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Short communication

Synthesis and biological evaluation of 4-alkylamino-6-(2-hydroxyethyl)-2-methylthiopyrimidines as new rubella virus inhibitors

Claudia Mugnaini [a], Elena Petricci [a], Maurizio Botta [a,**], Federico Corelli [a,*], Paola Mastromarino [b], Gianluca Giorgi [c]

[a] *Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, Via A. De Gasperi, 2, 53100 Siena, Italy*
[b] *Dipartimento di Scienze di Sanità Pubblica, Università ''La Sapienza'', Piazzale Aldo Moro 5, 00185 Roma, Italy*
[c] *Dipartimento di Chimica, Università degli Studi di Siena, Via Aldo Moro 53100 Siena, Italy*

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Abstract

In the search for new chemotherapeutic agents useful against Rubella virus (RV) infections, a solution-phase parallel approach for the synthesis of a small library of 4-alkylamino-6-(2-hydroxyethyl)-2-methylthiopyrimidines has been set up, based on previous results from our research group. Biological evaluation of the newly synthesized compounds pointed out their interesting properties as anti-RV agents with IC_{50} values in the micromolar range.

Keywords: Rubella virus; Parallel synthesis; Solid-supported scavengers; 2,4,6-Trisubstituted pyrimidines

1. Introduction

Rubella virus (RV) is the sole member of the *Rubivirus* genus of the *Togaviridae* and it is characterized by a positive-sense single stranded RNA genome consisting of 9762 nucleotides [1,2]. The virus typically causes a scarlatiniform rash, cervical lymphadenopathy, and mild constitutional symptoms, but in older children and adults, especially women, it may be more severe, with joint involvement and purpuric rash. Rubella is the first virus demonstrated to be teratogen, infection during the first 12 weeks of pregnancy resulting in congenital infection and/or miscarriage in 80–90% of cases. There is a high risk to develop congenital rubella syndrome (CRS) if the infection occurs in the first part of pregnancy, particularly in women without specific immunological protection. CRS involves multiple organ systems and has a long period of active infection and virus shedding in the postnatal period. It includes a configuration of anomalies, such as nerve deafness, cataracts, cardiac anomalies and mental retardation, with late complications including diabetes, thyroid disease, growth hormone deficiency, and progressive panencephalitis [3].

The development of live and attenuated vaccines and the expansion of vaccination strategies since 1970 have reduced but not eradicated the incidence of CRS [4]. In fact, in developing countries or where campaigns of rubella surveillance and preconceptional vaccination are inadequate, there are still cases of CRS registered.

Although natural and semisynthetic polysaccharides [5], acylated 1,2,4-triazole derivatives [6], Fungizone® (amphotericin B and sodium deoxycholate) [7] and mopyridone [8] (Chart 1) show inhibitory effects against RV, currently specific therapies to prevent CRS are not available.

Thus, based on the encouraging results previously obtained with the preparation of 2-alkoxy- and 2-alkylthiopyrimidines as anti-rubella compounds [9,10], we planned to prepare a number of new 2-alkylthiopyrimidines with the aim to increase potency and to improve their pharmacological profile.

* Corresponding author. Tel.: +39 577 234308; fax: +39 577 234333.
** Corresponding author. Tel.: +39 577 234306; fax: +39 577 234333.
E-mail addresses: botta@unisi.it (M. Botta), corelli@unisi.it (F. Corelli).

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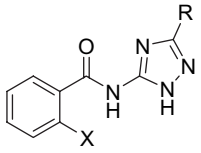
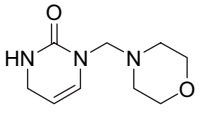
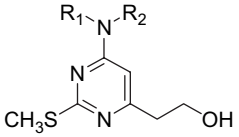


R = SCH_3, SC_2H_5
X = Br, Cl, SCH_3, NO_2, CH_3

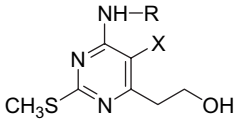
Triazole derivatives with anti-RV activity



Mopyridone



1



2

Chart 1. Compounds endowed with anti-rubella activity together with our target compounds **1** and **2**.

As a result, a small library of 4-dialkyl and 4-alkyl-2-thiopyrimidines **1** and **2** (Chart 1) was prepared through solution-phase parallel synthesis and evaluated for antiviral activity.

2. Results and discussion

2.1. Chemistry

For the synthesis of the target compounds, a general approach was followed, essentially based on the tosylation of a pyrimidinone scaffold followed by nucleophilic displacement with a series of different amines. Accordingly, a solution-phase parallel approach for the synthesis of 2-alkylthio-4-dialkylaminopyrimidines (Scheme 1) was developed starting from a model reaction between tosylate **3** and pyrrolidine in THF. A slight excess of pyrrolidine (1.5 equiv) and 10 h time at reflux temperature were sufficient to drive the reaction to completion. At the end of the reaction, the excess of amine and the by-product *p*-toluenesulfonic acid were removed from the reaction mixture using a polymer-bound isocyanate [11–13] and a P-TBD resin [14–16], respectively.

Once the best conditions for the substitution reaction had been set up, we went on performing the parallel synthesis. Thus, tosylate **4** (Scheme 1) was reacted in a Buchi Syncore® with 19 different secondary amines, according to the experimental conditions previously investigated. We observed that some reactions needed longer times (from 10 to 48 h) and larger amounts of reagents (from 1.5 to 2.5 equiv) to go to completion while amines characterized by high steric hindrance failed to react.

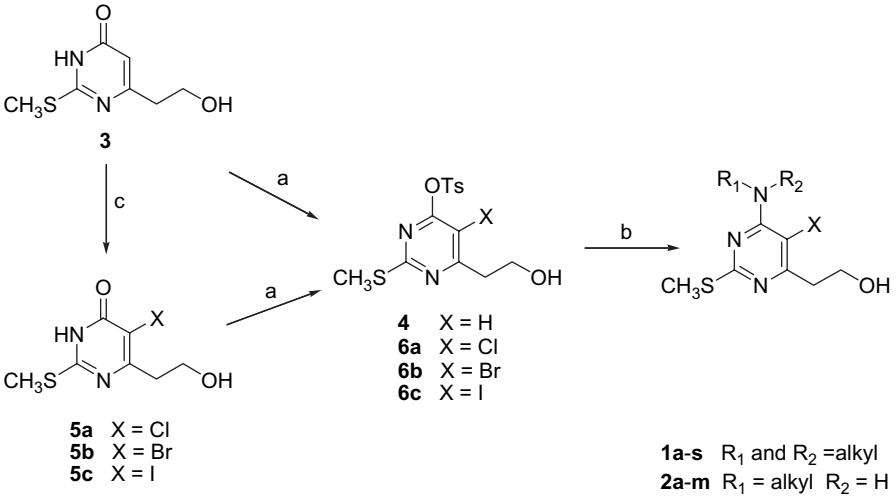
These results allowed us to find the optimal set of conditions to carry out the reactions in parallel: reaction time, 48 h; nucleophile, 2.5 equiv; temperature, 75 °C; stirring, 200 rpm.

In this way, most of the compounds **1a–s** were obtained in quantitative yield and high purity, as determined by ^{1}H NMR and HPLC analyses (Table 1).

In order to increase the chemical diversity of our library and to get new insight into the structure–activity relationships of this class of compounds, we further functionalized the heterocyclic ring introducing a halogen atom at C-5.

The bromination reaction was performed on intermediate **3** (Scheme 1) at room temperature using NBS in dry MeOH in the presence of 2,6-di-*tert*-butylpyridine (DTBP). In the case of chlorination and iodination, better results in terms of yield and purity of compounds were obtained with the aid of microwaves (50 °C, 15 min) in the presence of NCS and ICl [17], respectively. The halogenated derivatives **5a–c** were reacted with TsCl to give compounds **6a–c** in approximately 60% overall yield.

With the aim of synthesizing in parallel a small library of 4-alkylaminopyrimidines, a model reaction between tosylate **6a**



Scheme 1. Synthetic approach to the target compounds **1** and **2**. Reagents and conditions: (a) TsCl, DMAP, CH_2Cl_2; (b) for **1a–s** (i) R_1R_2NH (2.5 equiv), THF, 70 °C, 250 rpm, 48 h; (ii) P-TBD; (iii) PS-isocyanate. For **2a–m** (i) RNH_2 (1.5 equiv), THF, 70 °C, 250 rpm, 48 h; (ii) P-TBD; (iii) PB-4-benzyloxybenzaldehyde; (c) For **5a**: NCS, DMF, MW, 50 °C, 15 min; for **5b**: NBS, DTBP, MeOH, 0 °C to rt; for **5c**: ICl, DMF, MW, 50 °C, 15 min. For R_1, R_2 and X, see Table 1.

Table 1
Chemical data, activity and cytotoxicity of compounds **1a–s** and **2a–m**

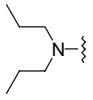
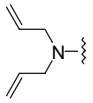
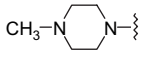
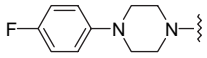
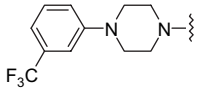
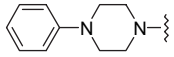
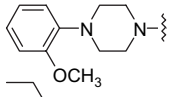
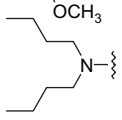
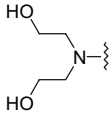
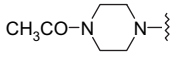
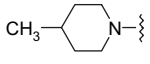
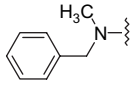
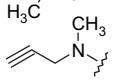
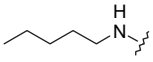
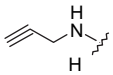
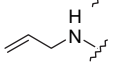
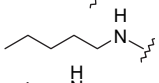
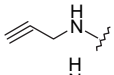
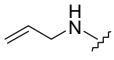
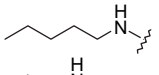
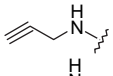
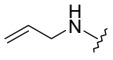
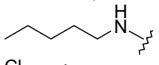
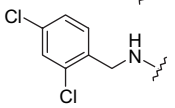
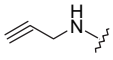
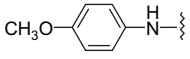
Compound	R_1 / R_2	R_1 / H	X	Yield (%)	IC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]	RV inhibition[d] (%)
1a		–	H	98	48.1	523.0	10.9	72.6
1b		–	H	100	>29.0	58.0	<2.0	0
1c		–	H	56	111.5	249.0	2.2	54.7
1d		–	H	99	>277.8	555.5	<2.0	35.6
1e		–	H	100	>233.2	466.4	<2.0	20.6
1f		–	H	99	>230.6	461.2	<2.0	45.2
1g		–	H	100	51.7	179.6	3.5	93.2
1h		–	H	100	17.6	39.2	2.2	58.9
1i		–	H	100	57.0	189.4	3.3	90.0
1j		–	H	98	27.8	173.6	6.2	96.1
1k		–	H	100	13.5	52.5	3.9	87.0
1l		–	H	99	169.7	1824.8	10.7	95.7
1m		–	H	100	49.0	422.3	8.6	65.6
1n		–	H	75	22.6	486.4	21.6	95.3
1o		–	H	99	47.8	233.2	4.9	96.5
1p		–	H	100	>54.0	108.0	<2.0	31.9
1q		–	H	99	25.3	518.7	20.5	94.2
1r		–	H	98	80.7	586.8	7.3	92.8
1s		–	H	75	113.9	1054.8	9.3	92.1
2a	–		Cl	67	16.2	107.6	6.6	66.4

Table 1 (*Continued*)

Compound	R_1 R_2 N–	R_1 H N–	X	Yield (%)	IC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]	RV inhibition[d] (%)
2b	–		Cl	37	135.8	485.0	3.6	85.4
2c	–		Cl	79	43.5	481.2	11.1	83.0
2d	–		I	49	27.5	81.8	3.0	95.1
2e	–		I	74	34.1	715.9	21.0	97.3
2f	–		I	100	15.7	355.9	22.7	93.9
2g	–		Br	57	37.4	93.30	2.5	60.0
2h	–		Br	65	148.9	827.3	5.6	94.4
2i	–		Br	62	50.9	410.9	8.1	86.9
2j	–		H	60	21.6	245.1	11.4	94.7
2k	–		H	55	48.0	181.7	3.8	76.1
2l	–		H	73	107.6	1121.1	10.4	98.8
2m	–		H	40	>214.8	429.5	<2.0	43.3

[a] Inhibitory concentration of compound required to inhibit virus yield by 50%.
[b] The minimal concentration of compound which affected one cytotoxicity parameter in 50% of cells.
[c] Selectivity index (CC_{50}/IC_{50}).
[d] Compounds at the maximal non-cytotoxic concentration were added to Vero cells after viral absorption (1 h, 37 °C) and maintained throughout the virus replication cycle. The yield of rubella virus was evaluated by plaque assay after a single round, high multiplicity infection (48 h, m.o.i. 3) Data represent the mean of two independent experiments conducted in duplicate. The standard deviations were <5% for all values.

and pentylamine was set up. We observed that 1.5 equiv of amine and 48 h time were necessary to drive the reaction to completion. As a result, tosylates **4** and **6a–c** were reacted in parallel with five different primary amines (Scheme 1) under the same conditions used for the model reaction. Surprisingly, most of the reactions did not go to completion and substantial amount of starting material was recovered at the end of the reaction together with by-products. Attempts to use a larger excess of amine in order to improve the reaction yield was unsuccessful. Conversely, the use of only 1.5 equiv of nucleophile was a good compromise to get the final compounds in good purity and acceptable yield. In any case, after treatment of the reaction mixture with P-TBD and PB-benzyloxybenzaldehyde to remove *p*-toluenesulfonic acid and the excess of primary amine, respectively, further purification on silica gel was necessary to get compounds **2a–m** as pure products (Table 1).

2.2. Anti-rubella virus activity

The antiviral activity of the synthesized compounds was evaluated in Vero cells starting from the highest non-cytotoxic concentration which did not affect any parameter (cell morphology, viability and growth) considered in 100% of the cells. After virus adsorption, the viral inoculum was removed, cell monolayers were washed three times with PBS and incubated in the presence or absence of two-fold dilutions of the compounds. Viral inhibition was evaluated by plaque assay after a single cycle of virus multiplication. The results are summarized in Table 1.

In general, the antiviral activity did not vary markedly among the tested compounds, the IC_{50} values spanning one order of magnitude, from 13.5 μM (compound **1k**) to 169.7 μM (compound **1l**) with most of the compounds showing IC_{50} values <60 μM. Therefore, despite the interesting antiviral properties of most of these molecules, it is difficult to highlight in detail their structure–activity relationships. Nevertheless, some general aspects merit to be commented. Excluding the most cytotoxic compounds **1b**, **1d–f**, **1p**, **2m**, the less active compounds **1c**, **1s**, **2b**, **2h**, **2l** are all characterized by a propargylamino or a diallylamino substituent. As an exception, **1l**, having a bis(2-hydroxyethyl)amino substituent, shows poor activity possibly due to the polar/hydrophilic nature of the substituent itself. However, **2e**, despite the presence of the propargyl group, is among the most active compounds.

On the other hand, derivatives **1a**, **1g–k**, **1m–o**, **1q**, **1r**, **2a**, **2c**, **2d**, **2f**, **2g**, **2i–k**, belonging to the cluster of the most active compounds, generally possess alkyl or cycloalkyl substituents bearing no more than one unsaturation. In particular, the allylamino derivative **2f** exhibits the best overall profile combining good antiviral activity ($IC_{50} = 15.7$ μM) with the best selectivity index (SI = 22.7).

3. Conclusions

A solution-phase parallel approach for the synthesis of a small library of 4-dialkylamino-6-(2-hydroxyethyl)-2-methylthiopyrimidines **1a–s** has been set up. In particular, the reaction of tosylate **4** with a number of secondary amines gave the corresponding derivatives in almost quantitative yield and high purity after removal of the excess of reagents and by-products by simple treatment with solid supported scavengers. On the other hand, the reaction between **4** and both saturated and unsaturated primary amines could not be driven to completion even by the use of an excess of reagent. In this case, a further purification by column chromatography was necessary to get the final compounds **2j–m** in good yield and purity. In order to get more information regarding the structural requirements necessary for the antiviral activity, the position 5 of the pyrimidine nucleus was also functionalized by introducing a halogen atom to give the tosylates **6a–c** by the use of microwave irradiation. Subsequent reaction of **6a–c** and primary amines yielded compounds **2a–i**.

Biological evaluation of compounds **1** and **2** pointed out their antiviral properties. Although the anti-RV activity is far from that commonly shown by clinically useful chemotherapeutic agents, nevertheless it lies in the micromolar range and hence it is in accordance with that required for new leads suitable for pharmaceutical development. This result is of interest since the identification of new lead compounds in this area is particularly demanding due to the lack of specific knowledge of possible viral targets as well as of general SAR information. In fact, only scattered examples of RV replication inhibitors endowed with modest antiviral activity have been reported in the literature [6,8]. Mopyridone itself, which is considered a highly active and broad spectrum inhibitor of togavirus replication [8], shows an IC_{50} value only one order of magnitude lower with respect to the most active compounds prepared by us. In conclusion, the new compounds **1** and **2** might be an interesting starting point for further structural optimization in the search for potential anti-RV inhibitors.

4. Experimental

4.1. Chemistry

All commercially available chemicals were used as purchased. CH_2Cl_2 was dried over calcium hydride, THF was dried over Na/benzophenone prior to use. Anhydrous reactions were run under a positive pressure of dry N_2. IR spectra were recorded on a Perkin–Elmer BX FTIR system, using KBr pellets. TLC was carried out using Merck TLC plates silica gel 60 F254. Chromatographic purifications were performed on columns packed with Merck 60 silica gel, 23–400 mesh, for flash technique. ^{1}H NMR spectra were recorded at 200 MHz on a Bruker AC200F spectrometer. Chemical shifts are reported relative to $CDCl_3$ at δ 7.24 ppm and tetramethylsilane at δ 0.00 ppm.

Büchi Syncore polyvap was used for parallel synthesis, filtration, and evaporation. Microwave reactions were conducted using a CEM Discover Synthesis Unit (CEM Corp., Matthews, NC). Elemental analyses (C, H, N) were performed in-house using a Perkin–Elmer Elemental Analyzer 240C.

4.1.1. 6-(2-Hydroxyethyl)-2-(methylthio)-4-pyrimidinyl 4-methyl-1-benzenesulfonate (*4*)

To a solution of **3** (1.00 g, 5.30 mmol) in dry CH_2Cl_2 (25 mL) *p*-toluenesulfonyl chloride (1.10 g, 6.40 mmol) and DMAP (0.77 g, 6.40 mmol) were added and the reaction mixture was stirred at room temperature for 24 h. The organic solution was washed with 2 N HCl, brine and then dried over anhydrous Na_2SO_4. Filtration and evaporation under reduced pressure gave **4** (1.44 g, 80%) as a white solid after recrystallization (AcOEt).

Mp 53–55 °C. ^{1}H NMR ($CDCl_3$): δ 7.80 (d, $J = 8.5$ Hz, 2H), 7.30 (d, $J = 8.5$ Hz, 2H), 6.56 (s, 1H), 3.89 (t, $J = 5.6$ Hz, 2H), 2.84 (t, $J = 5.6$ Hz, 2H), 2.39 (s, 3H), 2.30 (s, 3H). MS (ESI): *m/z* 341 $[M + 1]^+$. Anal Calcd for $C_{14}H_{16}N_2O_4S_2$: C, 49.40; H, 4.74; N, 8.23. Found: C, 49.20; H, 4.72; N, 8.26.

4.2. General procedure for the preparation of *1a–s*

A solution of **4** (50.00 mg, 0.15 mmol) in dry THF (5 mL) was introduced in 19 different vessels of Büchi Syncore® and the appropriate amines were added (0.37 mmol, 2.5 equiv). The reaction mixtures were heated overnight at reflux temperature at 250 rpm. The solvent was partially evaporated and the reaction mixtures were cooled down to room temperature. Each reaction mixture was diluted with CH_2Cl_2 (4 mL), then P-TBD (150 mg, 0.44 mmol) was added and the mixture was stirred for 1 h. Finally, PB-isocyanate (297 mg, 0.59 mmol) was added and the reaction mixtures were stirred for additional 24 h. The reaction mixtures were filtered in parallel and the scavengers were washed twice with CH_2Cl_2 (5 mL) and MeOH (5 mL). The final compounds (except when specified) were not further purified.

4.2.1. Examples

*4.2.1.1. 2-[2-(Methylthio)-6-tetrahydro-1H-1-pyrrolyl-4-pyrimidinyl]-1-ethanol (**1a**).* White solid (98%). Mp 110–112 °C. ^{1}H NMR ($CDCl_3$): δ 5.78 (s, 1H), 3.87 (t, $J = 5.3$ Hz, 2H), 3.65–3.17 (m, 4H), 2.69 (t, $J = 5.3$ Hz, 2H), 2.43 (s, 3H), 2.09–1.82 (m, 4H). MS (ESI): *m/z* 240

$[M+1]^+$. Anal Calcd for $C_{11}H_{17}N_3OS$: C, 55.20; H, 7.16; N, 17.56. Found: C, 55.13; H, 7.34; N, 17.42.

*4.2.1.2. 2-[6-(Diallylamino)-2-(methylthio)-4-pyrimidinyl]-1-ethanol (**1c**).* Purification on preparative TLC (eluent: $CHCl_3$/MeOH, 9/1). Colourless oil (56%). ^1H NMR ($CDCl_3$): δ 5.90 (s, 1H), 5.87–5.71 (m, 2H), 5.17–5.07 (m, 4H), 4.23–4.02 (m, 4H), 3.89 (t, $J = 5.2$ Hz, 2H), 2.71 (t, $J = 5.2$ Hz, 2H), 2.44 (s, 3H). MS (ESI): *m/z* 266 $[M+1]^+$. Anal Calcd for $C_{13}H_{19}N_3OS$: C, 58.84; H, 7.22; N, 15.83. Found: C, 58.66; H, 7.31; N, 15.94.

*4.2.1.3. 2-[2-(Methylthio)-6-(1,3-thiazolan-3-yl)-4-pyrimidinyl]-1-ethanol (**1n**).* Purification by column chromatography (eluent: $CHCl_3$/MeOH, 9/1).

Yellow solid (75%). Mp 77–79 °C. ^1H NMR ($CDCl_3$): δ 5.91 (s, 1H), 4.60 (s, 2H), 3.92 (t, $J = 5.4$ Hz, 2H), 3.76 (t, $J = 6.1$ Hz, 2H), 3.10 (t, $J = 6.1$ Hz, 2H), 2.75 (t, $J = 5.4$ Hz, 2H), 2.46 (s, 3H). MS (ESI): *m/z* 258 $[M+1]^+$. Anal Calcd for $C_{10}H_{15}N_3OS_2$: C, 46.67; H, 5.87; N, 16.33. Found: C, 46.54; H, 5.93; N, 16.42.

*4.2.2. 5-Chloro-6-(2-hydroxyethyl)-2-(methylthio)-3,4-dihydro-4-pyrimidinone (**5a**)*

To a solution of **3** (100 mg, 0.54 mmol) in DMF (2 mL) NCS (98.3 mg, 1.34 mmol) was added and the reaction mixture was irradiated at 50 °C for 15 min. The solvent was removed and the residue was purified by column chromatography (eluent: $CHCl_3$/MeOH, 9/1) giving **5a** (64 mg, 54%) as a white solid. Mp 168–170 °C. ^1H NMR (CD_3OD): δ 3.91 (t, $J = 6.5$ Hz, 2H), 2.96 (t, $J = 6.5$ Hz, 2H), 2.55 (s, 3H). MS (ESI): *m/z* 243 $[M+Na]^+$. Anal Calcd for $C_7H_9N_2O_2SCl$: C, 38.10; H, 4.11; N, 12.69. Found: C, 37.94; H, 4.12; N, 12.63.

*4.2.3. 5-Bromo-6-(2-hydroxyethyl)-2-(methylthio)-3,4-dihydro-4-pyrimidinone (**5b**)*

To a solution of **3** (1.60 g, 8.60 mmol) in dry MeOH (40 mL) DTBP (4.82 mL, 21.5 mmol) and NBS were added at 0 °C. After 1 h, the solvent was evaporated at reduced pressure and the residue was purified by column chromatography (eluent: $CHCl_3$/MeOH, 9/1) giving **5b** (1.58 g, 69%) as a white solid.

Mp 167–170 °C. ^1H NMR (CD_3OD): δ 3.91 (t, $J = 6.5$ Hz, 2H), 2.97 (t, $J = 6.5$ Hz, 2H), 2.55 (s, 3H). MS (ESI): *m/z* 266 $[M+1]^+$. Anal Calcd for $C_7H_9BrN_2O_2S$: C, 31.71; H, 3.42; N, 10.57. Found: C, 31.65; H, 3.42; N, 10.60.

*4.2.4. 6-(2-Hydroxyethyl)-5-iodo-2-(methylthio)-3,4-dihydro-4-pyrimidinone (**5c**)*

To a solution of **3** (300 mg, 1.61 mmol) in DMF (6 mL) a 1 M solution of ICl in CH_2Cl_2 (3.3 mL, 3.3 mmol) was added and the reaction mixture was irradiated at 50 °C for 15 min. After evaporation of the solvent, the residue was purified by column chromatography (eluent: $CHCl_3$/MeOH, 9/1) giving **5c** (302 mg, 60%) as a white solid.

Mp 201–202 °C (dec.). ^1H NMR (CD_3OD): δ 3.90 (t, $J = 6.7$ Hz, 2H), 3.03 (t, $J = 6.7$ Hz, 2H), 2.55 (s, 3H). MS (ESI): *m/z* 313 $[M+1]^+$. Anal Calcd for $C_7H_9IN_2O_2S$: C, 26.94; H, 2.91; N, 8.97. Found: C, 26.83; H, 2.92; N, 8.93.

*4.3. General procedure for the preparation of **6a–c***

To a solution of the appropriate alcohol **5** (0.23 mmol) in dry CH_2Cl_2 (10 mL) TsCl (51 mg, 0.27 mmol) and DMAP (33 mg, 0.27 mmol) were added and the reaction mixture was stirred overnight at room temperature. The organic solution was washed with 2 N HCl, brine and dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent, the residue was purified by column chromatography (eluent: $CHCl_3$/MeOH, 95/5) giving the corresponding tosylate **6a–c**.

4.3.1. Example

*4.3.1.1. 5-Chloro-6-(2-hydroxyethyl)-2-(methylthio)-4-pyrimidinyl 4-methyl-1-benzenesulfonate (**6a**).* White solid (70%). Mp 94–96 °C. ^1H NMR ($CDCl_3$): δ 7.90 (d, $J = 8.5$ Hz, 2H), 7.41 (d, $J = 8.5$ Hz, 2H), 3.90 (t, $J = 6.4$ Hz, 2H), 2.84 (t, $J = 6.4$ Hz, 2H), 2.41 (s, 3H), 2.32 (s, 3H). MS (ESI): *m/z* 398 $[M+Na]^+$. Anal Calcd for $C_{14}H_{15}ClN_2O_4S_2$: C, 44.86; H, 4.03; N, 7.47. Found: C, 45.03; H, 4.04; N, 7.49.

*4.4. General procedure for the preparation of **2a–m***

A solution of **6** (0.20 mmol, 1 equiv) in dry THF (8 mL) was introduced in 13 different vessels of Büchi Syncore® and the appropriate amines were added (0.30 mmol, 1.5 equiv). The reaction mixtures were heated overnight at reflux temperature at 250 rpm. The solvent was partially evaporated and the reaction mixtures were cooled down to room temperature. Each reaction mixture was diluted with CH_2Cl_2 (5 mL), then P-TBD (0.60 mmol, 3 equiv) was added and the mixture was stirred for 1 h. Finally, PB-4-benzyloxybenzaldehyde (0.90 mmol, 4.5 equiv) was added and the reaction mixtures were stirred for additional 24 h. The reaction mixtures were filtered in parallel and the scavengers were washed twice with CH_2Cl_2 (5 mL) and MeOH (5 mL). After evaporation of the solvents, the residues were purified by column chromatography (eluent: CH_2Cl_2/MeOH, 98/2) to give the final compounds **2a–m** as pure products.

4.4.1. Examples

*4.4.1.1. 2-[5-Chloro-2-(methylthio)-6-(pentylamino)-4-pyrimidinyl]-1-ethanol (**2a**).* White solid (67%). Mp 64–67 °C. ^1H NMR ($CDCl_3$): δ 3.92 (m, 2H), 3.50–3.44 (m, 2H), 2.87 (t, $J = 5.4$ Hz, 2H), 2.51 (s, 3H), 1.75–1.52 (m, 2H), 1.34–1.27 (m, 4H), 0.91–0.85 (m, 3H). MS (ESI): *m/z* 290 $[M+1]^+$. Anal Calcd for $C_{12}H_{20}N_3OSCl$: C, 49.73; H, 6.96; N, 14.50. Found: C, 49.85; H, 6.83; N, 14.42.

*4.4.1.2. 2-[6-(Allylamino)-5-iodo-2-(methylthio)-4-pyrimidinyl]-1-ethanol (**2f**).* White solid (100%). Mp 74–76 °C. ^1H NMR

($CDCl_3$): δ 6.02–5.81 (m, 1H), 5.27–5.13 (m, 2H), 4.16–4.10 (m, 2H), 4.10–3.96 (m, 2H), 2.96–2.90 (m, 2H), 2.52 (s, 3H).MS (ESI): *m/z* 352 $[M+1]^+$. Anal Calcd for $C_{10}H_{14}IN_3OS$: C, 34.20; H, 4.02; N, 11.96. Found: C, 34.35; H, 4.06; N, 11.96.

*4.4.1.3. 2-[2-(Methylthio)-6-(2-propynylamino)-4-pyrimidinyl]-1-ethanol (**2l**).* White solid (75%). Mp 119–121 °C. ^1H NMR ($CDCl_3$): δ 6.08 (s, 1H), 4.12 (t, $J = 2.3$ Hz, 2H), 3.79 (t, $J = 6.3$ Hz, 2H), 2.64 (t, $J = 6.3$ Hz, 2H), 2.53 (t, $J = 2.3$ Hz, 1H), 2.47 (s, 3H). MS (ESI): *m/z* 224 $[M+1]^+$. Anal Calcd for $C_{10}H_{13}N_3OS$: C, 53.79; H, 5.87; N, 18.82. Found: C, 53.83; H, 5.94; N, 18.68.

*4.4.1.4. 2-[6-(4-Methoxyanilino)-2-(methylthio)-4-pyrimidinyl]-1-ethanol (**2m**).* Yellow solid (40%). Mp 168–172 °C. ^1H NMR (CD_3OD): δ 7.47 (d, $J = 7.0$ Hz, 2H), 6.87 (d, $J = 7.0$ Hz, 2H), 6.21 (s, 1H), 3.80 (t, $J = 6.4$ Hz, 2H), 3.76 (s, 3H), 2.66 (t, $J = 6.4$ Hz, 2H), 2.47 (s, 3H). MS (ESI): *m/z* 292 $[M+1]^+$. Anal Calcd for $C_{14}H_{17}N_3OS$: C, 57.71; H, 5.88; N, 14.42. Found: C, 57.64; H, 5.79; N, 14.55.

4.5. Biology

4.5.1. Anti-rubella activity

4.5.1.1. Cells. Vero cells were cultured at 37 °C in a 5% CO_2 atmosphere in Eagle's Minimum Essential Medium (MEM) containing 2.0 mg/mL $NaHCO_3$ and supplemented with 6% (v/v) foetal 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).

4.5.1.2. Virus. Rubella virus (Therien strain) was grown in Vero cells in maintenance medium. Semi-confluent monolayers were inoculated with virus at a multiplicity of infection of 0.1 PFU/cell and incubated at 37 °C for 72 h. After infection, supernatant was collected, centrifuged at 1000*g* for 10 min to remove cellular debris, and then stored in small aliquots at −80 °C.

4.5.1.3. Cytotoxicity assays. The cytotoxicity of compounds was monitored by evaluating the effects on cell morphology, viability and growth. Vero cells in 24-well plates were cultured for 2 days at 37 °C in the presence or absence of two-fold serially diluted compounds. After 48 h incubation cytotoxicity was scored microscopically as morphological alterations (such as swelling, granularity, rounding up, shrinking and detachment). Cell morphology, viability and yield were 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 a hemocytometer after trypsinization.

4.5.1.4. Antiviral assay. For antiviral assays, confluent monolayers of Vero cells grown in 24-well plates were inoculated with RV (3 PFU/cell). After virus adsorption (1 h, 37 °C), the viral inoculum was removed. Cell monolayers were washed three times with phosphate-buffered saline (PBS) and incubated with maintenance medium in the presence or absence of the compounds. Virus yield was evaluated by plaque assay after 48 h.

4.5.1.5. Plaque assay. Serial ten-fold dilutions of virus were inoculated onto confluent Vero cell monolayers. After a 1 h adsorption period at 37 °C, the inoculum was removed and cells were washed three times with PBS before being overlaid with MEM containing 0.4% (w/v) agar (Oxoid). After 5 days of incubation at 37 °C, plaques were stained with 0.1% crystal violet solution.

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Appendix. Supplementary material

Analytical data of compounds **1b**, **1d–1m**, **6b–6c**, **2b–2e**, **2g–2k**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2006.09.002.

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