

INSTRUCTIONS
(a) If Convention
application insert
"Convention"

60805/86

(a) Convention

AUSTRALIA

Patents Act

(b) Delete one

APPLICATION FOR A (b) STANDARD ~~PATENT~~ PATENT

60 684 7

(c) Insert FULL
name(s) of
applicant(s)

/We (c)

NORWICH EATON PHARMACEUTICALS, INC.

(d) Insert FULL
address(es) of
applicant(s)

of (d)

17 Eaton Avenue,
Norwich, New York 13815,
United States of America

(e) Delete one

hereby apply for the grant of a (e) Standard ~~Patent~~ Patent for an invention entitled

(f) Insert TITLE
of invention

(f)

ACYLPHOSPHOROTRIAMIDES USEFUL AS LIPID-
ALTERING AGENTS

(g) Insert "complete"
or "provisional"
or "petty patent"

which is described in the accompanying (g) COMPLETE specification.

(Note: The following applies only to Convention applications)

Details of basic application(s)

(h) Insert number,
country and
filing date for
the or each
basic application

	Application No.	Country	Filing Date
(h)	761,992	United States of America	1 August 1985

APPLICATION ACCEPTED AND AMENDMENTS

ALLOWED 22-11-90

Address for Service:

PHILLIPS ORMONDE AND FITZPATRICK
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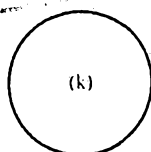
(i) Insert date
of signing

Dated (i) 31 July 1986

(j) Signature of
applicant(s)
(For body
corporate
see headnote*)

(j) PHILLIPS ORMONDE & FITZPATRICK
Attorneys for:
NORWICH EATON PHARMACEUTICALS, INC.

(k) Corporate seal
if any



Note: No legalization
or other witness
required

David B Fitzpatrick

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DECLARATION FOR A PATENT APPLICATION

▼ INSTRUCTIONS

- (a) Insert "Convention" if applicable
 (b) Insert FULL name(s) of applicant(s)

In support of the (a) Convention application made by
 (b) Norwich Eaton Pharmaceuticals, Inc.

- (c) Insert "of addition" if applicable
 (d) Insert TITLE of invention

(hereinafter called "applicant(s)" for a patent (c) for an
 invention entitled (d)
 ACYLPHOSPHOROTRIAMIDES USEFUL AS LIPID-ALTERING AGENTS

- (e) Insert FULL name(s) AND address(es) of declarant(s) (See headnote*)

I/We (e) Richard Charles Witte, Assistant Secretary
 Norwich Eaton Pharmaceuticals, Inc.
 17 Eaton Avenue
 Norwich, New York 13815, U.S.A.

do solemnly and sincerely declare as follows:

1. ~~I am/We are the applicant(s).~~
 (or, in the case of an application by a body corporate)
1. I am/We are authorized to make this declaration on behalf of the applicant(s).
2. ~~I am/We are the actual inventor(s) of the invention.~~
 (or, where the applicant(s) is/are not the actual inventor(s))

- (f) Insert FULL name(s) AND address(es) of actual inventor(s)

2. (f)
 Thomas John Moorehead Allan Vincent Bayless
 89 South Broad Street R. D. #3, Box 126A
 Norwich, New York 13815 Norwich, New York 13815
 both in the United States of America

- (g) Recite how applicant(s) derive(s) title from actual inventor(s) (See headnote**)

is/are the actual inventor(s) of the invention and the facts upon which the applicant(s) is/are entitled to make the application are as follows:

- (g) Applicant is the assignee of the invention from the actual inventor(s).

(Note: Paragraphs 3 and 4 apply only to Convention applications)

- (h) Insert country, filing date, and basic applicant(s) for the/or EACH basic application

3. The basic application(s) for patent or similar protection on which the application is based is/are identified by country, filing date, and basic applicant(s) as follows:
- (h) U.S.S.N. 761,992 filed August 1, 1985; Thomas John Moorehead and Allan Vincent Bayless, in the United States of America

4. The basic application(s) referred to in paragraph 3 hereof was/were the first application(s) made in a Convention country in respect of the invention the subject of the application.

- (k) Insert PLACE of signing

Declared at (k) Cincinnati, Ohio, U.S.A.

- (l) Insert DATE of signing

Dated August 18, 1986

- (m) Signature(s) of declarant(s)

(m)

Richard Charles Witte

Assistant Secretary

Note: No legalization or other witness required

To: The Commissioner of Patents

(12) PATENT ABRIDGMENT (11) Document No. AU-B-60805/86
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 606847

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ACYLPHOSPHOROTRIAMIDES

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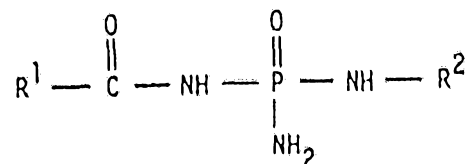
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(56) Prior Art Documents
US 4221730
US 4182881

(57) Claim

1. A method of altering the blood plasma lipid content of a mammal which comprises systemically administering to such mammal an effective but nontoxic amount of a composition comprising a compound conforming to the following chemical structure:



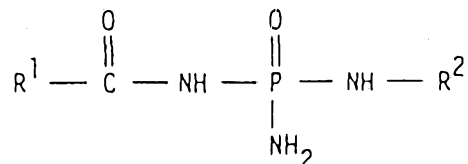
wherein R² is H and R¹ is phenyl or substituted phenyl, pyridyl or substituted pyridyl, furyl or substituted furyl, naphthyl or substituted naphthyl, benzyl or substituted benzyl, or phenyl(lower)alkyl or substituted phenyl(lower)alkyl and salts and/or hydrates thereof, wherein substituents for R¹ are selected from the group consisting of halo, lower straight or branched chain alkyl, lower straight or branched chain alkoxy, amino, nitro, lower straight or branched chain alkylsulfonyl, cyano and phenoxy.

(11) AU-B-60805/86

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(10) 606847

11. A compound of the class of acylphosphorotriamides which conform to the chemical structure:



wherein R^2 is H and R^1 is phenyl or substituted phenyl, pyridyl or substituted pyridyl, furyl or substituted furyl, naphthyl or substituted naphthyl, benzyl or substituted benzyl, or phenyl(lower)alkyl or substituted phenyl(lower)alkyl wherein substituents for R^1 are selected from the group consisting of halo, lower straight or branched chain alkyl, lower straight or branched chain alkoxy, amino, nitro, lower straight or branched chain alkylsulfonyl, cyano and phenoxy, except such compounds wherein R^2 is H and R^1 is 3-pyridyl or 2-furyl or 2-naphthyl or cinnamyl or benzyl or phenyl or phenyl substituted by 3-nitro or 4-nitro or 4-halo or 4-amino or 4-(lower)alkoxy or 4-(lower)alkyl or 2-methyl or 2,3-dimethyl or 2,4-dimethyl or 2,4,6-trimethyl or 3-trifluoromethyl or 3-[(4-aminophenyl)sulfonyl]amino or 4-cyano or 4-phenyl or 3-phenoxy; and salts and hydrates thereof.

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COMPLETE SPECIFICATION

(ORIGINAL)

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60805/86

Class

Int. Class

Lodged:

60 68 47

Complete Specification Lodged:

Accepted:

Published:

Priority

Related Art:

This document contains the
amendments made under
Section 49 and is correct for
printing.

APPLICANT'S REF.: N-483

Name(s) of Applicant(s):

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Thomas John Moorehead
Allan Vincent Bayless

Address for Service is:

PHILLIPS, ORMONDE AND FITZPATRICK
Patent and Trade Mark Attorneys
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Melbourne, Australia, 3000

Complete Specification for the invention entitled:

ACYLPHOSPHOROTRIAMIDES USEFUL AS LIPID-ALTERING AGENTS

The following statement is a full description of this invention, including the best method of performing it known to applicant(s):

11-483
Australia

21a-

ACYLPHOSPHOROTRIAMIDES USEFUL AS LIPID-ALTERING AGENTS

Thomas J. Moorehead
Allan V. Bayless

5

TECHNICAL FIELD

The present invention relates to compounds and compositions which are useful as blood plasma lipid-altering agents, and to the treatment of a host to alter the lipid content of the blood plasma of the host.

10

BACKGROUND OF THE INVENTION

It has been generally established that hyperlipidemia (high levels of lipids in blood plasma) is associated with several health related disfunctions such as atherosclerosis, diabetes, and obesity. Blood cholesterol levels have been particularly implicated in the incidence of atherosclerosis. More particularly, a high risk of cardiovascular disease has been shown to correlate with the ratio of low density lipoprotein (LDL) cholesterol to high density lipoprotein (HDL) cholesterol. Agents which can reduce the ratio of LDL/HDL have been shown to effectively lower the incidence of cardiovascular disease associated with atherosclerosis. While it is preferable to reduce the LDL/HDL ratio by reducing the level of LDL in blood plasma, reduction of the LDL/HDL ratio without significantly reducing the level of LDL has also been shown to correlate with lower risk of cardiovascular disease.

25

More recently, it has been shown that there is a relationship between blood levels of the enzyme pseudocholinesterase and

hyperlipidemia. The possible treatment of hyperlipidemia by inhibition of blood plasma pseudocholinesterase is suggested by Kutty, K.M., R. Redheendran & D. Murphy, "Serum Cholinesterase: Function in Lipoprotein Metabolism", Experientia, Vol. 33 (1977), pp. 420-422. A study published in Kutty, K.M., R. Jain, S.-N. Huang & K. Kean, "Serum Pseudocholinesterase: High Density Lipoprotein Cholesterol Ratio as an Index of Risk for Cardiovascular Disease", Clinica Chimica Acta, Vol. 115 (1981), pp. 55-61, indicates that there is a significant increase in the ratio of plasma pseudocholinesterase/HDL cholesterol in subjects classified as high risk for cardiovascular disease on the basis of the ratio of total cholesterol/HDL cholesterol. The results of this study of 290 subjects suggest that plasma pseudocholinesterase has a parallel relationship with LDL and a reciprocal relationship with HDL. Further studies reported in Jain, R., K.M. Kutty, S.-N. Huang & K. Kean, "Pseudocholinesterase/High-Density Lipoprotein Cholesterol Ratio in Serum of Normal Persons and of Hyperlipoproteinemics", Clinical Chemistry, Vol. 29 (1983), pp. 1031-1033, show that the ratio of plasma pseudocholinesterase/HDL cholesterol is particularly high in subjects with certain types of hyperlipidemia. Similar results showing a correlation between high blood levels of pseudocholinesterase and hyperlipidemia are disclosed in Cucuianu, M., T.A. Popescu & S. Jaragus, "Pseudocholinesterase in Obese and Hyperlipemic Subjects", Clinica Chimica Acta, Vol. 22 (1968), pp. 151-155; Cucuianu, M., T.A. Popescu, A. Opincaru & S. Jaragus, "Serum Pseudocholinesterase and Ceruloplasmin in Various Types of Hyperlipoproteinemia", Clinica Chimica Acta, Vol. 59 (1975), pp. 19-27; Cucuianu, M., A. Opincaru & D. Tapalaga, "Similar Behavior of Lecithin:Cholesterol Acyltransferase and Pseudocholinesterase in Liver Disease and

Hyperlipoproteinemia", Clinica Chimica Acta, Vol. 85 (1978), pp. 73-79; and Cucuianu, M.P., A. Cristea, S. Roman & I. Missits, "Increased Plasma Antithrombin III Level in Hyperlipidemic Subjects", Clinica Chimica Acta, Vol. 110 (1981), pp. 147-155. A study reported in Ando, M., S. Hirosaki, K. Tamura & T. Taya, "Multiple Regression Analysis of the Cholinesterase Activity with Certain Physiochemical Factors", Environmental Research, Vol. 33 (1984), pp. 96-105, shows a significant positive correlation between blood plasma pseudocholinesterase levels and serum total cholesterol content.

Animal studies have shown that agents which inhibit cholinesterase activity will significantly decrease plasma LDL and/or significantly increase plasma HDL levels as reported in Kutty, Redheendran & Murphy, and in Kutty, K.M., J.D. Jacob, C.J. Hutton, P.J. Davis & S.C. Peterson, "Serum Beta-Lipoproteins: Studies of a Patient and in Guinea Pigs After the Ingestion of Organophosphorus Compounds", Clinical Biochemistry, Vol. 8 (1975), pp. 379-383. In Kutty, Jacob, Hutton, Davis & Peterson, it is reported that in a patient accidentally poisoned with parathione, a cholinesterase inhibitor, the blood plasma levels of pseudocholinesterase and LDL were markedly decreased and HDL was increased.

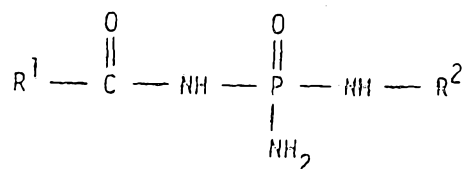
The physiological role of pseudocholinesterase has not been clearly established but in Kutty, K.M., "Review: Biological Function of Cholinesterase", Clinical Biochemistry, Vol. 13 (1980), pp. 239-243, evidence is presented that the enzyme is involved with lipid metabolism.

Several types of organophosphorus compounds are known to have cholinesterase inhibiting characteristics. Although they would

likely be effective at lowering blood plasma levels of pseudocholinesterase, they are highly toxic because they also inhibit acetylcholinesterase, an enzyme involved in neuro transmission.

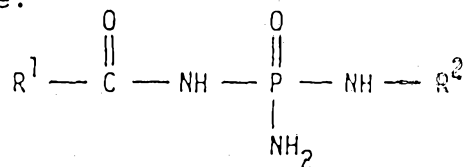
Certain acylphosphorotriamides are known, and it has been disclosed that some of these compounds have activity as inhibitors of the enzyme urease and therefore are useful in the treatment of certain urinary tract infections. U.S. Patent Nos. 4,182,881 issued to Bayless & Millner on January 8, 1980, and 4,221,730 issued to Alaimo, Storrin & Millner on September 9, 1980, disclose certain acylphosphorotriamides and methods of synthesizing them and are hereby incorporated by reference.

The present invention resides in a method of altering the blood plasma lipid content of a mammal which comprises systemically administering to such mammal an effective but nontoxic amount of a composition comprising a compound conforming to the following chemical structure:



wherein R^2 is H and R^1 is phenyl or substituted phenyl, pyridyl or substituted pyridyl, furyl or substituted furyl, naphthyl or substituted naphthyl, benzyl or substituted benzyl, or phenyl(lower)alkyl or substituted phenyl(lower)alkyl and salts and/or hydrates thereof, wherein substituents for R^1 are selected from the group consisting of halo, lower straight or branched chain alkyl, lower straight or branched chain alkoxy, amino, nitro, lower straight or branched chain alkylsulfonyl, cyano and phenoxy.

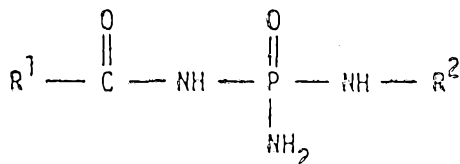
The present invention further resides in a compound of the class of acylphosphorotriamides which conform to the chemical structure:



wherein R^2 is H and R^1 is phenyl or substituted phenyl, pyridyl or substituted pyridyl, furyl or substituted furyl, naphthyl or substituted naphthyl, benzyl or substituted benzyl, or phenyl(lower)alkyl or substituted phenyl(lower)alkyl wherein substituents for the group R^1 are selected from the group consisting of halo, lower straight or branched chain alkyl, lower straight or branched chain alkoxy, amino, nitro, lower straight or branched chain alkylsulfonyl, cyano and phenoxy, except such compounds wherein R^2 is H and R^1 is 3-pyridyl or 2-furyl or 2-naphthyl or cinnamenyl or benzyl or phenyl or phenyl substituted by 3-nitro or 4-nitro or 4-halo or 4-amino or 4-(lower)alkoxy or 4-(lower)alkyl or 2-methyl or 2,3-dimethyl or 2,4-dimethyl or 2,4,6-trimethyl or 3-trifluoromethyl or 3-[(4-aminophenyl)sulfonyl]amino or 4-cyano or 4-phenyl or 3-phenoxy; and salts and hydrates thereof.

DETAILED DESCRIPTION OF THE INVENTION

In one of its aspects, the present invention is a method for altering the blood plasma lipid content of mammals which comprises systemically administering to such mammals an effective but nontoxic amount of a composition comprising certain acylphosphorotriamides as lipid-altering agents. The acylphosphorotriamides found to be useful as lipid-altering agents conform to the following chemical structure:



wherein R^2 is H or straight or branched chain lower alkyl and R^1 is phenyl or substituted phenyl, pyridyl or substituted pyridyl, furyl or substituted furyl, naphthyl or substituted naphthyl, benzyl or substituted benzyl, or phenyl(lower)alkyl or substituted phenyl(lower)alkyl wherein



substituents for the group R^1 are selected from the group consisting of halo, lower straight or branched chain alkyl, lower straight or branched chain alkoxy, amino, nitro, lower straight or branched chain alkylsulfonyl, cyano and phenoxy; and salts and/or hydrates thereof (Compounds A).



naphthyl or substituted naphthyl, benzyl or substituted benzyl, or phenyl(lower)alkyl or substituted phenyl(lower)alkyl. More preferred are such compounds wherein R^1 is phenyl or substituted phenyl, furyl, or pyridyl. Still more preferred are such compounds wherein R^1 is 2-furyl, 3-pyridyl, phenyl, or 4-substituted phenyl wherein said phenyl substituent is selected from the group consisting of halo, lower straight or branched chain alkyl, lower straight or branched chain alkoxy, amino, nitro, lower straight or branched chain alkylsulfonyl, cyano, phenoxy or substituted phenoxy. Especially preferred are such compounds wherein R^1 is phenyl, 4-methoxyphenyl, 4-fluorophenyl, 3-fluorophenyl, 2-fluorophenyl, 4-methylphenyl, or 4-aminophenyl.

Preferred acylphosphorotriamides useful in the present invention include compounds which conform to the chemical structure of Compounds A hereinabove, but wherein R^2 is H.

Compounds which are useful in the present invention are potent irreversible inhibitors of pseudocholinesterase which exhibit minimal inhibition of acetylcholinesterase at the same concentration. The following method was used to determine the relative inhibition of pseudocholinesterase and acetylcholinesterase by compounds of the present invention.

TEST METHOD 1

Plasma (Source of Pseudocholinesterase Enzyme)

Human plasma was obtained from blood which was collected in ethylenediaminetetraacetic acid (EDTA) treated tubes. Collected plasma was stored frozen at -20°C until use.

Lysed Red Blood Cells (RBC's) (Source of Acetylcholinesterase Enzyme)

Human blood which was collected in EDTA treated tubes was centrifuged at 1500 x g for 10 min. at 4°C. One volume of chilled
5 0.9% saline solution was gently mixed with one volume of packed RBC's. One volume of this suspension was then washed three times with ten volumes of chilled 0.9% saline solution (4°C). Each wash was centrifuged at 1500 x g for 10 min. at 4°C and supernatant was discarded. After the last wash the supernatant was replaced with
10 deionized, distilled water (treated by Milli-Q apparatus, Millipore Corp., Bedford, MA) (4°C). After 15 min. at 4°C the suspension was centrifuged at 1500 x g for 10 min. at 4°C. The supernatant (lysed RBC's) was stored frozen at -20°C until use.

Buffer/Chromogen

15 The buffer/chromogen solution was 0.25 mM 5,5'-dithiobis-[2-nitrobenzoic acid] (chromogen), 50 mM phosphate buffer, pH 7.2. In making up the buffer/chromogen solution and to keep it as colorless as possible, the phosphate buffer was first adjusted to pH 7.1 with NaOH; the chromogen was then added and dissolved; the
20 pH was adjusted to 7.2; and the final volume was adjusted with addition of Milli-Q water. 5,5'-dithiobis-(2-nitrobenzoic acid) was obtained from Sigma Chemicals, St. Louis, MO.

Substrate

The substrate solution for the pseudocholinesterase assay was
25 156 mM S-butyrylthiocholine iodide, and the substrate solution for the acetylcholinesterase assay was 156 mM S-acetylthiocholine iodide. Both substrate compounds were obtained from Sigma Chemicals.

Test Compound Stock Solutions

Solutions of all test compounds were made up at 2 μM , 20 μM , and 200 μM concentrations (in 0.9% saline solution) except as noted where lower concentrations were necessary because of decreased
5 solubility of the compound in saline solution.

Procedure

Pseudocholinesterase (PChE) Assay

In three separate tubes, a 100 μl aliquot of either 0.9% saline solution, 2 μM test compound solution, or 20 μM test
10 compound solution was mixed with a 100 μl aliquot of human plasma, and the tubes were placed in a 35°C water bath. Four cuvettes, each containing 3 ml buffer/chromogen solution mixed with 100 μl substrate solution (156 mM S-butyrylthiocholine iodide), had been placed in a heated cuvette holder (35°C) in a
15 Beckman DU-8B Spectrophotometer (Beckman Instruments). After the temperature of the buffer/chromogen/substrate solution in the cuvette had equilibrated for about 10 minutes, and after 0 or 30 minutes incubation of the plasma/test compound solution, a 25 μl aliquot of the plasma/test compound
20 solutions was added to the corresponding cuvettes numbered 2-4. Cuvette #1 remained a blank. After mixing, the net absorbance of each cuvette was determined at 405 nm every 35 seconds for ten readings. From a plot of net absorbance versus time, the initial linear portion of the curve was
25 determined, and a rate of absorbance change per minute was calculated. The positive control (100% PChE activity) was the plasma/saline solution.

Acetylcholinesterase (AChE) Assay

The acetylcholinesterase assay was performed identically to the pseudocholinesterase assay with the following exceptions. The concentrations of the stock solutions of test compounds were 20 μM and 200 μM , instead of 2 μM and 20 μM , and the substrate solution was 156 mM S-acetylthiocholine iodide instead of S-butyrylthiocholine iodide. The lysed RBC's/test compound solution was incubated for 0 and 60 minutes instead of 0 and 30 minutes before being added to the cuvette containing the buffer/chromogen/substrate solution. From a plot of net absorbance versus time there was an initial period before the curve reached a steady linear rate. From this linear portion of the curve a rate of absorbance change per minute was calculated.

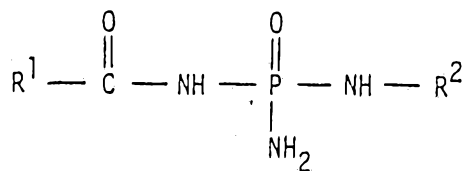
Results

Each compound was tested for its ability to inhibit plasma pseudocholinesterase activity and acetylcholinesterase activity. Because of variation in the time points in the linear portion of the curve, inhibition values below 10-15% and higher than 85-90% are nearly indistinguishable from 0% and 100%, respectively. The values exhibited after 30 minutes of preincubation of enzyme with test compound is considered the irreversible % PChE inhibition; for % AChE inhibition, the values exhibited after 60 minutes of preincubation of enzyme are used.

EXAMPLES I-28

Test compounds having the following chemical structure were tested for pseudocholinesterase and acetylcholinesterase inhibition

according to the above test method:



5 The results of the tests are provided in Table 1:

TABLE 1

Test Compound	R ¹	R ²	% PChE Inhibition		% AChE Inhibition	
			1 μ M	10 μ M	10 μ M	100 μ M
1	-phenyl	-H	92.7	99.1	4.1	37.3
2	-3-pyridyl	-H	84.1	97.6	-1.9	70.7
3	-4-nitrophenyl	-H	90.4	99.0	64.3	86.9
4	-4-fluorophenyl	-H	92.8	99.1	12.4	52.3
10 5	-4-aminophenyl	-H	57.7	98.2	1.8	32.1
6	-4-chlorophenyl	-H	87.7	98.7	5.4	86.5
7	-3-nitrophenyl	-H	65.6	98.9	23.9	86.6
8	-4-methoxyphenyl	-H	83.4	96.3	3.0	34.2
9	-4-methylphenyl	-H	73.7	98.1	9.6	46.0
15 10	-2-methylphenyl	-H	21.4	89.2	8.6	44.5
11	-2-chlorophenyl	-H	49.5	98.5	32.1	87.3
12	-4-cyanophenyl	-H	97.4	98.8	34.4	85.5
13	-3-fluorophenyl	-H	80.2	98.8	-4.0	41.3
14	-3-trifluoromethylphenyl	-H	25.0	94.1	25.7	85.9
20 15	-2-fluorophenyl	-H	97.4	99.3	14.7	82.1
16	-4-(1,1-dimethylethyl)phenyl	-H	46.3	97.9	3.1	38.7
17	-2-naphthyl	-H	27.4 ^a	95.1 ^a	16.0 ^a	87.2 ^a
18	-benzyl	-H	10.7	61.3	17.1	85.2
19	-3-phenoxyphenyl	-H	63.7	98.3	15.8	64.2

TABLE 1 (cont.)

5	Test Compound	R ¹	R ²	% PChE Inhibition		% AChE Inhibition	
				1 μ M	10 μ M	10 μ M	100 μ M
	20	-4-(methylsulfonyl)phenyl	-H	92.9	98.5	20.5	86.4
	21	-2,6-difluorophenyl	-H	74.4	98.7	51.1	93.7
	22	-4-butoxyphenyl	-H	28.8	94.9	12.6	57.4
	23	-phenylethenyl	-H	11.5	73.2	6.6	54.8
10	24	-4-biphenyl	-H	5.3 ^b	66.6 ^b	-3.7 ^b	34.8 ^b
	25	-3-[(4-aminophenyl)sulfonyl]amino]phenyl	-H	97.2	99.0	62.2	88.5
	26	-2-furyl	-H	91.0	98.7	7.7	73.0
	27	-4-fluorophenyl	-isopropyl	8.7 ^c	43.3 ^c	5.8 ^c	32.2 ^c
	28	-5-nitro-2-furyl	-H	21.0	90.8	46.9	93.1

15

a - 0.5 μ M and 5.0 μ M for PChE and 5 μ M and 50 μ M for AChEb - 0.25 μ M and 2.5 μ M for PChE and 2.5 μ M and 25 μ M for AChEc - 10 μ M and 100 μ M for PChE and 50 μ M and 500 μ M for AChE

All 28 of the test compounds showed substantially greater inhibition of pseudocholinesterase than acetylcholinesterase.

The above results show that compounds of the present invention, at certain concentrations, have the ability to strongly
5 inhibit pseudocholinesterase while having only a minimal inhibitory effect on acetylcholinesterase.

The activity of compounds of the present invention in reducing the ratio of LDL/HDL in hyperlipidemic guinea pigs is demonstrated by the following example:

10

TEST METHOD 2 AND EXAMPLE 29

Guinea Pigs

Male Hartley guinea pigs (Elm Hill Breeding Labs., Chelmsford, MA or Charles River, Wilmington, MA) were used in all experiments unless otherwise specified. The animals were maintained on a normal
15 diet of rabbit pellets (Purina Rabbit Chow 5321, Ralston Purina, St. Louis, MO) and water containing 0.1% ascorbic acid ad libitum. For special studies in the hyperlipidemic guinea pig model, the animals were maintained on the high cholesterol diet described in this report and water containing 0.1% ascorbic acid ad libitum.

20

Individual animals were identified by ear and cage markings. The dose of drug or placebo was adjusted daily for changes in the weight of the guinea pigs. Blood samples were collected in EDTA Vacutainers by open chest cardiac puncture under carbon dioxide anesthesia from guinea pigs which had been fasted overnight. The
25 animals were then sacrificed by CO₂ inhalation. Blood samples were centrifuged at 2000 rpm for 15 min. in an IEC centrifuge (International Equipment Co., Boston, MA) with a 240 rotor for isolation of plasma. Lipoprotein analyses of plasma were carried out

on the same day as the blood collection. Assays of pseudocholinesterase activity were carried out within a week of blood collection on plasma samples which had been stored at 4°C.

Pseudocholinesterase Assay

5 The assay method used was based on the procedure described in Ellman, G.L., K.D. Courtney, V. Andres & R.M. Featherstone, "A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity", Biochemical Pharmacology, Vol. 7 (1961), pp. 88-95. Reactions were carried out at 35°C in pH 7.2 NaH_2PO_4 50 mM buffer
10 containing 5 mM butyrylthiocholine iodide (substrate) and 0.25 mM 5,5'-dithiobis-[2-nitrobenzoic acid] (chromogen). The rate of change of absorbance at 405 nm with time was monitored by means of a Cary 16 spectrophotometer (Varian Associates, Walnut Creek, CA) or a Beckman DU-8B spectrophotometer (Beckman Instruments, Inc.,
15 Palo Alto, CA). Corrections were made for non-enzymic hydrolysis of substrate.

Reagents

Enzyme Buffer: 6.90 g (50 mmol) $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ + 0.9914 g (0.25 mmol) 5,5'-dithiobis-(2-nitrobenzoic acid). The reagents were
20 dissolved in Milli-Q purified distilled water, titrated to pH 7.2 with 1N NaOH, and diluted to one liter with water.

Substrate: 0.2476 g (0.780 mmol) S-butyrylthiocholine iodide (BTC). The substrate was dissolved in 5 ml of Milli-Q purified distilled water. [BTC] = 156 mM.

25 Procedure (Cary 16 Spectrophotometer)

Into a 1-cm pathlength cuvette was placed 3.0 ml of enzyme buffer. At least 10 min. was allowed for temperature equilibration. A 100 μl aliquot of substrate was added to the cuvette, the solution was mixed and about 3 min. was allowed for determination of the

non-enzymic rate of substrate hydrolysis before the enzymic reaction was initiated by addition of 25 μ l of plasma. The increase in absorbance at 405 nm was measured vs. a blank containing enzyme buffer.

5 Procedure (Beckman DU-8B Spectrophotometer)

Into a 1-cm pathlength cuvette was placed 3.0 ml of enzyme buffer and 100 μ l of substrate. The solution was mixed and about 10 min. was allowed for temperature equilibration. The reaction was initiated by addition of 25 μ l of plasma. The increase in absorbance
10 at 405 nm was measured vs. a blank containing enzyme buffer and substrate. No additional correction for non-enzymic substrate hydrolysis was necessary.

Lipoprotein Analysis

Freshly prepared samples of EDTA plasma were analyzed for
15 lipoproteins with the Redi-Disc Lipoprotein Electrophoresis Kit (Miles Laboratories, Elkhart, IN) by the procedure recommended in the package insert supplied in the kit.

Reagents (Stored at 4°C)

Redi-Disc Lipoprotein Gel Tubes, polyacrylamide 3%, buffer,
20 preservative.

Redi-Disc Lipoprotein Loading Gel, acrylamide 2.5 g/dl.,
N,N'-methylenebisacrylamide 0.6 g/dl, sudan black B 13 mg/dl,
catalyst, stabilizer, buffer.

Redi-Disc Lipoprotein Buffer, Trishydroxymethylaminomethane
25 27% w/w, boric acid 73% w/w, pH of solution 7.4-7.9 (11 g diluted to 1200 ml with water).

Procedure

The gel tubes were removed from the storage buffer and shaken to remove excess buffer. A 25 μ l aliquot of plasma was added to

each tube and mixed with 200 μ l of loading gel. The loading gel was photopolymerized for 30-40 min. under a fluorescent light. The gel tubes were placed in the electrophoresis apparatus (Buchler Instruments, Fort Lee, NJ) and Redi-Disc Lipoprotein Buffer was
 5 then added to the upper and lower chambers of the apparatus. The current was adjusted to 5 mA per gel tube. Electrophoresis was stopped when the HDL band was about 4-5 mm from the bottom of the gel tube.

The gels were stored in water for at least 30 min. following
 10 electrophoresis to allow the lipoprotein zones to spread. The gels were scanned at 615 nm in either a Beckman Acta CIII spectrophotometer or a Beckman DU-8B spectrophotometer. The areas of the lipoprotein peaks (VLDL, LDL, and HDL) were determined by gravimetric analysis (cut and weighed) on the Acta
 15 CIII or by electronic integration on the DU-8B.

High Cholesterol Diet

A 1:15 mixture of cholesterol:corn oil was prepared by adding
 25 g of USP cholesterol (Sigma Chemical Company, St. Louis, MO) to
 375 g of corn oil (Mazola, Best Foods, CPC International, Inc.,
 20 Englewood Cliffs, NJ). The mixture was stirred, sonicated, and heated in a hot water bath until the cholesterol completely dissolved. It was necessary to keep the mixture warm (50-60°C) in order to prevent congealing.

Batches of feed were prepared by adding 160 g of the
 25 cholesterol-corn oil solution to 840 g of Purina Rabbit Chow 5321. The pellets and lipid mixture were thoroughly mixed in a 2-liter or 3-liter Erlenmeyer flask to ensure uniform coating of the pellets. The feed was then stored at room temperature until needed.

Hyperlipidemia Induced by Cholesterol Feeding

A control group of five male Hartley guinea pigs was maintained on a normal diet of Purina Rabbit Chow 5321 and water containing 0.1% ascorbic acid. A second group of five male guinea pigs was given the high-cholesterol diet described in this report and water containing 0.1% ascorbic acid. After seven days on the respective diet, the animals were fasted overnight and allowed only water containing 0.1% ascorbic acid. The animals were anesthetized by CO₂ inhalation and blood was collected by open chest cardiac puncture into EDTA Vacutainers. Then the animals were sacrificed by CO₂ inhalation. Plasma was separated and analyzed for lipoproteins by electrophoresis and assayed for pseudocholinesterase activity.

Multiple Dose Studies (Hyperlipidemic Guinea Pigs)

Two groups of male Hartley Guinea pigs were maintained on the high cholesterol diet described above and water containing 0.1% ascorbic acid ad libitum. One group was treated once daily with a freshly prepared solution of test compound 4, N-(diaminophosphinyl)-4-fluorobenzamide, (1.0 mg/ml in 0.9% saline solution) at 2.5 mg/kg by intraperitoneal injection for 14 days. The second group was given 0.9% saline solution once daily at 2.5 ml/kg by intraperitoneal injection for 14 days. Blood samples were collected in EDTA Vacutainers by open chest cardiac puncture under carbon dioxide anesthesia from guinea pigs which had been fasted overnight. Blood samples were obtained from selected control (saline treated) animals before treatment on days 0, 1, 3, 7, 10, and 14, and from selected dosed (test compound 4) animals before treatment on days 1, 3, 7, 10, and 14. The animals were sacrificed by CO₂ inhalation after blood collection. Plasma was isolated by

centrifugation and analyzed for lipoprotein and for pseudocholinesterase activity.

Results

Hyperlipidemia Induced by Cholesterol Feeding

5 The LDL/HDL ratio of the cholesterol-fed guinea pigs, 4.36, was significantly ($p < 0.05$) greater than that of the normally fed guinea pigs, 1.98. The levels of pseudocholinesterase were not affected by the cholesterol feeding, 5500 and 5550 nmol/min/ml., respectively, for the two groups of guinea
10 pigs.

Multiple Dose Studies (Hyperlipidemic Guinea Pigs)

 Guinea pigs maintained on a high cholesterol diet and treated once daily with test compound 4 at 2.5 mg/kg i.p. had substantially lower plasma pseudocholinesterase activity than did
15 saline-treated control animals. On the 14th day of treatment the LDL/HDL ratio was significantly lower ($p < 0.05$) for the test compound 4-treated animals compared to that for control animals given the same diet but given daily injections of saline. The results are summarized in Table 2:

20

Table 2

Multiple Dose Studies (Hyperlipidemic Guinea Pigs)

	Day	% PChE Inhibition (Test Compound 4 Compared to Control)	LDL/HDL	
			Test Compound 4	Control
25	1	51.6	1.91	2.45
	3	45.0	2.43	2.28
	7	58.6	4.08	4.25
	10	65.0	2.75	3.23
	14	69.0	1.50 ^a	2.60

30 ^aSignificantly different from Control value ($p < 0.05$)

Another aspect of the present invention is certain acylphosphorotriamides which are novel compounds and are useful as lipid-altering agents.

Novel compounds of the present invention include compounds of the class of acylphosphorotriamides which conform to the chemical structure of Compounds A except for such acylphosphorotriamides which are disclosed in Bayless & Millner incorporated by reference hereinabove.

Novel compounds of the present invention include compounds of the class of acylphosphorotriamides which conform to the chemical structure of Compounds A, except such compounds wherein R^2 is H and R^1 is 3-pyridyl, 2-furyl, 2-naphthyl, cinnameryl, benzyl, phenyl, or phenyl substituted by 3- or 4-nitro, 4-halo, 4-amino, 4-(lower)alkoxy, 4-(lower)alkyl, 2-methyl, 2,3-dimethyl, 2,4-dimethyl, 2,4,6-trimethyl, 3-trifluoromethyl, 3-[(4-aminophenyl)sulfonyl]amino, 4-cyano, 4-phenyl, or 3-phenoxy (Compounds B).

Preferred novel compounds of the present invention include Compounds B, wherein R^1 is phenyl or substituted phenyl, pyridyl or substituted pyridyl, furyl or substituted furyl, naphthyl or substituted naphthyl, benzyl or substituted benzyl, or phenyl(lower)alkyl or substituted phenyl(lower)alkyl. More preferred compounds of the present invention include Compounds B wherein R^1 is phenyl or substituted phenyl, furyl, or pyridyl. More preferred still novel compounds of the present invention include Compounds B wherein R^1 is 2-furyl, 3-pyridyl, phenyl, or 4-substituted phenyl wherein said phenyl substituents are selected from the group consisting of halo, lower straight or branched chain alkyl, lower straight or branched chain alkoxy, amino, nitro, lower straight or branched chain alkylsulphonyl, cyano, phenoxy or

substituted phenoxy. Especially preferred novel compounds of the present invention include Compounds B wherein R^1 is phenyl, 4-methoxyphenyl, 3-fluorophenyl, 2-fluorophenyl, 4-fluorophenyl, 4-methylphenyl, or 4-aminophenyl.

5 Preferred novel compounds of the present invention include Compounds B, wherein R^2 is H.

Methods for preparing novel compounds of the present invention are generally analogous to the methods of preparing acylphosphorotriamides disclosed in U.S. Patent Nos. 4,182,881 and 4,221,730
10 which have been incorporated herein by reference. Further examples of methods for preparing novel compounds of the present invention are the following:

EXAMPLE 30

Test compound 20 above, N-(diaminophosphinyl)-4-(methylsulfonyl)benzamide, was prepared by the following method.
15

To a suspension of 5.04 g (0.025 mole) of p-methylsulfonylbenzamide in 50 ml of $CHCl_3$ was added 5.27 g (0.025 mole) of phosphorus pentachloride. The reaction was refluxed for 18 min., and was filtered to give 1.27 g of starting material.

20 To the filtrate was added .89 g of 97% formic acid. (This is .019 mole, which takes into account the starting material recovered.) Much gas was evolved (CO and HCl) and a precipitate formed. After 1 hr. of stirring at room temperature, the reaction was filtered to give a white solid.

25 This was suspended in 50 ml of tetrahydrofuran and was stirred in an ice bath for 1-1/4 hr. while NH_3 was blown in. The reaction was filtered, and the residue was washed with water to afford 4.4 g (84%) of white solid, m.p. 245-260° (some unmelted at 300°C).

Recrystallization from DMF gave 2.55 g of shiny, white platelets, m.p. 236-→310°C.

Anal. Calcd. for $C_8H_{12}N_3O_4PS$: C, 34.66; H, 4.36; N, 15.16

Found: C, 34.22; H, 4.38; N, 15.34

5

EXAMPLE 31

Test compound 27 above, N-[amino-(1-methylethylamino)phosphinyl]-4-fluorobenzamide, was prepared using the following method.

A mixture of 4-fluorobenzamide (9.98 g, 0.0717 mol) phosphorous pentachloride (14.98 g, 0.0719 mol, Aldrich Chemical Co.) and carbon tetrachloride (100 ml, A.R.) was stirred and heated at 60-70°C for 1/2 hour. Cooling to 25-30°C and adding formic acid (95-97%, 2.8 ml, 0.074 mol) dropwise while keeping the temperature below 10°C gave a white precipitate. Collecting and rinsing the solid with chloroform gave 15.15 g (83% yield) of acid chloride. The acid chloride (15.15 g, 0.0592 mol) was stirred in chloroform (100 ml, A.R.) and 90% of the theoretically required isopropylamine (9 ml, 6.2 g, 0.1057 mol) was added dropwise, keeping the temperature below 10°C. The reaction was allowed to warm to room temperature while stirring for 3 hours. This solution was added to an ammonia-saturated solution of chloroform (200 ml. A.R.) over a 1-1/2 hour time period and stirring was continued overnight. Filtering gave 9 g consisting of a mixture of by-products. The chloroform filtrate was washed with water (6 X 200 ml) and the aqueous layer then salted with sodium chloride and extracted with chloroform (8 X 225 ml). Combining the chloroform layers, drying over magnesium sulfate, and concentrating the filtrate gave 0.570 g (4%) of N-[amino-(1-methylethylamino)phosphinyl]-4-fluorobenzamide; m.p. 197-199°C.

Anal. Calcd. for $\text{CH}_{10}\text{H}_{15}\text{FN}_3\text{O}_2\text{P}$: C, 46.34; H, 5.83; N, 16.21

Found: C, 46.20; H, 6.05; N, 15.92

Another aspect of the present invention is a composition in dosage unit form for altering plasma lipids of mammals comprising an effective but nontoxic amount of the novel acylphosphorotriamides having lipid-altering activity disclosed hereinabove. The composition is preferably adapted to systemic administration to mammals.

As used herein, the term "pharmaceutical carrier" denotes a solid or liquid filler, diluent, or encapsulating substance. Some examples of the substances which can serve as pharmaceutical carriers are sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose and its derivatives, such as sodium carboxymethylcellulose, ethylcellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; stearic acid; magnesium stearate; calcium sulphate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil, and oil of thiobroma; polyols, such as propylene glycol, glycerine, sorbitol, mannitol, and polyethylene glycol; agar; alginic acid; pyrogen-free water; isotonic salines; and phosphate buffer solutions; as well as other nontoxic compatible substances used in pharmaceutical formulations. Wetting agents and lubricants, such as sodium lauryl sulphate, as well as coloring agents, flavoring agents and preservatives, can also be present.

The pharmaceutical carrier employed in conjunction with the lipid-altering acylphosphorotriamides is used at a concentration sufficient to provide a practical size-to-dosage relationship. Preferably, the pharmaceutical carrier comprises from about 0.1% to about 99% by weight of the total composition.

Preferred dosage unit forms of the compositions of the present invention include capsules, tablets, solutions and suspensions to be administered orally and solutions and suspensions to be administered perenterally. Preferred dosage unit forms include solutions and

5 suspensions to be administered perenterally comprising from about 2 mg to about 1000 mg of a novel lipid-altering acylphosphorotriamide and a suitable pharmaceutical carrier; more preferred is such a dosage unit form comprising from about 5 mg to about 250 mg of such compound; more preferred still is such a dosage unit form

10 comprising from about 10 mg to about 50 mg of such compound. Other preferred dosage unit forms includes capsules and tablets each comprising from about 2 mg to about to about 1000 mg of a novel lipid-altering acylphosphorotriamide and a suitable pharmaceutical carrier; more preferred is such a dosage unit form comprising from

15 about 5 mg to about 250 mg of such compound; more preferred still is such a dosage unit form comprising from about 10 mg to about 50 mg of such compound.

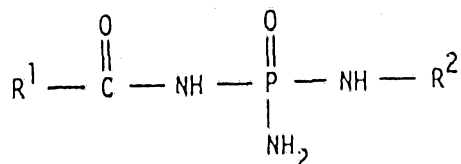
While particular embodiments of the present invention have been described, it will be obvious to those skilled in the art that various

20 changes and modifications to the compounds and compositions disclosed herein can be made without departing from the spirit and scope of the invention. It is intended to cover, in the appended claims, all such modifications that are within the scope of this invention.

25 WHAT IS CLAIMED IS:

The claims defining the invention are as follows:

1. A method of altering the blood plasma lipid content of a mammal which comprises systemically administering to such mammal an effective but nontoxic amount of a composition comprising a compound conforming to the following chemical structure:



wherein R^2 is H and R^1 is phenyl or substituted phenyl, pyridyl or substituted pyridyl, furyl or substituted furyl, naphthyl or substituted naphthyl, benzyl or substituted benzyl, or phenyl(lower)alkyl or substituted phenyl(lower)alkyl and salts and/or hydrates thereof, wherein substituents for R^1 are selected from the group consisting of halo, lower straight or branched chain alkyl, lower straight or branched chain alkoxy, amino, nitro, lower straight or branched chain alkylsulfonyl, cyano and phenoxy.

2. A method according to claim 1 wherein R^1 is phenyl or substituted phenyl, furyl or pyridyl.

3. A method of claim 1 or 2 wherein R^1 is 2-furyl, 3-pyridyl, phenyl, or 4-substituted phenyl.

4. A method according to any one of claims 1 to 3 wherein R^1 is phenyl.

5. A method according to any one of claims 1 to 3 wherein R^1 is 4-methoxyphenyl.

6. A method according to any one of claims 1 to 3 wherein R^1 is 4-fluorophenyl.

7. A method according to claim 1 or 2 wherein R^1 is 3-fluorophenyl.

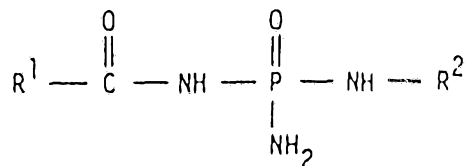


8. A method according to claim 1 or 2 wherein R^1 is 2-fluorophenyl.

9. A method according to any one of claims 1 to 3 wherein R^1 is 4-methylphenyl.

10. A method according to any one of claims 1 to 3 wherein R^1 is 4-aminophenyl.

11. A compound of the class of acylphosphorotriamides which conform to the chemical structure:



wherein R^2 is H and R^1 is phenyl or substituted phenyl, pyridyl or substituted pyridyl, furyl or substituted furyl, naphthyl or substituted naphthyl, benzyl or substituted benzyl, or phenyl(lower)alkyl or substituted phenyl(lower)alkyl wherein substituents for R^1 are selected from the group consisting of halo, lower straight or branched chain alkyl, lower straight or branched chain alkoxy, amino, nitro, lower straight or branched chain alkylsulfonyl, cyano and phenoxy, except such compounds wherein R^2 is H and R^1 is 3-pyridyl or 2-furyl or 2-naphthyl or cinnameryl or benzyl or phenyl or phenyl substituted by 3-nitro or 4-nitro or 4-halo or 4-amino or 4-(lower)alkoxy or 4-(lower)alkyl or 2-methyl or 2,3-dimethyl or 2,4-dimethyl or 2,4,6-trimethyl or 3-trifluoromethyl or 3-[(4-aminophenyl)sulfonyl]amino or 4-cyano or 4-phenyl or 3-phenoxy; and salts and hydrates thereof.

12. A compound according to claim 11 wherein R^1 is 4-substituted phenyl.



13. A compound according to claim 11 wherein R¹ is 3-fluorophenyl.

14. A compound according to claim 11 wherein R¹ is 2-fluorophenyl.

15. A composition in dosage unit form for altering the bloodplasma lipid content of a mammal comprising: (1) an effective but nontoxic amount of the compound according to any one of claims 11 to 14 and (2) a pharmaceutical carrier.

16. A method, according to claim 1, substantially as hereinbefore described with reference to any one of the examples.

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