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Synthesis and in vivo influenza virus-inhibitory effect of ester prodrug of 4-guanidino-7-*O*-methyl-Neu5Ac2en

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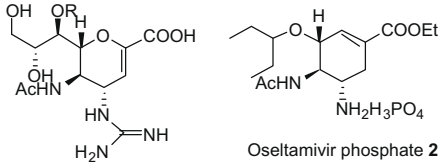
ABSTRACT

A series of ester prodrugs of 7-*O*-methyl derivative of Zanamivir (compound **3**) was synthesized and their efficacy was evaluated in an influenza infected mice model by intranasal administration. Compound **7c** (**CS-8958**), octanoyl ester prodrug of the C-9 alcohol of compound **3**, was found to be much longer-acting than Zanamivir. Furthermore, the in vivo efficacies of compounds **12a**, **12b**, and **12c**, the linear alkyl ester prodrug of the carboxylic acid, were comparable to that exerted by compound **7c**.

Influenza is a respiratory infection associated with significant morbidity in the general population and mortality in elderly and high-risk patients.[1] Influenza A and B viruses have two major membrane-associated surface glycoproteins, hemagglutinin and sialidase, which are both essential for infectivity.[2] Sialidase cleaves terminal sialic acid residues from glycoconjugates and promotes the release of newly formed virus particles from infected cells. Recently, several potent and specific inhibitors of this enzyme have been developed,[3] and two (Zanamivir **1**[4] and Oseltamivir phosphate **2**[5]) have been approved for human use (Fig. 1). Unlike amantadine[6] and rimantadine, which target the M2 protein of influenza A viruses, these drugs inhibit replication of both influenza A and B viruses. Zanamivir (Relenza™) is delivered by inhalation because of its low oral bioavailability, whereas Oseltamivir phosphate (Tamiflu™) is administered orally. Early treatment with either drug reduces the severity and duration of influenza symptoms and associated complications. Both agents are effective for chemprophylaxis. Because of a broader antiviral spectrum, better tolerance, and less potential for the emergence of resistance than is seen with the M2 inhibitors, the sialidase inhibitors represent an important advance in the treatment of influenza.



1 R : H (Zanamivir)
3 R : Me

Oseltamivir phosphate **2**

Figure 1.

In the course of our study on the synthesis of sialidase inhibitors, we focused our attention on a novel sialidase inhibitor administered by inhalation because an inhaled compound has the advantage of a rapid onset of action, very few side effects and the ability to use a lower dose than that administered orally. However, Zanamivir is administered twice daily in spite of the inhaled administration because of its low maintenance of activity. Therefore, our main goal is to develop a new inhaled sialidase inhibitor which has longer lasting activity than that of Zanamivir. Thus, it would have slower clearance of the drug from the target tissue and the advantage of a single inhalation for treatment.

We recently reported that the replacement of the 7-OH moiety of Zanamivir by small lipophilic groups (F, N_3, OMe, OEt) resulted in an enhancement of anti-viral activity in an influenza A virus plaque reduction assay.[7] In particular, compound **3**[8] effectively inhib-

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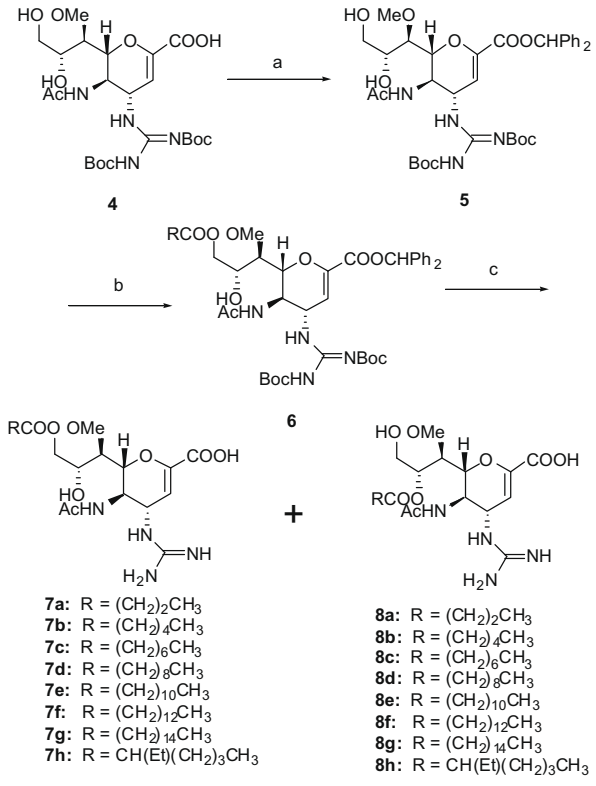
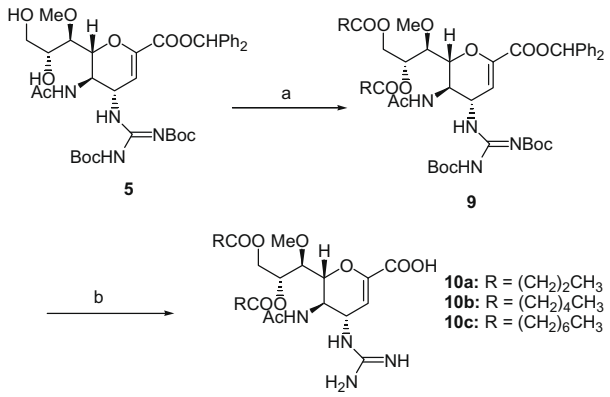
ited the enzymatic activities of several influenza A (H1N1, H2N2, H3N2) and B viruses including an avian influenza H5N1 virus and Tamiflu resistant virus. The IC50s against their influenza virus replication were better than that of Zanamivir. However, the efficacy of intranasally administered compound **3** showed the only slight increase (2–3-fold) compared to that of Zanamivir in an influenza infected mice model.[9] Therefore, in our attempt to synthesize a more potent sialidase inhibitor than compound **3** in vivo, we designed the ester prodrugs of compound **3** on the basis of our hypothesis that a lipophilic ester prodrug could suppress its clearance from lung tissue to plasma and the active form is hydrolyzed and retain in the lung for a long time. Herein, we report that the synthesis of the ester prodrug of compound **3** and their in vivo evaluations in an infected mice model.

The synthetic pathway to the ester prodrug at the C-9 position of compound **3** is shown in Scheme 1. Compound **4**[10] was treated with diphenyl diazomethane in THF to provide the diphenyl ester **5** in a 85% yield. Subsequently, the C-9 alcohol of compound **5** was selectively acylated with the appropriate linear alkyl carboxylic acid chlorides or 2-ethylhexanoyl chloride in the presence of TEA, followed by deprotection with CF_3COOH–CH_2Cl_2 gave the target compounds **7a–7g**, respectively, accompanied by the unseparable esters **8a–8g** at the C-8 position (**7**:**8** = 10:1–20:1) after purification by reverse phase chromatography.

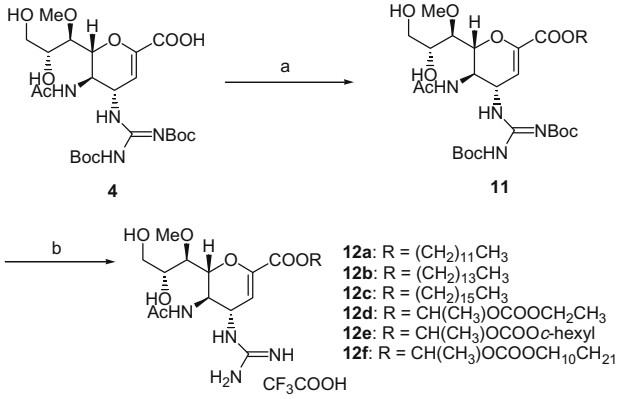
The diester formation at the C-8, 9 positions and the deprotection with CF_3COOH–CH_2Cl_2 gave compounds **10a–10c** as shown in Scheme 2. The synthetic route for the ester prodrug of the carboxylic acid of compound **3** is shown in Scheme 3. Compound **4** was treated with 1.1 equiv of KOH to afford the potassium salt of the carboxylic acid of compound **4**. The potassium salt was reacted with the appropriate linear alkyl bromides or carbonic acid alkyl ester 1-iodo-ethyl esters[11] to provide the corresponding ester **11**, respectively. Compounds **12a–12c** and **12d–12f** were obtained as trifluoroacetic acid salts by deprotection with CF_3COOH–CH_2Cl_2.



Scheme 1. Reagents: (a) Ph_2CN_2, THF (85%); (b) RCOCl (1.1 equiv), TEA (1.2 equiv), CH_2Cl_2, (50–70%); (c) CF_3COOH–CH_2Cl_2 (60–70%).



Scheme 2. Reagents: (a) RCOCl (3.0 equiv), TEA (3.0 equiv), 4-*N*,*N*-dimethylaminopyridine (3.0 equiv), CH_2Cl_2 (80–90%); (b) CF_3COOH–Cl_2Cl_2 (60–70%).



Scheme 3. Reagents and conditions: (a) (i) KOH (1.1 equiv), MeOH, (ii) **12a–12c**: RBr (1.1 equiv), DMF 50 °C, 4 h (50–80%); **12d–12f**: ICH(CH$_3$)OCOOR (1.5 equiv), DMF, 20 °C, 1 h (50–60%); (b) CF_3COOH–Cl_2Cl_2 (60–70%).

The efficacy of the intranasally administered ester prodrugs of compound **3** was evaluated on the basis of the survival rate measured for 20 days post infection in treated infected mice relative to untreated infected (control) mice. These prodrugs showed very weak or no inhibition of sialidase activity. They were administered intranasally 4 h before and 4 h, 17 h after the infection. All of the linear alkyl esters prepared at the C-9 alcohol position had longer lasting efficacy than Zanamivir and compound **3** (data not shown), as shown in Table 1. Their prolonged in vivo activities were supposed to be due to slower clearance of the active form from the lung. Thus, a high concentration of compound **3**, the active form of the esters, could be presumably maintained for a long time at the site of virus

Table 1
Survival rates of infected mice[a] intranasally administered compounds **7a–7g**[b] and Zanamivir[b]

	No. of survivors/total no. of mice	
	At 8 days after infection	At 8 days after infection
Zenamivir	2/12	0/12
7a	2/12	0/12
7b	3/11	1/11
7c	9/12	9/12
7d	10/11	6/11
7e	6/11	2/11
7f	8/12	6/12
7g	8/11	4/11

[a] Twelve mice were infected with influenza A/PR/8/34 (H1N1) virus.
[b] Compounds **7a–7g** and Zanamivir were intranasally administered at doses of 0.3 μmol/kg 4 h before and 4 h, 17 h after the infection.

Table 2
Survival rates of infected mice[a] intranasally administered compounds **7c**[b], **12a**,[b] **12b**,[b] **12c**,[b] and Zanamivir[b]

	No. of survivors/total no. of mice	
	At 8 days after infection	At 8 days after infection
Zenamivir	3/12	0/12
7c	12/12	12/12
12a	12/12	11/12
12b	12/12	10/12
12c	12/12	10/12

[a] Twelve mice were infected with influenza A/PR/8/34 (H1N1) virus.
[b] Compounds **7c**, **12a**, **12b**, **12c**, and Zanamivir were intranasally administered at doses of 0.3 μmol/kg 4 h before and 4 h, 17 h after the infection.

replication compared to Zanamivir and compound **3**. Their pharmacokinetic study is currently in progress. However, the alkyl branched ester **7h** resulted in a decreased activity[12] compared to the linear alkyl esters. The reason for this remains unclear: it could be due to the resistance of enzymatic hydrolysis of the prodrugs. Among the prodrugs of the C-9 position compound**7c** (**CS-8958**), octanoyl ester prodrug of compound **3**, was found to be most effective. On the other hand, the diacyl esters[13] at the C-8, 9 positions **10a**, **10b**, and **10c** showed a loss of activity compared to the former prodrugs. The evaluation of the linear alkyl esters of the carboxylic acid demonstrated that compounds **12a**, **12b**, and **12c** were much more potent than Zanamivir and were comparable to compound **7c** (Table 2). The shorter carbon chain in length than that of compound **12a** resulted in decreased activity. A series of 1-alkoxy carbonyloxy ethylester prodrugs[14] **12d**, **12e**, and **12f** were less effective than the linear alkyl ester prodrugs at the carboxylic acid.

In summary, a series of the ester prodrugs of compound **3** was synthesized and evaluated in a mouse model by intranasal administration. The linear alkyl esters at the C-9 position showed long lasting activity relative to Zanamivir and compound **3**. Among them compound **7c** (**CS-8958**), octanoyl ester prodrug at the C-9 position of **3**, was the most potent. Furthermore, the long efficacies of compounds **12a**, **12b**, and **12c**, the linear alkyl ester of carboxylic acid, were comparable to that of compound **7c** (**CS-8958**). In contrast to the currently available drugs, it is expected that a single inhalation of the prodrugs such as compound **7c** (**CS-8958**) would provide effective treatment for influenza virus infections. A phase III clinical trial by a single inhalation of **CS-8958** is currently underway to determine its potential as a LANI (long-acting NA inhibitor) for the treatment of seasonal influenza. Further in vivo evaluation of the ester prodrugs of compound **3**,[15] including a pharmacokinetic investigation, is currently in progress and the obtained results will be reported in due course.

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8. Sialidase inhibitory and plaque reduction activities of compound **3** against various influenza virus strains. IC_{50} (nM) sialidase inhibitory and plaque reduction activities of compounds were determined by previously reported methods.

Virus	Subtrain	Sialidase		Virus replication	
		3	Zanamivir	**3**	Zanamivir
A/PR/8/34	H1N1	5.97	3.62	2.2	11
A/Yamagata/32/89	H1N1	3.42	2.55	2.4	10
A/Singapore/1/57	H2N2	11.4	3.66	0.43	0.79
A/Aichi/2/68	H3N2	16.8	7.33	1.3	1.6
A/Kitakyushu/159/93	H3N2	23.5	9.30	1.7	3.3
B/Mie/1/93	B	31.3	12.0	4.2	9.2
A/R(duck/mongolia/54/01-duck/mongolia/47/01)	H5N1	4.54	2.99	0.34	0.79
A/Yokohama/67/2006(Tamiflu resistant virus)	H1N1	5.62	3.05	NT	NT

9. Compound **3** and Zanamivir were administered intranasally at doses of. 0.2 μmol/kg 4 h before and 4 h, 17 h after the infection in mice. Compound**3** was more effective than Zanamivir (2/10 survived in the **3** treated infected group while there was no survivor in the case of Zanamivir at 20 days after infection).
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12. Survival rates of infected mice[a] intranasally administered compounds **7h**[b] and Zanamivir[b]

	No.of survivors/total No.of mice	
	At 8 days after infection	At 20 days after infection
Zanamivir	3/10	0/10
7h	4/10	0/10

[a] Ten mice were infected with influenza A/PR/8/34 (H1N1) virus.
[b] Compounds **7h** and Zanamivir were intranasally administered at doses of 0.3 μmol/kg 4 h before and 4 h, 17 h after the infection.

13. Compounds **10a**–**10c** were administered intranasally at doses of 0.3 μmol/kg 4 h before and 4 h, 17 h after the infection in mice. There was no survivor in the **10a**, **10b**, and **10c**-treated infected groups at 20 days after infection.
14. Compounds **12d**, **12e**,**12f**, and Zanamivir were administered intranasally at doses of 0.3 μmol/kg 4 h before and 4 h, 17 h after the infection in mice. Their efficacies were found to be almost same as that of Zanamivir (there were no survivors in the **12d**, **12e**, **12f**, and Zanamivir-treated infected groups at 9 days after infection).
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