Industria y Comercio

SUPERINTENDENCIA

Delegatura de propiedad Industrial

División de Nuevas Creaciones

PCT

SOLICITUD

PATENTE DE INVENCIÓN

21. **EXPEDIENTE No.** 06-30143

54. **TÍTULO** Métodos de tratamiento de cáncer con inhibidores HDAC

51. **CLASIFICACIÓN INTERNACIONAL** A61K

71. **SOLICITANTE** Aton Pharma, Inc.

DOMICILIO Boston, MA, USA.

74. **APODERADO** Delia María Rodríguez D'Aleman

22. **BOGOTÁ, D. C.,** Marzo 27/06.

(FORMA 10)

90971 -Imprenta nacional de Colombia- 2004

SS

PETITORIO

SUPERINDUSTRIA Y COMERCIO
Radicacion : 06030143 00000000 Folios: 280
Fecha (AHD): 2006-03-27 17:41:38
Tramite : 011 PCT-PATENT 378 FASE NACI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

Industria y Comercio
SUPERINTENDENCIA

FORMULARIO ÚNICO DE SOLICITUD DE PATENTE
PCT
ENTRADA EN FASE NACIONAL

27-03-2006

1) SOLICITUD DE:

☒ Patente de invención ☐ Patente de Modelo de Utilidad

☒ Capitulo I. ☐ Capitulo II.

2) SOLICITANTE (71)	**Nombre: ATON PHARMA, INC.** **Dirección: 33 Avenue Louis Pasteur, Boston, MA 02115 Estados Unidos** **Nacionalidad o domicilio: Boston, MA, Estados Unidos** **Lugar de constitución: MA, Estados Unidos**	**IDENTIFICACIÓN** **C.C.** ☐ **NIT:** ☐ **C.E.** ☐ **OTRO** ☐ **Cual** _____ **NÚMERO** _____
	Teléfono: Fax: **E. Mail:**	
3) REPRESENTANTE O APODERADO	Nombre: DILIA MARÍA RODRÍGUEZ D´ALEMAN Dirección: Carrera 11 N°. 86-53 Piso 6 Bogotá D.C. Teléfono: 6181088 Fax: 6350824 E. Mail: info@clarkemodet.com.co	**IDENTIFICACIÓN** **C.C.** ☒ **NIT:** ☐ **C.E.** ☐ **OTRO** ☐ **NÚMERO:** 41.654.882 **TP:** 20824 CSJ
4) INVENTOR (ES) (72)	**Nombre: 1. BACOPOULOS, Nicholas, G. 2. CHIAO, Judy, H. 3. MILLER, Thomas, A.; 4. PARADISE, Carolyn, M.; 5. RICHON, Victoria, M.** **Dirección: 1. 2109 Broadway, Apartment 15-109, New York, NY 10023, USA; 2. 110 Cedar Green Lane, Berkeley Heights, NJ 07922 USA; 3. 517 West 113th Street, Number 82, New York, NY 10025 USA; 4. 6 Gilman Lane, Cortland Manor, NY 10567 USA; 5. 184 Highland Road, Rye, NY 10580 USA** **Nacionalidad o Domicilio: Todos los anteriores, estadounidenses**	

5) PCT (86)
Solicitud Internacional No. PCT/US2004/027943 Fecha: Agosto 26, 2004

Publicación Internacional No. WO 2005/018578 A2 Fecha: Marzo 3, 2005

6)Título (54) MÉTODOS DE TRATAMIENTO DE CÁNCER CON INHIBIDORES HDAC

7) Clasificación Internacional: A 61K31/185, A61P35/00

8) PRIORIDAD SI ☒ NO ☐	**33) País de Origen**	**(32) Fecha**	**(31) Número de Solicitud**
	ESTADOS UNIDOS	**AGO. 26/2003**	**10/650,025**
	ESTADOS UNIDOS	**SEPT. 16, 2003**	**10/665,079**

9) Comprobante de pago N°. 06-9,419 Fecha: FEB. 3, 2006

Instrucciones para el diligenciamiento del presente formulario. Ver reverso de esta pagina

61

8758 2

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

SUPERINTENDENCIA Y COMERCIO
Radicacion : 86830143 00030838 Folios: 280
Fecha (AHD): 2006-03-27 17:41:38
Tramite : 811 PCT-PATENT 378 FASE NACI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

RECIBO OFICIAL DE CAJA : 06 - 9,419
FECHA : FEBRERO 3 DE 2006

***** C O N S I G N A C I O N *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
CLARKE MODET Y CO. COLOMB	CONSIGNACION	BANCO POPULAR	050-00110-6	49044333	03/02/2006	12,853,500.00

***** C O N C E P T O *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	463,000.00
		TOTAL : $	463,000.00

SON: CUATROCIENTOS SESENTA Y TRES MIL PESOS

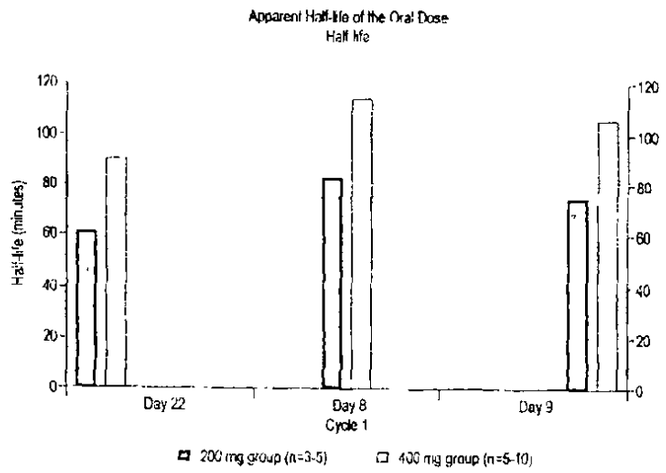
RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No._____

10) ANEXOS

- ☒ Comprobante de pago de la tasa de presentación de la solicitud
- ☐ Comprobante de pago por reivindicación de prioridad
- ☐ Documento que acredita la existencia y representación legal cuando el solicitante sea persona Jurídica
- ☐ Poderes, si fuera el caso
- ☐ Documento de cesión del inventor al solicitante o a su causante
- ☐ Declaraciones
- ☒ Copia de la solicitud Internacional. (Resumen, descripción, reivindicación, Dibujos).
- ☒ Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicación, Dibujos)
- ☐ Copia del examen preliminar efectuado por la IPEA
- ☐ De ser el caso, copia del contrato de acceso
- ☐ De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas
- ☐ De ser el caso, certificado de depósito del material biológico
- ☐ Arte final 12 x 12 y 6 x 6 cm
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionados parcial o totalmente con la invención de esta solicitud.
- ☒ Otros:
  1. Reporte internacional de búsqueda
  2. Solicitud PCT
  3. Notificación de la transmisión de la publicación o republicación internacional

11) FIGURA CARACTERÍSTICA:



12) Solicitud concesión de la patente,

NOMBRE: DILIA RODRÍGUEZ D´ALEMAN

FIRMA:

C.C. 41.654.882 de Bogota **TP** 20824 CSJ

Instrucciones para el diligenciamiento del presente formulario. Ver reverso de esta pagina

PATENT COOPERATION TRF Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
TRANSMITTAL OF COPY OF INTERNATIONAL
APPLICATION AS PUBLISHED OR REPUBLISHED

To:

ELRIFI, Ivor, R.
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P.C
.
666 Third Avenue
New York, NY 10017
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 03 March 2005 (03.03.2005)	
Applicant's or agent's file reference 24852-501CIP5 061	**IMPORTANT NOTICE**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2004/027943	26 August 2004 (26.08.2004)	26 August 2003 (26.08.2003)

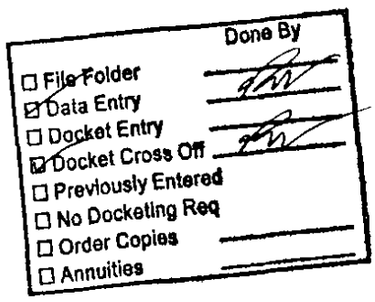
Applicant
ATON PHARMA, INC. et al

The International Bureau transmits herewith the following documents:

☑ copy of the international application as published by the International Bureau on 03 March 2005 (03.03.2005) under No. WO 2005/018578

☐ copy of international application as republished by the International Bureau on under No WO
For an explanation as to the reason for this republication of the international application, reference is made to INID codes (15), (48) or (88) *(as the case may be)* on the front page of the attached document.

RECEIVED
MAR 07 2005
Mintz Levin - NYC



The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Nora Lindner
Facsimile No.+41 22 740 14 35	Facsimile No.+41 22 338 89 65

Form PCT/IB/311 (January 2004)

METHODS OF TREATING CANCER WITH HDAC INHIBITORS

GOVERNMENT INTEREST STATEMENT

This invention was made in whole or in part with government support under grant number 1R21 CA 096228-01 awarded by the National Cancer Institute. The government may have certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to methods of treating cancers, e.g., mesothelioma or lymphoma. More specifically, the present invention relates to methods of treating mesothelioma, diffuse large B-cell lymphoma (DLBCL), or other cancers or tumors by administration of pharmaceutical compositions comprising HDAC inhibitors, e.g., suberoylanilide hydroxamic acid (SAHA). The oral formulations of the pharmaceutical compositions have favorable pharmacokinetic profiles such as high bioavailability and surprisingly give rise to high blood levels of the active compounds over an extended period of time.

BACKGROUND OF THE INVENTION

Throughout this application various publications are referenced by arabic numerals within parentheses. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Cancer is a disorder in which a population of cells has become, in varying degrees, unresponsive to the control mechanisms that normally govern proliferation and differentiation.

Mesothelioma is a rare form of cancer in which malignant (cancerous) cells are found in the mesothelium, a protective sac that covers most of the body's internal organs. The mesothelium is a membrane that covers and protects most of the internal organs of the body. It is composed of two layers of cells: one layer immediately surrounds the organ; the other forms a sac around it. The mesothelium produces a lubricating fluid that is released between these layers, allowing moving organs (such as the beating heart and the expanding and contracting lungs) to glide easily against adjacent structures.

The mesothelium has different names, depending on its location in the body. The peritoneum is the mesothelial tissue that covers most of the organs in the abdominal cavity. The pleura is the membrane that surrounds the lungs and lines the wall of the chest cavity. The pericardium covers and protects the heart. The mesothelial tissue surrounding the male internal reproductive organs is called the tunica vaginalis testis. The tunica serosa uteri covers the internal reproductive organs in women. Most cases of mesothelioma begin in the pleura or peritoneum. Malignant tumors arising from the pleural mesothelium are strongly linked to asbestos exposure.

Although reported incidence rates have increased in the past 20 years, mesothelioma is still a relatively rare cancer. About 2,000 new cases of mesothelioma are diagnosed in the United States each year. Mesothelioma occurs more often in men than in women and risk increases with age, but this disease can appear in either men or women at any age.

Shortness of breath and pain in the chest due to an accumulation of fluid in the pleura are often symptoms of pleural mesothelioma. Symptoms of peritoneal mesothelioma include weight loss and abdominal pain and swelling due to a buildup of fluid in the abdomen. Other symptoms of peritoneal mesothelioma may include bowel obstruction, blood clotting abnormalities, anemia, and fever. If the cancer has spread beyond the mesothelium to other parts of the body, symptoms may include pain, trouble swallowing, or swelling of the neck or face.

The prognosis for mesothelioma is dismal, with poor response to radical surgery, current chemotherapy, radiation therapy, and combination therapy. Microscopic spread of cancer cells into the chest wall and diaphragm are common even when such spread cannot be detected by routine tests.

METHODS OF TREATING CANCER WITH HDAC INHIBITORS

GOVERNMENT INTEREST STATEMENT

This invention was made in whole or in part with government support under grant number 1R21 CA 096228-01 awarded by the National Cancer Institute. The government may have certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to methods of treating cancers, e.g., mesothelioma or lymphoma. More specifically, the present invention relates to methods of treating mesothelioma, diffuse large B-cell lymphoma (DLBCL), or other cancers or tumors by administration of pharmaceutical compositions comprising HDAC inhibitors, e.g., suberoylanilide hydroxamic acid (SAHA). The oral formulations of the pharmaceutical compositions have favorable pharmacokinetic profiles such as high bioavailability and surprisingly give rise to high blood levels of the active compounds over an extended period of time.

BACKGROUND OF THE INVENTION

Throughout this application various publications are referenced by arabic numerals within parentheses. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Cancer is a disorder in which a population of cells has become, in varying degrees, unresponsive to the control mechanisms that normally govern proliferation and differentiation.

Mesothelioma is a rare form of cancer in which malignant (cancerous) cells are found in the mesothelium, a protective sac that covers most of the body's internal organs. The mesothelium is a membrane that covers and protects most of the internal organs of the body. It is composed of two layers of cells: one layer immediately surrounds the organ; the other forms a sac around it. The mesothelium produces a lubricating fluid that is released between these layers, allowing moving organs (such as the beating heart and the expanding and contracting lungs) to glide easily against adjacent structures.

The mesothelium has different names, depending on its location in the body. The peritoneum is the mesothelial tissue that covers most of the organs in the abdominal cavity. The pleura is the membrane that surrounds the lungs and lines the wall of the chest cavity. The pericardium covers and protects the heart. The mesothelial tissue surrounding the male internal reproductive organs is called the tunica vaginalis testis. The tunica serosa uteri covers the internal reproductive organs in women. Most cases of mesothelioma begin in the pleura or peritoneum. Malignant tumors arising from the pleural mesothelium are strongly linked to asbestos exposure.

Although reported incidence rates have increased in the past 20 years, mesothelioma is still a relatively rare cancer. About 2,000 new cases of mesothelioma are diagnosed in the United States each year. Mesothelioma occurs more often in men than in women and risk increases with age, but this disease can appear in either men or women at any age.

Shortness of breath and pain in the chest due to an accumulation of fluid in the pleura are often symptoms of pleural mesothelioma. Symptoms of peritoneal mesothelioma include weight loss and abdominal pain and swelling due to a buildup of fluid in the abdomen. Other symptoms of peritoneal mesothelioma may include bowel obstruction, blood clotting abnormalities, anemia, and fever. If the cancer has spread beyond the mesothelium to other parts of the body, symptoms may include pain, trouble swallowing, or swelling of the neck or face.

The prognosis for mesothelioma is dismal, with poor response to radical surgery, current chemotherapy, radiation therapy, and combination therapy. Microscopic spread of cancer cells into the chest wall and diaphragm are common even when such spread cannot be detected by routine tests.

Lymphoma is cancer of the lymphatic system, a network of lymph nodes, organs (including the spleen, thymus, and tonsils), and vessels that are part of the immune system. There are many different types of lymphoma, and they can be divided into two categories: Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL). The major difference between the two is the type of cells involved.

The lymphatic system is part of the body's immune system and helps fight infection. It is a complex system made up of organs, such as the bone marrow, the thymus, and the spleen, and the lymph nodes (or lymph glands), which are connected by a network of tiny lymphatic vessels. Lymph nodes are found all over the body.

Lymphocytes are white blood cells that circulate through the lymphatic system; they are essential components of the body's immune system. There are two main types of lymphocytes: B-cells and T-cells. Most lymphocytes start growing in the bone marrow. The B-cells continue to develop in the bone marrow, whereas the T-cells go from the bone marrow to the thymus gland and mature there. Once they are mature, both B-cells and T-cells help the body fight infections.

There are more than 20 different types of non-Hodgkin's lymphoma. Diffuse large B-cell lymphoma is a common type, making up about 40% of all cases. It is a cancer of the B-lymphocytes. Diffuse B-cell lymphoma can occur at any time from adolescence to old age. It is slightly more common in men than women.

Non-Hodgkin's lymphomas are also divided into one of two groups: low and high grade. Low-grade lymphomas are usually slowly growing and high-grade lymphomas tend to grow more quickly. Diffuse large B-cell lymphoma is a high-grade lymphoma and needs prompt treatment.

The main treatment for diffuse large B-cell lymphoma is chemotherapy. The type of chemotherapy depends on the extent of the lymphoma and other factors, such as age and general health. The two drugs that are usually given to treat diffuse large B-cell lymphoma, are called doxorubicin and cyclophosphomide. They are usually given together with other anti-cancer drugs. Currently the most widely used combination is called the 'CHOP' regime. This includes the drugs vincristine and prednisolone, as well as doxorubicin and cyclophosphomide. The chemotherapy can usually be given as an outpatient at hospital and continues for four to six months.

In addition, high dose chemotherapy with bone marrow or stem cell infusions has been effective in some patients whose lymphoma has relapsed. This type of treatment

involves having very intensive chemotherapy and sometimes radiotherapy. As side effects can be severe, some types of transplant are not given to people over the age of 45-50 and others can be given to people of up to 65 years who are fit enough to have treatment. The intensity of the treatment increases the risks of serious side effects for people over this age.

Radiotherapy may also be used when the lymphoma cells are contained in one or two areas of lymph nodes in the same part of the body (Stage 1 or 2). It may also be given in addition to chemotherapy.

Another treatment that has been tried is a monoclonal antibody called rituximab.

For many years there have been two principal strategies for chemotherapeutic treatment of cancer: 1) blocking hormone-dependent tumor cell proliferation by interference with the production or peripheral action of sex hormones; and 2) killing cancer cells directly by exposing them to cytotoxic substances, which injure both neoplastic and normal cell populations.

Despite many advances in the field of oncology, the majority of solid tumors remain incurable in the advanced stages. Cytotoxic therapy is used in most cases, however, it often causes significant morbidity to the patient without significant clinical benefit. Less toxic and more specific agents to treat and control advanced malignancies are needed.

An alternate approach for cancer chemotherapy is induction of terminal differentiation of the neoplastic cells (1). In cell culture models differentiation has been reported by exposure of cells to a variety of stimuli, including: cyclic AMP and retinoic acid (2,3), aclarubicin and other anthracyclines (4).

There is abundant evidence that neoplastic transformation does not necessarily destroy the potential of cancer cells to differentiate (1,5,6). There are many examples of tumor cells which do not respond to the normal regulators of proliferation and appear to be blocked in the expression of their differentiation program, and yet can be induced to differentiate and cease replicating. A variety of agents, including some relatively simple polar compounds (5,7-9), derivatives of vitamin D and retinoic acid (10-12), steroid hormones (13), growth factors (6,14), proteases (15,16), tumor promoters (17,18), and inhibitors of DNA or RNA synthesis (4,19-24), can induce various transformed cell lines and primary human tumor explants to express more differentiated characteristics.

Early studies identified a series of polar compounds that were effective inducers of differentiation in a number of transformed cell lines (8,9). Of these, the most effective inducer was the hybrid polar/apolar compound N,N'-hexamethylene bisacetamide (HMBA)

(9). The use of this polar/apolar compound to induce murine erythroleukemia cells (MELC) to undergo erythroid differentiation with suppression of oncogenicity has proved a useful model to study inducer-mediated differentiation of transformed cells (5,7-9). HMBA-induced MELC terminal erythroid differentiation is a multi-step process. Upon addition of HMBA to MELC (745A-DS19) in culture, there is a latent period of 10 to 12 hours before commitment to terminal differentiation is detected. Commitment is defined as the capacity of cells to express terminal differentiation despite removal of inducer (25). Upon continued exposure to HMBA there is progressive recruitment of cells to differentiate. The present inventors have reported that MELC cell lines made resistant to relatively low levels of vincristine become markedly more sensitive to the inducing action of HMBA and can be induced to differentiate with little or no latent period (26).

HMBA is capable of inducing phenotypic changes consistent with differentiation in a broad variety of cells lines (5). The characteristics of the drug-induced effect have been most extensively studied in the murine erythroleukemia cell system (MELC) (5,25,27,28). MELC induction of differentiation is both time and concentration dependent. The minimum concentration required to demonstrate an effect in vitro in most strains is 2 to 3 mM; the minimum duration of continuous exposure generally required to induce differentiation in a substantial portion (> 20%) of the population without continuing drug exposure is about 36 hours.

The primary target of action of HMBA is not known. There is evidence that protein kinase C is involved in the pathway of inducer-mediated differentiation (29). The in vitro studies provided a basis for evaluating the potential of HMBA as a cytodifferentiation agent in the treatment of human cancers (30). Several phase I clinical trials with HMBA have been completed (31-36). Clinical trials have shown that this compound can induce a therapeutic response in patients with cancer (35,36). However, these phase I clinical trials also have demonstrated that the potential efficacy of HMBA is limited, in part, by dose-related toxicity which prevents achieving optimal blood levels and by the need for intravenous administration of large quantities of the agent, over prolonged periods.

It has been reported that a number of compounds related to HMBA with polar groups separated by apolar linkages that, on a molar basis, are as active (37) or 100 times more active than HMBA (38). As a class, however, it has been found that the symmetrical dimers such as HMBA and related compounds are not the best cytodifferentiating agents.

It has unexpectedly been found that the best compounds comprise two polar end groups separated by a flexible chain of methylene groups, wherein one or both of the polar end groups is a large hydrophobic group. Preferably, the polar end groups are different and only one is a large hydrophobic group. These compounds are unexpectedly a thousand times more active than HMBA and ten times more active than HMBA related compounds.

Histone deacetylase inhibitors such as suberoylanilide hydroxamic acid (SAHA), belong to this class of agents that have the ability to induce tumor cell growth arrest, differentiation, and/or apoptosis (39). These compounds are targeted towards mechanisms inherent to the ability of a neoplastic cell to become malignant, as they do not appear to have toxicity in doses effective for inhibition of tumor growth in animals (40). There are several lines of evidence that histone acetylation and deacetylation are mechanisms by which transcriptional regulation in a cell is achieved (41). These effects are thought to occur through changes in the structure of chromatin by altering the affinity of histone proteins for coiled DNA in the nucleosome.

There are five types of histones that have been identified (designated H1, H2A, H2B, H3 and H4). Histones H2A, H2B, H3, and H4 are found in the nucleosomes and H1 is a linker located between nucleosomes. Each nucleosome contains two of each histone type within its core, except for H1, which is present singly in the outer portion of the nucleosome structure. It is believed that when the histone proteins are hypoacetylated, there is a greater affinity of the histone to the DNA phosphate backbone. This affinity causes DNA to be tightly bound to the histone and renders the DNA inaccessible to transcriptional regulatory elements and machinery.

The regulation of acetylated states occurs through the balance of activity between two enzyme complexes, histone acetyl transferase (HAT) and histone deacetylase (HDAC). The hypoacetylated state is thought to inhibit transcription of associated DNA. This hypoacetylated state is catalyzed by large multiprotein complexes that include HDAC enzymes. In particular, HDACs have been shown to catalyze the removal of acetyl groups from the chromatin core histones.

The inhibition of HDAC by SAHA is thought occur through direct interaction with the catalytic site of the enzyme as demonstrated by X-ray crystallography studies (42). The result of HDAC inhibition is not believed to have a generalized effect on the genome, but rather, only affects a small subset of the genome (43). Evidence provided by DNA microarrays using malignant cell lines cultured with a HDAC inhibitor shows that there are

a finite (1-2%) number of genes whose products are altered. For example, cells treated in culture with HDAC inhibitors show a consistent induction of the cyclin-dependent kinase inhibitor p21 (44). This protein plays an important role in cell cycle arrest. HDAC inhibitors are thought to increase the rate of transcription of p21 by propagating the hyperacetylated state of histones in the region of the p21 gene, thereby making the gene accessible to transcriptional machinery. Genes whose expression is not affected by HDAC inhibitors do not display changes in the acetylation of regional associated histones (45).

It has been shown in several instances that the disruption of HAT or HDAC activity is implicated in the development of a malignant phenotype. For instance, in acute promyelocytic leukemia, the oncoprotein produced by the fusion of PML and RAR alpha appears to suppress specific gene transcription through the recruitment of HDACs (46). In this manner, the neoplastic cell is unable to complete differentiation and leads to excess proliferation of the leukemic cell line.

U.S. Patent Numbers 5,369,108, 5,932,616, 5,700,811, 6,087,367 and 6,511, 990, issued to some of the present inventors, disclose compounds useful for selectively inducing terminal differentiation of neoplastic cells, which compounds have two polar end groups separated by a flexible chain of methylene groups or a by a rigid phenyl group, wherein one or both of the polar end groups is a large hydrophobic group. Some of the compounds have an additional large hydrophobic group at the same end of the molecule as the first hydrophobic group which further increases differentiation activity about 100 fold in an enzymatic assay and about 50 fold in a cell differentiation assay. Methods of synthesizing the compounds used in the methods and pharmaceutical compositions of this invention are fully described the aforementioned patents, the entire contents of which are incorporated herein by reference.

In addition to their biological activity as antitumor agents, the compounds disclosed in the aforementioned patents have recently been identified as useful for treating or preventing a wide variety of thioredoxin (TRX)-mediated diseases and conditions, such as inflammatory diseases, allergic diseases, autoimmune diseases, diseases associated with oxidative stress of diseases characterized by cellulora hyperproliferation (U.S. Application No. 10/369,094, filed February 15, 2003. Further, these compounds have been identified as useful for treating diseases of the central nervous system (CNS) such as neurodegenerative diseases and for treating brain cancer (See, U.S. Application No. 10.273,401, filed October 16, 2002).

The aforementioned patents do not disclose specific oral formulations of the HDAC inhibitors or specific dosages and dosing schedules of the recited compounds that are effective at treating cancer, e.g., mesothelioma or lymphoma. Importantly, the aforementioned patents do not disclose oral formulations that have favorable pharmacokinetic profiles such as high bioavailability which gives rise to high blood levels of the active compounds over an extended period of time.

There is an urgent need to discover suitable dosages and dosing schedules of these compounds, and to develop formulations, preferably oral formulations, which give rise to steady, therapeutically effective blood levels of the active compounds over an extended period of time, and which are effective at treating cancer.

SUMMARY OF THE INVENTION

The present invention relates to methods of treating cancers, e.g., mesothelioma or lymphoma. More specifically, the present invention relates to methods of treating mesothelioma or diffuse large B-cell lymphoma (DLBCL), by administration of pharmaceutical compositions comprising HDAC inhibitors, e.g., suberoylanilide hydroxamic acid (SAHA). In particular aspects, the methods of the invention are used to treat mesothelioma. In other aspects, the methods of the invention are used to treat lymphoma, e.g., DLBCL.

The oral formulations of the pharmaceutical compositions have favorable pharmacokinetic profiles such as high bioavailability and surprisingly give rise to high blood levels of the active compounds over an extended period of time. The present invention further provides a safe, daily dosing regimen of these pharmaceutical compositions, which is easy to follow, and which results in a therapeutically effective amount of the HDAC inhibitors *in vivo*.

In one embodiment, the present invention provides a method of treating mesothelioma or DLBCL in a subject in need thereof, by administering to the subject a pharmaceutical composition comprising an effective amount of suberoylanilide hydroxamic acid (SAHA) or a pharmaceutically acceptable salt or hydrate thereof, as described herein. SAHA can be administered in a total daily dose of up to 800 mg, preferably orally, once, twice, or three times daily, continuously (every day) or intermittently (e.g., 3-5 days a week).

Oral SAHA has been safely administered in phase I clinical studies to patients suffering from mesothelioma or DLBCL.

Furthermore, the present invention provides a method of treating mesothelioma or DLBCL in a subject in need thereof, by administering to the subject a pharmaceutical composition comprising an effective amount of an HDAC inhibitor as described herein, or a pharmaceutically acceptable salt or hydrate thereof. The HDAC inhibitor can be administered in a total daily dose of up to 800 mg, preferably orally, once, twice or three times daily, continuously (i.e., every day) or intermittently (e.g., 3-5 days a week).

The HDAC inhibitors and methods of the present invention are useful in the treatment of a wide variety of cancers, e.g., lymphoma, including Hodgkin's disease (HD) and Non-Hodgkin's lymphoma (NHL). In one embodiment, the HDAC inhibitors are useful at treating mesothelioma or a large cell lymphoma, including diffuse large B-cell lymphoma (DLBCL). As defined herein "large cell lymphoma" is a lymphoma that is characterized by unusually large cells.

HDAC inhibitors suitable for use in the present invention include but are not limited to hydroxamic acid derivatives, Short Chain Fatty Acids (SCFAs), cyclic tetrapeptides, benzamide derivatives, or electrophilic ketone derivatives, as defined herein. Specific non-limiting examples of HDAC inhibitors suitable for use in the methods of the present invention are:

A) **Hydroxamic acid derivatives** selected from m-carboxycinnamic acid bishydroxamide (CBHA), Trichostatin A (TSA), Trichostatin C, Salicylhydroxamic Acid, Azelaic Bishydroxamic Acid (ABHA), Azelaic-1-Hydroxamate-9-Anilide (AAHA), 6-(3-Chlorophenylureido) carpoic Hydroxamic Acid (3Cl-UCHA), Oxamflatin, A-161906, Scriptaid, PXD-101, LAQ-824, CHAP, MW2796, and MW2996;

B) **Cyclic tetrapeptides** selected from Trapoxin A (TPX)-cyclic tetrapeptide (cyclo-(L-phenylalanyl-L-phenylalanyl-D-pipecolinyl-L-2-amino-8-oxo-9,10-epoxy decanoyl); FR901228 (FK 228, Depsipeptide); FR225497 cyclic tetrapeptide; Apicidin cyclic tetrapeptide [cyclo(N-O-methyl-L-tryptophanyl-L-isoleucinyl-D-pipecolinyl-L-2-amino-8-oxodecanoyl)]; Apicidin Ia, Apicidin Ib, Apicidin Ic, Apicidin IIa, and Apicidin IIb; CHAP, HC-toxin cyclic tetrapeptide; WF27082 cyclic; and Chlamydocin;

C) **Short Chain Fatty Acids** (SCFAs) selected from Sodium Butyrate, Isovalerate, Valerate, 4 Phenylbutyrate (4-PBA), Phenylbutyrate (PB), Propionate,

Butyramide, Isobutyramide, Phenylacetate, 3-Bromopropionate, Tributyrin, Valproic Acid and Valproate and Pivanex™;

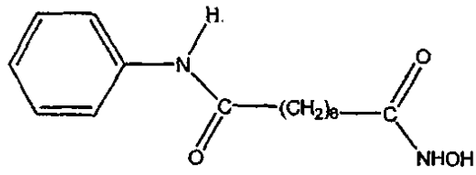
D) **Benzamide Derivatives** selected from CI-994, MS-27-275 (MS-275) [N- (2-aminophenyl)-4-[N-(pyridin-3-ylmethoxycarbonyl) aminomethyl] benzamide] and a 3'-amino derivative of MS-27-275;

E) **Electrophilic Ketone Derivatives** selected from a trifluoromethyl ketone and an α-keto amide such as an N-methyl-α-ketoamide; and

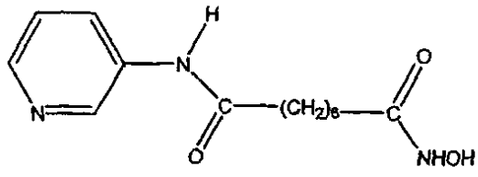
F) **Miscellaneous HDAC inhibitors** including natural products, psammaplins, and Depudecin.

Specific HDAC inhibitors include, for example:

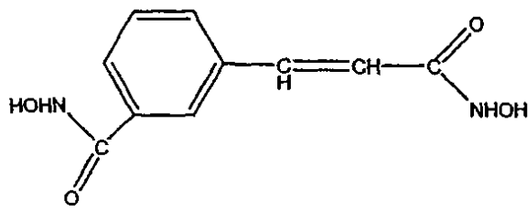
Suberoylanilide hydroxamic acid (SAHA), which is represented by the following structural formula:



Pyroxamide, which is represented by the following structural formula:

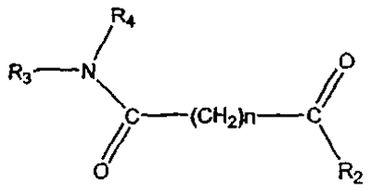


m-Carboxycinnamic acid bishydroxamide (CBHA), which is represented by the structural formula:



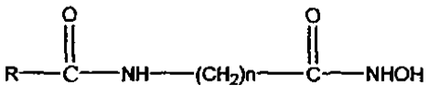
Other non-limiting examples of HDAC inhibitors that are suitable for use in the methods of the present invention are:

A compound represented by the structure:



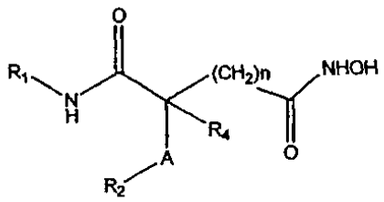
wherein R_3 and R_4 are independently a substituted or unsubstituted, branched or unbranched alkyl, alkenyl, cycloalkyl, aryl, alkyloxy, aryloxy, arylalkyloxy, or pyridine group, cycloalkyl, aryl, aryloxy, arylalkyloxy, or pyridine group, or R_3 and R_4 bond together to form a piperidine group; R_2 is a hydroxylamino group; and n is an integer from 5 to 8.

A compound represented by the structure:



wherein R is a substituted or unsubstituted phenyl, piperidine, thiazole, 2-pyridine, 3- pyridine or 4-pyridine and n is an integer from 4 to 8.

A compound represented by the structure:



wherein A is an amide moiety, R_1 and R_2 are each selected from substituted or unsubstituted aryl, arylalkyl, naphthyl, pyridineamino, 9-purine-6-amino, thiazoleamino, aryloxy, arylalkyloxy, pyridyl, quinolinyl or isoquinolinyl; R_4 is hydrogen, a halogen, a phenyl or a cycloalkyl moiety and n is an integer from 3 to 10.

In one embodiment, the pharmaceutical compositions comprising the HDAC inhibitor are administered orally, for example within a gelatin capsule. In a further embodiment, the pharmaceutical compositions are further comprised of microcrystalline cellulose, croscarmellose sodium and magnesium stearate.

The HDAC inhibitors can be administered in a total daily dose that may vary from patient to patient, and may be administered at varying dosage schedules. Suitable dosages are total daily dosage of between about 25-4000 mg/m^2 administered orally once-daily, twice-daily, or three times-daily, continuous (every day) or intermittently (e.g., 3-5 days a week). Furthermore, the compositions may be administered in cycles, with rest periods in

between the cycles (e.g., treatment for two to eight weeks with a rest period of up to a week between treatments).

In one embodiment, the composition is administered once daily at a dose of about 200-600 mg. In another embodiment, the composition is administered twice daily at a dose of about 200-400 mg. In another embodiment, the composition is administered twice daily at a dose of about 200-400 mg intermittently, for example three, four, or five days per week. In another embodiment, the composition is administered three times daily at a dose of about 100-250 mg.

In one embodiment, the daily dose is 200 mg, which can be administered once-daily, twice-daily, or three-times daily. In one embodiment, the daily dose is 300 mg, which can be administered once-daily or twice-daily. In one embodiment, the daily dose is 400 mg, which can be administered once-daily or twice-daily.

The present invention also provides methods for selectively inducing terminal differentiation, cell growth arrest and/or apoptosis of neoplastic cells, e.g., lymphoma cells in a subject, thereby inhibiting proliferation of such cells in said subject, by administering to the subject a pharmaceutical composition comprising an effective amount of an HDAC inhibitor, e.g., SAHA, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or diluent. An effective amount of an HDAC inhibitor in the present invention can be up to a total daily dose of 800 mg.

The present invention also provides methods for inhibiting the activity of a histone deacetylase in a subject, by administering to the subject a pharmaceutical composition comprising an effective amount of an HDAC inhibitor, e.g., SAHA, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or diluent. An effective amount of an HDAC inhibitor in the present invention can be up to a total daily dose of 800 mg.

The present invention also provides *in-vitro* methods for selectively inducing terminal differentiation, cell growth arrest and/or apoptosis of neoplastic cells, e.g., lymphoma cells, thereby inhibiting proliferation of such cells, by contacting the cells with an effective amount of a an HDAC inhibitor, e.g., SAHA, or a pharmaceutically acceptable salt or hydrate thereof.

The present invention also provides *in-vitro* methods for inhibiting the activity of a histone deacetylase, by the histone deacetylase with an effective amount of an HDAC inhibitor, e.g., SAHA, or a pharmaceutically acceptable salt or hydrate thereof.

The present invention further provides a safe, daily dosing regimen of the formulation of pharmaceutical compositions comprising an HDAC inhibitor that is easy to follow and to adhere to. These pharmaceutical compositions are suitable for oral administration and are useful for treating cancer, e.g., mesothelioma, lymphoma, or other cancers or tumors, by selectively inducing terminal differentiation, cell growth arrest, and/or apoptosis of neoplastic cells, and/or -by inhibiting histone deacetylase (HDAC).

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

FIG. 1 is a picture of a Western blot (top panel) showing the quantities of acetylated histone-4 (α-AcH4) in the blood plasma of patients following an oral or intravenous (IV) dose of SAHA. IV SAHA was administered at 200 mg infused over two hours. Oral SAHA was administered in a single capsule at 200 mg. The amount of α-AcH4 is shown at the indicated time points. Bottom panel: Coomassie blue stain.

FIG. 2 is a picture of a Western blot (top panels) showing the quantities of acetylated histone-4 (α-AcH4) in the blood plasma of patients having a solid tumor, following an oral or intravenous (IV) dose of SAHA. IV and Oral SAHA were administered as in Figure 1. The amount of α-AcH4 is shown at the indicated time points. The experiment is shown in duplicate (Fig 2A and Fig 2B). Bottom panels: Coomassie blue stain.

FIG. 3 is a picture of a Western blot (top panels) showing the quantities of acetylated histone-4 (α-AcH4) (Figure 3A) and acetylated histone-3 (α-AcH3) (Figures 3B-E) in the blood plasma of patients following an oral or intravenous (IV) dose of SAHA, on Day 1 and Day 21. IV and Oral SAHA were administered as in Figure 1. The amount of α-AcH4 or α-AcH3 is shown at the indicated time points. Bottom panels: Coomassie blue stain.

FIG. 4 is a picture of a Western blot (top panels) showing the quantities of acetylated histone-3 (α-AcH3) in the blood plasma of patients having a solid tumor, following an oral or intravenous (IV) dose of SAHA. IV and Oral SAHA were administered as in Figure 1. The amount of α-AcH3 is shown at the indicated time points. Bottom panel: Coomassie blue stain.

FIG. 5 is a picture of a Western blot (top panels) showing the quantities of acetylated histone-3 (α-AcH3) in the blood plasma of patients following an oral or intravenous (IV) dose of SAHA. IV SAHA was administered at 400 mg infused over two hours. Oral SAHA was administered in a single capsule at 400 mg. The amount of α-AcH4 is shown at the indicated time points. The experiment is shown in triplicate (Fig 5A and B). Bottom panels: Coomassie blue stain.

FIG. 6 is a picture of a Western blot (top panel) showing the quantities of acetylated histone-3 (α-AcH3) in the blood plasma of patients having a solid tumor, following an oral or intravenous (IV) dose of SAHA. IV and Oral SAHA were administered as in Figure 5. The amount of α-AcH3 is shown at the indicated time points. Bottom panel: Coomassie blue stain.

FIG. 7 is a picture of a Western blot (top panels) showing the quantities of acetylated histone-3 (α-AcH3) in the blood plasma of patients having a solid tumor following an oral or intravenous (IV) dose of SAHA, on Day 1 and Day 21. IV and Oral SAHA were administered as in Figure 4. The amount of α-AcH4 or α-AcH3 is shown at the indicated time points. The experiment is shown in triplicate (Fig 7 A-C). Bottom panels: Coomassie blue stain.

FIG. 8 is a picture of a Western blot (top panels) showing the quantities of acetylated histone-3 (α-AcH3) in the blood plasma of patients following an oral or intravenous (IV) dose of SAHA. IV and Oral SAHA were administered as in Figure 5. The amount of α-AcH3 is shown at the indicated time points. Bottom panels: Coomassie blue stain.

FIGS. 9A-C are graphs showing the mean plasma concentration of SAHA (ng/ml) at the indicated time points following administration. Fig 9A: Oral dose (200 mg and 400 mg) under fasting on Day 8. Fig 9B: Oral dose (200 mg and

400 mg) with food on Day 9. Fig 9C: IV dose on day 1.

FIG. 10 shows the apparent half-life of a SAHA 200 mg and 400 mg oral dose, on Days 8, 9 and 22.

FIG. 11 shows the AUC (ng/ml/hr) of a SAHA 200 mg and 400 mg oral dose, on Days 8, 9 and 22.

FIG. 12 shows the bioavailability of SAHA after a 200 mg and 400 mg oral dose, on Days 8, 9 and 22.

FIG. 13 is a CT Scan of a mesothelioma tumor of a patient, before (left) and after (right) six months of treatment with SAHA at a dose of 300 mg twice daily 3 days a week.

FIG. 14 is a CT scan taken from a patient with Diffuse Large Cell Lymphoma, before treatment (A) and after (B) 2 months of treatment with SAHA at a dose of 400 mg BID for 1 month followed 400 mg QD for one month.

FIG. 15 is a PET scan taken from a patient with Diffuse Large Cell Lymphoma, before treatment (A) and after (B) 2 months of treatment with SAHA at a dose of 400 mg BID for 1 month followed 400 mg QD for one month.

FIG. 16 is a CT scan taken from a patient with Diffuse Large Cell Lymphoma, before treatment (A) and after (B) 1 month of treatment with SAHA at a dose of 600 mg QD.

FIG. 17 is a PET scan taken from a patient with Diffuse Large Cell Lymphoma, before treatment (A) and after (B) 2 months of treatment with SAHA at a dose of 200 mg BID.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods of treating cancers, e.g., mesothelioma or lymphoma. More specifically, the present invention relates to methods of treating mesothelioma or diffuse large B-cell lymphoma (DLBCL), by administration of pharmaceutical compositions comprising HDAC inhibitors, e.g., suberoylanilide hydroxamic acid (SAHA). In specific aspects, the methods of the invention are used to treat mesothelioma. In other aspects, the methods of the invention are used to treat lymphoma, including DLBCL.

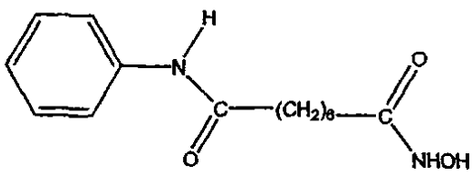
The oral formulations of the pharmaceutical compositions of the invention have favorable pharmacokinetic profiles such as high bioavailability and surprisingly give rise to

high blood levels of the active compounds over an extended period of time. Thus, the present invention further provides a safe, daily dosing regimen of these pharmaceutical compositions, which is easy to follow, and which results in a therapeutically effective amount of the HDAC inhibitors *in vivo*.

Accordingly, in one embodiment, the present invention provides a method of treating mesothelioma or DLBCL in a subject in need thereof, by administering to the subject a pharmaceutical composition comprising an effective amount of an HDAC inhibitor as described herein, or a pharmaceutically acceptable salt or hydrate thereof. The HDAC inhibitor can be administered in a total daily dose of up to 800 mg, preferably orally, once, twice or three times daily, continuously (i.e., every day) or intermittently (e.g., 3-5 days a week).

In one embodiment, the HDAC inhibitor is suberoylanilide hydroxamic acid (SAHA). In another embodiment, the HDAC inhibitor is a hydroxamic acid derivative as described herein. In another embodiment, the HDAC inhibitor is represented by any of the structure of formulas 1-51 described herein. In another embodiment, the HDAC inhibitor is a benzamide derivative as described herein. In another embodiment, the HDAC inhibitor is a cyclic tetrapeptide as described herein. In another embodiment, the HDAC inhibitor is a Short Chain Fatty Acid (SCFA) as described herein. In another embodiment, the HDAC inhibitor is an electrophilic ketone as described herein. In another embodiment, the HDAC inhibitor is depudecin. In another embodiment, the HDAC inhibitor is a natural product. In another embodiment, the HDAC inhibitor is a psammaplin.

In one particular embodiment, the present invention provides a method of treating mesothelioma or DLBCL in a subject in need thereof, by administering to the subject a pharmaceutical composition comprising an effective amount of suberoylanilide hydroxamic acid (SAHA) or a pharmaceutically acceptable salt or hydrate thereof, as described herein. SAHA can be administered in a total daily dose of up to 800 mg, preferably orally, once, twice, or three times daily, continuously (every day) or intermittently (e.g., 3-5 days a week). SAHA is represented by the following structure:



In another particular embodiment, the present invention relates to a method of treating mesothelioma or DLBCL in a subject, comprising the step of administering to the subject an effective amount of a pharmaceutical composition comprising a histone deacetylase (HDAC) inhibitor represented by any of the structure described herein as by formulas 1-51 described herein, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or diluent, wherein the amount of the histone deacetylase inhibitor is effective to treat mesothelioma or DLBCL in the subject.

The term "treating" in its various grammatical forms in relation to the present invention refers to preventing (i.e., chemoprevention), curing, reversing, attenuating, alleviating, minimizing, suppressing or halting the deleterious effects of a disease state, disease progression, disease causative agent (e.g., bacteria or viruses) or other abnormal condition. For example, treatment may involve alleviating a symptom (i.e., not necessary all symptoms) of a disease or attenuating the progression of a disease. Because some of the inventive methods involve the physical removal of the etiological agent, the artisan will recognize that they are equally effective in situations where the inventive compound is administered prior to, or simultaneous with, exposure to the etiological agent (prophylactic treatment) and situations where the inventive compounds are administered after (even well after) exposure to the etiological agent.

Treatment of cancer, as used herein, refers to partially or totally inhibiting, delaying or preventing the progression of cancer including cancer metastasis; inhibiting, delaying or preventing the recurrence of cancer including cancer metastasis; or preventing the onset or development of cancer (chemoprevention) in a mammal, for example a human.

As used herein, the term "therapeutically effective amount" is intended to encompass any amount that will achieve the desired biological response. In the present invention, the desired biological response is partial or total inhibition, delay, or prevention of the progression of cancer including cancer metastasis; inhibition, delay or prevention of the recurrence of cancer including cancer metastasis; or the prevention of the onset or development of cancer (chemoprevention) in a mammal, for example a human.

The method of the present invention is intended for the treatment or chemoprevention of human patients with cancer. However, it is also likely that the method would be effective in the treatment of cancer in other mammals.

Histone Deacetylases and Histone Deacetylase Inhibitors

Histone deacetylases (HDACs), as that term is used herein, are enzymes that catalyze the removal of acetyl groups from lysine residues in the amino terminal tails of the nucleosomal core histones. As such, HDACs together with histone acetyl transferases (HATs) regulate the acetylation status of histones. Histone acetylation affects gene expression and inhibitors of HDACs, such as the hydroxamic acid-based hybrid polar compound suberoylanilide hydroxamic acid (SAHA) induce growth arrest, differentiation and/or apoptosis of transformed cells *in vitro* and inhibit tumor growth *in vivo*. HDACs can be divided into three classes based on structural homology. Class I HDACs (HDACs 1, 2, 3 and 8) bear similarity to the yeast RPD3 protein, are located in the nucleus and are found in complexes associated with transcriptional co-repressors. Class II HDACs (HDACs 4, 5, 6, 7 and 9) are similar to the yeast HDA1 protein, and have both nuclear and cytoplasmic subcellular localization. Both Class I and II HDACs are inhibited by hydroxamic acid-based HDAC inhibitors, such as SAHA. Class III HDACs form a structurally distant class of NAD dependent enzymes that are related to the yeast SIR2 proteins and are not inhibited by hydroxamic acid-based HDAC inhibitors.

Histone deacetylase inhibitors or HDAC inhibitors, as that term is used herein are compounds that are capable of inhibiting the deacetylation of histones *in vivo, in vitro* or both. As such, HDAC inhibitors inhibit the activity of at least one histone deacetylase. As a result of inhibiting the deacetylation of at least one histone, an increase in acetylated histone occurs and accumulation of acetylated histone is a suitable biological marker for assessing the activity of HDAC inhibitors. Therefore, procedures that can assay for the accumulation of acetylated histones can be used to determine the HDAC inhibitory activity of compounds of interest. It is understood that compounds that can inhibit histone deacetylase activity can also bind to other substrates and as such can inhibit other biologically active molecules such as enzymes. It is also to be understood that the compounds of the present invention are capable of inhibiting any of the histone deacetylases set forth above, or any other histone deacetylases.

For example, in patients receiving HDAC inhibitors, the accumulation of acetylated histones in peripheral mononuclear cells as well as in tissue treated with HDAC inhibitors can be determined against a suitable control.

HDAC inhibitory activity of a particular compound can be determined *in vitro* using, for example, an enzymatic assays which shows inhibition of at least one histone deacetylase. Further, determination of the accumulation of acetylated histones in cells

treated with a particular composition can be determinative of the HDAC inhibitory activity of a compound.

Assays for the accumulation of acetylated histones are well known in the literature. See, for example, Marks, P.A. *et al.*, J. Natl. Cancer Inst., 92:1210-1215, 2000, Butler, L.M. *et al.*, Cancer Res. 60:5165-5170 (2000), Richon, V. M. *et al.*, Proc. Natl. Acad. Sci., USA, 95:3003-3007, 1998, and Yoshida, M. *et al.*, J. Biol. Chem., 265:17174-17179, 1990.

For example, an enzymatic assay to determine the activity of an HDAC inhibitor compound can be conducted as follows. Briefly, the effect of an HDAC inhibitor compound on affinity purified human epitope-tagged (Flag) HDAC1 can be assayed by incubating the enzyme preparation in the absence of substrate on ice for about 20 minutes with the indicated amount of inhibitor compound. Substrate ([^{3}H]acetyl-labeled murine erythroleukemia cell-derived histone) can be added and the sample can be incubated for 20 minutes at 37°C in a total volume of 30 μL. The reaction can then be stopped and released acetate can be extracted and the amount of radioactivity release determined by scintillation counting. An alternative assay useful for determining the activity of an HDAC inhibitor compound is the "HDAC Fluorescent Activity Assay; Drug Discovery Kit-AK-500" available from BIOMOL® Research Laboratories, Inc., Plymouth Meeting, PA.

In vivo studies can be conducted as follows. Animals, for example, mice, can be injected intraperitoneally with an HDAC inhibitor compound. Selected tissues, for example, brain, spleen, liver etc, can be isolated at predetermined times, post administration. Histones can be isolated from tissues essentially as described by Yoshida *et al.*, J. Biol. Chem. 265:17174-17179, 1990. Equal amounts of histones (about 1 μg) can be electrophoresed on 15% SDS-polyacrylamide gels and can be transferred to Hybond-P filters (available from Amersham). Filters can be blocked with 3% milk and can be probed with a rabbit purified polyclonal anti-acetylated histone H4 antibody (α-Ac-H4) and anti-acetylated histone H3 antibody (α-Ac-H3) (Upstate Biotechnology, Inc.). Levels of acetylated histone can be visualized using a horseradish peroxidase-conjugated goat anti-rabbit antibody (1:5000) and the SuperSignal chemiluminescent substrate (Pierce). As a loading control for the histone protein, parallel gels can be run and stained with Coomassie Blue (CB).

In addition, hydroxamic acid-based HDAC inhibitors have been shown to up regulate the expression of the $p21^{WAF1}$ gene. The $p21^{WAF1}$ protein is induced within 2 hours of culture with HDAC inhibitors in a variety of transformed cells using standard methods. The

induction of the $p21^{WAF1}$ gene is associated with accumulation of acetylated histones in the chromatin region of this gene. Induction of $p21^{WAF1}$ can therefore be recognized as involved in the G1 cell cycle arrest caused by HDAC inhibitors in transformed cells.

Typically, HDAC inhibitors fall into five general classes: 1) hydroxamic acid derivatives; 2) Short-Chain Fatty Acids (SCFAs); 3) cyclic tetrapeptides; 4) benzamides; and 5) electrophilic ketones.

Thus, the present invention includes within its broad scope compositions comprising HDAC inhibitors which are 1) hydroxamic acid derivatives; 2) Short-Chain Fatty Acids (SCFAs); 3) cyclic tetrapeptides; 4) benzamides; 5) electrophilic ketones; and/or any other class of compounds capable of inhibiting histone deacetylases, for use in inhibiting histone deacetylase, inducing terminal differentiation, cell growth arrest and/or apoptosis in neoplastic cells, and/or inducing differentiation, cell growth arrest and/or apoptosis of tumor cells in a tumor.

Non-limiting examples of such HDAC inhibitors are set forth below. It is understood that the present invention includes any salts, crystal structures, amorphous structures, hydrates, derivatives, metabolites, stereoisomers, structural isomers, and prodrugs of the HDAC inhibitors described herein.

A. **Hydroxamic Acid Derivatives** such as suberoylanilide hydroxamic acid (SAHA) (Richon *et al.*, Proc. Natl. Acad. Sci. USA 95,3003-3007 (1998)); m-carboxycinnamic acid bishydroxamide (CBHA) (Richon *et al.*, supra); pyroxamide; trichostatin analogues such as trichostatin A (TSA) and trichostatin C (Koghe *et al.* 1998. Biochem. Pharmacol. 56: 1359-1364); salicylhydroxamic acid (Andrews *et al.*, International J. Parasitology 30,761-768 (2000)); suberoyl bishydroxamic acid (SBHA) (U.S. Patent No. 5,608,108); azelaic bishydroxamic acid (ABHA) (Andrews *et al.*, supra); azelaic-1-hydroxamate-9-anilide (AAHA) (Qiu *et al.*, Mol. Biol. Cell 11, 2069-2083 (2000)); 6-(3-chlorophenylureido) carpoic hydroxamic acid (3Cl-UCHA); oxamflatin [(2E)-5-[3-[(phenylsufonyl) aminol phenyl]-pent-2-en-4-ynohydroxamic acid] (Kim *et al.* Oncogene, 18: 2461 2470 (1999)); A-161906, Scriptaid (Su *et al.* 2000 Cancer Research, 60: 3137-3142); PXD-101 (Prolifix); LAQ-824; CHAP; MW2796 (Andrews *et al.*, supra); MW2996 (Andrews *et al.*, supra); or any of the hydroxamic acids disclosed in U.S. Patent Numbers 5,369,108, 5,932,616, 5,700,811, 6,087,367 and 6,511, 990.

B. **Cyclic Tetrapeptides** such as trapoxin A (TPX)-cyclic tetrapeptide (cyclo-(L-phenylalanyl-L-phenylalanyl-D-pipecolinyl-L-2-amino-8-oxo-9,10-epoxy decanoyl))

(Kijima *et al.*, J Biol. Chem. 268,22429-22435 (1993)); FR901228 (FK 228, depsipeptide) (Nakajima *et al.*, Ex. Cell Res. 241,126-133 (1998)); FR225497 cyclic tetrapeptide (H. Mori *et al.*, PCT Application WO 00/08048 (17 February 2000)); apicidin cyclic tetrapeptide [cyclo(N-O-methyl-L-tryptophanyl-L -isoleucinyl-D-pipecolinyl-L-2-amino-8-oxodecanoyl)] (Darkin-Rattray *et al.*, Proc. Natl. Acad. Sci. USA 93,1314313147 (1996)); apicidin Ia, apicidin Ib, apicidin Ic, apicidin IIa, and apicidin IIb (P. Dulski *et al.*, PCT Application WO 97/11366); CHAP, HC-toxin cyclic tetrapeptide (Bosch *et al.*, Plant Cell 7, 1941-1950 (1995)); WF27082 cyclic tetrapeptide (PCT Application WO 98/48825); and chlamydocin (Bosch *et al.*, supra).

C. **Short chain fatty acid (SCFA) derivatives** such as: sodium butyrate (Cousens *et al.*, J. Biol. Chem. 254,1716-1723 (1979)); isovalerate (McBain *et al.*, Biochem. Pharm. 53: 1357-1368 (1997)); valerate (McBain *et al.*, supra) ; 4-phenylbutyrate (4-PBA) (Lea and Tulsyan, Anticancer Research, 15,879-873 (1995)); phenylbutyrate (PB) (Wang *et al.*, Cancer Research, 59, 2766-2799 (1999)); propionate (McBain *et al.*, supra); butyramide (Lea and Tulsyan, supra); isobutyramide (Lea and Tulsyan, supra); phenylacetate (Lea and Tulsyan, supra); 3-bromopropionate (Lea and Tulsyan, supra); tributyrin (Guan *et al.*, Cancer Research, 60,749-755 (2000)); valproic acid, valproate and Pivanex™.

D. **Benzamide derivatives** such as CI-994; MS-275 [N- (2-aminophenyl)-4- [N-(pyridin-3-yl methoxycarbonyl) aminomethyl] benzamide] (Saito *et al.*, Proc. Natl. Acad. Sci. USA 96, 4592-4597 (1999)); and 3'-amino derivative of MS-275 (Saito *et al.*, supra).

E. **Electrophilic ketone derivatives** such as trifluoromethyl ketones (Frey *et al*, Bioorganic & Med. Chem. Lett. (2002), 12, 3443-3447; U.S. 6,511,990) and α-keto amides such as N-methyl-α-ketoamides.

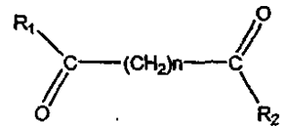
F. **Other HDAC Inhibitors** such as natural products, psammaplins, and Depudecin (Kwon *et al.* 1998. PNAS 95: 3356-3361).

Preferred hydroxamic acid based HDAC inhibitors are suberoylanilide hydroxamic acid (SAHA), m-carboxycinnamic acid bishydroxamide (CBHA), and pyroxamide. SAHA has been shown to bind directly in the catalytic pocket of the histone deacetylase enzyme. SAHA induces cell cycle arrest, differentiation, and/or apoptosis of transformed cells in culture and inhibits tumor growth in rodents. SAHA is effective at inducing these effects in both solid tumors and hematological cancers. It has been shown that SAHA is effective at inhibiting tumor growth in animals with no toxicity to the animal. The SAHA-induced

inhibition of tumor growth is associated with an accumulation of acetylated histones in the tumor. SAHA is effective at inhibiting the development and continued growth of carcinogen-induced (N-methylnitrosourea) mammary tumors in rats. SAHA was administered to the rats in their diet over the 130 days of the study. Thus, SAHA is a nontoxic, orally active antitumor agent whose mechanism of action involves the inhibition of histone deacetylase activity.

Preferred HDAC inhibitors are those disclosed in U.S. Patent Numbers 5,369,108, 5,932,616, 5,700,811, 6,087,367 and 6,511, 990, issued to some of the present inventors disclose compounds, the entire contents of which are incorporated herein by reference, non-limiting examples of which are set forth below:

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 1, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.

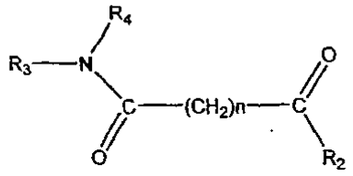


(1)

wherein R_1 and R_2 can be the same or different; when R_1 and R_2 are the same, each is a substituted or unsubstituted arylamino, cycloalkylamino, pyridineamino, piperidino, 9-purine-6-amine or thiazoleamino group; when R_1 and R_2 are different R_1=R_3-N-R_4, wherein each of R_3 and R_4 are independently the same as or different from each other and are a hydrogen atom, a hydroxyl group, a substituted or unsubstituted, branched or unbranched alkyl, alkenyl, cycloalkyl, aryl alkyloxy, aryloxy, arylalkyloxy or pyridine group, or R_3 and R_4 are bonded together to form a piperidine group, R_2 is a hydroxylamino, hydroxyl, amino, alkylamino, dialkylamino or alkyloxy group and n is an integer from about 4 to about 8.

In a particular embodiment of formula 1, R_1 and R_2 are the same and are a substituted or unsubstituted thiazoleamino group; and n is an integer from about 4 to about 8.

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 2, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



(2)

wherein each of R_3 and R_4 are independently the same as or different from each other and are a hydrogen atom, a hydroxyl group, a substituted or unsubstituted, branched or unbranched alkyl, alkenyl, cycloalkyl, arylalkyloxy, aryloxy, arylalkyloxy or pyridine group, or R_3 and R_4 are bonded together to form a piperidine group, R_2 is a hydroxylamino, hydroxyl, amino, alkylamino, dialkylamino or alkyloxy group and n is an integer from about 4 to about 8.

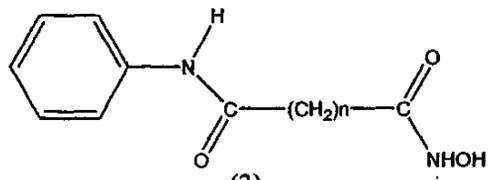
In a particular embodiment of formula 2, each of R_3 and R_4 are independently the same as or different from each other and are a hydrogen atom, a hydroxyl group, a substituted or unsubstituted, branched or unbranched alkyl, alkenyl, cycloalkyl, aryl, alkyloxy, aryloxy, arylalkyloxy, or pyridine group, or R_3 and R_4 bond together to form a piperidine group; R_2 is a hydroxylamino, hydroxyl, amino, alkylamino, or alkyloxy group; n is an integer from 5 to 7; and R_3-N-R_4 and R_2 are different.

In another particular embodiment of formula 2, n is 6. In yet another embodiment of formula 2, R_4 is a hydrogen atom, R_3 is a substituted or unsubstituted phenyl, and n is 6. In yet another embodiment of formula 2, R_4 is a hydrogen atom, R_3 is a substituted phenyl and n is 6, wherein the phenyl substituent is selected from the group consisting of a methyl, cyano, nitro, trifluoromethyl, amino, aminocarbonyl, methylcyano, chloro, fluoro, bromo, iodo, 2,3-difluoro, 2,4-difluoro, 2,5-difluoro, 3,4-difluoro, 3,5-difluoro, 2,6-difluoro, 1,2,3-trifluoro, 2,3,6-trifluoro, 2,4,6-trifluoro, 3,4,5-trifluoro, 2,3,5,6-tetrafluoro, 2,3,4,5,6-pentafluoro, azido, hexyl, t-butyl, phenyl, carboxyl, hydroxyl, methoxy, phenyloxy, benzyloxy, phenylaminooxy, phenylaminocarbonyl, methoxycarbonyl, methylaminocarbonyl, dimethylamino, dimethylamino carbonyl, or hydroxylaminocarbonyl group.

In another embodiment of formula 2, n is 6, R_4 is a hydrogen atom, and R_3 is a cyclohexyl group. In another embodiment of formula 2, n is 6, R_4 is a hydrogen atom, and R_3 is a methoxy group. In another embodiment of formula 2, n is 6 and R_3 and R_4 bond together to form a piperidine group. In another embodiment of formula 2, n is 6, R_4 is a hydrogen atom, and R_3 is a benzyloxy group. In another embodiment of formula 2, R_4 is a

hydrogen atom and R_3 is a γ-pyridine group. In another embodiment of formula 2, R_4 is a hydrogen atom and R_3 is a β-pyridine group. In another embodiment of formula 2, R_4 is a hydrogen atom and R_3 is an α-pyridine group. In another embodiment of formula 2, n is 6, and R_3 and R_4 are both methyl groups. In another embodiment of formula 2, n is 6, R_4 is a methyl group, and R_3 is a phenyl group.

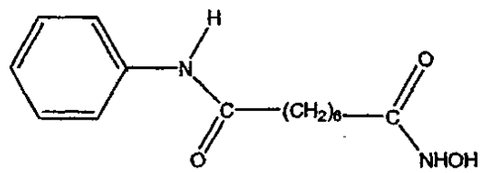
In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 3, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



(3)

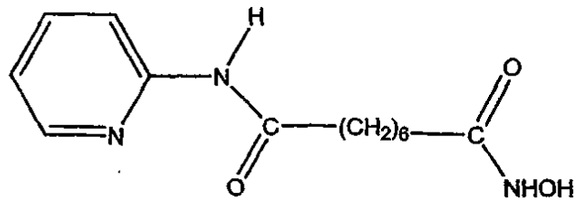
wherein n is an integer from 5 to about 8.

In a preferred embodiment of formula 3, n is 6. In accordance with this embodiment, the HDAC inhibitor is SAHA (4), or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient. SAHA can be represented by the following structural formula:



(4)

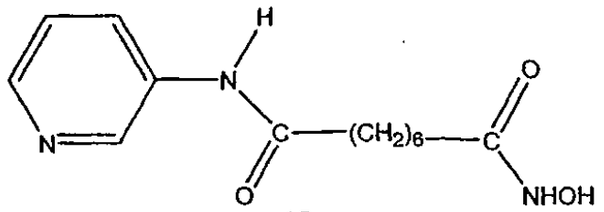
In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 5, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



(5)

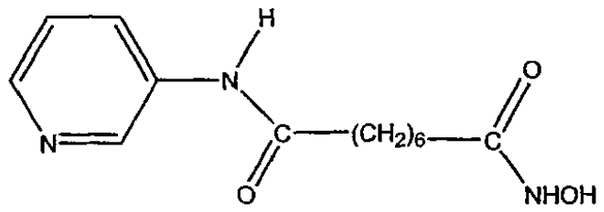
In one embodiment, the HDAC inhibitor useful in the methods of the present

invention is represented by the structure of formula 6 (pyroxamide), or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



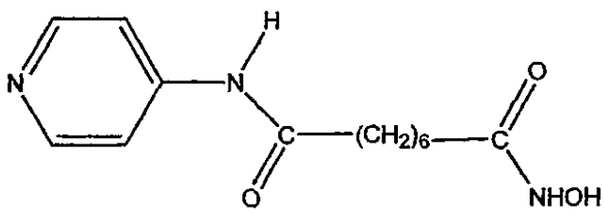
(6)

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 7, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



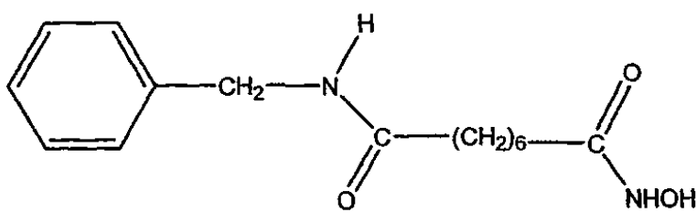
(7)

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 8, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



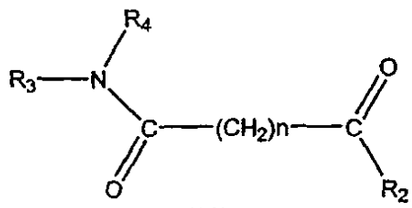
(8)

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 9, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



(9)

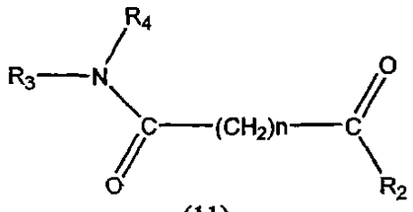
In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 10, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



(10)

wherein R_3 is hydrogen and R_4 cycloalkyl, aryl, aryloxy, arylalkyloxy, or pyridine group, or R_3 and R_4 bond together to form a piperidine group; R_2 is a hydroxylamino group; and n is an integer from 5 to about 8.

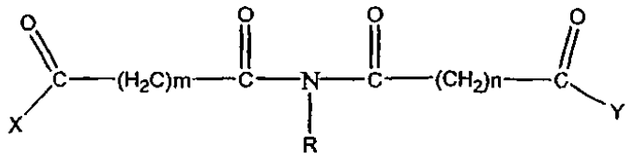
In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 11, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



(11)

wherein R_3 and R_4 are independently a substituted or unsubstituted, branched or unbranched alkyl, alkenyl, cycloalkyl, aryl, alkyloxy, aryloxy, arylalkyloxy, or pyridine group, cycloalkyl, aryl, aryloxy, arylalkyloxy, or pyridine group, or R_3 and R_4 bond together to form a piperidine group; R_2 is a hydroxylamino group; and n is an integer from 5 to about 8.

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 12, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.

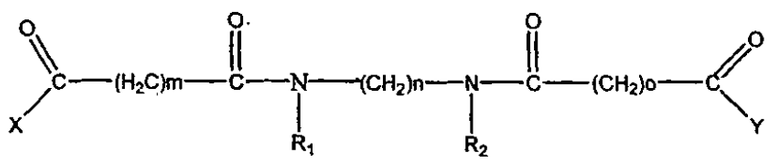


(12)

wherein each of X and Y are independently the same as or different from each other and are a hydroxyl, amino or hydroxylamino group, a substituted or unsubstituted alkyloxy, alkylamino, dialkylamino, arylamino, alkylarylamino, alkyloxyamino, aryloxyamino, alkyloxyalkylamino, or aryloxyalkylamino group; R is a hydrogen atom, a hydroxyl, group, a substituted or unsubstituted alkyl, arylalkyloxy, or aryloxy group; and each of m and n are independently the same as or different from each other and are each an integer from about 0 to about 8.

In a particular embodiment, the HDAC inhibitor is a compound of formula 12 wherein X, Y, and R are each hydroxyl and both m and n are 5.

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 13, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.

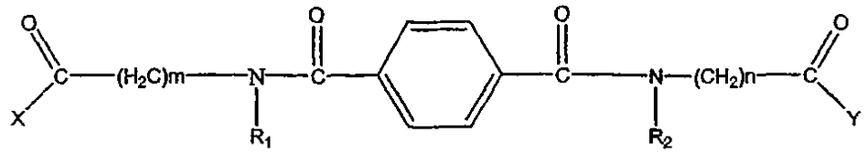


(13)

wherein each of X and Y are independently the same as or different from each other and are a hydroxyl, amino or hydroxylamino group, a substituted or unsubstituted alkyloxy, alkylamino, dialkylamino, arylamino, alkylarylamino, alkyloxyamino, aryloxyamino, alkyloxyalkylamino or aryloxyalkylamino group; each of R_1 and R_2 are independently the same as or different from each other and are a hydrogen atom, a hydroxyl group, a substituted or unsubstituted alkyl, aryl, alkyloxy, or aryloxy group; and each of m, n and o are independently the same as or different from each other and are each an integer from about 0 to about 8.

In one particular embodiment of formula 13, each of X and Y is a hydroxyl group and each of R_1 and R_2 is a methyl group. In another particular embodiment of formula 13, each of X and Y is a hydroxyl group, each of R_1 and R_2 is a methyl group, each of n and o is 6, and m is 2.

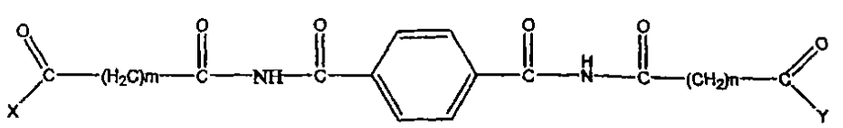
In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 14, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



(14)

wherein each of X and Y are independently the same as or different from each other and are a hydroxyl, amino or hydroxylamino group, a substituted or unsubstituted alkyloxy, alkylamino, dialkylamino, arylamino, alkylarylamino, alkyloxyamino, aryloxyamino, alkyloxyalkylamino or aryloxyalkylamino group; each of R_1 and R_2 are independently the same as or different from each other and are a hydrogen atom, a hydroxyl group, a substituted or unsubstituted alkyl, aryl, alkyloxy, or aryloxy group; and each of m and n are independently the same as or different from each other and are each an integer from about 0 to about 8.

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 15, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



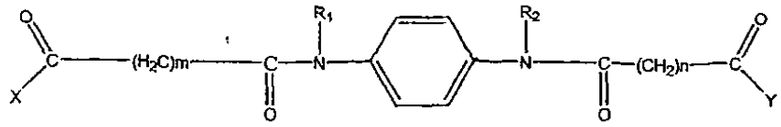
(15)

wherein each of X and Y are independently the same as or different from each other and are a hydroxyl, amino or hydroxylamino group, a substituted or unsubstituted alkyloxy, alkylamino, dialkylamino, arylamino, alkylarylamino, alkyloxyamino, aryloxyamino, alkyloxyalkylamino or aryloxyalkylamino group; and each of m and n are independently the same as or different from each other and are each an integer from about 0 to about 8.

In one particular embodiment of formula 15, each of X and Y is a hydroxyl group and each of m and n is 5.

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 16, or a pharmaceutically acceptable

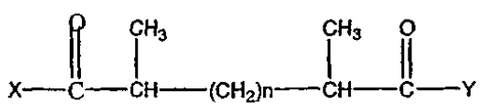
salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



(16)

wherein each of X and Y are independently the same as or different from each other and are a hydroxyl, amino or hydroxylamino group, a substituted or unsubstituted alkyloxy, alkylamino, dialkylamino, arylamino, alkylarylamino, alkyloxyamino, aryloxyamino, alkyloxyalkylamino or aryloxyalkylamino group; R_1 and R_2 are independently the same as or different from each other and are a hydrogen atom, a hydroxyl group, a substituted or unsubstituted alkyl, arylalkyloxy or aryloxy group; and each of m and n are independently the same as or different from each other and are each an integer from about 0 to about 8.

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 17, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.

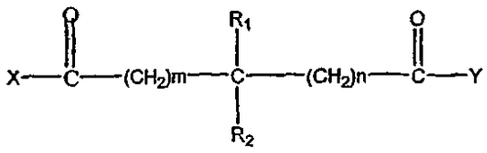


(17)

wherein each of X an Y are independently the same as or different from each other and are a hydroxyl, amino or hydroxylamino group, a substituted or unsubstituted alkyloxy, alkylamino, dialkylamino, arylamino, alkylarylamino, or aryloxyalkylamino group; and n is an integer from about 0 to about 8.

In one particular embodiment of formula 17, each of X and Y is a hydroxylamino group; R_1 is a methyl group, R_2 is a hydrogen atom; and each of m and n is 2. In another particular embodiment of formula 17, each of X and Y is a hydroxylamino group; R_1 is a carbonylhydroxylamino group, R_2 is a hydrogen atom; and each of m and n is 5. In another particular embodiment of formula 17, each of X and Y is a hydroxylamino group; each of R_1 and R_2 is a fluoro group; and each of m and n is 2.

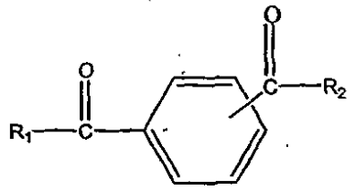
In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 18, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



(18)

wherein each of X and Y are independently the same as or different from each other and are a hydroxyl, amino or hydroxylamino group, a substituted or unsubstituted alkyloxy, alkylamino, dialkylamino, arylamino, alkylarylamino, alkyloxyamino, aryloxyamino, alkyloxyalkyamino or aryloxyalkylamino group; each of R_1 and R_2 are independently the same as or different from each other and are a hydrogen atom, a hydroxyl group, a substituted or unsubstituted alkyl, aryl, alkyloxy, aryloxy, carbonylhydroxylamino or fluoro group; and each of m and n are independently the same as or different from each other and are each an integer from about 0 to about 8.

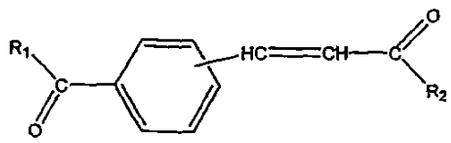
In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 19, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



(19)

wherein each of R_1 and R_2 are independently the same as or different from each other and are a hydroxyl, alkyloxy, amino, hydroxylamino, alkylamino, dialkylamino, arylamino, alkylarylamino, alkyloxyamino, aryloxyamino, alkyloxyalkylamino, or aryloxyalkylamino group. In a particular embodiment, the HDAC inhibitor is a compound of structural formula 19 wherein R_1 and R_2 are both hydroxylamino.

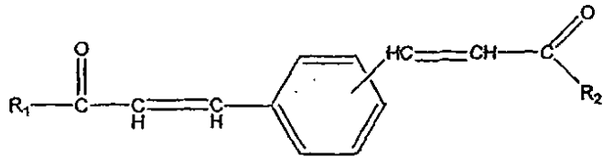
In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 20, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



(20)

wherein each of R_1 and R_2 are independently the same as or different from each other and are a hydroxyl, alkyloxy, amino, hydroxylamino, alkylamino, dialkylamino, arylamino, alkylarylamino, alkyloxyamino, aryloxyamino, alkyloxyalkylamino, or aryloxyalkylamino group. In a particular embodiment, the HDAC inhibitor is a compound of structural formula 20 wherein R_1 and R_2 are both hydroxylamino.

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 21, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.

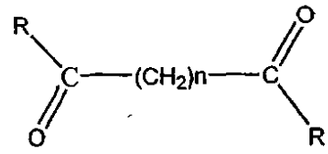


(21)

wherein each of R_1 and R_2 are independently the same as or different from each other and are a hydroxyl, alkyloxy, amino, hydroxylamino, alkylamino, dialkylamino, arylamino, alkylarylamino, alkyloxyamino, aryloxyamino, alkyloxyalkylamino, or aryloxyalkylamino group.

In a particular embodiment, the HDAC inhibitor is a compound of structural formula 21 wherein R_1 and R_2 are both hydroxylamino

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 22, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.

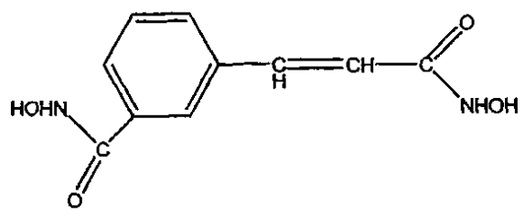


(22)

wherein R is a phenylamino group substituted with a cyano, methylcyano, nitro, carboxyl, aminocarbonyl, methylaminocarbonyl, dimethylaminocarbonyl, trifluoromethyl, hydroxylaminocarbonyl, N-hydroxylaminocarbonyl, methoxycarbonyl, chloro, fluoro, methyl, methoxy, 2,3-difluoro, 2,4-difluoro, 2,5-difluoro, 2,6-difuloro, 3,5-difluoro, 2,3,6-trifluoro, 2,4,6-trifluoro, 1,2,3-trifluoro, 3,4,5-trifluoro, 2,3,4,5-tetrafluoro, or 2,3,4,5,6-pentafluoro group; and n is an integer from 4 to 8.

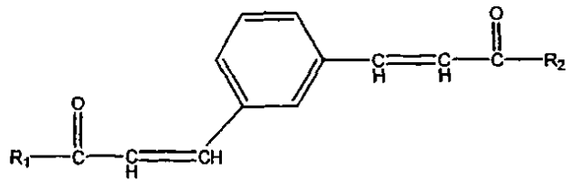
In one embodiment, the HDAC inhibitor useful in the methods of the present

invention is represented by the structure of formula 23 (m-carboxycinnamic acid bishydroxamide - CBHA), or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



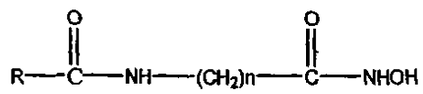
(23)

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 24, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



(24)

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 25, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



(25)

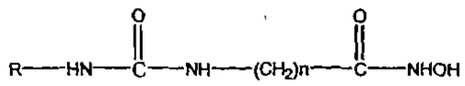
wherein R is a substituted or unsubstituted phenyl, piperidine, thiazole, 2-pyridine, 3-pyridine or 4-pyridine and n is an integer from about 4 to about 8.

In one particular embodiment of formula 25, R is a substituted phenyl group. In another particular embodiment of formula 25, R is a substituted phenyl group, where the substituent is selected from the group consisting of methyl, cyano, nitro, thio, trifluoromethyl, amino, aminocarbonyl, methylcyano, chloro, fluoro, bromo, iodo, 2,3-difluoro, 2,4-difluoro, 2,5-difluoro, 3,4-difluoro, 3,5-difluoro, 2,6-difluoro, 1,2,3-trifluoro, 2,3,6-trifluoro, 2,4,6-trifluoro, 3,4,5-trifluoro, 2,3,5,6-tetrafluoro, 2,3,4,5,6-pentafluoro, azido, hexyl, t-butyl, phenyl, carboxyl, hydroxyl, methyloxy, phenyloxy, benzyloxy,

phenylaminooxy, phenylaminocarbonyl, methyloxycarbonyl, methylaminocarbonyl, dimethylamino, dimethylaminocarbonyl, or hydroxylaminocarbonyl group.

In another particular embodiment of formula 25, R is a substituted or unsubstituted 2-pyridine, 3-pyridine or 4-pyridine and n is an integer from about 4 to about 8.

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 26, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



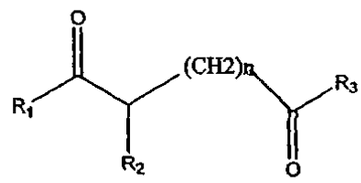
(26)

wherein R is a substituted or unsubstituted phenyl, pyridine, piperidine, or thiazole group and n is an integer from about 4 to about 8 or a pharmaceutically acceptable salt thereof.

In a particular embodiment of formula 26, R is a substituted phenyl group. In another particular embodiment of formula 26, R is a substituted phenyl group, where the substituent is selected from the group consisting of methyl, cyano, nitro, thio, trifluoromethyl, amino, aminocarbonyl, methylcyano, chloro, fluoro, bromo, iodo, 2,3-difluoro, 2,4-difluoro, 2,5-difluoro, 3,4-difluoro, 3,5-difluoro, 2,6-difluoro, 1,2,3-trifluoro, 2,3,6-trifluoro, 2,4,6-trifluoro, 3,4,5-trifluoro, 2,3,5,6-tetrafluoro, 2,3,4,5,6-pentafluoro, azido, hexyl, t-butyl, phenyl, carboxyl, hydroxyl, methyloxy, phenyloxy, benzyloxy, phenylaminooxy, phenylaminocarbonyl, methyloxycarbonyl, methylaminocarbonyl, dimethylamino, dimethylaminocarbonyl, or hydroxylaminocarbonyl group.

In another particular embodiment of formula 26, R is phenyl and n is 5. In another embodiment, n is 5 and R is 3-chlorophenyl.

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 27, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



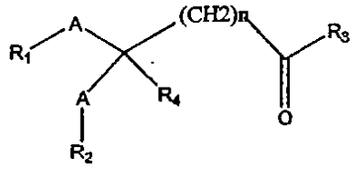
(27)

wherein each of R_1 and R_2 is directly attached or through a linker and is substituted or

unsubstituted, aryl (e.g., phenyl), arylalkyl (e.g., benzyl), naphthyl, cycloalkyl, cycloalkylamino, pyridineamino, piperidino, 9-purine-6-amino, thiazoleamino, hydroxyl, branched or unbranched alkyl, alkenyl, alkyloxy, aryloxy, arylalkyloxy, pyridyl, or quinolinyl or isoquinolinyl; n is an integer from about 3 to about 10 and R_3 is a hydroxamic acid, hydroxylamino, hydroxyl, amino, alkylamino or alkyloxy group. The linker can be an amide moiety, e.g., O-, -S-, -NH-, NR_5, $-CH_2-$, $-(CH_2)_m-$, -(CH=CH)-, phenylene, cycloalkylene, or any combination thereof, wherein R_5 is a substitute or unsubstituted C_1-C_5 alkyl.

In certain embodiments of formula 27, R_1 is -NH-R_4 wherein R_4 is substituted or unsubstituted, aryl (e.g., phenyl), arylalkyl (e.g., benzyl), naphthyl, cycloalkyl, cycloalkylamino, pyridineamino, piperidino, 9-purine-6-amino, thiazoleamino, hydroxyl, branched or unbranched alkyl, alkenyl, alkyloxy, aryloxy, arylalkyloxy, pyridyl, quinolinyl or isoquinolinyl

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 28, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or excipient.



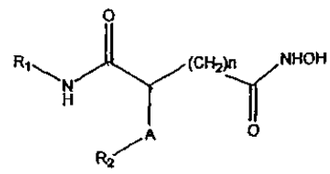
(28)

wherein each of R_1 and R_2 is, substituted or unsubstituted, aryl (e.g., phenyl), arylalkyl (e.g., benzyl), naphthyl, cycloalkyl, cycloalkylamino, pyridineamino, piperidino, 9-purine-6-amino, thiazoleamino, hydroxyl, branched or unbranched alkyl, alkenyl, alkyloxy, aryloxy, arylalkyloxy, pyridyl, quinolinyl or isoquinolinyl; R_3 is hydroxamic acid, hydroxylamino, hydroxyl, amino, alkylamino or alkyloxy group; R_4 is hydrogen, halogen, phenyl or a cycloalkyl moiety; and A can be the same or different and represents an amide moiety, O-, -S-, -NH-, NR_5, $-CH_2-$, $-(CH_2)_m-$, -(CH=CH)-, phenylene, cycloalkylene, or any combination thereof wherein R_5 is a substitute or unsubstituted C_1-C_5 alkyl; and n and m are each an integer from 3 to 10.

In further particular embodiment compounds having a more specific structure within the scope of compounds 27 or 28 are:

In one embodiment, the HDAC inhibitor useful in the methods of the present

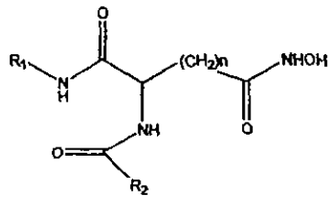
invention is represented by the structure of formula 29:



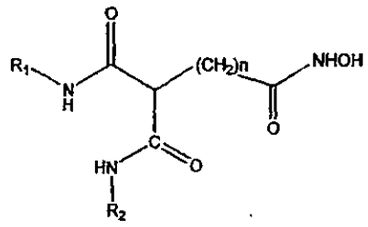
(29)

wherein A is an amide moiety, R_1 and R_2 are each selected from substituted or unsubstituted aryl (e.g., phenyl), arylalkyl (e.g., benzyl), naphthyl, pyridineamino, 9-purine-6-amino, thiazoleamino, aryloxy, arylalkyloxy, pyridyl, quinolinyl or isoquinolinyl; and n is an integer from 3 to 10.

For example, the compound of formula 29 can have the structure 30 or 31:



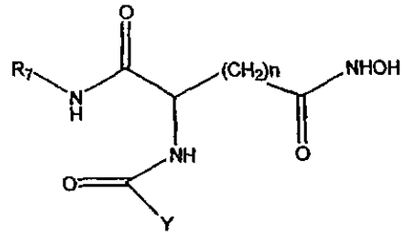
(30)



(31)

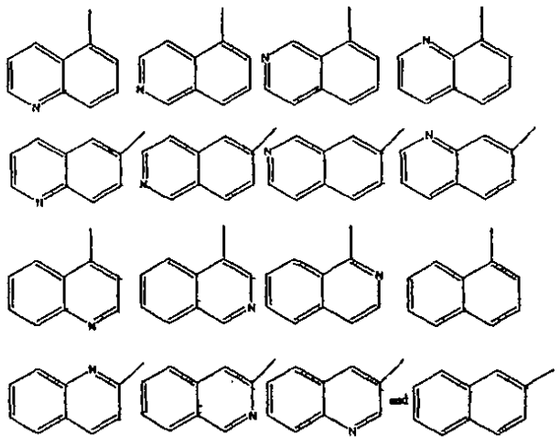
wherein R_1, R_2, and n have the meanings of formula 29.

In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 32:

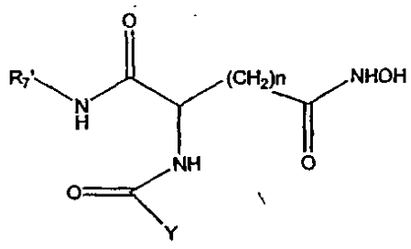


(32)

wherein R_7 is selected from substituted or unsubstituted aryl (e.g., phenyl), arylalkyl (e.g., benzyl), naphthyl, pyridineamino, 9-purine-6-amino, thiazoleamino, aryloxy, arylalkyloxy, pyridyl, quinolinyl, or isoquinolinyl; n is an integer from 3 to 10 and Y is selected from:

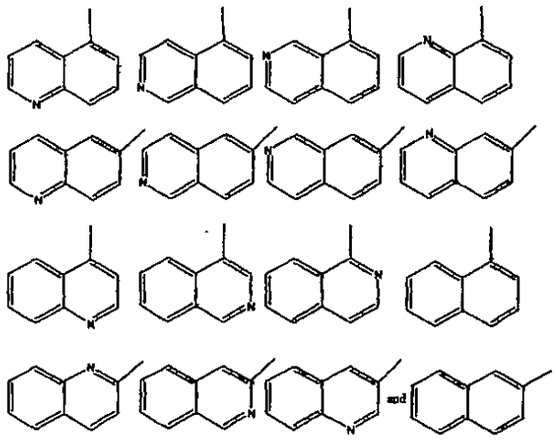


In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 33:

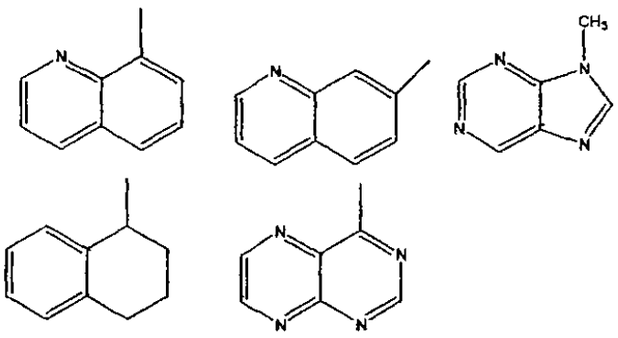


(33)

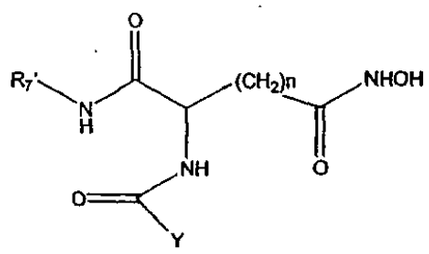
wherein n is an integer from 3 to 10, Y is selected from



and R_7' is selected from

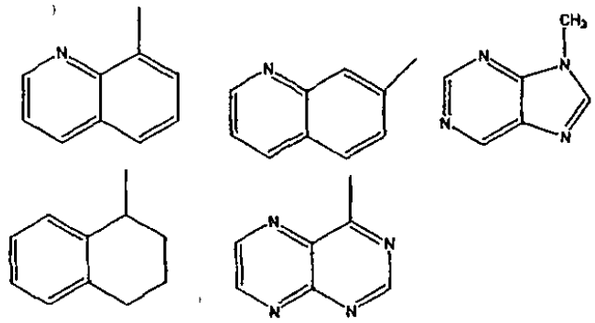


In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 34:

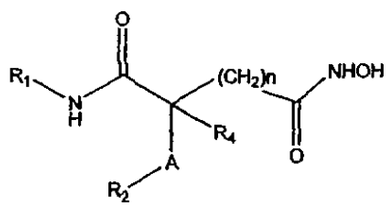


(34)

aryl (e.g., phenyl), arylalkyl (e.g., benzyl), naphthyl, pyridineamino, 9-purine-6-amino, thiazoleamino, aryloxy, arylalkyloxy, pyridyl, quinolinyl or isoquinolinyl; n is an integer from 3 to 10 and R_7' is selected from



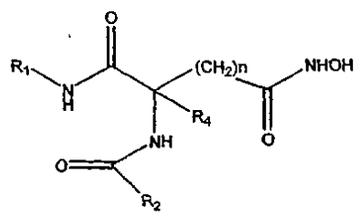
In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 35:



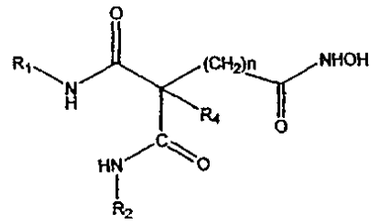
(35)

wherein A is an amide moiety, R_1 and R_2 are each selected from substituted or unsubstituted aryl (e.g., phenyl), arylalkyl (e.g., benzyl), naphthyl, pyridineamino, 9-purine-6-amino, thiazoleamino, aryloxy, arylalkyloxy, pyridyl, quinolinyl or isoquinolinyl; R_4 is hydrogen, a halogen, a phenyl or a cycloalkyl moiety and n is an integer from 3 to 10.

For example, the compound of formula 35 can have the structure 36 or 37:



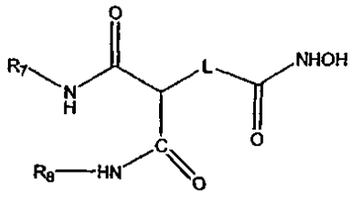
(36)



(37)

wherein R_1, R_2, R_4, and n have the meanings of formula 35.

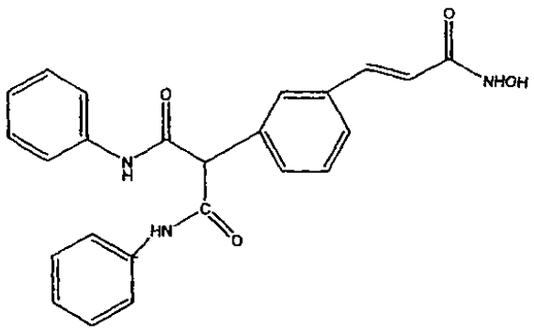
In one embodiment, the HDAC inhibitor useful in the methods of the present invention is represented by the structure of formula 38:



(38)

wherein L is a linker selected from the group consisting of an amide moiety, O-, -S-, -NH-, NR_5, $-CH_2-$, $-(CH_2)_m-$, -(CH=CH)-, phenylene, cycloalkylene, or any combination thereof wherein R_5 is a substitute or unsubstituted C_1-C_5 alkyl; and wherein each of R_7 and R_8 are independently a substituted or unsubstituted aryl (e.g., phenyl), arylalkyl (e.g., benzyl), naphthyl, pyridineamino, 9-purine-6-amino, thiazoleamino, aryloxy, arylalkyloxy, pyridyl, quinolinyl or isoquinolinyl; n is an integer from 3 to 10 and m is an integer from 0-10.

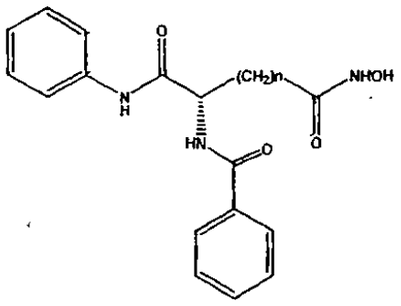
For example, a compound of formula 38 can be represented by the structure of formula (39):



(39)

Other HDAC inhibitors suitable for use in the methods of the present invention include those shown in the following more specific formulas:

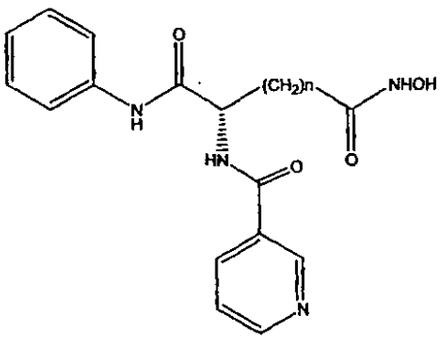
A compound represented by the structure:



(40)

wherein n is an integer from 3 to 10, or an enantiomer thereof. In one particular embodiment of formula 40, n=5.

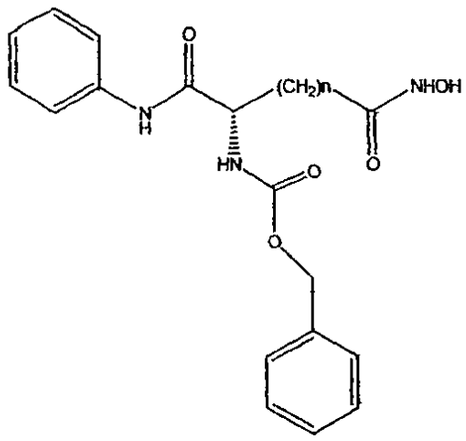
A compound represented by the structure:



(41)

wherein n is an integer from 3 to 10, or an enantiomer thereof. In one particular embodiment of formula 41, n=5.

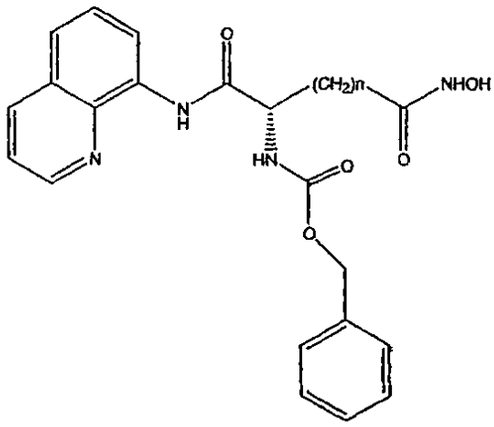
A compound represented by the structure:



(42)

wherein n is an integer from 3 to 10 or an enantiomer thereof. In one particular embodiment of formula 42, n=5.

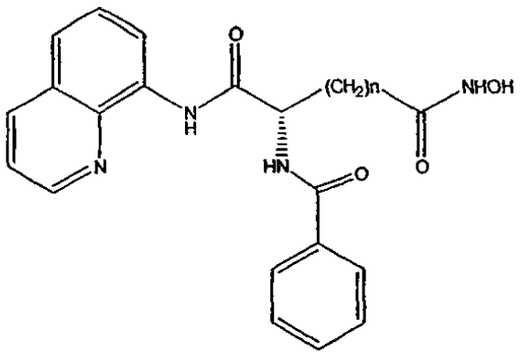
A compound represented by the structure:



(43)

wherein n is an integer from 3 to 10, or an enantiomer thereof. In one particular embodiment of formula 43, n=5.

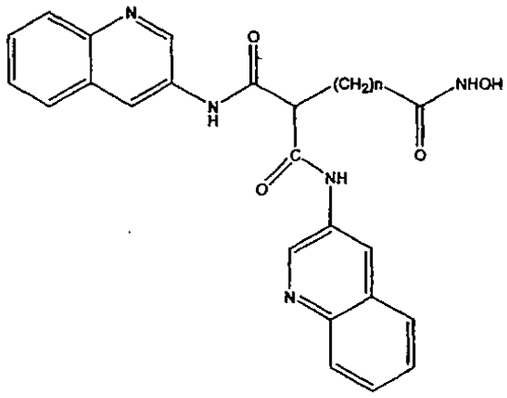
A compound represented by the structure:



(44)

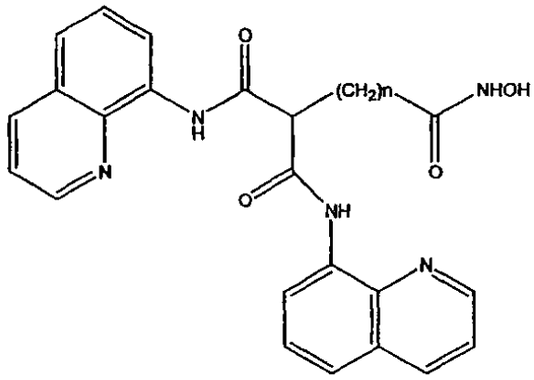
wherein n is an integer from 3 to 1,0 or an enantiomer thereof. In one particular embodiment of formula 44, n=5.

A compound represented by the structure:



(45)

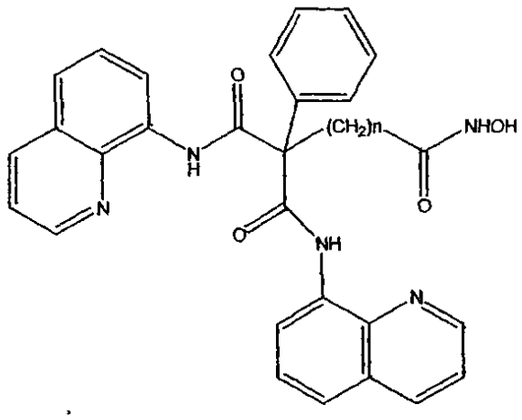
wherein n is an integer from 3 to 10, or an enantiomer thereof. In one particular embodiment of formula 45, n=5.



(46)

wherein n is an integer from 3 to 10 or an enantiomer thereof. In one particular embodiment of formula 46, n=5.

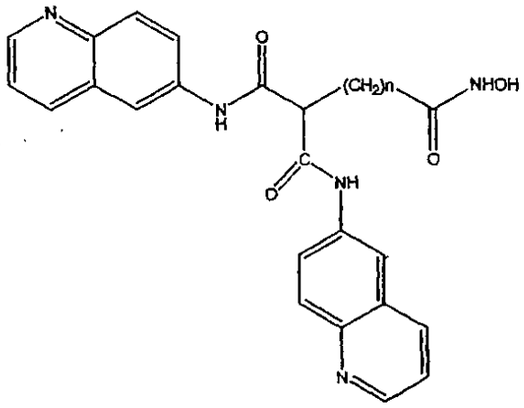
A compound represented by the structure:



(47)

wherein n is an integer from 3 to 10, or an enantiomer thereof. In one particular embodiment of formula 47, n=5.

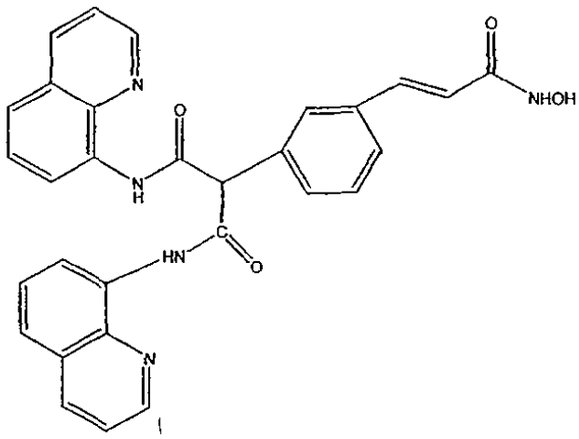
A compound represented by the structure:



(48)

wherein n is an integer from 3 to 10, or an enantiomer thereof. In one particular embodiment of formula 48, n=5.

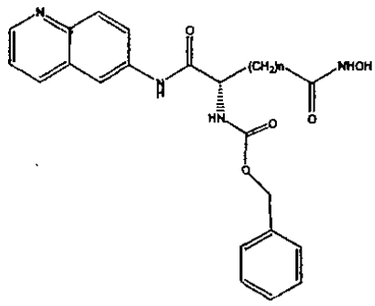
A compound represented by the structure:



(49)

wherein n is an integer from 3 to 10, or an enantiomer thereof. In one particular embodiment of formula 49, n=5.

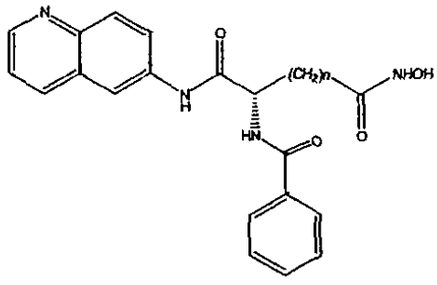
A compound represented by the structure:



(50)

wherein n is an integer from 3 to 10, or an enantiomer thereof. In one particular embodiment of formula 50, n=5.

A compound represented by the structure:



(51)

wherein n is an integer from 3 to 10, or an enantiomer thereof. In one particular embodiment of formula 51, n=5.

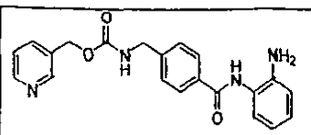
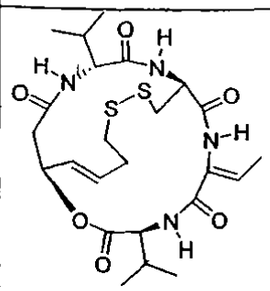
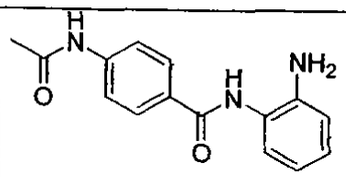
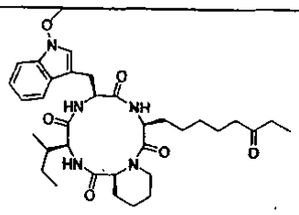
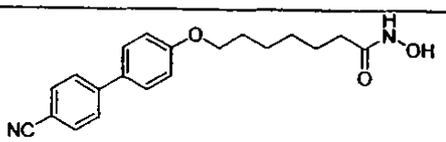
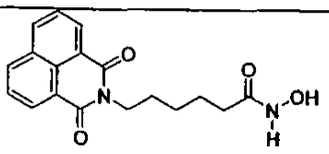
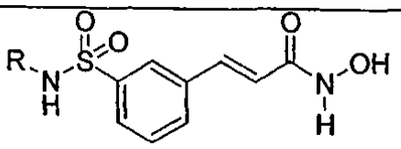
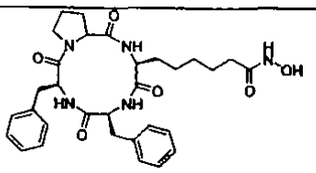
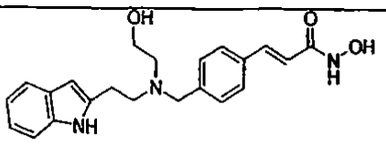
Other examples of such compounds and other HDAC inhibitors can be found in U.S.

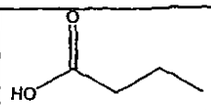
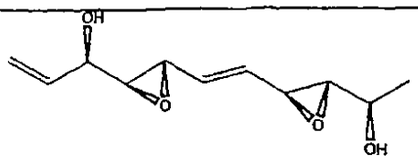
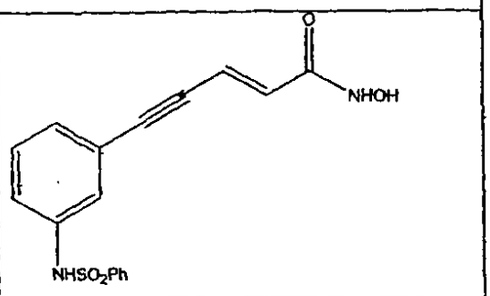
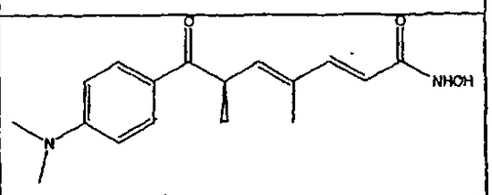
Patent No. 5,369,108, issued on November 29, 1994, U.S. Patent No. 5,700,811, issued on December 23, 1997, U.S. Patent No. 5,773,474, issued on June 30, 1998, U.S. Patent No. 5,932,616, issued on August 3, 1999 and U.S. Patent No. 6,511,990, issued January 28, 2003, all to Breslow *et al.*; U.S. Patent No. 5,055,608, issued on October 8, 1991, U.S. Patent No. 5,175,191, issued on December 29, 1992 and U.S. Patent No. 5,608,108, issued on March 4, 1997, all to Marks *et al.*; as well as Yoshida, M., *et al.*, Bioassays 17, 423-430 (1995); Saito, A., *et al.*, PNAS USA 96, 4592-4597, (1999); Furamai, R. *et al.*, PNAS USA 98 (1), 87-92 (2001); Komatsu, Y., *et al.*, Cancer Res. 61(11), 4459-4466 (2001); Su, G.H., *et al.*, Cancer Res. 60, 3137-3142 (2000); Lee, B.I. *et al.*, Cancer Res. 61(3), 931-934; Suzuki, T., *et al.*, J. Med. Chem. 42(15), 3001-3003 (1999); published PCT Application WO 01/18171 published on March 15, 2001 to Sloan-Kettering Institute for Cancer Research and The Trustees of Columbia University; published PCT Application WO02/246144 to Hoffmann-La Roche; published PCT Application WO02/22577 to Novartis; published PCT Application WO02/30879 to Prolifix; published PCT Applications WO 01/38322 (published May 31, 2001), WO 01/70675 (published on September 27, 2001) and WO 00/71703 (published on November 30, 2000) all to Methylgene, Inc.; published PCT Application WO 00/21979 published on October 8, 1999 to Fujisawa Pharmaceutical Co., Ltd.; published PCT Application WO 98/40080 published on March 11, 1998 to Beacon Laboratories, L.L.C.; and Curtin M. (Current patent status of HDAC inhibitors *Expert Opin. Ther. Patents* (2002) 12(9): 1375-1384 and references cited therein).

SAHA or any of the other HDACs can be synthesized according to the methods outlined in the Experimental Details Section, or according to the method set forth in U.S. Patent Nos. 5,369,108, 5,700,811, 5,932,616 and 6,511,990, the contents of which are incorporated by reference in their entirety, or according to any other method known to a person skilled in the art.

Specific non-limiting examples of HDAC inhibitors are provided in the Table below. It should be noted that the present invention encompasses any compounds which are structurally similar to the compounds represented below, and which are capable of inhibiting histone deacetylases.

Name	Structure

MS-275	
Depsipeptide	
CI-994	
Apicidin	
A-161906	
Scriptaid	
PXD-101	
CHAP	
LAQ-824	

Butyric Acid	
Depudecin	
Oxamflatin	
Trichostatin C	

The compounds of the present invention are useful for selectively inducing terminal differentiation, cell growth arrest and/or apoptosis of neoplastic cells and therefore aid in treatment of cancer in patients as described in detail herein.

Chemical Definitions

An "aliphatic group" is non-aromatic, consists solely of carbon and hydrogen, and can optionally contain one or more units of unsaturation, e.g., double and/or triple bonds. An aliphatic group can be straight chained, branched or cyclic. When straight chained or branched, an aliphatic group typically contains between about 1 and about 12 carbon atoms, more typically between about 1 and about 6 carbon atoms. When cyclic, an aliphatic group typically contains between about 3 and about 10 carbon atoms, more typically between about 3 and about 7 carbon atoms. Aliphatic groups are preferably C_1-C_{12} straight chained or branched alkyl groups (i.e., completely saturated aliphatic groups), more preferably C_1-C_6 straight chained or branched alkyl groups. Examples include methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, and tert-butyl.

An "aromatic group" (also referred to as an "aryl group") as used herein includes

carbocyclic aromatic groups, heterocyclic aromatic groups (also referred to as "heteroaryl") and fused polycyclic aromatic ring system as defined herein.

A "carbocyclic aromatic group" is an aromatic ring of 5 to 14 carbons atoms, and includes a carbocyclic aromatic group fused with a 5-or 6-membered cycloalkyl group such as indan. Examples of carbocyclic aromatic groups include, but are not limited to, phenyl, naphthyl, e.g., 1-naphthyl and 2-naphthyl; anthracenyl, e.g., 1-anthracenyl, 2-anthracenyl; phenanthrenyl; fluorenonyl, e.g., 9-fluorenonyl, indanyl and the like. A carbocyclic aromatic group is optionally substituted with a designated number of substituents, described below.

A "heterocyclic aromatic group" (or "heteroaryl") is a monocyclic, bicyclic or tricyclic aromatic ring of 5- to 14-ring atoms of carbon and from one to four heteroatoms selected from O, N, or S. Examples of heteroaryl include, but are not limited to pyridyl, e.g., 2-pyridyl (also referred to as α-pyridyl), 3-pyridyl (also referred to as β-pyridyl) and 4-pyridyl (also referred to as (γ-pyridyl); thienyl, e.g., 2-thienyl and 3-thienyl; furanyl, e.g., 2-furanyl and 3-furanyl; pyrimidyl, e.g., 2-pyrimidyl and 4-pyrimidyl; imidazolyl, e.g., 2-imidazolyl; pyranyl, e.g., 2-pyranyl and 3-pyranyl; pyrazolyl, e.g., 4-pyrazolyl and 5-pyrazolyl; thiazolyl, e.g., 2-thiazolyl, 4-thiazolyl and 5-thiazolyl; thiadiazolyl; isothiazolyl; oxazolyl, e.g., 2-oxazoyl, 4-oxazoyl and 5-oxazoyl; isoxazoyl; pyrrolyl; pyridazinyl; pyrazinyl and the like. Heterocyclic aromatic (or heteroaryl) as defined above may be optionally substituted with a designated number of substituents, as described below for aromatic groups.

A "fused polycyclic aromatic" ring system is a carbocyclic aromatic group or heteroaryl fused with one or more other heteroaryl or nonaromatic heterocyclic ring. Examples include, quinolinyl and isoquinolinyl, e.g., 2-quinolinyl, 3-quinolinyl, 4-quinolinyl, 5-quinolinyl, 6-quinolinyl, 7-quinolinyl and 8-quinolinyl, 1-isoquinolinyl, 3-quinolinyl, 4-isoquinolinyl, 5-isoquinolinyl, 6-isoquinolinyl, 7-isoquinolinyl and 8-isoquinolinyl; benzofuranyl, e.g., 2-benzofuranyl and 3-benzofuranyl; dibenzofuranyl, e.g., 2,3-dihydrobenzofuranyl; dibenzothiophenyl; benzothienyl, e.g., 2-benzothienyl and 3-benzothienyl; indolyl, e.g., 2-indolyl and 3-indolyl; benzothiazolyl, e.g., 2-benzothiazolyl; benzooxazolyl, e.g., 2-benzooxazolyl; benzimidazolyl, e.g., 2-benzoimidazolyl; isoindolyl, e.g., 1-isoindolyl and 3-isoindolyl; benzotriazolyl; purinyl; thianaphthenyl and the like. Fused polycyclic aromatic ring systems may optionally be substituted with a designated number of substituents, as described herein.

An "aralkyl group" (arylalkyl) is an alkyl group substituted with an aromatic group, preferably a phenyl group. A preferred aralkyl group is a benzyl group. Suitable aromatic groups are described herein and suitable alkyl groups are described herein. Suitable substituents for an aralkyl group are described herein.

An "aryloxy group" is an aryl group that is attached to a compound via an oxygen (e.g., phenoxy).

An "alkoxy group"(alkyloxy), as used herein, is a straight chain or branched C_1-C_{12} or cyclic C_3-C_{12} alkyl group that is connected to a compound via an oxygen atom. Examples of alkoxy groups include but are not limited to methoxy, ethoxy, and propoxy.

An "arylalkoxy group" (arylalkyloxy) is an arylalkyl group that is attached to a compound via an oxygen on the alkyl portion of the arylalkyl (e.g., phenylmethoxy).

An "arylamino group" as used herein, is an aryl group that is attached to a compound via a nitrogen.

As used herein, an "arylalkylamino group" is an arylalkyl group that is attached to a compound via a nitrogen on the alkyl portion of the arylalkyl.

As used herein, many moieties or groups are referred to as being either "substituted or unsubstituted". When a moiety is referred to as substituted, it denotes that any portion of the moiety that is known to one skilled in the art as being available for substitution can be substituted. For example, the substitutable group can be a hydrogen atom that is replaced with a group other than hydrogen (i.e., a substituent group). Multiple substituent groups can be present. When multiple substituents are present, the substituents can be the same or different and substitution can be at any of the substitutable sites. Such means for substitution are well known in the art. For purposes of exemplification, which should not be construed as limiting the scope of this invention, some examples of groups that are substituents are: alkyl groups (which can also be substituted, with one or more substituents, such as CF_3), alkoxy groups (which can be substituted, such as OCF_3), a halogen or halo group (F, Cl, Br, I), hydroxy, nitro, oxo, -CN, -COH, -COOH, amino, azido, N-alkylamino or N,N-dialkylamino (in which the alkyl groups can also be substituted), esters (-C(O)-OR, where R can be a group such as alkyl, aryl, etc., which can be substituted), aryl (most preferred is phenyl, which can be substituted), arylalkyl (which can be substituted) and aryloxy.

Stereochemistry

Many organic compounds exist in optically active forms having the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these compounds, called stereoisomers, are identical except that they are non-superimposable mirror images of one another.

A specific stereoisomer can also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture. Many of the compounds described herein can have one or more chiral centers and therefore can exist in different enantiomeric forms. If desired, a chiral carbon can be designated with an asterisk (*). When bonds to the chiral carbon are depicted as straight lines in the formulas of the invention, it is understood that both the (R) and (S) configurations of the chiral carbon, and hence both enantiomers and mixtures thereof, are embraced within the formula. As is used in the art, when it is desired to specify the absolute configuration about a chiral carbon, one of the bonds to the chiral carbon can be depicted as a wedge (bonds to atoms above the plane) and the other can be depicted as a series or wedge of short parallel lines is (bonds to atoms below the plane). The Cahn-Inglod-Prelog system can be used to assign the (R) or (S) configuration to a chiral carbon.

When the HDAC inhibitors of the present invention contain one chiral center, the compounds exist in two enantiomeric forms, and the present invention includes both enantiomers and mixtures of enantiomers, such as the specific 50:50 mixture referred to as a racemic mixtures. The enantiomers can be resolved by methods known to those skilled in the art, for example by formation of diastereoisomeric salts which may be separated, for example, by crystallization (see, CRC Handbook of Optical Resolutions via Diastereomeric Salt Formation by David Kozma (CRC Press, 2001)); formation of diastereoisomeric derivatives or complexes which may be separated, for example, by crystallization, gas-liquid or liquid chromatography; selective reaction of one enantiomer with an enantiomer-specific reagent, for example enzymatic esterification; or gas-liquid or liquid chromatography in a chiral environment, for example on a chiral support for example silica with a bound chiral ligand or in the presence of a chiral solvent. It will be appreciated that where the desired enantiomer is converted into another chemical entity by one of the

separation procedures described above, a further step is required to liberate the desired enantiomeric form. Alternatively, specific enantiomers may be synthesized by asymmetric synthesis using optically active reagents, substrates, catalysts, or solvents, or by converting one enantiomer into the other by asymmetric transformation.

Designation of a specific absolute configuration at a chiral carbon of the compounds of the invention is understood to mean that the designated enantiomeric form of the compounds is in enantiomeric excess (ee) or in other words is substantially free from the other enantiomer. For example, the "R" forms of the compounds are substantially free from the "S" forms of the compounds and are, thus, in enantiomeric excess of the "S" forms. Conversely, "S" forms of the compounds are substantially free of "R" forms of the compounds and are, thus, in enantiomeric excess of the "R" forms. Enantiomeric excess, as used herein, is the presence of a particular enantiomer at greater than 50%. For example, the enantiomeric excess can be about 60% or more, such as about 70% or more, for example about 80% or more, such as about 90% or more. In a particular embodiment when a specific absolute configuration is designated, the enantiomeric excess of depicted compounds is at least about 90%. In a more particular embodiment, the enantiomeric excess of the compounds is at least about 95%, such as at least about 97.5%, for example, at least 99% enantiomeric excess.

When a compound of the present invention has two or more chiral carbons it can have more than two optical isomers and can exist in diastereoisomeric forms. For example, when there are two chiral carbons, the compound can have up to 4 optical isomers and 2 pairs of enantiomers ((S,S)/(R,R) and (R,S)/(S,R)). The pairs of enantiomers (e.g., (S,S)/(R,R)) are mirror image stereoisomers of one another. The stereoisomers that are not mirror-images (e.g., (S,S) and (R,S)) are diastereomers. The diastereoisomeric pairs may be separated by methods known to those skilled in the art, for example chromatography or crystallization and the individual enantiomers within each pair may be separated as described above. The present invention includes each diastereoisomer of such compounds and mixtures thereof.

As used herein, "a," an" and "the" include singular and plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an active agent" or "a pharmacologically active agent" includes a single active agent as well a two or more different active agents in combination, reference to "a carrier" includes mixtures of two or more carriers as well as a single carrier, and the like.

This invention is also intended to encompass pro-drugs of the HDAC inhibitors disclosed herein. A prodrug of any of the compounds can be made using well known pharmacological techniques.

This invention, in addition to the above listed compounds, is intended to encompass the use of homologs and analogs of such compounds. In this context, homologs are molecules having substantial structural similarities to the above-described compounds and analogs are molecules having substantial biological similarities regardless of structural similarities.

The invention also encompasses pharmaceutical compositions comprising pharmaceutically acceptable salts of the HDAC inhibitors with organic and inorganic acids, for example, acid addition salts which may, for example, be hydrochloric acid, sulphuric acid, methanesulphonic acid, fumaric acid, maleic acid, succinic acid, acetic acid, benzoic acid, oxalic acid, citric acid, tartaric acid, carbonic acid, phosphoric acid and the like. Pharmaceutically acceptable salts can also be prepared from by treatment with inorganic bases, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The invention also encompasses pharmaceutical compositions comprising hydrates of the HDAC inhibitors. The term "hydrate" includes but is not limited to hemihydrate, monohydrate, dihydrate, trihydrate, and the like.

This invention also encompasses pharmaceutical compositions comprising any solid or liquid physical form of SAHA or any of the other HDAC inhibitors. For example, The HDAC inhibitors can be in a crystalline form, in amorphous form, and have any particle size. The HDAC inhibitor particles may be micronized, or may be agglomerated, particulate granules, powders, oils, oily suspensions, or any other form of solid or liquid physical form.

Therapeutic Uses of HDAC Inhibitors

1. Treatment of Cancer

As demonstrated herein, the HDAC inhibitors of the present invention are useful for the treatment of cancer. Accordingly, in one embodiment, the invention relates to a method of treating cancer in a subject in need of treatment comprising administering to said subject a therapeutically effective amount of a histone deacetylase inhibitor described herein.

The term "cancer" refers to any cancer caused by the proliferation of neoplastic cells, such as solid tumors, neoplasms, carcinomas, sarcomas, leukemias, mesotheliomas, lymphomas and the like. For example, cancers include, but are not limited to: mesotheliomas such as pleural mesothelioma, peritoneal mesothelioma, and benign fibrous mesothelioma; leukemias, including acute leukemias and chronic leukemias such as acute lymphocytic leukemia (ALL), acute nonlymphocytic leukemia, acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML) and hairy cell leukemia; lymphomas such as cutaneous T-cell lymphomas (CTCL), noncutaneous peripheral T-cell lymphomas, lymphomas associated with human T-cell lymphotrophic virus (HTLV) such as adult T-cell leukemia/lymphoma (ATLL), Hodgkin's disease and non-Hodgkin's lymphomas, large-cell lymphomas, diffuse large B-cell lymphoma (DLBCL); Burkitt's lymphoma; primary central nervous system (CNS) lymphoma; multiple myeloma; childhood solid tumors such as brain tumors, neuroblastoma, retinoblastoma, Wilm's tumor, bone tumors, and soft-tissue sarcomas, common solid tumors of adults such as head and neck cancers (e.g., oral, laryngeal and esophageal), genito urinary cancers (e.g., prostate, bladder, renal, uterine, ovarian, testicular, rectal and colon), lung cancer, breast cancer, pancreatic cancer, melanoma and other skin cancers, stomach cancer, brain tumors, liver cancer and thyroid cancer.

2. Treatment of Mesothelioma and Lymphoma

As demonstrated herein, the HDAC inhibitors are useful for the treatment of mesothelioma and various forms of lymphoma, including diffuse large B-cell lymphoma (DLBCL).

There are various types of mesothelioma. Pleural and peritoneal (malignant) mesotheliomas involve tumors of mesothelial tissue associated with asbestos exposure. Histologically, these tumors are composed of epitheloid, sarcomatoid, or fibrous, or mixed cell types (also called biphasic type). Benign fibrous mesothelioma is a rare solid tumor of the pleura that produces chest pain, dyspnea, fever, and hypertrophic osteoarthropathy.

One staging system used for mesothelioma is the Butchart system. This system is based mainly on the extent of the primary malignant tumor mass, and divides mesotheliomas into stages I through IV.

Stage I: Mesothelioma is present on one side of the chest only and isn't growing into the

chest wall.
Stage II: Mesothelioma invades the chest wall or involves the esophagus (food passage connecting the throat to the stomach), heart, or has grown into the pleura on the other side of the chest. The lymph nodes in the chest may also be involved.
Stage III: Mesothelioma has grown through the diaphragm into the peritoneum (lining of the abdominal cavity) or has spread to lymph nodes beyond those in the chest.
Stage IV: Mesothelioma has spread through the bloodstream to other organs (metastases).

Another staging system has recently been developed by the International Mesothelioma Interest Group. In this system, information about the tumor, lymph nodes, and metastasis is combined in a process called stage grouping.

Stage I: Disease confined within the capsule of the parietal pleura: ipsilateral pleura, lung, pericardium, and diaphragm.
Stage II: All of stage I with positive intrathoracic (N1 or N2) lymph nodes.
Stage III: Local extension of disease into the following: chest wall or mediastinum; heart or through the diaphragm, peritoneum; with or without extrathoracic or contralateral (N3) lymph node involvement.
Stage IV: Distant metastatic disease.

There are many different types of lymphoma, and they can be divided into two categories: Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL). The major difference between the two is the type of cells involved.

There are two main types of lymphocytes: B-cells and T-cells. Most lymphocytes start growing in the bone marrow. The B-cells continue to develop in the bone marrow, whereas the T-cells go from the bone marrow to the thymus gland and mature there. Once they are mature, both B-cells and T-cells help the body fight infections.

There are more than 20 different types of non-Hodgkin's lymphoma. Diffuse large B-cell lymphoma is a common type, making up about 40% of all cases. It is a cancer of the B-lymphocytes. Diffuse B-cell lymphoma can occur at any time from adolescence to old age. It is slightly more common in men than women. A large-cell lymphoma is a lymphoma that is characterized by unusually large cells.

Both Hodgkin's disease NHL are classified by the same categories of stages. Most lymphomas in HIV-positive people involve B-cells, as opposed to T-cells.

The stage of the lymphoma is very important and can help determine prognosis and the course of treatment. The four stages are:

Stage I: There is one cancer site. No bone marrow involvement.
Stage II: There are two sites; both are either above or below the diaphragm. No bone marrow involvement.
Stage III: There are sites above and below the diaphragm. No bone marrow involvement.
Stage IV: The bone marrow is affected or the cancer cells have spread outside the lymphatic system.

In Hodgkin's disease, staging is further classified as follows:

Hodgkin's Disease Classifications
B: the presence of fever, weight loss or night sweats
A: the absence of fever, weight loss or night sweats
E: the disease has spread to organs outside the lymph system

Grading

For practical purposes non-Hodgkin's lymphomas are also divided into one of two groups: low and high grade. Low-grade lymphomas are usually slowly growing and high-grade lymphomas tend to grow more quickly. Diffuse large B-cell lymphoma is a high-grade lymphoma.

As contemplated herein, the HDAC inhibitors of the present invention are useful at treating all of the stages of mesothelioma and lymphoma, i.e., stages I, II, III and IV stated above, as well as stages A, B and E of HD. In addition, the HDAC inhibitors are useful at treating low and high grade lymphomas.

As demonstrated herein, the HDAC inhibitors of the present invention are particularly useful at treating mesothelioma and diffuse large B-cell lymphoma (DLBCL).

3. Other uses of HDAC inhibitors

HDAC inhibitors are effective at treating a broader range of diseases characterized by the proliferation of neoplastic diseases, such as any one of the cancers described hereinabove. However, the therapeutic utility of HDAC inhibitors is not limited to the

treatment of cancer. Rather, there is a wide range of diseases for which HDAC inhibitors have been found useful.

For example, HDAC inhibitors, in particular SAHA, have been found to be useful in the treatment of a variety of acute and chronic inflammatory diseases, autoimmune diseases, allergic diseases, diseases associated with oxidative stress, and diseases characterized by cellular hyperproliferation. Non-limiting examples are inflammatory conditions of a joint including and rheumatoid arthritis (RA) and psoriatic arthritis; inflammatory bowel diseases such as Crohn's disease and ulcerative colitis; spondyloarthropathies; scleroderma; psoriasis (including T-cell mediated psoriasis) and inflammatory dermatoses such an dermatitis, eczema, atopic dermatitis, allergic contact dermatitis, urticaria; vasculitis (e.g., necrotizing, cutaneous, and hypersensitivity vasculitis); eosinphilic myositis, eosinophilic fasciitis; cancers with leukocyte infiltration of the skin or organs, ischemic injury, including cerebral ischemia (e.g., brain injury as a result of trauma, epilepsy, hemorrhage or stroke, each of which may lead to neurodegeneration); HIV, heart failure, chronic, acute or malignant liver disease, autoimmune thyroiditis; systemic lupus erythematosus, Sjorgren's syndrome, lung diseases (e.g., ARDS); acute pancreatitis; amyotrophic lateral sclerosis (ALS); Alzheimer's disease; cachexia/anorexia; asthma; atherosclerosis; chronic fatigue syndrome, fever; diabetes (e.g., insulin diabetes or juvenile onset diabetes); glomerulonephritis; graft versus host rejection (e.g., in transplantation); hemohorragic shock; hyperalgesia: inflammatory bowel disease; multiple sclerosis; myopathies (e.g., muscle protein metabolism, esp. in sepsis); osteoporosis; Parkinson's disease; pain; pre-term labor; psoriasis; reperfusion injury; cytokine-induced toxicity (e.g., septic shock, endotoxic shock); side effects from radiation therapy, temporal mandibular joint disease, tumor metastasis; or an inflammatory condition resulting from strain, sprain, cartilage damage, trauma such as burn, orthopedic surgery, infection or other disease processes. Allergic diseases and conditions, include but are not limited to respiratory allergic diseases such as asthma, allergic rhinitis, hypersensitivity lung diseases, hypersensitivity pneumonitis, eosinophilic pneumonias (e.g., Loeffler's syndrome, chronic eosinophilic pneumonia), delayed-type hypersensitivity, interstitial lung diseases (ILD) (e.g., idiopathic pulmonary fibrosis, or ILD associated with rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, systemic sclerosis, Sjogren's syndrome, polymyositis or dermatomyositis); systemic anaphylaxis or hypersensitivity responses, drug allergies (e.g., to penicillin, cephalosporins), insect sting allergies, and the like.

For example, HDAC inhibitors, and in particular SAHA, have been found to be useful in the treatment of a variety of neurodegenerative diseases, a non-exhaustive list of which is:

I. Disorders characterized by progressive dementia in the absence of other prominent neurologic signs, such as Alzheimer's disease; Senile dementia of the Alzheimer type; and Pick's disease (lobar atrophy).

II. Syndromes combining progressive dementia with other prominent neurologic abnormalities such as A) syndromes appearing mainly in adults (e.g., Huntington's disease, Multiple system atrophy combining dementia with ataxia and/or manifestations of Parkinson's disease, Progressive supranuclear palsy (Steel-Richardson-Olszewski), diffuse Lewy body disease, and corticodentatonigral degeneration); and B) syndromes appearing mainly in children or young adults (e.g., Hallervorden-Spatz disease and progressive familial myoclonic epilepsy).

III. Syndromes of gradually developing abnormalities of posture and movement such as paralysis agitans (Parkinson's disease), striatonigral degeneration, progressive supranuclear palsy, torsion dystonia (torsion spasm; dystonia musculorum deformans), spasmodic torticollis and other dyskinesis, familial tremor, and Gilles de la Tourette syndrome.

IV. Syndromes of progressive ataxia such as cerebellar degenerations (e.g., cerebellar cortical degeneration and olivopontocerebellar atrophy (OPCA)); and spinocerebellar degeneration (Friedreich's atazia and related disorders).

V. Syndrome of central autonomic nervous system failure (Shy-Drager syndrome).

VI. Syndromes of muscular weakness and wasting without sensory changes (motorneuron disease such as amyotrophic lateral sclerosis, spinal muscular atrophy (e.g., infantile spinal muscular atrophy (Werdnig-Hoffman), juvenile spinal muscular atrophy (Wohlfart-Kugelberg-Welander) and other forms of familial spinal muscular atrophy), primary lateral sclerosis, and hereditary spastic paraplegia.

VII. Syndromes combining muscular weakness and wasting with sensory changes (progressive neural muscular atrophy; chronic familial polyneuropathies) such as peroneal muscular atrophy (Charcot-Marie-Tooth), hypertrophic interstitial polyneuropathy (Dejerine-Sottas), and miscellaneous forms of chronic progressive neuropathy,

VIII. Syndromes of progressive visual loss such as pigmentary degeneration of the

retina (retinitis pigmentosa), and hereditary optic atrophy (Leber's disease).

Combination therapy

The methods of the present invention may also comprise initially administering to the subject an antitumor agent so as to render the neoplastic cells in the subject resistant to an antitumor agent and subsequently administering an effective amount of any of the compositions of the present invention, effective to selectively induce terminal differentiation, cell growth arrest and/or apoptosis of such cells, or to treat cancer or provide chemoprevention.

The antitumor agent may be one of numerous chemotherapy agents such as an alkylating agent, an antimetabolite, a hormonal agent, an antibiotic, colchicine, a vinca alkaloid, L-asparaginase, procarbazine, hydroxyurea, mitotane, nitrosoureas, or an imidazole carboxamide. Suitable agents are those agents that promote depolarization of tubulin. Preferably the antitumor agent is colchicine or a vinca alkaloid; especially preferred are vinblastine and vincristine. In embodiments where the antitumor agent is vincristine, the cells preferably are treated so that they are resistant to vincristine at a concentration of about 5 mg/ml. The treating of the cells to render them resistant to an antitumor agent may be effected by contacting the cells with the agent for a period of at least 3 to 5 days. The contacting of the resulting cells with any of the compounds above is performed as described previously. In addition to the above chemotherapy agents, the compounds may also be administered together with radiation therapy.

Dosages and Dosage Schedules

The dosage regimen utilizing the HDAC inhibitors can be selected in accordance with a variety of factors including type, species, age, weight, sex and the type of cancer being treated; the severity (i.e., stage) of the cancer to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to treat, for example, to prevent, inhibit (fully or partially) or arrest the progress of the disease.

Suitable dosages are total daily dosage of between about 25-4000 mg/m^2 administered orally once-daily, twice-daily or three times-daily, continuous (every day) or intermittently (e.g., 3-5 days a week). For example, SAHA or any one of the HDAC

inhibitors can be administered in a total daily dose of up to 800 mg. The HDAC inhibitor can be administered once daily (QD), or divided into multiple daily doses such as twice daily (BID), and three times daily (TID). The HDAC inhibitor can be administered at a total daily dosage of up to 800 mg, e.g., 150 mg, 200 mg, 300 mg, 400 mg, 600 mg or 800 mg, which can be administered in one daily dose or can be divided into multiple daily doses as described above. Preferably, the administration is oral.

In one embodiment, the composition is administered once daily at a dose of about 200-600 mg. In another embodiment, the composition is administered twice daily at a dose of about 200-400 mg. In another embodiment, the composition is administered twice daily at a dose of about 200-400 mg intermittently, for example three, four, or five days per week. In another embodiment, the composition is administered three times daily at a dose of about 100-250 mg.

In one embodiment, the daily dose is 200 mg, which can be administered once-daily, twice-daily, or three-times daily. In one embodiment, the daily dose is 300 mg, which can be administered once-daily, twice-daily, or three-times daily. In one embodiment, the daily dose is 400 mg, which can be administered once-daily or twice-daily. In one embodiment, the daily dose is 150 mg, which can be, administered twice-daily or three-times daily.

In addition, the administration can be continuous, i.e., every day, or intermittently. The terms "intermittent" or "intermittently" as used herein means stopping and starting at either regular or irregular intervals. For example, intermittent administration of an HDAC inhibitor may be administration one to six days per week or it may mean administration in cycles (e.g., daily administration for two to eight consecutive weeks, then a rest period with no administration for up to one week) or it may mean administration on alternate days.

SAHA or any of the HDAC inhibitors can be administered to the patient at a total daily dosage between 25-4000 mg/m^2.

A currently preferred treatment protocol comprises continuous administration (i.e., every day), once, twice, or three times daily at a total daily dose in the range of about 200 mg to about 600 mg.

Another currently preferred treatment protocol comprises intermittent administration of between three to five days a week, once, twice or three times daily at a total daily dose in the range of about 200 mg to about 600 mg.

In one particular embodiment, the HDAC inhibitor is administered continuously once daily at a dose of 400 mg or twice daily at a dose of 200 mg.

In another particular embodiment, the HDAC inhibitor is administered intermittently three days a week, once daily at a dose of 400 mg or twice daily at a dose of 200 mg.

In another particular embodiment, the HDAC inhibitor is administered intermittently four days a week, once daily at a dose of 400 mg or twice daily at a dose of 200 mg.

In another particular embodiment, the HDAC inhibitor is administered intermittently five days a week, once daily at a dose of 400 mg or twice daily at a dose of 200 mg.

In one particular embodiment, the HDAC inhibitor is administered continuously once daily at a dose of 600 mg, twice daily at a dose of 300 mg, or three times daily at a dose of 200 mg.

In another particular embodiment, the HDAC inhibitor is administered intermittently three days a week, once daily at a dose of 600 mg, twice daily at a dose of 300 mg, or three times daily at a dose of 200 mg.

In another particular embodiment, the HDAC inhibitor is administered intermittently four days a week, once daily at a dose of 600 mg, twice daily at a dose of 300 mg, or three times daily at a dose of 200 mg.

In another particular embodiment, the HDAC inhibitor is administered intermittently five days a week, once daily at a dose of 600 mg, twice daily at a dose of 300 mg, or three times daily at a dose of 200 mg.

In addition, the HDAC inhibitor may be administered according to any of the schedules described above, consecutively for a few weeks, followed by a rest period. For example, the HDAC inhibitor may be administered according to any one of the schedules described above from two to eight weeks, followed by a rest period of one week, or twice daily at a dose of 300 mg for three to five days a week. In another particular embodiment, the HDAC inhibitor is administered three times daily for two consecutive weeks, followed by one week of rest.

It should be apparent to a person skilled in the art that the various dosages and dosing schedules described herein merely set forth specific embodiments and should not be construed as limiting the broad scope of the invention. Any permutations, variations, and combinations of the dosages and dosing schedules are included within the scope of the present invention.

The present invention provides a safe, daily dosing regimen of these formulations, which is easy to follow and to adhere to. The formulations of the present invention are

therefore useful for selectively inducing terminal differentiation, cell growth arrest, and/or apoptosis of neoplastic cells and therefore aid in treatment of tumors in patients.

Pharmaceutical compositions

The compounds of the invention, and derivatives, fragments, analogs, homologs pharmaceutically acceptable salts or hydrate thereof, can be incorporated into pharmaceutical compositions suitable for oral administration, together with a pharmaceutically acceptable carrier or excipient. Such compositions typically comprise a therapeutically effective amount of any of the compounds above, and a pharmaceutically acceptable carrier. Preferably, the effective amount is an amount effective to selectively induce terminal differentiation of suitable neoplastic cells and less than an amount which causes toxicity in a patient.

The compositions of the present invention may be formulated in any unit dosage form (liquid or solid) suited for oral administration, for example, in the form of a pellet, a tablet, a coated tablet, a capsule, a gelatin capsule, a solution, a suspension, or a dispersion. In a currently preferred embodiment, the composition is in the form of a gelatin capsule.

Any inert excipient that is commonly used as a carrier or diluent may be used in the formulations of the present invention, such as for example, a gum, a starch, a sugar, a cellulosic material, an acrylate, or mixtures thereof. A preferred diluent is microcrystalline cellulose. The compositions may further comprise a disintegrating agent (e.g., croscarmellose sodium) and a lubricant (e.g., magnesium stearate), and in addition may comprise one or more additives selected from a binder, a buffer, a protease inhibitor, a surfactant, a solubilizing agent, a plasticizer, an emulsifier, a stabilizing agent, a viscosity increasing agent, a sweetener, a film forming agent, or any combination thereof. Furthermore, the compositions of the present invention may be in the form of controlled release or immediate release formulations.

One embodiment is a pharmaceutical composition for oral administration comprising a HDAC inhibitor or a pharmaceutically acceptable salt or hydrate thereof, microcrystalline cellulose, croscarmellose sodium and magnesium stearate. Another embodiment has SAHA as the HDAC inhibitor. Another embodiment comprises 50-70% by weight of a HDAC inhibitor or a pharmaceutically acceptable salt or hydrate thereof, 20-40% by weight microcrystalline cellulose, 5-15% by weight croscarmellose sodium and 0.1-5% by weight

magnesium stearate. Another embodiment comprises about 50-200 mg of a HDAC inhibitor.

In one embodiment, the pharmaceutical compositions are administered orally, and are thus formulated in a form suitable for oral administration, i.e., as a solid or a liquid preparation. Suitable solid oral formulations include tablets, capsules, pills, granules, pellets, and the like. Suitable liquid oral formulations include solutions, suspensions, dispersions, emulsions, oils, and the like. In one embodiment of the present invention, the composition is formulated in a capsule. In accordance with this embodiment, the compositions of the present invention comprise in addition to the HDAC inhibitor active compound and the inert carrier or diluent, a hard gelatin capsule.

As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration, such as sterile pyrogen-free water. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Solid carriers/diluents include, but are not limited to, a gum, a starch (e.g., corn starch, pregelatinized starch), a sugar (e.g., lactose, mannitol, sucrose, dextrose), a cellulosic material (e.g., microcrystalline cellulose), an acrylate (e.g., polymethylacrylate), calcium carbonate, magnesium oxide, talc, or mixtures thereof.

For liquid formulations, pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, emulsions, or oils. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions, or suspensions, including saline and buffered media. Examples of oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, mineral oil, olive oil, sunflower oil, and fish-liver oil. Solutions or suspensions can also include the following

components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide.

In addition, the compositions may further comprise binders (e.g., acacia, cornstarch, gelatin, carbomer, ethyl cellulose, guar gum, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, povidone), disintegrating agents (e.g., cornstarch, potato starch, alginic acid, silicon dioxide, croscarmellose sodium, crospovidone, guar gum, sodium starch glycolate, Primogel), buffers (e.g., tris-HCl, acetate, phosphate) of various pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), protease inhibitors, surfactants (e.g., sodium lauryl sulfate), permeation enhancers, solubilizing agents (e.g., glycerol, polyethylene glycerol), a glidant (e.g., colloidal silicon dioxide), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite, butylated hydroxyanisole), stabilizers (e.g., hydroxypropyl cellulose, hyroxypropylmethyl cellulose), viscosity increasing agents (e.g., carbomer, colloidal silicon dioxide, ethyl cellulose, guar gum), sweeteners (e.g., sucrose, aspartame, citric acid), flavoring agents (e.g., peppermint, methyl salicylate, or orange flavoring), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), lubricants (e.g., stearic acid, magnesium stearate, polyethylene glycol, sodium lauryl sulfate), flow-aids (e.g., colloidal silicon dioxide), plasticizers (e.g., diethyl phthalate, triethyl citrate), emulsifiers (e.g., carbomer, hydroxypropyl cellulose, sodium lauryl sulfate), polymer coatings (e.g., poloxamers or poloxamines), coating and film forming agents (e.g., ethyl cellulose, acrylates, polymethacrylates) and/or adjuvants.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal

suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

The daily administration can be repeated continuously for a period of several days to several years. Oral treatment may continue for between one week and the life of the patient. Preferably the administration takes place for five consecutive days after which time the patient can be evaluated to determine if further administration is required. The administration can be continuous or intermittent, i.e., treatment for a number of consecutive days followed by a rest period.

The compounds of the present invention may be administered intravenously on the first day of treatment, with oral administration on the second day and all consecutive days thereafter.

The compounds of the present invention may be administered for the purpose of preventing disease progression or stabilizing tumor growth.

The preparation of pharmaceutical compositions that contain an active component is well understood in the art, for example, by mixing, granulating, or tablet-forming processes. The active therapeutic ingredient is often mixed with excipients that are pharmaceutically acceptable and compatible with the active ingredient. For oral administration, the active agents are mixed with additives customary for this purpose, such as vehicles, stabilizers, or inert diluents, and converted by customary methods into suitable forms for administration, such as tablets, coated tablets, hard or soft gelatin capsules, aqueous, alcoholic or oily

solutions and the like as detailed above.

The amount of the compound administered to the patient is less than an amount that would cause toxicity in the patient. In the certain embodiments, the amount of the compound that is administered to the patient is less than the amount that causes a concentration of the compound in the patient's plasma to equal or exceed the toxic level of the compound. Preferably, the concentration of the compound in the patient's plasma is maintained at about 10 nM. In another embodiment, the concentration of the compound in the patient's plasma is maintained at about 25 nM. In another embodiment, the concentration of the compound in the patient's plasma is maintained at about 50 nM. In another embodiment, the concentration of the compound in the patient's plasma is maintained at about 100 nM. In another embodiment, the concentration of the compound in the patient's plasma is maintained at about 500 nM. In another embodiment, the concentration of the compound in the patient's plasma is maintained at about 1000 nM. In another embodiment, the concentration of the compound in the patient's plasma is maintained at about 2500 nM. In another embodiment, the concentration of the compound in the patient's plasma is maintained at about 5000 nM.

It has been found with HMBA that administration of the compound in an amount from about 5 gm/m^2/day to about 30 gm/m^2/day, particularly about 20 gm/m^2/day, is effective without producing toxicity in the patient. The optimal amount of the compound that should be administered to the patient in the practice of the present invention will depend on the particular compound used and the type of cancer being treated.

In a currently preferred embodiment of the present invention, the pharmaceutical composition comprises a histone deacetylase (HDAC) inhibitor; microcrystalline cellulose as a carrier or diluent; croscarmellose sodium as a disintegrant; and magnesium stearate as a lubricant. In another current preferred embodiment, the HDAC inhibitor is suberoylanilide hydroxamic acid (SAHA). Another currently preferred embodiment of the invention is a solid formulation of SAHA with microcrystalline cellulose, NF (Avicel Ph 101), sodium croscarmellose, NF (AC-Di-Sol) and magnesium stearate, NF, contained in a gelatin capsule.

The percentage of the active ingredient and various excipients in the formulations may vary. For example, the composition may comprise between 20 and 90%, preferably between 50-70% by weight of the histone deacetylase (HDAC). Furthermore, the composition may comprise between 10 and 70%, preferably between 20-40% by weight

microcrystalline cellulose as a carrier or diluent. Furthermore, the composition may comprise between 1 and 30%, preferably 5-15% by weight croscarmellose sodium as a disintegrant. Furthermore, the composition may comprise between 0.1-5% by weight magnesium stearate as a lubricant. In another preferred embodiment, the composition comprises about 50-200 mg of the HDAC inhibitor (e.g., 50 mg, 100 mg and 200 mg for the HDAC inhibitor, for example, SAHA). In a particularly preferred embodiment, the composition is in the form of a gelatin capsule.

A currently preferred embodiment is 200 mg of solid SAHA with 89.5 mg of microcrystalline cellulose, 9 mg of sodium croscarmellose and 1.5 mg of magnesium stearate contained in a gelatin capsule.

***In Vitro* Methods:**

The present invention also provides *in-vitro* methods for selectively inducing terminal differentiation, cell growth arrest and/or apoptosis of neoplastic cells, e.g., lymphoma cells, thereby inhibiting proliferation of such cells, by contacting the cells with an effective amount of a an HDAC inhibitor, e.g., SAHA, or a pharmaceutically acceptable salt or hydrate thereof.

The present invention also provides *in-vitro* methods for inhibiting the activity of a histone deacetylase, by the histone deacetylase with an effective amount of an HDAC inhibitor, e.g., SAHA, or a pharmaceutically acceptable salt or hydrate thereof.

Although the methods of the present invention can be practiced *in vitro*, it is contemplated that the preferred embodiment for the methods of selectively inducing terminal differentiation, cell growth arrest and/or apoptosis of neoplastic cells, and of inhibiting HDAC will comprise contacting the cells *in vivo*, i.e., by administering the compounds to a subject harboring neoplastic cells or tumor cells in need of treatment.

Thus, the present invention also provides methods for selectively inducing terminal differentiation, cell growth arrest and/or apoptosis of neoplastic cells, e.g., lymphoma cells in a subject, thereby inhibiting proliferation of such cells in said subject, by administering to the subject a pharmaceutical composition comprising an effective amount of an HDAC inhibitor, e.g., SAHA, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or diluent. An effective amount of an HDAC inhibitor in the present invention can be up to a total daily dose of 800 mg.

The present invention also provides methods for inhibiting the activity of a histone deacetylase in a subject, by administering to the subject a pharmaceutical composition comprising an effective amount of an HDAC inhibitor, e.g., SAHA, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or diluent. An effective amount of an HDAC inhibitor in the present invention can be up to a total daily dose of 800 mg.

EXAMPLES

The invention is illustrated in the Examples that follow. This section is set forth to aid in an understanding of the invention but is not intended to, and should not be construed to limit in any way the invention as set forth in the claims which follow thereafter.

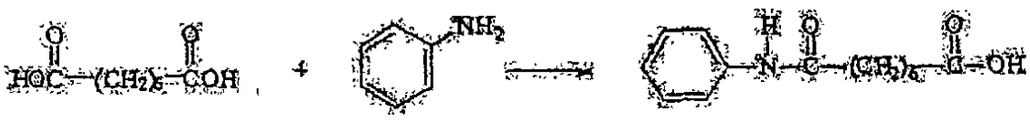
EXAMPLE 1

Synthesis of SAHA

SAHA can be synthesized according to the method outlined below, or according to the method set forth in US Patent 5,369,108, the contents of which are incorporated by reference in their entirety, or according to any other method.

Synthesis of SAHA

Step 1 - Synthesis of Suberanilic acid

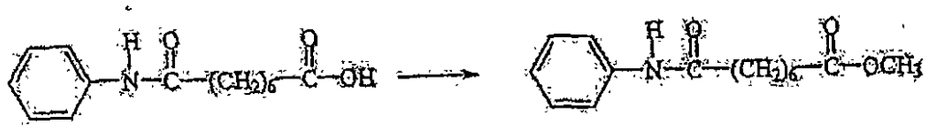


In a 22 L flask was placed 3,500 g (20.09 moles) of suberic acid, and the acid melted with heat. The temperature was raised to 175°C, and then 2,040 g (21.92 moles) of aniline was added. The temperature was raised to 190°C and held at that temperature for 20 minutes. The melt was poured into a Nalgene tank that contained 4,017 g of potassium hydroxide dissolved in 50 L of water. The mixture was stirred for 20 minutes following the addition of the melt. The reaction was repeated at the same scale, and the second melt was poured into the same solution of potassium hydroxide. After the mixture was thoroughly stirred, the stirrer was turned off, and the mixture was allowed to settle. The mixture was then filtered through a pad of Celite (4,200 g) (the product was filtered to remove the

neutral by-product (from attack by aniline on both ends of suberic acid). The filtrate contained the salt of the product, and also the salt of unreacted suberic acid. The mixture was allowed to settle because the filtration was very slow, taking several days). The filtrate was acidified using 5 L of concentrated hydrochloric acid; the mixture was stirred for one hour, and then allowed to settle overnight. The product was collected by filtration, and washed on the funnel with deionized water (4 x 5 L). The wet filter cake was placed in a 72 L flask with 44 L of deionized water, the mixture heated to 50°C, and the solid isolated by a hot filtration (the desired product was contaminated with suberic acid which is has a much greater solubility in hot water. Several hot triturations were done to remove suberic acid. The product was checked by NMR [D_6DMSO] to monitor the removal of suberic acid). The hot trituration was repeated with 44 L of water at 50°C. The product was again isolated by filtration, and rinsed with 4 L of hot water. It was dried over the weekend in a vacuum oven at 65°C using a Nash pump as the vacuum source (the Nash pump is a liquid ring pump (water) and pulls a vacuum of about 29 inch of mercury. An intermittent argon purge was used to help carry off water); 4,182.8 g of suberanilic acid was obtained.

The product still contained a small amount of suberic acid; therefore the hot trituration was done portionwise at 65°C, using about 300 g of product at a time. Each portion was filtered, and rinsed thoroughly with additional hot water (a total of about 6 L). This was repeated to purify the entire batch. This completely removed suberic acid from the product. The solid product was combined in a flask and stirred with 6 L of methanol/water (1:2), and then isolated by filtration and air dried on the filter over the week end. It was placed in trays and dried in a vacuum oven at 65°C for 45 hours using the Nash pump and an argon bleed. The final product has a weight of 3,278.4 g (32.7% yield).

Step 2—Synthesis of Methyl Suberanilate

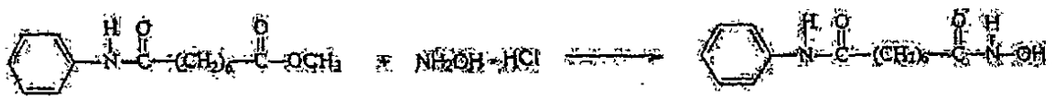


To a 50 L flask fitted with a mechanical stirrer, and condenser was placed 3,229 g of suberanilic acid from the previous step, 20 L of methanol, and 398.7 g of Dowex 50WX2-400 resin. The mixture was heated to reflux and held at reflux for 18 hours. The mixture was filtered to remove the resin beads, and the filtrate was taken to a residue on a rotary

evaporator.

The residue from the rotary evaporator was transferred into a 50 L flask fitted with a condenser and mechanical stirrer. To the flask was added 6 L of methanol, and the mixture heated to give a solution. Then 2 L of deionized water was added, and the heat turned off. The stirred mixture was allowed to cool, and then the flask was placed in an ice bath, and the mixture cooled. The solid product was isolated by filtration, and the filter cake was rinsed with 4 L of cold methanol/water (1:1). The product was dried at 45°C in a vacuum oven using a Nash pump for a total of 64 hours to give 2,850.2 g (84% yield) of methyl suberanilate, CSL Lot # 98-794-92-3 1.

Step 3 – Synthesis of Crude SAHA



To a 50 L flask with a mechanical stirrer, thermocouple, and inlet for inert atmosphere was added 1,451.9 g of hydroxylamine hydrochloride, 19 L of anhydrous methanol, and a 3.93 L of a 30% sodium methoxide solution in methanol. The flask was then charged with 2,748.0 g of methyl suberanilate, followed by 1.9 L of a 30% sodium methoxide solution in methanol. The mixture was allowed to stir for 16 hr and 10 minutes. Approximately one half of the reaction mixture was transferred from the reaction flask (flask 1) to a 50 L flask (flask 2) fitted with a mechanical stirrer. Then 27 L of deionized water was added to flask 1 and the mixture was stirrer for 10 minutes. The pH was taken using a pH meter; the pH was 11.56. The pH of the mixture was adjusted to 12.02 by the addition of 100 ml of the 30% sodium methoxide solution in methanol; this gave a clear solution (the reaction mixture at this time contained a small amount of solid. The pH was adjusted to give a clear solution from which the precipitation the product would be precipitated). The reaction mixture in flask 2 was diluted in the same manner; 27 L of deionized water was added, and the pH adjusted by the addition of 100 ml of a 30 % sodium methoxide solution to the mixture, to give a pH of 12.01 (clear solution).

The reaction mixture in each flask was acidified by the addition of glacial acetic acid to precipitate the product. Flask 1 had a final pH of 8.98, and Flask 2 had a final pH of 8.70. The product from both flasks was isolated by filtration using a Buchner funnel and

filter cloth. The filter cake was washed with 15 L of deionized water, and the funnel was covered and the product was partially dried on the funnel under vacuum for 15.5 hr. The product was removed and placed into five glass trays. The trays were placed in a vacuum oven and the product was dried to constant weight. The first drying period was for 22 hours at 60°C using a Nash pump as the vacuum source with an argon bleed. The trays were removed from the vacuum oven and weighed. The trays were returned to the oven and the product dried for an additional 4 hr and 10 minutes using an oil pump as the vacuum source and with no argon bleed. The material was packaged in double 4-mill polyethylene bags, and placed in a plastic outer container. The final weight after sampling was 2633.4 g (95.6%).

Step 4 – Recrystallization of Crude SAHA

The crude SAHA was recrystallized from methanol/water. A 50 L flask with a mechanical stirrer, thermocouple, condenser, and inlet for inert atmosphere was charged with the crude SAHA to be crystallized (2,525.7 g), followed by 2,625 ml of deionized water and 15,755 ml of methanol. The material was heated to reflux to give a solution. Then 5,250 ml of deionized water was added to the reaction mixture. The heat was turned off, and the mixture was allowed to cool. When the mixture had cooled sufficiently so that the flask could be safely handled (28°C), the flask was removed from the heating mantle, and placed in a tub for use as a cooling bath. Ice/water was added to the tub to cool the mixture to -5°C. The mixture was held below that temperature for 2 hours. The product was isolated by filtration, and the filter cake washed with 1.5 L of cold methanol/water (2:1). The funnel was covered, and the product was partially dried under vacuum for 1.75 hr. The product was removed from the funnel and placed in 6 glass trays. The trays were placed in a vacuum oven, and the product was dried for 64.75 hr at 60°C using a Nash pump as the vacuum source, and using an argon bleed. The trays were removed for weighing, and then returned to the oven and dried for an additional 4 hours at 60°C to give a constant weight. The vacuum source for the second drying period was an oil pump, and no argon bleed was used. The material was packaged in double 4-mill polyethylene bags, and placed in a plastic outer container. The final weight after sampling was 2,540.9 g (92.5%).

EXAMPLE 2

Oral dosing of suberoylanilide hydroxamic acid (SAHA)

Background: Treatment with hybrid polar cellular differentiation agents has resulted in the inhibition of growth of human solid tumor derived cell lines and xenografts. The effect is mediated in part by inhibition of histone deacetylase. SAHA is a potent histone deacetylase inhibitor that has been shown to have the ability to induce tumor cell growth arrest, differentiation, and apoptosis in the laboratory and in preclinical studies.

Objectives: To define a safe daily oral regimen of SAHA that can be used in Phase II studies. In addition, the pharmacokinetic profile of the oral formulation of SAHA was be evaluated. The oral bioavailability of SAHA in humans in the fasting vs. non-fasting state and anti-tumor effects of treatment were also monitored. Additionally, the biological effects of SAHA on normal tissues and tumor cells were assessed and responses with respect to levels of histone acetylation were documented.

Patients: Patients with histologically documented advanced stage, primary or metastatic adult solid tumors that are refractory to standard therapy or for which no curative standard therapy exists. Patients must have a Karnofsky Performance Status of ≥70%, and adequate hematologic, hepatic, and renal function. Patients must be at least four weeks from any prior chemotherapy, radiation therapy or other investigational anticancer drugs.

Dosing Schedule: On the first day, patients were first treated with 200 mg of intravenously-administered SAHA. Starting on the second day, patients were treated with daily doses of oral SAHA according to Table 1. Each cohort received a different dose of SAHA. "QD" indicates dosing once a day; "Q12 hours" indicates dosing twice a day. For example, patients in Cohort IV received two 800 mg doses of SAHA per day. Doses were administered to patients daily and continuously. Blood samples were taken on day one and on day 21 of oral treatment. Patients were taken off oral SAHA treatment due to disease progression, tumor regression, unacceptable side effects, or treatment with other therapies.

Table 1: Oral SAHA Dose Schedule

Cohort	Oral Dose (mg)	Number of Days	Daily Dosing Schedule
I	200	Continuous	QD
II	400	Continuous	QD
III	400	Continuous	Q12 hours
IV	800	Continuous	Q12 hours
V	1200	Continuous	Q12 hours

VI	1600	Continuous	Q12 hours
VII	2000	Continuous	Q12 hours

Results: Comparison of serum plasma levels shows high bioavailability of SAHA administered orally, both when the patient fasted and when the patient did not fast, compared to SAHA administered intravenously (IV SAHA). "AUC" is an estimate of the bioavailability of SAHA in (ng/ml)min, where 660 ng/ml is equal to 2.5 μM SAHA. The AUC taken together with the half-life ($t_{½}$) shows that the overall bioavailability of oral SAHA is better than that of IV SAHA. C_{max} is the maximum concentration of SAHA observed after administration. IV SAHA was administered at 200 mg infused over two hours. The oral SAHA was administered in a single capsule at 200 mg. Tables 2 and 3 summarize the results of an HPLC assay (LCMS using a deuterated standard) that quantitates the amount of SAHA in the blood plasma of the patients versus time, using acetylated histone-4 (α-AcH4) as a marker.

Table 2: Serum Plasma Levels of Oral SAHA – Patient #1

	IV	Oral (fasting)	Oral (nonfasting)
C_{max} (ng/ml)	1329	225	328
$t_{½}$ (min)	20	80	64
AUC (ng/ml)min	153,000	25,000	59,000

Table 3: Serum Plasma Levels of Oral SAHA – Patient #2

	IV	Oral (fasting)	Oral (nonfasting)
C_{max} (ng/ml)	1003	362	302
$t_{½}$ (min)	21	82	93
AUC (ng/ml)min	108,130	63,114	59,874

Figures 1 to 8 are HPLC slides showing the amount of α-AcH4 in patients in Cohorts I and II, measured at up to 10 hours after receiving the oral dose, compared with the α-AcH4 levels when SAHA was administered intravenously. Fig 9 shows the mean plasma concentration of SAHA (ng/ml) at the indicated time points following administration. Fig 9A: Oral dose (200 mg and 400 mg) under fasting on Day 8. Fig 9B: Oral dose (200 mg

and 400 mg) with food on Day 9. Fig 9C: IV dose on day 1. Fig 10 shows the apparent half-life of a SAHA 200 mg and 400 mg oral dose, on Days 8, 9 and 22. Fig 11 shows the AUC (ng/ml/hr) of a SAHA 200 mg and 400 mg oral dose, on Days 8, 9 and 22. Figure 12 shows the bioavailability of SAHA after a 200 mg and 400 mg oral dose, on Days 8, 9 and 22.

EXAMPLE 3

Oral dosing of suberoylanilide hydroxamic acid (SAHA) – Dose Escalation

In another experiment, twenty-five patients with solid tumors have been enrolled onto arm A, thirteen patients with Hodgkin's or non-Hodgkin's lymphomas have been enrolled onto arm B, and one patient with acute leukemia and one patient with myelodysplastic syndrome have been enrolled onto arm C, as shown in Table 4.

Table 4: Dose Escalation Scheme and Number of Patients on Each Dose Level

Cohort	Dose (mg/day)	Dosing Schedule	#Days of Dosing	Rest Period	#Patients Enrolled (arm A/arm B/arm C)*
I	200	Once a day	Continuous	None	6/0/0
II	400	Once a day	Continuous	None	5/4/2
III	400	q 12 hours	Continuous	None	6/3/0
IV	600	Once a day	Continuous	None	4/3/0
V	200	q 12 hours	Continuous	None	4/3/0
VI	300	q 12 hours	Continuous	None	-/-/-
					Sub-totals: 25/13/2
					Total = 40

*Arm A= solid tumor, arm B= lymphoma, arm C= leukemia

Results:

Among eleven patients treated in Cohort II, one patient experienced the DLT of grade 3 diarrhea and grade 3 dehydration during the first treatment cycle. Nine patients were entered into Cohort III. Two patients were not evaluable for the 28-day toxicity assessment because of early study termination due to rapid progression of disease. Of the seven remaining patients, five experienced DLT during the first treatment cycle: diarrhea/dehydration (n=1), fatigue/dehydration (n=1), anorexia (n=1), dehydration (n=1) and anorexia/dehydration (n=1). These five patients recovered in approximately one week after the study drug was held. They were subsequently dose reduced to 400 mg QD, which

appeared to be well tolerated. The median days on 400 mg BID for all patients in Cohort III was 21 days. Based on these findings the 400 mg q12 hour dosing schedule was judged to have exceeded the maximally tolerated dose. Following protocol amendment, accrual was continued in cohort IV at a dose of 600 mg once a day. Of the seven patients enrolled onto cohort IV, two were not evaluable for the 28-day toxicity assessment because of early study termination due to rapid progression of disease. Three patients experienced DLT during the first treatment cycle: anorexia/dehydration/fatigue (n=1), and diarrhea/dehydration (n=2). The 600 mg dose was therefore judged to have exceeded the maximally tolerated dose and the 400 mg once a day dose was defined as the maximally tolerated dose for once daily oral administration. The protocol was amended to evaluate additional dose levels of the twice a day dosing schedule at 200 mg BID and 300 mg BID administered continuously.

The interim pharmacokinetic analysis was based on 18 patients treated on the dose levels of 200 mg QD, 400 mg QD, and 400 mg BID. In general, the mean estimates of C_{max} and AUC_{inf} of SAHA administered orally under fasting condition or with food increased proportionally with dose in the 200 mg to 400 mg dose range. Overall, the fraction of AUC_{inf} due to extrapolation was 1% or less. Mean estimates for apparent half-life were variable across dose groups under fasting condition or with food, ranging from 61 to 114 minutes. The mean estimates of C_{max}, varies from 233 ng/ml (0.88 μM) to 570 ng/ml (2.3 μM). The bioavailable fraction of SAHA, calculated from the AUC_{inf} values after the IV infusion and oral routes, was found to be approximately 0.48.

Peripheral blood mononuclear cells were collected pre-therapy, immediately post-infusion and between 2 - 10 hours after oral ingestion of the SAHA capsules to assess the effect of SAHA on the extent of histone acetylation in a normal host cell. Histones were isolated and probed with anti-acetylated histone (H3) antibody followed by HRP-secondary antibody. Preliminary analysis demonstrated an increase in the accumulation of acetylated histones in peripheral mononuclear cells that could be detected up to 10 hours after ingestion of SAHA capsules at 400 mg per day dose level.

Thirteen patients continued treatment for 3-12 months with responding or stable disease: thyroid (n=3), sweat gland (n=1), renal (n=2), larynx (n=1), prostate (n=1), Hodgkin's lymphoma (n=2), non-Hodgkin's lymphoma (n=2), and leukemia (n=1).

Six patients had tumor shrinkage on CT scans. Three of these six patients meet the criteria of partial response (one patient with metastatic laryngeal cancer and two patients

with non-Hodgkin's lymphomas). These partial responses occurred at the dose levels of 400 mg BID (n=2) and 600 mg QD (n=1).

The dosages described above have also been administered twice daily intermittently. Patients have received SAHA twice daily three to five days per week. Patient response has been seen with administration of SAHA twice daily at 300 mg for three days a week.

EXAMPLE 4

Intravenous Dosing of SAHA

Table 5 shows a dosing schedule for patients receiving SAHA intravenously. Patients begin *in* Cohort I, receiving 300 mg/m^2 of SAHA for five consecutive days in a week for one week, for a total dose of 1500 mg/m^2. Patients were then observed for a period of two weeks and continued to Cohort II, then progressed through the Cohorts unless treatment was terminated due to disease progression, tumor regression, unacceptable side effects or the patient received other treatment.

Table 5: Standard Dose Escalation for Intravenously-Administered SAHA

Cohort	Dose (mg/m^2)	Number of Days/Week	Number of Consecutive Weeks	Observation Period (Weeks)	Total Dose (mg/m^2)
I	300	5	1	2	1500
II	300	5	2	2	3000
III	300	5	3	1*	4500
IV	600	5	3	1*	9000
V	800	5	3	1*	13500
VI	1200	5	3	1*	18000
VII	1500	5	3	1*	22500

*Hematologic patients started at dose level III.

EXAMPLE 5

Treatment of Mesothelioma with SAHA

Three patients with mesothelioma were enrolled in Phase I studies with SAHA. The patients were administered oral SAHA twice daily at a dose of 300 mg or 400 mg for three days a week. One partial response was observed following SAHA treatment according to the above regimen for 6 months.

Figure 13 is a CT scan of a mesothelioma tumor from a patient, before (PRE – left panel) and after (POST – right panel) treatment with SAHA twice daily at a dose of 300 mg three days a week for 6 months. The data demonstrates that SAHA is effective at treating mesothelioma tumors in patients.

EXAMPLE 6

Treatment of Diffuse Large B-cell Lymphoma (DLBCL) with SAHA

A phase I study of oral SAHA in sixty eight patients with advanced cancer including hematologic cancer and solid tumors was conducted. Patients received SAHA orally (po) 200, 400 or 600 mg QD daily, 200, 300 or 400 mg BID daily, 300 mg or 400 mg (BID) intermittently 3 days a week, or 100 mg TID (2 wks). Seven patients with Diffuse Large B-cell Lymphoma (DLBCL) enrolled in the study.

Results:

A. Complete Response (CR) to oral SAHA treatment:

One patient, a 66 y/o woman, was diagnosed with stage I small lymphocytic lymphoma (plasmacytoid); received bleomycin, CPT and local XRT with complete response; developed recurrent disease (breast/subcutaneous nodule, and lung nodules) and was treated with Fludarabine/mitoxantrone, rituximab, CEPT, liposomal doxorubicin. Transformed to DBLCL, subsequently treated with Rituximab, Anti-B1, CTX/liposomal doxorubicin/pred/vincristine.

The patient was referred to oral SAHA Phase I in with subcutaneous nodules, diffuse adenopathy, large gastrohepatic mass, bilateral lung nodules, bone marrow involvement by lymphoma. The patient received SAHA 400 mg BID for 1 month, was subsequently dose reduced to 400 mg QD, and was on SAHA treatment for a total of 1 year.

Figure 14 is a CT scan taken before (Figure 14A) and after (Figure 14B) 2 months of treatment with SAHA, demonstrating shrinkage of the Gastrohepatic mass. A complete resolution of the Gastrohepatic mass was observed after 2 cycles of treatment (4 months).

Figure 15 is a PET scan taken before (Figure 15A) and after (Figure 15B) 2 months of treatment with SAHA, demonstrating tumor regression following the treatment.

A complete response (CR) (meeting Cheson Criteria) was achieved following 4 months of SAHA treatment. The complete response (CR) has lasted thus far for 14 months, and the patient is still in CR.

B. Partial Response (PR) to oral SAHA treatment:

One patient with DLBCL received 600 mg QD of SAHA orally, and was on SAHA treatment for a total of five months.

Figure 16 is a CT scan taken before (Figure 16A) and after (Figure 16B) 1 month of treatment with SAHA, demonstrating tumor shrinkage following the treatment.

A partial response (RR) (meeting Cheson Criteria) was achieved following 5 months of SAHA treatment.

C. Tumor Regression following oral SAHA treatment:

One patient, a 75 year old woman, initially presented with follicular lymphoma with evidence of transformation. She was originally treated with 6 cycles of cyclophosphamide, doxorubicin, etoposide, and prednisone. She then underwent a splenectomy which showed DLBCL, for which she was treated with Zevalin. She subsequently received two courses of rituximab and eventually pentostatin, cyclophosphamide and rituximab.

The patient received 200 mg BID of SAHA orally, and was on SAHA treatment for six months.

Figure 17 is a PET scan taken before (Figure 17A) and after (Figure 17B) 2 months of treatment with SAHA. As seen, the patient achieved an excellent PET negative response after 2 months on SAHA with continued reduction in her disease.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the meaning of the invention described. The scope of the invention includes the subject matter of the claims that follow. All patents, patent applications, and publications cited herein are hereby incorporated by reference.

References

1. Sporn, M. B., Roberts, A. B., and Driscoll, J. S. (1985) in Cancer: Principles and Practice of Oncology, eds. Hellman, S., et al., Ed. 2, (J. B. Lippincott, Philadelphia), P. 49.

2. Breitman, T. R., Selonick, S. E., and Collins, S. J. (1980) Proc. Natl. Acad. Sci. USA 77: 2936-2940.

3. Olsson, I. L. and Breitman, T. R. (1982) Cancer Res. 42: 3924-3927.

4. Schwartz, E. L. and Sartorelli, A. C. (1982) Cancer Res. 42: 2651-2655.

5. Marks, P. A., Sheffery, M., and Rifkind, R. A. (1987) Cancer Res. 47: 659.

6. Sachs, L. (1978) Nature (Lond.) 274: 535.

7. Friend, C., Scher, W., Holland, J. W., and Sato, T. (1971) Proc. Natl. Acad. Sci. (USA) 68: 378-382.

8. Tanaka, M., Levy, J., Terada, M., Breslow, R., Rifkind, R. A., and Marks, P. A. (1975) Proc. Natl. Acad. Sci. (USA) 72: 1003-1006.

9. Reuben, R. C., Wife, R. L., Breslow, R., Rifkind, R. A., and Marks, P. A. (1976) Proc. Natl. Acad. Sci. (USA) 73: 862-866.

10. Abe, E., Miyaura, C., Sakagami, H., Takeda, M., Konno, K., Yamazaki, T., Yoshika, S., and Suda, T. (1981) Proc. Natl. Acad. Sci. (USA) 78: 4990-4994.

11. Schwartz, E. L., Snoddy, J. R., Kreutter, D., Rasmussen, H., and Sartorelli, A. C. (1983) Proc. Am. Assoc. Cancer Res. 24: 18.

12. Tanenaga, K., Hozumi, M., and Sakagami, Y. (1980) Cancer Res. 40: 914-919.

13. Lotem, J. and Sachs, L. (1975) Int. J. Cancer 15: 731-740.

14. Metcalf, D. (1985) Science, 229: 16-22.

15. Scher, W., Scher, B. M., and Waxman, S. (1983) Exp. Hematol. 11: 490-498.

16. Scher, W., Scher, B. M., and Waxman, S. (1982) Biochem. & Biophys. Res. Comm. 109: 348-354.

17. Huberman, E. and Callaham, M. F. (1979) Proc. Natl. Acad. Sci. (USA) 76: 1293-1297.

18. Lottem, J. and Sachs, L. (1979) Proc. Natl. Acad. Sci. (USA) 76: 5158-5162.

19. Terada, M., Epner, E., Nudel, U., Salmon, J., Fibach, E., Rifkind, R. A., and Marks, P. A. (1978) Proc. Natl. Acad. Sci. (USA) 75: 2795-2799.

20. Morin, M. J. and Sartorelli, A. C. (1984) Cancer Res. 44: 2807-2812.

21. Schwartz, E. L., Brown, B. J., Nierenberg, M., Marsh, J. C., and Sartorelli, A. C. (1983) Cancer Res. 43: 2725-2730.

22. Sugano, H., Furusawa, M., Kawaguchi, T., and Ikawa, Y. (1973) Bibl. Hematol. 39: 943-954.

23. Ebert, P. S., Wars, I., and Buell, D. N. (1976) Cancer Res. 36: 1809-1813.

24. Hayashi, M., Okabe, J., and Hozumi, M. (1979) Gann 70: 235-238.

25. Fibach, E., Reuben, R. C., Rifkind, R. A., and Marks, P. A. (1977) Cancer Res. 37: 440-444.

26. Melloni, E., Pontremoli, S., Damiani, G., Viotti, P., Weich, N., Rifkind, R. A., and Marks, P. A. (1988) Proc. Natl. Acad. Sci. (USA) 85: 3835-3839.

27. Reuben, R., Khanna, P. L., Gazitt, Y., Breslow, R., Rifkind, R. A., and Marks, P. A. (1978) J. Biol. Chem. 253: 4214-4218.

28. Marks, P. A. and Rifkind, R. A. (1988) Int. Journal of Cell Cloning 6: 230-240.

29. Melloni, E., Pontremoli, S., Michetti, M., Sacco, O., Cakiroglu, A. G., Jackson, J. F., Rifkind, R. A., and Marks, P. A. (1987) Proc. Natl. Acad. Sciences (USA) 84: 5282-5286.

30. Marks, P. A. and Rifkind, R. A. (1984) Cancer 54: 2766-2769.

31. Egorin, M. J., Sigman, L. M. VanEcho, D. A., Forrest, A., Whitacre, M. Y., and Aisner, J. (1987) Cancer. Res. 47: 617-623.

32. Rowinsky, E. W., Ettinger, D. S., Grochow, L. B., Brundrett, R. B., Cates, A. E., and Donehower, R. C. (1986) J. Clin. Oncol. 4: 1835-1844.

33. Rowinsky, E. L. Ettinger, D. S., McGuire, W. P., Noe, D. A., Grochow, L. B., and Donehower, R. C. (1987) Cancer Res. 47: 5788-5795.

34. Callery, P. S., Egorin, M. J., Geelhaar, L. A., and Nayer, M. S. B. (1986) Cancer Res. 46: 4900-4903.

35. Young, C. W. Fanucchi, M. P., Walsh, T. B., Blatzer, L., Yaldaie, S., Stevens, Y. W., Gordon, C., Tong, W., Rifkind, R. A., and Marks, P. A. (1988) Cancer Res. 48: 7304-7309.

36. Andreeff, M., Young, C., Clarkson, B., Fetten, J., Rifkind, R. A., and Marks, P. A. (1988) Blood 72: 186a.

37. Marks, P. A., Breslow, R., Rifkind, R. A., Ngo, L., and Singh, R. (1989) Proc. Natl. Acad. Sci. (USA) 86: 6358-6362.

38. Breslow, R., Jursic, B., Yan, Z. F., Friedman, E., Leng, L., Ngo, L., Rifkind, R. A., and Marks, P. A. (1991) Proc. Natl. Acad. Sci. (USA) 88: 5542-5546.

39. Richon, V.M., Webb, Y., Merger, R., et al. (1996) PNAS 93:5705-8.

40. Cohen, L.A., Amin, S., Marks, P.A., Rifkind, R.A., Desai, D., and Richon, V.M. (1999) Anticancer Research 19:4999-5006.

41. Grunstein, M. (1997) Nature 389:349-52.

42. Finnin, M.S., Donigian, J.R., Cohen, A., et al. (1999) Nature 401:188-193.

43. Van Lint, C., Emiliani, S., Verdin, E. (1996) Gene Expression 5:245-53.

44. Archer, S. Shufen, M. Shei, A., Hodin, R. (1998) PNAS 95:6791-96.

45. Dressel, U., et al. (2000) Anticancer Research 20(2A):1017-22.

46. Lin, R.J., Nagy, L., Inoue, S., et al. (1998) Nature 391:811-14.

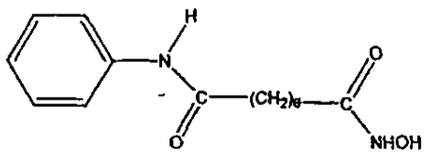
What is claimed is:

1. A method of treating mesothelioma or diffuse large B-cell lymphoma in a subject, said method comprising the step of administering to the subject an effective amount of a pharmaceutical composition comprising a histone deacetylase (HDAC) inhibitor, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or diluent, wherein the amount of histone deacetylase inhibitor is effective to treat said mesothelioma or diffuse large B-cell lymphoma in said subject.

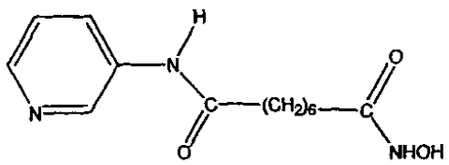
2. The method of claim 1, wherein the method is used to treat mesothelioma in said subject.

3. The method of claim 1, wherein the method is used to treat diffuse large B-cell lymphoma in said subject.

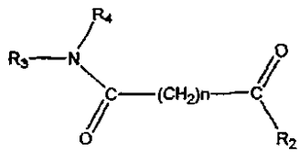
4. The method of claim 1, wherein the HDAC inhibitor is suberoylanilide hydroxamic acid (SAHA), represented by the structure:



5. The method of claim 1, wherein the HDAC inhibitor is pyroxamide, represented by the structure:

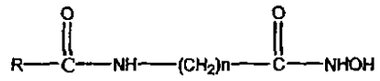


6. The method of claim 1, wherein the HDAC inhibitor is represented by the structure:



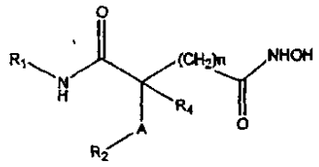
wherein R_3 and R_4 are independently a substituted or unsubstituted, branched or unbranched alkyl, alkenyl, cycloalkyl, aryl, alkyloxy, aryloxy, arylalkyloxy, or pyridine group, cycloalkyl, aryl, aryloxy, arylalkyloxy, or pyridine group, or R_3 and R_4 bond together to form a piperidine group; R_2 is a hydroxylamino group; and n is an integer from 5 to 8.

7. The method of claim 1, wherein the HDAC inhibitor is represented by the structure:



wherein R is a substituted or unsubstituted phenyl, piperidine, thiazole, 2-pyridine, 3- pyridine or 4-pyridine and n is an integer from 4 to 8.

8. The method of claim1, wherein the HDAC inhibitor is represented by the structure:



wherein A is an amide moiety, R_1 and R_2 are each selected from substituted or unsubstituted aryl, arylalkyl, naphthyl, pyridineamino, 9-purine-6-amino, thiazoleamino, aryloxy, arylalkyloxy, pyridyl, quinolinyl or isoquinolinyl; R_4 is hydrogen, a halogen, a phenyl or a cycloalkyl moiety and n is an integer from 3 to 10.

9. The method of claim 1, wherein said HDAC inhibitor is a hydroxamic acid derivative, a Short Chain Fatty Acid (SCFA), a cyclic tetrapeptide, a benzamide derivative, or an electrophilic ketone derivative.

10. The method of claim 1, wherein said HDAC inhibitor is a hydroxamic acid derivative selected from the group consisting of SAHA, Pyroxamide, CBHA, Trichostatin A (TSA), Trichostatin C, Salicylhydroxamic Acid, Azelaic

Bishydroxamic Acid (ABHA), Azelaic-1-Hydroxamate-9-Anilide (AAHA), 6-(3-Chlorophenylureido) carpoic Hydroxamic Acid (3Cl-UCHA), Oxamflatin, A-161906, Scriptaid, PXD-101, LAQ-824, CHAP, MW2796, and MW2996.

11. The method of claim 1, wherein said HDAC inhibitor is a cyclic tetrapeptide selected from the group consisting of Trapoxin A, FR901228 (FK 228 or Depsipeptide), FR225497, Apicidin, CHAP, HC-Toxin, WF27082, and Chlamydocin.

12. The method of claim 1, wherein said HDAC inhibitor is a Short Chain Fatty Acid (SCFA) selected from the group consisting of Sodium Butyrate, Isovalerate, Valerate, 4 Phenylbutyrate (4-PBA), Phenylbutyrate (PB), Propionate, Butyramide, Isobutyramide, Phenylacetate, 3-Bromopropionate, Tributyrin, Valproic Acid and Valproate.

13. The method of claim 1, wherein said HDAC inhibitor is a benzamide derivative selected from the group consisting of CI-994, MS-27-275 (MS-275) and a 3'-amino derivative of MS-27-275.

14. The method according to claim 1, wherein said HDAC inhibitor is an electrophilic ketone derivative selected from the group consisting of a trifluoromethyl ketone and an α-keto amide.

15. The method according to claim 1, wherein said HDAC inhibitor is a natural product, a psammaplin, or Depudecin.

16. The method of claim 1, wherein the pharmaceutical composition is administered orally.

17. The method of claim 16, wherein said composition is contained within a gelatin capsule.

18. The method of claim 17, wherein said carrier or diluent is microcrystalline cellulose.

19. The method of claim 18, further comprising sodium croscarmellose as a disintegrating agent.

20. The method of claim 19, further comprising magnesium stearate as a lubricant.

21. The method of claim 16, wherein said composition is administered to the subject at a total daily dosage of between about 25-4000 mg/m^2.

22. The method of claim 16, wherein said composition is administered once-daily, twice-daily, or three times-daily.

23. The method of claim 22, wherein said composition is administered once daily at a dose of about 200-600 mg.

24. The method of claim 22, wherein said composition is administered twice daily at a dose of about 200-400 mg.

25. The method of claim 22, wherein said composition is administered twice daily at a dose of about 200-400 mg intermittently.

26. The method of claim 25, wherein said composition is administered three to five days per week.

27. The method of claim 25, wherein said composition is administered three days a week.

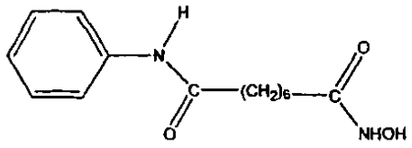
28. The method of claim 27, wherein said composition is administered at a dose of about 200 mg.

29. The method of claim 27, wherein said composition *is* administered at a dose of about 300 mg.

30. The method of claim 27, wherein said composition is administered at a dose of about 400 mg.

31. The method of claim 22, wherein said composition is administered three times daily at a dose of about 100-250 mg.

32. A method of treating mesothelioma or diffuse large B-cell lymphoma in a subject, said method comprising the step of administering to the subject an effective amount of a pharmaceutical composition comprising suberoylanilide hydroxamic acid (SAHA) or a pharmaceutically acceptable salt or hydrate thereof, represented by the structure:



and a pharmaceutically acceptable carrier or diluent, wherein the amount of SAHA is effective to treat said mesothelioma or diffuse large B-cell lymphoma in said subject.

33. The method of claim 32, wherein the method is used to treat mesothelioma in said subject.

34. The method of claim 32, wherein the method is used to treat diffuse large B-cell lymphoma in said subject.

35. The method of claim 32, wherein the pharmaceutical composition is administered orally.

36. The method of claim 35, wherein said composition is contained within a gelatin capsule.

37. The method of claim 36, wherein said carrier or diluent is microcrystalline cellulose.

38. The method of claim 37, further comprising sodium croscarmellose as a disintegrating agent.

39. The method of claim 38, further comprising magnesium stearate as a lubricant.

40. The method of claim 35, wherein said composition is administered to the subject at a total daily dosage of between about 25-4000 mg/m^2.

41. The method of claim 35, wherein said composition is administered once-daily, twice-daily, or three times-daily.

42. The method of claim 41, wherein said composition is administered once daily at a dose of about 200-600 mg.

43. The method of claim 41, wherein said composition is administered twice daily at a dose of about 200-400 mg.

44. The method of claim 41, wherein said composition is administered twice daily at a dose of about 200-400 mg intermittently.

45. The method of claim 44, wherein said composition is administered three to five days per week.

46. The method of claim 44, wherein said composition is administered three days a week.

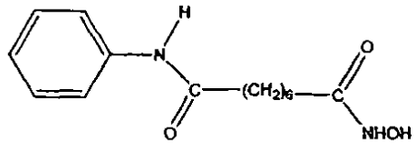
47. The method of claim 46, wherein said composition is administered at a dose of about 200 mg.

48. The method of claim 46, wherein said composition is administered at a dose of about 300 mg.

49. The method of claim 46, wherein said composition is administered at a dose of about 400 mg.

50. The method of claim 41, wherein said composition is administered three times daily at a dose of about 100-250 mg.

51. A method of treating mesothelioma or diffuse large B-cell lymphoma in a subject, said method comprising the step of administering to the subject a total daily dose of up to about 800 mg of a pharmaceutical composition comprising suberoylanilide hydroxamic acid (SAHA) or a pharmaceutically acceptable salt or hydrate thereof, represented by the structure:



and a pharmaceutically acceptable carrier or diluent, wherein the amount of SAHA is effective to treat said mesothelioma or diffuse large B-cell lymphoma in said subject.

52. The method of claim 51, wherein the method is used to treat mesothelioma in said subject.

53. The method of claim 51, wherein the method is used to treat diffuse large B-cell lymphoma in said subject.

54. The method of claim 51, wherein the pharmaceutical composition is administered orally.

55. The method of claim 54, wherein said composition is contained within a gelatin capsule.

56. The method of claim 55, wherein said carrier or diluent is microcrystalline cellulose.

57. The method of claim 56, further comprising sodium croscarmellose as a disintegrating agent.

58. The method of claim 57, further comprising magnesium stearate as a lubricant.

59. The method of claim 54, wherein said composition is administered once-daily, twice-daily, or three times-daily.

60. The method of claim 59, wherein said composition is administered once daily at a dose of about 200-600 mg.

61. The method of claim 59, wherein said composition is administered twice daily at a dose of about 200-400 mg.

62. The method of claim 59, wherein said composition is administered twice daily at a dose of about 200-400 mg intermittently.

63. The method of claim 62, wherein said composition is administered three to five days per week.

64. The method of claim 62, wherein said composition is administered three days a week.

65. The method of claim 64, wherein said composition is administered at a dose of about 200 mg.

66. The method of claim 64, wherein said composition is administered at a dose of about 300 mg.

67. The method of claim 64, wherein said composition is administered at a dose of about 400 mg.

68. The method of claim 59, wherein said composition is administered three times daily at a dose of about 100-250 mg.

FIG. 1

SAHA Patient: IV vs ORAL (200 mg/dose)

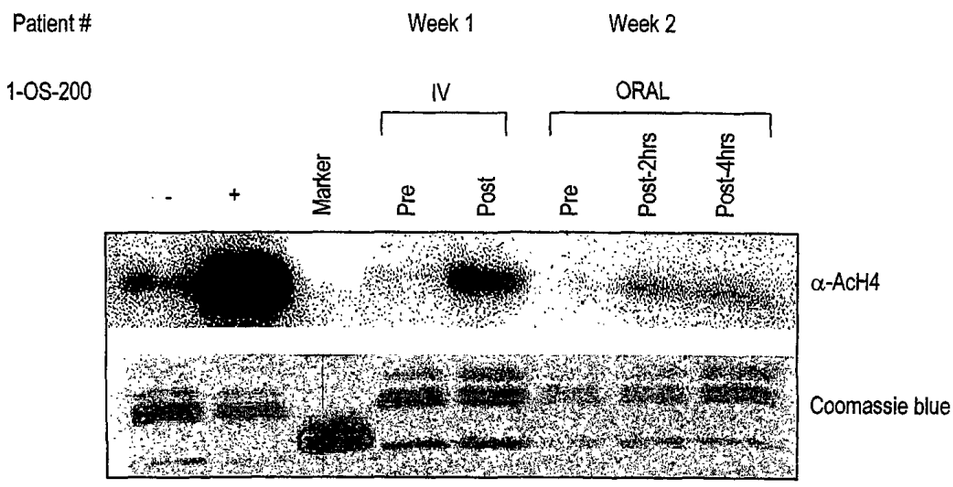


FIG. 2

Protocol 01-021 (ORAL SAHA) ARM A: SOLID TUMOR PATIENTS Cohort I (200 mg/dose)

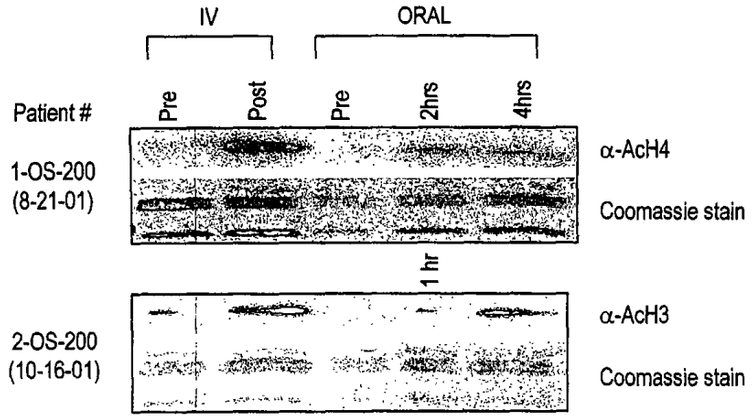


FIG. 3

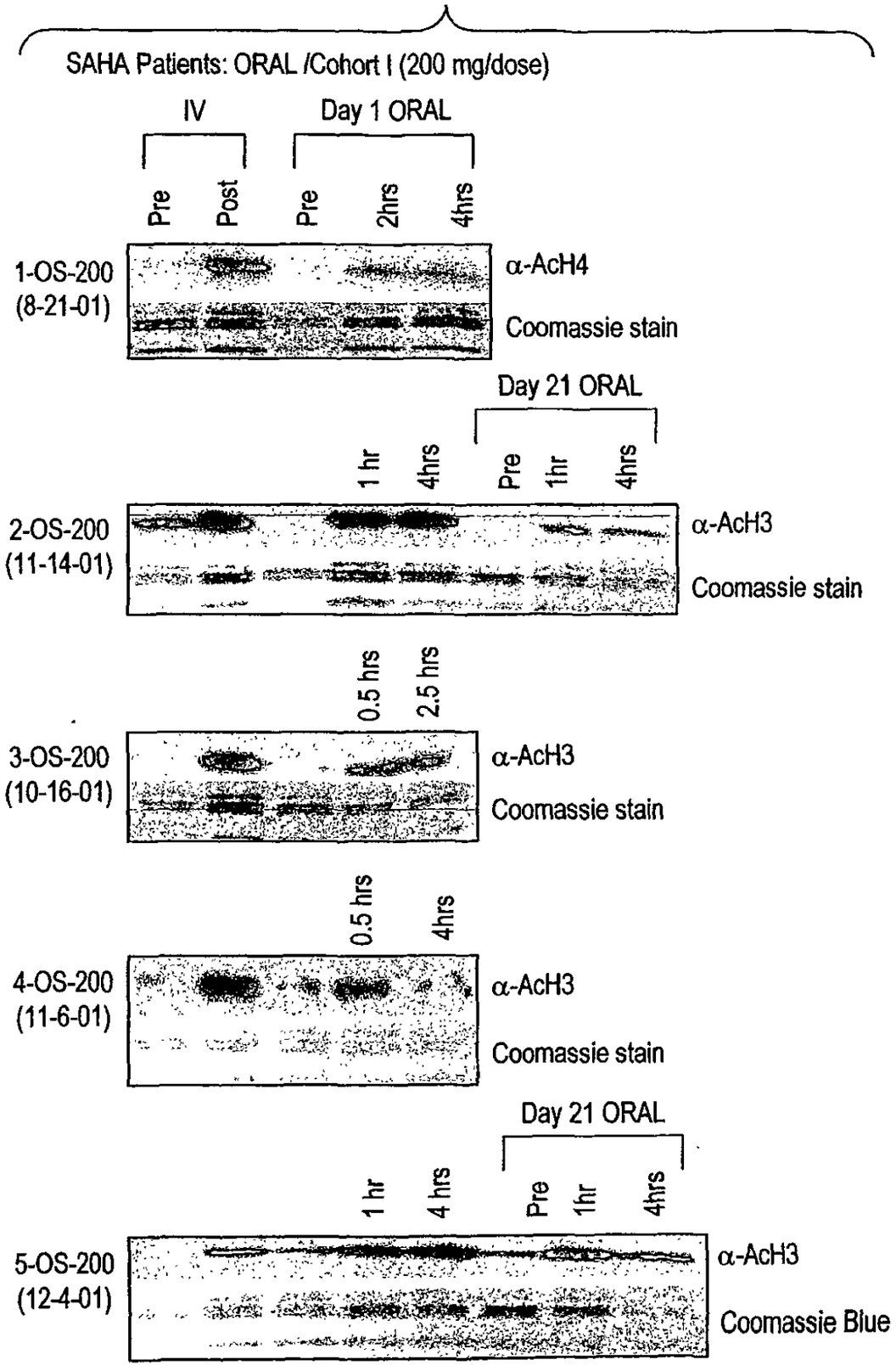

SAHA Patients: ORAL /Cohort I (200 mg/dose)
IV
Day 1 ORAL
Pre
Post
Pre
2hrs
4hrs
1-OS-200
(8-21-01)
α-AcH4
Coomassie stain
Day 21 ORAL
1 hr
4hrs
Pre
1hr
4hrs
2-OS-200
(11-14-01)
α-AcH3
Coomassie stain
0.5 hrs
2.5 hrs
3-OS-200
(10-16-01)
α-AcH3
Coomassie stain
0.5 hrs
4hrs
4-OS-200
(11-6-01)
α-AcH3
Coomassie stain
Day 21 ORAL
1 hr
4 hrs
Pre
1hr
4hrs
5-OS-200
(12-4-01)
α-AcH3
Coomassie Blue

FIG. 4

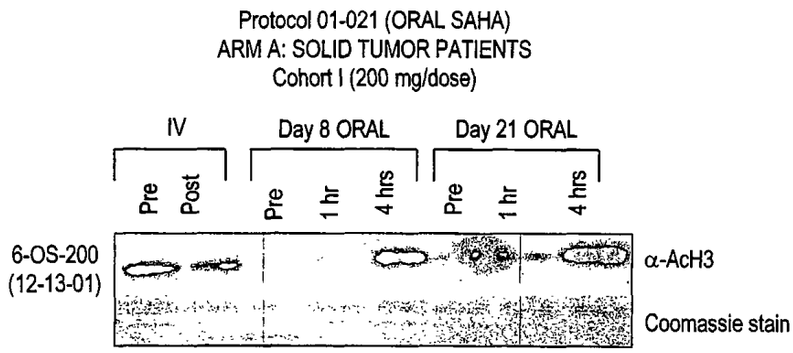

Protocol 01-021 (ORAL SAHA)
ARM A: SOLID TUMOR PATIENTS
Cohort I (200 mg/dose)
IV
Day 8 ORAL
Day 21 ORAL
Pre
Post
Pre
1 hr
4 hrs
Pre
1 hr
4 hrs
6-OS-200
(12-13-01)
α-AcH3
Coomassie stain

FIG. 5

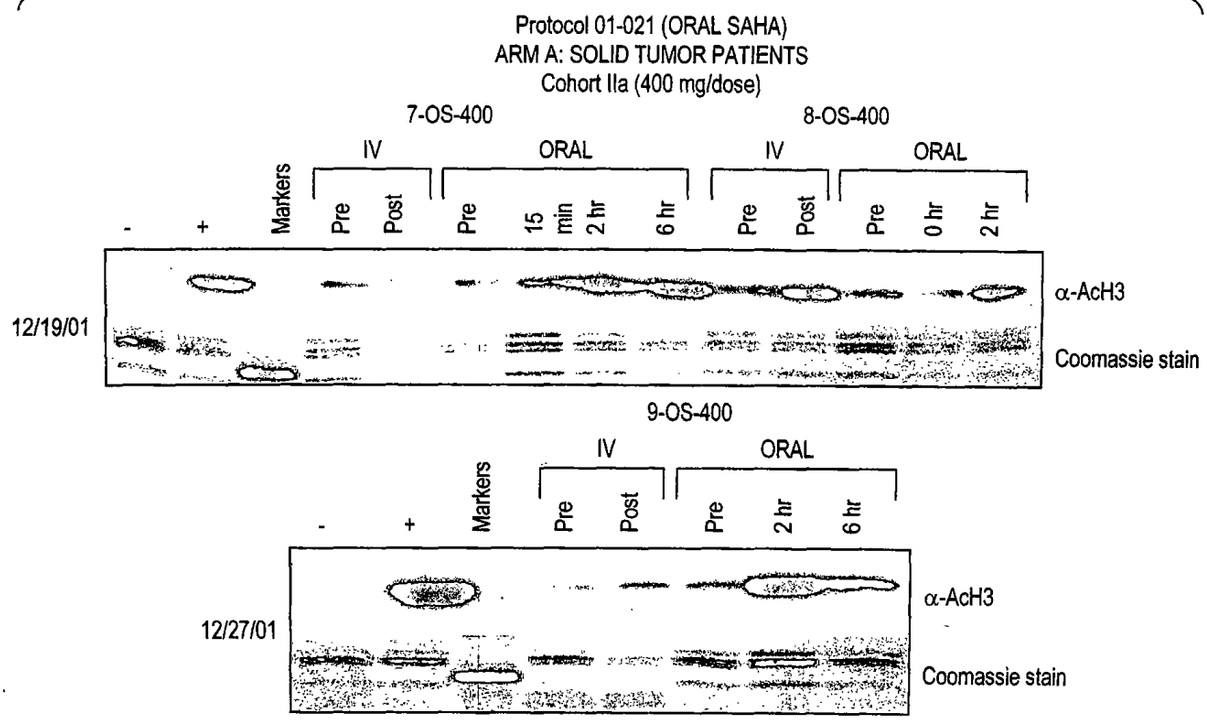
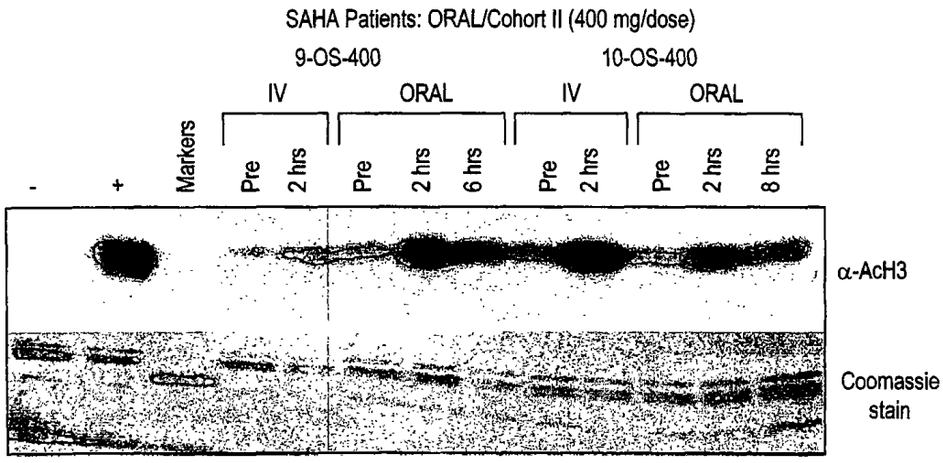

Protocol 01-021 (ORAL SAHA)
ARM A: SOLID TUMOR PATIENTS
Cohort IIa (400 mg/dose)
7-OS-400
8-OS-400
IV
ORAL
IV
ORAL
Markers
Pre
Post
Pre
15 min
2 hr
6 hr
Pre
Post
Pre
0 hr
2 hr
-
+
12/19/01
α-AcH3
Coomassie stain
9-OS-400
IV
ORAL
Markers
Pre
Post
Pre
2 hr
6 hr
-
+
12/27/01
α-AcH3
Coomassie stain

FIG. 6


SAHA Patients: ORAL/Cohort II (400 mg/dose)
9-OS-400
10-OS-400
IV
ORAL
IV
ORAL
-
+
Markers
Pre
2 hrs
Pre
2 hrs
6 hrs
Pre
2 hrs
Pre
2 hrs
8 hrs
α-AcH3
Coomassie stain

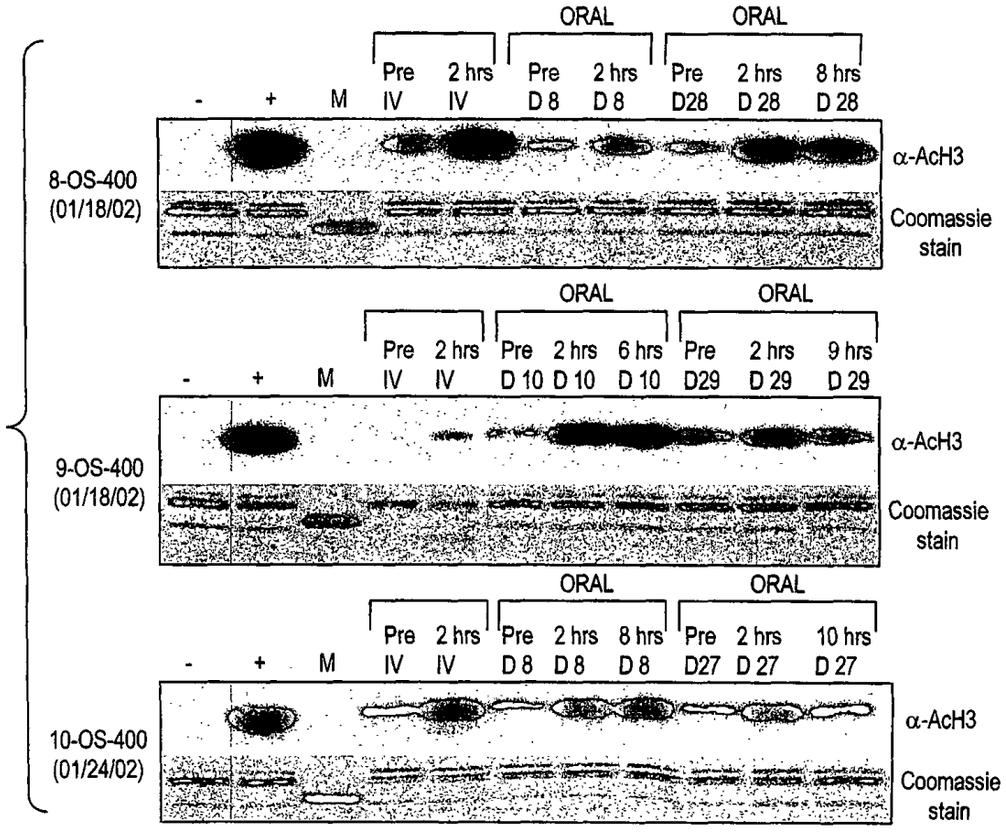


FIG. 7

Protocol 01-021
(ORAL SAHA)
ARM A: SOLID
TUMOR PATIENTS
Cohort IIa (400 mg/dose)

FIG. 8

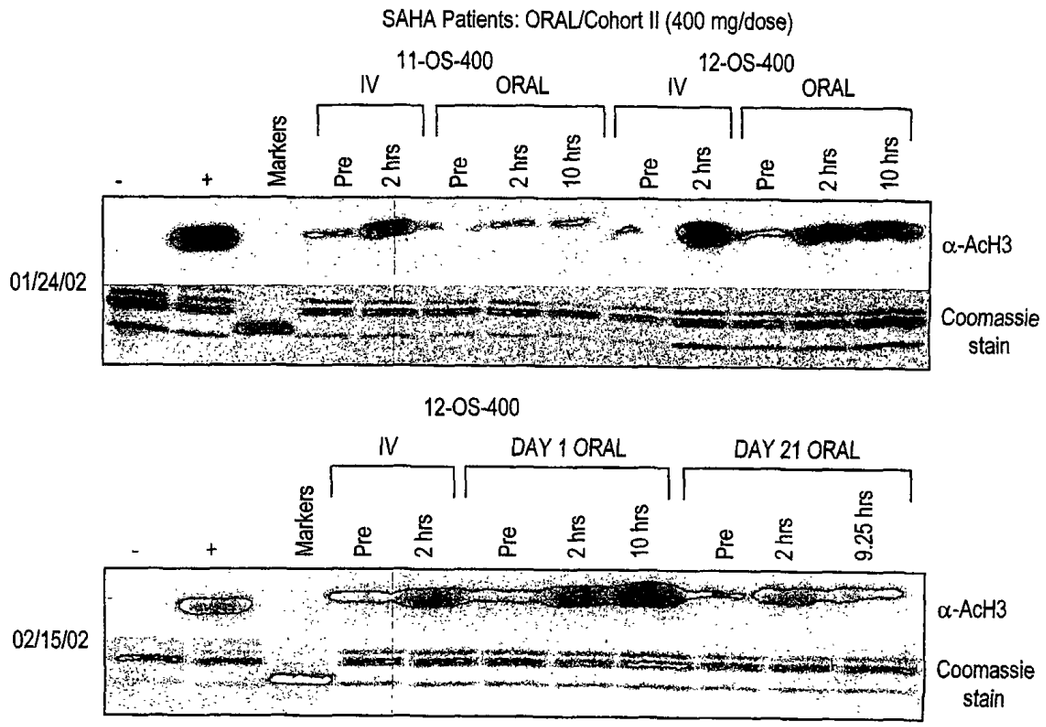

SAHA Patients: ORAL/Cohort II (400 mg/dose)
11-OS-400
12-OS-400
IV
ORAL
IV
ORAL
Markers
Pre
2 hrs
Pre
2 hrs
10 hrs
Pre
2 hrs
Pre
2 hrs
10 hrs
-
+
01/24/02
α-AcH3
Coomassie stain
12-OS-400
IV
DAY 1 ORAL
DAY 21 ORAL
Markers
Pre
2 hrs
Pre
2 hrs
10 hrs
Pre
2 hrs
9.25 hrs
-
+
02/15/02
α-AcH3
Coomassie stain

FIG. 9A

Oral 200 mg vs. 400 mg (Fasting)
Oral dose on Cycle 1 Day 8

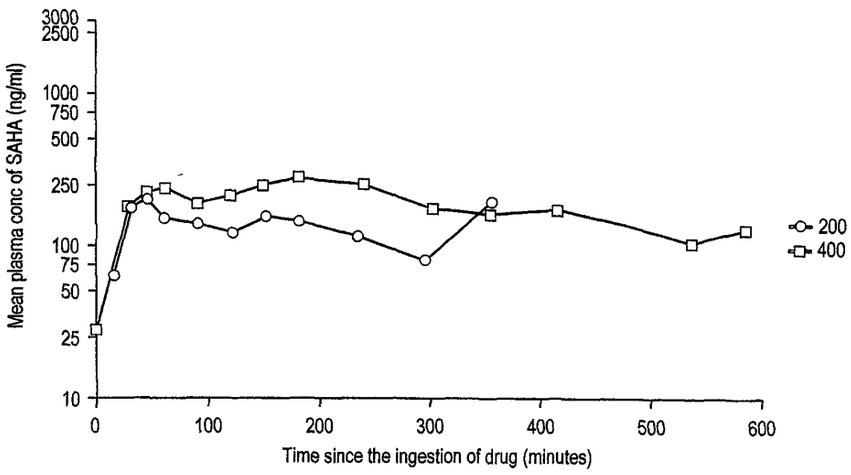


FIG. 9B

Oral 200 mg vs. 400 mg (no-fasting)
Oral Dose on Cycle 1 Day 9

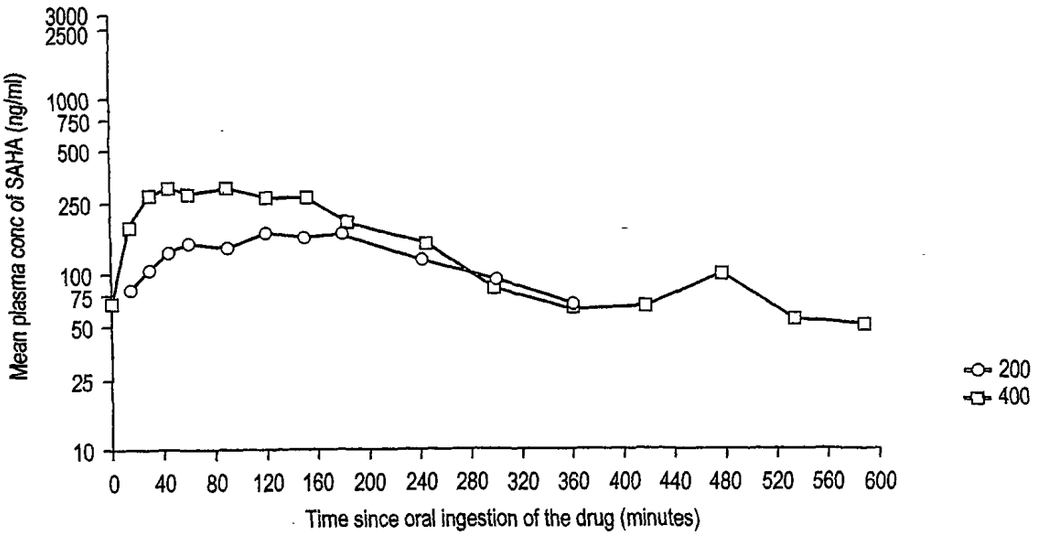


FIG. 9C

IV 200 mg vs. 400 mg
IV Dose on Cycle 1 Day 1

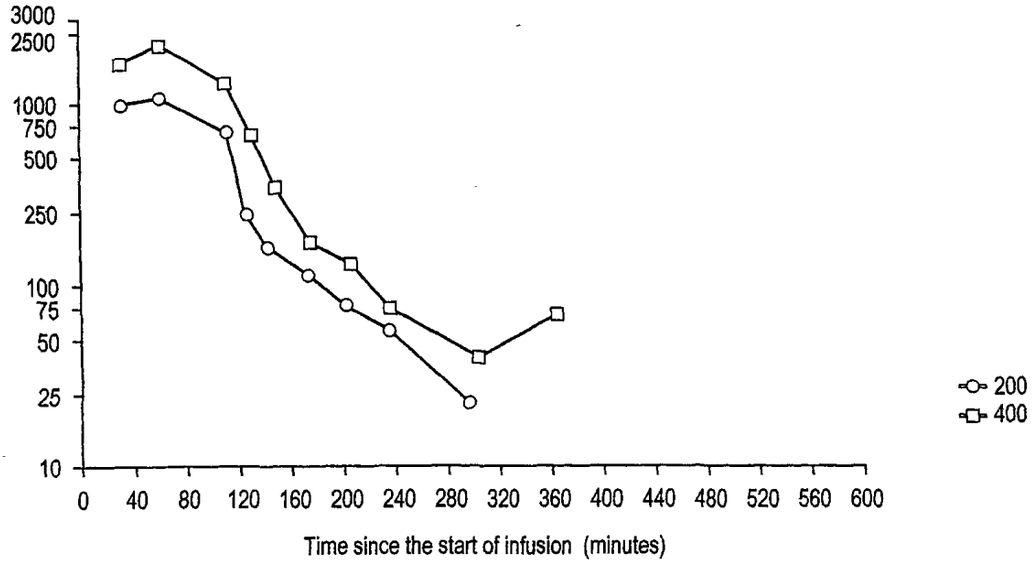


FIG. 10

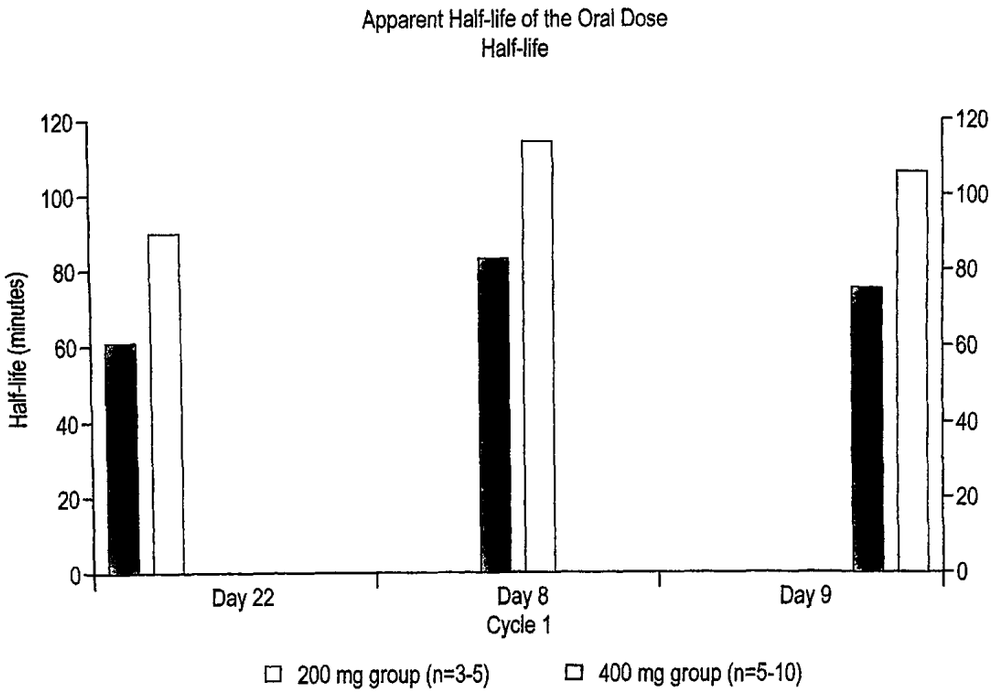

Apparent Half-life of the Oral Dose
Half-life
Half-life (minutes)
120
100
80
60
40
20
0
Day 22
Day 8
Cycle 1
Day 9
200 mg group (n=3-5)
400 mg group (n=5-10)

FIG. 11

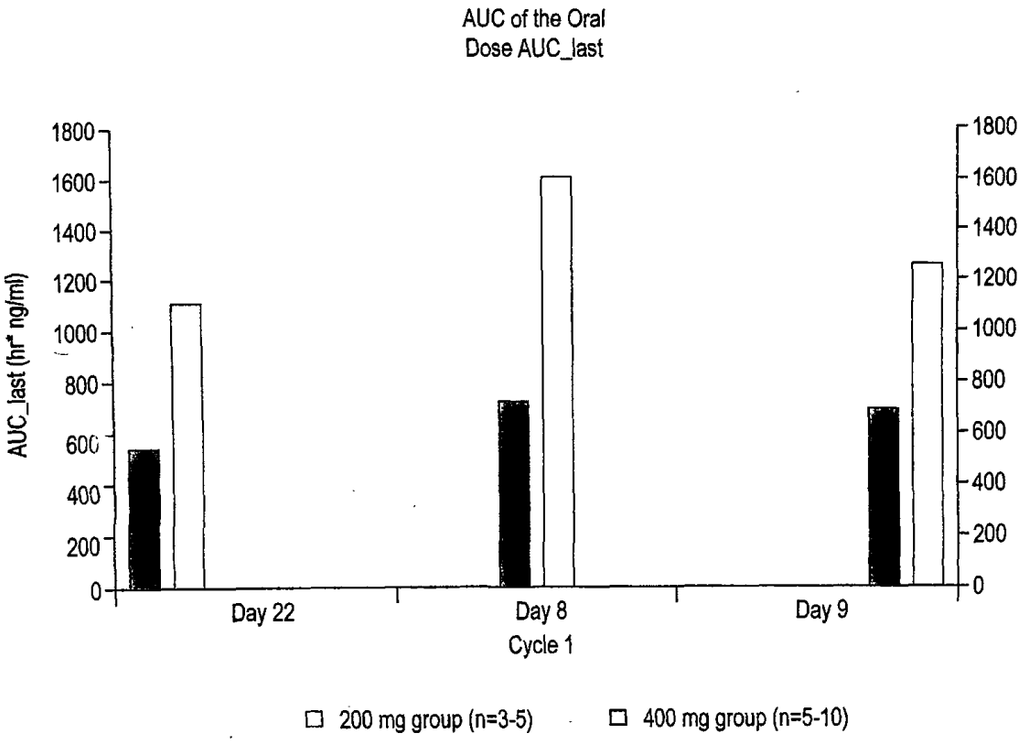
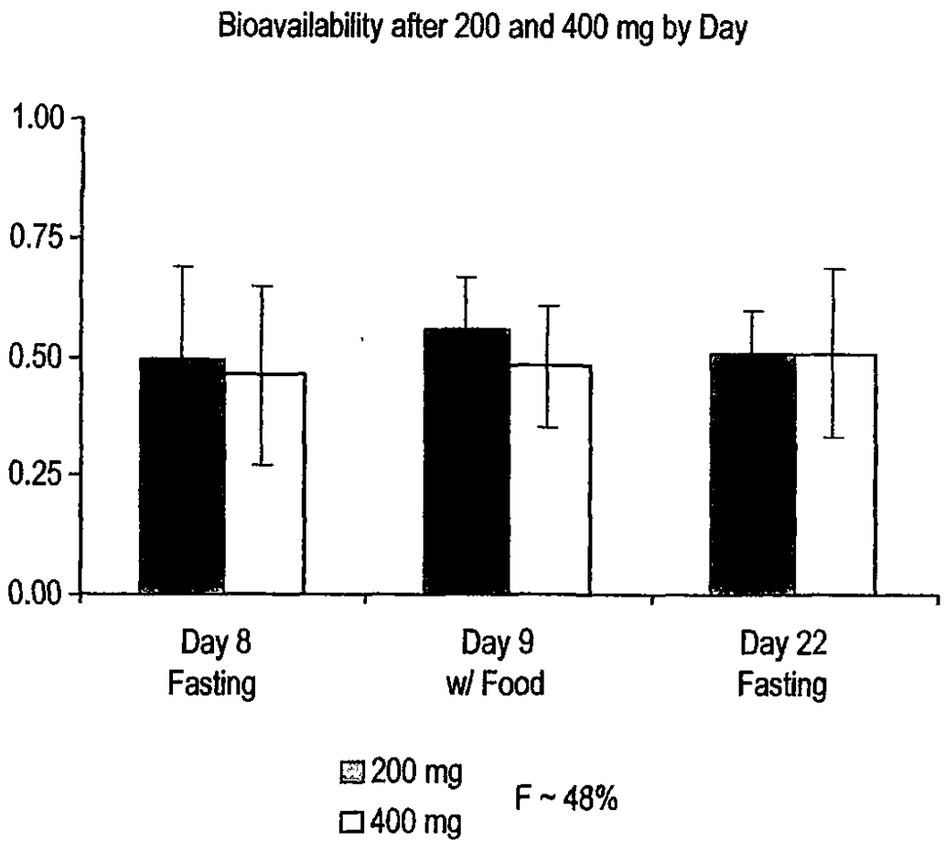
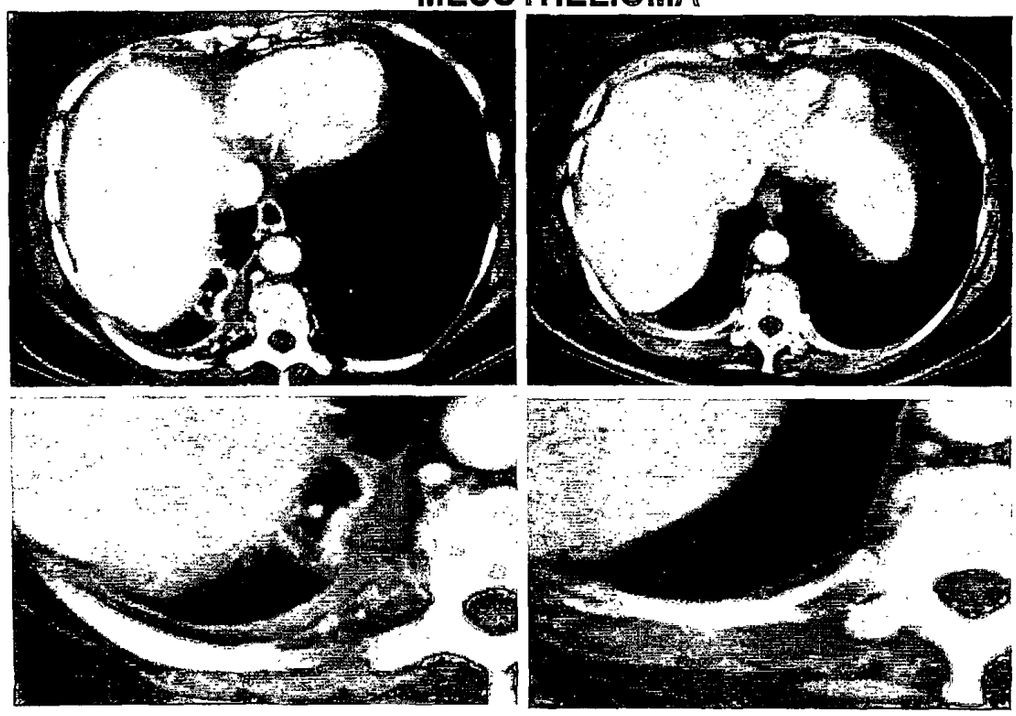

AUC of the Oral
Dose AUC_last
AUC_last (hr* ng/ml)
1800
1600
1400
1200
1000
800
600
400
200
0
Day 22
Day 8
Day 9
Cycle 1
200 mg group (n=3-5)
400 mg group (n=5-10)

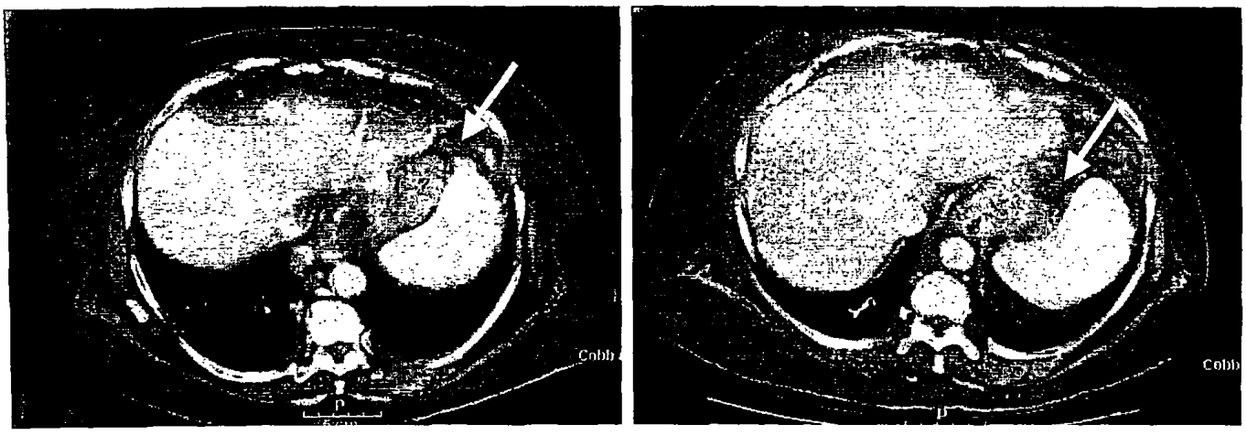
FIG. 12


Bioavailability after 200 and 400 mg by Day
1.00
0.75
0.50
0.25
0.00
Day 8
Fasting
Day 9
w/ Food
Day 22
Fasting
200 mg
400 mg
F ~ 48%

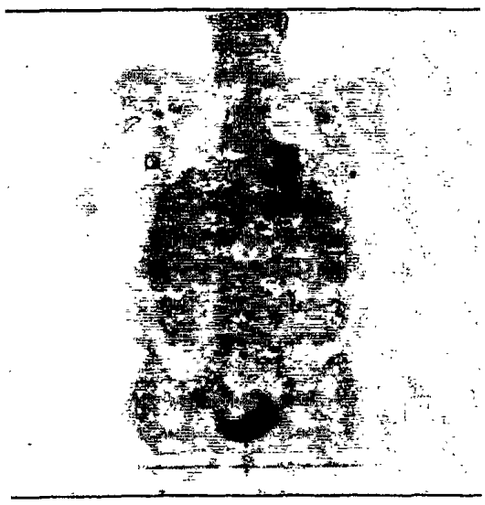
MESOTHELIOMA



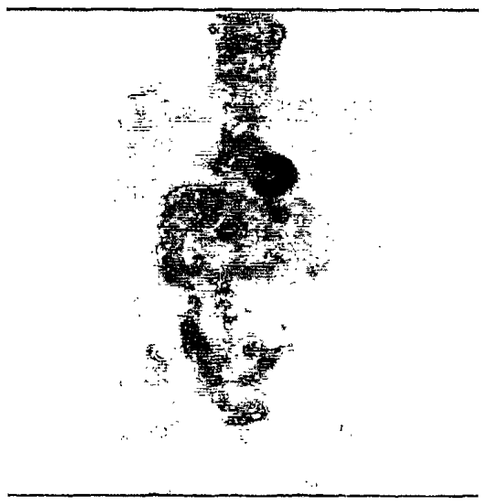
PRE FIG. 13 POST



A FIG. 14 B

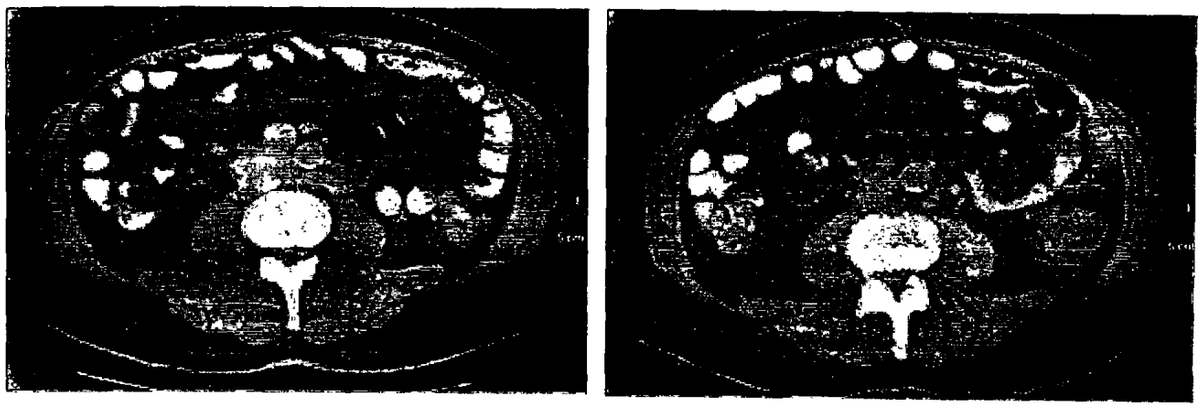


A



B

FIG. 15



A FIG. 16 B



A FIG. 17 B

MÉTODOS DE TRATAMIENTO DE CÁNCER CON INHIBIDORES HDAC

DECLARACIÓN DE INTERÉS DEL GOBIERNO

Esta invención se hizo en todo o en parte con ayuda del gobierno bajo el número de otorgamiento 1R21 CA 096228-01 premiado por el Instituto Nacional de Cáncer. El gobierno puede tener ciertos derechos en la invención.

CAMPO DE LA INVENCIÓN

La presente invención se relaciona con métodos para tratar cánceres, por ejemplo, mesotelioma o linfoma. Más específicamente, la presente invención se relaciona con métodos para ensayar mesotelioma, linfoma de célula B grande difusa (DLBCL), u otros cánceres o tumores mediante la administración de composiciones farmacéuticas que comprenden inhibidores HDAC, por ejemplo, ácido suberoilanilida hidroxámico (SAHA). Las formulaciones orales de las composiciones farmacéuticas tienen perfiles farmacocinéticos favorables tal como biodisponibilidad y sorprendentemente dan origen a altos niveles en la sangre de los compuestos activos durante un periodo extendido de tiempo.

ANTECEDENTES DE LA INVENCIÓN

A lo largo de esta solicitud se hace referencia a varias publicaciones mediante números arábigos dentro de paréntesis. Las citas completas de estas publicaciones se pueden encontrar al final de la especificación precediendo inmediatamente las reivindicaciones. Las descripciones de estas publicaciones se incorporan aquí dentro de esta solicitud en su totalidad como referencia con el fin de describir más completamente el estado de la técnica al cual pertenece la invención.

El cáncer es un desorden en el cual una población de células se ha vuelto, en varios grados, no respondiente a mecanismos de control que normalmente originan proliferación y diferenciación.

El mesotelioma es una forma rara de cáncer en el cual las células malignas (cancerosas) se encuentran en el mesotelio, un saco protector que cubre la mayoría de los órganos internos del cuerpo. El mesotelio es una membrana que cubre y protege la mayoría de los órganos internos del cuerpo. Se compone de dos capas de células: una capa que circunda inmediatamente el órgano, la otra forma un saco alrededor de éste. El mesotelio produce un fluido lubricante que se libera entre estas capas, que permite el movimiento de los órganos

(tal como el palpitar del corazón y la expansión y contracción de los pulmones) para deslizarse fácilmente contra las estructuras adyacentes.

El mesotelio tiene diferentes nombres, dependiendo de su localización en el cuerpo. El peritoneo es el tejido de mesotelio que cubre la mayoría de los órganos en la cavidad abdominal. La pleura es la membrana que circunda los pulmones y las líneas de la pared de la cavidad del pecho. El pericardio cubre y protege el corazón. El tejido mesotelial que circunda los órganos reproductivos en cuerpos masculinos se denomina la *tunica vaginalis testis*. La *tunica serosa uteri* cubre los órganos reproductores internos en la mujer. En la mayoría de los casos de mesotelioma inicia en la pleura o el peritoneo. Los tumores malignos que surgen en el mesotelio pleural están fuertemente ligados a exposición a asbestos.

Aunque las proporciones de incidencia reportadas se han incrementado en los pasados veinte años el mesotelioma es aún un cáncer relativamente raro. Aproximadamente 2,000 nuevos casos de mesotelioma son diagnosticados en los Estados Unidos cada año. El mesotelioma ocurre más a menudo en hombres que en mujeres y el riesgo se incrementa con la edad, pero esta enfermedad puede aparecer en hombres o en mujeres a cualquier edad.

La brevedad en la respiración y dolor en el pecho debido a una acumulación de fluido en la pleura son a menudo los síntomas del mesotelioma pleural. Los síntomas del mesotelioma peritoneal incluyen pérdida de peso y dolor abdominal e hinchamiento debido al desarrollo del fluido en el abdomen. Otros síntomas de mesotelioma peritoneal pueden incluir obstrucción del intestino, anormalidades de taponamiento en la sangre, anemia y fiebre. Si el cáncer se ha esparcido más allá del mesotelio a otras partes del cuerpo, los síntomas pueden incluir dolor, tragado problemático o hinchamiento del cuello o la cara.

El pronóstico del mesotelioma es triste, con pobre respuesta a la cirugía radical, quimioterapia habitual, terapia de radiación, y terapia de combinación. El esparcimiento microscópico de las células cancerígenas en la pared del pecho y del diafragma son comunes aun cuando tal esparcimiento puede no ser detectado mediante ensayos de rutina.

El linfoma es el cáncer del sistema linfático, una red de nodos linfáticos, órganos (que incluyen el bazo, el timo y las amígdalas), y vasos que son parte del sistema inmune. Existen muchos diferentes tipos de linfoma, y ellos se pueden dividir en dos categorías: enfermedad de Hodgkin (HD) y

linfoma no Hodgkin (NHL). La principal diferencia entre los dos es el tipo de célula involucrado.

El sistema linfático es parte del sistema inmune del cuerpo y ayuda a combatir la infección. Es un sistema complejo hecho de órganos, tal como la médula ósea, el timo, y el bazo, y los nodos linfáticos (o glándulas linfáticas), que están conectadas por una red de vasos linfáticos pequeños. Los nodos linfáticos se encuentran en todo el cuerpo.

Los linfocitos son células sanguíneas blancas que circulan a través del sistema linfático; ellos son componentes esenciales del sistema inmune del cuerpo. Existen dos tipos principales de linfocitos: las células B y las células T. La mayoría de los linfocitos inician el crecimiento en la médula ósea. Las células B continúan el desarrollo en la médula ósea, mientras que las células T van desde la médula ósea a la glándula del timo y maduran allí. Una vez ellas están maduras, tanto las células B como las células T ayudan al cuerpo a combatir las infecciones.

Existen más de 20 diferentes tipos de linfoma no-Hodgkin. El linfoma de célula B grande difusa es un tipo común, totalizando aproximadamente el 40% de todos los casos. Este es un cáncer de los linfocitos B. El linfoma de célula difuso puede ocurrir en cualquier momento desde la adolescencia a la

edad madura. Es ligeramente más común en hombres que en mujeres.

Los linfomas no Hodgkin están también divididos en uno de dos grupos: bajo y alto grado. Los linfomas de bajo grado son usualmente de crecimiento lento y los linfomas de alto grado tienden a crecer más rápidamente. Los linfomas de célula B grandes difusos son linfomas de alto grado y necesitan pronto tratamiento.

El tratamiento principal para el linfoma de célula B grande difusa es la quimioterapia. El tipo de quimioterapia depende de la extensión del linfoma y otros factores, tal como la edad y la salud en general. Las dos drogas que son usualmente dadas para tratar el linfoma de célula B grande difusa, se denominan doxorubicina y ciclofosfomida. Ellas son usualmente dadas con otras drogas anticancerígenas. Habitualmente la combinación más ampliamente utilizada se denomina régimen "CHOP". Este incluye las drogas vincristina y prednisolona, así como también doxorubicina y ciclofosfomida. La quimioterapia puede usualmente ser dada a un paciente externo en el hospital y continúa por cuatro a seis meses.

Además, la quimioterapia en altas dosis con infusiones de la médula ósea o las células madre ha sido efectiva en algunos pacientes cuyo linfoma ha reincidido. Este tipo de

tratamiento involucra tener quimioterapia muy intensiva y algunas veces radioterapia. Como algunos efectos colaterales pueden ser severos, algunos tipos de trasplante no son dados a personas de más de 45-50 años y otros pueden ser dados a personas de hasta 65 años quienes se ajustan suficientemente para tomar el tratamiento. La intensidad del tratamiento incrementa los riesgos de efectos colaterales serios para personas mayores de esta edad.

La radioterapia también se puede utilizar cuando las células de linfoma están contenidas en una o dos áreas de los nodos de linfoma en la misma parte del cuerpo (etapa 1 o 2). Este también se puede dar además de la quimioterapia.

Otro tratamiento que se ha tratado es un anticuerpo monoclonal denominado rituximab.

Durante muchos años ha habido dos estrategias principales para el tratamiento quimioterapéutico del cáncer: 1) bloqueo de proliferación de célula tumoral dependiente de hormona mediante la interferencia con la producción o la acción periférica de las hormonas sexuales; y 2) matar las células cancerosas directamente al exponerlas a sustancias citotóxicas, que dañan tanto la población de células neoplásicas como las normales.

A pesar de muchos avances en el campo de la oncología, la mayoría de los tumores sólidos permanecen incurables en las etapas avanzadas. La terapia citotóxica se utiliza en la mayoría de los casos, sin embargo, esta causa a menudo morbilidad significativa al paciente sin beneficio clínico significativo. Se necesitan agentes menos tóxicos y más específicos para tratar y controlar las malignidades avanzadas.

Una aproximación alternativa para la terapia de cáncer es la inducción de la diferenciación terminal de células neoplásicas (1). En los modelos de cultivo celular la diferenciación se ha reportado mediante la disposición de células a una variedad de estímulos, que incluyen: AMP cíclica y ácido retinoico (2,3) aclarubicina y otras antraciclinas.

Existe abundante evidencia de que las dosis de transformación neoplásica no necesariamente destruyen el potencial de células cancerígenas para diferenciarlas (1,5,6). Existen muchos ejemplos de células tumorales que no responden a los reguladores normales de proliferación y parecen estar bloqueados en la expresión de su programa de diferenciación, y se pueden aún introducir para diferenciar y cesar la replicación. Una variedad de agentes, que incluyen algunos compuestos polares relativamente simples (5,7-9), derivados

de vitamina D y ácido retinoico (10-12), hormonas esteroides (13), factores de crecimiento (6,14) proteasas (15,16) promotores tumorales (17,18), e inhibidores de síntesis de ADN o ARN (4,19-24), pueden inducir varios explantes tumorales de líneas celulares transformadas y de tumores humanos primarios para expresar características más diferenciadas.

Estudios recientes identificaron una serie de compuestos polares que son inductores efectivos de la diferenciación en un número de líneas celulares transformadas (8,9). De estos, el inductor más efectivo es el compuesto polar/apolar híbrido N,N'-hexametileno bisacetamida (HMBA) (9). El uso de este compuesto polar/apolar para inducir células de eritroleucemia de murino (MELC) para sufrir diferenciación de eritroide con supresión de oncogenicidad ha probado ser un modelo útil para estudiar la diferenciación mediada por inductor de células transformadas (5,7-9). La diferenciación eritroide terminal MELC inducida por HMBA es un proceso multietapa. Luego de la adición de HMBA a MELC (745A-DS19) en cultivo, existe un periodo latente de 10 a 12 horas antes que se detecte el compromiso para la diferenciación terminal. El compromiso se define como la capacidad de las células a expresar diferenciación terminal a pesar de la remoción del inductor (25). Luego de la exposición continúa a HMBA existe un reclutamiento progresivo de células para diferenciación. Los

presentes inventores han reportado que las líneas celulares MELC hacen resistencia a relativamente bajos niveles de vincristina se vuelve marcadamente más sensibles a la acción inductora del HMBA y se pueden inducir para diferenciarse como poco o ningún periodo latente (26).

El HMBA es capaz de inducir cambios fenotípicos consistentes con la diferenciación en una amplía variedad de líneas celulares (5). Las características del efecto inducido por la droga han sido más intensamente estudiadas en el sistema celular de eritroleucemia de murino (MELC) (5,25,27,28). La inducción de MELC de diferenciación es tanto dependiente del tiempo como de la concentración. La concentración mínima requerida para demostrar un efecto in vitro en la mayoría de las cepas es 2 a 3 mM; la duración mínima de la exposición continúa requiere generalmente inducir la diferenciación en una porción substancial (mayor 20%) de la población sin la exposición continúa a la droga es de aproximadamente 36 horas.

El objetivo primario de acción del HMBA no es conocido. Existe evidencia de que la proteína quinasa C está involucrada en la senda de diferenciación mediada por inductor (29). Los estudios in vitro suministran una base para evaluar el potencial del HMBA como agente de citodiferenciación en el tratamiento de cánceres humanos

(30). Varios ensayos clínicos fase I con HMBA se han completado (31-36). Los ensayos clínicos han mostrado que este compuesto puede inducir una respuesta terapéutica en pacientes con cáncer (35,36). Sin embargo, estos ensayos clínicos de fases I también han demostrado que la eficacia potencial del HMBA está limitada, en parte, por la toxicidad relacionada con dosis que evita lograr niveles sanguíneos óptimos y por la necesidad de la administración intravenosa de grandes cantidades del agente, durante periodos prolongados.

Se ha reportado que un número de compuestos relacionados con HMBA con grupos polares separados mediante enlaces apolares que, sobre una base molar, son tan activos (37) o 100 veces más activos que el HMBA (38). Como una clase, sin embargo, se ha encontrado que los dímeros simétricos tales como el HMBA y los compuestos relacionados no son los mejores agentes de citodiferenciación.

Se ha encontrado de manera inesperada que los mejores compuestos comprenden dos grupos de extremo polar separados por una cadena flexible de grupos metileno en donde uno o ambos grupos de extremo polar son grupos hidrófobos largos. Preferiblemente, los grupos de extremo polar son diferentes y solamente uno es un grupo hidrófobo grande. Estos compuestos

son inesperadamente miles de veces más activos que los HMBA y diez veces más activos que los compuestos relacionados HMBA.

Los inhibidores de histona desacetilasa tal como el ácido suberoilanilida hidroxámico (SAHA), pertenecen a esta clase de agentes que tienen la capacidad de inducir disminución del crecimiento de la célula tumoral, diferenciación, y/o apoptosis (39). Estos compuestos son objetivos hacia mecanismos inherentes a la capacidad de una célula neoplásica de convertirse maligna, en razón a que ellos no parecen tener toxicidad en dosis efectivas para inhibir el crecimiento tumoral en animales (40). Existen varias líneas de evidencia de que la acetilación y la desacetilación de histona son mecanismos por medio de los cuales se logra la regulación transcripcional en una célula (41). Estos efectos se cree que ocurren a través de cambios en la estructura de la cromatina al alterar la afinidad de las proteínas histona para el ADN helicoidal en el nucleosoma.

Existen cinco tipos de histonas que han sido identificadas (designadas H1, H2A, H2B, H3 y H4). Las histonas H2A, H2B, H3, y H4 se encuentran en los nucleosomas y el H1 es un ligador localizado entre los nucleosomas. Cada nucleosoma contiene dos de cada tipo de histona dentro de su núcleo, excepto para H1 que está presente sencillamente en la porción exterior de la estructura del nucleosoma. Se cree que cuando

las proteínas histona son hipoacetiladas, existe una mayor afinidad de la histona con la estructura fosfato del ADN. Existe una mayor afinidad de la histona con la estructura fosfato del ADN. Esta afinidad origina que el ADN este fuertemente unido a la histona y hace el ADN inaccesible a los elementos regulatorios transcripcionales y a la maquinaria.

La regulación de los estados acetilados ocurre a través del balance entre la actividad entre dos complejos de enzima, la acetiltranferasa de histona (HAT) y la desacetil histona (HDAC). El estado hipoacetilado se cataliza por complejos multiproteínas grandes que incluyen enzimas HDAC. En particular, los HDAC han mostrado que catalizan la remoción de los grupos acetilos provenientes de las histonas de núcleo de cromatina.

La inhibición del HDAC por el SAHA se cree que ocurre a través de la interacción directa con el sitio catalítico de la enzima como se demuestra por estudios de cristalografía de rayos X (42). El resultado de la inhibición del HDAC no se cree que tenga un efecto generalizado sobre el genoma, sino al contrario solo afecta un pequeño subconjunto del genoma (43). Se suministra evidencia mediante microarreglos de ADN utilizando líneas celulares malignas cultivadas con un inhibidor de HDAC que muestran que existe un número finito

(1-2%) de genes cuyos productos se alteran. Por ejemplo, las células tratadas en cultivo con inhibidores HDAC muestran una inducción consistente del inhibidor de quinasa dependiente de ciclina p21 (44). Esta proteína juega un papel importante en la disminución del ciclo celular. Los inhibidores HDAC se cree que incrementan la proporción de transcripción de p21 al propagar el estado hiperacetilado de histonas en la región del gen p21, haciendo de esta manera el gen accesible a la maquinaria transcripcional. Los genes cuya expresión no se afecta por los inhibidores HDAC no despliegan cambios en la acetilación de las histonas asociadas regionales (45).

Se ha mostrado en varios casos que la disrupción de la actividad HAT o HDAC está implicada en el desarrollo del fenotipo maligno. Por ejemplo, en leucemia promielocítica aguda, la oncoproteína producida por la fusión del PML y el RAR alfa parece suprimir la trascripción del gen específico a través del reclutamiento de los HDAC (46). De esta manera, la célula neoplásica es incapaz de completar la diferenciación y conduce a la proliferación en exceso de la línea celular de leucemia.

Los números de patentes estadounidenses 5,369,108, 5,932,616, 5,700,811, 6,087,367 y 6,511,990, elaboradas por algunos de los presentes inventores, describen los útiles para inducir selectivamente la diferenciación terminal de las células

neoplásicas, cuyos compuestos tienen dos grupos de extremos polares separados por una cadena flexible de grupos metileno o por un grupo fenilo rígido, en donde uno o ambos de los grupos de extremo polar son grupos hidrófobos grandes. Algunos de los compuestos tienen un grupo hidrófobo grande adicional en el mismo extremo de las moléculas en el primer grupo hidrófobo que además incrementa la actividad de diferenciación aproximadamente 100 veces en un ensayo enzimático y aproximadamente 50 veces en un ensayo de diferenciación celular. Los métodos para sintetizar los compuestos utilizados en los métodos y las composiciones farmacéuticas de esta invención se describen completamente en las patentes anteriormente mencionadas, cuyos contenidos completos se incorporan aquí como referencia.

Además de su actividad biológica como agentes antitumorales, los compuestos descritos en las patentes anteriormente mencionadas se han identificado recientemente como útiles para tratar o prevenir una amplía variedad de enfermedades y condiciones mediadas por tioredoxin (TRX), tal como enfermedades inflamatorias, enfermedades alérgicas, enfermedades autoinmunes, enfermedades asociadas con tensión oxidativa de enfermedades caracterizadas por hiperproliferación celular (solicitud Estadounidense No. 10/369,094, presentada en Febrero 15,2003). Además, estos compuestos se han identificado como útiles para tratar

enfermedades del sistema nervioso central (CNS) tal como enfermedades neurodegenerativas y para tratar cáncer de cerebro (ver, solicitud Estadounidense No. 10. 273,401, presentada en Octubre 16,2002).

Las patentes anteriormente mencionadas no describen formulaciones orales específicas de inhibidores HDAC de dosis específicas y programas de dosificación de los compuestos citados que son efectivos para tratar cáncer, por ejemplo, mesotelioma o linfoma. De manera importante, las patentes anteriormente mencionadas no describen formulaciones orales que tengan perfiles farmacocinéticos favorables tal como biodisponibilidad que da origen a altos niveles en la sangre de los compuestos activos durante un periodo de tiempo extendido.

Existe una necesidad urgente para descubrir drogas adecuadas y programas de dosificación de estos compuestos, y para desarrollar formulaciones, preferiblemente formulaciones orales, de compuestos activos que eleven constantemente los niveles de sangre terapéuticamente efectivos durante un periodo de tiempo extendido, y que sean efectivos en el tratamiento del cáncer.

RESUMEN DE LA INVENCIÓN

La presente invención se relaciona con métodos para tratar cánceres por ejemplo, mesotelioma o linfoma. Más específicamente, la presente invención se relaciona con métodos para tratar mesotelioma o linfoma de célula B grande difusa (DLBCL), mediante la administración de composiciones farmacéuticas que comprenden inhibidores HDAC, por ejemplo, ácido suberoilanilida hidroxámico (SAHA). En aspectos particulares, los métodos de la invención son utilizados para tratar mesotelioma. En otros aspectos, los métodos de la invención son utilizados para tratar linfoma, por ejemplo, DLBCL.

Las formulaciones orales de las composiciones farmacéuticas tienen perfiles farmacocinéticos favorables tales como alta biodisponibilidad y sorprendentemente dan origen a altos niveles en la sangre de los compuestos activos durante un periodo de tiempo extendido. La presente invención suministra además un régimen de dosificación seguro diario de estas composiciones farmacéuticas, que son fáciles de seguir y que dan como resultado una cantidad terapéuticamente efectiva de los inhibidores HDAC in vivo.

En una modalidad, la presente invención suministra un método para tratar mesotelioma o DLBCL en un sujeto necesitado de

ello, al administrarle al sujeto una composición farmacéutica que comprende una cantidad efectiva de ácido suberoilanilida hidroxámico (SAHA) o una sal farmacéuticamente aceptable de hidrato de este, como se describió aquí. El SAHA se puede administrar en una dosis diaria total de hasta 800 mg, preferiblemente oralmente, una vez, dos veces, o tres veces diariamente, continuamente (cada día) o intermitentemente (por ejemplo, 3-5 días en una semana).

El SAHA oral ha sido administrado de manera segura en estudios clínicos fase uno a pacientes que sufren de mesotelioma o DLBCL.

Adicionalmente, la presente invención suministra un método para tratar mesotelioma o DLBCL en un sujeto necesitado de ello, al administrarle al sujeto una composición farmacéutica que comprende una cantidad efectiva de un inhibidor HDAC como se describe aquí, o una sal farmacéuticamente aceptable o un hidrato de este. El inhibidor HDAC se puede administrar en una dosis diaria total de hasta 800 mg, preferiblemente de manera oral, una vez, dos veces, o tres veces diariamente, continuamente (es decir cada día) o intermitentemente (por ejemplo, 3-5 días en una semana).

Los inhibidores HDAC y los métodos de la presente invención son útiles en el tratamiento de una amplia variedad de

cánceres, por ejemplo, linfoma que incluyen enfermedad de Hodgkin (HD) y linfoma no Hodgkin (NHL). En una modalidad, los inhibidores HDAC son útiles para tratar mesotelioma o un linfoma de célula larga, que incluye linfoma de célula B grande difusa (DLBCL). Como se define aquí "linfoma de célula grande" es un linfoma que se caracteriza por células inusualmente largas.

Los inhibidores HDAC adecuados para uso en la presente invención incluyen pero no están limitados a derivados de ácido hidroxámico, Ácidos Grasos de Cadena Corta (SCFA), tetrapéptidos cíclicos, derivados de benzamida, o derivados de cetona electrofílica, como se definieron aquí. Los ejemplos no limitantes específicos de inhibidores HDAC adecuados para uso en los métodos de la presente invención son:

A) **Derivados de ácido hidroxámico** seleccionados de bishidroxamida de ácido m-carboxicinámico (CBHA), Tricostatina A (TSA), Tricostatina C, ácido Salicilhidroxámico, ácido Azelaico Bishidroxámico (ABHA), ácido Azelaic-1-Hidroxamato-9-Anilida (AAHA), 6-(3-Clorofenilureido) ácido carpóico Hidroxámico (3Cl-UCHA), Oxamflatina, A-161906, Scriptaid, PXD-101, LAQ-824, CHAP, MW2796, y MW2996;

B) **Tetrapéptidos cíclicos** seleccionados de tetrapéptido cíclico(TPX)-Trapoxin A (ciclo-(L-fenilalanil-L-fenilalanil-D-pipecolinil-L-2-amino-8-oxo-9,10-epoxi decanoilo); FR901228 (FK 228, Depsipéptido); FR225497 tetrapéptido cíclico; tetrapéptido Apicidina cíclico [ciclo(N-O-metil-L-triptofanil-L-isoleucinil-D-pipecolinil-L-2-amino-8-oxodecanoil)]; Apicidina Ia, Apicidina Ib, Apicidina Ic, Apicidina IIa, y Apicidina IIb ; CHAP, tetrapéptido cíclico de toxina HC; WF27082 cíclico; y Clamidocin;

C) **Ácidos Grasos de Cadena Corta** (SCFA) seleccionados de Butirato de Sodio, Isovalerato, Valerato, Fenilbutirato 4 (4-PBA), Fenilbutirato (PB), Propionato, Butiramida, Isobutiramida, Fenilacetato, 3-Bromopropionato, Tributirin, ácido Valproico y Valproato y Pivanex™;

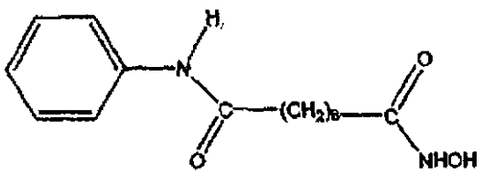
D) **Derivados de Benzamida** seleccionados de CI-994, MS-27-275(MS-275)[N-(2-aminofenil)-4-[N(piridin-3-ilmetoxicarbonil) aminomethil] benzamida] y un derivado 3'-amino de MS-27-275;

E) **Derivados de Cetona Electrofílica** seleccionada de cetona de trifluorometilo y una α-cetoamida tal como N-metil-a-cetoamida; y

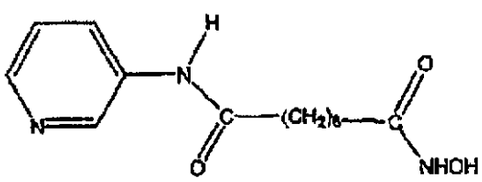
F) **Inhibidores HDAC Misceláneos** que incluyen productos naturales, psammaplinas, y Depudecina.

Los inhibidores HDAC específicos incluyen, por ejemplo:

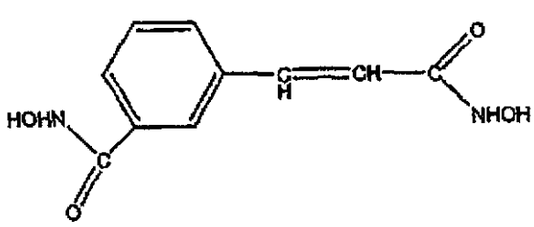
Ácido suberoilanilida hidroxámico (*SAHA*), que se representa por la siguiente fórmula estructural:



Piroxamida, que se representa por la siguiente fórmula estructural:

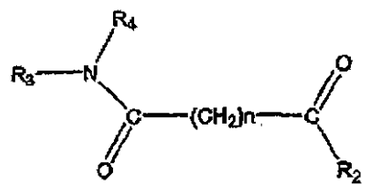


Ácido m-carboxinámico bishidroxámida (CBHA), que se representa por la siguiente fórmula estructural:



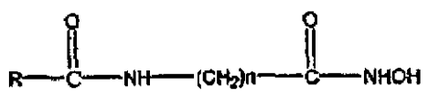
Otros ejemplos no limitantes de inhibidores HDAC que son adecuados para uso en los métodos de la presente invención son:

Un compuesto representado por la estructura



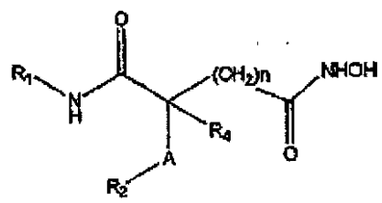
En donde R_3 y R_4 son independientemente un grupo alquilo, alquenilo, cicloalquilo, arilo, alquiloxi, ariloxi, arilalquiloxi, o piridina sustituido o no sustituido, ramificado o no ramificado, grupo cicloalquilo, arilo, ariloxi, arilalquiloxi, o o R_3 y R_4 se unen juntos para formar un grupo piperidina; R_2 es un grupo hidroxilamino; y n es un entero de 5 a 8.

Un compuesto representado por la estructura



Donde R es un fenilo sustituido o no sustituido, piperidina, tiazol, 2-piridina, 3-piridina o 4-piridina y n es un entero de 4 a 8.

Un compuesto representado por la estructura



En donde A es una porción amida, R_1 y R_2 son cada uno seleccionados de arilo, ariloalquilo, naftilo, piridinoamino, 9-purina-6-amino, tiazoloamino, ariloxi, ariloalquiloxi, piridilo, quinolinilo o isoquinolinilo sustituido o no sustituido; R_4 es hidrógeno, una porción halógena, una porción fenilo o una porción cicloalquilo y n es un entero de 3 a 10.

En una modalidad, las composiciones farmacéuticas que comprenden el inhibidor HDAC son administradas oralmente, dentro de una cápsula de gelatina. En una modalidad adicional, las composiciones farmacéuticas están adicionalmente comprendidas de celulosa microcristalina, croscarmelosa de sodio y estearato de magnesio.

Los inhibidores HDAC se pueden administrar en una dosis diaria total que pueden variar de paciente a paciente, y se puede administrar en varios programas de dosis. Las dosis adecuadas son dosis diarias totales de entre aproximadamente 25-4000 mg/m^2 administradas oralmente una vez al día, dos veces al día, o tres veces al día, continua (es decir cada día) o intermitentemente (por ejemplo, 3-5 días a la semana). Además, las composiciones se pueden administrar en ciclos con periodos de descanso entre los ciclos (por ejemplo, tratamiento de dos a ocho semanas con un periodo de descanso de hasta una semana entre tratamientos).

En una modalidad la composición se administra una vez diariamente en una dosis de aproximadamente 200-600 mg. En otra modalidad, la composición se administra dos veces diariamente en una dosis de aproximadamente 200-400 mg. En otra modalidad, la composición se administra dos veces diariamente a una dosis de aproximadamente 200-400 mg intermitentemente, por ejemplo, 3, 4 o 5 días por semana. En una modalidad, la composición se administra tres veces diariamente en una dosis de aproximadamente 100-250 mg.

En una modalidad, la dosis diaria es de 200 mg, y se puede administrar una vez diariamente, dos veces diariamente o tres veces diariamente. En una modalidad, la dosis diaria es de 300 mg, que se puede administrar una vez diariamente o dos

veces diariamente. En una modalidad, la dosis diaria es de 400 mg, que se puede administrar una vez diariamente o dos veces diariamente.

La presente invención también suministra métodos para inducir selectivamente la diferenciación terminal, disminución del crecimiento celular y/o apoptosis de las células neoplásicas, por ejemplo, las células de linfoma en un sujeto, inhibiendo de esta manera la proliferación de tales células en dicho sujeto al administrarle al sujeto una composición farmacéutica que comprende una cantidad efectiva del inhibidor HDAC, por ejemplo, SAHA o una sal farmacéuticamente aceptable o hidrato de esta, y un portador o diluyente farmacéuticamente aceptable. Una cantidad efectiva de un inhibidor HDAC en la presente invención puede ser hasta de una dosis diaria total de 800 mg.

La presente invención también suministra métodos para inhibir la actividad de una histona desacetilasa en un sujeto, al administrarle al sujeto una composición farmacéutica que comprende una cantidad efectiva de un inhibidor HDAC, por ejemplo, SAHA o una sal farmacéuticamente aceptable o hidrato de esta, y un portador o diluyente farmacéuticamente aceptable. Una cantidad efectiva de un inhibidor HDAC en la presente invención puede ser hasta de una dosis diaria total de 800 mg.

La presente invención también suministra métodos in vivo para inducir selectivamente la diferenciación terminal, la disminución del crecimiento celular y/o la apoptosis de células neoplásicas, por ejemplo, células de linfoma que inhiben de esta manera la proliferación de tales células, al poner en contacto las celas con una cantidad efectiva de un inhibidor HDAC por ejemplo, SAHA, o una sal farmacéuticamente aceptable o hidrato de esta.

La presente invención también suministra métodos in vivo para inhibir la actividad de una histona desacetilasa, mediante la histona desacetilasa con una cantidad efectiva de un inhibidor HDAC por ejemplo, SAHA, o una sal farmacéuticamente aceptable o hidrato de esta.

La presente invención suministra además un régimen de dosificación seguro, diario de las formulaciones de las composiciones farmacéuticas que comprenden un inhibidor HDAC que es fácil de seguir y adherirse. Estas composiciones farmacéuticas son adecuadas para administración oral y son útiles para tratar cáncer, mesotelioma, linfoma, otros cánceres o tumores al inducir selectivamente la diferenciación terminal, la disminución del crecimiento celular y/o la apoptosis de las células neoplásicas y/o al inhibir la histona desacetilasa (HADC).

BREVE DESCRIPCIÓN DE LOS DIBUJOS

Lo anterior y otros objetos, características y ventajas de la invención serán evidentes en las siguientes descripciones más particular de las modalidades preferidas de la invención, como se ilustran en las fotografías que la acompañan en los cuales los caracteres de referencia similares se refieren a las mismas partes a través de diferentes vistas. Las fotografías no son necesariamente a escala, siendo en su lugar ubicado el énfasis en ilustrar los principios de la invención.

La figura 1 es una fotografía de Western blot (panel superior) que muestran las cantidades de histona-4 acetilada (α-AcH4) en plasma sanguíneo de pacientes luego de una dosis oral o intravenosa (IV) de SAHA. El SAHA IV se administra en 200 mg infundido durante dos horas. El SAHA oral se administra en una cápsula simple a 200 mg. La cantidad de α-AcH4 se muestra en los puntos de tiempo indicados. Panel inferior: tinte azul Coomassie.

La figura 2 es una fotografía de un Western blot (paneles superiores) que muestran las cantidades de histona-4 acetilada (α-AcH4) en plasma sanguíneo de pacientes que tienen un tumor sólido, luego de una dosis oral o intravenosa

(IV) de SAHA. El SAHA IV oral se administra como en la figura 1. La cantidad de α-AcH4 se muestra en los puntos de tiempo indicados. El experimento es mostrado en duplicado (figura 2a y figura 2b). Paneles inferiores: tinte azul Coomassie.

La figura 3 es una fotografía de un Western blot (paneles superiores) que muestran las cantidades de histona-4 acetilada (α-AcH4) (figura 3A) y una histona-3 acetilada (α-AcH3) (figuras 3B-E) en el plasma sanguíneo de pacientes luego de una dosis oral o intravenosa (IV) de SAHA en el día 1 y en el día 21. El SAHA IV oral se administra como en la figura 1. La cantidad de α-AcH4 o α-AcH3 se muestra en los puntos de tiempo indicados. Paneles inferiores: tinte azul Coomassie.

La figura 4 es una fotografía de un Western blot (paneles superiores) que muestran las cantidades de histona-3 acetilada (α-AcH3) en plasma sanguíneo de pacientes que tienen un tumor sólido, luego de una dosis oral o intravenosa (IV) de SAHA. El SAHA IV y oral se administra como en la figura 1. La cantidad α-AcH3 se muestra en los puntos de tiempo indicados. Panel inferior: tinte azul Coomassie.

La figura 5 es una fotografía de un Western blot (paneles superiores) que muestran las cantidades de histona-3 acetilada (α-AcH3) en plasma sanguíneo de pacientes luego de

una dosis oral o intravenosa (IV) de SAHA. El SAHA IV se administra a 400 mg infundido durante dos horas. El SAHA oral se administra en una cápsula simple a 400 mg. La cantidad de α-AcH4 se muestra en los puntos de tiempo indicados. El experimento se muestra en triplicado (figura 5A y B). Paneles inferiores: tinte azul Coomassie.

La figura 6 es una fotografía de un Western blot (panel superior) que muestra las cantidades de histona-3 acetilada (α-AcH3) en el plasma sanguíneo de pacientes que tienen un tumor sólido, luego de una dosis oral o intravenosa (IV) de SAHA. El SAHA IV y oral se administra como en la figura 5. La cantidad de α-AcH3 se muestra en los puntos de tiempo indicados. Panel inferior: tinte azul Coomassie.

La figura 7 es una fotografía de un Western blot (paneles superiores) que muestran las cantidades de histona-3 acetilada (α-AcH3) en el plasma sanguíneo de pacientes que tienen un tumor sólido luego de una dosis oral o intravenosa (IV) de SAHA en el día 1 y en el día 21. El SAHA IV y oral se administra como en la figura 4. La cantidad de α-AcH4 o α-AcH3 se muestra en los puntos de tiempo indicados. El experimento se muestra en triplicado (figura 7A-C). Paneles de fondo: tinte azul Coomassie.

La figura 8 es una fotografía de un Western blot (paneles superiores) que muestran las cantidades de histona-3 acetilada (α-AcH3) en el plasma sanguíneo de pacientes que tienen un tumor sólido, luego de una dosis oral o intravenosa (IV) de SAHA. El SAHA IV y oral se administra como en la figura 5. La cantidad α-AcH3 se muestra en los puntos de tiempo indicados. Paneles inferiores: tinte azul Coomassie.

Figuras 9A-C son gráficas que muestran la concentración de plasma medio de SAHA (ng/ml) en los puntos de tiempo indicados luego de administración. Figura 9A: dosis oral (200 mg y 400 mg) bajo ayuno en el día 8. La figura 9B: dosis oral (200 mg y 400 mg) con alimentación en el día 9. Figura 9C: dosis IV en el día 1.

Figura 10 muestra la vida media aparente de SAHA con una dosis oral de 200 mg y 400 mg, en los días 8, 9 y 22.

Figura 11 muestra el AUC (ng/ml/hr) de un SAHA con una dosis oral de 200 mg y 400 mg, en los días 8, 9 y 22.

Figura 12 muestra la biodisponibilidad de SAHA después de una dosis oral de 200 mg y 400 mg en los días 8, 9 y 22.

Figura 13 es una exploración de un tumor de mesotelioma de un paciente, antes (izquierda) y después (derecha) seis meses de

tratamiento con SAHA a una dosis de 300 modalidad dos veces diariamente tres días a la semana.

Figura 14 es una exploración CT tomada de un paciente con linfoma de célula larga difusa, antes del tratamiento (A) y después (B) dos meses de tratamiento con SAHA a una dosis de 400 mg BID durante mes luego 400 mg QD durante un mes.

Figura 15 es una exploración PET tomada de un paciente con linfoma de célula larga difusa, antes del tratamiento (A) y después (B) y después de dos meses de tratamiento con SAHA a una dosis de 400 mg BID durante un mes seguido por 400 mg de QD durante un mes.

Figura 16 es una exploración CT tomada de un paciente con linfoma de célula larga difusa, antes del tratamiento (A) y después (B) un mes de tratamiento con SAHA a una dosis de 600 mg QD.

Figura 17 es una exploración PET tomada de un paciente con linfoma de célula larga difusa, antes del tratamiento (A) y después (B) dos meses de tratamiento con SAHA a una dosis de 200 mg BID.

DESCRIPCIÓN DETALLADA DE LA INVENCIÓN

La presente invención también suministra métodos para tratar cánceres, por ejemplo, mesotelioma o linfoma. Más específicamente, la presente invención se relaciona con métodos para tratar mesotelioma o linfoma de célula B larga difusa (DLBCL), mediante la administración de unas composiciones farmacéuticas que comprenden inhibidores de HDAC, por ejemplo, ácido suberoilanilida hidroxámico (SAHA). En aspectos específicos los métodos de la invención son utilizados para tratar mesotelioma. En otros aspectos los métodos de la invención son utilizados para tratar linfoma, que incluye DLBCL.

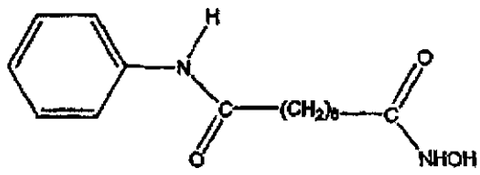
Las formulaciones orales de las composiciones farmacéuticas de la invención tienen perfiles farmacocinéticos favorables tales como alta biodisponibilidad y sorprendentemente dan orígenes a niveles sanguíneos altos de los compuestos activos durante un periodo de tiempo extendido. Así, la presente invención suministra además un régimen de dosificación seguro, diario de estas composiciones farmacéuticas, que son fáciles de seguir, los cuales dan como resultado una cantidad terapéuticamente efectiva de inhibidores HDAC in vivo.

De acuerdo con esto, en una modalidad, la presente invención suministra un método para tratar mesotelioma o DLBCL en un

sujeto necesitado de esto, al administrarle al sujeto una composición farmacéutica que comprende una cantidad efectiva de un inhibidor HDAC como se describe aquí, o una sal farmacéuticamente aceptable o hidrato de esta. El inhibidor HDAC se puede administrar en una dosis diaria total de hasta 800 mg, preferiblemente oralmente, una vez, dos veces o tres veces diariamente, continuamente (es decir, cada día) o intermitentemente (por ejemplo, 3-5 días a la semana).

En una modalidad, el inhibidor HDAC es ácido suberoilanilida hidroxámico (SAHA). En otra modalidad, el inhibidor HDAC es un derivado de ácido hidroxámico como se describe aquí. En otra modalidad, el inhibidor HDAC se representa con cualquiera de las estructuras de las fórmulas 1-51 descritas aquí. En otra modalidad, el inhibidor HDAC es un derivado de benzamida como se describió aquí. En otra modalidad, el inhibidor HDAC es un tetrapéptido cíclico como se describió aquí. En otra modalidad, el inhibidor HDAC es un Ácido Graso de Cadena Corta (SCFA) como se describió aquí. En otra modalidad, el inhibidor HDAC es una cetona electrofílica como se describe aquí. En otra modalidad, el inhibidor HDAC es depudecina. En otra modalidad, el inhibidor HDAC es un producto natural. En otra modalidad, el inhibidor HDAC es una psammaplina.

En una modalidad particular, la presente invención suministra un método para tratar mesotelioma o DLBCL en un sujeto necesitado de ello, al administrarle al sujeto una composición farmacéutica que comprende una cantidad efectiva de ácido suberoilanilida hidroxámico (SAHA) o una sal farmacéuticamente aceptable o hidrato de esta, como se describió aquí. El SAHA se puede administrar en una dosis diaria total de hasta 800 mg, preferiblemente de manera oral, una vez, dos veces, o tres veces diariamente, continuamente (cada día) o intermitentemente (por ejemplo, 3-5 días en una semana). El SAHA se representa por la siguiente fórmula estructural:



En otra modalidad particular la presente invención se relaciona con un método para tratar mesotelioma o DLBCL en un sujeto, que comprende la etapa de administrar al sujeto una cantidad efectiva de una composición farmacéutica que comprende un inhibidor de histona desacetilasa (HDAC) representado por cualquiera de las estructuras descritas aquí o por las fórmulas 1-51 descritas aquí, o una sal farmacéuticamente aceptable o hidrato de esta, y un portador

o diluyente farmacéuticamente en donde la cantidad de inhibidor de histona desacetilasa es efectiva para tratar mesotelioma o DLBCL en un sujeto.

El término "tratar" en sus varias formas gramaticales en relación con la presente invención se refiere a prevenir (es decir quimioprevención), cura, reversamiento, atenuación, alivio, minimización o supresión de efectos deletéreos de un estado de enfermedad, progresión de la enfermedad, agente causante de la enfermedad (por ejemplo bacteria o virus) u otra condición anormal. Por ejemplo, el tratamiento puede involucrar aliviar un síntoma (es decir no necesariamente todos los síntomas) de una enfermedad o atenuar la progresión de una enfermedad. En razón de que algunos de los métodos de la invención involucran la remoción física del agente etiológico, el experto reconocerá que ellos son igualmente efectivos en situaciones donde el compuesto inventivo se administra antes de, o simultáneamente con, la exposición al agente etiológico (tratamiento profiláctico) y situaciones donde los compuestos de la invención se administran después (aún bien después) de la exposición al agente etiológico.

El tratamiento de cáncer, como se utiliza aquí, se refiere a inhibir parcial o totalmente, retrasar o prevenir la progresión de cáncer que incluye metástasis de cáncer; inhibir, demorar o prevenir la recurrencia de cáncer que

incluye metástasis de cáncer; o prevenir el ataque o el desarrollo de cáncer (quimioprevención) en un mamífero, por ejemplo, un humano.

Como se utiliza aquí, el término "cantidad terapéuticamente efectiva" pretende comprender cualquier cantidad que logrará la respuesta biológica deseada. En la presente invención, la respuesta biológica deseada es inhibición parcial o total, demora o prevención de la progresión del cáncer que incluye metástasis del cáncer; inhibición, demora o prevención de la recurrencia de cáncer que incluye metástasis de cáncer; inhibición, demora o prevención del ataque o desarrollo de cáncer (quimioprevención) en un mamífero, por ejemplo un humano.

El método de la presente invención está destinado al tratamiento o quimioprevención de pacientes humanos con cáncer. Sin embargo, también es probable que sea efectivo en el tratamiento de cánceres en otros mamíferos.

Inhibidores de histona desacetilasa e histonas desacetilasa

Las histonas desacetilasas (HDAC) tal como el término se utiliza aquí, son enzimas que catalizan la remoción de los grupos acetilo proveniente de residuos de lisina en las colas amino terminales de las histonas de núcleo nucleosomal. Como

tales, los HDAC junto con las acetil transferasas de histona (HAT) regulan el estado de acetilación de las histonas. La expresión del gen afecta la acetilación de histona y los inhibidores de HDAC tal como el ácido suberoilanilida hidroxámico compuesto por híbrido con base en ácido hidroxámico (SAHA) induce disminución de crecimiento, diferenciación y/o apoptosis de células transformadas in vitro e inhiben el crecimiento tumoral in vivo. Los HDAC se pueden dividir en tres clases con base en la homología estructural. HDAC clase I (HDAC 1, 2, 3 y 8) tienen similitud con la proteína RPD3 de levadura, están localizados en el núcleo y se encuentran en complejos asociados con co-represores transcripcionales. Los HDAC clase II (HDAC 4, 5, 6, 7 y 9) son similares a la proteína HDA1 de levadura, y tiene ambos localización subcelular nuclear y citoplasmática. Ambos HDAC clase I y clase II son inhibidos por los inhibidores HDAC basados en ácido hidroxámico tales como el SAHA. Los HDAC clase III forman una clase estructuralmente distante de las enzimas dependientes de NAD que están relacionadas con las proteínas SIR2 de levadura y no son inhibidas por los inhibidores HDAC basados en ácido hidroxámico.

Los inhibidores de histona desacetilasa o los inhibidores HDAC, tal como el término se utiliza aquí son compuestos que son capaces de inhibir la desacetilación de histonas in vivo,

in vitro o ambos. Como tales, los inhibidores HDAC inhiben la actividad de al menos una histona desacetilasa. Como resultado de inhibir la desacetilación de al menos una histona, ocurre un incremento en la histona acetilada y la acumulación de histona desacetilasa en un marcador biológico adecuado para evaluar la actividad de los inhibidores HDAC. Por lo tanto, los procedimientos que se pueden ensayar para la acumulación de histonas desacetilasas se pueden utilizar para determinar la actividad inhibitoria de HDAC de los compuestos de interés. Se entiende que los compuestos que pueden inhibir la actividad de histona desacetilasa se pueden unir a otros sustratos y como tales pueden inhibir otras moléculas biológicamente activas tales como enzimas. Se debe entender que los compuestos de la presente invención son capaces de inhibir cualquiera de las histonas desacetilasas establecidas anteriormente, o cualquiera otra de las histonas desacetilasas.

Por ejemplo, en pacientes que reciben inhibidores HDAC, la acumulación de histonas acetilasas en células mononucleares periféricas así como también en tejidos tratados con inhibidores HDAC se pueden determinar contra un control adecuado.

La actividad inhibitoria de HDAC de un compuesto particular se puede determinar in vitro utilizando, por ejemplo, unos

ensayos enzimáticos que muestran la inhibición de al menos una histona desacetilasa. Además, la determinación de la acumulación de histonas acetilasas en células tratadas con una composición particular se puede determinar mediante la actividad inhibitoria de HDAC de un compuesto.

Los ensayos para la acumulación de histonas acetilasas son bien conocidos en la literatura. Ver, por ejemplo, Marks, P. A. *et al.*, J. Natl. Cancer Inst., 92: 1210-1215, 2000, Butler, L.M. *et al.*, Cancer Res. 60: 5165-5170 (2000), Richon, V. M. *et al.*, Proc. Natl. Acad. Sci., USA, 95: 3003-3007, 1998, y Yoshida, M. *et al.* J. Biol. Chem., 265: 17174-17179, 1990.

Por ejemplo, un ensayo enzimático para determinar la actividad de un compuesto inhibidor HDAC se puede conducir como sigue. En resumen, el efecto del compuesto inhibidor HDAC sobre el HDAC1 (Bandera) etiquetado con epítopo humano purificado de afinidad se puede ensayar al incubar la preparación de enzima en la ausencia de sustrato sobre hielo durante aproximadamente 20 minutos con una cantidad indicada del compuesto inhibidor. El sustrato (histona derivada de célula de eritroleucemia de murino marcada con [^{3}H] acetil) se puede agregar y la muestra se puede incubar durante 20 minutos a 37° C en un volumen total de 30 µL. La reacción puede se entonces detenida y liberada el acetato puede ser

extraído de la cantidad de radioactividad liberada determinada por conteo de centelleo. Un ensayo alternativo útil para determinar la actividad de un compuesto inhibidor HDAC es el "Ensayo de Actividad Fluorescente HDAC; Kit de Descubrimiento de Droga-AK-500"disponible de BIOMOL® Research Laboratories, Inc., Plymouth Meeting, PA.

Los estudios in vivo se puede conducir como sigue: Animales, por ejemplo, ratones, se pueden inyectar intraperitonealmente con un compuesto inhibidor HDAC. Los tejidos seleccionados, por ejemplo, cerebro, bazo, hígado etc., se puede aislar en momentos predeterminados, post administración. Las histonas se pueden aislar de tejidos esencialmente como se describe por Yoshida *et al.*, J. Biol. Chem. 265: 17174-17179, 1990. Cantidades iguales de histona (aproximadamente 1 µg) se pueden electroforizar sobre geles SDS-poliacrilamida 15% y se pueden transferir a filtros Hybond-P (disponibles de Amersham). Los filtros se pueden bloquear con 3% de leche y se pueden sondear con un anticuerpo H4 de histona anti-acetilada policlonal purificada de conejo (α-Ac-H4) y anticuerpo H3 de histona anti-acetilada (α-Ac-H3) (Upstate Biotechnology, Inc.). Los niveles de histona acetilada se pueden visualizar utilizando anticuerpo anti-conejo de cabra conjugado con peroxidasa de cola de caballo (1:5000) y substrato quimioluminiscente SuperSignal (Pierce). Como

control de carga para la proteína de histona, se pueden correr genes paralelos y teñir con azul Coomassie (CB).

Además, los inhibidores HDAC basados en ácido hidroxámico han mostrado que sobreregulan la expresión del gen $p21^{WAF1}$. La proteína $p21^{WAF1}$ se induce dentro de dos horas de cultivo con inhibidores HDAC en una variedad de células transformadas que utilizan métodos estándar. La inducción del gen $p21^{WAF1}$ se asocia con acumulación de histonas acetiladas en la región de cromatina de este gen. La inducción del $p21^{WAF1}$ se puede reconocer por lo tanto como que está involucrada en la detención del ciclo de la célula G1 originado por los inhibidores HDAC en células transformadas.

Típicamente, los inhibidores HDAC caen en cinco clases generales: 1) Derivados de ácido hidroxámico; 2) Ácidos Grasos de Cadena Corta (SCFA); 3) tetrapéptidos cíclicos; 4) benzamidas; y 5) cetonas electrofílicas.

Así, la presente invención incluye dentro de su amplio alcance composiciones que comprenden inhibidores HDAC que son 1) Derivados de ácido hidroxámico; 2) Ácidos Grasos de Cadena Corta (SCFA); 3) tetrapéptidos cíclicos; 4) benzamidas; y 5) cetonas electrofílicas; y/o cualquier otra clase de compuestos capaces de inhibir histonas desacetilasas , para uso en la inhibición de histona desacetilasa, que induce

diferenciación terminal, detención de crecimiento celular y/o apoptosis en células neoplásicas, y/o que inducen diferenciación, detención de crecimiento celular y/o apoptosis de células de tumor en un tumor.

Ejemplos no limitantes de tales inhibidores HDAC se establecen adelante. Se entiende que la presente invención incluye cualquier sal, estructuras de cristal, estructuras amorfas, hidratos, derivados, metabolitos, estereoisómeros, isómeros estructurales y prodrogas de los inhibidores HDAC descritos aquí.

A. Derivados de Ácido hidroxámico Tales como ácido suberoilanilida hidroxámico(SAHA) (Richon *et al.*, Proc. Natl. Acad. Sci. USA 95,3003-3007 (1998)); bishidroxamida de ácido m-carboxicinámico (CBHA) (Richon *et al.*, supra); piroxamida; análogos de tricostatina tales como tricostatina A (TSA) y tricostatina C (Koghe *et al.* 1998. Biochem. Pharmacol. 56: 1359-1364); ácido salicilhidroxámico (Andrews *et al.*, International J. Parasitology 30, 761-768 (2000)); ácido suberoil bishidroxámico(SBHA) (Patente estadounidense No. 5,608,108); ácido azelaico bishidroxámico(ABHA) (Andrews *et al.*, supra); azelaic-1-hidroxamato-9-anilida (AAHA) (Qiu *et al.*, Mol. Biol. Cell 11,2069-2083 (2000)); 6-(3-clorofenilureido) ácido carpoico hidroxámico(3Cl-UCHA); oxamflatin ácido [(2E)-5-[3-[(fenilsufonil)amino fenil]

ácido-pent-2-en-4-inohidroxámico] (Kim *et al.*, Oncogene, 18: 2461 2470 (1999)); A-161906, Scriptaid (Su *et al.* 2000 Cancer Research, 60: 3137- 3142); PXD-101 (Prolifix); LAQ-824; CHAP; MW2796 (Andrews *et al.*, supra); MW2996 (Andrews *et al.*, supra); o cualquiera de los ácidos hidroxámicos descritos en la Patentes estadounidenses Nos. 5,369,108, 5,932,616, 5,700,811, 6,087,367 y 6,511, 990.

B. **Tetrapéptidos cíclicos** tales como tetrapéptido cíclico (TPX)-trapoxin A (ciclo-(L- fenilalanil-L-fenilalanil-D-pipecolinil-L-2-amino-8-oxo-9,10-epoxi decanoilo)) (Kijima *et al.*, J Biol. Chem. 268,22429-22435 (1993)); FR901228 (FK 228, depsipeptido) (Nakajima *et al.*, Ex. Cell Res. 241, 126-133 (1998)); tetrapéptido cíclico FR225497 (H. Mori *et al.*, Solicitud PCT WO 00/08048 (17 Febrero 2000)); tetrapéptido cíclico apicidina [ciclo(N-O-metil-L-triptofanil-L-isoleucinil-D-pipecolinil-L-2-amino-8- oxodecanoil)] (Darkin-Rattrai *et al.*, Proc. Natl. Acad. Sci. USA 93,1314313147 (1996)); apicidina Ia, apicidina Ib, apicidina Ic, apicidina IIa, y apicidina IIb (P. Dulski *et al.*, Solicitud PCT WO 97/11366); CHAP, tetrapéptido cíclico de toxina HC (Bosch *et al.*, Plant Cell 7, 1941-1950 (1995)); tetrapéptido cíclico WF27082 (Solicitud PCT WO 98/48825); y clamidocin (Bosch *et al.*, supra).

C. **Derivados de Ácidos Grasos de Cadena Corta (SCFA)** tales como: butirato de sodio (Cousens *et al.*, J. Biol. Chem. 254,1716-1723 (1979)); isovalerato (McBain *et al.*, Biochem. Pharm. 53: 1357-1368 (1997)); valerato (McBain *et al.*, supra); 4-fenilbutirato (4-PBA) (Lea and Tulsyan, Anticancer Research, 15,879-873 (1995)); fenilbutirato (PB) (Wang *et al.*, Cancer Research, 59, 2766-2799 (1999)); propionato (McBain *et al.*, supra); butiramida (Lea and Tulsyan, supra); isobutiramida (Lea and Tulsyan, supra); fenilacetato (Lea and Tulsyan, supra); 3-bromopropionato (Lea and Tulsyan, supra); tributirin (Guan *et al.*, Cancer Research, 60,749-755 (2000)); ácido valpróico, valproato y Pivanex™.

D. **Derivados de Benzamida** tales como CI-994; MS-275 [N-(2-aminofenil)-4-[N-(piridin-3-il metoxicarbonil)aminometil] benzamida] (Saito *et al.*, Proc. Natl. Acad. Sci. USA 96, 4592-4597 (1999)); y derivados 3'-amino de MS-275 (Saito *et al.*, supra).

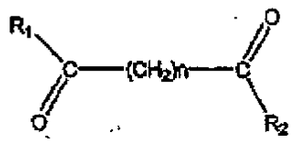
E. **Derivados de cetonas electrofílicas** tales como cetonas de trifluorometilo (Frey *et al*, Bioorganic & Med. Chem. Lett. (2002), 12, 3443-3447; U.S. 6,511,990) y α-cetoamidas tales como N-metil-α-cetoamidas.

F. Otros inhibidores de HDAC tales como productos naturales, psammaplinas, y Depudecina (Kwon *et al.* 1998. PNAS 95: 3356-3361).

Ácidos hidroxámicos preferidos basados en inhibidores HDAC son ácidos suberoilanilida hidroxámico (SAHA), bishidroxamida de ácido m-carboxicinámico (CBHA), y piroxamida. Se ha mostrado que el SAHA se une directamente en la cavidad catalítica de la enzima de histona acetilasa. El SAHA induce la detención del ciclo de la célula, diferenciación, y/o apoptosis de células transformadas en cultivo e inhibe el crecimiento del tumor en roedores. El SAHA es efectivo en la inducción de estos efectos en tumores sólidos y cánceres hematológicos. Se ha mostrado que el SAHA es efectivo en la inhibición del crecimiento de tumor en animales sin toxicidad al animal. El SAHA induce la inhibición del crecimiento de tumor se asocia con una acumulación de histona acetiladas en el tumor. El SAHA es efectivo para inhibir el desarrollo y el crecimiento continuado de tumores mamarios inducidos por carcinógenos (N-metilnitrosourea) en ratas. El SAHA se administra a las ratas en su dieta durante los 103 días del estudio. Así, el SAHA es un agente antitumoral no tóxico oralmente activo cuyo mecanismo de acción involucra la inhibición de la actividad de histona desacetilasa.

Los inhibidores HDAC preferidos son aquellos descritos en las patentes estadounidenses No. 5,369,108, 5,932,616, 5,700,811, 6,087,367 y 6,511,990, hecha por algunos de los presentes inventores describe los compuestos, cuyos contenidos totales se incorporan aquí como referencia, cuyos ejemplos no limitativos se establecen adelante:

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por la estructura de la fórmula 1 o una sal farmacéuticamente aceptable o un hidrato de esta, y un portador o excipiente farmacéuticamente aceptable.



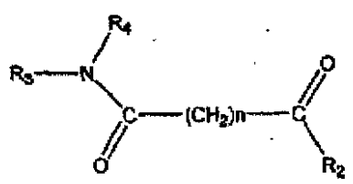
(1)

en donde R_1 y R_2 puede ser iguales o diferentes; cuando R_1 y R_2 son iguales, cada uno es un arilamino sustituido o no sustituido, cicloalquilamino, piridinoamino, piperidino, 9-purina-6-amina o grupo tiazoleamina; cuando R_1 y R_2 son diferentes R_1=R_3-N-R_4, en donde cada uno de R_3 y R_4 son independientemente los mismos o diferentes uno del otro y son un átomo de hidrógeno, un grupo hidroxilo, un grupo alquilo, alquenilo, cicloalquilo, arilo alquiloxi, ariloxi,

arilalquiloxi o piridina sustituido o no sustituido, ramificado o no ramificado, o R_3 y R_4 están unidos juntos para formar un grupo piperidina, R_2 es un grupo hidroxilamino, hidroxilo, amino, alquilamino, dialquilamino o un grupo alquiloxi y n es un entero de aproximadamente 4 a aproximadamente 8.

En una modalidad particular de la fórmula 1, R_1 y R_2 son iguales y son un grupo tiazoleamino sustituido o no sustituido; y n es un entero desde aproximadamente 4 a aproximadamente 8.

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por la estructura de la fórmula 2 o una sal farmacéuticamente aceptable o un hidrato de esta, y un portador o excipiente farmacéuticamente aceptable.



(2)

en donde cada uno de R_3 y R_4 son independientemente los mismos o diferentes uno del otro y son un átomo de hidrógeno, un

grupo hidroxilo, un grupo alquilo, alquenilo, cicloalquilo, arilalquiloxi, ariloxi, arilalquiloxi o piridina sustituido o no sustituido, ramificado o no ramificado, o R_3 y R_4 están unidos juntos para formar un grupo piperidino; R_2 es un grupo hidroxilamino, hidroxilo, amino, alquilamino, dialquilamino o alquiloxi; n es un entero de aproximadamente 4 a aproximadamente 8.

En una modalidad particular de la fórmula 2, cada uno de R_3 y R_4 son independientemente el *mismo* o diferente uno del otro y son un átomo de hidrógeno, un grupo hidroxilo un grupo alquilo, alquenilo, cicloalquilo, arilo, alquiloxi, ariloxi, arilalquiloxi o piridino sustituido o no sustituido, ramificado o no ramificado, o R_3 y R_4 unidos juntos forman un grupo piperidino; R_2 es un grupo hidroxilamino, hidroxilo, amino, alquilamino, o alquiloxi; n es un entero de 5 a 7, y R_3-N-R_4 y R_2 son diferentes.

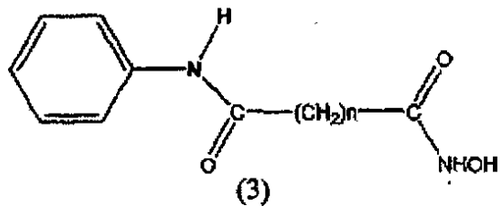
En otra modalidad particular de la fórmula 2, n es 6. En aun otra modalidad de la fórmula 2, R_4 es un átomo de hidrógeno, R_3 es un fenilo sustituido o no sustituido, y n en 6. En aún otra modalidad de la fórmula 2, R_4 es un átomo de hidrógeno, R_3 es un fenilo sustituido y n es 6, en donde el sustituyente fenilo se seleccionad del grupo que consiste de metilo, ciano, nitro, trifluorometilo, amino, aminocarbonilo, metilciano, cloro, fluoro, bromo, iodo, 2,3-difluoro, 2,4-

difluoro, 2,5-difluoro, 3,4-difluoro, 3,5-difluoro, 2,6-difluoro, 1,2,3-trifluoro, 2,3,6-trifluoro, 2,4, 6-trifluoro, 3,4,5-trifluoro, 2,3,5,6-tetrafluoro, 2,3,4,5,6-pentafluoro, azido, hexilo, t-butilo, fenilo, carboxilo, hidroxilo, metoxi, feniloxi, benziloxi, fenilaminooxi, fenilaminocarbonilo, metoxicarbonilo, metilaminocarbonilo, dimetilamino, dimetilamino carbonilo, o un grupo hidroxilaminocarbonilo.

En otra modalidad de la fórmula 2, n es 6, R_4 es un átomo de hidrógeno, y R_3 es un grupo ciclohexilo. En otra modalidad de la fórmula 2, n es 6, R_4 es un átomo de hidrógeno, y R_3 es un grupo metoxi. En otra modalidad de la fórmula 2, n es 6 y R_3 y R_4 se unen juntos para formar un grupo piperidino. En otra modalidad de la fórmula 2, n es 6, R_4 es un átomo de hidrógeno, y R_3 es un grupo benziloxi. En otra modalidad de la fórmula 2, R_4 es un átomo de hidrógeno y R_3 es un grupo γ-piridina. En otra modalidad de la fórmula 2, R_4 es un átomo de hidrógeno y R_3 es un grupo β-piridino. En otra modalidad de la fórmula 2, R_4 es un átomo de hidrógeno y R_3 es un grupo α-piridino. En otra modalidad de la fórmula 2, n es 6, y R_3 y R_4 son ambos grupo metilo. En otra modalidad de la fórmula 2, n es 6, R_4 es un grupo metilo, y R_3 es un grupo fenilo.

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por la estructura de la

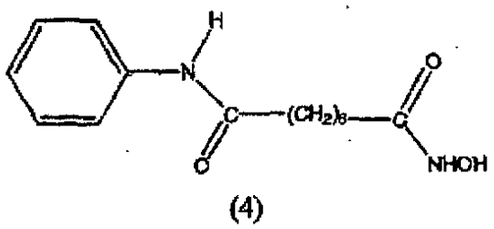
fórmula 3 o una sal farmacéuticamente aceptable o un hidrato de esta, y un portador o excipiente farmacéuticamente aceptable.



(3)

en donde n es un entero de 5 a 8.

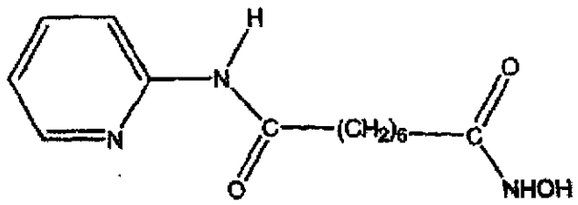
En una modalidad preferida de la fórmula 3, n es 6. De acuerdo con esta modalidad, el inhibidor HDAC es SAHA (4) o una sal farmacéuticamente aceptable o un hidrato de esta, y un portador o excipiente farmacéuticamente aceptable. SAHA se puede representar por medio de la siguiente fórmula estructural:



(4)

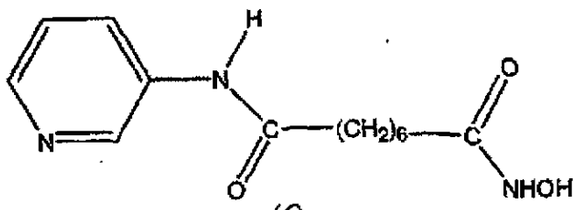
En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por la estructura de la fórmula 5 o una sal farmacéuticamente aceptable o un hidrato

de esta, y un portador o excipiente farmacéuticamente aceptable.



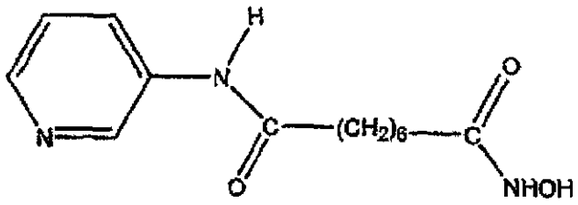
(5)

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por la estructura de la fórmula 6 (piroxamida) o una sal farmacéuticamente aceptable o un hidrato de esta, y un portador o excipiente farmacéuticamente aceptable.



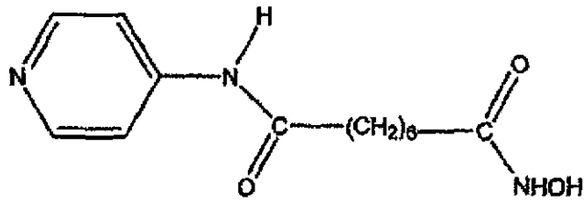
(6)

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por la estructura de la fórmula 7 o una sal farmacéuticamente aceptable o un hidrato de esta, y un portador o excipiente farmacéuticamente aceptable.



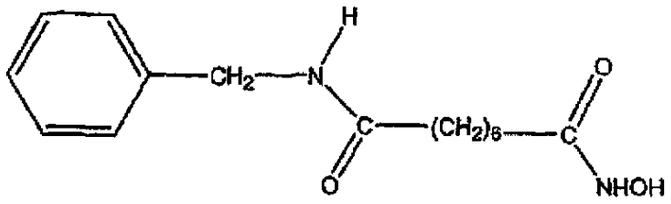
(7)

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por la estructura de la fórmula 8 o una sal farmacéuticamente aceptable o un hidrato de esta, y un portador o excipiente farmacéuticamente aceptable.



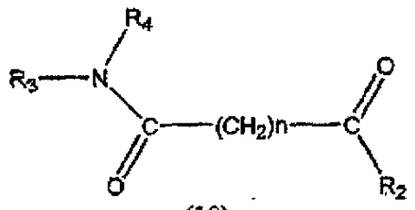
(8)

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por la estructura de la fórmula 9 o una sal farmacéuticamente aceptable o un hidrato de esta, y un portador o excipiente farmacéuticamente aceptable.



(9)

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 10, o una sal farmacéuticamente aceptable o hidrato de ésta, y un portador o excipiente farmacéuticamente aceptable.

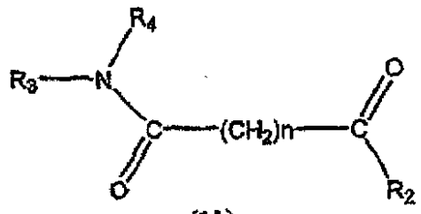


(10)

En donde R_3 es hidrógeno y R_4 es un grupo cicloalquilo, arilo, ariloxi, arilalquiloxi, o piridina, o R_3 y R_4 unidos juntos forman un grupo piperidina; R_2 es un grupo hidroxilamino; y n es un entero de 5 a aproximadamente 8.

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 11, o una sal o hidrato farmacéuticamente

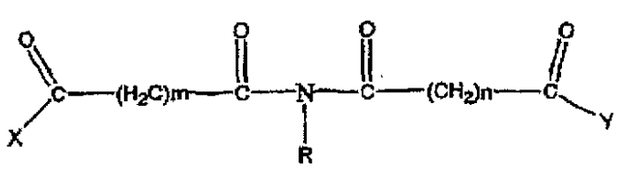
aceptable de éste, y un portador o excipiente farmacéuticamente aceptable.



(11)

En donde R_3 y R_4 son independientemente un grupo alquilo, alquenilo, cicloalquilo, arilo, alquiloxi, ariloxi, arilalquiloxi, o piridina, sustituido o no sustituido, ramificado o no ramificado, grupo cicloalquilo, arilo, ariloxi, arilalquiloxi, o piridina, o R_3 y R_4 forman juntos un grupo piperidina, R_2 es un grupo hidroxilamino; y n es un entero de 5 a aproximadamente 8.

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 12, o una sal farmacéuticamente aceptable o hidrato de ésta, y un portador o excipiente farmacéuticamente aceptable.

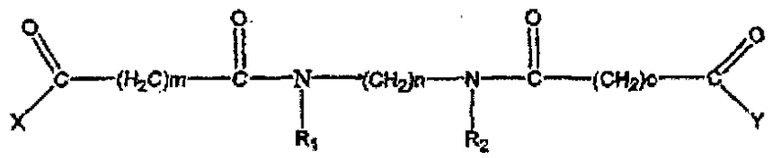


(12)

En donde cada uno de X y Y son independientemente los mismos o diferentes uno del otro y son un grupo hidroxilo, amino o hidroxilamino, un grupo alquiloxi, alquilamino, dialquilamino, arilamino, alquilarilamino, alquiloxiamino, ariloxiamino, alquiloxialquilamino, o ariloxialquilamino sustituido o no sustituido; R es un átomo de hidrógeno, un grupo hidroxilo, un grupo alquilo, arilalquiloxi, o ariloxi sustituido o no sustituido; cada uno de m y n son independientemente el mismo o diferente uno del otro y son cada uno un entero de aproximadamente 0 a aproximadamente 8.

En una modalidad particular, el inhibidor HDAC es un compuesto de fórmula 12 en donde X, Y y R son cada uno hidroxilo y ambos m y n son 5.

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 13, o una sal farmacéuticamente aceptable o hidrato de ésta, y un portador o excipiente farmacéuticamente aceptable.

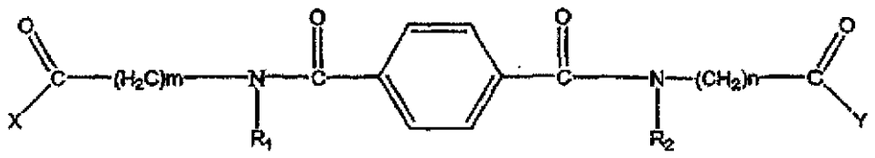


(13)

En donde cada uno de X y Y son independientemente los mismos o diferentes uno del otro y son un grupo hidroxilo, amino o hidroxilamino, un grupo alquiloxi, alquilamino, dialquilamino, arilamino, alquilarilamino, alquiloxiamino, ariloxiamino, alquiloxialquilamino, o ariloxialquilamino sustituido o no sustituido; cada uno de R_1 y R_2 son independientemente el mismo o diferente uno del otro y son un átomo de hidrógeno, un grupo hidroxilo, un grupo alquilo, arilo, alquiloxi, o ariloxi, sustituido o no sustituido; y cada uno de m, n y o son independientemente iguales o diferentes uno del otro y son cada uno un entero de aproximadamente 0 a aproximadamente 8.

En una modalidad particular de la fórmula 13, cada uno de X y Y es un grupo hidroxilo y cada uno de R_1 y R_2 es un grupo metilo. En otra modalidad particular de la fórmula 13, cada uno de X y Y es un grupo hidroxilo, cada uno de R_1 y R_2 es un grupo metilo, cada uno de n y o es 6, y m es 2.

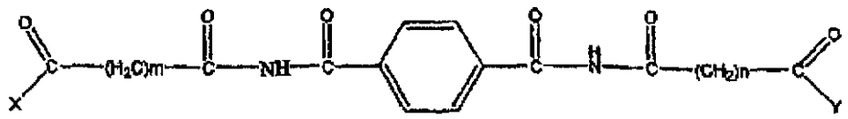
En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 14, o una sal farmacéuticamente aceptable o hidrato de éste, y un portador o excipiente farmacéuticamente aceptable.



(14)

En donde cada uno de X y Y son independientemente los mismos o diferentes uno del otro y son un grupo hidroxilo, amino o hidroxilamino, un grupo alquiloxi, alquilamino, dialquilamino, arilamino, alquilarilamino, alquiloxiamino, ariloxiamino, alquiloxialquilamino, o ariloxialquilamino sustituido o no sustituido; cada uno de R_1 y R_2 son independientemente el mismo o diferente uno del otro y son un átomo de hidrógeno, un grupo hidroxilo, un grupo alquilo, arilo, alquiloxi, o ariloxi, sustituido o no sustituido; y cada uno de m y n son independientemente iguales o diferentes uno del otro y son cada uno un entero de aproximadamente 0 a aproximadamente 8.

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 15, o una sal farmacéuticamente aceptable o hidrato de éste, y un portador o excipiente farmacéuticamente aceptable.

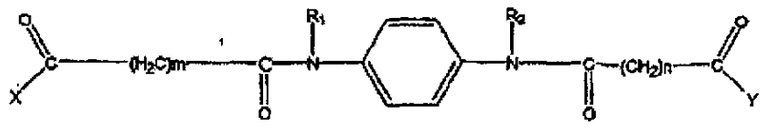


(15)

En donde cada uno de X y Y son independientemente los mismos o diferentes uno del otro y son un grupo hidroxilo, amino o hidroxilamino, un grupo alquiloxi, alquilamino, dialquilamino, arilamino, alquilarilamino, alquiloxiamino, ariloxiamino, alquiloxialquilamino, o ariloxialquilamino sustituido o no sustituido; y cada uno de m y n son independientemente el mismo o diferente uno del otro y son cada uno un entero de aproximadamente 0 a aproximadamente 8.

En una modalidad particular de la fórmula 15 cada uno de X y Y es un grupo hidroxilo y cada uno de m y n es 5.

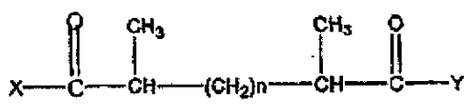
En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 16, o una sal farmacéuticamente aceptable o hidrato de éste, y un portador o excipiente farmacéuticamente aceptable.



(16)

En donde cada uno de X y Y son independientemente los mismos o diferentes uno del otro y son un grupo hidroxilo, amino o hidroxilamino, un grupo alquiloxi, alquilamino, dialquilamino, arilamino, alquilarilamino, alquiloxiamino, ariloxiamino, alquiloxialquilamino, o ariloxialquilamino sustituido o no sustituido; R_1 y R_2 son independientemente el mismo o diferente uno del otro y son un átomo de hidrógeno, un grupo hidroxilo, un grupo alquilo, arilo, alquiloxi, o ariloxi, sustituido o no sustituido; y cada uno de m y n son independientemente iguales o diferentes uno del otro y son cada uno un entero de aproximadamente 0 a aproximadamente 8.

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 17, o una sal farmacéuticamente aceptable o hidrato de éste, y un portador o excipiente farmacéuticamente aceptable.

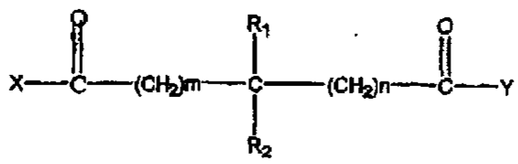


(17)

En donde cada uno de X y Y son independientemente los mismos o diferentes uno del otro y son un grupo hidroxilo, amino o hidroxilamino, un grupo alquiloxi, alquilamino, dialquilamino, arilamino, alquilarilamino, o ariloxialquilamino sustituido o no sustituido; y n es un entero desde aproximadamente 0 a aproximadamente 8.

En una modalidad particular de la fórmula 17, cada uno de X y Y es un grupo hidroxilamino, R_1 es un grupo metilo, R_2 es un átomo de hidrógeno; y cada uno de m y n es 2. En otra modalidad particular de la fórmula 17, cada uno de X y Y es un grupo hidroxilamino; R_1 es un grupo carbonilhidroxilamino, R_2 es un átomo de hidrógeno; y cada uno de m y n es 5. En otra modalidad particular de la fórmula 17, cada uno de X y Y es un grupo hidroxilamino; cada uno de R_1 y R_2 es un grupo fluoro; y cada uno de m y n es 2.

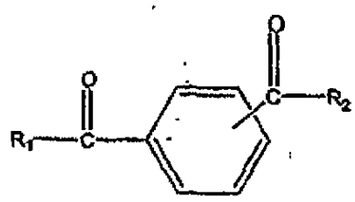
En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 18, o una sal farmacéuticamente aceptable o hidrato de éste, y un portador o excipiente farmacéuticamente aceptable.



(18)

En donde cada uno de X y Y son independientemente los mismos o diferentes uno del otro y son un grupo hidroxilo, amino o hidroxilamino, un grupo alquiloxi, alquilamino, dialquilamino, arilamino, alquilarilamino, alquiloxiamino, ariloxiamino, alquiloxialquilamino, o ariloxialquilamino sustituido o no sustituido; cada uno de R_1 y R_2 son independientemente el mismo o diferente uno del otro y son un átomo de hidrógeno, un grupo hidroxilo, un grupo alquilo, arilo, alquiloxi, o ariloxi, ariloxi, carbonilhidroxilamino o fluoro sustituido o no sustituido; y cada uno de m y n son independientemente iguales o diferentes uno del otro y son cada uno un entero de aproximadamente 0 a aproximadamente 8.

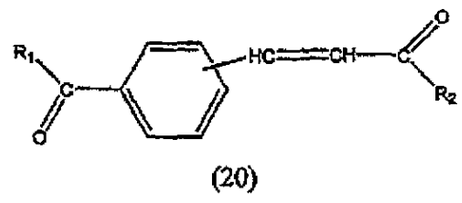
En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 19, o una sal farmacéuticamente aceptable o hidrato de éste, y un portador o excipiente farmacéuticamente aceptable.



(19)

En donde cada uno de R_1 y R_2 son independientemente los mismos o diferentes uno del otro y son un grupo hidroxilo, alquiloxi, amino, hidroxilamino, alquilamino, dialquilamino, arilamino, alquilarilamino, alquiloxiamino, ariloxiamino, alquiloxialquilamino, o ariloxialquilamino. En una modalidad particular, el inhibidor HDAC es un compuesto de fórmula estructural 19 en donde R_1 y R_2 son ambos hidroxilamino.

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 20, o una sal farmacéuticamente aceptable o hidrato de éste, y un portador o excipiente farmacéuticamente aceptable.

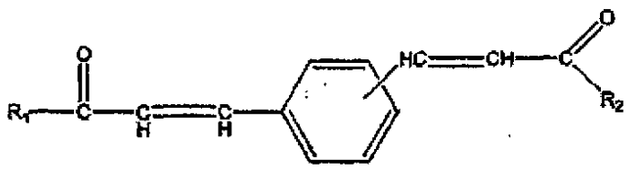


(20)

En donde cada uno de R_1 y R_2 son independientemente los mismos o diferentes uno del otro y son un grupo hidroxilo,

alquiloxi, amino, hidroxilamino, alquilamino, dialquilamino, arilamino, alquilarilamino, alquiloxiamino, ariloxiamino, alquiloxialquilamino, o ariloxialquilamino. En una modalidad particular, el inhibidor HDAC es un compuesto de fórmula estructural 20 en donde R_1 y R_2 son ambos hidroxilamino.

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 21, o una sal farmacéuticamente aceptable o hidrato de éste, y un portador o excipiente farmacéuticamente aceptable.

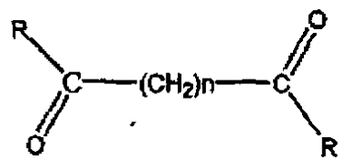


(21)

En donde cada uno de R_1 y R_2 son independientemente los mismos o diferentes uno del otro y son un grupo hidroxilo, alquiloxi, amino, hidroxilamino, alquilamino, dialquilamino, arilamino, alquilarilamino, alquiloxiamino, ariloxiamino, alquiloxialquilamino, o ariloxialquilamino.

En una modalidad particular, el inhibidor HDAC es un compuesto de fórmula estructural 21 en donde R_1 y R_2 son ambos hidroxilamino.

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 22, o una sal farmacéuticamente aceptable o hidrato de éste, y un portador o excipiente farmacéuticamente aceptable.

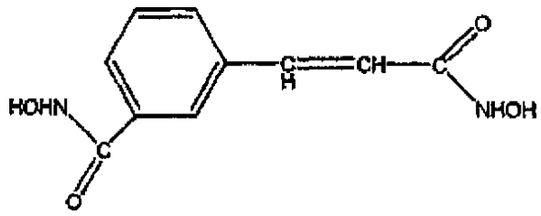


(22)

En donde R es un grupo fenilamina sustituido con un grupo ciano, metilciano, nitro, carboxilo, aminocarbonilo, metilaminocarbonilo, dimetilaminocarbonilo, trifluorometilo, hidroxilaminocarbonilo, N-hidroxilaminocarbonilo, metoxicarbonilo, cloro, fluoro, metilo, metoxi, 2,3-difluoro, 2,4-difluoro, 2,5-difluoro, 2,6-difluoro, 3,5-difluoro, 2,3,6-trifluoro, 2,4,6-trifluoro, 1,2,3-trifluoro, 3,4,5-trifluoro, 2,3,4,5-tetrafluoro, o 2,3,4,5,6-pentafluoro; y n es un entero de 4 a 8.

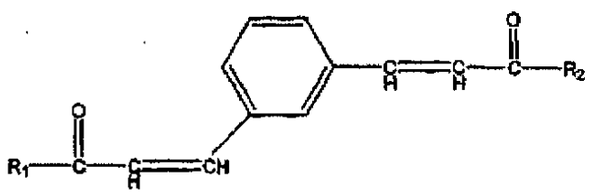
En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 23 (ácido m-carboxicinnámico bishidroxamida -

CBHA), o una sal farmacéuticamente aceptable o hidrato de éste, y un portador o excipiente farmacéuticamente aceptable.



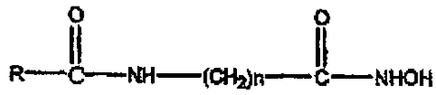
(23)

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 24, o una sal farmacéuticamente aceptable o hidrato de éste, y un portador o excipiente farmacéuticamente aceptable.



(24)

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 25, o una sal farmacéuticamente aceptable o hidrato de éste, y un portador o excipiente farmacéuticamente aceptable.



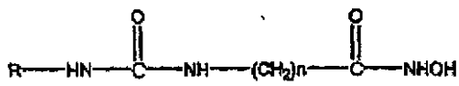
(25)

En donde R es un grupo fenilo, piperidina, tiazol, 2-piridina, 3-piridina o 4-piridina sustituido o no sustituido y n es un entero desde aproximadamente 4 a aproximadamente 8.

En una modalidad particular de la fórmula 25, R es un grupo fenilo sustituido. En otra modalidad particular de la fórmula 25, R es un grupo fenilo sustituido, donde el sustituyente se selecciona del grupo que consiste de metilo, ciano, nitro, tio, trifluorometilo, amino, aminocarbonilo, metilciano, cloro, fluoro, bromo, yodo, 2,3-difluoro, 2,4-difluoro, 2,5-difluoro, 3,4-difluoro, 3,5-difluoro, 2,6-difluoro, 1,2,3-trifluoro, 2,3,6-trifluoro, 2,4,6-trifluoro, 3,4,5-trifluoro, 2,3,5,6-tetrafluoro, 2,3,4,5,6-pentafluoro, azido, hexilo, t-butilo, fenilo, carboxilo, hidroxilo, metiloxi, feniloxi, benziloxi, fenilaminooxi, fenilaminocarbonilo, metiloxicarbonilo, metilaminocarbonilo, dimetilamino, dimetilaminocarbonilo, o hidroxilaminocarbonilo.

En otra modalidad particular de la fórmula 25, R es 2-piridina, 3-piridina o 4-piridina sustituido o no sustituido y n es un entero de aproximadamente 4 a aproximadamente 8.

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 26, o una sal farmacéuticamente aceptable o hidrato de éste, y un portador o excipiente farmacéuticamente aceptable.



(26)

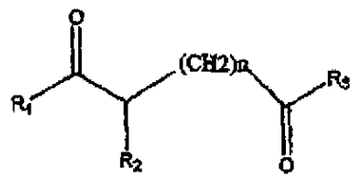
En donde R es un grupo fenilo, piridina, piperidina, o tiazol sustituido o no sustituido y n es un entero de aproximadamente 4 a aproximadamente 8 o una sal farmacéuticamente aceptable de éste.

En una modalidad particular de la fórmula 26, R es un grupo fenilo sustituido. En otra modalidad particular de la fórmula 26, R es un grupo fenilo sustituido, donde el sustituyente se selecciona del grupo que consiste de metilo, ciano, nitro, tio, trifluorometilo, amino, aminocarbonilo, metilciano, cloro, fluoro, bromo, yodo, 2,3-difluoro, 2,4-difluoro, 2,5-difluoro, 3,4-difluoro, 3,5-difluoro, 2,6-difluoro, 1,2,3-trifluoro, 2,3,6-trifluoro, 2,4,6-trifluoro, 3,4,5-trifluoro, 2,3,5,6-tetrafluoro, 2,3,4,5,6-pentafluoro, azido, hexilo, t-butilo, fenilo, carboxilo, hidroxilo, metiloxi, feniloxi, benziloxi, fenilaminooxi, fenilaminocarbonilo,

metiloxicarbonilo, metilaminocarbonilo, dimetilamino, dimetilaminocarbonilo, o un grupo hidroxilaminocarbonilo.

En otra modalidad particular de la fórmula 26, R es feniloo y n es 5. En otra modalidad, n es 5 y R es 3-clorofenilo.

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 27, o una sal farmacéuticamente aceptable o hidrato de éste, y un portador o excipiente farmacéuticamente aceptable.



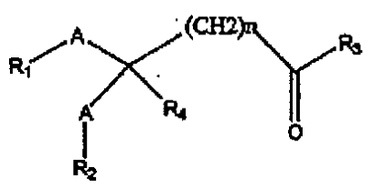
(27)

En donde cada uno de R_1 y R_2 está directamente unido o a través de un ligador y es un grupo arilo (por ejemplo, fenilo), arilalquilo (por ejemplo, benzilo), naftilo, cicloalquilo, cicloalquilamino, piridinoamino, piperidino, 9-purino-6-amino, tiazolamino, hidroxilo, sustituido o no sustituido, alquilo, alquenilo, alquiloxi, ariloxi, arilalquiloxi, piridilo o quinolinilo o isoquinolinilo ramificado o no ramificado; n es un entero desde aproximadamente 3 a aproximadamente 10 y R_3 es un grupo de

ácido hidroxámico, hidroxilamino, hidroxilo, amino, alquilamino o alquiloxi. El ligador puede ser una porción amida, por ejemplo, O-, S-, -NH-, NR_5, $-CH_2-$, $-(CH_2)_m-$, -(CH=CH)-, fenileno, ciclalquileno, o cualquier combinación de estos, en donde R_5 es un alquilo C_1-C_5 sustituido o no sustituido.

En ciertas modalidades de la fórmula 27, R_1 es $-NH-R_4$ en donde R_4 es arilo (por ejemplo, fenilo), arilalquilo (por ejemplo, benzilo), naftilo, cicloalquilo, cicloalquilamino, piridinoamino, piperidino, 9-purino-6-amino, tiazolamino, hidroxilo, sustituido o no sustituido, alquilo, alquenilo, alquiloxi, ariloxi, arilalquiloxi, piridilo o quinolinilo o isoquinolinilo ramificado o no ramificado.

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 28, o una sal farmacéuticamente aceptable o hidrato de éste, y un portador o excipiente farmacéuticamente aceptable.

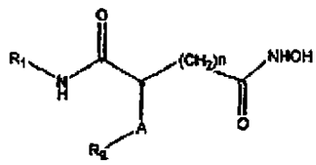


(28)

En donde cada uno de R_1 y R_2 es, arilo arilo (por ejemplo, fenilo), arilalquilo (por ejemplo, benzilo), naftilo, cicloalquilo, cicloalquilamino, piridinoamino, piperidino, 9-purino-6-amino, tiazolamino, hidroxilo, sustituido o no sustituido, alquilo, alquenilo, alquiloxi, ariloxi, arilalquiloxi, piridilo o quinolinilo o isoquinolinilo ramificado o no ramificado; R_3 es un grupo de ácido hidroxámico, hidroxilamino, hidroxilo, amino, alquilamino o alquiloxi; R_4 es hidrógeno, halógeno, fenilo o una porción cicloalquilo; y A puede ser igual o diferente y representa una porción amida, O-, S-, -NH-, NR_5, $-CH_2-$, $-(CH_2)_m-$, -(CH=CH)-, fenileno, ciclalquileno, o cualquier combinación de estos en donde R_5 es un alquilo C_1-C_5 sustituido o no sustituido; y n y m son cada uno un entero de 3 a 10.

En una modalidad particular adicional los compuestos que tienen una estructura más específica dentro del alcance de los compuestos 27 o 28:

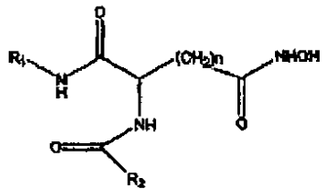
En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura 29:



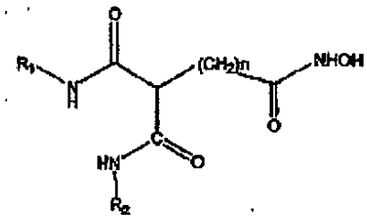
(29)

En donde A es una porción amida, R_1 y R_2 son cada uno seleccionados de arilo (por ejemplo, fenilo), arilalquilo (por ejemplo, benzilo), naftilo, piridinoamino, 9-purino-6-amino, tiazolamino, ariloxi, arilalquiloxi, piridilo, quinolinilo o isoquinolinilo sustituido o no sustituido; y n es un entero de 3 a 10.

Por ejemplo, el compuesto de fórmula 29 puede tener la estructura 30 o 31:



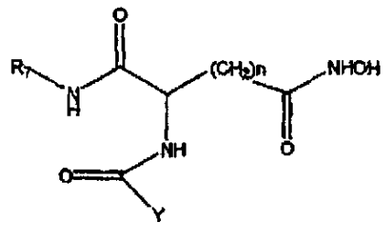
(30)



(31)

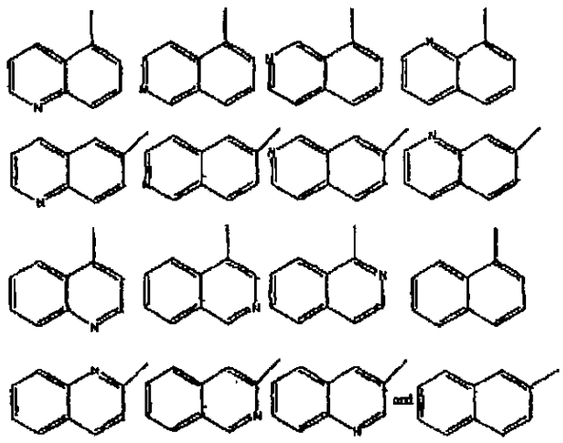
En donde R_1 y R_2, y n tienen los significados de la fórmula 29.

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 32:

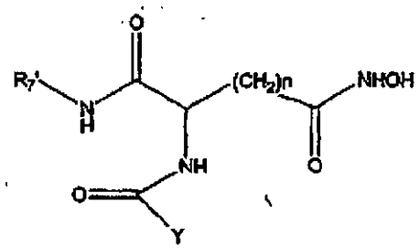


(32)

En donde R_7 se selecciona de arilo sustituido o no sustituido (por ejemplo, fenilo), arilalquilo (por ejemplo, benzilo), naftilo, piridinoamino, 9-purino-6-amino, tiazolamino, ariloxi, arilalquiloxi, piridilo, quinolinilo o isoquinolinilo; n es un entero de 3 a 10 y Y se selecciona de:

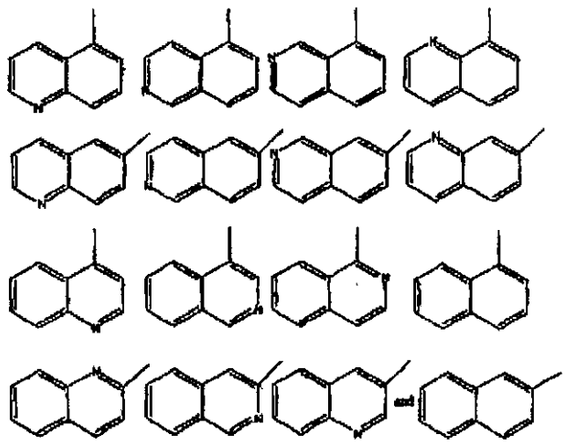


En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 33:

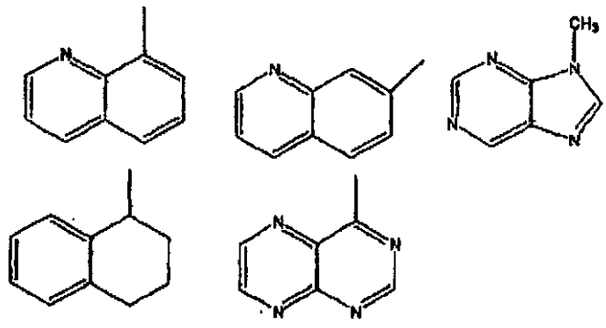


(33)

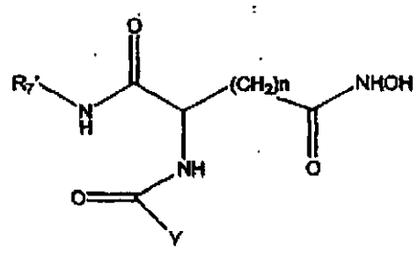
En donde n es un entero de 3 a 10, Y se selecciona de



Y R_7' se selecciona de

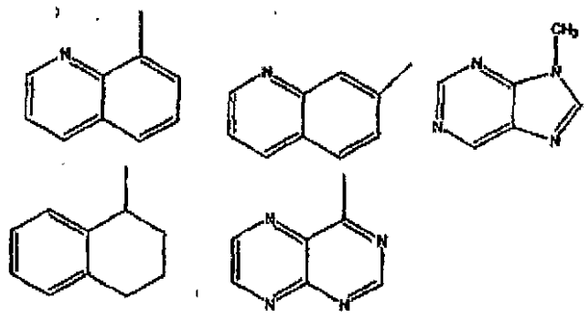


En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 34:

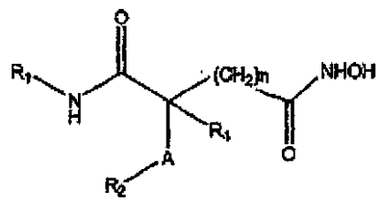


(34)

arilo (por ejemplo, fenilo), arilalquilo (por ejemplo, benzilo), naftilo, piridinoamino, 9-purino-6-amino, tiazolamino, ariloxi, arilalquiloxi, piridilo, quinolinilo o isoquinolinilo; n es un entero de 3 a 10 y R_7' se selecciona de



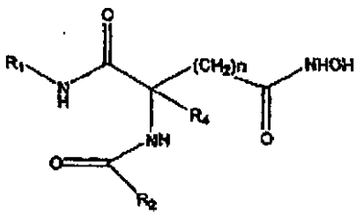
En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 35:



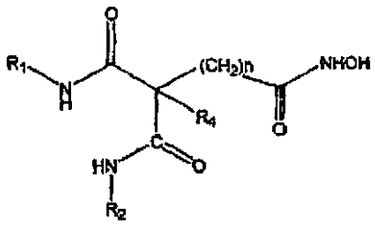
(35)

En donde A es una porción amida, R_1 y R_2 son cada uno seleccionados de arilo (por ejemplo, fenilo), arilalquilo (por ejemplo, benzilo), naftilo, piridinoamino, 9-purino-6-amino, tiazolamino, ariloxi, arilalquiloxi, piridilo, quinolinilo o isoquinolinilo; R_4 es hidrógeno, un halógeno, un fenilo o una porción cicloalquilo y n es un entero de 3 a 10.

Por ejemplo, el compuesto de fórmula 35 puede tener la estructura 36 o 37:



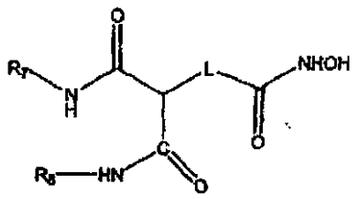
(36)



(37)

En donde R_1, R_2, R_4, y n tienen los significados de la fórmula 35.

En una modalidad, el inhibidor HDAC útil en los métodos de la presente invención se representa por medio de la estructura de fórmula 38:

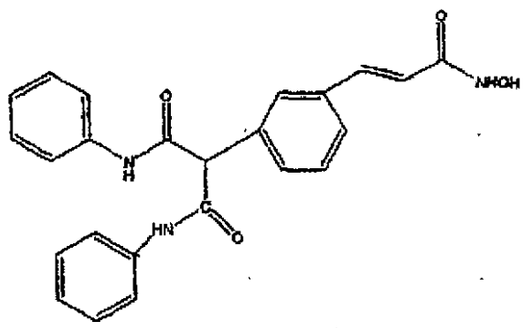


(38)

En donde L es un ligador seleccionado del grupo que consiste de una porción amido, O-, S-, -NH-, NR_5, $-CH_2-$, $-(CH_2)_m-$, -(CH=CH)-, fenileno, ciclalquileno, o cualquier combinación de estos en donde R_5 es un alquilo C_1-C_5 sustituido o no sustituido; y en donde cada uno de R_7 y R_8 son independientemente un arilo sustituido o no sustituido (por ejemplo, fenilo), arilalquilo (por ejemplo, benzilo),

naftilo, piridinoamino, 9-purino-6-amino, tiazolamino, ariloxi, arilalquiloxi, piridilo, quinolinilo o isoquinolinilo; n es un entero de 3 a 10 y m es un entero de 0 a 10.

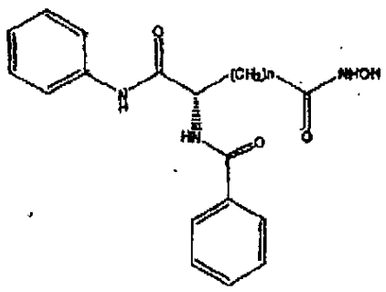
Por ejemplo, un compuesto de fórmula 38 se puede representar por medio de la estructura de fórmula (39):



(39)

Otros inhibidores HDAC adecuados para uso con los métodos de la presente invención incluyen aquellos mostrados en las siguientes fórmulas más específicas:

Un compuesto representado por la estructura:

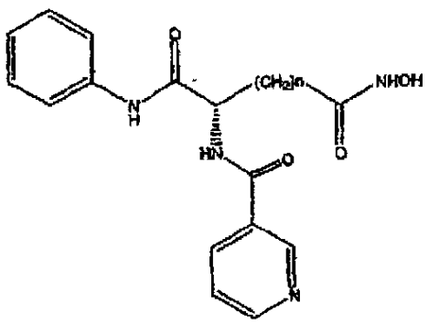


(40)

En donde n es un entero de 3 a 10, o un enantiómero de estos.

En una modalidad particular de la fórmula 40, n=5.

Un compuesto representado por la estructura:

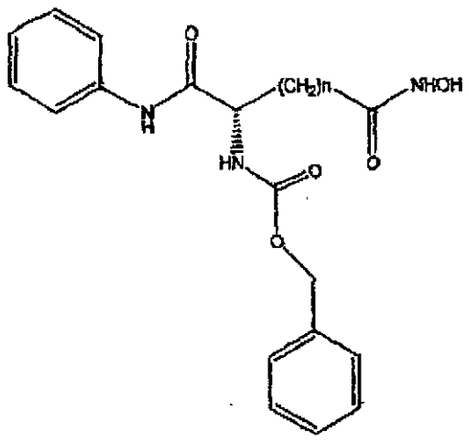


(41)

En donde n es un entero de 3 a 10, o un enantiómero de estos.

En una modalidad particular de la fórmula 41, n=5.

Un compuesto representado por medio de la estructura:

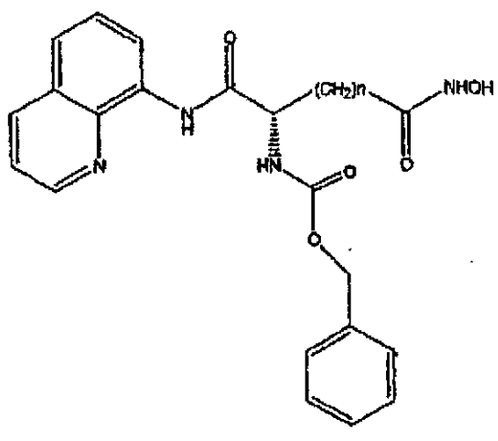


(42)

En donde n es un entero de 3 a 10 o un enantiómero de estos.

En una modalidad particular de la fórmula 42, n=5.

Un compuesto representado por la estructura:

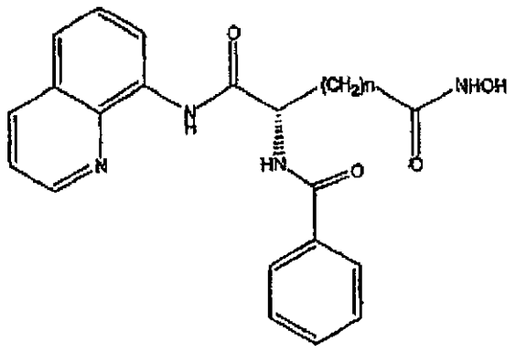


(43)

En donde n es un entero de 3 a 10, o un enantiómero de estos.

En una modalidad particular de fórmula 43, n=5.

Un compuesto representado por medio de la estructura:

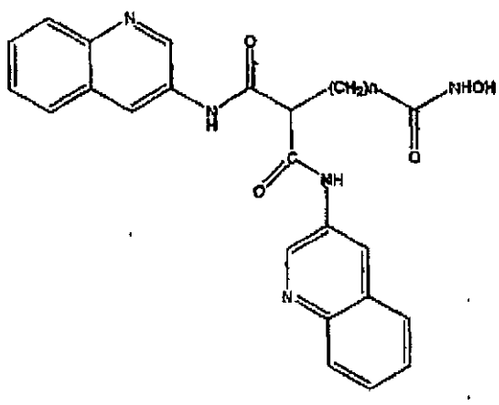


(44)

En donde n es un entero de 3 a 10, o un enantiómero de estos.

En una modalidad particular de la fórmula 44, n=5.

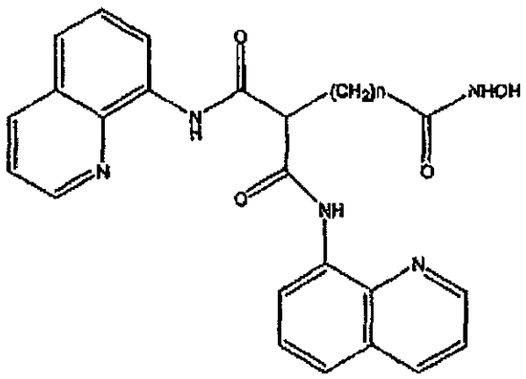
Un compuesto representado por la estructura:



(45)

En donde n es un entero de 3 a 10, o un enantiómero de estos.

En una modalidad particular de la fórmula 45, n=5.

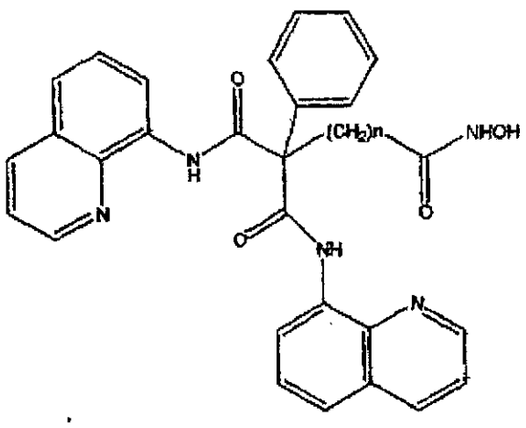


(46)

En donde n es un entero de 3 a 10, o un enantiómero de estos.

En una modalidad particular de la fórmula 46, n=5.

Un compuesto representado por medio de la estructura:

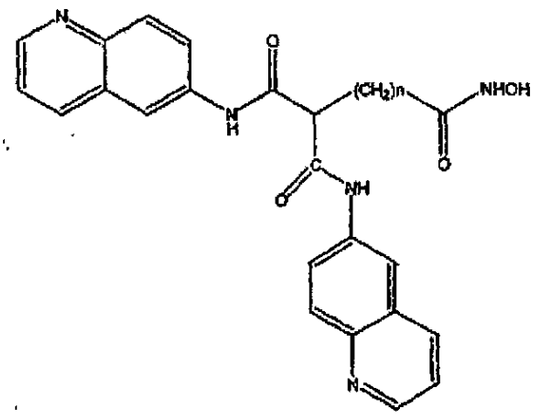


(47)

En donde n es un entero de 3 a 10, o un enantiómero de estos.

En una modalidad particular de la fórmula 47, n=5.

Un compuesto representado por medio de la estructura:

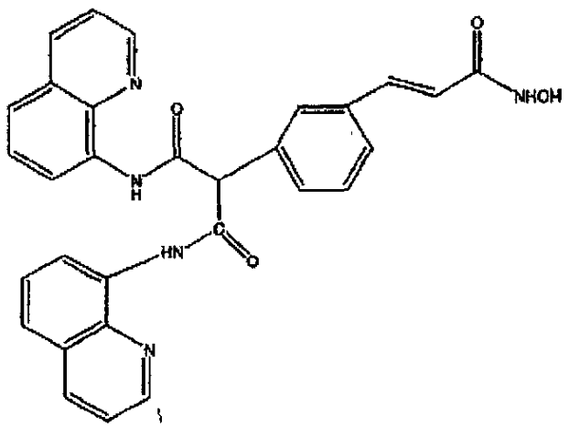


(48)

En donde n es un entero de 3 a 10, o un enantiómero de estos.

En una modalidad particular de la fórmula 48, n=5.

Un compuesto representado por medio de la estructura:

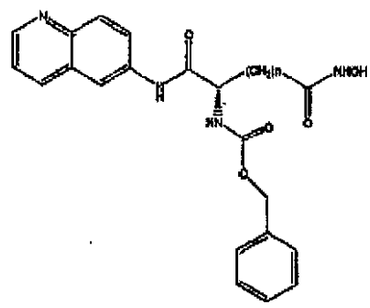


(49)

En donde n es un entero de 3 a 10, o un enantiómero de estos.

En una modalidad particular de la fórmula 49, n=5.

Un compuesto representado por medio de la estructura:

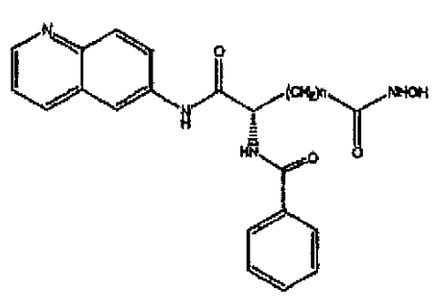


(50)

En donde n es un entero de 3 a 10, o un enantiómero de estos.

En una modalidad particular de la fórmula 50, n=5.

Un compuesto representado por medio de la estructura:



(51)

En donde n es un entero de 3 a 10, o un enantiómero de estos.

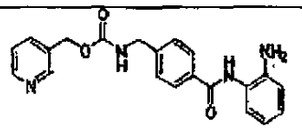
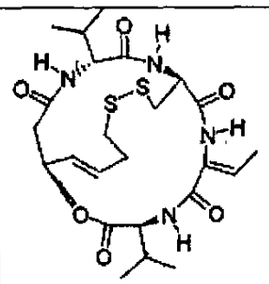
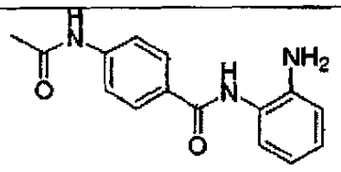
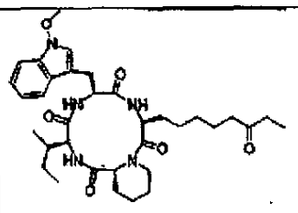
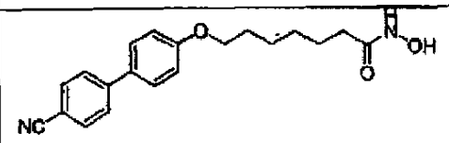
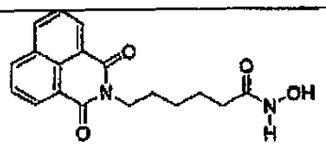
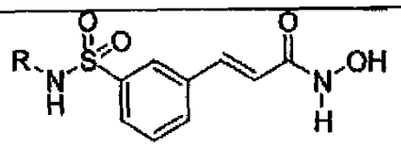
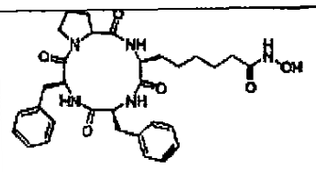
En una modalidad particular de la fórmula 51, n=5.

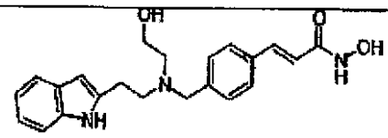
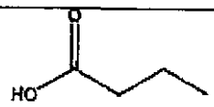
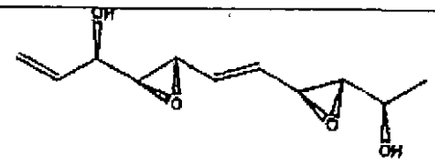
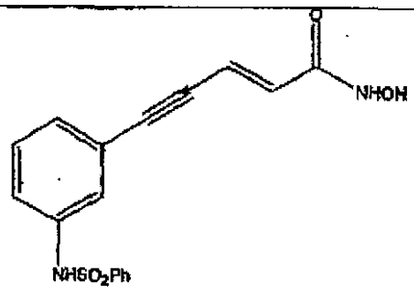
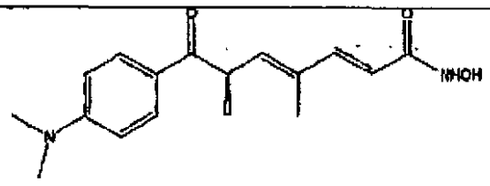
Otros ejemplos de tales compuestos y otros inhibidores HDAC se pueden encontrar en la Patente Estadounidense No. 5,369,108, publicada en Noviembre 29, 1994, Patente Estadounidense No. 5,700,811, publicada en Diciembre 23, 1997, Patente Estadounidense No. 5,773,474, publicada en Junio 30, 1998, Patente Estadounidense No. 5,932,616, publicada en Agosto 3, 1999 y Patente Estadounidense No. 6,511,990, publicada en Enero 28, 2003, todas a Breslow *et al.*; Patente Estadounidense No. 5,055,608, publicada en Octubre 8,1991, Patente Estadounidense No. 5,175,191, publicada en Diciembre 29, 1992 y Patente Estadounidense No. 5,608,108, publicada en Marzo 4, 1997, todas a Marks *et al.*; así como también Yoshida, M., *et al.*, Bioassays 17, 423-430 (1995); Saito, A., *et al.*, PNAS USA 96, 4592-4597, (1999); Furamai, R. *et al.*, PNAS USA 98 (1), 87-92 (2001); Komatsu, Y., *et al.*, Cancer Res. 61 (11), 4459-4466 (2001); Su, G. H., *et al.*, Cancer Res. 60,3137-3142 (2000); Lee, B. I. *et al.*, Cancer Res. 61 (3), 931-934; Suzuki, T., *et al.*, J. Med. Chem. 42 (15), 3001-3003 (1999); Solicitud PCT publicada WO 01/18171 publicada en Marzo 15, 2001 a Sloan-Kettering Institute for Cancer Research and The Trustees of Columbia University; Solicitud PCT publicada WO02/246144 a Hoffmann-La Roche; Solicitud PCT publicada W002/22577 a Novartis; Solicitud PCT publicada W002/30879 a Prolifix ; Solicitud PCT publicadas WO 01/38322 (publicada Mayo 31,2001), WO 01/70675 (publicada en Septiembre 27, 2001) y WO 00/71703 (publicada

en Noviembre 30, 2000) todas a Methylgene, Inc.; Solicitud PCT publicada WO 00/21979 publicada en Octubre 8, 1999 a Fujisawa Pharmaceutical Co., Ltd.; Solicitud PCT publicada WO 98/40080 publicada en Marzo 11, 1998 a Beacon Laboratories, L.L.C.; y Curtin M. (Current patent status of HDAC inhibitors *Expert Opina. Ihrer. Patents* (2002) 12 (9): 1375-1384 y referencias citadas aquí).

SAHA o cualquiera de los otros HDAC se puede sintetizar de acuerdo con los métodos resaltados en la Sección de Detalles Experimentales, o de acuerdo con el método establecido en las Patentes Estadounidenses Nos. 5,369,108, 5,700,811, 5,932,616 y 6,511,990, cuyos contenidos se incorporan como referencia en su totalidad, o de acuerdo con cualquier otro método conocido por una persona experta en la técnica.

Ejemplos no limitantes específicos de inhibidores HDAC se suministran en la Tabla de adelante. Se debe notar que la presente invención comprende cualquiera de los compuestos que son estructuralmente similares a los compuestos representados adelante, y que son capaces de inhibir desacetilasas histona.

Nombre	Estructura
MS-275	
Depsipéptido	
CI-994	
Apicidin	
A-161906	
Escriptaid	
PXD-101	
CHAP	

LAQ-824	
Ácido Butírico	
Depudecin	
Oxamflatin	
Tricostatin C	

Los compuestos de la presente invención son útiles para inducir selectivamente diferenciación terminal, detener el crecimiento celular y/o apoptosis de células neoplásicas y por lo tanto ayudar en el tratamiento de cáncer en pacientes como se describe en detalle aquí.

Definiciones Químicas

Un "grupo alifático" es no-aromático, consiste solamente de carbono e hidrógeno, y puede opcionalmente contener una o más

unidades de instauración, por ejemplo, enlaces dobles y/o triples. Un grupo alifático puede ser de cadena recta, ramificada o cíclica. Cuando es de cadena recta o ramificada, un grupo alifático típicamente contiene entre aproximadamente 1 y aproximadamente 12 átomos de carbono, más típicamente entre aproximadamente 1 y aproximadamente 6 átomos de carbono. Cuando es cíclico, un grupo alifático típicamente contiene entre aproximadamente 3 y aproximadamente 10 átomos de carbono, más típicamente entre aproximadamente 3 y aproximadamente 7 átomos de carbono. Los grupos alifáticos son preferiblemente grupos alquilo C_1-C_{12} de cadena recta o ramificada (es decir, grupos alifáticos completamente saturados), más preferiblemente grupos alquilo C_1-C_6 de cadena recta o ramificada. Ejemplos incluyen metilo, etilo, n-propilo, iso-propilo, n-butilo, secuencia-butilo, y terc-butilo.

Un "grupo aromático" (también referido como un "grupo arilo") como se utiliza aquí incluye grupos aromáticos carbocíclicos, grupos aromáticos heterocíclicos (también referidos como "heteroarilo") y sistema de anillo aromático policíclico fusionado como se define aquí.

Un "grupo aromático carbocíclico" es un anillo aromático de 5 a 14 átomos de carbono, e incluye un grupo aromático carbocíclico fusionado con un grupo cicloalquilo de 5 o 6

miembros tal como indano. Ejemplos de grupos aromáticos carbocíclicos incluyen, pero no están limitados a, fenilo, naftilo, por ejemplo, 1-naftilo y 2-naftilo; antracenilo, por ejemplo, 1-antracenilo, 2-antracenilo; fenantrenilo; fluorenonilo, por ejemplo, 9-fluorenonilo, indanilo y similares. Un grupo aromático carbocíclico es opcionalmente sustituido con un número designado de sustituyentes, descritos adelante.

Un "grupo aromático heterocíclico" (o "heteroarilo") es un anillo aromático monocíclico, bicíclico o tricíclico de anillos de 5 a 14 átomos de carbono y de uno a cuatro heteroátomos seleccionados de O, N, o S. Ejemplos de heteroarilo incluyen, pero no están limitados a piridilo, por ejemplo, 2-piridilo (también referido como α-piridilo), 3-piridilo (también referido como β-piridilo) y 4-piridilo (también referido como (γ-piridilo); tienilo, por ejemplo , 2-tienilo y 3-tienilo; furanilo, por ejemplo , 2-furanilo y 3-furanilo; pirimidilo, por ejemplo, 2-pirimidilo y 4-pirimidilo; imidazolilo, por ejemplo , 2-imidazolilo; piranilo, por ejemplo, 2-piranilo y 3-piranilo; pirazolilo, por ejemplo, 4-pirazolilo y 5-pirazolilo; tiazolilo, por ejemplo , 2-tiazolilo, 4-tiazolilo y 5-tiazolilo; tiadiazolilo; isotiazolilo; oxazolilo, por ejemplo , 2-oxazoilo, 4-oxazoilo y 5-oxazoilo; isoxazoilo; pirrolilo;

piridazinilo; pirazinilo y similares. Aromático heterocíclico (o heteroarilo) como se definió anteriormente puede ser opcionalmente sustituido con un número designado de sustituyentes, como se describe adelante para los grupos aromáticos.

Un sistema de anillo "aromático policíclico fusionado" es un grupo aromático carbocíclico o heteroarilo fusionado con uno o más de otros heteroarilos o anillos heterocíclicos no aromáticos. Ejemplos incluyen, quinolinilo y isoquinolinilo, por ejemplo, 2-quinolinilo, 3-quinolinilo, 4-quinolinilo, 5-quinolinilo, 6-quinolinilo, 7-quinolinilo y 8-quinolinilo, 1-isoquinolinilo, 3-quinolinilo, 4-isoquinolinilo, 5-isoquinolinilo, 6-isoquinolinilo, 7-isoquinolinilo y 8-isoquinolinilo; benzofuranilo, por ejemplo , 2-benzofuranilo y 3-benzofuranilo; dibenzofuranilo, por ejemplo, 2,3-dihidrobenzofuranilo; dibenzotiofenilo; benzotienilo, por ejemplo, 2-benzotienilo y 3-benzotienilo; indolilo, por ejemplo , 2-indolilo y 3-indolilo; benzotiazolilo, por ejemplo, 2-benzotiazolilo; benzooxazolilo, por ejemplo , 2-benzooxazolilo; benzimidazolilo, por ejemplo, 2-benzoimidazolilo; isoindolilo, por ejemplo, 1-isoindolilo y 3-isoindolilo; benzotriazolilo; purinilo; tianaftenilo y similares. Los sistemas de anillo aromático policíclico fusionado pueden ser opcionalmente sustituidos con un número designado de sustituyentes, como se describe aquí.

Un "grupo aralquilo" (arilalquilo) es un grupo alquilo sustituido con un grupo aromático, preferiblemente un grupo fenilo. Un grupo aralquilo preferido es un grupo benzilo. Grupos aromáticos adecuados son descritos aquí y grupos alquilo adecuados son descritos aquí. Sustituyentes adecuados para un grupo aralquilo son descritos aquí.

Un "grupo ariloxi" es un grupo arilo que se une a un compuesto por vía de un oxígeno (por ejemplo, fenoxi).

Un "grupo alcoxi" (alquiloxi), como se utiliza aquí, es un grupo alquilo C_1-C_{12} de cadena recta o ramificada o C_3-C_{12} cíclico que se conecta a un compuesto por vía de un átomo de oxígeno. Ejemplos de grupos alcoxi incluyen pero no están limitados a metoxi, etoxi, y propoxi.

Un "grupo arilalcoxi" (arilalquiloxi) es un grupo arilalquilo que se une a un compuesto por vía de un oxígeno en la porción alquilo del arilalquilo (por ejemplo, fenilmetoxi).

Un "grupo arilamino" como se utiliza aquí, es un grupo arilo que se une a un compuesto por vía de un nitrógeno.

Como se utiliza aquí, un "grupo arilalquilamino" es un grupo arilalquilo que se une a un compuesto por vía de un nitrógeno en la porción alquilo del arilalquilo.

Como se utiliza aquí, varias porciones o grupos son referidos como si fueran "sustituidos o no sustituidos". Cuando una porción es referida como sustituida, esto denota que cualquier porción de la porción que es conocida por un experto en la técnica como disponible para sustitución se puede sustituir. Por ejemplo, el grupo sustituible puede ser un átomo de hidrógeno que se reemplaza con un grupo diferente de hidrógeno (es decir, un grupo sustituyente). Se pueden presentar grupos de sustituyentes múltiples. Cuando se presentan sustituyentes múltiples, los sustituyentes pueden ser los mismos o diferentes y la sustitución puede ser en cualquiera de los sitios sustituibles. Tales medios para sustitución son bien conocidos en la técnica. Para propósitos de ejemplificación, los cuales no deben ser limitantes del alcance de la invención, algunos ejemplos de grupos que son sustituyentes son: grupos alquilo (que también pueden ser sustituidos, con uno o más sustituyentes, tales como CF_3), grupos alcoxi (que pueden ser sustituidos, tales como OCF_3), un grupo halógeno o halo (F, Cl, Br, I), hidroxi, nitro, oxo, -CN, -COH, -COOH, amino, azido, N-alquilamino o N,N-dialquilamino (en el cual los grupos alquilo también pueden ser sustituidos), ésteres (-C(O)-OR, donde R puede ser un

grupo tal como alquilo, arilo, etc., que puede ser sustituido), arilo (el más preferido es fenilo, que puede ser sustituido), arilalquilo (que puede ser sustituido) y ariloxi.

Estereoquímica

Muchos compuestos orgánicos existen en formas ópticamente activas que tienen la capacidad de rotar el plano de la luz polarizada plana. En la descripción de un compuesto ópticamente activo, los prefijos D y L o R y S se utilizan para denotar la configuración absoluta de la molécula cerca de su centro o centros quirales. Los prefijos d y l o (+) y (-) se emplean para designar la señal de rotación de la luz polarizada plana por el compuesto, con (-) o significando que el compuesto es levorotatorio. Un compuesto con prefijo (+) o d es dextrorotatorio. Para una estructura química dada, estos compuestos, llamados estereoisómeros, son idénticos excepto que ellos son imágenes de espejo no-superimpuestas de otro.

Un estereoisómero específico también puede ser referido como un enantiómero, y una mezcla de tales isómeros es a menudo llamada mezcla enantiomérica. Una mezcla 50:50 de enantiómeros es referida como una mezcla racémica. Muchos de los compuestos descritos aquí pueden tener uno o más centros quirales y por lo tanto pueden existir en diferentes formas

enantioméricas. Si se desea, un carbono quiral se puede designar con un asterisco (*). Cuando los enlaces al carbono quiral son descritos como líneas rectas en las fórmulas de la invención, se entiende que ambas configuraciones (R) y (S) del carbono quiral, y por lo tanto ambos enantiómeros y mezclas de estos, están contenidos dentro de la fórmula. Como se utiliza en la técnica, cuando se desea especificar la configuración absoluta cerca de un carbono quiral, uno de los enlaces al carbono quiral se puede describir como una cuña (enlaces con átomos sobre el plano) y el otro se puede describir como una serie o cuña de líneas paralelas cortas (enlaces con átomos debajo del plano). El sistema Cahn-Inglod-Prelog se puede utilizar para asignar la configuración (R) o (S) a un carbono quiral.

Cuando los inhibidores HDAC de la presente invención contienen un centro quiral, los compuestos existen en dos formas enantioméricas, y la presente invención incluye ambos enantiómeros y mezclas de enantiómeros, tal como la mezcla específica 50:50 referida como una mezcla racémica. Los enantiómeros se pueden resolver mediante métodos conocidos por aquellos expertos en la técnica, por ejemplo mediante la formación de sales diastereoisoméricas que pueden ser separadas, por ejemplo, mediante cristalización (ver, CRC Handbook of Optical Resolutions via Diastereomeric SALT Formation por David Kozma (CRC Press,2001)); la formación de

complejos o derivados diastereoisoméricos que se pueden separar, por ejemplo, mediante cristalización, cromatografía de líquido o gas-líquido; reacción selectiva de un enantiómero con un reactivo específico de enantiómero, por ejemplo esterificación enzimática; o cromatografía de líquido o gas-líquido en un ambiente quiral, por ejemplo en un soporte quiral por ejemplo sílice con un ligando quiral unido o en la presencia de un solvente quiral. Se puede apreciar que donde el enantiómero deseado se convierte en otra entidad química por uno de los procedimientos de separación descritos anteriormente, se requiere una etapa adicional para liberar la forma enantiomérica deseada. Alternativamente, los enantiómeros específicos se pueden sintetizar mediante síntesis asimétrica utilizando reactivos ópticamente activos, sustratos, catalizadores, o solventes, o al convertir un enantiómero en el otro mediante transformación asimétrica.

La designación de una configuración absoluta específica en un carbono quiral de los compuestos de la invención se entiende que significa que la forma enantiomérica designada de los compuestos es en exceso enantiomérica (ee) o en otras palabras está sustancialmente libre del otro enantiómero. Por ejemplo, las formas "R" de los compuestos están sustancialmente libres de las formas "S" de los compuestos y son, así, en exceso enantioméricas de las formas "S". Por el contrario, las formas "S" de los compuestos están

sustancialmente libres de las formas "R" de los compuestos y son, así, en exceso enantioméricas de las formas "R". Exceso enantiomérico, como se utiliza aquí, es la presencia de un enantiómero particular en más del 50%. Por ejemplo, el exceso enantiomérico puede ser aproximadamente 60% o más, tal como aproximadamente 70% o más, por ejemplo aproximadamente 80% o más, tal como aproximadamente 90% o más. En una modalidad particular cuando una configuración absoluta específica se designa, el exceso enantiomérico de los compuestos descritos es por lo menos aproximadamente 90%. En una modalidad particular adicional, el exceso enantiomérico de los compuestos es por lo menos aproximadamente 95%, tal como por lo menos aproximadamente 97.5%, por ejemplo, por lo menos 99% de exceso enantiomérico.

Cuando un compuesto de la presente invención tiene dos o más carbonos quirales este puede tener más de dos isómeros ópticos y puede existir en formas diastereoisoméricas. Por ejemplo, cuando hay dos carbonos quirales, el compuesto puede tener hasta 4 isómeros ópticos y 2 pares de enantiómeros ((S,S)/(R,R) y (R,S)/(S,R)). Los pares de enantiómeros (por ejemplo, (S,S)/(R,R)) son estereoisómeros de imagen de espejo del otro. Los estereoisómeros que no son imágenes-espejo (por ejemplo, (S,S) y (R,S)) son diastereómeros. Los pares diastereoisoméricos se pueden separar mediante métodos conocidos por aquellos expertos en la técnica, por ejemplo

cromatografía o cristalización y los enantiómeros individuales dentro de cada par se pueden separar como se describió anteriormente. La presente invención incluye cada diastereoisómero de tales compuestos y mezclas de estos.

Como se utiliza aquí, "un", "una" y "el" incluyen referentes singulares y plurales a menos que el contexto claramente dicte otra cosa. Así, por ejemplo, la referencia para "un agente activo" o "un agente activo farmacológicamente" incluye un agente activo único así como dos o más agentes activos diferentes en combinación, la referencia para "un portador" incluye mezclas de dos o más portadores así como un portador único, y similares.

La invención también pretende abarcar pro-drogas de los inhibidores HDAC descritos aquí. Una prodroga de cualquiera de los compuestos se puede fabricar utilizando técnicas farmacológicas bien conocidas.

Esta invención, además de los compuestos listados anteriormente, pretende abarcar el uso de homólogos y análogos de tales compuestos. En este contexto, los homólogos son moléculas que tienen semejanzas estructurales sustanciales con los compuestos descritos anteriormente y los análogos son moléculas que tienen semejanzas biológicas sustanciales con respecto a las semejanzas estructurales.

La invención también abarca composiciones farmacéuticas que comprenden sales farmacéuticamente aceptables de los inhibidores HDAC con ácidos orgánicos e inorgánicos, por ejemplo, sales de adición ácidas que pueden, por ejemplo, ser ácido clorhídrico, ácido sulfúrico, ácido metanosulfónico, ácido fumárico, ácido maleico, ácido succínico, ácido acético, ácido benzoico, ácido oxálico, ácido cítrico, ácido tartárico, ácido carbónico, ácido fosfórico y similares. Las sales farmacéuticamente aceptables también se pueden preparar mediante el tratamiento con bases inorgánicas, por ejemplo, sodio, potasio, amonio, calcio, o hidróxidos férricos, y tales bases orgánicas como isopropilamina, trimetilamina, 2-etilamino etanol, histidina, procaína, y similares.

La invención también abarca composiciones farmacéuticas que comprenden hidratos de los inhibidores HDAC. El término "hidrato" incluye pero no está limitado a hemihidrato, monohidrato, dihidrato, trihidrato, y similares.

La invención también abarca composiciones farmacéuticas que comprenden cualquier forma física sólida o líquida de SAHA o cualquiera de los otros inhibidores HDAC. Por ejemplo, los inhibidores HDAC pueden estar en forma cristalina, en forma amorfa, y tienen cualquier tamaño de partícula. Las partículas de inhibidor HDAC se pueden micronizar, o se

pueden aglomerar, gránulos, polvos, aceites, suspensiones aceitosas, o cualquier otra forma física sólida o líquida.

Usos Terapéuticos de Inhibidores HDAC

1. Tratamiento de Cáncer

Como se demuestra aquí, los inhibidores HDAC de la presente invención son útiles para el tratamiento de cáncer. De acuerdo con esto, en una modalidad, la invención se relaciona con un método para tratar el cáncer en un sujeto que necesite el tratamiento que comprende administrar a dicho sujeto una cantidad terapéuticamente efectiva de un inhibidor de desacetilasa histona descrito aquí.

El término "cáncer" se refiere a cualquier cáncer originado por la proliferación de células neoplásicas, tales como tumores sólidos, neoplasmas, carcinomas, sarcomas, leucemias, mesoteliomas, linfomas y similares. Por ejemplo, los cánceres incluyen, pero no están limitados a: mesoteliomas tales como mesotelioma pleural, mesotelioma peritoneal, mesotelioma fibroso benigno, leucemias, que incluyen leucemias agudas y leucemias crónicas tales como leucemia linfocítica aguda (ALL), leucemia no linfocítica aguda, leucemia mieloide aguda (AML), leucemia linfocítica crónica (CLL), leucemia mielógena crónica (CML) y leucemia por tricoleucitos; linfomas tales

como linfoma de células T cutáneo (CTCL), linfoma de célula T periférico no cutáneo, linfomas asociados con virus linfotrófico de células T humanas (HTLV) tal como linfoma/leucemia de célula T adulta (ATLL), enfermedad de Hodgkin y linfomas distintos a Hodgkin, linfomas de célula grande, linfoma de células B grandes difusas (DLBCL); linfoma de Burkitt; linfoma del sistema nervioso central (CNS) primario; mieloma múltiple, tumores sólidos de niñez tales como tumores cerebrales, neuroblastoma, retinoblastoma, tumor de Wilm, tumores óseos, y sarcomas de tejido blando, tumores sólidos comunes de adultos tales como cánceres de cabeza y cuello (por ejemplo, oral, laringe y esófago); cánceres genito urinarios (por ejemplo, próstata, vejiga, renal, uterino, de ovario, testicular, rectal y de colon), cáncer de pulmón, cáncer de seno, cáncer de páncreas, melanoma y otros cánceres de piel, cáncer de estómago, tumores cerebrales, cáncer de hígado y cáncer de tiroides.

2. Tratamiento de Mesotelioma y Linfoma

Como se demuestra aquí, los inhibidores HDAC son útiles para el tratamiento de mesotelioma y varias formas de linfoma, que incluyen linfoma de células B grande difuso (DLBCL).

Existen varios tipos de mesotelioma. Los mesoteliomas pleurales y peritoneales (malignos) involucran tumores de

tejido mesotelial asociados con la exposición a asbestos. Histológicamente, estos tumores se componen de tipos de células epiteloides, sarcomatoides, o fibrosas, o mezcladas (también se llaman tipo bifásico). El mesotelioma fibroso benigno es un tumor sólido raro de la pleura que produce dolor de pecho, disnea, fiebre, y osteoartropatía hipertrófica.

Un sistema de estadificación utilizado para el mesotelioma es el sistema Butchart. Este sistema se basa principalmente en el alcance de la masa de tumor maligno primario, y divide los mesoteliomas en etapas I a IV.

Etapa I: El mesotelioma está presente en un lado del pecho solo y no está creciendo en la pared del pecho.
Etapa II: El mesotelioma invade la pared del pecho o involucra el esófago (pasaje del alimento que conecta la garganta al estómago), corazón, o tiene crecimiento en la pleura u otro lado del pecho. Los nodos linfáticos en el pecho también pueden estar involucrados.
Etapa III: El mesotelioma ha crecido a través del diafragma dentro del peritoneo (que cubre la cavidad abdominal) o se ha esparcido a los nodos linfáticos más allá de aquellos en el pecho.
Etapa IV: El mesotelioma se ha esparcido a través del torrente sanguíneo a otros órganos (metástasis).

Otro sistema de estadificación se ha desarrollado recientemente por el International Mesothelioma Interest

Group. En este sistema, la información acerca del tumor, nodos linfáticos, y metástasis se combina en un proceso llamado etapa de agrupamiento.

Etapa I: Enfermedad confinada dentro de la cápsula de la pleura parietal: pleura ipsilateral, pulmón, pericardio, y diafragma.
Etapa II: Todas las de la etapa I con nodos linfáticos intratoráxicos positivos (N1 o N2).
Etapa 3: Extensión local de la enfermedad dentro de lo siguiente: pared del pecho o mediastino; corazón o a través del diafragma, peritoneo; con o sin involucrar el nodo linfático extratoráxico o contralateral (N3)
Etapa IV: Enfermedad metastático distante.

Existen muchos tipos diferentes de linfoma, y ellos se pueden dividir en dos categorías: enfermedad de Hodgkin (HD) y linfoma sin enfermedad de Hodgkin (NHL). La mayor diferencia entre las dos es el tipo de células involucradas.

Existen dos tipos principales de linfocitos: células T y células B. La mayoría de los linfocitos inician su crecimiento en la médula ósea. Las células B continúan el desarrollo en la médula ósea, mientras que las células T van de la médula ósea a la glándula timo y maduran allí. Una vez ellas se maduran, las células B y las células T ayudan a combatir las infecciones corporales.

Existen más de 20 diferentes tipos de linfoma de Hodgkin. El linfoma de célula B grande difusa es un tipo común, constituyendo cerca del 40% de todos los casos. Este es un cáncer de linfocitos B. El linfoma de célula B difuso puede ocurrir en cualquier tiempo desde la adolescencia a la edad adulta. Es ligeramente más común en los hombres que en las mujeres. Un linfoma de célula grande es un linfoma que se caracteriza por células inusualmente grandes.

La enfermedad de Hodgkin NHL se clasifica por las mismas categorías de etapas. La mayoría de linfomas en personas VIH positivas involucran células B, opuesto a las células T.

La etapa del linfoma es muy importante y puede ayudar a determinar el pronóstico y el curso de tratamiento. Las cuatro etapas son:

Etapa I: Existe un sitio con cáncer. No se involucra la médula ósea.
Etapa II: Existen dos sitios; ambos están arriba o abajo del diafragma. No se involucra la médula ósea.
Etapa III: Existen sitios arriba y abajo del diafragma. No se involucra la médula ósea.
Etapa IV: La médula ósea se afecta o las células de cáncer se han esparcido al exterior del sistema linfático.

En la enfermedad de Hodgkin, la estadificación se clasifica adicionalmente como sigue:

Clasificaciones de Enfermedad de Hodgkin
B: La presencia de fiebre, pérdida de peso o sudor nocturno
A: La ausencia de fiebre, pérdida de peso o sudor nocturno
E: La enfermedad se ha esparcido a los órganos exteriores al sistema linfático.

Clasificación

Para propósitos prácticos los linfomas sin la enfermedad de Hodgkin también se dividen en uno de dos grupos: clasificación alta y baja. Los linfomas de clasificación baja son usualmente linfomas de alto grado y crecimiento lento con tendencia a crecer más rápidamente. El linfoma de célula B grande difusa es un linfoma de grado alto.

Como se contempla aquí, los inhibidores HDAC de la presente invención son útiles en el tratamiento de todas las etapas de mesotelioma y linfoma, es decir, etapas I, II, III, y IV establecidas anteriormente, así como también las etapas A, B, y E de HD. Adicionalmente, los inhibidores HDAC son útiles en el tratamiento de linfomas de alto y bajo grado.

Como se demuestra aquí, los inhibidores HDAC de la presente invención son particularmente útiles en el tratamiento de mesotelioma y de linfoma de célula B grande difusa (DLBCL).

3. Otros usos de inhibidores HDAC

Los inhibidores HDAC son efectivos en el tratamiento de un rango más amplio de enfermedades caracterizadas por la proliferación de enfermedades neoplásicas, tales como uno cualquiera de los cánceres descritos aquí anteriormente. Sin embargo, la utilidad terapéutica de los inhibidores HDAC no se limita al tratamiento del cáncer. A diferencia, existe un amplio rango de enfermedades para los cuales los inhibidores HDAC se han encontrado útiles.

Por ejemplo, los inhibidores HDAC, en particular SAHA, se ha encontrado que son útiles en el tratamiento de una variedad de enfermedades crónicas y agudas inflamatorias, enfermedades autoinmunes, enfermedades alérgicas, enfermedades asociadas con estrés de oxidación, y enfermedades caracterizadas por la hiperproliferación celular. Ejemplos no limitativos son las condiciones inflamatorias de una articulación que incluye artritis reumatoide (RA) y artritis soriática; enfermedad inflamatoria del intestino tal como la enfermedad de Cronh y colitis ulcerativa; espondiloartropatías; escleroderma; soriasis (que incluye soriasis mediada por células T) y

dermatosis inflamatoria tal como dermatitis, eczema, dermatitis atópica, dermatitis de contacto alérgico, urticaria; vasculitis (por ejemplo, vasculitis necrotizante, cutánea, e hipersensible); miositis eosinfílica, fascitis eosinofílica; cánceres con infiltración de leucocitos de la piel u órganos, lesiones isquémicas, que incluyen isquemia cerebral (por ejemplo, lesión de cerebro como un resultado de trauma, epilepsia, hemorragia o apoplejía, cada una de las cuales conduce a neurodegeneración); VIH, falla cardiaca, enfermedad del hígado crónica, aguda o maligna, tiroiditis autoinmune; lupus eritematoso sistémico, síndrome de Sjorgren, enfermedades de pulmón (por ejemplo, ARDS); pancreatitis aguda; esclerosis lateral amiotrópica (ALS), enfermedad de Alzheimer; caquexia/anorexia; asma; ateroesclerosis; síndrome de fatiga crónica, fiebre; diabetes (por ejemplo, diabetes insulina o diabetes de ataque juvenil); glomerulonefritis; rechazo de huésped versus injerto (por ejemplo, en transplantes); choque hemorrágico; hiperalgesia; enfermedad inflamatoria del intestino; esclerosis múltiple, miopatías (por ejemplo, metabolismo de proteína de músculo, especialmente en sepsis); osteoporosis; enfermedad de Parkinson; dolor; labor pre-término; soriasis; reperfusión de lesión; toxicidad inducida por citoquina (por ejemplo, choque séptico, choque endotóxico); efectos colaterales de terapia de radiación, enfermedad de articulación temporal mandibular, metástasis de tumor; o una

condición inflamatoria que resulta de tensión, esguince, daño de cartílago, trauma tal como quemadura, cirugía ortopédica, infección u otros procesos de la enfermedad. Las enfermedades y condiciones alérgicas, incluye pero no están limitadas a enfermedades alérgicas respiratorias tales como asma, rinitis alérgicas, enfermedad de pulmón hipersensible, neumonitis hipersensible, neumonías eosinofílicas (por ejemplo, síndrome de Loeffler, neumonía eosinofílica crónica), hipersensibilidad de tipo retardada, enfermedad de pulmón intersticial (ILD) (por ejemplo, fibrosis pulmonar idiomática, o ILD asociado con artritis reumatoide, lupus eritematoso sistémico, espondilitis anquilosante, esclerosis sistémica, síndrome de Sjogren, polimiositis o dermatomiositis); anafilaxis sistémica o respuestas hipersensibles, alergias a drogas (por ejemplo, a penicilina, cefalosporinas), alergias a picaduras de insectos, y similares.

Por ejemplo, inhibidores HDAC, y en particular SAHA, se han encontrado que son útiles en el tratamiento de una variedad de enfermedades neurodegenerativas, una lista no exhaustiva la cual es:

I. Desórdenes caracterizados por demencia progresiva en ausencia de otros signos neurológicos prominentes, tales como

enfermedad de Alzheimer; demencia senil del tipo Alzheimer, y enfermedad de PicK (atrofia lobular).

II. síndromes que combinan la demencia progresiva con otras anormalidades neurológicas prominentes tales como A) síndromes que aparecen principalmente en adultos (por ejemplo, enfermedad de Huntington, atrofia de sistema Múltiple que combina demencia con ataxia y/o manifestaciones de la enfermedad de Parkinson, parálisis supranuclear Progresiva (Steel-Richardson-Olszewski), enfermedad de cuerpo de Lewy difuso, y degeneración corticodentatonigral); y B) síndromes que aparecen principalmente en niños o adultos jóvenes (por ejemplo, enfermedad Hallervorden-Spatz y epilepsia mioclónica familiar progresiva).

III. Síndromes que desarrollan gradualmente anormalidades de postura y movimiento tales como parálisis agitante (enfermedad de Parkinson), degeneración estriatonigral, parálisis supranuclear progresiva, distonía de torsión (espasmo de torsión, deformaciones musculares de distonía), torticolis espasmódica y otras disquinecias, temblor familiar, y síndrome Pilles de la Tourette.

IV. Síndromes de ataxia progresiva tal como degeneraciones cerebelares (por ejemplo, degeneración cortical cerebelar y atrofia olivopontocerebelar (OPCA)); y degeneración

espinocerebelar (ataxia de Friedreich y desórdenes relacionados).

V. Síndrome de falla de sistema nervioso autonómico central (síndrome Shy-Drager).

VI. Síndrome de debilidad muscular y emanación sin cambios sensoriales (enfermedad neuromotora tal como esclerosis lateral amiotrófica, atrofia muscular espinal (por ejemplo, atrofia muscular espinal infantil (Werdnig-Hoffman), atrofia muscular espinal juvenil (Wohlfart-Kugelberg-Welander) y otras formas de atrofia muscular espinal familiar), esclerosis lateral primaria, y paraplejia espástica hereditaria.

VII. Síndromes que combinan debilidad muscular y emanación con cambios sensoriales (atrofia muscular neural progresiva; polineuropatías familiares crónicas) tal como atrofia muscular perineal (Charcot-Marie-Tooth), polineuropatía intersticial hipertrófica (Dejerine-Sottas), y formas misceláneas de neuropatía progresiva crónica.

VIII. Síndromes de pérdida visual progresiva tal como degeneración pigmentaria de la retina (retinitis pigmentosa), y atrofia óptica hereditaria (enfermedad de Leber).

177

Terapia de Combinación

Los métodos de la presente invención también pueden comprender inicialmente administrar al sujeto un agente antitumor para dar a las células neoplásicas en el sujeto la resistencia y posteriormente administrar una cantidad efectiva de cualquiera de las composiciones de la presente invención, efectiva para inducir selectivamente la diferenciación terminal, detener el crecimiento celular y/o apoptosis de tales células, o tratar cáncer o suministrar quimioprevención.

El agente antitumor puede ser uno de numerosos agentes de quimioterapia tales como un agente alquilatante, un antimetabolito, un agente hormonal, un antibiótico, colchicina, un alcaloide vinca, L-asparaginasa, procarbazina, hidroxiurea, mitotano, nitrosoureas, o una carboxamida imidazol. Agentes adecuados son aquellos agentes que promueven la despolarización de tubulina. Preferiblemente el agente antitumor es colchicina o un alcaloide vinca; especialmente preferidos son la vinblastina y vincristina. En modalidades en donde el agente antitumor es vincristina, las células se tratan preferiblemente de tal forma que ellas son resistentes a la vincristina en una concentración de cerca de 5 mg/ml. El tratamiento de las células para darle resistencia a un agente antitumor se puede efectuar al poner en contacto

178

las células con el agente durante un periodo de por lo menos 3 a 5 días. El contacto de las células resultantes con cualquiera de los compuestos anteriores se desarrolla como se describió previamente. Adicionalmente a los agentes de quimioterapia anteriores, los compuestos también se pueden administrar juntos con terapia de radiación.

Dosis y Programación de Dosis

El régimen de dosis que utiliza los inhibidores HDAC se pueden seleccionar de acuerdo con una variedad de factores que incluyen tipo, especies, edad, peso, sexo y el tipo de cáncer a ser tratado; la severidad (es decir, etapa) del cáncer a ser tratado; la ruta de administración; la función hepática y renal del paciente; y el compuesto particular o sal de éste empleado. Un médico o veterinario medianamente versado en la materia puede fácilmente determinar y prescribir la cantidad efectiva de la droga requerida para tratar, por ejemplo, evitar, inhibir (completa o parcialmente) o detener el progreso de la enfermedad.

Dosis adecuadas son dosis diarias totales de entre cerca de 25-4000 mg/m^2 administrado oralmente una vez diariamente, dos veces diariamente o tres veces diariamente, continua (cada día) o intermitentemente (por ejemplo, 3-5 días por semana). Por ejemplo, SAHA o uno cualquiera de los inhibidores HDAC se

puede administrar en una dosis diaria total de hasta 800 mg. El inhibidor HDAC se puede administrar una vez diariamente (QD), o dividido en múltiples dosis diarias tal como dos veces diarias (BID), y tres veces diarias (TID). El inhibidor HDAC se puede administrar en una dosis diaria total de hasta 800 mg, por ejemplo, 150 mg, 200 mg, 300 mg, 400 mg, 600 mg, u 800 mg, que se pueden administrar en una dosis diaria o se puede dividir en múltiples dosis diarias como se describió anteriormente. Preferiblemente, la administración es oral.

En una modalidad, la composición se administra una vez diariamente en una dosis de cerca de 200-600 mg. En otra modalidad, la composición se administra dos veces diariamente en una dosis de cerca de 200-400 mg. En otra modalidad, la composición se administra dos veces diariamente en una dosis de cerca de 200-400 mg intermitentemente, por ejemplo tres, cuatro, o cinco días por semana. En otra modalidad, la composición se administra tres veces diariamente en una dosis de cerca de 100-250 mg.

En una modalidad, la dosis diaria es de 200 mg, que se puede administrar una vez diariamente, dos veces diariamente, o tres veces diariamente. En una modalidad, la dosis diaria es 300 mg, que se puede administrar una vez diariamente, dos veces diariamente, o tres veces diariamente. En una modalidad, la dosis diaria es de 400 mg, que se puede

administrar una vez diariamente o dos veces diariamente. En una modalidad, la dosis diaria es de 150 mg, que puede ser, administrada dos veces diariamente o tres veces diariamente.

Adicionalmente, la administración puede ser continua, es decir, cada día, o intermitentemente. Los términos "intermitente" o "intermitentemente" como se utiliza aquí significan detener e iniciar en intervalos regulares o irregulares. Por ejemplo, la administración intermitente de un inhibidor HDAC puede ser una administración de uno a seis días por semana o esta puede significar la administración en ciclos (por ejemplo, administración diaria durante dos a ocho semanas consecutivas, luego un periodo de descanso sin administración hasta una semana) o esta puede significar administración en días alternos.

El SAHA o cualquiera de los inhibidores HDAC se pueden administrar al paciente en un total de dosis diaria entre 25-4000 mg/m^2.

Un protocolo de tratamiento actualmente preferido comprende la administración continua (es decir, cada día), una vez, dos veces, o tres veces diariamente en una dosis diaria total en el rango de cerca de 200 mg a cerca de 600 mg.

Otro protocolo de tratamiento actualmente preferido comprende la administración intermitente de entre tres a cinco días por semana, una vez, dos veces o tres veces diariamente en un total de dosis diaria en el rango de cerca de 200 mg a cerca de 600 mg.

En una modalidad particular, el inhibidor HDAC se administra continuamente una vez diariamente en una dosis de 400 mg o dos veces diarias en una dosis de 200 mg.

En otra modalidad particular, el inhibidor HDAC se administra intermitentemente tres días por semana, una vez diaria en una dosis de 400 mg o dos veces diarias en una dosis de 200 mg.

En otra modalidad particular, el inhibidor HDAC se administra intermitentemente durante cuatro días por semana, una vez diaria en una dosis de 400 mg o dos veces diarias en una dosis de 200 mg.

En otra modalidad particular, el inhibidor HDAC se administra intermitentemente cinco días por semana, una vez diaria en una dosis de 400 mg o dos veces diarias en una dosis de 200 mg.

En otra modalidad particular, el inhibidor HDAC se administra continuamente una vez diaria en una dosis de 600 mg, dos

veces diarias en una dosis de 300 mg, o tres veces diarias en una dosis de 200 mg.

En otra modalidad particular, el inhibidor HDAC se administra intermitentemente tres días por semana, una vez diaria en una dosis de 600 mg, dos veces diarias en una dosis de 300 mg, o tres veces diarias en una dosis de 200 mg.

En otra modalidad particular, el inhibidor HDAC se administra intermitentemente cuatro días por semana, una vez diariamente en una dosis de 600 mg, dos veces diariamente en una dosis de 300 mg, o tres veces diariamente en una dosis de 200 mg.

En otra modalidad particular, el inhibidor HDAC se administra intermitentemente cinco días por semana, una vez diaria en una dosis de 600 mg, dos veces diarias en una dosis de 300 mg, o tres veces diarias en una dosis de 200 mg.

Adicionalmente, el inhibidor HDAC se puede administrar de acuerdo con cualquiera de los programas descritos anteriormente, consecutivamente durante unas pocas semanas, seguido por un periodo de descanso. Por ejemplo, el inhibidor HDAC se puede administrar de acuerdo con uno cualquiera de los programas descritos anteriormente de dos a ocho semanas, seguido por un periodo de descanso de una semana, o dos veces diarias en una dosis de 300 mg durante tres a cinco días por

183

semana. En otra modalidad particular, el inhibidor HDAC se administra tres veces diarias durante dos semanas consecutivas, seguido por una semana de descanso.

Es evidente para una persona experta en la técnica que varias dosis y programas de dosis descritos aquí se establecen únicamente como modalidades específicas y no se deben constituir como limitantes del amplio alcance de la invención. Cualquier permutación, variación y combinación de dosis y programas de dosis se incluyen dentro del alcance de la presente invención.

La presente invención suministra un régimen de dosis diario seguro de estas formulaciones, que es fácil de seguir y de adherir. Las formulaciones de la presente invención son por lo tanto útiles para inducir selectivamente la diferenciación terminal, detener el crecimiento celular, y/o apoptosis de células neoplásicas y por lo tanto ayudar en el tratamiento de tumores en pacientes.

Composiciones Farmacéuticas

Los compuestos de la invención, y derivados, fragmentos, análogos, homólogos, sales farmacéuticamente aceptables o hidratos de estos, se pueden incorporar en composiciones farmacéuticas adecuadas para administración oral, junto con

un portador farmacéuticamente aceptable o excipiente. Tales composiciones comprenden típicamente una cantidad y terapéuticamente efectiva de cualquiera de los compuestos anteriores, y un portador farmacéuticamente aceptable. Preferiblemente, la cantidad efectiva es una cantidad efectiva para inducir selectivamente la diferenciación terminal de células neoplásicas adecuadas y menos de una cantidad que origine toxicidad en un paciente.

Las composiciones de la presente invención se pueden formular en cualquier forma de dosis unitaria (líquida o sólida) adecuada para administración oral, por ejemplo, en la forma de una pastilla, una tableta, tableta recubierta, una cápsula, una cápsula de gelatina, una solución, una suspensión, o una dispersión. En una modalidad actualmente preferida, la composición está en la forma de una cápsula de gelatina.

Cualquier excipiente inerte que se utilice comúnmente como un portador o diluyente se puede utilizar en las formulaciones de la presente invención, tales como por ejemplo, una goma, un almidón, un azúcar, un material celulósico, un acrilato, o mezclas de estos. Un diluyente preferido es celulosa microcristalina. Las composiciones pueden comprender adicionalmente un agente desintegrante (por ejemplo, croscarmelosa sodio) y un lubricante (por ejemplo, estearato

de magnesio), y adicionalmente puede comprender uno o más aditivos seleccionados de un ligador, un amortiguador, un inhibidor de proteasa, un tensoactivo, un agente solubilizante, un plastificador, un emulsificador, un agente estabilizante, un agente que incrementa la viscosidad, un endulzante, un agente formador de película, o cualquier combinación de estos. Adicionalmente, las composiciones de la presente invención pueden estar en la forma de formulaciones de liberación inmediata o liberación controlada.

Una modalidad es una composición farmacéutica para administración oral que comprende un inhibidor HDAC o una sal farmacéuticamente aceptable o hidrato de ésta, celulosa microcristalina, croscarmelosa de sodio y estearato de magnesio. Otra modalidad tiene el SAHA como el inhibidor HDAC. Otra modalidad comprende 50-70% en peso de inhibidor HDAC o una sal farmacéuticamente aceptable o hidrato de ésta, 20-40% en peso de celulosa microcristalina, 5-15% en peso de croscarmelosa de sodio y 0.1-5% en peso de estearato de magnesio. Otra modalidad comprende cerca de 50-200 mg de un inhibidor HDAC.

En una modalidad, las composiciones farmacéuticas se administran oralmente, y se formulan así en una forma adecuada para administración oral, es decir, como un sólido o una preparación líquida. Las formulaciones orales sólidas

186

adecuadas incluyen tabletas, cápsulas, píldoras, gránulos, pastillas, y similares. Las formulaciones orales líquidas adecuadas incluyen soluciones, suspensiones, dispersiones, emulsiones, aceites, y similares. En una modalidad de la presente invención, la composición es formulada en una cápsula. De acuerdo con esta modalidad, las composiciones de la presente invención comprenden en adición al compuesto activo inhibidor HDAC y al portador inerte o diluyente, una cápsula de gelatina dura.

Como se utiliza aquí, "portador farmacéuticamente aceptable" se pretende incluir cualquiera y todos los solventes, medios de dispersión, recubrimientos, agentes antibacteriales y antifungosos, agentes retardantes de absorción e isotónicos, y similares, compatibles con la administración farmacéutica, tales como agua estéril libre de pirógenos. Portadores adecuados se describen en la edición más reciente de Remington's Pharmaceutical Sciences, un texto de referencia estándar en el campo, que se incorpora aquí como referencia. Ejemplos preferidos de tales portadores o diluyentes incluyen, pero no están limitados a, agua, salina, solución de Ringer, solución de dextrosa, y 5% de albúmina de suero humano. Los liposomas y vehículos no acuosos tales como aceites fijos también se pueden utilizar. El uso de tales medios y agentes para sustancias farmacéuticamente activas son bien conocidos en la técnica. Excepto en cuanto a

cualquier medio convencional o agente que es incompatible con el compuesto activo, el uso en las composiciones se contempla. Complementariamente los compuestos activos también se pueden incorporar en las composiciones.

Los diluyentes/portadores sólidos incluyen, pero no están limitados a, una goma, un almidón (por ejemplo, almidón de maíz, almidón pregelatinizado), un azúcar (por ejemplo, lactosa, manitol, sucrosa, dextrosa), un material celulósico (por ejemplo, celulosa microcristalina), un acrilato (por ejemplo, polimetilacrilato), carbonato de calcio, óxido de magnesio, talco, o mezclas de estos.

Para formulaciones líquidas, los portadores farmacéuticamente aceptables pueden ser soluciones acuosas o no acuosas, suspensiones, emulsiones, o aceites. Ejemplos de solventes no acuosos son propilenglicol, polietilenglicol, y ésteres orgánicos inyectables tales como etil oleato. Portadores acuosos incluyen agua, soluciones acuosas/alcohólicas, emulsiones, o suspensiones, que incluyen salina, y medios amortiguados. Ejemplos de aceite son aquellos de petróleo, animal, vegetal, o de origen sintético, por ejemplo, aceite de cacahuete, aceite de soya, aceite mineral, aceite de oliva, aceite de girasol, y aceite de hígado de pescado. Las soluciones o suspensiones también pueden incluir los siguientes componentes: un diluyente estéril tal como agua

para inyección, solución salina, aceites fijos, polietilenglicoles, glicerina, propilenglicol u otros solventes sintéticos; agentes antibacteriales tales como benzil alcohol o metil parabenos, antioxidantes tales como ácido ascórbico o bisulfito de sodio; agentes quelantes tales como ácido etilenodiaminatetraacético (EDTA), amortiguadores tales como acetatos, citratos o fosfatos, y agentes para el ajuste de tonicidad tales como cloruro de sodio o dextrosa. El pH se puede ajustar con ácidos o bases, tales como ácido clorhídrico o hidróxido de sodio.

Adicionalmente, la composición puede comprender además ligadores (por ejemplo, acacia, almidón de maíz, gelatina, carbomero, etil celulosa, goma guar, hidroxipropilcelulosa, hidroxipropilmetilcelulosa, povidona), agentes desintegrantes (por ejemplo, almidón de maíz, almidón de papa, ácido algínico, dióxido de silicio, croscarmelosa de sodio, crospovidona, goma guar, almidón de sodio, glicolato, Primogel), amortiguadores (por ejemplo, tris-HCI, acetato, fosfato) de varios pH y fuerza iónica, aditivos tales como albúmina o gelatina para evitar la absorción a las superficies, detergentes (por ejemplo, Tween 20, Tween 80, Plurónico F68, sales de ácido biliar), inhibidores de proteasa, tensoactivos (por ejemplo, sulfato de lauril sodio), mejoradores de permeación, agentes solubilizantes (por ejemplo, glicerol, polietilenglicerol), un glidante (por

ejemplo, dióxido de silicio coloidal), antioxidantes (por ejemplo, ácido ascórbico, metabisulfito de sodio, hidroxianisol butilado), estabilizantes (por ejemplo, hidroxipropil celulosa, hidroxipropilmetil celulosa), agentes que incrementan la viscosidad (por ejemplo, carbonero, dióxido de silicio coloidal, etil celulosa, goma guar), endulzantes (por ejemplo, sucrosa, aspartamo, ácido cítrico), agentes saborizantes (por ejemplo, hierbabuena, metil salicilato, o sabor de naranja), preservativos (por ejemplo, Timerosal, benzil alcohol, parabenos), lubricantes (por ejemplo, ácido esteárico, estearato de magnesio, polietilen glicol, lauril sulfato de sodio), ayudantes de flujo (por ejemplo, dióxido de silicio coloidal), plastificadores (por ejemplo, dietil ftalato, citrato de trietilo), emulsificadores (por ejemplo, carbómero, hidroxipropil celulosa, lauril sulfato de sodio), recubrimientos de polímero (por ejemplo, poloxámeros o poloxaminas), agentes de formación de película y recubrimiento (por ejemplo, etil celulosa, acrilatos, polimetacrilatos) y/o adyuvantes.

En una modalidad, los compuestos activos se preparan con portadores que protegerán el compuesto contra la rápida eliminación del cuerpo, tal como una formulación de liberación controlada, que incluye implantes y sistemas de administración microencapsulada. Se pueden utilizar polímeros biocompatibles, biodegradables, tal como vinil acetato de

etileno, polianhídridos, ácido poliglicólico, colágeno, poliortoésteres, y ácido poliláctico. Métodos para la preparación de tales formulaciones serán evidentes para aquellos expertos en la técnica. Los materiales también se pueden obtener comercialmente de Alza Corporation y Nova Pharmaceuticals, Inc. Las suspensiones liposomales (que incluyen liposomas objetivados para las células infectadas con anticuerpos monoclonales para antígenos virales) también se utilizan como portadores farmacéuticamente aceptables. Estos se pueden preparar de acuerdo con los métodos conocidos por aquellos expertos en la técnica, por ejemplo, como se describe en la Patente Estadounidense No. 4,522,811.

Es especialmente ventajoso formular composiciones orales en forma de dosis unitaria para facilidad de administración y uniformidad de dosis. La forma de dosis unitaria como se utiliza aquí se refiere a unidades físicamente discretas adaptadas como dosis unitarias para el sujeto a ser tratado, cada unidad contiene una cantidad predeterminada de compuesto activo calculada para producir el efecto terapéutico deseado en asociación con el portador farmacéuticamente requerido. La especificación para las formas de dosis unitaria de la invención se dictan directamente y dependiendo de las características únicas del compuesto activo y el efecto terapéutico particular a ser alcanzado, y las limitaciones

inherentes en la técnica de compuestos tal como un compuesto activo para el tratamiento de individuos.

Las composiciones farmacéuticas se pueden incluir en un contenedor, paquete, o dispensador junto con las instrucciones para administración.

La administración diaria se puede repetir continuamente durante un periodo de varios días a varios años. El tratamiento oral puede continuar entre una semana y la vida del paciente. Preferiblemente la administración tiene lugar durante cinco días consecutivos después de dicho tiempo el paciente se puede evaluar para determinar si se requiere administración adicional. La administración puede ser continua o intermitente, es decir, el tratamiento para un número de días consecutivos seguido por un periodo de descanso.

Los compuestos de la presente invención se pueden administrar intravenosamente en el primer día de tratamiento, con administración oral en el segundo día y todos los días consecutivos de ahí en adelante.

Los compuestos de la presente invención se pueden administrar para los propósitos de prevenir la progresión de la enfermedad o estabilizar el crecimiento de tumor.

La preparación de composiciones farmacéuticas que contienen un componente activo es bien entendida en la técnica, por ejemplo, al mezclar, granular, o procesos de formación de tabletas. El ingrediente terapéutico activo se mezcla a menudo con excipientes que son farmacéuticamente aceptables y compatibles con el ingrediente activo. Para administración oral, los agentes activos se mezclan con aditivos habituales para este propósito, tal como vehículos, estabilizantes, o diluyentes inertes, y convertido por métodos acostumbrados en formas adecuadas para administración, tal como tabletas, tabletas cubiertas, cápsulas de gelatina blanda o dura, suspensiones aceitosas, alcohólicas o acuosas, y similares como se detalló anteriormente.

La cantidad de compuesto administrado al paciente es menor que una cantidad que podría originar toxicidad en el paciente. En ciertas modalidades, la cantidad del compuesto que se administra al paciente es menor que la cantidad que origina la concentración del compuesto en el plasma del paciente o igual o excede el nivel tóxico del compuesto. Preferiblemente, la concentración del compuesto en el plasma del paciente se mantiene en cerca de 10 nM. En otra modalidad, la concentración del compuesto en el plasma del paciente se mantiene en cerca de 25 nM. En otra modalidad, la concentración del compuesto en el plasma del paciente se

193

mantiene en cerca de 50 nM. En otra modalidad, la concentración del compuesto en el plasma del paciente se mantiene en cerca de 100 nM. En otra modalidad, la concentración del compuesto en el plasma del paciente se mantiene en cerca de 500 nM. En otra modalidad, la concentración del compuesto en el plasma del paciente se mantiene en cerca de 1000 nM. En otra modalidad, la concentración del compuesto en el plasma del paciente se mantiene en cerca de 2500 nM. En otra modalidad, la concentración del compuesto en el plasma del paciente se mantiene en cerca de 5000 nM.

Se ha encontrado con el HMBA que la administración del compuesto en una cantidad de cerca de 5 gm/m^2/día a cerca de 30 gm/m^2/día, particularmente cerca de 20 gm/m^2/día, es efectiva sin producir toxicidad en el paciente. La cantidad óptima del compuesto que se debe administrar al paciente en la práctica de la presente invención dependerá del compuesto particular utilizado y el tipo de cáncer a ser tratado.

En una modalidad actualmente preferida de la presente invención, la composición farmacéutica comprende un inhibidor de desacetilasa histona (HDAC); celulosa microcristalina como un portador o diluyente; croscarmelosa de sodio como un desintegrante; y estearato de magnesio como un lubricante. En otra modalidad actualmente preferida, el inhibidor HDAC es

ácido suberoilanilida hidroxámico (SAHA). Otra modalidad actualmente preferida de la invención es una formulación sólida de SAHA con celulosa microcristalina, NF (Avicel pH 101), croscarmelosa de sodio, NF (AC-Di-Sol) y estearato de magnesio, NF, contenida en una cápsula de gelatina.

El porcentaje del ingrediente activo y varios excipientes puede variar en las formulaciones. Por ejemplo, la composición puede comprender entre 20 y 90%, preferiblemente entre 50-70% en peso de desacetilasa histona (HDAC). Adicionalmente, la composición puede comprender entre 10 y 70%, preferiblemente entre 20-40% en peso de celulosa microcristalina como un portador o diluyente. Adicionalmente, la composición puede comprender entre 1 y 30%, preferiblemente 1-15% en peso de croscarmelosa de sodio como un desintegrante. Adicionalmente, la composición puede comprender entre 0.1-5% en peso de estearato de magnesio como un lubricante. En otra modalidad preferida, la composición comprende cerca de 50-200 mg del inhibidor HDAC (por ejemplo, 50 mg, 100 mg y 200 mg para el inhibidor HDAC, por ejemplo, SAHA). En una modalidad particularmente preferida, la composición está en la forma de una cápsula de gelatina.

Una modalidad actualmente preferida es 200 mg de SAHA sólido con 89.5 mg de celulosa microcristalina, 9 mg de

croscarmelosa de sodio y 1.5 mg de estearato de magnesio contenida en una cápsula de gelatina.

Métodos *In Vitro*:

La presente invención también suministra métodos *in vitro* para inducir selectivamente la diferenciación terminal, detener el crecimiento celular y/o apoptosis de células neoplásicas, por ejemplo, células de linfoma, inhibiendo por lo tanto la proliferación de tales células, al poner en contacto las células con una cantidad efectiva de un inhibidor HDAC, por ejemplo, SAHA, o una sal farmacéuticamente aceptable o hidrato de ésta.

La presente invención también suministra métodos *in vitro* para inhibir la actividad de una desacetilasa histona, mediante la desacetilasa histona con una cantidad efectiva de un inhibidor HDAC, por ejemplo, SAHA, o una sal farmacéuticamente aceptable o hidrato de ésta.

Aunque los métodos de la presente invención se pueden practicar *in vitro*, se contempla que la modalidad preferida para los métodos de inducir selectivamente diferenciación terminal, detener el crecimiento celular y/o apoptosis de células neoplásicas, y de inhibir HDAC comprenderá poner en contacto las células *in vivo*, es decir, al administrar los

compuestos a un sujeto que posee células neoplásicas o células de tumor en necesidad de tratamiento.

Así, la presente invención también suministra métodos para inducir selectivamente diferenciación terminal, detener el crecimiento celular y/o apoptosis de células neoplásicas, por ejemplo, células de linfoma en un sujeto, inhibiendo por lo tanto la proliferación de tales células en dicho sujeto, al administrar al sujeto una composición farmacéutica que comprende una cantidad efectiva de un inhibidor HDAC, por ejemplo, SAHA, o una sal farmacéuticamente aceptable o hidrato de ésta, y un portador farmacéuticamente aceptable o diluyente. Una cantidad efectiva de un inhibidor HDAC en la presente invención puede ser de una dosis diaria total de 800 mg.

La presente invención también suministra métodos pata inhibir la actividad de una desacetilasa histona en un sujeto, al administrar al sujeto una composición farmacéutica que comprende una cantidad efectiva de un inhibidor HDAC, por ejemplo, SAHA, o una sal farmacéuticamente aceptable o hidrato de ésta, y un portador farmacéuticamente aceptable o diluyente. Una cantidad efectiva de un inhibidor HDAC en la presente invención puede ser de un total de dosis diaria de 800 mg.

197

EJEMPLOS

La invención se ilustra en los ejemplos que siguen. Esta sección se establece para ayudar en el entendimiento de la invención pero no se destina a, y no se debe constituir como limitante en ninguna forma de la invención como se establece en las reivindicaciones que siguen adelante.

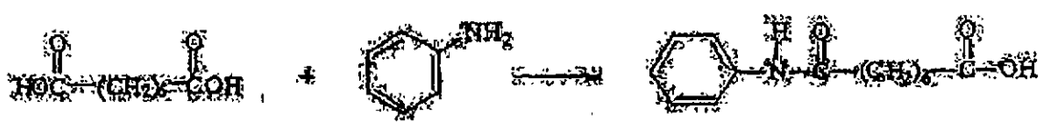
EJEMPLO 1

Síntesis de SAHA

El SAHA se puede sintetizar de acuerdo con el método destacado adelante, o de acuerdo con el método establecido en la Patente Estadounidense 5,369,108, el contenido de la cual se incorpora aquí en su totalidad como referencia, o de acuerdo con cualquier otro método.

Síntesis de SAHA

Etapa 1 - Síntesis de ácido Suberanílico



198

En un frasco de 22 L se coloca 3500 g (20.09 moles) de ácido subérico, y el ácido se funde con calor. La temperatura se eleva a 175° C, y luego se agrega 2040 g (21.92 moles) de anilina. La temperatura se eleva a 190° C y se mantiene en esa temperatura durante 20 minutos. La mezcla fundida se vierte en un tanque de Nalgeno que contiene 4017 g de hidróxido de potasio disuelto en 50 L de agua. La mezcla se agita durante 20 minutos luego de la adición de la fusión. La reacción se repite en la misma escala y la segunda fusión se vierte en la misma solución de hidróxido de potasio. Después la mezcla se agita vigorosamente, el agitador se apaga, y la mezcla se le permite decantarse. La mezcla luego se filtra a través de una almohadilla de Celita (4200 g) (el producto se filtra para remover los sub-productos neutrales (de ataque por anilina en ambos extremos del ácido subérico). El filtrado contenía la sal del producto, y también la sal de ácido subérico sin reacción. La mezcla se le permitió decantarse debido a que la filtración fue muy lenta, tomando varios días). El filtrado se acidificó utilizando 5 L de ácido clorhídrico concentrado; la mezcla se agitó durante una hora, y entonces se le permitió decantarse durante la noche. El producto se recolectó por filtración, y se lavo en un embudo con agua desionizada (4 x 5 L). La torta de filtro húmeda se coloca en un frasco de 72 L con 44 L de agua desionizada, la mezcla se calienta a 50° C, y el sólido aislado mediante una filtración caliente (el producto deseado

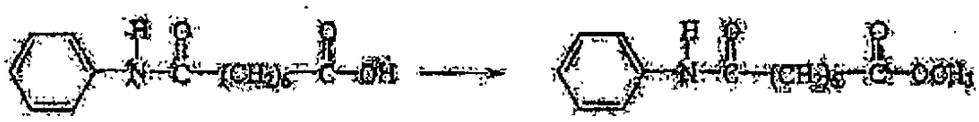
199

se contamina con ácido subérico que tiene mayor solubilidad en agua caliente. Varias titulaciones calientes se hicieron para remover el ácido subérico. El producto se revisó mediante RMN [D_6DMSO] para monitorear la remoción de ácido subérico). La titulación caliente se repitió con 44 L de agua a 50° C. El producto se aíslo de nuevo mediante filtración, y se enjuaga con 4 L de agua caliente. Se seca durante el fin de semana en un horno de vacío a 65° C que utiliza una bomba Nash como la fuente de vacío (la bomba Nash es una bomba de anillo líquido (agua) y producen un vacío de cerca de 29 pulgadas de mercurio. Se utiliza una purga de argón intermitente para ayudar a sacar el agua); se obtuvo 4182.8 g de ácido suberanílico.

El producto todavía contenía una pequeña cantidad de ácido subérico, por lo tanto la titulación caliente se hizo en forma de porciones a 65° C, utilizando cerca de 300 g de producto por una vez. Cada porción se filtra, se enjuaga vigorosamente con agua caliente adicional (un total de cerca de 6 L). Esto se repitió para purificar la tanda entera. El ácido subérico se removió completamente del producto. El producto sólido se combina en un frasco y se agita con 6 L de metanol/agua (1:2), y luego se aísla mediante filtración y se seca al aire en el filtro durante un fin de semana. Se coloca en bandejas y se seca en un horno de vacío a 65° C durante 45 horas utilizando la bomba Nash y una purga de argón. El

producto final tiene un peso de 3278.4 g (32.7% de rendimiento).

Etapa 2 - síntesis de Suberanilato de Metilo



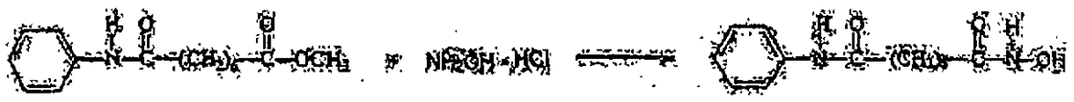
A un frasco de 50 L apretado con un agitador mecánico, y un condensador se colocan 3229 g de ácido suberanílico de la etapa anterior, 20 L de metanol, y 398.7 g de resina Dowex 50WX2-400. La mezcla se calienta hasta reflujo y se mantiene en reflujo durante 18 horas. La mezcla se filtra para remover las gotas de resina, y el filtrado se saca para un residuo sobre un evaporador rotatorio.

El residuo del evaporador rotatorio se transfiere en un frasco de 50 L ajustado con un condensador y agitador mecánico. Al frasco se agregan 6 L de metanol, y la mezcla se calienta para dar una solución. Luego se agregan 2 L de agua desionizada, y se apaga el calor. La mezcla agitada se permite enfriar, y luego se coloca el frasco en un baño de hielo, y se enfría la mezcla. El producto sólido se aísla por filtración, y la torta de filtro se enjuaga con 4 L de agua/metanol frío (1:1). El producto se seca a 45° C en un

201

horno de vacío que utiliza una bomba Nash para un total de 64 horas para dar 2850.2 g (84% de rendimiento) de suberanilato de metilo, CSL Lote # 98-794-92-3 1.

Etapa 3 - Síntesis de SAHA Crudo



A un frasco de 50 L con un agitador mecánico, termopar, y entrada para atmósfera inerte se agrega 1451.9 g de clorhidrato de hidroxilamina, 19 L de metanol anhidro, y 3.93 L de una solución de metóxido de sodio al 30% en metanol. El frasco se carga luego con 2748.0 g de suberanilato de metilo, seguido por 1.9 L de solución de metóxido de sodio al 30% en metanol. A la mezcla se le permite agitarse durante 16 horas y 10 minutos. Aproximadamente la mitad de la mezcla de reacción se transfiere del frasco de reacción (frasco 1) a un frasco de 50 L (frasco 2) ajustado con un agitador mecánico. Luego 27 L de agua desionizada se agregan al frasco 1 y la mezcla se agita durante 10 minutos. El pH se toma utilizando un pH metro; el pH era de 11.56. El pH de la mezcla se ajusta a 12.02 mediante la adición de 100 mL de solución de metóxido de sodio al 30% en metanol, esto da una solución clara (la mezcla de reacción en este tiempo contiene una pequeña cantidad de sólido. El pH se ajusta para dar una solución

202

clara de la cual la precipitación del producto se precipitaría). La mezcla de reacción en el frasco 2 se diluyó de la misma forma; 27 L de agua desionizada se agrega, y el pH se ajusta por la adición de 100 mL de solución de metóxido de sodio al 30% a la mezcla, para dar un pH de 12.01 (solución clara).

La mezcla de reacción en cada frasco se acidifica mediante la adición de ácido acético glacial para precipitar el producto. El frasco 1 tiene un pH final de 8.98, y el frasco 2 tiene un pH final de 8.70. El producto de ambos frascos se aísla mediante filtración utilizando un embudo Buchner y un filtro de tela. La torta de filtro se lava con 15 L de agua desionizada, y el embudo se cubre y el producto se seca parcialmente en el embudo bajo vacío durante 15.5 hr. El producto se remueve y se coloca en cinco bandejas de vidrio. Las bandejas se colocan en un horno de vacío y el producto se seca para peso constante. El primer periodo de secado era de 22 horas a 60° C utilizando una bomba Nash como la bomba fuente con una purga de argón. Las bandejas se remueven del horno de vacío y se pesan. Las bandejas se regresan al horno y el producto se seca durante 4 horas adicionales y 10 minutos utilizando una bomba de aceite como la bomba fuente y sin purga de argón. El material se empaca en bolsas de polietileno de 4 mill, y se colocan en un contenedor exterior

plástico. El peso final después de muestreo es 2633.4 g (95.6%).

Etapa 4 - Recristalización de SAHA Crudo

El SAHA crudo se recristaliza a partir de metanol/agua. Un frasco de 50 L con un agitador mecánico, termopar, condensador, y entrada para atmósfera inerte se carga con el SAHA crudo para ser cristalizado (2575.7 g), seguido por 2625 ml de agua desionizada y 15755 ml de metanol. El material se calienta hasta reflujo para dar una solución. Luego se agrega 5250 ml de agua desionizada a la mezcla de reacción. El calor se paga y a la mezcla se le permite enfriar. Cuando la mezcla se ha enfriado suficientemente de tal forma que el frasco se puede manejar en forma segura (28° C), el frasco se remueve del manto de calor, y se coloca en tubo para uso como una tanda de enfriamiento. Se agrega hielo/agua al tubo para enfriar la mezcla a -5° C. La mezcla se mantiene por debajo de esa temperatura durante 2 horas. El producto se aísla mediante filtración, y la torta de filtro se lava con 1.5 L de agua/metanol frío (2:1). El embudo se cubre, y el producto se seca parcialmente bajo vacío durante 1.75 hr. El producto se remueve del embudo y se coloca en 6 bandejas de vidrio. Las bandejas se colocan en un horno de vacío, y el producto se seca durante 64.75 hr a 60° C utilizando una bomba Nash como la bomba fuente, y utilizando una purga de argón. Las

204

bandejas se remueven para pesar, y luego se regresan al horno y se secan durante 4 horas adicionales a 60° C para dar un peso constante. La fuente de vacío para el segundo periodo de secado fue una bomba de aceite, y no se utilizó purga de argón. El material se empacó en bolsas de polietileno de 4-mill dobles, y se colocó en un contenedor exterior plástico. El peso final después de muestreo fue de 2540.9 g (92.5%).

EJEMPLO 2

Dosificación oral de ácido hidroxámico suberoilanilida (SAHA)

Antecedente: El tratamiento con agentes de diferenciación celular polar híbrida ha resultado en la inhibición del crecimiento de líneas de células derivadas de tumor sólido humano y xenoinjertos. El efecto se media en parte por la inhibición de desacetilasa histona. El SAHA es un potente inhibidor de desacetilasa histona que se ha mostrado por tener la capacidad de inducir detención de crecimiento de célula de tumor, diferenciación, y apoptosis en el laboratorio y en estudios preclínicos.

Objetivos: Definir un régimen oral diario seguro de SAHA que se puede utilizar en estudios de Fase II. Adicionalmente, el perfil farmacocinético de la formulación oral de SAHA se evalúa. La biodisponibilidad oral del SAHA en humanos en el

estado rápido vs. no rápido y efecto anti-tumor de tratamiento también se monitorea. Adicionalmente, los efectos biológicos del SAHA de tejidos normales y de células de tumor se evalúa y la respuestas con respecto a los niveles de acetilación de histona se documentan.

Pacientes: Los pacientes con tumores sólidos adultos metastáticos o primarios, de etapa avanzada histológicamente documentada que son refractarios para la terapia estándar o para los cuales no existe terapia estándar curativa. Los pacientes deben tener un Estatus de Desempeño Karnofsky de ≥70%, y función renal, hepática y hematológica adecuada. Los pacientes deben estar al menos cuatro semanas sin ninguna quimioterapia anterior, terapia de radiación u otras drogas anti-cáncer en investigación.

Programa de Dosificación: En el primer día, los pacientes se tratan primero con 200 mg de SAHA administrado intravenosamente. Partiendo en el segundo día, los pacientes se tratan con dosis diarias de SAHA de acuerdo con la Tabla 1. Cada cohorte recibe una dosis diferente de SAHA. "QD" indica la dosificación una vez al día; "Q12 horas" indica dosificación dos veces al día. Por ejemplo, los pacientes en Cohorte IV reciben dos dosis de 800 mg de SAHA por día. Las dosis se administran a pacientes diaria y continuamente. Se

tomas muestras de sangre en el día uno y en el día 21 del tratamiento oral. Los pacientes se retiran de tratamiento SAHA oral debido a la progresión de la enfermedad, regresión de tumor, efectos colaterales inaceptables, o tratamiento con otras terapias.

Tabla 1: Programa de Dosis SAHA Oral

Cohorte	Dosis Oral (mg)	Número de Días	Programa de Dosis Diaria
I	200	Continua	QD
II	400	Continua	QD
III	400	Continua	Q12 horas
IV	800	Continua	Q12 horas
V	1200	Continua	Q12 horas
VI	1600	Continua	Q12 horas
VII	2000	Continua	Q12 horas

Resultados: La comparación de los niveles de plasma de suero muestran alta biodisponibilidad de SAHA administrado oralmente, cuando el paciente fue rápido y cuando el paciente no fue rápido, comparado con el SAHA administrado intravenosamente (SAHA IV). "AUC" es un estimado de la biodisponibilidad de SAHA en (ng/ml)min, en donde 660 ng/ml es igual a 2.5 μM de SAHA. El AUC tomado junto con la vida media ($T_{1/2}$) muestra que la biodisponibilidad general del SAHA oral es mejor que el SAHA IV. El C_{max} es la concentración máxima de SAHA observada después de administración. El SAHA

IV se administra en 200 mg de infusión durante dos horas. El SAHA oral se administra en una cápsula sencilla de 200 mg. Las Tablas 2 y 3 resumen los resultados de un ensayo HPLC (LCMS que utiliza un estándar deuterizado) que cuantifica la cantidad de SAHA en el plasma sanguíneo de los pacientes versus el tiempo, utilizando histona-4 acetilada (α-AcH4) como un marcador.

Tabla 2: Niveles de Plasma de Suero de SAHA Oral - Paciente #1

	IV	Oral (rápido)	Oral (no rápido)
C_{max} (ng/ml)	1329	225	328
$T_{1/2}$ (min)	20	80	64
AUC (ng/ml)min	153000	25000	59000

Tabla 3: Niveles de Plasma de Suero de SAHA Oral - Paciente #2

	IV	Oral (rápido)	Oral (no rápido)
C_{max} (ng/ml)	1003	362	302
$T_{1/2}$ (min)	21	82	93
AUC (ng/ml)min	108130	63114	59874

Las Figuras 1 a 8 son láminas de HPLC que muestran la cantidad de α-AcH4 en pacientes en Cohortes I y II, medidas hasta 10 horas después de recibir la dosis oral, comparado con los niveles α-AcH4 cuando se administra intravenosamente

SAHA. La Figura 9 muestra la concentración de plasma media de SAHA (ng/ml) en los puntos de tiempo indicados luego de administración. La Fig. 9A: Dosis oral (200 mg y 400 mg) bajo acción rápida en el Día 8. La Fig. 9B Dosis Oral (200 mg y 400 mg) con alimentación en el Día 9. Fig. 9C: Dosis IV en el Día 1. La Fig. 10 muestra la vida media aparente de un SAHA de 200 mg y 400 mg de dosis oral, en los Días 8, 9 y 22. La Fig. 11 muestra el AUC (ng/ml/hr) de un SAHA de 200 mg y 400 mg de dosis oral, en los Días 8, 9 y 22. La Fig. 12 muestra la biodisponibilidad de SAHA después de una dosis oral de 200 mg y 400 mg en los días 8, 9 y 22.

EJEMPLO 3

Dosis oral de ácido suberoilanilida hidroxámico (SAHA) - Escalada de Dosis

En otro experimento, veinticinco pacientes con tumores sólidos que se han enrolado en el brazo A, trece pacientes con linfoma de Hodgkin o no-Hodgkin se han enrolado en el brazo B, y un paciente con leucemia aguda y un paciente con síndrome mielodisplásico se han enrolado en el brazo C, como se muestra en la Tabla 4.

209

Tabla 4: Esquema de Escalada de Dosis y Número de Pacientes en Cada Nivel de Dosis

Cohorte	Dosis (mg/día)	Programa de Dosis	#Días de Dosis	Periodo de descanso	#Pacientes Encontrados (Brazo A/brazo B/brazo C)*
I	200	Una vez al día	Continua	Ninguno	6/0/0
II	400	Una vez al día	Continua	Ninguno	5/4/2
III	400	Cada 12 horas	Continua	Ninguno	6/3/0
IV	600	Una vez al día	Continua	Ninguno	4/3/0
V	200	Cada 12 horas	Continua	Ninguno	4/3/0
VI	300	Cada 12 horas	Continua	Ninguno	-/-/-
					Subtotal: 25/13/2
					Total = 40

*Brazo A= tumor sólido, brazo B= linfoma, brazo C= leucemia

Resultados:

Entre los once pacientes tratados en la Cohorte II, un paciente experimenta el DLT de diarrea grado 3 y deshidratación grado 3 durante el primer ciclo de tratamiento. Nueve pacientes se encontraron en la Cohorte III. Dos pacientes no se evaluaron para la evaluación de toxicidad en el Día 28 en razón a su temprana terminación de estudio debido a la rápida progresión de la enfermedad. De los siete pacientes restantes, cinco experimentaron DLT durante el primer ciclo de tratamiento: diarrea/deshidratación (n=1), fatiga/deshidratación (n=1),

anorexia (n=1), deshidratación (n=1) y anorexia/deshidratación (n=1). Estos cinco pacientes se recuperaron en aproximadamente una semana después de que se suspendió el estudio de la droga. A ellos se les redujo posteriormente la dosis a 400 mg QD, que parece ser bien tolerado. Los días medianos en 400 mg BID para todos los pacientes en la Cohorte III fue de 21 días. Basado en estos hallazgos el programa de dosificación de cada 12 horas de 400 mg se juzgó que ha excedido el máximo de dosis tolerado. Siguiendo el protocolo corregido, se continuó la acumulación en la cohorte IV en una dosis de 600 mg una vez al día. De estos siete pacientes enlistados en la cohorte IV, dos no se evaluaron para evaluación de toxicidad en el día 28 debido a la terminación anticipada del estudio debido a la rápida progresión de la enfermedad. Tres pacientes experimentaron DLT durante el primer ciclo de tratamiento: anorexia/deshidratación/fatiga (n=1), y diarrea/deshidratación (n=2). La dosis de 600 mg se juzgo por lo tanto que excede la dosis máxima tolerada y una dosis de 400 mg al día se definió como la dosis máxima tolerada para una vez diaria de administración oral. El protocolo se corrigió para evaluar los niveles de dosis adicionales de la programación de dosificación de dos veces al día en 200 mg de BID y 300 mg de BID administrado continuamente.

El análisis farmacocinético provisional se basa en 18 pacientes tratados con niveles de dosis de 200 mg QD, 400 mg QD, y 400 mg BID. En general, los estimados medios de C_{max} y AUC_{inf} de SAHA administrado oralmente bajo condiciones de rapidez o con alimento incrementado proporcionalmente con dosis en los rangos de 200 mg a 400 mg. En general, la fracción de AUC_{inf} debido a la explotación fue de 1% o menos. Los estimados medios para la vida media aparente fueron variables a través de grupos de dosis bajo condiciones rápidas o con alimento, variando de 65 a 114 minutos. Los estimados de la media de C_{max}, varían de 233 ng/ml (0.88 μM) a 570 ng/ml (2.3 μM). La fracción biodisponible de SAHA, calculada de los valores AUC_{inf} después de la infusión IV y la ruta oral, se encontró que es de aproximadamente 0.48.

Las células mononucleares de sangre periférica se recolectaron pre-terapia, inmediatamente luego de la infusión y entre 2-10 horas después de ingestión oral de cápsulas de SAHA para evaluar en efecto de SAHA sobre el alcance de la acetilación de histona en una célula huésped normal. Las histonas se aislaron y se probaron con anticuerpo histona anti-acetilada (H3) seguido por anticuerpo HRP secundario. El análisis preliminar demostró un incremento en la acumulación de histonas acetiladas en células mononucleares periféricas

212

que se pueden detectar hasta 10 horas después de ingestión de cápsulas de SAHA a 400 mg por nivel de dosis diario.

Trece pacientes continúan el tratamiento durante 3-12 meses con respuesta o enfermedad estable: tiroide (n=3), glándula sudorípara (n=1), renal (n=2), laringe (n=1), próstata (n=1), linfoma de Hodgkin (n=2), linfoma no-Hodgkin (n=2), y leucemia (n=1).

Seis pacientes tuvieron retracción de tumor en exploraciones CT. Tres de estos seis pacientes presentan el criterio de respuesta parcial (un paciente con cáncer de laringe metastático y dos pacientes con linfoma no-Hodgkin). Estas respuestas parciales ocurren a niveles de dosis de 400 mg BID (n=2) y 600 mg QD (n=1).

Las dosis descritas anteriormente también se han administrado dos veces diariamente en forma intermitente. Los pacientes han recibido SAHA dos veces diarias tres a cinco días por semana. La respuesta de los pacientes se ha visto con la administración de SAHA dos veces diarias en 300 mg durante tres días por semana.

EJEMPLO 4

Dosificación Intravenosa de SAHA

213

La Tabla 5 muestra un programa de dosificación para pacientes que reciben SAHA en forma intravenosa. Los pacientes empiezan en la Cohorte I, recibiendo 300 mg/m^2 de SAHA durante cinco días consecutivos en una semana durante una semana, para una dosis total de 1500 mg/m^2. Los pacientes se observaron luego para un periodo de dos semanas y se continuó a la Cohorte II, entonces se progresa a través de Cohortes a menos que se termine el tratamiento debido a progresión de la enfermedad, la regresión del tumor, efectos colaterales inaceptables o que el paciente reciba otro tratamiento.

Tabla 5: Escalamiento de la Dosis Estándar para SAHA Administrado Intravenosamente

Cohorte	Dosis (mg/m^2)	Número de Días/Semana	Número de Semanas Consecutivas	Periodo de Observación (Semanas)	Dosis Total (mg/m^2)
I	300	5	1	2	1500
II	300	5	2	2	3000
III	300	5	3	1*	4500
IV	600	5	3	1*	9000
V	800	5	3	1*	13500
VI	1200	5	3	1*	18000
VII	1500	5	3	1*	22500

*Pacientes hematológicos iniciaron en el nivel de dosis III.

EJEMPLO 5

Tratamiento de Mesotelioma con SAHA

214

Tres pacientes con mesotelioma se enlistaron en los estudios de Fase I con SAHA. A los pacientes se les administró SAHA oral dos veces al día en una dosis de 300 mg o 400 mg durante tres días por semana. Una respuesta parcial se observó luego de tratamiento con SAHA de acuerdo con el régimen anterior durante 6 meses.

La Figura 13 es una exploración CT de un tumor mesotelioma de un paciente, antes (PRE-panel izquierdo) y después (POST-panel derecho) de tratamiento con SAHA dos veces diarias en una dosis de 300 mg tres días por semana durante 6 meses. Los datos demuestran que el SAHA es efectivo en el tratamientote tumores de mesotelioma en pacientes.

EJEMPLO 6

Tratamiento de Linfoma de Célula B Grande Difusa (DLBCL) con SAHA

Se condujo un estudio de Fase I de SAHA oral en sesenta y ocho pacientes con cáncer avanzado que incluyen cáncer hematológico y tumores sólidos. Los pacientes reciben SAHA en forma oral (po) 200, 400 o 600 mg QD diario, 200, 300 o 400 mg BID diario, 300 mg o 400 mg (BID) intermitentemente 3 días por semana, o 100 mg TID (2 semanas). Siete pacientes con

215

Linfoma de Célula B grande Difusa (DLBCL) se enlistaron en el estudio.

Resultados:

A. Respuesta Completa (CR) a Tratamiento SAHA oral:

Un paciente, una de 66 años de edad, se diagnosticó con linfoma linfocítico pequeño etapa I (plasmacitoide); recibe bleomicina, CPT y XRT local con respuesta completa; desarrolló enfermedad recurrente (seno/nódulo subcutáneo, y nódulos de pulmón) y se trató con Fludarabina/mitoxantrona, rituximab, CEPT, doxorubicina liposomal. Se transformó a DBLCL, posteriormente tratado con Rituximab, Anti-B1, CTX/doxorubicina liposomal/pred/vincristina.

El paciente se refirió a SAHA oral Fase I con nódulos subcutáneos, adenopatía difusa, masa gastrohepática grande, nódulos de pulmón bilateral, involucramiento de la médula ósea por linfoma. El paciente recibe 400 mg BID de SAHA durante 1 mes, se redujo la dosis posteriormente a 400 mg QD, y se trató con SAHA durante un periodo total de 1 año.

La Figura 14 es una exploración CT tomada antes (Figura 14A) y después (Figura 14B) de dos meses de tratamiento con SAHA demostrando la retracción de la masa gastrohepática. Una

resolución completa de la masa gastrohepática se observó después de dos ciclos de tratamiento (4 meses).

La Figura 15 es una exploración PET tomada antes (Figura 15A) y después (Figura 15B) de dos meses de tratamiento con SAHA, demostrando la regresión de tumor luego del tratamiento.

Una respuesta completa (CR) (que cumple los Criterios de Cheson) se alcanza luego de 4 meses de tratamiento SAHA. La respuesta completa (CR) ha durado 14 meses, y el paciente todavía está en CR.

B. Respuesta Parcial (PR) a Tratamiento SAHA oral:

Un paciente con DLBCL recibió 600 mg QD de SAHA oralmente, y estuvo en tratamiento con SAHA durante un periodo total de cinco meses.

La Figura 16 es una exploración CT tomada antes (16A) y después (Figura 16B) de 1 mes de tratamiento con SAHA, lo que demuestra la retracción del tumor luego del tratamiento.

Una respuesta parcial (RR) (que cumple los Criterios Cheson) se alcanza luego de 5 meses de tratamiento SAHA.

C. Regresión de Tumor luego de tratamiento SAHA oral:

Un paciente, una mujer de 75 años de edad, presentada inicialmente con linfoma folicular con evidencia de transformación. Ella se trató originalmente con 6 ciclos de ciclofosfamida, doxorubicina, etoposida, y prednisona. Ella se sometió entonces a una esplenectomía que mostró DLBCL, para el que ella se trató con Zevalin. Ella recibe posteriormente dos series de Rituximab y eventualmente pentostatina, ciclofosfamida y rituximab.

El paciente recibe 200 mg BID de SAHA oralmente, y el tratamiento con SAHA fue de seis meses.

La Figura 17 es una exploración PET tomada antes (Figura 17A) y después (Figura 17B) de dos meses de tratamiento con SAHA. Como se ve, el paciente alcanza una respuesta PET negativa excelente después de 2 meses en SAHA con reducción continuada en su enfermedad.

Aunque esta invención se ha mostrado y descrito particularmente con referencia a las modalidades preferidas de ésta, se entenderá por aquellos expertos en la técnica que varios cambios en la forma y detalles se pueden hacer en esta sin apartarse del significado de la invención descrita. El alcance de la invención incluye la materia objeto de las reivindicaciones que siguen. Todas las patentes, solicitudes

de patente, y publicaciones citadas aquí se incorporan como referencia.

Referencias

1. Sporn, M. B., Roberts, A. B., y Driscoll, J. S. (1985) in Cancer: Principles and Practice of Oncology, eds. Hellman, S., et al., Ed. 2, (J. B. Lippincott, Philadelphia), P. 49.

2. Breitman, T. R., Selonick, S. E., y Collins, S. J. (1980) Proc. Natl. Acad. Sci. USA 77: 2936-2940.

3. Olsson, I. L. y Breitman, T. R. (1982) Cancer Res. 42: 3924-3927.

4. Schwartz, E. L. y Sartorelli, A. C. (1982) Cancer Res. 42: 2651-2655.

5. Marks, P. A., Sheffery, M., y Rifkind, R. A. (1987) Cancer Res. 47: 659.

6. Sachs, L. (1978) Nature (Lond.) 274: 535.

7. Friend, C., Scher, W., Holland, J. W., y Sato, T. (1971) Proc. Natl. Acad. Sci. (USA) 68: 378-382.

8. Tanaka, M., Levy, J., Terada, M., Breslow, R., Rifkind, R. A., y Marks, P. A. (1975) Proc. Natl. Acad. Sci. (USA) 72: 1003-1006.

9. Reuben, R. C., Wife, R. L., Breslow, R., Rificind, R. A., y Marks, P. A. (1976) Proc. Natl. Acad. Sci. (USA) 73: 862-866.

10. Abe, E., Miyaura, C., Sakagami, H., Takeda, M., Konno, K., Yamazaki, T., Yoshika, S., y Suda, T. (1981) Proc. Natl. Acad. Sci. (USA) 78: 4990-4994.

11. Schwartz, E. L., Snoddy, J. R., Kreutter, D., Rasmussen, H., y Sartorelli, A. C. (1983) Proc. Am. Assoc. Cancer Res. 24: 18:

12. Tanenaga, K., Hozumi, M., y Sakagami, Y. (1980) Cancer Res. 40: 914-919.

13. Lotem, J. y Sachs, L. (1975) Int. J. Cancer 15: 731-740.

14. Metcalf, D. (1985) Science, 229: 16-22.

15. Scher, W., Scher, B. M., y Waxman, S. (1983) Exp. Hematol. 11: 490-498.

16. Scher, W., Scher, B. M., y Waxman, S. (1982) Biochem. & Biophys. Res. Comm. 109: 348-354.

17. Huberman, E. and Callaham, M. F. (1979) Proc. Natl. Acad. Sci. (USA) 76: 1293-1297.

18. Lottem, J. y Sachs, L. (1979) Proc. Natl. Acad. Sci. (USA) 76: 5158-5162.

19. Terada, M., Epner, E., Nudel, U., Salmon, J., Fibach, E., RiRind, R. A., y Marks, P. A. (1978) Proc. Natl. Acad. Sci. (USA) 75: 2795-2799.

20. Morin, M. J. y Sartorelli, A. C. (1984) Cancer Res. 44: 2807-2812.

21. Schwartz, E. L., Brown, B. J., Nierenberg, M., Marsh, J. C., y Sartorelli, A. C. (1983) Cancer Res. 43: 2725-2730.

22. Sugano, H., Furusawa, M., Kawaguchi, T., y Ikawa, Y. (1973) Bibl. Hematol. 39: 943-954.

23. Ebert, P. S., Wars, I., y Buell, D. N. (1976) Cancer Res. 36: 1809-1813.

24. Hayashi, M., Okabe, J., y Hozumi, M. (1979) Gann 70: 235-238.

25. Fibach, E., Reuben, R. C., Rifkind, R. A., y Marks, P. A. (1977) Cancer Res. 37: 440-444.

26. Melloni, E., Pontremoli, S., Damiani, G., Viotti, P., Weich, N., Rifkind, R. A., y Marks, P. A. (1988) Proc. Natl. Acad. Sci. (USA) 85: 3835-3839.

27. Reuben, R., Khanna, P. L., Gazitt, Y., Breslow, R., Rifkind, R. A., y Marks, P. A. (1978) J. Biol. Chem. 253: 4214-4218.

28. Marks, P. A. y Rifkind, R. A. (1988) Int. Journal of Cell Cloning 6: 230-240.

29. Melloni, E., Pontremoli, S., Michetti, M., Sacco, O., Cakiroglu, A. G., Jackson, J. F., Rifkind, R. A., y Marks, P. A. (1987) Proc. Natl. Acad. Sciences (USA) 84: 5282-5286.

30. Marks, P. A. y Rifkind, R. A. (1984) Cancer 54: 2766-2769.

31. Egorin, M. J., Sigman, L. M. VanEcho, D. A., Forrest, A., Whitacre, M. Y., y Aisner, J. (1987) Cancer. Res. 47: 617-623.

32. Rowinsky, E. W., Ettinger, D. S., Grochow, L. B., Brundrett, R. B., Cates, A. E., y Donehower, R. C. (1986) J. Clin. Oncol. 4: 1835-1844.

33. Rowinsky, E. L. Ettinger, D. S., McGuire, W. P., Noe, D. A., Grochow, L. B., y Donehower, R. C. (1987) Cancer Res. 47: 5788-5795.

34. Callery, P. S., Egorin, M. J., Geelhaar, L. A., y Nayer, M. S. B. (1986) Cancer Res. 46: 4900-4903.

35. Young, C. W. Fanucchi, M. P., Walsh, T. B., Blatzer, L., Yaldaie, S., Stevens, Y. W., Gordon, C., Tong, W., Rifkind, R. A., y Marks, P. A. (1988) Cancer Res. 48: 7304-7309.

36. Andreeff, M., Young, C., Clarkson, B., Fetten, J., Rifkind, R. A., y Marks, P. A. (1988) Blood 72: 186a.

37. Marks, P. A., Breslow, R., Rifkind, R. A., Ngo, L., y Singh, R. (1989) Proc. Natl. Acad. Sci. (USA) 86: 6358-6362.

38. Breslow, R., Jursic, B., Yan, Z. F., Friedman, E., Leng, L., Ngo, L., Rifkind, R. A., y Marks, P. A. (1991) Proc. Natl. Acad. Sci. (USA) 88: 5542-5546.

39. Richon, V. M., Webb, Y., Merger, R., et al. (1996) PNAS 93: 5705-8.

222

40. Cohen, L. A., Amin, S., Marks, P. A., Rifkind, R. A., Desai, D., y Richon, V. M. (1999) Anticancer Research 19: 4999-5006.

41. Grunstein, M. (1997) Nature 389: 349-52.

42. Finnin, M. S., Donigian, J. R., Cohen, A., et al. (1999) Nature 401: 188-193.

43. Van Lint, C., Emiliani, S., Verdin, E. (1996) Gene Expression 5: 245-53.

44. Archer, S. Shufen, M. Shei, A., Hodin, R. (1998) PNAS 95: 6791-96.

45. Dressel, U., et al. (2000) Anticancer Research 20 (2A): 1017-22.

46. Lin, R. J., Nagy, L., Inoue, S., et al. (1998) Nature 391: 811-14.

40. Cohen, L. A., Amin, S., Marks, P. A., Rifkind, R. A., Desai, D., y Richon, V. M. (1999) Anticancer Research 19: 4999-5006.

41. Grunstein, M. (1997) Nature 389: 349-52.

42. Finnin, M. S., Donigian, J. R., Cohen, A., et al. (1999) Nature 401: 188-193.

43. Van Lint, C., Emiliani, S., Verdin, E. (1996) Gene Expression 5: 245-53.

44. Archer, S. Shufen, M. Shei, A., Hodin, R. (1998) PNAS 95: 6791-96.

45. Dressel, U., et al. (2000) Anticancer Research 20 (2A): 1017-22.

46. Lin, R. J., Nagy, L., Inoue, S., et al. (1998) Nature 391: 811-14.

224

REIVINDICACIONES

Lo que se reindica es:

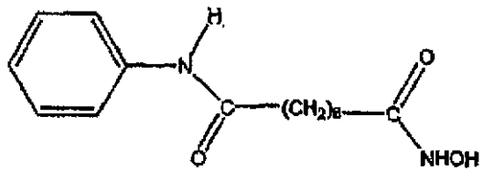
1. Un método para tratar linfoma de célula B grande difusa o mesotelioma en un sujeto, dicho método comprende la etapa de administrar al sujeto una cantidad efectiva de una composición farmacéutica que comprende un inhibidor de histona desacetilasa (HDAC), o una sal farmacéuticamente aceptable o hidrato de esta, y un portador farmacéuticamente aceptable o diluyente, en donde la cantidad de inhibidor de histona desacetilasa es efectiva para tratar dicho linfoma de célula B grande difusa o mesotelioma en dicho sujeto.

2. El método de la reivindicación 1, en donde el método se utiliza para tratar mesotelioma en dicho sujeto.

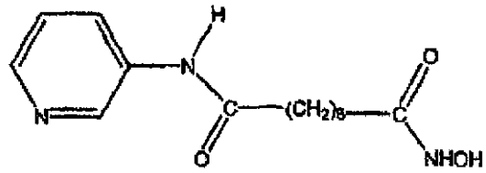
3. El método de la reivindicación 1, en donde el método se utiliza para tratar linfoma de célula B grande difusa en dicho sujeto.

4. El método de la reivindicación 1, en donde el inhibidor HDAC es ácido suberoilanilida hidroxámico (SAHA) representado por la estructura:

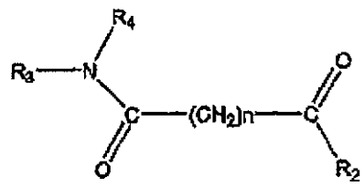
225



5. El método de la reivindicación 1, en donde el inhibidor HDAC es piroxamida, representado por la estructura:



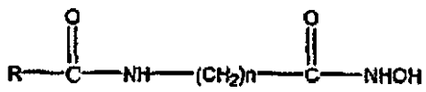
6. El método de la reivindicación 1, en donde el inhibidor HDAC se representa por la estructura:



en donde R_3 y R_4 son independientemente son un grupo alquilo, alquenilo, cicloalquilo, arilo, alquiloxi, ariloxi, arilalquiloxi o piridino sustituido o no sustituido, ramificado o no ramificado, un grupo cicloalquilo, arilo, ariloxi, arilalquiloxi o piridino, o R_3 y R_4 unidos juntos para formar un grupo

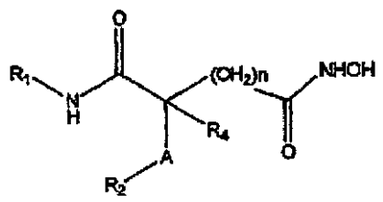
piperidina; R_2 es un grupo hidroxilamino; y n es un entero de 5 a 8.

7. El método de la reivindicación 1, en donde el inhibidor HDAC se representa por la estructura:



en donde R es un fenilo, piperidina, tiazol, 2-piridina, 3-piridina o 4-piridina sustituido o no sustituido y n es un entero de 4 a 8.

8. El método de la reivindicación 1, en donde el inhibidor HDAC se representa por la estructura:



en donde A es una porción amida, R_1 y R_2 son cada uno seleccionados de arilo, arilalquilo, naftilo, piridinoamino, 9-purino-6-amino, tiazoleamino, ariloxi, ariloalquiloxi, piridilo, quinolinilo o isoquinolinilo sustituidos o no sustituidos; R_4 es

hidrógeno, un halógeno, un fenilo o una porción cicloalquilo y n es un entero de 3 a 10.

9. El método de la reivindicación 1, en donde el inhibidor HDAC es un derivado de ácido hidroxámico, un Ácido Graso de Cadena Corta (SCFA), un tetrapéptido cíclico, un derivado de benzamida, o un derivado de cetona electrofílica.

10. El método de la reivindicación 1, en donde dicho inhibidor HDAC es un derivado de ácido hidroxámico seleccionado del grupo que consiste de SAHA, Piroxamida, CBHA, Tricostatina A (TSA), Tricostatina C, ácido Salicilhidroxámico, ácido Azelaico Bishidroxámico (ABHA), ácido Azelaic-1-Hidroxamato-9-Anilida (AAHA), 6-(3-Clorofenilureido) ácido carpoico Hidroxámico (3Cl-UCHA), Oxamflatina, A-161906, Scriptaid, PXD-101, LAQ-824, CHAP, MW2796, y MW2996.

11. El método de la reivindicación 1, en donde dicho inhibidor HDAC es un tetrapéptido cíclico seleccionado del grupo que consiste de Trapoxin A, FR901228 (FK 228, o Depsipéptido); FR225497, Apicidina, CHAP, toxina HC; WF27082, y Clamidocina.

228

12. El método de la reivindicación 1, en donde dicho inhibidor HDAC es un Ácido Graso de Cadena Corta (SCFA) seleccionado del grupo que consiste de Butirato de Sodio, Isovalerato, Valerato, 4 Fenilbutirato (4-PBA), Fenilbutirato (PB), Propionato, Butiramida, Isobutiramida, Fenilacetato, 3-Bromopropionato, Tributirin, ácido Valproico y Valproato.

13. El método de la reivindicación 1, en donde dicho inhibidor HDAC es un derivado de benzamida seleccionado del grupo que consiste de CI-994, MS-27-275(MS-275) y un derivado 3'-amino de MS-27-275.

14. El método de acuerdo con la reivindicación 1, en donde dicho inhibidor HDAC es una cetona electrofílica derivada seleccionada a partir del grupo que consiste de una cetona de trifluorometilo y una α-cetoamida.

15. El método de acuerdo con la reivindicación 1, en donde dicho inhibidor HDAC es un producto natural, una psammaplina, y Depudecina.

16. El método de la reivindicación 1, en donde la composición farmacéutica se administra oralmente.

229

17. El método de la reivindicación 16, en donde dicha composición está contenida dentro de una cápsula de gelatina.

18. El método de la reivindicación 17, en donde dicho portador o diluyente es celulosa microcristalina.

19. El método de la reivindicación 18, que comprende adicionalmente croscarmelosa de sodio como un agente desintegrante.

20. El método de la reivindicación 19, que comprende además estearato de magnesio como un lubricante.

21. El método de la reivindicación 16, en donde dicha composición se administra al sujeto en una dosis diaria total de entre cerca de 25-4000 mg/m^2.

22. El método de la reivindicación 16, en donde dicha composición se administra una vez diariamente, dos veces diariamente, o tres veces diariamente.

23. El método de la reivindicación 22, en donde dicha composición se administra una vez diariamente en una dosis de cerca de 200-600 mg.

230

24. El método de la reivindicación 22, en donde dicha composición se administra dos veces diariamente en una dosis de cerca de 200-400 mg.

25. El método de la reivindicación 22, en donde dicha composición se administra dos veces diariamente en una dosis de cerca de 200-400 mg intermitentemente.

26. El método de la reivindicación 25, en donde dicha composición se administra tres a cinco días por semana.

27. El método de la reivindicación 25, en donde dicha composición se administra tres días una semana.

28. El método de la reivindicación 27, en donde dicha composición se administra en una dosis de cerca de 200 mg.

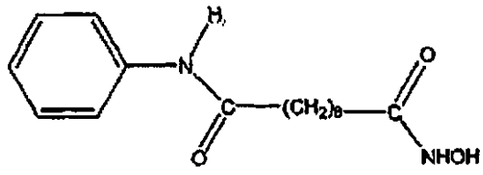
29. El método de la reivindicación 27, en donde dicha composición se administra en una dosis de cerca de 300 mg.

30. El método de la reivindicación 27, en donde dicha composición se administra en una dosis de cerca de 400 mg.

231

31. El método de la reivindicación 22, en donde dicha composición se administra tres veces diariamente en una dosis de cerca de 100-250 mg.

32. Un método para tratar linfoma de célula B grande difusa o mesotelioma en un sujeto, dicho método comprende la etapa de administrar al sujeto una cantidad efectiva de una composición farmacéutica que comprende ácido suberoilanilida hidroxámico (SAHA) o una sal farmacéuticamente aceptable o hidrato de esta, representado por la estructura:



Y un portador farmacéuticamente aceptable o diluyente, en donde la cantidad de SAHA es efectiva para tratar dicho linfoma de célula B grande difusa o mesotelioma en dicho sujeto.

33. El método de la reivindicación 32, en donde el método se utiliza para tratar mesotelioma en dicho sujeto.

232

34. El método de la reivindicación 32, en donde el método se utiliza para tratar linfoma de célula B grande difusa en dicho sujeto.

35. El método de la reivindicación 32, en donde la composición farmacéutica se administra oralmente.

36. El método de la reivindicación 35, en donde dicha composición está contenida dentro de una cápsula de gelatina.

37. El método de la reivindicación 36, en donde dicho portador o diluyente es celulosa microcristalina.

38. El método de la reivindicación 37, que comprende adicionalmente croscarmelosa de sodio como un agente desintegrante.

39. El método de la reivindicación 38, que comprende además estearato de magnesio como un lubricante.

40. El método de la reivindicación 35, en donde dicha composición se administra al sujeto en una dosis diaria total de entre cerca de 25-4000 mg/m^2.

41. El método de la reivindicación 35, en donde dicha composición se administra una vez diariamente, dos veces diariamente, o tres veces diariamente.

42. El método de la reivindicación 41, en donde dicha composición se administra una vez diariamente en una dosis de cerca de 200-600 mg.

43. El método de la reivindicación 41, en donde dicha composición se administra dos veces diariamente en una dosis de cerca de 200-400 mg.

44. El método de la reivindicación 41, en donde dicha composición se administra dos veces diariamente en una dosis de cerca de 200-400 mg intermitentemente.

45. El método de la reivindicación 44, en donde dicha composición se administra tres a cinco días por semana.

46. El método de la reivindicación 44, en donde dicha composición se administra tres días una semana.

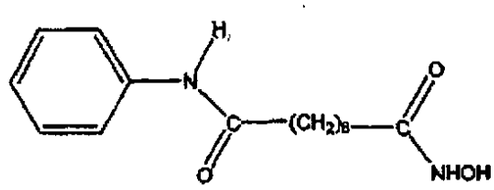
47. El método de la reivindicación 46, en donde dicha composición se administra en una dosis de cerca de 200 mg.

48. El método de la reivindicación 46, en donde dicha composición se administra en una dosis de cerca de 300 mg.

49. El método de la reivindicación 46, en donde dicha composición se administra en una dosis de cerca de 400 mg.

50. El método de la reivindicación 41, en donde dicha composición se administra tres veces diariamente en una dosis de cerca de 100-250 mg.

51. Un método para tratar linfoma de célula B grande difusa o mesotelioma en un sujeto, dicho método comprende la etapa de administrar al sujeto una dosis diaria total de hasta cerca de 800 mg de una composición farmacéutica que comprende ácido suberoilanilida hidroxámico (SAHA) o una sal farmacéuticamente aceptable o hidrato de esta, representado por la estructura:



y un portador farmacéuticamente aceptable o diluyente, en donde la cantidad de SAHA es efectiva para tratar dicho linfoma de célula B grande difusa o mesotelioma en dicho sujeto.

52. El método de la reivindicación 51, en donde el método se utiliza para tratar mesotelioma en dicho sujeto.

53. El método de la reivindicación 51, en donde el método se utiliza para tratar linfoma de célula B grande difusa en dicho sujeto.

54. El método de la reivindicación 51, en donde la composición farmacéutica se administra oralmente.

55. El método de la reivindicación 54, en donde dicha composición está contenida dentro de una cápsula de gelatina.

56. El método de la reivindicación 55, en donde dicho portador o diluyente es celulosa microcristalina.

57. El método de la reivindicación 56, que comprende adicionalmente croscarmelosa de sodio como un agente desintegrante.

236

58. El método de la reivindicación 57, que comprende adicionalmente estearato de magnesio como un lubricante.

59. El método de la reivindicación 54, en donde dicha composición se administra una vez diariamente, dos veces diariamente, o tres veces diariamente.

60. El método de la reivindicación 59, en donde dicha composición se administra una vez diariamente en una dosis de cerca de 200-600 mg.

61. El método de la reivindicación 59, en donde dicha composición se administra dos veces diariamente en una dosis de cerca de 200-400 mg.

62. El método de la reivindicación 59, en donde dicha composición se administra dos veces diariamente en una dosis de cerca de 200-400 mg intermitentemente.

63. El método de la reivindicación 62, en donde dicha composición se administra tres a cinco días por semana.

64. El método de la reivindicación 62, en donde dicha composición se administra tres días una semana.

65. El método de la reivindicación 64, en donde dicha composición se administra en una dosis de cerca de 200 mg.

66. El método de la reivindicación 64, en donde dicha composición se administra en una dosis de cerca de 300 mg.

67. El método de la reivindicación 64, en donde dicha composición se administra en una dosis de cerca de 400 mg.

68. El método de la reivindicación 59, en donde dicha composición se administra tres veces diariamente en una dosis de cerca de 100-250 mg.

FIG. 1

Paciente de SAHA: IV vs ORAL (200 mg/dosis)

Protocolo 01-021 (SAH

Paciente #

1-OS-200
(8-21-01)

2-OS-200
(10-16-01)

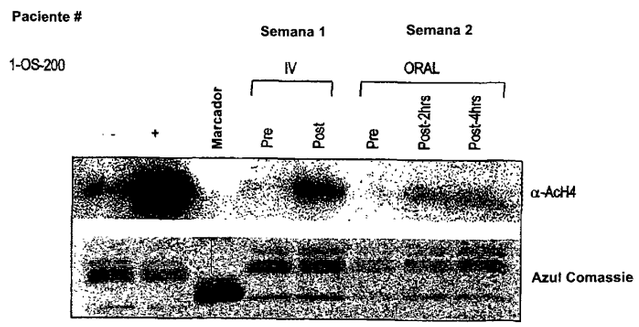


FIG. 3

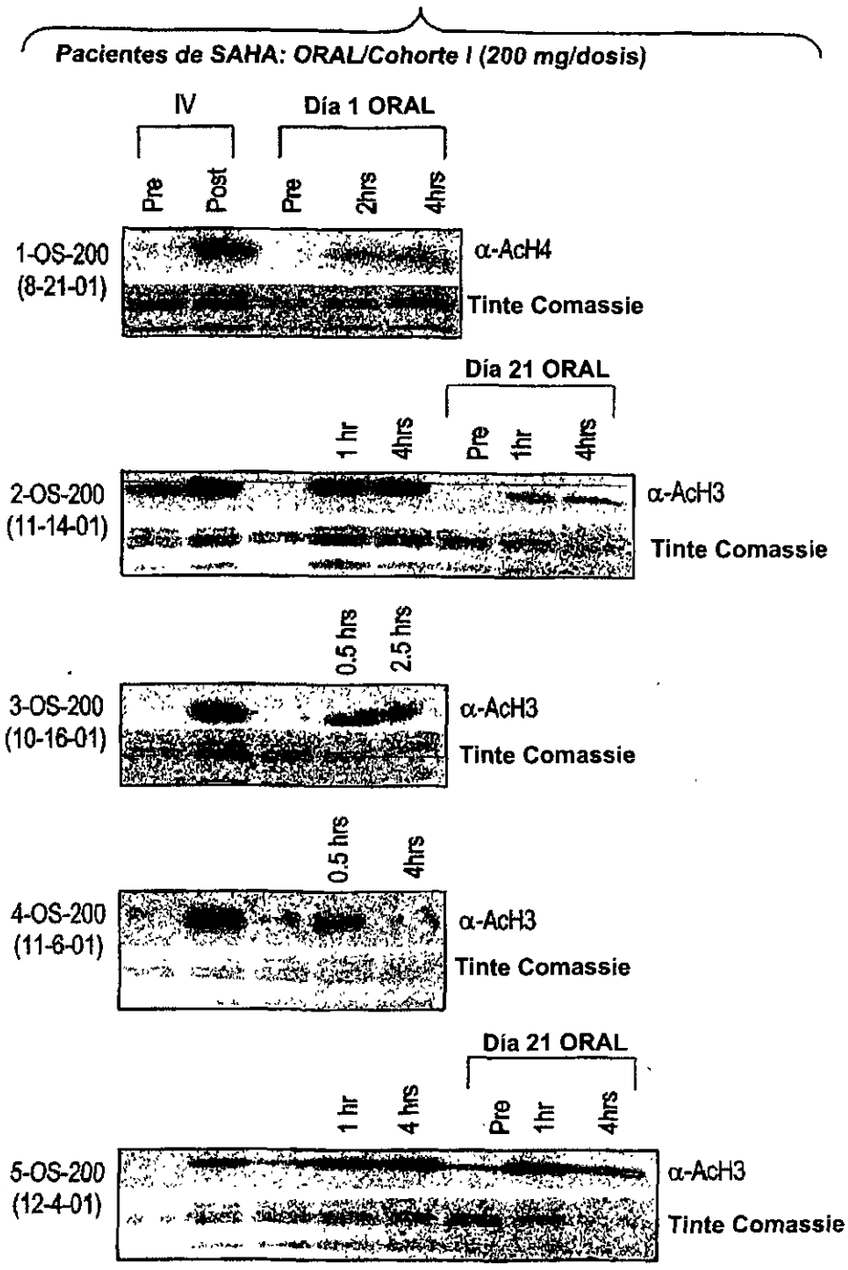

Pacientes de SAHA: ORAL/Cohorte I (200 mg/dosis)
IV
Día 1 ORAL
Pre
Post
Pre
2hrs
4hrs
1-OS-200
(8-21-01)
α-AcH4
Tinte Comassie
Día 21 ORAL
1 hr
4hrs
Pre
1hr
4hrs
2-OS-200
(11-14-01)
α-AcH3
Tinte Comassie
0.5 hrs
2.5 hrs
3-OS-200
(10-16-01)
α-AcH3
Tinte Comassie
0.5 hrs
4hrs
4-OS-200
(11-6-01)
α-AcH3
Tinte Comassie
Día 21 ORAL
1 hr
4 hrs
Pre
1hr
4hrs
5-OS-200
(12-4-01)
α-AcH3
Tinte Comassie

FIG. 4

Protocolo 01-021 (SAHA ORAL)
BRAZO A: PACIENTES CON TUMOR SÓLIDO
Cohorte I (200 mg/dosis)

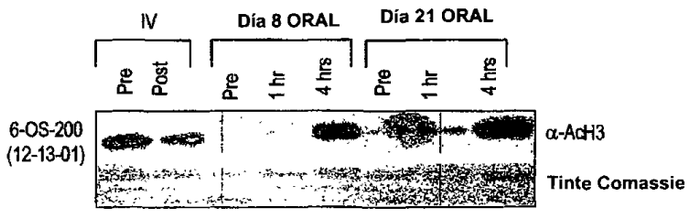


FIG. 5

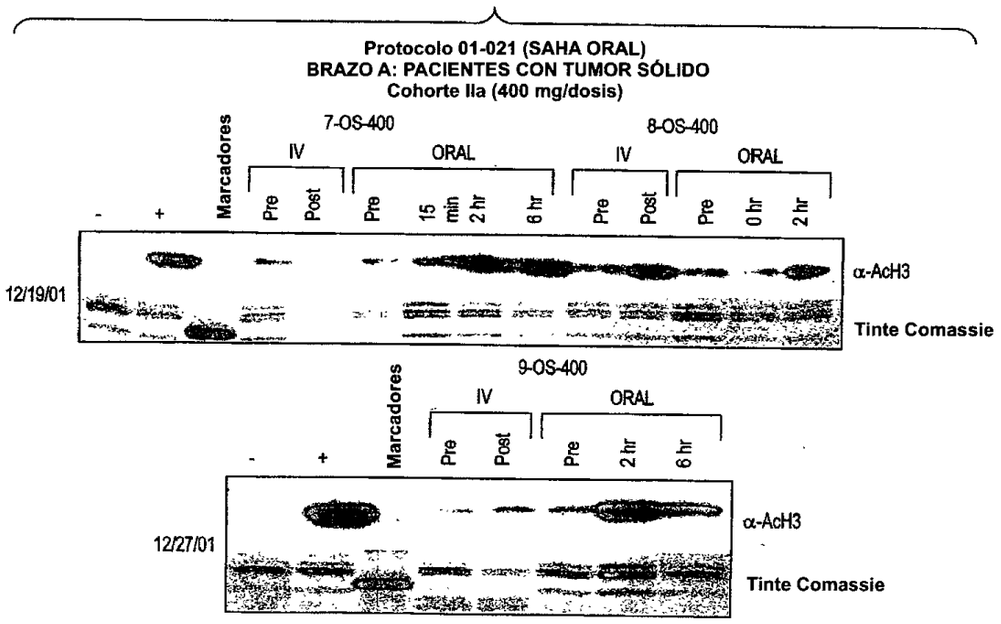

Protocolo 01-021 (SAHA ORAL)
BRAZO A: PACIENTES CON TUMOR SÓLIDO
Cohorte IIa (400 mg/dosis)
7-OS-400
8-OS-400
IV
ORAL
IV
ORAL
Marcadores
- + Pre Post Pre 15 min 2 hr 6 hr Pre Post Pre 0 hr 2 hr
12/19/01
α-AcH3
Tinte Comassie
9-OS-400
IV
ORAL
Marcadores
- + Pre Post Pre 2 hr 6 hr
12/27/01
α-AcH3
Tinte Comassie

FIG. 6

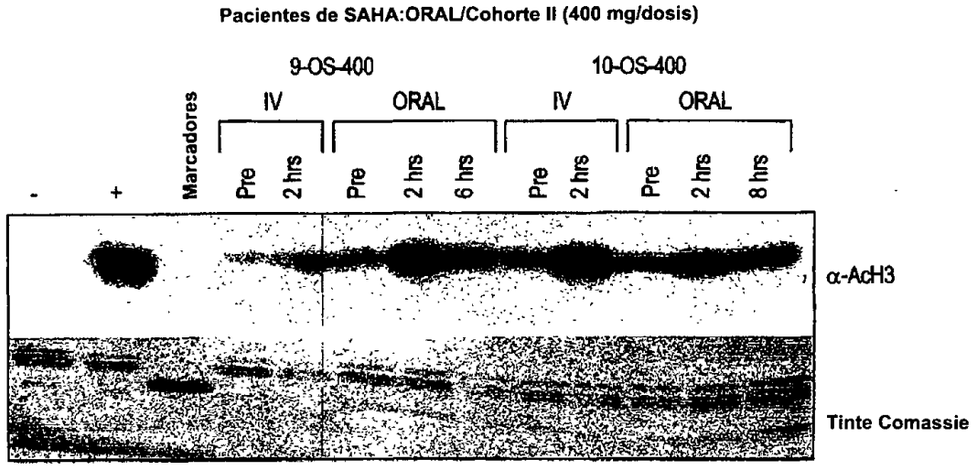
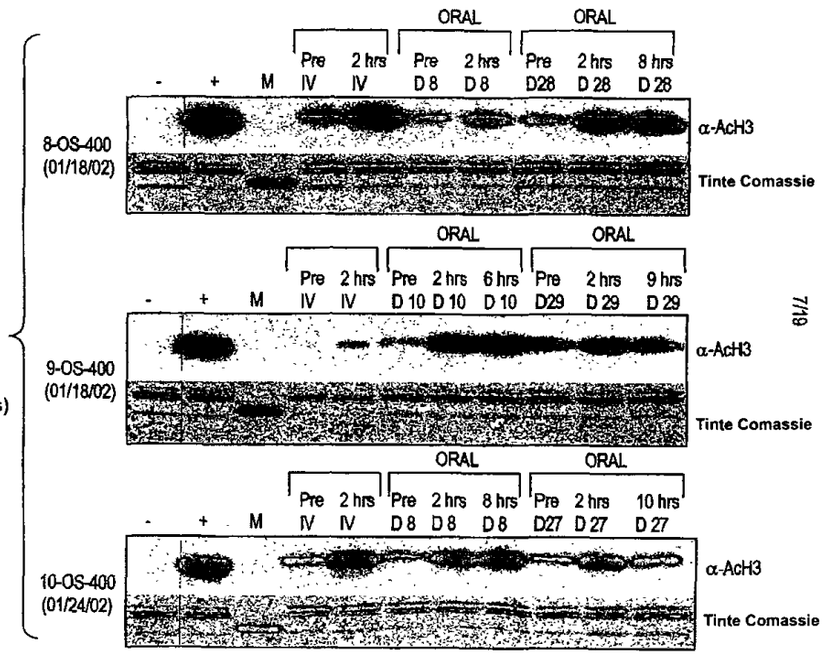

Pacientes de SAHA:ORAL/Cohorte II (400 mg/dosis)
9-OS-400
10-OS-400
IV
ORAL
IV
ORAL
Marcadores
-
+
Pre
2 hrs
Pre
2 hrs
6 hrs
Pre
2 hrs
Pre
2 hrs
8 hrs
α-AcH3
Tinte Comassie

FIG. 7

Protocolo 01-021 (SAHA ORAL) BRAZO A: PACIENTES CON TUMOR SÓLIDO Cohorte IIa (400 mg/dosis)



WO 2005/018578
7/19
PCT/US2004/027043

FIG. 8

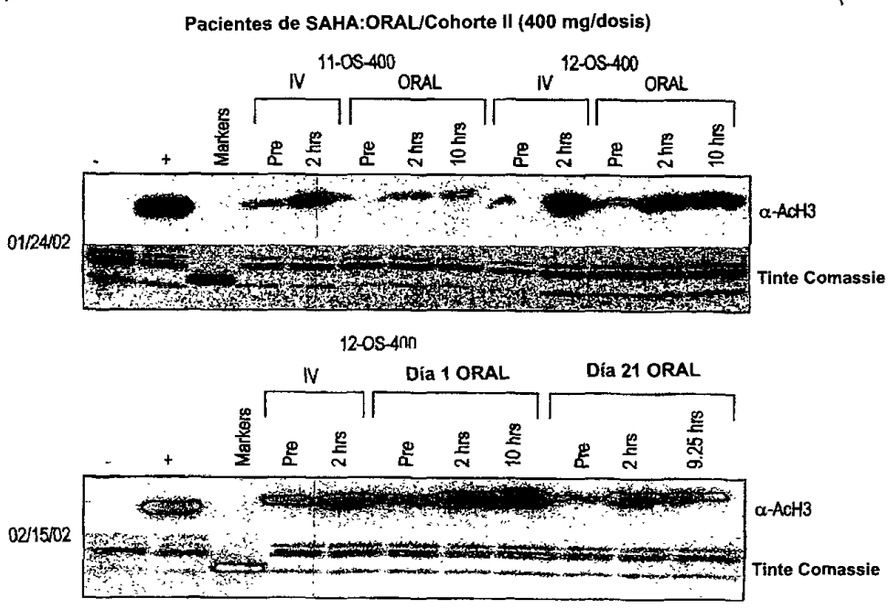

Pacientes de SAHA:ORAL/Cohorte II (400 mg/dosis)
11-OS-400
IV
ORAL
12-OS-400
IV
ORAL
Markers
Pre
2 hrs
Pre
2 hrs
10 hrs
Pre
2 hrs
Pre
2 hrs
10 hrs
01/24/02
α-AcH3
Tinte Comassie
12-OS-400
IV
Día 1 ORAL
Día 21 ORAL
Markers
Pre
2 hrs
Pre
2 hrs
10 hrs
Pre
2 hrs
9.25 hrs
02/15/02
α-AcH3
Tinte Comassie

FIG. 9A

Oral 200 mg vs 400 mg (Rápido)
Dosis oral en el Ciclo 1 Día 8

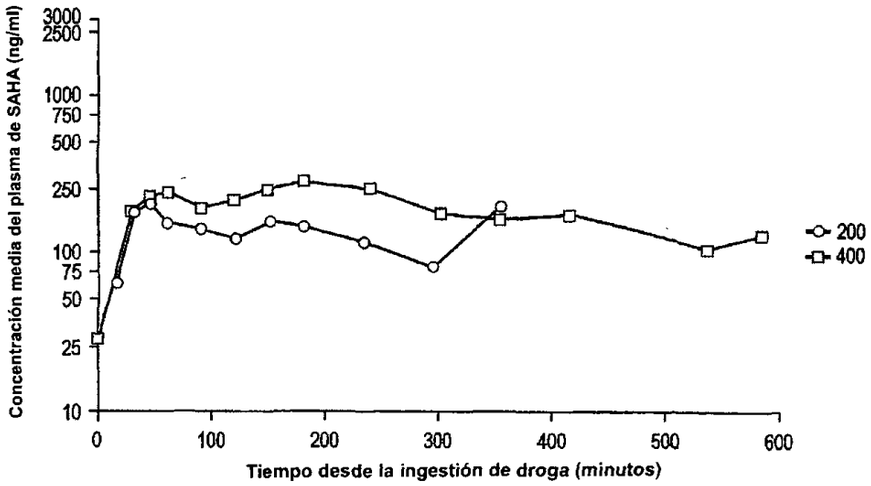


FIG. 9B

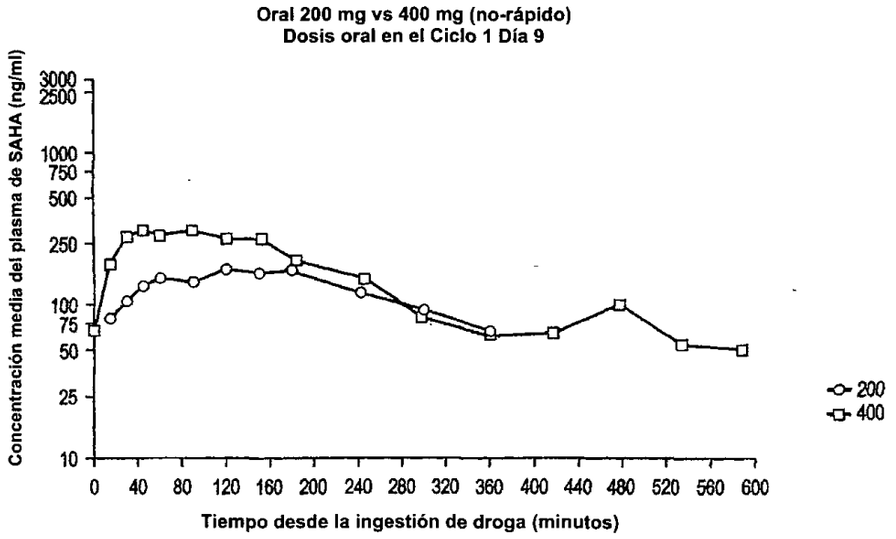

Oral 200 mg vs 400 mg (no-rápido)
Dosis oral en el Ciclo 1 Día 9
Concentración media del plasma de SAHA (ng/ml)
3000
2500
1000
750
500
250
100
75
50
25
10
0
40
80
120
160
200
240
280
320
360
400
440
480
520
560
600
Tiempo desde la ingestión de droga (minutos)
200
400

FIG. 9C

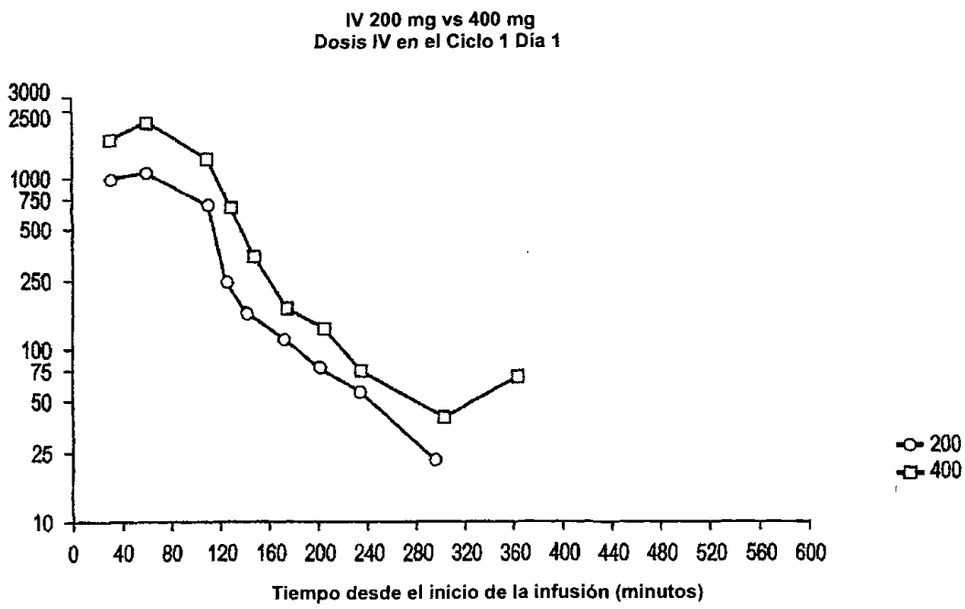

IV 200 mg vs 400 mg
Dosis IV en el Ciclo 1 Día 1
3000
2500
1000
750
500
250
100
75
50
25
10
0 40 80 120 160 200 240 280 320 360 400 440 480 520 560 600
Tiempo desde el inicio de la infusión (minutos)
200
400

FIG. 10

Vida Media aparente de la Vida Media de Dosis Oral

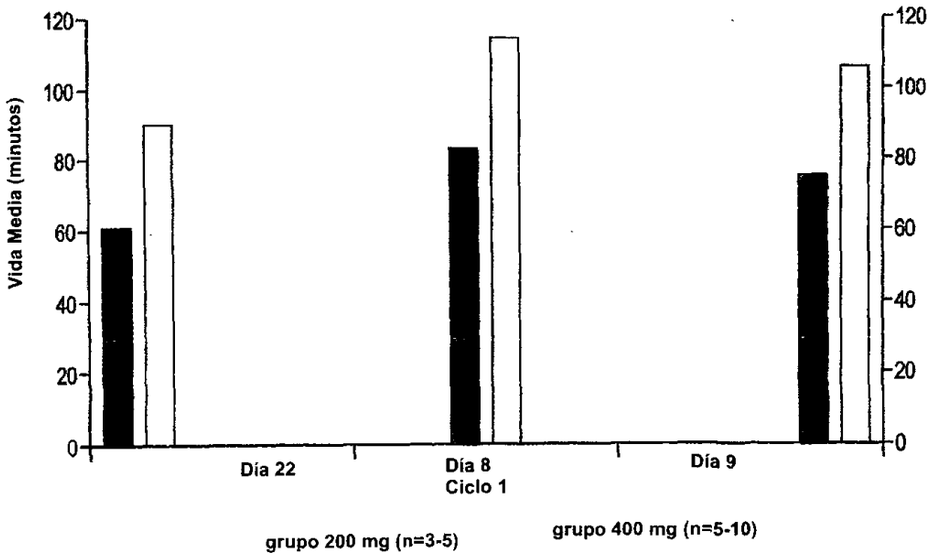


FIG. 11

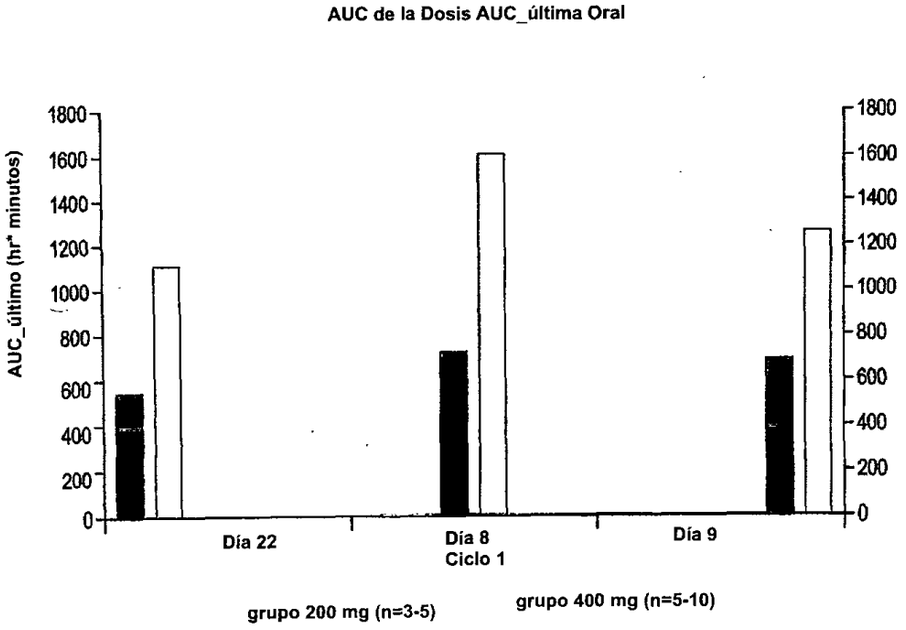
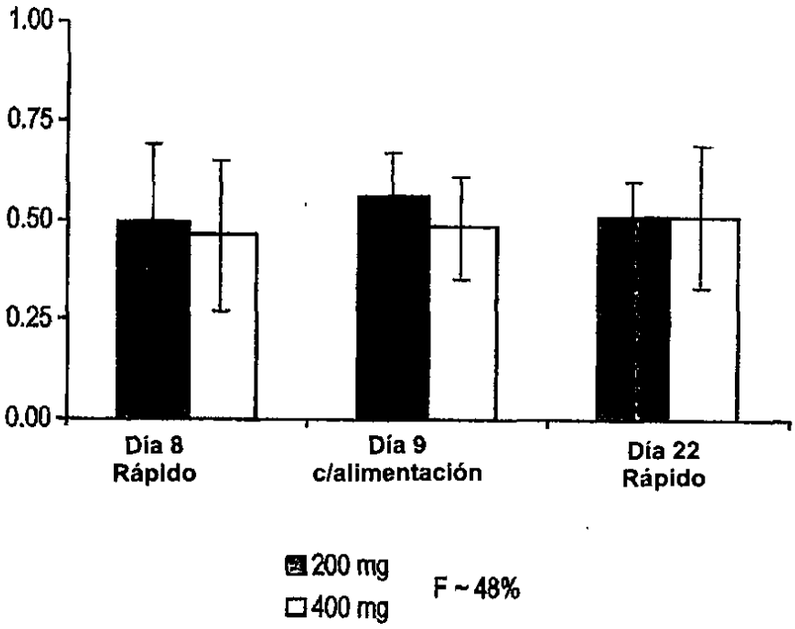

AUC de la Dosis AUC_última Oral
AUC_último (hr* minutos)
1800
1600
1400
1200
1000
800
600
400
200
0
Día 22
Día 8
Ciclo 1
Día 9
grupo 200 mg (n=3-5)
grupo 400 mg (n=5-10)

FIG. 12

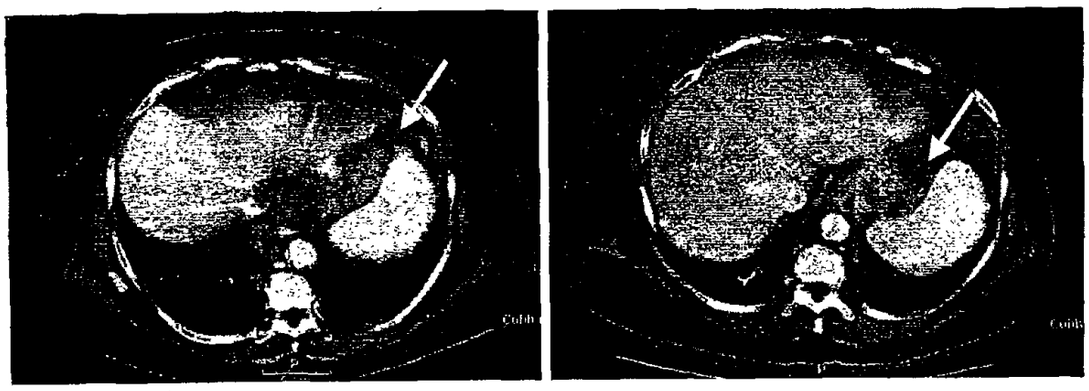
Biodisponibilidad después de 200 y 400 mg por día



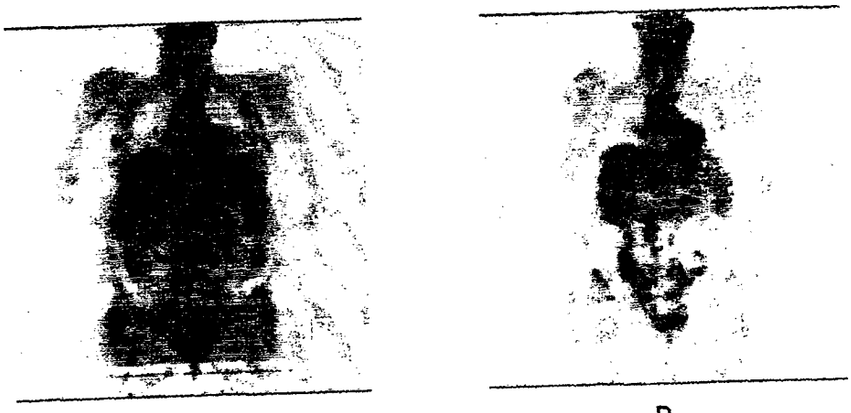
MESOTELIOMA



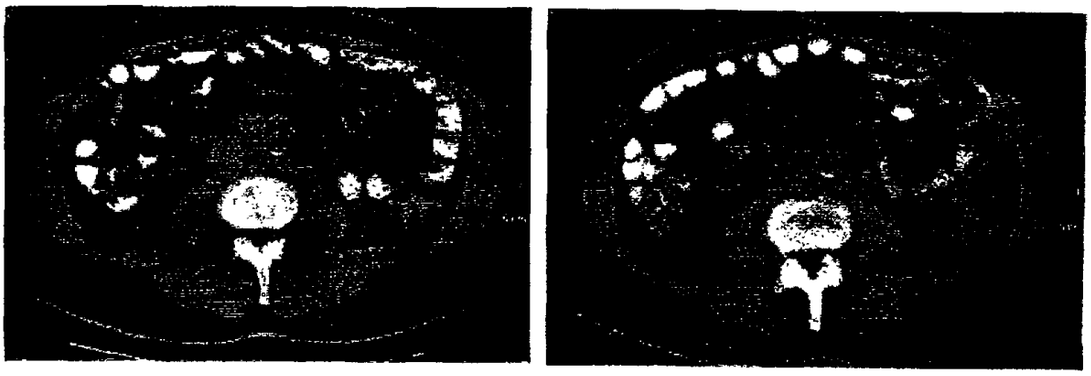
PRE FIG. 13 POST



A FIG. 14 B



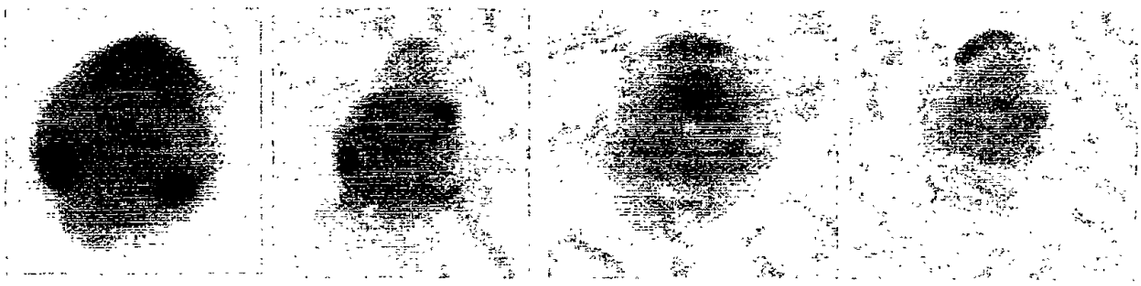
A FIG. 15 B



A FIG. 16 B

Pre-Terapia

2 Meses después de SAHA



A

FIG. 17

B

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
IVOR R. ELRIFI
MINTZ, LEVIN, COHN, FERRIS, GLOVSKY & POPEO, PC
666 THIRD AVENUE
NEW YORK, NY 10017

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing (*day/month/year*)
Applicant's or agent's file reference 24852-501CIP	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US04/27943	International filing date (*day/month/year*) 26 August 2004 (26.08.2004)
Applicant ATON PHARMA, INC.	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46).

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 740 14 35

   For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the ISA/ US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Phyllis G. Spivack Telephone No. 571.272.1600

Form PCT/ISA/220 (January 2004) *(See notes on accompanying sheet)*

RECEIVED
MAR 10 2005
Mintz Levin - NYC

Done By	
☐ File Folder	
☐ Data Entry	
☑ Docket Entry	
☑ Docket Cross Ref	
☐ Previously Entered	
☐ No Docketing Req	
☐ Order Copies	
☐ Annuities	

256

RECEIVED
MAR 10 2005
Mintz Levin - NYC

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
IVOR R. ELRIFI
MINTZ, LEVIN, COHN, FERRIS, GLOVSKY & POPEO, PC
666 THIRD AVENUE
NEW YORK, NY 10017

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing (*day/month/year*) 07 MAR 2005
Applicant's or agent's file reference 24852-501CIP	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US04/27943	International filing date (*day/month/year*) 26 August 2004 (26.08.2004)
Applicant ATON PHARMA, INC.	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 740 14 35

   For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the ISA/ US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Phyllis G. Spivack Telephone No. 571.272.1600

Form PCT/ISA/220 (January 2004) *(See notes on accompanying sheet)*

Done By
☐ File Folder
☐ Data Entry
☑ Docket Entry
☑ Docket Cross Ref
☐ Previously Entered
☐ No Docketing Req
☐ Order Copies
☐ Annuities

259

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 24852-501CIP	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US04/27943	International filing date (*day/month/year*) 26 August 2004 (26.08.2004)	(Earliest) Priority Date (*day/month/year*) 26 August 2003 (26.08.2003)
Applicant ATON PHARMA, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the Report**
   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
      ☐ The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.
2. ☐ **Certain claims were found unsearchable** (See Box No. II)
3. ☐ **Unity of invention is lacking** (See Box No. III)
4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,
   a. the figure of the **drawings** to be published with the abstract is Figure No. ____
      ☐ as suggested by the applicant.
      ☐ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☒ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/27943

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 31/185
US CL : 514/616
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/616

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database Caplus on STN, Accession No. DN134:293668, "Negative regulation of bcl-2 expression by p53 in hematopoietic cells." abstract, Wu et al, 2001, Vol. 20, No. 2, pages 240-251.	1-68
A,P	US 2004/0127523 A1 (BACOPOULOS et al.) 01 July 2004, see entire document.	1-68
A,P	US 2003/0161830 A1 (JACKSON et al.) 28 August 2003(28.08.2003), see entire document.	1-68

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

* 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 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

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

"&" document member of the same patent family

Date of the actual completion of the international search
21 January 2005 (21.01.2005)

Date of mailing of the international search report
07 MAR 2005

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703) 305-3230

Authorized officer
Phyllis G. Spivack

Telephone No. 571-272-1600

Form PCT/ISA/210 (second sheet) (January 2004)

261

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
IVOR R. ELRIFI
MINTZ, LEVIN, COHN, FERRIS, GLOVSKY & POPEO, PC.
666 THIRD AVENUE
NEW YORK, NY 10017

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	07 MAR 2005
Applicant's or agent's file reference 24852-501CIP	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US04/27943	26 August 2004 (26.08.2004)	26 August 2003 (26.08.2003)

International Patent Classification (IPC) or both national classification and IPC

IPC(7): A61K 31/185 and US Cl.: 514/616

Applicant

ATON PHARMA, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I — Basis of the opinion
- ☐ Box No. II — Priority
- ☐ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV — Lack of unity of invention
- ☒ Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI — Certain documents cited
- ☐ Box No. VII — Certain defects in the international application
- ☐ Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703) 305-3230

Authorized officer
Phyllis G. Spivack

Telephone No. 571.272.1600

262

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/27943

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☐ in written format

      ☐ in computer readable form

   c. time of filing/furnishing

      ☐ contained in international application as filed.

      ☐ filed together with the international application in computer readable form.

      ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/27943

Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-68	YES
	Claims none	NO
Inventive step (IS)	Claims 1-68	YES
	Claims NONE	NO
Industrial applicability (IA)	Claims 1-68	YES
	Claims NONE	NO

2. Citations and explanations:

Claims 1-68 meet the criteria of PCT 33(2) as the prior art fails to teach each limitation of the present claims.

Claims 1-68 meet the criteria of PCT Article 33(3) as having an inventive step over the prior art.

Claims 1-68 meet the criteria of PCT Article 33(4) as they are directed to methods of treating mesothelioma or diffuse large B-cell lymphoma.

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under Article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*, a publication of WIPO.

In these Notes, "Article," "Rule" and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended ?

Under Article 19, only the claims may be amended

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Preliminary Examining Authority

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When ? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments ?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2)

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments ?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below under "Statement under Article 19(1)")

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;

(ii) the claim is cancelled,

(iii) the claim is new,

(iv) the claim replaces one or more claims as filed,

(v) the claim is the result of the division of a claim as filed

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51].
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." **or**
"Claims 7 to 13 cancelled, new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged, claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17, new claims 20 and 21 added."

"Statement under Article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1))

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated elected Office, see the *PCT Applicant's Guide*, Volume II.

TRANSMITTAL LETTER TO THE UNITED STATES RECEIVING OFFICE

PTO-1382 (Rev. 04-2003) (Modified) PCTUS2.FRP /REV03

Date	26 August 2004 (26.08.04)
International Application No.	
Attorney Docket No.	24852-501 CIP5 061

I. Certification under 37 CFR 1.10 (if applicable)

EV287338188	26 August 2004 (26.08.04)
Express Mail mailing number	Date of Deposit

I hereby certify that the application/correspondence attached hereto is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Signature of person mailing correspondence	Typed or printed name of person mailing correspondence

II. ☒ New International Application

TITLE	METHODS OF TREATING CANCER WITH HDAC INHIBITORS	Earliest priority date (Day/Month/Year): 26 August 2003 (26.08.03)

SCREENING DISCLOSURE INFORMATION: In order to assist in screening the accompanying international application for purposes of determining whether a license for foreign transmittal should and could be granted and for other purposes, the following information is supplied. (Note: check as many boxes as apply):

A. ☐ The invention disclosed was **not** made in the United States.

B. ☐ There is no prior U.S. application relating to this invention.

C. ☒ The following prior U.S. application(s) contain subject matter which is related to the invention disclosed in the attached international application. *(NOTE: priority to these applications may or may not be claimed on form PCT/RO/101 (Request) and this listing does not constitute a claim for priority).*

application no.	10/650,025	filed on	26 August 2003 (26.08.03)
application no.	10/665,079	filed on	16 September 2003 (16.09.03)

D. ☒ The present international application contains additional subject matter not found in the prior U.S. application(s) identified in paragraph C. above. The additional subject matter is found on pages Thoughout and ☒ **DOES NOT ALTER** ☐ **MIGHT BE CONSIDERED TO ALTER** the general nature of the invention in a manner which would require the U.S. application to have been made available for inspection by the appropriate defense agencies under 35 U.S.C. 181 and 37 CFR 5.1. See 37 CFR 5.15.

III. ☐ A Response to an Invitation from the RO/US. The following document(s) is (are) enclosed:

A. ☐ A Request for An Extension of Time to File a Response

B. ☐ A Power of Attorney (General or Regular)

C. ☐ Replacement pages:

pages		of the request (PCT/RO/101)	pages		of the figures
pages		of the description	pages		of the abstract
pages		of the claims			

D. ☐ Submission of Priority Documents

Priority document		Priority document	

E. ☐ Fees as specified on attached Fee Calculation sheet form PCT/RO/101 annex

IV. ☐ A Request for Rectification under PCT 91 ☐ **A Petition** ☐ **A Sequence Listing Diskette**

V. ☒ Other (please specify): Request, Check, Postcard

The person signing this form is the:		
☐	Applicant	Caryn DeHoratius
☒	Attorney/Agent (Reg. No.) 45,881	Typed name of signer
☐	Common Representative	Signature

U.S. Department of Commerce: Patent and Trademark Office

EV287338188US

266

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 24852-501CIP5 061

Box No. I TITLE OF INVENTION

METHODS OF TREATING CANCER WITH HDAC INHIBITORS

Box No. II APPLICANT ☐ This person is also inventor

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

ATON PHARMA, INC.
33 Avenue Louis Pasteur
Boston, Massachusetts 02115
United States of America

Telephone No.

Facsimile No.

Teleprinter No.

Applicant's registration No. with the Office

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH
1275 York Avenue
New York, New York 10021
United States of America

This person is:
☒ applicant only
☐ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

Applicant's registration No. with the Office

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

ELRIFI, Ivor R.
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P.C.
666 Third Avenue
New York, New York 10017
United States of America

Telephone No.
(212) 935-3000

Facsimile No.
(212) 983-3115

Teleprinter No.

Agent's registration No. with the Office
39,529

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

267

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BACOPOULOS, Nicholas G.
2109 Broadway
Apartment 15-109
New York, New York 10023
United States of America

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office:

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant ☐ all designated States ☐ all designated States except the United States of ☒ the United States of America only ☐ the States indicated in the Supplemental

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

CHIAO, Judy H.
110 Cedar Green Lane
Berkeley Heights, New Jersey 07922
United States of America

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office:

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the ☐ all designated States ☐ all designated States except the United States of ☒ the United States of America only ☐ the States indicated in the Supplemental

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

MILLER, Thomas A.
517 West 113th Street, Number 82
New York, New York 10025
United States of America

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office:

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the ☐ all designated States ☐ all designated States except the United States of ☒ the United States of America only ☐ the States indicated in the Supplemental

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

PARADISE, Carolyn M.
6 Gilman Lane
Cortland Manor, New York 10567
United States of America

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office:

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the ☐ all designated States ☐ all designated States except the United States of ☒ the United States of America only ☐ the States indicated in the Supplemental

☒ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

RICHON, Victoria M.
184 Highland Road
Rye, New York 10580
United States of America

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office:

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant
- ☐ all designated States
- ☐ all designated States except the United States of
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office:

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the
- ☐ all designated States
- ☐ all designated States except the United States of
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office:

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the
- ☐ all designated States
- ☐ all designated States except the United States of
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office:

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the
- ☐ all designated States
- ☐ all designated States except the United States of
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

269

Supplemental Box *If the Supplemental Box is not used, this sheet should not be included in the request.*

1. If, in any of the Boxes, except Boxes Nos VIII(i) to (v) for which a special continuation box is provided, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No...." (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

(i) If more than two persons are to be indicated as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;

(ii) If, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;

(iii) If, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;

(iv) If, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;

(v) If, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box VI.

2. If the applicant intends to make an indication of the wish that the international application be treated, in certain designated States, as an application for a patent of addition, certificate of addition, inventor's certificate of addition or utility certificate of addition: in such a case, write the name or two-letter code of each designated State concerned and the indication "patent of addition," "certificate of addition," "inventor's certificate of addition" or "utility certificate of addition," the number of the parent application or parent patent or other patent grant and the date of grant of the parent patent or other patent grant or the date of filing of parent application (Rules 4.11(a)(iii) and 49bis.1(a) or (b)).

3. If the applicant intends to make an indication of the wish that the international application be treated, in the United States of America, as a continuation or continuation-in-part of an earlier application: in such a case, write "United States of America" or "US" and the indication "continuation" or "continuation-in-part" and the number and the filing date of the parent application (Rules 4.11(a)(iv) and 49.bis.1(d)).

Continuation of U.S. Patent Application Numbers:

Application Number:	File Date:
10/650,025	26 August 2003 (26.08.03)
10/655,079	16 September 2003 (16.09.03)

270

Box No.V DESIGNATIONS

The filing of this request **constitutes under Rule 4.9(a), the designation** of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.

However,

☐ DE Germany **is not designated** for any kind of national protection

☐ KR Republic of Korea **is not designated** for any kind of national protection

☐ RU Russian Federation **is not designated** for any kind of national protection

(The check-boxes above may be used to exclude (irrevocably) the designations concerned in order to avoid the ceasing of the effect, under the national law, of an earlier national application from which priority is claimed. See the Notes to Box No. V as to the consequences of such national law provisions in these and certain other States.)

Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is:		
		national application: country or Member	regional application:* regional Office	international application: receiving Office
item (1) 26 August 2003 (26.08.03)	10/650,025	US		
item (2) 16 September 2003 (16.09.03)	10/655,079	US		
item (3)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office)* identified above as:

☒ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ other, see Supplemental Box

** Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)):* .....

.....

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA/US .....

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII DECLARATIONS

The following **declarations** are contained in Boxes Nos. VIII (i) to (v) *(mark the applicable check-boxes below and indicate in the right column the number of each type of declaration)*:		Number of declarations
☐ Box No. VIII (i)	Declaration as to the identity of the inventor :	
☐ Box No. VIII (ii)	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent :	
☐ Box No. VIII (iii)	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application :	
☐ Box No. VIII (iv)	Declaration of inventorship (only for the purposes of the designation of the United States of America) :	
☐ Box No. VIII (v)	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty :	

271

Sheet No. ...6...

Box No. IX CHECK LIST; LANGUAGE OF FILING

This international application **contains:**		This international application **is accompanied by** the following item(s) *(mark the applicable check-boxes below and indicate in right column the number of each item)*:	Number of items
(a) **in paper form**, the following number of sheets:			
request (including declaration sheets)	: 6	1. ☒ fee calculation sheet	: 1
description (excluding sequence listing and/or tables related thereto)	: 78	2. ☐ original separate power of attorney	:
claims	: 8	3. ☐ original general power of attorney	:
abstract	: 1	4. ☐ copy of general power of attorney; reference number, if any:	:
drawings	: 19	5. ☐ statement explaining lack of signature	:
Sub-total number of sheets	112	6. ☐ priority document(s) identified in Box No. VI as item(s):	:
sequence listing	:	7. ☐ translation of *international application into (language)*:	:
tables related thereto	:	8. ☐ separate indications concerning deposited microorganism or other biological material	:
(for both, actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (c)		9. ☐ sequence listing in computer readable form *(indicate type and number of carriers)*	
Total number of sheets	: 112	(i) ☐ copy submitted for the purposes of international search under Rule 13*ter* only (and not as part of the international application)	:
(b) ☐ **only in computer readable form** (Section 801(a)(i))		(ii) ☐ *(only where check-box (b)(i) or (c)(i) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Rule 13*ter*	:
(i) ☐ sequence listing		(iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing mentioned in left column	:
(ii) ☐ tables related thereto		10. ☐ tables in computer readable form related to sequence listing *(indicate type and number of carriers)*	
(c) ☐ **also in computer readable form** *(Section 801(a)(ii))*		(i) ☐ copy submitted for the purposes of international search under Section 802(b-*quater*) only (and not as part of the international application)	:
(i) ☐ sequence listing		(ii) ☐ *(only where check-box (b)(ii) or (c)(ii) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Section 802(b-*quater*)	:
(ii) ☐ tables related thereto		(iii) ☐ together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column	:
Type and number of carriers (diskette, CD-ROM, CD-R or other) on which are contained the		11. ☒ other *(specify)*: Postcard, Transmittal, Check	: 3
☐ sequence listing:			
☐ tables related thereto:			
(additional copies to be indicated under items 9(ii) and/or 10(ii), in right column)			

Figure of the drawings which should accompany the abstract: FIG 10	**Language of filing** of the international application: English

Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Caryn DeHoratius

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA/	6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

272

PCT

FEE CALCULATION SHEET

Annex to the Request

For receiving Office use only

International Application No.

Date stamp of the receiving Office

Applicant's or agent's file reference: 24852-501CIP5 061

Applicant: ATON PHARMA, ET AL.

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE ..... 300.00 [T]

2. SEARCH FEE ..... 1,000.00 [S]

International search to be carried out by US

(If two or more International Searching Authorities are competent to carry out the international search, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FILING FEE

Where item (b) and/or (c) of Box No. IX apply, enter **Sub-total number of sheets**
Where item (b) and (c) of Box No. IX do not apply, enter **Total number of sheets** } 112

[i1] first 30 sheets ..... 1,134.00 [i1]

[i2] 82 (number of sheets in excess of 30) x 12.00 (fee per sheet) = 984.00 [i2]

[i3] additional component (only if sequence listing and/or tables related thereto are filed in computer readable form under Section 801(a)(i), or both in that form and on paper, under Section 801(a)(ii)):

400 x _____ (fee per sheet) = _____ [i3]

Add amounts entered at i1, i2 and i3 and enter total at I ..... 2,118.00 [I]

(Applicants from certain States are entitled to a reduction of 75% of the international filing fee. Where the applicant is (or all applicants are) so entitled,

4. FEE FOR PRIORITY DOCUMENT *(if applicable)* ..... 40.00 [P]

5. TOTAL FEES PAYABLE ..... 3,458.00 TOTAL

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

MODE OF PAYMENT

- ☒ authorization to charge deposit account (see below)
- ☐ postal money order
- ☐ cash
- ☐ coupons
- ☒ cheque
- ☐ bank draft
- ☐ revenue stamps
- ☐ other *(specify)*:

AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT

(This mode of payment may not be available at all receiving Offices)

- ☐ Authorization to charge the total fees indicated above.
- ☒ *(This check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit)* Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.
- ☒ Authorization to charge the fee for priority document.

Receiving Office: US

Deposit Account No.: 50-0311

Date: 26 August 2004 (26.08.04)

Name: Caryn DeHoratius

Signature: [signature]

PATENT COOPERATION TREAT

From the INTERNATIONAL BUREAU 273

PCT

SECOND AND SUPPLEMENTARY NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION (TO DESIGNATED OFFICES WHICH APPLY THE 30 MONTH TIME LIMIT UNDER ARTICLE 22(1))

(PCT Rule 47.1(c))

To:

ELRIFI, Ivor, R.
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P.C

666 Third Avenue
New York, NY 10017
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 29 December 2005 (29.12.2005)	
Applicant's or agent's file reference 24852-501CIP5 061	**IMPORTANT NOTICE**
International application No. PCT/US2004/027943	International filing date *(day/month/year)* 26 August 2004 (26.08.2004)
Priority date *(day/month/year)* 26 August 2003 (26.08.2003)	
Applicant ATON PHARMA, INC. et al	

1. **ATTENTION**: For any designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002 (30 months from the priority date), **does not apply**, please see Form PCT/IB/308(First Notice) issued previously.

2. Notice is hereby given that the following designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002, **does apply**, has/have requested that the communication of the international application, as provided for in Article 20, be effected under Rule 93*bis*.1. The International Bureau has effected that communication on the date indicated below:
03 March 2005 (03.03.2005)

AU, AZ, BY, CN, CO, DZ, EP, HU, KG, KP, KR, MD, MK, MZ, NA, RU, SY, TM, US

In accordance with Rule 47.1(c-*bis*)(i), those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

3. The following designated Offices, for which the time limit under Article 22(1), as in force from 1 April 2002, **does apply**, have not requested, as at the time of mailing of the present notice, that the communication of the international application be effected under Rule 93*bis*.1 :

AE, AG, AL, AM, AP, AT, BA, BB, BG, BR, BW, BZ, CA, CR, CU, CZ, DE, DK, DM, EA, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, ID, IL, IN, IS, JP, KE, KZ, LC, LK, LR, LS, LT, LV, MA, MG, MN, MW, MX, NI, NO, NZ, OA, OM, PG, PH, PL, PT, RO, SC, SD, SG, SK, SL, TJ, TN, TR, TT, UA, UZ, VC, VN, YU, ZA, ZW

In accordance with Rule 47.1(c-*bis*)(ii), those Offices accept the present notice as conclusive evidence that the Contracting State for which that Office acts as a designated Office does not require the furnishing, under Article 22, by the applicant of a copy of the international application.

4. **TIME LIMITS for entry into the national phase**

For the designated or elected Office(s) listed above, the applicable time limit for entering the national phase will, **subject to what is said in the following paragraph,** be **30 MONTHS** from the priority date.

In practice, **time limits other than the 30-month time limit** will continue to apply, for various periods of time, in respect of certain of the designated or elected Office(s) listed above. For **regular updates on the applicable time limits** (30 or 31 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at http://www.wipo.int/pct/en/index.html.

It is the applicant's **sole responsibility** to file and obtain these time limits.

Done By
☐ File Folder
☒ Data Entry
☐ Docket Entry
☐ Docket Cross Off
☐ Previously Entered
☐ No Docketing Req
☐ Order Copies
☐ Annuities

RECEIVED
JAN 05 2006
Mintz Levin - NYC

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Nora Lindner
Facsimile No.+41 22 740 14 35	Facsimile No.+41 22 338 89 65

Form PCT/IB/308(Second and Supplementary Notice) (January 2004)

PATENT COOPERATION TREATY

Date by
- ☐ File Folder
- ☒ Data Entry
- ☐ Docket Entry
- ☐ Docket Cross Off
- ☐ Previously Entered
- ☐ No Docketing Req
- ☐ Order Copies
- ☐ Annuities

274

From the RECEIVING OFFICE

PCT

To

IVOR R. ELRIFI
MINTZ, LEVIN, COHN, FERRIS, GLOVSKY & POPEO, PC.
666 THIRD AVENUE
NEW YORK, NEW YORK 10017

NOTIFICATION RELATING TO PRIORITY CLAIM

(PCT Rules 26*bis*.1 and 26*bis*.2 and Administrative Instructions, Sections 302 and 314)

Date of mailing *(day/month/year)*	05 NOV 2004
Applicant's or agent's file reference: 24852-501CIP5 061	**IMPORTANT NOTIFICATION**
International application No.: PCT/US2004/027943	International filing date *(day/month/year)*: 26 Aug 2004
Applicant: ATON PHARMA, INC.	

The applicant is hereby **notified** of the following in respect of the priority claim(s) made in the international application.

1. ☐ **Correction of priority claim.** In accordance with the applicant's notice received on : the following priority claim has been corrected to read as follows.

   ☐ even though the indication of the number of the earlier application is missing.
   ☐ even though the following indication in the priority claim is not the same as the corresponding indication appearing in the priority document·

2. ☐ **Addition of priority claim.** In accordance with the applicant's notice received on _____ the following priority claim has been added·

   ☐ even though the indication of the number of the earlier application is missing.
   ☐ even though the following indication in the priority claim is not the same as the corresponding indication appearing in the priority document:

3. ☐ **As a result of the correction and/or addition** of (a) priority claim(s) under items 1 and/or 2, **the (earliest) priority date** is:

4. ☐ The priority claim *(see also item 5, below, if applicable)* is **considered not to have been made** because:

   ☐ the applicant failed to respond to the invitation under Rule 26*bis* 2(a) (Form PCT/RO/110) within the prescribed time limit
   ☐ the applicant's notice was received after the expiration of the prescribed time limit under Rule 26*bis* 1(a)
   ☐ the applicant's notice failed to correct the priority claim so as to comply with the requirements of Rule 4.10.

   The applicant may, before the technical preparations for international publication have been completed and subject to the payment of a fee, request the International Bureau to publish, together with the international application, information concerning the priority claim See Rule 26*bis* 2(c) and the *PCT Applicant's Guide*, Volume I, Annex B2(IB).

5. ☒ In case where **multiple priorities** have been claimed, the above item(s) relate to the following priority claim(s):
   10/665,079, FILED 16 SEPT 03

6. A copy of this notification has been sent to the International Bureau and
   ☒ to the International Searching Authority

Name and mailing address of the receiving Office	Authorized officer
Mail Stop PCT, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450	Mamie Holmes
Facsimile No 703-305-3230	Telephone No 703-305-3664

Form PCT/RO/111 (July 1998, reprint January 2004)

RECEIVED

NOV 09 2004

Mintz Levin - NYC

Done By
- ☐ File Folder
- ☒ Data Entry
- ☐ Docket Entry
- ☐ Docket Cross Off
- ☐ Previously Entered
- ☐ No Docketing Req
- ☐ Order Copies
- ☐ Annuities

Fax 275

PATENT COOPERATION TREATY

PCT

NOTIFICATION RELATING TO PRIORITY CLAIM

(PCT Rules 26bis.1 and 26bis.2 and Administrative Instructions, Sections 402(c) and 409)

From the INTERNATIONAL BUREAU

To:

ELRIFI, Ivor, R.
Mintz, Levin, Cohn, Ferris,
Glovsky and Popeo, P.C
.
666 Third Avenue
New York, NY 10017
United States of America

Date of mailing (day/month/year) 16 November 2004 (16.11.2004)	
Applicant's or agent's file reference 24852-501CIP5 061	**IMPORTANT NOTIFICATION**
International application No. PCT/US2004/027943	International filing date (day/month/year) 26 August 2004 (26.08.2004)
Applicant ATON PHARMA, INC. et al	

The applicant is hereby **notified** of the following in respect of the priority claim(s) made in the international application.

1. ☒ **Correction of priority claim.** In accordance with the applicant's notice received on: 27 October 2004 (27.10.2004), the following priority claim has been corrected, according to paragraph 176 of the PCT Receiving Guidelines where application, to read as follows:

   US 16 September 2003 (16.09.2003) 10/665,079

   ☐ even though the indication of the number of the earlier application is missing.
   ☐ even though the following indication in the priority claim is not the same as the corresponding indication appearing in the priority document:

2. ☐ **Addition of priority claim.** In accordance with the applicant's notice received on: , the following priority claim has been added:

   ☐ even though the indication of the number of the earlier application is missing.
   ☐ even though the following indication in the priority claim is not the same as the corresponding indication appearing in the priority document:

3. ☐ As a **result of the correction and/or addition** of (a) priority claim(s) under items 1 and/or 2, the (earliest) priority date is:

4. ☐ **Priority claim considered not to have been made.**
   ☐ The applicant failed to respond to the Invitation under Rule 26bis.2(a) (Form PCT/IB/316) within the prescribed time limit.
   ☐ The applicant's notice was received after the expiration of the prescribed time limit under Rule 26bis.1(a).
   ☐ The applicant's notice failed to correct the priority claim so as to comply with the requirements of Rule 4.10.

   The applicant may, before the technical preparations for international publication have been completed and subject to the payment of a fee, request the International Bureau to publish, together with the international application, information concerning the priority claim. See Rule 26bis.2(c) and the PCT Applicant's Guide, Volume I, Annex B2(IB).

5. ☒ Where **multiple priorities** have been claimed, the above item(s) relate(s) to the following priority claim(s):

   US 16 September 2003 (16.09.2003) 10/665,079

RECEIVED
NOV 29 2004
Mintz Levin - NYC

6. A copy of this notification has been sent to the receiving Office and
   ☒ to the International Searching Authority (where the international search report and the written opinion of the International Searching Authority have not yet been issued).
   ☐ the designated Offices (in accordance with Rule 93bis).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Anman QIU
Facsimile No. (41-22) 338.89.65	Telephone No. (41-22) 338 9098

Form PCT/IB/318 (January 2004)

006506739

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To

ELRIFI, Ivor, R.
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P C

666 Third Avenue
New York, NY 10017
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 20 October 2004 (20 10.2004)	
Applicant's or agent's file reference 24852-501CIP5 061	**IMPORTANT NOTIFICATION**
International application No. PCT/US04/027943	International filing date *(day/month/year)* 26 August 2004 (26.08.2004)
International publication date *(day/month/year)*	Priority date *(day/month/year)* 26 August 2003 (26.08.2003)
Applicant ATON PHARMA, INC et al	

1. By means of this Form, which replaces any previously issued notification concerning submission or transmittal of priority documents, the applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to all earlier application(s) whose priority is claimed Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to a date of receipt, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17 1(a) or (b)

2 *(If applicable)* The **letters "NR"** appearing in the right-hand column denote **a priority document which, on the date of mailing of this Form, had not yet been received by the International Bureau** under Rule 17 1(a) or (b) Where, under Rule 17 1(a), the priority document must be submitted by the applicant to the receiving Office or the International Bureau, but the applicant fails to submit the priority document within the applicable time limit under that Rule, **the attention of the applicant is directed** to Rule 17 1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances

3 *(If applicable)* **An asterisk (*)** appearing next to a date of receipt, in the right-hand column, denotes **a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b)** (the priority document was received after the time limit prescribed in Rule 17.1(a) or the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17 1(b)) Even though the priority document was not furnished in compliance with Rule 17.1(a) or (b), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17 1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
26 August 2003 (26 08 2003)	10/650,025	US	04 October 2004 (04.10.2004)

RECEIVED
NOV 08 2004
Mintz Levin - NYC

Done By
- ☐ File Folder
- ☑ Data Entry
- ☐ Docket Entry
- ☐ Docket Cross Off
- ☐ Previously Entered
- ☐ No Docketing Req
- ☐ Order Copies
- ☐ Annuities

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No +41 22 740 14 35

Authorized officer
Smahi Abderrezak

Facsimile No +41 22 338 89 65
Telephone No +41 22 338 8021

WO 2005/018578
PCT/US2004/027943

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

CDH-277

PCT

NOTIFICATION CONCERNING
TRANSMITTAL OF COPY OF INTERNATIONAL
APPLICATION AS PUBLISHED OR REPUBLISHED

To.

ELRIFI, Ivor, R.
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P.C
.
666 Third Avenue
New York, NY 10017
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 12 May 2005 (12.05.2005)	
Applicant's or agent's file reference 24852-501CIP5 061	**IMPORTANT NOTICE**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2004/027943	26 August 2004 (26.08.2004)	26 August 2003 (26.08.2003)

Applicant
ATON PHARMA, INC. et al

The International Bureau transmits herewith the following documents:

☐ copy of the international application as published by the International Bureau on under No. WO

☑ copy of international application as republished by the International Bureau on 12 May 2005 (12.05.2005) under No. WO 2005/018578
For an explanation as to the reason for this republication of the international application, reference is made to INID codes (15), (48) or (88) (*as the case may be*) on the front page of the attached document.

RECEIVED
MAY 17 2005
Mintz Levin - NYC

Done By
☐ File Folder
☑ Data Entry
☐ Docket Entry
☐ Docket Cross Off
☐ Previously Entered
☐ No Docketing Req
☐ Order Copies
☐ Annuities

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.+41 22 740 14 35

Authorized officer
Nora Lindner

Facsimile No.+41 22 338 89 65

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 March 2005 (03.03.2005)

PCT

(10) International Publication Number
WO 2005/018578 A3

(51) International Patent Classification[7]: A61K 31/185

(21) International Application Number:
PCT/US2004/027943

(22) International Filing Date: 26 August 2004 (26.08.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

10/650,025	26 August 2003 (26.08.2003)	US
10/665,079	16 September 2003 (16.09 2003)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US	10/650,025 (CON)
Filed on	26 August 2003 (26.08.2003)
US	10/655,079 (CON)
Filed on	16 September 2003 (16.09.2003)

(71) Applicant: ATON PHARMA, INC. [US/US], 33 Avenue Louis Pasteur, Boston, MA 02115 (US).

(71) Applicant *(for all designated States except US)*: SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH [US/US]; 1275 York Avenue, New York, NY 10021 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: BACOPOULOS, Nicholas, G. [US/US]; 2109 Broadway, Apartment 15-109, New York. NY 10023 (US). CHIAO, Judy, H. [US/US], 110 Cedar Green Lane, Berkeley Heights, NJ 07922 (US). MILLER, Thomas, A. [US/US], 517 West 113th Street, Number 82. New York, NY 10025 (US). PARADISE, Carolyn, M. [US/US]; 6 Gilman Lane, Cortland Manor, NY 10567 (US). RICHON, Victoria, M. [US/US]; 184 Highland Road, Rye, NY 10580 (US).

(74) Agent: ELRIFI, Ivor, R.; Mintz, Levin, Cohn, Ferris, Glovsky and Popeo. P.C. .. 666 Third Avenue, New York. NY 10017 (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, HR, HU, ID, IL, IN, IS. JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU. LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO. NZ, OM, PG. PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, 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, IT, LU, 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).

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
12 May 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD OF TREATING CANCER WITH HDAC INHIBITORS

(57) Abstract: The present invention relates to methods of treating cancers, e g., mesothelioma or lymphoma. More specifically, the present invention relates to methods of treating mesothelioma or diffuse large B-cell lymphoma (DLBCL), by administration of pharmaceutical compositions comprising HDAC inhibitors, e.g., suberoylanilide hydroxamic acid (SAHA) The oral formulations of the pharmaceutical compositions have favorable pharmacokinetic profiles such as high bioavailability and surprisingly give rise to high blood levels of the active compounds over an extended period of time. The present invention further provides a safe, daily dosing regimen of these pharmaceutical compositions, which is easy to follow, and which results in a therapeutically effective amount of the HDAC inhibitors *in vivo*

WO 2005/018578 A3

279

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/27943

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 31/185
US CL : 514/616
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/616

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database Caplus on STN, Accession No. DN134:293668, "Negative regulation of bcl-2 expression by p53 in hematopoietic cells." abstract, Wu et al, 2001, Vol. 20, No. 2, pages 240-251.	1-68
A,P	US 2004/0127523 A1 (BACOPOULOS et al.) 01 July 2004, see entire document.	1-68
A,P	US 2003/0161830 A1 (JACKSON et al.) 28 August 2003(28.08.2003), see entire document.	1-68

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

* 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 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

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

"&" document member of the same patent family

Date of the actual completion of the international search
21 January 2005 (21.01.2005)

Date of mailing of the international search report
07 MAR 2005

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703) 305-3230

Authorized officer
Phyllis G. Spivack

Telephone No. 571-272-1600

Form PCT/ISA/210 (second sheet) (January 2004)

FORMATO DE DIGITALIZACION
RELACION DE ANEXOS

Royal Technologies S.A.

280

Item		Expediente No 06030143	No de Folio
		No de unidades	√
1	CD		
2	DVD		
3	REVISTA		
4	LIBRO		
5	PERIODICO		
6	DISQUETES		
7	SOBRE DE MANILA	1	1
8	SOBRE BLANCO TAMAÑO CARTA		
9	SOBRE BLANCO TAMAÑO OFICIO		
10	OTROS:		

Observaciones: Sobre con Artes Finales ff 3

281

SUPERINDUSTRIA Y COMERCIO
Radicacion : 06030143 00000000 Folios: 268
Fecha (AMD): 2006-03-27 17:41:38
Tramite : 811 PCT-PATENT 378 FASE NACI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

Industria y Comercio

SUPERINTENDENCIA

Delegatura de Propiedad Industrial
División de Nuevas Creaciones

REQUISITOS NECESARIOS PARA PRESENTACIÓN Y AGILIZACIÓN DEL TRAMITE

	USUARIO	RADICADOR
PATENTE DE INVENCIÓN (✓) MODELO DE UTILIDAD ( )	( )	( )
-Indicación de que se solicita la concesión de una patente. ✓	( )	( )
-Datos de identificación del solicitante o de la persona que se presenta la solicitud. ✓	( )	( )
-Descripción de la invención ✓	( )	( )
-Dibujos de ser estos pertinentes ✗	( )	( )
-Comprobante de Pago de las tasas establecidas. ✓	( )	( )
COMPLETA ( ) INCOMPLETA ( )	( )	( )
DISEÑO INDUSTRIAL ( )		
-Indicación de que se solicita el registro de Diseño Industrial.	( )	( )
-Datos de identificación del solicitante o de la persona que se presenta la solicitud.	( )	( )
-Representación gráfica y fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño.	( )	( )
-Comprobante de Pago de las tasas establecidas.	( )	( )
COMPLETA ( ) INCOMPLETA ( )	( )	( )
ESQUEMA DE TRAZADO ( )		
-Indicación de que se solicita el registro de Diseño Industrial.	( )	( )
-Datos de identificación del solicitante o de la persona que se presenta la solicitud.	( )	( )
-Representación gráfica del Esquema Trazado.	( )	( )
-*Comprobante de Pago de las tasas establecidas.*	( )	( )
COMPLETA ( ) INCOMPLETA ( )	( )	( )

Firma Responsable: _____

Fecha: marzo 27 de 2006.