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NOTICE OF ENTITLEMENT

We SCHERING CORPORATION

of 2000 Galloping Hill Road, Kenilworth, New Jersey 07033, U.S.A.

being the Applicant and Nominated Person, in respect of Application No. 62679/94, entitled "USE OF 2-CHLORO-1-[[4-[(2,6-DICHLOROPHENOXY)METHYL]-PHENYL]METHOXY]-4-METHOXY-BENZENE FOR THE SELECTIVE TREATMENT OF ENTEROVIRAL INFECTIONS IN HUMANS" state the following:

Edward J. Rozhon; John F. O'Connell; Peter Buontempo; Stuart A. Cox; Jason L. Demartino and Jacquelyn M. Wright are the actual inventors of the invention the subject of the Application.

The applicant and nominated person is the assignee of the invention from the actual inventors.

The applicant and nominated person is entitled to rely on the application listed in the declaration under Article 8 of the PCT.

Convention priority is claimed from the following basic application referred to in the declaration under Article 8 of the PCT:

Basic Applicants	Application Number	Application Date	Country	Country Code
Edward J. Rozhon; John F. O'Connell; Peter Buontempo; Stuart A. Cox; Jason L. Demartino and Jacquelyn M. Wright	08/023,527	26 February 1993	United States of America	US

The basic application referred to in the declaration under Article 8 of the PCT was the first application made in a Convention country in respect of the invention the subject of the Application.

DATED this 25th day of February 1998

SCHERING CORPORATION
By their Patent Attorney

GRIFFITH HACK

I:16104GX

AU9462679

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USE OF 2-CHLORO-1-{[4-{(2,6-DICHLOROPHENOXY)METHYL}-PHENYL}METHOXY}-4-METHOXY-BENZENE FOR THE SELECTIVE TREATMENT OF ENTEROVIRAL INFECTIONS IN HUMANS

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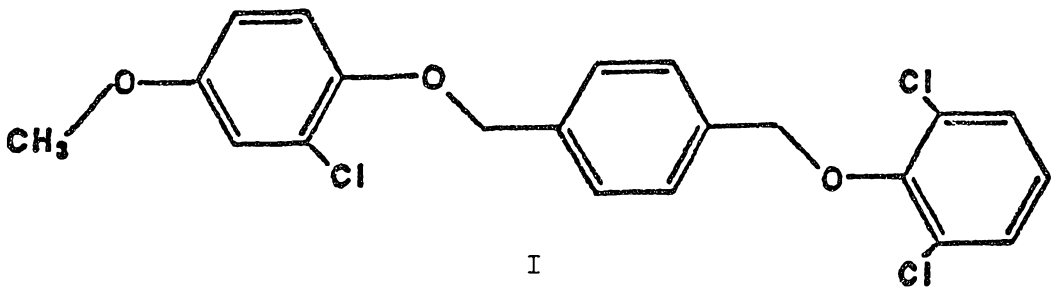
(56) Prior Art Documents
EP 0159702
WO 92/22520

(57) Claim

1. A method of treatment and/or prevention of acute hemorrhagic conjunctivitis in humans caused by enterovirus-70 comprising administering an effective amount of 2-chloro-1-[[4-[(2,6-dichlorophenoxy)methyl]-phenyl]methoxy]-4-methoxybenzene represented by formula I



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to a patient in need of such treatment.

2. A method according to claim 1 wherein the 2-chloro-1-[[4-[(2,6-dichlorophenoxy)methyl]-phenyl]methoxy]-4-methoxybenzene is administered orally.

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(54) Title: USE OF 2-CHLORO-1-[[4-[(2,6-DICHLOROPHENOXY)METHYL]-PHENYL]METHOXY]-4-METHOXY-BENZENE FOR THE SELECTIVE TREATMENT OF ENTEROVIRAL INFECTIONS IN HUMANS

(57) Abstract

The use of 2-chloro-1-[[4-[(2,6-dichlorophenoxy)methyl]phenyl]methoxy]-4-methoxybenzene for the preparation of a medicament for the selective therapeutic and prophylactic treatment of enteroviral-caused infections in humans is disclosed.

Use of 2-chloro-1-[[4-[(2,6-dichlorophenoxy)methyl]-phenyl]methoxy]-4-methoxy-benzene for the selective treatment of enteroviral infections in humans.

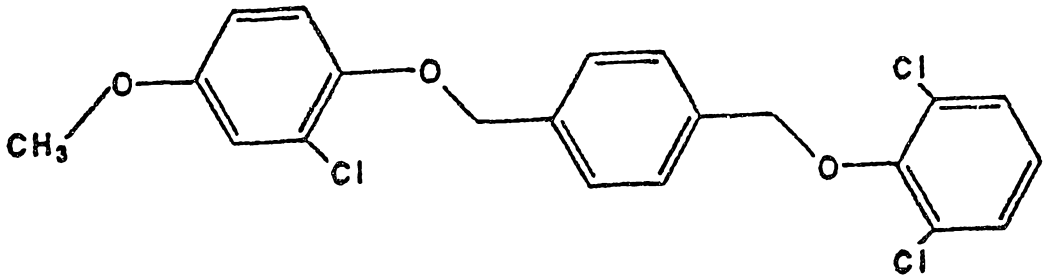
Introduction to the Invention

This invention relates to use of 2-chloro-l-[[4-[(2,6-dichlorophenoxy)methyl]-phenyl]methoxy]-4-methoxybenzene for the preparation of a medicament for the selective treatment and/or prevention of enteroviral-caused infections in humans.

Enteroviruses are members of the RNA-containing Picornavirus Family listed in Table 1 and are known to cause a wide variety of diseases and deaths in humans . A drug may exhibit in vitro and in vivo activity against enteroviruses. However, to be clinically useful, such a drug must have broad activity within the enterovirus genus since the same disease may be caused by a variety of different enteroviruses (see Table 2). Although about 70 immunotypes of enteroviruses are known, surveillance data from the Centers for Disease Control (CDC) on nonpolio-enteroviruses from 1970 to 1983 show that enteroviruses from the following 15 common immunologic groups account for 65-89% of the isolates for a given year in the United States: echoviruses (echo) 3,4,5,6,7,9,11,24 and 30; coxsackievirus A (CVA) 9; and coxsackieviruses B (CVB) 1,2,3,4, and 5. Most recent surveillance data indicate that the following 6 of the original common 15 enteroviral immunotypes represented 64% of all enterovirus isolates in 1988: echo 11 (18.6%), echo 9 (14.1%), CVB4 (10.6%), CVB2 (9.2%), echo 6 (6.2%), and CVB5 (5.1%). As recently as the summer of 1991, the CDC reported an epidemic in the eastern United States of aseptic meningitis associated with echo 30 which was isolated from patients at a frequency 14 times greater than the normal frequency of isolation. There is no commercially available medicament for treating and/or preventing diseases caused by enterovirus. Thus, there is a need for a medicament for the treatment and even prevention of enteroviral-caused infections in humans.

Summary of the Invention

This invention provides the use of 2-chloro-l-[[4-[(2,6-dichlorophenoxy)methyl]-phenyl]methoxy]-4-methoxybenzene represented by formula I for the preparation of a medicament for the of treatment and/or prevention of enteroviral infections in humans.



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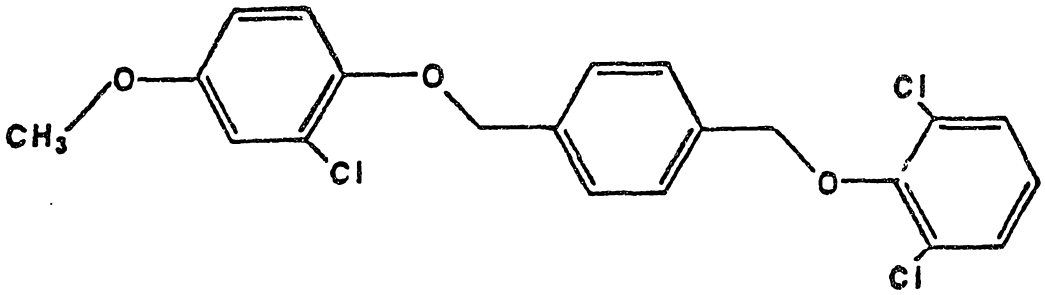
The present invention also provides a pharmaceutical composition for selectively treating and/or preventing enteroviral infections in humans which comprises an anti-enterovirally effective amount of 2-chloro-1-[[4-[(2,6-dichlorophenoxy)methyl]phenyl]methoxy]-4-methoxybenzene represented by formula I suspended in a vegetable oil and optionally containing at least one pharmaceutically acceptable excipient.

This invention also provides a method of selectively treating and/or preventing enteroviral infections in humans caused by an enterovirus selected from the group consisting of coxsackieviruses A and B, echoviruses, enteroviruses and polioviruses comprising administering to a human in need of such treating and/or preventing an anti-enterovirally effective amount of 2-chloro-l-[[4-[(2,6-dichlorophenoxy)methyl]-phenyl]methoxy]-4-methoxybenzene represented by
formula I



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or a pharmaceutical composition thereof.

This invention further provides a method of selectively treating and/or preventing an enteroviral infection in humans selected from the group consisting of enteroviral respiratory diseases, enteroviral aseptic meningitis, enteroviral

meningoencephalitis, chronic enteroviral infections in a gammaglobulinemics and acute hemorrhagic conjunctivitis caused by enterovirus-70 which comprises administering to humans in need of such treating and/or preventing an antienterovirally effective amount of 2-chloro-l-[[4-[(2,6-dichlorophenoxy)methyl]methoxy]-4-methoxybenzene and represented by formula I

Detailed Description of the Invention

To survey the enteroviral spectrum of the compound of formula I, recent human isolates of enteroviruses representing the above-listed 15 common enteroviral immunotypes were tested for susceptibility using the plaque assay. Older laboratory strains of enteroviruses and less frequently isolated enteroviruses as well as hepatitis A virus also were surveyed. A list of the antiviral activities of the compound of formula I against 154 enteroviruses from the 15 common enteroviral immunotypes is presented in Table 3. Again, eighty percent of the 154 enteroviruses (123/154) the compound of formula I exhibited IC_{50} values equal to or less than 0.90 µg/ml (2.12 µM) (EC_{80} = 0.90 µg/Ml). While the average IC_{50} value (arithmetic mean) for the compound of formula I against all 154 enteroviral immunotypes was less than 1 µg/ml, the number of the isolates tested in two enteroviral immunotypes (echo 9 and echo 24) was too few (2 isolates) to obtain an accurate representation of the average IC_{50} value.

Based on results in a variety of relevant cell culture-based test systems, the compound of formula I is a potent, broad spectrum, orally-active anti-enteroviral agent which has significant activity against enteroviruses believed to be responsible for most symptomatic enteroviral infections in the United States. Similar activity is expected against enteroviral infections found in other countries.

Enteroviruses from the 15 common immunotypes, listed in Table 3, were categorized according to the enteroviral disease diagnosed at the time of isolation. The majority of these isolates (71% or 110/154) were isolated from patients diagnosed with asceptic meningitis and/or meningoencephalitis, and the average IC_{50} value of the compound of formula I for this group was 0.98 µg/ml (2.31 µM). The next largest category (18% or 28/155) comprised enterovirus isolated from undiagnosed individuals, and the remaining viral isolates were obtained from patients with enteroviral diseases other than those associated with the central nervous system (CNS). For the latter groups, the average IC_{50} values for the compound of formula I were based on too few viruses to represent accurate estimations.

Ninety-three isolates of the aseptic meningitis/meningoencephalitis were associated exclusively with patients diagnosed as having aseptic meningitis. Of these 93 isolates, 47% (44/93) comprised isolates from the cerebrospinal fluid, while the remaining isolates were derived from the throat, rectum, stool, or urine. Since the frequency of enterovirus isolation in asymptomatic individuals in this study was 3%, it was concluded that viruses isolated from non-CNS sites in patients with aseptic meningitis likely represented the virus responsible for CNS disease.

The antiviral activities of the compound of formula I were determined against laboratory strains of enteroviruses and less frequently isolated enteroviruses as well as hepatitis A virus (HAV), which is classified in its own genus. The average IC_{50} of the compound of formula I against 30 enteroviruses (including polio 1 and polio 2) was 0.97 µg/ml (2.29 µM) and an EC_{80} 0.8 µg/ml (1.89 µM) was obtained for these viruses. Although some of the viruses are less frequently isolated in the United States, several of these viruses are of considerable importance to other countries and especially those in tropical climates. For example, poliomyelitis in populations not receiving the poliovirus vaccine remains a major health problem. Another example is enterovirus 70, a major cause of acute hemorrhagic conjunctivitis. For both polio 1, polio 2, and enterovirus (entero) 70, the compound of formula I is inhibitory: five strains of polio 1 (including recent human isolates) had IC_{50} values ranging from 0.009 to 0.04 mg/ml (0.021-0.094 µM); the average IC_{50} for the polio 2_{MEF1} strain in HeLa cells was 0.02 µg/ml (0.047 µM); and 4 strains of entero 70 were inhibited in a range of 0.1 to 0.4 µg/ml (0.24-0.9 µM). Unlike the situation with the common isolates in which the compound of formula I was inactive against only 2% (3/158 at an IC_{50} > 10 µg/ml) of viruses tested, the compound of formula I was inactive against 19% (7/37) of less frequent isolates and laboratory strains at the maximum concentration tested. This result is largely due to the inactivity of the compound of formula I against isolates of coxsackievirus A. Without testing additional coxsackievirus A imunotypes, it is not possible to establish whether the activity of the compound of formula I against these viruses is representative of other viruses in these immunotypes. Thus, based on the antiviral activities against the 15 common and less common enterovirus isolates as well as activities against laboratory strains of enteroviruses, it is anticipated that the compound of formula I will be active against the majority of enteroviruses encountered in the clinical setting.

Either HeLa (human), RD (human rhabdsarcoma), or BGMK (buffalo green monkey) cells were used to determine the antiviral activities of the compound of formula I against all enteroviruses which were tested. Thus, a therapeutic index ("TI's")for the compound of formula I with each virus was calculated by measuring adverse effects of the compound of formula I in these cell lines using the CPE-based MTT assay. The term "therapeutic index" as used herein is defined as cytotoxicity IC_{50} value divided by the antiviral IC_{50} value. Of 184 enteroviruses exhibiting measurable activity in the plaque assay, only 8.7% had TIs $\leq$10 and 5.4% had TIs $\geq$ 11 and $\leq$ 25, while 75.1% has TIs $\geq$ 51. Therefore, for the majority of enteroviruses tested, the antiviral activity of the compound of formula I is independent of possible adverse effects of the compound of formula i on cells and cytotoxicity does not contribute to the antiviral activity of the compound of the formula I.

The compound of formula I is efficacious against both echovirus-4 ("echo 4") and coxsackievirus A9 ("CVA9") viral infections when orally administered to mice in a therapeutic (+3.5 hr.) regimen at 20 mg/kg/day (4 doses of 5 mg/kg). The compound of formula I orally administered in this therapeutic regimen to mice intracranially infected with echo 4 at 980 PFU, exhibited an area under the curve (AUC) activity at day 21 of 75.2%.

Mice treated with the compound of the formula I in a therapeutic regimen infected intracranially with CVA9 at 1,200 PFU have an AUC activity of 71.7%. In echo 4 infected mice, the AUC activity decreases from 75.2% to 34.4% as the infectious challenge increases 3 orders of magnitude from 980 PFU to 980,000 PFU. Similarly, in CVA9 infected mice, the AUC activity decreases from 71.7% to 35.8% as the infection challenge increase 3 orders of magnitude from 1,200 PFU to 1,200,000 PFU.

The compound of formula I was also effacious against the polio-2-viral infection in the murine model in a therapeutic regimen when oral administration of the compound of formula I is 3.5, 6, and 24 hours after the mice were infected intracranially with polio-2 virus.

Prophylactic treatment of mice by the oral administration of the compound of formula I, 24 hours before infection with echo-4, CVA9 and poliovirus-2 produced a similar effacious result compared to those in the three therapeutic regimens described above. In two prophylactic treatment experiments (-24 hr) with 2 oral doses of 30 mg/kg (60 mpk/day) of the compound of formula I, mice infected with 98,000 PFU of

Echo-4 and 1,200,000 PFU of CVA9 exhibited AUC activity of 59.0% and 50%, respectively.

Prophylactic treatment with the compound of formula I, begun 24 hours (-24 hr) before intracranial infection into mice of 520,000 PFU of poliovirus-2, provided similar efficacy when the compound was orally administered BID (2 x 20 mg/kg (40 mpk/day), AUC = 46.4%), TID (3 x 6.7 mg/kg (20 mpk/day), AUC = 29.2%) and QID (4 x 5.0 mg/kg (20 pmk/day), AUC = 37.2%).

Serum levels after oral administration of the compound of formula I in corn oil at dosages of 1, 5, 20 and 30 mg/kg were measured in mice, rats, dogs, and monkeys. All species showed a dose-related increase in AUC, although the AUC did not increase at the higher dose. Similarly, the CMAX increases with the dose of the compound of formula I in all species. Among the four species, the highest serum levels of the compound of formula I occurred in beagles. The serum half-life of the compound of formula I was 4.7 hours in mice, but was dramatically greater in the higher species: 26 hours and > 24 hours in beagles and monkeys, respectively. The time to CMAX (i.e., TMAX) had a similar value in mice, rates and dogs (i.e., 2-4 hrs.), but it was longer in monkeys (8-12 hrs.). The shorter TMAX in dogs suggests that the serum life of 26 hrs. is independent of absorption in this species. On the basis of these pharmacokinetic results in dogs, the expected human levels of the compound of formula I should achieve antiviral levels (1 mg/ml) for 24 hours at a single dose of 10 mg/kg administered orally to humans.

A two week oral pilot toxicity study was conducted with the compound of formula I in rats and dogs. The LD_{50} value was in excess of 1000 mg/Kg/day in the rat and was in excess of 750 mg/Kg/day in the dog after two weeks of oral dosing.

Materials and Methods

Enteroviruses, representing the 15 most commonly isolated immunotypes in the USA were obtained from Centers for Disease Control (CDC), Atlanta, Georgia. Normally, five viruses from each immunotype were obtained. Other, less frequently isolated enteroviruses and the rhinoviruses were obtained from the American Type Culture Collection (ATCC), Rockville, Maryland. Enteroviruses and rhinoviruses were propagated in an appropriate cell line, eg HeLa, (human), BGMK (Buffalo greemn monkey), or RD (human rhabdosarcoma) cells and working stocks were prepared as cellular lysates. The standard procedures used for quantitating antiviral activity of the compoundd of formula I in vitro and in vivo are given herein below.

Pre-mix plaque assay: 150 plaque forming units (PFU; infectious particles) were mixed with test compound (0.0001-50 mg/ml) in Eagles' modified minimal essential medium (EMEM) for 45 min. and added to monolayers of appropriate cells. After 45 min. at 33°C, the inoculum was aspirated, the cells were washed, and plaque analysis was performed. In accordance with the methods desclosed by Trousdale, M.D., et al., (1977) in Biochem. Biophys. Res. Commun. 76:368-375 and Rueckert, R.R., et al., (1981) in Methods in Enzymology 78:315-325. The number PFU was determined at each concentration and plotted as a percentage of control PFU using Cricket Graph® or Wingz® graphics programs and the individual values were connected by a line through each value. To determine the IC_{50} concentration, a perpendicular line from the intersection of the PFU curve and a horizontal line representing 50% inhibition were connected to the abscissa. The value on the abscissa which intersects the perpendicular line represents the IC_{50} value. Values indicative of percentage inhibition (ordinate) are represented linearly, while the concentrations of test molecule (abscissa) are represented logarithimically.

Pre-mix cytopathic effect (CPE) assay: This assay, based on viral cytopathology, has a colorimetric endpoint dependent on light absorption by MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; SigmaChemical Co.) formazan. Briefly, test compound and virus were premixed for 45 min. at 22°C and used to infect cells {multiply of infection (MOI) = 1.0} in 96 well microelisa plates. Following 45 min. incubation (37°C), the culture fluid is aspired and the monolayers washed once with phospate buffered saline (PBS), EMEM with 1% fetal calf serum (FCS) is added, and incubation is continued for 18-24 hr. prior to measuring cellu®r graphics programs as described for the pre-mix plaque assay.

CPE cytotoxicity assay using MTT: The 50% inhibitory concentration (IC_{50}), referred to as the cytotoxic IC_{50} was determined as follows: Uninfected monolayers of cells in 96-well microelisa plates were treated with the compound of formula I (0.025-50 mg/ml) for prescribed periods of time prior to lysis of the monolayer and treatment with MTT. The IC_{50} value is determined as described for the pre-mix plaque assay

Therapeutic Index: The therapeutic index "TI" of the compound of formula I, which is dependent on the enterovirus tested as well as the type of cell in which the test was performed, was determined by two procedures: (i) The antiviral IC_{50} was determined by pre-mix plaque assay and the cytotoxic IC_{50} was determined by the MTT assay. In the latter, test molecule was present for 45 min. prior to removal by aspiration and the period of incubation prior to MTT treatment was identical with that of cells used in the plaque assay. (ii) The antiviral IC_{50} and the cytotoxic IC_{50} values were determined by CPE assay using MTT to determine the endpoints. Since enteroviruses normally adversely effect cellular metabolic processes to result in a cytopathic effect during their replication, the MTT assay is suitable for quantitating the antiviral effect of a test agent by indirectly assessing cellular metabolic functions. Thus, at concentrations in which an antiviral agent inhibits viral replication, relatively more cells remain viable as measured by the MTT assay compared to a less effective antiviral agent that permits comparatively more viral replication and subsequent killing of cells. The MTT assay is particularly advantageous for determination of TI since the virus and the antiviral molecule are tested under identical conditions with an equivalent end-point methodology.

Activity in Animals: Studies with mice (as well as other animals) were performed as recomended in the Guide for the Care and Use of Laboratory Animals (NIH Publications 85-23). Groups of 15-25 male mice (16-20 g; Harland Sprague Dawley) were infected intracranially with poliovirus 2, echovirus 4, or coxsackievirus A9 as described in McKinlay and Steinberg (1986 Antimicrob. Agents Chemothen, 29: 30-32). Treatment with the compound of formula I was by oral gavage (0.3 ml in corn oil) or other oleogacious materials such as peanut oil could also be used. Survival was monitered daily for 21 days. Results were evaluated for statistical significance using Chi square analysis, and protection was considered to be significant if results met a probability of $p < 0.05$ for at least 5 days during the study compared to the placebo-treated group.

An additional means of efficacy called area under the curve activity ("AUC activity") could also be used. AVC activity is calculated from the formula: AUC activity = [(experimental AUC-placebo AUC) divided by (maximum AUC-placebo AUC) x 100].

Dosing Formulation: In a preferred aspect of the present invention, a 2 mg/ml stock of the compound of formula I was prepared as a suspension in corn oil for studies in which the compound was administered orally. On each day of the study, the stock was diluted with the correct amount of corn oil to give the appropriate mg/kg dosage based on the average weight of the mice for that day.

In another preferred aspect of the present invention, the amount of the compound of formula I which may be suspended in one milliliter of corn or peanut oil to form a pharmaceutical compositions is in the range of about 100mg to about 1 gram. The pharmaceutical compositions of the present invention may also contain the typical pharmaceutically acceptable excipients, e.g., stabilizers, surfactants and antioxidants. Typical antioxidants include butylated hydroxyanisole "BHA" and butylated hydroxytoluene "BHT" which are used to prevent vegetable oils from becoming rancid. Stabilizers, e.g., lecithin and surfactants, e.g., polysorbate 60 or Arlacel 186 (a mixture of glyceryl oleate and propylene glycol available from ICI Americas, Inc., Wilimington, Delaware) may also be added.

In other studies where methycellulose (0.4% methylcellulose, 0.9% benzyl alcohol, 0.9% NaCl & 0.5% polysorbate-80), 1% gum tragacanth, or 1% lecithin were used as the oral dosing vehicle, the compound of formula I was added as a powder to the vehicle, mixed by homogenization (one min) and the resulting suspension orally administered to the animals.

Food matrix was prepared by mixing the compound of formula I in 1% lechitin and then adding it to a hot solution of 2x Jello® cherry flavored gelatin (but any gelatin could be used) plus sifted ground food (Purina® Mouse chow). The final concentration of the compound of formula I in the matrix for a 10 mg/kg dose was calculated based on a retriction of 10 grams of food a day per mouse (a 1 mg/kg dosage was present in each g of food matrix). In the efficacy studies, food matrix was made every four days and corrected for the weight change in the groups. Food was placed in trays at the bottom of cages for consumption.

The compound of formula I was prepared by the below-described Williamson ether synthesis as described in Example 15a of Orally Active Antiviral Compounds, International Publication No. WO 92/22520, published 23 Dec. 1992, International Appln. No. PCT/US92/04961.

A. Add 5g of 2-chloro-4-methoxyphenol to a mixture of 17g of α,α'-dibromo-p-xylene to a stirred mixture of 2.5g of 50:50 (w/w) NaOH:H_2O and 50mL of DMF. Stir the so-formed mixture for 4 hours. Partition the reaction mixture with methylene chloride and water. Separate the organic layer and remove the solvent by rotary evaporation to obtain a solid residue. Purify the residue on a silica gel chromatography column using pure hexane to pure methylene chloride as the eluants to provide 8g of 1-[4-bromomethylbenzyloxy]-2-chloro-4-methoxyphenyl ether.

B. To a solution of 0.50g of 2,6-dicholorophenol in a mixture of 0.12g of NaOH in 5mL of DMF, add 0.50g of the phenyl ether from Step (A) and stir for 4 hours at room temperature. Add 50mL of water to the reaction mixture and filter the crude precipitate so-formed. Purify the crude precipitate by crystallization from methylene chloride to provide 350 mg of 2-chloro-1-[[4-[(2,6-dichlorophenoxy)methyl]-phenyl]methoxy]-4-methoxybenzene, the compound of formula I, as a pure solid.

The compounds of formula I may be administered by any conventional mode of administration by employing an antienterovirally effective amount of a compound of formula I for such mode. The dosages may be varied depending upon the requirements of the patient in the judgement of the attending clinician, the severity of the condition being treated and the particular mode of administration being employed. Determination of the proper dosage for a particular situation is within the skill of the art. Treatment can be initiated with similar dosages which are less than the optimum dose of the compound. Thereafter, the dosage should be increased by small increments until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day if desired.

The compound of formula I may be administered for example, orally, intravenously, intraventricularly, subcutaneously, intramuscularly, or via rectal suppository; intravenous dosage should preferably comprise continuous drip infusion.

The pharmaceutical composition of this invention contains an anti-enterovirally effective amount of the compound of formula I suspended in a vegetable oil, e.g., corn oil or peanut oil and optionally a pharmaceutically aceptable excipient. The preparation of pharmaceutical compositions and the selction of pharmaceutically acceptable excipients are well known to those skilled in the art; see for example Remington's Pharmaceutical Sciences, Mark Publishing Co., Easton, Pa., 15th edition (1975) or International Publication WO 92/2250, published 23 Dec. 1992. The preferred carrier is corn oil which may be used alone or in combination with stabilizers, e.g., lecithin, or surfactants, e.g., polysorbate 60 or Arlacel 186.

The anti-enterovirally effective amount of the compound of formula I is in the range of about 1 to 90 mg/kg of body weight per day. A dose of about 10 to 15 mg/kg given one to six times a day, in equal or divided doses is preferred. A dose of 10 mg/kg/day is more preferred.

The exact dosage of the compounds of this invention which is administered is dependent, in the judgement of the attending clinician, upon a variety of factors, e.g. the age and weight of the individual being treated, the mode of administration, the potency of the administered compound, the indication for which the durg is administered and the severity of the ailment being treated.

Because of the broad anti-enteroviral activity of the compound of formula I, the high degree of potency of the molecule against enteroviruses, the efficacy afforded to enterovirus-infected mice following oral administration of the molecule, and measurable blood levels in dogs and primates following oral dosage of the molecule, we propose that the compound of formula I will have utility in treating and/or preventing human enterovirus infections, including the diseases listed in Table 2.

In a preferred aspect of this invention, the effective therapeutic and/or prophylactic treatment of the following enteroviral diseases in humans by the compound of formula I or a pharmaceutical composition thereof is expected: enteroviral respiratory disease (ie., summer cold, summer grippe), enteroviral aseptic meningitis, enteroviral meningoencephalitis, chronic enterovirus infection in agammaglobulinemics, or acute hemorrhagic conjunctivitis caused by enterovirus-70.

Patient Population: Selection of a patient population experiencing enterovirus disease would be based on: (i) pathognomic symptomatology associated with some enterovirus diseases (e.g., acute rhinitis/cold symptoms during the summer, skin lesions in hand-foot-and-mouth diseases, vesicle in pharynx, or myalgia associated with pleurodynia); (ii) confirmation of enterovirus infection by isolation of virus using cell culture techniques ; (iii) PCR (polymerase chain reaction) diagnosis of viral genetic material (e.g., in cerebral spinal fluid for enteroviral aseptic meningitis); immunological techniques such as fluorescent antibody enzyme-linked imunoabsorbant technolgy; or a combination of the above.

Efficacy Evaluation: Evaluation of the efficacy of the compound of formula I in humans affected with enteroviral disease is measured by reduction of symptomatology and/or the viral tissue load associated with a particular enteroviral illness. For example, parameters that could be measured to assess efficacy include: (i) decrease of symptoms associated with summer cold; decrease in shed virus,

decrease in mucous weight, decrease in fever, and decrease in myalgia compared to placebo-treated individuals; (ii) for aseptic meningitis: reduction of time in hospital, reduction of headache & fever, decrease in time to normal neurological findings compared to placebo patients; (iii) for hemorrhagic conjunctivitis: improvement in sight, clearing of conjunctiva more rapidly compared to placebos. Other parameters to assess the efficacy of the compound of formula I may need to be established for each enteroviral disease under clinical testing, in the opinion of the attending clinician.

Summary of Results

Against 154 enteroviruses representing 15 common isolated enteroviral immunotypes, the compound of formula I exhibits an average IC_{50} (50% inhibitory concentration) of 0.98 µg/ml (2.31 µM) in the plaque assay (Table 3). The average IC_{50} for 30 less frequently isolated enteroviruses and laboratory strains of enteroviruses, including poliovirus (polio) types 1 and 2, is 0.97 µg/ml (2.29 µM). Using the MTT assay as a measure of adverse effects produced by the compound of formula I in uninfected cells, no adverse effects were observed in cell cultures at concentrations of the compound of formula I equal to or greater than 51 times the antiviral IC_{50} concentrations for 75% of the enteroviruses tested (138 of 184 viruses, including common clinical isolates and laboratory strains of enteroviruses). Thus, the antiviral activity of the compound of formula I is not due to cytotoxic effects of the compound. Against rhinoviruses, which represent another genus of picornaviruses, the compound of formula I exhibited an activity (IC_{50}) equal to or less than 10 µg/ml (23.6 µM) agains 38% of rhinoviruses which were surveyed. Representing a third genus of picornaviruses, a single strain of hepatitis A virus was inhibited by the compound of formula I at an IC_{50} value of 10.1 µg/ml (23.8 µM). The compound of formula I was inactive against other RNA viruses or DNA viruses comprising ten viral families which were tested, including influenza virus, human immunodeficiency virus, herpes simplex virus, and adenovirus.

The compound of formula I was orally active prophylactically (24 hrs. before infection) and therapeutically (3.5 hrs. after infection) against Echovirus 4, Coxsackievirus A9, and poliovirus 2 infections in murine models. The compound of formula I also exhibited increased activity in vivo as the size of the viral inoculum decreased and it reduced viral tissue titers in mice in both therapeutic and proprophylactic regimens.

The compound of formula I is orally bioavailable after a single oral dose: In mice receiving 1 mg/kg, oral absorption was 78% and absolute oral bioavailability was

50%. After oral administration to mice, the compound of formula I reaches inhibitory levels in the brain, the primary site of viral infection in the murine models. Blood levels of the compound of formula I are also achieved in mice, rats, dogs, and monkeys following oral dosage. The half-life of the molecule in the serum increases from 4.7 hours in mice, to 26 hours in dogs.

Based on the antiviral activity of the compound of formula I in cell culture and in animal models and the ability to achieve significant blood and tissue levels in mice, rats, beagles, and cynomolgus monkeys following oral administration of the molecule, efficacious clinical behavior in humans for the therapeutic and prophylactic treatment of enteroviral infections in humans is expected.

Table 1. Taxonomy of the the Picornavirus Family.

Genus	Group or Viral Immunotypes	Subgroup	No. of[a] Viruses	Member[b] Viruses
Enterovirus[c]	coxsackieviruses	A B	23 6	CVA1-22, 24 CVB1-6
	echoviruses	--[d]	30	Echo 1-9, 11-27, 29-34
	enteroviruses	--[d]	4	Entero 68-71
	polioviruses	--[d]	3	Polio 1-3
	Vilyuisk virus[e]	--[d]	1	Vilyuisk
Rhinovirus	human rhinovirus	A B	33 67	--[f] --[f]
Hepatitis A	hepatitis A virus	--[d]	1	Hepatitis A
Apthovirus	foot-and-mouth-[g] disease virus	--[d]	7	FMDV A,C,O, SAT 1,3,3, Asia-1
Cardiovirus	encephalomyo-[h] carditis (EMC) virus	EMC	6	EMC, MM mengo, ME, SK, Columbia
	Theiler's murine-[i] encephalo-myelitis virus (TMEV)	TMEV	7	GDVII, FA DA, WW, TO4, Yale, BeAn 8366

Legend to Table 1.

a Number refer to the number of viruses or number of viral immunotypes in a subgroup.

b Abbreviations for the names of viruses are shown. In instances where numbers or viruses are missing (e.g., CVA23), viruses having these numbers originally were incorrectly classified in the enterovirus genus. Upon reclassification, the numbers were dropped from the classification system foe enteroviruses (Rueckert, 1990).

c Enteroviruses which infect non-human species as their natural host are not included in this listing.

d Viral subgroups do not exist.

e Vilyuisk virus is associated with a neurodegenerative disease occurring in humans in Siberia (Casals, 1963; Lipton et al., 1983).

Legend to Table 1. (continued)

f Although 100 immunotypes of human rhinoviruses are officially recognized by the International Committee on the Taxonomy of Viruses, at least 10 more rhinoviruses have been reported. Classification of individual rhinoviruses into groups A and B can be found in Andries et al. (1992).

g Foot-and-mouth-disease viruses do not infect humans.

h EMC viruses have rarely been isolated from humans, however they are not associated with any apparent disease (Lipton & Rozhon, 1986).

i Theliler's murine encephalitis viruses infect only mice (Lipton & Rozhon, 1986).

Table 2. Clinical Spectrum of Enteroviral Disease[a]

Disease	Principal[b] Viral Immunotypes
Diseases of Proven Enterovirus Etiology	
respiratory disease (summer colds; summer grippe)	many coxsackievirus A & B and echovirus immunotypes
pneumonia, pneumonitis, bronchiolitis	CVA 9 & 16, CVB 4 & 5; echo 68
aseptic meningitis	CVA 1-11, 14, 16-18, 22, 24; CVB 1-6; all echo except types 24, 26, 29, 32; polio 1-3
meningoencephalitis	CVB 1-5; enterovirus types 68-72
encephalitis	CVA 2, 5, 6, 7, 9; CVB 1-3, 5, 6; echo 2, 6, 9, 19, others less frequently
chronic infection in agamma-globulinemics	echo 2, 3, 5, 9, 11, 19, 24, 25, 30, 33
poliomyelitis, paralysis, paralytic myelitis	polio 1-3; CVA 4, 6, 7, 9, 11, 14, 21; CVB 1-6; echo 1-4, 6, 7, 9, 11, 16, 18, 19, 30; entero 70, 71
myocarditis, pericarditis, dilated cardiomyopathy	CVB 1-5
exanthems (rubelliform, roseoliform, herpetiform)	CVA 2, 4, 5, 9, 16; CVB 1, 3, 4, 5; echo 9, 16; other less frequent echo types
sepsis, systemic infection in neonates & infants	CVB 1-5; echo 6, 9, 11, 14, 19, 31; CVA 2, 9, 16 less frequently
Guillian-Barre syndrome	echo 2, 6, 9, 19; possible other echo types
hepatitis, pancreatitis	CVA 4, 9; CVB 5; echo 4 & 9; also same viruses that cause systemic infection in infants
herpangina	CVA 2-6, 8, 10, 22
hemorrhagic conjunctivitis	enterovirus-70; CVA 24 variant
epidemic pleurodynia, myalgia, Bornholm disease	CVB 1-5; CVA 4, 6, 10 sporadically

Table 2. (continued). Clinical Spectrum of Enteroviral Disease[a]

hand, foot & mouth disease	CVA 5, 7, 9, 10, 16; enterovirus 71
lymphonodular pharyngitis	CVA 10
epidemic myalagia	echo 1, 6, 9
Disease of Possible Enterovirus Etiology	
neonatal diarrhea, general diarrhea	CVA 18, 20-22, 24; many echo types
postviral fatigue syndrome	CVB immunotypes
adult onset (insulin-dependent) diabetes mellitus	CVB immunotypes

a.Abelman, W. H. 1988. The etiology, pathogenesis and physiology of dilated cardiomyopathies. In: New concepts in Viral Heart Disease (H.P. Schultheiss, ed.), pp. 3-21. Springer-Verlag, Berlin, Germany.

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Wagenknecht, L.E., Rosemon, J.M., and Herman, W. H. 1991. Increased incidence of insulin-dependent diabetes-mellitus following an epidemic of coxsackievirus B5. Am. J. Epidemiology 133: 1024-1031.

Wright, P. W., Strauss, G. H., and Langford, M.P. 1992. Acute hemorrhagic conjunctivitis. American Family Physician 45: 173-178.

b Viruses shown in this column represent the most common enteroviruses isolated from individuals diagnosed with each disease, however other enteroviruses, which are not listed as being associated with the disease, may be capable of producing the same disease.

Table 3. Anti-Viral Activity of the Compound of Formula I Against 15 Common Clinical Enterovirus Isolates[1]

Entereoviral Immunotype[1]	Mean[2] IC_{50} (µg/mL)
E3	0.40
E4	0.15
E5	0.93
E6	0.30
E7	0.16
E9	1.45
E11	0.08
E24	0.15
E30	0.42
CA9	0.73
CB1	3.02
CB2	0.31
CB3	0.58
CB4	0.19
CB5	2.80

1. E =Echorirus Immunotype.
   CA =Coxsackievirus A Immunotype.
   CB =Coxsackievirus B Immunotype.
2. Antiviral Activity was determined by per-mix plaque assay (see Materials and Methods Section).

Table 4. Summary of Cell Culture-Based Activies of the Compound of Formula I Against 15 Common Enteroviruses and Laboratory Strains of Enteroviruses.

Enterovirus Group	No. of Viruses Tested	No. with IC_{50} ≤ 1 µg/ml	No. with IC_{50} ≤ 10 µg/ml	Average[a] IC_{50}	EC_{80}[b]
15 Common Isolates[c]	158	125	155	0.98 µg/ml (2.31 µM)	0.9 µg/ml (2.12 µM)
Lab Strains/ Infrequent Isolates[d]	37	24	30	0.97µg/ml (2.29 µM)	0.8 µg/ml (1.89 µM)

a Average IC_{50} value was calculated from the antiviral activities (pre-mix plaque assay) of 128 viruses from the 15 common enteroviruses isolate group and 30 viruses from the lab strain group.

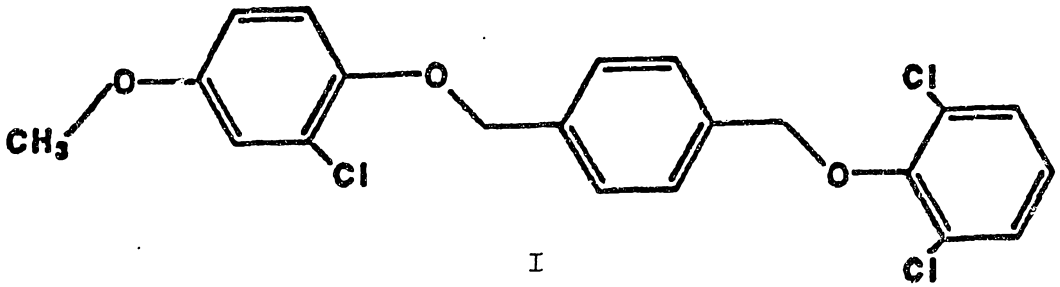
b EC_{80} represents the concentration of the compund formula I that inhibits 80% of the viruses tested based on their IC_{50} values.

c The 15 common enteroviral isolates are listed in Table 3.

d Includes polioviruses 1-3 and enterovirus 70.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of treatment and/or prevention of acute hemorrhagic conjunctivitis in humans caused by enterovirus-70 comprising administering an effective amount of 2-chloro-1-[[4-[(2,6-dichlorophenoxy)methyl]-phenyl]methoxy]-4-methoxybenzene represented by formula I



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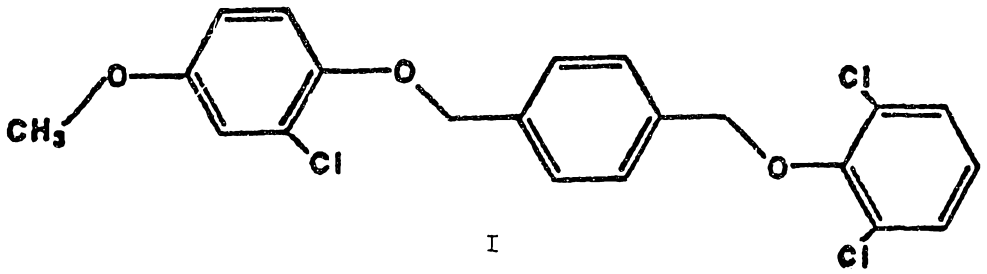
to a patient in need of such treatment.

2. A method according to claim 1 wherein the 2-chloro-1-[[4-[(2,6-dichlorophenoxy)methyl]-phenyl]methoxy]-4-methoxybenzene is administered orally.

3. A method according to claim 1 or claim 2 wherein the 2-chloro 1-[[4-[(2,6-dichlorophenoxy)methyl]-phenyl]methoxy]-4-methoxybenzene is administered at a rate of 1-60 mg/kg of body weight per day.

4. A method of treatment and/or prevention of acute hemorrhagic conjunctivitis caused by enterovirus-70 comprising administering an effective amount of 2-chloro-1-[[4-[(2,6-dichlorophenoxy)methyl]-phenyl]methoxy]-4-methoxybenzene represented by formula I to a patient in need of such treatment, the method being substantially as herein described with reference to the accompanying examples.

5. 2-chloro-1-[[4-[(2,6-dichlorophenoxy)methyl]-phenyl]methoxy]-4-methoxybenzene represented by formula I



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when used in the treatment and/or prevention of acute hemorrhagic conjunctivitis in humans caused by enterovirus-70, by administering an effective amount of the 2-chloro-1-[[4-[(2,6-dichlorophenoxy)methyl]-phenyl]methoxy]-4-methoxybenzene to a patient in need of such treatment.

DATED this 23rd day of March 1998
SCHERING CORPORATION

By their Patent Attorneys
GRIFFITH HACK

INTERNATIONAL SEARCH REPO! .T

Int ional Application No
PCT/US 94/01605

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/085

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)
IPC 5 A61K

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, w... practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	EP,A,0 519 702 (SHERING CORP.) 23 December 1992 cited in the application see page 5, column 442 - column 49 see page 8, line 26 - line 34 see page 9, line 30 - line 35 see page 20, line 25 see page 21; example 15a	1-10
X	& WO,A,92 22520 (SHERING CORP.) --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance

'E' earlier document but published on or after the international filing date

'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 May 1994	16.06.94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Gerli, P

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

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 94/01605

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANTIMICROB.AGENTS CHEMOTHER. vol. 28, no. 4 , 1985 pages 578 - 80 'Activity of Arildone with or without Interferon against acute hemorragic conjunctivitis viruses in cell culture' see page 578, left column, line 6-9 see page 578, left column, line 35 - line 41 -----	1-10

INTERNATIONAL SEARCH REPORT

Int. ..ational application No.
PCT/US94/01605

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 3, (partially) 9-10 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the COMPOUND/COMPOSITION.

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

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

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

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

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

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

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ional Application No
PCT/US 94/01605

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0519702	23-12-92	AU-A-	2221392	12-01-93
		CA-A-	2111854	23-12-92
		EP-A-	0590026	06-04-94
		WO-A-	9222520	23-12-92
WO-A-9222520	23-12-92	AU-A-	2221392	12-01-93
		CA-A-	2111854	23-12-92
		EP-A-	0519702	23-12-92
		EP-A-	0590026	06-04-94