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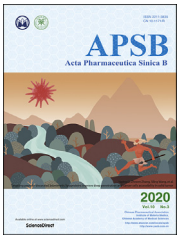

ORIGINAL ARTICLE

Novel small molecule retrograde transport blocker confers post-exposure protection against ricin intoxication

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KEY WORDS

Ricin toxin;
Ribosome-inactivating proteins;
Retrograde transport;
Post-exposure antidote;
Ricin antibody

Abstract Ricin is a highly toxic type 2 ribosome-inactivating protein (RIP) which is extracted from the seeds of castor beans. Ricin is considered a potential bioterror agent and no effective antidote for ricin exists so far. In this study, by structural modification of a retrograde transport blocker Retro-2^{cycl}, a series of novel compounds were obtained. The primary screen revealed that compound **27** has an improved anti-ricin activity compare to positive control. *In vitro* pre-exposure evaluation in Madin–Darby Canine Kidney (MDCK) cells demonstrated that **27** is a powerful anti-ricin compound with an EC_{50} of 41.05 nmol/L against one LC (lethal concentration, 5.56 ng/mL) of ricin. Further studies surprisingly indicated that **27** confers post-exposure activity against ricin intoxication. An *in vivo* study showed that 1 h post-exposure administration of **27** can improve the survival rate as well as delay the death of ricin-intoxicated mice. A drug combination of **27** with monoclonal antibody mAb4C13 rescued mice from one LD (lethal dose) ricin challenge and the survival rate of tested animals is 100%. These results represent, for the first time, indication that small molecule retrograde transport blocker confers both *in vitro* and *in vivo* post-exposure protection against ricin and therefore provides a promising candidate for the development of anti-ricin medicines.

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1. Introduction

Ricin is a type 2 ribosomal inactive protein[1] that is abundant in *Ricinus communis* seeds (castor beans)[2]. The LD_{50} of ricin for human adults is approximately 5−25 μg/kg if the exposure is from injection or inhalation and the oral lethal dose (LD) is 1 mg/kg[3]. After injection of ricin, victim would develop multiple organ dysfunction syndrome (MODS) which eventually caused death within 3 days. The inhalation of ricin could cause acute respiratory distress syndrome at 18−24 h, death generally occurs three days after exposure[4]. The ingestion of ricin can cause toxin induced liver and kidney failure, which lead to death 3−5 days after exposure[5]. Owing to its high toxicity, high stability and relatively easy purification techniques, ricin is considered a potential bioterrorism agent[6]. To date, neither effective clinical treatment nor specific medicine for ricin intoxication exists. Supportive care remains the only measure when treating ricin-poisoned patients. Therefore, research and discovery of an effective ricin antidote is of great importance to public health.

Ricin consists of two subunits: a catalytically active A (RTA)-chain joined by one disulfide bond to a B (RTB) chain that acts as an *N*-acetylgalactosamine-specific lectin[7]. By virtue of RTB, ricin toxins can be internalized by endocytosis. Once inside the cell, ricin are transported from early endosomes to the endoplasmic reticulum (ER) *via* the Golgi apparatus[8] and this transport pathway has been termed retrograde transport[9,10]. Resorting to retrograde transport, the RTA chain is able to travel into the cytoplasm and depurinate a specific adenine residue in 28S ribosomal RNA, which disables the protein synthesis ability of ribosomes[11,12] and eventually causes cell death[13].

Many attempts have been made to identify small molecule ricin inhibitors[14−16]. A retrograde transport blocker Retro-2 (Fig. 1), discovered by Julien Barbier and coworkers[17], proved to be a potential small molecule inhibitor of type 2 ribosome-inactivating proteins (RIPs) such as ricin and shiga toxins. In their experiments they reported that a 1 h pre-intoxication administration of 200 mg/kg Retro-2 can protect mice from a 2 μg/kg ricin exposure *via* the nasal route with a survival rate of 100%. Study of the mechanism revealed that Retro-2 does not inhibit the catalytically active site of type 2 RIPs; instead, Retro-2 blocks the retrograde transport of toxins from early endosomes to the ER through the *trans*-Golgi network (TGN)[18]. Later, Park et al.[19] reported that the bioactive compound was not Retro-2 but the cyclized product Retro-2^{cycl} (Fig. 1). Structural−activity relationship instructed structural modification of Retro-2^{cycl} produced a more powerful shiga toxin inhibitor compound **94** (Fig. 1) with an *in vitro* EC_{50} of 300 nmol/L in HeLa cells[20]. Anti-ricin data has not been reported for compound **94**.

So far, all reported Retro-2 series compounds need to be administered pre-intoxication to achieve their anti-type 2 RIPs activity. Therefore, the compound can only be used as a prophylactic agent, which limits compounds' practicability. In this study, we modified the structure of Retro-2^{cycl} in order to obtain bioactivity improved and post-exposure effective compounds against ricin intoxication.

2. Results and discussion

2.1. *Structural modification of Retro-2^{cycl}*

The optimization work of this study was focused on three main substitutable parts of Retro-2^{cycl}: the benzene ring of dihydroquinazolinone (R_1), the substituent group on 3-N (R_2) and the substituent group on 5-position of thiophene (R_3) (Fig. 2). The Madin−Darby Canine Kidney (MDCK) cell line was used to assess the anti-ricin ability of new compounds at a concentration of 50 μmol/L. MDCK cell line is sensitive to ricin intoxication and a good correlation between cell survival rate and compound activity has been observed in the preliminary experiments, so in this section compounds activity were quantified by cell survival rate. Retro-2^{cycl} was used as the positive control in primary screen of new compound.

First, by replace 7-C or 8-C with N, we substitute the benzene ring with a more electrophilic pyridine ring. Then R_3 was subsequently substitute with various groups to explore the bioactivity of pyridine ring system, the results were shown in Table 1. Replace 8-C of Retro-2^{cycl} with N resulted in **1** and the compound retained the activity of Retro-2^{cycl}, which indicated the modification in 8-C is tolerable. Replacement of 7-C, however, resulted in a loss of activity. The substitution of R_3 with bromo retained the activity compare to Retro-2^{cycl}. Substitution with phenyl or heterocyclic 5-methylthiazole decreased the activity compare to Retro-2^{cycl} but still demonstrated a mild anti-ricin ability.

Then we probed the influence of substituent R_2 on activity, results were shown in Table 2. In the 8-N replaced series compounds, substitution of R_2 with *o*-chlorobenzene resulted in **9**−**12**, they retained or slightly increased the anti-ricin activity. It was evidenced by **7** and **8** compare to **3** and **4** respectively that when replace benzene on R_2 with *o*-methoxybenzene, the activity increased. Certified that the *o*-substitution on 3-phenyl could benefit to the *in vitro* anti-ricin activity of compounds. The substitution of R_2 and R_3 did not improve the activity of 7-N series

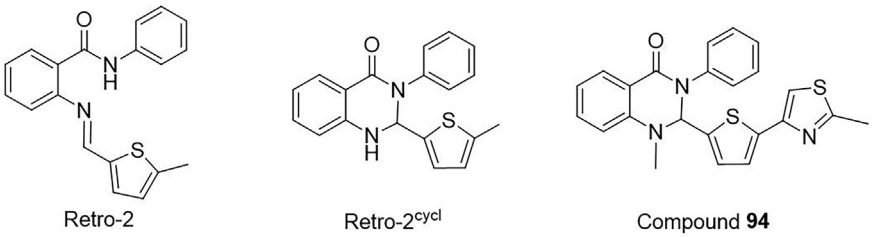


Figure 1 The structures of Retro-2, Retro-2^{cycl} and compound **94**.

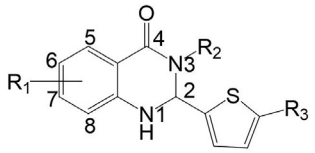
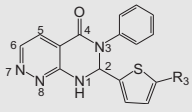


Figure 2 Optimization domains of Retro-2^{cycl}.

compounds. Compounds **18**−**20** substitute benzene on R_2 with 5-substituedthiazole, the modification showed little influence on activity compare to Retro-2^{cycl}.

Based on the results of 8-N series compounds we concluded that the increase of electrophilic on dihydroquinazolinone is a feasible modification strategy. Based on that we then explored the influence of a more electrophilic ring system on compounds' activity. A series of compounds with 6-F or 7-F dihydroquinazolinone structure were synthesized and evaluated, results shown in Table 3. Compounds with 7-F ring system demonstrated little difference in activity compare to Retro-2^{cycl}. But 6-F substituted compounds had a significant improvement of anti-ricin activity evidenced by activities of **25**−**27**. Therefore, electrophilic substitute in 6-H increased compounds' activity. Additionally, substitute R_2 with *o*-methoxybenzene also improved the bioactivity of compounds as **24** possess the best activity among 7-F series compounds. The methylation on 1-N is a tolerable modification and may benefit to the pharmacokinetic quality of

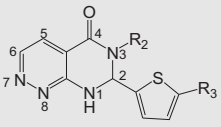
Table 1 Structure and anti-ricin activity of compounds **1**−**5**.



Compd.	N substitute position	R_3	Cell survival rate (%)[a]
1	8		74.16±7.47
2	8		68.82±7.98
3	8		47.09±1.85
4	8		53.23±7.15
5	7		35.57±2.58
Ricin	−	−	41.69±5.22
Retro-2^{cycl}	−	−	68.76±5.24

−Not applicable.
[a]Data are expressed as mean ± SD.

Table 2 Structure and anti-ricin activity of compounds **6**−**20**.



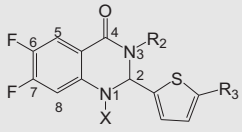
Compd.	N substitute position	R_2	R_3	Cell survival rate (%)[a]
6	8			68.79±13.01
7	8			62.83±5.56
8	8			60.81±1.79
9	8			68.53±5.65
10	8			68.91±9.63
11	8			72.51±5.16
12	8			47.84±3.51
13	8			58.49±5.77
14	7			43.26±5.59
15	7			41.05±5.54
16	7			56.47±11.31
17	7			42.88±3.16
18	−			59.15±7.89
19	−			60.35±9.19

Table 2 (*continued*)

Compd.	N substitute position	R_2	R_3	Cell survival rate (%)[a]
20	–			55.24±8.61
Ricin	–	–	–	41.69±5.22
Retro-2^{cycl}	–	–	–	68.76±5.24

–Not applicable.
[a]Data are expressed as mean ± SD.

Table 3 Structure and anti-ricin activity of compounds **21**–**27**.



Compd.	F substitute position	R_2	R_3	X	Cell survival rate (%)[a]
21	6			H	64.31±7.47
22	7			CH_3	54.60±3.69
23	7			H	60.50±6.98
24	7			H	64.98±3.80
25	6			H	81.14±6.11
26	6			CH_3	75.16±9.54
27	6			CH_3	94.31±10.02
Ricin	–	–	–	–	52.18±6.13
Retro-2^{cycl}	–	–	–	–	63.39±9.23

–Not applicable.
[a]Data are expressed as mean ± SD.

compounds as it stabilized the secondary amine of dihydroquinazolinone. Compound **27**, which combined the optimized structure discussed above, conferred a significant improvement in anti-ricin activity compare to Retro-2^{cycl}.

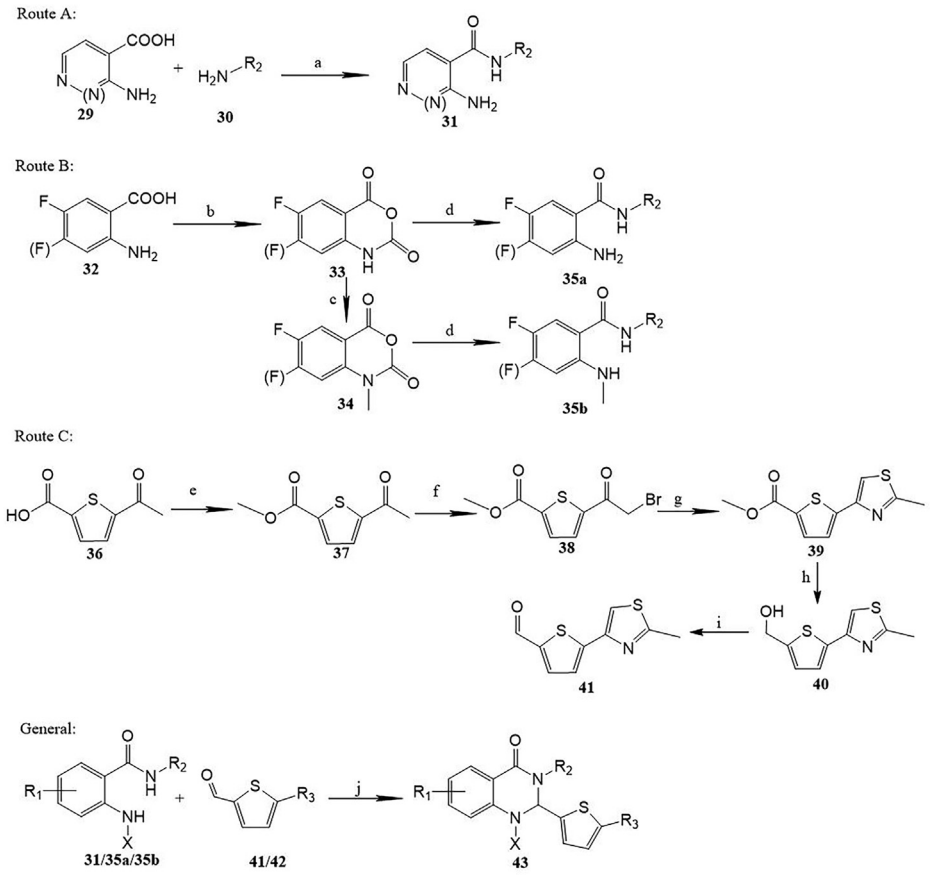
2.2. Chemistry

As outlined in Scheme 1, the general synthesize strategy of designed compounds was by condensation of amino intermediate **35a/35b** with aldehyde intermediate **41/42**. The required amino intermediate of each compound was synthised follow two routes: Route A produce the required nicotinamide intermediates for compounds **1**–**17** by condensation of nicotinic acid/isonicotinic acid **29** with amide **30**, the condensation reagents were 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt). Route B produce fluro substituted amide intermediates for compounds **21**–**27** from starting material F-substituted *o*-amide benzoic acid, by cyclization with thiposigene. F-substituted lsatoic anhydride **33** was produced, then **33** was retact with required amide in acid to form **35a** or methylated by MeI to form **34,** then **34** reacted with amide to form **35b**. The required amino intermediates for **18**–**20** were also synthesized according to Route B by replace the starting material with *o*-amide benzolic acid. The aldehyde intermediates are mostly commercial available with one exception: 5-(2-methylthiazol-4-yl)thiophene-2-carbaldehyde was synthesized from 5-acetylthiophene-2-carboxylic acid (**36**), the carboxylic group of starting material was protected by esterification with MeOH to form **37**, then **37** was brominated by cupric bromide and cyclized with thiacettamide to form **39**. Intermediate **39** was subjected to a reduction by $LiAlH_4$ and then oxidized by MnO_2 to form **41** (Route C).

2.3. *In vitro* pre-intoxication anti-ricin activity of 27

The anti-ricin ability of **27** against ricin intoxication in MDCK cells was evaluated to determine *in vitro* pre-intoxication anti-ricin activity of the compound. Compound **94**, a powerful anti-shiga Retro-2^{cycl} series compound developed by Romain and co-workers[20], demonstrated better anti-ricin ability compare to Retro-2^{cycl} in our pre-experiments. Therefore, compound **94** was used as a positive control in further evaluation experiments.

In this assay, MDCK cells were pretreated with different concentrations of **27** (0.01, 0.03, 0.10, 0.30 or 0.90 μmol/L) or compound **94** (0.01, 0.15, 0.31, 0.62, 1.25, 2.50, 5.00, 10.00, 20.00, 30.00 or 40.00 μmol/L), incubated for 12 h and then challenged with 1 LC (5.56 ng/mL) of ricin. Then, cells were incubated for another 12 h, and then, the survival rate of cells in each group and the EC_{50} values of tested compounds were calculated (results shown in Fig. 3). The results indicated that **27** was effective against ricin at a concentration higher than 0.01 μmol/L suggested by the improvement of cell survival rate, and **27** can protect cells against a ricin challenge with the maximum survival rate of 100.95±6.72% at a concentration of 0.9 μmol/L, while compound **94** was effective at a concentration higher than 5 μmol/L and could convey a maximum cell survival rate of 66.21±2.57% at the highest tested concentration of 40 μmol/L. The pre-exposure treatment EC_{50} value of **27** was 41.05 nmol/L and the EC_{50} value of compound **94** was 16.13 μmol/L. In summary, **27** have stronger anti-ricin activity compared to compound **94**.



Scheme 1 Reaction conditions and reagents: a) EDCI, HOBt, DIPEA, DCM, 0 °C−r.t., Yield 60%−90%; b) thioposigene, THF, DMF, 66 °C, Yield 90%; c) NaH, MeI, DMF, 0 °C−rt, Yield 45%; d) required amide, AcOH, 60 °C, Yield 70%−80%; e) $SOCl_2$, MeOH, DMF, 50 °C, Yield 95%; f) $CuBr_2$, ethyl acetate, reflux, Yield 60%; g) thiacettamide, DMF, 65 °C, Yield 55%; h) $LiAlH_4$, THF, 0 °C, Yield 68%; i) MnO_2, DCM, rt, Yield 90%; j) AcOH, rt, Yield 40%−90%.

Then, the protection ability of **27** against ricin was assessed. In this assay, MDCK cells were pretreated with different concentrations of **27** (0.011, 0.033, 0.10, 0.30 or 0.90 μmol/L), incubated for 2 h and then challenged with different concentrations of ricin (0.06, 0.20, 0.60, 1.80, 5.50, 16.70, 50.00 or 100.00 ng/mL). The rest of the procedures were the same as in the above assay (results shown in Fig. 4). The results showed that **27** at a concentration as low as 0.033 μmol/L could protect cells from 1 LC (5.56 ng/mL) ricin challenge, with a cell survival rate of 102.68±15.47%, the significant difference compare to ricin control group were determined by *t*-test with a result of $P < 0.01$. At a concentration of

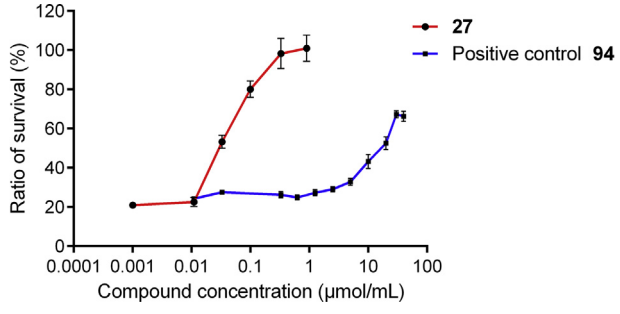


Figure 3 Cell survival curve in pre-exposure anti-ricin experiments. The Madin−Darby Canine Kidney (MDCK) cell survival rate when pre-treated with **27** or positive control **94** before ricin challenge was calculated to compare the protect ability of tested compounds. Data are expressed as mean ± SD ($n = 6$).

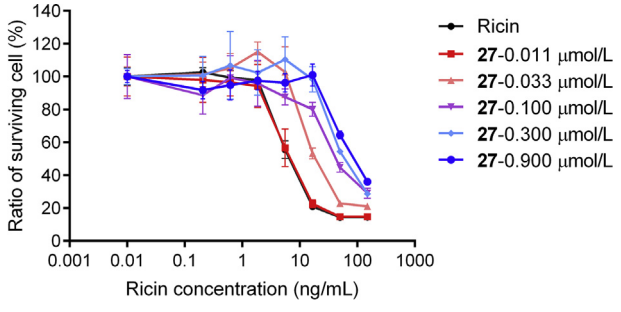


Figure 4 Maximum protection ability cell survival rate of **27**. The MDCK cell survival rate when pre-treated with different concentrations of **27** before variety lethal concentration (LC) of ricin challenge was calculated to determine the protection ability of **27**. Data are expressed as mean ± SD ($n = 6$).

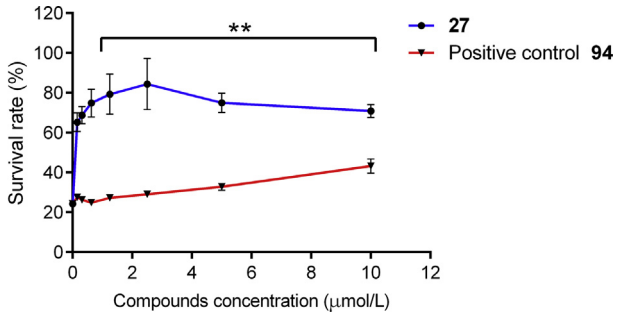


Figure 5 Survival rate of cell treated with **27** or positive control **94** after ricin intoxication. The MDCK cell survival rate when treated with **27** or positive control **94** after one LC ricin challenge was calculated to compare the protect ability of tested compounds. Data are expressed as mean ± SD ($n = 6$); $^{**}P < 0.01$.

0.9 μmol/L, **27** could protect cells from up to 3 LC (16.7 ng/mL) of ricin challenge, with a cell survival rate of 100.95±6.72% ($P < 0.01$ compare to ricin control group). In conclusion, **27** is an effective protector of MDCK cells when administrated 2 h before ricin intoxication and the protect ability of **27** is dose related.

2.4. *In vitro post-intoxication anti-ricin activity of 27*

The *in vitro* post-exposure protecting ability of **27** against ricin was subsequently evaluated. First, the dose-effect relationship of **27** and positive control compound **94** in MDCK cells was evaluated. In this assay, MDCK cells were challenged with 1 LC of ricin and incubated for 1 h and then treated with different concentrations of **27** or compound **94** (0.16, 0.32, 0.63, 1.25, 2.50, 5.00 or 10.00 μmol/L), then the cells were incubated for another 12 h, and the survival rates were calculated (results shown in Fig. 5). The results indicated significant anti-ricin activity of **27** compared to that of compound **94**. Compound **27** at the lowest tested concentration of 0.16 μmol/L demonstrated a cell survival rate of 65.24±4.66%, improved the cell survival rate to 84.39±12.84% at a concentration of 2.50 μmol/L compared to that of ricin controls

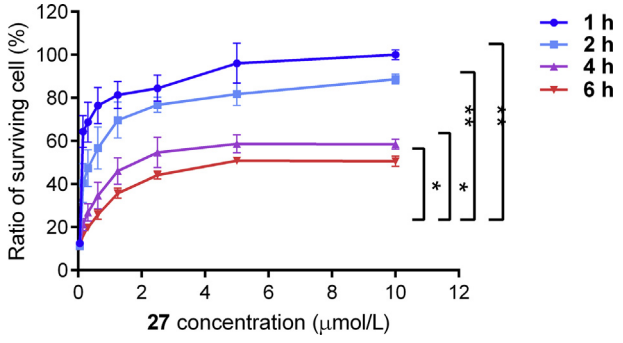


Figure 6 Time-effect relationship of cells treated with **27** after ricin intoxication. The MDCK cell survival rate when treated with **27** after variety periods of 1 LC ricin challenge was calculated to compare the protect ability of tested compounds. Data are expressed as mean ± SD ($n = 6$); $^{**}P < 0.05$, $^{**}P < 0.01$.

(24.29±0.97%). The EC_{50} value of **27** in a 1 h post-exposure treatment assay was 144 nmol/L, indicating that after ricin challenge, post-exposure treatment with **27** can rescue cells from ricin induced cell death. Compound **94** showed weak anti-ricin activity with a survival rate of 43.21±3.54% at the highest tested concentration of 10.00 μmol/L; therefore, the EC_{50} for compound **94** cannot be calculated. The survival rate of **27** in all tested concentrations had a statistical significance compared to the survival rate of the ricin control group and compound **94** group ($P < 0.001$), therefore the therapeutic effect of **27** was significant.

The time-effect relationship of **27** was evaluated to determine the therapeutic window of the compound. In this assay, MDCK cells were intoxicated with one LC of ricin, and then, after 1, 2, 4 or 6 h, the cells were respectively treated with different concentrations of **27** (0.16, 0.31, 0.63, 1.25, 2.50, 5.00 or 10.00 μmol/L); the rest of the procedures were the same as in dose-effect assay (results shown in Fig. 6). Among the four sets of experiments, a 10 μmol/L administration of **27** in the 1 h post-exposure treatment groups conferred the best survival rate of the cells (100±2.23%) and a lowest value of 0.16 μmol/L for **27** in 1 h group still conferred a survival rate of 64.40±7.40%, which showed significant improvement compared to the survival rate of the ricin control group (12.57±0.28%). The 2 h post-exposure treatment groups showed a milder yet still significant anti-ricin activity with the highest survival rate of 88.68 ± 2.28% and lowest rate of 40.43 ± 9.02%. In the 4 and 6 h groups, however, **27** demonstrated weaker anti-ricin activity. The highest survival rate in 4 h group was 58.64 ± 4.19% when given 5 μmol/L of **27**, and an increasing of dose conferred no improvement (58.42 ± 2.31%), which indicated that the best survival rate that **27** could achieve when administered 4 h post-intoxication was approximately 58%. A similar result was observed in the 6 h group, with the best survival rate of approximately 50%. The results indicated that the therapeutic ability of **27** was correlated with the administration time and that an early treatment is crucial for **27** to achieve a better therapeutic effect.

2.5. *In vivo pre-intoxication anti-ricin activity of 27*

In vitro bioactivity evaluation results of **27** encouraged us to continue exploring the *in vivo* bioactivity of compound **27**. Pre-experiments were performed to determine the toxicity of **27** on mice. The results indicated that at a concentration of 50 mg/kg, **27** has no influence on the viability of female CD-1 mice. A model of ricin intoxication by i.p. administration was developed to mimic

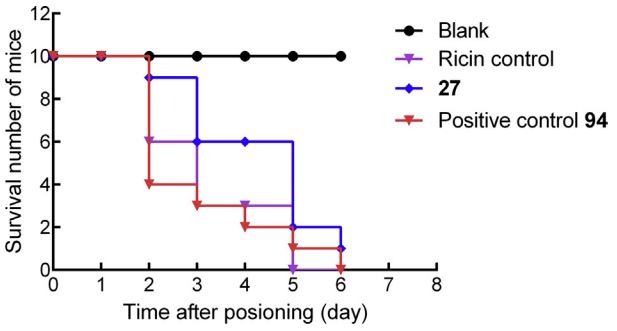


Figure 7 Pre-intoxication administration of **27** mice survival curve.

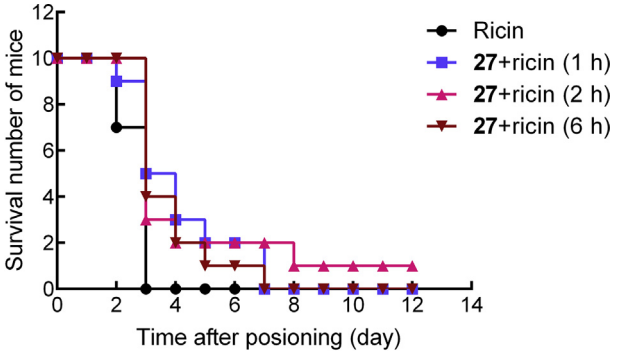


Figure 8 Post-intoxication administration of **27** mice survival curve.

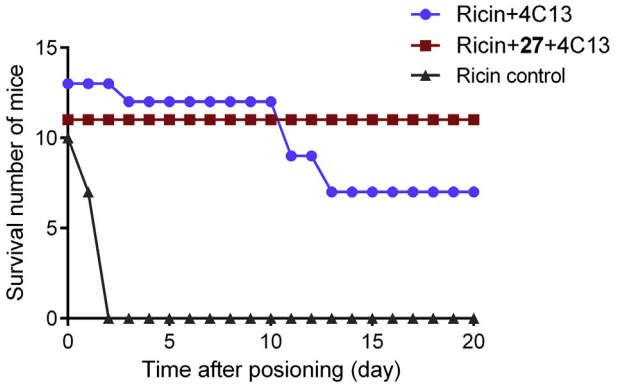


Figure 9 Compound **27** and mAb4C13 against post-intoxication of ricin in mice.

exposure through injection. This is one of the most lethal approaches of ricin intoxication. A dose of 13 μg/kg for ricin was used in this model. Compound **94** was used as a positive control. Mice were randomly divided into the blank control group, ricin control group, positive control group and **27** group. The test groups of mice were respectively treated with 50 mg/kg of **27** or compound **94** 1 h prior to intoxication with one LD (13 μg/kg) of ricin; then the number of surviving mice were recorded every day until 6 days after intoxication. The survival curve is shown in Fig. 7.

The results indicated that the death of mice started from day 3, with four deaths occurring in the ricin control group and six deaths in the compound **94** group, but only one mouse died in the **27** group. At day 4, only three mice were still alive in the ricin control group and the compound **94** group, while six survived in the **27** group. At the end of this experiment, all mice in the ricin control group and compound **94** group died, but one mouse survived in the **27** group and a regain of bodyweight was observed in that mouse. The survival curves of **27** group and ricin control group were analyzed by Log-rank (Mantel−Cox) Test, the result indicated that the survival curve of **27** is significant different compared with that of ricin control group ($P = 0.0374$). The pre-treatment of mice with **27** could increase the survival rate of mice and prolong their survival time while no such delayed effect was observed in the positive control group.

2.6. *In vivo post-intoxication anti-ricin activity of 27*

The post-exposure anti-ricin activity of **27** in mice was also evaluated. The intoxicated animal model and ricin dose were the same as Section 2.5. Due to the poor activity of compound **94** in the pre-exposure assay, no positive control group was set in this assay. Mice were respectively treated with 50 mg/kg of **27** at 1, 2 or 6 h after intoxication. The behavior and survival rate of mice were observed every day for 12 days. The survival rate curve is shown in Fig. 8. Ricin control group mice started to die from day 2, three deaths occurred that day, and all the rest died the day after. The test group, however, revealed a delay of total death compared to that of the control group. Total death in the 1 and 4 h group occurred 4 days later than that of the control group. The same delaying effect was also observed in the 2 h group and one mouse survived the ricin challenge. The survival curve of **27** groups was significant different compared with that of ricin control group, tested by Log-rank (Mantel−Cox) Test ($P < 0.05$). Collectively, post-intoxication administration of **27** can prolong the survival time of mice.

2.7. *Combined pharmacotherapy of 27 with mAb4C13 against ricin intoxication*

It has been reported by Na Dong and co-workers[21] that monoclonal antibody mAb4C13 can recognize the specific epitope of ricin with very high affinity and the antibody demonstrated potent anti-ricin efficiency in both *in vitro* and *in vivo* experiments. Therefore, we explored the efficacy of a combined administration of **27** and mAb4C13 against ricin intoxication in mice.

The experiments were performed on the same intoxication model as in Section 2.5. The antibody group were treated with vehicle 0.5 h after intoxication and then treated with 300 μg/kg mAb4C13 2 h after intoxication. The combined group were treated with 50 mg/kg of **27** 0.5 h after intoxication then treated with 300 μg/kg of mAb4C13 2 h after intoxication. Ricin control group were challenged with 1 LD (13 μg/kg) of ricin and then given vehicle at 0.5 and 2 h after intoxication. The results were shown in Fig. 9. The single use of mAb4C13 protected mice from ricin challenge to some extent and achieved a 53.85% survival rate 20 days after intoxication. The combination of **27** and mAb4C13 strongly protected mice from one LD ricin challenge with a survival rate of 100% at the end of the experiment. Log-Rank test confirmed that the combination curve was significantly different

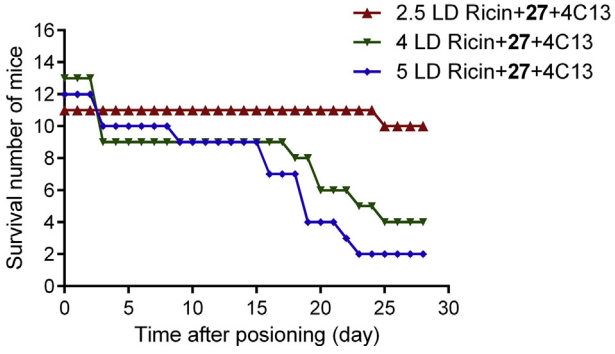


Figure 10 Anti-ricin ability of **27** and mAb4C13.

from antibody curve ($P < 0.05$). Therefore, a combination of **27** with mAb4C13 is an effective therapeutic approach against ricin intoxication in mice.

Then the anti-ricin ability of this combination was also evaluated. In this experiment, each group of mice was challenged respectively with 2.5, 4 or 5 LD (32.5, 52 or 65 μg/kg) of ricin. Then each group was treated with 50 mg/kg of **27** 0.5 h after intoxication and 300 μg/kg of mAb4C13 1.5 h after the administration of **27**. Results were shown in Fig. 10. In the 2.5 LD group, only one mouse died 25 days after intoxication and the final survival rate is 90.90%, indicated that the combination is effective against 2.5 LD challenge of ricin. The survival curve of 4 and 5 LD group began decrease from day 3, and the final survival rate are 44.44% (4 LD) and 20% (5 LD), therefore the combination was less effective against a intoxication dose higher than 4 LD of ricin.

Former studies revealed that the inhibition bio-mechanism of Retro-2^{cycl} series compounds is to block the intracellular retrograde trafficking of toxins from early endosomes to the ER through the *trans*-Golgi network[17], so intuitively, an advanced deliver of compounds is crucial for the anti-toxin activity of Retro-2^{cycl} series compounds. However, in our post-intoxication treatment experiments, Retro-2^{cycl} series compound **27** demonstrated specific anti-ricin activity when given after cell intoxication. These results of our study expanded the practicability of Retro-2^{cycl} series compounds against ricin. The reason of the improvement could be resulted from the stronger retrograde transport block ability of **27**. The intracellular trafficking of ricin was proved to be sustained, so after intoxication, it took a period of time for ricin to transport inside the cell and accumulate RTA into cytoplasm to reach the required toxic concentration. Therefore, the administration of a transport blocker during that period can still protect the cell, however, a fairly effective blocker is required, as a considerable portion of RTA is already inside the cytoplasm, and the block of the rest should be timely and efficient. The structure modification promotes **27** to be a stronger retrograde transport blocker of ricin (which can be concluded from the *in vitro* experiments) and would be more efficient when blocking the intracellular trafficking of ricin.

Retro-2 was discovered by high throughput screen, it countered ricin intoxication by block the retrograde transport of the toxin from early endosome to ER. However, as the detailed molecular level mechanism of retrograde transport was not thoroughly explained, no specific protein could be used to explore the anti-ricin mechanism of **27**. But as **27** possesses the same dihydroquinazoline structure of Retro-2 series compounds, the compound should share the same target protein as other Retro-2 series compounds. In the future when key proteins and the mechanism of retrograde transport route are studied, the mechanism study of **27** can be performed to interpret the specific target and binding sites of the compound to provide guidance for future developments of Retro-2 series compounds.

3. Conclusions

In this study, a novel compound **27** with a 12 h pre-exposure EC_{50} of 41.05 nmol/L against ricin was discovered. Later experiments showed the compound had significant post-exposure therapeutic activity against ricin intoxication with a 1 h post-exposure EC_{50} of 144 nmol/L. This is the first time a retrograde transport blocker conferred post-exposure anti-ricin activity. The combined administration of **27** with mAb4C13 effectively rescued mice from LD ricin challenge and therefore provides a possible therapeutic approach for treatment of ricin intoxication.

4. Experimental

4.1. Chemistry

Melting points were determined with an XRC-1 melting point apparatus (Beijing, China) and are reported as uncorrected. ^{1}H NMR spectra were measured on a Bruker AVANCE spectrometer (Bremen, Germany) at 400 MHz using tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were recorded by an HP 5989B spectrometer with an electrospray ionization (ESI) source (Palo Alto, CA, USA). High-resolution mass spectra were obtained with a Varian spectrometer (Palo Alto, CA, USA). All chemicals and solvents were of analytical or reagent grade. They were purchased commercially and used as received, unless otherwise stated.

4.2. Compound synthesis

4.2.1. Synthesis of 5-(2-methylthiazol-4-yl)thiophene-2-carbaldehyde (intermediate *41*)

To a solution of 5-acetylthiophene-2-carboxylic acid (5 g, 29.4 mmol) in methanol (120 mL) and DMF (1.5 mL) was added dropwise $SOCl_2$ (12.24 g, 102.9 mmol) at room temperature, and the mixture was heated to reflux for 5 h. Next, the pH of the mixture was adjusted to 7 by a saturated $NaHCO_3$ solution. The mixture was filtered, and the precipitates were collected, washed with water and dried under a vacuum to produce 5-acetylthiophene-2-methylacetate (intermediate **6**) as a gray solid (4.75 g, Yield 87.4%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.77 (s, 1H), 7.65 (s, 1H), 3.93 (s, 3H), 2.60 (s, 3H).

Methyl-5-acetylthiophene-2-carboxylate (4.75 g, 25.7 mmol), cupric bromide (11.48 g, 51.4 mmol) and ethyl acetate (150 mL) were added to a 250 mL round flask, and then, the mixture was stirred and heated to reflux for 8 h. Next, the mixture was filtered, and the filtrates were washed with water and dried over $MgSO_4$. The solution was then evaporated to produce 5-(2-bromoacetyl) thiophene-2-methylacetate (intermediate **7**) as a light green solid (6.2 g, Yield 91.2%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.77 (d, J = 13.1 Hz, 2H), 7.65 (s, 1H), 4.36 (s, 2H), 3.94 (s, 3H).

To a 250 mL round flask were added **7** (6.2 g, 23.5 mmol), thioacetamide (1.95 g, 25.9 mmol) and DMF (100 mL), and the mixture was heated to reflux for 6 h. Then, the mixture was cooled to room temperature and quenched with saturated NH_4Cl water solution. The mixture was filtered to collect the precipitate, and the filtered cakes were washed with water and dried to produce 5-(2-methylthiazol-4-yl)thiophene-2-methylacetate (intermediate **8**) as a yellow solid (3.85 g, Yield 68.2%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.74 (d, J = 3.9 Hz, 1H), 7.39 (d, J = 3.9 Hz, 1H), 7.33 (s, 1H), 3.89 (s, 3H), 2.76 (s, 3H).

To a suspension of $LiAlH_4$ (1.83 g, 48.3 mmol) in anhydrous THF (35 mL) at 0 °C was added dropwise **8** (3.85 g, 16.1 mmol). The mixture was then stirred at 0 °C for 90 min, the reaction quenched with water, and the solution filtered; the filtrate was dried over $MgSO_4$. Next, the solution was evaporated to produce

(5-(2-methylthiazol-4-yl)thiophen-2-yl)methanol (intermediate **9**) as a yellow oil (3.28 g, Yield 97%), and the product was used without purification.

Intermediate **9** (3.28 g, 15.6 mmol) was dissolved in DCM (85 mL), and MnO_2 (27.1 g, 312.4 mmol) was added to the solution. The resulting mixture was stirred at room temperature for 2 h, and after completion, the mixture was filtered and the filtrate evaporated to produce 5-(2-methylthiazol-4-yl)thiophene-2-carbaldehyde (intermediate **10**) as a yellow solid (1.73 g, Yield 53.4%). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm), 9.91 (1H, m), 8.15 (1H, s), 8.02 (1H, d, J = 3.9 Hz), 7.55 (1H, d, J = 3.9 Hz), 2.70 (3H, s). ESI-MS (*m/z*), 210.01 $[M+H]^+$.

*4.2.2. General procedure for the preparation of compound **1**−**17***

2-Amino nicotinic acid or 3-amino isonicotinic acid (7.24 mmol), *N,N*-diisopropylethylamine (DIPEA, 8.69 mmol) and required amine (7.24 mmol) were disolved in DCM. Then EDCI (8.69 mmol) and 1-hydroxybenzotriazole (HOBt, 7.24 mmol) were added to the solution at 0 °C. Then the mixture was stirred at room temperature overnight. After completion, DCM was evaporated under reduced pressure and the residue was purified with silica gel chromatography elution with hexane/ethyl acetate = 2:1 to produce required amino intermediate **31**. Then **31** (1.4 mmol) and required aldehyde intermediate **41** or **42** (2 mmol) were disloved in AcOH (5 mL) and then stirred at room temperature for 12−24 h, the solution was evaporated under reduced pressure and the residue was purified with silica gel chromatography to produced pure products **1**−**17**.

*4.2.2.1. 2-(5-Methylthiophen-2-yl)-3-phenyl-2,3-dihydropyrido [2,3-d]pyrimidin-4(1H)-one (**1**).* Yield: 45.4%, mp: 202−204 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (d, J = 3.1 Hz, 1H), 8.22 (dd, J = 4.8, 1.9 Hz, 1H), 8.02 (dd, J = 7.6, 1.7 Hz, 1H), 7.40−7.20 (m, 5H), 6.79 (dd, J = 7.6, 4.9 Hz, 1H), 6.68 (d, J = 3.4 Hz, 1H), 6.54 (dd, J = 3.4, 1.1 Hz, 1H), 6.36 (d, J = 3.1 Hz, 1H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 161.94, 156.71, 152.75, 141.10 (d, J = 9.3 Hz), 140.01, 137.87, 129.24, 127.46, 127.15, 126.64, 124.61, 115.50, 111.40, 70.47, 15.53. ESI-MS (*m/z*), 322.1 $[M+H]^+$. HRMS: Calcd. for $C_{18}H_{15}N_3OS$: 321.0936; Found: 322.0857 $[M+H]^+$.

*4.2.2.2. 2-(5-Bromothiophen-2-yl)-3-phenyl-2,3-dihydropyrido [2,3-d]pyrimidin-4(1H)-one (**2**).* Yield: 46.3%, mp: 188−190 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (d, J = 2.9 Hz, 1H), 8.29 (dd, J = 4.8, 1.9 Hz, 1H), 8.08 (dd, J = 7.6, 1.8 Hz, 1H), 7.46−7.25 (m, 5H), 7.03 (d, J = 3.8 Hz, 1H), 6.87 (dd, J = 7.6, 4.9 Hz, 1H), 6.80 (d, J = 3.8 Hz, 1H), 6.51 (d, J = 3.1 Hz, 1H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 161.56, 156.25, 152.67, 145.09, 139.68, 138.22, 129.44, 127.72, 127.04, 115.94, 113.35, 111.45, 70.26. ESI-MS (*m/z*), 386.0 $[M+H]^+$.

*4.2.2.3. 3-Phenyl-2-(5-phenylthiophen-2-yl)-2,3-dihydropyrido [2,3-d]pyrimidin-4(1H)-one (**3**).* Yield: 42.2%, mp: 234−236 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (d, J = 3.2 Hz, 1H), 8.24 (dd, J = 4.9, 1.9 Hz, 1H), 8.06 (dd, J = 7.6, 1.9 Hz, 1H), 7.52−7.47 (m, 2H), 7.40−7.29 (m, 6H), 7.28−7.21 (m, 3H), 6.92 (d, J = 3.7 Hz, 1H), 6.82 (dd, J = 7.6, 4.9 Hz, 1H), 6.49 (d, J = 3.2 Hz, 1H). ESI-MS (*m/z*), 384.1 $[M+H]^+$. HRMS: Calcd. for $C_{23}H_{17}N_3OS$: 383.1092; Found: 384.1165 $[M+H]^+$.

*4.2.2.4. 2-(5-(2-Methylthiazol-4-yl)thiophen-2-yl)-3-phenyl-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (**4**).* Yield: 44.1%, mp: 204−206 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (d, J = 3.2 Hz, 1H), 8.24 (dd, J = 4.8, 1.9 Hz, 1H), 8.06 (dd, J = 7.6, 1.8 Hz, 1H), 7.71 (s, 1H), 7.42−7.20 (m, 6H), 6.90−6.77 (m, 2H), 6.47 (d, J = 3.1 Hz, 1H), 2.59 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 166.52, 156.06, 148.77, 142.70, 139.84, 139.23, 138.07, 129.38, 127.65, 127.20, 123.16, 112.05, 70.51, 19.28. ESI-MS (*m/z*), 405.1 $[M+H]^+$. HRMS: Calcd. for $C_{21}H_{16}N_4OS_2$: 404.0766; Found: 405.0838 $[M+H]^+$.

*4.2.2.5. 3-Phenyl-2-(5-phenylthiophen-2-yl)-2,3-dihydropyrido [3,4-d]pyrimidin-4(1H)-one (**5**).* Yield: 42.0%, mp: 194−196 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (s, 1H), 8.02 (d, J = 4.9 Hz, 2H), 7.57 (d, J = 5.0 Hz, 1H), 7.50 (dd, J = 8.2, 1.0 Hz, 2H), 7.41−7.20 (m, 9H), 6.95 (d, J = 3.7 Hz, 1H), 6.65 (d, J = 2.9 Hz, 1H). ESI-MS (*m/z*), 405.1 $[M+H]^+$. HRMS: Calcd. for $C_{21}H_{16}N_4OS_2$: 404.0766; Found: 405.0838 $[M+H]^+$.

*4.2.2.6. 3-(2-Methoxyphenyl)-2-(5-methylthiophen-2-yl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (**6**).* Yield: 45.3%, mp: 178−180 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (d, J = 2.8 Hz, 1H), 8.20 (dd, J = 4.9, 1.9 Hz, 1H), 7.98 (dd, J = 7.6, 1.9 Hz, 1H), 7.30−7.22 (m, 1H), 7.09 (d, J = 7.4 Hz, 1H), 6.99 (d, J = 6.5 Hz, 1H), 6.86 (td, J = 7.6, 1.2 Hz, 1H), 6.77 (dd, J = 7.6, 4.9 Hz, 1H), 6.61 (d, J = 3.5 Hz, 1H), 6.49 (dd, J = 3.4, 1.1 Hz, 1H), 6.11 (d, J = 2.8 Hz, 1H), 3.76 (s, 3H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 166.21, 154.52, 143.64, 138.10, 134.65, 129.22 (d, J = 23.5 Hz), 126.86, 126.13, 124.95, 120.87 (d, J = 8.8 Hz), 115.12, 112.87, 112.33, 19.42. ESI-MS (*m/z*), 352.0 $[M+H]^+$. HRMS: Calcd. for $C_{19}H_{17}N_3O_2S$: 351.1041; Found: 352.1114 $[M+H]^+$.

*4.2.2.7. 3-(2-Methoxyphenyl)-2-(5-phenylthiophen-2-yl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (**7**).* Yield: 40.1%, mp: 160−162 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (d, J = 2.7 Hz, 1H), 8.23 (dd, J = 4.9, 1.9 Hz, 1H), 8.02 (dd, J = 7.7, 1.8 Hz, 1H), 7.54−7.44 (m, 2H), 7.36−7.18 (m, 5H), 7.09 (dd, J = 7.4, 3.3 Hz, 2H), 6.90−6.76 (m, 3H), 6.25 (d, J = 2.6 Hz, 1H), 3.77 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 157.22, 152.45, 145.57, 142.00, 137.98, 133.93, 129.51, 129.05, 128.06 (d, J = 3.0 Hz), 127.29, 125.89, 122.06, 120.86, 115.39, 111.87, 110.98, 69.18, 55.84. ESI-MS (*m/z*), 413.9 $[M+H]^+$. HRMS: Calcd. for $C_{24}H_{19}N_3O_2S$: 413.1198; Found: 414.1271 $[M+H]^+$.

*4.2.2.8. 3-(2-Methoxyphenyl)-2-(5-(2-methylthiazol-4-yl)thiophen-2-yl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (**8**).* Yield: 45.1%, mp: 188−190 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (d, J = 2.8 Hz, 1H), 8.23 (dd, J = 4.9, 1.9 Hz, 1H), 8.02 (dd, J = 7.6, 1.8 Hz, 1H), 7.71 (s, 1H), 7.32−7.20 (m, 2H), 7.10 (d, J = 8.3 Hz, 1H), 7.01 (d, J = 7.0 Hz, 1H), 6.91−6.75 (m, 3H), 6.21 (d, J = 2.7 Hz, 1H), 3.77 (s, 3H), 2.60 (s, 3H). ESI-MS (*m/z*), 435.0 $[M+H]^+$. ^{13}C NMR (101 MHz, chloroform-*d*) δ 166.42, 157.07, 152.44, 148.97, 142.24, 129.48, 127.75, 127.23, 122.89, 120.86,

115.34, 111.92, 110.83, 69.10, 55.83, 19.29. HRMS: Calcd. for $C_{22}H_{18}N_4O_2S_2$: 434.0871; Found: 435.0944 $[M+H]^+$.

*4.2.2.9. 3-(2-Chlorophenyl)-2-(5-methylthiophen-2-yl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (**9**).* Yield: 40.2%, mp: 196−198 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H), 8.24 (dd, J = 4.8, 1.5 Hz, 1H), 8.02 (d, J = 6.8 Hz, 1H), 7.60 (d, J = 7.4 Hz, 1H), 7.32 (dt, J = 27.6, 7.4 Hz, 2H), 6.99 (d, J = 7.4 Hz, 1H), 6.80 (dd, J = 7.5, 4.9 Hz, 1H), 6.69 (s, 1H), 6.54 (s, 1H), 6.12 (s, 1H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 156.73, 152.79, 141.80, 139.36, 138.06, 136.31, 132.55, 132.31, 130.28, 129.64, 127.45, 124.55, 115.75, 110.74, 69.18, 15.60. ESI-MS (*m/z*), 405.1 $[M+H]^+$. HRMS: Calcd. for $C_{21}H_{16}N_4OS_2$: 404.0766; Found: 405.0838 $[M+H]^+$.

*4.2.2.10. 2-(5-Bromothiophen-2-yl)-3-(2-chlorophenyl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (**10**).* Yield: 41.0%, mp: 174−176 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (d, J = 2.5 Hz, 1H), 8.28 (dd, J = 4.9, 1.9 Hz, 1H), 8.09−7.97 (m, 1H), 7.62 (d, J = 7.7 Hz, 1H), 7.34 (dt, J = 15.0, 7.0 Hz, 2H), 7.02 (dd, J = 19.5, 5.7 Hz, 2H), 6.87−6.77 (m, 2H), 6.25 (d, J = 2.7 Hz, 1H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 161.76, 156.95, 152.81, 143.74, 138.22, 136.06, 132.33, 130.40, 129.84, 129.34, 127.69, 115.84, 114.19, 110.56, 68.97. ESI-MS (*m/z*), 419.7 $[M+H]^+$. HRMS: Calcd. for $C_{17}H_{11}BrClN_3OS$: 418.9495; Found: 419.9567 $[M+H]^+$.

*4.2.2.11. 3-(2-Chlorophenyl)-2-(5-phenylthiophen-2-yl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (**11**).* Yield: 40.2%, mp: 206−208 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (d, J = 2.5 Hz, 1H), 8.27 (dd, J = 4.8, 1.8 Hz, 1H), 8.04 (dd, J = 11.9, 10.5 Hz, 1H), 7.62 (d, J = 7.6 Hz, 1H), 7.51 (t, J = 10.8 Hz, 2H), 7.41−7.22 (m, 6H), 7.09 (d, J = 6.5 Hz, 1H), 6.95 (d, J = 3.6 Hz, 1H), 6.83 (dt, J = 12.5, 6.3 Hz, 1H), 6.26 (d, J = 2.6 Hz, 1H). ESI-MS (*m/z*), 418.1 $[M+H]^+$. ^{13}C NMR (101 MHz, chloroform-*d*) δ 156.89, 152.91, 145.94, 141.09, 138.12, 136.25, 133.70, 132.47 (d, J = 20.0 Hz), 130.33, 129.73, 129.08, 128.28 (d, J = 10.9 Hz), 127.57, 125.93, 122.23, 115.81, 69.15. HRMS: Calcd. for $C_{23}H_{16}ClN_3OS$: 417.0703; Found: 418.0775 $[M+H]^+$.

*4.2.2.12. 3-(2-Chlorophenyl)-2-(5-(2-methylthiazol-4-yl)thiophen-2-yl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (**12**).* Yield: 41.1%, mp: 176−178 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (s, 1H), 8.27 (dd, J = 4.7, 1.7 Hz, 1H), 8.06 (d, J = 7.7 Hz, 1H), 7.75 (s, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.40−7.23 (m, 3H), 7.01 (d, J = 7.3 Hz, 1H), 6.90 (d, J = 3.7 Hz, 1H), 6.84 (dd, J = 7.6, 4.9 Hz, 1H), 6.23 (d, J = 2.7 Hz, 1H), 2.60 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 166.58, 156.78, 153.00, 148.79, 141.39, 138.09, 132.41, 130.31, 129.71, 128.03, 127.60, 122.98, 115.79, 112.17, 69.15, 19.29. ESI-MS (*m/z*), 439.2 $[M+H]^+$. HRMS: Calcd. for $C_{21}H_{15}ClN_4OS_2$: 438.0376; Found: 439.0449 $[M+H]^+$.

*4.2.2.13. 2-(5-Bromothiophen-2-yl)-3-(2-methoxyphenyl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (**13**).* Yield: 46.0%, mp: 194−196 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (d, J = 2.7 Hz, 1H), 8.24 (dd, J = 4.9, 1.9 Hz, 1H), 8.00 (dd, J = 7.6, 1.8 Hz, 1H), 7.31−7.24 (m, 1H), 7.07 (dd, J = 14.2, 7.8 Hz, 2H), 6.94 (d, J = 3.8 Hz, 1H), 6.89 (td, J = 7.7, 1.2 Hz, 1H), 6.81 (dd, J = 7.6, 4.9 Hz, 1H), 6.70 (d, J = 3.8 Hz, 1H), 6.22 (d, J = 2.6 Hz, 1H), 3.74 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 156.90, 152.53, 144.44, 138.04, 129.65, 129.08, 127.36, 120.94, 115.72, 113.77, 111.94, 111.01, 69.05, 55.83. ESI-MS (*m/z*), 416.0 $[M+H]^+$. HRMS: Calcd. for $C_{18}H_{14}BrN_3O_2S_2$: 414.9990; Found: 418.0043 $[M + H$, with $^{81}Br]^+$.

*4.2.2.14. 3-(2-Methoxyphenyl)-2-(5-methylthiophen-2-yl)-2,3-dihydropyrido[3,4-d]pyrimidin-4(1H)-one (**14**).* Yield: 48.1%, mp: 186−188 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (s, 1H), 7.99 (d, J = 5.0 Hz, 1H), 7.79 (s, 1H), 7.50 (d, J = 5.0 Hz, 1H), 7.25 (ddd, J = 8.3, 7.4, 1.7 Hz, 1H), 7.02 (dd, J = 30.1, 7.9 Hz, 2H), 6.84 (td, J = 7.6, 1.3 Hz, 1H), 6.67 (d, J = 3.5 Hz, 1H), 6.55−6.43 (m, 1H), 6.24 (d, J = 2.1 Hz, 1H), 3.74 (s, 3H), 2.28 (d, J = 0.9 Hz, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 143.74, 141.35, 140.71, 138.57, 130.70, 129.77, 129.16, 127.55, 127.01, 121.12 (d, J = 26.5 Hz), 113.65, 112.00, 69.85, 55.84. ESI-MS (*m/z*), 352.0 $[M+H]^+$. HRMS: Calcd. for $C_{19}H_{17}N_3O_2S$: 351.1041; Found: 352.1114 $[M+H]^+$.

*4.2.2.15. 2-(5-Bromothiophen-2-yl)-3-(2-methoxyphenyl)-2,3-dihydropyrido[3,4-d]pyrimidin-4(1H)-one (**15**).* Yield: 47.3%, mp: 182−184 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (s, 1H), 8.02 (d, J = 5.0 Hz, 1H), 7.90 (d, J = 1.9 Hz, 1H), 7.52 (d, J = 5.0 Hz, 1H), 7.32−7.20 (m, 1H), 7.14−6.98 (m, 2H), 6.94 (d, J = 3.8 Hz, 1H), 6.87 (td, J = 7.6, 1.1 Hz, 1H), 6.76 (d, J = 3.8 Hz, 1H), 6.36 (s, 1H), 3.73 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 141.26, 140.71, 139.57, 138.30, 130.83, 129.51, 127.24, 124.35, 122.26, 121.18, 120.76, 111.88, 70.00, 55.81, 15.52. ESI-MS (*m/z*), 416.0 $[M+H]^+$. HRMS: Calcd. for $C_{18}H_{14}BrN_3O_2S_2$: 414.9990; Found: 418.0042 (M + H, with $^{81}Br)^+$.

*4.2.2.16. 3-(2-Methoxyphenyl)-2-(5-phenylthiophen-2-yl)-2,3-dihydropyrido[3,4-d]pyrimidin-4(1H)-one (**16**).* Yield: 36.6%, mp: 208−210 °C. 1H NMR (400 MHz, DMSO-d_6) δ 8.24 (s, 1H), 8.02 (d, J = 5.0 Hz, 1H), 7.92 (d, J = 1.8 Hz, 1H), 7.60−7.45 (m, 3H), 7.39−7.15 (m, 5H), 7.06 (d, J = 7.9 Hz, 2H), 6.86 (ddd, J = 13.9, 8.6, 2.4 Hz, 2H), 6.38 (d, J = 1.5 Hz, 1H), 3.75 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 145.47, 141.16 (d, J = 15.2 Hz), 138.36, 133.76, 129.65, 129.05, 128.15 (d, J = 9.6 Hz), 125.86, 122.48, 122.09, 121.29, 120.91, 111.96, 70.03, 55.86. ESI-MS (*m/z*), 414.3 $[M+H]^+$. HRMS: Calcd. for $C_{24}H_{19}N_3O_2S$: 413.1198; Found: 414.1271 $[M+H]^+$.

*4.2.2.17. 3-(2-MMethoxyphenyl)-2-(5-(2-methylthiazol-4-yl)thiophen-2-yl)-2,3-dihydropyrido[3,4-d]pyrimidin-4(1H)-one (**17**).* Yield: 36.2%, mp: 222−224 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, J = 0.5 Hz, 1H), 8.02 (d, J = 5.0 Hz, 1H), 7.90 (d, J = 2.0 Hz, 1H), 7.71 (s, 1H), 7.54 (d, J = 5.0 Hz, 1H), 7.31−7.18 (m, 2H), 7.07 (d, J = 8.0 Hz, 1H), 7.01 (d, J = 7.0 Hz, 1H), 6.91−6.79 (m, 2H), 6.34 (d, J = 2.0 Hz, 1H), 3.75 (s, 3H), 2.60 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 166.53, 161.14, 148.83, 141.20, 140.83, 139.32, 138.35, 129.62, 127.93, 122.90, 122.55, 121.34, 120.94, 111.97, 69.94, 55.87, 19.27. ESI-MS (*m/z*), 435.0 $[M+H]^+$. HRMS: Calcd. for $C_{22}H_{18}N_4O_2S_2$: 434.0871; Found: 435.0944 $[M+H]^+$.

*4.2.3. General procedure for the preparation of compounds **18**−**27***

Required fluro substituted or unsubstituted 2-aminobenzoic acid (21.6 mmol) was dissolved in THF (50 mL) and DMF (0.75 mL);

triphosgene (28.1 mmol) was dissolved in 25 mL of THF and added dropwise to the system at 50 °C. Then, the mixture was stirred at 60 °C for 8 h. The solutions were evaporated and recrystallized in ethyl acetate (30 mL) to produce **33** as a yellow solid. **33** (18.9 mmol) was added dropwise to a suspension of 60% sodium hydride (*w*/*w*) in mineral oil (22.68 mmol) in DMF (35 mL) at 0 °C. After stirring at 0 °C for 30 min, the mixture was warmed to room temperature, and methyl iodide (22.68 mmol) was added. The mixture was allowed to stir overnight and then was quenched in an ice bath. The precipitates were collected by filtration to produce **34** as a white power (2.46 g, Yield 66.7%). Then **33** or **34** (12.6 mmol) and required amine (13.87 mmol) were dissolved in acetic acid (7 5 mL). The mixture was then stirred at 60 °C for 1 h. Then, the reaction mixture was purified with silica gel chromatography. The solvent was evaporated to produce required intermediate **35a** or **35b**. Then **35a** or **35b** (1.4 mmol) and required aldehyde intermediate **41** or **42** (2 mmol) were disloved in AcOH (5 mL) and then stirred at room temperature for 24 h, the solution was evaporated under reduced pressure and the residue was purified with silica gel chromatography to produced pure products**18**−**27**.

*4.2.3.1. 2-Phenyl-3-(5-bromothiazol-2-yl)-2,3-dihydroquinazolin-4(1H)-one (**18**).* Yield: 57.7%, mp: 206−208 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (d, J = 3.9 Hz, 1H), 7.72 (dt, J = 11.4, 5.7 Hz, 1H), 7.64 (d, J = 8.1 Hz, 1H), 7.39−7.12 (m, 7H), 6.84 (d, J = 8.2 Hz, 1H), 6.74 (dd, J = 11.1, 4.0 Hz, 1H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 138.14, 135.30, 129.22, 128.86, 125.97, 120.39, 116.10, 68.55. HRMS: Calcd. for $C_{17}H_{12}BrN_3OS$: 384.9884; Found: 385.9957 $[M+H]^+$.

*4.2.3.2. 3-(5-Bromothiazol-2-yl)-2-(5-(2-methylthiazol-4-yl)thiophen-2-yl)-2,3-dihydroquinazolin-4(1H)-one (**19**).* Yield: 44.2%, mp: 228−230 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (d, J = 3.8 Hz, 1H), 7.77 (dd, J = 7.9, 1.5 Hz, 1H), 7.73 (s, 1H), 7.66 (s, 1H), 7.49−7.40 (m, 2H), 7.24 (d, J = 3.7 Hz, 1H), 6.95 (d, J = 7.8 Hz, 1H), 6.89 (dd, J = 3.8, 0.9 Hz, 1H), 6.87−6.80 (m, 1H), 2.57 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 158.52, 154.60, 151.20, 143.20, 142.00, 137.33, 130.57, 129.38, 127.85 (d, J = 15.8 Hz), 123.93, 122.05, 121.13 (d, J = 23.3 Hz), 116.29, 115.56, 115.01 (d, J = 6.9 Hz), 112.38, 56.02, 24.36. ESI-MS (*m*/*z*), 491.0 $[M+H]^+$. HRMS: Calcd. for $C_{19}H_{13}BrN_4OS_3$: 487.9435; Found: 488.9508 $[M+H]^+$.

*4.2.3.3. 3-(5-Methylthiazol-2-yl)-2-(5-(2-methylthiazol-4-yl)thiophen-2-yl)-2,3-dihydroquinazolin-4(1H)-one (**20**).* Yield: 65.2%, mp: 246−248 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (d, J = 3.8 Hz, 1H), 7.75 (dd, J = 7.9, 1.4 Hz, 1H), 7.65 (s, 1H), 7.48 (d, J = 3.3 Hz, 1H), 7.44−7.37 (m, 1H), 7.27 (t, J = 4.0 Hz, 1H), 7.23 (t, J = 4.3 Hz, 1H), 6.92 (t, J = 8.0 Hz, 1H), 6.86 (dt, J = 5.8, 2.9 Hz, 1H), 6.84−6.78 (m, 1H), 2.63−2.50 (m, 3H), 2.36 (d, J = 1.1 Hz, 3H). ESI-MS (*m*/*z*), 425.2 $[M+H]^+$. HRMS: Calcd. for $C_{20}H_{16}N_4OS_3$: 424.0486; Found: 425.0559 $[M+H]^+$.

*4.2.3.4. 6-Fluoro-3-(5-methylthiazol-2-yl)-2-(5-methylthiophen-2-yl)-2,3-dihydroquinazolin-4(1H)-one (**21**).* Yield: 50.2%, mp: 182−184 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (d, J = 4.0 Hz, 1H), 7.71 (s, 1H), 7.56−7.25 (m, 3H), 6.97 (dd, J = 9.0, 4.4 Hz, 1H), 6.68 (d, J = 3.5 Hz, 1H), 6.51 (dd, J = 3.5, 1.1 Hz, 1H), 2.23 (s, 3H). ESI-MS (*m*/*z*), 423.5 $[M+H]^+$. HRMS: Calcd. for $C_{16}H_{11}BrFN_3OS_2$: 422.9511; Found: 423.9584 $[M+H]^+$.

*4.2.3.5. 7-Fluoro-1-methyl-2-(5-(2-methylthiazol-4-yl)thiophen-2-yl)-3-phenyl-2,3-dihydroquinazolin-4(1H)-one (**22**).* Yield: 80.6%, mp: 164−166 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (dd, J = 8.6, 6.7 Hz, 1H), 7.72 (s, 1H), 7.40−7.34 (m, 2H), 7.31−7.24 (m, 4H), 6.93 (d, J = 3.7 Hz, 1H), 6.69 (td, J = 8.6, 2.4 Hz, 1H), 6.60 (dd, J = 11.5, 2.3 Hz, 1H), 6.49 (s, 1H), 2.92 (s, 3H), 2.59 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 183.13, 166.70, 161.75, 142.95, 140.28, 131.92, 129.27, 126.92, 123.25, 120.77, 111.98, 107.77, 101.85, 71.12, 19.20. ESI-MS (*m*/*z*), 435.6 $[M+H]^+$. HRMS: Calcd. for $C_{23}H_{18}FN_3OS_2$: 435.0875; Found: 436.0947 $[M+H]^+$.

*4.2.3.6. 7-Fluoro-2-(5-(2-methylthiazol-4-yl)thiophen-2-yl)-3-phenyl-2,3-dihydroquinazolin-4(1H)-one (**23**).* Yield: 85.4%, mp: 155−156 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (dd, J = 7.6, 5.7 Hz, 1H), 7.73 (s, 1H), 7.69−7.51 (m, 2H), 7.47 (s, 1H), 7.45−7.35 (m, 2H), 7.28 (d, J = 7.5 Hz, 1H), 7.18 (m, 1H), 6.86 (td, J = 9.1, 3.2 Hz, 1H), 6.79 (s, 1H), 6.68 (dd, J = 8.9, 2.0 Hz, 1H), 6.29 (s, 1H), 2.80 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 166.60, 160.77, 148.82, 143.21, 139.61, 138.25, 130.58, 129.39, 127.89, 127.63, 122.95, 121.24, 120.99, 115.60, 115.36, 114.95, 114.88, 112.36, 111.82, 19.23. HRMS: Calcd. for $C_{22}H_{16}FN_3OS_2$: 421.0719; Found: 422.0792 $[M+H]^+$.

*4.2.3.7. 7-Fluoro-3-(2-methoxyphenyl)-2-(5-(2-methylthiazol-4-yl)thiophen-2-yl)-2,3-dihydro quinazolin-4(1H)-one (**24**).* Yield: 65.6%, mp: 232−234 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 (d, J = 1.9 Hz, 1H), 7.79−7.66 (m, 2H), 7.28−7.17 (m, 2H), 7.05 (d, J = 8.2 Hz, 1H), 6.97 (d, J = 7.3 Hz, 1H), 6.87−6.77 (m, 2H), 6.62−6.49 (m, 2H), 6.26 (d, J = 1.7 Hz, 1H), 3.75 (s, 3H), 2.60 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 166.61, 148.70, 144.78, 143.09, 138.84, 138.57, 132.13, 131.40, 129.50, 127.79, 127.50, 122.90, 121.76, 121.53, 115.67, 115.43, 114.79, 114.72, 112.09, 36.54, 19.26. ESI-MS (*m*/*z*), 451.6 $[M+H]^+$. HRMS: Calcd. for $C_{23}H_{18}FN_3O_2S_2$:451.0824; Found: 452.0897 $[M+H]^+$.

*4.2.3.8. 6-Fluoro-2-(5-(2-methylthiazol-4-yl)thiophen-2-yl)-3-phenyl-2,3-dihydroquinazolin-4(1H)-one (**25**).* Yield: 52.3%, mp: 176−178 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (d, J = 4.8 Hz, 2H), 7.48−7.16 (m, 8H), 6.93−6.81 (m, 2H), 6.52 (d, J = 2.7 Hz, 1H), 2.60 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 135.52, 129.28, 129.03, 127.64, 127.24, 126.56, 125.03, 124.25, 123.16, 121.47 (d, J = 23.8 Hz), 120.81, 117.80, 115.05, 114.81, 114.25, 111.89, 71.23, 19.23. ESI-MS (*m*/*z*), 422.0 $[M+H]^+$. HRMS: Calcd. for $C_{22}H_{16}FN_3OS_2$: 421.0719; Found: 422.0792 $[M+H]^+$.

*4.2.3.9. 6-Fluoro-3-(2-methoxyphenyl)-1-methyl-2-(5-phenylthiophen-2-yl)-2,3-dihydroquinazolin-4(1H)-one (**26**).* Yield: 52.1%, mp: 158−160 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56−7.45 (m, 3H), 7.39−7.18 (m, 6H), 7.13 (d, J = 8.1 Hz, 1H), 7.04 (d, J = 7.6 Hz, 1H), 6.90 (dd, J = 17.2, 5.6 Hz, 2H), 6.79 (dd, J = 9.0, 4.2 Hz, 1H), 6.11 (d, J = 13.7 Hz, 1H), 3.75 (s, 3H), 2.89 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 139.23, 133.84, 130.53, 129.42, 128.99, 127.88 (d, J = 6.8 Hz), 125.86, 121.80, 121.23, 121.01, 119.05, 114.92, 112.40, 56.03,

36.93. ESI-MS (*m/z*), 444.6 $[M+H]^+$. HRMS: Calcd. for $C_{26}H_{21}FN_2O_2S$: 444.1308; Found: 445.1380 $[M+H]^+$.

*4.2.3.10. 6-Fluoro-3-(2-methoxyphenyl)-1-methyl-2-(5-(2-methylthiazol-4-yl)thiophen-2-yl)-2,3-dihydroquinazolin-4(1H)-one (**27**).* Yield: 66.2%, mp: 168−170 °C ^{1}H NMR (400 MHz, DMSO-d_6), δ 7.74 (1H, s), 7.57 (1H, dd, J = 8.8 Hz, 3.1 Hz), 7.42−7.28 (3H, m), 7.17 (1H, d, J = 8.1 Hz), 7.01 (1H, d, J = 5.9 Hz), 6.92 (2H, t, J = 6.6 Hz), 6.82 (1H, dd, J = 9.0, 4.2 Hz), 6.15 (1H, s), 3.79 (3H, s), 2.90 (3H, s), 2.63 (3H, s). ^{13}C NMR (DMSO-d_6, 101 MHz) δ 166.55, 160.06, 157.10, 154.76, 148.34, 143.94, 139.40, 138.21, 130.34, 129.78, 129.10, 128.11, 123.18, 121.97, 121.74, 120.80, 118.31, 115.79, 114.41, 113.34, 75.99, 56.28, 36.01, 19.12. ESI-MS (*m/z*), 466.1 $[M+H]^+$. HRMS: Calcd. for $C_{24}H_{20}FN_3O_2S_2$: 465.0981; Found: 466.1054 $[M+H]^+$. HPLC (99.6188%).

4.3. Pharmacology

Materials: the ricin used in this study was supplied by the Laboratory of Toxicant Analysis, Beijing Institute of Pharmacology and Toxicology, China. The purity of ricin was more than 95% analyzed by SDS-PAGE (Shanghai, China), the molecular weight of ricin was determined by MALDI-TOF/MS (Autoflex III Smartbeam, Bruker Daltonics Inc. Leipzig, Germany). MDCK cells were obtained from the American Type Culture Collection (ATCC, The Institute of Laboratory Animal Science, Beijing, China). Cellular Counting Kits (CCK8) were purchased from Dojindo (Shanghai, China). Mouse ascites containing mAb4C13 were produced in the Beijing Institute of Basic Medical Science, China. The antibodies were purified using protein G sepharose 4 Fast Flow (Amersham, USA). Female CD1 mice were supplied by Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The mice were housed in a controlled environment (21 ± 2 °C; 55 ± 5% humidity; 12 h dark and light cycle with light provided between 6 am and 6 pm). Food and water were given ad libitum. All procedures involving the use of animals were conducted in facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

Cell culture: cells were cultured in a MEM/EBSS medium supplemented with fetal bovine serum (10% FBS), 1% nonessential amino acids, 100 units/mL penicillin and 100 μg/mL streptomycin and incubated at 37 °C in the presence of 5% CO_2. Cells were subcultured when they reached approximately 80% confluence.

4.3.1. Anti-ricin activity screen of novel compounds ***1*−*27***

MDCK cells were maintained at 37 °C under 5% CO_2 in DMEM supplemented with 10% fetal bovine serum and 100 U/mL penicillin and 100 μg/mL streptomycin. The cells were seeded at 20,000 cells per well in 96-well plates and incubated for 24 h at 37 °C under 5% CO_2. Then, the cells were washed twice with PBS to remove the serum. Then cells were respectively treated with 50 μmol/L DMSO solution of compounds **1**−**27**; blank controls were also applied to cells. Cells were then cultured for 6 h and challenged with 5.56 ng/mL ricin. Next, cells were incubated for another 12 h, and the medium was removed by rinsing cells using PBS. Finally, 0.1 mL of 10% CCK8 working solution was added into each well for 2 h of further incubation. The absorbance value (A_{450}) of each well was measured in a microplate reader (Varioskan FlashVersion2.4.3, Thermo Scientific, Waltham, MA, USA) at 450 nm wavelength. The survival rates of the cells were estimated by Eq. (1):

$$\text{Cell survival rate } (\%) = (A_{450} \text{ in the experiment group}/A_{450} \text{ in blank control group}) \times 100 \quad (1)$$

The experiments in each group were repeated six times.

4.3.2. MDCK cell pre-exposure treatment assay

The cell seeding, data retrieval and survival rate calculation methods were the same as those in Section 4.3.1. After the removal of serum, cells were treated with gradient concentrations of **27** or compound **94** (0.01, 0.03, 0.10, 0.30 or 0.90 μmol/L for **27**; 0.01, 0.15, 0.31, 0.62, 1.25, 2.50, 5.00, 10.00, 20.00, 30.00 or 40.00 μmol/L for compound **94**); blank controls were also applied to cells. Cells were then cultured for 12 h and challenged with 5.56 ng/mL ricin. Next, cells were incubated for another 12 h and data were collected the same as that in Section 4.3.1. The experiments in each group were repeated six times.

4.3.3. MDCK cells maximum protection assay

The cell seeding, data retrieval and survival rate calculation methods were the same as those in Section 4.3.1. After the removal of serum, cells were treated with gradient concentrations of **27** (0.011, 0.033, 0.10, 0.30 or 0.90 μmol/L or blank solution as control), cultured for 12 h and then challenged with gradient concentrations of ricin (0.06, 0.20, 0.60, 1.80, 5.50, 16.70, 50.00 or 100.00 ng/mL); next, cells were incubated for another 12 h, and data were collected the same as that in Section 4.3.1. The experiments in each group were repeated six times.

4.3.4. MDCK cells post-exposure treatment assay

The cell seeding, data retrieval and survival rate calculation methods were the same as those in Section 4.3.1. After removal of serum, cells were challenged with 5.56 ng/mL ricin, incubated for 1 h and then treated with gradient concentrations of **27** or compound **94** (0.16, 0.32, 0.63, 1.25, 2.50, 5.00 or 10.00 μmol/L and blank solution as ricin control), blank controls were also applied to cells. Next, cells were incubated for another 12 h, and data were collected the same as that in Section 4.3.1. The experiments in each group were repeated six times.

4.3.5. MDCK cells post-exposure time-effect relationship assay

The cell seeding, data retrieval and survival rate calculation methods were the same as those in Section 4.3.1. After the removal of serum, cells were challenged with 5.56 ng/mL ricin, incubated separately for 1, 2, 4 or 6 h, and then treated with gradient concentrations of **27** (0.16, 0.31, 0.63, 1.25, 2.50, 5.00 or 10.00 μmol/L and blank solution as ricin control); blank controls were also applied to cells. Each group of cells was incubated for another 12 h, and data were collected the same as that in Section 4.3.1. The experiments in each group were repeated six times.

4.3.6. Ricin-intoxicated mice pre-exposure treatment assay

Animal experiments were approved by the Ethics Committee of Beijing Institute of Pharmacology and Toxicology. Female CD1

mice weighing from 20 to 22 g were randomly separated into four groups that were labeled the blank control group, ricin control group, **27** group and compound **94** group, with 10 mice each group. The **27** and compound **94** groups were injected i.p. with a 2 mg/mL solution of the tested compound, the final administration dose of compound was 50 mg/kg. The blank control and ricin control groups were given 25 mL/kg of blank solution. Then, after 1 h, the **27** group, compound **94** group and ricin control group were injected i.p. with a 1.3 μg/mL ricin normal saline (NS) solution; the final intoxication dose was 13 μg/kg. The blank control group was treated with 10 mL/kg NS. Survival was recorded daily until 6 days after intoxication.

4.3.7. Ricin-intoxicated mice post-exposure treatment assay

The separation of groups was the same as that in Section 4.3.6. The groups were labeled the ricin control group, 1, 2 and 6 h treatment groups. The compound solution, ricin solution and administration assay were the same as that in Section 4.3.6. All four groups of mice were challenged with ricin and given **27** separately at 1, 2 or 6 h after ricin intoxication, and the final dose of **27** was 50 mg/kg; the ricin control group was given 25 mL/kg blank solution 1 h after intoxication. Survival was recorded daily until 12 days after intoxication.

*4.3.8. Ricin-intoxicated mice **27** and mAb4C13 combined theraputic assay*

The separation of groups was the same as that in Section 4.3.6. The groups were labeled ricin control group, antibody group and combined group. The compound solution, ricin solution and administration assay were the same as that in Section 4.3.6. All groups of mice were challenged with 13 μg/kg of ricin and then combined group was given 50 mg/kg of **27** 0.5 h after ricin intoxication while antibody group received corresponding amount of vehicle, then at 2 h both group were treated with 300 μg/kg of mAb4C13. The ricin control group was given vehicle at 0.5 and 2 h. Survival was recorded daily until 20 days after intoxication. When evaluating the anti-ricin ability of the combination, mice were divided into 2.5, 4 and 5 LD group and treated with 32.5, 52 or 65 μg/kg of ricin respectively, then all groups received 50 mg/kg of **27** at 0.5 h and 300 μg/kg of mAb4C13 at 2 h. Survival was recorded daily until 28 days after intoxication.

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Author contributions

Xu Zhao was responsible for compounds synthesis, data investigation and original draft preparation. Haixia Li was responsible for pharmacology experiments and data analysis. Jia Li was responsible for the synthesis of some compounds synthesis. Kunlu Liu and Bo Wang were responsible for pharmacology experiments and data curation. Wu Zhong, Xingzhou Li and Yuxia Wang were responsible for conceptualization, methodology, draft editing and funding acquisition.

Conflicts of interest

The authors have no conflicts of interest to declare.

Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2019.08.005.

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