5'-THIOADENOSINE DERIVATIVES AS POTENT AND SELECTIVE INHIBITORS OF HISTAMINE N-METHYLTRANSFERASE

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SUMMARY

Several new analogues of adenosine bearing a lipophilic side chain at the 5'-position have been synthesized and investigated for their ability to inhibit histamine N-methyltransferase (HNMT). The 5'-deoxy-5'- [4-(3-indolyl)but-1-yl] thio}adenosine (*2e*), exhibited a pI_{50} of 5.00 against guinea pig brain HNMT. Interestingly, the polar methyl sulphonium analogue (*1c*) was a more potent inhibitor of this enzyme (pI_{50}=5.26). Both compounds were relatively ineffective inhibitors of rabbit adrenal phenylethanolamine N-methyltransferase (PNMT), rabbit lung indoleamine N-methyltransferase (INMT), and rat brain catechol O-methyltransferase (COMT). 5 -[N(4-phenylbutyl)] -amino-5 deoxy-adenosine (*2a*) and 5 -[N-methyl,N-(4-phenylbutyl] -amino-5 deoxy-adenosine (*2b*) also exhibited potent and selective inhibition against guinea pig brain HNMT. Results from kinetic studies indicate that the above compounds are inhibitors that compete for both the histamine and the S-adenosylmethionine (SAM) binding sites of HNMT. Compound *1c* is one of the most potent adenosine analogue inhibitors of HNMT known.

I. INTRODUCTION

The enzyme histamine N-methyltransferase catalyzes the S-adenosyl-methionine (SAM)-dependent N^{τ}-methylation of histamine (Figure 1), which is one of the major routes of metabolism of this putative neurotransmitter /1/. In brain, histamine N^{τ}-methylation appears to be the only significant route of metabolism for histamine in humans /2/. Several analogues of SAM and S-adenosylhomocysteine (SAH), the feed-back inhibitor of HNMT, have been examined for inhibitor activity against HNMT (for a review see reference /3/). Generally, such compounds, if active, exhibit inhibitory activity against other related

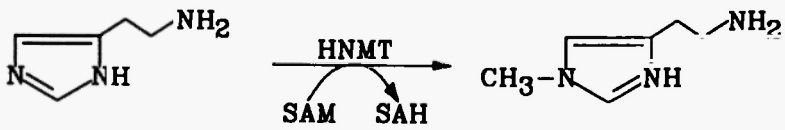


Fig. 1: The histamine N-methyltransferase-catalyzed reaction.

SAM-dependent enzymes. Some notable exceptions are D-SAM(Ki^{HNMT} = 92.2μM), and S-SAH(Ki^{HNMT} = 10.5μM) /4,5/.

The purpose of the present study was to examine a series of 5′-substituted adenosine derivatives bearing lipophilic 5′-substituents (see Figure 2) for their selective inhibitory properties against purified guinea pig brain HNMT. It was thought that such compounds, if they were effective, might be good candidates for studying the role of histamine in brain function.

II. EXPERIMENTAL SECTION

Melting points were determined on a Thomas-Hoover Unit-Melt apparatus (A.H. Thomas Company, Philadelphia, PA, USA) and are reported uncorrected. Microanalyses were performed by Desert Analytics Organic Microanalysis (Tucson, AZ, USA). Infrared spectra (IR) were determined as films, or as Nujol mulls on a Perkin-Elmer 567 grating infrared spectrophotometer (Norwalk, CT, USA). Proton magnetic resonance spectra (^{1}H-NMR) were carried out at 200MHz on a Varian Model XL-200 spectrometer (Varian Associates, Palo Alto, CA, USA); carbon magnetic resonance spectra (^{13}C-NMR) were carried out at 50.3MHz on a Varian Model XL-200 spectrometer. Chemical shifts are expressed in δ-values (ppm) downfield from tetramethylsilane (TMS) as an internal standard, or as an external standard where indicated, and signals are defined as singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), and multiplet (m); solvent residues determined from microanalytical data were confirmed by NMR spectroscopy but are not included in the line data for such compounds. Mass spectra (MS) were determined in either the electron impact (E.I.) or chemical ionization (C.I.) mode on a Finnigan MAT 8230 reverse geometry double focussing mass spectrometer (Bremen, West Germany) (high resolution), or on a Hewlett-Packard Model 5985a mass spectrometer (Rolling Meadows, IL, USA) (low resolution). Thin layer chromatographic (TLC) analyses were carried out on Kodak chromogram silica gel sheets with fluorescent indicator (Eastman-Kodak Company, Rochester, NY, USA). Preparative TLC separations were carried out on Whatman pkF_6 silica gel 60A TLC plates (Whatman Labsales, Inc., Hillsboro, OR, USA) of 1000μm thickness. All reaction products separated by TLC were visualized using UV light at 254nm. HPLC analyses and preparative

a) R = $C_6H_5-CH(OH)(CH_2)_3-$, X = I

b) R = 3-Indolyl-$(CH_2)_3-$, X = I

c) R = 3-Indolyl-$(CH_2)_4-$, X = I

a) R = $C_6H_5-(CH_2)_4-$, Y = NH

b) R = $C_6H_5-(CH_2)_4-$, Y = NCH_3

c) R = $C_6H_5-CH(OH)CH_2)_3-$, Y = S

d) R = 3-Indolyl-$(CH_2)_3-$, Y = S

e) R = 3-Indolyl-$(CH_2)_4-$, Y = S

Fig. 2: Synthetic adenosine analogues possessing histamine N-methyltransferase inhibitory properties.

separations were carried out on an Altex programmable HPLC unit consisting of an Altex Model 110A solvent metering pump, an Altex 420 system controller, and an Altex 153 single wavelength UV detector comprising a 254nm filter and either an analytical or preparative flow-through cell (Altex, Berkeley, CA, USA). Samples were introduced onto the appropriate column via a Rheodyne Model 7125 loop injector (Rheodyne, Cotati, CA, USA). Chromatograms were recorded on an Omniscribe Model 5000 dual channel recorder (Houston Instruments, Austin, TX, USA).

Compounds

The sulphur-containing compounds *1b*, *1c*, *2d*, and *2e* were prepared as previously described by the method of Benghiat and Crooks /6/ (see Figure 3). Compounds *1a*, *2a*, *2b*, and *2c* were prepared as outlined in Figures 4 and 5. Compounds *1a-1c* were each isolated as racemic mixtures of their respective epimers at the sulphonium centre. Compound *1a* was also a racemic mixture of the epimers at the benzylic carbon. Details of synthetic procedures for new compounds are given below.

Synthetic Procedures

Synthesis of 5′-[(4-hydroxy-4-phenylbut-1-yl)thio]-5′-deoxyadenosine (2c) and S-(5′-deoxyadenosyl), S-(4-hydroxy-4-phenylbut-1-yl), S-methylsulfonium iodide (1a) (Figure 4).

4-Bromo-1-phenylbutan-1-ol (3). 4-Bromo-1-phenylbutan-1-one /7/ (7.5g, 0.033mol) in absolute ethanol (100mL) was added dropwise over 5 min to a stirred solution of sodium borohydride (1 2g 0.033mol) in absolute ethanol (150mL) at 25°C. An immediate reaction occurred with effervescence. Stirring was continued for 3 hr at 25°C and the excess of sodium borohydride was then destroyed by the addition of acetone (50ml). The organic solvents were evaporated under reduced pressure and the resulting oil was partitioned between aqueous hydrochloric acid solution (1% v/v, 250ml) and chloroform (250ml). The chloroform layer was dried over magnesium sulphate,

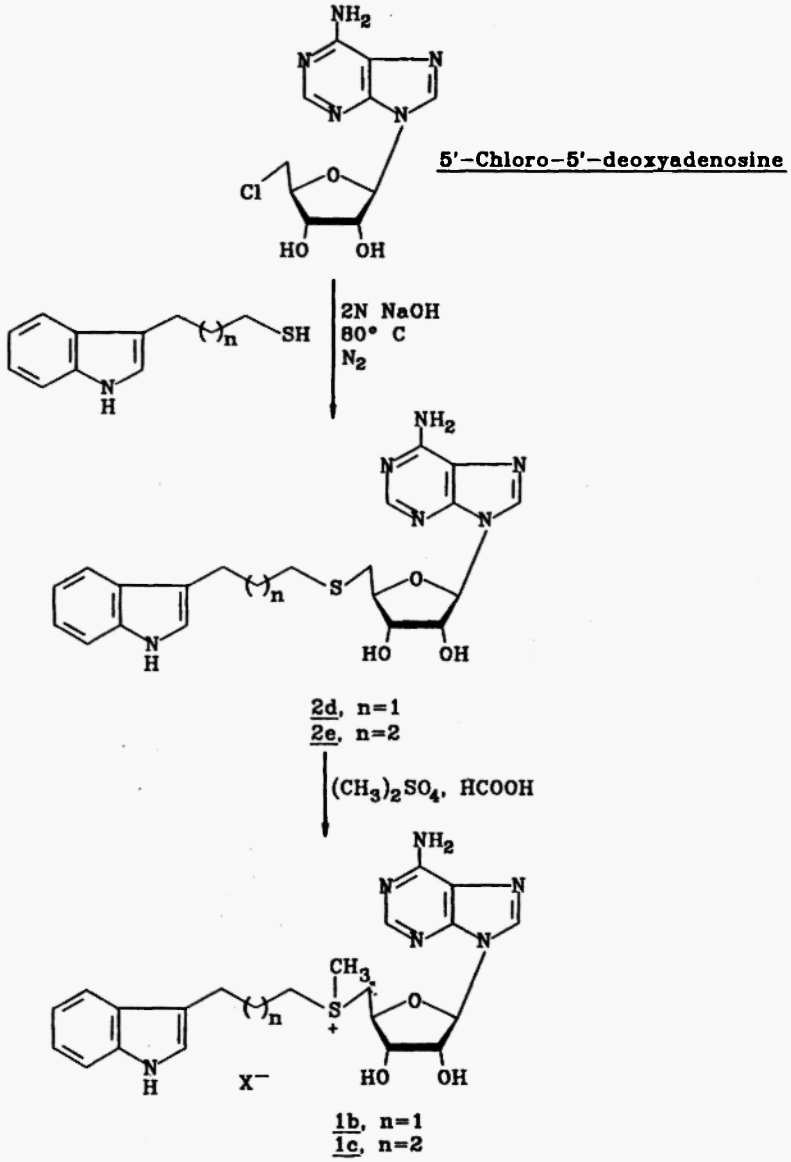


Fig. 3: Synthetic scheme for the preparation of compounds *1b*, *1c*, *2d*, and *2e*.

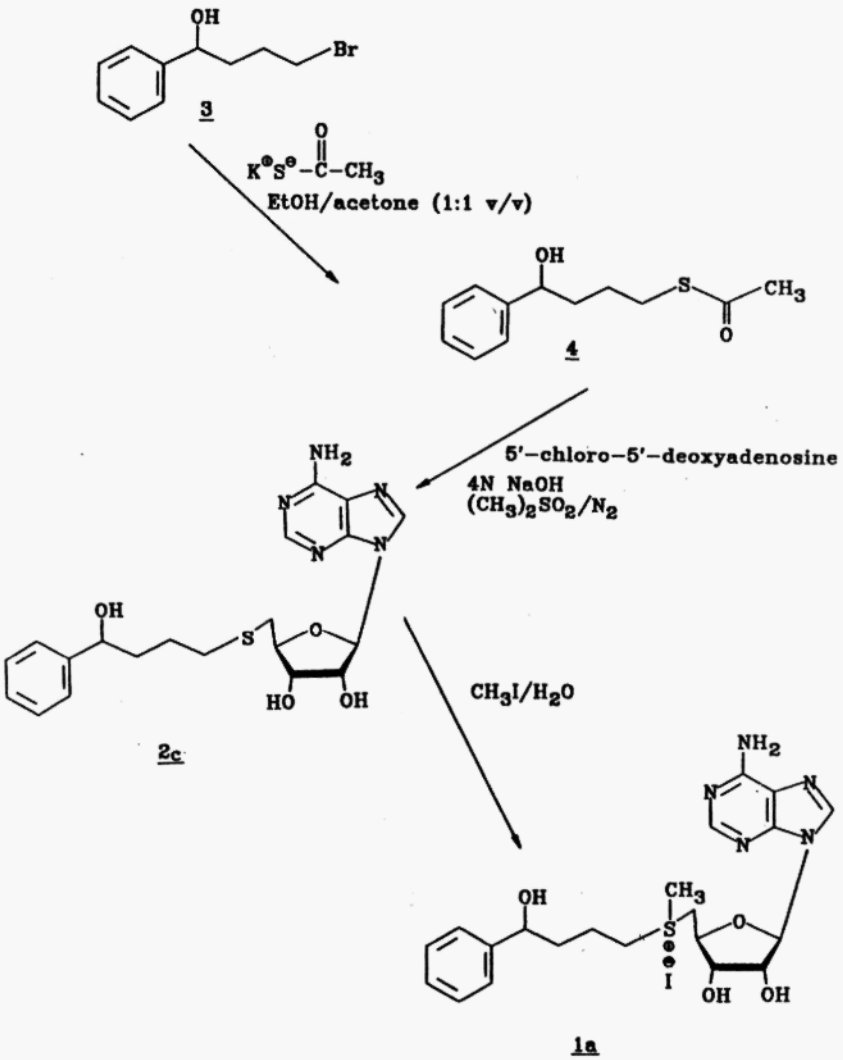


Fig. 4: Synthetic scheme for the preparation of compounds *1a* and *2c*.

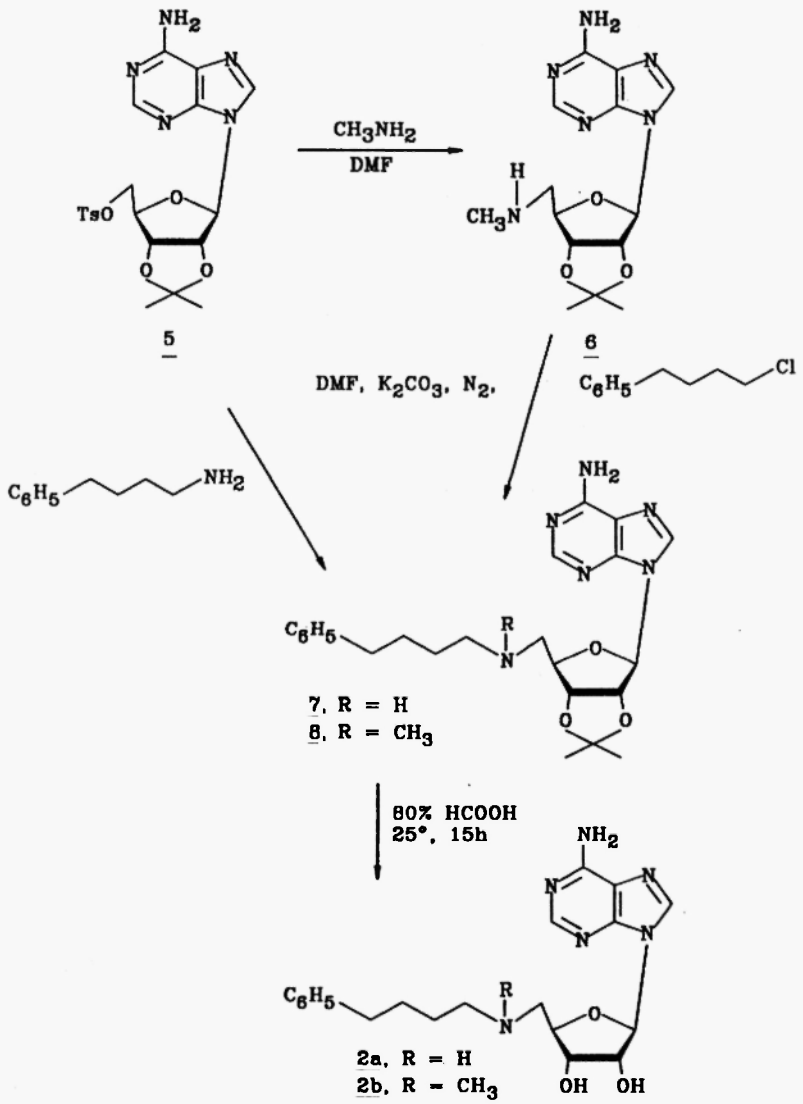


Fig. 5: Synthetic scheme for the preparation of compounds *2a* and *2b*.

filtered and evaporated to dryness to give *4-bromo-1-phenylbutan-1-ol (3)* as a colorless oil (6.5g, 0.028mol, yield 86%). IR ν_{max} (film) 3680-3140 (br, OH group), absence of stretching at 1740 and 1690cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 7.57-7.33 (5H, m, aromatic protons) 4.93 (1H, t, $J \approx$ 6Hz, CH_2Br), 2.27-1.83 (4H, m,-CH_2-CH_2-CH_2Br) ppm; MS (E.I., 70eV) m/z (% relative intensity) 212 {[(M + 1) – H_2O], 14%}, 210 {[(M – 1) – H_2O, 10%}, 149 {[(M – 1) – Br], 10%}, 148 {[(M – 1)HBr], 86% , 147}{[(M – 1) – (Br + 2H)], 99% , 131 {[(M – 1) – (H_2O + Br)], 71%}, 107 (PhCHOH, 86%), 105 (PhCO, 98%), 91 ($PhCH_2$, 100%), 77 (Ph, 57%); High resolution MS (E.I. 70eV) for $C_{10}H_{13}BrO$. Found, M + 1: 230.0130; M – 1: 228.0150. Calculated, M + 1: 230.0130; M – 1: 228.0150 (relative intensity ratio of M1/M – 1≅1). Elemental analysis for $C_{10}H_{13}BrO$: Found: C, 52.62; H, 5.91; Br, 35.03, Calculated: C, 52.60; H, 5.75, Br, 35.04.

S-Acetyl-4-hydroxy-4-phenylbutan-1-thiol (4). A mixture of 4-bromo-1-phenylbutan-1-ol (*3*) (1.0g, 4.30mmol) and potassium thioacetate (0.98g, 8.60mmol) in a solution of acetone and absolute ethanol (30mL, 1:1 v/v) was heated under reflux for 1.5hr. The dark yellow mixture was cooled and filtered, and the filtrate was evaporated to dryness at 40°C. Water (15mL) was added to the resulting residue and the mixture was extracted into ether (2X30mL). The combined organic extracts were washed with water (2X30mL), dried over magnesium sulphate, filtered, and the solvent removed under reduced pressure to give an oily residue. The residue (0.80g) was dissolved in ether (1.5mL) and applied to a dry-packed chromatography column (20g, silica gel 35-70 mesh). Elution of the column was carried out first with ether: petroleum ether (bp 60-80°C) 1:1 (v/v), then with ether alone, and all fractions that showed one spot on TLC with R_f 0.76 [ether/petroleum ether (bp 60-80°C) (1:1, v/v)] were combined. Removal of the solvent gave *S-acetyl-4-hydroxy-4-phenylbutan-1-thiol (4)* as a yellowish oil (0.42g, yield 44%). IR ν_{max} (film) 3650-3150 (br, OH group), 1700 (CO) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.57-7.33 (5H, m, aromatic protons), 4.80 (1H, t, J = 6Hz, PhC*H*) 2.97 (2H, t, J = 6Hz, CH_2-S), 2.56 (1H, brs, OH, exchangeable with D_2O, 2.36 (3H, s, CH_3), 1.97-1.67 (4H, m,-CH_2CH_2-CH_2-S) ppm; MS (E.I. 70eV) m/z (relative intensity) 224 [M^+, 6%], 181 [(M – $COCH_3$), 41%], 180 {[M – ($COCH_3$ + H)] 31%}, 163 {[M – ($COCH_3$ + H_2O)], 18%}, 148 {[M – ($SCOCH_3$ +

H)], 23%}, 147{[M + ($SCOCH_3$ + 2H)], 50%}, 131 {[M – ($SCOCH_3$ + H_2O)], 14%}, 107 (PhCHOH, 100%), 105 (PhCO, 41%), 91 ($PhCH_2$, 23%), 77 (Ph, 36%); High resolution MS (E.I., 70eV) for $C_{12}H_{16}O_2S$. Found, M:224.0870. Calculated, M:224.0872. Elemental analysis for $C_{12}H_{16}O_2S$: Found: C, 64.55, H, 7.20; S, 14.53. Calculated: C, 64.25; H, 7.19; S, 14.29.

5'- [(4- Hydroxy-4-phenylbut-1-yl)- thio] -5'-deoxyadenosine (2c). A stream of nitrogen was bubbled through a solution of S-acetyl-4-hydroxy-4-phenylbutan-1-thiol (*4*) (0.41g, 1.85mmol) in dimethyl sulfoxide (7mL) for 1 hr, after which time 5'-chloro-5'-deoxyadenosine /8/ (0.5g, 1.75mmol) was added, followed by 4N aqueous sodium hydroxide solution (1.4mL). The resulting solution was stirred at 25°C for 17 hr and then poured into water (53mL) to form a gummy deposit, after neutralization with glacial acetic acid. The reaction mixture was kept at 4°C overnight, the water decanted, and the gummy deposit then dissolved in hot absolute ethanol (~5mL). On cooling to 25°C, some colloidal material precipitated which was removed by filtration using a 0.45μ micropore filter. The filtrate was evaporated to dryness and ether was added to give a precipitate of *5'- [(4- hydroxy- 4-phenylbut- 1-yl)-thio]- 5'-deoxyadenosine (2c)* (400mg, 0.928mmol, yield 50%); mp 243-245°C; ^{1}H-NMR (DMSO-d_6 and D_2O, 3:1 v/v) δ 8.50 (1H, s, adenyl H-2), 8.37 (1H, s, adenyl H-8), 7.60-7.23 (5H, m, phenyl protons), 6.03 (1H, d, *J* = 5Hz, ribosyl H-1'), 4.86 (1H, t, *J* = 6Hz, PhC*H*), 4.73-4.40 (1H, m, ribosyl H-2'), 4.40-4.10 (1H, m, ribosyl H-3'), 4.10-3.80 (1H, m, ribosyl H-4'), 2.90 (2H, d, *J* = 6Hz, ribosyl H-5'), 2.63 (2H, t, *J* = 6Hz, CH_2CH_2-S), 1.77-1.40 (4H, m, -CH_2-CH_2-CH_2-S); ^{13}C-NMR (proton-decoupled, DMSO-d_6) 156.12 (adenyl C-6), 153.08 (adenyl C-2), 149.73 (adenyl C-4), 146.13 (adenyl C-8), 140.37 (phenyl C-1), 128.39 (phenyl C-2 and C-6), 127.09 (phenyl C-4), 126.10 (phenyl C-3 and C-5), 119.35 (adenyl C-5), 87.86 (ribosyl C-1'), 854.36 (PhCH), 72.94, 72.77, 72.26 (3 X s, ribosyl C-2', C-3', C-4'; specific assignments not determined), 34.34 (ribosyl C-5'), 32.45 ($CH_2CH_2CH_2S$), 25.85 ($CH_2CH_2CH_2$-S), 18.61 ($CH_2CH_2CH_2S$) ppm; MS (C.I., CH_4) m/z (relative intensity) 432 [(M + H), 50%], 165 {[(M + 2H) – (H_2O + 5'deoxyadenosine)], 40%}, 164 [(adenine + 30), 22%], 147 {M – [PhCHOHCH$_2$CH$_2$CH$_2$) + (adenine + H)], 20%}, 136 [(adenine + 2), 100%], 131 [M – (H_2O + 5'deoxy-5'-thioadenosine), 40%]; High resolution MS (E.I., 70eV) for $C_{20}H_{25}N_5O_4S$. Found,

M:431.1619. Calculated, M:431.1622. Elemental analysis for $C_{20}H_{25}N_5O_4S$: Found: C, 55.51; H, 5.83; N, 16.63. Calculated: C, 55.67; H, 5.85; N, 16.32.

S-(5'-Deoxyadenosyl), S-(4-hydroxy-4-phenylbut-1-yl), S-methylsulfonium iodide and its corresponding acetate salt (1a). Methyl iodide (37mg, 0.262mmol) was added to a mixture of 5'- [(4-hydroxy-4-phenylbut-1-yl)thio]- 5'-deoxyadenosine (*2c*) (50mg, 0.116 mmol) and water (5mL) warmed to 30°C. The mixture was stirred at 50°C for 4 hr (additional amounts of methyl iodide, 0.116mmol, were added every hour during the first 4 hr). Stirring was continued for an additional 12 hr at 25°C, during which time the reaction mixture was fitted with a cold water condenser (approximately 5°C) by connecting the inlet to the main water tap *via* a copper coil immersed in an ice-salt bath in order to prevent loss of iodomethane. The reaction mixture was then extracted with ether (10mL) and the aqueous layer lyophilized to give a mixture of the four possible diastereomeric forms of*S-(5'deoxyadenosyl), S-(4-hydroxy-4-phenylbut-1-yl), S-methylsulphonium iodide* (*1a*) as a slightly impure and very hygroscopic, colorless powder (75.2mg). ^{1}H-NMR (D_2O, external TMS standard) δ 8.20 (1H, adenyl H-2), 8.12 (1H, s, adenyl H-8), 7.32-7.02 (5H, m, phenyl protons), 5.98-5.92 (1H, superimposed d's of all the four diastereomers, ribosyl H-1'), 4.86-4.72 (1H, m, ribosyl H-2'), 4.54-4.32 (3H, m, PhC*H*, and ribosyl H-3' and H-4'), 3.82-3.66 (2H, superimposed d's of all the four diastereomers, ribosyl H-5'), 3.30-3.08 (2H, superimposed t's of all the four diastereomers, CH_2-CH_2-CH_2-S^+), 2.78, 2.76, 2.74, 2.72 (3H, 4 X s, diastereomeric forms of S^+-CH_3), 1.72-1.40 (4H, m, CH_2-CH_2-CH_2-S^+); ^{13}C-NMR (APT, (+) indicates positive absorption, (-) indicates negative absorption, D_2O) 156.75 [(+), adenyl C-6], 151.75 [(+), adenyl C-4], 146.06 [(+), phenyl C-1], 131.60 [(-), phenyl C-2 and C-6], 131.30 [(-), phenyl C-4], 129.51 [(-), phenyl C-3 and C-5], 92.00 [(-), ribosyl C-1'], 81.70 [(-), PhCH], 81.30 [(-), ribosyl C-2'], 75.85 [(-), CH_3S^+], 75.60 [(-), ribosyl C-3'], 75.45 [(-), ribosyl C-4'], 46.80 [(+), ribosyl C-5'], 45.00 [(+), CH_2-CH_2CH_2-S^+], 38.60 [(+), $CH_2CH_2CH_2S^+$], 22.70 [(+), CH_2-CH_2-CH_2S^+] ppm. The adenyl C-2 and C-8 signals could not be observed because of their unusually large line separation (J = 200Hz). Their signals were supressed by the APT delay which is usually used to see the signals of the rest of the C-atoms in the molecule. The adenyl C-5 signal could not be seen because of its

relative long relaxation time. The above product when applied to an analytical HPLC column [Partisil 10 SCX (25 X 0.04cm), mobile phase methanol:water 50:50 (v/v) + 1% triethylamine, adjusted to pH=6 with acetic acid, 2mL/min] gave three peaks with t_R's 2.0 min (10%), 3.9 min (87%), and 7.9 min (3%). These peaks were separated by preparative HPLC as follows. The reaction mixture (75.2mg) was dissolved in water (0.3mL) and applied to a preparative HPLC column [Partisil Magnum 9 SCX (50 X 0.9cm), mobile phase methanol:water 50:50 (v/v) + 0.1% triethylamine, adjusted to pH=6 with acetic acid 4mL/min], and the major fraction eluting at t_R 13.0 min was collected. This fraction was reinjected onto the analytical HPLC column, and was shown to be a single peak with t_R = 3.9 min. The above fraction from the preparative HPLC separation was evaporated under reduced pressure to a small volume (~ 3mL) at 25°C, and the resulting solution was then lyophilized to give a mixture of all the four possible diastereomers of *S-(5'-deoxyadenosyl), S-(4-hydroxy-4-phenylbut-1-yl), S-methylsulphonium acetate* as a colorless and very hygroscopic powder (20mg, 0.034mmol, yield 34%), mp 210-215°C (dec.); ^{1}H-NMR (D_2O, external TMS standard δ 8.10 (1H, s, adenyl H-2), 8.06, (1H, s, adenyl H-8), 7.34-6.98 (5H, m, phenyl protons), 5.92-5.86 (1H, superimposed d's of all the four diastereomers, ribosyl H-1'), 4.90-4.70 (1H, m, ribosyl H-2'), 4.54-4.32 (3H, m, PhC*H*, ribosyl H-3' and H-4') 3.84-3.66 (2H, superimposed d's of all the four diastereometers, ribosyl H-5'), 3.30-3.08 (2H, superimposed t's of all the four diastereomers, CH_2-CH_2-CH_2S^+), 2.78, 2.76, 2.74, 2.72 (3H, 4 X s, diastereomeric forms of S^+-CH_3), 1.78 (3H, s, CH_3COO^-), 1.72-1.30 (4H, m, CH_2-CH_2-CH_2-S^+) ppm; MS (C.I., CH_4) m/z (relative intensity) 449 [(M + 3H), 2%], 435 {[(M + 3H) – CH_2], 82%}, 428 [(M-H_2O), 8%], 322 {[(M+H) – (PhCHOH + H_2O)], 28%}, 298 {[(M+H) – PhCHOH-$(CH_2)_3$], 26%}, 235 {[M – (adenine + Ph), 100%]}, 199 {[(M + 3H) – 5'-deoxyadenosine], 34%}, 194 [(adenine + 60), 5%], 178 [(adenine + 44), 12%], 164 (adenine + 30) or {[(M + H) – (H_2O + CH_3 + 5'-deoxyadenosine)], 20%}, 149 {M – [5'-(S-methyl)-5'-deoxyadenosine], 28% , 148 M-[adenine + CH_3 + PhCHOH$(CH_2)_3$], 18%}, 136 [(adenine + 2H), 44%], 121 (PhCHOHCH$_2$, 80%).

Synthesis of 5'- [N-(4-phenylbut-1-yl)-amino]- 5'deoxyadenosine (2a) and 5'- [N-methyl, N-(4-phenylbut-1-yl)-amino]-5'-deoxyadenosine (2b) (Figure 5).

5'- 0- Tosyl-2', 3'- 0-isopropylidene adenosine (5). This compound was prepared *via* a modification of a literature procedure /9/. 2',3'-O-Isopropylidene adenosine /10/ (1g, 3.26mmol; dried over P_2O_5 at 110°C for 12 hr) was dissolved in anhydrous pyridine (6mL, dried over sodium hydroxide pellets for 12 hr and distilled from fresh sodium hydroxide pellets), p-toluene-sulphonyl chloride (1g, 5.26mmol) recrystallized from chloroform and petroleum ether (bp 30-60°C) was then added and the mixture was shaken well and left at -25°C for 22 hr. Cold water (4°C) (10mL) was added to the reaction mixture and the mixture was shaken. Cold chloroform (4°C) (2 X 50mL) was then added, the mixture was shaken, and the chloroform layers were separated and combined. The chloroform solution was then washed with cold aqueous (4°C) sulphuric acid (2N, 2 X 10mL) to remove all the pyridine. The chloroform layer was separated, washed with cold water (10mL), and dried over magnesium sulphate and filtered. The chloroform was removed under high vacuum (5-10mm Hg) at 4°C to give a yellow gummy residue (1.75g), which was dissolved in methanol (10mL). Petroleum ether (bp 30-60°C) was then added to the solution and the mixture was left at -20°C for 24 hr. The resulting solid was filtered to give *5'-0-Tosyl-2', 3'- 0-isopropylidene adenosine (5)* as a yellow powder (1.35g, 2.93mmol, yield 89.8%); mp 250°C (dec.) [Lit. /11/ 252°C (dec.)] ; physical properties were identical to those previously described /9/.

5'- Methylamino- 5'- deoxy-2', 3'- 0- isopropylideneadenosine (6). 5'- 0-Tosyl-2', 3'-0-isopropylidene adenosine (5) (430mg, 0.93mmol) was added to a solution of methylamine in absolute ethanol (20mL, 33%) and the mixture was stirred for 3 days at 25°C. The reaction mixture was then evaporated to dryness under reduced pressure and the resulting residue was partitioned between chloroform (100mL) and 10% potassium carbonate solution (50mL). The organic layer was separated, washed with water (50mL), dried over magnesium sulphate, filtered and evaporated to dryness to give *5'- methylamino- 5'- deoxy-2', 3'-0-isopropylidene adenosine (6)* as shiny colorless crystals (247mg, 0.77mmol, 83%); mp 48-52°C; ^{1}H-NMR ($CDCl_3$) δ 8.37 (1H, s, adenyl

H-2), 7.97 (1H, s, adenyl H-8), 6.13 (2H, br s, adenyl NH_2, exchangeable with D_2O), 6.06 (1H, d, J = 5Hz, ribosyl H-1'), 5.67-5.40 (1H, m, ribosyl H-2'), 5.20-4.97 (1H, m, ribosyl H-3'), 4.67-4.20 (1H, m, ribosyl H-4'), 2.90 (2H, d, J = 6Hz, ribosyl H-5'), 2.83-2.57 (1H, br s, NH, exchangeable with D_2O), 2.50 (3H, s, CH_3N), 1.63 (3H s, one of ketal CH_3 groups), 1.43 (3H, s, one of ketal CH_3 groups) ppm; MS (C.I., CH_4) m/z (relative intensity) 349 [M + (C_2H_5), 25%], 321 [(M + H), 100%], 290 [(M – CH_3NH), 6%], 277 {[(M + H) – (CH_3NHCH_2)], 8%}, 236 {[(M + 2H) – (CH_3NHCH_2 + C_3H_6)], 10%}, 186 [(M – adenine), 40%], 176 [(adenine + 42), 4%], 164 [(adenine + 30), 36%], 148 (adenine + 14), 2%], 136 [(adenine + 2H), 82%]; High resolution MS (E.I., 70 eV) for $C_{14}H_{20}N_6O_3$. Found: 320.1595. Calculated: 320.1593; Elemental analysis for $C_{14}H_{20}N_6O_3 . H_2O$. Found, C 49.82; H, 6.62; N, 24.55. Calculated: C, 49.69; H, 6.60; N, 24.84%.

5'-[N-(4-Phenylbut-1-yl)-amino]-5'-deoxy-2', 3'-0-isopropylidene adenosine (7). To a stirred solution of 5'-0-tosyl-*2', 3'*-0-isopropylidene adenosine (5) (0.150mg, 0.32mmol) in dry N, N-dimethylformamide (1mL), was added 1-amino-4-phenylbutane (0.94g, 6.3mmol) and stirring was continued at 25°C until the reaction ceased (5 days). The reaction mixture was then evaporated to dryness under reduced pressure and the resulting residue was partitioned between chloroform (120mL) and water (80mL). The organic layer was separated, dried over magnesium sulphate, filtered, and evaporated to dryness to give an oily residue. The residue afforded three components on TLC [chloroform/methanol 12:1 (v/v)] at R_f = 0.87, 0.75, and 0.50, respectively. The components could not be separated after several attempts using preparative HPLC; they were therefore separated by preparative TLC. The mixture (1.2g) was dissolved in chloroform (1mL) and applied as a band to preparative TLC plates. The approximate loading levels were 50-100mg per plate. The plates were dried, developed in chloroform:-methanol 12:1 (v/v), and the desired band (R_f = 0.32) was isolated. The silica was treated with methanol (100mL) and the suspension stirred for 1-2 hr. The silica gel was removed by filtration and washed with methanol (50mL) and then chloroform (2 X 40mL). The combined organic filtrates were evaporated to dryness and partitioned between chloroform (50mL) and water (20mL). The organic extract was dried over magnesium sulphate, filtered and evaporated to dryness to give *5'-[(4-phenylbut-1-yl) amino]-5'-deoxy-2', 3'-0-isopropylidene adenosine*

(7) as a colorless oil (66mg, 0.15mmol, 47%); R_f = 0.75 [analytical TLC, chloroform:methanol, 12:1 (v/v)]; ^{1}H-NMR δ 8.23 (1H, s, adenyl H-2), 7.83 (1H, s, adenyl H-8), 7.27-6.90 (5H, m, phenyl protons), 5.97 (3H, d superimposed on br s, J = 5Hz, ribosyl H-1′ and NH_2, reduced to 1H on exchange with D_2O), 5.53-5.30 (1H, m, ribosyl H-2′), 5.10-4.83 (1H, m, ribosyl H-3′), 4.47-4.10 (1H, m, ribosyl H-4′), 2.83 (2H, d, J = 6Hz, ribosyl H-5′), 2.57 (4H, apparent t, J = 8Hz, CH_2CH_2N superimposed on $PhCH_2$), 1.60 (3H, s, one of ketal CH_3 groups), 1.53-1.20 (5H, m, $PhCH_2CH_2CH_2$ and NH, reduces to 4H on exchange with D_2O), and 1.37 (3H, s, one of ketal CH_3 groups), ppm; MS (C.I., CH_4) m/z (relative intensity) 468 [(M + C_2H_5), 18%], 440 [(M + 2H), 32%], 439 [(M + H), 100%], 395 {[M – $C(CH_3)_2$], 5%}, 305 {[M – $Ph(CH_2)_4$], 8%}, 304 [(M – adenine, 34%], 164 [(adenine + 30), 8%], 136 [(adenine + 2H), 20%]; High resolution MS (E.I., 70 eV) for $C_{23}H_{30}N_6O_3$. Found 438.2378. Calculated: 438.2380.

5′-[N-Methyl, N-(4-Phenylbut-1-yl)-amino]-5′-deoxy- 2′, 3′- 0-isopropylidene adenosine (8). 5′-Methylamino- 5′- deoxy- 2′, 3′- 0-isopropylidene adenosine (6) (170mg, 0.53mmol) was stirred with anhydrous potassium carbonate (100mg, 0.7mmol) in dry N,N-dimethylformamide (5mL) at 90-100°C under a nitrogen atmosphere for 1 hr. 1-Chloro-4-phenylbutane (100mg, 0.595mmol) was then added (*via* a syringe) in one portion, and the stirring was continued at 90°C for an additional 24 hr. The reaction mixture was then evaporated to dryness under reduced pressure and the resulting residue was partitioned between chloroform (30mL) and water (20mL). The organic extract was separated, dried over magnesium sulphate, filtered and evaporated to dryness to give an oily residue. ^{1}H-NMR spectroscopic analysis of the residue ($CDCl_3$) indicated multiple components. The residue also showed five components by analytical TLC [chloroform/methanol 12:1 (v/v), at R_f = 0.95, 0.78, 0.67, 0.22 and 0.00. These components were separated by preparative TLC. The 5-component mixture (237mg) was dissolved in chloroform (0.5mL) and applied as a band to preparative TLC plates. The approximate loading levels were 50-100mg per plate. The plates were dried and developed in chloroform:methanol, 12:1 (v/v), and the desired band (R_f = 0.42) was isolated. The silica was treated with methanol (100mL) and the suspension stirred for 1-2 hr. The silica gel

was filtered off, washed with methanol (50mL) and then chloroform (2 X 40mL). The combined organic filtrates were evaporated to dryness, and partitioned between chloroform (50mL) and water (20mL). The organic extract was dried over magnesium sulphate, filtered and evaporated to dryness to give *5'- [N-Methyl, N- (4-phenylbut-1-yl) amino]- 5'- deoxy- 2', 3'- 0- isopropylidene adenosine (8)* as an oil (66.7mg, 0.15mmol, yield 28%); R_f = 0.78 [analytical TLC], ^{1}H-NMR ($CDCl_3$) δ 8.34 (1H, s, adenyl H-2), 7.94 (1H, s, adenyl H-8), 7.34-7.10 (5H, m, phenyl protons), 6.06 (1H, d, *J* = 5Hz, ribosyl H-1'), 5.78 (2H, br s, NH_2, exchangeable with D_2O), 5.54-5.44 (1H, m, ribosyl H-2'), 5.00-4.90 (1H, m, ribosyl H-3'), 4.46-4.32 (1H, m, ribosyl H-4'), 2.70-2.50 (4H, m, ribosyl H-5' and PhCH_2), 2.36 (2H, t, *J* = 8Hz, CH_2CH_2N), 2.24 (3H, s, CH_3-N), 1.6 (3H, s, one of ketal CH_3 groups), 1.58-1.24 (4H, m, $PhCH_2CH_2CH_2$), 1.40 (3H, s, one of ketal CH_3 groups) ppm; MS (C.I., CH_4) m/z (relative intensity) 481 [(M + C_2H_5), 20%], 454 [(M + 2), 30%], 453 [(M + H), 100%], 452 [M+, 10%], 437 [(M − CH_3), 5%], 346 {[M − (PhCH_2 + CH_3)], 10%}, 319 {[M − $Ph(CH_2)_4$], 18%}, 318 [(M − adenine), 56%], 136 [(adenine + 2H), 2H), 8%]; Elemental analysis for $C_{24}H_{32}N_6O_3.H_2O$; Found: C, 61.19; H, 7.06; N, 17.40. Calculated: C, 61.24; H, 7.29; N, 17.86.

5'- [N- (4- Phenylbutyl)- amino]- 5' -deoxyadenosine formate salt (2a). 5'-[(4-Phenylbut-1-yl)-amino]-5'-deoxy-2', 3'-0-isopropylidene adenosine (7) (52mg, 0.12mmol) was dissolved in formic acid (1mL, 80%). The reaction mixture was stirred for 22 hr at 25°C; addition of methanol and evaporation of the solvent under high vacuum (5-10mm Hg) gave a thick oil which solidified upon standing (54.2mg). The solid was recrystallized from methanol-ether (1:30, 15mL) to give *5'- [N-(4-phenylbut-1-yl)- amino]- 5'- deoxyadenosine (2a)* as its formate salt (37.7mg, 0.08mmol, 71%); mp 140-142°C; ^{1}H-NMR (D_2O, external TMS standard) δ 8.32 (1H, br s, *H*COO$^-$), 8.10 (1H, br s, adenyl H-2), 8.06 (1H, s, adenyl H-8), 7.30-6.94 (5H, m, phenyl), 5.94 (1H, d, *J* = 5Hz, ribosyl H-1'), 4.80-4.68 (1H, m, ribosyl H-2'), 4.40-4.20 (2H, m, ribosyl H-3' and H-4'), 3.42-3.20 (2H, m, ribosyl H-5'), 2.96 (2H, t, *J* = 8Hz, CH_2-CH_2-N), 2.40 (2H, t, *J*=8Hz, Ph-CH_2), 1.64-1.36 (4H, m, Ph-$CH_2CH_2CH_2$) ppm; MS (C.I., CH_4) m/z (relative intensity 427 [(M + C_2H_5), 18%], 399 [(M + H), 98%], 264 [(M − adenine), 30%], 228 {[M − adenine + H_2O)], 18%}, 204 [M −

(adenine + CHOHCHOH), 8%], 194 [(adenine + 60), 5%], 178 [(adenine + 44), 16%], 164 [(adenine + 30), 30%], 150 [(adenine + H + 14), 8%], 136 [(adenine + 2H), 100%], 91 (Ph-CH_2, 6%); High resolution MS (E.I., 70 eV) for $C_{20}H_{26}N_6O_3$. Found: 398.2066. Calculated: 398.2067; Elemental analysis for $C_{20}H_{26}N_6O_3$. 2HCOOH: Found: C, 53.90; H, 6.19; N, 16.84. Calculated: C, 53.87; H, 6.17; N, 17.13.

5'- [N-Methyl, N- (4-phenylbut-1-yl)- amino] - 5'- deoxyadenosine (2b), formate salt. 5'- [N-Methyl, N- (4-phenylbut-1-yl)- amino] - 5'- deoxy- 2', 3'-isopropylidene adenosine (68mg, 0.15mmol) was dissolved in formic acid (2mL, 80%). The reaction mixture was stirred for 22 hr at 25°C. Addition of methanol and evaporation of the solvent under reduced pressure gave a thick oil which solidified upon standing (54mg). The solid was recrystallized from methanol-ether (1:30, 15ml) to give *5'- [N- methyl, N- (4-phenylbut-1-yl)- amino] - 5' -deoxyadenosine (2b)* as its formate salt (46mg, 0.1mmol, yield 66.9%); mp 65-70°C; ^{1}H-NMR (D_2O, external TMS standard) δ 8.10 (2H, br s, adenyl H-2 and *H*COO^-), 8.03 (1H, s, adenyl H-8), 7.27-6.93 (5H, m, phenyl protons), 5.93 (1H, d, *J*=5Hz, ribosyl H-1'), 4.90-4.67 (1H, m, ribosyl H-2'), 4.47-4.27 (2H, m, ribosyl H-3' and H-4'), 3.60-3.43 (2H, m, ribosyl H-5'), 3.10 (2H, t, *J* = 8Hz, CH_2CH_2N), 2.93 (3H, s, CH_3-N), 2.33 (2H, t, *J* = 8Hz, PhCH_2), 1.77-1.27 (4H, m, Ph$CH_2CH_2CH_2$) ppm; MS (C.I., CH_4) m/z (relative intensity) 441 [(M + C_2H_5), 20%], 414 [(M + 2H), 30%], 413 [(M + H), 100%], 278 [(M – adenine), 44%], 218 {[M – (adenine + 60)], 10%}, 164 [(adenine + 60), 16%], 136 [(adenine + 2H), 30%], 133 [Ph$(CH_2)_4$, 10%], 91 (PhCH_2, 10%); Elemental analysis for $C_{21}H_{28}N_6O_3$. HCOOH.1.6H_2O. Found: C, 53.95; H, 6.51; N, 17.09. Calculated: C, 54.22; H, 6.87; N, 17.24.

2.3 Enzyme Inhibition Studies

Rabbit Adrenal Phenylethanolamine N-Methyltransferase (PNMT) The method of assay involved the use of ^{14}C-SAM (53.9 Ci/mol) as the methyl donor and *L*-norepinephrine as the methyl acceptor. In this procedure, a selective precipitation of ^{14}C-SAM after incubation was conducted, and the ^{14}C-*N*-methylated product (^{14}C-epinephrine) remaining in the supernatant was measured utilizing the Reineckate Method /12/. The assay procedure was as follows.

Just prior to the assay, a buffer-substrate solution was prepared by mixing the following quantities of reagents: sodium phosphate buffer (25μL, 0.5M, pH7.9), water (55μL), partially purified enzyme (10μL, 120μg protein), and ^{14}C-SAM (10μL, 300μM). Incubation mixtures (15μL) contained buffer-substrate solution (10μL), *L*-norepinephrine (2μL, 40μM) and inhibitor solution (3μL). Incubations were carried out in duplicate at 37°C for 30 min and terminated by the addition of a quenching reagent (10μL) containing unlabelled SAM (12.5M) in hydrochloric acid (2.5M) [2 parts of water and 1 part SAM (21.7mg/mL)+ 1 part hydrochloric acid (10M)]. A saturated Reineckate solution (ammonium tetrathiocyanodiammoniochromate) (20μM) was then added. The unlabelled SAM serves as a carrier in the precipitation step. The incubates were mixed and centrifugated in the cold for 5 min. Aliquots (15μL) of the supernatant fraction containing the methylated product were added to a mixture of methanol (1mL) and scintillation cocktail (10 mL) {15.2g PPO (2,5-diphenyloxazole) and 0.38g POPOP [1,4- bis- 2- (5-phenyloxazoyl) benzene] dissolved in 1 gal toluene}, and the radioactivity of the samples was determined. A blank incubation was also performed containing no enzyme preparation. A control experiment without inhibitor was conducted, and was considered to represent 100% enzyme activity. Percent inhibition exhibited by the test compound at various concentrations was calculated by determining the quantity of epinephrine (pmol/30 min) formed in the presence of the inhibitor. These values were related to those obtained in the control experiment (i.e., no inhibitor). Inhibitory properties were expressed as pI_{50} values (see Figure 6 and Table 1), representing the negative logarithm of the molar concentration required for 50% inhibition of enzyme activity.

Guinea Pig Brain Histamine N-Methyltransferase (HNMT). HNMT activity was determined using ^{14}C-SAM (59.8mCi/mmol) as the methyl donor and histamine as the methyl acceptor. After incubation, the ^{14}C-labelled *N*-methylhistamine (product) was selectively extracted into chloroform using a modification of the procedure of Brown et al. /13/. The assay procedure is described below.

A buffer-substrate solution was prepared by mixing the following quantities of reagent: sodium phosphate buffer (255μL 0.2M, pH7.4), HNMT enzyme (255μL, 120mg) and ^{14}C-SAM (20μM). The incubation mixture (15μL) contained buffer-substrate solution (10μL), histamine

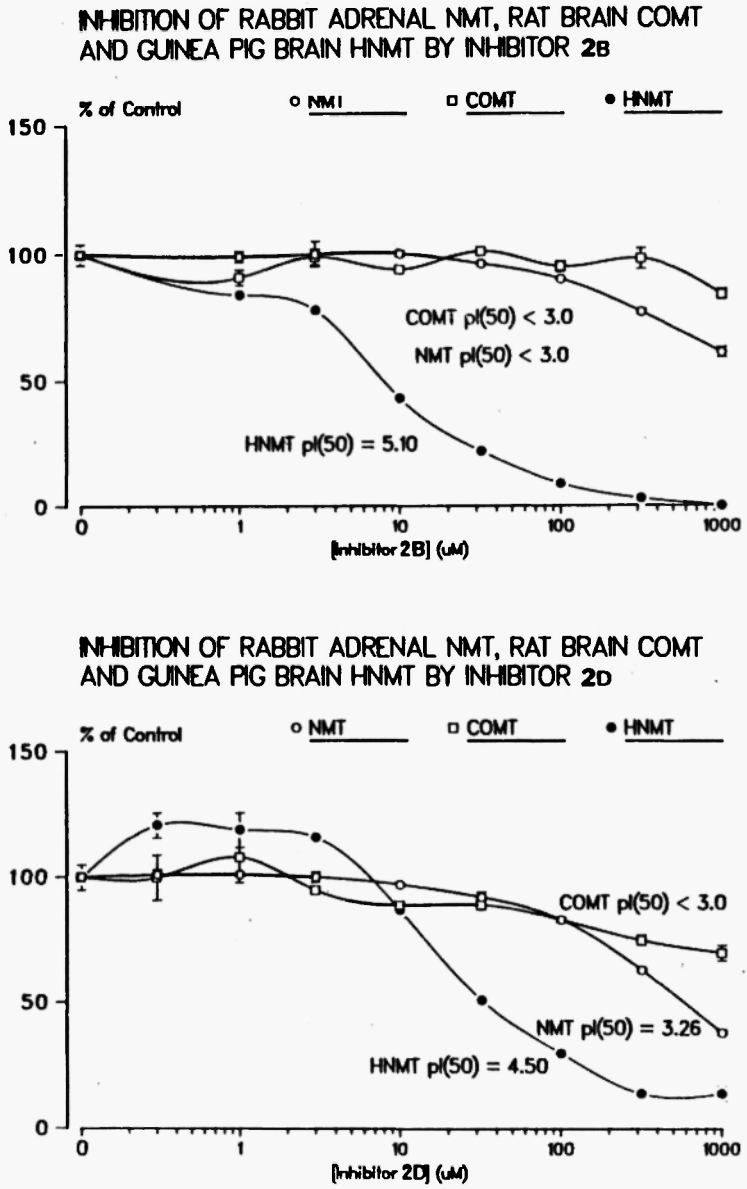
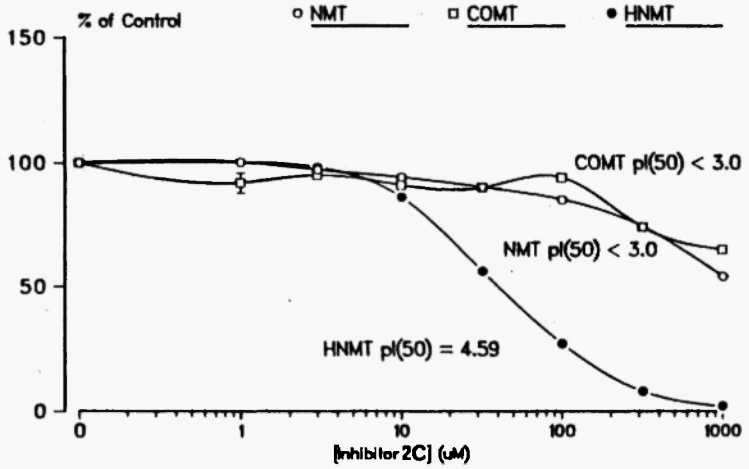
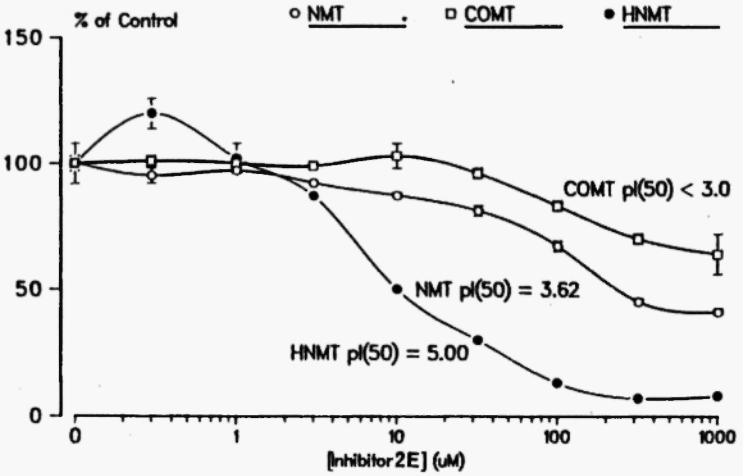


Fig. 6: Comparative dose-response curves for compounds *1a-1c* and compounds *2a-2e* against guinea pig brain histamine N-methyltransferase, rabbit adrenal phenylethanolamine N-methyltransferase, and rat brain catechol *O*-methyltransferase.

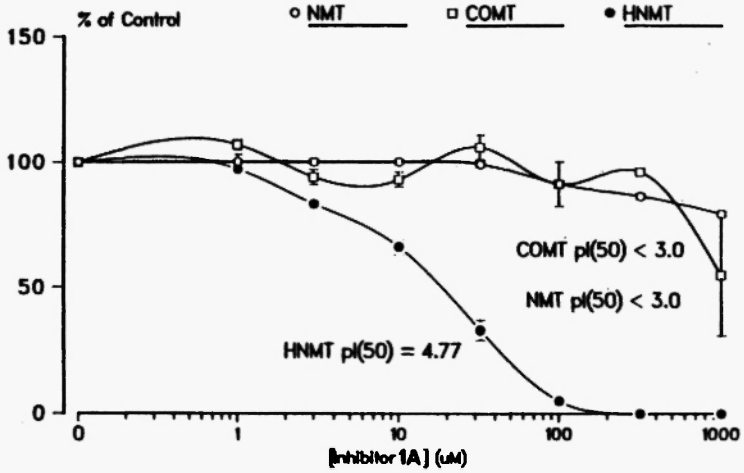
INHIBITION OF RABBIT ADRENAL NMT, RAT BRAIN COMT AND GUINEA PIG BRAIN HNMT BY INHIBITOR **2c**



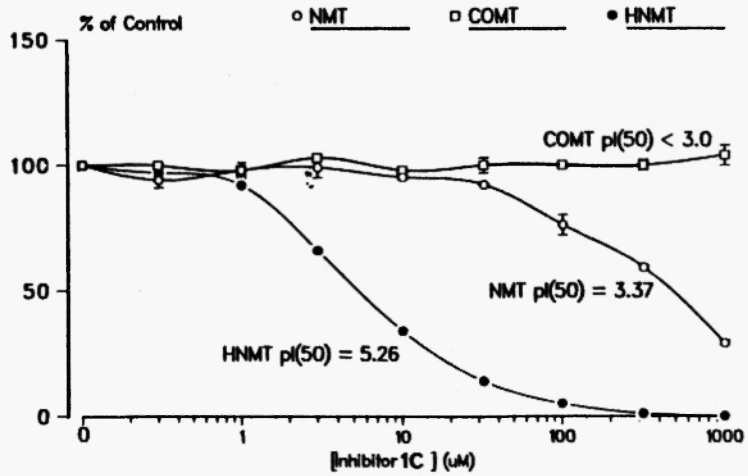
INHIBITION OF RABBIT ADRENAL NMT, RAT BRAIN COMT AND GUINEA PIG BRAIN HNMT BY INHIBITOR **2E**



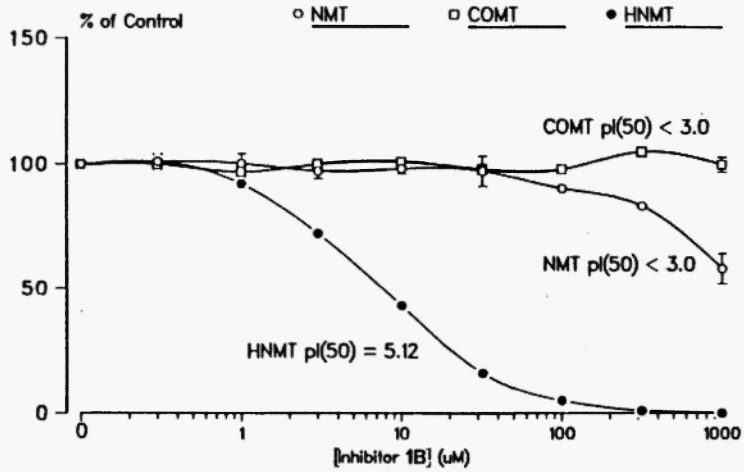
INHIBITION OF RABBIT ADRENAL NMT, RAT BRAIN COMT AND GUINEA PIG BRAIN HNMT BY INHIBITOR 1A



INHIBITION OF RABBIT ADRENAL NMT, RAT BRAIN COMT AND GUINEA PIG BRAIN HNMT BY INHIBITOR 1C



INHIBITION OF RABBIT ADRENAL NMT, RAT BRAIN COMT AND GUINEA PIG BRAIN HNMT BY INHIBITOR **1B**



INHIBITION OF RABBIT ADRENAL NMT, RAT BRAIN COMT AND GUINEA PIG BRAIN HNMT BY INHIBITOR **2A**

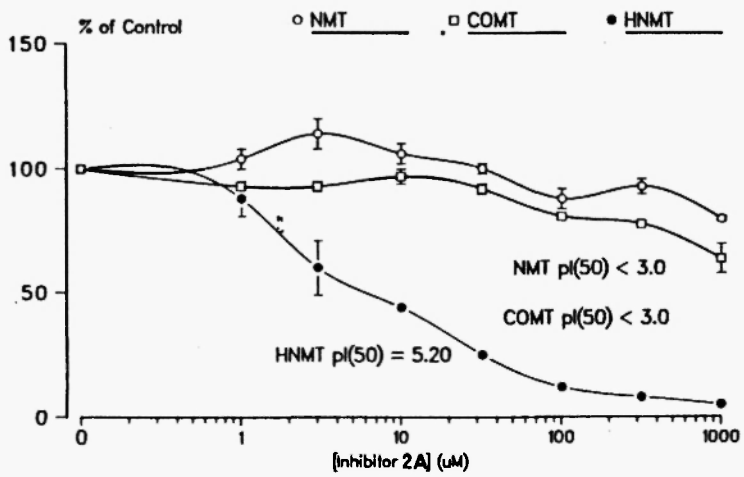


TABLE 1

pI_{50} values for adenosyl derivatives *1a-1c* and *2a-2e* against four methyltransferases

Compound	HNMT	COMT	Enzyme Inhibition (pI_{50})	PNMT	INMT*
1a	4.77	<3.0		<3.0	ND**
1b	5.12	<3.0		<3.0	4.42
1c	5.26	<3.0		<3.7	3.90
2a	5.20	<3.0		<3.0	ND
2b	5.10	<3.0		<3.0	ND
2c	4.59	<3.0		<3.0	ND
2d	4.50	<3.0		<3.26	<3.0
2e	5.00	<3.0		<3.62	<3.0

* Data from reference 6.
** ND = not determined.

2μL, 43μM) and inhibitor solution (3μL); incubations were carried out in duplicate at 37°C for 30 min. The reaction was terminated by the addition of sodium hydroxide (5μL, 1M), and the ^{14}C-labelled methylated product was then extracted into chloroform (3x15μL). The measurement of the radioactivity and quantitation of the assay were identical to those described previously for the inhibition of rabbit PNMT, except that the chloroform layer was mixed with the liquid scintilation cocktail and the radioactivity was then determined.

Rat Brain Catechol O-Methyltransferase (COMT). This assay was carried out utilizing the procedure of Axelrod and Tomchick /14/, which is essentially similar to the methodology described for rabbit adrenal PNMT, with the following exceptions, a) the product of the reaction was ^{14}C-labelled O-methylnorepinephrine, b) the *L*-norepinephrine concentration was 50μM.

Kinetic Analysis. The analysis of inhibition of guinea pig brain HNMT with compounds *1c* and *2a* was carried out varying the concentration of both SAM (at 43μM histamine) and histamine (at 20μM SAM) in

separate experiments. Individual values of velocity are means of triplicates. The values of Km and Vmax were determined by linear regression analysis and Ki values were determined by the formula:

$$Ki = [I] / [Km(I)/Km(no\ I))-1]$$

Since both *1c* and *2a* showed competitive inhibition toward both substrates the Vmax values were approximately equal (within experimental variations) thus, the above formula for Ki is shortened from:

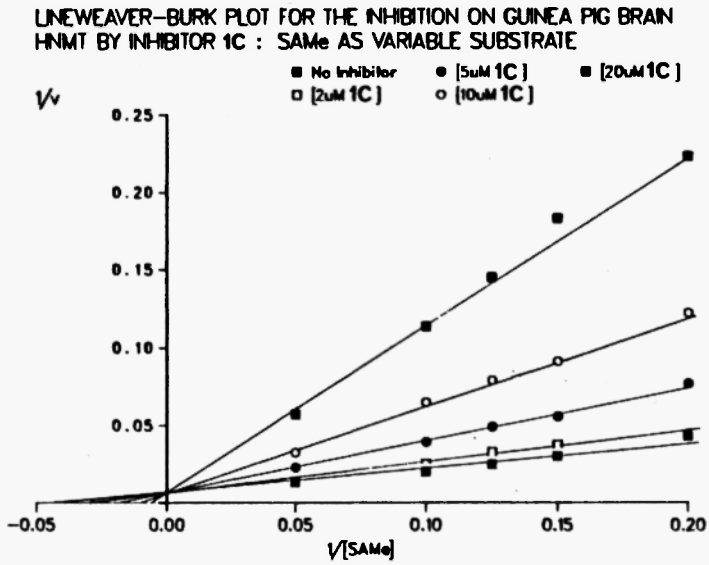
$$Ki = [I] / [(Km(I)/Vmax(I)/Km(No\ I)/Vmax(No\ I)-1)] .$$

Lineweaver Burk and Dixon plots were constructed for the above four sets of data and are illustrated in Figures 7 and 8.

III. RESULTS AND DISCUSSION

The preparation of the indolyl derivatives *1b* and *1c*, and *2d* and *2c* has been described previously /6/ and is summarized in Figure 3. The thioethers *2d* and *2e* were obtained *via* the coupling of 3- (3-indolyl)-prop-1-yl) thiol and 4- (3-indolyl) -but-1-yl) thiol, respectively, with 5'-chloro-5'-deoxyadenosine in aqueous alkaline solution under a nitrogen atmosphere. The resulting thioethers could each be converted efficiently into the sulphonium salts *1b* and *1c* by reaction with dimethyl sulphate in formic acid. The syntheses of compounds *2c* and *1a* were patterned on similar chemistry (see Figure 4). Synthesis of 4-bromo-1-phenylbutan-1-ol was achieved *via* the sodium borohydride reduction of 4-bromo-1-phenylbutan-1-one. Reaction of 4-bromo-1-phenylbutan-1-ol with potassium thioacetate afforded S- acetyl- 4- hydroxy- 4- phenylbutan-1-thiol, which could then be coupled with 5'-chloro-5'-deoxyadenosine in alkaline aqueous dimethyl sulphoxide and under nitrogen to give *2c*. S-Methylation with iodomethane in water afforded the sulphonium salt, *1a*.

The compound *2a* was prepared by reaction of 5'-0-tosyl-2', 3'-0-isopropylidene adenosine with 1-amino-4-phenylbutane followed by deprotection of the resulting product with 80% aqueous formic acid. Compound *2b* was synthesized by initial formation of 5'- methylamino-5'-deoxy-2', 3'-0-isopropylidene adenosine *via* the reaction of methyl-



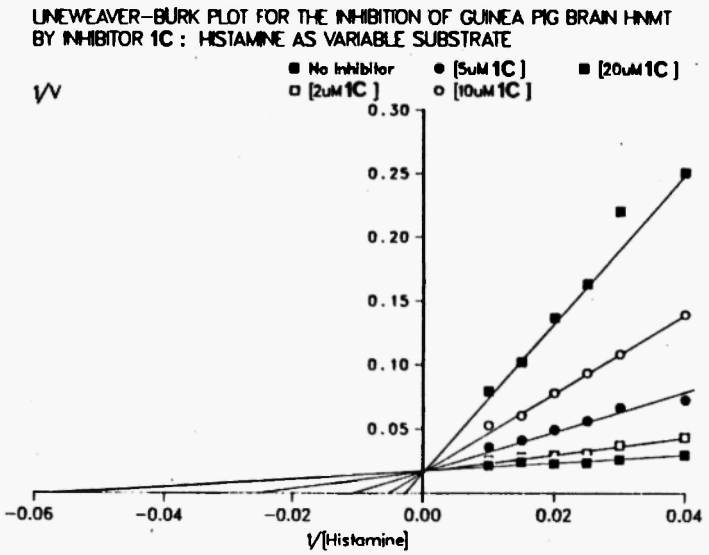
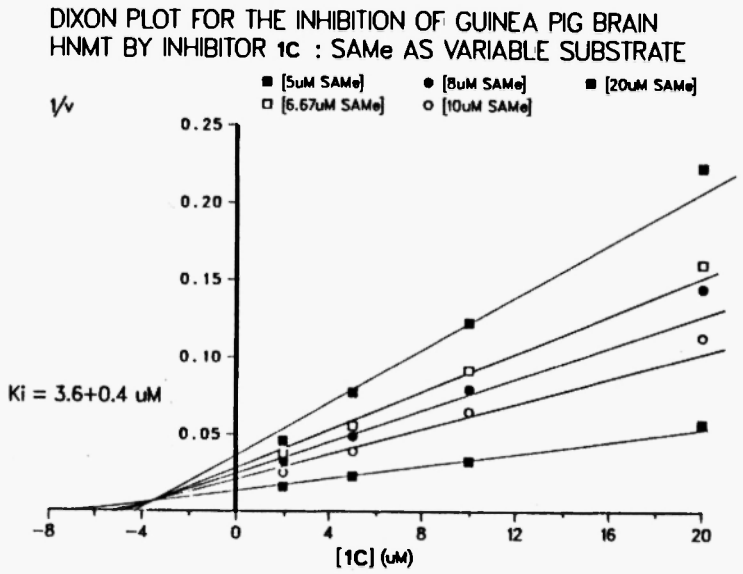
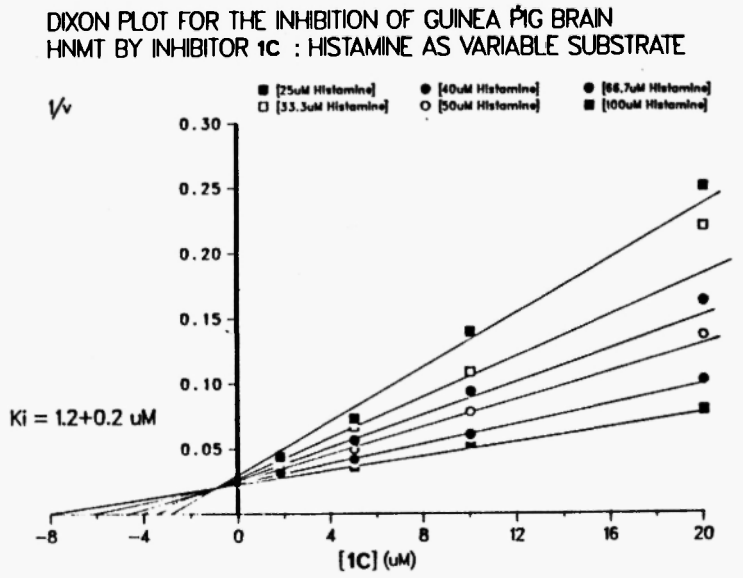
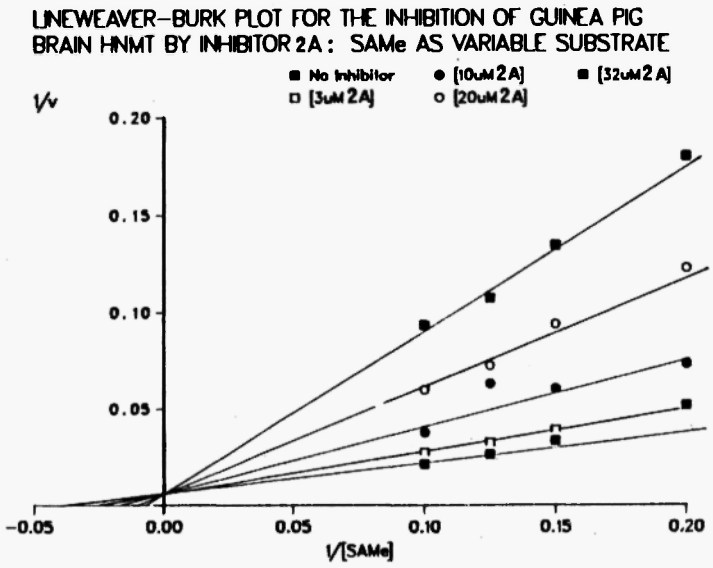


Fig. 7: Lineweaver-Burk and Dixon plots for compound *1c* against guinea pig brain histamine N-methyltransferase.







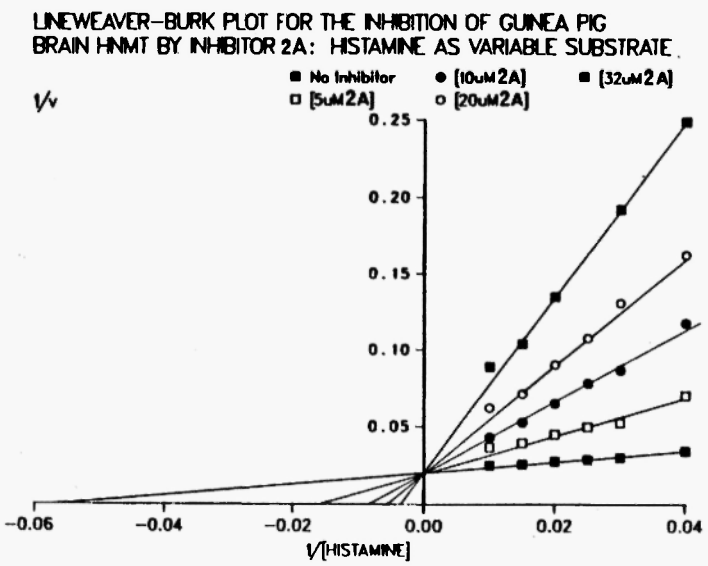
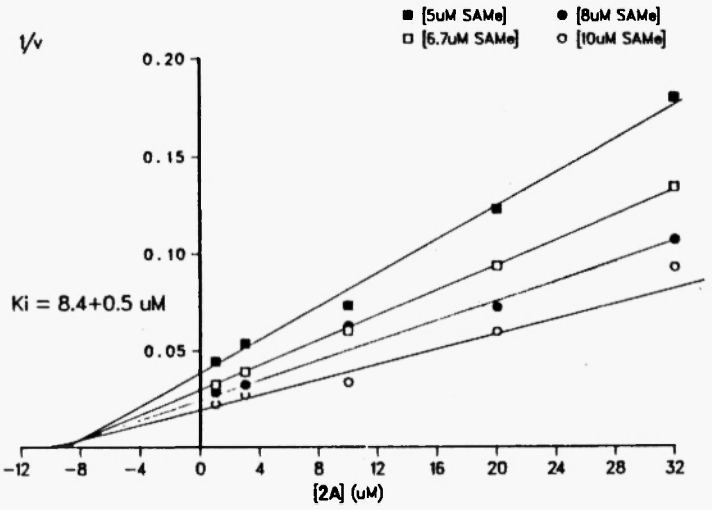
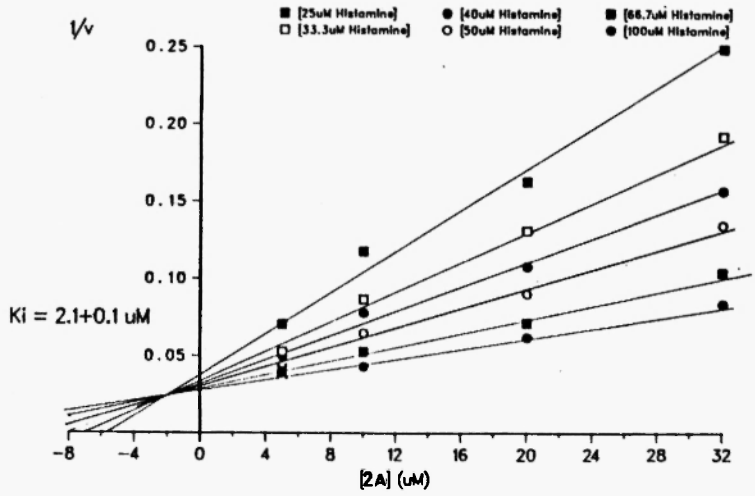


Fig. 8: Lineweaver-Burk and Dixon plots for compound *2a* against guinea pig brain histamine N-methyltransferase.

DIXON PLOT FOR THE INHIBITION OF GUINEA PIG BRAIN HNMT BY INHIBITOR **2A**: SAMe AS VARIABLE SUBSTRATE



DIXON PLOT FOR THE INHIBITION OF GUINEA PIG BRAIN HNMT BY INHIBITOR **2A**: HISTAMINE AS VARIABLE SUBSTRATE



amine with 5′-0-tosyl-2′, 3′-0-isopropylidene adenosine. The product was then reacted with 1-chloro-4-phenylbutane and the resulting N-phenylbutyl derivative deprotected with 80% aqueous formic acid to give *2b* (Figure 5).

The pI_{50} values for the eight compounds tested are shown in Table 1. Comparative dose-response curves for the compounds against HNMT, PNMT and COMT are also illustrated in Figure 6. The data show that all the adenosine derivatives exhibited inhibitory properties against guinea pig brain HNMT. Compounds *1b* and *1c* have been previously reported as multisubstrate analogue inhibitors of rabbit lung PNMT, exhibiting pI_{50} values of 4.42 and 3.90 respectively /6/; however, both compounds were considerably more effective as inhibitors towards HNMT.

Kinetic analysis data were obtained for compounds *1c* and *2a*. Both these compounds were shown to be competitive inhibitors of HNMT with respect to both histamine and SAM. The Ki values are shown in Table 2, and Figures 7 and 8 illustrate the kinetic data from both Dixon and Lineweaver-Burk plots. As can be seen, both compounds are very potent and selective inhibitors of guinea pig brain HNMT. Compound *1c* ($Ki^{histamine}$ = 1.2μM) is one of the most potent HNMT inhibitors of its class.

TABLE 2

Ki values for compounds *1c* and *2a* against guinea pig brain histamine N-methyltransferase

	Inhibition of HNMT	
Compound	Ki^{SAM}(μM)	Ki^{Hist}(μM)
1c	3.6 ± 0.4	1.2 ± 0.2
2a	8.4 ± 0.5	2.1 ± 0.1
L-SAH		18.5*
D-SAH		10.5*

* Data taken from reference 5.

IV.CONCLUSIONS

This study has shown that a series of 5'-substituted adenosine derivatives bearing relatively lipophilic 5'-substituents possess potent and specific inhibitory activity against guinea pig brain HNMT. Hopefully, these compounds may find utility in studying the role of histamine in brain function. In this respect, it is interesting to note that sensitivity to seizures has recently been correlated inversely to brain histamine concentrations /15/. It remains to be seen whether the above compounds will be of use as anticonvulsants.

V. REFERENCES

1. Schayer, R.W. and Reilly, M.A. Histamine catabolism in guinea pigs, rats and mice. *Eur. J. Pharmacol.*, 1974; **25**:101-107.
2. Beaven, M.A. Factors regulating availability of histamine at tissue receptors In: Ganellin, C.R. and Parsons, M.E., eds., Pharmacology of histamine receptors. Boston: Wright PSG, 1982; 103-145.
3. Crooks, P.A. Interactions of sulphur xenobiotics with S-adenosyl-L-methionine-dependent methyltransferase reactions. In: Damani, L.A. ed. Sulphur drugs and related organic chemicals - chemistry, biochemistry and toxicology. Chichester: Ellis Horwood Ltd., 1989; vol. 2: in press.
4. Borchardt, R.T., Wu, Y.S., Huber, J.A. and Wycpalek, A.F. Potential inhibitors of S-adenosyl-L-methionine-dependent methyltransferases. 6 Structural modifications of S-adenosylmethionine. *J. Med. Chem.*, 1976; **19**:1104-1110.
5. Borchardt, R.T., Huber J.A. and Wu, Y.S. Potential inhibitors of S-adenosyl-L-methionine-dependent methyltransferases. 4. Further modifications of the amino acid and base portions of S-adenosyl-L-homocysteine. *J. Med. Chem.* 1976; **19**:1094-1099.
6. Benghiat, E. and Crooks, P.A. Multisubstrate adducts as potential inhibitors of S-adenosylmethionine-dependent methylases: inhibition of indole N-methyltransferase by (5'-deoxyadenosyl)[3-(3-indolyl)prop-a-yl] methylsulfonium and 5'-deoxyadenosyl) [4-(3-indolyl)but-l-yl] methylsulfonium salts. *J. Med. Chem.*, 1983; **26**:1470-1477.
7. Ahlbrecht, H., Raab, W. and Vonderheid, C. Dehydrocyanation of α-aminonitriles; a versatile and convenient enamine and dieneamine synthesis. *Synthesis*, 1979; 127-129.
8. Crooks, P.A., Dreyer, R.N. and Coward, J.K. Metabolism of S-adenosylhomocyteine and S-tubercidinylhomocysteine in neuroblastoma cells *Biochemistry*, 1979; **18**:2601-2609.

9. Kuhn, R. and Jahn, W. Thioethers and sulfoxides derived from adenosine. *Chem. Berichte*, 1965; **98**:1699-1704.
10. Hampton, A. Nucleotides. II. A new procedure for the conversion of ribonucleosides to 2', 3'-0-isopropylidene derivatives. *J. Amer. Chem. Soc*, 1961; **83**:3640-3645.
11. Clark, V.M., Todd, A.R. and Zussman, J. Nucleosides. Part VIII. cyclo nucleoside salts. A novel rearrangement of some toluene-p-sulphonyl-nucleosides. *J. Chem. Soc.*, 1951; 2952-2957.
12. Fuller, R.W. and Hunt, J.M. A method for the assay of phenylethanolamine N-methyltransferase. *Analyt. Biochem.*, 1966; **16**:349-354.
13. Brown, D.D., Tomchick, R. and Axelrod, J. The distribution and properties of a histamine-methylating enzyme. *J. Biol. Chem.*, 1959; **234**:2948-2950.
14. Axelrod, J. and Tomchick, R. Enzymatic O-methylation of epinephrine and other catechols. *J. Biol. Chem.*, 1958; **233**:702-705.
15. Tuomisto, L. and Tacke, U. Is histamine an anti-convulsive inhibitory transmitter? *Neuropharmacology*, 1986; **25**:955-958.