Bioorganic & Medicinal Chemistry Letters, Vol.2, No.10, pp. 1309-1314, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

STRUCTURAL ANALYSIS OF 2-ARYL-1,3-DIONE COMPOUNDS AS INHIBITORS OF 5-LIPOXYGENASE

Dee W. Brooks*, Steven P. Schmidt, Richard D. Dyer, Patrick R. Young, and George W. Carter
Immunoscience Research, Dept. 47K, Abbott Laboratories, Abbott Park, Illinois 60064

(*Received* 16 *July* 1992)

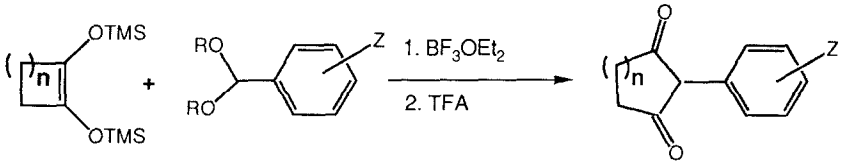
Abstract. The 2-phenylindane-1,3-dione system was observed to inhibit RBL-1 supernatant 5-lipoxygenase (5-LO) activity (IC_{50} = 15 μM). The structure-activity relationships for 5-LO inhibition were examined. Novel 2-phenylcycloheptan-1,3-diones and substituted 2-fluorophenylindane-1,3-dione inhibitors were prepared.

The enzyme 5-lipoxygenase catalyzes the oxidation reaction of arachidonic acid which ultimately leads to the biosynthesis of the leukotrienes.[1] Numerous studies support the premise that the leukotrienes are potent pathological mediators in a variety of conditions including asthma, arthritis, psoriasis, inflammatory bowel disease, and allergy.[2] Preventing or modulating the biosynthesis of leukotrienes represents a potentially useful therapeutic intervention.[3]

In the course of evaluating compounds for *in vitro* 5-lipoxygenase in the 20,000Xg supernatant of homogenized RBL-1 cells, we identified 2-phenylindan-1,3-dione (**1**) as a moderately active inhibitor, IC_{50} = 15 μM. This class of compound with a rich pharmacological background[4a] was also an early lead in the discovery research leading to the oxicam class of antiinflammatory agents.[4b] Several congeners are used clinically as orally administered anticoagulants[5] while other analogs are anti-inflammatory agents.[6] The ability to separate these two major activities by chemical modification has been demonstrated.[7] Other pharmacological properties have been reported for this series.[8] Our objective was to determine if we could utilize this lead to design novel 5-lipoxygenase inhibitors with oral bioavailability as already demonstrated for this series in man.[9] A major shortcome of many potent 5-lipoxygenase inhibitors commonly referenced is a lack of oral bioavailability.[10] In view of our discovery of the 5-lipoxygenase inhibitory activity of the phenindandione series, it was interesting to speculate that perhaps some of the antiinflammatory activity observed might be due in-part to inhibition of leukotriene biosynthesis.

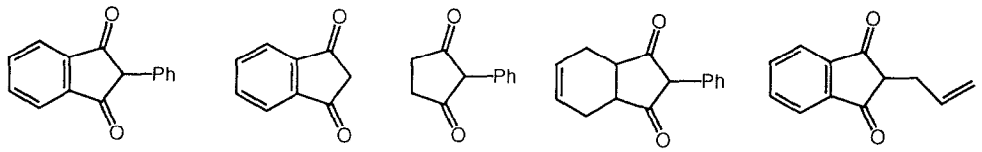
Chemistry. The synthesis of the novel compounds of this study are described as follows. The geminal acylation method developed by Kuwajima and coworkers[11] proved useful for the preparation of the cyclopentanoid analog **4**. Extension of this method to provide larger cyclic-1,3-diones using the requisite homologous bis-silylated acyloin and benzaldehyde acetal (Scheme 1) provided the 6-, 7- and 8-membered cycloalkan-1,3-diones **35-39**. The yield of products was modest (15-30%) but the reaction conditions were not optimized.[12] This method provided rapid access to the desired compounds for evaluation of 5-lipoxygenase inhibitory activity.

Scheme 1.



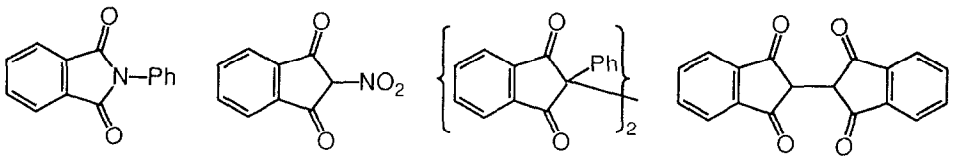
The substituted phenylindan-1,3-diones evaluated in this study, several of which are known compounds, were prepared according to the method reported by Shapiro.[13] The 2-fluorosubstituted analogs **24**, **27**, and **29** were prepared by displacement of the corresponding 2-bromo analogs with AgF.[14] The 2-thiomethyl **25** and 2-thiophenyl **26** analogs were prepared by treatment of **1** with the corresponding thiolsuccinamide in the presence of triethylamine.[15]

Structure-Activity Relationships. An initial objective was to elucidate the structural basis for 5-lipoxygenase inhibition by 2-phenylindan-1,3-dione (**1**).[16] Two partial structures of **1** were examined, indan-1,3-dione (**2**)[16] and 2-phenylcyclopentan-1,3-dione (**3**)[11] and found to be inactive.[17] The cyclohexene analog **4** and the allyl analog **5** were both inactive



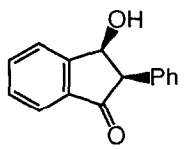
1 **2** **3** **4** **5**

Substituting a nitrogen for carbon at C2 as in N-phenylphthalimide (**6**)[16] resulted in an inactive compound. The 2-nitro derivative **7** which has been reported to have antiallergic activity[18] did not inhibit 5-lipoxygenase. The dimeric phenindandione **8**, a common oxidation product of **1**,[19] was inactive, whereas the dimer **9** was a weak inhibitor (IC_{50} = 28 μM).

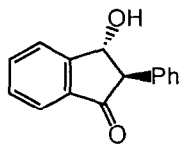


6 **7** **8** **9**

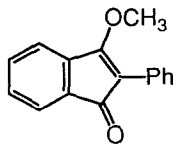
Reduction of one of the carbonyl groups in **1** resulted in a mixture of *syn* and *anti* racemic diastereoisomers **10** and **11**[20] which were both inactive. The enol ether analog **12**[21] was also inactive. The vinylogous analogs **13** and **14**[22] were also surprisingly inactive. From this brief survey of structure-activity relationships it appeared that an intact 2-phenylindan-1,3-dione system (**1**) was required for 5-lipoxygenase inhibitory activity.



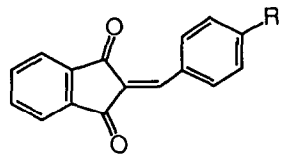
10



11



12

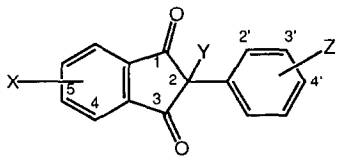


13 R = H

14 R = OCH_3

Substituted 2-phenylindan-1,3-diones were then examined and the results are summarized in Table I.

Table I. Inhibition of RBL-1 supernatant lipoxygenase activity[23] by 2-phenyl-1,3-indanediones.

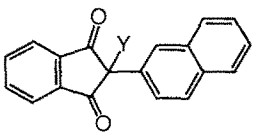


Compound	X	Y	Z	mp (lit.), °C	Formula[a]	*In vitro* inhibn: 5-LO IC_{50} [b] (μM)
1	H	H	H	148-149[c]		15 (12-18)
16	H	H	4'-CO_2H	229-231[d]	$C_{16}H_{10}O_4$	NA[e]
17	H	H	4'-nC_4H_9	88-89	$C_{19}H_{18}O_2$	1.7 (1.5-1.8)
18	H	H	4'-Cl	142-143[f]	$C_{15}H_9ClO_2$	5.0 (4.0-5.9)
19	H	H	4'-F	115-116[g]	$C_{15}H_9FO_2$	9.6 (7.6-12)
20	H	H	4'-OCH_3	142-144[h]	$C_{16}H_{12}O_3$	9.1 (8.4-9.9)
21	H	H	4'-SCH_3	153-155	$C_{16}H_{12}O_2S$	2.7 (1.9-3.3)
22	H	H	4'-$N(CH_3)_2$	185-187	$C_{17}H_{15}NO_2$	4.5 (4.0-4.9)
23	H	H	3',5'-t-C_4H_9	131-134[i]	$C_{23}H_{26}O_2$	2.8 (2.4-3.1)
24	H	F	H	87-88	$C_{15}H_9FO_2$	1.7 (1.1-2.2)
25	H	SCH_3	H	110-111	$C_{16}H_{12}O_2S$	15 (13-18)
26	H	SC_6H_5	H	98-99	$C_{21}H_{14}O_2S$	4.8 (4.0-5.8)
27	5-Br	F	H	129-131	$C_{15}H_8BrFO_2$	2.3 (2.1-2.6)
phenidone[24]						1.9 (1.5-2.8)

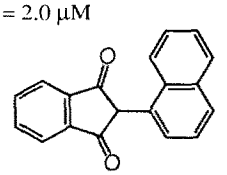
[a] Elemental analysis (C,H,N) within ± 0.4 of the theoretical value; [b] IC_{50} with 95% confidence limits indicated in parentheses; [c] lit. [13a] mp148-149; [d] lit. [8a] mp 221-225; [e] NA indicates no significant inhibitory activity up to 30 μM. [f] lit.[13a] mp.142-144 ; [g] lit.[13a] mp 116-117; [h] lit.[8a] mp 143-146; [i] lit.[8c] compound no data reported.

It was observed that inhibitory activity against 5-lipoxygenase could be enhanced by substitution on the 2-phenyl ring. A variety of *para* substituents were examined. No obvious correlation between electron-donating or electron-withdrawing properties and inhibitory activity was apparent in this small but representative group of examples. The carboxyl derivative **16** was inactive at 30 μM whereas the other examples studied were all more active than the parent compound **1**. The lipophilic n-butyl substituted derivative **17** was a potent inhibitor with an IC_{50} = 1.7 μM.

It was of interest to evaluate the role of acidic proton at the C2 position of the 1,3-dione system with respect to inhibitory activity. Enolization might be an important factor for inhibition. Another possiblity was that electron transfer from the 2-phenyl-1,3-dione system could reduce the active Fe^{+3} form of the enzyme to the inactive Fe^{+2} state with the generation of a fairly stable delocalized 2-phenyl-1,3-dione radical. Substitution of hydrogen at C2 in **1** with fluorine as in **24** provided a nine-fold increase in inhibitory activity (IC_{50} = 1.7 μM). The 2-thioether derivatives **25** and **26** also retained inhibitory activity. The 2-fluoro derivative **27** (IC_{50} = 2.3 μM) of the known uricosuric agent,[25] 5-bromo-2-phenylindan-1,3-dione was also a potent inhibitor. These observations indicated that the acidic C2 proton of 2-phenylindan-1,3-dione was not required for 5-lipoxygenase inhibitory activity and that the 2-fluoro analogs were more potent inhibitors. Replacing the 2-phenyl group in **1** with a naphthyl group provided an interesting observation of steric effects, in that, the 2-naphthyl derivative **28**[7] (IC_{50} = 3.2 μM) was much more potent than the 1-naphthyl compound **30**[7](14% inhibition at 30μM). The 2-fluoro-2-naphthyl analog **29** was also an active inhibitor with an IC_{50} = 2.0 μM

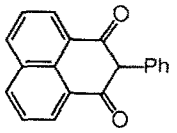


28 Y = H, **29** Y = F

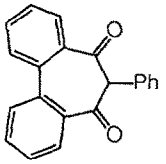


30

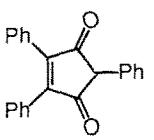
Since 2-phenylindan-1,3-diones are well-known and patented for a variety of applications it was our interest to explore related structural systems in search of more potent 5-lipoxygenase inhibitory activity. The naphthylcyclohexadione system **31**[26] was found to be inactive, whereas the biphenylcycloheptandione system **32**[27] (50% inhibition at 20 μM) and the diphenylcyclopentendione system **33**[28] (45% inhibition at 30 μM) were both moderately active. The acyclic 1,3-dione analog **34**[29] was inactive.



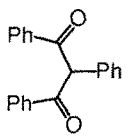
31



32



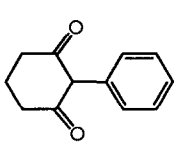
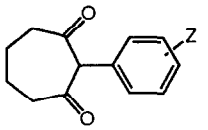
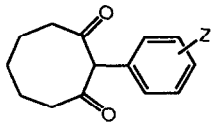
33



34

We next tested the hypothesis that inhibitory activity might be influenced by the ring size of the 1,3-dione system. The five-membered **3**[11] and the six-membered **35**[30] were both inactive. The novel seven-membered analog **36** was a moderately active inhibitor (IC_{50} = 41 μM). Substitution on the aryl ring further improved inhibitory activity as shown by the 3,4-methylenedioxy derivative **37** (IC_{50} = 25 μM) and the 4-n-butyl

derivative **38** (IC_{50} = 12 μM) with inhibitory activity comparable to **1**. Further ring enlargement as in the eight-membered analog **39** (45% inhibition at 100 μM) resulted in a decrease in inhibitory activity.

35 **36** Z = H, **37** Z = 3,4-OCH_2O, **38** Z = 4-nC_4H_9 **39**

Summary. The 2-phenylindane-1,3-dione system was observed to have inhibitory activity against RBL-1 supernatant 5-lipoxygenase activity. The structural features which define the requirements for *in vitro* inhibitory activity have been presented. Novel 2-phenylcycloheptan-1,3-diones were also found to have comparable 5-lipoxygenase inhibitory activity *in vitro*. Several well-known 2-phenylindane-1,3-dione compounds have been shown to have significant inhibitory activity against 5-lipoxygenase which may contribute to their pharmacological activity as antiinflammatory agents.

References.

1. a) Samuelsson, B. *Science* **1983**, *120*, 568. b) Matsumoto, T.; Funk, C. D.; Radmark, O.; Hoog, J.-O.; Jornvall, H.; Samuelsson, B. *Proc. Natl. Acad. Sci. USA* **1988**,*85*, 26.
2. Lewis, R.A.; Austen, F.; Soberman, R.J. *New England J. of Medicine* **1990**,*323* ,645.
3. 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. d) Gillard, J. W.; Guindon, Y. *Drugs of the Future*, **1987**, *12*, 455.
4. a) Fanelli, O. *Arzneim.- Forsch.(Drug Res.)* **1975**, *25*, 873. b) Lombardino, J.G. *Nonsteroidal Antiinflammatory Drugs* ; Lombardino, Ed.; Wiley: New York, 1985; p 379.
5. Levine, W. G. In "The Pharmacological Basis of Therapeutics", 5th ed., Goodman, L.S.; Gilman, A., Ed.; New York: MacMillan, 1975.
6. a) Badin, J.; Merle, A.; Descotes, G.; Tinland, B.; Bacques, C. *Eur. J. Med. Chem.* 1976, **11**, 533., b) Van der Goot, H.; Eriks, J. C.; Van Rhijn, P. J.; Zuiderveld, O. P.; Nauta, W. T. *Eur. J. Med. Chem.* **1978**, *13*, 425.
7. Lombardino, J. G.; Wiseman, E. H. *J. Med. Chem.* **1968**, *11*, 342.
8. a) Murthy, A. R.; Wyrick, S. D.; Hall, I. H. *J. Med. Chem.* 1985, **28**, 1591. b) Van der Goot, H.; Timmerman, H.; Asghar, S. S.; Siddiqui, A. H. *Agents and Actions*, **1984**, *15*, 371. c) Van den Berg, G.; Nauta, W. T. *Biochemical Pharmacology* **1975**, *24*, 815. d) Van den Berg, G.; Bultsma, T.; Rekker, R. F.; Nauta, W. T. *Eur. J. Med. Chem.* **1975**, *10*, 242.
9. Schulert, A. R.; Weiner, M. *J. Pharmacol.* **1954**, *110*, 451.
10. Summers, J. B.; Gunn, B. P.; Mazdiyasni, H.; Goetze, A. M.; Young, P. R.; Bouska, J. B.; Dyer, R. D.; Brooks, D. W. ; Carter, G. W. *J. Med. Chem.* **1987**, *30*, 2121.
11. Shimada, J.; Hashimoto. K.; Kim, B. H.; Nakamura, E.; Kuwajima, I. *J. Am. Chem. Soc.* **1984**, *106*, 1759.

12. Representative experimental procedure: 2-phenylcycloheptan-1,3-dione (**36**). To a solution of $BF_3{\cdot}OEt_2$ (2.5 mL, 20.4 mmol) in dichloromethane (20 mL) at -78°C was added dropwise benzaldehyde dimethylacetal (4 g, 22 mmol) followed by a solution of 1,2-bis-trimethylsilyloxycyclohex-1-ene (5.5 g, 21 mmol) in dichloromethane (10 mL). After stirring at -78°C for 2h, a solution of 10% $NaHCO_3$ (20 mL) was added and the mixture was allowed to warm to room temperature. The organic phase was separated and washed with saturated aqueous NaCl (20 mL), dried over $MgSO_4$, filtered and evaporated to provide a liquid residue. The residue was dissolved in dichloromethane (20 mL) and cooled to 5° C. Methanesulfonic acid (1.6 mL, 25 mmol) was added dropwise and the mixture was stirred at room temperature for 16h . Water (20 mL) was added and the organic phase was separated and washed with aqueous saturated NaCl (2x 20 mL), dried over $MgSO_4$, filtered and evaporated to provide an oil. Purification by chromatography (silica gel, dichloromethane) gave the desired product **37** (1.1 g, 28% yield). ^1H NMR ($CDCl_3$) 1 91 (4H, m), 2.27 (2H, m), 2.5 (2H, m), 6.2 (1H, br s), 7.1-7.4 (5H, m); MS· M^+, 202.
13. a) Shapiro, S. I.; Geiger, K.; Freedman, L. *J. Org. Chem.* **1960**, *25*, 1860, b) Shapiro, S. I.; Geiger, K.; Youlus, J.; Freedman, L. *J. Org. Chem* **1961**, *26*, 3580.
14. Bhandari, K. S.; Pincock, R. E. Synthesis **1974**, 655.
15. Buchel, K. H., Conte, A. *Chem. Ber* **1967**, *100*, 1248.
16. Commercially available material.
17. Inactive is defined as non-significant inhibitory activity at > 30 μM.
18. Buckle, D. R.; Morgan, N. J.; Ross, J. W.; Smith, H.; Spicer, B. A. *J. Med. Chem.* **1973**, *16*, 1334.
19. a) DeVries, J.; Engel, D. J. C.; Koekkoek, P. H. *J Chrom.* **1975**, *108*, 117. b) DeVries, J.; Verboom, C. N.; Van der Heijden, P. J. C. M. *Eur. J. Med. Chem.* **1976**, *11*, 317.
20. Lacey, P. H.; Smith, D. C. C. *J. Chem. Soc Perkin Trans 1*, **1974**, *23*, 2617.
21. House, H. O.; Rasmusson, G. H. *J. Org. Chem.* **1963**, *28*, 27.
22. Inayama, S.; Mamoto, K.; Shibata, T.; Hirose, T. *J. Med. Chem.* **1976**, *19*, 433.
23. Determination of 5-LO inhibitory potency. Adherent rat basophilic leukemia (RBL-1) cells (2H3 subline) were harvested by trypsinization, suspended (3.0 x 10^7 cells/mL) in pH 6.8 buffer (10 mM BES, 10 mM PIPES and 1 mM EDTA) and lysed by sonication. The lysate was centifuged at 20,000 x g for 20 min and the supernatant containing 5-LO activity was stored frozen until used. Compounds were evaluated for 5-LO inhibitory activity in 100 μL incubations containing 12.5 μL of RBL-1 supernatant in pH 6.8 buffer (as above with additional 0.7 mM $CaCl_2$ and 100 mM NaCl). Compounds were dissolved in DMSO and preincubated with the enzyme for 20 min at 37°C before initiation of the 5-LO catalysis by addition of 6.6 nmol of arachidonic acid and 25 nCi of [^{14}C]arachidonic acid (55.8 mCi/mmol) in 3 μL of aqueous NH_4OH (0.028%). The internal recovery standard [^{3}H]-5-HETE, 3nCi was also added. Reactions were terminated after 5 min by acidification with HCl to pH 3.5 and 100 μg triphenylphosphine was added to reduce any remaining 5-HPETE to 5-HETE. The eicosanoid products were extracted with 150 μL of acetone and evaluated by thin layer chromatography on silica gel-impregnated glass fiber sheets (85:15:0.25, hexane-ethylacetate-acetic acid) after adding 20 μg each of reference standards 5-HETE and arachidonic acid for visualization by iodine vapor. The 5-HETE radioactivity was measured by a liquid scintillation counter and corrected for recovery of [^{3}H]-5-HETE.
24. Reference inhibitor of 5-LO. Blackwell, G.J.; Flower, R.J *Prostaglandins* **1978**, *16*, 417.
25. Fanelli, O; Mazzoncini, V.; Ferri, S. *Drug Res.* **1974**, *24*, 1609.
26. Aly, O. M.; Awad, W. I.; Islam, A. M. *J. Org. Chem.* **1958**, *23*, 1624.
27. Meksiev, B.; Milosev, M. *Chem. Ber.* **1967**, *100*, 701.
28. a) Allen, C. F. H.; Massey, E. E.; Nicholls, R. V. V. *J. Am. Chem. Soc.* **1937**, *59*, 679. b) Koelsch, C. F.; Wawzonek, S. *J. Org Chem.* **1941**, *6*, 684.
29. Politizer, I. R.; Griggin, G. W. *Tetrahedron Lett.* **1972**, 4775.
30. Wheeler, T. N. *J. Org. Chem* **1979**, *26*, 4906.