United States Patent [19]

Ackerman et al.

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[45] **Date of Patent: Nov. 6, 1990**

[54] **4-QUINOLINE CARBOXYLIC ACID DERIVATIVES USEFUL AS IMMUNOSUPPRESSIVE AGENTS**

[75] Inventors: **Neil R. Ackerman**, Greenville; **Richard R. Harris**, Wilmington; **Scott E. Loveless**, Newark, all of Del.; **Russell H. Neubauer**, West Chester, Pa.

[73] Assignee: **E. I. Du Pont de Nemours and Company**, Wilmington, Del.

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[51] **Int. Cl.**[5] **A61K 31/47**

[52] **U.S. Cl.** **514/312**; 514/311; 514/314; 514/825; 514/885

[58] **Field of Search** 514/311, 314, 312, 825, 514/885

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,973,022	8/1976	Goschke	514/825
4,407,803	10/1983	Haviv et al.	514/825
4,680,299	7/1987	Hesson	514/311
4,847,381	7/1989	Sutherland et al.	548/485

FOREIGN PATENT DOCUMENTS

0190722	8/1986	European Pat. Off.	514/312

Primary Examiner—Douglas W. Robinson
Assistant Examiner—Zohreh A. Fay

[57] **ABSTRACT**

4-Quinolinecarboxylic acids and derivatives thereof, such as 2-(2'-fluoro-1,1'-biphenyl-4-yl)-6-fluoro-3-methyl-4-quinolinecarboxylic acid, are useful as immunomodulatory and anti-inflammatory agents. Pharmaceutical formulations containing such compounds are useful for the treatment of autoimmune diseases, organ transplantation rejection, graft vs. host disease, multiple sclerosis, and chronic inflammatory diseases such as rheumatoid arthritis.

8 Claims, No Drawings

4-QUINOLINE CARBOXYLIC ACID DERIVATIVES USEFUL AS IMMUNOSUPPRESSIVE AGENTS

BACKGROUND OF THE INVENTION

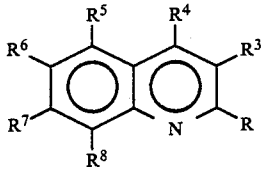
This invention relates to methods of treating immunological and inflammatory diseases and more particularly to methods of treating such diseases with 4-quinoline carboxylic acids and derivatives thereof.

U.S. Pat. No. 4,680,299, granted July, 14, 1987, to Hesson describes phenylquinoline caboxylic acids and their derivatives as tumor inhibiting agents.

It has now been found that the compounds described in U.S. Pat. No. 4,680,299 are useful as immunomodulatory and antiinflammatory agents.

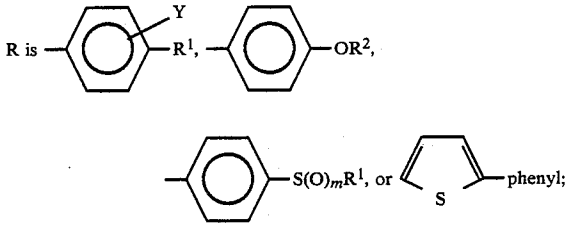
SUMMARY OF THE INVENTION

According to the present invention there is provided a method of treating an autoimmune disease in a mammal comprising administering to the mammal an immunosuppressive amount of a compound having the formula:

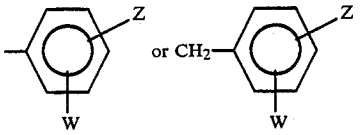


(I)

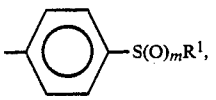
wherein



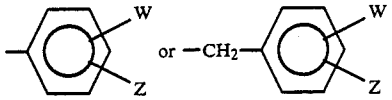
R^1 is $CH_3CH_2(CH_3)CH$, alkyl of 5–12 carbon atoms, cyclohexyl,



when R is



R^1 can be in addition alkyl of 3–4 carbon atoms; R^2 is



R^3 is H, alkoxy of 1–3 carbon atoms, or alkyl of 1–2 carbon atoms;

R^4 is CO_2H or CO_2R^{11};

R^5, R^6, R^7 and R^8 are independently H, F, Cl, Br, I, CH_3, CF_3, SCH_3 or CH_2CH_3, at least two of R^5, R^6, R^7 and R^8 being H;

R^9 and R^{9A} are independently H or alkyl of 1 to 3 carbon atoms;

R^{11} is $(CH_2)_{2-4}NR^9R^{9A}$;

W, Y and Z are independently H, F, Cl, Br, alkyl of 1–5 carbon atoms, NO_2, OH, CF_3 or OCH_3;

m is 0 or 1; or

a pharmaceutically suitable salt thereof;

with the following provisos:

(1) R^5, R^6 and R^7 cannot all be H;

(2) when R^4 is $CO_2CH_2CH_2N(CH_3)_2$, R^6 is CH_2CH_3, or R^7 is Cl, R^1 cannot be cyclohexyl;

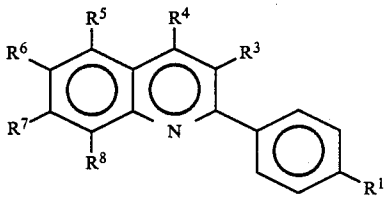
(3) when R^1 is cyclohexyl and R^3 is H R^6 must be Cl or F, but R^6 and R^8 cannot both be Cl; and

(4) when R^6 is CH_3, then R^7 cannot be Cl.

Additionally provided is the above-described method wherein the compound is administered in combination with a nonsteroidal antiinflammatory drug.

PREFERRED EMBODIMENTS

Preferred compounds useful in the method have the formula:



(II)

wherein

R^1 is cyclohexyl; phenyl; phenyl substituted with one halogen; alkyl or 1–5 carbon atoms or CF_3; phenoxy; or phenoxy substituted with one halogen or alkyl of 1–5 carbon atoms;

R^3 is H or alkyl of 1–2 carbon atoms;

R^4 is CO_2H, a sodium or potassium salt thereof; or CO^2R^{11};

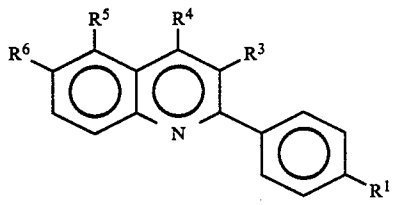
R^5 and R^6 are independently H, halogen, CH_3 or CF_3;

R^7 and R^8 are independently H or halogen;

R^{11} is $(CH_2)_{2-4}NR^9R^{9A}$; and R^9 and R^{9A} are independently alkyl of 1 to 3 carbon atoms,

or a pharmaceutically suitable salt thereof; provided that R^5, R^6 and R^7 cannot all be H and that when R^1 is cyclohexyl and R^3 is H, R^6 must be Cl or F, but R^6 and R^8 cannot both be Cl, and when R^6 is CH_3, then R^7 cannot be Cl.

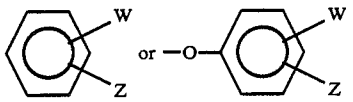
More preferred compounds useful in this invention have the formula:



(III)

wherein

R^1 is cyclohexyl,



R^3 is H or alkyl of 1–2 carbon atoms;

R^4 is CO_2H, a sodium or potassium salt thereof, or CO_2R^{11};

R^5 and R^6 are independently H, halogen or CF_3 provided that both R^5 and R^6 are not hydrogen;

R^{11} is $(CH_2)_{2-4}NR^9R^{9A}$; and

R^9 and R^{9A} are independently alkyl or 1 to 3 carbon atoms, and

W and Z are independently H, halogen, alkyl of 1–5 carbon atoms or CF_3;

provided that when R^1 is phenyl or phenoxy, and R^5 is H, then R^6 cannot be Br; and that when R^1 is cyclohexyl and R^3 is H, R^6 must be Cl or F. Specifically preferred compounds useful in this invention are:

(1) 2-(1,1′-biphenyl-4-yl)-5-chloro-3-methyl-4-quinoline carboxylic acid, sodium or potassium salt

(2) 2-(1,1′-biphenyl-4yl)-6-fluoro-3-methyl-4-quinoline carboxylic acid, sodium or potassium salt

(3) 6-fluoro-3-methyl-2-(4-phenoxyphenyl)-4-quinoline carboxylic acid, sodium or potassium salt

(4) 2-(4′-bromo-1,1′-biphenyl-4yl)-6-fluoro-3-methyl-4-quinoline carboxylic acid, sodium or potassium salt

(5) 2-(2′-fluoro-1,1′-biphenyl-4yl)-6-fluoro-3-methyl-4quinoline carboxylic acid, sodium o r potassium salt.

DETAILED DESCRIPTION OF THE INVENTION

The compounds useful in this invention are described in and prepared by methods set forth in U.S. Pat. No. 4,680,299, the disclosure, synthesis, and synthesis example of which are hereby incorporated by reference.

The invention can be further understood by the following example in which parts and percentages are by weight unless otherwise indicated; all temperatures are in degrees centigrade.

EXAMPLE 1

Part A:

2-(1,1′-Biphenyl-4yl)-5-chloro-3-methylguinoline-4carboxylic acid

A mixture of 4-chloroisatin (7.28 g, 0.04 mol), [*J. Am. Chem. Soc.*, 1251 (1956)], 4-henylpropiophenone (8.8 g, 0.04 mol), diethylamine (4 ml, 0.04 mol) and ethanol (200 ml) was stirred for a period of 18 hours at room temperature. The precipitated solids where collected by filtration, washed with ice-cold ethanol and air dried to yield the adduct (9.1 g, 58%) m.p. 209°–214° dec.

Part B

The above described adduct (9.1 g) was added to a mixture of tetrahydrofuran (200 ml), and concentrated HCl (200 ml) and heated at reflux for 24 hr. The reaction mixture was cooled, water (300 ml) was added and most of the tetrahydrofuran removed by evaporation in vacuo. The aqueous residue was cooled and the sticky solids collected by filtration. Trituration in 150 mol of boiling methanol yielded (4.8 g. 55%) m.p. 295°–297° dec.

$C_{23}H_{16}ClNO_2$HRMS: 373.0869 Calcd, measured m/e 373.0814.

^{1}H NMR (DMSO-d_6):δ8.5(m,1H), 7.7–7.85(m,7H), 7.35–7.55(m,4H), 2.45(s,3H).

Part C: Sodium 2-(1,1′-Biphenyl-4-yl)-5-chloro-3-methyl-guinoline-4-carboxylate

To a suspension of the above acid (3.7 g, 0.01 mol) in ethanol 100 ml, sodium hydroxide (1N, 10 ml, 0.01 mol) was added, and gently warmed. The clear solution was then filtered and evaporated to dryness to yield (4.0 g) m.p. 320°–330° dec.

EXAMPLE 2

Part A:

2-(2-Flouro-1,1′-biphenyl-4-yl)-6-fluoro-3-methyl-4-quinoline carboxylic acid

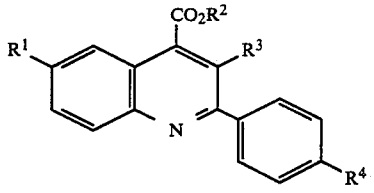
5-Fluoroisatin (72.6 g, 0.44 mole) and 4-(2-fluorophenyl)propiophenone (100 g, 0.44 mole) were suspended in 720 ml of ethanol and stirred mechanically as a solution of KOH (147.8 g, 2.64 mole) in 300 ml of water was added dropwise over 15 minutes. The reaction mixture was heated at reflux for 12 hours, cooled and the ethanol evaporated under reduced pressure. The resulting solid was dissolved in water and washed with ethyl ether. The aqueous layer was cooled to 5° and acidified with glacial acetic acid. The resulting precipitate was filtered, washed 2 times with 300 ml of ethyl ether and dried. Recrystallization from dimethylformamide and water gave 84 g of a white 2-(2′-Fluoro-1,1′-biphenyl-4yl)-6-fluoro-3-methyl-4quinoline carboxylic acid, m.p. 315°–317°.

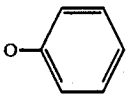
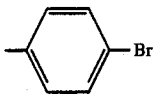
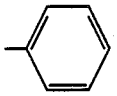
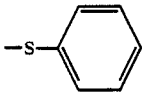
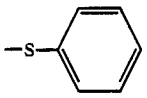
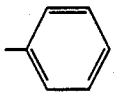
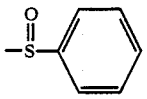
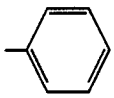
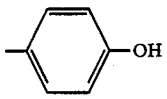
Part B: Sodium 2-(2′-Fluoro-1,1′-biphenyl-4-yl)-6-fluoro-3-methylg-quinoline-4-carboxylate

The compound of Part A (37.5 g, 0.10 mole) was suspended in 1,000 ml of ethanol and treated with 1N NaOH (100 ml, 0.10 mole). The mixture was warmed and stirred until clear; the ethanol and water were evaporated at reduced pressure to give 39.6 g of the white solid sodium 2-(2′-fluoro-1,1′-biphenyl-4-yl)6-fluoro-3fluoro-3-methylquinoline-4-carboxylate, m.p. >360°.

Following the procedures of Example 1 and 2 or the synthesis procedures described in U.S. Pat. No. 4,680,299, the compounds set forth in Table 1 were prepared.

TABLE 1



Ex. No.	R^1	R^2	R^3	R^4	m.p.(°)
3	F	Na	CH_3		>350
4	F	Na	CH_3		>350
5	CH_3	Na	CH_3		>350
6	F	Na	CH_3	$-S-CH(CH_3)_2$	339–343
7	Cl	Na	CH_3		319–324
8	Cl	K	CH_3		310–325
9	F	Na	H		>360
10	F	Na	CH_3		251–260
11	F	Na	OCH_3		345–349
12	Cl	Na	CH_3		>360

UTILITY

Results of the biological tests-described below establish that the compounds useful in this invention have the ability of suppress/inhibit: the contact sensitivity response to 2,4-dinitrofluorobenzene (DNFB) in mice, the human mixed lymphocte reaction, and adjuvant-induced arthritis in rats.

Contact sensitivity of DNFB has been extensively studied and characterized in the mouse to determine the regulatory mechanisms involved in cell mediated immune responses (Claman, et al., Immunol Rev 50:105, 1980). this is an antigen-specific T-cell mediated inflammatory response that represents delayed-type hypersensitivity reactions seen in both humans and other mammals. The primary use of the human mixed lymphocyte reaction is for the determination of transplantation compatibility between the donor (graft) and the recipient (Park and Good. p. 71. In Yunis, et al., Tissue typing and organ transplantation. 1973 Academic Press Inc., N.Y.).

Rat adjuvant-induced arthritis represents a systemic inflammatory disease with bone and cartilage changes similar to that observed in rheumatoid arthritis, but in an accelerated time span (Pearson, Arth Rheum 7:80, 1964).

Most clinically effective drugs exhibit activity in these biological tests similar to that observed with the compounds useful in this invention (Fenichel and Chirigos, ed, Immune modulation agents and their mechanisms, 1984 Dekker, Inc., N.Y. , and Billingham, 21:389, 1983).

Contact Sensitivity Response to DNFB in Mice

Balb/c female mice (≃20 g, Charles River) were sensitized on the shaved abdomen with 25 μl of 0.5% 2,4-dinitrofluorobenzene (DNFB, Eastmen Kodak Co.) in a vehicle of 4:1 acetone:olive oil on days 0 and 1. Mice were are challenged with 20 μl of 0.2% DNFB in a vehicle of 4:1 acetone:olive oil on day 5. A constant area of the ears was measured immediately before challenge and 24 hours later with an engineer's micrometer. Ear swelling was expressed as the difference in ear thickness before and after challenge in units of 10^{-4} inches±SEM. Percent suppression was calculated as:

$$\% \text{ Suppression} = 1 - \frac{\text{compound treated} - \text{negative control}}{\text{positive control} - \text{negative control}} \times 100$$

Compounds were administered orally from days—2 through day 6 and were prepared in 0.25% Methocel ® (Dow chemical Co.). Control animals received only vehicle (0.25% Methocel ®). Negative controls were not sensitized on days 0 and 1 but were ear challenged on day 5. Ten mice were used per group. Results with compounds of invention and drugs used clinically are shown in Tables 2 and 3.

TABLE 2

Treatment	Dose (mg/kg)	Ear Swelling[a] (units ± SEM)	% Suppression	ED_{50}
Negative	Vehicle	0.74 ± 0.52	—	—
Positive	Vehicle	74.11 ± 3.78	0	—
Dexamethasone	0.2	52.95 ± 3.39	28.84	1.50
	1.0	41.60 ± 2.46	44.31	
	5.0	23.79 ± 2.71	68.58	
	10.0	15.50 ± 2.10	79.88	
Cyclosporin A	2.0	56.15 ± 3.74	24.48	70.00
	10.0	66.58 ± 3.75	10.27	
	50.0	47.90 ± 3.76	35.72	
	100.0	7.80 ± 2.04	90.37	
Methotrexate	0.4	71.30 ± 2.96	3.83	9.00
	2.0	60.80 ± 1.99	18.14	
	10.0	36.10 ± 3.23	51.80	
	20.0	27.45 ± 4.99	63.59	
Example 1	0.4	66.05 ± 4.32	10.99	3.50
	2.0	56.94 ± 4.80	23.40	
	10.0	6.10 ± 0.75	92.69	
	20.0	5.20 ± 1.17	93.92	
Example 2	0.4	51.95 ± 2.33	30.20	0.95
	2.0	25.61 ± 3.39	66.10	

TABLE 2-continued

Treatment	Dose (mg/kg)	Ear Swelling[a] (units ± SEM)	% Suppression	ED_{50}
	10.0	6.40 ± 1.09	92.28	
	20.0	4.75 ± 1.20	94.53	

[a]Increase in ear thickness from day 5 to day 6, unit = 10^{-4} inches

TABLE 3

Treatment	Dose (mg/kg)	Ear Swelling[a] (units ± SEM)	% Suppression
Negative	Vehicle	2.60 ± 0.73	—
Positive	Vehicle	73.11 ± 3.69	0
Dexamethasone	1.0	42.20 ± 2.61	43.83
Cyclosporin A	20.0	74.30 ± 2.86	—1.69
Methotrexate	20.0	16.94 ± 2.10	79.66
Example 3	20.0	14.25 ± 1.49	83.48
Example 4	20.0	11.80 ± 1.03	86.95
Example 5	20.0	35.47 ± 2.31	53.37
Example 6	20.0	58.20 ± 4.63	21.14
Example 7	20.0	62.95 ± 3.40	14.40
Example 8	20.0	63.25 ± 3.58	13.98
Example 9	20.0	42.60 ± 2.68	43.27
Example 10	20.0	57.28 ± 2.36	22.45
Example 11	20.0	20.85 ± 2.53	74.12
Example 12	20.0	54.58 ± 3.21	26.28

[a]increase in ear thickness from day 5 to day 6, unit = 10^{-4}

Human Mixed Lymphocyte Reaction

Blood was obtained by venipunture from two non-related human donors. Peripheral blood mononuclear cells (PBMC) were isolated from these samples by using the Leuco Prep procedure (Becton-Dickinson). PBMC were washed twice in phosphate buffered saline (without calcium and magnesium) and the separate cell isolations were adjusted to the appropriate concentrations in media (PRMI 1640) supplemented with 10% human AB serum and 50 μl/ml gentamicin. Cells from donor A (2×10^5) were incubated with cells from donor B (2×10^5) in 96 well round bottom microliter plates at 37° C., 5% CO_2 for 6 days. Eighteen hours prior to harvesting cells from the plates, all wells were pusled with 1 μCi of ^{3}H-thymidine. Cells from the plates were harvested on day 6 and ^{3}H-thymidine incorporation was determined using a scintillation counter. Test results are shown in Table 4.

TABLE 4

Compound	IC_{50} (M)
Indomethacin	$>10^{-6}$
Cyclosporin A	1.6×10^{-8}
Methotrexate	2.5×10^{-9}
Example 1	9.6×10^{-9}
Example 2	2.5×10^{-8}

Adjuvant-Induced Arthritis

Male Lewis rats (Charles River) weighing 160–210 grams were injected subcutaneously with 0.1 ml of Freund's Complete Adjuvant containing 5 mg of M. butyricum/ml of paraffin oil (Difco Laboratories) into the planter region of the right hind paw. Paraffin oil was injected for non-arthritic controls. Ten rate were used per group. Compounds were prepared in 0.25% Methocel ® (Dow Chemical Co) with one drop of Tween ® 80 per 10 ml of Methocel ®. Animals were dosed every day beginning on the day of paw injection until day 18. the weight of each animal was recorded ever other day beginning on the day of the paw injections. On day **18** the animals were weighed, and the non-injected hind paw volume was measured using a Ugo Basile Volume Differential Plethysmometer. The results are shown in Table 5.

TABLE 5

Group (AA)	Compound (mg/kg)	Weight Gain (g)	Non-Injected Hind-Paw Volume (ml)	% Suppression
A −	Vehicle	85.6 ± 4.8	1.12 ± 0.01	—
B +	Vehicle	−20.3 ± 2.9	1.88 ± 0.05	—
C +	Example 1 (10.00)	−14.0 ± 4.2	1.87 ± 0.08	1.4
D +	Example 1 (17.5)	2.8 ± 5.3	1.72 ± 0.08	20.8
E +	Example 1 (25.0)	20.6 ± 6.3	1.34 ± 0.10	70.6
F +	Example 2 (2.0)	−1.5 ± 3.6	1.62 ± 0.05	34.5
G +	Example 2 (10.0)	65.6 ± 5.2	1.15 ± 0.02	96.2
H +	Example 2* (25.0)			

Example 1: ED_{50} = 21 mg/kg
Example 2: ED_{50} < 10 mg/kg
*Toxic by day 7

In summary, test results show that the compounds useful in this invention have both immunomodulating an anti-inflammatory effectiveness. Based on these data, the compounds useful in this invention should be efficacious in treating autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematious, multiple sclerosis, and myastenia gravis; all of which involve T lymphocyte mediated components similar to those known in the contact sensitivity model. Activities in the human mixed lymphocte reaction indicate that the compounds of invention should be effective in preventing organ transplantation rejection and graft vs. host disease. These compounds were also effective n the adjuvant-induced arthritis model and should therefore be useful anti-inflammatory agents for the treatment of chronic inflammatory diseases such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease.

DOSAGE FORMS

the antitumor compounds (active ingredients) of this invention can be administered to inhibit tumors by any means that produces contact of the active ingredient with the agent's site of action in the body of a mammal. They can be administered by any conventional means available for use in conjunction with pharmaceuticals; either as individual therapeutic active ingredients or in a combination of therapeutic active ingredients. They can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

The dosage administered will be a tumor-inhibiting amount of active ingredient and will, of course, vary depending upon known factors such as the pharmacodynamic characteristic of the particular active ingredient, and its mode and route of administration; age, health, and weight of the recipient; nature an extend of symptoms; kind of concurrent treatment, frequency of treatment, and the effect desired, usually a daily dosage of active ingredient can be about 0.1 to 400 milligrams per kilogram of body weight. Ordinarily 1 to 100, and preferably 10 to 50 milligrams per kilogram per day is effective to obtain desired results.

Dosage forms (compositions) suitable for internal administration contain from about 10–500 milligrams to about 500 milligrams of active ingredient per unit. In

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