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(12) # EUROPEAN PATENT APPLICATION

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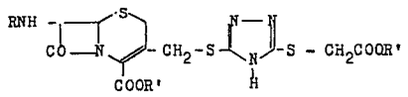
(54) **7-Acylamino-3-((3-(carboxymethyl) thio-1H-1,2,4-triazol-5-yl) thiomethyl)-3-cephem-4-carboxylic acids, processes for their preparation, compositions containing them, and intermediates for their preparation.**

(57) Cephalosporins of the formula



in which R represents various acyl substituents;

R' represents hydrogen or an alkali metal salt, have antibacterial activity.

They can be prepared by reacting 4,5-dihydro-5- thioxo-1H- 1,2,4- triazol-3-yl thioacetic acid or an alkali metal salt thereof with the corresponding 3-acetoxymethyl cephalosporin. The thioacetic acids used as intermediates in preparing cephalosporins of the invention are also part of the present invention.

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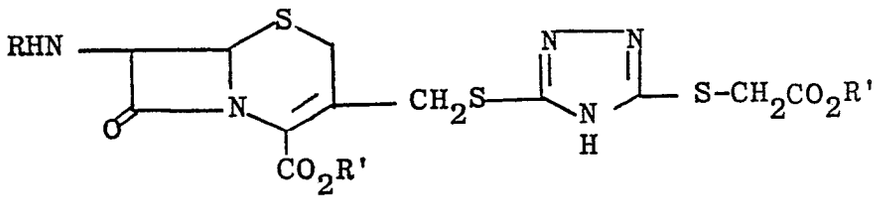
7-ACYLAMINO-3-[[3-(CARBOXYMETHYL)THIO-1H-1,2,4-TRIAZOL-5-YL]THIOMETHYL]-3-CEPHEM-4-CARBOXYLIC ACIDS, PROCESSES FOR THEIR PREPARATION, COMPOSITIONS CONTAINING THEM, AND INTERMEDIATES FOR THEIR PREPARATION.

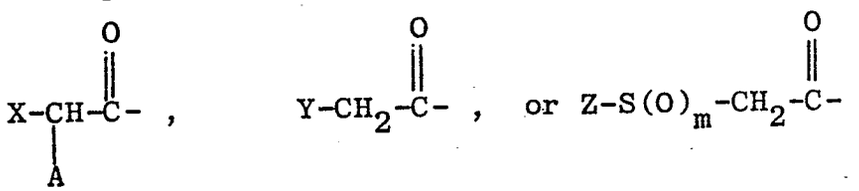
This invention relates to cephalosporin compounds having antibacterial activity, to intermediates for preparing them, to compositions containing them, and to processes for their preparation.

According to the present invention there are provided compounds of the formula:



FORMULA I

in which R represents an acyl substituent of formula



where

X is thienyl, furyl, phenyl or phenyl mono-substituted by hydroxy, hydroxymethyl, formamido or ureido;

A is NH_2, OH, COOH, SO_3H, formyloxy or, when the α-C-hydrogen is absent, methoxyimino;

Y is cyano, sydnone, pyridone, thienyl, o-amino-methylphenyl, phenyl or tetrazolyl;

Z is methyl, trifluoromethyl, trifluoroethyl, pyridyl or cyanomethyl; and

m is zero to two;

R' is hydrogen or an alkali metal salt such as sodium or potassium.

The acyl group R is preferably a group known to be of utility as a substituent on the 7-amino group in the structures of known or prior art cephalosporins or on the 6-amino group in the structures of known or prior art penicillins.

Representative 7-acylamino substituents of the compounds of Formula 1 are listed below:

α-hydroxyphenylacetamido
α-aminophenylacetamido
α-amino-4-hydroxyphenylacetamido
trifluoromethylthioacetamido
2,2,2-trifluoroethylsulfinylacetamido
2,2,2-trifluoroethylthioacetamido
cyanoacetamido
α-carboxythienylacetamido
α-carboxyphenylacetamido
α-sulfophenylacetamido
methylsulfonylacetamido
cyanomethylthioacetamido
3-sydnoneacetamido
1-tetrazolylacetamido
2-thienylacetamido
α(Z)-(methoxyimino)-2-furanacetamido
4-pyridylthioacetamido
o-aminomethylphenylacetamido

Others together with N-acylation procedures may be found in Cephalosporins and Penicillins, Flynn, Academic Press, 1972; U. S. Patent Nos. 2,721,196 and 3,953,424; Belgian Patent No. 832,725; German Patent Nos. 2,127,285 and 2,406,165.

It will be recognized that the 4-carboxylic acid group of the compounds of Formula 1 may be readily esterified by methods well known to the art. 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 such as indanyl, pivaloyloxymethyl, acetoxymethyl, propionyloxymethyl, glycyloxymethyl, phenylglycyloxymethyl and thienylglycyloxymethyl esters and others. Of course, when A is COOH, this group may be similarly esterified. All such ester derivatives are included within the scope of this invention.

Also covered in this invention are pharmaceutically-acceptable, non-toxic derivatives of the compounds of Formula 1, e.g.salts; as stated above easily split ester or ther derivatives of either a carboxy or hydroxy function; amide derivatives of an amino group in a 7-phenylglycyl-amino group, for example the furyl-, pyranyl-, oxolanyl or oxiranyl-carbonyl amides (i.e., Belgian Patent No. 835,295); and solvates such as hydrates, glycolates or alcoholates. As examples of these, one skilled in the art would be able to prepare and use the alkali metal salts such as the sodium or potassium salts (for example using sodium or potassium 2-ethyl hexanoate), ammonium salts, and organic amine salts such as those with procaine or dibenzylethylenediamine.

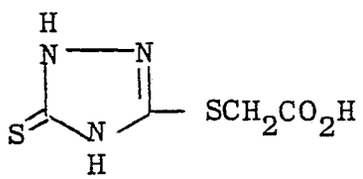
Other known cephalosporin modifications can be made by known synthetic procedures such as introduction of an α-methoxy group at position 7, preferably at the stage of the 7-aminocephalosporanic acid reactants described below (IV), prior to N-acylation. Optical isomers are also possible, such as with mandeloyl or phenylglycyl substituents at 7. The D-forms of these subgeneric groups are preferred.

The compounds of this invention are conveniently prepared by a displacement of the acetoxy group of a known 7-acylaminocephalosporanic acid (II) by [(4,5-dihydro-5-thioxo-1H-1,2,4-triazol-3-yl)thio]acetic acid (III). Alternatively, a similar displacement with the above acetic acid can be effected on 7-aminocephalosporanic acid to give 7-amino-3-[[3-(carboxymethyl)thio-1H-1,2,4-triazol-

5-yl]-thiomethyl]-3-cephem-4-carboxylic acid (IV) which may then be N-acylated as known to the art as described above. Suitable protective groups may be used in either method as is known to the art (see "Protective Groups in Organic Chemistry", J.F.W. McOmie, Plenum Press, 1973, Chapters 2 and 3 for use of amino, carboxy, sulfo or hydroxyl protective groups).

For example, the t-butyl (for COOH) or t-butoxy-carbonyl (for NH_2) groups are easily removed by treatment with trifluoroacetic acid.

The compound of Formula III, which can exist in several tautomeric forms, is a new compound and is part of this invention.



III

This invention also includes the alkali metal and ammonium salts of the compound of Formula III.

The compounds of Formula I have antibacterial activity against both Gram positive and Gram negative bacteria, and minimum inhibitory concentrations (MIC's) in vitro of from 0.2 to greater than 200 µg/ml have been observed. Test results for 7-D-mandelamino-3-[[3-(carboxymethyl)thio-1H-1,2,4-triazol-5-yl]thiomethyl]-3-cephem-4-carboxylic acid, disodium salt, hydrate (A) are:

	A	Cephalothin	Cefazolin
S. aureus HH 127	1.6	0.4	0.4
S. aureus SK 23390	0.8	0.2	0.4
S. aureus villaluz SK 70390	200	50	200
Strep. faecalis HH 34358	100	12.5	6.3
E. coli SK 12140	0.8	3.1	1.6
E. coli HH 33779	0.8	6.3	1.6
Kleb. pneumo. SK 4200	0.4	3.1	1.6
Kleb. pneumo. SK 1200	0.2	1.6	0.8
Salmonella ATCC 12176	0.8	1.6	0.8
Pseudo. aeru. HH 63	200	200	200
Serratia marc. ATCC 13880	12.5	200	200
Proteus morgani 179	1.6	200	200
Entero. aerog. ATCC 13048	1.6	12.5	1.6
Entero. cloacae HH 31254	0.8	6.3	0.8

Compound A showed an ED_{50} in mice of 1.56 against E. coli as well as 1.02 mg/kg against Kleb. pneumo. (s.c.).

These results are superior to those for a related compound, 7-D-mandelamino-3-(2-carboxymethylthio-1,3,4-thiadiazol-5-yl-thiomethyl)-3-cephem-4-carboxylic acid, sodium salt hydrate which showed an ED_{50} against E. coli of 6.4 and as well as 4.4 mg/kg against Kleb. pneumo.

Pharmaceutical compositions having antibacterial activity which comprise a pharmaceutical carrier containing a compound of Formula I as well as methods of combatting bacterial infections by administering such a composition in a nontoxic amount sufficient to combat such infections are also objects of this invention. The administration may be orally or by parenteral injection such as subcutaneously, intramuscularly or intravenously. The injection of suitably prepared sterile solutions or suspensions containing an effective, non-toxic amount of a cephalosporin compound of the invention is the preferred route of administration.

The compounds of Formula I are preferably formulated and administered in the same manner as other prior art cephalosporins such as cephazolin of cephalothin. The dosage regimen preferably 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 about 250 mg. to 600 mg. with the total daily dosage regimen being from about 750 mg. to 6 g. The precise dosages are dependent upon the age and weight of the subject and on the susceptibility of the infection being treated to each individual. These can be determined by those skilled in the art based on the data disclosed herein compared with that available to the art attained with the known cephalosporins outlined herebefore.

The following examples illustrate the invention. Temperatures are in degrees Centigrade (^{o}C.) unless otherwise stated.

EXAMPLE 1

To a suspension of 5.48 g. (40 mmol) of 1,2,4-triazolidine-3,5-dithione, monohydrazine salt in 80 ml. of tetrahydrofuran and 80 ml. of dimethylformamide was added a solution of 6.7 (40 mmol) of ethyl bromoacetate in 20 ml. of tetrahydrofuran. The mixture was stirred at room temperature for 1-1/2 hours, and then at 50^{o}C. for 1/2 hour. The solution was filtered and the filtrate was concentrated to 80 ml., diluted with 250 ml. of water and extracted with diethyl ether. The extract was dried ($MgSO_4$) and evaporated to dryness to give a residue which was crystallized from methanol and water to give 5.76 g. (65% yield) of ethyl [(4,5-dihydro-5-thioxo-1H-1,2,4,-triazol-3-yl)thio]acetate. mp 134-6^{o}C.

A solution of 2.19 g. (10 mmol) of ethyl [4,5-dihydro-5-thioxo-1H-1,2,4-triazol-3-yl)thio]acetate in 50 ml. of 5% sodium hydroxide was heated at reflux for 1 hour. After thorough cooling, the mixture was filtered, and the filtrate was washed with ethyl acetate. The

aqueous layer was separated, acidified to pH 1 and extracted with ethyl acetate. The extract was evaporated in vacuo to dryness. The residue was crystallized from chloroform to give 1.43 g. (75% yield) of [(4,5-dihydro-5-thioxo-1H-1,2,4-triazol-3-yl)thio]acetic acid.

To a solution of 1.16 g. (13.8 mmol) of sodium bicarbonate in 30 ml. of water was added 1.32 g. (6.9 mmol) of [4,5-dihydro-5-thioxo-1H-1,2,4-triazol-3-yl)thio]acetic acid. After CO_2 gas evolution had ceased, 2.12 g. (5 mmol) of 7-mandelamido-cephalosporanic acid sodium salt was added to the solution. The mixture was heated at 80°C. for 3.5 hours. After cooling, the solution was filtered. The filtrate was applied to an XAD-7 (200 ml.) resin column, eluting with water. The fractions containing product by thin layer chromatography were pooled and evaporated to dryness. The residue was triturated with absolute ethanol and filtered. The filtrate was diluted with isopropanol, and the solid formed was filtered, air-dried, dissolved in de-ionized water and lyophilized to give 1.02 g. (15.6 % yield) of 7-D-mandelamido-3-[[3-(carboxymethyl)thio-1H-1,2,4-triazol-5-yl]thiomethyl]-3-cephem-4-carboxylic acid, disodium salt hydrate, mp 220-230°C. dec. Anal. calculated for $C_{20}H_{17}N_5O_7S_3Na_2 \cdot 4H_2O$ = C, 36.75; H, 3.85; N, 10.71. Found: C, 36.47; H, 3.53; N, 10.11. IR-(NUJOL) = 5.65 .

EXAMPLE 2

An aqueous solution (100 ml.) of 4.27 g (0.0096 mol) of 7-[α(Z)-(methoxyimino)-2-furanacetamido]-cephalosporanic acid sodium salt, 1.78 g. (0.0212 mol) of sodium bicarbonate and 2.02 g. (0.0106 mol) of [(4,5-dihydro-5-thioxo-1H-1,2,4-triazol-3-yl)thio]acetic acid is heated at 65°C. for 6 hours during which time the pH is maintained at 7.6-7.8 with dilute sodium bicarbonate. After cooling, the reaction mixture is purified on an XAD-7 column as described in Example 1 to give a lyophilized product, 7-[α(Z)-(methoxyimino)-2-furanacetamido]-3-[[3-(carboxymethyl) thio-1H-1,2,4-

triazol-5-yl]thiomethyl]-3-cephem-4-carboxylic acid, disodium salt.

EXAMPLE 3

A mixture of 5.22 g. (10.0 mmol) of 7-(D--t-butoxycarbonylamino-4-hydroxyphenylacetamido)cephalosporanic acid and an excess (15.0 mmol) of [(4,5-dihydro-5-thioxo-1H-1,2,4-triazol-3-yl)thio]acetic acid in 75 ml. of pH 6.4 phosphate buffer solution is treated with sufficient sodium bicarbonate togive a pH of 6.4. The mixture is heated at 70° for 3 hours, cooled, acidified with dilute hydrochloric acid to pH 2 and extracted with ethyl acetate. Removal of the ethyl acetate in vacuo gives the t-boc derivative of the desired compound. This derivative is stirred at 25°C. with 25 ml. of trifluoroacetic acid and 25 ml. of 1,3-dimethoxybenzene for 2 hours. The mixture is evaporated to dryness in vacuo, ethyl acetate is added to the residue and the precipitated salt is collected. This is dissolved in water and treated with Amberlite IR-45 weakly basic ion-exchange resin. The solution is lyophilized to give 7-(D-α-amino-4-hydroxyphenylacetamido)-3-[[3-carboxymethyl)thio-1H-1,2,4-triazol-5-yl]thiomethyl]-3-cephem-4-carboxylic acid.

EXAMPLE 4

A mixutre of an excess (12.2 mmol) of [(4,5-dihydro-5-thioxo-1H-1,2,4-triazol-3-yl)thio]acetic acid, 32.5 mmol of sodium bicarbonate and 8.1 mmol of 7-trifluoromethylthioacetamidocephalosporanic acid in 50 ml. of water is stirred at 70° for 5 hours. The reaction mixture is cooled and applied to an XAD-2 column and eluted with water and then methanol. The product containing effluent is evaporated to dryness to give a residue which is dissolved in a small amount of water and lyophilized to give 7-trifluoromethylthioacetamido-3-[[3-(carboxymethyl)thio-1H-1,2,4-triazol-5-yl]thiomethyl]-3-cephem-4-carboxylic acid disodium salt. Substituting 7-(2-thienylacetamido)-cephalosporanic acid gives 7-(2-thienylacetamido)-3-[[3-(carboxymethyl)thio-1H-1,2,4-

triazol-5-yl]thiomethyl]-3-cephem-4-carboxylic acid sodium salt.

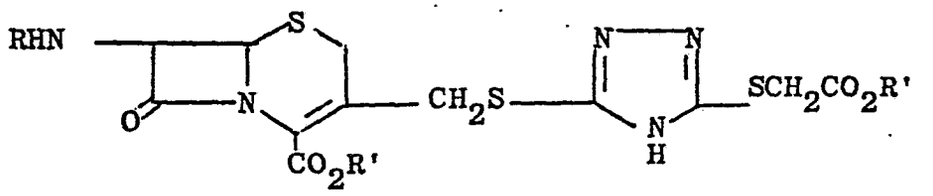
Stoichiometric quantities of cephalosporanic acids having the individual 7-acylamino substituent listed hereabove may be substituted in Examples 1-3 with variations which will be obvious to those skilled in this art.

EXAMPLE 5

An injectable pharmaceutical composition is formed by adding sterile saline solution (2 ml.) to 500 mg. of the product of Example 1. This material is injected parenterally four times daily to a human patient infected with susceptible bacteria. Other compounds of this invention may be similarly used.

Claims

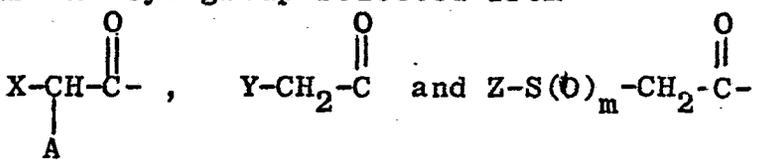
1. A compound of the formula:



I

in which:

R is an acyl group selected from



where:

X is thienyl, furyl, phenyl or phenyl monosubstituted with hydroxy, hydroxymethyl, formamido or ureido;

A is NH_2, OH, COOH, SO_3H, formoxyl or, when the α-carbon hydrogen is absent, methoxyimino;

Y is cyano, sydnone, pyridone, thienyl, α-aminomethylphenyl, phenyl or tetrazolyl;

Z is methyl, trifluoromethyl, trifluoroethyl, pyridyl, or cyanomethyl;

m is zero to two,

R' is hydrogen or an alkali metal salt.

2. A pharmaceutical composition in dosage unit form having antibacterial activity characterized in that it comprises a pharmaceutical carrier and a compound as defined in Claim 1.

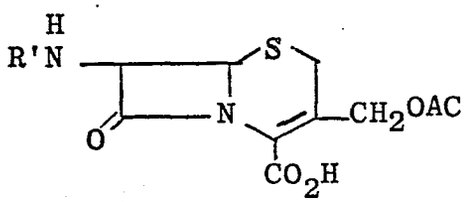
3. [(4,5-Dihydro-5-thioxo-1H-1,2,4-triazol-3-yl)thio]-acetic acid and its alkali metal and ammonium salts.

4. [(4,5-Dihydro-5-thioxo-1H-1,2,4-triazol-3-yl)thio]acetic acid.

5. The compound of Claim 4 in the form of its sodium salt.

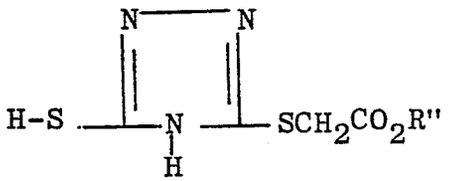
6. 7-[α(Z)-(Methoxyimino)-2-furanacetamido]-3-[[3-(carboxymethyl)thio-1H-1,2,4-triazol-5-yl]thiomethyl]-3-cephem-4-carboxylic acid.

7. A process for preparing a compound of the formula I, as defined in Claim 1, characterized in that a compound of the formula:



II

(where R' is hydrogen or an acyl group R as defined in Claim 1) is reacted with a compound of the formula:



III

(where R" is hydrogen or an alkali metal salt) and when R' is hydrogen the product is acylated with an acylating agent or activated derivative of formula R'"COOH where R'" is X-CH(A), $Y-CH_2$ and $Z-S(O)_m-CH_2$, where A, X, Y, Z and m are defined in Claim 1.

European Patent Office

EUROPEAN SEARCH REPORT

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Application nu

EP 78 30 0103

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim
	FR - A - 2 266 507 (YAMANOUCHI) * Page 1, paragraphs 1-2; pages 3,4 *	1-7
	DE - A - 2 655 717 (CARLO EIBA) * Pages 1-19; claims 1,98,99 *	1,2,6, 7
	JP - A - 72 05550 (FUJISAMA) * Derwent *	1,2,6, 7
	CHEMICAL ABSTRACTS 85 (1976) 21397e. & JP - A - 75 131981 (YAMANOUCHI)	1,2,6, 7
	DE - A - 2 016 082 (EASTMAN KODAK) * Pages 23-24: claim 1 *	3,4,5

CLASSIFICATION OF THE APPLICATION (Int. Cl.²)

C 07 D 501/36
C 07 D 249/12
A 61 K 31/545

TECHNICAL FIELDS SEARCHED (Int.Cl.²)

C 07 D 501/36
C 07 D 249/12

CATEGORY OF CITED DOCUMENTS

X: particularly relevant
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention
E: conflicting application
D: document cited in the application
L: citation for other reasons

&: member of the same patent family, corresponding document

X The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	15-09-1978	LUYTEN