6 h, with a solid separating after a short time. The yellow solid was collected and washed with a small amount of DMF and then with ethanol, yielding 225 mg (70%) of **10a**: mp 250–254 °C; TLC ($CHCl_3$–MeOH, 50:1) 0.8. A small amount was recrystallized from DMF to give an analytically pure sample, mp 251–256 °C. Anal. ($C_{45}H_{34}N_6O_{10}\cdot H_2O$) C, H, N.

1,7-Bis[3-(3,8-diamino-5-methylphenanthridinium-6-yl)-phenoxy]heptane Dibromide (1). To a solution of 964 mg (1.17 mmol) of **10a** (dried iv, 24 h) in 20 mL of freshly distilled DMF, at 150 °C under N_2, was added 6 mL of Me_2SO_4, causing separation of a small amount of yellow solid, which quickly dissolved; heating under N_2 was continued for 2 h. The light brown solution was concentrated to a syrup, which was dissolved in 25 mL of hot ethanol; upon addition of 50 mL of H_2O, an orange-red oil separated and then solidified. This material was separated and extracted several times with a total of 300 mL of boiling ethanol. The filtered ethanol solution was concentrated to about one-third its volume and cooled with separation of 716 mg of an orange-red solid. Further concentration of the mother liquors yielded 210 mg of solid.

Without further characterization, this material was subjected to reduction of the nitro groups; 485 mg was mixed with 32 mL of 60% ethanol, 0.2 mL of 48% HBr, and 500 mg of Fe powder. The mixture was heated under reflux for 1.5 h and then filtered hot. The pH of the dark red filtrate was adjusted to 8.5 with NH_4OH. After the solution was filtered through Celite, concentrated to about 10 mL, and chilled overnight, a dark red solid product, 100 mg, separated. Retreatment of the solids that had been collected by filtration from the reduction reaction mixture, by mixing with aqueous ethanol, HBr, and Fe, refluxing for 3 h, and working up as previously described, yielded an additional 126 mg of product. These combined materials were purified on a Sephadex LH-20 column (2.5 × 30 cm) using methanol solvent, collecting 2-mL fractions. Examination by TLC showed that the initial fractions contained largely impurities; subsequent fractions, still slightly impure, were combined and repurified on the same column. Concentration of like fractions yielded a slightly sticky residue that solidified on trituration with acetone, yielding 235 mg of 1 (43%, based on **10a**) of dark red amorphous solid: mp 214–221 °C (?); TLC (MeOH–0.1 N HCl, 10:1) R_f 0.38; TLC (BuOH–HOAc–H_2O, 4:1:1) R_f 0.18, bright orange-red fluorescent spot under UV light; NMR (CD_3OD) δ 8.40 (t, 4, H_1, H_{10}), 7.0–7.8 (m, 14, Ar protons), 6.58 (d, 2, H_7), 4.0–4.15 (s + m, 10, *N*-Me, OCH_2), 1.3–1.9 [m, 10, $(CH_2)_5$]. Anal. ($C_{47}H_{48}N_6O_2Br_2\cdot 2H_2O$) C, H, N, Br.

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Anticoccidial Derivatives of 6-Azauracil. 4. A 1000-fold Enhancement of Potency by Phenyl Sulfide and Phenyl Sulfone Side Chains[1]

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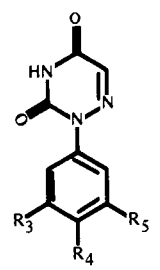
We report further progress in exploiting our earlier discovery that the anticoccidial activity of 6-azauracil increases markedly when appropriately substituted benzyl or phenyl groups are attached at N-1. With guidance from previous structure–activity relationships and a multiple linear regression analysis, 6-azauracils containing phenyl sulfone or phenyl sulfide side chains were prepared. These prevented a broad spectrum of coccidial infections in chickens at minimum inhibitory concentrations by weight in feed as low as 0.25 ppm, a 4000-fold increase in potency over 6-azauracil, and had shorter plasma half-lives than earlier potent analogues. Sulfides were more potent than sulfones, although they were oxidized rapidly to sulfones in vivo.

This paper is part of a series of publications on anticoccidial derivatives of 6-azauracil. Earlier papers in the series described how the anticoccidial activity of 6-azauracil can be markedly increased by attaching at the "ribosylation position", N-1, certain meta-substituted benzyl or phenyl groups.[2-4] The lower potencies of **2–6** relative to **1** seemed to indicate that either electron-donating or electronegative groups at the para position in the side chain had an undesirable effect on activity. On the other hand, it was recognized very early that activity was enhanced by a strong para-oriented, exocyclic-vectored dipole (as in **1**), which increases the acidity of the imide hydrogen,[2] and **7** was equipotent with **1**, suggesting that further variations in the physical–chemical properties of R_4 should be tried.



	R_3	R_4	R_5
1	Cl	H	Cl
2	Cl	CH_3	Cl
3	Cl	OCH_3	Cl
4	Cl	SCH_3	Cl
5	H	OC_6H_5	H
6	Cl	SO_2CH_3	Cl
7	Cl	Cl	Cl
8	H	COC_6H_5	H

The difficulty of correlating the group electropolarity (σ), group lipophilicity (π), and group dipole moment (μ)

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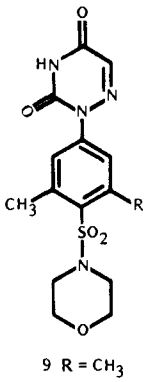
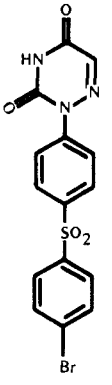
contributions among the many analogues with their anticoccidial activities called for a multiple linear regression analysis. The analysis was carried out by the Hansch method, using his compiled group values for π.[5] The Hammett group σ values[6] were used, and group dipole moment data were extracted from McClellan's tables.[7] Although the resulting equation accounted for only about 50% of the variance, it suggested that the lipophilicity was an important factor and that the optimum value for π was around 1.1; significant or not, this value served as a useful guide for the design of additional analogues.

The linear regression analysis still had to be related to our experience with steric requirements. While it had been shown repeatedly that chloro and methyl substituents were potency enhancing when introduced into the meta positions of phenyl side chains, bulkier meta substituents did not appear to be compatible with activity. On the other hand, we found that 8—containing a bulky *p*-benzoyl substituent—although devoid of the meta substituents usually regarded as requisite for potency, retained a considerable degree of activity (60 ppm). Furthermore, the *p*-(morpholinylsulfonyl) analogues **9** and **10** were highly active,[4] with MECs in feed in the range of 10–15 ppm, again suggesting not only that much larger substituents were tolerated in the para than in the meta positions, but also that a second ring in the side chain might, in fact, help to boost activity.



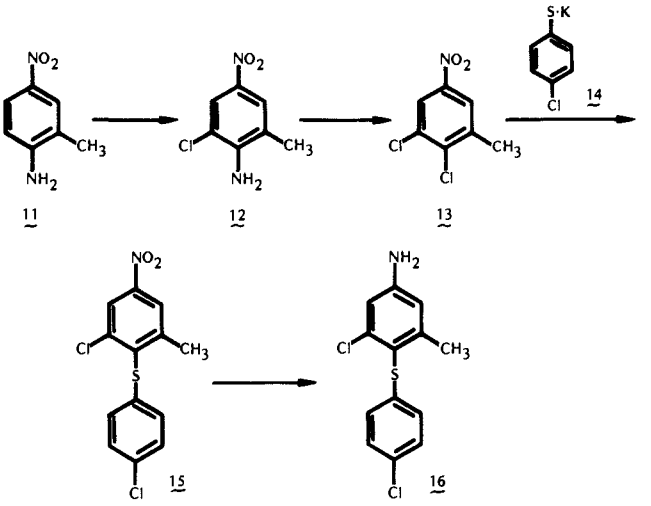
9 R = CH_3
10 R = Cl



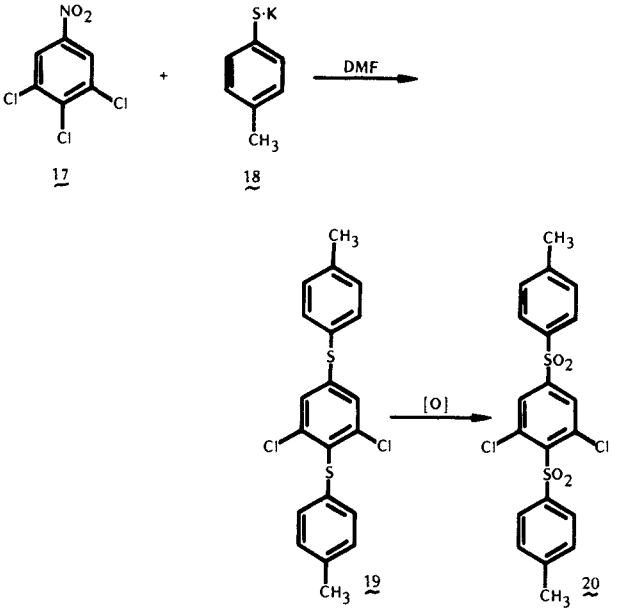
48

Such considerations led to the design of **48**. This compound lacked the desirable meta substituents in the central ring, but it was easily synthesized from a commercially available intermediate. While not a sulfonamide, the sulfone incorporated the electron-withdrawing character of the sulfonamide, together with more lipophilicity. The additional *p*-bromophenyl group was reminiscent of the *p*-chlorophenyl groups which are features of several antimalarial drugs, e.g., cycloguanil and pyrimethamine. Structure–activity overlaps in the antimalarial and anti-

Scheme I



Scheme II



coccidial areas are well-known, and both infections are caused by evolutionarily related intracellular protozoan parasites.

Compound **48**, the outgrowth of our analysis, was a potent anticoccidial in chickens, with an MEC (*Eimeria tenella*) of 8 ppm. Moreover, this potency was maintained across a broad spectrum of *Eimeria* species pathogenic to broilers, in contrast to the sulfonmorpholides,[4] which were less potent against other coccidial species than against *E. tenella*. This test result prompted the synthesis of a series of 6-azauracils substituted at the 1 position by phenyl sulfone, phenyl sulfoxide and diphenyl sulfide side chains.

Chemistry. The complex aniline side-chain precursors were prepared in various ways. In several instances, a route through a 4-chloronitrobenzene was used. The activated halogen was displaced by a thiophenol, the resulting sulfide was adjusted to the desired oxidation state (this adjustment could be done also after formation of the 6-azauracil), and the nitro group was reduced to the amine (Scheme I).

In one instance, a double displacement was noticed when proper care was not given to the order of reagent addition. The displacement of aromatic nitro groups in high dipole moment solvents has been reviewed[8-10] (Scheme II).

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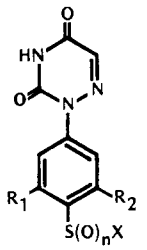
Table I. 2-[4-(Phenylthio)phenyl]-*as*-triazine-3,5(2*H*,4*H*)-diones (1-[4-(Phenylthio)phenyl]-6-azauracils), Related Structures, and Their Anticoccidial Activities[a]



no.	R_1	R_2	*n*	X	mol formula	pH $^1/_2$[b]	mp, °C	MEC,[c] ppm, in feed	plasma half-life, h
4	Cl	Cl	0	CH_3	$C_{10}H_7Cl_2N_3O_2S$		182-185	30	
6	Cl	Cl	2	CH_3	$C_{10}H_7Cl_2N_3O_4S$		235-237	>125[d]	
25	Cl	Cl	1	CH_3	$C_{10}H_7Cl_2N_3O_3S$		288-290	>125	
26	H	H	2	$CH_2C_6H_5$	$C_{16}H_{13}N_3O_4S$		257-259	>125	
27	H	H	2	$CH(CH_3)C_6H_5$	$C_{17}H_{15}N_3O_4S$	6.84[e]	214-216	>125	
28	H	H	2	$CH_2C_6H_4$-*p*-Cl	$C_{16}H_{12}ClN_3O_4S$	7.64	261-263	250	
29	H	H	0	C_6H_4-*p*-Cl	$C_{15}H_{10}ClN_3O_2S$	7.84[f]	195-197	4	
30	H	H	0	C_6H_4-*p*-NO_2	$C_{15}H_{10}N_4O_4S$	7.50	186-188	30	
31	Cl	H	0	C_6H_4-*p*-Br	$C_{15}H_9BrClN_3O_2S$		190-191	2	
32	Cl	H	0	C_6H_4-*p*-Cl	$C_{15}H_9Cl_2N_3O_2S$	7.08	177-179	2	38B[g]
33	Cl	H	0	C_6H_4-*p*-*t*-Bu	$C_{19}H_{18}ClN_3O_2S$		166-168	>60	
34	Me	H	0	C_6H_4-*p*-Cl	$C_{16}H_{12}ClN_3O_2S$		149-151	2	
35	NO_2	H	0	C_6H_4-*p*-Cl	$C_{15}H_9ClN_4O_4S$	8.11[f]	243-245	60	
36	Br	Br	0	C_6H_4-*p*-NO_2	$C_{15}H_8Br_2N_4O_4S$		205-207	8	
37	Cl	Cl	0	C_6H_5	$C_{15}H_9Cl_2N_3O_2S$		136-139	8	
38	Cl	Cl	0	C_6H_4-*p*-Cl	$C_{15}H_8Cl_3N_3O_2S$	7.93	177-179	0.25	47B
39	Cl	Cl	0	C_6H_4-*p*-(SO_2-4-morph)	$C_{19}H_{16}Cl_2N_4O_5S_2$[h]		294-296	>60	
40	Cl	Me	0	C_6H_4-*p*-Cl	$C_{16}H_{11}Cl_2N_3O_2S$	7.64	148-150	0.25	
41	Me	Me	0	C_6H_4-*p*-Cl	$C_{17}H_{14}ClN_3O_2S$	7.74	139-140	0.5	16B
42	Me	Me	0	C_6H_4-*p*-I	$C_{17}H_{14}IN_3O_2S$		161-163	15	
43	Me	Et	0	C_6H_4-*p*-Cl	$C_{18}H_{16}ClN_3O_2S$[h]		90-95	60	
44	Cl	H	0	2-naphthyl	$C_{19}H_{12}ClN_3O_2S$		159-161	125	
45	Me	H	1	C_6H_4-*p*-Cl	$C_{16}H_{12}ClN_3O_3S$	7.71[e]	155-157	4	
46	Me	Me	1	C_6H_4-*p*-Cl	$C_{17}H_{14}ClN_3O_3S$	7.67[e]	153-155	1	
47	H	H	2	C_6H_5	$C_{15}H_{11}N_3O_4S$	7.56[f]	300-305	30	
48	H	H	2	C_6H_4-*p*-Br	$C_{15}H_{10}BrN_3O_4S$	7.76[f]	284-287	8	19C
49	H	H	2	C_6H_4-*p*-Cl	$C_{15}H_{10}ClN_3O_4S$	7.53	272-274	4	
50	H	H	2	C_6H_4-*o*-Me	$C_{16}H_{13}N_3O_4S$	7.26	268-270	>125	
51	H	H	2	C_6H_4-*p*-Me	$C_{16}H_{13}N_3O_4S$	7.94[f]	295	250	
52	H	H	2	C_6H_4-*p*-*t*-Bu	$C_{19}H_{19}N_3O_4S$		321-325	>250	
53	H	H	2	C_6H_4-*p*-NO_2	$C_{15}H_{10}N_4O_6S$	6.76[e]	230-235	>250	
54	Cl	H	2	C_6H_4-*p*-Cl	$C_{15}H_9Cl_2N_3O_4S$	7.80	253-255	2	
55	Me	H	2	C_6H_4-*p*-Br	$C_{16}H_{12}BrN_3O_4S$	7.23	250-251	15	
56	Me	H	2	C_6H_4-*p*-Cl	$C_{16}H_{12}ClN_3O_4S$	7.15	230-231	2	10C, 14.5B
57	MeO	H	2	C_6H_4-*p*-Cl	$C_{16}H_{12}ClN_3O_5S$		268-270	>125	
58	Cl	Cl	2	C_6H_4-*p*-Cl	$C_{15}H_8Cl_3N_3O_4S$		269.5-271	0.5	
59	Cl	Me	2	C_6H_4-*p*-Cl	$C_{16}H_{11}Cl_2N_3O_4S$		237-239	15	26B
60	Me	Me	2	C_6H_4-*p*-Br	$C_{17}H_{14}BrN_3O_4S$		186-190	NT	
61	Me	Me	2	C_6H_4-*p*-Cl	$C_{17}H_{14}ClN_3O_4S$	8.25	192-193	1	

[a] Compound 35 was prepared from the (4-chloro-3-nitrophenyl)triazinedione (made by the modified Slouka method[3]) by displacement of the halogen with *p*-chlorothiophenol. The rest of the compounds were prepared from the appropriate *p*-(phenylthio)anilines. Compound 39 was prepared by chlorosulfonation of 37, followed by reaction with morpholine. [b] Because of their low solubility in water, pH $^1/_2$ determinations of the compounds were made in 2:1 DMF/H_2O, unless otherwise noted. [c] These values are the minimum amounts (parts per million by weight in feed mixtures) of drug required to prevent formation of detectable disease induced by experimental *Eimeria tenella* infections in chickens. [d] This value was incorrectly reported in ref 3. [e] Titrated in 1:1 DMF/H_2O. [f] Titrated in 3:1 DMF/H_2O. [g] The letter B indicates that the measurements were made in broiler chickens (6-8 weeks old); the letter C indicates that the measurements were made in cockerels or 2-week-old chickens. Sulfides 32, 38, and 41 are readily converted to the sulfones in vivo; half-life values are for the oxidized form. [h] Compound 39, monohydrate; 43, hemihydrate.

Conversion of the anilines to the 6-azauracils was based on the modified Slouka synthesis used in the earlier papers in this series. Use of the symmetrical reagent, malonyldiurethane, instead of cyanoacetylurethane improved yields in the coupling reaction with the diazonium salt. Combining this improvement with the sodium acetate-

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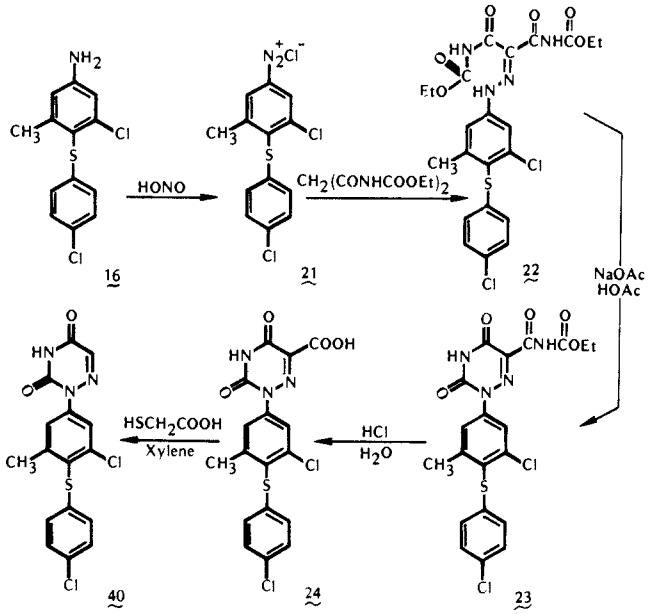
Scheme III



acetic acid cyclization method and the high-yield decarboxylation catalyzed by thioglycolic acid permitted the synthesis of **40** in 80% yield in a "one-pot" process without isolation of intermediates (Scheme III).

Results and Discussion

The derivatives of 6-azauracil prepared by these methods are listed in Table I. They were tested for their ability to protect chickens against infections caused by *E. tenella*. The minimum effective concentrations in feed (MECs) given in parts per million (ppm) were determined by methods designated in the earlier publications of this series.

As anticipated, addition of meta substituents to the central phenyl ring added an increment to potency. As found in the earlier series, methyl or chloro groups were best. Sulfides proved to be the best dosage oxidation state, perhaps because they were more lipophilic and better absorbed than the sulfones. However, they were rapidly oxidized to sulfones in vivo, and we speculate that the latter may be the better enzyme inhibitors. It has been reported that there is more through-conjugation in phenyl sulfones than in phenyl sulfides,[11-14] so that better molecular polarizability (and adaptability to the active site of the target enzyme) might be expected in the sulfones.

Substituents in the terminal benzene ring ortho to the sulfide bond reduced potency. This could be ascribed to steric intolerance, either of the substituent itself or of its deformation of the normal angular sulfide bond. The phenyl sulfide or phenyl sulfone group is a salient feature of drugs claimed to have activity against mycobacteria,[15] helminths,[16,17] plasmodia (malaria),[18-20] cancer,[21] viruses,[22] Marek's disease,[23] weeds,[24] bacteria,[25] mites,[26] and hyperlipemia–hypercholesterolemia.[27] Binding of groups of this kind to enzymes, and to proteins in general, usually is attributed to the "stacking" of benzene rings against like groups in the aromatic amino acids of the protein.[28,29] The binding of dapsone [bis(4-aminophenyl)sulfone] to serum albumen, for example, has been studied in some detail.[30] Conformations of phenyl sulfides and phenyl sulfones, as well as bridged diphenyls in general, have been studied,[31-36] and biological effects have in certain cases been shown to be conformation dependent.[37] Recently, 6-azauracils bearing tricyclic side chains, which are conformationally restrained and related structurally to this series, were reported to have considerable anticoccidial activity.[38]

In an earlier paper we had postulated that phenylazauracils may exert their anticoccidial action through inhibition of pyrimidine synthesis by binding the enzyme orotidylate decarboxylase in a manner similar to that of 6-azauridine.[3] The possibility that another enzyme target may also be involved is suggested by a recent report that 6-azauridine interferes with purine biosynthesis by inhibiting inosinate dehydrogenase.[39] A related example of interference with both purine and pyrimidine biosynthesis is found in the azapurine analogue, allopurinol, which in addition to being a purine antagonist has been shown to inhibit orotidylate decarboxylase,[40-45] probably as a false

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nucleotide. The behavior of the 6-azauridine-related structures of this series toward inosinate dehydrogenase is not known.

The most active anticoccidials of this series were **38**, **40**, and **41**, all with MECs for *E. tenella* below 1 ppm, compared with an MEC of about 1000 ppm for 6-azauracil. Of these, **41** was selected for further evaluation because it had a desirably moderate plasma half-life of 16 h in broiler age (6-week old) chickens. By contrast, **38** had a plasma half-life of 47 h, and potent 1-phenyl-6-azauracils[3] had shown half-lives as long as 160 h.

Compound **41** controlled all the major species of poultry coccidia at remarkably low concentrations and showed a wide range of safety when administered to broiler chicks for the full length of their usual 8-week growth period. During consumer safety studies, however, when **41** was administered to laboratory animals over long periods of time, even low doses elicited toxicological symptoms suggesting interferences with nucleic acid synthesis in higher species, reminiscent of the inhibition of RNA synthesis by 6-azauracil.[46]

Experimental Section

Melting points were determined on a calibrated Kofler hot stage microscope. Solvents used were analytical reagent grade and, where pertinent, were protected from water by storage over molecular sieves. Mass spectra were obtained with Hitachi Model RMU-6E. Nuclear magnetic resonance spectra were obtained for selected compounds with a Perkin-Elmer/Hitachi Model R-20. Thin-layer chromatography was performed with Uniplate (Analtech) precoated TLC plates (silica gel GF, 250 μm) in a variety of solvent systems.

General Method. Scheme I. 2-Chloro-6-methyl-4-nitroaniline (12). To a stirred solution of 76 g (0.50 mol) of 2-methyl-4-nitroaniline in 445 mL of concentrated HCl was added 1 kg of ice. Chlorine gas was introduced below the surface of the weighed reaction mixture until a constant weight was attained. The yellow solid present was collected by filtration and washed successively with H_2O, 50% ethanol, and H_2O. The crude product weighed 68.2 g (73%). It was recrystallized from acetonitrile to give pure product, mp 167 °C.[47]

2,3-Dichloro-5-nitrotoluene (13). To a stirred solution of 75 mL of concentrated H_2SO_4 was added 9.0 g (0.13 mol) of sodium nitrite in portions. The temperature rose to 70 °C, and then the solution was cooled to 20 °C and held at that level for the remainder of the experiment. In small portions, 22.4 g (0.12 mol) of **12** was added with stirring. After 10 min, 400 mL of acetic acid was added dropwise to the thick black slurry. After stirring 30 min longer, the mixture was poured into an equal volume of ice–water. Insoluble impurities were removed by filtration, and the cold diazonium solution was added dropwise to a stirred solution of 25 g (0.25 mol) of cuprous chloride in 300 mL of concentrated HCl at room temperature during 90 min. After the solution was stirred for about 24 h, the tan solid was collected by filtration, washed with water, and dried at 60 °C under vacuum to give 18.7 g (76%) of crude product, which melted at 85–85.5 °C after recrystallization from ethanol.[48]

3-Chloro-4-[(*p*-chlorophenyl)thio]-5-methylaniline (16). A solution of 3.3 g (0.05 mol) of 85% KOH pellets in 35 mL of absolute methanol was cooled in an ice bath. With stirring, 7.5 g (0.05 mol) of 4-chlorothiophenol was added in small portions during 20 min, and the mixture was stirred for another 20 min at room temperature. The solvent was removed at reduced pressure, and the residue was triturated with ether and filtered to give 9.1 g (0.05 mol) of potassium 4-chlorothiophenolate (**14**). A solution of this material in 50 mL of dimethylformamide was added dropwise to a cooled solution of 10.3 g (0.05 mol) of **13** in 50 mL of DMF. After stirring overnight, the mixture was chilled, and ice–water was added. The solid that separated was collected and washed with water to give 14.9 g of crude 3-chloro-4-[(*p*-chlorophenyl)thio]-5-methylnitrobenzene (**15**), mp 86–89 °C.

A solution of 45 g (0.2 mol) of stannous chloride dehydrate in 85 mL of concentrated HCl was added to 14.9 g (0.047 mol) of crude **15** in 85 mL of ethanol. The mixture was refluxed for 35 min on a steam bath and allowed to stand overnight at room temperature. The resulting solid was collected and slurried in a mixture of 300 mL of $CHCl_3$ and 100 mL of H_2O. The mixture was made alkaline with 10% NaOH solution and was filtered through cellulose. The organic phase was separated, washed with H_2O, treated with activated charcoal, and dried over Na_2SO_4. Removal of solvent at reduced pressure left 11.3 g (85%) of **16**, white crystals, mp 140–142 °C after recrystallization from ethanol–water.

2,6-Dichloro-1,4-bis(*p*-tolylsulfonyl)benzene (20). A solution of 24.3 g (0.15 mol) of potassium *p*-thiocresol (**18**) in 90 mL of DMF was cooled to 0–5 °C, and 33.9 g (0.15 mol) of 3,4,5-trichloronitrobenzene (**17**) was added in small portions with stirring during 35 min. The mixture was stirred for 1 h at room temperature, for 16 h at 50 °C, and for 1 h at 100 °C. It was next poured into 1 L of cold H_2O, and the product was extracted with $CHCl_3$. The $CHCl_3$ solution was washed successively with H_2O, 10% KOH, 10% Na_2SO_3, and H_2O. After treatment with activated charcoal, the solution was dried over Na_2SO_4. The solvent was removed at reduced pressure, and the resulting oil (35.8 g) was dissolved in 200 mL of acetic acid. After 150 mL of 30% H_2O_2 was added, the mixture was refluxed for 14 h. The solid which formed on cooling was collected and washed with H_2O.

It was slurried in hot ethanol and, after cooling, was collected by filtration and dissolved in hot acetonitrile. After charcoal treatment and cooling, 11.5 g of crystalline product (16.6%), mp 200–202 °C, was obtained. The structure was confirmed by NMR and by C, H, N, and S analyses.

2-[3,5-Dimethyl-4-[(*p*-chlorophenyl)thio]phenyl]-*as*-triazine-3,5(2*H*,4*H*)-dione (41). This and related structures could be prepared by the modified Slouka process used for preparing the triazines reported in the preceding papers of this series.

Oxidation of 41 to the Sulfone 61. A solution of 43.0 g (0.2 mol, corrected for purity) of 80% *m*-chloroperbenzoic acid in 100 mL of ethyl acetate was added dropwise, with stirring, to a solution of 36.0 g (0.1 mol) of **41** in 1150 mL of ethyl acetate, the rate of addition being adjusted to maintain a temperature of 50–55 °C. After the addition was completed, the mixture was stirred at ambient temperature for 30 min and then refluxed for 1 h. Enough additional ethyl acetate was added to dissolve the crystalline precipitate. The solution was washed with 10% sodium bisulfite solution and with saturated $NaHCO_3$ solution and then dried over $MgSO_4$. After filtering, the solution was concentrated to one-fourth volume and allowed to crystallize. The product was removed by filtration, washed with hexane, and vacuum-dried to yield 35.7 g (92%), mp 206–208 °C. The other sulfones and sulfoxides reported were prepared similarly.

Scheme III. 2-[3-Chloro-4-[(*p*-chlorophenyl)thio]-5-methylphenyl]-6-carboxy-*as*-triazine-3,5(2*H*,4H)-dione (24). A solution of 0.77 g (0.011 mol) of sodium nitrite in 2 mL of H_2O was added to a chilled (10 °C) slurry of 2.85 g (0.01 mol) of 3-chloro-4-[(*p*-chlorophenyl)thio]-5-methylaniline (**16**) in a mixture of 35 mL of glacial acetic acid and 28 mL of concentrated HCl, and the mixture was stirred for 15 min at 10 °C. After the addition of 2.05 g (0.025 mol) of sodium acetate and 2.7 g (0.011 mol) of malonyldiurethane,[49] the reaction mixture was allowed to warm to room temperature and stirred for 1 h. An additional 0.82 g

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(46) These included teratogenicity in the rat and rabbit and atrophy of the prostate gland and testicles in dogs detected in 90-day toxicology studies.
(47) W. P. Wynne and A. Greeves, *Chem. News*, **72**, 58 (1895).
(48) W. P. Wynne and A. Greeves, *Proc. Chem. Soc., London*, **no. 154** (1922); cf. "Beilsteins Handbuch der Organischen Chemie", 4th ed., Vol. 5, Springer-Verlag, Berlin, 1922, p 332.
(49) M. Conrad and A. Schulze, *Chem. Ber.*, **42**, 729 (1909), cf. also S. Basterfield, E. L. Woods, and M. S. Whelan, *J. Am. Chem. Soc.*, **49**, 2942 (1927).

(0.01 mol) of sodium acetate was added, and the mixture was refluxed for 2 h. A solution of 5 mL of concentrated H_2SO_4 in 5 mL of H_2O was added, and the mixture was refluxed for an additional 4 h. Water (50 mL) was added to the cooled mixture, and stirring was continued at room temperature for 90 min. The solid was collected and washed with H_2O. The wet cake was taken up in ethyl acetate and extracted twice with 2% $NaHCO_3$ solution. The extracts were washed with hexane, and the bicarbonate solution was adjusted to pH 2.0 with concentrated HCl. After the solution was stirred at room temperature for 1 h, the solid was collected and washed well with H_2O to give 4.2 g (95%) of **24**, mp 203–218 °C dec. Elemental analyses indicated that the product was the monohydrate. In large-scale runs, the decarboxylation step was accomplished without isolation of **24**, using heat and a catalyst, such as thioglycolic acid. A high-yield decarboxylation of **24** is described below.

2-[3-Chloro-4-[(*p*-chlorophenyl)thio]-5-methylphenyl]-*as*-triazine-3,5(2*H*,4*H*)-dione (40). A mixture of 10.6 g (0.025 mol) of **24** in 10 mL of 98% thioglycolic acid was stirred under N_2 at 140 °C for 3 h. The thioglycolic acid was removed at reduced pressure. The residue was quenched with H_2O, granulated for 30 min, filtered, washed with H_2O, and vacuum-dried. The yield of crude **40** was 9.5 g (100%). Recrystallization from glacial acetic acid with activated charcoal treatment, followed by pumping to constant weight, yielded 8.9 g of pale yellow crystals (93.6%), mp 148.5–150.5 °C. In some instances, a combination of a high-boiling solvent (e.g., xylene) and a catalytic amount of thioglycolic acid was used for the decarboxylation.

2-[3-Nitro-4-[(*p*-chlorophenyl)thio]phenyl]-*as*-triazine-3,5(2*H*,4*H*)-dione (35). To a stirred slurry of 132 mg (0.002 mol) of 85% KOH (crushed pellets) in 5 mL of dimethylformamide at 80 °C was added in one portion 419 mg (0.0016 mol) of 2-(3-nitro-4-chlorophenyl)-*as*-triazine-3,5(2*H*,4*H*)-dione. The mixture was stirred for 10 min, and 285 mg (0.0016 mol) of potassium *p*-chlorothiophenolate (**14**) added. The mixture was heated at 90–95 °C for 4.5 h and then was quenched in 200 mL of H_2O. After the mixture was acidified with 10 mL of concentrated HCl, the yellow solid was collected and washed with H_2O. The crude dry yield was 500 mg. Recrystallization from ethanol–acetonitrile with activated charcoal treatment yielded 324 mg (55%) of product, mp 243–245 °C.

3,5-Dibromo-4-[(*p*-nitrophenyl)thio]aniline (Precursor of 36). A solution of 13.5 g (0.07 mol) of stannous chloride dihydrate in 75 mL of concentrated HCl was added to a stirred suspension of 9.5 g (0.015 mol) of bis(2,6-dibromo-4-nitrophenyl) disulfide.[4] The mixture was refluxed for 3 h and then poured into cold water. The solid was collected and suspended in $CHCl_3$. Water was added, and the pH was adjusted to 11 with 40% NaOH solution. After it was washed several times with $CHCl_3$, the alkaline solution was acidified and the mixture was extracted with $CHCl_3$. Evaporation of solvent gave 4.5 g (53%) of crude bis(4-amino-2,6-dibromophenyl) disulfide. The product was combined with 40 mL of hypophosphorous acid and refluxed for 2 h. The mixture was cooled, and the solid that separated was collected and washed with H_2O to yield 4.4 g (89%) of crude 4-amino-2,6-dibromothiophenol.

The thiophenol (4.4 g, 0.015 mol) was added to a solution of 1.02 g (0.015 mol) of 85% KOH pellets in 25 mL of methanol. The mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure, and the residue was triturated with a little ethyl ether and removed by filtration to give 5.1 g of potassium 4-amino-2,6-dibromothiophenolate. This salt was dissolved in 20 mL of DMF and added to a stirred solution of 2.44 g (0.015 mol) of *p*-chloronitrobenzene in 20 mL of DMF under N_2. The mixture was stirred for 90 min at room temperature. Ice–water (40 mL) was added dropwise, and an oil separated and later solidified. Collected solids were washed with H_2O to give 5.2 g (83%) of 3,4-dibromo-4-[(*p*-nitrophenyl)thio]aniline. This product was not rigorously characterized but was used in the synthesis of **36**, which is well-characterized.

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Side-Chain Effects on Phenothiazine Cation Radical Reactions

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The cation radical of each of the phenothiazine tranquilizers is a likely intermediate in the metabolism of the drugs to at least two of the three major metabolic classes, the sulfoxides and the hydroxylated derivatives. Previous work has shown that the reactions of the radical are highly dependent on the environment, particularly the presence of nucleophiles. The present report discusses the effect of cation radical structure on the formation of sulfoxide and hydroxylated metabolites in vitro. Cyclic voltammetry, spectrophotometry, and liquid chromatography were used to examine reactions of various phenothiazine radicals in aqueous buffers. A radical with a three-carbon aliphatic side chain (e.g., chlorpromazine) forms solely sulfoxide and parent unless amine nucleophiles are present, in which case hydroxylation occurs. A shorter side chain (e.g., promethazine) causes radical dimerization and pronounced hydroxylation, regardless of external nucleophiles. A piperazine side chain (e.g., fluphenazine) promotes hydroxylation, with some sulfoxide observed. The results indicate that a deprotonated amine is necessary for hydroxylation and that the amine may be present in the original drug rather than an external nucleophile. In addition to information about cation radical reactions, the redox properties of several different phenothiazines are presented.

The metabolism of the phenothiazine major tranquilizers is complex and has been studied in detail in several animal species and in humans.[1-3] For chlorpromazine (**1**), at least 77 metabolites have been observed, with 35 of them being identified.[4] While the metabolic profile varies greatly with animal species and dosage regimen,[5] three major pathways are observed: formation of sulfoxide, hydroxylation of the ring, and degradation of the side chain on the 10 position of the phenothiazine nucleus. A phenothiazine cation radical is generally assumed to be a metabolic intermediate in the formation of sulfoxide and hydroxylated products in vivo,[1,2] and work from this laboratory has demonstrated that these metabolites can be formed from cation radical reactions in aqueous buffers.[6,7] It was shown that the

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