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DESIGN, SYNTHESIS AND EVALUATION OF ANTITUMOR AND ANTIVIRAL ACTIVITIES OF 5-AMINO-1*H*-[1,2,3]TRIAZOLO[4,5-*d*]-PYRIMIDIN-7(6*H*)-ONES (8-AZAGUANINES) AND 7-AMINO-1*H*-[1,2,3]-TRIAZOLO[4,5-*d*]PYRIMIDIN-5(4*H*)-ONES (8-AZAISOGUANINES)

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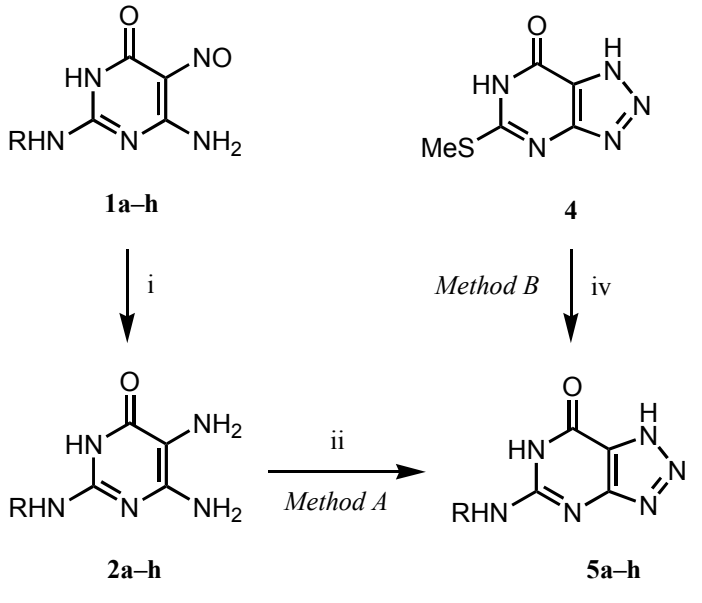
Abstract – Preparation of 5-amino-1*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-ones (**5a–h**) and 7-amino-1*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-5(4*H*)-ones (**8a–n**) was accomplished by nitrosative cyclization of the desired 2,5,6-triamino-pyrimidine (**2a–h**) and 4,5,6-triaminopyrimidine derivatives (**7a–n**) with nitrous acid, respectively. Compounds **5a–h** were also prepared by nucleophilic replacement of the methylthio group of 5-methylthio-1*H*-[1,2,3]triazolo[4,5-*d*]-pyrimidin-7(6*H*)-one (**4**) by appropriate amines. Similarly, some 7-amino derivatives (**10a–i**) were synthesized by replacement of the thiol groups of the 7-thiol derivative (**9**) by appropriate amines. Antitumor and antiviral activities of the synthesized compounds were evaluated *in vitro*.

INTRODUCTION

Currently some drugs are in use for the treatment of neoplastic and viral infected diseases, but unfortunately patients are still suffering from adverse side effects. Therefore, synthesis of an effective and selective anticancer and antiviral agent is an extending challenge to date. To accomplish the challenge, tremendous efforts have been focused on the modification of established biologically active substances for enhancing their activities leading to the ideal drugs. The introduction as well as the positional alternation of different functional groups on biologically active substances is an important approach to them. Among the established biologically active substances, naturally occurring purine

derivatives and purine analogues have attracted great attention because of their broad biomedical values[1–7] as therapeutics and molecular tools as well as probes for investigating the biological systems. The introduction of substituents onto purine ring can significantly affect the steric effect, hydrogen-bonding and hydrophobicity that results in altered interactions with nucleic acids and proteins. Diaminopurines,[2,8] aza analogue of purine (8-azapurine),[8] some oxopurines[6] and oxo-8-azapurines[2] have been evaluated for antitumor and antiviral activities. The combination of amino and oxo groups at the 2- and/or 6-position of purine and 8-azapurine is an effective tool to enhance their biological activities.[2,8–9] Synthesis and characterization of 8-azaguanine derivatives, especially the 8-azaisoguanine derivatives, have not been inquired sufficiently for biological evaluation in this area. Thus, in connection with our continuous research on fused pyrimidines,[10–17] we have designed, synthesized, and evaluated antitumor and antiviral activities for 5-amino-1*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-ones (8-azaguanines) and 7-amino-1*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-5(4*H*)-ones (8-azaisoguanines), and report herein.



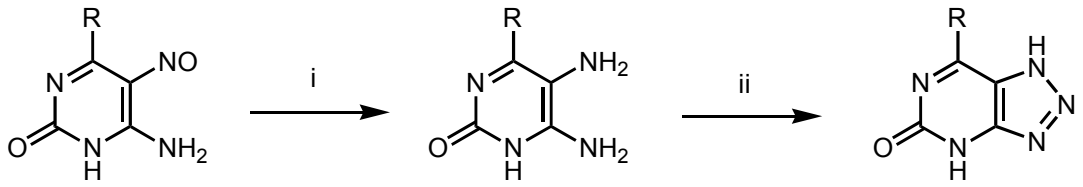
Compd	R
1, **2**, **5 a**	Pr
b	*i*-Pr
c	Bu
d	*i*-Bu
e	cyclohexyl
f	C_8H_{17}
g	$HOCH_2CH_2CH_2$
h	$PhCH_2CH_2$

Reagents and conditions: i, $Na_2S_2O_4$, 5% AcOH (aq), 70–80 °C; aq. NH_3; ii, $NaNO_2$, 25% AcOH (aq), rt; iii, $NaNO_2$, 10% HCl, rt; iv, amine, 1-BuOH, reflux.

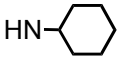
Scheme 1

RESULTS AND DISCUSSION

Reduction of 2-monoalkylamino-6-amino-5-nitrosopyrimidin-4(3*H*)-ones (**1a–h**)[16] with sodium dithionite afforded the requisite key intermediates, 2,5,6-triaminopyrimidine derivatives (**2a–h**), which were used without isolation and characterization for the preparation of 5-monoalkylamino-1*H*-[1,2,3]triazolo-[4,5-*d*]pyrimidin-7(6*H*)-ones (8-azaguanines) (**5a–h**). That is, nitrosative cyclization of the 2,5,6-



6a–n **7a–n** **8a–n**

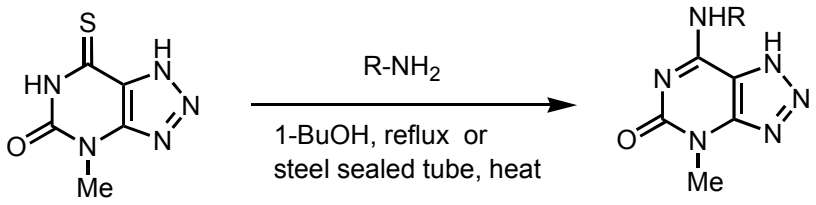
Compd	R	Compd	R
6, 7, 8 a	NHMe	**6, 7, 8 h**	NHC_8H_{17}
b	NHEt	**i**	$NHCH_2CH_2OH$
c	NHPr	**j**	NHPh
d	NH*i*-Pr	**k**	$NHCH_2Ph$
e	NHBu	**l**	$NHCH_2CH_2Ph$
f	NH*i*-Bu	**m**	
g		**n**	

Reagents and conditions: i, $Na_2S_2O_4$, 5% AcOH (aq), 70–80 °C; aq. NH_3; ii, $NaNO_2$, 25% AcOH (aq), rt.

Scheme 2

triaminopyrimidine derivatives (**2a–h**) with nitrous acid at room temperature afforded the corresponding 8-azaguanine derivatives (**5a–h**) in good yields as shown in the Scheme 1 and Tables 1 and 4 (*Method A*). The synthesis of 8-azaguanines (**5a–h**) was also attained by replacement of the methylthio group of 5-methylthio-1*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-one (**4**) by appropriate amines in 1-butanol under reflux (*Method B*). The nucleophilic replacement of the methylthio group at the 5-position of **4** by amines was smooth, however these reactions were not expeditious. On the other hand, 7-Amino-1*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-5(4*H*)-ones (8-azaisoguanines) were prepared starting from 4-alkylamino-6-amino-5-nitrosopyrimidin-2(1*H*)-ones (**6a–n**).[16] Reduction of the 5-nitroso group with sodium dithionite gave the corresponding 4,5,6-triaminopyrimidines (**7a–n**), which without isolation and characterization were cyclized nitrosatively with nitrous acid to afford the corresponding 8-azaisoguanine derivatives (**8a–n**) as shown in Scheme 2 and Tables 2 and 4. Similarly, when 4-methyl-1*H*-[1,2,3]-triazolo[4,5-*d*]pyrimidin-5(4*H*)-one-7(6*H*)-thione (**9**)[17] was treated with different amines under reflux in 1-butanol, the corresponding 7-amino derivatives (**10c**, **e–i**) were obtained in good yields as shown in the Scheme 3 and Tables 3 and 4. The reactions of **9** with methyl, ethyl and isopropyl amines were carried out in steel sealed tube under heating to afford the **10a**,**b**,**d**. This simple and convenient preparation suggests that the conversion of thiol into alkylthio group is not essential for the introduction of amino

groups by nucleophilc attack. It is noteworthy that the substitution of thiol by amines at the 7-position takes place much more readily than the replacement of methylthio group at the 5-position. The replacement of thiol or methylthio group of 8-azapurine by amino groups has rarely been used. The IR, ^{1}H-NMR and microanalyses of the synthesized compounds were quite consistent with the structures as shown in the Table 4.



9 **10a–i**

Compd	R
a	Me
b	Et
c	Pr
d	*i*-Pr
e	Bu
f	C_8H_{17}
g	CH_2CH_2OH
h	CH_2Ph
i	CH_2CH_2Ph

Scheme 3

BIOLOGICAL ACTIVITY

Antitumor activity: The synthesized 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 [3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay[19] for cellular growth and survival application method developed by Mosmann.[20] The results, i.e., IC_{50} (μg/mL) of each compound against both cells are summarized in Table 5. Most of the tested compounds, e.g., **5a**,**c**,**d**,**f**,**h** and **8j** showed 50% inhibitory activity against CCRF-HSB-2 and KB cancer cells at the concentration 26–67 μg/mL. Other compounds showed the activities 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*.[21,22] The results are summarized in Table 5. Compounds **5f** and **8j–l** showed their activities against both viruses at the concentration >20 μg/mL and other compounds did not exhibit any activity up to 100 μg/mL.

Table 1. Yields and analytical data for compounds **(5a–h)**

Compd No.	Yield (%) *(Method)*	Appearance (shape of crystal)	Mp (°C)	Recrystn. solvent	Formula (R_f)[a]	Analysis (%) Calcd (Found)		
						C	H	N
5a	74 (*A*) 71 (*B*)	colorless (powder)	286–288	EtOH	$C_7H_{10}N_6O$ (0.52)	43.29 (43.17	5.19 5.09	43.28 43.37)
5b	67 (*A*)	pale yellow (powder)	280–282	EtOH	$C_7H_{10}N_6O\cdot 3/5H_2O$ (0.52)	41.01 (40.81	5.51 5.32	40.99 41.28)
5c	73 (*A*) 68 (*B*)	colorless (powder)	278–279	EtOAc-EtOH	$C_8H_{12}N_6O$ (0.54)	46.15 (46.03	5.81 5.52	40.36 40.18)
5d	69 (*A*) 63 (*B*)	colorless (powder)	270–271	EtOAc-EtOH	$C_8H_{12}N_6O$ (0.56)	46.15 (45.85	5.81 5.64	40.36 39.98)
5e	71 (*A*) 49 (*B*)	pale yellow (powder)	261–262	EtOAc-EtOH	$C_{10}H_{14}N_6O$ (0.56)	51.27 (51.04	6.02 6.00	35.88 35.90)
5f	77 (*A*) 68 (*B*)	pale yellow (powder)	226–227	EtOAc-EtOH	$C_{12}H_{20}N_6O\cdot 1/5H_2O$ (0.62)	53.79 (53.46	7.67 7.27	31.37 31.21)
5g	63 (*A*) 61 (*B*)	colorless (powder)	274–276	H_2O	$C_7H_{10}N_6O_2$ (0.26)	40.00 (39.80	4.80 4.69	39.98 39.88)
5h	78 (*A*) 69 (*B*)	pale yellow (powder)	256–257	EtOH	$C_{12}H_{12}N_6O$ (0.54)	56.24 (56.43	4.72 4.84	32.79 32.56)

[a] Solvent system for TLC is EtOAc : EtOH (4:1, v/v).

CONCLUSION

5-Amino-1*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-ones (8-azaguanines) and 7-amino-1*H*-[1,2,3]-triazolo[4,5-*d*]pyrimidin-5(4*H*)-ones (8-azaisoguanines) were synthesized smoothly by different synthetic methods, *e.g.*, nitrosative cyclization of diaminopyrimidines and nucleophilic replacement of methylthio or thiol with amines. Synthesized compounds were screened against different cancer and viral cells *in vitro*. However, these compounds were found 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 by a JASCO FT/IR-200 spectrophotometer in Nujol mulls and the absorption value in italic refers to wave number at which shoulder or inflexion occurs in the absorption. ^{1}H-NMR spectra were recorded 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; br, broad; m, multiplet. Microanalyses were measured using a Yanako CHN Corder MT-5-apparatus. Reaction progress was monitored by analytical thin-layer chromatography (TLC) on pre-coated glass plates (silica

Table 2. Yields and analytical data for compounds **(8a–n)**

Compd No.	Yield (%) *(Method)*	Appearance (shape of crystal)	Mp (°C)	Recrystn. solvent	Formula (R_f)[a]	Analysis (%) Calcd (Found)		
						C	H	N
8a	68	pale yellow (leaves)	>300	DMF-H_2O	$C_5H_6N_6O$ (0.32)	36.15 (36.21	3.64 3.75	50.58 50.29)
8b	70	pale yellow (powder)	>300	DMF-H_2O	$C_6H_8N_6O$ (0.34)	40.00 (39.79	4.48 4.37	46.65 46.56)
8c	88	pale yellow (needles)	>300	H_2O	$C_7H_{10}N_6O$ (0.43)	43.29 (43.00	5.19 4.92	43.28 43.06)
8d	84	colorless (powder)	>300	H_2O	$C_7H_{10}N_6O\cdot 7/10H_2O$ (0.45)	40.65 (40.54	5.56 5.26	40.64 40.94)
8e	86	colorless (powder)	>300	H_2O	$C_8H_{12}N_6O$ (0.48)	46.15 (46.24	5.81 5.62	40.36 40.60)
8f	79	pale yellow (needles)	>300	H_2O	$C_8H_{12}N_6O\cdot 1/7H_2O$ (0.49)	45.58 (45.72	5.87 5.71	39.87 39.62)
8g	82	pale yellow (powder)	>300	EtOH	$C_{10}H_{14}N_6O$ (0.50)	51.27 (50.97	6.02 5.87	35.88 35.78)
8h	77	yellow (powder)	>300	EtOH	$C_{12}H_{20}N_6O$ (0.59)	54.53 (54.25	7.63 7.33	31.79 31.60)
8i	71	pale yellow (prisms)	>300	H_2O	$C_6H_8N_6O_2\cdot 3/7H_2O$ (0.29)	35.35 (35.23	4.38 4.42	41.22 42.29)
8j	87	pale yellow (powder)	>300	EtOH	$C_{10}H_8N_6O$ (0.51)	52.63 (52.44	3.53 3.63	36.83 36.44)
8k	83	pale yellow (powder)	>300	EtOH	$C_{11}H_{10}N_6O\cdot 1/8H_2O$ (0.46)	54.04 (53.96	4.23 4.13	34.37 34.28)
8l	83	yellow (prisms)	>300	EtOH	$C_{12}H_{12}N_6O$ (0.50)	56.24 (56.30	4.72 4.82	32.79 32.80)
8m	78	colorless (powder)	>300	H_2O	$C_9H_{12}N_6O\cdot 1/10H_2O$ (0.36)	48.68 (48.62	5.54 5.49	37.85 38.02)
8n	74	colorless (needles)	>300	H_2O	$C_8H_{10}N_6O_2\cdot 2/5H_2O$ (0.31)	41.88 (41.77	4.75 4.63	36.63 36.71)

[a] Solvent system for TLC is EtOAc : EtOH (4:1, v/v).

gel 60 F_{254} Plate-Merck) and products were visualized by UV light.

Preparation of 5-methylthio-1*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-one (4)

A solution of 5,6-diamino-2-methylthiopyrimidin-4(3*H*)-one (**3**, 1.50 g, 8.71 mmol) in 10% HCl (20 mL) was cooled in ice-water and to it was added a solution of $NaNO_2$ (0.97 g, 14 mmol) dropwise with stirring over 15 min. Then, the reaction mixture was brought to rt and stirred for 2 h. After the reaction was complete, the solid deposited was collected by filtration and washed with water to give the triazolopyrimidine derivative (**4**)[18] as pale yellow prisms; yield: 1.29 g (81%); mp 262–263 °C

Table 3. Yields and analytical data for compounds **(10a–i)**

Compd No.	Yield (%) *(Method)*	Appearance (shape of crystal)	Mp (°C)	Recrystn. solvent	Formula (R_f)[a]	Analysis (%) Calcd (Found)		
						C	H	N
10a	69	colorless (powder)	>300	DMF-H_2O	$C_6H_8N_6O\cdot$ 1/6 H_2O (0.27)	39.34 (39.16	4.59 5.08	45.88 46.17
10b	59	pale yellow (powder)	286–287	DMF-H_2O	$C_7H_{10}N_6O$ (0.30)	43.29 (43.17	5.19 4.93	43.28 43.37)
10c	65	pale yellow (powder)	281–282	DMF-H_2O	$C_8H_{12}N_6O$ (0.37)	46.15 (45.73	5.81 5.65	40.36 40.50)
10d	67	colorless (powder)	296–297	DMF-H_2O	$C_8H_{12}N_6O\cdot$ 1/5H_2O (0.39)	45.36 (45.53	5.90 5.72	39.67 39.38)
10e	63	pale yellow (powder)	277–278	DMF-H_2O	$C_9H_{14}N_6O\cdot$ 2/5H_2O (0.42)	47.11 (47.42	6.50 6.23	36.63 36.31)
10f	68	colorless (powder)	268–269	DMF-H_2O	$C_{13}H_{22}N_6O$ (0.48)	56.09 (55.74	7.97 7.72	30.19 30.24)
10g	41	colorless (powder)	274–275	DMF-H_2O	$C_7H_{10}N_6O_2\cdot$ 1/5H_2O (0.27)	39.32 (39.49	4.90 4.82	39.31 38.98)
10h	78	pale yellow (powder)	270–271	DMF-H_2O	$C_{12}H_{12}N_6O$ (0.43)	56.24 (56.09	4.72 4.67	32.79 32.57)
10i	73	colorless (powder)	297–298	DMF-H_2O	$C_{13}H_{14}N_6O\cdot$ 1/4H_2O (0.45)	56.82 (56.79	5.32 5.19	30.58 30.26)

[a] Solvent system for TLC is EtOAc : EtOH (4:1, v/v).

(EtOH); R_f= 0.5 (*n*-hexane : EtOAc, 1:2); IR (Nujol) υ_{max}/cm^{-1}: 3160, *3100* (NH), 1680 (C=O); ^{1}H-NMR [$(CD_3)_2SO$]: δ 2.56 (3H, s, S-CH_3), 12.78 (1H, br s, 6-NH), 15.96 (1H, br s, 1-NH); *Anal*. Calcd for $C_5H_5N_5OS$: C, 32.78; H, 2.75; N, 38.23. Found: C, 32.93; H, 2.79; N, 38.02.

General procedure for 5-amino-1*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-ones (5a–h)

Method A: To a suspension of 2,6-diamino-5-nitrosopyrimidine derivatives[16] (**1a–h**, 6.0 mmol) in 5% aqueous acetic acid (25 mL) was added $Na_2S_2O_4$ (20–24 mmol) by portions with stirring at 70–80 °C. Then, the mixture was stirred at 80 °C for 0.5–1.0 h. After the reaction was complete, the solution was cooled to rt and neutralized with 28% aqueous ammonia. Upon keeping in refrigerator for several hours, the solid deposited was collected by filtration to give the corresponding 2,5,6-triamino intermediates (**2a–h**). It was dissolved in 25% aqueous acetic acid (25 mL) and cooled in ice-water. A cooled solution of $NaNO_2$ (8.0 mmol) in water (4 mL) was added to the triamino solution dropwise with stirring over 15 min. The mixture was stirred for 30 min at 0–10 °C. Then, it was brought to rt and stirred for 2 h. Thus, the solid deposited was collected by filtration to give the corresponding 5-amino-triazolopyrimidine derivatives (8-azaguanines, **5a–h**) as shown in the Tables 1 and 4.

Table 4. IR and ^{1}H-NMR spectroscopic data for compounds (**5a–h, 8a–n** and **10a–i**)

Compd No.	[ν_{max} (Nujol)/cm^{-1}][a]	δ_H [300 MHz; $(CD_3)_2SO$; Me_4Si)][b]
5a	3240, 3130, *3040* (NH), 1680 (C=O)	0.90 (3H, t, *J* = 7.2 Hz, CH_3), 1.55 (2H, sext, *J* = 7.2 Hz, N-$CH_2C\mathit{H}_2$), 3.23 (2H, q, *J* = 6.9 Hz, N-CH_2), 6.64 (1H, s, 5-C-NH), 10.69 (1H, s, 6-NH), 15.27 (1H, br s, 1-NH)
5b	3340, 3135, 3065 (NH), 1700 (C=O)	1.20 (6H, d, *J* = 6.3 Hz, 2 x CH_3), 3.97–4.07 (1H, m, N-CH), 6.35 (1H, d, *J* = 6.3 Hz, 5-C-NH), 10.40 (1H, br s, 6-NH), 15.08 (1H, br s, 1-NH)
5c	3250, 3130, *3040* (NH), 1690 (C=O)	0.94 (3H, t, *J* = 7.2 Hz, CH_3), 1.37 (2H, sext, *J* = 7.2 Hz, $C\mathit{H}_2CH_3$), 1.54 (2H, quin, *J* = 7.2 Hz, N-$CH_2C\mathit{H}_2$), 3.29 (2H, q, *J* = 6.9 Hz, N-CH_2), 6.59 (1H, br s, 5-C-NH), 10.61 (1H, br s, 6-NH), 15.05 (1H, br s, 1-NH)
5d	3250, 3120, 3040 (NH), 1685 (C=O)	0.90 (6H, d, *J* = 6.6 Hz, 2 x CH_3), 1.78–192 (1H, m, CH), 3.12 (2H, t, *J* = 6.6 Hz, N-CH_2), 6.65 (1H, br s, 5-C-NH), 10.61 (1H, br s, 6-NH), 15.28 (1H, br s, 1-NH)
5e	3320, 3170, 3080 (NH), 1700 (C=O)	1.19–1.44 (5H, m, cyclohexyl-H), 1.59–1.78 (3H, m, cyclohexyl-H), 1.95–2.02 (2H, m, cyclohexyl-H), 3.80 (1H, br s, N-CH), 6.16 (1H, br s, 5-C-NH), 10.40 (1H, br s, 6-NH), 14.57 (1H, br s, 1-NH)
5f	3250, 3125, 3040 (NH), 1690 (C=O)	0.88 (3H, t, *J* = 6.9 Hz, CH_3), 1.30 (10H, br s, $CH_2[C\mathit{H}_2]_5CH_3$), 1.53–1.64 (2H, m, N-$CH_2C\mathit{H}_2$), 3.35 (2H, q, *J* = 6.6 Hz, N-CH_2), 6.17 (1H, br s, 5-C-NH), 10.52 (1H, br s, 6-NH), 14.57 (1H, br s, 1-NH)
5g	3385 (OH), 3320, *3140,* 3070 (NH), 1710 (C=O)	1.69 (2H, quin, *J* = 6.3 Hz, N-$CH_2C\mathit{H}_2$), 3.37 (2H, q, *J* = 6.6 Hz, N-CH_2), 3.49 (2H, q, *J* = 5.7 Hz, O-CH_2), 4.45 (1H, br s, OH), 6.55 (1H, br s, 5-C-NH), 10.67 (1H, br s, 6-NH), 15.08 (1H, br s, 1-NH)
5h	3250, 3130, *3040* (NH), 1685 (C=O)	2.92 (2H, t, *J* = 6.9 Hz, Ph-$C\mathit{H}_2$), 3.65 (2H, q, *J* = 6.9 Hz, N-CH_2), 6.27 (1H, s, 5-C-NH), 7.19–7.35 (5H, m, Ph-H), 10.53 (1H, br s, 6-NH), 14.75 (1H, br s, 1-NH)
8a	3340, 3145 (NH), 1690 (C=O)	3.87 (3H, s, CH_3), 7.80 (1H, br s, 7-C-NH), 8.85 (1H, br s, 4-NH), 11.16 (1H, br s, 1-NH)
8b	3340, 3160 (NH), 1685 (C=O)	1.40 (3H, t, *J* = 7.2 Hz, CH_3), 3.29 (2H, q, *J* = 7.2 Hz, CH_2), 7.95 (1H, br s, 7-C-NH), 8.88 (1H, br s, 4-NH), 11.29 (1H, br s, 1-NH)
8c	3345, 3150 (NH), 1685 (C=O)	0.89 (3H, t, *J* = 7.5 Hz, CH_3), 1.82 (2H, sext, *J* = 7.2 Hz, N-$CH_2C\mathit{H}_2$), 4.20 (2H, q, *J* = 6.9 Hz, N-CH_2), 8.20–9.60 (2H, br s, 7-C-NH and 4-NH)
8d	3380, 3130 (NH), 1705 (C=O)	1.51 (6H, d, *J* = 6.3 Hz, 2 x CH_3), 4.72–4.79 (1H, m, N-CH), 8.95–9.40 (2H, br s, 7-C-NH and 4-NH)
8e	3350, 3155 (NH), 1690 (C=O)	0.96 (3H, t, *J* = 7.5 Hz, CH_3), 1.35 (2H, sext, *J* = 7.5 Hz, $C\mathit{H}_2CH_3$), 1.83 (2H, quin, *J* = 7.5 Hz, N-$CH_2C\mathit{H}_2$), 4.25 (2H, q, *J* = 6.9 Hz, N-CH_2), 7.91 (1H, br s, 7-C-NH), 8.92 (1H, br s, 4-NH), 11.20 (1H, br s, 1-NH)
8f	3365, *3140* (NH), 1660 (C=O)	0.92 (6H, d, *J* = 6.9 Hz, 2 x CH_3), 2.05–2.21 (1H, m, CH), 4.27 (2H, d, *J* = 7.2 Hz, N-CH_2), 9.41 (1H, br s, 7-C-NH), 10.72 (1H, br s, 4-NH)
8g	3400, 3145 (NH), 1670 (C=O)	1.27–1.48 (3H, m, cyclohexyl-H), 1.70–1.98 (7H, m, cyclohexyl-H), 4.35–4.43 (1H, m, cyclohexyl-H), 7.95 (1H, br s, 7-C-NH), 8.91 (1H, br s, 4-NH), 11.21 (1H, br s, 1-NH)
8h	3350, 3165 (NH), 1690 (C=O)	0.88 (3H, br s, CH_3), 1.28 (10H, br s, $CH_2[C\mathit{H}_2]_5CH_3$), 1.83 (2H, br s, N-$CH_2C\mathit{H}_2$), 4.23 (2H, s, N-$CH_2$), 8.02 (1H, br s, 7-C-NH), 8.91 (1H, br s, 4-NH), 11.30 (1H, br s, 1-NH)
8i	*3395* (OH), *3320*, 3200 (NH), 1710 (C=O)	3.81 (2H, br s, O-CH_2), 4.33 (2H, t, *J* = 5.4 Hz, N-CH_2), 4.89 (1H, br s, OH), 8.06 (1H, br s, 7-C-NH), 8.83 (1H, br s, 4-NH), 11.29 (1H, br s, 1-NH)
8j	3230, 3100 (NH), 1670 (C=O)	7.55-7.86 (5H, m, Ph-H), 9.04 (1H, br s, 7-C-NH), 10.37 (1H, br s, 4-NH)
8k	3395, 3100 (NH), 1665 (C=O)	5.47 (2H, s, CH_2), 7.32–7.41 (5H, m, Ph-H), 7.94 (1H, br s, 7-C-NH), 9.03 (1H, br s, 4-NH), 11.26 (1H, br s, 1-NH)
8l	3310, 3120 (NH), 1690 (C=O)	3.14 (2H, t, *J* = 7.5 Hz, Ph-$C\mathit{H}_2$), 4.68 (2H, t, *J* = 7.5 Hz, N-CH_2), 7.19–7.29 (5H, m, Ph-H), 9.37 (1H, br s, 7-C-NH), 10.67 (1H, br s, 4-NH)

8m	3105, 3060 (NH), 1650 (C=O)	1.69 (6H, br s, 3 x CH_2), 3.89 and 4.45 (each 2H, each s, 2 x CH_2), 11.28 (1H, br s, 4-NH), 14.15 (1H, br s, 1-NH)
8n	3200, *3100* (NH), 1635 (C=O)	3.74 (4H, s, 2 x CH_2), 3.91 and 4.47 (each 2H, each s, 2 x CH_2), 11.21 (1H, br s, 4-NH), 14.92 (1H, br s, 1-NH)
10a	3250, 3175 (NH), 1735 (C=O)	2.94 (3H, s, NH-C*H*$_3$), 3.37 (3H, s, 4-N-CH_3), 8.94 (1H, br s, 7-C-NH)
10b	3250, 3160 (NH), 1730 (C=O)	1.17 (3H, t, *J* = 7.2 Hz, CH_2C*H*$_3$), 3.37 (3H, s, 4-N-CH_3), 3.45 (2H, q, *J* = 7.2 Hz, CH_2), 8.73 (1H, br s, 7-C-NH)
10c	3250, 3160 (NH), 1730 (C=O)	0.91 (3H, t, *J* = 7.2 Hz, CH_2C*H*$_3$), 1.54–1.69 (2H, m, N-CH_2C*H*$_2$), 3.36 (3H, s, 4-N-CH_3), 3.41 (2H, t, *J* = 7.2 Hz, N-CH_2), 8.88 (1H, br s, 7-C-NH)
10d	*3230*, 3170 (NH), 1735 (C=O)	1.21 (6H, d, *J* = 6.6 Hz, C*H*$_3$CHC*H*$_3$), 3.36 (3H, s, 4-N-CH_3), 4.25–4.42 (1H, m, N-CH), 8.73 (1H, br s, 7-C-NH)
10e	3250, 3160 (NH), 1730 (C=O)	0.91 (3H, t, *J* = 7.5 Hz, CH_2C*H*$_3$), 1.28–1.42 (2H, m, C*H*$_2$CH$_3$), 1.53–1.65 (2H, m, N-CH_2C*H*$_2$), 3.36 (3H, s, 4-N-CH_3), 3.41 (2H, t, *J* = 6.9 Hz, N-CH_2), 8.86 (1H, br s, 7-C-NH)
10f	*3240*, 3170 (NH), 1740 (C=O)	0.86 (3H, t, *J* = 6.6 Hz, CH_2C*H*$_3$), 1.26 (10H, br s, CH_2[C*H*$_2$]$_5$CH$_3$), 151–1.68 (2H, m, N-CH_2C*H*$_2$), 3.37 (3H, s, 4-N-CH_3), 3.42 (2H, s, N-CH_2), 8.75 (1H, br s, 7-C-NH)
10g	3380 (OH), 3240, 3120 (NH), 1735 (C=O)	3.37 (3H, s, 4-N-CH_3), 3.52 (2H, t, *J* = 5.4 Hz, O-CH_2), 3.58 (2H, t, *J* = 5.4 Hz, N-CH_2), 5.00 (1H, br s, OH), 8.79 (1H, br s, 7-C-NH)
10h	3250, 3155 (NH), 1730 (C=O)	3.35 (3H, s, 4-N-CH_3), 4.65 (2H, s, CH_2), 7.20–7.48 (5H, m, Ph-H), 9.16 (1H, br s, 7-C-NH)
10i	3250, 3165 (NH), 1730 (C=O)	2.93 (2H, t, *J* = 7.5 Hz, Ph-C*H*$_2$), 3.36 (3H, s, 4-N-CH_3), 3.67 (2H, t, *J* = 7.5 Hz, N-CH_2), 7.18–7.33 (5H, m, Ph-H), 8.91 (1H, br s, 7-C-NH)

[a] IR absorption value in italic refers to wave numbers at which shoulders or inflexions occur in the absorption.
[b] ^{1}H-NMR spectra of compounds **5e, f, h** were measured in $CDCl_3$.

Method B: A mixture of 5-methylthio-1*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-one (**4**; 0.50 g, 2.73 mmol) and an appropriate amine (8–15 mmol) in 1-butanol (50 mL) was heated under reflux for 1–2 days. After the reaction was complete, the solution was evaporated to dryness under reduced pressure. The resulting residue was treated with dilute aqueous acetic acid to give the crystals, which were collected by filtration. For purification, the crystals were dissolved in dilute NaOH and the resulting solution was neutralized with acetic acid. Thus, the solid deposited was collected by filtration to give the corresponding 5-aminotriazolopyrimidine derivatives (**5a**, **c–h**), which were in all respects identical with authentic samples prepared by the above general method A (Tables 1 and 4).

General procedures for 7-amino-1*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-5(4*H*)-ones (8a–n)

To a suspension of 4,6-diamino-5-nitrosopyrimidine derivatives[16] (**6a–n**, 6.0 mmol) in 5% aqueous acetic acid (25 mL) was added $Na_2S_2O_4$ (20-24 mmol) by portions with stirring at 70–80 °C. Then, the mixture was stirred at 80 °C for 0.5–1.0 h. After the reaction was complete, the solution was cooled to rt and neutralized with 28% aqueous ammonia. Upon keeping the mixture in refrigerator for several hours, the solid deposited was collected by filtration to give the corresponding 4,5,6-triamino intermediates (**7a–n**), which were dissolved in 25% aqueous acetic acid (25 mL) and cooled in ice-water. A cooled solution of

Table 5. Evaluation of antitumor and antiviral activities *in vitro* of the compounds (**5** and **8**)

Compd No.	Inhibitory concentration against tumor cell lines [IC_{50} (μg/mL)]		Inhibitory concentration against Herpes Simplex Virus [ED_{50} (μg/mL)]	
	CCRF-HSB-2	KB	HSV-1	HSV-2
5a	64.4	66.2	>100	>100
5c	63.3	57.2	>100	>100
5d	64.0	62.2	>100	>100
5e	96.0	79.2	>100	>100
5f	38.5	26.6	>20	>20
5g	>100	>100	>100	>100
5h	59.1	61.4	>100	>100
8c	>100	98.6	>100	>100
8d	>100	>100	>100	>100
8e	>100	63.4	>100	>100
8f	>100	58.2	>100	>100
8g	89.5	69.3	>100	>100
8h	>100	86.6	>100	>100
8i	>100	>100	>100	>100
8j	59.1	60.5	>20	>20
8k	89.4	50.6	>20	>20
8l	>100	>100	>20	>20
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.

$NaNO_2$ (8.0 mmol) in water (4 mL) was added to the triamino solution dropwise with stirring over 15 min. The mixture was stirred for 30 min at 0–10 °C. Then, it was brought to rt and stirred for 2 h. The solid deposited was collected by filtration to give the corresponding 7-aminotriazolopyrimidine derivatives (8-azaisoguanines, **8a–n**) as shown in the Tables 2 and 4.

General procedure for 7-amino-4-methyl-1*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-5(4*H*)-ones (10a–i)

A solution of 4-methyl-1*H*-[1,2,3]triazolo[4,5-*d*]pyrimidine-5(4*H*)-one-7(6*H*)-thione (**9**; 0.50 g, 2.73 mmol) and an appropriate amine (8–15 mmol) in 1-butanol (40 mL) was heated under reflux for 10–15 h. In the case of methyl, ethyl, and isopropyl amines, the reactions were carried out in steel sealed tube at 140 °C for overnight. After the reaction was complete, the solution was evaporated to dryness under reduced pressure. The resulting residue was triturated with ethyl acetate to give crystals, which were dissolved in dilute NaOH and the solution was neutralized with acetic acid. Thus, the solid deposited was collected by filtration to give the corresponding 7-amino-triazolopyrimidine derivatives (**10a–i**) as shown in the Tables 3 and 4.

Growth inhibitory activities of compounds 5 and 8 against human tumor cell lines

The synthesized 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 [3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay[19] for cellular growth and survival application method developed by Mosmann.[20] The results, i.e., IC_{50} (μg/mL) of each compound against both cells are summarized in Table 5.

Antiviral activities of compounds 5 and 8

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.*[21,22] The results are summarized in Table 5.

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