Synthesis and Biological Evaluation of Substituted Flavones as Gastroprotective Agents

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Flavone (**1**) was found to protect against ethanol-induced gastric damage in rats; however, it is known that certain compounds in the flavone class, including flavone itself, are inducers of hepatic drug metabolizing enzymes. With the hope of identifying gastroprotective flavones that have minimal effects on drug metabolizing enzymes, we have synthesized and evaluated selected flavone analogs. Gastroprotective potency in the ethanol model was retained by methoxy substitution in the 5-position (**4**) and by methoxy (**12**) or methyl (**14**) substitution in the 7-position. A number of substituted analogs of the potent molecule 5-methoxyflavone (**4**) were also synthesized, and in many cases, these substitutions provided gastroprotective molecules. In order to assess liver enzyme induction potential, two of the gastroprotective flavones, 7-methoxyflavone (**12**) and 5-methoxy-4′-fluoroflavone (**26**), were examined for their effect on liver microsomal cytochrome P450 and 7-ethoxyresorufin *O*-dealkylase (CYP1A) activity. These two compounds caused minimal changes in the cytochrome P450 concentration and were considerably less potent than β-naphthoflavone as inducers of CYP1A enzyme activity. Furthermore, following oral administration to rats, 5-methoxy-4′-fluoroflavone (**26**) was found to protect against indomethacin-induced gastric damage. These results indicate that, through appropriate substitution, flavones can be obtained that are gastroprotective but have minimal effects on drug-metabolizing enzymes.

Introduction

Over 30 million people in the world take nonsteroidal antiinflammatory drugs (NSAID's).[1] Many patients with rheumatoid arthritis or osteoarthritis routinely use this medication on a long-term basis. The major side effect of this class of drugs is the development of gastric ulcers and their associated complications,[2–5] a problem which can affect as many as 10–25% of all patients on chronic NSAID therapy.[6] For the most part, standard antiulcer medications are ineffective in preventing NSAID-induced gastric damage.[5,7–9] The prostaglandin E_1 analog, misoprostol, has been shown to be effective,[10] but its use is limited by side effects including diarrhea, abdominal pain, and an increase in uterine contractions, the latter leading to misoprostol being contraindicated in pregnant women.[11] For these reasons, the development of a safe and effective drug, capable of preventing stomach damage induced by NSAID's or other gastric-damaging substances, represents an important area of medicinal research.

As a result of natural product isolation work, we identified gastroprotective properties in the flavone class of compounds. Flavones are a group of oxygen-containing heterocycles that contain the 2-phenylbenzopyrone ring system. Many flavones are naturally occurring, and they comprise a major subclass of a more broadly defined family of ubiquitous, low molecular weight plant products known as the flavonoids. Other subclasses of flavonoids, such as flavanones[12] and chalcones,[13] have been shown to possess gastroprotective properties. Additionally, quercetin and other polyhydroxy flavonoids have been evaluated for antiulcer properties in the acetic acid and acidified ethanol damage models.[14,15] In our initial set of experiments, we found the simple unsubstituted molecule flavone (**1**) to be a potent gastroprotective agent in rats, with an ED_{50} of 6.9 mg/kg in an ethanol-induced gastric damage model.[16] Although substituted flavonoids can serve as useful therapeutic agents,[17] it is known that molecules in the flavone class, such as β-naphthoflavone and flavone itself, have the potential to induce hepatic drug-metabolizing enzymes,[18,19] a property that can increase the clearance and thereby decrease the therapeutic action of concomitantly administered drugs. Since a flavone-based gastroprotective drug would, by definition, be coadministered with at least one other therapeutic agent (the NSAID), we established as a goal the synthesis of flavone analogs that would be gastroprotective but have minimal effects on drug metabolizing enzymes. In this report, we describe the synthesis of a number of substituted flavones, as well as the gastroprotective properties of these and related commercially available flavones in the ethanol damage model. We also describe the enzyme induction properties of two of the more promising gastroprotective flavones. Finally, the gastroprotective properties of one of these compounds in the indomethacin damage model is presented.

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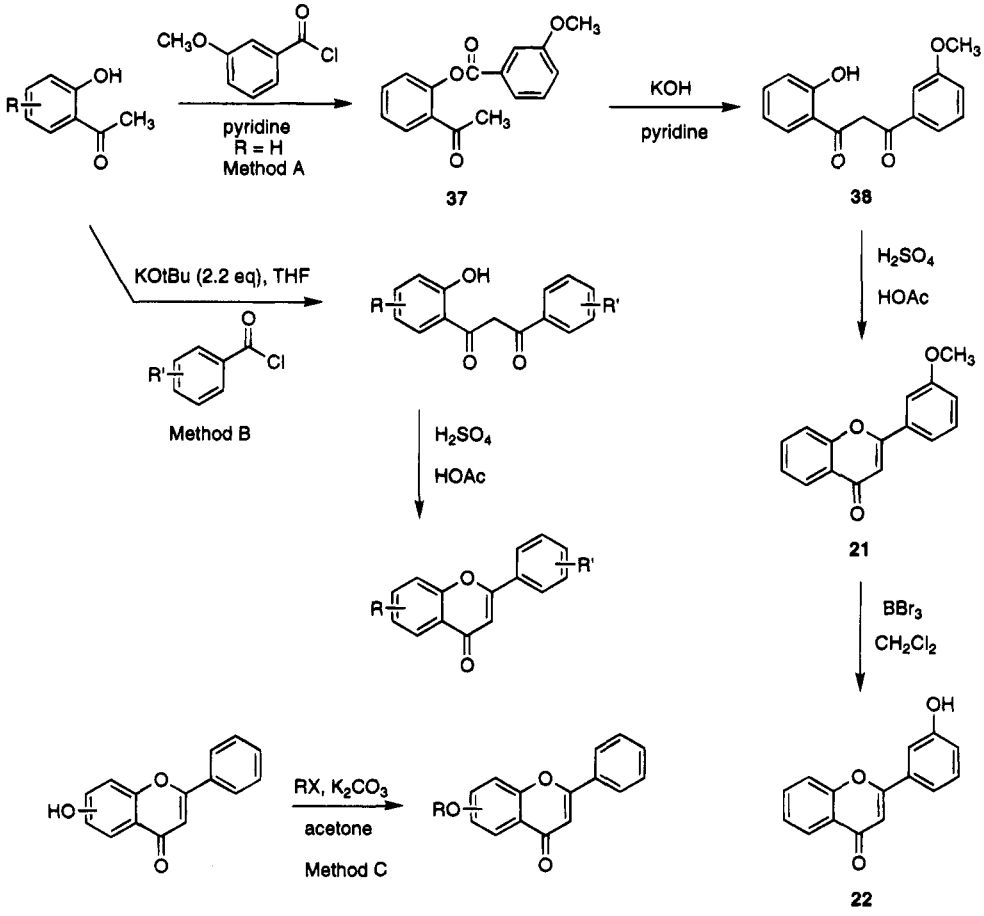
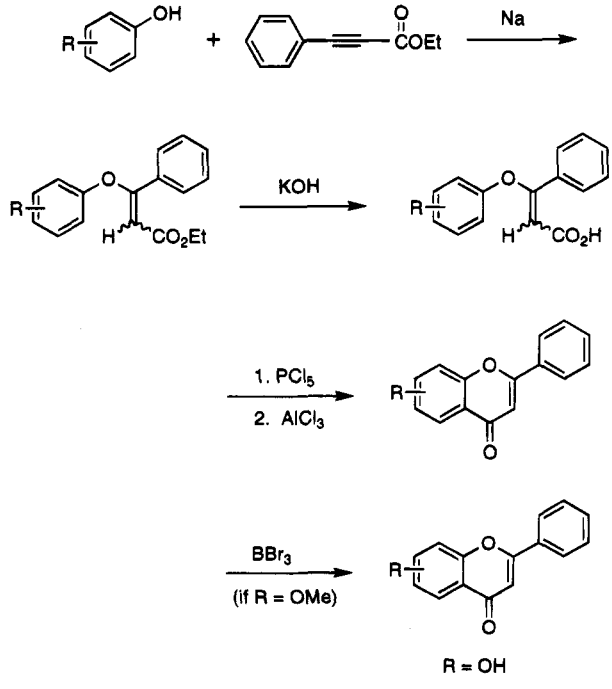
Scheme 1



Scheme 2. Method D



Chemistry

Four methods (methods A–D), indicated in Schemes 1 and 2, were employed to prepare analogs of interest in this study. The most common method of synthesizing flavones is known as the Baker–Venkataraman transformation.[20–22] In this process, a hydroxyacetophenone is first converted into a benzoyl ester, and this species is then treated with base, forming a 1,3-diketone. Treatment of this diketone with acid leads to generation of the desired flavone. Method A (Scheme 1) illustrates an application of the conventional Baker–Venkataraman synthesis. 3′-Methoxyflavone (**21**), prepared in this way, was demethylated with boron tribromide to provide 3′-hydroxyflavone (**22**). Recently, we described a new, modified version of the Baker–Venkataraman synthesis employing a potassium *tert*-butoxide-mediated diketone synthesis as the key step.[23] As indicated in method B (Scheme 1), a hydroxyacetophenone was treated with a total of 2.2 equiv of potassium *tert*-butoxide and an appropriately substituted benzoyl chloride in tetrahydrofuran to form the diketone. We have found diketone formation can be accomplished in one of two ways. One can either treat the hydroxyacetophenone initially with the entire 2.2 equiv of base and then add the acid chloride, or alternatively, one may treat the hydroxyacetophenone sequentially with 1.1 equiv of potassium *tert*-butoxide, the benzoyl chloride, and a second 1.1 equiv of potassium *tert*-butoxide. In either case, following heating the diketone is obtained in a satisfactory manner.[23] Acid-catalyzed cyclization then provided the desired flavone.

In method C (Scheme 1), A-ring alkoxy-substituted flavones were obtained by treatment of the corresponding hydroxyflavones with an alkyl halide and potassium carbonate in acetone. Finally, method D (Scheme 2) was utilized to synthesize certain other A-ring-substituted flavones. In this process, conjugate addition of the sodium salt of an appropriately substituted phenol to ethyl phenylpropiolate, followed by saponification and

intramolecular Friedel–Crafts acylation, afforded the desired flavone. Ruhemann[24,25] had previously utilized this process to prepare 8-methoxyflavone (**16**) as well as an inseparable mixture of 5-methylflavone (**6**) and 7-methylflavone (**14**). In our hands, the synthesis of 8-methoxyflavone (**16**) proceeded extremely well, and this material was converted into 8-hydroxyflavone (**17**) with boron tribromide. Moreover, we also used the Ruhemann process to generate the methylflavone mixture and then chromatographically separated these components. It should be noted that the conjugate addition produced the unsaturated ester as an *E,Z*-mixture of regioisomers. This mixture was carried through to the acid chloride stage, but upon generation of the conjugated acylium ion, stereochemical integrity was lost, and both isomers ultimately afforded the desired flavone.

Pharmacological Results and Discussion

It has been established through feeding experiments in rats that methoxy substitution in the flavone ring can reduce the enzyme-inducing properties of these compounds.[26] Therefore, we established as a strategy the generation of a database of mono- and disubstituted flavones, with particular emphasis on methoxy and related substituents. These compounds were initially screened in the rat ethanol-induced damage model, a well-established and convenient model for the evaluation of gastric cytoprotection.[27,28] Intraperitoneal administration was employed in this initial screen to minimize the likelihood that bioavailability limitations might contribute to a compound's low activity. Following this initial screen, a limited number of the most promising compounds were examined for their effects on liver microsomal cytochrome P450 and 7-ethoxyresorufin *O*-dealkylase (CYP1A) activity, as well as gastroprotective properties following oral administration in an indomethacin damage model.

Flavone gastroprotection data from the rat ethanol damage model is presented in Table 1. Data for cimetidine and PGE_2 are also included for comparison. In the benzopyrone portion of the flavone system, substitution with methoxy or hydroxy groups in the 3-, 6-, or 8-positions led to reduction in gastroprotective activity. In the 5-position, substitution with a methoxy group (**4**) provided a high level of gastroprotection that was comparable to that of flavone (**1**). All other substitutions in the 5-position led to reduction in activity. In the 7-position, two of the substituents examined (methoxy **12** and methyl **14**) produced a potent level of gastroprotection. All substitutions on the isolated aromatic B ring (compounds **18**–**25**) led to reduction in activity. One compound, 2′-methylflavone **20**, did show a good level of efficacy at 20 mg/kg, but a desirable dose–response relationship was not obtained. A number of novel gastroprotective compounds were synthesized by using the promising 5-methoxyflavone (**4**) as a starting point and adding additional substituents to the isolated B ring. For example, addition of a fluorine to the 4′-position (**26**) or addition of a trifluoromethyl to the 3′-position (**32**) produced levels of gastroprotective activity comparable to the parent compound. This was also the case when the 4′-fluoro group was added to the 7-methoxyflavone nucleus (**12**), forming compound **34**.

Since we had found that a number of substituted flavones could retain the gastroprotective properties of the parent compound, we sought to conduct a detailed examination of the enzyme-induction potential of a limited number of these gastroprotective flavone derivatives. It is well established that certain compounds in the flavone class are inducers of liver microsomal cytochrome P450 1A (CYP1A).[18,19] In this study, two of the gastroprotective flavones, 7-methoxyflavone (**12**) and 5-methoxy-4′-fluoroflavone (**26**), were examined for their effects on liver microsomal cytochrome P450 in mature male rats. Equimolar doses of the compounds were administered orally to rats for 4 consecutive days, and 24 h after the last dose, liver microsomes were prepared by differential centrifugation. These microsomal samples were analyzed for the concentration of the total cytochrome P450 and cytochrome b_5, as well as the activities of NADPH–cytochrome *c* reductase and 7-ethoxyresorufin *O*-dealkylation (EROD), a measure of CYP1A activity. The results are shown in Table 2. Neither of the gastroprotective flavones caused a statistically significant change in the concentrations of cytochrome P450 and cytochrome b_5 or in the activity of NADPH–cytochrome *c* reductase. 5-Methoxy-4′-fluoroflavone (**26**) caused a 4.4-fold increase in EROD activity, indicating that this compound is a weak inducer of CYP1A, whereas 7-methoxyflavone (**12**) had no significant effect on this marker for CYP1A activity. In comparison, β-naphthoflavone (BNF), a strong inducer of CYP1A, produced a 20-fold increase in EROD activity and a 1.4-fold increase in P450 content. These data indicate that appropriate substitution can attenuate the P450 induction properties of flavones while retaining their gastroprotective characteristics.

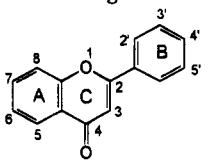
Finally, we examined the ability of the novel compound 5-methoxy-4′-fluoroflavone (**26**) to provide gastroprotection following oral administration in the acute indomethacin damage model. A favorable result here would indicate not only that the compound could provide protection from NSAID-induced damage but also that the compound was sufficiently bioavailable for oral administration. The result is shown in Table 3. At a 30 mg/kg dose, the 4′-fluoro derivative **26** provided 76% gastroprotection. These data suggest that compound **26**, although not nearly as potent as PGE_2, is capable of providing a reasonable level of efficacy in the indomethacin model.

In summary, we have found that, through appropriate substitution, flavones can be obtained which are gastroprotective in the ethanol damage model but have minimal effects on CYP1A activity in rat liver microsomes. One of these compounds, 5-methoxy-4′-fluoroflavone **26**, has also shown gastroprotective activity following oral administration in the rat indomethacin damage model. Efforts to examine the effect of changes on the flavone ring, as well as efforts to understand the mechanism of action of these compounds, are underway and will be reported in due course.

Experimental Section

Reagents were purchased from the Aldrich Chemical Co. unless otherwise indicated. Liquid chromatography was performed (a) using flash chromatography conditions (silica gel, 50–60 μm), (b) filtering through a fritted-glass funnel packed with a layer of sand/flash silica gel/sand using a water-aspirator vacuum (hereafter this method will be referred to as a vacuum column), or (c) using a Chromatotron (Harrison Research, Palo Alto, CA) on 1-, 2-, and 4-mm silica gel rotors

Table 1. Evaluation of Substituted Flavones as Gastroprotective Agents in Rat Ethanol Damage Model



compound	substituent	source[a]	% protection[b]	ED_{50}[c]
1	none (Flavone)	Aldrich	93	6.9 (4.4–16.9)
2	3-OMe	Spectrum	41	
3	3-OH	Indofine	47	
4	5-OMe	Indofine	97	2.6 (1.4–5.0)
5	5-OH	Indofine	58	
6	5-Me	method D	52	
7	5-OEt	method C	36	
8	5-O-allyl	method C	57	
9	5-OBu	method C	75	
10	6-OMe	Indofine	29	
11	6-OH	Indofine	57	
12	7-OMe	Indofine	89	5.9 (3.1–14.6)
13	7-OH	Indofine	75	
14	7-Me	method D	90	5.6 (3.4–13.8)
15	7-OEt	method C	87	
16	8-OMe	method D	53	
17	8-OH	method D	46	
18	2′-OMe	Indofine	66	22.4 (13.2–81.2)
19	2′-OH	Indofine	78	15.6 (8.2–120.4)
20	2′-Me	method B	92	
21	3′-OMe	method A	48	
22	3′-OH	method A	71	
23	4′-OMe	Indofine	21	
24	4′-OH	Indofine	63	
25	4′-Me	method B	68	
26	5-OMe, 4′-F	method B	97	5.5 (4.0–7.0)
27	5-OMe, 4′-Me	method B	93	
28	5-OMe, 4′-NO_2	method B	52	
29	5-OMe, 4′-Cl	method B	94	
30	5,4′-$(OMe)_2$	method B	33	
31	5-OMe, 4′-CF_3	method B	65	
32	5-OMe, 3′-CF_3	method B	90	3.3 (2.1–4.7)
33	5-OMe, 2′-Cl	method B	89	
34	7-OMe, 4′-F	method B	67	8.9 (6.3–14.3)
35	7-OMe, 3′-CF_3	method B	67	
36	7-OMe, 2′-Cl	method B	80	
cimetidine (52 mg/kg, oral administration)			14	
PGE_2 (0.1 mg/kg, oral administration)			82	

[a] Commercial supplier or synthetic method utilized. [b] Percent protection from gastric damage relative to vehicle control following intraperitoneal administration of drug (20 mg/kg). [c] Dose in mg/kg required to produce a 50% gastroprotection level relative to control, as calculated from dose–response experiments; 95% confidence limits are shown in parentheses.

Table 2. Effects of Substituted Flavones on Rat Liver Microsomal Cytochrome P450

treatment group[a]	daily dose (mg/kg)	cytochrome P450 (nmol/mg of protein)[b]	cytochrome b_5 (nmol/mg of protein/min)[b]	NADPH cytochrome *c* reductase (nmol/mg of protein/min)[b]	EROD[c] (pmol/mg of protein/min)[b]
vehicle control		1.48 ± 0.12	0.63 ± 0.11	295 ± 76	253 ± 33
β-naphthoflavone (positive control)	100	2.02 ± 0.15	0.79 ± 0.05	166 ± 11	5010 ± 500
12	250	1.42 ± 0.23	0.73 ± 0.11	339 ± 68	442 ± 59
26	268	1.17 ± 0.17	0.65 ± 0.06	275 ± 5	1110 ± 220*

[a] Male rats (n = 4/group) were treated with vehicle or compound as described in the Experimental Section. [b] Values are mean ± standard deviation of four determinations. * Significantly different from the control value as determined by Dunnett's test ($\alpha < 0.05$). No statistical comparisons were made between β-naphthoflavone and control. [c] EROD, 7-ethoxyresorufin *O*-dealkylase, is a measure of CYP1A enzyme activity.[19]

which were prepared according to the manufacturer's instructions. Thin layer chromatography (TLC) was accomplished using silica gel GHLF (250 μm) on prescored plates (10 × 20 cm) obtained from Analtech, Inc. Tetrahydrofuran (THF) was dried over sodium/benzophenone. Proton and carbon-13 nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra were recorded using a GE QE-300 spectrometer or a Bruker AC-300 Quad-nuclei probe system using $CDCl_3$ as a solvent except where noted. Mass spectra (MS) were obtained on a Finnigan Model TSQ700, TSQ46CH, or TSQ4500. Melting points were run on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were obtained from Galbraith Laboratories, Knoxville, TN, or Oneida Research Services, Whitesboro, NY.

Table 3. Evaluation of Gastroprotective Agents in Rat Indomethacin Damage Model

compound	dose (mg/kg)	% protection[a]
26	30	76
PGE_2	0.25	61

[a] Percent protection from gastric damage relative to vehicle control following oral administration of compound.

Compounds **1**–**5**, **10**–**13**, **18**, **19**, **23**, and **24** were purchased from commercial sources, which are indicated in the Table 1. The synthesis, physical properties, and spectral characteristics of compounds **20**, **25**–**27**, **29**, **31**, and **34**–**36** have been previously described.[23] Compounds **28**, **30**, **32**, and **33** were prepared by method B in 32–57% yield according to known procedures.[23] Their physical and spectral properties are noted as follows.

2-[3-(Trifluoromethyl)phenyl]-5-methoxy-4*H*-1-benzopyran-4-one (32, 5-methoxy-3′-(trifluoromethyl)flavone): mp 184–185 °C; ^{1}H NMR (300 MHz) δ 3.98 (s, 3 H, OCH_3), 6.75 (s, 1 H), 6.83 (d, 1 H, J = 8 Hz), 7.14 (d, 1 H, J = 8 Hz), 7.59 (m, 2 H), 7.75 (d, 1 H, J = 7 Hz), 8.03 (d, 1 H, J = 7 Hz), 8.13 (s, 1 H); ^{13}C NMR (75 MHz) δ 56.47, 106.85, 109.92, 110.06, 115.25, 121.94, 122.87, 125.46, 127.67, 129.10, 129.52, 131.63, 131.99, 132.56, 133.90, 158.21, 159.28, 159.92, 177.60; MS (CI) m/z 321 $[M + H]^+$. Anal. ($C_{17}H_{11}F_3O_3$) C, H.

2-(2-Chlorophenyl)-5-methoxy-4*H*-1-benzopyran-4-one (33, 5-methoxy-2′-chloroflavone): mp 148–149 °C; ^{1}H NMR (300 MHz) δ 3.98 (s, 3 H, OCH_3), 6.55 (s, 1 H), 6.82 (d, 1 H, J = 8 Hz), 7.05 (d, 1 H, J = 8 Hz), 7.39 (m, 2 H), 7.53 (m, 3 H); ^{13}C NMR (75 MHz) δ 56.48, 106.66, 110.17, 114.47, 114.71, 126.93, 130.51, 130.71, 131.49, 131.67, 132.95, 133.70, 158.61, 159.93, 160.26, 177.60; MS (CI) m/z 287 $[M + H]^+$. Anal. ($C_{16}H_{11}ClO_3$) C, H.

2-(4-Nitrophenyl)-5-methoxy-4*H*-1-benzopyran-4-one (28, 5-methoxy-4′-nitroflavone): mp >237 °C; ^{1}H NMR (300 MHz, acetic acid-d_4) δ 3.97 (s, 3 H, CH_3), 6.99 (d, 1 H, J = 8 Hz), 7.24 (s, 1 H), 7.30 (d, 1 H, J = 8 Hz), 7.75 (apparent t, 1 H), 8.24 (m, 2 H), 8.39 (m, 2 H); ^{13}C NMR (75 MHz, acetic acid-d_4) δ 55.43, 106.99, 109.80, 110.00, 123.95, 127.30, 135.26, 136.67, 149.61, 158.31, 159.69, 159.95, 179.73; MS (CI) m/z 298 $[M + H]^+$. Anal. ($C_{16}H_{11}NO_5$) C, H.

2-(4-Methoxyphenyl)-5-methoxy-4*H*-1-benzopyran-4-one (30, 5,4′-dimethoxyflavone): mp 159–160 °C; ^{1}H NMR (300 MHz) δ 3.87 (s, 3 H, OCH_3), 3.98 (s, 3 H, OCH_3), 6.66 (s, 1 H), 6.78 (d, 1 H, J = 8 Hz), 6.99 (d, 2 H, J = 8 Hz), 7.13 (d, 1 H, J = 8 Hz), 7.56 (apparent t, 1 H), 7.81 (d, 2 H, J = 8 Hz); ^{13}C NMR (75 MHz) δ 55.47, 56.50, 106.34, 107.72, 110.11, 114.37, 114.52, 123.70, 127.73, 133.53, 158.23, 159.72, 161.08, 162.17, 178.34; MS (CI) m/z 283 $[M + H]^+$. Anal. ($C_{17}H_{14}O_4$) C, H.

2-Phenyl-8-methoxy-4*H*-1-benzopyran-4-one (16, 8-Methoxyflavone). Flavone **16** was prepared in 63% yield from guaiacol (Eastman Kodak Co.) and ethyl phenylpropiolate according to the procedure of Ruhemann,[24] mp 198–199 °C (lit. mp 199–200 °C).

2-Phenyl-8-hydroxy-4*H*-1-benzopyran-4-one (17, 8-Hydroxyflavone). A solution of **16** (1.0 g, 3.97 mmol) in methylene chloride (8 mL) was cooled in an ice-water bath, and boron tribromide (8 mL of a 1 M solution in methylene chloride, 8 mmol) was added. The mixture was allowed to warm to room temperature and stir over a weekend. The reaction mixture was poured into ice water with stirring. The gummy solid that formed was filtered and vacuum dried. The residue remaining was partitioned between water and methylene chloride. The layers were separated, and the organic layer was dried and evaporated to afford a solid. The two batches of crude product were combined and recrystallized from ethanol to afford the product (0.77 g, 82% yield): mp >220 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.01 (s, 1 H), 7.21 (m, 2 H), 7.45 (m, 1 H), 7.58 (m, 3 H), 8.11 (m, 2 H), 10.49 (br s, 1 H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 107.03, 114.56, 119.80, 125.08, 125.71, 126.80, 129.49, 131.72, 132.13, 145.75, 147.38, 162.47, 177.74; MS (CI) m/z 239 $[M + H]^+$. Anal. ($C_{15}H_{10}O_3$) C, H.

2-Phenyl-5-methyl-4*H*-1-benzopyran-4-one (6, 5-Methylflavone) and 2-Phenyl-7-methyl-4*H*-1-benzopyran-4-one (14, 7-Methylflavone). Flavones **6** and **14** were prepared from *m*-cresol and ethyl phenylpropiolate by the procedure of **16**, which yielded two isomers. These were purified by chromatotron (eluting with 15% ethyl acetate in hexane), yielding 12% of 5-methylflavone and 26% of 7-methylflavone.

5-Methylflavone (6): mp 125–127 °C (lit.[29] mp 129–130 °C).

7-Methylflavone (14): mp 120–121 °C (lit.[29] mp 120 °C).

2-Phenyl-5-(2-proprenoxy)-4*H*-1-benzopyran-4-one [8, 5-(Allyloxy)flavone]. A mixture of 5-hydroxyflavone (1.0 g, 4.2 mmol, Indofine Chemicals), 3-iodo-1-propene (3.52 g, 1.92 mL, 21 mmol), potassium carbonate (5.8 g, 42 mmol), and acetone (25 mL) was heated at reflux for 6 h. The reaction mixture was filtered hot, and the potassium carbonate cake was washed well with acetone. Evaporation of the filtrate afforded a yellow solid which was purified by flash column chromatography (eluting with 25% ethyl acetate in hexane) to afford a white solid (820 mg, 70% yield): mp 128–129 °C; ^{1}H NMR (300 MHz) δ 4.78 (m, 2 H, OCH_2), 5.38 (m, 1 H), 5.71 (m, 1 H), 6.08–6.24 (m, 1 H), 6.74 (s, 1 H), 6.85 (d, 1 H, J = 8.5 Hz), 7.17 (d, 1 H, J = 8.5 Hz), 7.55 (m, 4 H), 7.92 (m, 2 H); ^{13}C NMR (75 MHz) δ 69.88, 108.10, 109.07, 110.28, 114.95, 117.73, 126.02, 128.92, 131.29, 131.46, 132.36, 133.55, 158.25, 158.62, 160.95, 178.04; MS (CI) m/z 279 $[M + H]^+$. Anal. ($C_{18}H_{14}O_3$) C, H.

2-Phenyl-7-ethoxy-4*H*-1-benzopyran-4-one (15, 7-Ethoxyflavone). Flavone **15** was prepared in 61% yield from 7-hydroxyflavone (Indofine Chemicals) and iodoethane by the procedure of **8**. The material was recrystallized from 2-propanol: mp 138–139 °C (lit.[30] mp 138–140 °C).

2-Phenyl-5-ethoxy-4H-1-benzopyran-4-one (7) (5-ethoxyflavone). Flavone **7** was prepared in 56% yield from 5-hydroxyflavone (Indofine Chemicals) and iodoethane by the procedure of **8**. The material was purified by flash column chromatography (gradient elution of 60% ethyl acetate in hexane, 80% ethyl acetate in hexane, and 100% ethyl acetate): mp 111–113 °C; ^{1}H NMR (300 MHz) δ 1.61 (t, 3 H, CH_3, J = 8 Hz), 4.25 (q, 2 H, CH_2, J = 8 Hz), 6.72 (s, 1 H), 6.84 (d, 1 H, J = 9 Hz), 7.12 (d, 1 H, J = 9 Hz), 7.51 (m, 4 H), 7.92 (m, 2 H); ^{13}C NMR (75 MHz) δ 14.60, 65.12, 107.68, 109.08, 109.93, 114.95, 126.04, 128.91, 131.26, 131.52, 133.61, 158.30, 159.12, 160.90, 178.21; MS (CI) m/z 267 $[M + H]^+$. Anal. ($C_{17}H_{14}O_3$) C, H.

2-Phenyl-5-butoxy-4*H*-1-benzopyran-4-one (9, 5-Butoxyflavone). Flavone **9** was prepared in 39% yield from 5-hydroxyflavone (Indofine Chemicals) and 1-iodobutane by the procedure of **8**. The material was purified by vacuum column (eluting with 25% ethyl acetate in hexane) and flash column chromatography (20% ethyl acetate in hexane) and then recrystallized from ethyl acetate: mp 96–97 °C; ^{1}H NMR (300 MHz) δ 1.04 (t, 3 H, CH_3, J = 7 Hz), 1.65 (m, 2 H, CH_2), 1.94 (m, 2 H, CH_2), 4.15 (t, 2 H, OCH_2, J = 7 Hz), 6.69 (s, 1 H), 6.81 (d, 1 H, J = 9 Hz), 7.11 (d, 1 H, J = 9 Hz), 7.53 (m, 4 H), 7.89 (m, 2 H); ^{13}C NMR (75 MHz) δ 13.89, 19.22, 31.07, 69.25, 107.61, 109.12, 109.79, 114.86, 126.05, 128.91, 131.23, 131.59, 133.58, 158.31, 159.39, 160.87, 178.12; MS (CI) m/z 295 $[M + H]^+$. Anal. ($C_{19}H_{18}O_3$) C, H.

2-[(3-Methoxybenzoyl)oxy]acetophenone (37). To *m*-anisic acid (2.0 g, 13.15 mmol) in methylene chloride (28 mL) was added oxalyl chloride (2.08 g, 1.43 mL, 16.44 mmol) followed by dimethylformamide (0.09 g, 0.1 mL, 1.31 mmol), resulting in gas evolution. This reaction mixture was stirred at room temperature for 2 h and then evaporated to dryness. Methylene chloride was added again, and the solution was evaporated to dryness to afford the crude acid chloride. To a stirring solution of this acid chloride in pyridine (10 mL) in an ice-water bath, was added 2-hydroxyacetophenone (1.19 g, 8.77 mmol). The reaction mixture was left in the ice bath for 15 min, stirred at room temperature for 30 min, and poured into a 3% aqueous HCl/ice solution with vigorous stirring. The precipitate which formed was filtered and washed with water. The crude material was recrystallized from methanol, yielding the acetophenone **37** (1.75 g, 74% yield): ^{1}H NMR (300 MHz) δ 2.57 (s, 3 H,), 3.91 (s, 3 H), 7.25 (m, 2 H), 7.45 (m, 2 H), 7.63

(m, 1 H), 7.75 (m, 1 H), 7.89 (m, 2 H); ^{13}C NMR (75 MHz) δ 29.69, 55.48, 114.70, 120.37, 122.68, 123.82, 126.09, 129.67, 130.14, 130.56, 131.46, 133.23, 149.36, 159.81, 164.93, 197.36; MS (CI) m/z 271 $[M + H]^+$.

1-(2-Hydroxyphenyl)-3-(3-methoxyphenyl)propane-1,3-dione (38). A solution of acetophenone **37** (2.78 g, 10.29 mmol) and pyridine (9.5 mL) was heated to 50 °C, and to it was added pulverized potassium hydroxide (0.86 g, 15.44 mmol). The reaction mixture was stirred for 15 min, and upon cooling, 14 mL of a 10% aqueous acetic acid solution was added. The pale yellow precipitate which formed was filtered. This crude material (2.72 g) was used in the next step without purification.

2-(3-Methoxyphenyl)-4*H*-1-benzopyran-4-one (21, 3′-Methoxyflavone). A mixture of the diketone **38** (2.72 g, 10 mmol), concentrated sulfuric acid (0.51 mL), and glacial acetic acid (13 mL) was heated at reflux for 1 h and cooled to room temperature. The mixture was poured onto crushed ice (75 g), and the resulting precipitate was filtered. Recrystallization from hexane/ethyl acetate afforded the product (1.65 g, 65% yield): mp 127–129 °C (lit.[31] mp 130–131 °C).

2-(3-Hydroxyphenyl)-4*H*-1-benzopyran-4-one (22, 3′-Hydroxyflavone). Flavone **22** was prepared in 86% yield from **21** and boron tribromide by the procedure of **17**. The crude material was recrystallized from ethanol: mp 209–211 °C (lit.[31] mp 209–211 °C).

Gastroprotection Evaluation: Animals. Male CD Sprague–Dawley rats were supplied from Charles River Breeding Laboratories, Kingston, New York. Upon receipt, rats were double housed in stainless steel mesh-bottom cages with ad libitum access to food (Laboratory Rodent Diet no. 5001 from PMI Feeds, Inc.) and water. Animals were allowed to acclimate for a minimum of 5 days prior to use.

Test Suspensions. Flavonoids were prepared 30–60 min prior to dosing in a poly(ethylene glycol)-200/1% methyl cellulose (PEG/MC) vehicle. A 5% weight/weight solution of the flavonoid in the PEG-200 (Sigma Chemical Co., St. Louis, MO) was first made. This was heated to solubilize the compound into the PEG-200 solvent. The required amount of this solution was weighed into 1% MC (4000 cps, Fisher Chemical Co., Fair Lawn, NJ) in deionized water to achieve the desired mg/kg dose in a 1 mL volume. The compound/PEG/MC mixture was probe sonicated to achieve a uniform suspension. Suspensions were kept homogeneous during dosing by magnetically stirring. Prostaglandin E_2 (PGE_2) (Sigma Chemical Co.) was prepared for dosing from a stock solution of 1 mg/mL in 100% ethanol which was kept in the −20 °C freezer between uses. An aliquot of the stock was added (vol/vol) to saline, on ice, and purged with nitrogen gas, to make the required dosing solution. These dosing solutions were mixed by inversion and kept on ice prior to and throughout the dosing period. Cimetidine (Sigma Chemical Co.) was prepared in the same manner as the flavonoids.

Damaging Agents. Gastric damage was induced with either ethanol or indomethacin according to the procedures outlined below. 100% Ethanol (AAPER Alcohol and Chemical Co., Shelbyville, KY) was used in a volume of 1 mL. Indomethacin (Sigma Chemical Co.) was prepared weight/volume in a vehicle of 0.5% methyl cellulose made in 2.0% Tween 20 (Sigma Chemical Co.) by weighing the appropriate amount of the compound into a vial and diluting it to a concentration of 20 mg/kg. The suspension was bath-sonicated for 10–20 min prior to dosing and magnetically stirred during the dosing period.

Animal Preparation. For both gastric damage protocols, rats were weighed, tailcupped to prevent coprophagy, single-housed, and food-fasted overnight prior to dosing. Each treatment group consisted of 7–10 rats. The mean weight of all rats was used to calculate dose concentrations. Rats were used in a range of 225–350 g, with no more than a 50 g range of weights in a single experiment. Rats received water ad libitum but remained food-fasted for the entire period of the experiment.

Ethanol Damage Model Protocol. In the morning after initiation of the fast, rats were administered the test compounds by intraperitoneal injection (1 mL/rat). One hour later, rats received 1 mL of 100% ethanol by oral gavage with an 8 French nelathon catheter. One hour after ethanol challenge, the rats were anesthetized by carbon dioxide inhalation and euthanized by cervical dislocation. The abdomen was opened, the stomach quickly removed and cut opened along the greater curvature. Stomach contents were rinsed away by dipping the open stomach into two consecutive beakers of saline. Each stomach was then placed in an individual vial of saline until all of the stomachs had been collected.

Iodomethacin Damage Model Protocol. In the morning after initiation of the fast, rats were administered the test compounds by oral gavage (1 mL/rat) with an 8 French nelathon catheter. Thirty minutes later, indomethacin was administered, also by oral gavage. Rats were sacrificed 4 h after the indomethacin dosing and stomachs removed in the same manner as described for the ethanol model.

Stomach Preparation and Lesion Analysis. Prior to analysis, stomachs were removed from saline, blotted dry and spread flat on small prelabeled cards for individual identification, mucosal surface up. When stored for later analysis, mounted stomachs were placed face down on saran wrap, wrapped to seal the edges and placed in a −20 °C freezer. Gastric damage was determined by one of two systems. The Zeiss interactive digitizing analysis system (ZIDAS) was used for the majority of ethanol damage studies and all indomethacin damage studies. The Optimas system was used for some of the ethanol damage studies. With the ZIDAS system, thawed stomachs were viewed under a dissecting microscope equipped with a drawing tube and digitizing pad on which the observer traced the length of each lesion with a lighted cursor. The ZIDAS converted cursor-traced lesions to millimeters using an internal conversion analysis. The ZIDAS summed the lesions for the total length of lesions for each stomach. Stomachs to be analyzed by the Optimas were not frozen but analyzed immediately. The Optimas Image Analysis System acquires and stores an image of the whole stomach with a video camera. Lesion areas were identified by differences in light/dark contrast, converted to mm^2 by the Optimas program using an internal conversion factor. The total area of damage was determined for each stomach and the treatment group mean used for comparison.

Statistics. For gastroprotection studies, gastric damage measurements were placed in an Excel spreadsheet for initial statistical analysis. Dixon's criteria for testing extreme observations in a single sample was used to identify and exclude outliers. The mean, standard deviation, and standard error were calculated for each treatment group. The percent protection was determined for each treatment group compared with vehicle control group with the formula: 1− (treatment mean damage/control mean damage) × 100. Standard regression analysis in a SAS program was used to determine the dose required to provide 50% protection (ED_{50}) in the ethanol damage model. A line was generated with the formula $y = mx + b$, where b = the constant, m = slope, y = % protection. The value for x (mg/kg) was determined at 50% protection. Total area was plotted vs the log dose, and the straight line was generated using the least squares method; 95% confidence limits were calculated according to the method of Snedecor and Cochran.[32]

Cytochrome P450 Evaluation: Animals. Sprague–Dawley CD mature male rats were supplied from Charles River Breeding Laboratories, Portage, MI. During the 6 day acclimation period and throughout the course of the study, rats were housed individually in stainless-steel mesh-bottom cages with free access to food (Purina Rat Chow no. 5002) and water. At study initiation, rats were approximately 8 weeks of age and body weights ranged from 224 to 275 g.

Test Suspensions. 7-Methoxyflavone (**12**) and 5-methoxy-4′-fluoroflavone (**26**) were micronized (Micron-Master Jet Pulverizer, Jet Pulverizer Co., Morristown, NJ) with Avicel PH102 (FMC Corporation, Philadelphia, PA) prior to preparing dose suspensions with 0.5% methyl cellulose/0.5% polysorbate 80 (ICI Americas, Inc., Wilmington, DE) in water. The positive control, β-naphthoflavone (BNF, Sigma), was prepared as a suspension in corn oil (The Procter & Gamble Co.). The vehicle control was prepared as a solution of 0.5% methyl cellulose/

0.5% polysorbate 80 in water. Dose suspensions/solutions were prepared fresh on the first day of dosing and were administered throughout the study. All dosing suspensions/solutions were stored at 4 °C between dosing periods.

Experimental Procedures. Each treatment group contained four rats. Compounds **12** and **26** were administered perorally at 250 and 268 mg/kg, respectively. The dosages were the highest dosages considered to be nontoxic to test animals. BNF was chosen as a positive control on the basis of its well-recognized ability to induce CYP1A[19] and was administered intraperitoneally at 100 mg/kg, a maximal effective dosage for the induction of CYP1A. All treatment groups were dosed once a day for 4 consecutive days. Twenty-four hours following the last dose, rats were terminated by CO_2 asphyxiation, livers were harvested, and microsomes were prepared according to the method of Guengerich.[33] The concentration of cytochrome P450 was determined by the method of Omura and Sato[34] from the carbon monoxide difference spectrum of dithionite-reduced microsomes on the basis of an extinction coefficient of 91 mM^{-1} cm^{-1}. The concentration of cytochrome b_5 was determined from the difference spectrum between oxidized and reduced cytochrome b_5 on the basis of an extinction coefficient of 185 mM^{-1} cm^{-1}.[34] NADPH–cytochrome *c* reductase activity was determined by the method of Phillips and Landon.[35] The rate of reduction of cytochrome *c* was calculated from the rate of increase in absorbance at 550 nm on the basis of an extinction coefficient of 19.1 mM^{-1} cm^{-1} for reduced vs oxidized cytochrome *c*. The 7-ethoxyresorufin *O*-dealkylase assay, which monitors the induction of CYP1A, was measured by the fluorimetric method of Burke et al.[19]

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References

(1) Gibson, T. Nonsteroidal Anti-Inflammatory Drugs – Another Look. *Br. J. Rheumatol.* **1988**, *27*, 87–90.
(2) Duggan, J. M.; Dobson, A. J.; Johnson, H.; Fahey, P. Peptic Ulcer and Non-Steroidal Anti-Inflammatory Agents. *Gut* **1986**, *27*, 929–933.
(3) Somerville, K.; Faulkner, G.; Langman, M. Non-Steroidal Anti-Inflammatory Drugs and Bleeding Peptic Ulcer. *Lancet* **1986**, No. I, 462–464.
(4) Hawkey, C. J. Non-Steroidal Anti-Inflammatory Drugs and Peptic Ulcers. *Br. Med. J.* **1990**, *300*, 278–284.
(5) Collier, D. S.; Pain, J. A. Non-Steroidal Anti-Inflammatory Drugs and Peptic Ulcer Perforation. *Gut* **1985**, *26*, 359–363.
(6) Fries, J. F.; Williams, C. A.; Bloch, D. A. Nonsteroidal Anti-Inflammatory Drug-Associated Gastropathy: Incidence and Risk Factor Models. *Am. J. Med.* **1991**, *91*, 213–222.
(7) McCarthy, D. M. Nonsteroidal Anti-Inflammatory Drug-Induced Ulcers: Management by Traditional Therapies. *Gastroenterology* **1989**, *96*, 662–674.
(8) Lanza, F.; Robinson, M.; Bowers, J.; Griffin, J.; Kogut, D.; Kogan, F.; Warner, C. A Multi-Center Double-Blind Comparison of Ranitidine vs. Placebo in the Prophylaxis of Nonsteroidal Anti-Inflammatory Drug (NSAID) Induced Lesions in Gastric and Duodenal Mucosae (Abstract). *Gastroenterology* **1988**, *94*, A250.
(9) Lanza, F. L.; Graham, D. Y.; Davis, R. E.; Rack, M. F. Endoscopic Comparison of Cimetidine and Sucralfate for Prevention of Naproxen-Induced Acute Gastroduodenal Injury. *Dig. Dis. Sci.* **1990**, *35*, 1494–1499.
(10) Graham, D. Y.; Agrawal, N. M.; Roth, S. H. Prevention of NSAID-Induced Gastric Ulcer with Misoprostol: Multicentre, Double-Blind, Placebo-Controlled Trial. *Lancet* **1988**, No. 2, 1277–1280.
(11) Monk, J. P.; Clissold, S. P. Misoprostol: A Preliminary Review of Its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Efficacy in the Treatment of Peptic Ulcer Disease. *Drugs* **1987**, *33*, 1–30.
(12) Konturek, S. J.; Brzozowski, T.; Radecki, T.; Kitler, M. E. Gastric Protection by Meciadanol, A New Synthetic Flavonoid Inhibiting Histidine Decarboxylase. *Gastroenterology* **1985**, *88*, 1452.
(13) Konturek, S. J.; Radecki, T.; Brzozowski, T.; Drozdowicz, D.; Piastucki, I.; Muramatsu, M.; Tanaka, M.; Aihara, H. Antiulcer and Gastroprotective Effects of Solon, A Synthetic Flavonoid Derivative of Sophoradin. Role of Endogenous Prostaglandins. *Eur. J. Pharmacol.* **1986**, *125*, 185–192.
(14) Motilva, V.; Alarcon de la Lastra, C.; Martin Calero, M. J. Effects of Naringenin and Quercetin on Experimental Chronic Gastric Ulcer in Rat. Studies on the Histological Findings. *Phytother. Res.* **1992**, *6*, 168–170.
(15) Izzo, A. A.; Di Carlo, G.; Mascolo, N.; Capasso, F.; Autore, G. Antiulcer Effect of Flavonoids. Role of Endogenous PAF. *Phytother. Res.* **1994**, *8*, 179–181.
(16) Ares, J. J.; Outt, P. E.; Kakodkar, S. V.; Randall, J. L.; Murray, P. D.; Weisshaar, P. S.; O'Brien, L. M.; Ems, B. L. Synthesis and Biological Evaluation of Substituted Flavones as Gastroprotective Agents. 207th National Meeting of the American Chemical Society, San Diego, 1994, Abstract No. MEDI 112.
(17) Gambacciani, M.; Spinetti, A.; Cappagli, B.; Taponeco, F.; Felipetto, R.; Parrini, D.; Cappelli, N.; Fioretti, P. Effects of Ipriflavone Administration on Bone Mass and Metabolism in Ovariectomized Women. *J. Endocrinol. Invest.* **1993**, *16*, 333–337.
(18) Brouard, C.; Seiss, M. H.; Vernevaut, M. F.; Suschetet, M. Comparison of the Effects of Feeding Quercetin or Flavone on Hepatic and Intestinal Drug-Metabolizing Enzymes of the Rat. *Food Chem. Toxicol.* **1988**, *26*, 99–103.
(19) Burke, M. D.; Thompson, S.; Elcombe, C. R.; Halpert, J.; Haaparanta, T.; Mayer, R. T. Ethoxy-, Pentoxy- and Benzyloxyphenoxazones and Homologues: A Series of Substrates to Distinguish Between Different Induced Cytochromes P-450. *Biochem. Pharmacol.* **1985**, *34*, 3337–3345.
(20) Baker, W. Molecular Rearrangement of Some o-Acyloxyacetophenones and the Mechanism of the Production of 3-Acylchromones. *J. Chem. Soc.* **1933**, 1381–1388.
(21) Mahal, H. S.; Venkataraman, K. Synthetical Experiments in the Chromone Group. Part XIV. The Action of Sodamide on 1-Acyloxy-2-Acetonaphthones. *J. Chem. Soc.* **1934**, 1767–1769.
(22) Wheeler, T. S. Flavone. *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. IV, pp 478–481.
(23) Ares, J. J.; Outt, P. E.; Kakodkar, S. V.; Buss, R. C.; Geiger, J. C. A Convenient Large-Scale Synthesis of 5-Methoxyflavone and Its Application to Analog Preparation. *J. Org. Chem.* **1993**, *58*, 7903–7905.
(24) Ruhemann, S. Uber ein einfaches Verfahren zur Darstellung der Flavone und uber die Synthese des Thioflavones. (A Simple Procedure for the Preparation of Flavones and the Synthesis of Thioflavone.) *Ber.* **1913**, *46*, 2188–2197.
(25) Ruhemann, S.; Stapleton, H. E. Condensation of Phenols with Esters of the Acetylene Series. Part III. Synthesis of Benzo-γ-Pyrone. *J. Chem. Soc.* **1900**, *77*, 1179–1185.
(26) Siess, M.-H.; Le Bon, A. M.; Suschetet, M. Dietary Modification of Drug-Metabolizing Enzyme Activities: Dose-Response Effect of Flavonoids. *J. Toxicol. Environ. Health* **1992**, *35*, 141–152.
(27) Robert, A.; Nezamis, J. E.; Lancaster, C.; Hanchar, J. Cytoprotection by Prostaglandins in Rats. *Gastroenterology* **1979**, *77*, 433–443.
(28) Kinney, W. A.; Lee, N. E.; Blank, R. M.; Demerson, C. A.; Sarnella, C. S.; Scherer, N. T.; Mir, G. N.; Borella, L. E.; DiJoseph, J. F.; Wells, C. N-Phenyl-2-pyridinecarbothioamides as Gastric Mucosal Protectants. *J. Med. Chem.* **1990**, *33*, 327–336.
(29) Robertson, A.; Waters, R. B.; Jones, E. T. Hydroxy-carbonyl Compounds. Part VII. Coumarins and 1:4-Benzopyrones Derived from m-Cresol. *J. Chem. Soc.* **1932**, 1681–1688.
(30) Klosa, J. Synthesis of Ethers of 7-Hydroxyflavone. *J. Prakt. Chem.* **1963**, *22*, 259–266.
(31) Looker, J. H.; Hanneman, W. W. Physical and Chemical Properties of Hydroxyflavones. I. Infrared Absorption Spectra of Monohydroxyflavones and Their O-Methyl and O-Acetyl Derivatives. *J. Org. Chem.* **1962**, *27*, 381–389.
(32) Snedecor, G. W.; Cochran, W. G. *Statistical Methods*, 8th ed.; Iowa State University Press: Ames, IA, 1989.
(33) Guengerich, F. P. Analysis and Characterization of Enzymes. In *Principles and Methods of Toxicology*; Hayes, A. W., Ed.; Raven Press: New York, 1989; pp 784–785.
(34) Omura, T.; Sato, R. The Carbon Monoxide-Binding Pigment of Liver Microsomes. Evidence for its Hemoprotein Nature. *J. Biol. Chem.* **1964**, *239*, 2379–2385.
(35) Phillips, A. H.; Landon, R. G. Hepatic Triphosphopyridine Nucleoside-Cytochrome c Reductase: Isolation, Characterization and Kinetic Studies. *J. Biol. Chem.* **1962**, *237*, 2652–2660.

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