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Communications to the Editor

Acetohydroxamic Acids as Potent, Selective, Orally Active 5-Lipoxygenase Inhibitors

Sir:

In the search for novel inhibitors of the 5-lipoxygenase (5-LO) pathway of arachidonic acid metabolism, we have had occasion to look at several series of hydroxamic acids, two examples of which are described within.

The 5-LO pathway leads to several compounds with extremely potent biological activities: leukotriene B_4 (LTB_4) has been shown[1] to be a potent chemotactic agent in vivo, while the peptido leukotrienes LTC_4 and LTD_4 are powerful bronchoconstrictors[2] and lead to an increase in vascular permeability.[3] Furthermore, elevated levels of leukotrienes have been found[4] in certain disease states such as asthma, rheumatoid arthritis, and psoriasis.

We[5] and others have developed compounds that inhibit the 5-LO or 5-LO *and* cyclooxygenase (CO) pathways of arachidonic acid metabolism, while the alternative approach of leukotriene antagonists has also been extensively investigated.[6] Unfortunately, many of the compounds so far developed suffer from toxicity problems or lack of oral bioavailability. More recently, several groups have prepared analogues of arachidonic acid,[7] 5-HETE,[8] and 15-HETE[9] which contain a hydroxamic acid moiety. In these examples, the hydroxamic acid portion of the molecule is thought to bind to Fe^{3+} at the catalytic site of the enzyme.

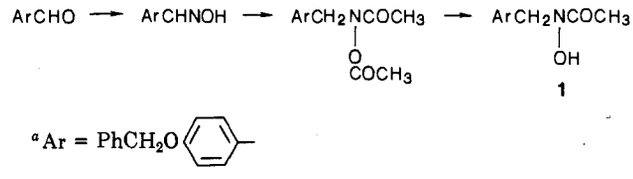
On this basis, a number of hydroxamic acids were prepared as potential 5-LO inhibitors. Of these, compounds 1 (BW A137C), *N*-[4-(benzyloxy)benzyl]acetohydroxamic acid, and 2 (BW A4C), *N*-[(*E*)-3-(3-phenoxyphenyl)prop-2-enyl]acetohydroxamic acid, were found to be potent, selective inhibitors of human leukocyte 5-LO (Table I) and to demonstrate significant oral bioavailability in animals. Compound 1 was prepared from the oxime of 4-(benzyloxy)benzaldehyde by reduction with sodium cyanoborohydride in acetic acid followed by in situ treatment with acetic anhydride; selective removal of the *O*-acetyl group then gave 1 in high yield (Scheme I).

Table I. In Vitro Inhibition of 5-LO and CO from Human Polymorphs

compd	mp, °C	IC_{50}, μM:[a] 5-LO	CO
1	121–122	0.77 ± 0.16 (9)[b] 0.2–2.3[c]	22 ± 4 (9)
2	84	0.14 ± 0.03 (7)[b] 0.06–0.36[c]	3.2 ± 0.8
4	124–125	0.05 ± 0.01 (3)[b]	5 ± 3 (3)

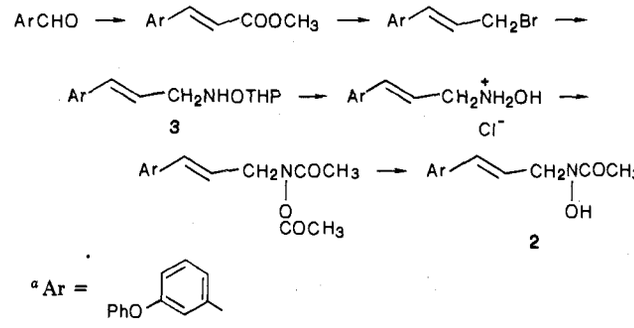
[a] Homogenates of human polymorphs were preincubated with inhibitor (added in DMSO) for 5 min at 37 °C before initiating reaction by addition of arachidonic acid and $CaCl_2$ (final concentrations 5 μM and 2 mM, respectively). After a further 5 min, incubation reaction was stopped by boiling, and LTB_4 and TXB_2 were assayed by RIA.[15] [b] Mean ± SEM for (*n*) experiments. [c] Minimum and maximum IC_{50}'s.

Scheme I[a]



[a] Ar = $PhCH_2O$

Scheme II[a]



[a] Ar = PhO

The preparation of 2 proved somewhat more problematic; reduction of the appropriate unsaturated oxime, as above, gave the unsaturated hydroxylamine, which is unstable at the pH of the reaction medium. Although 2 *could* be obtained by this route, extensive purification was required and yields were low. A preferable synthesis is shown in Scheme II. Reaction of 3-phenoxybenzaldehyde under standard conditions (pyridine, malonic acid, piperidine) gave the cinnamic acid, which was esterified, reduced (DIBAL), and converted to the (*E*)-allylic bromide (1:1 Et_2O/hexane, 48% HBr). Reaction with 3 equiv of *O*-tetrahydropyranylhydroxylamine[10] in DMF gave the

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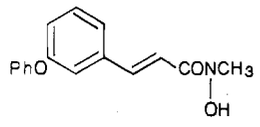
monoalkylated hydroxylamine 3 together with about 10% bisalkylated product. Hydrolysis of the crude reaction mixture with concentrated HCl in MeOH gave the deprotected hydroxylamine hydrochloride, which was purified by crystallization (EtOAc). Acetylation followed by O-deacetylation, as above, gave 2 in good overall yield. Acetylation of 3 followed by deprotection (PPTS, MeOH) gave less pure product.

Both 1 and 2 have so far been found to be devoid of toxicity problems and to be nonmutagenic[11] in the Ames Salmonella test. Furthermore, 1 and 2 selectively inhibit the ex vivo Ca^{2+} ionophore stimulated production of LTB_4 in whole rat blood for well over 6 h after a single oral dose of 50 mg/kg; compound 2, in fact, has an ED_{50} at 6 h of 9 mg/kg. In contrast, compound 4, which is structurally



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similar to and in vitro is equipotent with 2 shows no ex vivo activity at 6 h after 50 mg/kg orally in rats. It should be noted that 4 is structurally related to the hydroxamic acid based inhibitors recently disclosed by several other groups.[12]

Compound 2 has also demonstrated its ability to block the "leukotriene-dependent" anaphylactic bronchospasm[13] in anesthetized guinea pigs in a dose-related manner. In the 6-h carrageenin sponge implant model of inflammation,[14] 2 selectively inhibits the formation of LTB_4 over PGE_2 in the sponge exudates with an ED_{50} of 2.6 mg/kg. This inhibition was accompanied by a decrease in the number of leukocytes in the sponge exudate, but there was no *direct* correlation between the two values. Further extensive biological observations with compounds 1 and 2 will appear in due course.[15]

Thus, with the development of potent, selective, orally active inhibitors of 5-LO, it should be possible to determine the relevance of lipoxygenase products in human disease states.

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Registry No. 1, 106328-28-3; 1 (*O*-acetyl deriv), 106328-89-6; 2, 106328-57-8; 2 (*O*-acetyl deriv), 112270-90-3; 3, 112270-88-9; 5-LO, 80619-02-9; 4-(benzyloxy)benzaldehyde oxime, 76193-67-4; 3-phenoxybenzaldehyde, 39515-51-0; malonic acid, 141-82-2; methyl 3-[(4-phenoxy)phenyl]propenoate, 87087-33-0; 3-bromo-1-[(4-phenoxy)phenyl]propene, 112270-87-8; *N*-hydroxy-3-(4-phenoxyphenyl)-2-propenamine hydrochloride, 112270-89-0.

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9-(*trans*-2′,*trans*-3′-Dihydroxycyclopent-4′-enyl) Derivatives of Adenine and 3-Deazaadenine: Potent Inhibitors of Bovine Liver *S*-Adenosylhomocysteine Hydrolase

Sir:

Neplanocin A (NpcA, Chart I), a cytotoxic, cyclopentenyl analogue of adenosine, is a naturally occurring antitumor antibiotic, which was isolated from the bacterium *Ampullariella regularis*.[1-4] NpcA possesses antitumor activity in vivo against murine L1210 leukemia in mice[2,5] and antiviral activity in cell culture against vaccinia virus,[6] herpes simplex-1,[7] herpes simplex-2,[7] and vesicular stomatitis virus.[7] Our laboratory has shown that NpcA is a potent, irreversible inhibitor of *S*-adenosylhomocysteine (AdoHcy) hydrolase (E.C. 3.3.1.1) isolated from bovine liver[6] and *Alcaleigenes faecalis*.[8] This enzyme, which catalyzes the reversible hydrolysis of AdoHcy to adenosine and homocysteine, is the only metabolic route for the removal of AdoHcy in eukaryotic cells.[9] Subsequently, inhibition of AdoHcy hydrolase by NpcA in eukaryotic cells (e.g., mouse L929 and neuroblastoma N2a cells) leads to elevation of cellular levels of AdoHcy and inhibition of *S*-adenosylmethionine (AdoMet) dependent methylations.[10,11] The inhibition of AdoHcy hydrolase has been correlated with the antiviral activity of NpcA,[12] and

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