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Design, Synthesis, and Anti-influenza Viral Activities of 1,3-Diarylprop-2-en-1-ones: A Novel Class of Neuraminidase Inhibitors

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A series of 1,3-diarylprop-2-en-1-one derivatives **3a-v** have been synthesized and evaluated for their ability to inhibit neuraminidase (NA). Among the prepared compounds, the less lipophilic derivative **3k** showed the most potent *in vitro* inhibitory activity against NA with an IC_{50} value of 1.5 μM.

Key words: Anti-influenza activity, Synthesis, Neuraminidase, 1,3-Diarylprop-2-en-1-ones

INTRODUCTION

The pandemic influenza virus has been identified as the cause of a widespread outbreak of febrile respiratory infection (Jamieson et al., 2009). It is particularly dangerous to the very young, the elderly and debilitated patients, and those who have suppressed immune systems are at an especially high risk to develop severe complications that lead to high morbidity and mortality rates (Hien et al., 2004). The influenza virus is an RNA virus belonging to the Orthomyxoviridae family, which is subdivided into three serologically distinct types: A, B and C. Among these, only influenza viruses A and B are responsible for the spread of seasonal flu epidemics every year (Von Itzstein, 2007).

Advances in understanding the molecular and cellular biology of influenza have led to the identification of several molecular targets to combat these pathogens. Neuraminidase (NA;[a] EC 3.2.1.18) is one of two major surface glycoproteins of both type A and B influenza viruses and is essential for viral replication *in vitro* (Air and Laver, 1989). The NA protein plays a key role not only in the release of virions from infected host cells but also in their movement through the upper respiratory tract by taking charge of catalyzing the cleavage of neuraminic acid residues (Von Itzstein, 2007). NA cleaves α-ketosidic linkages between sialic acid and the adjacent sugar residues. The removal of sialic acid lowers the viscosity of the virus particle, thus permitting the entry of the virus into epithelial cells. The replication of virus particles is blocked by the application of NA inhibitors, which have the ability to fit into the active site of the enzyme and thus inhibit the cleavage of the connection between the host cell and newly built virions (Moscona, 2005). Therefore, it is possible to block an influenza virus infection by inhibiting NA. The current anti-influenza drugs oseltamivir (Tamiflu) and zanamivir are successful examples, and both exert their antiviral effects through the inhibition of NA in the influenza A and B viruses (Kim et al., 1997). The identification of novel structures that can be potentially useful in designing new, potent, selective and less toxic anti-viral agents is still a major challenge to researchers.

In the present investigation, we began our studies by focusing on 1,3-diarylprop-2-en-1-one compounds. The class of compounds with a 1,3-diarylprop-2-en-1-one framework has been known for over a century. These natural compounds occur mainly as petal pigments and are found in variety of trees and plants, and have been found to show good inhibitory activity against the influenza virus. Recently, Grienke et al.

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isolated and investigated six candidates from the seed extract of *Alpinia katsumadai* and found an active compound against influenza virus that contains a prop-2-en-1-one moiety (Grienke et al., 2010). In a related study, Ryu et al. isolated several polyphenols, comprising chalcones, flavonoids, coumarins, and polybenzofuran responsible for NA inhibitory activity found in extracts of *Glycyrrhiza uralensis* (Ryu et al., 2010). In this article, we describe our initial optimization studies for a series of new 1,3-diarylprop-2-en-1-ones to determine their antiviral activities and identify the compound with the highest NA inhibition potency.

MATERIALS AND METHODS

General experimental procedures

^{1}H-NMR and ^{13}C-NMR were measured downfield relative to tetramethylsilane in $CDCl_3$ unless otherwise noted (value in ppm); coupling constants J are reported in hertz, and conducted on a JNM-ECA 500 spectrometer (Jeol). IR spectra (KBr) were recorded with a Bruker-Vector22 instrument (Bruker). High resonance mass spectra (HRMS) were recorded on JMS-700 (Jeol) and measured at Gyeongsang National University (Chinju). The stationary phases used for column chromatography (Silica gel 60, 70-230 mesh), and TLC plates (Silical-gel 60 F_{254}) were purchased form Merck KGaA. Spots were detected under UV radiation. All the reagent-grade chemicals were purchased from the Sigma-Aldrich.

General procedure for synthesis of 1,3-diarylprop-2-en-1-ones derivatives 3a-v

To a solution of appropriate ketone (10 mmol) and aldehyde (10 mmol) in ethanol was added aqueous sodium hydroxide (12 mmol) at 0ºC. The reaction mixture was stirred for 7-8 h at room temperature until the disappearance of the starting materials. On completion, the reaction mixture was poured into ice cold water and adjusted to pH 6 with 0.1 N HCl. The precipitate was filtered, and washed with ethanol. The crude product was subjected to purification through silica gel column chromatography (*n*-hexane-ethyl acetate = 3:1) to afford **3a-v** in 53-95% yields.

1-(Thien-2-yl)-3-(4-dimethylaminophenyl)prop-2-en-1-one (3a)

Yield 92%; IR (ν_{max}, KBr): 1636 cm^{-1} (C=O); HRMS calcd for $C_{15}H_{15}NOS$: 257.0874. Found: 257.0873; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 3.03 (6H, s), 6.67 (2H, d, J = 8.8 Hz), 7.14 (1H, dd, J = 4.8, 3.7 Hz), 7.21 (1H, d, J = 15.4 Hz), 7.53 (2H, d, J = 8.8 Hz), 7.60 (1H, dd, J = 4.8, 1.1 Hz), 7.80 (2H, m); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 40.2, 111.8, 116.3, 122.5, 128.1, 130.5, 130.9, 132.9, 145.0, 146.4, 152.1, 182.2.

1-(Thien-2-yl)-3-(2,6-difluorophenyl)prop-2-en-1-one (3b)

Yield 95%; IR (ν_{max}, KBr): 1635 cm^{-1} (C=O); HRMS calcd for $C_{13}H_{18}F_2OS$: 250.0264. Found: 250.0262; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 6.96 (2H, m), 7.18 (1H, dd, J = 4.8, 3.7 Hz), 7.32 (1H, m), 7.71 (2H, m), 7.85 (1H, dd, J = 3.7, 1.1 Hz), 7.91 (1H, d, J = 16.0 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 111.9, 127.2, 128.3, 129.9, 131.2, 132.2, 134.3, 145.4, 161.0, 163.0, 182.2.

1-(Thien-2-yl)-3-(4-fluorophenyl)prop-2-en-1-one (3c)

Yield 68%; IR (ν_{max}, KBr): 1636 cm^{-1} (C=O); HRMS calcd for $C_{13}H_9OSF$: 230.0358. Found: 230.0355; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 7.09 (2H, m), 7.16 (1H, dd, J = 4.8, 3.7 Hz), 7.32 (1H, d, J = 15.7 Hz), 7.62 (2H, m), 7.66 (1H, m), 7.79 (1H, d, J = 15.7 Hz), 7.84 (1H, m); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm); 116.0, 121.2, 128.3, 130.5, 131.0, 131.9, 134.0, 142.8, 145.5, 163.0, 181.9.

1-(Thien-2-yl)-3-(phenyl)prop-2-en-1-one (3d)

Yield 70%; IR (ν_{max}, KBr): 1635 cm^{-1} (C=O); HRMS calcd for $C_{13}H_{10}OS$: 214.0452. Found: 214.0453; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 7.18 (1H, dd, J = 4.8, 3.7 Hz), 7.41 (4H, m), 7.64 (2H, m), 7.67 (1H, dd, J = 4.8, 1.1 Hz), 7.85 (2H, m); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm); 121.6, 128.3, 128.6, 129.0, 130.7, 131.9, 134.0, 134.7, 144.1, 147.5, 182.

1-(Thien-2-yl)-3-(2,4-dichlorophenyl)prop-2-en-1-one (3e)

Yield 68%; IR (ν_{max}, KBr): 1637 cm^{-1} (C=O); HRMS calcd for $C_{13}H_8OSCl_2$: 281.9673. Found: 281.9705; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 7.18 (1H, dd, J = 4.8, 4.0 Hz), 7.29 (1H, m), 7.35 (1H, d, J = 15.7 Hz), 7.46 (1H, d, J = 2.4 Hz), 7.66 (1H, d, J = 8.6 Hz), 7.70 (1H, dd, J = 5.1, 1.1 Hz), 7.85 (1H, dd, J = 3.8, 1.1 Hz), 8.13 (1H, d, J = 15.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm); 124.7, 127.6, 128.4, 128.6, 130.2, 131.7, 132.2, 134.4, 136.2, 136.6, 138.7, 145.1, 181.6.

1-(Thien-2-yl)-3-(4-chlorophenyl)prop-2-en-1-one (3f)

Yield 92%; IR (ν_{max}, KBr): 1636 cm^{-1} (C=O); HRMS calcd for $C_{13}H_9OSCl$: 248.0063. Found: 248.0068; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 7.11 (2H, m), 7.18 (1H, dd, J = 5.2, 3.7 Hz), 7.34 (1H, d, J = 15.4 Hz), 7.63

(2H, m), 7.68 (1H, dd, J = 4.8, 1.1 Hz), 7.81 (1H, d, J = 15.5 Hz), 7.86 (1H, dd, J = 4.0, 1.1 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm); 122.1, 128.4, 129.3, 129.7, 132.0, 133.2, 134.2, 136.5, 142.6, 145.4, 181.8.

1-(Thien-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (3g)

Yield 68%; IR (ν_{max}, KBr): 1635 cm^{-1} (C=O); HRMS calcd for $C_{14}H_{12}O_2S$: 244.0558. Found: 244.0556; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 3.84 (3H, s), 6.92 (2H, d, J = 8.8 Hz), 7.16 (1H, dd, J = 4.8, 3.7 Hz), 7.29 (1H, d, J = 15.4 Hz), 7.59 (2H, d, J = 8.6 Hz), 7.64 (1H, dd, J = 4.8, 1.1 Hz), 7.82 (2H, m); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 55.3, 114.5, 119.3, 127.5, 128.2, 130.3, 131.5, 133.6, 144.0, 145.8, 161.8, 182.1.

1-(Thien-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one (3h)

Yield 68%; IR (ν_{max}, KBr): 1636 cm^{-1} (C=O); HRMS calcd for $C_{13}H_9OSNO_2$: 259.0303. Found: 259.0300; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 7.31 (1H, m), 7.77 (1H, d, J = 15.7 Hz), 8.05 (2H, m), 8.13 (2H, d, J = 8.9 Hz), 8.25 (2H, d, J = 8.3 Hz), 8.36 (1H, d, J = 3.7 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 124.4, 126.3, 129.6, 130.4, 135.0, 136.9, 140.8, 141.5, 145.6, 148.6, 181.9.

1-(Thien-2-yl)-3-(4-methylphenyl)prop-2-en-1-one (3i)

Yield 85%; IR (ν_{max}, KBr): 1636 cm^{-1} (C=O); HRMS calcd for $C_{14}H_{12}OS$: 228.0609. Found: 228.0611; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.38 (3H, s), 7.17 (1H, dd, J = 4.9, 4.0 Hz), 7.22 (2H, d, J = 8.0 Hz), 7.37 (1H, d, J = 15.4 Hz), 7.53 (2H, d, J = 8.0 Hz), 7.66 (1H, dd, J = 4.8, 1.1 Hz), 7.83 (2H, m); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm); 21.6, 120.6, 128.3, 128.6, 129.8, 131.7, 132.1, 133.8, 141.2, 144.2, 145.7, 182.2.

1-(Thien-2-yl)-3-(naphtha-2-yl)prop-2-en-1-one (3j)

Yield 94%; IR (ν_{max}, KBr): 1637 cm^{-1} (C=O); HRMS calcd for $C_{17}H_{12}OS$: 264.0609. Found: 264.0601; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 7.20 (1H, dd, J = 4.8, 3.7 Hz), 7.53 (3H, m), 7.69 (1H, dd, J = 4.9, 1.1 Hz), 7.78 (1H, m), 7.87 (4H, m), 8.02 (2H, m); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 121.8, 123.7, 126.8, 127.5, 127.8, 128.3, 128.7, 128.8, 130.8, 131.9, 132.2, 133.4, 133.9, 134.5, 144.2, 145.7, 182.1.

1-(5-Methylfuran-2-yl)-3-(4-dimethylaminophenyl)prop-2-en-1-one (3k)

Yield 86%; IR (ν_{max}, KBr): 1635 cm^{-1} (C=O); HRMS calcd for $C_{14}H_{12}O_3$: 228.0786. Found: 228.0786; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.42 (3H, s), 3.03 (6H, s), 6.18 (1H, m), 6.68 (2H, d, J = 8.9 Hz), 7.17 (2H, m), 7.55 (2H, d, J = 8.6 Hz), 7.80 (1H, d, J = 15.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 14.2, 40.2, 109.1, 111.8, 116.1, 118.4, 122.7, 130.4, 144.2, 152.0, 153.0, 157.4, 177.7.

1-(5-Methylfuran-2-yl)-3-(3-hydroxyphenyl)prop-2-en-1-one (3l)

Yield 60%; IR (ν_{max}, KBr): 1635 cm^{-1} (C=O); HRMS calcd for $C_{16}H_{17}O_2N$: 255.1259. Found: 255.1257; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.38 (3H, s), 6.17 (1H, m), 6.88 (1H, dd, J = 8.0, 1.4 Hz), 7.10 (2H, m), 7.19 (2H, m), 7.30 (1H, d, J = 15.7 Hz), 7.73 (1H, d, J = 15.7 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 14.3, 109.6, 115.2, 117.8, 120.1, 121.0, 121.5, 130.2, 136.4, 143.5, 152.4, 156.3, 159.2, 179.0.

1-(5-Methylfuran-2-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one (3m)

Yield 58%; IR (ν_{max}, KBr): 1638 cm^{-1} (C=O), 3279 cm^{-1} (OH); HRMS calcd for $C_{14}H_{12}O_3$: 228.0786. Found: 228.0786; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.30 (3H, s), 6.07 (1H, m), 6.76 (2H, d, J = 8.6 Hz), 7.09 (2H, m), 7.38 (2H, d, J = 8.6 Hz), 7.64 (1H, d, J = 15.4 Hz), 9.14 (1H, s); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 14.25, 109.86, 116.3, 118.9, 121.0, 126.1, 131.0, 143.0, 152.6, 158.5, 160.5, 176.4.

1-(5-Methylfuran-2-yl)-3-(4-methylphenyl)prop-2-en-1-one (3n)

Yield 72%; IR (ν_{max}, KBr): 1635 cm^{-1} (C=O); HRMS calcd for $C_{15}H_{14}O_2$: 226.0994. Found: 226.0992; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.38 (3H, s), 2.43 (3H, s), 6.21 (1H, m), 7.22 (3H, m), 7.34 (1H, d, J = 15.7 Hz), 7.54 (2H, d, J = 8.3 Hz), 7.83 (1H, d, J = 15.7 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 14.3, 21.6, 109.3, 119.5, 120.3, 128.5, 129.7, 132.2, 141.0, 143.4, 152.6, 158.1, 177.4.

1-(5-Methylfuran-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (3o)

Yield 67%; IR (ν_{max}, KBr): 1636 cm^{-1} (C=O); HRMS calcd for $C_{15}H_{14}O_3$: 242.0943. Found: 242.0940; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.44 (3H, s), 3.85 (3H, s), 6.20 (1H, m), 6.93 (2H, d, J = 8.8 Hz), 7.24 (2H, m), 7.60 (2H, d, J = 8.8 Hz), 7.82 (1H, d, J = 15.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 14.2, 55.4, 109.3, 114.4, 119.0, 119.2, 127.6, 130.3, 143.1, 152.7, 157.9, 161.6, 177.4.

1-(5-Methylfuran-2-yl)-3-(naphtha-2-yl)prop-2-en-1-one (3p)

Yield 96%; IR (ν_{max}, KBr): 1635 cm^{-1} (C=O); HRMS calcd for $C_{18}H_{14}O_2$: 262.0994. Found: 262.0992; ^{1}H-

NMR (500 MHz, $CDCl_3$, δ, ppm): 2.46 (3H, s), 6.23 (1H, m), 7.29 (1H, d, J = 3.45 Hz), 7.50 (2H, m), 7.83 (4H, m), 8.02 (3H, m); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 14.3, 109.4, 119.6, 121.5, 123.8, 126.8, 127.4, 127.8, 128.7, 130.7, 132.4, 133.4, 134.4, 143.4, 152.6, 158.3, 177.3.

1-(5-Methylfuran-2-yl)-3-(4-chlorophenyl)prop-2-en-1-one (3q)

Yield 94%; IR (ν_{max}, KBr): 1635 cm^{-1} (C=O); HRMS calcd for $C_{15}H_{14}O_2Cl$: 246.0448. Found: 246.0446; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.44 (3H, s), 6.22 (1H, m), 7.24 (1H, m), 7.36 (3H, m), 7.56 (2H, d, J = 8.6 Hz), 7.78 (1H, d, J = 15.7 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 14.3, 109.5, 119.8, 121.8, 129.2, 129.6, 133.4, 136.3, 141.8, 152.5, 158.4, 177.0.

1-(5-Methylfuran-2-yl)-3-(2,4-dichlorophenyl) prop-2-en-1-one (3r)

Yield 75%; IR (ν_{max}, KBr): 1636 cm^{-1} (C=O); HRMS calcd for $C_{14}H_{10}O_2Cl_2$: 280.0058. Found: 280.0058; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.43 (3H, s), 6.21 (1H, m), 7.26 (2H, m), 7.32 (1H, d, J = 15.7 Hz), 7.45 (1H, m), 7.67 (1H, d, J = 8.6 Hz), 8.12 (1H, d, J = 15.7 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 14.3, 109.6, 120.1, 124.4, 127.5, 128.5, 130.1, 131.9, 136.1, 136.3, 137.8, 152.3, 158.5, 176.7.

1-(5-Methylfuran-2-yl)-3-(4-fluorophenyl)prop-2-en-1-one (3s)

Yield 71%; IR (ν_{max}, KBr): 1637 cm^{-1} (C=O); HRMS calcd for $C_{15}H_{11}O_2F$: 230.0743. Found: 230.0745; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.43 (3H, s), 6.20 (1H, m), 7.08 (2H, m), 7.24 (1H, m), 7.30 (1H, d, J = 15.7 Hz), 7.61 (2H, m), 7.79 (1H, d, J = 15.7 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 14.2, 109.4, 116.0, 119.6, 121.1, 130.3, 131.2, 142.0, 152.5, 158.2, 163.0, 177.1.

1-(5-Methylfuran-2-yl)-3-(2-(2-hydroxyethoxy) phenyl)prop-2-en-1-one (3t)

Yield 54%; IR (ν_{max}, KBr): 1638 cm^{-1} (C=O), 3432 cm^{-1} (OH); HRMS calcd for $C_{16}H_{16}O_4$: 272.1049. Found: 272.1049; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.41 (3H, s), 4.04 (2H, m), 4.15 (2H, m), 6.16 (1H, m), 6.90 (1H, d, J = 8.3 Hz), 6.96 (1H, m), 7.24 (1H, m), 7.31 (1H, m), 7.51 (1H, d, J = 16.0 Hz), 7.60 (1H, dd, J = 7.7, 1.7 Hz), 8.12 (1H, d, J = 15.7 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 14.2, 61.4, 69.9, 109.4, 112.2, 119.8, 121.1, 122.1, 124.0, 129.4, 131.8, 138.8, 152.7, 158.0, 158.2, 178.0.

1-(5-Methylfuran-2-yl)-3-(3-(2-hydroxyethoxy) phenyl)prop-2-en-1-one (3u)

Yield 53%; IR (ν_{max}, KBr): 1638 cm^{-1} (C=O), 3436 cm^{-1} (OH); HRMS calcd for $C_{16}H_{16}O_4$: 272.1049. Found: 272.1048; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.45 (3H, s), 3.99 (2H, m), 4.12 (2H, m), 6.22 (1H, m), 6.96 (1H, m), 7.25 (5H, m), 7.79 (1H, d, J = 15.7 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 14.2, 61.4, 69.4, 109.5, 114.2, 115.2, 119.9, 120.6, 121.7, 130.0, 136.3, 143.1, 152.5, 158.4, 159.0, 177.2.

1-(5-Methylfuran-2-yl)-3-(4-(2-hydroxyethoxy) phenyl)prop-2-en-1-one (3v)

Yield 56%; IR (ν_{max}, KBr): 1637 cm^{-1} (C=O), 3432 cm^{-1} (OH); HRMS calcd for $C_{16}H_{16}O_4$: 272.1049. Found: 272.1050; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.45 (3H, s), 3.97 (2H, m), 4.11 (2H, m), 6.18 (1H, m), 6.92 (2H, d, J = 8.3 Hz), 7.24 (2H, m), 7.57 (2H, d, J = 8.3 Hz), 7.79 (1H, d, J = 15.7 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 14.2, 61.3, 69.4, 109.4, 115.0, 116.2, 119.2, 127.9, 130.3, 143.1, 152.6, 158.1, 160.7, 177.5.

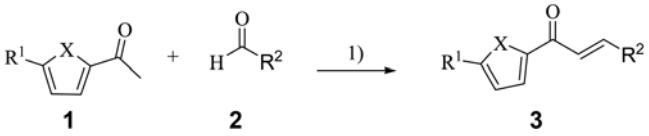
Neuraminidase (*Clostridium perfringens*) inhibition assay

The enzyme assay was performed as previously reported with slight modifications (Potier et al., 1979). In general, 4-methylumbelliferyl-a-$_D$-*N*-acetylneuraminic acid sodium salt hydrate (SIGMA, M8639) 0.125 mM in 50 mM sodium acetate buffer (pH 5.0) was used as a substrate. Neuraminidase 0.1 U/mL in acetate buffer was used as the enzyme source. The isolated compounds were dissolved in MeOH and diluted to appropriate concentrations in acetate buffer. Enzyme (15 μL) was added to 15 μL of sample solution mixed with buffer (510 μL) in a cuvette, and 60 μL of substrate was added at 37°C. 4-Methylumbelliferone was immediately quantified by fluorometrically by LS-55 luminescence. The excitation wavelength was 365 nm, with the excitation slits set at 2.5 nm, and the emission wavelength was 450 nm, with the emission slits set at 20 nm. For the determination of enzyme activity, we used a time-driven protocol with initial velocity recorded over a range of concentrations and the data analyzed using a nonlinear regression program (Sigma Plot; SPCC Inc.).

$$\text{Activity (\%)} = 100[1/(1 + [I]/IC_{50})]]$$

RESULTS AND DISCUSSION

In this study, the 1,3-diarylprop-2-en-1-one derivatives **3a-v** were synthesized according to the method shown in Scheme 1. To prepare the target compounds, the appropriate commercially available ketones and aldehydes were suspended in ethanol with sodium



Scheme 1. Synthesis of derivatives **3a-v**. Reagents and conditions: (1) EtOH, NaOH, 0°C

Table I. Neuraminidase inhibitory activities of 1,3-diarylprop-2-en-1-one derivatives **3a-v**

Compd	X	R^1	R^2	% Inhibition at 25 μM	IC_{50} (μM)[a]
3a	S	H	4-$N(CH_3)_2$-Ph	78.8	3.2 ± 0.31
3b	S	H	2,6-di-F-Ph	7.4	
3c	S	H	4-F-Ph	8.6	
3d	S	H	Ph	3.8	
3e	S	H	2,4-di-Cl-Ph	6.7	
3f	S	H	4-Cl-Ph	9.9	
3g	S	H	4-OCH_3-Ph	54.6	23.2 ± 1.82
3h	S	H	4-NO_2-Ph	1.6	
3i	S	H	4-CH_3-Ph	25.4	
3j	S	H	Naphtha-2-yl	8.0	
3k	O	CH_3	4-$N(CH_3)_2$-Ph	85.1	1.5 ± 0.28
3l	O	CH_3	3-OH-Ph	30.0	
3m	O	CH_3	4-OH-Ph	62.5	18.4 ± 1.21
3n	O	CH_3	4-CH_3-Ph	18.5	
3o	O	CH_3	4-OCH_3-Ph	33.3	
3p	O	CH_3	Naphtha-2-yl	11.1	
3q	O	CH_3	4-Cl-Ph	18.0	
3r	O	CH_3	2,4-di-Cl-Ph	9.4	
3s	O	CH_3	4-F-Ph	29.4	
3t	O	CH_3	2-$O(CH_2)_2OH$-Ph	21.6	
3u	O	CH_3	3-$O(CH_2)_2OH$-Ph	35.8	
3v	O	CH_3	4-$O(CH_2)_2OH$-Ph	54.4	22.3 ± 2.5

[a]All compounds were examined in a set of experiments repeated three times; IC_{50} values of compounds represent the concentration that caused 50% enzyme activity loss; Oseltamivir was used as a positive control (IC_{50} value = 1.59 nM).

hydroxide at 0°C. The chemical structures of these compounds were determined using several spectroscopic analyses including IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry.

The prepared compounds **3a**-**v** were tested for their enzymatic inhibitory activities against NA from *Clostridium perfringens*. The enzyme assay was based on a previously described procedure with some modifications (Potier et al., 1979). We examined the inhibitory effects of 1,3-diarylprop-2-en-1-one derivatives on the release of 4-methylumbelliferone from 20-(4-methylumbelliferyl)-a-D-*N*-acetylneuraminic acid. Oseltamivir, a well known NA inhibitor, was used as a positive control (IC_{50} = 1.59 nM). We initially examined the effect

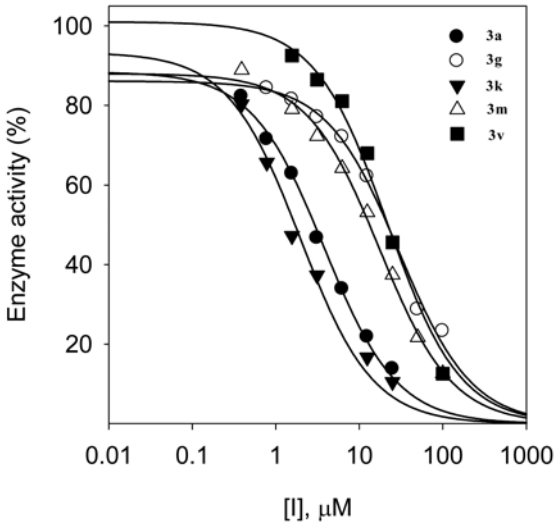


Fig. 1. Effects of compounds **3a**, **3g**, **3k**, **3m** and **3v** on neuraminidase hydrolysis of neuraminic acid (compound **3a**, ●; compound **3g**, ○; compound **3k**, ▼; compound **3m**, ▽; compound **3v**, ■).

of the substituent on the phenyl ring. As shown in Table I, the lead compound **3a** with *N*,*N*-dimethylamino at *para* position of the phenyl group showed good potency (IC_{50} = 3.2 μM), but replacing the substituents at R^2 resulted in a decrease in activity. Among the compounds, the 4-methoxy-substituted **3g** showed moderate activity (IC_{50} = 23.2 μM), whereas fluoro-, chloro-, methyl-, NO_2-, and naphthyl-substituted analogues resulted in a significant loss of potency. The analogues that had more lipophilic groups generally showed greater decrease in potency compared with the corresponding analogues containing less lipophilic groups. Based on the above results, further simple modification of the structure did not show further enhancements in activity. We envisioned that more potent inhibitors would be obtained by replacing the thiophene to the 5-methylfuran ring with substitutions at R^2. Various functional groups, such as methyl, *N*,*N*-dimethylamino, hydroxyl, chloro, naphthyl, and fluoro groups were introduced at R^2 to give compounds **3k**-**s**. The analogues containing more lipophilic groups, such as methyl (**3n**), 4-chloro (**3q**), 2,4-dichloro (**3r**), 4-fluoro (**3s**), and naphthyl (**3p**), resulted in a significant loss in potency. The position effect of the hydroxyl group was examined. The introduction of a hydroxyl group at the 4-position resulted in a moderate potency (**3m**, IC_{50} value of 18.4 μM). However, the 3-hydoxyl-substituted derivative **3l** resulted in decreased potency compared with **3m**. For the 4-methoxy group at the R^2 position, compound **3o** demonstrated decreased potency compared with the corresponding analogues containing thiophene. However, the introduction of an *N*,*N*-dimethylamino group

at *para* position of the phenyl group in compound **3k** resulted in a two-fold improvement in inhibitory activities compared with **3a**, with an IC_{50} values of 1.5 μM. Next, attention was focused on changing the hydroxyl group to 2-hydroxyethyl ether because of the result from compound **3m**. Introduction of 2-hydroxyethyl ether at the 2-, 3-, or 4-positions of the phenyl ring resulted in decreased potency. However, compound **3v**, containing a 2-hydroxylethyl ether at the 4-position of the phenyl ring, was equipotent to **3m**, with an IC_{50} value of 22.3 μM. This suggests that the 4-position of the substituent on the phenyl ring is crucial and plays an important role in the inhibitory effect of these compounds.

Previous reports have suggested that naturally isolated diarylheptanoids containing a prop-2-en-1-one core moiety were able to inhibit NA in an enzyme test (Grienke et al., 2010). The report revealed that the two peripheral phenyl groups, with a spacer represented by the heptyl chain, seemed to contribute to NA inhibitory activity. In this study, we examined the NA and influenza virus inhibitory activities of our series of 1,3-diarylprop-2-en-1-one derivatives (**3a**-**v**). We conducted a SAR study with the compounds to determine the optimal structure and position of the substituent for significant and specific NA inhibitory activities. To our knowledge, this is the first demonstration of a small-molecule inhibitor of NA. Compound **3k**, with an *N*,*N*-dimethylamino at the 4-position on the phenyl ring, was found to have especially effective inhibitory activities (an IC_{50} value of 1.5 μM). The analogues that have more lipophilic groups generally showed decreased potency compared with the corresponding, less lipophilic analogues. This would indicate that the increase in potency of the NA inhibitory activity is related to a more substantial interaction with the enzyme and influenced by lipophilicity. Jeong and co-workers also suggested that the presence of the less lipophilic group in the B-ring of flavonoids is essential for their potent inhibitory activity against NA (Jeong et al., 2009). In summary, these preliminary data demonstrate that 1,3-diarylprop-2-en-1-ones derivatives might be considered as a potential therapeutic agent in the treatment of influenza virus infections.

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