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Synthesis and Biological Evaluation of 2-Methoxy- and 2-Methylthio-6-[(2′-alkylamino)ethyl]-4(3*H*)-pyrimidinones with Anti-Rubella Virus Activity

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Abstract—The synthesis of a new family of antiviral compounds, 2-methoxy-, and 2-methylthio-6-[(2′-alkylamino)ethyl]-4(3*H*)-pyrimidinones, has been accomplished. The activity of these agents against positive strand (rubella virus and sindbis virus) and negative strand (vesicular stomatitis virus) RNA viruses is reported. Some of these compounds are efficient and selective inhibitors of rubella virus.

Introduction

Pyrimidine derivatives and their nucleosides have a great biological significance because they exhibit a wide range of antiviral and anticancer activities. In contrast to extensive studies about 5-substituted pyrimidines less attention has been devoted to 6-substituted isomers, probably because of their no easy synthetic availability, and their supposed biological inactivity.[1] In the last few years some uracil and pyrimidinone derivatives substituted either at C-5 and C-6 position have emerged in the field of antiviral chemotherapy.[2,3] Among the important 6-substituted uracil derivatives, 1-[(2-hydroxyethyl)methyl]-6-(phenylthio)thymine (HEPT[4,5]) and its analogues[6,7] (of which 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil has been chosen as a candidate for clinical trials[6,7]), and 3,4-dihydro-2-alkoxy-6-benzyl-4-oxopyrimidines (DABOs[8]), showed a potent and selective activity against human immunodeficiency virus type-1 (HIV-1). Thus, the excellent biological activities exhibited by these 6-substituted uracil derivatives provides a new emphasis to explore chemistry and biological activities of these pyrimidine derivatives. In this context, the finding that C-6 substituted uracil and pyrimidinone derivatives showed selective antitumor[9] and antiviral activity against other type of viruses, as for example, african swine fever virus (ASFV[10]) and parainfluenza 1 virus (sendai virus[11]), suggests the importance of testing this family of compounds as broad-spectrum drugs. In this context, in the course of our studies on the chemistry and biological evaluation of antiviral activity of C-6 substituted uracil and pyrimidinone derivatives,[12] we found that 1,3-dimethyl-6-(1′-hydroxy-2′-diethylamino)-uracil **1** posseses a low but selective activity against vesicular stomatitis virus (VSV).[13] We are reporting in this communication the synthesis of a new class of antiviral agents, compounds **6a**–**e**, **7a**,**b**, **8**, and **11a**,**b** that are structurally related to **1**, together with their biological evaluation against positive strand (rubella virus (RV) and sindbis virus (SV)) and negative strand (vesicular stomatitis virus (VSV)) RNA viruses. Some of these compounds represent, to the best of our knowledge, the first reported effective inhibitors of RV; a virus that is responsible for important medical and social problems, for which, at moment, no efficient chemotherapy is available.[14]

Chemistry

Compounds **6a**–**e** and **7a**,**b** were prepared in a step-wise manner starting from commercially available acetone

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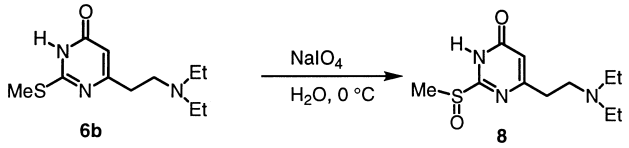
dicarboxylic acid diethyl ether **2** and *O*-methylisourea hydrogen sulphate **3a** or *S*-methylisourea hydrogen sulphate **3b** (Scheme 1). Cyclization was performed in the presence of $Ca(OH)_2$ in ethanol:water mixture (1.0:3.0 v/v) at 25°C for 48 h. In these experimental conditions, 4(3*H*)-pyrimidinone derivatives **4a** and **4b** were obtained in 75 and 78% yields, respectively. The reduction of the ester moiety in compounds **4a**,**b** was performed, after many failures, with $NaBH_4$ in refluxing isopropanol to give the corresponding alcohols **5a**,**b** in reproducible yields of 60 and 57%, respectively. Compounds **5a**,**b** were treated with a small excess of *p*-toluenesulfonyl chloride (TsCl; 1.2 equiv/mol) in the presence of 1.10 equiv/mol of 4-(dimethylamino)pyridine (DMAP) in $CHCl_3$ at 25°C. After the work up of the reaction the crude was reacted without further purification with an excess (2 equiv/mol) of nitrogen nucleophiles (diethylamine, piperidine, morpholine, and piperazine) in refluxing THF to give compounds **6a**–**e** in yields ranging from 53 to 91%. In the latter two steps, the possible formation of an O^4-tosyl imidate intermediate (formed by reaction of the amide function with TsCl)[15] followed by undesired C-4 nucleophilic displacement was ruled out on the basis of the characteristic amide absorption band, in the range of 1735–1740 cm^{-1}, presents in the IR spectra of compounds **6a**–**e**. The presence of the C-5 proton resonance at ca. 6 ppm in the ^1H NMR spectra, typical of 4(3*H*)-pyrimidinones, is also diagnostic.[16] Moreover, compounds **6a** and **6b** were also treated with H_2SO_4 (2 N water solution) at 80°C to give the corresponding 6-substituted uracil and 2-thiouracil derivatives, compounds **7a** and **7b**, in 96 and 94% yields, respectively (Scheme 1).

Compounds **8** and **11a**,**b** were further synthesized to evaluate a possible role of both the sulfur oxidation state and the size of the C-6 side chain on the antiviral activity. Compound **8** was prepared in 76% yield by sodium periodate ($NaIO_4$) oxidation of **6b** performed in H_2O at 0°C (Scheme 2).

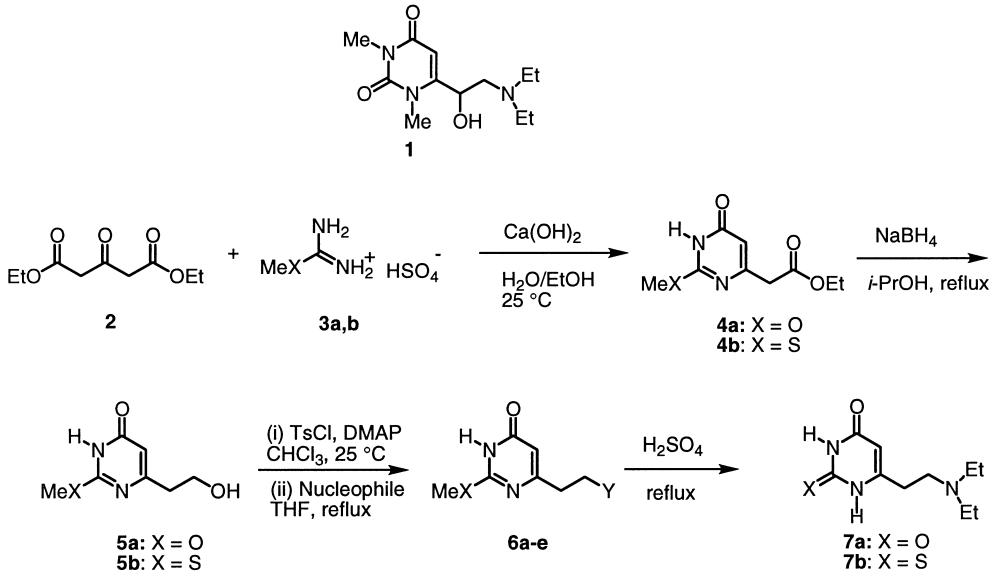
Compounds **11a,b** were prepared in a step-wise manner starting from commercially available ethyl 4-chloroacetoacetate **9** and **3b** in H_2O:MeOH (v/v) at 25°C in the presence of a large excess of $Ca(OH)_2$ to give **10** in 75% yield (Scheme 3). Chlorine nucleophilic displacement with diethylamine and morpholine, performed in refluxing THF gave compounds **11a**,**b** in 63 and 59% yields, respectively.

Biology

Confluent vero cell monolayers were exposed to compounds **6a**–**e**, **7a**,**b**, **8**, and **11a**,**b** for 48 h at 37°C. Cell morphology, viability and yield were then examined. Cell viability was assessed on the basis of vital dye exclusion test, using Trypan Blue, and cell yield was determined by counting cells with an hemocytometer after trypsinization. For antiviral assay, the compounds were tested starting from the highest non-cytotoxic concentration which did not affect any parameter considered in 100% of the cells. After virus adsorption (1 h, 37°C), the viral inoculum was removed. Cell monolayers were washed three times with PBS and incubated with maintenance medium in the presence or absence of

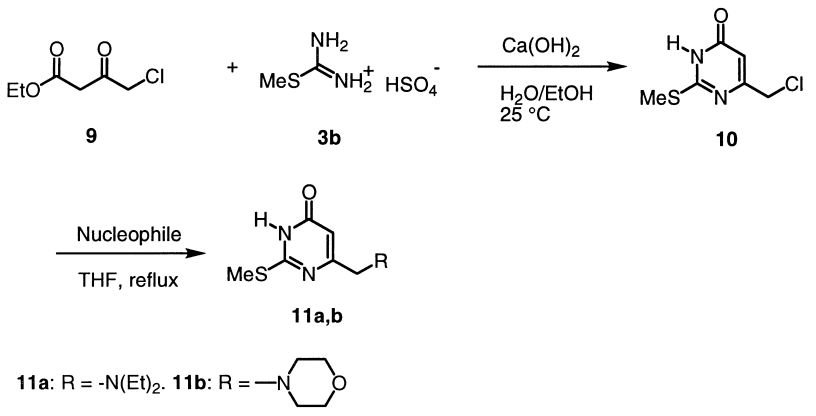


Scheme 2.



6a: X = O, Y = -N(Et)$_2$. **6b**: X = S, Y = -N(Et)$_2$. **6c**: X = S, Y = piperidino. **6d**: X = S, Y = morpholino. **6e**: X = S, Y = piperazino.

Scheme 1.



Scheme 3.

fourfold dilutions of the compounds. Virus yield was evaluated by plaque assay after a single cycle of virus multiplication at high multiplicity infection (SNV and VSV, 8 h; RV, 48 h) and after multiple rounds at low multiplicity infection (SNV and VSV, 24 h; RV 96 h). The compounds were tested in two independent experiments conducted in triplicate.

Results

The effect of compounds **6a–e**, **7a**,**b**, **8**, and **11a**,**b** on RV, SV, and VSV replication in Vero cells was studied under one-step or multi-step multiplication conditions. After viral adsorption (1 h, 37°C) the drugs or control diluent were added to the culture medium and viral yield was determined by plaque assay. As shown in Table 1, compounds **6b–d** were found to strongly inhibit VSV production, whereas a second set of substances (**6e**, **7b**, **8**, and **11b**) produced an inhibition of ca. 1 log. Compounds **6b–d** were also effective towards the replication of SNV, whereas **6b**, and to a lesser extent **8**, inhibit the multiplication of RV. Furthermore, compounds **6e**, **8**, and **11b** significantly reduced the production of SNV in multi-round, low multiplicity infection, whereas only **8** was active on RV replication in these experimental conditions.

The 50% inhibitory concentrations of virus yield have been calculated on a dose–response line obtained by plotting the percentage of plaque reduction, with respect to the control plaque count, versus the logarithm of compound dose. Triplicate wells were utilized for each concentration tested. The standard deviation was less than 5% of the mean value. The reduction of viral yield was found to be concentration-dependent (data not shown). The CC_{50} and IC_{50} of compounds are shown in Table 2. Analogues **6b** and **8** were the most active substances against all the viruses tested. Among the other compounds, **6c** was also effective, although at higher concentrations, whereas **6e** and **11b** showed an inhibitory activity only towards SNV and VSV. The other products showed only a weak effect. In order to determine the selectivity indices (SI) of the products, the ratio between 50% drug cytotoxicity (CC_{50}) and the concentration required to inhibit viral multiplication by 50% was calculated. The data obtained, reported in Table 2, showed a good SI for compound **6b** towards RV replication. All the other compounds showed low SIs, indicating a limited selectivity.

Table 1. Effect of compounds **6a–e**, **7a**,**b**, **8**, and **11a**,**b** on VSV, SNV, and RV virus multiplication[a]

Compound	VSV[b]		SNV[b]		RV[c]	
	moi 10	moi 0.1	moi 10	moi 0.1	moi 3.0	moi 0.1
6a	100	ND[d]	27	66	78	54
6b	0.03	0.2	2.3	2.6	16	1.3
6c	0.09	0.5	8.0	12	36	3.7
6d	1.0	23	16	56	100	41
6e	8.7	7.2	29	3.4	60	69
7a	100	ND	27	66	88	58
7b	3.5	100	74	100	71	28
8	6.7	9.4	90	2.9	8.3	11
11a	100	47	50	61	69	100
11b	7.2	11	48	7.2	91	63

[a] Compounds were added at the concentration of 125 μg/mL (**6c**: 62 μg/mL; **7**: 31 μg/mL) to vero cells after viral adsorption (1 h, 37°C) and maintained throughout the incubation. The values represent the % of plaque forming units relative to to an untreated control. Data represent the mean of three independent experiments; each experiment was conducted in duplicate. The standard deviations were < 5% for all values.

[b] Titers were determined by plaque assay 8 h post-infection (pi) with SNV and VSV at high multiplicity of infection (moi 10) or 24 h pi using a low moi (0.1 PFU/cell).

[c] The yield of rubella virus was evaluated after a single-round, high multiplicity infection (48 h, moi 3.0) as well as in multi-round, low multiplicity infection (96 h, moi 0.1).

[d] ND, not determined.

Table 2. Antiviral activity of compounds **6a**–**e**, **7a**,**b**, **8**, and **11a**,**b**, against VSV, SNV, and RV infection in vero cells[a]

Compound	CC_{50}[b]	IC_{50}[c]			SI[d]		
		RV	SNV	VSV	RV	SNV	VSV
6a	250	125	> 125	> 125	2.0	—	—
6b	250	3.9	44.3	29	64	5.6	8.5
6c	125	38.5	40.7	37	2.7	3.1	3.4
6d	1000	125	> 125	80	8.0	—	12.5
6e	1000	> 125	63	81.7	—	15.9	12.2
7a	> 1000	125	> 125	> 125	> 8.0	—	—
7b	> 1000	90	> 125	> 125	> 11.1	—	—
8	62.5	3.9	19	24	16	3.3	2.6
11a	> 1000	> 125	125	125	—	> 8	> 8
11b	> 1000	> 125	72.5	83.5	—	> 13.8	> 12

[a] Virus yield was determined by plaque assay. The antiviral activity of compounds was evaluated in comparison with ribavirin towards SNV and VSV (IC_{50}: 100 μg/mL, and 300 μg/mL, respectively).
[b] The minimal concentration of compound (μg/mL) which affected one cytotoxicity parameter in 50% of cells.
[c] Inhibitory concentration of compound (μg/mL) required to inhibity virus yield by 50%.
[d] Selectivity index (CC_{50}/IC_{50}).

At the moment, compound **6b** is a very efficient inhibitor of RV, and it could be used as a reference structure for the synthesis of more active and selective C-6 substituted pyrimidinone derivatives.

Experimental

^{1}H NMR spectra were recorded on a Bruker (200) MHz spectrometer and are reported in δ value. IR spectra were recorded on a Perkin–Elmer 298 spectrophotometer using NaCl plates. Microanalyses were performed with a C. Erba 1106 analyser. MS spectra were recorded on a VG 70/250S spectrometer with an electron beam of 70 eV. Melting points were obtained on Mettler apparatus and are uncorrected. All solvents were ACS reagent grade and when necessary were redistilled and dried according to standard procedures. Chromatographic purifications were performed on columns packed with Merck silica gel, 230–400 mesh for flash-technique. TLC was carried out using Merck platten Kieselgel 60 F254.

2-Methoxy-6-[(1′-carboxyethyl)methan-1′-yl]-4(3*H*)-pyrimidinone (4a). *O*-Methylisourea hydrogen sulfate (0.93 g, 5.4 mmol), diethyl 1,3-acetonedicarboxylate (1 g, 4.9 mmol), and $Ca(OH)_2$ (0.42 g, 5.4 mmol), were dissolved in H_2O (6 mL) and EtOH (18 mL), and stirred at 25°C for 48 h. The reaction mixture was neutralized with HCl (2 N water solution) and extracted with $CHCl_3$. The extract, washed with NaCl (saturated solution), was dried successively over Na_2SO_4. Evaporation under reduced pressure gave 2-methoxy-6-[(1′-carboxyethyl)methan-1′-yl]-4(3*H*)pyrimidinone (**4a**) (0.78 g, 75%). Mp 118–122°C (EtOAc), IR (CH_2Cl_2) ν_{max} 1730, 1715, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 7.5 Hz, CH_3), 3.48 (2H, s, CH_2), 3.95 (3H, s, O-CH_3), 4.15 (2H, q, J = 7.5 Hz, CH_2), 6.08 (1H, s, CH); ^{13}C NMR (CDCl3) δ 13.84 (CH_3), 43.02 (CH_3), 55.12 (CH_2), 61.04 (CH_2), 157.44 (CH), 162.33 (C), 166.58 (C), 169.30 (C), 171.45; MS: *m*/*e* 212 (M^+), 197, 181, 169, 141. Anal. calcd for $C_9H_{12}N_2O_4$: C, 50.94; H, 5.70; N, 13.20. Found: C, 50.86; H, 5.70; N, 13.11.

2-Methylthio-6-[(carboxyethyl)methyl]-4(3*H*)-pyrimidinone (4b). *S*-Methyl-2-thiopseudourea sulfate (1.5 g, 5.4 mmol), diethyl 1,3-acetonedicarboxylate (1 g, 4.9 mmol), and $Ca(OH)_2$ (0.22 g, 5.9 mmol), were dissolved in H_2O (6 mL) and EtOH (18 mL), and stirred at 25°C for 48 h. The reaction mixture was neutralized with HCl (2 N water solution) and extracted with $CHCl_3$. The extract, washed with NaCl (saturated solution), was dried successively over Na_2SO_4. Evaporation under reduced pressure gave 2-methylthio-6-[(carboxyethyl)-methyl]-4(3*H*)pyrimidinone (**4b**) (0.89 g, 78%). Mp 112–114°C (EtOAc), IR (CH_2Cl_2) ν_{max} 1735, 1720, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t, J = 7.2 Hz, CH_3), 2.50 (2H, s, S-CH_3), 3.30 (2H, s, CH2), 4.15 (2H, q, J = 7.2 Hz, CH_2), 6.25 (1H, s, CH); MS: *m*/*e* 228 (M^+), 213, 185, 181, 157. Anal. calcd for $C_9H_{12}N_2SO_3$: C, 47.36; H, 5.30; N, 12.27. Found: C, 47.40; H, 5.31; N, 12.35.

2-Methoxy-6-[(2′-hydroxy)ethan-1′-yl]-4(3*H*)-pyrimidinone (5a). 2-Methoxy-6-[(1′-carboxyethyl)methan-1′-yl]-4(3*H*)-pyrimidinone (**4a**) (1.45 g, 6.8 mmol) and $NaBH_4$ (0.5 g, 13.6 mmol) were dissolved in dry *i*-PrOH (100 mL) under nitrogen atmosphere and the mixture was stirred at 25°C for 48 h. The reaction mixture was treated with glacial acetic acid (0.1 mL) and successively evaporated under reduced pressure. Flash chromatography ($CHCl_3$:MeOH, 9.5:0.5) purification of the crude gave 2-methoxy-6-[(2′-hydroxy)ethan-1′-yl]-4(3*H*)pyrimidinone (**5a**) (0.68 g, 60%). Mp 137–140°C (MeOH/EtOAc), IR (CH2Cl2) ν_{max} 3200, 1720, 1665 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.48 (2H, t, J = 6.8 Hz, CH_2), 3.68 (2H, t, J = 6.8 Hz, CH_2), 4.0 (3H, s, O-CH_3), 5.80 (1H, s, CH); MS: *m*/*e* 170 (M^+), 152, 139, 127, 99. Anal. calcd for $C_7H_{10}N_2O_3$: C, 49.41; H, 5.92; N, 16.46. Found: C, 49.39; H, 5.92; N, 16.50.

2-Methylthio-6-[(2′-hydroxy)ethan-1′-yl]-4(3*H*)-pyrimidinone (5b). 2-Methylthio-6-[(carboxyethyl)methyl]-4(3*H*)pyrimidinone (**4b**) (0.4 g, 1.75 mmol) and $NaBH_4$ (0.13 g, 3.5 mmol) were dissolved in dry *i*-PrOH (100 mL) under nitrogen atmosphere and the mixture was stirred at 25°C for 48 h. The reaction mixture was treated with

glacial acetic acid (0.1 mL) and successively evaporated under reduced pressure. Flash-chromatography ($CHCl_3$:MeOH, 9.5:0.5) purification of the crude gave 2-methylthio-6-[(2′-hydroxy)ethan-1′-yl]-4(3*H*)pyrimidinone (**5b**) (0.19 g, 57%). Mp 137–140°C (MeOH/EtOAc), IR (CH_2Cl_2) ν_{max} 3200,1725, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) (2.30 (3H, s, S-CH_3), 2.48 (2H, t, $J=6.6$ Hz, CH_2), 3.68 (2H, t, $J=6.6$ Hz, CH_2), 5.80 (1H, s, CH); MS: *m/e* 186 (M^+), 168, 143, 139, 115. Anal. calcd for $C_7H_{10}N_2SO_2$: C, 45.15; H, 5.41; N, 15.04. Found: C, 45.20; H, 5.43; N, 15.05.

2-Methoxy-6-[(2′-diethylamino)ethan-1′-yl]-4(3*H*)-pyrimidinone (6a) and 2-methylthio-6-[(2′-alkylamino)ethan-1′-yl]-4(3*H*)-pyrimidinones (6b–e). General procedure. Compound (**5a**) (or (**5b**)) (1 mmol), *p*-toluenesulfonyl chloride (1.1 equiv/mol), and 4-dimethylaminopyridine (1.1 equiv/mol), were dissolved in dry $CHCl_3$ (70 mL) under nitrogen atmosphere and the reaction stirred at 25°C for 24 h. The reaction mixture, washed with HCl (2 N water solution) and NaCl (saturated solution), was dried over Na_2SO_4 and succesively evaporated under vacuum. The crude was dissolved in dry THF (20 mL) in the presence of the appropriate amine (1.2 equiv/mol) and the reaction mixture heated at 50°C for 12 h. The cooled solution was diluted with $CHCl_3$ (100 mL), washed with NaCl (saturated solution), dried over Na_2SO_4 and succesively evaporated under reduced pressure. Purification of the crude by flash chromatography ($CHCl_3$:MeOH, 9.5:0.5) gave products (**6a**) and (**6b–e**) in acceptable yields.

6a. (0.12 g, 53%), oil, IR (CH_2Cl_2) ν_{max} 1730, 1715, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.13 (6H, t, $J=7.0$ Hz, CH_3), 2.69 (2H, t, $J=6.8$ Hz, CH_2), 3.43 (4H, m, CH_2), 3.88 (2H, t, $J=6.8$ Hz, CH_2), 4.01 (3H, s, O-CH_3), 5.88 (1H, s, CH); MS: *m/e* 225 (M^+), 210, 201, 182. Anal. calcd for $C_{11}H_{19}N_3O_2$: C, 58.65; H, 8.50; N, 18.65. Found: C, 58.70; H, 8.50; N, 18.70.

6b. (0.15 g, 64%), oil, IR (CH_2Cl_2) ν_{max} 1725, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.16 (6H, m, CH_3), 2.43 (3H, s, CH_3), 2.69 (2H, t, $J=6.5$ Hz, CH_2), 3.45 (4H, m, CH_2), 3.87 (2H, t, $J=6.5$ Hz, CH_2), 5.85 (1H, s, CH); MS: *m/e* 241 (M^+), 240, 226, 198, 194. Anal. calcd for $C_{11}H_{19}N_3SO$: C, 54.74; H, 7.93; N, 17.41. Found: C, 54.75; H, 7.93; N, 17.50.

6c. (0.2 g, 79%), mp 90–92°C (EtOAc), IR (CH_2Cl_2) ν_{max} 1735, 1668 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49–1.60 (6H, m, CH_2), 2.41 (3H, s, CH_3), 2.68 (2H, t, $J=6.5$ Hz, CH_2), 3.54 (4H, m, CH_2), 3.87 (2H, t, $J=6.4$ Hz, CH_2), 6.0 (1H, s, CH); MS: *m/e* 253 (M^+), 225, 210, 206, 225, 155. Anal. calcd for $C_{12}H_{19}N_3SO$: C, 56.89; H, 7.56; N, 16.58. Found: C, 56.90; H, 7.63; N, 16.50.

6d. (0.18 g, 71%), mp 97–99°C (MeOH:EtOAc), IR (CH_2Cl_2) ν_{max} 1740, 1668 cm^{-1}; ^1H NMR (CD_3SOCD_3) δ 2.48 (3H, s, CH3), 2.67 (2H, t, $J=6.4$ Hz, CH2), 2.79 (2H, t, $J=6.4$ Hz, CH_2), 2.85 (4H, m, CH2), 3.63 (4H, m, CH_2), 5.99 (1H, s, CH); MS: *m/e* 255 (M^+), 227, 212, 208, 155. Anal. calcd for $C_{11}H_{17}N_3SO_2$: C, 51.74; H, 6.71; N, 16.46. Found: C, 51.80; H, 7.70; N, 16.40.

6e. (0.23 g, 91%), mp 89–91°C (MeOH:EtOAc), IR (CH_2Cl_2) ν_{max} 3250, 1735, 1668 cm^{-1}; ^1H NMR (CD_3SOCD_3) δ 2.46 (3H, s, CH_3), 2.72 (2H, t, $J=6.4$ Hz, CH_2), 2.88 (2H, t, $J=6.4$ Hz, CH_2), 3.60 (4H, m, CH_2), 3.91 (4H, m, CH_2), 6.10 (^1H, s, CH); MS: *m/e* 254 (M^+), 226, 211, 207, 155. Anal. calcd for $C_{11}H_{18}N_4SO$: C, 51.94; H, 7.13; N, 22.03. Found: C, 51.96; H, 7.12; N, 22.0.

6-[(2′-Diethylamino)ethan-13-yl]uracil (7a) and 2-thio-6-[(2′-diethylamino)ethan-1′-yl]uracil (7b). General procedure. Compound (**6a**) (or (**6b**)) (1 mmol) was dissolved in H_2SO_4 (2 N water solution) and the reaction mixture stirred at 25°C for 48 h. The mixture was carefully neutralized with $NaHCO_3$ (saturated solution) and extracted with $CHCl_3$. The organic layer was dried under Na_2SO_4 and evaporated under reduced pressure. The crude was crystallised from methanol to give (**7a**) (or (**7b**)).

7a. (0.20 g, 96%), mp 188–190°C (MeOH:EtOAc), IR (CH_2Cl_2) ν_{max} 3250, 1735, 1710, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.13 (6H, m, CH_3), 2.71 (2H, m, CH_2), 3.45 (4H, m, CH_2), 3.88 (2H, m, CH_2), 5.83 (1H, s, CH); MS: *m/e* 211 (M^+), 196, 168, 140, 125. Anal. calcd for $C_{10}H_{17}N_3O_2$: C, 56.85; H, 8.11; N, 19.89. Found: C, 56.93; H, 8.20; N, 19.90

7b. (0.21 g, 94%), mp 111–113°C (MeOH:EtOAc), IR (CH_2Cl_2) ν_{max} 3250, 1735, 1660, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.13 (6H, m, CH_3), 2.69 (2H, m, CH_2), 3.43 (4H, m, CH_2), 3.88 (2H, m, CH_2), 5.88 (1H, s, CH); MS: *m/e* 227 (M^+), 212, 184, 140, 141. Anal. calcd for $C_{10}H_{17}N_3SO$: C, 52.84; H, 7.54; N, 18.48. Found: C, 52.90; H, 7.80; N, 18.61.

2-Methylsulfoxide-6-[(2′-alkylamino)ethan-1′-yl]-4(3*H*)-pyrimidinone (8). 2-Methylthio-6-[(2′-diethylamino)-ethan-1′-yl]-4(3*H*)pyrimidinones (**6b**) (0.13 g, 0.54 mmol) and sodium periodate ($NaIO_4$) (0.10 g, 0.54 mmol) were dissolved in H_2O (10 mL) and the reaction mixture was stirred at 0°C for 16 h. The mixture was filtered and succesively extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude was purified by preparative TLC ($CHCl_3$:MeOH, 9.0:1.0) to give (**8**) (0.11 g, 76%). Oil, IR (CH_2Cl_2) ν_{max} 1725, 1660, 1060 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.19 (6H, m, CH_3), 2.60 (3H, s, CH_3), 2.71 (2H, t, $J=6.5$ Hz, CH_2), 3.48 (4H, m, CH_2), 3.89 (2H, t, $J=6.5$ Hz, CH_2), 5.90 (1H, s, CH); MS: *m/e* 257 (M^+), 242, 214, 194, 171. Anal. calcd for $C_{11}H_{19}N_3SO_2$: C, 51.34; H, 7.44; N, 16.33. Found: C, 51.38; H, 7.44; N, 16.24.

2-Methylthio-6-chloromethyl-4(3*H*)-pyrimidinone (10). 2-Methyl-2-thiopseudourea sulfate (1 g, 3.6 mmol), ethyl 4-chloroacetoacetate (0.64 g, 3.9 mmol), and $Ca(OH)_2$ (0.3 g, 4 mmol), were dissolved in H_2O (6 mL) and EtOH (18 mL), and stirred at 25°C for 48 h. The reaction mixture was neutralized with HCl (2 N water solution) and extracted with EtOAc. The extract, washed with NaCl (saturated solution), was dried successively over Na_2SO_4 and evaporated under reduced pressure.

The crude was purified by flash chromatography ($CHCl_3$:MeOH, 9.0:1.0) to give (**10**) as the only recovered product (0.56 g, 75%). Mp 155–157°C (MeOH: EtOAc), IR (CH_2Cl_2) ν_{max} 1725, 1661 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.55 (3H, s, CH_3), 4.33 (2H, s, CH_2), 6.40 (1H, s, CH); MS: *m*/*e* 190 (M^+), 175, 162, 147, 143. Anal. calcd for $C_6H_7ClN_2SO$: C, 37.80; H, 3.70; N, 14.69. Found: C, 37.85; H, 3.70; N, 14.75.

2-Methylthio-6-[(1′-alkylamino)methan-1′-yl]-4(3*H*)-pyrimidinones (11a,b). General procedure. Compound **10** (1 mmol) and the appropriate amine (diethyl amine or morpholine) (1 equiv/mol) were dissolved in dry THF (20 mL) and the reaction mixture heated at 90°C for 12 h. The cooled solution was diluted with $CHCl_3$ (100 mL), washed with NaCl (saturated solution), dried over Na_2SO_4 and successively evaporated under reduced pressure. Purification of the crude by flash chromatography ($CHCl_3$:MeOH, 9.2:0.8) gave products (**11a**,**b**) in acceptable yields.

11a. (0.14 g, 63%), oil, IR (CH_2Cl_2) ν_{max} 1732, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.0 (6H, t, $J = 7.2$ Hz, CH_3), 2.52 (7H, m, CH_3 and CH_2), 3.42 (2H, s, CH_2), 6.41 (1H, s, CH); MS: *m*/*e* 227 (M^+), 212, 199, 184, 141. Anal. calcd for $C_{10}H_{17}N_3SO$: C, 52.83; H, 7.53; N, 18.58. Found: C, 52.90; H, 7.53; N, 18.65.

11b. (0.14 g, 59%), mp 95–97°C (MeOH:EtOAc), IR (CH_2Cl_2) ν_{max} 1745, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.48 (3H, s, CH_3), 2.67 (2H, t, $J = 6.8$ Hz, CH_2), 2.79 (2H, t, $J = 6.8$ Hz, CH_2), 2.85 (4H, m, CH_2), 3.65 (4H, m, CH_2), 5.99 (1H, s, CH); MS: *m*/*e* 241 (M^+), 226, 198, 194. Anal. calcd for $C_{10}H_{15}N_3SO_2$: C, 49.77; H, 6.26; N, 17.41. Found: C, 49.80; H, 6.26; N, 17.50.

Cells. Vero cells were cultured at 37°C in a 5% CO_2 atmosphere in Eagle's minimum essential medium (MEM) containing 1.2 mg/mL $NaHCO_3$ and supplemented with 6% (v/v) fetal bovine serum (FBS), 2 mM glutamine, 100 IU/mL penicillin and 100 μg/mL streptomycin. For cell maintenance the serum concentration was lowered to 2% (v/v).

Viruses. RV (Therien strain), SNV (AR 339 strain) and VSV (Indiana strain) were grown in vero cells in Eagle's MEM supplemented with 2% FBS. Semiconfluent monolayers were inoculated with viruses at a multiplicity of infection of 0.1 PFU/cell and incubated at 37°C for 24 h (SNV, VSV) or 72 h (RV). After infection, supernatants were collected, centrifuged at 1000 g for 10 min to remove cellular debris, and then stored in small aliquots at −80°C.

Cytotoxicity assays. Cytotoxicity was evaluated by incubating twofold serial dilutions of the compounds in maintenance medium with confluent vero cell monolayers grown in 96-well plates (Falcon). Cell morphology, viability and yield were examined after 48 h incubation at 37°C. Cell viability was assessed on the basis of vital dye exclusion test, using Trypan Blue, and cell yield determined by counting cells with an hemocytometer trypsinization.

Plaque assay. Serial tenfold dilutions of RV, SV, and VSV were inoculated on to confluent vero cell monolayers. After a 1 h adsorption period at 37°C, the inoculum was removed and cells were washed three times with phosphate-buffered saline (PBS) before being overlaid with MEM containing 0.4% (w/v) agar (Oxoid). After two days (SNV, VSV) or five days (RV) incubation at 37°C, plaques were stained with 0.1% crystal violet solution.

Antiviral assay. For antiviral assays, confluent monolayers of vero cells grown in 24-well plates were inoculated with SNV and VSV (10 or 0.1 PFU/cell) and with RV (3 or 0.1 PFU/cell). After virus adsorption (1 h, 37°C), the viral inoculum was removed. Cell monolayers were washed three times with PBS and incubated with maintenance medium in the presence or absence of the compounds. Virus yield was evaluated by plaque assay after a single cycle of virus multiplication at high multiplicity infection (SNV and VSV, 24 h; RV, 96 h).

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16. ^{1}H NMR spectra of the tosylates produced from **5a** and **5b** were obtained by analytical TLC purification of a small amount of the corresponding crude. Tosylate from **5a**: ^{1}H NMR ($CDCl_3$) δ 2.43 (3H, s, CH_3), 2.88 (2H, t, J = 7.0 Hz, CH_2), 3.95 (2H, t, J = 7.0 Hz, CH_2), 4.01 (3H, s, CH_3), 6.58 (1H, s, CH), 7.33 (2H, d, J = 9.0 Hz, CH), 7.93 (2H, d, J = 9.0 Hz, CH). Tosylate from **5b**: ^{1}H NMR ($CDCl_3$) δ 2.32 (3H, s, CH_3), 2.43 (3H, s, CH_3), 2.88 (2H, t, J = 7.1 Hz, CH_2), 3.95 (2H, t, J = 7.1 Hz, CH_2), 6.58 (1H, s, CH), 7.33 (2H, d, J = 9.0 Hz, CH), 7.90 (2H, d, J = 9.0 Hz, CH).