1.68-1.72 (m, 1 H, CH_2), 1.86-2.01 (m, 1 H, CH_2), 2.04-2.27 (m, 5 H, CH_2), 2.35-2.64 (m, 4 H, CH_2), 2.91-3.05 (m, 1 H, CH), 3.23-3.34 (m, 1 H, CH_2), 3.60-3.68 (m, H, 5-CH), 7.55 (d, J = 8.5 Hz, 2 H, aromatic CH), 7.73 (br s, 2 H, aromatic CH), 12.5 (br s, 1 H, NH^+). Anal. ($C_{14}H_{16}BrN$·HCl) C, H, N.

***trans*-3-Cyclohexyloctahydroindolizine Hydrochloride (12).** A solution of 20 g of **3b** in 96 mL of glacial HOAc was hydrogenated over 2.95 g of 5% Rh on C at 60 psi. Daily additions of 2.95 g of 5% Rh on C were made for 4 days. On day 5, 5.9 g of catalyst was added. After 13 days total, the catalyst was filtered and the solvent evaporated. The residue was partitioned between NaOH solution and Et_2O. The Et_2O solution was washed with brine, dried (K_2CO_3), and evaporated. The residue was distilled in a Kugelrohr at 110-160 °C (1.2 Torr). The distillate was purified by preparative HPLC using 5% EtOAc in hexane as eluant. The solvent was evaporated and a salt prepared from MeOH/EtOAc/HCl. The salt was recrystallized twice from CH_2Cl_2/THF to give 2.74 g (14% yield) of white solid: mp 199-202 °C; ^1H NMR ($CDCl_3$) δ 1.0-2.30 (m, 19 H, CH_2), 2.45-2.90 (m, 5 H, CH, CH_2), 3.8-3.92 (m, 1 H, CH), 11.6 (s, 1 H, NH^+). Anal. ($C_{14}H_{25}$·HCl) C, H, N.

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Supplementary Material Available: Tables listing atomic coordinates for non-hydrogen and hydrogen atoms in crystalline $BrC_{14}H_{19}NCl$, bond lengths, and band angles (5 pages). Ordering information is given on any current masthead page.

New Antiinflammatory Agents. 2.† 5-Phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-ones: A New Class of Nonsteroidal Antiinflammatory Agents with Potent Activity Like Glucocorticoids

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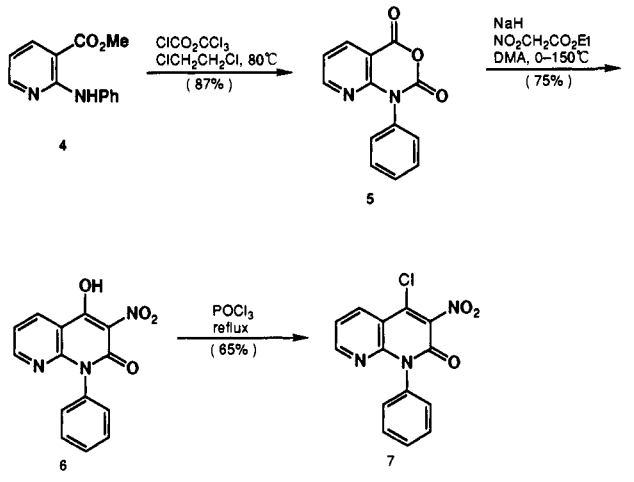
We previously described new antiinflammatory agents, 4-hydroxy-2-oxo-1-phenyl-1*H*-1,8-naphthyridine-3-carboxamides 1. Further modification of the compounds bearing 1-phenyl-1,8-naphthyridin-2-one as a mother skeleton led to 5-phenylimidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one derivatives **2** and **3**. Regioselective synthesis of these compounds bearing a substituent at the 1- or 3-position was conducted according to the method shown in Schemes I and II. In this series of compounds, antiinflammatory activities were greatly influenced by the position and nature of substituents on imidazole. 3-Alkyl or 3-benzyl substitution resulted in the potent activity, but 1-substitution did not. Minor modification of the benzyl group reduced or eliminated the activity. Detailed examination of structure–activity relationships led to 3-benzyl-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (**22**), which exhibited potent oral antiinflammatory activities in carrageenan-, zymosan-, and reversed passive Arthus reaction-induced rat paw edemas (ED_{40} = 5.3, 0.37 mg/kg, ED_{50} = 0.47 mg/kg, respectively). This broad activity of **22** was like that of glucocorticoids. Compound **22** did not affect activities of CO and 5-LO enzymes and receptor binding of various ligands. As one of the mechanisms of action, induction of release of glucocorticoids was postulated. These results suggest that **22** represents a novel class of antiinflammatory agents.

Introduction

Glucocorticosteroids are used as useful therapeutic drugs toward patients with severe arthritis, but with systemic side effects often preclude chronic use at efficacious doses.[1] Since the discovery of aspirin, much effort has been devoted to the development of nonsteroidal acidic antiinflammatory drugs (NSAIDs). Although these drugs reduce symptoms of chronic inflammatory diseases and do not show glucocorticosteroid-like side effects, none of them prevent progression of arthritis. This limited effectiveness may be attributed to their mechanism of action, mainly only cyclooxygenase (CO) inhibition.[2] This situation has caused many laboratories to develop a new type of antiinflammatory agents. For example, dual cyclooxygenase-/5-lipoxygenase (CO/5-LO) inhibitors have been discovered,[3] some of which are under clinical evaluation. In addition, as other instances, a new class of antiinflammatory agent, *N*-[(fluorenyl-9-methoxy)carbonyl]amino acids has been recently reported by Burch et al.[4] to possess a broad spectrum of antiinflammatory activity with no CO inhibition and very weak LO inhibition. Gans et al. reported that Dup 697 is a potent orally effective antiinflammatory agent with less toxicity due to tissue selective inhibition of PG synthesis.[5] NE-19550 and NE-28345 are orally active antiinflammatory analgesics derived from the capsaicin class and do not act like conventional NSAIDs via suppression of arachidonic acid metabolism.[6]

Scheme I



† Part 1 in a series of New Antiinflammatory Agents is ref 7.

(1) Silber, R. H.; Busch, R. D.; Oslaps, R. Practical Procedure for Estimation of Corticosteron or Hydrocortisone. *Clin. Chem.* **1958**, *4*, 278-285.
(2) (a) Vane, J. R. Inhibition of Prostaglandin Synthesis as a Mechanism of Action for Aspirin Like Drugs. *Nature (New Biol.)* **1971**, *231*, 232-235. (b) Flower, R. J. Drugs which Inhibit Prostaglandin. Biosynthesis. *Pharmacol. Rev.* **1974**, *26*, 33-71.

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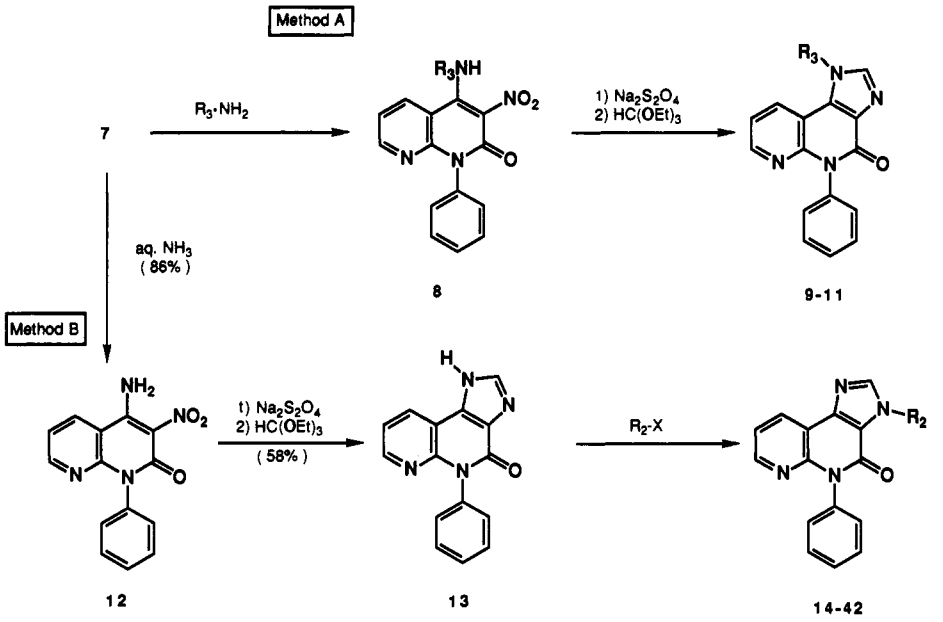
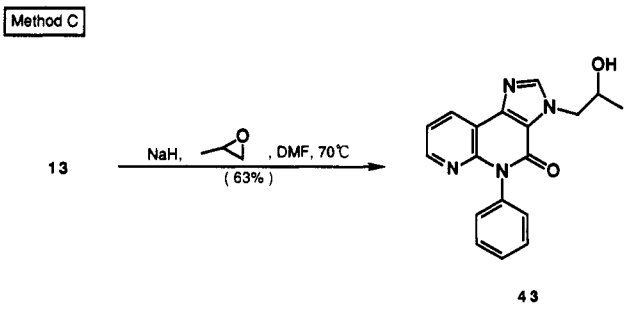
Scheme II



In our search for new antiinflammatory agents different from classical NSAIDs, we found that 4-hydroxy-2-oxo-1-phenyl-1*H*-1,8-naphthyridine-3-carboxamides **1** exhibited a broad spectrum of antiinflammatory activity in animal models.[7] Though their pharmacological profile satisfied us, their potencies did not. Even the antiinflammatory activity of KF 17515 (**1**: R_1 = 3-pyridyl), which was the most potent compound among **1**, was almost equipotent to that of tenidap,[8] a dual inhibitor. Therefore, we have continued to discover more potent compounds. During the modification of the 1,8-naphthyridin-2(1*H*)-one skeleton,[9] 3-substituted 5-phenyl-3*H*-imidazo[4,5-*c*][1,8]-naphthyridin-4(5*H*)-ones **2** were found to show potent inhibition in a broad range of animal models of inflammation. Among these derivatives, compound **22** ($R_2 = CH_2Ph$, KF 18280) exhibited the extremely potent activity and behaved like glucocorticoids with respect to the antiinflammatory profile.

Now we describe here the synthesis and the structure–activity relationships of new tricyclic heterocycles, imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-ones (**2** and **3**).

Scheme III



Chemistry

5-Phenyl-1*H*- or -3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-ones **2** or **3** were synthesized by the methods de-

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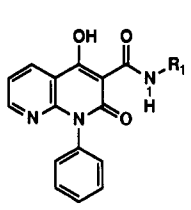

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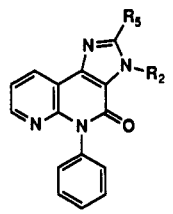

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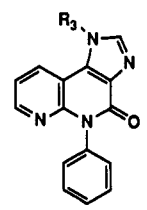

3

scribed earlier[9] which are outlined in Schemes I and II (methods A and B). The key intermediate 7, 4-chloro-1-phenyl-1,8-naphthyridin-2(1*H*)-one, was prepared from methyl 2-anilinonicotinate (4) in three steps (Scheme I). Regioselective introduction of substituents into the imidazole moiety was carried out according to methods A and B in Scheme II. 3-Substituted compounds (14–42) synthesized by method B are listed in Table I. Besides the compounds mentioned in Table I, the 3-(2-hydroxy)propyl derivative **43** was obtained by the reaction of the sodium salt of **13** with propylene oxide instead of alkyl halide (method C, Scheme III). Furthermore, the acetic acid derivative **44** was prepared by the reaction of anion of **13** with *tert*-butyl bromoacetate, followed by the treatment of trifluoroacetic acid (TFA) (Scheme IV, method D). Treatment of anion of **13** with 1-bromo-3-chloropropane afforded **45**, whose chlorine was transformed to iodine with sodium iodide, followed by amination with diethylamine or morpholine to give **46** or **47**, respectively (Scheme IV, method E). With respect to introduction of substituents into the 2-position of imidazole, **12** was reduced, acylated with acetyl chloride or benzoyl chloride, and dehydrated to afford **48** or **49**. Compounds **48** and **49** were regioselectively benzylated to afford **50** and **51**, respectively (Scheme V, method F).

Pharmacological Result and Discussion

In order to examine antiinflammatory activities of 5-phenylimidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one derivatives, we used three different types of rat paw edema models, such as carrageenan-induced paw edema (CPE), zymosan-induced paw edema (ZPE), and reversed passive Arthus reaction (RPAR)-induced paw edema. The characteristics of these screening models were previously reported.[7] The antiinflammatory activity of compounds was examined at the oral doses of 5 (or 10), 25 (or 50), and 50 (or 100) mg/kg, respectively, and the results of active compounds in edema assays are expressed as the ED_{40} (or ED_{50}), together with data of acute lethal toxicity in mice, in Tables II and III.[10]

Unsubstituted or 1-substituted 5-phenylimidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one derivatives showed weak inhibitory activities in three paw edema assays (**9**–**11** and **13**, Table II). In contrast with 1-substitution, 3-substitution dramatically affected the profiles and potencies of activities (Table III). Compound **14** showed moderate antiinflammatory activities in the CPE and RPAR assays. Introduction of longer alkyl groups into the 3-position of imidazole produced potent activity. Interestingly, increasing length of low alkyl groups in R_2 appeared to decrease acute lethal toxicity. There is a bulk tolerance in several lipophilic alkyl groups at the 3-position except for **17** to maintain potent activities (**14**–**20**). *n*-Hexyl-substituted compound **21** decreased inhibitory effects on CPE and ZPE, but maintained potent activity in the RPAR assay. Among above-mentioned compounds, the maximum activity in three assays was observed when R_2 was the isobutyl group (**20**). Its antiinflammatory activity was noteworthily potent, but it also showed acute lethal toxicity at 300 mg/kg (po). On the other hand, benzyl substitution in R_2 (**22**) decreased lethal toxicity with retention of the potent antiinflammatory activities in three paw edema assays. Thus, more extensive modification of **22** was undertaken. Replacement of benzyl hydrogen by the methyl group and insertion of the methylene or vinyl group between methylene and phenyl in the benzyl group diminished or reduced antiinflammatory activities (**23**–**25**). A hexahydro derivative **26** also decreased activity. Para substitution on the benzyl group in **22** had a great influence on the antiinflammatory activity. Compounds **35** (4-methoxybenzyl) and **34** (4-methylbenzyl) exhibited potent activities in the ZPE and RPAR assays, but showed less activity in the CPE assay. However, **32** (4-chlorobenzyl) and **33** (4-nitrobenzyl) diminished activity in the RPAR assay. Furthermore, **36** (4-(methoxycarbonyl)benzyl) abolished antiinflammatory activities. Thus, para substitution of the electron-donating group retained but that of the electron-attracting group reduced the activity associated with **22**. On the other hand, ortho or metha substitution on the benzyl group in **22** diminished or reduced the activity (**37**–**42**). Methyl or phenyl substitution at the 2-position of imidazole in **22** diminished biological activities (**50** and **51**). Compounds containing alcohol, ketone, and ether groups at the 3-position (**27**–**29** and **43**) exhibited potent inhibition against CPE, ZPE, and RPAR, but their potencies were lower than those of **22**. Ester derivatives **30** and **31** reduced inhibitory effects on the paw edemas. Introduction of acidic or basic parts into the 3-position of **2** abolished biological activities (**44**, **46**, and **47**). Structure–activity relationships of these compounds are very narrow and summarized as follows. In order to exhibit a broad range of potent antiinflammatory activity and less acute lethal toxicity, the lipophilic substituent such as the benzyl group must be present at the 3-position of 5-phenylimidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one. Activities are very sensitive to change of the environment around the benzyl group. Slight minor modification of the benzyl group and the presence of substituent at the 2-position on imidazole reduced or abolished activities. Thus, **22** was selected for further studies.

As previously described,[7] a typical acidic NSAID such as piroxicam, which is a CO inhibitor, was active in only CPE assay among these assays. Although a nonacidic NSAID, tiaramide inhibited ZPE and RPAR, its inhibition dose was very high (100 mg/kg, po). As shown in Table III, tenidap, which was reported to be a dual CO/5-LO inhibitor, exhibited moderate activities in the CPE and ZPE assays, but was inactive in the RPAR assay. Prednisolone, which is a modified corticosteroid, exhibited more potent antiinflammatory activities in the CPE and ZPE assays than in the RPAR assay. Though the activity of **22** in the CPE assay was similar to that of prednisolone, its inhibitory effects on ZPE and especially on RPAR were superior to those of prednisolone. Since **22** possesses more potent and broader range of antiinflammatory activities than other NSAIDs and prednisolone, it may be a valuable therapeutic agent for inflammatory diseases.

We then studied mechanisms of these antiinflammatory activities. Compound **22** did not show significant inhibition against CO and 5-LO enzymes at 100 μM and did not affect receptor binding of a variety of ligands including histamine, muscarine, adenosine, and catecholamines at 10 μM. These results indicate that mechanisms of antiinflammatory activities of **22** differ from those of the

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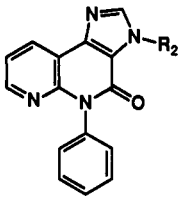
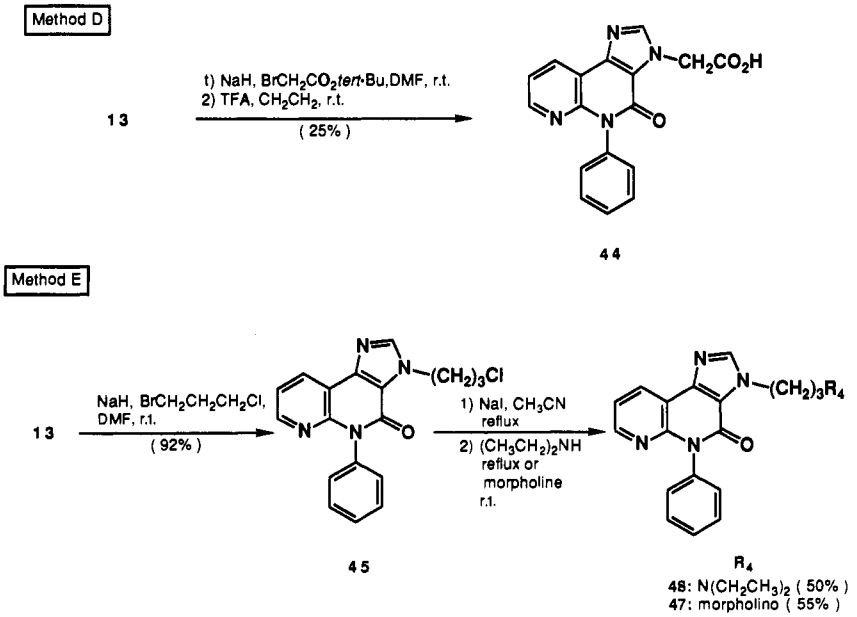
Table I. 3-Substituted 5-Phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-ones



compd	R_2	X[a]	yield, %	mp, °C	recrystn solvent	formula[b]
14	CH_3	I	72	>300	EtOH–*i*-Pr_2O	$C_{16}H_{12}N_4O$
15	C_2H_5	I	96	233–234	$CHCl_3$–*i*-Pr_2O	$C_{17}H_{14}N_4O$
16	*n*-C_3H_7	I	71	194–204	EtOAc–hexane	$C_{18}H_{16}N_4O$
17	*i*-C_3H_7	I	41	192–193	*i*-PrOH–H_2O	$C_{18}H_{16}N_4O$
18	CH_2CHCH_2	Br	74	186–189	$CHCl_3$–*i*-Pr_2O	$C_{18}H_{14}N_4O$
19	*n*-C_4H_9	I	70	192–194	EtOAc–*i*-Pr_2O	$C_{19}H_{18}N_4O$
20	*i*-C_4H_9	I	71	255–267	*i*-Pr_2O	$C_{19}H_{18}N_4O \cdot {}^1/_{10}H_2O$
21	*n*-C_6H_{13}	I	83	166–168	$CHCl_3$–*i*-Pr_2O	$C_{21}H_{22}N_4O$
22	$CH_2C_6H_5$	Br	78	189–192	EtOH–H_2O	$C_{22}H_{16}N_4O$
23	$CH(CH_3)C_6H_5$	Br	75	221–226	$CHCl_3$–*i*-Pr_2O	$C_{23}H_{18}N_4O$
24	$CH_2CH_2C_6H_5$	Br	75	232–233	*i*-PrOH–EtOH–H_2O	$C_{23}H_{18}N_4O$
25	$CH_2CHCHC_6H_5$	Br	81	231–233	$CHCl_3$–*i*-Pr_2O	$C_{24}H_{18}N_4O \cdot {}^3/_{10}H_2O$
26	CH_2-cyclohexyl	Br	74	236	$CHCl_3$–hexane	$C_{22}H_{22}N_4O$
27	CH_2COCH_3	Br	60	275–276	EtOAc	$C_{18}H_{14}N_4O_2$
28	$CH_2CH_2CH_2OH$	Br	30	248–252	$CHCl_3$–*i*-Pr_2O	$C_{18}H_{16}N_4O_2 \cdot {}^3/_{10}H_2O$
29	$CH_2CH_2OCH_2CH_3$	Br	95	209–210	$CHCl_3$–*i*-Pr_2O	$C_{19}H_{18}N_4O_2$
30	$CH_2CO_2C_2H_5$	Cl	77	228–229	$CHCl_3$–*i*-Pr_2O	$C_{19}H_{16}N_4O_3$[c]
31	$CH_2CH_2OCOCH_3$	Br	57	198	EtOAc	$C_{19}H_{16}N_4O_3$[d]
32	CH_2-4-ClC_6H_4	Br	93	287–290	DMF–H_2O	$C_{22}H_{15}N_4OCl$
33	CH_2-4-$NO_2C_6H_4$	Cl	98	256–259	DMF–MeOH	$C_{22}H_{15}N_5O_3 \cdot H_2O$[e]
34	CH_2-4-$CH_3C_6H_4$	Br	94	238–243	DMF–MeOH	$C_{23}H_{18}N_4O \cdot {}^4/_5H_2O$[f]
35	CH_2-4-$CH_3OC_6H_4$	Cl	56	267–268	DMF–H_2O	$C_{23}H_{18}N_4O_2$[g]
36	CH_2-4-$CH_3O_2CC_6H_4$	Br	51	160–162	DMF–H_2O	$C_{24}H_{18}N_4O_3$
37	CH_2-3-BrC_6H_4	Br	80	244–246	DMF–H_2O	$C_{22}H_{15}N_4OBr$
38	CH_2-3-$NO_2C_6H_4$	Br	78	284–289	DMF–MeOH	$C_{22}H_{15}N_5O_3$
39	CH_2-3-$CH_3C_6H_4$	Br	87	238–239	EtOH–MeOH	$C_{23}H_{18}N_4O$
40	CH_2-3-$CH_3OC_6H_4$	Cl	76	209–210	DMF–H_2O	$C_{23}H_{18}N_4O_2$
41	CH_2-2-ClC_6H_4	Br	75	257–262	DMF–H_2O	$C_{22}H_{15}N_4OCl$
42	CH_2-2,5-$(CH_3)_2C_6H_3$	Cl	72	237–238	DMF–H_2O	$C_{24}H_{20}N_4O$[h]

[a] X refers to R_2–X shown in Scheme II. [b] All compounds were analyzed for C, H, and N. [c] Calcd: C, 65.51. Found: C, 64.97. [d] Calcd: C, 65.51. Found: C, 65.03. [e] Calcd: N, 16.86. Found: N, 16.28. [f] Calcd: N, 14.71. Found: N, 14.20. [g] Calcd: H, 4.74. Found: H, 4.32. [h] Calcd: N, 14.73. Found: N, 14.12.

Scheme IV



typical NSAIDs. Since the antiinflammatory potency and profile of **22** appear to be similar to those of corticosteroids such as prednisolone, its activity might be partly mediated by endogenous corticosteroids. Therefore, serum levels of corticosterone in rats after oral administration of **22** and prednisolone were measured, respectively.[1] The serum levels of corticosterone decreased 1 h after oral administration of prednisolone at 100 mg/kg (53 ± 8 ng/mL, significant differences from that of the placebo at $P < 0.05$ determined by the Dunnett test ($n = 5$); the placebo: 188

Scheme V

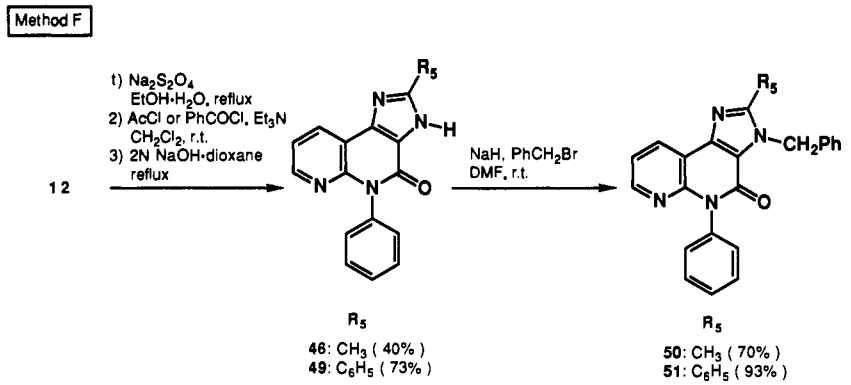
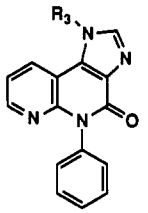


Table II. Effects of 5-Phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one Derivatives on Carrageenan-, Zymosan-, and Reversed Passive Arthus Reaction-Induced Rat Paw Edemas by the Oral Route



compd	R_3	CPE:[a] % inhibn at 100 mg/kg	ZPE:[a] % inhibn at dose 50 mg/kg	ZPE:[a] % inhibn at dose 25 mg/kg	RPAR:[a] % inhibn at 5 mg/kg	MLD: acute lethal[b] toxicity in mouse (mg/kg po)
9	C_2H_5	20.6		13.5	3.5	>300
10	*i*-C_3H_7	20.0			32.0	>300
11	$CH_2C_6H_5$			4.7	1.5	>300
13	H	13.0	27.1		15.4	>300

[a] Five animals were used in each experiment. [b] Three animals were used.

± 45 ng/mL). On the contrary, the concentration of corticosterone significantly increased 1 h after oral administration of **22** even at 1 mg/kg (571 ± 49 ng/mL, significant differences from that of the placebo at $P < 0.01$ determined by the Dunnett test ($n = 5$); the placebo: 116 ± 26 ng/mL).[11] Thus, the antiinflammatory activity of **22** appears to be deeply concerned in the promotion of serum levels of the endogenous glucocorticoid. This hypothesis was also supported by the decreased antiinflammatory activity of **22** in adrenalectomized rats.[12] Inhibition of swelling of paws produced by zymosan (ZPE) was 15% after oral administration of **22** at 1 mg/kg in adrenalectomized rats ($n = 5$) which was smaller than that (38%) in sham-operated rats ($n = 5$). Detailed mechanistic studies will be reported elsewhere.

In conclusion, the synthesis and antiinflammatory activity of 5-phenylimidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one derivatives have been described. Structure–activity relationships in this series have led to 3-benzyl-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (**22**), which possesses a broad spectrum of potent antiinflammatory activity like glucocorticoids. Compound **22** (KF 18280) was potently active in the CPE, ZPE, and RPAR assays. It did not affect activities of the CO and 5-LO enzymes and receptor binding of a variety of ligands. Its antiinflammatory activity seemed to be partly due to increase in serum levels of glucocorticoids. These results indicate that compound **22** represents a new class of antiinflammatory agent. Further investigations on other biological activities and action mechanisms of compound **22** are currently underway.

Experimental Section

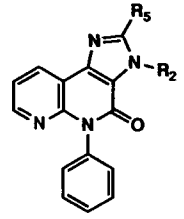
Melting points were determined on a Yanagimoto hot plate micro melting point apparatus and are uncorrected. Infrared (IR) spectra were measured on a JASCO IR-810 spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a JEOL JNM GX-270 spectrometer or a Hitachi R-90H spectrometer with tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were determined on a JEOL JMS-D300 instrument at an ionization potential of 70 eV. Elemental analyses were performed with a Perkin-Elmer 2400CHN. For column chromatography, silica gel 60 (E. Merck, 0.063–0.200 mm) was used. The reactions were usually carried out under nitrogen. Organic extracts were dried over anhydrous sodium sulfate and concentrated by a rotary evaporator. The general synthesis of 5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-ones has been described elsewhere.[9]

Method B. 3-(2-Methylpropyl)-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (20). To a solution of 0.80 g (3.1 mmol) of **13** in 30 mL of dry DMF was added 0.18 g (4.6 mmol) of 60 wt % sodium hydride at 0 °C in portions. When the evolution of hydrogen ceased, 0.72 mL (6.3 mmol) of isobutyl iodide was added. After stirring at room temperature for 12 h, 2 mL of aqueous saturated ammonium chloride was added with cooling. The solvent was evaporated under reduced pressure and water was added to the residue. The aqueous mixture was extracted with $CHCl_3$. The organic phase was washed with water, dried, and evaporated under reduced pressure. The residue was chromatographed on silica gel using $CHCl_3$ to afford the product 0.39 g (41%) of colorless crystals **20**. An analytical sample was

(11) Other pharmacological results of compound **22** will be soon reported elsewhere.

(12) Villar, A.; Gasco, M. A.; Alcaraz, M. J. Some Aspects of the Inhibitory Activity of Hypolaetin-8-glucoside in Acute Inflammation. *J. Pharm. Pharmacol.* **1987**, *39*, 502–507.

Table III. Effects of 5-Phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one Derivatives on Carrageenan-, Zymosan-, and Reversed Passive Arthus Reaction-Induced Rat Paw Edemas by the Oral Route



			CPE[a]				ZPE[a]				RPAR[a]				acute lethal toxicity mouse[b]
			% inhibn; dose (mg/kg)			95% confidence	% inhibn; dose (mg/kg)			95% confidence	% inhibn; dose (mg/kg)			95% confidence	MLD
compd	R_2	R_5	100	50	ED_{40}[c]	interval	50	25	ED_{40}[c]	interval	10	5	ED_{50}[c]	interval	(mg/kg po)
14	CH_3	H		40.0								45.0			100
15	C_2H_5	H		58.2	13.9	9.2–21.1		77.5	1.6	0.88–2.8	86.9		1.9	1.1–3.3	100
16	*n*-C_3H_7	H		57.3	15.9	9.8–25.9		61.2	1.3	0.66–2.6		74.4	2.0	1.9–2.1	200
17	*i*-C_3H_7	H	43.1					62.4	12.7	6.3–25.3		13.3			200
18	CH_2CHCH_2	H		67.8	21.9	6.6–72.4		67.3	2.7	1.8–3.9		22.7			>300
19	*n*-C_4H_9	H		45.8	15.4	9.7–24.5		55.5	2.1	1.3–3.4		49.9	4.8	2.6–8.8	300
20	*i*-C_4H_9	H		57.4	9.1	7.0–10.0		69.7	0.19	0.11–0.33		61.8	0.97	0.49–1.9	300
21	*n*-C_6H_{13}	H		35.2				33.5				51.1	4.9	4.4–5.5	>300
22	$CH_2C_6H_5$	H		63.0	5.3	4.2–6.7		68.3	0.37	0.25–0.55		79.7	0.47	0.31–0.72	>300
23	$CH(CH_3)C_6H_5$	H	3.5					1.6				14.9			>300
24	$CH_2CH_2C_6H_5$	H	2.2					25.7			5.6				>300
25	$CH_2CHCHC_6H_5$	H	26.3					34.2				17.5			>300
26	CH_2-cyclohexyl	H	34.7					49.8	11.8	8.9–15.5	62.9		5.7	4.1–7.8	>300
27	CH_2COCH_3	H		41.3	40.0	26.5–60.7		53.7	3.9	2.8–5.6		57.7	4.5	3.0–6.7	200
28	$CH_2CH_2CH_2OH$	H		42.6	33.5	9.0–122		58.1	4.8	2.5–9.2		41.3	3.7	2.3–6.0	>300
29	$CH_2CH_2OCH_2CH_3$	H		56.7	15.5	10.9–22.1		57.4	6.0	4.4–8.1		50.3	3.2	2.2–4.7	>300
30	$CH_2CO_2C_2H_5$	H		23.1				5.0				9.9			
31	$CH_2CH_2OCOCH_3$	H		42.2	40.3	24.2–67.3		41.6	19.4	12.4–30.4	11.8				>300
32	CH_2-4-ClC_6H_4	H	21.3					42.8	20.3	15.5–26.5		15.1			>300
33	CH_2-4-$NO_2C_6H_4$	H	35.9					45.6	3.5	1.7–7.2		22.2			>300
34	CH_2-4-$CH_3C_6H_4$	H	39.2					56.2	3.5	1.7–7.3		67.4	1.9	1.1–3.2	>300
35	CH_2-4-$CH_3OC_6H_4$	H	37.8					66.9	0.93	0.63–1.4		59.2	2.3	1.4–3.8	>300
36	CH_2-4-$CH_3O_2CC_6H_4$	H		8.7			9.9					9.2			
37	CH_2-3-BrC_6H_4	H	26.9				36.3				17.2				
38	CH_2-3-$NO_2C_6H_4$	H	33.1					35.7				43.9			
39	CH_2-3-$CH_3C_6H_4$	H		18.4				33.3			33.5				
40	CH_2-3-$CH_3OC_6H_4$	H	13.0					43.5			24.0				
41	CH_2-2-ClC_6H_4	H	14.9					27.3				20.8			
42	CH_2-2,5-$(CH_3)_2C_6H_3$	H	1.2					10.1				−3.5			
43	$CH_2CH(OH)CH_3$	H		48.0	7.8	4.4–14.0		74.7	4.5	2.9–7.0		61.8	1.9	0.89–4.0	300
44	CH_2CO_2H	H	10.8					5.1				3.3			
46	(diethylamino)propyl	H	7.2					17.4				11.1			
47	morpholinopropyl	H		9.7				22.4				−3.5			
50	$CH_2C_6H_5$	CH_3		4.7			19.2					6.5			
51	$CH_2C_6H_5$	C_6H_5		0			−4.0					1.2			
tenidap					14.8	7.8–28.3			41.2	16.2–105.0			(4.8%)[d]		>300
prednisolone					4.7	3.6–6.0			1.2	0.91–1.5			74.2	58.7–83.8	>300

[a] See footnote *a* in Table II. [b] See footnote *b* in Table II. [c] ED_{40} (or ED_{50}) as determined by linear-regression analysis. [d] Inhibition percent at 100 mg/kg po.

crystallized from isopropyl ether: mp 255–267 °C; IR (KBr) 1671 cm^{-1}; NMR (DMSO-d_6) δ 0.86 (d, 6 H, J = 7 Hz), 2.10–2.25 (m, 1 H), 4.26 (d, 2 H, J = 7 Hz), 7.26–7.60 (m, 6 H), 8.36 (dd, 1 H, J = 4, 2 Hz), 8.40 (s, 1 H), 8.53 (dd, 1 H, J = 8, 2 Hz). Anal. ($C_{19}H_{16}N_4O \cdot {}^1/_{10}H_2O$) C, H, N.

Method C. 3-(2-Hydroxypropyl)-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (43). To a solution of 2.0 g (7.6 mmol) of **13** in 40 mL of dry DMF was added 0.34 g (8.4 mmol) of 60 wt % sodium hydride at 0 °C in portions. When the evolution of hydrogen ceased, 1.1 mL (15 mmol) of propylene oxide was added. After stirring at 100 °C for 1.5 h, aqueous saturated ammonium chloride was added with cooling. The solvent was evaporated under reduced pressure and water was added to the residue. The aqueous mixture was extracted with $CHCl_3$. The organic phase was washed with water, dried, and evaporated under reduced pressure. The residue was chromatographed on silica gel using $CHCl_3$ to afford 1.6 g (63%) of colorless crystals **43**. An analytical sample was crystallized from $CHCl_3$–isopropyl ether: mp 95–98 °C; IR (KBr) 1662 cm^{-1}; NMR (DMSO-d_6) δ 1.10 (d, 3 H, J = 6 Hz), 3.45–3.70 (m, 1 H), 3.78–3.92 (m, 1 H), 4.40–4.60 (m, 2 H), 7.27–7.57 (m, 6 H), 8.30–8.37 (m, 2 H), 8.53 (dd, 1 H, J = 8, 2 Hz). Anal. ($C_{16}H_{16}N_4O_2 \cdot {}^2/_5H_2O$) H, N; C: calcd, 17.10, found, 16.64.

Method D. 3-(Carboxymethyl)-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (44). To a solution of 4.5 g (17 mmol) of **13** in 150 mL of dry DMF was added 1.0 g (26 mmol) of 60 wt % sodium hydride at 0 °C in portions. When the evolution of hydrogen ceased, 5.5 mL (34 mmol) of *tert*-butyl bromoacetate was added. After stirring at room temperature for 2 h, aqueous saturated ammonium chloride was added with cooling. The solvent was evaporated under reduced pressure and water was added to the residue. The aqueous mixture was extracted with $CHCl_3$. The organic phase was washed with water, dried, and evaporated under reduded pressure. The residue was chromatographed on silica gel using $CHCl_3$ to afford 3.6 g (55%) of *tert*-butyl ester of **13**. Without analysis, to a solution of 3.6 g of *tert*-butyl ester of **13** in 180 mL of CH_2Cl_2 was added 80 mL of trifluoroacetic acid with ice cooling. The mixture was stirred at room temperature for 6 h, and the solvent was evaporated under reduced pressure. The residue was suspended in water and 4 N NaOH was added to dissolve the residue. HCl (2 N) was added to the solution to give the resulting precipitate, which was collected by filtration. Recrystallization from DMF–water gave 1.4 g (45%) of colorless crystals **44**: mp > 300 °C; IR (KBr) 1722, 1709, 1687, 1662 cm^{-1}; NMR (DMSO-d_6) δ 5.27 (s, 2 H), 7.15–7.57 (m, 6 H), 8.30–8.40 (m, 2 H), 8.54 (dd, 1 H, J = 8, 2 Hz). Anal. ($C_{17}H_{12}N_4O_3$) C, H, N.

Method E. 3-[3-(Diethylamino)propyl]-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one Hydrochloride (46 Hydrochloride). (a) 3-(3-Chloropropyl)-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (45). To a solution of 15 g (0.057 mol) of **13** in 400 mL of dry DMF was added 2.78 g (0.068 mol) of 60 wt % sodium hydride at 0 °C in portions. When the evolution of hydrogen ceased, 8.8 mL (0.086 mmol) of 3-bromo-1-chloropropane was added. After stirring at room temperature for 3 h, aqueous saturated ammonium chloride was added with cooling. The solvent was evaporated under reduced pressure and water was added to the residue. The aqueous mixture was extracted with $CHCl_3$. The organic phase was washed with water, dried, and evaporated under reduced pressure. The residue was chromatographed on silica gel using $CHCl_3$ to afford 18 g (92%) of colorless crystals **45**. An analytical sample was crystallized from ethyl acetate–hexane: mp 186–190 °C; IR (KBr) 1650 cm^{-1}; NMR (DMSO-d_6) δ 2.30–2.55 (m, 2 H), 3.53 (t, 2 H, J = 7 Hz), 4.67 (t, 2 H, J = 7 Hz), 7.11–7.62 (m, 6 H), 8.01 (s, 1 H), 8.41 (dd, 1 H, J = 4, 2 Hz), 8.62 (dd, 1 H, J = 8, 2 Hz). Anal. ($C_{18}H_{15}N_4OCl \cdot {}^1/_5H_2O$) C, H, N.

(b) 3-[3-(Diethylamino)propyl]-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one Hydrochloride (46 Hydrochloride). A mixture of 18 g (0.052 mol) of **45** and 12 g (0.078 mol) of sodium iodide in 200 mL of acetonitrile was refluxed for 24 h. During reflux, 7.8 g (0.052 mol) of additional sodium iodide was added to the mixture. After cooling, the solvent was evaporated under reduced pressure and water was added to the residue. The mixture was extracted with $CHCl_3$. The organic phase was washed with water, dried, and concentrated under reduced pressure. A suspension of the residue in 50 mL of diethylamine was refluxed for 1 h. After cooling, the solvent was evaporated under reduced pressure and water was added to the residue. The aqueous phase was extracted with $CHCl_3$. The organic phase was extracted with 2 N HCl. The aqueous solution was adjusted to pH 11 with 8 N NaOH, followed by extraction with $CHCl_3$. The organic phase was washed with a saturated aqueous sodium chloride solution and dried. To this solution was added ethyl acetate saturated with HCl. The resulting precipitate was filtered and dried to afford 12 g (50%) of colorless crystals **46** as the HCl salt: mp 152–153 °C; IR (KBr) 1650 cm^{-1}; NMR (DMSO-d_6) δ 1.18 (t, 6 H, J = 7 Hz), 2.20–2.35 (m, 2 H), 2.91–3.14 (m, 6 H), 4.60 (t, 2 H, J = 7 Hz), 7.22–7.60 (m, 6 H), 8.39 (dd, 1 H, J = 4, 2 Hz), 8.60 (dd, 1 H, J = 8, 2 Hz), 8.68 (s, 1 H), 10.57 (br s, 2 H). Anal. ($C_{22}H_{25}N_5O \cdot 2HCl \cdot {}^{13}/_{10}H_2O$) C, H, N.

3-Morpholino-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one Hydrochloride (47 Hydrochloride). Compound **47** as the HCl salt was obtained in a 55% yield from **45** according to the same procedure as the synthesis of **46** except that morpholine was used instead of diethylamine: mp 294–297 °C; IR (KBr) 1695 cm^{-1}; NMR (DMSO-d_6) δ 2.28–2.58 (m, 2 H), 2.92–3.22 (m, 4 H), 3.32–3.49 (m, 2 H), 3.77–4.02 (m, 4 H), 4.45–4.80 (m, 2 H), 7.28–7.63 (m, 6 H), 8.40 (dd, 1 H, J = 4, 2 Hz), 8.67 (dd, 1 H, J = 8, 2 Hz), 8.87 (s, 1 H), 11.51 (br s, 2 H). Anal. ($C_{22}H_{23}N_5O_2 \cdot 2HCl \cdot {}^7/_5H_2O$) C, H, N.

Method F. 3-Benzyl-2-methyl-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (50). (a) 2-Methyl-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (48). A suspension of 12 g (0.043 mol) of **12** and 30 g (0.17 mol) of sodium hydrosulfite in a mixture of 10 mL of ethanol and 14 mL of water was refluxed for 10 min. After cooling, the solid was filtered and dried. To the suspension of this solid in 100 mL of CH_2Cl_2 was added 3.1 mL (0.022 mol) of triethylamine and 1.4 mL (0.019 mol) of acetyl chloride with ice cooling. The mixture was stirred at room temperature for 1.5 h. Then methanol was added to the mixture and the solvent was evaporated under reduced pressure. To the residue was added 10 mL of dioxane and 10 mL of 2 N NaOH, and the mixture was refluxed for 1.5 h. After the mixture was cooled with ice, concentrated HCl was added to neutralize. The resulting precipitate was collected by filtration and dried. Recrystallization from methanol gave 2.0 g (40%) of colorless crystals **48**: mp > 300 °C; IR (KBr) 1657 cm^{-1}; NMR (DMSO-d_6) δ 2.51 (s, 3 H), 7.18–7.61 (m, 6 H), 8.33 (dd, 1 H, J = 4, 2 Hz), 8.57 (dd, 1 H, J = 8, 2 Hz), 13.5 (br s, 1 H). Anal. ($C_{16}H_{12}N_4O \cdot {}^1/_2H_2O$) C, H, N.

(b) 3-Benzyl-2-methyl-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (50). To a solution of 0.66 g (2.4 mmol) of **48** in 30 mL of dry DMF was added 0.11 g (2.9 mmol) of 60 wt % sodium hydride at 0 °C in portions. When the evolution of hydrogen ceased, 0.43 mL (3.6 mmol) of benzyl bromide was added. After stirring at room temperature for 1 h, aqueous saturated ammonium chloride was added with cooling. The solvent was evaporated under reduced pressure and water was added to the residue. The resulting precipitate was recrystallized from ethanol–water to afford 0.61 g (70%) of pale brown crystals **50**: mp 267–269 °C; IR (KBr) 1615 cm^{-1}; NMR (DMSO-d_6) δ 2.52 (s, 3 H), 5.79 (s, 2 H), 7.19–7.56 (m, 11 H), 8.39 (dd, 1 H, J = 4, 2 Hz), 8.51 (dd, 1 H, J = 8, 2 Hz). Anal. ($C_{23}H_{16}N_4O$) C, H; N: calcd, 15.29; found, 14.84.

3-Benzyl-2,5-diphenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (51). (a) 2,5-Diphenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (49). Compound **49** was prepared in a 73% yield from **12** according to the same procedure as the synthesis of **48** except that benzoyl chloride was used instead of acetyl chloride: mp >300 °C ($CHCl_3$); IR (KBr) 1651 cm^{-1}; NMR (DMSO-d_6) δ 7.22–7.72 (m, 10 H), 8.29–8.41 (m, 3 H), 8.5–8.7 (br s, 1 H). Anal. ($C_{21}H_{14}N_4O$) C, H, N. **(b) 3-Benzyl-2,5-diphenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (51).** Compound **51** was prepared in a 93% yield from **49** according to the same procedure as the synthesis of **50** from **48**: mp 283–287 °C (DMF–water); IR (KBr) 1664 cm^{-1}; NMR (DMSO-d_6) δ 5.88 (s, 2 H), 6.97 (dd, 2 H, J = 7, 2 Hz), 7.18–7.61 (m, 12 H), 7.71 (dd, 2 H, J = 7, 2 Hz), 8.41 (dd, 1 H, J = 4, 2 Hz), 8.62 (dd, 1 H, J = 8, 2 Hz). Anal. ($C_{28}H_{20}N_4O$) C, H, N.

Rat Paw Edema Induced by Carrageenan and Zymosan. Carrageenan- and zymosan-induced paw edemas were conducted

by the methods of Winter et al.[13] and Gemmell et al.,[14] respectively. Male Wistar rats weighing about 150 g were used. Test compounds suspended in 5% arabic gum solution were administered orally 1 h before 1% λ-carrageenan (picnin A, Zushi Kagaku) and 1% zymosan (zymosan A, Sigma) in 0.1 mL of physiological saline (saline) were injected subplantarly into the hind paw. The paw volumes were measured by a plethsmograph immediately before the drugs were administered, and 3 and 4 h after carrageenan and zymosan were injected, respectively. The percentage of inhibition was calculated from the difference in mean swelling values between the test compounds treated animals and the control animals. This was then plotted versus the log of drug concentration and the value of ED_{40} or ED_{50} was estimated by linear-regression analysis.

Rabbit Anti-EA Antiserum. Rabbit anti-EA (egg albumin) antiserum was prepared according to the method of Koda et al.[15] 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.

Reversed Passive Arthus Reaction-Induced Rat Paw Edema. This assay was conducted as described by Terasawa et al.[16] Male Wister rats weighing about 150 g were used. Test compounds suspended in 5% arabic gum solution were administered orally 1 h before EA injection. Thirty minutes after the administration of the compounds, 0.5 mL of rabbit anti-EA antiserum was injected into the tail vein. Thirty minutes later, 0.1 mL of saline containing 0.025 mg of EA was injected subplantarly into the hind paw. The paw volumes were measured by a plethsmograph immediately, before the drugs were administered and 2 h after EA injection. The percentage of inhibition was calculated from the difference in mean swelling values between the test compounds treated animals and the control group. This was then plotted versus the log of drug concentration, and the value of ED_{50} was estimated by linear-regression analysis.

Acute Toxicity. The compounds were orally administered to male dd mice weighing 20–25 g (n = 3). Minimum lethal doses (MLD) was determined by observing the mortality for 7 days after the administration.

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Quenched Molecular Dynamics Simulations of Tuftsin and Proposed Cyclic Analogues

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We have used high-temperature quenched molecular dynamics calculations to investigate the conformational properties of tuftsin (Thr-Lys-Pro-Arg) in solution. Conformers obtained after quenching of the dynamical structures were sorted into families depending on their relative energies and backbone conformations. By examination of these families, several cyclic analogues of tuftsin were proposed and examined theoretically by further quenched dynamics simulations. Two of the four proposed analogues were found to adopt essentially identical conformations to that of linear tuftsin. It is suggested that these two derivatives (cyclo[Thr-Lys-Pro-Arg-Gly] and cyclo[Thr-Lys-Pro-Arg-Asp]) may be biologically active, and that the introduction of cyclic conformational constraints should help to reduce the entropic penalty to peptide binding.

Introduction

Tuftsin is a linear tetrapeptide (Thr-Lys-Pro-Arg) which is involved in the stimulation of the phagocytosis of polymorphogranulocytes and phagocytes.[1] Tuftsin is located between residues 289 and 292 of the heavy chain of leukokinin (a cytophilic γ-globulin),[2] in the sequence -Val-His-Asn-Ala-Lys-*Thr-Lys-Pro-Arg*-Glu-Gln-Gln-Tyr-Asx-, and in close proximity to the carbohydrate binding region.[3]

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