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Lipid methyl transferase inhibitory activity of novel α-amino alkylic acid derivatives

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Summary

S-Adenosylhomocysteine (SAH) is a natural inhibitor of *S*-adenosylmethionine (SAM) methylation in biological processes. Analogues were synthesised, where the nucleoside moiety was replaced with a long alkyl chain substituent. Lipid methylation inhibitory activities of SAM/SAH analogues **1–8** were determined on microsomes. Compound **4**, with the highest inhibitory activity, showed 40% inhibition at a concentration of 10^{-7} M, while SAH required 3 orders of magnitude higher concentration for the same effect.

S-Adenosylmethionine (SAM) is the major methyl donor in biological processes. It is involved in lipid, steroid, protein, nucleic acid and catecholamine methylations. Phospholipid methylation in particular has been implicated in a large number of receptor mediated biological processes, induction of cAMP, Ca^{2+} flux and release of mediators (Hirata et al., 1981). There is evidence that simple ligands (dopamine, GABA, bradykinin, zymosan, concanavalin A, isoproterenol, etc.), steroids, peptide hormones and growth factors affect these processes in many cell lines and such modifications correlated well with changes in phospholipid methylation. Vance (1990) has recently shown that newly synthesised phosphatidylethanolamine (PE) is quickly converted to phosphatidylcholine (PC) in hepatocytes by SAM-dependent methylation. It was reported that PE molecules in the presence of unsaturated fatty acid moieties are preferentially methylated (Tacconi and Wurtman, 1985; Lakher and Wurtman, 1987). The turnover of methylated PE is considerably faster than that of bulk PE. Methylation may therefore generate a specific PC pool for the release of arachidonate, diglycerides and choline in cells and hence is important for cell regulation. *S*-Adenosylhomocysteine (SAH) is an endogenous inhibitor of biological methylation. Other known inhibitors include 3-deazaadenosine-(C^3-Ado), 3′-deazaaristeromycin (C^3-Ari) and 5-deoxy-5′-isobutylthio-3-deazaadenosine

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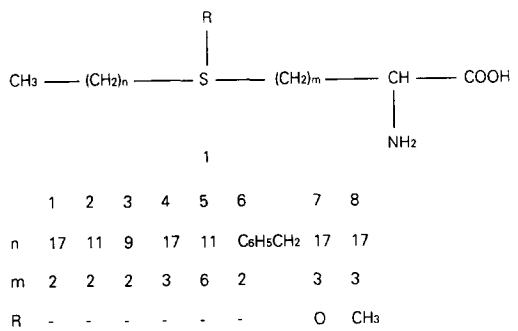

Scheme 1. General structure of SAM/SAH analogues **1–8**.

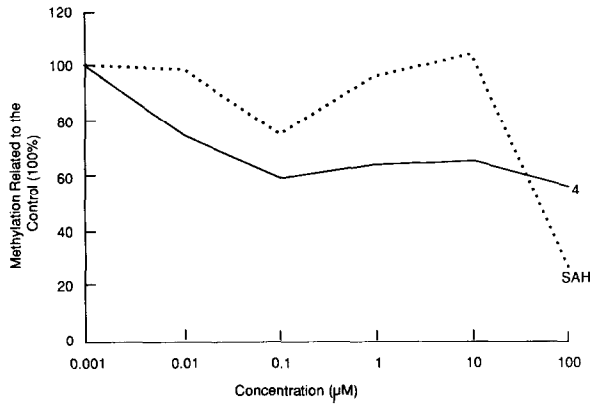

Fig. 1. Effects of heteroalkyl amino acids and SAH on lipid methylation.

(C^3-Siba). C^3-Ado and C^3-Ari inhibit SAH-hydrolase and C^3-Ado has been shown to inhibit SAH-decarboxylase and cAMP-phosphodiesterase (Aksamit et al., 1981; Garcia-Castro et al., 1983). C^3-Siba, an analogue of SAH, inhibits thymidine incorporation into mouse lymphocytes in response to concanavalin A (Hirata et al., 1979), and is also a potent inhibitor of the de novo 1,2-diacylglycerol phosphocholine transferase (CDP-choline) pathway for PC biosynthesis in rat basophil leucocytes (RBL) cells (Morita et al., 1982) and neuroblastoma cells (De Blas et al., 1984). Because these inhibitors are structurally similar to SAM, SAH and purines in general, their effects in cells are wide-spread and difficult to interpret.

To develop more specific inhibitors of membrane-bound lipid methylases, SAH and SAM analogues were synthesised with a modified nucleoside moiety (Toth et al., 1992) (see Scheme 1). Here we report the biological activity of the compounds, where the sugar-base part of SAH/SAM was replaced by a long alkyl chain.

To determine the inhibition of lipid methylation, compounds **1–8** were incubated with microsomes in the presence of radiolabelled SAM ([*methyl*-^{3}H]SAM). The concentration of the compounds varied between 10^{-9} and 10^{-4} M and the extents of inhibition were compared with that of SAH (Table 1). Compound **4** showed the strongest inhibition at 10^{-7} M (40%) which was 3 orders of magnitude higher than the natural compound SAH (Fig. 1) and compound **1** displayed weaker inhibition (25%), whereas the remaining compounds (**2, 3, 5, 6,** sulphoxide **7** and sulphonium salt **8**) exhibited no inhibition (Table 1 and Fig. 1). Although some of the compounds **2, 3,** and **5–8** were not found to inhibit SAM-dependent lipid methylation, they are analogues of the methionine and homocysteine moieties of SAM

TABLE 1

Effects of α-alkyl amino acid SAH / SAM congeners on lipid methylation (%)

Concentration (μM)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	SAH
0.001	100	100	100	100	100	100	100	100	100
0.01	79	104	100	75	101	99	104	109	99
0.1	75	109	104	59	101	105	95	112	75
1	77	114	109	64	99	103	100	107	96
10	77	123	110	65	107	102	109	120	103
100	63	126	109	55	143	107	117	122	25

All experiments were performed in triplicate. The average error was 5%.

and SAH. Experiments are underway to investigate their possible inhibitory effects on other enzymes involved in SAM metabolism.

Preparation of hepatic membranes: Wistar rats were intoxicated by CO_2 and their liver dissected immediately, and homogenized in 4 vols of 25 mM Tris (pH 7.4) containing 0.25 M sucrose and 0.1 mM phenylmethylsulphonyl fluoride (PMSF, buffer A). The homogenate was centrifuged twice (3000 rpm, $1000 \times g$) and the resultant supernatant spun at 15000 rpm ($12000 \times g$) to obtain pellets P2 and supernatant S2. The supernatant S2 containing microsomes and soluble proteins was spun at 39000 rpm ($100000 \times g$) for 1 h to obtain microsomal pellets which were resuspended in buffer A (4–5 mg protein/ml determined by the method of Bradford (1976)). Both supernatant S2 and microsomal pellets were used for investigating the effects of synthetic compounds on lipid methyl transferase (LMTase) activity.

Determination of lipid methyltransferase activity: S2 or microsomes were incubated with [*methyl*-^{3}H]SAM (0.5 μCi, 100 μM) in a final volume of 420 μl without (control) or with varying concentrations (0.001–100 μM) of synthetic compounds **1–8** and SAH at 30°C for 30 min. The compounds were dispersed in buffer A by sonication for 30 min prior to addition to the enzyme suspension. The enzyme reaction was terminated and lipids extracted by the addition of 2 ml $CHCl_3/CH_3OH$/2 M HCl (6:3:1) and 1 min vortexing. The lipid-rich chloroform lower phase was extracted and washed with 1 ml of 0.5 M KCl in 50% methanol. Aliquots of this extract (200 μl) were put into scintillation vials, dried under N_2. Aquaphase scintillant was added to each vial and the amount of activity incorporated into lipids determined by scintillation counting.

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