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Synthesis and biological evaluation of nucleoside analogues having 6-chloropurine as anti-SARS-CoV agents

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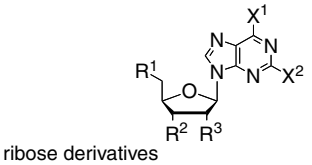
Abstract—Nucleoside analogues that have 6-chloropurine as the nucleobase were synthesized and evaluated for anti-SARS-CoV activity by plaque reduction and yield reduction assays in order to develop novel anti-SARS-CoV agents. Among these analogues, two compounds, namely, **1** and **11**, exhibited promising anti-SARS-CoV activity that was comparable to those of mizoribine and ribavirin, which are known anti-SARS-CoV agents. Moreover, we observed several SAR trends such as the antiviral effects of the 6-chloropurine moiety, unprotected 5′-hydroxyl group and benzoylated 5′-hydroxyl group.

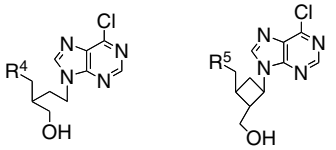
Severe acute respiratory syndrome (SARS) is an emerging infectious disease of the 21st century and poses a global threat to public health, mainly leading to fatal infection with influenza-like symptoms such as high fever, dry cough, pneumonia and dyspnoea.[1] This disease appeared in the Guandong province of southern China in late 2002, and subsequently spread to 29 countries in early 2003, affecting approximately 8000 persons. The overall mortality rate of SARS has amounted to approximately 10%. Following the identification of the causative pathogen—a new coronavirus (CoV) named SARS-CoV—in 2003, several compounds have been reported to exhibit antiviral activity against SARS-CoV.[2] However, thus far, no effective treatment has been developed.

In our previous studies, several nucleoside derivatives having 6-chloropurine as the nucleobase showed potent antiviral activity against some types of viruses.[3] We presume that the 6-chloropurine moiety could play an important role in the antiviral activity; in fact, several 6-chloropurine analogues are known to inhibit bacterial RNA polymerases.[4] Therefore, we expected that nucleoside analogues that have 6-chloropurine would be efficacious against SARS-CoV. Thus, several nucleoside analogues, namely, **1**–**11**, which could be readily prepared or are commercially available, were designed as anti-SARS-CoV agents (Fig. 1). In this report, we



ribose derivatives
1: R^1 = OH, R^2 = OH, R^3 = OH, X^1 = Cl, X^2 = H
2: R^1 = OBz, R^2 = OH, R^3 = OH, X^1 = Cl, X^2 = H
3: R^1 = OH, R^2 = OH, R^3 = OH, X^1 = OMe, X^2 = H
4: R^1 = OH, R^2 = OH, R^3 = OH, X^1 = SMe, X^2 = H
5: R^1 = OH, R^2 = OH, R^3 = OH, X^1 = Cl, X^2 = NH_2

2' or 3'-deoxyribose derivatives
6: R^1 = OH, R^2 = OH, R^3 = H, X^1 = Cl, X^2 = H
7: R^1 = OH, R^2 = H, R^3 = OH, X^1 = Cl, X^2 = H



8: R^4 = OH
9: R^4 = OBz
10: R^5 = OH
11: R^5 = OBz

Figure 1. Structures of the nucleoside analogues.

Keywords: SARS-CoV; Nucleoside; 6-Chloropurine; Antiviral.
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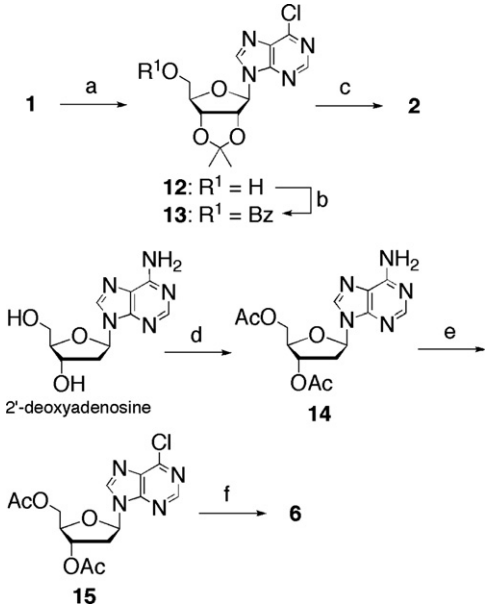
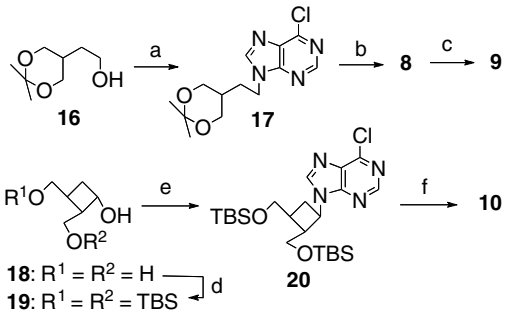
describe the syntheses and anti-SARS-CoV activities of the nucleoside analogues **1**–**11**.

Compounds **1**, **4** and **5** were purchased from chemical companies, and **3**, **7** and **11** were prepared according to previous literatures.[5] Syntheses of the (deoxy)ribonucleoside derivatives **2** and **6** are illustrated in Scheme 1. Regioselective protection of the 2′,3′-diol moiety of **1** yielded acetonide **12**,[6] which was subsequently benzoylated to afford **13** with a 91% yield over two steps. Cleavage of the acetonide group produced the desired product **2** with a 61% yield. Acetylation of both hydroxyl groups of 2′-deoxyadenosine afforded **14**,[7] which was transformed to 6-chloropurine **15** with a 57% yield over two steps. The deprotection of the acetyl groups was accomplished at ice-water temperature to avoid decomposition of the 6-chloropurine moiety, thereby producing the desired product **6** with a 76% yield. Syntheses of the acyclic analogues **8** and **9** and the carbocyclic oxetanocin analogue **10** are outlined in Scheme 2. The Mitsunobu reaction of known alcohol **16**[8] with 6-chloropurine, followed by the removal of the acetonide group in **17**, resulted in the desired diol **8** with a 66% yield over two steps. Subsequently, monobenzoylation of **8** was carried out via hydrolysis of the corresponding orthobenzoate[9] to afford the desired benzoate **9** with a 67% yield. Triol **18**, which was prepared as described in a previous literature,[10] was converted to bis-silyl ether **19**; this compound was then subjected to a Mitsunobu reaction to yield the 6-chloropurine derivative **20**. Finally, cleavage of the TBS groups produced the desired product**10** with a 96% yield. We could not directly convert benzoate **11**[5c] to **10** because of the instability of the 6-chloropurine moiety under basic conditions.



Scheme 1. Reagents and conditions: (a) $Me_2C(OMe)_2$, *p*-TsOH, acetone, rt, 4 h, 91%; (b) BzCl, Et_3N, DMAP, CH_2Cl_2, 0 °C to rt, 2 h, quant; (c) AcOH/water (4:1), 60 °C, 5 h, 61%; (d) Ac_2O, DMAP, Pyr, 4 h; (e) *t*-BuONO, Et_4NCl, CCl_4, CH_2Cl_2, 0 °C to 50 °C, 4.5 h, 57% from 2′-deoxyadenosine; (f) 2 M NH_3–MeOH, 0 °C, 9 h, 76%.



Scheme 2. Reagents and conditions: (a) 6-Chloropurine, PPh_3, 1, 1′-azobis(*N*,*N*-dimethylformamide), THF, rt, 2 days; (b) *p*-TsOH, MeOH, rt, 24 h, 66% from **16**; (c) $PhC(OMe)_3$, *p*-TsOH, MeCN, rt, 4 h, followed by water, rt, 3 h, 67%; (d) TBSCl, Pyr, −20 °C to rt, 18 h, 37%; (e) 6-chloropurine, PPh_3, 1,1′-azobis(*N*,*N*-dimethylformamide), THF, rt to 45 °C, 18 h, 44% (recovery of **19**, 40%); (f) *p*-TsOH, MeOH, rt, 18 h, 96%.

The antiviral effect of the prepared nucleoside analogues was evaluated by a plaque reduction assay and a yield reduction assay with SARS-CoV Frankfurt-1 strain in Vero E6 cells as described in a previous report.[11] The anti-SARS-CoV activities determined by the plaque reduction assay are shown in Figure 2 and Table 1. Compounds **1** and **11** showed potent activity (IC_{50}: 48.7 μM and 14.5 μM, respectively), and compound **2** showed weak activity (IC_{50}: 108 μM), while the other analogues **3**–**10** did not show any significant activity (IC_{50} > 300 μM). Notably, the inhibitory activities of compounds **1** and **11** against SARS-CoV were comparable to those of mizoribine and ribavirin, which were reported as potential anti-SARS-CoV agents by our group,[11] although the antiviral indices of **1** and **11** were smaller than those of mizoribine and ribavirin. Figure 3 illustrates the anti-SARS-CoV activities of **1**, **2**, and **11** determined by the yield reduction assay. The inhibitory effect of **1** was the greatest among the three compounds; the virus yield at a concentration of approximately 20 μM decreased to one-hundredth or less of the control (Fig. 3, left). It is important to note that this inhibitory effect was superior to those of

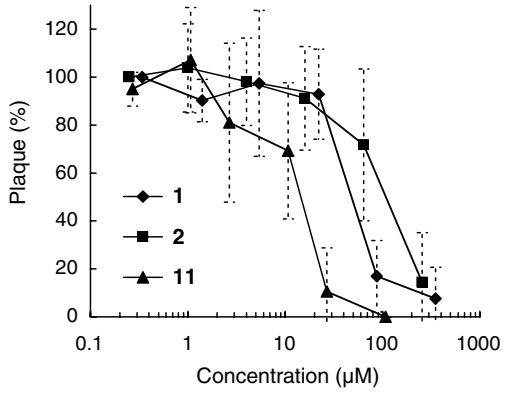


Figure 2. Inhibitory effect of compounds **1**, **2** and **11** on plaque formation of SARS-CoV Frankfurt-1 strain in the plaque reduction assay.

Table 1. Inhibitory and cytotoxic concentrations of nucleoside analogues **1**, **2**, **3**–**10** and **11**; ribavirin; and mizoribine in the plaque reduction assay[a]

Compound	IC_{50} (μM)[b]	CC_{50} (μM)[c]	Antiviral index[d]
1	48.7	279	5.7
2	108	174	1.6
3–**10**	>300	—	—
11	14.5	78	5.4
Ribavirin[e]	82	>850	>10
Mizoribine[e]	13.5	>700	>52

[a] The same experiment was performed at least three times independently with SARS-CoV Frankfurt-1 strain and Vero E6 cells.
[b] Average of 50% inhibitory concentrations or concentration required to reduce virus plaque formation by 50%.
[c] Average of 50% cytotoxic concentrations (CC) or concentration required to reduce cell growth by 50%. The CC was measured using the WST-1 cytotoxicity assay kit.
[d] Antiviral index was defined as the 50% toxic dose divided by the 50% inhibitory dose.
[e] Extracts obtained from a previous study.[11]

mizoribine and ribavirin since approximately 35 μM of mizoribine or 200 μM of ribavirin was required to decrease the virus yield to one-tenth of the control.[11] In the case of compounds **2** and **11**, approximately 50–70 μM of each compound was required to reduce the virus yield to one-hundredth of the control (Fig. 3, middle and right).

These results revealed several structure-activity relationship (SAR) trends. A chlorine atom at the 6-position of the purine base was considered to be important for the anti-SARS-CoV activity (compound **1** vs **3** and **4**); however, an introduction of the amino group at the 2-position of 6-chloropurine, which corresponds to a guanine derivative, was unfavourable for the antiviral activity (compound **5**). Although the reason for this trend is unclear, there is a possibility that due to its electrophilic nature,[12] the 6-chloropurine moiety can form a covalent bond with the target enzyme and can induce an effective irreversible inhibition.[13] The substitution of weak leaving groups (e.g., –OMe (compound **3**) or –SMe (compound **4**)) for the chlorine atom at the 6-position that led to the diminution of the antiviral activity also supports this possibility. In the case of ribofuranosyl structure, unprotected 5′-hydroxyl group was important in the antiviral effect (compound **1** vs **2**); this would indicate that following the intracellular phosphorylation of the 5′-hydroxyl group to the corresponding triphosphates, compound **1** exhibited antiviral activity in the same manner as common nucleoside antivirals.[14] The type of sugar moiety also influenced the anti-SARS-CoV activity. For example, the 2′-deoxy- and 3′-deoxyribonucleoside derivatives **6** and **7** showed weak antiviral activity as compared to the ribonucleoside derivative **1**. The conversion to an acyclic backbone that imitates the antiviral agent penciclovir (i.e., 9-[4-Hydroxy-3-(hydroxymethyl)butyl]guanine) also decreased the activity (compound **1** vs **8** and **9**). Interestingly, the carbocyclic oxetanocin analogue **11** whose hydroxyl group was protected by benzoylation exhibited potential activity as compared to unprotected **10** that exhibited almost no activity.[15] This trend seems to be different from those of the common nucleoside antivirals (including compound **1**), and could indicate that compound **11** acts on SARS-CoV through another pathway that does not involve the intracellular phosphorylation.

In conclusion, we have synthesized several nucleoside analogues having 6-chloropurine as the nucleobase. Among these analogues, two compounds, namely, **1** and **11**, were found to be efficacious against SARS-CoV and showed antiviral activities comparable to those of mizoribine and ribavirin. This study revealed several curious SAR trends such as the antiviral effects of the 6-chloropurine moiety (compounds **1** and **11**), unprotected 5′-hydroxyl group (compound **1**), and protected (benzoylated) 5′-hydroxyl group (compound **11**). Although some issues such as reduction of cytotoxicity remain to be resolved, we hope that the results of the present study will contribute to further development of antiviral agents against SARS-CoV. Studies that focus on further optimisation of the synthesized compounds and their biological evaluation will be reported in due course.

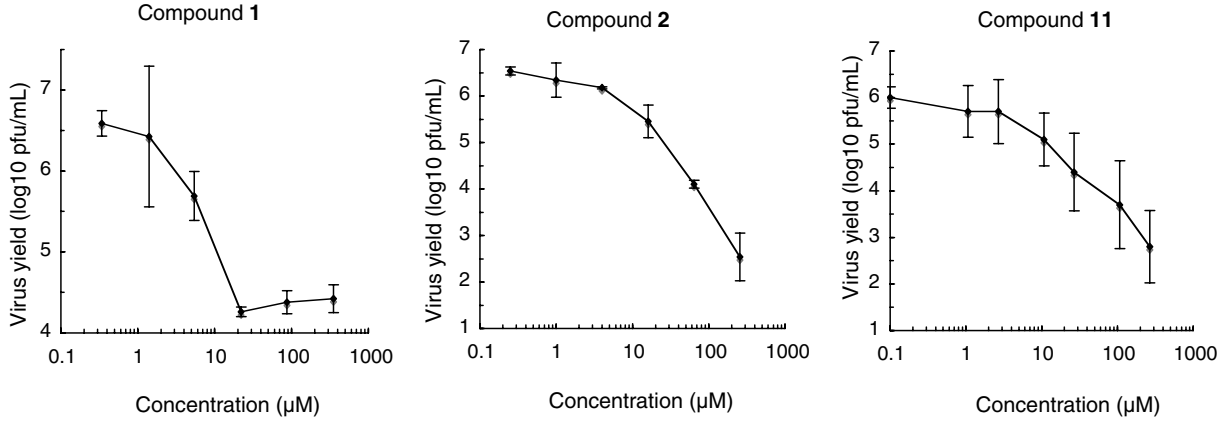


Figure 3. Inhibitory effect of nucleoside analogues **1**, **2** and **11** in the yield reduction assay. The same experiment was performed at least three times independently with SARS-CoV Frankfurt-1 strain and Vero E6 cells.

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