Magda N. Nasr,
Magdy M. Gineinah

Department of Medicinal Chemistry
Faculty of Pharmacy,
University of Mansoura,
Mansoura 35516, Egypt

Pyrido[2,3-*d*]pyrimidines and Pyrimido[5′,4′:5,6]pyrido[2,3-*d*]pyrimidines as New Antiviral Agents: Synthesis and Biological Activity

A series of 7-amino- and 7-oxo-5-aryl-6-cyanopyrido[2,3-*d*]pyrimidines, **4** and **11**, respectively, and pyrimido[5′,4′:5,6]pyrido[2,3-*d*]pyrimidine derivatives **6** and **7** was synthesized and investigated as antiviral agents. Different synthetic strategies for the preparation of the target compounds were explored. A synthetic procedure for **4** and **11** starting with 6-amino-1,2,3,4-tetrahydro-2,4-dioxopyrimidine, proper aldehyde, and malononitrile or ethyl cyanoacetate, respectively, in a one-pot reaction proved to be the method of choice for preparation of compounds of such type. Construction of another pyrimidine ring on the pyridine nucleus of compound **4** was achieved either by reaction with phenyl iso(thio)cyanate or with formic acid to yield **6** and **7**, respectively. The structure of the prepared compounds was confirmed through elemental analysis and spectral investigation. Most of the newly synthesized compounds were subjected to antiviral activity testing against herpes simplex virus (HSV) where some of them show good activities.

Keywords: 7-Amino-6-cyanopyrido[2,3-*d*]pyrimidine; Pyrimido[5′,4′:5,6]pyrido-[2,3-*d*]pyrimidine; Antiviral activity; Cytotoxicity; Aphidicholin

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Introduction

Antiviral and antimicrobial agents are among the most commonly prescribed pharmaceuticals worldwide. Efforts to improve the therapeutic significance of these agents have resulted in identification of more potent compounds. Pyrido[2,3-*d*]pyrimidine and some of its derivatives display several potentially useful biological activities [1, 2]. They show dihydrofolate reductase inhibition and antitumor [3, 4] as well as diuretic properties [5]. Moreover, some of them possess antimicrobial [6], antiviral, and cytotoxic activities [7, 8]. On the other hand, 4,6-disubstituted 2-amino-3-cyanopyridines have been prepared and evaluated extensively as antimicrobial agents [9, 10]. Furthermore, substituted 3-cyano-2-oxopyrido[2,3-*d*]pyridine was found to be highly cytotoxic to the infected T4 cell at a concentration which showed marginal antiviral activity in the infected culture [7]. In the light of these observations, we have synthesized 7-amino-6-cyanopyrido[2,3-*d*]pyrimidines in addition to other derivatives with the 7-amino group being replaced with an oxo functionality. Furthermore, some pyrimido-[5′,4′:5,6]pyrido[2,3-*d*]pyrimidines were prepared in order to obtain novel pyrido[2,3-*d*]pyrimidine derivatives with antiviral and cytotoxic properties.

Correspondence: Magdy M. Gineinah, Department of Medicinal Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt. Phone: +20 50 2247496, Fax: +20 50 2247496, e-mail: maggineinah@yahoo.com

Results and discussion

Chemistry

The synthetic pathways to the target compounds are outlined in Schemes 1 and 2. Preparation of the requisite 7-amino-5-aryl-6-cyanopyrido[2,3-*d*]pyrimidines **4** for the construction of pyrimido[5′,4′:5,6]pyrido[2,3-*d*]pyrimidine derivatives **6** and **7** (Table 1) began with 6-amino-1,2,3,4-tetrahydro-2,4-dioxopyrimidine (**1**). Synthesis of the key intermediate **4** from compound **1** was accomplished through two procedures. Firstly, an ethanolic suspension of compound **1** was allowed to react with equimolar amount of benzylidene derivatives of malononitrile **2** [11, 12] as α,β-unsaturated compounds (Method A). The 5-phenyl analogue of compounds **4** was previously reported [13]. In the second procedure, a mixture of compound **1**, the proper aromatic aldehyde **3**, malononitrile, and ethanol was heated under reflux in a one-pot reaction technique (Method B). However, the one-pot reaction technique is considered to be superior over Method A in terms of product yields. Structural assignment of **4** through IR spectroscopy gave the characteristic nitrile absorption at 2218–2226 cm^{-1} while bands

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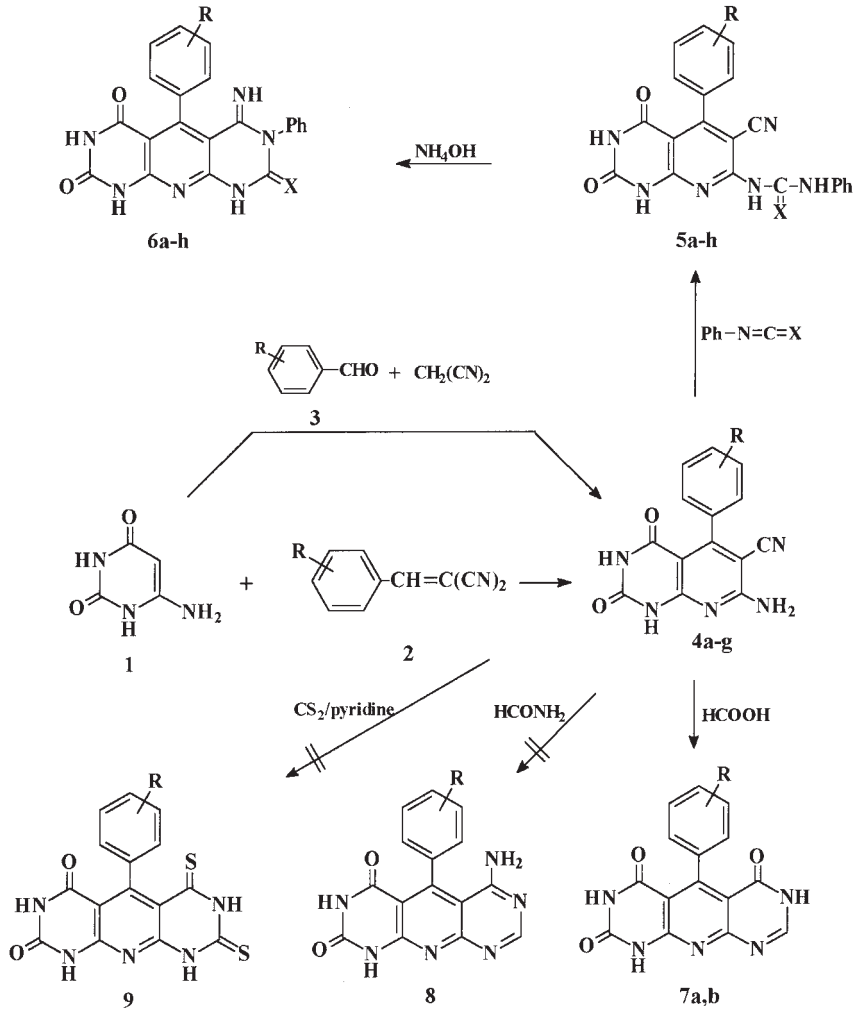
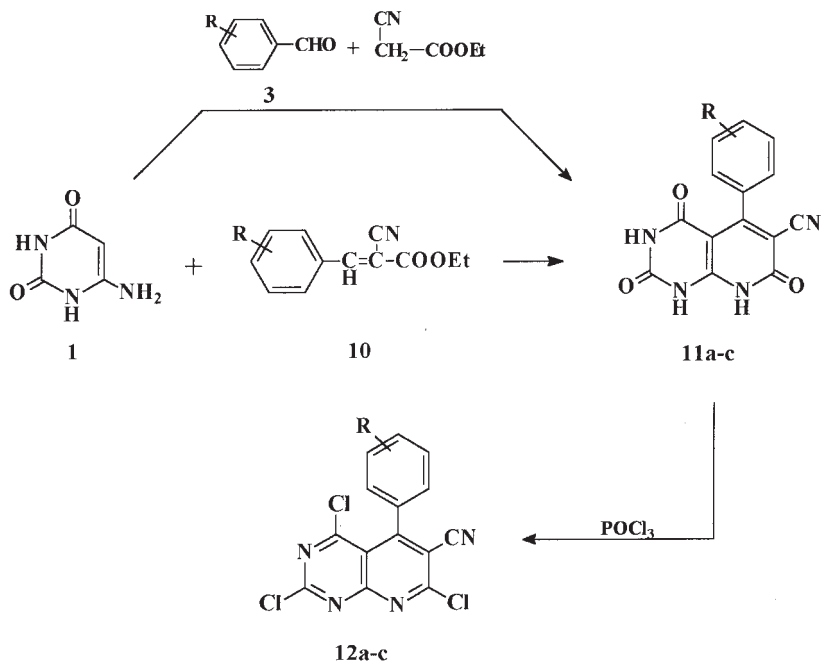


Scheme 1



Scheme 2

Table 1. Physicochemical data of the new compounds.

Comp.	R	X	Mp (°C)	Recryst. solvent[a]	Yield (%)	Molecular formula
4a	4-Cl		>300	M	88	$C_{14}H_8ClN_5O_2$
4b	2-Br		>300	M	90	$C_{14}H_8BrN_5O_2$
4c	4-Br		>300	M	85	$C_{14}H_8BrN_5O_2$
4d	3-OH		>300	AM	79	$C_{14}H_9N_5O_3$
4e	3-NO_2		>300	AM	93	$C_{14}H_8N_6O_4$
4f	2,4-$(OCH_3)_2$		>300	M	84	$C_{16}H_{13}N_5O_4$
4g	3,4-$(OCH_3)_2$		>300	M	89	$C_{16}H_{13}N_5O_4$
5a	4-Cl	S	285–287	E	79	$C_{21}H_{13}ClN_6O_2S$
5b	4-Br	S	296–298	E	75	$C_{21}H_{13}BrN_6O_2S$
5c	2,4-$(OCH_3)_2$	S	>300	E	82	$C_{23}H_{18}N_6O_4S$
5d	3,4-$(OCH_3)_2$	S	293–295	AE	77	$C_{23}H_{18}N_6O_4S$
5e	4-Cl	O	>300	AE	86	$C_{21}H_{13}ClN_6O_3$
5f	4-Br	O	>300	AE	90	$C_{21}H_{13}BrN_6O_3$
5g	2,4-$(OCH_3)_2$	O	>300	E	74	$C_{23}H_{18}N_6O_5$
5h	3,4-$(OCH_3)_2$	O	284–286	E	78	$C_{23}H_{18}N_6O_5$
6a	4-Cl	S	>300	E	69	$C_{21}H_{13}ClN_6O_2S$
6b	4-Br	S	>300	M	71	$C_{21}H_{13}BrN_6O_2S$
6c	2,4-$(OCH_3)_2$	S	>300	M	73	$C_{23}H_{18}N_6O_4S$
6d	3,4-$(OCH_3)_2$	S	>300	E	68	$C_{23}H_{18}N_6O_4S$
6e	4-Cl	O	>300	M	81	$C_{21}H_{13}ClN_6O_3$
6f	4-Br	O	>300	E	78	$C_{21}H_{13}BrN_6O_3$
6g	2,4-$(OCH_3)_2$	O	>300	E	82	$C_{23}H_{18}N_6O_5$
6h	3,4-$(OCH_3)_2$	O	>300	M	83	$C_{23}H_{18}N_6O_5$
7a	4-Cl		>300	E	66	$C_{15}H_8ClN_5O_3$
7b	3-NO_2		>300	M	75	$C_{15}H_8N_6O_5$
11a	4-Br		294–296	M	85	$C_{14}H_7BrN_4O_3$
11b	3-OH		292–294	M	89	$C_{14}H_8N_4O_4$
11c	2,4-$(OCH_3)_2$		>300	M	93	$C_{16}H_{12}N_4O_5$
12a	4-Br		238–240	E	82	$C_{14}H_4BrCl_3N_4$
12b	3-OH		284–286	M	79	$C_{14}H_5Cl_3N_4O$
12c	2,4-$(OCH_3)_2$		164–166	E	91	$C_{16}H_9Cl_3N_4O_2$

[a] Recrystallisation solvent: AE: aqueous ethanol, AM: aqueous methanol, E: ethanol, M: methanol.

of the NH groups came at 3243–3485 cm^{-1}. The 7-amino-5-aryl-6-cyanopyrido[2,3-*d*]pyrimidine derivatives **4** were subjected to reaction with phenyl iso(thio)cyanate neat at room temperature to furnish the corresponding 7-(3-phenyl(thio)ureido) derivatives **5**. Cyclization of these urea and thiourea derivatives was achieved through the use of ammonia solution to give the tricyclic structure **6**. Efforts for the transformation of **4** into pyrimidopyridopyrimidines **7**–**9** with formic acid, formamide, or carbon disulfide, respectively, under reflux were unsuccessful. The IR spectra of the products illustrated the typical absorption peak at 2217–2223 cm^{-1} indicative of the unreacted cyano group. Similarly, trials for preparation of **8** by refluxing **4** with formamide even in the presence of catalytic amount of formic acid or a small amount of dimethylformamide did not succeed. However, similar cyclization reactions were successful when the ring bearing the α-amino-β-cyano groups is a π-excessive heterocyclic ring [14, 15]. In contrast, heating of **4** with formic acid in a sealed tube gave compounds **7 a, b**. Analogous conditions of **4** with formamide or carbon disulfide/pyridine did not give **8** or **9**, respectively. However, formation of **7** might be attributed, in part, to the acidity of the cyclizing agent that catalyzes the hydrolysis of the cyano group as an initial step prior to cyclization.

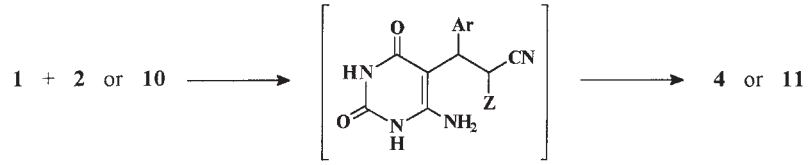
The 6-cyano-7-oxopyrido[2,3-*d*]pyrimidine derivatives **11** were obtained using the procedure described in Scheme 2. They were prepared by a synthetic approach similar to that used for preparation of **4**. The synthetic pathways utilized were either reaction of **1** with the benzylidene derivatives of ethyl cyanoacetate **10** [12] (Method A) or a one-pot reaction procedure using equimolar ratios of **1**, the proper aldehyde **3**, and ethyl cyanoacetate (Method B). The same advantage of Method B over Method A is noted here as well. The phenyl analogue of **11** was synthesized applying a procedure more or less similar to that described in Method A [13]. However, compounds **11** were subjected to chlorination using phosphoryl chloride to give the trichloro derivatives of pyrido[2,3-*d*]pyrimidine structure **12**. The formation of compounds **4** and **11** in the current work is assumed to proceed via a sequence similar to that discussed earlier [11, 12]. These reactions take place through 1,4-addition of the nucleophilic carbon atom C-5 of the pyrimidine ring of **1** towards the α,β-unsaturated system of **2** or **10** followed by cyclization and autooxidation (Scheme 3).

An unequivocal structure proof of the previously reported similar compounds was achieved by examination of the crystal structure through X-ray analysis [12].

Biological screening

Virtually all of the newly synthesized compounds were evaluated for their antiviral as well as their cytotoxic activities. The goal of the current study is to define the optimum substituents on the pyrido[2,3-*d*]pyrimidine skeleton to identify compounds with high potency. The antiviral activity was assayed against herpes simplex virus (HSV) type 1. The activity was determined by plaque reduction assays in Vero cells using aphidicholine as a reference standard [16]. Cytotoxicity measurements were based on the inhibition of cultured mammalian cell growth [17]. The biological screening data for the active compounds are presented in Table 2.

From the active compounds, **4** with 7-amino-5-aryl-6-cyanopyrido[2,3-*d*]pyrimidine structure are among the least active group of compounds. Significant improvements resulted when modifications were made to the pyridine ring. Replacement of the 7-amino group in 4 with a thioureido functionality, sharply increased the antiviral activity. It is conceivable that the halo substituent on the 5-aryl group in compounds **5** creates better activity. This may be attributed to the greater lipophilicity of these compounds. Thus, compound **5 b** with 4-bromo group was found to be the most potent of the test compounds while compound **5 a** with 4-chloro substituent comes at the second rank in terms of antiviral and cytotoxic activities. Furthermore, regarding compound **5 b**, the selectivity index [ratio of the 50 % cytotoxic concentration (CC_{50}) to the 50 % virus inhibitory concentration (IC_{50})] is about 12. This indicates a fairly selective antiviral action. Within the thioureido series, compounds **5 c** and **5 d** with isomeric dimethoxyphenyl moieties are of comparable activities and less potent than the halo analogs. On the other hand, replacement of the thioureido group with a ureido functionality afforded compounds **5 f** and **5 g**. The resulting compounds retain considerable antiviral potencies. However, these compounds are less potent than the corresponding thioureido analogs. Cyclization of **5** with ammonium hydroxide gave **6** with one more pyrimidine ring fused to the pyridine nucleus. The antiviral activities of these tricyclic compounds are comparable to



Scheme 3

Table 2. Anti-HSV (type 1) activity and cytotoxic properties of tested compounds.

Comp.	Antiviral activity IC_{50} (μg/mL)[a]	Cytotoxicity CC_{50} (μg/mL)[b]	Selectivity index
4 c	40	200	5
4 e	50	170	3.4
4 g	50	200	4
5 a	8	40	5
5 b	4	47	11.75
5 c	12	50	4.17
5 d	14	50	3.57
5 e	20	80	4
5 f	18	70	3.89
5 g	20	80	4
6 b	10	50	5
6 d	16	80	5
6 f	30	80	2.67
7 b	10	50	5
11 a	40	150	3.75
12 a	50	190	3.8
APC[c]	2.1	50	23.81

[a] 50 % inhibitory concentration, or concentration required to reduce virus plaque formation by 50 %. Virus input was 40 plaque forming units (PFU);
[b] 50 % cytotoxic concentration, or concentration required to reduce cell growth by 50 %;
[c] APC: Aphidicholin. Assays were performed in duplicate.

activities of compounds **5**, which are the most active ones of the congeneric series studied. The thioxo derivatives of **6** are more potent than the oxo analogs. On the other hand, replacement of the 4-bromophenyl group in **6 b** with 3,4-dimethoxyphenyl in **6 d** resulted in attenuation of the antiviral activity and cytotoxicity as well. Compound **7 b**, with analogous structure to **6**, being tricyclic with the same ring system, has been found to possess potent antiviral activity. So we can say that the presence of another pyrimidine ring fused to the pyridine nucleus or the presence of a substituent with two nitrogen atoms separated by one carbon (ureido and thioureido groups) shifted the antiviral activity toward better potency. Moreover, compound **11 a**, being substantially less potent than compounds **5**, **6**, and **7** and possessing comparable activity with **4** (both have the same ring system), may confirm the previous conclusion. However, chlorination of the three oxo groups of **11** was achieved to obtain compounds with greater number of halogen atoms in the hope of getting more active compounds. Unexpectedly, compound **12 a** was less active than **11 a**. This might suggest that the halo groups are not tolerated at all the positions of the fused system.

In conclusion, 7-substituted 5-aryl-6-cyanopyrido[2,3-*d*]pyrimidine derivatives exhibit antiviral and cytotoxic activities. Both electron-withdrawing and donating groups are tolerated on the 5-phenyl ring. Compounds possessing moieties with hydrophobic properties show obviously increased activities. Nevertheless, it should be stated that most halo derivatives exhibit a tendency for higher antiviral activity compared to the non-halogenated compounds. The introduction of a thioureido group at the 8-position proved to furnish the maximum antiviral activity. Annelation of another pyrimidine ring at the pyridine side gave compounds with good potency. The isosteric replacement of a sulfur atom with oxygen resulted in a slight decrease in activity of both cyclized and non-cyclized structures.

Finally, in view of the promising activities displayed by various compounds described in this study, further improvements on their potency could lead to novel and safe antiviral agents with potential therapeutic utilities.

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Experimental part

Chemistry

Melting points were determined on a Fischer-Johns apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian EM-360 (90 MHz) instrument using TMS as internal standard (chemical shifts in ppm, δ units). IR spectra were carried out on a PYE UNICAM SP 1000 spectrophotometer on KBr8 disk (ν in cm^{-1}) The results of elemental analyses (C,H,N) were within ± 0.4 % of the theoretical values. Thin-layer chromatography was performed on silica gel GLF plates, 250 μm.

*7-Amino-5-aryl-6-cyano-1,2,3,4-tetrahydro-2,4-dioxopyrido-[2,3-d]*pyrimidines (**4 a**–**g**)

Method A. A mixture of 6-amino-1,2,3,4-tetrahydro-2,4-dioxopyrimidine (**1**) (0.25 g, 2 mmol), the corresponding arylidene derivative of malononitrile **2** (2 mmol) and absolute ethanol (15 mL) was heated under reflux for 2 h. After cooling to room temperature, the product was collected by filtration, washed with ethanol, dried, and recrystallized to give the desired product.

Method B. A mixture of **1** (0.25 g, 2 mmol), the corresponding substituted benzaldehyde **3** (2 mmol), malononitrile (2 mmol), and absolute ethanol (20 mL) was heated under reflux for 4 h. The reaction mixture was allowed to cool, the product was collected by filtration, washed with ethanol, dried, and recrystal-

lized. IR, **4 a**: 3415, 3320 (NH), 2218 (CN), 1705, 1640 (C=O). **4 b**: 3487, 3352 (NH), 2226 (CN), 1716, 1637 (C=O). **4 d**: 3243, 3410 (NH and OH), 2219 (CN), 1720, 1619 (C=O). **4 e**: 3518, 3364 (NH), 2224 (CN), 1724, 1639 (C=O), 1345, 1518 (NO_2). **4 g**: 3424, 3243 (NH), 2221 (CN), 1725, 1642 (C=O). ^{1}H-NMR (DMSO-d_6), **4 a**: 5.94 (br s, 2 H, NH_2; D_2O exchangeable), 7.41–7.63 (m, 4 H, Ar-H), 12.31 (br s, 2 H, 2 NH; D_2O exchangeable), **4 b**: 6.03 (br s, 2 H, NH_2; D_2O exchangeable), 7.47–7.72 (m, 4 H, Ar-H), 12.35 (br s, 2 H, 2 NH; D_2O exchangeable). **4 d**: 5.02 (s, 1 H, OH; D_2O exchangeable), 5.89 (br s, 2 H, NH_2; D_2O exchangeable), 6.77–7.61 (m, 4 H, Ar-H), 12.19 (br s, 2 H, 2 NH; D_2O exchangeable). **4 e**: 6.11 (br s, 2 H, NH_2; D_2O exchangeable), 7.48–7.82 (m, 4 H, Ar-H), 12.51 (br s, 2 H, 2 NH; D_2O exchangeable). **4 f**: 4.11 (s, 6 H, 2 OCH_3), 5.84 (br s, 2 H, NH_2; D_2O exchangeable), 6.62–7.62 (m, 3 H, Ar-H), 12.63 (br s, 2 H, 2 NH; D_2O exchangeable).

*5-Aryl-6-cyano-7-(3-phenyl(thio)ureido)-1,2,3,4-tetrahydro-2,4-dioxopyrido[2,3-d]*pyrimidines (**5 a**–**h**)

A mixture of **4** (2 mmol), phenyl iso(thio)cyanate (2 mmol), and absolute ethanol (20 mL) was heated under reflux for 30–40 h. The reaction mixture was cooled to room temperature, the product was collected by filtration, dried, and recrystallized from the proper solvent. IR, **5 a**: 3428, 3291 (NH), 2223 (CN), 1707, 1625 (C=O). **5 d**: 3470, 3284 (NH), 2216 (CN), 1715, 1642 (C=O). **5 f**: 3392, 3218 (NH), 2221 (CN), 1704, 1631 (C=O). ^{1}H-NMR (DMSO-d_6), **5 a**: 7.21–7.68 (m, 9 H, Ar-H), 7.81 (br s, 1 H, NH; D_2O exchangeable), 8.32 (br s, 1 H, NH; D_2O exchangeable), 12.34 (br s, 2 H, 2 NH; D_2O exchangeable). **5 c**: 4.21 (br s, 6 H, 2 OCH_3), 6.64–7.57 (m, 8 H, Ar-H), 7.73 (br s, 1 H, NH; D_2O exchangeable), 8.41 (br s, 1 H, NH; D_2O exchangeable, 12.41 (br s, 2 H, 2 NH; D_2O exchangeable). **5 f**: 7.14–7.58 (m, 9 H, Ar-H), 8.01 (br s, 1 H, NH; D_2O exchangeable), 8.52 (br s, 1 H, NH; D_2O exchangeable), 12.81 (br s, 2 H, 2 NH; D_2O exchangeable). **5 h**: 4.33 (s, 6 H, 2 OCH_3), 6.79–7.61 (m, 8 H, Ar-H), 7.97 (br s, 1 H, NH; D_2O exchangeable), 8.49 (br s, 1 H, NH; D_2O exchangeable), 12.79 (br s, 2 H, NH; D_2O exchangeable).

*5-Aryl-4-imino-3-phenyl-1,2,3,4,6,7,8,9-octahydro-6,8-dioxo-2-(thio)oxopyrimido-[5′,4′:5,6]pyrido[2,3-d]*pyrimidines (**6 a**–**h**)

A mixture of compound **4** (1 mmol), ethanol (10 mL), and aqueous ammonia solution (25 %, 10 mL) was stirred at room temperature for 24 h. The reaction mixture was refluxed for 1 h, the product was collected by filtration, washed with water, dried, and recrystallized from the proper solvent. ^{1}H-NMR (DMSO-d_6), **6 a**: 7.22–7.74 (m, 9 H, Ar-H), 8.94 (br s, 1 H, NH; D_2O exchangeable), 11.42 (br s, 1 H, NH; D_2O exchangeable), 12.69 (br s, 2 H, 2 NH; D_2O exchangeable). **6 b**: 7.25–7.82 (m, 9 H, Ar-H), 8.99 (br s, 1 H, NH; D_2O exchangeable), 11.44 (br s, 1 H, NH; D_2O exchangeable), 12.71 (br s, 2 H, 2 NH; D_2O exchangeable). **6 d**: 4.27 (s, 6 H, 2 OCH_3), 6.68–7.74 (m, 8 H, Ar-H), 8.89 (br s, 1 H, NH; D_2O exchangeable), 11.43 (br s, 1 H, NH; D_2O exchangeable, 12.68 (br s, 2 H, 2 NH; D_2O exchangeable). **6 f**: 7.31–7.82 (m, 9 H, Ar-H), 9.02 (br s, 1 H, NH; D_2O exchangeable), 11.95 (br s, 1 H, NH; D_2O exchangeable, 12.84 (s, 2 H, 2 NH; D_2O exchangeable). **6 g**: 4.35 (s, 6 H, 2 OCH_3), 6.61–7.76 (m, 8 H, Ar-H), 8.91 (br s, 1 H, NH; D_2O exchangeable), 12.02 (br s, 1 H, NH; D_2O exchangeable), 12.74 (br s, 2 H, 2 NH; D_2O exchangeable).

5-Aryl-1,2,3,4,6,7-hexahydro-2,4,6-trioxopyrimido[5′,4′:5,6]-pyrido[2,3-d]pyrimidines (**7 a, b**)

A mixture of **4** (0.5 mmol) and formic acid (10 mL) was heated in a sealed tube at 140–150 °C for 5 h. The reaction mixture was then allowed to cool, poured onto crushed ice, and neutralized with ammonium hydroxide. The product was collected by filtration, washed with water, dried, and recrystallized. IR, **7 a**: 3460, 3233 (NH), 1715, 1646 (C=O). ^{1}H-NMR (DMSO-d_6), **7 a**: 7.31–7.85 (m, 4 H, Ar-H), 8.65 (s, 1 H, Ar-H), 10.92 (br s, 1 H, NH; D_2O exchangeable), 12.81 (br s, 2 H, 2 NH; D_2O exchangeable). **7 b**: 7.44–7.93 (m, 4 H, Ar-H), 8.66 (s, 1 H, Ar-H), 10.89 (br s, 1 H, NH; D_2O exchangeable), 12.75 (br s, 2 H, 2 NH; D_2O exchangeable).

5-Aryl-6-cyano-1,2,3,4,7,8-hexahydro-2,4,7-trioxopyrido[2,3-d]pyrimidines (**11 a**–**c**)

These compounds were prepared following the same procedures (Methods A and B) as were used for preparation of compounds **4** provided that malononitrile was replaced by ethyl cyanoacetate. IR, **11 a**: 3465, 3296 (NH), 2223 (CN), 1732, 1645 (C=O). ^{1}H-NMR (DMSO-d_6), **11 a**: 7.32–7.81 (m, 4 H, Ar-H), 12.42 (br s, 3 H, 3 NH; D_2O exchangeable). **11 c**: 4.17 (s, 6 H, 2 OCH_3), 6.75–7.71 (m, 3 H, Ar-H), 12.72 (s, 3 H, 3 NH; D_2O exchangeable).

5-Aryl-2,4,7-trichloro-6-cyanopyrido[2,3-d]pyrimidines (**12 a**–**c**)

A mixture of **11** (5 mmol), phosphoryl chloride (40 mL), and *N,N*-dimethylaniline (0.5 mL) was heated under reflux for 8 h. The reaction mixture was cooled to room temperature, poured onto ice, the product was collected by filtration, washed with water, dried, and extracted with ether. The ethereal extract was washed with water, dried over sodium sulfate, the solvent was evaporated, and the product was recrystallized. ^{1}H-NMR (DMSO-d_6), **12 b**: 5.12 (s, 1 H, OH; D_2O exchangeable), 6.82–7.64 (m, 4 H, Ar-H). **12 c**: 4.21 (s, 6 H, 2 OCH_3), 6.68–7.63 (m, 3 H, Ar-H).

Biological screening

1. Antiviral testing

Confluent Vero cells grown in 96-well microtiter trays were infected with 40 plaque forming units (PFU) of Herpes simplex virus-type 1 (HSV-1) in 100 µL medium containing 10 % (*v/v*) calf serum. A serial dilution of the test compounds (in duplicate) dissolved in the medium was added (100 µL in each well). After 66 h of incubation at 37 °C in 5 % CO_2 atmosphere, the trays were inverted onto a pad of paper towels and remaining cells were rinsed with the medium and fixed with 3.7 % (*v/v*) formaldehyde in saline solution for 20 min. The cells were then rinsed with water, stained with 0.5 % (*w/v*) crystal violet solution in 20 % ethanol for 30 min and rinsed with water. The trays were examined visually. The antiviral activity is expressed as IC_{50} which represents the compound concentration required to reduce virus plaque formation by 50 %.

2. Cytotoxicity assay

Cytotoxicity measurements were based on the inhibition of 3T3 cell growth. 3T3 fibroblasts were seeded at a rate of 2×10^3 cells/well microtiter plates. Different concentrations of the test compounds were then added (in duplicate), and after 3–4 days of incubation at 37 °C in 5 % CO_2 atmosphere, the cell number was determined using a hemocytometer. Cytotoxicity is expressed as CC_{50}, which represents the compound concentration required to reduce cell growth by 50%.

References

[1] L. Prakash, M. Shaihla, R.L. Mital, *Pharmazie* **1989**, *44*, 490.

[2] L. K. A. Rahman, S. R. Chhabra, *Med. Res. Rev.* **1988**, *8*, 95.

[3] E. M. Grivsky, S. Lee, C. W. Sigel, D. S. Duch, C. A. Nichol, *J. Med. Chem.* **1987**, *23*, 327.

[4] A. Gangjee, A. Vasudevan, F. Queener, R. Kisliuk, *J. Med. Chem.* **1996**, *39*, 1438.

[5] A. Monge, V. Martinez, C. San Martin, M. A. Simon, *Spanish Patent ES* 2,056,742. **1994** [Chem. Abstr. **1995**, 122, 105912q].

[6] S. A. K. Sharma, L. Prakash, *Heterocyclic Commun.* **1994**, *1*, 89.

[7] H. I. El-Subbagh, S. M. Abuzaid, M. A. Mahran, F. A. Badria, A. M. Al-Obaid, *J. Med. Chem.* **2000**, *43*, 2915.

[8] I. Lorand, J. Deli, D. Szabo, A. Foldesi, Pharmazie **1985**, *40*, 530.

[9] A. Attia, A. Michael, *Pharmazie* **1982**, *37*, 551.

[10] P. Koeckkritz, C. Ruhmann, D. Fieblinger, C. Schroeder, B. Joksch, H. Heider, B. Weiher, J. Liebscher, *Ger. Offen. DE* 4,117,802 [Chem. Abstr. **1993**, 118, 191550s].

[11] J. Quiroga, M. Alvarado, B. Insuasty, M. Nogueras, A. Sanchez, J. Cobo, *J. Heterocyclic Chem.* **1998**, *35*, 1309.

[12] J. Quiroga, M. Alvarado, B. Insuasty, R. Moreno, E. Ravina, I. Estevez, *J. Heterocyclic Chem.* **1999**, *36*, 1311.

[13] A. Geies, *J. Chin. Chem. Soc.* **1999**, *46*, 69.

[14] C. G. Dave, R. D. Shah, *J. Heterocyclic Chem.* **1998**, *35*, 1295.

[15] C. G. Dave N. D. Desia, *J. Heterocyclic Chem.* **1999**, *36*, 729.

[16] M. Abou-Karam, W. T. Shier, *J. Nat. Prod.* **1990**, *53*, 340.

[17] L. D. Hafford, F. A. Badria, M. Abou-Karam, W. T. Shier, R. D. Rogers, *J. Nat. Prod.* **1991**, *54*, 1543.