

A Novel Synthesis and Potent Antiinflammatory Activity of 4-Hydroxy-2(1*H*)-oxo-1-phenyl-1,8-naphthyridine-3-carboxamides

Takeshi Kuroda, Fumio Suzuki,* Tadafumi Tamura, Kenji Ohmori, and Hisashi Hosoe

Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Shizuoka 411, Japan. Received August 27, 1991

New antiinflammatory agents 4-hydroxy-2(1*H*)-oxo-1-phenyl-1,8-naphthyridine-3-carboxamides **7** were designed and synthesized via a valuable intermediate, 1-phenyl-2*H*-pyrido[2,3-*d*][1,3]oxazine-2,4(1*H*)-dione (**9**). The nature of substituents on the amide nitrogen had a pronounced effect on antiinflammatory activity. Studies of structure-activity relationships led to compounds **33** and **34** bearing a pyridine ring on the amide nitrogen. Compounds **33** and **34** were active against carrageenin-, zymosan-, and arachidonic acid-induced rat paw edemas and also potently inhibited the reversed passive Arthus reaction in rats. Thus, they possess a broader spectrum of antiinflammatory activity than the classical nonsteroidal antiinflammatory drugs (NSAIDs) such as indomethacin and piroxicam.

Introduction

There is currently a considerable therapeutic interest in novel antiinflammatory drugs with a mode of action different from that of the classical acidic nonsteroidal antiinflammatory drugs (NSAIDs), mainly for use in patients with arthritis of varying degrees of severity. The classical NSAIDs do not prevent progression of such a disease and are subject to irritant side effects on gastric mucosa.¹ The rat carrageenin-induced paw edema model has been used as a popular assay for such antiinflammatory agents² which are primarily cyclooxygenase (CO) inhibitors.³ In order to screen new antiinflammatory agents, other inflammatory models mechanistically different from the carrageenin model are necessary as the primary assay.

Injection of zymosan in rat paw provides a local, acute inflammatory reaction. It causes activation of the alternative pathway of complement⁴ and anaphylatoxins C_{3a} and C_{5a} which are capable of inducing histamine release from mast cells and basophils and the synthesis of derivatives of arachidonic acid metabolism. During phagocytosis of zymosan particles by polymorphonuclear leukocytes⁵ or by macrophages,⁶ leukotrienes are considered to be one of the important mediators. Thus zymosan-induced inflammation has been regarded as an interesting model to discover new antiinflammatory agents.⁷

Rheumatoid arthritis is associated with defects in the responsiveness of the immune system,⁸ and growing evidence indicates that defects in immunoregulation involving lymphocyte-macrophage interactions may be implicated in the pathogenesis of this disease.⁹ Reversed passive Arthus reaction (RPAR) is an immunologically induced inflammatory response characterized by immune complex deposition, complement fixation, polymorphonuclear leukocyte infiltration, and tissue damage. Many of these same pathological tissue alternations are found in the lesions of rheumatoid arthritis (RA). The similarities between RA and the Arthus reaction suggest its usefulness in the search for more effective antiinflammatory and antirheumatic agents.¹⁰

The parallel application of the above three models in rats allows for the opportunity to obtain new types of antiinflammatory agents. In this report we describe design, syntheses, and structure-activity relationships of 4-hydroxy-2(1*H*)-oxo-1-phenyl-1,8-naphthyridine-3-carboxamides.

Design

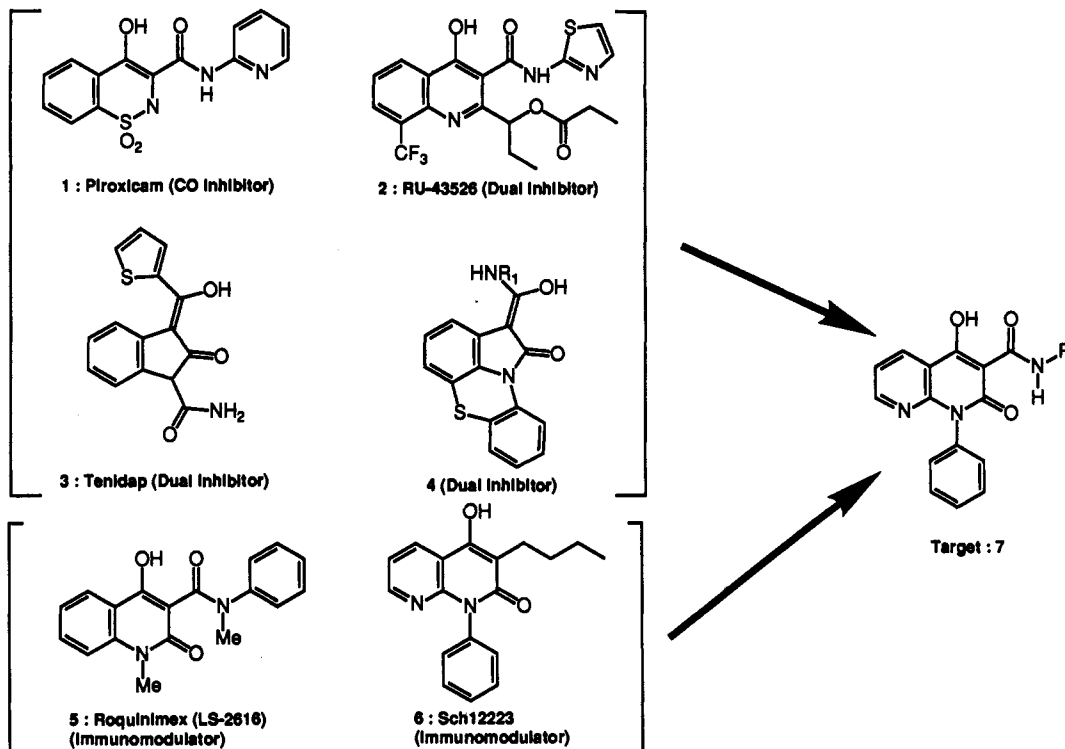
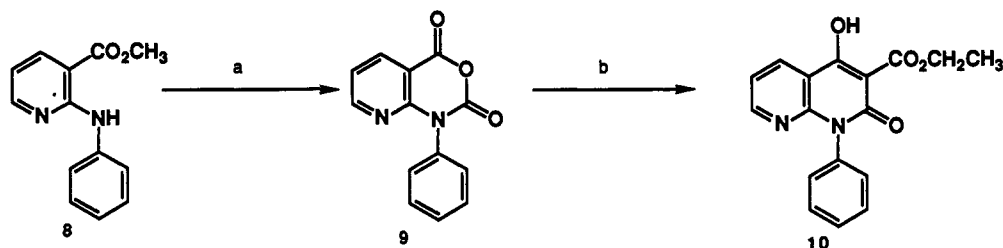
The classical NSAIDs possess an acidic proton and can be divided into two classes. One is the group containing a carboxylic acid function (indomethacin, etc.). The other contains a β -ketocarboxamide moiety (acidic amide; pi-

roxicam (1), tenoxicam, etc.). Both functional groups play an important role in activity.¹¹ Recently several interesting antiinflammatory agents bearing the β -keto-carboxamide moiety, such as RU-43526 (**2**),¹² tenidap (**3**),¹³

- (1) Gemmell, D. K.; Cottney, J.; Lewis, A. J. Comparative Effects of Drugs on Four Paw Oedema Models in the Rat. *Agents Actions* 1979, 9, 107-116.
- (2) Van Arman, C. G. Oedema and Increased Vascular Permeability. *Handb. Exp. Pharmacol.* 1979, 50, 75-91.
- (3) Winter, C. A.; Risley, E. A.; Nuss, G. W. Carrageenin-Induced Edema in Hind Paw of the Rat as an Assay for Antiinflammatory Drugs. *Proc. Soc. Exp. Biol. Med.* 1962, 111, 544-547.
- (4) Pillemer, L.; Blum, L.; Lepow, J. H.; Ross, O. A.; Todd, E. W.; Wardlaw, A. C. The Properdin System and Immunity: Demonstration and Isolation of a New Serum Protein, Properdin, and Its Role in Immune Phenomena. *Science* 1954, 120, 279-285.
- (5) Claesson, H. E.; Lundberg, H.; Malmstam, C. Serum-Coated Zymosan Stimulates the Synthesis of Leukotriene B₄ in Human Polymorphonuclear Leukocytes. Inhibition by Cyclic AMP. *Biochem. Biophys. Res. Commun.* 1981, 99, 1230-1237.
- (6) Scott, W. A.; Zrike, J. M.; Hamill, A. L.; Kempe, J.; Cohn, Z. A. Regulation of Arachidonic Acid Metabolites in Macrophages. *J. Exp. Med.* 1980, 152, 324-335.
- (7) Tarayre, J. P.; Delhon, A.; Aliaga, M.; Barbara, M.; Bruniquel, F.; Caillol, V.; Puech, L.; Consul, N.; Tisne-Versailles, J. Pharmacological Studies on Zymosan Inflammation in Rats and Mice. 1: Zymosan-Induced Paw Oedema in Rats and Mice. *Pharmacol. Res.* 1989, 21, 375-384.
- (8) Paget, S. A.; Gibofsky, A. Immunopathogenesis of Rheumatoid Arthritis. *Am. J. Med.* 1979, 67, 961-970.
- (9) Janossy, G.; Duke, O.; Poulter, L. W.; Panayi, G.; Bofill, M.; Goldstein, G. Rheumatoid Arthritis: A Disease of T-Lymphocyte/Macrophage Immunoregulation. *Lancet* 1981, 2, 839-842.
- (10) Pflum, L. R.; Graeme, M. L. The Arthus Reaction in Rats, a Possible Test for Antiinflammatory and Antirheumatic Drugs. *Agents Actions* 1979, 9, 184-189.
- (11) (a) Lambordino, J. G.; Wiseman, E. H. Antiinflammatory 3,4-Dihydro-2-alkyl-3-oxo-2*H*-1,2-benzothiazine-4-carboxamide 1,1-Dioxides. *J. Med. Chem.* 1971, 14, 973-977. (b) Lambordino, J. G.; Wiseman, E. H.; Mclamore, M. W. Synthesis and Antiinflammatory Activity of Some 3-Carboxamides of 2-Alkyl-4-hydroxy-2*H*-1,2-benzothiazine 1,1-Dioxide. *J. Med. Chem.* 1971, 14, 1171-1175. (c) Lambordino, J. G.; Wiseman, E. H.; Chiaini, J. Potent Antiinflammatory *N*-Heterocyclic 3-Carboxamides of 4-Hydroxy-2-methyl-2*H*-1,2-benzothiazine 1,1-Dioxide. *J. Med. Chem.* 1973, 16, 493-496. (d) Wiseman, E. H.; Chiaini, J.; Mcmanus, J. M. Studies with Antiinflammatory Oxindolecarboxanilides. *J. Med. Chem.* 1973, 16, 131-134.
- (12) Clemence, F.; Martret, O. L.; Delevallee, F.; Benzoni, J.; Jouanen, A.; Jouquey, S.; Mouren, M.; Deraedt, R. 4-Hydroxy-3-quinolinecarboxamides with Antiarthritic and Analgesic Activities. *J. Med. Chem.* 1988, 31, 1453-1462.
- (13) Moilanen, E.; Alanko, J.; Asmawi, M. Z.; Vapaatalo, H. CP-66,248, a New Anti-inflammatory Agent, is a Potent Inhibitor of Leukotriene B₄ and Prostanoid Synthesis in Human Polymorphonuclear Leukocytes in Vitro. *Eicosanoids* 1988, 1, 35-39.

* To whom all correspondence should be addressed.

Scheme I

Scheme II^a

^a (a) $\text{ClCH}_2\text{CH}_2\text{Cl}$, TCF, 80 °C, 3 h; (b) $(\text{CH}_3)_2\text{NCOCH}_3$, $\text{CH}_2(\text{CO}_2\text{CH}_2\text{CH}_3)_2$, NaH, 0–150 °C.

and 1,2-dihydro-1-oxopyrrolo[3,2,1-*kl*]phenothiazine-2-carboxamides (4),¹⁴ have been synthesized. However, these compounds were reported to inhibit not only cyclooxygenase (CO) but also 5-lipoxygenase (5-LO) and are regarded as new types of dual inhibitors.

On the other hand, several immunomodulators such as roquinimex (5)¹⁵ and Sch 12223 (6)¹⁶ which contain a bicyclic heterocycle and a β -ketocarboxamide moiety have been reported.

Appreciation of the above results stimulated our interest in drugs designed to incorporate some immunological activity into pharmacophores of the existing dual inhibitors. A 1,8-naphthyridine skeleton in 6 is known to be a bioisostere of quinoline. Thus, we designed a target compound

7, 4-hydroxy-2(1*H*)-oxo-1-phenyl-1,8-naphthyridine-3-carboxamide which was regarded as a hybrid compound of the β -ketocarboxamide (dual inhibition) and the 1-phenyl-1,8-naphthyridin-2(1*H*)-one moiety (immunomodulation and dual inhibition) as shown in Scheme I.

Critical to the early success of this approach would be (1) evidence of antiinflammatory activity in relevant animal models and (2) demonstration of some unique pharmacological profiles as a result of incorporating an acidic amide into the 1,8-naphthyridine skeleton.

Chemistry

4-Hydroxy-2(1*H*)-oxo-1-phenyl-1,8-naphthyridine-3-carboxamides were mainly synthesized by using method A and method B described in Scheme III. *N*-(2-Pyridyl)-4-hydroxy-2(1*H*)-oxo-1-phenyl-1,8-naphthyridine-3-carboxamide (14) was prepared according to Scheme IV (method C).

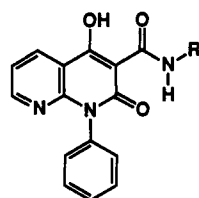
1-Phenyl-2*H*-pyrido[2,3-*d*][1,3]oxazine-2,4(1*H*)-dione (9) was prepared by treatment of methyl 2-anilinonicotinate (8)¹⁷ with trichloromethyl chloroformate (TCF) in 1,2-dichloroethane.¹⁸ Compound 9 was reacted with the anion

(14) Mylari, B. L.; Carty, T. J.; Moore, P. F.; Zembrowski, W. J. 1,2-Dihydro-1-oxopyrrolo[3,2,1-*kl*]phenothiazine-2-carboxamides and Congeners. Dual Cyclooxygenase/5-Lipoxygenase Inhibitors with Antiinflammatory Activity. *J. Med. Chem.* 1990, 33, 2019–2024.

(15) (a) Ilback, N. G.; Fohlman, J.; Slorach, S.; Friman, G. Effects of the Immunomodulator LS 2616 on Lymphocyte Subpopulations in Murine Coxsackievirus B3 Myocarditis. *J. Immunol.* 1989, 142, 3225–3228. (b) Bjork, J.; Kleinau, D. Paradoxical Effects of LS-2616 (Linomide) Treatment in the Type II Collagen Arthritis Model in Mice. *Agents Actions* 1989, 27, 319–321.

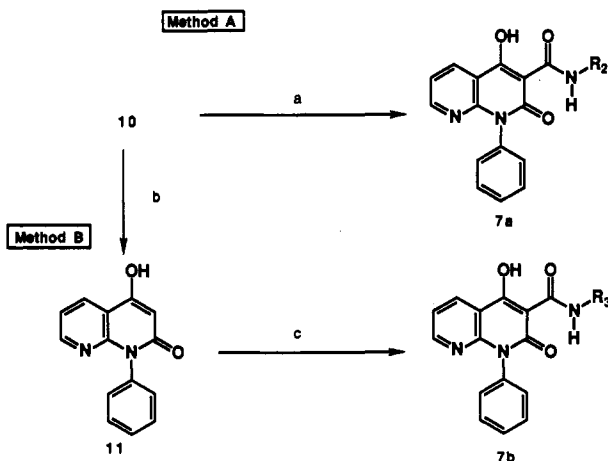
(16) Randol, S. S.; Ira, S. M. Pharmaceutical Compositions Containing Azanaphthalenes. JP Patent 63 500 100, 1988.

(17) Serlock, M. H.; Kaminsky, J. J.; Tom, W. C.; Lee, J. F.; Wong, S. H.; Kreutner, W.; Bryant, R. W.; McPhail, A. T. Antiallergy Agents. 1. Substituted 1,8-Naphthyridin-2(1*H*)-ones as Inhibitors of SRS-A Release. *J. Med. Chem.* 1988, 31, 2108–2121.

Table I. 4-Hydroxy-2(1*H*)-oxo-1-phenyl-1,8-naphthyridine-3-carboxamide Derivatives

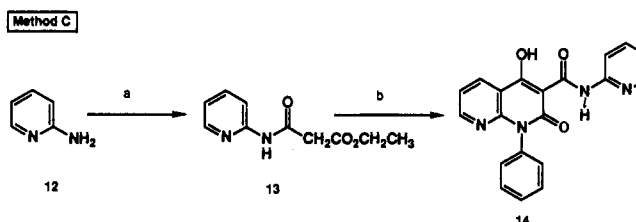
compd	R	method	yield, %	mp, °C	recrystn solvent	formula ^a
15	CH ₃	A	70	>300	C ₂ H ₅ OH	C ₁₆ H ₁₃ N ₃ O ₃
16	<i>n</i> -C ₄ H ₉	A	62	181–184	C ₂ H ₅ OH–H ₂ O	C ₁₉ H ₁₉ N ₃ O ₃
17	CH ₂ C ₆ H ₅	A	66	219–220	DMSO–H ₂ O	C ₂₂ H ₁₇ N ₃ O ₃
18	CH ₂ CH ₂ N(CH ₃) ₂	A	66	240–242	C ₂ H ₅ OH	C ₁₉ H ₂₀ N ₄ O ₃
19	1-benzyl-4-piperidinyl	A	97	205–206	C ₂ H ₅ OH–CH ₃ OH	C ₂₇ H ₂₀ N ₄ O ₃
20	4-morpholyl	A	87	263–266	xylene	C ₁₉ H ₁₈ N ₄ O ₄
21	C ₆ H ₅	B	50	>300	DMSO–H ₂ O	C ₂₁ H ₁₅ N ₃ O ₃
22	4-CH ₃ C ₆ H ₄	B	45	>300	DMSO–H ₂ O	C ₂₂ H ₁₇ N ₃ O ₃
23	4-CH ₃ OC ₆ H ₄	B	42	>300	DMSO–H ₂ O	C ₂₂ H ₁₇ N ₃ O ₄
24	4-ClC ₆ H ₄	B	56	281–286	DMSO–H ₂ O	C ₂₁ H ₁₄ N ₃ O ₃ Cl
25	4-NO ₂ C ₆ H ₄	B	42	>300	DMSO–H ₂ O	C ₂₁ H ₁₄ N ₃ O ₅
26	4-NH ₂ C ₆ H ₄	A	90	>300	xylene	C ₂₁ H ₁₆ N ₄ O ₃
27	3-CH ₃ C ₆ H ₄	A	74	229–230	DMSO–H ₂ O	C ₂₂ H ₁₇ N ₃ O ₃
28	3-CH ₃ OC ₆ H ₄	B	48	275–280	DMSO–H ₂ O	C ₂₂ H ₁₇ N ₃ O ₄
29	3-ClC ₆ H ₄	A	75	295–298	DMSO–H ₂ O	C ₂₁ H ₁₄ N ₃ O ₃ Cl
30	3-NO ₂ C ₆ H ₄	A	77	>300	DMSO–H ₂ O	C ₂₁ H ₁₄ N ₃ O ₅
31	2-CH ₃ C ₆ H ₄	A	74	280–283	DMSO–H ₂ O	C ₂₂ H ₁₇ N ₃ O ₃
32	2-CH ₃ OC ₆ H ₄	B	52	>300	DMSO–H ₂ O	C ₂₂ H ₁₇ N ₃ O ₄ ^{1/10} H ₂ O
14	2-pyridyl	C	30	>300	DMF	C ₂₀ H ₁₄ N ₄ O ₃
33	3-pyridyl	A	74	>300	DMSO	C ₂₀ H ₁₄ N ₄ O ₃ ^{3/10} H ₂ O
34	4-pyridyl	A	51	>300	DMSO	C ₂₀ H ₁₄ N ₄ O ₃ ^{2/5} H ₂ O
35	2-thiazolyl	A	81	>300	DMF–H ₂ O	C ₁₈ H ₁₂ N ₄ O ₃ S
36	2-pyrazyl	A	38	>300	xylene	C ₁₉ H ₁₃ N ₅ O ₃
37	CH ₂ -3-pyridyl	A	65	222–224	xylene	C ₂₁ H ₁₆ N ₄ O ₃
38	CH ₂ -4-pyridyl	A	73	232–234	xylene	C ₂₁ H ₁₆ N ₄ O ₃
39	3-CH ₃ -4-pyridyl	A	62	276–279	DMF	C ₂₁ H ₁₆ N ₄ O ₃ ^{2/5} H ₂ O
40	2-CH ₃ O-5-pyridyl	A	71	285–287	DMF–H ₂ O	C ₂₁ H ₁₆ N ₄ O ₄ ^{2/5} H ₂ O
41	2-Cl-5-pyridyl	A	64	282–283	CHCl ₃	C ₂₀ H ₁₃ N ₄ O ₃ Cl

^a All compounds were analyzed for C, H, and N and results agreed to $\pm 0.4\%$ of theoretical values.

Scheme III^a

^a (a) xylene, R₂NH, Δ ; (b) aqueous NaOH, Δ ; (c) DMSO, R₃NCO, (CH₃CH₂)₃N.

of diethyl malonate to give compound 10, regarded as a common intermediate, in order to obtain 4-hydroxy-2-(1*H*)-oxo-1-phenyl-1,8-naphthyridine-3-carboxamide derivatives (Scheme II). Exchange reaction of the ester 10 with a primary amine in refluxing xylene afforded the target compound 7a (Scheme III, method A). Hydrolysis of 10 followed by decarboxylation afforded 11. Treatment

Scheme IV^a

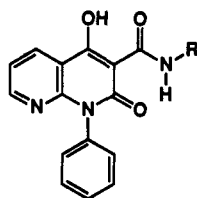
^a (a) THF, CH₃CH₂O₂CH₂COCl, (CH₃CH₂)₃N, DMAP; (b) (C-H₃)₂NCOCH₃, 9, NaH, 0–100 °C.

of 11 with an isocyanate in DMSO in the presence of triethylamine gave the target compound 7b (Scheme III, method B). Since *N*-(2-pyridyl)-4-hydroxy-2(1*H*)-oxo-1-phenyl-1,8-naphthyridine-3-carboxamide (14) could not be synthesized by using the methods described in Scheme III, an alternative route was developed (Scheme IV, method C). Acylation of 2-aminopyridine (12) with ethyl malonyl chloride in the presence of triethylamine and catalytic amounts of 4-(dimethylamino)pyridine (DMAP) gave compound 13. Compound 14 was obtained by the reaction of 9 with the anion of 13. Chemical data of obtained target compounds are shown in Table I.

Pharmacological Results and Discussion

The pharmacological activity of the compounds was determined by the oral route in the rats in carrageenin-induced paw edema (CPE), in zymosan-induced paw edema (ZPE), and in the reversed passive Arthus reaction (RPAR)-induced pleurisy.

(18) Suzuki, F.; Kuroda, T.; Kitamura, S.; Ohmori, K. Imidazo-naphthyridine Derivatives. EP Patent 91 10 8908. 4 (JP Patent Appl. 143460, June 1, 1990).

Table II. Effects of 4-Hydroxy-2(1*H*)-oxo-1-phenyl-1,8-naphthyridine-3-carboxamide Derivatives on Carrageenin- and Zymosan-Induced Rat's Paw Edema at 100 mg/kg po

compd	R	CPE		ZPE	
		swelling, ^a %	inhibn, %	swelling, ^a %	inhibn, %
15	CH ₃	50.7 ± 3.8 ^{b,c}	21.0	65.9 ± 3.9 ^m	9.6
16	<i>n</i> -C ₄ H ₉	51.1 ± 2.1 ^{b,c}	20.4	62.5 ± 1.0 ⁿ	12.8
17	CH ₂ C ₆ H ₅	60.4 ± 4.1 ^d	11.3	60.3 ± 10.0 ^o	1.6
18	CH ₂ CH ₂ N(CH ₃) ₂	52.4 ± 3.4 ^b	18.4	55.5 ± 5.8 ⁿ	22.6
19	1-benzyl-4-piperidinyl	80.8 ± 9.0 ^e	1.2	51.3 ± 2.4 ^p	1.7
20	4-morpholyl	82.8 ± 4.3 ^f	-26.2	66.2 ± 2.0 ^q	-8.5
21	C ₆ H ₅	73.8 ± 5.3 ^b	-15.0	68.4 ± 5.5 ⁿ	4.6
22	4-CH ₃ C ₆ H ₄	66.6 ± 7.8 ^d	2.2	65.0 ± 7.0 ^r	-3.5
23	4-CH ₃ OC ₆ H ₄	69.4 ± 5.8 ^d	-1.9	62.6 ± 5.1 ^r	0.3
24	4-ClC ₆ H ₄	66.4 ± 7.1 ^d	2.5	54.8 ± 5.4 ^o	11.6
25	4-NO ₂ C ₆ H ₄	85.7 ± 9.7 ^d	-25.8	67.6 ± 6.0 ^r	-7.6
26	4-NH ₂ C ₆ H ₄	79.0 ± 2.2 ^g	0	76.9 ± 3.0 ^m	-5.5
27	3-CH ₃ C ₆ H ₄	61.3 ± 2.8 ^b	4.5	61.5 ± 4.3 ⁿ	14.2
28	3-CH ₃ OC ₆ H ₄	54.0 ± 6.7 ^d	20.7	59.2 ± 3.8 ^r	5.7
29	3-ClC ₆ H ₄	77.9 ± 0.4 ^h	13.4	79.0 ± 2.1 ^g	7.6
30	3-NO ₂ C ₆ H ₄	83.6 ± 2.6 ^h	7.4	80.6 ± 2.3 ^g	5.7
31	2-CH ₃ C ₆ H ₄	81.2 ± 4.2 ^h	9.8	75.5 ± 3.4 ^g	11.7
32	2-CH ₃ OC ₆ H ₄	70.7 ± 3.4 ^b	-10.1	65.9 ± 3.4 ⁿ	8.1
14	2-pyridyl	54.8 ± 4.0 ^b	14.6	81.4 ± 3.1 ⁿ	-13.5
33	3-pyridyl	44.4 ± 2.9 ^{b,i}	30.8	50.0 ± 2.2 ^{n,i}	30.3
34	4-pyridyl	48.6 ± 2.8 ^{b,i}	24.3	46.7 ± 2.6 ^{n,i}	34.9
35	2-thiazolyl	69.9 ± 1.6 ^j	2.5	66.3 ± 5.2 ^t	4.6
36	2-pyrazyl	50.6 ± 2.6 ^{b,c}	21.2	50.5 ± 3.5 ^{n,i}	29.6
37	CH ₂ -3-pyridyl	62.8 ± 4.0 ^f	14.7	54.3 ± 4.3 ^{m,i}	25.5
38	CH ₂ -4-pyridyl	54.3 ± 3.7 ^b	15.4	64.5 ± 3.9 ⁿ	10.0
39	3-CH ₃ -4-pyridyl	64.6 ± 2.8 ^h	8.8	53.3 ± 2.8 ^{m,i}	26.9
40	2-CH ₃ O-5-pyridyl	72.3 ± 5.0 ^g	-10.2	53.0 ± 1.3 ^q	13.1
41	2-Cl-5-pyridyl	66.8 ± 3.3 ^b	-4.0	65.1 ± 1.7 ⁿ	9.2
10		63.1 ± 4.2 ⁱ	15.5	47.2 ± 2.0 ^u	23.5

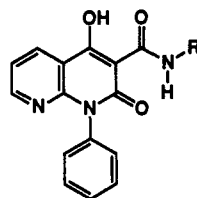
^a Test compounds were administered orally 1 h before the subplantar injection of the irritants. Edema density of the paw was calculated as [(the volume at 3 h) - (the volume at 0 h)] / (the latter volume). Each value represents the mean ± SEM in groups of rats. ^b Control value of swelling was 64.2 ± 1.6%. ^c Significant differences from the control at *P* < 0.05. ^d Control value of swelling was 68.1 ± 5.3%. ^e Control value of swelling was 81.0 ± 3.5%. ^f Control value of swelling was 65.6 ± 2.7%. ^g Control value of swelling was 73.7 ± 3.6%. ^h Control value of swelling was 90.0 ± 3.6%. ⁱ Significant differences from the control at *P* < 0.01. ^j Control value of swelling was 63.7 ± 4.0%. ^k Control value of swelling was 71.9 ± 7.9%. ^l Control value of swelling was 74.7 ± 3.7%. ^m Control value of swelling was 72.9 ± 3.5%. ⁿ Control value of swelling was 71.7 ± 1.4%. ^o Control value of swelling was 62.0 ± 2.3%. ^p Control value of swelling was 52.2 ± 3.5%. ^q Control value of swelling was 61.0 ± 3.9%. ^r Control value of swelling was 62.8 ± 2.3%. ^s Control value of swelling was 85.5 ± 3.2%. ^t Control value of swelling was 69.5 ± 6.9%. ^u Control value of swelling was 62.0 ± 2.3%.

The antiinflammatory activity of compounds at 100 mg/kg is summarized in Tables II and III. Ethyl ester 10, which is a starting material of 7, did not exhibit antiinflammatory activity in the CPE and ZPE assays (Table II). On the other hand, the nature of substituents (R) on the amide nitrogen of 7 had a pronounced effect on antiinflammatory activity. Compounds which possessed alkyl groups (R) were slightly active only against CPE (15 and 16). Increasing the size of the lipophilic group reduced activity (17). Introduction of an aliphatic base into the amide moiety did not improve antiinflammatory activity in CPE, ZPE, and RPAR assays (18–20). Substitution of the amide function with phenyl derivatives produced less active or totally inactive compounds (21–32). However, introduction of basic aromatic rings such as pyridine and pyrazole improved antiinflammatory activity (33, 34, and 36). 2-Pyrazyl derivative 36 had moderate antiinflammatory activity in both CPE and ZPE assays. 3-Pyridyl and 4-pyridyl derivatives (33 and 34) exhibited potent inhibition against CPE, ZPE, and RPAR. On the other hand, the 2-pyridyl derivative 14 lost activity in CPE and ZPE assays. This result is greatly contrasted with that of piroxicam (2-pyridyl derivative).¹⁹ Introduction of sub-

stituents into the 3-pyridyl or 4-pyridyl ring abolished or reduced biological activity (37, 38, 40, and 41). It is interesting to note that the 3-methyl-4-pyridyl derivative 39 inhibited ZPE and RPAR except CPE. Structure-activity relationships of substituents on the amide nitrogen were narrow, and only two pyridyl derivatives (33 and 34) exhibited broad antiinflammatory activity in these three assays.

Selected compounds from the primary assays such as 33 and 34 were analyzed further in detail (Table IV and Table V). Arachidonic acid-induced paw edema (APE) is known to be involved by lipoxygenase products and mast cell mediators.²⁰ Thus the APE assay was added in these assays. A typical CO inhibitor piroxicam (1), which is an acidic NSAID, appears to be active only in the CPE assay

- (19) Lombardino, J. G.; Wiseman, E. H. Sudoxicam and Related N-Heterocyclic Carboxamides of 4-Hydroxy-2*H*-1,2-benzothiazine 1,1-Dioxide. Potent Nonsteroidal Antiinflammatory Agents. *J. Med. Chem.* 1972, 15, 848–849.
- (20) Dimartino, M. J.; Campbell, G. K.; Wolff, Jr., C. E.; Hanna, N. The Pharmacology of Arachidonic Acid-Induced Rat Paw Edema. *Agents Actions* 1987, 27, 303–309.

Table III. Effects of 4-Hydroxy-2(1H)-oxo-1-phenyl-1,8-naphthyridine-3-carboxamide Derivatives on Rat Reversed Passive Arthus Pleurisy at 100 mg/kg po

compd	R	exudate vol, ^a mL	inhibn, %	no. of cells, ^a × 10 ⁶	inhibn, %
15	CH ₃	0.42 ± 0.10 ^b	22.3	2.23 ± 0.10 ^k	3.2
16	<i>n</i> -C ₄ H ₉	0.72 ± 0.01 ^c	-36.7	3.82 ± 0.18 ^l	7.7
17	CH ₂ C ₆ H ₅	0.61 ± 0.09 ^c	-14.3	3.37 ± 0.26 ^l	9.6
18	CH ₂ CH ₂ N(CH ₃) ₂	0.94 ± 0.12 ^d	-10.4	4.35 ± 0.06 ^l	-16.6
19	1-benzyl-4-piperidinyl	0.33 ± 0.10 ^e	32.4	1.69 ± 0.09 ^m	22.1
20	4-morpholyl	0.43 ± 0.08 ^f	14.6	3.02 ± 0.17 ⁿ	10.4
22	4-CH ₃ C ₆ H ₄	0.65 ± 0.07 ^g	22.4	4.66 ± 1.15 ^o	21.5
23	4-CH ₃ OC ₆ H ₄	0.80 ± 0.09 ^g	5.1	4.47 ± 0.46 ^o	25.4
24	4-ClC ₆ H ₄	0.62 ± 0.09 ^g	-16.3	3.43 ± 0.11 ^l	18.8
25	4-NO ₂ C ₆ H ₄	0.71 ± 0.07 ^g	16.7	4.81 ± 0.21 ^o	18.5
27	3-CH ₃ C ₆ H ₄	0.65 ± 0.06 ^d	27.3	3.02 ± 0.16 ^p	28.5
28	3-CH ₃ OC ₆ H ₄	0.63 ± 0.04 ^g	26.9	4.63 ± 0.22 ^o	22.1
29	3-ClC ₆ H ₄	0.82 ± 0.04 ^d	5.2	3.22 ± 0.23 ^q	21.7
30	3-NO ₂ C ₆ H ₄	0.66 ± 0.05 ^h	19.1	3.36 ± 0.24 ^q	15.4
31	2-CH ₃ C ₆ H ₄	0.73 ± 0.82 ^d	16.9	3.37 ± 0.24 ^p	16.6
32	2-CH ₃ OC ₆ H ₄	0.71 ± 0.05 ^g	16.7	4.40 ± 0.21 ^o	26.8
33	3-pyridyl	0.28 ± 0.06 ^{d,i}	75.3	2.96 ± 0.16 ^p	30.5
34	4-pyridyl	0.08 ± 0.01 ^{d,i}	100	2.13 ± 0.24 ^{p,r}	58.6
35	2-thiazolyl	0.64 ± 0.03 ^h	18.1	3.77 ± 0.24 ^q	15.4
36	2-pyrazyl	0.37 ± 0.03 ^e	24.3	1.59 ± 0.13 ^m	26.7
37	CH ₂ -3-pyridyl	0.58 ± 0.03 ^j	29.3	3.44 ± 0.52 ^r	53.5
38	CH ₂ -4-pyridyl	0.64 ± 0.06 ^j	22.0	3.74 ± 0.61 ^s	49.5
39	3-CH ₃ -4-pyridyl	0.28 ± 0.04 ^{j,i}	65.9	1.19 ± 0.07 ^{s,i}	83.9
40	2-CH ₃ O-5-pyridyl	0.66 ± 0.11 ^j	19.5	5.76 ± 1.86 ^s	22.2
41	2-Cl-5-pyridyl	0.65 ± 0.04 ^j	20.7	6.01 ± 0.71 ^s	18.8

^a Test compounds were administered orally 1 h before rabbit anti-EA antiserum was injected intrapleurally. Each value represents the mean ± SEM in groups of rats. ^b Control value of exudate volume was 0.52 ± 0.05 mL. ^c Control value of exudate volume was 0.54 ± 0.08 mL. ^d Control value of exudate volume was 0.86 ± 0.11 mL. ^e Control value of exudate volume was 0.46 ± 0.10 mL. ^f Control value of exudate volume was 0.49 ± 0.08 mL. ^g Control value of exudate volume was 0.84 ± 0.01 mL. ^h Control value of exudate volume was 0.79 ± 0.05 mL. ⁱ Significant differences from the control at *P* < 0.01. ^j Control value of exudate volume was 0.82 ± 0.15 mL. ^k Control value of no. of cells was 2.28 ± 0.08 × 10⁶ cells. ^l Control value of no. of cells was 3.62 ± 0.25 × 10⁶ cells. ^m Control value of no. of cells was 2.17 ± 0.22 × 10⁶ cells. ⁿ Control value of no. of cells was 3.27 ± 0.37 × 10⁶ cells. ^o Control value of no. of cells was 5.72 ± 0.34 × 10⁶ cells. ^p Control value of no. of cells was 3.86 ± 0.33 × 10⁶ cells. ^q Control value of no. of cells was 3.86 ± 0.33 × 10⁶ cells. ^r Significant differences from the control at *P* < 0.05. ^s Control value of no. of cells was 7.40 ± 1.17 × 10⁶ cells.

Table IV. Effects of Antiinflammatory Drugs on the Development of Carrageenin-, Zymosan-, and Arachidonic Acid-Induced Rat's Paw Edema

compd	dose (mg/kg po)	CPE		ZPE		APE	
		swelling, ^a %	inhibn, %	swelling, ^a %	inhibn, %	swelling, ^a %	inhibn, %
33	100	44.4 ± 2.9 ^{b,c}	30.8	50.0 ± 2.2 ^{e,c}	30.3		
	30	50.2 ± 1.8 ^{d,c}	33.8	38.2 ± 7.2 ^{h,c}	43.9	30.6 ± 2.9 ^{b,c}	40.7
	10	58.6 ± 2.6 ^{d,e}	22.7	50.8 ± 2.6 ^{h,e}	25.4	28.9 ± 3.8 ^{h,c}	44.0
34	3	60.5 ± 5.5 ^d	20.2	53.2 ± 6.4 ^h	21.9	30.5 ± 2.8 ^{h,c}	40.9
	100	48.6 ± 2.8 ^{b,c}	24.3	46.7 ± 2.6 ^{g,c}	34.9		
	30	58.8 ± 3.3 ^{d,e}	22.4	48.5 ± 3.0 ^{h,e}	28.8	29.1 ± 1.6 ^{h,c}	43.6
piroxicam (1)	10	71.3 ± 4.5 ^d	5.9	54.3 ± 3.4 ^h	20.3	25.5 ± 2.1 ^{h,c}	50.6
	3	72.1 ± 5.1 ^d	4.9	60.6 ± 1.6 ^h	11.9	33.0 ± 2.4 ^{h,c}	36.0
	30	25.8 ± 5.2 ^{d,c}	66.0	50.3 ± 2.0 ⁱ	8.7		
tiaramide	10	34.4 ± 4.2 ^{d,c}	54.6	61.7 ± 3.9 ⁱ	9.4	42.7 ± 2.9 ^h	17.2
	3	60.6 ± 2.7 ^f	13.3	53.9 ± 2.6 ⁱ	2.2		
	100	64.0 ± 3.6 ^f	8.4	51.5 ± 3.3 ^{j,e}	23.0	26.7 ± 2.1 ^{h,c}	48.3
tenidap (3)	30	29.7 ± 3.0 ^{f,c}	57.5	56.8 ± 4.8 ^f	15.1	32.1 ± 2.1 ^l	5.0
	10	51.0 ± 5.0 ^{f,c}	27.0	40.1 ± 2.1 ^{j,e}	40.0	35.5 ± 3.0 ^{m,e}	19.8
	3	64.5 ± 6.7 ^f	7.7	49.4 ± 3.0 ^{h,c}	26.2	32.0 ± 2.3 ^{m,c}	27.7
				50.8 ± 2.2 ^{j,c}	24.1	38.8 ± 2.1 ^m	12.4

^a See footnote a in Table II. ^b Control value of swelling was 64.2 ± 1.6%. ^c Significant differences from the control at *P* < 0.01. ^d Control value of swelling was 75.8 ± 3.8%. ^e Significant differences from the control at *P* < 0.05. ^f Control value of swelling was 69.9 ± 2.9%. ^g Control value of swelling was 64.2 ± 1.6%. ^h Control value of swelling was 68.1 ± 2.9%. ⁱ Control value of swelling was 55.1 ± 4.4%. ^j Control value of swelling was 66.9 ± 6.0%. ^k Control value of swelling was 51.6 ± 2.0%. ^l Control value of swelling was 33.8 ± 2.1%. ^m Control value of swelling was 44.3 ± 2.3%.

among three paw edema assays. The CO inhibitor indomethacin was inactive in the RPAR assay. Although a nonacidic NSAID, tiaramide inhibited ZPE, APE, and

RPAR at a high dose (100 mg/kg), it was inactive in the CPE assay. Tenidap (3), which is a dual inhibitor, exhibited antiinflammatory activity in the CPE and ZPE

Table V. Effects of Antiinflammatory Drugs on Rat Reversed Passive Arthus Pleurisy

compd	dose (mg/kg po)	Exudate vol, ^a mL	inhibn, %	no. of cells, ^a × 10 ⁶	inhibn, %
33	100	0.28 ± 0.06 ^{b,c}	75.3	2.96 ± 0.16 ^j	30.5
	25	0.32 ± 0.07 ^{d,e}	62.9	4.25 ± 0.10 ^{k,e}	42.1
	10	0.37 ± 0.07 ^{d,e}	55.7	4.30 ± 0.41 ^{k,e}	41.2
	5	0.40 ± 0.04 ^{f,e}	50.0	3.54 ± 0.06 ^{l,e}	33.3
34	100	0.08 ± 0.01 ^{b,c}	100	2.13 ± 0.24 ^{j,e}	58.6
	25	0.40 ± 0.07 ^d	51.4	4.74 ± 0.24 ^k	33.3
	10	0.53 ± 0.05 ^d	32.9	4.60 ± 0.25 ^k	35.8
	5	0.73 ± 0.03 ^d	-3.2	2.79 ± 0.19 ^j	26.0
tiaramide	100	0.12 ± 0.03 ^{b,c}	94.9	1.52 ± 0.57 ^{m,c}	67.2
	30	0.33 ± 0.07 ^e	59.3	2.79 ± 0.18 ^m	9.8
	10	0.44 ± 0.07 ^e	40.7	3.18 ± 0.22 ^m	2.3
	3	0.60 ± 0.04 ^e	13.6	3.17 ± 0.27 ^m	2.7
indomethacin	10	0.44 ± 0.03 ^h	23.9	3.20 ± 0.15 ⁿ	-20.9
	3	0.59 ± 0.06 ^h	-8.7	4.01 ± 0.47 ⁿ	-60.2
tenidap (3)	30	0.39 ± 0.06 ⁱ	37.1	4.06 ± 0.53 ^o	-4.1
	10	0.33 ± 0.08 ⁱ	46.8	4.37 ± 0.41 ^o	-12.1

^a See footnote a in Table III. ^b Control value of exudate volume was 0.86 ± 0.11 mL. ^c Significant differences from the control at $P < 0.01$. ^d Control value of exudate volume was 0.76 ± 0.12 mL. ^e Significant differences from the control at $P < 0.05$. ^f Control value of exudate volume was 0.71 ± 0.18 mL. ^g Control value of exudate volume was 0.68 ± 0.05 mL. ^h Control value of exudate volume was 0.55 ± 0.04 mL. ⁱ Control value of exudate volume was 0.62 ± 0.06 mL. ^j Control value of no. of cells was 3.86 ± 0.33 × 10⁶ cells. ^k Control value of no. of cells was 6.58 ± 0.65 × 10⁶ cells. ^l Control value of no. of cells was 3.54 ± 0.17 × 10⁶ cells. ^m Control value of no. of cells was 3.24 ± 0.29 × 10⁶ cells. ⁿ Control value of no. of cells was 2.77 ± 0.22 × 10⁶ cells. ^o Control value of no. of cells was 3.90 ± 0.44 × 10⁶ cells.

assays and moderate activity in the APE assay. Thus tenidap is proven to be a new type of NSAID. However, it had little effect on RPAR. On the other hand, compounds 33 and 34 inhibit CPE, ZPE, APE, and RPAR. Thus they possess a broader antiinflammatory profile than that of tenidap or other NSAIDs in these assays. In addition, with respect to the potency of inhibition against CPE and APE, difference was observed between 33 (or 34) and tenidap. Tenidap is more potent in the CPE assay than in the APE assay. On the other hand, 33 and 34 are more potent in the APE assay than in the CPE assay. Furthermore, 33 and 34 did not show any acute lethal toxicity in mice at 300 mg/kg (po).

The mechanism by which 33 and 34 exhibit antiinflammatory activity is unclear. Inhibitory doses for RPAR were almost equal to those for zymosan-mediated reactions (ZPE). These results suggest that the inhibitory effect of 33 and 34 on RPAR or ZPE is due to inhibition of complement-induced vascular permeability. Compounds 33 and 34 potently suppressed APE and RPAR which might be inflammatory reactions induced by leukotrienes. Thus their effects on 5-LO and CO enzymes were examined. In contrast to in vitro inhibitory activity of tenidap against CO enzyme (IC₅₀ = 0.01 μM) and 5-LO enzyme (IC₅₀ = 9.0 μM),¹⁴ 33 and 34 did not show significant inhibition against these enzymes at 100 μM. Furthermore, 33 and 34 did not suppress immunoreactions such as the plaque-forming reaction²¹ at 10 μM and do not have an effect on receptor binding by a variety of ligands including histamine, muscarine, adenosine, and catecholamines. These results suggest that the antiinflammatory mechanism of 33 and 34 is different from those of classical NSAIDs, dual inhibitors, immunomodulators, and antagonists of mediators.

We described the design, syntheses, and antiinflammatory activity of 4-hydroxy-2(1H)-oxo-1-phenyl-1,8-naphthyridine-3-carboxamides. Structure-activity relationships have led to 4-hydroxy-2(1H)-oxo-1-phenyl-N-(3-pyridyl)-1,8-naphthyridine-3-carboxamide (33, KF17515), which exhibited potent antiinflammatory activity in a broad range of animal models (CPE, ZPE, APE, and RPAR assays) but did not show significant inhibitory

activity against CO and LO enzymes. Current research is dedicated toward further mechanistic studies and examining the in vivo metabolism/pharmacokinetics and the toxicology of these agents. These studies will make clear whether compound 33 is an interesting agent for therapeutic use.

Experimental Section

Melting points were determined on a Yanagimoto hot plate micro melting point apparatus and are uncorrected. Infrared (IR) spectra were measured on a JASCO IR-810 spectrometer. Proton nuclear magnetic resonance (¹H-NMR) spectra were measured on a JEOL JNM GX-270 spectrometer or a Hitachi R-90H spectrometer with tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were determined on a JEOL JMS-D300 instrument at an ionization potential of 70eV. Elemental analyses were performed with a Perkin-Elmer 2400CHN. For column chromatography, Silica gel 60 (E. Merck, 0.063–0.200 mm) was used, and elution was carried out with 1–10% MeOH-chloroform unless otherwise noted. The reactions were usually carried out under nitrogen. Organic extracts were dried over anhydrous sodium sulfate and concentrated by a rotary evaporator.

1-Phenyl-2H-pyrido[2,3-d][1,3]oxazine-2,4(1H)-dione (9). To a solution of 7.0 g (0.031 mol) of methyl 2-anilino nicotinate 8 in 150 mL of dry 1,2-dichloroethane was slowly added 11 mL (0.092 mol) of TCF at 80 °C. The solution was stirred for 3 h at this temperature. After cooling, 0.25 g of activated carbon was added, and the solution was refluxed for 30 min. After cooling, the solvent was evaporated under reduced pressure. The residue was recrystallized from dichloromethane-isopropyl ether to give 6.5 g (87%) of colorless crystals 9: mp 196–198 °C; IR (KBr) 1791, 1727 cm⁻¹; NMR (CDCl₃) δ 7.10–7.70 (m, 6 H), 8.47 (dd, 1 H, J = 6, 2 Hz), 8.57 (dd, 1 H, J = 5, 2 Hz). Anal. (C₁₃H₉N₂O₃) C, H, N.

3-(Ethoxycarbonyl)-4-hydroxy-1-phenyl-1,8-naphthyridin-2(1H)-one (10). To a mixture of 25 mL of dimethylacetamide and 25 mL (0.16 mol) of diethyl malonate was added 0.80 g (0.020 mol) of 60 wt % sodium hydride under ice-cooling. After evolution of hydrogen ceased, 4.0 g (0.017 mol) of 9 was added to the solution. The temperature was gradually elevated, and the solution was heated at 150 °C for 2.5 h. After the solution was cooled, 100 mL of ethyl acetate was added. The resulting precipitate was obtained by filtration and dissolved in 100 mL of water. The solution was made acidic with concentrated hydrochloric acid, and the resulting precipitate was filtered, washed with water, and dried. Recrystallization from isopropyl alcohol-ethyl alcohol gave 4.3 g (88%) of 10 as colorless crystals: mp 247–252 °C; IR (KBr) 1670, 1615 cm⁻¹; NMR (DMSO-d₆) δ 1.29 (t, 3 H, J = 7 Hz), 4.31 (q, 2 H, J = 7 Hz), 7.21–7.26 (m, 2

(21) Yamamoto, I.; Ohmori, H.; Sasano, M. CCA: An Immunopharmacological Profile in Vivo and in Vitro. *Drugs Exp. Clin. Res.* 1982, 8, 5–10.

H), 7.32 (dd, 1 H, $J = 8, 5$ Hz), 7.38–7.56 (m, 3 H), 8.44 (dd, 1 H, $J = 8, 2$ Hz), 8.49 (dd, 1 H, $J = 5, 2$ Hz). Anal. ($C_{17}H_{14}N_2O_4$) C, H, N.

Method A. *N*-Butyl-4-hydroxy-2(1*H*)-oxo-1-phenyl-1,8-naphthyridine-3-carboxamide (16). A mixture of 7.5 g (5.1 mmol) of 10 and 0.27 mL (15 mmol) of butylamine in 30 mL of xylene was refluxed for 2 h. After cooling, the resulting precipitate was filtered, washed with ethyl acetate, and dried. Recrystallization from water–ethyl alcohol gave 1.1 g (62%) of 16 as colorless crystals: mp 181–184 °C; IR (KBr) 1622, 1554 cm^{-1} ; NMR ($C_2F_5CO_2D$) δ 1.02 (t, 3 H, $J = 7$ Hz), 1.40–1.57 (m, 2 H), 1.69–1.81 (m, 2 H), 3.63 (t, 2 H, $J = 7$ Hz), 7.50–7.54 (m, 2 H), 7.77–7.93 (m, 4 H), 8.63 (dd, 1 H, $J = 5, 2$ Hz), 9.40 (dd, 1 H, $J = 8, 2$ Hz). Anal. ($C_{19}H_{19}N_3O_3$) C, H, N.

Method B. 4-Hydroxy-1-phenyl-1,8-naphthyridin-2(1*H*)-one (11). A suspension of 2.0 g (0.068 mol) of 10 in 70 mL of 2 N sodium hydroxide solution was refluxed for 1 h. After cooling, 2 N hydrochloric acid was added to neutralize the mixture. The resulting precipitate was filtered, washed with water, and dried. Recrystallization from water–dimethyl sulfoxide gave 1.4 g (86%) of 11 as colorless crystals: mp 300 °C; IR (KBr) 1680, 1641, 1615 cm^{-1} ; NMR (DMSO- d_6) δ 5.95 (s, 1 H), 7.18–7.28 (m, 3 H), 7.37–7.53 (m, 3 H), 8.26 (dd, 1 H, $J = 8, 2$ Hz), 8.40 (dd, 1 H, $J = 5, 2$ Hz), 11.79 (br s, 1 H). Anal. ($C_{19}H_{19}N_3O_3 \cdot \frac{1}{5}H_2O$) C, H, N.

***N*-(4-Chlorophenyl)-4-hydroxy-2(1*H*)-oxo-1-phenyl-1,8-naphthyridine-3-carboxamide (24).** To a mixture of 1.5 g (6.3 mmol) of 11 and 0.92 mL (6.6 mmol) of triethylamine in 20 mL of dimethyl sulfoxide was added 0.85 mL (6.0 mmol) of 4-chlorophenyl isocyanate. The mixture was stirred at room temperature for 12 h. After the mixture was poured into 150 mL of 4 N hydrochloric acid solution, the resulting precipitate was filtered and washed with ethyl acetate. Recrystallization from water–dimethyl sulfoxide gave 1.4 g (56%) of 24 as colorless crystals: mp 281–286 °C; IR (KBr) 1660, 1593, 1548 cm^{-1} ; NMR (CF_3CO_2D) δ 7.42–7.57 (m, 6 H), 7.81–7.86 (m, 3 H), 7.94 (dd, 1 H, $J = 8, 6$ Hz), 8.69 (dd, 1 H, $J = 6, 2$ Hz), 9.43 (dd, 1 H, $J = 8, 2$ Hz). Anal. ($C_{21}H_{14}N_3O_3Cl$) C, H, N.

Method C. 2-(Ethoxycarbonyl)-*N*-(2-pyridyl)acetamide (13). To a mixture of 4.0 g (43 mmol) of 2-aminopyridine 12, 7.7 mL (55 mmol) of triethylamine, and 0.52 g (4.2 mmol) of 4-(dimethylamino)pyridine in 50 mL of tetrahydrofuran was added 5.4 mL (55 mmol) of ethyl malonyl chloride at 0 °C. The mixture was stirred at room temperature for 4 h. Water was added, and the mixture was extracted with ethyl acetate. The organic phase was reextracted with 2 N hydrochloric acid solution. The aqueous mixture was neutralized with 4 N sodium hydroxide solution and extracted with ethyl acetate. The organic phase was washed with water, dried, and concentrated under reduced pressure. The residue was chromatographed on a column of silica gel using chloroform–methyl alcohol = 50/1 to give 3.9 g (44%) of 13 as a colorless oil; MS m/z 208 (M^+); NMR ($CDCl_3$) δ 1.30 (t, 3 H, $J = 7$ Hz), 3.52 (s, 2 H), 4.24 (q, 2 H, $J = 7$ Hz), 7.03–7.08 (m, 1 H), 7.67–7.74 (m, 1 H), 8.20 (d, 1 H, $J = 6$ Hz), 8.29–8.31 (m, 1 H), 9.83 (br s, 1 H).

4-Hydroxy-2(1*H*)-oxo-1-phenyl-*N*-(2-pyridyl)-1,8-naphthyridine-3-carboxamide (14). To a solution of 1.9 g (9.2 mmol) of 13 in 40 mL of dry dimethylacetamide was added 1.0 g (25 mmol) of 60 wt % sodium hydride at 0 °C in portions. When the evolution of hydrogen ceased, 2.0 g (8.3 mmol) of 9 was added. The temperature was raised slowly to 110 °C and kept there for 1 h (carbon dioxide evolution occurred). The solvent was evaporated under reduced pressure, and 50 mL of water and 50 mL of ethyl acetate were added to the residue. The resulting precipitate was filtered, washed with water, and recrystallized from dimethylformamide to give 0.88 g (30%) of 14 as yellow crystals: mp >300 °C; IR (KBr) 1652, 1520, 1491 cm^{-1} ; NMR (CF_3CO_2D) δ 7.53–7.56 (m, 2 H), 7.82–7.87 (m, 3 H), 7.98–8.05 (m, 2 H), 8.56–8.63 (m, 2 H), 8.77 (d, 1 H, $J = 6$ Hz), 9.45–9.48 (m, 1 H). Anal. ($C_{20}H_{14}N_4O_3$) C, H, N.

Rat Paw Edema Induced by Carrageenin, Zymosan, and Arachidonic Acid. Carrageenin-, zymosan-, and arachidonic acid-induced paw edemas were conducted by the methods of Winter et al.,³ Gemmell et al.,⁴ and DiMartino et al.,²⁰ respectively. Male Wistar rats weighing about 150 g were used. Test com-

pounds suspended in 5% arabic gum solution were administered orally 1 h before 1% λ -carrageenin (picnin A, Zushi Kagaku), 1% zymosan (zymosan A, sigma), or 1% arachidonic acid (arachidonic acid sodium salt, sigma) in 0.1 mL physiological saline (saline) were injected subplantarily into the hind paw. The paw volumes were measured by a plethamograph immediately before the drugs were administered, and 3, 4, and 1 h after carrageenin, zymosan, and arachidonic acid were injected, respectively. The edema volume was assessed by the difference between the paw volume before and that after the injection of irritants. The percentage of inhibition was calculated from the difference in mean swelling values between the test compound-treated animals and the control group.

Rabbit Anti-EA Antiserum. Rabbit anti-EA (egg albumin) antiserum was produced by the method of Koda et al.²² Rabbits were immunized with an injection of a 1 mL suspension with an equal volume of saline containing EA (2 mg/mL) and FCA (Freund complete adjuvant) into each gluteus muscle at weekly intervals for 4 weeks. One week after the last immunization, the serum was obtained and pooled.

Rat Reversed Passive Arthus Pleurisy. This assay was conducted as described by Yamamoto et al.²³ Male Wistar rats weighing about 250 g were anesthetized with ether and 0.2 mL of rabbit anti-EA antiserum injected into the pleural cavity. Thirty minutes later, 0.1 mL of saline containing 15 mg of EA/100 g body weight was injected intravenously. The rats were killed by decapitation 4.5 h after the pleurisy was induced. The thoracic cavity was rinsed with 5 mL of saline. The volume of exudate and the number of infiltrated inflammatory cells were measured. The test compounds were orally administered 30 min before intrapleural injection of rabbit anti-EA antiserum. The percentage inhibition of exudate volume and number of cells in the pleurisy cavity of rats was the indicator for antiinflammatory activity.

Statistical Analysis. The values obtained were expressed as means \pm standard errors. Scheffe's multiple range test²⁴ was used for statistical comparisons.

Acknowledgment. We thank Dr. I. Miki, Dr. H. Nakajima, and Dr. S. Sato for their assistance in biochemical and pharmacological assays. We also thank K. Takada and Y. Watanabe for their technical assistance, H. Hayashi and J. Shimada for their careful reading of the manuscript, and Dr. T. Hirata for encouragement and support.

Registry No. 8, 51269-84-2; 9, 138305-19-8; 10, 138305-20-1; 11, 89109-17-1; 12, 504-29-0; 13, 138305-21-2; 14, 138305-18-7; 15, 138304-88-8; 16, 138304-87-7; 17, 138304-89-9; 18, 138304-93-5; 19, 138305-07-4; 20, 138305-13-2; 21, 138305-06-3; 22, 138305-03-0; 23, 138305-02-9; 24, 138305-00-7; 25, 138305-01-8; 26, 138305-16-5; 27, 138304-95-7; 28, 138305-04-1; 29, 138304-94-6; 30, 138304-97-9; 31, 138304-96-8; 32, 138305-05-2; 33, 138304-90-2; 34, 138304-91-3; 35, 138304-98-0; 36, 138305-09-6; 37, 138305-15-4; 38, 138305-14-3; 39, 138305-17-6; 40, 138305-11-0; 41, 138305-12-1; TCF, 503-38-8; PhNCO, 103-71-9; *p*- $CH_3C_6H_4NCO$, 622-58-2; *p*- $CH_3OC_6H_4NCO$, 5416-93-3; *p*- $NO_2C_6H_4NCO$, 100-28-7; *m*- $CH_3OC_6H_4NCO$, 18908-07-1; *o*- $CH_3OC_6H_4NCO$, 700-87-8; ethylmalonyl chloride, 36239-09-5; diethyl malonate, 105-53-3; 4-chlorophenyl isocyanate, 104-12-1; 1-(phenylmethyl)-4-piperidinamine, 50541-93-0; 4-morpholinamine, 4319-49-7; 1,4-benzenediamine, 106-50-3; 3-pyridinamine, 462-08-8; 4-pyridinamine, 504-24-5; 2-thiazolamine, 96-50-4; 2-aminopyrazine, 5049-61-6; 3-pyridomethylamine, 3731-52-0; 4-pyridinemethanamine, 3731-53-1; 3-methyl-4-pyridinamine, 1990-90-5; 6-methoxy-3-pyridinamine, 6628-77-9; 6-chloro-3-pyridinamine, 5350-93-6.

(22) Koda, A.; Nagai, H.; Wada, H. *Pharmacological Actions of Baicalin and Baicalein. II. Active Anaphylaxis*. *Nippon Yakurigaku Zasshi* 1970, 66, 237–247.

(23) Yamamoto, S.; Dunn, C. J.; Deporter, D. A.; Capasso, F.; Willoughby, D. A.; Huskisson, E. C. A Model for the Quantitative Study of Arthus (Immunologic) Hypersensitivity in Rats. *Agents Actions* 1975, 5, 374–377.

(24) Wallestein, S.; Zucker, C. L.; Fleiss, J. L. Some Statistical Methods Useful in Circulation Research. *Circ. Res.* 1980, 47, 1–9.