Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2427–2430

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Quinoline-3-carbothioamides and Related Compounds as Novel Immunomodulating Agents

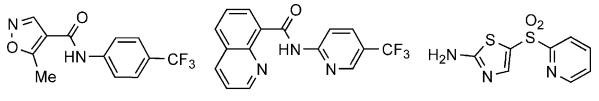
Takashi Tojo,[a] Glen W. Spears,[a] Kiyoshi Tsuji,[a,*] Hiroaki Nishimura,[a] Takashi Ogino,[a] Nobuo Seki,[c] Aiko Sugiyama[b] and Masaaki Matsuo[b]

[a]*Medicinal Chemistry Research Laboratories, Fujisawa Pharmaceutical Co., Ltd, 2-1-6, Kashima, Yodogawa-ku, Osaka 532-8514, Japan*
[b]*Research Information Management, Fujisawa Pharmaceutical Co., Ltd, 2-1-6, Kashima, Yodogawa-ku, Osaka 532-8514, Japan*
[c]*Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd, 5-2-3, Tokodai, Tsukuba, Ibaraki 300-2698, Japan*

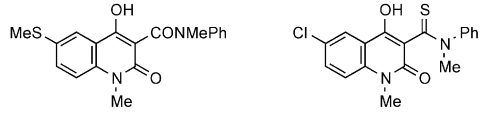
Received 17 April 2002; accepted 16 May 2002

Abstract—A series of quinoline-3-carbothioamides and their analogues was prepared via four synthetic routes and evaluated for their antinephritic and immunomodulating activities. The optimal compound **9g** strongly inhibited the T-cell independent antibody production in mice immunized with TNP-LPS and was highly effective in two nephritis models, namely chronic graft-versus-host disease and autoimmune MRL/l mice.

Typical T-cell immunosuppressants such as cyclosporin A and FK506 cannot prevent the antibody-mediated rejection process of xenotransplantation. Leflunomide and the quinoline-8-carboxamide derivative **1** have been shown to inhibit antibodies produced by B-cells elicited by T-cell independent antigens like the trinitrophenyl-lipopolysaccharide (TNP-LPS), and this class of B-cell immunosuppressants is emphasized to have the substantial therapeutic potential in antibody-mediated diseases including xenograft rejection and autoimmune diseases.[1]



Leflunomide **1** FR115092



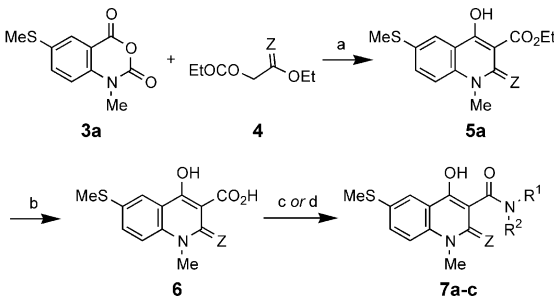
2 **9g**

We have reported on the 2-aminothiazole derivative FR115092[2] and the quinoline-3-carboxamide FR137316 (**2**)[3] as novel antinephritic agents, which are effective in chronic graft-versus-host disease (GVHD) and autoimmune W/BF_1 mice and MRL/l mice. These compounds suppress not only proteinuria and histological changes but also the anti-DNA antibody production in these models. Recent finding that **2** is also an effective inhibitor of anti-TNP antibody production in TNP-LPS immunized mice prompted us to further optimize the structure of **2**. Reported here are the chemical modification of **2** and the identification of the quinoline-3-carbothioamide derivative FR165009 (**9g**) as the optimal compound.[4,5]

*Corresponding author. Tel.: +81-6-6390-1142; fax: +81-6-6304-5435; e-mail: kiyoshi_tsuji@po.fujisawa.co.jp

Chemistry

The quinoline-3-carboxamide derivatives **7a–c** were synthesized as shown in Scheme 1. Isatoic anhydride **3a**[3] was treated with an appropriate acetate **4** (Z = O or S) in



3a **4** **5a**

6 **7a-c**

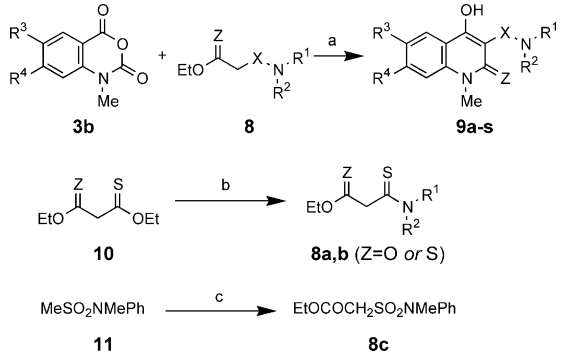
Scheme 1. Synthesis of compounds **7a–c**. Reagents and conditions: (a) NaH, DMA, 120 °C; (b) HBr, AcOH, 70 °C; (c) HNR^1R^2, PCl_3, toluene, 100 °C; (d) HNR^1R^2, DCC, toluene, 90 °C.

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00377-3

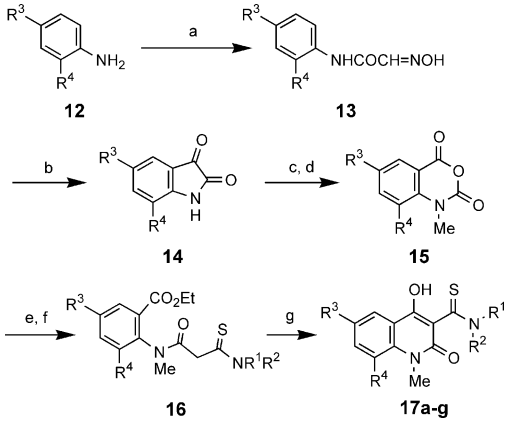
the presence of sodium hydride in *N,N*-dimethylacetamide to give quinoline-3-carboxylates **5a**. The carboxylates were hydrolyzed and condensed with various amines using PCl_3[6] or 1,3-dicyclohexylcarbodiimide[7] to afford **7a**–**c**.

A series of quinoline-3-carbothioamide and sulfonamide derivatives **9a**–**s** was prepared as depicted in Scheme 2. Reaction of isatoic anhydrides **3b** and an appropriate acetate or thioacetate **8** by using the same procedure for the preparation of **5a** gave **9a**–**s**.[8] Thioamides **8a**,**b** were obtained by selective amidation of **10**.[9] Sulfonamide **11** was treated with *n*-BuLi in THF at −30 °C, followed by quenching with $(EtO)_2CO$ to afford **8c**.

Preparation of some quinoline-3-carbothioamides **17a**–**g** is shown in Scheme 3. Heating of a mixture of **12**, chloral hydrate, sodium sulfate, concd HCl and hydroxylamine hydrochloride in water gave oximes **13**, which were treated with sulfuric acid, followed by methylation and oxidation by *m*-CPBA to give isatoic anhydrides **15**. Compounds **17a**–**g** were synthesized by anionic cyclization of **16**, which were derived from ring opening reaction of **15** and subsequent condensation with the (thiocarbamoyl)acetic acid derivatives.[9]



Scheme 2. Synthesis of compounds **9a**–**s**. Reagents and conditions: (a) NaH, DMA, 120 °C; (b) HNR^1R^2, 180 °C; (c) *n*-BuLi, $(EtO)_2CO$, THF, −30 °C to rt.



Scheme 3. Synthesis of compounds **17a**–**g**. Reagents and conditions: (a) H_2NOH, $Cl_3CCH(OH)_2$, Na_2SO_4, HCl, H_2O, reflux; (b) H_2SO_4, 80 °C; (c) MeI, NaH, DMA, rt; (d) *m*-CPBA, CH_2Cl_2, rt; (e) NaOEt, EtOH, reflux; (f) $HOCOCH_2C(S)NR^1R^2$, MS, pyridine, *tert*-BuCOCl, CH_2Cl_2, rt; (g) NaOEt, EtOH, rt.

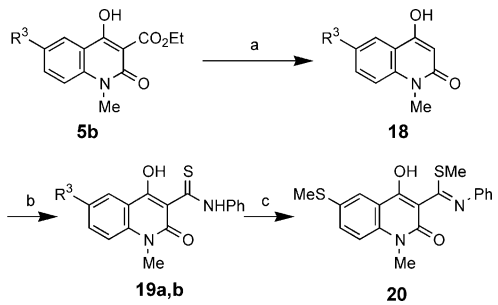
Mono-substituted thioamide **19a** and thioimidate **20** were prepared as shown in Scheme 4. Quinoline-3-carboxylates **5b** were hydrolyzed, decarboxylated, and treated with phenyl isothiocyanate to give **19a**,**b**. Careful methylation of **19b** (R^3 = MeS) afforded **20** in 87% yield.

Biological Results and Discussion

Nephritis is a disease that affects millions of people worldwide, but is poorly treated with current therapies. The chronic GVHD model is thought to be a good model for nephritis disease, because its etiology and symptoms resemble human lupus nephritis.[10] In addition, the production of autoantibodies can be an indication of the immunomodulating activity of the drug.[11] The effects of the compounds on the in vivo T-cell independent antibody production were also assessed after immunization of mice with TNP-LPS.[12] The test results,% inhibition of proteinuria in the GVHD model and% inhibition of anti-TNP antibody in the TNP-LPS model, are summarized in Tables 1–3.

Introduction of a sulfur-containing moiety such as MeS generated potent antinephritic agents in the series of **2**-related compounds.[3] It has been reported that replacement of the acetamide to thioacetamide or thiourea derivatives resulted in enhanced antibacterial activities.[13] There is also a thiocarbamate type of TNF-α inhibitor.[14] Accordingly, we investigated various sulfur-containing structures as a first step of the optimization of **2** (Table 1). Incorporation of a MeS moiety on the *N*-methyl-*N*-phenylcarboxamide group (**7a**,**b**) and the sulfonamide analogue (**9b**) resulted in significant losses of potency. On the other hand, the thioamide analogues (**7c** and **9a**) showed good activities. However, bis(thioamide) derivative **9c** lost the activity. Interestingly, some activity was maintained in the structurally different thioimidate **20**. The quinoline-3-carbothioamide derivative **9a** displayed the most potent inhibition and thus was studied further.

Substitutions on the quinoline ring of **9a** were optimized as shown in Table 2. Replacement of the MeS group with MeO resulted in a similar potency (**9e**). Halogen substitution afforded more potent compounds (**9g**,**h**) in the TNP-LPS model. The unsubstitution (**9d**) and other



Scheme 4. Synthesis of compounds **19a** and **20**. Reagents and conditions: (a) NaOH, H_2O, reflux; (b) PhNCS, Et_3N, DMSO, rt; (c) MeI, NaH, DMF, −20 °C.

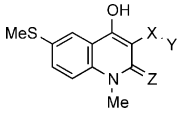
substitutions (**9f** and **17a**,**b**) both resulted in decreased activities. Substituents on the 7 or 8 position of the quinoline ring seemed to diminish the activity (**9i** and **17c**).

Maintaining the 6-Cl moiety of **9g**, we varied the *N*-substituents (R^1 and R^2) of the thioamide part (Table 3). The methoxy, trifluoromethoxy and halogen substituted phenyl provided a series of highly potent compounds (**9j**–**q**). However, the alkylphenyl and thienyl substitutions exhibited decreased activities (**17d**–**f**). As a surrogate for the methyl in R^2, both unsubstitution (**19a**) and allyl and 3-methoxypropyl substitutions (**9r**,**s**) resulted in fairly good activities, but the bulky *tert*-butyl derivative (**17g**) was less potent.

One of the most active compounds, **9g**, inhibited anti-TNP antibody dose-dependently from an oral dose of 100 mg/kg down to 0.1 mg/kg in the TNP-LPS model (Table 4). This potency was comparable with the lead compound **2**, and nearly 10-fold superior to linomide, which has been under clinical investigation for the treatment of various autoimmune diseases and cancer.[15] In addition, compound **9g** exhibited a favorable half-life (9.3 h) in dog, while **2** showed a half-life of 128 h. Moreover, **9g** was free of the mutagenicity that plagued both **2** and linomide.

The antinephritic and immunomodulating activities of **9g** were further evaluated against the spontaneous

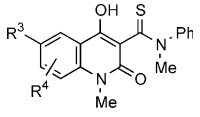
Table 1. Antinephritic activity of **2**-related compounds containing additional sulfur atom(s)



Compd	X	Y	Z	% Inhibition of proteinuria[a]
2	CO	NMePh	O	100**
7a	CO	NMe(4-MeS–Ph)	O	87*
7b	CO	NPh(CH_2CH_2SMe)	O	65
7c	CO	NMePh	S	96**
9a	CS	NMePh	O	99**
9b	SO_2	NMePh	O	53
9c	CS	NMePh	S	37
20	C=NPh	SMe	O	84**

[a]Chronic GVHD, 32 mg/kg po; $^{**}p<0.01$, $^{*}p<0.05$ versus control (Student's *t*-test). See ref 11 for experimental detail.

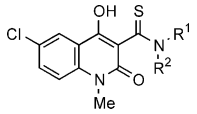
Table 2. Optimization of quinoline substitution of **9a**



Compd	R^3	R^4	% Inhibition of proteinuria[a]	% Inhibition of anti-TNP antibody[b]
9a	MeS	H	99**	32**
9d	H	H	85	
9e	MeO	H	99*	31*
9f	Me	H		20
9g	Cl	H	100**	64**
9h	Br	H	98*	56**
9i	H	7-Cl	−23	
17a	CF_3O	H		19
17b	1-Pyrrolyl	H		−10
17c	Cl	8-Me		17*

[a]Chronic GVHD, 32 mg/kg po; $^{**}p<0.01$, $^{*}p<0.05$ versus control (Student's *t*-test).
[b]TNP-LPS immunization in mice, 10 mg/kg po; $^{**}p<0.01$, $^{*}p<0.05$ versus control (Student's *t*-test). See ref 12 for experimental detail.

Table 3. Optimization of *N*-substitution of the thioamide part of **9g**



Compd	R^1	R^2	% Inhibition of proteinuria[a]	% Inhibition of anti-TNP antibody[b]
9j	4-MeO–Ph	Me	97**	45**
9k	3-MeO–Ph	Me	74**	30**
9l	4-CF_3O–Ph	Me		39**
9m	4-F–Ph	Me		54**
9n	3,4-diF–Ph	Me		62**
9o	4-Cl–Ph	Me		47**
9p	3-Cl–Ph	Me		63**
9q	4-Br–Ph	Me		50**
17d	4-Me–Ph	Me		23*
17e	3-CF_3–Ph	Me		29
17f	2-Thienyl	Me		19
19a	Ph	H		38*
9r	Ph	Allyl	96**	
9s	Ph	$MeO(CH_2)_3$		31**
17g	Ph	*tert*-Bu		11

[a]Chronic GVHD, 32 mg/kg po; $^{**}p<0.01$, $^{*}p<0.05$ versus control (Student's *t*-test).
[b]TNP-LPS immunization in mice, 10 mg/kg po; $^{**}p<0.01$, $^{*}p<0.05$ versus control (Student's *t*-test).

Table 4. Effects of **9g**, **2** and linomide on TNP-LPS immunized mice

Compd	Dose (mg/kg po)	% Inhibition of anti-TNP antibody[a]
9g	0.01	−3
	0.1	22*
	1	33**
	10	64**
	100	77**
2	0.1	18
	1	50**
	10	67**
Linomide	1	13
	10	41**
	100	49**

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

Table 5. Effects of **9g** on the autoimmune disease MRL/l mice

Dose (mg/kg po)	% Inhibition of histological score[a]	% Inhibition of anti-DNA antibody[b]
0.32	67**	52
1	85**	73**
3.2	85**	83**

[a]Nephritis in the kidneys $^{**}p<0.02$ versus control (Mann–Whitney test).
[b]$^{**}p<0.01$ versus control (Cochran–Cox test).

autoimmune disease MRL/l mice.[16] Compound **9g** strongly suppressed the development of glomerulonephritis in the kidneys[17] as shown in Table 5. In addition, the production of autoantibodies, that has been shown to play an important role in the development of lupus nephritis,[18] was also reduced.

In conclusion, it has been demonstrated that the quinoline-3-carbothioamide derivatives are potent immunomodulating agents and have potential for treating various kind of autoimmune diseases and nephritis. Our recent results have suggested that **9g** and related compounds inhibit autoimmune responses and potentiate normal immune responses by augmentation of NKT cells,[19] which play a crucial role in controlling the development of autoimmune diseases.[20]

Acknowledgements

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

References and Notes

1. Papageorgiou, C.; von Matt, A.; Joergensen, J.; Andersen, E.; Wagner, K.; Beerli, C.; Than, T.; Borer, X.; Florineth, A.; Rihs, G.; Schreier, M. H.; Weckbecker, G.; Heusser, C. *J. Med. Chem.* **2001**, *44*, 1986.
2. 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.
3. Tsuji, K.; Spears, G. W.; Nakamura, K.; Tojo, T.; Seki, N.; Sugiyama, A.; Matsuo, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 85.
4. 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%). **9g**: mp 219–223 °C (dec); IR (Nujol) 1630, 1595, 1575, 1495 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.42 (3H, s), 3.73 (3H, s), 7.1–7.7(7H, m), 7.81 (1H, d, $J=2$ Hz), 11.17 (1H, s); MS m/z 359 $(M+H)^+$. Anal. calcd for $C_{18}H_{15}ClN_2O_2S$: C,60.24; H,4.21; N,7.81. Found: C,60.64; H,4.07; N,7.74. Complete physicochemical data and experimental details are disclosed in refs 6–8.
5. Abbreviations: DCC, 1,3-dicyclohexylcarbodiimide; DMA, *N,N*-dimethylacetamide; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; *m*-CPBA, *m*-chloroperbenzoic acid; MS, molecular sieves 4A; THF, tetrahydrofuran; TNF, tumor necrosis factor.
6. Matsuo, M.; Tsuji, K.; Nakamura, K.; Spears, G.W. WO92–18483, 1992; *Chem. Abstr.* **1993**, *118*, 212903b.
7. Matsuo, M.; Tsuji, K.; Spears, G.W.; Tojo, T. Japanese Patent JP07224040, 1995; *Chem. Abstr.* **1996**, *124*, 29618x.
8. Matsuo, M.; Tsuji, K.; Spears, G.W.; Ogino, T.; Nishimura, H.; Tojo, T. WO95–24395, 1995; *Chem. Abstr.* **1996**, *124*, 117102e.
9. Spears, G. W.; Tsuji, K.; Tojo, T.; Nishimura, H.; Ogino, T. *Synth. Commun.* **2000**, *30*, 565.
10. 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.
11. 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.
12. Female (C57BL/6 × DBA/2) F_1 mice (6 weeks old) were immunized intravenously with TNP-LPS (10 μg/mice) on day 0. Mice were bled on day 4 after immunization, and anti-TNP IgM levels in each serum were determined by ELISA. To test the inhibitory activity of the compound, mice were randomly divided after immunization (7 mice/group) and were treated orally with the compound on day 0 and day 1. The activity of the compound was expressed as% inhibition of anti-TNP IgM level.
13. Tokuyama, R.; Takahashi, Y.; Tomita, Y.; Suzuki, T.; Yoshida, T.; Iwasaki, N.; Kado, N.; Okezaki, E.; Nagata, O. *Chem. Pharm. Bull.* **2001**, *49*, 347.
14. Fischer, R; Mueller, U; Handke, G; Petesch, N; Schmeck, C. German Patent DE10034628, 2002.
15. Robinson, C.; Castaner, J. *Drugs Future* **1995**, *20*, 19.
16. Fujitsu, T.; Sakuma, S.; Seki, N.; Senoh, H.; Mori, J.; Kikuchi, H. *Int. J. Immunopharmacol.* **1986**, *8*, 897.
17. Evaluated by histopathological examination in a blinded fashion.
18. Okumura, M.; Kanayama, Y.; Amatsu, K.; Negoro, N.; Kohda, S.; Takeda, T.; Inoue, T. *Ann. Rheum. Dis.* **1993**, *52*, 14.
19. Masunaga, T.; Sasagawa, Y.; Seki, N.; Tsuji, K.; Spears, G.W.; Tojo, T. WO99–30711, 1999; *Chem. Abstr.* **1999**, *131*, 44748.
20. Mieza, M. A.; Itoh, T.; Cui, J. Q.; Makino, Y.; Kawano, T.; Tsuchida, K.; Koike, T.; Shirai, T.; Yagita, H.; Matsuzawa, A.; Koseki, H.; Taniguchi, M. *J. Immunol.* **1996**, *156*, 4035.