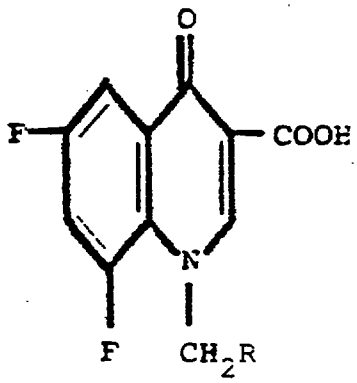
Quinoline Derivatives

The invention relates to quinoline derivatives, to a process for their preparation and to pharmaceutical compositions containing them.

The invention provides 1,4-dihydro-3-carboxy-6,8-difluoro--4-oxo-quinolines having the general formula I



I

wherein R represents a methyl, fluoromethyl, 2-hydroxyethoxy, cyclopropyl, 5-nitro-2-furyl, cyclohexyl or 2,6-dichlorophenyl group.

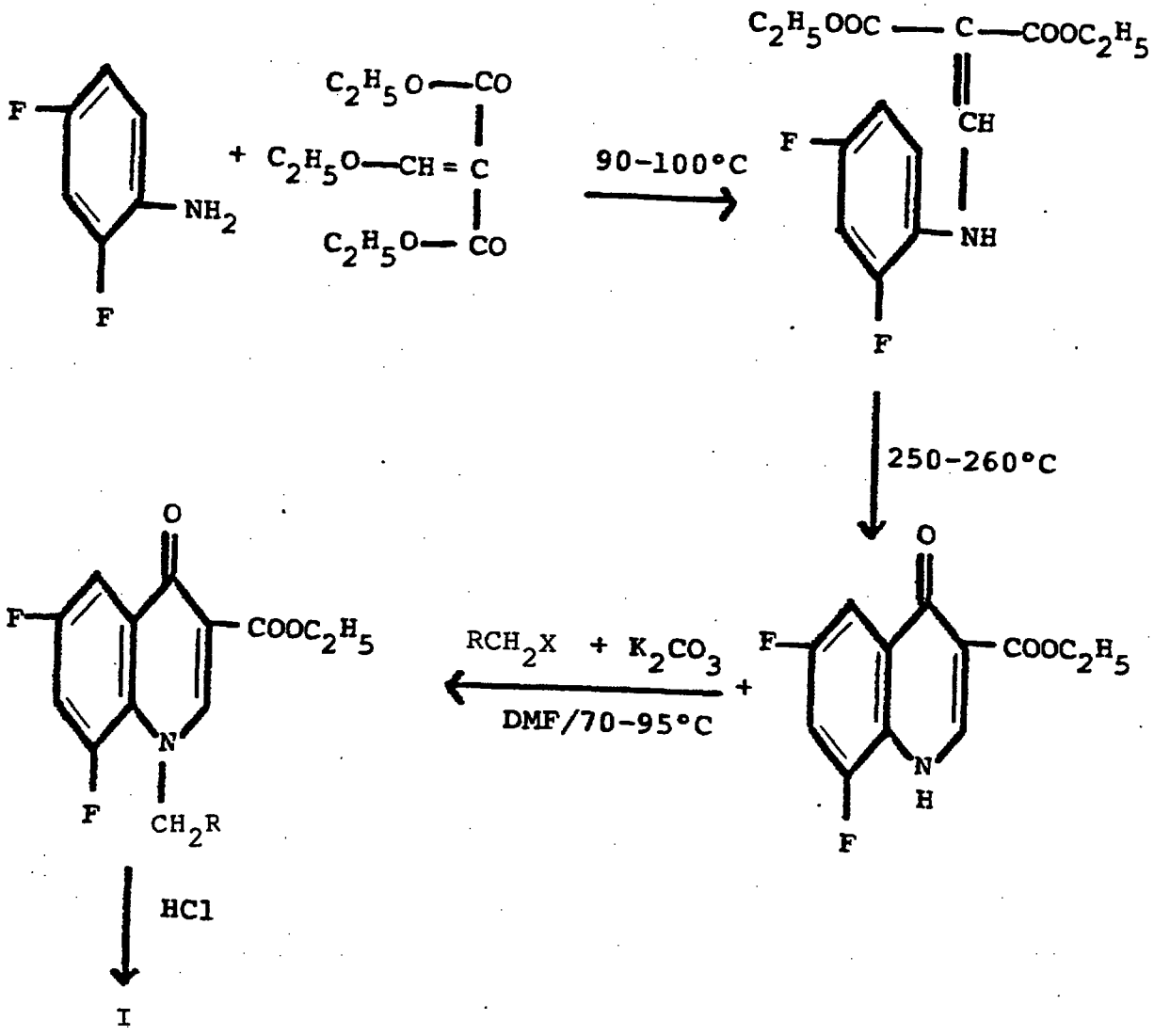
These compounds belong to a rather important group of quinoline derivatives, some of which are known to possess antibacterial activity whereas most of them are completely inactive. In various searches effected in this group of derivatives it has been commonly found that if a product with a certain substituent in a determined position possesses an antibacterial activity, minor changes in the nature of the substituent or in the position generally lead to completely inactive products. For this reason it is completely impossible to forecast whether a particular compound would be active or inactive. The compounds of the invention have been found to have an antibacterial power against a large family of bacteria, either of gram positive or of gram negative nature.

The invention further provides a process for the preparation of the 1,4-dihydro-3-carboxy-6,8-difluoro-4--oxo-quinolines having the general formula I as above defined, the process comprising reacting, in a solvent in the presence of a base, 1,4-dihydro-3-ethoxycarbonyl-6,8-difluoro-4-oxo-quinoline with an excess of a compound of the general formula RCH_2X wherein R is as above defined and X represents a chlorine, bromine or iodine atom, and hydrolysing the 3-ethoxycarbonyl group of the resultant quinoline derivative. The reaction is preferably conducted in dimethylformamide at 70°C to 95°C for from

12 to 24 hours. Suitably from 2 to 5 moles of the compound RCH_2X are used per mole of the quinoline derivative. A suitable base is potassium carbonate. Hydrolysis is preferably effected with an inorganic acid under reflux. 2N Hydrochloric acid is particularly suitable.

The 1,4-dihydro-3-ethoxycarbonyl-6,8-difluoro-4-oxo--quinoline starting material may easily be prepared from 2,4-difluoroaniline. This is condensed with diethyl ethoxymethylenemalonate, leading to 1-(2,2-diethoxy-carbonyl-vinylamino)-2,4-difluorobenzene which is cyclised by warming.

The whole process, from 2,4-difluoroaniline through to the compounds I is illustrated by the following reaction scheme:



The invention also provides a pharmaceutical composition comprising a 1,4-dihydro-3-carboxy-6,8--difluoro-4-oxo-quinoline having the general formula I as above defined in admixture with a pharmaceutically acceptable diluent or carrier.

The invention is illustrated by the following Examples.

EXAMPLE 1 I: R=CH_3

Into an appropriate reactor fitted with warming, cooling and stirring means, were poured 152.5 ml (1.5 mol) of 2,4-difluoroaniline and 425 ml (2.1 mol) of diethyl ethoxymethylenemalonate. The mixture was warmed at 90 to 100°C for about three hours with evacuation of ethanol appearing in the condensation. This led to a solid product which was treated with hexane, washed, crystallized from hexane and dried to give 356.5 g (yield 79.5%) of 1-(2,2-diethoxycarbonyl-vinylamino)-2,4-difluoro-benzene. Elemental analysis of the product showed a good correspondence with the formula $C_{14}H_{15}NO_4F_2$.

350 g (1.17 mol) of this product were poured into a reactor with 1 litre of diphenyloxide. The mixture was refluxed at 265 - 270°C for 1½ hours with a subsequent evacuation of the ethanol produced during the cyclisation. After cooling to ambient temperature, there was obtained a residue which was treated with benzene and then with hexane, dried and recrystallized from methanol. This gave 239 g (yield 80.7%) of 1,4-dihydro-3-ethoxycarbonyl--6,8-difluoro-4-oxo-quinoline, elemental analysis of which showed a good correspondence with the formula $C_{12}H_9NO_3F_2$.

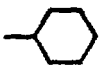
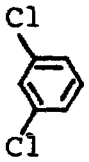
For the fixation of an ethyl group on the nitrogen atom, 210 g (0.84 mol) of this product, 287 g (2.1 mol)

of potassium carbonate and 337 ml (4.2 mol) of ethyl iodide were treated for 24 hours at about 90°C in the presence of 1.5 litres of dimethylformamide. After evacuation of the dimethylformamide under reduced pressure, the mixture was treated with 1 litre of water and stirred at about 0°C. The resultant precipitate was washed with water, dried and recrystallized from isopropanol at the boil. After separation and drying there were obtained 220 g (yield 93.2%) of 1,4-dihydro-3-ethoxycarbonyl-6,8-difluoro-1-ethyl-4-oxo-quinoline, elemental analysis of which showed a good correspondence with the formula $C_{14}H_{13}NO_3F_2$.

To obtain the corresponding acid, 210 g (0.74 mol) of this product was treated with 2N hydrochloric acid under reflux. This gave 179 g (yield 95%) of 1,4-dihydro-3-carboxy-6,8-difluoro-1-ethyl-4-oxo-quinoline, elemental analysis of which showed a good correspondence with the formula $C_{12}H_9NO_3F_2$.

Examples 2 to 7

Example 1 was repeated, replacing the ethyl iodide with 2-fluoroethyl chloride, 2-benzoyloxyethoxymethyl bromide, cyclopropylmethyl bromide, 5-nitro-furfuryl bromide, cyclohexylmethyl bromide and 2,6-dichloro-benzyl chloride respectively. The characteristics of and yields of the products obtained were as follows

Example	Product I, R=	Yield % (condensation)	Yield % (hydrolysis)	m.p. °C (Tottoli)	Elemental Analysis	Soluble IN DMSO
2	CH_2F	90.3	96.2	268*	$C_{12}H_8F_3NO_3$	no
3	$HOCH_2CH_2O$	56	73.5	218	$C_{13}H_{11}F_2NO_5$	yes
4		67	88.4	204	$C_{14}H_{11}F_2NO_3$	yes
5		58	77	210-215	$C_{15}H_8F_2N_2O_6$	yes
6		44	76	241	$C_{17}H_{17}F_2NO_3$	no
7		50	86.6	272	$C_{17}H_9Cl_2F_2NO_3$	no

* with sublimation

All the compounds were white powders, except in Example 5 which gave a beige powder. All the compounds were insoluble in water. In Example 3, 2-benzoyloxyethoxymethyl bromide was used rather than 2-hydroxyethoxymethyl bromide for practical reasons; the benzoyl group was removed during the hydrolysis step.

TOXICITY

The toxicity of the compounds of the invention has been determined per os, by the usual methods on rats and mice. LD_{50} values were from 1320 to 1940 mg/kg on rats

and from 910 to 1430 mg/kg on mice.

Usual tests for checking mutagenesis and clastogenesis (Ames test, micronucleus test and lymphocytes cultures) were negative.

5 BACTERIOLOGY

A) The bactericidal activity of the compounds of the invention has been determined on various microorganisms, as hereinafter described.

Serial dilutions of the test and reference compounds
10 are prepared in Brain Heart Infusion Broth (Oxoid (RTm) CM225) in two-fold steps from 1000 µg/ml down to 0.5 µg/ml.

The test organisms are cultured on Tryptone Soy Agar (TSA, Oxoid CM131) and checked for viability and purity. Standardized inocula are then ppepared by inoculating slopes of TSA and incubating at 37°C for 24 hours. The resultant bacterial growth is then removed by the addition of sterile physiological saline and shaking with glass beads. The suspensions of organisms are then standardized to give 50 % transmission at 520 nM on an SP600 Spectrophotometer. Calibration curves show this to yield approximately 10^8 colony forming units per ml.

0.1 ml aliquots of these suspensions are then used to inoculate each of the prepared serial dilutions of the compounds in Brain Heart Infusion Broth.

The sets of dilutions are incubated at 37°C for 24 hours and then inspected for the presence or absence of growth as shown by turbidity of the medium. The lowest concentration showing no growth of the test organism is recorded as the Minimal Inhibitory Concentration (MIC) of the compound for that organism.

Each of the tubes showing no growth are then sub-cultured onto plates of Tryptone Soy Agar and incubated at 37°C for 24 hours. The plates are then inspected for the presence or absence of growth at the inoculation points. The lowest concentration of the test compound which shows no growth on sub-culture is recorded as the Minimal Microbiocidal Concentration (MMC). MIC and MMC are expressed in µg/ml.

The results are presented in the following table, in comparison with those for two reference compounds, nitrofuroxazide and pipemidic acid. The abreviations used in the table have the following meanings :

S.a.	:	Staphylococcus aureus	ATCC 6538
S.p.	:	Streptococcus pyogenes	NCTC 8198
P.a.	:	Pseudomonas aeruginosa	ATCC 9027
E.c.	:	Escherichia coli	NCTC 8196
P.m.	:	Proteus mirabilis	NCTC 8559
S.e.	:	Salmonella enteritidis	NCTC 6676
S.b.	:	Shigella boydii	NCTC 9328
Nif.	:	Nifuroxazide	
Pip.	:	Pipemidic acid	
T.M.	:	Tested microorganism	

B) The bacteriostatic activity of the compounds of the invention has also been searched against microorganisms more particularly related with gastro-enteritis.

This experiment was conducted in Mueller - Hinton gelose with tested compounds dissolved in dimethylsulfoxide (control dimethylsulfoxide was provided) at increasing concentrations from 0.01 to 100 µg. The strains used were Vibrio Cholerae (3 strains respectively from Europe, Africa and Conth-East-Asia), Vibrio parahemolyticus, Vibrio alginolyticus, Aeromonas hydrophila Sobria and Salmonella. Minimum inhibitory concentration was found for the 7 compounds of the invention, between 0.5 and 1 µg for the Salmonella strain and between 0.1 and 0.5 µg for the other strains, which denotes a strong bacteriostatic activity.

C) This experiment was completed by an in vivo test on batches of each 10 SWISS mice weighing about 20 g and administered orally with the compounds of the invention. Three doses were used for each compound and the urines of each batch were collected for evaluation of their bactericidal activity on cultures of Vibrio Cholerae Ogawa (10^6 per ml) on Mueller - Hinton gelose. The activity was appreciated, after a 24 hours incubation at 37°C, by the diameter in mm of the inhibition of the strain around the point of introduction in the gelose. A negative control (Nifuroxazide) and a positive control

(Pipemidic acid) were used as references. The doses administered to mice were 0.5, 1 and 2.5 µg for the compounds of examples 1 to 5 and 1, 2.5 and 5 µg for the compounds of examples 6 and 7 which appeared less active in preceding experiment.

In this experiment the activity of the compounds of the invention was found at about 1 µg for the compounds of examples 1 to 5 and between 1 and 2.5 µg for the compounds of examples 6 and 7.

PRESENTATION - POSOLOGY

In human therapy the compounds of the invention may be presented in tablets of gelatine capsules, containing 0.1 to 0.5 g of active ingredient per dose unit, for oral administration. Usual posology is 0.5 to 2 g per diem.

Injectable forms include phials of each 0.1 g of active ingredient to be suspended extemporeanously before injection. Posology : 1 to 3 phials per diem.

T A B L E

Compound	T.M.	S.a	S.p.	P.a.	E.c.	P.m.	S.e.	S.b.
Nif.	MIC	8	1	500	4	500	500	8
	MMC	8	1	500	4	500	500	8
Pip.	MIC	2	4	1	4	4	4	4
	MMC	250	125	16	4	8	8	4
1	MIC	32	16	32	8	1	2	1
	MMC	32	32	64	8	2	4	1
2	MIC	4	32	8	32	4	4	8
	MMC	8	32	8	32	8	8	16
3	MIC	4	2	8	4	32	32	8
	MMC	8	4	16	8	32	32	8
4	MIC	64	4	8	64	1	2	32
	MMC	64	4	16	64	2	4	32
5	MIC	16	8	2	64	16	125	32
	MMC	64	32	2	64	32	125	32
6	MIC	8	16	4	64	8	4	8
	MMC	16	16	8	64	8	4	8
7	MIC	8	32	8	1	2	2	16
	MMC	8	64	8	2	4	4	32

* C L M S *

CLAIMS:

1. A quinoline derivative having the general formula I as herein defined.

2. A process for the preparation of a quinoline derivative having the general formula I as herein defined, the process comprising reacting, in a solvent in the presence of a base, 1,4-dihydro-3-ethoxycarbonyl--6,8-difluoro-4-oxo-quinoline with an excess of a compound of the general formula RCH_2X wherein R is as above defined and X represents a chlorine, bromine or iodine atom, and hydrolysing the 3-ethoxycarbonyl group of the resultant quinoline derivative.

3. A process according to claim 2 in which the solvent is dimethylformamide and the reaction is conducted at from 70°C to 95°C for from 12 to 24 hours.

4. A process according to claim 2 or claim 3 in which from 2 to 5 moles of the compound RCH_2X are used per mole of the quinoline derivative.

5. A process according to any of claims 2 to 4 in which the base is potassium carbonate.

6. A process according to any of claims 2 to 5 in which the hydrolysis is effected with an inorganic acid under

* C L M S *

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reflux.

7. A process according to claim 6 in which the inorganic acid is hydrochloric acid.

8. A process according to any of claims 2 to 7 in which the 1,4-dihydro-3-ethoxycarbonyl-6,8-difluoro-4-oxo-quinoline used has been prepared by condensing 2,4--difluoroaniline with diethyl ethoxymethylenemalonate and heating the condensate.

9. A process for the preparation of a quinoline derivative having the general formula I as herein defined, the process being substantially as described herein with reference to any of the Examples.

10. A pharmaceutical composition comprising a quinoline derivative having the general formula I as herein defined in admixture with a pharmaceutically acceptable diluent or carrier.