Water-Soluble Antitumor Agents. I. Synthesis and Biological Activity of 6-*S*-Aminoacyloxymethyl Mercaptopurine Derivatives

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In an attempt to improve the effectiveness and bioavailability of 6-mercaptopurine, various kinds of water-soluble analogues, such as 6-*S*-aminoacyloxymethyl mercaptopurine derivatives (3a—m) and 6-*S*,9-disubstituted derivatives (7a, b and 9a, b), were synthesized. These compounds were evaluated for activity to augment antitumor immunity by using a double grafted tumor system. Antitumor activities against solid tumors (sarcoma 180 and colon 26) were also evaluated. Many compounds exhibited potent activities in both test systems. In particular, the aminopropionate derivative (3a) and the L-glutamate derivative (3f) showed significant enhancement of antitumor immunity together with potent antitumor activities.

Key words 6-mercaptopurine derivative; water-soluble; tumor immunity; antitumor activity; double grafted tumor system

6-Mercaptopurine (6-MP, **1**, X = H) was synthesized by Elion *et al.*[1] in 1952 and employed in the treatment of lymphoblastic leukemia in children.[2] Since then, many purine derivatives and analogues have been synthesized for evaluation of their biological activities. Some of them showed considerable antitumor activity with reduced toxicity.[3]

However, 6-MP and its derivatives, especially azathiopurine, which has been employed clinically, are immunosuppressants.

In the previous paper we showed that 6-MP and 6-MP ribonucleoside 2′,3′,5′-*O*-triacetate(6-MPR acetate, **5**) augmented antitumor immunity in a double grafted tumor system.[4] That is, they had a moderate (6-MP) or slight (**5**) effect on the growth of the primary tumor, but strongly inhibited the growth of the secondary tumor. On the other hand, adriamycin (ADM), 5-fluorouracil (5-FU), cisplatin (CDDP) *etc.* strongly inhibited growth of the primary tumor, but had no effect on the secondary one. Immunomodulators such as PSK (crestine) had no effect on either tumor.

Since 6-MP and **5** are insufficiently soluble in water to administer by injection, we planned to synthesize water-soluble 6-MP derivatives with improved bioavailability and strong antitumor and antitumor immunity-augmenting activities. In this paper we describe the synthesis of water-soluble 6-MP and 6-thioguanine derivatives using chloromethyl esters of N-blocked amino acids,[5] as well as their immunostimulating activity and antitumor activity against solid tumors.

Chemistry

First, to prepare the thioacetal compounds **2**, we examined the reaction of 6-MP with chloromethyl esters **4**, which were readily obtained by our method[5] from the corresponding amino acids. The reaction of 6-MP and **4** in the presence of K_2CO_3 and NaI in acetone–*N*,*N*-dimethylformamide (DMF)[6] gave a complex mixture, from which the unexpected 6-*S*,9-disubstituted derivative was isolated in 19% yield as a major product without formation of the desired product **2**. Therefore, we examined other bases. Among various bases ($NaHCO_3$, pyridine, NH_4OH, Et_3N, *etc.*), calcium carbonate ($CaCO_3$) was found to be the best, giving **2a**—**i** in good yields (34—74%) without formation of the disubstituted derivative. $CaCO_3$ is poorly soluble in DMF–acetone, and may act as a proton sponge under the reaction conditions, preventing the formation of the disubstituted product and the decomposition of **2**. Removal of protecting groups (Boc and *tert*-butyl ester) from **2a**—**i** readily proceeded under acidic conditions to give water-soluble compounds **3a**—**i**, which were obtained as amorphous powders in good yields, as shown in Table 1 and Chart 1. Similarly, thioguanine (6-TG) derivatives **3j, k** and dipeptide derivatives **3l, m** were obtained in moderate yields.

The HCl salts of **3** were soluble to the extent of more than 40 mg/ml in water, and in the case of betaine-type forms, the solubilities in water exceeded 10 mg/ml.

Next, we prepared water-soluble thiopurine nucleoside analogues **7** in an attempt to improve the bioavailability of the 6-MPR acetate **5** (Chart 2). The 6-*S*,9-disubstituted

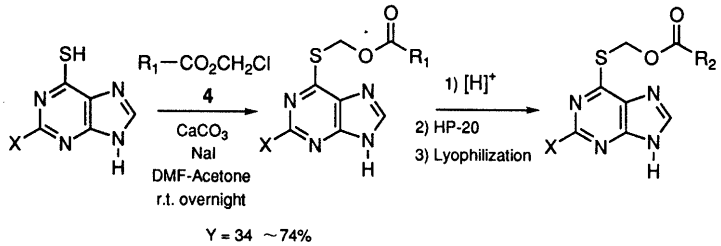

Chart 1

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derivatives **6a, b** were readily prepared from **5** and **4** in the presence of K_2CO_3 and NaI.[7] Conversion to **7a, b** was accomplished under acidic conditions.

Finally, the 9-pivaloyloxymethyl derivatives **9** were prepared (Chart 3) to evaluate the influence of the lipophilic group on the biological activity. Treatment of **2a, i** with chloromethyl pivalate in the presence of K_2CO_3 in acetone afforded **8a, b** in good yields. The resulting **8a, b** were deprotected to provide **9a, b** in good yields.

Table 1. 6-*S*-Substituted Water-Soluble 6-MP Derivatives **3a—m**

No.	X	Method[a]	R_2	Yield (%)
3a	H	A	$NH_2 \cdot HCl$	65
3b	H	A	NH·HCl	79
3c	H	A	$NH_2 \cdot HCl$	50
3d	H	A	$NH_2 \cdot HCl$	58
3e	H	A	$NH_2 \cdot HCl$, CO_2Et	70
3f	H	B	NH_2, COOH	71
3g	H	B	NH_2, COOH	61
3h	H	B	NH_2, COOH	34
3i	H	B	NH_2, COOH	63
3j	NH_2	A	$NH_2 \cdot HCl$	75
3k	NH_2	B	NH_2, COOH	58
3l	H	A	HCl·H-L-Val-β-Ala-	45
3m	H	A	HCl·H-L-Val-L-Ala-	44

a) See Experimental section.

Biological Results and Discussion

Inhibitory activities of water-soluble 6-MP derivatives against the growth of the secondary tumor in a double grafted tumor system are summarized in Table 2, together with those of 6-MP, 5-FU, ADM and CDDP.

6-MP and various water-soluble derivatives showed strong inhibitory effects on the growth of the secondary tumor, although 5-FU, CDDP and ADM augmented or only slightly inhibited its growth. The derivatives in which a β- or γ-amino acid was introduced at the S-6 position (**3a, e** or **f**) were more potent than the α-amino acid derivatives **3c, d**. Dipeptide derivatives showed strong inhibitory activity with a similar tendency (**3l** > **3m**). 6-*S*,9-disubstituted derivatives **7b**, **9a** and thioguanine derivative **3k** had poor activity. Compound **3a** caused stronger inhibition when it was administered intratumorally (i.t.) or intraperitoneally (i.p.) than intravenously (i.v.).

Next, we tested antitumor activities against sarcoma 180 and colon 26 in mice (Tables 3 and 4). All the 6-*S*,9-disubstituted derivatives (**7a, b** and **9b**) had weak activity, while the 6-*S* monosubstituted derivatives showed potent antitumor activities. The compounds which have a β- or γ-amino acid at the 6-*S* position showed more potent antitumor activity than α-amino acid derivatives. In particular, 3-aminopropionate derivatives **3a, l** aspartate derivatives **3h, i** and glutamate derivatives **3f, g** showed complete inhibition of the growth of colon 26 at the maximum tolerated dose (MTD). Moreover, they were effective over a wide range of dose. These compounds showed larger therapeutic ratio (TR)s than 6-MP. The absolute configuration of the amino acid moiety had no influence on the antitumor activity (**3f** *vs.* **3g, h** *vs.* **i**). 6-TG derivatives **3j, 3k** had potent antitumor activity. However, they showed smaller TR values than the 6-MP derivatives because of their severe acute toxicity.

In conclusion, we have synthesized water-soluble 6-*S*-aminoacyloxymethyl mercaptopurine derivatives having potent antitumor activity. In particular, **3a** and **3f** showed strong augmentation of antitumor immunity in a double grafted tumor system and also strong direct antitumor activities against sarcoma 180 and colon 26 in mice, and they have been selected for further testing.

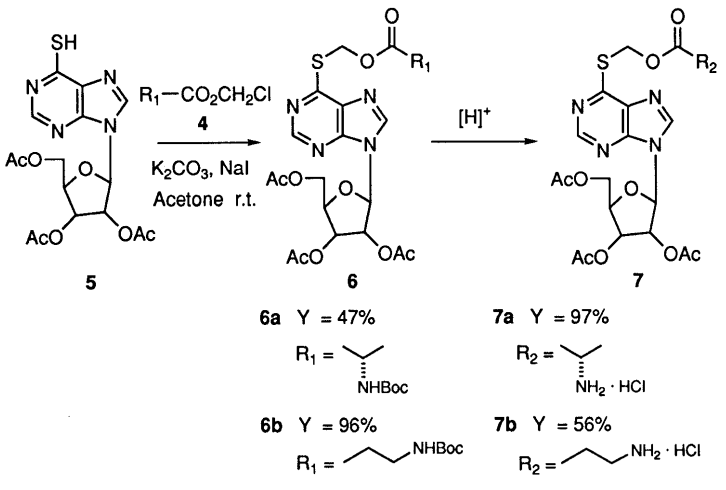

Chart 2

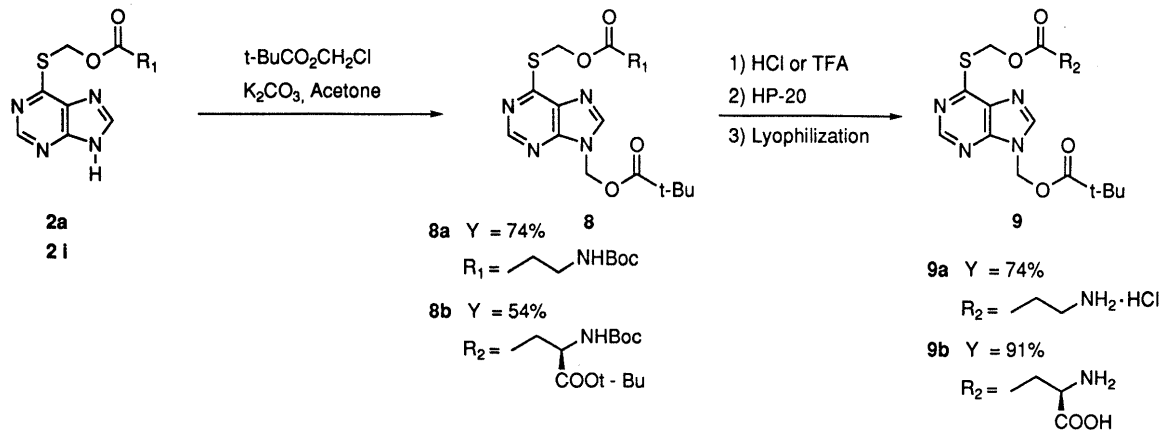

Chart 3

Table 2. Inhibitory Effect of 6-MP Derivatives on Growth of the Secondary Tumor in a Double Grafted Tumor System (Anti Meth A)[a]

No.	Admin. route	Inhibition (%)[b]	No.	Admin. route	Inhibition (%)[b]
3a	i.t.	81.5	**3k**	i.p.[c]	21.3
	i.p.	81.7	**3l**	i.p.	84.0
	i.v.	62.6	**3m**	i.p.	60.7
3b	i.t.	59.2	**7b**	i.p.	55.0
3c	i.t.	45.9	**9a**	i.p.	49.3
3d	i.p.	59.3			
3e	i.p.	75.8	6-MP	i.t.	77.0
3f	i.p.	82.2	5-FU	i.t.	−31.3[d]
3h	i.p.	24.6	ADM	i.t.	15.6
3i	i.p.	49.6	CDDP	i.t.	−19.7[d]

a) One million Meth A fibrosarcoma cells were inoculated intradermally at the right inguinal region of BALB/C mice on day 0 (primary tumor), and three million cells at the left on day 10 (secondary tumor). Drugs (50 mg/kg) were administered intratumorally (i.t.), intraperitoneally (i.p.) or intravenously (i.v.) on days 3 to 7. *b*) The size of secondary tumors was measured with a caliper on day 24. The tumor size was expressed as the mean of the long and short diameters (mm). The significance of the difference in tumor size between the control and experimental groups was statistically analyzed using Student's *t* test. *c*) Ten mg/kg of the drug was administered because of its strong toxicity. *d*) The size of secondary tumors in the experimental groups was larger than that of the control groups.

Table 3. Antitumor Activity against Sarcoma 180[a]

No.	Admin. route	MTD (mg/kg/d)[b]	Inhibition (%)[c]	TR[d]
3a	*p.o.*	400	99.9	8.7
3b	*p.o.*	400	94.9	3.9
3j	*p.o.*	25	88.7	3.7
3l	*p.o.*	400	86.1	8.5
7a	i.p.	200	49.5	—
7b	i.p.	200	77.4	2.6
6-MP	*p.o.*	200	94.2	5.6

a) Two million sarcoma 180 cells were inoculated intradermally at the right inguinal region of ICR mice on day 0. Drugs were administered perorally (*p.o.*) or i.p. on days 1 to 5. *b*) Maximum tolerated dose. *c*) The tumors were removed and weighed on day 10. Inhibition (%) = (1 − mean tumor weight of treated group/that of control group) · 100. *d*) Therapeutic ratio = MTD/ED_{50}. ED_{50}; daily dose providing 50% inhibition of the tumor growth compared to the control.

Experimental

IR spectra were obtained with an Analect FX-6200 FT-IR spectrophotometer. ^{1}H-NMR were measured with a JEOL JNM-FX-200 spectrometer. Mass spectra (MS) were recorded with a Hitachi RMU-6 or a JEOL JMS-HX 100 mass spectrometer. Microanalyses were performed on a Perkin–Elmer 240B CHN analyzer. Silica gel 60K-230 (230—430 mesh) (Katayama) and Highpolarous polymer HP-20 (Mitsubishi Kasei) was used for column chromatography.

Table 4. Antitumor Activity against Colon 26[a]

No.	Admin.[b] route	MTD (mg/kg/d)[c]	Inhibition (%)[c,d]	TR[c]
3a	A	100	100	>4
	B	200	98.4	9.5
	C	200	100	>8
3c	B	200	98.2	5.8
3e	A	200	99.2	4.2
3f	A	200	100	>8
	C	200	98.9	5.7
3g	A	100	100	6.6
3h	A	200	100	6.3
3i	A	200	100	6.9
3k	A	25	92.4	3.3
3l	A	200	100	>4
9b	A	100	89.6	1.6
6-MP	A	200	98.0	6.3

a) One million colon 26 cells were inoculated intradermally at the right inguinal region of CDF_1 mice on day 0. Drugs were administered following schedule A, B or C. *b*) A, *p.o.* on days 1 to 7; B, *p.o.* on days 1, 3, 5, 7 and 9; C, i.v. on days 1 to 7. *c*) See Table 3. *d*) The tumors were removed and weighed on day 16.

General Procedure for the Synthesis of 3 A suspension of a chloromethyl ester **4** (14.5 mmol) and NaI (17.4 mmol) in acetone was stirred at room temperature for 1 h. To the reaction mixture was added a solution of 6-MP (12.0 mmol) in DMF (60 ml) and $CaCO_3$ (60 mmol). The whole was stirred at room temperature for 18 h. The precipitate was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (AcOEt), washed twice with water and brine, and dried. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on SiO_2 using $CHCl_3$–MeOH (20 : 1) as an eluent to give **2** as a foam.

Method A: To a cold solution of **2** (3.0 mmol) in dry dioxane (15 ml) was added 15% HCl/dioxane (15 ml) with stirring on an ice bath, then the mixture was allowed to come to ambient temperature and stirred for 2 h. After addition of Et_2O (50 ml), the reaction mixture was stirred vigorously for 1 h. The resulting precipitate was collected by filtration, and purified by column chromatography on HP-20 using H_2O and 10% MeOH as eluents. The 10% MeOH fractions were concentrated *in vacuo* and lyophilized to give **3** as an amorphous powder.

Method B: To a cold solution of **2** (3.0 mmol) in dry CH_2Cl_2 (10 ml) was added trifluoroacetic acid (TFA) (20 ml) with stirring on an ice bath. The reaction mixture was allowed to come to ambient temperature and stirred for 5 h. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on HP-20 using H_2O and 15% EtOH as eluents. The 15% EtOH fractions were concentrated *in vacuo* and lyophilized to give **3** as an amorphous powder.

7a, b and **9a, b** were each obtained as an amorphous powder in the same manner as described for the preparation of **3**. Analytical and physical data of **3**, **7**, **9** are summarized in Table 5.

Table 5. Analytical and Physical Data for **3**, **7**, **9**

No.	IR cm^{-1}	FAB-MS *m/z*	^{1}H-NMR (D_2O) δ	Formula	Analysis (%) Calcd (Found)		
					C	H	N
3a	3400, 1740	254 (MH^+)	3.0 (2H, t, J=6.6 Hz), 3.44 (2H, t, J=6.6 Hz), 6.03 (2H, s), 8.41 (1H, s), 8.64 (1H, s)	$C_9H_{11}N_5O_2S \cdot HCl \cdot 0.8H_2O$	35.54 (35.53)	4.51 4.30	23.03 23.08)
3b	3380, 1730	294 (MH^+)	1.92 (2H, m), 2.21 (2H, m), 2.90 (1H, m), 3.13 (2H, m), 3.45 (2H, m), 6.01 (2H, s), 8.41 (1H, s), 8.66 (1H, s)	$C_{12}H_{15}N_5O_2S \cdot HCl \cdot H_2O$	41.44 (41.69)	5.22 5.02	20.14 20.36)
3c	3160, 1750	282 (MH^+)	[a] 0.90 (6H, d, J=6.8 Hz), 2.2 (1H, m), 3.9 (1H, m), 6.12 (1H, d, J=11.2 Hz), 6.25 (1H, d, J=11.2 Hz), 6.0—7.7 (1H, br, D_2O-exch.), 8.54 (1H, s), 8.73 (6H, br, D_2O-exch.), 8.78 (1H, s)	$C_{11}H_{15}N_5O_2S \cdot HCl \cdot 0.9H_2O$	39.56 (39.68)	5.37 5.33	20.97 20.79)
3d	3400, 1750	268 (MH^+)	1.57 (6H, s), 6.13 (2H, s), 8.45 (1H, s), 8.70 (1H, s)	$C_{10}H_{13}N_5O_2S \cdot HCl \cdot 1.5H_2O$	36.31 (36.33)	5.18 5.01	21.17 21.03)
3e	3350, 1740	326 (MH^+)	1.11 (3H, t, J=7.3 Hz), 3.18 (1H, dd, J=5.4, 18.1 Hz), 3.32 (1H, dd, J=5.4, 18.1 Hz), 4.12 (2H, q, J=7.3 Hz), 4.53 (1H, m), 6.04 (2H, s), 8.42 (1H, s), 8.67 (1H, s)	$C_{12}H_{15}N_5O_4S \cdot HCl \cdot H_2O$	37.95 (37.87)	4.78 4.70	18.44 18.27)
3f	3600—2000, 1740	312 (MH^+)	2.20 (2H, m), 2.66 (2H, t, J=7.3 Hz), 3.80 (1H, t, J=6.6 Hz), 5.93 (2H, s), 8.33 (1H, s), 8.57 (1H, s)	$C_{11}H_{13}N_5O_4S \cdot 0.6H_2O$	41.01 (40.99)	4.44 4.46	21.74 21.58)
3g	1750, 1735	312 (MH^+)	2.20 (2H, m), 2.67 (2H, m), 3.81 (1H, t, J=6.4 Hz), 5.92 (2H, s), 8.32 (1H, s), 8.56 (1H, s)	$C_{11}H_{13}N_5O_4S \cdot H_2O$	40.12 (40.08)	4.59 4.58	21.27 21.37)
3h	3400—2600, 1730	298 (MH^+)	3.13 (2H, m), 4.15 (1H, dd, J=5.4, 6.4 Hz), 5.96 (2H, s), 8.33 (1H, s), 8.57 (1H, s)	$C_{10}H_{11}N_5O_4S \cdot H_2O$	38.09 (38.20)	4.16 4.15	22.21 22.08)
3i	3500—2600, 1735	298 (MH^+)	3.12 (1H, dd, J=6.4, 18.1 Hz), 3.13 (1H, dd, J=5.4, 18.1 Hz), 4.12 (1H, dd, J=5.4, 6.4 Hz), 5.97 (2H, s), 8.32 (1H, s), 8.58 (1H, s)	$C_{10}H_{11}N_5O_4S \cdot 0.8H_2O$	38.53 (38.51)	4.07 4.16	22.47 22.32)
3j	3600—2200, 1740	269 (MH^+)	2.95 (2H, t, J=6.2 Hz), 3.40 (2H, t, J=6.2 Hz), 5.94 (2H, s), 7.98 (1H, s)	$C_9H_{12}N_6O_2S \cdot HCl \cdot 1.7H_2O$	32.23 (32.26)	4.93 4.74	25.06 24.87)
3k	3500, 1735	313 (MH^+)	3.11 (2H, d, J=6.0 Hz), 4.11 (1H, t, J=6.0 Hz), 5.84 (1H, d, J=11.0 Hz), 5.91 (1H, d, J=11.0 Hz), 7.91 (1H, s)	$C_{10}H_{12}N_6O_4S \cdot 1.3H_2O$	35.78 (35.77)	4.38 4.35	25.03 24.84)
3l	3210, 1740, 1670	353 (MH^+)	0.81 (6H, m), 2.01 (1H, m), 2.72 (2H, m), 3.43 (1H, m), 3.68 (2H, m), 5.98 (2H, s), 8.45 (1H, s), 8.68 (1H, s)	$C_{14}H_{20}N_6O_3S \cdot HCl \cdot 1.5H_2O$	40.43 (40.50)	5.82 5.63	20.21 20.02)
3m	3200, 1750, 1675	353 (MH^+)	0.78 (3H, d, J=6.8 Hz), 0.80 (3H, d, J=6.8 Hz), 1.43 (3H, d, J=7.3 Hz), 1.96 (1H, m), 3.75 (1H, d, J=5.4 Hz), 4.55 (1H, q, J=7.3 Hz), 6.06 (2H, s), 8.43 (1H, s), 8.69 (1H, s)	$C_{14}H_{20}N_6O_3S \cdot HCl \cdot H_2O$	41.33 (41.48)	5.70 5.62	20.65 20.48)
7a	3400, 1750	512 (MH^+)	1.53 (3H, d, J=7.0 Hz), 2.11 (3H, s), 2.17 (3H, s), 2.24 (3H, s), 4.27 (1H, q, J=7.0 Hz), 4.50 (2H, m), 4.72 (1H, m), 5.79 (1H, t, J=5.0 Hz), 6.03 (1H, t, J=5.0 Hz), 6.15 (2H, d, J=2.0 Hz), 6.46 (1H, d, J=4.0 Hz), 8.60 (1H, s), 8.80 (1H, s)	$C_{20}H_{25}N_5O_9S \cdot HCl \cdot 0.6H_2O$	42.99 (42.85)	4.91 5.04	12.53 12.44)
7b	3380, 1740	512 (MH^+)	2.12 (3H, s), 2.17 (3H, s), 2.24 (3H, s), 2.90 (2H, t, J=6.0 Hz), 3.34 (2H, t, J=6.0 Hz), 4.50 (2H, m), 4.67 (1H, m), 5.78 (1H, t, J=5.0 Hz), 6.01 (1H, t, J=5.0 Hz), 6.03 (2H, s), 6.43 (1H, d, J=5.0 Hz), 8.57 (1H, s), 8.74 (1H, s)	$C_{20}H_{25}N_5O_9S \cdot HCl \cdot H_2O$	42.44 (42.24)	4.99 4.74	12.37 12.19)
9a	3400, 1735	368 (MH^+)	[a] 1.10 (9H, s), 2.75 (2H, t, J=7.0 Hz), 3.01 (2H, m), 6.06 (2H, s), 6.24 (2H, s), 8.08 (3H, br, D_2O-exch.), 8.66 (1H, s), 8.88 (1H, s)	$C_{15}H_{21}N_5O_4S \cdot HCl \cdot 0.8H_2O$	43.07 (43.14)	5.69 5.86	16.74 16.59)
9b	3410, 1740	412 (MH^+)	1.13 (9H, s), 3.10 (1H, dd, J=6.0, 18.1 Hz), 3.12 (1H, dd, J=6.0, 18.1 Hz), 4.11 (1H, m), 6.03 (2H, s), 6.29 (2H, s), 8.59 (1H, s), 8.75 (1H, s)	$C_{16}H_{21}N_5O_6S \cdot 0.8H_2O$	43.13 (45.30)	5.35 5.26	16.45 16.27)

a) DMSO-d_6.

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