Bioorganic & Medicinal Chemistry Letters, Vol.2, No.11, pp. 1357-1360, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

STRUCTURE-ACTIVITY RELATIONSHIPS OF THE PYRIDAZINONE SERIES OF 5-LIPOXYGENASE INHIBITORS

Dee W. Brooks*, Anwer Basha, Francis A. J. Kerdesky, James H. Holms, James D. Ratajcyk, Pramila Bhatia, Jimmie L. Moore, Jonathan G. Martin, Steven P. Schmidt, Daniel H. Albert, Richard D. Dyer, Patrick Young, George W. Carter

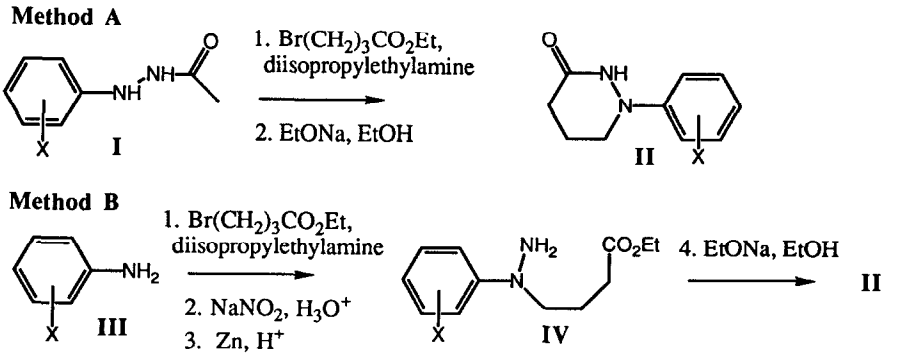
Immunosciences Research Area, Department 47K, Abbott Laboratories, Abbott Park, Illinois 60064

(*Received* 30 *June* 1992)

Abstract. Structure activity analysis of the pyridazinone series as represented by the initial lead compound A-53612 revealed that the 1-phenyl-2H-tetrahydropyridazin-3-one structure was necessary for inhibitory activity as several modifications diverging from this structure led to a dramatic loss of inhibitory activity. Substituents on the phenyl ring had a marked effect on inhibitory activity and methemoglobinemia toxicity.

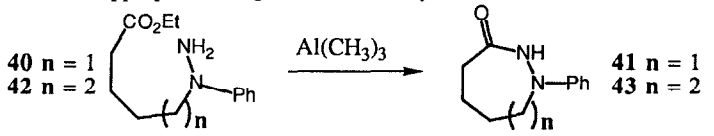
Inhibition of leukotriene biosynthesis by inhibition of the enzyme 5-lipoxygenase (5-LO) represents a promising new therapeutic intervention.[1] The discovery of A-53612, 1-phenyl-[2H]-tetrahydropyridazin-3-one as a selective orally active 5-LO inhibitor provided a lead compound for further optimization.[2] This report describes the structure-activity relationships of this series of 5-LO inhibitors.

Chemistry. The syntheses of the novel compounds evaluated in this investigation are described as follows.[3] The phenyl substituted pyridazinones, **1-21** were prepared by either Method A: condensation of the appropriate substituted acetylphenylhydrazide **I** with ethyl 4-bromobutyrate in the presence of ethyldiisopropylamine followed by treatment with sodium ethoxide in ethanol to provide the pyridazinone **II** or Method B: heating the corresponding substituted aniline **III** with methyl 4-bromobutyrate in the presence of diisopropylethylamine followed by nitrosation and reduction to the intermediate hydrazine **III** which was cyclized by treatment with sodium ethoxide in ethanol to yield the desired pyridazinone **II**. The carboxyl analog **22** was prepared by saponification of the ester **20**. The 1-phenylpyrazolidinones **23-29** were prepared by known methods[9] involving condensation of the requisite phenylhydrazine with acrylonitrile followed by acid hydrolysis.



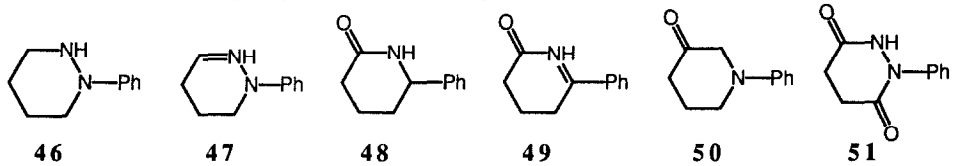
Compounds **30-32** were prepared by treatment of **1** with methyliodide, propionylchloride, and benzoylchloride, respectively. The cyclohexyl analog **34** was prepared by catalytic hydrogenation of **1** using 5% rhodium on alumina as catalyst. Compounds **35-39** were prepared by reaction of the hydrazide **52** with pentylbromide, benzylbromide, benzoylchloride, octylbromide, and dodecylbromide, respectively.

The novel seven- and eight-membered hydrazides **41** and **43** were prepared by intramolecular cyclization of the corresponding hydrazine esters **40** and **42** by treatment with trimethylaluminum. Hydrazines **40** and **42** were prepared from aniline and the appropriate *omega*-bromo ester by Method B.



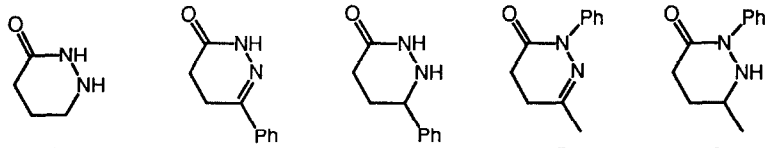
Identification of the Pharmacophore for 5-Lipoxygenase Inhibitory Activity. A survey of the structural requirements for inhibitory activity was conducted using the 5-lipoxygenase activity in the 20,000xg supernatant from sonicated RBL-1 cells.[4] Several structural modifications described as follows supported the premise that 1-phenyl-2H-tetrahydropyridazin-3-one (**1**) was the pharmacophore for 5-LO inhibitory activity.

The following structural modifications resulted in inactive compounds: 1) removal of the carbonyl function as in **46**[5] and **47**[6], 2) replacement of the nitrogen atom at position 1 with carbon as in **48**[7] and **49**[8], and 3) at position 2 as in **50**[9]. A carbonyl at position 6 as in **51**[10] provided weak activity (24% at 30 μM).



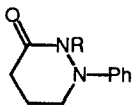
46 **47** **48** **49** **50** **51**

The phenyl group at N1 proved to be essential for 5-LO inhibitory activity as the following analogs were inactive: 1) removal of the phenyl group as in 2H-tetrahydropyridazinone (**52**)[11], 2) repositioning the phenyl group at carbon 6 as in **53**[12] and **54**, and 3) placing the phenyl group at N2 as in **55**[13] and **56**[14].

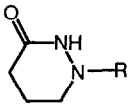


52 **53** **54** **55** **56**

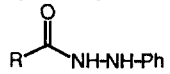
Substitution on Nitrogen. N2-alkyl analogs such as N-methyl **30**, N-phenyl **33**[15] and N2-acyl analogs **31** and **32** were inactive. Thus, an unsubstituted 2-NH group was required for 5-LO inhibitory activity.



30 R = CH_3
31 R = $COCH_2CH_3$
32 R = COPh
33 R = Ph



34 R = C_6H_{12}
35 R = nC_5H_{12}
36 R = CH_2Ph
37 R = COPh
38 R = nC_8H_{18}
39 R = $nC_{12}H_{26}$



44 R = CH_3
45 R = n C_3H_7

Replacement of the Phenyl Group. Replacement of the phenyl group at N1 was investigated as follows. The 1-cyclohexyl congener **34** and the 1-n-pentyl analog **35** were both inactive. The benzyl analog **36** and the benzoyl analog **37** were inactive. Appending a lipophilic hydrocarbon chain at N1, as in the octyl **38** and dodecyl analogs **39**, provided modest inhibitors, IC_{50}'s = 23 and 6 μM, respectively.

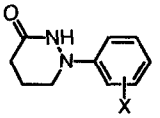
Effect of Ring Size and Conformation. The effect of the ring size of the cyclic hydrazide was examined. The five-membered analog, phenidone had similar *in vitro* activity as the six-membered analog, A-53612[2]. Surprisingly, the seven-membered analog **41** was devoid of 5-lipoxygenase inhibitory activity at concentrations less than 32 μM and the eight-membered analog **43** was marginally active, 8% at 32 μM. This dramatic difference in inhibitory activity indicated that the conformational configuration of the cyclic N-phenylhydrazide unit was important for effective inhibition of 5-LO. The conformation requirements of the hydrazide unit were further explored by examining simple acyclic phenylhydrazides such as **44** (IC_{50} = 65 μM) and **45** (IC_{50} = 52 μM) which showed more than a ten-fold reduction in inhibitory activity compared to A-53612 (**1**). On the basis of this survey of structural modifications we concluded that A-53612 was a minimal structural unit for satisfactory 5-lipoxygenase inhibitory activity.

Substitution on the Phenyl Group. The structure-activity relationships of the pyridazinone series with a variety of substituents on the phenyl ring were evaluated. A very interesting pattern of inhibitory activity was observed and the results are summarized in Table 1. Substitution in the *ortho* or *para* positions resulted in a significant loss of inhibitory activity as illustrated for methyl **2, 4**; ethyl **5, 7**; chloro **8, 10** and methoxy **11, 13** derivatives. Similar substitution with these same groups at the *meta* position **3, 6, 9, 12** only slightly modulated the inhibitory activity compared to the parent system **1**. This phenomenon is in contrast to that observed for the substituted five-membered phenidone system, shown in Table 2. As demonstrated for the methyl **24-26** and chloro **27-29** substituted phenidone analogs, the position of the substituent did not change inhibitory activity as dramatically as in the pyridazinone system. The pyridazinone series was much more sensitive to substituent effects which suggests a more specific binding and alignment with the enzyme.

Further analysis of various substituents at the *meta* position in the pyridazinone series was conducted in a effort to maximize inhibitory activity (Table 1). Only small improvements in inhibitory activity were found with the methyl **3**, ethyl **6**, chloro **9**, bromo **14** derivatives compared to the parent **1**. The more polar substituents resulted in pronounced loss of inhibitory activity .

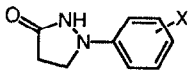
Structure-Activity Relationships With Respect to Methemoglobinemia. The removal of the toxicity associated with the methemoglobinemia expressed by A-53612 *in vivo* [2] was an important objective in order to identify a suitable therapeutic agent. Several analogs were evaluated by daily oral doses of 400 mg/kg in rats and measuring the proportion of red blood cells containing Heinz bodies. Of the active pyridazinone inhibitors, the nitrile analog **18** (A-63640) was unique as it exhibited no Heinz bodies after 11 days at 400 mg/kg per day. The plasma level of **18** achieved in the rat after oral dosing was similar to **1** (A-53612). However this inhibitor did not have adequate *in vivo* potency in the rat anaphylaxis assay[16] (ED_{50} = 23 mg/kg) to merit further development.

Summary. A-53612, 1-phenyl-2H-tetrahydropyridazin-3-one (**1**) offered a lead for the development of selective, orally active 5-LO inhibitors. The position of substituents on the phenyl ring had a marked effect on inhibitory activity indicating a specific binding interaction with the enzyme. Substituents in the *ortho* or *para* positions resulted in a loss of inhibitory activity whereas non-polar substituents in the *meta* position had minor influence on inhibitory activity. The observation that A-63640 (**18**) did not cause methemoglobinemia after eleven daily oral doses provided support that this toxic effect could be overcome in this series. The SAR elucidated in this study indicated a narrow range of productive modifications for further optimization of potency.

Table 1. Substituted 1-Phenyl-[2H]-tetrahydropyridazin-3-ones 

Compound	X	IC_{50},[a] μM	Compound	X	IC_{50},[a] μM
1	H	3.8 (3.1-4.6)	**12**	3 - OCH_3	6.7 (6.1-7.4)
2	2 - CH_3	57 (56-58)	**13**	4 - OCH_3	156 (140-173)
3	3 - CH_3	3.4 (2.8-4.0)	**14**	3 - Br	1.8 (1.6-2.1)
4	4 - CH_3	277 (261-294)	**15**	3 - CH_2OH	53 (45-60)
5	2 - CH_2CH_3	39 (33-47)	**16**	3 - $COCH_3$	19 (16-21)
6	3 - CH_2CH_3	3.0 (2.5-3.8)	**17**	3 - $COtC_4H_9$	15 (13-17)
7	4 - CH_2CH_3	125 (116-136)	**18**	3 - CN	9.3 (8.1-11)
8	2 - Cl	99 (90-108)	**19**	3 - CF_3	5.2 (4.6-5.7)
9	3 - Cl	2.5 (2.2-3.0)	**20**	3 - $COOCH_2CH_3$	5.6 (4.9-6.6)
10	4 - Cl	79 (60-99)	**21**	3 - F	4.5 (4.2-4.8)
11	2 - OCH_3	204 (176-237)	**22**	3 - COOH	NA [b] up to 300 μM

a. IC_{50} with 95% confidence limits indicated in parentheses. b. NA indicates no significant inhibitory activity.

Table 2. Substituted 1-Phenylpyrazolidin-3-ones 

Compound	X	IC_{50}[a], μM	Compound	X	IC_{50}[a], μM
23	H	1.9 (1.5-2.8)			
24	2 - CH3	8.2 (7.2-9.4)	**27**	2 - Cl	3.7 (3.4-4.1)
25	3 - CH_3	1.3 (1.0-1.7)	**28**	3 - Cl	0.73 (0.62-0.87)
26	4 - CH_3	3.5 (3.1-3.9)	**29**	4 - Cl	1.1 (0.86-1.4)

a. IC_{50} with 95% confidence limits indicated in parentheses.

References

1. Lewis, R.A.; Austen, F.; Soberman, R.J. *New England J. of Medicine* **1990**, *323*, 645.
2. Brooks, D.W.; Albert, D.H.; Dyer, R.D.; Bouska, J.B.; Young, P; Carter, G.W. preceeding article.
3. Detailed procedures for the preparation of the active inhibitors are reported in: Brooks, D.W.; Carter, G.W.; Basha, A.; Gunn, B.P.; Dyer R.D. U.S. Patent 5,086,052.
4. The term inactive is defined as no significant inhibition up to 30 μM in the 5-LO inhibition assay reported by Dyer, R. D.; Bornemeier, D. A.; Haviv, F.; Carter, G. W. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1985**, *44*, 904.
5. Eberle, M.K.; Kahle, G. G.; Talati, S. M. *Tetrahedron* **1973**, *29*, 4045.
6. Aubaynac, J. L.; Elguero, J.; Jacquier, R.; Robert, R. *C. R. Acad. Sci.*, Ser. C, **1970**, *270*, 1829.
7. DeGraaff, B. R.; Melger,W. C.; Van de Kolk, G. *Rec. Trav. Chim.* **1962**, *81*, 786.
8. Diller, D.; Bergmann, F. *J. Org. Chem.* **1972**, *37*, 2147.
9. Taylor, E. C., Skotnicki, J. S. *Synthesis* **1981**, 606.
10. Pirkle, W. H., Gravel, P.L. *J. Org. Chem.* **1977**, *42*, 1367.
11. Gut, J., Novacek, A. Fiedler, P. *Collect. Czech. Chem. Commun.* **1968**, *33*, 2087.
12. Delaby, R. Chabrier, P. Danton S. *Compt. Rend.* **1953**, *66*, 237.
13. Lui, H.; Warkentin, J. *Can J. Chem.* **1972**, *59*, 1767.
14. Ege, S.; Carter, M.L.C.; Ortwine, D.F.; Chow, S.; Shing, P.; Richman, J.F. *J. Chem. Soc. Perkin Trans. I*, **1977**, *11*, 1252.
15. Degrand, C; Jacquin, D. *Tetrahedron Letters* **1978**, 4955.
16. 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.