(C_7). Anal. ($C_4H_4N_6S$) C, H, N.

(b) Powdered **25a** (20 mg) in a stoppered vial under N_2 was heated in an oil bath at 165° for 3 h. The resulting solid was triturated with 1 N NaOH (1 ml), collected, washed with H_2O, and dried in vacuo (P_2O_5): yield 6.1 mg (31%). This product was the same (TLC, uv) as that described in (a).

5-Amino-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidine-7-thione (25a). A suspension of **24a** (500 mg, 2.98 mmol) in 1 N NaOH (30 ml) was heated under N_2 at ~98° until complete solution occurred (20 min). The solution was heated an additional 10 min, cooled to 25°, filtered under N_2, and acidified with 6 N HCl to pH 6. The white precipitate was collected, washed with cold H_2O, and dried in vacuo (P_2O_5): yield 434 mg (87%); mp 258° dec (Mel-Temp); λ_{max}, nm ($\epsilon \times 10^{-3}$) (Me_2SO–MeOH, 2:23), (0.1 N HCl) 224 (14.1), 258 (7.41), 338 (17.3); λ_{max} (pH 7) 231 (19.5), 257 (5.04), 282 (sh) (34.7), 343 (16.8); λ_{max} (0.1 N NaOH) 224 (19.6), 279 (8.40), 328 (14.9); ^1H NMR δ 6.95 (s, 2, NH_2), 10–16 (2, NH); ^{13}C NMR δ (±0.02) 154.02 (C_5), 148.90 (C_{3a}), 132.82 (C_{7a}), 177.62 (C_7). Anal. ($C_4H_4N_6S$) C, H, N.

Reaction of 8c with Adenosine Deaminase. A solution of 22 units of a crystalline suspension of adenosine deaminase from calf intestinal mucosa in 0.01 ml of 3.2 M $(NH_4)_2SO_4$ (Sigma Chemical Co.) was added to a solution of **8c** (4.1 mg) in 50 ml of 0.05 M phosphate buffer at pH 7.5. A uv spectrum of this solution after 16 min showed complete conversion to **7c**.

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Inhibitors of tRNA Methyltransferases. *S*-Adenosylsulfonium Salts

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Three new compounds have been synthesized and tested as in vitro inhibitors of normal and tumor tRNA methyltransferases. These compounds are 5′-methylethyl(5′-adenosyl)sulfonium chloride (MEAS), 5′-methylpropyl-(5′-adenosyl)sulfonium chloride (MPAS), and 5′-ethylpropyl(5′-adenosyl)sulfonium chloride (EPAS). They were prepared by reacting an alkyl iodide with the appropriate alkyladenosyl thioether. Inhibition assays revealed all three compounds to be inhibitors of normal and tumor tRNA methyltransferases. The propyl compounds were slightly better inhibitors of the tumor tRNA methyl transferases. MPAS, EPAS, and MEAS had K_I's of 58.5, 61.7, and 24.5, respectively, for the normal tRNA methyltransferases and 15.3, 13.8, and 44.3, respectively, for the tumor tRNA methyltransferases.

The discovery by Magee and Farber[1] that the chemical carcinogen dimethylnitrosamine would methylate tRNA to a greater extent than DNA led Borek[2] to postulate that chemical alkylation might result in aberrant methylation in tumor cells. In a wide variety of tumor and oncogenic systems the tRNA methyltransferase activity is increased

Table I. Paper Chromatography of Synthesized Sulfonium Compounds and Adenosyl Thioethers[a]

Compd	R_f values (x100) in solvent systems A	B	C
MPAS	45	72	23
EPAS	48	74	21
MEAS	36	69	27
MTA	67	79	6
ETA	76	81	14
PTA	81	87	4
Adenine	56	60	13
SAH	17	40	15
SAM	5	30	37

[a] Solvent A, butanol–acetic acid–water (12:3:5); solvent B, ethanol–acetic acid–water (65:1:34); solvent C, 1 l. of 4.55 M $(NH_4)_2SO_4$–0.1 M sodium phosphate, pH 6.8, + 20 ml of 2-propanol. Chromatography was performed using the ascending technique on Whatman No. 1 paper sheets; spots were detected by ultraviolet light (254 nm).

several times over that of the normal counterpart.[3] Both qualitative and quantitative changes in the tRNA methyltransferases have been found in extracts from several tumors such as those induced by SV-40 virus[4] or Marek's disease virus.[5]

Several natural inhibitors of the tRNA methyltransferases have been identified in normal tissue. One such inhibitor, the glycine methyltransferase enzyme, discovered by Kerr[6] is apparently absent from some tumor tissues. This could explain the increased tRNA methyltransferase activity observed in these tissues. In fact, Wainfain and Borek[7] found that the adenosine analogue, tubercidin, which is used in cancer chemotherapy, inhibits the tRNA methyltransferases. Isopentenyladenosine, a naturally occurring cytokinin, inhibits the tRNA methyltransferases and is used in short-term therapy of leukemias.[8]

In the present work, inhibitors of the tRNA methyltransferases were synthesized for use as potential anticarcinogenic agents. The compounds were sulfonium analogues of the substrate, *S*-adenosylmethionine, and were tested as inhibitors of tRNA methyltransferases from livers of normal rats and the livers of rats with tumors induced by the carcinogen DAB.[9] Kinetic experiments were performed using the normal and tumor tRNA methyltransferases to determine the type of inhibition exhibited by the sulfonium compounds.

Results

Inhibitors. The new analogues of SAM were synthesized as follows. 2′,3′-*O*-Isopropylideneadenosine was converted to 2′,3′-*O*-isopropylidene-5′-*O*-toluene-*p*-sulfonyladenosine by treatment with *p*-toluenesulfonyl chloride in pyridine. The 5-tosyl group was displaced using the sodium salt of the appropriate thiol in liquid ammonia. The 2′,3′-*O*-isopropylidene group was then removed with dilute sulfuric acid and the resulting alkyl 5′-adenosyl sulfide was converted to the desired 5′-dialkyl(5′-adenosyl)sulfonium halide by treatment with the appropriate alkyl iodide in formic acid. The structures for the three new sulfonium halide inhibitors [5′-methylpropyl(5′-adenosyl)sulfonium iodide (MPAS), 5′-ethylpropyl(5′-adenosyl)sulfonium iodide (EPAS), and 5′-ethylmethyl(5′-adenosyl)sulfonium iodide (MEAS)] were supported by elemental and NMR analysis. In addition, the compounds migrated as single bands toward the cathode upon electrophoresis in 0.2 M sodium acetate buffer, pH 4.8 (8 V/cm), and appeared as single spots on paper in three different solvent systems. The R_f values and the structural units in three solvent systems are given in Table I. The sulfonium compounds, like *S*-adenosylmethionine, were degraded completely to adenine in 0.1 N NaOH within 10 min at 25°. The three inhibitors were prepared as the sulfonium iodides but were converted to the chloride salts for inhibition studies.

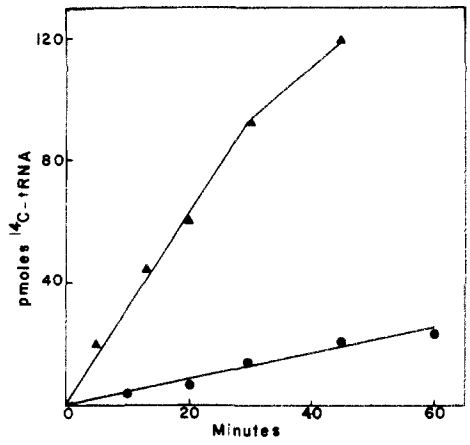

Figure 1. Comparison of tRNA methyltransferase activity of normal and tumor liver extracts. Methyltransferase activity of dialyzed high-speed supernatant fluid of normal (●) and tumor (▲) livers at a protein concentration of 1 mg/ml was measured using the standard assay as described in Methods. [$^{14}CH_3$]-SAM (0.05 μCi) was used in each assay.

Enzyme Purification. The ammonium sulfate fractionation of the high-speed supernatant fluid from normal rat liver gave a twofold increase in the specific activity, from 0.26 pmol/min/mg of protein to 0.58 pmol/min/mg of protein. The specific activity of the high-speed supernatant fluid from tumor livers (1.2 pmol/min/mg of protein) was much greater than that from the normal liver preparation. Figure 1 illustrates the increased activity of the tumor extract using the 10^5g supernatant fluids. However, the fractionation procedure used for the normal enzymes gave only a small increase in the specific activity for the tumor extract and a large (71%) loss in total activity. This may indicate the presence of isozymes in the tumor extract.

The synthesized sulfonium compounds were tested for their inhibitory activity against normal and tumor tRNA methyltransferases in the presence of [*methyl*-^{14}C]-*S*-adenosylmethionine. The inhibition of both types of enzyme extracts by *S*-adenosylhomocysteine, which is known to be strong inhibitor of many methylating enzyme,[10-12] was also measured.

The sulfonium compounds are inhibitors of both normal and tumor tRNA methyltransferases as shown in Table II. Consideration of the inhibition of the normal enzymes by the sulfonium compounds reveals MEAS to be the strongest inhibitor at the two concentrations of *S*-adenosylmethionine tested. The propyl compounds exhibit similar but lesser degrees of inhibition, with MPAS showing slightly stronger inhibition than EPAS. The inhibition shown by the compounds against the tumor enzymes again follows a definite pattern but one reversed from that of the normal enzymes. The data of Table II reveal that at both concentrations of *S*-adenosylmethionine tested, MPAS is the strongest sulfonium inhibitor of the tumor methyltransferases. With this enzyme system, MEAS seems to be a much weaker inhibitor.

S-Adenosylhomocysteine is the strongest inhibitor against both normal and tumor enzymes. Inhibition of normal and tumor methyltransferases by the alkyl thioethers was determined and the results are also given in Table II. These thioethers showed only weak inhibition

Table II. Inhibition of Normal and Tumor tRNA Methyltransferases by the Synthesized Sulfonium Compounds and Adenosyl Thioethers[a]

Compd	Inhibitor concn, M	Percent inhibition[b] N	T	N	T
MPAS	10^{-3}	96	98	64	89
	10^{-4}	76	76	48	49
	10^{-5}	28	31	15	33
	10^{-6}	10	20	10	12
	10^{-7}	0	10	0	0
EPAS	10^{-3}	98	95	56	62
	10^{-4}	69	60	30	27
	10^{-5}	17	36	14	25
	10^{-6}	10	10	10	21
	10^{-7}	0	0	0	0
MEAS	10^{-3}	99	95	81	72
	10^{-4}	91	68	61	54
	10^{-5}	57	23	25	31
	10^{-6}	22	10	11	20
	10^{-7}	0	0	0	0
SAH	10^{-4}	97	94	89	86
	10^{-5}	91	76	80	77
	10^{-6}	54	57	31	32
	10^{-7}	37	40	13	20
MTA	10^{-3}	65	32		
ETA	10^{-3}	64	37		
PTA	10^{-3}	0	21		

[a] The dialyzed high-speed supernatant fluid of normal and tumor liver was assayed as described in Methods. Assays with the normal supernatant fluid were incubated at 37° for 15 min; 1.0 mg/ml of protein was used in each assay. Assays with the tumor supernatant fluid were incubated for 20 min at 37° using a protein concentration of 0.5 mg/ml. [$^{14}CH_3$]-SAM (57 mCi/mmol) was added in the given concentrations. Experiments were performed in duplicate and the results had less than 10% standard deviation. [b] N and T refer to the source of the enzyme: N = normal liver, T = tumor liver. The values reported in the first two columns under percent inhibition were determined at a SAM concentration of 0.1×10^{-6} M while the values in the other two columns were determined at a SAM concentration of 1×10^{-6} M.

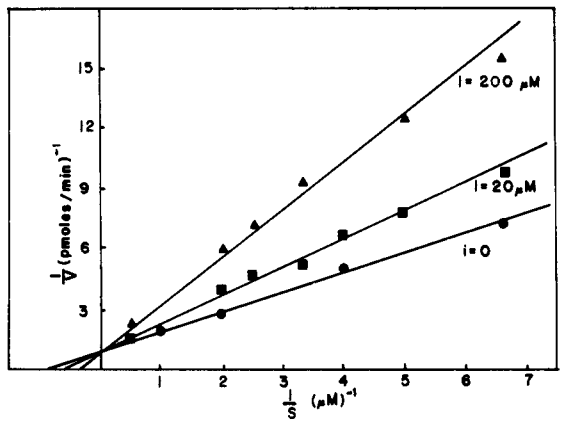

Figure 2. Lineweaver-Burk plot showing inhibition of normal liver tRNA methyltransferases by MPAS. The enzyme was assayed as described in Methods. The reaction mixture contained 0.5 mg of protein per milliliter from the fractionated normal liver extract, 250 μg of *E. coli* B tRNA, and [$^{14}CH_3$]-SAM as indicated. The assay was incubated for 30 min. The lines were drawn according to least-squares values.

when tested at high concentrations (10^{-3} M).

Kinetic assays reveal all three sulfonium compounds, MPAS, EPAS, and MEAS, to be competitive type inhibitors of both normal and tumor tRNA methyltransferases with respect to *S*-adenosylmethionine. Using a Lineweaver–Burk plot the kinetic pattern of inhibition of

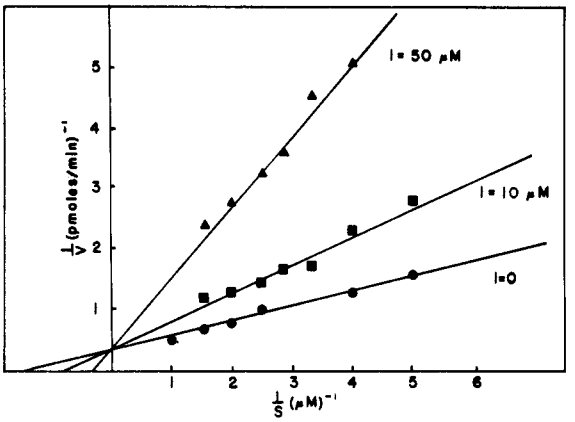

Figure 3. Lineweaver-Burk plot showing inhibition of tumor liver tRNA methyltransferases by MPAS. The enzyme was assayed as described in Methods. The reaction mixture contained 0.5 mg of protein per milliliter from the tumor dialyzed high-speed supernatant fluid, 250 μg of *E. coli* B tRNA, and [$^{14}CH_3$]-SAM as indicated. The assay was incubated for 20 min. The lines were drawn according to least-squares values.

Table III. Kinetic Constants for the Inhibition of Normal and Tumor Liver tRNA Methyltransferases

Inhibitor	Kinetic constants, μM ($K_I \pm$ SD)[a] Normal	Tumor
None (K_m)	2.3 ± 0.5	0.56 ± 0.16
MPAS	58.5 ± 4.8	15.3 ± 3.0
EPAS	61.7 ± 9.5	13.8 ± 1.5
MEAS	24.5 ± 4.5	44.3 ± 3.6

[a] The kinetic and inhibition constants were determined on a computer by the Wilkinson hyperbolic regression analysis.[26] Each value is the result of at least two separate experiments.

MPAS against normal methyltransferases is shown in Figure 2. For EPAS and MEAS a linear competitive pattern of inhibition was also observed. The same linear competitive type inhibition was seen for MPAS, EPAS, and MEAS as inhibitors of the tumor tRNA methyltransferases. The inhibition of the tumor extract by MPAS is shown in Figure 3.

The kinetic constants for the normal and tumor methyltransferases and the K_I's of the propyl compounds, MPAS and EPAS, are one-third that of MEAS (44.3 μM). The propyl compounds are, therefore, much stronger inhibitors of the tumor enzymes than MEAS (Table III).

Discussion

The data presented above indicate that the analogues of *S*-adenosylmethionine in general are good inhibitors of both the normal and tumor tRNA methyltransferases even though these analogues lack the amino acid moiety of the substrate *S*-adenosylmethionine. The sulfonium group which is common to both the analogues and *S*-adenosylmethionine appears to be a very important binding group in this system. In contrast, Zappia et al.[13] reported that *S*-adenosyl-L-(2-hydroxy-4-methylthio)butyric acid was a poor inhibitor of histamine *N*-methyltransferase and acetylserotonin methyltransferase while 5′-*S*-adenosyl-3-methylthiopropylamine was a good inhibitor. From their inhibition studies they suggested that the sulfonium pole is not a major factor in enzyme protein binding. One might infer from their results that the 6-amino group of the adenine portion of *S*-adenosylmethionine and the 2′,3′-hydroxyl groups of the sugar moiety would be more im-

portant in binding than the sulfonium group in our system. However, examination of Table II indicates that the thioethers which possess all of the above-mentioned groups except the sulfonium group are not as good inhibitors as the sulfonium analogues. However, it should be kept in mind that the methyltransferase systems do vary in their ability to bind substrates. Thus, Borchardt and Wu[14] as well as Coward and Slisz[11] found that the sulfur atom was not necessary for optimum binding in the case of catechol *O*-methyltransferase. However, the former investigators did find the sulfur atom necessary for optimum binding in the case of histamine *N*-methyltransferase and phenylethanolamine *N*-methyltransferase. The hydroxyl groups of the sugar moiety have been shown to be essential in some cases while nonessential in other.[15]

The dialkylsulfonium analogues used in this study showed competitive type inhibition of normal and tumor methyltransferase with respect to *S*-adenosylmethionine. However, it is interesting to note the apparent patterns of inhibition of both types of enzymes by the sulfonium compounds. The compound that inhibited the normal enzymes to the greatest extent, MEAS, was the weakest inhibitor of the tumor methyltransferases. This seems to indicate a difference between the two enzyme systems and not merely inhibition due to structural similarity to *S*-adenosylmethionine since MPAS would be expected to be a better inhibitor because its structure more closely resembles *S*-adenosylmethionine.

The reason for the increased tumor tRNA methyltransferase activity is not known at the present time. The selective inhibition of the sulfonium compounds does not appear to indicate a general increase of all tRNA methyltransferases during carcinogenesis but rather a selective increase of certain enzyme activities or perhaps synthesis of new methyltransferases with a differing specificity. The presence of tRNA methyltransferases with specificities altered from their normal counterpart has been detected in several tumor systems.[4,16,17]

The results of the present work suggest that inhibitors can be synthesized which might be more effective against tumor tRNA methylases than normal tRNA methyltransferases. Furthermore, our results and those of Zappia et al.,[13] Borchardt and Wu,[15] Coward and Slisz,[11] and others suggest the possible synthesis of inhibitors specific for one type of methyltransferase. Thus, an inhibitor for tumor tRNA methyltransferase might be synthesized which would not interfere with normal physiological reactions involving other methyltransferases. Additional studies are underway to determine if further modifications will improve the specificity of these inhibitors.

Experimental Section

Elemental analyses were conducted by Galbraith Laboratories, Knoxville, Tenn. NMR data were recorded on a Varian EM-360 spectrophotometer. Scintillation counting was done on a Packard Tri-carb Model 3003 scintillation counter. Kinetic constants were calculated on a PDP 12/40 digital computer, Digital Equipment Corp., using a FOCAL program. Least-squares values were determined on a Wang electronic calculator. Ultraviolet data were recorded on a Beckman DU-2 spectrophotometer. Spots from thin-layer and paper chromatography were detected by visual examination under uv light, 254 nm.

2′,3′-*O*-Isopropylidene-5-*O*-toluene-*p*-sulfonyladenosine and 5′-*S*-adenosyl-L-homocysteine were prepared according to the method of Sakami.[18,19] Methyl 5′-adenosyl sulfide, ethyl 5′-adenosyl sulfide, and propyl 5′-adenosyl sulfide were prepared according to the method of Kuhn and Jahn.[20]

5′-Methylethyl(5′-adenosyl)sulfonium Iodine (MEAS). MEAS was prepared according to the method of Baddiley[21] for the synthesis of dimethyl(5′-adenosyl)sulfonium iodide. Ethyl 5′-adenosyl sulfide (0.5 g, 1.38 mmol) was dissolved in 5 ml of formic acid. Iodomethane (11.6 g, 80 mmol) was added, the flask stoppered, and the reaction stirred in the dark at 25° for 5 days. The mixture was then lyophilized and the residue taken up in 10 ml of water and extracted with diethyl ether until all color was removed. The aqueous phase was relyophilized and taken up in methanol. Ether was added dropwise until the iodide precipitated as a hygroscopic white powder. The precipitate (40%) was dissolved in water and stored at −20°: NMR (D_2O) δ 1.56 (t, 3 H, CH_3CH_2S–), 3.00 (s, 3 H, CH_3S–), 3.40 (m, 2 H, CH_3CH_2S–), 3.95 (d, 1 H, 5′-H), 4.26 (m, 1 H, 3′-H), 5.03 (m, 1 H, 2′-H), 6.10 (d, 1 H, 1′-H), 8.16 and 8.26 (2 s, 2 H, H_2 and H_8, pur). Anal. ($C_{13}H_{20}IN_5O_3S$) C, H, S.

5′-Methylpropyl(5′-adenosyl)sulfonium Iodide (MPAS). MPAS was prepared as described for MEAS, by the alkylation of propyl 5′-adenosyl sulfide (0.5 g, 1.42 mmol) by iodomethane (11.7 g, 80 mmol) in 5 ml of formic acid. The iodide was isolated as an extremely hygroscopic white powder (30%), dissolved in water, and stored at −20°: NMR (D_2O) δ 1.05 (t, 3 H, $CH_3CH_2CH_2S$–), 1.85 (m, 2 H, $CH_3CH_2CH_2S$–), 3.0 (s, 3 H, CH_3S–), 3.43 (t, 2 H, $CH_3CH_2CH_2S$–), 4.06 (d, 2 H, 5′-H), 4.33 (m, 1 H, 4′-H), 4.73 (m, 1 H, 3′-H), 5.20 (m, 1 H, 2′-H), 6.10 (d, 1 H, 1′-H), 8.23 and 8.46 (2 s, 2 H, H_2 and H_8, pur). Anal. ($C_{14}H_{22}IN_5O_3S$) C, H, S.

5′-Ethylpropyl(5′-adenosyl)sulfonium Iodide (EPAS). EPAS was prepared as described for MEAS and MPAS by the alkylation of propyl 5′-adenosyl sulfide (0.5 g, 80 mmol) in 5 ml of formic acid. The iodide was isolated as an extremely hygroscopic white powder (23%), dissolved in water, and stored at −20°: NMR (D_2O) δ 0.90 (t, 3 H, CH_3CH_2S–), 1.40 (m, 3 H, CH_3CH_2S–), 1.66 (m, 2 H, $CH_3CH_2CH_2S$–), 3.36 (m, 2 H and 2 H, CH_3CH_2S– and $CH_3CH_2CH_2S$–), 3.90 (d, 2 H, 5′-H), 4.56 (m, 1 H, 4′-HO), 4.64 (m, 1 H, 3′-H), 5.06 (m, 1 H, 2′-H), 6.13 (d, 1 H, 1′-H), 8.23 and 8.36 (2 s, 2 H, H_2 and H_8, pur). Anal. ($C_{15}H_{24}IN_5O_3S$) C, H, S.

Purification of MEAS, MPAS, and EPAS as Chloride Salts. The aqueous phase resulting from the first ether extraction was relyophilized and dissolved in 0.01 N HCl. The sulfonium compounds were separated from their unreacted thioethers by preparative thin-layer chromatography on silica gel PF-254 plates (20 × 20 cm, 0.5 mm) in a solvent system of butanol–acetic acid–water (25:3:5). Complete separation was accomplished in 2 h after which the areas containing the sulfonium compounds were scraped and eluted into 0.01 N HCl. Excess silica was removed by centrifugation and millipore filtration (0.25 μ pore size). The solution was lyophilized, redissolved in 0.01 N HCl, and stored at −20° until use.

Quantitation of the compounds was accomplished spectrophotometrically at 260 nm (E_m = 15400, pH 7.0). In acidic solutions, E_m (254 nm) = 14700.[22]

Enzyme Purification and Assay. Normal Liver Extracts. tRNA methyltransferases were prepared according to the method of Rodeh et al.[23] Albino male rats (250 g) were decapitated; the livers were removed and homogenized in 4 vol of a buffer containing 0.01 M Tris–HCl (pH 7.4), 0.01 M $MgCl_2$, 0.005 M β-mercaptoethanol, and 0.25 M sucrose. The tissue was used fresh or frozen at −20°. No appreciable change of activity was noted using frozen tissue. The homogenate was centrifuged at 18000g for 15 min and then at 105000g for 1 h. Before use, the high-speed supernatant fluid was dialyzed against 100 vol of 0.01 M Tris–HCl buffer (pH 8.0) for 2 h, with two changes of buffer. For the kinetic experiments, the high-speed supernatant was brought to 29–42% saturation using saturated (0°) ammonium sulfate. The pellet was dissolved in a minimal volume of 0.01 M Tris–HCl buffer (pH 8.0) and dialyzed against this buffer as described above. The enzyme concentration was adjusted to 10 mg/ml and made 5 mM in β-mercaptoethanol before use.

Tumor Liver Extracts. Male albino rats fed a diet containing 0.29 mg % of the hepatocarcinogen 3′-methyl-4′-dimethylaminoazobenzene for 16 weeks were sacrificed; the livers were removed and used as a source of tumor extract. The liver was frozen quickly at −60° and stored at −20°. The tissue when used was minced and homogenized and the high-speed supernatant fluid prepared as described above for the normal liver. In all experiments, the high-speed supernatant fluid which was dialyzed for 2 h against 0.01 M Tris–HCl buffer (pH 8.0) was used as a source of enzyme. To retain constant methyltransferase activity

one tumor liver was used for all assays.

Protein was determined by the method of Lowry et al.[24] using bovine serum albumin as a standard.

Enzyme Assay. The enzyme was assayed using [*methyl*-^{14}C]-*S*-adenosyl-L-methionine and *E. coli* B tRNA as substrates. The incubation mixture (0.5 ml) contained 50 μmol of Tris–HCl buffer (pH 8.2), 5 μmol of $MgCl_2$, 2.5 μmol of β-mercaptoethanol, 0.25 M ammonium acetate, 250 μg of *E. coli* B tRNA, various amounts of [$^{14}CH_3$]-SAM, and enzyme as indicated. Incubation was carried out at 37°. In the inhibition assays various amounts of the synthesized sulfonium compounds were added. Methylation was determined by subtracting a blank containing no tRNA from the assay value.

At completion of the reaction a 0.1-ml aliquot was withdrawn, pipetted onto a Whatman 3 MM filter disk, dried, and immersed immediately in cold 5% TCA. After 30 min the disks were washed further with 500 ml of cold TCA, ethanol–ether, and ether in 50-ml portions.[25] The disks were dried, placed in 5 ml of scintillation solution containing PPO, POPOP, and toluene, and counted.

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(9) Abbreviations used are SAM, *S*-adenosyl-L-methionine; SAH, *S*-adenosyl-L-homocysteine; MPAS, 5′-methylpropyl(5′-adenosyl)sulfonium iodide; EPAS, 5′-ethylpropyl(5′-adenosyl)sulfonium iodide; MEAS, 5′-ethylmethyl(5′-adenosyl)sulfonium iodide; MTA, methyl 5′-adenosyl sulfide; ETA, ethyl 5′-adenosyl sulfide; PTA, propyl 5′-adenosyl sulfide; DAB, 3′-methyl-4-dimethylaminoazobenzene; PPO, 2,5′-diphenyloxazole; POPOP; 1,4-bis[2-(5-phenyloxazolyl)]benzene.
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(2-Piperidine)- and (2-Pyrrolidine)ethanones and -ethanols as Inhibitors of Blood Platelet Aggregation

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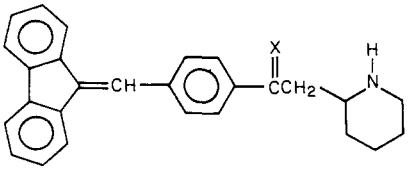
(*E*)-4-[4-(Methylthio)phenyl]-1-(2-piperidinyl)-3-buten-2-one hydrochloride (**44**, RMI 14 133A) was found to inhibit ADP-induced aggregation of blood platelets. It was selected from a large series of (2-piperidinyl)- and (2-pyrrolidinyl)ethanones synthesized by a modified Schopf reaction from enolate magnesium salts of β-keto acids and 2,3,4,5-tetrahydropyridine trimer or 3,4-dihydro-2*H*-pyrrole trimer, respectively. Evaluation of the compounds was carried out in vitro on human blood platelets. Structure–activity relationships are discussed. **44** also inhibited platelet aggregation ex vivo in guinea pigs. Subacute toxicity evaluation in dogs and guinea pigs showed it to have an unfavorable therapeutic ratio. 1-[4′-Chloro(1,1′-biphenyl)-4-yl]-2-(2-piperidinyl)ethanone hydrochloride (**18**, RMI 12436A) was found to lower serum cholesterol levels in rats with concurrent accumulation of (3β)-cholesta-5,7-dien-3-ol, suggesting inhibition of 7-dehydrocholesterol Δ^7-reductase.

We reported earlier on the blood platelet aggregation inhibitory activity of α-[*p*-(fluoren-9-ylidenemethyl)-phenyl]-2-piperidineethanol (**1**).[1] The synthesis of **1** was accomplished by a novel modification of the Schopf reaction via **2**.[2,3] This piperidinemethyl ketone **2** was found to also inhibit blood platelet aggregation.[2] Since relatively few such ketones had been prepared prior to the development of our new synthetic method, we set out to synthesize additional analogues and to evaluate their effects on adenosine diphosphate (ADP) induced aggregation of human blood platelets. Physiologically, platelet aggregation precedes blood clot formation, and it is therefore felt that inhibition of platelet aggregation may also inhibit arterial thrombosis.[4] Inhibitors of platelet aggregation may



1, X = H, OH
2, X = O

Scheme I