Short communication

Antiviral activity of nucleoside analogues against SARS-coronavirus (SARS-CoV)

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The recent outbreak of severe acute respiratory syndrome (SARS), which is an acute respiratory illness, is caused by newly discovered SARS coronavirus (SARS-CoV). Herein we describe the antiviral activity of several classes of nucleoside analogues evaluated against SARS-CoV in Vero 76 cells, some of which exhibited moderate activity.

Keywords: antiviral activity, nucleoside analogues, SARS-CoV

Introduction

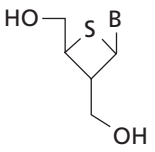
Severe acute respiratory syndrome (SARS) is a new form of non-typical pneumonia, which is caused by a new member of the *coronaviridae* family, the SARS-coronavirus (SARS-CoV; Drosten *et al.,* 2003; Ksiazek *et al.,* 2003; Peiris *et al.,* 2003; Poutanen *et al.,* 2003). Common symptoms of the infectious disease include fever (a measured temperature of more than 100.4°F [38°C]) that may be accompanied by chills, headache, malaise, body aches, dry and non-productive cough and dyspnea. This febrile respiratory illness first originated in the Guangdong Province of Southern China and Hong Kong in late 2002, and then rapidly spread to over 32 other countries or regions in five continents. This disease infected approximately 8,459 patients and resulted in over 800 deaths (Cumulative number of reported probable cases of SARS as of June 23, 2003 [homepage on internet] Geneva, Switzerland: World Health Organization [last accessed 30 October 2006]. Available from: http://www.who.int/csr/sars/coutry/2003_06_23/en/).
SARS has thus become an emerging serious and contagious illness of the 21st century which, in turn, has created a potentially serious health crisis and economic disruption worldwide (Lee *et al.,* 2003).

To date, there are no approved or universally recommended therapies for SARS. Therefore, intensive efforts have been made throughout the world to discover clinically effective antiviral agents to combat any future outbreaks. Although corticosteroids, antibiotics and antiviral agents have been used empirically for the treatment of this disease, these agents have not demonstrated any clinical efficacy (Wenzel *et al.,* 2003). A synthetic nucleoside, ribavirin, has been studied in combination with corticosteroids and interferon-α for the treatment of SARS (Peiris *et al.,* 2003; Koren *et al.,* 2003; Morgenstern *et al.,* 2005). However, ribavirin has been shown to be only weakly active *in vitro* against SARS-CoV, and to even enhance and prolong viral replication in mice (Barnard *et al.,* 2006). Other agents such as isatine derivatives (Chen *et al.,* 2005; Wu *et al.,* 2005), small interfering RNA (Wu *et al.,* 2005), glycyrrhizic acid derivatives (Hoever *et al.,* 2005), peptide inhibitors (Wu *et al.,* 2004; Zhang *et al.,* 2006) and natural compounds like Chinese medical herb extracts (Xiao *et al.,* 2003; Zhong *et al.,* 2003; Li *et al.,* 2005) have been reported as effective SARS-CoV inhibitors. Since ribavirin has empirically been studied in combination therapy for the treatment of SARS, it was of interest to evaluate the biological activity of the nucleoside library from our laboratory against SARS-CoV.

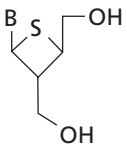
The nucleoside analogues listed (Figure 1) were synthesized as described elsewhere (Choo *et al.,* 2006; Gadthula *et al.,* 2005; Chen *et al.,* 2004; Chun *et al.,* 2000; Song *et al.,* 2001; Chu *et al.,* 1991; Liang *et al.,* 1997; Sureyya *et al.,* 2001). For the synthesis of compound **3**, experimental procedure and analysis data are provided as supplemental information (see additional file).

Different classes of synthesized nucleoside analogues in our library were evaluated for their inhibitory activity

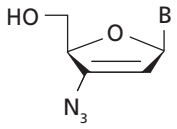
Figure 1. Library of compounds evaluated for their anti-SARS-CoV activity



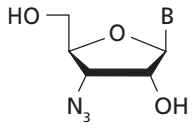
B = thymine (**4**)
uracil (**5**)
adenine (**6**)
inosine (**7**)
guanine (**8**)



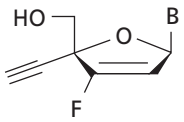
B = uracil (**9**)
cytosine (**10**)
adenine (**11**)
inosine (**12**)
guanine (**13**)



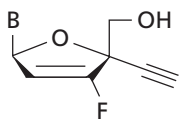
B = thymine (**14**)
cytosine (**15**)
adenine (**16**)
guanine (**17**)



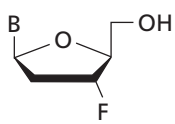
B = thymine (**18**)
adenine (**19**)
guanine (**20**)



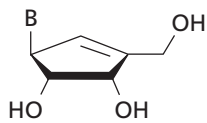
B = cytosine (**21**)
adenine (**22**)
thymine (**23**)



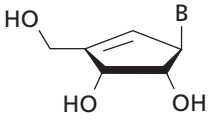
B = adenine (**24**)
thymine (**25**)
cytosine (**26**)



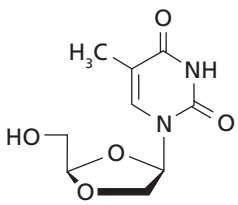
B = 5-C1-uracil (**27**)
thymine (**28**)
cytosine (**29**)
uracil (**30**)
adenine (**31**)



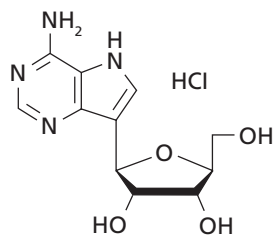
B = thymine (**32**)
inosine (**33**)



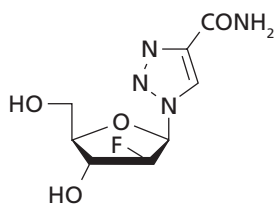
B = thymine (**34**)
N-OH-cytosine (**35**)
adenine (**36**)
inosine (**37**)



DOT (**38**)



39



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CoV, coronavirus; SARS, severe acute respiratory syndrome.

against SARS-CoV *in vitro* in African green monkey kidney (Vero 76) cells. The median antiviral potency and growth inhibition of the nucleoside analogues were expressed as 50% viral inhibitory concentration (EC_{50}) and cytotoxic concentration (IC_{50}), respectively. The selectivity index (SI) was calculated using the formula: $SI=IC_{50}/EC_{50}$. Alferon (interferon) N, provided by David Strayer (Hemispherx Biopharma, Philadelphia, PA, USA) was used as a positive control and potently inhibited SARS-CoV replication in cell culture with an EC_{50}=1,000 IU and displayed no toxicity up to 100,000 IU. Four-membered ring D-(**4–8**) and L-(**9–13**) thionucleosides were evaluated for their anti-SARS-CoV activity in neutral red and visual assays (Table 1) as described previously (Barnard *et al.*, 2006). Among these compounds, the D-isomer of thymine analogue (**4**) exhibited good anti-SARS-CoV activity (EC_{50}=20 μM) without any toxicity at dosages up to 100 μM, which was the highest evaluated. The L-cytosine analogue (**10**) was also found to be active (EC_{50}=20 μM) in a neutral red assay, but toxicity (IC_{50}=20

Table 1. Inhibition of the SARS-CoV replication in African green monkey kidney (Vero 76) cells by nucleoside analogues

Nucleoside	Assay	EC_{50}, μM	IC_{50}, μM	SI*	Reference†
4	Visual	20	>100	>5	Choo *et al*.
	NR	20	>100	>5	
5	Visual	>100	>100	0	Choo *et al*.
	NR	>100	>100	-	
6	Visual	>100	>100	0	Choo *et al*.
	NR	>100	>100	0	
7	Visual	>100	>100	0	Choo *et al*.
	NR	>100	>100	0	
8	Visual	>100	>100	0	Choo *et al*.
	NR	>100	50	0	
9	Visual	>100	>100	0	Choo *et al*.
	NR	>100	40	0	
10	Visual	>100	100	0	Choo *et al*.
	NR	20	20	1	
11	Visual	>100	>100	0	Choo *et al*.
	NR	>100	40	0	
12	Visual	>100	>100	0	Choo *et al*.
	NR	>100	40	0	
13	Visual	>100	>100	0	Choo *et al*.
	NR	>100	40	0	
14	NR	10.3	16.1	1.6	Gadthula *et al*.
15	NR	>100	18	0	Gadthula *et al*.
16	Visual	52	52	1	Gadthula *et al*.
	NR	72	17	0	
17	Visual	10	>10	0	Gadthula *et al*.
	NR	50	52	1	
18	Visual	>100	>100	0	Gadthula *et al*.
	NR	>100	>100	0	
19	Visual	>100	3.2	0	Gadthula *et al*.
	NR	11.5	3.5	0	
20	Visual	>100	>100	0	Gadthula *et al*.
	NR	>100	>100	0	
21	NR	22.2	>100	>4.5	Chen *et al*.
22	NR	11.1	14.5	1.3	Chen *et al*.
23	NR	>100	>100	0	Chen *et al*.
24	NR	11.9	25.6	2.2	Chen *et al*.
25	NR	>100	21	0	Chen *et al*.
26	NR	>100	75	0	Chen *et al*.
27	NR	<10	28	2.8	Chun *et al*.
28	NR	>100	>100	0	Chun *et al*.
29	NR	>100	>100	0	Chun *et al*.
30	NR	>100	>100	0	Chun *et al*.
31	NR	>100	41	0	Chun *et al*.
3	NR	65	>100	1.5	
32	NR	>100	>100	0	Song *et al*.
33	NR	>100	>100	0	Song *et al*.
34	NR	>100	>100	0	Song *et al*.
35	NR	>100	<10	0	Song *et al*.
36	NR	>100	<10	0	Song *et al*.
37	NR	>100	>100	0	Song *et al*.
DOT (38)	NR	24.6	>100	>4.1	Chu *et al*.
39	NR	28.6	18.8	0	Liang *et al*.
40	NR	76.8	>100	>1.3	Sureyya *et al*.
Alferon N‡		1,000 IU	100,000 IU	100	–

*Selectivity index (SI)=cytotoxic concentration (IC_{50})/50% viral-inhibitory concentration (EC_{50}). †References of compound's chemical synthesis. ‡Positive control. NR, neutral red.

μM) was also detected with this compound. The other analogues in these series did not show any significant antiviral activity.

The 3′-azido-2′,3′-unsaturated thymine analogue (**14**) showed good anti-SARS-CoV activity (EC_{50}=10.3 μM) but with significant toxicity. The adenine analogue (**16**) exhibited moderate antiviral activity in both the visual and neutral red assays (EC_{50}=52 and 72 μM, respectively), but it also exhibited some cytotoxicity. The guanine analogue (**17**) also showed good anti-SARS-CoV activity (EC_{50}=10 μM) in the visual assay with accompanying toxicity; however, it was less active (EC_{50}=50 μM) in the neutral red assay and toxicity was once again observed (IC_{50}=52 μM).

β-D-3′-Azido-3′-deoxyribo-furanosyladenine (**19**) exhibited moderate antiviral activity (EC_{50}=11.5 μM) by neutral red assay. It also displayed significant toxicity. The other compounds did not exhibit any significant antiviral activity up to 100 μM.

The 3′-fluoro-2′,3′-dideoxy-2′,3′-didehydro-4′-ethynyl-D-and L-furanosyl nucleosides (**21–26**) were evaluated against SARS-CoV activity in a neutral red assay. Among these series, the D-cytosine analogue (**21**) displayed moderate anti-SARS activity (EC_{50}=22.2 μM) without any toxicity at the highest concentration tested, whereas its L-isomer (**26**) did not show any antiviral activity. In the same series, both D-and L-adenine derivatives (**22** and **24**) showed moderate antiviral activity (EC_{50}=11.1 and 11.9 μM, respectively); however, they were also toxic. The other compounds (**23** and **25**) in the same series did not show any activity. A series of 2′,3′-dideoxy-3′-fluoro-L-ribonucleosides (**27–31**) were evaluated, among which only 5-chloro 2′,3′-dideoxy-3′-fluoro-β-L-uridine (**27**) showed moderate antiviral activity (EC_{50} <10 μM), but with significant toxicity. None of the other series exhibited any SARS-CoV activity. The D- and L-cyclopentenyl carbocyclic nucleosides (**3** and **32–37**) were evaluated, among which only **3** exhibited a weak inhibitory activity (EC_{50}=65 μM) without any toxicity.

The other carbocyclic analogues did not show any efficacy against the SARS-CoV. However, it was observed that dioxalane-thymine (DOT; **38**) showed moderate antiviral activity (EC_{50}=24.6 μM) without significant cytotoxicity. Interestingly, the C-nucleoside, 4-amino-7-(β-L-ribofuranosylpyrrolo[3,2-D]pyrimidine hydrochloride (**39**) inhibited SARS-CoV replication (EC_{50}=28.6 μM), but was also cytotoxic. Compound **40** exhibited weak antiviral activity (EC_{50} 76.8 μM) without any cytotoxicity.

In summary, we have tested several classes of nucleoside analogues against SARS-CoV *in vitro* from which several nucleoside analogues have been found to exhibit moderate antiviral activity. However, no clear structure-activity relationships have emerged.

Acknowledgments

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Additonal files

The additional file 'Synthesis of compound **(2)** and **(3)** can be accessed via the Volume 17 Issue 5 contents page of *Antiviral Chemistry and Chemotherapy*, which can be found at www.intmedpress.com (by clicking on 'Antiviral Chemistry and Chemotherapy' then Journal PDFs).

References

Balakrishna Pai S, Liu S-H, Zhu Y-L, Chu CK & Cheng Y-C (1996) Inhibition of hepatitis B virus by a novel L-nucleoside, 2′-fluoro-5- methyl-β-L-arabinofuranosyl uracil. *Antimicrobial Agents & Chemotherapy* **40:**380–386.

Barnard DL, Day CW, Bailey K, Heiner M, Montgomery R, Lauridsen L, Winslow S, Hoopes J, Li JK, Lee J, Carson DA, Cottam HB & Sidwell RW (2006) Enhancement of the infectivity of SARS-CoV in BALB/c mice by IMP dehydrogenase inhibitors, including ribavirin. *Antiviral Research* **71:** 53–63.

Chen LR, Wang YC, Lin YW, Chou SY, Chen SF, Liu LT, Wu YT, Kuo CJ, Chen TSS, & Juang SH (2005) Synthesis and evaluation of isatin derivatives as effective SARS coronavirus 3CL protease inhibitors. *Bioorganic Medicinal Chemistry Letters* **15:**3058–3062.

Chen X, Zhou W, Schinazi RF & Chu CK (2004) Synthesis of 3′-fluoro-2′,3′-dideoxy-2′,3′-didehydro-4′-ethynyl-D- and -L-furanosyl nucleosides. *Journal of Organic Chemistry* **69:**6034–6041.

Choo H, Chen X, Yadav V, Wang J, Schinazi RF & Chu CK (2006) Synthesis and anti-HIV activity of D- and L-thietanose nucleosides. *Journal of Medicinal Chemistry* **49:**1635–1647.

Chun BK, Schinazi RF, Cheng YC & Chu CK (2000) Synthesis of 2′,3′-dideoxy-3′-fluoro-L-ribonucleosides as potential antiviral agents from D—sorbitol *Carbohydrate Research* **328:**49–59.

Drosten C, Gunther S, Preiser W, Van der Werf S, Brodt HR, Becker S, Rabenau H, Panning M, Kolesnikova L, Fouchier RA, Berger A, Burguiere AM, Cinatl J, Eickmann M, Escriou N, Grywna K, Kramme S, Manuguerra JC, Muller S, Rickerts V, Sturmer M, Vieth S, Klenk HD, Osterhaus AD, Schmitz H & Doerr HW (2003) Identification of a novel coronavirus in patients with Severe Acute Respiratory Syndrome. *The New England Journal of Medicine* **348:**1967–1976.

Gadthula S, Schinazi RF & Chu CK (2005) Synthesis and anti-HIV activity of β-D-3′-azido-2′,3′-unsaturated nucleosides and β–D-3′-azido-3′-deoxyribofuranosyl nucleosides. *Nucleosides Nucleotides & Nucleic Acids* **24:**1707–1727.

Hoever G, Baltina L, Michaelis M, Kondratenko Baltina L, Tolstikov GA, Doerr HW & Cinatl Jr. J (2005) Antiviral activity of glycyrrhizic acid derivatives against SARS-Coronavirus. *Journal of Medicinal Chemistry* **48:**1256–1259.

Koren G, King S, Knowles S & Phillips E (2003) Ribavirin in the treatment of SARS: A new trick for an old drug? *Canadian Medical Association Journal* **168:**1289–1292.

Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, Tong S, Urbani C, Comer JA, Lim W, Rollin PE, Dowell SF, Ling

AE, Humphrey CD, Shieh WJ, Guarner J, Paddock CD, Rota P, Fields B, DeRisi J, Yang JY, Cox N, Hughes JM, LeDuc JW, Bellini WJ & Anderson LJ (2003) A novel coronavirus associated with Severe Acute Respiratory Syndrome. *The New England Journal of Medicine.* **348:**1953–1966.

Lee N, Hui D, Wu A, Chan P, Cameron P, Joynt GM, Ahuja A, Yung MY, Leung CB, To KF, Lui SF, Szeto CC, Chung S & Sung JJ (2003) A Major Outbreak of Severe Acute Respiratory Syndrome in Hong Kong. *The New England Journal of Medicine.* **348:**1986–1994.

Liang C, Ma T, Cooperwood JS, Du J & Chu CK (1997) Synthesis of L-ribofuranosyl *C*-nucleosides *Carbohydrate Research* **303:**33–38.

Morgenstern B, Michaelis M, Baer PC, Doerr HW & Cinatl Jr. (2005) Ribavirin and interferon-(synergistically inhibit SARS-associated coronavirus replication in animal and human cell lines. *Biochemical & Biophysical Research Communications* **326:**905–908.

Peiris JS, Lai ST, Poon LL, Guan Y, Yam LY, Lim W, Nicholls J, Yee WK, Yan WW, Cheung MT, Cheng VC, Chan KH, Tsang DN, Yung RW, Ng TK & Yuen KY (2003) Coronavirus as a possible cause of severe acute respiratory syndrome. *The Lancet* **361:**1319–1325.

Peiris JS, Chu CM, Cheng VC, Chan KS, Hung IF, Poon LL, Law KI, Tang BS, Hon TY, Chan CS, Chan KH, Ng JS, Zheng BJ, Ng WL, Lai RW, Guan Y & Yuen KY (2003) Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *The Lancet* **361:**1767–1772.

Poutanen SM, Low DE, Henry B, Finkelstein S, Rose D, Green K, Tellier R, Draker R, Adachi D, Ayers M, Chan AK, Skowronski DM, Salit I, Simor AE, Slutsky AS, Doyle PW, Krajden M, Petric M, Brunham RC & McGeer AJ (2003) Identification of Severe Acute Respiratory Syndrome in Canada. *The New England Journal of Medicine.* **348:**1995–2005.

Song GY, Paul V, Choo H, Morrey J, Sidwell RW, Schinazi RF & Chu CK (2001) Enantiomeric synthesis of D- and L-cyclopentenyl nucleosides and their antiviral activity against HIV and West Nile Virus. *Journal of Medicinal Chemistry* **44:**3985–3993.

Sureyya O & Chu CK*Z* (2001) Synthesis and antiviral activity of 2′-deoxy-2′-fluoro-L-arabinofuranosyl 1,2,3-triazole derivatives. *Naturforsch. B* **56:**804–811.

Wenzel RP & Edmond MB (2003) Managing SARS amidst uncertainty. *The New England Journal of Medicine.* **348:**1947–1948.

Wu C-J, Huang H-W, Liu C-Y, Hong C-F & Chan Y-L (2005) Inhibition of SARS-CoV replication by siRNA. *Antiviral Research* **65**:45–48.

Xiao Z, Li Y, Chen R, Li S, Zhong S & Zhong N (2003) A retrospective study of 78 patients with severe acute respiratory syndrome. *Chinese Medical Journal* **116:**805–810

Zhang H-Z, Zhang H, Kemnitzer W, Tseng B, Cinatl Jr J, Michaelis M, Doerr HW & Cai SX (2006) Design and synthesis of dipeptidyl glutaminyl fluoromethyl ketones as potent Severe Acute Respiratory Syndrome Coronovirus (SARS-CoV) Inhibitors. *Journal of Medicinal Chemistry* **49:**1198–1201.

Zhong NS & Zeng GQ (2003) Our strategies for fighting Severe Acute Respiratory Syndrome (SARS) *American Journal of Respiratory & Critical Care Medicine* **168:**7–9.

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