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FACILE SYNTHSIS AND EVALUATION OF ANTITUMOR AND ANTIVIRAL ACTIVITIES OF [1,2,5]THIADIAZOLO[3,4-*d*]-PYRIMIDINES (8-THIAPURINES) AND 4–β–D-RIBOFURANOSYL-[1,2,5]THIADIAZOLO[3,4-*d*]PYRIMIDINES

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Abstract – Synthesis of 5-amino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidin-7(6*H*)-ones (8-thiaguanine) (**4a–m**), 7-amino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidin-5(4*H*)-ones (8-thiaisoguanine) (**6a–k**), 5,7-diamino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidines (**8a–d**) and some other thiadiazolopyrimidine derivatives were prepared by treating 6-amino-5-nitrosopyrimidine derivatives with sodium thiosulfate in aqueous acid media. 4-β-D-Ribofuranosyl-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (8-thiaxanthosine) (**18**) was also prepared by the reliable method. Moderate antitumor and antiviral activities of the synthesized compounds have been evaluated in vitro.

INTRODUCTION

Modified purine derivatives have aroused great attention for their potentiality as therapeutic agents.[1–5] Therefore, much attention has been devoted for the synthesis of analogues of naturally occurring purine bases and closely related heterocyclic systems as well as their ribonucleosides in the view of producing unique selective antimetabolites, which would be useful in the treatment of neoplastic and viral diseases. Since [1,2,5]thiadiazolo[3,4-*d*]pyrimidine has a structural resemblance to purine, it seemed desirable to prepare and evaluate the biological activities of a series of [1,2,5]thiadiazolo[3,4-*d*]pyrimidines in connection with our continuous research on the fused pyrimidines.[6–12] The [1,2,5]thiadiazolo[3,4-*d*]pyrimidines nucleus was prepared first in 1951 by Schrage and Hitchings.[13] Hitherto, only one compound of guanine analogue (8-thiaguanine) has been prepared from 4,5-diaminopyrimidine and

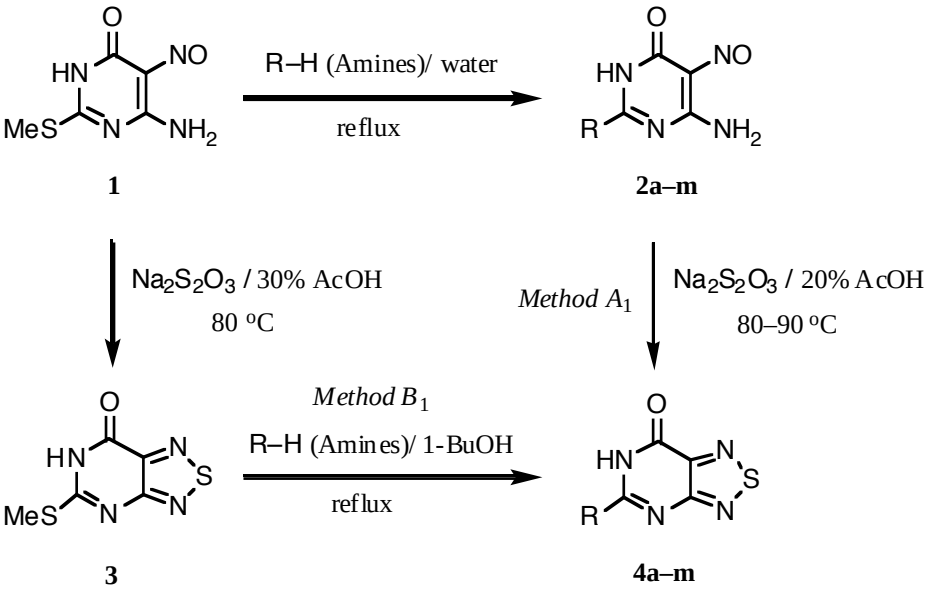
N-sulfinylaniline,[14] and other derivatives as well as isoguanine analogues have not been prepared yet. Besides, the synthesis for necleosides of this class has not been still challenged. In recent years there has been an increasing interest in the synthesis of bicyclic nucleosides with a ribofuranosyl moiety residing in the pyrimidine ring. This interest has been generated to a large extent by the isolation and identification of 3-ribofuranosyluric acid from beef blood.[15,16] Thus, much effort has been directed to a large extent toward 3-ribofuranosyl purines and other ring systems, but 4-ribofuranosyl-[1,2,5]-thiadiazolo[3,4-*d*]pyrimidine has not been investigated yet.

Therefore, we report here a facile synthesis of 5-amino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidin-7(6*H*)-ones (8-thiaguanines), 7-amino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidin-5(4*H*)-ones (8-thiaisoguanines) and 5,7-diamino derivatives similar to guanine, isoguanine and 2,6-diaminopurine, respectively, as well as the ribonucleoside similar to xanthosine with the thought that these compounds might possess potential biological activities.

RESULTS AND DISCUSSION

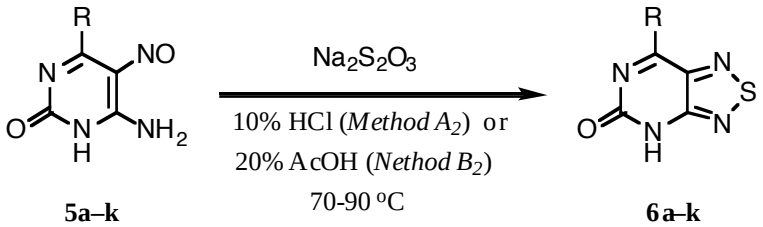
5-Amino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidin-7(6*H*)-ones (8-thiaguanines) were prepared by two synthetic routes. The requisite starting materials for the first route, 2,6-diamino-5-nitrosopyrimidin-4(3*H*)-ones (**2a–m**), were prepared from 6-amino-2-methylthio-5-nitrosopyrimidin-4(3*H*)-one (**1**)[17] according to the literature procedure.[18] Treatment of **2a–m** with sodium thiosulfate in aqueous acetic acid at 80–90 °C led to the formation of the desired 5-amino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidin-7(6*H*)-ones (**4a–m**) in 46–84% yields. The second route was replacement of the methylthio group of 5-methylthio-[1,2,5]thiadiazolo[3,4-*d*]pyrimidin-7(6*H*)-one (**3**) by heating with an appropriate amine in 1-butanol to give the corresponding 5-amino derivatives (**4b**,**c**, **e–k**) (Scheme 1 and Tables 1 and 2). The 5-methylthio derivative (**3**) was prepared according to the modified procedure outlined previously.[19] The reported yield of this compound was only 31%, whereas we carried out the preparation of **3** by treating **1** with sodium thiosulfate in 30% aqueous acetic acid at 80 °C in 82% yield.

7-Amino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidin-5(4*H*)-ones (8-thiaisoguanines) (**6a–k**) were prepared by heating 4,6-diamino-5-nitrosopyrimidin-2(1*H*)-ones (**5a–k**) with sodium thiosulfate in aqueous acid in 43–78% yields (Scheme 2 and Tables 1 and 2). Similarly, heating 4,6-diamino-2-methylthio-5-nitrosopyrimidine (**7**) with amines in water gave the corresponding 2,4,6-triamino-5-nitrosopyrimidines, which were treated with sodium thiosulfate in aqueous acid medium without purification to afford 5,7-diamino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidines (**8a–d**) (Scheme 3). Compound **7** was also directly treated with sodium thiosulfate in aqueous acetic acid to give 7-amino-5-methylthio-[1,2,5]thiadiazolo[3,4-*d*]-



Compd	R	Compd	R
2, 4 a	NHOH	**2, 4 h**	$NH(CH_2)_7CH_3$
b	NHMe	**i**	$NHCH_2CH_2CH_2OH$
c	NHPr	**j**	$NHCH_2Ph$
d	NH*i*-Pr	**k**	$NHCH_2CH_2Ph$
e	NHBu	**l**	N⟨ ⟩
f	NH*i*-Bu	**m**	N⟨ O⟩
g	HN⟨ ⟩		

Scheme 1

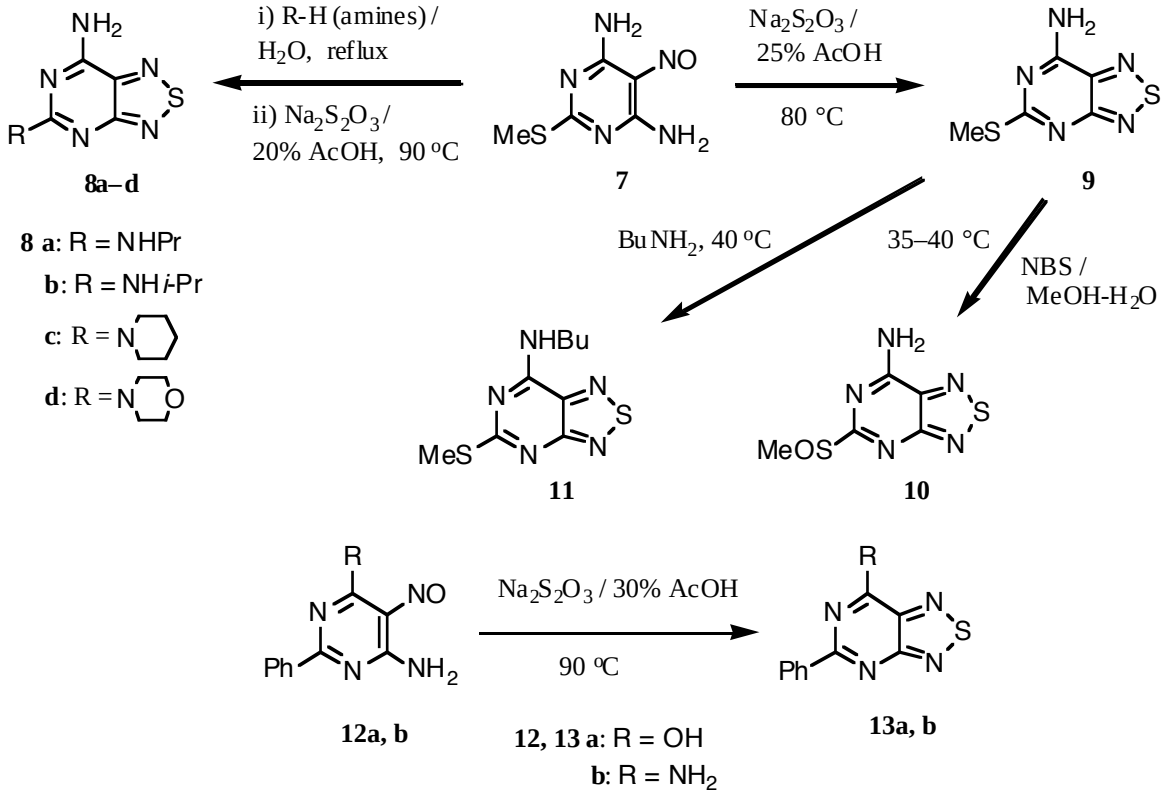


Compd	R	Compd	R
5, 6 a	NH_2	**5, 6 g**	$NH(CH_2)_7CH_3$
b	NHMe	**h**	$NHCH_2Ph$
c	NHPr	**i**	$NHCH_2CH_2Ph$
d	NHBu	**j**	N⟨ ⟩
e	NH*i*-Bu	**k**	N⟨ O⟩
f	HN⟨ ⟩		

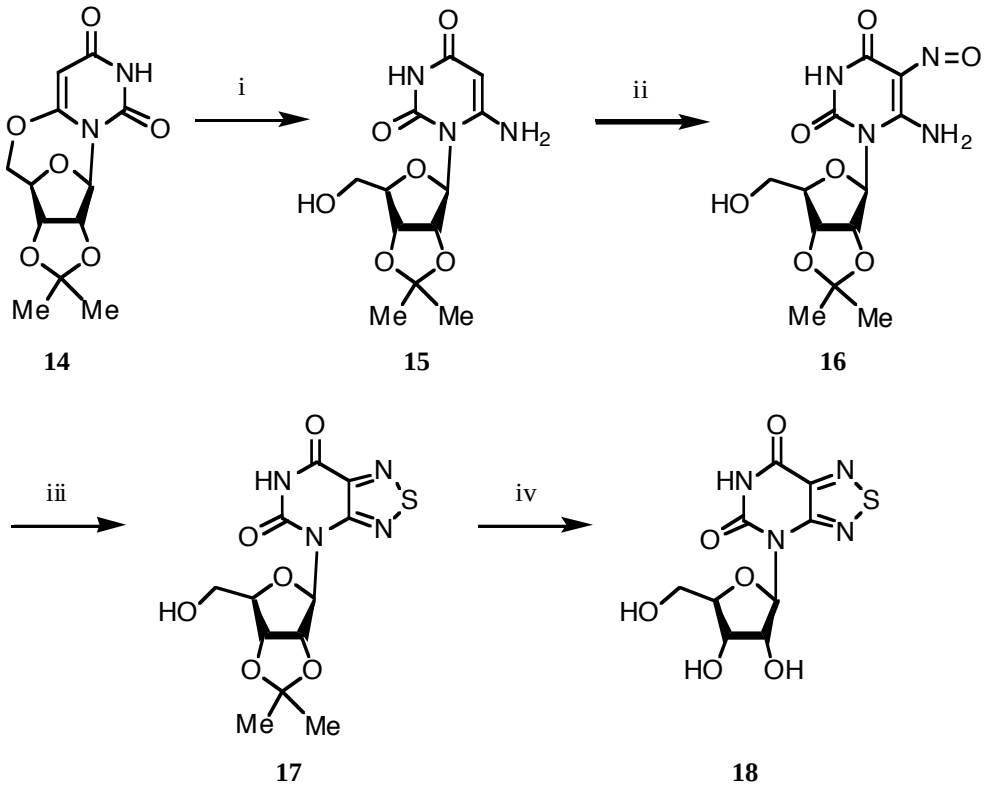
Scheme 2

pyrimidine (**9**).[14] Oxidation of **9** was accomplished with *N*-bromosuccinimide (NBS), *i.e*. treating **9** with NBS in a mixture of methanol and water (4:1) at 35–40 °C for two hours gave the 5-methylsulfinyl derivative (**10**). The ^{1}H-NMR spectrum of **10** showed a singlet signal at δ 2.85 attributable to the C*H*$_3$ protons of $SOCH_3$ group. Usually the chemical shift for methylthio (SCH_3) protons appears comparatively in the little higher field, *e.g*. δ 2.67 for **3** and δ 2.70 for **9**. Microanalysis data of **10** undoubtedly proved that the oxidation product (**10**) was not the 2-methylsulfonyl (SO_2CH_3) derivative but it was the 2-methylsulfinyl ($SOCH_3$) derivative. Reaction of **9** with butylamine without the presence of any solvent at 40 °C yielded the 7-butylamino-5-methylthio-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine (**11**) in 68% yield. In this reaction, it was observed that amine exchange reaction at the 7-position of **9** took place exclusively at lower temperature rather than replacement reaction of the methylthio group of **9** by amine. The ^{1}H-NMR spectrum of **11** apparently exhibited the presence of signals at δ 0.99 (triplet), δ 1.47 (sextet), δ 1.72 (quintet) and δ 3.68 (quartet) assigned to the butyl group, and simultaneously the presence of singlet signal at δ 2.63 attributable to the S-C*H*$_3$ protons. The part integral values of these peaks indicated the presence of only one NH proton at δ 6.34. Therefore, it was evident that amino group (NH_2) of **9** was replaced by butylamino group (NHBu) to yield the 7-butylamino-5-methylthio derivative (**11**). The microanalysis data of **11** was also quite satisfactory. Further, 7-oxo-5-phenyl (**13a**) and 7-amino-5-phenyl derivatives (**13b**) were prepared from 6-amino-5-nitroso-2-phenylpyrimidine derivatives (**12a**,**b**) using the same reagent in acid medium as discussed above.

The thiadiazole ring of 8-thiaxanthosine (**18**) was constructed by work-up in a similar manner as above. Thus, the reaction of 5',6-*O*-anhydro-2',3'-*O*-isopropylidene-6-hydroxyuridine (**14**)[20] with liquid ammonia in the presence of ammonium chloride at 50 °C afforded the 6-amino-2',3'-*O*-isopropylideneuridine (**15**)[21] in 40% yield. Probably, the lower yield of the amination reaction was due to cleavage of the sugar-pyrimidine linkage. Nitrosation of **15** with sodium nitrite in aqueous acid medium did not proceed with ease. Thereupon, the nitrosation was accomplished with amyl nitrite in the presence of catalytic amount of hydrochloric acid in a mixture of ethanol and ethyl acetate to give the 5-nitroso derivative (**16**) in excellent yield (88%). Conversion of **16** into the 4-(2',3'-*O*-isopropyridene-β-D-ribofuranosyl)-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (**17**) was carried out with sodium thiosulfate in a mixture of acetic acid, methanol and water at 50 °C in 61% yield. Finally, the hydrolysis of **17** with dilute hydrochloric acid in methanol afforded the intended 4-β-D-ribofuranosyl-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (8-thiaxanthosine) (**18**) in 86% yield. The IR, ^{1}H-NMR and microanalyses data of all the synthesized new compounds were quite consistent with the structures.



Scheme 3



Reagents and conditions: i, liq. NH_3, NH_4Cl, 50 °C; ii, amyl nitrite, EtOH-EtOAc, rt; iii, $Na_2S_2O_3$, AcOH-MeOH-water, 50 °C; iv, 0.5N HCl, 50 °C.

Scheme 4

Table 1. Yields and analytical data for compounds (**4a–m** and **6a–k**)

Compd No.	Yield (%) (*Method*)	Shape of crystal[a]	Mp (°C)	Recrystn solvent	Formula (R_f)[b]	Analysis (%) Calcd (Found) C	H	N
4a	67 (A_1)	powder	>300	DMF-H_2O	$C_4H_3N_5O_2S$ 0.49 (B)	25.95 (26.01	1.63 1.99	37.82 37.76)
4b	46 (A_1) 71 (B_1)	powder	>300	H_2O	$C_5H_5N_5OS$ 0.53 (B)	32.78 (32.80	2.75 2.78	38.23 37.90)
4c	52 (A_1) 67 (B_1)	plates	258–259	EtOAc-EtOH	$C_7H_9N_5OS$ 0.42 (A)	39.80 (39.48	4.29 4.14	33.15 33.13)
4d	75 (A_1)	prisms	237–238	H_2O	$C_7H_9N_5OS$ 0.44 (A)	39.80 (39.84	4.29 4.19	33.15 32.95)
4e	62 (A_1) 71 (B_1)	powder	243–244	EtOAc-EtOH	$C_8H_{11}N_5OS$ 0.48 (A)	42.65 (42.64	4.92 4.82	31.09 31.06)
4f	69 (A_1) 66 (B_1)	powder	255–256	EtOAc-EtOH	$C_8H_{11}N_5OS$ 0.48 (A)	42.65 (42.91	4.92 4.93	31.09 30.82)
4g	60 (A_1) 63 (B_1)	needles	286–288	EtOAc-EtOH	$C_{10}H_{13}N_5OS$ 0.48 (A)	47.79 (47.64	5.21 5.09	27.87 27.70)
4h	84 (A_1) 78 (B_1)	powder	117–118	EtOAc	$C_{12}H_{19}N_5OS$ 0.56 (A)	51.22 (51.26	6.81 6.63	24.89 24.54)
4i	57 (A_1) 54 (B_1)	needles	250–251	H_2O	$C_7H_9N_5O_2S$ 0.40 (B)	37.00 (37.12	3.99 3.90	30.82 31.05)
4j	69 (A_1) 78 (B_1)	needles	261–263	EtOH	$C_{11}H_9N_5OS$ 0.46 (A)	50.95 (50.85	3.50 3.57	27.01 26.68)
4k	62 (A_1) 74 (B_1)	powder	258–259	EtOH	$C_{12}H_{11}N_5OS$ 0.47 (A)	52.73 (52.52	4.06 4.07	25.62 25.47)
4l	68 (A_1)	needles	224–226	EtOAc	$C_9H_{11}N_5OS$ 0.62 (A)	45.56 (45.39	4.67 4.86	29.51 29.34)
4m	56 (A_1)	needles	294–296	H_2O	$C_8H_9N_5O_2S$ 0.61 (A)	40.16 (40.20	3.79 3.77	29.27 29.11)
6a	69 (A_2)	powder	>300	DMF-H_2O	$C_4H_3N_5OS$ 0.36 (B)	28.40 (28.39	1.79 1.96	41.40 41.29)
6b	43 (A_2)	needles	>300	H_2O	$C_5H_5N_5OS$ 0.43 (B)	32.78 (32.41	2.75 2.72	38.23 38.35)
6c	67 (A_2)	powder	232–234	EtOAc	$C_7H_9N_5OS$ 0.31 (A)	39.80 (39.47	4.29 4.12	33.15 32.96)
6d	64 (A_2)	needles	210–211	EtOAc	$C_8H_{11}N_5OS$ 0.34 (A)	42.65 (42.61	4.92 4.77	31.09 31.16)
6e	62 (A_2)	powder	208–209	EtOAc	$C_8H_{11}N_5OS$ 0.34 (A)	42.65 (43.01	4.92 4.95	31.09 30.75)
6f	69 (A_2)	powder	261–262	EtOAc	$C_{10}H_{13}N_5OS$ 0.39 (A)	47.79 (47.48	5.21 5.11	27.87 27.54)
6g	62 (A_2) 67 (B_2)	powder	187–188	*n*-hexane-EtOAc	$C_{12}H_{19}N_5OS$ 0.48 (A)	51.22 (51.01	6.81 6.42	24.89 24.71)
6h	78 (A_2) 72 (B_2)	powder	215–216	EtOAc	$C_{11}H_9N_5OS$ 0.38 (A)	50.95 (51.06	3.50 3.57	27.01 27.02)
6i	74 (A_2) 65 (B_2)	needles	179–180	EtOAc	$C_{12}H_{11}N_5OS$ 0.39 (A)	52.73 (52.79	4.06 4.25	25.62 25.62)
6j	59 (B_2)	prisms	245–247	H_2O	$C_9H_{11}N_5OS$ 0.37 (A)	45.56 (45.66	4.67 4.66	29.51 29.65)
6k	54 (B_2)	prisms	>300	H_2O	$C_8H_9N_5O_2S$ 0.50 (B)	40.16 (39.89	3.79 3.81	29.27 28.93)

[a]Appearance of almost all compounds are yellow except for **4c**,**e**,**f**,**i**, which are either pale yellow or pale orange.
[b] Solvent system for TLC: (A) AcOEt; (B) EtOAc : EtOH (4:1 v/v).

Table 2. IR and ^{1}H-NMR spectroscopic data for compounds (**4a–m** and **6a–k**)

Compd No.	[ν_{max} (Nujol)/cm^{-1}][a]	δ_H [300 MHz; ($CDCl_3$; Me_4Si)][b]
4a	3530 (OH), *3270*, 3130 (NH), 1710 (C=O)	9.51 (1H, br s, N-OH), 11.18 (2H, br s, 2 x NH)
4b	3300, 3140 (NH), 1715 (C=O)	2.89 (3H, d, *J* = 4.8 Hz, CH_3), 6.61 (1H, br s, 5-C-NH), 11.45 (1H, s, 6-NH)
4c	3290, 3125 (NH), 1705 (C=O)	1.0 (3H, t, *J* = 7.2 Hz, CH_3), 1.66 (2H, sext, *J* = 7.2 Hz, N-$CH_2C\mathit{H}_2$), 3.44 (2H, q, *J* = 6.9 Hz, N-CH_2), 6.32 (1H, br s, 5-C-NH), 11.01 (1H, br s, 6-NH)
4d	3305, 3110 (NH), 1705 (C=O)	1.27 (6H, d, *J* = 6.6 Hz, 2 x CH_3), 4.25-4.34 (1H, m, N-CH), 6.10 (1H, br s, 5-C-NH), 10.93 (1H, br s, 6-NH)
4e	3285, 3120 (NH), 1700 (C=O)	0.96 (3H, t, *J* = 7.2 Hz, CH_3), 1.40 (2H, sext, *J* = 7.2 Hz, $C\mathit{H}_2CH_3$), 1.61 (2H, quin, *J* = 7.2 Hz, N-$CH_2C\mathit{H}_2$), 3.47 (2H, q, *J* = 7.2 Hz, N-CH_2), 6.29 (1H, s, 5-C-NH), 11.0 (1H, s, 6-NH)
4f	3290, 3100 (NH), 1700 (C=O)	0.98 (6H, d, *J* = 6.6 Hz, 2 x CH_3), 1.88-1.97 (1H, m, CH), 3.32 (2H, t, *J* = 5.7 Hz, N-CH_2), 6.32 (1H, br s, 5-C-NH), 10.98 (1H, br s, 6-NH)
4g	3320, 3105 (NH), 1710 (C=O)	1.18-1.32 (3H, m, cyclohexyl-H), 1.38-1.47 (2H, m, cyclohexyl-H), 1.61-1.75 (3H, m, cyclohexyl-H), 1.98-2.06 (2H, m, cyclohexyl-H), 3.97-4.04 (1H, m, cyclohexyl-H), 6.19 (1H, d, *J* = 7.5 Hz, 5-C-NH), 10.91 (1H, s, 6-NH)
4h	3295, 3120 (NH), 1705 (C=O).	0.88 (3H, t, *J* = 6.6 Hz, CH_3), 1.28 (10H, br s, $[C\mathit{H}_2]_5CH_3$), 1.58-1.67 (2H, m, N-CH_2-$C\mathit{H}_2$), 3.47 (2H, q, *J* = 6.6 Hz, N-CH_2), 6.30 (1H, br s, 5-C-NH), 11.07 (1H, br s, 6-NH)
4i	3465 (OH), 3295, 3110 (NH), 1710 (C=O)	1.69 (2H, quin, *J* = 6.3 Hz, N-$CH_2C\mathit{H}_2$), 3.38 (2H, q, *J* = 6.6 Hz, N-CH_2), 3.49 (2H, br s, O-CH_2), 4.44 (1H, br s, OH), 6.54 (1H, br s, 5-C-NH), 10.63 (1H, br s, 6-NH)
4j	3280, 3120 (NH), 1700 (C=O)	4.68 (2H, d, *J* = 5.1 Hz, CH_2), 6.67 (1H, br s, 5-C-NH), 7.30-7.38 (5H, m, Ph-H), 10.87 (1H, br s, 6-NH)
4k	3290, 3120 (NH), 1710 (C=O)	2.96 (2H, t, *J* = 6.9 Hz, Ph-CH_2), 3.78 (2H, q, *J* = 6.9 Hz, N-CH_2), 6.33 (1H, br s, 5-C-NH), 7.24-7.35 (5H, m, Ph-H), 11.05 (1H, br s, 6-NH)
4l	3150 (NH), 1700 (C=O)	1.75 (6H, br s, 3 x CH_2), 3.82 (4H, br s, 2 x CH_2), 10.39 (1H, s, NH)
4m	3125 (NH), 1690 (C=O)	3.68 (8H, br s, 4 x CH_2), 11.72 (1H, s, NH)
6a	3360, *3290*, *3150* (NH), 1670 (C=O)	8.35 (2H, s, NH_2), 11.63 (1H, br s, NH)
6b	*3170*, 3110 (NH), 1660 (C=O)	2.97 (3H, d, *J* = 4.5 Hz, CH_3), 8.84 (1H, d, *J* = 4.2 Hz, 7-C-NH), 11.58 (1H, s, 4-NH)
6c	3240, 3120 (NH), 1670 (C=O)	1.02 (3H, t, *J* = 7.5 Hz, CH_3), 1.75 (2H, sext, *J* = 7.5 Hz, $CH_2C\mathit{H}_2$), 3.64 (2H, q, *J* = 6.3 Hz, N-CH_2), 7.05 (1H, br s, 7-C-NH), 11.03 (1H, br s, 4-NH)
6d	3240, 3105 (NH), 1655 (C=O)	0.98 (3H, t, *J* = 7.2 Hz, CH_3), 1.46 (2H, sext, *J* = 7.2 Hz, $CH_2C\mathit{H}_2$), 1.67-1.76 (2H, m, N-$CH_2C\mathit{H}_2$), 3.71 (2H, q, *J* = 7.5 Hz, N-CH_2), 6.54 (1H, br s, 7-C-NH), 10.47 (1H, br s, 4-NH)
6e	3230, 3130 (NH, 1660 (C=O)	1.03 (6H, d, *J* = 6.6 Hz, 2 x CH_3), 1.90-2.11 (1H, m, CH), 3.55 (2H, t, *J* = 6.6 Hz, N-CH_2), 6.59 (1H, br s, 7-C-NH), 10.60 (1H, br s, 4-NH)
6f	3245, 3110 (NH), 1650 (C=O)	0.88 (2H, m, cyclohexyl-H), 1.21-1.47 (4H, m, cyclohexyl-H), 1.64-1.82 (2H, m, cyclohexyl-H), 2.07-2.11 (2H, m, cyclohexyl-H), 4.29-4.36 (1H, m, N-CH), 6.60 (1H, d, *J* = 8.1 Hz, 7-C-NH), 10.81 (1H, br s, 4-NH)
6g	3180, *3120* (NH), 1630 (C=O)	0.88 (3H, t, *J* = 6.6 Hz, CH_3), 1.25-1.40 (10H, br s, $[(C\mathit{H}_2)_5CH_3]$, 1.67-1.77 (2H, m, N-$CH_2C\mathit{H}_2$), 3.69 (2H, q, *J* = 6.6 Hz, N-CH_2), 6.61 (1H, br s, 7-C-NH), 10.20 (1H, br s, 4-NH)
6h	3225, 3100 (NH), 1660 (C=O)	4.85 (2H, d, *J* = 5.7 Hz, CH_2), 7.28-7.43 (5H, m, Ph-H), 8.25 (1H, s, 7-C-NH), 11.47 (1H, s, 4-NH)
6i	3240, 3085 (NH), 1655 (C=O)	3.03 (2H, t, *J* = 7.2 Hz, Ph-$C\mathit{H}_2$), 3.91 (2H, q, *J* = 6.9 Hz, N-CH_2), 7.20-7.40 (5H, m, Ph-H), 7.59 (1H, s, 7-C-NH), 11.35 (1H, s, 4-NH)
6j	3120 (NH), 1640 (C=O)	1.70 (6H, br s, 3 x CH_2), 3.98, 4.58 (each 2H, each br s, 2 x CH_2), 11.68 (1H, s, NH)
6k	3120 (NH), 1640 (C=O)	3.76 (4H, t, *J* = 4.5 Hz, 2 x CH_2), 4.0, 4.64 (each 2H, each br s, 2 x CH_2), 11.79 (1H, s, NH)

[a] The IR absorption value in italic refers to wave numbers at which shoulders or inflexions occur in the absorption.
[b] ^{1}H-NMR spectra for compounds (**4a**,**b**,**i**,**m** and **6a**,**b**,**j**,**k**) were measured in $(CD_3)_2SO$.

BIOLOGICAL EVALUATION

Antitumor activity: The synthesized new compounds were evaluated in vitro for the growth inhibitory effects against CCRF-HSB-2 (human T-cell acute lymphoblastoid leukemia) and KB (human oral epidermoid carcinoma) cells by the modified MTT [3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay[22] for cellular growth and survival application method developed by Mosmann.[23] The results, *i.e.* IC_{50} (μg/ml) of each compound against the both cells, are summarized in Table 3. Among the tested compounds, 7-amino-5-methylsulfinyl-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine **(10)** showed 50% inhibitory activity against CCRF-HSB-2 and KB cancer cells at the concentration 6.8 μg/ml and 8.5 μg/ml, respectively. Compound **4k** showed better activity against KB cells (8.9 μg/ml) than against CCRF-HSB-2 cells (23.0 μg/ml). Other compounds showed the activity at higher concentration.

Antiviral activity: The synthesized compounds were also evaluated for antiviral activity in vitro against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) according to the methods developed by Machida H., *et al*.[24,25] As summarized in Table 3, compounds **4m** and **10** showed their activity at the concentration >4 μg/ml and compound **4k** was potential at the concentration of >0.8 μg/ml against both viruses. Some compounds (**4f**, **h-j**, **8c**,**d**, **13a**,**b**) showed activity against both herpes virus at the concentration >20 μg/ml and the others did not exhibited any activity up to 100 μg/ml.

CONCLUSION

The synthesis of novel 8-thiaguanines, 8-thiaisoguanines, 5,7-diamino and other 8-thiapurines was accomplished easily from reaction of 6-amino-5-nitrosopyrimidines with sodium thiosulfate in aqueous acid media. The efficient methodology for the direct preparation of 4-β-D-ribofuranosyl-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (8-thiaxanthosine) was also established. Antitumor and antiviral activities of 8-thiapurine analogues have been evaluated in vitro. Only a few compounds showed moderate activity and others were very low toxic to exhibit potential activity.

EXPERIMENTAL

Mps were determined on a Yanagimoto micro-melting point hot stage apparatus and were uncorrected. IR spectra were obtained using a JASCO FT/IR-200 spectrophotometer in Nujol mulls. ^{1}H-NMR spectra were measured using a VXR 300 MHz spectrometer and chemical shift values were expressed in δ values (ppm) relative to TMS as an internal standard. Coupling constants are given in Hz and signals are quoted as follows: s, signlet; d, doublet; t, triplet; q, quartet; quin, quintet; sext, sextet; sept, septet; br, broad; m, multiplet. UV spectra were recorded with a Beckman DU-68 spectrophotometer. Microanalyses were measured by a Yanako CHN Corder MT-5 apparatus. Reaction progress was monitored by analytical thin-layer chromatography (TLC) on pre-coated glass plates (silica gel 60 F_{254}

Plate-Merck) and products were visualized by UV light. Column chromatography was accomplished on Daisogel IR-60 (63/210 μm, Daiso Co.).

Table 3. Evaluation of antitumor and antiviral activities in vitro for compounds (**4a–m**, **6a–k**, **8a–d**, **10**, **11**, **13a**,**b**, **17** and **18**)

Compd No.	Inhibitory concentration gainst tumor cell lines [IC_{50} (mg/ml)]		Inhibitory concentration against Herpes Simplex Virus [ED_{50} (mg/ml)]	
	CCRF-HSB-2	KB	HSV-1	HSV-2
4a	79.6	68.9	>100	>100
4b	92.6	82.1	>100	>100
4c	84.5	66.4	>100	>100
4d	>100	82.6	>100	>100
4e	67.5	74.2	>100	>100
4f	62.0	44.2	>20	>20
4g	71.9	68.5	>100	>100
4h	83.2	10.3	>20	>20
4i	93.5	87.5	>20	>20
4j	65.7	44.0	>20	>20
4k	23.0	8.9	>0.8	>0.8
4l	>100	>100	>100	>100
4m	>100	38.2	>4	>4
6a	>100	32.1	>100	>100
6b	76.1	>100	>100	>100
6c	n.d.	n.d.	n.d.	n.d.
6d	>100	>100	>100	>100
6e	84.7	>100	>100	>100
6f	>100	>100	>100	>100
6g	84.9	91.7	>100	>100
6h	n.d.	n.d.	n.d.	n.d.
6i	62.8	82.0	>100	>100
6j	64.7	93.7	>100	>100
6k	71.3	78.4	>100	>100
8a	64.3	76.9	>100	>100
8b	n.d.	n.d.	n.d.	n.d.
8c	85.1	58.3	>20	>20
8d	94.5	>100	>20	>20
10	6.8	8.5	>4	>4
11	>100	>100	>100	>100
13a	50.6	49.0	>20	>20
13b	35.1	36.6	>20	>20
17	>100	>100	>100	>100
18	>100	>100	>100	>100
Ara-C[a]	0.07	0.09	n.d.	n.d.
ACV[b]	n.d.	n.d.	0.16	0.16

[a]Ara-C: arabinosylcytidine. [b]ACV: acyclovir. The n.d. means not done.

5-Methylthio-[1,2,5]thiadiazolo[3,4-*d*]pyrimidin-7(6*H*)-one (3)

A suspension of 6-amino-2-methylthio-5-nitrosopyrimidin-4(3*H*)-one (**1**)[17] (2.0 g, 10.74 mmol) in 30% aqueous AcOH (50 mL) was heated at 80 °C and to the suspension was added $Na_2S_2O_3 \cdot 5H_2O$ (5.33 g, 21.48 mmol) by portions with stirring over 15 min. The heating was continued at 80 °C for additional 30 min. Upon cooling to rt, the solid deposited was collected by filtration followed by washing with water to give **3** (1.77 g, 82%) as pale yellow needles, mp 276–277 °C (EtOAc)[19]; R_f (EtOAc) 0.67; IR (Nujol) ν_{max}/cm^{-1}: 3160 (NH), 1700 (C=O); ^{1}H-NMR ($CDCl_3$): δ 2.67 (3H, s, CH_3), 12.84 (1H, br s, NH); *Anal*. Calcd for $C_5H_4N_4OS_2$: C, 29.99; H, 2.01; N, 27.98. Found: C, 30.13; H, 2.14; N, 27.94.

General Procedure for the Preparation of 5-Amino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidin-7(6*H*)-ones (4a-m)

Method A$_1$: To a suspension of 2,6-diamino-5-nitrosopyrimidin-4(3*H*)-ones[18] (**2a–m**, 3.5 mmol) in 20% aqueous AcOH (15–20 mL) was added $Na_2S_2O_3 \cdot 5H_2O$ (6–7 mmol) by portions with stirring at 70–80 °C and the mixture was stirred at 80–90 °C for 0.5–1.0 h. Upon keeping the reaction mixture in refrigerator for overnight, the solid deposited was collected by filtration and washed with cold water to afford the corresponding 5-amino-[1,2,5]thiadiazolopyrimidine derivatives (**4a–m**) as shown in the Tables 1 and 2.

Method B$_1$: A suspension of **3** (0.50 g, 2.50 mmol) and an appropriate amine (10–12 mmol) in 1-BuOH (40 mL) was heated under reflux for 12–24 h. After cooling to rt, the resulting solution was acidified with AcOH and evaporated to dryness *in vacuo*. The residue was triturated with water to give crystals, which were collected by filtration, washed with cold water and recrystallized from appropriate solvent to afford the corresponding 5-amino derivatives (**4b**,**c**, **e–k**) in 54–78% yields (Tables 1 and 2).

General Procedure for the Preparation of 7-Amino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidin-5(4*H*)-ones (6a–k)

Method A$_2$: A purple suspension of 4,6-diamino-5-nitrosopyrimidin-2(1*H*)-ones[26, 27] (**5a–i**, 4.0 mmol) in 10% HCl (12 mL) was heated to *ca*. 70–90 °C and to the suspension was added $Na_2S_2O_3 \cdot 5H_2O$ by portions with stirring until the purple color of the reaction mixture disappeared completely to produce the light yellow solution. The solution was cooled to rt and neutralized with 28% aqueous NH_3. Then, the solution was kept in refrigerator for several hours. Thus, the solid deposited was collected by filtration and washed with cold water to afford the corresponding 7-amino-[1,2,5]thiadiazolopyrimidine derivatives (**6a–i**) as shown in the Tables 1 and 2.

Method B$_2$: To a suspension of 4,6-diamino-5-nitrosopyrimidin-2(1*H*)-ones (**5g–k**, 3.0 mmol) in 20% aqueous AcOH (15 mL) was added $Na_2S_2O_3 \cdot 5H_2O$ (*ca*. 6 mmol) by portions with stirring at 80–90 °C until the red or violet color of the reaction mixture disappeared completely. Then, the heating was

continued for additional 45 min and the reaction mixture was kept at rt for 1 d. Thus, the solid deposited was collected by filtration and washed with cold water to give the corresponding 7-amino-[1,2,5]thiadiazolopyrimidine derivatives (**6g–k**) as shown in the Tables 1 and 2.

General Procedure for the Preparation of 5-Alkylamino-7-amino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidines (8a-d)

A mixture of 4,6-diamino-2-methylthio-5-nitrosopyrimidine[28] (**7**, 1.0 g, 5.40 mmol) and an appropriate amine (20–25 mmol) in water (15 mL) was heated under reflux for 25–45 min. After cooling to rt, the solution was acidified with AcOH. The solid deposited was filtered and transferred into 20% aqueous AcOH (25 mL). To the resulting solution was added $Na_2S_2O_3 \cdot 5H_2O$ (*ca*. 8.0 mmol) by portions with stirring at 80–90 °C and the reaction mixture was heated at 90 °C for 30–45 min. After the reaction was complete, the solutoion was kept in refrigerator for overnight. The solid deposited was collected by filtration and washed with cold water to give the corresponding 5,7-diamino-[1,2,5]thiadiazolopyrimidine derivatives (**8a–d**).

7-Amino-5-propylamino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine (8a)

Yield: 0.50 g (44%); yellow powdery crystals; mp 152–153 °C (*n*-hexane-EtOAc); R_f (EtOAc) 0.60; IR (Nujol) ν_{max}/cm^{-1}: 3460, 3360, 3330 (NH); ^{1}H-NMR ($CDCl_3$): δ 0.99 (3H, t, J = 7.5, CH_3), 1.67 (2H, sept, J = 7.2 Hz, N-CH_2CH_2), 3.49 (2H, q, J = 6.3 Hz, N-CH_2), 5.24 (1H, br s, NH), 5.78 (2H, br s, NH_2); *Anal*. Calcd for $C_7H_{10}N_6S$: C, 39.99; H, 4.79; N, 39.97. Found: C, 40.21; H, 4.77; N, 40.01.

7-Amino-5-isopropylamino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine (8b)

Yield: 0.67 g (59%); yellow powdery crystals; mp 184–185 °C (*n*-hexane-EtOAc); R_f (EtOAc) 0.59; IR (Nujol) ν_{max}/cm^{-1}: 3445, 3340, 3280 (NH); ^{1}H-NMR ($CDCl_3$): δ 1.27 (6H, d, J = 6.6 Hz, 2 x CH_3), 4.33 (1H, br s, CH), 5.13 (1H, br s, NH), 5.83 (2H, br s, NH_2); *Anal*. Calcd for $C_7H_{10}N_6S$: C, 39.99; H, 4.79; N, 39.97. Found: C, 39.68; H, 4.69; N, 39.65.

7-Amino-5-piperidino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine (8c)

Yield: 0.66 g (52%); yellow needles; mp 215–216 °C (H_2O); R_f(EtOAc) 0.60; IR (Nujol) ν_{max}/cm^{-1}: 3400, 3300 (NH); ^{1}H-NMR ($CDCl_3$): δ 1.63 [6H, br s, $(CH_2)_3$], 3.90 (4H, t, J = 6.0 Hz, CH_2NCH_2), 5.75 (2H, br s, NH_2); *Anal*. Calcd for $C_9H_{12}N_6S$: C, 45.75; H, 5.12; N, 35.57. Found: C, 45.48; H, 5.00; N, 35.69.

7-Amino-5-morpholino-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine (8d)

Yield: 0.71 g (55%); yellow needles; mp 244–245 °C (H_2O); R_f(EtOAc) 0.73; IR (Nujol) ν_{max}/cm^{-1}: 3430, 3320 (NH); ^{1}H-NMR ($CDCl_3$): δ 3.76 (4H, t, J = 5.1 Hz, 2 x -CH_2-), 3.95 (4H, t, J = 5.1 Hz, 2 x -CH_2-), 5.81 (2H, br s, NH_2); *Anal.* Calcd for $C_8H_{10}N_6OS$: C, 40.33; H, 4.23; N, 35.27. Found: C, 40.12; H, 4.14; N, 35.46.

7-Amino-5-methylthio-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine (9)

This compound **9** was prepared from **7** (2.0 g, 10.80 mmol) in an analogous way to the compound **3** and obtained as yellow needles; yield: 1.6 g (74%); mp 203–204 °C (*n*-hexane-EtOAc) (Lit.[14] 204–206 °C); R_f (EtOAc) 0.56; IR (Nujol) ν_{max}/cm^{-1}: 3420, 3290 (NH); ^{1}H-NMR ($CDCl_3$): δ 2.70 (3H, s, CH_3), 7.43 (2H, s, NH_2). *Anal.* Calcd for $C_5H_5N_5S_2$: C, 30.14; H, 2.53; N, 35.15. Found: C, 30.46; H, 2.57; N, 35.01.

7-Amino-5-methylsulfinyl-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine (10)

A mixture of **9** (0.3 g, 1.51 mmol) and NBS (0.27 g, 1.52 mmol) in a mixture of MeOH (6 mL) and water (1.5 mL) was stirred at 35–40 °C for 2 h. The resulting solid was collected by filtration and washed with ethyl acetate to give **10** as yellow powdery crystals; yield: 0.19 g (59%); mp >300 °C (MeOH); R_f (EtOAc) 0.25; IR (Nujol) ν_{max}/cm^{-1}: 3455, 3290 (NH); ^{1}H-NMR [$(CD_3)_2SO$]: δ 2.85 (3H, s, CH_3), 9.17 (2H, d, J = 7.8 Hz, NH_2); *Anal.* Calcd for $C_5H_5N_5OS_2$: C, 27.90; H, 2.34; N, 32.53. Found: C, 27.68; H, 2.43; N, 32.83.

7-Butylamino-5-methylthio-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine (11)

A solution of **9** (0.4 g, 2.01 mmol) in butylamine (10 mL) was stirred at 40 °C for 2 d. Excess butylamine was evaporated to dryness *in vacuo*. The resulting residue was purified by column chromatography on silica gel using *n*-hexane-EtOAc (2:3) as eluting solvent to give **11** as yellow needles; yield: 0.35 g (68%); mp 97–98 °C (*n*-octane-EtOAc); R_f(*n*-hexane : EtOAc, 1:4) 0.54; IR (Nujol) ν_{max}/cm^{-1}: 3390 (NH); ^{1}H-NMR ($CDCl_3$): δ 0.99 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.47 (2H, sext, J = 7.2 Hz, CH_2CH_3), 1.72 (2H, quin, J = 7.2 Hz, N-CH_2CH_2), 2.63 (3H, s, S-CH_3), 3.68 (2H, q, J = 7.2 Hz, N-CH_2), 6.34 (1H, br s, NH); *Anal*. Calcd for $C_9H_{13}N_5S_2$: C, 42.33; H, 5.13; N, 27.43. Found: C, 42.13; H, 5.04; N, 27.25.

5-Phenyl-[1,2,5]thiadiazolo[3,4-*d*]pyrimidin-7(6*H*)-one (13a)

A suspension of 6-amino-5-nitroso-2-phenylpyrimidin-4(3*H*)-one[29] (**12a**, 0.50 g, 2.31 mmol) in 30% aqueous AcOH (30 mL) was treated with $Na_2S_2O_3 \cdot 5H_2O$ (1.29 g, 5.20 mmol) at 90 °C for 1 h. Upon cooling the mixture to rt, the solid deposited was collected by filtration and recrystallized from EtOAc to afford **13a** as orange needles; yield: 0.30 g (56%); mp 260–261 °C; R_f(EtOAc) 0.74; IR (Nujol) ν_{max}/cm^{-1}: 3160 (NH), 1700 (C=O); ^{1}H-NMR ($CDCl_3$) δ: 7.59–7.67 (3H, m, Ph-*m,p*H), 8.21 (2H, d, J = 6.1 Hz,

Ph-*o*H), 10.77 (1H, br s, OH); *Anal*. Calcd for $C_{10}H_6N_4OS$: C, 52.16; H, 2.63; N, 24.33. Found: C, 52.39; H, 2.91; N, 23.98.

7-Amino-5-phenyl-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine (13b)

This compound **13b** was prepared from 4,6-diamino-5-nitroso-2-phenylpyrimidine[28] (**12b**, 0.40 g, 1.86 mmol) following the above procedure and obtained as orange powdery crystals; yield: 0.27 g (63%); mp 231–232 °C (*n*-octane-EtOAc); R_f(EtOAc) 0.75; IR (Nujol) ν_{max}/cm^{-1}: 3450, 3330 cm^{-1} (NH); ^{1}H-NMR ($CDCl_3$) δ: 6.39 (2H, br s, NH_2), 7.46–7.52 (3H, m, Ph-*m,p*H), 8.55 (2H, dd, $J_{o,p}$ = 1.8 Hz, $J_{o,m}$ = 7.5 Hz, Ph-*o*H); *Anal*. Calcd for $C_{10}H_7N_5S$: C, 52.39; H, 3.08; N, 30.55. Found: C, 52.38; H, 3.27; N, 30.17.

6-Amino-2',3'-*O*-isopropylideneuridine (15)

A mixture of 5',6-*O*-anhydro-2',3'-*O*-isopropylidene-6-hydroxyuridine (**14**,[20] 2.0 g, 7.09 mmol), NH_4Cl (0.35 g) and liquid ammonia (40 mL) in steel sealed tube was heated at 50 °C for 1.5 d. Then, ammonia solution was removed *in vacuo* and the residue was purified by column chromatography on silica gel using EtOAc-EtOH (10:1) as eluting solvent to give **15** as colorless powdery crystal; yield: 0.85 g (40 %); mp 229–230 °C (EtOAc-EtOH); R_f(EtOAc-EtOH, 4:1) 0.43; IR (Nujol) ν_{max}/cm^{-1}: 3405 (OH), 3300, 3260, 3210 (NH), 1735, 1650 (C=O); ^{1}H-NMR [$(CD_3)_2SO$] δ: 1.29 and 1.49 (each 3H, each s, 2 x CH_3), 3.61 (2H, t, J = 4.5 Hz, 5'-CH_2), 4.00 (1H, q, J = 3.9 Hz, 4'-H), 4.62 (1H, d, J = 2.1 Hz, 5-H), 4.80 (1H, dd, $J_{3',4'}$ = 3.9 Hz, $J_{2',3'}$ = 6.9 Hz, 3'-H), 5.07 (1H, dd, $J_{1',2'}$ = 3.3 Hz, $J_{2',3'}$ = 6.9 Hz, 2'-H), 5.36 (1H, t, J = 4.8 Hz, OH, exchangeable with D_2O), 6.16 (1H, d, $J_{1',2'}$ = 3.3 Hz, 1'-H), 6.81 (2H, s, NH_2, exchangeable with D_2O), 10.57 (1H, s, NH, exchangeable with D_2O); UV λ_{max} (EtOH) nm (log ε): 271 (4.52); *Anal*. Calcd for $C_{12}H_{17}N_3O_6$: C, 48.16; H, 5.73; N, 14.04. Found: C, 48.00; H, 5.55; N, 13.93.

6-Amino-5-hydroxyimino-6-imino-2',3'-*O*- isopropylidene-5,6-dihydorouridine (16)

The compound **15** (1.0 g, 3.34 mmol) was dissolved in a warming solution of EtOH (20 mL) and EtOAc (20 mL). After cooling to rt, amyl nitrite (2.5 mL) was added to the solution dropwise with stirring over 15 min. After one drop of concd HCl was added to the solution, the purple crystals deposited immediately. The mixture was stirred at rt for more 15 min and kept in refrigerator for several hours. The solid deposited was filtered and washed with EtOAc to afford the 5-nitroso derivative **16** as purple powdery crystals; yield: 0.97 g (88%); mp >300 °C (EtOAc-EtOH); R_f (EtOAc-EtOH, 4:1) 0.54; IR (Nujol) ν_{max}/cm^{-1}: 3335 (OH), 3200, 3150, 3090 (NH), 1740, 1700 (C=O); ^{1}H-NMR [$(CD_3)_2SO$] δ: 1.29 and 1.50 (each 3H, each s, 2 x CH_3), 3.56 (2H, t, J = 4.5 Hz, 5'-CH_2), 4.09 (1H, q, J = 3.6 Hz, 4'-H), 4.78 (1H, dd, $J_{3',4'}$ = 3.6 Hz, $J_{2',3'}$ = 6.3 Hz, 3'-H), 5.14 (1H, dd, $J_{1',2'}$ = 2.4 Hz, $J_{2',3'}$ = 6.3 Hz, 2'-H), 5.39 (1H, br s, OH, exchangeable with D_2O), 6.16 (1H, d, $J_{1',2'}$ = 2.4 Hz, 1'-H), 9.52 (1H, br s, N-OH, exchangeable

with D_2O), 11.63 (1H, s, 3-NH, exchangeable with D_2O), 13.83 (1H, br s, =NH, exchangeable with D_2O); UV λ_{max} (EtOH) nm (log ε): 316 (4.59); *Anal*. Calcd for $C_{12}H_{16}N_4O_7 \cdot 1/10\ H_2O$: C, 43.66; H, 4.95; N, 16.97. Found: C, 43.78; H, 4.84; N, 16.59.

4-(2',3'-*O*-Isopropylidene-β-D-ribofuranosyl)-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (17)

A solution of **16** (1.0 g, 3.05 mmol) in a mixture of water (12 mL), MeOH (40 mL) and AcOH (6 mL) was heated to *ca*. 50 °C and to the solution was added a solution of $Na_2S_2O_3 \cdot 5H_2O$ (1.40 g, 5.64 mmol) in water (5 mL) dropwise. The stirring was continued at 55–60 °C until the color of the solution changed completely from purple to light yellow. Then, the solution was evaporated to dryness *in vacuo* at low temperature. The product cropped was extracted with absolute EtOH from the residue and the extract was evaporated to dryness *in vacuo*. The residue was subjected to column chromatography on silica gel using *n*-hexane-EtOAc (3:5) as eluent to afford the compound **17** as colorless powdery crystals; yield: 0.64 g (61%); mp 126–127 °C (EtOAc); R_f(EtOAc) 0.52; IR (Nujol) ν_{max}/cm^{-1}: 3450 (OH), 3200 (NH), 1720 (C=O); ^{1}H-NMR ($CDCl_3$) δ: 1.38 and 1.63 (each 3H, each s, 2 x CH_3), 2.93 (1H, br s, OH, exchangeable with D_2O), 3.82 (1H, br dd (dd, $J_{4',5'a}$ = 3.82 Hz, J_{gem} = 12.3 Hz), 5'-H_a), 3.92 (1H, dd, $J_{4',5'b}$ = 2.7 Hz, J_{gem} = 12.3 Hz, 5'-H_b), 4.33 (1H, q, J = 3.6 Hz, 4'-H), 5.07 (1H, dd, $J_{3',4'}$ = 3.9 Hz, $J_{2',3'}$ = 6.6 Hz, 3'-H), 5.32 (1H, dd, $J_{1',2'}$ = 3.6 Hz, $J_{2',3'}$ = 6.6 Hz, 2'-H), 6.54 (1H, d, $J_{1',2'}$ = 3.6 Hz, 1'-H), 9.03 (1H, br s, NH, exchangeable with D_2O); UV λ_{max} (EtOH) nm (log ε): *248* (3.73), 314 (4.13); *Anal*. Calcd for $C_{12}H_{14}N_4O_6S$: C, 42.10; H, 4.12; N, 16.37. Found: C, 41.90; H, 4.03; N, 16.22.

4-β-D-Ribofuranosyl-[1,2,5]thiadiazolo[3,4-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (18)

A solution of **17** (0.5 g, 1.46 mmol) in a mixture of MeOH (50 mL) and 0.5N HCl (20 mL) was heated at 50 °C for 7 h. After cooling to rt, the solution was neutralized with triethylamine and evaporated *in vacuo*. Finally, co-evaporation of the solution with EtOH was achieved to complete dryness. The dry residue was dissolved in absolute ethanol and the insoluble material was filtered off. The filtrate was evaporated *in vacuo* and the residue was purified by column chromatography using EtOAc as eluting solvent to give the compound **18** as colorless powdery crystals; yield 0.38 g (86%); mp 115 °C (amorphous, EtOAc); R_f (EtOAc-MeOH, 8:1) 0.45; IR (Nujol) ν_{max}/cm^{-1}: 3385 (OH), 3190 (NH), 1720 (C=O); ^{1}H-NMR [$(CD_3)_2SO$] δ: 3.47 [1H, ddd (dd after addition of D_2O, $J_{4',5'a}$ = 6.0 Hz, J_{gem} = 11.7 Hz), $J_{4',5'a}$ = 6.0 Hz, J_{gem} = 11.7 Hz, $J_{5'a,OH}$ = 5.7 Hz, 5'-H_a], 3.65 [1H, ddd (dd after addition of D_2O, $J_{4',5'b}$ = 4.2 Hz, J_{gem} = 11.7 Hz), $J_{4',5'b}$ = 4.8 Hz, J_{gem} = 11.7 Hz, $J_{5'b,OH}$ = 5.7 Hz, 5'-H_b], 3.78 [1H, q (dd after addition of D_2O, $J_{4',5'}$ = 4.2 Hz, $J_{3',4'}$ = 5.4 Hz), J = 5.7 Hz, 4'-H], 4.18 [1H, q (dd after addition of D_2O, $J_{2',3'}$ = 5.7 Hz, $J_{3',4'}$ = 5.4 Hz), J = 5.7 Hz, 3'-H], 4.60 (1H, t, J = 5.7 Hz, 5'-OH, exchangeable with D_2O), 4.67 [1H,

q (dd after addition of D_2O, $J_{2',3'}$ = 5.7 Hz, $J_{1',2'}$ = 4.8 Hz), J = 5.4 Hz, 2'-H], 5.03 (1H, d, $J_{3',OH}$ = 6.0 Hz, 3'-OH, exchangeable with D_2O), 5.17 (1H, $J_{2',OH}$ = 5.1 Hz, 2'-OH, exchangeable with D_2O), 6.12 (1H, d, $J_{1',2'}$ = 4.8 Hz, 1'-H), 12.03 (1H, s, NH, exchangeable with D_2O). UV λ_{max} (EtOH) nm: (log ε): *249* (3.71), 315 (4.09); *Anal*. Calcd for $C_9H_{10}N_4O_6S$: C, 35.76; H, 3.33; N, 18.54. Found: C, 36.00; H, 3.40; N, 18.22.

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