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Research Letter

Synthesis and Preliminary Biologic Evaluation of 5-Substituted-2-(4-substituted phenyl)-1,3-Benzoxazoles as A Novel Class of Influenza Virus A Inhibitors

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The diversity-oriented chemistry synthesis together with the random screening approach has permitted the discovery and optimization of novel antiviral lead compounds. In this paper, a series of novel 5-substituted-2-(4-substituted phenyl)-1,3-benzoxazoles was synthesized and evaluated for their *in vitro* anti-influenza A virus and anti-influenza B virus activity. The activity was monitored by the MTS assay in the Madin–Darby canine kidney cells. Compound 7h showed excellent inhibitory activity and selective index against A/H3N2 (EC_{50} = 37.03 μM, SI > 5), which were all higher than that of the reference drug oseltamivir (EC_{50} > 59.00 μM, SI > 1). However, no compound displays inhibitory activity against influenza B virus.

Key words: anti-influenza agents, antiviral activity, benzoxazoles, influenza A, influenza virus

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Influenza virus (IFV) is an RNA virus that infects avian and mammalian cells with the assistance of three transmembrane proteins: hemagglutinin (HA), sialidase [neuraminidase (NA)], and M2 (1–3). Hemagglutinin plays a key role in cell recognition and initiating infection by binding sialic acid-containing receptors on host cells, which then mediates the subsequent membrane fusion and viral entry (4,5). It recognizes specific sugar chain structures including sialic acids at the non-reducing termini of *N*- and *O*-glycans (6,7). Neuraminidase is responsible for the release of newly formed virions by hydrolyzing sialic acids from sialoglycoconjugates at the surface of the host cell as well as infected cells, preventing aggregation of the progeny virus particles (1). M2 is very important for the maturation of the viral particles. The proteins HA, NA, and M2 are folded and glycosylated (except for M2) in the endoplasmic reticulum, and then HA, NA, and M2 are assembled the former in a trimer and the two latter into tetramers (8). At present, five anti-influenza agents have been approved by the FDA for the treatment of influenza (Figure 1), including two M2 protein ion channel inhibitors (9): amantadine and rimantadine, and three neuraminidase inhibitors: zanamivir (10–12), oseltamivir (13–16), and peramivir (17,18). However, the emergence of drug resistance during the treatment of influenza infections has been widely reported (19). It is also unclear whether these drugs will be sufficient to deal with larger influenza epidemics, so there is an urgent need to develop new antiviral agents that act on novel influenza virus targets.

Heterocyclic structures continue to play an increasingly important role in lead discovery and biologic activities in the pharmaceutical industry and academic research (20,21). Among all the aromatic heterocycles, the benzo-fused heterocycle (i.e., benzimidazole, benzoxazole) structure is one of the classical examples of privileged scaffolds present in a number of pharmaceutically active compounds (22).

Recently, in the antiviral drug research field, substituted benzoxazoles have attracted considerable attention from the medicinal chemists, owing to the high number of positive hits encountered with this heterocycle (23–26). Despite this, the medicinal chemistry effort concentrated on structural modifications on the benzoxazole platform as anti-influenza virus agents intact.

To fill the gaps in the field of heterocycle-based antiviral drug discovery, in this paper, we reported the synthesis and antiviral evaluation of a series of novel 5-substituted-2-(4-substituted phenyl)-1,3-benzoxazoles as potent anti-influenza agents, which provides promising lead compounds for the further optimization and development.

Experimental Section

Chemistry

All melting points were determined on a micromelting point apparatus and are uncorrected. Infrared spectra (IR) were recorded with a Nexus 470FT-IR Spectrometer. ^{1}H-NMR spectra were obtained on a Brucker Avance-600 NMR-spectrometer in the indicated solvents.

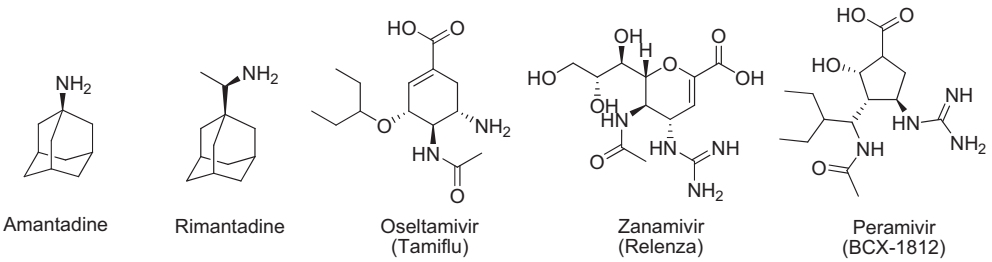


Figure 1: Anti-influenza drugs have been approved by the FDA for the treatment of influenza.

Chemical shifts are expressed in δ units and TMS as internal reference. Mass spectra were taken on a LC Autosampler Device: Standard G1313A instrument. Solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of the reaction solutions involved the use of rotary evaporator at reduced pressure.

Preparation of 5-amine-2-(4-amino phenyl)-1,3-benzoxazole (6)

A suspension of 4-nitrobenzoic acid (**1**) (6.68 g, 40 mmol) in 30 mL of $SOCl_2$ was stirred at reflux for 2 h. The solvent was removed by distillation under vacuum to obtain 4-nitrobenzoyl chloride (**2**). To a solution of 2-amino-4-nitrophenol (**3**) (3.08 g, 20 mmol) and triethylamine (4.04 g, 40 mmol) in 50 mL dioxane, **2** was added dropwise under an ice bath. After finishing dropping, the mixture was stirred at reflux for 2 h. The solvent was removed under vacuum, and then an appropriate amount of ice water was added, and the separated yellow solid **4** was filtered off and washed with water. The crude product was recrystallized from DMF-ethanol to obtain yellow needle crystals and was dried at 90 °C under vacuum. Yield: 85.4%.

A mixture of **4** (2.8 g, 6 mmol) and 4-methylbenzenesulfonic acid (2.0 g, 12 mmol) in xylene (100 mL) was stirred at reflux for 4 h. After the reaction mixture was cooled, crude product of 5-nitro-2-(4-nitrophenyl)-1,3-benzoxazole (**5**) was precipitated, which was filtered and washed with cold ethanol, then recrystallized from DMF-ethanol to obtain the off-white needle crystals. Yield: 76.0%. M.p. 261–262 °C.

To a solution of compound 5 (0.89 g, 3 mmol) in 30 mL of DMF, Pd-C (0.09 g, 10%) was added and stirred under hydrogen gas at room temperature for 24 h. Appropriate amount of water was added dropwise to obtain the 5-amine-2-(4-aminophenyl)-1,3-benzoxazole (**6**) in pale pink needle crystals. Yield: 81.5%. M.p. 230–231 °C.

General procedure for the preparation of compounds (7a–7v)

A suspension of substituted benzoic acid (2.5 mmol) in $SOCl_2$ (30 mL) was stirred at reflux for 2 h. The solvent was removed by distillation under vacuum, the residue was diluted with dry DMF (10 mL), and then a solution of 5-amine-2-(4-amino phenyl)-1,3-benzoxazole **6** (0.23 g, 1 mol), triethylamine (0.25 g, 2.5 mmol) and 20 mL dry DMF was added dropwise to the mixture at 0 °C. After 2 h of stirring at room temperature, an appropriate amount of water was added to the mixture, and the crude product was separated by filtering off the solution *in vacuo*. The crude product was further purified by recrystallization from DMF-H_2O to yield compounds **7a–7v**.

4-Nitro-*N*-(4-(5-(4-nitrobenzamido)benzo[d]oxazol-2-yl)phenyl)benzamide (**7a**) (61%) as a yellow needle crystal having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.92 (s, 1H, N-H), 10.76 (s, 1H, N-H), 8.40 (d, 4H, J = 7.8 Hz, Ar-H), 8.28 (s, 1H, Ar-H), 8.22–8.24 (m, 6H, Ar-H), 8.06 (d, 2H, J = 7.8 Hz, Ar-H), 7.79 (d, 1H, J = 8.4 Hz, Ar-H), 7.75 (d, 1H, J = 8.4 Hz, Ar-H); IR (KBr, ⁄cm): 3309 (ν_{NH}), 1664 ($\upsilon_{C=O}$), 1598, 1499, 1479 ($\upsilon_{C=C}$), 1525, 1347 (ν_{NO2}), 1320, 1172 (υ_{C-N}); EI-MS: *m⁄z* 524.2 [M+H]. $C_{27}H_{17}N_5O_7$ (523.11).

4-Fluoro-*N*-(4-(5-(4-fluorobenzamido)benzo[d]oxazol-2-yl)phenyl)benzamide (**7b**) (57%) as an off-white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.63 (s, 1H, N-H), 10.46 (s, 1H, N-H), 8.25 (s, 1H, Ar-H), 8.21 (d, 2H, J = 8.4 Hz, Ar-H), 8.04–8.09 (m, 6H, Ar-H), 7.77 (d, 1H, J = 9.0 Hz, Ar-H), 7.38–7.42 (m, 4H, Ar-H); IR (KBr, ⁄cm): 3310 (ν_{NH}), 1654 ($\upsilon_{C=O}$), 1534, 1506, 1412 ($\upsilon_{C=C}$), 1327, 1142 (υ_{C-N}); EI-MS: *m⁄z* 470.5 [M+H]. $C_{27}H_{17}F_2N_3O_3$ (469.12).

4-Chloro-*N*-(4-(5-(4-chlorobenzamido)benzo[d]oxazol-2-yl)phenyl)benzamide (**7c**) (62%) as an off-white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.68 (s, 1H, N-H), 10.51 (s, 1H, N-H), 8.25 (s, 1H, Ar-H), 8.21 (d, 2H, J = 9.0 Hz, Ar-H), 8.02–8.05 (m, 6H, Ar-H), 7.77 (d, 1H, J = 9.0 Hz, Ar-H), 7.74 (d, 1H, J = 9.0 Hz, Ar-H), 7.63–7.66 (m, 4H, Ar-H); IR (KBr, ⁄cm): 3243 (ν_{Ar-H}), 1647 ($\upsilon_{C=O}$), 1594, 1526, 1485, 1410 ($\upsilon_{C=C}$), 1321, 1175 (υ_{C-N}); EI-MS: *m⁄z* 502.2 [M+H]. $C_{27}H_{17}Cl_2N_3O_3$ (501.06).

4-Bromo-*N*-(4-(5-(4-bromobenzamido)benzo[d]oxazol-2-yl)phenyl)benzamide (**7d**) (47%) as a light yellow crystal having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.67 (s, 1H, N-H), 10.50 (s, 1H, N-H), 8.26 (s, 1H, Ar-H), 8.21 (d, 2H, J = 9.0 Hz, Ar-H), 8.05 (d, 2H, J = 9.0 Hz, Ar-H), 7.95 (d, 4H, J = 8.4 Hz, Ar-H), 7.73–7.80 (m, 6H, Ar-H); IR (KBr, ⁄cm): 3285 (ν_{NH}), 1647 ($\upsilon_{C=O}$), 1590, 1526, 1502, 1482 ($\upsilon_{C=C}$), 1325, 1174 (υ_{C-N}); EI-MS: *m⁄z* 590.1 [M+H]. $C_{27}H_{17}Br_2N_3O_3$ (588.96).

4-Iodo-*N*-(4-(5-(4-iodobenzamido)benzo[d]oxazol-2-yl)phenyl)benzamide (**7e**) (53%) as an off-white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.64 (s, 1H, N-H), 10.48 (s, 1H, N-H), 8.26 (s, 1H, Ar-H), 8.21 (d, 2H, J = 9.0 Hz, Ar-H), 8.04 (d, 2H, J = 9.0 Hz, Ar-H), 7.94–7.97 (m, 4H, Ar-H), 7.72–7.79 (m, 6H, Ar-H);

IR (KBr, /cm): 3297, 3202 (ν_{NH}), 1656 ($\upsilon_{C=O}$), 1605, 1531, 1493 ($\upsilon_{C=C}$), 1324, 1173 (υ_{C-N}); EI-MS: *m/z* 686.4 [M+H]. $C_{27}H_{17}I_2N_3O_3$ (684.94).

4-Methoxy-*N*-(4-(5-(4-methoxybenzamido)benzo[d]oxazol-2-yl)phenyl) benzamide (**7f**) (61%) as an off-white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.50 (s, 1H, N-H), 10.31 (s, 1H, N-H), 8.28 (s, 1H, Ar-H), 8.19 (d, 2H, *J* = 9.0 Hz, Ar-H), 7.99 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.74 (d, 1H, *J* = 9.0 Hz, Ar-H), 7.64–7.69 (m, 3H, Ar-H), 7.51–7.55 (m, 2H, Ar-H), 7.20 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.09 (t, 2H, Ar-H), 3.92 (s, 6H, -CH_3); IR (KBr, /cm): 3295 (ν_{NH}), 2932, 2837 ($\upsilon_{CH(CH3)}$), 1648 ($\upsilon_{C=O}$), 1605, 1505, 1478 ($\upsilon_{C=C}$), 1315, 1176 (υ_{C-N}); EI-MS: *m/z* 494.5 [M+H]. $C_{29}H_{23}N_3O_5$ (493.16).

3,4-Dichloro-*N*-(4-(5-(3,4-dichlorobenzamido)benzo[d]oxazol-2-yl) phenyl)benzamide (**7g**) (57%) as an off-white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.73 (s, 1H, N-H), 10.58 (s, 1H, N-H), 8.24–8.26 (m, 3H, Ar-H), 8.22 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.04 (d, 2H, *J* = 9.0 Hz, Ar-H), 7.94–7.97 (m, 4H, Ar-H), 7.72–7.79 (m, 6H, Ar-H); IR (KBr, /cm): 3257 (ν_{NH}), 1645 ($\upsilon_{C=O}$), 1595, 1526, 1500 ($\upsilon_{C=C}$), 1320, 1177 (υ_{C-N}); EI-MS: *m/z* 572.1 [M+H]. $C_{27}H_{15}Cl_4N_3O_3$ (571.24).

N-(4-(5-(3,4-dimethoxybenzamido)benzo[d]oxazol-2-yl)phenyl)-3,4-dimethoxybenzamide (**7h**) (47%) as a white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.42 (s, 1H, N-H), 10.26 (s, 1H, N-H), 8.25 (s, 1H, Ar-H), 8.21 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.04 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.75 (d, 1H, *J* = 9.0 Hz, Ar-H), 7.72 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.67 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.57 (s, 2H, Ar-H), 7.12 (t, 2H, Ar-H), 3.86 (s, 12H, OCH_3); IR (KBr, /cm): 3277 (ν_{NH}), 2934, 2837 (υ_{CH}), 1647 ($\upsilon_{C=O}$), 1599, 1506 ($\upsilon_{C=C}$), 1315, 1176 (υ_{C-N}); EI-MS: *m/z* 554.5 [M+H]. $C_{31}H_{27}N_3O_7$ (553.18).

4-Chloro-*N*-(4-(5-(4-chloro-2-nitrobenzamido)benzo[d]oxazol-2-yl)phenyl)-2-nitrobenzamide (**7i**) (49%) as a light yellow powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 11.10 (s, 1H, N-H), 10.92 (s, 1H, N-H), 8.31 (d, 2H, *J* = 7.8 Hz, Ar-H), 8.23 (d, 2H, *J* = 9.0 Hz, Ar-H), 8.16 (s, 1H, *J* = 7.8 Hz, Ar-H), 8.01 (t, 2H, Ar-H), 7.89–7.92 (m, 4H, Ar-H), 7.79 (d, 1H, *J* = 9.0 Hz, Ar-H), 7.61 (d, 1H, *J* = 9.0 Hz, Ar-H); IR (KBr, /cm): 3246 (ν_{NH}), 1654 ($\upsilon_{C=O}$), 1534, 1350 (ν_{NO2}), 1350, 1173 (υ_{C-N}); EI-MS: *m/z* 592.2 [M+H]. $C_{27}H_{15}Cl_2N_5O_7$ (591.03).

N-(4-(5-benzamidobenzo[d]oxazol-2-yl)phenyl)benzamide (**7j**) (64%) as an off-white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.63 (s, 1H, N-H), 10.45 (s, 1H, N-H), 8.27 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.21 (d, 2H, *J* = 9.0 Hz, Ar-H), 8.18 (d, 1H, *J* = 9.0 Hz, Ar-H), 8.06 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.98–8.00 (m, 5H, Ar-H), 7.76 (d, 3H, *J* = 3.6 Hz, Ar-H), 7.61–7.65 (m, 3H, Ar-H), 7.72–7.79 (m, 6H, Ar-H); IR (KBr, /cm): 3262 (ν_{NH}), 1653 ($\upsilon_{C=O}$), 1533, 1493, 1410 ($\upsilon_{C=C}$), 1325, 1176 (υ_{C-N}); EI-MS: *m/z* 434.6 [M+H]. $C_{27}H_{19}N_3O_3$ (433.14).

4-Amino-*N*-(4-(5-(4-aminobenzamido)benzo[d]oxazol-2-yl)phenyl)benzamide (**7k**) (44%) as a sandy beige solid having mp >300 °C. ^{1}H-NMR (DMSO-d_6, p.p.m.) 600 MHz, δ: 10.12 (s, 1H, N-H), 9.46 (s, 1H, N-H), 8.22 (s, 1H, Ar-H), 8.14–8.18 (m, 3H, Ar-H), 8.02 (d, 2H, *J* = 9.0 Hz, Ar-H), 7.95 (s, 1H, Ar-H), 7.83 (d, 1H, *J* = 9.0 Hz, Ar-H), 7.74–7.77 (m, 5H, Ar-H), 7.70 (d, 2H, *J* = 1.8 Hz, Ar-H); IR (KBr, /cm): 3335, 3221 (ν_{NH}), 1653 ($\upsilon_{C=O}$), 1604, 1508, 1408 ($\upsilon_{C=C}$), 1314, 1179 (υ_{C-N}); EI-MS: *m/z* 464.5 [M+H]. $C_{27}H_{21}N_5O_3$ (463.16).

4-Methyl-*N*-(4-(5-(4-methylbenzamido)benzo[d]oxazol-2-yl)phenyl)benzamide (**7l**) (67%) as an off-white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.57 (s, 1H, N-H), 10.39 (s, 1H, N-H), 8.27 (s, 1H, Ar-H), 8.21 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.05 (d, 2H, *J* = 9.0 Hz, Ar-H), 7.76–7.81 (m, 6H, Ar-H), 7.43–7.46 (m, 4H, Ar-H), 2.51 (s, 6H, Ph-CH_3); IR (KBr, /cm): 3282 (ν_{NH}), 2919 (υ_{CH}), 1654 ($\upsilon_{C=O}$), 1592, 1500, 1478 ($\upsilon_{C=C}$), 1321, 1165 (υ_{C-N}); EI-MS: *m/z* 462.4 [M+H]. $C_{29}H_{23}N_3O_3$ (461.17).

4-Tert-butyl-*N*-(4-(5-(4-tert-butylbenzamido)benzo[d]oxazol-2-yl) phenyl)benzamide (**7m**) (57%) as an off-white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.55 (s, 1H, N-H), 10.37 (s, 1H, N-H), 8.27 (s, 1H, Ar-H), 8.20 (d, 2H, *J* = 9.0 Hz, Ar-H), 8.05 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.92 (d, 4H, *J* = 8.4 Hz, Ar-H), 7.75 (s, 2H, Ar-H), 7.58 (t, 4H, Ar-H), 1.34 (s, 18H, CH_3); IR (KBr, /cm): 3304 (ν_{NH}), 2961, 2868 ($\upsilon_{CH(CH3)}$), 1638 ($\upsilon_{C=O}$), 1592, 1500, 1479 ($\upsilon_{C=C}$), 1318, 1167 (υ_{C-N}); EI-MS: *m/z* 546.5 [M+H]. $C_{35}H_{35}N_3O_3$ (545.27).

4-Propyl-*N*-(4-(5-(4-propylbenzamido)benzo[d]oxazol-2-yl)phenyl)benzamide (**7n**) (63%) as an off-white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.54 (s, 1H, N-H), 10.36 (s, 1H, N-H), 8.26 (s, 1H, Ar-H), 8.20 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.05 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.92 (d, 4H, *J* = 7.8 Hz, Ar-H), 7.75 (s, 2H, Ar-H), 7.38 (t, 4H, Ar-H), 2.65 (t, 4H, Ar-CH_2), 1.62–1.66 (m, 4H, CH_2), 0.92 (t, 6H, CH_3); IR (KBr, /cm): 3309 (ν_{NH}), 2957, 2929, 2870 (υ_{CH}), 1652 ($\upsilon_{C=O}$), 1526, 1500, 1479 ($\upsilon_{C=C}$), 1318, 1179 (υ_{C-N}); EI-MS: *m/z* 518.5 [M+H]. $C_{33}H_{31}N_3O_3$ (517.24).

3-Fluoro-*N*-(4-(5-(3-fluorobenzamido)benzo[d]oxazol-2-yl)phenyl)benzamide (**7o**) (64%) as an off-white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.68 (s, 1H, N-H), 10.51 (s, 1H, N-H), 8.26 (s, 1H, Ar-H), 8.22 (d, 2H, *J* = 9.0 Hz, Ar-H), 8.04–8.06 (m, 2H, Ar-H), 7.85 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.81 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.78 (d, 1H, *J* = 9.0 Hz, Ar-H), 7.69 (d, 1H, *J* = 9.0 Hz, Ar-H), 7.60–7.65 (m, 2H, Ar-H), 7.46–7.51 (m, 2H, Ar-H); IR (KBr, /cm): 3320 (ν_{NH}), 1656 ($\upsilon_{C=O}$), 1587, 1531, 1482 ($\upsilon_{C=C}$), 1323, 1179 (υ_{C-N}); EI-MS: *m/z* 470.5 [M+H]. $C_{27}H_{17}F_2N_3O_3$ (469.12).

3-Chloro-*N*-(4-(5-(3-chlorobenzamido)benzo[d]oxazol-2-yl)phenyl)benzamide (**7p**) (42.9%) as an off-white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.70 (s, 1H, N-H), 10.54 (s, 1H, N-H), 8.26 (s, 1H, Ar-H), 8.22 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.04–8.06 (m, 4H, Ar-H), 7.96 (d, 2H, *J* = 7.2 Hz, Ar-H), 7.78 (d, 1H, *J* = 9.0 Hz, Ar-H), 7.74 (d, 1H, *J* = 9.0 Hz, Ar-H), 7.69 (t, 2H, Ar-H), 7.59–7.62 (m, 2H, Ar-H); IR (KBr, /cm): 3312 (ν_{NH}), 1651 ($\upsilon_{C=O}$), 1596, 1524, 1501 ($\upsilon_{C=C}$), 1324, 1178 (υ_{C-N}); EI-MS: *m/z* 502.2 [M+H]. $C_{27}H_{17}Cl_2N_3O_3$ (501.06).

3-Methyl-*N*-(4-(5-(3-methylbenzamido)benzo[d]oxazol-2-yl)phenyl)benzamide (**7q**) (67%) as an off-white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.57 (s, 1H, N-H), 10.40 (s, 1H, N-H), 8.26 (s, 1H, Ar-H), 8.21 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.05 (d, 2H, *J* = 9.0 Hz, Ar-H), 7.75–7.81 (m, 6H, Ar-H), 7.43–7.46 (m, 4H,

Ar-H), 2.51 (s, 6H, Ph-CH_3); IR (KBr, ⁄cm): 3276 (ν_{NH}), 2918, 2860 (υ_{CH}), 1694 ($\upsilon_{C=O}$), 1603, 1530, 1501, 1478 ($\upsilon_{C=C}$), 1320, 1178 (υ_{C-N}); EI-MS: *m/z* 462.4 [M+H]. $C_{29}H_{23}N_3O_3$ (461.17).

3-Nitro-*N*-(4-(5-(3-nitrobenzamido)benzo[d]oxazol-2-yl)phenyl)benzamide (**7r**) (40%) as a yellow powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.90 (s, 1H, N-H), 10.77 (s, 1H, N-H), 8.26 (s, 1H, Ar-H), 8.22 (d, 2H, *J* = 9.0 Hz, Ar-H), 8.04–8.06 (m, 2H, Ar-H), 7.85 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.81 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.78 (d, 1H, *J* = 9.0 Hz, Ar-H), 7.69 (d, 1H, *J* = 9.0 Hz, Ar-H), 7.60–7.65 (m, 2H, Ar-H), 7.46–7.51 (m, 2H, Ar-H); IR (KBr, ⁄cm): 3307 (ν_{NH}), 1667 ($\upsilon_{C=O}$), 1596, 1499, 1479 ($\upsilon_{C=C}$), 1525, 1348 (ν_{NO2}), 1323, 1172 (υ_{C-N}); EI-MS: *m/z* 524.2 [M+H]. $C_{27}H_{17}N_5O_7$ (523.11).

2-Methoxy-*N*-(4-(5-(2-methoxybenzamido)benzo[d]oxazol-2-yl)phenyl) benzamide (**7s**) (52%) as an off-white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.50 (s, 1H, N-H), 10.31 (s, 1H, N-H), 8.28 (s, 1H, Ar-H), 8.19 (d, 2H, *J* = 9.0 Hz, Ar-H), 8.00 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.74 (d, 2H, *J* = 9.0 Hz, Ar-H), 7.64–7.70 (m, 3H, Ar-H), 7.51–7.55 (dd, 2H, J_1 = 15.0 Hz, J_2 = 8.4 Hz, Ar-H), 7.21 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.09 (t, 2H, Ar-H), 3.92 (s, 6H, OCH_3); IR (KBr, ⁄cm): 3342 (ν_{NH}), 2944, 2840 (υ_{CH}), 1665 ($\upsilon_{C=O}$), 1596, 1533, 1502, 1482 ($\upsilon_{C=C}$), 1316, 1163 (υ_{C-N}); EI-MS: *m/z* 494.5 [M+H]. $C_{29}H_{23}N_3O_5$ (493.16).

N-(4-(5-acetamidobenzo[d]oxazol-2-yl)phenyl)acetamide (**7t**) (79%) as a light yellow powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.32 (s, 1H, N-H), 10.12 (s, 1H, N-H), 8.12 (d, 1H, *J* = 9.0 Hz, Ar-H), 8.09 (s, 1H, Ar-H), 7.81 (d, 2H, *J* = 9.0 Hz, Ar-H), 7.67 (d, 1H, *J* = 9.0 Hz, Ar-H), 7.48 (d, 2H, *J* = 9.0 Hz, Ar-H), 2.11 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$); IR (KBr, ⁄cm): 3302 (ν_{NH}), 1666 ($\upsilon_{C=O}$), 1600, 1532, 1500, 1481 ($\upsilon_{C=C}$), 1315, 1176 (υ_{C-N}); EI-MS: *m/z* 310.1 [M+H]. $C_{17}H_{15}N_3O_3$ (309.11).

2-Chloro-*N*-(4-(5-(2-chloroacetamido)benzo[d]oxazol-2-yl)phenyl)acetamide (**7u**) (82%) as a white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.69 (s, 1H, N-H), 10.50 (s, 1H, N-H), 8.17 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.10 (s, 1H, Ar-H), 7.84 (d, 2H, *J* = 9.0 Hz, Ar-H), 7.74 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.51–7.53 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.4 Hz, Ar-H), 4.31 (s, 4H, $COCH_2Cl$); IR (KBr, ⁄cm): 3278 (ν_{NH}), 2948 (υ_{CH}), 1673 ($\upsilon_{C=O}$), 1602, 1534, 1500, 1480 ($\upsilon_{C=C}$), 1338, 1178 (υ_{C-N}); EI-MS: *m/z* 378.4 [M+H]. $C_{17}H_{13}Cl_2N_3O_3$ (377.03).

2-Chloro-*N*-(4-(5-(2-chloropropanamido)benzo[d]oxazol-2-yl)phenyl)propanamide (**7v**) (83%) as an off-white powder having mp >300 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, p.p.m.) δ: 10.71 (s, 1H, N-H), 10.53 (s, 1H, N-H), 8.18 (d, 2H, *J* = 9.0 Hz, Ar-H), 8.13 (s, 1H, Ar-H), 7.86 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.74 (d, 1H, *J* = 9.0 Hz, Ar-H), 7.53–7.55 (dd, 1H, J_1 = 1.8 Hz, J_2 = 9.0 Hz, Ar-H), 4.72 (t, 2H, COCHCl), 1.65 (dd, 6H, J_1 = 3.0 Hz, J_2 = 6.6 Hz, CH_3); IR (KBr, ⁄cm): 3273 (ν_{NH}), 2981, 2931 (υ_{CH}), 1666 ($\upsilon_{C=O}$), 1600, 1538, 1499 ($\upsilon_{C=C}$), 1251, 1198 (υ_{C-N}); EI-MS: *m/z* 406.5 [M+H]. $C_{19}H_{17}Cl_2N_3O_3$ (405.06).

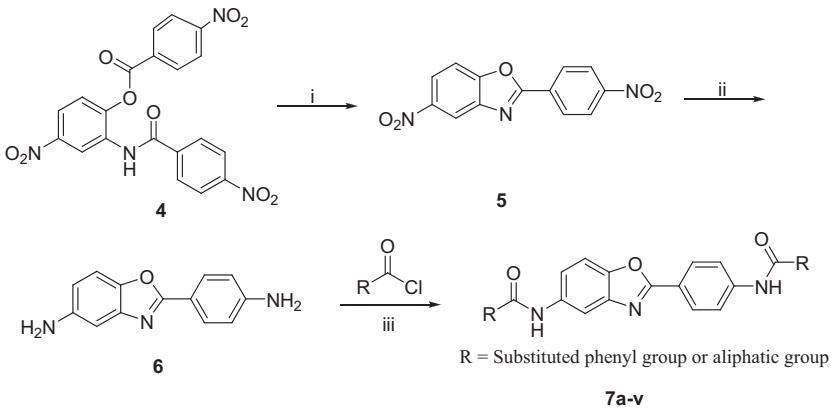
Anti-IFV activity assays (27–28)

The human influenza virus strains were A⁄Puerto Rico⁄8⁄34(A⁄H1N1), A⁄HK⁄7⁄87 (A⁄H3N2), and B⁄Hong Kong⁄5⁄72. The following control compounds were included: ribavirin (Virazole), obtained from ICN Pharmaceuticals (15 Morgan, Irvine, CA, USA); amantadine (Sigma-Aldrich, Bornem, Belgium); rimantadine (Sigma-Aldrich, Bornem, Belgium); and oseltamivir carboxylate (GS-4071) (a kind gift from T. Cihlar, Gilead Sciences, Foster City, CA, USA). All the compounds were evaluated for anti-influenza virus activities by the formazan-based 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*- tetrazolium (MTS) cell viability assay in the Madin–Darby canine kidney (MDCK) cells (CellTiter 96 AQueous One Solution Cell Proliferation Assay; Promega, Madison, WI, USA), and the spectrophotometric data were used to calculate the EC_{50} and 50% cytotoxic concentration (CC_{50}).

Results and Discussion

Synthesis of the compounds 7a–7v

The synthetic route of the target compounds **7a–7v** is outlined in Scheme 1. 4-Nitro-2-(4-nitrobenzamido) phenyl-4-nitrobenzoate (**4**) was readily prepared in excellent yields by the reaction of 2-amino-4-nitrophenol (**3**) with 4-nitrobenzoyl chloride (**2**), generated *in situ*



Reagents: (i) p-TSOH,xylene,140°C,8h; (ii) Pd-C, H_2; (iii) Et_3N,DMF

Scheme 1: The synthetic route of the benzoxazole derivatives **7a–v**.

from 4-nitrobenzoic acid (**1**). The key step for the formation of the benzoxazole backbone **5** was accomplished *via* the intramolecular cyclization reaction of intermediate **4**, using 4-methylbenzenesulfonic acid as the catalyst in refluxing xylene (29). The parent nucleus 5-amine-2-(4-aminophenyl)benzoxazole (**6**) was then obtained *via* reduction of **5** under H_2 (Pd-C) (30). Finally, condensation of **6** with various substituted aryl chloride gave the corresponding benzoxazoles **7a–7v** (Table 1 and Scheme 1). The synthesized compounds **7a–7v** were characterized by physicochemical and the MS, IR, and NMR spectral data that are in agreement with the assigned molecular structures (see Experimental Section).

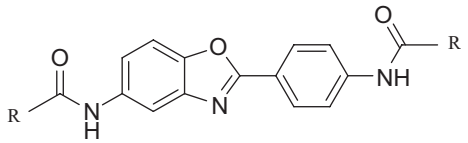
Anti-IFV activities of compounds 7a–7v

Compounds **7a–7v** were evaluated for their anti-IFV activity *in vitro* and monitored by the MTS assay in the MDCK cells (27,28). The reference drugs of oseltamivir carboxylate, ribavirin, amantadine, and rimantadine were used for positive control. The results were listed in Table 1, showing that only two compounds, **7h** and **7t**, from the series exhibited potent inhibitory effects against IFV-A replication in cell culture. The most potent and selective compound was found to be **7h** having an EC_{50} value of 37.03 μM against A/H3N2 subtype with a selectivity index (SI) of >5, which were all higher than that of the positive control drug oseltamivir (EC_{50} > 59.00 μM, SI > 1). The activity of **7h** against A/H1N1 (EC_{50} = 112.00 μM) was also higher than the activity of positive control drug amantadine (EC_{50} = 149.00 μM); however, the SI value was lower (SI > 1 and >3 for **7h** and amantadine, respectively). In addition, no compound exhibits inhibitory activity against influenza B virus.

Conclusions

In this paper, we have synthesized a series of novel 5-substituted-2-(4- substituted phenyl)-1,3-benzoxazole derivatives, which were structurally confirmed by IR, ^{1}H-NMR, and MS spectral analysis and

Table 1: Anti-IFV activities and cytotoxicities of the benzoxazoles **7a–7v**



Compound no.	R	Antiviral EC_{50} (μM)[a]			CC_{50}[b] (μM)	Selective index (SI)[c]		
		Influenza A H1N1	Influenza A H3N2	Influenza B		H1N1	H3N2	Influenza B
7a	4-Nitrophenyl	>191.04	>191.04	>191.04	>191.04	X1	X1	X1
7b	4-Fluorophenyl	>213.02	>213.02	>213.02	>213.02	X1	X1	X1
7c	4-Chlorophenyl	>199.06	>199.06	>199.06	>199.06	X1	X1	X1
7d	4-Bromophenyl	>169.13	>169.13	>169.13	>169.13	X1	X1	X1
7e	4-Iodophenyl	>145.93	>145.93	>145.93	>145.93	X1	X1	X1
7f	4-MeOphenyl	>202.63	>202.63	>202.63	>202.63	X1	X1	X1
7g	3,4-Cl_2 phenyl	>175.06	>175.06	>175.06	>175.06	X1	X1	X1
7h	3,4-$(MeO)_2$Ph	112.00	37.03	>180.64	>180.64	>1	>5	X1
7i	4-Cl-2-NO_2Ph	>168.82	>168.82	>168.82	>168.82	X1	X1	X1
7j	Phenyl	>230.70	>230.70	>230.70	37.60	<1	<1	<1
7k	4-Aminophenyl	>215.75	>215.75	>215.75	6.26	<1	<1	<1
7l	4-Methylphenyl	>216.68	>216.68	>216.68	19.72	<1	<1	<1
7m	4-t-butylphenyl	>183.26	>183.26	>183.26	1.10	<1	<1	<1
7n	4-Propylphenyl	>193.19	>193.19	>193.19	>193.19	X1	X1	X1
7o	3-Fluorophenyl	>213.02	>213.02	>213.02	>213.02	X1	X1	X1
7p	3-Chlorophenyl	>199.06	>199.06	>199.06	>199.06	X1	X1	X1
7q	3-Methylphenyl	>216.08	>216.08	>216.08	>216.08	X1	X1	X1
7r	3-Nitrophenyl	>191.04	>191.04	>191.04	1.15	<1	<1	<1
7s	2-MeOphenyl	>202.63	>202.63	>202.63	5.67	<1	<1	<1
7t	Methyl	202.06	74.68	>323.29	>323.29	>1	>4	X1
7u	Chloromethyl	>264.40	>264.40	>264.40	0.26	<1	<1	<1
7v	2-Chloroethyl	>246.15	>246.15	>246.15	>246.15	X1	X1	X1
Oseltamivir carboxylate		32.50	>59.00	27.50	>100.00	>3	>1	>3
Ribavirin		9.70	9.00	8.40	>100.00	>10	>11	>12
Amantadine		149.00	3.95	>500.00	>500.00	>3	>126	X1
Rimantadine		33.60	0.05	>500.00	258.00	7	5160	<1

[a]EC_{50}: concentration of compound required to achieve 50% protection of MDCK cell against IFV-induced cytotoxicity, as determined by the colorimetric formazan-based MTS assay.
[b]CC_{50}: 50% cell toxicity concentration determined by MTS assay.
[c]SI: selectivity index (CC_{50}/ EC_{50}). The SI values: X1 stand for ≥1 or <1.

evaluated for their anti-IFV (IFV-A H3N2, IFV-A H1N1, and IFV-B) activities by the MTS assay in the MDCK cells. The results showed that two of the derivatives exhibited excellent anti-IFV-A activities, and none of them was potent against IFV-B. Particularly, **7h** showed the highest activity and SI against A/H3N2 (EC_{50} = 37.03 μM, SI > 5), which was all higher than that of the reference drug oseltamivir (EC_{50} > 59.00 μM, SI > 1). Further studies including the elucidation of the mechanism of action on this novel family of potential antiviral inhibitors are ongoing in our laboratories and will be reported in due course.

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