Romazarit: A Potential Disease-Modifying Antirheumatic Drug

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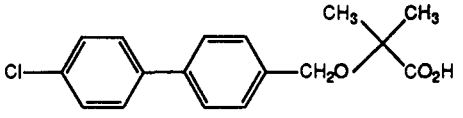
The synthesis of a series of substituted heterocyclic alkoxypropionic acids is described. They were evaluated for antiinflammatory effects in two animal models of chronic inflammation; adjuvant arthritis and type II collagen arthritis in the rat. The desired profile of biological activity was characterized by the reduction of inflammation with the coincident restoration toward normal levels of the biochemical markers (acute phase proteins) associated with the inflammatory response, an effect that was not shared by classical nonsteroidal antiinflammatory agents. Romazarit, (Ro 31-3948, **7**), 2-[[2-(4-chlorophenyl)-4-methyl-5-oxazolyl]methoxy]-2-methylpropionic acid, was selected for further evaluation. In contrast to NSAIDs, romazarit was inactive in animal models of acute inflammation, and furthermore it did not inhibit the cyclooxygenase enzyme in vitro or in vivo. Inhibition of interleukin-1-mediated events in vitro has been observed.

Introduction

The disease modifying antirheumatic drugs (DMARDs) which are available in current clinical practice are considerably limited by their narrow therapeutic ratio and by the severity of their side effects.[1] Therefore there is a demonstrable need for DMARDs of increased potency coupled with reduced adverse effects. The development of such agents by rational means has been hampered by the paucity of detailed information on the mechanisms of action of the clinically accepted DMARDs.

Clobuzarit (ICI 55897), originally developed as an antihyperlipidemic agent, was observed to lower plasma concentrations of the acute phase protein (APP) fibrinogen in patients with atherosclerosis.[2] At this time McConkey[3]



Clobuzarit

and colleagues were stressing the value of the determination of APP's (such as C-reactive protein and haptoglobin) for evaluating the effects of gold and penicillamine in rheumatoid arthritis. Furthermore, remission-inducing drugs could clearly be differentiated from the nonsteroidal antiinflammatory drugs (NSAIDs), in that substantial clinical benefit occurred coincident with a fall in relevent plasma APP concentrations; NSAIDs did not lower these biochemical markers of disease activity and progression.

Therefore, clobuzarit was evaluated in man for the treatment of rheumatoid arthritis where it was observed to possess a similar profile of effects to accepted DMARDs. This distinction between DMARDs and NSAIDs in the clinic, in the case of clobuzarit, was reflected in animal models of chronic inflammation, thereby providing the opportunity to identify potential DMARDs in animal models of inflammation.

As part of our program to develop novel entities that affect the course of the rheumatic diseases we regarded clobuzarit of sufficient promise to merit the investigation of analogues. While the clinical and laboratory data supported the effectiveness of the compound, adverse effects in man such as Stevens Johnson syndrome, and hepatomas and peroxisomal proliferation in rodents were observed. We concluded that such adverse effects might be ameliorated by modification of the physicochemical properties of the molecule, and therefore engaged upon a program to synthesize and evaluate analogues of clobuzarit.

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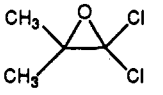
Chemistry

Compounds (Table I) were prepared utilizing one of two synthetic methodologies. The 2-methylpropionic acid side chain was introduced via one of the coupling reactions with the appropriate aryl heterocycle (Scheme I, method A or B) or incorporated during construction of the heterocyclic ring via a [1,3]-dipolar cycloaddition reaction (Scheme II).

In method A (Scheme I) the requisite halogenomethylarylheterocycles (obtained by standard literature procedures) were coupled with the alkoxide derivative of methyl 2-hydroxy-2-methylpropionate. Reaction of an appropriate hydroxymethylheterocyclic compound with acetone and chloroform in the presence of powdered sodium hydroxide[4] (method B) could also be utilized to incorporate the desired 2-methylpropionate side chain; the reaction is assumed to proceed via the intermediacy of epoxide **20** with subsequent trapping by the alcohol. It



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was observed that in cases where the alkoxide displacement reaction (method A) had failed (compounds **2**, **3**, **15**, and **16**) the acetone/chloroform/sodium hydroxide procedure (method B) afforded the required compound.

[1,3]-Dipolar cycloadditions afforded other ring systems (method C, Scheme II). Reaction of 4-chlorophenylcarbonitrile oxide with methyl 2-(propynyloxy)-2-methylpropionate afforded a single regioisomer as the only product. Subsequent hydrolysis afforded the isoxazole acid **10**. In contrast the [1,3]-dipole generated from *N*-nitroso-4-(chlorophenyl)glycine afforded two regioisomers in a 1:1 ratio upon reaction with the same propargyl ether. These were separated by chromatography and subsequently hydrolyzed to the carboxylic acids. The regioisomers were assigned upon the basis of standard spectral data (proton and carbon NMR).

Results and Discussion

The initial biological evaluation of compounds **1–19** was carried out in a developing adjuvant arthritis model[5] in

(1) Goddard, D.; Butler, R. *Rheumatoid Arthritis: The Treatment Controversy*; MacMillan: New York, 1984.
(2) Billingham, M. E. J.; Rushton, A. R. *Anti-Inflammatory and Anti-rheumatic Drugs*; CRC Press: Boca Raton, FL, 1985, Vol. III, pp 31–63.
(3) (a) McConkey, B.; Crockson, R. A.; Oliver, M. F. *Q. J. Med.* **1972**, *41*, 115. (b) McConkey, B.; Crockson, R. A.; Oliver, M. F.; Wilkinson, A. R. *Q. J. Med.* **1973**, *42*, 785.
(4) (a) Lai, J. T. *Synthesis* **1984**, 122. (b) Lai, J. T. *Synthesis* **1984**, 124.

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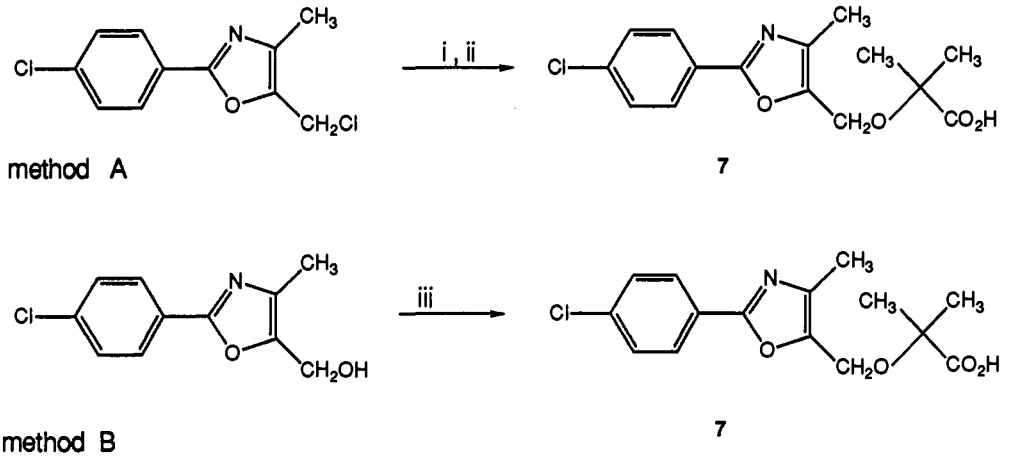
Table I. Aryl and [[(1-Carboxy-1-methylethyl)oxy]methyl] Disubstituted Heterocycles

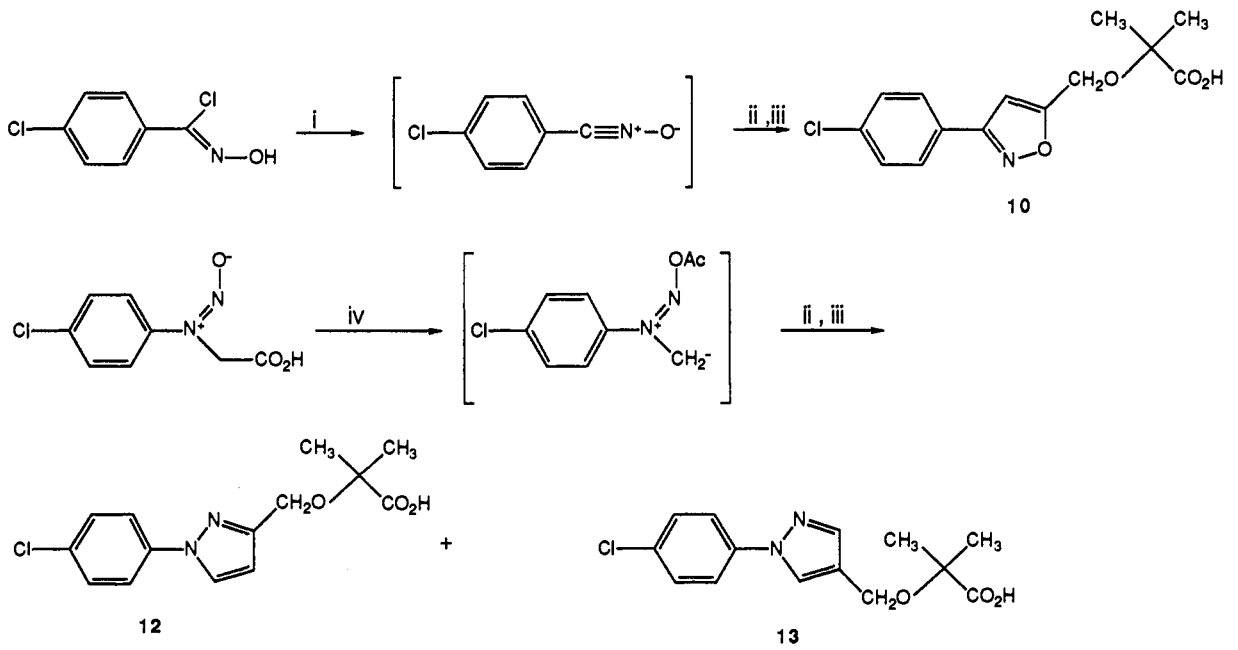
no.	heterocycle	substituent position: 4-Cl-C_6H_4	substituent position: $-CH_2OC(CH_3)_2CO_2H$	mp, °C	method	yield, %	formula	anal.	log *P'*
1	thiazole	2	4	130–140	A	50	$C_{14}H_{14}ClNO_3S$	C,H,N	0.66
2	thiazole	4	5	119–123	B	49	$C_{14}H_{14}ClNO_3S$	C,H,N	0.23
3	thiazole	5	4	208–210	B	5	$C_{14}H_{14}ClNO_3S$	C,H,N	0.23
4	oxazole	2	4	144.5–145	A	57	$C_{14}H_{14}ClNO_4$	C,H,N	0.23
5	oxazole	2	5	151.5–153	B	37	$C_{14}H_{14}ClNO_4$	C,H,N	0.11
6	5-methyloxazole	2	4	123–124	B	17	$C_{15}H_{16}ClNO_4$	C,H,N	0.52
7	4-methyloxazole	2	5	166–167	B (A)	33 (60)	$C_{15}H_{16}ClNO_4$	C,H,N	0.34
8	oxazole	4	2	115.5–118	B	39	$C_{14}H_{14}ClNO_4$	C,H,N	0.15
9	oxazole	5	2	143–146	B	14	$C_{14}H_{14}ClNO_4$	C,H,N	0.15
10	isoxazole	3	5	130–134	C	26	$C_{14}H_{14}ClNO_4$	C,H,N	0.36
11	isoxazole	5	3	115–118	B	15	$C_{14}H_{14}ClNO_4$	C,H,N	0.46
12	pyrazole	1	3	135–137	C	10	$C_{14}H_{15}ClN_2O_3$	C,H,N	0
13	pyrazole	1	4	98–100	C	8	$C_{14}H_{15}ClN_2O_3$	C,H,N	0.04
14	pyrazole	3	5	211–212	C	6	$C_{14}H_{15}ClN_2O_3$	C,H,N	0.36
15	*N*-methylimidazole	4	2	192–193	B	19	$C_{15}H_{17}ClN_2O_3$	C,H,N	0.08
16	pyridazine	6	3	158–159	B	28	$C_{15}H_{15}ClN_2O_3$	C,H,N	−0.15
17	pyridine	6	2	117–119	A	35	$C_{16}H_{16}ClNO_3$	C,H,N	0.69
18	5-chloro benzofuran	–	2	81	A	36	$C_{13}H_{13}ClO_4$	C,H	−0.57
19	5-chloro benzofuran	–	3	102–103	A	53	$C_{13}H_{13}ClO_4$	C,H	−0.54
	Clobuzarit								1.52

Scheme I[a]



[a] Reagents: (i) $Na^+{}^-OC(CH_3)_2CO_2CH_3$, DMF, DME or THF, room temp/reflux; (ii) aqueous NaOH, EtOH, reflux, H_3O^+; (iii) CH_3COCH_3, $NaOH_{(s)}$, $CHCl_3$ reflux.

Scheme II[a]



[a] Reagents: (i) Et_3N, Et_2O, 0–5 °C; (ii) $HC{\equiv}CCH_2OC(CH_3)_2CO_2CH_3$, room temp; (iii) aqueous NaOH, EtOH reflux; H_3O^+; (iv) Ac_2O reflux.

Table II. Developing Adjuvant Arthritis[a]

no.	% inhibition paw edema[b]	% decrease serum haptoglobin[c]
1	81*	16*
2	NS[f]	NS
3	NS	NS
4	84*	40*
5	33*	NS
6	67*	NS
7	73*,[d]	26*,[d,e]
8	69*	9*
9	40*	17*
10	72*	37*
11	64*	34*
12	46*	30*
13	76*	37*
14	NS	NS
15	NS	NS
16	NS	NS
17	NS	NS
18	39*	NS
19	NS	NS
indomethacin (1 mg/kg)	84*	NS
clobuzarit	48*	31*

*p <0.05 student's t test. [a]Reference 5; five rats per group dosed at 100 mg/kg po unless otherwise stated. Results are the mean of five rats/dose. [b]Percent inhibition = [arthritic control − test]/[arthritic control] × 100; calculated from the area under the curve of paw volume changes measured on days 1–4. [c]Determined at day 4; percent decrease = [arthritic control − treated]/[arthritic control − normal] × 100. [d]Figures refer to a mean of 10 experiments with five rats/dose group per experiment. [e]Plasma levels of haptoglobin (expressed as the mean ± SEM) for nonarthritic animals were 0.3 ± 0.1 g/L and for arthritic controls 1.4 ± 0.04 g/L. [f]NS = no significant effect.

the rat. This model allowed the clear distinction between DMARDs and NSAIDs, clobuzarit being readily distinguishable from classical cyclooxygenase inhibitors, such as indomethacin, by comparison of their effects on the profile of acute phase proteins. The effect of compounds on both edema and APP levels in this animal model are given in Table II. The desired activity was characterized by a late onset of antiinflammatory action and decreases in serum haptoglobin toward control levels.[5]

The compounds described herein were derived by replacement of the aromatic B ring of clobuzarit with heterocyclic systems. The effects of variation of the phenyl A ring and alkoxy propionate side chain in conjunction with specific heterocycles will be reported in a later article. From the present results it was apparent that a 1,2-orientation of 4-chlorophenyl and [[(1-carboxy-1-methylethyl)oxy]alkly] substituents resulted in a complete loss of biological activity (e.g. compounds **2** and **3**]. Compounds which possessed a 1,3- or 1,4-orientation of substituents in the majority of examples retained biological activity. This retention of the desired profile of activity was observed with a wide range of heterocyclic systems, thus providing compounds with a broad range of partition coefficients (Table I).

Compounds which exhibited the required biological activities in the developing adjuvant arthritis model were evaluated further in a model of chronic inflammation, the type II collagen arthritis[6] in the rat (Table III). In this model, assessment of bone changes were made by radiographic measurement, and hepatic effects were assessed

Table III. Rat Type II Collagen Arthritis[a]

compd (dose, mg/kg)	radiographic score % inhibition[b] talus	radiographic score % inhibition[b] tarsus	hepatic effects[c] % change liver weight	hepatic effects[c] % change catalase
4	59+	44+	19*	52*
5	42+	41+	16*	11
6	65+,[e]	54+,[e]	2	60*
7 (20)	33	7	−5	−51*,[d]
7 (40)	40+	35+	−3	−34*,[d]
7 (60)	63+	46+	4	−7[d]
7 (100)	82+	56+	13*	19[d]
8	63+	39+	19*	68*
9	36	24	3	−10
10	60+	47+	47*	73*
11	24+	42+	41*	140*
12 (50)	48+	32+	13*	90*
13 (50)	15	18	70*	180*
clobuzarit (60)	64+	41+	19*	164*
indomethacin (1)	48	45+	ND[f]	ND
dexamethasone (0.5, day 1–5) (0.25, day 6–15)	95+	98+	ND	ND

+p < 0.05 Mann Whitney U test. *p < 0.05 student's t test. [a]Reference 6; eight rats per group at 100 mg/kg per day po unless otherwise stated. Results are the mean of eight rats/dose. [b]Radiographic change was judged in the area of the tibia/talus joint and in the tarsus region by using an arbitrary scoring system upon termination of the test (14 days dosing). [c]Liver weights and catalase levels were determined upon termination of the test (14 days dosing), and the results are expressed as percentage change compared to normals. [d]Catalase activity (mean ± SEM) defined by the first-order rate constant K for nonarthritic animals was 153 ± 19 K/s per g and for arthritic controls 116 ± 10 K/s per g. [e]Overtly toxic, dosed 100 mg/kg days 1–7, reduced to 50 mg/kg day 8–14. [f]ND Not determined.

Table IV. Rat Type II Collagen Arthritis–Haptoglobin Levels[a]

	haptoglobin, g/L
normals	0.29 ± 0.04
arthritic controls	0.54 ± 0.06
7 20 mg/kg	0.31 ± 0.04**
7 40 mg/kg	0.43 ± 0.06
7 60 mg/kg	0.32 ± 0.05*
7 100 mg/kg	0.34 ± 0.02**

*p < 0.05 Student's t test. **p < 0.01 Student's t test. [a]Reference 6; eight rats per group dosed as stated per day po. Results are the mean of eight rats/dose. Haptoglobin levels were determined upon termination of the test (14 days dosing).

by increases in liver weight and induction of the enzyme catalase.

The majority of oxazole and isoxazole variants tested in the type II collagen arthritis (at 100 mg/kg per day po unless otherwise stated) had beneficial effects on bone erosions. However, the effects of compounds on the livers of the test animals varied widely. Dramatic induction of the enzyme catalase (an indicator of peroxisomal proliferation linked to hepatoma formation in rodents[7]) occurred with compounds **4**, **6**, **8**, **10**, and **11**. Romazarit (**7**) was identified as the lead compound, exhibiting beneficial effects on the course of the chronic inflammation over a range of dose levels (see Table III for dose response).

The observed biological activities of **7** in this model of chronic inflammation have remained consistent over a large number of tests, and from these data the minimum effective dose was determined to be 25 mg/kg.

In both the developing adjuvant arthritis (Table II) and the type II collagen arthritis (Table IV) models **7** restored levels of the acute phase protein haptoglobin toward those

(5) Lewis, E. J.; Bishop, J.; Cashin, C. H. *J. Pharmcol. Meth.* **1989**, *21*, 183.
(6) (a) Jones, S. A.; Kennedy, A. J.; Roberts, N. A. *Agents Actions* **1982**, *12*, 650. (b) Bloxham, D. P.; Bradshaw, D.; Cashin, C. H.; Dodge, B. B.; Lewis, E. J.; Westmacott, D.; Self, C. R. *J. Pharcol. Exp. Ther.* **1990**, *252*(3), 1331–1340.
(7) Reddy, J. K.; Lalwani, N. D. *CRC Crit. Rev. Toxicol.* **1983**, *12*, 1–58.

Table V. Inhibition of Prostaglandin Synthesis

compd	human MNC PGE_2 production	rat renal medulla PG synthetase: IC_{50}, μM	sheep seminal vesicle PG synthetase
7	>400	≫300	6500
clobuzarit	ND[a]	ND	950
indomethacin	0.3	1.8	1.2

[a] ND = not determined.

found in normal animals. In contrast to 7, and the other compounds in Table II, indomethacin as expected, while exhibiting potent antiinflammatory effects in both animal models, had no normalizing effect upon serum haptoglobin levels. In order to exclude the possibility of a direct effect on protein synthesis by the liver, romazarit (7) was dosed to normal animals. No effect on APP levels was observed.

In the developing adjuvant arthritis model in adrenalectomized rats, 7 exhibited similar activity on APP's and inflammation to that observed in intact animals,[6] indicating that its therapeutic effects are not attributable to stimulation of the pituitary/adrenal axis.

In models of acute inflammation such as carrageenan pleurisy in the rat, rabbit skin edema and UV erythema in the guinea pig, 7 had no effect, which was in marked contrast to the potent antiinflammatory effects observed with NSAIDs. The compound was not ulcerogenic in the stressed rat[8] at 100 mg/kg compared with piroxicam, naproxen, and indomethacin (ED_{50} values 0.6, 4.0, and 2.9 mg/kg respectively). Essentially no effect on prostaglandin synthetase from sheep seminal vesicles was observed (Table V). Furthermore in normal rats dosed with the compound at 100 mg/kg per day po for 7 days no significant reduction in the urinary PGE_2 output was observed, and therefore inhibition of renal cyclooxygenase by the compound or its metabolites was unlikely. In the case of classical NSAID's there is a clear correlation between reduction of urinary PGE_2 output and the antiinflammatory effects observed in carrageenan edema in the rat.[9]

Clearly the biological profile of romazarit is very different to that from classical NSAIDs. While possessing none of the conventional acute antiinflammatory and analgesic effects of NSAIDs, romazarit (7) markedly inhibited polyarthritis in the rat. Effects were also clearly seen on the biochemical markers of inflammation, and therefore the biological activities of romazarit clearly parallel the observed effects of DMARD therapy in man. At this point in the mechanism of action of the compounds described herein is unknown; however, the recent reports on the role of cytokines (interleukin-1, interleukin-6, tumour necrosis factor α) in the stimulation of the acute-phase response[10] and the pathogenesis of rheumatoid arthritis[11] led us to investigate the possibility that these compounds might elicit some of their observed actions by influencing biological processes at or near the cytokine level.

It was clear from receptor studies that 7 affected neither binding of interleukin-1 (IL-1) to cells nor the internalization of the receptor ligand complex.[12]

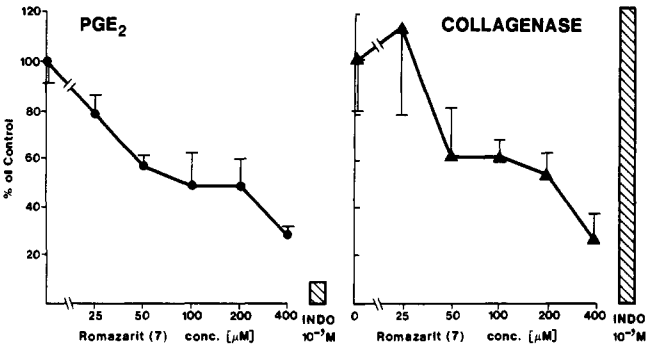


Figure 1. Effect of 7 on interleukin-1-stimulated mediator release from fibroblasts.

However, we have observed in vitro inhibition of cellular events induced by IL-1 which may have some relevance. Romazarit (7) inhibited IL-1-stimulated release of prostaglandin E_2 and the degradative enzyme collagenase from human fibroblasts with an IC_{50} of 150 μM (Figure 1) without affecting cell viability as determined by protein synthesis and release of enzyme markers (see Experimental Section). In contrast, indomethacin prevents prostaglandin release via its inhibition of PG synthetase, but has no effect upon the release of the degradative enzyme collagenase.

The mean plasma levels after 14 days of dosing 7 to arthritic rats (type II collagen arthritis model) at 20 mg/kg per day were determined to be 200 μM (see Experimental Section) which is similar to the observed in vitro IC_{50} for IL-1-stimulated events.

Further studies to identify the molecular mechanism of action of 7 are in progress.

Conclusion

2-[[2-(4-Chlorophenyl)-4-methyl-5-oxazolyl]methoxy]-2-methylpropionic acid [7; generic name, romazarit] represents a novel type of antiinflammatory drug, the biological profile of which is consistent with that of a potential DMARD. While it exhibits no significant activity in models of acute inflammation, it is orally active in models of chronic inflammation where it has beneficial effects on both the clinical (swelling and bone changes) and biochemical (APP) components. The compound does not inhibit PG synthetase, nor is it ulcerogenic.

The compound is currently in phase 2 clinical trial in rheumatoid arthritis patients.

Experimental Section

The animal assays referred to above were carried out as described below.

5-Day Developing Adjuvant Arthritis. Adjuvant was prepared by homogenizing heat-killed *Mycobacterium tuberculosis* (human strains C, DT, and PN from Central Veterinary Laboratory, Weybridge, Surrey, England) in paraffin oil (5 mg/mL). The right hind paws of groups of female Allen and Hanbury hooded rats were injected subcutaneously with 0.1 mL of adjuvant on day 0. Paw volumes of both left (noninjected) and right hind paws were measured on days 1, 2, 3, and 4, 1 h after administration of compounds (prepared as their sodium salts in aqueous solution). The difference in paw volume between the hind paws was integrated and expressed as the area under the curve of paw-volume changes. After the final paw-volume measurement on day 4, the animals were anaesthetized with ether and blood withdrawn from the dorsal tail artery for analysis of plasma haptoglobin levels which were measured with use of a colorometric assay adapted for use on the COBAS-BIO centrifugal analyzer.[5]

Type II Collagen Arthritis. Female Alderley Park strain 1 rats were sensitized by intradermal injection of a type II col-

(8) Müller, R. K. M. Unpublished results.
(9) Matsuda, K.; Ohnishi, K.; Misaka, E.; Yamakazi, M. *Biochem. Pharmacol.* **1983**, *32*, 1347.
(10) (a) Sipe, J. D. *Adv. Intern. Med.* **1989**, *34*, 1–20. (b) Andus, T.; Geiger, T.; Hirano, T.; Kishimoto, T.; Hienrich, P. C. *Eur. J. Immunol.* **1988**, *18*, 739. (c) Billingham, M. E. J. *Br. Med. Bull.* **1987**, *43*(2), 350–370.
(11) (a) Eastgate, J. A.; Wood, N. C.; DiGiovine, F. S.; Symons, J. A.; Grinlinton, F. M.; Duff, G. W. *Lancet* **1988**, ii, 706. (b) Buchan, G.; Barrett, K.; Turner, M.; Chantry, D.; Maini, R. N.; Feldmann, M. *Clin. Exp. Immunol.* **1988**, *73*(3), 449. (c) Fontana, A.; Hengarther, H.; Weber, E.; Fehr, K.; Grob, P. J.; Cohen, G. *Rheumatol. Int.* **1982**, 49.
(12) Kilian, P.; Westmacott, D. Unpublished results.

lagen/Freunds incomplete adjuvant emulsion. Fifteen days after sensitization animals which had developed an arthritic response were placed in groups of eight animals distributed to give similar mean hind paw volumes and body weights. Oral administration of the compounds, prepared as their sodium salts in aqueous solution, was started on the day of grouping (designated day 1) and was continued daily until day 15, radiologic assessment was made on the hind limbs of the animals postmortem. The degree of damage was judged in the area of the tibia/talus joint and in the tarsus region and scored by using a 0–3 score for each foot were 0 = normal, 1 = slight, 2 = moderate, and 3 = severe damage. The radiographs were scored by two observers and for each foot the mean of the two scores was calculated. A combined score for each animal was determined by summating the scores for left and right feet (maximum possible score per animal = 6). The radiographic scores of the treated animals were compared to the scores of the arthritic controls and the significance of the result determined by Mann Whitney U test. Results are expressed as the percentage change from arthritic relative to the normals.

Samples of the rat liver (2 g) were homogenized in ice-cold 0.25 M sucrose (20 mL). The homogenate was centrifuged at 2000 rpm for 10 min to remove cell debris. Catalase activity was determined spectrophotometrically by following the decline in absorbance of 240 nm due to the decomposition of H_2O_2 substrate. Since catalase does not follow normal enzyme kinetics, enzyme activity was defined by the first-order rate constant K ($K = (2.303/t)(\log E_1/E_2)$ s^{-1}) where t is time, E_1 is absorbance 0.08, and E_2 is absorbance 0.05.

Plasma levels of **7** were determined after 14 days of dosing by an HPLC method. To an aliquot of plasma (0.2 mL) was added an internal standard (2-[[2-[4-(trifluoromethyl)phenyl]-4-methyl-5-oxazolyl]methoxy]-2-methylpropionic acid) followed by 0.1% trifluoroacetic acid in acetonitrile (1 mL) to precipitate protein, which was removed by centrifugation. The supernatant was blown down to dryness under a stream of nitrogen and redissolved in 0.2 mL of eluant (42% acetonitrile in water containing 0.1% trifluoroacetic acid). This solution was analyzed by HPLC on a C-18 reverse-phase column with UV detection at 282 nm.

dose, mg/kg	plasma level, μg/mL (mean ± SD)
0	0
20	64 ± 27
40	102 ± 18
60	172 ± 27
100	267 ± 41

Ulcer Formation in Stressed Rats. Female SPF rats (150–170 g) were starved for 24 h and then dosed with compound suspended in a gel of 0.5% traganth administered orally in a volume of 10 mL/kg. Animals were exposed to a cold-water stress (22.5 °C) for 2 h. The rats were not restrained but freely moving in a bath of 7 cm depth. (Such cold-water stress leads by itself to erosions in the gastric mucosa within 6 h. After 2 h the stress by itself does not produce macrosopically visible lesions of the stomach mucosa, but enhances the ulcerogenic effect of drugs.) The rats were sacrificed and animals with recognizable lesions of the gastric mucosa counted. The ED_{50} is defined as the dose which produced gastric mucosal erosions in 50% of animals.

Cyclooxygenase Inhibition Assay.[6b] Sheep seminal vesicle enzyme (2.5 mg/mL) was preincubated for 5 min at 30 °C with or without compounds (prepared as their sodium salts in aqueous solution) in the presence of glutathione (1 mM) and adrenaline (625 μM). The reaction was started with the addition of [^{14}C]-arachidonate (0.05 μCi, 58.3 mCi/mmol) in a total volume of 0.5 mL. The reaction was terminated after 30 min with 0.1 mL of 2 M citric acid/3.3 M sodium chloride. The mixture was then extracted with ethyl acetate (0.2 mL). A portion (20 μL) of the organic phase was subjected to TLC on silica gel with 1% formic acid in ethyl acetate as solvent and counted on a Berthold radiochromatoscanner. The integrated area of the prostaglandin/thromboxane was determined and compared with the control (no drug).

The above assay was carried out in a similar manner using a homogenate of rat renal medulla.

Mononuclear cells were isolated from blood of healthy volunteers, treated with 2.5 μg/mL phytohaemagglutinin, and incubated with compounds (prepared as their sodium salts) for 24 h before determination of PGE_2 by RIA.

Inhibition of Interleukin-1-Stimulated Cellular Events.[6b] Human fibroblasts were plated out in 48-well multidish plates and 1 day later the adherent cells treated with recombinant human IL-1α at 1.4×10^{-12}M (1 unit mL^{-1}) and romazarit (**7**) or indomethacin. After 24 h PGE_2 levels in media were determined by RIA (control level 89 ng × 10^5 cells^{-1}). Trypsin-activated collagenase was measured after 4 days incubation with use of a collagen microfibril assay (control level 21 μg collagen degraded × h^{-1} 10^5 cell^{-1}).

In experiments where Il-1-treated fibroblasts were treated with **7** at levels up to 300 μM there was no significant effect on cell viability as measured by release of lactate dehydrogenase, on protein synthesis measured by [^{35}S]methionine incorporation and solubilized cellular protein as estimated by the method of Bradford,[13] or on mitochondrial activity measured by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan.[14] Furthermore, fructose 2,6-bisphosphate, which is a stimulator of the glycolysis regulating 6-phosphofructo-1-kinase is not inhibited by romazarit (**7**) at a concentration of 300 μM (unpublished results).

Chemistry. Melting points were determined with a Büchi melting point apparatus and are uncorrected. NMR spectra were obtained on a Bruker 300 MHZ spectrometer for $CDCl_3$ solutions and are consistent with the assigned structure. Microanalyses were performed by the Physical Methods Department, Roche Products Limited, and carbon, hydrogen, and nitrogen results were within ±0.4% of theory. Reagents, solvents, and other chemicals obtained commercially were used without further purification unless otherwise indicated. The starting materials were prepared as described in the cited literature references: (halomethyl)-heterocyclic precursors to **1**,[15] **4**,[16] **7**,[16] **17**,[17] **18**,[18] **19**,[19] (hydroxymethyl)heterocyclic precursors to **2**,[20] **3**,[21] **5**,[16], **6**,[16], **7**,[16], **8**,[16] **9**,[16] **11**,[16] **15**,[22] **16**.[23]

Determination of log P'. Log P' data were generated by dissolving the compound in aqueous buffer (10 mL, disodium hydrogen phosphate/citric acid) at pH 7.4, of which 5 mL were partitioned against octan-1-ol (5 mL). The absorbance in the buffer phase was measured before and after partitioning by UV spectrophotometry. The apparent partition coefficient P' was calculated from the ratio of absorbance of the octan-1-ol to the aqueous buffer in the region of the λ_{max} for the compound under study.

Method A. 2-[[2-(4-Chlorophenyl)-4-methyl-5-oxazolyl]-methoxy]-2-methylpropionic Acid (7). A solution of methyl 2-hydroxy-2-methylpropionate (1.95 g, 30.2 mmol) in dry DMF

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(10 mL) was added during 1 h to a stirred suspension of sodium hydride (1.16 g of a 60% suspension in oil, 30.2 mmol) in DMF (20 mL). The solution was stirred at room temperature for 1 h and was then slowly added to a solution of 2-(4-chlorophenyl)-4-methyl-5-(chloromethyl)oxazole (5.2 g, 21.5 mmol) in dry DMF (10 mL) at 0 °C. The mixture was stirred at room temperature overnight and saturated aqueous ammonium chloride (5 mL) then added. The solvent was removed under reduced pressure and the residue partitioned between diethyl ether (100 mL) and water (100 mL). The organic phase was washed with water (2 × 100 mL) and dried ($MgSO_4$). The solvent was removed and the solid residue dissolved in ethanol (200 mL) and sodium hydroxide (1 g, 25 mmol) in water (10 mL) added. The solution was heated to reflux for 1 h and cooled and the solvent removed at reduced pressure. The residue was dissolved in water (400 mL) and extracted with diethyl ether (2 × 100 mL). The aqueous phase was acidified to pH 2 with concentrated HCl and extracted with dichloromethane (3 × 100 mL). The dichloromethane solution was dried ($MgSO_4$) and the solvent removed at reduced pressure to afford a crude solid which was recrystallized from ethyl acetate: NMR ($CDCl_3$) δ 7.95 (2 H, m), 7.47 (2 H, m), 4.58 (2 H, s), 2.27 (3 H, s), and 1.59 (6 H, s).

Method B. 2-[[2-(4-Chlorophenyl)-4-methyl-5-oxazolyl]methoxy]-2-methylpropionic Acid (7). 2-(4-Chlorophenyl)-4-methyl-5-oxazolemethanol (6.9 g, 30.8 mmol) was combined with powdered sodium hydroxide (6.2 g, 155 mmol) in acetone (45 mL) and the suspension heated to reflux. Chloroform (4.9 g, 41.1 mmol) in acetone (10 mL) was added dropwise over 30 min and the suspension heated at reflux for 4 h. The solvent was removed at reduced pressure and the residue partitioned between diethyl ether (150 mL) and water (400 mL). The aqueous phase was extracted with diethyl ether (2 × 100 mL) and acidified to pH 2 with concentrated HCl and extracted with dichloromethane (3 × 50 mL). The dichloromethane solution was dried ($MgSO_4$) and the solvent removed at reduced pressure to afford a crude solid which was recrystallized from ethyl acetate.

Method C. [1,3]-Dipolar Cycloadditions. 2-[[3-(4-Chlorophenyl)-5-isoxazolyl]methoxy]-2-methylpropionic Acid (10). A solution of triethylamine (3.57 g, 37 mmol) in dry DMF (30 mL) was added to a solution of *N*-hydroxy 4-chlorobenzenecarboximidoyl chloride (6.33 g, 33.3 mmol) in dry DMF (60 mL) at 10 °C over 10 min. After 1 h at room temperature the solution of 4-chlorobenzenecarbonitrile oxide was poured onto water and the solid isolated by filtration. The moist solid was dissolved in diethyl ether (150 mL) and dried (3A molecular sieves). The organic solution was filtered and the filtrate added to a solution of methyl 2-(propynyloxy)-2-methylpropionate[16] (6.56 g, 36.6 mmol) in diethyl ether (50 mL) at 5 °C. After 24 h the solvent was removed and the residue purified by column chromatography on silica gel with 4:1 hexane/ethyl acetate as eluant. Upon removal of the solvent there was obtained 4.67 g of methyl ester.

The methyl ester (0.31 g, 1 mmol) was dissolved in methanol (10 mL) and treated with aqueous sodium hydroxide (1.3 mL of a 1 M solution, 1.3 mmol) and stirred for 20 h at room temperature. The solvent was removed at reduced pressure and the residue treated with hydrochloric acid (5 mL of a 2 M solution) and extracted with diethyl ether (3 × 50 mL). Upon removal of the solvent a crude solid was obtained which was recrystallized from toluene/hexane.

2-[[1-(4-Chlorophenyl)-3-pyrazolyl]methoxy]-2-methylpropionic Acid (12) and 2-[[1-(4-Chlorophenyl)-4-pyrazolyl]methoxy]-2-methylpropionic Acid (13). In a manner analogous to that described above (Method C) there was obtained from *N*-(4-chlorophenyl)sydnone[24] and methyl 2-(propynyloxy)-2-methylpropionate two regioisomeric dipolar cycloaddition products in a 1:1 ratio which were separated by chromatography on silica gel with 4:1 hexane/ethyl acetate as eluant. Subsequent base hydrolysis and recrystallization afforded the acids 12 and 13. Acid 12: NMR ($CDCl_3$) δ 7.8 (1 H, d), 7.6 (2 H, m), 7.41 (2 H, m), 6.52 (1 H, d), 4.68 (2 H, s), and 1.6 (6 H, s). Acid 13: NMR ($CDCl_3$) δ 7.92 (1 H, s), 7.73 (1 H, s), 7.6 (2 H, m), 7.41 (2 H, m), 4.5 (2 H, s), and 1.58 (6 H, s).

2-[[3-(4-Chlorophenyl)-5-pyrazoyl]methoxy]-2-methylpropionic Acid (14). The requisite [1,3]-dipole was generated by base treatment of 4-methylbenzenesulfonic acid [(4-chlorophenyl)methylene]hydrazide[25] and reaction with methyl 2-(propynyloxy)-2-methylpropionate carried out as described in general method C. The desired regioisomer was isolated as the major project by chromatography on silica gel and subsequent hydrolysis to afforded the acid 14.

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