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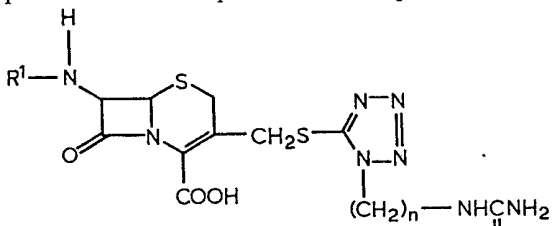
(54) NEW CEPHALOSPORIN COMPOUNDS

(71) We, SMITHKLINE CORPORATION of 1500 Spring Garden Street, Philadelphia, Pennsylvania 19101, United States of America, a corporation organized under the laws of the Commonwealth of Pennsylvania, one of the United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-

This invention relates to a new series of cephalosporin compounds, to a process for their preparation to pharmaceutical compositions containing them and to a method of treating bacterial infections in non-human animals using these compounds.

Accordingly the present invention provides a compound of formula (I) :-



(I)

in which :

R^1 is $X\text{-}\underset{\underset{A}{|}}{CH}\text{-}\overset{\overset{O}{\|}}{C}-$, $Y\text{-}CH_2\text{-}\overset{\overset{O}{\|}}{C}-$ or $Z\text{-}S(O)_m\text{-}CH_2\text{-}\overset{\overset{O}{\|}}{C}-$

where X is thienyl, dihydrophenyl, phenyl or phenyl monosubstituted with hydroxy, hydroxymethyl, formamido, ureido or carboxymethylamino; A is NH_2, OH, COOH or SO_3H; or formyloxy when X is phenyl; Y is cyano, aminomethylphenyl, sydnonyl, thienyl or tetrazolyl; Z is methyl, trifluoromethyl, trifluoroethyl, pyridyl or cyanomethyl; m is zero, one or two; and n is two to five, and esters thereof and pharmaceutically acceptable salts thereof.

It will be recognized that the cephem-4-carboxylic acid group of the compounds of formula (I) and the group A when A is COOH may be esterified. Accordingly, by an ester of a compound of formula (I) we mean an ester of the 4-carboxylic acid group and/or A. These esters include, for example, simple alkyl and aryl esters as well as esters which are easily cleaved, within the body, to the parent acid, for example, indanyl, pivolyloxymethyl, acetoxymethyl, propionyloxymethyl, glycyloxymethyl, phenylglycyloxymethyl and thienylglycyloxymethyl esters.

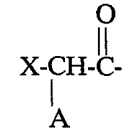
Compounds of formula (I) having one or more carboxylic acid groups can form salts with salt forming cations and compounds of formula (I) having a free amino group can form acid addition salts. Thus, by a salt of a compound of formula (I), we mean a salt with a salt forming cation or an acid addition salt as the context requires.

Compounds of formula (I) which have a free amino and carboxylic acid group can also exist as a zwitter-ion.

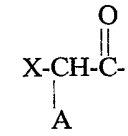
Examples of salts of compounds of formula (I) include those with alkali metals, for example, sodium and potassium, alkaline earths, for example, calcium, and with the ammonium cation.

Preferred compounds of this invention are represented by formula (I) in which n is two.

Advantageous compounds of this invention are represented by formula (I) in which n is two and R^1 is



Most advantageous are the compounds represented by formula (I) where n is two, R^1 is



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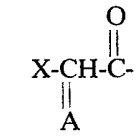
X is phenyl or hydroxyphenyl and A is NH_2 or OH.

Examples of the most preferred 7-acylamino substituents (R^1NH-) of the compounds of the formula (I) are listed below:

α-hydroxyphenylacetamido
α-aminophenylacetamido
α-amino-4-hydroxyphenylacetamido
trifluoromethylthioacetamido
methylthioacetamido
2,2,2-trifluoroethylsulfinylacetamido
cyanoacetamido
α-carboxythienylacetamido
α-carboxyphenylacetamido
α-sulfophenylacetamido
methylsulfonylacetamido
cyanomethylthioacetamido
α-amino-4-carboxymethylaminophenylacetamido
2-aminomethylphenylacetamido
3-sydnonylacetamido
1-tetrazolylacetamido
2-thienylacetamido
2-pyridonyl-N-acetamido
4-pyridonyl-N-acetamido
4-pyridylthioacetamido.

When R^1 is



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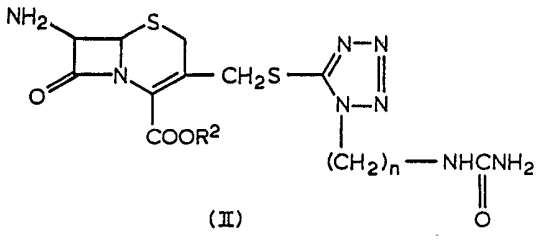
the carbon atom of the 7-acylamido group is asymmetric and will exist as optical isomers. Reference to a compound of formula (I) having such a group R^1 is to be understood as a reference to a racemic mixture and to optically pure enantiomers.

Particularly preferred is the compound 7-D-mandelamido-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid.

The compounds of formula (I) can be prepared by acylation of an appropriate 7-amino-3-ureidoalkyltetrazolylthiomethyl-cephalosporin of formula (II) :-



(II)

in which :

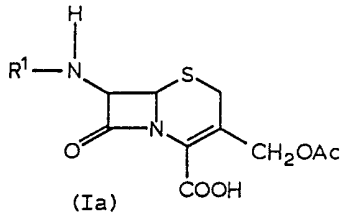
n is two to five; and R^2 is hydrogen or an ester-forming group, with an acylating agent capable of supplying the group R^1 as defined with reference to formula (I) provided that A also represents a protected amino group, removing any protecting group, optionally removing any ester-forming group, optionally converting a compound of formula (I) so obtained having a free carboxyl group into an ester or pharmaceutically acceptable salt and optionally converting a compound of formula (I) so obtained having a free amino group into a pharmaceutically acceptable salt.

The carboxylic acid group of the acylating agent is activated by any of the standard methods such as conversion to the mixed anhydride, acid chloride, acid imidazolide or activated ester. In addition, a reagent such as dicyclohexylcarbodiimide can be used provided that the carboxyl group on the cephem nucleus is protected with an easily removable protecting group, for example, benzhydryl, *t*-butyl, trichloroethyl, benzyl, benzyloxymethyl, *p*-methoxybenzyl or *p*-nitrobenzyl group. When A is NH_2, the α-amino group of the acylating agent is, preferably, protected prior to acylation with an easily removable protective group known in the art, for example, *t*-butoxycarbonyl, trichloroethoxycarbonyl, benzyloxycarbonyl, or similar groups commonly used in the synthesis of peptides.

The acylating agents used as starting materials are either known or can be prepared by known methods.

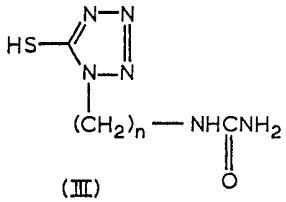
Alternatively, the compounds of formula (I) can be prepared from an acylated 7-aminocephalosporanic acid derivative by displacing the 3-acetoxy group with a ureidoalkyltetrazolyl-thiol.

Accordingly the invention provides a process for preparing a compound of formula (I) as previously defined or an ester thereof or a pharmaceutically acceptable salt thereof which comprises reacting a compound of formula (Ia) :-



(Ia)

in which R^1 is as defined with reference to formula (I) provided that A also represents a protected amino group, or a salt thereof, with a thiol of formula (III):-



(III)

in which n is two to five, or a salt thereof; removing any protecting group, optionally removing any ester-forming group, optionally converting a compound of formula (I) so obtained having a free carboxylic acid group into an ester or a pharmaceutically acceptable salt and optionally converting a compound of formula (I) so obtained having a free amino group into a pharmaceutically acceptable salt.

Compounds of formula (Ia) can be prepared by acylating 7-aminocephalosporanic acid or a salt or an ester thereof by standard methods using an acylating agent as previously described.

The protecting groups referred to above can be removed according to methods well known to the art, for example, with trifluoroacetic acid when the protecting group is *t*-butyl or *t*-butoxycarbonyl. The resulting salt is converted to the zwitterionic product or to the free acid by means of a basic ion exchange resin, for example, polystyreneamine ion exchange resin (Amberlite IR-45; Amberlite is a Registered Trade Mark) or else by basification of an aqueous solution of the salt.

The 7-amino-3-ureidoalkyltetrazolylthiomethyl-cephalosporan starting materials of formula (II) can be prepared by reacting 7-aminocephalosporanic acid, or a salt or ester thereof, with a ureidoalkyltetrazole-thiol of formula (III) and thereafter optionally esterifying or salifying a compound of formula (II) so obtained having a free carboxyl group.

The ureidoalkyltetrazole-thiols of formula (III) can be prepared by reacting the corresponding 1-aminoalkyl-5-(2,4-dinitrophenylthio)tetrazole compounds, by displacement of the acetamido moiety, with cyanic acid and subsequent cleavage of the 2,4-dinitrophenyl protecting group. In this process the cyanic acid is preferably prepared *in situ*, for example, from potassium cyanate and acetic acid. The 5-(2,4-dinitrophenylthiol)tetrazole compound can be prepared from 2,4-dinitrofluorobenzene and a 1-acetamidoalkyltetrazole-5-thiol. The 1-acetamidoalkyltetrazole-5-thiols can be prepared by reacting an acetamidoalkyldithiocarbamate, for example, methyl 2-acetamidoethyldithiocarbamate with an azide, for example, sodium azide. The acetamidoalkyldithiocarbamates are prepared by treatment of an N-aminoalkylacetamide, for example, N-(2-aminoethyl)acetamide with carbon disulfide and an alkyl halide, for example, methyl iodide in the presence of a base, for example, triethylamine.

Compounds of formulae (II) and (III) are disclosed and claimed in our copending Applications Nos. 24429/79 (Serial No. 1573006) and 24430/79 (Serial No. 1573005).

Pharmaceutically acceptable salts of the compounds (I) can be prepared by standard methods using a wide variety of acids and bases known in the art to form such salts.

Compounds of formula (I) can be esterified by known methods.

Compounds of formula (I) having an asymmetric α-carbon atom in the 7-acylamido group can be obtained as racemic mixture or resolved products depending upon whether a racemic or resolved acylating agent is used. The resolved acylating agents can be prepared from resolved acids which in turn can be obtained from the racemic compounds by resolution according to well known methods, including fractional crystallization of a salt formed with an optionally active acid or base.

The compounds of formula (I) have antibacterial activity against both Gram-positive and Gram-negative organisms. Minimum inhibitory concentrations (MIC's) range from 0.1 to >200 μg./ml. in *in vitro* testing. Test results for the compound 7-D-mandelamido-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid are given below:

Bacteria	MIC (μg./ml.)
S. aureus HH 127	3.1, 1.6
S. aureus SK 23390	0.4, 0.4
S. villaluz SK 70390	25, 25
Strep. faecalis HH 34358	25, 25
E. coli SK 12140	0.8, 0.8
E. coli HH 33779	1.6, 1.6
Kleb. pneumo. SK 4200	0.8, 0.8
Kleb. pneumo. SK 1200	0.4, 0.4
Salmonella ATCC 12176	0.8, 0.2
Shigella HH 117	0.1, 0.2
Pseudo. aerug. HH 63	>200, >200
Serratia Marc. ATCC 13880	25, 25
Proteus morganii 179	0.8, 0.8
Entero aerog. ATCC 13048	1.6, 6.3
Entero. cloacae HH 31254	0.8, 0.8

In the *in vivo* mouse protection test, 7-D-mandelamido-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid exhibited ED_{50} s of 0.86 mg./kg. against E. coli 12140 and 1.34 mg./kg. against Kleb. pneumo. 4200 upon subcutaneous injection; and 17.5 mg./kg. against E. coli 12140 and 25.5 mg./kg. against Kleb. pneumo. 4200 upon oral administration.

The invention also provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of formula (I) or a pharmaceutically acceptable salt or

ester thereof which is easily cleaved within the body to the parent acid.

The compositions of this invention can be adapted for oral or parenteral administration, although parenteral administration is preferred. Compositions for parenteral administration are adapted for subcutaneous, intramuscular or intravenous injection. Injectable compositions are sterile solutions or suspensions containing an effective non-toxic amount of the compound in a liquid carrier.

The above composition can be made by mixing compounds of formula (I) with a pharmaceutically acceptable carrier in the same manner as other cephalosporins.

Accordingly the invention also provides a process for preparing a pharmaceutical composition which comprises mixing a compound of formula (I) or a pharmaceutically acceptable salt or ester thereof which is easily cleaved within the body of the parent acid with a pharmaceutically acceptable carrier.

Compounds of formula (I) can be administered in the same was as other cephalosporins. The dosage regimen comprises administration, preferably by injection, of an active but non-toxic quantity of a compound of formula (I) selected from the dosage unit range of from 100 to 1000 mg. with the total daily dosage regimen being from 400 mg. to 6 g. The precise dosages are dependent upon the age and weight of the subject and on the infection being treated and can be determined by those skilled in the art based on the data disclosed herein compared with that available to the art attained with known cephalosporins.

The invention further provides a method for treating a bacterial infection in a non-human animal which comprises administering a dose of a compound of formula (I) or a pharmaceutically acceptable salt or ester thereof which is easily cleaved in the body to the parent acid, alone or in admixture with a pharmaceutically acceptable carrier.

The following Examples illustrate the invention. Temperatures are in degrees Centigrade (°C) unless otherwise stated.

EXAMPLE 1

7-D-Mandelamido-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid

To a solution of 20.4 g. (0.20 mol.) of N-(2-aminoethyl)acetamide in 200 ml. of 95% ethanol were added 27.9 ml. (0.20 mol.) of triethylamine and 12.0 ml. (0.20 mol.) of carbon disulfide. The exothermic reaction reached reflux and then the mixture was cooled to ambient temperature over a 1.5 hour period. Methyl iodide (28.4 g., 0.20 mol.) was added, which again produced an exothermic reaction. After 1.75 hours the reaction mixture was evaporated to dryness and the solid residue was dissolved in 200 ml. of water. The aqueous solution was extracted twice with 250 ml. portions of ethyl acetate. The extracts were combined, shaken with sodium thiosulfate, dried ($MgSO_4$) and evaporated to dryness to give methyl 2-acetamidoethyldithiocarbamate.

To a solution of 38.4 g. (0.198 mol.) of methyl 2-acetamidoethyldithiocarbamate in 100 ml. of 95% ethanol was added a solution of 13.5 g. (0.208 mol.) of sodium azide in 100 ml. of water. The reaction mixture was refluxed for 24 hours, then cooled and concentrated under reduced pressure to about half volume. The solution was cooled to 15°, 50 ml. of 6N sulfuric acid were added and the acidic solution was filtered. The filtrate was concentrated to about 100 ml., chilled at 5° and 1-(2-acetamidoethyl)tetrazole-5-thiol was collected, m.p. 139-139.5°. Additional amounts of the product were obtained by continuous extraction of the filtrate with ethyl acetate.

A solution of 9.3 g (0.050 mol.) of 2,4-dinitrofluorobenzene in 50 ml. of acetone was added to a solution of 9.35 g. (0.050 mol.) of 1-(2-acetamidoethyl)tetrazole-5-thiol and 6.85 ml. (0.050 mol.) of triethylamine in 100 ml. of acetone and the reaction mixture was stirred for 1 hour. The solid was collected and recrystallized from acetonitrile to give 1-(2-acetamidoethyl)-5-(2,4-dinitrophenylthio)tetrazole, m.p. 197-198°.

A mixture of 6.5 g. (0.02 mol.) of 1-(2-acetamidoethyl)-5-(2,4-dinitrophenylthio)tetrazole, 100 ml. of 12 N hydrochloric acid and 100 ml. of 95% ethanol was refluxed for 4.5 hours. The mixture was evaporated to dryness to give a gummy residue which crystallized upon addition of ethanol to give 1-(2-aminoethyl)-5-(2,4-dinitrophenylthio)tetrazole hydrochloride, m.p. 217-219° (d).

To a solution of 0.84 g. (0.010 mol.) of sodium bicarbonate and 0.81 g. (0.010 mol.) of potassium cyanate in 35 ml. of water containing 2.5 ml. of glacial acetic acid were added 3.15 g. (0.010 mol.) of 1-(2-aminoethyl)-5-(2,4-dinitrophenylthio)tetrazole hydrochloride. The mixture was refluxed for 2.5 hours, then it was filtered and the solid product was washed with water and recrystallized from methanol-acetone to give 5-(2,4-dinitrophenylthio)-1-(2-ureidoethyl)tetrazole, m.p. 190-191° (d).

A mixture of 10 g. (0.031 mol.) of 5-(2,4-dinitrophenylthio)-1-(2-ureidoethyl)tetrazole and 60 ml. (0.055 mol.) of 5% sodium methoxide in methanol was stirred for 2.5 hours. An additional 35 ml. (0.031 mol.) of sodium methoxide solution was added and the mixture was stirred at 25° for 12 hours. Ether (ca. 2 l.) was added to the reaction mixture and the

crystallized product was collected, washed with ethyl acetate and recrystallized from methanol-ether to give 1-(2-ureidoethyl)tetrazole-5-thiol sodium salt, m.p. 131-134°.

$$C_4H_7N_6OS \cdot Na \cdot 1\ H_2O$$

Calculated:	21.05% C;	3.98% H;	36.83% N
Found:	20.97% C;	4.04% H;	36.76% N

A solution of 1-(2-ureidoethyl)tetrazole-5-thiol sodium salt in water is passed through an Amberlite IR-120H ion exchange resin column to give, after lyophilization, 1-(2-ureidoethyl)tetrazole-5-thiol.

A mixture of 1.5 g. (0.071 mol.) of 1-(2-ureidoethyl)tetrazole-5-thiol sodium salt and 2.14 g. (0.005 mol.)of 7-D-mandelamidocephalosporanic acid sodium salt in 25 ml. of water was heated at ca. 80° for 2.5 hours. The reaction mixture was passed through an Amberlite XAD-8 resin column while eluting with water and then methanol. The product-containing aqueous fractions were lyophilized to give 7-D-mandelamido-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid sodium salt.

$$C_{20}H_{21}N_8O_6S_2 \cdot Na \cdot 2.5\ H_2O$$

Calculated:	39.89% C;	4.32% H;	18.61% N
Found:	39.98% C;	3.86% H;	18.51% N

An aqueous solution of 7-D-mandelamido-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid sodium salt is acidifed with 3 N HCl to pH 2.5 and extracted with ethyl acetate. The extract is dried over anhydrous magnesium sulfate and evaporated to dryness to give the title compound.

EXAMPLE 2

7-(D-α-Aminophenylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid

A solution of 7.58 g. (0.015 mol.) of 7-(D-α-*t*-butoxycarbonylaminophenylacetamido)-cephalosporanic acid, 1.88 g. (0.01 mol.) of 1-(2-ureidoethyl)tetrazole-5-thiol and 2.52 g. (0.03 mol.) of sodium bicarbonate in 125 ml. of water is stirred at 60° for 5 hours while maintaining the pH at 7.0-7.2 by addition of sodium bicarbonate. The mixture is cooled and extracted with ethyl acetate. The aqueous phase is acidifed to pH 2.5 with 3N hydrochloric acid and the acidic solution is extracted again with ethyl acetate. The extract is dried ($MgSO_4$), filtered and evaporated to dryness to give 7-(D-α-*t*-butoxycarbonylaminophenylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid.

7-(D-α-*t*-Butoxycarbonylaminophenylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid (ca. 1 g.) is stirred at 25° with 25 ml. of trifluoroacetic acid and 25 ml. of 1,3-dimethoxybenzene for 2.25 hours. The mixture is evaporated to dryness, ether is added to the residue and the precipitate is collected, washed with ether, stirred in acetonitrile for 2 hours, then collected and dried *in vacuo* to give the title compound as its trifluoroacetic acid salt. The salt is dissolved in water and the solution is stirred with Amberlite IR-45 ion exchange resin then lyophilized to give the title compound.

EXAMPLE 3

Reaction of the N-*t*-butoxycarbonyl derivative of the following cephalosporanic acids:
7-(α-amino-4-hydroxyphenylacetamido)cephalosporanic acid
7-(α-amino-4-formamidophenylacetamido)cephalosporanic acid
7-(α-amino-3-formamidophenylacetamido)cephalosporanic acid
7-(α-amino-4-ureidophenylacetamido)cephalosporanic acid
7-(α-amino-3-ureidophenylacetamido)cephalosporanic acid
7-(α-amino-4-hydroxymethylphenylacetamido)cephalosporanic acid
7-(α-amino-1,4-cyclohexadienylacetamido)cephalosporanic acid
7-(α-amino-4-carboxymethylaminophenylacetamido)cephalosporanic acid

with 1-(2-ureidoethyl)tetrazole-5-thiol as described in the procedure of Example 2, followed by removal of the protective group and conversion of the trifluoroacetic acid salt to the free acid as described therein gives the following compounds of this invention:
7-(α-amino-4-hydroxyphenylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
7-(α-amino-4-formamidophenylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-

3-cephem-4-carboxylic acid
7-(α-amino-3-formamidophenylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
7-(α-amino-4-ureidophenylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
7-(α-amino-3-ureidophenylacetamido-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
7-(α-amino-4-hydroxymethylphenylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
7-(α-amino-1,4-cyclohexadienylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
7-(α-amino-4-carboxymethylaminophenylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid

EXAMPLE 4

7-(4-Hydroxymandelamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid is prepared by reaction of 7-(4-hydroxymandelamido)cephalosporanic acid sodium salt and 1-(2-ureidoethyl)tetrazole-5-thiol sodium salt as described in the procedure of Example 1, followed by conversion of the product sodium salt to the free acid as described herein.

EXAMPLE 5

When the sodium salt of a cephalosporanic acid listed below:
7-(3-sydnonylacetamido)cephalosporanic acid
7-(2-thienylacetamido)cephalosporanic acid
7-(1-tetrazolylacetamido)cephalosporanic acid
is reacted with 1-(2-ureidoethyl)tetrazole-5-thiol sodium salt by the procedure described in Example 1 and the product is converted to the free acid as described therein, the following compounds of this invention are obtained, respectively:
7-(3-sydnonylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
7-(2-thienylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
7-(1-tetrazolylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid.

EXAMPLE 6

7-(2-Aminomethylphenylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid

When 7-(2-aminomethylphenylacetamido)cephalosporanic acid sodium salt is reacted with 1-(2-ureidoethyl)tetrazole-5-thiol sodium salt by the procedure described in Example 1 and the product is converted to the free acid as described therein, the title compound is obtained.

EXAMPLE 7

7-Trifluoromethylthioacetamido-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid

A solution of 1.88 g. (10.0 mmol.) of 1-(2-ureidoethyl)tetrazole-5-thiol, 0.840 g. of sodium bicarbonate and 5.45 g. (12.5 mmol.) of 7-trifluoromethylthioacetamido-cephalosporanic acid sodium salt in 60 ml. of water is stirred at 70-75° for 5 hours while maintaining the pH at 6.8 by addition of 5% aqueous sodium carbonate solution. The reaction mixture is cooled and diluted with water. Ethyl acetate is added and the mixture is acidified to pH 2.0 with 6N hydrochloric acid. The combined aqueous phases are further extracted with ethyl acetate and the extracts are dried ($MgSO_4$) and evaporated to dryness to give the title compound.

EXAMPLE 8

Reaction of the sodium salt of a cephalosporanic acid listed below:
7-(2,2,2-trifluoroethylthioacetamido)cephalosporanic acid
7-trifluoromethylsulfinylacetamidocephalosporanic acid
with 1-(2-ureidoethyl)tetrazole-5-thiol as described in the procedure of Example 7 gives the following compounds of this invention as final products:
7-(2,2,2-trifluoroethylthioacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
7-trifluoromethylsulfinylacetamido-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-

cephem-4-carboxylic acid.

EXAMPLE 9

7-Amino-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid

A solution of 11.29 g. (0.06 mol.) of 1-(2-ureidoethyl)tetrazole-5-thiol in 120 ml. of acetone is added to a warm (45°) solution of 10.9 g. (0.04 mol.) of 7-aminocephalosporanic acid in a mixture of 220 ml. of water, 50 ml. of acetone and 8.4 g (0.01 mol.) of sodium bicarbonate. The temperature is raised to 65° and the pH maintained at 7.4-7.6 by addition of aqueous sodium carbonate solution. After 3 hours, the acetone is removed *in vacuo* and the reaction mixture is cooled to 10° and adjusted to pH 3.5 by addition of dilute hydrochloric acid. The product is collected and washed with water and then acetone to give the title compound.

EXAMPLE 10

When an equivalent amount of an N-aminoalkylacetamide listed below:

N-(3-aminopropyl)acetamide
N-(4-aminobutyl)acetamide
N-(5-aminopentyl)acetamide

is used in the procedure of Example 1 in place of N-(2-aminoethyl)acetamide and the resulting dithiocarbamates are treated with sodium azide to produce the corresponding 1-acetamidoalkyltetrazole-5-thiols which are converted to the 1-ureidoalkyl derivatives, all as described therein, the following 1-ureidoalkyltetrazole-5-thiols are obtained:

1-(3-ureidopropyl)tetrazole-5-thiol
1-(4-ureidobutyl)tetrazole-5-thiol
1-(5-ureidopentyl)tetrazole-5-thiol.

Reaction of the sodium salt of a 1-ureidoalkyltetrazole-5-thiol listed above with 7-D-mandelamidocephalosporanic acid sodium salt as described in the procedure of Example 1, followed by conversion of the salt formed to the free acid, gives the following compounds of this invention:

7-D-mandelamido-3-[1-(3-ureidopropyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
7-D-mandelamido-3-[1-(4-ureidobutyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
7-D-mandelamido-3-[1-(5-ureidopentyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid.

Likewise, reaction of the substituted tetrazole-thiols or the corresponding sodium salts listed above with any of the 7-acyl-cephalosporanic acids mentioned herein or their corresponding salts according to procedures described herein gives the corresponding compounds of this invention.

EXAMPLE 11

7-(2,2,2-Trifluoroethylsulfinylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid

To a stirred solution of 5.7 g. (0.03 mol.) of 2,2,2-trifluoroethylsulfinylacetic acid and 3.45 g. (0.03 mol.) of N-hydroxysuccinimide in 50 ml. of tetrahydrofuran at 0° is added 6.2 g. (0.031 mol.) of dicyclohexylcarbodiimide. The reaction mixture is stirred at 0° for 1 hour, then at 25° for 12 hours. The precipitate is filtered and washed with tetrahydrofuran and the filtrate is evaporated to dryness to give the activated ester of 2,2,2-trifluoroethylsulfinylacetic acid.

A suspension of 4.0 g. (0.01 mol.) of 7-amino-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid in 50 ml. of dry dimethylformamide is treated with 2 ml. of triethylamine and the mixture is stirred for 15 minutes at 25°. A slight excess of 0.01 mol. of the activated ester of 2,2,2-trifluoroethylsulfinylacetic acid is added to the mixture and it is stirred for an additional hour. The reaction mixture is evaporated to dryness and water and ethyl acetate are added to the residue. The layers are separated, the ethyl acetate layer is discarded, fresh ethyl acetate is added to the aqueous phase and it is acidified to pH 2.5 by addition of 6N hydrochloric acid. The mixture is filtered, the layers are separated and the aqueous phase is extracted with ethyl acetate. The combined extracts are washed with water, dried ($MgSO_4$) and evaporated to dryness to give the title compound.

In like manner, the 7-(2,2,2-trifluoroethylsulfinylacetamido) derivatives of other 3-ureidoalkyltetrazolylthiomethyl cephalosporins described above may be prepared.

EXAMPLE 12

7-Methylthioacetamido-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid

To a stirred, cooled (−20°) solution of 10.4 g. (0.026 mol.) of 7-amino-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid in 220 ml. of 3% sodium bicarbonate and 220 ml. of acetone is added dropwise a solution of 3.66 g. (0.029 mol.) of methylthioacetyl chloride in 52 ml. of acetone, during which time the pH of the reaction mixture is maintained at 8.0 by addition of 10% sodium hydroxide. After addition the reaction mixture is stirred for an additional 20 minutes at −15°, then is warmed to 25° and extracted with ether. The remaining aqueous phase is cooled, 250 ml. of ethyl acetate is added and the slurry is acidified with 3N hydrochloric acid. The layers are separated and the aqueous phase is extracted twice more with ethyl acetate. The combined extracts are dried ($MgSO_4$) and evaporated to dryness to yield the title compound.

EXAMPLE 13

7-(D-α-Formyloxyphenylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid

A mixture of 4.0 g. (0.01 mol.) of 7-amino-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid, 3.97 g. (0.02 mol.) of the formate ester of D-mandeloyl chloride and 5 g. of sodium bicarbonate in 100 ml. of water and 140 ml. of acetone is stirred in the cold for 1 hour, then at 25° for 2 hours. The acetone is evaporated *in vacuo* and the remaining aqueous mixture is extracted with ethyl acetate. The aqueous solution is added with stirring to a cold mixture of 100 ml. of water and 200 ml. of ethyl acetate and the pH of the resulting mixture is adjusted to 2.0 by addition of 6N hydrochloric acid. The mixture is filtered, the layers of the filtrate are separated and the ethyl acetate layer is washed with water, dried ($MgSO_4$) and evaporated to dryness to give the title compound.

EXAMPLE 14

Acylation of 7-amino-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid with an activated derivative of an acid listed below:

cyanoacetic acid
cyanomethylthioacetic acid
4-pyridylthioacetic acid
2-pyridone-N-acetic acid
4-pyridone-N-acetic acid

as described in the procedure of Example 11 gives the following compounds of this invention:

7-cyanoacetamido-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid

7-cyanomethylthioacetamido-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid

7-(4-pyridylthioacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cepham-4-carboxylic acid

7-(2-pyridonyl-N-acetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cepham-4-carboxylic acid

7-(4-pyridonyl-N-acetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid.

EXAMPLE 15

Reaction of a cephalosporanic acid listed below or its corresponding salt:

7-(α-hydroxy-2-thienylacetamido)cephalosporanic acid
7-(α-carboxy-2-thienylacetamido)cephalosporanic acid
7-(α-sulfophenylacetamido)cephalosporanic acid

with 1-(2-ureidoethyl)tetrazole-5-thiol sodium salt by procedures described hereinabove gives, after conversion of the product to the free acid, the following compounds of this invention:

7-(α-hydroxy-2-thienylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid

7-(α-carboxy-2-thienylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid

7-(α-sulfophenylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid.

EXAMPLE 16

7-(2,2,2-Trifluoroethylsulfonylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid

To a solution of 8.6 g. (0.019 mol.) of 7-amino-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid *t*-butyl ester and 3.9 g. (0.019 mol.) of 2,2,2-trifluoroethylsulfonylacetic acid in tetrahydrofuran is added dropwise a solution of 3.9 g. (0.019 mol.) of dicyclohexylcarbodiimide in 100 ml. of tetrahydrofuran. The reaction mixture is stirred at 25° for 12 hours, then filtered and concentrated to about 10 ml. The residue is filtered and evaporated to dryness to give 7-(2,2,2-trifluoroethylsulfonylacetamido)-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid *t*-butyl ester.

The ester is dissolved in acetonitrile and trifluoroacetic acid is added. The solution is stirred for 3 hours, then evaporated to dryness to give the title compound.

Likewise, 7-(2,2,2-trifluoroethylsulfonylacetamido) derivatives of the other 3-substituted tetrazolylthiomethyl-cephalosporins disclosed herein are prepared.

EXAMPLE 17

An injectable pharmaceutical composition is formed by adding sterile water or sterile saline solution (2 ml.) to 500 mg. of 7-D-mandelamido-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid sodium salt.

Pharmaceutical compositions of the other antibacterial compounds disclosed above may be formulated in a similar manner.

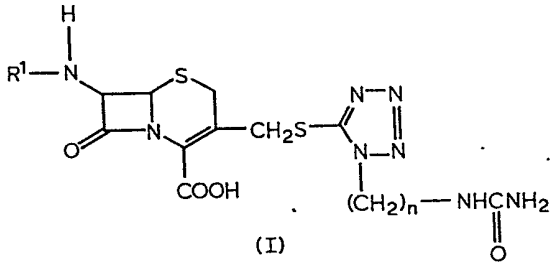
EXAMPLE 18

A tablet or capsule is formed from 500 mg. of 7-D-mandelamido-3-[1-(2-ureidoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid, 250 mg. of lactose and 75 mg. of magnesium stearate.

Tablets or capsules of the other antibacterial compounds disclosed above may be formulated in a similar manner.

WHAT WE CLAIM IS :

1. A compound of the formula (I) :-



(I)

in which:

R^1 is $X\text{-}\underset{\displaystyle A}{\underset{|}{CH}}\text{-}\overset{\displaystyle O}{\overset{\|}{C}}\text{-}$, $Y\text{-}CH_2\text{-}\overset{\displaystyle O}{\overset{\|}{C}}\text{-}$ or $Z\text{-}S(O)_m\text{-}CH_2\text{-}\overset{\displaystyle O}{\overset{\|}{C}}\text{-}$

where X is thienyl, dihydrophenyl, phenyl or phenyl monosubstituted with hydroxy, hydroxymethyl, formamido, ureido or carboxymethylamino; A is NH_2, OH, COOH or SO_3H; or formyloxy when X is phenyl; Y is cyano, aminomethylphenyl, sydnonyl, thienyl, pyridonyl, or tetrazolyl; Z is methyl, trifluoromethyl, trifluoroethyl, pyridyl or cyanomethyl; m is zero, one or two; and n is two to five, and esters thereof and pharmaceutically acceptable salts thereof.

2. A compound as claimed in claim 1 in which n is two.

3. A compound as claimed in claim 1 in which R^1 is

$$Z\text{-}S(O)_m\text{-}CH_2\text{-}\overset{\displaystyle O}{\overset{\|}{C}}\text{-}$$

and Z is trifluoromethyl or cyanomethyl.

4. A compound as claimed in claim 1 in which R^1 is

$$X\text{-}\underset{\displaystyle A}{\underset{|}{CH}}\text{-}\overset{\displaystyle O}{\overset{\|}{C}}\text{-},$$

X is phenyl or hydroxyphenyl and A is NH_2 or OH.

5. 7-Cyanomethylthioacetamido-3-[1-(2-ureidoethyl)-tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid.

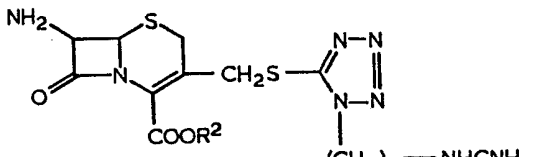
6. 7-Trifluoromethylthioacetamido-3-[1-(2-ureidoethyl)-tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid.

7. 7-(D-α-Aminophenylacetamido)3-[1-(2-ureidoethyl)-tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid.

8. 7-(α-Amino-4-hydroxyphenylacetamido)-3-[1-(2-ureido-ethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid.

9. 7-D-Mandelamido-3-[1-(2-ureidoethyl)-tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid.

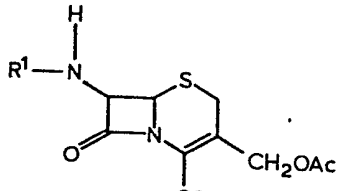
10. A process for preparing a compound as claimed in any one of claims 1 to 9 which comprises reacting a compound of formula (II):-



(II)

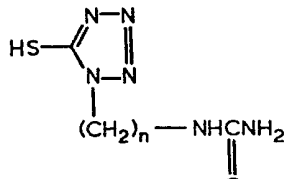
in which n is two to five and R^2 is hydrogen or an ester-forming group with an acylating agent capable of supplying the group R^1 as defined with reference to formula (I) provided that A also represents a protected amino group, removing any protecting group, optionally removing any ester-forming group, optionally converting a compound of formula (I) so obtained having a free carboxyl group into an ester or pharmaceutically acceptable salt and optionally converting a compound of formula (I) so obtained having a free amino group into a pharmaceutically acceptable salt.

11. A process for preparing a compound as claimed in any one of claims 1 to 9 which comprises reacting a compound of formula (Ia):-



(Ia)

in which R^1 is as defined with reference to formula (I) provided that A also represents a protected amino group, or a salt thereof with a thiol of formula (III):-



(III)

in which n is as defined with reference to formula (I), or a salt thereof, removing any protecting group, optionally removing any ester-forming group, optionally converting a compound of formula (I) so obtained having a free carboxylic acid group into an ester or a pharmaceutically acceptable salt, and optionally converting a compound of formula (I) so obtained having a free amino group into a pharmaceutically acceptable salt.

12. A process as claimed in claim 10 substantially as described herein with reference to any one of Examples 11 - 14 and 16.

13. A process as claimed in claim 11 substantially as described herein with reference to any one of Examples 1 - 10 and 15.

14. A compound as claimed in claim 1 whenever prepared by a process as claimed in any one of claims 10 - 13.

15. A compound as claimed in claim 1 as specifically described herein with reference to any one of the Examples.

16. A pharmaceutical composition comprising a compound of formula (I) as defined in claim 1 or a pharmaceutically acceptable salt or ester thereof which is easily cleaved within the body to the parent acid and a pharmaceutically acceptable carrier.

17. A composition as claimed in claim 16 suitable for oral administration.

18. A composition as claimed in claim 16 suitable for parenteral administration.

19. A composition as claimed in any one of claims 16 to 18 containing a compound as claimed in claim 9.

20. A process for preparing a composition as claimed in any one of claims 16 to 19 which comprises mixing a compound of formula (I) as defined in claim 1 or a pharmaceutically acceptable salt or ester thereof which is easily cleaved in the body to the parent acid with a pharmaceutically acceptable carrier.

21. A method for treating a bacterial infection in a non-human animal which comprises administering to an animal so infected a dose of a compound of formula (I) as defined in claim 1 or a pharmaceutically acceptable salt or ester thereof which is easily cleaved within the body to the parent acid, alone or in admixture with a pharmaceutically acceptable carrier.

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