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6-Amino-2-thio- and 6-Aminouracils as Precursors for the Synthesis of Antiviral and Antimicrobial Methylenebis(2-thiouracils), Tricyclic Pyrimidines, and 6-Alkylthiopurine-2-ones

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Summary. Several derivatives of 5-arylmethylenebis(1-methyl-6-amino-2-thiouracils) and 5-aryldipyrimidopyridines were prepared by stirring of 6-amino-1-methyl-2-thiouracil and 6-amino-1-benzyluracil with different aromatic aldehydes in ethanol in the presence of HCl or refluxing with *Ac*OH. On the other hand, 6-alkylthio-3,9-dimethylpurine-2-ones were synthesized by the alkylation of 3,9-dimethyl-6-thioxanthine which was prepared by treatment of 3,9-dimethylxanthine with P_2S_5. The structures of the novel compounds were characterized by spectroscopic methods. The effects of the novel compounds on both HAV and HSV type 1 were investigated. Also, some compounds showed inhibitory effects on *Gram*-positive and *Gram*-negative bacteria as well as yeast and fungi.

Keywords. 6-Amino-1-methyl-2-thiouracil; 5-Arylmethylenebis(1-methyl-6-amino-2-thiouracils); 5-Aryldipyrimidopyridines; 6-Alkythiopurines; Antiviral and antimicrobial activity.

Introduction

In 1943, *Astwood* discovered the high antithyroid activity and low toxicity of 2-thiouracil [1] and many derivatives of this compound have been prepared and tested for physiological activity [2–5]. Amongst a variety of compounds known to interfere with the metabolism in proliferating cells, antagonists of nucleic acids and their constituents have gained special significance since some of them exhibit considerable cytostatic and antiviral activities [6]. In particular, sulfur-containing analogous of purine bases, such as 6-mercaptopurine and its derivatives, have been widely used as drugs in cancer chemotherapy [7]. Unfortunately, few data are available to evaluate the putative altered function of nucleic acids in cells treated with thiopurines. Several reports have described effects of 6-thioguanine on *RNA* metabolism and protein synthesis, which may be a result of incorporation of the drug into *RNA* [8]. It has been reported that 6-mercaptopurine reduced the transforming activity. *Bacillus subtilis* UTH-8505 has been grown in the presence of 6-thioguanine and 6-mercaptopurine. *DNA* isolated from these and control cultures were used to transform *B. subtilis* T_3, a strain lacking tryptophan synthetase, to prototrophy. 6-Mercaptopurine exposure decreased the transforming activity by 80 and 6-thioguanine by 20% [9].

Due to the importance of N-substituted 6-amino-2-thiouracils with respect to their antitumor activity, and in extension to our work [10, 11], we look to prepare new fused thiouracils and study their antimicrobial and antiviral activities. Herpes simplex virus, HSV (Herpesviridae is a family of enveloped *DNA* viruses that occurs in man, cold–blooded vertebrates, and in vertebrates. HSV-1 and HSV-2 are the most common causes of genital ulceration in developed

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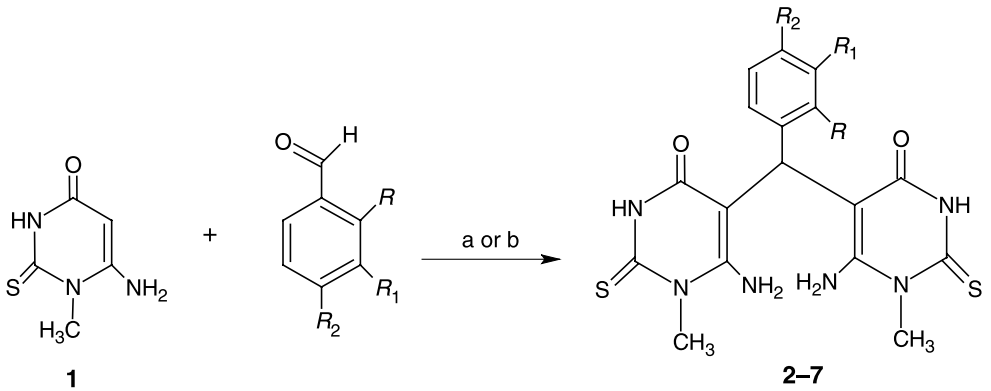
countries. In the United States only, approximately 500000 new cases of herpes are reported each year. Genital herpes is life long and may result in painful and recurrent lesions and systemic complications. However, the contribution of asymptomatic viral shedding to the genital transmission of herpes in the population may account for as much as 50 to 90% of cases [12]. Also it has been reported that HSV-1 and HSV-2 are neurotropic viruses which may spread to the brain during primary or recurrent infections. HSV-1 is the most usual cause of sporadic encephalitis, except in neonates, with a mortality rate of 75% and severe permanent sequelae in survivors [13]. Hepatitis A Virus (HAV) is classified with the enterovirus group of the picornaviridae family. HAV has a single molecule of *RNA* surrounded by a small (27 nm diameter) protein capsid. HAV causes the disease Hepatitis A, it is usually a mild illness characterized by sudden onset of fever, malaise, nausea, and abdominal discomfort. HAV is excreted in feces of infected people and can produce clinical diseases when susceptible individuals consume contaminated water or food.

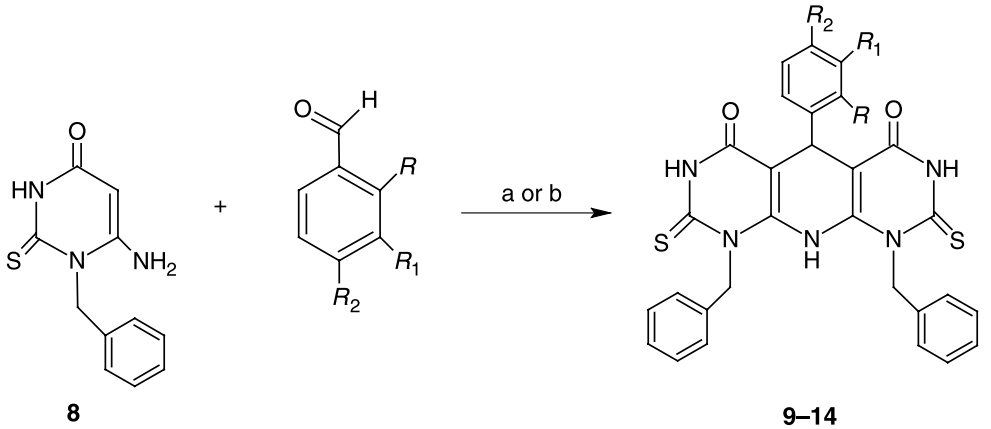
Results and Discussion

Chemistry

In the presence of hydrochloric acid at room temperature or refluxing with glacial acetic acid, stirring of 1-methyl-6-amino-2-thiouracil (**1**) in absolute ethanol with different aromatic aldehydes afforded the 5-arylmethylenebis(6-amino-1-methyl-2-thiouracils) **2–7** in 44–69% yield and starting material. Under

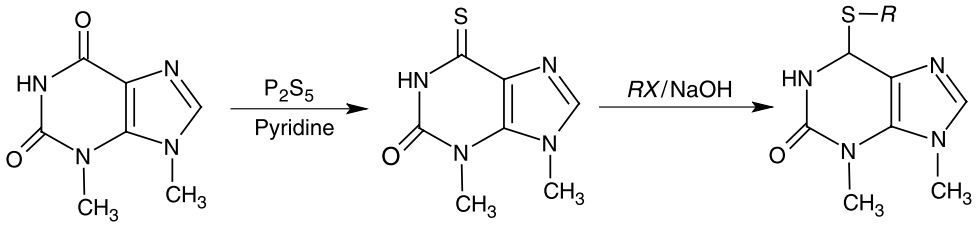


Reagents: a) Abs. ethanol/HCl, r.t.; b) CH_3COOH, reflux



Reagents: a) Abs. ethanol/HCl, r.t.; b) CH_3COOH, reflux

Scheme 1



15 **16** **17–20**

Scheme 2

the same conditions the treatment of 6-amino-1-benzyluracil (**8**) with different aromatic aldehydes afforded the cyclic pyrido[*2,3-d*][*6,5-d*]dipyrimidines **9–14** in 36–49% yield and starting material as shown in Scheme 1. An explanation for the formation of the open-chain compounds **2–7** may come from the presence of sulfur in position 2, which deactivates the uracil ring, increases the π-bond character of C=NH_2, and prevents cyclization contrary to the case of oxygen in compounds **9–14**. ^{1}H NMR for compounds **2–7** showed the absence of $\delta = 4.53$ ppm characteristic for CH-5 of uracil **1** and presence of $\delta = 7.56$–7.59 ppm characteristic for $2NH_2$ at C-6,6′. ^{13}C NMR showed ten signals for compound **2**. The ^{1}H NMR spectrum for compounds **9–14** showed the absence of $\delta = 7.56$–7.59 ppm characteristic for NH_2-6 and presence of $\delta = 11.23$–11.98 ppm characteristic for NH-10.

It has been reported that 6-thioguanine acts as an inhibitor of the growth of *Lactobacillus casei* [14, 15], embryonic tissue [16], and a number of neoplasms, and 2-alkylthiopurines act as amplifiers of the antibiotic activity of phleomycin [17]. In addition, 6-mercaptopurines and their analogues have been a synthetic target for therapeutic areas of cancer [18], viral infections [19] in CNS, as ligands for benzodiazepine receptors [20], and it is clinically used in the treatment of leukaemia [18]. We look to synthesize 6-mercaptopurine analogues which might exhibit biological activities. In this work, we used 3,9-dimethylxanthine (**15**) [21, 22] as a starting material. Several 6-alkylthio-3,9-dimethylpurine-2-ones **17–20** were prepared by refluxing **15** with phosphorous pentasulfide in dry pyridine to afford 6-thioxanthine **16**. This was followed by the treatment with different alkyl halides, such as methyl, ethyl, and butyl iodide, and benzyl bromide in 2 *N* NaOH affording **17–20** as shown in Scheme 2. The structures of compounds **17–20** were corroborated by ^{1}H NMR and UV spectra. ^{1}H NMR for compound **17** showed the disappearance of the signal of the NH-1 proton at $\delta = 12.3$ ppm for compound **16** and the presence of a signal at $\delta = 4.08$ ppm characteristic of S–CH_3-6.

Pharmacology

The antiviral activities of the synthesized compounds are shown in Table 1. The first group of the compounds **2–7** has various virucidal effects. Their activities were modified by substituent variations, and certain trends are apparent. The unsubstituted compound **2** showed a virucidal effect with 45% at concentration of 20 μg/cm^3 against HSV-1, where a methoxy substituted, $R^1 = OCH_3$ (**5**), showed no change in the antiviral activity against HSV but decreased the activity against HAV-27. A halogen substituent, $R^2 = F$ (**3**), showed also lower activity against both HSV and HAV-27 than **2**. Concurrent a methoxy substitution, $R = OCH_3$ (**4**), resulted in the best activity with a concentration of 20 μg/cm^3 against HSV-1 with a higher inhibition percentage of 63%. Secondly, compounds of 5-aryldipyrimidopyridines **9–14** showed promising results. The unsubstituted compound **9** showed moderate antiviral activity of 44.5%, while substitution with methoxy, R^1=OCH_3 as in **12**, and with halogen, R and R^2=Cl (**14**), resulted in disappearance of the virucidal activity of 0% against HSV-1. The most promising activity appeared with OH substituted compound **13** where the virucidal activity was 95.7 and 100% at concentrations of 10 and 20 μg/cm^3, its EC_{50} was <5 μg/cm^3. This compound is comparable with the first antivirally active nucleoside analogue [23]. It has been reported that 2-amino-7-[(1,3-dihydroxy-2-propoxy)methyl]purine (S2242) represents the first antivirally active nucleoside analogue with the side chain attached to the N-7 position of the purine ring.

Table 1. Antiviral activities of the 5-arylmethylenebis(6-amino-1-methyl-2-thiouracils) **2–7** and 5-aryldipyrimidopyridines **9–14**

Compound	*Ar*	HAV-27			HSV-1		
		% of virucidal effect		50% inhibitory concentration	% of virucidal effect		50% inhibitory concentration
		10 $\mu g/cm^3$	20 $\mu g/cm^3$		10 $\mu g/cm^3$	20 $\mu g/cm^3$	
S2242				ND			0.1–0.2
2	C_6H_5	0	26	>20	ND	45	>20
3	C_6H_4-4-F	0	6	–	ND	32	>20
4	C_6H_4-2-OCH_3	0	23	–	54	63	9.25
5	C_6H_4-3-OCH_3	0	0	–	31	45	22.22
6	C_6H_4-4-OH	0	36	>20	ND	27	>20
7	C_6H_3-2,4-Cl	6	43	>20	0	35.5	>20
9	C_6H_5	36	43	23.25	0	44.5	>20
10	C_6H_4-4-F	23	40	>20	27	31	>20
11	C_6H_4-2-OCH_3	0	0	–	27	50	20
12	C_6H_4-3-OCH_3	23	33	>20	0	0	–
13	C_6H_4-4-OH	0	10	–	95.7	100	<5
14	C_6H_3-2,4-Cl	36	36	–	0	0	–

ND, Not determined. Each value is the mean of triplicate assays

Compound S2242 strongly inhibits the *in vitro* replication of both herpes simplex viruses, HSV-1 and HSV-2.

Concerning the antibacterial and antifungal activity, all synthesized compounds were tested against *Gram*-positive and -negative bacteria, yeast, and fungi. The first group of the synthesized compounds **2–7** showed various activities dependent on substitution. The unsubstituted compound **2** showed no effect on both *Gram*-positive and -negative bacteria whereas compounds **2–5** showed a moderate activity against both *C. tropicalis* and *A. niger*. The substitution $R^2 = F$ resulted in compound **3** with a broad spectrum of antimicrobial activity. It showed a good antimicrobial activity against all tested microorganisms. The other important observation was the substitution with Cl, which resulted in the only antibacterial compound **7**. Compounds **4–6** showed inhibition activity only against the *Gram*-positive bacterium *B. subtilis*. The second group of the synthesized compounds showed no activity against *C. tropicalis*. On the other hand, compound **10** showed activity against *B. subtilis*, whereas *E. coli* was affected only by **13** and *A. niger* was affected only by **14**. These results showed that the second group of the synthesized compounds has poor antimicrobial activity and the most promising compounds were **6** and **7** which showed the best inhibitory effect against *B. subtilis*. Thus the *MIC* was determined for both compounds and the results showed that the inhibitory effect of **6** extended until the concentration of 5 $\mu g/cm^3$ and for **7** to 10 $\mu g/cm^3$ demonstrating the high activity of these two compounds against *B. subtilis*. These results are comparable with the reported data [24] for other nucleoside analogues, 6-(3-ethyl-4-methylanilino)uracil (*EMAU*) and 6-[(3,4-trimethylene)anilino]uracil (*TMAU*), which showed inhibitory effect against *B. subtilis* BD54 with *MIC* of 9 $\mu g/cm^3$. Compounds **17–20** showed

Table 2. Antimicrobial activity of the newly synthesized compounds **2–19**

Compound	Diameter of the inhibition zone/mm			
	B. subtilis	*E. coli*	*C. tropicalis*	*A. niger*
2	0.0	0.0	10.0	10.0
3	11.0	8.0	12.0	10.0
4	12.0	0.0	12.0	8.0
5	10.0	0.0	8.0	10.0
6	14.0	0.0	0.0	0.0
7	13.0	11.0	0.0	0.0
9	0.0	0.0	0.0	0.0
10	8.0	0.0	0.0	0.0
11	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0
13	0.0	8.0	0.0	0.0
14	0.0	0.0	0.0	10.0
17	10.0	10.0	0.0	8.0
18	15.0	10.0	0.0	0.0
19	12.0	10.0	0.0	0.0
20	10.0	10.0	0.0	0.0

a mild antimicrobial activity, *i.e.* they are non-promising as synthesis targets.

Depending on the above mentioned results, it could be concluded that both compound **13** as potent antiviral and **3** as a broad-spectrum antimicrobial could be considered to be viable choice for further investigations. Of course, the compounds would need to show superiority in other factors, such as bioavailability, oral absorption, and toxicity.

Experimental

Melting points were determined with an electro thermal Mel-Temp II apparatus. IR spectra were obtained in the solid state in form of KBr discs using a Perkin-Elmer model 1430 spectrometer. The UV spectra were determined with Perkin Elmer, Lambda 5 or 15 spectrometers. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 MHz spectrometer in *DMSO*-d_6 as the solvent and *TMS* as internal standard. Elemental analyses were performed at the Micro-analytical Centre, Cairo University, Giza, Egypt, and were found to be matching the calculated values properly.

5-Arylmethylenebis(6-amino-1-methyl-2-thiouracils) ***2–7*** *and 5-Substituted 1,9-Dibenzyl-3,5,7,10-tetrahydro-2,4,6,8-tetraoxopyrido[2,3-d][6,5-d]dipyrimidines* ***9–14***

Method A: To a solution of 1.96 mmol 6-amino-1-methyl-2-thiouracil (**1**) [25] or 1.96 mmol 6-amino-1-benzyluracil (**8**) [26] in hot absolute ethanol, 0.98 mmol of an appropriate aromatic aldehyde was added in presence of 1.0 cm^3 conc. HCl. The reaction mixture was stirred for 1.5–2.5 h at room temperature. The formed product was filtered off, washed with ethanol, dried, and recrystallized from 50% acetic acid.

Method B: A mixture of 3.2 mmol **1** or **8** and 1.6 mmol of an appropriate aromatic aldehyde in 15 cm^3 glacial acetic acid was heated under reflux for 2–7 h. After cooling the formed product was filtered off and recrystallized from 50% acetic acid.

5-Phenylmethylenebis(6-amino-1-methyl-2-thiouracil) (**2**, $C_{17}H_{18}N_6O_2S_2$)
Yield 1.8 g (47%); mp 270°C; yellow crystals; IR (KBr): $\bar{\nu} = 3380$ (NH_2), 3270 (NH_2), 1630 (C=O), 1612 (NH_2) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.25$ (s, 2NH), 7.59 (bs, $2NH_2$), 7.22–7.19 (m, 2H-arom), 7.14–7.10 (m, 3H-arom), 5.52 (s, CH), 3.78 (s, $2NCH_3$) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 28.21$ (CH), 39.4 (CH_3 (1)), 89.7, 112.2, 117.8, 126.5, 128.3, 131.2, 162.5 (2C=O), 171.8 (2C=S) ppm.

5-(4-Fluorophenyl)methylenebis(6-amino-1-methyl-2-thiouracil) (**3**, $C_{17}H_{17}FN_6O_2S_2$)
Yield 1.8 g (45%); mp 286°C; yellow crystals; IR (KBr): $\bar{\nu} = 3420$ (NH_2), 3315 (NH_2), 1665 (C=O), 1614 (NH_2) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.25$ (bs, 2NH), 7.59 (bs, $2NH_2$), 7.16–7.11 (tt, $J = 5.7$, 2.7 Hz, 2H-arom), 7.04–6.98 (t, $J = 9$ Hz, 2H-arom), 5.48 (s, CH), 3.77 (s, $2NCH_3$) ppm.

5-(2-Methoxyphenyl)methylenebis(6-amino-1-methyl-2-thiouracil) (**4**, $C_{18}H_{20}N_6O_3S_2$)
Yield 1.72 g (62%); mp 270°C; yellowish crystals; IR (KBr): $\bar{\nu} = 3370$ (NH_2), 3273 (NH_2), 1630 (C=O), 1610 (NH_2) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.15$ (s, 2NH), 7.59 (bs, $2NH_2$), 7.14–7.08 (m, 2H-arom), 6.87–6.81 (m, 2H-arom), 5.41 (s, CH), 3.78 (s, $2NCH_3$), 3.62 (s, OCH_3) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 28.3$ (CH), 39.2 (CH_3), 55.4 (OCH_3), 91.3, 112.4, 117.8, 126.4, 128.2, 129.8, 131.1, 131.9, 162.4, 171.6 ppm.

5-(3-Methoxyphenyl)methylenebis(6-amino-1-methyl-2-thiouracil) (**5**, $C_{18}H_{20}N_6O_3S_2$)
Yield 1.89 g (69%); mp 278°C; yellowish crystals; IR (KBr): $\bar{\nu} = 3350$ (NH_2), 3265 (NH_2), 1628 (C=O), 1617 (NH_2) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.25$ (s, 2NH), 7.57 (bs, $2NH_2$), 7.16–7.11 (m, 3H-arom), 6.71 (s, 1H-arom), 5.48 (s, CH), 3.77 (s, OCH_3), 3.67 (s, $2NCH_3$) ppm.

5-(4-Hydroxyphenyl)methylenebis(6-amino-1-methyl-2-thiouracil) (**6**, $C_{17}H_{18}N_6O_3S_2$)
Yield 1.75 g (44%); mp 276°C; yellowish crystals; IR (KBr): $\bar{\nu} = 3640$ (br, OH), 3400 (NH_2), 3290 (NH_2), 1638 (C=O), 1600 (NH_2) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.24$ (s, 2NH), 7.57 (bs, $2NH_2$), 7.13–6.97 (m, 2H-arom), 6.72–6.69 (m, 2H-arom), 6.63 (s, OH), 5.48 (s, CH), 3.78 (s, $2NCH_3$) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 28.3$ (CH), 39.2 (CH_3), 91.5, 113.1, 117.6, 126.5, 128.3, 131.2, 162.32, 171.4 ppm.

5-(2,4-Dichlorophenyl)methylenebis(6-amino-1-methyl-2-thiouracil) (**7**, $C_{17}H_{16}Cl_2N_6O_2S_2$)
Yield 1.60 g (53%); mp 272°C; yellowish crystals; IR (KBr): $\bar{\nu} = 3410$ (NH_2), 3280 (NH_2), 1635 (C=O), 1607 (NH_2) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 12.33$ (s, 2NH), 7.56 (bs, $2NH_2$), 7.44–6.83 (m, 3H-arom), 5.41 (s, CH), 3.78 (s, $2NCH_3$) ppm.

1,9-Dibenzyl-5-phenyl-3,5,7,10-tetrahydro-2,4,6,8-tetraoxopyrido[2,3-d][6,5-d]dipyrimidine (**9**, $C_{29}H_{23}N_5O_4$)
Yield 1.13 g (49%); mp 268°C; white crystals; IR (KBr): $\bar{\nu} = 3120$ (NH), 1680 (C=O) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 11.99$ (bs, NH), 10.95 (s, 2NH), 7.40–7.05 (m, 15H-arom), 5.48 (s, CH), 5.14 (s, $2CH_2$) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 34.16$ (CH), 43.19 (CH_2), 124.71, 125.42, 126.35, 127.39, 128.29, 128.52, 128.46, 130.25, 131.77, 133.28, 140.22, 149.80 ppm.

1,9-Dibenzyl-5-(4-fluorophenyl)-3,5,7,10-tetrahydro-2,4,6,8-tetraoxopyrido[2,3-d][6,5-d]dipyrimidine (**10**, $C_{29}H_{22}FN_5O_4$)
Yield 1.09 g (45%); mp 278°C; white crystals; IR (KBr): $\bar{\nu} = 3120$ (NH), 1670 (C=O) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 11.53$ (s, NH), 10.93 (s, 2NH), 7.39–6.96 (m, 14H-arom), 5.24 (s, CH), 5.12 (s, $2CH_2$) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 33.98$ (CH), 43.27 (CH_2), 125.18, 127.27, 128.43, 128.67, 129.19, 129.52, 131.20, 133.62, 133.78, 138.38, 149.90, 151.17 ppm.

1,9-Dibenzyl-5-(2-methoxyphenyl)-3,5,7,10-tetrahydro-2,4,6,8-tetraoxopyrido[2,3-d][6,5-d]dipyrimidine
(**11**, $C_{30}H_{25}N_5O_5$)
Yield 1.10 g (45%); mp 226°C; yellowish crystals; IR (KBr): $\bar{\nu} = 3170$ (NH), 1660 (C=O) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 11.62$ (s, NH), 10.78 (s, 2NH), 7.41-7.23 (m, 10H-arom), 7.12-7.09 (t, 1H-arom), 6.94–6.91 (d, 1H-arom), 6.84–6.82 (d, $J = 6.0$ Hz, 1H-arom), 6.71–6.67 (t, $J = 6.0$ Hz, 1H-arom), 5.43 (s, CH), 5.13 (s, $2CH_2$), 3.62 (s, OCH_3) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 33.41$ (CH), 43.16 (CH_2), 54.62 (OCH_3), 114.11, 125.28, 126.61, 127.13, 127.35, 128.60, 128.96, 129.43, 129.72, 131.15, 133.61, 149.86, 157.09 ppm.

1,9-Dibenzyl-5-(3-methoxyphenyl)-3,5,7,10-tetrahydro-2,4,6,8-tetraoxopyrido[2,3-d][6,5-d]dipyrimidine
(**12**, $C_{30}H_{25}N_5O_5$)
Yield 1.0 g (41%); mp 252°C; yellow crystals; IR (KBr): $\bar{\nu} = 3130$ (NH), 1655 (C=O) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 11.23$ (s, 2NH), 8.06 (bs, NH), 7.38–7.24 (m, 10H-arom), 7.08–7.02 (t, 1H-arom), 6.56–6.58 (tt, 3H-arom), 5.41 (s, CH), 5.13 (s, $2CH_2$), 3.60 (s, OCH_3) ppm.

1,9-Dibenzyl-5-(4-hydroxyphenyl)-3,5,7,10-tetrahydro-2,4,6,8-tetraoxopyrido[2,3-d][6,5-d]dipyrimidine
(**13**, $C_{29}H_{23}N_5O_5$)
Yield 1.06 g (45%); mp 258°C; yellowish crystals; IR (KBr): $\bar{\nu} = 3620$ (br, OH), 3190 (NH), 1680 (C=O) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 10.88$ (s, 2NH), 8.97 (s, OH), 7.63 (bs, NH), 7.38–7.25 (m, 10H-arom), 6.80–6.83 (d, $J = 8.4$ Hz, 2H-arom), 6.57–6.54 (d, $J = 8.7$ Hz, 2H-arom), 5.37 (s, CH), 5.16 (s, $2CH_2$) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 33.36$ (CH), 43.09 (CH_2), 116.12, 118.72, 124.02, 125.32, 127.41, 128.62, 129.01, 129.22, 129.63, 131.52, 133.39, 150.17, 154.67 ppm.

1,9-Dibenzyl-5-(2,4-dichlorophenyl)-3,5,7,10-tetrahydro-2,4,6,8-tetraoxopyrido[2,3-d][6,5-d]dipyrimidine
(**14**, $C_{29}H_{21}Cl_2N_5O_4$)
Yield 0.94 g (36%); mp 250°C; yellow crystals; IR (KBr): $\bar{\nu} = 3175$ (NH), 1687 (C=O) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 11.98$ (s, NH), 11.06 (s, NH), 10.76 (s, NH), 7.43–7.17 (m, 13H-arom), 5.39 (s, CH), 5.20 (s, $2CH_2$) ppm.

3,9-Dimethylxanthine (**15**)
This compound was prepared according to the reported method [17, 18]; mp 336°C (Ref. [17] 322°C).

6-Mercapto-3,9-dimethylxanthine (**16**, $C_7H_8N_4OS$)
3,9-Dimethylxanthine (5.0 g) and 15 g P_2S_5 were refluxed in 45 cm^3 dry pyridine for 4 h in an oil bath. The mixture was evaporated under vacuum to dryness and the residue was boiled with 160 cm^3 water and filtered. The filtrate was evaporated *in vacuo* to a third of its volume and the solution was leaved to stand overnight. The formed precipitate was collected by filtration, dried at 100°C, and recrystallized from 50 cm^3 H_2O to yield 0.38 g yellowish-white needles (70%); R_f (SG) = 0.47 in methanol:chloroform = 2:8; mp 308°C; UV (methanol): λ_{max} (ε) = 340.8 (4.30), 255 (3.68), 224 (3.94), 205 (4.17) nm ($mol^{-1}\,dm^3\,cm^{-1}$); ^1H NMR (*DMSO*-d_6): $\delta = 12.3$ (s, NH-1), 7.70 (s, CH-8), 3.90 (s, NCH_3-9), 3.62 (s, NCH_3-3) ppm.

6-Alkylmercapto-3,9-dimethylxanthines **17–20**
3,9-Dimethyl-6-thioxanthine (0.5 g) was dissolved in 6.0 cm^3 2 *N* NaOH and an excess of alkyl halides, 7.5 mmol methyl, ethyl, butyl iodide, and 2.5 mmol benzyl bromide were added dropwise with stirring for 2 h. The reaction mixture was cooled in an ice bath and acidified using acetic acid till *pH* adjusted 5–6. The mixture was evaporated *in vacuo* to a half amount and the formed precipitate was collected, washed with water, and recrystallized from methanol.

3,9-Dimethyl-6-methylthiopurine-2-one (**17**, $C_8H_{10}N_4OS$)
Yield 0.42 g (79%); mp 263°C; UV (methanol): λ_{max} (ε) = 273.0 (3.92), 213.0 (4.32) nm ($mol^{-1}\,dm^3\,cm^{-1}$); ^1H NMR (*DMSO*-d_6): $\delta = 7.68$ (s, CH-8), 4.08 (s, SCH_3), 3.43 (s, NCH_3-9), 3.37 (s, NCH_3-3) ppm.

3,9-Dimethyl-6-ethylthiopurine-2-one (**18**, $C_9H_{12}N_4OS$)
Yield 0.49 g (86%); mp 250°C; UV (methanol): λ_{max} (ε) = 272.0 (3.97), 215.0 (4.21) nm ($mol^{-1}\,dm^3\,cm^{-1}$); ^1H NMR (*DMSO*-d_6): $\delta = 7.68$ (s, CH-8), 4.05 (q, SCH_2), 3.41 (s, NCH_3-9), 3.36 (s, NCH_3-3), 1.28 (t, CH_3) ppm.

3,9-Dimethyl-6-butylthiopurine-2-one (**19**, $C_{11}H_{16}N_4OS$)
Yield 0.53 g (82%); mp 214°C; ^1H NMR (*DMSO*-d_6): $\delta = 7.75$ (s, CH-8), 4.11 (t, SCH_2), 3.55 (s, NCH_3-9), 3.37 (s, NCH_3-3), 1.82–1.93 (m, $2CH_2$), 0.88 (t, CH_3) ppm.

6-Benzylthio-3,9-dimethylpurine-2-one (**20**, $C_{14}H_{14}N_4OS$)
Yield 0.61 g (84%); mp 279°C; ^1H NMR (*DMSO*-d_6): $\delta = 7.82$ (s, CH-8), 7.34–7.45 (m, 5H-arom), 4.21 (s, SCH_2), 3.38 (s, NCH_3-9), 3.36 (s, NCH_3-3) ppm.

Pharmacology
The newly synthesized compounds were tested for their antimicrobial activity against some microorganisms. The following microorganisms were used: HSV-1, HAV-27 viruses (obtained from Environmental Virology Lab. Department of Water Pollution Res., National Research Centre) and Hepatitis A (cell culture adapted strain HAV-MBB, kindly provided by Prof. Dr. *Verena Gauss-Müller*, Lübeck University of Medicine, Institute of Molecular Virology, Germany), *B. subtilis* as *Gram*-positive and *E. coli* as *Gram*-negative bacteria, *C. tropicalis* as yeast, and *A. niger* as fungus. All bacterial, yeast and fungal strains were obtained from Microbial Biotechnology department (NRC), Egypt.

Antiviral Bioassay

Preparation of the Synthesized Compounds Solutions
The solutions of the compounds were prepared by dissolving 100 mg each in 1 cm^3 of 10% *DMSO* in water. The final concentration was 100 $\mu g/mm^3$ (stock solution). The stock solutions thus prepared were sterilized by addition of anti-

biotic-antimycotic mixture: 10000 U penicillin G sodium, 10000 μg streptomycin sulfate, and 250 μg amphotericin B or 50 $\mu g/cm^3$ Gentamicin. The cells used were derived from African green monkey kidney (Vero) and were propagated in *Dulbecco* as well as the D in *D*MEM (Minimum essential medium) supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic mixture. The *pH* was adjusted to 7.2–7.4 with saturated $NaHCO_3$ solution.

Plaque Reduction Assay for Rapid Screening of Antiviral Activity

Plaque reduction assay [27–29] was carried out as follows: a 6-well plate was cultivated with Vero cell culture (10^5 cell/cm^3) and incubated for 2 days at 37°C. HSV-1 and HAV-27 were diluted to give 10^4 PFU/cm^3 final concentrations for each virus and mixed with the test compound with two concentrations, 10 $\mu g/cm^3$ and 20 $\mu g/cm^3$, and incubated overnight at 4°C. Growth medium was removed from the multiwell plate and virus-compound mixture was inoculated (100 mm^3/well). After 1 h contact time, the inoculum was aspirated and 3 cm^3 MEM with 1% agarose were overlaid the cell sheets. The plates were left to solidify and incubated at 37°C until the development of virus plaques. Cell sheets were fixed in 10% formalin solution for 2 h and stained with crystal-violet. Control virus and cells were treated identically without chemical compound. Virus plaques were counted and the percentage of reduction was calculated. A 50% inhibitory concentration EC_{50} was calculated as the concentration required reducing virus yield by 50%, in the compounds-treated cultures compared with the untreated ones.

The Antimicrobial (Bacteria, Yeast, and Fungi) Bioassay

Growth Media

a) *Nutrient Agar Medium for Bacteria* [30]

The medium was prepared by dissolving the following ingredients in 1 dm^3 distilled water and autoclaved for 15 min, beef extract (1 g), yeast extract (2 g), peptone (5 g), sodium chloride (5 g), agar (2 g), and Tween-60 (10 cm^3) the *pH* adjusted to 7.4.

b) *Malt Extract Agar Medium for Yeast and Fungi* [30]

The medium consisted of the following ingredients in 1 dm^3, malt extract (3 g), peptone (5 g), agar (20 g), and Tween-60 (10 cm^3), the *pH* was adjusted to 5.4.

Broth Media

For preparation of the inoculum of each microorganism the broth of both media was prepared with the same method but without agar.

Modified Agar Diffusion Cylinder Method [31]

1) *Preparation of Inoculum*

The broth media were used for about 18–20 h till the optical density (OD) becomes in the range 0.2–0.5 for bacteria and yeast. The broth media were used for about 48 h and the mycelial fragments were removed and the solution was used for inoculation of fungi.

2) *Preparation of Plates and Inoculation*

Inoculum (0.5 cm^3) was added to 24 cm^3 of agar malted media (50°C) and mixed by simple inversion. The malted medium was poured into each 150 mm sterile *Petri* plates and 4–6 mm wells were cut into the harden seeded plates by *pasteur* pipette and finally, the wells were filled with the various compounds (stock solution, 5 mg/cm^3 *DMSO*). The plates were incubated at 37°C for 24 h for bacteria and yeast and for 48–72 h for fungi. The diameters of the inhibition zones (mm) were measured and recorded.

Determination of the Minimum Inhibitory Concentration (MIC)

Serial dilutions of the promising compounds were subjected to *MIC* determination, they were prepared in the following pattern: 1000 $\mu g/cm^3$, 200 $\mu g/cm^3$, 100 $\mu g/cm^3$, 50 $\mu g/cm^3$, 10 $\mu g/cm^3$, and 5 $\mu g/cm^3$. *Bacillus subtilis* was selected to be the test organism because it was the most sensitive organism. The different concentrations of each compound were tested with the Modified Agar Diffusion Cylinder method as described before.

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