Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 85–88

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Antinephritic Activities of Quinoline-3-carboxamides and Related Compounds

Kiyoshi Tsuji,[a,*] Glen W. Spears,[a] Katsuya Nakamura,[a] Takashi Tojo,[a] Nobuo Seki,[c] Aiko Sugiyama[b] and Masaaki Matsuo[b]

[a]*Medicinal Chemistry Research Laboratories, Fujisawa Pharmaceutical Co., Ltd, 1-6, Kashima 2-chome, Yodogawa-ku, Osaka 532-8514, Japan*

[b]*Research Information Management, Fujisawa Pharmaceutical Co., Ltd, 1-6, Kashima 2-chome, Yodogawa-ku, Osaka 532-8514, Japan*

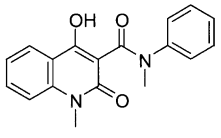
[c]*Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd, 2-3, Tokodai 5-chome, Tsukuba, Ibaraki 300-2698, Japan*

Received 22 August 2001; accepted 1 October 2001

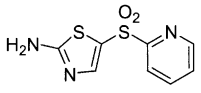
Abstract—A series of linomide-related quinoline-3-carboxamides and their analogues was prepared and evaluated for antinephritic activities. The 6-MeS derivative **7a** was highly effective in two nephritis models, namely chronic graft-versus-host disease and autoimmune MRL/l mice.

Linomide (roquinimex, **1**), a novel immunomodulator, has been shown to be effective against various types of cancers[1–3] and autoimmune diseases such as MRL/l mice,[4] (NZB×NZW)F_1 hybrid mice[5] and experimental autoimmune encephalomyelitis.[6,7] Recent clinical trials suggest that **1** has potential for the treatment of multiple sclerosis,[8,9] rheumatoid arthritis,[10] systemic lupus erythematosis,[10] cancer[11] and leukemia.[12,13] Interestingly, **1** increases lymphocyte proliferation, interleukin-2 production and natural killer cell activity,[2,14] and antagonizes the immunosuppressive effect of cyclosporin.[15] This indicates that **1** is classified as an immunostimulant, different from the typical immunosuppressants that have similar experimental and clinical activities shown above. Recently, **1** also has been shown to inhibit the process of angiogenesis critical for tumor metastases.[16]

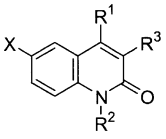
In our work to discover antinephritic agents safer than steroids or immunosuppressants, we recently reported on the 2-aminothiazole derivative FR115092(**2**) as a novel antinephritic agent.[17] Our continuing effort to find more potent antinephritic agents led us to test the effect of **1** against chronic graft-versus-host disease (GVHD), which is a model for human lupus nephritis,[18,19] and we found that **1** and **2** had almost equal potency. Consequently, although the precise mechanism for the efficacy of **1** in nephritis remained obscure, we started the chemical modification of **1**, represented by the general structure of **3**, to clarify the structure–activity relationships (SAR) and find more effective compounds. This approach led to the discovery of FR137316 (**7a**), which exhibited a nearly 100-fold increase in antinephritic activity.[20,21]



1



2



3

A series of 1,2-dihydro-4-hydroxy-2-oxoquinoline derivatives **7a–j** (Tables 1–3, Scheme 3) was prepared as shown in Scheme 1. Various thio and phenoxy substituents (X) were introduced into the 5-position of 2-nitrobenzoic acid. Then compounds **5** were reduced, treated with either ethyl chloroformate[22] or phosgene,[23] and alkylated with halides to afford the isatoic anhydrides **6**. Reaction of **6** with the appropriate acetates gave compounds **7a–j**.

The sulfide **7a** was oxidized with *m*-CPBA to give the sulfoxide **8a** and the sulfone **8b** as depicted in Scheme 2.

*Corresponding author. Tel.: +81-6-6390-1286; fax: +81-6-6304-5435; e-mail: kiyoshi_tsuji@po.fujisawa.co.jp

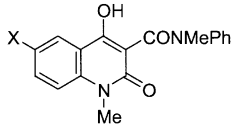
0960-894X/02/$ - see front matter
PII: S0960-894X(01)00671-0

Treatment of **7a** with $POCl_3$ gave the 4-chloro derivative **9**, which was allowed to react with various nucleophiles to form compounds **10a–d** (Table 2).

The 1-unsubstituted derivative **12a** (Table 2) and the ketones **12b,c** (Table 3) were synthesized by anionic cyclization of **11**, which were derived from ring opening reaction of **6′** (R^2 = H,Me) and subsequent condensation with the acetic acid derivatives (Scheme 3). The amides **13a–c** (Table 3) were prepared by direct amidation of the ester **7j** with various amines, while less nucleophilic amines were condensed with the acid **14** using PCl_3[24] to give the amides **15a–f**.

Severe immune complex glomerulonephritis is a major symptom of chronic GVHD in mice. The animals have elevated protein excretion in the urine, hypoalbuminemia, and frequently, ascites and edema. Since all of the histological patterns occurring in human lupus nephritis can be seen in these animals, murine GVHD can be used as an experimental model for human lupus nephritis.[19] The disease can be induced experimentally and develops relatively rapidly.[18] We, therefore, chose this GVHD model in the search for novel antinephritic agents.[25] The test results, % inhibition of proteinuria in the GVHD model, are summarized in Tables 1–3.

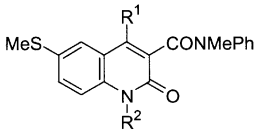
Table 1. Investigation of substituents on 6-position



Compd	X	% Inhibition of proteinuria[a]
1[b]	H	86**
7a	MeS	100**
7b	EtS	100**
7c	PhS	100*
7d	PhO	26
8a	MeS(O)	100**
8b	$MeSO_2$	0

[a]Chronic GVHD, 32 mg/kg po; $^{**}p<0.01$, $^{*}p<0.05$ versus control (Student's *t*-test). See ref 25 for experimental detail.
[b]Synthesized according to ref 24.

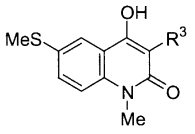
Table 2. Investigation of substituents on 1- and 4-positions



Compd	R^1	R^2	% Inhibition of proteinuria[a]
9	Cl	Me	−1
10a	NH_2	Me	22
10b	NHMe	Me	83
10c	OMe	Me	94
10d	SH	Me	19
12a	OH	H	88
7e	OH	Et	83
7f	OH	CH_2OMe	78
7g	OH	CH_2Ph	−66

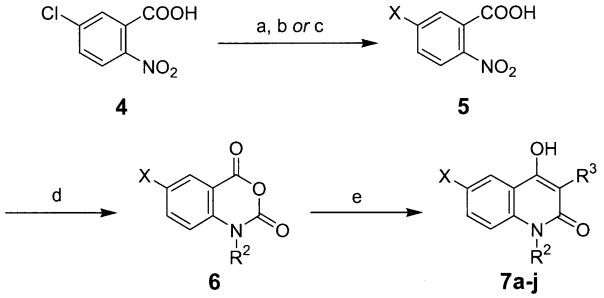
[a]Chronic GVHD, 32 mg/kg po; $^{**}p<0.01$, $^{*}p<0.05$ versus control (Student's *t*-test).

Table 3. Investigation of amide isosters and amide derivatives on 3-position

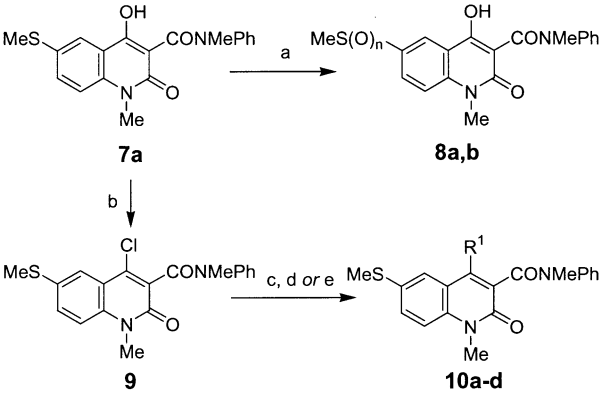


Compd	R^3	% Inhibition of proteinuria[a]
7h	SO_2CH_2Ph	−49[b]
7i	SO_2NMePh	53
12b	COPh	42[b]
12c	CO(1-phenylcyclopropyl)	29[b]
13a	CO(1-indolinyl)	48[b]
13b	CONHPh	24
13c	CONMe(cyclohexyl)	43
15a	CONEtPh	89*
15b	CONMe(2-pyridyl)	65*
15c	CONMePh(4-F)	100**
15d	CONMePh(4-OMe)	100**
15e	CONMePh(4-Me)	100**
15f	CONMePh(3-CF_3)	99*

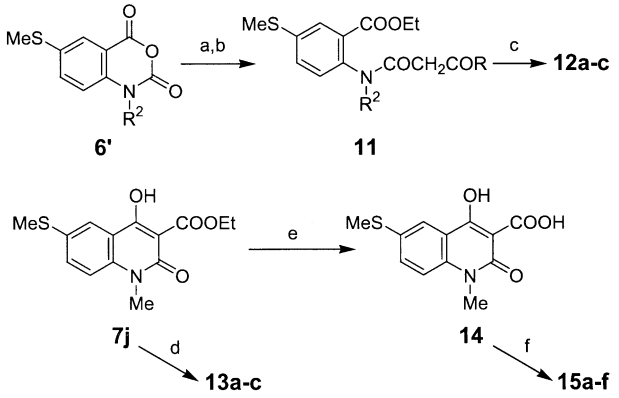
[a]Chronic GVHD, 32 mg/kg po unless otherwise noted; $^{**}p<0.01$, $^{*}p<0.05$ versus control (Student's *t*-test).
[b]100 mg/kg po.



Scheme 1. Synthesis of compounds **7a–j**. Reagents and conditions: (a) (1) Na_2S, NaOH, H_2O, 60 °C; (2) Me_2SO_4 or Et_2SO_4, reflux; (b) PhSH, K_2CO_3, DMF, 100 °C; (c) PhOH, KOH, DMA, reflux; (d) (1) NaOH, H_2O, NH_2NH_2, $FeCl_3$, reflux; (2) ClCOOEt, xylene, reflux; or $COCl_2$, rt; (3) NaH, R^2-halide, DMF, rt; (e) $EtOCOCH_2$-R^3, NaH, DMA, 120 °C.



Scheme 2. Synthesis of compounds **8a,b**, **9** and **10a–d**. Reagents and conditions: (a) *m*-CPBA, CH_2Cl_2, 5 °C or rt; (b) $POCl_3$, 80 °C; (c) H_2NR, MeOH, 100 °C; (d) NaOMe, MeOH, 40 °C; (e) (1) KSCOMe, NaI, Me_2CO, reflux; (2) aq $NaHCO_3$, rt.



Scheme 3. Synthesis of compounds **12a–c**, **13a–c** and **15a–f**. Reagents and conditions: (a) NaOEt, EtOH, reflux; (b) $HOCOCH_2CO$-R, WSCD, DMAP, CH_2Cl_2, rt; (c) NaOEt, EtOH, rt; (d) HNRR′, pyridine, reflux; (e) HBr, AcOH, 70 °C; (f) HNRR′, PCl_3, toluene, 100 °C.

Table 4. Effects of **1**, **7a** and steroid on chronic GVHD

Compd	Dose (mg/kg po)	% Inhibition of proteinuria[a]	% Inhibition of anti-DNA antibody[a]
1	10	9	−19
	32	86**	36
	100	98**	37
7a	0.1	66	41
	1	82*	50
	10	100**	88**
	100	100**	94**
Prednisolone	0.1	53	39
	1	75*	39*
	10[b]	100**	52*

[a]**$p<0.01$, *$p<0.05$ versus control (Student's *t*-test).
[b]Toxic (losing weight, etc.).

Introduction of a sulfur atom generated desirable antinephritic agents in the series of **2**-type compounds.[17] We, therefore, investigated various sulfur-containing substituents on the 6-position of the quinoline ring (Table 1). The sulfide and sulfoxide derivatives (**7a–c** and **8a**) completely suppressed the proteinuria. On the other hand, the oxy and sulfone analogues (**7d** and **8b**) were inactive. Through the dose-response and metabolic studies,[26] **7a** was selected as a lead compound for further modification.

Replacement of the hydroxy group on the 4-position of the quinoline ring of **7a** with chloro, amino, methoxy or mercapto resulted in decreased activity (Table 2; **9** and **10a–d**). The 1-methyl substitution appears to be important for activity as well. The unsubstitution (**12a**) and other substitutions (**7e–g**) both resulted in significant losses of potencies (Table 2).

In the modification of the 3-position (Table 3), we first aimed at finding chemically more stable surrogates of the *N*-methyl-*N*-phenylcarboxamide group. However, the sulfone (**7h**), sulfonamide (**7i**), ketone (**12b**,**c**) and stable carboxamide (**13a–c**) derivatives were all inactive. Even minor modifications such as *N*-ethyl or *N*-pyridyl analogues (**15a**,**b**) resulted in decreased activity. Whereas some substitutions on the phenyl ring afforded a series of highly potent compounds (**15c–f**).

Table 5. Effects of **1** and **7a** on the autoimmune disease MRL/l mice

Compd	Dose (mg/kg po)	% Inhibition of proteinuria[a]	Histology (nephritis)[a]	% Inhibition of anti-DNA antibody[a]
1	1	Inactive	Inactive	24
	3.2	Inactive	Inactive	35
	10	60	*	51*
	32	100	**	52*
	100	100	**	76**
7a	0.1	100	**	49**
	0.32	100	**	69**
	1	100	**	76**
	3.2	100	**	88**
	10	100	**	93**

[a]**$p<0.01$, *$p<0.05$ significant improvement versus control (Student's *t*-test).

One of the most active compounds, **7a**, inhibited proteinuria as effectively as prednisolone and much more effectively than **1** in the GVHD mice (Table 4). Both **7a** and prednisolone also suppressed anti-DNA antibody in a similar manner, suggesting **7a** to be an immunomodulating agent. While prednisolone showed toxic symptoms at 10 mg/kg, **7a** was well tolerated up to 100 mg/kg.

The antinephritic activity of **7a** was further evaluated against the spontaneous autoimmune disease MRL/l mice.[27] Compound **7a** strongly suppressed both the proteinuria and the development of glomerulonephritis in the kidneys[28] (Table 5). In addition, the production of autoantibodies, which has been shown to play a crucial role in the development of lupus nephritis,[29] was also reduced. The activities of **7a** were more than 100 times stronger than those of **1**.

In conclusion, it has been demonstrated that the quinoline-3-carboxamide derivatives are potent and safe immunomodulating agents, which have potential for treating various kind of nephritis. The activities of the 6-MeS derivative **7a** are nearly 100 times more potent than **1** and comparable with prednisolone, a representative steroid for the nephritis treatment. Further SAR studies and pharmacological investigations on the related compounds will be reported subsequently.

Acknowledgements

The authors are grateful to Drs. Kazuo Sakane, Hachiro Senoh and Takashi Fujii for their continuing guidance and encouragement.

References and Notes

1. Kalland, T. *Cancer Res.* **1986**, *46*, 3018.
2. Robinson, C.; Castaner, J. *Drugs Future* **1995**, *20*, 19.
3. Ichikawa, T.; Lamb, J. C.; Christensson, P. I.; Hartley-Asp, B.; Isaacs, J. T. *Cancer Res.* **1992**, *52*, 3022.
4. Tarkowski, A.; Gunnarsson, K.; Nilson, L.-A.; Lindholm, L.; Stalhandske, T. *Arthr. Rheum.* **1986**, *29*, 1405.

5. Tarkowski, A.; Gunnarsson, K.; Stalhandske, T. *Immunology* **1986**, *59*, 589.
6. Karussis, D. M.; Lehmann, D.; Slavin, S.; Vourka-Karussis, U.; Mizrachi-Koll, R.; Ovadia, H.; Kalland, T.; Abramsky, O. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6400.
7. Karussis, D. M.; Lehmann, D.; Slavin, S.; Vourka-Karussis, U.; Mizrachi-Koll, R.; Ovadia, H.; Ben-Num, D.; Kalland, T.; Abramsky, O. *Ann. Neurol.* **1993**, *34*, 654.
8. Anderson, O.; Lycke, J.; Tollesson, P. O.; Svenningsson, A.; Runmarker, B.; Linde, A. S.; Astrom, M.; Gjorstrup, P.; Ekholm, S. *Neurology* **1996**, *47*, 895.
9. Karussis, D. M.; Meiner, Z.; Lehmann, D.; Gomori, J.-M.; Schwaz, A.; Linde, A.; Abramsky, O. *Neurology* **1996**, *47*, 341.
10. Nived, O.; Sturfelt, G.; Nilsson, B.; Termander, B.; Gunnarsson, P. O.; Strandgarden, K.; Wollheim, F. *Int. J. Immunother.* **1994**, *10*, 49.
11. Bergh, J.; Totterman, T.; Nilsson, S.; Ahlbom, H.; Larsson, S.-G.; Jansson, T.; Letocha, H.; Termander, B.; Svedberg; Strandgarden, K.; Gunnarsson, P. O.; Nilsson, B. *Eur. J. Cancer* **1991**, *27* (Suppl. 2), 195.
12. Bengtsson, M.; Simonsson, B.; Carlsson, K.; Nilsson, B.; Smedmyr, B.; Termander, B.; Oberg, G.; Totterman, T. *Transplantation* **1992**, *53*, 882.
13. (a) Rowe, J.; Nilsson, B.; Simonsson, B. *Leuk. Lymph.* **1993**, *11*, 321. (b) Rowe, J.; Ryan, D.; Dipersio, J.; Gaspari, A.; Nilsson, B.; Larsson, L.; Liesveld, J.; Kouides, P.; Simonsson, B. *Stem Cells* **1993**, *11* (Suppl. 3), 34.
14. Kalland, T.; Alm, G.; Stalhandske, T. *J. Immunol.* **1985**, *134*, 3956.
15. Wanders, A.; Vogt, P.; Karlsson-Parra, A.; Wonigkeit, K.; Gerdin, B.; Tufveson, G. *Transplantation* **1991**, *52*, 234.
16. Khan, S. R.; Mhaka, A.; Pili, R.; Isaacs, J. T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 451.
17. Ogino, T.; Tsuji, K.; Tojo, T.; Igari, N.; Seki, N.; Sudo, Y.; Manda, T.; Nishigaki, F.; Matsuo, M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 75.
18. Bruijn, J. A.; van Elven, E. H.; Hogendoorn, P. C. W.; Corver, W. E.; Hoedemaeker, P. J.; Fleuren, G. J. *Am. J. Pathol.* **1988**, *130*, 639.
19. Bruijn, J. A.; Hogendoorn, P. C. W.; Corver, W. E.; van den Broek, L. J. C. M.; Hoedemaeker, P. J.; Fleuren, G. J. *Clin. Exp. Immunol.* **1990**, *79*, 115.
20. All new compounds reported herein showed satisfactory spectral data (^{1}H NMR, IR and MS). The purity of all target compounds was further confirmed by combustion analysis (C, H, N within 0.4%). **7a**: mp 200–202 °C (dec); IR (Nujol) 1650, 1635, 1615, 1590, 1565, 1500 cm^{-1}; ^{1}H NMR(DMSO-d_6) δ 2.49 (3H,s), 3.29 (3H,s), 3.42 (3H,s), 7.0–7.8 (8H,m), 11.3 (1H,s); MS m/z 354 (M^+), 247. Anal. calcd for $C_{19}H_{18}N_2O_3S$: C, 64.39; H, 5.12; N, 7.91. Found: C, 64.54; H, 5.07; N, 7.69. Complete physicochemical data and experimental details are disclosed in the following patent: Matsuo, M.; Tsuji, K.; Nakamura, K.; Spears, G. W. WO92-18483, 1992; *Chem. Abstr.* **1993**, *118*, 212903b.
21. Abbreviations : DMA, *N*,*N*-dimethylacetamide; DMF, *N*,*N*-dimethylformamide; DMAP, 4-(dimethylamino)pyridine; m-CPBA, m-chloroperbenzoic acid; WSCD, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide.
22. Heilbron, I. M.; Kitchen, F. N.; Parkes, E. B.; Sutton, G. O. *J. Chem. Soc.* **1925**, 2171.
23. Hardtmann, G. E.; Koletar, G.; Pfister, O. R. *J. Heterocycl. Chem.* **1975**, *12*, 565.
24. Eriksoo, E.; Sandberg, E. B. M.; Stalhandske, T. EP59698, 1982; *Chem. Abstr.* **1983**, *98*, 34513h.
25. Six-week-old female (C57BL/6×DBA/2)F_1 and DBA/2 mice were used. Chronic GVHD was induced in (C57BL/6×DBA/2)F_1 mice with two injections of DBA/2 spleen cells given 5 days apart. Each injection contained 5×10^7 cells. From 3 days after the second cell injection, drug was administered orally once a day for 8 weeks. To assess the renal disease, proteinuria were measured after the last drug administration. The concentration of serum albumin in the urine was determined by the single radial immunodiffusion method using rabbit anti-mouse serum albumin antiserum. Ten mice were used per group. The activity was expressed as a % inhibition of proteinuria.

As an indication of autoimmune disease, 4 weeks after the last cell injection anti-DNA antibodies were measured by ELISA.[27]
26. The sulfoxide **8a** was easily metabolized to the corresponding sulfide **7a** in mice, while **7a** was unexpectedly stable in vivo (mice, rats and dogs).
27. Fujitsu, T.; Sakuma, S.; Seki, N.; Senoh, H.; Mori, J.; Kikuchi, H. *Int. J. Immunopharmacol* **1986**, *8*, 897.
28. Evaluated by histopathological examination in a blinded fashion.
29. Okumura, M.; Kanayama, Y.; Amatsu, K.; Negoro, N.; Kohda, S.; Takeda, T.; Inoue, T. *Ann. Rheum. Dis.* **1993**, *52*, 14.