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Design, synthesis, and biological evaluation of novel 4-hydro-quinoline-3-carboxamide derivatives as an immunomodulator

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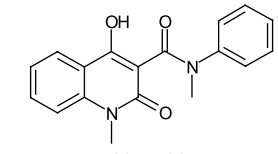
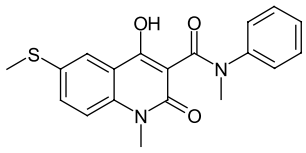
Abstract—A series of novel quinoline-3-carboxamide derivatives were synthesized and evaluated for their immunomodulatory activity. The compounds were tested in vitro for effects on spleen lymphocyte proliferation and TNF-α production by macrophage. Three compounds showed immunomodulatory profiles similar to and more potent than those of linomide and **FR137316** and were selected for further pharmacological studies in vivo.

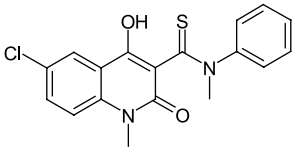
1. Introduction

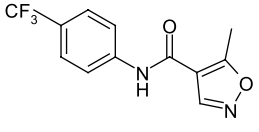
Linomide (*N*-phenylmethyl-1,2-dihydro-4-hydroxyl-1-methyl-2-oxo-quinoline-3-carboxamide) has been shown to be effective against various types of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosis, and lupus nephritis.[1–3] Recently, linomide and its derivatives **FR137316** and **FR165009** have also been reported to have antinephritic activity.[4,5] Although the mechanism of action for linomide is still not completely understood, the results from several studies have suggested that linomide may exert its effect via the modulation of antigen presenting cell (APC) function.[6,7]

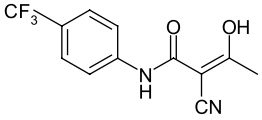
Leflunomide, an isoxazol derivative structurally unrelated to other known antirheumatic drugs, is a prodrug rapidly converted in vivo to its active metabolite (**A-771726**), which is a potent noncytotoxic inhibitor of the dihydroorotate dehydrogenase (DHODH), a key enzyme in the de novo synthesis of uridine monophosphate (UMP). Activated lymphocytes depend on the pyrimidine de novo syntheses to fulfill their metabolic needs for clonal expansion and terminal differentiation into effector cells.[8,9] The SAR study of leflunomide indicated that the active pharmacophore responsible for the immunosuppressive effects of **A-771726** was a β-keto amide with the enolic hydroxy group fixed in a cis configuration to the amide moiety.[10] Interestingly, linomide and **FR137316** have the same enolic hydroxy group fixed in a cis configuration to the amide moiety. Thus we pre-

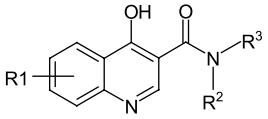

Linomide


FR137316


FR165009


Leflunomide


A-771726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Keywords: Immunomodulator; Quinoline-3-carboxamide; Linomide; SAR.
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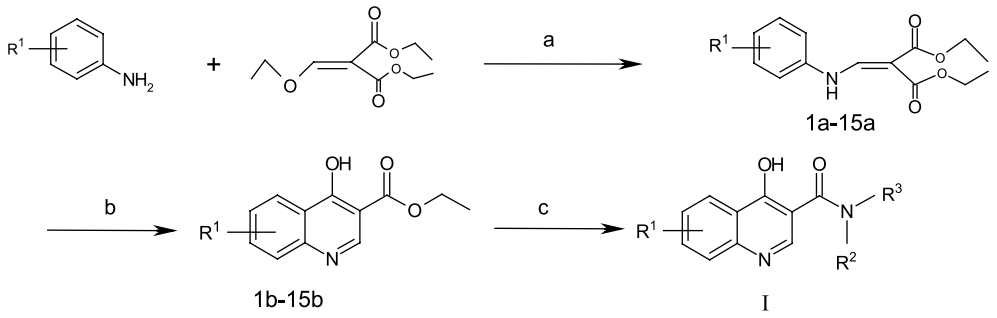


Scheme 1. Reagents and conditions: (a) toluene, 105 °C; (b) diphenyl oxide, 250 °C; (c) R^2R^3NH, diphenyl oxide, 210 °C.

sume that this structural element of the enolic hydroxy group is necessary for the immunomodulatory activity of these amide compounds. In addition, the immunomodulatory SAR studies of 4-hydroxyl-2-oxo-quinoline-3-carboxamide derivatives have been published,[11–13] and the action of 2-oxo group at the quinoline ring is not clear. In this letter, we report the synthesis and biological evaluation of novel 4-hydroxyl-quinoline-3-carboxamides(I) whose scaffolds are free of 2-oxo group.

2. Chemistry

Compounds were synthesized as outlined in Scheme 1.

The syntheses of the target compounds required access to a number of different substituted 4-hydroyl-3-quinolinecarboxylic ester derivatives. In detail, both the condensation of the substituted aniline with diethyl ethoxymethylenemalonate (EMME) in toluene at refluxing temperature and the subsequent thermal cyclization of anilinomethylenemalonate in diphenyl ether at about 250 °C gave good yields.[14,15] The target compounds could be prepared by condensation of the 4-hydroyl-3-quinolinecarboxylic ester with the appropriate amine in diphenyl ether at about 210 °C while the formed ethanol is distilled off. The products were purified by chromatography on silica gel column (dichloromethane–methanol, 20:1) or by recrystallization from ethanol.

3. Results and discussion

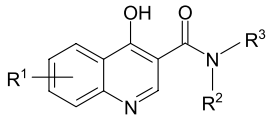
Linomide and **FR137316** were inhibitors of T-cell proliferation induced by ConA and TNF-α secreted by macrophage in vitro. Thus, the synthesized quinoline-3-carboxamide derivatives were tested for their effects on spleen lymphocyte proliferation and TNF-α production by macrophage in preliminary screens.[16] The test results rate % inhibition of spleen lymphocyte and TNF-α are summarized in Tables 1 and 2.

It can be seen from Tables 1 and 2 that the immunomodulatory activity was either preserved or enhanced with the introduction of substituents at the 7-position, compared to linomide and **FR137316**; this means that the substituents position is related with the immunomodulatory activity. Although it is not valid at the 6- and 8-position, the activity could be preserved when two methyl groups were simultaneously introduced at the 6- and 8-position. For example, the immunomodulatory activity of compounds **6**, **7**, **12**, and **19** was higher than that of compounds **5**, **9**, and **11**. Tables 1 and 2 show that the activities of compounds **6** and **7** substituted with electron-donating substituents (MeS, MeO) were remarkably higher than those of compounds **8** and **10** substituted with electron-withdrawing substituents (CF_3, F), indicating that the class of substituents is an important factor to immunomodulatory activity. It is surprising to find that the introduction of carboxy group at 6-position of quinoline ring strongly enhanced the inhibition of spleen lymphocyte proliferation reaction but there was no evident effect on TNF-α production by macrophage compared to linomide and **FR137316**, which supposes that compounds **15** and **16** might have different immunomodulatory activities from those of linomide and **FR137316**. We can also see in the table that the activities were maintained for most of the compounds with R^2 and R^3 substituted by methyl and aromatic groups, except for compound **18** in which R^2 and R^3 were hydrogen and 2-hydroxyethyl group, having a high immunomodulatory activity; it means the substituents at the N atom of amide (R^2, R^3) also influence the immunomodulatory activity.

4. Conclusion

In this paper, we have described the synthesis and immunomodulatory activity of a series of novel quinoline-3-carboxamide derivatives. The SAR of those compounds demonstrated that the 2-oxo group of the quinoline ring in linomide and **FR137316** might be unimportant for their immunomodulatory activities; the type and position of quinoline ring substitution played an important role in sustaining the activity, and furthermore the *N*-carboxamide substitution has an evident effect. Mostly, introduction of electron-donating substituents at the 7-position of quinoline ring is advantageous to sustain the activity. The introduction of carboxy group into quinoline ring may change the immunomodulatory type. Compounds **6**, **18**, and **19**[17] showed immunomodulatory profiles similar to and more potent than those of linomide and **FR137316** and were selected for further pharmacological and

Table 1. 4-Hydroxyl-quinoline-3-carboxamide and its effect on spleen lymphocyte proliferation



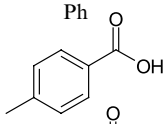
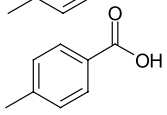
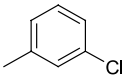
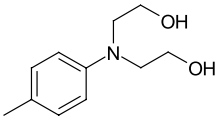
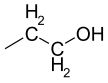
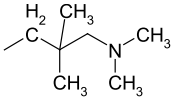
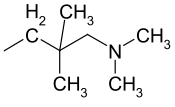
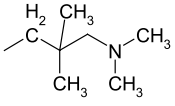
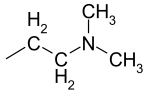
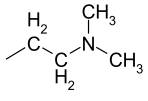
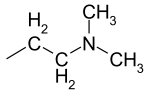
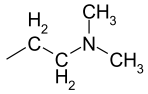
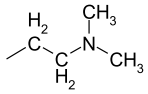
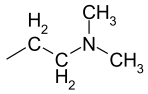
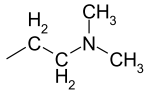
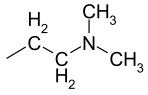
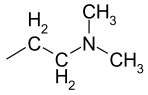
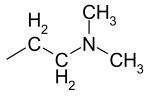
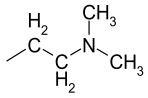
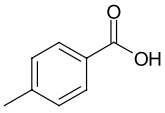
Compound	R^1	R^2	R^3	%Inhibition of T-cell proliferation induced by ConA			%Inhibition of B-cell proliferation reaction induced by LPS		
				Dose (μg/mL)			Dose (μg/mL)		
				1	10	100	1	10	100
Linomide				58.1[a]	70.6[a]	78.5[a]	0.00	24.6	62.7[a]
FR137316				52.0[a]	75.3[b]	79.6[b]	13.6	30.3	37.8[a]
3	7-Cl	CH_3	Ph	−24.7[a]	−27.6[a]	41.2[b]	−4.33	−26.7	14.1
4	H	CH_3	Ph	−7.55	24.9	−9.33	17.7	8.46	32.4
5	8-MeS	CH_3	Ph	11.4	52.4[a]	81.7[b]	−2.46	36.5[a]	71.7[a]
6	7-MeS	CH_3	Ph	26.2[b]	11.8	76.0[b]	31.1	17.0	83.5
7	7-MeO	CH_3	Ph	36.5[a]	29.3	53.7[b]	41.3[a]	24.9	57.5[b]
8	7-CF_3	CH_3	Ph	54.7[b]	4.11	93.6[b]	39.8[a]	6.41	64.9[b]
9	6-MeS	CH_3	Ph	−209[b]	−155[a]	96.3[b]	−47.8	−114[a]	65.6[b]
10	7-F	CH_3	Ph	−46.8[a]	−21.5	17.1	4.00	12.5	−126.7[a]
11	6-CH_3	CH_3	Ph	−59.3	−20.0	−1.70			
12	6-CH_3, 8-CH_3	CH_3	Ph	1.84	12.9	47.1[a]			
13	H	H		28.6[b]	38.8[b]	−20.9	−1.94	4.33	28.0
14	6-Cl	H		12.7	61.5[b]	98.9[b]	17.8	52.9[b]	87.0[a]
15	6-CO_2H	H	Ph	71.3[b]	57.5[a]	39.8	82.5[b]	80.6[b]	48.5[a]
16	6-CO_2H	H		87.6[b]	84.8[b]	73.4[b]	78.5[b]	94.3[b]	94.7[b]
17	H	H		45.8[a]	61.0[b]	99.1[b]	38.0	75.8[b]	88.5[b]
18	7-Cl	H		42.5[a]	49.8[a]	93.8[b]	21.3	29.1	53.3[a]
19	6-CH_3, 8-CH_3	Et	Ph	31.9[b]	35.5[b]	64.2[b]	4.49	36.0	87.5[b]
20	7-Cl	H	Ph	16.7[a]	32.6[a]	22.3	−2.65	12.3	27.1
21	7-Cl	n-C_3H_7	n-C_3H_7	42.1[a]	49.5[a]	51.2[b]	−3.57	18.1	12.6
22	6-F	H		−30.7	32.9	87.4[b]	21.3	17.8	70.5[b]
23	7-MeO	H		32.6	29.4	98.5[b]	48.8[b]	72.3[b]	91.6[b]
24	7-F	H		20.8	37.2	96.3[a]			
25	6-MeS	H		−181.6	−42.6	98.2[b]	−4.33	−9.69	87.7[b]
26	8-MeS	H		32.0[b]	69.1[b]	99.9[b]			

Table 1 (*continued*)

Compound	R^1	R^2	R^3	%Inhibition of T-cell proliferation induced by ConA			%Inhibition of B-cell proliferation reaction induced by LPS		
				Dose (μg/mL)			Dose (μg/mL)		
				1	10	100	1	10	100
27	7-MeS	H		30.3[a]	38.5[b]	100[b]			
28	6-NO_2	H		41.5[a]	53.2[b]	99.9[b]			
29	7-Cl	H		14.0	33.7[b]	99.9[b]			
30	H	H		−14.4[a]	−6.94[a]	73.6[b]			
31	6-CH_3	H		−27.5	−11.2	94.9[b]			
32	6-Br	H		−19.6[b]	31.6[b]	99.7[b]			
33	6-CH_3, 8-CH_3	H		0.95	38.5[b]	99.6[b]			
34	7-MeO	H		−40.7	−15.3	92.8[b]			
35	7-F	H		−24.6	−9.00	87.2[b]			

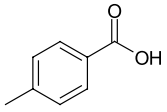
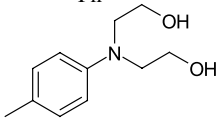
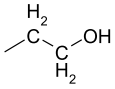
[a] $P < 0.05$ versus control (Student's *t*-test).
[b] $P < 0.01$.

Table 2. 4-Hydroxyl-quinoline-3-carboxamide and its effect on TNF-α production by macrophage

Compound	R^1	R^2	R^3	%Inhibition of TNF-α secreted by macrophage		
				Dose (μg/mL)		
				1	10	100
Linomide				9.95	18.2	27.3
FR137316				14.7	11.2	29.7
3	7-Cl	CH_3	Ph	18.6	5.61	25.6
4	H	CH_3	Ph	7.59	−17.4	−1.33
5	8-MeS	CH_3	Ph	2.08	−22.5	−9.52
6	7-MeS	CH_3	Ph	14.6	22.2	52.7
7	7-MeO	CH_3	Ph	12.1	35.7	35.7
8	7-CF_3	CH_3	Ph	−10.9	−5.45	−3.57
13	H	H		31.2	5.61	27.5

(*continued on next page*)

Table 2 (*continued*)

Compound	R^1	R^2	R^3	%Inhibition of TNF-α secreted by macrophage		
				Dose (μg/mL)		
				1	10	100
14	6-Cl	H		0.78	32.1	−5.35
15	6-CO_2H	H	Ph	15.2	15.5	−27.1
17	H	H		1.82	12.1	30.4
18	7-Cl	H		−10.9	6.06	77.7
19	6-CH_3, 8-CH_3	Et	Ph	46.3	29.7	47.6
20	7-Cl	H	Ph	10.5	10.4	23.1
21	7-Cl	*n*-C_3H_7	*n*-C_3H_7	14.7	18.4	−4.03

druglike/pharmacokinetic studies in vivo, and the results will be presented elsewhere.

Acknowledgments

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References and notes

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16. (i) **Spleen lymphocyte preparation an proliferation**. Spleen cells were passed through a 200 gauge nylon sieve to get a single cell suspension. Red blood cells were lysed with 0.16 M NH_4Cl–Tris buffer. Then cells were washed twice and resuspended in RPMI-1640 medium containing 10% fetal bovine serum. The cells were seeded in a 96-well microtiter plate (Nunc) at 2.5×10^6/mL and were stimulated with ConA at 0.5 μg/mL or with LPS at 10 μg/mL or left nonstimulated. The final concentration of the drug was 1, 10, and 100 μg/mL, respectively. The final volume per well was 200 μL and all treatment regimens were run in triplicates. The cells were cultured at 37 °C for 72 h and during the final 16 h of culture 0.5 μCi of [^{3}H]thymidine was added to each well. The cells were harvested onto glass–fiber filters, processed and counted in a β-counter (Perkin–Elmer, USA). The results are expressed as the mean ± SD of counts per minute (cpm). The activity was expressed as a %inhibition of T cell or B cell proliferation. The %inhibition = (the mean of control well − the mean of administered drug well)/the mean of control well. (ii) **TNF-α production by macrophage cultures**. RAW264.7 cells were grown in RPMI 1640 medium, supplemented with 50 μg/mL gentamycin, 2 mM glutamine, and 10% fetal calf serum (FCS; Hyclone). Cells (2×10^5/mL) were seeded into each well in 96-well plates (Nunc, Roskilde, Denmark) for collection of supernatants, and incubated in 37 °C in 5% CO_2 and 95% humidity. The experiments were started when the cells were growing confluently (after

approx. 3 days).To stimulate the macrophages, lipopolysaccharide (LPS; Sigma–Aldrich) was added at 10 μg/mL. The final concentration of drug was 1, 10, and 100 μg/mL, respectively. After 24 h incubation, supernatants were collected to measure the TNF-α level. The level of TNF-α in the supernatants was measured with commercial ELISA kits according to the instructions of the producer and the results were expressed as mean ± SD pg/mL. The activity was expressed as a %inhibition of TNF-α production. The %inhibition = (the mean of control well – the mean of administered drug well)/the mean of control well.

17. All new compounds reported herein showed satisfactory spectral data (^{1}H NMR, MS). Compound **6**: mp 238–240 °C. MS *m*/*z* 325(M+1, ESI). Anal. Calcd for $C_{18}H_{16}N_2O_2S_2$: C, 66.65; H, 4.97; N, 8.64. Found: C 66.26; H, 5.03; N, 8.70. ^{1}H NMR (DMSO-d_6, δ): 11.71 (s, 1H), 7.93 (s, 1H), 7.85 (d, 1H, J = 8.7 Hz), 7.20–7.30 (m, 5H), 7.07–7.14 (m, 2H), 3.30 (s, 3H), 2.50 (s, 3H). Compound **18**: mp 200 °C (dec). MS *m*/*z* 267 (M+1, ESI). Anal. Calcd for $C_{12}H_{11}ClN_2O_3$: C, 54.05; H, 4.16; N, 10.50. Found: C, 53.67; H, 4.20; N, 10.37. ^{1}H NMR (DMSO-d_6, δ): 10.37 (br, 1H), 8.77 (s, 1H), 8.20 (d, 1H, J = 8.7 Hz), 7.64 (s, 1H), 7.35 (d, 1H, J = 8.7 Hz), 3.50 (m, 2H), 3.37 (q, 2H, J = 5.7Hz), 2.78 (t, 1H, J = 5.6 Hz). Compound **19**: mp 216–218 °C. MS *m*/*z* 321 (M+1, ESI). Anal. Calcd for $C_{20}H_{20}N_2O_2$: C, 74.98; H, 6.29; N, 8.74. Found: C, 74.62, H, 6.28; N, 8.62. ^{1}HNMR (DMSO-d_6, δ): 11.07 (s, 1H), 7.79 (d, 1H, J = 6.2 Hz), 7.62 (s, 1H), 7.18–7.29 (m, 5H), 7.08–7.11 (m, 1H), 3.79 (q, 2H, J = 7.0 Hz), 2.38 (s, 3H), 2.31 (s, 3H), 1.07 (t, 3H, J = 7.0 Hz).