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Anti-influenza Virus Activities of 2-Alkoxyimino-*N*-(2-isoxazolin-3-ylmethyl)acetamides

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Abstract—A series of 2-alkoxyimino-*N*-(2-isoxazolin-3-ylmethyl)acetamides and related compounds were synthesized and their antiviral activities against human influenza A virus were assessed. Studies of the structure–activity relationships revealed the strongest antiviral activity when position-5 of the isoxazoline ring was substituted with a *tert*-butyl group. When the alkoxyimino moiety was substituted with a methyl, ethyl, isopropyl or allyl group, good antiviral activity was obtained. Among the geometrical isomers at the oxime moiety, the *E*-isomers were more active than the *Z*-isomers. Among the compounds examined, (*E*)-2-allyloxyimino-2-cyano-*N*-(5-*tert*-butyl-2-isoxazolin-3-ylmethyl)acetamide (**1j**) was the most active inhibitor with an EC_{50} of 3 μg/mL in vitro.

Introduction

Influenza is caused by a group of enveloped RNA viruses that belong to the *Orthomyxoviridae* family. There are three types of influenza viruses: A, B, and C. The first two types are responsible for annual epidemics or pandemic outbreaks, while influenza C is endemic. The most severe pandemic human influenza outbreak of recent times occurred in 1918–1919, when 'Spanish' influenza killed more than 20 million people worldwide.[1] Vaccination provides limited protection based on the ability to predict the exact strains of influenza virus that will predominate a year in advance of the influenza season. The antiviral agents, Amantadine and Rimantadine, are the only two drugs approved for the prophylactic treatment of human influenza A virus infection.[2] Recently, a novel series of neuraminidase inhibitors, exemplified by Zanamivir and Oseltamivir, have emerged.[3] These agents are effective against both type A and B human influenza infections and their success has spurred a new wave of research in fighting the infection.

To discover novel antiviral compounds with different pharmacophores from currently used antiviral agents, many compounds from our institute's chemical library were randomly screened. Certain 2-alkoxyimino-*N*-(2-isoxazolin-3-ylmethyl)acetamides (**1**, Fig. 1) were found to show antiviral activities against human influenza virus. Since potent antiviral activities of isoxazolines were observed, a series of isoxazoline derivatives were synthesized with various substituents to find the optimal structure exhibiting the most potent antiviral activity against human influenza virus.

Chemistry

The 3-aminomethyl-2-isoxazolines **6a–e** were prepared as outlined in Scheme 1. The 3-hydroxymethyl-2-isoxazolines **4** were prepared by 1,3-dipolar cycloaddition of the chloro oxime **2** and the alkenes followed by reduction with sodium borohydride. The alcohols **4** were converted via the modified Gabriel method to their amines **6a–e** by successive treatment with thionyl chloride, potassium phthalimide and hydrazine.[4]

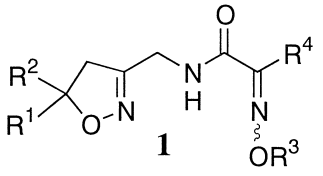

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Figure 1.

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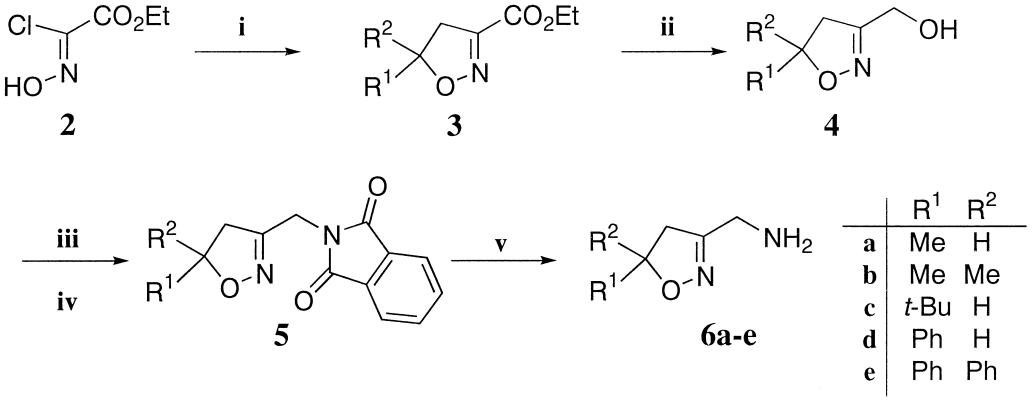
The 2-cyano-*N*-(2-isoxazolin-3-ylmethyl)acetamides **1a–n** were prepared as outlined in Scheme 2. The *E*-isomer esters **8** were prepared by alkylation of the oxime **7**.[4] The *Z*-isomer ester **11** was prepared by amidation of the dicarboxylate **10** followed by dehydration.[5] The isoxazoline derivatives **1a–m** were prepared by reaction of the esters (**8** or **11**) and the amines **6a–e**.

The 2-allyloxyimono-*N*-(5-*tert*-butyl-2-isoxazolin-3-ylmethyl)acetamides **1n–p** were prepared as outlined in Scheme 3. The 2-hydroxyimonoacetates **12** were synthesized as described in the literature.[6–8] The esters **13** were prepared by alkylation of the oximes **12**. The isoxazoline derivatives **1n–p** were prepared by reaction of the esters **13** and the amines **6c**.

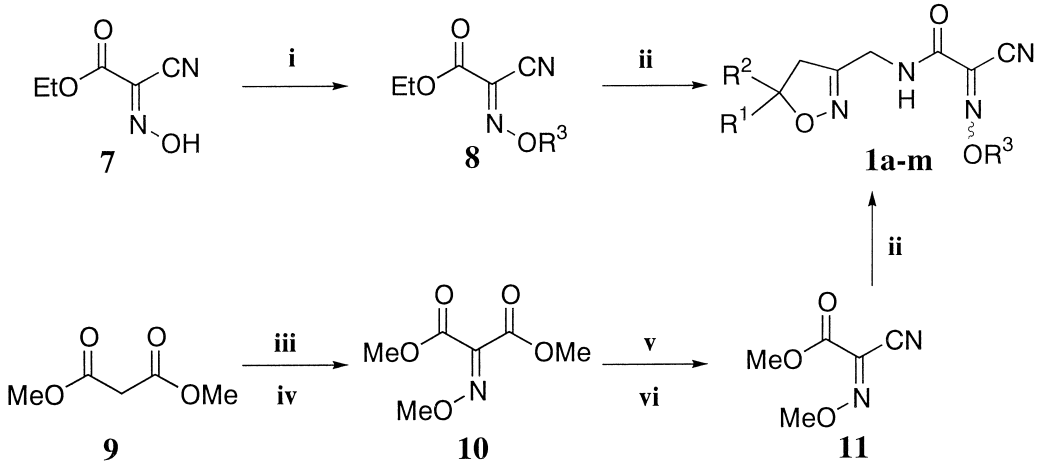
The regio isomer **15** was prepared as outlined in Scheme 4. The amide **14** was prepared by reaction of the ester **8a** and allylamine. Compound **15** was prepared by 1,3-dipolar cycloaddition of *tert*-butyl oxime chloride[9] and the amide **14**. The isoxazole analogue **16** was prepared as described for the synthesis of **1**. Compounds **17–21** were synthesized as described in the literature.[4,10,11]

Results and Discussion

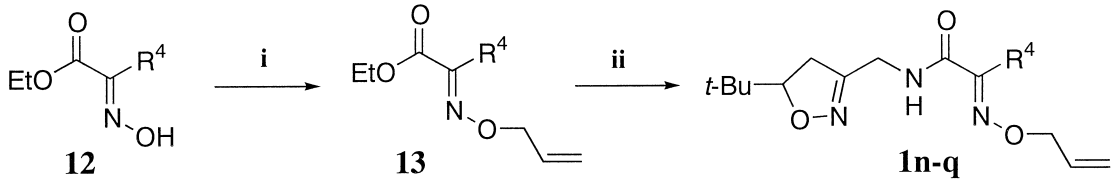
Antiviral activity and cytotoxicity of the 2-alkoxyimino-*N*-(5-substituted 2-isoxazolin-3-ylmethyl)acetamides and related compounds are shown in Tables 1 and 2. Antiviral activity (or cytotoxicity) is expressed as an index of



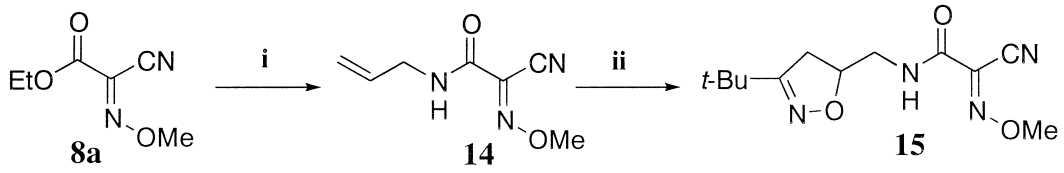
Scheme 1. Synthesis of 3-aminomethyl-2-isoxazolines (**6**). Reagents and conditions: (i) $R^1(R^2)C{=}CH_2$, $NaHCO_3$, *i*-PrOH, 40 °C 24 h; (ii) $NaBH_4$, MeOH, reflux 1 h; (iii) $SOCl_2$, PhH, rt 1 h, reflux 1 h; (iv) PhtNK, DMF, rt overnight; (v) NH_2NH_2, MeOH, 60 °C 2 h (PhtNK = potassium phthalimide).



Scheme 2. Synthesis of 2-cyano-*N*-(2-isoxazolin-3-ylmethyl)acetamides (**1a–m**). Reagents and conditions: (i) alkyl halides (Me_2SO_4), K_2CO_3, DMF, rt 5 h; (ii) amines (**6**), Et_3N, MeOH, rt overnight; (iii) $NaNO_2$, AcOH, H_2O, rt overnight; (iv) Me_2SO_4, K_2CO_3, acetone, reflux 4 h; (v) 28% NH_3 aq, MeOH, rt 3 h; (vi) $(CF_3CO)_2O$, pyridine, rt 0.5 h.



Scheme 3. Synthesis of 2-allyloxyimino-*N*-(5-*tert*-butyl-2-isoxazolin-3-ylmethyl)acetamides (**1n–q**). Reagents and conditions: (i) allyl chloride, K_2CO_3, DMF, rt 5 h; (ii) 3-aminomethyl-5-*tert*-butyl-2-isoxazoline (**6c**), 150 °C 3 h.



Scheme 4. Synthesis of the *regio* isomer **15**. Reagents and conditions: (i) allylamine, Et_3N, MeOH, rt 24 h; (ii) *t*-butyl oxime chloride, $NaHCO_3$, *i*-PrOH, rt 65 h.

+++, ++, +, or −, corresponding to 1–10, 10–100, 100, and >100 ppm EC_{50} (or CC_{50}) values, respectively. The EC_{50} of A/WSN/33 strain of influenza virus (H1N1 subtype) in cell protection assay in MDBK cells is measured in μg/mL. The CC_{50} of the compound in MDBK cells is given.[12,13]

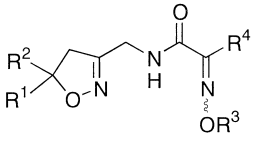
Table 1 shows some 2-alkoxyimino-*N*-(2-isoxazolin-3-ylmethyl)acetamides and their anti-influenza virus activities. When position-5 on the isoxazoline ring was substituted with a *tert*-butyl (**1c**) group, good antiviral activity was obtained. Methyl (**1a**), dimethyl (**1b**) and phenyl (**1d**) derivatives showed weak activity, whereas phenyl (**1d**) and diphenyl (**1e**) derivatives showed cytotoxicity. These results suggested that the physicochemical characteristics or steric effects of the *tert*-butyl group might lead to enhancement of the activity, although no definitive explanations for these observations have yet been obtained.

The effects of substituent (R^3) on the oxime moiety were examined. Among the compounds **1c** and **1h**–**m**, methyl (**1c**), ethyl (**1h**), isopropyl (**1i**), and allyl (**1j**) derivatives were the most active, whereas larger substituent groups (benzyl; **1l**, pyridylmethyl; **1m**) or nitrogen-containing groups (cyanomethyl, **1k**), reduced the activity. The benzyl (**1l**) derivative showed weak cytotoxicity. Among the geometrical isomers at the oxime moiety of dimethyl and *tert*-butyl derivatives, *E*-isomers (**1b** and **1c**) were more active than *Z*-isomers (**1f** and **1g**).

The effects of substituent (R^4) at position-2 on the acetamide were examined. Among the compounds (**1j** and **1n**–**q**), cyano (**1j**) and methyl (**1o**) derivatives were most active. In the case of smaller (H, **1n**) or larger (Et, **1p**; **Ph**, **1q**) substituents, the activity was decreased.

Since 2-alkoxyimino-*N*-(5-*tert*-butyl-2-isoxazolin-3-ylmethyl)acetamides were found to be favorable for the antiviral activity, the 5-*tert*-butyl-2-isoxazolin-3-ylmethyl moiety of compound **1c** was modified structurally. Table 2 shows the effects of group Z on the antiviral activity examined with various kinds of derivatives. Isomeric isoxazoline ring (**15**) or isoxazole analogues (**16**) had lower activity than the corresponding compound **1c**. Compound **17**, produced by deletion of $–CH_2–$ between the isoxazoline ring and amide moiety of compound **1c**, was inactive. Thienylmethyl (**18**), pyridylmethyl (**19**), ethylcarbamoyl (**20**) and *tert*-butoxycarbonyl (**21**) derivatives were inactive. These results

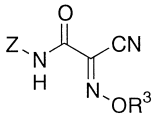
Table 1. 2-Alkoxyimino-*N*-(2-isoxazolin-3-ylmethyl)acetamides and their antiviral activities

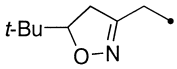
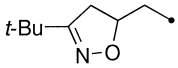
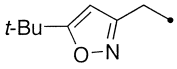
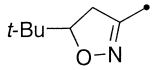
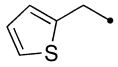
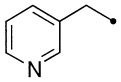


Compound no.	R^1	R^2	R^3	R^4	*E*/*Z*	Antiviral activity[a]	Cytotoxicity[a]
1a	Me	H	Me	CN	*E*	++	–
1b	Me	Me	Me	CN	*E*	++	–
1c	*t*-Bu	H	Me	CN	*E*	+++	–
1d	Ph	H	Allyl	CN	*E*	++	++
1e	Ph	Ph	Allyl	CN	*E*	ND[b]	++
1f	Me	Me	Me	CN	*Z*	–	–
1g	*t*-Bu	H	Me	CN	*Z*	++	–
1h	*t*-Bu	H	Et	CN	*E*	+++	–
1i	*t*-Bu	H	*i*-Pr	CN	*E*	+++	–
1j	*t*-Bu	H	Allyl	CN	*E*	+++	–
1k	*t*-Bu	H	CH_2CN	CN	*E*	++	–
1l	*t*-Bu	H	Benzyl	CN	*E*	++	+
1m	*t*-Bu	H	3-Pyridylmethyl	CN	*E*	–	–
1n	*t*-Bu	H	Allyl	H	*E*	+	–
1o	*t*-Bu	H	Allyl	Me	*E*	+++	–
1p	*t*-Bu	H	Allyl	Et	*E*	+	–
1q	*t*-Bu	H	Allyl	Ph	*E*	+	–

[a]Antiviral activity (or Cytotoxicity) is expressed as an index of +++, ++, or −, corresponding to 1–10, 10–100, 100, and >100 ppm EC_{50} (or CC_{50}) values, respectively. The EC_{50} of a A/WSN/33 strain of influenza virus (H1N1 subtype) in cell protection assay in MDBK cells in μ/mL. The CC_{50} of the compound in MDBK cells.
[b]ND, $>CC_{50}$.

Table 2. 2-Alkoxyiminoacetamides and their antiviral activities



Compound no.	Z	R^3	Antiviral activity[a]	Cytotoxicity[a]
1c		Me	+ + +	–
15		Me	+ +	–
16		Allyl	+ +	+
17		Me	–	–
18		Me	–	–
19		Me	–	–
20	EtNHCO	Me	–	–
21	*t*-BuOCO	Me	–	–

[a]Antiviral activity or cytotoxicity are expressed as in Table 1.

Table 3. Antiviral activities of selected compounds

Compound no.	EC_{50} (μg/mL)[a]	CC_{50} (μg/mL)[b]
1j	3	> 100
1o	6	> 100
Amantadine	10–100	> 100

[a]The EC_{50} of A/WSN/33 strain of influenza virus (H1N1 subtype) in cell protection assay in MDBK cells in μg/mL.
[b]The CC_{50} of the compound in MDBK cells.

suggested that the 2-isoxazolin-3-ylmethyl group on the amide nitrogen atom of 2-alkoxyiminoacetamides played a major role in the potent antiviral activity.

Based on the results described above, compounds **1j** and **1o** were selected for further evaluation (Table 3). Compound **1j** showed the most potent activity among this series of derivatives. It showed excellent control at a dose of 3 μg/mL, with stronger activity than Amantadine. However, these compounds showed less or no antiviral activities against influenza B and respiratory syncytial virus (unpublished data).

In conclusion, our studies indicated that these 2-alkoxyimino-*N*-(5-*tert*-butyl-2-isoxazolin-3-ylmethyl)-acetamides, a new class of antiviral agents, are highly effective against human influenza A. However, the mechanisms of action of the isoxazoline derivatives are unknown at present.

Acknowledgements

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12. Human influenza virus A/WSN/33 (H1N1) strain was grown in 10-day-old chick embryonated eggs and its infectious titer was assayed on Mardy–Darby kidney (MDBK) cells using the 50% tissue culture infectious dose ($TCID_{50}$) method.[13] Anti-influenza virus activity of each compound was determined as described previously.[13] The 50% effective dose (EC_{50}) and 50% cytotoxic dose (CC_{50}) were determined as described previously.[13]
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