Bioorganic & Medicinal Chemistry Letters, Vol.2, No.11, pp. 1353-1356, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

1-PHENYL-[2H]-TETRAHYDROPYRIDAZIN-3-ONE, A-53612, A SELECTIVE ORALLY ACTIVE 5-LIPOXYGENASE INHIBITOR

Dee W. Brooks*, Daniel H. Albert, Richard D. Dyer, Jennifer B. Bouska,
Patrick Young, Gary Rotert, Joseph M. Machinist and George W. Carter
Immunosciences Research Area, Department 47K, Abbott Laboratories, Abbott Park, Illinois 60064

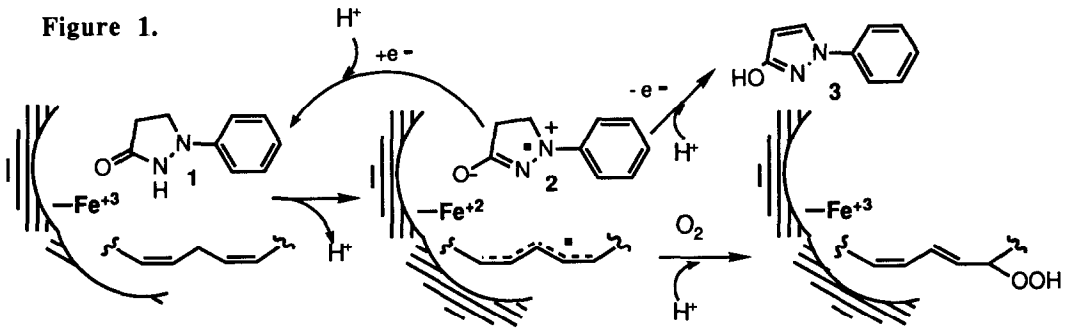
(*Received* 30 *June* 1992)

Abstract. Ring homologation of the known lipoxygenase inhibitor, phenidone to 1-phenyl-[2H]-tetrahydropyridazin-3-one provided A-53612, which was discovered to be a selective, orally active 5-lipoxygenase inhibitor. In contrast to phenidone, A-53612 did not cause significant inhibition of platelet 12-lipoxygenase, soybean 15-lipoxygenase, or intact rat PMNL cyclo-oxygenase at concentrations up to 100 μM.

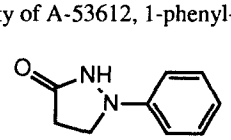
The biosynthetic products of the arachidonate cascade have been the subject of intense research due to the variety of important biological activities they express.[1] The enzyme 5-lipoxygenase initiates the pathway leading to the biosynthesis of the leukotrienes.[2] The leukotrienes have been shown to have numerous biochemical effects and present evidence indicates that they are important mediators in a variety of conditions including, asthma, ulcerative colitis, and rheumatoid arthritis.[3] Inhibition of 5-lipoxygenase represents a promising therapeutic target and an important means to further elucidate the role of leukotrienes in pathological or physiological processes.[4]

The simple heterocyclic compound phenidone (**1**), used commercially as a photographic developer,[5] was one of the first inhibitors of 5-lipoxygenase (5-LO) to be discovered.[6] The redox properties of phenidone led to the general hypothesis that the inhibition of 5-LO could involve an electron transfer from phenidone to an activated Fe(III) site in the enzyme resulting in a deactivated Fe(II) enzyme and a stabilized phenidone derived radical intermediate **2** as illustrated in Figure 1. The radical intermediate **2** could react with an electron donor and thus be recycled or it could be further oxidized irreversibly to the stable 1-phenyl-3-hydroxypyrazole (**3**). A recent study of several phenidone analogs as 5-LO inhibitors by the Sterling Research Group provides an excellent account of this series of 5-LO inhibitors.[7] The Wellcome Research Institute using phenidone as an initial lead, extensively studied the aminopyrazoline series, represented by BW755C (**4**).[8]

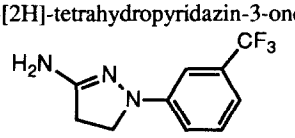
Figure 1.



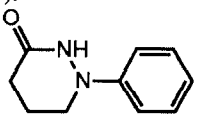
Our 5-lipoxygenase inhibitors program also considered phenidone as a lead compound. One objective of our study was to examine the effect on 5-LO inhibitory activity of structural analogs of phenidone where the redox potential was reduced. We proposed to examine the six-membered ring homolog of phenidone as a 5-LO inhibitor. It was rationalized that a methylene insertion in phenidone would result in reduction of the favorable thermodynamics for oxidization to the stable heteroaromatic, 1-phenyl-3-hydroxypyrazole (**3**) by precluding the heteroaromatic delocalization of electrons. Support for reduced redox potential was previously reported[9] for **5** in that it was first synthesized and evaluated as a photographic developer and found to be much less effective than phenidone with respect to super-additivity with hydroquinone. This report describes the 5-lipoxygenase inhibitory activity of A-53612, 1-phenyl-[2H]-tetrahydropyridazin-3-one (**5**).[10]



phenidone (1)



BW 755C (4)



A-53612 (5)

Chemistry. The previously reported synthesis of **5**[9] was improved to a simple two step process involving, first condensation of acetylphenylhydrazide with ethyl 4-bromobutyrate in the presence of ethyldiisopropylamine followed by treatment with sodium ethoxide in ethanol providing **5** in 40% yield overall.

Inhibitory Evaluation *In Vitro*.[10] The inhibition of 5-LO activity for A-53612 (**5**) was evaluated in several assays and compared to the reference inhibitors, phenidone (**1**) and BW-755C (**4**). The results are shown in Table 1. A-53612 inhibited the 5-lipoxygenase activity in the 20,000x g supernatant of broken RBL-1 cells with a potency similar to phenidone and BW-755C. A significant difference in biological properties of A-53612 is evident in the level of selectivity of the inhibitory activity toward related oxygenase enzymes. A-53612 does not inhibit platelet 12-lipoxygenase at 100 μM and only weakly inhibits soybean 15-lipoxygenase at 100 μM whereas phenidone is a potent inhibitor of both of these enzymes. The selectivity of A-53612 was evaluated in ionophore stimulated intact rat polymorphonuclear leukocytes. In this assay, A-53612 inhibited LTB_4 biosynthesis with an IC_{50} of 1 μM but did not inhibit the formation of the cyclooxygenase product PGE_2 at concentrations up to 100 μM whereas phenidone and BW-755C inhibited both LTB_4 and PGE_2 formation in this intact leukocyte assay. In calcium ionophore stimulated whole human blood, A-53612 inhibited LTB_4 biosynthesis with an IC_{50} of 0.7 μM and had little effect on the formation of the cyclooxygenase product TXB_2 at 100 μM. These results indicate that A-53612 exhibits specificity for 5-lipoxygenase and good inhibitory potency in broken cell preparations, intact cells or even in human whole blood.

Plasma Levels by Oral Administration.[11] A-53612 provided satisfactory plasma levels in the rat, dog, and monkey as shown in Table 2. A-53612 was rapidly absorbed and exhibited a similar oral half-life in each of the three species studied. Additional studies in the rat showed that the i.v. half-life was 1.1 hours. Comparison of the areas under the p.o. and i.v. plasma concentration curves indicated that A-53612 was 93% bioavailable in the rat.

Inhibition of Leukotriene Biosynthesis *In Vivo*.[12] The *in vivo* activity of A-53612 was evaluated in a rat anaphylaxis model involving antigen-antibody stimulated leukotriene generation in the peritonium. A-53612 exhibited a dose related inhibition of leukotriene biosynthesis with an ED_{50} of 6 mg/kg by oral administration. In this same assay phenidone had an ED_{50} of 17 mg/kg and BW-755C had an ED_{50} of 36 mg/kg.

Table 1. Enzyme Inhibitory Evaluation *In Vitro*

Assay	Phenidone	BW-755C	A-53612
	IC_{50}[a] (μM)		
5-lipoxygenase (RBL broken cell-supernatant)	1.9 (1.5-2.8)	2.0 (1.8-2.3)	4.3 (4.1-4.5)
12-lipoxygenase (human platelet)	0.10 (0.09-0.10)	5% at 100 μM[b]	7% at 100 μM[b]
15-lipoxygenase (soybean)	0.42 (0.34-0.51)	213 (113-535)	25% at 100 μM[b]
cyclo-oxygenase (sheep seminal vesicle)	94 (78-115)	97 (85-111)	5% at 300 μM[b]
5-lipoxygenase (intact rat PMNL, LTB_4)	0.52 (0.33-0.74)	12 (10-14)	1.2 (0.8-1.9)
5-lipoxygenase (intact human PMNL, LTB_4)	1.3 (1.1-1.6)	4.9 (3.8-6.0)	1.0 (0.1-2.3)
cyclo-oxygenase (intact rat PMNL, PGE_2)	13 (11-16)	0.8 (0.6-1.0)	3% at 300 μM[b]
5-lipoxygenase (human whole blood, LTB_4)	0.3 (0.25-0.4)	7.6 (7.0-8.1)	0.7 (0.1-1.3)
cyclo-oxygenase (human whole blood, TxB_2)	12 (4-20)	5.8 (1.8-9.0)	13% at 100 μM[b]

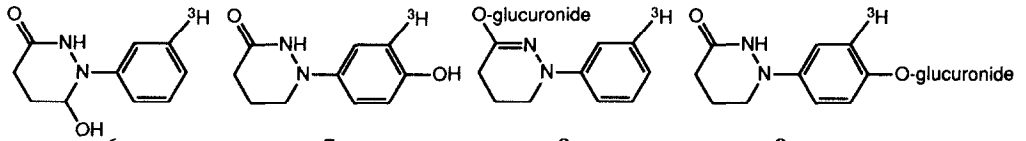
[a] 95% confidence limits shown in parenthesis [b] Percent inhibition at highest tested concentration.

Table 2. Plasma Levels Upon Oral Administration of 30 mg/kg of A-53612

Species	Time Max	Plasma Conc Max	p.o.Est. $T_{1/2}$
	hours	μM	hours
dog	0.3	124	0.32
monkey	1.0	90	0.75
rat	0.4	22	1.20

Pilot Toxicity Evaluation. The promising pharmacological properties of this compound prompted pilot toxicity evaluation. A-53612 tested negative in the Ames test. In contrast to phenidone and BW-755C, A-53612 did not convert hemoglobin to methemoglobin *in vitro* in whole blood. However it was surprising and very disappointing to observe that repeated daily administration of A-53612 in pilot toxicity studies in the rat resulted in methemoglobinemia as indicated by the appearance of Heinz bodies in red blood cells within 24 h following a second 400 mg/kg dose. The extent of methemoglobinemia observed was dose related. A metabolite was presumed responsible for this toxicity since A-53612 did not produce methemoglobin *in vitro*.

Metabolism. In order to better understand the source of the *in vivo* toxicity associated with A-53612, a metabolism study was conducted in the rat. A-53612 was labelled with tritium at the 3-phenyl position by tritiation of the corresponding 3-bromophenyl derivative. From the bile and urine, 89% of the radiolabel was recovered 24 h after oral dosing. Along with parent compound, four metabolites were identified which accounted for about 92% of the radioactivity recovered from the bile and urine. The structures were determined to be the 6-hydroxy derivative **6**, the 4-hydroxyphenyl derivative **7**, the O-glucuronide of parent compound **8**, and the O-glucuronide of the 4-hydroxyphenyl metabolite **9**. None of these metabolites showed activity to convert hemoglobin to methemoglobin *in vitro* up to 400 μM. Thus, the source of toxicity remained elusive.



6 **7** **8** **9**

Summary. A-53612 (**5**) is a selective, orally active 5-lipoxygenase inhibitor which can serve as a useful pharmacological tool in elucidating the role of leukotrienes in animal studies. The increased inhibitory selectivity for 5-LO of A-53612 compared to phenidone supported the original hypothesis of reducing the non-specific antioxidant properties by ring expansion. Efforts to further understand and eliminate the *in vivo* methemoglobinemia observed in addition to further enhancement of the inhibitory potency for this class of 5-lipoxygenase inhibitors is required in order to realize potential clinical candidates.

References.

1. Samuelsson, B. *Science* **1983**, *120*, 568.
2. Samuelsson, B.; Dahlen, S. -E., Lindgren, J. A.; Rouzer, C. A.; Serhan, C. N. *Science* **1987**, *237*, 1171.
3. Lewis, R.A.; Austen, F.; Soberman, R.J. *New England Journal of Medicine* **1990**, *323* ,645.
4. a) Brooks, D.W.; Bell, R.L.; Carter, G.W. *Annual Reports in Medicinal Chemistry*; Allen, R., Ed.; Academic Press; New York, 1988; Vol. 22, p 69. b) Salmon, J.A.; Garland, L.G. *Progress in Drug Research*, **1991**, *37*, 9. c) Howard R. Knapp *New England Journal of Medicine* **1990**, *323* ,1745. d) Isreal, E.; Dermarkarian, R.; Rosenberg, M.; Sperling, R.; Taylor, G.; Rubin, P.; Drazen, J.M. *New England Journal of Medicine* **1990**, *323* ,1740.
5. Kendall, J. D.; Duffin, G. F.; Axford, A. J. U.S. Patent 2,688,024, Aug. 31, 1954.
6. a) Blackwell, G. J.; Flower, R. J. *Br. J. Pharmacol.* **1978**, *63*, 360P, b). Blackwell, G. J.; Flower, R. J. *Prostaglandins* **1978**, *16*, 417.
7. Hlasta, D.J.; Casey, F.B.; Ferguson, E.W.; Gangell, S.J.; Heimann, M.R.; Jaeger, E.P.; Kullnig, R.K.; Gordon, R.J. *J. Med. Chem.* **1991**, *34*, 1560.
8. a) Higgs, G. A.; Flower, R. J.; Vane, J. R. *Biochem. Pharmacol.* **1979**, *28*, 1959, b) Copp, F. C.; Islip, P. J.; Tateson, J. E. *Biochem. Pharmacol.* **1984**, *33*, 339.
9. a) Ficken, G. E.; Sanderson, B. G. *Photo. Sci.* **1963**, *11*, 157, b) Walther, W. *Veroeffentl. Wiss. Photo-Lab.* **1965**, *10*, 159.
10. Brooks, D.W.; Kerdesky, F.A.J.; Holms, J.H.; Schmidt, S.P.; Basha, A.; Ratajczyk, J.D.; Martin, J.G.; Young, P.R.; Albert, D.H.; Dyer, R.D.; Bouska, J.B.; Carter, G.W. Abstracts of the ACS, Fall 1988, MEDI 121.
11. The procedures for the biological assays are described in detail in: Carter, G. W.; Young, P.R.; Albert, D.H.; Bouska, J.; Dyer, R.; Bell, R.L.; Summers, J.B.; Brooks, D.W. *J. Pharm. and Exper. Ther.* **1991**, *256*; 929.
12. The procedure for the rat anaphylaxis model is described in detail in: Young, P.R.; Bell, R.L.; Lanni, C.; Summers, J.B.; Brooks, D.W.; Carter, G.W. *Eur. J. Pharm.* **1991**, *205*, 259.