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Studies in the Heterocyclic Series. XVI.[1] Open Azaphenothiazines as New Central Nervous System Depressants

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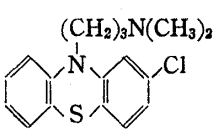
Acid-catalyzed condensation of 2-amino-3-mercapto-6-methylpyridine and 3-aminopyridine-2[1*H*]-thiones with 4-chloropyrimidines having free 5-carbon centers gave *N*-(3-mercapto-2-pyridyl)-6-pyrimidinylamines and *N*-(2-thioxo-3-pyridyl)-6-pyrimidinylamines, which we have described as open 1,3,9-triaza- and 1,3,6-triaza-phenothiazines, respectively. A newly developed method of reducing nitro groups was used for preparing the aminopyridine precursors. Eight new and five related compounds including an open 1,9-diazaphenoxazine were tested in rats and mice and found to display central nervous system(CNS)-depressant activities. The most active compound in the series is *N*-(6-chloro-2[1*H*]-thioxo-3-pyridyl)-2,4-diamino-6-pyrimidinylamine, an open 1,3,6-triaza-phenothiazine derivative. Structure–activity correlations are discussed on the basis of the biological data.

Keywords——Acid catalysis; open 1,3,9-triazaphenothiazine; open 1,3,6-triazaphenothiazine; reduction of nitro groups; CNS-depressant activity; anticonvulsant activity; structure–activity relationships

One approach to the development of new and more effective psychotropic drugs is to synthesize compounds which resemble chlorpromazine (**1**), the prototype of phenothiazine tranquilizers.[3] These compounds may differ from promazine in the following ways: the presence of side chains,[4] absence[5] or a modification of the 10-alkylaminoalkyl group,[6–8] incorporation of one[9,10] or more annular heteroatoms,[11] the expansion of the central thiazine ring leading to dibenzothiazepine (**2**)[12–17] and dibenzothiazocine (**3**)[18–20] rings of various types, expansion of the side rings[21] in phenothiazine and the replacement of ring sulfur with oxygen (**4**)[22,23] to give phenoxazine ring systems. It is also noteworthy from the work of Karreman, Isenberg and Szent-Gyorgyi,[24] and Phillips and Mehta[25,26] that the 10-alkylaminoalkyl group in phenothiazinoid drugs may not be a prerequisite for tranquilizing activity.



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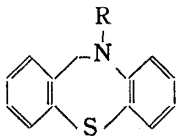

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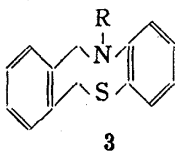

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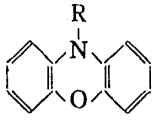

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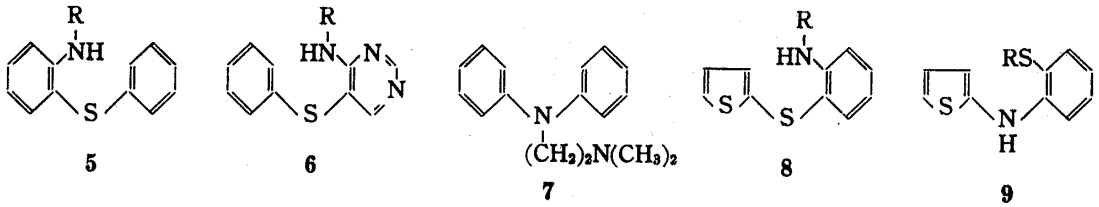
In some previous papers in this series, we reported the incorporation of two,[27] three and four nitrogen atoms[27–33] in the first and third rings in phenothiazine, and in two cases, the replacement of thiazine sulfur with oxygen[34–36] was achieved. These modifications led to novel 3,6-diaza-, 1,3,9-triaza-, 1,3,6-triaza- and 1,4,7,9-tetraazabenzo-phenothiazines and the parent 1,9-diaza- and 1,4,9-triazaphenoxazines. The pharmacological evaluation of these compounds was subsequently undertaken.[37,38] Derivatives of these closed systems were found to show appreciable central nervous system (CNS)-depressant activities in mice and rats. These activities are comparable to but less than that of chlorpromazine (Largactil).

In addition to all these structural changes in phenothiazine, there is a further modification which is least developed, but which is of topical interest. It involves the opening of the

central thiazine ring[39] while retaining the rest of the structural features. Gatti,[40] Burger and Stanmyer[41] had earlier prepared and tested some diaryl sulfides (**5**) related to promazine and chlorpromazine.

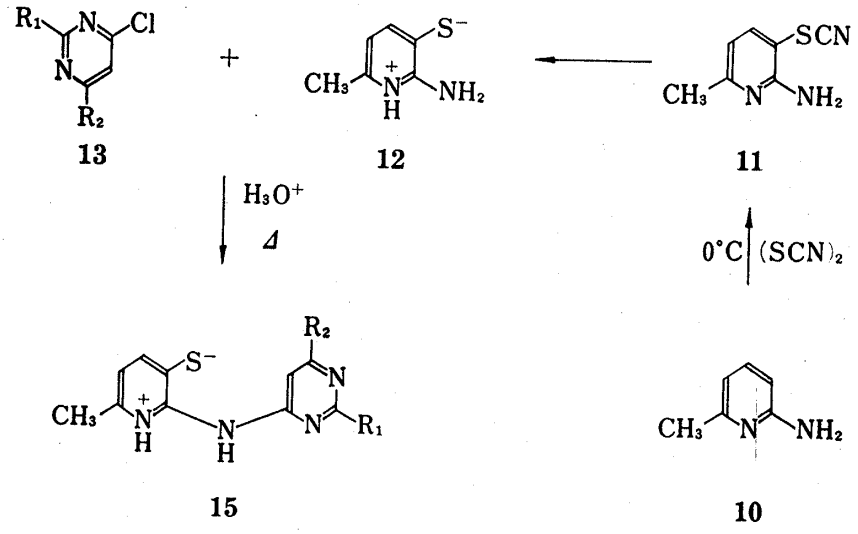


5 **6** **7** **8** **9**

The open 1,3-diazaphenothiazine (**6**) was reported by Roth and Hitchings[42] as showing marked activities as hypotensives and serotonin antagonists. Surprisingly, an open dethia-1-azaphenothiazine (**7**)[43] showed antihistaminic activity, although lower than that of isothipendyl (Nilergex).[44] Some *o*-aminophenyl 2-thienyl sulfides (**8**) and *N*-(*o*-mercaptopenyl)-2-thienylamines (**9**) were also prepared and tested by Schuetz and Okafor.[45] As a further extension of these investigations, we have now opened the central ring of the novel 1,3,9-triaza-, and 1,3,6-triazaphenothiazines and have obtained the open analogs of these rings. In this article, we therefore wish to present the synthesis and pharmacological activity of these compounds.

Chemistry

2-Amino-6-methylpyridine (**10**) was converted to the 3-thiocyanato derivative (**11**) and hydrolyzed in dilute sodium hydroxide, followed by acidification to yield the dipolar salt of 2-amino-3-mercapto-6-methylpyridine (**12**).

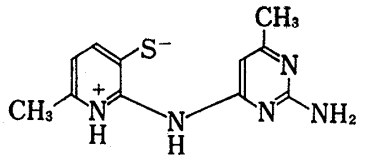


13 **12** **11** **15** **10**

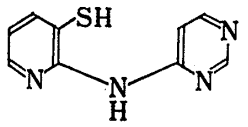
Chart 1

An equimolar reaction of an acidic mixture of this product with 2-amino-4-chloro-6-methylpyrimidine obtained by chlorination of the 4-hydroxy analog, **14**, led to a white crystalline compound having the molecular formula $C_{11}H_{13}N_5S$ and melting at 226°C (Chart 1). As the reaction is catalyzed by acid, the product is more likely to be a diarylamine[31,46] than a diarylsulfide. Furthermore, its solubility in sodium hydroxide and insolubility in acid are in agreement with an *o*-mercaptodiarylamine structure. The proton magnetic resonance (PMR) and infrared (IR) spectra further support this conclusion. Owing to the absence of SH group absorption at 2550 cm^{-1} even in concentrated solutions, a zwitterionic structure[47] was assigned to this product, which is now formulated as 2-(2-amino-4-methylpyrimidin-6-ylamino)-6-

methyl-1*H*-pyridinium-3-thiolate (**15**, $R_1=NH_2$, $R_2=CH_3$), a derivative of open 1,3,9-triazaphenothiazine (**16**),



15 : $R_1=NH_2$, $R_2=CH_3$



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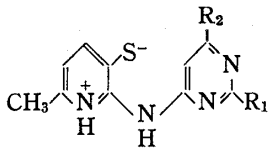
Derivatives of structure **16** were prepared by reacting compound **12** with 4-chloropyrimidines having free 5-carbon centers, as shown in Chart 1. The products were characterized and their properties are presented in Table I.

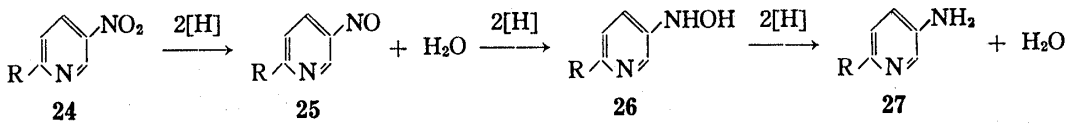
TABLE I.



Compd.	R_1	R_2	Reflux time (h)[a]	Recrystallization solvent	mp (°C)[b]	Yield %	Formula	Analyses
15	NH_2	CH_3	4	Water	226—227	82	$C_{11}H_{13}N_5S$	C,H,N,S.
17	NH_2	NH_2	3	H_2O–EtOH	276—277	66	$C_{10}H_{12}N_6S$	C,H,N,S.
18	OCH_3	OCH_3	4	dil. AcOH	260	62	$C_{12}H_{14}N_4O_2S$	c)
19	NH_2	OH	3	Me_2CO/H_2O/EtOH	263	87	$C_{10}H_{11}N_5OS$	c)
20	Cl	Cl	5	H_2O/MeOH	159—160	60	$C_{10}H_8Cl_2N_4S$	C,H,N,S,Cl.
21	SMe	NH_2	5	H_2O/EtOH	244—245	65	$C_{11}H_{13}N_5S_2$	C,H,N,S.
22	NH_2	Cl	3	H_2O/EtOH	250	52	$C_{10}H_{10}ClN_5S$	C,H,N,S,Cl.

a) These compounds are stable to heat and light.
b) All compounds melt with decomposition.
c) C.O. Okafor, *J. Org. Chem.*, **40**, 2753 (1975).

3-Amino-6-substituted pyridine-2[1*H*]-thiones (**23**), required for the synthesis of the open 1,3,6-triazaphenothiazines, were conveniently prepared from 3-nitropyridines by reduction of the nitro group using iron and a small amount of calcium chloride in 70—80% aqueous ethanol. The nascent hydrogen required for the reduction is produced *in situ* by hydrolysis of the calcium chloride in the presence of aqueous ethanol. Thus the use of excessive amounts of acid,[48] which will involve the production of large amounts of ferric hydroxide, is avoided. This method is cheaper, more convenient, less hazardous and easier in the work-up stage than the previously described methods,[49,50] which often involve several successive extractions with ether[28] after neutralization of the stannic chloride salt of the amine, followed by saturation with hydrogen sulfide.[51]



24 **25** **26** **27**

An added advantage is the readiness with which the intermediate nitrosopyridine and pyridine hydroxylamine can be isolated when limited amounts of calcium chloride are used.

The 3-aminopyridines obtained in these reactions were thiocyanated followed by base-catalyzed hydrolysis of the resulting 2-aminothiazolo[5,4-*b*]-pyridines (**28**). The 3-aminopyridine-2[1*H*]-thiones (**23**), thus formed, were condensed with the 4-chloropyrimidines (**13**) as shown in Chart 2.

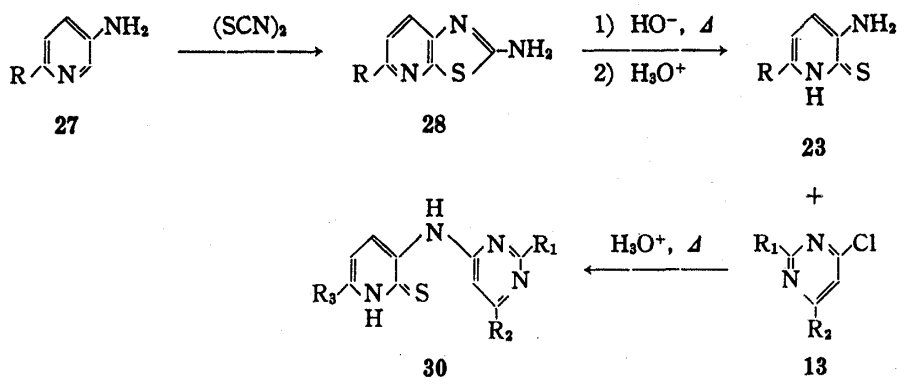
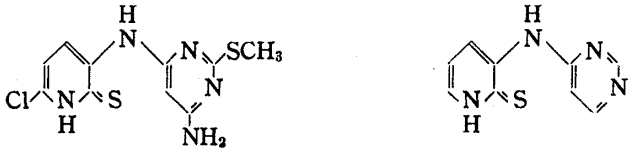


Chart 2

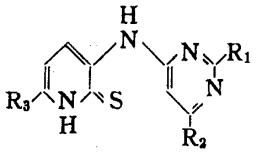
In a typical reaction, 3-amino-6-chloropyridine-2[1*H*]-thione (**23**, R=Cl) was condensed with 4-amino-6-chloro-2-methylthiopyrimidine (**29**) in dilute sulfuric acid. The product obtained was soluble in dilute base and showed strong infrared absorption between 1470 and 1500 cm^{-1} indicating the presence of an >N–C=S group as in thioamides. Furthermore, SH group absorption in the IR spectrum was absent while the C=S band in the region of 1090—1145 cm^{-1} [52] was observed thereby supporting the assigned thione structure. Confirmatory evidence was obtained from the PMR spectrum, which showed two separate singlet peaks due to ring NH and 3-NH groups. The product was therefore formulated as *N*-(6-chloro-2-[1*H*]-thioxo-3-pyridyl)-4-amino-2-methylthio-6-pyrimidinylamine (**30**, $R_1=SCH_3$, $R_2=NH_2$, $R_3=Cl$), which is a derivative of open 1,3,6-triazaphenothiazine (**31**).



30: $R_1=SCH_3$, $R_2=NH_2$, $R_3=Cl$ **31**

Some other derivatives of this open system were similarly prepared and their properties are summarized in Table II. It was again observed in the mass spectra of these compounds that the parent peaks appeared consistently at two mass units below the expected value, due

TABLE II.



Compd.	R_1	R_2	R_3	Reflux time (h)	Recrystallization solvent	mp (°C)[a]	Yield (%)	Formula	Analyses
30	SCH_3	NH_2	Cl	5	Me_2CO/H_2O	250—252	60	$C_{10}H_{10}ClN_5S_2$	C,H,N,S,Cl.
32	NH_2	NH_2	OCH_3	1.75	Me_2CO/H_2O	270—272	94	$C_{10}H_{12}N_6OS$[b]	c)
33	Cl	Cl	Cl	3	$Me_2CO/MeOH/H_2O$	>300	47	$C_9H_5Cl_3N_4S$[b]	C,H,N,S,Cl.
34	Cl	Cl	OCH_3	3	$Me_2CO/H_2O/MeOH$	>300	73	$C_{10}H_8Cl_2N_4OS$	C,H,N,S,Cl.
35	NH_2	NH_2	Cl	1.5	Me_2CO/H_2O	>300	91	$C_9H_9ClN_6S$	c)
36	NH_2	OH	Cl	4.5	Me_2CO/H_2O	>300	74	$C_9H_8ClN_5OS$[d]	C,H,N,S,Cl.
37	NH_2	OH	OCH_3	5	Me_2CO/H_2O	>300	69	$C_{10}H_{11}N_5O_2S$[b,d]	C,H,N,S.

a) Melts with decomposition.
b) Oven-dried at 60°C for 6 h.
c) C.O. Okafor, *J. Org. Chem.*, **38**, 4386 (1973).
d) Not among the compounds examined for pharmacological activity.

TABLE III. Effect of Chloropromazine (CPZ) and 13 Open Azaphenothiazine and Azaphenoxazine Derivatives on Forced Motor Activity in Mice

Drug	Dose mg/kg *i.p.*	3rd trial SEC $\bar{X}\pm$S.E.	4th trial SEC $\bar{X}\pm$S.E.	% of control	Dose mg/kg *i.p.*	3rd trial SEC $\bar{X}\pm$S.E.	4th trial SEC $\bar{X}\pm$S.E.	% of control	Dose mg/kg *i.p.*	3rd trial SEC $\bar{X}\pm$S.E.	4th trial SEC $\bar{X}\pm$S.E.	% of control
CPZ	1	209.98 ±13.94	200.26 ±26.11	95.4	2	225.50 ±19.30	184.33 ±37.46	81.7	4	212.33 ±42.28	57.36 ±36.38	27.0
15	6.25	112.34 ±23.60	74.63[a] ±14.69	66.4	12.5	160.68 ±15.85	125.72[a] ±18.30	78.2	25	200.69 ±34.75	136.64[a] ±20.03	68.1
17	25	139.67 ±19.91	99.00 ±16.96	70.9	50	160.84 ±16.24	94.90 ±11.79	59.0	100	170.70 ±13.43	123.14[a] ±15.89	72.1
18	1.5	136.93 ±12.88	91.00 ±12.90	66.5	3.0	115.69 ±21.35	106.94 ±17.62	92.4	6.0	140.63 ±16.06	139.48 ±28.40	99.2
19	3.13	141.89 ±8.28	133.47 ±23.43	94.1	6.25	123.72 ±12.54	81.44 ±18.92	65.8	12.5	141.54 ±14.37	126.89 ±14.37	89.7
20	25	233.83 ±27.62	183.93 ±27.89	78.7	50	205.88 ±23.91	115.34 ±28.55	56.0	100	99.14 ±11.23	13.54[a] ±4.14	13.6
21	3.13	115.54 ±17.12	81.23 ±19.09	70.3	6.25	117.72 ±7.48	100.00 ±13.83	85.0	12.5	145.24 ±20.51	110.22 ±16.64	75.9
22	25	167.00 ±15.87	107.24[a] ±20.53	64.2	50	171.28 ±11.91	103.54[a] ±21.23	60.4	100	124.83 ±17.00	118.81 ±19.57	95.2
30	12.5	184.23 ±15.50	154.40 ±30.41	83.8	25.0	173.51 ±30.04	155.43 ±7.12	89.6	50	187.54 ±13.10	185.00 ±19.89	98.7
32	25	225.04 ±34.37	210.11 ±9.04	93.4	50	224.92 ±20.23	242.97 ±24.60	108.0	100	216.79 ±9.46	182.24[a] ±12.62	84.1
33	25	128.05 ±25.87	97.27 ±22.38	76.0	50	111.05 ±9.00	90.38[a] ±21.84	81.4	100	105.86 ±5.47	66.32[a] ±19.76	58.9
34	25	139.32 ±22.46	138.70 ±21.61	99.5	50	127.82 ±14.54	108.80 ±11.50	85.1	100	131.03 ±14.48	106.42 ±22.93	81.2
35	25	215.00 ±20.95	197.28 ±20.98	91.8	50	200.62 ±26.56	186.48 ±24.76	92.9	100	190.37 ±10.35	144.14[a] ±14.48	75.7
38	25	188.04 ±13.90	194.49 ±21.03	103.8	50	182.88 ±16.63	166.64 ±20.89	91.1	100	236.94 ±26.45	198.00 ±37.02	83.6

a) $p<0.05$.

Table IV. Effect CPZ and 13 Open Azaphenothiazine and Azaphenoxazine Derivatives on Spenotaneous Motor Activity in Mice

Drug	Dose mg/kg *i.p.*	Control $\bar{X}\pm$S.E.	Drug $\bar{X}\pm$S.E.	% of control	Dose mg/kg *i.p.*	Control $\bar{X}\pm$S.E.	Drug $\bar{X}\pm$S.E.	% of control	Dose mg/kg *i.p.*	Control $\bar{X}\pm$S.E.	Drug $\bar{X}\pm$S.E.	% of control
CPZ	1	444.66 ±96.48	396.23 ±71.15	89.1	2	449.66 ±87.76	109.00[a] ±41.63	24.2	4	449.66 ±87.76	86.00[a] ±61.94	19.1
15	6.25	945.74 ±74.41	735.68 ±60.03	77.8	12.50	876.00 ±102.35	596.56 ±89.88	68.1	25	810.00 ±53.15	364.30[a] ±16.38	45.0
17	25.0	857.00 ±169.87	703.33 ±80.48	82.1	50	1091.73 ±105.85	720.71 ±61.19	66.0	100	967.68 ±128.50	415.00 ±191.75	42.9
18	1.5	948.62 ±183.38	394.58[a] ±124.29	41.6	3.13	948.62 ±183.38	716.00 ±207.60	75.5	6.25	809.72 ±68.92	849.71 ±122.50	104.9
19	3.13	805.74 ±90.93	674.68 ±119.50	83.7	6.25	805.74 ±90.93	780.69 ±205.55	96.9	12.5	694.00 ±169.23	540.00 ±81.43	77.8
20	25	836.54 ±60.25	613.47 ±86.09	73.3	50	836.54 ±60.25	327.92[a] ±49.12	39.2	100	945.74 ±74.41	122.95 ±83.80	13.0
21	3.13	944.34 ±111.48	791.28 ±140.72	83.8	6.25	944.34 ±111.48	938.00 ±120.53	99.3	12.5	945.74 ±74.41	961.68 ±98.62	101.7
22	25	967.68 ±128.50	654.00 ±106.17	67.7	50	981.32 ±117.89	606.31 ±86.62	61.8	100	981.32 ±117.89	462.20[a] ±84.34	47.1
30	12.5	542.68 ±29.49	335.34 ±101.40	61.8	25	643.74 ±92.77	238.33[a] ±42.16	37.0	50	766.78 ±57.41	304.00[a] ±160.07	39.7
32	25.0	562.74 ±40.86	384.00 ±131.36	68.2	50	563.00 ±130.40	425.00 ±100.31	75.5	100	447.00 ±41.08	112.33 ±3.75	25.1
33	25.0	602.38 ±93.75	424.00 ±109.61	70.4	50	602.38 ±93.75	319.67[a] ±108.41	53.1	100	602.38 ±93.75	89.33 ±26.03	14.8
34	25.0	725.67 ±111.25	411.67 ±59.47	56.7	50	725.67 ±111.25	290.00[a] ±55.08	40.0	100	725.67 ±111.25	87.67[a] ±23.68[a]	12.1
35	25.0	447.00 ±41.08	380.32 ±53.35	85.1	50	562.74 ±40.86	197.33[a] ±42.26	35.1	100	542.68 ±29.49	97.34[a] ±42.26	17.9
38	25	743.33 ±139.80	409.00 ±139.47	55.0	50	743.33 ±139.80	403.67 ±50.24	54.3	100	444.66 ±96.48	186.33 ±63.26	41.9

a) $p<0.05$.

probably to loss of hydrogen in agreement with an earlier observation[29] that in the excited states of these compounds, the molecules are in steric arrangements which favor the loss of hydrogen and cyclization of the ring.

Related to these open phenothiazines is 3-hydroxy-3′-nitro-2,2′-dipyridylamine (**38**), the diarylamine precursor of 1,9-diazaphenoxazine. It was prepared by acid-catalyzed condensation of 2-amino-3-hydroxypyridine and 2-chloro-3-nitropyridine. Compounds **15**, **17**—**22**, **30**, **32**—**36** and **38** were tested for pharmacological activities in laboratory animals.

Pharmacology, Results and Discussion

One, five and seven derivatives of open 1,9-diazaphenoxazine, 1,3,6-triazaphenothiazine and 1,3,9-triazaphenothiazine, respectively, were tested in rats and mice in order to determine their effects on the central nervous system using chlorpromazine as the reference compound. Since these compounds closely resemble the closed systems, it is of interest to determine the effect of these modifications on the reported activities of the latter compounds.[37]

Forced and Spontaneous Motor Activity

The effects of chlorpromazine and the thirteen test compounds on forced and spontaneous motor activity were studied and the results are presented in Tables III and IV. At doses of 1, 2 and 4 mg/kg *i.p.* chlorpromazine was more effective than any of the compounds tested. Compounds **17**, **20** and **33** were the only compounds showing any significant dose-dependent decrease in forced motor activity. All of the compounds except compound **21** produced significant inhibition of spontaneous motor activity in the doses used. When toxic doses were administered, the animals suffered clonic-tonic convulsions and died of respiratory arrest. Compound **18** showed no significant effect in doses 3.13, 6.25 and 25 mg/kg *i.p.*, but lower doses (1.5 mg/kg *i.p.*) produced a significant decrease in spontaneous motor activity of approximately 60%, but no effect on forced motor activity, suggesting that the decrease in spontaneous activity was due to an action on the subcortical center. Table V shows the ED_{50} and LD_{50} values of the compounds. The ED_{50} is the dose (calculated from the spontaneous motor activity data) producing a 50% decrease in spontaneous activity (Table V).

Table V. ED_{50}[a] of CPZ and 13 Open Azaphenothiazine and Azaphenoxazine Derivatives

Compounds	LD_{50} mg/kg *i.p.*	ED_{50} mg/kg *i.p.*
CPZ	119	1.20
15	82.40	23.30
17	$200<LD_{50}<300$	80.10
18	26.70	1.86
19	17.60	>12.50
20	$100<LD_{50}<200$	41.50
21	89.70	No effect
22	$200<LD_{50}<300$	85.00
30	>500	24.80
32	>500	52.80
33	>500	53.30
34	>500	33.00
35	>500	46.60
38	>500	48.10

a) Calculated from effect on spontaneous motor activity.

Gross Behavior

Only one dose was used for each of the experimental compounds, usually the highest dose used in the activity studies in mice. Chlorpromazine and twelve of the test compounds

decreased motor activity, but only chlorpromazine and compounds **20** and **33** produced significant effects. All compounds decreased heart rate except **21**, which increased heart rate by 25%. Compounds **33**, **35** and **38** decreased heart rate by 25—30% while **15** only decreased heart rate by 10%. The body temperature was decreased by all compounds except **17**, **18** and **21**. Compounds **15**, **20**, **35** and **38** decreased the rectal temperature by 2°C while **19**, **32** and **34** decreased rectal temperature by only 0.2°C. Ptosis was induced by all compounds except **17**—**19** and **38**, but only chlorpromazine, **33** and **35** produced an effect greater than 50%.

Compound **38** is a deep red powder which produced a dark yellow urine in rats and mice 30 minutes after *i.p.* administration; the urine remained colored for more than 4 h. This compound produced only moderate effects in mice, but when administered to rats (100 mg/kg *i.p.*) it produced significant decreases in heart rate, respiration and body temperature.

Barbiturate Sleeping Time

It is well-known that the short duration of action of hexobarbital sodium is due to its inactivation by the liver microsomal enzyme system, which is influenced by a number of factors including drugs.[53] It is possible for a compound to prolong hexobarbital sleeping time by inhibiting the enzyme system rather than by acting directly on the CNS.

Barbital sodium—unlike other barbiturates—is not biotransformed by liver microsomal enzymes, but is excreted unchanged in the urine,[54] and its duration of action is not influenced

Table VI. Potentiation of Barbital-Sodium Hypnosis

Compound	HD_{80} (mg/kg *i.p.*) with 95% confidence limit
CPZ	3.4 (1.91—6.05)
15	22.5 (13.1—35.3)
17	100.5 (65.8—151.9)
18	18.8 (9.9—35.7)
19	15.8 (19.1—37.2)
20	25.0 (6.4—97.9)
21	12.0 (8.2—17.6)
22	25.3 (HD_{80}—no significance)
30	350 (HD_{80}—no significance)
32	No effect
33	300 (HD_{80}—no significance)
34	No effect
35	108.0 (69.7—167.4)
38	No effect

Table VII. Potentiation of Hexobarbital Sodium Hypnosis

Compound	HD_{80} (mg/kg *i.p.*) with 95% confidence limit
CPZ	1.35 (0.61—2.98)
15	25.50 (10.10—54.80)
17	No effect
18	94.00 (57.40—96.30)
19	No effect
20	45.50 (23.80—87.10)
21	25.00 (14.20—43.80)
22	47.30 (24.40—100.30)
30	60.00 (30.00—120.00)
32	320.00 (133.30—760.00)
33	200.00 (180.00—250.30)
34	80.00 (56.50—150.30)
35	180.00 (60.00—540.00)
38	269.20 (HD_{80}—no significance)

by changes in liver microsomal enzyme activity. A compound which potentiates barbital sodium is more likely to be acting directly on the CNS.

Tables VI and VII illustrate the effects of chlorpromazine and the 13 open azaphenothiazine derivatives on barbital and hexobarbital sleeping time. All compounds except **30**, **32**, **34** and **38** prolonged barbital sleeping time, with chlorpromazine and compounds **15**, **18** and **21** being the most effective. All compounds except **17**, **19**, **32**, **33** and **35** produced an increase in hexobarbital sleeping time.

Anticonvulsant Activity

None of the compounds protected the animals against strychinine or maximal electroshock seizure (M.E.S.) but chlorpromazine, **18**, **19** and **35** prolonged the time for the onset of convulsions due to strychnine while **15**, **19** and **33** prolonged that due to M.E.S. significantly.

Pentylenetetrazole produced both clonic and tonic convulsions, the former occurring approximately 2—3 min after injection, whereas tonic extension of the hind limbs and arching of the back began approximately 10—15 min after the pentylenetetrazole administration, and were usually followed by death. Only compounds **33** and **34** partially protected (40% and 50%) the animals from tonic convulsion and **30** prologed the time for the onset of convulsion (Table VIII).

Table VIII. Effect of CPZ and 13 Open Azaphenothiazine and Azaphenoxazine Derivatives on Onset of Tonic Convulsion in Mice induced by either Strychinine, Pentylenetetrazole or Maximal Electroshock Seizure

Drug	Dose mg/kg *i.p.*	N	Strychinine 2 mg/kg *i.p.* SEC Mean±S.E.	Pentylenetetrazole 100 mg/kg *i.p.* SEC Mean±S.E.	M.E.S. SEC Mean±S.E.
CPZ	1.2	10	256.8[d] ±20.03	1278.81[d] ±128.41	1.99±0.10
30	24.8	10	144.04±12.42	1974.52[d] ±73.10	2.24±0.17
32	53.8	10	152.04±11.08	1197.91±138.16	2.49±0.14
35	46.6	10	126.82[d] ±7.71	1092.31±134.78	2.17±0.14
Vehicle	10 ml	10	173.74±13.65	1146.83±95.36	2.04±0.12
17	80.2	10	186.82±12.77	890.45±37.39	1.26[d] ±0.04
18	3.1	10	283.10±14.78	679.79±128.43	1.32±0.89
19	12.5	10	205.80[d] ±8.43	805.83±49.80	1.50[d] ±0.06
15	23.1	10	163.84±11.40	841.82±11.40	1.56[d] ±0.09
Vehicle	10 ml	10	164.00±8.38	871.17±50.68	1.35±0.02
20	41.5	10	198.04±16.32	233.87[d] ±48.25	1.24±0.10
21	12.5	10	143.89±14.64	757.24±87.63	1.16±0.04
22	91.5	10	95.88[d] ±8.48	461.83±142.74	1.22±0.06
33	5.0	10	153.00±14.55	1362.0[d] ±180.48[a]	1.44[d] ±0.07
34	33.0	10	141.00±12.93	1393.89[d] ±184.03[b]	1.40±0.10
Vehicle	10 ml	10	180.08±14.94	829.91±75.28	1.16±0.04
38	48.1	10	126.00±12.54	964.17±42.00[c]	1.65±0.14
Vehicle	10 ml	10	136.00±12.51	774.63±111.99	1.81±0.20

a) 40% protected.
b) 50% protected.
c) 20% protected.
d) $p<0.05$.

Structure-Activity Relationships

The compounds evaluated in this study are all open triazaphenothiazine and diazaphenoxazine structures, whereas the previous derivatives[37] were the closed systems.

Twelve of the thirteen compounds were divided into two groups as contained in Tables I and II. Group A includes compounds **30**, **32**, **35**, **33** and **34**, which are derivatives of the open 1,3,6-triazaphenothiazine structure. Group B includes compounds **15**, **17**—**21** and **22**, which are open 1,3,9-triazaphenothiazine derivatives. It can be seen from Table V that

both the 6′-methyl and 3′-mercapto groups in the open 1,3,9-triazaphenothiazine systems contribute to the toxicity of the compounds, with compounds **18** and **19** being the most toxic (LD_{50} 's$\leq$30 mg/kg *i.p.*).

All the compounds in group A have *i.p.* LD_{50} 's$\geq$500 mg/kg showing thas the open 1,3,6-triazaphenothiazines are less toxic than the 1,3,9-triaza analogs.

Compounds **20** and **33** produced the greatest decrement in spontaneous and forced moter activity, suggesting cortical depression. Both have chloro groups at R_1 and R_2 while compound **33** has a third chloro group at R_3. Compounds **17**, **32** and **35**, in which $R_1=R_2=NH_2$ were relatively inactive.

Compounds **30** and **35** showed similar effects on gross behavior, even though the dose of **35** was twice as high as that of **30**. They are similar in structure except for the side group R_1 where compound **30** has an SMe group while **35** has an NH_2 group. 50 mg/kg *i.p.* of compound **30** produced a 60% decrease in spontaneous motor activity with no effect on forced motor activity, while the same dose of **35** produced a 65% decrease in spontaneous motor activity and a 5% decrease in forced motor activity. The ED_{50} for **30** was 24.8 mg/kg *i.p.* and that for **35** was 46.6 mg/kg *i.p.* suggesting that replacement of the NH_2 group by SMe increases the central nervous system activity of the compound.

Compound **32** was the only compound that did not prolong either barbital or hexobarbital sleeping time. Compounds **17**, **19**, **33** and **35** were the only other compounds not affecting hexobarbital sleeping time, but they prolonged barbital sleeping time, suggesting a direct effect on the central nervous system and indicating the importance of a negatively charged 3′-mercapto group for producing compounds (**17**, **18** and **19**) with a direct depressant effect on the CNS. Except for compounds **17** and **19**, tho rest of the compounds in groups A and B did affect hexobarbital sleeping time, suggesting that these compounds have an inhibiting effect on liver microsomal enzymes, thereby decreasing the rate of metabolism of hexobarbital.

Compounds **33** and **34** were the only compounds that provided any protection against pentylenetetrazole tonic convulsion and death (40% and 50%). Here a 2,4-dichloro-3′-mercapto combination seems to be of importance, since neither compound **20** (2,4-dichloro-3′-mercapto) nor any of the **12** compounds tested in an earlier study[37] (2,4-dichloro-1,3,9- and 2,4-dichloro-1,3,6-triazaphenothiazine) showed any protection against pentylenetetrazole.

The most effective and one of the least toxic of the compounds tested here is compound **35**. This compound has $LD_{50}>500$ mg/kg *i.p.* and $ED_{50}=46.6$ mg/kg *i.p.* which gives a safety index (LD_{50}/ED_{50}) greater than 10. Compound **35** prolonged both barbital sodium and hexobarbital sleeping times. It produced bradycardia and decreased respiration and body temperature, besides increasing pupil size.

Compounds which differ from one another by being either an open or a closed[37] triaza structure can now be compared as follows.

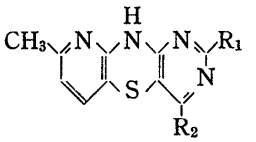
Both open and closed 1,3,6-triazaphenothiazines tested have $LD_{50}>300$ mg/kg *i.p.* while open and closed 1,3,9-triaza-analogs have $LD_{50}<300$ mg/kg *i.p.* The most toxic and least effective compounds were 2-amino-4-hydroxy-8-methyl-1,3,9-triazaphenothiazine (**39**) (Chart 3) and compound **19**, both being inactive and having *i.p.* LD_{50}'s$<$35 mg/kg. Compounds **30** and **40** have almost identical LD_{50}'s (Table IX). In compound **40**, a closed 1,3,6-triaza-compound, $R_1=H$ while in the open system, **30**, it is a methylthio group, suggesting that SMe at the R_1 position does not change either toxicity or effect on spontaneous motor activity. Unfortunately, this is in conflict with the observation made for the 1,3,9-triazaphenothiazine system, since compounds **41** ($R_1=H$) and **21** ($R_1=SMe$) had *i.p.* LD_{50}'s of 30 and 90 mg/kg, respectively. The ED_{50} of **41** was 5.6 mg/kg *i.p.* whereas **21** did not alter spontaneous motor activity. The ED_{50} was higher for all open 1,3,9-triaza structures.

For example, compounds **42** and **22**, which have 2-amino-4-chloro structures, show ED_{50} values of 25.1 and 85.0 mg/kg *i.p.*, respectively. Among the six open and closed 1,3,6-triaza-phenothiazines, (**32**, **35**, **34**, **43**, **44** and **45**) the closed structures, **43** and **45**, have higher ED_{50}'s

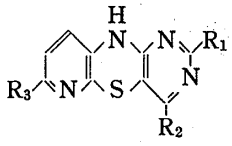
TABLE IX. Comparative ED_{50} Values of Open and Closed Triazaphenothiazines and Phenoxazines

Compound	ED_{50} mg/kg *i.p.*	Compound[b]	ED_{50} mg/kg *i.p.*
A. Open 1,3,6-triazaphenothiazines		Closed 1,3,6-triazaphenothiazines	
30[a]	24.80	**40**[b,c]	22.31
32	52.80	**43**[b]	109.90
33	53.30	**47**[b]	No effect
34	33.00	**45**[b]	46.79
35	46.60	**44**[b]	23.30
B. Open 1,3,9-triazaphenothiazines		Closed 1,3,9-triazaphenothiazines	
17	80.10	**48**[b]	35.50
19	$ED_{50}>LD_{50}$	**39**[b]	$ED_{50}>LD_{50}$
21	No effect	**41**[b]	5.58
22	85.00	**42**[b]	25.14
38[d]	48.10	**46**[b,e]	155.00

a) $R_1=SCH_3$.
b) In the previous paper (C.O. Okafor, M.L. Steenberg and J.P. Buckley, *European J. Chem.*, **12**, 249 (1977)) compounds **39**, **40**, **41**, **42**, **43**, **44**, **45**, **46**, **47** and **48**, were numbered **16**, **21**, **11**, **15**, **27**, **26**, **22**, **28**, **24** and **4**, respectively.
c) $R_1=H$.
d) An open 1,9-diazaphenoxazine.
e) A closed 1,9-diazaphenoxazine.



Compound[a]	R_1	R_2
39:	NH_2	OH
41:	H	NH_2
42:	NH_2	Cl



Compound[a]	R_1	R_2	R_3
40:	H	NH_2	Cl
43:	NH_2	NH_2	OMe
44:	NH_2	NH_2	Cl
45:	Cl	Cl	OMe

a) In our previous paper, (C.O. Okafor, M.L. Steenberg and J.P. Buckley, *European J. Med. Chem.*, **12**, 249 (1977)) compounds **39**, **40**, **41**, **42**, **43**, **44** and **45**, were numbered **16**, **21**, **11**, **15**, **27**, **26** and **22**, respectively.

Chart 3

TABLE X. Effect of Open and Closed Triazaphenothiazine and Phenoxazine Derivatives on Sleeping Time induced by Barbital Sodium

Compound	HD_{80} mg/kg *i.p.*	Compound[a]	HD_{80} mg/kg *i.p.*
A. Open 1,3,6-triazaphenothiazines		Closed 1,3,6-triazaphenothiazines	
32	No effect	**43**[a]	35.0
33	300.00	**47**[a]	No effect
34	No effect	**45**[a]	140.0
35	108.00	**44**[a]	2100
B. Open 1,3,9-triazaphenothiazines		Closed 1,3,9-triazaphenothiazines	
17	100.50	**48**[a]	35.0
19	15.80	**39**[a]	No effect
21	12.00	**41**[a]	13.0
22	23.30	**42**[a]	No effect
38[b]	No effect	**46**[a,c]	300.0

a) In the previous paper (C.O. Okafor, M.L. Steenberg and J.P. Buckley, *European J. Med. Chem.*, **12**, 249 (1977)) compounds **39**, **40**, **41**, **42**, **43**, **44**, **45**, **46**, **47** and **48** were numbered **16**, **21**, **11**, **15**, **27**, **26**, **22**, **28**, **24** and **4**, respectively.
b) An open 1,9-diazaphenoxazine.
c) A closed 1,9-diazaphenoxazine.

than compounds **32** and **34**, while compound **44** has a lower ED_{50} than compound **35** (Table IX).

When compounds with open structures were compared with the closed analogs for effect on sleeping time in mice induced by either barbital or hexobarbital sodium, the following observations were made (Tables X and XI):

TABLE XI. Effect of Open and Closed Triazaphenothiazine and Phenoxazine Derivatives on Sleeping Time induced by Hexobarbital Sodium

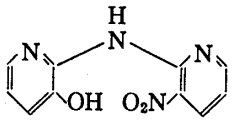
Compound	HD_{80} mg/kg *i.p.*	Compound[a)]	HD_{80} mg/kg *i.p.*
A. Open 1,3,6-triazaphenothiazines		Closed 1,3,6-triazaphenothiazines	
32	320.0	**43**[a)]	200.0
33	200.0	**47**[a)]	100.0
34	80.0	**45**[a)]	77.0
35	180.0	**44**[a)]	41.5
B. Open 1,3,9-triazaphenothiazines		Closed 1,3,9-triazaphenothiazines	
17	No effect	**48**[a)]	210.0
19	No effect	**39**[a)]	No effect
21	25.0	**41**[a)]	41.5
22	47.3	**42**[a)]	133.0
38[b]	269.2	**46**[a,c)]	55.0

a) In the previous paper (C.O. Okafor, M.L. Steenberg and J.P. Buckley, *European J. Med. Chem.*, **12**, 249 (1977)) compounds **39**, **40**, **41**, **42**, **43**, **44**, **45**, **46**, **47** and **48**, were numbered **16**, **21**, **11**, **15**, **27**, **26**, **22**, **28**, **24** and **4**, respectively.
b) An open 1,9-diazaphenoxazine.
c) A closed 1,9-diazaphenoxazine.

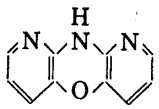
An open 1,3,9-triazaphenothiazine with an —S group in position 3′ does have a greater CNS depressant effect producing a lower HD_{80} for barbital sodium than the corresponding closed 1,3,9-structure, but there is less difference between open and closed 1,3,6-triazaphenothiazines since only compounds **35** and **45** produced a significant HD_{80}. The hypnotic dose HD_{80} for hexobarbital sodium is lower for closed 1,3,6-triazaphenothiazines than for open 1,3,6-triaza-systems, and no general conclusion can be drawn by comparison of the closed with the open 1,3,9-triazaphenothiazines.

None of the closed triazaphenothiazines protected rats against pentylenetetrazole convulsions, whereas compounds **33** and **34** (both open 1,3,6-triaza derivatives) produced 40% and 50% protection, respectively.

As Tables X, XI show, a comparison can be made between compound **38** (the open 1,9-diazaphenoxazine) and the closed 1,9-diazaphenoxazine (**46**).



38



46

Compound **38** has a lower ED_{50} than compound **46** (Table IX). None of the compounds showed any effect on forced motor activity and only compound **38** decreased heart rate, respiration and body temperature. Neither of the compounds had any significant effect on sleeping time induced by barbital sodium, and only compound **46** showed a significant effect on hexobarbital sodium sleeping time. Neither of the compounds showed any anticonvulsant activity. In summary, compound **38** showed greater CNS effects than compound **46**.

Experimental

All melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. UV spectra were taken with a Pye Unicam SP 8000 spectrophotometer using matched 1 cm quartz cells.

The solvent used in all measurements was methanol. The absorption maxima and minima are always given in nanometers; the figures in parenthesis represent the log ε values. IR spectra were obtained on a Perkin Elmer model 137 spectrophotometer using KBr discs unless otherwise stated. NMR spectra were determined on a Varian Associates T-60 instrument. Chemical shifts are reported on the τ scale relative to tetramethylsilane (TMS) used as an internal standard. The letters b, s, d, t, sh and m are used to indicate broad, singlet, doublet, triplet, shoulder and multiplet peaks, respectively. The mass spectra (MS) were obtained with a Perkin-Elmer Hitachi RMU-6E instrument at 70 eV.

2-(2-Amino-4-methylpyrimidin-6-ylamino)-6-methyl-1*H*-pyridinium-3-thiolate (15; $R_1=NH_2$, $R_2=CH_3$)——An intimate mixture of 2-amino-4-chloro-6-methylpyrimidine (13; $R_1=NH_2$, $R_2=CH_3$) (2.87 g, 20 mmol) and 2-amino-3-mercapto-6-methylpyridine (**12**; 2.80 g, 20 mmol) was suspended in 200 ml of water to which 4 ml of concentrated sulfuric acid and 2 g of sodium sulfite had been added. The mixture was then refluxed for 4 h. At the end of the reflux period, the pH of the clear hot solution was raised to 5 by the addition of concentrated ammonia. The solution was cooled in a refrigerator for 2 d. The dull white product that separated out was collected by filtration and crystallized twice from boiling water after addition of Norit A each time. Glistening white plates of 2-(2-amino-4-methylpyrimidin-6-ylamino)-6-methyl-1*H*-pyridinium-3-thiolate (**15**; $R_1=NH_2$, $R_2=CH_3$) were collected, dried and weighed (4.05 g; 82% yield): UV nm (log ε): λ_{max} 205 (4.22), λ_{min} 215 (4.13), λ_{max} 252 (4.28), λ_{min} 280 (3.89), λ_{max} 304 (4.15); IR ν_{max} cm^{-1}: 3330, 3130, 1650, 1635, 1570, 1535, 1330, 1280, 1221, 1195, 868, 820, 785. NMR (in CF_3COOD) τ: 8.07 (s, 6′-CH_3), 7.90 (s, 4-CH_3), 3.87 (s, 5-CH), 3.62 (d, $J=9.2$ Hz, 5′-CH), 2.99 (s, b, 6-NH), 2.75 (d, $J=9.2$ Hz, 4′-CH). MS *m/e* (relative intensity): 51 (31), 54 (14), 66 (12), 67 (13), 80 (4), 93 (3), 95 (4), 106 (3), 107(3), 108(4), 112 (2), 139 (13), 140 (4), 141 (1), 190 (4), 214 (26), 215 (4), 232 (100), 233 (16), 234 (6), 246 (2), 247 [M^+, 32], 248 (4), 249(1), *Anal.* Calcd for $C_{11}H_{13}N_5S$: C, 53.44; H, 5.26; N, 28.34; S, 12.96. Found: C, 53.39; H, 5.20; N, 28.41; S, 13.01.

2-(2,4-Diaminopyrimidin-6-ylamino)-6-methyl-1*H*-pyridinium-3-thiolate (17)——2-Amino-3-mercapto-6-methylpyridine (**12**; 0.7 g, 5 mmol) and 4-chloro-2,6-diaminopyrimidine (0.72 g, 5 mmol) were ground together in a mortar and placed in a reaction flask, to which 100 ml of water and 2 ml of concentrated sulfuric acid were added. The mixture was refluxed with vigorous stirring for 3 h. Upon cooling, a white crystalline precipitate was obtained. The entire mixture was neutralized to pH 8 with concentrated ammonia. The resulting white precipitate dissolved in the basic medium and was reprecipitated by addition of glacial acetic acid and cooling overnight. The solid material was then collected by filtration and recrystallized from aqueous ethanol after treatment with activated charcoal to give glistening white crystals of 2-(2,4-diaminopyrimidin-6-ylamino)-6-methyl-1*H*-pyridinium-3-thiolate, **17** (0.82 g, 66% yield). UV nm (log ε): λ_{max} 209 (4.58), λ_{min} 225 (4.36), λ_{max} 239 (4.38), λ_{min} 265 (3.79), λ_{max} 292 (4.12). IR ν_{max}^{Nujol} cm^{-1}: 3350, 1660, 1620, 1560, 1540, 1285, 1150, 1100, 1055, 1000, 973, 907, 790. NMR (DMSO-d_6) τ: 7.72 (s, 6′-CH_3), 3.45 (d, $J=$ 9.4 Hz, 5′-CH), 2.67 (s, 5-CH), 2.35 (b, 2-NH_2 and 4-NH_2), 2.09 (d, $J=9.4$ Hz, 4′-CH) and 1.30 (s, b, 6-NH). *Anal.* Calcd for $C_{10}H_{12}N_6S$: C, 48.39; H, 4.84; N, 33.87; S, 12.90. Found: C, 48.48; H, 4.99; N, 33.65; S, 12.77.

2-(2,4-Dimethoxypyrimidin-6-ylamino)-6-methyl-1*H*-pyridinium-3-thiolate (18)——A mixture of 2-amino-3-mercapto-6-methylpyridine (**12**; 1.4 g, 10 mmol) and 4-chloro-2,6-dimethoxypyrimidine (1.92 g, 11 mmol) was refluxed in dilute sulfuric acid as described for 2-(2,4-diaminopyrimidin-6-ylamino)-6-methyl-1*H*-pyridinium-3-thiolate (**17**), except that the solution was not made alkaline before the product precipitated out. It was later washed with methanol to remove the unreacted starting materials. The white residue was recrystallized from water–ethanol–acetone mixture (Norit A) to give glistening white plates of 2-(2,4-dimethoxypyrimidin-6-ylamino)-6-methyl-1*H*-pyridinium-3-thiolate (**18**) (1.72 g, 62% yield).

2-(2,4-Dichloropyrimidin-6-ylamino)-6-methyl-1*H*-pyridinium-3-thiolate (20)——2,4,6-Trichloropyrimidine (2 ml) was added to 0.7 g (5 mmol) of 2-amino-3-mercapto-6-methylpyridine (**12**) in 100 ml of water. Concentrated sulfuric acid (2 ml) was gradually added with stirring followed by addition of sodium sulfite, which was used as an antioxidant. The acidic mixture was then refluxed with continuous mechanical agitation for 5 h (complete dissolution occurred as soon as refluxing started). The mixture was cooled to room temperature, and an oily liquid settled at the bottom. Upon cooling at 5°C overnight, the oil solidified and was collected by vacuum filtration. After purification by chromatography, this oil was identified as the excess 2,4,6-trichloropyrimidine. The filtrate after removal of the solidified oil was neutralized with cold concentrated ammonia to pH 8 and chilled overnight. The white product was collected by filtration and recrystallized twice from aqueous methanol (Norit A). Light white glistening plates of 2-(2,4-dichloropyrimidin-6-ylamino)-6-methyl-1*H*-pyridinium-3-thiolate (**20**) were obtained (0.86 g, 60% yield). UV (log ε): λ_{max} 206 (4.39), λ_{min} 217 (4.02), λ_{max} 237 (4.42), λ_{min} 255 (3.94), λ_{max} 262 (3.95), λ_{min} 273 (3.90), λ_{max} 296 (4.05). IR ν_{max}^{Nujol} cm^{-1}: 3340, 3120, 1650, 1575, 1526, 1405, 1250, 1233, 814, 775. NMR (CF_3COOD) τ: 7.56 (s, 6′-CH_3), 3.20 (d, $J=9.4$ Hz, 5′-CH), 3.17 (s, b, 1′-NH, 6-NH), 2.90 (s, 5-CH), 2.25 (d, $J=9.4$ Hz, 4′-CH). NMR (DMSO-d_6) τ: 7.68 (s, 6′-CH_3), 3.51 (d, $J=9.4$ Hz, 5′-CH), 3.49 (s, 1′-NH), 3.01 (s, 5-CH), 2.50 (d, $J=9.4$ Hz, 4′-CH), 2.27 (s, b, 6-NH). *Anal.* Calcd for $C_{10}H_8Cl_2N_4S$: C, 41.81; H, 2.79; Cl, 24.74; N, 19.51; S, 11.15. Found: C, 41.64; H, 2.88; Cl, 24.59; N, 19.56; S, 11.30.

2-(4-Amino-2-methylthiopyrimidin-6-ylamino)-6-methyl-1*H*-pyridinium-3-thiolate (21)——An intimate mixture of 4-amino-6-chloro-2-methylthiopyrimidine (3.86 g, 22 mmol) and 2-amino-3-mercapto-6-methyl-

pyridine (12; 2.8 g, 20 mmol) was refluxed for 5 h in 100 ml of water to which 3 ml of concentrated sulfuric acid and 2 g of sodium sulfite had been added (complete dissolution occurred as soon as boiling commenced). The mixture was cooled to room temperature by allowing it to stand overnight. The white product which precipitated out was collected by vacuum filtration. Neutralization of the acidic filtrate with a little concentrated ammonia led to the recovery of 30 mg of 4-amino-6-chloro-2-methylthiopyrimidine. The white residue was recrystallized from aqueous ethanol (Norit) to yield 2-(4-amino-2-methylthiopyrimidin-6-ylamino)-6-methyl-1*H*-pyridinium-3-thiolate (**21**) as glistening white microneedles (3.63 g, 65% yield). UV nm (log ε): λ_{max} 205 (4.06), λ_{min} 228 (3.39), λ_{max} 260 (4.10), λ_{min} 295 (3.80), λ_{max} 302 (3.81). IR ν_{max}^{Nujol} cm^{-1}: 3330, 3150, 1653, 1555, 1518, 1250, 1106, 823. NMR (CCl_3COOH) τ: 8.20 (s, 6′-CH_3), 5.34 (d, J=7.2 Hz, 5′-CH), 4.95 (s, 5-CH), 4.70 (d, J=7.2 Hz, 4′-CH). *Anal.* Calcd for $C_{11}H_{13}N_5S_2$: C, 47.31; H, 4.66; N, 25.09; S, 22.94. Found: C, 47.22; N, 4.65; N, 25.28; S, 22.99.

2-(2-Amino-4-chloropyrimidin-6-ylamino)-6-methyl-1*H*-pyridinium-3-thiolate (**22**)——2-Amino-3-mercapto-6-methylpyridine (**12**; 0.7 g, 5 mmol) was mixed with 2-amino-4,6-dichloropyrimidine (0.82 g, 5 mmol) and refluxed in dilute sulfuric acid for 3 h as described for 2-(4-amino-2-methylthiopyrimidin-6-ylamino)-6-methyl-1*H*-pyridinium-3-thiolate (**21**). The reaction mixture was cooled, neutralized with concentrated ammonia and filtered. The residue was recrystallized from aqueous ethanol (Norit) to yield 2-(2-amino-4-chloropyrimidin-6-ylamino)-6-methyl-1*H*-pyridinium-3-thiolate (**22**) as yellow microneedles (0.70 g; 52% yield). UV nm (log ε): λ_{max} 204 (4.24), λ_{min} 215 (4.13), λ_{max} 247 (4.34), λ_{min} 280 (3.85), λ_{max} 306 (4.09). IR ν_{max}^{Nujol} cm^{-1}: 3330 d, 3110, 1650, 1636, 1575, 1548, 1520, 1430, 1400, 1225, 1018, 818, 780. NMR (DMSO-d_6) τ: 7.65 (s, 6′-CH_3), 3.37 (d, J=9.4 Hz, 5′-CH), 3.52 (s, 1′-NH), 2.92 (s, 5-CH), 2.43 (b, 2-NH_2), 2.15 (d, J=9.4 Hz, 4′-CH) and 1.88 (s, b, 6-NH). *Anal.* Calcd for $C_{10}H_{10}ClN_5S$: C, 44.86; H, 3.74; Cl, 12.27; N, 26.17; S, 11.96. Found: C, 44.84; H, 3.93; Cl, 12.26; N, 26.09; S, 12.02.

Reduction of 2-Methoxy-5-nitropyridine and Conversion to 2-Amino-5-methoxythiazolo[5,4-*b*]pyridine (**28**, **R=OMe**)——This compound was obtained by extensive modification of the previously described method. Reduction of 2-methoxy-5-nitropyridine was conveniently carried out by using iron dust and calcium chloride in 80% ethanol.

2-Methoxy-5-nitropyridine (30.8 g, 200 mmol) was refluxed on a steam bath in 160 ml of 85% ethanol in the presence of 100 g of iron dust and 10 g of calcium chloride for 2 h. The dark brown solution was filtered hot to remove excess iron. The resulting clear solution was heated with activated charcoal, boiled for 15 min and filtered. The solvent was removed by evaporation on a steam bath leaving 3-amino-6-methoxypyridine as a deep red oil after chromatography (24.06 g, 97%), n_D^{20} 1.5728. This product was used satisfactorily in the next stage of the reaction without further purification.

3-Amino-6-methoxypyridine thus obtained was converted to 2-amino-5-methoxythiazolo[5,4-*b*]-pyridine (**28**; R=OMe) by the action of potassium thiocyanate and bromine in glacial acetic acid at 0°C to 5°C as described previously.[28]

2-Amino-5-chlorothiazolo[5,4-*b*]-pyridine (**28**; **R=Cl**)——2-Chloro-5-nitropyridine (15.85 g, 100 mmol) was converted to 3-amino-6-chloropyridine using iron dust (80 g) and calcium chloride (5 g) in 80 ml of 85% ethanol, as described for 3-amino-6-methoxypyridine. The product was converted to 2-amino-5-chlorothiazolo[5,4-*b*]-pyridine (**28**; R=Cl) by the previously described method.[28]

***N*-6′-Chloro-2′[1*H*]-thioxo-3′-pyridyl-4-amino-2-methylthio-6-pyrimidinylamine** (**30**; **R_1=SMe, R_2=NH_2, R_3=Cl**)——To a suspension of 4-amino-6-chloro-2-methylthiopyrimidine (**29**) (1.76 g, 10 mmol) and 3-amino-6-chloropyridine-2[1*H*]-thione (**23**; R=Cl) (1.61 g, 10 mmol) in 100 ml of water was added 1 ml of concentrated sulfuric acid and 1 g of sodium sulfite. The mixture was refluxed on a steam bath at 92°C for 5 h. Complete dissolution was achieved within 10 min. The greenish-yellow solution was treated with activated charcoal, boiled for an additional 15 min, and filtered hot. The filtrate was cooled, neutralized with concentrated ammonia, and cooled further overnight. The yellow product was collected by filtration and crystallized from aqueous acetone (Norit A) to yield *N*-6′-chloro-2′[1*H*]-thioxo-3′-pyridyl-4-amino-2-methylthio-6-pyrimidinylamine (**30**; R_1=SMe, R_2=NH_2, R_3=Cl) as a yellow powder (1.80 g; 60% yield). UV nm (log ε): λ_{max} 221 (4.49), λ_{min} 246 (4.18), λ_{max} 269 (4.32), λ_{min} 287 (4.00), λ_{max} 297 (4.04). IR ν_{max}^{KBr} cm^{-1}: 3430, 3280, 3130, 1610, 1576, 1545, 1520, 1480, 1425, 1395, 1380, 1340, 1312, 1266, 1250, 1190, 1120, 1110, 1090, 1062, 950, 871, 850, 822, 760. NMR (DMSO-d_6) τ: 7.57 (s, 2-SMe), 4.14 (s, 5-CH), 3.47 (b, d, 4-NH_2), 3.13 (s, b, 6-NH), 2.40 (d, J=9.2 Hz, 5′-CH), 1.73 (J=9.2 Hz, 4′-CH), 1.24 (s, b, 1′-NH). MS *m/e* (rel. intensity): 91 (56), 94 (32), 105 (100), 129 (28), 133 (44), 175 (38), 255 (12), 257 (4), 284 (8), 297 [M^+, 40], 299 (16). *Anal.* Calcd for $C_{10}H_{10}ClN_5S_2$: C, 40.07; H, 3.34; Cl, 11.85; N, 23.37; S, 21.37. Found: C, 39.96; H, 3.40; Cl, 11.70; N, 23.31; S, 21.38.

2′[1*H*]-Thioxo-2,4,6′-trichloro-3′-pyridyl-6-pyrimidinylamine (**33**)——A mixture of 3-amino-6-chloropyridine-2[1*H*]-thione (**23**; R=Cl) (3.21 g, 20 mmol) and 2,4,6-trichloropyrimidine (4.04 g, 22 mmol) in 250 ml of water, to which was added 3 ml of concentrated sulfuric acid and 2 g of sodium sulfite, was refluxed on a steam bath for 3 h. A greenish-yellow precipitate formed which remained solid until the end of the reflux period. This product was collected by filtration and recrystallized from acetone–methanol–water mixture to give 2′[1*H*]-thioxo-2,4,6′-trichloro-3′-pyridyl-6-pyrimidinylamine (**33**) as a yellow powder (2.89 g; 47% yield). UV nm (log ε): λ_{max} 290 (3.60), λ_{min} 273 (3.55), λ_{max} 250 (3.68), λ_{min} 240 (3.66). IR ν_{max}^{KBr} cm^{-1}: 1660, 1575, 1540, 1520, 1460, 1418, 1376, 1365, 1266, 1212, 1140, 1120, 1075, 990, 827 cm^{-1}. NMR (DMSO-d_6) τ:

3.15 (d, J=8.6 Hz, 5′-CH), 2.67 (d, J=8.6 Hz, 4′-CH), 1.89 (s, 5-CH) and 0.73 (s, b, 6-NH). *Anal.* Calcd for $C_9H_5Cl_3N_4S$: C, 35.24; H, 1.63; Cl, 34.42; N, 18.27; S, 10.44. Found: C, 35.35; H, 1.56; Cl, 34.49; N, 18.22; S, 10.50.

***N*-6′-Methoxy-2′[1*H*]-thioxo-3′-pyridyl-2,4-dichloro-6-pyrimidinylamine** (**34**)——This compound was obtained by refluxing an equimolar acidic mixture of 5-amino-6-methoxypyridine-2[1*H*]-thione (**23**; R=OMe) (17.16 g; 11 mmol) and 2,4,6-trichloropyrimidine (2.02 g; 11 mmol) as described for compound **33**. *N*-6′-Methoxy-2′[1*H*]-thioxo-3′-pyridyl-2,4-dichloro-6-pyrimidinylamine (**34**) was obtained as a yellowish powder (2.43 g, 73% yield). UV nm (log ε): λ_{max} 295 (3.86), λ_{min} 275 (3.83), λ_{max} 250 (3.97), λ_{min} 230 (3.88). IR ν_{max}^{KBr} cm^{-1}: 1570 b, 1468, 1419, 1377, 1365, 1310, 1260, 1210, 1175, 1116, 1067, 1022, 890, 830. NMR (DMSO-d_6) τ: 6.17 (s, 6′-OMe), 3.00 (d, J=8.8 Hz, 5′-CH), 2.57 (d, J=8.8 Hz, 4′-CH), 2.39 (s, 5-CH) and 0.90 (s, b, 6-NH). *Anal.* Calcd for $C_{10}H_8Cl_2N_4OS$: C, 39.60; H, 2.64; Cl, 23.43; N, 18.48; S, 10.56. Found: C, 39.44; H, 2.73; Cl, 23.60; N, 18.49; S, 10.62.

***N*-6′-Chloro-2′[1*H*]-thioxo-3′-pyridyl-2-amino-4-hydroxy-6-pyrimidinylamine** (**36**)——This compound was obtained from a mixture of 2-amino-4-chloro-6-hydroxypyrimidine (1.46 g, 10 mmol) and 3-amino-6-chloropyridine-2[1*H*]-thione (**23**; R=Cl) (1.61 g, 10 mmol) as described for *N*-6′-chloro-2′[1*H*]-thioxo-3′-pyridyl-4-amino-2-methylthio-6-pyrimidinylamine (**30**), except that the reaction mixture was not neutralized with concentrated ammonia. The product was recrystallized from aqueous acetone (Norit A) to yield *N*-6′-chloro-2′[1*H*]-thioxo-3′-pyridyl-2-amino-4-hydroxy-6-pyrimidinylamine (**36**) as a yellowish-green powder (1.99 g, 74% yield). UV nm (log ε): λ_{max} 220 (4.48), λ_{min} 235 (4.16), λ_{max} 263 (4.50), λ_{min} 310 (3.32), λ_{max} 370 (3.77). IR ν_{max}^{Nujol} cm^{-1}: 3280, 3110, 1650, 1550, 1475, 1370, 1280, 1241, 1110, 1090, 1040, 960, 878, 822, 755. NMR (DMSO-d_6) τ: 3.19 (d, J=8.8 Hz, 5′-CH), 2.70 (s, 2-NH$_2$), 2.43 (d, J=8.8 Hz, 4′-CH), 2.00 (s, 5-CH), 1.64 (s, 6-NH) and 0.05 (s, b, 4-OH). *Anal.* Calcd for $C_9H_8N_5ClOS$: C, 40.07; H, 2.97; Cl, 13.18; N, 25.97; S, 11.88. Found: C, 40.21; H, 2.96; Cl, 13.03; N, 26.00; S, 11.77.

***N*-6′-Methoxy-2′[1*H*]-thioxo-3′-pyridyl-2-amino-4-hydroxy-6-pyrimidinylamine** (**37**)——An acidic mixture of 3-amino-6-methoxypyridine-2[1*H*]-thione (**23**; R=OMe) (1.56 g, 10 mmol) and 2-amino-4-chloro-6-hydroxypyrimidine (1.60 g, 11 mmol) was refluxed for 5 h as described for *N*-6′-chloro-2′[1*H*]-thioxo-3′-pyridyl-4-amino-2-methylthio-6-pyrimidinylamine (**30**) but without neutralization with ammonia. Extensive frothing which occurred during the reaction was checked by the use of a large reaction flask and the addition of water from the condenser. The yellowish-green product was recrystallized from aqueous acetone to yield *N*-6′-methoxy-2′[1*H*]-thioxo-3′-pyridyl-2-amino-4-hydroxy-6-pyrimidinylamine (**37**) as a yellowish-green powder (1.83 g, 69% yield). UV nm (log ε): λ_{min} 210 (4.16), λ_{max} 217 (4.18), λ_{min} 233 (4.03), λ_{max} 253 (4.11), λ_{min} 287 (3.85), λ_{max} 293 (3.84), λ_{min} 339 (3.47), λ_{max} 350 (3.50). IR ν_{max}^{Nujol} cm^{-1}: 3300, 3110, 1680, 1625, 1570, 1478, 1417, 1520, 1258, 1200, 1100, 1021, 813. NMR (DMSO-d_6) τ: 6.20 (s, 6′-OMe), 3.23 (d, J=8.8 Hz, 5′-CH), 2.97 (s, 2-NH$_2$), 2.50 (d, J=8.8 Hz, 4′-CH), 2.04 (s, 5-CH), 1.55 (s, b, 6-NH) and 0.06 (s, b, 4-OH). *Anal.* Calcd for $C_{10}H_{11}N_5O_2S$: C, 45.29; H, 4.15; N, 26.42; S, 12.08. Found: C, 45.50; H, 4.16; N, 26.33; S, 12.11.

3-Hydroxy-3′-nitro-di-2-pyridylamine (**38**)——This compound was prepared from 2-amino-3-hydroxypyridine and 2-chloro-3-nitropyridine according to method A described in the previous paper.[36]

Pharmacological Methods and Materials——Male Swiss-Webster mice[55] weighing 20—25 g and Wistar Descentent rats[55] weighing 200—250 g were used after being acclimatized to laboratory conditions for 3—4 d. Each dose was usually tested in 6 mice and 5 rats, which were permitted food and water *ad libitum*. Since only chlorpromazine (CPZ) was soluble in saline, the 13 test compounds were suspended in 5% Tween-80+95% isotonic saline and administered in doses of 10 ml/kg *i.p.* to mice and 1 ml/kg *i.p.* to rats. The injection time was over a period of 15 s. Results that differed from control values at the $p<0.05$ level (student t-test) were considered to be statistically significant.

Forced and Spontaneous Motor Activity——The effects of chlorpromazine and the 13 open phenothiazine and phenoxazine compounds on forced motor activity of mice were studied by the use of a rotarod. The wooden rod rotated at 4 rpm for the first 30 s, 6 rpm during the next 30 s and at progressively increasing speeds thereafter at 30 s intervals (maximum 50 rpm) until the mouse fell off.[56,57] Six animals were tested simultaneously and were given 4 trials with two spaced 4—6 h apart on each of two consecutive days. The fourth trial was preceded by an interval of 50 min for the administration of vehicle or one of the selected doses of a test compound. The drug or placebo effect for each animal was computed as performance time on the fourth trial divided by performance time on the third trial.

The effects of these compounds on the spontaneous motor activity in mice were measured in three photocell cages (Actophotometer, Woodard Research Corporation) in which two animals treated with identical doses of the same compound were placed in each photocell cage 10 min after the start of the rotarod test, and a 15 min count was initiated 5 min after the animals were placed in the Actophotometer cages. Each dose was tested in a factorial design in each of the three activity cages in order to negate differences in sensitivity among units. Control animals were tested simultaneously at the same time intervals after administration of an equal volume of vehicle, and the ED_{50} of each compound (defined as the dose which decreased the level of performance to 50% of the control scores) was calculated.

The percent of control activity (spontaneous and forced motor activity) at each dose level of the com-

pounds used was plotted in an attempt to make an early evaluation of the possible types of activity of each compound.

Determination of LD_{50}'s——The LD_{50} was calculated by determining the number of animals alive and dead 24 h following administration of the compound *i.p.* The percentage of dead animals was plotted for each dose and the dose killing 50% of the animals (LD_{50}) was determined for each compound. A minimum of 3 doses was used for each compound.[37,58]

Gross Behavior in Rats——All fourteen compounds were evaluated according to a gross observation rating scale as described by Watzman *et al.*[59] The time-course of the drug effect was ascertained by checking items on the scale at 15 min prior to and 30, 60, 120 and 180 min following drug administration with special emphasis on behavioral and autonomic effects. Only one dose was used for each compound and this dose was the same as the highest dose used to determine the ED_{50}.

Potentiation of Barbital Sodium and Hexobarbital Sodium Sleeping Time——Using a method described by Fujimori,[60] HD_{20} was determined for both barbital sodium and hexobarbital sodium. Male mice (20—25 g) in groups of 10 received various doses of barbital sodium or hexobarbital sodium intraperitoneally in order to estimate the dose causing 20% of the mice to lose their righting reflux for at least 5 min during the first hour following injection of one of the two barbiturates. The HD_{20} for barbital sodium was 124 (100.8—142.8) mg/kg *i.p.* and that for hexobarbital sodium was 34.0 (27.4—42.4 mg/kg *i.p.*), determined according to the method of Litchfield and Wilcoxon.[53] Male mice (20—25 g) in groups 10 received either saline or one of three doses of each compound—the same doses as used to determine ED_{50}—60 min prior to the administration of the HD_{20} of either barbital sodium of hexobarbital sodium.

The dose of each compound which would produce hypnosis in 80% of the animals (HD_{80}) in combination with HD_{20} of the barbiturate was determined, using the Litchfield and Wilcoxon method.[61]

Anticonvulsant Activity——The effects of the compounds on convulsions and death produced by strychnine sulfate (2 mg/kg *i.p.*) and pentylenetetrazole (100 mg/kg *s.c.*) were studied in mice as described by Watzman *et al.*[59] and those on maximal electroschock seizure (M.E.S.) (60 mA intensity and 0.2 s duration) were studied as described by Swinyard, Brown and Goodman.[62] The doses of chlorpromazine and of the experimental compounds were identical to the ED_{50} values found in the spontaneous motor activity studies.

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