Journal of Medicinal Chemistry

Volume 31, Number 3 March 1988

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.

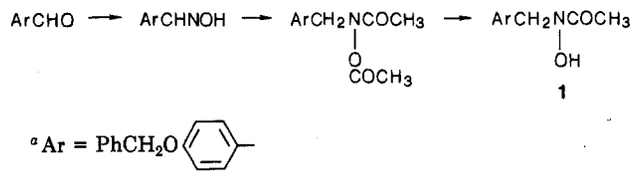
On this basis, a number of hydroxamic acids were prepared as potential 5-LO inhibitors. Of these, compounds 1 (BW A137C), *N*-[4-(benzyloxy)benzyl]acetohydroxamic acid, and 2 (BW A4C), *N*-[(*E*)-3-(3-phenoxyphenyl)prop-2-enyl]acetohydroxamic acid, were found to be potent, selective inhibitors of human 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)

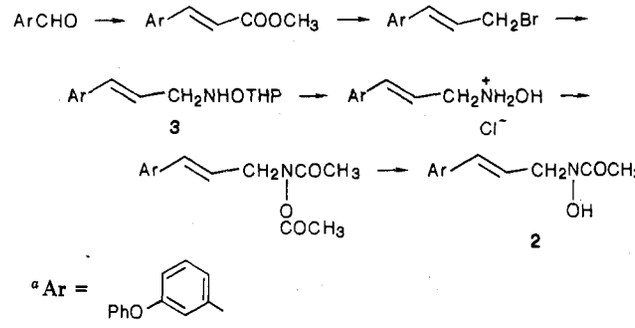
[a] Homogenates of human polymorphs were preincubated with inhibitor (added in DMSO) for 5 min at 37 °C before initiating reaction by addition of arachidonic acid and $CaCl_2$ (final concentrations 5 μM and 2 mM, respectively). After a further 5 min, incubation reaction was stopped by boiling, and LTB_4 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

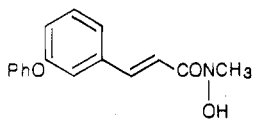
The preparation of 2 proved somewhat more problematic; reduction of the appropriate unsaturated oxime, as above, gave the unsaturated hydroxylamine, which is unstable at the pH of the reaction medium. Although 2 *could* be obtained by this route, extensive purification was required and yields were low. 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

(1) Bray, M. A. *Br. Med. Bull.* **1983**, *39*, 249.
(2) Weiss, J. W.; Drazen, J. M.; Coles, N.; McFadden, E. R.; Weller, P. W.; Corey, E. J.; Lewis, R. A.; Austen, K. F. *Science (Washington, D.C.)* **1982**, *216*, 196.
(3) Bisgaard, H.; Kirstensen, J.; Sodengaard, J. *Prostaglandins* **1982**, *23*, 801.
(4) Bray, M. A. *Agents Actions* **1986**, *19*, 87.
(5) Higgs, G. A.; Flower, R. J.; Vane, J. R. *Biochem. Pharmacol.* **1979**, *28*, 1959.
(6) Gardiner, P. J. In *Drugs Affecting Leukotrienes and Lipoxygenase Products; A Potential Fulfilled?* IBC Technical Services Ltd.: London, 1987; Cashman, J. R. *Pharm. Res.* **1985**, 253.
(7) Corey, E. J.; Cashman, J. R.; Kantner, S. S.; Wright, S. W. *J. Am. Chem. Soc.* **1984**, *106*, 1503.
(8) Kerdesky, F. A. J.; Holms, J. H.; Schmidt, S. P.; Dyer, R. D.; Carter, G. W. *Tetrahedron Lett.* **1985**, 2143.
(9) Nicolaou, K. C.; Ladduwahetty, T.; Elisseou, E. M. *J. Chem. Soc., Chem. Commun.* **1985**, 1580.

0022-2623/88/1831-0499$01.50/0

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



4

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.

Acknowledgment. We thank our biological collaborators for their dedicated support in this project.

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.

(10) Warrener, R. N.; Cain, E. N. *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 511.
(11) Unpublished observations from the Wellcome Biological Control Laboratories, Dartford, UK.
(12) Summers, J. B.; Mazdiyasni, H.; Holms, J. H.; Ratajczyk, J. D.; Dyer, R. D.; Carter, G. W. *J. Med. Chem.* **1987**, *30*, 574; Brit. UK Pat. Appl. 2183637; Eur. Pat. Appl. 0196674; U.S. Pat. Appl. 4604407; U.S. Pat. Appl. 4607053. Summers, J. B.; Gunn, B. P.; Mazdiyasni, H.; Goetze, A. M.; Young. P. R; Bouska, J. B.; Dyer, R. D.; Brooks, D. W. *J. Med. Chem.* **1987**, *30*, 2121.
(13) Payne, A. N.; Garland, L. G.; Lees, I. W.; Salmon, J. A. *Br. J. Pharmacol.*, in press.
(14) Higgs, G. A.; Follenfant, R. L.; Garland, L. G. *Br. J. Pharmacol.*, in press.
(15) Tateson, J. E.; Randall, R. W.; Reynolds, C. H.; Jackson, W. P.; Bhattacherjee, P.; Salmon, J. A.; Garland, L. G. *Br. J. Pharmacol.*, in press.

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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

(1) Hayashi, M.; Yuginuma, S.; Yoshioka, H.; Nakatsu, K. *J. Antibiot.* **1981**, *34*, 675.
(2) Yaginuma, S.; Muto, N.; Tsujino, M.; Sudate, Y.; Hayashi, M.; Otani, M. *J. Antibiot.* **1981**, *34*, 359.
(3) Hayashi, M.; Yaginuma, S.; Muto, N.; Tsujino, N. *Nucleic Acids Symp. Ser.* **1980**, *8*, 65.
(4) Yaginuma, S.; Tsujino, N.; Muto, N.; Fujii, T.; Hayano, K.; Matsuda, T.; Watanbe, T.; Abe, J. *Current Chemotherapy and Infectious Disease*, Proceedings of the 11th International Conference of Chemotherapy; Nelson, J. P., Grassi, C., Eds.; American Society for Microbiology: Washington, DC, 1980; Vol. 2, p 1559.
(5) Tsujino, M.; Yaginuma, S.; Fujii, T.; Hagaso, K.; Matsuda, T.; Watanbe, T.; Abe, J. *Current Chemotherapy and Infectious Disease*, Proceedings of the 11th International Conference of Chemotherapy; Nelson, J. P., Grassi, C., Eds.; American Society for Microbiology: Washington, DC, 1980; Vol. 2, p 1558.
(6) Borchardt, R. T.; Keller, B. T.; Patel-Thombre, U. *J. Biol. Chem.* **1984**, *259*, 4353.
(7) De Clercq, E. *Antimicrob. Agents Chemother.* **1985**, *28*, 84.
(8) Matuszewska, B.; Borchardt, R. T. *Arch. Biochem. Biophys.* **1987**, *256*, 50.
(9) De la Haba, G.; Cantoni, G. L. *J. Biol. Chem.* **1959**, *234*, 603.
(10) Keller, B. T.; Borchardt, R. T. *Biological Methylation and Drug Design*; Borchardt, R. T.; Creveling, C. R.; Ueland, P. M. Eds.; Humana: Clifton, NJ, 1986, p 385.
(11) Ramakrishnan, V.; Borchardt, R. T. *Neurochem. Int.* **1987**, *10*, 423.
(12) De Clercq, E.; Cools, M. *Biochem. Biophys. Res. Commun.* **1985**, *129*, 306.

0022-2623/88/1831-0500$01.50/0