PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:
A61K 31/215	A1	WO 93/12782
		(43) International Publication Date: 8 July 1993 (08.07.93)

(21) International Application Number: PCT/US92/09377

(22) International Filing Date: 29 October 1992 (29.10.92)

(30) Priority data:
07/785,982 31 October 1991 (31.10.91) US

(71)(72) Applicants and Inventors: ELFORD, Howard, L. [US/US]; 3313 Gloucester Road, Richmond, VA 23227 (US). VAN'T RIET, Bartholomeus [US/US]; 3419 Noble Avenue, Richmond, VA 23222 (US).

(74) Common Representative: ELFORD, Howard, L.; 3313 Gloucester Road, Richmond, VA 23227 (US).

(81) Designated States: JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG).

Published
With international search report.

(54) Title: METHOD OF TREATING VIRAL DISEASES

(57) Abstract

A therapeutic process for treating disease in mammals caused by DNA viruses or retroviruses, in which the infected mammal is treated with a polyhydroxy benzoic, mandelic or phenylacetic acid derivative, such as N,3,4-trihydroxybenzamide.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SK	Slovak Republic
CI	Côte d'Ivoire	KZ	Kazakhstan	SN	Senegal
CM	Cameroon	LI	Liechtenstein	SU	Soviet Union
CS	Czechoslovakia	LK	Sri Lanka	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	UA	Ukraine
DK	Denmark	MG	Madagascar	US	United States of America
ES	Spain	ML	Mali	VN	Viet Nam
FI	Finland	MN	Mongolia		

METHOD OF TREATING VIRAL DISEASES

BACKGROUND OF THE INVENTION

This invention relates to a method of suppressing the growth or reproduction of DNA viruses and of retroviruses in mammals infected with one or more of said viruses. The term "DNA virus" is defined as being a virus with a DNA genome and the term "retrovirus" is defined as being a RNA virus having a reverse transcriptase (RNA-dependent DNA polymerase) within the virion, according to the TEXTBOOK OF HUMAN VIROLOGY, 2nd ed. R. B. Belshe Ed., (Mosby, St. Louis 1991). Among the DNA viruses whose growth is suppressed in vivo by the therapeutic processes of this invention are included the Paroviruses which can infect both rodents (Kilham's virus, Aleutian mink disease), feline, dogs and humans (Parovirus strain B19 and RA-1 virus), the Papovaviruses which include the Papilloma viruses which can cause human papilloma (warts) and infect rabbits (Shope papillomavirus), and the Polyomaviruses which infect primates (vacuolating virus of Rhesus monkeys and JC and BK viruses of man). JC virus is associated with progressive multifocal leukoencephalopathy (PML). Other DNA viruses which can have their in vivo growth in mammals suppressed by the processes of this invention include the Adenoviruses which cause acute respiratory diseases, febrile catarrhs, pharyngitis and conjunctivitis of man, the Herpes viruses which causes both latent and persistent infection in man including Epstein-Barr herpesvirus (Lymphocryptovirus) associated with human Burkitt's lymphoma, nasopharyngeal carcinoma and causes infectious mononucleosis in man; Herpes simplex which causes both fever

SUBSTITUTE SHEET

blisters and genital herpes among other human diseases as well as chicken pox, Simian B virus, often fatal to man; Herpes zoster (Varicellovirus) which causes shingles in man; Betaherpes viruses which are associated with cytomegalic inclusion disease and infection of T and B cell leukocytes; the hepadnaviruses which, upon infection in man are associated with hepatitis, hepatocellular carcinoma and immunecomplex-mediated extrahepatic injury including both hepatitis B and C, and, in domestic animals, swine pseudorabies as well as other viruses which cause disease in both cattle and horses. Retroviral diseases which are treatable by the therapeutic processes of this invention include the RNA tumor viruses (oncoviruses) and the slow viruses (lentiviruses). Specific oncovirus diseases include, in man, T-cell leukemia, human T-cell lymphotropic viruses (HTLV-I and II) as well as the murine viruses including Friend leukemia virus and Rauscher and Moloney murine leukemia viruses. In addition, there are included leukemic diseases of cats, birds, hamsters and primates other than man. Among the lentivirus diseases, HIV which causes AIDS in man is the most prominent.

One of the major problems in finding treatment methods for persons infected with one or more DNA or retroviruses is the occurrence of an asymptomatic viremia lasting from several weeks to several years or, in the case of Herpes simplex infections, of recurrent episodes after periods of dormancy while the human subject still harbors the virus. Thus, the ideal method of treating a retroviral disease would be to use a drug that can destroy all cells harboring latent virus, rather

SUBSTITUTE SHEET

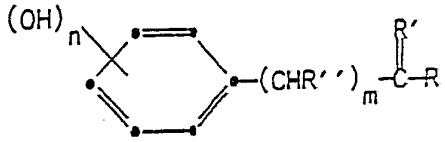
than simply finding a drug which is only useful after an initial exposure and acts by preventing implanting of the virus or provirus into host chromosomal cells or by inhibiting other early steps in the viral replication cycle. Another therapeutic approach would be to find a drug capable of stimulating the immune system of the host, AZT, the first drug currently approved for the treatment of AIDS, is targeted against reverse transcriptase, an enzyme with a critical function in the early part of the retroviral life cycle. Unfortunately, AZT can cause anemia after prolonged administration.

A second major problem which has hindered the search for effective anti-AIDS drugs has been the lack of an animal model. Ruprecht et al CANCER RESEARCH (SUPPL.) 50 5618a Sept. 1, 1990 describe and evaluate the various models then available for evaluating antiretroviral therapy. Johnson et al ibid 5682 have described a useful murine model which utilizes the retrovirus, Friend leukemia virus, in what is described as a Friend Virus-induced ErythroLeukemia Model. This system for the first time enables investigators to evaluate various anti-retroviral treatment methods and drugs useful in such methods as candidates for the therapy of AIDS and other retroviral diseases.

SUBSTITUTE SHEET

SUMMARY OF THE INVENTION

This invention provides a therapeutic process for the treatment of diseases caused by DNA viruses or retroviruses in mammals which comprises administering to a mammal suffering from one or more of said viral diseases and in need of treatment, an amount of a drug represented by the following formula sufficient to suppress the growth of said virus in said mammal



wherein n is 2-5, m is 0 or 1, R is NH_2, OC_{1-3} alkyl or O-phenyl, R' is O, NH or NOH and R" is H or OH. Also included are the pharmaceutically-acceptable salts of compounds according to the above formula where chemically feasible. Also included within the scope of this invention are the phenolic acetyl derivatives of compounds according to the above formula. Such acetyl derivatives act as "pro-drugs" in that they are converted by the mammalian body to the corresponding compound having entirely unesterified phenolic hydroxyls, which are the therapeutically active drugs.

Illustrative of the polyhydroxy-substituted phenyl ring in the above formula are included 2,3-dihydroxyphenyl, 3,4-dihydroxyphenyl, 2,5-dihydroxyphenyl, 2,6-dihydroxyphenyl, 2,3,4-trihydroxyphenyl, 2,3,5-trihydroxyphenyl, 3,4,5-trihydroxyphenyl, 2,4,5-trihydroxyphenyl, pentahydroxyphenyl, 2,3,4,5-tetrahydroxyphenyl and the like groups.

In the above formula, when m is 1 and R" is H, a

phenylacetic acid derivative is denominated. When m is 1 and R" is OH, a mandelic acid derivative is represented. When m is O, R is NHOH and R' is O, an N-hydroxybenzamide (formerly, a benzohydroxamic acid) is represented; when R is NH_2 and R' is NH, a benzimidamide (formerly a benzamidine) is represented; when R is NHOH and R' is NH, an N-hydroxy benzimidamide (formerly a benzamidoxime) is shown; when R is NHOH and R' is NOH, an N,N'-dihydroxy benzimidamide (formerly, an hydroxyamidoxime) is represented; and when R is O-alkyl or O-phenyl and R' is NH, the resulting compounds are benzimidates (rather than benzamidates as previously). In the above formula, when R is OC_{1-3} alkyl, the alkyl groups represented include methyl, ethyl, isopropyl and n-propyl.

Compounds represented by the above formula are fully illustrated in United States Patents 4,253,322, 4,623,659, 2,848,430 and 3,629,443. Methods for their preparation are also fully disclosed in those patents as well as in the many references cited therein. In particular, the compounds listed in Cols. 2 and 3 of United States Patent 4,623,659 illustrate the scope of the compounds represented by the above formula (always remembering that the approved nomenclature for these structures has changed since 1983 when the application which resulted in that patent was filed) and the disclosure of United States Patent 4,623,659 is incorporated herein, and made a part of, by reference.

It will be apparent to those skilled in the art that the therapeutic processes of this invention are directed to the treatment of diseases caused by DNA viruses and by one class of

RNA viruses, the retroviruses, other viruses containing an RNA genome among those listed in THE TEXTBOOK OF HUMAN VIROLOGY (loc. cit.) may be successfully treated and their replication prevented in vivo by administration of a drug according to the above formula.

It will also be apparent to those skilled in the art that other phenolic blocking groups, such as the acetyl group specified above, may be employed, including esters of other lower alkanolic acids, as well as phenacyl esters and the like, to yield potential pro-drugs, only provided that the ester group on a phenolic hydroxyl is removable by the mammalian host to whom it was administered.

As previously stated, the therapeutic processes disclosed by this invention are useful in suppressing the growth or reproduction of DNA viruses and retroviruses in mammals, including primates such as new-world monkeys, and the great apes including man, in rodents, ungulates (both cattle and sheep), in horses, swine, mink, etc. An illustration of such therapeutic process, utilizing the retrovirus Friend leukemia virus in mice, is illustrated in the next section.

SUBSTITUTE SHEET

BIOLOGICAL TESTING

To test the ability of the compounds of the above formula to suppress the growth of retroviruses in vivo, the following protocol was used: six week old female mice (B6D2/F1 hybrids) were infected with Friend leukemia virus (FLV) on day 0. The FLV employed is classified as a murine leukosis sarcoma complex retrovirus that causes red blood cell tumors in the spleen resulting in splenomegaly and was obtained from the American Type Culture Collection, ATCC No. VR-245. The drug was administered parenterally (via the intraperitoneal route) daily for 12 days starting on day 1. The mice were sacrificed on day 14 for collection of spleen, liver and blood. In an initial experiment, the treatment groups included the following: Didox (N,3,4-trihydroxybenzamide) at 450 mg/kg, Amidox (N,3,4-trihydroxybenzimidamide as the HCl salt) at 225 mg/kg, FLV-infected saline control, saline control. There were four mice per treatment group. Table 1 below gives the spleen and liver weights for the four groups.

TABLE 1

Relative Spleen and Liver Weights, Didox and Amidox

TREATMENT GROUP	SPLEEN WT.**	LIVER WT.**
	Mean $\pm$ S.E.	
Saline Control	0.33 ± 0.1*	$4.76 \pm .11$
FLV-Saline	6.20 ± 1.73	$5.62 \pm .40$
FLV-Didox	$0.47 \pm .06$*	$4.88 \pm .26$
FLV-Amidox	$1.48 \pm .48$	$4.76 \pm .11$

* = statistically significant at alpha = 0.05

** = % of body weight

SUBSTITUTE SHEET

Blood cell count data on the above groups plus a Didox and an Amidox uninfected group (at the same dose rate as with the infected groups) as a further control gave the results given in Table 2 below.

TABLE 2

Blood Count and Percent Reticulocytes for Table 1 Groups

TREATMENT GROUP		WBC	RBC	HGB	PLT	% RET
FLV-Saline		40.99	9.28	13.83	514.57	10.48
	±	11.27	0.31	0.48	112.97	3.48
Saline		5.00**	10.83*	17.04**	1360.6**	2.70
	±	0.92	0.06	0.16	27.44	0.17
FLV-Didox 450		3.13**	4.23**	6.83**	97.60	1.68
	±	0.73	0.76	1.05	25.94	0.81
FLV-Amidox 225		8.18**	8.79	13.52	438.98	2.60
	±	1.32	0.29	0.54	115.20	0.95
Didox 450		2.84**	8.87	14.60	333.74	1.65
	±	0.34	0.33	0.42	53.40	0.32
Amidox 225		4.92**	10.56	16.98*	1158.2**	1.74
	±	0.60	0.18	0.21	103.97	0.13

* and ** correspond to alpha levels of .05 and .01 respectively.

As can be seen from the data presented in Tables 1-2, Didox reduced the splenomegaly associated with murine FLV infection to control levels, indicating an almost total suppression of the retrovirus. Liver weights were not significantly increased over control weights whereas the FLV-infected mice showed a large increase in liver weight. Both these results demonstrate the effectiveness of Didox in

SUBSTITUTE SHEET

suppressing FLV since FLV infection in mice invariably gives an increase in both spleen and liver weights. Amidox gave a qualitatively similar effect. In the blood, the Didox-treated FLV-infected mice showed an essentially normal WBC (compared to the tremendous increase found in FLV infected mice). RBC and hemoglobin values were decreased compared to FLV-saline controls. Amidox gave similar, but not as pronounced, results. Both Didox and Amidox in the uninfected controls decreased platelet counts, as well as in the infected and the drug-alone control groups. All of the treatment and control groups with Didox and Amidox gave essentially normal reticulocyte levels whereas the FLV-saline controls showed a marked increase in the percentage of reticulocytes.

It is apparent from the above data that Didox, and to a lesser extent, Amidox, at the dose levels tested, are able to suppress growth of the retrovirus, FLV, in FLV-infected mice.

In a further exploration of the ability of Didox to suppress the growth of the retrovirus, FLV, Didox was administered as before to groups of FLV-infected hybrid mice at dose levels varying from 28-450 mg/kg using the previous protocol. In addition, one group of mice treated at the 450 mg/kg level were sacrificed on day 28, rather than day 14 (although treatment was terminated on day 12 as before) as with the other groups of infected mice, in order to determine whether the positive effects seen at 14 days would persist. The results of that and the other experiments are given in Table 3 below.

SUBSTITUTE SHEET

TABLE 3

Relative Spleen and Liver Weights for Didox Treated Mice

TREATMENT GROUP	SPLEEN WT.	LIVER WT.
	Mean $\pm$ S.E.	
FLV-Saline	$3.84^{\pm}.180$	$5.77^{\pm}0.53$
FLV-450 Didox	$0.35^{\pm}0.03$	$5.71^{\pm}0.27$
FLV-450 Didox (28 day)	$1.50^{\pm}0.52$	$5.76^{\pm}0.27$
FLV-225 Didox	$1.89^{\pm}0.69$	$5.67^{\pm}0.19$
FLV-112.5 Didox	$4.39^{\pm}1.56$	$6.12^{\pm}0.51$
FLV-56.25 Didox	$5.40^{\pm}1.81$	$5.95^{\pm}0.57$
FLV-28 Didox	$4.94^{\pm}1.59$	$5.51^{\pm}0.36$
Saline	$0.35^{\pm}0.01$	$4.75^{\pm}0.06$

All numbers in col. 1 refer to mg/kg of Didox for 12 days. Blood studies, as in Table 2, were carried out on the mouse groups in Table 3. Although not statistically significant, WBC, platelet and % reticulocytes were generally lower in the FLV-infected, Didox treated mice. The higher dose levels (450 and 225 mg/kg) gave the most drastic reductions.

It is also apparent that, in FLV-infected mice, only the higher dose levels of Didox were effective to suppress the growth of the virus in vivo. In addition, the 450 mg/kg dose level of Didox demonstrated some long-lasting positive effects in FLV-infected mice long after the therapy had been terminated.

Although two of the compounds represented by the above formula, Didox and Amidox, were administered in saline to retrovirus infected mice by the intraperitoneal route, other parenteral routes (intravenous, intramuscular, intradermal or

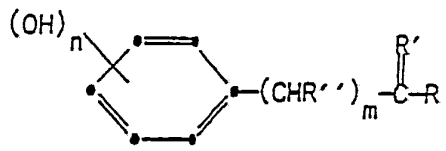
SUBSTITUTE SHEET

intrathecal) could also be used and the compounds formulated for such parenteral routes in accordance with the skill of the art, preferably in isotonic solution. The same considerations apply to oral medications containing Didox or Amidox, i.e., tablets, filled gelatin capsules, or liquid formulations. As will be apparent also to those skilled in the art, the effective dose levels will vary according to the mode of administration. For example, oral dose levels would be higher, and intravenous or intramuscular levels lower in general than intraperitoneal dose levels. Drug carriers may also be employed and the viral suppressive agents of this invention can be combined in a co-ination dosage form with, or be administered at the same time as, other viral suppressive agents, immune stimulators and the like. Phenol-acetylated compounds according to the above formula, although called "pro-drugs" herein, can also be considered as a special type of drug carrier.

SUBSTITUTE SHEET

We claim:

1. A therapeutic procedure for treating mammalian diseases caused by DNA viruses or retroviruses which comprises administering to a DNA virus or retrovirus infected mammal in need of treatment an amount of a compound of the following formula sufficient to at least partially suppress the growth or reproduction of said virus in said mammal



wherein n is 2-5, m is 0 or 1, R is NOH, NH_2, C_{1-3} alkyl or O-phenyl, R' is O, NH, or NOH, R" is H or OH and pharmaceutically-acceptable acid-addition salts and acetylated phenol derivatives thereof.

2. A procedure according to Claim 1 in which the anti-viral drug is N,3,4-trihydroxybenzamide.
3. A procedure according to Claim 1 in which the anti-viral drug is N,3,4,5-tetrahydroxybenzimidamide.
4. A procedure according to Claim 1 in which the infection being treated is caused by a retrovirus.
5. A process according to Claim 1 in which the infection being treated is caused by a DNA virus.
6. A procedure according to Claim 4 in which the retrovirus is HIV-1.
7. A procedure according to Claim 5 in which the DNA virus is a herpes virus.

8. A procedure according to Claim 7 in which the herpes virus is Herpes simplex.

9. A procedure according to Claim 5 in which the DNA virus infection being treated is caused by a hepadnavirus.

10. A procedure according to Claim 9 in which the disease caused by virus is Hepatitis B or C.

11. A procedure according to Claim 1 in which the viral disease is caused by Friend leukemia virus and the infected mammal is a mouse.

12. A procedure according to Claim 1 in which the viral disease being treated is caused by HIV-1 and the infected mammal is man.

13. A procedure according to Claim 1 in which the viral disease being treated is caused by HIV-2 and the infected mammal is man.

14. A procedure according to Claim 4 in which the retrovirus is HIV-2.

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

PCT/US92/09377

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/215
US CL :514/508
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. : 514/544, 575, 678, 637, 640, 641

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)

STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	N, (Chemical Abstracts, Volume 108, no.1, 6009e, issued 1988, January 4, (Columbus, Ohio, U.S.A), Ogata et al, Preparation of N-vinylazoles as antimycotics, cardiotonics and viricides, (Shionogi and Co., Ltd) Eur. Pat. Appl. EP 277,100.	1-14

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date		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
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		document 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
"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	"&"	document member of the same patent family

Date of the actual completion of the international search 11 MARCH 1993	Date of mailing of the international search report 26 MAR 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer RUSSELL TRAVERS Telephone No. (703) 308-1235

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/09377

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	N, (Chemical Abstracts, Volume 97, no. 14, 106184f, issued 1982, April 6 (Columbus, Ohio, U.S.A.), Kjoeller et al, Characterization of the active site and ribonucleotide reductase of Escherichiacoli, bacteriophage T4 and mammalian cells by inhibition studies with hydroxyurea analogs, (Dept. Chem. BC, R. Dan. Sch. Pharm., Copenhagen, DK-2100 Den.). Eur. J. Biochem. 1982, 125, no. 1 pp. 75-81.	1-14
Y	USSR, A, 0,647,940 (BELO EPIDEMOLOGY) 08 January 1976, See entire document.	1-14
Y	US, A, 2,952,583 (FRITTS), 13 September 1960, See entire document.	1-14
Y	US, A, 1,715,251 (SABALITSCHKA) 28 May 1929, See entire document.	1-14
Y	US, A, 4,946,868 (DEMARNE ET AL) 07 August 1990, See entire document.	1-14