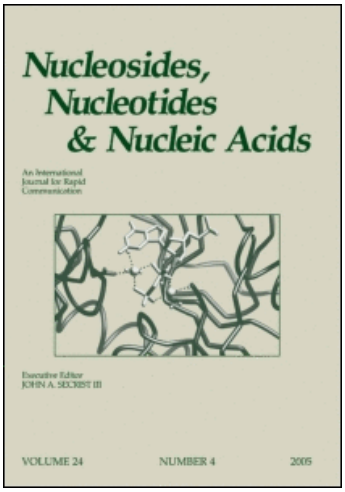

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SYNTHESIS AND BIOLOGICAL EFFECTS OF 5'-DEOXY-5'-(CYCLOPROPYLMETHYLTHIO)ADENOSINE

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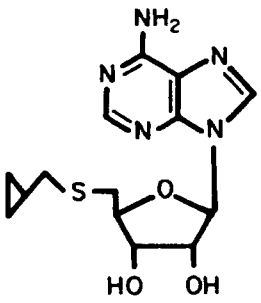
Abstract: A novel synthesis of the nucleoside analog, 5'-deoxy-5'-(cyclopropylmethylthio)adenosine (CPMTA, 1) has been developed. CPMTA is a closely related structural analog of 5'-deoxy-5'-(isobutylthio)-adenosine (SIBA, 2), which has been widely studied and shown to exert a multitude of biological effects. The in vitro and in vivo antitumor (L1210 leukemia) activity of CPMTA has been found to be comparable to that of SIBA, whereas its in vitro antiviral (HSV and VSV) activity is diminished. These agents are being developed as inhibitors of methylation and/or polyamine synthesis.

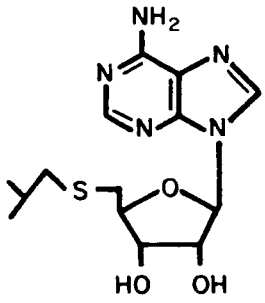
The development of compounds that interfere with the biosynthesis and/or metabolism of S-adenosylmethionine (AdoMet) as potential chemotherapeutic agents is an area of active investigation.[1,2] Biological methylation reactions and polyamine biosynthesis, both of which utilize AdoMet, are critically involved in cellular growth and function. Thus, chemotherapeutic strategies targeting AdoMet have been variously explored for their antitumor, antiviral, antibacterial and/or antiparasitic potential.[2] Among the most interesting and widely studied compounds to emerge in this area of pharmacological development is 5'-deoxy-5'-(isobutylthio)adenosine (SIBA),[3] a nucleoside analog which is structurally related to two biologically active AdoMet metabolites: S-adenosylhomocysteine (AdoHcy), a product of all AdoMet-

mediated methylation reactions, and 5'-deoxy-5'-(methylthio)adenosine (MTA), a product of spermidine (Spd) and spermine (Spm) biosynthesis and of ethylene biosynthesis in plants. SIBA has been found to have potent effects on a number of AdoMet metabolic enzymes which include Spd synthase,[4] Spm synthase,[4] MTA phosphorylase[4,5] and AdoHcy hydrolase.[6] It is also an inhibitor of cyclic AMP phosphodiesterase[7] and of cellular nucleoside and sugar transport.[8] The potential interaction of SIBA with these diverse cellular targets undoubtedly contributes to the wide spectrum of *in vivo* biological effects observed for this compound. 5'-Deoxy-5'-(cyclopropylmethylthio)adenosine (CPMTA), a sterically constrained analog of SIBA, has been synthesized by a novel route and has been evaluated, in comparison with SIBA, for its antiviral and antitumor activity. It was hoped that introduction of this structural modification would produce a SIBA analog with enhanced biological activity.



CPMTA

1



SIBA

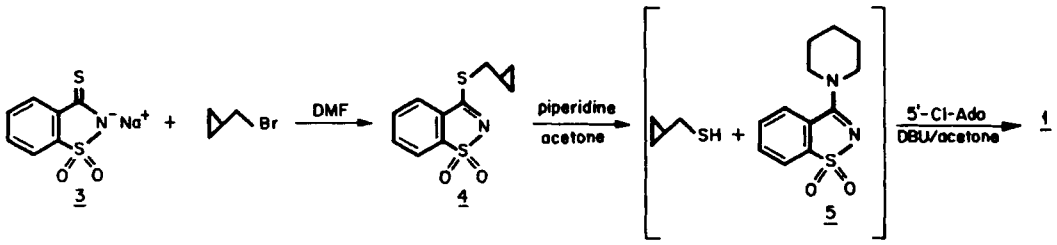
2

RESULTS

Chemistry.

The most commonly used synthetic route for the preparation of SIBA and related 5'-deoxy-5'-(alkythio)adenosine analogs, involves the reaction of 5'-deoxy-5'-chloroadenosine (5'-ClAdo) with a specific alkyl mercaptan under a variety of basic conditions. A limitation of this otherwise straightforward synthetic route is its reliance on the commercial availability of suitable alkyl mercaptans as starting materials. Laboratory precautions associated with the handling of mercaptans may have discouraged the preparation of unusual alkyl mercaptans for use in the synthesis of 5'-deoxy-5'-(alkylthio)adenosine analogs.

A novel synthesis of CPMTA is reported here which involves the facile, in situ generation of cyclopropylmethyl mercaptan. This methodology is potentially applicable to the formation of a variety of previously unprepared 5-deoxy-5'-(alkylthio)adenosine analogs. The synthesis has been adapted from the methods of Yamada et al.[9] who have developed 3-(alkylthio)-1,2-benzisothiazole 1,1-dioxide (BID-SR) reagents as odorless thiol equivalents and have used them in the preparation of corresponding sulfides. As illustrated for the synthesis of CPMTA in Scheme 1, BID-SR reagents are easily prepared by the reaction of a suitable alkyl halide (i.e. cyclopropylmethyl bromide) with the sodium salt of thiosaccharin. In situ generation of



3 4 5 1

Scheme 1

the corresponding alkanethiol (i.e. cyclopropylmethyl mercaptan) was accomplished by treatment with piperidine followed by treatment with DBU to provide the reactive alkylthiolate anion which, upon further addition of an electrophilic alkyl halide (i.e. 5'-ClAdo) gave the corresponding sulfide (i.e. CPMTA). Although the yield (23%) of CPMTA was modest, the attractive feature of the synthesis is facile generation of the required alkanethiol from a corresponding alkyl halide. The yield was improved slightly by using 5'-deoxy-5'-toluenesulfonyladenosine in place of 5'-ClAdo, but similar attempts, using 5'-deoxy-5'-iodoadenosine, met with limited success.

Biological Studies

Antiviral Activity

The comparative abilities of CPMTA and SIBA to inhibit the replication of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in infected CV-1 monkey kidney cells and of vesicular stomatitis virus

Table 1. Cytotoxic and Antiviral Activity of CPMTA versus SIBA in CV-1 and BHK Cell Cultures.

		Cytotoxicity		Antiviral Activity		
Compound	mM	CV-1 cells[1]	BHK cells[2]	HSV-1	HSV-2	VSV
CPMTA	0.1	-	-	-	-	-
	0.3	PT(7)[3]	-	+[5]	+	-
	1.0	T[4]	-	nd[7]	nd	+
SIBA	0.1	PT(8)	-	+	+	-
	0.3	PT(12)	-	++[6]	++	+
	1.0	PT(10)	PT(5)	++	++	++

1 CV-1 cells are infected with HSV-1, strain KOS and HSV-2, strain 333.
2 BHK cells are infected with VSV, vesicular stomatitis virus.
3 PT, partially toxic, 7 mm diameter zone of dead cells.
4 T, toxic to all cells in well.
5 +, plaque diameter reduced relative to controls.
6 ++, no plaques detectable
7 nd, not determined

(VSV) in BHK cells, were evaluated by use of a disk assay. As summarized in Table 1, SIBA, whose antiviral effects are well-documented,[3] was highly active in our assays at a 1 mM concentration. In contrast, CPMTA was found not only to be less effective than SIBA as an antiviral agent, but also more cytotoxic. Since a close correlation between antiviral activity and potency as S-adenosylhomocysteine hydrolase inhibitors has been observed for a series of adenosine analogs,[10] the diminished antiviral effects of CPMTA may be a reflection of this phenomenon.

Antitumor Activity

The growth inhibitory activity of SIBA, a substrate of MTA phosphorylase, has been found to be significantly affected by the presence or absence of this enzyme in tumor cell lines.[11] Accordingly, the antitumor effects of CPMTA and SIBA were studied in two murine leukemia cell lines, L1210 (MTA phosphorylase-containing) and L5178Y (MTA phosphorylase-deficient).

Table 2. Effects of CPMTA and SIBA on Growth and Polyamine Pools of Cultured L1210 and L5178Y Cells.

Cell Line	Treatment (48 hr)	Growth (% Control)	Polyamine Pools* Put (nmol/10^6 cells)	Spd	Spm
L1210	None	100	0.46	2.92	0.84
	CPMTA (300 μM)	54	0.67	2.34	0.68
	SIBA (300 μM)	47	0.80	2.52	0.69
	MTA (600 μM)	51	3.01	1.31	0.15
L5178Y	None	100	0.35	2.13	1.45
	CPMTA (100 μM)	59	0.36	2.12	1.13
	(300 μM)	31	0.39	1.87	1.03
	SIBA (100 μM)	47	0.30	1.98	0.97
	(300 μM)	26	0.22	1.30	0.87
	MTA (40 μM)	55	0.32	2.40	0.62

*Average of at least 2 separate experiments

In L1210 cells the IC_{50} values for both SIBA and CPMTA were approximately 300 μM, whereas for L5178Y cells their IC_{50} values were approximately 100 μM (Table 2). This difference in sensitivity between the two cell lines was seen to a greater extent in the IC_{50} values obtained for MTA: 600 μM (L1210) versus 40 μM (L5178Y). To determine the possible effects of CPMTA and SIBA on Spd and Spm synthases, polyamine pools were analyzed at the IC_{50} values and compared to the levels in cells similarly treated with MTA, known to inhibit both of these polyamine biosynthetic enzymes. In L1210 cells,

CPMTA increased putrescine (Put) to 145%, decreased Spd to 80% and decreased Spm to 81% of control. SIBA-treated L1210 cells responded similarly. For comparison, after MTA treatment, Put increased to 654%, Spd decreased to 45%, and Spm decreased to 18% of control values. In L1210 cells, it appeared that CPMTA and SIBA had little effect on the polyamine pool profiles as compared to MTA. The L5178Y cells showed no significant changes in Put and Spd pools when treated with either CPMTA or SIBA at 100 μM, whereas at this concentration, SIBA decreased Spm pools to 67% of control suggesting some inhibition of Spm synthase in this cell line. However, MTA at similar growth inhibitory doses, depleted Spm to a greater extent than either analog. These results again suggest that the effects of SIBA and CPMTA on polyamine pools were not as dramatic as those seen with MTA. Overall, the in vitro responses to treatment by CPMTA or SIBA were comparable, whether examined in L1210 or L5178Y tumor cells.

Evaluation of the in vivo antitumor activity of these nucleoside analogs in mice bearing L1210 leukemia produced minimal effects, giving at best, an increase in life span of 14% at dosages of 25 mg/kg, daily x 5 (i.p.) for both CPMTA and SIBA. SIBA, itself, was found to be nontoxic in nontumor bearing mice given 500 mg/kg, daily x 5. This latter observation is of interest in light of recent studies exploring its in vivo antimetastatic properties.[12]

EXPERIMENTAL SECTION

Chemistry

Melting points were determined on a Mel-Temp apparatus and are uncorrected. IR spectra were determined on a Perkin-Elmer 710B spectrometer. ^{1}H NMR spectra were taken on a 90 MHz Varian EM-390 spectrometer using tetramethylsilane as an internal standard. Mass spectra were recorded on a Finnegan 4000 mass spectrometer. Elemental analyses were performed by Robertson Laboratory, Madison, NJ.

Thiosaccharin, Sodium Salt (3). Thiosaccharin was prepared as described by Scheibye et al.[13] Saccharin (165 g, 0.9 mol) and

Lawesson's Reagent (182.2 g, 0.45 mol) were refluxed in dry toluene (800 ml) for 11 h. After cooling to room temperature, the toluene was removed *in vacuo* to give a deep yellow residue which was extracted exhaustively with ether-petroleum ether (1:3). Concentration of the ether solution gave thiosaccharin (156.6 g, 0.79 mol, 87%) as a yellow orange solid. This material was dissolved in MeOH (800 ml), to which was added a suspension of $NaHCO_3$ (66 g, 0.79 mol) in MeOH (200 ml). After stirring overnight at room temperature, the solution was filtered to remove traces of undissolved material, and then evaporated *in vacuo* to give a light yellow solid. The solid was washed extensively with CH_2Cl_2, filtered and dried to give 150.5 g (86.5%) 3, whose infrared spectrum was identical to that of an authentic sample of 3 kindly provided by Dr. H. Kotake, Department of Chemistry, Kanazawa University, Kanazawa, Japan. IR (KBr) 3550, 3240-3150, 1610, 1460, 1345, 1250, 1240, 1160, 1120, 1010, 840, 750 cm^{-1}.

3-(Cyclopropylmethylthio)-1,2-benzisothiazole 1,1-Dioxide (4). Compound 4 was prepared according to a general procedure of Yamada *et al.*[9] Cyclopropylmethyl bromide (1.18 g, 8.8 mmol) was injected into a solution of 3 (1.73 g, 7.8 mmol) in DMF (13 ml), under nitrogen at room temperature. The mixture was stirred 4 h at 48°C then cooled to room temperature and diluted with water. The resulting precipitate was filtered and recrystallized from CH_2Cl_2-petroleum ether to give 1.24 g (63%) 4; mp 95-96°. ^1H NMR ($CDCl_3$) δ7.72 (m, 2H), 3.30 (d, 2H, J = 7.5 Hz), 1.22 (m, 1H), 0.68 (m, 2H), 0.40 (m, 2H); mass spectrum (EI-direct probe), m/e 254 (MH^+). Anal. Calcd. for $C_{11}H_{11}NO_2S_2$: C, 52.15; H, 4.38; N, 5.53; S, 25.31. Found: C, 52.20; H, 4.46; N, 5.60; S, 25.22.

5'-Deoxy-5'-(cyclopropylmethylthio)adenosine (CPMTA) (1). Compound 1 was prepared with modifications to the general procedure of Yamada *et al.*[9] A solution of piperidine (0.339 ml, 3.4 mmol) in dry acetone (6 ml) was added to 4 (775 mg, 3.1 mmol) in dry acetone (9 ml) and then stirred at room temperature for 3 h under nitrogen. To this was added a solution of 5'-deoxy-5'-chloroadenosine[14] (790 mg, 2.76 mM) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.504 ml, 3.4 mmol) in dry acetone (5 ml), followed by stirring at room temperature for 2

wks. The reaction mixture was evaporated to dryness in vacuo. The crude product, containing a mixture of 1, unreacted 5'-ClAdo, 3-(piperidinyl)-1,2-benzisothiazole 1,1-dioxide 5 and DBU, was purified either by preparative TLC [Analtech 1000 micron silica gel GF plates (Cat No. 02013); CH_2Cl_2-MeOH (85:15)] or silica gel column chromatography, eluting first with CH_2Cl_2 to remove 5, and then with CH_2Cl_2-MeOH (85:15) to separate CPMTA from the slightly more polar, unreacted starting material, 5'-ClAdo. In this manner, 210 mg (23%) CPMTA and 563 mg (71% recovery) 5'-deoxy-5-chloroadenosine were obtained. Recrystallization of 1 from MeOH/Et_2O/petroleum ether gave an analytically pure sample with mp 185-185.5°. ^1H NMR (DMSO-d_6) δ 8.30 (s, 1H), 8.13 (s, 1H), 7.20 (s, 2H); 5.83 (d, 1H, J = 6 Hz), 5.40 (d, 1H, J = 6 Hz), 5.20 (d, 1H, J = 5 Hz), 4.70 (m, 1H), 4.03 (m, 2H), 2.86 (m, 2H), 2.45 (d, 2H, J = 7 Hz), 0.87 (m, 1H), 0.42 (m, 2H), 0.12 (m, 2H); mass spectrum (EI-direct probe), 337 (M). Anal. Calcd. for $C_{14}H_{19}N_5O_3S.1/2H_2O$: C, 48.54; H, 5.82; N, 20.22; S, 9.26. Found: C, 48.63; H, 5.86; N, 20.01; S, 9.53.

Biological Methods

Antiviral Assays. The detailed procedures for evaluating antiviral activity have been described by us previously.[15]

Antitumor Activity. Murine lymphocytic leukemia L1210 and L5178Y cell lines were grown in RPMI 1640 media supplemented with 10% Nu Serum (Collaborative Research, Inc., Lexington, MA) and HEPES/MOPS buffer as previously described.[16]. Cell cultures (0.3 x 10^5 cells per ml) were treated at 0 hr with SIBA (Sigma Chemical Co.), CPMTA or MTA (Sigma Chemical Co.) at concentrations up to 600 μM. All compounds were dissolved in 0.1 N HCl. After 48 h, cells were removed and cell number was determined by electronic particle counting (Cell Dyne 300, Sequoia-Turner Corp., Mountain View, CA). The dose which resulted in 50% growth inhibition (IC_{50} at 48 h) was determined. For therapeutic studies, groups of eight DBA/2J female mice (18-20 g, Jackson Laboratory, Bar Harbor, ME) were inoculated i.p. with 10^6 L1210 leukemia cells on day 0. Drugs (15-200 mg/kg) were administered daily (day 1 through 5) i.p. in 0.2 ml Tween 80. Lifespan was checked daily. Statistical analysis was performed as described.[18]

Polyamine Pools. Cells were treated for 48 h with CPMTA, SIBA or MTA at their approximate IC_{50} doses, and were then extracted with 0.6 M perchloric acid (10^7 cells per 0.5 ml acid). The extracts (50 µl) were analyzed by HPLC by using a system based on cation exchange and post column derivatization with o-phthalaldehyde as described[16].

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