Synthesis and Pharmacological Properties of 4-Piperazino-5-methylthiopyrimidines. Selection of New Antiemetic Agents

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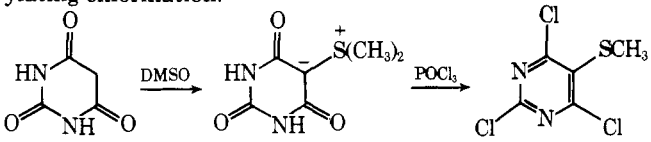
We have synthetized a series of 22 new 4-piperazinopyrimidines bearing a methylthio substituent in the 5 position of the pyrimidine ring. These compounds have been obtained by separation of the isomers formed during nucleophilic attack of the corresponding 2,4,6-trichloropyrimidine by amines. Pharmacological screening has shown that this chemical series displays a very interesting profile, which includes antiemetic, tranquilizing, analgesic, antiserotonin, and musculotropic-spasmolytic properties. We have particularly selected for clinical investigations two compounds with powerful antiemetic activity: 2-methylamino-4-(*N*-methylpiperazino)-5-methylthio-6-chloropyrimidine and 2-isopropylamino-4-(*N*-methylpiperazino)-5-methylthio-6-chloropyrimidine.

Derivatives of 5-methylthiobarbituric acid have only recently been obtained. 2,4,6-Trichloro-5-methylthiopyrimidine was prepared by Razavi, using the dimethyl sulfoxide (DMSO) reaction on barbituric acid, followed by a demethylating chlorination.[1]



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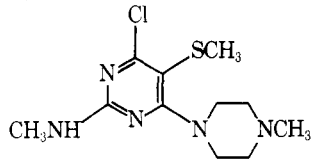
Nucleophilic attack of cyclic carbons bearing activated chlorine atoms, in particular by amines, led us to suppose that it would be possible to synthesize, on the basis of this compound, molecules with antifungal or antimalarial properties[2,3] like those of pyrimethamine (4753 RP) and diaveridine. During the course of pharmacological screening, however, our attention was first attracted by the psychotropic properties of **3**.



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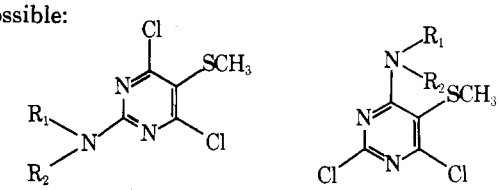
This observation encouraged us to pursue the study of the 4-piperazinopyrimidine structure. The initial results are reported in this article.

Chemistry. The synthesis of 2,4,6-trichloro-5-methylthiopyrimidine (1) is reported in the Experimental Section.[1] Obtainance of monosubstituted derivatives by reaction of primary or secondary amines with the trichlorinated molecule gives rise to a mixture of isomers bearing the amino substituent in either position 2 or 4. As will be established below, the isomer ratio varies little as a function of the nucleophilic agent used. This ratio is approximately 60% of the product substituted in position 4, the amount being determined by gas–liquid chromatography (GC). The separation of these isomers can in certain cases occur spontaneously during the reaction, in which the less soluble 2-substituted isomer is partly precipitated (procedure A). In other cases the mixture of the two isomers was dissolved in concentrated hydrochloric acid solution. Following dilution with a small quantity of water, the 2-substituted isomer was precipitated as a result of its less basic character (procedure B). Subsequent condensations with various piperazines were carried out starting from the 4,6-dichloro-2-aminopyrimidines. A third case is one in which the isomers, formed during the first substitution, are present in the liquid state and cannot be separated by procedures A or B. It is possible in this case to separate the isomers, obtained after a second substitution by a piperazinyl group, because their solubilities are different in the solvents. The 4-piperazino derivative is, in general, less soluble (procedure C).

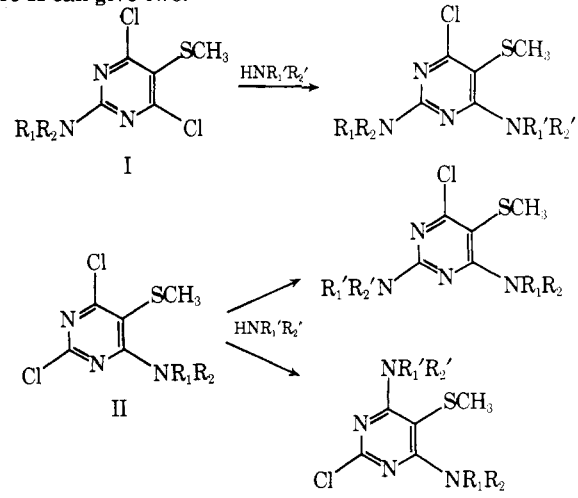
Two monosubstitution isomers of 1 by one amino group are possible:



I II

In fact, in all the cases studied, two products which can, in general, be isolated in the pure state and have the overall formula and the NMR signals expected, are obtained. It is necessary to determine which one of them corresponds to formula I and which to formula II. First of all, the physical and chemical properties of the pairs of isomers obtained differentiate them so clearly that they can be classed without ambiguity into two categories, A and B. Thus, the demonstration of the structure could be limited to a single example. Nevertheless, an unambiguous structure determined was made in two cases; one of these will be reported here.

Three independent proofs of this structure will be given. The substitution of each of the two monoamino isomers with a second amino residue permits the determination of the position of the first amino group, since structure I can only give one disubstituted amino isomer, whereas structure II can give two:



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II

Table I. Chemical Shifts of ^1H in Monoamino Isomers[a]

	Type A	Type B
Methylamino	2.35	2.30
Ethylamino	2.37	2.30
Isopropylamino	2.34	2.29
Dimethylamino	2.35	2.32
Diethylamino	2.32	2.30
Di-*n*-propylamino	2.33	2.31
(Trichloro-5-methylthiopyrimidine, 2.57)		

[a] δ in $CDCl_3$ (ppm) for ^1H in CH_3S group.

Table II. Chemical Shifts of ^1H in Monoamino Isomers[a]

	Type A	Type B
Methylamino δ CH_3NH	3.06	3.11
Ethylamino δ CH_2NH	3.50	3.55
δ CH_3CH_2NH	1.24	1.28
Isopropylamino δ $CHNH$	4.20	4.25
δ $(CH_3)_2CHNH$	1.23	1.28
Dimethylamino δ $(CH_3)_2N$	3.20	3.35
Diethylamino δ $(CH_3CH_2)_2N$	3.61	3.75
δ $(CH_3CH_2)_2N$	1.20	1.27
Di-*n*-propylamino δ $(CH_3CH_2CH_2)_2N$	3.54	3.65

[a] δ in $CDCl_3$ (ppm) for ^1H in alkylamino groups.

If, therefore, two diamino-substituted isomers are isolated and identified without ambiguity when starting from one of the two monoamino products, the precursor monoamino must have had structure II. This work has been carried out on two examples, i.e., the pair of methylamino and ethylamino isomers. Only the first of these will be described. Another proof was deduced from the ^{13}C NMR spectra; carbons 4 and 6 of the pyrimidine nucleus should be magnetically equivalent for structure I (symmetrical formula) and nonequivalent for structure II. A third proof was furnished by a unequivocal synthesis of the I methylamino isomer.

The isomer pairs which can be isolated in the pure form by hydrolysis of hydrochlorides and recrystallization, or by GC, in all cases studied have constant differences in their properties, which enable them to be immediately allocated to one of categories A or B. Thus, isomers of type A are weaker bases (hydrochlorides which more easily undergo hydrolysis than those of type B). Secondly, it is possible to observe ir absorptions which are characteristic of one or the other type (apart from the bands reliable to the radicals R_1 and R_2). Particularly, an intensive band between 860 and 825 cm^{-1} is specific to isomers of type B. Finally, NMR chemical shifts (in the same solvent, Tables I and II) show consistent differences. The protons of the methylthio group are deshielded to a greater extent for isomers of type A. On the other hand, the protons of the hydrocarbon groups upon nitrogen are less deshielded for these same isomers: $\delta_{(A)}$ $CH_3S > \delta_{(B)}$ CH_3S; $\delta_{(A)}$ $CH_nN < \delta_{(B)}$ CH_nN.

In compounds where the amino group is secondary, differences also arise in the physical and spectroscopic properties due to the existence—in one of the isomers only—of intermolecular hydrogen bonds. These differences distinguish type A and B isomers even more clearly. Type A products have in fact higher melting points than type B products. They are also less soluble in organic solvents, and in GC their retention times are increased to a greater extent than type B compounds, when passing from a nonpolar to a polar stationary phase. The ir spectra in the condensed state of products of type A have ν N–H band, which is enlarged and of lower frequency (toward 3280–3250 cm^{-1}; NH "bound"), in contrast to that of the type B products, where the ν N–H band is present at about 3360 cm^{-1} (N–H free or "weakly bound"). By dilution in nonpolar solvents, for compounds of type A a sharp band appears at high frequency (~3450 cm^{-1}; "free" NH) whereas the broad band at about 3280–3250 cm^{-1} diminishes in intensity, which shows clearly the intermolecular nature of the H bonds of the compounds of type A. On the contrary, the NH band of the compounds of type B is little affected by dilution in nonpolar solvents; it remains as a single absorption band and shifts only from 3360 to ~3390 cm^{-1}, which shows the absence of a form associated by strong intermolecular H bonds (Table II).

Table III. Chemical Shifts of ^1H in Di(amino-substituted) Isomers[a]

Isomers	A_1 (2-methylamino-4-isopropylamino)		B_1 (4-methylamino-2-isopropylamino)		B_2 (4-methylamino-6-isopropylamino)
δ CH_3S	2.18	=	2.18	>	2.08
δ CH_3NH	2.98	<	3.02	≃	3.017
δ $CHNH$	~4.29	>	4.15	<	4.31

The following describes our study of the bis(amino) derivatives obtained from a pair of monoamino isomers. We describe the introduction on the two monomethylamino isomers (types A and B) of an isopropylamino group and the complete identification of the three dialkylamino isomers obtained as described in the Experimental Section. The methylaminodichloro-5-methylthiopyrimidine of type A gave a single product A_1, isolated practically pure at the onset, by precipitation during the reaction with isopropylamine. Examination by GC of the precipitate and of the mother liquor of the reaction at the same time did not reveal a detectable quantity of other reaction products. The methylaminodichloro-5-methylthiopyrimidine of type B gave two products, the one preponderant B_1, the other in a much lower proportion B_2 (~7%). There was no spontaneous separation in this case. These two products were separated and purified by GC. It was checked spectroscopically (ir and NMR) that the GC separation did not alter the structure of these compounds. The three products thus isolated, A_1, B_1, and B_2, correspond to the formula expected for the methylaminoisopropylaminochloromethylthiopyrimidines, $C_9H_{15}N_4SCl$. They possess, in GC, relative retention times corresponding, according to our observation in the series, to di(alkylamino) derivatives of this molecular weight. Their ir absorption bands are characteristic of the expected groups and their NMR spectra show quantitatively all the types of protons expected for only these types. The chemical shifts observed in the NMR of the three products permitted us to forecast the substitution positions (Table III).

These results dismiss the hypothesis of a possible transamination, which would give either an isopropylaminodichloro-5-methylthiopyrimidine or, more probably, a di(isopropylamino)chloro-5-methylthiopyrimidine. Further support of the assigned structures was afforded by unambiguous synthesis of one of the products.

In conclusion, the fact of production of two bis(amino) isomers B_1 and B_2 of well-established structure from the methylamino derivative of type B shows that the amino group of this derivative is at position 4. Consequently, the amino group in the derivative of type A must be in position

Table IV. ^{13}C Chemical Shifts of Studied Pyrimidines

Position of C	Pyrimidine	2,4,6-Trichloro-5-methylthio	Monomethylamino of type A	Monomethylamino of type B
C(5)	122.1	129.6	113.9	109.4
C(2)	159.5	156.0	167.0	159.2
C(4)	157.5	165.0	160.6	164.7
C(6)	157.5	165.0	160.6	163.3

2. The bis(amino) product A_1 obtained from A is therefore 2-methylamino-4-isopropylamino-6-chloro-5-methylthiopyrimidine. For the products B_1 and B_2 obtained from B (4-methylamino), it remains to be shown which is the 2,4-bis(amino) and which the 4,6-bis(amino); consideration of the ir and NMR spectra permits this point to be established.

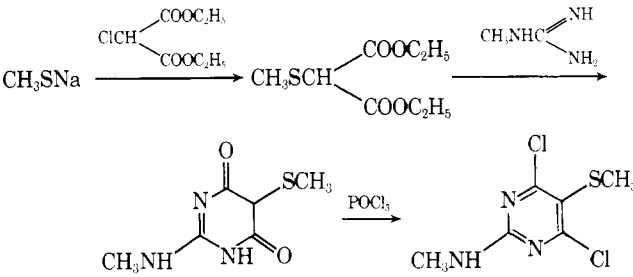
The product formed in lower proportion B_2 shows only one NH band at about 3380 cm^{-1} ("free" or "weakly associated" NH). It therefore does not form associations by H bonds, and the substitution positions of the RNH groups appear to be of the same type (mixed NH bands), therefore in 4 and 6. On the contrary, the preponderant product B_1 possesses at the same time a sharp "free NH" band at 3365 cm^{-1} and a wide "bonded NH" band at about 3265 cm^{-1}; it thus appears entirely similar to the product A_1 previously identified, of which the 2-methylamino-4-isopropylamino groups occupy positions of different types. This analogy leads us to attribute to the compound B_1 the structure 4-methylamino-2-isopropylamino-6-chloro-5-methylthiopyrimidine. Moreover, this reasoning is consistent with our many observations on monoamines in the condensed state; i.e., a "free NH" band is observed in 4-RNH-substituted pyrimidine of this type whereas a "bonded NH" band is noted in 2-RNH-substituted analogs. In particular, the nature of the radical R does not introduce irregularities in these observations for the cases studied.

The comparison of the NMR chemical shifts of the signals of the protons CH_3S, CH_3NH, and $CHNH$ leads to the same conclusion, for, if it is adopted, they are classed strictly in the order which could be foreseen in terms of their positions on the pyrimidine ring, according to our observations on all the monoamine derivatives which we have studied (as well as on the trialkoxymethylthiopyrimidines, unpublished work).

The ^{13}C NMR of the methylaminodichloromethylthiopyrimidine isomers of types A and B spectra were determined on the solutions in $CDCl_3$ at 100 MHz, with the Varian XL-100 apparatus, by Fourier transform, with decoupling of protons (and also without decoupling), in solution in $CDCl_3$, CD_3COCD_3, or $CDCl_3$ + DMSO-d_6. Observations to the signals of ^{13}C in the pyrimidine nucleus we noted were three signals at 113.9, 160.5, and 167.0 ppm relative to TMS for the methylaminopyrimidine of type A and four signals at 109.4, 159.2, 163.3, and 164.7 ppm for the methylaminopyrimidine of type B. The comparison with the chemical shifts of ^{13}C in the nonsubstituted pyrimidine and in the 2,4,6-trichloro-5-methylthiopyrimidine, as well as the coupling with ^1H or D in the spectra of nondecoupled ^{13}C (Table IV), permits their identification.

Thus, the isomer of type B has four different ^{13}C, whereas for the isomer of type A, two ^{13}C are equivalent. These observations can only be compatible with the methylamino group being in the 2 position of type A and in the 4 position for the product of type B. Thus, compounds of type A clearly have the structure I and those of type B have structure II.

Final proof of structure was established by unambiguous synthesis of the type I isomer. In the case where R_1 = H, R_2 = CH_3, the isomers of types I (mp 158–160°) and II (mp 108–110°) are isolated. The unequivocal synthesis of the isomer of structure I fully confirmed the structure assigned on the basis of the spectral data. The unambiguous synthesis, which is described in the Experimental Section, was carried out as follows.



The 2-amino-4,6-dichloro-5-methylthiopyrimidines (type I monoamino isomers) were condensed with a piperazine in the presence of a tertiary base, in order to give the products described in Table V. The isomers of type II were condensed with a piperazine give a series of products which did not display good results during the preliminary pharmacological screening. For this reason, these results are not reported here. The last chlorine atom of these molecules cannot be substituted by a third amino group under the conditions employed to prepare mono- and diamino derivatives. On the other hand, the action of alcoholates enables alkoxy derivatives to be easily obtained. A similar homolog with no sulfur atom was prepared for comparative purposes.

Results and Discussion

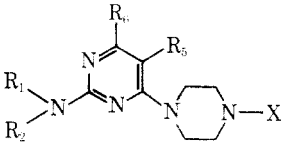
Pharmacology. These new 4-piperazino-5-methylthiopyrimidine derivatives have been subjected to a wide range of pharmacological screening including approximate determination of the acute toxicity in mice and a series of tests intended to reveal possible pharmacological and metabolic effects.

In this paper, we adopted only a limited number of methods to define the pharmacological spectrum of these products and to illustrate the effect of modification of the nature and position of the substituents introduced on pyrimidine ring on pharmacological activity. The toxico-pharmacological results are recorded in Table VI, on the basis of the chemical structure.

Taken overall, the oral and intravenous acute toxicities in the mouse of these 4-piperazino-5-methylthiopyrimidine derivatives are moderate and fairly homogeneous. Intravenously the LD_{50} of the compounds which are soluble in water or in 0.9% NaCl solution are within the range 30–125 mg/kg. Orally the LD_{50} are within the range 200–600 mg/kg, apart from **19** where the LD_{50} was found to be above the highest dose of 900 mg/kg po.

The basic chemical structure itself implies a potential musculotropic–spasmolytic activity. Its intensity appears to be modified by the nature of the substituents of the $-NH_2$ function in position 2, the piperazinyl group in position 4, and the nature of the 6-substituent of the pyrimidine ring. The introduction on the –NHR function in position 2 on the ring of a branched or oxidized aliphatic chain produces a definite increase in this activity (**4, 5, 6,** and **13**), as does also the introduction of a hydroxyethyl chain on the piperazine (**20**) or the substitution of the –Cl in posi-

Table V. Physicochemical Properties of the Prepared 4-Piperazinopyrimidines[h]



No.	R_1R_2N–	X	R_5	R_6	Salt	Mp, °C (Kofler)	Solvent
2	Amino	CH_3	CH_3S	Cl	*a*	192	*d*
3	Methylamino	CH_3	CH_3S	Cl	*a*	121–122	*d*
4	Ethylamino	CH_3	CH_3S	Cl	*a*	101	*d*
5	Isopropylamino	CH_3	CH_3S	Cl	*a*	145–146	*d*
6	*tert*-Butylamino	CH_3	CH_3S	Cl	*a*	157	*d*
7	Anilino	CH_3	CH_3S	Cl	*a*	129–130	*d*
8	*N*-Methylanilino	CH_3	CH_3S	Cl	*b*	220 dec	*d*
9	Diethylamino	CH_3	CH_3S	Cl	*c*	248 dec	*d*
10	Piperidino	CH_3	CH_3S	Cl	*b*	215 dec	*d*
11	Morpholino	CH_3	CH_3S	Cl	*a*	102	*d*
12	*N*-Methylpiperazino	CH_3	CH_3S	Cl	*b*	>250	
13	γ-Methoxypropylamino	CH_3	CH_3S	Cl	*a*	80	*e*
14	β-Hydroxyethylamino	CH_3	CH_3S	Cl	*a*	142	*d*
15	*N*-Methylacetamido	CH_3	CH_3S	Cl	*b*	188	*f*
16	*N*-Methylbenzamido	CH_3	CH_3S	Cl	*b*	210	*d*
17	Acetylamino	CH_3	CH_3	Cl	*a*	202	*d*
18	Methylamino	H	CH_3S	Cl	*a*	>200 dec	
19	Methylamino	C_6H_5	CH_3S	Cl	*a*	163–164	*d*
20	Methylamino	$OHCH_2CH_2$	CH_3S	Cl	*a*	120–122	*d*
21	Methylamino	CH_3	CH_3S	CH_3O	*b*	245 dec	
22	Methylamino	CH_3	CH_3S	*n*-C_3H_7O	*a*	90	*g*
23	Methylamino	CH_3	CH_3S	$C_2H_5CH_2CH_2O$	*b*	136	*d*
24	Methylamino	CH_3	CH_3S	$CH_2{=}CHCH_2O$	*b*	174 dec	*d*

[a] Base. [b] Monohydrochloride. [c] Dihydrobromide. [d] Ethyl alcohol. [e] Hexane. [f] Acetone-ethyl alcohol. [g] Petroleum ether. [h] The percentage analyses of the quoted products are in conformity with normal standards (±0.3%).

tion 6 on the ring by a straight alkyloxy or allyloxy chain (**22** and **24**).

Only the 4-(*N*-methylpiperazino) and 5-methylthio- (or 5-methyl-) 6-chloropyrimidine derivatives exhibit a marked analgesic activity in the phenylbenzoquinone (PBQ) test[13] in mice po, provided that position 2 is occupied by a secondary alkylamine, possibly after acylation (**3–6, 13, 17,** and **18**).

The prototype compound **2,** which heads this chemical series, and its derivatives, **3** and **4,** possess clear central antiserotonin activities,[5] which are nevertheless 6–7 times weaker than that of a specific antiserotonin agent, such as methysergide, the oral ED_{50} of which is only 5 mg/kg in the head-twitch technique. A few examples tend to prove that branching of the alkyl chain on the secondary amine group in position 2 of the ring (**5** and **6**), like demethylation of the *N*-methylpiperazine in position 4 (**18**), alters or eliminates this property.

Most compounds of the series are virtually inactive in the traction test[6] in mice following oral administration, with the exception of **13** which exhibits a certain sedative activity. The initial product, **2,** and its immediate derivatives, **3, 5,** and **18,** inhibit the vomiting, induced by apomorphine in dogs,[4] in very low oral doses, i.e., 0.1–0.2 mg/kg. This powerful antiemetic activity appears to be linked to the 2-amino-6-chloro-4-piperazino-5-methylthiopyrimidine structure, provided that the amino group at position 2 is primary or secondary with a short or only slightly branched alkyl chain. The methyl substituent of the piperazine in position 4 is not essential for the activity (**18**). These molecules, with the exception of **5,** exhibit cataleptic activity[7] which appears together with the antiapomorphine property. Its intensity is of the same order as that of metoclopramide, but it is very much weaker than that of major antipsychotic agents of the type of thioproperazine or fluphenazine. Lastly, a few products (**2–4, 9, 13, 15, 17, 18,** and **21**) possess, like metoclopramide and chlorpromazine, a far from negligible anorexigenic activity[9] in the rat, although this activity is weaker than that of conventional drugs in this therapeutic field such as fenfluramine or fenproporex. However, unlike the reference anorectic agents, these pyrimidine derivatives have no psychoanaleptic effect and their action on food consumption in the rat could be a reflection of a slight sedative effect on the central nervous system rather than a specific anorexigenic activity.

In summary, this new chemical series, derived from 4-piperazino-5-methylthiopyrimidine, possesses pharmacological profile which includes at the same time antiemetic, tranquilizing, analgesic, antiserotonin, and musculotropic-spasmolytic effects. Pharmacological screening has in particular revealed the low neuroleptic activity of **13** and the powerful antiemetic properties of **3** and **5.** This latter product is less sedative than **3** and exhibits a relaxant effect on the smooth muscle fiber which is a useful complement to its central action. For this reason these compounds, which also possess a high degree of safety, merit clinical pharmacological study.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer IR 125 spectrophotometer and NMR spectra on Varian spectrometers A-60 and XL-100. Gas–liquid chromatographic determinations were made using a Varian Aerograph 1520 A.

Table VI. Pharmacological Properties

No.	Acute toxicity, LD_{50}, mg/kg,[a] mouse		Pharmacological activity						
	iv	Oral	Sedative: traction,[b] mouse, ED_{50}, mg/kg, oral	Analgesic: PBQ,[c] mouse, ED_{50}, mg/kg oral	Antiserotonin: head-twitch,[d] mouse, ED_{50}, mg/kg, oral	Anorexigen: rat,[e] ED_{50}, mg/kg, oral	Cataleptic: rat,[f] ED_{50}, mg/kg, oral	Antiemetic: antiapomorphine,[g] dog, ED_{50}, mg/kg, oral	Spasmolytic: spasm $BaCl_2$,[h] duodenum rat, EC_{50}, mg/l.
2	33	~225	>100	>100	30	6	~100	0.10	>10
3	30.5 << 37.5	280 << 390	Inactive at 100	38	35	9	60	0.20	>10
4	41	~200	>33	33	28	5	80	0.35	1.6
5	75 << 95	455 << 590	>100	35	Inactive at 100	45	>300	0.10	1.2
6	66.5	~600	Inactive at 300	75	~300	>100	Inactive at 300	>5	0.5
7	Insoluble	~600	Inactive at 100	>100	60	>100	Inactive at 100	~5	
8	112	~525	Inactive at 100	~100	~100	~100	Inactive at 300	>5	2.2
9	123	~600	Inactive at 300	260	Inactive at 300	13	Inactive at 300	Inactive at 5	4
10	115	525	>100	>100	>100	30	Inactive at 300	>5	2.2
11	110	~400	Inactive at 100	~100	~70	37	Inactive at 100	>5	1.7
12	83	~675	Inactive at 300	150	~300	>100	Inactive at 300	Inactive at 5	3.6
13	55	200	30	33	92	14	175	1.7	1.25
14	89	600	Inactive at 100	~100	>100	~100	Inactive at 100	0.85	>10
15	Insoluble	~525	Inactive at 100	>100	>100	10	Inactive at 100	>5	
16	91.5	~600	Inactive at 300		~300	~100	Inactive at 300	1.9	2.6
17	Insoluble	~265	Inactive at 100	33	Inactive at 100	23	Inactive at 300	3.7	
18	57	~225	Inactive at 100	67	>150	6.5	73	0.065	Inactive at 10
19	Insoluble	Atoxic at 900	Inactive at 900	Inactive at 400	~400	>50	Inactive at 400	>5	>10
20	124	~525	Inactive at 100	Inactive at 100	Inactive at 100	38	Inactive at 100	0.30	1.6
21	57	~400	Inactive at 100	Inactive at 100	Inactive at 100	22	Inactive at 300	1.4	>10
22	63	~525	Inactive at 100	>100	>100	>50	Inactive at 300	3.9	2.1
23	Insoluble	~270	>100	>100	Inactive at 100	44	Inactive at 100	Inactive at 5	
24	56	~400	Inactive at 100	>100	~100	37	Inactive at 400	1.5	3
Chlorpromazine	80	380	12	1	4.5	6	60	1.2	8
Metoclopramide (maxolon)	55 << 65	280 << 370	Inactive at 100	40	30	5	40	0.10	>10

[a]The acute toxicity was determined intravenously and orally in the CD 1 mouse. The LD_{50} values were calculated after 3 days of observation by the cumulative quantal method.[12] In view of the pharmacological interest of **3** and **5**, their LD_{50} values were determined more accurately after 5 days of observation, using the statistical method of Litchfield and Wilcoxon.[10] [b]The traction test[6] in mice which indicates the sedative effect on the central nervous system. [c]The phenylbenzoquinone (PBQ) technique according to Siegmund et al.[13] [d]Antiserotonin activity: head-twitch test according to Corne et al.[5] The intraperitoneal injection of 400 mg/kg of 5-hydroxytryptophan (5-HTP), a precursor of serotonin, induces in the mouse the onset of very rapid shaking of the head: the head-twitch syndrome which correlates to a marked increase of the brain serotonin level. The ED_{50} of a drug is the dose which suppresses, in 50% of the mice, the shaking of the head induced by 5-HTP. [e]Anorexia in the rat: a technique adapted from the procedure described by Le Douarec.[9] CD rats are accustomed to take their daily food during a fixed period of 4 hr. When their food consumption has become stable over a period of several successive days, the inhibiting effect produced by the test compounds is determined. The ED_{50} of a drug is the dose which decreases by 50% the amount of food taken by the experimental animals, in relation to the controls. [f]The catalepsy test in the rat[7] which covers neuroleptic agents and has a certain predictive value for assessment of their extrapyramidal effects under clinical conditions.[8] [g]The apomorphine-induced vomiting test in the dog[4] which covers the centrally acting antiemetics. [h]The musculotropic-spasmolytic activity was examined in vitro using barium chloride induced contractions of the isolated rat duodenum, according to Magnus.[11]

2,4,6-Trichloro-5-methylthiopyrimidine.[1] Barbituric acid (520 g), 360 ml of DMSO, 1.6 l. of acetic acid, and 580 ml of acetic anhydride were heated progressively to 90–100° (during 1.5 hr). This temperature was maintained for 4 hr and then 2.6 l. of water was added. After cooling and filtering the precipitate, it was washed with acetone. The dimethylsulfonium-substituted barbituric acid weighed 762 g (94.5%). The above product (660 g) was allowed to react with 1.760 ml of phosphorus oxychloride and 176 ml of dimethylaniline. After heating for 24 hr under reflux, hydrolysis was carried out in iced salt water. This was followed by filtration and washing of the solid with water. After drying, the solid, mp 78°, was distilled to give 720 g (90%) of a colorless liquid, bp 105–107° (2 Torr).

Example of the Preparation of 2-Amino-4,6-dichloro-5-methylthiopyrimidines (Type I). Procedure A. 1 (1 mol) was dissolved in 740 g of methyl ethyl ketone, 650 g of ice was added, and 1 mol of amine in 20–40% aqueous solution, or pure if the amine is insoluble, was added over a period of 30 min, the temperature being maintained below 5°. After allowing the temperature to rise to 20°, NaOH (1 mol) in 30% solution was added over a period of 1 hr with subsequent vigorous stirring for 6 hr at ambient temperature (+20°). In the most favorable case the desired isomer (type I) was in part precipitated, whereas the 4-substituted isomer remained in the methyl ethyl ketone solution; under these conditions the required product was recovered from the reagent mixture after cooling at +10° for 15 hr.

Procedure B. Where no crystallization was observed but the products were solid, the organic phase was isolated and the methyl ethyl ketone was distilled under vacuum. The residue was redissolved in amounts of 1 g per 5 ml of 10 *N* hydrochloric acid. After solution, the derivative substituted in the 2 position was precipitated with 2.2 ml of H_2O; the isomer of type II which remained in solution was isolated by subsequent dilution and crystallization from ethanol.

Procedure C. Where the isomers were liquid, the following reaction was carried out on the mixture of the two isomers and it was sometimes possible in this way to separate the isomeric products (type III). The products of type I were used in the following step after recrystallization in ethanol and their purity determined by GC.

Example of the Preparation Procedure for 2-Amino-4-piperazino-5-methylthiopyrimidines (Type III). (a) The mixture of 0.14 mol of 2-amino-4,6-dichloro-5-methylthiopyrimidine, 400 ml of ethanol, and 43 g of *N*-methylpiperazine was refluxed for 3 hr. The product III was precipitated by cooling to −30 to −40° and crystallized from ethanol (**4, 2, 3, 8,** and **9**). If no precipitate was formed as a result of the cooling, the mixture was concentrated under vacuum. After washing the residue in water, it was recrystallized from ethanol to yield the product III (**14**). If the distillation residue did not completely crystallize it was redissolved in 300 ml of Et_2O to precipitate the *N*-methylpiperazine hydrochloride, which was collected by filtration. The ethereal solution was washed with water and evaporated under vacuum; the residue crystallized to yield III (**12, 11, 13,** and **7**).

(**b**) The mixture of 0.14 mol of the two isomers, 2(4)-amino-4(2)-6-dichloro-5-methylthiopyrimidine, 400 ml of ethanol, and 43 g of *N*-methylpiperazine was refluxed for 3 hr. Cooling to −30° caused crystallization of the product III, whereas its isomer remained in solution. The crude compound **6** was recrystallized from ethanol. If no crystallization occurred in the cold, the solvent was evaporated and the residue was redissolved in 300 ml of Et_2O; the product III was precipitated along with *N*-methylpiperazine hydrochloride, which was removed by washing with water after filtration of the precipitate. The crude product thus obtained was recrystallized from ethanol. Its isomer (**5**) remained in solution. The products **18, 19,** and **20** were obtained from the corresponding piperazines.

Chloroisopropylaminomethylamino-5-methylthiopyrimidines. The 2- and 4-methylamino dervatives (5 g) in ethanol were treated with an excess of isopropylamine (6 g) at 50° for 4 hr and then at reflux for 3 hr. Precipitates and the reaction liquids were analyzed by GC and then isolated for spectroscopic studies which led to the determination of their structures and of the substitution positions of the methylamino derivatives.

Ethyl Methylthiomalonate. Sodium methylmercaptide (35 g) was suspended in 500 ml of toluene at 100°; then 133 g of ethyl chloromalonate was introduced and the mixture was refluxed for 3 hr. After cooling, filtrating, and distillating under vacuum, 68 g of material, bp 135–137° (2 Torr), was obtained.

4,6-Dioxo-2-methylamino-5-methylthiopyrimidine. A mixture of 32.4 g of ethyl methylthiomalonate, 45.7 g of methylguanidine hydrochloride, and 500 ml of ethanol in which 23.7 g of sodium had previously been dissolved was refluxed for 3 hr. After cooling and then neutralization with 60 ml of acetic acid, 40 g of the product which recrystallized from 1.650 ml of water was obtained, mp >250° dec.

2-Methylamino-5-methylthio-4,6-dichloropyrimidine. A mixture of 12.4 g of 4,6-dioxo-2-methylamino-5-methylthiopyrimidine plus 51 ml of phosphorus oxychloride was refluxed for 1 hr. After cooling, followed by hydrolysis in a mixture of 225 ml of 10 *N* NaOH and 500 g of ice, crystals which recrystallized from ethanol were obtained: mp 158° (Köfler). These crystals were identical in their physical and spectra properties with the product obtained by procedure A.

Example of the Preparation of 2-Amino-4-piperazino-5-methylthio-6-alkoxypyrimidines (Type IV). 6-Chloro-2-methylamino-4-(*N*-methylpiperazino)-5-methylthiopyrimidine (20 g) was dissolved in 130 ml of 1-propanol in the presence of 7 g of potassium hydroxide pellets. The reaction was exothermic. Refluxing was continued for 2 hr, followed by dilution with water, after which an oil separated upon addition of Et_2O. The oil was washed with water, dried, and evaporated. The residue became solid in petroleum ether and **22** was obtained after recrystallization. The remaining products (IV) were obtained by using the appropriate alcohols (**21, 23,** and **24**).

2-(*N*-Methyl-*N*-acetyl)amino-4-(*N*-methylpiperazino)-5-methylthio-6-chloropyrimidine (15). A mixture of 20 g of **3** and 60 ml of acetic anhydride was refluxed for 2 hr. After distillation of the excess anhydride and washing with a dilute NaOH solution, the separated oil was extracted with chloroform. Evaporation of the solvent yielded an ethanol-soluble residue from which the monohydrochloride precipitated. It was recrystallized in acetone–ethanol.

2-(*N*-Methyl-*N*-benzoyl)amino-4-(*N*-methylpiperazino)-5-methylthio-6-chloropyrimidine (16). Benzoyl chloride (13.7 g) was added during 30 min at ambient temperature to 28 g of **3** in solution in 150 ml of THF and 10.5 g of triethylamine. Reflux heating was carried out for 5 hr, followed by dilution with water and ether extraction. The solution was dried and evaporated. This treatment was repeated twice, after which the hydrochloride was formed in ether by the addition of the calculated quantity of an alcoholic solution of 2 *N* hydrochloric acid. Recrystallization was carried out in ethanol.

2-Acetylamino-4-(*N*-methylpiperazino)-5-methyl-6-chloropyrimidine (17) was obtained from 2-amino-4,6-dioxo-5-methylpyrimidine by heating at reflux for 8 hr in a 5:1 acetic acid–acetic anhydride mixture. After filtration and washing with ether, 2-acetylamino-4,6-dioxo-5-methylpyrimidine was obtained. To a solution of this product (38 g), 46.5 g of triethylamine, and 255 ml of tetrahydrofuran was added dropwise at 0° 108 g of phosphorus oxychloride. After 6 hr of stirring at ambient temperature the mixture was poured onto ice–water. The mixture was extracted with chloroform. The organic phase was washed with water, dried, and evaporated, and the residue, consisting mainly of 2-acetylamino-4,6-dichloro-5-methylpyrimidine, was crystallized in ethanol. This latter product (8.8 g) was refluxed in a mixture of 100 ml of THF, 4 g of triethylamine, and 4 g of *N*-methylpiperazine for 6 hr. The mixture was poured into water and the insoluble residue was filtered and recrystallized from ethanol.

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Synthesis and Biological Activity of Some 8-Substituted Selenoguanosine Cyclic 3',5'-Phosphates and Related Compounds

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8-Bromoguanosine cyclic 3',5'-monophosphate, 8-bromoguanosine 5'-monophosphate, and 8-bromoguanosine served as intermediates for the chemical synthesis of a series of 8-substituted seleno cyclic nucleotides, nucleotides, and their nucleosides. Selenourea was found to be a useful reagent in synthesizing these seleno-substituted nucleoside, nucleotide, and cyclic nucleotide. A nucleic acid analyzer was used to study the hydrolysis of these cyclic nucleotides by phosphodiesterase. It was found that all of the 8-substituted selenoguanosine cyclic 3',5'-phosphates synthesized, except 8-MeSe-cGMP, were resistant to hydrolyze by phosphodiesterase. These 8-substituted seleno cyclic GMP derivatives showed some antitumor activities against murine leukemic cells (L5178Y) in vitro and in vivo.

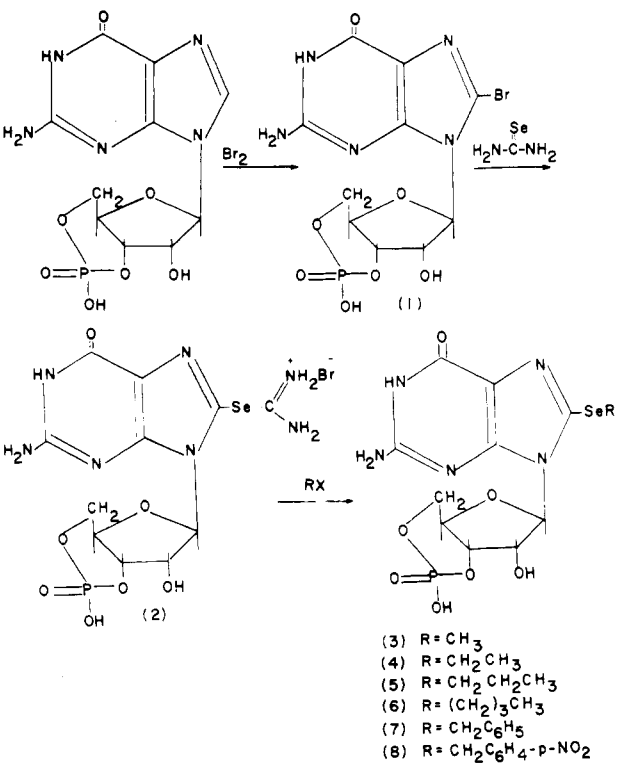
Guanosine cyclic 3',5'-phosphate (cGMP), like cAMP, has been well established as a mediator of many hormonal effects in biological regulating processes.[1] George et al.[2] first found that perfusion of acetylcholine resulted in elevation of cGMP concentration in the rat heart. Subsequently, a number of investigators have found the same results in calf heart,[3] rabbit cerebral cortex,[3] mouse cerebellum,[4] and human lymphocytes.[5] Goldberg[6] also found that the administration of oxytocin or serotonin promoted cGMP accumulation 2.5- and 3-fold in uteri (in vitro) from diethylstilbestrol-treated rats while no change in cAMP levels; clonal proliferation of lymphocytes resulted in 10–50-fold increase in cGMP levels, while cAMP levels were unaffected.[7] Recently, Miller et al.[8] have synthesized a number of 8-substituted derivatives of cGMP, cIMP, and cXMP. It was found that 8-bromo, 8-hydroxy, 8-methylthio, 8-benzylthio, and 8-*p*-chlorophenylthio analogs were more effective than cGMP in activating lobster muscle cGMP-dependent protein kinase and retained their specificity for this kinase.[8] In continuation of our interest in synthesized cyclic nucleotide analogs and study of their biological activity,[9] we have synthesized a series of 8-seleno-cGMP analogs.

Direct bromination of cGMP gave 8-bromo-cGMP (**1**)[8,10] in good yield. Treatment of the free acid of 8-bromo-cGMP with selenourea in refluxing methanol gave cGMP 8-isoselenouronium hydrobromide **2** as an intermediate.

Alkylation of compound **2** in situ with methyl iodide, ethyl bromide, *n*-propyl bromide, *n*-butyl bromide, benzyl bromide, and *p*-nitrobenzyl bromide yielded 8-methylseleno- (**3**), 8-ethylseleno- (**4**), 8-*n*-propylseleno- (**5**), 8-*n*-butylseleno- (**6**), 8-benzylseleno- (**7**), and 8-*p*-nitrobenzylseleno-cGMP (**8**), respectively (Scheme I). The 8-substituted guanosine cyclic 3',5'-phosphates were readily purified by crystallization from water at pH 2 with or without the aid of methanol or from preparative Avicel plates (see the Experimental Section). The physical properties of the nucleotides are shown in Table I. A comparison of the ultraviolet spectra of these 8-substituted seleno cyclic nucleotides with known 8-substituted thio analogs[8] confirmed the position of substitution. Furthermore, the cyclic structures of these nucleotides were verified by enzymatic studies (see later).

For the identification of the cyclic structure of 8-substituted seleno cyclic nucleotides several 8-substituted seleno-

Scheme I



guanosine 5'-monophosphates and selenoguanosines were synthesized. Bromination of 5'-GMP gave 8-bromo-5'-GMP (**9**) as a sodium salt. Treatment of the free acid of 8-bromo-5'-GMP with selenourea in refluxing methanol gave 5'-GMP 8-isoselenouronium hydrobromide (**10**) as an intermediate. Alkylation of compound **10** in situ with methyl iodide yielded 8-methylseleno-GMP (**11**). Treatment of 8-bromoguanosine with selenourea in refluxing ethanol gave 8-selenoguanosine (**12**). Likewise, treatment of 8-bromo-2',3',5'-tri-*O*-acetylguanosine with selenourea in refluxing ethanol gave 8-seleno-2',3',5'-tri-*O*-acetylguanosine (**13**) as an intermediate. Alkylation of either compound **12** or **13** with alkyl halides gave the corresponding alkylated nu-