Synthesis and Rat Lens Aldose Reductase Inhibitory Activity of Some Benzopyran-2-ones[1]

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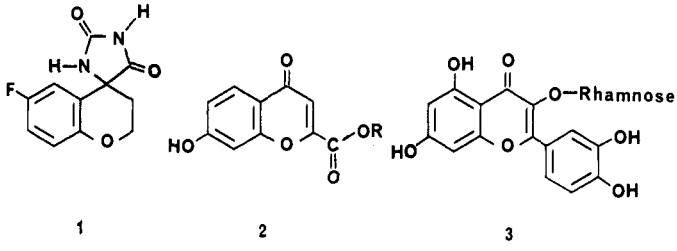
A number of 4,7-disubstituted benzopyran-2-ones (**4–24**) were synthesized and evaluated for crude rat lens aldose reductase inhibitory activity. Substituents on position 4 included CH_3, CO_2H, CH_2CO_2H, $CH{=}CHCO_2H$, and $CH_2CH_2CO_2H$. The aromatic substituents included OH, OCH_3, $OCOCH_3$, CH_2CH_3, and Cl. Also included in the study were 3-oxo-3*H*-naphtho[2,1-*b*]pyran-1-acetic (**25**), 2-oxo-2*H*-naphtho[1,2-*b*]pyran-4-acetic (**26**), and 1-naphthylacetic (**30**) acids. The benzopyran and naphthopyran derivatives were prepared by the classical von Pechmann reaction. General structure–activity relationships reveal that optimal enzyme inhibitory activity is displayed by those compounds possessing the acetic acid moiety. For example, the most potent derivative, 3-oxo-3*H*-naphtho-[2,1-*b*]pyran-1-acetic acid (**25**) with an IC_{50} of 0.020 μM, is as potent as sorbinil (IC_{50} = 0.017 μM) in the crude rat lens aldose reductase assay.

The intracellular accumulation of the polyol sorbitol, in tissues (lens, nerve, retina, kidney) that do not require insulin for glucose uptake, is the common basis of the pathophysiological mechanisms resulting in diabetic complications such as cataracts, peripheral neuropathy, retinopathy, and nephropathy.[2-4] During chronic hyperglycemia, the enzyme aldose reductase may convert excess intracellular glucose in these tissues to sorbitol. The poor membrane penetrability of this polar molecule, in addition to its slow conversion to fructose by sorbitol dehydrogenase, leads to an accumulation of this polyol intracellularly, and this disrupts the normal osmotic integrity of the cell, resulting eventually in cell damage. The prevention or delay of the onset of some diabetic complications by treatment of diabetic test animals with aldose reductase inhibitors (ARI) such as sorbinil (**1**) provides further evidence to support the role of sorbitol in these diabetic complications.

A prior paper[5] from this laboratory described the aldose reductase inhibitory activity of some quinazolinones. The most potent aldose reductase inhibitor of the series, 6,7-dimethoxy-2-[(4-carboxyphenyl)amino]-4(3*H*)-quinazolinone, suggested to us that the presence of an acidic moiety on the quinazolinone nucleus contributed significantly to the inhibitory potency of the quinazolinones studied, and this prompted us to investigate the aldose reductase inhibitory activity of some 7-substituted 2-oxo-2*H*-1-benzopyran-4-acetic acids that were prepared for an unrelated project. Moreover, the benzopyran-2-ones of interest contain a structural feature common to many known inhibitors of aldose reductase such as sorbinil (**1**), 7-hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid (**2**), and quercetin (**3**): all of these compounds contain the benzopyran moiety. A survey of the literature revealed that several other benzopyran-2-ones[6,7] had been screened for



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aldose reductase inhibitory activity, and the aldose reductase activity of a limited series of substituted 2-oxo-2*H*-1-benzopyran-4-acetic acids is reported in the patent literature.[8] However, IC_{50} values are not available for all benzopyran-2-ones reported to inhibit aldose reductase, and the limited number of compounds that has been reported does not permit a true assessment of how key functional groups may influence inhibitory activity within this structural class.

The potent inhibitory activity demonstrated by the lead compound **14** in our assay, coupled with the ease of preparing an extensive series of 2-oxo-2*H*-benzopyran-4-acetic acids in which the electronic nature of the substituent on the aromatic ring is varied, suggested to us that these compounds could serve as excellent probes to investigate the steric and electronic requirements of the common inhibitor binding site[4] present on aldose reductase. The fact that the proposed benzopyran-2-ones possess a lactone carbonyl that could conceivably participate in a reversible charge-transfer interaction[9] also makes these derivatives attractive as probes to study the inhibitory site of aldose reductase since it has been established that the chemical reactivity of the lactone ring of benzopyran-2-ones is dependent on both the electronic nature and the position of the substituent on the benzene ring of benzopyran-2-ones.[10]

This report describes the synthesis of a number of benzopyran-2-one derivatives and their aldose reductase inhibitory activity.

Chemistry

The methods used for the synthesis of the benzopyran-2-ones **4–26** are outlined in Scheme I. The 7-substituted 4-methyl-2*H*-1-benzopyran-2-ones **6**, **7**, and **9–12** and the 2-oxo-2*H*-1-benzopyran-4-acetic acids **13**, **15–18**, and **22–26** were prepared by the classical von Pechmann

(1) Presented in part: Joint Southeast-Southwest Regional Meeting of the American Chemical Society, Memphis, TN, Oct 1985.
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(9) Kador, P. F.; Sharpless, N. E. *Mol. Pharmacol.* **1983**, *24*, 521.
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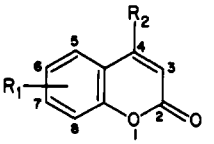
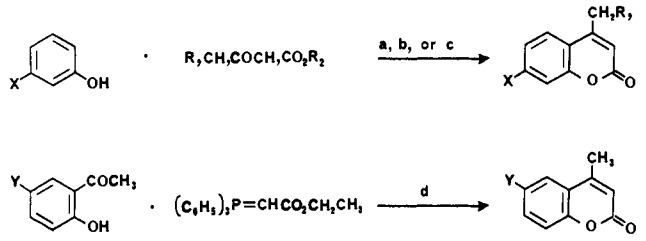
Table I. 2*H*-1-Benzopyran-2-ones



compd	R_1	R_2	mp,[a] °C	lit. mp, °C	method of synth[b]	recryst solvent[c]	yield,[d] %	formula[e]	anal.
4	H	CH_3	75–78	82[f]	D	A	69	$C_{10}H_8O_2$	C, H
5	6-OH	CH_3	245–247	243[g]	D	A	21	$C_{10}H_8O_3$	C, H
6	7-OH	CH_3	185	185–186[h]	B	B	39	$C_{10}H_8O_3 \cdot {}^1/_9H_2O$	C, H
7	7-OCH_3	CH_3	158–160	161–162[h]		C	87	$C_{11}H_{10}O_3$	C, H
8	7-OAc	CH_3	149–150	159[i]		C	97	$C_{12}H_{10}O_4$	C, H
9	7-CH_2CH_3	CH_3	75–78		B	B	77	$C_{12}H_{12}O_2$	C, H
10	7-Cl	CH_3	135–139	144[j]	B	B	33	$C_{10}H_7ClO_2$	C, H
11	5,7-$(OH)_2$	CH_3	284–292	282–294[k]	B	B	74	$C_{10}H_8O_4 \cdot {}^3/_2H_2O$	C, H
12	7,8-$(OH)_2$	CH_3	240–242	235[h]	B	B	76	$C_{10}H_8O_4$	C, H
13	H	CH_2CO_2H	182–185	168[l]	C	B	2	$C_{11}H_8O_4$	C, H
14	7-OH	CH_2CO_2H	200	201–202[m]	A	D	64	$C_{11}H_8O_5$	C, H
15	7-OH	$CH_2CO_2CH_3$	210–216	220[n]		E	75	$C_{12}H_{10}O_5$	C, H
16	7-OH	CH_2CONH_2	263–264			B	96	$C_{11}H_9NO_4$	C, H, N
17	7-OAc	CH_2CO_2H	168–170	175[j]		C	80	$C_{13}H_{10}O_6$	C, H
18	7-OCH_3	CH_2CO_2H	167–170	175–176[m]	C	D	85	$C_{12}H_{10}O_5$	C, H
19	7-OCH_3	CO_2H	217–221	219[o]		B	54	$C_{11}H_8O_5$	C, H
20	7-OCH_3	$CH{=}CHCO_2H$	223–231			B	58	$C_{13}H_{10}O_5$	C, H
21	7-OCH_3	$CH_2CH_2CO_2H$	177–179			D	91	$C_{13}H_{12}O_5$	C, H
22	7-Cl	CH_2CO_2H	167–168	206[p]	C	D	10	$C_{11}H_7ClO_4 \cdot {}^1/_9H_2O$	C, H
23	7-CH_2CH_3	CH_2CO_2H	165–166	183–184[m]	C	D	30	$C_{13}H_{12}O_4$	C, H
24	5,7-$(OH)_2$	CH_2CO_2H	234–236	204–205[q]	C	D	30	$C_{11}H_8O_6 \cdot {}^2/_3H_2O$	C, H
25	5,6-$(CH)_4$	CH_2CO_2H	169–171	191[q]	C	B	17	$C_{15}H_{10}O_4$	C, H
26	7,8-$(CH)_4$	CH_2CO_2H	181–183	181[q]	C	B	59	$C_{15}H_{10}O_4$	C, H

[a] All melting points were determined in open capillary tubes and are uncorrected. [b] See Scheme I. [c] Key: A, compound precipitated by concentrating chromatographic fractions containing only the final product; B, EtOH; C, compound precipitated from the reaction mixture by the addition of H_2O; D, compound precipitated from 5% $NaHCO_3$ by addition of concentrated HCl. [d] The values represent the yield of analytically pure product, and no effort was made to optimize yields. [e] All products exhibited, IR, UV, and ^1H NMR spectra consistent with the assigned structures. All products gave satisfactory combustion analyses. The presence of H_2O was demonstrated in the ^1H NMR spectra of **6**, **22**, and **24**. [f] Reference 13. [g] Reference 21. [h] Reference 23. [i] Reference 24. [j] Reference 25. [k] Reference 26. [l] Reference 27. [m] Reference 12. [n] Reference 28. [o] Reference 22. [p] Reference 29. [q] Reference 20.

Scheme I[a]



[a] Key: (a) $R_1 = CO_2H$; $R_2 = H$; X = OH; concentrated H_2SO_4. (b) $R_1 = H$; $R_2 = CH_2CH_3$; X = OH, CH_2CH_3, or Cl; 70% H_2SO_4. (c) $R_1 = CO_2CH_2CH_3$; $R_2 = CH_2CH_3$; X = H, Cl, CH_2CH_3, OCH_3, 2,3-$(CH)_4$, or 1,2-$(CH)_4$; 70% H_2SO_4. (d) Y = OH or H; C_6H_6.

reaction[11] from a meta-substituted phenol and ethyl acetoacetate for the former and a meta-substituted phenol and diethyl acetone-1,3-dicarboxylate for the latter. For both methods, the use of 70% H_2SO_4 as catalyst gave better yields of the desired product than when concentrated H_2SO_4 was used. Compound **14** was prepared from resorcinol and acetone-1,3-dicarboxylic acid (prepared in situ from citric acid) using concentrated H_2SO_4 according to the method reported by Laskowski and Clinton.[12]

4-Methyl-2*H*-1-benzopyran-2-one (**4**) and its 6-hydroxy analogue **5** were prepared by treatment of 2-hydroxyacetophenone and 2,5-dihydroxyacetophenone, respectively, with [(ethoxycarbonyl)methylene]triphenylphosphorane as described by Mali and Yadav.[13]

The 7-methoxy derivative **7** was synthesized by methylation of 7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one using KOH and $(CH_3)_2SO_4$, and the acetoxy derivatives **8** and **17** were prepared from the corresponding hydroxy compounds by acetylation with acetic anhydride using standard conditions.

The amide derivative **16** was prepared by treating methyl 7-hydroxy-2-oxo-2*H*-benzopyran-4-acetate (**15**), prepared from the acid **14** using the Fischer procedure, with concentrated NH_4OH.

The 4-carboxylic, 4-propenic, and 4-propanoic acids, **19–21**, respectively, were synthesized from 4-formyl-7-methoxy-2*H*-1-benzopyran-2-one as outlined in Scheme II. The formyl compound **27**, prepared by oxidation of 7-methoxy-4-methyl-2*H*-1-benzopyran-2-one (**7**) using SeO_2,[14] was converted to its aldoxime derivative **28**. Treatment of **28** with acetic anhydride gave 4-cyano-7-methoxy-2*H*-1-benzopyran-2-one (**29**). Hydrolysis of **29** with 50% H_2SO_4 gave the 4-carboxylic acid derivative **19**.

The propenic acid derivative **20** was prepared by treating **27** with malonic acid in pyridine using piperidine as the catalyst, and the coupling constant obtained from NMR analysis of **20** revealed that only the trans isomer was present. Catalytic hydrogenation of **20** with H_2 over Pd/C gave propanoic acid **21**.

Results and Discussion

The compounds **4–27** were screened for their ability to inhibit crude aldose reductase obtained from rat lens as described previously.[15] IC_{50} values were determined for

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(12) Laskowski, S. C.; Clinton, R. O. *J. Am. Chem. Soc.* **1950**, *72*, 3987.
(13) Mali, R. S.; Yadav, U. J. *Synthesis* **1977**, 464.
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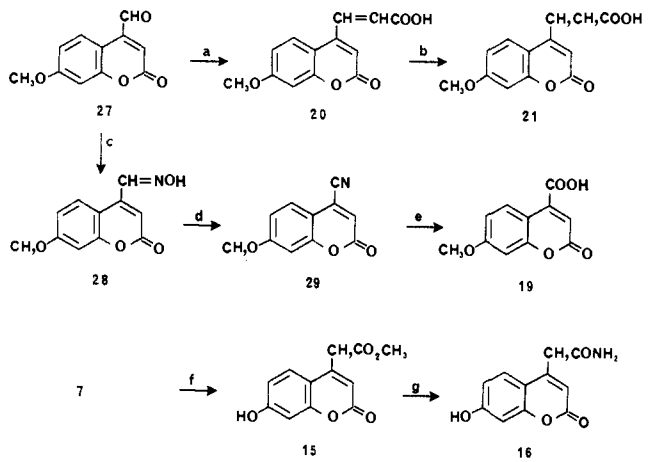
Table II. Aldose Reductase Inhibitory Activity of 2*H*-1-Benzopyran-2-ones

compd	R_1	R_2	inhibn[a] type	inhibn,[b] % (SEM)	IC_{50},[c] μM
4	H	CH_3		19 (4.12)	
5	6-OH	CH_3		22 (2.00)	
6	7-OH	CH_3	noncomp		62
7	7-OCH_3	CH_3		21 (3.25)	
8	7-OAc	CH_3		13 (0.75)	
9	7-CH_2CH_3	CH_3		16 (0.0)	
10	7-Cl	CH_3		10 (2.10)	
11	5,7-$(OH)_2$	CH_3	noncomp		17
12	7,8-$(OH)_2$	CH_3	uncomp		10
13	H	CH_2CO_2H	uncomp		0.60
14	7-OH	CH_2CO_2H	noncomp		0.15
			noncomp (NADPH)		
15	7-OH	$CH_2CO_2CH_3$	uncomp[d]		38
16	7-OH	CH_2CONH_2	uncomp		10
17	7-OAc	CH_2CO_2H	noncomp		61
18	7-OCH_3	CH_2CO_2H	noncomp		0.37
19	7-OCH_3	CO_2H	uncomp[e]		11
20	7-OCH_3	$CH{=}CHCO_2H$	uncomp		4.4
21	7-OCH_3	$CH_2CH_2CO_2H$	noncomp		2.1
22	7-Cl	CH_2CO_2H	uncomp		0.56
23	7-CH_2CH_3	CH_2CO_2H	uncomp		0.24
24	5,7-$(OH)_2$	CH_2CO_2H	noncomp		0.26
25	5,6-$(CH)_4$	CH_2CO_2H	uncomp		0.020
			noncomp (NADPH)		
26	7,8-$(CH)_4$	CH_2CO_2H	uncomp		0.14
30	1-naphthylacetic acid		noncomp		32
1	sorbinil		uncomp		0.017

[a] Double-reciprocal plots were linear, and the significance level for the least-squares fit was <0.01 unless indicated otherwise. [b] Percent inhibition produced at 10^{-4} M followed by the standard error of the mean (SEM). [c] IC_{50} values represent the concentration required to produce 50% inhibition. Values were determined from least-squares analysis of log dose–response curves, and each curve was generated using at least four concentrations of inhibitor with four replicates at each concentration. The significance level for the least-squares fit was <0.01. [d] $p < 0.02$. [e] $p < 0.4$.

Scheme II[a]



[a] Key: (a) $CH_2(CO_2H)_2/C_5H_5N/C_5H_{11}N$; (b) H_2/Pd/C/EtOH; (c) NH_2OH/EtOH; (d) Ac_2O; (e) 50% H_2SO_4; (f) MeOH/concentrated H_2SO_4; (g) concentrated NH_4OH.

those compounds displaying greater than 50% inhibition at 100 μM by least-squares analysis of log dose–response curves (Table II). Kinetic experiments were also performed to determine the type of inhibition produced by the benzopyran-2-ones when substrate or cofactor concentration was varied in the enzyme reaction mixture (Table II). These kinetic studies reveal that the more potent benzopyran-2-ones **6** and **11–26** and 1-naphthylacetic acid (**30**) produce either noncompetitive or uncompetitive inhibition. These findings are consistent with those observed for other ARI and supports the suggestion that the compounds in this study may also interact with the enzyme by a mechanism(s) similar to the Kador and Sharpless[4] proposal for other ARI.

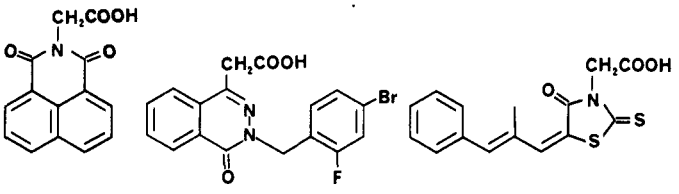
Examination of the enzyme inhibition data reveals one general structure–activity trend: the 2-oxobenzopyran-4-acetic acids **13**, **14**, **18**, and **22–26** are significantly more potent than the 4-methyl (**4–12**), 4-carboxylic acid (**19**), or 4-propenic and propanoic acid (**20**, **21**) derivatives. Also, the free acid **14** is more potent than its corresponding amide (**16**) or ester (**15**) derivative. These data suggest two basic SAR points: (a) an ionizable carboxyl moiety is necessary for optimal aldose reductase inhibitory activity; (b) the number of atoms between the carboxyl group and benzopyran-2-one ring is a critical determinant of potency. The dependence of an ionizable functional group for optimal enzyme inhibitory activity in this series of benzopyran-2-ones is consistent with the observation that virtually all known ARI possess an acidic moiety. Moreover, Kato et al.[16] reported that ARI activity in a series of thiazolidinecarboxylic acids was decreased when the carboxylic acid was converted to the primary amide derivative. The conversion of **14** to the ester **15** results in 300-fold decrease in potency. This was not anticipated since an analogous structural change in the isomeric 4-oxochromene-2-carboxylic acids did not alter ARI potency in that series. For example, conversion of **2** to its ester derivatives produces no change in inhibitory potency.[4]

The observation that the number of atoms between the carboxyl group and the benzopyran-2-one ring is critical for activity is best illustrated by the 7-OMe series **18–21**. These compounds have no (**19**), one (**18**), or two (**20**, **21**) atoms between the carboxyl group and the benzopyran-2-one ring. Here, the 4-acetic acid derivative **18** displays approximately 10 times the potency of the *trans*-propanoic (**21**) or propenic acid (**20**) derivatives, and 30 times the

(15) Kador, P. F.; Sharpless, N. E. *Biophys. Chem.* **1978**, *8*, 81.

(16) Kato, E.; Yamamoto, K.; Baba, T.; Watanabe, T.; Kawashima, Y.; Masuda, H.; Horiuchi, M.; Oya, M.; Iso, T.; Iwao, J. *Chem. Pharm. Bull.* **1985**, *33*, 74.

potency of the 4-carboxyl analogue **19**. It is interesting to note the "heterocycle CH_2CO_2H" moiety, determined to be required for optimal activity for benzopyran-2-ones, is also found in other ARI such as alrestatin (**31**), ICI-128,436 (**32**), and ONO-2235 (**33**).[4]



31 **32** **33**

Another significant observation is the dependence of ARI activity on the electronic nature and position of the benzene ring substituent for the benzopyran-4-acetic acids. In these derivatives, the order of potency is **25** > **26** > **14** > **23**, **24** > **18** > **13** and **22**. Other workers[9] have noted that the addition of a phenolic OH to the structure of known AR inhibitors generally produces an increase in ARI activity. Furthermore, masking of the hydroxyl by methylation generally results in loss of ARI potency. We also noted that addition of the hydroxyl group to the benzopyran-4-acetic acids produced an increase in potency (**13** → **14**) but methylation of the 7-OH (**18**) produced only a modest decrease in inhibitory potency. Other researchers[6,7] have also noted variable effects on inhibitory potency when phenolic groups on flavonoids are methylated.

The annulated benzopyran-2-one derivatives **25** and **26** are the most potent of the ring-substituted benzopyran-4-acetic acids, indicating that addition of a lipophilic moiety may increase binding to the AR inhibitory site. This is consistent with the proposed model[4] of the inhibitor site that suggests that addition of a secondary hydrophobic moiety on an ARI may enhance inhibitory potency by enhancing its affinity for the inhibitor site on the enzyme.

To determine the contribution of the benzopyran-2-one moiety to ARI activity, we included 1-naphthylacetic acid (**30**) in our study and found nearly a 55-fold difference in potency between 2-oxo-2*H*-1-benzopyran-4-acetic acid (IC_{50} = 0.60 μM) and 1-naphthylacetic acid (IC_{50} = 32 μM). On the basis of these findings, it is appealing to speculate that the benzopyran-2-one lactone carbonyl or perhaps, the 4-position of benzopyran-2-one ring, may be a chemically reactive site and may be susceptible to nucleophilic attack by a 1,2-addition at the former and a Michael attack at the latter. Nucleophilic attack at the 4-position of the benzopyran-2-one ring seems less plausible though than nucleophilic attack at the carbonyl carbon of the lactone ring. Although benzopyran-2-one undergoes the Michael reaction with a variety of nucleophiles, alkyl groups in the 3- or 4-position inhibit this type of addition reaction.[17] A recent report[18] that benzopyran-2-one is attacked preferentially at the 2-position by the anion of diethyl malonate under phase-transfer reaction conditions further supports what has been predicted by theoretical calculations.[19]

In their model of the AR inhibitor site, Kador and Sharpless have proposed that those ARI that possess a suitably reactive carbonyl group may form a reversible tetrahedral intermediate with the inhibitor site. This has been suggested to occur via nucleophilic attack of the carbonyl by the hydroxyl group of the tyrosine residue, which has been detected at the inhibitor site by using protein-modification agents.

If the lactone ring of the benzopyran-2-ones is participating as an electrophilic site in this type of mechanism, then the ability of the electropositive carbonyl to chemically react with a nucleophile should be influenced by the electronic nature of 7-substituent. Bowden et al.[10] have demonstrated that the chemical reactivity of the carbonyl carbon of benzopyran-2-ones is dependent on the electronic nature and position of the substituent on the benzene ring. A similar correlation between the electronic nature of the substituent on the benzene ring of benzopyran-2-one and enzyme inhibitory activity is not observed in this study. Moreover, from the data, it appears that the opposite is true. Benzopyran-4-acetic acids possessing electron-withdrawing substituents are less potent than those possessing electron-donating substituents; the 7-OAc derivative **17** is significantly less potent then the 7-OMe derivative **18** (IC_{50} = of 61 vs. 0.15 μM). We are continuing studies to assess in greater detail the exact nature of the contribution of this moiety in producing potent ARI activity.

In summary, the key SAR points for aldose reductase inhibitory activity of benzopyran-2-ones that are evident from this study, are as follows: (a) the free carboxylic acid moiety is required; (b) the relationship between the acidic moiety and the benzopyran-2-one nucleus is important for maximal inhibitory activity in this structural class of compounds.

Experimental Section

Melting points were determined in open glass capillaries with a Thomas-Hoover melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian T-60 spectrometer. Chemical shifts are reported in δ relative to Me_4Si. IR spectra were recorded with a Beckmann 4230 spectrometer. Absorptions are expressed in units of frequency (cm^{-1}). UV spectra and enzyme reaction rates were recorded on a Perkin-Elmer (Hitachi 200) double-beam spectrometer. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and are within 0.4 of theoretical percentages. Common reagent-grade chemicals and starting materials were purchased from commercial sources and were used as received. DL-Glyceraldehyde and NADPH (type I) were purchased from Sigma Chemical Co.

The syntheses of compounds listed in Table I, except **9**, **16**, **20**, and **21**, have been reported (see references listed in Table I), and the Experimental Section includes procedures only for the preparation of those compounds that have not been reported previously. Also, one example of each method used to prepare the benzopyran-2-ones **4**–**26** is outlined in this section, except the preparation of **14** using method A, which was performed precisely as described in the literature.[12] 1-Naphthylacetic acid (**30**) is available from Mann Research Laboratories, Inc., New York, NY.

Method B. 7-Ethyl-4-methyl-2*H*-1-benzopyran-2-one (9). A mixture of 3-ethylphenol (6.1 g, 50 mmol) and ethyl acetoacetate (6.95 g, 53 mmol) in 70% H_2SO_4 (50 mL) was stirred at 25 °C for 2 days and then was poured onto crushed ice. The crude solid was collected and recrystallized from EtOH to afford 7.28 g (77%) of **9**: mp 75–78 °C; ^1H NMR ($CDCl_3$) δ 1.25 (t, 3 H), 2.38 (d, 3 H), 2.73 (q, 2 H), 6.17 (m, 1 H), 7.0–7.6 (m, 3 H); IR (KBr) 2985, 1740, 1625 cm^{-1}; UV (MeOH) 312, 276, 213 nm. Anal. Calcd for $C_{12}H_{12}O_2$: C, H.

Method C. 5,7-Dihydroxy-2-oxo-2*H*-1-benzopyran-4-acetic Acid (24). A mixture of phloroglucinol dihydrate (8.1 g, 50 mmol) and diethyl acetone-1,3-dicarboxylate (11.13 g, 55 mmol) in 70% H_2SO_4 (50 mL) was stirred at 25 °C for 2 days and then was poured onto crushed ice. The solid was collected and dissolved in a minimum volume of 5% $NaHCO_3$, the solution filtered, and the filtrate acidified with concentrated HCl to cause precipitation of **24**. The precipitate was collected and dried over P_2O_5, to yield 3.7 g (30%) of **24**: mp 234–236 °C (lit.[20] mp 204–205 °C); ^1H NMR

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(Me_2SO-d_6) δ 3.4 (br, H_2O), 3.82 (s, 2 H), 5.93 (s, 1 H), 6.20 (s, 2 H), 10.3 (s, 1 H), 10.8 (br s, 1 H); IR (KBr) 3480, 3080 (br), 1660, 1600 cm^{-1}; UV (MeOH) 324, 257, 250 (sh), 212 nm. Anal. Calcd for $C_{11}H_8O_6 \cdot {}^2/_3H_2O$: C, H.

Method D. 6-Hydroxy-4-methyl-2*H*-1-benzopyran-2-one (5). A mixture of 2',5'-dihydroxyacetophenone (2.5 g, 16.7 mmol) and [(ethoxycarbonyl)methylene]triphenylphosphorane (8.7 g, 25 mmol) in benzene (50 mL) was stirred at reflux for 54 h. Evaporation of the solvent under reduced pressure yielded a brown oil. Chromatography using silica gel and eluting with a gradient of benzene to 20% $CHCl_3$ in benzene gave 0.62 g (21%) of **5**: mp 245–247 °C (lit.[21] mp 243 °C); ^1H NMR ($CDCl_3$/$(CD_3)_2SO$) δ 2.35 (s, 3 H), 6.18 (s, 1 H), 6.84–7.14 (m, 3 H), 9.37 (s, H exchanges in D_2O).

3-(7-Methoxy-2-oxo-2*H*-1-benzopyran-4-yl)propenoic Acid (20). A mixture of 4-formyl-7-methoxy-2*H*-1-benzopyran-2-one[14] (1.27 g, 6.2 mmol), $CH_2(CO_2H)_2$ (0.97 g, 9.3 mmol), and piperidine (5 drops) in pyridine (5 mL) was stirred at 110 °C (oil bath) under a head of N_2. The reaction was stirred an additional 2 h after evolution of gas had ceased (2 h) and was then poured into H_2O. The aqueous solution was acidified with concentrated HCl, and the precipitate was collected and recrystallized from EtOH to yield 0.88 g (58%) of **20** as a yellow powder: mp 223–231 °C; ^1H NMR ($(CD_3)_2SO$) δ 3.88 (s, 3 H), 6.57 (s, 1 H), 6.71 (d, 1 H, J = 17 Hz), 6.91 (m, 1 H), 6.98 (s, 1 H), 7.72 (m, 1 H), 7.79 (d, 1 H, J = 17 Hz); IR (KBr) 3060, 3000 (br), 1710, 1610 cm^{-1}; UV (MeOH) 340, 244, 218 nm. Anal. Calcd for $C_{13}H_{10}O_5$: C, H.

3-(7-Methoxy-2-oxo-2*H*-1-benzopyran-4-yl)propanoic Acid (21). A mixture of **20** (0.246 g, 1 mmol) and Pd/C (0.05 g) in EtOH (50 mL) was hydrogenated at an initial H_2 pressure of 50 psi. After 1 h, the reaction mixture was filtered through a Celite pad, and the volatiles were removed with vacuum to yield an oil. The oil was dissolved in a minimum volume of 5% $NaHCO_3$, the aqueous solution then filtered, and the filtrate acidified with concentrated HCl. The white precipitate was collected and dried to yield 0.225 g (91%) of **21**: mp 177–179 °C; ^1H NMR ($(CD_3)_2SO$) δ 2.63 (t, 2 H, J = 6 Hz), 3.0 (t, 2 H, J = 6 Hz), 3.83 (s, 3 H), 6.10 (s, 1 H), 6.8–7.1 (m, 2 H), 7.6–7.8 (m, 1 H); IR (KBr) 3100 (br), 1735, 1690, 1620 cm^{-1}; UV (MeOH) 320, 260 (sh), 230 nm. Anal. Calcd for $C_{13}H_{12}O_5$: C, H.

7-Methoxy-2-oxo-2*H*-1-benzopyran-4-aldoxime (28). A solution of 4-formyl-7-methoxy-2*H*-1-benzopyran-2-one (**27**; 2.04 g, 10 mmol) in EtOH (50 mL) was added with stirring in one portion to an aqueous solution of hydroxylamine hydrochloride (0.77 g, 11 mmol) and NaOAc (0.902 g, 11 mmol). The reaction mixture was heated to reflux during which time a cream-colored precipitate formed. After 15 min, the reaction mixture was diluted with H_2O (50 mL) and the solid collected and washed with H_2O, to yield 2.04 g (93%) of **28** as a powder: mp 240–242 °C dec; ^1H NMR ($(CD_3)_2SO$) δ 3.90 (s, 3 H), 6.53 (s, 1 H), 7.0–8.4 (m, 4 H), 12.33 (s, 1 H); IR (KBr) 3100, 3050, 3000, 1695, 1610 cm^{-1}. Anal. Calcd for $C_{11}H_9NO_4$: C, H, N.

7-Methoxy-2-oxo-2*H*-1-benzopyran-4-carboxylic Acid (19). Compound **27** (1.0 g, 4.6 mmol) in Ac_2O (35 mL) was heated at reflux overnight, and after cooling, the volatiles were removed with vacuum to yield 0.925 g of a yellow solid (**29**) that was used without further purification for the next step: ^1H NMR ($CDCl_3$) δ 3.87 (s, 3 H), 6.6 (s, 1 H), 6.8 (s, 1 H), 6.9 (m, 1 H), 7.7 (m, 1 H); IR (KBr) 3060, 1720, 1610, 1590 cm^{-1}. The crude **29** (0.75 g, 3.73 mmol) in 50% H_2SO_4 (30 mL) was stirred at 100 °C (oil bath) for 12 h and then diluted with H_2O (70 mL), and the solid that was collected was dissolved in a minimum volume of 5% $NaHCO_3$. This solution was filtered and the filtrate acidified with concentrated HCl. The yellow solid was collected and recrystallized from EtOH to yield 0.44 g (54%) of **19** as thick dark needles: mp 217–221 °C (lit.[22] mp 219 °C); ^1H NMR ($(CD_3)_2SO$) δ 3.9 (s, 3 H), 6.65 (s, 1 H), 6.8–8.2 (m, 3 H); IR (KBr) 2900 (br), 1735, 1680, 1610 cm^{-1}. Anal. Calcd for $C_{11}H_8O_5$: C, H.

7-Hydroxy-2-oxo-2*H*-1-benzopyran-4-acetamide (16). Compound **15** (5 g, 21.4 mmol), prepared from **14** by the Fischer method using concentrated H_2SO_4 as the catalyst, was added to concentrated NH_4OH (25 ml) and the resultant mixture stirred overnight at 25 °C during which time a yellow solid formed. The reaction was diluted with H_2O (100 mL), and the aqueous solution was acidified with concentrated HCl. The white solid was collected and dried to yield 4.5 g (96%) of **16**: mp 255–259 °C (recrystallization in EtOH gave a solid with mp 263–264 °C); ^1H NMR ($(CD_3)_2SO$) δ 3.5 (br s, H_2O), 3.65 (s, 2 H), 6.37 (s, 1 H), 6.73 (s, 1 H), 6.9 (m, 1 H), 7.2 (br s, 1 H), 7.6–7.8 (br s and m, 2 H), 10.53 (br s, 1 H), exchanges with D_O); IR (KBr) 3420, 3360, 3100 (br), 1720, 1680, 1630, 1610, 1565 cm^{-1}. Anal. Calcd for $C_{11}H_9NO_4$: C, H, N.

Enzyme Assay. Frozen rat eyes were purchased from Charles River Breeding Laboratories, Inc., and the lenses were dissected from the partially thawed eyes and were then kept at −6 °C until used for the assay. Crude enzyme supernatant was prepared by homogenizing 50 lenses in distilled H_2O (10 mL) and then centrifuging the crude homogenate at 10000 rpm for 10 min, maintaining an ambient temperature of 0–5 °C.

Aldose reductase activity of the freshly prepared supernatant was assayed spectrophotometrically by determining the decrease in NADPH concentration at 340 nm in a Perkin-Elmer Hitachi 200 double-beam spectrometer. The reaction mixture contained 0.1 M phosphate buffer, pH 6.2, 0.104 mM NADPH (Sigma type I); 10 mM DL-glyceraldehyde, and 0.2 mL of the enzyme supernatant, in a total volume of 2 mL. The reference blank contained all of the above reagents except glyceraldehyde, to correct for nonspecific reduction of NADPH. The reaction was initiated by the addition of substrate, and it was monitored spectrophotometrically for 5 min. Enzyme activity was adjusted by diluting the supernatant with distilled H_2O so that 0.2 mL of the enzyme supernatant gave an average reaction rate for the control sample of 0.055 ± 0.005 absorbance unit/5 min. The effects of inhibitors on the enzyme activity were determined by including in the reaction mixture 0.2 mL of each inhibitor solution at desired concentrations and including inhibitors in the reference blanks to correct for absorbance of the inhibitor. The percent inhibition of each compound was calculated by comparing the reaction rate of the solution containing both substrate and inhibitor with that of control solutions containing only the substrate. Inhibitor IC_{50} values were obtained from least-square analysis of the log dose–response curves using the LINEFIT program by R. B. Barlow, Elsevier-Biosoft, Cambridge, UK.

A 10^{-4} M solution of each inhibitor was prepared in 0.2 M $NaHCO_3$, 10% Me_2SO, or distilled H_2O. These stock solutions were diluted with distilled H_2O to the desired concentrations of the inhibitors, and 0.2-mL aliquots of the diluted solutions were added to the reaction mixtures and reference blanks. The concentration of Me_2SO in the reaction mixture was never greater than 1%, and appropriate controls were run to compensate for the inhibition produced by Me_2SO, which was insignificant. Compound **17** was dissolved in Me_2SO and 25-μL aliquots of the diluted Me_2SO solutions were added to the reaction mixture to give Me_2SO in a final concentration of <0.1%. Kinetic experiments were performed with all inhibitors, for which an IC_{50} value was determined, by using five concentrations of substrate (0.0625–1.0 mM). Two inhibitors (**14**, **25**) that displayed different types of inhibition vs. substrate were selected for kinetic experiments to determine the type of inhibition vs. cofactor (NADPH). For this experiment, five cofactor concentrations were used (0.104–0.0065 mM) while the substrate was present in a concentration of 10 mM. The type of inhibition was determined by analyzing double-reciprocal plots of experimental results. Double-reciprocal plots were generated by least-squares fit of the

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data. The effect of the inhibitor on K_m and V_{max} was verified by analyzing the data using the nonlinear least-squares HYPMIC program of R. B. Barlow, Elsvevier-Biosoft, Cambridge, UK.

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Antiinflammatory Activity of Substituted 6-Hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-triones. Atypical Nonsteroidal Antiinflammatory Agents

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A novel class of antiinflammatory drugs, which are substituted derivatives of the fused tricyclic system 6-hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-trione (see, for instance, Table III), is described. Synthetic procedures and structure determination with the assistance of X-ray crystallography are discussed. Semiempirical molecular orbital calculations are used to investigate the relative stability of the possible isomers and tautomers of the title compounds. A biological profile of the class, and of several of the more potent analogues, in several antiinflammatory models, including the adjuvant-induced arthritis and the collagen II models, is defined. Several members of the class are shown to possess extremely low ulcerogenic effects in spite of exhibiting cyclooxygenase inhibition. A preliminary bioavailability study of two of the lead structures is presented. The compounds **6–72** appear to constitute a class of drugs that shows interesting potential antiarthritic activity and also exhibits an activity profile different from that of the standard classical NSAI drugs, as determined by a comparison of the profile of this class of drug with that of several standard agents. Certain findings from toxicological studies have precluded the further development of compounds within this group, although related structural types are being investigated.

A large amount of work has been performed, in many laboratories, over the past several years, to develop new types of antiinflammatory agents, mainly for use in patients with arthritis of varying degrees of severity.[1] Although numerous products have been introduced over the years, almost all contain a carboxylic acid function[1a] or equivalent[1b]. These products are subject to the serious, limiting side effects of gastrointestinal irritation and ulceration, probably due to their commonly shared ability to inhibit the cyclooxygenase enzyme in the GI tract.[2] In addition, they do not affect the long-term prognosis of the disease state. In our continuing attempts to discover new and useful drugs for the treatment of inflammatory diseases,[3] we have examined a series of tricyclic pyrimido-[2,1-*f*]purines (Table III) which has been found to possess an interesting profile of antiinflammatory activity, different in several respects from that of the known, standard agents, and, in some ways, exhibiting the profile of a disease-modifying drug.

The compounds described lack significant analgesic effects, acute toxicity, and GI side effects. After a single oral dose, significant plasma levels have been observed for two of the most interesting compounds. Finally, single-crystal X-ray analyses,[4] performed on three analogues (**32**, **67**, **76**), confirm unequivocally the direction of cyclization and demonstrate that an intramolecular O–H···O hydrogen-bonded interaction is a consistent feature of the 6-hydroxypyrimido[2,1-*f*]purinetrione system.

Chemistry

The basic ring system was entered by a modification of the classical pyrimidine-forming reaction between a 1,1-diamino derivative (in this case a substituted 8-aminopurine, Table I) and a malonate ester (Table II).[5] However, the typical conditions used for this type of reaction, i.e. NaOEt in refluxing EtOH, failed to produce the desired tricyclic products. A useful modification was found by using a catalytic quantity of NaOMe or NaOEt and employing the malonate as solvent at approximately 190–210 °C, as shown in Scheme I, method A. Use of a Dean–Stark separator to remove the volatile products often improved the yield.

An alternate procedure, using the same starting materials, utilized NaH in DMF to cause condensation and cyclization, as shown in Scheme I, method C.

Alkylation of the 7-unsubstituted derivative **6** occurred exclusively at carbon-7 and is the subject of a separate report[6] (Scheme I, method B).

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