and water, and dried ($MgSO_4$). The $CHCl_3$ layer was evaporated in vacuo to give 920 mg of an oil (97% yield). The oil (750 mg) was placed in a saturated HCl solution of MeOH, the solution was evaporated in vacuo, and the resulting solid was recrystallized from EtOH–EtOAc–Et_2O to give 450 mg of 7 (HCl): mp 184–185 °C. Anal. ($C_{33}H_{36}NO_5Cl$) C, H, N.

1-(2′,4′,5′-Trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (HCl, 3). A solution of 400 mg (0.7 mmol) of 6 and 300 mg of 10% Pd/C in 200 mL of ethanol was hydrogenated on a Parr apparatus at 40 psi for 12 h. The reaction mixture was filtered, the solvent was removed in vacuo to a small volume and ether was added, and the resulting solid, 190 mg (71% yield), was collected. A small portion was recrystallized from ethanol–ether to give crystals, mp 256 °C. Anal. ($C_{19}H_{24}NO_5Cl$) C, H, N.

Biological Testing. Isolated Tracheal Strip Preparation.[5] Guinea pigs of either sex weighing 300–500 g were killed by a sharp blow on the head. The trachea of each animal was isolated and cleaned free of fatty tissue. From each guinea pig two spiral tracheal strips were prepared and mounted in a 12-mL jacketed muscle chamber containing a physiological solution maintained at 37 °C, through which a mixture of 95% O_2–5% CO_2 was bubbled. Drug-induced effects were recorded on a Grass polygraph (Model 7C) via a force-displacement transducer. Strips were allowed to equilibrate for 1–1.5 h before each experiment under a tension of 1 g. Carbachol (3×10^{-7} M) was used to increase the tone of each preparation, and cumulative dose–response curves were obtained for each drug. Individual plots of tracheal relaxation, expressed as a percent of the maximum relaxation obtained with 10^{-5} M (–)-isoproterenol added at the end of each experiment vs. log molar concentration of each drug, were prepared and the ED_{50} values determined individually. In all biological experiments, the ED_{50} values represent the concentration of each agonist required to produce a response equal to one-half of its maximal response in the appropriate system.

Isolated Right Atrial Preparation.[5] The atrium was dissected from extraneous tissue and placed in a 12-mL jacketed muscle bath. The atrium was allowed to equilibrate for a 1-h period in a physiological solution maintained at 37 °C, through which a mixture of 95% O_2–5% CO_2 was bubbled. The increase in atrial rate was recorded on a Grass polygraph (Model 7C) via a force-displacement transducer.

In each experiment, the atrium was exposed to a test dose of a drug and the atrial rate recorded during a 3-min period. Individual recordings were made at 1- and 3-min intervals. Cumulative dose–response curves were obtained for each analogue. The data were plotted on a log scale and the chronotropic responses expressed in terms of the maximum response obtained in the presence of 10^{-5} M (–)-isoproterenol added at the end of each experiment. ED_{50} values were determined from individual plots.

Platelet Aggregation. Human blood was taken by venipuncture from volunteers who reported being free of aspirin-containing medication for at least 14 days. Rabbit blood was collected by arterial puncture from the ear. The whole blood was combined and mixed with 3.8% trisodium citrate (9:1, v/v). Platelet-rich plasma was then prepared by centrifugation at 200*g* for 10 min at room temperature and used within 2 h of isolation. Platelet-poor plasma was obtained by centrifuging platelet-rich plasma for 10 min at 4000*g*. Platelet aggregation was monitored at 37 °C by nephelometry in a Chrono-log aggregometer (Model 330; Haverton, PA) with constant stirring at 1100 rpm. Platelets were incubated for 2 min at 37 °C prior to the initiation of aggregation. This time period also served as the incubation interval for modulators of the system. In all experiments, the minimal concentration of aggregating agent that produced irreversible aggregation was used.

Drugs. Stock solutions of arachidonic acid (NuChek Prep, Elysian, MN) were prepared in absolute ethanol and all other drugs in 0.05 M potassium phosphate buffer, pH 7.4, containing 0.05% metabisulfite.

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Central Nervous System Activity of 7-Substituted 1-Azaphenoxathiin Analogues and Their Oxidation Products

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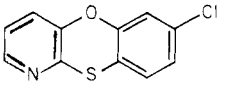
A number of 7-substituted 1-azaphenoxathiins and their sulfone oxidation products have been synthesized and screened for central nervous system activity. Some of the compounds have antidepressant activity, with the most active, 7-(trifluoromethyl)-1-azaphenoxathiin 10,10-dioxide (8), having similar potency to imipramine.

Recently, Martin et al.[1-6] reported the synthesis and central nervous system (CNS) depressant activity of 7-chloro-1-azaphenoxathiin (1) and related compounds. The



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claim of phenothiazine-like antipsychotic activity for 1 was made on the basis of loss of spontaneous motor activity and drug-induced hypothermia in mice.[1,5] We report here our studies on this interesting ring system and the results obtained in five biological screens designed to uncover CNS activity.

Scheme I

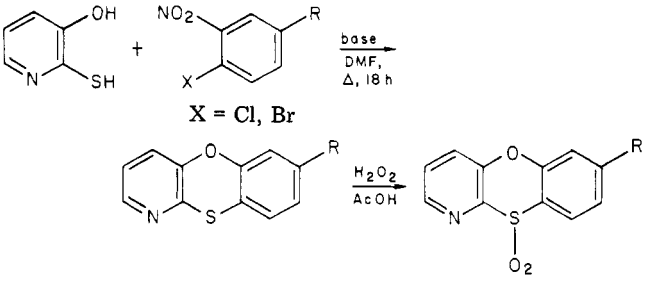

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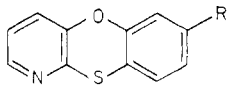
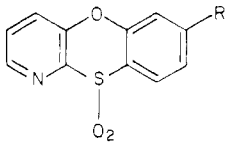
Table I



no.	R	base[a]	solv[b]	mp, °C	yield, %	emp formula	anal.[c]	TBZ[d]	muricide[e]
1	Cl	NEt_3	EtOH	128-129[f]	40	$C_{11}H_6NOSCl$	C, H, N	>30	>10
2	CF_3	NEt_3	95% EtOH	87-88	54	$C_{12}H_6NOSF_3$	C, H, N	16	>10
3	NO_2	NEt_3	EtOH	163[g]	32	$C_{11}H_6N_2O_3S$	C, H, N	>30	7
4	SO_2NH_2	NEt_3	EtOH	219-222	2	$C_{22}H_8N_2O_3S_2$	C, H, N	>30	8
5	H	NaH	hexane	69-70[h]	19	$C_{11}H_7NOS$	C, H, N	8	>10
6	Br	NEt_3	EtOH	155-156	37	$C_{22}H_6NOSBr$	C, H, N	>30	>10
7	OCH_3	NaH	hexane	75-76	22	$C_{12}H_9NO_2S$	C, H, N	>30	>10

[a] Base used for cyclization. [b] Crystallization solvent. [c] Analytical results were within ±0.4% of theoretical. [d] ED_{50}, mg/kg po (mouse), to reverse tetrabenazine ptosis. [e] ED_{50}, mg/kg ip (rat), to block muricidal behavior. [f] Lit. (ref 1) mp 122-124 °C. [g] Lit. (ref 1) mp 166-167 °C. [h] Lit. (ref 2) mp 66-68 °C.

Table II



no.	R	solv[a]	mp, °C	yield, %	emp formula	anal.[c]	TBZ[c]	muricide[c]
8	CF_3	EtOH	180-181	33	$C_{12}H_6NO_3SF_3$	C, H, N	1	17
9	Cl	EtOAc	201-202	63	$C_{11}H_6NO_3SCl$	C, H, N	18	>10
10	NO_2	HOAc	229-231	69	$C_{11}H_6N_2O_5S$	C, H, N	10	12
11	NHOH	EtOH[b]	>240	46	$C_{11}H_8N_2O_3S$	C, H, N	2	>10
12	H	EtOH/EtOAc	223-224	51	$C_{11}H_7NO_3S$	C, H, N	12	>10
13	Br	EtOH/EtOAc	231-233	28	$C_{11}H_6NO_3SBr$	C, H, N	15	7
14	OCH_3	HOAc	275-277	31	$C_{12}H_9NO_4S$	C, H, N	>30	>10
imipramine							2	20

[a] Crystallization solvent. [b] Obtained from **10** by hydrogenation (5% Pd) in acetic acid, followed by evaporation and trituration with ethanol. [c] See footnote *c*, Table I.

Chemistry. The 7-substituted 1-azaphenoxathiins were prepared by a modification of the reported[1] method. The general reaction is shown in Scheme I.

In systems activated toward cyclization (e.g., R = CF_3), the base used was triethylamine. Where no activation was present (e.g., R = OCH_3), 2 equiv of NaH was used. Details are given in Table I.

In the earlier work,[1] proof that the substituent was at the 7 position (no Smiles rearrangement) was made by ^{13}C NMR. In an attempt to prove this by ^1H NMR, the azaphenoxathiins were oxidized to the sulfones using H_2O_2 in refluxing acetic acid (Table II). In these sulfones, the proton adjacent to the SO_2 group (9 proton) is shifted considerably downfield and appears as a doublet with ortho coupling (J = 8 Hz). This confirms the earlier proof that the 8 position is unoccupied.

Pharmacology. Compounds 1–14 were screened for activity as follows: antagonism of methamphetamine aggregate toxicity (MAT), acetic acid induced writhing, pentylenetetrazole-induced convulsions and tetrabenazine (TBZ) induced ptosis in mice, and inhibition of muricidal activity in rats.

Discussion

In general, the compounds exhibited only weak CNS activity. None of the compounds was active in the MAT, writhing, or pentylenetetrazole tests (ED_{50} > 30 mg/kg). The lack of activity in MAT at this high dose makes it unlikely that these compounds have significant phenothiazine-like neuroleptic activity. Several of the compounds blocked TBZ-induced ptosis in mice and/or muricidal behavior in rats, indicating antidepressant potential (Tables I and II).

The sulfones appeared to have antidepressant potential when R was electron withdrawing, and 8 showed activity of the same order as imipramine. This activity seemed unusual in a compound of this structural type, and 8 was investigated in more detail.

At concentrations of 10^{-4} to 10^{-7} M, 8 did not block norepinephrine or serotonin uptake in rat synaptosomal preparations. Since antihistamines can give false positives in TBZ and muricide tests[7], 8 was submitted for antihistamine screening and was inactive.

In an acute behavioral and toxicity study, mice were treated orally with 8 at doses from 10 to 300 mg/kg. At all doses, a slight decrease in motor activity was observed and slight ptosis was seen at 100 mg/kg and higher. There were no lethalities after 24 h. However, in a 5-day chronic toxicity study in rats treated po with 50 mg/kg, severe weight loss and toxicity was observed. This precluded the further development of 8.

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian CFT-20 spectrometer, IR spectra were recorded on a Perkin-Elmer 221 spectrophotometer, and mass spectra were determined with a Varian MAT CH5. Microanalyses were performed by the Physical Analytical Services Department of the Schering-Plough Corp.

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2-Mercapto-3-pyridinol and the substituted nitrohalobenzenes were obtained commerically, with one exception. 4-Chloro-3-nitrobenzenesulfonamide was obtained from 4-chloro-3-nitrobenzenesulfonyl chloride (Aldrich) by reaction with concentrated aqueous ammonia. The following examples illustrate the general methods.

7-(Trifluoromethyl)-1-azaphenoxathiin (2). Triethylamine (50 mL) was added to a stirred mixture of 2-mercapto-3-pyridinol (12.7 g 0.1 mol), 4-chloro-3-nitrobenzotrifluoride (22.56 g, 0.1 mol), and dry DMF (200 mL), and the mixture was boiled under reflux for 18 h. The cooled mixture was poured into H_2O (700 mL) and extracted with Et_2O (2 × 600 mL). The combined extracts were washed with H_2O, dried ($MgSO_4$), and evaporated in vacuo. The residue was crystallized from 95% EtOH as pale yellow needles: mp 87–88 °C; IR (Nujol) 1610, 1570, 1500 cm^{-1}; NMR ($CDCl_3$) δ 8.35 (dd, J = 4.5 and 1.5 Hz, H-2), 7.17 (m, 5 H); MS M^+ 269 (100%). Anal. ($C_{12}H_6NOSF_3$) C, H, N.

7-Methoxy-1-azaphenoxathiin (7). 2-Mercapto-3-pyridinol (12.7 g, 0.1 mol) in dry DMF (50 mL) was added dropwise to a suspension of NaH (4.8 g, 0.2 mol) in DMF (200 mL) at 5 °C. 4-Chloro-3-nitroanisole (18.8 g, 0.1 mol) was added, and the mixture was stirred and heated under reflux for 18 h. The cooled mixture was poured into H_2O (900 mL) and extracted with Et_2O (2 × 600 mL). The combined extracts were washed with H_2O, dried ($MgSO_4$), and evaporated. The residual brown oil solidified overnight and was crystallized twice from hexane (charcoal) to give the product as fawn needles: mp 75–76 °C; NMR (Me_2SO-d_6) δ 8.38 (dd, J = 4.5 and 1.5 Hz, H-2), 7.5–6.8 (m, 5 H), 3.78 (s, 3 H); MS M^+ 231 (100%). Anal. ($C_{12}H_9NO_2S$) C, H, N.

7-(Trifluoromethyl)-1-azaphenoxathiin 10,10-Dioxide (8). Compound **2** (2.69 g, 0.01 mol) was added to a mixture of HOAc (50 mL) and 12% H_2O_2 (10 mL), and the mixture was stirred and boiled under reflux for 15 min. The reaction mixture was diluted with H_2O (50 mL) and allowed to cool. The colorless product was collected by filtration and crystallized from EtOH as needles: mp 180–181 °C; IR (Nujol) 1580, 1560 cm^{-1}; NMR (Me_2SO-d_6) δ 8.74 (dd, J = 4.5 and 1.5 Hz, H-2), 8.35 (d, J = 8 Hz, 1 H), 8.0 (m, 4 H); MS M^+ 301 (100%). Anal. ($C_{12}H_6NO_3SF_3$) C, H, N.

Pharmacological Methods. Compounds were administered either intraperitoneally (ip) or orally (po) to male, albino CFI mice (18–22 g), male Charles River (CD) rats (200–300 g, behavioral and toxicity studies), or male Long–Evans rats (200–300 g, muricide) in a methylcellulose suspension. The initial screening dose was 30 mg/kg, except for muricide (10 or 20 mg/kg).

(a) Antagonism of Methamphetamine Aggregate Toxicity.[10] Groups of 10 mice were kept in a plastic cage (11 × 26 ×13 cm) and treated with test drug po 30 min prior to ip administration of 15 mg/kg methamphetamine hydrochloride. ED_{50}, the dose required to protect 50% of the animals from death, was determined from mortality 4 h after methamphetamine injection.

(b) Reversal of Tetrabenazine-Induced Ptosis.[11] Groups of five mice were treated po with test drug 30 min prior to ip administration of 30 mg/kg tetrabenazine methanesulfonate. The size of the palpebral opening was evaluated at 30 min. The ED_{50} represents the dose required for half the mice to show a 50% increase in size of palpebral opening with respect to control.

(c) Inhibition of Muricidal Behavior.[12] Test drug was administered ip to groups of five rats, and mice were presented at 30 min. The ED_{50} reflects the dose at which half the rats failed to kill.

(d) Antagonism of Pentylenetetrazole-Induced Convulsions.[13] Groups of five mice were treated po with test drug 30 min before ip treatment with 150 mg/kg of pentylenetetrazole. Animals were observed for convulsions for 60 min. The ED_{50} reflects a 50% decrease in the number of animals convulsing.

(e) Antagonism of Acetic Acid Writhing.[14,15] Groups of five mice were treated po with test drug 15 min before ip dosing with 10 mL of 0.6% aqueous HOAc solution. The animals were observed for writhing behavior for a 10-min period beginning 3 min after treatment with HOAc. An animal was considered protected if the number of writhes was decreased 50% or more from control. The ED_{50} is expressed as the dose required to protect 50% of the treated mice.

(f) Biochemical Studies. The method of Hendley[9] was used.

(g) Behavioral and Acute Toxicity Studies. The test drugs were evaluated by the method of Irwin.[8] Groups of three mice were treated with test drug at 10, 30, 100, and 300 mg/kg and observed in comparison with controls 60 min later. Delayed toxicity was assessed at 24 h.

Acknowledgment. We thank Dr. D. C. Clody for helpful discussions and Dr. F. H. Leitz for the biochemical measurements.

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Inhibition of Histamine-Induced Gastric Secretion by Flavone-6-carboxylic Acids[1]

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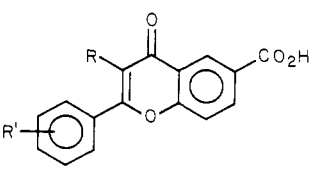
Twenty-five flavone-6-carboxylic acids were synthesized and tested as to their ability to inhibit histamine-induced gastric acid secretion in the rat. 3-Isopropoxy-4′-methoxyflavone-6-carboxylic acid (**41**) showed consistent oral activity while being devoid of any other noteworthy pharmacological effects. In vitro, this compound was found to be inactive as a histamine H_2 antagonist, and its mode of action remains unknown.

During the course of our investigation into the antiallergic activity of xanthone-2-carboxylic acids,[2,3] we became interested in substituted flavone-6-carboxylic acids (**1**), in



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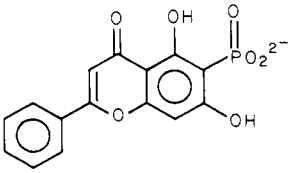

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which the relative positions of the pyrone carbonyl and the carboxy group would be the same as in xanthone-2-

(1) Contribution No. 533 from the Institute of Organic Chemistry, Syntex Research.

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