AUSTRALIA

Patents Act

APPLICATION FOR A STANDARD PATENT

We Merrell Dow Pharmaceuticals Inc

of 2110 East Galbraith Road,
Cincinnati,
Ohio,
UNITED STATES OF AMERICA.

hereby apply for the grant of a standard patent for an invention entitled:

NOVEL ASPARTATE TRANSCARbamylase INHIBITORS

which is described in the accompanying complete specification.

Details of basic application

Number of basic application: 88 400 348.4

Convention country in which basic application was filed: EUROPEAN PATENT OFFICE

Date of basic application: 16 February 1988

Address for Service:

PHILLIPS ORMONDE & FITZPATRICK
Patent and Trade Mark Attorneys
367 Collins Street
Melbourne 3000 AUSTRALIA

Our Ref: 121843
POF Code: 1432/1432

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DECLARATION FOR A PATENT APPLICATION

In support of the (o) convention application made by (b) MERRELL DOW PHARMACEUTICALS INC.

(see subdivision) (hereinafter called "applicant(s) for a patent") for an invention entitled (o)

NOVEL ASPARTATE TRANSCARBAMYLASE INHIBITORS

I/we (o) Gary D. Street
of MERRELL DOW PHARMACEUTICALS INC.
2110 East Galbraith Road
Cincinnati, Ohio 45215, United States of America

do solemnly and sincerely declare as follows:

(a) Insert "Convention" if applicable
(b) Insert FULL name(s) of applicant(s)
(c) Insert "of addition" if applicable
(d) Insert TITLE of invention
(e) Insert FULL name(s) AND address(es) of declarant(s)
(see subdivision(s))

(1) Insert "The basic application(s) for patent or similar protection on which the application is based is/are identified by country, filing date, and basic application number as follows:

(a) France, 16 February 1988
MERRELL DOW Pharmaceuticals Inc.

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 application number as follows:

(a) France, 16 February 1988
MERRELL DOW Pharmaceuticals Inc.

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.

Declared at (a) Cincinnati, Ohio
Dated (0) June 7, 1989

By (m) MERRELL DOW PHARMACEUTICALS INC.

To: The Commissioner of Patents

P10/7/78
PHILLIPS ORMONDE & FITZPATRICK
Patent and Trade Mark Attorneys
367 Collins Street
Melbourne, Australia
NOVEL ASPARTATE TRANSCARBAMYLASE INHIBITORS

1. A compound of the formula

\[
\begin{align*}
\text{R}_1 \text{O} & \quad \text{O} \quad \text{Z} \quad \text{X} \quad \text{OR}_3 \\
\text{R}_2 \text{O} & \quad \text{O} \quad \text{I} 
\end{align*}
\]

or a pharmaceutically acceptable salt thereof, wherein
- \(Z\) is NH or CH\(_2\), \(Q\) is O and when \(Z\) is CH\(_2\), \(Q\) is also \((\text{H}, \text{OH})\),
- \(X\) is H, F or Cl and \(Y\) is F or Cl,
- \(R_1\) and \(R_2\) each are H or \(C_{1-6}\) lower alkyl, and
- \(R_3\) and \(R_4\) each are H or \(C_{1-6}\) lower alkyl.

13. A process for the treatment of cancers amenable to treatment with chemotherapeutic agents selected from the group consisting of 5-fluorouracil, 1-\(\beta\)-D-arabinofuranosylcytosine, methotrexate, acivicin and 4'-\((9\text{-acridinylamino})\)-methanesulfon-\(m\)-aniside, which comprises utilizing said chemotherapeutic agents in conjunctive therapy with a compound of Claim 1.
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COMPLETE SPECIFICATION
(ORIGINAL)

Application Number:
Lodged:

Complete Specification Lodged:
Accepted:
Published:

Priority

Related Art:

APPLICANT'S REFERENCE: M01317A AU

Name(s) of Applicant(s):
Merrell Dow Pharmaceuticals Inc

Address(es) of Applicant(s):
2110 East Galbraith Road,
Cincinnati, 
Ohio, 
UNITED STATES OF AMERICA.

Address for Service is:

PHILLIPS ORMONDE & FITZPATRICK
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367 Collins Street
Melbourne 3000 AUSTRALIA

Complete Specification for the invention entitled:

NOVEL ASPARTATE TRANSCARBAMYLASE INHIBITORS

Our Ref : 121843
POF Code: 1432/1432

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

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NOVEL ASPARTATE TRANSCARBAMYLASE INHIBITORS

This invention relates to halogenated derivatives of certain phosphonates having the ability to inhibit aspartate transcarbamylase and therefore are useful in the treatment of certain cancers.

More specifically this invention relates to novel halogenated derivatives of phosphonates having the structural formula

![Chemical Structure]

or a pharmaceutically acceptable salt thereof, wherein Z is NH or CH$_2$, Q is O, or when Z is CH$_2$ then Q can also be (H,OH), X is H, F or Cl, and Y is F or Cl, $R_1$ and $R_2$ each are H or C$_{1-6}$ lower alkyl, and $R_3$ and $R_4$ each are H or C$_{1-6}$ lower alkyl.

It is to be noted that the compounds may exist in their enantiomeric forms and thus the individual enantiomers as well as the mixtures thereof are contemplated as part of the scope of this invention.
The compounds of this invention are useful both in the free base form and in the form of acid addition salts. The acid addition salts are simply a more convenient form for use and, in practice, use of the salt amounts to use of the free base. The expression "pharmaceutically acceptable acid addition salts" is intended to apply to any non-toxic organic or inorganic acid addition salts of the base compounds of formula I. Illustrative inorganic acids which form suitable salts include hydrochloric, hydrobromic, sulfuric and phosphoric acids and acid metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids which form suitable salts include the mono-, di-, and tricarboxylic acids. Illustrative of such acids are, for example, acetic, glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, hydroxymaleic, benzoic, hydroxybenzoic, phenylacetic, cinnamic, salicylic and 2-phenoxybenzoic acids. Other organic acids which form suitable salts are the sulfonic acids such as methane sulfonic acid and 2-hydroxyethane sulfonic acid. Either the mono- or the di-acid salts can be formed, and such salts can exist in either a hydrated or a substantially anhydrous form. The acid salts are prepared by standard techniques such as by dissolving the free base in aqueous or aqueous-alcohol solution or other suitable solvent containing the appropriate acid and isolating by evaporating the solution, or by reacting the free base in an organic solvent in which case the salt separates directly or can be obtained by concentration of the solution. In general the acid addition salts of the compounds of this invention are crystalline materials which are soluble in water and various hydrophilic organic solvents and which, in comparison to their free base forms, demonstrate higher melting points and an increased solubility.

In general the compounds of formula I may be prepared by methods and techniques which are analogously known in the art. Of course the specific route followed for preparation
of any one specific compound will depend upon many factors (e.g. availability and costs of starting materials, protecting groups and such other factors generally appreciated by the skilled artisan) but, in general, two generic routes of synthesis are available depending primarily upon the definition of the moiety defined by Z.

In those instances wherein Z is nitrogen, it is convenient to utilize derivatives of phosphono acetic acid and aspartic acid according to the following reaction scheme

**Reaction Scheme A**

![Reaction Scheme A Diagram](image)

wherein R'₃ and R'₄ are C₁₋₆ alkyl, and Pg is a suitable protecting group, preferably benzyl, methyl or ethyl, but also including C₁₋₆ lower alkyl.

The initial condensation reaction of the acid chloride (3) with suitably protected derivatives of aspartic acid (2) is readily achieved by contacting equivalent quantities of the reactants together at temperatures of about -30°C to
30°C, preferably at about 0°C in the presence of a base such as dimethylaminopyridine (DMAP), N-methyl morpholine (NMM), triethylamine (TEA) and the like; the reaction taking place under anhydrous conditions in a polar aprotic solvent, preferably dichloromethane. Of course, the acid chloride derivatives of formula 3 are readily prepared from appropriate OR'₃, OR'₄ phosphono-X,Y-substituted acetic acid derivatives by standard procedures well known in the art. In general, these acids are reacted with oxalyl chloride in the presence of a base (e.g. DMAP, NMM, TEA and the like) at temperatures of about -10°C to 20°C, preferably at 0°C under an inert atmosphere (argon or nitrogen being preferred) in a polar aprotic solvent under anhydrous conditions according to well known conditions.

Following the above-described condensation the R'₃, R'₄ protecting groups (i.e. a C₁₋₆ alkyl, preferably methyl and ethyl) are removed by reaction with trimethylsilyl bromide (TMSBr) in an anhydrous polar aprotic solvent (preferably CH₂Cl₂) in an inert atmosphere (argon or nitrogen) at temperatures of about 0°C to room temperature, followed by hydrolysis with water, and the resulting products are deprotected (e.g. de-benzylated) by hydrogenolysis, preferably by treatment with hydrogen in the presence of a catalyst, such as palladium on carbon in water. In the event the protecting groups (represented by Pg) are lower alkyl, then they are cleaved by basic hydrolysis.

The preparation of the generic class of compounds of formula I wherein Z represents a CH₂ moiety is achieved by a nucleophilic addition of an appropriate aldehyde with a lithio derivative of a diester of an X,Y-substituted methyl phosphonate to form an alcohol which is oxidized to its corresponding ketone. Following this oxidation the hydroxyl-protecting groups are removed as described above. This sequence is depicted by the following reaction scheme
wherein Pg, X, Y, R'₃ and R'₄ are as previously described.

The nucleophilic addition of the aldehydes (6) is effected with 2 equivalents of the R'₃, R'₄, X, Y-substituted lithio methanephosphonates at low temperatures of about -50°C to -110°C, preferably about -78°C in a THF/ether (3/1) solvent mixture in the presence of 1 equivalent of lithium bromide.

The reaction is preferably effected under an inert atmosphere, preferably using nitrogen or argon, under anhydrous conditions in an aprotic polar solvent. Following completion the reaction is generally quenched with aqueous ammonium chloride and the resulting product isolated according to standard procedures. The so-obtained alcohols (8) are oxidized to their corresponding ketones using...
standard procedures well known in the art, such as, for example, the use of pyridinium dichromate, the Dess-Matin reagent and preferably the Swern oxidation. The Swern oxidation is conducted in an aprotic solvent under an inert atmosphere (nitrogen or argon preferred) under anhydrous conditions using dimethylsulfoxide (DMSO) in the presence of an agent activating DMSO as for example oxalychloride or trifluoroacetic anhydride, followed by the addition of a base, preferably TEA. Following this oxidation the hydroxyl-protecting groups of the compounds of formula (9) are removed by sequential treatment with trimethylsilyl bromide and hydrogenolysis by the procedures described for Reaction Scheme A. In those instances wherein Pg is a C₁₋₆ lower alkyl, preferably methyl or ethyl, then those protecting groups are removed by basic hydrolysis. Of course, in those instances wherein it is desired to obtain final compounds leaving a C₁₋₆ lower alkyl radical (i.e., R₁, R₂, R₃ and R₄ are other than H), then the need to remove those groups is obviated.

The necessary aldehydes of formula 6 are readily prepared by subjecting 3-cyclopentene-1-carboxylate esters (benzyl, methyl or ethyl) to ozonolysis using gaseous ozone according to the procedure outlined in Organic Synthesis, Vol. 64, A.S. Kende (editor), pp. 150-156 in an article submitted by R.E. Claus and S.L. Schreiber. In this procedure the ozonolytic cleavage is effected in the presence of an alcohol to produce a compound having an aldehyde and a methoxy hydroperoxide group at the termini. (Of course, if benzyl alcohol is used a benzyloxy hydroperoxide is formed instead of the methoxy hydroperoxide. Thus, it is preferred to use that alcohol which will best accommodate its removal after the Swern oxidation). Following the ozonolysis, in situ-dehydration, preferably with acetic anhydride in the presence of base (preferably TEA), affords the desired starting materials (6).
It is preferred to produce compounds of formula I in their L-enantiomeric form and by the use of L-aspartic acid derivatives (such as in the Reaction Scheme A) such compounds are readily prepared. Those compounds produced by Reaction Scheme B will be produced as racemic mixtures which can readily be separated by procedures well known in the art. In general it is preferred to separate the enantiomeric forms of the aldehydes of formula 6 rather than after they have been converted to compounds (5).

In practice it is preferred to utilize phosphono esters wherein R'3 and R'4 are methyl or ethyl. Reactants utilizing other alkyl esters may be utilized and such esters may be prepared by methods analogous to those used in the preparation of intermediates (3) and (7).

The following examples are illustrative of the above-described procedures for the preparation of the compounds of formula I.

EXAMPLE 1

PREPARATION OF 2-[(2,2-DIFLUORO-1-OXO-2-PHOSPHONO ETHYL)AMINO]BUTANEDIOIC ACID

Step A:

2-[(2-DIETHOXYPHOSPHINYL-2,2-DIFLUORO-1-OXO-ETHYL)AMINO]-BUTANEDIOIC ACID, BIS(PHENYLMETHYL) ESTER

1.75 ml of oxalyl chloride (20 mmol) are slowly added to a stirred solution of diethyl phosphono-difluoro-acetic acid (20 mmol, 4.64 g) and 2.02 g of N-methylmorpholine (20 mmol) dissolved in 50 ml of anhydrous dichloromethane at 0°C under argon. The reaction mixture is cooled to 0°C and 9.7 g of aspartic acid tosylate (20 mmol) and 4.5 g of N-methylmorpholine (43 mmol) dissolved in 100 ml of anhydrous dichloromethane are added. After stirring for 18 hours at 20°C the reaction mixture is hydrolyzed with saturated aqueous bicarbonate (30 ml), washed with 1 N HCl (30 ml), bicarbonate (30 ml) and brine (30 ml). The organic layer is dried.

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over Na₂SO₄, filtered and purified by flash chromatography on silica gel giving 4.8 g of 2-[(2-diethoxyphosphinyl-2,2-difluoro-1-oxo-ethyl)amino]butanedioic acid bis(phenylmethyl) ester.

5 **Step B:**

2-[(2,2-DIFLUORO-1-OXO-2-PHOSPHONO ETHYL)AMINO]BUTANEDIOIC ACID, BIS(PHENYLMETHYL) ESTER

10 ml of trimethylsilyl bromide (75 mmol) are slowly added to a stirred solution of 2-[(2-diethoxyphosphinyl-2,2-difluoro-1-oxo-ethyl)amino]butanedioic acid bis(phenylmethyl) ester (4 g, 7.5 mmol) dissolved in 50 ml of anhydrous dichloromethane at 0°C under argon. The reaction mixture is stirred at 20°C for 18 hours and evaporated to dryness. The residue is dissolved in 25 ml of acetonitrile and hydrolyzed by 1.5 ml of water. The reaction mixture is evaporated to dryness to give 3.4 g of 2-[(2,2-difluoro-1-oxo-2-phosphono ethyl)amino]butanedioic acid, bis(phenylmethyl) ester which will be used in the next step without further purification.

15 **Step C:**

2-[(2,2-DIFLUORO-1-OXO-2-PHOSPHONO ETHYL)AMINO]BUTANEDIOIC ACID

3.4 g of 2-[(2,2-difluoro-1-oxo-2-phosphonoethyl)amino]butanedioic acid, bis(phenylmethyl) ester (7.2 mmol) are dissolved in water (70 ml) and stirred under atmospheric pressure of hydrogen in the presence of 0.6 g of Palladium dispersed on charcoal. After 18 hours, the reaction mixture is filtered and 2-[(2,2-difluoro-1-oxo-2-phosphonoethyl)-amino]butanedioic acid is obtained on crystallization from THF (tetrahydrofuran).
EXAMPLE 2
PREPARATION OF
(3,3-DICHLORO-2-OXO-3-PHOSPHONO PROPYL)BUTANEDIOIC ACID

Step A:
3,4-BUTANAL DICARBOXYLIC ACID, BIS(PHENYL METHYL) ESTER

A round-bottomed flask equipped with a glass tube, a calcium chloride drying tube and a glass stopper is charged with 20 g of methyl-3-cyclopentene-1-carboxylate (158 mmol), 500 ml of dichloromethane, 100 ml of methanol and 4 g of anhydrous sodium bicarbonate. Ozone is bubbled through the solution at -78°C until a blue color appears. Nitrogen is passed through the solution until the blue color is discharged and the mixture is allowed to be stirred at 20°C. The solution is filtered and 150 ml of benzene are added. The volume is reduced to approximately 100 ml by evaporation. After dilution with 450 ml of dichloromethane, the flask is cooled to 0°C and 32 ml of triethylamine and 43 ml of acetic anhydride are added dropwise and the solution is stirred under a nitrogen atmosphere for 30 min. The ice-bath is removed and stirring is continued for 4 hours. The solution is washed with 150 ml-portions of aqueous 0.1 N HCl, aqueous 10% NaOH and water. The organic layer is dried (Na₂SO₄), filtered and evaporated to give 25 g of crude material which is purified by flash chromatography on silica gel affording 18 g of 3,4-butanal dicarboxylic acid, bis-(phenylmethyl)ester.

Step B:
2-(3-DIETHOXYPHOSPHINYL-3,3-DICHLORO-2-HYDROXYPROPYL)-BUTANEDIOIC ACID, DIMETHYL ESTER

A solution of diethyl trichloromethane phosphonate (25.6 g) together with 5 g of lithium bromide in ether (90 ml) and THF (70 ml) is placed in a four-necked flask equipped with a stirrer, an addition funnel, a low-T° thermometer and a nitrogen inlet tube. A 1.5 N n-butyllithium solution (72 ml) is added over a 10 min period, dropwise at -100°C under N₂ atmosphere. After the addition is complete the tem-
perature of the mixture is allowed to rise to \(-85^\circ C\) for 10 min. Then a solution of 10 g of 3,4-butanal dicarboxylic acid, bis(phenylmethyl)ester in 30 ml of THF and 20 ml of ether is slowly added to the reaction mixture kept between \(-95^\circ C\) to \(-110^\circ C\). The mixture is stirred at \(-100^\circ C\) for 30 min, hydrolyzed with rapid addition of 1 N H\(_2\)SO\(_4\), warmed up and extracted with ethyl-acetate (3 times 100 ml). The extract is washed with 0.1 N H\(_2\)SO\(_4\), brine, dried over sodium sulfate, filtered and evaporated to give a residue which is purified by flash chromatography on silica gel giving 11 g of 2-(3-diethoxyphosphinyl-3,3-dichloro-2-hydroxypropyl)-butanedioic acid, dimethyl ester.

**Step C:**

2-(3,3-DICHLORO-3-DIETHOXYPHOSPHINYL-2-OXO-PROPYL)-BUTANEDIOIC ACID, DIMETHYL ESTER

DMSO (3.4 ml) is added slowly to a stirred solution of oxalylchloride (2.1 ml) in 20 ml of anhydrous dichloromethane at \(-78^\circ C\) under argon. The reaction mixture is stirred at \(-50^\circ C\) for 10 min, cooled again to \(-78^\circ C\) and a solution of 2-(3-diethoxyphosphinyl-3,3-dichloro-2-hydroxypropyl)butanedioic acid, dimethyl ester (8 g) in 30 ml of anhydrous dichloromethane is added dropwise. After 15 min at \(-40^\circ C\) and 1 hour at 20°C, a saturated aqueous ammonium chloride solution is added. The organic layer is separated, the aqueous phase is extracted twice with 80 ml of dichloromethane, the organic phases are combined, washed with brine, dried (Na\(_2\)SO\(_4\)), filtered and evaporated to give 8.7 g of crude material which is purified by flash chromatography on silica gel affording 6.7 g of 2-(3,3-dichloro-3-diethoxyphosphinyl-2-oxo-propyl)butanedioic acid, dimethyl ester.

**Step D:**

(3,3-DICHLORO-2-OXO-3-PHOSPHONYL PROPYL)BUTANEDIOIC ACID

12 ml of trimethylsilylbromide are slowly added to a stirred solution of 2-(3,3-dichloro-3-diethoxyphosphinyl-2-oxo-propyl)butanedioic acid, dimethyl ester (6 g), dissolved in 75 ml of anhydrous dichloromethane at 0°C under argon.
The reaction mixture is stirred at 20°C for 18 hours and evaporated to dryness. The residue is dissolved in 30 ml of acetonitrile and hydrolyzed by 2 ml of water. The reaction mixture is evaporated to dryness, affording 4.4 g of product which is dissolved in MeOH (methanol) (30 ml) and water (10 ml) and treated with 0.5 g of LiOH (lithium hydroxide) at 20°C for 15 hours. The reaction mixture is evaporated to dryness. The residue is dissolved in 0.5 N HCl (50 ml), concentrated to a few ml and extracted 5 times by ethyl acetate. The organic layers are combined, washed with brine, dried (Na₂SO₄), filtered and evaporated to give 4 g of (3,3-dichloro-2-oxo-3-phosphono propyl)butanedioic acid which is recrystallized from hot THF.

EXAMPLE 3
PREPARATION OF
2-(3-DIETHOXYPHOSPHINYL-3,3-DIFLUORO-2-HYDROXY-PROPYL)-
BUTANEDIOIC ACID, DIMETHYL ESTER

30 mmol of 1.5 N n-butyllithium in hexane (200 ml) are added at -35°C to a stirred solution of 33 mmol of diisopropylamine (33.3 g) in 300 ml of anhydrous THF. The reaction mixture is stirred at 0°C for 30 min, cooled to -78°C and added in 30 min to a stirred solution of 52.5 g of diethyl difluoromethane phosphonate in 300 ml of THF cooled at -78°C under argon. Butanal 3,4-dicarboxylic acid bis methyl ester (34 g) dissolved in 250 ml of THF is then added at -78°C and the reaction mixture is slowly warmed up to 20°C. The brown solution cooled to 0°C is quenched with 0.5 l of saturated aqueous ammonium chloride and extracted with ethyl acetate, washed with 0.5 N HCl, brine, dried (Na₂SO₄), filtered and evaporated. The crude product is purified by flash chromatography on silica gel, giving 38 g of 2-(3-diethoxyphosphinyl-3,3-difluoro-2-hydroxypropyl)butanedioic acid, dimethyl ester.
EXAMPLE 4

PREPARATION OF

2-(3-DIETHOXYPHOSPHINYL-3-FLUORO-2-HYDROXY-PROPYL)-
BUTANEDIOIC ACID, BIS METHYL ESTER

200 mmol of 1.5 N n-butyllithium in hexane (130 ml) are added at -35°C to a stirred solution of diisopropylamine (21 g) in 200 ml of anhydrous THF. The reaction mixture is stirred at 0°C for 30 min. To this solution cooled at -78°C is added dropwise diethylfluoromethane phosphonate (34 g) dissolved in 60 ml of anhydrous THF. After stirred for 15 min, butanal 3,4-dicarboxylic acid, bis methyl ester (22 g) dissolved in 50 ml of THF, was added dropwise to the reaction mixture at -78°C. The reaction mixture was slowly warmed up to 20°C, quenched with saturated aqueous ammonium chloride, extracted with ethyl acetate (3 x 250 ml), washed with 0.1 N HCl and brine, dried (Na₂SO₄), filtered and evaporated. The crude product was purified by flash chromatography on silica gel affording 20 g of 2-(3-diethoxyphosphinyl-3-fluoro-2-hydroxy-propyl)butanedioic acid, bis methyl ester.

By following the foregoing procedures there may also be prepared the following compounds:

2-(2,2-dichloro-1-oxo-2-phosphono ethyl)amino)butanedioic acid,
2-(fluoro-1-oxo-2-phosphono ethyl)aminobutanedioic acid,
2-(chloro-1-oxo-2-phosphono ethyl)aminobutanedioic acid,
(3,3-difluoro-2-oxo-3-phosphono propyl)butanedioic acid,
(3-fluoro-2-oxo-3-phosphono propyl)butanedioic acid,
(3-chloro-2-oxo-3-phosphono propyl)butanedioic acid.

The rational design of effective antineoplastic agents has long been an important adjuvant to classical approaches to medicinal chemistry and biochemical pharmacology. Indeed, this approach has led to the discovery of such clinically active antineoplastic agents as 5-fluorouracil, cyclophosphamide and malphalan. Following such successes,
Efforts have also been expended to discover antineoplastic agents based on their ability to serve as transition-state inhibitors of aspartate transcarbamylase, an early enzyme in the pathway of de novo pyrimidine nucleotide biosynthesis. This is a particularly desirable approach because some drugs which inhibit this pathway have proven useful in the treatment of a number of human tumor types.

Studies have shown that N-(phosphonoacetyl)-L-aspartate is an effective inhibitor of aspartate transcarbamylase and has been found to be useful in the conjunctive treatment of cancer with such agents as 5-fluorouracil (5-FU), 1-β-D-arabinofuranosylcytosine (ARA-C), methotrexate (MTX) (alone or with 5-FU), acivicin (alone or with 5-FU) and 4'-(9-acridinylamino)methanesulfon)-m-aniside (AMSA) in such cancer disease states as colorectal cancers, malignant melanoma, ovary tumors, neurofibrosarcoma, metastatic adenocarcinoma, and brain tumors such as meningioma and glioblastoma.

N-(Phosphonoacetyl)L-asparate (PALA) is effective in conjunctive therapy in the treatment of the cancer disease states at doses of about 100 mg/m² to 3000 mg/m² (e.g., 100 milligrams per square meter of body surface), preferably at 1 to 2 grams/m² in a single dose scheduled or in 0.5 mg/m² per day for five days (repeated every 2 weeks) in a multiple dose schedule with the dose schedules of 5-FU, ARA-C, MTX, acivicin and AMSA. For example, at the aforementioned dose levels for PALA, effective conjunctive therapy in the treatment of the foregoing cancer disease states can be achieved with 5-FU when 5-FU is intravenously (i.v.) administered between 200 and 500 mg/m² in a single weekly dose, with ARA-C when ARA-C is i.v. administered at 200 to 500 mg/m², with AMSA when AMSA is i.v. administered at a monthly dose of 90 to 120 mg/m².

In their use in conjunctive therapy with known antineoplastic agents, (e.g., 5-FU, ARA-C, MTX, acivicin and...
AMSA) the compounds of formula I may be administered at
doses of about 1/5 to 1/1 the doses of PALA when used in
conjunctive therapy with the above-mentioned dose regimes
for 5-FU, ARA-C, MTX, acivicin and AMSA.

The amount of the active ingredient to be administered
can vary widely according to the particular dosage unit
employed, the period of treatment, the age and sex of the
patient treated and the nature and extent of the disorder
treated. The total amount of the active ingredient to be
administered will generally range from about 1 mg/kg to 50
mg/kg and preferably from 3 mg/kg to 25 mg/kg. A unit
dosage may contain from 25 to 500 mg of active ingredient,
and can be taken one or more times per day. The active
compound of formula I can be administered with a pharmaceu-
tical carrier using conventional dosage unit forms either
orally, parenterally or topically.

The preferred route of administration is oral adminis-
tration. For oral administration the compounds can be
formulated into solid or liquid preparations such as cap-
sules, pills, tablets, troches, lozenges, melts, powders,
solutions, suspensions or emulsions. The solid unit dosage
forms can be a capsule which can be of the ordinary hard- or
soft-shelled gelatin type containing, for example, surfac-
tants, lubricants and inert fillers such as lactose, suc-
rose, calcium phosphate and cornstarch. In another embodi-
ment the compounds of this invention can be tableted with
conventional tablet bases such as lactose, sucrose and
cornstarch in combination with binders such as acacia,
cornstarch or gelatin, disintegrating agents intended to
assist the break-up and dissolution of the tablet following
administration such as potato starch, alginic acid, corn
starch and guar gum, lubricants intended to improve the flow
of tablet granulations and to prevent the adhesion of tablet
material to the surfaces of the tablet dies and punches, for
example, talc, stearic acid or magnesium, calcium or zinc
stearate, dyes, coloring agents and flavoring agents
intended to enhance the aesthetic qualities of the tablets and make them more acceptable to the patient. Suitable excipients for use in oral liquid dosage forms include diluents such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant, suspending agent or emulsifying agent.

The compounds of this invention may also be administered parenterally, that is, intravenously or intramuscularly, as injectable dosages of the compound in a physiologically acceptable diluent with a pharmaceutically carrier which can be a sterile liquid or a mixture of liquids such as water, saline, aqueous dextrose and related sugar solutions, an alcohol such as ethanol, isopropanol or hexadecyl alcohol, glycols such as propylene glycol or polyethylene glycol, glycerol ketals such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers such as poly(ethyleneglycol) 400, an oil, a fatty acid, a fatty acid ester or glyceride, or an acetylated fatty acid glyceride with or without the addition of a pharmaceutically acceptable surfactant such as soap or a detergent, suspending agent such as pectin, carbomers, methylcellulose, hydroxypropyl methylcellulose or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants. Illustrative of oils which can be used in the parenteral formulations of this invention are those of petroleum, animal, vegetable or synthetic origin, for example, peanut oil, soybean oil, sesame oil, cottonseed oil, corn oil, olive oil, petrolatum and mineral oil. Suitable fatty acids include oleic acid, stearic acid, and isostearic acid. Suitable fatty acid esters are, for example, ethyl oleate and isopropyl myristate. Suitable soaps include fatty alkali metal, ammonium and triethanolamine salts, and suitable detergents include cationic detergents, for example, dimethyl dialkyl ammonium halides, alkyl pyridinium halides, and alkylamines acetates; anionic detergents, for example, alkyl, aryl and olefin sulfonates, alkyl, olefin, ether and monoglyceride sulfates and sulfo-
succinates; non-ionic detergents, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylene-polypropylene copolymers; and amphoteric detergents, for example alkyl-beta-aminopropionates and 2-alkylimidazoline quaternary ammonium salts, as well as mixtures. The parenteral compositions of this invention will typically contain from about 0.5 to about 25% by weight of the active ingredient in solution. Preservatives and buffers may also be used advantageously. In order to minimize or eliminate irritation at the site of injection, such compositions may contain a non-ionic surfactant having a hydrophilic-lipophilic balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations ranges from about 5 to about 15% by weight. The surfactant can be a single component having the above HLB or can be a mixture of two or more components having the desired HLB. Illustrative of surfactants used in parenteral formulations are the class of polyethylene sorbitan fatty acid esters, for example, sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by condensation of propylene oxide with propylene glycol.

The active ingredient may also be administered by means of a sustained release system whereby the compound of formula I is gradually released at a controlled, uniform rate to form an inert or bioerodible carrier by means of diffusion, osmosis or disintegration of the carrier during the treatment period. Controlled release drug delivery systems may be in the form of a patch or bandage applied to the skin or to the buccal, sublingual or intranasal membranes, an ocular insert placed in the cul de sac of the eye, or a gradually eroding tablet or capsule or a gastrointestinal reservoir administered orally. Administration by means of such sustained release delivery systems permits the tissues of the body to be exposed constantly for a prolonged time period to a therapeutically or prophylactically effective dosage of a compound of formula I. The unit dosage of the compound administered by means of a sustained release
system will approximate the amount of an effective daily dosage multiplied by the maximum number of days during which the carrier is to remain on or in the body of the host. The sustained release carrier may be in the form of a solid or porous matrix or reservoir and may be formed from one or more natural or synthetic polymers, including modified or unmodified cellulose, starch, gelatin, collagen, rubber, polyolefins, polyamides, polyacrylates, polyalcohols, polyethers, polyesters, polyurethanes, polysulphones, polysiloxanes and polyimides as well as mixtures and copolymers of these polymers. The compounds of formula I may be incorporated in the sustained release carrier in a pure form or may be dissolved in any suitable liquid or solid vehicle, including the polymer of which the sustained release carrier is formed.

In the use of the compounds it is preferred to use compounds wherein both X and Y are fluoro or chloro. When one of X or Y is hydrogen it is preferred that the other is fluoro.
CLAIMS
The claims defining the invention are as follows:

1. A compound of the formula

   \[
   \begin{align*}
   &\text{R}_1\text{O} - \text{O} - \text{Z} - \text{X} - \text{Y} - \text{OR}_3 \\
   &\text{R}_2\text{O} - \text{O} - \text{I}
   \end{align*}
   \]

   or a pharmaceutically acceptable salt thereof, wherein
   \(Z\) is NH or CH\(_2\), \(Q\) is O and when \(Z\) is CH\(_2\), \(Q\) is also
   (H,OH),
   \(X\) is H,F or Cl and \(Y\) is F or Cl,
   \(R_1\) and \(R_2\) each are H or C\(_{1-6}\) lower alkyl, and
   \(R_3\) and \(R_4\) each are H or C\(_{1-6}\) lower alkyl.

2. Compounds of Claim 1 wherein \(Q\) is oxygen.

3. Compounds of Claim 2 wherein \(Z\) is CH\(_2\).

4. Compounds of Claim 2 wherein \(Z\) is NH.

5. Compounds of Claim 3 wherein \(R_1\), \(R_2\), \(R_3\) and \(R_4\) are
   H.

6. Compounds of Claim 4 wherein \(R_1\), \(R_2\), \(R_3\) and \(R_4\) are
   H.

7. Compounds of Claim 5 wherein \(X\) and \(Y\) are F.
8. Compounds of Claim 5 wherein X and Y are Cl.

9. Compounds of Claim 5 wherein X is H and Y is F.

10. Compounds of Claim 6 wherein X and Y are F.

11. Compounds of Claim 6 wherein X and Y are Cl.

12. Compounds of Claim 6 wherein X is H and Y is F.

13. A process for the treatment of cancers amenable to treatment with chemotherapeutic agents selected from the group consisting of 5-fluorouracil, 1-β-D-arabinofuranosylcytosine, methotrexate, acivicin and 4'-(9-acridinylamino)methanesulfonyl)-m-aniside, which comprises utilizing said chemotherapeutic agents in conjunctive therapy with a compound of Claim 1.

14. A process for preparing a compound of the formula

![Formula I]

which comprises treating a compound of the formula

![Formula II]

(a) with trimethylsilyl bromide in an anhydrous polar aprotic solvent under an inert atmosphere at temperatures of about...
0°C to room temperature, followed by hydrolysis with water to remove the R'₃ and R'₄ protecting groups, and (b) by hydrogenolysis or basic hydrolysis to remove the Pg groups, said Pg being C₁₋₆ alkyl or benzyl protecting group and R'₃ and R'₄ being C₁₋₆ alkyl, X is H, F or Cl, Y is Cl or F, Z is NH OR CH₂, Q is O, or when Z is CH₂ then Q can also be (H,OH).

15. A compound substantially as hereinbefore described with reference to any one of the examples.

16. A process substantially as hereinbefore described with reference to any one of the examples.

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END