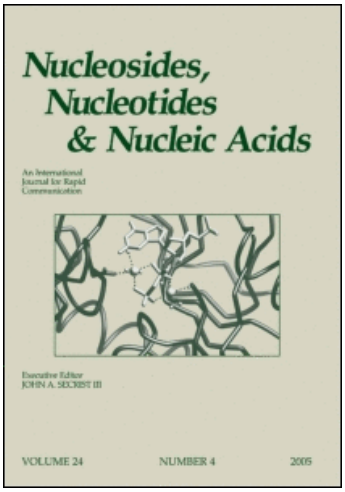

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Synthesis and Biological Activities of Some New 5'-Substituted S-Adenosylhomocysteine Analogues*

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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF SOME NEW 5'-SUBSTITUTED S-ADENOSYLHOMOCYSTEINE ANALOGUES*

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Abstract: The synthesis of new S-adenosyl-homocysteine (SAH) analogues substituted at the 5'-position is described. Their activity on cell transformation induced by Rous sarcoma virus (RSV) as well as their effect on cell growth and on replication of polyoma virus were also studied. The best results were obtained with compounds having a phenyl group at the 5'-position of adenosine. These molecules inhibit very strongly uridine uptake by cells.

In previous work we have reported some interesting biological activities of synthetic analogues of S-adenosyl-L-homocysteine (SAH) in cell culture. The analogues have been screened for their ability to interfere with oncogenic transformation of chick embryo fibroblasts (CEF) by Rous sarcoma virus (RSV)[1-3] and for their capacity to inhibit various S-adenosyl-L-methionine (SAM) mediated methyl transferases,[4-5] SAH being the natural inhibitor of these enzymes.

In order to study further the relationship between the chemical structure of the analogues and their ability

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** Dedicated to the memory of F. Sorm.

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to inhibit cell transformation and virus replication, a new series of compounds was synthesized with various modifications at the 5'-side chain.

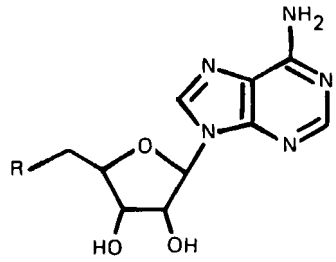
Our results show that analogues with S-phenyl substitution at the 5' position of adenosine strongly inhibit RSV induced cell transformation as well as polyoma virus (PyV) replication in mouse cells.

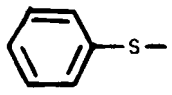
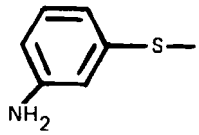
RESULTS AND DISCUSSION

As shown in Table 1 compounds having the S-phenyl group at the 5' position : 1,2,3 are highly potent inhibitors of

TABLE 1

EFFECT OF SAH ANALOGUES ON RSV INDUCED CELL TRANSFORMATION AND ON POLYOMA VIRUS REPLICATION

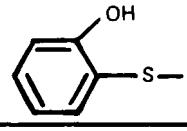
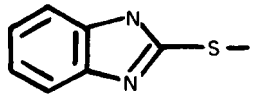
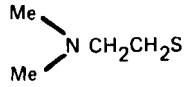
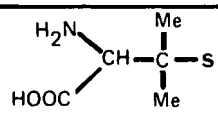
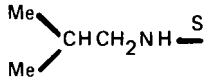
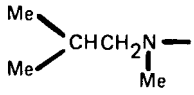
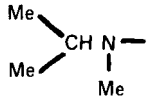


	R	CONCENTRATION μM	% INHIBITION CELL TRANSFORMATION	% INHIBITION POLYOMA REPLICATION
1		100 500 1000	 80 99	94 98 100
2		250 500	0 77	0 87
3		250 500	34 92	0 87

both cell transformation induced by RSV and of polyoma virus replication. The analogue 4 with an 5'-S-benzimidazole group is inactive . The other compounds with aliphatic side chains (6 to 10) showed no significant activity against the two viruses.

Table 2 shows that when cells are treated with 500μM of one of the compounds described above for 24 hours, the cell growth was not significantly affected. Thus the observed inhibition of cell transformation was not due to cytotoxicity.

TABLE 1 (continued)

4		500 1000	35 90	–
5		500	0	–
6		500 1000	0 51	50
7		1000	0	50
8		100 500	50 • TOXIC	–
9		100 500	30 60	–
10		500	24••	–

• SEE REF. 2

•• SEE REF. 3

Table 2

Effect of SAH Analogues on Cell Growth and Incorporation of Macromolecular Precursors into CEF.

Compound 500µM 24 hour	Cell growth % of control	Uptake % of control			Uptake % of control		
		leu	U	T	leu	U	T
2	72	114	6	63	82	12	15
3	76	143	10	76	66	16	32
4	93	204	50	140	94	113	65
6	96	146	85	126	89	102	77
7	118	78	143	108	106	150	70
8	86	211	81	121	122	71	192
9	89	150	87	70	94	101	73
10	84	85	126	347	80	259	389

CEF were cultivated for 24 hours with 500µM of the various compounds and then labelled for 60 min at 37° with 5µCi leucine, uridine or thymidine. The medium was discarded and cells washed twice with ice cold buffer. Uptake was measured in cold TCA soluble fraction and incorporation in 0.5N NaOH hydrolysate . Cell growth was estimated as percentage of protein content with respect to untreated cells. All measurements were done in duplicate. Uptake and incorporation were calculated in cpm/mg protein and results are expressed as percentages with respect of control cells . 100% uptake were respectively , for leu, U and T : 27820 , 1468987 and 94580 cpm/mg protein , 100 % incorporation were respectively 74690 , 291510 and 322000 cpm/mg protein for the same precursors.

Uptake and incorporation of macromolecular precursors were then examined in cells treated for 24 hours with 500µM of the various analogues and the results compared to that of untreated cells. The uptake of leucine was not significantly inhibited by any of the molecules ,and in most cases it was even stimulated. A slight inhibition of leucine incorporation into protein was observed in cells treated with 2 and 3 (18% and 34%). The most strik-

ing effect was observed on Uridine uptake which was inhibited by 90-94% in cells treated with 2 and 3 and to a lesser degree in those treated with 4 . It seems that nevertheless the incorporation of Uridine into RNAs was not affected , since the percentage of radioactivity in the TCA insoluble fraction was higher than that in the TCA soluble pool. Thus, the apparent inhibition of RNA synthesis is only the consequence of inhibition of the uptake.

Results obtained with thymidine were much less clear cut. Thymidine uptake and incorporation were inhibited by the two molecules (2 and 3) with a phenyl group at position 5', while 4 having a pyridinyl group stimulated the uptake and inhibited the DNA synthesis. With molecules having an aliphatic side chain at the 5' position of adenosine different patterns were observed . Stimulation of uptake without effect on synthesis : 8 and 10 , stimulation of uptake and inhibition of synthesis : 6 , or no effect on uptake and inhibition of synthesis : 7 and inhibition of uptake and no effect on synthesis : 9 .

EXPERIMENTAL

Melting points were determined with a Reichert melting point apparatus and are uncorrected . Pmr spectra were obtained on a Varian T60 or a Varian EM 360 instrument using tetramethylsilane as internal standard. Mass spectra were measured on a MS-50 AEI spectrometer (electron impact) ; all the compounds showed the classical fragmentation of adenosyl moiety [6]. Thin layer chromatography was performed on Schleicher and Schüll F 1500 LS 254 silica gel plates. Short column chromatography was carried out on Merck silicagel, finer than 230 mesh . All compounds gave satisfactory elemental analyses.

5'-Deoxy-5'S-(phenyl)-5'-thioadenosine (1)

A mixture of 5'-O-tosyl-2',3'-O-isopropylidene adenosine (4.6 g, 10 mmole), benzenethiol (10 ml, 12 mmole) and sodium hydride (1.0 g) in dimethylformamide (20 ml)

was stirred for 3 days at 20°C. After neutralization with dilute acetic acid, the solvents were removed under reduced pressure , the residue dissolved in dichloromethane and purified by short column chromatography . Elution was performed with hexane, followed by dichloromethane. Fractions containing homogeneous products were combined, evaporated and the resulting solid crystallized from methanol to give 1.4 g (35%) of colourless crystals . Nmr (d6-DMSO , δ : 1.4 and 1.6 (6H,2s, CH_3), 3.2 (2H,d,H5') 4.4 (1H,m,H4') , 5.0(1H, dd, H3'), 5.45(1H,dd,H2'),5.95 (1H,dH1'), 6.15 (2H,s,NH_2) ,7.15 (5H,m,C_6H_5), 7.75 (1H,s, H_2), 8.2 (1H,s,H_8).

The 2',3'-isopropylidene group was removed by dissolving the crystals in 80% formic acid, the resulting solution being left for 20 hours at 20° C. The solvents were removed by evaporation leaving a white powder in quantitative yield.M.pt 139°C (lit [7] 137-8°C) R_f: 0.36 (dichloromethane : methanol 4:1) ; MS m/e=359 (M^{+}) , m/e = 282 (M-77), m/e=250 (M-109); NMR (d6-DMSO, δ TMS=0) δ : 3.30(2H,d,H_5,), 4.10 (2H,m,H_4,,H_3,), 4.70 (1H,dd,H_4,), 5.8 (1H,d,H_1'), 7.10 (7H,m,6-NH_2, aromatic), 7.95 (1H,s,H_2) 8.10 (1H,s,H_8)

5'-Deoxy-5'S-(4-aminophenyl)-5'-thioadenosine (2)

A mixture of 4-aminothiophenol (2.50g, 20mmoles) and sodium hydride (0.3g, 12.5 mmoles) in dimethylformamide (20ml) was purged with nitrogen. 5'-Chloro-5'-deoxyadenosine (85g, 10 mmole) was added, the mixture again purged with nitrogen, then agitated with a magnetic stirrer for 3 hours. Thin-layer chromatography (ethyl acetate/methanol 8/2) showed the reaction to be complete.

HCl solution was added to neutralise the solution which was subsequently evaporated to dryness at 30°C. Water and ethyl acetate were added to the residue, a voluminous white precipitate immediately forming . The product was filtered , washed with ethyl acetate and water, and

recrystallized from water which had been degassed and saturated with nitrogen. Yield 3.02g (80.7%) M.p 122°C Rf : 0.21 (dichloromethane:methanol 4:1);
NMR (DMSO-d6, δTMS=0) 3.0 (2H, d, H_5'), 4.1 (2H, m, H_4', H_3') 4.7 (1H, dd, H_2'), 5.1 (2H br.s ϕ-NH_2), 5.3 (4H, dd, OH_2', OH_3') 5.8 (1H, d, H_1'), 6.9 (4H, dd, phenyl), 7.1 (2H, 5,6-NH_2), 7.95 (1H, s, H_2), 8.15 (H, s, H_8) MS m/e= 374 (molecular ion), m/e=280 (M^+-94).

5'-Deoxy-5'S-(3-aminophenyl)-5'-thioadenosine (3)

Prepared exactly as 2 ; yield 3.15g (84.2%) M.p. 106°C Rf: 0.22 (dichloromethane:methanol 4:1) NMR (DMSO-D_6, δ TMS=0) : exactly as 2 except for the aromatic signals MS m/e=374 (M^+), m/e=248 (M-126).

5'-Deoxy-5'S-(3-hydroxy-2-pyridinyl)-5'-thioadenosine (4)

5'-Chloro-5'-deoxyadenosine (1.0g, 3.50 mmoles) and 3-hydroxy-2-mercaptopyridine (1.0g, 7.87 mmoles) were dissolved in dimethylformamide (10ml) and sodium hydride (100mg) added. After stirring the mixture for 24 hours, dilute acetic acid was added to neutralise and the solvents were evaporated in vacuum. The residue was partitioned between water and chloroform and the aqueous phase was evaporated, the crude product dissolved in a little methanol and purified by short column chromatography on silicagel, the product was eluted with dichloromethane:methanol(4:1). Recrystallisation from propanol-2 gave 860mg (65%) of pale yellow crystals M.pt 119°C. R_F: 0.25 (dichloromethane:methanol 4:1) MS m/e = 375 (molecular ion), m/e = 250 (M^+ -125); NMR (DMSO-d6, δTMSO=0) δ: 3.45 (2H, d, H_5'), 4.10 (2H, m H_4', H_3'), 4.75 (3H, m, H_2', OH_2', OH_3'), 5.85 (1H, d, H_1'), 7.0 (3H, m, aromatic), 7.15 (2H, s, 6-NH_2), 8.10 (1H, s, H_2), 8.30 (1H, s, H_8).

5'-Deoxy-5'S-(2-benzimidazolyl)-5'-thioadenosine (5)

A mixture of 2-mercaptobenzimidazole (300mg ; 2 mmoles), 5'-chloro-5'-deoxyadenosine (285mg : 1 mmole) and sodium

hydride (30 mg) in DMF (2 ml) was stirred under nitrogen for two days at room temperature. Thin-layer chromatography indicated approximatively 50% reaction. The mixture was neutralized with dilute acetic acid and the solvents evaporated under reduced pressure. The residue was partitioned between dichloromethane and water and the organic phase was evaporated to a small volume before placing it on a short column of compacted silicagel. Elution with ethyl acetate : methanol 95:5 gave a mixture of product and starting materials . Some product (50mg) was purified by high-performance liquid chromatography on a column of LiChrosorb Si 60-10 (250 x 9 mm) with dichloromethane : methanol 92:8 using a flow rate of 10 ml min^{-1}. Retention time as detected at 260 nm. was 8.8 min. The elution pattern confirmed approximately 50% reaction. Evaporation of the solvents gave a powder. M. pt 145-6°C . R_F: 0.22 (dichloromethane: methanol 4:1) MS m/e=398 (molecular ion), m/e=304 M-94), m/e=273 (M-125) ; NMR (DMSO-d6 , : 3.30 (2H,d,H_5'), 4.15 (2H,m;H_4',H_3'), 4.75 (1H,dd,H_2'), 5.35 (2H,dd,OH_2"OH_3'), 5.8 (1H,d,H_1'), 7.1 (2H,s,6-NH_2), 7.1 (4H,m,aromatic), 8.0 (1H,s,H_2), 8.15 (1H,s,H_8)

5'-Deoxy-5'S-(N,N-dimethylaminoethyl)-5'-thioadenosine (6)

2'3'-Isopropylidene-5'-tosyladenosine (1.0g; 2.17 mmoles) was added to 2-dimethylaminoethanethiol hydro-chloride (1,0g; 7 mmoles) and sodium hydride (0.30g) in dimethylformamide (10ml) . According to tlc reaction was complete within 60 min. The mixture was neutralized with solid CO_2, filtered and the filtrate evaporated to an oil. The oil was dissoved in absolute ethanol and the resulting solution applied to a short column (4 x 1.5cm) of Kieselgel 60. The product was eluted with chloroform saturated with ammonia . The solvent was removed under reduced pressure and the resulting oil evaporated several times with absolute ethanol . The product (65 mg, 1.65 mmole, 76%) was deprotected by treatment for 24 hours with 80 % formic acid . After evaporation of the formic acid the residue

was dissolved in water and passed through a column (4x1 cm) of Amberlite IRA-400 (OH^-) . The column was washed with water to neutrality and the eluent and washing evaporated to dryness. The product was recrystallized from acetone. Two crops were obtained giving 380mg (49%) of white crystals. Mp 157-8°C . R_F : 0.23 (chloroform : methanol 9 : 1 saturated with ammmonia); MS m/e=355 (M^+), m/e=283 (M-72), m/e = 250 (M-104), NMR (DMSO-d6, δ TMS=0) δ : 2.65 (6H,s,$(CH_3)_2$ N), 2.7-3 (4H,m,CH_2CH_2), 2.90 (2H,d,H_5'), 4.20 (2H,m,H_4',H_3') 4.80 (1H,dd,H_2'), 5.10(2H,m,OH_2', OH_3'), 5.95 (1H,d,H_1') , 7.25 (2H,s,6-NH_2), 8.20(1H,s,H_2) , 8.35(1H,s,H_8).

5'-Deoxy-5'S-(DL-penicillamino)-5'-thioadenosine (7)

A mixture of 5'-deoxy-5'-chloroadenosine (285mg ; 1mmole), DL-penicillamine (149mg ; 1mmole) and sodium hydride 30mg in DMF (5ml) was stirred for 3 days at room temperature .A precipitate which contained all the nucleosidic material was formed . This was filtered, dissolved in methanol : acetonitrile (3 : 1) and purified by HPLC on a column of 190g silicagel, the same solvent mixture being used for the elution . The fractions containing the major product were combined and evaporated to give a pale yellow powder (255mg 64%). M.pt 180°C (dec.) R_F : 0.38 (ethanol : water 4 : 1) MS m/e=399 (M^+) ,m/e= 282 (M-117), m/e=250 (M-149), NMR (DMSO-d6 , δ TMS=0) δ : 1,20, 1.45 (6H, two s, CH_3), 2.10 (1H,s,CH), 2.80 (2H,d,H_5'), 4.10 (2H,m,H_4',H_3'), 4.70 (1H,dd,H_2'), 5.90 (1H,d,H_1'), 7.25 (2H,s,6-NH_2), 8.15(1H,s,H_2), 8.35(1H,s,H_8).

BIOLOGICAL ASSAYS

Cell transformation: Secondary CEF were plated as described earlier , [8] and infected one day later with 100 focus forming units of RSV. Cultures were then overlaid with 0.8% Difco agar. Various concentrations of compounds in 1.5 ml were added on top of the gelled underlayer , immediately after virus adsorption . Control cultures were overlaid with compound free medium . After two days of ex-

posure, the liquid overlayer was replaced by the standard growth medium. Foci of transformed cells were counted 5 days later and their number compared with the number of foci from the control cultures.

Polyoma virus replication : The concentration of the virus particles in control and in treated cells was determined by the hemagglutination method[9] using guinea pig erythrocytes.

Macromolecular synthesis : Following a 24 hours treatment in the presence of the desired concentration of the various compounds , [4,5-^{3}H] -leucine (452mCi/mM), [5-^{3}H] uridine (121 mCi/mM) and [methyl-^{3}H] thymidine were respectively added to the media (5 µCi/ml). The labelled cultures were further incubated at 37° for one hour. After washing the cells twice with ice cold buffer, uptake and incorporation into macromolecules were measured in cold trichloroacetic acid (TCA) 5% and in hot (80°) 0.5N NaOH hydrolysate respectively [10]. The radioactivity was counted in 0.1ml Bray solution[11] with an intertechnic SL 30 scintillation counter. All assays were carried out in duplicate. Protein concentration in cells was determined by the method of Lowry et al[12] with bovine serum albumin as standard.

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