Abstract:

Hypoxia activated prodrug compounds of bis-alkylating agents are useful in the treatment of cancer and other hyperproliferative diseases.
HYPOXIA ACTIVATED PRODRUGS OF BIS-ALKYLATING AGENTS

CROSS-REFERENCES TO RELATED APPLICATIONS
[0001] This application claims priority to U.S. Provisional Application No. 60/970,364 filed 6 September 2007 which is incorporated herein by reference.

FIELD OF THE INVENTION
[0002] The present invention provides compositions and methods for the treatment of cancer, and generally relates to the fields of medicinal chemistry, medicine, pharmacology, molecular biology, and biology.

BACKGROUND OF THE INVENTION
[0003] Hypoxia activated prodrugs of anticancer agents, or HAP compounds, are useful for tumor therapy. A HAP compound contains a bioreductive group, a linker, and an anticancer agent and is less cytotoxic than the corresponding anticancer agent under normoxic conditions or normoxia, such as those existing in a normal cell. Under hypoxia, however, the bioreductive group present in the HAP compound is reduced, and the cytotoxic anticancer agent is generated and/or released. In hypoxic regions, such as those existing in solid tumors, a HAP compound generates and/or releases a cytotoxin and kills cancer cells selectively in and around the hypoxic tumor zone. HAP compounds are described for example in PCT Patent Application Publication Nos. WO 00/64864; 04/85361; 04/85421; 04/87075; 06/57946; and 07/002931.

[0004] Certain HAP compounds comprise an anticancer agent covalently bonded to a bioreductive group and are large molecules. Diffusion of a large HAP compound into hypoxic tumor zones is problematic due to, for example, poor vascularization of the hypoxic tumor zone. Smaller compounds wherein an N,N-bis-2-chloroethyl moiety is covalently bonded to a nitroimidazole moiety have been reported, but, none of these compounds have been approved for clinical use (see, Lee et al., 1998, Bioorg. Med. Chem. Lett., 8: 1741-44. There remains a need for additional HAP compounds for the treatment of cancer, including smaller HAP compounds that are more toxic to hypoxic cells than normoxic cells, and/or can diffuse into the hypoxic tumor zone. The present invention meets such needs.
BRIEF SUMMARY OF THE INVENTION

[0005] The present invention provides HAP compounds of bis-alkylating agents comprising a bioreductive group and two alkylating moieties that can alkylate DNA and/or other biologically relevant nucleophiles wherein each alkylating moiety is covalently bonded to the bioreductive group. In one aspect the present invention provides HAP compounds having a structure of formula HyP-(L-X)₂ wherein Hyp is a bioreductive group, L is a linker selected from a bond and a Ci-C₆ alkylene moiety, and each X is an alkylating group, for example, a leaving group. Leaving groups can be replaced by DNA and/or other biologically relevant nucleophiles leading to alkylation of the DNA and/or the biologically relevant nucleophile; generally, one of the leaving groups is replaced slower than the other leaving groups. In one embodiment, the bioreductive group is a 2-nitroimidazole moiety. In another embodiment, L is Ci-C₆ alkylene. In another embodiment, L is methylene.

[0006] In another embodiment, the present invention provides a compound having a structure of formula:

![Chemical Structure](image)

wherein R¹ is selected from the group consisting of hydrogen, Ci-C₆ alkyl, Ci-C₆ heteroalkyl, C3-C8 cycloalkyl, heterocyclyl, aryl, and heteroaryl; L is methylene; and X¹ and X² is an alkylating group. In another embodiment, R¹ is methyl.

[0007] In another embodiment, the present invention provides a compound having a structure of formula:
wherein $X^1$ is a leaving group and $X^2$ is a different leaving group. In another embodiment, $X^1$ is selected from a carbamate. In another embodiment, $X^i$ is $-\text{OCONR}^2\text{R}^3$ wherein $\text{R}^2$ and $\text{R}^3$ is selected from the group consisting of hydrogen, $\text{C}_1-\text{C}_6$ alkyl, $\text{C}_1-\text{C}_6$ heteroalkyl, $\text{C}_3-\text{C}_8$ cycloalkyl, heterocyclyl, aryl, and heteroaryl. In another embodiment, $X^2$ is chloro. In another embodiment, $\text{R}^2$ is selected from hydrogen and hydrogen. In another embodiment, $\text{R}^3$ is methyl.

[0008] In another embodiment, the present invention provides a compound having a structure of formula:

\[ \text{O}_2\text{N} \quad \text{N} \quad \text{R}^1 \quad \text{L} \quad X^1 \quad \text{O} \]

wherein $\text{R}^1$ is selected from the group consisting of hydrogen, $\text{C}_1-\text{C}_6$ alkyl, $\text{C}_1-\text{C}_6$ heteroalkyl, $\text{C}_3-\text{C}_8$ cycloalkyl, heterocyclyl, aryl, and heteroaryl; $\text{L}$ is methylene; and $X^1$ is selected from a leaving group and a pro-leaving group.

[0009] In another embodiment, the present invention provides a compound having the structure of formula:

\[ \text{O}_2\text{N} \quad \text{N} \quad \text{L} \quad X^3 \quad \text{N} \quad \text{L} \quad X^3 \]

wherein $\text{R}^1$ is selected from the group consisting of hydrogen, $\text{C}_1-\text{C}_6$ alkyl, $\text{C}_1-\text{C}_6$ heteroalkyl, $\text{C}_3-\text{C}_8$ cycloalkyl, heterocyclyl, aryl, and heteroaryl; $\text{L}$ is methylene; and $X^3$ is sulfonyl.
In another embodiment, the present invention provides the HAP compounds of the present invention in substantially pure forms.

In another aspect, the present invention provides methods of synthesizing HAP compounds of the present invention.

In another aspect, the present invention provides a pharmaceutically acceptable formulation comprising a HAP compound of the present invention and pharmaceutically acceptable carriers, diluents, and/or excipients.

In another aspect, the present invention provides a method of treating cancer and other hyperproliferative diseases comprising administering a therapeutically effective amount of a HAP compound of the present invention to a patient in need of such treatment.

DETAILED DESCRIPTION OF THE INVENTION

This detailed description of the different aspects and embodiments of the present invention is organized as follows: Section I provides useful definitions; Section II describes the HAP compounds of the present invention and methods of their synthesis; Section III describes therapies provided by the present invention; and Section IV provides illustrative examples for synthesizing HAP compounds of the present invention, and demonstrates in vitro efficacy of HAP compounds of the present invention. This detailed description is organized into sections only for the convenience of the reader, and disclosure found in any section is applicable to disclosure elsewhere in the specification.

I. Definitions

The following definitions are provided to assist the reader. Unless otherwise defined, all terms of art, notations, and other scientific or medical terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the chemical and medical arts. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not be
construed as representing a substantial difference over the definition of the term as generally understood in the art.

[0016] "∆∆∆" refers to a position on a moiety which is covalently bonded to the rest of the molecule via a single bond.

[0017] "Acyl" refers to a moiety having a structure of formula -CO-RX wherein RX is selected from the group consisting of hydrogen, Ci-C₆ alkyl, Ci-C₆ heteroalkyl, C₃-C₈ cycloalkyl, heterocyclyl, aryl, and heteroaryl.

[0018] "C₂-C₆ Alkenyl" refers to a linear monovalent hydrocarbon radical or a branched monovalent hydrocarbon radical having 1-6 carbon atoms and containing at least one double bond, but no more than three double bonds. C₂-C₆ alkenyl group includes, for example, ethenyl, propenyl, and 1,3-butadienyl.

[0019] "Ci-C₆ alkoxy" refers to a substituted or unsubstituted alkyl group of 1-6 carbon atoms covalently bonded to an oxygen atom. In other words, a Ci-C₆ alkoxy group has the general structure -O-(Ci-C₆)alkyl. Ci-C₆ alkoxy groups include, for example, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, sec-butoxy, tert-butoxy, n-pentoxy, 2-pentoxy, 3-pentoxy, isopentoxy, neopentoxy, hexoxy, 2-hexoxy, 3-hexoxy, and 3-methylpentoxy.

[0020] "Ci-C₆ alkoxy carbonyl" refers to an alkoxy group covalently bonded to a carbonyl. In other words, a Ci-C₆ alkoxy carbonyl group has the general structure -C(=O)-O-(Ci-C₆)alkyl.

[0021] "Ci-C₆ alkyl" refers to a substituted or unsubstituted straight or branched chain alkyl groups having 1-6 carbon atoms. Ci-C₆ alkyl groups include, for example, methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, pentyl, 2-pentyl, isopentyl, neopentyl, hexyl, 2-hexyl, 3-hexyl and 3-methylpentyl. A Ci-C₆ alkyl substituent may be covalently bonded to an atom within a molecule of interest via any chemically suitable portion of the Ci-C₆ alkyl group.

[0022] "Ci-C₆ alkyamino," refers to a substituted or unsubstituted alkyl group of 1-6 carbon atoms covalently bonded to an -NH- moiety. In other words, a Ci-C₆ alkyamino group has the general structure -NH-(Ci-C₆)alkyl. Similarly a di(Ci-C₆)alkyamino group has the general structure -N-(Ci-C₆)alkyl.
structure -N-[(Ci-C6)alkyl] 2. Ci-C6 alkylamino groups include, for example, methylamino, ethylamino, propylamino and butylamino.

[0023] "Ci-C6 alkylene" refers to a linear saturated divalent substituted or unsubstituted hydrocarbon radical or a branched saturated divalent hydrocarbon radical having 1 - 6 carbon atoms. Alkylene groups include, for example, methylene, ethylene, propylene, butylene, 2-methylpropylene, pentylene. A substituted alkylene can be substituted, among other groups, with Ci-C6 alkyl and aryl groups.

[0024] "C2-C6 alkyl ether" refers to a substituent with an oxygen atom and 2 - 6 carbon atoms positioned such that at least one carbon atom is located on either side of the oxygen atom.

[0025] "C2-C6 Alkynyl" refers to a linear monovalent hydrocarbon radical or a branched monovalent hydrocarbon radical having 1 - 6 carbon atoms and containing a triple bond, but no more than three double bonds. C2-C6 alkenyl group includes, for example, ethynyl, propynyl, and butynyl.

[0026] "Aryl" refers to a substituted or unsubstituted cyclic moiety that includes one or more monocyclic or fused ring aromatic systems. Such moieties include any moiety that has one or more monocyclic or bicyclic fused ring aromatic systems, including but not limited to phenyl and naphthyl.

"Carbamate" refers to a moiety having a structure of formula -O-CO-NR\textsuperscript{x}R\textsuperscript{y} wherein R\textsuperscript{x} is selected from the group consisting of hydrogen, Ci-C\textsubscript{6} alkyl, Ci-C\textsubscript{6} heteroalkyl, C\textsubscript{3}-Cs cycloalkyl, heterocyclyl, aryl, and heteroaryl. R\textsuperscript{y} is selected from the group consisting of Ci-C\textsubscript{6} alkyl, Ci-C\textsubscript{6} heteroalkyl, C\textsubscript{3}-Cs cycloalkyl, heterocyclyl, aryl, and heteroaryl. Examples of carbamates include, for example, -OCONHMe and -OCONMe\textsubscript{2}.

"Cycloalkyl" or "carbocycle" by themselves or in combination with other terms, refers to, unless otherwise stated, cyclic versions of "alkyl", "alkenyl" and "alkynyl" in which all ring atoms are carbon. "Cycloalkyl" or "carbocycle" refers to a mono- or polycyclic group. When used in connection with cycloalkyl substituents, the term "polycyclic" refers herein to fused and non-fused alkyl cyclic structures. "Cycloalkyl" or "carbocycle" may form a bridged ring or a spiro ring. The cycloalkyl group may have one or more double or triple bond(s). The term "cycloalkeny" refers to a cycloalkyl group that has at least one site of alkenyl unsaturation between the ring vertices. The term "cycloalkynyl" refers to a cycloalkyl group that has at least one site of alkynyl unsaturation between the ring vertices. When "cycloalkyl" is used in combination with "alkyl", as in C\textsubscript{3}-8cycloalkylC\textsubscript{3}-8alkylene-, the cycloalkyl portion is meant to have the stated number of carbon atoms (e.g., from three to eight carbon atoms), while the alkylene portion has from one to eight carbon atoms. Typical cycloalkyl substituents have from 3 to 8 ring atoms. Examples of cycloalkyl include cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like.

"Halogen" or "halo" refers to by themselves or as part of another substituent, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as "haloalkyl," are meant to include alkyl in which one or more hydrogen is substituted with halogen atoms which can be the same or different, in a number ranging from one up to the maximum number of halogens permitted e.g., for alkyl (2m'+l), where m' is the total number of carbon atoms in the alkyl group. For example, the term "haloCi\textsubscript{6}alkyl" is meant to include trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like. The term "perhaloalkyl" means, unless otherwise stated, alkyl substituted with (2m'+l) halogen atoms, where m' is the total number of carbon atoms in the alkyl group. For example, the term "perhaloCi\textsubscript{6}alkyl," is meant to include trifluoromethyl, pentachloroethyl, 1,1,1-trifluoro-2-bromo-2-chloroethyl, and the like. Additionally, the term "haloalkoxy" refers to an alkoxy
radical substituted with one or more halogen atoms. "Halide" refers to the acid or anionic form of a halo group.

[0031]  "Heteroalkyl" means an alkyl radical as defined herein with one, two or three substituents independently selected from cyano, -ORw, -NRxRy, and -S(O)nRz (where n is an integer from 0 to 2), with the understanding that the point of attachment of the heteroalkyl radical is through a carbon atom of the heteroalkyl radical. Rw is hydrogen, alkyl, cycloalkyl, cycloalkyl-alkyl, aryl, araalkyl, alkoxy carbonyl, aryloxycarbonyl, carboxamido, or mono- or di-alkylcarbamoyl. Rx is hydrogen, alkyl, cycloalkyl, cycloalkyl-alkyl, aryl or araalkyl. Ry is hydrogen, alkyl, cycloalkyl, cycloalkyl-alkyl, aryl, araalkyl, alkoxy carbonyl, aryloxycarbonyl, carboxamido, mono- or di-alkylcarbamoyl or alkylsulfonyl. Rz is hydrogen (provided that n is 0), alkyl, cycloalkyl, cycloalkyl-alkyl, aryl, araalkyl, amino, mono-alkylamino, di-alkylamino, or hydroxyalkyl. Representative examples include, for example, 2-hydroxyethyl, 2,3-dihydroxypropyl, 2-methoxyethyl, benzoxymethyl, 2-cyanoethyl, and 2-methylsulfonyl-ethyl. For each of the above, Rw, Rx, Ry, and Rz can be further substituted by amino, fluorine, alkylamino, di-alkylamino, OH or alkoxy. Additionally, the prefix indicating the number of carbon atoms (e.g., C1-C4) refers to the total number of carbon atoms in the portion of the heteroalkyl group exclusive of the cyano, -ORw, -NRxRy, or -S(O)nRz portions.

[0032]  "Heteroaryl" refers to a substituted or unsubstituted monocyclic aromatic system having 5 or 6 ring atoms, or a fused ring bicyclic aromatic system having 8 - 20 atoms, in which the ring atoms are C, O, S, SO, SO2, or N and at least one of the ring atoms is a heteroatom, i.e., O, S, SO, SO2, or N. Heteroaryl groups include, for example, acridinyl, azocinyl, benzimidazolyl, benzofuranyl, benzothio-furanyl, benzo thiophenyl, benzoxazolyl, benzothiazolyl, benzotriazolyl, benzotetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazoliny, carbazolyl, NH-carbazolyl, carbolinyl, chromanyl, chromenyl, cinnolinyl, dithiazinyl, furanyl, furazanyl, imidazolidinyl, imidazoliny, imidazolyl, indazolyl, indolenyl, indoliny, indoliziny, indoly, isobenzofuranyl, isochromany, isoindazolyl, isoindoliny, isoindolyl, isoquinoliny, isothiazolyl, isoxazolyl, naphthyridinyl, octahydroisoquinoliny, oxadiazolyl, oxazolinyl, oxazolyl, oxazolidinyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxathiinyl, pheno xazinyl, phthalazinyl, piperazinyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyrido oxazolyl,
pyridoimidazolyl, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolyl, quinazolinyl, quinoliny, quinoxalinyl, quinuclidinyl, tetrahydroisoquinolynyl, tetrahydroquinolynyl, tetrazolyl, thiadiazinyl, thia diazolyl, thianthrenyl, thiazolyl, thieryl, thienothiazolyl, thienooxazolyl, thienomidazolyl, thiophenyl, triazinyl and xanthenyl. Unless indicated otherwise, the arrangement of the heteroatoms within the ring may be any arrangement allowed by the bonding characteristics of the constituent ring atoms.

[0033] "Heterocyclyl" refers to a monocyclic or fused ring multicyclic cycloalkyl group at least a portion of which is not aromatic and in which one or more of the carbon atoms in the ring system is replaced by a heteroatom selected from O, S, SO, SO₂, P, or N. Examples of heterocyclyl groups include but are not limited to imidazoliny1, morpholinyl, piperidinyl, piperidin-2-only, piperezinyl, pyrrolidinyl, pyrrolidin-2-onyl, tetrahydrofurany1, and tetrahydroimidazo [4,5-c] pyridinyl.

[0034] "Ci-C₆ heteroalkylene" refers to a Ci-C₆ alkylene as defined above wherein 1 - 3 carbon atoms in the hydrocarbon radical or a branched saturated divalent hydrocarbon radical is replaced with a hetero atom. Ci-C₆ heteroalkylene groups include, for example, -CH₂CH₂-O-CH₂CH₂- and -CH₂CH₂-S-CH₂CH₂-.

[0035] "Leaving group" refers to a moiety or an atom that can be replaced by a nucleophile. Examples of leaving groups include but are not limited to halo and sulfonate.

[0036] "Michael acceptor" refers to a C₂-C₆ alkenyl or a C₂-C₆ alkynyl group covalently bonded to an electron withdrawing moiety.

[0037] "Substituted" refers to a group as defined herein in which one or more bonds to a carbon(s) or hydrogen(s) are replaced by a bond to non-hydrogen and non-carbon atom "substituents" such as, but not limited to, a halogen atom such as F, Cl, Br, and I; an oxygen atom in groups such as hydroxyl groups, alkoxy groups, aryloxy, and acyloxy groups; a sulfur atom in groups such as thiol groups, alkyl and aryl sulfide groups, sulfone groups, sulfonyl groups, and sulfoxide groups; a nitrogen atom in groups such as amino, alkylamines, dialkylamines, arylamines, alkylarylamines, diarylamines, alkoxyamino, hydroxyamino, acylamino, sulfonylamino, N-oxides, imides, and enamines; and other heteroatoms in various other groups. "Substituents" also include groups in which one or more bonds to a carbon(s) or
hydrogen(s) atom is replaced by a higher-order bond (e.g., a double- or triple-bond) to a heteroatom such as oxygen in oxo, acyl, amido, alkoxy carbonyl, aminocarbonyl, carboxyl, and ester groups; nitrogen in groups such as imines, oximes, hydrazones, and nitriles.

[0038] "Substituents" further include groups in which one or more bonds to a carbon(s) or hydrogen(s) atoms is replaced by a bond to a cycloalkyl, heterocyclyl, aryl, and heteroaryl groups. Representative "substituents" include, among others, groups in which one or more bonds to a carbon or hydrogen atom is/are replaced by one or more bonds to fluoro, chloro, or bromo group. Another representative "substituent" is the trifluoromethyl group and other groups that contain the trifluoromethyl group. Other representative "substituents" include those in which one or more bonds to a carbon or hydrogen atom is replaced by a bond to an oxygen atom such that the substituted alkyl group contains a hydroxy, alkoxy, or aryloxy group. Other representative "substituents" include alkyl groups that have an amine, or a substituted or unsubstituted alkylamine, dialkylamine, arylamine, (alkyl)(aryl)amine, diarylamine, heterocyclylamine, diheterocyclylamine, (alkyl)(heterocyclyl)amine, or (aryl)(heterocyclyl)amine group. Still other representative "substituents" include those in which one or more bonds to a carbon(s) or hydrogen(s) atoms is replaced by a bond to an alkyl, cycloalkyl, aryl, heteroaryl, or heterocyclyl group.

[0039] The herein-defined groups may include prefixes and/or suffixes that are commonly used in the art to create additional well-recognized substituent groups. As examples,

"alkylamino" refers to a group of the formula \(-\text{NR}^a\text{R}^b\). Unless stated otherwise, for the following groups containing \(\text{R}^a\), \(\text{R}^b\), \(\text{R}^c\), \(\text{R}^d\) and \(\text{R}^e\): \(\text{R}^a\) and \(\text{R}^b\) are each independently selected from H, alkyl, alkoxy, thioalkoxy, cycloalkyl, aryl, heteroaryl, or heterocyclyl or are optionally joined together with the atom(s) to which they are attached to form a cyclic group. When \(\text{R}^a\) and \(\text{R}^b\) are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6- or 7-membered ring. For example, \(-\text{NR}^a\text{R}^b\) is meant to include 1-pyrrolidinyl and 4-morpholinyl. \(\text{R}^c\), \(\text{R}^d\), \(\text{R}^e\) and \(\text{B}^f\), unless otherwise indicated, are each independently selected from alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, heteroaryl, heterocyclyl or alkylenearyl, as defined herein.
Typically, a particular radical will have 0, 1, 2 or 3 substituents, with those groups having two or fewer substituents being preferred in the present invention. More preferably, a radical will be unsubstituted or monosubstituted. Most preferably, a radical will be unsubstituted.

In some embodiments, "substituents" refers to an atom or group, including, for example, amino, Cj.Cgalkylamino or di(Cj .Chalky lamino, Cj.Cgalkoxy, C^Cgalkylthio, aryl, -COOH, -CONH2, cyano, ethenyl, ethynyl, halo, heteroaryl, hydroxy, mono- or di(C\C
6)alkylcarboxamido, mono or di(Ci_C6)alkylsulfonamido, nitro, -OS\O\2_Ry, and -SO2NH2.

"Substituents" for the alkyl and heteroalkyl radicals (as well as those groups referred to as alkyne, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycl) can be a variety of groups selected from: - OR^a, =0, =NR^a, =N-OR^b, -SR^a, halogen, -SiR^aR^bR^c, -OC(O)R^a, -C(O)R^a, -CONR^aR^b, -OC(O)NR^aR^b, -NR^bC(O)R^a,
-NR^a-C(O)NR^bR^c, -NR^a= SO2NR^bR^c, -NR^bCO2R^a, -NH-C(NH2)=NH, -NR^a C(NH2)=NH,
-NH-C(NH2)=NR^a, -S(O) R^a, -SO2R^a, -SO2NR^aR^b, -NR^bSO2R, -CN and -NO2, in a number ranging from zero to three, with those groups having zero, one or two substituents being particularly preferred.

In some embodiments, "substituents" for the alkyl and heteroalkyl radicals are selected from: -OR^a, =0, -NR^aR^b, -SR^a, halogen, -SiR^aR^bR^c, -OC(O)R^a, -C(O)R^a, -CO2R^a,
-CONR^aR^b, -OC(O)NR^aR^b, -NR^bC(O)R^a, -NR^bCO2R^a, -NR^a-SO2NR^bR^c, -S(O)R^a, -SO2R^a,
-SO2NR^aR^b, -NR^cSO2R, -CN and -NO2, where R^a and R^b are as defined above. In some embodiments, substituents are selected from: -OR^a, =0, -NR^aR^b, halogen, -OC(O)R^a, -CO2R^a,
-CONR^aR^b, -OC(O)NR^aR^b, -NR^bC(O)R^a, -NR^bCO2R^a, -NR^a-SO2NR^bR^c, -SO2R^a,
-SO2NR^aR^b, -NR"SO2R, -CN and -NO2.

Examples of substituted alkyl are: -(CH2)3NH2, -(CH2)3NH(CH3),
-(CH2)3NH(CH3)2, -CH2C(=CH2)CH2NH2, -CH2C(=O)CH2NH2, -CH2S(=O)2CH3,
-CH2OCH2NH2, -CO2H. Examples of substituents of substituted alkyl are: CH2OH, - OH,
-OCH₃, -OC₂H₅, -OCF₃, -OC(O)CH₃, -OC(O)NH₂, -OC(O)N(CH₃)₂, -CN, -NO₂,
-C(O)CH₃, -CO₂H, -CO₂CH₃, -CONH₂, -NH₂, -N(CH₃)₂, -NHSO₂CH₃, -NHCOCCH₃,
-NHC(O)CH₃, -NHSO₂CH₃, -SO₂CH₃, -SO₂NH₂, and halo.

[0045] Similarly, "substituents" for the aryl and heteroaryl groups are varied and are selected
from: -halogen, -OR a, -OC(O) R a, -NR a R b, -SR a, -R a, -CN, -N(O2), -C(O) R a, -C02R a, -C0NR a R b,
-C(O) R a, -OC(O)NR a R b, -NR b C(O) R a, -NR b C(O) 2R a, -NR a-C(O)NR b R c, -NH-C(NH₂)
=NH, -NR a C(NH₂)=NH, -NH-C(NH₂)=NR a, -S(O) R a, -S(O) 2 R a, -S(O) 2 NR b, -N³,
-CH(Ph)₂, perfluoroC j.galkoxy, and perfluoroCl-βalkyl, in a number ranging from zero to the
total number of open valences on the aromatic ring system; and where R a, R b and R c are
independently selected from hydrogen, C j.galkyl and heteroalkyl, unsubstituted aryl and
heteroaryl, (unsubstituted aryl)-C j.galkyl, and (unsubstituted aryl)oxy-C i.galkyl.

[0046] Two of the "substituents" on adjacent atoms of the aryl or heteroaryl ring may
optionally be replaced with a substituent of the formula -T-C(O)-(CH₂)q-U-, wherein T and U
are independently -NH-, -O-, -CH₂- or a single bond, and q is o, 1 or 2. Alternatively, two of the
substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a
substituent of the formula -A-(CH₂)r-B-, wherein A and B are independently -CH₂-, -O-, -NH-, -S-, -S(O)-, -S(O) 2-, -S(O) 2NR a- or a single bond, and r is 1, 2 or 3. One of the single bonds of
the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the
substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a
substituent of the formula -(CH₂)s-X-(CH₂)t-, where s and t are independently integers of from
Oₚ to 3, and X is -O-, -NR a-, -S-, -S(O)-, -S(O) 2-, or -S(O) 2NR a-. The substituent R a in -NR a-
and -S(O) 2NR a- is selected from hydrogen or unsubstituted Cl-galkyl. Otherwise, R is as
defined above.

[0047] Unless indicated otherwise, the nomenclature of substituents that are not explicitly
defined herein are arrived at by naming the terminal portion of the functionality followed by the
adjacent functionality toward the point of attachment. For example, the substituent
"arylalkyloxy Carbonyl" refers to the group (aryl)-(alkyl)-O-C(O)-.
"Sulfonyloxy" refers to a moiety having a structure of formula \(-\text{OSO}_2\) wherein \(R\) is selected from the group consisting of \(\text{Ci-C}_6\) alkyl, \(\text{Ci-C}_6\) heteroalkyl, \(\text{C}_3\text{C}_8\) cycloalkyl, heterocyclyl, aryl, and heteroaryl.

"Sulfonyl" refers to a moiety having a structure of formula \(-\text{SO}_2\) wherein \(R\) is selected from the group consisting of \(\text{Ci-C}_6\) alkyl, \(\text{Ci-C}_6\) heteroalkyl, \(\text{C}_3\text{C}_8\) cycloalkyl, heterocyclyl, aryl, and heteroaryl.

"Administering" or "administration of a drug to a patient" (and grammatical equivalents of these phrases) refers to direct administration, which may be administration to a patient by a medical professional or may be self-administration, and/or indirect administration, which may be the act of prescribing a drug. For example, a physician who instructs a patient to self-administer a drug and/or provides a patient with a prescription for a drug is administering the drug to the patient.

"Anoxia" and anoxic condition refers to a zero and immeasurably low oxygen concentration.

"Hypoxia" and hypoxic condition refers to oxygen concentration lower than that observed in air including oxygen concentration lower than that observed in oxygenated tissue and anoxia.

"Normoxia" and normoxic condition refers to oxygen concentration observed in air and for example in a liquid media equilibrated with air.

"Pharmaceutically acceptable carrier, excipient, or diluent" refers to a carrier, excipient, or diluent that is useful in preparing a pharmaceutical composition that is generally safe, nontoxic and neither biologically nor otherwise undesirable, and includes a carrier, excipient, or diluent that is acceptable for veterinary use as well as human pharmaceutical use. A "pharmaceutically acceptable carrier, excipient, or diluent" includes both one and more than one such carrier, excipient, or diluent.

"Pharmaceutically acceptable salts" refers to salts of the active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present invention contain
relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of salts derived from pharmaceutically-acceptable inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic, manganous, potassium, sodium, zinc and the like. Salts derived from pharmaceutically-acceptable organic bases include salts of primary, secondary and tertiary amines, including substituted amines, cyclic amines, naturally-occurring amines and the like, such as arginine, betaine, caffeine, choline, N,N'-dibenzylethlenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine and the like. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, malonic, benzoic, succinic, suberic, fumaric, mandelic, phthalic, benzenesulfonic, /?-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, e.g., Berge, S.M. et al., "Pharmaceutical Salts," Journal of Pharmaceutical Science, 66:1-19, 1977). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

[0056] The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.
"Prodrug" refers to a compound that, after administration, is metabolized or otherwise converted to an active or more active form with respect to at least one property. A prodrug, relative to the corresponding drug is modified chemically in a manner that renders it, relative to the drug, less active or inactive, but the chemical modification is such that the corresponding drug is generated by metabolic or other biological processes after the prodrug is administered. A prodrug may have, relative to the corresponding active drug, altered metabolic stability or transport characteristics, fewer side effects or lower toxicity, or improved flavor (for example see the reference Nogrady, 1985, Medicinal Chemistry A Biochemical Approach, Oxford University Press, New York, pages 388-392, incorporated herein by reference).

"Reduction" of a symptom or symptoms (and grammatical equivalents of this phrase) refers to decreasing the severity or frequency of the symptom(s), or elimination of the symptom(s).

"Therapeutically effective amount" of a drug refers to an amount of a drug that, when administered to a patient with cancer or another hyperproliferative disease, will have the intended therapeutic effect, e.g., alleviation, amelioration, palliation or elimination of one or more manifestations of cancer or another hyperproliferative disease in the patient. The full therapeutic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a therapeutically effective amount may be administered in one or more administrations.

"Treating" or "treatment of" a condition or patient refers to taking steps to obtain beneficial or desired results, including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation or amelioration of one or more symptoms of cancer or another hyperproliferative disease; diminishment of extent of disease; delay or slowing of disease progression; amelioration, palliation, or stabilization of the disease state, or other beneficial results.

II. Compounds and Synthetic Methods

The present invention provides HAP compounds of bis-alkylating agents comprising a bioreductive group and two alkylating moieties that can alkylate DNA and/or other biologically relevant nucleophiles wherein each of the alkylating moieties is covalently bonded to the
bioreductive group. Suitable alkylating moieties include, but are not limited to Michael acceptors, and C\textsubscript{i} alkyl groups substituted with a leaving group or a pro leaving group. In one aspect the present invention provides HAP compounds having a structure of formula HyP-(L-X)\textsubscript{2} wherein HyP is a bioreductive group, L is a linker selected from a bond and a C\textsubscript{i}-C\textsubscript{6} alkylene moiety, and each X is an alkylating group. In one embodiment, the bioreductive group is a 2-nitroimidazole moiety. In another embodiment, L is C\textsubscript{i}-C\textsubscript{6} alkylene. In another embodiment, L is methylene. In another embodiment, L is a monoalkyl substituted C\textsubscript{i} alkylene moiety. In one embodiment, the alkylating group is selected from the group consisting of -OCONR\textsubscript{2}R\textsubscript{3}, halogen, -OH, H, -C\textsubscript{2}-C\textsubscript{6}alkenyl, -C\textsubscript{2}-C\textsubscript{6}alkynyl, -CHO, sulfonyloxy, -(+)NR\textsubscript{2}, -NR\textsubscript{2}haloC\textsubscript{i}-C\textsubscript{6}alkyl, -CH=NSulfonyloxy, -N=N, and R\textsubscript{2} and R\textsubscript{3} is independently selected from the group consisting of hydrogen, C\textsubscript{i}-C\textsubscript{6}alkyl, C\textsubscript{i}-C\textsubscript{6}heteroalkyl, C\textsubscript{s}-C\textsubscript{cycloalkyl}, heterocyclyl, aryl, heteroaryl, - C\textsubscript{i}-C\textsubscript{6}alkylheterocyclyl, or combined to form heterocyclyl. In another group of embodiments, X is independently selected from the group consisting of -OCONMe\textsubscript{2}, -Cl, -OCONHMe, -OH, H, CH\textsubscript{2}Cl, CH=CH\textsubscript{2}, -CHO, -OTs, -C(Me)=CH\textsubscript{2} and - C\equiv CH, -Cl.

In one embodiment, X is a leaving group.
In another embodiment, the present invention provides a compound having a structure of formula:

wherein \(R^1\) is selected from the group consisting of hydrogen, \(\text{Ci-C}_6\) alkyl, \(\text{Ci-C}_6\) heteroalkyl, \(\text{C}_3\text{-C}_8\) cycloalkyl, heterocyclyl, aryl, and heteroaryl; \(L\) is methylene; and \(X^1\) and \(X^2\) is an alkylating group. In another embodiment, \(R^1\) is methyl. In another embodiment \(X^1\) and \(X^2\) is a leaving group.

In another embodiment, the present invention provides a compound having a structure of formula:

wherein \(X^1\) is a leaving group and \(X^2\) is a different leaving group. In another embodiment, \(X^1\) is a carbamate. In another embodiment, \(X^1\) is \(-\text{OCONR}^2\text{R}^3\) wherein \(R^2\) and \(R^3\) is selected from the group consisting of hydrogen, \(\text{Ci-C}_6\) alkyl, \(\text{Ci-C}_6\) heteroalkyl, \(\text{C}_3\text{-Cs}\) cycloalkyl, heterocyclyl, aryl, and heteroaryl. In another embodiment, \(X^2\) is chloro. In another embodiment, \(R^2\) is selected from the group consisting of hydrogen and methyl. In another embodiment, \(R^3\) is methyl.

In another embodiment, the present invention provides a compound having a structure of formula:
wherein $R^1$ is selected from the group consisting of hydrogen, $C_6$ alkyl, $C_6$ heteroalkyl, $C_3$-$C_8$ cycloalkyl, heterocyclyl, aryl, and heteroaryl; $L$ is methylene; and $X^1$ is a leaving group.

[0066] In another embodiment, the present invention provides a compound having a structure of formula:

![Structural formula](image)

wherein $R^1$ is selected from the group consisting of hydrogen, $C_6$ alkyl, $C_6$ heteroalkyl, $C_3$-$C_8$ cycloalkyl, heterocyclyl, aryl, and heteroaryl; $L$ is methylene; and $X^3$ is sulfonyl.

[0067] In other embodiments, the present invention provides HAP compounds having structures of formulas selected from the group consisting of:

![Additional structural formulas](image)
wherein $R^4$ is selected from the group consisting of $\text{C}_i-\text{C}_6$ alkyl and $\text{C}_i-\text{C}_h$ heteroalkyl and $R^5$ is selected from the group consisting of $\text{C}_i-\text{C}_6$ alkyl, $\text{C}_i-\text{C}_h$ heteroalkyl, $\text{C}_3-\text{C}_8$ cycloalkyl, heterocyclyl, aryl, heteroaryl, and acyl.

[0068] Without being bound by a particular mechanism, the bis-alkylating HAP compounds of the present invention are inactive or less active with respect to alkylation cellular DNA and/or protein under normoxic conditions compared to hypoxic conditions. The leaving groups of certain HAP compounds of the present invention are not replaced under normoxic conditions by DNA and/or other biologically relevant nucleophiles. In the hypoxic tumor zone, as the HAP compounds of the invention are reduced to a hydroxylamino compound, the flow of electron pair from the hydroxylamino group enhances the alkylation ability of the alkylation moiety covalently bonded to the 5-position of the imidazole ring and alkylates cellular DNA and/or another biologically relevant nucleophile. Once the imidazole moiety of the bis-alkylating agent is attached to the cellular DNA and/or the biologically relevant nucleophile, the alkylation moiety covalently bonded to the 4-position of the imidazole can alkylate the DNA and/or the biologically relevant nucleophile.

[0069] Following reductive activation in a hypoxic tumor as described above, a HAP compound having a structure of formula:

\[
\begin{align*}
\text{O}_2\text{N} & \quad \text{R}^1 \\
\text{N} & \quad \text{L} \\
\text{N} & \quad \text{L} \\
\text{N} & \quad \text{X}^3 \\
\text{N} & \quad \text{X}^3
\end{align*}
\]

can generate a carbocation adjacent to the 4-position of the imidazole moiety:
and bis-alkylate DNA and/or other biologically relevant nucleophiles.

[0070] Examples of compounds of the present invention include, but are not limited to, the following compounds:

<table>
<thead>
<tr>
<th>Cmpd No.</th>
<th>R¹</th>
<th>S₁</th>
<th>S₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Me</td>
<td>-CH₂OCONMe₂</td>
<td>-CH₂Cl</td>
</tr>
<tr>
<td>3</td>
<td>Me</td>
<td>-CH₂OCONMe₂</td>
<td>-CH=CH₂</td>
</tr>
<tr>
<td>4</td>
<td>Me</td>
<td>-CH₂OCONMe₂</td>
<td>-CH₂OCONMe₂</td>
</tr>
<tr>
<td>5</td>
<td>Me</td>
<td>-CH₂OCONMe₂</td>
<td>-CH₂OCONMe₂</td>
</tr>
<tr>
<td>6</td>
<td>Me</td>
<td>-CH₂Cl</td>
<td>-CH=CH₂</td>
</tr>
<tr>
<td>Cmpd No.</td>
<td>$R^1$</td>
<td>$S_1$</td>
<td>$S_2$</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
<td>--------------------</td>
<td>-------</td>
</tr>
<tr>
<td>7</td>
<td>Me</td>
<td>-CH$_2$OCONMe$_2$</td>
<td>-CHO</td>
</tr>
<tr>
<td>8</td>
<td>Me</td>
<td>-CH$_2$OCONMe$_2$</td>
<td>-CH$_2$OTs</td>
</tr>
<tr>
<td>9</td>
<td>Me</td>
<td>-CH$_2$OCONHMe</td>
<td>-CH=CH$_2$</td>
</tr>
<tr>
<td>10</td>
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</tr>
<tr>
<td>11</td>
<td>Me</td>
<td>-CH$_2$OCONMe$_2$</td>
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<tr>
<td>12</td>
<td>Me</td>
<td>-CH$_2$OCONMe$_2$</td>
<td><img src="image2.png" alt="Structure" /></td>
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<tr>
<td>13</td>
<td>Me</td>
<td>-CH$_2$OCONHMe</td>
<td><img src="image3.png" alt="Structure" /></td>
</tr>
<tr>
<td>14</td>
<td>Me</td>
<td>-CH$_2$OCONHMe</td>
<td>-CH$_2$Cl</td>
</tr>
<tr>
<td>15</td>
<td>Me</td>
<td>-CH$_2$OH</td>
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</tr>
<tr>
<td>16</td>
<td>Me</td>
<td>-CH$_2$OCONHMe</td>
<td><img src="image4.png" alt="Structure" /></td>
</tr>
<tr>
<td>17</td>
<td>Me</td>
<td>-CH$_2$OCONHMe</td>
<td><img src="image5.png" alt="Structure" /></td>
</tr>
<tr>
<td>18</td>
<td>Me</td>
<td>-CH$_2$OCONHMe</td>
<td><img src="image6.png" alt="Structure" /></td>
</tr>
<tr>
<td>19</td>
<td>CH$_2$CO$_2$CMe$_3$</td>
<td>-H</td>
<td>-CH=CH$_2$</td>
</tr>
<tr>
<td>20</td>
<td>Me</td>
<td>-CH$_2$OCONHMe</td>
<td>-CH(Me)Cl</td>
</tr>
<tr>
<td>21</td>
<td>Me</td>
<td>-H</td>
<td>-CH=CH$_2$</td>
</tr>
<tr>
<td>22</td>
<td>Me</td>
<td>-CH$_2$OCONHMe</td>
<td>H</td>
</tr>
<tr>
<td>23</td>
<td>Me</td>
<td>-CH$_2$OCONMe$_2$</td>
<td><img src="image7.png" alt="Structure" /></td>
</tr>
<tr>
<td>24</td>
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<td>-CH$_2$OCONMe$_2$</td>
<td><img src="image8.png" alt="Structure" /></td>
</tr>
<tr>
<td>25</td>
<td>Me</td>
<td>-CH$_2$OCONMe$_2$</td>
<td><img src="image9.png" alt="Structure" /></td>
</tr>
<tr>
<td>26</td>
<td>Me</td>
<td>-CH$_3$Cl</td>
<td>-C≡CH</td>
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</table>
The HAP compounds of the present invention may be synthesized following the novel methods described in this specification, and/or methods known to one skilled in this art, upon appropriate substitution of reactants. The HAP compounds of the present invention are synthesized according to the present methods starting with a vinyl and pronenyl nitroimidazole compound as schematically shown below:

<table>
<thead>
<tr>
<th>Cmpd No.</th>
<th>R →</th>
<th>S₁</th>
<th>S₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>CH₂CONHMe</td>
<td>-H</td>
<td>-CH=CH₂</td>
</tr>
<tr>
<td>28</td>
<td>Me</td>
<td>-CH₂OCONHMe</td>
<td>H₃C</td>
</tr>
<tr>
<td>29</td>
<td>Me</td>
<td>-CH₂OCONHMe</td>
<td>-C(Me)=CH₂</td>
</tr>
<tr>
<td>30</td>
<td>-CH₂CF₃</td>
<td>-H</td>
<td>-CH=CH₂</td>
</tr>
<tr>
<td>31</td>
<td>Me</td>
<td>-CH₂OCONHMe</td>
<td>-CH₂S(Me)₂I</td>
</tr>
<tr>
<td>32</td>
<td>Me</td>
<td>-CH₂OCONMe₂</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>Me</td>
<td>-CH₂OCONMe₂</td>
<td>-CH₂OSO₂Me</td>
</tr>
<tr>
<td>34</td>
<td>Me</td>
<td>-CH₂OCONMe₂</td>
<td>-CH₂S(Me)₂I</td>
</tr>
<tr>
<td>35</td>
<td>Me</td>
<td>-CH₂OCONMe₂</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>Me</td>
<td>-CH₂C₁</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>Me</td>
<td>-CH₂C₁</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>Me</td>
<td>-CH₂OCONMe₂</td>
<td>-CH₂OH</td>
</tr>
<tr>
<td>39</td>
<td>Me</td>
<td>-CH₂OCONMe₂</td>
<td>-CH₂Br</td>
</tr>
</tbody>
</table>

* X₂ is a leaving group
wherein $R^1$ is selected from the group consisting of hydrogen, $\text{C}_1-\text{C}_6$ alkyl, $\text{C}_1-\text{C}_6$ heteroalkyl, $\text{C}_3-\text{C}_8$ cycloalkyl, heterocyclyl, aryl, and heteroaryl. Methods for synthesizing various 1-N-$R^1$-2-nitroimidazole-5-methanol compounds are described in PCT Patent Application Publication No. WO 07/002931. Bromonitroimidazole compounds can be synthesized generally according to the method described below in Example 1. The vinyl nitroimidazole compound is converted to various HAP compounds of the present invention as shown below:

wherein $R^2$ is selected from the group consisting of hydrogen, $\text{C}_1-\text{C}_6$ alkyl, $\text{C}_1-\text{C}_6$ heteroalkyl, $\text{C}_3-\text{C}_8$ cycloalkyl, heterocyclyl, aryl, and heteroaryl and $X^2$ is selected from the group consisting
of chloro and bromo. In addition to employing an isocyanate \((R_2\text{NCO})\) as provided in the scheme above, the alcohol:

\[
\begin{array}{c}
\text{O}_2\text{N} \\
\text{N} \\
\text{N} \\
\text{Br} \\
\text{OH}
\end{array}
\]

is suitably activated to react with an amine by reacting the alcohol with \(A\)-nitrophenylchloroformate to yield the corresponding 4-nitrophenylcarbonate and reacting the \(A\)-nitrophenyl carbonate with the amine to yield a carbamate HAP of the present invention.

Without limitation, this method of the present invention is exemplified in Example IE.

[0072] The propenyl nitroimidazole can be converted to HAP compounds of the present invention using methods described above for the vinyl nitroimidazole compound.

[0073] A HAP compound of the present invention having the structure of formula:

\[
\begin{array}{c}
\text{O}_2\text{N} \\
\text{N} \\
\text{N} \\
\text{L} \\
\text{X}^3
\end{array}
\]

wherein \(R^1\) is selected from the group consisting of hydrogen, \(\text{Ci-C}_6\) alkyl, \(\text{Ci-C}_6\) heteroalkyl, \(\text{C}_3\text{-C}_g\) cycloalkyl, heterocyclyl, aryl, and heteroaryl; \(L\) is methylene; and \(X^3\) is sulfanyl, is synthesized according to the present methods as described schematically below:
In one aspect the present invention provides a method of synthesizing a compound having a structure of formula:

wherein $R^1$ is selected from the group consisting of hydrogen, $\text{C}_1-\text{C}_6$ alkyl, $\text{C}_1-\text{C}_6$ heteroalkyl, $\text{C}_3-\text{C}_8$ cycloalkyl, heterocyclyl, aryl, and heteroaryl comprising reacting a bromonitroimidazole compound having a structure of formula:
vinyltributyltin (BUsSnCH=CH₂); and a heavy metal catalyst, to obtain a compound having structure of formula:

\[
\begin{align*}
\text{O}_2\text{N} & \quad \text{N} \\
\text{R}^1 & \quad \text{OH}
\end{align*}
\]

[0075] In one embodiment, \( R^1 \) is methyl. In another embodiment, the heavy metal catalyst is selected from the group consisting of a Pd(0), Pd(II), and a Cu(I) catalyst. In another embodiment, the heavy metal catalyst is Pd(PPh₃)₄.

[0076] In another embodiment, the present invention provides a method of synthesizing a compound having structure of formula:

\[
\begin{align*}
\text{O}_2\text{N} & \quad \text{N} \\
\text{R}^1 & \quad \text{OCONHR}^2
\end{align*}
\]

wherein \( R^* \) is selected from the group consisting of hydrogen, \( \text{C}_6\text{H}_5 \) alkyl, \( \text{C}_6\text{H}_5 \) heteroalkyl, \( \text{C}_3\text{C}_g \) cycloalkyl, heterocyclyl, aryl, and heteroaryl and \( R^2 \) is selected from the group consisting of hydrogen, \( \text{C}_6\text{H}_5 \) alkyl, \( \text{C}_6\text{H}_5 \) heteroalkyl, \( \text{C}_3\text{C}_g \) cycloalkyl, heterocyclyl, and aryl, comprising reacting a compound having a structure of formula:

\[
\begin{align*}
\text{O}_2\text{N} & \quad \text{N} \\
\text{R}^1 & \quad \text{OH}
\end{align*}
\]

and an isocyanate having the structure of formula \( \text{R}^2\text{NCO} \); and optionally a Lewis acid catalyst to yield the compound having structure of formula:
In one embodiment, $R_1$ is Me. In another embodiment, $R_2$ is Me. In another embodiment, the Lewis acid catalyst is $\text{Bu}_2\text{Sn(OAc)}_2$. One of skill in the art will appreciate that the steps of the methods are carried out in appropriate solvents as described, for example, in the EXAMPLES section below.

In another embodiment, the present invention provides a method of synthesizing a HAP compound of the present invention having the structure of formula:

wherein $R_1$ is selected from the group consisting of hydrogen, $\text{C}_1$-$\text{C}_6$ alkyl, $\text{C}_1$-$\text{C}_6$ heteroalkyl, $\text{C}_3$-$\text{C}_8$ cycloalkyl, heterocyclyl, aryl, and heteroaryl; $R_2$ is selected from the group consisting of hydrogen, $\text{C}_1$-$\text{C}_6$ alkyl, $\text{C}_1$-$\text{C}_6$ heteroalkyl, $\text{C}_3$-$\text{Cs}$ cycloalkyl, heterocyclyl, aryl, and heteroaryl; and $X^2$ is selected from the group consisting of chloro and bromo, comprising the steps of (i) reacting a compound having a structure of formula:

with an oxidizing agent to obtain a compound having structure of formula
(ii) reacting the compound obtained in step (i) and a reducing agent to yield a compound having a structure of formula:

![Chemical Structure](image)

and (iii) reacting the compound obtained in step (ii) and SO(X₂)₂ wherein X₂ is selected from the group consisting of chloro and bromo to obtain the HAP compound having the structure of formula:

![Chemical Structure](image)

[0079] In another embodiment, R¹ is Me. In another embodiment, R² is Me. In another embodiment, X² is chloro. In another embodiment, the one or more oxidizing agents are selected from the group consisting of OsO₄ and NaIO₄. In another embodiment, the reducing agent is a borohydride. In another embodiment, the borohydride is NaBH₄.
III. Therapies

[0080] In other aspects, the present invention provides methods of treating cancer and other hyperproliferative diseases comprising administering a therapeutically effective amount of a HAP compound of the present invention to a patient in need of such treatment. In one embodiment, the HAP compound administered has a structure of formula:

wherein $R^1$ is selected from the group consisting of hydrogen, $\text{Ci-C}_6$ alkyl, $\text{Ci-C}_6$ heteroalkyl, $\text{C}_3$-$\text{Cs}$ cycloalkyl, heterocyclyl, aryl, and heteroaryl; $L$ is methylene; and $X_1$ and $X_2$ is a leaving group. In another embodiment, $R^1$ is methyl. In another embodiment, the HAP compound administered has a structure of formula:

wherein $X^1$ is a leaving group and $X_2$ is a weaker leaving group. In another embodiment, $X^1$ is selected from a carbamate. In another embodiment, $X^1$ is $-\text{OCONR}_2\text{R}_3$ wherein $\text{R}_2$ and $\text{R}_3$ is selected from the group consisting of hydrogen, $\text{Ci-C}_6$ alkyl, $\text{Ci-C}_6$ heteroalkyl, $\text{Cs}$ cycloalkyl, heterocyclyl, aryl, and heteroaryl. In another embodiment, $R^2$ is hydrogen. In another embodiment, $R^3$ is methyl. In another embodiment, the HAP compound administered is selected from Compound 2 and Compound 13.

[0081] In another embodiment, the HAP compound administered has a structure of formula:
wherein \( R^1 \) is selected from the group consisting of hydrogen, \( \text{C}_6 \text{alkyl} \), \( \text{C}_6 \text{heteroalkyl} \), \( \text{C}_3-\text{C}_g \text{cycloalkyl} \), \( \text{heterocyclyl} \), \( \text{aryl} \), and \( \text{heteroaryl} \); \( L \) is methylene; and \( X^1 \) is a leaving group.

5 In another embodiment, the HAP compound administered is Compound 14.

[0082] In another embodiment, the HAP compound administered has a structure of formula:

\[
\begin{array}{c}
\text{O}_2\text{N} \\
\text{R}^1 \\
\text{L} \\
\text{X}^1 \\
\text{O}
\end{array}
\]

wherein \( R^1 \) is selected from the group consisting of hydrogen, \( \text{C}_6 \text{alkyl} \), \( \text{C}_6 \text{heteroalkyl} \), \( \text{C}_3-\text{C}_8 \text{cycloalkyl} \), \( \text{heterocyclyl} \), \( \text{aryl} \), and \( \text{heteroaryl} \); \( L \) is methylene; and \( X^3 \) is sulfonyl.

[0083] In another embodiment, the therapeutically effective amount of the HAP compound is administered as a pharmaceutically acceptable formulation comprising a HAP compound of the present invention and pharmaceutically acceptable diluents or excipients.

[0084] In another embodiment, the therapeutically effective amount is administered in a daily dose. The therapeutically effective daily dose can be administered by employing suitable unit dose forms of the HAP compounds of the present invention. In another embodiment, the daily dose is administered from once every day, once every two weeks, up to, once every month. In another embodiment, the daily dose is administered parenterally or orally.

[0085] Various cancers can be treated according to the methods of the present invention by administering the HAP compounds the present invention. In certain embodiment, the cancer treated is selected from the group consisting of cancer of the adrenal gland, bone, brain, breast,
bronchi, colon and/or rectum, gallbladder, head and neck, kidneys, larynx, liver, lung, neural tissue, pancreas, prostate, parathyroid, skin, stomach, and thyroid. In another embodiment, the cancer treated is selected from the group consisting of acute and chronic lymphocytic and granulocytic tumors, adenocarcinoma, adenoma, basal cell carcinoma, cervical dysplasia and in situ carcinoma, Ewing's sarcoma, epidermoid carcinomas, giant cell tumor, glioblastoma multiforma, hairy-cell tumor, intestinal ganglioneuroma, hyperplastic corneal nerve tumor, islet cell carcinoma, Kaposi's sarcoma, leiomyoma, leukemias, lymphomas, malignant carcinoid, malignant melanomas, malignant hypercalcemia, marfanoid habitus tumor, medullary carcinoma, metastatic skin carcinoma, mucosal neuroma, myeloma, mycosis fungoides, neuroblastoma, osteosarcoma, osteogenic and other sarcoma, ovarian tumor, pheochromocytoma, polycythemia vera, primary brain tumor, small-cell lung tumor, squamous cell carcinoma of both ulcerating and papillary type, hyperplasia, seminoma, soft tissue sarcoma, retinoblastoma, rhabdomyosarcoma, renal cell tumor, topical skin lesion, vetriculum cell sarcoma, and Wilm's tumor.

[0086] In one embodiment, the HAP compound of the present invention is administered for the treatment of cancer in combination with other anticancer agents or other anticancer therapies. Suitable anticancer therapies useful in accordance with the present methods include radiation therapy and surgery. Methods for treating cancer employing other hypoxia activated prodrugs are described, for example, in PCT Patent Application Publication Nos. WO 07/002931 and WO06/57946, and U.S. Patent Application Publication No. 2006/0258656 (each of which is incorporated herein by reference) and can be used for the treatment of cancer according to the present methods upon appropriate substitution of the other hypoxia activated prodrugs with the HAP compounds of the present invention.

[0087] In certain embodiments, the present invention provides methods of treating non-cancer hyperproliferative diseases characterized by cellular hyperproliferation (e.g., an abnormally increased rate or amount of cellular proliferation) in accordance with the present methods. In certain embodiments, the hyperproliferative disease is selected from the group consisting of allergic angitis and granulomatosis (Churg-Strauss disease), asbestosis, asthma, atrophic gastritis, benign prostatic hyperplasia, bullous pemphigoid, coeliac disease, chronic bronchitis and chronic obstructive airway disease, chronic sinusitis, Crohn's disease, demyelinating
neuropathies, dermatomyositis, eczema including atopic dermatitis, eustachean tube diseases, giant cell arteritis, graft rejection, hypersensitivity pneumonitis, hypersensitivity vasculitis (Henoch-Schonlein purpura), irritant dermatitis, inflammatory hemolytic anemia, inflammatory neutropenia, inflammatory bowel disease, Kawasaki’s disease, multiple sclerosis, myocarditis, myositis, nasal polyps, nasolacrimal duct diseases, neoplastic vasculitis, pancreatitis, pemphigus vulgaris, primary glomerulonephritis, psoriasis, periodontal disease, polycystic kidney disease, polyarteritis nodosa, polyangitis overlap syndrome, primary sclerosing cholangitis, rheumatoid arthritis, serum sickness, surgical adhesions, stenosis or restenosis, scleritis, scleroderma, strictures of bile ducts, strictures (of duodenum, small bowel, and colon), silicosis and other forms of pneumoconiosis, type I diabetes, ulcerative colitis, ulcerative proctitis, vasculitis associated with connective tissue disorders, vasculitis associated with congenital deficiencies of the complement system, vasculitis of the central nervous system, and Wegener’s granulomatosis.

[0088] In one embodiment, the hyperproliferative disease treated is psoriasis, a disease characterized by the cellular hyperproliferation of keratinocytes which builds up on the skin to form elevated, scaly lesions. In another embodiment, the hyperproliferative disease treated is multiple sclerosis, a disease characterized by progressive demyelination in the brain. In another embodiment, the hyperproliferative diseases treated is rheumatoid arthritis, a multisystem chronic, relapsing, inflammatory disease that can lead to destruction and ankylosis of joints affected. In another embodiment, a HAP compound of the present invention is administered to prevent a hyperproliferative disease resulting from cellular proliferation on a prosthesis implanted in a patient by coating the prosthesis with a composition containing a HAP compound of the present invention.

[0089] The invention, having been described in summary and in detail, is illustrated but not limited by the Examples below, which describe methods for synthesizing HAP compounds of the present invention and testing them under hypoxia/anoxia and normoxia.
IV. EXAMPLES

Example 1: Synthesis of HAP Compounds of the Present Invention

A. Synthesis of Compound 14

[0090] Example IA describes the synthesis of Compound 14, a HAP compound of the present invention, according to the novel synthetic method of the present invention described above, starting from 1-methyl-4-bromo-2-nitroimidazole methanol.

\[
\text{i. } \xrightarrow{\text{NBS/DMF}} \text{ii.} \]

[0091] To a solution of Compound i (1 g) in DMF (15 mL) was added N-bromosuccinimide (NBS, 1.25 g) and the reaction mixture stirred at 60°C for 3 h. Then, the reaction mixture was diluted with brine, extracted with EtOAc, the EtOAc portion dried, and concentrated to yield a residue that was separated by column chromatography using 0 - 80% EtOAc/Hexanes to yield Compound ii (1.3 g).
A solution of l-methyl-4-bromo-2-nitroimidazolemethanol (compound (ii), 3 g) in dimethylformamide (DMF, 100 mL) was degassed by evacuation and purged three times with argon. Tetrakis triphenylphosphine (1.47 g) was added to the reaction mixture and the ensuing solution purged with argon followed by the addition of vinyl tributyltin (11.1 mL). The solution was again purged with argon and the reaction mixture was heated to 110 °C and stirred under argon for 16 h. The solution was cooled and diluted with water (100 mL), filtered through celite, and the celite pad washed twice with hexanes. The aqueous portion of the filtrate was diluted with saturated aqueous NaCl (100 mL) and extracted with EtOAc (250 mL) four times. The combined organic portions was washed twice with brine, dried over MgSO₄, and separated by column chromatography on silica gel using EtOAc/Hexane (0-100%) as eluent to yield compound (ii) (479 mg) as yellow crystals that was used in the next step.

Bu₂Sn(OAc)₂ (50 µL) was added to a solution of compound (iii) (470 mg) in dichloromethane (DCM, 12.5 mL) at 0 °C followed by the addition of MeNCO (197 µL). The initial suspension became a solution that was allowed to warm to rt (2 h) then evaporated until dry and the residue was separated by column chromatography on silica gel using EtOAc/Hexane (0-100%) as eluent to yield compound (567 mg) as a yellow powder that was used in the next step.

A solution OfOsO₄ (427 mL) and deionized water (10 mL) was added to a solution of compound (iv) (322 mg) in dioxane (10 mL) followed by the addition OfNaIO₄ (860 mg) in
portions and stirred at room temperature (rt) for 3 h. The reaction mixture was evaporated under vacuum to yield a residue to which was added water (20 mL) and extracted with DCM (30 mL) four times. The DCM solution was dried over NaSO₄ and evaporated to dryness. The residue was co-evaporated with absolute EtOH (10 mL), taken up in absolute EtOH (10 mL), and cooled to 0 °C. NaBH₄ (56 mg) was added and the solution was stirred for 1 h. Acetone (1 mL) and silica (2 g) were added and the reaction mixture and the adsorbed reaction mixture separated by column chromatography on silica gel using MeOH/DCM (5%) as eluent to yield compound (v) (282 mg) as a colorless solid that was used in the next step.

SOCl₂ was added to a solution of compound (v) (49 mg) suspended in DCM (1 mL) at 0 °C. The solution was allowed to warm to rt (40 min), poured into ice water (50 mL), and extracted with EtOAc (50 mL). The organic portion was washed with brine and evaporated to dryness and the residue was separated by chromatography on silica gel using EtOAc/Hexanes (0:100%) as eluent to yield Compound 14 (27.3 mg) as a colorless solid. ¹H-NMR (CDCl₃): δ 5.20 (s, 2H), 4.72 (s, 1H), 4.71 (s, 2H), 4.06 (s, 3H), 2.93 (d, J = 4.8, 3H).

B. Synthesis of Compound 2

Example IB describes the synthesis of Compound 2, a HAP compound of the present invention, according to the novel synthetic methods of the present invention and starting from compound (iii).
N,N-Dimethylcarbamoyl chloride (181 µL), TEA (367 µL), and DMAP (40 mg) were added to a solution of compound (iii) (241 mg) in dichloroethane (DCE, 5 mL). The mixture was stirred at rt overnight and diluted with EtOAc. Then, the EtOAc solution was washed with water and brine, dried, and concentrated and the residue separated by column chromatography on silica gel using EtOAc/Hexanes (0-60%) to yield compound (vi) (150 mg) as a yellow solid. Compound vi was converted to Compound 2 following the methods described in Example IA for the synthesis of Compound 14. \(^1\)H-NMR (CDCl\(_3\)): \(\delta\) 5.22 (s, 2H), 4.72 (s, 2H), 4.08 (s, 3H), 2.93 (s, 3H), 2.92 (s, 3H).

### C. Synthesis of Compound 13

Example 1C describes the synthesis of Compound 13, a HAP compound of the present invention according to the novel synthetic method of the present invention and starting from compound (iv).
Meta-chloroperoxybenzoic acid (mCPBA, 144 mg) was added to a solution of compound (iv) (100 mg) in dry DCM at 0°C and the reaction mixture warmed to rt. After 4 h, additional mCPBA (144 mg) was added. After an additional 4 h at rt, cyclohexene (200 µL) was added and stirred (20 min) until no mCPBA remained according to a thin layer chromatography (TLC) analysis. Then, DCM (100 mL) was added to the reaction mixture, the organic layer separated, washed with saturated aqueous NaHCO₃ (25 mL), dried, evaporated, and the residue separated by column chromatography on silica gel using EtOAc/Hexanes (0-100%) as eluent to yield Compound 13. 

\[
\text{\(^1\)H-NMR \((\text{CDCl}_3)\): } \delta 5.30 (d, J = 13.9, \text{ IH}), 5.18 (d, J = 13.9, \text{ IH}), 4.72 (s, \text{ IH}), 4.06-4.08 (m, \text{ IH}), 4.04 (s, \text{ 3H}), 3.35-3.33 (m, \text{ IH}), 3.18-3.15 (m, \text{ IH}), 2.83 (d, J = 4.9, \text{ 3H}).
\]

D. Synthesis of a Propenyl Nitroimidazole Intermediate

Example ID describes the synthesis of a propenyl nitroimidazole intermediate useful in the synthesis of HAP compounds of the present invention according to the present methods.
A solution of 1-methyl-4-bromo-2-nitroimidazolemethanol (compound (ii) R<sub>1</sub> is CH<sub>3</sub>, 500 mg) in dimethylformamide (DMF, 20 mL) was degassed by evacuation and purged three times with argon. Palladium-1,2-bis(diphenylphosphinoethane)-dichloride (DPPF, 155 mg) and K<sub>3</sub>PO<sub>4</sub> (900 mg) were added to the reaction mixture and the ensuing solution purged with argon then stirred at room temperature for 10 minutes. Next, isopropenylboronic acid pinacol ester was added to the solution, the solution purged with argon, and the reaction mixture was stirred at 60°C overnight. The reaction mixture was diluted with a water brine mixture (1:1) and extracted with EtOAc three times. The combined organic portions was dried, concentrated, and separated by column chromatography on silica gel using EtOAc/Hexane (0-100%) as eluent to yield compound (vii) (350 mg) as a yellow solid. <sup>1</sup>H-NMR (DMSO): δ 5.52 (t, J = 5.2, 1H), 5.37 (s, 1H), 5.26 (s, 1H), 4.58 (d, J = 4.8, 2H), 3.95 (s, 3H), 2.07 (s, 3H).

**E. Synthesis of Compound 37**

Example 1E describes the synthesis of Compound 37, a HAP compound of the present invention, according to the novel synthetic methods of the present invention and starting from compound (ii).
[0102] A solution of 1-methyl-4-bromo-2-nitroimidazolesmethanol (compound (ii) \( R_1 \) is CH3, 1.66 g) in dimethylformamide (DMF, 60 mL) was degassed by evacuation and purged three times with argon. Palladium-1,2-bis(diphenylphosphinoethane)-dichloride (DPPF, 515 mg) and K3PO4 (2.99 g) were added to the reaction mixture and the ensuing solution purged with argon then stirred at room temperature for 10 minutes. Next, trans-2-phenylvinylboronic acid pinacol ester was added to the solution, the solution purged with argon, and the reaction mixture was stirred at 60 °C overnight. The reaction mixture was concentrated under high vacuum and the resulting residue diluted with hot acetone and filtered through a silica plug which was washed with additional hot acetone. The combined acetone fractions were concentrated until precipitate formed, heated to re-dissolve the solid, water was added and the solution cooled to effect crystallization. The crystals were collected by filtration and washed with acetone and the filtrate concentrated to provide additional product which was washed with water and EtOAc. The combined product was dried under high vacuum to yield compound (viii) (1.49 g, 82%) as a yellow solid.
[0103] To a 0°C solution of compound (viii) (700 mg) and pyridine (240 µL) in DCM (14 mL) and DMF (6 mL) was added dropwise a solution of /?-nitrophenylchloroformate (600 mg) in DCM (3 mL). The mixture was stirred at rt overnight. Pyridine (109 µL) and p-nitrophenylchloroformate (273 mg) in DCM (1.5 mL) was added and the reaction mixture stirred 5 h then diluted with water. The aqueous solution was washed with DCM (5 x) and the combined organic dried over, concentrated and the residue separated by column chromatography on silica gel using EtOAc/Hexanes (0-10%) followed by acetone/DCM (0-10%) to yield compound (ix) (607 mg) as a yellow solid.

[0104] To a suspension of compound (ix) (100 mg) in THF (2 mL) was added morpholine (45 µL) followed by pyridine (10 µL). The reaction mixture was stirred at rt 2 h, concentrated and the residue separated by column chromatography on silica gel using 1:1 DCM/Hexanes to 1:4:5 acetone/DCM/Hexanes (0-100%) to yield compound (x) (75 mg) as a yellow solid.

[0105] Compound (x) was converted to compound (xi) following the methods described in Example 1A for the synthesis of compound (v).

[0106] To a solution of compound (xi) (24 mg) in acetonitrile (3 mL) was added dichlorodiphenylphosphorane (86 mg). The reaction mixture was stirred at rt 30’concentrated and the residue separated by column chromatography on silica gel using 1:1 DCM/Hexanes to 1:10 acetone/DCM (0-100%) to yield compound 37 (19 mg) as a colorless oil. 1H NMR (CDCl₃) δ 5.26 (s, 2H), 4.71 (s, 2H), 4.08 (s, 3H), 3.68-3.65 (m, 4H), 3.55-3.40 (m, 4H).

Example 2: Demonstration of Cytotoxicities of the HAP Compounds

[0107] This example describes methods for determining cytotoxicities of HAP compounds of the present invention by employing an AlamarBlue fluorescence intensity based detection of cell survival, and demonstrate that HAP compounds of the present invention are more cytotoxic under hypoxic conditions than under normoxic conditions. H460 cells (10,000 - 15,000 cells/well/500 µL, ATCC HTB-177) were seeded in glass inserts on 24-well plates in RPMI 1640 medium supplemented with 10% FBS and 1% Penicillin/Streptomycin (Invitrogen Corporation, Carlsbad, CA). The cells were incubated overnight at 37°C in 5% CO₂, 95% air and 100% relative humidity (these incubation conditions were used throughout the experiment unless
otherwise mentioned) and divided into 2 groups: a "control group" (no test compound), and "treatment groups" (in which the cells were kept in contact with the test compound at various concentrations for 2 h).

[0108] The control fluorescence intensity, or $F_0$, proportional to the cell population of the control group at the beginning of the experiment, was determined following an AlamarBlue assay ($\lambda_{ex} = 550$ nm and $\lambda_{em} = 590$ nm) (See, Biosource International Inc., Tech Application Notes, Use of Alamar Blue in the measurement of Cell Viability and Toxicity, Determining IC50). The cells in the treatment groups were incubated for 2 hours with 6 different concentrations of a test compound, under hypoxia (5% CO$_2$, 5% H$_2$, 90% N$_2$) or normoxia (5% CO$_2$, 95% air), media containing the test compound removed, fresh media added, and the cells incubated for 3 days. The fluorescence intensities of the various treatment group cells incubated with different concentrations of the test compound and having different cell populations, and the control group cells at the end of the experiment ($F_t$) having the highest cell population among all the groups, was determined following an AlamarBlue assay. The fluorescence intensities determined were background corrected by subtracting $F_0$, and normalized by dividing with $F_t-F_0$.

[0109] The background corrected and normalized fluorescence intensities of the control group after 3 days of incubation, and the various treatment groups after 3 days of incubation, were plotted against the corresponding concentrations of the test compound. The IC50 value for the test compound, i.e., the concentration of the test compound that killed, or made unviable, 50% of the cells, was calculated based on a best-fit plot using an F test (GraphPad Prism4 software, San Diego, CA). The results are tabulated below.
<table>
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<tr>
<th>Compound No.</th>
<th>Cytotoxicity (IC₅₀, µM)</th>
<th></th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>Normoxia</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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</table>
The results demonstrate that the HAP compounds of the present invention, Compound 2 and Compound 14, are about 25 times more cytotoxic under hypoxia than under normoxia. Thus, in one embodiment of the present invention, Compound 2 and Compound 14 is administered to treat cancer according to the present methods by selectively killing hypoxic tumor cells and not killing or killing fewer of the normoxic, normal cells. The cytotoxicities of Compound 12 and Compound 17 are estimates because under the conditions tested they did not yield an IC50. Compounds which did not show enhanced cytotoxicity in an H460 cell line under hypoxia over normoxia can be more cytotoxic under hypoxia over normoxia when different test conditions and/or cell lines are used.

***

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes can be made and equivalents can be substituted without departing from the scope of the invention. In addition, many modifications can be made to adapt a particular situation, material, composition of matter, process, process step or steps, to achieve the benefits provided by the present invention without departing from the scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

All publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents is not intended as an indication that any such document is pertinent prior art, nor does it constitute any admission as to the contents or date of the same.
WHAT ISCLAIMED IS:

1. A compound having a structure of the formula Hyp-(L-X)₂ wherein Hyp is a 2-nitroimidazole moiety, L is a linker independently selected from the group consisting of a bond and C₁-C₆ alkylene, and each X is independently selected from an alkylating group; or a pharmaceutically acceptable salt thereof.

2. A compound of claim 1 having a structure of the formula:

   ![](image)

   wherein R¹ is selected from the group consisting of H, C₁-C₆ alkyl, C₁-C₆ heteroalkyl, C₃-C₆ cycloalkyl, heterocyclyl, aryl and heteroaryl;

   each L is independently selected from the group consisting of a bond and C₁-C₆ alkylene,

   X¹ and X² is independently selected from the group consisting of -OCONR₂R³, halo, -OOH, H, -CC₆, -CC₆ alkynyl, -C₂-C₆ alkenyl, -CHO, sulfonyloxy, -(+)NR₂R³, -NR₂haloC₁-C₆ alkyl, -CH=N-sulfonyloxy,

   R² and R³ is independently selected from the group consisting of hydrogen, C₁-C₆ alkyl, C₁-C₆ heteroalkyl, C₃-C₆ cycloalkyl, heterocyclyl, aryl, heteroaryl, -C₁-C₆ alkylene.
C₆alkylheterocyclyl, or combined to form heterocyclyl; and the wavy line indicates a covalent linkage to the rest of the molecule.

3. The compound of claim 1 having a structure of formula:

![Chemical structure](image)

wherein R¹ is selected from the group consisting of hydrogen, C₁-C₆ alkyl, C₁-C₆ heteroalkyl, C₃-C₈ cycloalkyl, heterocyclyl, aryl, and heteroaryl; each L is selected from the group consisting of a bond, CH₂ or CHMe; and X¹ and X² is independently selected from the group consisting of -OCONMe₂, -Cl, -OCONHMe, -OH, H, CH₂Cl, CH=CH₂, CHO, -OTs, -C(Me)=CH₂, -C≡CH, -Cl,

![Chemical structures](image)

and the wavy line indicates a covalent linkage to the rest of the molecule.

4. The compound of claim 1 having a structure of formula:
wherein R₁ is selected from the group consisting of hydrogen, C₆ alkyl, C₁₋₆ heteroalkyl, C₃₋₆ cycloalkyl, heterocycl, aryl, and heteroaryl; L is methylene; and X₁ is selected from the group consisting of -OCONMe₂, -Cl, -OCONHMe, -OH, H, CH₂Cl, CH=CH₂, -CHO, -OTs, -C(Me)=CH₂, -C≡CH; -Cl.

5. The compound of any of claims 2-4 wherein X₁ is -OCONR²R³; R² and R³ is selected from the group consisting of hydrogen, C₆ alkyl, C₁₋₆ heteroalkyl, C₃₋₆ cycloalkyl, heterocycl, aryl, and heteroaryl and X² is chloro.

6. The compound of claim 5 wherein X₁ is -OCONHMe.

7. The compound of any of the preceding claims wherein R² is selected from hydrogen and methyl and R³ is methyl.

8. A compound having a structure of formula:
R\(^1\) is selected from the group consisting of hydrogen, \(\text{Ci-C}_6\) alkyl, \(\text{Ci-C}_6\) heteroalkyl, \(\text{C}_3\text{-Cs}\) cycloalkyl, heterocyclyl, aryl, and heteroaryl; L is methylene; and \(X^3\) is sulfonyl; or a pharmaceutically acceptable salt thereof.

9. The compound of any of the previous claims wherein \(R^1\) is methyl.

10. A pharmaceutically acceptable formulation comprising a compound of any of the preceding claims, or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier, excipient, or diluent.

11. A method of synthesizing a compound having a structure of formula:

\[
\text{O}_2\text{N} \quad \begin{array}{c}
\text{R}^1
\end{array}
\text{N} \quad \begin{array}{c}
\text{OH}
\end{array}
\]

or a pharmaceutically acceptable salt thereof, wherein \(R^1\) is selected from the group consisting of \(\text{Ci-C}_6\) alkyl, \(\text{Ci-C}_6\) heteroalkyl, \(\text{C}_3\text{-C}_8\) cycloalkyl, heterocyclyl, aryl, and heteroaryl comprising reacting a bromonitroimidazole compound having structure of formula:

\[
\text{O}_2\text{N} \quad \begin{array}{c}
\text{R}^1
\end{array}
\text{N} \quad \begin{array}{c}
\text{Br}
\end{array}
\text{OH}
\]
vinyltributyltin (Bu₃SnCH=CH₂); and a Pd(O) catalyst, to obtain a compound having a structure of formula:

12. A method of treating cancer comprising administering a therapeutically effective amount of a compound of any of claims 1-9; thereof to a patient in need of such treatment.
INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/75615

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) ... Box 1450, Alexandria, Virginia 22313-1450 PCT Helpdesk. 571-272-4300
Facsimile No. 571-273-3201 PCTOSP 571-272-7774

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 514/385

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/387-388, 392-393 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic Databases Searched: USPTO WEST (PGPUB, EPAB, JPAB, USPT), Google patent, Google Scholar
Search Terms Used: 2-nitroimidazole, alkylating group, linker, alkylene, pharmaceutO, salt

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

Further documents are listed in the continuation of Box C.

- Special categories of cited documents
  - "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier application or patent but published on or after the international filing date
  - "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  - "O" document referring to an oral disclosure, use, exhibition or other means
  - "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- "Z" document member of the same patent family

Date of the actual completion of the international search
11 November 2008 (11.11.2008)

Date of mailing of the international search report
18 NOV 2008

Authorized officer: Lee W. Young

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Facsimile No. 571-273-3201
INTERNATIONAL SEARCH REPORT

International application No. PCT/US 08/75615

Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. □ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

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

3. □S□ Claims Nos.: 7, 9-10 and 12 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

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

2. □ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

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

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

Remark on Protest

□ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

□ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

□ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (April 2007)