For analysis, the product was dissolved in water by the addition of a minimal amount of 1 N KOH, followed by precipitation with HCl and workup as described above: ^{1}H NMR (D_2O) δ 2.36 (3 H, s, H-2'), 3.2 (2 H, m, H-β), 4.0 (1 H, m, H-α), 4.91 (2 H, d, H-5', ^{31}P–^{1}H coupling constant = 10 Hz), 5.77 (1 H, s, H-4'), 6.20 (1 H, s, H-5''), 6.69 (1 H, s, H-2''), 7.74 (1 H, s, H-6); UV λ_{max} (pH 7.0) 250 (ϵ 6400, s), 292 (ϵ 4630), 326 nm (ϵ 8780). Anal. ($C_{17}H_{19}N_2O_9P\cdot H_2O$) C, H, N, P.

Acknowledgment. The research was supported by Public Health Service Grant 5429-16-9 (5-23323).

References and Notes

(1) (a) Abbreviations used are: Dopa, 3,4-dihydroxyphenylalanine; dopamine, 3,4-dihydroxyphenylethylamine; Boc, *tert*-butoxycarbonyl; OMe, methyl ester; OEt, ethyl ester; TFA, trifluoroacetic acid; NMR nuclear magnetic resonance.
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Cyclic Amidine Inhibitors of Indolamine *N*-Methyltransferase

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Syntheses of a large number of mono- and bicyclic, as well as a few tricyclic, amidine derivatives related to 2,3,4,6,7,8-hexahydropyrrolo[1,2-*a*]pyrimidine (DBN) are reported. In vitro potencies for inhibition of the enzyme indolamine *N*-methyltransferase (INMT) from rabbit and human lung are presented. Four bicyclic amidine derivatives and 11 monocyclic derivatives were found to be equal or superior to DBN in in vitro potencies. With the bicyclic amidines, increasing ring size or introduction of substituents reduced activity. Among the monocyclic analogues, the most potent representatives were five- or six-membered systems with an exocyclic imino group, combined with methyl or ethyl substituents on the endocyclic nitrogen. Introduction of additional substituents decreased inhibitory potency. 2,3,5,6-Tetrahydro-8*H*-imidazo[2,1-*c*][1,4]thiazine and 3-methyl-2-iminothiazolidine have been shown to cause inhibition of lung INMT when administered orally to rabbits.

Although the current treatment of schizophrenia involves the use of neuroleptic agents which are dopamine antagonists, interest persits in other approaches to the therapy of this disease which are based on the theory that the illness is caused by an endogenously produced chemical toxin. There is considerable evidence that *N*,*N*-di-

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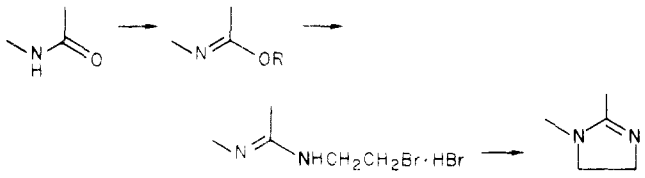
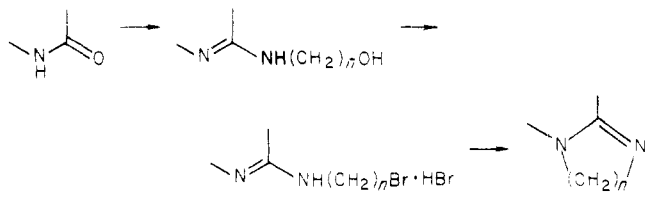
Scheme I

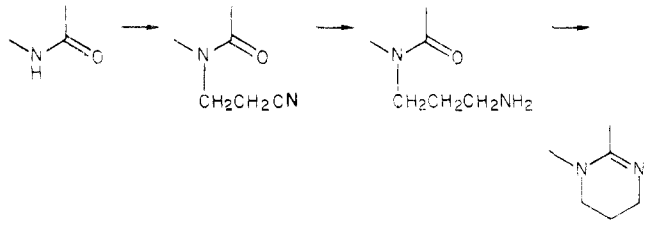
Method A



Method B



Method C

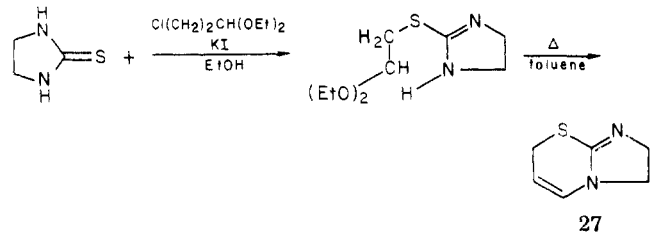


methyltryptamine (DMT) may be such a causative agent.[1,2] The existence of enzymatic pathways for its formation in animals and the fact that it is hallucinogenic in man have been cited in support of this theory.[3,4] In addition, the administration of the amino acid precursors of DMT, methionine and tryptophan, has been found to exacerbate schizophrenic symptomatology.[5] Furthermore, the DMT precursor tryptamine is present in human brain[4] and has been reported to be elevated in the urine of some schizophrenics.[6] Thus, the inhibition of DMT biosynthesis as a possible therapeutic approach to schizophrenia is both rational and attractive to the medicinal chemist.

The availability of indolamine *N*-methyltransferase (INMT), a lung enzyme which has been found to catalyze the synthesis of DMT by transfer of the methyl group from *S*-adenosylmethionine to *N*-methyltryptamine (NMT), provided the in vitro assay required to search for an appropriate DMT synthesis inhibitor.[7] This assay has been used in our laboratory as a primary screen and served to identify 2,3,4,6,7,8-hexahydropyrrolo[1,2-*a*]pyrimidine (DBN) as a potent, specific inhibitor of INMT from rabbit, monkey, and human lung tissue.[8] This suggested the design and synthesis of related structures in a search for more effective agents. Presented in this paper are the syntheses and in vitro enzyme inhibitory activities of a variety of bicyclic and monocyclic amidine analogues related to DBN. Positive results from in vivo experiments in which rabbits were fed 2,3,5,6-tetrahydro-8*H*-imidazo[2,1-*c*][1,4]thiazine (**22**) and 3-methyl-2-iminothiazolidine (**29**) in the drinking water are described. Inhibition of the biosynthesis of DMT from NMT in rabbits by **22** is also reported.

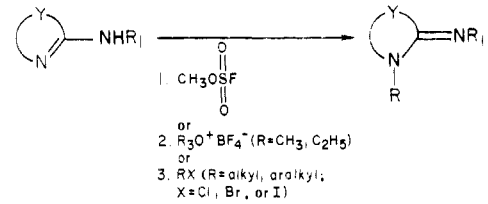
Chemistry. The bicyclic amidines prepared as analogues of DBN were prepared, in general, from the appropriate lactams using one of the three synthetic methods outlined in Scheme I. The lactams were either commercially available or were prepared by known procedures. These methods have been described previously and do not require further comment.[9,10] In each case, the intermediates did not require rigorous purification, since the bicyclic amidines could be purified by simple distillation

Scheme II

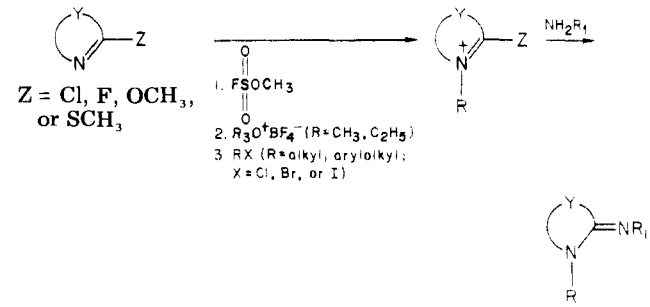


Scheme III

Method D



Method E



Z = Cl, F, OCH_3, or SCH_3

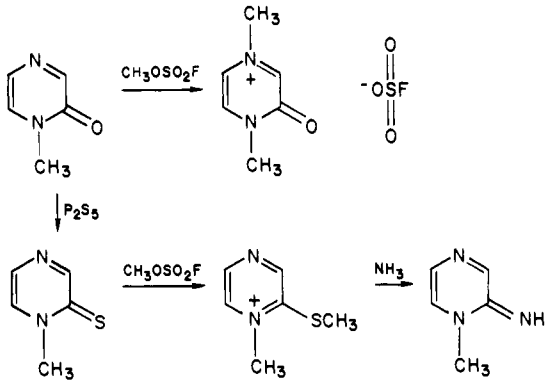
Y = $-CHR_3CH_2-$, $-SCH{=}CH-$, $-SN{=}CH-$, $-(CH_2)_2O-$, $-CH_2CH_2SCH_2-$, $-CR_6{=}CHSCH_2-$, $-CH_2CH_2CH_2S-$, $-CH{=}CHCH_2S-$, $-CR_6{=}CR_5CR_4{=}CR_3-$, $-CH_2CH_2CH{=}CH-$, $-CH{=}CHN{=}CH-$.

and then further characterized as crystalline, stable hydrogen fumarates or other salts. Compounds **5**–**7** and **16** were synthesized by different, but straightforward, procedures as described under the Experimental Section. Compound **27** was prepared as shown in Scheme II.

The two most generally employed routes to the monocyclic amidine analogues of interest in this study are shown in Scheme III. In method D, a high degree of regioselectivity of alkylation on the ring nitrogen of the α-amino cyclic imine normally was observed. In method E, alkylation on the ring nitrogen of compounds with Z substituents resulted in a great enhancement of the rate of displacement of Z by amines. Frequently for method E the cyclic amide was available as starting material. The amide could be either chlorinated (with $POCl_3$ or PCl_5) to give the chloro intermediate (Z = Cl) or alternatively alkylated using trimethyl- or triethyloxonium fluoroborate or methyl fluorosulfonate to give the imino ether intermediate (Z = OCH_3, OC_2H_5). As a variation of the latter, it was sometimes found necessary to convert the amide to the thioamide using P_2S_5, which was alkylated (on sulfur) with greater ease than the corresponding cyclic amide (on oxygen).

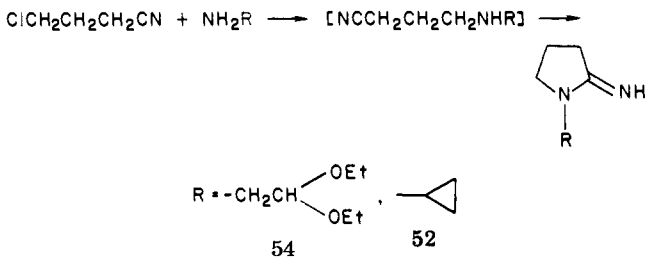
6-Fluoro-1-methyl-2-imino-1,2-dihydropyridine (**74**), prepared from 2,6-difluoropyridine by method E (1), was readily isolated as such. However, exposure to K_2CO_3 in ethanol resulted in the displacement of the fluoride ion by an ethoxy group to give **78**. Reaction of 6-fluoro-1-methyl-2-fluoropyridinium fluorosulfate with excess NH_3 or $MeNH_2$ gave the 6-amino-1-methyl-2-imino-1,2-dihydropyridine derivatives **76** and **77** directly as the

Scheme IV



80

Scheme V



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products. 5,6-Dihydropyridinimines of the type **79** have not been described previously. The intermediate 1-methyl-2-oxo-1,2,5,6-tetrahydropyridine was prepared using the literature conditions reported for the corresponding 1,6-dimethyl compound.[11]

When 1-methyl-1,2-dihydropyrazin-2-one was allowed to react with methyl fluorosulfate, reaction occured on the second nitrogen in preference to the carbonyl oxygen.[12] Replacement of oxygen by sulfur[13] permitted methylation to occur as desired, giving an intermediate suitable for conversion to the desired imine **80** (Scheme IV).

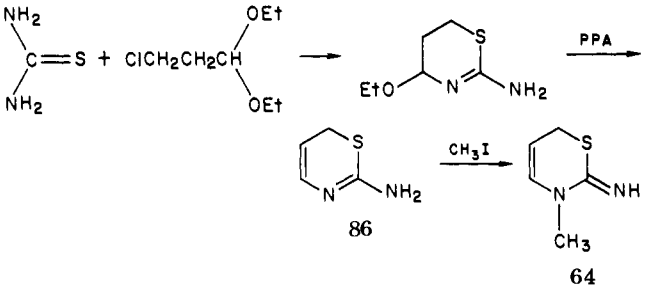
Pyrrolidine derivatives with an N substituent not readily introduced by the general procedure could be prepared starting from 4-chlorobutyronitrile, by the general procedure of Kwok and Pranc (Scheme V).[14]

Preparation of the intermediate thione **87** required for the syntheses of 4-methyl-3,4-dihydro-3-imino-2*H*-1,4-thiazines **62** and **63** was accomplished by the method of Johnson and Thanawalla,[15] except that chloroacetaldehyde dimethyl acetal was employed. The same intermediate served for the preparation of bicyclic compound **25**. The synthesis of 3-methyl-3,4-dihydro-2-imino-6*H*-1,3-thiazine (**64**) was accomplished by a different route (see Scheme VI).

Syntheses of 3-amino- and 3-methylamino-2-iminothiazolidines **37** and **38** were accomplished using 2,4,6-trimethylphenylsulfonyloxyamine[16] and its *N*-methyl derivative, respectively. Methylation of both 3- and 5-aminoisothiazoles resulted in methylation exclusively on the ring nitrogen (compounds **43**, **44**, **84**, and **85**.) In contrast, when methyl fluorosulfonate was reacted with 3-amino-1,2,5-thiadiazole, a mixture of exo- and endo-N-methylated products resulted. 2-Methyl-3-(methylimino)-1,2,5-thiadiazole (**45**) was prepared by a novel procedure which will be detailed in another publication.[17] The 2-alkyl-3-iminothiadiazole and isothiazole derivatives proved unstable on conversion to the free base forms.

Biological Results and Discussion. The primary in vitro assay to which compounds were submitted was inhibition of INMT enzyme from rabbit lung.[8] Many of the active compounds from this assay were then tested for

Scheme VI



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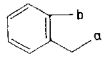
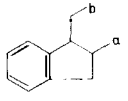
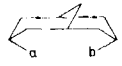
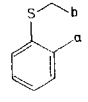
inhibition of human lung INMT. The latter was less readily available and of a lower state of purity, which may account for the IC_{50} values being consistently higher than with the rabbit enzyme. The data for the bicyclic and monocyclic amidine derivatives are presented in Tables I and II, respectively. The detailed assay procedure is described under the Experimental Section.

In the early stage of this investigation, the effect of ring size on enzyme inhibition by bicyclic amidine derivatives was examined. A comparison of the IC_{50} values obtained for DBN, 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (DBU), **1**, **2**, **9**, **19**, and **20** indicated that increasing the ring size decreased the capacity of such compounds to inhibit indolamine *N*-methyltransferase. Thus, only 2,3,5,6-tetrahydro-7*H*-pyrrolo[1,2-*a*]imidazole (**9**) and 2,3,5,6,-7,8-hexahydroimidazo[1,2-*a*]pyridine (**20**), bicyclic amidines with both nitrogens in the five-membered ring, were selected for further structural modification. It was found with these structures that increasing the size of the molecule either by addition of large bulky alkyl groups (**11** and **13**) or aromatic rings (**14**, **15**, **17**, and **18**) markedly decreased enzyme inhibitory activity. While the placement of a methyl group on the five-membered ring (**10** and **12**) only moderately affected biological activity, as was the case with DBN (compare **3** and **4**), the placement of substituents on the six-membered ring of these structures or DBN (compare **8** and **6**) appeared to lower significantly INMT inhibitory activity. Quaternization of the nitrogen (**7**) in DBN also lowered inhibitory activity.

The consequences of making an isosteric substitution for C-7 in **20** was also examined. The results in Table I for **21**–**24** clearly indicated that only the thio analogue **22** was comparable to DBN and **20** in relative potency. Increasing the degree of unsaturation (**25** and **26**), adding an aromatic ring (**28**), and changing the location of the sulfur atom (**27**) appeared to be undesirable modifications.

Although tricyclic analogues of DBN were essentially inactive (see Table I), the monocyclic analogue **49**, in which two carbon atoms are excised from the tetrahydropyrimidine moiety of DBN, was more potent than DBN as an inhibitor against both rabbit and human INMT. This finding prompted an examination of the variety of five- and six-membered monocyclic amidine derivatives, bearing an exocyclic imine group, shown in Table II. With the exception of pyrazine **80**, monocyclic amidine derivatives from all other five- and six-membered ring systems, which possessed methyl groups on the endocyclic N and no substituents on the exocyclic N, showed significant in vitro enzyme inhibitory activity. While the corresponding compounds lacking a substituent on the ring N (in which the double bond was endocyclic) showed measurable activity, the level was consistently lower. Increases in the size of the alkyl substituent on the ring nitrogen beyond unsubstituted ethyl resulted in considerable loss of activity, with large or polar substituents rendering the compounds essentially inactive. Compounds with a methyl substituent

Table I. Inhibition of Rabbit and Human Lung Indolamine *N*-Methyltransferase by Bicyclic Amidine Derivatives

compd	structure[a] a, b	structure[a] c	method (lit. ref)[b]	bp, °C	mp, °C, hydrogen fumarate	recryst solvent	% yield	formula[c]	IC_{50} ($M \times 10^{-6}$) for inhibn of INMT in vitro[d] rabbit lung enz	IC_{50} ($M \times 10^{-6}$) for inhibn of INMT in vitro[d] human lung enz
DBN	a-$(CH_2)_3$-b	-$(CH_2)_3$-		80 (10 mm)	159-160	*i*-PrOH		$C_{11}H_{16}N_2O_4$	1.67 (1.0-2.5)[e]	13.4 (7.0-28.0)[e]
DBU	a-$(CH_2)_5$-b	-$(CH_2)_3$-		127-128 (14 mm)				$C_9H_{16}N_2$	8.0	25.7
1	a-$(CH_2)_3$-b	-$(CH_2)_4$-		108-110 (14 mm)			8	$C_8H_{14}N_2$	74.8	852
2	a-$(CH_2)_4$-b	-$(CH_2)_3$-	C	110-112 (10 mm)	128-130	*i*-PrOH	56	$C_{12}H_{18}N_2O_4$	0.7	3.3
3	a-$CH_2CH(CH_3)CH_2$-b	-$(CH_2)_3$-	B (18)	90-93 (10 mm)	114-116	*i*-PrOH-acetone	39	$C_{12}H_{18}N_2O_4$	7.0	
4	a-$CH(CH_3)CH_2CH_2$-b	-$(CH_2)_3$-	C (18)	89-93 (10 mm)	102	*i*-PrOH-acetone	34	$C_{12}H_{18}N_2O_4$	13.2	
5	a-$C(=CHC_6H_5)CH_2CH_2$-b	-$(CH_2)_3$-	*h*		207-209	*i*-PrOH		$C_{18}H_{20}N_2O_4$	37.1	
6	a-$(CH_2)_3$-b	-$CH_2C(=CH_2)CH_2$-	*h*		148-152	EtOH		$C_{12}H_{16}N_2O_4$	43.3	
7	DBN methiodide		*h*					$C_{18}H_{15}IN_2$	38.7	
8	a-$(CH_2)_3CH(Cl)$-b	-$(CH_2)_2$-	A (28)		105-108	*i*-PrOH-MeOH		$C_{11}H_{15}ClN_2O_4 \cdot 1\text{-}2H_2O$	52.2	
9	a-$(CH_2)_3$-b	-$(CH_2)_2$-	A	80-81 (10 mm)	171-172	EtOH	39	$C_{10}H_{14}N_2O_4$	0.04	1.0
10	a-$CH_2CH(CH_3)CH_2$-b	-$(CH_2)_2$-	A (18)	81-83 (10 mm)	113-114	*i*-PrOH-acetone	36	$C_{11}H_{16}N_2O_4$	3.8	
11	a-$CH_2C[(CH_3)_2]CH_2$-b	-$(CH_2)_2$-	B (18)	80-83 (10 mm)	122-123	acetone	27	$C_{12}H_{18}N_2O_4$	54.2	
12	a-$CH(CH_3)(CH_2)_3$-b	-$(CH_2)_2$-	A (18)	88-90 (15 mm)	144-145	*i*-PrOH-acetone	39	$C_{11}H_{16}N_2O_4$	9.3	
13	a-$C(c\text{-}C_5H_{10})(CH_2)_2$-b	-$(CH_2)_2$-	A (20)	120-124 (4 mm)	124-125	*i*-PrOH	38	$C_{15}H_{22}N_2O_4$	141.0	
14	a-$CH_2CH(Ph)CH_2$-b	-$(CH_2)_2$-	B (18)		159-162	*i*-PrOH	48	$C_{16}H_{18}N_2O_4$	>330	
15	a-$CH_2C(Ph)_2CH_2$-b	-$(CH_2)_2$-	B (19)		199-201	*i*-PrOH	52	$C_{22}H_{22}N_2O_4$	>330	
16	a-$(CH_2)_3$-b	-CH=CH-	*h*					$C_8H_{10}N_2O_4$	83.1	
17		-$(CH_2)_2$-	A (25)		170-171	*i*-PrOH-MeOH	26	$C_{14}H_{14}N_2O_4$	>330	
18		-$(CH_2)_2$-	A (26)	148-151 (0.15 mm)	101-103[f]	hexane[g]	48	$C_{13}H_{14}N_2$	16.4	
19		-$(CH_2)_2$-	A (27)	105-108 (0.3 mm)	131-133	*i*-PrOH-acetone	35	$C_{12}H_{18}N_2O_4$	>330	
20	a-$(CH_2)_4$-b	-$(CH_2)_2$-	B	88 (10 mm)	149-151	*i*-PrOH	35	$C_{11}H_{16}N_2O_4$	0.17	0.8
21	a-$(CH_2)_2OCH_2$-b	-$(CH_2)_2$-	A (21)	107-109 (18 mm)	121-123	EtOH	36	$C_{10}H_{15}N_2O_5$	13.8	
22	a-$(CH_2)_2SCH_2$-b	-$(CH_2)_2$-	A	114-116 (5 mm)	179-181	*i*-PrOH	39	$C_{10}H_{14}N_2O_4S$	0.5	6.0
23	a-$CH_2CH(CH_3)SCH_2$-b	-$(CH_2)_2$-	A (24)	117 (6 mm)	181-182	*i*-PrOH	39	$C_{11}H_{16}N_2O_4S$	30.3	
24	a-$(CH_2)_2S(\rightarrow O)CH_2$-b	-$(CH_2)_2$-		140 (0.2 mm)	166-168	*i*-PrOH	30	$C_{10}H_{14}N_2O_5S$	3.6	18.8
25	a-$CH=CHSCH_2$-b	-$(CH_2)_2$-	A (22)		163	*i*-PrOH	25	$C_{10}H_{12}N_2O_4S$	18.0	35.0
26	a-$C(CH_3)=CHSCH_2$-b	-$(CH_2)_2$-	A (23)		160-162	*i*-PrOH	25	$C_{11}H_{14}N_2O_4S$	45.4	
27	a-$CH=CHCH_2S$-b	-$(CH_2)_2$-	*h*					$C_8H_{10}N_2O_4S$		24.5
28		-$(CH_2)_2$-	A (29)	60-62 mp	173 dec	MeOH-*i*-PrOH	34.7	$C_{14}H_{14}N_2O_4S$	>330	

[a] All compounds except DBU, 1, and 7 were tested as hydrogen fumarates or oxalates. [b] Literature reference is for starting lactam. [c] C, H, and N analyses for all compounds were within ±0.4% of the calculated values. [d] Values were estimated by analysis of regression line in reverse using two to six data points per compound. [e] 95% confidence limits obtained by Fieller's theorem. [f] Melting point for base. [g] Recrystallization solvent for base. [h] See Experimental Section.

on the exocyclic imine (and methyl or ethyl on the endocyclic nitrogen) retained activity but at a reduced level. Introduction of substituents larger than methyl on the exocyclic nitrogen generally resulted in a major loss of activity. Thus, the most active compounds had no substituent on the exocyclic nitrogen and only methyl or ethyl on the ring nitrogen. The rings could be saturated or partially or fully unsaturated (except pyrazine). Introduction of substituents on carbon consistently caused a reduction in activity, as seen with bicyclic analogues of DBN. In all, ten of the monocyclic amidine analogues in Table II were more potent than DBN in the in vitro assay systems (**29**, **31**, **38**, **41**, **49**, **50**, **60**, **61**, **64**, and **79**). It is noteworthy that in seven of these compounds there is a ring sulfur adjacent to the exocyclic imine group.

Compound **22**, which was the most chemically novel of the active bicyclic analogues of DBN, was submitted to more detailed biological evaluation as an inhibitor of the INMT enzyme. The effect of **22** on the methylation of other amines was studied. Thus, at 1.76×10^4 M, **22** inhibited the methylation of *N*-methylserotonin (88%), tryptamine (84%) and serotonin (85%). This is of interest, since it would suggest that **22** also should block the conversion of serotonin to bufotenin, a known hallucinogen. To determine whether or not **22** inhibited INMT in vivo, experiments were carried out by maintaining rabbits on drinking water containing 0.5% (v/v) **22** for several days. Determination of INMT activity in the lungs of sacrificed animals showed 75% inhibition of enzyme activity (Table III). The ability of **22** to inhibit the biosynthesis of [^{14}C]DMT was also determined in vivo in rabbits. Pretreatment of rabbits with **22** resulted in a 64% average inhibition in the conversion of [^{14}C]NMT to [^{14}C]DMT in the lung and 57% inhibition in the brain (Table IV). Compound **29**, one of the most potent monocyclic analogues of DBN, in vitro, produced a 76% inhibition of INMT activity from the lungs of rabbits fed a 0.02% level in the drinking water for 12 days (Table III).

Thus, the data presented in this paper demonstrate that a variety of mono- and bicyclic amidine analogues related to DBN have comparable or superior in vitro activity as inhibitors of the enzyme INMT from rabbit and human lung. One representative monocyclic and one representative bicyclic analogue have both been shown to be active in vivo in inhibiting the lung enzyme when administered in the drinking water of rabbits. Compounds of this type may prove of value in testing the transmethylation hypothesis of schizophrenia in man.

Experimental Section

Chemical Syntheses. Melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR 60-MHz spectra were recorded with Varian Associates EM-360 and T-60 instruments. Chemical shifts were recorded in ppm (δ) relative to Me_4Si as internal standard. IR spectra were determined on a Perkin-Elmer 257 spectrophotometer. Elemental analyses were performed by Mr. K. B. Streeter and associates, Merck Sharp and Dohme Research Laboratories, and by Dr. C. Daessle, Organic Microanalyses, Montreal.

2,3,5,6-Tetrahydro-8*H*-imidazo[2,1-*c*][1,4]thiazine (22). Method A. A solution of 23.4 g (0.20 mol) of 3-thiomorpholinone in 150 mL of dry CH_2Cl_2 was added dropwise with stirring to a solution of 44 g (0.23 mol) of triethyloxonium fluoroborate in 100 mL of dry CH_2Cl_2 maintained at 0.5 °C. The resulting solution was stirred for 6 h as the temperature was allowed to rise to room temperature. The CH_2Cl_2 solution was treated with 46 g of solid K_2CO_3, and the organic solution was decanted and dried over anhydrous K_2CO_3. Filtration and evaporation of the solvent gave a concentrate which was distilled to yield 17.9 g (0.12 mol) of the lactam ether, bp 75–80 °C (4 mm). The lactam ether (17.9) was added to a solution of 24.6 g (0.12 mol) of 2-bromoethylamine hydrobromide in 150 mL of EtOH, and the resulting solution was warmed on the steam bath for 2 h. NaOMe (4.9 g, 0.09 mol) was added, and the solution was stirred and heated under reflux for 2 h. The solvent was evaporated and the residue dissolved in 75 mL of H_2O. This solution was saturated with K_2CO_3 and extracted with $CHCl_3$ (3 × 100 mL). The combined extracts were dried over anhydrous Na_2SO_4 and filtered, and the filtrate was concentrated under reduced pressure. Distillation of the concentrate gave 11.3 g (39%) of **22**: bp 114–116 °C (5 mm); IR (neat) 1620, 1420, 1270, 1180 and 990 cm^{-1}; NMR ($CDCl_3$) δ 2.85 (m, 2 H, SCH_2CH_2), 3.1–3.8 (m, 6 H, $CH_2NCH_2CH_2$), 3.4 (s, 2 H, SCH_2).

6-Methyl-2,3,5,6-tetrahydro-7*H*-pyrrolo[1,2-*a*]imidazole (10). Method B. A solution of 4-methyl-2-pyrrolidinone (25 g, 0.25 mol) in CH_2Cl_2 (100 mL) was added dropwise to a stirred solution of triethyloxonium fluoroborate (56 g, 0.32 mol) in CH_2Cl_2 (200 mL), and the resulting solution was stirred for 16 h. The reaction mixture was washed with saturated aqueous K_2CO_3 solution, dried over anhydrous K_2CO_3, and filtered, and the filtrate was concentrated under reduced pressure. Distillation of the concentrate gave the lactam ether: bp 85–87 °C (90 mm); yield 24 g. The lactam ether (24 g) and ethanolamine (12 g) were heated under reflux for 2 h. A 10 M solution (30 mL) of HCl in C_2H_5OH was added, and the solution was evaporated to dryness. The residue was dissolved in $CHCl_3$ (100 mL), $SOCl_2$ (20 mL) was added, and the solution was heated under reflux for 1 h. The solvent was evaporated, and the residue was slurried with 25% NaOH solution and extracted with Et_2O. The Et_2O was evaporated and the residue dissolved in EtOH (50 mL). After heating under reflux for 0.5 h, this solution was concentrated under reduced pressure. The residue was slurried with 25% NaOH solution and extracted with $CHCl_3$. The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and filtered, and the filtrate was concentrated under reduced pressure. Distillation of the concentrate gave 12 g (39%) of **10**, bp 80–83 °C (10 mm).

6-Methyl-2,3,4,6,7,8-hexahydropyrrolo[1,2-*a*]pyrimidine (4). Method C. A solution of 5-methyl-2-pyrrolidinone (25 g, 0.25 mol) and acrylonitrile (15 g) was stirred and heated at 80 °C for 3 h. The reaction mixture was distilled, and the fraction, bp 110–120 °C (0.2 mm), was collected and dissolved in MeOH (75 mL) containing concentrated aqueous NH_3 (150 mL). This solution was hydrogenated over Raney Nickel at 100 °C and 2000 psi. Filtration, evaporation of the solvent, and distillation of the residue gave 1-(3-aminopropyl)-5-methyl-2-pyrrolidinone: bp 100 °C (0.2 mm); yield 29 g. A solution of this material in xylene (170 mL) containing *p*-toluenesulfonic acid (1 g) was heated under reflux with H_2O separation (Dean-Stark trap) for 36 h. Distillation of the reaction mixture gave 20.7 g (60%) of 4, bp 91–93 °C (10 mm).

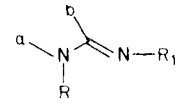
2,3,4,5,7,8-Hexahydro-9*H*-pyrrolo[1,2-*a*][1,3]diazepine (1). A mixture of α-butyrolactone (18.9 g) and 1,4-diaminobutane (19.6 g) was heated in a stainless-steel bomb for 7 h at 280 °C. The reaction mixture was distilled, and the fraction, bp 115–120 °C (0.1 mm), was collected and redistilled at 280 °C (200 mm). This distillate was redistilled to yield 2.3 g of 1, bp 108–110 °C (14 mm), lit.[10] 108–110 °C (14 mm).

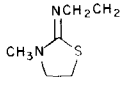
DBN hydrogen fumarate was prepared by dissolving redistilled DBN, bp 88 °C (10 mm) (23.2 g, 0.2 mol), and fumaric acid (24.8 g, 0.2 mol) in hot *i*-PrOH, cooling, and collection of the DBN hydrogen fumarate, mp 159–60 °C, by filtration.

2,3,5,6-Tetrahydro-8*H*-imidazo[2,1-*c*][1,4]thiazine Sulfoxide Hydrogen Fumarate (24). A solution of **22** (2.35 g, 0.0166 mol) and $NaIO_4$ (3.4 g, 0.016 mol) in 80% aqueous MeOH (90 mL) was stirred overnight. The reaction mixture was extracted with $CHCl_3$ (3 × 100 mL). The combined extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated. The concentrate was distilled to yield 1.1 g of colorless liquid, bp 140 °C (0.2 mm), which was dissolved in EtOAc (5 mL) and added to a solution of fumaric acid (0.76 g) in *i*-PrOH (100 mL). On cooling, 1.4 g of **24**, mp 166–168 °C, was collected by suction filtation with Me_2CO washing.

8-Benzylidine-2,3,4,6,7,8-hexahydropyrrolo[1,2-*a*]pyrimidine (5). A solution of DBN (2.48 g, 0.02 mol) and benzaldehyde (2.12 g, 0.02 mol) in ethyl formate (2 mL) was stirred at 45 °C for 22 h. Evaporation of the solvent and recrystallization of the residue from cyclohexane gave 1.0 g (21%) of 8-(α-hydroxylbenzyl)-2,3,4,6,7,8-hexahydropyrrolo[1,2-*a*]pyrimidine,

Table II. Inhibition of Rabbit and Human Lung Indolamine N-Methyltransferase by Monocyclic Amidine Derivatives



compd	R	R_1	a, b	method and/or lit. ref[a]	% yield	mp, °C	recrystn solvent	formula (anal.)[b]	IC_{50} value ($M \times 10^{-6}$) for inhibn of INMT in vitro[c] rabbit lung enz	human lung enz
29	CH_3	H	a-$(CH_2)_2$S-b	30, 31				$C_4H_8N_2S$·HI	0.8	2.2
30	CH_3	CH_3	a-$(CH_2)_2$S-b	D (1)[o]	36	159-161	EtOH-Et_2O	$C_5H_{10}N_2S$·HI (C, H, N, I)[e]	8.1	
31	CH_3CH_2	H	a-$(CH_2)_2$S-b	32		80-83	*i*-PrOH-Et_2O	$C_5H_{10}N_2S·C_4H_4O_4$	0.2	2.6
32	H	CH_3	a-$(CH_2)_2$S-b	30				$C_4H_8N_2S$·HCl	7.7	15.2
33	$CH_3(CH_2)_2$	H	a-$(CH_2)_2$S-b	D (1)[p]	31	121-123	*i*-PrOH-Et_2O	$C_6H_{12}N_2S$·HBr (C, N, S, Br)[f]	9.9	
34	CH_2=$CHCH_2$	H	a-$(CH_2)_2$S-b	D (1)[q]	65	112-116	*i*-PrOH-Et_2O	$C_6H_{10}N_2S$·HBr (C, H, N, S, Br)	7.1	
35	HC≡CCH_2	H	a-$(CH_2)_2$S-b	D (1)[r]	35	143-145	*i*-PrOH-Et_2O	$C_6H_8N_2S$·HBr (C, H, N, S, Br)	13.8	
36	(4-Py)CH_2	H	a-$(CH_2)_2$S-b	D (1)[s]	10	295 dec	*i*-PrOH	$C_9H_{11}N_3S$·2HBr (H, N, S)[g]	>6.9[t]	
37	NH_2	H	a-$(CH_2)_2$S-b	*u*	6	122-123	*i*-PrOH-MeOH-Et_2O	$C_3H_7N_3S·C_4H_4O_4$[h] (C, H, N, S)		46.8 (16.2-703)[d]
38	CH_3NH	H	a-$(CH_2)_2$S-b	*u*	21	93-95	*i*-PrOH	$C_4H_9N_3S·C_4H_4O_4$[i] (H, N)	1.5	6.4 (5.1-7.9)[d]
39	CH_3	H	a-$CH_2CH(CH_3)$S-b	E (1)[33,v]	73	133-136	EtOH-Et_2O	$C_5H_{10}N_2S·C_4H_4O_4$[j] (C, H, N, S)	7.9	
40	CH_3		a-$(CH_2)_2$S-b	*u*	86	292 dec	EtOH-Et_2O	$C_{10}H_{20}N_4S_2$·2HCl (C, H, N, S, Cl)		>75.1[x]
41	CH_3	H	a-CH=CHS-b	34				$C_4H_6N_2S$·HI	2.0	6.7
42	CH_3	H	a-CH=$C(CF_3)$S-b	D (1)[35]	80	177-180	CH_2Cl_2	$C_5H_5F_3N_2S·FSO_3H$ (C, H, F, N, S)	>88.6[u]	>86.6[y]
43	CH_3	H	a-SCH=CH-b	17, 36		147-151	*i*-PrOH	$C_4H_6N_2S$·HCl (C, H, N, S, Cl)		3283
44	CH_3	CH_3	a-SCH=CH-b	17		208 dec	*i*-PrOH-Et_2O	$C_5H_8N_2S$·HCl (C, H, N, S, Cl)	6.9	19.2 (13.7-27)[d]
45	CH_3	CH_3	a-SN=CH-b	17		205-212 dec	MeOH-*i*-PrOH	$C_4H_7N_3S$·HCl (C, H, N, S, Cl)	5.2	12.0 (6.6-20.5)[d]
46	CH_3	H	a-$(CH_2)_2$O-b	D (3)[38,o]		165-167	*i*-PrOH	$C_4H_8N_2O$·HI (C, H, N, I)	40.0	
47	H	H	a-$(CH_2)_3$-b	14, 39				$C_4H_8N_2$·HCl	33.6	>8300
48	H	CH_3	a-$(CH_2)_3$-b	14, 39				$C_5H_{10}N_2$·HI	27.1	>4425
49	CH_3	H	a-$(CH_2)_3$-b	14	51.5	169-172	EtOH-Et_2O	$C_5H_{10}N_2$·HI	1.5	4.3
50	C_2H_5	H	a-$(CH_2)_3$-b	D (3)[z]	40	124-128	*i*-PrOH-Et_2O	$C_6H_{12}N_2·C_2H_2O_4$	1.6	2.8
51	$CH_3(CH_2)_4$	H	a-$(CH_2)_3$-b	D (3)[aa]	44	68-75	*i*-PrOH-Et_2O	$C_9H_{18}N_2$·HBr (C, H, N, Br)	>106[t]	>106[dd]
52	c-C_3H_5[n]	H	a-$(CH_2)_3$-b	*u*	100	oil		$C_7H_{12}N_2$ (C, H, N)	67.0	
53	$(CH_3)_2CHCH_2$	H	a-$(CH_2)_3$-b	D (3)[bb]	4.7	134-138	*i*-PrOH-Et_2O	$C_8H_{16}N_2·C_2H_2O_4$	19.4	
54	$(EtO)_2CHCH_2$	H	a-$(CH_2)_3$-b	*u*		132-133		$C_{10}H_{20}N_2O_2$·HCl (C, H, N, Cl)	>106[t]	
55	$PhCH_2CH_2$	H	a-$(CH_2)_3$-b	D (3)	61	144-147 dec	*i*-PrOH-Et_2O	$C_{12}H_{16}N_2$·HBr (C, H, N)[k]	3717	
56	CH_3	CF_3CH_2	a-$(CH_2)_3$-b	E (1)[cc]	87.6	96-99 dec	Et_2O	$C_7H_{11}F_3N_2·C_2H_2O_4$ (C, H, F, N)	>92.6[t]	

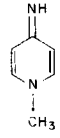
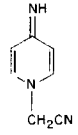
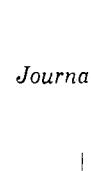
57	CH_3	CH_3	a-$(CH_2)_3$-b	D (3)[14]		142–145			31.2	
58	CH_3	c-C_4H_7-	a-$(CH_2)_3$-b	E (1)[cc]	54	108–111	*i*-PrOH-Et_2O	$C_9H_{16}N_2 \cdot C_2H_2O_4$ (C, H, N)	>10.3[gg]	
59	CH_3	H	a-$(CH_2)_2SCH_2$-b	E (2)[ee]		111–113	*i*-PrOH-MeOH	$C_5H_{10}N_2S \cdot HCl \cdot H_2O$ (C, H, N, S)	6.3	32.3
60	CH_3	H	a-$(CH_2)_3S$-b	E (2)[33,v]	54.5	168–171	*i*-PrOH	$C_5H_{10}N_2S \cdot HBr$ (C, H, N, S, Br)	2.0	3.7
61	C_2H_5	H	a-$(CH_2)_3S$-b	40				$C_6H_{12}N_2S \cdot HBr$	1.8	9.9
62	CH_3	H	a-CH=CHSCH_2-b	E (2)[ff]	11.5	124–125	*i*-PrOH-Et_2O	$C_5H_8N_2S \cdot HBr$ (C, H, N, S, Br)	3.0	12.7 (7.2–25.9)[d]
63	CH_3	CH_3	a-CH=CHSCH_2-b	E (2)[ff]	35	147–148	MeOH-*i*-PrOH	$C_6H_{10}N_2S \cdot C_4H_4O_4$[j] (C, H, N, S)	9.8	
64	CH_3	H	a-CH=CHCH_2S-b	D (3)	33	133–136	*i*-PrOH	$C_5H_8N_2S \cdot HCl \cdot H_2O$ (C, H, N, S, Cl)	2.0	4.0 (2.0–7.0)[d]
65	CH_3	H	a-$(CH=CH)_2$-b	41				$C_6H_8N_2 \cdot HCl$	1.6	15.0 (9.7–23.3)[d]
66	CH_2=$CHCH_2$	H	a-$(CH=CH)_2$-b	42				$C_8H_{10}N_2 \cdot HBr$	1.58	>11.6[jj]
67	CF_3CH_2	H	a-$(CH=CH)_2$-b	E (1)		204–206.5	EtOH-Et_2O	$C_7H_7F_3N_2 \cdot HBr$ (C, H, N, F, Br)	>9.7[t]	
68	CH_3	CF_3CH_2	a-$(CH=CH)_2$-b	E (1)[hh]	23.4	176–178	*i*-PrOH	$C_8H_9F_3N_2 \cdot HBr$ (C, H, Br, F, N)	>9.2[t]	
69	CH_3	$HO(CH_2)_2$	a-$(CH=CH)_2$-b	A (3)[43]	42	158–159	*i*-PrOH	$C_8H_{12}N_2O \cdot HI$ (C, H, N, I)	>8.9[ii]	
70	CH_3	c-C_3H_5-	a-$(CH=CH)_2$-b	E (3)[hh,o]	69	92.5–94.5	*i*-PrOH-Et_2O	$C_9H_{12}N_2 \cdot HBr \cdot H_2O$ (H, N)[l]	>10.1[t]	
71	CH_3	H	a-CH=CHCH=C(CN)-b	D (3)	22.2	222–225	H_2O	$C_7H_7N_3 \cdot HI$ (C, H, N, I)	179.5	
72	CH_3	H	a-CH=CHC(CH_3)=CH-b	44				$C_7H_{10}N_2 \cdot HI$	2.8	
73	CH_3	H	a-CH=CFCH=CH-b	E (1)	27.4	181–185	*i*-PrOH-H_2O	$C_6H_7FN_2 \cdot HBr$ (C, H, N, Br)	69.8	
74	CH_3	H	a-CF=CHCH=CH-b	E (1)[u]	52	163–180	*i*-PrOH	$C_6H_7FN_2 \cdot FSO_3H$ (C, H, N, S, F)	5.3	
75	CH_3	CH_3	a-CF=CHCH=CH-b	E (1)	32.5	123–125	*i*-PrOH	$C_7H_9FN_2 \cdot FSO_3H$ (C, H, N, S, F)	74.0	
76	CH_3	H	a-C(NH_2)=CHCH=CH-b	*u*	51	166–168	*i*-PrOH	$C_6H_9N_3 \cdot C_2H_2O_4$ (C, H, N)	6.2	
77	CH_3	CH_3	a-C$(NHCH_3)$=CHCH=CH-b	*u*	21.7	130–133	*i*-PrOH	$C_8H_{13}N_3 \cdot FSO_3H$ (H, N, S)[m]	13.1	
78	CH_3	H	a-C(OC_2H_5)=CHCH=CH-b	*u*	78.9	160–162	*i*-PrOH	$C_8H_{12}N_2O \cdot C_2H_2O_4$[n]	>10.3[t]	
79	CH_3	H	a-$(CH_2)_2$CH=CH-b	E (2) *u*	25.2	120–121	EtOH-Et_2O	$C_6H_{10}N_2 \cdot C_4H_4O_4$[j] (C, H, N)	0.9	7.9 (6.0–10.4)
80	CH_3	H	a-CH=CHN=CH-b	E (1)[13,v]	24.6	239–242	H_2O	$C_5H_7N_3 \cdot HBr$ (C, H, N, Br)	>5263	
	Related Compds									
81				45				$C_6H_8N_2 \cdot HI$	6.2	63.8
82				D (3)[kk]	51.5	273–275	*i*-PrOH	$C_7H_7N_3 \cdot HCl$ (C, H, N, Cl)	>147.5[ii]	

Table II (*Continued*)



compd	R	R_1	a, b	method and/or lit. ref[a]	% yield	mp, °C	recrystn solvent	formula (anal.)[b]	IC_{50} value ($M \times 10^{-6}$) for inhibn of INMT in vitro[c]: rabbit lung enz	human lung enz
83				D (3)[o]	44.4	209-211	EtOH-Et_2O	$C_8H_8N_2$·HBr (C, H, N, Br)	>11.7[t]	
84				D (3)[u]		160-164 dec	acetone-MeOH	$C_4H_6N_2S$·HCl (C, H, N, S, Cl)		21.9
85				D (3)[37,o]	14	200-202.5	*i*-PrOH	$C_5H_8N_2S$·HI (C, H, N, S, I)	19.6	45.41

[a] Literature reference is to preparation of intermediate or to end product. [b] Compounds were analyzed for the elements shown in parentheses and were within 0.4% of the calculated value unless otherwise indicated. [c] Values were estimated by analysis of regression line in reverse; there were two to four data points per compound. [d] For eight compounds, 95% confidence limits were obtained by Fieller's theorem; there were five to eight data points for these compounds. [e] S: calcd, 12.42; found, 12.94. [f] H: calcd, 5.82; found, 5.33. [g] Br: calcd, 45.01; found, 43.98. [h] Acid maleate salt. [i] C: calcd, 38.86; found, 39.46. S: calcd, 12.97; found, 13.57. [j] Acid fumarate salt. [k] Br: calcd, 29.68; found, 30.11. [l] Br: calcd, 32.33; found, 31.76. C: calcd, 43.74; found, 42.99. [m] C: calcd, 38.24; found, 37.60. [n] Anal. calcd: C, 49.58; H, 5.82; N, 11.56. Found: C, 48.33; H, 6.27; N, 12.05. [o] RX = CH_3I. [p] RX = $CH_3CH_2CH_2I$. [q] RX = CH_2=$CHCH_2Br$. [r] RX = HC≡CCH_2Br. [s] RX = (4-Py)CH_2Br. [t] There is 0% inhibition at this value. [u] See Experimental Section. [v] Z = SCH_3. [w] 29% inhibition at this value. [x] 28% inhibition at this value. [y] 16% inhibition at this value. [z] RX = C_2H_5I. [aa] RX = $CH_3(CH_2)_4Br$. [bb] RX = $(CH_3)_2CHCH_2Br$. [cc] Z = OCH_3. [dd] 43% inhibition at this value. [ee] Z = OEt. [ff] Z = SEt. [gg] 17% inhibition at this value. [hh] Z = Cl. [ii] 14% inhibition at this value. [jj] 13% inhibition at this value. [kk] RX = $ClCH_2CN$. [ll] 40% inhibition at this value.

Table III. Inhibition of Rabbit Lung INMT in Vivo by 22 and 29

drug treatment	av sp act.[a]	av % inhibn
expt 1. control	44 967 ± 2521	
0.5% 22 in drinking water for 5 days	11 367 ± 2900	75 ($p < 0.001$)[b]
expt 2. control	65 290 ± 4486	
0.02% 29 in drinking water for 12 days	15 671 ± 1717	76 ($p < 0.001$)[b]

[a] Cpm/mg of enzyme protein (after 1 h incubation at 37 °C) in toluene–isoamyl alcohol phase. All data are average values (±SEM). [b] Student's *t* test.

Table IV. In Vivo Inhibition of the Conversion of [^{14}C]NMT to [^{14}C]DMT in the Rabbit by 22[a]

drug treatment[b]	av [^{14}C]DMT cpm/g of lung tissue	av [^{14}C]DMT cpm/g of brain tissue
control	5342	69.3
22	1841 (64% inhibn, $p < 0.05$[c])	30 (57% inhibn, $p < 0.05$[d])

[a] Results are averaged for three animals. [b] Animals were given 0.5% 22 in the drinking water for 5 days. The average total consumption of 22 was approximately 2 g. [c] Student's *t* test ($t = 4.15$). [d] Student's *t* test ($t = 3.89$).

mp 207–209 °C. A solution of this alcohol (2.3 g, 0.01 mol) in 3 N HCl in MeOH (50 mL) was stirred for 0.5 h and then evaporated to dryness. The residue was dissolved in *i*-PrOH (50 mL) and treated with fumaric acid (1.2 g) in hot *i*-PrOH (50 mL). On cooling, **5** hydrogen fumarate was obtained (2.8 g, 80%).

3-Methylene-2,3,4,6,7,8-hexahydropyrrolo[1,2-*a*]pyrimidine Fumarate (6). A mixture of 2-amino-Δ^1-pyrroline (1.22 g, 0.14 mol) and 3-chloro-2-(chloromethyl)-1-butene (0.9 g, 0.072 mol) in EtOH (20 mL) was refluxed for 6 h. Following evaporation, the residue was triturated with Et_2O–*i*-PrOH. After removal of insoluble aminopyrroline hydrochloride, the solvent was evaporated, and the residue was dissolved in MeOH, to which was added K_2CO_3 (1.5 g). When neutralization was complete, the filtered solution was evaporated. The oily residue was dissolved in $CHCl_3$, and the $CHCl_3$ solution was washed with H_2O and evaporated. Addition of excess fumaric acid in EtOH gave **6** (0.24 g, 12.9%).

DBN Methiodide (7). Methyl iodide (2.8 g, 0.02 mol) was added dropwise to a solution of DBN (2.5 g, 0.2 mol) in benzene (100 mL). The solution was stirred for 1 h and then evaporated to dryness. The residue was recrystallized from $CHCl_3$-Et_2O to yield **7** (2 g, 37%), mp 248–250 °C.

6,7-Dihydro-5*H*-pyrrolo[1,2*a*]imidazole Oxalate (16). After standing for 4 weeks, a mixture of 3-chloropropionitrile (1.03 g, 0.01 mol) and aminoacetaldehyde diethyl acetal (1.33 g, 0.01 mol) was dissolved in $CHCl_3$. Ammonium chloride was removed by filtration, and the filtrate was evaporated. The residue treated with Et_2O gave 1-(1,1-diethoxyethyl)-2-iminopyrrolidine hydrochloride (**54**) (0.4 g, 16.9%).

A solution of **54** (0.2 g, 0.8 mmol) in concentrated HCl (10 mL) was refluxed for 4 h. The colorless solution was evaporated to dryness, H_2O was added and the solution was reevaporated. The residue was dissolved in EtOH, and excess K_2CO_3 was added. After several hours, the solids were removed, and oxalic acid was added to generate **16** oxalate (0.10 g, 62%), mp 98–104 °C.

2,3-Dihydro-7*H*-imidazo[2,1-*b*][1,3]thiazine Oxalate (27). A mixture of imidazolidine-2-thione (35.7 g, 0.35 mol), 3-chloropropionaldehyde diethyl acetal (87.4 g, 0.525 mol), and KI (11.6 g, 0.07 mol) in EtOH (1400 mL) was heated at reflux for 4 days. Following evaporation, the residue was dissolved in H_2O and extracted with Et_2O. The aqueous solution was made weakly basic with dilute NaOH. After removal of solids by filtration, the filtrate was extracted repeatedly with $CHCl_3$. The combined $CHCl_3$ solutions were extracted with an excess of aqueous oxalic acid. The aqueous solution was made weakly basic with dilute NaOH and reextracted with $CHCl_3$. Evaporation of the $CHCl_3$ solution gave an oil (31.3 g). A portion (21.3) was dissolved in toluene (1200 mL) with warming. After decanting from a small amount of insolubles, molecular sieves 4A (200 g) were added.

and the mixture was refluxed for 2 days. Filtration, followed by evaporation, gave 10 g of crude product, which was dissolved in EtOH (250 mL), to which, after filtration, was added with warming excess oxalic acid. Addition of ether to the cloud point resulted in crystallization of **27** oxalate (6.2 g, 11% overall), mp 115 °C dec.

General Procedures for Methods D and E (Scheme III). Method D. Generally exothermic reactions of the amines with methyl fluorosulfonate were carried out neat or in inert solvent using excess methylating agent.[46,47] Alkylations employing the less reactive trialkyloxonium fluoroborates or alkyl and aralkyl halides were carried out in inert solvent with heating when necessary. The resulting imine salts could in some cases be isolated in crystalline form directly. Frequently, the salts were treated with dilute NaOH to generate the free base, which was then converted to a more acceptable salt form. In other cases, metathesis to another salt form was accomplished by the use of an anion-exchange resin.

Method E. Alkylation reactions on the halo-, methoxy-, or methylthio-substituted intermediates were carried out as described for method D. The product salts were then allowed to react with aqueous or alcoholic amine solutions, generally at room temperature, to generate the desired imine derivatives. Evaporation, followed by treatment with dilute NaOH, provided the imines as free bases, which were then converted to the desired salt forms.

6-Fluoro-1-methyl-2-imino-1,2-dihydropyridine Fluorosulfonate (74) [Method E (1)]. 2,6-Difluoropyridine (5.0 g, 0.043 mol) and FSO_2OCH_3 (15.0 g, 0.13 mol) were mixed and warmed to above 70 °C. After 2 min, the mixture had solidified. After an additional 2 min, CH_2Cl_2 (40 mL) was added, and the mixture was stirred and then filtered. A yield of 9.9 g (99%) of the hygroscopic white fluorosulfonate salt was isolated. Without further purification, the salt (2.75 g, 0.012 mol) was mixed with ice, and dilute NH_3 [14.6 mL of concentrated NH_3 diluted to 146 mL (0.024 mol)] was added. After standing for 0.5 h, the mixture was evaporated to dryness. The residue was stirred with MeCN (50 mL), and inorganic salts were removed by filtration. After evaporation of the MeCN solution, the residue was taken up in $CHCl_3$ (50 mL) and, after 1 h, filtered, giving 1.44 g of pale yellow crystals: IR (KBr) 1685, 1588, 1300 cm^{-1}; NMR (CF_3COOD) δ 3.73 (d, CH_3, J = 3 Hz), 6.42 (2 d, H_5, $J_{H_5-H_4}$ = 8, J_{H_5-F} = 5 Hz), 6.89 (d, H_3, $J_{H_3-H_4}$ = 9 Hz), 7.87 (q, H_4, J = 8 H).

6-Amino-1-methyl-2-imino-1,2-dihydropyridine Oxalate (76). To 2,6-difluoro-1-methylpyridinium fluorosulfonate (2.0 g, 0.0087 mol) mixed with ice was added an excess of concentrated NH_4OH. The mixture was evaporated to dryness, and the residue was slurried in MeCN (50 mL) and filtered. The filtrate was evaporated, and the residue was slurried with $CHCl_3$ (20 mL) and filtered to give 1.08 g (55.6%) of yellow-green 6-amino-1-methyl-2-imino-1,2-dihydropyridinefluorosulfonic acid salt. To an EtOH (50 mL) solution of the salt was added powdered K_2CO_3 (6.0 g). After 3 h, the mixture was evaporated to dryness and the residue slurried with *i*-PrOH (25 mL). Following removal of solids by filtration, oxalic acid (1.0 g) was added to the filtrate. The precipitated solid oxalate was dissolved in hot ethanol and charcoaled. The yellow solution was evaporated and the residue triturated with *i*-PrOH to give 0.95 g of crystalline **76**: IR (KBr) 1670, 1655, 1590 cm^{-1}; NMR (D_2O) δ 3.37 (s, CH_3), 6.07 (d, H_3 and H_5, J = 8 Hz). 7.39 (t, H_4, J = 8 Hz). Repeating the above procedure using CH_3NH_2 in place of NH_3 afforded the corresponding 2,6-dimethyl derivative **77** as the FSO_3H salt: IR (KBr) 1648, 1595, 1300 cm^{-1}; NMR (D_2O) δ 2.87 (s, CH_3), 3.40 (s, CH_3), 6.05 (d, H_3 and H_5, J = 8 Hz), 7.68 (t, H_4, J = 8 Hz).

6-Ethoxy-1-methyl-2-imino-1,2-dihydropyridine Oxalate (78). To a solution of 6-fluoro-1-methyl-2-imino-1,2-dihydropyridine fluorosulfonate (0.20 g, 0.0009 mol) in EtOH (20 mL) was added powdered K_2CO_3 (1.0 g). After stirring for 2 h, the solvent was evaporated and the residue slurried with $CHCl_3$ (10 mL). The mixture was filtered and the filtrate evaporated. To the residue dissolved in *i*-PrOH (5 mL) was added oxalic acid in *i*-PrOH to form crystalline oxalate: IR (KBr) 1672, 1640, 1600 cm^{-1}; NMR ($CDCl_3$) δ 1.40 (t, CH_3), 3.43 (s, CH_3), 4.02 (q, CH_2), 5.08 (d, H_5, J = 7 Hz), 5.53 (s, NH), 5.97 (d, H_3, J = 8 Hz), 6.76 (2 d, H_4, J = 7.5 and 8 Hz).

1,2-Bis[(3-methylthiazolidinyliden-2-yl)amino]ethane Dihydrochloride (40). To a solution of 2-(methylthio)-Δ^2-thiazoline (19.0 g, 0.143 mol) in CH_2Cl_2 (120 mL), cooled in ice, was added CH_3OSO_2F (18.0 g, 0.158 mol) in several portions. After stirring for 15 min, the mixture was evaporated to dryness and the residue flushed with C_6H_6. To the residue dissolved in MeCN (200 mL) was added ethylenediamine (4.3 g, 0.072 mol). After standing at room temperature for 2 days, the mixture was evaporated to give a solid. The solid, dissolved in water, was passed through a column of Amberlite AGl-X8 resin on the chloride cycle. The eluate was evaporated to dryness, and the residue was crystallized from EtOH–Et_2O.

3-Amino-2-iminothiazolidine Hemimaleate (37). To a solution of 2-amino-Δ^2-thiazoline (2.04 g, 0.02 mol) in CH_2Cl_2 (20 mL) cooled in an acetone–ice bath was added dropwise a solution of 2,4,6-trimethylbenzenesulfonyloxyamine[16] (4.3 g, 0.02 mol) over 30 min. After another 30 min at 0 °C, the mixture was permitted to warm to room temperature. Ether (60 mL) was added, and the solids were removed by filtration (4.5 g). Recrystallization from EtOH–Et_2O gave 2.5 g of crude salt, which was converted to free base with dilute NaOH and extracted into $CHCl_3$. After evaporation of the $CHCl_3$ solution, the residue was redissolved in $CHCl_3$, and undissolved solids were removed by filtration. To the residue from the $CHCl_3$ dissolved in *i*-PrOH was added maleic acid (0.33 g). Evaporation followed by trituration with *i*-PrOH–Et_2O gave **37**. **38** was obtained when the above reaction was carried out using 2,4,6-trimethylbenzenesulfonyloxymethylamine. The latter was prepared by the procedure[16] reported for 2,4,6-trimethylbenzenesulfonyloxyamine (mp 83–84 °C, Et_2O–petroleum ether, 30–60 °C), except that *N*-methylhydroxylamine was used.

5-Imino-2-methyl-2,5-dihydroisothiazole Hydrochloride (84). A mixture of isothiazole-5-carboxylic acid[48] (3.6 g, 0.028 mol) and $SOCl_2$ (30 mL) was heated at reflux until complete solution occurred (30 min). Following evaporation, THF (28 mL) was added to the residue, followed by NaN_3 (1.82 g, 0.028 mol) in H_2O (14 mL). The mixture was stirred for 1 h, and the THF was removed under vacuum. The aqueous residue was extracted with CH_2Cl_2. To the residue from the extract was added toluene (60 mL), and the mixture was refluxed for 10 min. After cooling the mixture, the intense yellow solids (2.0 g) were removed by filtration. (This material showed a carbonyl band at 1615 cm^{-1} and a parent ion in the mass spectrum of 128 corresponding to the desired isothiazol-5-yl isocyanate. The structure of the insoluble product, which was not fully characterized, presumably was a trimer or a low-molecular-weight polymer and not monomeric isocyanate.) The solid (2.0 g) was heated at reflux in 6 N HCl (50 mL) for 6 h. Following filtration and evaporation, a semisolid residue was obtained, which was dissolved in H_2O. After making strongly basic with dilute NaOH, the aqueous solution was extracted with $CHCl_3$, which gave 0.75 g of solid product on evaporation: NMR ($CDCl_3$, acetone-d_6) δ 6.52 (d, 1 H, J = 2 Hz), 8.23 (d, 1 H, J = 2 Hz); MS (M^+ 100). To the crude 5-aminoisothiazole (0.75 g, 0.0065 mol) dissolved in *i*-PrOH (10 mL) was added MeI (2 mL). The solution was refluxed for 1.5 h. Following evaporation to a small volume, acetone was added, and the mixture was triturated until a solid material was obtained (0.40 g). The solids were dissolved in water and passed through a column of Amberlite AG1-X8 (100–200 mesh, Cl^- cycle) ion-exchange resin. Evaporation of the aqueous eluate, followed by trituration with MeOH–acetone, gave pure **84**.

1-Cyclopropyl-2-imino-2,3,4,5-tetrahydropyrrolidine (52). A solution of 4-chlorobutyronitrile (2.07 g, 0.02 mol) and cyclopropylamine (4.50 g, 0.08 mol) in EtOH (10 mL) was heated at 90–100 °C in a 200-mL pressure bottle for 18 h. After cooling, the reaction mixture was filtered and the filtrate evaporated to dryness. To the residue dissolved in MeOH (20 mL) was added an excess of K_2CO_3, and the mixture was allowed to stand for 24 h. The solvent was evaporated, and the residue was extracted with $CHCl_3$ to yield 2.8 g of a brown oil. Attempts to prepare crystalline salts were unsuccessful.

1-(2,2,2-Trifluoroethyl)-1,2-dihydropyridin-2-one. To a solution of 2-pyridone (4.75 g, 0.05 mol) in DMF (50 mL) was added NaH (1.2 g, 0.05 mol). After 0.5 h, CF_3CH_2I (25.0 g, 0.125 mol) was added. The mixture was heated at 150 °C for 2 h and then for an additional 1 h at 185 °C in a pressure bottle. The DMF was removed under vacuum, and the residue was dissolved in ether (100 mL). Evaporation afforded 3.39 g of an oil. When

3.09 g was chromatographed on silica gel, using ether as eluant, 1-(2,2,2-trifluoroethyl)-1,2-dihydropyridin-2-one was obtained as an oil. This material was treated directly with FSO_2OCH_3, followed by ammonia according to method E (1) (Scheme III) to give **67**.

1-Methyl-2-oxo-1,2,5,6-tetrahydropyridine. Using the procedure of M. Shamma and P. D. Rosenstock,[11] a mixture of vinylacrylic acid (20 g, 0.2 mol), 40% aqueous $MeNH_2$ (400 mL), and hydroquinone (0.5 g) was heated at about 170 °C overnight in a stainless-steel pressure vessel. Following cooling and evaporation of the reaction mixture, the residue was distilled. Two main fractions were obtained, 8.0 g, bp 86-125 °C at 1.8–0.5 mm, and 4.5 g, bp 130 °C at 0.4 mm. The NMR of both fractions showed the absorption bands corresponding to the expected structure: NMR ($CDCl_3$) δ 2.47 (m, 5-CH_2), 3.0 (s, CH_3), 3.48 (d, 6-CH_2, J = 7 Hz), 5.93 (d of m, H_4, J = 10 Hz), 6.63 (d of t, 3 H, J_t = 7, J_d = 10 Hz). Both fractions were suitable for conversion to **79**.

2-Amino-6*H*-1,3-thiazine (86). A mixture of thiourea (10.0 g, 0.13 mol), 3-chloropropionaldehyde diethyl acetal (50 g, 0.30 mol), and KI (2 g) in EtOH (75 mL) was heated at reflux for 2 days. Following evaporation and partitioning between H_2O–Et_2O, the aqueous phase was made basic with Na_2CO_3 solution. Extraction with ether (250 mL) afforded 8.0 g of crude product. The solid was redissolved in Et_2O, and the solution was charcoal treated and then evaporated to a small volume (15 mL). The crystals which formed were filtered, giving 6.8 g (32%) of solid 2-amino-4-ethoxy-5,6-dihydro-6*H*-1,3-thiazine: NMR ($CDCl_3$) δ 1.24 (t, CH_3, J = 7 Hz), 2.0 (m, 6-CH_2), 3.09 (m, 5-CH_2), 3.70 (m, CH_2O), 4.65 (q, 4-CH), NH_2 variable. The ethoxythiazine, without purification (6.38 g, 0.04 mol), was mixed with freshly prepared polyphosphoric acid [P_2O_5 (50 g) and 85% H_3PO_4 (40 mL)], and the mixture was heated at 80–90 °C for 15 min. Following cooling and quenching with ice, the mixture was made basic with concentrated NaOH solution, and then extracted four times with $CHCl_3$ (100 mL). The $CHCl_3$ extract gave a yellow oil (36 g, 79.5%) of **86** suitable for use in the preparation of **64**: NMR ($CDCl_3$) δ 3.42 (2 d, CH_2, $J_{CH_2\text{-}H_5}$ = 5, $J_{CH_2\text{-}H_4}$ = 1 Hz), 5.50 (2 t, H_5, $J_{H_5\text{-}H_4}$ = 8, $J_{H_5\text{-}CH_2}$ = 5 Hz), 6.53 (2 t, H_4, $J_{H_4\text{-}H_5}$ = 8, $J_{H_4\text{-}CH_2}$ = 1 Hz), NH_2 variable.

2,3-Dihydro-4*H*-1,4-thiazine-3-thione (87). A mixture of thioacetamide (25 g, 0.27 mol), 2-chloroacetaldehyde dimethyl acetal (55 mL, 0.46 mol), KI (1.0 g), and NaOH (11.5 g, 0.29 mol) in methanol (250 mL) was heated at reflux overnight. After neutralization to pH 7 with dilute HCl, the mixture was filtered and the filtrate again evaporated. The oily residue was pyrolyzed, starting at approximately 1 mm and 170 °C, giving 11.0 g of crude product in the condensate. Chromatography on silica gel with ethyl acetate–benzene (1:4) as eluant provided 8.0 g (25%) of purified 2,3-dihydro-4*H*-1,4-thiazin-3-one, mp 65–68 °C. Anal. (C_4H_5NOS) C, H, N, S. Treatment with P_2S_5 in pyridine[15] gave **87** in 30% yield, mp 87–89 °C. Anal. ($C_4H_5NS_2$) C, H, N, S.

Biological Procedures. *S*-[*methyl*-^{14}C]Adenosylmethionine (sp act. 42–53 mCi/mmol) was purchased from the New England Nuclear Corp. *N*-[*methyl*-^{14}C]Methyltryptamine was synthesized by the method of Horner and Skinner.[49] Pheniprazine was kindly donated by Lakeside Laboratories. The rabbits employed in these studies were New Zealand White (NZW) males, purchased from H.A.R.E., West Milford, N.J. Human lung tissue samples were post mortem specimens stored at −20 °C.

Rabbit Lung in Vitro INMT Inhibition Assay.[8] For the enzyme preparation, NZW male rabbits were exsanguinated and the lungs homogenized in 3–5 mol of 0.15 M KCl containing 10^{-4} M dithiothreitol and 10^{-4} M EDTA. The homogenate was centrifuged at 50 000*g* for 20 min at 0 °C. The supernatant was fractionated with ammonium sulfate. The 40–60% ammonium sulfate fraction was dialyzed against 100–400 volumes of 0.15 M KCl for 18 h. The preparation was centrifuged and, for most studies, used at this state of purity. For some of the screening, the enzyme was purified further by Sephadex chromatography. A 12-mL aliquot of the dialyzed enzyme preparation, containing about 150 mg of protein, was placed on a Sephadex G-150 column equilibrated with 1×10^{-3} M potassium phosphate buffer (pH 7.0) containing 5×10^{-5} M EDTA and 1×10^{-4} M dithiothreitol. The column was eluted with this buffer at a rate of 12 mL/h, and the active fractions (150–200 mL eluate) were pooled and concentrated tenfold by ultrafiltration. The protein concentration at this stage was about 5 mg/mL.

The in vitro incubations contained 0.028 mL of 1 M potassium phosphate buffer (pH 7.9), 0.017 mL of 0.002 M *N*-methyltryptamine, 0.005 mL of *S*-[*methyl*-^{14}C]adenosylmethionine (ca. 50 μCi/μmol, 50 μCi/2.5 mL), 0.04–0.06 mL of enzyme, and 0.005 mL of test compound (in H_2O or 5% Me_2SO) in a total volume of 0.105 mL. Samples were incubated for 60–90 min at 37 °C. The reaction was terminated by the addition of 0.20 mL of 0.12 M sodium tetraborate (pH 10), and the ^{14}C-labeled products (dimethyltryptamine) were extracted into 2 mL of H_2O-saturated isoamyl alcohol (or 97% toluene–3% isoamyl alcohol). Following centrifugation, the radioactivity of a 1-mL aliquot was determined in a Packard liquid scintillation counter.

Boiled enzyme fractions, omission of substrate, and addition of borate at the outset of incubation were procedures used to assess nonenzymatic incorporation of radioactivity into the organic phase. The indolamines present after incubation with enzyme were identified by cochromatography with known standards on silica gel TLC plates using MeOH–1 N NH_4OH (5:1) or *n*-BuOH–HOAc–H_2O (72:18:30) as developing solvents. Dimethyltryptamine was further characterized by gas–liquid chromatography and mass spectrometry.[50]

Human Lung in Vitro INMT Inhibition Assay. INMT was prepared from human lung through the Sephadex G-150 step as outlined above. The in vitro reaction mixtures contained 0.03 mL of 1 M potassium phosphate buffer (pH 7.9), 0.015 mL of 0.007 M *N*-methyltryptamine, 0.01 mL of *S*-[*methyl*-^{14}C]adenosylmethionine (ca. 50 μCi/μmol; 50 μCi/2.5 mL), 0.03–0.06 mL of enzyme, and 0.005 mL of test solution (5 mg/10 mL–5 mg/1000 mL in H_2O) in a total of 0.105 mL. Samples were incubated for 90 min at 37 °C. Appropriate controls were included. The reaction was terminated and worked up as outlined above for rabbit lung enzyme.

Effect of in Vivo Administration of 22 and 29 on the Activity of Rabbit Lung INMT. Rabbits (three per group) were administered the test compound in the drinking water (pH 7) for the duration of the experiment. Controls were given water. At the end of the experiment, the animals were decapitated and bled out, the lungs were removed, and a crude preparation (54 000*g* supernatant) of INMT was made and assayed by the usual in vitro procedure. Rabbits treated with **22** consumed an estimated 1.5–2.0 g over a 5-day period. The animals treated with **29** over 12 days consumed about one-tenth of that quantity.

Effect of Oral Administration of 22 on the Conversion in Vivo of [^{14}C]NMT to [^{14}C]DMT. Male NZW rabbits (400–500 g) were given 0.5% (v/v) **22** in the drinking water for 5 days; water was given to the controls. On day 5 the animals (three per group) were given the monoamine oxidase inhibitor pheniprazine (5 mg/kg iv). Six hours later they were given 25 μCi (0.5 mg) of ^{14}C-labeled *N*-methyltryptamine iv, and 5 min after injection of the label the animals were decapitated and bled out and the lungs and brains were removed and frozen at once in liquid nitrogen. [^{14}C]DMT was isolated from the tissues by standard solvent methods and quantitated by TLC. The data in Table IV gives evidence for the in vivo biosynthesis of [^{14}C]DMT in both lung and brain. Earlier experiments gave no evidence for the occurrence of [^{14}C]DMT in the blood.[50] Pretreatment with **22** gave variable results, but there was a 64% inhibition (avg) in the conversion of [^{14}C]NMT to [^{14}C]DMT in the lung and 57% inhibition in the brain. The cpm are expressed on a per gram basis, but 5–7 g of brain tissue was analyzed. Although the brain data were statistically significant ($p < 0.05$), it is not known if the results are meaningful in terms of DMT biosynthesis in view of the extremely low counts obtained.

Inhibition of the Methylation of *N*-Methylserotonin, Tryptamine and Serotonin by 22. The assay procedure for these experiments was as described above for *N*-methyltryptamine as substrate. After termination of the reactions by the addition of 0.2 mL of 0.125 M sodium tetraborate (pH 10), the ^{14}C-methylated products were extracted into 97% toluene–3% isoamyl alcohol containing 50–100 μg of carrier methylated indolamine. Following centrifugation, the radioactivity of a 1-mL aliquot was determined by liquid scintillation counting. For identification of the dimethylated products, aliquots of the organic phase were taken to dryness in vacuo at room temperature, and the residues

were examined by thin-layer chromatography on silica gel as described earlier.[51]

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Synthesis and Activities of Antitumor Agents

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Received August 2, 1978

N-(2-Chloroethyl)-*N*-nitrosocarbamoyl derivatives of glycosylamines have been prepared. Six *N*-(2-chloroethyl)-*N*-nitrosoureas, including three disaccharide derivatives, were submitted to a determination of antitumor activity. All the compounds tested exhibited strong antitumor activity against leukemia L1210 in mice.

Streptozotocin (NSC 85998) is a unique antitumor antibiotic produced by a fermentation of *Streptomyces achromogenes* var 128,[2-4] and its structure has been established as 2-(3-methyl-3-nitrosoureido)-2-deoxy-D-glucopyranose.[5,6] By synthetic studies of its methyl glycoside[7] and analogues,[8-11] it was found that the *N*-methyl-*N*-nitrosoureido group was an essential functional group for antitumor activity.

Prior to this finding, the research group at the Southern Research Institute had demonstrated that *N*-methyl-*N*-nitrosoureas were effective against leukemia L1210.[12] Soon after, it was discovered that replacements of the methyl group on the nitrosated nitrogen atom of these agents by a 2-chloroethyl group achieved an improved antitumor activity against leukemia L1210,[13,14] and three *N*-(2-chloroethyl)-*N*-nitrosoureas [*N*,*N*′-bis(2-chloroethyl)-(BCNU: NSC 409962),[13] *N*-(2-chloroethyl)-*N*′-cyclohexyl-(CCNU: NSC 79037),[13] and *N*-(2-chloroethyl)-*N*′-(*trans*-4-methylcyclohexyl)-*N*-nitrosourea (MeCCNU: NSC 95441)][14] have been prepared and used for clinical trials.

When *N*′-substituted *N*-(2-chloroethyl)-*N*-nitrosoureas decompose in a buffer solution, a (2-chloroethyl)diazo hydroxide is probably formed, which alkylates biological materials.[15,16,30] On the other hand, isocyanates that are generated on the decomposition of the nitrosoureas also

0022-2623/79/1822-0247$01.00/0