Synthesis and Anti-HIV-1 Activity of Novel 10-Thiaisoalloxazines, a Structural Analog of C-5 and/or C-6 Substituted Pyrimidine Acyclonucleoside

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A series of novel 10-thiaisoalloxazine derivatives bearing an alkoxymethyl or benzyloxymethyl moiety at the *N*-1 position has been synthesized through the bromination of 1-substituted-5-hydroxyuracils and subsequent condensation with aminobenzenethiol in a one-pot reaction. Contrary to the previous report, the formation of intermediary 5,6-diethoxy-5-hydroxy-5,6-dihydrouracil seems to be not the necessary factor for the formation of the thiaisoalloxazines, since the reaction proceeds in tetrahydrofuran (THF) or acetonitrile far more smoothly than in ethanol. The anti-human immunodeficiency virus (HIV)-1 activity of the resulted thiaisoalloxazine derivatives was evaluated in lymphocyte cells based on the inhibitory activity against the viral-induced cytopathic activity. Among the derivatives, compounds 6, 7, and 8 bearing an alkoxymethyl moiety at the *N*-1 position exhibited modest inhibitory activity towards the cytotopathic effect of HIV-1.

Key words thiaisoalloxazine; human immunodeficiency virus; pyrimidine acyclonucleoside; 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT)

10-Thiaisoalloxazine, pyrimido[4,5-*b*][1,4]thiazin-2,4-(1*H*, 3*H*)-dione, is the thia analogue of isoalloxazines in which the nitrogen atom at the 10th position of isoalloxazine is substituted by a sulfur atom. It is known that some thiaisoalloxazine derivatives exhibit anti-allergy or anti-inflammation activity as lipoxygenase inhibitor.[3,4)] Meanwhile, Miyasaka *et al.* reported that C-5 and/or C-6 substituted pyrimidine acyclonucleoside analogues, such as 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) and 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil (MKC-442), have considerable anti-human immunodeficiency virus (HIV) activity (Fig. 1).[5—7)] These compounds exhibit the activity as a selective HIV-1 reverse transcriptase inhibitor. From the structural point of view, 10-thiaisoalloxazine can be regarded as the C-6 phenylthio derivative of uracil and, therefore, it shows some structural similarity with HEPT.[8)] These facts prompted us to synthesize and evaluate the anti-HIV activity of 10-thiaisoalloxazine derivatives. In this study, a series of novel acyclonucleoside analogs of 10-thiaisoalloxazines bearing an alkoxymethyl moiety or benzyloxymethyl moiety at the *N*-1 position was prepared and their anti-HIV activity was examined. The anti-HIV-1 assay using MT-4 cells indicated that some of the analogs possess inhibitory effect against the cytotoxicity of HIV-1 towards lymphocyte cells. Here, we would like to describe the synthesis and the first report of the anti-HIV-1 activity of the 10-thiaisoalloxazine derivatives.

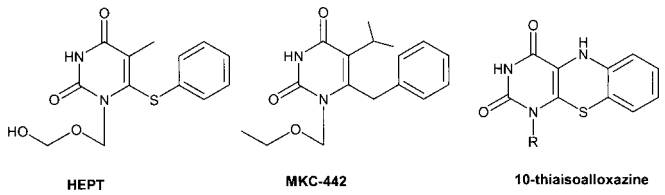

Fig. 1

Chemistry

1-Substituted 10-thiaisoalloxazine derivatives **6**—**14** were synthesized by following the procedure of Sako *et al.*[9,10)] The key intermediates, 1-substituted-5-hydroxy uracils (**2**—**4**), except 5-hydroxyuridine (**5**), were synthesized from 5-hydroxyuracil (**1**) (Chart 1). Thus, compound **1** was treated with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMS-Cl) in acetonitrile (CH_3CN), followed by alkoxy chlorides alone or a mixture of 2-acetoxyethyl acetoxymethyl ether and tin(IV) chloride ($SnCl_4$) to give 1-substituted-5-hydroxyuracils (**2**—**4**) in modest yields. In order to prepare 1-substituted-10-thiaisoalloxazines (**6**—**12**, **14**), 1-substituted-5-hydroxyuracils (**2**—**5**) were treated with *N*-bromosuccinimide (NBS) in ethanol. Subsequently, 3-substituted or non-substituted analogs of 2-aminobenzenethiol[10)] were added to the mixture, and the mixture was heated under reflux. This one-pot condensation reaction was effective for the preparation of 1-ethoxymethyl (**6**—**8**), 1-acetoxyethoxymethyl (**12**) and 1-ribosyl (**14**) substituted 10-thiaisoalloxazines from the corresponding uracil derivatives. The products were obtained in modest yields (Chart 2a, 40—69%) (Table 1, entries 1, 3, 5, 18, 19). Deacetylation of **12**

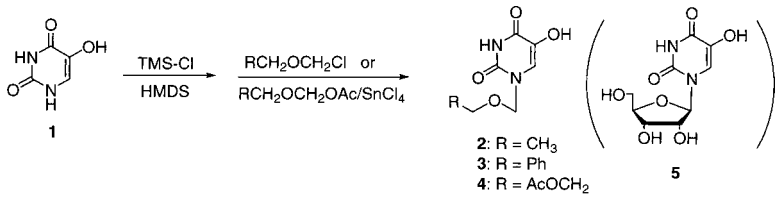

Chart 1

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Table 1.

Entry	Substrate	R	R′	Solvent	Condition	Yield (%)	Product	By-product (yield)
1	**2**	Me	H	EtOH	reflux	62	**6**	
2	**2**	Me	H	THF	r.t.	79	**6**	
3	**2**	Me	Me	EtOH	reflux	60	**7**	
4	**2**	Me	Me	THF	r.t.	64	**7**	
5	**2**	Me	Cl	EtOH	reflux	40	**8**	
6	**2**	Me	Cl	THF	r.t.	70	**8**	
7	**3**	Ph	H	EtOH	reflux	27	**9**	**6** (10%)
8	**3**	Ph	H	THF	r.t.	70	**9**	
9	**3**	Ph	H	$CHCl_3$	r.t.	64	**9**	
10	**3**	Ph	H	CH_3CN	r.t.	57	**9**	
11	**3**	Ph	H	DMF	r.t.	47	**9**	
12	**3**	Ph	Me	EtOH	reflux	1	**10**	**7** (16%)
13	**3**	Ph	Me	BnOH	80 °C	5	**10**	
14	**3**	Ph	Me	THF	r.t.	63	**10**	
15	**3**	Ph	Cl	EtOH	reflux	8	**11**	**8** (22%)
16	**3**	Ph	Cl	BnOH	80 °C	4	**11**	
17	**3**	Ph	Cl	THF	r.t.	65	**11**	
18	**4**	$AcOCH_2$	H	EtOH	reflux	69	**12**	
19	**5**			EtOH	reflux	59	**14**	
20	**1**			EtOH	reflux	75	**15**	
21	**1**			THF	reflux	22	**15**	

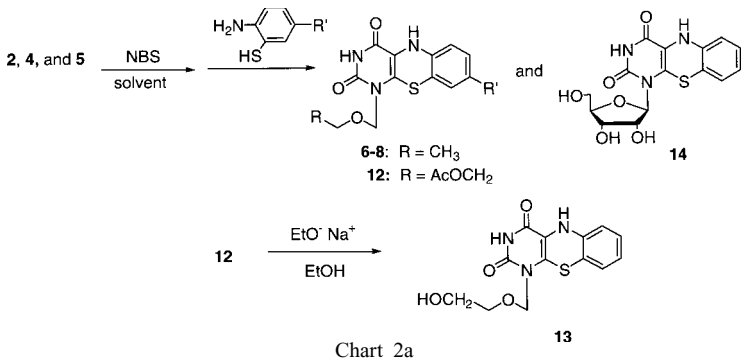

Chart 2a

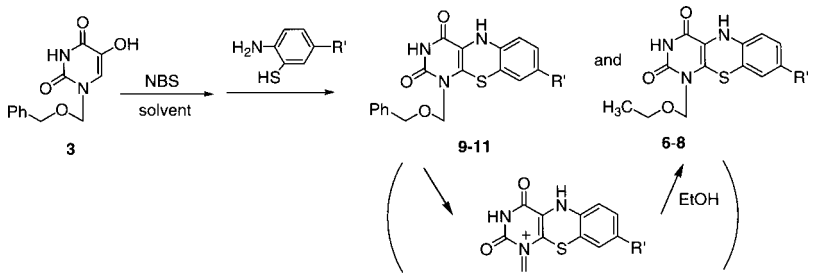

Chart 2b

using sodium ethoxide in ethanol gave compound **13**, almost quantitatively (Chart 2a).

The same reaction procedure using 1-benzyloxymethyl substituted uracil (**3**) as the starting material, however, resulted in a considerable amount (10 to 22%) of 1-ethoxymethyl derivatives (**6**—**8**) as by-products while the yields of the desirable compounds (**9**—**11**) were quite low (Chart 2b), as indicated in Table 1 (entries 7, 12, 15). The formation of the by-products may be due to an alcohol exchange reaction (Chart 2b) since ethanol was used as the reaction solvent. Using benzyl alcohol (BnOH) instead of ethanol as the reaction solvent to prevent the possible alcohol exchange reaction gave the desirable products in only low yields (4 to 5%, Table 1, entries 13, 16).

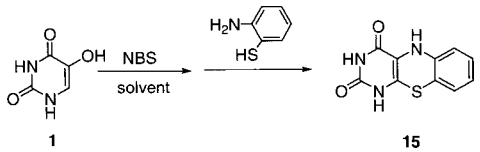

Chart 2c

In further attempts, we employed other reaction solvents such as tetrahydrofuran (THF), chloroform ($CHCl_3$), CH_3CN, or *N*,*N*-dimethylformamide (DMF). These aprotic solvents worked far better than ethanol. The reaction proceeded even at room temperature and the yields of the desirable compounds were generally higher than in the cases using ethanol (Table 1, entries 8—11). THF was the most effective solvent among the solvents tested here. Compounds **9**, **10** and **11** were successfully obtained in 70, 63 and 65% yield, respectively, from the corresponding 1-benzyloxymethyl hydroxyuracil (**3**) and 2-aminobenzenethiol derivatives in THF (Chart 2b) (Table 1, entries 8, 14, 17). Under this reaction condition, we could not detect the formation of 1-ethoxymethyl derivatives (**6**—**8**) at all. Using THF as the reaction solvent also gave better results for the preparation of 1-ethoxymethyl thiaisoalloxazines (**6**—**8**) (Chart 2a) (Table 1, entries 2, 4, 6). In the same manner, compound **15**[9] was obtained directly from compound **1** (Chart 2c), although the yield was not very good (Table 1, entry 21).

Biological Activity

The newly synthesized 1-substituted-10-thiaisoalloxazines were evaluated for their anti-HIV-1 activities in the human lymphoblastoid cell line MT-4 based on the inhibition of HIV-1-induced cytopathic effect, as previously described.[12] Briefly, MT-4 cells were suspended in culture medium at 3×10^4 cells/ml and infected HIV at a multiplicity of infection (MOI, ratio of CCID50 (50% cell culture infective dose) to cell number) of 0.02. Immediately after virus infection, 100 μl of the cell suspension was brought into each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. After 5 d of incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[13] Cytotoxicity of the 1-substituted 10-thiaisoalloxazines was also assessed based on the viability of mock-infected host cells as determined by the MTT method. The experiments were performed in parallel with an antiviral activity experiment undertaken for comparison purposes for comparability. The results are summarized in Table 2. As shown in Table 2, compound **13** having a hydroxyethoxymethyl moiety at the *N*-1 position was the least toxic among the test compounds, whereas it displayed a modest antiviral activity against HIV-1 replication. Like compound **13**, compounds **6**—**8** bearing an ethoxymethyl moiety at the *N*-1 position inhibited HIV-1 replication in MT-4 cells, though their selectivity indices were not high enough. On the other hand, compounds **9**—**11** bearing a benzyloxymethyl moiety at the *N*-1 position tend to show considerable cytotoxicity. Cytotoxic effect of compounds **6**—**8** bearing an ethoxymethyl moiety at the *N*-1 position was relatively lower than that of the other compounds. Compounds **6**—**8** exhibited inhibitory activity towards the cytotopathic effect of HIV-1, although the activity was modest. Attaching a substitution group (chloro group or methyl group) at the benzene ring of the alloxazine moiety seems to increase the cytotoxicity, although it does not have much effect on the anti-HIV activity of the compounds.

Table 2. Inhibitory Effects of the Test Compounds on HIV-1 Induced Cytopathicity in MT-4 Cells

Compound	EC50 (μg/ml)	CC50 (μg/ml)	S.I.
6	6.9	50	7.2
7	1.4	9.5	6.8
8	1.4	28	20
9	>23	23	>1
10	>1.9	1.9	>1
11	>3	3	>1
13	23	276	12
14	>53	53	>1
3′-Azido-3′-deoxythymidine (AZT)	0.0022	8.3	3773

50% effective concentration or compound concentration required to inhibit HIV-1-induced cytopathicity in MT-4 cells by 50%. 50% cytotoxic concentration or compound concentration required to reduce MT-4 cell viability by 50%. Selectivity index or ratio of CC50 to EC50.

Discussion

Synthesis of 10-thiaisoalloxazine and related compounds starting from readily available 5-hydroxyuracil (**1**) was first reported by Sako *et al.*[9] In the report, it was suggested that 5-hydroxyuracil reacts with NBS reagent in the presence of ethanol to form an intermediary 5,6-diethoxy-5-hydroxy-5,6-dihydrouracil (**17**) (Chart 3, path B, R=H). Subsequently, the intermediate reacts with 2-aminobenzenethiol to result in the corresponding 10-thiaisoalloxazine. In this mechanism, the presence of ethanol plays a crucial role to form the intermediate.

In our study, however, the procedure gave good results only for the cases in which 1-ethoxymethyl-, 1-acetoxymethyl-, and 1-ribosyl-5-hydroxyuracil were used as the starting materials. Modest amounts of the corresponding 1-substituted 10-thiaisoalloxazins were obtained by the procedure. Attempts to prepare 1-benzyloxymethyl-10-thiaisoalloxazines starting from the corresponding 5-hydroxyuracil, however, led to the formation of a considerable amount of 1-ethoxymethyl-10-thiaisoalloxazines as by-products, while the yields of the desirable compounds were quite low. An increment of the formation of the desirable products was achieved by switching the reaction solvent from ethanol to an aprotic solvent such as THF, $CHCl_3$, or CH_3CN. Among these, THF seems to be the most suitable solvent. The formation of the products was observed even without heating and the yields were quite satisfactory. Interestingly, this is also the case in which 1-ethoxymetyl-5-hydroxyuracil was used as the starting material. These results indicate that the formation of the 5,6-diethoxy type of intermediate **17** is not the necessary factor for the formation of the 1-substituted-10-thiaisoalloxazines (Chart 3, path A). Alternatively, 2-aminobenzenethiol may react with the possible bromonium intermediate of 1-subsituted 5-hydroxyuracil directly in THF, as was mentioned before (Table 1, entries 2, 4, 6). The fact that 10-thiaisoalloxazine (**15**)[9] was successfully obtained from 5-hydroxyuracil (**1**) with 2-aminobenzenethiol in THF would support this mechanism. However, the yield of the product in this case was considerably lower than that of the reaction using ethanol as the solvent (Table 1, entries 20, 21). This result and those mentioned above indicate that the substituent of the *N*-1 position of 5-hydroxyuracils may affect the stability and/or the reactivity of the bromonium intermediate (**16**).[9]

In the biological study, the thiaisoalloxazines without the terminal hydroxyl group at the N^1-substituent (compounds

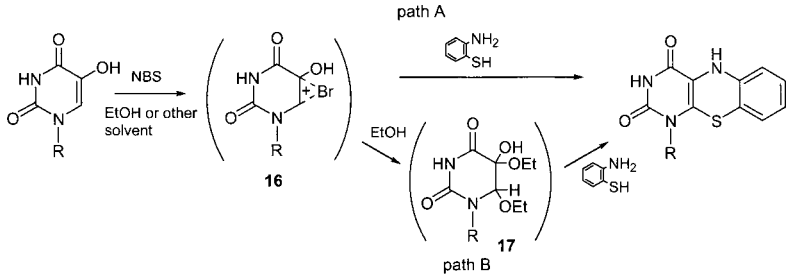

Chart 3

6—**8**) exhibited inhibitory activity towards the virus-induced cytotopathic effect of HIV-1 in human lymphocyte cells. This precludes the possibility that phosphorylation of the thiaisoalloxazines is required for the activity. The results coincided with those of HEPT and other related compounds of Tanaka's report.[7] Although compounds **6**—**8** selectively inhibited HIV-1-induced cytopahtic effect in MT-4 cells, their activity was not comparable to that of HEPT derivatives including MKC-442. These compounds are classified as non-nucleoside HIV-1 reverse transcriptase inhibitors, which interact with amino acids located near the catalytic site of the enzyme.[14] In these compounds, the bulky alkyl substituent at the C-5 position of the pyrimidine ring reportedly forces Tyr 181 of the enzyme to induce some conformational change of the enzyme so that the amino acid residue interacts with the 6-benzyl group.[14] The relatively low anti-HIV activity of our compounds could be attributed to the fact that the current 10-thiaisoalloxazines may not be able to interact with Tyr 181 residue to trigger the necessary conformational change of the enzyme. The possibility that the rigid tricyclic structure of the thiaisoalloxazines was not favorable to fit the reverse transcriptase pocket is, however, not precluded.

Conclusion

We have synthesized a new series of structural analogs of C-5 and/or C-6 substituted pyrimidine acyclonucleoside, namely, 1-substituted 10-thiaisoalloxazines, from the corresponding 5-hydroxy uracils. It was found that the formation of the 5,6-diethoxy type of intermediate is not the necessary factor for the formation of the thiaisoalloxazines. Some of the thiaisoalloxazines were found to be active against HIV-1 replication in cell cultures. Thus, this class of compounds should be further pursued for their antiviral activity and structure–activity relationship.

Experimental

General Procedures Thin-layer chromatography (TLC) analyses were carried out on Merck silica gel 60 F254-precoated plates. Column chromatography was performed with silica gel 60 (Merk 7734). ^{1}H-NMR spectra were recorded on JEOL GSX-400 NMR spectrometer at 400 MHz, using tetramethylsilane as the internal standard. FAB mass spectra were recorded with JEOL JMS-AX500 spectrometer. Melting temperatures were determined with Yanagimoto micro melting apparatus and are uncorrected. Elemental analyses were performed on a Yanagimoto MT-5 elemental analyzer.

1-[(Ethoxy)methyl]-5-hydroxyuracil (2) To a suspension of 5-hydroxyuracil (**1**, 1.0 g, 8.0 mmol) in acetonitrile (5 ml), chlorotrimethylsilane (1.6 ml, 12 mmol) and 1,1,1,3,3,3-hexamethyldisilazane (2.5 ml, 12 mmol) were added and the mixture was stirred at room temperature for 5 h. Chloromethyl ethyl ether (0.6 ml, 8.0 mmol) was added to the mixture and allowed to react at room temperature for 20 h. After the reaction was completed, sat. $NaHCO_3$ was added and the resulted precipitates were collected by filtration to give 0.84 g (58%) of **2**; mp 187—190 °C; FAB$^+$MS 187 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6) δ: 11.45 (1H, bs, NH), 7.15 (1H, s, 6-H), 4.99 (2H, s, CH_2), 3.48 (2H, q, J=7.0 Hz, $\underline{CH_2}CH_3$), 1.11 (3H, t, J=7.0 Hz, $CH_2\underline{CH_3}$). *Anal.* Calcd for ($C_7H_{10}N_2O_3$): C, 45.16; H, 5.41; N, 15.05. Found: C, 45.06; H, 5.36; N, 15.05.

1-[(Phenylmethoxy)methyl]-5-hydroxyuracil (3) To a suspension of 5-hydroxyuracil (**1**, 10.2 g, 80 mmol) in acetonitrile (40 ml), chlorotrimethylsilane (15.6 ml, 120 mmol) and 1,1,1,3,3,3-hexamethyldisilazane (25.2 ml, 120 mmol) were added, and the mixture was stirred at room temperature for 5 h. Benzyl chloromethyl ether (11.2 ml, 81 mmol) was added to the mixture and allowed to react at room temperature for 20 h. After the reaction was completed, sat. $NaHCO_3$ was added and the resulted precipitates were collected by filtration to give 12.0 g (61%) of **3**; mp 157—159 °C; MS *m/z* 248 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 11.45 (1H, bs, NH), 8.71 (1H, bs, OH) 7.36—7.27 (5H, m, Ph) 7.18 (1H, s, 6-H), 5.11 (2H, s, $PhCH_2$), 4.54 (2H, s, CH_2). *Anal.* Calcd for ($C_{12}H_{12}N_2O_3$): C, 58.06; H, 4.87; N, 11.28. Found: C, 58.01; H, 4.80; N, 11.34.

1-[[(2-Acetoxy)ethoxy]methyl]-5-hydroxyuracil (4) A mixture of 5-hydroxyuracil (**3**, 1.28 g, 10 mmol), chlorotrimethylsilane (2.6 ml, 20 mmol) and 1,1,1,3,3,3-hexamethyldisilazane (4.2 ml, 20 mmol) was stirred at room temperature for 5 h. After cooling to 0 °C, 2-acetoxyethyl acetoxymethyl ether (1.8 ml, 10 mmol) and $SnCl_4$ (0.47 ml, 4.0 mmol) were added. The mixture was stirred at room temperature for 20 h, then sat. $NaHCO_3$ was added and the resulted precipitates were collected by filtration to give 0.70 g (29%) of **4**; mp 195—197 °C (MeOH); FAB$^+$MS 245 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6) δ: 11.48 (1H, bs, NH), 8.75 (1H, bs, OH) 7.16 (1H, s, 6-H), 5.04 (2H, s, CH_2), 4.10—4.08 (2H, m, CH_2), 3.68—3.66 (2H, s, CH_2), 1.99 (3H, s, OAc). *Anal.* Calcd for ($C_9H_{12}N_2O_6\cdot 1/4H_2O$): C, 43.48; H, 5.07; N, 11.26. Found: C, 43.63; H, 4.82; N, 10.97.

General Procedure for the Preparation of 10-Thiaisoalloxazines. Procedure A NBS (392 mg, 2.2 mmol) was added to 1-substituted-5-hydroxyuracil (2.0 mmol) in THF (5 ml), and the mixture was stirred at room temperature for 30 min. To this mixture was added 5-subsituted-2-aminobenzenethiol (4.0 mmol), and the mixture was stirred at room temperature for 1 h.

Procedure B NBS (196 mg, 1.1 mmol) was added to 1-substituted-5-hydroxyuracil (1.0 mmol) in ethanol (10 ml), and the mixture was stirred at room temperature for 30 min. To this mixture was added 2-aminobenzenethiol (250 mg, 2.0 mmol), and the mixture was heated under reflux for 1 h.

1-(Ethoxymethyl)-1,5-dihydro-2*H*-pyrimido[4,5-*b*][1,4]benzothiadine-2,4(3*H*)-dione (6) 1-Ethoxymethyl-5-hydroxyuracil (**2**, 372 mg, 2.0 mmol) was allowed to react with 2-aminobenzenethiol (500 mg, 4.0 mmol) in THF according to procedure A. The reaction mixture was purified by silica gel column chromatography and the obtained material was crystallized from ethanol to give compound **6**: yield 460 mg (79%): mp 193 °C (EtOH); MS *m/z* 292 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 11.69 (1H, s), 7.80 (1H, s), 7.09—6.79 (4H, m), 5.27 (2H, s), 3.52 (2H, q, J=7.0 Hz, $\underline{CH_2}CH_3$), 1.13 (3H, t, J=7.0 Hz, $CH_2\underline{CH_3}$). *Anal.* Calcd for ($C_{13}H_{13}N_3O_3S$): C, 53.60; H, 4.50; N, 14.42. Found: C, 53.32; H, 4.29; N, 14.34.

1-(Ethoxymethyl)-1,5-dihydro-8-methyl-2*H*-pyrimido[4,5-*b*][1,4]benzothiadine-2,4(3*H*)-dione (7) 1-Ethoxymethyl-5-hydroxyuracil (**2**, 372 mg, 2.0 mmol) was reacted with 2-amino-5-methylbenzenethiol (556 mg, 4.0 mmol) in the same manner as above. The reaction mixture was purified by silica gel column chromatography and the obtained material was crystallized from ethanol to give compound **7**: yield 391 mg (64%): mp 194—197 °C (EtOH); MS *m/z* 306 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 11.67 (1H, s), 7.64 (1H, s), 7.35—6.85 (3H, m), 5.26 (2H, s), 3.52 (2H, q, J=7.0 Hz, $\underline{CH_2}CH_3$), 2.14 (3H, s, Ph-Me), 1.13 (3H, t, J=7.0 Hz, $CH_2\underline{CH_3}$). *Anal.* Calcd for ($C_{14}H_{15}N_3O_3S$): C, 55.07; H, 4.95; N, 13.76. Found: C, 55.22; H,

4.91; N, 14.01.

8-Chloro-1-(ethoxymethyl)-1,5-dihydro-2*H*-pyrimido[4,5-*b*][1,4]benzothiadine-2,4(3*H*)-dione (8) 1-Ethoxymethyl-5-hydroxyuracil (**2**, 372 mg, 2.0 mmol) was reacted with 2-amino-5-chlorobenzenethiol (640 mg, 2.0 mmol) in the same manner as above. The reaction mixture was purified by silica gel column chromatography and the obtained material was crystallized from ethanol to give compound **8**: yield 456 mg (70%): mp 178—180 °C (EtOH); FAB$^+$MS 326 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6) δ: 11.72 (1H, s), 8.01 (1H, s), 7.35—7.05 (3H, m), 5.26 (2H, s), 3.52 (2H, q, J=7.0 Hz, $\underline{CH_2}CH_3$), 1.13 (3H, t, J=7.0 Hz, $CH_2\underline{CH_3}$). *Anal.* Calcd for ($C_{13}H_{12}N_3O_3ClS$): C, 47.93; H, 3.71; N, 12.90. Found: C, 48.02; H, 4.00; N, 13.15.

1,5-Dihydro-1-[(phenylmethoxy)methyl]-2*H*-pyrimido[4,5-*b*][1,4]benzothiadine-2,4(3*H*)-dione (9) 1-[(Phenylmethoxy)methyl]-5-hydroxyuracil (**3**, 496 mg, 2.0 mmol) was reacted with 2-aminobenzenthiol (500 mg, 4.0 mmol) in the same manner as above. The reaction mixture was purified by silica gel column chromatography and the obtained material was crystallized from ethanol to give compound **9**: yield 494 mg (70%): mp 173 °C (EtOH); FAB$^+$MS 353 (M^+), 354 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6) δ: 11.68 (1H, s), 7.79 (1H, s), 7.35—6.80 (9H, m), 5.38 (2H, s), 4.57 (2H, s). *Anal.* Calcd for ($C_{18}H_{15}N_3O_3S$): C, 61.18; H, 4.28; N, 11.89. Found: C, 61.24; H, 4.28; N, 11.92.

1,5-Dihydro-8-methyl-1-[(phenylmethoxy)methyl]-2*H*-pyrimido[4,5-*b*][1,4]benzothiadine-2,4(3*H*)-dione (10) 1-[(Phenylmethoxy)methyl]-5-hydroxyuracil (**3**, 496 mg, 2.0 mmol) was reacted with 2-amino-5-methylbenzenthiol (556 mg, 4.0 mmol) in the same manner as above. The reaction mixture was purified by silica gel column chromatography and the obtained material was crystallized from ethanol to give compound **10**: yield 459 mg (63 %): mp 164—167 °C (EtOH); MS m/z 368 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 11.65 (1H, s), 7.65 (1H, s), 7.35—6.85 (8H, m), 5.37 (2H, s), 4.57 (2H, s), 2.14 (3H, s, Me). *Anal.* Calcd for ($C_{19}H_{17}N_3O_3S$): C, 62.11; H, 4.66; N, 11.44. Found: C, 62.10; H, 4.66; N, 11.16.

8-Chlro-1,5-dihydro-1-[(phenylmethoxy)methyl]-2*H*-pyrimido[4,5-*b*][1,4]benzothiadine-2,4(3*H*)-dione (11) 1-[(Phenylmethoxy)methyl]-5-hydroxyuracil (**3**, 496 mg, 2.0 mmol) was reacted with 2-amino-5-chlorobenzenethiol (640 mg, 4.0 mmol) in the same manner as above. The reaction mixture was purified by silica gel column chromatography and the obtained material was crystallized from ethanol to give compound **11**: yield 505 mg (65%): mp 132—135 °C (EtOH); FAB$^+$MS 387 (M^+), 388 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6) δ: 11.71 (1H, s), 8.01 (1H, s), 7.36—6.87 (8H, m), 5.37 (2H, s), 4.56 (2H, s). *Anal.* Calcd for ($C_{18}H_{14}N_3O_3ClS$): C, 55.74; H, 3.64; N, 10.83. Found: C, 55.52; H, 3.55; N, 10.88.

1-[[(2-Acetoxy)ethoxy]methyl]-1,5-dihydro-2*H*-pyrimido[4,5-*b*][1,4]-benzothiadine-2,4(3*H*)-dione (12) 1-[[(2-Acetoxy)ethoxy]methyl]-5-hydroxyuracil (**4**, 244 mg, 1.0 mmol) was reacted with 2-aminobenzenthiol (250 mg, 2.0 mmol) in ethanol according to procedure B. The reaction mixture was purified by silica gel column chromatography and the obtained material was crystallized from ethanol to give compound **12**: yield 241 mg (69%): mp 141 °C (EtOH); MS m/z 350 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 11.72 (1H, s), 7.81 (1H, s), 7.09—6.80 (4H, m), 5.30 (2H, s), 4.11 (2H, m), 3.71 (2H, s), 2.01 (3H, s). *Anal.* Calcd for ($C_{15}H_{15}N_3O_5S$): C, 51.57; H, 4.33; N, 12.03. Found: C, 51.84; H, 4.28; N, 11.78.

1,5-Dihydro-1-β-D-ribosyl-2*H*-pyrimido[4,5-*b*][1,4]benzothiadine-2,4(3*H*)-dione (14) This compound was synthesized from 5-hydroxyuridine (**5**, 260 mg, 1.0 mmol) with 2-aminobenzenthiol (250 mg, 2.0 mmol) in the same manner as above. The reaction mixture was purified by silica gel column chromatography and the obtained material was crystallized from ethanol to give compound **14**: yield 223 mg (59%); mp 203—207 °C (EtOH); FAB$^+$MS 366 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6) δ: 11.76 (1H, s), 7.87 (1H, s), 6.80—7.12 (4H, m), 5.73 (1H, d, J=4.0Hz), 4.61 (1H, dd, J=2.0, 5.0 Hz), 4.11 (1H, t, J=7.0 Hz), 3.31—3.82 (5H, m). *Anal.* Calcd for ($C_{15}H_{15}N_3O_6S\cdot 3/4H_2O$): C, 47.55; H, 4.39; N, 11.09. Found: C, 47.42; H, 4.15; N, 11.02.

1,5-Dihydro-1-[(2-hydroxyethoxy)methyl]-2*H*-pyrimido[4,5-*b*][1,4]-benzothiadine-2,4(3*H*)-dione (13) To a solution of sodium ethoxide (680 mg, 10 mmol) in ethanol (20 ml), compound **12** (349 mg, 1.0 mmol) was added and the mixture was stirred at room temperature for 2 h. Glacial acetic acid was occasionally added to the reaction mixture to maintain the pH at about 4. After evaporation to dryness, water was added to the mixture and the insoluble materials were collected by filtration, then crystallized from ethanol to give 255 mg (83%) of **13**: mp 195 °C (EtOH); MS m/z 324 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 11.68 (1H, s), 7.79 (1H, s), 7.09—6.79 (4H, m), 5.30 (2H, s), 3.51 (5H, m). *Anal.* Calcd for ($C_{13}H_{13}N_3O_5S$): C, 50.81; H, 4.26; N, 13.67. Found: C, 50.59; H, 4.29; N, 13.38.

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