United States Patent [19]

Hamanaka

[11] **3,933,797**

[45] **Jan. 20, 1976**

[54] **6-[α-(ω-QUANIDINOALKANOYLAMIDO)ACYLAMIDO]PENICILLANIC ACIDS**

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[21] Appl. No.: **508,070**

Related U.S. Application Data

[62] Division of Ser. No. 228,344, Feb. 22, 1972, Pat. No. 3,870,709.

[52] **U.S. Cl.** **260/239.1**

[51] **Int. Cl.²** **C07D 499/44**

[58] **Field of Search** 260/239.1

[56] **References Cited**

UNITED STATES PATENTS

3,433,784	3/1969	Long et al.	260/239.1
3,634,405	1/1972	Holdrege	260/239.1
3,711,471	1/1973	Kaplan	260/239.1

Primary Examiner—Gerald A. Schwartz

Attorney, Agent, or Firm—Connolly and Hutz

[57] **ABSTRACT**

6-[α-(ω-guanidinoalkanoylamido)acylamido]penicillanic acids, the non-toxic salts and esters thereof are useful as antibacterial agents, therapeutic agents in animals, including man, of particular value against gram-negative bacteria, and as animal feed nutritional supplements.

7 Claims, No Drawings

6-[α-(ω-QUANIDINOALK-ANOYLAMIDO)ACYLAMIDO]PENICILLANIC ACIDS

CROSS REFERENCE TO RELATED APPLICATION

This application is a division of application Ser. No. 228,344 filed Feb. 22, 1972 and now U.S. Pat. No. 3,870,709.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to novel antibacterial agents; namely, (substituted acyl)derivatives of α-aminoacyl penicillins. More particularly, it is directed to 6[α-(ω-guanidinoalkanoylamido)acylamido]penicillanic acids, the non-toxic salts and esters thereof, which are especially useful in the treatment of gram-negative infections and, particularly, Pseudomonas infections.

2. Description of the Prior Art

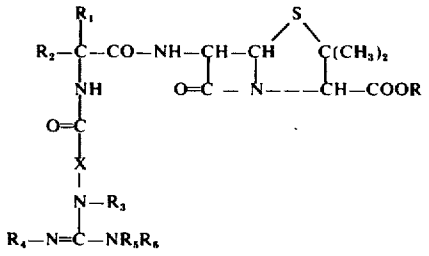
A large number of 6-[(α-aminoacylamido)penicillanic acids wherein the acyl moiety is alkanoyl or substituted alkanoyl wherein the substituent is an aryl, cycloalkyl or heterocyclic group are disclosed in U.S. Pats. 2,985,648; 3,007,920; 3,192,198; 3,485,819; 3,342,677; 3,538,083; 3,553,201; British Patents 873,049; 903,785; 991,586, 1,033,257 and 1,189,990. Further, 6-[α-substituted amino)acylamido]penicillanic acids are described in U.S. Patents 3,198,788; 3,248,387; 3,308,023; 3,320,240; 3,325,477; 3,340,252; 3,381,001; 3,433,784; 3,518,253; British patents 891,977; 894,457; 985,688; 1,048,907; 1,051,675; 1,057,697; 1,064,893; 1,066,107; 1,125,339; 1,180,745; 1,210,472; Belgian Patent 593,295 and Japanese Patent 7,116,994. Additionally, 6-(α-ureido acylamido)penicillanic acids are disclosed in U.S. Pat. 3,352,851 and German Patent 2,054,772; 6-(α-guanidinoacylamido)penicillanic acids in U.S. Pats. 3,454,557 and 3,406,185; and a variety of p-quanidinoaroyl-,p-quanidinomethylaroyl-and p-guanidinoarylalkanoylamidopenicillanic acids are disclosed in U.S. Pat. 3,453,265. British Patent 1,061,335 discloses 6-(D-α-hydrazinocarbonylamino-α-phenylacetamido)penicillanic acid, and British Patent 1,053,818 describes esters of 6-(α-oxalamidoacylamido)penicillanic acids.

A wide variety of 6-[α-(3-substituted ureido)acylamido]penicillanic acids and 6-[α-(3-substituted thioureido) acylando-]penicillanic acids are reported in the recent literature. U.S. Patents 3,479,339; 3,481,922; Netherlands Patents 69,01646; 69,08909; and Japanese Patent 7,112,732 describe such compounds wherein the 3-substituent is a carbamoyl group; the compounds being referred to as 6-[α-(3-allophanamido)acylamido]penicillanic acids. U.S. Pat. 3,579,501 discloses 6-[α-(3-guanylureido)acylamido]-penicillanic acids; that is, such compounds wherein the 3-substituent is a guanyl moiety.

The above described products are active as antibacterial agents against a variety of gram-positive and gram-negative bacteria. However, while they are active in vitro and in vivo via the intraperitoneal route of administration, they are inactive or, at best, poorly active in vivo via the oral route of administration. Additionally, their pharmaco-kinetics, as evidenced by levels of the compound in the blood, are poor.

SUMMARY OF THE INVENTION

There has now been found a novel series of antibacterial agents; namely, 6-[α-(ω-guanidinoalkanoylamido)acylamido]penicillanic acids of the formula



I

and the pharmaceutically acceptable acid addition salts thereof wherein R is selected from the group consisting of hydrogen and acyloxy lower alkyl wherein acyloxy is selected from the group consisting of lower alkanoyloxy, benzoyloxy and substituted benzoyloxy wherein the substitutent is selected from the group consisting of chloro, bromo, fluoro, lower alkyl, lower alkoxy and trifluoromethyl;

R_1 is selected from the group consisting of hydrogen, alkyl of 1 to 14 carbon atoms, cycloalkyl of 3 to 6 carbon atoms, 1,4-cyclohexadienyl, naphthyl, benzyl phenethyl, indolylmethyl, furyl, thienyl, ω-ethylthio(-lower alkyl and

wherein Y is selected from the group consisting of hydrogen, nitro, di(lower alkyl)amino, lower alkanoylamino, lower alkyl, lower alkoxy, hydroxy, sulfamyl, chloro, bromo, fluoro, iodo and trifluoromethyl;

R_2 is selected from the group consisting of hydrogen and lower alkyl;

R_1 and R_2 when taken together with the carbon atom to which they are attached are cycloalkylidene of 3 to 10 carbon atoms;

each of R_3, R_4, R_5 and R_6 is selected from the group consisting of hydrogen, lower alkyl, benzyl and phenyl;

R_4 and R_5 when taken together with the guanyl moiety to which they are attached form a 5- or 6-membered heterocyclic ring selected from the group consisting of 2-imidazolyl, 2-(2-imidazolinyl), 2-(1,4,5,6-tetrahydropyrimidinyl) and 2-pyrimidinyl;

X is selected from the group consisting of alkylene having from 1 to 7 carbon atoms, phenylene cycloalkylene having from 3 to 9 carbon atoms, propenylene whose $-CH_2$ group is bound to the adjacent nitrogen vinylenephenylene, methylene oxyphenylene and phenylenemethylene, each of whose phenylene group is bound to the adjacent nitrogen;

X and $N-R_3$ when taken together form a 5- or 6-membered heterocyclic ring selected from the group consisting of pyrrolidyl and piperidyl;

X and R_5 when taken together with the guanidino moiety to which they are attached form a 5- or 6-membered heterocyclic ring selected from the group consisting of 2-amino-2-imidazolinyl and 2-amino-2-(1,4,5,6-tetrahydropyrimidinyl);

R_3 and R_4 when taken together with the guanidino moiety to which they are attached form a 5- or 6-mem-

bered heterocyclic ring selected from the group consisting of 2-imidazolino and 2-(1,4,5,6-tetrahydropyrimidino); and

R_3 and R_5 when taken together with the guanidino moiety to which they are attached form a 5- or 6-membered heterocyclic ring selected from the group consisting of imidazolidino and hexahydropyrimidino.

Included among the pharmaceutically acceptable acid addition salts of this invention are inorganic and organic acid addition salts. Typical salts include hydrochloride, hydrobromide, hydroiodide, sulfate, phosphate, acetate, citrate, benzoate, maleate, succinate, malate, fumarate, ascorbate, glycolate, tartrate, oxalate and mandelate.

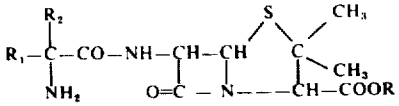
Also included within the scope of this invention are the optically active isomeric forms and mixtures thereof which arise by virtue of the asymmetric α-carbon atom of the acyl side chain. These are the D- and the L-diastereoiosmers and the DL-form which is a mixture of the two optically active isomers. The D-form of these compounds is the preferred form because of its greater activity relative to that of the L- or the DL-forms.

Other isomers of the herein described compounds in addition to those arising from the asymmetric α-carbon of the acyl side chain are, of course, possible because of the presence of asymmetric carbon atoms in the 6-aminopenicillanic acid nucleus.

The novel antibacterial products of this invention are of value as additives to materials such as fuels and cutting oils which are subject to bacterial deterioration and are useful in soaps and shampoos and in topical compositions for treatment of wounds. They are also remarkably effective in treating a number of infections caused by susceptible gram-negative and gram-positive bacteria in poultry and animals, including man.

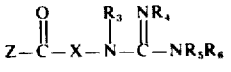
DETAILED DESCRIPTION OF THE INVENTION

The novel and valuable compounds of this invention are prepared by reacting an appropriate 6-[(α-amino-substituted)acylamido]penicillanic acid, or a suitable ester thereof, of the formula



II

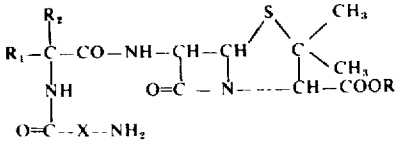
with a reactive functional derivative of the carboxy group of an appropriate guanidino substituted acid of the formula



III

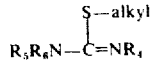
wherein the variables X, R and R_1 – R_6 are as defined above, and Z is hydroxy or a halo group.

Alternatively, compounds of formula I, especially those wherein X is alkylene, can be prepared by reacting a compound of formula III-A



III-A

with an appropriate S-alkylisothiourea of formula III-B



III-B

according to standard procedures.

The terms "lower alkyl, lower alkoxy and lower alkanoyloxy" as used herein are intended to include those alkyl, alkoxy and alkanoyloxy groups having from one to four carbon atoms.

Suitable esters of the formula II reactants are those wherein R is acyloxy lower alkyl as defined above and those wherein R is a group which can readily be removed as, for example, by catalytic hydrogenation (benzyl, cyanomethyl, phenacyl, allyl and diphenylmethyl).

Suitable reactive functional derivatives of acids of formula III are the acid chlorides or bromides (Z = Cl, Br). The acid reactant can be reacted with a "condensing" agent such as a carbodiimide, an alkoxyacetylene, N,N'-carbonyldiimidazole, N,N'-carbonylditriazole and hexahalocyclotriphosphatriazines to give a reactive intermediate which is coupled to the 6-[(α-amino substituted)acylamido]penicillanic acid. Additionally, the appropriate acid azide or an active ester or thio ester of the formula III reactant with, for example, N-hydroxyphthalimide, N-hydroxysuccinimide, a phenol or thiophenol can be used as acylating agent.

The preferred acylation processes of this invention comprise the reaction of the appropriate 6-[(α-amino substituted)acylamido]penicillanic acid compound (formula II) with the acid chloride of an acid of formula III, or with the acid form of a formula III reactant in the presence of a carbodiimide for reasons of convenience, availability of reactants and overall yield of product.

The 6-[(α-amino substituted)acylamido]penicillanic acid reactant can be used in a variety of forms. It can, for example, be used as the free acid or as an alkali metal or amine salt thereof. The use of a salt form of the penicillanic acid reactant is frequently of advantage since the solubility can be manipulated by judicious choice of the salt to permit the use of aqueous or non-aqueous systems. Alkali metal salts are valuable for use in aqueous systems. In non-aqueous systems, an amine salt such as a tertiary lower alkylamine salt, e.g., triethylamine, or an N-alkyl piperidine salt is generally used. Alternatively, an ester of the 6-[(α-amino substituted-)acylamido]penicillanic acid is used, especially in non-aqueous systems. In those instances wherein the final product (formula I) is desired in the form of an ester (R is other than hydrogen), it is obvious and practical to use that ester form of the penicillanic acid reactant.

The acylation process is conducted in a reaction-inert solvent system which can be aqueous or non-aqueous. Aqueous or non-aqueous solvent systems can be used when a carbodiimide is the condensing agent. When using a carbodiimide in an aqueous system, the pH is desirably adjusted to the range of about 5 to about 8, and preferably to about 6 to 7. In a typical procedure, the formula III reactant and carbodiimide are mixed in equimolar proportions in a suitable sol-

vent (tetrahydrofuran, dioxan) and a water-water-miscible organic solvent solution (water plus dioxan or tetrahydrofuran) containing the 6-[α-amino substituted)acylamido]penicillanic acid is added at room temperature and the mixture stirred for several hours until reaction is complete. Temperatures of from about −5° to 30° C. are generally used. In most instances, an excess up to about 10% of the condensing agent is used. The penicillin product is recovered by methods known to the art.

Acylation with an acid halide (formula III) can also be conducted in aqueous or non-aqueous solvent systems. In aqueous systems, the reaction is generally carried out at a pH of from about 6 to about 9 and a temperature of from about 0° C. to about 50° C. It can also be preformed in unstable emulsions of water and a water-immiscible organic solvent such as methyl isobutyl ketone and lower alkyl acetates over the pH range of from about 2 to about 4.

In addition to the above purely chemical technique of acylation, a sonochemical technique; that is, the application of vibrations of ultrasonic frequency (35,000 to 90,000 cycles per second), as described in U.S. Pat. 3,079,314, issued Feb. 26, 1963, can also be used to achieve acylation of the 6-[(α-amino substituted-)acylamido]penicillanic acid, especially acylation with an acid halide. Acylation under such conditions is rapid and permissive of a wide range of reaction media, aqueous and nonaqueous alike, homogeneous and non-homogeneous, including emulsified systems.

The esters of this invention, compounds of formula I wherein R is acyloxy(lower alkyl), can be prepared by reacting an alkali metal salt (sodium, potassium, lithium) of a compound of formula I wherein R is hydrogen and NR_5R_6 is $NHNO_2$ with the appropriate acyloxy(lower alkyl) halide (chloride or bromide). The reaction is normally conducted in a reaction-inert solvent such as tetrahydrofuran, dimethylformamide, dimethylsulfoxide or hexamethylphosphoramide. In practice, the halide is added, usually dropwise, to a solution or suspension of an alkali metal salt of the nitroguanidino compound. At least one equivalent of the halide reactant is added but, in certain cases, it may be advantageous to employ as much as a 50 percent excess. The reaction is carried out at temperatures of from 0° to 50°C. and preferably from 20° to 30° C. Reaction time will vary according to the temperature employed and the reactivity of the appropriate starting materials. Normally, the reaction period will range from one to twenty hours. The nitroguanidino ester derivative thus produced is then catalytically hydrogenated to the corresponding guanidino ester of formula I. The nitroguanidino derivative of I (R=H, NR_5R_6=$NHNO_2$) is prepared by acylating the appropriate α-aminoacyl-penicillin with the appropriate nitroguanidino carboxylic acid by method described herein.

Alternatively, and preferably, the acyloxy(lower alkyl) esters of formula I compounds are prepared by the above described acylation procedures but using the appropriate acyloxy(lower alkyl) ester of the appropriate 6-[(α-amino substituted)acylamido]penicillanic acid in place of the non-esterified 6-[(α-amino substituted)acylamido]penicillanic acid. The acyloxy(-lower alkyl) esters of the 6-[(α-amino substituted-)acylamido]penicillanic acids are prepared according to methods described in Belgian 721,515 and by Daehne et al., J. Med. Chem. 13, 607-612 (1970).

The acyloxy(lower alkyl) halides are synthesized from the corresponding acid chlorides and aldehydes or ketones in accordance with the general procedures of Ulich et al., J. Am. Chem. Soc. 43, 660 (1921) and Euranto et al., Acta. Chem. Scand. 20, 1273 (1966). The formation of esters from acid salts and alkyl halides is well documented in the chemical literature (Zook and Wagner, "Synthetic Organic Chemistry," John Wiley and Sons, Inc., New York, 1956, p. 484).

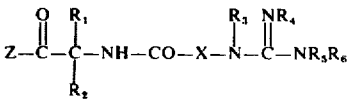
The 6-[(α-amino substituted)acylamido]penicillanic acid reactants are described in the art cited above.

Many of the guanidino substituted carboxylic acid reactants of the formula $HOOC—X—NR_3—C(=NR_4)-NR_5R_6$ are described in the literature. Particular reference is made to U.S. Pats. 3,257,411; 3,406,185 and 3,479,401 which describe such acids wherein X is phenylene, methylenephenylene, alkylene and cyclohexylene (see formula II). Those guanidino substituted acid reactants which are not known in the art are prepared from the corresponding amino substituted carboxylic acids by reacting the amino acid with S-methylisothionitrourea. The intermediate nitroguanidinocarboxylic acid is then catalytically hydrogenated to the desired guanidino substituted carboxylic acid.

An alternative and favored method for preparing guanidino-substituted carboxylic acid comprises reaction of the appropriate amino substituted carboxylic acid with benzoylcyanamid followed by hydrolysis of the benzoyl guanidino substituted reaction product.

A further method for preparing the guanidino-substituted carboxylic acids involves reaction of the appropriate amino substituted carboxylic acid with O-methylisourea, S-methylisothiourea or appropriate substituted isothiourea in alkaline solution.

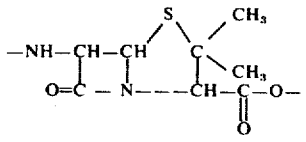
Still another method for preparing compounds of formula I comprises acylating 6-aminopenicillanic acid or an appropriate ester thereof with a reactive functional derivative of an acid of formula IV according to the above described procedures



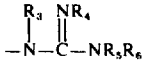
IV

wherein X, R_1–R_6 and Z are as defined above. This method is not generally favored because the reactants are less readily available than are those of formula III. They can, of course, be prepared by methods known to those skilled in the art.

Examples illustrating the preparation of compounds within the scope of this invention are given below. In the formulae accompanying the examples, "-APA-" represents the moiety



The guanidino moiety of the acyl side chain, for convenience, employs the following numbering system



1 2 3

Tautomeric forms of the guanidino moiety wherein at least one of R_3, R_4, R_5 and R_6 is other than hydrogen are also embraced within this invention.

The novel penicillins described herein exhibit in vitro activity against a wide variety of both gram-positive and gram-negative bacteria, including indole-positive Proteus. Their useful activity can readily be demonstrated by in vitro tests against various organisms in a brain-heart infusion medium by the usual two-fold serial dilution technique. The in vitro activity of the herein-described compounds renders them useful for topical application in the form of ointments, creams and the like, or for sterilization purposes, e.g., sick room utensils.

The in vitro spectra of a number of 6-[α-(ω-guanidinoalkanoylamido)acylamido]penicillanic acids of this invention against certain gram-negative bacteria are presented in Table I below. D-α-aminobenzylpenicillin and 6-[D-2-phenyl-2-(3-guanylureido)acetamido]penicillanic acid are included for the purpose of comparison. The compounds of Table I have the formula

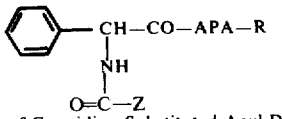
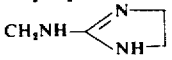
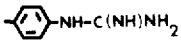
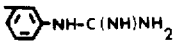
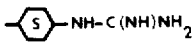
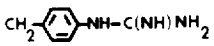
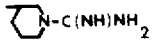


TABLE I.

In Vitro Spectra of Guanidino-Substituted Acyl Derivatives of α-Aminobenzylpenicillin Against Certain Gram-negative organisms (MIC'S)

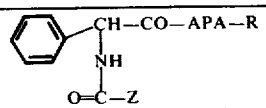
Z	*E.coli* 266	*Pseudo.aeruginosa* 104	173	490	*vul-garis* A059	*Proteus rett-geri* B006	*mira-bilis* C015	*mira-bilis* C020	*Serr-atia mar-cesens* A001	R*
$CH_2NH-C(NH)NH_2$	1.56	1.56	1.56	1.56	1.56	12.5	1.56	1.56	6.25	H
$CH_2N(CH_3)-C(NH)NH_2$	6.25	50	12.5	3.12	6.25	100	6.25	6.25	3.12	H
$CH_2CH_2NH-C(NH)NH_2$	3.12	6.25	3.12	3.12	3.12	50	0.78	12.5	6.25	H
	12.5	6.25	3.12	3.12	3.12	100	3.12	6.25	25	H
	3.12	0.78	0.78	0.78	6.25	25	3.12	12.5	3.12	H
	3.12	1.56	1.56	0.78	3.12	12.5	6.25	25	12.5	H
	6.25	3.12	1.56	6.25	50	50	6.25	25	12.5	H
	6.25	6.25	3.12	1.56	12.5	—	12.5	—	25	H
	6.25	12.5	6.25	1.56	6.25	—	3.12	—	6.25	H
$CH_2-NH-C(NH)NHCH_3$	1.56	1.56	3.12	0.78	3.12	—	1.56	—	3.12	H
$CH_2-N-C(NH)-NHCH_2CH_2$	6.25	12.5	12.5	3.12	6.25	—	3.12	—	12.5	H
$-NH-C(NH)NH_2$	3.1	2.5	1.5	1.5	1.56	—	0.78	—	6.25	H
$CH_3NH-C(NH)NH_2$	25	—	—	0.78	—	—	50	—	—	POM
$CH_3N(CH_3)-C(NH)NH_2$	12.5	—	—	3.12	—	—	12.5	—	—	POM
$CH_2CH_2NH-C(NH)NH_2$	6.25	—	—	6.25	—	—	0.39	—	—	POM
Ampicillin	3.1	200	200	0.78	6	—	1.5	—	200	H

*POM = hydrochloride salt of pivaloyloxymethyl ester.

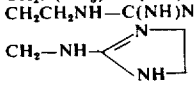
Additionally, the compounds of this invention are active versus gram-positive and gram-negative bacteria in vivo via the parenteral route of administration in animals, including man. Their in vivo activity in animals, including man, by the oral route of adminstration is more limited as regards susceptible organisms. Nevertheless, oral in vivo activity against *Escherichia col.* and *Staphylococcus aureus* is a common property of many compounds of this invention. Table II below presents the comparative in vivo spectra of several compounds within the scope of this invention against D-α-aminobenzylpenicillin (Ampicillin). Table III presents comparative PD_{50} values of 6-]D-2-phenyl-2-(guanidinoacetamido)acetamido]penicillanic acid [A], 6-[D-2-phenyl-2-(3-guanylureido)acetamido]-penicillanic acid [B] and D-α-aminobenzylpenicillin versus *E. coli*, *S. aureus* and *Pseudomonas aeruginosa*.

The compounds of TAble II have the formula:

Table II.



In Vivo Spectrum of Guanidino-Substituted Acyl Derivatives of α-Aminobenzylpenicillin Versus *Escherichia coli* 266. % Protection at 50 mg./kg. in Mice Via Subcutaneous Route

Z	R=H % Protection	R=POM* % Protection
$CH_2NH-C(NH)NH_2$	100	90
$CH_2N(CH_3)-C(NH)NH_2$	60	70
$CH_2CH_2NH-C(NH)NH_2$	70	100
	90	

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