to rats for 50 days at 10 mg/kg po, 1 did not alter the neutrophil arachidonic acid composition, nor did 1 alter the profile of lipid mediators generated by ionophore stimulation of human neutrophils (thus, 1 is not a phospholipase inhibitor).

Certain sulfamate esters have been reported[18] to possess carbonic anhydrase inhibiting activity. Carbonic anhydrase has been implicated in the mechanism of bone resorption,[19] which is an important component of rhematoid arthritis.[20] For this reason 1, 2, and 3 were assayed against carbonic anhydrase[21] but were found to be inactive ($IC_{50} > 16\ \mu M$). Also, the urine of rats receiving 10 mg/kg of 1 daily for 25 days did not inhibit carbonic anhydrase, indicating that no appreciable amount (<2% of dose) of metabolites which inhibited carbonic anhydrase[22] were present. Compound 1 was also devoid of any of the effects associated with carbonic anhydrase inhibitors such as diuretic[23] (10 mg/kg po, rats) or anticonvulsant activity (inactive at 100 mg/kg ip, mice, in the maximal electroshock assay[24]).

Summarily, 1 represents a new chemical class (the sulfamate esters) of antiarthritic agents which acts by a mechanism distinct from known therapeutic agents. This compound is active in the AA model in rats when given therapeutically or prophylactically. Unlike NSAIDs, steroids, or immunomodulators, 1 did not inhibit the generation of the arthritic state (day 18 edema, Figure 1). Compound 1 lacks nonspecific activity since it failed to inhibit carrageenan-induced, serotonin-induced, and PAF-induced edema or the passive-foot anaphylaxis response in rats, even at doses 10-fold in excess of those required for antiarthritic activity. This compound is active in a chronic animal model of inflammation but not active in acute models. Compound 1 has no effect on a DTH reaction, on arachidonic acid release, on the 5-lipoxygenase pathway, on cycloxygenase activity, or on PAF-mediated events. The fact that the S-(−)-isomer (2) is more potent than the R-(+)-isomer (3) in the AA rat assay suggests that a specific binding site, heretofore unreported to have an influence on the inflammatory process, may be mediating the diminution of the edema and bone changes.

Studies to discern the mechanism of action of this chemical class of compounds (over 400 sulfamate ester derivatives tested to date possess varying degrees of activity in the AA assay) are ongoing and will be reported in due course.

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(15) Human blood neutrophils were isolated by dextran sedimentation[17] and centrifugation on Ficoll/Hypaque.[18] Neutrophil cell suspensions in 1.0 mL of Dulbecco's medium (1.7×10^7 cells/mL) were supplemented with 1 mM $CaCl_2$ and preincubated for 5 min at 37 °C with either ethanol or an ethanolic solution of 1, 2, or 3 (1–100 μM). Reactions were initiated by addition of an ethanolic solution containing a mixture of arachidonic acid and/or calcium ionophore (A23187). The final concentration of arachidonic acid and A23187 was 80 and 5 μM, respectively. At appropriate time periods, incubations were terminated by addition of 2 mL of acetone and assayed for 5-HETE, 12-HETE, 15-HETE, and LTB_4 formation. One nanomole of PGB_2 was added to the terminated reaction, and arachidonic acid metabolites were extracted on Baker-10 C_{28} sample-preparation columns. One nanomole of 13-hydroxyoctadeca-9,11,15-trienoic acid was added to each sample before reconstituting them in 300 μL of 75% methanol in water. One hundred microliter aliquots were analyzed by HPLC (Ultrasphere 3u-ODS column). The column was eluted isocratically for 10 min with a solvent mixture composed of 30% solvent A [methanol/acetonitrile/water/phosphoric acid (25:25:50:0.02 v/v)] and 70% solvent B [methanol/acetonitrile/water/phosphoric acid (35:35:30:0.02 v/v)], followed by elution with a linear gradient that increased the component of solvent B from 70 to 90% over the next 15 min. From 25 to 45 min, the elution was continued isocratically with a mixture of 10% of solvent A and 90% of solvent B. Elution of hydroxy fatty acids, leukotriene B, and PGB_2 was monitored with a UV detector.

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Nonsteroidal Antiinflammatory Drug Hydroxamic Acids. Dual Inhibitors of Both Cyclooxygenase and 5-Lipoxygenase

Sir:

The broad class of nonsteroidal antiinflammatory drugs (NSAIDs) are invaluable in the mainline treatment of rheumatoid arthritis and osteoarthritis.[1] The inhibition of arachidonate cyclooxygenase (CO) is a hallmark feature of virtually all marketed NSAIDs, and this property is believed to play an important role in their therapeutic efficacy.[2] However, the consequences of CO inhibition by NSAIDs result in certain mechanism-based side effects[3,4] including dyspepsia, gastrointestinal ulceration/bleeding, and nephrotoxicity, especially in the elderly population who use them most frequently.[1] The antisecretory and cytoprotective properties of prostaglandins have been well-studied,[5] and their role in cytoprotection

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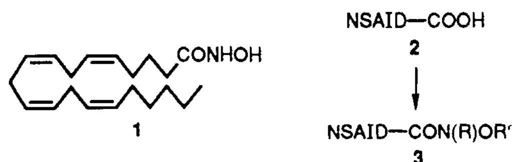
Scheme I



is currently an important area of clinical research. Thus CO inhibition by NSAIDs removes a natural cytoprotective mechanism. In searching for NSAID classes with a broader mechanistic profile and reduced side effect liability, we have noted that evidence has accumulated which implicates uninhibited or increased leukotriene production in addition to prostaglandin inhibition as a more complete explanation for these NSAID-induced side effects.[6-8] Additionally, various 5-lipoxygenase products have also been shown to exhibit proinflammatory/immune properties in vitro and in vivo.[9]

Appreciation of the above findings stimulated our interest in a research program designed to incorporate 5-lipoxygenase (5-LO) inhibition into the wide range of existing NSAID pharmacophores. The initial report by Corey[10] that arachidonate hydroxamic acid 1 is a potent inhibitor of 5-LO prompted us to consider the conversion of various nonsteroidal antiinflammatory drugs 2 to their corresponding hydroxamic acids 3 (Scheme I).[11] Critical to the early success of this approach would be (1) realization of 5-LO inhibitory activity in NSAID-derived hydroxamates, (2) retention of CO inhibitory activity, (3) evidence of antiinflammatory activity in relevant animal models, and (4) demonstration of some unique profile as a result of incorporating 5-LO inhibitory activity into the NSAID pharmacophore.

Initially, our attention focused on the preparation of various hydroxamic acids of meclofenamic acid (4) (X = OH). Treatment of meclofenamic acid with oxalyl chloride/DMF (methylene chloride) followed by the appropriate hydroxylamine hydrochloride (triethylamine/THF/water) afforded the corresponding hydroxamic acids **5a–c**. All compounds were evaluated in an intact RBL-1 cell line for inhibition of cyclooxygenase and 5-lipoxygenase activities (Table I).[12] While sodium meclofenamate (4) itself is a 240-fold selective inhibitor of CO vs 5-LO, *O*-methyl hydroxamate **5a** is a more balanced dual inhibitor (29-fold CO selective). The hydroxamates bearing a free *N*-hydroxyl group are more potent 5-LO inhibitors. The unsubstituted hydroxamic acid (**5b**) exhibits balanced dual inhibition of both CO (IC_{50} = 1.1 μM) and 5-LO (IC_{50} = 3.9 μM), while *N*-methylhydroxamate **5c** *exhibits an inverted selectivity favoring 5-LO inhibition* (5-LO IC_{50} = 1.5 μM, 10-fold selectivity vs CO).

A similar trend was observed in a series of indomethacin hydroxamates 7, prepared in analogous fashion to the meclofenamate hydroxamates. Thus indomethacin (**6**) itself is an extremely selective CO inhibitor (>200-fold selectivity) in the intact RBL-1 cell assay, *while the N-alkylated hydroxamic acid analogues **7c** and **7d** are, in fact, approximately 3-fold selective as 5-LO inhibitors.* The same trend in 5-LO inhibitory potency is present in a series of sulindac hydroxamates **9a–c**. Finally, a series of ibuprofen hydroxamates were investigated. Compared to ibuprofen (**10**), the hydroxamic acids **11b** and **11c** are more balanced inhibitors of both CO and 5-LO. While **11b** and **11c** have been reported as inhibitors of 5-lipoxygenase,[13a] no mention was made of their effects on the

Table I. In Vitro Biochemistry of NSAID Hydroxamates

4/5 6/7 8/9 10/11

no.	X	IC_{50}, μM[a] CO	5-LO	5-LO/CO IC_{50} ratio
4	O^-Na^+	0.10	24	240
5a	NHOMe	0.55	16	29
5b	NHOH	1.1	3.9	3.5
5c	N(Me)OH	15	1.5	0.10
6	OH	0.50	>100	>200
7a	NHOMe	<0.20	24	>120
7b	NHOH	1.1	7.5	68
7c	N(Me)OH	5.2	1.4	0.27
7d	N(iPr)OH	2.7	0.90	0.33
8	OH	N[b] (32)*	>100	
9a	NHOMe	N[b] (32)*	26	
9b	NHOH	N[b] (32)*	13	
9c	N(Me)OH	N[b] (32)*	1.0	
10	OH	2.0	N[b] (32)	≫16
11a	NHOMe	5.8	24% (32)	>5.5
11b	NHOH	10	20	2.0
11c	N(Me)OH	20	1.2	0.06

[a] IC_{50} from regression analysis of percent inhibition vs inhibitor concentration or percent inhibition at highest tested concentration. [b] N = no significant inhibition at indicated concentration (μM). *Sulindac requires metabolic reduction for CO inhibition.[18]

Table II. In Vivo Pharmacology of NSAID Hydroxamates

series	no.	5-LO/CO IC_{50} ratio	CFE[a]	MFE[b]	UD_{50}[c]
fenamate	**4**	240	8.2	0.39	36.0
	5a	29	1.3	24.0	N[f] (200)
	5b	3.5		27.0	N[f] (200)
	5c	0.1	0.2	2.5	N[f] (200)
indomethacin	**6**	>200	36 ± 3.2% (5)[d]	0.21	5.4
	7a	>120	40 ± 4.3% (10)[d]		42.0
	7b	6.8	46 ± 4.9% (30)[d]		103.0
	7c	0.3	29 ± 4.3% (10)[d]		10% (100)
	7d	0.3	27 ± 5.1% (10)[e]	0.82	N[f] (200)

[a] ID_{25} or percent inhibition of edema at indicated dose (mg/kg po); n = 7–14 per experimental group. [b] ID_{40} of induced edema at indicated dose (mg/kg po); n = 7–14 per experimental group. [c] Dose (mg/kg po) producing ulcers in 50% of rats tested. [d] $p < 0.001$ compared to controls. [e] $p < 0.02$ compared to controls. [f] N = no significant ulceration.

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cyclooxygenase pathway. Our finding extends the utility of arylhydroxamic acids as 5-lipoxygenase inhibitors[13,14] with application to the wide range of NSAID pharmacophores, thus converting CO-selective NSAIDs to analogues possessing dual CO/5-LO inhibitory properties.

The various nonsteroidal antiinflammatory hydroxamates were evaluated for in vivo activity in models of efficacy [carrageenin footpad edema (CFE)[15] and mycobacterium footpad edema (MFE)[16]] and in a model of NSAID-induced side effects (ulcerogenicity in a rat stress model[17]). Table II illustrates the in vivo profiles of these agents compared to those of the parent NSAIDs. In the fenamate NSAID series, the meclofenamic hydroxamates **5a–c** are efficacious in both acute (CFE) and subacute (MFE) models of inflammation as is sodium meclofenamate (**4**) itself. However, whereas sodium meclofenamate is ulcerogenic in the rat (UD_{50} = 36 mg/kg), none of the dual inhibitor hydroxamate analogues **5a–c** display ulcerogenicity.

A more discriminating trend is seen in the series of indomethacin hydroxamates **7a–d**. While these analogues exhibit potency as antiinflammatory agents in CFE and MFE, there is an inverse correlation of CO/5-LO selectivity and induced ulceration in the rat stress model. Thus as more 5-LO inhibitory activity is introduced into the NSAID pharmacophore, a commensurate decrease in ulceration is realized. While indomethacin (**6**, >200-fold selective CO inhibitor) exhibits a UD_{50} of 5.4 mg/kg, *N*-isopropyl hydroxamate **7d** (3-fold selective 5-LO inhibitor) is not ulcerogenic at oral doses up to 200 mg/kg. Additionally, **7d** retains excellent potency in CFE (27% inhibition at 10 mg/kg) and MFE (ID_{40} = 0.82 mg/kg).

While these observations are worthy of further mechanistic studies, it is noted that Peskar et al. have reported that the leukotrienes play a role in ethanol-induced gastric damage in the rat,[6] and Konturak et al. have demonstrated that leukotriene antagonists partially suppress ulceration induced by ASA.[8b] It is possible that the lack of ulcerogenicity by the NSAID hydroxamates is due to the presence of intact hydroxamates in the gut, where 5-LO inhibition plays a mucosal-protective role in preventing ulceration. The antiinflammatory activities of the NSAID hydroxamates may be in part due to systemic metabolic conversion to the parent NSAID carboxylic acids. In this scenario the NSAID hydroxamates serve to deliver relevant 5-LO inhibition to the gut (protection from NSAID ulceration), while relevant CO inhibition is delivered systemically (antiinflammatory activity). In support of this interpretation, Summers et al.[13a] have demonstrated that hydroxamic acids of this structural type are readily metabolized to the parent carboxylic acids in vivo in rats, dogs, and monkeys. However, the intact NSAID hydroxamates retain potent CO inhibitory activity; hence, it is possible that a portion of the antiinflammatory activity observed is due to systemic NSAID hydroxamate. Further insight into these mechanistic considerations awaits further investigation.

Thus 5-LO inhibitory activity may be readily designed into the NSAID pharmacophore while CO inhibition and antiinflammatory activity is maintained in animal models which correlate with human efficacy.[1] The degree of 5-LO inhibitory activity may be modulated easily by varying the substituents on the hydroxamate function. Toward this end, NSAID hydroxamates have been shown to be either partially or totally devoid of ulcerogenicity in a rat stress model, depending on the degree of 5-LO inhibitory potency of the analogue and the inherent ulcerogenicity of the parent NSAID. This study suggests that a number of the clinically used NSAIDs may be converted into useful antiinflammatory agents possessing reduced ulcerogenicity by rationally incorporating 5-lipoxygenase inhibitory activity. Current research is dedicated toward other NSAID hydroxamates and studying the in vivo metabolism/pharmacokinetics of these agents.

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