Pure & Appl. Chem., Vol. 66, Nos 10/11, pp. 2197–2200, 1994.
Printed in Great Britain.

Second generation leukotriene biosynthesis inhibitors

Anwer Basha,* Dee W. Brooks, Andrew O.Stewart, Randy L. Bell, and George W. Carter.
Immunoscience Research Area, D-47K AP10, Abbott Laboratories, Abbott Park, IL 60064-3500

Abstract: Zileuton (A-64077) was the first 5-lipoxygenase inhibitor of the N-hydroxyurea class selected as an investigational new drug for the treatment of leukotriene mediated diseases such as asthma, inflammatory bowel disease and rheumatoid arthritis. Clinical results with zileuton in asthma have demonstrated efficacy for this new therapeutic approach. Continuing research has focused on the discovery of second generation inhibitors with improved potency and duration of action. A strategy was implemented for the N-hydroxyurea series involving substantial synthetic throughput to evaluate key structural elements of a simple inhibitor model with respect to inhibitory activity, oral bioavailability, and rate of metabolism. This approach culminated in the identification of A-78773, an optimized second-generation 5-lipoxygenase inhibitor with improved *in vivo* potency and significantly longer plasma half-life in several species.

Leukotrienes have been implicated as mediators in a number of human diseases such as asthma, inflammatory bowel disease and rheumatoid arthritis (1,2). 5-Lipoxygenase is the pivotal enzyme which catalyzes the first step leading to the biosynthesis of leukotrienes (3,4). Therefore, the discovery of compounds which would prevent the synthesis of leukotrienes potentially provides a novel therapeutic approach for these diseases.

The generally accepted mechanism for the catalytic oxidation of arachidonic acid by 5-LO is derived from the analogous proposal for the soybean 15-LO in which Fe^{+3} is involved (5). Both 5- and 15-LO are known to contain a non-heme iron (6).

Since no X-ray or NMR structural information for 5-LO is available, the strategy to design the inhibitors involved an iterative process of intuitive medicinal chemistry guided by biological evaluation in various inhibition assays . Rational inhibitor design for 5-LO incorporated one or more structural entity complementary to those of enzyme active site selected from the following:

a. a lipophilic binding region which interacts with the unsaturated fatty acid chain of the substrate;
b. a proton acceptor which removes the 7-pro-S hydrogen;
c. an iron atom and accompanied ligands positioned for stereospecific interaction at the 5-unsaturated position;
d. a binding site for the carboxylate group of arachidonic acid.

The working hypothesis of interacting with a catalytically important iron co-factor stimulated investigation of compounds that contained functional groups that were known to bind with Fe^{+3}. Corey and coworkers have published the corresponding hydroxamic acid analog of the enzyme's substrate arachidonic acid and found it to be a potent *in vitro* inhibitor (7). Early research at Abbott demonstrated that the hydroxamate group served as a potent inhibitory ligand when attached to a 5-HETE template (8). Many aryl hydroxamates were found to be potent *in vitro* inhibitors of 5-LO activity (9). Evaluation of the pharmacokinetic disposition of these hydroxamate in the rat revealed that they were readily absorbed but metabolized rapidly to the corresponding carboxylic acid which possess no 5-LO inhibory activity. Thus this metabolic inactivation accounted for the poor *in vivo* activity observed (10). In preventing hydrolytic cleavage or other routes of metabolism, the N-hydroxyurea functionality appeared most promising. After oral administration in several species, N-hydroxyureas exhibited potent *in vivo* inhibition, provided greater plasma concentrations and longer plasma half-lives.

N-hydroxyureas represented a very broad class of potent direct 5-LO inhibitors. The following simple model defines arbitrary components of this series of 5-LO inhibitors.

TEMPLATE LINK UNIT PHARMACOPHORE

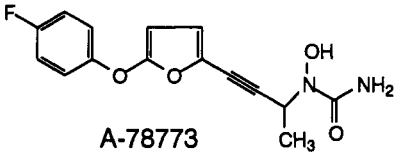
Several combinations are possible for the template and link unit which are further multiplied by any modifying substituents at either component. After studying hundreds of compounds we found that the template served to provide the hydrophobic component for enzyme interaction. Increased hydrophobicity was correlated to increase inhibition potency *in vitro* (11). However, specific structural parameters beyond mere lipophilicity were necessary to provide improved potency. Aryl templates were often superior to saturated ring systems. Heterocyclic templates consisting of groups such as furan, thiophene and fused systems such as benzofuran and benzothiophene provided more potent templates than nitrogen containing heterocycles.

The linking group was designed to insulate the hydroxylamine derived pharmacophore from the aryl template to prevent formation of arylhydroxylamine metabolites. The template and link unit mutually defined the orientation of the pharmacophore. The judicious selection of the combination of components to maximize Van der Waals interaction in the inhibitor-enzyme complex and positioning the putative iron-binding pharmacophone optimized the potency.

The benzothiophene template and ethyl link unit led to the discovery of A-64077, N-(1-benzo[b]thien-2-ylethyl)-N-hydroxyurea, which subsequently became Abbott's first 5-LO clinical candidate, zileuton (12-14). Zileuton is a potent *in vitro* inhibitor of 5-LO activity in the 20,000xg supernatant of sonicated RBL-1 cells (IC_{50} = 0.6 μM), intact neutrophils from rats (IC_{50} = 0.2 μM) and humans (IC_{50} = 0.7μM). Zileuton is very effective inhibitor *in vivo* in the rat anaphylaxis model (ED_{50} = 5 mg/kg). The plasma half-life in the dog, mouse, sheep, rat and monkey was 7.5, 0.9, 4.0, 2.3, and 0.3 h respectively. The acute oral LD_{50} in mice is approximately 1.8 g/kg.

Our next goal was to optimize potency and duration of action of N-hydroxyureas while maintaining satisfactory oral bioavailability. The major route of metabolism elucidated by the clinical

studies of zileuton was glucuronidation of the hydroxyurea function and subsequent elimination. *In vitro* assays (15) with liver microsomes proved useful in identifying structural features which reduced the rate of glucuronidation. Tandem to these evaluations, new templates providing enhanced potency were prepared. These studies culminated in the discovery of A-78773, [N-[3-[5-(4-fluorophenoxy)-2-furanyl]1methyl-2-propynyl]-N-hydroxyurea (16). The 1-methylpropynyl link unit was a key factor in reducing the rate of glucuronidation while 4-fluorophenoxyfuran provided a highly potent template with excellent bioavailability.



A-78773

A-78773 is about 11-fold more potent than zileuton in inhibiting the *in vitro* formation of LTB_4 in human whole blood (IC_{50} = 85 nM) and has 35-fold greater potency in preventing LTB_4 in human neutophils (IC_{50} = 20 nM). This compound was about 300-fold more selective for 5-LO than CO as measured by thromboxane formation in human whole blood. The duration of action of the compound was impressive in the monkey with a 2 mg/kg oral dose providing essentially 100% inhibition of LTB_4 for over a 24 h period. The *in vivo* activity of A-78773 in the rat anaphylaxis model was about 6-fold better than zileuton (ED_{50} = 0.8 mg/kg).

Conclusion: The demonstration that leukotrienes have pathological properties relevant to various diseases has stimulated the development of various new chemical entities to block the production of leukotrienes. The clinical trials with zileuton have provided the first evidence of efficacy by blocking leukotrienes biosynthesis (17). Future clinical studies with zileuton and other more potent compounds will further support the therapeutic applicability of this novel mode of intervention.

1. P. Sirois. *Advances in Lipid Research*, p.79, Academic Press, New York (1985).
2. R.A. Lewis R.A. Lewis *et al. New Engl.J.Med.* **323** 645 (1991).
3. B. Samuelsson. *Science* **120** 568 (1983).
4. E.J. Corey *et al. J.Amer.Chem.Soc.* **102** 1436 (1980).
5. M.R. Gibian and R.A. Galaway. In *Bio-organic Chemistry* p.117, Academic Press, New York (1977).
6. D.L. Sloane *et al. Biochen.Biophys.Res.Com.* **173** 507 (1990) and M.D. Percival. *J.Biol.Chem.* **266** 10058 (1991).
7. E.J. Corey. *J.Amer.Chem.Soc.* **106** 503 (1984).
8. F.A.J. Kerdesky *et al. J.Med.Chem.* **30** 1177 (1987).
9. J.B. Summers *et al. J.Med.Chem.* **30** 574 (1987).
10. J.B. Summers *et al. J.Med.Chem.* **30** 2121 (1987).
11. J.B. Summers *et al. J.Med.Chem.* **33** 992 (1990).
12. J.B. Summers *et al. U.S.Patent 4,873,259* Oct. 10, 1991.
13. D.W. Brooks *et al. International Chemical Congress of Pacific Basin Societies* **BIOS 34** Dec. 18, 1989.

14. G.W. Carter *et al. J.Pharmacol.Exp.Therp.* **256** 929 (1991).
15. D.J. Sweeny *et al. Drug Metab.Disp.* **20** 328 (1992).
16. R.L.Bell *et. al . J .Lipid Med.* **6** 259 (1993).
17. E. Isreal *et al. Ann. Intern. Med.* **119** 1059 (1993)