Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 545–548

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Structure–Activity Relationships of Quinazoline Derivatives: Dual-Acting Compounds with Inhibitory Activities Toward Both TNF-α Production and T Cell Proliferation

Masanori Tobe, Yoshiaki Isobe, Hideyuki Tomizawa, Mitsuhiro Matsumoto, Fumihiro Obara, Takahiro Nagasaki and Hideya Hayashi*

Pharmaceuticals and Biotechnology Laboratory, Japan Energy Corporation, Toda-shi, Saitama 335-8502, Japan

Received 4 October 2000; accepted 8 December 2000

Abstract—We synthesized 4-chlorophenethylaminoquinazoline derivatives and evaluated their inhibitory activities toward both TNF-α production and T cell proliferation responses. Compound **2f**, containing a piperazine ring at the C(7)-position of the quinazoline ring, exhibited more potent inhibitory activities toward both than the lead compound **1a**. A smaller *N*-substituent in the piperazine ring was required for inhibition of TNF-α production.

Introduction

Rheumatoid arthritis (RA) is considered to be characterized by joint destruction as a result of chronic synovial inflammation, and many reseachers have described the importance of the T cell mediated autoimmune response in the initiation and maintenance of this inflammation.[1] In particular, T cell (especially Th1) proliferation has been shown to be a possible mechanism by which inflammation is enhanced in RA.[2]

Tumor necrosis factor-α[3] (TNF-α), among a variety of cytokines, predominantly functions as the principal mediator in RA. TNF-α is mainly produced by activated macrophages/monocytes and induces the production of several key inflammatory cytokines such as IL-1β, IL-6, and GM-CSF,[4] and thereby enhances inflammation. Recently, TNF soluble receptor (Enbrel™)[5] and TNF antibody (Remicade™)[6] have been reported to have an excellent efficacy in RA patients. However, these biopharmaceuticals are very expensive and difficult to administer orally. On the other hand, it has been reported that many low-molecular-weight compounds have an inhibitory activity toward the production of TNF-α.[7] Among them, pentoxifylline has shown a weak efficacy to RA patients under the recent clinical trial.[8] According to this evidence, we considered that it is essential for anti-rheumatic agents to have the property of the correction of T cell mediated abnormal immune responses accompanied with TNF-α inhibitory activity. Glucocorticoids show the inhibitory activities toward both TNF-α production and T cell proliferation, but long-time use of glucocorticoids is limited because of its many side effects, such as atrophia cutis and infection.

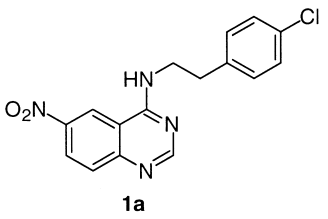
Therefore, nonsteroidal agents having inhibitory activities toward both TNF-α production and T cell proliferation would be expected to be effective in the treatment of RA. To develop this type of nonsteroidal compound, we conducted a random screening and found lead compound **1a** (Fig. 1). In this study, we describe and discuss the SARs of 4-chlorophenethylaminoquinazoline derivatives as a dual inhibitor.

Chemistry and Biology

The compounds described in this study are listed in Tables 1 and 2, and the methods used for their synthesis are outlined in Schemes 1 and 2. Compounds **1a**–**1t** were prepared from the corresponding 2-aminobenzamides as shown in Scheme 1. Catalytic hydrogenation of **1a**, **1k** and **1m** gave **1f**, **1j** and **1n**, respectively. Compounds **1g** and **1h** were obtained by the alkylation of **1f** with the corresponding alkyl halides. Formylation of **1f** with acetic anhydride in formic acid afforded **1i**. The reaction of **1s** with sodium methoxide provided **1t**.

*Corresponding author. Tel.: +81-48-433-2194; fax: +81-48-433-1605; e-mail: hhayashi@j-energy.co.jp

0960-894X/01/$ - see front matter
PII: S0960-894X(00)00718-6



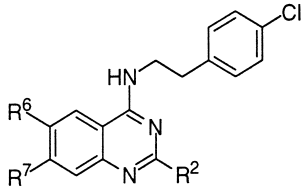
1a

Figure 1. Structure of compound **1a**.

Synthetic routes of **2a**–**2m** are shown in Scheme 2. Nitration of **3**, followed by removal of the unwanted 8-nitro isomer by recrystallization from acetic acid, gave the key intermediate 7-chloro-6-nitro-4-quinazolone (**4**).[9] Compound **5** was prepared by the chlorination of **4** with phosphorus oxychloride. Subsequently, compound **5** was converted to **2a** by selective amination at the 4-position of the quinazoline ring. Then, compounds **2b**–**2m** were obtained from **2a** by the displacement of the activated chlorine with the desired nucleophile such as the amines or the sodium alkoxides (Method A). This selectivity described above was confirmed by another route of synthesis, method B. The same compounds were afforded by method A and method B in several cases.

A series of these compounds was evaluated for their abilities to inhibit both TNF-α production and T cell proliferation. Inhibition of TNF-α production was measured using human peripheral blood mononuclear cells (PBMCs) stimulated by LPS as previously reported.[10,11] The cytotoxicity for human PBMCs was measured by MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt] assay.[12] T cell proliferatrion was determined by MTS assay using concanavalin A (Con A)-stimulated mice spleen cells according to the previous report.[13,14]

Table 1. Inhibition of TNF-α production and T cell proliferation by compounds **1a**–**1t**



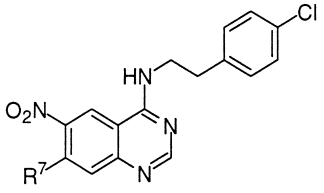
Compound	R^2	R^6	R^7	IC_{50} (μM) TNF-α[a]	Con A[b]	MTS[c]
1a	H	NO_2	H	14.6	3.2	>30
1b	H	H	H	>30	0.5	>30
1c	H	Cl	H	>30	>10	>30
1d	H	Br	H	>30	4.8	>30
1e	H	Me	H	>30	0.8	>30
1f	H	NH_2	H	>30	2.6	>30
1g	H	NHMe	H	>30	>10	>30
1h	H	NHBn	H	>30	>10	>30
1i	H	NHCHO	H	>30	>10	>30
1j	H	OH	H	>30	1.4	>30
1k	H	OBn	H	>30	>10	>30
1l	H	H	Cl	>30	>10	>30
1m	H	H	NO_2	>30	5.6	>30
1n	H	H	NH_2	>30	3.4	>30
1o	H	F	F	>30	nt	>30
1p	H	OMe	OMe	>30	1.4	>30
1q	Me	H	H	>30	0.2	>30
1r	Ph	H	H	>30	>10	>30
1s	Cl	H	H	>30	1.2	>30
1t	OMe	H	H	>30	>10	>30

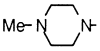
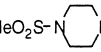
nt: not tested.
[a]IC_{50} of TNF-α production from human PBMCs stimulated by LPS.
[b]IC_{50} of Con A-induced proliferation of mice spleen cells.
[c]IC_{50} of the growth in human PBMCs stimulated by LPS.

Results and Discussion

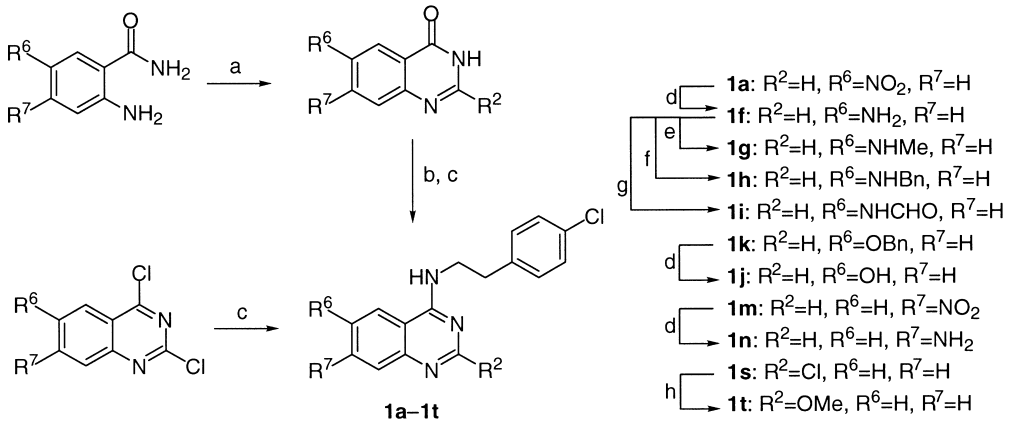
We synthesized various compounds to optimize the substituents on the quinazoline ring. As shown in Table 1, 6- or 7-substituted quinazoline derivatives (**1a**, **1b**, **1d**–**1f**, **1j**, **1m**, **1n**, **1p**) inhibited the T cell proliferation, but only **1a** also showed inhibitory activity toward TNF-α production. The methyl group (**1q**) or hydrogen atom (**1b**) were preferable as C(2) substituents for blocking T cell proliferation, but other groups (**1r**–**1t**) were not suitable.

Table 2. Inhibition of TNF-α production and T cell proliferation by compounds **2a**–**2m**

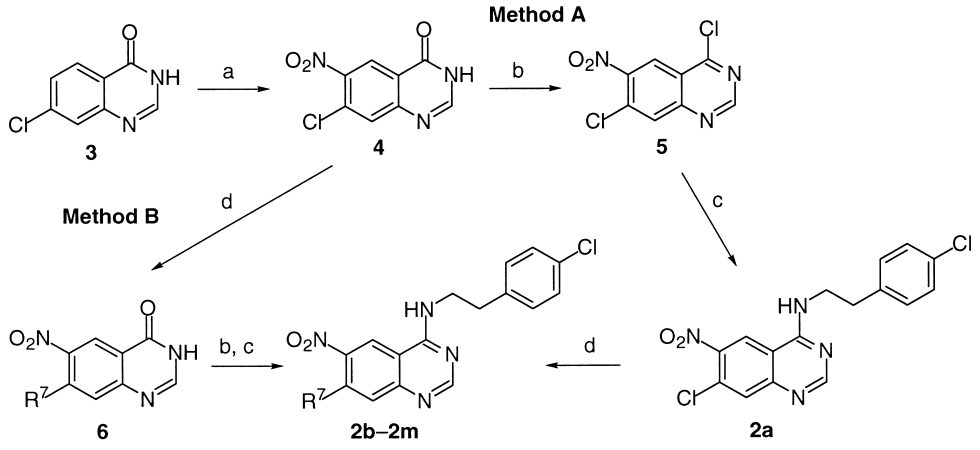


Compound	R^7	IC_{50} (μM) TNF-α[a]	Con A[b]	MTS[c]
2a	Cl	>30	>10	>30
2b	EtNH	>30	>10	>30
2c	Me_2N	19.4	8.6	27.3
2d		>30	>10	>30
2e		24.8	8.3	22.5
2f		0.8	1.1	12.1
2g		2.1	0.9	9.8
2h		1.4	1.2	22.1
2i		>30	4.3	4.5
2j		>30	>10	>30
2k		>30	>10	>30
2l	MeO	>30	nt	>30
2m	EtO	>30	nt	>30
Auranofin		>30	nt	>30
D-Pencillamine		>30	nt	>30
Salazosulfapyridine		>30	>10	>30
Rolipram		0.5	>10	>30
Dexamethasone		0.01	0.005	0.04

nt: not tested.
[a]IC_{50} of TNF-α production from human PBMCs stimulated by LPS.
[b]IC_{50} of Con A-induced proliferation of mice spleen cells.



Scheme 1. Synthesis of compounds **1a**–**1t**. Reagents and conditions: (a) R^2CO_2Et, EtONa, EtOH, reflux or $HC(OMe)_3$, 12 N HCl; (b) phosphorus oxychloride, 90 °C; (c) 4-chlorophenethylamine, Et_3N, *i*-PrOH; (d) H_2, Pd–C, EtOH; (e) MeI, K_2CO_3, DMF; (f) benzyl bromide, K_2CO_3, DMF; (g) HCO_2H, Ac_2O, 60 °C; (h) MeONa, MeOH, reflux.



Scheme 2. Synthesis of compounds **2a**–**2m**. Reagents and conditions: (a) HNO_3/H_2SO_4 (1:1, v/v), 80 °C; (b) phosphorus oxychloride, 90 °C; (c) 4-chlorophenethylamine, Et_3N, *i*-PrOH; (d) amines, *i*-Pr_2NEt, *n*-BuOH, reflux or sodium alkoxides, alcohols, reflux.

Next, we investigated the effect of substituents at the C(7)-position in the 6-nitroquinazolines. The results are shown in Table 2. Compound **2f**, having a piperazine ring, exhibited inhibitory activities toward both TNF-α production ($IC_{50}=0.8$ μM) and T cell proliferation ($IC_{50}=1.1$ μM). In addition, this compound did not show cytotoxicity at 5 μM toward human PBMCs stimulated by LPS. In contrast, both inhibitory activities of compounds **2c**, **2d** and **2e** containing dimethylamine, piperidine, and morpholine, respectively, were drastically decreased. Compound **2h**, having an *N*-methylpiperazine ring, showed almost the same activity as **2f**.

Moreover, compounds **2i**–**2k**, in which the NH group of the piperazine ring was blocked by bulky substituents, had diminished activities. These results suggest that not only the presence of a piperazine ring but also a smaller *N*-substituent in piperazine are necessary to inhibit TNF-α production. Compounds **2l** and **2m**, containing a methoxy group and ethoxy one, respectively, at the C(7)-position did not show an activity toward TNF-α production.

Furthermore, anti-rheumatic drugs such as auranofin, D-penicillamine, and salazosulfapyridine were not effective for TNF-α inhibitory activity. Therefore, the biological property of compound **2f** could be considered as a novel unique anti-inflammatory agent. A substitution study at the C(4)-position of **2f** and elucidation of the mechanism of action of this compound are underway.

Conclusion

We conducted a study to optimize lead compound **1a**, which showed moderate inhibitory activity toward TNF-α production and suppression of T cell proliferation. As a result, we found that compound **2f**, containing a piperazine ring at the C(7)-position of the quinazoline ring, exhibited more potent inhibitory activities than **1a** toward both parameters. Compound **2f** is thus a new candidate having inhibitory potency toward both TNF-α production and T cell proliferation. At present, we are now investigating the SARs of 4-substituted-7-piperazinyl-quinazolines.

Acknowledgements

The authors are grateful to Dr. Satoru Misawa and Mr. Tsutomu Mimoto for useful suggestions and encouragement during the course of this research.

References and Notes

1. (a) Klareskog, L.; Forsum, U.; Scheynius, A.; Kabelitz, D.; Wigzell, H. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 3632. (b) Panayi, G. S.; Lanchbury, J. S.; Kingsley, G. H. *Arthritis Rheum.* **1992**, *35*, 729.
2. (a) Quayle, A. J.; Chomarat, P.; Miossec, P.; Kjeldsen-Kragh, J.; Forre, O.; Natvig, J. B. *Scand. J. Immunol.* **1993**, *38*, 75. (b) Simon, A. K.; Seipelt, E.; Sieper, J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8562. (c) Schulze-Koops, H.; Lipsky, P. E.; Kavanaugh, A. F.; Davis, L. S. *J. Immunol.* **1995**, *155*, 5029. (d) Van Roon, J. A. G.; Van Roy, J. L. A. M.; Duits, A.; Lafeber, F. P. J. G.; Bijlsma, J. W. J. *Ann. Rheum. Dis.* **1995**, *54*, 836.
3. Bemelmans, M. H. A.; Van Tits, L. J. H.; Buurman, W. A. *Crit. Rev. Immunol.* **1996**, *16*, 1.
4. (a) Dinarello, C. A.; Cannon, J. G.; Wolff, S. M.; Bernheim, H. A.; Beutler, B.; Cerami, A.; Figari, I. S.; Palladino, M. A., Jr.; O'Connor, J. V. *J. Exp. Med.* **1986**, *163*, 1433. (b) Brennan, F. M.; Chantry, D.; Jackson, A.; Maini, R. N.; Feldmann, M. *Lancet* **1989**, *2*, 244. (c) Brennan, F. M.; Maini, R. N.; Feldmann, M. *Br. J. Rheumatol.* **1992**, *31*, 293.
5. (a) Moreland, L. W.; Baumgartner, S. W.; Schiff, M. H.; Tindall, E. A.; Fleischmann, R. M.; Weaver, A. L.; Ettlinger, R. E.; Cohen, S.; Koopman, W. J.; Mohler, K.; Widmer, M. B.; Blosch, C. M. *N. Eng. J. Med.* **1997**, *337*, 141. (b) Moreland, L. W. *Cleveland Clin. J. Med.* **1999**, *66*, 367.
6. (a) Elliott, M. J.; Maini, R. N.; Feldmann, M.; Kalden, J. R.; Antoni, C.; Smolen, J. S.; Leeb, B.; Breedveld, F. C.; Macfarlane, J. D.; Bijl, H.; Woody, J. N. *Lancet* **1994**, *344*, 1105. (b) Maini, R. N.; Elliott, M. J.; Brennan, F. M.; Feldmann, M. *Clin. Exp. Immunol.* **1995**, *101*, 207. (c) Feldmann, M.; Brennan, F. M.; Paleolog, E.; Taylor, P.; Maini, R. N. *Eur. Cytokine Netw.* **1997**, *8*, 297.
7. (a) Cottam, H. B.; Shih, H.; Tehrani, L. R.; Wasson, D. B.; Carson, D. A. *J. Med. Chem.* **1996**, *39*, 2. (b) Borcherding, D. R.; Peet, N. P.; Munson, H. R.; Zhang, H.; Hoffman, P. F.; Bowlin, T. L.; Edwards, C. K., III *J. Med. Chem.* **1996**, *39*, 2615. (c) Chao, Q.; Deng, L.; Shih, H.; Leoni, L. M.; Genini, D.; Carson, D. A.; Cottam, H. B. *J. Med. Chem.* **1999**, *42*, 3860. (d) Fujiwara, N.; Fujita, H.; Iwai, K.; Kurimoto, A.; Murata, S.; Kawakami, H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1317.
8. Dubost, J. J.; Soubrier, M.; Ristori, J. M.; Beaujon, G.; Oualid, T.; Bussiere, J. L.; Sauvezie, B. *Rev. Rheum. Engl. Ed.* **1997**, *64*, 789.
9. Leonard, N. J.; Morrice, A. G.; Sprecker, M. A. *J. Org. Chem.* **1975**, *40*, 356.
10. (a) Henry, J. R.; Rupert, K. C.; Dodd, J. H.; Turchi, I. J.; Wadsworth, S. A.; Cavender, D. E.; Fahmy, B.; Olini, G. C.; Davis, J. E.; Pellegrino-Gensey, J. L.; Schafer, P. H.; Siekierka, J. J. *J. Med. Chem.* **1998**, *41*, 4196. (b) Henry, J. R.; Rupert, K. C.; Dodd, J. H.; Turchi, I. J.; Wadsworth, S. A.; Cavender, D. E.; Schafer, P. H.; Siekierka, J. J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3335.
11. TNF-α inhibition assay was modified from previously described methods.[10] Human PBMCs from healthy volunteers were seeded (3×10^6 cells/mL RPMI-1640 10% fetal calf serum/well) into 96-well culture plates and 100 μL of medium containing 20 ng/mL LPS and test compounds was added. Cultures were incubated for 16 h at 37 °C, an amount of TNF-α in supernatant was determined by ELISA system.
12. In the purpose of evaluating the cytotoxicity of the compounds, we assessed cell viability by MTS assay[13] with the assay of TNF-α production.
13. (a) Cory, A. H.; Owen, T. C.; Barltrop, J. A.; Cory, J. G. *Cancer Commun.* **1991**, *3*, 207. (b) Riss, T. L.; Moravec, R. A. *Mol. Biol. Cell* **1991**, *3*(suppl.), 184a. (c) Yamahara, J.; Shimoda, H.; Matsuda, H.; Yoshikawa, M. *Biol. Pharm. Bull.* **1996**, *19*, 1241.
14. The assay of the inhibition of T cell proliferation was modified from previously described methods.[13] Briefly, mice spleen cells were seeded (8×10^6 cells/mL RPMI-1640 10% fetal calf serum/well) into 96-well culture plates and incubated with test compounds and Con A (10 μg/mL) at 37 °C under a 5% CO_2 atmosphere for 3 days. MTS assay was performed using a commercial kit and formazan dye products were measured by absorbance at 490 nm using a microplate reader.