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PII: S0223-5234(20)30678-4

DOI: https://doi.org/10.1016/j.ejmech.2020.112706

Reference: EJMECH 112706

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 25 April 2020

Revised Date: 28 July 2020

Accepted Date: 28 July 2020

Please cite this article as: G. Zhang, M. Wang, J. Zhao, Y. Wang, M. Zhu, J. Wang, S. Cen, Y. Wang, Design, synthesis and in vitro anti-influenza A virus evaluation of novel quinazoline derivatives containing S-acetamide and NH-acetamide moieties at C-4, *European Journal of Medicinal Chemistry*, https://doi.org/10.1016/j.ejmech.2020.112706.

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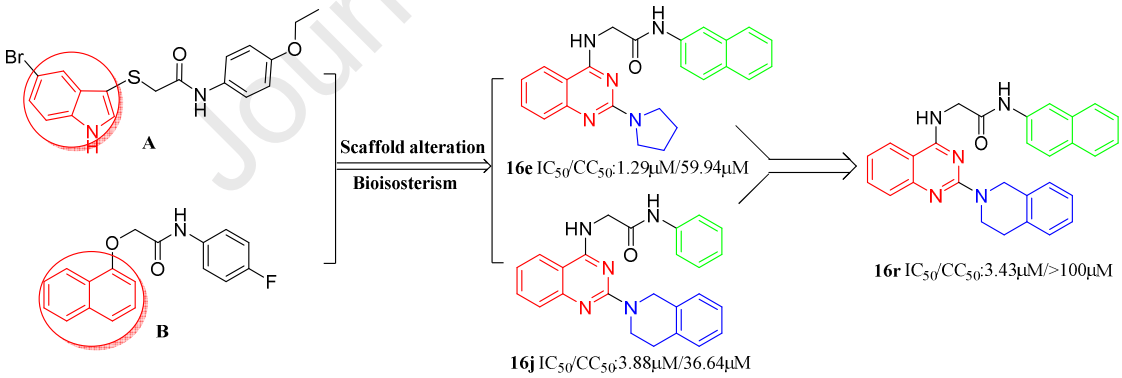

# Design, synthesis and in vitro anti-influenza A virus evaluation of novel quinazoline derivatives containing S-acetamide and NH-acetamide moieties at C-4

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**Abstract:** It is an urgent need to develop more effective anti-influenza agents due to the emergence of highly pathogenic and drug-resistant influenza viruses. Herein, a series of 2,4-disubstituted quinazoline derivatives were designed, synthesized and their antiviral activities against influenza A virus were evaluated. Nine compounds (**10a2**, **16a**, **16e**, **16i**, **16j**, **16n**, **16o**, **16p** and **16r**) showed potent activity against influenza A virus (IAV) with $IC_{50}$ at the low-micromole level (1.29–9.04 μM). Particularly, **16e** and **16r** possess good anti-IAV activity ($IC_{50}$: 1.29 μM and 3.43 μM, respectively) and acceptable cytotoxicity, and inhibit the transcription and replication of viral RNA. Together with reasonable PK profiles of **16e**, these results suggest their promising potential as candidates for further investigation.

**Key words**: Anti-influenza A virus activity, quinazoline derivatives, synthesis, structure-activity relationships

## 1. Introduction

Influenza, or “flu”, is a highly contagious respiratory infectious diseases caused by the influenza virus and is responsible for approximately 3–5 million cases of severe disease, many hospitalisations and about 290,000 to 650,000 respiratory deaths worldwide every year [1–5]. Influenza virus can be divided into four types: influenza A, B, C and D [6]. Among them, influenza A virus (IAV), a linear, negative-sense, single-stranded RNA virus, possesses high pathogenicity and is the main cause of seasonal epidemics and occasional pandemics of respiratory diseases worldwide, which belongs to the *Orthomyxoviridae* family[7–11].

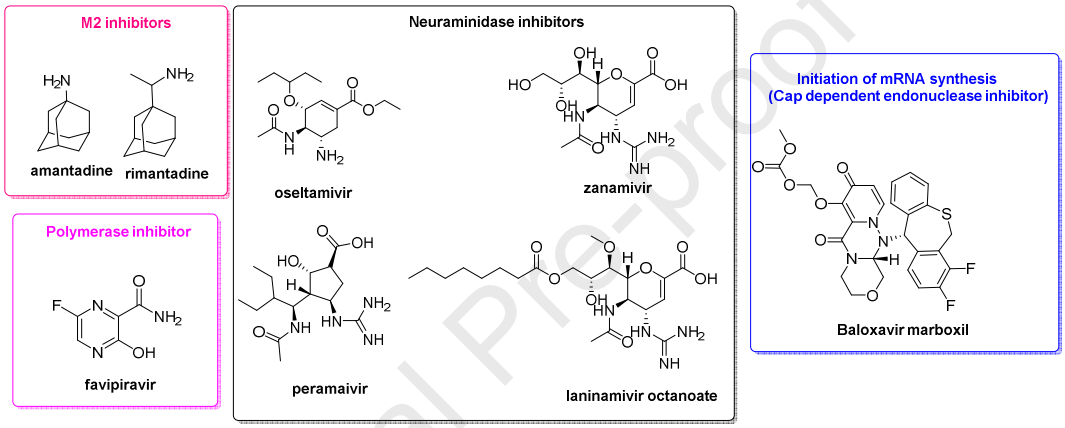



**Fig. 1**. Four classes of approved antiviral agents for the treatment of influenza virus infection

However, there are limited options in the clinic treatment of influenza infections. Up to now, only two classes of anti-influenza drugs are approved by the FDA, namely, M2 ion-channel blockers (amantadine and rimantadine) and neuraminidase inhibitors (oseltamivir, zanamivir, peramivir and laninamivir octanoate)(Fig. 1)[12]. In addition, as a RNA polymerase inhibitor, Favipiravir(Fig. 1) was approved by Japan against influenza infection in 2014(Fig. 1)[13] and Baloxavir marboxil (BXM)(Fig. 1), an orally available small molecule inhibitor of cap-dependent endonuclease (CEN), was approved for the treatment of uncomplicated influenza in Japan and the United States of America in 2018(Fig. 1)[14]. 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 [15–19]. Safe and effective therapy for IAV infection is still a high unmet medical

need. Therefore, we are constantly required to develop new antiviral agents with new scaffold and novel mechanism of action.

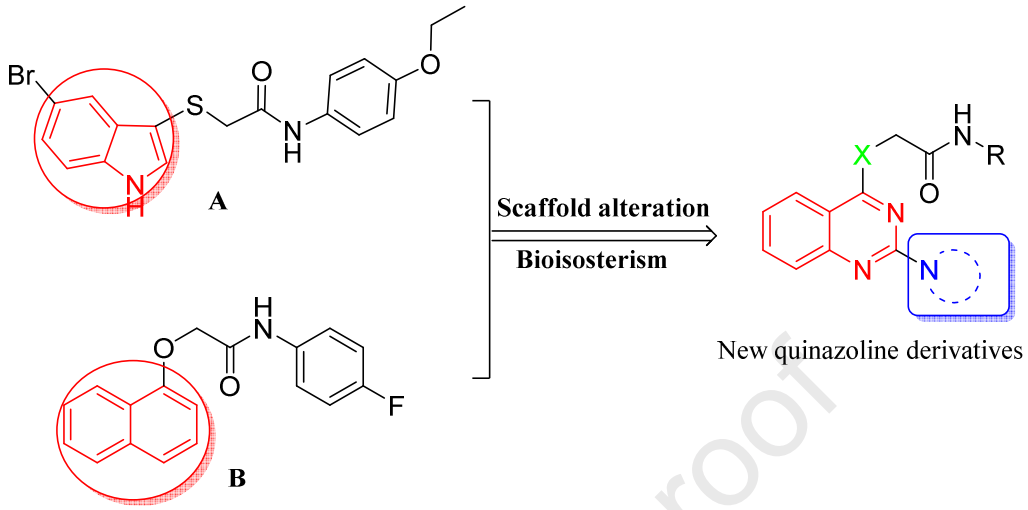



**Fig. 2**. Design of new quinazoline derivatives

In our previous studies [20], a series of substituted indole derivatives were synthesized and most of them displayed significant activity against IAV A/WSN/33 (H1N1) strain. Among them, two compounds **A** and **B** with indole and naphthalene ring displayed more potential *in vitro* anti-IAV activity ($IC_{50}$: 4.18 μM and 5.22 μM, respectively, Fig.2). We also reported that 3-substituted indole derivatives inhibited IAV replication at the post-entry stage and might target viral RNA transcription and replication [21].

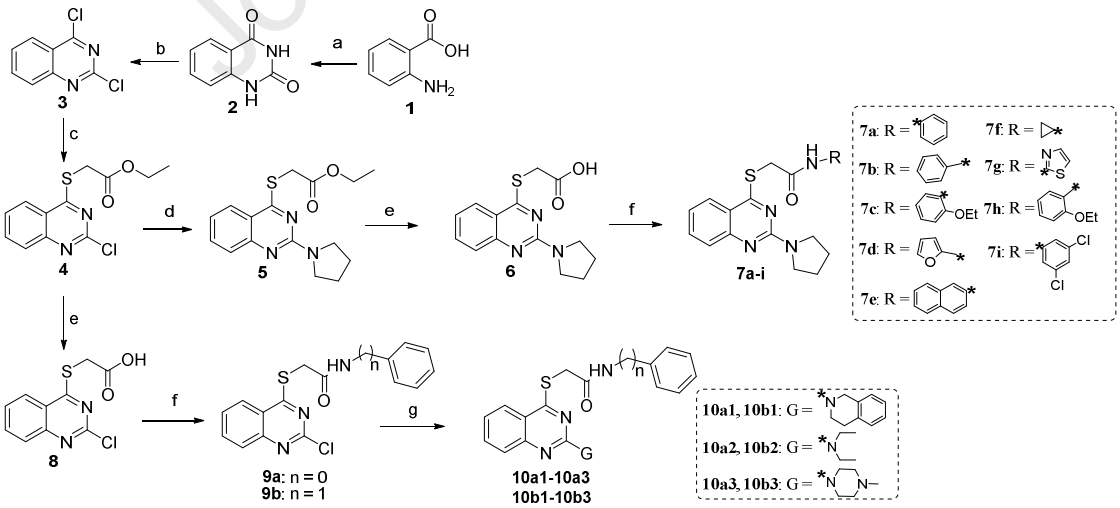
Based on the above research results, and as part of our persistent efforts to develop potential antiviral candidates, we intended to replace the indole and naphthalene scaffold with quinazoline scaffold employing the strategies of bioisosterism and scaffold alteration to explore the structure-activity relationship (SAR) (Fig. 2). Here, we designed and synthesized a series of 2,4-substitued quinazoline derivatives containing S-acetamide and NH-acetamide moieties and evaluated their anti-IAV activity in this study, aiming at developing these compounds into a new class of anti-IAV drug candidates.

## 2. Results and Discussion

### *2.1. Chemistry*

The target compounds **7a–i** and **10a–b** were synthesized by following the pathway

described in Scheme 1. The quinazoline-2,4(1H,3H)-dione core was constructed by reacting anthranilic acid **1** with urea at 180□. Subsequently, the two carbonyl groups in compound **2** were subjected to chlorination with phosphoryl chloride ($POCl_3$) to produce the 2,4-dichloroquinazoline **3**. Under basic conditions, the 4-position chlorine of **3** was selectively substituted with ethyl thioglycolate to give the key intermediate **4**. To confirm the regioselectivity of this reaction, we did HMBC spectrum analysis of compound **4** and found that there is a coupling between the newly introduced methylene hydrogen and the 4-position carbon atom (Fig. 3). Nucleophilic substitution of intermediate **4** with pyrrolidine in the presence of N,N-diisopropylethylamine (DIPEA) yielded the corresponding compound **5**, which was hydrolyzed subsequently to give acid **6**. In the presence of HATU and DIPEA, intermediate **6** reacted with different amines to afford the target compounds **7a–7i**. On the other hand, the key intermediate **4** was hydrolyzed in the presence of LiOH to give the corresponding acid **8**. Amidation of acid **8** with aniline or benzylamine in the presence of HATU and DIPEA afforded the amides **9a–9b**. Finally, the chlorine at the 2-position of **9a–b** was substituted with available piperazine or piperidine derivatives to provide the target compounds **10a–b**.




Reagents and conditions: a) urea, 180℃, 4 h; b) $POCl_3$, DIPEA, $CH_3CN$, 100℃, 6 h; c) DIPEA, ethyl thioglycolate,THF, RT, 2 h; d) pyrrolidine, DIPEA,THF, 80℃, 1 h; e) LiOH, $CH_3CH_2OH$/THF/$H_2O$, 40 min; f) $RNH_2$, HBTU, DIPEA, DCM, 4 h; g) amines, DIPEA, THF, 110℃, 10 h.

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

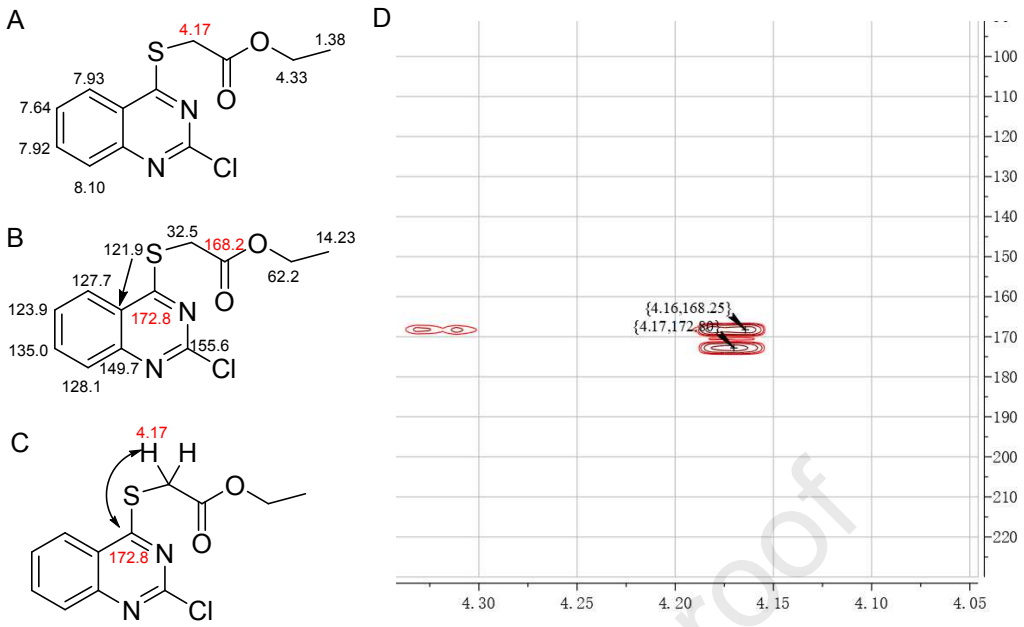
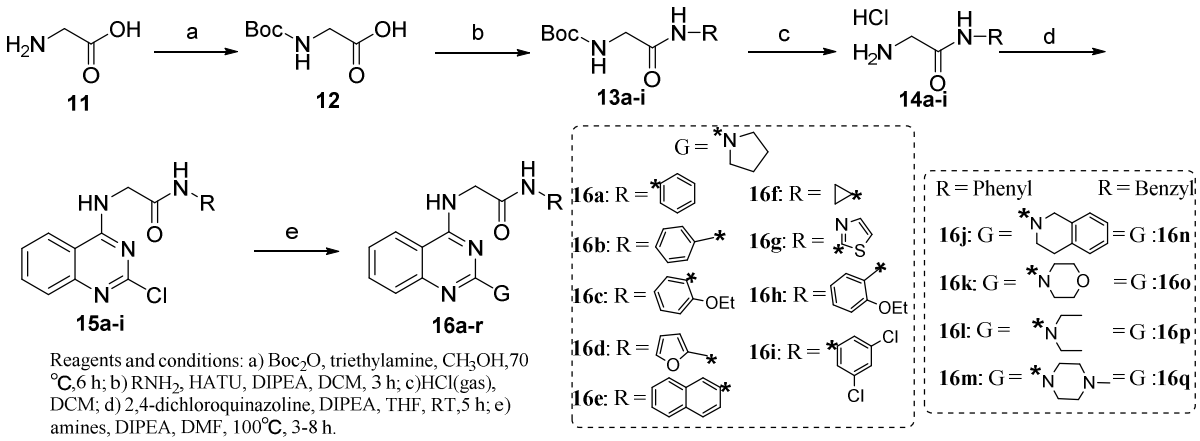



**Fig. 3**. Structure and HMBC spectrum of compound **4** ( A: Chemical shift of hydrogen atom in $^{1}$HNMR; B: Chemical shift of carbon atom in $^{13}$CNMR; C: Coupling between the newly introduced methylene hydrogen and the 4-position carbon atom; D: Part of HMBC-spectrum of compound **4**)

The synthesis of quinazoline derivatives **16a–r** are shown in Scheme 2. Commercially available glycine **11** was protected by ditertbutyl dicarbonate to afford the corresponding Boc-glycine **12**. Amidation of **12** with different amines in the presence of HATU and DIPEA afforded the amides **13**. The subsequent deprotection afforded the desired intermediate **14**, which underwent a coupling reaction with 2,4-dichloroquinazoline **3** to generate the key intermediate **15**. Finally, compounds **15** were reacted with appropriate amines in the presence N,N-diisopropylethylamine (DIPEA) to obtain the desired products **16a–r** in moderate to good yields.




**Scheme 2.** Synthesis of the target compounds **16**

*2.2 Anti-influenza virus activity*

The target compounds **7**, **10**, and **16** were initially screened for their inhibitory effect against IAV A/WSN/33 (H1N1) at a concentration of 10 μM in HEK293T-Gluc cells and the results were shown in Fig.1. Notably, most of the tested compounds exhibited a moderate to high inhibition ratio to IAV infection. Among them, compounds **16a**, **16e** and **16j** showed the most potent inhibition ratio (95.84%, 99.84%, 98.21%, respectively), which was higher than compounds A and B (90.21% and 78.04%, respectively). Overall, the potency of the quinazoline derivatives in this study depends on both of the groups at C-2 and C-4 positions and the NH-acetamide derivatives (**16**) are more active than the S-acetamides (**7**, **10**).

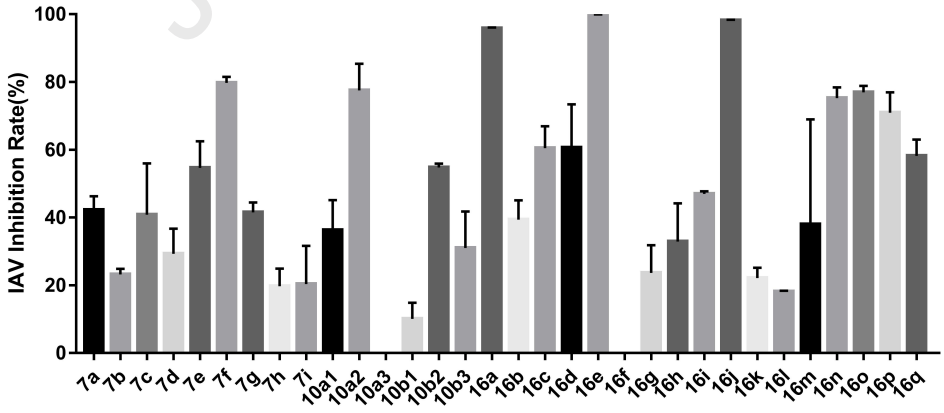



**Fig. 4.** Inhibition rate of the target compounds on IAV.

An exploration on C-4 (R) of quinazoline core revealed a flat SAR (Fig.1). All the modifications on R are less beneficial to anti-IAV activity, even decreased the antiviral activity, except for **7e** and **7f**, suggesting that naphthalen-2-yl and

cyclopropyl are acceptable. Furthermore, insertion of a methylene between the benzene ring and amide bond leads to decreased activity. For example, N-phenyl acetamide derivatives provided better anti-IAV activity than N-benzyl acetamide derivatives (**7a** *vs* **7b**, **7c** *vs* **7h**). Additionally, replacement of the pyrrolidin-1-yl at C-2 with other amino groups has a significant effect on the activity and diethylamino group (**10a2**, **10b2**) is optimal for the C-2 position.

In the next round of optimization, we replaced the sulfur atom with nitrogen atom employing the strategy of bioisosterism with the aim of improving the antiviral potency. To our delight, we observed the NH-acetamide derivatives (**16**) are much more active than the S-acetamides (**7**, **10**), indicating that nitrogen atom is preferred over sulfur atom. The naphthalen-2-yl and phenyl at C-4 were identified to be crucial for antiviral activity. Compound **16a** and compound **16e** were optimal for excellent anti-IAV activity (IR = 99.84% and 95.84%, respectively). On the other hand, replacing the pyrrolidin-1-yl by other secondary amines (from **16j** to **16q**) significantly enhances the anti-IAV activity, except for **16k**, **16l** and **16m**. The anti-IAV activity of the quinazoline derivatives in this study depends on both of the groups at the C-4 and C-2 positions and the optimal combination needs further discussion in the future.

Thirteen compounds identified with high inhibition rates in the initial screening were selected for the dose response assays (Table 1). The $IC_{50}$ of ribavirin used as a positive control was 15.36 μM. Among them, eight compounds (**10a2**, **16a**, **16e**, **16i**, **16j**, **16n**, **16o** and **16p**) showed stronger anti-influenza A/WSN/33 (H1N1) activities with $IC_{50}$ at micromole level (1.29–9.04 μM). Compound **16e** showed the strongest inhibitory activity with $IC_{50}$ value of 1.29 μM in this study. Subsequently, compound **16r** synthesized by replacing the pyrrolidin-1-yl of **16e** with 3,4-dihydroisoquinolin-2(1H)-yl of **16j** remains excellent activity ($IC_{50}$: 3.43±0.54 μM) and exhibited lower cytotoxicity with $CC_{50}$ values of up to and beyond 100 μM.

**Tale 1** Anti-influenza virus activity and cytotoxicity of the selected compounds in HEK293T-Gluc cells

| Cpd. | $IC_{50}$（μM） | $CC_{50}$（μM） | SI |
|---|---|---|---|
| **7e** | 36.10±7.88 | >100 | >2.77 |
| **7f** | 38.24±7.40 | >100 | >2.61 |
| **10a2** | 7.18±1.89 | >100 | >13.93 |
| **10b2** | 19.15±0.73 | >100 | >5.22 |
| **16a** | 1.88±0.10 | 23.28±2.91 | 12.38 |
| **16d** | 39.43±0.93 | >100 | >2.54 |
| **16e** | 1.29±0.01 | 59.94±3.04 | 46.46 |
| **16i** | 9.04±0.57 | 15.86±0.58 | 1.75 |
| **16j** | 3.88±0.47 | 36.64±2.24 | 9.44 |
| **16n** | 6.84±0.68 | 29.43±0.95 | 4.30 |
| **16o** | 3.83±0.15 | >100 | >26.11 |
| **16p** | 5.00±1.37 | >100 | >20.00 |
| **16q** | 11.47±0.54 | >100 | >8.72 |
| **16r** | 3.43±0.54 | >100 | >29.15 |
| **A** | 4.18±0.12 | 43.98±2.17 | 10.52 |
| **B** | 5.22±0.34 | >100 | >19.15 |
| **ribavirin** | 15.36±0.93 | >100 | >6.51 |

As shown in Table 1, the cytotoxicity of the fourteen compounds was also investigated in HEK293T-Gluc cells using a cell counting kit-8 (CCK-8) assay. Most compounds displayed low cytotoxic ($CC_{50}$ > 100 μM), whereas the introduction of nitrogen atom at C-4 resulted in increased cytotoxicity (**16a**, **16i** and **16n**). Considering both of the activity and cytotoxicity, compound **16e** and **16r,** both of that hold the higher selective index (SI >29.15) among these analogues, could be selected as a lead compound for further modification.

Encouraged by their strong potency against influenza virus IAV A/WSN/33 (H1N1) strain, eight compounds were evaluated against influenza virus IAV

A/PR/8/1934 (H1N1) strain in HEK293T-Gluc cells. As shown in Table 2, all tested compounds turned out to be potent inhibitors against influenza virus IAV A/PR/8/1934 (H1N1) with $IC_{50}$ values from 0.12 to 6.05 μM, suggesting their promising potential antiviral activity. In addition, we assessed the cytotoxicity of these compounds on A549 cells, a cell line that is widely used in IAV study. The results showed that most of the compounds displayed low to medium toxicity on A549 cells (Table 3), similar to what found in 293T cells. Despite of an increasing cytotoxicity of **16e**, **16i**, **16j** and **16n** on A549 compared with 293T, much higher values of $CC_{50}$ than $IC_{50}$ indicate that their antiviral activities are not due to cytotoxicity. Furthermore, the similar cytotoxicity profile of these compounds on both cell lines would help us to improve their safety by further optimization in the future.

**Table 2** Anti-influenza virus activity of the selected compounds in 293T-GLUC cells

| Cpd. | $IC_{50}$ (μM) | Cpd. | $IC_{50}$ (μM) |
|---|---|---|---|
| **10a2** | 1.69±0.21 | **16n** | 0.12±0.03 |
| **16a** | 1.47±0.12 | **16o** | 6.05±0.32 |
| **16e** | 0.63±0.02 | **16p** | 5.80±0.68 |
| **16i** | 0.95±0.08 | **A** | 0.33±0.01 |
| **16j** | 0.41±0.09 | | |

**Table 3** The cytotoxicity of selected compounds on A549 cells

| Cpd. | $CC_{50}$（μM） | Cpd. | $CC_{50}$（μM） |
|---|---|---|---|
| **10a2** | **>** 100 | **16n** | 12.84±0.03 |
| **16a** | 19.04±1.34 | **16o** | **>** 100 |
| **16e** | 4.77±0.13 | **16p** | **>** 100 |
| **16i** | 5.92±0.14 | **A** | 71.41±3.4 |
| **16j** | 10.72±0.18 | | |

*2.3 Primary Mechanism of compound **16e** against IAV*

Next, compound **16e** was selected for further mechanisms study against the influenza A virus. We initially carried out a time-of-addition experiment for **16e** to

investigate the possible time-dependent inhibitory effects on influenza replication, and the results were shown in Figure 4. When compound **16e** was added before viral infection (from -1 to 0 h), no reduction in viral yield was observed. However, **16e** displayed a significant inhibitory effect on influenza virus when added after viral infection, particularly in the early stage (from 2 to 4 h), suggesting that **16e** affects the early steps of the replication cycle.

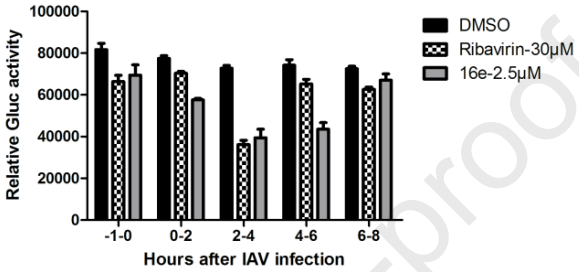



**Fig.5.** Times-of-addition of **16e** after infected with IAV**.** A549 cells were inoculated with influenza single-cycle A/WSN/33(H1N1) virus at a multiplicity of infection of 0.2 and compound **16e** (2.5 μM) was added. Viral yields were determined at 11 h post infection by measuring Gluc activity. In this experiment, DMSO was used as the negative control and ribavirin (30 μM) as the positive control.

In order to further confirm the mode of action of these compounds against influenza virus, we investigated the effect on IAV RNA transcription and replication using an IAV mini-genome replicon system. As shown in Fig.5, compared with the negative control, compound **16e** could significantly inhibited the activity of luciferase in a dose-dependent manner, and the activity of luciferase was inhibited by more than 80% at 2.5 μM. These results suggest that these compounds may target viral RNA transcription and replication.

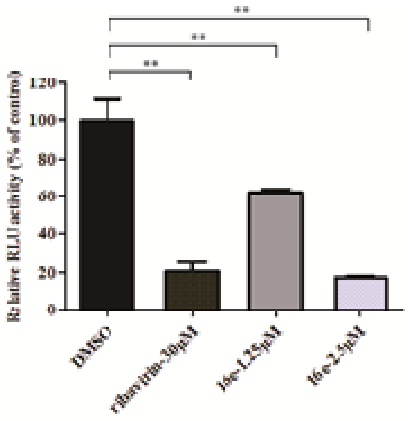



**Fig.6.** Inhibition of compound **16e** on influenza viral vRNP. HEK239T cells were transfected with pCAGGS expression plasmids encoding PB2、PB1、PA、NP、pol-LUC and SV40-Relina in the absence or presence of compound **16e** (1.25 μM or 2.5 μM). Effect of vRNA transcription was evaluated by measuring luciferase and relina at 24 h post-transfection. ** indicates $p<0.01$ as compared to negative control.

*2.4 Lipinski's rules and in vivo PK profiles*

Lipinski's rules are important guidelines for determining drug-likeness compounds [22]. The related theoretical values of most ten potent compounds were obtained using Molinspiration free online software (https://www.molinspiration.com). As shown in Table 4, none violation of Lipinski's rule-of-five was found among compounds **10a2**, **16a**, **16n**, **16o**, **16p** and **16q**. The logP of compounds **16e**, **16i**, **16j** and **16r** are out of the recommended range ($0 \leqslant \log P \leqslant 5$), which indicates they have a bad hydrophilicity ratio to be bioavailable compounds. However, they are still incorporate with the Lipinski's rule-of-five (violations $\leqslant 1$). The calculated polar surface area values were much lower than 140, predicting adequate intestinal absorption.

**Table 4** Parameters of Lipinski's rules

| Cpd. | LogP[1](≤5) | TPSA[2] | nON[3] (≤10) | nOHNH[4] (≤5) | Vol (Å$^3$) | MW[5] (≤500) | Lipinski's violations (≤1) |
|---|---|---|---|---|---|---|---|
| **10a2** | 4.77 | 58.12 | 5 | 1 | 336.96 | 366.49 | 0 |
| **16a** | 4.05 | 70.15 | 6 | 2 | 320.87 | 347.42 | 0 |
| **16e** | 5.24 | 70.15 | 6 | 2 | 364.86 | 397.48 | 1 |
| **16i** | 5.34 | 70.15 | 6 | 2 | 347.94 | 416.31 | 1 |
| **16j** | 5.04 | 70.15 | 6 | 2 | 375.48 | 409.49 | 1 |
| **16n** | 4.88 | 70.15 | 6 | 2 | 392.28 | 423.52 | 0 |
| **16o** | 3.33 | 79.38 | 7 | 2 | 346.66 | 377.45 | 0 |
| **16p** | 4.24 | 70.15 | 6 | 2 | 348.03 | 363.46 | 0 |
| **16q** | 3.38 | 73.39 | 7 | 2 | 367.02 | 390.49 | 0 |
| **16r** | 6.22 | 70.15 | 6 | 2 | 419.47 | 459.55 | 1 |

[1] LogP, logarithm of compound partition coefficient between n-octanol and water.

[2] TPSA, topological polar surface area.

[3] nON, number of hydrogen bond acceptors.

[4] nOHNH, number of hydrogen bond donors.

[5] MW, molecular weight.

In order to identify the oral bioavailability of these compounds, compound **16e** was further evaluated for *in vivo* PK profiles in male SD rats (n = 3), following a single oral dose (25 mg/kg, n = 3) and an intravenous dose administrations (1.25 mg/kg, n = 3). As shown in Table 5, compound **16e** shows reasonable oral exposure and bioavailability ($AUC_{0\text{-}inf}$: 183 h·ng·mL$^{-1}$, F: 22.5%), but a relative high Vss (26.6 L/kg) might lead to unexpected accumulation and potential toxicity risk for basic molecule. The oral $T_{1/2}$ of 25 mg/kg was approximate 2.25 h, suggesting a need for further optimization to improve the exposure. Thus far, all the above results support compound **16e** to be worth of further investigation.

**Table 5** Rat PK profiles of compound **16e**

| | | Dose (mg/kg) | |
|---|---|---|---|
| | Parameter | 1.25 (iv) | 25 (po) |
| Plasma | $K_{el}$ ($h^{-1}$) | 1.03±0.12 | 0.32±0.07 |
| | $T_{1/2}$ (h) | 0.68±0.07 | 2.25±0.5 |
| | $t_{max}$ (h) | - | 0.33±0.14 |
| | $C_{max}$ (ng/mL) | 58.0±16 | 78.6±16 |
| | $C_0$ (ng/mL) | 80.8±27 | - |
| | $AUC_{0-t}$ (h·ng/mL) | 34.9±8.9 | 157±62 |
| | $AUC_{0-inf}$ (h·ng/mL) | 39.2±9.4 | 183±55 |
| | $AUMC_{0-t}$ (h·h·ng/mL) | 18.0±4.2 | 313±111 |
| | $AUMC_{0-inf}$ (h·h·ng/mL) | 30.6±6.4 | 553±85 |
| | CL (mL/min/kg) | 557±155 | - |
| | MRT (h) | 0.79±0.08 | 3.18±0.99 |
| | Vss (L/kg) | 26.6±9.16 | - |
| | F(%) | - | 22.5±8.8 |

The PK study in male SD rats was carried out according to the standard procedures (iv, 1.25 mg/kg; po, 25 mg/kg). Major parameters, including plasma clearance (CL), volume of distribution at steady state (Vss), $T_{1/2}$, area under the cure (AUC), and oral bioavailability (F), are reported.

## 3. Conclusions

In summary, we reported the synthesis and characterization of a series novel quinazoline derivatives containing S-acetamide and NH-acetamide moieties as new anti-IAV agents. Most of them exhibit potent in vitro anti-IAV activity. The subsequent SAR studies showed that nine compounds (**10a2**, **16a**, **16e**, **16i**, **16j**, **16n**, **16o**, **16p** and **16r**) showed much stronger anti-influenza A activities with $IC_{50}$ at micromole level (1.29–9.04 μM). Particularly, **16e** and **16r** exhibited excellent anti-IAV activity ($IC_{50}$:1.29 μM and 3.43 μM, respectively) and displayed acceptable cytotoxicity. The preliminary mechanism studies imply that these quinazoline derivatives exert their antiviral activity at the viral post-entry stage. In addition, compound **16e** with reasonable PK profiles has been selected as a potential candidate for further investigation.

## 4. Experimental section

### 4.1. *Chemistry*

Melting points were obtained from an X4 micromelting point meter and the temperature was uncorrected. $^{1}$H NMR and $^{13}$C NMR spectra were determined on a on a Bruker AVANCE III 400 MHz, 500 MHz or 600 MHz spectrometer (Bruker Inc) in DMSO-$d_6$, Methanol-$d_4$ or $CDCl_3$ using tetramethylsilane as an internal standard. High resolution mass spectra were obtained on an Autospee Ultima-TOF spectrometer. The reagents were all of analytical grade or chemically pure. TLC was performed on silica gel plates (Merck, ART5554 60F254).

### *4.2. Synthesis*

#### *4.2.1. Ethyl 2-((2-chloroquinazolin-4-yl)thio)acetate **4***

Quinazoline-2,4(1H,3H)-dione (**2**) and 2,4-diichloroquinazoline (**3**) were synthesized according to previously reported methods[23-24].

To a stirred solution of **3** (2.6 g, 13 mmol) and DIPEA (3.4 g, 26 mmol) in THF (25 mL), ethyl thioglycolate (1.56 g, 13 mmol) was added dropwise. The reaction mixture was stirred at r.t. for 1.5 h. The reaction mixture was concentrated under reduced pressure and purified by column chromatography (silica gel) eluting with petroleum ether and ethyl acetate (15:1, v / v) to get the title compound **4** (2.9 g, 80% from **3**) as light yellow solid. $^{1}$H NMR (400 MHz, Chloroform-*d*) δ 8.13 – 8.08 (m, 1H), 7.97 – 7.88 (m, 2H), 7.64 (ddd, *J* = 8.2, 6.2, 1.9 Hz, 1H), 4.32 (q, *J* = 7.1 Hz, 2H), 4.17 (s, 2H), 1.38 (t, *J* = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, Chloroform-*d*) δ 172.83, 168.24, 155.59, 149.69, 135.01, 128.08, 127.75, 123.88, 121.88, 62.18, 32.53, 14.23.

#### *4.2.2. General procedure for the synthesis of compounds **7a–7i***

Pyrrolidine (0.6 g, 8.4 mmol) was added to the suspension of **4** (1.2 g, 4.2 mmol) and DIPEA (1.1 g, 8.4mmol) in THF (15 mL) and then the system was heated to 80□ for 1 h. The solvent was removed by evaporation and the residue was purified by column chromatography (silica gel) eluting with petroleum ether and ethyl acetate (10:1, v / v) to get the title compound **5** (1.2 g, 90% from 4) as yellow solid. The solid was then dissoved in THF (10 mL) and lithium hydroxide (2 eq.) in water (10 mL) was added. The reaction mixture was stirred for 1.5 h at room temperature and evaporated under vacuum. The residue in water (20 mL) was adjusted to pH 2 with 1

N HCl and the precipitated solid was filtered, washed with distilled water (20 mL) to give compound **6** as an off-white solid.

A solution of compound **6** (99.8 mg, 0.34 mmol), 2-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) (157.7 mg, 0.42 mmol) in DMF (2 mL) was stirred at room temperature under the atmosphere of nitrogen for 5 min. The substituted amines (0.42 mmol) and N,N-diisopropylethylamine (DIPEA) (0.70 mmol) was added to the mixture and stirred for 4 h at room temperature. The mixture was diluted with water and extracted with ethyl acetate. The combined extracts were washed by saturated brine, dried over $Na_2SO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel) eluting with hexanes/EtOAc 1 : 1 to give the target compounds **7a–7i**.

*4.2.2.1. N-phenyl-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)thio)acetamide **7a.*** According to the general procedure, employing **6** and aniline afforded compound **7a** as a light yellow solid, 67% yield, mp: 227–229□. $^1$H NMR (500 MHz, Chloroform-*d*) δ 9.10 (s, 1H), 7.82 (d, *J* = 8.0 Hz, 1H), 7.63 (p, *J* = 9.0, 8.3 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.32 – 7.23 (m, 2H), 7.16 (t, *J* = 7.5 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 4.08 (s, 2H), 3.73 (d, *J* = 6.0 Hz, 4H), 2.00 (d, *J* = 6.0 Hz, 4H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.95, 166.28, 156.17, 151.09, 139.57, 134.51, 129.24, 125.99, 124.18, 123.76, 122.13, 119.48, 117.69, 46.88, 34.65, 25.36. HRMS-ESI (*m/z*): Calcd. for $C_{20}H_{21}N_4OS(M+H)^+$: 365.1431, Found: 365.1428.

*4.2.2.2. N-benzyl-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)thio)acetamide **7b.*** According to the general procedure, employing **6** and benzylamine afforded compound **7b** as a yellow solid, 55% yield, mp: 190–192□. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.67 – 8.57 (m, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.66 (t, *J* = 7.0 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.31 – 7.09 (m, 5H), 4.29 (d, *J* = 6.0 Hz, 2H), 4.07 (d, *J* = 4.0 Hz, 2H), 3.52 (t, *J* = 6.0 Hz, 4H), 1.87 (s, 4H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.77, 167.49, 156.15, 139.66, 134.48, 128.63, 127.74, 127.24, 125.97, 124.26, 122.04, 117.80, 46.83, 43.13, 33.32, 25.43. HRMS-ESI (*m/z*): Calcd. for $C_{21}H_{23}N_4OS$ $(M+H)^+$:

379.1587, Found: 379.1593.

*4.2.2.3. N-(2-ethoxyphenyl)-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)thio)acetamide* ***7c.*** According to the general procedure, employing **6** and o-phenetidine afforded compound **7c** as a yellow solid, 57% yield, mp: 176–178□. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 9.27 (s, 1H), 8.08 (d, *J* = 8.0 Hz, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.74 – 7.62 (m, 1H), 7.47 (d, *J* = 8.5 Hz, 1H), 7.28 – 7.17 (m, 1H), 7.07 – 6.94 (m, 2H), 6.93 – 6.84 (m, 1H), 4.26 (s, 2H), 3.95 (d, *J* = 7.5 Hz, 2H), 3.57 (d, *J* = 6.5 Hz, 5H), 1.87 (s, 5H), 1.12 – 1.03 (m, 3H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.31, 166.58, 156.12, 151.30, 148.10, 134.76, 127.84, 126.05, 124.53, 124.18, 122.27, 120.81, 120.58, 117.66, 112.36, 64.27, 46.85, 34.09, 25.39, 14.58. HRMS-ESI (*m/z*): Calcd. for $C_{22}H_{25}N_4O_2S$ $(M+H)^+$: 409.1693, Found: 409.1702.

*4.2.2.4. N-(furan-2-ylmethyl)-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)thio)acetamide* ***7d.*** According to the general procedure, employing **6** and furfurylamine afforded compound **7d** as a yellow solid, 60% yield, mp: 178–180℃. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 8.61 (t, *J* = 6.0 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.65 (t, *J* = 8.0 Hz, 1H), 7.56 (s, 1H), 7.44 (d, *J* = 8.5 Hz, 1H), 7.17 (t, *J* = 7.5 Hz, 1H), 6.38 (s, 1H), 6.21 (d, *J* = 3.5 Hz, 1H), 4.29 (d, *J* = 5.5 Hz, 2H), 4.04 (s, 2H), 3.53 (d, *J* = 6.0 Hz, 4H), 1.91 (s, 4H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.80, 167.35, 156.10, 152.42, 151.12, 142.62, 134.46, 125.94, 124.19, 122.04, 117.73, 110.88, 107.52, 46.82, 36.47, 33.30, 25.47. HRMS-ESI (*m/z*): Calcd. for $C_{19}H_{21}N_4O_2S$ $(M+H)^+$: 369.1380, Found: 369.1382.

*4.2.2.5. N-(naphthalen-2-yl)-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)thio)acetamide* ***7e.*** According to the general procedure, employing **6** and 2-naphthylamine afforded compound **7e** as an off-white solid, 66% yield, mp: 236–238℃. $^{1}$H NMR (500 MHz, Chloroform-*d*) δ 9.35 (s, 1H), 8.15 (s, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.78 – 7.72 (m, 3H), 7.67 (s, 2H), 7.44 (t, *J* = 7.5 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.20 (s, 1H), 4.15 (s, 2H), 3.76 (s, 4H), 1.98 (s, 4H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.99, 166.57,

156.14, 151.06, 137.15, 134.55, 133.90, 130.23, 128.90, 127.94, 127.74, 126.93, 125.96, 125.10, 124.20, 122.18, 120.22, 117.68, 115.60, 46.88, 34.73, 25.32. HRMS-ESI (*m/z*): Calcd. for $C_{24}H_{23}N_4OS$ $(M+H)^+$: 415.1587, Found: 415.1619.

*4.2.2.6. N-cyclopropyl-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)thio)acetamide* ***7f.***

According to the general procedure, employing **6** and cyclopropylamine afforded compound **7f** as an off-white solid, 72% yield, mp: 244–246□. $^1$H NMR (500 MHz, Chloroform-*d*) δ 7.78 (d, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.13 (t, *J* = 7.5 Hz, 1H), 6.94 (s, 1H), 3.90 (s, 2H), 3.69 (d, *J* = 6.0 Hz, 4H), 2.65 (dq, *J* = 7.5, 3.9 Hz, 1H), 2.03 (d, *J* = 6.0 Hz, 4H), 0.73 (d, *J* =7.0 Hz, 2H), 0.39 (d, *J* = 4.5 Hz, 2H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.96, 168.31, 156.17, 151.11, 134.44, 125.98, 124.18, 122.06, 117.74, 46.86, 33.49, 25.44, 23.18, 6.01. HRMS-ESI (*m/z*): Calcd. for $C_{17}H_{21}N_4OS$ $(M+H)^+$: 329.1431, Found: 329.1420.

*4.2.2.7. N-(thiazol-2-yl)-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)thio)acetamide* ***7g.***

According to the general procedure, employing **6** and 2-aminothiazole afforded compound **7g** as a brown solid, 63% yield, mp: 234–236℃. $^1$H NMR (500 MHz, Chloroform-*d*) δ 11.02 – 10.71 (m, 1H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.65 (d, *J* = 6.0 Hz, 2H), 7.39 (d, *J* = 4.0 Hz, 1H), 7.16 (t, *J* = 7.5 Hz, 1H), 6.96 (d, *J* = 4.0 Hz, 1H), 4.15 (s, 2H), 3.76 (t, *J* = 6.0 Hz, 4H), 2.01 (d, *J* = 6.0 Hz, 4H). HRMS-ESI (*m/z*): Calcd. for $C_{17}H_{18}N_5OS_2$ $(M+H)^+$: 372.0948, Found: 372.0932.

*4.2.2.8. N-(2-ethoxybenzyl)-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)thio)acetamide* ***7h.***

According to the general procedure, employing **6** and 2-ethoxybenzylamine afforded compound **7h** as a light yellow solid, 89% yield, mp: 169–171℃. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.29 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.18 (t, *J* = 8.0 Hz, 2H), 7.08 (d, *J* = 7.5 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 6.82 – 6.76 (m, 1H), 4.25 (d, *J* = 6.0 Hz, 2H), 4.06 (s, 2H), 3.90 (q, *J* = 7.0z Hz, 2H), 3.49 (s, 4H), 1.94 – 1.77 (m, 4H), 1.22 (t, *J* = 7.0 Hz, 3H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.60, 167.46, 156.43, 156.09, 151.23, 134.50, 128.58, 128.43, 125.98,

124.23, 122.04, 120.28, 117.78, 111.76, 63.60, 46.77, 38.52, 33.23, 25.40, 15.05. HRMS-ESI (*m/z*): Calcd. for $C_{23}H_{27}N_4O_2S$ $(M+H)^+$: 423.1849, Found: 423.1866.

*4.2.2.9. N-(3,5-dichlorophenyl)-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)thio)acetamide* ***7i.*** According to the general procedure, employing **6** and 3,5-dichloroaniline afforded compound **7i** as a pink solid, 65% yield, mp: 240–241℃. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 10.67 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.65 (s, 3H), 7.44 (d, *J* = 8.5 Hz, 1H), 7.29 (s, 1H), 7.19 (t, *J* = 8.0 Hz, 1H), 4.23 (s, 2H), 3.52 – 3.43 (m, 4H), 1.82 (s, 4H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.72, 167.17, 156.15, 151.09, 141.81, 134.62 (d, *J* = 7.1 Hz), 126.02, 124.15, 123.04, 122.21, 117.63, 46.90, 34.64, 25.31. HRMS-ESI (*m/z*): Calcd. for $C_{20}H_{19}Cl_2N_4OS$ $(M+H)^+$: 433.0651, Found: 433.0675.

*4.2.3. General procedure for the synthesis of compounds* ***10a,10b***

To a solution of **4** (1.3 g, 4.5 mmol) in THF (10 mL) was added lithium hydroxide (0.38 g, 9.0 mmol) in 10 mL water and stirred for 1 h at room temperature. The solvent was removed by evaporation, and then the residue was re-dissolved in water (10 mL) and adjusted to pH 2 with 1 N HCl. The precipitated solid was filtered, washed with distilled water (10 mL) to give compound **8** as a white solid .

A solution of compound **8** (0.46 g, 1.8 mmol), 2-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) (0.81 g, 2.1 mmol) in DCM (10 mL) was stirred at room temperature under the atmosphere of nitrogen for 5 min. Aniline or benzylamine (2.1 mmol) and N,N-diisopropylethylamine (DIPEA) (3.6 mmol) was added to the mixture and stirred for 4 h at the same temperature. The mixture was washed by water, saturated brine and dried over $Na_2SO_4$. After filtration, the solvent was removed under reduced pressure and the residue was recrystallized from ethyl acetate to obtain a yellow solid **9a** and an off-white solid **9b**.

Secondary amines (2 eq.) was added to the suspension of **9a** or **9b** ( 0.3 mmol) and DIPEA (3 eq.) in THF (5 mL) and then the system was heated to 110℃ for 8-12 h. The mixture was concentrated under reduced pressure and then re-dissolved in DCM/MeOH(10:1,v/v), washed by water, saturated brine and dried over $Na_2SO_4$. After filtration, the solvent was removed under reduced pressure and the residue was

was recrystallized from ethyl acetate to give the target compounds **10a**, **10b**.

*4.2.3.1.*

*N-phenyl-2-((2-(3,4-dihydroisoquinolin-2(1H)-yl)quinazolin-4-yl)thio)acetamide* ***10a1.*** According to the general procedure, employing **9a** and 1,2,3,4-tetrahydroisoquinoline afforded compound **10a1** as a yellow solid, 59% yield, mp: 192–194□. $^{1}$H NMR (500 MHz, Chloroform-*d*) δ 8.79 (s, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 7.0 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.25 – 7.19 (m, 4H), 7.18 – 7.13 (m, 2H), 7.11 (d, *J* = 6.5 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 5.05 (s, 2H), 4.17 (t, *J* = 6.0 Hz, 2H), 4.10 (s, 2H), 2.91 (t, *J* = 6.0 Hz, 2H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 170.59, 166.13, 157.17, 150.71, 139.66, 135.29, 134.74, 134.49, 129.29, 128.79, 126.90, 126.64, 126.34, 126.22, 124.18, 123.79, 122.87, 119.46, 118.00, 46.27, 41.81, 34.74, 28.65. HRMS-ESI (*m/z*): Calcd. for $C_{25}H_{23}N_4OS$ $(M+H)^+$: 427.1587, Found: 427.1600.

*4.2.3.2. N-phenyl-2-((2-(diethylamino)quinazolin-4-yl)thio)acetamide* ***10a2.*** According to the general procedure, employing **9a** and diethylamine afforded compound **10a2** as an off-white solid, 47% yield, mp: 180–181℃. $^{1}$H NMR (500 MHz, Chloroform-*d*) δ 8.66 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.63 (t, *J* = 7.5 Hz, 1H), 7.57 (s, 1H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.15 (t, *J* = 7.5 Hz, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 4.08 (s, 2H), 3.74 (q, *J* = 7.0 Hz, 4H), 1.20 (t, *J* = 7.0 Hz, 6H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.94, 166.01, 156.78, 151.16, 139.66, 134.47, 129.21, 126.09, 124.08, 123.71, 122.16, 119.42, 117.64, 41.74, 34.40, 13.71. HRMS-ESI (*m/z*): Calcd. for $C_{20}H_{23}N_4OS$ $(M+H)^+$: 367.1587, Found: 367.1597.

*4.2.3.3. N-phenyl-2-((2-(4-methylpiperazin-1-yl)quinazolin-4-yl)thio)acetamide* ***10a3.*** According to the general procedure, employing **9a** and 1-methylpiperazine afforded compound **10a3** as an off-white solid, 56% yield. mp: 228–230℃. $^{1}$H NMR (500 MHz, Chloroform-*d*) δ 8.73 (s, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.28 (d, *J* = 8.0 Hz, 2H), 7.21 (t, *J* = 7.5

Hz, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 4.04 (s, 2H), 3.98 (t, *J* = 5.0 Hz, 4H), 2.48 (t, *J* = 5.0 Hz, 4H), 2.32 (s, 3H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 170.58, 166.07, 157.26, 150.63, 139.55, 134.73, 129.23, 126.21, 124.13, 123.78, 122.95, 119.41, 118.01, 54.79, 46.04, 43.84, 34.67. HRMS-ESI (*m/z*): Calcd. for $C_{21}H_{24}N_5OS$ $(M+H)^+$: 394.1696, Found: 394.1704.

*4.2.3.4.*

*N-benzyl-2-((2-(3,4-dihydroisoquinolin-2(1H)-yl)quinazolin-4-yl)thio)acetamide* ***10b1.*** According to the general procedure, employing **9b** and 1,2,3,4-tetrahydroisoquinoline afforded compound **10b1** as an off-white solid, 53% yield, mp: 230–232□. $^1$H NMR (500 MHz, Chloroform-*d*) δ 7.82 (d, *J* = 8.0 Hz, 1H), 7.66 (s, 1H), 7.24 – 7.10 (m, 8H), 7.07 (d, *J* = 6.5 Hz, 2H), 7.01 (s, 1H), 4.96 (s, 2H), 4.40 (d, *J* = 6.0 Hz, 2H), 4.08 (s, 2H), 4.02 (s, 2H), 2.88 (s, 2H), 1.56 (s, 2H). HRMS-ESI (*m/z*): Calcd. for $C_{26}H_{25}N_4OS$ $(M+H)^+$: 441.1744, Found: 441.1764.

*4.2.3.5. N-benzyl-2-((2-(diethylamino)quinazolin-4-yl)thio)acetamide* ***10b2.*** According to the general procedure, employing **9b** and diethylamine afforded compound **10b2** as an off-white solid, 50% yield, mp: 189–191□. $^1$H NMR (500 MHz, Chloroform-*d*) δ 7.78 (d, *J* = 8.1 Hz, 1H), 7.58 (d, *J* = 8.0 Hz, 2H), 7.22 (d, *J* = 7.0 Hz, 3H), 7.12 (d, *J* = 7.0 Hz, 3H), 6.97 (s, 1H), 4.43 (d, *J* = 6.0 Hz, 2H), 4.00 (s, 2H), 3.67 (q, *J* = 7.0 Hz, 4H), 1.19 (t, *J* = 7.0 Hz, 6H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.79, 167.34, 156.76, 151.26, 139.66, 134.42, 128.67, 127.74, 127.25, 126.09, 124.13, 122.08, 117.73, 43.13, 41.80, 33.16, 13.74. HRMS-ESI (*m/z*): Calcd. for $C_{21}H_{25}N_4OS$ $(M+H)^+$: 381.1744, Found: 381.1739.

*4.2.3.6. N-benzyl-2-((2-(4-methylpiperazin-1-yl)quinazolin-4-yl)thio)acetamide* ***10b3.*** According to the general procedure, employing **9b** and 1-methylpiperazine afforded compound **10b3** as an off-white solid, 38% yield. mp: 180–181℃. $^1$H NMR (500 MHz, Chloroform-*d*) δ 7.80 (d, *J* = 8.0 Hz, 1H), 7.63 (t, *J* = 7.5 Hz, 1H), 7.53 (d, *J* = 8.5 Hz, 1H), 7.23 – 7.15 (m, 4H), 7.10 – 7.05 (m, 2H), 7.01 (d, *J* = 6.0 Hz, 1H), 4.42 (d, *J* =

6.0 Hz, 2H), 3.97 (s, 2H), 3.92 (s, 4H), 2.44 (s, 4H), 2.32 (s, 3H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 170.44, 167.40, 157.21, 150.71, 139.66, 134.70, 128.69, 127.65, 127.25, 126.20, 124.19, 122.89, 118.09, 54.74, 46.01, 43.77, 43.06, 33.48. HRMS-ESI (*m/z*): Calcd. for $C_{22}H_{26}N_5OS$ $(M+H)^+$: 408.1853, Found: 408.1841.

*4.2.4. General procedure for the synthesis of compounds **16a–r***

Glycine (30.84 g, 0.41 mol) was dissolved in methanol (200 mL) and triethylamine (180 mL). Di-tert-butyl dicarbonate (179.11 g, 0.82 mol) was added and the mixture was stirred at 70℃ for 5 h. The solvent was evaporated and water (100 mL) and EtOAc (150 mL) were added to the residue. The two-phase mixture was transferred to a separatory funnel. The aqueous phase was extracted with EtOAc (2 X 80 mL). The organic phases were combined, dried over $MgSO_4$, filtered and the solvent was removed by rotary evaporation to yield **12** as a light yellow solid.

HATU(1.2 eq.) was added to a solution of Boc-Gly-OH and DIPEA(2.0 eq.) in DCM (20 mL), and various primary amines (1.2 eq.) followed by stirring for 3 hours at room temperature. The mixture was washed with brine (two times for each), and the organic layer was dried over sodium sulfate. After filtration, the filtrate was concentrated under reduced pressure obtaining the crude amide **13a–i**. The obtained amide compound was deprotected of N-Boc by hydrogen chloride gas to give **14a–i**.

To a solution of **3** (0.5 g, 2.5 mmol) and DIPEA (0.97 g, 7.5 mmol) dissolved in THF (5 mL) was added **14a–i** (1 eq.) and stirred for 3-5 h at room temperature. The mixture was concentrated under reduced pressure and then dissolved in ethyl acetate (20 mL), washed by water, saturated brine and dried over $Na_2SO_4$. After filtration, the solvent was removed under reduced pressure to obtain the target compounds **15a–i**.

To a solution of **15a–i** (1 e.q.) and DIPEA (3 e.q.) dissolved in DMF (4 mL) was added secondary amines (2 e.q.) and stirred for 3-8 h at 100℃. After cooling to room temperature, the mixture was diluted with ethyl acetate (20 mL), washed by water, saturated brine and dried over $Na_2SO_4$, and concentrated under reduced pressure. The residue was recrystallized from ethyl acetate to get the target compound **16a–r**.

*4.2.4.1. N-phenyl-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)amino)acetamide* ***16a.*** According to the general procedure, employing **15a** and pyrrolidine afforded compound **16a** as an off-white solid, 75% yield, mp: 250–252□. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 10.24 – 10.20 (m, 1H), 8.42 (q, *J* = 5.5 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.32 – 7.23 (m, 3H), 7.03 (q, *J* = 8.0 Hz, 2H), 4.19 (d, *J* = 5.5 Hz, 2H), 3.49 – 3.40 (m, 4H), 1.79 (s, 4H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.05, 160.18, 157.79, 152.63, 139.69, 132.71, 129.09, 125.21, 123.52, 123.47, 120.19, 119.65, 110.85, 46.51, 45.33, 25.43. HRMS-ESI (*m/z*): Calcd. for $C_{20}H_{22}N_5O$ $(M+H)^+$: 348.1819, Found: 348.1846.

*4.2.4.2. N-benzyl-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)amino)acetamide* ***16b.*** According to the general procedure, employing **15b** and pyrrolidine afforded compound **16b** as a light yellow solid, 70% yield, mp: 161–162□. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 8.44 (t, *J* = 6.0 Hz, 1H), 8.37 – 8.29 (m, 1H), 8.00 (d, *J* = 8.0 Hz, 1H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.33 – 7.16 (m, 5H), 7.03 (t, *J* = 7.5 Hz, 1H), 4.29 (d, *J* = 6.0 Hz, 2H), 4.07 (d, *J* = 6.0 Hz, 2H), 3.47 (t, *J* = 6.0 Hz, 4H), 1.85 (s, 4H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 170.11, 160.25, 157.84, 152.66, 140.13, 132.65, 128.50, 127.54, 127.05, 125.18, 123.62, 120.07, 111.00, 46.53, 44.73, 42.42, 25.53. HRMS-ESI (*m/z*): Calcd. for $C_{21}H_{24}N_5O$ $(M+H)^+$: 362.1976, Found: 362.1997.

*4.2.4.3. N-(2-ethoxyphenyl)-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)amino)acetamide* ***16c.*** According to the general procedure, employing **15c** and pyrrolidine afforded compound **16c** as a white solid, 72% yield, mp: 147–148□. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 9.03 (s, 1H), 8.59 (t, *J* = 6.0 Hz, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.56 – 7.47 (m, 1H), 7.31 (d, *J* = 8.5 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 7.02 – 6.96 (m, 1H), 6.93 (d, *J* = 8.0 Hz, 1H), 6.88 (t, *J* = 7.5 Hz, 1H), 4.25 (d, *J* = 6.0 Hz, 2H), 3.88 (q, *J* = 7.0 Hz, 2H), 3.54 – 3.40 (m, 4H), 1.83 (d, *J* = 6.0 Hz, 4H), 0.95 (t, *J* = 7.0 Hz, 3H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 168.48, 160.05, 150.35, 148.01 (d, *J* = 18.3 Hz), 140.72, 133.53, 133.14, 127.75, 124.34, 124.17, 123.48, 120.34,

112.34, 110.41, 64.29, 46.80, 45.96, 25.36, 17.55, 14.48. HRMS-ESI (*m/z*): Calcd. for $C_{22}H_{26}N_5O_2$ $(M+H)^+$: 392.2081, Found: 392.2104.

*4.2.4.4. N-(furan-2-ylmethyl)-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)amino)acetamide* ***16d.*** According to the general procedure, employing **15d** and pyrrolidine afforded compound **16d** as a light yellow solid, 80% yield, mp: 199–201□. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.69 (s, 1H), 8.46 (t, *J* = 6.0 Hz, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 7.55 (d, *J* = 7.0 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.12 (t, *J* = 8.0 Hz, 1H), 6.37 (s, 1H), 6.19 (d, *J* = 3.0 Hz, 1H), 4.27 (d, *J* = 6.0 Hz, 2H), 4.05 (d, *J* = 6.0 Hz, 2H), 3.47 (s, 4H), 1.88 (s, 4H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.44, 160.05, 152.85, 142.46, 133.40, 123.86, 123.13, 121.36, 110.82, 110.65, 107.26, 46.89, 44.64, 35.92, 25.42. HRMS-ESI (*m/z*): Calcd. for $C_{19}H_{22}N_5O_2$ $(M+H)^+$: 352.1768, Found: 352.1795.

*4.2.4.5. N-(naphthalen-2-yl)-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)amino)acetamide* ***16e.*** According to the general procedure, employing **15e** and pyrrolidine afforded compound **16e** as a white solid, 69% yield, mp: 169–171℃. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 10.32 (s, 1H), 8.40 (t, *J* = 6.0 Hz, 1H), 8.28 (d, *J* = 2.0 Hz, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.86 (d, *J* = 9.0 Hz, 1H), 7.82 (d, *J* = 8.0 Hz, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.63 (dd, *J* = 9.0, 2.1 Hz, 1H), 7.50 (t, *J* = 8.0 Hz, 1H), 7.45 (t, *J* = 7.5 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 4.25 (d, *J* = 5.5 Hz, 2H), 3.50 – 3.40 (m, 4H), 1.76 (s, 4H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.35, 160.23, 157.78, 152.66, 137.23, 133.92, 132.75, 130.16, 128.75, 127.92, 127.67, 126.85, 125.27, 124.96, 123.44, 120.52, 120.25, 115.67, 110.85, 46.51, 45.48, 25.40. HRMS-ESI (*m/z*): Calcd. for $C_{24}H_{24}N_5O$ $(M+H)^+$: 398.1976, Found: 398.2012.

*4.2.4.6. N-cyclopropyl-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)amino)acetamide* ***16f.*** According to the general procedure, employing **15f** and pyrrolidine afforded compound **16f** as an off-white solid, 74% yield, mp: 210–212℃. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.14 (t, *J* = 6.0 Hz, 1H), 8.00 (d, *J* = 4.0 Hz, 1H), 7.95 (d, *J* = 8.0 Hz,

1H), 7.51 – 7.42 (m, 1H), 7.26 (d, *J* = 8.5 Hz, 1H), 7.02 (t, *J* = 7.5 Hz, 1H), 3.93 (d, *J* = 6.0 Hz, 2H), 3.56 – 3.44 (m, 4H), 2.63 (m, 1H), 1.88 (d, *J* = 6.0 Hz, 4H), 0.60 (dt, *J* = 7.0, 3.4 Hz, 2H), 0.45 – 0.31 (m, 2H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 170.94, 160.13, 157.61, 152.23, 132.71, 124.98, 123.47, 120.25, 110.87, 46.57, 44.45, 25.48, 22.73, 6.03. HRMS-ESI (*m/z*): Calcd. for $C_{17}H_{22}N_5O$ $(M+H)^+$: 312.1819, Found: 312.1828.

*4.2.4.7. N-(thiazol-2-yl)-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)amino)acetamide* ***16g.*** According to the general procedure, employing **15g** and pyrrolidine afforded compound **16g** as a light yellow solid, 65% yield, mp: 155–157□. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 12.31 (s, 1H), 8.51 (q, *J* = 6.0 Hz, 1H), 8.03 (dd, *J* = 8.0, 3.5 Hz, 1H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.46 (d, *J* = 3.5 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 3.5 Hz, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 4.28 (d, *J* = 5.5 Hz, 2H), 3.43 – 3.37 (m, 4H), 1.77 (d, *J* = 6.5 Hz, 4H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.30, 160.08, 158.58, 157.63, 152.61, 138.09, 132.81, 125.21, 123.55, 120.26, 113.69, 110.75, 46.44, 44.52, 25.40. HRMS-ESI (*m/z*): Calcd. for $C_{17}H_{19}N_6OS$ $(M+H)^+$: 355.1336, Found: 355.1360.

*4.2.4.8. N-(2-ethoxybenzyl)-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)amino)acetamide* ***16h.*** According to the general procedure, employing **15h** and pyrrolidine afforded compound **16h** as an off-white solid, 60% yield, mp: 170–172□. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.32 (t, *J* = 6.0 Hz, 1H), 8.11 (t, *J* = 6.0 Hz, 1H), 8.03 – 7.93 (m, 1H), 7.52 – 7.44 (m, 1H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.20 – 7.15 (m, 1H), 7.14 – 7.11 (m, 1H), 7.03 (t, *J* = 7.5 Hz, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 6.78 (t, *J* = 7.5 Hz, 1H), 4.24 (d, *J* = 6.0 Hz, 2H), 4.07 (d, *J* = 6.0 Hz, 2H), 3.94 (q, *J* = 7.0 Hz, 2H), 3.45 (s, 4H), 1.84 (s, 4H), 1.23 (t, *J* = 7.0 Hz, 3H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 170.09, 160.22, 157.79, 156.32, 152.70, 132.71, 128.29, 128.01, 127.47, 125.22, 123.53, 120.18, 120.11, 111.61, 110.93, 63.58, 46.49, 44.86, 37.78, 25.50, 15.05. HRMS-ESI (*m/z*): Calcd. for $C_{23}H_{28}N_5O_2$ $(M+H)^+$: 406.2238, Found: 406.2249.

*4.2.4.9.*

*N-(3,5-dichlorophenyl)-2-((2-(pyrrolidin-1-yl)quinazolin-4-yl)amino)acetamide* ***16i.*** According to the general procedure, employing **15i** and pyrrolidine afforded compound **16i** as an off-white solid, 66% yield, mp: 185–187□. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 10.44 (s, 1H), 8.44 (t, *J* = 5.5 Hz, 1H), 8.00 (d, *J* = 8.0 Hz, 1H), 7.68 (d, *J* = 2.0 Hz, 2H), 7.50 (t, *J* = 8.0 Hz, 1H), 7.36 – 7.22 (m, 2H), 7.06 (t, *J* = 7.5 Hz, 1H), 4.16 (d, *J* = 5.5 Hz, 2H), 3.41 (t, *J* = 6.0 Hz, 4H), 1.79 (s, 4H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.90, 160.13, 157.52, 152.37, 141.92, 134.53, 132.87, 125.12, 123.44, 122.78, 120.40, 117.72, 110.73, 46.55, 45.59, 25.38. HRMS-ESI (*m/z*): Calcd. for $C_{20}H_{19}Cl_2N_5O$ $(M+H)^+$: 416.1040, Found: 416.1063.

*4.2.4.10.*

*N-phenyl-2-((2-(3,4-dihydroisoquinolin-2(1H)-yl)quinazolin-4-yl)amino)acetamide* ***16j.*** According to the general procedure, employing **15a** and 1,2,3,4-tetrahydroisoquinoline afforded compound **16j** as an off-white solid, 56% yield, mp: 151–153□. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 10.26 (s, 1H), 8.53 (t, *J* = 5.4 Hz, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 7.67 (d, *J* = 8.0 Hz, 2H), 7.54 (t, *J* = 8.0 Hz, 1H), 7.37 – 7.26 (m, 3H), 7.15 – 6.97 (m, 6H), 4.84 (s, 2H), 4.21 (d, *J* = 5.5 Hz, 2H), 3.96 (t, *J* = 6.0 Hz, 2H), 2.69 (s, 2H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 168.97, 160.46, 158.65, 152.24, 139.74, 135.43, 135.17, 132.96, 129.17, 128.88, 126.78, 126.39, 126.16, 125.48, 123.55, 123.43, 121.00, 119.59, 111.06, 46.24, 45.57, 41.51, 28.60. HRMS-ESI (*m/z*): Calcd. for $C_{25}H_{24}N_5O$ $(M+H)^+$: 410.1976, Found: 410.1984.

*4.2.4.11. N-phenyl-2-((2-morpholinoquinazolin-4-yl)amino)acetamide* ***16k.*** According to the general procedure, employing **15a** and morpholine afforded compound **16k** as a light yellow solid, 60% yield, mp: 228–230□. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 10.24 (t, *J* = 7.7 Hz, 1H), 8.56 (q, *J* = 5.5 Hz, 1H), 8.07 (dd, *J* = 8.0, 3.2 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.53 (t, *J* = 8.0 Hz, 1H), 7.29 (t, *J* = 7.5 Hz, 3H), 7.12 (t, *J* = 7.5 Hz, 1H), 7.03 (t, *J* = 7.5 Hz, 1H), 4.16 (d, *J* = 5.5 Hz, 2H), 3.66 (t, *J* = 5.0 Hz, 4H),

3.47 (t, *J* = 5.0 Hz, 4H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 168.90, 160.46, 158.78, 152.09, 139.59, 132.98, 129.13, 125.54, 123.56, 123.49, 121.23, 119.61, 111.22, 66.57, 45.50, 44.55. HRMS-ESI (*m/z*): Calcd. for $C_{20}H_{22}N_5O_2$ $(M+H)^+$: 364.1768, Found: 364.1779.

*4.2.4.12. N-phenyl-2-((2-(diethylamino)quinazolin-4-yl)amino)acetamide* ***16l.*** According to the general procedure, employing **15a** and diethylamine afforded compound **16l** as a light yellow solid, 49% yield, mp: 142–144□. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 10.07 (s, 1H), 8.32 (t, *J* = 6.0 Hz, 1H), 8.06 – 7.92 (m, 1H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.53 – 7.44 (m, 1H), 7.27 (q, *J* = 8.0 Hz, 3H), 7.03 (dt, *J* = 12.5, 8.0 Hz, 2H), 4.16 (d, *J* = 6.0 Hz, 2H), 3.54 (q, *J* = 7.0 Hz, 4H), 1.00 (s, 6H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 168.91, 160.33, 158.20, 152.67, 139.75, 132.69, 129.09, 125.33, 123.45, 123.32, 120.21, 119.54, 110.72, 45.25, 41.32, 13.99. HRMS-ESI (*m/z*): Calcd. for $C_{20}H_{24}N_5O$ $(M+H)^+$: 350.1976, Found: 350.1999.

*4.2.4.13. N-phenyl-2-((2-(4-methylpiperazin-1-yl)quinazolin-4-yl)amino)acetamide* ***16m.*** According to the general procedure, employing **15a** and 1-methylpiperazine afforded compound **16m** as an off-white solid, 65% yield, mp: 189–191□. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 10.21 (d, *J* = 6.0 Hz, 1H), 8.50 (q, *J* = 5.0 Hz, 1H), 8.05 (d, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.52 (t, *J* = 8.0 Hz, 1H), 7.33 – 7.23 (m, 3H), 7.09 (t, *J* = 7.5 Hz, 1H), 7.03 (t, *J* = 7.5 Hz, 1H), 4.16 (d, *J* = 5.5 Hz, 2H), 3.68 (t, *J* = 5.0 Hz, 4H), 2.17 (t, *J* = 5.0 Hz, 4H), 2.09 (s, 3H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 168.87, 160.43, 158.71, 152.17, 139.57, 132.94, 129.11, 125.48, 123.57, 123.39, 121.06, 119.65, 111.07, 54.94, 46.15, 45.48, 43.72. HRMS-ESI (*m/z*): Calcd. for $C_{21}H_{25}N_6O$ $(M+H)^+$: 377.2085, Found: 377.2090.

*4.2.4.14.*

*N-benzyl-2-((2-(3,4-dihydroisoquinolin-2(1H)-yl)quinazolin-4-yl)amino)acetamide*

***16n.*** According to the general procedure, employing **15b** and 1,2,3,4-tetrahydroisoquinoline afforded compound **16n** as a yellow solid, 58% yield,

mp: 102–104□. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 8.52 (t, *J* = 6.0 Hz, 1H), 8.42 (t, *J* = 6.0 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.57 – 7.50 (m, 1H), 7.34 (d, *J* = 8.5 Hz, 1H), 7.27 – 7.13 (m, 9H), 7.09 (t, *J* = 7.5 Hz, 1H), 4.90 (s, 2H), 4.31 (d, *J* = 6.0 Hz, 2H), 4.11 (d, *J* = 6.0 Hz, 2H), 4.00 (t, *J* = 6.0 Hz, 2H), 2.81 (t, *J* = 6.0 Hz, 2H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 170.07, 160.51, 158.64, 152.21, 140.02, 135.51, 135.22, 132.93, 129.04, 128.57, 127.53, 127.07, 126.87, 126.49, 126.32, 125.39, 123.57, 120.90, 111.20, 46.25, 44.86, 42.52, 41.52, 28.74. HRMS-ESI (*m/z*): Calcd. for $C_{26}H_{26}N_5O$ $(M+H)^+$: 424.2132, Found: 424.2129.

*4.2.4.15. N-benzyl-2-((2-morpholinoquinazolin-4-yl)amino)acetamide* ***16o.*** According to the general procedure, employing **15b** and morpholine afforded compound **1o** as a pink solid, 46% yield, mp: 224–225℃; $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 8.47 (q, *J* = 6.0 Hz, 2H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.58 – 7.45 (m, 1H), 7.33 – 7.29 (m, 1H), 7.27 (d, *J* = 7.0 Hz, 2H), 7.24 – 7.19 (m, 3H), 7.11 (t, *J* = 7.5 Hz, 1H), 4.29 (d, *J* = 6.0 Hz, 2H), 4.06 (d, *J* = 6.0 Hz, 2H), 3.69 (t, *J* = 5.0 Hz, 4H), 3.60 (t, *J* = 5.0 Hz, 4H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.95, 160.54, 158.89, 152.08, 140.08, 132.92, 128.61, 127.49, 127.09, 125.52, 123.60, 121.12, 111.38, 66.65, 44.82, 44.58, 42.42. HRMS-ESI (*m/z*): Calcd. for $C_{21}H_{24}N_5O_2$ $(M+H)^+$: 378.1925, Found: 378.1939.

*4.2.4.16. N-benzyl-2-((2-(diethylamino)quinazolin-4-yl)amino)acetamide* ***16p.*** According to the general procedure, employing **15b** and diethylamine afforded compound **16p** as an off-white solid, 40% yield, mp: 222–224℃; $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 12.13 (s, 1H), 9.90 (s, 1H), 8.74 – 8.66 (m, 1H), 8.38 – 8.30 (m, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.81 (t, *J* = 8.0 Hz, 1H), 7.44 (t, *J* = 8.0 Hz, 1H), 7.34 – 7.30 (m, 2H), 7.29 – 7.24 (m, 3H), 4.32 (d, *J* = 5.5 Hz, 2H), 4.21 (d, *J* = 5.5 Hz, 2H), 3.70 (s, 4H), 1.18 (s, 6H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 168.34, 159.97, 139.77, 135.10, 128.66, 128.62, 127.75, 127.68, 127.26, 127.23, 124.56, 110.09, 44.89, 42.66, 38.16, 13.35. HRMS-ESI (*m/z*): Calcd. for $C_{21}H_{26}N_5O$ $(M+H)^+$: 364.2132, Found: 364.2162.

*4.2.4.17. N-benzyl-2-((2-(4-methylpiperazin-1-yl)quinazolin-4-yl)amino)acetamide* ***16q.*** According to the general procedure, employing **15b** and 1-methylpiperazine afforded compound **16q** as a light yellow solid, 68% yield, mp: 129–131□. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 8.46 (t, *J* = 6.0 Hz, 1H), 8.38 (t, *J* = 6.0 Hz, 1H), 8.00 (d, *J* = 8.0 Hz, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.27 (dd, *J* = 11.0, 8.0 Hz, 3H), 7.21 (d, *J* = 8.0 Hz, 3H), 7.08 (t, *J* = 7.5 Hz, 1H), 4.29 (d, *J* = 6.0 Hz, 2H), 4.05 (d, *J* = 6.0 Hz, 2H), 3.73 (t, *J* = 5.0 Hz, 4H), 2.31 (t, *J* = 5.0 Hz, 4H), 2.20 (s, 3H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 170.03, 160.48, 158.82, 152.21, 140.04, 132.88, 128.62, 127.46, 127.09, 125.44, 123.53, 120.91, 111.20, 55.11, 46.36, 44.79, 43.81, 42.43. HRMS-ESI (*m/z*): Calcd. for $C_{22}H_{27}N_6O$ $(M+H)^+$: 391.2241, Found: 391.2229.

*4.2.4.17.*

*N-(naphthalen-2-yl)-2-((2-(3,4-dihydroisoquinolin-2(1H)-yl)quinazolin-4-yl)amino)ac etamide* ***16r.*** According to the general procedure, employing **15e** and 1,2,3,4-tetrahydroisoquinoline afforded compound **16r** as a light yellow solid, 62% yield, mp: 237–239℃. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 10.72 (s, 1H), 8.68 (t, *J* = 5.7 Hz, 1H), 8.36 (s, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 7.85 (dd, *J* = 19.0, 8.5 Hz, 2H), 7.76 (t, *J* = 7.0 Hz, 2H), 7.53 (t, *J* = 8.0 Hz, 1H), 7.45 (t, *J* = 7.5 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.11 (t, *J* = 7.5 Hz, 1H), 7.00 (t, *J* = 7.0 Hz, 1H), 6.97 – 6.81 (m, 3H), 4.82 (s, 2H), 4.29 (d, *J* = 5.5 Hz, 2H), 3.95 (t, *J* = 6.0 Hz, 2H), 2.66 (s, 2H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 169.35, 160.47, 158.65, 152.16, 137.50, 135.39, 135.17, 133.96, 132.93, 130.16, 128.77, 128.70, 127.91, 127.64, 126.80, 126.78, 126.31, 126.02, 125.40, 124.87, 123.62, 120.97, 120.50, 115.52, 111.11, 46.22, 45.70, 41.50, 28.60. HRMS-ESI (*m/z*): Calcd. for $C_{29}H_{26}N_5O$ $(M+H)^+$: 460.2132, Found: 460.2128.

*4.3 Viruses, cells and cytotoxicity assay*

HEK293T-Gluc cells were generated by transfection of plasmid DNA pLenti6-Gluc constitutively expressing the negative-strand RNA of Gaussia luciferase (Gluc) gene, that is converted into the positive strand upon IAV infection, and

expresses the Gluc enzyme. A cell-based high-throughput approach to identify inhibitors of influenza A virus[25].

Cell viability was evaluated by cell counting kit-8 (CCK-8) assay. Briefly, 293T cells were cultured in a 96-well plate and incubated with compounds. Six concentrations of each compound, ranging from 12.5 μM to 200 μM, were used to treat cells for 48 h. Cells cultured in DMSO only were used as the control. After a 48-h incubation, 10 μL CCK-8 solution was added to each well and incubated for an additional 1 h at 37□. Optical density (OD) of each well at 450 nm was recorded on a Microplate Reader (Thermo, Varioskan Flash).

*4.4 Pharmacokinetic Profiles determination*

Pharmacokinetic Profiles determination of compound **16e**. SD male rats (obtained from JOINN (Suzhou) were used in the pharmacokinetic study. Every treatment group contain 3 rats for i.v. groups and 3 rats for p.o. groups. Rats were dosed with the tested compounds suspension at 1.25 mg/kg (i.v.) and 25 mg/kg (p.o.). Blood was collected from the eye socket of each animal at the following times after administration of drugs: 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after a single oral dosing. All blood samples were centrifuged at 5000 r/min for 10 min to obtain serum. 150 mL of the serum was added to 500 mL of acetonitrile and the mixture was centrifuged at 13000 r/min for 10 min to remove protein. The supernatant was dried and dissolve in 100 mL of acetonitrile, the solution was centrifuged at 13000 r/ min for 10 min. The supernatant was moved to a sample bottle for HPLC analysis. Total area under the concentration time curve (AUC), the elimination half-time ($T_{1/2}$), the peak concentration ($C_{max}$) and the time to reach peak concentration ($T_{max}$) of samples were determined directly from the experimental data using WinNonlin V6.3.

**Conflicts of interest**

The authors declare no conflict of interest.

**Funding sources**

This work was supported by the National Natural Science Foundation of China (No.

81703366), CAMS Innovation Fund for Medical Sciences (Nos. 2016-I2M-1-011, and 2016-I2M-3-014) and National Science & Technology Major Project "Key New Drug Creation and Manufacturing Program", China (Nos. 2019ZX09201001-003-007, 2019ZX09721001-004-006).

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Tale 1. Anti-influenza virus activity and cytotoxicity of the selected compounds in HEK293T-Gluc cells

| Cpd. | $IC_{50}$（μM） | $CC_{50}$（μM） | SI |
|---|---|---|---|
| **7e** | 36.10±7.88 | >100 | >2.77 |
| **7f** | 38.24±7.40 | >100 | >2.61 |
| **10a2** | 7.18±1.89 | >100 | >13.93 |
| **10b2** | 19.15±0.73 | >100 | >5.22 |
| **16a** | 1.88±0.10 | 23.28±2.91 | 12.38 |
| **16d** | 39.43±0.93 | >100 | >2.54 |
| **16e** | 1.29±0.01 | 59.94±3.04 | 46.46 |
| **16i** | 9.04±0.57 | 15.86±0.58 | 1.75 |
| **16j** | 3.88±0.47 | 36.64±2.24 | 9.44 |
| **16n** | 6.84±0.68 | 29.43±0.95 | 4.30 |
| **16o** | 3.83±0.15 | >100 | >26.11 |
| **16p** | 5.00±1.37 | >100 | >20.00 |
| **16q** | 11.47±0.54 | >100 | >8.72 |
| **16r** | 3.43±0.54 | >100 | >29.15 |
| **A** | 4.18±0.12 | 43.98±2.17 | 10.52 |
| **B** | 5.22±0.34 | >100 | >19.15 |
| **ribavirin** | 15.36±0.93 | >100 | >6.51 |

**Table 2** Anti-influenza virus activity of the selected compounds in 293T-GLUC cells

| Cpd. | $IC_{50}$ (μM) | Cpd. | $IC_{50}$ (μM) |
|---|---|---|---|
| **10a2** | 1.69±0.21 | **16n** | 0.12±0.03 |
| **16a** | 1.47±0.12 | **16o** | 6.05±0.32 |
| **16e** | 0.63±0.02 | **16p** | 5.80±0.68 |
| **16i** | 0.95±0.08 | **A** | 0.33±0.01 |
| **16j** | 0.41±0.09 | | |

**Table 3** The cytotoxicity of selected compounds on A549 cells

| Cpd. | $CC_{50}$（μM） | Cpd. | $CC_{50}$（μM） |
|---|---|---|---|
| **10a2** | **>** 100 | **16n** | 12.84±0.03 |
| **16a** | 19.04±1.34 | **16o** | **>** 100 |
| **16e** | 4.77±0.13 | **16p** | **>** 100 |
| **16i** | 5.92±0.14 | **A** | 71.41±3.4 |
| **16j** | 10.72±0.18 | | |

**Table 4** Parameters of Lipinski's rules

| Cpd. | LogP[1](≤5) | TPSA[2] | nON[3] (≤10) | nOHNH[4] (≤5) | Vol ($Å^3$) | MW[5] (≤500) | Lipinski's violations (≤1) |
|---|---|---|---|---|---|---|---|
| **10a2** | 4.77 | 58.12 | 5 | 1 | 336.96 | 366.49 | 0 |
| **16a** | 4.05 | 70.15 | 6 | 2 | 320.87 | 347.42 | 0 |
| **16e** | 5.24 | 70.15 | 6 | 2 | 364.86 | 397.48 | 1 |
| **16i** | 5.34 | 70.15 | 6 | 2 | 347.94 | 416.31 | 1 |
| **16j** | 5.04 | 70.15 | 6 | 2 | 375.48 | 409.49 | 1 |
| **16n** | 4.88 | 70.15 | 6 | 2 | 392.28 | 423.52 | 0 |
| **16o** | 3.33 | 79.38 | 7 | 2 | 346.66 | 377.45 | 0 |
| **16p** | 4.24 | 70.15 | 6 | 2 | 348.03 | 363.46 | 0 |
| **16q** | 3.38 | 73.39 | 7 | 2 | 367.02 | 390.49 | 0 |
| **16r** | 6.22 | 70.15 | 6 | 2 | 419.47 | 459.55 | 1 |

[1] LogP, logarithm of compound partition coefficient between n-octanol and water.

[2] TPSA, topological polar surface area.

[3] nON, number of hydrogen bond acceptors.

[4] nOHNH, number of hydrogen bond donors.

[5] MW, molecular weight.

**Table 5** Rat PK profiles of compound **16e**

| | | Dose (mg/kg) | |
|---|---|---|---|
| | Parameter | 1.25 (iv) | 25 (po) |
| Plasma | $K_{el}$ ($h^{-1}$) | 1.03±0.12 | 0.32±0.07 |
| | $T_{1/2}$ (h) | 0.68±0.07 | 2.25±0.5 |
| | $t_{max}$ (h) | - | 0.33±0.14 |
| | $C_{max}$ (ng/mL) | 58.0±16 | 78.6±16 |
| | $C_0$ (ng/mL) | 80.8±27 | - |
| | $AUC_{0-t}$ (h·ng/mL) | 34.9±8.9 | 157±62 |
| | $AUC_{0-inf}$ (h·ng/mL) | 39.2±9.4 | 183±55 |
| | $AUMC_{0-t}$ (h·h·ng/mL) | 18.0±4.2 | 313±111 |
| | $AUMC_{0-inf}$ (h·h·ng/mL) | 30.6±6.4 | 553±85 |
| | CL (mL/min/kg) | 557±155 | - |
| | MRT (h) | 0.79±0.08 | 3.18±0.99 |
| | Vss (L/kg) | 26.6±9.16 | - |
| | F(%) | - | 22.5±8.8 |

The PK study in male SD rats was carried out according to the standard procedures (iv, 1.25 mg/kg; po, 25 mg/kg). Major parameters, including plasma clearance (CL), volume of distribution at steady state (Vss), $T_{1/2}$, area under the cure (AUC), and oral bioavailability (F), are reported

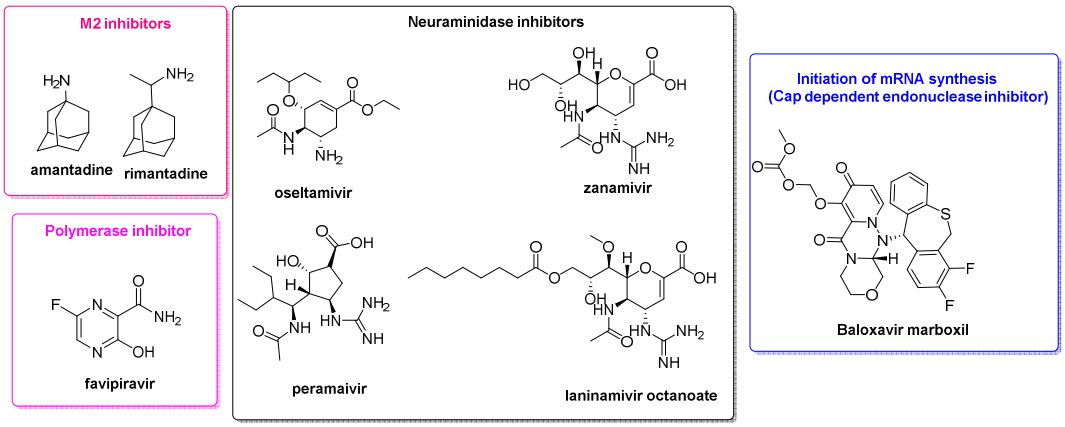



Fig. 1. Four classes of approved antiviral agents for the treatment of influenza virus infection

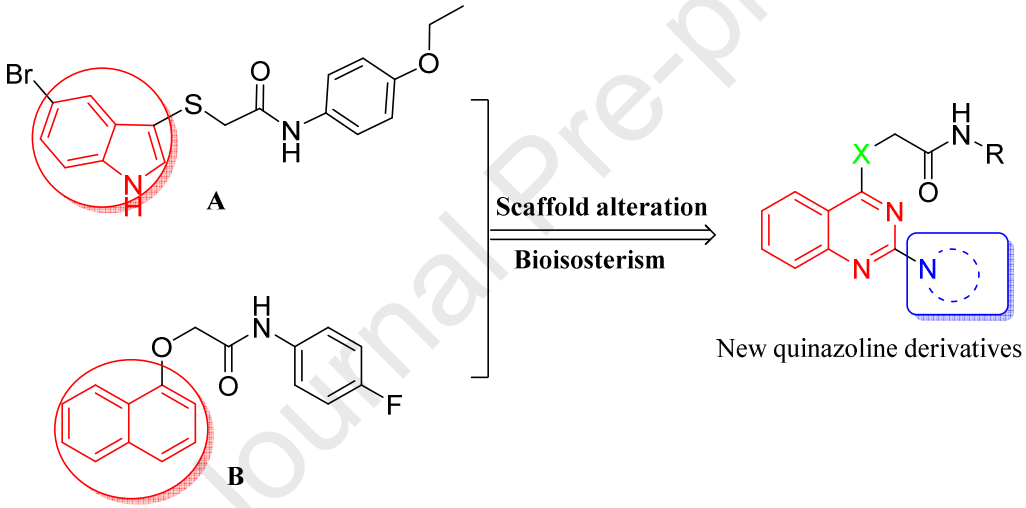



Fig. 2. Design of new quinazoline derivatives.

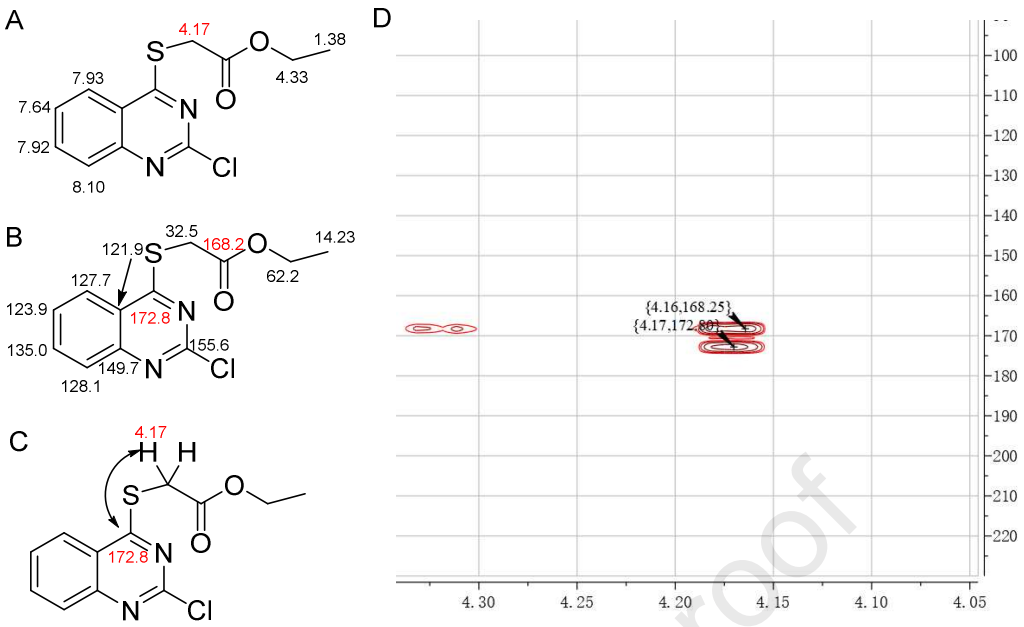



**Fig. 3**. Structure and HMBC spectrum of compound **4** ( A: Chemical shift of hydrogen atom in $^{1}$HNMR; B: Chemical shift of carbon atom in $^{13}$CNMR; C: Coupling between the newly introduced methylene hydrogen and the 4-position carbon atom; D: Part of HMBC-spectrum of compound **4**)

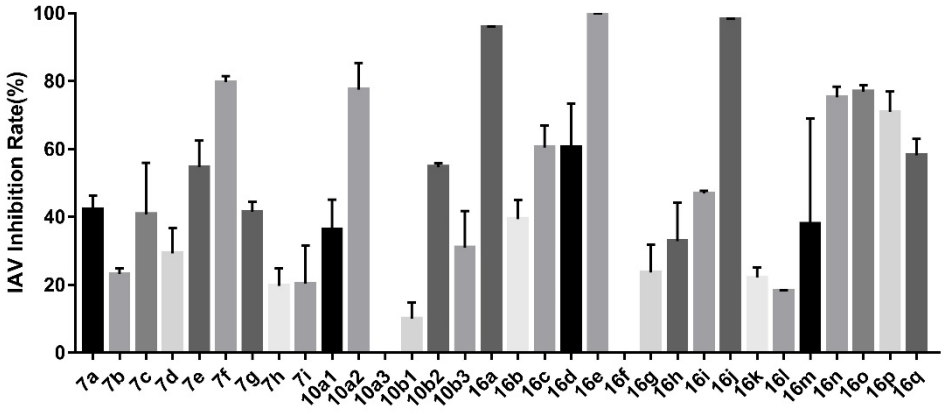



**Fig. 4**. Inhibition rate of the target compounds on IAV.

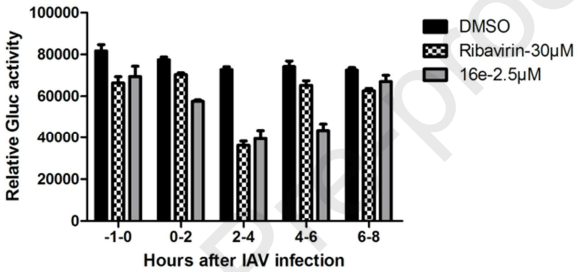



**Fig.5.** Times-of-addition of **16e** after infected with IAV**.** A549 cells were inoculated with influenza single-cycle A/WSN/33(H1N1) virus at a multiplicity of infection of 0.2 and compound **16e** (2.5 μM) was added. Viral yields were determined at 11 h post infection by measuring Gluc activity. In this experiment, DMSO was used as the negative control and ribavirin (30 μM) as the positive control.

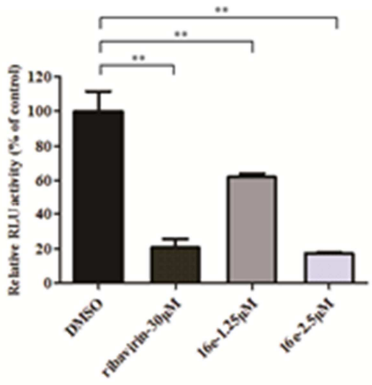
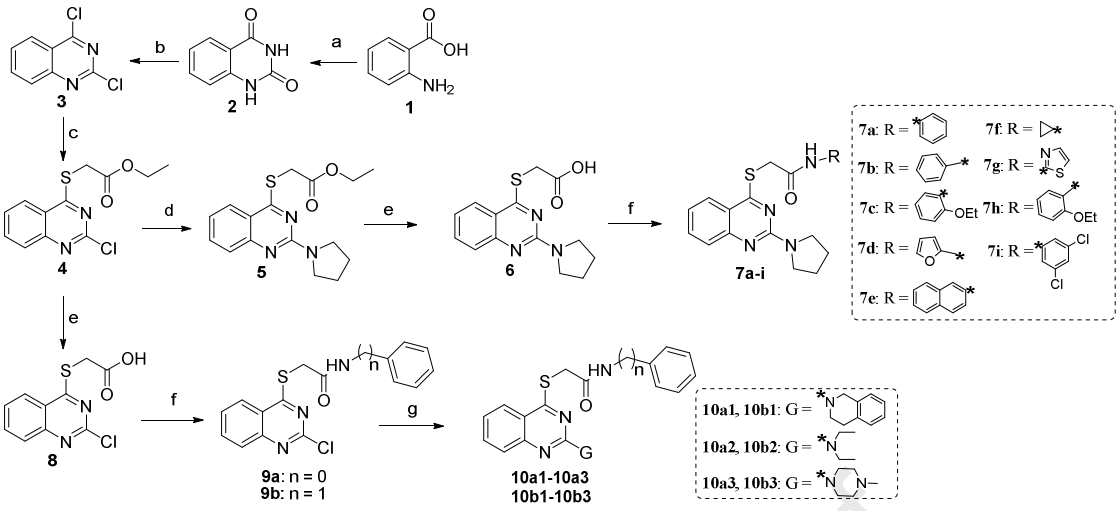
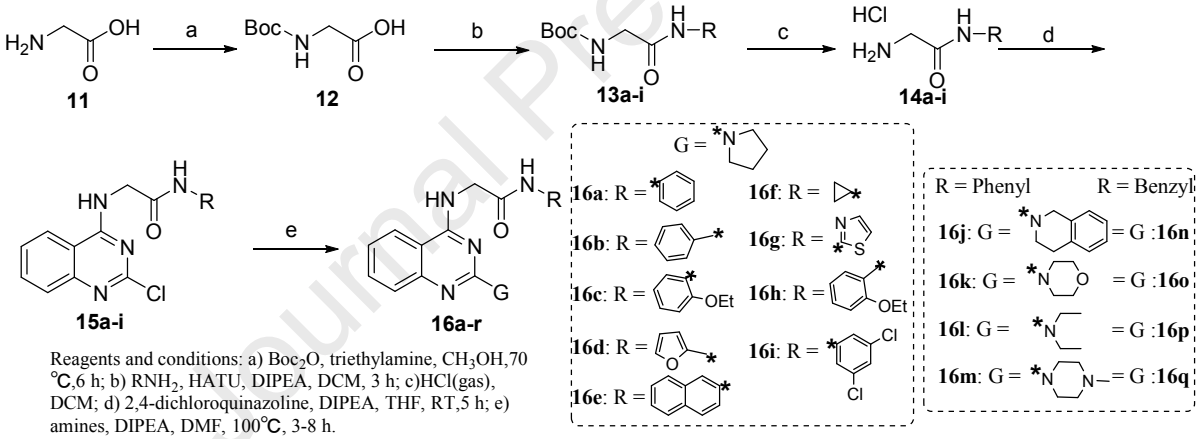



**Fig.6.** Inhibition of compound **16e** on influenza viral vRNP. HEK239T cells were transfected with pCAGGS expression plasmids encoding PB2、PB1、PA、NP、pol-LUC and SV40-Relina in the absence or presence of compound **16e** (1.25 μM or 2.5 μM). Effect of vRNA transcription was evaluated by measuring luciferase and relina at 24 h post-transfection. ** indicates $p<0.01$ as compared to negative control.




Reagents and conditions: a) urea, 180°C, 4 h; b) $POCl_3$, DIPEA, $CH_3CN$, 100°C, 6 h; c) DIPEA, ethyl thioglycolate,THF, RT, 2 h; d) pyrrolidine, DIPEA,THF, 80°C, 1 h; e) LiOH, $CH_3CH_2OH/THF/H_2O$, 40 min; f) $RNH_2$, HBTU, DIPEA, DCM, 4 h; g) amines, DIPEA, THF, 110°C, 10 h.

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




Reagents and conditions: a) $Boc_2O$, triethylamine, $CH_3OH$,70 °C,6 h; b) $RNH_2$, HATU, DIPEA, DCM, 3 h; c)HCl(gas), DCM; d) 2,4-dichloroquinazoline, DIPEA, THF, RT,5 h; e) amines, DIPEA, DMF, 100°C, 3-8 h.

**Scheme 2.** Synthesis of the target compounds **16**

## Highlights

- Novel quinazolines containing S-acetamide and NH-acetamide moieties were synthesized.
- Most of compounds showed strong anti-influenza A activities and low cell cytotoxicity.
- These compounds might inhibit the transcription and replication of viral RNA.
- Compounds **16e** with reasonable PK profiles shows better anti-IAV activity and acceptable cytotoxicity.

**Declaration of interests**

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: