcinomas such as epithelial cell, acidic cell, alveolar cell, basal cell, basal squamous cell, cervical, renal, liver, Hurthle,

Lucke, mucinous and Walker, and sarcomas such as Abernathy's, alveolar soft part, angiolithic, botyroid, encephaloid, endometria stroma, Ewing's fascicular, giant cell, lymphatic, Jensen's, juxtacortical osteogenic, Kaposi's, medullary, and synovial. Specific examples of tumors that have been sensitized with other radiosensitizers are reported in Adams, G.E., Cancer: A Comprehensive Treatise (F. Becker, Ed) vol 6, pp 181-223, Plenum, New York, 1977.

The compounds may be administered to patients orally or parenterally (intravenously, subcutaneously, intramuscularly, intraspinally, intraperitoneally, and the like). When administered parenterally the compounds will normally be formulated in a unit dosage injectable form (solution, suspension, emulsion) with a pharmaceutically acceptable vehicle. Such vehicles are typically nontoxic and nontherapeutic. Examples of such vehicles are water, aqueous vehicles such as saline, Ringer's solution, dextrose solution, and Hank's solution and nonaqueous vehicles such as fixed oils (e.g., corn, cottonseed, peanut, and sesame), ethyl oleate, and isopropyl myristate. Sterile saline is a preferred vehicle and the compounds are sufficiently water soluble to provide a solution for all foreseeable needs. The vehicle may contain minor amounts of additives such as substances that enhance solubility, isotonicity, and chemical stability, e.g., antioxidants, buffers, and preservatives. When administered orally (or rectally) the compounds will usually be formulated into a unit dosage form such as a tablet, capsule, suppository or cachet. Such formulations typically include a solid, semisolid or liquid carrier or diluent. Exemplary diluents and vehicles are lactose, dextrose,

sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, mineral oil, cocoa butter, oil of theobroma, aginates, tragacanth, gelatin, syrup, methylcellulose, polyoxyethylene sorbitan monolaurate, methyl hydroxybenzoate, propyl hydroxybenzoate, talc, and magnesium stearate.

The amount of compound administered to the subject is sufficient to radiosensitize or to produce cytotoxicity in the malignant neoplasm to be treated but below that which may elicit toxic effects. This amount will depend upon the type of tumor, the species of the subject being treated, the indication dosage intended and the weight or body surface of the subject. The radiation may be administered to humans in a variety of different fractionation regimes, i.e., the total radiation dose is given in portions over a period of several days to several weeks. These are most likely to vary from daily (i.e., five times per week) doses for up to six weeks, to once weekly doses for four to six weeks. An individual dose of the benzotriazine will be given before or after each radiation treatment and is likely to be in the range of 0.01 to 20 mmol/kg and usually in the range of 0.1 to 2 mmol/kg.

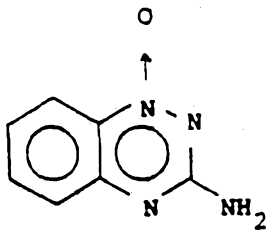
For use as selective cytotoxic agents, the compounds of the invention can be administered alone, with radiation or other cancer cytotoxic agents, with vasoactive drugs (e.g., hydralazine), or with procedures which reduce the amount of available oxygen carried by the blood such as anemia or drugs which increase the binding of oxygen to hemoglobin, all of which can enhance selectively the degree of hypoxia in the tumor. As noted above, while all of the compounds encompassed by Formula 1 are generally useful as radiosensitizers

herein, only those compounds which are 3-substituted-1,2,4-benzotriazine 1,4-dioxides (i.e., X=H, hydrocarbyl (1-4C), NH_2, NHR or NR_2 with R as defined above and n is 1) are useful as selective cytotoxic agents.

Examples

The following examples further illustrate the compounds of the invention and methods for synthesizing and using them, and are not intended to limit the invention in any manner.

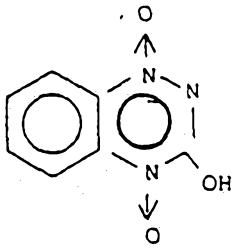
Example 1: Preparation of 3-Hydroxy-1,2,4-Benzotriazine 1,4-Dioxide



1

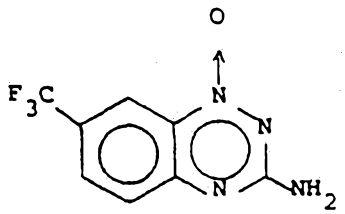
A stirred mixture of 1.50g (9.25 mmole) of 3-amino-1,2,4-benzotriazine 1-oxide (1), 100.0 ml acetic acid, and 30.0 ml of 30% hydrogen peroxide was treated with 3.05 g (9.25 mmole) of Na_2WO_4 $2H_2O$. The mixture was stirred in an oil bath at 60°C for 4 days. The yellowish orange mixture was cooled to about 30° and filtered to remove a light yellow non-UV absorbing solid that was presumably tungstic acid. The orange solution of hydrogen peroxide in acetic acid was evaporated to semi-dryness carefully with several additions of water

and acetic acid to remove most of the peroxide. The concentrated solution was allowed to stand at room temperature to afford four crops of an orange solid, 0.87g (42% yield of the sodium salt of 2). UV_{max} (20% CH_3OH/H_2O): 262.2 (ε 39,460); 477 (ε 7,030). IR (neat): 3530μ, 3150μ, 2650μ, 2180μ and 1635μ. Anal. (calculated for the sodium salt): $C_7H_4N_3O_3Na$ $1.25H_2O$, 223.64: C,37.6; H,2.93; N, 18.79. Found: C, 37.8; H,2.75; N,18.65.



2

Example 2: Preparation of 3-Amino-7-Trifluoromethyl-1,2,4-Benzotriazine 1-Oxide:

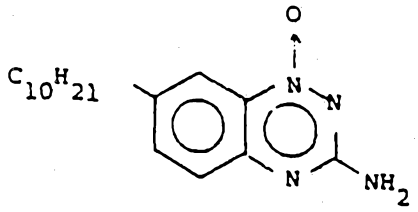


3

A solution of Na (1.13g, 49.2 mmole) in ethanol (50 ml) was added to a solution of guanidine hydrochloride (4.93g, 51.6 mmole) in ethanol (50 ml). After 1h, the mixture was filtered and the filtrate was

combined with a solution of 4-chloro-3-nitro-benzo trifluoride (Aldrich, 5.5g, 24.4 mmole) in ethanol (25 ml). The mixture was stirred and refluxed for 5 h, cooled to 0-5°C, and the precipitated solid collected. The solid was washed with water and ethanol and air-dried to give 0.48g (9%) of 3 as a light yellow solid, mp½300°C. TLC: R_f 0.60 (9:1 methylene chloride: methanol on silica gel plates). Mass. Spec.: M^+=230 (q = 100).

Example 3: Preparation of 3-Amino-7-Decyl-1,2,4-Benzotriazine 1-Oxide



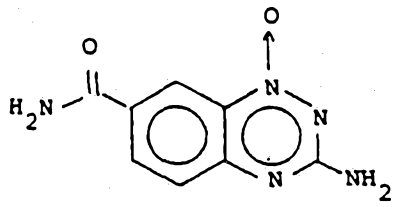
4

Preparation of 4-(1-decyl)-2-nitroaniline: Acetic anhydride (400 ml) was added over a 30-minute period to a stirred solution of 4-decylaniline (Aldrich, 80g, 0.34 mole) in hexanes (2.4l). After stirring for 1h, the mixture was cooled and treated over 30 min. at 5-10°C with 70% nitric acid (34 ml). Stirring was continued at 5-10°C for 1h and at 25°C for 16h. The mixture was diluted with H_2O (1l), stirred for 5h, poured into an open dish and allowed to stand for 16h. After further dilution with H_2O (1.5l), the solid was collected and recrystallized from an 85% ethanol solution (in water) to give 92g (84%) of the intermediate as an orange solid, m.p. 64°C.

A solution (100 ml) of 85% KOH (19g, 0.288 mole) in H_2O was combined with a suspension of 4-(1-decyl)-2-nitroaniline (89g, 0.28 mole), prepared above, in methanol (900 ml). The mixture was stirred for 6h, neutralized to pH 7-8 with concentrated HCl, and evaporated in vacuo to near dryness. After dilution with H_2O (400 ml), the solid was collected and air-dried to give 77g (100%) of the intermediate as an orange solid, mp 59°C.

1.0g (8.7 mmole) of chloroamidine hydrochloride (previously prepared for use by treating an ether solution of cyanamide with HCl gas and collecting the precipitated solid) was added portionwise over 10 min to a preheated melt (190°C) of 4-(1-decyl)-2-nitroaniline prepared in the preceding step (500 mg, 1.8 mmole). The reaction mixture was heated at 190°C for 5 min, cooled to 25°C, treated with 6N KOH (10 ml), and heated at 9095°C for 1h. After cooling to 25°C, the solid was collected, washed with H_2O and ethanol and air-dried to give 0.25g (46%) of compound 4 as a light yellow solid, m.p. 177°C (dec). Mass. spec. M^+=285 (q=100), 302 (q=13).

Example 4: Preparation of 3-Amino-7-Carbamyl-1,2,4-Benzotriazine 1-Oxide



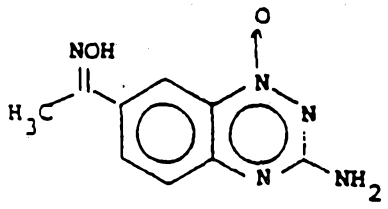
5

Preparation of 4-chloro-3-nitrobenzamide: 20.2g (0.1 mole) of 4-chloro-3-nitrobenzoic acid (Aldrich) and thionyl chloride (20 ml) were combined, allowed to stand for 16h, and refluxed for 4h to give a clear red solution. The solution was evaporated in vacuo and azeotroped with benzene. The residue was dissolved in acetonitrile (20 ml) and added over 30 min to cold (-10°C) concentrated ammonium hydroxide (100 ml). After 3h at -10°C and 16h at 25°C the mixture was poured into an open dish and allowed to evaporate to dryness. The residue was slurried in H_2O and the solid was collected and air-dried to give 19.8g (98%) of the intermediate as a light yellow solid, m.p. 153°C.

A solution of Na (3.45g, 0.15 mole) in ethanol (75 ml) was added to a solution of guanidine hydrochloride (15.8g, 0.165 mole) in ethanol (75 ml). After 1h the mixture was filtered and the filtrate was combined with a suspension of 4-chloro-3-nitrobenzamide (10g, 0.05 mole) prepared above, in ethanol (50 ml). The mixture was stirred and refluxed for 16h, cooled to 0-5°C, and acidified with concentrated HCl (8 ml). The collected solid was combined with K_2CO_3 (28g, 0.2 mole) and H_2O (40 ml) and the mixture was stirred and heated at 100°C for 8h. After cooling to 25°C, the solid was collected, washed with H_2O, and air-dried. The solid was suspended in boiling ethyl acetate, collected and washed with hot ethyl acetate. The solid was repeatedly suspended in boiling dioxane and collected (6x100ml). The combined filtrate was evaporated in vacuo to a solid. The solid was suspended in 95% ethanol, collected and air-dried to give 0.44g (4.3%) of compound 5 as a light yellow solid, m.p.>300°C. TLC: R_f=0.23

(methylene chloride: acetone of 2:1, silica gel plates). Mass. Spec.: M^+ 205 (q= 100).

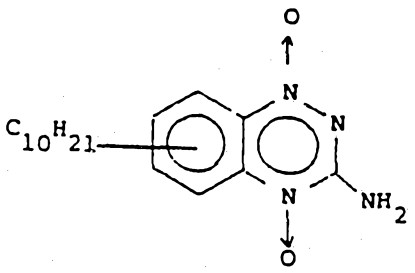
Example 5: Preparation of 7-Acetyl-3-Amino-1,2,4-Benzotriazine 1-Oxide Oxime



6

A combined mixture of 7-acetyl-3-amino-1,2,4-benzotriazine 1-oxide (prepared in Example 5; 50 mg, 0.25 mmole), hydroxylamine hydrochloride (200 mg, 2.88 mmole), pyridine (1 ml) , and ethanol (1 ml) was heated at 90-95°C for 1h and then cooled to 25°C. The mixture was diluted with 95% ethanol (5 ml) and the solid was collected and air-dried to give 30 mg (56%) of compound 6 as a light yellow solid, m.p. 278°C (dec). TLC: R_f=0.60 (9:1 methylene chloride: methanol). Mass Spec.: M^+=219 (q=100).

Example 6: Preparation of 3-Amino-6(7)-Decyl-1,2,4-Benzotriazine 1,4-Dioxide



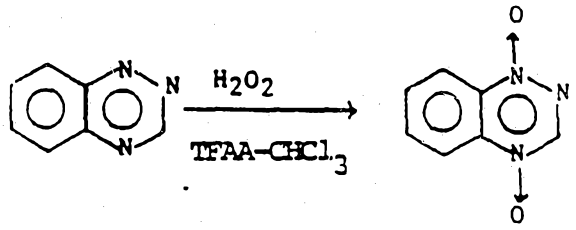
7

5-(1-decyl)-benzofuroxan: A combined mixture of 4-(1-decyl)-2-nitroaniline (77g, 0.28 mole), 5.25% NaOCl in H_2O (476g, 0.34 mole), 85% KOH (20.3g, 0.31 mole), Bn_4NHSO_4 (4.7g, 0.014 mole), and CH_2Cl_2 (2.28 l) was stirred rapidly for 6h and diluted with H_2O (500 ml) and CH_2Cl_2 (1 l). The separated organic phase was washed successively with 1N HCl (1 l) and brine (2 x 1 l)), dried (Na_2SO_4), and concentrated in vacuo to yield a red oil, 70 g (92%).

A solution of 5-(1-decyl)-benzofuroxan as prepared above (10 g, 0.036 mole) and benzyltriethyl ammonium chloride (0.36 g, 0.0016 mole) in DMSO (180 ml) was treated gradually over several hours with cyanamide (13.0 g, 0.31 mole) and K_2CO_3 (36.8 g, 0.27 mole). The mixture was stirred for 48h and filtered. The filtrate was diluted with H_2O (6 l) and glacial acetic acid (40 ml) and extracted with CH_2Cl_2 (4 x 500 ml). The combined organic solution was washed successively with 5% $NaHCO_3$ solution (1 x 500 ml) and brine (2 x 500 ml), dried (Na_2SO_4), and evaporated in vacuo to dryness. The crude product was purified by chromatography on silica

gel using CH_2Cl_2: methanol (98:2) to give 1.8g (16%) of compound 7 as a red solid, m.p. 155°C (dec). Mass. Spec.: M^+=318 (q=4), 285 (q=100).

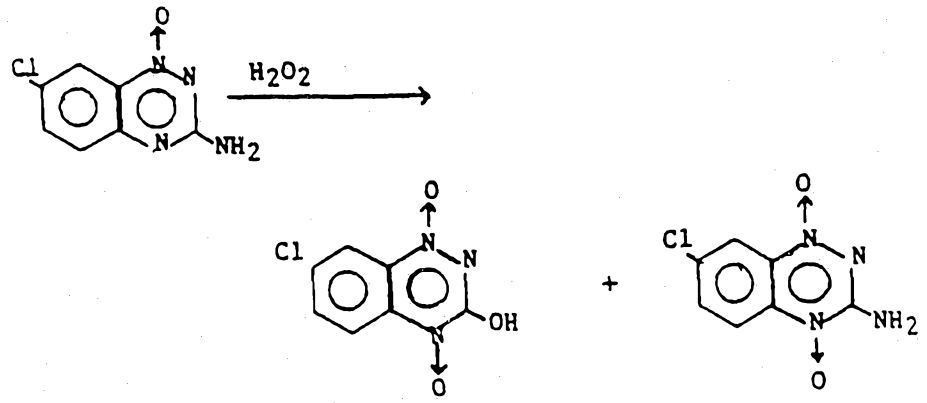
Example 7: Preparation of 1,2,4-Benzotriazine 1,4-Dioxide



8 9

A mixture of 1.80 g (13.73 mmole) of 8, 90% H_2O_2 (9 ml), trifluoroacetic anhydride (13.5 ml) and $Na_2WO_4.2H_2O$ (12.50g, 38 mmole) in $CHCl_3$ (170 ml) was stirred at room temperature for 5 days. The reaction mixture was diluted with H_2O (100 ml) and extracted with $CHCl_3$ (100 ml). The organic layer was washed with H_2O (50 ml), dried (Na_2SO_4), and the solvent removed in vacuo. The residue was chromatographed on silica gel using EtOAc-CH_2Cl_2 (1:1) to give 0.30 g (13.4%) of compound 9 as a yellow solid, m.p. 204-205°C. Anal. Calcd. for $C_7H_5N_3O_2$ (163.13): C, 51.5; H, 3.09; N, 25.76. Found: C, 51.6; H, 3.36; N, 26.01. Mass Spec. M^+=163 (q=100), 147 (q=50). TLC: R_f=0.27 (EtOAc-CH_2Cl_2, 1:1, silica gel plates). IR (nujol): 1600μ, 1460μ, 1300μ, 1230μ. UV_{max} (H_2O): 227 (ε 22,900) 252 (ε 12,950); 392 (ε 4,080).

Example 8: Preparation of 7-Chloro-3-Hydroxy-1,2,4-Benzotriazine 1,4-Dioxide

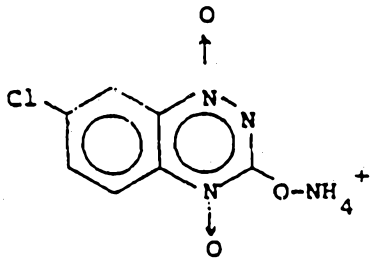


10 11 12

A mixture of 1.50 g (7.63 mmole) of 10 in 100 ml acetic acid was treated with 2.52 g (7.63 mmole) of $Na_2WO_4 \cdot 2H_2O$ and 30 ml of 30% H_2O_2. The mixture was stirred and heated for 6 days at 50°C, then slowly evaporated to dryness to remove H_2O_2. The residue was boiled in 250 ml H_2O and filtered to remove about 25 mg of starting material 12. The aqueous solutions were then extracted with 2 x 250 ml portions of ethyl acetate. A deep red crystalline material that was characterized as 12 by TLC and Mass. Spec. analysis formed in the partitioning mixture above and was collected by filtration to afford 60.0 mg of a yellowish orange solid (3.7% yield), characterized as follows as 12, which showed good solubility in a mixture of hot isopropyl alcohol and water. Mass. Spec.: M^+=212 (q=100)(compound 10); TLC: R_f= 0.34 (acetone, silica gel plates).

The ethyl acetate solutions above, separated from the H_2O layer after the filtration to remove 12,

were evaporated to dryness. The residue was then treated with isopropyl alcohol at room temperature to afford a dull orange solid, 0.41g (25% yield) of 11. Mass. Spec.: M^+=213 (q=70); TLC: R_f=0.22 (acetone, silica gel plates). Compound 11 was characterized as the ammonium salt, $C_7H_4ClN_3O_3 \cdot NH_3$, m.w. 230.61, as follows. The free acid 11 was dissolved in concentrated NH_4OH and then chilled in ice and filtered to remove a trace of insoluble 12. The red filtrate and washings were evaporated to dryness, leaving a reddish-orange solid. The solid was treated with 50 ml of boiling 1,2-dimethoxyethane, collected on a filter and washed with an additional 25 ml of hot 1,2-dimethyl ether. The solid was dried over P_2O_5 at 56°C/1.0 mm, leaving 0.244 g (87% yield) of 13



13

Anal. Calcd. for $C_7H_4ClN_3O_3$ NH_3 (230.61): C, 36.5; H, 3.06; N, 24.30. Found: C, 36.5; H, 3.07; N, 23.94. UV_{max} (H_2O): 219 (ε 12,580); 265.4 (ε 40,000); 4830486 (ε 6,640).

Example 9: In Vivo Assay for Activity in Combination with Radiation

The compounds of the invention were tested in vivo for activity by the assay of Brown, J.M., Radiation Res (1975) 64:633-47, incorporated herein by reference.

For this assay, SCCVII carcinomas in female C3H mice weighing 20-25 g were used. These mice were bred under specific pathogen-free conditions and were 3-4 months old at the beginning of each experiment. The SCVIII tumor was grown intradermally in the flank from an inoculation of 2 x 10^5 tumor cells taken from the 2nd-8th *in vitro* passage of the tumor cells after removal from the previous *in vivo* tumor. Two tumors per mouse were implanted, and were used as subject tumors when they reached a volume of approximately 100 ml. At this point the tumors contained approximately 20% hypoxic cells.

The test compound was tested at a fixed injected dose of either 5 mmol/kg or 2/3 of the LD_{50} (whichever is lower). Suitable controls of test compoundinjected but nonirradiated and saline-injected and irradiated mice were also included. A fixed radiation dose of 20 Gy was applied at variable intervals of 2 hr after to 3 hr before injection of the drug. By using these intervals, the results give an indication of both the optimum irradiation time and the extent of extra cell killing compared to radiation alone. The results of such time-course experiments using 3-amino-1,2,4-benzotriazine 1,4-dioxide are shown in Figure 2. They show enhanced cell killing compared to radiation only, more than would have been expected on the basis of additivity of the two individual cytotoxicities. The similar increased cytotoxicity when the drug is given before or after radiation indicates selective toxicity to the hypoxic cells rather than a radiosensitizing effect of the benzotriazine dioxide.

Irradiation of the SCCVII tumors was done by irradiating nonanaesthetized tumor-bearing mice in a

Plexiglas box. Irradiation conditions were 250 kVp X-rays, 15 mA, FSC 33 cm, added filtration of 0.35 mm Cu, half value layer 1.3 mm Cu, and a dose rate of 317 rad/min.

The amount of cell killing was judged by survival rate of dissected and cultured tumor cells as follows. The tumor-bearing mice were killed 24 hr after irradiation, and tumors were dissected from the skin, cut into several pieces, and made into a fine brei by high-speed chopping with a razor blade attached to a jigsaw. The brei was added to 30 ml of Hank's buffered salt solution (HBSS) containing 0.02% DNase, 0.05% promase, and 0.02% collagenase. The suspension was stirred for 30 min at 37°C, filtered, and centrifuged at 1,600 rmp for 10 min at 4°C. The cell pellet was resuspended in complete Waymouth's medium plus 15% fetal calf serum (FCS) and an aliquot mixed with trypan blue and counted with the use of a hemacytometer. Suitable dilutions of this serum plated into 60- or 100-mm polystyrene petri dishes (Lux Scientific Corp) in 5 or 15 ml of medium. After incubation for 13 days, the colonies were fixed and stained, and those containing 50 cells or more were counted. The dilution yielding an average count of 25-100 colonies in a 60 mm dish was used in calculation of results.

Example 10: Cytotoxicity Tests

Cytotoxicity tests were carried out using 3-amino-1,2,4-benzotriazine 1,4-dioxide and a variety of aerobic and hypoxic cells in culture (human, mouse, and hamster). The cells in spinner flasks were gassed for one hour at 37°C with either air or nitrogen containing 5% CO_2 prior to adding the specified amounts of the

drug. Figures 1A, 1B and 1C show the results for cell survival of mouse, hamster and human cells at various concentrations of 3-amino-1,2,4-benzotriazine 1,4-dioxide. It was found that only 1 to 2% of the drug concentration under aerobic conditions was required to get equal cell killing under hypoxia. This ratio of selective hypoxic toxicity (50-100) is higher than that for any compound so far reported in the literature.

Example 11: Determination of LD_{50}

LD_{50} is determined in BALB/c female mice (weighing 20-25 g) following intraperitoneal (ip) injection, unless the compound tested has low lipophilicity and is very soluble, wherein intravenous (iv) administration is used. LD_{50} values at 1, 2, 5, and 60 days are determined by administering graded doses of the drug dissolved in physiological saline immediately prior to injection.

Example 12: Radiosensitivity in Vitro

The results of assays to determine the concentration of drug necessary to produce a sensitizer enhancement ratio of hypoxic cells in culture of 1.6 are as follows:

Compound	$C_{1.6}$ (mM)
7-chloro-3-amino-1,2,4-benzotriazine 1-oxide	3.3
6(7)-methoxy-3-amino-1,2,4-benzotriazine 1,4-dioxide	~1.0
3-hydroxy-1,2,4-benzotriazine 1,4-dioxide	~2.0

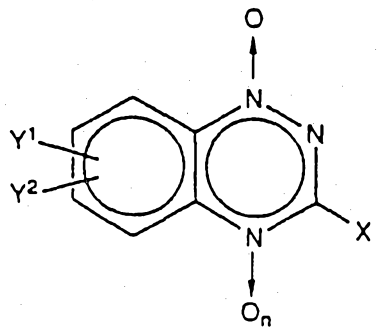
Modifications of the above described modes for carrying out the invention that are apparent to those of skill in the chemical, pharmaceutical, medical, and related arts are intended to be within the scope of the following claims.

Example 13: Enhanced Tumor Cell Toxicity Using Hydralazine

Hydralazine is an antihypertensive drug which acts by relaxing the smooth muscle around blood vessels. This has the effect of preferentially shunting blood flow into normal tissues and away from tumors, which process produces immediate hypoxia in the tumors. If 3-amino-1,2,4-benzotriazine 1,4-dioxide is given in conjunction with this agent, there is a massive increase in tumor cell killing. In this experiment, neither hydralazine nor the aforementioned benzotriazine compound produced any significant cell killing in the SCCVII tumor, whereas the combination of the two reduced survival by a factor of 10^3 (i.e., only 1 cell in every 1000 was left viable). The experimental procedures are the same as described in Example 9, and the results are shown in Figure 3.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of selectively killing hypoxic tumor cells comprising administering to said cells a pharmaceutical composition comprising a compound of the formula



wherein X is H or a hydrocarbyl (1-4C);

n is 1; and

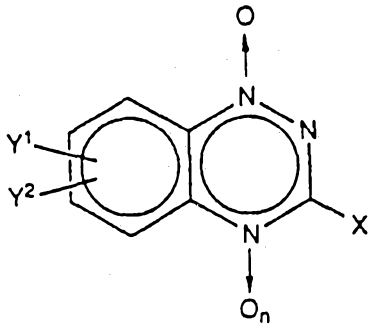
Y^1 and Y^2 are independently either H; halogen; hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, if hydrocarbyl, either unsubstituted or substituted with 1 or 2 substituents selected from the group consisting of halogen, hydroxy, epoxy, alkoxy (1-4C), alkylthio (1-4C), primary amino (NH_2), morpholino, acyloxy (1-4C), acylamido (1-4C) and thio analogs thereof, carboxy, alkoxycarbonyl (1-4C), carbamyl, alkylcarbamyl (1-4C), and wherein the hydrocarbyl may be interrupted by a single ether (-O-)

linkage; or wherein Y^1 and Y^2 are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR')R' in which R' is a hydrocarbyl as defined above,

~~with the proviso that if X is NH_2, Y^1 and Y^2 are other than H,~~

or a pharmacologically acceptable salt of said compound.

2. A method of selectively killing hypoxic tumor cells comprising administering to said cells a pharmaceutical composition comprising a compound of the formula



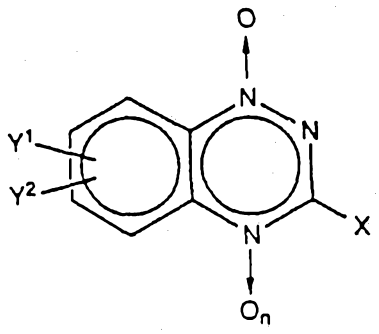
wherein X is H or hydrocarbyl (1-4C), and, if hydrocarbyl, may be substituted with OH, NH_2, alkoxy (1-4C), or halogen substituents;

n is 1; and

Y^1 and Y^2 are independently either H; halogen; hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, if hydrocarbyl, either unsubstituted or substituted with 1 or 2 substituents selected from the group consisting of halogen, hydroxy, epoxy, alkoxy (1-4C), alkylthio (1-4C), primary amino (NH_2), morpholino, acyloxy (1-4C), acylamido (1-4C) and thio analogs thereof, carboxy, alkoxycarbonyl (1-4C), carbamyl, alkylcarbamyl (1-4C), and wherein the hydrocarbyl may be interrupted by a single ether (-O-)

linkage; or wherein Y^1 and Y^2 are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR')R' in which R' is a hydrocarbyl as defined above, or a pharmacologically acceptable salt of said compound.

3. A method of radiosensitizing hypoxic tumor cells comprising administering to said cells a pharmaceutical composition comprising a compound of the formula:



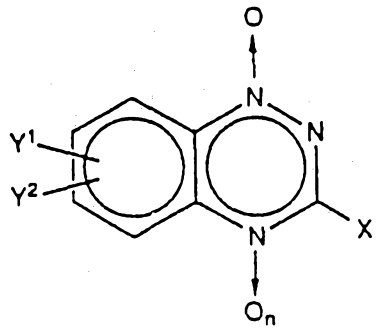
wherein X is H or a hydrocarbyl (1-4C);

wherein n is 0 or 1; and

Y^1 and Y^2 are independently either H; halogen; hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, if hydrocarbyl, either unsubstituted or substituted with 1 or 2 substituents selected from the group consisting of halogen, hydroxy, epoxy, alkoxy (1-4C), alkylthio (1-4C), primary amino (NH_2), morpholino, acyloxy (1-4C), acylamido (1-4C) and thio analogs thereof, carboxy, alkoxycarbonyl (1-4C), carbamyl, alkylcarbamyl (1-4C), and wherein the hydrocarbyl may be interrupted by a single ether (-O-)

linkage; or wherein Y^1 and Y^2 are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR')R' in which R' is a hydrocarbyl as defined above, or a pharmacologically acceptable salt of said compound.

4. A method of radiosensitizing hypoxic tumor cells, comprising administering to said cells a pharmaceutical composition comprising a compound of the formula:



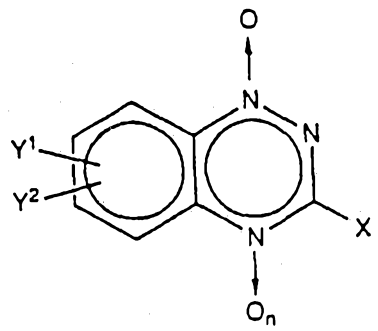
wherein X is H; hydrocarbyl (1-4C); or hydrocarbyl (1-4C) substituted with OH, NH_2, NHR, NRR, alkoxy (1-4C) and halogen, wherein the R groups are independently selected from alkyl (1-4C) and morpholino;

wherein n is 0 or 1; and

Y^1 and Y^2 are independently either H; halogen; hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, if hydrocarbyl, either unsubstituted or substituted with 1 or 2 substituents selected from the group consisting of halogen, hydroxy, epoxy, alkoxy (1-4C), alkylthio (1-4C), primary amino (NH_2), morpholino, acyloxy (1-4C), acylamido (1-4C) and thio analogs thereof, carboxy, alkoxycarbonyl (1-4C), carbamyl, alkylcarbamyl (1-4C), and wherein the hydrocarbyl may be interrupted by a single ether (-O-) linkage; or wherein Y^1 and Y^2 are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR')R' in which

R′ is a hydrocarbyl as defined above, or a pharmacologically acceptable salt of said compound.

5. A compound having the structural formula:

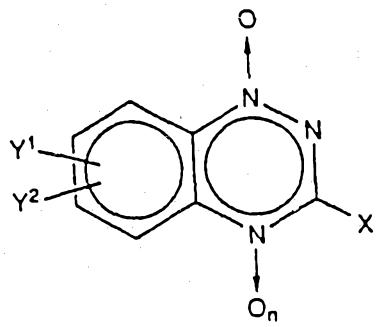


wherein X is H or a hydocarbyl (1-4C);

n is 1; and

Y^1 and Y^2 are independently either H; halogen; hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, if hydrocarbyl, either unsubstituted or substituted with 1 or 2 substituents selected from the group consisting of halogen, hydroxy, epoxy, alkoxy (1-4C), alkylthio (1-4C), primary amino (NH_2), morpholino, acyloxy (1-4C), acylamido (1-4C) and thio analogs thereof, carboxy, alkoxycarbonyl (1-4C), carbamyl, alkylcarbamyl (1-4C), and wherein the hydrocarbyl may be interrupted by a single ether (-O-) linkage; or wherein Y^1 and Y^2 are independently either NHR′, O(CO)R′, NH(CO)R′, O(SO)R′, or O(POR′)R′ in which R′ is a hydrocarbyl as defined above, or a pharmacologically acceptable salt of said compound.

6. A compound having the structural formula:

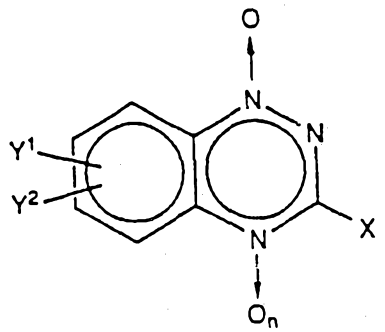


X is H or a hydrocarbyl (1-4C);

n is 1; and

Y^1 and Y^2 are independently either saturated hydrocarbyl of 7-14C or unsaturated hydrocarbyl of 2-14C, including cyclic hydrocarbyl, either unsubstituted or substituted with 1 or 2 substituents selected from the group consisting of halogen, hydroxy, epoxy, alkoxy (1-4C), alkylthio (1-4C), primary amino (NH_2), morpholino, acyloxy (1-4C), acylamido (1-4C) and thio analogs thereof, carboxy, alkoxycarbonyl (1-4C), carbamyl, alkylcarbamyl (1-4C), and wherein the hydrocarbyl may be interrupted by a single ether (-O-) linkage; or wherein Y^1 and Y^2 are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR')R' in which R' is a hydrocarbyl as defined above, or a pharmacologically acceptable salt of said compound.

7. A compound having the structural formula:



X is hydrogen or a hydrocarbyl (2-4C) optionally substituted with OH, NH_2, alkoxy (1-4C) or halogen substituents;

n is 1; and

Y^1 and Y^2 are independently either H; halogen; hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, if hydrocarbyl, either unsubstituted or substituted with 1 or 2 substituents selected from the group consisting of halogen, hydroxy, epoxy, alkoxy (1-4C), alkylthio (1-4C), primary amino (NH_2), morpholino, acyloxy (1-4C), acylamido (1-4C) and thio analogs thereof, carboxy, alkoxycarbonyl (1-4C), carbamyl, alkylcarbamyl (1-4C), and wherein the hydrocarbyl may be interrupted by a single ether (-O-) linkage; or wherein Y^1 and Y^2 are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR')R' in which R' is a hydrocarbyl as defined above, or a pharmacologically acceptable salt of said compound.

Dated this 15th day of March 1993

SRI INTERNATIONAL
Patent Attorney for the
Applicant:

F B RICE & CO

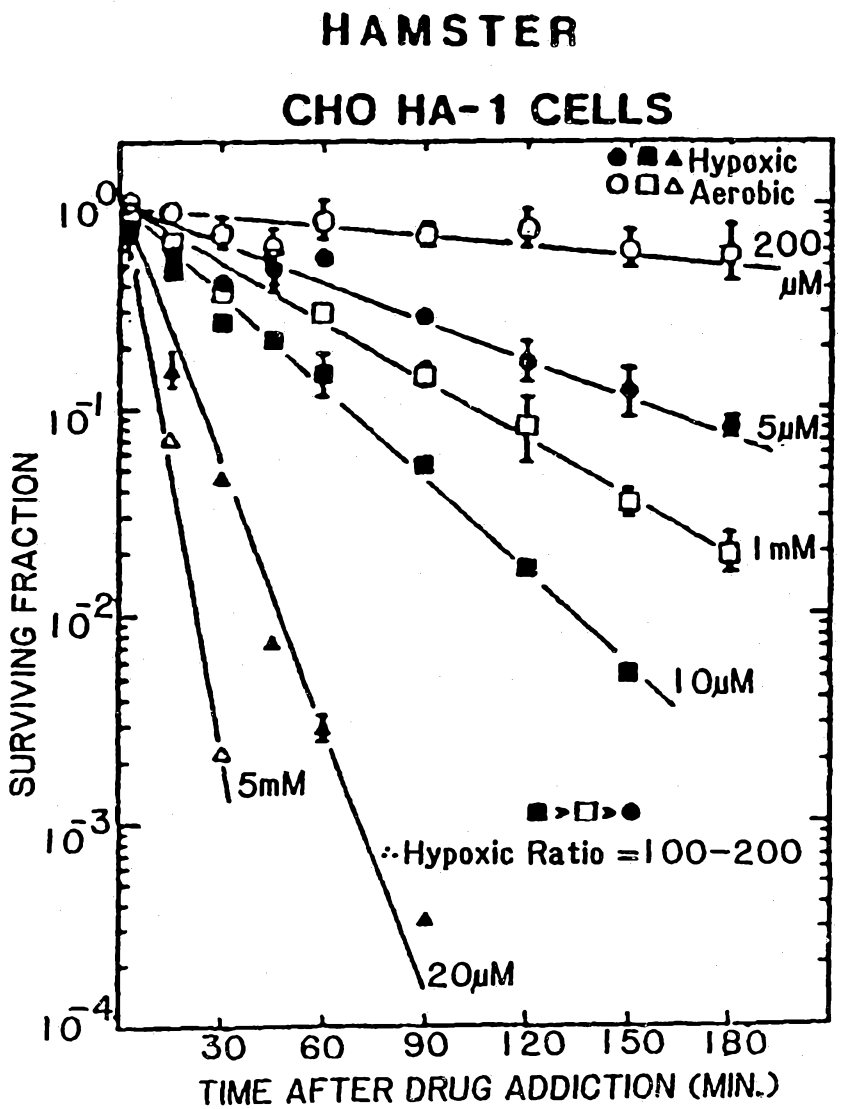


FIG. 1A

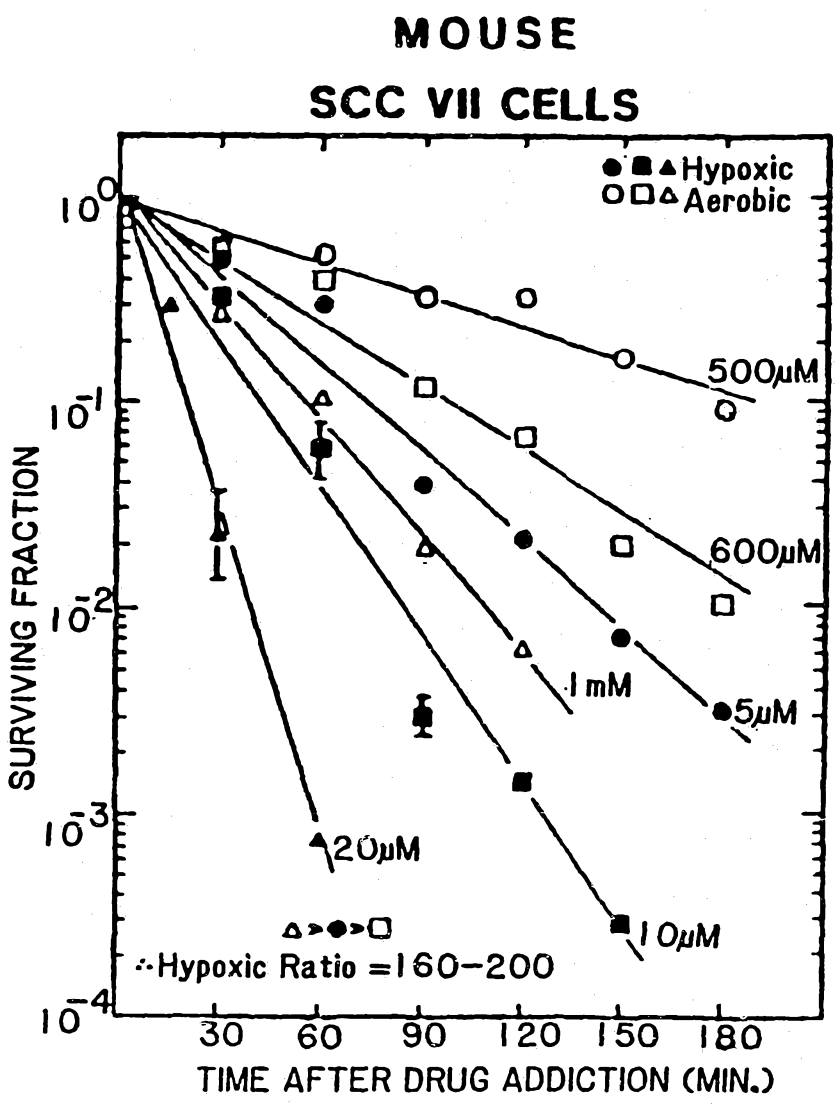


FIG. 1B

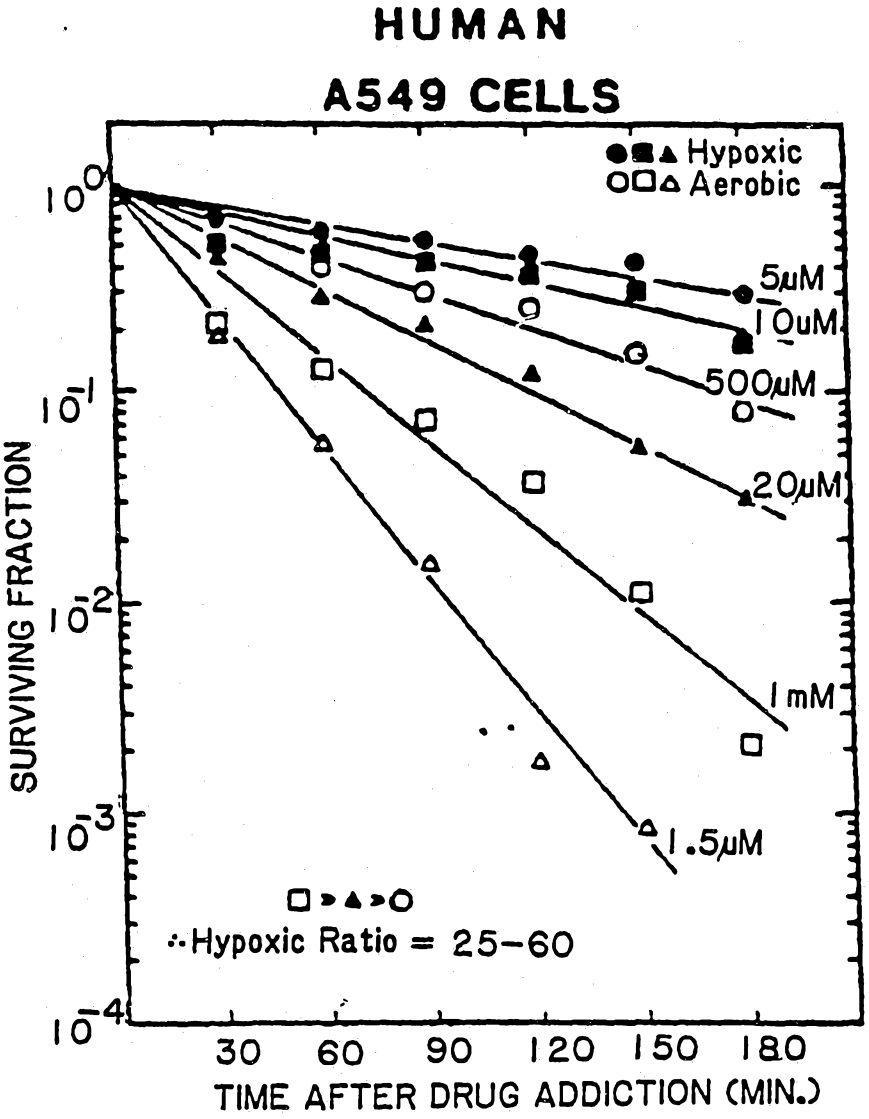

HUMAN
A549 CELLS
●■▲ Hypoxic
○□△ Aerobic
5μM
10μM
500μM
20μM
1mM
1.5μM
□>▲>○
∴Hypoxic Ratio = 25–60
SURVIVING FRACTION
10^0
10^-1
10^-2
10^-3
10^-4
30 60 90 120 150 180
TIME AFTER DRUG ADDICTION (MIN.)

FIG. 1C

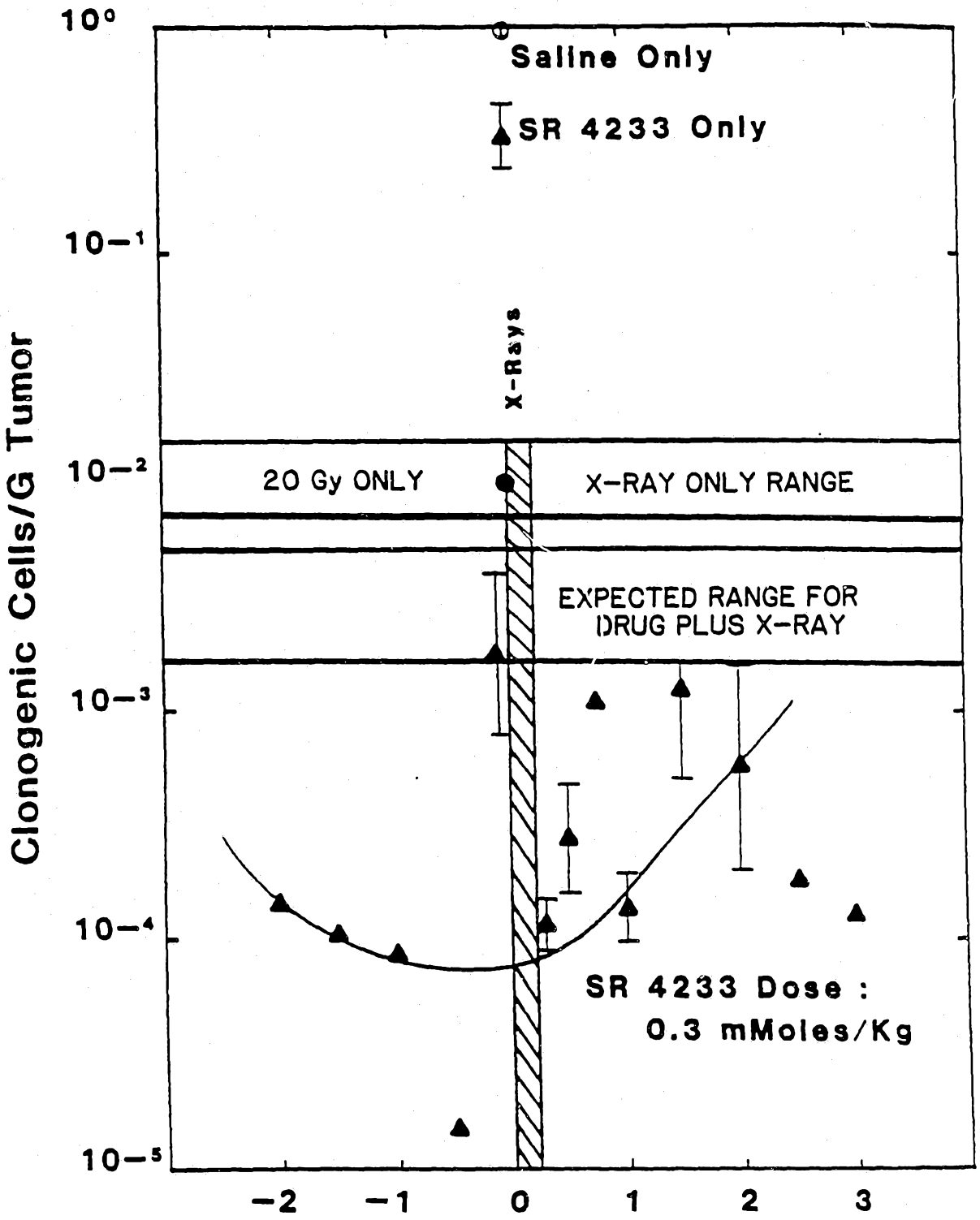

Saline Only
SR 4233 Only
X-Rays
20 Gy ONLY
X-RAY ONLY RANGE
EXPECTED RANGE FOR DRUG PLUS X-RAY
SR 4233 Dose : 0.3 mMoles/Kg
Clonogenic Cells/G Tumor
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
10⁻⁵
-2
-1
0
1
2
3
Time Between Drug and X-Rays (Hours)

FIG. 2

CYTOTOXICITY OF HYDRALAZINE AND SR 4233 IN SCCVII TUMORS

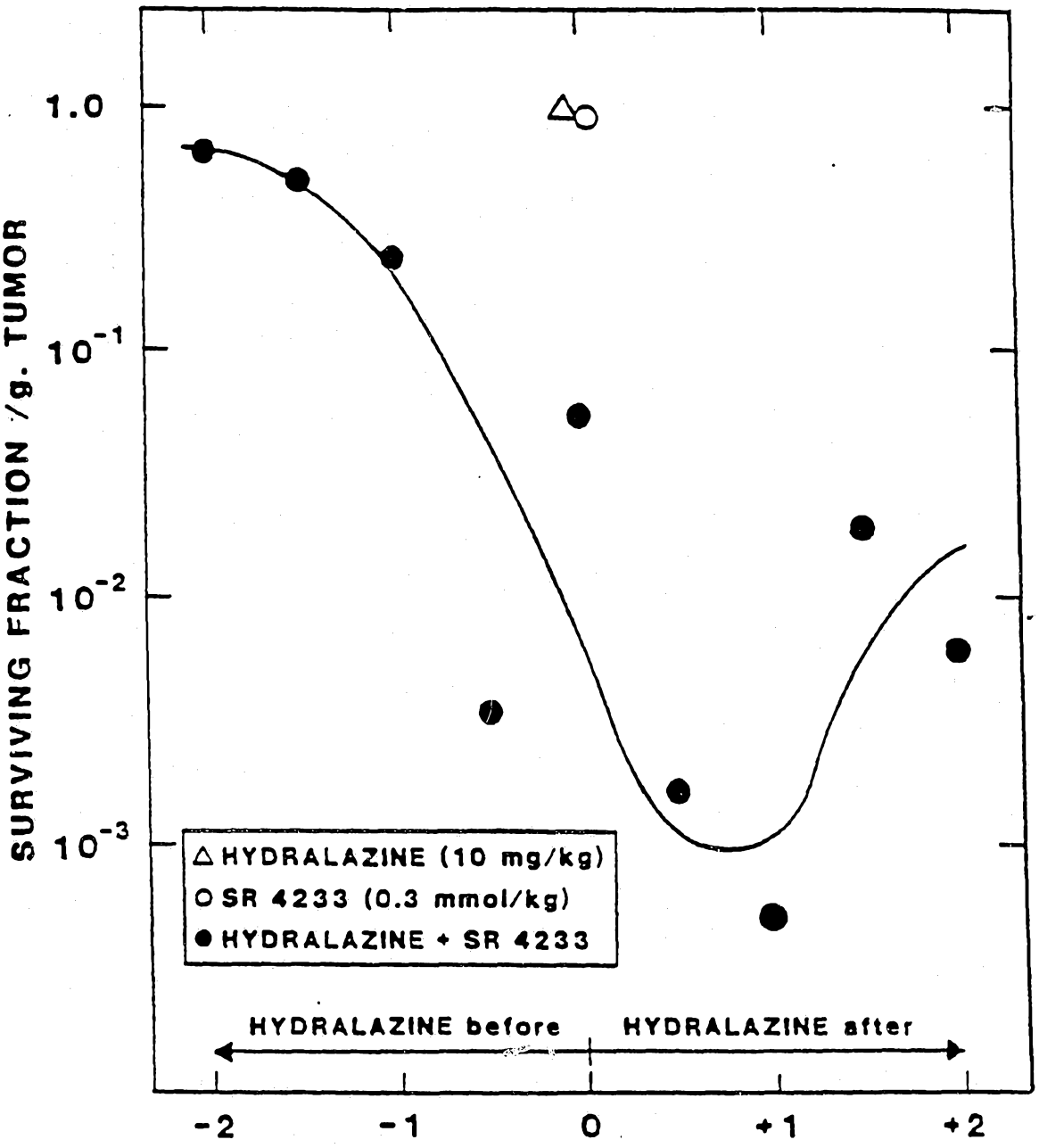


FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/01037

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC4: C 07 D 253/08, A 61 K 31/53

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC4	C 07 D; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
P,X	WO, A1, 88/02366 (SRI INTERNATIONAL) 7 April 1988, see the whole document --	3
X	The Journal of Organic Chemistry, Vol. 24, No. 6, 1959 (American Chemical Society, (US),) J. Jiu et al: "Syntheses in the 1,2,4-benzotriazine series ", see page 813 - page 818 see page 814, tables I,II; page 815 table IV --	3
X	US, A, 2489359 (F.J. WOLF) 29 November 1949, see column 2, line 35 - line 45; column 3, line 13 - line 37 --	3

* Special categories of cited documents: [14]

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

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

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

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

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

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

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

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

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
19th June 1989	06. 07. 89
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	US, A, 2489352 (F J WOLF) 29 Novembe. 1949, see column 3, line 38 - line 40 --	3
X	Journal of the American Chemical Society, Vol. 76, No. 18, 1954 (American Chemical Society, (US),) F J WOLF et al: "Benzotriazines.II. Synthesis of 3-amino-7-halo-1,2,4-benzotriazine-1-oxides ", see page 4611 - page 4613 see page 4612, table I --	3
X	Journal of the American Chemical Society, Vol. 76, No. 13, 1954 (American Chemical Society, (US),) F J Wolf et al: "Benzotriazines.I. A new series of compounds having antimalarial activity ", see page 3551 - page 3553 see page 3552, table I --	3
X	FR, A, 2322140 (BAYER) 25 March 1977, see page 27, line 10 - line 22 --	3
X	EP, A, 0001090 (BAYER) 21 March 1979, see page 24, line 3 - page 25, line 13 --	3
X	Journal of Chromatography, Vol. 131, 1977 (Amsterdam) M S F Ross: "Chromatographic analysis of azapropazone and related benzotriazines ", see page 448 - page 452 see page 448, compound IV --	3
X	US, A, 3482024 (I MOLNAR) 2 December 1969, see column 3, lines 20-22,34-37,69-75; column 4, lines 61-64; column 5, lines 5-17 --	3
X	GB, A, 1234845 (BAYER) 9 June 1971, see page 11, line 5 - line 40; page 12, line 1 - line 20 --	3
X	DE, A, 2404375 (CIBA-GEIGY) 8 August 1974, see page 4, line 16 - line 22; page 22; page 28 --	3

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Chemical Abstracts, volume 106, no. 13, 30 March 1987, (Columbus, Ohio, US), E M Zeman et al: "SR-4233: a new bio-reductive agent with high selective toxicity for hypoxic mammalian cells", see page 29, abstract 95684v, & Int.J.Radiat.Oncol.,Biol.,Phys. 1986, 12(7), 1239-42	3

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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 1-2, because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv):

Methods for treatment of the human or animal body, by means of surgery or therapy, as well as diagnostic methods.

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. PCT/US 89/01037

SA. 27862

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 03/03/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 88/02366	07/04/88	DE-T- 3790581	25/08/88
		GB-A- 2203151	12/10/88
US-A- 2489359	29/11/49	NONE	
US-A- 2489352	29/11/49	NONE	
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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82