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(54) Title: CYTOTOXIC COMPOUNDS

(57) Abstract

Compounds are disclosed which display cytotoxic effects against estrogen-independent tumors. These compounds may be represented by the formula X-R_1 wherein X is a phenyl ring which may be substituted with one or more nitro, methyl or trihalomethyl groups and R_1 is a benzophenone hydrazone, a xanthone hydrazone, a thioxanthone hydrazone, a fluoren hydrazone, an anthraquinone hydrazone, an anthraquinone phenylhydrazone, an indano [1,2,3-*de*]-2*H*-phtalazinone, a tetralin [1,2,3-*de*]-2*H*-phtalazinone, an acridone hydrazone, or a dibenzosuberenone hydrazone. The R_1 moiety may be substituted with one or more hydroxy, methoxy or halogen groups. These compounds are useful as chemotherapeutic antineoplastic agents.

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CYTOTOXIC COMPOUNDS

FIELD OF THE INVENTION

This invention concerns cytotoxic compounds useful as antineoplastic and anti-viral agents.

DISCUSSION OF THE BACKGROUND OF THE INVENTION

Approximately 55% of all human breast cancers are dependent on estrogen hormones for cell replication and the remaining 45% are non-estrogen-dependent. A number of antiestrogenic compounds have been successfully used to treat estrogen-dependent breast cancers. These compounds include tamoxifen, which is especially useful in the palliative treatment of advanced carcinoma of the breast in post-menopausal women. However, tamoxifen is usually ineffective against non-estrogen-dependent tumors and is generally less effective in pre-menopausal women. Additionally, tamoxifen undergoes an isomerization under physiological conditions from a therapeutically useful antiestrogenic compound to an estrogenic isomer which can stimulate the growth of estrogen-dependent tumor cells.

U.S. Patent No. 4,732,904, which is incorporated herein by reference, discloses a number of hydrazone compounds that have antiestrogenic activity. These antiestrogenic hydrazone compounds do not undergo isomerization to estrogenic compounds under physiological conditions and the estrogenic side effects observed for tamoxifen are therefore absent. These hydrazone compounds have been proposed as alternative treatments for estrogen-dependent breast cancers. Among these, the substituted benzophenone nitrophenyl hydrazones, such as 4,4'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone (A-007) are superior anti-cancer agents when compared to the acetophenone and propriophenone nitrophenyl hydrazones.

Antiestrogens such as tamoxifen and the hydrazone-based compounds are thought to bind to estrogen or antiestrogen receptors. The complex of the receptor and the antiestrogen may then bind to nuclear

chromatin in an atypical manner and for a longer time than the normal hormone receptor complex. Furthermore, antiestrogens may deplete the cytoplasm of free receptor. Either or both of these effects could severely impair the continued growth of an estrogen-dependent tumor.

Of the 55% of breast cancers that are estrogen-dependent, most will eventually become estrogen-independent. This may be accounted for by a natural loss of differentiation by the tumor cells. Estrogen-dependent cancer cells have often been observed to eventually lose their ability to produce estrogen-binding protein receptors and degenerate into much more aggressive estrogen-independent life-threatening cancers. Alternatively, a few estrogen-dependent cells originally present in the tumor mass may outgrow the estrogen-independent cells and become predominant. Indeed, the use of antiestrogens to treat estrogen-dependent tumors may lead to the clonal selection of estrogen-independent tumor cells and therefore may promote the conversion of an estrogen-dependent breast cancer to a non-estrogen-dependent breast cancer.

Cancers of other organs, such as lung and colon, do not possess estrogen-binding protein receptors and are considered independent of estrogens for cell replication. Such estrogen-independent tumors are not susceptible to the antiestrogenic properties of drugs such as tamoxifen and thus other chemotherapeutic agents must be used to treat such tumors.

Many compounds have been documented to be effective to varying degrees against estrogen-independent tumors. These compounds are reviewed in Calabresi and Parks, "Chemotherapy of Neoplastic Diseases," *Pharmacological Basis of Therapeutics* (Gilman et al., Eds.) (1986) pp. 1240-1306, McMillan Publishing Co., New York. These compounds are cytotoxic, and differ from antiestrogens in not requiring interaction with an estrogen receptor to exert their therapeutic

action. An example of such a cytotoxic compound is the anthracycline antibiotic doxorubicin, also known as ADRIAMYCIN. This compound exerts its antineoplastic activity by preferentially affecting rapidly dividing tumor cells. The underlying principle of chemotherapy using general cytotoxic agents is based upon the observation that malignant tumor cells replicate at a higher rate than normal body cells and are therefore correspondingly more susceptible to these compounds.

Because clonogenic malignant cells can give rise to sufficient progeny to kill the host, it is necessary to destroy every such malignant cell in order to achieve a cure. The cell-kill caused by antineoplastic agents follows first order kinetics; that is, a constant percentage, rather than a constant number, of cells is killed by a given therapeutic application. This finding has had a profound impact on clinical cancer chemotherapy. For instance, a patient with advanced acute lymphocytic leukemia might harbor 10^{12} or about 1 kg of malignant cells. A drug capable of killing 99.99% of these cells would reduce the tumor mass to about 100 mg, and this would be apparent as a complete clinical remission. However, 10^{8} malignant cells would remain, any of which could cause a relapse in the disease. The logical outgrowth of these concepts has been the attempt to achieve total cell-kill by the use of several chemotherapeutic agents concurrently or in rational sequences.

Many of the most potent cytotoxic agents have activity only against cells that are in the process of division. Accordingly, human malignancies that are currently most susceptible to chemotherapeutic agents are those with a large growth fraction, that is, a high percentage of cells in the process of division. Similarly, normal tissues that proliferate rapidly (for example, bone marrow and intestinal epithelium) are subject to damage by some of these potent cytotoxic drugs, and such toxicity often limits utility. On the

other hand, slow growing tumors with a small growth fraction, for example carcinomas of the colon or lung, are often unresponsive to cytotoxic drugs.

While each of the presently known anti-cancer drugs may be effective in particular circumstances, problems associated with broad cytotoxicity and resistance of tumors to a particular drug may prevent the use of that drug under certain circumstances. For example, the anthracycline antibiotic doxorubicin is one of the most effective agents described to date for the treatment of estrogen-independent breast tumors. A number of important biochemical effects have been described for doxorubicin and related anthracycline antibiotics, any one or all of which could play a role in the therapeutic and toxic effects of such drugs. These include intercalation into DNA which may inhibit DNA and RNA synthesis, mutagenic scission of DNA, and the generation of highly destructive free radicals. Furthermore, these compounds can also disrupt cell membrane function.

Unfortunately, while doxorubicin is relatively effective against a number of solid tissue tumors, including breast carcinoma, some tumors may be resistant or may develop resistance to this compound. Pleiotropic drug resistance to the anthracycline antibiotics appears to result from acceleration of the efflux of anthracyclines and other agents from the cell. A membrane associated glycoprotein, synthesized in high quantity as a result of gene amplification, has been implicated (Myers et al. Antitumor Antibiotics I: Anthracyclines. In, *The Cancer Pharmacology Annual II* (Chabner and Pinedo, EDS) Elsevier, Amsterdam, 1984, pp. 66-79).

In addition, the administration of doxorubicin can result in serious toxic manifestations. Total dosage of doxorubicin as low as 250 mg/m^2 can cause myocardial toxicity. Such chronic, cumulative dose-related toxicity is manifested by congestive heart

failure that is unresponsive to digitalis and a mortality rate in excess of 50%. The risk of myocardial toxicity is greater than 20% of patients at total doses higher than 550 mg/m^2.

The selection of a particular drug for antineoplastic chemotherapy is therefore based on a number of considerations including estrogen receptor status of the tumor, the potential for drug resistance and the side effects of administering the drug. If a treatment regime includes multi-drug therapy, the potential for complex side effects is greatly increased. The current drug of choice for treating estrogen-dependent breast cancer is tamoxifen; this drug can present problems caused by the isomerization of the drug to a tumor-promoting estrogenic form. The antiestrogenic hydrazones disclosed in U.S. Patent No. 4,732,904 offers an alternative to tamoxifen. However, neither these hydrazones nor tamoxifen are known to be effective against estrogen-independent tumors.

The need for new and improved cancer chemotherapeutic is demonstrated by the intense research and development activity in this area. In particular, there is a need for drugs that can be used to treat both estrogen-dependent and estrogen-independent tumors and which do not carry the risk of myocardial toxicity associated with doxorubicin.

The increasing importance of viral disease in clinical medicine has also resulted in a search for improved anti-viral drugs. Each new anti-viral agent adds another element to the pharmaceutical armamentarium available to treat such diverse diseases as AIDS, herpes and hepatitis.

It is an object of the present invention to provide improved cytotoxic compounds useful in the treatment of estrogen-dependent and estrogen-independent tumors.

Another object of the present invention is to provide an improved method for the treatment of

estrogen-independent tumors, which compounds are not associated with doxorubicin induced cardiotoxicity.

Yet another object is to provide improved compounds that have an anti-viral activity.

Finally, it is also an object of the present invention to provide such compounds having minimal toxic effects.

SUMMARY OF THE INVENTION

U.S. Patent No. 4,732,904 describes the antiestrogenic activity of 4,4'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone (DHBDNP). That patent states that the antiestrogenic effect of DHBDNP is due to the prevention of the interaction of the estrogen receptor with DNA in the nucleus. The hydrazones disclosed in that patent are said to have only an antiestrogenic activity, and no indication is given that any of the hydrazones have any general cytotoxic activity. That is, there is no indication that these compounds would be effective against estrogen-independent tumors.

It has now unexpectedly been found that DHBDNP has both antiestrogenic and non-estrogen receptor dependent cytotoxic activity. Thus, it is now shown that DHBDNP may be used to treat estrogen-independent tumors in addition to estrogen-dependent tumors. Also included in the present invention are compounds that show even more potent cytotoxicity against estrogen-independent tumors. These compounds are not antiestrogens, and have a cytotoxic activity unrelated to estrogen receptor mediated modes of action.

The present invention provides a novel method of impairing growth of neoplastic cells (which are not necessarily estrogen dependent cells) by exposing the neoplastic cells to an effective amount of a compound sufficient to inhibit growth of the neoplastic cells. The compound has the formula X - R^1 wherein X is phenyl substituted with one or more substituents selected from the group consisting of nitro, lower alkyl (preferably methyl), and trihalomethyl; and R^1 is selected from the

group consisting of (a) a benzophenone hydrazone, with one or more substituents on the benzophenone selected from the group consisting of halogen, hydroxy, acetoxy and lower alkoxy, wherein a benzophenone substituted with only lower alkoxy or only hydroxy has at least one of the alkoxy or hydroxy substituents in an *ortho* position on the benzophenone; (b) a substituted or unsubstituted xanthone hydrazone, wherein the substituted xanthone hydrazone is substituted on the xanthone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy and halogen; (c) a substituted or unsubstituted fluoren hydrazone, wherein the substituted fluoren hydrazone is substituted on the fluoren with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy and halogen; (d) a substituted or unsubstituted anthraquinone hydrazone, wherein the substituted anthraquinone hydrazone is substituted on the anthraquinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy and halogen; (e) a substituted or unsubstituted anthraquinone phenylhydrazone, wherein the substituted anthraquinone phenylhydrazone is substituted on the anthraquinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen, and the phenylhydrazone is selected from the group consisting of nitrophenylhydrazone, dinitrophenylhydrazone, methyl phenylhydrazone and dimethyl phenylhydrazone; (f) a substituted or unsubstituted indano [1,2,3-*de*]-2*H*-phthalazinone, wherein the substituted indano [1,2,3-*de*]-2*H*-phthalazinone is substituted on the indano [1,2,3-*de*]-2*H*-phthalazinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy and halogen; (g) a substituted or unsubstituted tetralin [1,2,3-*de*]-2*H*-phthalazinone, wherein the substituted tetralin [1,2,3-*de*]-2*H*-phthalazinone is substituted on the tetralin

[1,2,3-*de*]-2*H*-phthalazinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy and halogen; (h) a substituted or unsubstituted acridone hydrazone, wherein the substituted acridone hydrazone is substituted on the acridone with one or more substitutents selected from the group consisting of hydroxy, lower alkoxy, acetoxy and halogen; (i) a substituted or unsubstituted thioxanthone hydrazone, wherein the substituted thioxanthone hydrazone is substituted on the thioxanthone with one of more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy and halogen; and (j) a substituted or unsubstituted dibenzosuberenone, wherein the substituted dibenzosuberenone is substituted on the dibenzosuberenone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy and halogen.

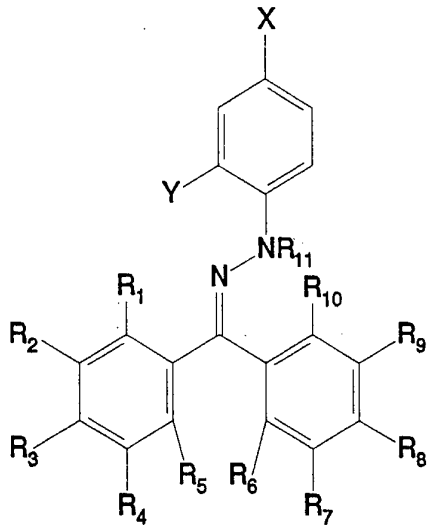
The present invention includes any one of these groups (a) through (j), or any combination of (a) through (j), for R^1. The lower alkoxy substitution on R^1 may be methoxy or ethoxy, but is preferably methoxy. The lower alkyl substituent on X is methyl or ethyl.

In another embodiment, the present invention provides a method of impairing growth of neoplastic cells by exposing the neoplastic cells to an effective amount of a compound sufficient to inhibit growth of the neoplastic cells. The compound has the formula X - R^1 wherein X is phenyl substituted with one or more substituents selected from the group consisting of methyl and trihalomethyl, and R^1 is benzophenone hydrazone, substituted on the benzophenone with one or more substituents selected from the group consisting of halogen, hydroxy and lower alkoxy, such as methoxy.

In particularly preferred embodiments, the method of impairing growth of neoplastic cells comprises exposing the neoplastic cells to an effective amount of a compound sufficient to inhibit growth of the

neoplastic cells. The compound is selected from the group consisting of 4-fluoro-4'-hydroxybenzophenone-2,4-dinitrophenylhydrazone; 2,6-dihydroxyanthraquinone-bis-2,4-dinitrophenylhydrazone; 1,8-dihydroxyanthraquinone-10-(2,4-dinitrophenylhydrazone); 1,2-dihydroxyanthroquinone-10-2-(2,4-dinitrophenylhydrazone); 6,9-diacetoxyanthraquinone-10-(2,4-dinitrophenylhydrazone); 2,4-dihydroxybenzophenone-2,4-dinitrophenylhydrazone; 2,7-dihydroxyfluoren-9-one-2,4-dinitrophenylhydrazone; 2,7-difluorofluoren-9-one-2,4-dinitrophenylhydrazone; 2,7-dichlorofluoren-9-one-2,4-dinitrophenylhydrazone; 1,4-dihydroxyfluoren-9-one-2,4-dinitrophenylhydrazone;4,4-dimethoxy-2,2'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone; 4,4'-dihydroxybenzophenone-2,4-dimethylphenylhydrazone; 2-hydroxyfluoren-9-one-2,4-dinitrophenylhydrazone; xanthone-2,4-dinitrophenylhydrazone; 2,7-difluoroxanthone-2,4-dinitrophenylhydrazone; 2,4,4'-trihydroxybenzophenone-2,4-dinitrophenylhydrazone; 4,4'-dihydroxybenzophenone-2-nitrophenylhydrazone; 2,2',4,4'-tetrahydroxybenzophenone-2,4-dinitrophenylhydrazone; 4,4'-difluorobenzophenone-2,4-dinitrophenylhydrazone; 2,2'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone; 4-hydroxy-4'-chlorobenzophenone-2,4-dinitrophenylhydrazone; 2-(2,4-dinitrophenyl) indano [1,2,3-*de*]-2*H*-phthalazinone; 2-(2,4-dinitrophenyl)-5,10-dihydroxyindano-[1,2,3-*de*]-2*H*-pthalazinone; 4,4'-dihydroxybenzophenone-2,4-dimethylphenylhydrazone; 2-(2,4-dinitrophenyl) tetralin [1,2,3-*de*] 2*H*-phthalazinone; acridone-2,4-dinitrophenylhydrazone; dihydroxydibenzosuberenone-2,4-dinitrophenylhydrazone; difluorodibenzosuberenone-2,4-dinitrophenylhydrazone; thioxanthone-2,4-dinitrophenylhydrazone.

A number of novel hydrazones, which are suitable for use in the method of treatment or in compositions that include a pharmaceutically inert carrier, the hydrazones may be represented by the formula



wherein

X = H, NO_2, lower alkyl, or trihalomethyl and Y = H, NO_2, lower alkyl, or trihalomethyl; and wherein at least one of X and Y is selected from the group consisting of NO_2, lower alkyl, and trihalomethyl, and wherein

(a) R^1 = H, OCOCH3 or OH; R^2 = H, halogen, $OCOCH_3$, or OH; R^3 = H, OH, halogen, $OCOCH_3$, or lower alkoxy; R^4 = H, $OCOCH_3$ or OH; R^7 = H, OH, or $OCOCH_3$; R^8 = H, OH, halogen, $OCOCH_3$, or lower alkoxy; R^9 = H, halogen, $OCOCH_3$, or OH; R^{10} = H or OH; R^5 = R^6 = R^{11} = H; and wherein at least one of R^1 and R^{10} is hydroxy; or

(b) each of R^1-R^4 and R^7-R^{10} is independently selected from the group consisting of hydrogen, hydroxy, acetoxy, lower alkoxy and halogen, and R^{11} is hydrogen, and R^5 and R^6 together form

i) a single bond of a five membered ring, or

ii) an oxygen in a six membered ring, or

iii) a carbonyl in a six membered ring, or

iv) a carbon in a six membered ring; or

v) a nitrogen with or without substitution in a six membered ring; or

vi) a sulfur in a six membered ring; or

vii) two carbons connected by a double bond in a seven membered ring; or

(c) each of R^1-R^4 and R^7-R^9 is independently selected from the group consisting of hydrogen, hydroxy, acetoxy, lower alkoxy and halogen, and R^{10} and R^{11} together form a carbonyl of a six membered ring, and R^5 and R^6 together form

i) a single bond of a five membered ring, or

ii) an oxygen in a six membered ring, or

iii) a carbonyl in a six membered ring, or

iv) a carbon in a six membered ring; or

v) a nitrogen with or without substitution in a six membered ring; or

vi) a sulfur in a six membered ring; or

vii) two carbons connected by a double bond in a seven membered ring; or

(d) each of R^1-R^4 and R^7-R^{10} is independently selected from the group consisting of hydrogen, hydroxy, acetoxy, lower alkoxy and halogen, R^{11} is hydrogen and R^5 and R^6 together form a carbon in a six membered ring linked to phenylhydrazone substituted with one or more substituents selected from the group consisting of nitro, methyl and trihalomethyl; or

(e) R^{11} is H and each of R^1-R^{10} is independently selected from the group consisting of hydrogen, hydroxy, acetoxy, lower alkoxy and a halogen and wherein at least one of R^1-R^{10} is a halogen and wherein at least one of R^1-R^{10} is a hydroxy.

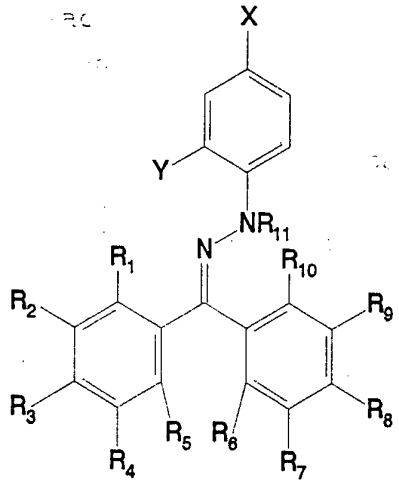
The halogen substitutions in (a) - (e) are preferably chlorine or fluorine. The lower alkoxy is methoxy or ethoxy. The present invention also includes the substitution patterns of any one of (a) through (e), or any combination of these groups.

A subset of the hydrazones of the present invention comprises: 4-fluoro-4'-hydroxybenzophenone-2,4-dinitrophenylhydrazone; 2,6-dihydroxyanthraquinone-bis-2,4-dinitrophenylhydrazone; 1,8-dihydroxyanthraquinone-10-(2,4-dinitrophenylhydrazone);

2,4-dihydroxybenzophenone-2,4-dinitrophenylhydrazone; 2,4-dihydroxyfluoren-9-one-2,4-dinitrophenylhydrazone; 4,4'-dimethoxy-2,2'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone; 2-hydroxyfluoren-9-one-2,4-dinitrophenylhydrazone; xanthone-2,4-dinitrophenylhydrazone; 2,4,4'-trihydroxybenzophenone-2,4-dinitrophenylhydrazone; 2,2', 4,4'-tetrahydroxybenzophenone-2,4-dinitrophenylhydrazone; 2,2'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone; 4-hydroxy-4'-chlorobenzophenone-2,4-dinitrophenylhydrazone; or 2-(2',4'-dinitrophenyl) indano [1,2,3-*de*]-2*H*-phthalazinone; 2-(2,4-dinitrophenyl) tetralin [1,2,3-*de*] 2*H*-phthalazinone.

In preferred embodiments, a therapeutically effective amount of the compound is suspended in a pharmaceutically inert carrier such as peanut oil. In other embodiments, the compounds can be made into a pill for oral administration. The compound could also be combined with an aqueous vehicle for injection for systemic use. In other important embodiments, the compound can be placed in suitable pharmaceutical solvents, such as propylene glycol, dimethylsulfoxide (DMSO), inert materials or mixtures thereof, and applied topically to tumor recurrences, of the kind that occur on the chest wall after breast tumors are surgically removed.

The present invention also includes compounds of the formula



wherein

X = H, NO_2, lower alkyl, or trihalomethyl and Y = H, NO_2, lower alkyl, or trihalomethyl; and wherein at least one of X and Y is selected from the group consisting of NO_2, lower alkyl, and trihalomethyl, and wherein

(a) R^1 = H, OCOCH3, or OH; R^2 = H, halogen, $OCOCH_3$, or OH; R^3 = H, OH, halogen, $OCOCH_3$, or lower alkoxy; R^4 = H, OCOCH3 or OH; R^7 = H, OH, or $OCOCH_3$; R^8 = H, OH, halogen, $OCOCH_3$, or lower alkoxy; R^9 = H, halogen, $OCOCH_3$, or OH; R^{10} = H or OH; R^5 = R^6 = R^{11} = H; and wherein at least one of R^1 and R^{10} is hydroxy; or

(b) each of R^1-R^4 and R^7-R^{10} is independently selected from the group consisting of hydrogen, hydroxy, acetoxy, lower alkoxy and halogen and R^{11} is hydrogen, and R^5 and R^6 together form

i) a carbonyl in a six membered ring; or

ii) a carbon in a six membered ring; or

iii) a nitrogen with or without substitution in a six membered ring; or

iv) a sulfur in a six membered ring; or

v) two carbons connected by a double bond in a seven membered ring; or

(c) each of R^1-R^4 and R^7-R^9 is independently selected from the group consisting of hydrogen, hydroxy, acetoxy, lower alkoxy and halogen and R^{10} and R^{11} together form a carbonyl of a six membered ring, and R^5 and R^6 together form

i) a single bond of a five membered ring; or

ii) an oxygen in a six membered ring; or

iii) a carbonyl in a six membered ring; or

iv) a carbon in a six membered ring; or

v) a nitrogen with or without substitution in a six membered ring; or

vi) a sulfur in a six membered ring; or

vii) two carbons connected by a double bond in a seven membered ring; or

(d) each of R^1-R^4 and R^7-R^{10} is independently selected from the group consisting of hydrogen, hydroxy, acetoxy, lower alkoxy and halogen, R^{11} is hydrogen and R^5 and R^6 together form a carbon in a six membered ring linked to phenylhydrazone substituted with one or more substituents selected from the group consisting of nitro, methyl and trihalomethyl.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the x-ray crystallographic structure of A-007 and a schematic diagram of this compound, showing hydrogen bonding as a dotted line.

FIG. 2 is a graph showing the percentage of growth inhibition of two cell lines, FDCP-1 (a bone marrow cell line) and MDA-435 (a human breast cancer cell line) with exposure to increasing concentrations of A-007.

FIG. 3 is a graph showing the percentage of growth inhibition of two cell lines, FDCP-1 (a bone marrow cell line) and MDA-435 (a human breast cancer cell line) with exposure to increasing concentrations of doxorubicin.

FIG. 4 is a graph showing serum levels of A-007 in a 507 g male Sprague Dawley rat dosed once orally with A-007 (1 g/kg). The curve 1 used

acrylonitrile:water 85:15 as an eluant; curve 3 used acrylonitrile:water 70:30 as an eluant; curve 2 is an average curve of 1 and 2.

DETAILED DESCRIPTION

U.S. Patent No. 4,732,904, which is incorporated herein by reference, discloses the compound 4,4'-dihydroxy benzophenone-2,4-dinitrophenyl hydrazone. That patent teaches that the disclosed hydrazone has potent antiestrogenic activity. The inventor has determined by x-ray crystallography that this compound (which is shown in FIG. 1 herein) has *gem*-diphenyl rings that are perpendicular to each other, resulting in a non-planar ring system through the aryl moieties of the molecule. Intramolecular hydrogen bonding exists between an oxygen of the *ortho* nitro group and the -NH- of the -HN-N=C- linkage which introduces partial planarity to the molecule around the hydrazone moiety. By empirical studies, the present inventor has determined that certain related compounds that have enhanced molecular planarity possess potent cytotoxic activity, that is unrelated to the antiestrogenic activity disclosed in U.S. Patent No. 4,732,904. This unexpected cytotoxic activity is apparently a result of the increased molecular planarity. This increased planarity is produced by planar ring structures, enhanced intramolecular hydrogen bonding and/or the inclusion of polar groups that promote hydrogen bonding. The described compounds share aryl-based structures that have been made more or less polar through substitutions or the aryl structures, or have been made more co-planar through intramolecular hydrogen bonding or fusion of the rings. Additional changes have been introduced into the hydrazone moiety to influence electrostatic distributions and/or polarity.

Assay for Anti-Tumor Activity

Cell Culture and Growth Conditions

The assay of the present invention is described more fully in U.S. Patent Application Serial No.

07/692,240, filed April 26, 1991, which is incorporated herein by reference.

Samples (1 cm) of sterile fresh human cancer tissues with histologies of malignant cancers were obtained from surgical biopsies or resections. Such tissue can be stored in 20-50 cc RPMI-1640 tissue culture medium (Gibco Laboratories) at 5-10°C for up to five days. Tissue samples were transferred under sterile conditions to a laboratory where, under sterile conditions, the tissue was removed from the holding solution and minced by scalpel and scissors. Cellular suspensions were made by cutting the specimen into 0.1 mm fragments in petri dishes with 60 ml of RPMI-1640 tissue culture medium (Gibco Laboratories) containing 10% fetal bovine serum (FBS) (Gibco Laboratories), 100 units/ml streptomycin (Sigma Chemical Co.) and 100 units/ml penicillin (Sigma Chemical Co.).

One (1) ml of the above cellular suspensions was added to NUNC SlideFlasks which are available from Nunc, Inc. (Catalog No. 170920 or Flask style 177453). These flasks are described more fully in United States Patent No. 3,726,764. The bottom edge of the flask is sealed to a baseplate slide by a thermoplastic or adhesive seal which is substantially fluid impervious. The seal between the bottom edge of the housing and the baseplate can be selectively broken by prying the baseplate away from housing. This may be achieved by inserting the supplied SlideFlask opener below the flange and prying the housing away from the base by exerting pressure as described by the manufacturer's instructions. Once the baseplate slide is removed from the housing, the baseplate acts as a conventional microscope slide that can be examined under a microscope.

The SlideFlask is used as an ordinary small-size culture flask with a culture area of 9 cm^2. After culturing, the contents are poured out, the bottom of the flask (the slide) is removed by using the SlideFlask opener, air dried, sprayed with Pro-Fixx™ (fixation,

staining, etc.). The bottom of the flask has standard slide microscopy dimensions and is handled exactly as a standard microscopy slide. Employing light microscopy, the cell growth on each slide is counted.

Determination of Cytotoxicity

The cultured human cancer cells prepared as described above, were used in determinations of the cytotoxicity of 4,4'-dihydroxybenzophenone-2,4-dinitrophenyl hydrazone and other compounds described herein. The cellular suspension of human tumor cells in each flask was diluted using the test compounds in concentrations of 0.005-20 μg/ml as described below:

Control Flasks (No Test Compound) Flasks were diluted to 6 ml with RPMI-1640 medium containing 10% FBS, 100 μg/ml streptomycin and 100 units/ml penicillin.

Test Flasks Flasks were diluted as in the control flasks to 6 ml with RPMI-1640 medium plus FBS, penicillin and streptomycin and test compounds were added to a final concentration of 0.005-20 μg/ml. A few of the compounds (KT-II-27, I-75, B-181 and I-147) were dissolved in 1N sodium hydroxide (5μg/20μl), then dilution with 2 ml dimethyl sulfoxide (DMSO), followed by dilution with RPMI medium to obtain the required concentration. This appears to be a unique method of dissolving, for physiological use, the compounds of the present invention.

The flasks were incubated for seven (7) to twenty-one (21) days in a 5% CO_2 incubator at 37°C. In some instances, incubation time was extended to obtain sufficient cell numbers to count. Sufficient cell growth was arbitrarily determined to be present when at least 10 cells per high power field (cells/HPF) or ten clumps of cells/HPF were present in the control culture. A minimum of 5 HPFs were counted per flask.

After sufficient cell growth in the flasks, the medium and contents were poured out and the slide bottom peeled off using the SlideFlask opener. The slides, which were previously labelled, were air dried

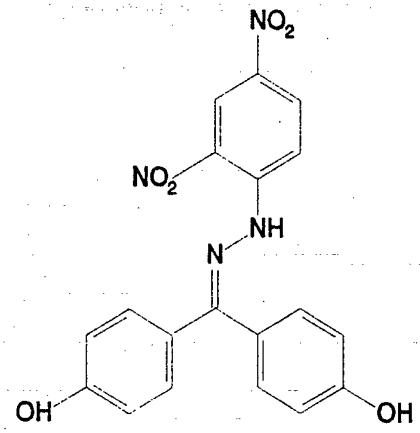
for a few minutes and sprayed with a cytofixative (Pro-Fixx™, Lerner Labs). They were then examined under a conventional light microscope as any cytological or histological slides would ordinarily be examined. If the laboratory has an inverted field microscope, the above slide preparation can be eliminated and the flasks containing cells and liquid content can be counted directly, without preparing and staining the slides. High power fields were scanned, counted and compared in all tissue culture systems. Ten (10) cells/HPF in 5 HPF were considered 100% growth, because sufficient cell growth in the control culture was set at 10 cells/HPF. The results of this assay were expressed as the dose of the compound required to produce a 50% inhibition of cell growth (IC_{50}). This IC_{50} dose was determined to be the dose required to produce 5 or fewer cells/HPF (compared to the 10 cells/HPF control), i.e. inhibition of growth of 50% of the cells.)

EXAMPLE I

Cytotoxicity of 4,4'-Dihydroxybenzophenone-2,4-dinitrophenylhydrazone (A-007)

U.S. Patent No. 4,732,904 discloses that 4,4'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone (A-007), the structure of which is shown below, has antiestrogenic activity.

A-007



The fact that A-007 has antiestrogenic activity means that the growth of estrogen-dependent tumor cells is inhibited by this compound. The determination of estrogen-dependent status for tumors, and therefore of potential sensitivity to antiestrogen therapy, may be made by determining the concentration of both estrogen and progesterone receptors on the surface of tumor cells. Tumors are classified as estrogen receptor plus or estrogen receptor minus (ER+/ER-) and progesterone receptor plus or progesterone receptor minus (PgR+/PgR-) depending on the concentration of receptors present. An ER+ cell is generally considered to be estrogen dependent, but the presence of PgR and ER together appears to be a better indicator of susceptibility to antiestrogen treatment than ER status alone. PgR is detected in approximately two-thirds of ER+ tumors, and is only occasionally found in ER- tumors.

Quantitative methods, including ligand binding assays, monoclonal antibody assays, and enzyme immunochemical analyses have been used to detect and quantitate the ER and PgR, as disclosed in ACTA Oncologica, 27:1-19 (1988). Using such techniques it has been determined that breast cancer tissue containing ER concentrations greater than 5 fentomoles/ng and PgR concentrations greater than 3 fentomoles/ng is considered sensitive to antiestrogen therapy (J. Clin. Oncol., 1:227-241 (1985)). Generally speaking, the higher the ER/PgR concentrations, the more sensitive the tissue should be to the antiestrogen therapy.

The data shown in Table I was generated in a continuing study of the activity of A-007. Cells from human breast tumors were cultured and assayed for sensitivity to A-007 as described above. In addition the estrogen and progesterone receptor concentrations of these cell lines were quantified by conventional methods. As shown in Table I, it was found that those breast cancer cells with higher ER/PgR values had a

lower IC_{50} for A-007 and were therefore more sensitive. This finding is in agreement with previous observations. Surprisingly, however, breast cancer cells which had ER/PgR values below those considered to be the threshold level for antiestrogen sensitivity also showed sensitivity to A-007, albeit at a reduced level. The IC_{50} for A-007 was 10 μg/ml for breast cancer cells and tissue which had less than 5 fmol/μg protein of ER and less than 3 fmol/μg protein of PgR. These ER/PgR levels would therefore classify the tissue as being derived from non-estrogen-dependent tumors, yet A-007 had some activity against it. This suggests that A-007 has cytotoxic activity against non-estrogen-dependent tumor cells and that therefore A-007 may have an additional anti-tumor modality in addition to its antiestrogenic mode of action. Cytotoxic activity as used in this context refers to antineoplastic activity that is not mediated by interaction with estrogen receptors or interference with interaction between estrogen and cellular components such as DNA.

TABLE I

CORRELATION OF BREAST CANCER ER/PgR VALUES WITH SENSITIVITIES (IC_{50}) TO A-007

n (75)	ER/PgR** (Range)	A-007 IC_{50}*** (Mean +/- SEM)
20	<5/<3	10.0 ± 1.3
15	5-100 &/or 3-50	8.8 ± 1.8
7	<50/>50	8.7 ± 2.7
6	>50<100/>50<100	5.0 ± 1.5
7	>50 <100/>100	5.3 ± 1.8
4	>100/<100	4.0 ± 0.7
16	>100/>100	3.4 ± 0.8

** ER & PgR: estrogen and progesterone receptor values are in fmol/μg protein. Tumors with ER and PgR values

<3 and <5 respectively are considered negative for these receptors.

*** IC_{50} in μg/ml.

EXAMPLE II

X-Ray Crystallographic structure of A-007

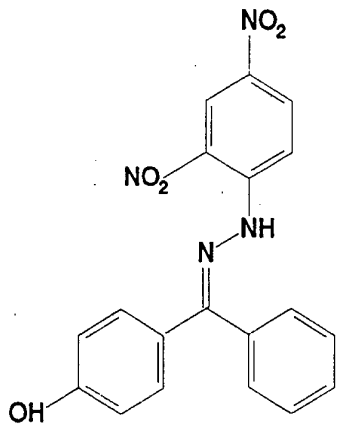
X-ray crystallography data for A-007 shows that the *gem*-diphenyl rings are perpendicular to each other, resulting in a non-planar ring system through the aryl moieties of the molecule. However, intramolecular hydrogen bonding between an oxygen of the *ortho* nitro group and the -NH- of the -HN-N=C- moiety confers partial planarity on the phenyl portion of the molecule and reduces rotation around the hydrazine linkage (N-NH). This x-ray crystallographic structure, and the hydrogen bonds are illustrated in FIG. 1.

EXAMPLE III

Modified Hydrazones

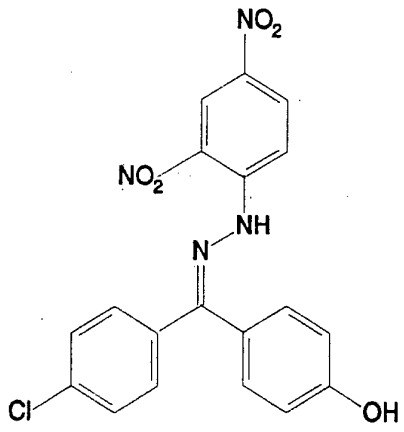
Other hydrazones have been synthesized and tested for activity against tumor cells in accordance with this invention. The molecular structures of these compounds are shown below:

4-Hydroxybenzophenone-2,4-dinitrophenylhydrazone (I-23) wherein the benzophenone is substituted with a single hydroxy *para* to the hydrazone linkage.



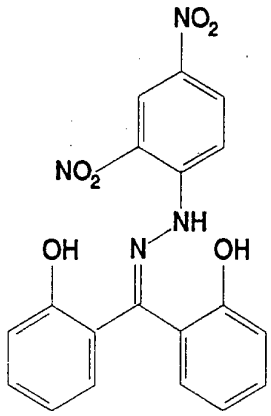
Empirical Formula: $C_{19}H_{14}N_4O_5$
Molecular Weight: 378
Melting Point: 224 - 225° (lit 226-227°C)
Solubility: ethanol, acetone, acetic acid
Physical Description: red crystals
Ref: G.D. Johnson, J. Amer. Chem. Soc., 75, 2720, (1953)

4-Hydroxy-4'-chlorobenzophenone-2,4-dinitrophenylhydrazone (I-25) wherein the benzophenone is substituted with a halogen and a hydroxy.



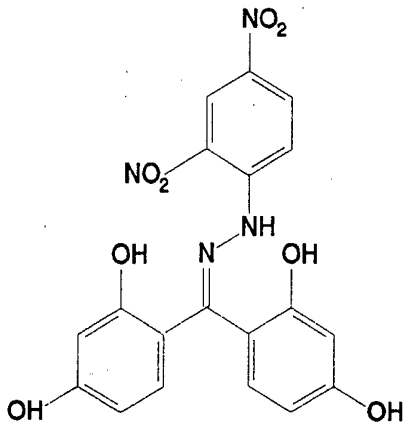
Empirical Formula: $C_{19}H_{13}O_5N_4Cl$
Molecular Weight: 412.5
Melting Point: 192 - 193°
Solubility: acetic acid, acetone, ethanol
Physical Description: red crystals
Ref: V.P. Malik and G.S. Sharma, J. Sci. Ind. Res. (India) 1513, 633-5 (1956).

2,2'-Dihydroxybenzophenone-2,4-dinitrophenylhydrazone (I-29) wherein the benzophenone is substituted on each ring of the benzophenone with a hydroxy in the *ortho* position.



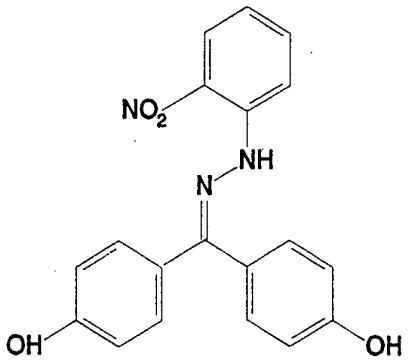
Empirical Formula: $C_{19}H_{14}N_4O_6$
Molecular Weight: 394
Melting Point: 270 - 271°
Solubility: ethanol, acetone, methanol
Physical Description: red needles
Anal. calc.: C, 57.86; H, 3.55; N, 14.21 Found: C, 57,85; H, 3.54; N, 14.34

2,2', 4,4'-Tetrahydroxybenzophenone-2,4-dinitrophenylhydrazone (I-37) wherein the benzophenone is substituted with hydroxy at both the *ortho* (2,2') and *para* (4,4') positions relative to the hydrazine linkage.



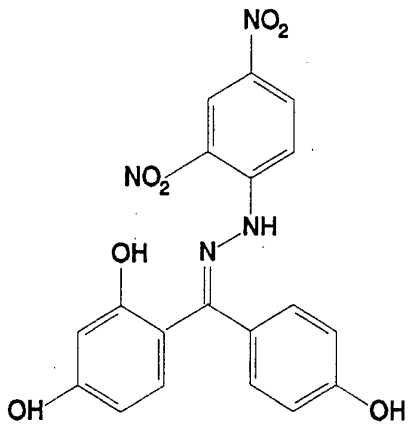
Empirical Formula: $C_{19}H_{14}O_8N_4$
Molecular Weight: 426
Melting Point: 292 - 293°
Solubility: acetic acid, ethanol, dimethylsulfoxide
Physical Description: red needles
Anal. calc.: C, 53.52; H, 3.28; N, 13.14 3.25; N, 13.73.
Found: C, 53.22; H, 3.25; N, 13.73

4,4'-Dihydroxybenzophenone-2-nitrophenylhydrazone (I-41) wherein the phenyl has only a single 2-nitro substitution, and the benzophenone is hydroxy *para* substituted.



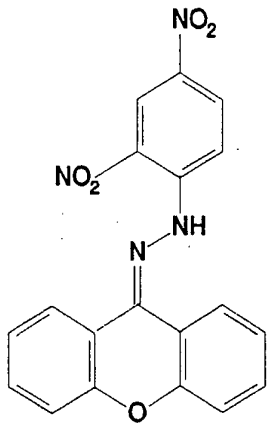
Empirical Formula: $C_{19}H_{15}N_3O_4$
Molecular Weight: 349
Melting Point: 234 - 236°
Solubility: acetic acid, acetone, ethanol
Physical Description: red crystals
Anal. calc.: C, 64.09; H, 4.45; N, 12.46 Found: C, 64.15; H, 4.51; N, 12.67.

2,4,4'-Trihydroxybenzophenone-2,4-dinitrophenylhydrazone (I-49)



Empirical Formula: $C_{19}H_{14}N_4O_7$
Molecular Weight: 410
Melting Point: 285°
Solubility: acetic acid, acetone, ethanol
Physical Description: red crystals
Anal. calc.: C, 55.60; H, 3.41; N, 13.65 Found: C, 54.89; H, 3.55; N, 13.73

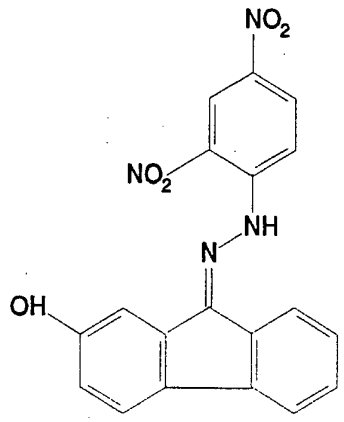
Xanthone-2,4-dinitrophenylhydrazone (I-75) wherein the aryl structure is an unsubstituted xanthone.



Empirical Formula: $C_{19}H_{12}N_4O_5$
Molecular Weight: 376
Melting Point: 276 - 277°C (lit 278°C)
Solubility: dimethylsulfoxide
Physical Description: Red crystals

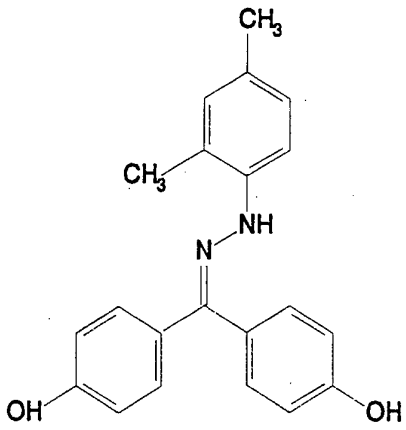
N. Campbell, S.R. Mc Callum and D.J. MacKenzie, J. Chem. Soc., 1957, 1922.

2-Hydroxyfluoren-9-one-2,4-dinitrophenylhydrazone (I-79) wherein the aryl ring structure is a fluoren substituted with a hydroxy on one of six membered rings of the fluoren.



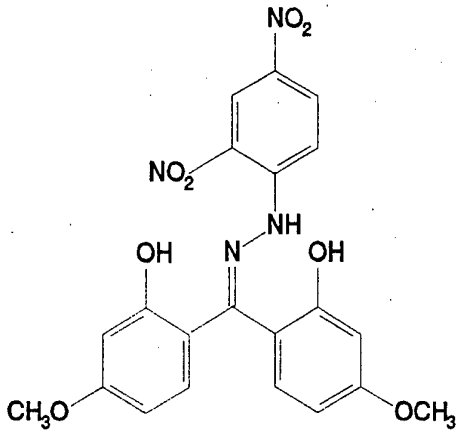
Empirical Formula: $C_{19}H_{12}N_4O_5$
Molecular Weight: 376
Melting Point: >270°
Solubility: dimethylsulfoxide
Physical Description: red crystals
Anal. calc. for: C, 60.63; H, 3.19; N, 14.89 Found: C, 60.60; H, 3.10; N, 14.64

4,4'-Dihydroxybenzophenone-2,4-dimethylphenylhydrazone (I-87) wherein the phenyl ring is substituted with the lower alkyl methyl.



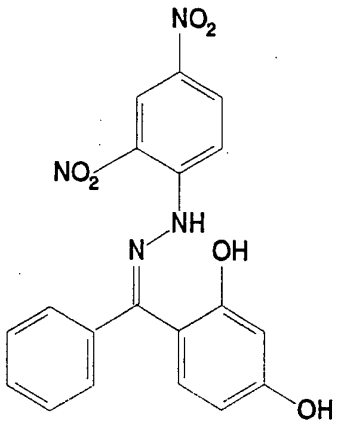
Empirical Formula: $C_{21}H_{20}N_2O_2$
Molecular Weight: 332
Melting Point: 145 - 146°
Solubility: acetic acid, dimethylsulfoxide, ethanol
Physical Description: yellow powder
Anal. calc.: C, 75.90; H, 6.02; N, 8.43 Found: C, 75.74; H, 6.02; N, 8.25

4,4'-Dimethoxy-2,2'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone (I-111) wherein the benzophenone is substituted with both hydroxy and lower alkoxy (methoxy) on each of the six membered benzophenone rings.



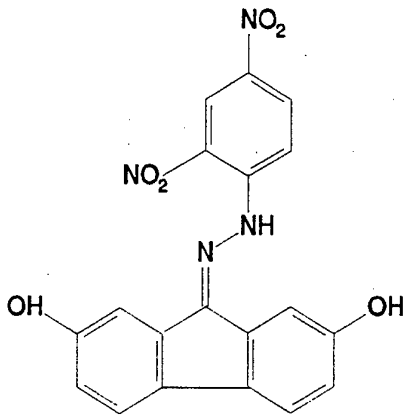
Empirical Formula: $C_{21}H_{18}N_4O_8$
Molecular Weight: 454
Melting Point: 218 - 219°
Solubility: acetic acid, acetone, ethanol
Physical Description: red crystals
Anal. calc.: C, 55.50; H, 3.96; N, 12.33 Found: C, 55.43; H, 3.95; N, 12.28

2,4-Dihydroxybenzophenone-2,4-dinitrophenylhydrazone (I-131) wherein the benzophenone is substituted with more than one hydroxy on only one of the six membered rings of the benzophenone.



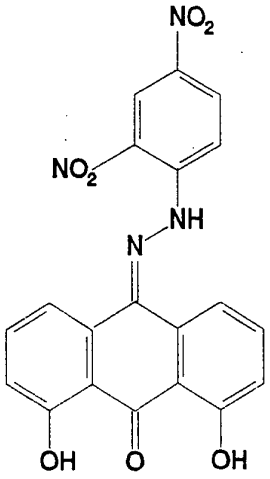
Empirical Formula: $C_{19}H_{14}N_4O_6$
Molecular Weight: 394
Melting Point: 304 - 304°
Solubility: acetone, ethanol, methanol
Physical Description: red crystals
Anal. calc.: C, 57.86; H, 3.55; N, 14.21 Found: C, 57.73; H, 3.51; N, 14.19

2,7-Dihydroxyfluoren-9-one 2,4-dinitrophenylhydrazone (I-147) wherein the fluoren is substituted on each of the six membered rings of the fluoren, in this case with a hydroxy substitution.



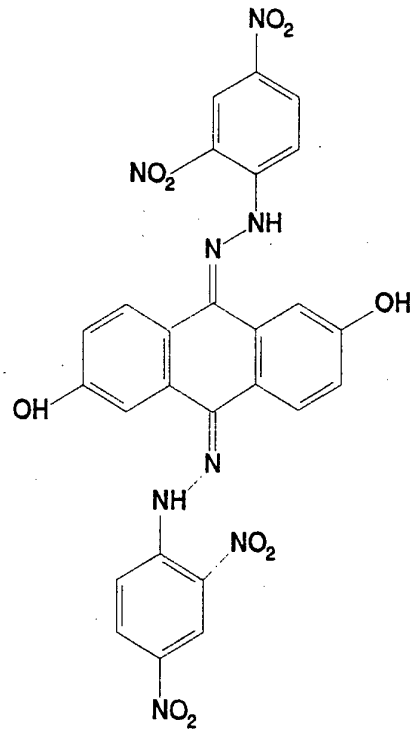
Empirical Formula: $C_{19}H_{14}N_4O_6$
Molecular Weight: 394
Melting Point: 304 - 304°
Solubility: acetone, ethanol, methanol
Physical Description: red crystals
Anal. calc.: C, 57.86; H, 3.55; N, 14.21 Found: C, 57.79; H, 3.51: N, 14.19

1,9-Dihydroxyanthraquinone-10-(2,4-dinitrophenylhydrazone) (KT-II-27) wherein the anthraquinone is substituted with more than one hydroxy, in this example on different rings of the anthraquinone.



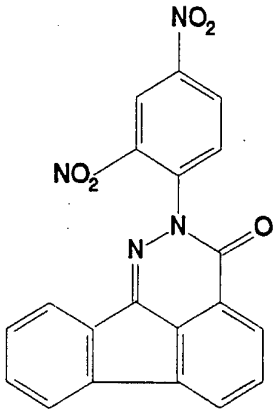
Empirical Formula: $C_{20}H_{12}N_4O_7$
Molecular Weight: 420
Melting Point: >280°
Solubility: dimethylsulfoxide
Physical Description: reddish-brown
Anal calc.: C, 57.86; N, 3.55; N, 14.21 Found: C, 57.78; H, 3.51; N, 14.19

2,7-Dihydroxyanthraquinone-bis-2,4-dinitrophenylhydrazone (B-193)



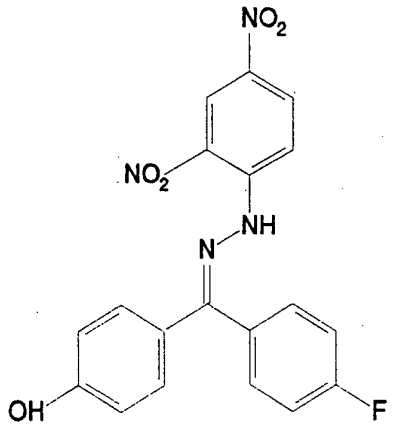
Empirical Formula: $C_{26}H_{16}N_8O_{10}$
Molecular Weight: 600
Melting Point: 348°C (dec)
Solubility: dimethylsulfoxide, dimethylformamide
Physical Description: red crystals
Anal. calc.: C, 52.00; H, 2.66; N, 18.66 Found: C, 51.25; H, 2.59; N, 18.05

2-(2,4-Dinitrophenyl)indano[1,2,3-*de*]-2*H*-phthalazinone (B-181)



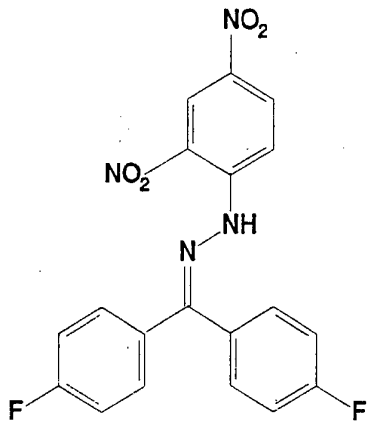
Empirical Formula: $C_{20}H_{10}N_4O_5$
Molecular Weight: 386
Melting Point: 273 - 274°C
Solubility: dimethylsulfoxide
Physical Description: deep red crystals
Anal. calc.: C, 62.17; H, 2.59; N, 14.50 Found: C, 61.63; H, 2.64; N, 14.17

4-Fluoro-4'-hydroxybenzophenone-2,4-dinitrophenylhydrazone (II-61) wherein the benzophenone is substituted with both hydroxy and halogen.

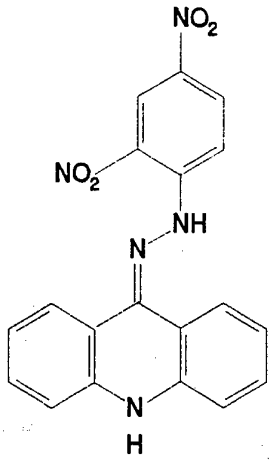


Empirical Formula: $C_{19}H_{13}N_4FO_5$
Molecular Weight: 396
Melting Point: 231 - 232°
Solubility: ethanol, acetonitrile, acetone
Physical Description: red crystals
Anal. calc.: C, 57.57; H, 3.28; N, 14.14; F, 4.79
Found: C, 57.59; H, 3.20; N, 14.00; F, 4.76

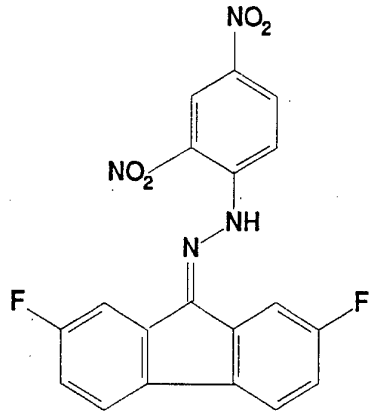
4,4'-Difluorobenzophenone-2,4,dinitrophenylhydrazone (I-26) wherein the benzophenone is substituted with at least two halogens, one on each of the benzophenone rings.



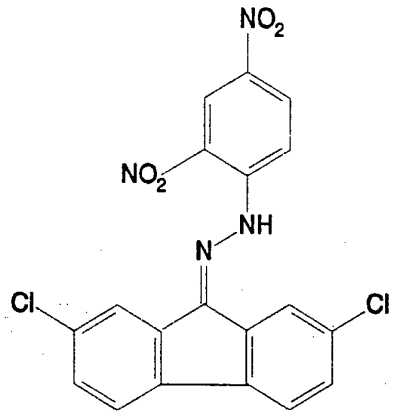
Acridone 2,4-dinitrophenylhydrazone (B-194)



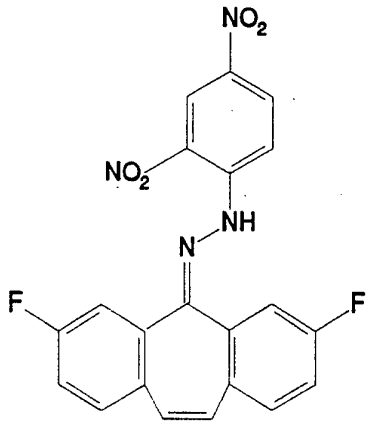
2,7-Difluorofluoren-9-one-2,4-dinitrophenylhydrazone (B-192) wherein the fluoren is substituted with at least two halogens.



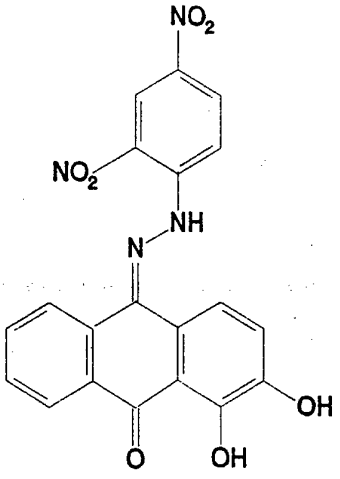
2,7-Dichlorofluoren-9-one-2,4-dinitrophenylhydrazone (B-197)



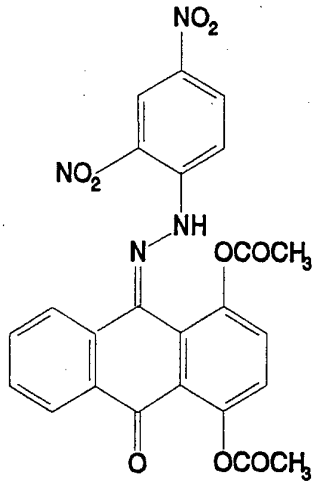
Difluorodibenzosuberenone-2,4-dinitrophenylhydrazone (B-198) wherein the dibenzosuberenone is substituted with at least two halogens, in this instance fluorine.



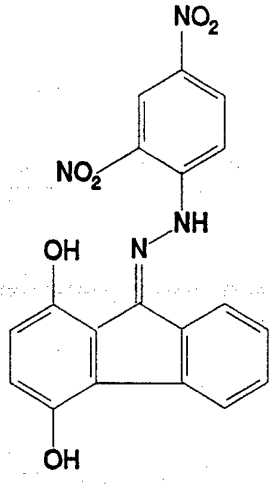
1,2-Dihydroxyanthraquinone-10-(2,4-dinitrophenylhydrazone) (B-207) wherein the anthraquinone is dihydroxy substituted, in this example on the same ring of the anthraquinone structure.



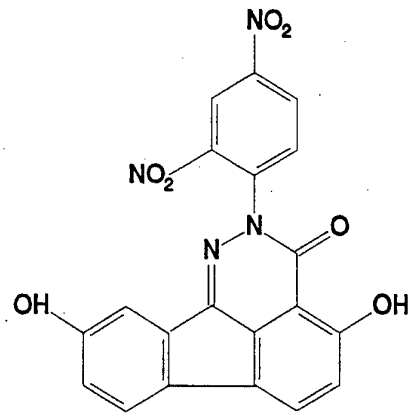
6,9-Diacetoxyanthraquinone-10-(2,4-dinitrophenylhydrazone) (KT-II-95) wherein the anthraquinone is substituted with at least one acetoxy, in this example two acetoxys on the same ring of the anthraquinone.



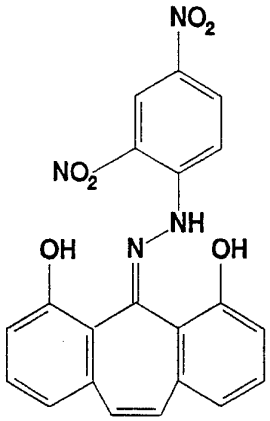
1,4-Dihydroxyfluoren-9-one-2,4-dinitrophenylhydrazone (B-200)



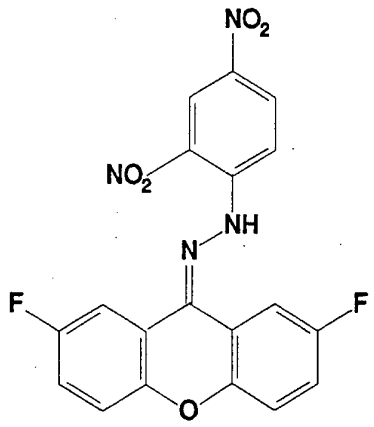
2-(2,4-Dinitrophenyl)-5,10-dihydroxy-indano[1,2,3-*de*]-2*H*-phthalazinone
(B-187)



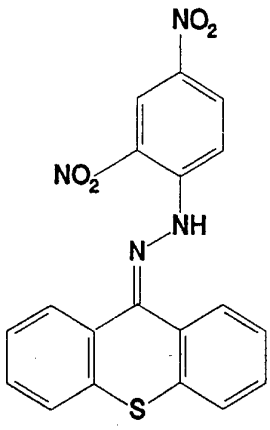
Dihydroxydibenzosuberenone-2,4,dinitrophenylhydrazone
(B-197)



2,7-Difluoro-xanthone-2,4-dinitrophenylhydrazone
(I-76)



Thioxanthone-2,4-dinitrophenylhydrazone
(B-195)



SYNTHESIS OF MODIFIED HYDRAZONES

The following ketone and hydrazine starting materials were obtained from Aldrich or Sigma Chemical Companies.

TABLE IIA

COMPOUND	STARTING MATERIALS
I-23	4-Hydroxybenzophenone 2,4-dinitrophenylhydrazine
I-25	4-Hydroxy-4'-chlorobenzophenone 2,4-dinitrophenylhydrazine
I-29	2,2'-Dihydroxybenzophenone 2,4-dinitrophenylhydrazine
I-37	2,2'4,4'-Tetrahydroxybenzophenone 2,4-dinitrophenylhydrazine
I-41	4,4'-Dihydroxybenzophenone 2-nitrophenylhydrazine
I-49	2,4,4'-Trihydroxybenzophenone 2,4-nitrophenylhydrazine
I-75	Xanthone 2,4-nitrophenylhydrazine
I-79	2-Hydroxyfluoren-9-one 2,4-nitrophenylhydrazine
I-87	4,4'-Dihydroxybenzophenone 2,4-dimethylphenylhydrazine
I-111	4,4'-Dimethoxy-2,2'-dihydroxy-benzophenone 2,4-dinitrophenylhydrazine
I-131	2,4-Dihydroxybenzophenone 2,4-dinitrophenylhydrazine
I-147	2,7-Fluoren-9-onediol diacetate 2,4-dinitrophenylhydrazine
KT-II-27	1,9-Dihydroxyanthraquinone 2,4-dinitrophenylhydrazine
B-181	Fluoren-9-one-1-carboxylic acid 2,4-dinitrophenylhydrazine

(TABLE IIA cont'd)

B-193	2,7-Dihydroxyanthraquinone 2,4-dinitrophenylhydrazine
II-61	4-Fluoro-4'-hydroxybenzophenone 2,4-dinitrophenylhydrazine

Examples of other starting materials are shown in Table IIB:

TABLE IIB

COMPOUND	STARTING MATERIALS
I-26	4,4'-Difluorobenzophenone 2,4-dinitrophenylhydrazine
B-194	Acridone 2,4-dinitrophenylhydrazine
B-198	Difluorodibenzosuberenone 2,4-dinitrophenylhydrazine
KT-II-95	6,9-Diacetoxyanthraquinone 2,4-dinitrophenylhydrazine
B-197	Dihydroxydibenzosuberenone 2,4-dinitrophenylhydrazine
I-76	2,7-difluoroxanthone 2,4-dinitrophenylhydrazine
B-195	Thioxanthone 2,4-dinitrophenylhydrazine

The following general procedures were employed for the synthesis of the phenylhydrazones unless described otherwise:

Procedure 1: 4-Nitro- or 2,4-dinitrophenyl hydrazine (0.0016 moles) was suspended in 5 ml of methanol. The 4-nitrophenyl hydrazine was used if the 4-nitrophenylhydrazone product was being prepared while the 2,4-dinitrophenylhydrazine was used if a 2,4 dinitrophenylhydrazone was being prepared. Dropwise, 0.4-0.5 ml of concentrated sulfuric acid was cautiously added with stirring and the warm solution filtered. A

solution of the carbonyl compound (0.0016 moles) in 5-20 ml of methanol or ethanol was added dropwise to twice the above phenylhydrazine solution. If no solid separated from the reddish colored solution within 10 minutes, the solution was carefully diluted with 5-20 ml of 2N sulfuric acid. The solid was collected by suction filtration and washed with a little cold methanol. The derivative was recrystallized from ethanol, methanol, dimethylformamide or dilute acetic acid.

Procedure 2: 4-Nitro- or 2,4-dinitrophenyl hydrazine 2.5 g (0.016 moles) was dissolved in 30 ml of 85% phosphoric acid. The solution was diluted with 20 ml of 95% ethanol, allowed to stand and then filtered.

The carbonyl compound (0.008 moles) was dissolved in 10-40 ml of ethanol and the calculated volume of the above reagent added, to produce a 2:1 ratio of the 4-nitro- or 2,4-dinitrophenyl hydrazine and ketone, respectively. If a precipitate did not form immediately, it was diluted with a little water. The derivative was collected and recrystallized.

General Procedure for compounds: I-23, I-25, I-29, I-37, I-41, I-49, I-75, I-79, I-131, II-61, II-27, B-193. 2-Nitro or 2,4-dinitro or 2,4-dimethylphenyl hydrazine (0.03 mol) was suspended in methanol (60 ml). To this was added concentrated sulfuric acid (4 ml) cautiously with stirring and heated at 65°C until a homogeneous solution occurred. A solution of the carbonyl compound (0.022 mol) (See Table II) in methanol (60 ml) was added dropwise for 10 minutes and heated at 65°C for 1 hour. It was then cooled, concentrated, water (200 ml) was added, filtered, washed with 5% aqueous sodium bicarbonate solution (100 ml), washed with water (200 ml). The solid obtained was recrystallized from either ethanol or dimethyl formamide. (Note: For compounds KT-II-27 and B-193, the reaction mixture was heated for 24 hours).

Procedure for Making I-111

2,4-Dinitrophenylhydrazine (4.8 g, 0.024 mol) was dissolved in 85% phosphoric acid (60 ml). The solution was diluted with ethanol (40 ml) and heated at 80°C. The solution of 2,2′-dihydroxy-4,4′-dimethoxybenzophenone (5.5 g, 0.02 mol) in ethanol (40 ml) was added for 10 minutes and the heating was continued at 80°C for an additional 4 hours then cooled. The solvent was evaporated and the slurry was diluted with water (200 ml). The solid was filtered, washed with 5% aqueous sodium bicarbonate (100 ml), washed with water and recrystallized from ethanol.

2,7-Dihydroxyfluoren-9-one-2,4-dinitrophenylhydrazone (I-147)

A mixture of 2,7-fluoren-9-onediol diacetate (3.0 g, 0.01 mol), 2,4-dinitrophenylhydrazine (2.52 g, 0.13 mol) and concentrated sulfuric acid (5 ml) in methyl alcohol (250 ml) was refluxed for 5 hours. The mixture was then cooled and the solvent was evaporated. The solution was poured into water (250 ml) filtered and recrystallized from dimethyl formamide, yield: 3.0 g (76%). (Ref: R. Grosserode, P.S. Tobin and M.S. Wheeler, Synthetic Communications, 377, (1976))

Synthesis of (B-181)

A mixture of fluoren-9-one-1-carboxylic acid (1.12 g, 0.005 mol), 2,4-dinitrophenylhydrazine (1.25 g, 0.00625 mol) and pyridine (5 ml) was refluxed for 5 hours and then cooled. The mixture was poured into ice-water, filtered and dried. The crude product was then treated with thionyl chloride (50 ml) and refluxed for 4 hours. Excess thionyl chloride was distilled off and the solid obtained was recrystallized from dimethyl formamide. Yield: 0.7 g (58%), m.p. 273 - 274°C.

EXAMPLE IV

Cytotoxicity of Compounds

The cytotoxic activity of many of the compounds of the present invention was tested against tumor cells derived from a variety of human cancers by the test assay described in Example I above. As described in Example I, the IC_{50} for A-007 against non-estrogen-dependent tumors derived from human breast cancers was determined to be 10 μg/ml. The data shown in Table III indicates that some of the compounds disclosed herein exhibited even more potent cytotoxic activity than A-007 itself.

TABLE III

COMPOUND	Cytotoxic Activity TYPE OF CANCER	IC_{50} μg/ml(AVG)
I-23	Colon (1)	>5.0
	Melanoma (1)	>5.0
	Ovarian (1)	>5.0
	Breast (1)	>5.0
I-131	Breast (1)	0.2
	Kidney (1)	2
	Lymphoma (1)	>5
I-25	Breast (8)	8.8
	Colon (3)	15
	Thyroid (2)	7
	Stomach (1)	5
I-29	Breast (12)	6.8
	Colon (5)	8
	Ovary (1)	5
	Stomach (1)	20
	Endometrium (1)	5
	Sarcoma (1)	5
	Pancreas (3)	5
	Melanoma (1)	5
	Lung (2)	5
	Thyroid (2)	5
I-37	Lung (1)	7
	Breast (1)	>10
	Colon (7)	7

(TABLE III cont'd)

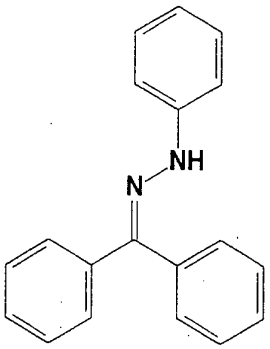
II-61	Sarcoma (1)	2
	Ovarian (2)	>5
	Lung (2)	4
	Breast (5)	3.4
	Colon (3)	>5
	Endometrium (1)	>5
I-147	Ovarian (1)	0.08
	Colon (1)	0.05
	Breast (1)	<0.005
I-79	Breast (6)	7.5
	Stomach (1)	5
	Pancreas (1)	3
	Thyroid (1)	>5
	Nasal (1)	>5
	Colon (1)	>5
I-75	Ovarian (1)	0.005
	Colon (1)	0.005
	Breast (1)	<0.005
KT-II-27	Lung (1)	0.05
	Breast (9)	0.012
	Lymphoma (2)	0..09
	Colon (6)	0.015
	Renal (1)	0.01
I-111	Renal (1)	0.05
	Breast (13)	2
	Colon (4)	4.5
	Sarcoma (1)	>5
B-193	Lung (1)	1
	Colon (1)	0.5
	Lymphoma (1)	5
	Renal (1)	>0.5
	Breast (5)	>1.5
I-41	Colon (4)	4.8
	Breast (16)	3.9
	Sarcoma (1)	5
I-87	Breast (2)	10

The specific compounds disclosed above are effective in inhibiting the growth of estrogen-independant tumor cells. The data shown in Table III show that the IC_{50} values for the compounds against cultured tumor cells derived from various human cancers were in the range of 0.05-20 μg/ml. The most potent

compounds included KT-II-27, I-111 and B-193. The amount of KT-II-27 required to produce 50% inhibition of the lung tumor cells tested was 0.05μg/ml. Because these compounds are cytotoxic, it will be apparent to one skilled in the art that any amount of one of these compounds will be an effective amount sufficient to inhibit growth of tumor cells. Increasing the dosage of the compound will produce a greater inhibition of growth. The therapeutic index for a particular hydrazone may be determined as described in Example V. As described in Example VI, the topical application of an ointment containing 0.25% of A-007 was effective in inhibiting growth of human tumors.

While not wishing to be bound by theory, it is believed that the compounds of the present invention have their unexpected cytotoxic effect against tumor cells because of their structural similarities. The mechanism(s) of action of these compounds is presently unknown, however it is believed that the compounds may exert their cytotoxic effect by directly intercalating into DNA. Antiestrogenic compounds (including A-007) are thought to disrupt estrogen utilization and to interact with the DNA of the cell, disrupting normal cell function. Certainly, the hydrazones described herein are effective against tumor cells (such as those derived from human kidney, stomach and lung cancers) which do not have estrogen receptors, and so the formation of an ER/hydrazone complex is not required for cytotoxicity.

The compounds of the present invention comprise three primary moieties: (1) a phenyl ring joined through (2) a hydrazine linkage to (3) an aryl ring structure which comprises at least a bicylic ring structure. Thus the common structure may be represented as



The steric bulk of the aryl ring (3) is believed to inhibit free rotation about the N-N bond (2), which diminishes movement of the phenyl ring (1) relative to the aryl ring. Although not wanting to be bound by theory, the inventor has unexpectedly found that this spatial stabilization appears to enhance the cytotoxic activity of these compounds.

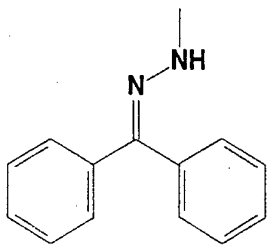
The present invention also encompasses a number of variations on the basic structure shown above. The variant compounds include those with modified aryl ring structures which may include heterocyclic ring structures, modified hydrazine linkages and substitutions on both the phenyl and aryl-based moieties.

The compounds of the present invention may thus be represented as:

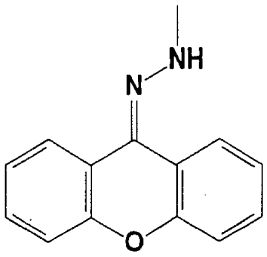
$$X - R^1$$

wherein X is the phenyl ring and R^1 is selected from the following groups:

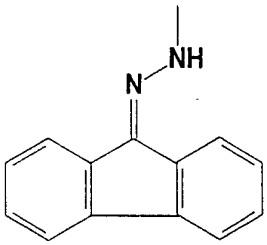
benzophenone hydrazone



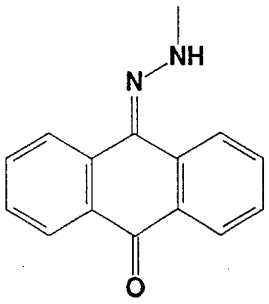
xanthone hydrazone



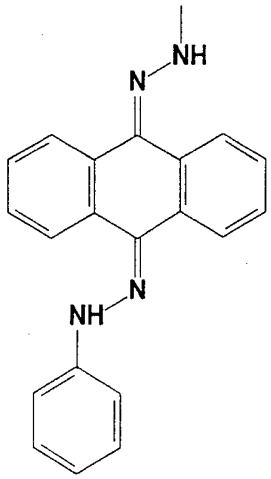
fluoren hydrazone



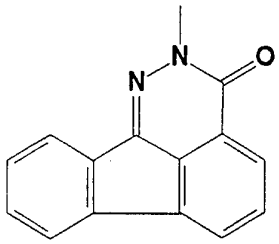
anthraquinone hydrazone



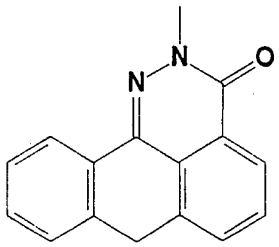
anthraquinone dihydrazone



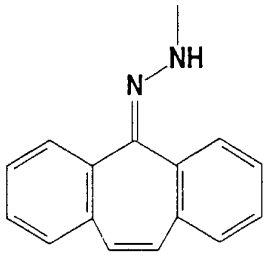
indano [1,2,3-*de*]-2*H*-phthalazinone



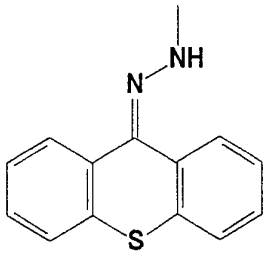
tetralin [1,2,3-*de*]-2*H*-phthalazinone



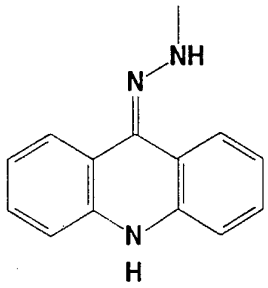
dibenzosuberenone hydrazone



thioxanthone hydrazone



acridone hydrazone



In preferred embodiments of the present invention, the phenyl ring may be substituted with one or more of the following groups: nitro, lower alkyl and trihalomethyl, preferably nitro and trihalomethyl, most preferably nitro. Suitable combinations also include lower alkyl and trihalomethyl, lower alkyl alone, or triahlomethyl alone. At least some of these groups are believed to have a polarizing effect on the molecule and to influence the resonance of the phenyl ring structure. Thus, the compounds of the present invention may include nitrophenyl rings (for example in

I-41), dinitrophenyl rings (for example in KT-II-27) and dimethylphenyl rings (for example in I-87).

In other preferred embodiments, the aryl ring structure (R^1) is substituted with one or more hydroxy, lower alkoxy, acetoxy or halogen groups. In other embodiments the aryl ring structure is substituted with one or more substituents selected from the following groups: hydroxy alone; hydroxy and lower alkoxy; hydroxy, lower alkoxy, and acetoxy; lower alkoxy alone; lower alkoxy and acetoxy; lower alkoxy, acetoxy and halogen; acetoxy alone; acetoxy and halogen; and halogen alone. The lower alkoxy is methoxy or ethoxy, and the halogen is preferably F or Cl. Thus, for example, the hydrazone or phthalazinone structure may be substituted with a single hydroxy group (I-23), two hydroxy groups (I-29) or four hydroxy groups (I-37). In other examples, the aryl moiety may be substituted with one or two alkoxy groups (such as methoxy, which may further be combined with one or more hydroxy groups as in I-111) one or more halogens (for example, two halogens in I-26, B-192, B-197, and B-198), or a single halogen and a single hydroxy (II-61). The aryl moiety may be substituted with a substituted or unsubstituted phenylhydrazone (B-193). In yet other embodiments, the aryl group is substituted with one or more acetoxy groups (for example, two acetoxy groups as in KT-II-95).

While not wishing to be bound by theory, it is believed that the co-planarity between at least part of the aryl-based moiety (for example, the ring structures which form part of the hydrazone or phthalazinone moiety) and the phenyl ring contributes to the cytotoxicity of the claimed compounds. In some embodiments (such as I-25 and as typified by A-007) the component rings of the aryl moiety may be non-planar. In other embodiments, where the aryl-based moiety comprises a tricyclic ring structure, such as in B-181 and KT-II-27, the aryl ring groups are co-planar. The

co-planarity of the phenyl and aryl-based moieties may be enhanced by electrostatic and hydrogen bonds which serve to reduce rotation about the =N-N- linkage. Thus, in preferred embodiments, the inclusion of polarizing groups such as nitro, methyl and trifluoromethyl, on the phenyl ring may influence the electron distribution of the phenyl ring and the =N-N- linkage. Polar substitutions on the phenyl ring may also influence electrostatic interactions between the ring and the =N-N- linkage and/or the aryl-based structure.

Substitution of a hydrogen bonding group, such as hydroxy or acetoxy, in the 2 or 2′ position on benzophenone (as in I-29, I-111 or I-131) or in the 6 position on anthraquinone (KT-II-95) is also believed to increase molecular planarity by optimizing a hydrogen bonding interaction with the NH of the hydrazine linkage. This is a substitution *ortho* to the ring carbon that is bonded to the carbon that forms a double bond with the hydrazine nitrogen. For example, in the benzophenone of I-29, the aryl ring is substituted at the 2 position on the benzophenone, which is the position *ortho* to the 1 position, which is bonded to the carbon (formerly of a carbonyl) that forms the hydrazone linkage. The acetoxy substitution on the anthraquinone in KT-II-95 occupies an analogous position *ortho* to the -C=N-NH- linkage.

The planarity of the aryl structure is even more evident when the aryl group is an indano phthalazinone (B-187). The six-membered ring that includes N-N-C=O covalently planarizes the aryl structure, instead of relying on hydrogen bonding interactions.

In other preferred embodiments, the aryl-based structure is substituted with polar groups that facilitate hydrogen bonding of the ring with the =N-N- linkage as typified by the hydrogen bonding drawn for A-007 in Figure 1. This hydrogen bonding stabilizes

the co-planarity between the aryl and phenyl moieties by reducing rotation around the =N-N- linkage which joins these two components of these molecules. Particularly preferred embodiments of this invention which show enhanced cytotoxic activity possess substitutions on both the phenyl and aryl-based ring structures, leading to especially enhanced co-planarity between these moieties.

It will be appreciated by one skilled in the art that the specific compounds shown above are only examplary and do not limit the present invention. The present invention encompasses a variety of compounds not specifically shown here which possess similar cytotoxic properties.

EXAMPLE V

The cytotoxicities of the compounds of the present invention were evaluated in granulocyte macrocyte colony forming cells (FDCP-1), and in MDA-MB-435 human estrogen-independent breast cancer cells.

The well characterized FDCP-1 cell line was provided by Dr. Joel S. Greenberger, University of Massachusetts Medical Center. Interleukin 3, necessary for the growth of this cell line, was obtained from conditioned medium of murine myelomonocytic leukemia cell lines. Briefly, murine FDCP-1 cells were grown in RPMI 1640 medium without glutamine, with 10% FBS at 37°C for ten (10) to fourteen (14) days. When roller cultures reached a density of approximately 10^6 cells/ml, cells were removed by centrifugation, dialyzed for 48 hours in distilled water, and then concentrated 5-fold by an Aquacide dehydration procedure (Calbiochem, San Diego, California). The concentrated conditioned medium was passed through a 0.2 μm Millipore sterilization filter into McCoy's Medium 5A supplemented with 10% FBS (Flow Laboratories, Rockville, Maryland). The final concentration of conditioned medium was 15%. The FDCP-1 cell lines were incubated at 37°C in an atmosphere of 5% CO_2 and air.

The MDA-MB-435 human breast carcinoma cell line was obtained from Dr. R. Callieau, Department of Medicine, M.D. Anderson Cancer Center (Houston, TX). This cell line was originally isolated from a pleural effusion sample, and is estrogen receptor negative. A-007 was tested against the MDA-MD-435 and FDCP-1 cell lines. Cells tested were maintained on plastic in complete Eagle's minimal essential medium supplemented with 5% FBS, sodium pyruvate, nonessential amino acids, L-glutamine, and vitamins. Cells that had been cultured for long periods were discarded, renewing the cultures from frozen stocks after every ten in vitro passages. Unless otherwise specified, all tissue culture reagents were obtained from GIBCO Laboratories (Grand Island, NY).

Assay for In Vitro Antiproliferative Effect in Human Tumor Cell Lines

MDA-MB-435 cells were suspended in medium and seeded at 2,000 to 3,000 cells/well in 96-well tissue culture plates. After an attachment period of eighteen (18) hours for the anchoring dependent lines, the cells were fed with fresh medium (controls) or with medium containing different concentrations of A-007. After an additional 96 hours, the antiproliferative activity was determined by monitoring the number of viable cells. This was accomplished by the tetrazolium **(MTT, N2128)** assay (Alley et al., Cancer Research 48:589-601, 1988.) The MTT procedure has been shown to correlate with cellular protein, dye exclusion, and clonogenic assays under a variety of culture and assay conditions. After incubation for 2-4 hours with 40 μl of MTT, (0.2% in phosphate buffer saline) the cells were lysed in DMSO, and the conversion of MTT to formazan by metabolically viable cells was monitored by a Titertek Multiskan 96-well microliter plate reader at 570 nm (Flow Laboratories, Helsinki, Finland). Inhibition of cell growth by A-007 was calculated by the formula:

$$\text{cytostasis (\%)} = [1 - A/B)] \times 100$$

where A= the absorbance at 570nm of treated cells and B= the absorbance at 570nm of control cells.

Assay for *In Vitro* Myelotoxicity

The FDCP-1 cells were harvested during exponential growth. Cell counts were performed using a hemocytometer. The MTT assay was performed as previously described above. Briefly, 2,000 cells were plated in each well of a 96-well microwell flat bottomed plate. This seeding density was chosen to ensure that the cells would be in an exponential growth phase at the end of the four (4) day incubation period. Cells were inoculated with 0.2 ml of McCoy's Medium 5A, supplemented with 10% FBS and 15% WEHI-3 conditioned medium. A-007 and doxorubicin were tested each at five (5) to ten (10) concentrations, covering a 1- to 2-log concentration range, to determine IC_{50} data for A-007 as compared to doxorubicin. After four (4) days of incubation, 0.2 mg (40 μl of 5 mg/ml) of MTT was added to each well and incubated at 37°C for an additional two (2) hours. Plates were then centrifuged at 450 x g for five (5) minutes. The medium was then aspirated gently from all plates, taking care not to disturb the cells at the bottom of the wells. Dimethyl sulfoxide (150 μl) was finally added to each well, and the plates were placed on a shaker for ten (10) minutes to solubilize the formazan product. The plates were read immediately thereafter, at 570 nm on an MR 5,000 Micro titer plate reader. Absorbance levels from drug tested cells were compared with untreated control absorbance values. Each test incorporated a cell dose inoculum, and the true control was determined by an extrapolation.

Drug Survival Curves

Drug dose response data were obtained by adding drugs to the cultures after twenty-four (24) hours of incubation, resulting in a four (4) day exposure. A 6- to 8-fold drug concentration range was used. Drug survival curves (FIG. 2 and 3) were

generated by plotting surviving fractions against drug concentrations using a linear scale. Drug sensitivity was measured by determining the dose response curve.

Results

These experiments allowed pre-clinical therapeutic indexes for A-007 to be determined relative to the IC_{50} for the FDCP-1 murine hematopoietic progenitor granulocyte macrophage colony forming cell (GMCFC) line. As shown in FIG. 2, the IC_{50} for A-007 on FDCP-1 cells was determined to be 9.52μg/ml. The IC_{50} for A-007 on MDA-435 was determined to be 2.52μg/ml. The therapeutic index (TI) (IC_{50} FDCP-1/IC_{50} MDA-435) for A-007 on MDA-435 cells was therefore determined to be 3.77 (versus FDCP-1) as shown in FIG. 2. Doxorubicin has a T.I. of 0.17 in the MDA-MB-435 cell line, as shown in FIG. 3. In contrast, A-007 has a T.I. of 3.77. A-007's T.I. supports its relatively non-myelotoxic and yet useful therapeutic activities toward breast cancer.

Calculated and plotted values for growth inhibition in doxorubicin are expressed as the mean value plus or minus the standard error of the mean in FIG. 3.

EXAMPLE VI

Clinical Responses

Sixteen (16) evaluable patients with advanced breast cancer spread to scalp or chest wall, who had failed on radiation therapy and systemic chemotherapy, and/or tamoxifen therapy, were treated with a 0.25% (0.25 g/100g) ointment of A-007 applied topically twice daily to the involved sites. The composition of the ointment was 0.25 g of A-007, 2 ml benzyl alcohol, and 98.2 g of 1,2-propylene glycol to provide the 0.25% concentration of drug in the ointment. This ointment was applied in a sufficient amount to cover the affected area, and treatment was continued for 6 weeks to 8 months, depending on the subject response.

The results are summarized in Table IV:

TABLE IV

Patients (16)	Age (Avg)	Menopause Status	ER/PgR Status	Response (Avg. Response Time in Months)
2	33	Pre	-/-	NR; CR (5)
5	67	Post	+/+	PR (5.2)
2	54	Post	-/-	NR
5	64	Post	-/-	PR (4.3)
2	66	Post	-/-	CR (6)

Responses occurred within 2-6 weeks and lasted 2-8 months. A response was a complete response (CR) if the treated lesions disappeared; a partial response (PR) if $\geq 50\% < 100\%$ of the treated lesions disappeared; and no response (NR) if the treated lesion demonstrated <50% disappearance. Only the treated lesions were measured. No toxicities (hematological or blood chemistry) or allergies have been noted from topical A-007. As noted in Table IV, eleven treated patients were classified as ER-/PgR- (<3/<5) and therefore as having estrogen-independent breast cancer. Of these patients, three showed a complete response to treatment with A-007, five showed a partial response to the treatment, and three showed no response. This data indicates that A-007 is active in estrogen-independent breast cancers, as well as estrogen-dependent breast cancers.

The ointment is preferably made with 0.25-10% active ingredient, in a base such as propylene glycol that allows the active ingredient to be applied topically to the skin.

EXAMPLE VII

The described compounds may be included in inert preparations such as gelatin capsules, and pressed tablets with sucrose, cellulose or other inert substances for dissolution and absorption. The

materials may also be included in inert oils for injection or solubilized as salts for aqueous infusions.

These compounds may be administered on a daily basis to patients. Studies have shown that A-007 is absorbed orally from a propylene glycol solution (100 mg/ml) to produce a peak serum level of 210 ng/ml after about 20 hours. Plasma levels of A-007 versus time in hours is shown in FIG. 4.

A subject (such as a patient or a test animal) would be treated systemically either by injecting the drug (such as KT-II-27) in doses of 0.2 g/m^2 intravenously, or by administering an oral dose of 250 mg/kg. This dose would be administered once per day for a period of 10 days. This procedure would then be repeated every 21 days. This dose can vary depending on the condition of the patient.

EXAMPLE VIII

Antiviral Activity of the Compounds

The compounds of the present invention can be shown to exhibit antiviral activity in addition to the cytotoxic activity demonstrated above. A CEM-SS human lymphocytic target cell line was maintained in RPMI-1640 medium (Gibco, Grand Island, NY) without phenol red and supplemented with 5% fetal bovine serum, 2mM L-glutamine and 50 μg/ml gentamicin (complete medium). Exponentially growing cells were pelleted and resuspended at a concentration of 2.0×10^5 cells/ml in complete medium. The Hatian variant of HIV, $HTLV\text{-}III_{RF}$ (3.54×10^6 SFU/ml) was used throughout. Frozen virus stock solutions were thawed immediately before use and resuspended in complete medium to yield 1.2×10^5 SFU/ml. The appropriate amounts of the pure compounds for anti-HIV evaluations were dissolved in 100% DMSO and then diluted in complete medium to the desired initial concentration (and with final DMSO content not exceeding 1%). All serial drug dilutions, reagent additions, and plate-to-plate transfers were carried

out with an automated Biomek 1000 Workstation (Beckman Instruments, Palo Alto, CA).

Additional testing details are as follows: Uninfected CEM-SS cells were plated at a density of 1 x 10^4 cells in 50 μl of complete medium. Diluted HIV-1 virus was then added to appropriate wells in a volume of 50 μl to yield a multiplicity of infection of 0.6. Appropriate cell, virus, and drug controls were incorporated in each experiment; the final volume in each microtiter well was 200 μl. Quadruplicate wells were used for virus-infected cells, and duplicate wells were used for uninfected cells. Plates were incubated at 37°C in an atmosphere containing 5% CO_2 for six (6) days. Subsequently, aliquots of cell-free supernatant were removed from each well using the Biomek, and analyzed for reverse transcriptase activity, p24 antigen production, and synthesis of infectious virions as described. Cellular growth or viability then was estimated on the remaining contents of each well using the MTT assay.

EXAMPLE IX

Estrogenic activity of the claimed compounds in the uterus was determined by employing the wet uterine weight of the immature (3 week old) Sprague Dawley rat. Rats were sacrificed after three consecutive days of daily intraperitoneal (i.p.) administration of test compounds at the specified doses. The mean control (unstimulated) uterine weight for a 21 day old rat was averaged at 74 mg. Five to ten animals were employed at each dose level. The purpose of this test was to determine the estrogenic activity of each of the test compounds. Estrogenic activity can be determined by comparing the net uterine weight of the test animals to which the compounds were administered with the mean weight of rats not being injected with the drug. All drugs and estrogens were dissolved in peanut oil. Controls received peanut oil alone.

Antiestrogen activity in rat uterii was determined by giving 0.64 μg of estradiol benzoate plus the test drug to immature Sprague Dawley rats at specified doses daily concomitantly i.p. for three days. Rats were sacrificed and uterii weighed. Estradiol benzoate alone produced a mean weight of 145 mg in this system. Five to ten animals were employed at each dose level.

The data in Table I concerning uterotrophic activity demonstrate the relative activities of the test compounds in stimulating uterine growth. Such stimulation of uterine growth is a measurement of the estrogenic activity of the test compounds. Such activity may be undesirable because it can cause side effects and may satisfy the estrogenic requirements of estrogen dependent tumor cells

The data in Table V also shows antiuterotrophic activity, and demonstrates the inability of the test compounds to inhibit stimulation of uterii, which have been already stimulated by administration of 0.64 μg of estradiol benzoate to the test animal. A Sprague Dawley rat receiving this dose of estradiol benzoate has a mean wet weight uterus of 145 mg. Percent inhibition of stimulation is measured by comparing the stimulated uterine weight (145 mg) with the uterine weight wherein estradiol benzoate and the test compound are administered concomitantly. Substantially no inhibition of stimulation occurs.

TABLE V

ANIMAL STUDIES I-29
UTEROTROPHIC ACTIVITIES IN IMMATURE FEMALE
(3 week old) SPRAGUE DAWLEY RATS

Drug Dose (μg)	UTEROTROPHIC ACTIVITY Mean wet wt. uterus (mg)	Ratio Drug/Estradiol
1	67	67/145
16	46	46/145

64	59	59/145
128	52	52/145

I-29

ANTI-UTEROTROPHIC ACTIVITY

In the presence of 60 μg of Estradiol Benzoate

Drug Dose (ug)	Mean wet wt. uterus (mg)	Control (mg)	Percent Inhibition
1	136	145	6
16	125	145	13
64	139	145	4
128	127	145	12

Having illustrated and described the principles of the present invention in many preferred embodiments, it should be apparent to those skilled in the art that the invention can be modified in arrangement and detail without departing from such principles. We claim all modifications coming within the spirit and scope of the following claims.

CLAIMS

1. A method of impairing growth of neoplastic cells, comprising the steps of:

exposing the neoplastic cells to an effective amount of a compound sufficient to inhibit growth of the neoplastic cells, wherein the compound is wherein X is phenyl substituted with one or more substituents selected from the group consisting of nitro, lower alkyl, and trihalomethyl, and R^1 is selected from the group consisting of

(a) benzophenone hydrazone, substituted on the benzophenone with one or more substituents selected from the group consisting of halogen, hydroxy, acetoxy, and lower alkoxy, wherein a benzophenone substituted with only lower alkoxy or only hydroxy has at least one of said lower alkoxy or hydroxy substituents in an *ortho* position on the benzophenone;

(b) a substituted or unsubstituted xanthone hydrazone, wherein the substituted xanthone hydrazone is substituted on the xanthone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(c) a substituted or unsubstituted fluoren hydrazone, wherein the substituted fluoren hydrazone is substituted on the fluoren with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(d) a substituted or unsubstituted anthraquinone hydrazone, wherein the substituted anthraquinone hydrazone is substituted on the anthraquinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(e) a substituted or unsubstituted anthraquinone phenylhydrazone, wherein the substituted anthraquinone phenylhydrazone is substituted on the anthraquinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy,

acetoxy, and halogen, and the phenylhydrazone is selected from the group consisting of nitrophenylhydrazone, dinitrophenylhydrazone, methyl phenylhydrazone and dimethyl phenylhydrazone;

(f) a substituted or unsubstituted indano [1,2,3-*de*]-2*H*-phthalazinone, wherein the substituted indano [1,2,3-*de*]-2*H*-phthalazinone is substituted on the indano [1,2,3-*de*]-2*H*-phthalazinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(g) a substituted or unsubstituted tetralin [1,2,3-*de*]-2*H*-phthalazinone, wherein the substituted tetralin [1,2,3-*de*]-2*H*-phthalazinone is substituted on the tetralin [1,2,3-*de*]-2*H*-phthalazinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(h) a substituted or unsubstituted acridone hydrazone, wherein the substituted acridone hydrazone is substituted on the acridone with one or more substitutents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(i) a substituted or unsubstituted thioxanthone hydrazone, wherein the substituted thioxanthone hydrazone is substituted on the thioxanthone with one of more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen; and

(j) a substituted or unsubstituted dibenzosuberenone, wherein the substituted dibenzosuberenone is substituted on the benzosuberenone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen.

2. The method of claim 1 wherein X is selected from the group consisting of: a 2-nitrophenyl, a 4-nitrophenyl, a 2,4-dinitrophenyl, a 2-methylphenyl, a 4-methylphenyl, a 2,4-dimethylphenyl, a

2-trifluoromethylphenyl, a 4-trifluoromethylphenyl, a 2,4-bis-trifluoromethylphenyl.

3. The method of claim 1 wherein R^1 is selected from the group consisting of: a hydroxychlorobenzophenone hydrazone, a 4-hydroxy-4'-chlorobenzophenone hydrazone, a dihydroxybenzophenone hydrazone, a trihydroxybenzophenone hydrazone, a tetrahydroxybenzophenone hydrazone, a 2,2'-dihydroxybenzophenone hydrazone, a difluorobenzophenone, a 4,4'-difluorobenzophenone, a 2,2', 4,4'-tetrahydroxybenzophenone hydrazone, a 2,4-dihydroxybenzophenone hydrazone, a fluorohydroxybenzophenone hydrazonea a substituted or unsubstituted xanthone hydrazone, a fluoren hydrazone, substituted with one or more hydroxyls on the fluoren, a methoxyhydroxybenzophenone hydrazone, a hydroxyanthraquinone hydrazone, a dihydroxyanthraquinone hydrazone, a 1,8-dihydroxyanthraquinone hydrazone, an indano [1,2,3-*de*]-2*H*-phthalazinone.

4. The method of claim 1 wherein R^1 is an anthraquinone dinitrophenylhydrazone that is substituted or unsubstituted on the anthraquinone with one or more substituents selected from the group consisting of hydroxy, methoxy, and halogen.

5. The method of claim 4 wherein R^1 is a 2,6-dihydroxyanthraquinone-2,4-dinitrophenylhydrazone.

6. A method of impairing growth of neoplastic cells, comprising the steps of:

exposing the neoplastic cells to an effective amount of a compound sufficient to inhibit growth of the neoplastic cells, wherein the compound is

$X - R^1$

wherein X is phenyl substituted with one or more substituents selected from the group consisting of methyl and trihalomethyl, and R^1 is benzophenone hydrazone, substituted on the benzophenone with one or

more substituents selected from the group consisting of halogen, hydroxy and lower alkoxy.

7. The method of claim 6 wherein R^1 is a dihydroxybenzophenone.

8. The method of claim 7 wherein R^1 is a 4,4'-dihydroxybenzophenone.

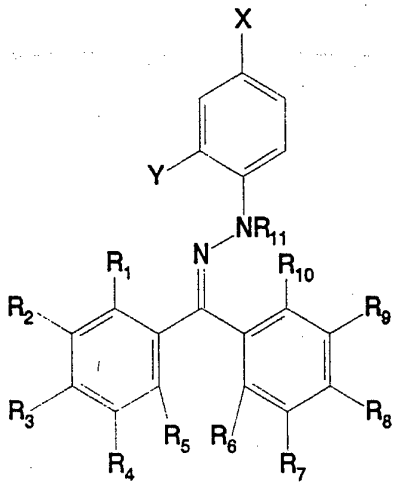
9. The method of claim 8 wherein the compound is 4,4'-dihydroxybenzophenone-2,4-dimethylphenyl hydrazone.

10. The method of claim 1 wherein the compound is selected from the group consisting of 4-fluoro-4'-hydroxybenzophenone-2,4-dinitrophenylhydrazone; 2,6-dihydroxyanthraquinone-bis-2,4-dinitrophenylhydrazone; 1,8-dihydroxyanthraquinone-10-(2,4-dinitrophenylhydrazone); 1,2-dihydroxyanthroquinone-10-2-(2,4-dinitrophenylhydrazone); 6,9-diacetoxyanthraquinone-10-(2,4-dinitrophenylhydrazone); 2,4-dihydroxybenzophenone-2,4-dinitrophenylhydrazone; 2,7-dihydroxyfluoren-9-one-2,4-dinitrophenylhydrazone; 2,7-difluorofluoren-9-one-2,4-dinitrophenylhydrazone; 2,7-dichlorofluoren-9-one-2,4-dinitrophenylhydrazone; 1,4-dihydroxyfluoren-9one-2,4-dinitrophenylhydrazone;4,4-dimethoxy-2,2'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone; 4,4'-dihydroxybenzophenone-2,4-dimethylphenylhydrazone; 2-hydroxyfluoren-9-one-2,4-dinitrophenylhydrazone; xanthone-2,4-dinitrophenylhydrazone; 2,7-difluoroxanthone-2,4-dinitrophenylhydrazone; 2,4,4'-trihydroxybenzophenone-2,4-dinitrophenylhydrazone; 4,4'-dihydroxybenzophenone-2-nitrophenylhydrazone; 2,2',4,4'-tetrahydroxybenzophenone-2,4-dinitrophenylhydrazone; 4,4'-difluorobenzophenone-2,4-dinitrophenylhydrazone; 2,2'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone; 4-hydroxy-4'-chlorobenzophenone-2,4-dinitrophenylhydrazone; 2-(2,4-dinitrophenyl) indano [1,2,3-*de*]-2*H*-phthalazinone; 2-(2,4-dinitrophenyl)-5,10-dihydroxyindano [1,2,3-de]-2H-pthalazinone; 4,4'-

dihydroxybenzophenone-2,4-dimethylphenylhydrazone; 2-(2,4-dinitrophenyl) tetralin [1,2,3-*de*] 2*H*-phthalazinone; acridone-2,4-dinitrophenylhydrazone; dihydroxydibenzosuberenone-2,4-dinitrophenylhydrazone; difluoro-dibenzosuberenone-2,4-dinitrophenylhydrazone; thioxanthone-2,4-dinitrophenylhydrazone.

11. A compound selected from the group consisting of 4-fluoro-4'-hydroxybenzophenone-2,4-dinitrophenylhydrazone; 2,6-dihydroxyanthraquinone-bis-2,4-dinitrophenylhydrazone; 1,9-dihydroxyanthraquinone-10-(2,4-dinitrophenylhydrazone); 2,4-dihydroxybenzophenone-2,4-dinitrophenylhydrazone; 2,4-dihydroxyfluoren-9-one-2,4-dinitrophenylhydrazone; 4,4'-dimethoxy-2,2'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone; 2-hydroxyfluoren-9-one-2,4-dinitrophenylhydrazone; xanthone-2,4-dinitrophenylhydrazone; 2,4,4'-trihydroxybenzophenone-2,4-dinitrophenylhydrazone; 2,2', 4,4'-tetrahydroxybenzophenone-2,4-dinitrophenylhydrazone; 2,2'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone; 4-hydroxy-4'-chlorobenzophenone-2,4-dinitrophenylhydrazone; or 2-(2',4'-dinitrophenyl) indano [1,2,3-*de*]-2*H*-phthalazinone; 2-(2,4-dinitrophenyl) tetralin [1,2,3-*de*] 2*H*-phthalazinone.

12. A compound of the formula



wherein

X = H, NO_2, lower alkyl, or trihalomethyl and Y = H, NO_2, lower alkyl, or trihalomethyl; and wherein at least one of X and Y is selected from the group consisting of NO_2, lower alkyl, and trihalomethyl, and wherein

(a) R^1 = H, OCOCH3, or OH; R^2 = H, halogen, $OCOCH_3$, or OH; R^3 = H, OH, halogen, $OCOCH_3$, or lower methoxy; R^4 = H, OCOCH3 or OH; R^7 = H, OH, or $OCOCH_3$; R^8 = H, OH, halogen, $OCOCH_3$, or lower methoxy; R^9 = H, halogen, $OCOCH_3$, or OH; R^{10} = H or OH; R^5 = R^6 = R^{11} = H; and wherein at least one of R^1 and R^{10} is OH; or

(b) each of R^1-R^4 and R^7-R^{10} is independently selected from the group consisting of hydrogen, hydroxy, acetoxy, lower alkoxy and halogen and R^{11} is hydrogen, and R^5 and R^6 together form

i) a carbonyl in a six membered ring, or

ii) a carbon in a six membered ring; or

iii) a nitrogen with or without substitution in a six membered ring

iv) a sulfur in a six membered ring

v) two carbons in a seven membered ring; or

(c) each of R^1-R^4 and R^7-R^9 is independently selected from the group consisting of hydrogen, hydroxy, lower alkoxy and halogen and R^{10} and R^{11} together form a carbonyl of a six membered ring, and R^5 and R^6 together form

i) a single bond of a five membered ring, or

ii) an oxygen in a six membered ring, or

iii) a carbonyl in a six membered ring, or

iv) a carbon in a six membered ring; or

v) a nitrogen with or without substitution in a six membered ring; or

vi) a sulfur in a six membered ring; or

vii) two carbons connected by a double bond in a seven membered ring; or

(d) each of R^1-R^4 and R^7-R^{10} is independently selected from the group consisting of hydrogen, hydroxy, lower alkoxy and halogen, R^{11} is hydrogen and R^5 and R^6 together form a carbon in a six membered ring linked to phenylhydrazone substituted with one or more substituents selected from the group consisting of nitro, lower alkyl and trihalomethyl; or

(e) R^{11} is H and each of R^1-R^{10} is independently selected from the group consisting of hydrogen, hydroxy, lower alkoxy, acetoxy, and a halogen, and wherein in at least one of R^1-R^{10} is a halogen and wherein at least one of R^1-R^{10} is hydroxy.

13. The compound of claim 12 wherein at least one of R^2, R^3, R^4, R^7, R^8, and R^9 is selected from the group consisting of halogen, hydroxy, and lower alkoxy.

14. The compound of claim 12 wherein at least one of R^1-R^4 or R^7-R^{10} is hydroxy.

15. The compound of claim 12 wherein R^1-R^4 and R^7-R^{10} are hydrogen.

16. A compound of the formula

$$X\text{-}R^1$$

wherein X is nitrophenyl, dinitrophenyl, methylphenyl, dimethylphenyl, trihalomethylphenyl, or bis-trihalomethylphenyl, and R^1 is selected from the group consisting of *ortho*-hydroxybenzophenone hydrazone, 2,2′-dihydroxybenzophenone hydrazone, 2,2′,4,4′-tetrahydoxybenzophenone hydrazone, 2,4,4′-trihydroxybenzophenone hydrazone, 4,4′-dimethoxy-2,2′-dihydroxybenzophenone hydrazone, 2,4-dihydroxybenzophenone hydrazone, an anthraquinone hydrazone, a hydroxyanthraquinone hydrazone, a dihydroxyanthraquinone hydrazone, an anthraquinone nitrophenyl hydrazone, an anthraquinone dinitrophenyl hydrazone, an indano phthalazinone, a hydroxy substituted indano phthalazinone, a tetralin [1,2,3-*de*] 2*H*-phthalazinone, a dibenzosuberenone hydrazone, a thioxanthone hydrazone, and an acridone hydrazone.

17. A compound of the formula

$X-R^1$

wherein X is phenyl substituted with one or more substituents selected from the group consisting of nitro, lower alkyl, and trihalomethyl, and R^1 is selected from the group consisting of

(a) anthraquinone;

(b) hydroxy substituted anthraquinone;

(c) anthraquinone phenyl hydrazone; and

(d) anthraquinone phenyl hydrazone wherein the anthraquinone is substituted with one or more hydroxys, and the phenyl ring is substituted with

(i) one or more methyls; or

(ii) one or more nitros.

18. The compound of claim 17 wherein R^1 is an hydroxy substituted anthraquinone, an anthraquinone phenyl hydrazone, or an anthraquinone phenyl hydrazone wherein the anthraquinone is substituted with one or more hydroxys, and the phenyl ring is substituted with

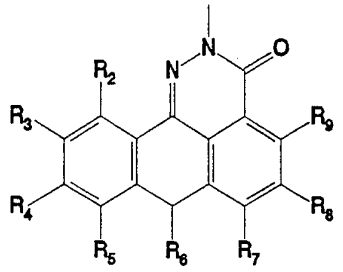
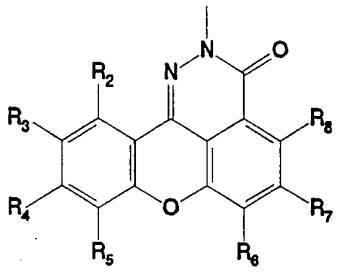
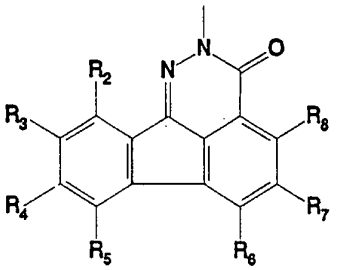
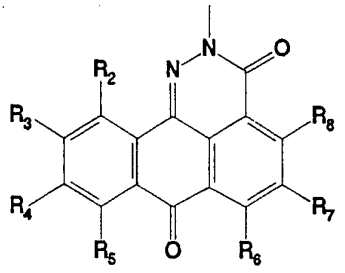
(i) one or more methyls; or

(ii) one or more nitros.

19. The compound of claim 18 wherein R^1 is anthraquinone phenyl hydrazone or anthraquinone dinitrophenyl hydrazone.

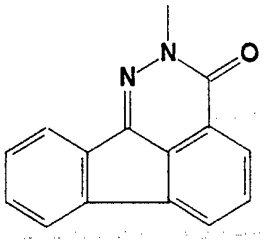
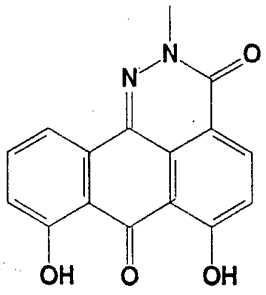
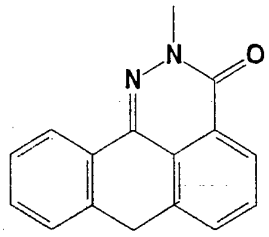
20. The compound of claim 19 wherein R^1 is hydroxy or dihydroxy anthraquinone dinitrophenyl hydrazone.

21. A compound of the formula $X - R^1$ wherein X is phenyl substituted with one or more substituents selected from the group consisting of nitro, lower alkyl, and trihalomethyl and R^1 is selected from the group consisting of:

wherein R_2-R_9 are selected from the group consisting of hydrogen, hydroxy, lower alkoxy and halogen.

22. The compound of claim 21 wherein R^1 is selected from the group consisting of

23. The compound of claim 22 wherein X is dinitrophenyl.

24. A composition comprising a pharmaceutically inert carrier and, in an amount sufficient to impair growth of a neoplastic tumor cell, a compound of the formula:

$$X - R^1$$

wherein X is phenyl substituted with one or more substituents selected from the group consisting of nitro, lower alkyl, and trihalomethyl, and R^1 is selected from the group consisting of

(a) benzophenone hydrazone, substituted on the benzophenone with one or more substituents selected from the group consisting of halogen, hydroxy, acetoxy, and lower alkoxy, wherein a benzophenone substituted with only lower alkoxy or only hydroxy has at least one of said lower alkoxy or hydroxy substitutions in an *ortho* position on the benzophenone;

(b) a substituted or unsubstituted xanthone hydrazone, wherein the substituted xanthone hydrazone is substituted on the xanthone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(c) a substituted or unsubstituted fluoren hydrazone, wherein the substituted fluoren hydrazone is substituted on the fluoren with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(d) a substituted or unsubstituted anthraquinone hydrazone, wherein the substituted anthraquinone hydrazone is substituted on the anthraquinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(e) a substituted or unsubstituted anthraquinone phenylhydrazone, wherein the substituted anthraquinone phenylhydrazone is substituted on the anthraquinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen, and the phenylhydrazone is

selected from the group consisting of nitrophenylhydrazone, dinitrophenylhydrazone, methyl phenylhydrazone and dimethyl phenylhydrazone;

(f) a substituted or unsubstituted indano [1,2,3-*de*]-2*H*-phthalazinone, wherein the substituted indano [1,2,3-*de*]-2*H*-phthalazinone is substituted on the indano [1,2,3-*de*]-2*H*-phthalazinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(g) a substituted or unsubstituted tetralin [1,2,3-*de*]-2*H*-phthalazinone, wherein the substituted tetralin [1,2,3-*de*]-2*H*-phthalazinone is substituted on the tetralin [1,2,3-*de*]-2*H*-phthalazinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(h) a substituted or unsubstituted acridone hydrazone, wherein the substituted acridone hydrazone is substituted on the acridone with one or more substitutents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(i) a substituted or unsubstituted thioxanthone hydrazone, wherein the substituted thioxanthone hydrazone is substituted on the thioxanthone with one of more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen; and

(j) a substituted or unsubstituted dibenzosuberenone, wherein the substituted dibenzosuberenone is substituted on the dibenzosuberenone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen.

25. The composition of claim 24 wherein the compound is 2,6-dihydroxyanthraquinone-bis-2,4-dinitrophenylhydrazone.

26. The composition of claim 24 wherein the compound is 1,8-dihydroxyanthraquinone-10-(2,4-dinitrophenylhydrazone).

27. A method of impairing growth of non-estrogen dependent neoplastic cells having less than 5 fentomoles/ng of estrogen receptors, comprising the steps of:

exposing the cells to an effective amount of a compound sufficient to inhibit growth of the neoplastic cells, wherein the compound is

$$X - R^1$$

wherein X is phenyl substituted with one or more substituents selected from the group consisting of nitro, lower alkyl, and trihalomethyl, and R^1 is selected from the group consisting of

(a) benzophenone hydrazone, substituted on the benzophenone with one or more substituents selected from the group consisting of halogen, hydroxy, acetoxy, and lower alkoxy, wherein a benzophenone substituted with only lower alkoxy or only hydroxy has at least one of said lower alkoxy or hydroxy substituents in an *ortho* position on the benzophenone;

(b) a substituted or unsubstituted xanthone hydrazone, wherein the substituted xanthone hydrazone is substituted on the xanthone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(c) a substituted or unsubstituted fluoren hydrazone, wherein the substituted fluoren hydrazone is substituted on the fluoren with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(d) a substituted or unsubstituted anthraquinone hydrazone, wherein the substituted anthraquinone hydrazone is substituted on the anthraquinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(e) a substituted or unsubstituted anthraquinone phenylhydrazone, wherein the substituted anthraquinone phenylhydrazone is substituted on the

anthraquinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen, and the phenylhydrazone is selected from the group consisting of nitrophenylhydrazone, dinitrophenylhydrazone, methyl phenylhydrazone and dimethyl phenylhydrazone; and

(f) a substituted or unsubstituted indano [1,2,3-*de*]-2*H*-phthalazinone, wherein the substituted indano [1,2,3-*de*]-2*H*-phthalazinone is substituted on the indano [1,2,3-*de*]-2*H*-phthalazinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(g) a substituted or unsubstituted tetralin [1,2,3-*de*]-2*H*-phthalazinone, wherein the substituted tetralin [1,2,3-*de*]-2*H*-phthalazinone is substituted on the tetralin [1,2,3-*de*]-2*H*-phthalazinone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen.

(h) a substituted or unsubstituted acridone hydrazone, wherein the substituted acridone hydrazone is substituted on the acridone with one or more substitutents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen;

(i) a substituted or unsubstituted thioxanthone hydrazone, wherein the substituted thioxanthone hydrazone is substituted on the thioxanthone with one of more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen; and

(j) a substituted or unsubstituted benzosuberone, wherein the substituted dibenzosuberone is substituted on the dibenzosuberone with one or more substituents selected from the group consisting of hydroxy, lower alkoxy, acetoxy, and halogen.

28. The method of claim 27 wherein the compound is a compound of the formula X-R^1 wherein X is nitrophenyl, dinitrophenyl, methylphenyl, dimethylphenyl, trihalomethylphenyl, or bis-

trihalomethylphenyl, and R^1 is selected from the group consisting of *ortho*-hydroxybenzophenone hydrazone, hydroxychlorobenzophenone hydrazone, hydroxyfluorobenzophenone hydrazone, 2,2'-dihydroxybenzophenone hydrazone, 2,2',4,4'-tetrahydoxybenzophenone hydrazone, 2,4,4'-trihydroxybenzophenone hydrazone, xanthone hydrazone, fluoren hydrazone, a hydroxy substituted fluoren hydrazone, 4,4'-dimethoxy-2,2'-dihydroxybenzophenone hydrazone, 2,4-dihydroxybenzophenone hydrazone, an anthraquinone hydrazone, a hydroxyanthraquinone hydrazone, a dihydroxyanthraquinone hydrazone, an anthraquinone nitrophenyl hydrazone, an anthraquinone dinitrophenyl hydrazone, an indano phthalazinone, a hydroxy substituted indano phthalazinone and a tetralin [1,2,3-*de*] 2*H*-phthalazinone.

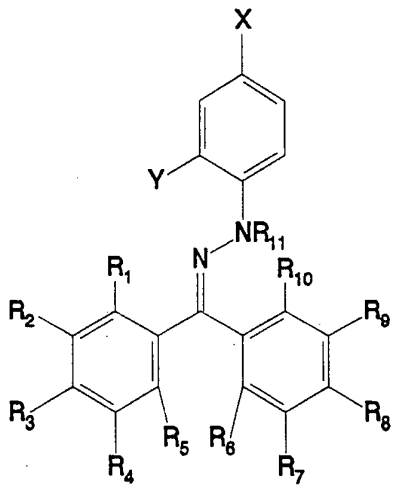
29. The method of claim 27 wherein the compound is a compound of the formula

$$X\text{-}R^1$$

wherein X is phenyl substituted with one or more substituents selected from the group consisting of nitro, methyl, and trihalomethyl, and R^1 is selected from the group consisting of (a) anthraquinone; (b) hydroxy substituted anthraquinone; (c) anthraquinone phenyl hydrazone; and (d) anthraquinone phenyl hydrazone wherein the anthraquinone is substituted with one or more hydroxys, and the phenyl ring is substituted with (i) one or more methyls; or (ii) one or more nitros.

30. A method of impairing growth of non-estrogen dependent neoplastic cells having less than 5 fentomoles/ng of estrogen receptors, comprising the steps of:

exposing the cells to an effective amount of a compound sufficient to inhibit growth of the neoplastic cells, wherein the compound is a compound of the formula:



wherein

X = H, NO_2, CH_3, or trihalomethyl and Y = H, NO_2, CH_3, or trihalomethyl wherein at least one of X and Y is selected from the group consisting of NO_2, CH_3, and trihalomethyl, and wherein

(a) R^1 = H, $OCOCH_3$, or OH; R^2 = H or OH; R^3 = H, OH, F, or OCH_3; R^4 = H or OH; R^7 = H or OH; R^8 = H, OH or OCH_3; R^9 = H or OH; R^{10} = H or OH; R^5 = R^6 = R^{11} = H; and wherein at least one of R^1 and R^{10} is hydroxy; or

(b) each of R^1-R^4 and R^7-R^{10} is independently selected from the group consisting of hydrogen, hydroxy, methoxy and halogen and R^{11} is hydrogen, and R^5 and R^6 together form

i) a single bond of a five membered ring, or

ii) an oxygen in a six membered ring, or iii) a carbonyl in a six membered ring, or iv) a carbon in a six membered ring, or

v) a nitrogen with or without substitution in a six membered ring, or

vi) a sulfur in a six membered ring, or

vii) two carbons in a seven membered ring; or

(c) each of R^1-R^4 and R^7-R^9 is independently selected from the group consisting of hydrogen, hydroxy, methoxy and halogen and R^{10} and R^{11} together

form a carbonyl of a six membered ring, and R^5 and R^6 together form

i) a single bond of a five membered ring, or

ii) an oxygen in a six membered ring, or

iii) a carbonyl in a six membered ring, or

iv) a carbon in a six membered ring; or

v) a nitrogen with or without substitution in a six membered ring, or

vi) a sulfur in a six membered ring, or

vii) two carbons in a seven membered ring; or

(d) each of R^1-R^4 and R^7-R^{10} is independently selected from the group consisting of hydrogen, hydroxy, methoxy and halogen, R^{11} is hydrogen and R^5 and R^6 together form a carbon in a six membered ring linked to phenylhydrazone substituted with one or more substituents selected from the group consisting of nitro, methyl and trihalomethyl; or

(e) R^{11} is H and each of R^1-R^{10} is independently selected from the group consisting of hydrogen, hydroxy, methoxy and a halogen and wherein in at least one of R^1-R^{10} is a halogen and wherein at least one of R^1-R^{10} is a hydroxy.

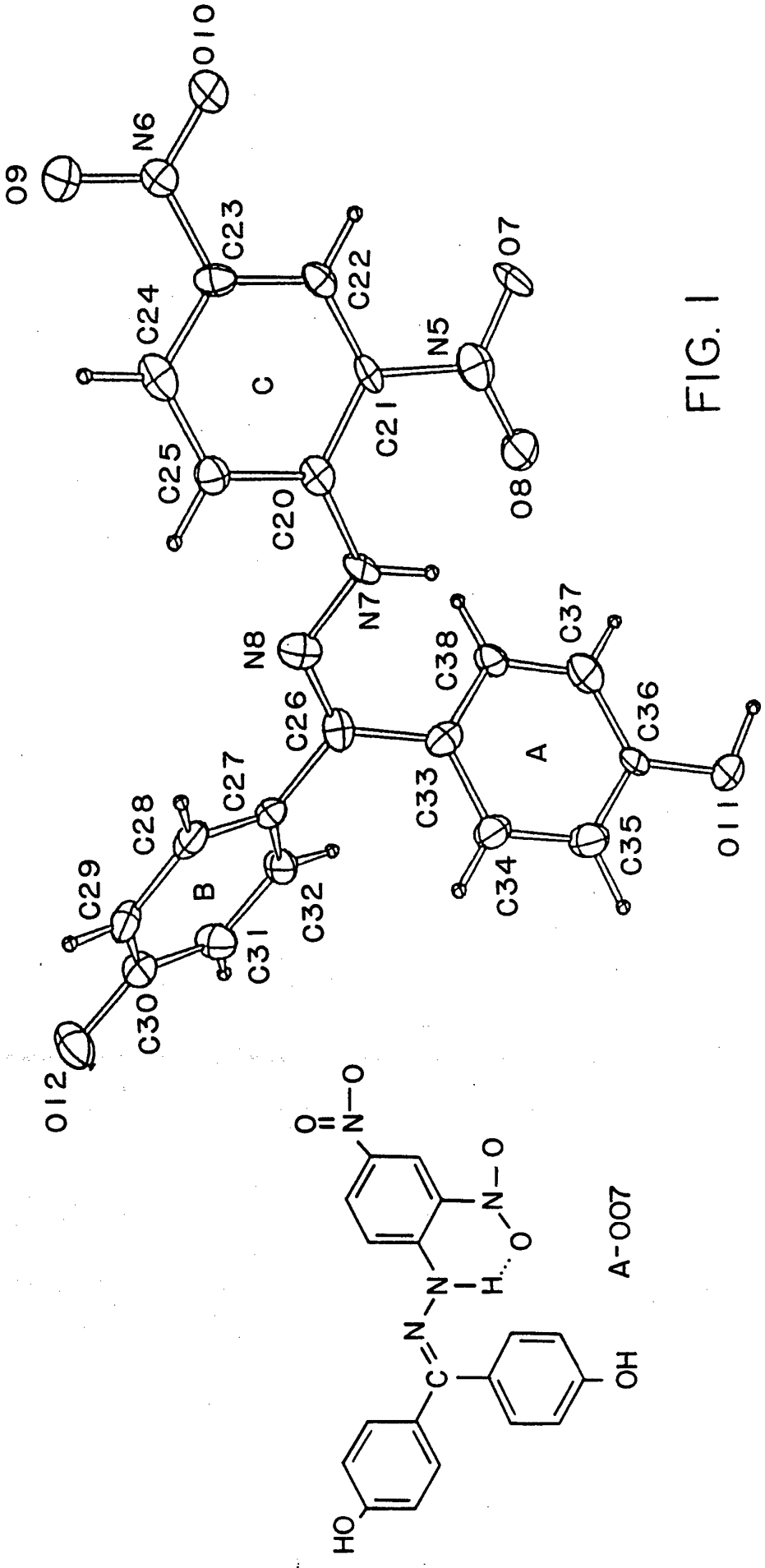

O10
N6
O9
C23
C22
O7
C24
N5
C
C21
C25
O8
C20
N7
N8
C37
C38
C26
C36
A
C27
C33
O11
C28
C35
C29
B
C34
C32
C31
C30
O12
O
N
O
N
O
H
A-007
N
C
OH
HO

FIG. 1

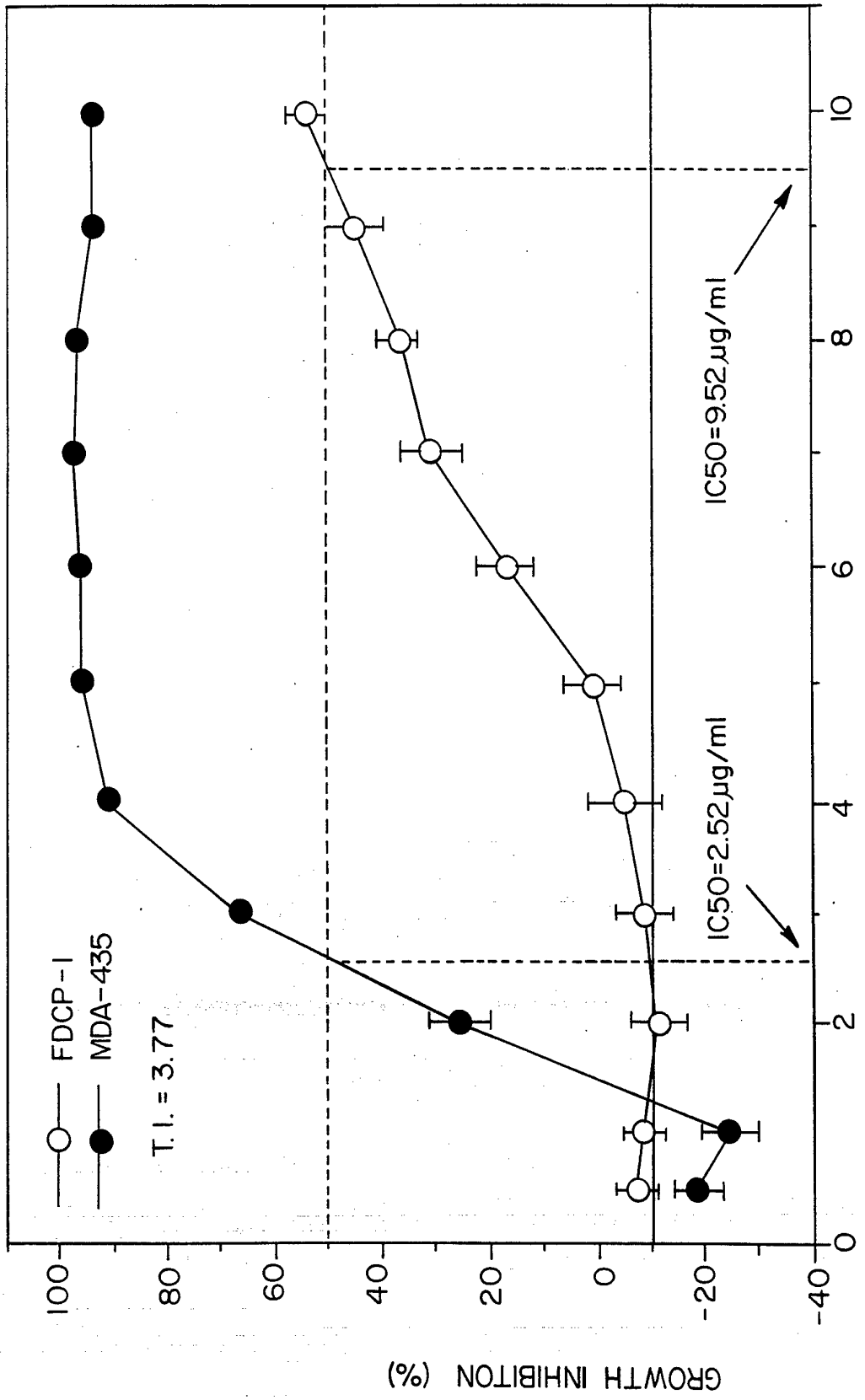

GROWTH INHIBITION (%)
100
80
60
40
20
0
-20
-40
0
2
4
6
8
10
007 (μg/ml)
IC50=2.52μg/ml
IC50=9.52μg/ml
FDCP-1
MDA-435
T.I. = 3.77

FIG. 2

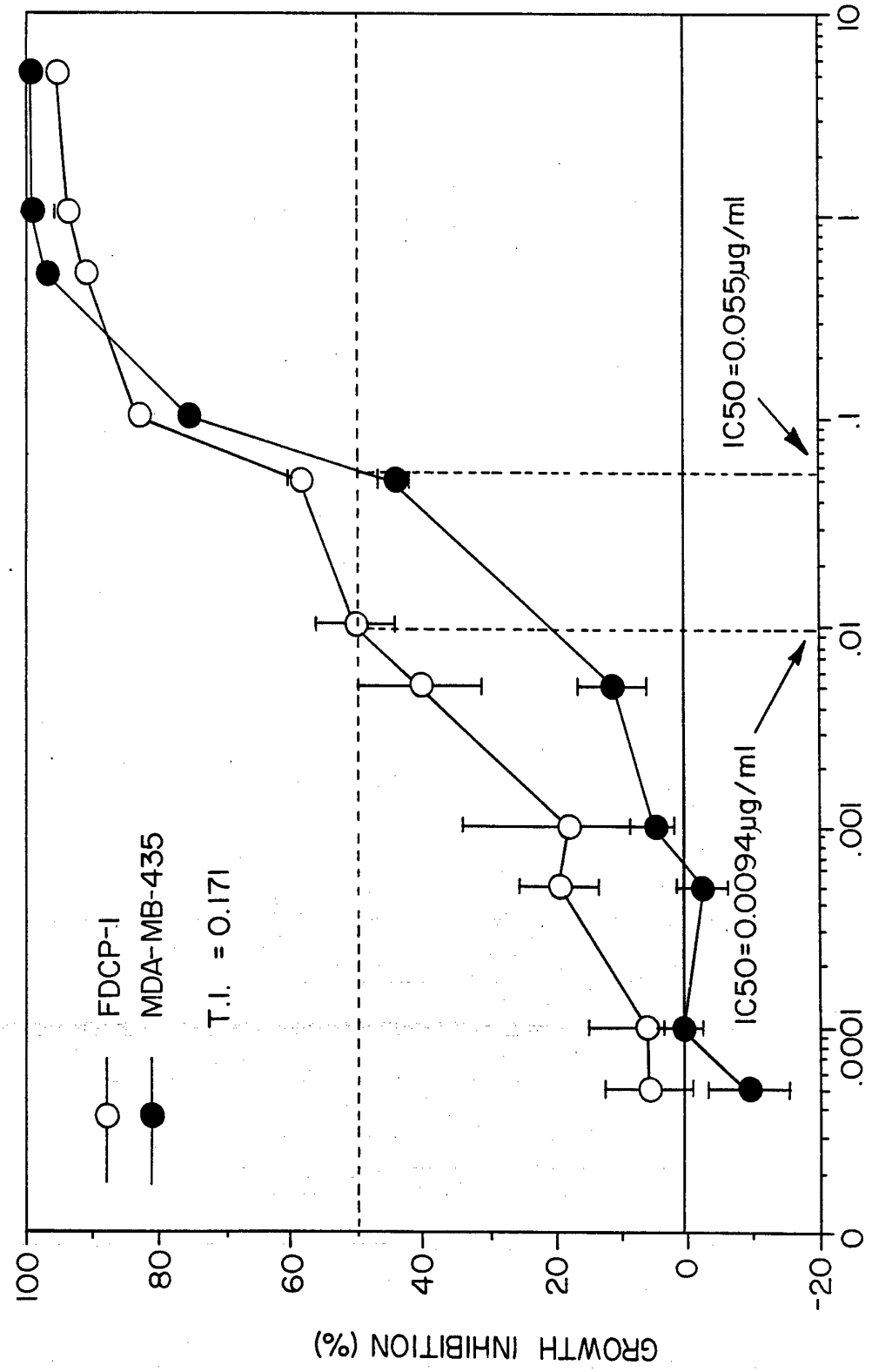
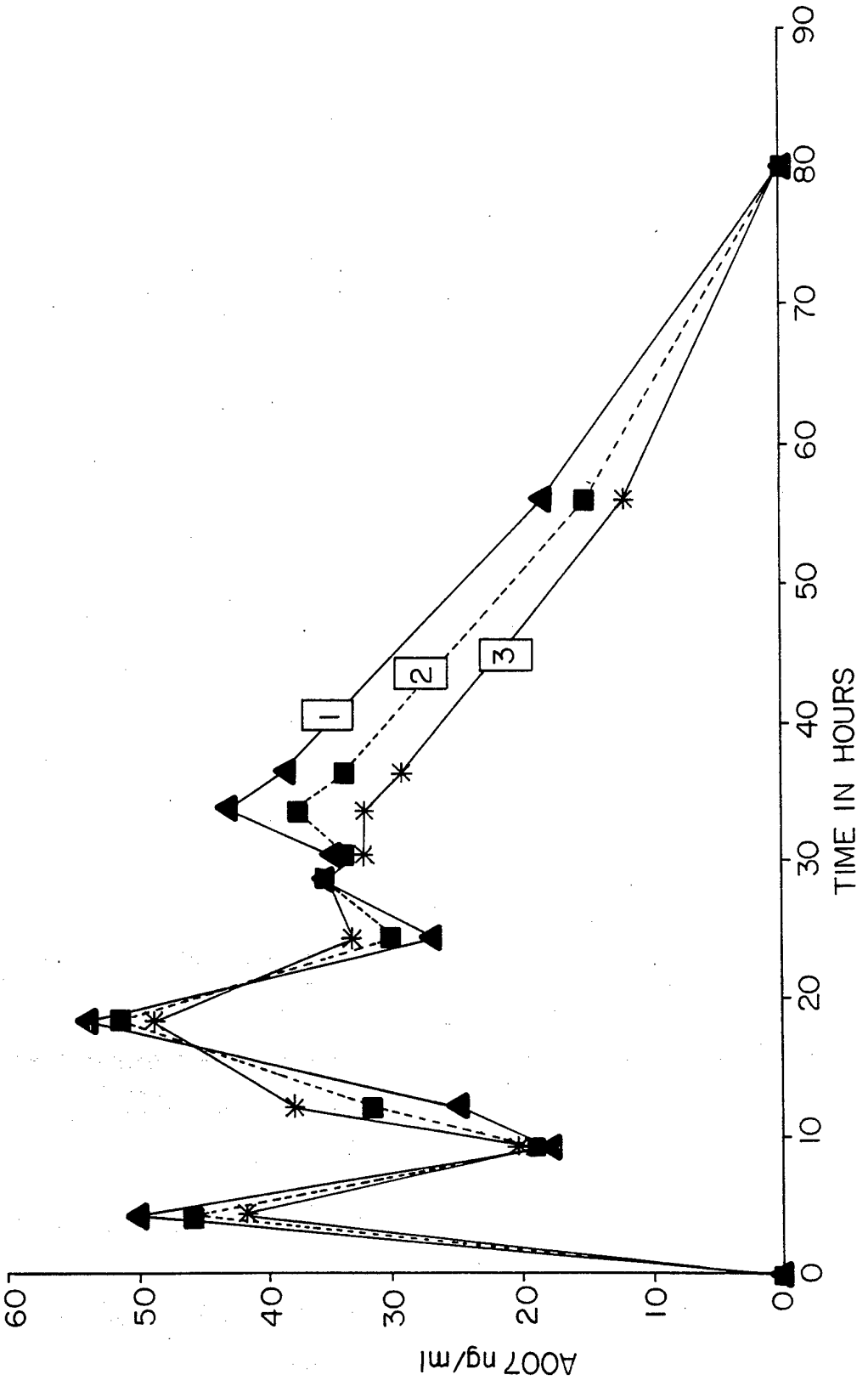

GROWTH INHIBITION (%)
100
80
60
40
20
0
-20
0
.0001
.001
.01
.1
1
10
DOXORUBICIN (µg/ml)
IC50=0.055µg/ml
IC50=0.0094µg/ml
FDCP-1
MDA-MB-435
T.I. = 0.171

FIG. 3

FIG. 4


AOO7 ng/ml
0
10
20
30
40
50
60
0
10
20
30
40
50
60
70
80
90
TIME IN HOURS
1
2
3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08427

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 534/653,654; 544/233; 552/210,302; 564/251; 546/300,332; 514/351, 357,639,847

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

Please See Extra Sheet.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A, 3,053,678 (MIX) 11 SEPTEMBER 1962, ENTIRE DOCUMENT.	11-26
X	US,A, 4,673,692 (SUZUKI ET AL) 16 JUNE 1987, ENTIRE DOCUMENT.	11-26
X	US,A, 4,732,904 (MORGAN) 22 MARCH 1988, ENTIRE DOCUMENT.	1-30

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 08 December 1993	Date of mailing of the international search report 16 DEC 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer FLOYD HIGEL Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08427

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A61K 31/15, 31/44, 49/02; C07C 243/22, 249/16, 251/14, 251/06; C07D 213/53, 213/65, 487/22

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

534/653,654; 544/233; 552/210,302; 564/251; 546/300,332; 514/351, 357,639,847

B. FIELDS SEARCHED
Documentation other than minimum documentation that are included in the fields searched:

CHEMICAL ABSTRACTS
CURRENT ABSTRACTS OF CHEMISTRY
INDEX CHEMICUS