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Original article

Regioselective synthesis of 6-substituted-2-amino-5-bromo-4(3*H*)-pyrimidinones and evaluation of their antiviral activity

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ABSTRACT

A series of 2-amino-5-bromo-4(3*H*)-pyrimidinone derivatives bearing different substituents at the C-6 position were synthesized using a highly regioselective lithiation–substitution protocol, and the effect of structural variation at the C-6 position on their antiviral activity in cell culture was evaluated. Although some of the derivatives were found to be active against various virus strains, they were effective only close to their toxicity threshold.

1. Introduction

The efficacy of interferons in mediating an antiviral response in the virus-infected host has been well established [1]. One of the most important approaches adopted in antiviral therapy is the use of interferon inducers [2,3]. Agents capable of stimulating interferon production range from viruses and bacterial cell walls to synthetic compounds or biopolymers [4] along with a small group of low-molecular-weight compounds (Fig. 1). Some of the compounds which have been reported to induce interferon include *N*,*N*-dioctadecyl-*N*′,*N*′-bis(2-hydroxyethyl)propanediamine **1** [5], *N*,*N*′-dihexadecyl-*m*-xylylenediamine **2** [6], bis(diethy1amino)fluorenone (**3**, tilorone) [7], 1,5-bis[[(diethylamino)ethyl]amino]-9,10-anthraquinone **4** [8] and nucleobase derived 9-benzyl-8-hydroxy adenines **5** [9].

Although the compound 2-amino-5-bromo-6-methyl-4(3*H*)-pyrimidinone **6** (Fig. 1) has been reported to induce interferon in rodents and cats when administered orally or intraperitoneally [10], it has been observed to exhibit toxicity-limiting crystal deposition in the renal papillae of rats upon chronic administration [11]. Subsequent studies have demonstrated that the analogue of ABMP, 6-phenylpyrimidinones does not possess this toxicity and exhibits enhanced interferon-inducing antiviral potency and activity [12]. Further biological evaluation of an initial lead candidate in this second-generation pyrimidinone series, 2-amino-5-bromo-6-phenyl-4(3*H*)-pyrimidinone **7**, revealed an intriguing spectrum of immunomodulatory activity which may be related to its antiviral [13] and antitumour activity [14]. In the present study, a highly regioselective and efficacious protocol to prepare C-6 elaborated 2-amino-5-bromo-4(3*H*)-pyrimidinones required for the elucidation of the structure–activity relationship (SAR) in this pyrimidinone series has been reported. In addition, a rather elaborated antiviral assay of this series of new compounds along with examination of their cytotoxicity has been carried out.

2. Chemistry

The compound 2-amino-6-methyl-4(3*H*)-pyrimidinone **8** was readily synthesized by condensation of the β-ketoester, ethylacetoacetate with guanidine carbonate in ethanol–toluene mixture (Scheme 1), obtaining 65% yield following a reported procedure [13].

In order to append different substituents at the C-6 position of compound **8**, our synthesis scheme was commenced by protecting the NH_2 group of compound **8** with a *tert*-butyloxycarbonyl (Boc)

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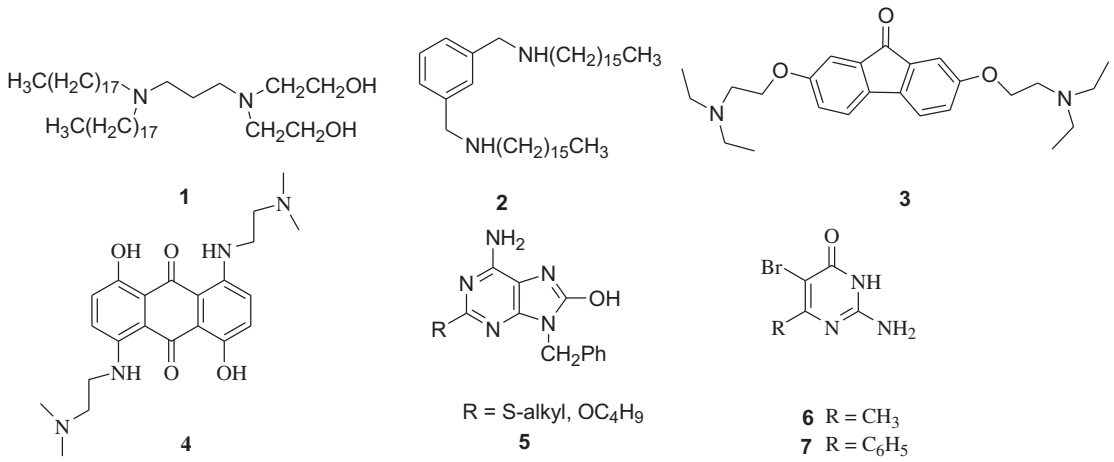
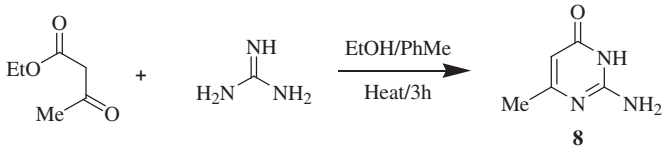


Fig. 1. Selected examples of low molecular weight interferon inducers.

group using Et_3N as the base in THF at 40 °C to obtain the desired compound **9** with a yield of 47% (Scheme 2) [15]. A metallation–alkylation sequence was used for the synthesis of 6-substituted derivatives of compound **8**. In order to fully exploit the synthesis potential of this procedure, the reaction of the lithium derivative of compound **8** with several electrophiles was investigated (Scheme 2). All the compounds exhibited satisfactory characteristic data (*vide experimental*).

The anion intermediate **10** was generated *in situ* by treating compound **9** with *n*-BuLi (3.1 equiv) in anhydrous THF at 0 °C in an atmosphere of dry nitrogen gas. The treatment of **10** with an appropriate electrophile **11** such as ethyl bromide, *n*-propyl bromide and *n*-butyl bromide (1.2 equiv, Table 1) at 0 °C led to the substitution at the more nucleophilic C-6 (vinylogous) methyl. Thus, the formation of the corresponding O/N-alkylated product was not observed. Subsequent quenching with a saturated aqueous solution of NH_4Cl at 0 °C, afforded 6-substituted derivatives **12a–c** (Scheme 2). These derivatives were then treated with trifluoroacetic acid: methylene chloride (2:8 v/v) mixture at room temperature without further purification to obtain compounds **13a–c** in good yields (Table 1, entries 1–3).

Similarly, compound **9** was subjected to the above-mentioned metallation reaction, followed by quenching with 2-bromoethyl benzene to obtain compound **12d**, and subsequent deprotection of **12d** produced the desired compound **13d** in 70% yield (Table 1). Likewise, reaction of the anionic intermediate **10** with acetone followed by deprotection of the corresponding **12e** produced a good yield of compound **13e**. To achieve further diversification at the C6-position of compound **8**, reactions of the anionic intermediate **10** were performed with a few aldehydes. Thus, the reaction of compound **9** with *n*-BuLi followed by reaction with 4-chlorobenzaldehyde and 2-trifluoromethylbenzaldehyde and subsequent deprotection of the corresponding intermediates **12f** and **12g** with TFA produced good yields of compounds **13f** and **13g** (Table 1, entries 6 and 7). Likewise, reaction of the anionic species **10** with 2,4,6-trimethylbenzaldehyde and 3,4-dimethoxybenzaldehyde



Scheme 1. Synthesis of 2-amino-6-methyl-4(3*H*)-pyrimidinone.

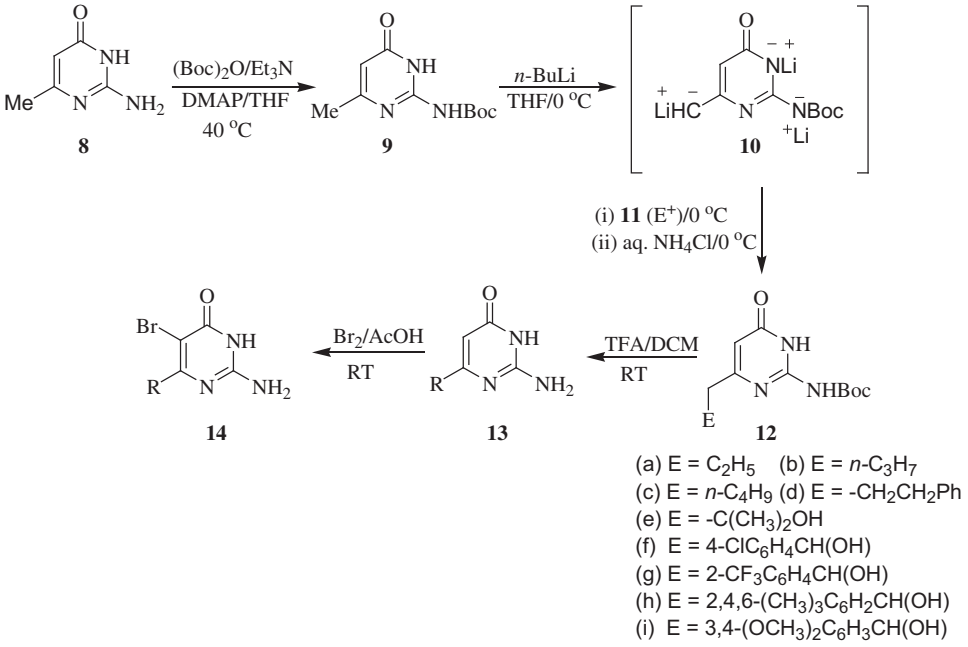
and subsequent deprotection with TFA produced good yields of compounds **13h** and **13i** (Table 1, entries 8 and 9). Evidently, the formation of **13h** and **13i** products suggests *in situ* elimination of water from the corresponding carbinols **12h** and **12i**, under the acid catalyzed conditions used in the deprotection reaction.

The 5-bromo substituted analogues of compound **13a–i**, namely, compounds **14a–i**, were obtained in good to excellent yields (Table 1, entries 10–18) through the reaction of the respective compounds **13a–i**, with bromine in acetic acid at room temperature (Scheme 2).

3. Biology

The antiviral assays were based on the inhibition of virus-induced cytopathicity in CRFK [Feline corona virus (FIPV) and Feline herpes virus], HEL [herpes simplex virus (HSV) type-1 (KOS), HSV-2 (G), vaccinia virus, vesicular stomatitis virus (VSV), and HSV-1 (TK^- KOS ACV^R)], Vero (parainfluenza-3, reovirus-1, Sindbis virus B4, Coxsackie B4, and Punta Toro virus), HeLa (VSV, Coxsackie virus B4, and respiratory syncytial virus) and MDCK [(influenza A (H1N1, H3N2) and B virus)] cell cultures. Confluent cell cultures in 96-well microtiter plates were inoculated with 100 cell culture inhibitory dose-50 (CCID50) of virus (1 CCID50 is the virus dose to infect 50% of the cell cultures), along with varying concentrations of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Table S1 (see Supporting information) shows the activity and cytotoxicity data of compounds **14a–i** against various virus strains. The anti-VSV results observed in HeLa cell cultures (Table S1, entry 5) could not be observed in HEL cell cultures (Table S1, entry 4).

Compound **14h** (R = 2,4,6-$(CH_3)_3C_6H_2CH{=}CH$) showed activity against the AD-169 (EC_{50} = 11 μM) and Davis strains (EC_{50} = 8.9 μM) of cytomegalovirus in HEL cell cultures, but its activity was close to its toxicity threshold (Table S1, entry 1). Similarly, compound **14h** also exhibited activity against varicella-zoster virus (VZV) strains [TK^+VZV OKA (EC_{50} = 2.5 μM) and TK^-VZV 07-1 (EC_{50} = 5.3 μM)] in HEL cell cultures, which was close to its toxicity threshold (Table S1, entry 2). In CRFK cell cultures, compound **14h** presented activity against Feline Herpes Virus at a concentration well below its cellular toxicity threshold (Table S1, entry 3). However, none of the compounds presented any activity against HSV-1, HSV-2, vaccinia virus and VSV in HEL cell cultures (Table S1, entry 4). Compounds **14b**, **14c**, **14f** and **14i** possessing, respectively, a *n*-butyl, *n*-pentyl, 4-$ClC_6H_4CH(OH)CH_2$ and 3,4-$(OCH_3)_2C_6H_3CH{=}CH$ substituents at C-6



Scheme 2. Synthesis of C6-substituted 2-amino-6-methyl-4(3*H*)-pyrimidinone **13** and 5-bromo derivatives **14**.

positions showed activity against VSV in HeLa cell cultures with EC_{50} values of 10, 45, 45 and 45 μM, respectively. Although, compound **14c** with an *n*-pentyl substituent at the C-6 position had presented pronounced antiviral activity (EC_{50} = 2 μM), it was close to its toxicity threshold (Table S1, entry 5). Furthermore, the anti-VSV activity was not observed in HEL cell cultures, in which the toxicity of the compound was also lower. In Vero cell cultures, none of the compounds showed any activity against parainfluenza virus, reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus (Table S1, entry 6). In MDCK cell cultures, all the compounds were found to be inactive against influenza virus (Table S1, entry 7).

4. Conclusions

A new series of 2-amino-5-bromo-4(3*H*)-pyrimidinone derivatives bearing different substituents at the C-6 position were synthesized from the corresponding 2-amino-6-methyl-4(3*H*)-pyrimidinones in a synthetically useful manner. The obtained derivatives were evaluated for antiviral activity in a series of cell cultures. Although some of the derivatives were found to be active against various virus strains in different cell cultures, they were effective only close to their toxicity threshold.

Table 1
Synthesis of C6-substituted derivatives of 2-amino-4(3*H*)-pyrimidinone **13** and 2-amino-5-bromo-4(3*H*)-pyrimidinone **14**.

Entry	Electrophile **11**	Compound	R	Yield (%)
1.	C_2H_5Br	**13a**	*n*-C_3H_7	65
2.	*n*-C_3H_7Br	**13b**	*n*-C_4H_9	69
3.	*n*-C_4H_9Br	**13c**	*n*-C_5H_{11}	62
4.	$BrCH_2CH_2Ph$	**13d**	*n*-C_3H_6Ph	70
5.	$(CH_3)_2CO$	**13e**	$CH_2C(CH_3)_2OH$	75
6.	4-ClC_6H_4CHO	**13f**	4-$ClC_6H_4CH(OH)CH_2$	58
7.	2-$CF_3C_6H_4CHO$	**13g**	2-$CF_3C_6H_4$ $CH(OH)CH_2$	61
8.	2,4,6-$(CH_3)_3C_6H_2CHO$	**13h**	2,4,6-$(CH_3)_3C_6H_2CH{=}CH$	71
9.	3,4,-$(OCH_3)_2C_6H_3CHO$	**13i**	3,4-$(OCH_3)_2C_6H_3CH{=}CH$	69
10.	–	**14a**	*n*-C_3H_7	88
11.	–	**14b**	*n*-C_4H_9	82
12.	–	**14c**	*n*-C_5H_{11}	90
13.	–	**14d**	*n*-C_3H_6Ph	83
14.	–	**14e**	$CH_2C(CH_3)_2OH$	92
15.	–	**14f**	4-$ClC_6H_4CH(OH)CH_2$	78
16.	–	**14g**	2-$CF_3C_6H_4$ $CH(OH)CH_2$	73
17.	–	**14h**	2,4,6-$(CH_3)_3C_6H_2CH{=}CH$	87
18.	–	**14i**	3,4-$(OCH_3)_2C_6H_3CH{=}CH$	76

5. Experimental section

5.1. General

All liquid reagents were dried/purified using recommended drying agents and/or distilled over 4 Å molecular sieves. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded in DMSO-d_6 and $CDCl_3$ on a multinuclear Jeol FT-AL-300 instruments with chemical shifts being reported in parts per million (δ) relative to internal tetramethylsilane (TMS, δ 0.0, ^{1}H NMR or $CDCl_3$, δ 77.0, ^{13}C NMR). Mass spectra were recorded at The Indian Institute of Integrative Medicine (CSIR), Jammu, India, under electron impact at 70 eV on a Bruker Daltonics Esquire 3000 spectrometer. Elemental analysis was performed on FLASH EA 112 (Thermoelectron Corporation) analyzer at the Department of Chemistry, Guru Nanak Dev University, Amritsar, India and the results were presented in percentage (%). IR was recorded on FTIR Shimadzu 8400 Fourier-transform spectrophotometer in the range of 400–4000 cm^{-1} using KBr. The melting points were determined in open capillaries and were not corrected. For monitoring the progress of a reaction and for comparison purpose, thin layer chromatography was performed on pre-coated aluminium sheets (Merck; $60F_{254}$, 0.2 mm) using an appropriate solvent system. The chromatograms were visualized under UV light. For column chromatography silica gel (60–120 mesh) was employed and the eluents were ethyl acetate/hexane mixtures.

5.2. General procedure for the synthesis of tert-butyl (4-methyl-6-oxo-1,6-dihydropyrimidin-2-yl) carbamate **9**

A mixture of compound **8** (3.99 mmol), Boc_2O (4.79 mmol), triethylamine (5.98 mmol), and DMAP (catalytic amount) in THF

(20 mL) was stirred at 40 °C for 2 days. The solvent was evaporated and the residue was purified by column chromatography on silica gel using ethyl acetate and hexane as the eluent to obtain the pure compound. The characteristic data of the compound are as follows.

Viscous oil. *Rf*: 0.4 (Ethyl acetate). Yield: 47%. IR (KBr): ν_{max} 1090, 1615, 1680, 3320 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ 1.48 (s, 12H, 3× CH_3), 2.12 (s, 3H, C6–CH_3), 5.85 (s, 1H, C5–H), 7.55 (br, 2H, D_2O exchangeable, 2× NH). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C) δ 22.3, 27.5, 84.0, 101.2, 147.4, 153.9, 154.6, and 165.4. Anal. Calcd. for $C_{10}H_{15}N_3O_3$: C, 53.32; H, 6.71; N, 18.66; Found: C, 53.21; H, 6.32; N, 18.44. MS: *m*/*z* 248 (M^++23).

5.3. General procedure for the synthesis of C6-substituted derivatives **13**

To a suspension of compound **9** (5.0 mmol) in dry THF (50 mL) under a blanket of dry N_2, 2.1 N *n*-BuLi (15.5 mmol) was added drop wise at 0 °C. After the addition, reaction mixture was stirred at room temperature for 0.5 h and quenched at 0 °C with electrophile **11** (7.5 mmol), dissolved in dry THF (10 mL). The reaction was stirred at room temperature for 0.5 h. After completion of reaction, a cold saturated aqueous solution of NH_4Cl (30 mL) was introduced at 0 °C. The reaction contents were extracted with ethyl acetate (3 × 25 mL), treated with brine, washed with water (2 × 25 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain crude compound **12** (60–75%). This compound was further treated with TFA:DCM (2:8) at room temperature for 0.5 h. After completion of reaction, solvent was removed under reduced pressure and reaction residue was neutralized with base. The reaction contents were extracted with ethyl acetate (3 × 20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Required compounds **13** were purified by column chromatography using silica gel-G (230–400 mesh) and mixtures of ethyl acetate/hexane as the eluent. The characteristic data of the compounds are as follows.

5.3.1. 2-Amino-6-propylpyrimidin-4(3H)-one (**13a**)

Brown solid. *Rf*: 0.4 (methanol:ethyl acetate/20:80). Yield: 65%. M.p. 192–195 °C (methanol/DCM). IR (KBr): ν_{max} 1010, 1313, 1635, 1699, 3353 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 0.99 (t, J = 7.2 Hz, 3H, CH_3), 1.62–1.75 (m, 2H, CH_2), 2.47 (t, J = 7.5 Hz, 2H, CH_2), 4.11 (br, 2H, D_2O exchangeable, NH_2), 5.62 (s, 1H, CH), 5.84 (br, 1H, D_2O exchangeable, NH). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 12.7, 19.8, 33.7, 101.5, 153.2, 156.2 and 161.0. Anal. Calcd. for $C_7H_{11}N_3O$: C, 54.89; H, 7.24; N, 27.43; Found: C, 54.55; H, 7.00; N, 27.23. MS: *m*/*z* 154 (M^++1).

5.3.2. 2-Amino-6-butylpyrimidin-4(3H)-one (**13b**)

White crystalline solid. *Rf*: 0.6 (methanol:ethyl acetate/20:80). Yield: 69%. M.p. 205–207 °C (methanol/DCM). IR (KBr): ν_{max} 1112, 1330, 1655, 1705, 3220 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 0.92 (t, J = 6.9 Hz, 3H, CH_3), 1.31–1.39 (m, 2H, CH_2), 1.52–1.59 (m, 2H, CH_2), 2.40 (t, J = 7.2 Hz, 2H, CH_2), 5.62 (s, 1H, CH), 8.25 (br, 2H, D_2O exchangeable, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 11.7, 19.7, 27.1, 30.2, 99.7, 151.6, 155.4 and 159.5. Anal. Calcd. for $C_8H_{13}N_3O$: C, 57.46; H, 7.84; N, 25.13; Found: C, 57.32; H, 7.51; N, 24.90. MS: *m*/*z* 168 (M^++1).

5.3.3. 2-Amino-6-pentylpyrimidin-4(3H)-one (**13c**)

White crystalline solid. *Rf*: 0.2 (methanol:ethyl acetate/10:90). Yield: 62%. M.p. 150–152 °C (methanol). IR (KBr): ν_{max} 1250, 1630, 1635, 1699, 3244 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 0.89 (t, J = 6.9 Hz, 3H, CH_3), 1.28–1.33 (m, 4H, 2× CH_2), 1.53–1.65 (m, 2H, CH_2), 2.40 (t, J = 7.2 Hz, 2H, CH_2), 5.59 (s, 1H, CH), 7.71 (br, 2H, D_2O exchangeable, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 12.0, 20.1, 25.0, 28.9, 32.2, 99.1, 152.8, 154.9 and 161.3. Anal. Calcd. for $C_9H_{15}N_3O$: C, 59.64; H, 8.34; N, 23.19; Found: C, 59.23; H, 8.11; N, 22.94. MS: *m*/*z* 182 (M^++1).

5.3.4. 2-Amino-6-phenethylpyrimidin-4(3H)-one (**13d**)

Brown solid. *Rf*: 0.4 (methanol:ethyl acetate/20:80). Yield: 70%. M.p. 85–87 °C (DCM). IR (KBr): ν_{max} 1255, 1465, 1625, 1715, 3450 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 2.71 (t, J = 7.2 Hz, 2H, CH_2), 2.89 (t, J = 7.2 Hz, 2H, CH_2), 5.73 (s, 1H, CH), 7.18–7.33 (m, 5H, ArH), 8.51 (br, 2H, D_2O exchangeable, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 32.8, 34.1, 102.0, 126.5, 128.5, 140.0, 153.3 and 161.5. Anal. Calcd. for $C_{12}H_{13}N_3O$: C, 66.96; H, 6.09; N, 19.52; Found: C, 66.75; H, 5.84; N, 19.19. MS: *m*/*z* 216 (M^++1).

5.3.5. 2-Amino-6-(2-hydroxy-2-methylpropyl)pyrimidin-4(3H)-one (**13e**)

Brown solid. *Rf*: 0.5 (methanol:ethyl acetate/10:90). Yield: 75%. M.p. 165–167 °C (methanol/DCM). IR (KBr): ν_{max} 1110, 1245, 1345, 1655, 1702, 3253 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ 1.28 (s, 6H, 2× CH_3), 2.59 (s, 2H, CH_2), 5.67 (s, 1H, CH), 8.99 (br, 1H, D_2O exchangeable, NH). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ 28.0, 44.1, 68.4, 103.3, 151.6 and 152.0. Anal. Calcd. for $C_8H_{13}N_3O_2$: C, 52.45; H, 7.15; N, 22.94; Found: C, 52.11; H, 6.91; N, 22.60. MS: *m*/*z* 184 (M^++1).

5.3.6. 2-Amino-6-(2-(4-chlorophenyl)-2-hydroxyethyl)pyrimidin-4(3H)-one (**13f**)

White solid. *Rf*: 0.2 (methanol:ethyl acetate/10:90). Yield: 58%. M.p. 206–207 °C (methanol/DCM). IR (KBr): ν_{max} 1067, 1287, 1344, 1615, 1709, 3140, 3644 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ 2.56 (dd, J = 3.6 Hz, 1.8 Hz, 2H, CH_2), 4.94–4.98 (m, 1H, CH), 5.56 (s, 1H, CH), 7.29 (d, J = 8.2 Hz, 2H, ArH), 7.40 (d, J = 8.2 Hz, 2H, ArH), 7.89 (br, 2H, D_2O exchangeable, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 42.1, 69.5, 103.1, 127.3, 127.8, 131.4, 143.0, 152.9 and 161.0. Anal. Calcd. for $C_{12}H_{12}N_3O_2Cl$: C, 54.25; H, 4.55; N, 15.82; Found: C, 54.10; H, 4.21; N, 15.43. MS: *m*/*z* 288 (M^++23).

5.3.7. 2-Amino-6-(2-hydroxy-2-(2-(trifluoromethyl)phenyl)ethyl) pyrimidin-4(3H)-one (**13g**)

Brown solid. *Rf*: 0.5 (methanol:ethyl acetate/20:80). Yield: 61%. M.p. 186–187 °C (methanol/DCM). IR (KBr): ν_{max} 1142, 1224, 1633, 1688, 3332 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ 2.59 (d, J = 8.7 Hz, 2H, CH_2), 5.31 (t, J = 4.8 Hz, 1H, CH), 5.47 (s, 1H, CH), 6.57 (br, 1H, D_2O exchangeable, NH), 7.39 (t, J = 7.5 Hz, 1H, ArH), 7.58–7.63 (m, 2H, ArH), 7.88 (d, J = 8.1 Hz, 1H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ 44.4, 66.0, 99.8, 123.5, 123.9, 125.7, 126.6, 130.8, 142.4 and 154.0. Anal. Calcd. for $C_{13}H_{12}F_3N_3O_2$: C, 52.18; H, 4.04; N, 14.04; Found: C, 51.88; H, 3.90; N, 13.76. MS: *m*/*z* 322 (M^++23).

5.3.8. 2-Amino-6-(2,4,6-trimethylstyryl)pyrimidin-4(3H)-one (**13h**)

Light yellow solid. *Rf*: 0.7 (methanol:ethyl acetate/10:90). Yield: 71%. M.p. 158–160 °C (methanol/DCM). IR (KBr): ν_{max} 1030, 1210, 1299, 1629, 1710, 3455 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 2.33 (s, 9H, 3× CH_3), 5.76 (s, 1H, CH), 6.29 (d, J = 16.5 Hz, 1H, CH), 6.88 (s, 2H, ArH), 7.68 (d, J = 16.5 Hz, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 20.0, 100.6, 126.2, 128.1, 130.9, 134.4, 135.4, 136.6, 153.8 and 163.2. Anal. Calcd. for $C_{15}H_{17}N_3O$: C, 70.56; H, 6.71; N, 16.46; Found: C, 70.23; H, 6.51; N, 16.40. MS: *m*/*z* 256 (M^++1).

5.3.9. 2-Amino-6-(3,4-dimethoxystyryl)pyrimidin-4(3H)-one (**13i**)

Yellow solid. *Rf*: 0.4 (methanol:ethyl acetate/10:90). Yield: 69%. M.p. 220–222 °C (methanol). IR (KBr): ν_{max} 1122, 1267, 1356, 1678, 1315, 3467 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ 3.90 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 5.79 (s, 1H, CH), 6.62 (d, J = 16.2 Hz, 1H,

CH), 6.90 (d, $J = 8.1$ Hz, 1H, ArH), 7.11 (s, 1H, ArH), 7.13 (d, $J = 8.7$ Hz, 1H, ArH), 7.63 (d, $J = 16.2$ Hz, 1H, CH), 8.07 (br, 1H, D_2O exchangeable, NH). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 55.5, 55.6, 100.9, 109.5, 111.8, 121.8, 128.2, 136.1, 149.0, 150.2, 154.3 and 162.3. Anal. Calcd. for $C_{14}H_{15}N_3O_3$: C, 61.53; H, 5.53; N, 15.38; Found: C, 61.20; H, 5.32; N, 15.10. MS: m/z 296 (M^++23).

5.4. General procedure for the synthesis of C5-bromo substituted derivatives **14**

To a suspension of compound **13** (1.0 equiv) in glacial acetic acid (10 mL), bromine (1.1 equiv) was added drop wise and reaction mixture was allowed to stir at room temperature for 0.5 h. After completion of reaction (TLC), solvent was removed under reduced pressure. Solid residue was washed with water to obtain required compound. The characteristic data of the compounds are as follows.

5.4.1. 2-Amino-5-bromo-6-propylpyrimidin-4(3H)-one (**14a**)

Light brown solid. *Rf*: 0.6 (methanol:ethyl acetate/20:80). Yield: 88%. M.p. 200–202 °C (methanol/DCM). IR (KBr): ν_{max} 550, 1120, 1212, 1373, 1695, 1745, 3166 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 0.93 (t, $J = 7.2$ Hz, 3H, CH_3), 1.54–1.66 (m, 2H, CH_2), 2.54 (t, $J = 7.2$ Hz, 2H, CH_2), 7.29 (br, 2H, D_2O exchangeable, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 13.3, 20.1, 35.4, 98.9, 152.0 and 157.3. Anal. Calcd. For $C_7H_{10}BrN_3O$: C, 36.23; H, 4.34; N, 18.11; Found: C, 36.02; H, 4.22; N, 17.90. MS: m/z 232 and 234 (M^++1).

5.4.2. 2-Amino-5-bromo-6-butylpyrimidin-4(3H)-one (**14b**)

Light brown solid. *Rf*: 0.6 (methanol:ethyl acetate/10:90). Yield: 82%. M.p. 210–212 °C (methanol/DCM). IR (KBr): ν_{max} 523, 1016, 1249, 1346, 1679, 1721, 3264 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 0.89 (t, $J = 7.2$ Hz, 3H, CH_3), 1.26–1.38 (m, 2H, CH_2), 1.49–1.59 (m, 2H, CH_2), 2.50 (t, $J = 7.2$ Hz, 2H, CH_2), 6.62 (br, 2H, D_2O exchangeable, NH_2), 11.18 (br, 1H, D_2O exchangeable, NH). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 14.1, 22.2, 29.2, 34.0, 99.2, 152.6 and 158.0. Anal. Calcd. for $C_8H_{12}BrN_3O$: C, 39.04; H, 4.91; N, 17.07; Found: C, 38.82; H, 4.70; N, 16.90. MS: m/z 246 and 247 (M^++1).

5.4.3. 2-Amino-5-bromo-6-pentylpyrimidin-4(3H)-one (**14c**)

White solid. *Rf*: 0.5 (methanol:ethyl acetate/10:90). Yield: 90%. M.p. 195–197 °C (methanol). IR (KBr): ν_{max} 610, 1120, 1255, 1344, 1677, 1712, 3453 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 0.85 (t, $J = 6.0$ Hz, 3H, CH_3), 1.26–1.30 (m, 4H, 2× CH_2), 2.04–2.15 (m, 2H, CH_2), 6.81 (br, 1H, D_2O exchangeable, NH), 11.05 (br, 2H, D_2O exchangeable, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 13.8, 21.8, 26.6, 30.8, 35.9, 97.3, 153.6 and 158.5. Anal. Calcd. for $C_9H_{14}BrN_3O$: C, 41.55; H, 5.42; N, 16.15; Found: C, 41.34; H, 5.33; N, 15.90. MS: m/z 260 and 262 (M^++1).

5.4.4. 2-Amino-5-bromo-6-phenethylpyrimidin-4(3H)-one (**14d**)

White solid. *Rf*: 0.7 (methanol:ethyl acetate/20:80). Yield: 83%. M.p. 220–222 °C (methanol/DCM). IR (KBr): ν_{max} 615, 1130, 1278, 1369, 1699, 1755, 3283 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 2.82 (d, $J = 8.4$ Hz, 4H, 2× CH_2), 6.71 (br, 2H, D_2O exchangeable, NH_2), 7.21–7.31 (m, 5H, ArH), 11.25 (br, 1H, D_2O exchangeable, NH). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 32.8, 126.0, 128.2, 128.4, 141.0 and 154.2. Anal. Calcd. for $C_{12}H_{12}BrN_3O$: C, 49.00; H, 4.11; N, 14.29; Found: C, 48.78; H, 3.90; N, 14.10. MS: m/z 316 and 318 (M^++23).

5.4.5. 2-Amino-5-bromo-6-(2-hydroxy-2-methylpropyl)pyrimidin-4(3H)-one (**14e**)

White solid. *Rf*: 0.7 (methanol:ethyl acetate/10:90). Yield: 92%. M.p. 180–182 °C (methanol/DCM). IR (KBr): ν_{max} 597, 1123, 1345, 1395, 1695, 1725, 3353 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 1.14 (s, 6H, 2× CH_3), 2.70 (s, 2H, CH_2), 5.29 (br, 1H, D_2O exchangeable, OH), 6.75 (br, 2H, D_2O exchangeable, NH_2), 11.35 (br, 1H, D_2O exchangeable, NH). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 29.4, 45.4, 100.9, 151.4, 153.2 and 157.4. Anal. Calcd. for $C_8H_{12}BrN_3O_2$: C, 36.66; H, 4.61; N, 16.03; Found: C, 36.45; H, 4.33; N, 15.78. MS: m/z 262 and 264 (M^++1).

5.4.6. 2-Amino-5-bromo-6-(2-(4-chlorophenyl)-2-hydroxyethyl) pyrimidin-4(3H)-one (**14f**)

Light yellowish solid. *Rf*: 0.3 (methanol:ethyl acetate/10:90). Yield: 78%. M.p. 255–257 °C (methanol). IR (KBr): ν_{max} 598, 710, 1066, 1343, 1685, 1729, 3183 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 2.78–2.93 (m, 2H, CH_2), 3.87 (br, 1H, D_2O exchangeable, OH), 5.43–5.44 (m, 1H, CH), 6.72 (br, 2H, D_2O exchangeable, NH_2), 7.35–7.52 (m, 4H, ArH). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 40.1, 67.5, 103.7, 126.3, 127.8, 132.4, 144.0, 156.9 and 161.9. Anal. Calcd. for $C_{12}H_{11}BrClN_3O_2$: C, 41.83; H, 3.22; N, 12.19; Found: C, 41.54; H, 3.11; N, 11.90. MS: m/z 344 and 346 (M^++1).

5.4.7. 2-Amino-5-bromo-6-(2-hydroxy-2-(2-(trifluoromethyl) phenyl)ethyl)pyrimidin-4(3H)-one (**14g**)

White solid. *Rf*: 0.7 (methanol:ethyl acetate/20:80). Yield: 73%. M.p. 177–179 °C (methanol/DCM). IR (KBr): ν_{max} 615, 1210, 1342, 1513, 1735, 3443 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 2.75 (dd, $J = 13.8$ Hz, 9.3 Hz, 1H, CH), 2.96 (dd, $J = 13.8$ Hz, 9.3 Hz, 1H, CH), 3.90 (br, 1H, D_2O exchangeable, OH), 5.40 (d, $J = 7.2$ Hz, 1H, CH), 6.76 (br, 2H, D_2O exchangeable, NH_2), 7.48 (t, $J = 7.2$ Hz, 1H, ArH), 7.64–7.74 (m, 2H, ArH), 7.89 (d, $J = 7.5$ Hz, 1H, ArH). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 39.7, 66.9, 99.7, 123.6, 124.5, 126.5, 130.0, 134.6, 154.0, 155.6 and 162.4. Anal. Calcd. for $C_{13}H_{11}BrF_3N_3O_2$: C, 41.29; H, 2.93; N, 11.11; Found: C, 40.99; H, 2.56; N, 10.87. MS: m/z 378 and 380 (M^++1).

5.4.8. 2-Amino-5-bromo-6-(2,4,6-trimethylstyryl)pyrimidin-4(3H)-one (**14h**)

Light yellow solid. *Rf*: 0.2 (ethyl acetate). Yield: 87%. M.p. 178–180 °C (methanol/DCM). IR (KBr): ν_{max} 611, 1030, 1262, 1364, 1653, 1679, 3433 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 2.23 (s, 3H, CH_3), 2.31 (s, 6H, 2× CH_3), 6.85–6.93 (m, 3H, CH & ArH), 7.76 (d, $J = 16.2$ Hz, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 20.7, 21.0, 98.3, 128.2, 129.2, 131.8, 136.2, 137.3, 153.3 and 158.9. Anal. Calcd. for $C_{15}H_{16}BrN_3O$: C, 53.91; H, 4.83; N, 12.57; Found: C, 53.67; H, 4.45; N, 12.19. MS: m/z 334 and 336 (M^++1).

5.4.9. 2-Amino-5-bromo-6-(3,4-dimethoxystyryl)pyrimidin-4(3H)-one (**14i**)

Yellow solid. *Rf*: 0.7 (methanol:ethyl acetate/10:90). Yield: 76%. M.p. 211–213 °C (methanol/DCM). IR (KBr): ν_{max} 613, 1120, 1255, 1353, 1655, 1711, 3329 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 3.86 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 5.88 (d, $J = 11.4$ Hz, 1H, CH), 6.18 (d, $J = 11.4$ Hz, 1H, CH), 6.77 (br, 2H, D_2O exchangeable, NH_2), 7.11 (d, $J = 8.1$ Hz, 2H, ArH), 8.06 (s, 1H, ArH). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 51.5, 56.0, 56.2, 104.5, 121.8, 128.2, 129.2, 136.2, 148.6, 151.2, 155.3 and 164.7. Anal. Calcd. for $C_{14}H_{14}BrN_3O_3$: C, 47.74; H, 4.01; N, 11.93; Found: C, 47.54; H, 3.91; N, 11.70. MS: m/z 350 and 352 (M^-−1).

6. Antiviral activity assays

The compounds were evaluated against the following viruses: herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK^-) HSV-1 KOS strain resistant to ACV (ACV^r), herpes simplex virus type 2 (HSV-2) strains Lyons and G, cytomegalovirus (CMV) strains AD-169 and Davis, varicella-zoster virus (VZV) strains OKA and 07-1, vaccinia virus Lederle strain, respiratory syncytial virus (RSV) strain Long, vesicular stomatitis virus (VSV), Coxsackie

B4, Parainfluenza 3, Influenza virus A (subtypes H1N1, H3N2), influenza virus B, Reovirus-1, Sindbis and Punta Toro virus. The antiviral assays were based on inhibition of virus-induced cytopathicity or plaque formation in human embryonic lung (HEL) fibroblasts, African green monkey cells (Vero), human cervix carcinoma epithelial cells (HeLa) or Madin–Darby canine kidney cells (MDCK). Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or 100 or 20 plaque forming units (PFU) (for CMV or VZV, respectively) in the presence of varying concentrations of the test compounds. Viral cytopathicity or plaque formation was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50} or compound concentration required to reduce virus-induced cytopathogenicity or viral plaque formation by 50%.

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Appendix A. Supporting information

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.06.036.

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