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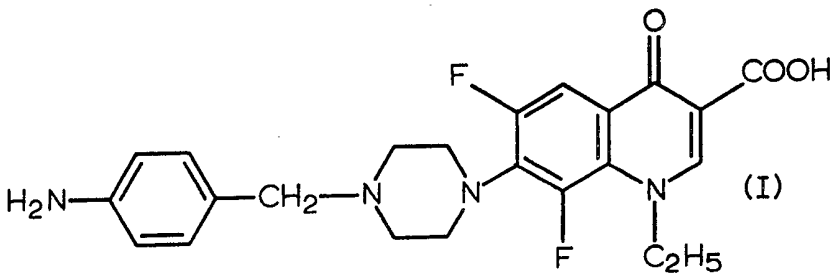
(54) Quinoline carboxylic acid derivative

(57) A novel compound is described of formula



and its pharmaceutically acceptable salts. Processes for the preparation of the compound are described. The compound possesses antibacterial activities against both gram-positive and gram-negative bacteria and it may be formulated in conventional manner as a pharmaceutical composition.

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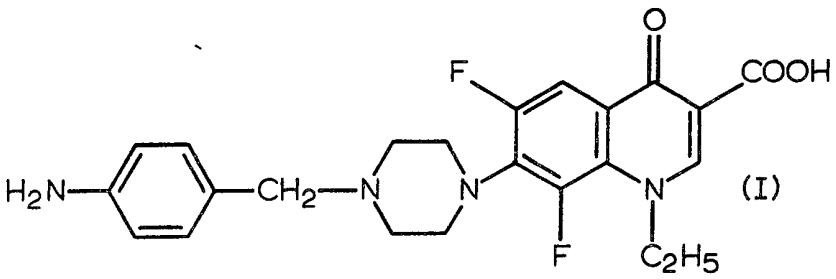
SPECIFICATION

Quinoline carboxylic acid derivative

This invention relates to a quinoline carboxylic acid derivative, to processes for its preparation and to its use as an antibacterial agent.

Antibacterial agents such as nalidixic acid, pyiromidic acid and pipemidic acid have been proved highly effective in the therapy of infections due to gram-negative bacteria, but such agents suffer the serious disadvantage of having only weak activities against most gram-positive bacteria. We have, therefore, sought a compound which possesses potent antibacterial activities against both gram-positive and gram-negative bacteria.

Thus the present invention provides a quinoline carboxylic acid derivative having the formula (I):

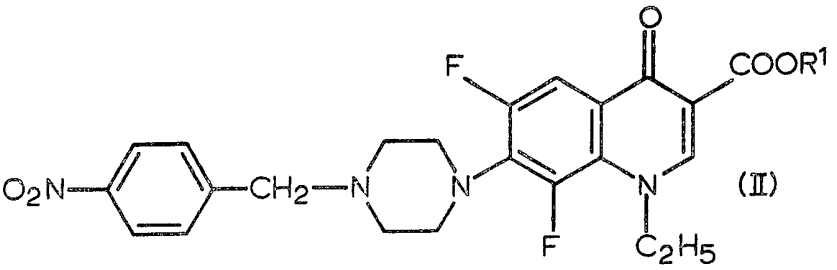


(I)

and its pharmaceutically acceptable salts.

The compound (I) is highly effective in the therapy of infections due to both gram-positive and gram-negative bacteria and it has been found that the compound (I) is fortunately metabolized in part to 1-ethyl-6,8-difluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)quinoline-3-carboxylic acid having superior activity against gram-negative bacteria when administered to animals.

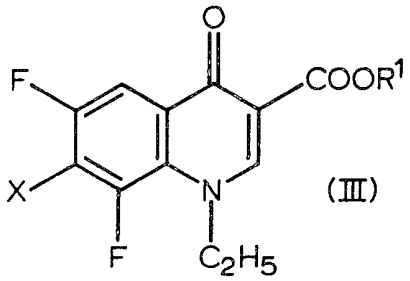
According to one embodiment of the invention, the compound (I) can be prepared by the reduction of a compound of the formula (II):



(II)

wherein R^1 is hydrogen or a lower alkyl group, if necessary, followed by hydrolysis by a conventioal method. The reduction may be accomplished, for example, by catalytic hydrogenation using palladium on charcoal, Raney Nickel or platinum oxide, as catalyst in an inert solvent, for example, an alcohol ether or an organic acid, or by the reaction with a metal, for example, iron, tin or zinc, or a halide or sulfate thereof in the presence of an acid such as hydrochloric acid, sulfuric acid or acetic acid.

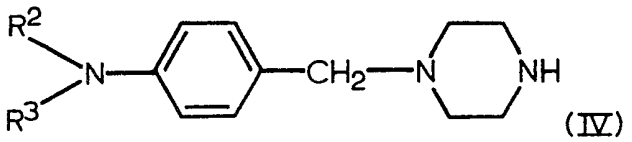
According to another embodiment of the invention, the compound (I) can be obtained by the amination of a compound of the formula (III):



(III)

wherein R^1 is as defined for formula (II) and X is a leaving group such as halogen or sulfonyloxy

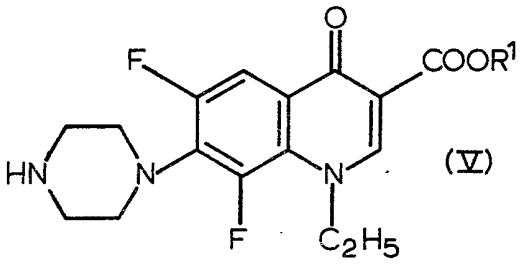
group, with a piperazine derivative of the formula (IV):



(IV)

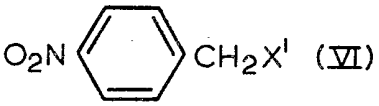
wherein R^2 and R^3 are independently hydrogen or a protecting group such as an acyl group, in an inert solvent, for example, water, an alcohol, ether, amine or nitrile or dimethylformamide or dimethylsulfoxide, preferably in the presence of an organic or inorganic base, at a temperature in the range of room temperature to 170°C, if desired, following by hydrolysis with an acid or alkali by a conventional method.

The starting material (II) can be obtained by the reaction of a compound (III) with N-(p-nitrogbenzyl)piperazine or by the treatment of a compound of the formula (V):



(V)

wherein R^1 is as defined for formula (II), with a compound of the formula (VI):



(VI)

wherein X′ is halogen.

The salts, for example, methanesulfonate, benzenesulfonate, acetate, maleate, citrate, malate, lactate, hydrochloride, sulfate, phosphate, sodium salt, potassium salt and amine salts, of the compound (I) are obtained by conventional techniques.

The compound (I) or a salt thereof is administered to human or animals, generally in the range of 1–100 mg/kg/day by an oral or parenteral route. The compound (I) or the salt may be used in the form of pharmaceutical compositions such as, tablets, capsules, syrups, injections, granules, powder, suppositories or emulsions. The pharmaceutical compositions may contain the compound in admixture with an adjuvant and are formed by conventional methods.

The following Examples serve to illustrate the invention.

EXAMPLE 1

A mixture of 1-ethyl-6,8-difluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)quinoline-3-carboxylic acid hydrochloride (6.7 g), triethylamine (5.45 g), p-nitrobenzylbromide (5.8 g) and dimethylformamide (200 ml) was stirred at 90°C for 10.5 hrs. The solvent was evaporated off and the residue was treated with water. The solid was filtered, washed with water, dried, and recrystallized from a mixture of dimethylformamide and ethanol to give 6.9 g of 1-ethyl-6, 8-difluoro-1,4-dihydro-7-[4-(p-nitrobenzyl)-1-piperazinyl]-4-oxoquinoline-3-carboxylic acid.
m.p. : 241 – 242°C.

			C	H	N
Anal.	Calcd.	for $C_{23}H_{22}F_2N_4O_5$	: 58.47	4.69	11.86
	Found		: 58.50	4.59	11.95

EXAMPLE 2

A mixture of 1-ethyl-6,8-difluoro-1,4-dihydro-7-[4-*p*-nitrobenzyl)-1-piperazinyl]-4-oxoquinoline-3-carboxylic acid (6.0 g), acetic acid (150 ml), and 5% palladium on charcoal (1.0 g) was hydrogenated. The resulting slurry was filtered and the filtrate concentrated to dryness. The residue was treated with water, neutralized with aqueous sodium hydroxide solution and extracted with dichloromethane. The organic layer was dried and evaporated. The residue was chromatographed on silica gel. Elution with a mixture of chloroform and ethanol (20:1) and

recrystallization from a mixture of chloroform and ethanol gave 7-[4-(*p*-aminobenzyl)-1-piperazinyl]-1-ethyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid.
m.p. : 220 – 221°C.

			C	H	N
Anal.	Calcd.	for $C_{23}H_{24}F_2N_4O_3$	: 62.43	5.47	12.66
	Found		: 62.53	5.36	12.68

Experiment 1: Antibacterial activity (*in vitro*)

The minimum inhibitory concentration (MIC) of the compound (I) was determined by an agar dilution technique (the standard method of Japan Society of Chemotherapy) against standard strains of gram-positive and gram-negative bacteria.

As shown in Table 1, nalidixic acid and pipemidic acid exerted antibacterial activity mainly on gram-negative bacteria, and were inactive on many strains of gram-positive bacteria.

On the other hand, the compound (I) was more active than nalidixic acid and pipemidic acid against both of gram-positive and gram-negative bacteria. In particular, the antibacterial activity of the compound (I) was more potent against gram-positive bacteria contained *Streptococcus spp.* which were not susceptible to nalidixic acid and pipemidic acid.

Experiment 2 : Antibacterial activity (*in vivo*)

The *in vivo* antibacterial activity of the compound (I) was determined in systemic infection in mice. The systemic infections were produced in male mice ICR (body weight, 19 ± 2 g) by inoculating intraperitoneally with *Staphylococcus aureus* Smith and *E. coli* ML 4707. The compounds were administered orally in divided doses at 0 and 4 hrs. after infection. The therapeutic effect of drugs was judged from the number of mice surviving after 7 days of observation. A comparison of *in vivo* antibacterial acitivity was made on the basis of the mean effective dose (ED_{50}) calculated by the method of Litchfield and Wilcoxin.

As shown in Table 2, the *in vivo* antibacterial activity of the compound (I) was significantly more effective than that of nalidixic acid and pipemidic acid against *S. aureus* Smith. The potency of the compound (I) was superior 172 times than that of nalidixic acid, and 62 times than that of pipemidic acid.

Experiment 3: Tissue levels of the compound (I) after a single oral administration of 50 mg/kg in mice and rats.

Tissue levels of the compound (I) were determined by micro-biological assay which employed thin layer cup method with *Bacillus subtilis* ATCC 6633 as the test organism. Serum and tissue levels of the compound (I) were calculated with the standard curve prepared in normal serum of the test animal species and M/15 phosphate buffer (pH 7.5), respectively.
The results are shown in Table 3.
After a single oral administration of 50 mg/kg of the compound (I) in mice and rats, a peak serum level was reached at 8.6 and 5.3 μg/ml, respectively, within 30–60 minutes. The level of the compound (I) in lung, liver and kidney were higher than those in serum with both species. The transferability of the compound (I) in tissues was excellent.

Experiment 4: Acute toxicity of the compound (I)

Acute toxicity of the compound (I) was examined in mice (ICR strain, 7 weeks old). Observation period was 7 days after a single oral and intravenous administration.

As shown in Table 4, the compound (I) has a low toxicity.

Table 1 *In vitro* antibacterial activity of the present compound

Table 1 *In vitro* antibacterial activity of the present compound

Organism	Gram	MIC (μg/ml) Present compound	Metabolite*	NA**	PPA***
Bacillus subtilis PCI 219	+	0.1	0.2	6.25	6.25
Staphylococcus aureus 209P	+	0.1	0.78	100	25
S. aureus IID670 (Terajima)	+	0.2	0.78	>100	25
S. epidermidis IID866	+	0.2	0.78		
Streptococcus pyogenes IID692	+	0.78	3.13	>100	>100
S. pyogenes S-8	+	0.78	12.5	>100	>100
S. pneumoniae IID552	+	0.39	6.25	>100	>100
S. faecalis IID682	+	0.78	3.13	>100	>100
E. coli NIHJ JC-2	−	0.20	0.05	3.13	1.56
E. coli ATCC10536	−	0.39	0.05	3.13	1.56
Haemophilus influenzae IID986	−	0.20	0.025	1.56	3.13
Klebsiella pneumoniae IFO3512	−	0.1	0.05	1.56	1.56
Proteus vulgaris IFO3167	−	1.56	0.05	3.13	3.13
P. mirabilis IID994	−	1.56	0.05		
P. morganii IID602	−	1.56	0.1		
Enterobacter cloacae IID977	−	1.56	0.1		
Citrobacter freundii IID976	−	1.56	0.1		
Shigella sonnei IID969	−	0.39	0.05	1.56	1.56
Salmonella enteritidis IID604	−	1.56	0.1	12.5	12.5
Yersinia enterocolitica IID981	−	1.56	0.1		
Serratia marcescens IID618	−	3.13	0.1		
Pseudomonas aeruginosa V-1	−	12.5	0.78	100	12.5
P. aeruginosa IFO12689	−	12	1.56	>100	25
Acinetobacter enitratus IID876	−	0.78	0.78		
Alcaligenes faecalis 0104002	−	3.13	0.78		

Inoculum size: 10^8 cells/ml

* : 1-Ethyl-6,8-difluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)quinoline-3-carboxylic acid
** : Nalidixic acid
***: Pipemidic acid

Table 2 *In vivo* antibacterial activity of the present compound

Strain	Challenge dose (cells/animal)	Compound	MIC (μg/ml)	ED_{50} (mg/kg)
Staphylococcus aureus Smith	2.4×10^5 (in BHI* containing mucin)	Present compound	0.05	3.7
		NA**	25	635
		PPA***	12.5	231
E. coli ML4707	1.2×10^7 (in saline)	Present compound	0.39	13.8
		NA**	3.13	38.3
		PPA***	1.56	38.9

* : Brain heart infusion
** : Nalidixic acid
***: Pipemidic acid

Table 3 Tissue levels of the present compound

Animal	Tissue	Concentration (μg/ml) Time after administration (hour) 0.5	1.0	2.0	4.0	6.0
Mouse	Serum	8.6	6.5	5.0	2.0	1.8
	Lung	12.8	9.5	5.5	4.3	2.8
	Liver	22.5	17.5	12.0	9.0	5.8
	Kidney	13.0	13.0	7.8	4.6	4.6
Rat	Serum	4.8	5.3	1.2	0.2	0.2
	Lung	6.0	8.6	2.1	0.6	ND*
	Liver	14.0	15.4	6.2	2.2	0.8
	Kidney	6.6	6.8	2.7	1.0	0.3

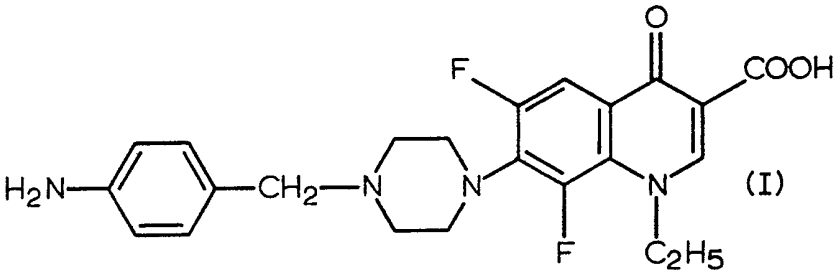
*: not detected

Table 4 Acute toxicity of the present compound in male mice.

Route of administration	LD_{50} (mg/kg)
iv	250–300
po	>4,000

CLAIMS

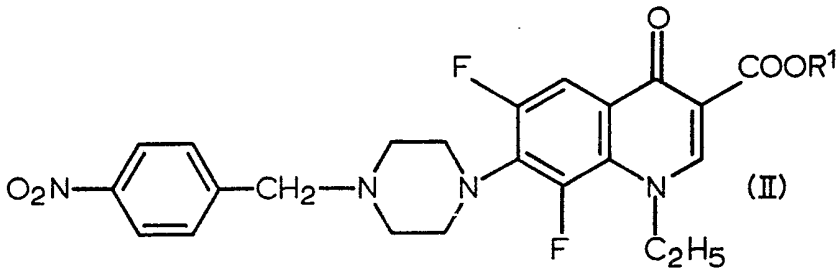
1. A compound of the formula (I):



(I)

and the salts thereof.

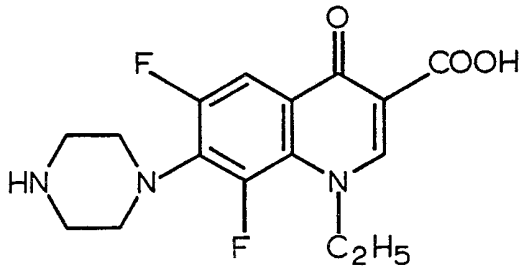
2. A process for preparing a compound of formula (I) as defined in Claim 1 or a salt thereof which comprises hydrogenating a compound of the formula (II):



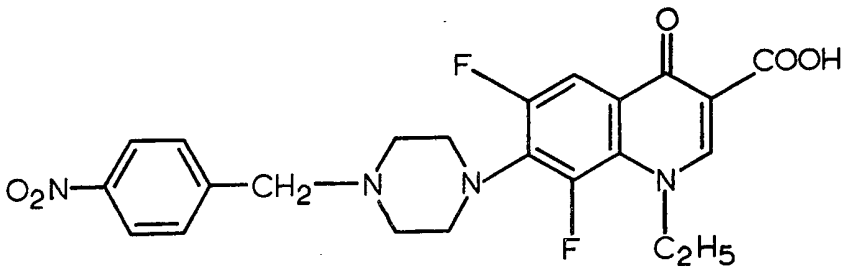
(II)

wherein R^1 is hydrogen or a lower alkyl group and, if necessary, hydrolising the resulting compound and recovering the resulting compound of formula (I) optionally in the form of a salt thereof.

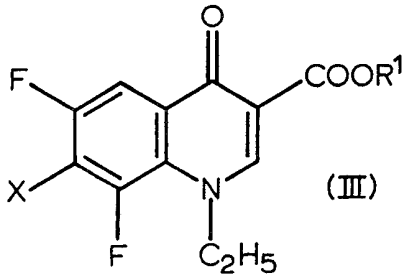
3. A process for preparing a compound of formula (I) as defined in Claim 1 or a salt thereof which comprises reacting a compound having the following formula



with *p*-nitrobenzyl halide, followed by hydrogenation of the compound having the following formula which is obtained by the above reaction

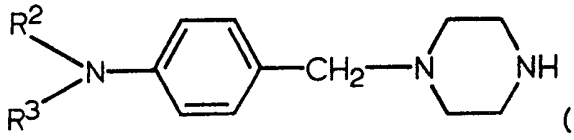


4. A process for preparing a compound of formula (I) as defined in Claim 1 or a salt thereof which comprises aminaling a compound of formula (III):



(III)

wherein R^1 is hydrogen or a lower alkykl group and X is a leaving group with a piperazine derivative of formula (IV):



(IV)

wherein R^2 and R^3 are independently hydrogen or a protecting group and, if necessary, hydrolysing the resulting compound and recovering the resulting compound of formula (I) optionally in the form of a salt thereof.

5. A compound of formula (I) or a salt thereof when prepared by a process according to any of Claims 2 to 4.

6. A pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable adjuvant.

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