Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2642717 C 2015/08/18

(11)(21) **2 642 717**

(12) **BREVET CANADIEN**
CANADIAN PATENT

(13) **C**

(86) **Date de dépôt PCT/PCT Filing Date:** 2007/02/20
(87) **Date publication PCT/PCT Publication Date:** 2007/08/30
(45) **Date de délivrance/Issue Date:** 2015/08/18
(85) **Entrée phase nationale/National Entry:** 2008/08/18
(86) **N° demande PCT/PCT Application No.:** US 2007/004252
(87) **N° publication PCT/PCT Publication No.:** 2007/098091
(30) **Priorité/Priority:** 2006/02/17 (US60/774,204)

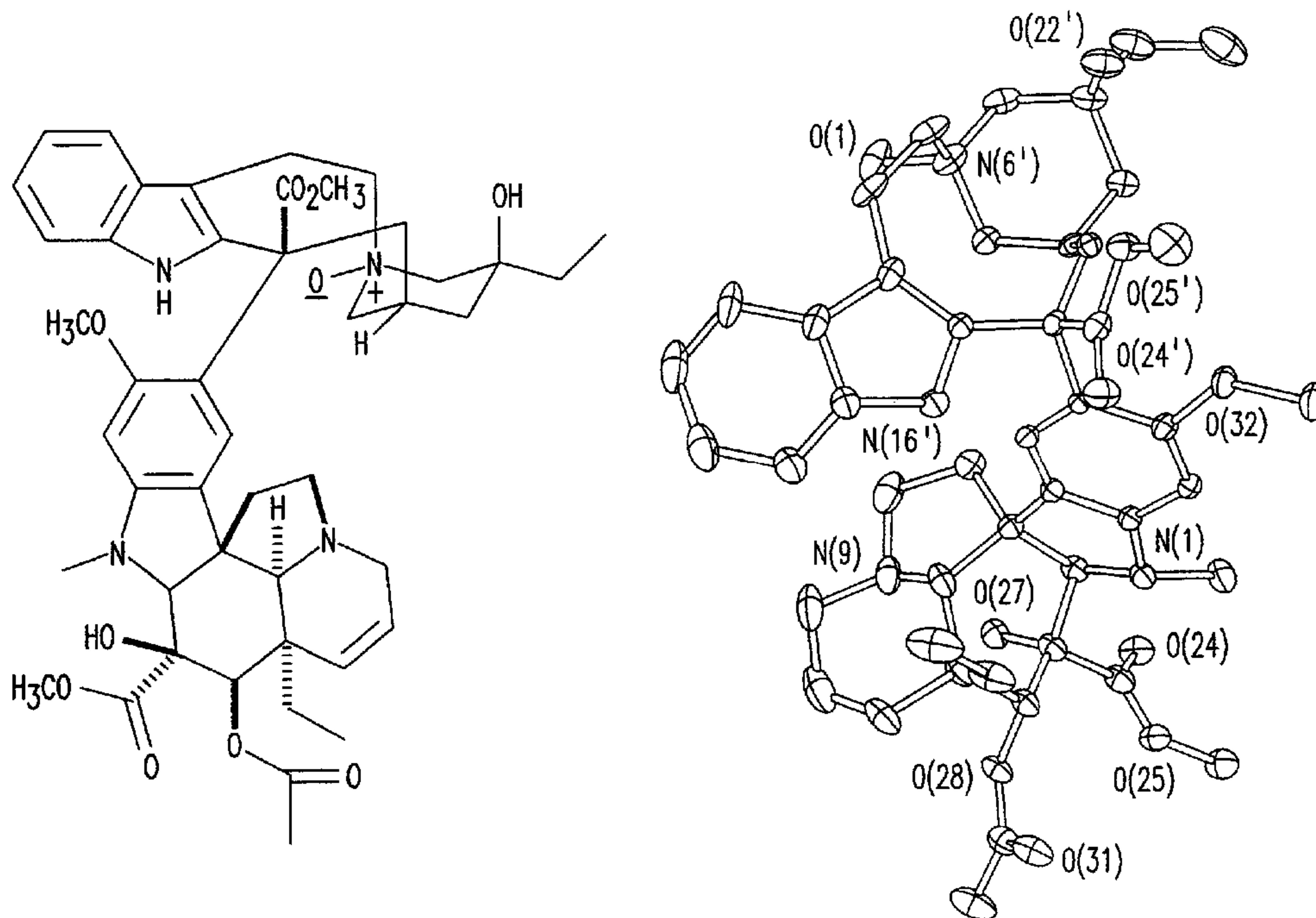
(51) **Cl.Int./Int.Cl.** ***C07D 519/04*** (2006.01), ***A61K 31/475*** (2006.01), ***A61P 35/00*** (2006.01)

(72) **Inventeurs/Inventors:**
CURD, JOHN G., US;
KEANA, JOHN F.W., US;
LALANI, ALSHAD S., US;
WESTBERG, PAUL B., US;
GOODWIN, BRADFORD S., US;
HENNER, WILLIAM DAVID, US

(73) **Propriétaire/Owner:**
CASCADE PRODRUG INC., US

(74) **Agent:** MBM INTELLECTUAL PROPERTY LAW LLP

(54) **Titre : TRAITEMENT DE MALADIES HYPERPROLIFERATIVES AVEC UN N-OXYDE D'ALCALOIDE DE LA PERVENCHE ET DES ANALOGUES CORRESPONDANTS**
(54) **Title: TREATMENT OF HYPERPROLIFERATIVE DISEASES WITH VINCA ALKALOID N-OXIDE AND ANALOGS**



(57) **Abrégé/Abstract:**
The present invention relates to vinca alkaloid and analog N-oxides having activity for treating hyperproliferative disorders. Further, the invention relates to pharmaceutical compositions and methods of using vinca alkaloid and analog N-oxides, alone or in combination with one or more other active agents or treatments, to treat hyperproliferative disorders.

CA 02642717 2008-08-18

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 August 2007 (30.08.2007)

PCT

(10) International Publication Number
WO 2007/098091 A3

(51) International Patent Classification:
C07D 519/04 (2006.01) *A61P 35/00* (2006.01)
A61K 31/475 (2006.01)

(21) International Application Number:
PCT/US2007/004252

(22) International Filing Date:
20 February 2007 (20.02.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/774,204 17 February 2006 (17.02.2006) US

(71) Applicant *(for all designated States except US)*: **NOVACEA, INC.** [US/US]; 601 Gateway Blvd., Suite 800, South San Francisco, CA 94080 (US).

(71) Applicants and
(72) Inventors: **CURD, John, G.** [US/US]; 128 Reservoir Road, Hillsborough, CA 94010 (US). **KEANA, John, F.W.** [US/US]; 3854 Onyx Street, Eugene, OR 97405 (US). **LALANI, Alshad, S.** [CA/US]; 1647 21st Avenue, Apt. A, San Francisco, CA 94122 (US). **WESTBERG, Paul, B.** [US/US]; 2684 Manson Lane, San Mateo, CA 94403 (US). **GOODWIN, Bradford, S.** [US/US]; 216 Amherst Avenue, San Mateo, CA 94402 (US). **HENNER, William, David** [US/US]; 777 SW 48th Drive, Portland, OR 97221 (US).

(74) Agents: **ESMOND, Robert, W.** et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., 1100 New York Avenue, N.W., Washington, DC 20005-3934 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY,

[Continued on next page]

(54) Title: TREATMENT OF HYPERPROLIFERATIVE DISEASES WITH VINCA ALKALOID N-OXIDE AND ANALOGS

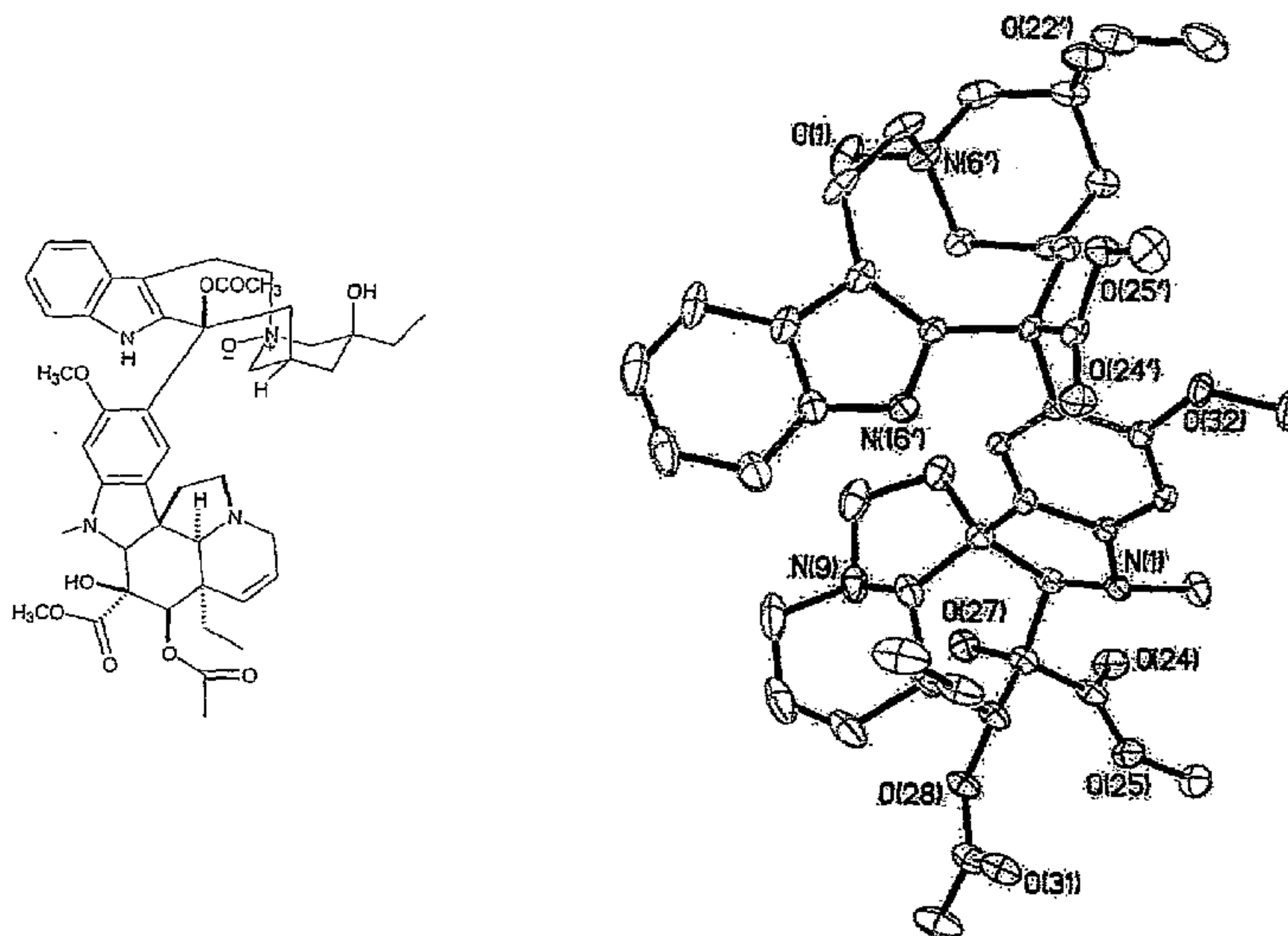


Figure 1

(57) Abstract: The present invention relates to vinca alkaloid and analog N-oxides having activity for treating hyperproliferative disorders. Further, the invention relates to pharmaceutical compositions and methods of using vinca alkaloid and analog N-oxides, alone or in combination with one or more other active agents or treatments, to treat hyperproliferative disorders.

WO 2007/098091 A3

WO 2007/098091 A3

MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report*

(88) Date of publication of the international search report:
28 August 2008

TREATMENT OF HYPERPROLIFERATIVE DISEASES WITH VINCA ALKALOID N-OXIDE AND ANALOGS

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates to vinca alkaloid N-oxides having activity for treating hyperproliferative disorders. Further, the invention relates to pharmaceutical compositions and methods of using vinca alkaloid N-oxides, alone or in combination with one or more other active agents or treatments, to treat hyperproliferative disorders.

Related Art

[0002] One in every four deaths in the United States is due to cancer, and cancer is the second leading cause of death. U.S. Cancer Statistics Working Group; *United States Cancer Statistics: 2000 Incidence*, Atlanta (GA): Department of Health and Human Services, Centers for Disease Control and Prevention, and National Cancer Institute (2003). The National Cancer Institute reports that almost 10 million Americans have a history of invasive cancer, while the American Cancer Society estimates that in the year 2004, over 1.3 million Americans will receive a diagnosis of invasive cancer with over a half million cases resulting in death. American Cancer Society, *Cancer Facts & Figures 2004*. These statistics exclude the 1 million cases of basal and squamous cell skin cancers that are expected to be diagnosed in the United States.

[0003] Cancers are classified based on the organ and cell tissue from which the cancer originates, including: (i) carcinomas (most common kind of cancer which originates in epithelial tissues, the layers of cells covering the body's surface or lining internal organs and various glands); (ii) leukemias (origination in the blood-forming tissues, including bone marrow, lymph nodes and the spleen); (iii) lymphomas (originates in the cells of the lymph system); (iv) melanomas (originates in the pigment cells located among the

epithelial cells of the skin); and (v) sarcomas (originates in the connective tissues of the body, such as bones, muscles and blood vessels). (*See* Molecular Biology of the Cell: Third Edition, "Cancer," Chapter 24, pp.1255-1294, B. Alberts *et al.*, (eds.), Garland Publishing, Inc., New York (1994); and Stedman's Pocket Medical Dictionary; Williams and Wilkins, Baltimore (1987)). Within these broad cancer classifications, there are over one hundred cancer subclassifications, such as breast, lung, pancreatic, colon, and prostate cancer, to name a few.

[0004] Cancer cells develop as a result of damage to a cell's DNA (*i.e.*, altered DNA sequence or altered expression pattern) from exposure to various chemical agents, radiation, viruses, or when some not-yet-fully-understood internal, cellular signaling event occurs. Most of the time when a cell's DNA becomes damaged, the cell either dies or is able to repair the DNA. However, for cancer cells, the damaged DNA is not repaired and the cell continues to divide, exhibiting modified cell physiology and function.

[0005] Neoplasms, or tumors, are masses of cells that result from an aberrant, accelerated rate of growth (*i.e.*, hyperproliferative cell growth). As long as the tumor cells remain confined to a single mass, the tumor is considered to be benign. However, a cancerous tumor has the ability to invade other tissues and is termed malignant. In general, cancer cells are defined by two heritable properties: the cells and their progeny 1) reproduce in defiance of normal restraints, and 2) invade and colonize the territories of other cells.

[0006] Cancerous tumors are comprised of a highly complex vasculature and differentiated tissue. A large majority of cancerous tumors have hypoxic components, which are relatively resistant to standard anti-cancer treatment, including radiotherapy and chemotherapy. Brown, *Cancer Res. 59*:5863 (1999); and Kunz, M. *et al.*, *Mol. Cancer 2*:1 (2003). Thomlinson and Gray presented the first anatomical model of a human tumor that describes a 100 to 150 μm thick hypoxic layer of tissue located between the blood vessels and necrotic tumor tissues.

[0007] Research has shown that the hypoxic tissues within a number of cancerous tumors promote the progression of the cancer by an array of complex mechanisms. *See*, Brown., *supra*, and Kunz *et al.*, *supra*. Among

these are activation of certain signal transduction pathways and gene regulatory mechanisms, induction of selection processes for gene mutations, tumor cell apoptosis and tumor angiogenesis. Most of these mechanisms contribute to tumor progression. Therefore, tissue hypoxia has been regarded as a central factor for tumor aggressiveness and metastasis. Therapies that target hypoxic tissues within a tumor would certainly provide improved treatments to patients suffering from tumor-related cancers and/or disorders.

[0008] In addition to cancer, there exist a number of hyperproliferative diseases and/or disorders that are associated with the onset of hypoxia in a given tissue. For example, Shweiki *et al.* explains that inadequate oxygen levels often lead to neovascularization in order to compensate for the needs of the hypoxic tissue. Neovascularization is mediated by expression of certain growth factors, such as vascular endothelial growth factor (VEGF). Shweiki *et al.*, *Nature 359*:843 (1992). However, when certain tissues or growth factors are either directly or indirectly upregulated in response to hypoxia without sufficient feedback mechanisms for controlling tissue expression, various diseases and/or disorders may ensue (*i.e.*, by hypoxia-aggravated hyperproliferation). By way of example, hypoxia-aggravated hyperproliferative diseases and/or disorders having over- expressed levels of VEGF include ocular angiogenic diseases, such as age-related macular degeneration and diabetic retinopathy, as well as cirrhosis of the liver. *See* Frank, *Ophthalmic Res. 29*:341 (1997); Ishibashi *et al.*, *Graefe's Archive Clin. Exp. Ophthamol. 235*:159 (1997); Corpechot *et al.*, *Hepatology 35*:1010 (2002).

[0009] Vinca alkaloids are a class of chemotherapeutic agents originally discovered in the Madagascar periwinkle. Currently known vinca alkaloids include vinblastine, vincristine, vindesine and vinorelbine. Vinca alkaloids act by inhibiting mitosis in metaphase. These alkaloids bind to tubulin, thus preventing the cell from making the spindles it needs to be able to move its chromosomes around as it divides. These alkaloids also seem to interfere with cells' ability to synthesize DNA and RNA. They are all administered intravenously in their sulfate form once a week; these solutions are fatal if they

are administered incorrectly, and can cause considerable tissue irritation if they leak out of the vein. See U.S. patent 6,555,547 for further detail.

[0010] U.S. patent 6,365,735 discloses a process for preparing vinca alkaloids of the general formula (I):

in which:

R'_1 represents a hydrogen atom or an alkoxy, acyl, formyl or halogenoacyl group,

R'_2 represents a hydrogen atom or an alkyl group,

R'_3 and R''_3 are identical or different and each independently represents a hydrogen atom or a hydroxyl or alkanoyloxyl group, or else R'_3 and R''_3 together form a carbonyl group or else R'_3 and R'_5 together form an epoxy bridge or a double bond,

R'_4 represents a hydrogen atom or an alkyloxycarbonyl, hydroxymethyl or alkanoyloxymethyl group, preferably an alkyloxycarbonyl group,

R'_5 and R''_5 are identical or different and each independently represents a hydrogen atom or a hydroxyl, alkanoyloxyl, ethyl or 2-hydroxyethyl group,

R'_6 represents a hydrogen atom or an ethyl, 2-hydroxyethyl or acetyl group,

R'_7 represents a hydrogen atom or a cyanide group,

R_1 represents a hydrogen atom or an alkyl, formyl or acyl group, preferably hydrogen or an alkyl group,

R_2 represents a hydrogen atom or an alkoxy group,

R_3 represents a hydrogen atom or a hydroxyl or alkanoyloxyl group, or else R_3 and R_4 together form an epoxy bridge or a double bond,

R_4 represents a hydrogen atom or a hydroxyl or alkanoyloxyl group, or else R_4 and R_5 together form an epoxy bridge,

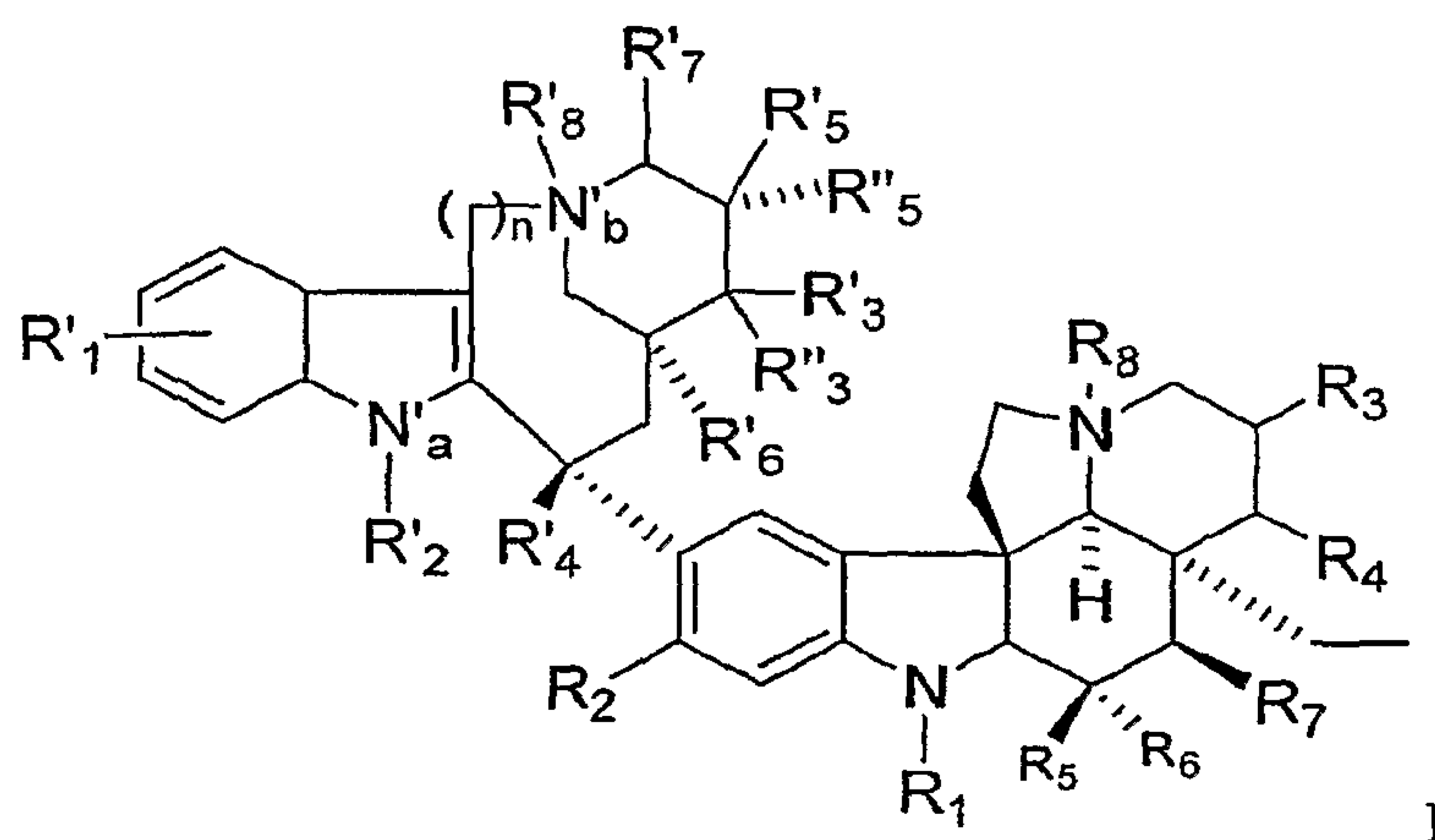
R_6 represents an alkyloxycarbonyl, hydrazido, acetamido, hydroxymethyl or alkanoyloxymethyl group,

R_5 and R_7 represent a hydrogen atom or a hydroxyl or alkanoyloxyl group, as well as their addition salts with acids and their quaternary ammonium salts.

[0011] Disclosed also are additional classes of vinca alkaloids as well specific vinca alkaloids such as vinblastine, vincristine, anhydrovinblastine and vinorelbine.

BRIEF SUMMARY OF THE INVENTION

[0012] The present invention is related to compounds, compositions and methods for treating hyperproliferative disorders, such as cancer and inflammation. One aspect of the invention is drawn to vinca alkaloid N-oxides having Formula I:



I

or a pharmaceutically acceptable salt thereof, wherein:

R'_1 represents hydrogen, alkyl, alkoxy, acyl, formyl or halogenoacyl group;

R'_2 represents hydrogen or alkyl group;

R'_3 and R''_3 are identical or different and each independently represents hydrogen, hydroxy or alkanoyloxy group, or else R'_3 and R''_3 together form a

carbonyl group or else R'_3 and R'_5 together form an epoxy bridge or a double bond;

R'_4 represents hydrogen, alkyloxycarbonyl, hydroxymethyl or alkanoyloxymethyl group, preferably an alkyloxycarbonyl group;

R'_5 and R''_5 are identical or different and each independently represents hydrogen, hydroxy, alkoxycarbonyl, C_1-C_7 alkyl optionally substituted with 1-3 halogen atoms, alkanoyloxy, or 2-hydroxyethyl group;

R'_6 represents hydrogen, ethyl, 2-hydroxyethyl or acetyl group;

R'_7 represents hydrogen or cyano;

R_1 represents hydrogen, alkyl, formyl or acyl group, preferably hydrogen or an alkyl group,

R_2 represents hydrogen or alkoxy;

R_3 represents hydrogen, hydroxy or alkanoyloxy group, or else R_3 and R_4 together form an epoxy bridge or a double bond;

R_4 represents hydrogen, hydroxy or alkanoyloxy group, or else R_4 and R_5 together form an epoxy bridge;

R_6 represents an alkyloxycarbonyl, hydrazido, acetamido, hydroxymethyl, alkanoyloxymethyl group or -C(=O)-A-NH-P, where -A- is one of -NH-, -NH-alk-COO- or -NH-alk-COONH-, alk is a straight chain or branched C_1-C_7 alkyl group and -NH-P is a peptide residue, or R_5 and R_6 together form an oxazolidine-2,4-dione ring;

R_5 and R_7 represent hydrogen, hydroxy or alkanoyloxy group, as well as their addition salts with acids and their quaternary ammonium salts;

each of R_8 and R'_8 is O or is absent provided that at least one of R_8 and R'_8 is O; and

n is 1 or 2.

[0013] In one embodiment, when n=2 and one of R'_5 and R''_5 forms a double bond together with R'_3 or R''_3, then the other is not ethyl.

[0014] In one embodiment, the compound having formula I is vinblastine N-oxide, desacetyl vinblastine N-oxide, vinorelbine N-oxide, vincristine N-oxide or vinflunine N-oxide, or a pharmaceutically acceptable salt thereof.

In one embodiment, the compound of formula I is selected from the group consisting of vinblastine N-oxide, desacetyl vinblastine N-oxide, vinorelbine N-oxide, vincristine N-oxide, desacetyl vinflunine N-oxide, desacetyl vinorelbine N-oxide, vindesine N-oxide and vinflunine N-oxide or a pharmaceutically acceptable salt or prodrug thereof.

[0015] Another aspect of the present invention is related to methods for treating hyperproliferative disorders. In certain aspects of the invention, the

hyperproliferative disorder is cancer. In one embodiment, the cancer is a solid tumor. In another embodiment, the cancer is selected from the group consisting of colon cancer, brain cancer, glioma, multiple myeloma, head and neck cancer, hepatocellular cancer, melanoma, ovarian cancer, cervical cancer, renal cancer, and non-small cell lung cancer. In a further embodiment, the cancer is acute and chronic lymphocytic leukemia, acute granulocytic leukemia, adrenal cortex carcinoma, bladder carcinoma, breast carcinoma, cervical carcinoma, cervical hyperplasia, choriocarcinoma, chronic granulocytic leukemia, chronic lymphocytic leukemia, colon carcinoma, endometrial carcinoma, esophageal carcinoma, essential thrombocytosis, genitourinary carcinoma, hairy cell leukemia, head and neck carcinoma, Hodgkin's disease, Kaposi's sarcoma, lung carcinoma, lymphoma, malignant carcinoid carcinoma, malignant hypercalcemia, malignant melanoma, malignant pancreatic insulinoma, medullary thyroid carcinoma, melanoma, multiple myeloma, mycosis fungoides, myeloid and lymphocytic leukemia, neuroblastoma, non-Hodgkin's lymphoma, osteogenic sarcoma, ovarian carcinoma, pancreatic carcinoma, polycythemia vera, primary brain carcinoma, primary macroglobulinemia, prostatic carcinoma, renal cell carcinoma, rhabdomyosarcoma, skin cancer, small-cell lung carcinoma, soft-tissue sarcoma, squamous cell carcinoma, stomach carcinoma, testicular carcinoma, thyroid carcinoma, or Wilms' tumor.

[0016] In further aspects of the invention the hyperproliferative disorder is any one of age-related macular degeneration, Crohn's disease, cirrhosis, chronic inflammatory-related disorders, proliferative diabetic retinopathy, proliferative vitreoretinopathy, retinopathy of prematurity, granulomatosis, immune hyperproliferation associated with organ or tissue transplantation, an immunoproliferative disease or disorder, *e.g.*, inflammatory bowel disease, psoriasis, rheumatoid arthritis, systemic lupus erythematosus (SLE), vascular hyperproliferation secondary to retinal hypoxia, or vasculitis.

[0017] In one embodiment the invention is drawn to methods of treating, ameliorating, or preventing hyperproliferative disease in a subject comprising administering to said subject a therapeutically effective amount of an N-oxide of vinca alkaloid or analog thereof. In another embodiment, the vinca alkaloid

analog is selected from the group consisting of vinblastine N-oxide, desacetyl vinblastine N-oxide, vinorelbine N-oxide, vincristine N-oxide and vinflunine N-oxide, or a pharmaceutically acceptable salt thereof.

[0018] In certain embodiments, a metronomic dosing regime for an N-oxide of vinca alkaloid or analog thereof comprises administration of the N-oxide at a dose below an established maximum tolerated dose (MTD) for the N-oxide, which upon repeated administration inhibits tumor growth and produces less toxic side effects as compared to administration of the maximum tolerated dose of the N-oxide. While not being bound to a particular mechanism, it is believed that metronomic dosing with an N-oxide of vinca alkaloid or analog thereof may target cells of the vasculature which form the blood vessels of the tumor as opposed to the tumor cells themselves. Accordingly, inhibition of tumor growth may result from the inability of the tumor cells to establish the functional microvasculature critical for tumor growth and dissemination.

[0019] An additional aspect of the present invention is a method for treating, ameliorating, or preventing hyperproliferative disorders in an animal comprising administering to the animal a therapeutically effective amount of a compound having Formula I in combination with one or more active agents or treatments. In one embodiment, the one or more active agent or treatment is a chemotherapeutic agent, a radiotherapeutic agent/treatment, an anti-angiogenesis agent, a vascular targeting agent, a hypoxia-inducible factor 1 (HIF1) inhibitor, an Hsp90 inhibitor, a tyrosine kinase inhibitor, a serine/threonine kinase inhibitor, a proteasome inhibitor, an HDAC inibitor, a caspase inducer, a CDK inhibitor, and a proapoptotic molecule. In another embodiment, the one or more active agent or treatment is used, has been used, or is known to be useful for the treatment of the hyperproliferative disorder.

[0020] In one embodiment, the method of treating, ameliorating, or preventing hyperproliferative disorder in an animal comprises administering to the animal a therapeutically effective amount of vinca alkaloid N-oxide or analog thereof. In particular embodiments, the vinca alkaloid analog N-oxide is selected from the group consisting of vinblastine N-oxide, desacetyl vinblastine N-oxide, vinorelbine N-oxide, vincristine N-oxide and vinflunine N-oxide, or a pharmaceutically acceptable salt thereof, in combination with one or more

active agents or treatments, for example, chemotherapeutic agents or radiotherapeutic agents/treatments.

[0021] In preferred embodiments of the invention, the one or more chemotherapeutic agents can be any chemotherapeutic agent which is used, has been used, or is known to be useful for the treatment of hyperproliferative disorders.

[0022] In preferred embodiments of the invention, the one or more radiotherapeutic agents or treatments can be external-beam radiation therapy, brachytherapy, thermotherapy, radiosurgery, charged-particle radiotherapy, neutron radiotherapy, photodynamic therapy, or radionuclide therapy.

[0023] In one embodiment of the invention, the compound having Formula I can be administered prior to, during, and/or beyond administration of the one or more chemotherapeutic agents or radiotherapeutic agents or treatments. In another embodiment of the invention, the method of administering a compound having Formula I in combination with one or more chemotherapeutic agents or radiotherapeutic agents or treatments is repeated more than once.

[0024] The combination of a compound having Formula I and one or more chemotherapeutic agents or radiotherapeutic agents or treatments of the present invention will have additive potency or an additive therapeutic effect. The invention also encompasses synergistic combinations where the therapeutic efficacy is greater than additive. Preferably, such combinations will reduce or avoid unwanted or adverse effects. In certain embodiments, the combination therapies encompassed by the invention will provide an improved overall therapy relative to administration of a compound having Formula I or any chemotherapeutic agent or radiotherapeutic agent or treatment alone. In certain embodiments, doses of existing or experimental chemotherapeutic agents or radiotherapeutic agents or treatments will be reduced or administered less frequently which will increase patient compliance, thereby improving therapy and reducing unwanted or adverse effects.

[0025] Further, the methods of the invention will be useful not only with previously untreated patients but also will be useful in the treatment of patients partially or completely refractory to current standard and/or experimental

cancer therapies, including but not limited to radiotherapies, chemotherapies, and/or surgery. In a preferred embodiment, the invention will provide therapeutic methods for the treatment or amelioration of hyperproliferative disorders that have been shown to be or may be refractory or non-responsive to other therapies.

[0026] While not wishing to be bound by any theory, it is believed that some of the N-oxide compounds of the invention will function as prodrugs with greatly diminished cytotoxicity. It is believed that these N-oxide compounds will be activated under hypoxic conditions within the target tissues (*i.e.*, reduced at the nitrogen atom), followed by inhibition of microtube formation in the mitotic spindle resulting in arrest of dividing cells at the metaphase stage, diminishing cells' ability to replicate. Other N-oxide compounds of the invention may have intrinsic cytotoxic activity. Since a number of pathological tissues have significant hypoxic components which promote hyperproliferation, it is believed that this portion of tissue will be preferentially targeted.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0027] Figure 1 shows x-ray single crystal structure of vinblastine N-oxide. The chemical structure of the compound is also shown for comparison purposes.

[0028] Figure 2A-2D show hypoxia-activated cytotoxicity of vinblastine N-oxide against multiple human solid tumor cell lines *in vitro*.

[0029] Figures 3A-3D show hypoxia-activated cytotoxicity of vincristine N-oxide against multiple human solid tumor cell lines *in vitro*.

[0030] Figure 4 shows differential cytotoxic activity of vinblastine N-oxide against viable H460 lung adenocarcinoma tumor colonies under hypoxic vs. normoxic conditions *in vitro*.

[0031] Figure 5 shows activation of cytotoxicity of Vinblastine N-oxide against H460 lung carcinoma cells is oxygen dependent. The HCR was calculated as the IC_{50} under normoxic conditions/IC_{50} hypoxic condtions

[0032] Figures 6A-6D show chromatograms from LC/MS-MS analysis of the extracellular medium from 200 nM vinblastine N-oxide treated H460 lung adenocarcinoma tumor cells demonstrating that vinblastine N-oxide is converted to vinblastine (parent drug) following hypoxia exposure.

[0033] Figures 7A and 7B show LC/MS-MS analysis of vinblastine N-oxide (filled bars) or bioreduced vinblastine (unfilled bars) from the lysates (7A) or extracellular medium (7B) of H460 cells treated with 200 nM vinblastine N-oxide exposed to normoxic or hypoxic conditions.

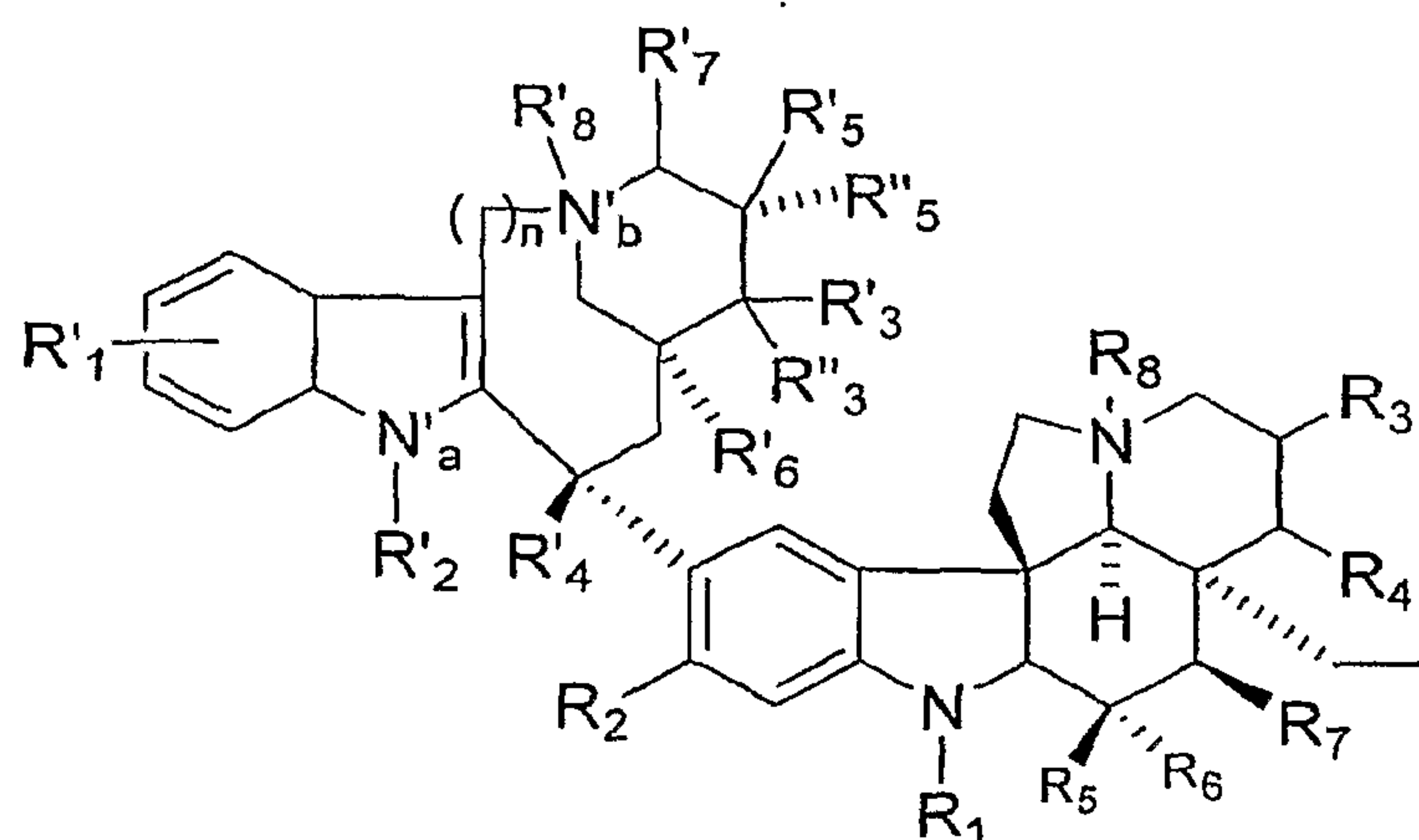
[0034] Figures 8A and 8B show LC/MS-MS analysis of vincristine N-oxide (filled bars) or bioreduced vincristine (unfilled bars) from the lysates (8A) or extracellular medium (8B) of H460 cells treated with 7 mM vincristine N-oxide exposed to normoxic or hypoxic conditions.

[0035] Figure 9 shows the effects of vinblastine n-oxide analog (VBL-NO) or vinblastine treatment on mean percentage change in body weight loss in immunodeficient mice (n=5 group). All agents were i.p. administered on a q3d X 5 schedule (pink arrowheads) at the indicated dosages.

[0036] Figure 10 shows the effects of vincristine n-oxide analog (VCR-NO) or vincristine sulfate treatment on mean percentage change in body weight loss in tumor-bearing immunodeficient mice (n=5 group). All agents were i.v. administered on a q3d X 5 schedule (pink arrowheads) at the indicated dosages.

DETAILED DESCRIPTION OF THE INVENTION

[0037] One aspect of the invention is drawn to vinca alkaloid N-oxides having Formula I:



I

wherein R_1-R_8 R'_1-R'_8, R''_3, R''_5, and n are as defined above.

[0038] According to another aspect of the invention, a therapeutically effective amount of a compound having Formula I, or a pharmaceutically acceptable salt thereof, and at least one other active agent is provided in the form of a pharmaceutical composition having at least one pharmaceutically acceptable carrier. In certain instances, the at least one other active agent is a chemotherapeutic agent (including an active vitamin D compound). Compounds having Formula I may be formulated in a single formulation with the other active agent(s), or formulated independently.

[0039] According to one aspect of the invention, methods for treating, ameliorating, or preventing hyperproliferative disorders are provided, wherein a therapeutically effective amount of a compound having Formula I, or a pharmaceutically acceptable salt thereof, is administered to an animal in need thereof. In certain aspects of the invention, the hyperproliferative disorder is cancer.

[0040] A further aspect of the invention relates to methods for treating, ameliorating, or preventing a hyperproliferative disorder comprising administering a therapeutically effective amount of a compound having Formula I, or a pharmaceutically acceptable salt thereof, in combination with at least one other active agent or treatment to a patient in need thereof. In certain embodiments, combinations of a compound having Formula I with a chemotherapeutic agent are administered. In one embodiment, the chemotherapeutic agent is selected from gemcitabine and irinotecan.

[0041] Hyperproliferative disorders which can be treated with the compounds having Formula I include any hypoxia-aggravated hyperproliferative disease and/or disorder, such as any number of cancers. Generally, such cancers include, without limitation, cancers of the bladder, brain, breast, cervix, colon, endometrium, esophagus, head and neck, kidney, larynx, liver, lung, oral cavity, ovaries, pancreas, prostate, skin, stomach, and testis. Certain of these cancers may be more specifically referred to as acute and chronic lymphocytic leukemia, acute granulocytic leukemia, adrenal cortex carcinoma, bladder carcinoma, breast carcinoma, cervical carcinoma, cervical hyperplasia, choriocarcinoma, chronic granulocytic leukemia, chronic lymphocytic leukemia, colon carcinoma, endometrial carcinoma, esophageal carcinoma, essential thrombocytosis, genitourinary carcinoma, hairy cell leukemia, head and neck carcinoma, Hodgkin's disease, Kaposi's sarcoma, lung carcinoma, lymphoma, malignant carcinoid carcinoma, malignant hypercalcemia, malignant melanoma, malignant pancreatic insulinoma, medullary thyroid carcinoma, melanoma, multiple myeloma, mycosis fungoides, myeloid and lymphocytic leukemia, neuroblastoma, non-Hodgkin's lymphoma, osteogenic sarcoma, ovarian carcinoma, pancreatic carcinoma, polycythemia vera, primary brain carcinoma, primary macroglobulinemia, prostatic carcinoma, renal cell carcinoma, rhabdomyosarcoma, skin cancer, small-cell lung carcinoma, soft-tissue sarcoma, squamous cell carcinoma, stomach carcinoma, testicular carcinoma, thyroid carcinoma, and Wilms' tumor. In one embodiment, the cancer is a solid tumor. In another embodiment, the cancer is selected from the group consisting of colon cancer, brain cancer, glioma, multiple myeloma, head and neck cancer hepatocellular cancer, melanoma, ovarian cancer, cervical cancer, renal cancer, and non-small cell lung cancer.

[0042] Animals which may be treated according to the present invention include all animals which may benefit from administration of compounds having Formula I. Such animals include humans, pets such as dogs and cats, and veterinary animals such as cows, pigs, sheep, goats and the like.

[0043] The term "alkyl" as used herein refers to an unsaturated acyclic hydrocarbon radical. The term "lower alkyl" refers to acyclic hydrocarbon radicals containing from about 2 to about 10 carbon atoms, preferably from

about 2 to about 8 carbon atoms and more preferably 1 to about 6 carbon atoms. Examples of suitable alkyl radicals include methyl, ethyl, propyl, butyl, isobutyl, pentyl, 2-methylbutyl, 3-methylbutyl, hexyl, heptyl, and octyl, and the like.

[0044] The term "alkoxy" means a straight, branched or cyclic hydrocarbon configuration and combinations thereof, including from 1 to 20 carbon atoms, preferably from 1 to 8 carbon atoms, more preferably from 1 to about 4 carbon atoms, and an oxygen atom at the point of attachment. Suitable alkoxy groups include methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, iso-butoxy, sec-butoxy, tert-butoxy, cyclopropyloxy, cyclohexyloxy, and the like. "Lower alkoxy" refers to alkoxy groups having from 1 to 4 carbon atoms.

[0045] The term "acyl" or "alkanoyl means an alkyl group attached to a carbonyl group.

[0046] The term "halogenoacyl" means an acyl group substituted with one or more halogen groups (e.g. F, Cl, Br and I groups), including trifluoroacetyl, pentafluoropropionyl and the like.

[0047] The term "non-N-oxide" as used herein refers to an amine compound that is not oxidized at the nitrogen atom. As an example, vinblastine is the non-N-oxide form of vinblastine N-oxide.

[0048] The term "pharmaceutical composition" as used herein, is to be understood as defining compositions of which the individual components or ingredients are themselves pharmaceutically acceptable, *e.g.*, where oral administration is foreseen, acceptable for oral use; where topical administration is foreseen, topically acceptable; and where intravenous administration is foreseen, intravenously acceptable.

[0049] As used herein, the term "therapeutically effective amount" refers to that amount of the therapeutic agent sufficient to result in amelioration of one or more symptoms of a disorder, or prevent advancement of a disorder, or cause regression of the disorder. For example, with respect to the treatment of cancer, a therapeutically effective amount preferably refers to the amount of a therapeutic agent that decreases the rate of tumor growth, decreases tumor mass, decreases the number of metastases, increases time to tumor progression, or increases survival time by at least 5%, preferably at least 10%,

at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100%.

[0050] The terms "prevent," "preventing," and "prevention," as used herein, refer to a decrease in the occurrence of pathological cells (*e.g.*, hyperproliferative or neoplastic cells) in an animal. The prevention may be complete, *e.g.*, the total absence of pathological cells in a subject. The prevention may also be partial, such that the occurrence of pathological cells in a subject is less than that which would have occurred without the present invention.

[0051] Compounds having Formula I can be provided as pharmaceutically acceptable salts. Examples of pharmaceutically acceptable salts (*i.e.*, addition salts) include inorganic and organic acid addition salts such as hydrochloride, hydrobromide, phosphate, sulphate, citrate, lactate, tartrate, maleate, fumarate, mandelate, benzoate and oxalate; and inorganic and organic base addition salts with bases such as sodium hydroxy, Tris(hydroxymethyl)aminomethane (TRIS, tromethane) and N-methyl-glucamine. Although the salts typically have similar physiological properties compared to the free base, certain acid addition salts may demonstrate preferred physicochemical properties, *e.g.*, enhanced solubility, improved stability. One particular pharmaceutically acceptable salt is derived from maleic acid, the salt being either a hydrogen maleate or a dimaleate salt.

[0052] Certain of the compounds of the present invention may exist as stereoisomers including optical isomers. The invention includes all stereoisomers and both the racemic mixtures of such stereoisomers as well as the individual enantiomers that may be separated according to methods that are well known to those of ordinary skill in the art. Certain of the compounds of the present invention may also exist as diasteroisomers wherein one or more substituents on the vinca alkaloid analog contain one or more chiral centers.

[0053] In certain embodiments, the N-oxide formation creates a new chiral center with the formation of individual enantiomers (e.g., in a stereoselective N-oxidation of an achiral vinca alkaloid or analog), mixture of enantiomers

(e.g., in a non-stereoselective oxidation of an achiral vinca alkaloid or analog), individual diasteroisomers (e.g., in a stereoselective N-oxidation of an enantiomerically pure vinca alkaloid or analog), or mixtures of diasteroisomers (e.g., in a non-stereoselective N-oxidation of an enantiomeric mixture of a vinca alkaloid or analog). Thus, the invention includes all N-oxide mixtures of enantiomers and diasteroisomers as well as individual diasteroisomers and enantiomers that may be prepared using stereoselective reactions or separated according to methods that are well known to those of ordinary skill in the art.

[0054] In certain embodiments of the invention, compounds having Formula I are administered in combination with one or more other active agents (*e.g.*, chemotherapeutic agents) or treatments. By way of non-limiting example, a patient may be treated for a hyperproliferative disorder, such as cancer, by the administration of a therapeutically effective amount of a compound having Formula I in combination with radiotherapy agent/treatment or the administration of a chemotherapeutic agent.

[0055] In other embodiments, compounds of the invention are administered in combination with agents, such as anti-angiogenic agents, that block inhibit or modulate tumor neovascularization. In preferred embodiments, anti-angiogenesis agents can be any anti-angiogenesis agent which is used, has been used, or is known to be useful for the treatment of hyperproliferative disorders. Examples of anti-angiogenesis agents include bevacizumab (Avastin™), VEGF-TRAP, anti-VEGF-receptor antibodies, angiostatin, endostatin, batimastat, captopril, cartilage derived inhibitor, genistein, interleukin 12, lavendustin, medroxypregesterone acetate, recombinant human platelet factor 4, tecogalan, thrombospondin, TNP-470, VEGF antagonists, anti-VEGF monoclonal antibody, soluble VEGF-receptor chimaeric protein, antisense oligonucleotides, antisense oligodexoynucleotides, siRNAs, anti-VEGF aptamers, pigment epithelium derived factor, a tyrosine kinase inhibitor, an inhibitor of epidermal-derived growth factor, an inhibitor of fibroblast-derived growth factor, an inhibitor of platelet derived growth factor, an MMP (matrix metalloprotease) inhibitor, an integrin blocker, interferon-α, pentosan polysulfate, a cyclooxygenase inhibitor, carboxyamidotriazole, combretastatin A-4, squalamine, 6-O-chloroacetyl-carbonyl)-fumagillol,

thalidomide, troponin-1, indolinethiones, pyridopyrimidines, quinoazolines, phenyl-pyrrolo-pyrimidines, trastuzumab, calcium influx inhibitor (CAI), neomycin, squalamine, marimastat, prinomastat (AG-3340), metastat (COL-3) and cinnoline derivatives. Additional anti-angiogenic compounds that may be administered in combination with the compounds of the present invention are described in U.S. Patent Nos. 5,192,744, 5,426,100, 5,733,876, 5,840,692, 5,854,205, 5,990,280, 5,994,292, 6,342,219, 6,342,221, 6,346,510, 6,479,512, 6,719,540, 6,797,488, 6,849,599, 6,869,952, 6,887,874, 6,958,340 and 6,979,682.

[0056] In certain embodiments, the compounds of the present invention are administered in combination with a vascular targeting agent (also known as vascular damaging agents). In one embodiment, the vascular targeting agent is for the treatment of malignant or non-malignant vascular proliferative disorders. In other embodiments, vascular targeting agents can be any vascular targeting agent which is used, has been used, or is known to be useful for the treatment of hyperproliferative disorders. Examples of vascular targeting agents that may be administered in combination with the compounds of the present invention include DMXAA 5,6-dimethylxanthenone-4-acetic acid, ZD6126, (5S)-5-(acetylamino)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[a,c]cyclohepten-3-yl dihydrogen phosphate, also known as *N*-acetylcolchinol-O-phosphate (see, for example, U.S. Patent No. 6,906,048); functionalized stilbene derivatives such as combretastatin A4 and its prodrugs (see, e.g., U.S. Patent Nos. 6,919,324 and 6,773,702); dioleoyltrimethyl-ammonium propane (DOTAP), N-[1-(2,3-dioleoyloxy)-propyl]-N,N,N-trimethylammonium chloride (DOTMA), dimethyldioctadecylammonium bromide (DDAB), 1,2-dimyristyloxypropyl-3-dimethylhydroxyethyl (DMRIE), dioleoyl-3-dimethylammonium propane (DODAP), N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), or N-(1-(2,3-dioleyloxy)propyl)-N-(2-(sperminecarboxamido)ethyl)-N,N-dimethyl ammonium trifluoroacetate (DOSPA), or any other natural or synthetic cationic lipids, including, for example, dioleoylphosphatidyl-choline (DOPC), dipalmitoylphosphatidylcholine (DPPC), disteroylphosphatidylcholine (DSPC), dimyristoylphosphatidylcholine (DMPC), or 1,2-sn-

dioleoylphosphatidylcholine (DOPE), or any other natural or synthetic electrostatically neutral lipids (see, for example, U.S. patent No. 6,680,068); vascular targeting agents which incorporate benzo[*b*]thiophene, indole, and benzofuran molecular skeletons such as those described in U.S. Patent No. 6,593,374.

[0057] In other embodiments, the compounds of the present invention are administered in combination with a hypoxia-inducible factor 1 (HIF1) inhibitor. In one embodiment, the HIF1 inhibitor is for the treatment of malignant or non-malignant vascular proliferative disorders. In other embodiments, HIF1 inhibitors can be any HIF1 inhibitor which is used, has been used, or is known to be useful for the treatment of hyperproliferative disorders. Examples of HIF1 inhibitors suitable for use in combination with compounds of the present invention include topotecan, P13 kinase inhibitors; LY294002; rapamycin; histone deacetylase inhibitors such as [(E)-(1S,4S,10S,21R)-7-[(Z)-ethylidene]-4,21-diisopropyl-2-oxa-12,13-dithia-5,8,20,23-tetraazabicyclo-[8,7,6]-tricos-16-ene-3,6,9,19,22-pentanone (FR901228, depsipeptide); heat shock protein 90 (Hsp90) inhibitors such as geldanamycin, 17-allylamino-geldanamycin (17-AAG), and other geldanamycin analogs, and radicicol and radicicol derivatives such as KF58333; genistein; indanone; staurosporin; protein kinase-1 (MEK-1) inhibitors such as PD98059 (2'-amino-3'-methoxyflavone); PX-12 (1-methylpropyl 2-imidazolyl disulfide); pleurotin PX478; quinoxaline 1,4-dioxides; sodium butyrate (NaB); sodium nitropurruside (SNP) and other NO donors; microtubule inhibitors such as novobiocin, panzem (2-methoxyestradiol or 2-ME2), vincristines, taxanes, epothilones, discodermolide, and derivatives of any of the foregoing; coumarins; barbituric and thiobarbituric acid analogs; camptothecins; and YC-1. See U.S. Patent No. 6,979,675.

[0058] In certain embodiments, the compounds of the present invention are administered in combination with an Hsp90 inhibitor. In one embodiment, the Hsp90 inhibitor is for the treatment of malignant or non-malignant vascular proliferative disorders. In other embodiments, Hsp90 inhibitors can be any Hsp90 inhibitor which is used, has been used, or is known to be useful for the

treatment of hyperproliferative disorders. Examples of Hsp90 inhibitors that may be combined with the compounds of the present invention include geldanamycin, 17-allylamino-17-demethoxygeldanamycin, geldanamycin derivatives such as those described in U.S. patent No. 6,890,917, dexamethasone and benzoquinone ansamycins such as those described in U.S. patent No. 6,872,715. Additional Hsp90 inhibitors are disclosed in U.S. Patent Nos. 6,613,780, 6,281,229 and 6,903,116.

[0059] In other embodiments, the compounds of the present invention are administered in combination with an inhibitor of tyrosine and/or serine/threonine kinases and tyrosine kinase receptors involved in cellular signaling. These include tyrosine kinase inhibitors of Src, Abl, Platelet Derived Growth Factor Receptors, Vascular Endothelial Growth Factor Receptors, c-Met, Fibroblast Growth Factor receptors, Epidermal Growth Factor Receptors, Insulin Growth Factor Receptors, mTOR, Flt-3, CSF-1 Receptor, AKT, Polo kinases, Aurora Kinases, STAT-3, PI-3 Kinase, Ras, Raf and Mitogen Activated Kinases, MEK, ERK. Examples of tyrosine kinase and serine/threonine kinase inhibitors include (but not limited to): AMG706, ZA6474, BAY 43-9006, Dasatinib, CEP-701, XL647, XL999, Lapatinb, MLN518/CT53518, PKC412, ST1571, AMN107, AEE 788, OSI-930, OSI-817, SU11248, AG-03736, GW-786034m , CEP-7055.

[0060] In other embodiments, the compounds of the present invention are administered in combination with HDAC inhibitors. Examples include (but not limited to) SAHA, MS-275, MGCD0103, LBH589, PXD101, FK228.

[0061] In other embodiments, the compounds of the present invention are administered in combination with proteasome inhbitors such as Velcade.

[0062] In other embodiments, the compounds of the present invention are administered in combination with pro-apoptotic agents such as TRAIL, anti-DR4/DR5 (TRA8) antibodies, IAP, Survivin or small molecules that stimulate caspase activation.

[0063] In other embodiments, the compounds of the present invention are administered in combination with inhibitors of cell cycle regulators such as CDK inhibitors.

[0064] "In combination" refers to the use of more than one treatment. The use of the term "in combination" does not restrict the order in which treatments are administered to a subject being treated for a hyperproliferative disorder. A first treatment can be administered prior to, concurrently with, after, or within any cycling regimen involving the administration of a second treatment to a subject with a hyperproliferative disorder. For example, the first treatment can be administered 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before a treatment; or the first treatment can be administered 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after a second treatment. Such treatments include, for example, the administration of compounds having Formula I in combination with one or more chemotherapeutic agents or radiotherapeutic agents/treatments.

[0065] The term "chemotherapeutic agent," as used herein, is intended to refer to any chemotherapeutic agent known to those of skill in the art to be effective for the treatment, prevention or amelioration of hyperproliferative disorders such as cancer. Chemotherapeutic agents include, but are not limited to, small molecules, synthetic drugs, peptides, polypeptides, proteins, nucleic acids (*e.g.*, DNA and RNA polynucleotides including, but not limited to, antisense nucleotide sequences, triple helices and nucleotide sequences encoding biologically active proteins, polypeptides or peptides), antibodies, synthetic or natural inorganic molecules, mimetic agents, and synthetic or natural organic molecules. Any agent which is known to be useful, or which has been used or is currently being used for the treatment or amelioration of a hyperproliferative disorder can be used in combination with a compound having Formula I. See, *e.g.*, Hardman *et al.*, eds., 2002, Goodman & Gilman's The Pharmacological Basis Of Therapeutics 10th Ed, Mc-Graw-Hill, New York, NY for information regarding therapeutic agents which have been or are currently being used for the treatment or amelioration of a hyperproliferative disorder.

[0066] Particular chemotherapeutic agents useful in the methods and compositions of the invention include alkylating agents, antimetabolites, anti-mitotic agents, epipodophyllotoxins, antibiotics, hormones and hormone antagonists, enzymes, platinum coordination complexes, anthracenediones, substituted ureas, methylhydrazine derivatives, imidazotetrazine derivatives, cytoprotective agents, DNA topoisomerase inhibitors, biological response modifiers, retinoids, therapeutic antibodies, differentiating agents, immunomodulatory agents, angiogenesis inhibitors and anti-angiogenic agents.

[0067] Certain chemotherapeutic agents include, but are not limited to, abarelix, aldesleukin, alemtuzumab, alitretinoin, allopurinol, altretamine, amifostine, anastrozole, arsenic trioxide, asparaginase, BCG live, bevaceizumab, bexarotene, bleomycin, bortezomib, busulfan, calusterone, camptothecin, capecitabine, carboplatin, carmustine, celecoxib, cetuximab, chlorambucil, cinacalcet, cisplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, darbepoetin alfa, daunorubicin, denileukin diftitox, dexrazoxane, docetaxel, doxorubicin, dromostanolone, Elliott's B solution, epirubicin, epoetin alfa, estramustine, etoposide, exemestane, filgrastim, floxuridine, fludarabine, fluorouracil, fulvestrant, gemcitabine, gemtuzumab ozogamicin, gefitinib, goserelin, hydroxyurea, ibritumomab tiuxetan, idarubicin, ifosfamide, imatinib, interferon alfa-2a, interferon alfa-2b, irinotecan, letrozole, leucovorin, levamisole, lomustine, meclorethamine, megestrol, melphalan, mercaptopurine, mesna, methotrexate, methoxsalen, methylprednisolone, mitomycin C, mitotane, mitoxantrone, nandrolone, nofetumomab, oblimersen, oprelvekin, oxaliplatin, paclitaxel, pamidronate, pegademase, pegaspargase, pegfilgrastim, pemetrexed, pentostatin, pipobroman, plicamycin, polifeprosan, porfimer, procarbazine, quinacrine, rasburicase, rituximab, sargramostim, streptozocin, talc, tamoxifen, tarceva, temozolomide, teniposide, testolactone, thioguanine, thiotepa, topotecan, toremifene, tositumomab, trastuzumab, tretinoin, uracil mustard, valrubicin, vinblastine, vincristine, vinorelbine, and zoledronate. In certain embodiments, chemotherapeutic agents are selected from gemcitabine and irinotecan.

[0068] Chemotherapeutic agents may be administered at doses that are recognized by those of skill in the art to be effective for the treatment of the

hyperproliferative disorder. In certain embodiments, chemotherapeutic agents may be administered at doses lower than those used in the art due to the additive or synergistic effect of the compounds having Formula I.

[0069] Therapeutic agents useful in the methods and compositions of the invention include active vitamin D compound or mimics thereof, antineoplastic agents (*e.g.*, actinomycin D, irinotecan, vincristine, vinorelbine, SN-38, azacitidine (5-azacytidine, 5AzaC), thalidomide vinblastine, methotrexate, azathioprine, fluorouracil, doxorubicin, mitomycin, docetaxel, paclitaxel), angiogenic inhibitors (e.g., VEGF-TRAP, angiostatin, endostatin, aptamer antogonist of VEGF, batimastat, captopril, cartilage derived inhibitor, genistein, interleukin 12, lavendustin, medroxypregesterone acetate, recombinant human platelet factor 4, tecogalan, thrombospondin and TNP-470), serine/threonine kinase inhibitors, tyrosine kinase inhibitors, HDAC inhibitors, Proteasome inhibitors, CDK inhibitors, HSP inhibitors, vasodilators (*e.g.*, nitrates, calcium channel blockers), anticoagulants (*e.g.*, heparin), anti-platelet agents (*e.g.*, aspirin, blockers of IIb/IIIa receptors, clopidogrel), anti-thrombins (*e.g.*, hirudin, iloprost), immunosuppressants (*e.g.*, sirolimus, tranilast, dexamethasone, tacrolimus, everolimus, A24), collagen synthetase inhibitors (*e.g.*, halofuginone, propyl hydroxylase, C-proteinase inhibitor, metalloproteinase inhibitor), anti-inflammatories (*e.g.*, corticosteroids, non-steroidal anti-inflammatory drugs), 17β-estradiol, angiotensin converting enzyme inhibitors, colchicine, fibroblast growth factor antagonists, histamine antagonists, lovastatin, nitroprusside, phosphodiesterase inhibitors, prostaglandin inhibitors, suramin, serotonin blockers, thioprotease inhibitors, platelet-derived growth factor antagonists, nitric oxide, and angiopeptin. In one embodiment, the therapeutic agent is a taxane, *e.g.*, paclitaxel or docetaxel.

[0070] In certain embodiments, patients are subjected to a hypoxia imaging technique prior to administration of the compositions comprising the compounds of the present invention. Examples of imaging techniques suitable for the determination of the presence of hypoxic tumor cells include computed tomography (CT), magnetic resonance imaging (MRI), single photon emission computer tomography (SPECT), and positron emission tomography (PET).

Use of such visualization methods can advantageously be used to select a subset of patients that are particularly suitable for treatment with hypoxia activated antiproliferative compositions of the present invention.

[0071] In this embodiment, the invention is directed to a method of treating, preventing or ameliorating a hyperproliferative disease in an animal in need thereof, comprising determining whether said hyperproliferative disease is characterized by hypoxic tissue, and treating said animal with an effective amount of a compound of the invention.

[0072] The term "radiotherapeutic agent," as used herein, is intended to refer to any radiotherapeutic agent known to one of skill in the art to be effective to treat or ameliorate cancer, without limitation. For instance, the radiotherapeutic agent can be an agent such as those administered in brachytherapy or radionuclide therapy. Such methods can optionally further comprise the administration of one or more additional cancer therapies, such as, but not limited to, chemotherapies, surgery, and/or another radiotherapy.

[0073] In certain embodiments involving radiotherapeutic agents or treatments, the present invention relates to a method for treating cancer comprising the administration of vinca alkaloid or analog N-oxide having Formula I, in combination with a treatment comprising a therapeutically effective dose of brachytherapy. The brachytherapy can be administered according to any schedule, dose, or method known to one of skill in the art to be effective in the treatment or amelioration of cancer, without limitation. In general, brachytherapy comprises insertion of radioactive sources into the body of a subject to be treated for cancer, preferably inside the tumor itself, such that the tumor is maximally exposed to the radioactive source, while preferably minimizing the exposure of healthy tissue.

[0074] In certain embodiments, the brachytherapy can be intracavitary brachytherapy. In other embodiments, the brachytherapy can be interstitial brachytherapy. Whether the brachytherapy is intracavitary brachytherapy or interstitial brachytherapy, the brachytherapy can be administered at a high dose rate, a continuous low dose rate, or a pulsed dose rate. For example, and not by way of limitation, a high dose rate brachytherapy regimen can be a dose of 60 Gy administered in ten fractions over six days, while a continuous low dose

rate brachytherapy regimen can be a total dose of about 65 Gy, administered continuously at about 40 to 50 cGy per hour. Other examples of high, continuous low, and pulsed dose rate brachytherapy are well known in the art. See, e.g., Mazeron *et al.*, *Sem. Rad. Onc.* 12:95-108 (2002).

[0075] Representative radioisotopes that can be administered in any of the above-described brachytherapies include, but are not limited to, phosphorus 32, cobalt 60, palladium 103, ruthenium 106, iodine 125, cesium 137, iridium 192, xenon 133, radium 226, californium 252, or gold 198. Other radioisotopes may be selected for administration in brachytherapy according to the desirable physical properties of such a radioisotope. One of skill in the art will readily recognize that many properties will affect a radioisotope's suitability for use in brachytherapy, including, but not limited to, the radioisotope's half-life, the degree to which emitted radiation penetrates surrounding tissue, the energy of emitted radiation, the ease or difficulty of adequately shielding the radioisotope, the availability of the radioisotope, and the ease or difficulty of altering the shape of the radioisotope prior to administration.

[0076] Additional methods of administering and apparatuses and compositions useful for brachytherapy are described in U.S. Patent Nos. 6,319,189, 6,179,766, 6,168,777, 6,149,889, and 5,611,767.

[0077] In certain embodiments involving radiotherapeutic agents or treatments, the present invention relates to a method for treating cancer comprising the administration of vinca alkaloid or analog N-oxide having Formula I, in combination with a treatment comprising a therapeutically effective dose of a radionuclide. The radionuclide therapy can be administered according to any schedule, dose, or method known to one of skill in the art to be effective in the treatment or amelioration of cancer, without limitation. In general, radionuclide therapy comprises systemic administration of a radioisotope that preferentially accumulates in or binds to the surface of cancerous cells. The preferential accumulation of the radionuclide can be mediated by a number of mechanisms, including, but not limited to, incorporation of the radionuclide into rapidly proliferating cells, specific

accumulation of the radionuclide by the cancerous tissue without special targeting (*e.g.*, iodine 131 accumulation in thyroid cancer), or conjugation of the radionuclide to a biomolecule specific for a neoplasm.

[0078] Representative radioisotopes that can be administered in radionuclide therapy include, but are not limited to, phosphorus 32, yttrium 90, dysprosium 165, indium 111, strontium 89, samarium 153, rhenium 186, iodine 131, iodine 125, lutetium 177, and bismuth 213. While all of these radioisotopes may be linked to a biomolecule providing specificity of targeting, iodine 131, indium 111, phosphorus 32, samarium 153, and rhenium 186 may be administered systemically without such conjugation. One of skill in the art may select a specific biomolecule for use in targeting a particular neoplasm for radionuclide therapy based upon the cell-surface molecules present on that neoplasm. For example, hepatomas may be specifically targeted by an antibody specific for ferritin, which is frequently over-expressed in such tumors. Examples of antibody-targeted radioisotopes for the treatment of cancer include ZEVALIN™ (ibritumomab tiuxetan) and BEXXAR™ (tositumomab), both of which comprise an antibody specific for the B cell antigen CD20 and are used for the treatment of non-Hodgkin lymphoma.

[0079] Other examples of biomolecules providing specificity for particular cell are reviewed in an article by Thomas, *Cancer Biother. Radiopharm.* *17*:71-82 (2002).

Furthermore, methods of administering and compositions useful for radionuclide therapy may be found in U.S. Patent Nos. 6,426,400, 6,358,194, 5,766,571, and 5,563,250.

[0080] In certain embodiments involving radiotherapeutic agents or treatments, the present invention relates to a method for treating cancer comprising the administration of vinca alkaloid or analog N-oxide having Formula I, in combination with a treatment comprising a therapeutically effective dose of external-beam radiation therapy. The external-beam radiation therapy can be administered according to any schedule, dose, or method known to one of skill in the art to be effective in the treatment or amelioration of cancer, without limitation. In general, external-beam radiation

therapy comprises irradiating a defined volume within a subject with a high energy beam, thereby causing cell death within that volume. The irradiated volume preferably contains the entire cancer to be treated, and preferably contains as little healthy tissue as possible.

[0081] In certain embodiments, the external-beam radiation therapy can be three-dimensional conformal radiotherapy. In other embodiments, the external-beam radiation therapy can be continuous hyperfractionated radiotherapy. In still other embodiments, the external-beam radiation therapy can be intensity-modulated radiotherapy. In yet other embodiments, the external-beam radiation therapy can be helical tomotherapy. In still other embodiments, the external-beam radiation therapy can be three-dimensional conformal radiotherapy with dose escalation. In yet other embodiments, the external-beam radiation therapy can be stereotactic radiotherapy, including, but not limited to, single fraction stereotactic radiotherapy, fractionated stereotactic radiotherapy, and fractionated stereotactically guided conformal radiotherapy.

[0082] The external-beam radiation therapy can be generated or manipulated by any means known to one of skill in the art. For example, the photon beam used in external-beam radiation therapy can be shaped by a multileaf collimator. Other examples of suitable devices for generating a photon beam for use in external-beam radiation therapy include a gamma knife and a linac-based stereotactic apparatus. In certain embodiments, administration of the external-beam radiation therapy is controlled by a computer according to a three-dimensional model of the patient in the treatment position. Such a model can be generated, for example, by computed tomography (CT), magnetic resonance imaging (MRI), single photon emission computer tomography (SPECT), and positron emission tomography (PET). Use of such visualization methods can advantageously minimize the volume of healthy tissue treated, thereby allowing higher total doses of radiation to be administered to the patient.

[0083] In addition, healthy tissues can optionally be protected from the effects of the external-beam radiation therapy by placing blocking devices such as, *e.g.*, lead shields, in locations where such protection is needed. Alternatively

or additionally, metal reflecting shields can optionally be located to reflect the photon beam in order to concentrate the radiation on the cancerous tissue to be treated and protect healthy tissue. Placement of either shield is well within the knowledge of one of skill in the art.

[0084] Methods of administering and apparatuses and compositions useful for external-beam radiation therapy can be found in U.S. Patent Nos. 6,449,336, 6,398,710, 6,393,096, 6,335,961, 6,307,914, 6,256,591, 6,245,005, 6,038,283, 6,001,054, 5,802,136, 5,596,619, and 5,528,652.

[0085] In certain embodiments involving radiotherapeutic agents or treatments, the present invention relates to a method for treating cancer comprising the administration of vinca alkaloid or analog N-oxide having Formula I, in combination with a treatment comprising a therapeutically effective dose of thermotherapy. The thermotherapy can be administered according to any schedule, dose, or method known to one of skill in the art to be effective in the treatment or amelioration of cancer, without limitation. In certain embodiments, the thermotherapy can be cryoablation therapy. In other embodiments, the thermotherapy can be hyperthermic therapy. In still other embodiments, the thermotherapy can be a therapy that elevates the temperature of the tumor higher than in hyperthermic therapy.

[0086] Cryoablation therapy involves freezing of a neoplastic mass, leading to deposition of intra- and extra-cellular ice crystals; disruption of cellular membranes, proteins, and organelles; and induction of a hyperosmotic environment, thereby causing cell death. Cryoablation can be performed in one, two, or more freeze-thaw cycles, and further the periods of freezing and thawing can be adjusted for maximum tumor cell death by one of skill in the art. One exemplary device that can be used in cryoablation is a cryoprobe incorporating vacuum-insulated liquid nitrogen. See, *e.g.*, Murphy *et al.*, *Sem. Urol. Oncol. 19*:133-140 (2001). However, any device that can achieve a local temperature of about -180°C to about -195°C can be used in cryoablation therapy. Methods for and apparatuses useful in cryoablation therapy are described in U.S. Patent Nos. 6,383,181, 6,383,180, 5,993,444, 5,654,279,

5,437,673, and 5,147,355.

[0087] Hyperthermic therapy typically involves elevating the temperature of a neoplastic mass to a range from about 42°C to about 44°C. The temperature of the cancer may be further elevated above this range; however, such temperatures can increase injury to surrounding healthy tissue while not causing increased cell death within the tumor to be treated. The tumor may be heated in hyperthermic therapy by any means known to one of skill in the art without limitation. For example, and not by way of limitation, the tumor may be heated by microwaves, high intensity focused ultrasound, ferromagnetic thermoseeds, localized current fields, infrared radiation, wet or dry radiofrequency ablation, laser photocoagulation, laser interstitial thermic therapy, and electrocautery. Microwaves and radiowaves can be generated by waveguide applicators, horn, spiral, current sheet, and compact applicators.

[0088] Other methods of and apparatuses and compositions for raising the temperature of a tumor are reviewed in an article by Wust *et al.*, Lancet Oncol. 3:487-97 (2002), and described in U.S. Patent Nos. 6,470,217, 6,379,347, 6,165,440, 6,163,726, 6,099,554, 6,009,351, 5,776,175, 5,707,401, 5,658,234, 5,620,479, 5,549,639, and 5,523,058.

[0089] In certain embodiments involving radiotherapeutic agents or treatments, the present invention relates to a method for treating cancer comprising the administration of vinca alkaloid or analog N-oxide having Formula I, in combination with a treatment comprising a therapeutically effective dose of radiosurgery. The radiosurgery can be administered according to any schedule, dose, or method known to one of skill in the art to be effective in the treatment or amelioration of cancer, without limitation. In general, radiosurgery comprises exposing a defined volume within a subject to a manually directed radioactive source, thereby causing cell death within that volume. The irradiated volume preferably contains the entire cancer to be treated, and preferably contains as little healthy tissue as possible. Typically, the tissue to be treated is first exposed using conventional surgical techniques, then the radioactive source is manually directed to that area by a surgeon.

Alternatively, the radioactive source can be placed near the tissue to be irradiated using, for example, a laparoscope. Methods and apparatuses useful for radiosurgery are further described in Valentini *et al.*, Eur. J. Surg. Oncol. 28:180-185 (2002) and in U.S. Patent Nos. 6,421,416, 6,248,056, and 5,547,454.

[0090] In certain embodiments involving radiotherapeutic agents or treatments, the present invention relates to a method for treating cancer comprising the administration of vinca alkaloid or analog N-oxide having Formula I, in combination with a treatment comprising a therapeutically effective dose of charged-particle radiotherapy. The charged-particle radiotherapy can be administered according to any schedule, dose, or method known to one of skill in the art to be effective in the treatment or amelioration of cancer, without limitation. In certain embodiments, the charged-particle radiotherapy can be proton beam radiotherapy. In other embodiments, the charged-particle radiotherapy can be helium ion radiotherapy. In general, charged-particle radiotherapy comprises irradiating a defined volume within a subject with a charged-particle beam, thereby causing cellular death within that volume. The irradiated volume preferably contains the entire cancer to be treated, and preferably contains as little healthy tissue as possible. A method for administering charged-particle radiotherapy is described in U.S. Patent No. 5,668,371.

[0091] In certain embodiments involving radiotherapeutic agents or treatments, the present invention relates to a method for treating cancer comprising the administration of vinca alkaloid or analog N-oxide having Formula I, in combination with a treatment comprising a therapeutically effective dose of neutron radiotherapy. The neutron radiotherapy can be administered according to any schedule, dose, or method known to one of skill in the art to be effective in the treatment or amelioration of cancer, without limitation.

[0092] In certain embodiments, the neutron radiotherapy can be a neutron capture therapy. In such embodiments, a compound that emits radiation when bombarded with neutrons and preferentially accumulates in a neoplastic mass is administered to a subject. Subsequently, the tumor is irradiated with a low

energy neutron beam, activating the compound and causing it to emit decay products that kill the cancerous cells. Such compounds are typically boron containing compounds, but any compound that has a significantly larger neutron capture cross-section than common body constituents can be used. The neutrons administered in such therapies are typically relatively low energy neutrons having energies at or below about 0.5 eV. The compound to be activated can be caused to preferentially accumulate in the target tissue according to any of the methods useful for targeting of radionuclides, as described below, or in the methods described in Laramore, *Semin. Oncol.* *24*:672-685 (1997) and in U.S. Patents Nos. 6,400,796, 5,877,165, 5,872,107, and 5,653,957.

[0093] In other embodiments, the neutron radiotherapy can be a fast neutron radiotherapy. In general, fast neutron radiotherapy comprises irradiating a defined volume within a subject with a neutron beam, thereby causing cellular death within that volume. The irradiated volume preferably contains the entire cancer to be treated, and preferably contains as little healthy tissue as possible. Generally, high energy neutrons are administered in such therapies, with energies in the range of about 10 to about 100 million eV. Optionally, fast neutron radiotherapy can be combined with charged-particle radiotherapy in the administration of mixed proton-neutron radiotherapy.

[0094] In certain embodiments involving radiotherapeutic agents or treatments, the present invention relates to a method for treating cancer comprising the administration of vinca alkaloid or analog N-oxide having Formula I, in combination with a treatment comprising a therapeutically effective dose of photodynamic therapy. The photodynamic therapy can be administered according to any schedule, dose, or method known to one of skill in the art to be effective in the treatment or amelioration of cancer, without limitation. In general, photodynamic therapy comprises administering a photosensitizing agent that preferentially accumulates in a neoplastic mass and sensitizes the neoplasm to light, then exposing the tumor to light of an appropriate wavelength. Upon such exposure, the photosensitizing agent

catalyzes the production of a cytotoxic agent, such as, *e.g.*, singlet oxygen, which kills the cancerous cells.

[0095] Representative photosensitizing agents that may be used in photodynamic therapy include, but are not limited to, porphyrins such as porfimer sodium, 5-aminolaevulanic acid and verteporfin; chlorins such as temoporfin; texaphyrins such as lutetium texephyrin; purpurins such as tin etiopurpurin; phthalocyanines; and titanium dioxide. The wavelength of light used to activate the photosensitizing agent can be selected according to several factors, including the depth of the tumor beneath the skin and the absorption spectrum of the photosensitizing agent administered. The period of light exposure may also vary according to the efficiency of the absorption of light by the photosensitizing agent and the efficiency of the transfer of energy to the cytotoxic agent. Such determinations are well within the ordinary skill of one in the art.

[0096] Methods of administering and apparatuses and compositions useful for photodynamic therapy are disclosed in Hopper, *Lancet Oncol. 1*:212-219 (2000) and U.S. Patent Nos. 6,283,957, 6,071,908, 6,011,563, 5,855,595, 5,716,595, and 5,707,401.

[0097] It will be appreciated that both the particular radiation dose to be utilized in treating a hyperproliferative disorder and the method of administration will depend on a variety of factors. Thus, the dosages of radiation that can be used according to the methods of the present invention are determined by the particular requirements of each situation. The dosage will depend on such factors as the size of the tumor, the location of the tumor, the age and sex of the patient, the frequency of the dosage, the presence of other tumors, possible metastases and the like. Those skilled in the art of radiotherapy can readily ascertain the dosage and the method of administration for any particular tumor by reference to Hall, E. J., Radiobiology for the Radiologist, 5th edition, Lippincott Williams & Wilkins Publishers, Philadelphia, PA, 2000; Gunderson, L. L. and Tepper J. E., eds., Clinical Radiation Oncology, Churchill Livingstone, London, England, 2000; and Grosch, D. S., Biological Effects of Radiation, 2nd edition, Academic Press,

San Francisco, CA, 1980. In certain embodiments, radiotherapeutic agents and treatments may be administered at doses lower than those known in the art due to the additive or synergistic effect of the compound having Formula I.

[0098] Compositions in accordance with the present invention may be employed for administration in any appropriate manner, *e.g.*, oral or buccal administration, *e.g.*, in unit dosage form, for example in the form of a tablet, in a solution, in hard or soft encapsulated form including gelatin encapsulated form, sachet, or lozenge. Compositions may also be administered parenterally or topically, *e.g.*, for application to the skin, for example in the form of a cream, paste, lotion, gel, ointment, poultice, cataplasm, plaster, dermal patch or the like, or for ophthalmic application, for example in the form of an eye-drop, -lotion or -gel formulation. Readily flowable forms, for example solutions, emulsions and suspensions, may also be employed *e.g.*, for intralesional injection, or may be administered rectally, e.g., as an enema or suppository, or intranasal administration, *e.g.*, as a nasal spray or aerosol. Microcrystalline powders may be formulated for inhalation, *e.g.*, delivery to the nose, sinus, throat or lungs. Transdermal compositions/devices and pessaries may also be employed for delivery of the compounds of the invention. The compositions may additionally contain agents that enhance the delivery of the compounds having Formula I (or other active agents), *e.g.*, liposomes, polymers or co-polymers (e.g., branched chain polymers). Preferred dosage forms of the present invention include oral dosage forms and intravenous dosage forms.

[0099] Intravenous forms include, but are not limited to, bolus and drip injections. In preferred embodiments, the intravenous dosage forms are sterile or capable of being sterilized prior to administration to a subject since they typically bypass the subject's natural defenses against contaminants. Examples of intravenous dosage forms include, but are not limited to, Water for Injection USP; aqueous vehicles including, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles including, but not limited to, ethyl alcohol, polyethylene glycol and polypropylene glycol; and non-aqueous vehicles including, but not limited to,

corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate and benzyl benzoate.

[00100] The pharmaceutical compositions of the present invention may further comprise one or more additives. Additives that are well known in the art include, *e.g.*, detackifiers, anti-foaming agents, buffering agents, antioxidants (*e.g.*, ascorbic acid, ascorbyl palmitate, sodium ascorbate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate, malic acid, fumaric acid, potassium metabisulfite, sodium bisulfite, sodium metabisulfite, and tocopherols, *e.g.*, α-tocopherol (vitamin E)), preservatives, chelating agents, viscomodulators, tonicifiers, flavorants, colorants, odorants, opacifiers, suspending agents, binders, fillers, plasticizers, lubricants, and mixtures thereof. The amounts of such additives can be readily determined by one skilled in the art, according to the particular properties desired, and can be formulated such that compounds having Formula I are stable, *e.g.*, not reduced by antioxidant additives.

[00101] The additive may also comprise a thickening agent. Suitable thickening agents may be of those known and employed in the art, including, *e.g.*, pharmaceutically acceptable polymeric materials and inorganic thickening agents. Exemplary thickening agents for use in the present pharmaceutical compositions include polyacrylate and polyacrylate co-polymer resins, for example poly-acrylic acid and poly-acrylic acid/methacrylic acid resins; celluloses and cellulose derivatives including: alkyl celluloses, *e.g.*, methyl-, ethyl- and propyl-celluloses; hydroxyalkyl-celluloses, *e.g.*, hydroxypropyl-celluloses and hydroxypropylalkyl-celluloses such as hydroxypropyl-methyl-celluloses; acylated celluloses, *e.g.*, cellulose-acetates, cellulose-acetatephthallates, cellulose-acetatesuccinates and hydroxypropylmethyl-cellulose phthallates; and salts thereof such as sodium-carboxymethyl-celluloses; polyvinylpyrrolidones, including for example poly-N-vinylpyrrolidones and vinylpyrrolidone co-polymers such as vinylpyrrolidone-vinylacetate co-polymers; polyvinyl resins, *e.g.*, including polyvinylacetates and alcohols, as well as other polymeric materials including gum traganth, gum arabicum, alginates, *e.g.*, alginic acid, and salts thereof, *e.g.*, sodium alginates; and inorganic thickening agents such as atapulgite, bentonite and

silicates including hydrophilic silicon dioxide products, *e.g.*, alkylated (for example methylated) silica gels, in particular colloidal silicon dioxide products.

[00102] Such thickening agents as described above may be included, *e.g.*, to provide a sustained release effect. However, where oral administration is intended, the use of thickening agents may not be required. Use of thickening agents is, on the other hand, indicated, *e.g.*, where topical application is foreseen.

[00103] In one embodiment of the invention, compounds having Formula I are formulated as described, for example, in U.S. patent 4,923,876.

[00104] Although the dosage of the compound having Formula I will vary according to the activity and/or toxicity of the particular compound, the condition being treated, and the physical form of the pharmaceutical composition being employed for administration, it may be stated by way of guidance that a dosage selected in the range from 0.1 to 20 mg/kg of body weight per day will often be suitable, although higher dosages, such as 0.1 to 50 mg/kg of body weight per day may be useful. Those of ordinary skill in the art are familiar with methods for determining the appropriate dosage. Methods for assessing the toxicity, activity and/or selectivity of the compounds having Formula I may be carried out using any of the methods known in the art, including the antiproliferative activity test.

[00105] In certain instances, the dosage of the compounds having Formula I will be lower, *e.g.*, when used in combination with at least a second hyperproliferative disorder treatment, and may vary according to the activity and/or toxicity of the particular compound, the condition being treated, and the physical form of the pharmaceutical composition being employed for administration.

[00106] When the composition of the present invention is formulated in unit dosage form, the compound having Formula I will preferably be present in an amount of between 0.01 and 2000 mg per unit dose. More preferably, the amount of compound having Formula I per unit dose will be about 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100,

1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900, 1950, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3500, 3750, 4000, 4250, 4500, 4750, 5000, 5250, 5500, 5750, 6000, 6250, 6500, 6750, 7000, 7250, 7500, 7750, 8000, 8250, 8500, 8750, 9000, 9250, 9500, 9750, or 10000 mg or any amount therein.

[00107] When the unit dosage form of the composition is a capsule, the total quantity of ingredients present in the capsule is preferably about 10-1000 μL. More preferably, the total quantity of ingredients present in the capsule is about 100-300 μL. In another embodiment, the total quantity of ingredients present in the capsule is preferably about 10-1500 mg, preferably about 100-1000 mg.

[00108] In certain embodiments, the dosage of an N-oxide of vinca alkaloid or analog thereof will be administered according to a metronomic dosing. As used here, the term "metronomic dosing" refers to a dosing regimen, which uses a dose lower than the maximum tolerated doses (MTD) to minimize toxic side effects, administered at constant intervals without rest periods. For purposes of the present invention, the desired pharmacological effect of metronomic dosing with an N-oxide of vinca alkaloid or analog thereof is inhibition of tumor growth. "Inhibition of tumor growth" means causing a suppression of tumor growth and/or causing a regression in tumor size. It is believed that metronomic dosing elicits repeated waves of apoptosis of tumor endothelial cells by targeting cells of the vasculature which form the blood vessels of the tumor as opposed to the tumor cells themselves. Thus, metronomic dosing appears to abrogate tumor cells' apparent capability to repair and recover during the usual rest periods between episodic application of a cytotoxic drug at or near MTD, followed by periods of rest to allow normal tissues to recover.

[00109] The term "MTD" as used here for N-oxides of vinca alkaloids and analogs may be identified as part of the clinical evaluation of the N-oxide. For example, phase I trials can include a determination of the maximum tolerated dose, dose-limiting toxicities (DLT) and pharmacokinetics of an N-oxides of vinca alkaloids or analog. Thus, the MTD for any Food and Drug

Administration (FDA) approved therapeutic compound can be determined by those of ordinary skill in the art. The MTD for any particular therapeutic compound may vary according to its formulation (e.g., injectable formulation, implantable bioerodible polymer formulation, oral formulation), route of delivery (e.g., intravenous, oral, intratumoral), manner of delivery (e.g., infusion, bolus injection), dosing schedule (e.g., hourly, daily, weekly) and the like. MTD frequently is defined as the highest dose level at which 50 percent of subjects administered with the drug develop a dose-limiting toxicity. Other definitions which are clinically relevant and generally accepted will be known to one of ordinary skill in the art.

[00110] The present invention also provides metronomic therapy regimes. In some embodiments, there is provided a metronomic dosing of an N-oxide of vinca alkaloid or analog, wherein the N-oxide is administered over a period of at least 1, 2, 3, 4, 5, 6, 7, or 8 weeks or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 30, or 36 months wherein the interval between each administration is no more than about 1, 2, 3, 4, 5, 6 or 7 days, and wherein the dose of the N-oxide of vinca alkaloid or analog at each administration is about 0.25% to about 80% of its MTD. In other embodiments, the vinca alkaloid N-oxide is administered at no more than 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 23%, 20%, 18%, 16%, 14%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% of its MTD, which is predicted to be 100 mg/m^2 to 700 mg/m^2. In some embodiments, there is provided a method of administering a composition comprising an N-oxide of vinca alkaloid or analog, wherein the composition is administered over a period of at least one week, wherein the interval between each administration is no more than about a day, and wherein the dose of the N-oxide of vinca alkaloid or analog at each administration is about 0.25% to about 80% of its MTD or other doses mentioned herein. In some embodiments, there is provided a method of administering a composition comprising an N-oxide of vinca alkaloid or analog, wherein the composition is administered over a period of at least one month, wherein the interval between each administration is no more than about a week, and wherein the dose of the N-oxide at each administration is about 0.25% to about 80% of MTD or other doses mentioned herein. In some embodiments, the composition is

administered at least about any of 1x, 2x, 3x, 4x, 5x, 6x, 7x (i.e., daily) a week.

[00111] In some embodiments, the compositions may be administered continuously over a pre-specified period, for example continuously transfusing the compositions for about 0.25 to about 80% of the MTD or other doses mentioned herein, wherein the pre-specified period is at least 1, 2, 3, 4, 5, 6, 7, or 8 weeks. In some embodiments, the intervals between successive continuous administration (e.g., infusion) sessions are at least about 1 day, 2 days, 3 days, 4 days, 5 days, six days, one week, 2 weeks, 3 weeks, 4 weeks, or 5 weeks after which a new continuous administration session is started.

[00112] In some embodiments, one or more patches applied at one or more parts of the body may be used to deliver a dose that does not exceed about 0.25 to about 80% of the MTD of the N-oxide per application period, or other doses mentioned herein. In some embodiments, the intervals between each administration by patch are less than about any of 7 days, 6 days, 5 days, 4 days, 3 days, 2 days, and 1 day. In some embodiments, the composition is administered by a patch over a period of at least about any of 1 day, 2 days, 3 days, 4 days, 5 days, six days, one week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, or 8 weeks. In some embodiments, the intervals between successive patch administrations are at least about 1 day, 2 days, 3 days, 4 days, 5 days, six days, one week, 2 weeks, 3 weeks, 4 weeks, or 5 weeks after which one or more new patches are applied. Examples of transdermal patches that may be suitable to deliver metronomic doses of vinca alkoloid N-oxides and analogs thereof may be found in U.S. Patent Nos. 6,977,070, 7,022,340, 6,004,581, 5,939,094, 5,624,677, 7,001,609, 6,632,522, 6,630,238, 6,482,871, 6,086,911, 5,698,217, 5,639,469, 5,244,677, 4,451,260, 6,173,851, 5,223,261, 5,192,548, 6,645,528, 5,700,480, 6,974,588, 6,238,693, 6,000,548, 4,726,951, 4,721,619, 5,225,196, 4,983,392, 6,103,257, 6,110,486, 5,955,098, 5,985,317, and 5,952,000.

[00113] In some embodiments, a controlled release formulation may be used to control release rate of the N-oxide of vinca alkaloid or analog from preparations so that a metronomic dosing regimen is maintained for a pre-determined period of time. In some embodiments, the metronomic dosing of

the N-oxide of vinca alkaloid or analog may be maintained by using an erodable polymer matrix, reservoir device, microparticles, nanoparticles, osmotic pumps, or pH dependant coatings. In these embodiments, the N-oxide of vinca alkaloid or analog may be delivered at a dose that does not exceed about 0.25 to about 80% of the MTD of the N-oxide per application period, or other doses mentioned herein.

[00114] In some embodiments, there is provided a metronomic dosing of an N-oxide of vinca alkaloid or analog, wherein the N-oxide is administered over a period of at least 1, 2, 3, 4, 5, 6, 7, or 8 weeks or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 30, or 36 months, wherein the interval between each administration is no more than about a week, and wherein the dose of the N-oxide at each administration is about 25 mg/m^2 to about 500 mg/m^2. In some embodiments, there is provided a metronomic dosing of an N-oxide of vinca alkaloid or analog, wherein the N-oxide is administered over a period of at least three months, wherein the interval between each administration is no more than about a week, and wherein the dose of the N-oxide at each administration is about 25 mg/m^2 to about 500 mg/m^2. In some embodiments, there is provided a metronomic dosing of an N-oxide of vinca alkaloid or analog, wherein the N-oxide is administered over a period of at least one month, wherein the interval between each administration is no more than about one day, two days, three days, four days, five days, six days, or one week, and wherein the dose of the N-oxide at each administration is about 25 mg/m^2 to about 500 mg/m^2. In some embodiments, there is provided a metronomic dosing of an N-oxide of vinca alkaloid or analog, wherein the N-oxide is administered over a period of at least one week, wherein the interval between each administration is no more than about 12, 24, 36, 48, 60, 72, 84, 96, 108, or 120 hours, and wherein the dose of the N-oxide at each administration is about 25 mg/m^2 to about 500 mg/m^2. In some embodiments, the dose of the N-oxide of vinca alkaloid or analog per administration is less than about any of 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, and 500 mg/m^2. In some embodiments, the composition is administered at least about any of 1x, 2x, 3x, 4x, 5x, 6x, and 7x (i.e., daily) a week. In some

embodiments, the intervals between each administration are less than about any of 7 days, 6 days, 5 days, 4 days, 3 days, 2 days, and 1 day.

[00115] The relative proportion of ingredients in the compositions of the invention will, of course, vary considerably depending on the particular type of composition concerned. The relative proportions will also vary depending on the particular function of ingredients in the composition. The relative proportions will also vary depending on the particular ingredients employed and the desired physical characteristics of the product composition, *e.g.*, in the case of a composition for topical use, whether this is to be a free flowing liquid or a paste. Determination of workable proportions in any particular instance will generally be within the capability of a person of ordinary skill in the art. All indicated proportions and relative weight ranges described below are accordingly to be understood as being indicative individually inventive teachings only and not as limiting the invention in its broadest aspect.

[00116] The amount of compound having Formula I in compositions of the invention will of course vary, *e.g.*, depending on the intended route of administration and to what extent other components are present. In general, however, the compound having Formula I will suitably be present in an amount of from about 0.005% to 20% by weight based upon the total weight of the composition. In certain embodiments, the compound having Formula I is present in an amount of from about 0.01% to 15% by weight based upon the total weight of the composition.

[00117] In addition to the foregoing, the present invention also provides a process for the production of a pharmaceutical composition as hereinbefore defined, which process comprises bringing the individual components thereof into intimate admixture and, when required, compounding the obtained composition in unit dosage form, for example filling said composition into tablets, gelatin, *e.g.*, soft or hard gelatin, capsules, or non-gelatin capsules.

[00118] The starting materials of the N-oxides of the present invention are known and described, for example, in U.S. patent Nos. 6,555,547, 6,365,735, RE37,449, 6,127,377, 5,369,111, 5,030,620, 5,024,835, 4,831,038, 4,765,972, RE30,561 and 4,160,767.

[00119] In certain situations, more than one of the nitrogen atoms of the vinca alkaloid analog may be oxidized simultaneously. In certain cases, one or more of the multiple N-oxide groups may be reduced selectively, leaving one or more of the other N-oxide groups in place. Thus, the present invention contemplates the preparation of N-oxide analogs in which one or more of the nitrogen atoms that are suitable for N-oxide formation are present as the N-oxide without regard to the susceptibility of a particular nitrogen atom to N-oxide formation or the susceptibility of a particular N-oxide group to reduction. It is envisaged to employ a combination of suitable protecting groups (see: Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, second edition, Wiley Interscience, 1991) to protect those nitrogen atoms not undergoing oxidation.

[00120] By way of an example, primary and secondary amines that may be present in a vinca alkaloid analog may be protected using, for example, *tert*-butyl sulfonyl (BUS) group. Jarowicki, K. and Kocienski, P., *J. Chem. Soc., Perkin Trans 1*, 4005-4037, 4029 (1998); Sun, P. and Weinreb, S. M. *J. Org. Chem. 62*:8604-08 (1997). The BUS protecting group is introduced by reaction of the amine with *tert*-butylsulfinyl chloride followed by oxidation of the sulfinyl amide with, for example, dimethyldioxirane, *m*-chloroperbenzoic acid or $RuCl_3$ catalyzed $NaIO_4$. The oxidation step in the preparation of the BUS-protected primary or secondary amines may also oxide any tertiary amine and heteroaromatic nitrogen present in the compounds. Thus, this protecting group may be introduced into primary and secondary amines while simultaneously oxidizing tertiary and heteroaromatic nitrogen atoms.

[00121] The BUS protecting group is stable towards strong reagents such as alkyllithium, Grignard reagents, 0.1M HCl in MeOH (20° C, 1 hr), 0.1M TFA in dichloromethane (20° C, 1 hr) and pyrolysis at 180° C. The BUS-protected secondary amines can be cleaved with 0.1 M triflic acid in dichloromethane containing anisole as a cation scavenger at 0° C for 15-30 minutes while primary amines are released more slowly at room temperature. If desired, both BUS-protected primary and secondary amines may be deprotected with 0.1 M triflic acid in dichloromethane containing anisole as a cation scavenger at 25° C for 2.5 hours. Thus, the BUS protecting group may allow protecting

primary and secondary amines simultaneously while also oxidizing tertiary amines and heteroaromatic nitrogen atoms to the N-oxides. Moreover, the BUS protecting group may allow protecting primary and secondary amines simultaneously, oxidizing tertiary amines and heteroaromatic nitrogen atoms to N-oxides, deprotect the secondary amine selectively, alkylate the secondary amine to a tertiary amine, oxidize the resulting tertiary amine and deprotect the primary amine. Alternatively, a primary and a secondary amine may be protected with BUS protecting group, the secondary amine may be deprotected selectively, the secondary amine may be protected with, for example, Boc protecting group, and then the primary amine may be deprotected selectively followed by alkylation and oxidation. Thus, when a primary amine and a secondary amine are present in a vinca alkaloid analog, a BUS protecting group may be used to transform one of amines to an N-oxide without affecting the other.

[00122] Recent development in the use of Boc group to protect amines allows introduction and removal of the group under mild conditions. For example, a vinca alkaloid analog amine group may be protected with Boc group by simply mixing the analog and Boc-ON (2-(Boc-oxyimino)-2-phenylacetonitrile, available from Aldrich Co.) in benzene at 25° C for 20 minutes (or 6 hours if the amine is an electron deficient aniline) in the presence of powdered zinc. *See* Spivey, A. C. and Maddaford A. *Annu. Rep. Prog. Chem., Sect. B, 95*:83-95 (1999). Alkyl esters are tolerated.

[00123] Boc-protected amines are generally deprotected using triflic acid although recent developments generally use mildly acidic conditions that leave acid-labile groups unaffected. For example, heating Boc protected p-anisidines at 180° C in the presence of 4-chlorophenol deprotects the amine group without affecting acid sensitive methoxy enols (-CH=C(OCH_3)-). Jarowicki, K. and Kocienski, P., *J. Chem. Soc., Perkin Trans 1*, 4005-4037, 4025 (1998). Thus, primary and secondary amines in vinca alkaloid may be protected with Boc group followed by oxidation of the tertiary amines and deprotection of the primary and secondary amines.

[00124] It has also recently been reported a new base-sensitive amino protecting group 1,1-dioxobenzo[*b*]thiophene-methoxycarbonyl (Bsmoc).

Bsmoc is introduced via its chloroformate or N-hydroxy-succinimide derivative. The Bsmoc group is stable towards tertiary amines for 24 hours but is removed within 3-5 minutes using piperidine. Jarowicki, K. and Kocienski, P., *J. Chem. Soc., Perkin Trans 1*, 4005-4037, 4027 (1998). Thus, primary and secondary amines present in vinca alkaloid analogs may conveniently be protected with Bsmoc protecting group followed by oxidation of the tertiary amines and removal of the protecting group under mild conditions.

[00125] It has also been reported that certain heteroaromatic nitrogen atoms can be oxidized selectively in the presence of certain aromatic primary amines and certain secondary amines adjacent to a double bond. Delia, T. J. et al. *J. Org. Chem. 30*:2766-68 (1965). For example, oxidation of cytosine with *m*-chloroperbenzoic acid results in cytosine 3-N-oxide despite the presence of aromatic primary amine and a secondary amine. Thus, heteroaromatic nitrogen atoms and tertiary amines may be oxidized in the presence of certain aromatic primary amines and secondary amines.

[00126] The following examples are illustrative, but not limiting, of the method and compositions of the present invention. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered in clinical therapy and which are obvious to those skilled in the art are within the spirit and scope of the invention.

EXAMPLES

[00127] ^{1}H NMR spectra were obtained from INOVA-300 MHz or INOVA™-500 MHz spectrometers using D_2O or $CDCl_3$ as a solvent (s, d, t, dd and m indicate singlet, doublet, triplet, doublet of doublet, and multiplet, respectively). Analytical thin-layer chromatography was performed on polyester-backed plates from EMD Chemicals, Inc., precoated with silica gel 60 F_{254}. Radial thin-layer chromatography was performed on a Harrison Research Chromatron (7924T) using 2 mm thick silica plates coated in our laboratory with silica gel 60 PF_{254} containing gypsum (EMD Chemicals, Inc.). Analytical scale high-performance liquid chromatography (HPLC) was performed on a 4.6 mm x 250 mm MICROSORB™ C_{18} column using a pressure

of 1800-2100 psi. The procedure for the synthesis for the N-oxides of vinblastine, vincristine, and vinorelbine was adapted from P. Mangeny et al. for the procedure for oxidation of vinorelbine. The formation of vinblastine N-oxide leads to the formation of a new stereocenter. We have determined the configuration of the newly introduced oxygen atom in vinblastine N'_b-oxide synthesized below to be cis to the nearby ethyl group by single crystal x-ray analysis of the maleate salt (Figure 1). Both epimers of the *N*-oxide on either the R_8 or R'_8 nitrogen atom in Formula I are contemplated in this invention. Vinblastine N-oxide is an intermediate for the synthesis of vinorelbine. See Mangeny, P. et al. *Tetrahedron*. *35*:2175-2179 (1979).

EXAMPLE 1

SYNTHESIS OF VINBLASTINE N'_b-OXIDE

[00128] Vinblastine sulfate was purchased from Asian Talent. A 610-mg sample of vinblastine sulfate was dissolved in 20 mL of dichloromethane and then it was shaken with 20 mL of 5% aqueous sodium carbonate. The organic layer was separated and aqueous solution was extracted with dichloromethane (2 × 20 mL) and then with chloroform (20 mL). The combined organic layer and extracts were dried over sodium sulfate and evaporated to dryness, affording 526 mg (97%) of vinblastine free base as a sticky white solid. The purity of vinblastine free base was checked by ^1H 300 MHz NMR and HPLC (99.1% AUC) using a Varian™ CHROMSEP™ HPLC column SS 250 X 4.6 mm including holder with guard column, MICROSORB 100-5 C18; gradient: 30/70 (0.1% trifluoroacetic acid in acetonitrile)/(0.1% trifluoroacetic acid in water) to 100/0 (0.1% trifluoroacetic acid in acetonitrile)/(0.1% trifluoroacetic acid in water); detection at 254 nm and 270 nm.

[00129] To a stirred solution of vinblastine free base (526 mg, 0.65 mmol) in CH_2Cl_2 (5 mL) at 0°C was added dropwise a chilled solution of *m*-chloroperoxybenzoic acid (168 mg, 0.97 mmol) in 6 mL of CH_2Cl_2. The progress of the reaction was followed by silica gel TLC (eluent: 5:0.7 $CHCl_3$-MeOH; vinblastine R_f = 0.65; vinblastine *N*-oxide R_f = 0.25). The reaction was complete after ~2 min. The solution was washed with aqueous sodium

carbonate (10 mL, 5g/100 mL) to remove *m*-chlorobenzoic acid and any remaining *m*-chloroperoxybenzoic acid. The organic layer was dried over sodium sulfate and the solvent was evaporated under reduced pressure, yielding crude vinblastine *N*-oxide (600 mg). Chromatography over a short column of silica gel (eluent: 5:0.5 $CHCl_3$-MeOH) followed by radial chromatography (eluent: 5:0.5 $CHCl_3$-MeOH) gave vinblastine *N*-oxide (340 mg, 64%) as a beige powder. For larger scale preparations, the *N*-oxide may be obtained in comparable purity and yield by column chromatography over silica gel (eluent: 5:0.3 $CHCl_3$-MeOH to 5:0.7 $CHCl_3$-MeOH). The silica gel should be deactivated with methanol prior to the chromatography. The 500 MHz ^1H NMR spectrum of vinblastine *N'$_b$*-oxide in $CDCl_3$ is consistent with the assigned stucture. HPLC, >98% AUC.

[00130] Hydrogen Chloride Salt of Vinblastine *N*-oxide. A 55-mg (0.066 mmol) sample of vinblastine *N*-oxide was dissolved in 4 mL of methanol. The solution was cooled to -30°C and then 33 μL (0.066 mmol) of 2N HCl in water was added. The resulting solution was stirred for 5 min. Then solvent was evaporated and the residue was dissolved in deionized water (4 mL), filtered and lyophilized to give the HCl salt of vinblastine *N*-oxide as a white somewhat hygroscopic powder. The 300 MHz ^1H NMR spectrum in D_2O is consistent with the assigned structure.

[00131] Maleic Acid salt of Vinblastine *N*-oxide. A 15-mg (0.018 mmol) sample of vinblastine *N*-oxide was dissolved in 2 mL of methanol. To this solution was added 2 mg (0.018 mmol) of maleic acid dissolved in 0.170 mL of methanol at room temperature. The solution was stirred for 5 min and then evaporated. Dichloromethane (5 mL) was added to the residue and then the solution was concentrated to dryness to give 17 mg of the maleic acid salt of vinblastine *N*-oxide. The sample was dissolved in D_2O (0.7 mL) for an NMR spectrum, which was consistent with the assigned structure. The D_2O solution was allowed to stand at room temperature overnight. White plate-like crystals separated. Single crystal x-ray analysis (Figure 1) confirmed that the newly introduced oxygen atom is attached to the *N*'$_b$ nitrogen atom and that this oxygen atom is cis to the ethyl group at the 20' position as seen in Figure 1. The configuration of the *N*-oxide oxygen atom in vinblastine *N*'$_b$-oxide is the

same as that of the methiodide methyl group in leurocristine methiodide dehydrate as described in the x-ray crystal structure reported by Moncrief and Lipscomb (Moncrief, J. W.; Lipscomb, W. N. *Acta Cryst.* 1966, *21*, 322).

EXAMPLE 2

SYNTHESIS OF VINORELBINE N'$_b$-OXIDE.

[00132] Vinorelbine free base was purchased from Asia Talent. Purification, when necessary, was accomplished by radial chromatography on silica gel using 9:1 $CHCl_3$-MeOH as eluent.

[00133] Vinorelbine *N'*$_b$-oxide was synthesized using essentially the same procedure used to prepare vinblastine *N'*$_b$-oxide. *m*-Chloroperoxybenzoic acid (45 mg, 0.26 mmol) in $CHCl_3$ (2.5 mL) was added at 0° to a stirred solution of purified vinorelbine (150 mg, 0.20 mmol) in $CHCl_3$ (4.5 mL) under nitrogen. After 15 min, the mixture was poured into an aqueous sodium carbonate (27 mL, 40g/L) and was extracted by $CHCl_3$ (20 mL). After drying over sodium sulfate and filtration, the $CHCl_3$ extract was evaporated under reduced pressure affording crude vinorelbine *N*-oxide (125 mg, 83%). Thin-layer chromatography (silica; 9:1 $CHCl_3$/MeOH) indicated the presence of a small amount of starting vinorelbine (vinorelbine $R_f = 0.37$, vinorelbine *N'*$_b$-oxide $R_f = 0.18$). Radial chromatography on silica gel (eluent, 9:1 $CHCl_3$/MeOH) gave vinorelbine *N'*$_b$-oxide as an off-white solid (100 mg; 75%). The sample was dissolved in ethyl acetate/hexanes and then the open flask was placed in a sealed container containing an open flask of hexanes. As the hexanes diffused slowly into the vinorelbine *N'*$_b$-oxide solution, vinorelbine *N'*$_b$-oxide precipitated out as an off-white powder (80 mg; 53%). The 300 MHz ^{1}HNMR spectrum was consistent with the assigned structure. The configuration of the *N'*$_b$-oxide oxygen atom is assigned the same stereochemistry as that found in vinblastine *N*-oxide by x-ray crystallography.

EXAMPLE 3

SYNTHESIS OF VINCRISTINE N'$_b$-OXIDE.

[00134] Vincristine sulfate was purchased from Asian Talent. A 480-mg sample of vincristine sulfate was dissolved in 10 mL of 7:3 chloroform/methanol and then it was shaken with a 15 mL of 5% aqueous sodium carbonate. The organic layer was separated and aqueous solution was then extracted with dichloromethane (2 × 20 mL) followed by chloroform (2 x 20 mL). The combined organic layer and extracts were dried over sodium sulfate and concentrated to dryness, affording 420 mg (98%) of vincristine free base as a sticky yellowish-white solid. The purity of vincristine free base was checked by ^{1}H 300 MHz NMR and HPLC (99.0% AUC).

[00135] *m*-Chloroperoxybenzoic acid (132 mg, 0.76 mmol) in 2 mL of dichloromethane was added dropwise at 0°C to a stirred solution of vincristine free base (420 mg, 0.51 mmol) in dichloromethane (4 mL). The progress of the reaction was followed by silica gel TLC (eluent: 5:0.5 $CHCl_3$–MeOH; vincristine $R_f = 0.65$; vincristine *N*-oxide $R_f = 0.30$) and was complete after ~2 min. The solution was washed with aqueous sodium carbonate (8 mL, 5g/100 mL) to remove *m*-chlorobenzoic acid and any remaining *m*-chloroperoxybenzoic acid. The organic layer was dried over sodium sulfate and the solvent was evaporated under reduced pressure, yielding crude vincristine *N*-oxide (450 mg). Chromatography over a short column of silica gel (eluent: 5:0.5 $CHCl_3$-MeOH) followed by radial chromatography over silica gel (eluent: 5:0.5 $CHCl_3$-MeOH) gave vincristine *N*-oxide (288 mg, 67%) as an off-white powder. The 300 MHz ^{1}H NMR spectrum in $CDCl_3$ was consistent with the assigned structure. Reverse phase HPLC showed a purity of >98% (AUC). The configuration of the *N*-oxide oxygen atom is assigned the same stereochemistry as that found in vinblastine *N*-oxide by x-ray crystallography.

EXAMPLE 4

Cytotoxicity Of Vinca-alkaloids And Analog N-Oxides Thereof In Lymphoma, Leukemia, Multiple Myeloma and Cells Derived From Hemtalogical Malignancies

[00136] The cytotoxicity of vinca alkaloids and analog N-oxides thereof on different lymphoma, leukemia, and multiple myeloma cell lines will be tested *in vitro* under normoxic as well as 0.2% O_2 hypoxic conditions. Standard viability assays using MTT or Alamar Blue dye will be conducted to determine the 50% Inhibitory Concentration (IC_{50}) for each compound. Cultured tumor cells will be treated with the compounds for 8 hr or greater under normoxic or hypoxic conditions, and viability will be measured 24-48 hr later. In certain cases, enzyme inhibitors to bioreductive enzymes will be co-cultured with the cells to verify mechanism of action. Positive controls will use chemotherapeutic agents at doses shown in the art to be effective. The results should indicate that vinca alkaloids and its related N-oxide analogs are cytotoxic to many of the cell lines derived from hematological malignancies, with IC_{50} values in the nanomolar to sub-nanomolar range. Vinca alkaloid analog N-oxides are expected to be increased or differential cytotoxicity profiles than the parent compounds under normoxic or euoxic conditions.

EXAMPLE 5

Anti-Tumor Activity Of Vinca-alkaloids And Analog N-Oxides Thereof In Lymphoma, Leukemia, Multiple Myeloma and Cells Derived From Hemtalogical Malignancies

[00137] The *in vivo* antitumor efficacy of vinca alkaloids and analog N-oxides will be evaluated using syngeneic and xenotransplant experimental murine models of hematological malignancies. For example, DBA/2 mice (n=12/group) will be inoculated intraperitoneally (ip) with 1 x 10^5 murine L1210 or 1 x 10^6 P388 leukemic tumor cells. Following tumor implantation, mice will be treated with 10-1000 mg/kg of vinca alkaloids and analog N-oxides by intravenous administration. Agents may be administered on a

single or multiple treatment regimen (ie. once weekly, q3d) to yield optimal dose schedule benefit. The effects of treatment with vinca alkaloids and N-oxide analogs compared to vehicle control treated mice on prolonging the survival of tumor bearing mice will be compared using a pre-determined survival endpoint. Animals will also be monitored for symptoms of acute toxicity such as body weight loss, neurotoxicities, lymphopenia and neutropenia. As a positive control, treatment will be compared with a standard agent.

[00138] In a related example, the *in vivo* antitumor efficacy of vinca alkaloids and analog N-oxides will be evaluated using a Namalwa human lymphoma xenograft model in nude mice. Female nu/nu mice (n= 10/group) will be implanted with human Namalwa lymphoma cells by subcutaneous injection. Pair-matched mice will be randomized to different treatment groups when their tumors are approximately 50-100 mm^3 in size as determined by caliper measurements. Mice will be treated with 10-1000 mg/kg of vinca alkaloids and analog N-oxides by intravenous administration on a weekly or once every 3 day schedule. The anti-tumor effects of the compounds will be assessed as tumor growth inhibition (TGI) and tumor growth delay (TGD) by established criteria and practices known in the art. Treatment effects will also be compared to a standard agent known in the art such as mitoxantrone or doxorubicin.

[00139] In a related example, the *in vivo* antitumor efficacy of vinca alkaloids and analog N-oxides will be demonstrated in a human xenotransplantion model of acute lymphocytic leukemia (ALL) in immunodeficient mice. NOD/SCID mice will be implanted with primary human ALL tumor cells. Tumor grafting and burden will be monitored by flow cytometry using standard leukemia identification markers and pair-matched animals will be randomized into control or treatment groups. Seven to 10 days following transplant, mice will be injected intravenously (iv) with 10-1000 mg/kg of vinca alkaloids and analog N-oxides by intravenous administration on a weekly or once every 3 day schedule. Mice will be bled routinely and assessed for tumor burden by FACS analysis. The number of leukemic tumor cells per ml of blood prior and post-treatment will be compared to evaluate compound efficacy. Animals will also be monitored for symptoms of acute toxicity such

as body weight loss, neurotoxicities, lymphopenia and neutropenia. As a positive control, treatment will be compared with a standard agent.

[00140] Further, the *in vivo* antitumor activity of vinca alkaloids and analog N-oxide in combination with chemotherapeutic agents and/or radiotherapy will be evaluated using a xenograft model in nude mice.

[00141] Further, the *in vivo* antitumor activity of vinca alkaloids and analog N-oxide in combination with therapeutic monoclonal antibodies such as anti-CD52 (Campath), anti-CD20 (Rituxan, Zevalin, Bexxar), anti-CD22 (LymphoCide) anti-CD33 (MyloTarg), or HLA-DR (Lym-1, Oncolym) will be evaluated using a xenograft model in nude mice.

[00142] Further, the *in vivo* antitumor activity of vinca alkaloids and analog N-oxide in combination with small molecular weight inhibitors of kinases such as imatanib (Gleevec) will be evaluated using a xenograft model in nude mice.

EXAMPLE 6

Cytotoxicity Of Vinca-alkaloid Analogs And N-Oxides Thereof In Solid Tumor Lines

[00143] The differential cytotoxicity of vinca-alkaloids and analog N-oxides thereof on different solid tumor cell lines are demonstrated *in vitro* under normoxic conditions and 0.2% O_2 hypoxic conditions. Shown in Figs. 2A-2D and 3A-3D and Tables 1 and 2 are results obtained from treatment effects of vinblastine N-oxide and vincristine N-oxide, against human H460 lung adenocarcinoma, HT29 colorectal adenocarcinoma, A549 non-small cell lung carcinoma, FaDU head and neck tumor cell lines *in vitro*. Tumor cell line were cultured in DMEM-10%FBS under standard conditions. One-hundred mm plastic culture dishes were seeded with 1 x 10^6 tumor cells and treated with vinca alkaloids or N-oxide analogs at a dose range of 0.01 – 100 nM. Cells were exposed with gentle rocking to a constant level of low oxygen (0.2% O_2–5% CO_2-balance N_2) in a hypoxia apparatus (INVIVO2400 Hypoxia Workstation, Ruskin Technology) for 14 hr at 37°C. Identically treated cells were incubated under normoxia (air-5% CO_2) at 37°C. Cells were harvested

and replated in fresh medium at a density of 1 x 10^3 cells/well in 48-well plastic culture plates to assess viability 24-72 hr later. Briefly, Alamar Blue was added to replicate wells on Day 0 and Day 3, and cells were incubated for a further 3-6 hr at 37°C before fluorescence readings using a plate reader (530-560 nm excitation, 590 emission). The percent inhibition of proliferation was calculated and plotted against control-treated cells at each drug concentration. The 50% growth inhibitory concentration (IC_{50}) values were calculated for the paired normoxic and hypoxic treatments. The Hypoxia Cytotoxicity Ratio (HCR) was determined as the ratio of the IC_{50} of the compound under normoxic conditions vs. hypoxic conditions.

[00144] As shown in Figures 2A-2D and Table 1, it was demonstrated that vinblastine N-oxide has decreased cytotoxic activity under normoxic conditions compared to the parent vinblastine compound against multiple human solid tumor cell lines including H460 lung, HT29 colorectal and A549 lung adenocarcinoma cells. Upon hypoxia exposure, vinblastine N-oxide treatment was demonstrated to have potent cytotoxic activity with comparable activity to vinblastine as shown in Fig. 2D and Table 1. The HCR values of 3-21 for vinblastine N-oxide as shown in Table 1 demonstrates hypoxia-induced cytotoxicity of this compound against multiple human solid tumor cell line *in vitro*.

[00145] Table 1: Cytotoxicity and 50% inhibitory concentrations (IC50) of vinblastine N-oxide or vinblastine on human tumor cell lines exposed to normoxic vs. hypoxic conditions.

Compound	Cell Line	IC50 Air (mM)	IC50 Hypoxia (mM)	Hypoxia Cytotoxicity Ratio (HCR)*
Vinblastine N-oxide	H460 H460 H460	0.15 0.2 0.2	0.07 0.02 0.03	21.4 10 6.7
Vinblastine	H460	0.02	0.017	1
Vinblastine N-oxide	A549 A549	0.15 0.45	0.035 0.10	4.3 4.5
Vinblastine N-oxide	HT29 HT29	0.15 0.2	0.05 0.07	3 2.9
Vinblastine N-oxide	SiHa	4	0.7	4.3

Compound	Cell Line	IC50 Air (mM)	IC50 Hypoxia (mM)	Hypoxia Cytotoxicity Ratio (HCR)*
Vinblastine N-oxide	FaDu	0.15	0.02	7.5

*HCR is defined as (IC50 air)/(IC50 hypoxia)

[00146] As shown in Figures 3A-3D and Table 2, it was demonstrated that vincristine N-oxide has decreased cytotoxic activity under normoxic conditions compared to the parent vincristine compound against multiple human solid tumor cell lines. Upon hypoxia exposure, vincristine N-oxide treatment was demonstrated to have potent cytotoxic activity with comparable activity to vincristine (Fig. 3D and Table 2). The HCR values of 5-25 for vincristine N-oxide as shown in Table 2 demonstrates hypoxia-induced cytotoxicity of this compound against multiple human solid tumor cell line *in vitro*.

[00147] Table 2. Cytotoxicity and 50% inhibitory concentrations (IC50) of vincristine N-oxide or vincristine on human tumor cell lines exposed to normoxic vs. hypoxic conditions.

Compound	Cell Line	IC50 Air (mM)	IC50 Hypoxia (mM)	Hypoxia Cytotoxicity Ratio (HCR)*
Vincristine N-oxide	H460 H460 H460	1.5 0.9 1	0.06 0.065 0.2	25 14 5
Vincristine	H460	0.017	0.015	1.1
Vincristine N-oxide	A549	0.9	0.08	11.2
Vincristine N-oxide	FaDu	0.09	0.015	6

*HCR is defined as (IC50 air)/(IC50 hypoxia)

EXAMPLE 7

Cytotoxicity Of Vinca Alkaloid Analogs And N-Oxides Thereof In Solid Tumor Lines Using Clonogenic Assays

[00148] The differential cytotoxicity of vinca alkaloids and analog N-oxides thereof on different solid tumor cell lines are demonstrated *in vitro* under

normoxic conditions and 0.2% O_2 hypoxic conditions using colony formation (clonogenic) assays. Shown are results in Fig. 4 obtained from treatment effects of vinblastine N-oxide on the growth of viable colonies of H460 lung carcinoma tumor cells following exposure to normoxic (20% O_2) vs. hypoxic (0.2% O_2) conditions.

[00149] Human H460 lung adenocarcinoma tumor cells were cultured in DMEM-10%FBS under standard conditions and seeded in 100 mm plastic culture dishes at a density of 1 x 10^6 cells/dish. Cells were then treated with vinblastine N-oxide at a 0.01 – 100 nM dose range. Cells were exposed with gentle rocking to a constant level of low oxygen (0.2% O_2–5% CO_2-balance N_2) in a hypoxia apparatus (INVIVO2400 Hypoxia Workstation, Ruskin Technology) for 14 hr at 37°C. Identically treated cells were incubated under normoxia (air-5% CO_2) at 37°C. Cells were harvested and replated in fresh medium at a density of 1 x 10^5 cells/well in fresh plastic culture plates to assess the growth of viable colonies 7 days later. Colonies were fixed, stained with 0.4% crystal violet and enumerated 7 days later. The 50% Inhibitory concentration to block the number of viable colonies compared to control-treated cells under normoxic and hypoxic conditions are calculated. As shown in Fig. 4, vinblastine N-oxide had decreased cytotoxic activity against H460 lung tumor cells under normoxic conditions compared to its potent cyotoxic activity under hypoxic conditions. Vinblastine N-oxide demonstrates a Hypoxia Cytotoxicity Ratio of ~20 against the growth of viable H460 tumor colonies *in vitro* (Fig. 4). The results demonstrated that vinblastine N-oxide is a prodrug that is activated under hypoxia to a potent cyototoxin with low nanomolar IC_{50} inhibitory activity against human solid tumor cell lines *in vitro*.

EXAMPLE 8

Activation of Cytotoxicity Of Vinca Alkaloid N-Oxides Analogs Against Human Solid Tumor Cell Lines Is Oxygen Dependent

[00150] Our results demonstrate the vinca alkaloid N-oxide analogs have decreased cytotoxic activity under normoxic conditions but are activated to

potent cytotoxic agents under conditions of hypoxia. Human H460 lung adenocarcinoma tumor cells were cultured in DMEM-10%FBS under standard conditions. One-hundred mm plastic culture dishes were seeded with 1 X 10^6 tumor cells/dish and treated with vinca alkaloids or N-oxide analogs at a 0.01 – 100 nM dose range. Cells were exposed with gentle rocking to a constant level of low oxygen (0.2, 1% or 5% O_2–5% CO_2-balance N_2) in a hypoxia apparatus (INVIVO2400 Hypoxia Workstation, Ruskin Technology) for 14 hr at 37°C. Identically treated cells were incubated under normoxia (air-5% CO_2) at 37°C. Cells were harvested and replated in fresh medium at a density of 1 x 10^3 cells/well in 48-well plastic culture plates to assess viability 24-72 hr later. Briefly, Alamar Blue was added to replicate wells on Day 0 and Day 3, and cells were incubated for a further 3-6 hr at 37°C before fluorescence readings using a plate reader (530-560 nm excitation, 590 emission). The percent inhibition of proliferation was calculated and plotted against control-treated cells at each drug concentration. The 50% growth inhibitory concentration (IC_{50}) values were calculated for the paired normoxic and hypoxic treatments. The Hypoxia Cytotoxicity Ratio (HCR) was defined as ((IC_{50} in Hypoxia)/(IC_{50} in Normoxia)). In Fig. 5 we demonstrate that the activation of cytotoxicity against human H460 lung adenocarcinoma cells is oxygen dependent. Increased cytotoxicity of vinblastine N-oxide against cultured H460 lung tumor cells was observed as the oxygen concentrations were decreased. Shown in Fig. 5 are the Hypoxia Cytotoxicity Ratio (HCR) of vinblastine N-oxide plotted against the partial O_2 concentrations of 0.2%, 1%, and 5% O_2 that were tested.

EXAMPLE 9

Bioreduction of Vinca-alkaloid N-Oxides To Their Respective Parent Compounds In Hypoxic Cancer Cells As Measured by LC/MS-MS Analysis

[00151] Here we demonstrate that vinblastine N-oxide and vicristine N-oxide are bioreduced under (0.2% O_2) hypoxic conditions to their respective parent compounds as detected by chromatography and mass spectrometric analysis. Human H460 lung adenocarcinoma tumor cells were seeded in 100 mm dishes

at a density of 1 X 10^6 cells/dish and untreated or treated with vinca alkaloid N-oxide analogs at a dose range of 0.02 – 7 μM. Cells were exposed with gentle rocking to a constant level of low oxygen (0.2% O_2–5% CO_2-balance N2) in a hypoxia apparatus (INVIVO2400 Hypoxia Workstation, Ruskin Technology) for 12 hr at 37°C. Identically treated cells were incubated under normoxia (air-5% CO_2) at 37°C. Cells were harvested and lysed in DNA lysis buffer (20 mM Tris-HCL, pH 8.0, 1 mM EDTA, 0.1% Triton X-100) and stored at -80°C for analysis of DNA content and LC/MS/MS analysis. Lysates were thawed and analysed for DNA using Hoescht 33258dye and fluorescence ($\lambda_{excitation}$ 350 nm, $\lambda_{emmision}$ 455 nm) in order to normalize the measured vinca alkaloid or N-oxide analog concentrations to amounts of cells lysed in the experiment. Lysates were mixed with 150 ml of methanol containing 0.1% acetic acid and vinca alkaloid internal standard, and vortexed for 10 min, and centrifuged for 5 min at 18000 g. Clarified supernatants were transferred to HPLC vials fitted with glass inserts and analyzed by LC-MS/MS. Chromatography was performed on an Acquity UPLC system fitted with an Acquity BEH C18 colum, and used 0.1% (v:v) formic acid in water for mobile phase A, and 0.1% (v:v) formic acid in acetonitrile for mobile phase B. Mass spectral analysis was performed using a Micromass Quattro Micro triple quadupole mass spectrometer.

[00152] In Figs. 6A-6D are shown the chromatograms of vinblastine and vinblastine N-oxide analog of the HPLC/MS-MS analysis of extracellular medium from vinblastine N-oxide treated H460 tumor cells under normoxia and hypoxia conditions. The vinblastine chromatogram distinctly illustrates the increased liberation of vinblastine and a decreased peak of vinblastine N-oxide under hypoxia exposure. In Figs. 7A and 7B are shown the quantitative amounts of vinblastine N-oxide and the vinblastine parent compound measured using mass spectral analysis from lysates or the extracellular medium of 0.2 μM vinblastine N-oxide treated cells under hypoxic or normoxic conditions. The amounts of each compound were calculated from standard curves. These results clearly demonstrate that vinblastine N-oxide is bioreduced to vinblastine under hypoxia exposure and is detected in both the lysates and extracellular medium of treated cells. The extracellular liberation

of vinblastine upon hypoxia activation in treated cells indicate that vinblastine N-oxide analog may have potential bystander cytotoxic effects.

[00153] In Figs. 8A and 8B are shown the quantitative amounts of vincristine N-oxide and the vincristine parent compound measured using mass spectral analysis from lysates or the extracellular medium of 7 μM vincristine N-oxide treated H460 lung adenocarcinoma cells under hypoxic or normoxic conditions. The amounts for each compound were calculated from standard curves. These results clearly demonstrate that vincristine N-oxide is bioreduced to vincristine under hypoxia exposure and is detected in both the lysates and extracellular medium of treated cells. The extracellular liberation of vincristine upon hypoxia activation in treated cells indicate that vincristine N-oxide analog may have potential bystander cytotoxic effects.

EXAMPLE 10

Vinca-alkaloid N-oxide Analogs Have Reduced Systemic and Lethal Toxicity Compared To Their Parental Vinca-alkaloids *In Vivo*

[00154] We demonstrate here that vinblastine N-oxide and vincristine N-oxide analogs have decreased systemic and lethal toxicity compared to their respective parent compounds, vinblastine and vincristine, in rodents *in vivo*.

[00155] In Fig. 9 are shown the results of a 28-day acute toxicity study of female athymic nu/nu mice that were injected intraperitoneally (ip) with 6 – 60 mg/kg vinblastine N-oxide once every three days for a total of 5 treatments (q3d X 5). Body weights were monitored twice weekly and up to 2 weeks following treatment. Mice were also injected ip with 4 or 6 mg/kg vinblastine using the same q3d x 5 schedule as a positive control. Shown in Fig. 9 are the % Body Weight Loss of mice (n=5/group) treated with vinblastine N-oxide at a dose range of 6 – 60 mg/kg or vinblastine at 4 – 6 mg/kg. Mice treated with vinblastine at a lethal dose of 4 or 6 mg/kg succumbed with a median day of death of 10.8 or 9.8 days, respectively. In contrast, mice treated with vinblastine N-oxide at doses of up to 15 times the lethal dose of vinblastine showed no evidence of significant body weight loss or gross symptoms of toxicity at 14 days following the last treatment.

[00156] In Fig. 10 are shown the results of a 28-day acute toxicity study of female athymic nu/nu tumor-bearing mice that were injected intravenously (iv) with 0.6 – 10 mg/kg vincristine N-oxide once every three days for a total of 5 treatments (q3d X 5). Body weights were monitored twice weekly and up to 2 weeks following treatment. Mice were also injected iv with 0.6 or 1 mg/kg vincristine using the same q3d x 5 schedule as a positive control. Shown in Fig. 10 are the % Body Weight Loss of mice (n=5/group) treated with vincristine N-oxide at a dose range of 0.6 – 10 mg/kg or vincristine at 0.6 – 1 mg/kg. Mice treated with vincristine showed significant body weight loss during the course of treatment. In contrast, mice treated with vincristine N-oxide at doses of up to 10 times the maximum tolerated dose of vincristine showed no evidence of significant body weight loss or gross symptoms of toxicity at 14 days following the last treatment.

EXAMPLE 11

Anti-Tumor Activity Of Vinca-alkaloids And Analog N-Oxides Thereof In Solid Tumor Models in Mice

[00157] The *in vivo* antitumor efficacy of vinca-alkaloid and analog N-oxides will be evaluated using xenograft murine models. For example, human H460 lung adenocarcinoma, HT29 colorectal adenocarcinoma, or A549 lung carcinoma tumor fragments (approximately 1 mm^3) will be implanted subcutaneously (sc) into female nude *(nu/nu)* mice. When the tumors reach approximately 100 mm^3 in size (25 days following implantation), the animals will be pair-matched into 10 mice per group. Mice will be injected iv with vinca-alkaloid N-oxide analog at a dose of 1–200 mg/kg on a q3d X 5 schedule. Mice will also be treated with vehicle as a negative control and the bioreduced parent compound at the maximal tolerated dose as a positive control using the same schedule. Tumors will be measured with calipers twice weekly and tumor volume calculated using the formula: Tumor Volume (mm^3) = $((\text{width})^2 \times \text{length})/2$. Animals will be monitored for signs of toxicity and weighed daily for the first 5 days of the study and then twice-weekly until the study end. As pre-defined in the protocol, each animal will be actively euthanized when its tumor reached the pre-determined endpoint size of

$1200mm^3$ or at the conclusion of the study (day 60) whichever comes first. The time to endpoint (TTE) will be calculated for each mouse as TTE (days) = (log_{10}(endpoint volume mm^3) –b)/m where b is the intercept and m is the slope of the line obtained by linear regression of a log-transformed tumor growth data set. Treatment outcome will be determined from tumor growth delay (TGD) which is defined as the increase in median TTE in a treatment (T) group as compared to the control (C) group (TGD=T-C) expressed in days or as a percentage of the median TTE of the control group %TGD = ((T-C)/C) x 100. Kaplan-Meier plots will be constructed to demonstrate the percentage animals remaining in the study as a function of time following treatment. Statistical significance between the treated vs. control groups will be evaluated by logrank analysis.

[00158] In another example, female nude mice (nu/nu) will be implanted sc with fragments of human BxPC-3 pancreatic tumors. When the tumors reach approximately 60-80 mm^3 in size, the animals will be pair-matched into treatment and control groups containing ten mice per group. Mice will be injected iv with vinca-alkaloid N-oxide analog at a dose of 1 – 200 mg/kg on a q3d X 5 schedule. Mice will also be treated with vehicle as a negative control and the bioreduced parent compound at the maximal tolerated dose as a positive control using the same schedule. Tumors will be measured with calipers twice weekly and tumor volume calculated using the formula: Tumor Volume (mm^3) = ((width)^2 x length)/2. Animals will be monitored for signs of toxicity and weighed daily for the first 5 days of the study and then twice-weekly until the study end. As pre-defined in the protocol, the experiment will be terminated when the vehicle control group tumor size reaches an average of ~2000 mm^3 (~27 days). Upon termination, the mice will be weighed, sacrificed, tumors excised, and the mean tumor volume per group calculated. Tumor growth inhibition (TGI), defined as the change in mean tumor volume of the treated groups/the change in mean tumor volume of the control group x 100 ($\Delta T/\Delta C$) will be calculated for each group. Statistical comparisons will be carried out using ANOVA followed by the Dunnett multiple comparisons test.

[00159] The anti-tumor activity of vinca-alkaloid N-oxides will also be assessed in an orthotopic (ot) solid tumor xenograft model. In one

embodiment, female athymic mice will injected with 2.5 x 10^6 human BxPC-3 pancreatic cells directly into the pancreas parenchyma. Treatment with vehicle (negative control), 10 – 200 mg/kg vinca-alkaloid N-oxide analog (iv), or 40 mg/kg gemcitabine (ip) will be initiated on day 14 post-tumor implantation at a q3dX5 schedule. Treatment groups will consist of 12 mice/group; an extra 5 mice be added to each group for histological analysis. Mice will be monitored twice daily for symptoms of disease and actively euthanized based on the criteria outlined by the Institutional Ethical Committee; the day of sacrifice wil be considered the day of cancer death. Prolongation of survival, a primary endpoint in the study, will be evaluated using Kaplan-Meier plots and statistical significance of treatment responses compared to control groups will be analyzed by logrank analysis. For histological analysis, mice will be sacrificed at multiple days throughout treatment course and blinded tissue sections from resected tumors will be analysed by standard immunohistochemical techniques for detection of tumor proliferation (BrDUrd incorporation), hypoxia (pimonidazole staining), vasculature (CD31 staining), apoptosis (TUNEL staining) and necrosis. The effects of vinca-alkaloid N-oxide analogs in comparison to vehicle control treated on the tumor microenvironment will be qualitatively and quantitatively assessed. In addition, other target organs such as the lung and liver that demonstrate metastases formation will be resected and analysed for treatment effects on metastatic spread. Five μm-thick formalin-fixed paraffin-embedded tissue sections will be stained with hematoxylin and eosin (H&E) gross histopathological analysis will be performed in a blinded fashion to compute the metastatic tumor burden. The total percentage of space occupied by invasive tumor cells was enumerated and will be expressed as a percentage of total tissue evaluated. Statistical analysis will be computed using the non-parametric Mann-Whitney U-test. The effects of vinca-alkaloid N-oxides on preventing the growth of metastatic tumor cells and preventing the metastatic spread of hypoxic tumor cells will be evaluated.

[00160] Further, the *in vivo* antitumor activity of vinca-alkaoid and analog N-oxide in combination with chemotherapeutic agents and/or radiotherapy will be evaluated using a xenograft model in nude mice.

[00161] Further, the *in vivo* antitumor activity of vinca-alkaoid and analog N-oxide in combination with biological agents, such as therapeutic monoclonal antibodies (anti-VEGF, anti-EGF) will be evaluated using a xenograft model in nude mice.

[00162] Further, the *in vivo* antitumor activity of vinca-alkaoid and analog N-oxide in combination with small molecules tyrosine kinase inhibitors will be evaluated using a xenograft model in nude mice.

[00163] Further, the *in vivo* antitumor activity of vinca-alkaoid and analog N-oxide in combination with vascular disrupting, vascular damaging, or anti-angiogenic agents will be evaluated using a xenograft model in nude mice.

[00164] Further, the *in vivo* antitumor activity of vinca-alkaoid and analog N-oxide in combination with Hypoxia-Inducible Factor-1 or -2 (HIF-1 or HIF-2) antagonists will be evaluated using a xenograft model in nude mice.

[00165] Further, the *in vivo* antitumor activity of vinca-alkaoid and analog N-oxide in combination with other therapeutic agents that target euoxic tumor cells will be evaluated using a xenograft model in nude mice.

[00166] Further, the *in vivo* antitumor activity of vinca-alkaoid and analog N-oxide in combination with other agents that decrease tumor oxygenation or increase tumor consumption will be evaluated using a xenograft model in nude mice.

EXAMPLE 12

Tumor Selectivity Of Vinca-alkaloids N-Oxide Analogs

[00167] The systemic and local tumor concentrations of vinca-alkaloid N-oxide analogs and their bioreduced parent compound metabolites will be monitored in tumor bearing mice using quantitative analytical procedures such as HPLC/MS-MS.

[00168] In one example, female athymic nude mice will be sc injected with 1 x 10^7 H460 lung adenocarcinoma tumor cells. Animals will be dosed when the mean tumor volume reached 300 mm^3. Mice will be randomized into treatment groups of 5 mice/group. The mice will receive a single iv bolus injection of vinca alkaloid N-oxide at 10 – 200 mg/kg by tail-vein injections.

Three mice per dose will be sacrificed at 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr and 24 hr post-treatment. Plasma and dried tumor samples will be collected and stored at -80°C for further analysis using HPLC/MS/MS. Quantitation of vinca-alkaloid N-oxides and its bioreduced metabolites, from plasma and tumor samples will be determined following liquid-liquid extraction in methanol containing 0.1% (v/v) acetic acid. Following liquid extraction, plasma samples will be spiked with known concentrations of the internal standard, and subjected to centrifugation at 15000 x rpm for 10 min at 4°C. The resulting supernatants will be analyzed by chromatography using an Acquity UPLC system fitted with an Acquity BEH C18 colum, and used 0.1% (v:v) formic acid in water for mobile phase A, and 0.1% (v:v) formic acid in acetonitrile for mobile phase B. Mass spectral analysis will be performed using a Micromass Quattro Micro triple quadupole mass spectrometer. Resected tumor samples (~300 mg) will be homogenized in cold water, spiked with known internal standard and subjected to liquid-liquid extraction. Following the centrifugation of tissue homogenates, the supernatants will be collected and concentrated to a final volume of 0.4 ml using a vacuum concentrator prior to HPLC/MS/MS analysis as described above. The amounts of vinca-alkaloid N-oxides and its bioreduced parent metabolite will be analysed from plasma and tumor samples at various times post-treatment to demonstrate increased tumor selectivity and decreased systemic exposure following drug administration.

[00169] Having now fully described this invention, it will be understood by those of ordinary skill in the art that the same can be performed within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or any embodiment thereof.

THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. Use of an effective amount of vinblastine N-oxide, desacetyl vinblastine N-oxide, vinorelbine N-oxide, vincristine N-oxide, desacetyl vinflunine N-oxide, desacetyl vinorelbine N-oxide, vindesine N-oxide, or vinflunine N-oxide, or a pharmaceutically acceptable salt thereof, in the treatment or amelioration of a hyperproliferative disorder in an animal in need thereof.

2. Use of vinblastine N-oxide, desacetyl vinblastine N-oxide, vinorelbine N-oxide, vincristine N-oxide, desacetyl vinflunine N-oxide, desacetyl vinorelbine N-oxide, vindesine N-oxide, or vinflunine N-oxide, or a pharmaceutically acceptable salt thereof, in the preparation of a medicament for the treatment or amelioration of a hyperproliferative disorder in an animal.

3. The use of claim 1 or 2, in conjunction with an imaging technique selected from the group consisting of computed tomography, magnetic resonance imaging, single photon emission computer tomography and positron emission tomography prior to or during administration of vinblastine N-oxide, desacetyl vinblastine N-oxide, vinorelbine N-oxide, vincristine N-oxide, desacetyl vinflunine N-oxide, desacetyl vinorelbine N-oxide, vindesine N-oxide, or vinflunine N-oxide, or a pharmaceutically acceptable salt thereof.

4. The use of claims 1 or 2, wherein said hyperproliferative disorder is cancer.

5. The use of claim 4, wherein the cancer is of the bladder, brain, breast, cervix, colon, endometrium, esophagus, head and neck, kidney, larynx, liver, lung, oral cavity, ovaries, pancreas, prostate, skin, stomach, or testis.

6. The use of claim 4, wherein the cancer is selected from the group consisting of acute and chronic lymphocytic leukemia, acute granulocytic leukemia, adrenal cortex carcinoma, bladder carcinoma, breast carcinoma, cervical carcinoma, cervical hyperplasia, choriocarcinoma, chronic granulocytic leukemia, chronic lymphocytic leukemia, colon carcinoma, endometrial carcinoma, esophageal carcinoma, essential thrombocytosis, genitourinary carcinoma, hairy cell

leukemia, head and neck carcinoma, Hodgkin's disease, Kaposi's sarcoma, lung carcinoma, lymphoma, malignant carcinoid carcinoma, malignant hypercalcemia, malignant melanoma, malignant pancreatic insulinoma, medullary thyroid carcinoma, melanoma, multiple myeloma, mycosis fungoides, myeloid and lymphocytic leukemia, neuroblastoma, non-Hodgkin's lymphoma, osteogenic sarcoma, ovarian carcinoma, pancreatic carcinoma, polycythemia vera, primary brain carcinoma, primary macroglobulinemia, prostatic carcinoma, renal cell carcinoma, rhabdomyosarcoma, skin cancer, small-cell lung carcinoma, soft-tissue sarcoma, squamous cell carcinoma, stomach carcinoma, testicular carcinoma, thyroid carcinoma, and Wilms' tumor.

7. The use of claim 1 or 2, wherein said hyperproliferative disorder is age-related macular degeneration, Crohn's disease, cirrhosis, chronic inflammatory-related disorders, diabetic retinopathy, granulomatosis, immune hyperproliferation associated with organ or tissue transplantation, inflammatory bowel disease, psoriasis, rheumatoid arthritis, systemic lupus erythematosus, vascular hyperproliferation secondary to retinal hypoxia, or vasculitis.

8. The use of claim 1 or 2, further comprising administration of one or more other active agents or treatments to the animal.

9. The use of claim 8, wherein the one or more other active agents are selected from the group consisting of abarelix, aldesleukin, alemtuzumab, alitretinoin, allopurinol, altretamine, amifostine, anastrozole, arsenic trioxide, asparaginase, BCG live, bevaceizumab, bexarotene, bleomycin, bortezomib, busulfan, calusterone, camptothecin, capecitabine, carboplatin, carmustine, celecoxib, cetuximab, chlorambucil, cinacalcet, cisplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, darbepoetin alfa, daunorubicin, denileukin diftitox, dexrazoxane, docetaxel, doxorubicin, dromostanolone, Elliott's B solution, epirubicin, epoetin alfa, estramustine, etoposide, exemestane, filgrastim, floxuridine, fludarabine, fluorouracil, fulvestrant, gemcitabine, gemtuzumab ozogamicin, gefitinib, goserelin, hydroxyurea, ibritumomab tiuxetan, idarubicin, ifosfamide, imatinib, interferon alfa-2a, interferon alfa-2b, irinotecan, letrozole, leucovorin, levamisole, lomustine, meclorethamine, megestrol, melphalan, mercaptopurine, mesna, methotrexate, methoxsalen, methylprednisolone, mitomycin C, mitotane, mitoxantrone, nandrolone, nofetumomab, oblimersen, oprelvekin,

oxaliplatin, paclitaxel, pamidronate, pegademase, pegaspargase, pegfilgrastim, pemetrexed, pentostatin, pipobroman, plicamycin, polifeprosan, porfimer, procarbazine, quinacrine, rasburicase, rituximab, sargramostim, streptozocin, talc, tamoxifen, tarceva, temozolomide, teniposide, testolactone, thioguanine, thiotepa, topotecan, toremifene, tositumomab, trastuzumab, tretinoin, uracil mustard, valrubicin, vinblastine, vincristine, vinorelbine, and zoledronate.

10. The use of claim 8, wherein the one or more other active agents are selected from the group consisting of bevacizumab, angiostatin, endostatin, batimastat, captopril, cartilage derived inhibitor, genistein, interleukin 12, lavendustin, medroxypregesterone acetate, recombinant human platelet factor 4, tecogalan, thrombospondin, TNP-470, anti-VEGF monoclonal antibody, soluble VEGF-receptor chimaeric protein, anti-VEGF receptor antibodies, anti-PDGF receptors, inhibitors of integrins, tyrosine kinase inhibitors, serine/threonine kinase inhibitors, antisense oligonucleotides, antisense oligodexoynucleotides, siRNAs, anti-VEGF aptamers and pigment epithelium derived factor.

11. The use of claim 8, wherein vinblastine N-oxide, desacetyl vinblastine N-oxide, vinorelbine N-oxide, vincristine N-oxide, desacetyl vinflunine N-oxide, desacetyl vinorelbine N-oxide, vindesine N-oxide, or vinflunine N-oxide, or a pharmaceutically acceptable salt thereof, is for administration prior to the administration of said active agents or treatments.

12. The use of claim 8, wherein vinblastine N-oxide, desacetyl vinblastine N-oxide, vinorelbine N-oxide, vincristine N-oxide, desacetyl vinflunine N-oxide, desacetyl vinorelbine N-oxide, vindesine N-oxide, or vinflunine N-oxide, or a pharmaceutically acceptable salt thereof, is for administration concurrently with the administration of said active agents or treatments.

13. The use of claim 8, wherein vinblastine N-oxide, desacetyl vinblastine N-oxide, vinorelbine N-oxide, vincristine N-oxide, desacetyl vinflunine N-oxide, desacetyl vinorelbine N-oxide, vindesine N-oxide, or vinflunine N-oxide, or a pharmaceutically acceptable salt thereof, is for administration after the administration of said active agents or treatments.

14. A pharmaceutical composition comprising a compound selected from the group consisting of vinblastine N-oxide, desacetyl vinblastine N-oxide, vinorelbine N-oxide, vincristine N-oxide, desacetyl vinflunine N-oxide, desacetyl vinorelbine N-oxide, vindesine N-oxide, and vinflunine N-oxide, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

15. The pharmaceutical composition of claim 14, wherein said compound is vinblastine N-oxide, or a pharmaceutically acceptable salt thereof.

16. The pharmaceutical composition of claim 14, wherein said compound is desacetyl vinblastine N-oxide, or a pharmaceutically acceptable salt thereof.

17. The pharmaceutical composition of claim 14, wherein said compound is vinorelbine N-oxide, or a pharmaceutically acceptable salt thereof.

18. The pharmaceutical composition of claim 14, wherein said compound is vincristine N-oxide, or a pharmaceutically acceptable salt thereof.

19. The pharmaceutical composition of claim 14, wherein said compound is desacetyl vinflunine N-oxide, or a pharmaceutically acceptable salt thereof.

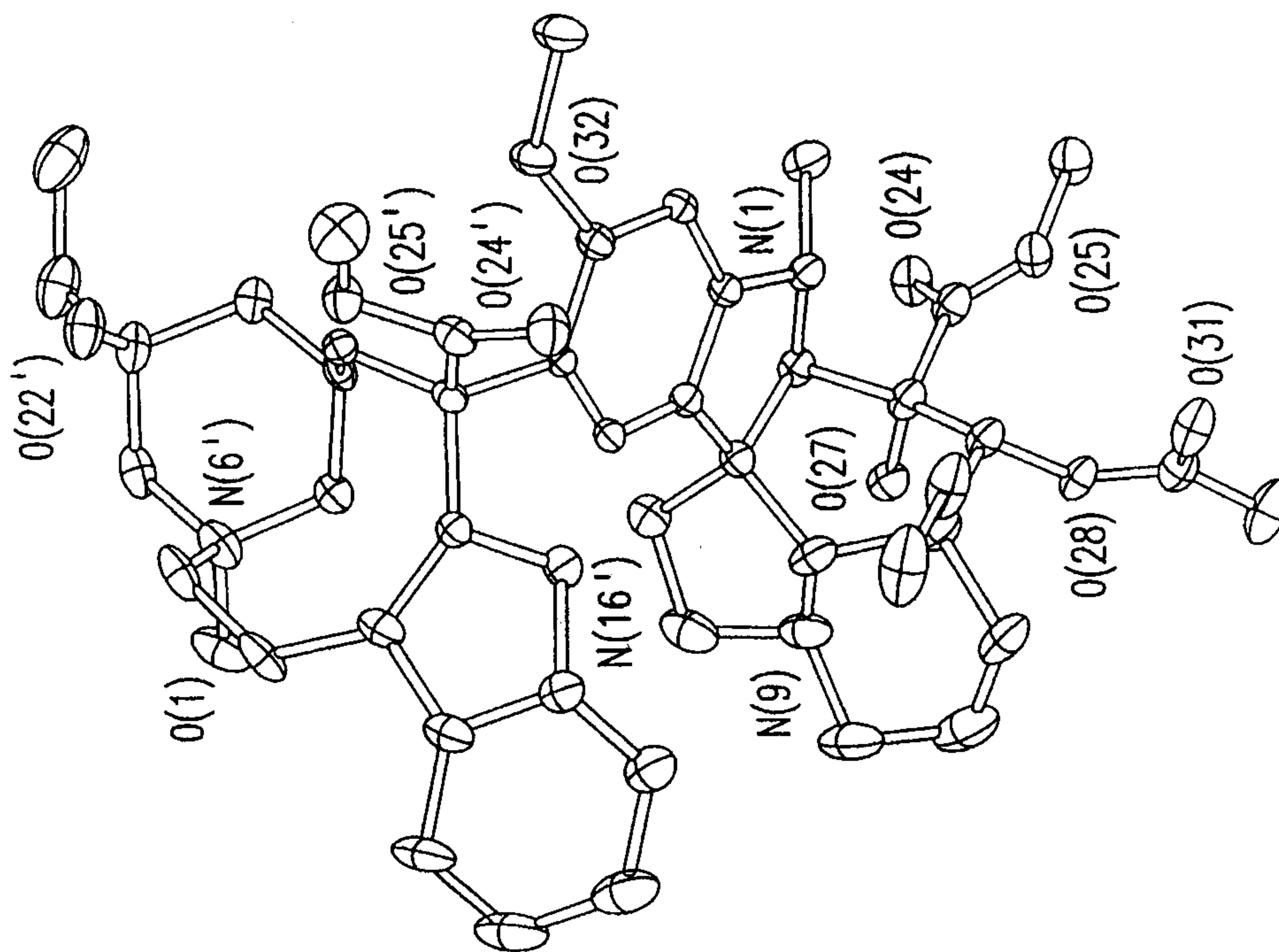
20. The pharmaceutical composition of claim 14, wherein said compound is desacetyl vinorelbine N-oxide, or a pharmaceutically acceptable salt thereof.

21. The pharmaceutical composition of claim 14, wherein said compound is vindesine N-oxide, or a pharmaceutically acceptable salt thereof.

22. The pharmaceutical composition of claim 14, wherein said compound is vinflunine N-oxide, or a pharmaceutically acceptable salt thereof.

23. The pharmaceutical composition of any one of claims 14-22, further comprising one or more active agents selected from the group consisting of abarelix, aldesleukin, alemtuzumab, alitretinoin, allopurinol, altretamine, amifostine, anastrozole, arsenic trioxide, asparaginase, BCG live, bevaceizumab, bexarotene, bleomycin, bortezomib, busulfan, calusterone, camptothecin, capecitabine, carboplatin, carmustine, celecoxib, cetuximab, chlorambucil, cinacalcet, cisplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, darbepoetin alfa, daunorubicin, denileukin diftitox, dexrazoxane, docetaxel, doxorubicin, dromostanolone, Elliott's B solution, epirubicin, epoetin alfa, estramustine, etoposide, exemestane, filgrastim, floxuridine, fludarabine, fluorouracil, fulvestrant, gemcitabine, gemtuzumab ozogamicin, gefitinib, goserelin, hydroxyurea, ibritumomab tiuxetan, idarubicin, ifosfamide, imatinib, interferon alfa-2a, interferon alfa-2b, irinotecan, letrozole, leucovorin, levamisole, lomustine, meclorethamine, megestrol, melphalan, mercaptopurine, mesna, methotrexate, methoxsalen, methylprednisolone, mitomycin C, mitotane, mitoxantrone, nandrolone, nofetumomab, oblimersen, oprelvekin, oxaliplatin, paclitaxel, pamidronate, pegademase, pegaspargase, pegfilgrastim, pemetrexed, pentostatin, pipobroman, plicamycin, polifeprosan, porfimer, procarbazine, quinacrine, rasburicase, rituximab, sargramostim, streptozocin, talc, tamoxifen, tarceva, temozolomide, teniposide, testolactone, thioguanine, thiotepa, topotecan, toremifene, tositumomab, trastuzumab, tretinoin, uracil mustard, valrubicin, vinblastine, vincristine, vinorelbine, and zoledronate.

24. The pharmaceutical composition of any one of claims 14-22, further comprising one or more active agents selected from the group consisting of bevacizumab, angiostatin, endostatin, batimastat, captopril, cartilage derived inhibitor, genistein, interleukin 12, lavendustin, medroxypregesterone acetate, recombinant human platelet factor 4, tecogalan, thrombospondin, TNP-470, anti-VEGF monoclonal antibody, soluble VEGF-receptor chimaeric protein, anti-VEGF receptor antibodies, anti-PDGF receptors, inhibitors of integrins, tyrosine kinase inhibitors, serine/threonine kinase inhibitors, antisense oligonucleotides, antisense oligodexoynucleotides, siRNAs, anti-VEGF aptamers and pigment epithelium derived factor.



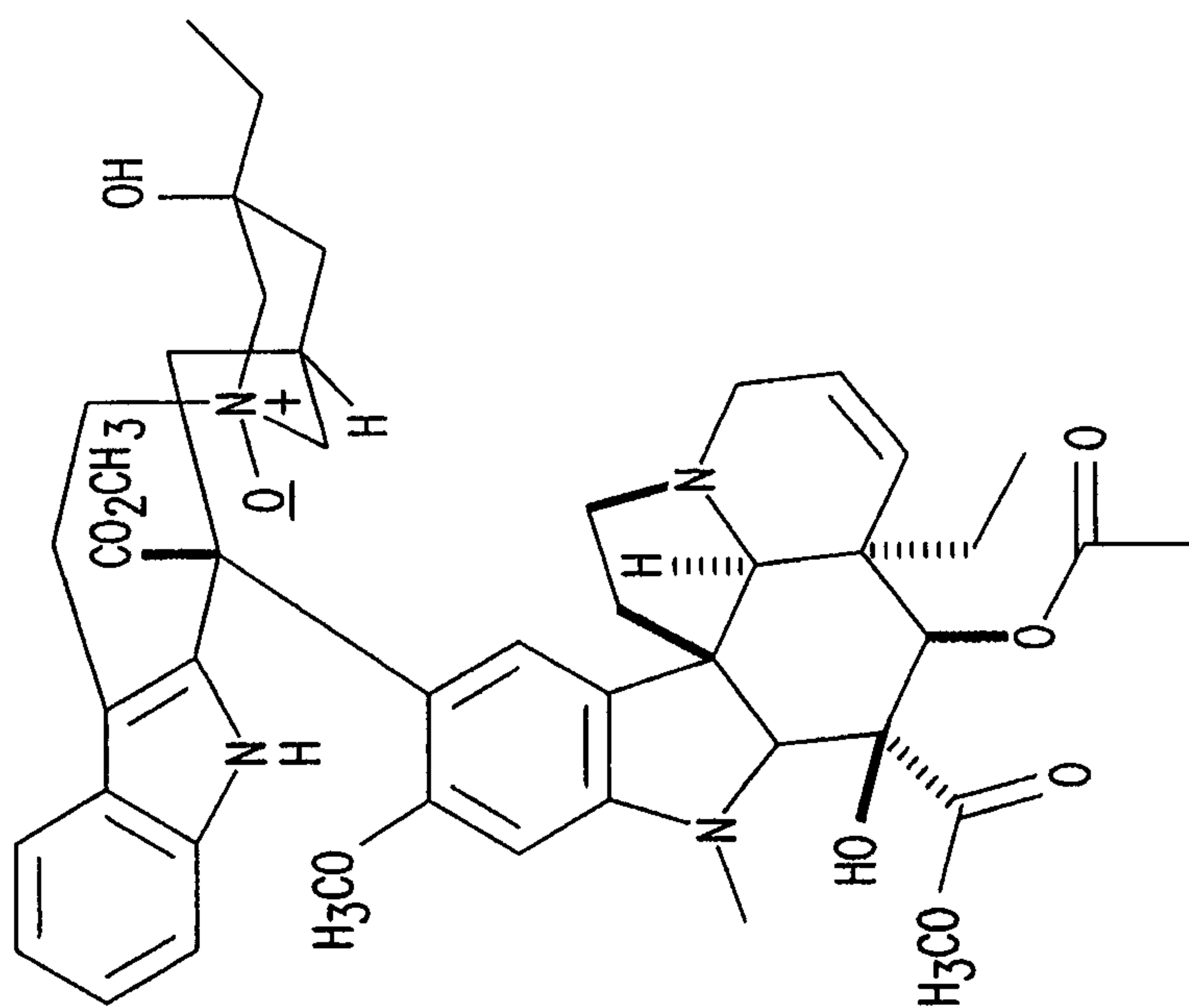
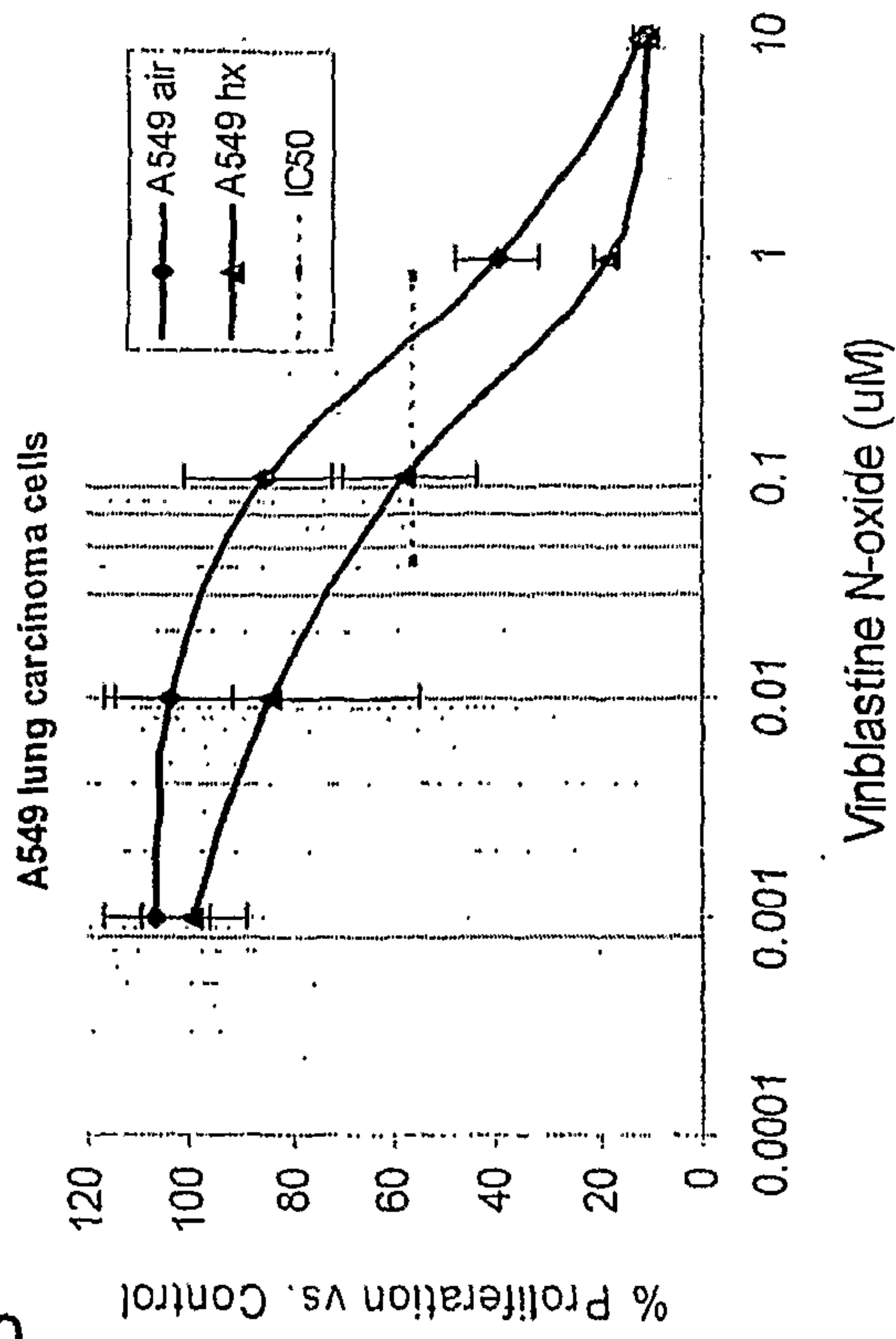
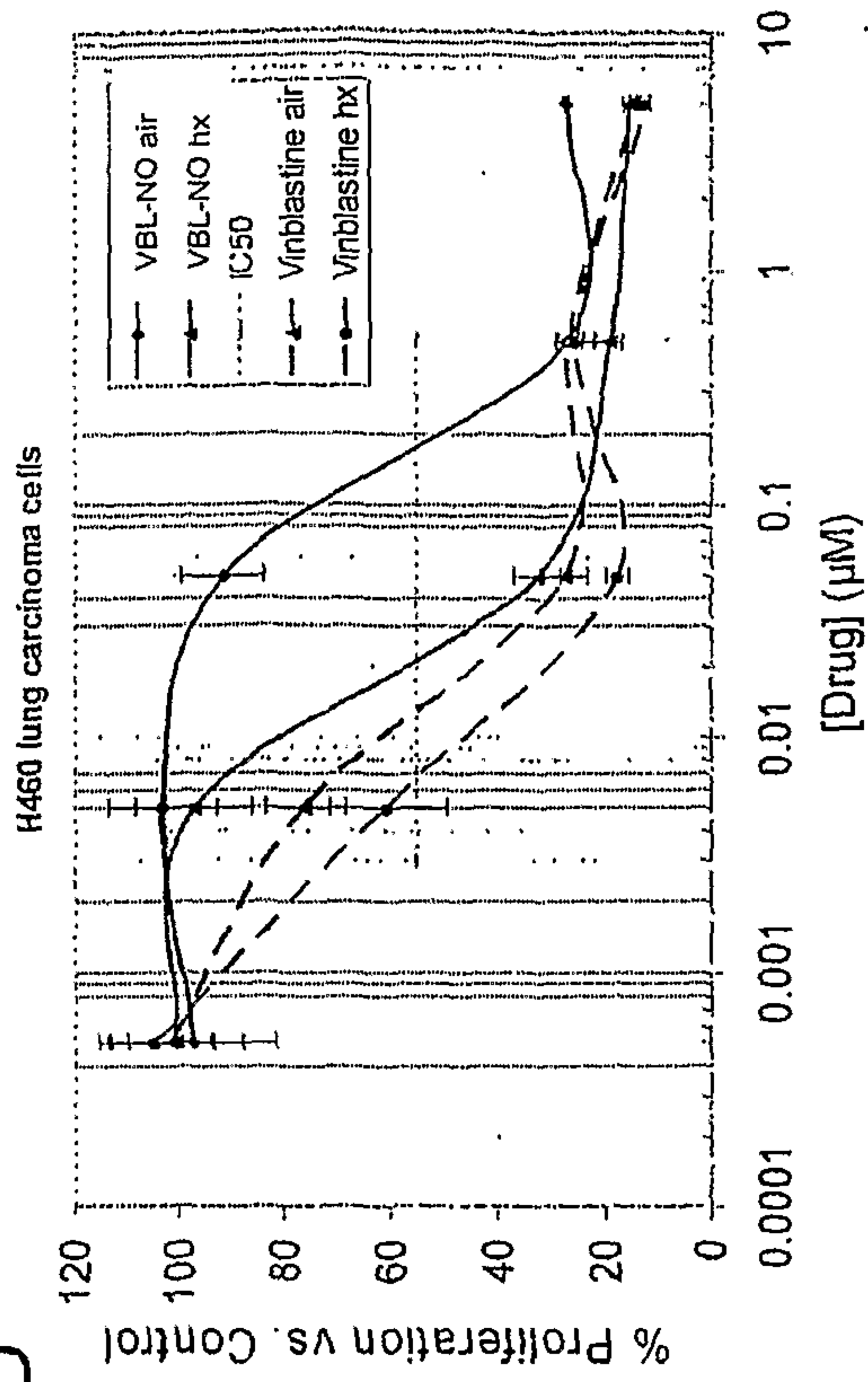
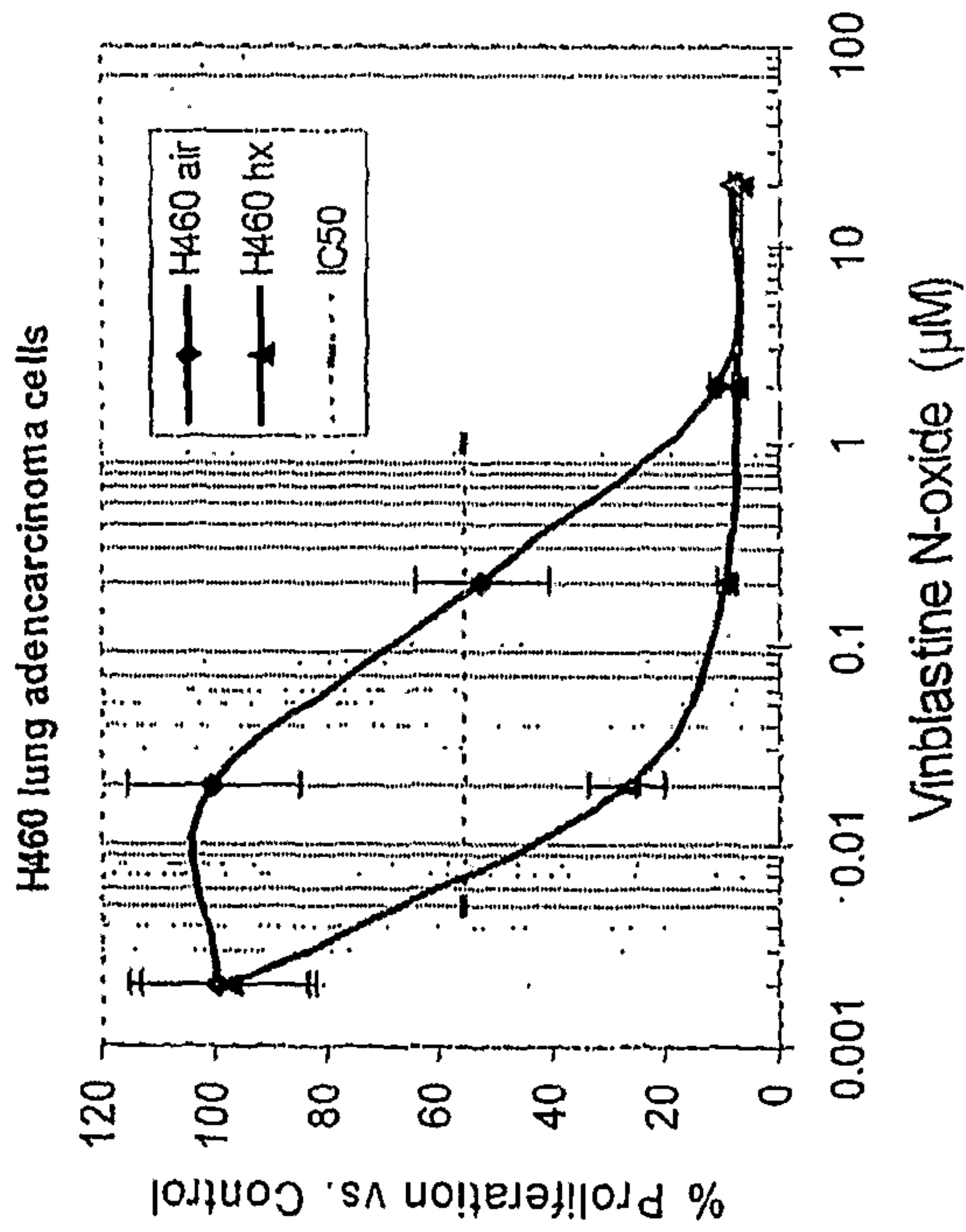
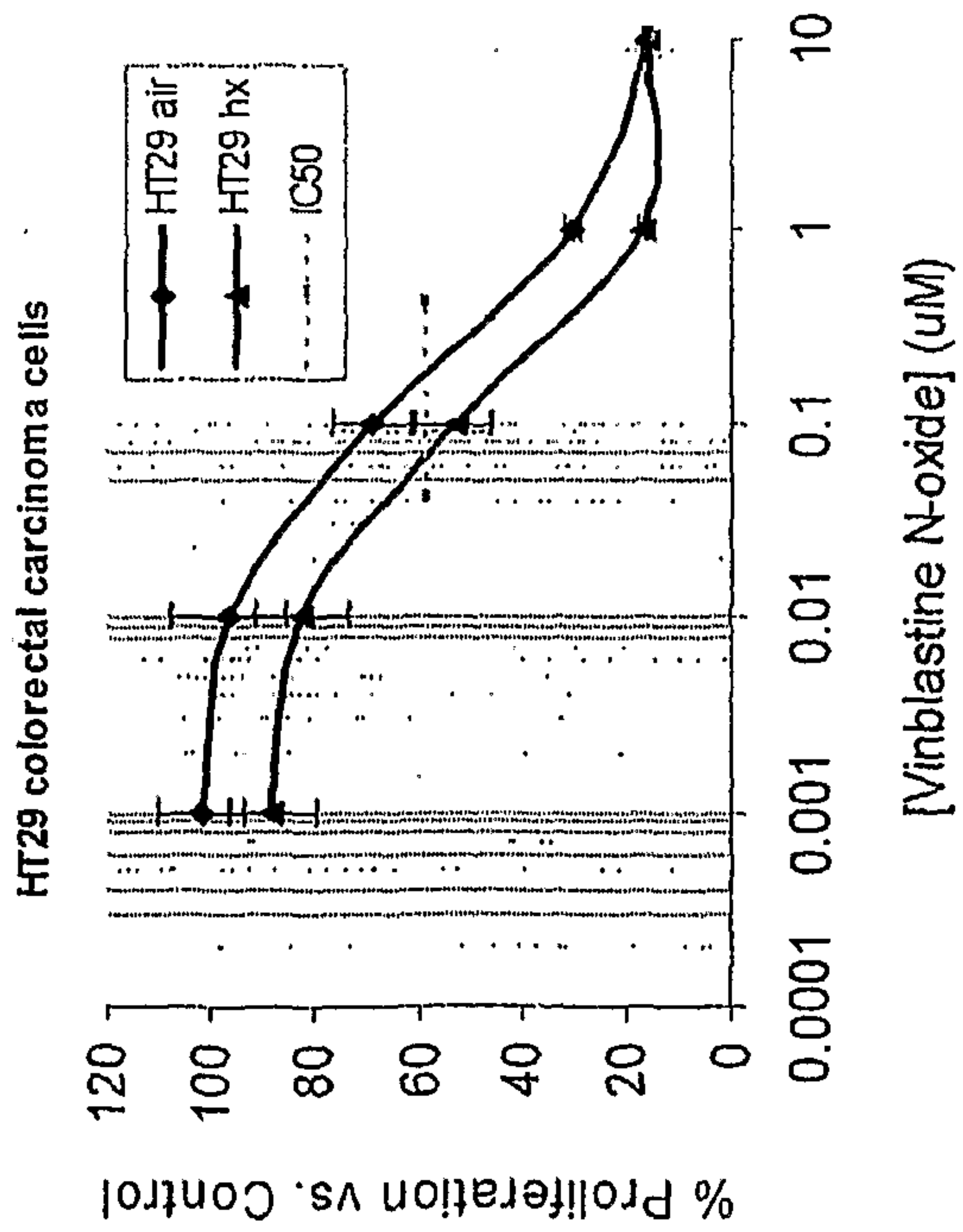


Figure 1



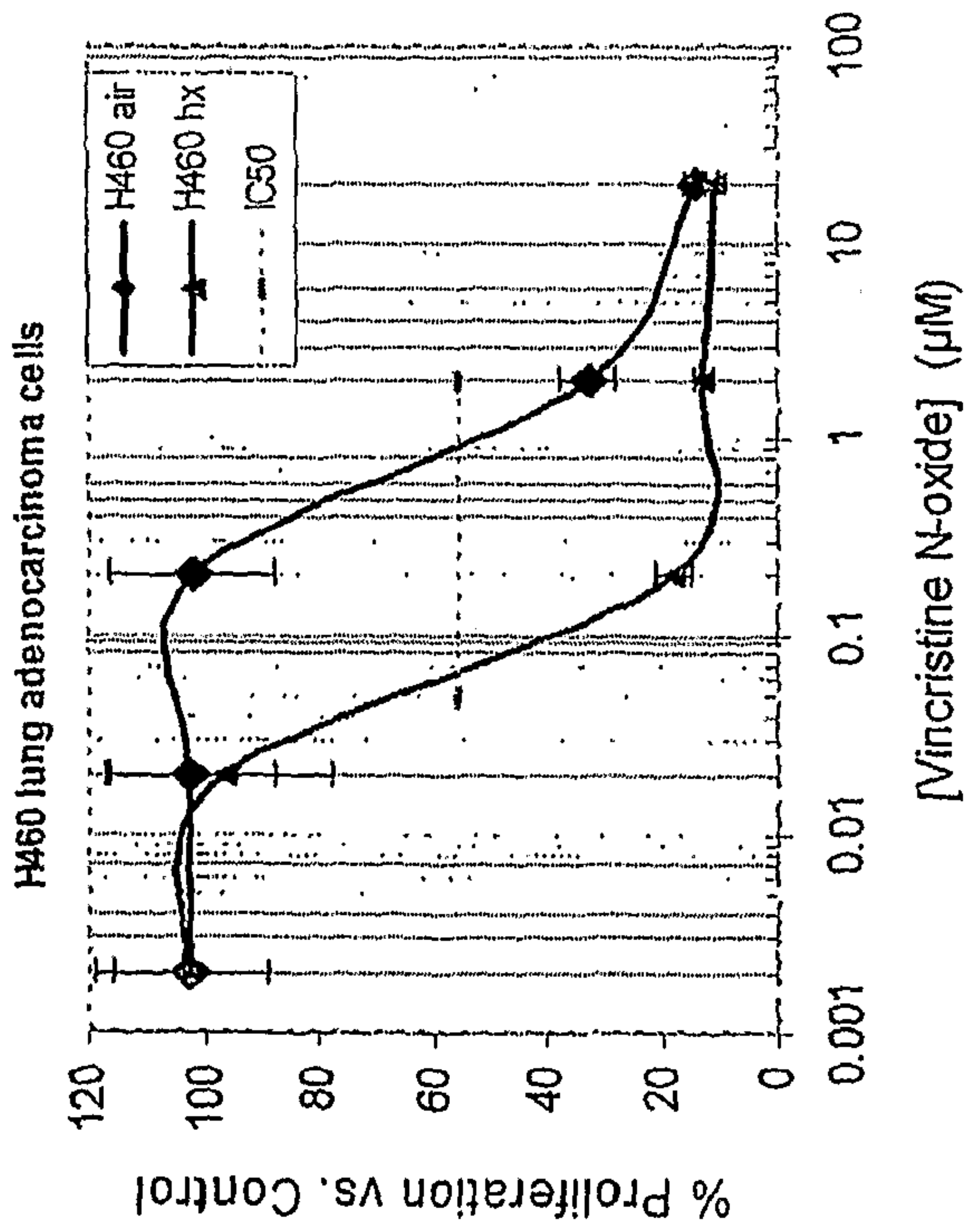




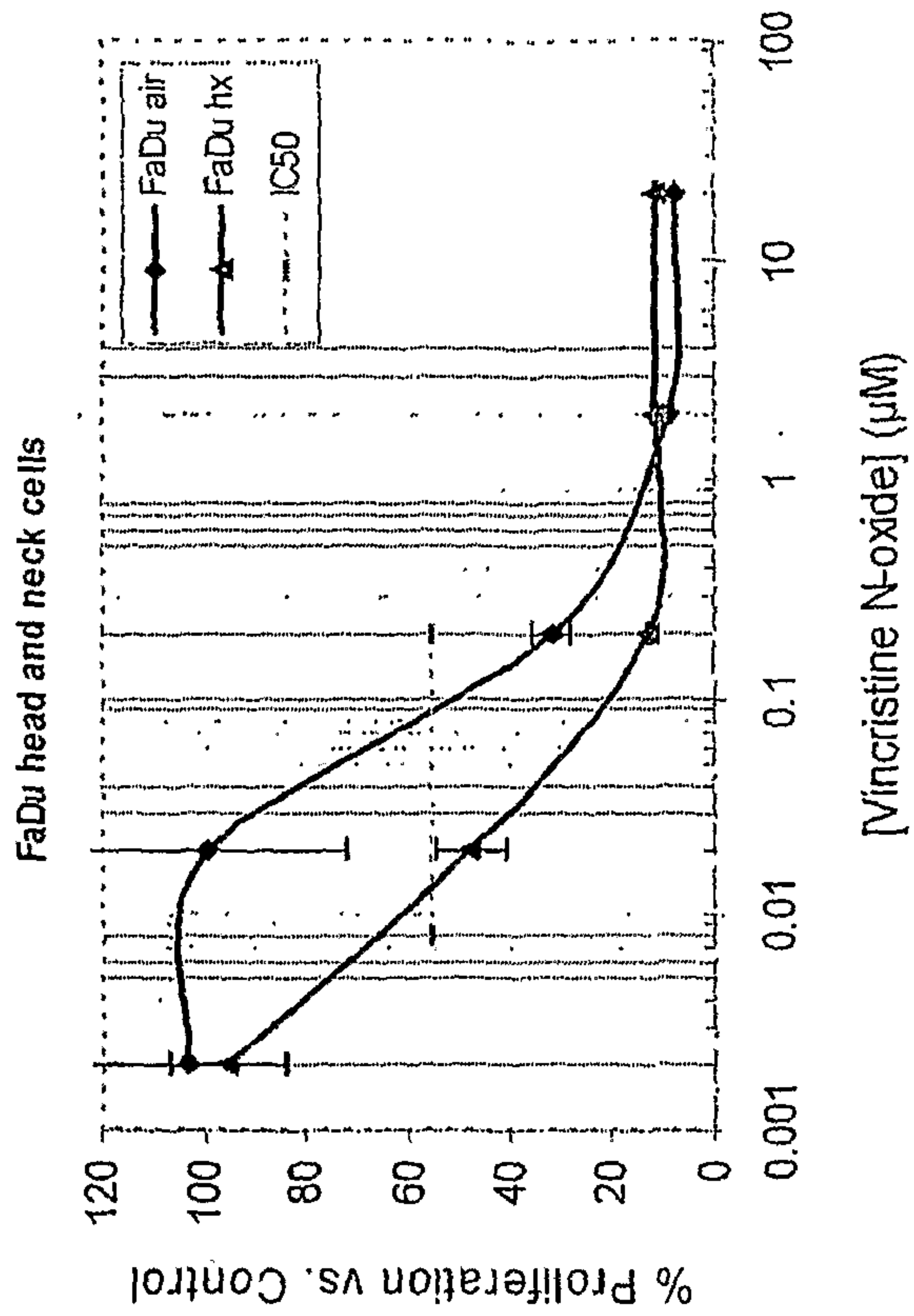


Figures 2A-2D

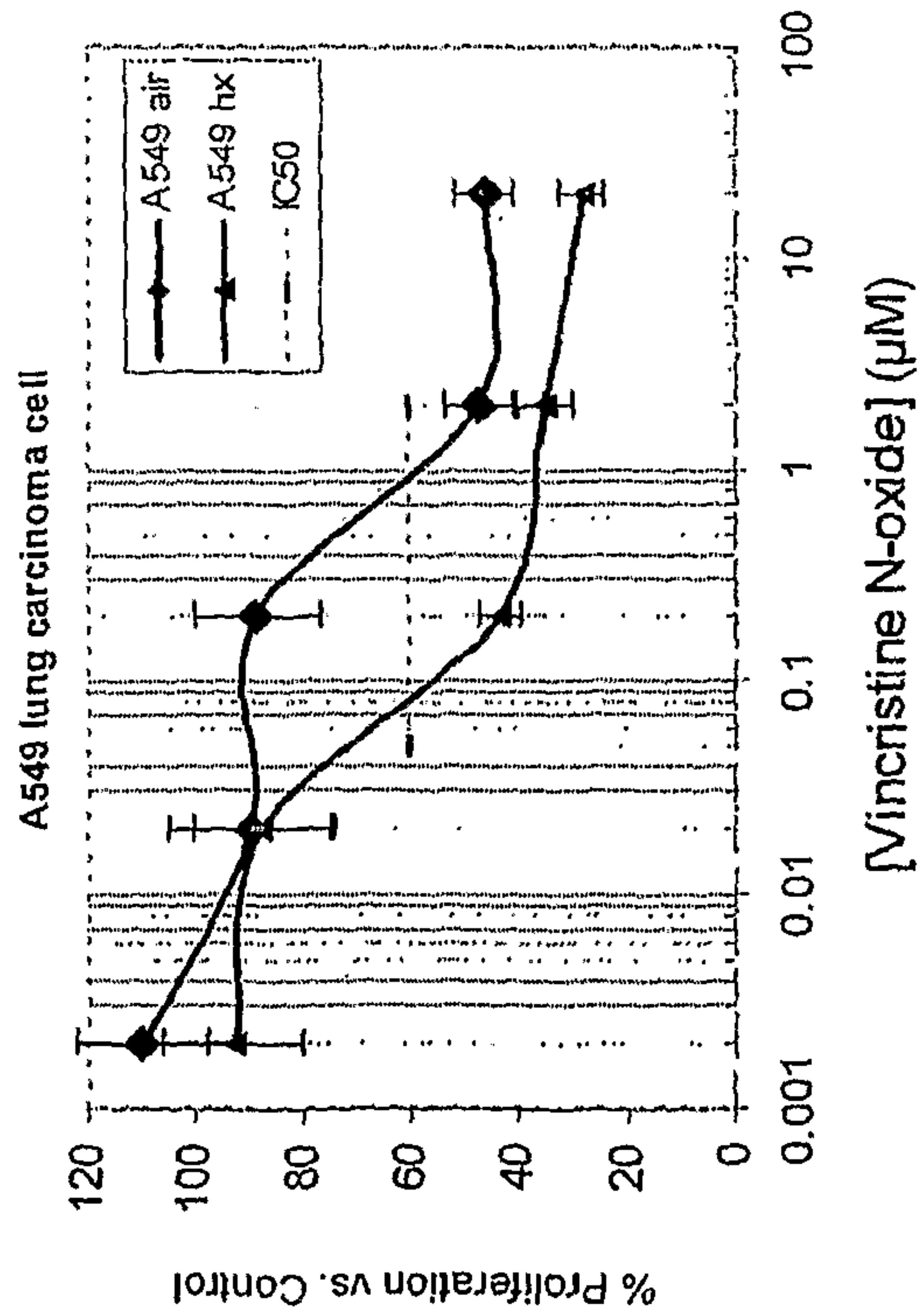
3A



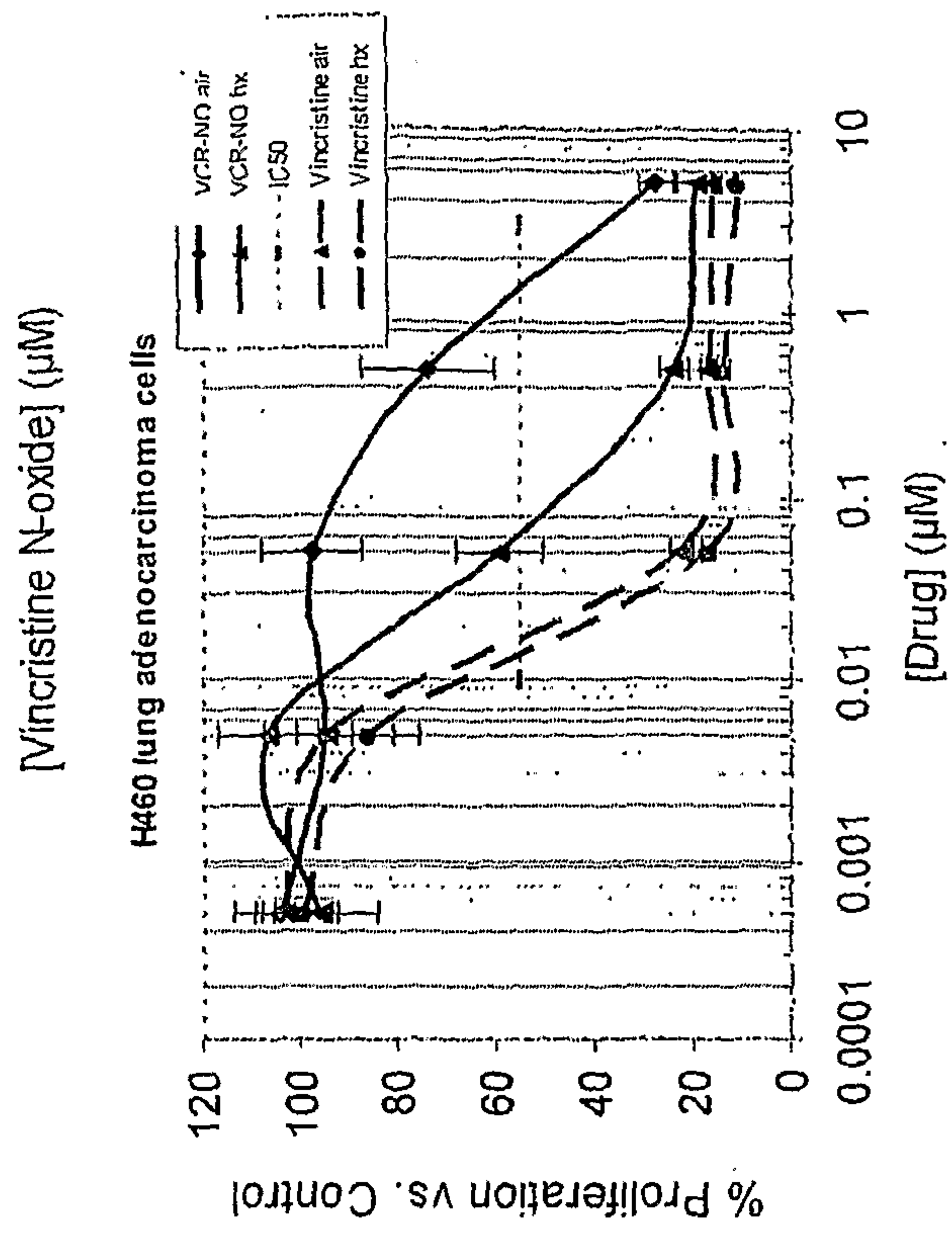
3B



3C



3D



Figures 3A-3D

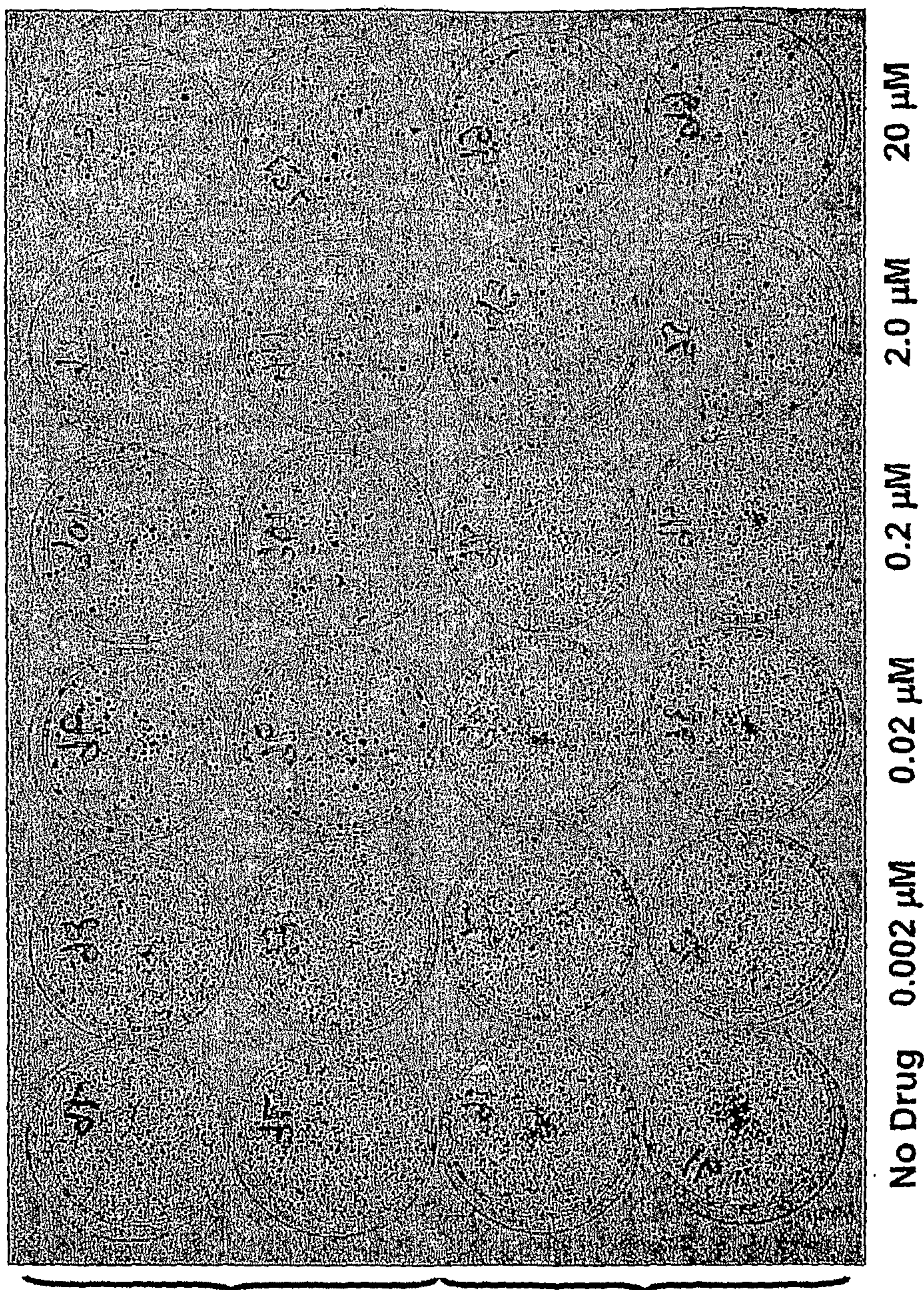


Figure 4

IC_{50} Normoxia = 0.15 µM
IC_{50} Hypoxia = 0.008 µM
HCR = 19

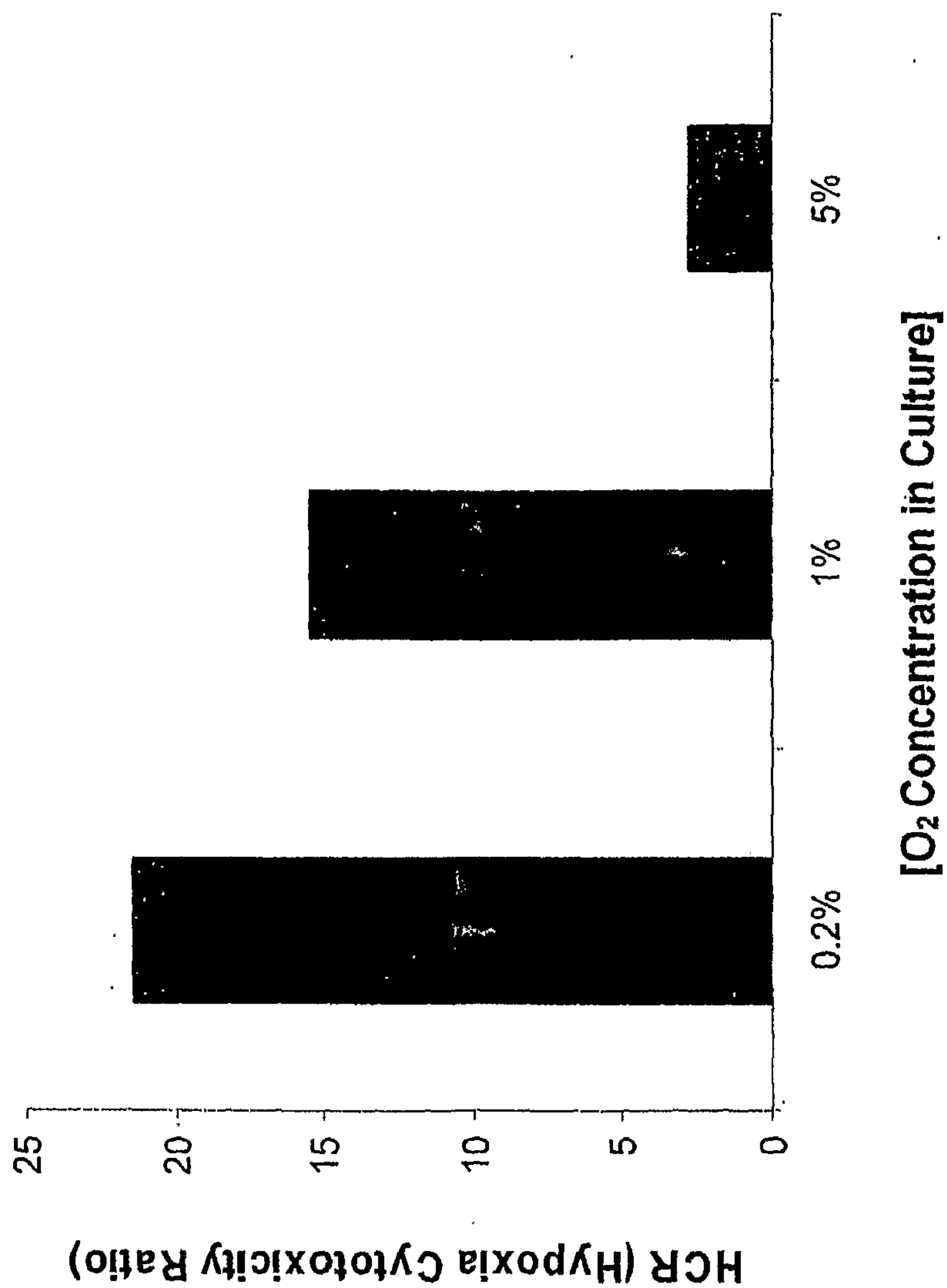
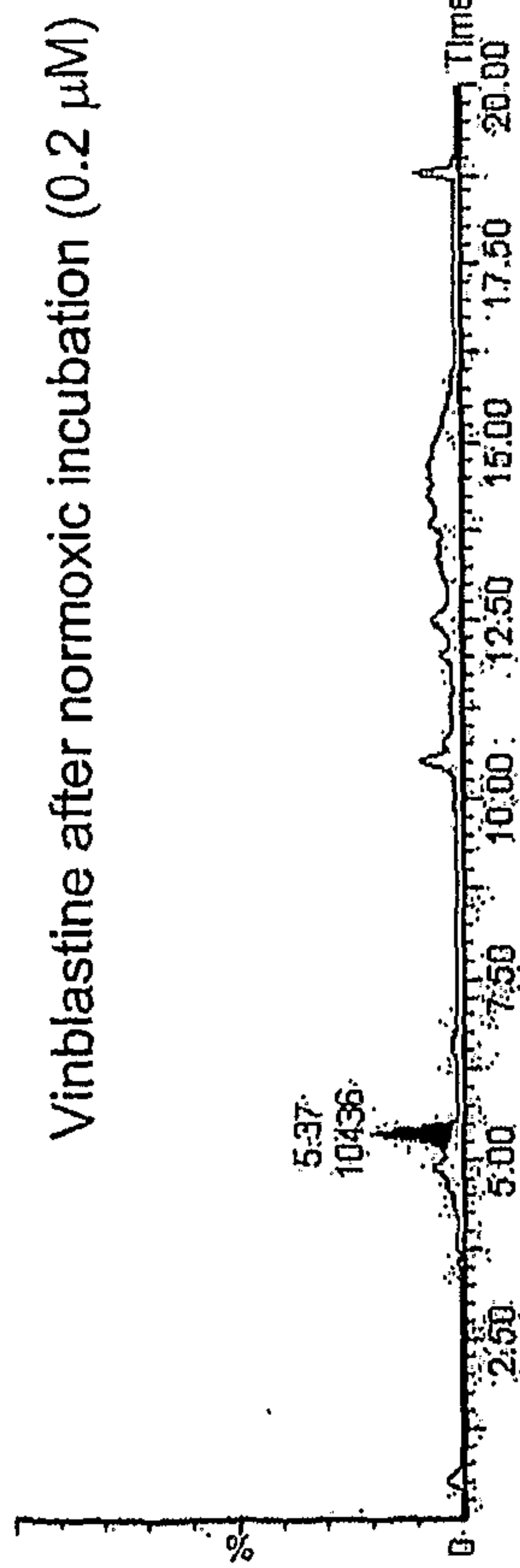
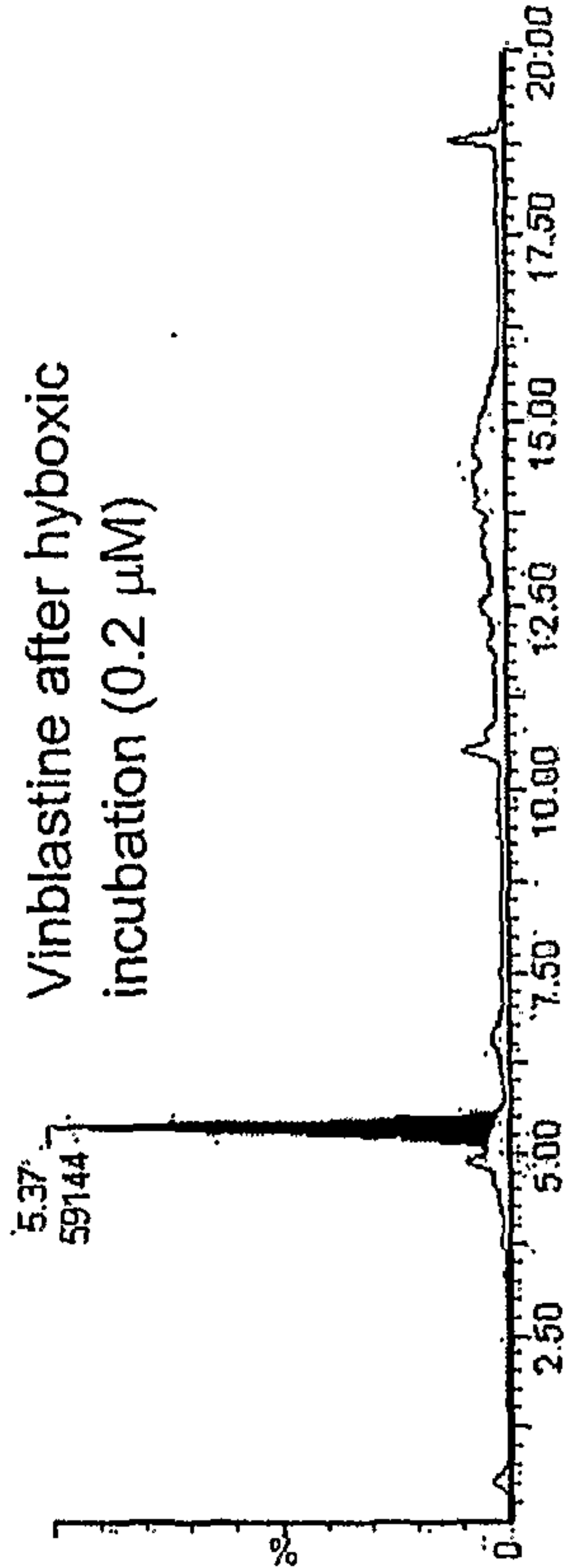
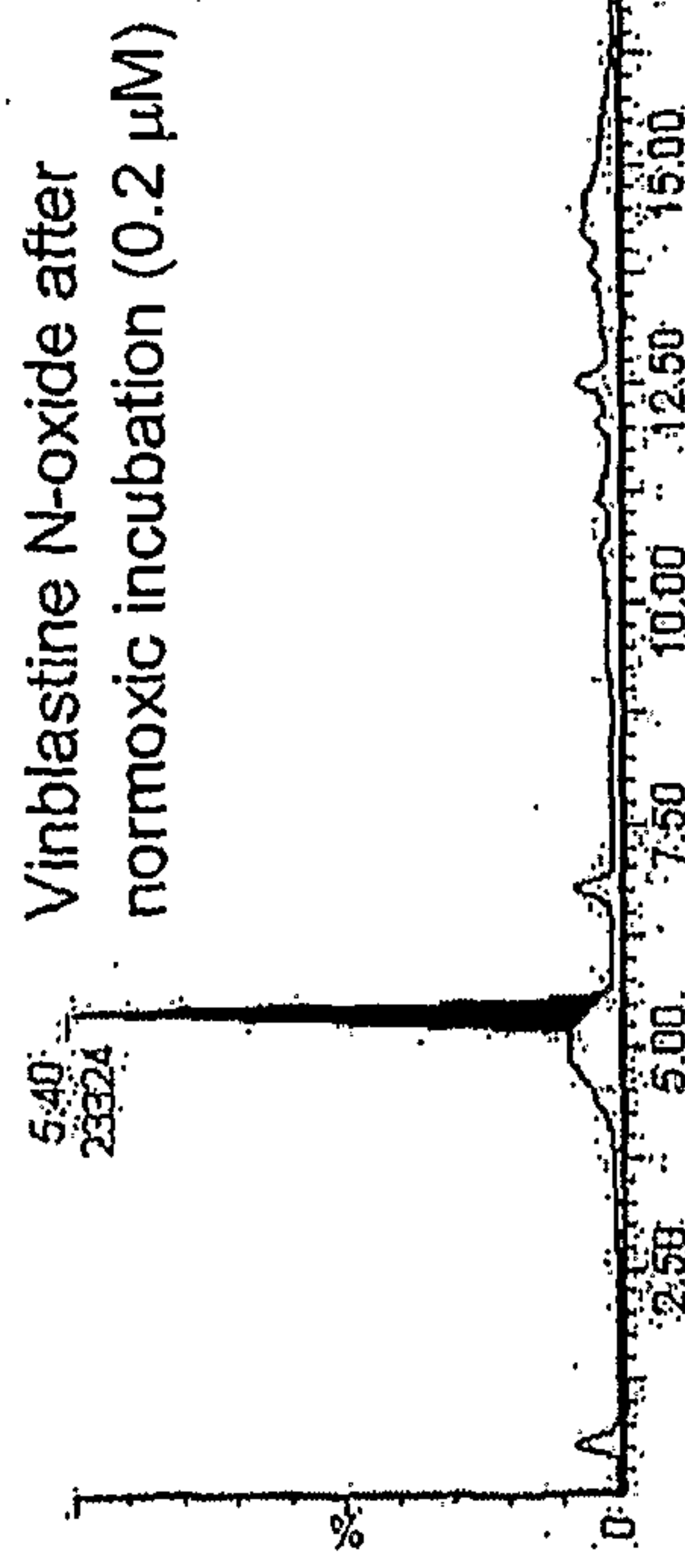


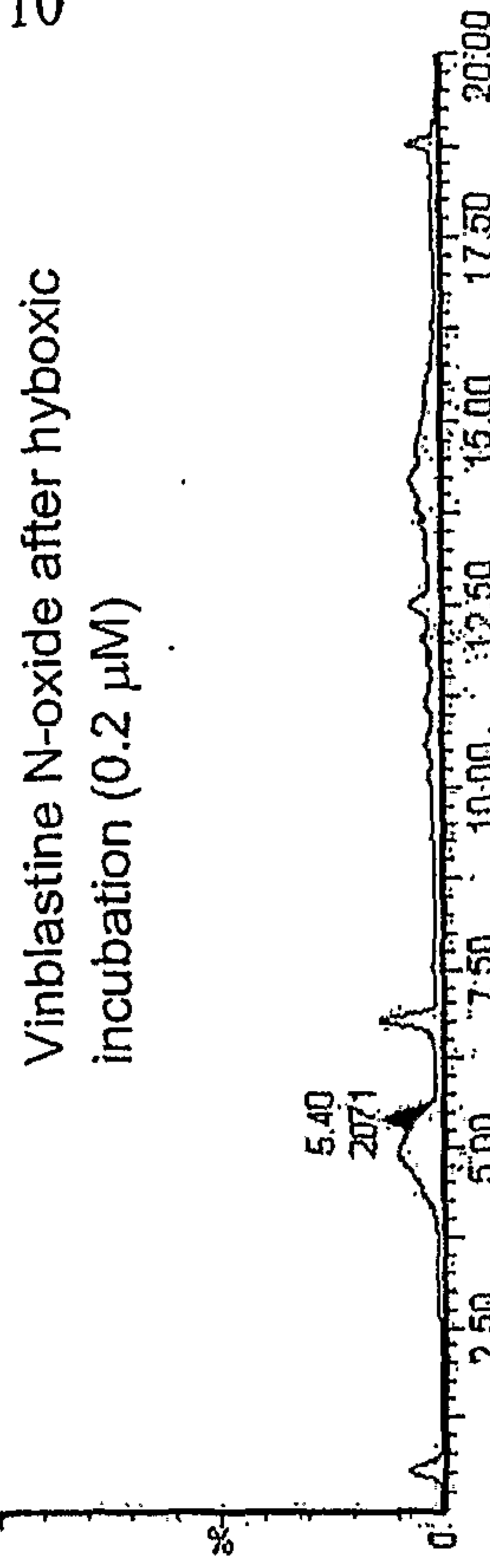
Figure 5

Normoxia → Hypoxia





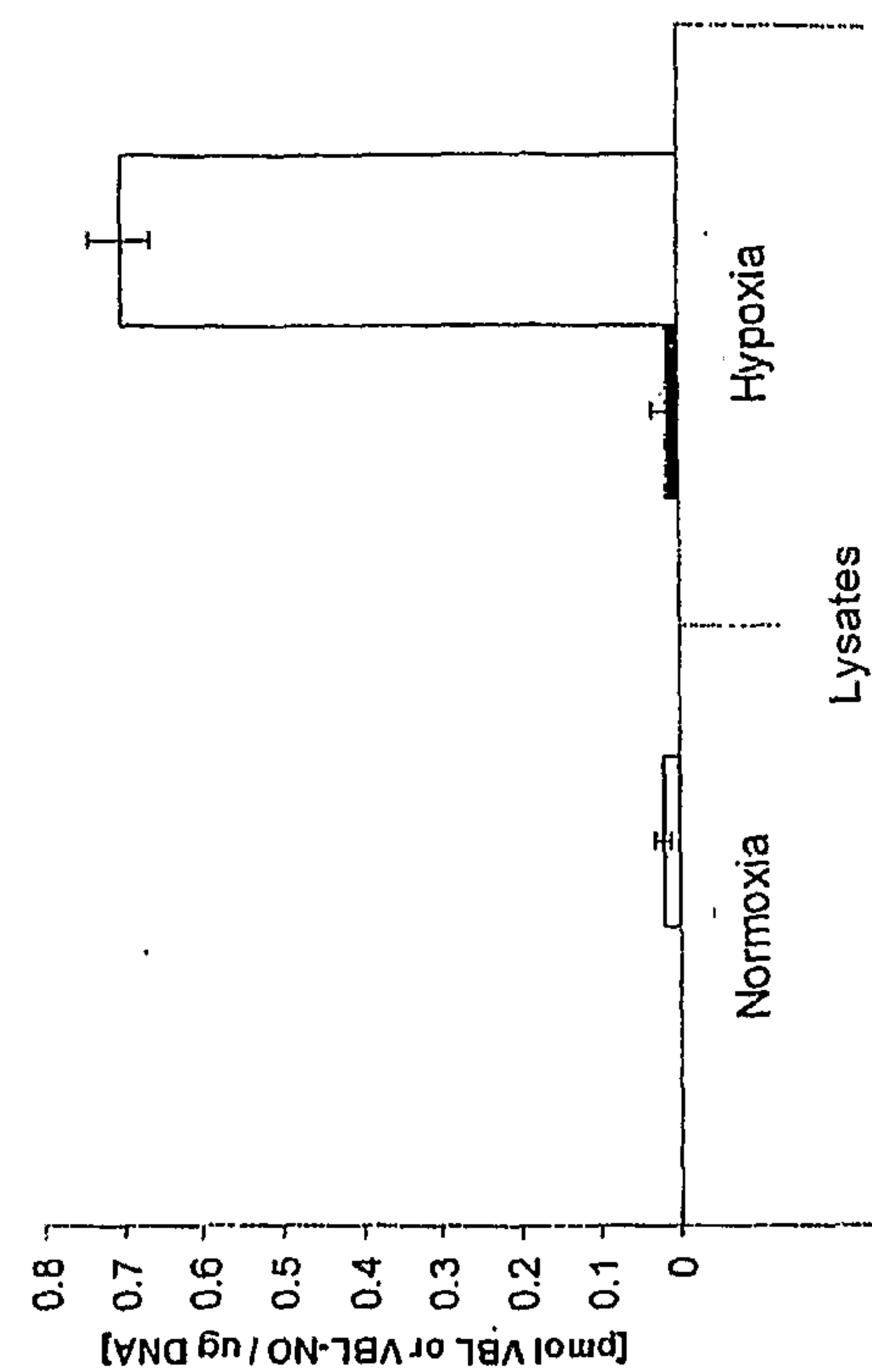




Figures 6A-6D

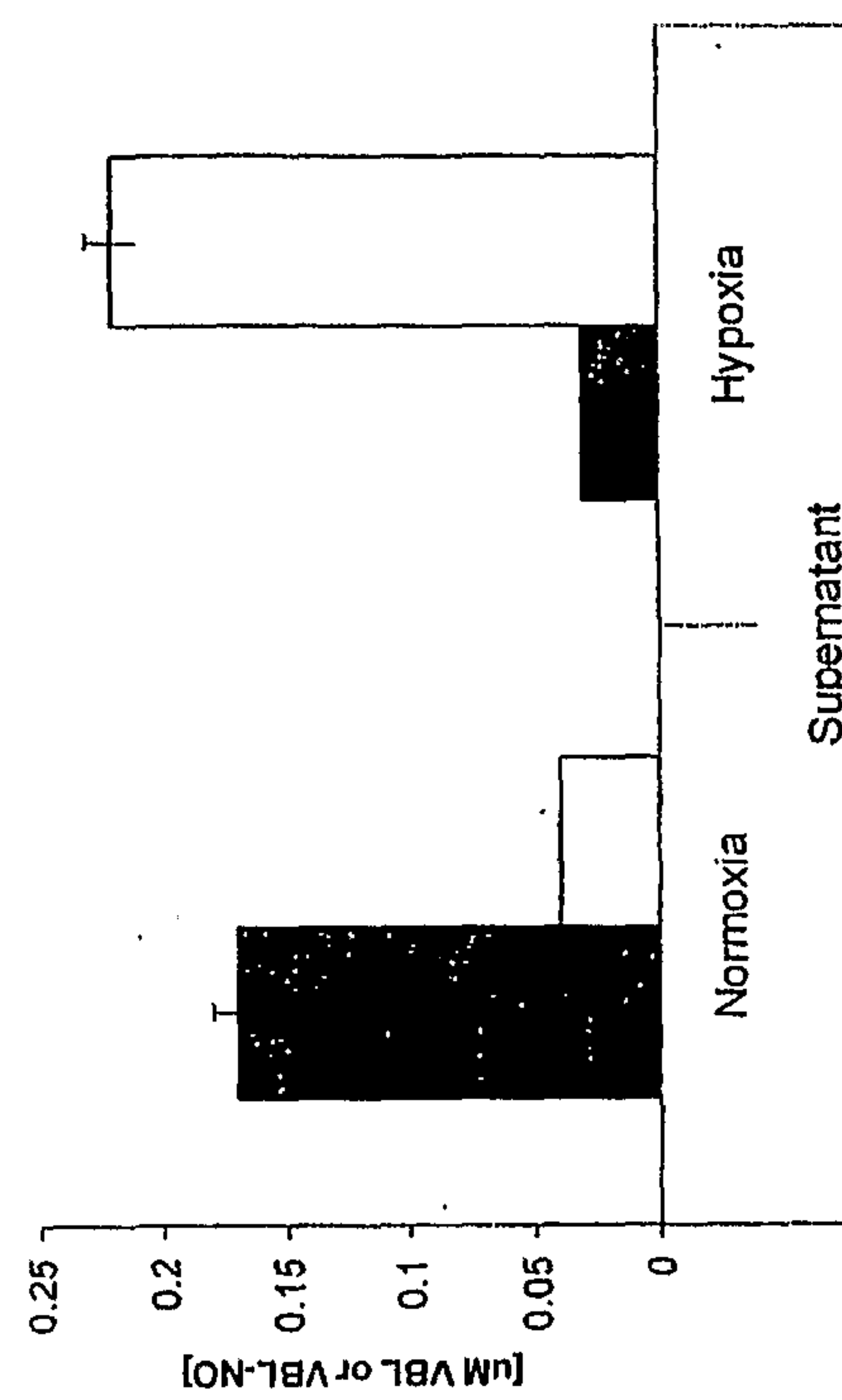
7A

Lysates



7B

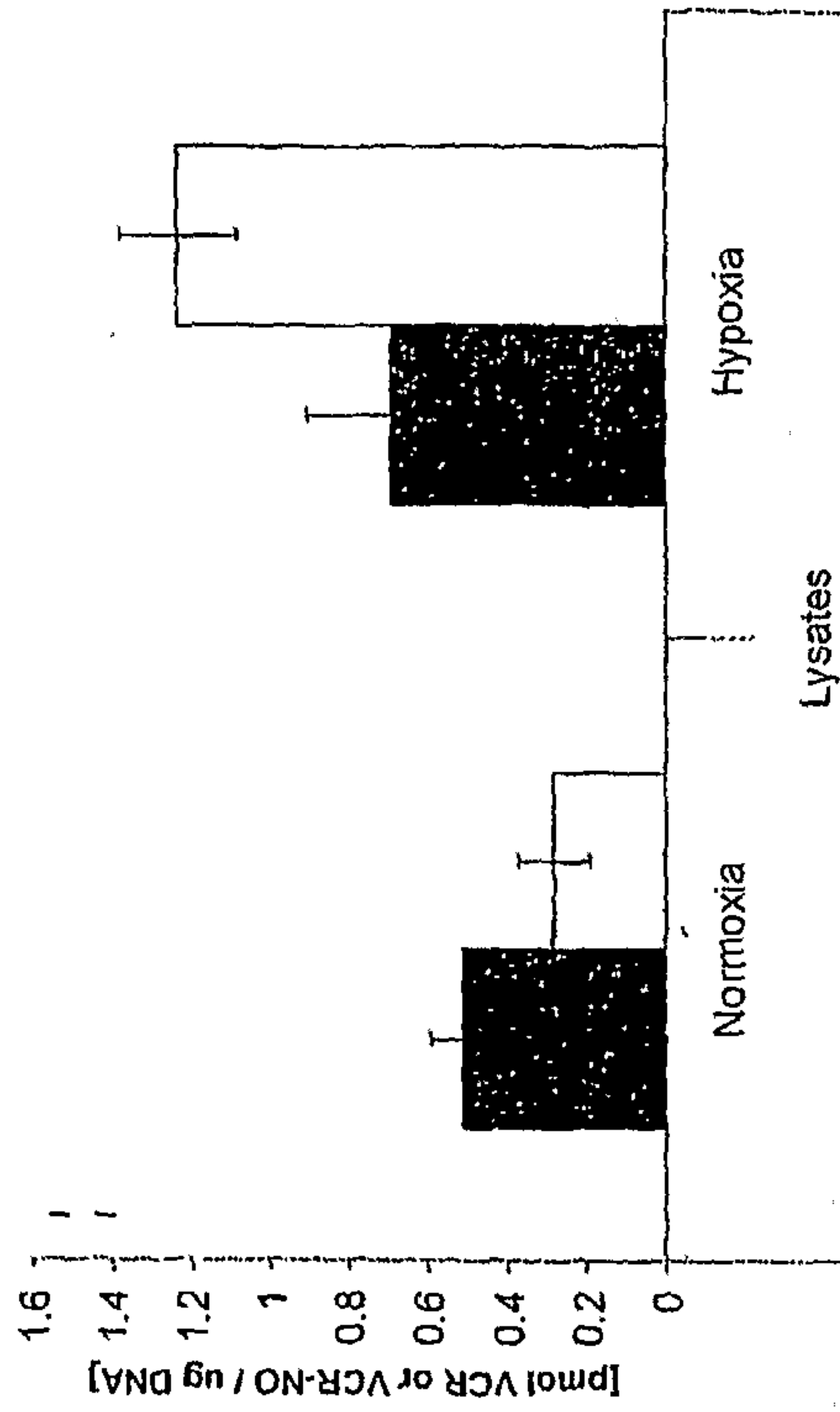
Extracellular medium



Figures 7A and 7B

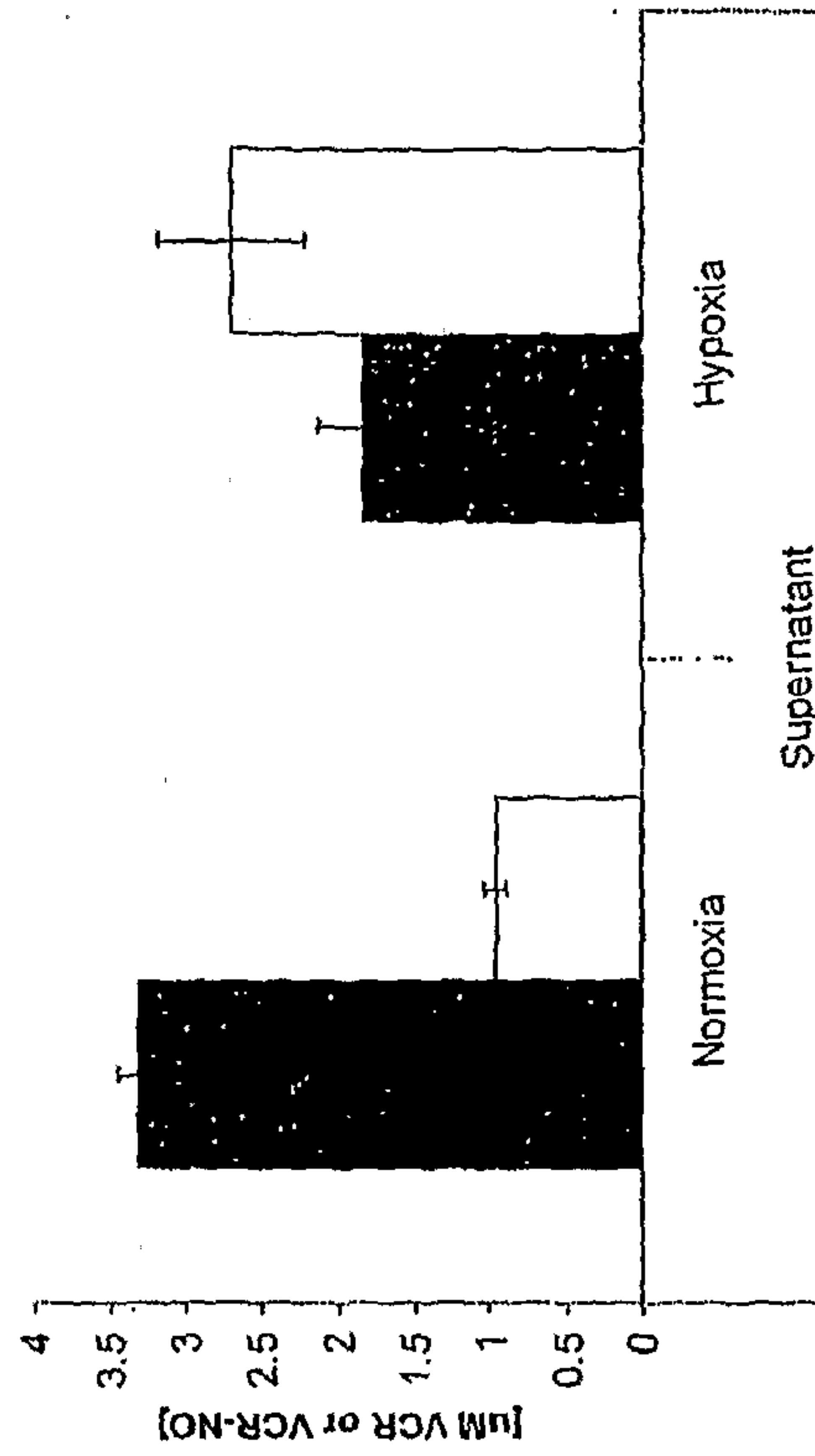
8A

Lysates



8B

Extracellular medium



Figures 8A and 8B

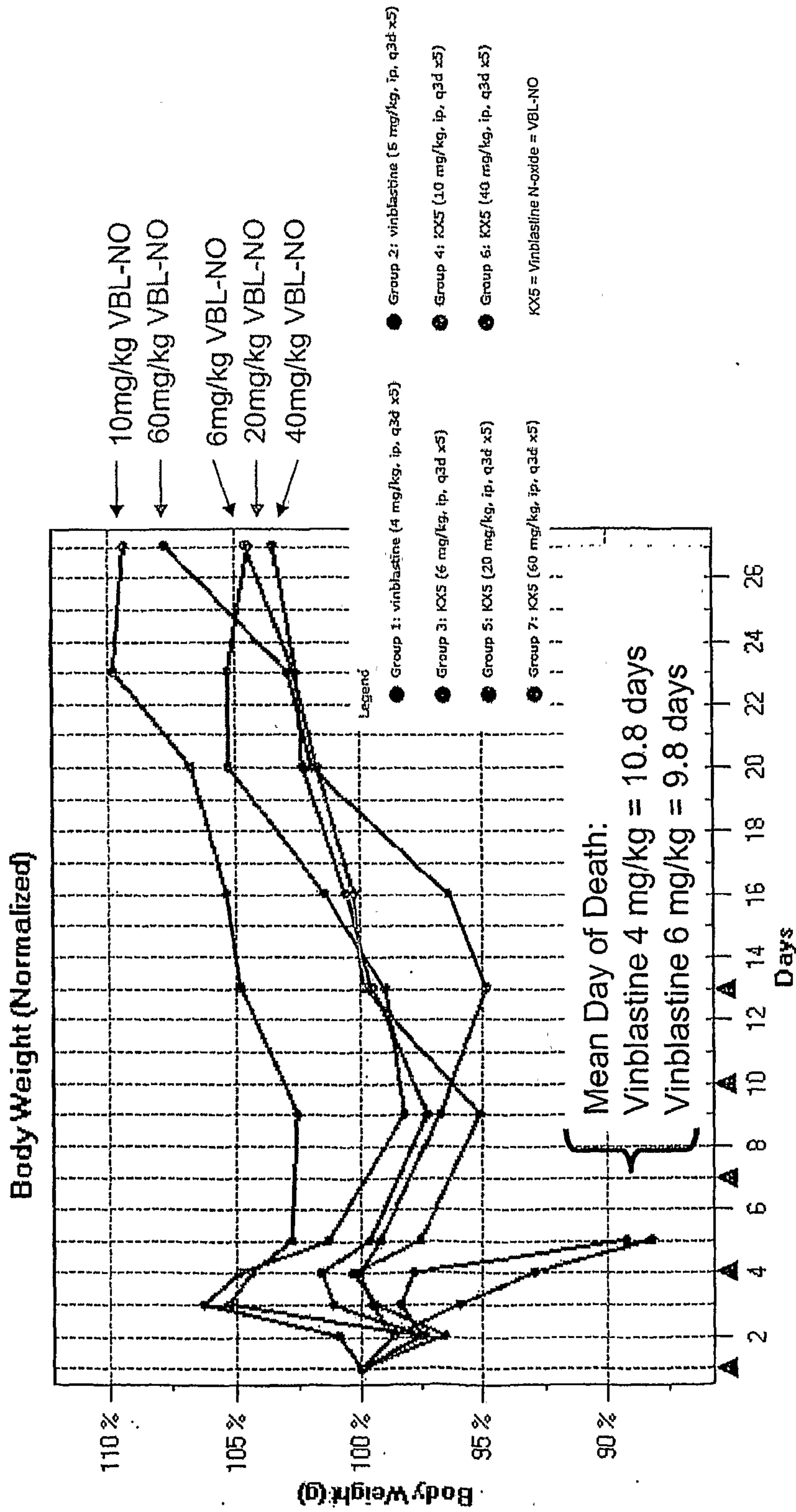


Figure 9

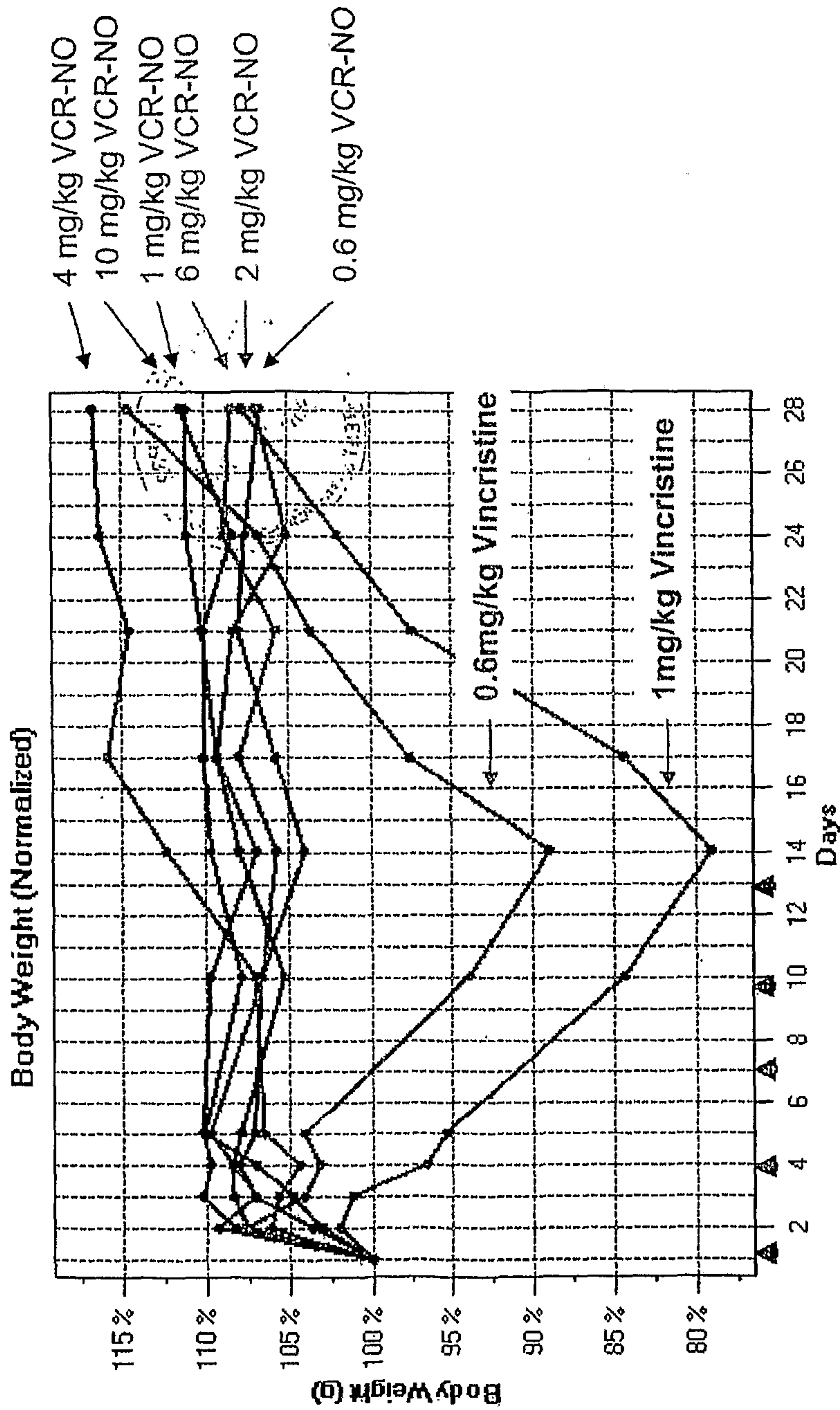
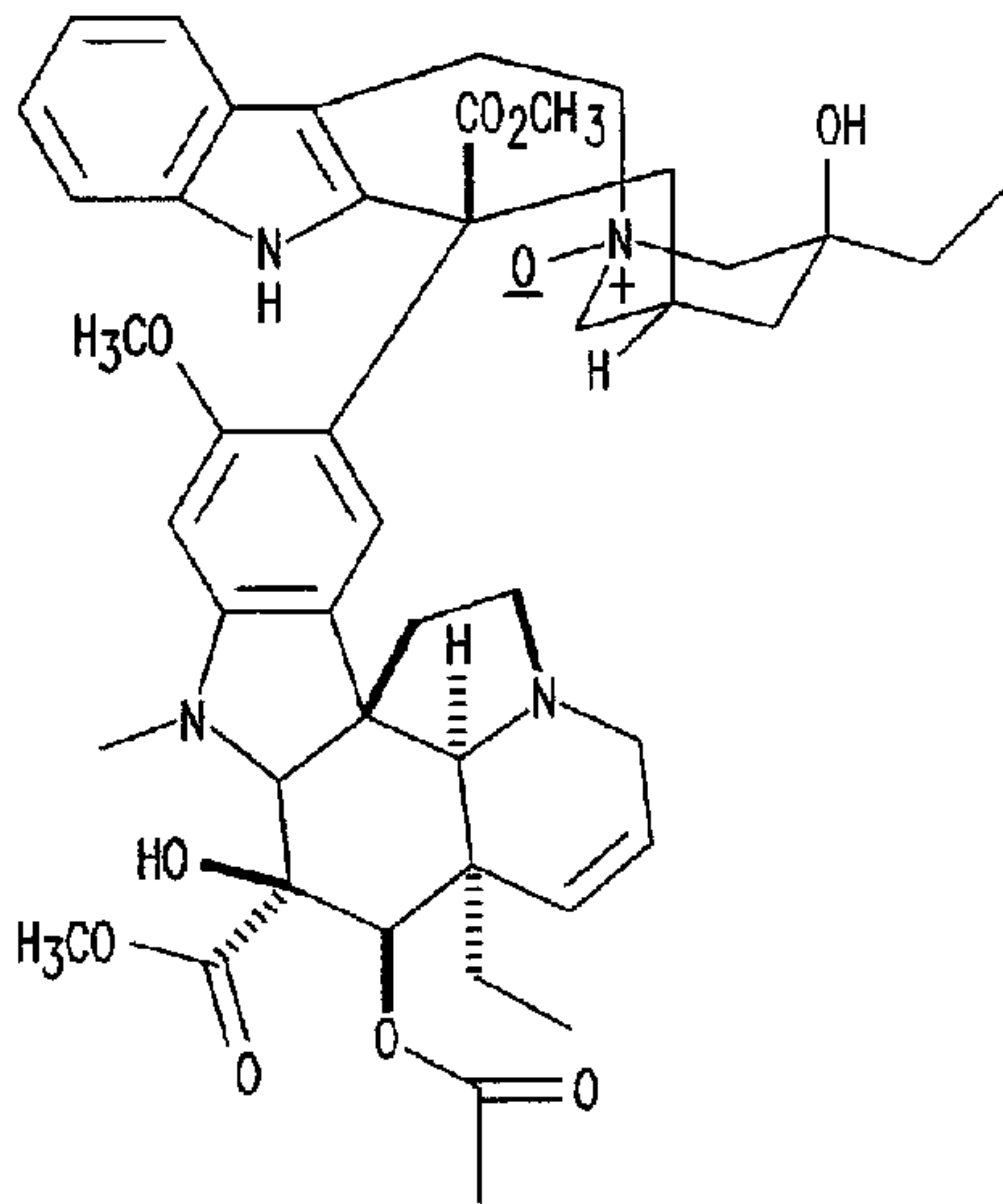


Figure 10


CO2CH3
OH
N
H
O
N
+
H
H3CO
H
N
N
HO
H3CO
O
O
O
0(22')
0(1)
N(6')
0(25')
0(24')
0(32)
N(16')
N(1)
N(9)
0(27)
0(24)
0(28)
0(25)
0(31)