5 rpm at 60 min after dosing, and the number of animals falling off the rod within 2 min was counted. The ED_{50} was obtained by graphical interpolation.

Antipentylenetetrazol Activity.[43] The test was performed with a group of five mice. The animals were challenged with a subcutaneous injection of 125 mg/kg pentylenetetrazol at 60 min after dosing. The dose required to prevent convulsion and death in 50% (ED_{50}) of the animals during a 2 h observation was obtained by graphical interpolation.

Antifighting Activity.[44] A pair of mice was confined during foot shock (5 Hz, 2 ms, DC 50 V) by being placed under an inverted circular glass enclosure (1-L beaker). Pairs showing 15–20 fighting episodes in 3 min were selected. Five pairs of mice were used for each dose, and the number of responses before and at 60 min after dosing was counted. The dose causing 50% inhibition of the response (ED_{50}) was obtained by graphical interpolation.

Potentiation of Thiopental Sodium. Five mice per group were used. Sixty minutes after administration of the test compound, the animals were challenged with an intravenous injection of 35 mg/kg thiopental sodium. The minimum effective doses of the compounds for potentiation of thiopental sodium induced anesthesia were compared.

Acute Toxicity. The acute toxicity of each compound was determined with groups of one to three mice on the 7th day after oral administration. The LD_{50} was obtained by graphical interpolation.

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Inhibitors of Indoleethylamine *N*-Methyltransferase. Derivatives of 3-Methyl-2-thiazolidinimine. In Vitro, in Vivo, and Metabolic Studies

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A variety of substituent groups has been attached to the exocyclic imine function of 2-imino-3-methylthiazolidine (1) in a search for metabolic precursors of this potent inhibitor of the enzyme indoleethylamine *N*-methyltransferase (INMT) which would exhibit superior pharmacodynamic properties in animals. It has been determined that chemically stable derivatives of 1 based on succinic, nicotinic, and N-acylated amino acids, although they lack in vitro efficacy, are potent inhibitors of INMT when administered orally or intravenously to rabbits. Metabolic studies carried out with ^{14}C-labeled *N,N'*-bis(3-methyl-2-thiazolidinylidene)succinamide (3) have established that conversion of this compound to 1 occurs both in the whole rabbit and in the isolated rabbit liver. 1 itself has been shown to be metabolically inert in rabbits, being excreted primarily in the urine.

The possibility that methylated indoleethylamine derivatives, such as *N,N*-dimethyltryptamine (DMT), may play a role in schizophrenia continues to attract attention.[1,2] The enzyme indoleethylamine *N*-methyltransferase (INMT), which catalyzes the methylation of indoleethylamines,[3] has been reported to be present in a number of species and tissues.[4] Although DMT has been shown to produce psychotomimetic effects in man,[5-7] there is as yet no convincing evidence for elevated DMT levels in schizophrenics, perhaps because of its rapid metabolism in vivo.[8,9] Inhibitors of INMT, which would effectively block the biosynthesis of dimethylindoleethylamines in man, may permit one to obtain more conclusive evidence concerning the possible contribution of indoleethylamine derivatives to schizophrenia.

We have reported recently on a series of monocyclic amidine derivatives which were shown to be potent inhibitors of INMT.[10] With these amidines (in which one of the nitrogens existed as an exocyclic imine function) the only permissible substituents, if high in vitro potencies were to be realized, were methyl or ethyl groups on the annular nitrogen. Substituents on the exocyclic nitrogen, or on other ring atoms, consistently caused a decrease in potency. Because it was anticipated that these small basic molecules might be of short duration of action in vivo, due to rapid elimination, it became of interest to explore the effect on in vivo activity of the introduction of substituents at the exocyclic nitrogen, which would be expected to show metabolic lability. Substituents of this type might modify

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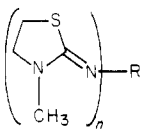
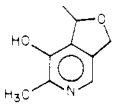
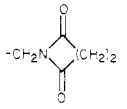
0022-2623/80/1823-0773$01.00/0

Table I. Derivatives of 3-Methyl-2-thiazolidinimine (1)



no.	n	R	% inhibn of human lung INMT in vitro: 25 μg/mL	% inhibn of human lung INMT in vitro: 2.5 μg/mL	approx LD_{50} (mice), mg/kg: ip	approx LD_{50} (mice), mg/kg: iv	approx LD_{50} (mice), mg/kg: po
1	1	-H	91	73		118	432
2	1	$-C(=O)CH_2CH_2COOH$	20			1280	>8 g
3	2	$-C(=O)CH_2CH_2C(=O)-$	+12				
			+74		1000-1500		1460
4	1	$-C(=O)CH_2NHC(=O)CH_3$	51	24		408	936
5	1	$-C(=O)CH_2NH_2$	54	11		549	4666
6	1	$-C(=O)CHNHC(=O)CH_3$ | $CH_2C_6H_5$ (*R,S*)	23	8	>2000		173
7	1	$-C(=O)C_5H_4N$-3	5	0		279	570
8	1	$-C(=O)CH_2C(=O)CH_3$	51	24			
9	2	-C(=O)-	0[c]		1140		~2000
10	1	$-C(=O)NHC_2H_5$	0[c]			1110	203
11	1	$-C(=O)OC(CH_3)_3$	6			68	306
12	1	$-SO_3Na$				>2.5 g	>5 g
13	2	$>CH_2$	*a*				
14	2	$>CHC_6H_5$	*a*				
15	2	$>CHC_5H_4N$-4	*b*				
16	2	$>CHC_6H_3(OCH_3)_2$-3,4	*b*				
17	1		*b*				
18	1	$-CH_2NHC(=O)C_6H_5$	0[c]			122	534
19	1		*b*				

[a] Percent inhibition values obtained with these compounds are not reported, as it is believed the activities seen can be ascribed to hydrolysis during the assay to generate 1. [b] Assay not done. [c] Results obtained with rabbit lung INMT.

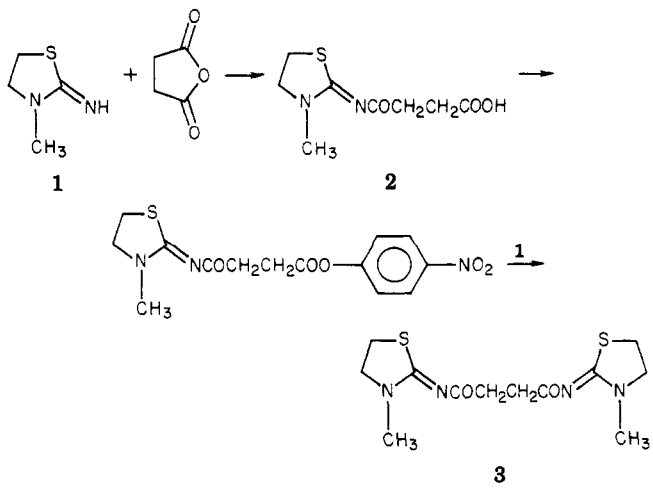
Table II. Inhibition of Lung INMT in Rabbits

no.	dose, mg/kg[a]: po	dose, mg/kg[a]: iv	time of sacrifice, h	sp act. of lung INMT[b]: controls	sp act. of lung INMT[b]: test animals	% inhibn
3	25		2	58 857	14 085	76.1 ($p < 0.01$)
3		5	0.67	103 280	37 043	64 ($p < 0.05$)
4	25 × 2[c]		2	77 050	11 635	84.9 ($p < 0.02$)
4		4[c]	0.5	84 375	33 250	60.6 ($p < 0.001$)
(*R,S*)-6	25 × 2		2	77 050	24 135	69 ($p < 0.05$)
(*R,S*)-6		4	0.5	84 375	60 000	28.9 ($p < 0.008$)
(*S*)-6		10	0.5	56 250	8 625	84.7 ($p < 0.013$)
7	25 × 2		2	77 050	18 350	76.2 ($p < 0.026$)
		4	0.5	84 375	52 500	37.8 ($p < 0.027$)
2	20		2	38 300	33 950	11.4[d]
		10	0.5	56 250	36 125	35.8[d]
9	25		2	90 000	61 250	31.9[d]

[a] Compounds were dissolved in, or if insoluble slurried with, water. For oral administration, compounds were given by stomach tube. Intravenous dosing was into the ear vein. [b] The specific activity is defined as the dpm in the [^{14}C]DMT fraction/mg of protein. The values were obtained from the slopes of the titration curves. There were two animals per group. [c] Made acidic to pH 2 with dilute HCl. [d] Not statistically significant at the $p = 0.05$ level (Student's t test).

properties such as rate and extent of absorption from the gastrointestinal tract, tissue distribution, duration of action and toxicity. In this paper, the syntheses of a variety of derivatives of 3-methyl-2-thiazolidinimine (1) are presented, all substitutions being at the imine function. Compound 1 was selected as the preferred representative from the series of monocyclic amidine derivatives because of its chemical stability and its pK_a (9.65), which we judged to be favorable for absorption and distribution in tissues. In vitro data are presented in Table I, which indicate, as expected, that derivatives of 1, hydrolytically stable to the assay conditions, exhibit low in vitro potencies. In vivo data in rabbits are reported in Table II for six such compounds. It is believed that the activities of these compounds reflect metabolic conversion to the parent 1. Studies in rabbits with *N,N'*-bis(3-methyl-2-thiazolidi-

Scheme I

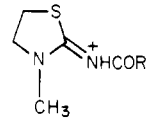


nylidene)succinamide (3) are described which provide positive evidence for its metabolic conversion to 1. Compound 1 was shown to be inert metabolically in rabbits, being excreted primarily in the urine.

Chemistry. The basic exocyclic imine moiety in 1 permits the introduction of a variety of potentially labile substituents by direct reaction with activated acyl groups, aldehydes and aldehyde derivatives, etc. Thus, the acylation of 1, employing standard procedures, produces amide derivatives of good chemical stability. The succinamic acid 2 was prepared by condensation of 1 with succinic anhydride. Conversion of 2 into its *p*-nitrophenyl ester, followed by a displacement with a second molecule of 1, afforded the bisamide 3 (Scheme I).

Amino acyl derivatives of 1 were considered to be of interest because of the possibility that such compounds might serve as substrates for proteolytic enzymes and be efficiently cleaved. The amide 4 derived from *N*-acetylglycine was prepared from the *p*-nitrophenyl ester of the latter. The amides 5, 6, and 7 (from glycine, *N*-acetylphenylalanine, and nicotinic acid, respectively) were prepared using the ethyl or isobutyl chloroformate mixed anhydride procedure. Compound 5 was isolated as the crystalline dihydrochloride salt on HCl cleavage of the *tert*-butyloxycarbonyl protecting group from the amine; attempts to obtain the free base were unsuccessful, as decomposition occurred at basic pH.

The IR spectra of the hydrochlorides of the acyl derivatives 5 and 6 showed unusually high carbonyl absorption bands at 1755 and 1745 cm^{-1}, respectively. Peresleni and co-workers[11] ascribed the high IR frequency values, observed in the case of protonated *N*-acetyl- and *N*-benzoyl-3-methyl-2-thiazolidinimines, to the strong elec-



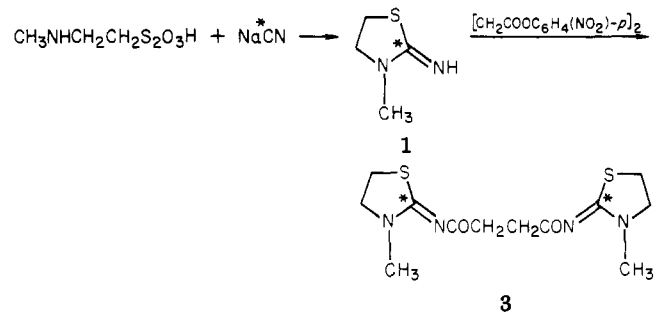
R = CH_3, C_6H_5

tron-acceptor properties of the quadrivalent, positively charged, exocyclic nitrogen. In the same way, the high absorption of the carbonyl band observed in compound 2, 1740 cm^{-1}, is attributed to the zwitterionic form.

The acetoacetylamide derivative 8 resulted from the action of diketene on 1, while reaction of phosgene with

(11) Peresleni, E. M.; Sheinker, Yu. N.; Zosimova, N. P.; Pomerantsev, Yu. I. *Zh. Fiz. Khim.* **1965**, *39*, 92; *Chem. Abstr.* **1965**, *62*, 16224*b*.

Scheme II



1 afforded the bisadduct 9; ethyl isocyanate and *tert*-butylcarbonyl azide yielded, respectively, 10 and 11. Sulfuryl chloride reacted with 1, affording the chlorosulfonyl intermediate which was unstable and reverted back to 1 on standing at room temperature. This intermediate, when immediately hydrolyzed in a mixture of water and dioxane, followed by treatment with sodium bicarbonate, yielded the sulfonate salt 12.

Reaction of 1 with paraformaldehyde gave the bis derivative 13, whereas 14 was readily prepared from benzylidenebis(dimethylamine). The analogous compounds 15 and 16 were obtained from reactions with isonicotinaldehyde and 3,4-dimethoxybenzaldehyde. Pyridoxal formed monoadduct 17, based on the NMR spectrum. The carboxamidomethyl derivative 18 and the succinimidomethyl derivative 19 were easily prepared by amidoalkylation reactions. Examination by NMR and TLC of hydrolytic stabilities in aqueous solution (pH 2–10) indicated that the aldehyde-based compounds as a group were too readily hydrolyzed to be of use.

Synthesis of labeled 1 (2-^{14}C) for metabolic studies was accomplished by reaction of $Na^{14}CN$ with 2-(methylamino)ethanethiosulfuric acid.[12] This intermediate was converted to doubly labeled 3 by displacement of the bis(*p*-nitrophenyl ester) of succinic acid (Scheme II). This approach, used for labeled 3, has the advantage of being carried out in one step from labeled 1, as compared to the three-step synthesis of unlabeled 3.

Biological Results and Discussion

Most of the compounds described here were tested against INMT preparations from human lung for their ability to inhibit the conversion of *N*-methyltryptamine (NMT) to DMT. As was expected from previous structure–activity data,[10] the chemically stable amide, urea, and carbamate derivatives of 1 were either of reduced activity or inactive in the in vitro assay. Since many of the aldehyde-based derivatives were insufficiently stable to hydrolysis under the conditions of the assay to provide reliable results, the data for these compounds are omitted. One exception is compound 18, which was stable and which proved inactive in the assay.

The amide derivatives 2–4, 6, and 7 were tested in the rabbit by both oral and intravenous routes for their ability to inhibit lung INMT (the urea 9 was tested orally only). In these determinations, the animals were sacrificed 2 h after po administration or 0.5 h (0.66 h for 3) after iv administration, and the INMT activity of the lungs was determined in comparison to control rabbits. As can be seen from the data in Table II, significant activity was observed for 3, 4, 6, and 7 by both routes of administration. Compound 2, while inactive orally at 20 mg/kg, showed evidence of moderate inhibition (~36%) at 10 mg/kg iv.

(12) Klayman, D. L.; Milne, G. W. A. *J. Org. Chem.* **1966**, *31*, 2349.

Table III. Excretion of Radioactivity Following Oral (25 mg/kg) or Intravenous (10 mg/kg) Administration of [^{14}C]1 to Rabbits

	% of dose in excreta			
	urine		feces	
time, h	iv[a]	po[b]	iv[a]	po[b]
0-6	46.5	46.4		
6-24	27.7	38.8		
0-24			3.1	3.2
24-48	5.3	2.9	0.9	0.6
48-72	3.3	1.6	0.3	0.1
total	82.8	89.7	4.3	3.9

[a] The iv dosing solution was prepared by dissolving 2.5 mg of [^{14}C]1·HCl·H_2O (17.6 μCi/mg) and 33.2 mg of 1 (unlabeled) in 2.4 mL of saline (10 mg of free base/mL, specific activity 4093 dpm/μg). [b] The po dosing solution was prepared by dissolving 2 mg of [^{14}C]1·HCl·H_2O (17.6 μCi/mg) and 88.0 mg of 1 (unlabeled) in 30 mL of distilled water (2 mg of free base/mL, specific activity 1183 dpm/μg).

Acute LD_{50} data in mice (Table I) for **2** are indicative of poor oral absorption in that species (po/iv ratio > 7). Possibly the more polar and acidic nature of this derivative is responsible for its apparent poor oral absorption in mice and rabbits. A comparison of the iv LD_{50} values for **2** and **1** (1280 vs. 118 mg/kg in mice) suggests that **2** may not be cleaved efficiently to **1** systemically in rabbits. Compound **9** did not show sufficient activity on oral administration to be of further interest.

In order to obtain unequivocal evidence bearing on the question of the metabolic conversion of amide derivatives of **1** to **1**, detailed studies were carried out in rabbits using radiolabeled **1** and **3**. Compound **3** was selected as the preferred amide derivative based on its oral potency as an inhibitor of INMT in the rabbit, its low acute toxicity in mice, and its chemical stability over a reasonable pH range. Using [^{14}C]1 it was established that **1** was efficiently absorbed in rabbits from the gastrointestinal tract and was eliminated, primarily in the urine, without significant metabolic modification of the thiazolidine ring system. Thus, administration of 25 mg/kg of **1** orally (by gavage) or 10 mg/kg ip resulted in the recovery of greater than 70% of the dose via the urine in the first 24-h period (Table III). More than half of this amount was excreted in the first 6 h. Overall, >83% of the administered radioactivity was recovered in the urine in 3 days, with fecal specimens accounting for an additional 4%. Methylene chloride extraction (1 × 5 volumes) at an alkaline pH removed 78–86% of the urinary radioactivity (iv or po). A similar extraction profile (~80% recovered) was obtained when authentic [^{14}C]1 was extracted from control rabbit urine under the same conditions. Thin-layer chromatography (silica gel) of the urinary extracts (iv and po) indicated the presence of only one radioactive component which possessed R_f values similar to that of authentic **1** in six solvent systems. If significant metabolism had occurred (e.g., heteroatom oxidation), the products would have been expected to display chromatographic behavior different to that of **1**.

Liver perfusion studies were conducted with radiolabeled **3** in order to gain an idea of the rate of modification, as well as the nature of metabolites produced by this tissue. In an experiment where **3** (25 mg) was administered, while the radioactivity levels (determined as microgram equivalents of **3**) remained relatively constant, the concentration of **3** present in the perfusate, as determined by TLC, decreased rapidly. Fifteen minutes after drug administration only 75% of perfusate radioactivity was unchanged **3**. This value decreased to 39% at 0.5 h, 21% at 1 h, and 9% at 1.5 h. Qualitatively similar results were obtained with a 100-mg dose, but here the values were less consistent, possibly as a result of tissue storage and subsequent release. The major radioactive components in the liver and remaining perfusate from the 100-mg experiment were determined separately. The liver contained 31.5% and the remaining perfusate 55.5% of the original radioactive dose. Methylene chloride extraction of alkalinized liver homogenate and perfusate resulted in approximately 65% of the radioactivities being extracted into the organic phase. Less than 1% was extracted into methylene chloride under acidic conditions. Thin-layer chromatography in four different solvent systems gave evidence for the presence of at least three radioactive components in both perfusate and liver homogenate fractions. The majority of radioactivity behaved chromatographically similar to **1** (>90% in liver homogenate; >75% in perfusate). About 10% of the perfusate radioactivity behaved similarly to **3**, whereas a negligible amount of the liver homogenate radioactivity was due to **3**. When the entire perfusate and liver extracts were chromatographed (separately) on silica gel TLC plates, using $CHCl_3$–MeOH–NH_4OH (95:5:1) as the developing solvent, four radioactive fractions were isolated (R_f 0.16, 0.39, 0.52, and 0.74). Three of the radioactive components were identified by R_f values as **1** (R_f 0.16), **3** (R_f 0.39), and 3-methyl-2-oxothiazolidine (R_f 0.52). The identity of the fourth metabolite (R_f 0.74) was not established. Confirmation of the identity of **1** as the major metabolite was obtained upon direct comparison with authentic material following trimethylsilylation and GLC analysis on a 3% OV-210 (4 ft) column. Probe mass spectrometric analysis of the isolated extracts substantiated the GLC and TLC findings. Therefore, **1** was the principal metabolite (>75%) present in the perfusate and liver from administration of **3** in the liver perfusion experiment.

In vivo studies in rabbits were conducted by administering 100 or 200 mg/kg of labeled **3** by gavage and determining the rate of elimination of radioactivity in urine and feces, as well as levels of radioactivity and metabolites in blood, liver, lung, kidney, and brain tissues. As indicated in Table IV, the radioactivity was excreted rapidly via the urine following oral dosing—about 50% of the dose being recovered in 2 h and 86% in 24 h. Overall, greater than 90% of the dose was recovered in 2 days. After 2 and 4 h, the liver, lung, and kidney all exhibited higher levels of radioactivity than the plasma, with the kidney showing the highest tissue/plasma ratio (9–14:1). The high concentrations of radioactivity in the kidney probably reflected the rapid rate of excretion at the 2- and 4-h time points. These data indicate rapid and near quantitative absorption of radioactivity following oral administration of **3**. Analysis of the components by the GLC method verified that **3** was extensively metabolized in the rabbit. There was no evidence of unchanged **3** in urine or tissue samples. As shown in Table V, about 50% of the urinary radioactivity was accounted for as **1**, based on GLC analyses. The nature of the nearly 50% of the radioactivity in the urine which was not due to **1** remains to be determined. Tissue data confirmed that extensive metabolism occurred; 27–93% of the radioactivity present in tissues (at the 2- and 4-h time points) was attributable to **1** (Tables IV, V, and VI). Plasma and brain levels of radioactivity were comparable. The lung appeared to concentrate (twofold) the radio-

(13) For additional biological data on compounds **1** and **3**, see Mandel, L. R.; Rokach, J.; Rooney, C. S.; Cragoe, E. J., Jr. *Mol. Pharmacol.* **1978**, *14*, 930.

Table IV. Summary of Radioactivity Content in Selected Rabbit Tissues, Urine, and Feces Following Oral Administration of [^{14}C]3

excreta data	time, h	% of administered dose: rabbit 1[a]	rabbit 2[b]	rabbit 3	rabbit 4
urine	0-2	48.8			
	0-4		24.0		
	0-24			85.9	87.1
	24-48			0.8	0.8
	48-72			0.2	0.1
	total			86.9	88.0
feces	0-48			3.8	6.0
	total			90.7	94.0

tissue data	rabbit 1 A[c]	rabbit 1 B[d]	rabbit 2 A[c]	rabbit 2 B[d]
liver	70.3	3.0	112.6	3.7
lung	36.3	1.5	102.3	3.3
kidney	211.1	9.0	445.8	14.5
plasma	23.5	1.0	30.7	1.0

[a] Rabbits 1 and 3 received 100 mg/kg of [^{14}C]3 (204.6 dpm/μg). [b] Rabbits 2 and 4 received 2 × 100 mg/kg of [^{14}C]3 (204.6 dpm/μg). The second dose was administered 2 h after the first dose. [c] A: calculated as microgram equivalents of 3 per gram of tissue. [d] B: tissue to plasma ratio.

Table V. Summary of GLC Analyses for 1, the Major Metabolite of 3, Following Oral Administration of [^{14}C]3 to Rabbits

urine specimen	Urinary Data[a] time, h	A[b]	B[c]
rabbit 1	0-2	57.2	27.9
rabbit 2	0-4	44.7	10.8
rabbit 3	0-24	51.3	41.2
rabbit 4	0-24	48.7	41.3

tissue	Tissue Data[a] A[b]: rabbit 1, 2 h	rabbit 2, 4 h
liver	37.9	34.4
lung	92.9	75.4
kidney	72.8	65.3
plasma	26.5	48.9

[a] Values represent averages of two determinations. [b] A: percent of radioactivity content as 1. [c] B: percent of administered dose as 1.

labeled material relative to plasma. At 2 h, 50% of the plasma radioactivity was identified as 1, whereas this metabolite represented >75% of the 2-h lung tissue radioactivity.

Conclusions

The data presented here establish that amide derivatives of 1, based on succinic, nicotinic, and N-acylated amino acids, are chemically stable compounds which effectively inhibit lung INMT following in vivo administration to rabbits. Since the compounds themselves are at best weak inhibitors of this enzyme in vitro, it was logical to assume that they must be metabolized in vivo to 1. This conclusion has been documented in the case of 3, which has been shown to be metabolized efficiently after oral administration in rabbits to yield at least 50% of 1. Liver perfusion experiments indicated that this conversion occurred rapidly in that organ. The nature of the nearly 50% of radioactivity in the urine of rabbits treated with radiolabeled 3, which was not attributable to 1, has not yet been established. No evidence was found for the accumulation of 2, a likely metabolite of 3 in which only one of the 3-methyl-2-thiazolidinimine moieties has been hydrolyzed. Nor do the data presented permit an estimate of the extent of metabolic conversion of 3 to 1 taking place in the gastrointestinal tract vs. that occurring subsequent to absorption. Although 3 has physical and chemical properties quite different to those of 1, the time courses for excretion of radioactivity after administration of labeled forms of the two compounds to rabbits are similar. 1 is excreted unchanged in rabbit urine to an extent exceeding 80% following both ip and po dosing. On the other hand, while greater than 80% of the radioactivity appears in the urine following administration of labeled 3 to rabbits, only about 50% of this can be accounted for as the biologically active metabolite 1.

Table VI. Summary of Content of 3 in Plasma and Tissues Based on Radioactivity Measurements Following Oral Administration of [^{14}C]3 to Rabbits at a Dose of 100 mg/kg

rabbit	time, h	radioactivity levels[a]: plasma	lung	brain
5	2	14.2	36.4 (2.5)[b]	17.8 (1.3)
6	2	21.1	46.7 (2.2)	19.8 (0.9)
7	6	4.1	9.5 (2.3)	4.1 (1.0)
8	6	4.0	7.2 (1.8)	3.5 (0.9)

[a] Values are expressed as microgram equivalents of 3 per gram or milliliter of tissue. [b] Values in parentheses represent calculated tissue to plasma ratios.

It is hoped that amide derivatives of 1, such as 3, may prove useful for testing in man the transmethylation hypothesis of schizophrenia.

Experimental Section

Chemical Procedures. Melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected. The ^{1}H NMR 60-MHz spectra were recorded with a Varian Associates EM-360 instrument. Chemical shifts were recorded in parts per million (δ) relative to Me_4Si as internal standard. The IR spectra were determined on a Perkin-Elmer 257 spectrophotometer, and elemental analyses were carried out by Dr. C. Daessle, Montreal.

3-Carboxy-*N*-(3-methyl-2-thiazolidinylidene)propionamide (2). A mixture of 1 (25.5 g, 0.22 mol) and succinic anhydride (20 g, 0.20 mol) in CH_2Cl_2 (700 mL) was refluxed for 3 h. Following filtration, the solvent was evaporated and the residue was triturated with Et_2O to yield 40 g (84.2%) of 2: mp 104–108 °C; ^{1}H NMR (D_2O + DCl) δ 2.90 [4 H, m, $(COCH_2)_2$], 3.42 (3 H, s, CH_3), 3.55 (2 H, t distorted, SCH_2), 4.27 (2 H, t distorted, NCH_2); IR (KBr) 1740 cm^{-1} (C=O), 1590 (w), 1560 cm^{-1} (>=NH$^+$– and COO^-). Anal. ($C_8H_{12}N_2O_3S$) C, H, N, S.

***N*,*N*'-Bis(3-methyl-2-thiazolidinylidene)succinamide (3).** A mixture of 2 (25 g, 0.116 mol), *p*-nitrophenol (16.09 g, 0.116 mol), and *N*,*N*'-dicyclohexylcarbodiimide (23.84 g, 0.116 mol) in ethyl acetate (800 mL) was stirred at room temperature for 2 days. Following filtration, the solution was washed with aqueous sodium carbonate solution and then evaporated down to an oily residue. After extraction several times with boiling ether, crude *p*-nitrophenyl ester was obtained, which was used without further purification. Crude ester was refluxed with 15 g of 1 (0.13 mol) in chloroform (600 mL) for 3 h; after cooling, the mixture was washed with aqueous sodium carbonate solution, dried, and stripped to a solid residue, which was triturated in ether and filtered. The solid was then crystallized from methanol (500 mL), affording 18.9 g (52%) of 3: mp 181–183 °C; ^{1}H NMR ($CDCl_3$) δ 2.80 [4 H, s, $(COCH_2)_2$], 3.13 (6 H, s, 2 CH_3), 3.10 (4 H, t distorted, 2 SCH_2), 3.61 (4 H, t distorted, 2 NCH_2); IR (KBr) 1645 cm^{-1} (C=O), 1550 cm^{-1} (C=N). Anal. ($C_{12}H_{18}N_4O_2S_2$) C, H, N, S.

2-Acetamido-*N*-(3-methyl-2-thiazolidinylidene)acetamide (4). A mixture of 1 (6.2 g, 0.053 mol) and *N*-acetylglycine *p*-nitrophenyl ester (12.7 g, 0.053 mol) in $CHCl_3$ (60 mL) was stirred at room temperature for 2 h. The solution was washed with aqueous K_2CO_3 solution and then with water, dried, and evapo-

rated, affording 9.5 g (82.7%) of **4**: mp 138–139 °C; ^{1}H NMR ($CDCl_3$) δ 2.02 (3 H, s, $COCH_3$), 3.15 (3 H, s, CH_3), 3.20 (2 H, t distorted, SCH_2), 3.68 (2 H, t distorted, NCH_2), 4.11 (2 H, d, $NHCH_2$, J_{CH_2NH} = 4.5 Hz, collapses to a singlet on D_2O exchange), 6.46 (1 H, br, NH); IR (KBr) 3320 (NH), 1660 (C=O), 1550 cm^{-1} (C=N). Anal. ($C_8H_{13}N_3O_2S$) C, H, N, S.

2-Amino-*N*-(3-methyl-2-thiazolidinylidene)acetamide (5). To a solution of *N*-(*tert*-butyloxycarbonyl)glycine (35 g, 0.204 mol) and triethylamine (40 mL) in CH_2Cl_2 (500 mL), at −5 °C, was added slowly ethyl chloroformate (25 mL); the mixture was stirred for 5 min and then a mixture of **1**·HI (61 g, 0.25 mol) and triethylamine (100 mL) in CH_2Cl_2 (500 mL) was added slowly. The resulting mixture was stirred in the cold for 15 min and then at room temperature for 18 h. After the mixture was washed with 20% aqueous citric acid solution, saturated aqueous $NaHCO_3$ solution, and then water, the organic phase was evaporated to dryness. The residue was triturated with petroleum ether (30–60 °C) and filtered; the solid was slurried in a small volume of ether and filtered, affording 20 g (36%) of 2-[(*tert*-butyloxycarbonyl)amino]-*N*-(3-methyl-2-thiazolidinylidene)acetamide: mp 129–130 °C. Anal. ($C_{11}H_{19}N_3O_3S$) C, H, N, S.

This intermediate (2.0 g, 0.073 mol) was dissolved in $CHCl_3$ (100 mL), and HCl gas was passed through the solution for 2 h. Filtration afforded 1.66 g (92%) of **5**·2HCl: mp 190–194 °C; ^{1}H NMR (D_2O) δ 3.38 (3 H, s, CH_3), 3.57 (2 H, t distorted, SCH_2), 4.24 (2 H, t distorted, NCH_2), 4.26 (2 H, s, $COCH_2$); IR (KBr) 1755 (C=O), 1655 cm^{-1} (>=NH$^+$–). Anal. ($C_6H_{11}N_3O_6$·2HCl) C, H, Cl, N, S.

2-Acetamido-3-phenyl-*N*-(3-methyl-2-thiazolidinylidene)propionamide (6). Racemic **6** was prepared in the same manner as **5**, from **1**·HI and (*RS*)-*N*-acetylphenylalanine, in 79% yield: mp 154–156 °C; ^{1}H NMR ($CDCl_3$) δ 1.92 (3 H, s, $COCH_3$), 2.95–3.25 (4 H, m, SCH_2, $CH_2C_6H_5$), 3.06 (3 H, s, CH_3), 3.65 (2 H, t distorted, NCH_2), 4.88 (1 H, m, CH), 6.46 (1 H, d, NH), 7.13 (5 H, s, C_6H_5); IR (KBr) 3340 (NH), 1690, 1625 (C=O), 1560 cm^{-1} (C=N). Anal. ($C_{15}H_{19}N_3O_2S$) C, H, N, S.

A hydrochloride salt was prepared in MeOH with HCl gas. After evaporation and treatment with EtOAc, **6**·HCl was obtained: mp 182–184 °C dec; IR (KBr) 1745 (C=O), 1680 [C(=O)CH_3], 1615 cm^{-1} (>=NH$^+$–).

Optically active (*S*)-**6** was prepared as follows: To a mixture of **1**·HI (5.38 g, 0.023 mol) and (*S*)-*N*-acetylphenylalanine (4.14 g, 0.02 mol) in DMF (100 mL), at 0 °C, was added diphenylphosphoryl azide (6.06 g, 0.022 mol), followed by triethylamine (4.44 g, 0.044 mol). The mixture was stirred in the cold for 1 h, then diluted with water (1 L), and extracted five times with $CHCl_3$. Combined organics were washed with 20% aqueous citric acid solution, saturated aqueous $NaHCO_3$ solution, and water and dried, and the $CHCl_3$ was removed. The residual DMF solution was diluted with ether and petroleum ether (30–60 °C) and allowed to crystallize. Recrystallization from $CHCl_3$–petroleum ether afforded 1.05 g (16%) of (*S*)-**6**: mp 142–149 °C; $[\alpha]_D^{RT}$ 10.71° (*c* 2, EtOH). Anal. ($C_{15}H_{19}N_3O_2S$) C, H, N, S.

***N*-(3-Methyl-2-thiazolidinylidene)nicotinamide (7).** This compound was prepared in a manner analogous to **5**, from **1**·HI and nicotinic acid; in this case, isobutyl chloroformate was used to generate the mixed anhydride. The yield of **7** was 56.6%: mp 108–109 °C; ^{1}H NMR ($CDCl_3$) δ 3.17 (2 H, t distorted, SCH_2), 3.23 (3 H, s, CH_3), 3.70 (2 H, t distorted, NCH_2); aromatics 7.34 (1 H, dd, H_5), 8.35–8.85 (2 H, m, H_4, H_6), 9.45 (1 H, br, H_2); IR (KBr) 1625 (C=O), 1565 cm^{-1} (C=N). Anal. ($C_{10}H_{11}N_3OS$) C, H, N, S.

3-Oxo-*N*-(3-methyl-2-thiazolidinylidene)butyramide (8). To a solution of **1** (18 g, 0.155 mol) in EtOH (100 mL), cooled in an ice bath, was added dropwise diketene (13 g, 0.155 mol) in EtOH (100 mL). The reaction mixture was allowed to come to room temperature and then stripped free of solvent, affording crude product which was triturated in ether and filtered, yielding 18.6 g (60%) of **8**: mp 43–45 °C; ^{1}H NMR ($CDCl_3$) δ 2.28 (3 H, s, $COCH_3$), 3.12 (3 H, s, CH_3), 3.15 (2 H, t distorted, SCH_2), 3.54 (2 H, s, $COCH_2$), 3.69 (2 H, t distorted, NCH_2). The enol form is also observed: δ 1.95 (3 H, s, CH_3), 5.14 (1 H, br s, CH); IR (thin film) 1730, 1645 (C=O), 1575 cm^{-1} (C=N). Anal. ($C_8H_{12}N_2O_2S$) C, H, N, S.

***N*,*N*'-Bis(3-methyl-2-thiazolidinylidene)urea (9).** Phosgene was passed through a solution of **1** (20 g, 0.17 mol) in CH_2Cl_2 (300 mL) for 3 h at room temperature. The mixture was stirred for another 2 h, after which it was flushed with N_2. The solids, after filtration, were dissolved in H_2O, basified with aqueous saturated $NaHCO_3$ solution, and extracted with CH_2Cl_2, affording, after evaporation, a solid residue which was slurried with Et_2O and filtered, yielding 6.08 g (27.3%) of **9**: mp 210–211 °C; ^{1}H NMR ($CDCl_3$) δ 3.07 (4 H, t distorted, 2 SCH_2), 3.13 (6 H, s, 2 CH_3), 3.61 (4 H, t distorted, 2 NCH_2); IR (KBr) 1660 cm^{-1} (C=O, C=N). Anal. ($C_9H_{14}N_4OS_2$) C, H, N, S.

***N*-Ethyl-*N*'-(3-methyl-2-thiazolidinylidene)urea (10).** To a mixture of **1**·HI (30 g, 0.123 mol) and triethylamine (50 mL) in CH_2Cl_2 (500 mL) was added, at room temperature, ethyl isocyanate (12 g, 0.17 mol); after stirring for 1 h, the mixture was washed three times with water and dried, and the solvent was evaporated. CH_2Cl_2 was added and the mixture reevaporated. The residue was slurried with ether, affording 18 g (78.3%) of **10**: mp 79–80 °C; ^{1}H NMR ($CDCl_3$) δ 1.13 (3 H, t, CH_2CH_3), 3.03 (3 H, s, CH_3), 2.85–3.75 (6 H, m, CH_2CH_2, CH_2CH_3), 5.40 (1 H, br, NH); IR (KBr) 3330 (NH), 1630 (C=O), 1595 cm^{-1} (C=N). Anal. ($C_7H_{13}N_3OS$) C, H, N, S.

***tert*-Butyl *N*-(3-Methyl-2-thiazolidinylidene)carbamate (11).** A solution of **1** (23.2 g, 0.20 mol) and tetramethylguanidine (46.0 g, 0.40 mol) in DMF (500 mL) was cooled in an ice bath and *tert*-butyloxycarbonyl azide (42.9 g, 0.30 mol) was added dropwise. The resulting mixture was stirred at room temperature for 3 days, and then the solvent was evaporated under reduced pressure. The residue was dissolved in ether, and the solution was washed with water, 20% aqueous citric acid, and again with water. After the solvent was dried and evaporated, the residue was slurried with petroleum ether (30–60 °C) and filtered, affording 18.9 g (43.7%) of **11**: mp 81–83 °C; ^{1}H NMR ($CDCl_3$) δ 1.53 [9 H, s, $C(CH_3)_3$], 3.13 (3 H, s, CH_3), 3.18 (2 H, t distorted, SCH_2), 3.68 (2 H, t distorted, NCH_2); IR (KBr) 1665 (C=O), 1580 cm^{-1} (C=N). Anal. ($C_9H_{16}N_2O_2S$) C, H, N, S.

***N*-(3-Methyl-2-thiazolidinylidene)sulfamic Acid Sodium Salt (12).** To a solution of SO_2Cl_2 (40 g, 0.296 mol) in CH_2Cl_2 (800 mL) at 0–5 °C was added **1** (40 g, 0.345 mol) in portions. After stirring for 1 h, the mixture was washed with H_2O and dried, and the solvent was evaporated. The residue was stirred with Et_2O and filtered to give the unstable monochlorosulfonyl intermediate, mp 120–122 °C, which was immediately suspended in dioxane (180 mL) and warmed gently until dissolution occurred. Water (15 mL) was then added, and the mixture was heated to 50 °C until crystallization took place (about 10 min). After cooling to ice temperature, the solids were filtered and washed with MeCN. The product was slurried in MeOH (100 mL) and filtered to yield 16 g (27.6%) of **12** free acid, mp 240–242 °C dec. For conversion to the sodium salt, the above solid (15 g, 0.076 mol) was dissolved in water (300 mL) containing $NaHCO_3$ (6.38 g, 0.076 mol). After evaporation, the residue was slurried with EtOH and filtered, affording 15.8 g (94.7%) of **12**: mp 280–285 °C dec; ^{1}H NMR (D_2O) δ 3.10 (3 H, s, CH_3), 3.44 (2 H, t distorted, SCH_2), 3.97 (2 H, t distorted, NCH_2); IR (KBr) 1590 cm^{-1} (C=N). Anal. ($C_4H_7N_2O_3S_2Na$) C, H, N, S.

Methylenebis(3-methyl-2-thiazolidinimine) (13). A mixture of **1** (25 g, 0.215 mol), paraformaldehyde (6.5 g, 0.072 mol), and 4Å molecular sieves (75 g) in benzene (250 mL) was stirred and heated at reflux temperature; after 5 h, a further 6.5 g of paraformaldehyde and 30 g of molecular sieves were added, and heating was continued for 19 h. The mixture was filtered, and the sieves were washed well with benzene. The filtrate was evaporated to a pale yellow oil, which crystallized on standing. The solids were slurried in petroleum ether and filtered, yielding 20 g (76%) of **13**: mp 57–59 °C; ^{1}H NMR ($CDCl_3$) δ 2.90 (6 H, s, 2 CH_3), 3.12 (4 H, t distorted, 2 SCH_2), 3.34 (4 H, t distorted, 2 NCH_2), 4.60 (2 H, s, CH_2); IR (thin film) 1620 cm^{-1} (C=N). Anal. ($C_9H_{16}N_4S_2$) C, H, N, S.

Benzylidenebis(3-methyl-2-thiazolidinimine) (14). A mixture of **1** (10 g, 0.086 mol) and benzylidenebis(dimethylamine) (7.7 g, 0.043 mol) in benzene (50 mL) was heated at reflux for 7 h; after cooling, the mixture was evaporated to one-third its volume and then diluted with ether. The precipitated solid was filtered and washed with ether, affording 8.0 g (58.2%) of **14**: mp 167–169 °C; ^{1}H NMR ($CDCl_3$) δ 2.91 (6 H, s, 2 CH_3), 3.08 (4 H, t distorted, 2 SCH_2), 3.39 (4 H, t distorted, 2 NCH_2), 5.22 (1 H, s, CH), 7.10–7.65 (5 H, m, C_6H_5); IR (KBr) 1660 (w), 1620 cm^{-1} (C=N).

Anal. ($C_{15}H_{20}N_4S_2$) C, H, N, S.

4-(Pyridylmethylene)bis(3-methyl-2-thiazolidinimine) (15). A mixture of pyridine-4-carboxaldehyde (1.07 g, 0.01 mol), 1 (2.32 g, 0.02 mol), and 4Å molecular sieves (15 g) in toluene (80 mL) was refluxed for 42 h. After filtration and evaporation of the mixture, the residue was triturated with Et_2O and filtered, affording 2.02 g (62.9%) of **15**: mp 110–113 °C; ^1H NMR ($CDCl_3$) δ 3.10 (6 H, s, 2 CH_3), 3.31 (4 H, t distorted, 2 SCH_2), 3.55 (4 H, t distorted, 2 NCH_2), 5.48 (1 H, s, CH); aromatics: 7.85 (2 H, d, H_3, H_5), 8.98 (2 H, d, H_2, H_6); IR (KBr) 1655 (w), 1620 cm^{-1} (C=N). Anal. ($C_{14}H_{19}N_5S_2$) C, H, N, S.

3,4-Dimethoxybenzylidenebis(3-methyl-2-thiazolidinimine) (16). When the procedure for **15** was carried out using 3,4-dimethoxybenzaldehyde, **16** was obtained in 51.8% yield: mp 144–145 °C; ^1H NMR ($CDCl_3$) δ 3.12 (6 H, s, 2 CH_3), 3.28 (4 H, t distorted, 2 SCH_2), 3.60 (4 H, t distorted, 2 NCH_2), 4.04 and 4.10 (6 H, 2 s, 2 OCH_3), 5.48 (1 H, s, CH), 7.00–7.65 (3 H, m, C_6H_3); IR (KBr) 1655 (w), 1625 cm^{-1} (C=N). Anal. ($C_{17}H_{24}N_4O_2S_2$) C, H, N, S.

1,3-Dihydro-6-methyl-1-[(3-methyl-2-thiazolidinylidene)amino]furo[3,4-c]pyridin-7-ol (17). When the procedure for **15** was carried out, using pyridoxal as the starting aldehyde, **17** was obtained in 80% yield: mp 188–189 °C dec; ^1H NMR ($CDCl_3$) δ 2.50 (3 H, s, CH_3), 2.97 (3 H, s, NCH_3), 3.20 (2 H, t distorted, SCH_2), 3.60 (3 H, t distorted, NCH_2), 5.13 (2 H, br s, CH_2), 6.33 (1 H, br s, CH), 6.57 (1 H, br, OH), 8.97 (1 H, s, Ar); IR (KBr) 1590 cm^{-1} (C=N). Anal. ($C_{12}H_{15}N_3O_2S$) C, H, N, S.

***N*-(Benzamidomethyl)-3-methyl-2-thiazolidinimine (18).** To a mixture of 1·HI (20 g, 0.082 mol) and triethylamine (20 g) in CH_2Cl_2 (500 mL), cooled with an ice bath, was added slowly a solution of *N*-(chloromethyl)benzamide (16.7 g, 0.099 mol) in CH_2Cl_2 (250 mL). After the solution was stirred for 2 h at room temperature, the solvent was evaporated. The residue was redissolved in CH_2Cl_2; the solution was washed with water, and the product was extracted into dilute aqueous HCl. The aqueous extract was made basic with dilute NaOH solution, and the product was extracted into CH_2Cl_2 to afford an oily residue, which was triturated with ether and filtered. The yield was 12.0 g (58.8%) of **18**: mp 132–135 °C; ^1H NMR ($CDCl_3$) δ 2.85 (3 H, s, CH_3), 3.12 (2 H, t distorted, SCH_2), 3.43 (2 H, t distorted, NCH_2), 4.83 (2 H, d, CH_2NH, J_{CH_2NH} = 5 Hz, collapses to a singlet on D_2O exchange), 7.10 (1 H, br, NH), 7.15–8.00 (5 H, m, C_6H_5); IR (KBr) 3330 (NH), 1650 cm^{-1} (C=O and C=N). Anal. ($C_{12}H_{15}N_3OS$) C, H, N, S.

A maleate salt was prepared in MeOH and crystallized from MeOH–ether: mp 120–121 °C. Anal. ($C_{12}H_{15}N_3OS \cdot C_4H_4O_4$) C, H, N, S.

When the above procedure was carried out using *N*-(bromomethyl)succinimide and the crude product crystallized from benzene, **19** was obtained in 21% yield: mp 142–144 °C; ^1H NMR ($CDCl_3$) δ 2.73 [4 H, s, $(COCH_2)_2$], 2.84 (3 H, s, CH_3), 3.21 (2 H, t distorted, SCH_2), 3.50 (2 H, t distorted, NCH_2), 4.82 (2 H, s, CH_2); IR (KBr) 1785 (w), 1720 (C=O), 1645 cm^{-1} (C=N). Anal. ($C_9H_{13}N_3O_2S$) C, H, N, S.

^{14}C Labeling of 1 and 3. For the preparation of 2-^{14}C-labeled **1**, the procedure of Klayman and Milne[11] was followed:

A solution of $Na^{14}CN$ (150 mg, 3.06 mmol), 2-(methylamino)ethanethiosulfuric acid (1.154 g containing 2 mol of NaBr per mol of acid, 3.06 mmol), and 1.0 N sodium hydroxide (3.2 mL) in water (12 mL) was stirred at room temperature for 4 h. The mixture was then extracted with chloroform (6 × 6 mL), and the combined extracts were dried over Na_2SO_4 and evaporated at 25 °C under reduced pressure to yield 299 mg of labeled **1** (84%) as a light yellow oil.

Fifty milligrams of this oil was dissolved in 2-propanol (1 mL) and 37% aqueous HCl (0.04 mL) was added, followed by ether (1 mL). The mixture deposited crystals of 1·HCl·H_2O (54 mg), mp 72–74 °C. The specific activity of the hydrochloride hydrate was ~17.6 μCi/mg. The radioactive purity as measured by TLC was greater than 99%.

^{14}C-Labeled **3** was prepared by refluxing 2-^{14}C-labeled **1** (160 mg, 1.4 mmol) and succinic acid bis(*p*-nitrophenyl ester) (233 mg, 0.62 mmol) in chloroform (15 mL) for 4 h; after cooling, the solution was washed with aqueous sodium carbonate solution and then stripped to a solid residue, which was crystallized from methanol (4 mL) affording 136 mg (63%) of doubly labeled **3**. Its specific activity was ~19.0 μCi/mg. Radioactive purity as measured by TLC was greater than 99%.

Biological Procedures. *S*-[*methyl*-^{14}C]Adenosylmethionine (specific activity 42–53 mCi/mmol) was purchased from the New England Nuclear Corp. The rabbits utilized in these studies were New Zealand White (NZW) males, purchased from H.A.R.E., West Milford, N.J. Human lung tissue samples were obtained postmortem and stored at −20 °C.

Human Lung in Vitro INMT Inhibition Assay. Human lung tissue was homogenized in 3–5 volumes of 0.15 M KCl containing 10^{-4} M dithiothreitol and 10^{-4} M EDTA. The homogenate was centrifuged at 50000*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. The enzyme was purified further by Sephadex chromatography. A 12-mL aliquot of the dialyzed enzyme preparation 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 of eluate) were pooled and concentrated 10-fold by ultrafiltration. The in vitro assay 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/mol; 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/100 mL in water) in a total of 0.105 mL. Samples were incubated for 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. This assay procedure has been described in detail in previous papers.[10,14]

Effect of in Vivo Administration of Amide Derivatives of 1 on the Activity of Rabbit Lung INMT. Rabbits were administered the test compounds in aqueous solution by iv administration and orally by gavage. Controls were given water. At the end of the experiment, the animals were decapitated and the lungs removed. The lungs were homogenized in 3–5 volumes of 0.15 M KCl containing 10^{-4} M dithiothreitol and 10^{-4} M EDTA. The homogenate was centrifuged at 50000g for 20 min at 0 °C. The supernatant was assayed by the standard in vitro procedure.

Metabolism Studies with 1 and 3. GLC studies were performed with a Hewlett-Packard 5830A instrument with a flame-ionization detector (FID). Radioactivities of ^{14}C-labeled compounds were determined with a Packard Tri-carb spectrometer operated at a setting optimal for carbon-14. An LKB 9000S instrument was employed for mass spectrometric measurements.

pH Distribution. ^{14}C-Labeled **1** and **3** were partitioned, in separate experiments, between equal volumes of organic solvent (benzene, heptane, ethyl acetate, methylene chloride, and chloroform) and aqueous phase at pH values 1, 3, 5, 7, 9, and 11. Optimum extraction (>95%) of **1** was obtained with chloroform and methylene chloride at a pH of 11, whereas **3** was extracted with equal efficiency (>99%) over the pH range 5–9 with the same two solvents.

Analytical Procedures for the Determination of 1 and 3. The best conditions for GLC analysis of **3** were with a 1% OV-17 column [3 ft; 4 mm (i.d.); Gas Chrom Q, 80–100 mesh] operated at 280 °C with a carrier flow rate of 98 mL/min. Using racemic **6** as an internal standard (concentration 125 μg/mL in H_2O), the following procedure was followed: flow rate, 105 mL/min; temperature programmed from 240 to 280 °C with a rate of 5 °C/min starting at time 0.0. After 9 min, the temperature was decreased to 240 °C at a rate of 30 °C/min. The retention times of **3** and **6** were 6.5 and 2.1 min, respectively. The detailed procedure for recovery from biological media was as follows: To a 50-mL glass-stoppered centrifuge tube was added 1 mL of urine or plasma; 1.0 mL of an appropriate amount of **3** (100, 50, 25, 10, and 5 μg); 1.0 mL of internal standard **6** (125 μg); 1 mL of 0.2 M

(14) Mandel, L. R. *Biochem. Pharmacol.* **1976**, *25*, 2251.

phosphate buffer, pH 7; and 25 mL of CH_2Cl_2. The contents of the tube were shaken and centrifuged. The aqueous phase was saved. The organic phase was transferred to a clean tube and evaporated to dryness under N_2. The residue was dissolved in 2 mL of pH 3 buffer (0.2 M phosphate) and 15 mL of benzene. After shaking and centrifugation of the mixture, the organic phase was aspirated and the pH of the aqueous phase adjusted to about 8 by the addition of 0.5 mL of 1 N NaOH. The compounds were subsequently extracted into 15 mL of CH_2Cl_2 and the organic solvent was evaporated to dryness under nitrogen. The residue was dissolved in 100 μL of EtOAc, and an appropriate aliquot, usually 5 μL, was injected into the gas chromatograph using the previously described conditions. When tissue homogenates (8–16 mL) were analyzed, the compounds were initially extracted with CH_2Cl_2 (2 × 40 mL). The pooled extract was washed with 2 mL of pH 6 buffer and then treated as described for plasma and urine.

Standard peak height ratio (**3**/**6**) vs. weight ratio curves were plotted following analysis of recovery samples, which were run concurrently with sample analysis (no **3** added). The level of drug in the sample was then calculated from the recovery curve using the peak height ratio obtained for the sample.

GLC analysis of **1** involved prior trimethylsilylation with BSA/pyridine, followed by analysis on a 3% OV-210 column [4 ft, 4 mm (i.d.)]. The procedure was as follows: to an aliquot (1 mL) of urine, plasma, or tissue homogenate (as described for the analysis of **3**) was added 125 μg of the internal standard, 3-ethyl-2-thiazolidinimine (**20**). The mixture was made alkaline (pH >11) by the addition of 1 mL of 1 N NaOH and then extracted with CH_2Cl_2 (60 mL × 2 for tissues, and 25 mL for plasma or urine). After shaking and centrifugation of the mixture, the CH_2Cl_2 phase was transferred to a clean tube and evaporated to ~0.5 mL (in the case of tissues the CH_2Cl_2 extract was washed with 0.1 volume of 0.1 N NaOH prior to concentration of the CH_2Cl_2). The residue from the organic phase was subsequently reacted with 200 μL of Tri-syl BSA in pyridine at 70 °C for 12–16 h. The resulting trimethylsilylated derivatives were injected into the GLC column, maintained at 95 °C, with a carrier gas flow rate of 54 mL/min. The standard peak height ratio (**1**/**20**) vs. weight ratio curves were determined as described for **3**. The level of drug in a given sample was calculated from the standard recovery curve.

In actual practice, both **1** and **3** were analyzed from the same sample. After extraction of **3** at pH 7, the aqueous solution was handled as described above for compound **1**.

Metabolic Profile of 1 in Rabbits. A male white New Zealand rabbit, weighing 825 g, was dosed intravenously with [^{14}C]**1** at a dose of 10 mg/kg. A second rabbit (825 g) was given **1** orally (25 mg/kg) by gavage. Urine was collected at 6, 24, 48, and 72 h; fecal specimens were collected at 24, 48, and 72 h postdose. A summary of the excretion results is presented in Table III. Thin-layer chromatography of the methylene chloride extract (pH 11) of the urine indicated only one radioactive component which possessed R_f values similar to unchanged **1** in six solvent systems ($CHCl_3$/MeOH/concentrated NH_4OH, 95:5:1, 90:9:1, 10:1:1, 90:5:5; *n*-BuOH/HOAc/H_2O, 4:1:1; *n*-BuOH/concentrated NH_4OH/H_2O, 4:1:1). These results indicated that the thiazolidinimine system of **1** was metabolically inert in the rabbit.

Rabbit Liver Perfusion Experiments with 3. Two fasted male white New Zealand rabbits (1.2–1.4 kg) were used as liver donors. The perfusates were prepared with 50 mL of heparinized rabbit whole blood and 250 mL of Gey and Gey buffer. An Ambec extracorporal perfusion unit was used to oxygenate and circulate the perfusion media at a flow rate of 3 mL/min at 37 °C. The usual operative procedures were performed on the nembutal-anesthetized animals. Following a 10–15-min equilibration time, the dosing solution was added through the injection manifold at the rate of 3–4 mL/min with a 0.3 mL saline rinse. In the first experiment 13.6 mL of solution (acidified to just above pH 4 with 0.1 N HCl) containing 25 mg of [^{14}C]**3** (340 dpm/μg) was employed, while in the second experiment 13.0 mL of solution of a similar pH containing 100 mg/kg of [^{14}C]**3** (130 dpm/μg) was used.

Venous aliquots were removed at appropriate times up to 2 h after drug addition to the liver. The perfusate samples were centrifuged, and appropriate aliquots were assayed for radioactivity and unchanged drug content. The liver and remaining perfusate medium from the 100-mg experiment were frozen until analyzed by procedures described above for metabolite profile (the liver was homogenized in H_2O). TLC on silica gel was carried out in four systems ($CHCl_3$/MeOH/concentrated NH_4OH, 95:5:1; BuOH/HOAc/H_2O, 4:1:1; $CHCl_3$/MeOH/HOAc, 95:5:5; $CHCl_3$/MeOH/NH_4OH, 95:5:5). The results are described under Biological Results and Discussion.

Metabolism of 3 in Rabbits. Four male white New Zealand rabbits, weighing 950–1050 g, were dosed orally with [^{14}C]**3**. Rabbit 1 received 100 mg/kg of [^{14}C]**3** (15 mL) by gavage. Two hours later the animal was sacrificed and blood was collected along with lung, liver, and kidney tissues. Rabbit 2 received a second 100 mg/kg dose 2 h after the first; blood, liver, lung, and kidney tissues were obtained 2 h after the last dose. Rabbits 3 and 4 were dosed similarly to no. 1 and 2, respectively. These animals were not sacrificed but instead were used for obtaining samples of urine and feces. The dosing solution was prepared by dissolving 3.4 mg of [^{14}C]**3** (19.0 μCi/mg) and 696.5 mg of **3** (unlabeled) in 60 mL of water and 25 mL 0.1 N HCl. The final volume was adjusted to 105 mL with water (6.66 mg/mL) to give a final specific activity of 204.6 dpm/μg. Radioactivity content and drug and metabolite levels were determined using methods described above.

Four additional male rabbits (5–8), weighing 900–1060 g, were dosed with [^{14}C]**3** (100 mg/kg) orally by gavage so that brain levels of radioactivity, drug, and metabolite could be determined. At the appropriate time period (2 or 6 h postdrug), two animals were sacrificed by injecting air into the ear vein. Blood was obtained via cardiac puncture using heparinized syringes. The brain and lung tissues were removed, weighed, and homogenized with water (1 g = 10 mL). Radioactivity content and drug and metabolite levels were determined using the methods previously described. The dosing solution was prepared by dissolving 3.3 mg of [^{14}C]**3** (19.0 μCi/mg) and 547 mg of unlabeled **3** in 50 mL of water and 20 mL of 0.1 N HCl (pH of solution was about 4.5). The final volume was adjusted with water to a concentration of 6.66 mg/mL to give a final specific activity of 251.7 dpm/μg. The results of the above experiments are recorded in Tables IV–VI.

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