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Design, synthesis and anti-influenza A virus activity of novel 2,4-disubstituted quinazoline derivatives

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ABSTRACT

Four 2,4-disubstituted quinazoline series containing various amide moieties were designed and synthesized as new anti-influenza A virus agents using the strategies of bio-isosterism and scaffold hopping. Many of them exhibit potent in vitro anti-influenza A virus activity and low cytotoxicity (CC_{50}: > 100 μM). Particularly, compounds **10a5** and **17a** show better activity (IC_{50}: 3.70–4.19 μM) and higher selective index (SI: > 27.03, > 23.87, respectively) against influenza A/WSN/33 virus (H1N1), opening a new direction for quinazoline derivatives in anti-influenza A virus field.

Influenza, which is caused by the influenza virus, remains one of occasional pandemics of respiratory diseases worldwide with approximately 3–5 million cases of severe disease, many hospitalisations and about 290,000 to 650,000 respiratory deaths every year.[1–2] As major respiratory disease pathogens, influenza A viruses (IAV) cause seasonal epidemics or spread worldwide in a pandemic when novel strains

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emerge, such as four influenza pandemics worldwide in history: Spanish influenza in 1918, Asian influenza in 1957, Hong Kong influenza in 1968, Mexico influenza in 2009.[3–5]

Currently, three classes of anti-influenza drugs are available for the treatment of flu, namely, M2 ion-channel blockers (amantadine and rimantadine), neuraminidase inhibitors (oseltamivir, zanamivir, peramivir and laninamivir octanoate),[6] and RNA polymerase inhibitor (Favipiravir).[7] Encouragingly, baloxavir marboxil (BXM) has been approved for the treatment of flu, which inhibits the cap-dependent endonuclease (CEN).[8] Unfortunately, the M2 ion-channel inhibitors are no longer recommended for treatment of influenza and NA inhibitors have several limitations in clinical practice due to their drug resistance and severe side effects.[9–13] Therefore, it is still an urgent demand to develop new antiviral agents for IAV infection with new scaffold and novel mechanism of action.

In our precious studies,[14,15] 3-substituted indole derivatives have been identified as anti-IAV agents with significant activity against A/WSN/33 (H1N1) virus. We found that these compounds inhibited IAV replication at the post-entry stage and might target viral RNA transcription and replication. On the other hand, quinazoline is a biologically imperative scaffold known to be linked with a wide range of pharmacological activities like anticancer,[16] anti-viral,[17] analgesic,[18] anti-inflammatory,[19] anti-hypertensive,[20] antitubercular[21] and antibacterial[22] activities. It is well-known that bio-isosterism and scaffold hopping are the strategies for discovering structurally novel compounds.[23,24] However, 2,4-disubstituted quinazoline anti-IAV agents have not been reported in the literature.

Based on the above facts and as part of our persistent efforts to develop potential antiviral candidates, we planned to take place of the indole core with quinazoline scaffold and meanwhile did structural modifications at the 2-position of the quinazoline core and amide part of the 4-position (Fig.1). Thus, we designed and synthesized a series of 2,4-disubstitued quinazoline derivatives and evaluated their anti-IAV activity in this study, aiming at finding new quinazoline derivatives with potent anti-IAV activity and facilitate the further development of these compounds.

The target compounds **7a–j**, **10a–b**, **14a–l** and **17a–c** were synthesized by following the pathway described in Scheme 1. The quinazoline core **3** was easily obtained via cyclization of anthranilic acid **1** and urea, and then chlorination.[25,26] Nucleophilic substitution of **3** with ethyl glycolate and pyrrolidine successively,[27] and then hydroxlysis gave 2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)oxy)acetic acid **6**. Coupling of acid **6** with various amines achieved quinazoline derivatives **7a–j**. The hydroxlysis of key intermediate **4** with LiOH, and then amidation with aniline or benzylamine afforded the amides **9a–b**. The chlorine at the 2-position of **9a–b** was substituted with available secondary amines to provide the target compounds **10a–b**. The synthesis of the compounds **14a–l** and **17ac** were similar to that of **7a–j** and **10a–b**. The structures of all the newly synthesized target compounds were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS (Supplementary material).

For preliminary screening of anti-IAV candidates, the target compounds **7**, **10**, **14**, **17** and several intermediates were first investigated for their inhibitory effect on IAV A/WSN/33 (H1N1) at 10 μM using the *Gaussia luciferase* (Gluc) reporter assay in 293T-Gluc cells[28] and the results are shown in Fig. 2. Contrary to our predictions, the results indicated that all the newly synthesized quinazoline derivatives exhibited a moderate to low inhibition ratio to IAV infection.

According to the result of preliminary screening, the IC_{50} values of the selected quinazoline derivatives along with ribavirin for comparison were summarized in Table 1. Overall, the antiviral activity of these compounds was equivalent to or weaker than that of the previous indole derivatives. However, some compounds still were identified as potential anti-IAV agents which deserved further structural modification. The selected compounds had potent in vitro anti-IAV activity and showed low cytotoxicity (CC_{50}: > 100 μM) except for **7i** (CC_{50}: 64.8 μM). Compared with ribavirin, compounds **10a5**, **14c** and **17a** (IC_{50}s: 3.70–8.64 μM) showed significantly better activity against IAV and higher selective indexes (SI > 11.57–27.03). Moreover, the most active compound **10a5** (IC_{50}: 3.17 ± 0.82 μM) was found to be 4.1-fold more potent than the reference drug, suggesting that the application of quinazoline scaffold in anti-IAV is promising. For the action mechanism, it may also act on viral RNA transcription and replication, similar to previous indole compounds, which needs to be further verified.

As shown in Fig. 2 and Table 1, the anti-IAV activity of the quinazoline derivatives in this study depends on both of the groups at the C-2 and C-4 positions. For O-acetamide groups at C-4, the nature of the substituents greatly influence activity. The introduction of naphthalen-2-yl (**7f**), cyclopropyl (**7g**) and adamantan-1-yl (**7j**) groups markedly reduces or even loses activity, which may be attributed to the size of rigid structures. Compounds with phenyl, benzyl and furfuryl groups have similar activities, while compounds with thiazol-2-yl (**7h**) and a 2-ethoxyl substitution on the benzene ring (**7i**) show relatively higher activity. Although a simple SAR is difficult to explore, aromatic ring is crucial for their anti-IAV effect.

In further modifications, we kept phenyl or benzyl groups at the amide terminal on the C-4 positions and investigated the effect of the substituent groups at C-2 position. The relative contribution of the substituents at the C-2 position to activity is as follows: dimethylamino (**10a5**) > 3,4-dihydroisoquinolin-2(1H)-yl (**10a1** and **10b1**) > tetrahydro-2H-pyran-4-yl (**10a3**) > pyrrolidin-1-yl (**7a**) ≈ diethylamino (**10a4** and **10b2**) > 4-methylpiperazin-1-yl (**10a2** and **10b3**).

On the other hand, switching the sense of the amide group at C-4 (O-ethylamines) leads to some increase in potency compared to O-acetamides at C-4 (i.e. **14a** vs **7g**, **14k** vs **7b**). Surprisingly, introduction of cyclohexyl (**14d**) and 4-acetylphenyl (**14j**) lead to the loss inhibitory activity. Although there are small differences in activity between the electron-donating groups and the electron-withdrawing groups on benzene ring, 4-trifluoromethyl (**14c**, Table 1) is preferred. For heterocyclic groups at C-2 position, 3,4-dihydroisoquinolin-2(1H)-yl makes more contribution to anti-IAV activity than diethylamino (**17a** vs **17b** vs **17c**, Fig.2), while 4-methylpiperazin-1-yl is the lowest (Table 1)

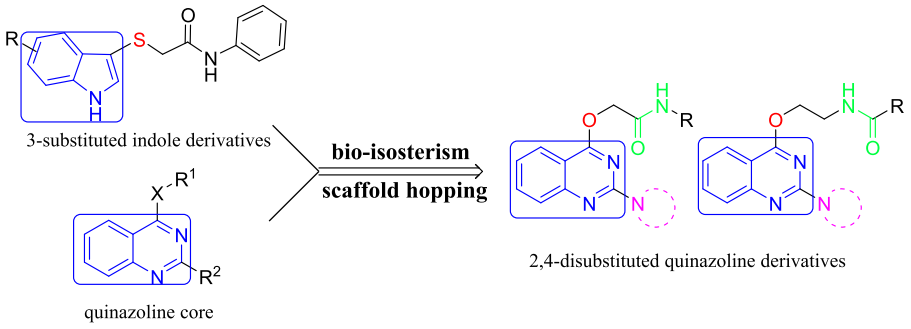
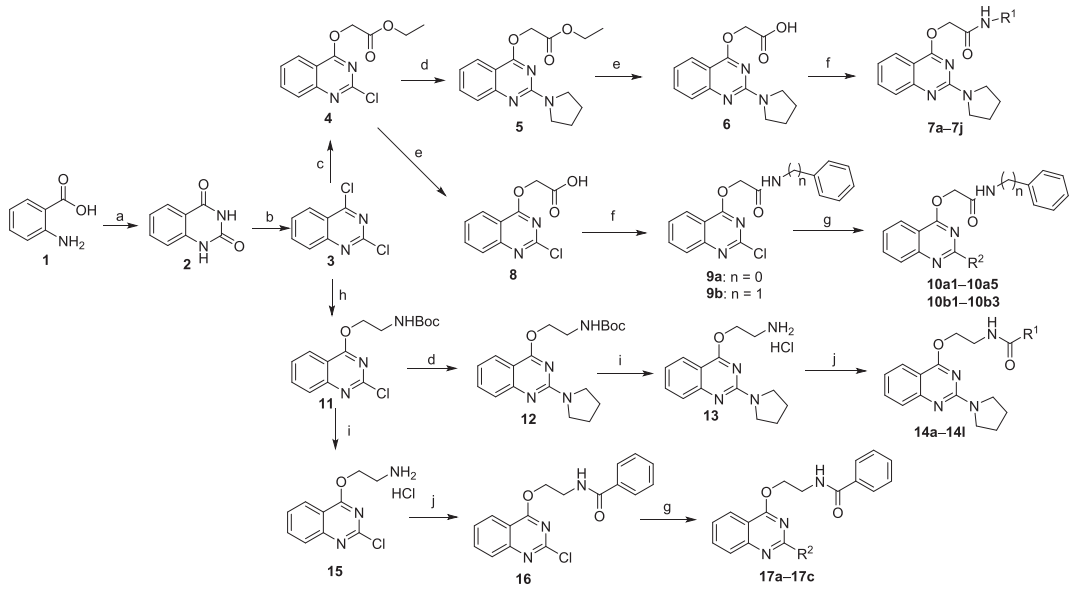


Fig. 1. Design of new quinazoline derivatives.



Scheme 1. Synthesis of the target compounds **7**, **10**, **14** and **17**.

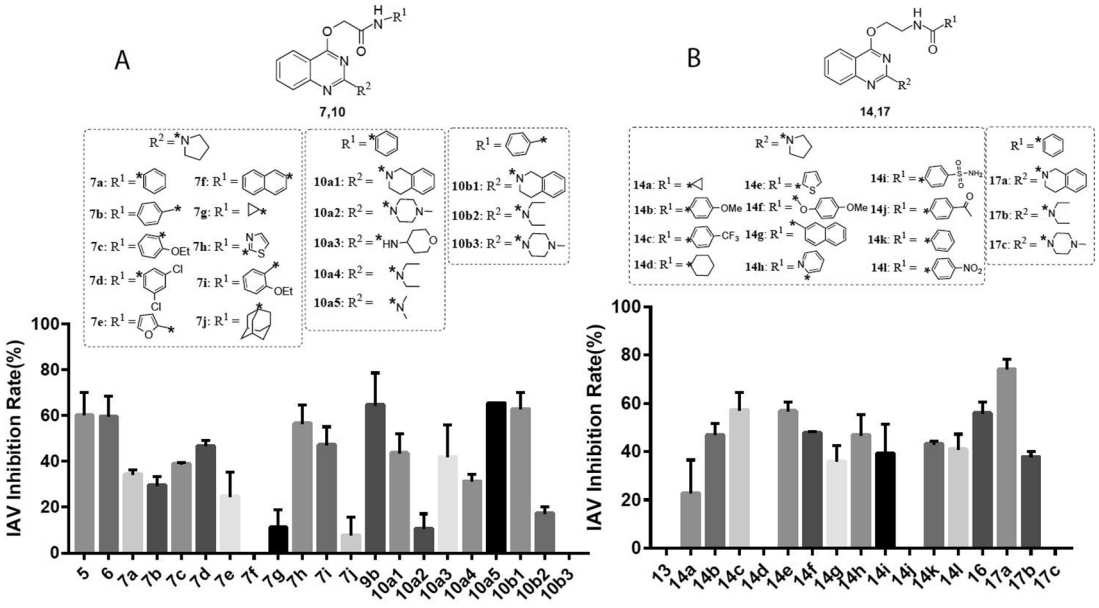


Fig. 2. Inhibition rate of the target compounds on IAV. (A: O-acetamides at C-4, B: O-ethylamines at C-4; the IAV inhibition rates were calculated by GraphPad Prism 7.)

which is similar as above in the O-acetamide derivatives.

Subsequently, intermediates **5**, **6**, **9b**, **13** and **16** were tested for their anti-IAV activity (Fig. 2 and Table 1). Intermediate **13** have no activity (Fig. 2), but **5**, **6**, **9b** and **16** (IC_{50} = 16.62–60.10 μM) fortunately display potent anti-IAV activity, which indicates that a ester or amide group is permitted at the C-4 position and the substituents at C-2 is essential for activity.

In conclusion, we reported the design and synthesis of four 2,4-disubstituted quinazoline series containing various amide moieties and tested their anti-influenza A/WSN/33 (H1N1) virus activities. Many of them exhibit potent in vitro anti-IAV activity and low cytotoxicity (CC_{50}: > 100 μM). The subsequent SAR studies showed that three compounds **10a5**, **14c** and **17a** exhibited the highest anti-influenza virus activity with IC_{50} of less than 10 μM (IC_{50}: 3.70–8.64 μM). The results reported here demonstrate the promising application of quinazoline scaffold in anti-IAV and provide a foundation for discovering more potent novel 2,4-disubstituted quinazoline anti-IAV agents. Further modifications on of these compounds are in progress.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 1
Anti-IAV A/WSN/33 (H1N1) activity and cytotoxicity of the selected compounds.

Cpd.	R^1	R^2	IC_{50}(μM)	CC_{50}(μM)	SI
5	–	pyrrolidin-1-yl	16.62 ± 1.61	> 100	> 6.0
6	–	pyrrolidin-1-yl	60.10 ± 3.78	> 100	> 1.66
7h	thiazol-2-yl	pyrrolidin-1-yl	39.44 ± 9.65	> 100	> 2.53
7i	2-ethoxybenzyl	pyrrolidin-1-yl	17.66 ± 4.23	64.80 ± 3.22	3.67
9b	benzyl	Cl	26.50 ± 2.12	> 100	> 3.77
10a5	phenyl	dimethylamino	3.70 ± 0.82	> 100	> 27.03
10b1	benzyl	3,4-dihydroisoquinolin-2(1H)-yl	17.71 ± 1.07	> 100	> 5.65
14c	4-trifluoromethylphenyl	pyrrolidin-1-yl	8.64 ± 1.76	> 100	> 11.57
14e	thiophene-2-yl	pyrrolidin-1-yl	18.18 ± 0.32	> 100	> 5.50
14f	4-methoxyphenoxymethyl	pyrrolidin-1-yl	55.33 ± 5.69	> 100	> 1.81
16	phenyl	Cl	48.04 ± 7.38	> 100	> 2.08
17a	phenyl	3,4-dihydroisoquinolin-2(1H)-yl	4.19 ± 0.43	> 100	> 23.87
ribavirin			15.36 ± 0.93	> 100	> 6.51

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127143. These data include MOL files and InChiKeys of the most important compounds described in this article.

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