2,4-Diamino-6-[(aralkyl and alicyclic)thio]quinazolines VIII (10–16, Table III). Procedure IV. A mixture of 2.3 g (0.01 mol) of 2-amino-5-(cyclohexylthio)benzonitrile (**6**) and 1.7 g (0.015 mol) of chloroformamidine hydrochloride[8] in 5 mL of dry diglyme was stirred and heated in an oil bath at 150 °C (bath temperature) for 0.5 h. During this time hydrogen chloride was evolved, a solution formed, and a new solid precipitated. The mixture was cooled and the solid was collected, washed with ether, and dried. It was recrystallized once from 90% aqueous ethanol containing an excess of ammonium hydroxide and then reprecipitated from 95% ethanol containing 0.5 mL of 2 N sodium hydroxide by addition of water. The pale yellow crystals were collected and dried to give 2.0 g (71%) of 2,4-diamino-6-(cyclohexylthio)-quinazoline (**10**), mp 190–192 °C with preliminary softening.

Procedure V. A mixture of 1.0 g (0.0032 mol) of 6-chloro-5-[(*p*-chlorobenzyl)thio]anthranilonitrile (**7**), 0.75 g (0.0065 mol) of chloroformamidine hydrochloride,[8] and 4.0 g of dimethyl sulfone was heated for 1 h in an oil bath that had been preheated to 160 °C. The dark solution was poured into water and the resulting cloudy solution was warmed on the steam bath and made basic with 50% sodium hydroxide. The precipitate that formed was collected, washed with water, and recrystallized from *N,N*-dimethylformamide–water to give 0.86 g (77%) of 2,4-diamino-5-chloro-6-[(*p*-chlorobenzyl)thio]quinazoline (**11**), mp 236–240 °C.

2,4-Diamino-6-[(benzyl)sulfinyl- and sulfonyl]quinazolines IX and X (17–21, Table IV). Procedure VI. A mixture of 0.64 g (0.0018 mol) of 2,4-diamino-5-chloro-6-[(*p*-chlorobenzyl)thio]quinazoline (**11**), 4.4 mL of 30% hydrogen peroxide, and 8 mL of glacial acetic acid was stirred at room temperature for 4 h, and the resulting solution was poured into a mixture of ice and 12 mL of 50% sodium hydroxide. The precipitate that formed was collected, washed with water, and recrystallized from *N,N*-dimethylformamide–water to give 0.37 g (55%) of 2,4-diamino-5-chloro-6-[(*p*-chlorobenzyl)sulfinyl]quinazoline (**17**), mp 256–258 °C dec.

Procedure VII. A mixture of 1.0 g (0.0028 mol) of 2,4-diamino-5-chloro-6-[(*p*-chlorobenzyl)thio]quinazoline (**11**), 8 mL of 30% hydrogen peroxide, and 15 mL of glacial acetic acid was stirred at room temperature for 48 h, and the resulting solution was poured into a mixture of ice and 23 mL of 50% aqueous sodium hydroxide. The precipitate that formed was collected, washed with water, and combined with 0.41 g of crude product which had been obtained in a similar manner from 0.5 g (0.0014 mol) of starting material. Recrystallization from *N,N*-dimethylformamide–H_2O and drying at 100 °C gave 0.86 g (42%) of 2,4-diamino-5-chloro-6-[(*p*-chlorobenzyl)sulfonyl]quinazoline (**18**), mp 274–276 °C.

Procedure VIII. A mixture of 3.2 g (0.01 mol) of 2,4-diamino-6-[(*p*-chlorobenzyl)thio]quinazoline (**12**) and 2.3 g (0.0053 mol) of the bromine complex of 1,4-diazabicyclo[2.2.2]octane[9] in 100 mL of 70% aqueous acetic acid was stirred at room temperature for 18 h. The mixture was poured into a stirred ice–water mixture containing 67 mL of 50% aqueous sodium hydroxide. The pale yellow solid which precipitated was collected and dried in vacuo. Recrystallization from ethanol followed by drying in vacuo (50 °C) yielded 2.5 g (74%) of 2,4-diamino-6-[(*p*-chlorobenzyl)sulfinyl]quinazoline (**19**), mp 234–236 °C. The infrared spectrum displayed sulfoxide absorption at 1040 cm^{-1}.

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References and Notes

(1) This is communication 40 of a series on antimalarial drugs. For paper 39, which is also the previous paper on folate antagonists, see L. M. Werbel, J. Johnson, E. F. Elslager, and D. F. Worth, *J. Med. Chem.*, **21**, 337 (1978).
(2) This investigation was supported in part by U.S. Army Medical Research and Development Command Contracts DA-49-193-MD-2754 and DADA-17-72-C-2077. This is Contribution No. 1481 to the Army Research Program on Malaria.
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(10) The parenteral antimalarial screening was carried out by Dr. Leo Rane of the University of Miami, and test results were supplied through the courtesy of Dr. David P. Jacobus, Dr. T. R. Sweeney, and Dr. E. A. Steck of the Walter Reed Army Institute of Research.
(11) For a description of the test method, see T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).
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(15) Eastman Organic Chemicals, Rochester, N.Y.
(16) British Patent 1 020 058.
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A New Class of Antimalarial Drugs: Derivatives of Benzothiopyrans[1]

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A series of substituted benzothiopyrans was synthesized and examined for antimalarial activity. Some were found to be active and curative at dose levels of 160–360 mg/kg against *Plasmodium berghei* in mice. A few observations concerning structure–activity relationships were made. The benzothiopyrans were prepared by treatment of either the *gem*-dichloro- or the thionothioflavone intermediate with various primary amines. The thionothioflavone intermediates were made from thioflavones. Condensation of thiophenols with benzoyl acetates gave the thioflavones.

We wish to report some derivatives of benzothiopyrans as a new class of antimalarial drugs[3] that are active in mice (against *Plasmodium berghei*) and chicks (against *Plasmodium gallinaceum*) in the Rane screen.[4] Some of these benzothiopyrans were curative at dose levels of 160–360 mg/kg in mice; a limited study of structure modification, however, did not result in compounds with any greater potency. In the same test, some of the most active known

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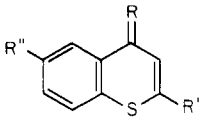
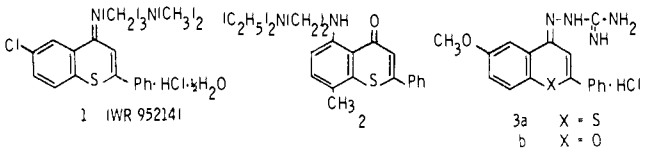
Table I. Thio- and Thionothioflavones 6 and 7



Compd	R	R'	R''	Mp, °C	Yield, %	Purifn solvent	Formula	Analyses
6a	O	C_6H_5	Cl	188-189[a]	42	Me_2CO		
7a	S	C_6H_5	Cl	149-151	24	*i*-PrOH	$C_{15}H_9ClS_2$	C, H
6b	O	C_6H_4-*p*-Cl	Cl	196-197[b]	57	Me_2CO	$C_{15}H_8Cl_2OS$	C, H
7b	S	C_6H_4-*p*-Cl	Cl	207-209 dec	61	C_6H_6-hexane	$C_{15}H_8Cl_2S_2$	C, H, S
6c	O	C_6H_4-*p*-Cl	H	163-165	81	Me_2CO	$C_{15}H_9ClOS$	C, H
7c	S	C_6H_4-*p*-Cl	H	192-194	7.2	Me_2CO	$C_{15}H_9ClS_2$	C, H; S[c]
6d	O	C_6H_5	Br	193-194	39	Me_2CO	$C_{15}H_9BrOS$	C, H, Br
7d	S	C_6H_5	Br	174-175	64	*i*-PrOH	$C_{15}H_9BrS_2$	C, H, S
6e	O	C_6H_4-*p*-Cl	Br	190-191	51	*i*-PrOH	$C_{15}H_8BrClOS$	C, H, S
7e	S	C_6H_4-*p*-Cl	Br	219-222	75	$C_6H_5CH_3$		*d*
6f	O	C_6H_3-3,4-Cl_2	Br	301-303	44	*e*	$C_{15}H_7BrCl_2OS$	C, H, S
6g	O	C_6H_4-*p*-F	Br	171-173	49	*i*-PrOH	$C_{15}H_8BrFOS$	C, H, S
6h	O	1-Adamantyl	Br	222-223	46	Dioxane	$C_{19}H_{19}BrOS$	C, H, S
6i	O	1-Adamantyl	Cl	204-206	51	$C_6H_5CH_3$	$C_{19}H_{19}ClOS$	C, H, S
6j	O	C_6H_4-*p*-F	F	190-192	40	C_6H_6	$C_{15}H_8F_2OS$	C, H, S
6k	O	C_6H_3-3,4-Cl_2	F	219-220	50	$C_6H_5CH_3$	$C_{15}H_7Cl_2FOS$	C, H, S
6l	O	C_6H_3-3,4-Cl_2	OCH_3	184-187	39	*i*-PrOH-$C_6H_5CH_3$	$C_{16}H_{10}Cl_2O_2S$	C, H, S

[a] Lit.[17] 189-190 °C. [b] Lit.[8] 202-204 °C. [c] S: calcd, 22.20; found, 21.43. [d] The crude material was used in subsequent steps without analyses. [e] The material was very insoluble and was purified by Soxhlet extraction with toluene for 3 days.

antimalarials, such as phenanthrenemethanols, are active at dose levels of <10 mg/kg.[5] One of us (Z.B.P.) first prepared a potential antimalarial, benzothiopyran (compound 1, WR 95214), on the basis of its relationship to a known[6] antischistosomal agent 2. When compound



1 WR 95214

2

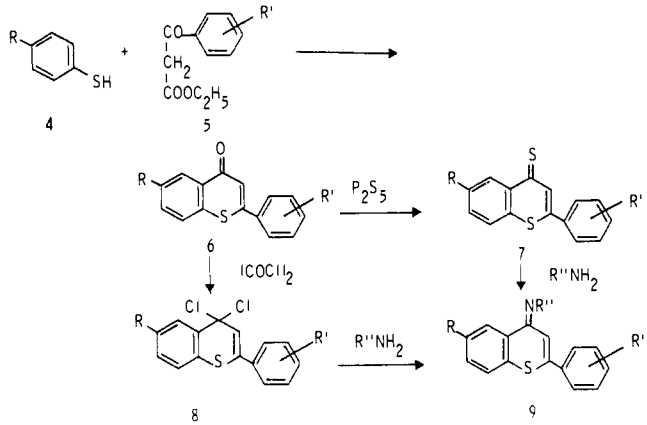
3a X = S
b X = O

1 showed antimalarial activity in mice at 640 mg/kg, the synthesis of other benzothiopyran derivatives as novel antimalarials was undertaken. In this paper, we describe their synthesis and antimalarial activity and discuss their structure–activity relationship (SAR).

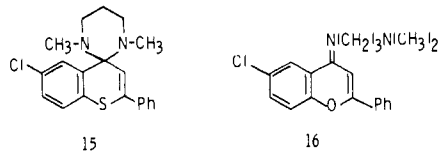
While our work was in progress, Bossert and Haberkorn[7] reported that compounds **3a,b** showed antimalarial activity. They are closely related to our compounds but, as far as we are aware, no further reports have appeared in the literature.

Chemistry. The various derivatives of benzothiopyrans of type **9** were synthesized according to the general Scheme I. The appropriately para-substituted thiophenols **4** were condensed with the various benzoylacetates **5** in the presence of polyphosphoric acid to give the corresponding thioflavones **6**[8] (Table I). These were converted to the thionothioflavones **7**[9] (Table I) by refluxing with P_2S_5 in xylene, which were then treated with the appropriate amine to give **9**. In an alternate route,[10] oxalyl chloride converted the thioflavones **6** to their *gem*-dichloro derivatives **8**. The latter were not purified or characterized but were treated with the amine to form the desired compounds **9**. In general, we preferred the *gem*-dichloro route, inasmuch as the condensation with the amines proceeded much more smoothly and at lower temperatures, and the isolation of the final products was simpler. The various compounds of type **9** which we prepared are listed in Table II (**10a–n**, **11a–h**, and **12a–c**), Table III (**13a–h**), and Table IV (**14a–e**). On several occasions the final compounds crystallized with moles of solvent that were difficult to remove, even with vacuum drying at 110 °C.

Scheme I



To extend our SAR studies in benzothiopyrans we also prepared (i) the spiro compound **15** for comparison with **10k** and (ii) a flavone analogue **16** of compound **1**. The



15 16

spiro compound **15** was obtained upon refluxing in chloroform the *gem*-dichloro derivative of type **8** with excess *N,N'*-dimethyl-1,3-propanediamine. The flavone derivative **16** was prepared from the known 6-chloroflavone.[11]

Biological Activity and Discussion of Results. The compounds were screened for antimalarial activity in mice and chicks according to Rane's test procedure.[4] In the primary test, five mice were infected with a lethal dose of *P. berghei* 3 days prior to administration of the compound, which was administered subcutaneously in peanut oil. The mean survival time of control mice was 6.2 ± 0.5 days and the increase in mean survival time was an indication of antimalarial activity. This value was not computed in those cases where any mice survived for 60 days, in which

case the compound was said to be *curative*. When the mean survival time in treated mice was more than twice that of the control group, the compound was said to be *active*.

Chicks (9–12 days old) were infected with a standard inoculum of *P. gallinaceum* (Strain B), which was fatal to 100% of untreated controls within 3–4 days. Compounds under evaluation were dissolved or suspended in peanut oil and administered subcutaneously or per os immediately after infection of the chicks. An increase of 100% in survival time was considered to be the minimum effective response to the antimalarial activity of the compound. Chicks that survived for 30 days were recorded as cured.

Of all the benzothiopyrans tested (Tables II–IV) nine were active and 10 were curative in the mouse screen at dose levels of 160–640 mg/kg. In the chick screen, five compounds were active and one was curative at dose levels of 160–320 mg/kg. None of the intermediates (Table I) showed any activity.

The five most active compounds were **10c**, **11a,b**, **12a**, and **13a**. The flavone analogue **16** of compound **1** was active in mice at a dose of 320 and 640 mg/kg, whereas the spiro compound **15** was inactive at 640 mg/kg.

An examination of the data led to a few general conclusions concerning SAR in this series.

1. A necessary, but not sufficient, condition for significant activity is the presence of a strongly basic nitrogen atom separated by three to four carbon atoms from the imino nitrogen atom at the 4 position. The exact nature of the alkyl groups attached to the terminal nitrogen atom is not important nor is the presence of a second strongly basic nitrogen atom (as in the compounds containing piperazine groups).

2. An aryl substitution at C-2 seems important for activity. The activity was lost when the 2-aryl group of **11h** was replaced by a 2-adamantyl group (**13h**). (Although in the examples cited the halogen substituents in the 6 position are different, in themselves they appear to have no effect on activity—see paragraphs 3 and 4 below.)

3. Among the compounds prepared by us a chlorine or bromine atom at either or both the 4′ and 6 position is necessary but not sufficient in itself for activity. Fluorine at the 6 position causes a complete loss of activity. In a single example of a 6-bromo-4′-fluoro compound **13g**, activity was significantly decreased from that of the nonfluorine analogue **13a**.

4. By restricting comparisons to those series where the amine side chain is held constant, chlorine or bromine in the 6 position seems to contribute more to activity than does that in the 4′ position, and the 4′,4-dihalogeno compounds are less active than the monohalogeno analogues. However, in a series containing a piperazine side chain, the dichloro compound **11a** was more active than either of the monochloro analogues, **10a** or **12b**.

5. When structures of active compounds are modified by placing chlorine atoms at both the 3′ and 4′ positions, activity is decreased. A possible exception is the single case of a 6-methoxy-3′,4′-dichloro compound (**14e**) that gave two cures at 640 mg/kg. Since this was the only methoxy compound prepared we cannot draw a firm conclusion concerning the effect of methoxy groups.

6. Substitution of sulfur by oxygen retains activity; compare **1** with **16**.

Experimental Section[12]

Melting points are uncorrected and were determined on a Thomas-Hoover or Mel-Temp capillary melting point apparatus. Elemental analyses were performed by Spang Microanalytical Laboratories or Galbraith Laboratories, Inc. The UV, IR, and NMR spectra were as expected for the assigned structures. The neutralization equivalent was determined by potentiometric titration with perchloric acid in glacial AcOH. Mercuric acetate solution was added to samples of the hydrochlorides prior to titration.

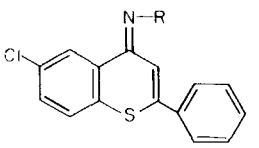
The various thiophenols, benzoylacetates, and amines were commercially available except for the following. Ethyl *p*-chlorobenzoylacetate was prepared according to a literature procedure.[13] Ethyl 3,4-dichlorobenzoylacetate was synthesized by the standard procedure used for the preparation of ethyl benzoylacetate.[14] After solvent was removed on a rotary evaporator, the volatile impurities were eliminated by vacuum distillation at 30–145 °C (1 mm). The residue (31%) was the desired β-keto ester in at least 90% purity (NMR). It was used without further purification in subsequent steps. 4-(2-Aminoethyl)-1-piperazineethanol [bp 158–162 °C (0.3 mm), n^{20}_D 1.5090; lit.[15] bp 200 °C (1.0 mm)] was prepared by the reaction of aziridine and *N*-(β-hydroxyethyl)piperazine according to the procedure of Clapp.[16] 4-(4-Aminobutyl)-1-piperazineethanol was prepared by refluxing a mixture of 20.0 g (0.07 mol) of *N*-(ω-bromobutyl)-phthalimide, 20.0 g (0.16 mol) of piperazineethanol, and 100 mL of xylene for 15 h. When the mixture was cooled to room temperature there was a hard layer of piperazineethanol hydrobromide on the bottom of the flask and a more fluffy, white precipitate of the desired material, which was collected and dried to yield 17.9 g (76%), mp 128.5–131 °C. The phthalimide was hydrolyzed by refluxing in a mixture of 30 mL of concentrated HCl and 10 mL of H_2O for 6 h. Phthalic acid was removed by filtration after the reaction mixture had been cooled to 5 °C. The filtrate was concentrated to dryness and then dried over KOH at 110 °C (0.1 mm) to give the hydrochloride salt: 16.0 g; off-white solid; mp 224–228 °C. The free base was liberated by stirring 16.0 g of the salt in 200 mL of MeOH containing 8 g of NaOH for 10 h. The inorganic salts were removed by filtration and the filtrate was concentrated. The residue was taken up in 100 mL of Et_2O–15% C_2H_5OH and filtered. The filtrate was concentrated and dried over KOH under vacuum to give 12.5 g of the desired amine, mp 75–118 °C. The crude amine was used without purification in subsequent steps.

Thioflavones (Table I). Typical Procedure. 4′,6-Dichlorothioflavone (6b). Ethyl *p*-chlorobenzoylacetate[13] (33.0 g, 0.15 mol), mp 37–42 °C, was melted with 17.4 g (0.12 mol) of *p*-chlorothiophenol. The solution was added to 200 g of polyphosphoric acid (preheated to 85 °C) with vigorous stirring at a rate sufficient to maintain the temperature at 85 °C (ca. 30 min). The mixture was heated at 95 °C for an additional 30 min and poured into 2 L of ice–water with stirring. The solid precipitate was filtered after the mixture had been stirred for 1 h and washed thoroughly with water and ether. The vacuum-dried product weighed 21.0 g (57%), mp 184–188 °C. It was recrystallized from Me_2CO as needles, mp 196–197 °C.

Thionothioflavones (Table I). Typical Procedure.[9] **4′,6-Dichloro-4-thionothioflavone (7b).** A mixture of 19.0 g (0.62 mol) of **6b** and 38.0 g of P_2S_5 (obtained by the continuous extraction of commercial P_2S_5 with CS_2) in 500 mL of toluene was heated as reflux for 2.5 h with occasional swirling. The mixture was filtered hot; the filtrate was treated with charcoal and diluted with an equal volume of hexane. The product, which crystallized as brown fluffy needles on cooling the solution at 0 °C for 3 days, was recrystallized from a C_6H_6–hexane mixture. It was then refluxed with *i*-PrOH to remove some P_2S_5 and again recrystallized from a C_6H_6–hexane mixture to give olive-yellow needles, mp 207–209 °C dec.

Imines of Type 9 (Tables II–IV). Typical Procedure. (a) From Thiono Derivatives 7. 4-[3-[6-Chloro-2-(4-chlorophenyl)-4*H*-1-benzothiopyran-4-ylideneamino]propyl]-1-methylpiperazine (11a). A mixture of 2.8 g (0.0086 mol) of **7b** (Table I), 9 mL (ca. 0.057 mol) of 4-(3-aminopropyl)-1-methylpiperazine, and 75 mL of absolute C_2H_5OH was stirred and refluxed under N_2 for 4 h. The hot solution was filtered and the filtrate was evaporated to a red oil which was dissolved in Et_2O. The solution was washed with H_2O until neutral, followed by saturated brine. The dried ($MgSO_4$) Et_2O solution was evaporated to a yellow solid, which was recrystallized from 100 mL of hexane. The product, which was mostly a crystalline yellow solid containing a small amount of crystalline red solid, was triturated with 50

Table II. Derivatives of Benzothiopyrans



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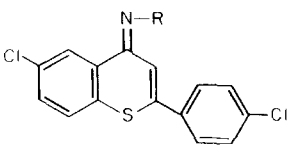

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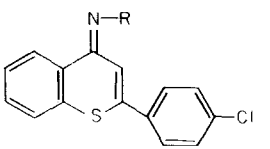

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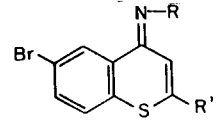
Compd	R	Reaction conditions[m]	Mp, °C	Yield, %	Purifn solvent	Formula	Analyses	Antimalarial activity: Mouse Dose, mg/kg	Mouse Increase in MST,[o] days	Chick Dose, mg/kg	Chick Increase in MST, days
1	$-(CH_2)_3N(CH_3)_2\cdot HCl\cdot 0.5H_2O$	A	115–120	68	EtOH-Et_2O	$C_{20}H_{21}ClN_2S\cdot HCl\cdot 0.5H_2O$	C, H, N	640	13.6 active		
10a	$-(CH_2)_3$-c-$N(CH_2CH_2)_2N$-CH_3	A	86–89	35	Hexane	$C_{23}H_{26}ClN_3S$	C, H, Cl	320	7.3 active	320	Inactive
								640	2 cures		
10b	$-(CH_2)_4$-c-$C_6H_{11}\cdot HCl\cdot 0.25H_2O$	A	135–138	41	EtOH-Et_2O	$C_{25}H_{28}ClNS\cdot HCl\cdot 0.25H_2O$	H, Cl; C[a]	640	Inactive	320	Inactive
10c	$-CH(CH_3)(CH_2)_3N(C_2H_5)_2\cdot 2HCl$	A	200–205	45	EtOH–Et_2O	$C_{24}H_{30}ClN_2S\cdot 2HCl$	C, H, Cl	160	8.0 active		
								320	10.6 active	320	1 cure (T)[n]
								640	2 cures		
10d	$-NHC(=NH)NH_2\cdot 0.5H_2O$	A	111–145	57	EtOH-H_2O	$C_{16}H_{13}ClN_4S\cdot 0.5H_2O$	C, H, N	640	6.2 active		
10e	$-CH_2CH_2N(CH_3)_2$	A	91–93	54	Hexane	$C_{19}H_{19}ClN_2S$	C, H, N	640	Inactive		
10f		A	138–140	57	EtOH-H_2O	$C_{22}H_{21}ClN_2S$	C, H, Cl	640	Inactive	320	Inactive
10g	$-(CH_2)_3NH\cdot COOCH_2Ph$	A	121–122	68	EtOH (95%)	$C_{26}H_{23}ClN_2O_2S$	C, H, N	640	Inactive		
10h	$-(CH_2)_3NH_2\cdot 2HCl\cdot 0.5H_2O\cdot 0.25C_2H_5OH$	A	210–260	52	EtOH	$C_{18}H_{17}ClN_2S\cdot 2HCl\cdot 0.5H_2O\cdot 0.25H_2O\cdot 0.25C_2H_5OH$	C, H, Cl, S[b]	640	Inactive		
10i	-2-$(CH_2)_2$-c-C_4H_7N-CH_3	A	84–86	28	Hexane	$C_{22}H_{23}ClN_2S$	C, H, S	320	7.0 active		
								640	1 cure		
10j	1-Adamantyl	B	126–129	29	Hexane	$C_{25}H_{24}ClNS$	C, H, S	640	Inactive	320	Inactive
10k	$-(CH_2)_3N(C_2H_5)_2\cdot 2H_2NSO_3H\cdot H_2O$	C	179–180	13	H_2O–EtOH	$C_{22}H_{26}ClN_2S\cdot 2H_2NSO_3H\cdot H_2O$	C, H, N, S	320	9.1 active	320	Inactive
								640	10.3 active		
10l	$-(CH_2)_3$-c-$N(CH_2CH_2)_2O\cdot 2HCl\cdot H_2O\cdot C_2H_5OH$	A	255–259	50	MeOH–Et_2O	$C_{22}H_{23}ClN_2OS$[c]	C, H, N, S	640	6.8 active		
10m	$-(CH_2)_2$-c-$NC_4H_8\cdot 2HCl$	C	247–250	37	*d*	$C_{21}H_{21}ClN_2S$[e]	C, H, N, S	640	Inactive		
10n	3-Pyridyl	A	134–136	13	*f*	$C_{20}H_{13}ClN_2S$	C, H, N, S	320	Inactive	320	Inactive
11a	$-(CH_2)_3$-c-$N(CH_2CH_2)_2N$-CH_3	A	110–116	52	Hexane	$C_{23}H_{25}Cl_2N_3S$	C, H, Cl	160	6.2 active	320	3.4 active
								320	11.2 active		
								640	4 cures		
11b	$-(CH_2)_3N(CH_3)_2$	A	78–79	56	Hexane	$C_{20}H_{20}Cl_2N_2S$	C, H, Cl	160	7.0 active	160	3.4 active
								320	9.8 active	320	4.4 active
								640	1 cure		
11c	$-(CH_2)_4$-c-$C_6H_{11}\cdot HCl$	A	205–210	65	EtOH-Et_2O	$C_{25}H_{27}Cl_2NS\cdot HCl$	C, H, Cl	640	Inactive	320	Inactive
11d	$-CH(CH_3)(CH_2)_3N(C_2H_5)_2\cdot 2HCl\cdot H_2O$	A	224–226	58	*g*	$C_{24}H_{28}Cl_2N_2\cdot 2HCl\cdot H_2O$	C, H, Cl	640	Inactive	320	4.7 active
11e	$-(CH_2)_2$-c-$N(CH_2CH_2)_2N$-$(CH_2)_2$-$OH\cdot H_2O$	A	55 dec	26	EtOAc-hexane	$C_{23}H_{25}Cl_2N_3OS\cdot H_2O$	C, H, S; Cl[h]	640	Inactive	320	Inactive
11f	-2-CH_2-C_5H_4N	D	222–226 dec	20	CH_2Cl_2	$C_{21}H_{14}Cl_2N_2S$	N, S; C, H[i]	320	Inactive	320	Inactive

11g	$-(CH_2)_3$-c-$NC_5H_{10}\cdot 2HCl$	A	270-274	46	*i*-PrOH	$C_{23}H_{24}ClN_2S\cdot 2HCl$	C, H, N, S	640	Inactive	320	5.2 active
11h	$-(CH_2)_4$-c-$NC_5H_{10}(CH_2)_2OH\cdot 3HCl$	C	265 dec	80	*j*	$C_{25}H_{29}Cl_2N_3OS\cdot 3HCl$	C, H, N, S	320 640	7.4 active 7.9 active (T)	320	(T)
12a	$-(CH_2)_3N(CH_3)_2\cdot 2HCl\cdot 2HCl\cdot 2H_2O$	A	237-241 dec	53	*g*	$C_{20}H_{21}ClN_2S\cdot 2HCl\cdot 2H_2O$	C, Cl, S; H[k]	320 640	3 cures (T)		
12b	$-(CH_2)_3$-c-$N(CH_2CH_2)_2N$-CH_3	A	105-107	58	Hexane	$C_{23}H_{26}ClN_3S$	C, H, N, S	320 640	6.9 active 3 cures		
12c	$-CH(CH_3)(CH_2)_3N(C_2H_5)_2\cdot 2HCl\cdot H_2O$	C	244-247	25	EtOH-Et_2O	$C_{24}H_{29}ClN_2S\cdot 2HCl\cdot H_2O$	H, S; C[l]	320 640	Inactive (T)	80 160	Inactive (T)

[a] Cl: calcd, 15.72; found, 16.30. [b] Neutralization equivalent (NE): calcd, 211; found, 204. [c] Analyzed as the free base. NE of the salt: calcd, 268; found, 265. [d] Precipitated from Et_2O by gaseous HCl. [e] Analyzed as the free base. NE: calcd, 220.4; found, 220.0. [f] Purified by column chromatography on silica gel. Elution with $CHCl_3$ gave some starting thioflavone. This was followed by elution with Et_2O which gave the desired material. [g] Precipitated with ethanolic HCl-Et_2O. [h] Cl: calcd, 14.76; found, 14.33. [i] C: calcd, 63.48; found, 62.87. H: calcd, 3.55; found, 3.05. [j] Precipitated with ethanolic HCl. [k] H: calcd, 5.84; found, 5.39. [l] C: calcd, 57.20; found, 56.60. [m] A = thiono intermediate was condensed with the amine in refluxing EtOH. B = *gem*-dichloro intermediate in CH_2Cl_2 was added to a solution of the amine in benzene containing triethylamine. The solution was refluxed for 3 h and the triethylamine hydrochloride was removed by filtration. The *gem*-dichloro intermediate was prepared from the corresponding thioketone by refluxing in CH_2Cl_2 with oxalyl chloride. C = thiono intermediate was condensed with the amine in refluxing toluene. D = thiono intermediate was condensed with the amine in refluxing *i*-PrOH for 2 days. [n] T = toxic. [o] MST = mean survival time.

Table III. Derivatives of Benzothiopyrans



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Compd	R	R'	Reaction conditions[d]	Mp, °C	Yield, %	Purifn solvent	Formula	Analyses	Antimalarial activity: Mouse Dose, mg/kg	Mouse Increase in MST, days	Chick Dose, mg/kg	Chick Increase in MST, days
13a	$-CH(CH_3)(CH_2)_3N(C_2H_5)_2\cdot 2HCl$	C_6H_5	A	210-215	73	CH_3CN	$C_{24}H_{29}BrN_2S\cdot 2HCl$	C, H, N, S	160 320 640	9.5 active 3 cures 5 cures	160	Inactive
13b	$-(CH_2)_3N(CH_3)_2\cdot 2HCl\cdot H_2O$	C_6H_5	B	251-252	47	EtOH	$C_{20}H_{21}BrN_2S\cdot 2HCl\cdot H_2O$	C, H, Br	320 640	12.6 active 3 cures		
13c	$-CH(CH_3)(CH_2)_3N(C_2H_5)_2\cdot 2HCl$	C_6H_4-*p*-Cl	A	198-200	50	EtOH-Et_2O	$C_{24}H_{28}BrClN_2S\cdot 2HCl$	C, H, N, S	320	Inactive	160	5.0 active
13d	$-(CH_2)_3$-c-$N(CH_2CH_2)_2N$-$CH_3\cdot 3HCl$	C_6H_4-*p*-Cl	A	202-206 dec	17	MeOH	$C_{23}H_{25}BrClN_3S$	C, N, S; H[a]	320	Inactive	160	Inactive
13e	$-CH(CH_3)(CH_2)_3N(C_2H_5)_2\cdot 2HCl$	C_6H_3-3,4-Cl_2	C	138-145 dec	18	EtOH-Et_2O	$C_{24}H_{27}BrCl_2N_2S$	C, H, N, S	320	Inactive	160	Inactive
13f	$-(CH_2)_3$-c-$N(CH_2CH_2)_2O$	C_6H_3-3,4-Cl_2	D	126-128	45	Cyclohexane	$C_{22}H_{21}Cl_2BrNOS$	C, H, N, S	640	10.5 active	320	Inactive
13g	$-CH(CH_3)(CH_2)_3N(C_2H_5)_2\cdot 2HCl$	C_6H_4-*p*-F	E	135-138	46	CH_2Cl_2	$C_{24}H_{28}BrFN_2S\cdot 2HCl$	C, H, N; S[b]	320 640	6.9 active 10.1 active	160 320	Inactive (T)
13h	$-(CH_2)_4$-c-$N(CH_2CH_2)_2N$-$(CH_2)_2OH$	1-Adamantyl	E	67-71	16	Cyclohexane	$C_{29}H_{40}BrNO_3S$	H, N, S; C[c]	320	Inactive	120	Inactive (T)[e]

[a] H: calcd, 4.70; found, 5.21. [b] S: calcd, 5.86; found, 4.95. [c] C: calcd, 62.34; found, 61.41. [d] A = thiono intermediate was condensed with the amine in refluxing EtOH. B = thiono intermediate was condensed with the amine in refluxing toluene. C = *gem*-dichloro intermediate in C_6H_6 was added to the amine in C_6H_6 and the mixture was refluxed for 2.5 h. The *gem*-dichloro intermediate was prepared from the corresponding thioketone by refluxing with neat oxalyl chloride for 2.5 h. D = *gem*-dichloro intermediate was refluxed with the amine for 3 h without solvent. The *gem*-dichloro intermediate was prepared from the thioketone by refluxing in $CHCl_3$ with oxalyl chloride for 2 h. E = *gem*-dichloro intermediate was refluxed with the amine in $CHCl_3$ for 1 h. The *gem*-dichloro intermediate was prepared as in D. [e] T = toxic.

Table IV. Derivatives of Benzothiopyrans

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Compd	R	R′	R″	Reaction conditions[b]	Mp, °C	Yield, %	Purifn solvent	Formula	Analyses	Antimalarial activity: Mouse: Dose, mg/kg	Mouse: Increase in MST, days	Chick: Dose, mg/kg	Chick: Increase in MST, days
14a	$-CH(CH_3)(CH_2)_3N(C_2H_5)_2 \cdot 2HCl \cdot H_2O$	1-Adamantyl	Cl	A	161–163	7	*i*-PrOH–Et_2O	$C_{28}H_{39}ClN_2S \cdot 2HCl \cdot H_2O$	C, H, N, S	640	Inactive	160	Inactive (T)[c]
14b	$-CH(CH_3)(CH_2)_3N(C_2H_5)_2 \cdot 2HCl$	C_6H_4-*p*-F	F	A	260–261	37	EtOH–Et_2O	$C_{24}H_{28}F_2N_2S \cdot 2HCl$	C, H, N, S	640	Inactive	120	(T)
14c	$-CH(CH_3)(CH_2)_3N(C_2H_5)_2 \cdot 2HCl \cdot H_2O$	C_6H_3-3,4-Cl_2	F	B	142–145	16	Ppt EtOH/HCl–Et_2O	$C_{24}H_{27}Cl_2FN_2S \cdot 2HCl \cdot H_2O$	H, N, S; C[a]	640	Inactive	120	Inactive
14d	$-2-(CH_2)_2$-c-C_4H_7N-$CH_3 \cdot 2HCl \cdot 2H_2O$	C_6H_3-3,4-Cl_2	F	A	160 dec	45	Ppt EtOH/HCl–Et_2O	$C_{22}H_{24}Cl_2FN_2S \cdot 2HCl \cdot H_2O$	C, H, N, S	640	Inactive	320	Inactive
14e	$-CH(CH_3)(CH_2)_3N(C_2H_5)_2 \cdot 2HCl$	C_6H_3-3,4-Cl_2	OCH_3	A	239–241 dec	36	Leached with 10:1 CH_3CN–*i*-PrOH	$C_{25}H_{30}Cl_2N_2OS \cdot 2HCl$	C, H, N, S	640	2 cures	120	Inactive

[a] C: calcd, 51.81; found, 51.31. [b] A = *gem*-dichloro intermediate was refluxed with the amine in $CHCl_3$ for 1 h. The *gem*-dichloro intermediate was prepared as by refluxing in $CHCl_3$ with oxalyl chloride for 2 h. B = *gem*-dichloro intermediate was refluxed with the amine in C_6H_6 for 1 h. The *gem*-dichloro intermediate was prepared as in A. [c] T = toxic.

mL of CCl_4 and filtered rapidly. The red solid was mostly insoluble. The CCl_4 solution was evaporated to dryness and the residue was recrystallized twice more from hexane to give 2.0 g (52%) of yellow crystals, mp 110–116 °C.

(b) From *gem*-Dichloro Derivatives 8. *N′,N′*-Diethyl-*N*⁴-[2-(3,4-dichlorophenyl)-6-methoxy-4*H*-1-benzothiopyran-4-ylidene]-1,4-pentanediamine Dihydrochloride (14e). A slurry of 6.5 g (0.019 mol) of **61** (Table I) in 30 mL of $CHCl_3$ was treated with 15 mL of oxalyl chloride. There was a vigorous gas evolution that lasted for about 10 min. The mixture was then refluxed for 1 h and concentrated to dryness. The yellow solid residue was treated with toluene, which was subsequently removed under reduced pressure. The residue was dissolved in 125 mL of $CHCl_3$ at 0–5 °C and 6.5 g (0.04 mol) of 2-amino-5-diethylaminopentane was added with stirring. The mixture was refluxed for 1 h and then allowed to stand at room temperature overnight. The solvent was removed under vacuum and the residue was partitioned between 200 mL of C_6H_6 and 150 mL of 12% Na_2CO_3 solution. The organic layer was washed with H_2O until neutral, followed by saturated brine solution. It was dried (Na_2SO_4), decolorized with carbon, and evaporated to dryness. The residue was extracted with hexane. Removal of hexane left an oil, which was dried in vacuo over NaOH. A solution of this oil in 15 mL of absolute C_2H_5OH was treated with a solution of about 1.2 g of HCl in 12 mL of C_2H_5OH. Addition of 60 mL of Et_2O and cooling at 0 °C gave 6.74 g of a crude hydrochloride salt, mp 223–228 °C dec. It was recrystallized from a hot solution of 300 mL of CH_3CN and 30 mL of *i*-PrOH upon addition of excess Et_2O to give 3.8 g (36%) of a crystalline solid, mp 239–241 °C dec.

6-Chloro-1′,3′-dimethyl-1′,2′,3′,4′,5′,6′-hexahydro-2-phenylspiro[4*H*-1-benzothiopyran-4,2′-pyrimidine] (15). 6-Chlorothioflavone[17] (**6a**, Table I) (10.0 g, 0.037 mol) was converted to its *gem*-dichloro derivative by the oxalyl chloride procedure described above. It was mixed with 250 mL of $CHCl_3$ and the slurry was added to 100 mL of $CHCl_3$ that was stirred and maintained at 10–15 °C. Concurrently, there was also added a solution of 10.0 g (0.098 mol) of *N,N′*-dimethyl-1,3-propanediamine in 250 mL of $CHCl_3$; the reaction was slightly exothermic. The mixture was stirred at room temperature for 2 h and then filtered from a solid. The $CHCl_3$ was removed under vacuum and the residue was stirred with 125 mL of *n*-hexane, filtered and air-dried to give 5.35 g of a solid, mp 155–158 °C. A second crop, 3.19 g, was obtained from the hexane filtrate. The combined solids were recrystallized several times from hexane to give 1.38 g (10%) of a crystalline solid, mp 175–177 °C dec. Anal. ($C_{20}H_{21}ClN_2S$) C, H, Cl, N, S.

***N,N*-Dimethyl-*N′*-(6-chloro-2-phenyl-4*H*-1-benzopyran-4-ylidene)-1,3-propanediamine (16).** 6-Chloro-4-thionoflavone was prepared from 6-chloroflavone[11] and P_2S_5 as described above. The hot toluene filtrate was evaporated to dryness and the residue was recrystallized twice from *i*-PrOH to give 6-chloro-4-thionoflavone (69%) as dark red needles, mp 141–142 °C. Anal. ($C_{15}H_9ClOS$) C, H, S.

A mixture of 30.0 g (0.11 mol) of 6-chloro-4-thionoflavone and 90 mL (ca. 0.9 mol) of *N,N*-dimethyl-1,3-propanediamine in 700 mL of absolute C_2H_5OH was refluxed with stirring under N_2 for 4 h. The cooled solution was evaporated to dryness and the liquid residue was dissolved in Et_2O. The solution was washed with eight 250-mL portions of H_2O, dried, and evaporated. The residual oil was allowed to precipitate and then crystallize from hexane by cooling to −15 °C. Further purification was accomplished by repeated trituration with warm petroleum ether (bp 37.5–46.8 °C), filtering to remove the higher melting impurity, concentration of the filtrate, and cooling to crystallize the product. This procedure gave 11.2 g (30%) of **16** as orange crystals, mp 50–54 °C. Anal. ($C_{20}H_{21}ClN_2O$) C, H, Cl, N.

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References and Notes

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Synthesis of *N*-Hydroxyacetaminophen, a Postulated Toxic Metabolite of Acetaminophen, and Its Phenolic Sulfate Conjugate

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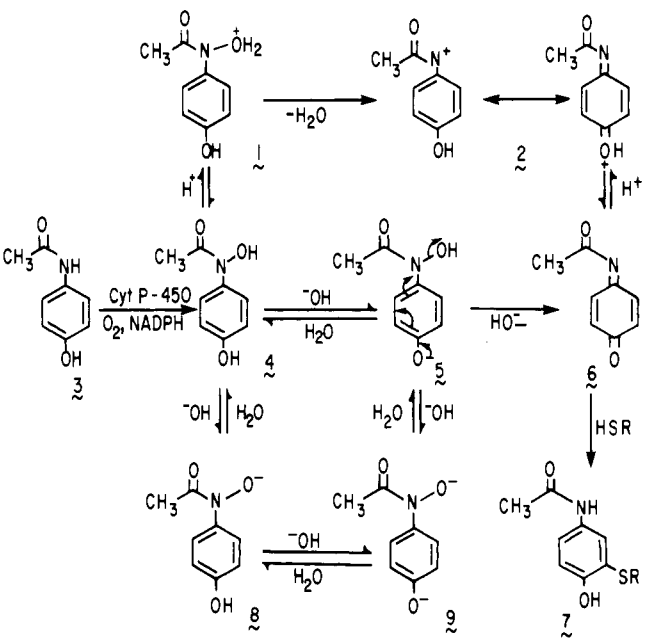
The synthesis of *N*-hydroxyacetaminophen (*N*-acetyl-*N*-hydroxy-*p*-aminophenol, 4), a postulated toxic metabolite of acetaminophen (*N*-acetyl-*p*-aminophenol, 3), and its phenolic sulfate conjugate (potassium *N*-acetyl-*N*-hydroxy-*p*-aminophenyl sulfate) (13) is described. Potassium *p*-nitrophenyl sulfate was reduced to the hydroxylamine, acetylated, and treated with sulfatase to yield *N*-hydroxyacetaminophen. The structures assigned are supported by the spectral data (IR, UV, MS, ^{1}H NMR, and ^{13}C NMR). *N*-Hydroxyacetaminophen was found to be moderately unstable at physiological pH and temperature, whereas its phenolic sulfate conjugate was stable.

Acetaminophen (*N*-acetyl-*p*-aminophenol, 3) is a widely used mild analgesic. It is largely metabolized to glucuronide and sulfate conjugates which are readily excreted by the kidney and are considered nontoxic. The possibility that a small fraction of the administered dose of acetaminophen might also be metabolized to a toxic metabolite arose from the high incidence of severe hepatotoxicity resulting from suicidal overdosage in man.[1] The probable mechanism of toxicity has emerged from studies in animals in which it has been shown that acetaminophen is converted to a metabolite which depletes hepatic glutathione and then covalently binds to tissue macromolecules.[2] Depletion of hepatic glutathione and the subsequent covalent binding of radio-labeled acetaminophen to hepatic protein are enhanced by pretreatment with agents known to stimulate the Cyt P-450 mixed function oxidase system and decreased by inhibitors of drug metabolism.[2] The pathway that has been postulated to account for the covalent binding is shown in Scheme I and involves the formation of *N*-hydroxyacetaminophen (*N*-acetyl-*N*-hydroxy-*p*-aminophenol, 4) or its dehydration product, *N*-acetyl-*p*-benzoquinone imine (6), which then reacts with the sulfhydryl group of glutathione (7) or other cellular nucleophiles. More recently it has been proposed that the same biochemical mechanism may underly acetaminophen-induced acute renal necrosis as well as the nephropathy of chronic analgesic abuse.[3,4] Although *N*-hydroxyacetaminophen is a key compound in the proposed mechanism of toxicity, it has not been synthesized. Many questions involving either hepatic or renal toxicity require the availability of *N*-hydroxyacetaminophen for toxico-

Scheme I



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