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Design, synthesis, and bioassay of 4-thiazolinone derivatives as influenza neuraminidase inhibitors

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ABSTRACT

A series of 4-thiazolinone derivatives (**D1-D58**) were designed and synthesized. All of the derivatives were evaluated *in vitro* for neuraminidase (NA) inhibitory activities against influenza virus A (H1N1), and the inhibitory activities of the five most potent compounds were further evaluated on NA from two different influenza viral subtypes (H3N2 and B), and then their *in vitro* anti-viral activities were evaluated using the cytopathic effect (CPE) reduction assay. The results showed that the majority of the target compounds exhibited moderate to good NA inhibitory activity. Compound **D18** presented the most potent inhibitory activity with IC_{50} values of 13.06 μM against influenza H1N1 subtype. Among the selected compounds, **D18** and **D41** turned out to be the most potent inhibitors against influenza virus H3N2 subtype (IC_{50} = 15.00 μM and IC_{50} = 14.97 μM, respectively). **D25** was the most potent compound against influenza B subtype (IC_{50} = 16.09 μM). In addition, **D41** showed low toxicity and greater potency than reference compounds Oseltamivir and Amantadine against N1−H275Y variant in cellular assays. The structure-activity relationship (SAR) analysis showed that introducing 4-CO_2H, 4-OH, 3-OCH_3-4-OH substituted benzyl methylene can greatly improve the activity of 4-thiazolinones. Further SAR analysis indicated that 4-thiazolinone and ferulic acid fragments are necessary fragments of target compounds for inhibiting NA. Molecular docking was performed to study the interaction between compound **D41** and the active site of NA. This study may providing important information for new drug development for anti-influenza virus including mutant influenza virus.

1. Introduction

Influenza viruses remain a major threat to human health due to its high morbidity and mortality rates. According to statistics from World Health Organization, seasonal influenza virus epidemics are estimated to cause 2−5 million cases of severe illness and up to 250,000−500,000 deaths per year worldwide [1]. These seasonal flu epidemics cause acute contagious respiratory infections, especially in children, the elderly and people with chronic diseases. Influenza virus neuraminidase (NA) is a sialidase that helps releasing of the newly formed virions from the infected cells and circulating to infect surrounding cells. Inhibition of NA can protect the host from viral infection and retard its propagation [2]. Because of the highly conserved structure of the active site of NA, neuraminidase has been an attractive target for development of novel anti-influenza drugs. Currently, four neuraminidase inhibitors (NAIs) were approved and are currently in clinical use for influenza (Fig. 1A): Relenza™ (zanamivir), Tamiflu™ (the phosphate salt of oseltamivir) are approved worldwide, wheras Inavir™ (laninamivir octanoate) and Rapivab™ (peramivir) are regionally approved for

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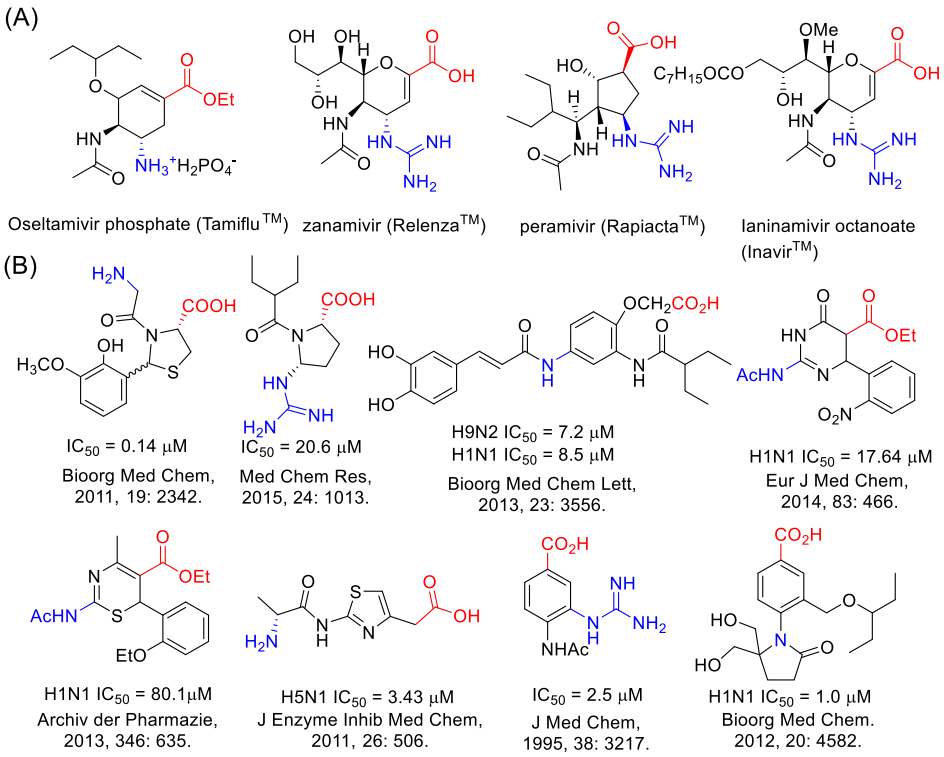


Fig. 1. (A) Approved NAIs; (B) Novel NAIs containing amino/guanidino and carboxyl/carboxylate groups.

human use [3]. However, the drug resistance caused by the high mutability of influenza virus has become increasingly severe [4,5]. In addition, these classic NA inhibitors contain multiple chiral centers in their structure, which is difficult to synthesize; and most of them are polar and have low oral bioavailability. Therefore, it is of great significance to explore new types of NA inhibitors with simple structure and good pharmacokinetic properties.

The catalytic cavity of neuraminidase contains a basic region composed of three guanidine groups and an acidic region composed of three carboxyl groups [6]. Acidic groups and basic groups are the two important active pharmacophores of NA inhibitors. In recent years, some novel influenza NA inhibitors containing amino/guanidino and carboxyl/carboxylate groups have been discovered (Fig. 1B) [7–14].

In our preliminary work, inspired by the studies on guanidinobenzoic acid type NA inhibitors [14–17], some amino/guanidinothiazole carboxylic acid derivatives (**S6**–**S9**, more details in supporting information) were synthesized with lower NA inhibition rate, but after being modified into 4-thiazolinone derivatives, they showed a significant improvement in activity (Fig. 2). These active compounds have a different scaffold from conventional NAIs in chemical space and shape, which indicated that they represent a novel class of NA inhibitors. 4-thiazolinone, also known as thiazolidin-4-one, have a wide range of biological activities [18–21], such as anti-inflammatory [22–24], anti-cancer [25–28], anti-fungal [29,30], anti-bacterial [31], antiviral [32,33] and so on. Rosiglitazone and pioglitazone, both marketed drugs containing 4-thiazolinone fragments, are used to treat type 2 diabetes [34,35]. In this study, 18 4-thiazolinone derivatives (**C1**–**C18**), 58 5-benzyl-4-thiazolinone derivatives (**D1**-**D58**), two 4-thiazolinone analogues (**S1**–**S2**) and three 4-thiazolinone ring-opening analogues (**S3**–**S5**) were designed, synthesized, and evaluated for their H1N1 NA inhibitory activity. In addition, the five most potent compounds were further evaluated with a neuraminidase (NA) activity assay using two different influenza viral strains (H3N2 and B), and a cytopathic effect (CPE) reduction assay. Molecular docking was further performed to study the interactions between the most potent compound and the active site of NA.

2. Results and discussion

2.1. Chemistry

The synthetic route for 4-thiazolinone derivatives (**D1-D58**) is outlined in Scheme 1. Using thiazole-2-amine with different substituents as raw materials, the intermediates **C1**–**C18** were obtained in two steps, including chloroacetylation and cyclic rearrangement reactions in sequence, and then the intermediates are condensed with different benzaldehydes to obtain target compounds **D1**-**D5**, **D7**-**D31**, **D33**-**D39**, and **D41**-**D58**. The nitro-substituted compound **D5**, **D31** and **D39** was reducted into its corresponding amino-substituted compounds **D6**, **D32** and **D40**, respectively.

To enrich the structure-activity relationship studies, two 4-thiazolinone analogues (**S1**–**S2**) and three 4-thiazolinone ring-opening analogues (**S3**–**S5**) were synthesized as illustrated in Scheme 2. The compound **S1** and **S2** was synthesized with the method similar to that of compounds **D**. Compounds **S3**–**S4** were synthesized by refluxing ethyl 2-amino-4-methylthiazole-5-carboxylate **A1** and cinnamoyl isothiocyanates **G3**-**G4**. Compound **S4** was hydrolyzed to target compound **S5**. The intermidieate cinnamoyl isothiocyanates **F3**–**F4** were obtained by reacting substituted cinnamic acid and thionyl chloride, which was further treated with ammonium thiocyanate to get **G3**-**G4**. The synthesized compounds were characterized by elemental analysis, ^{1}H NMR, ^{13}C NMR and mass spectrum.

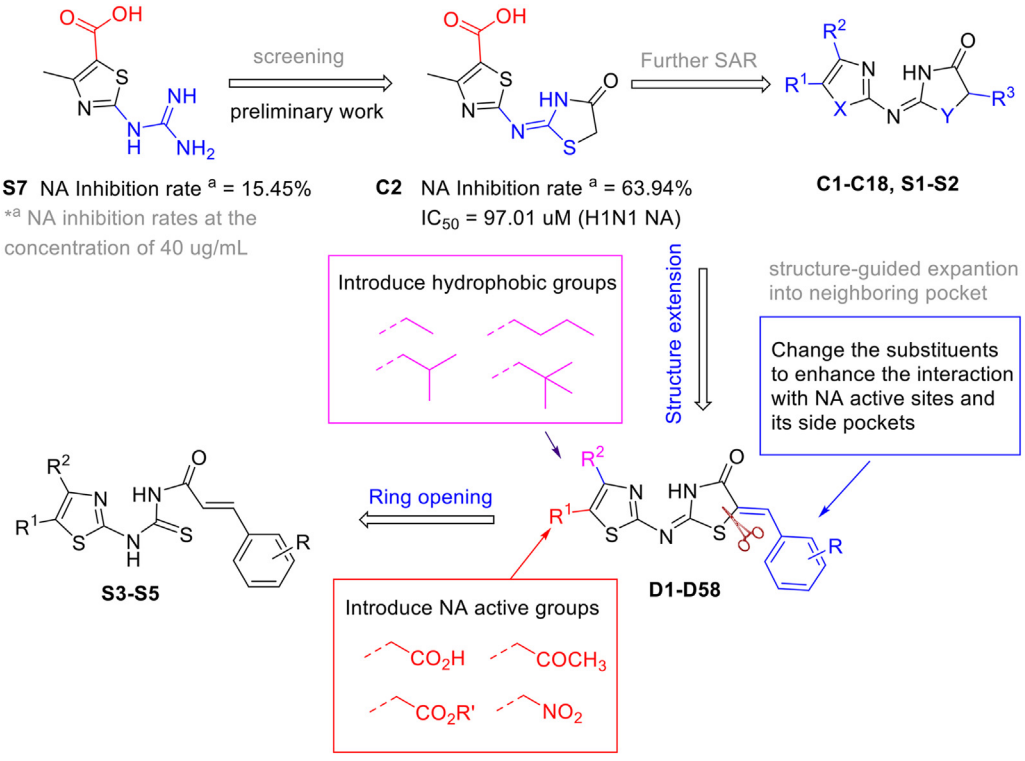
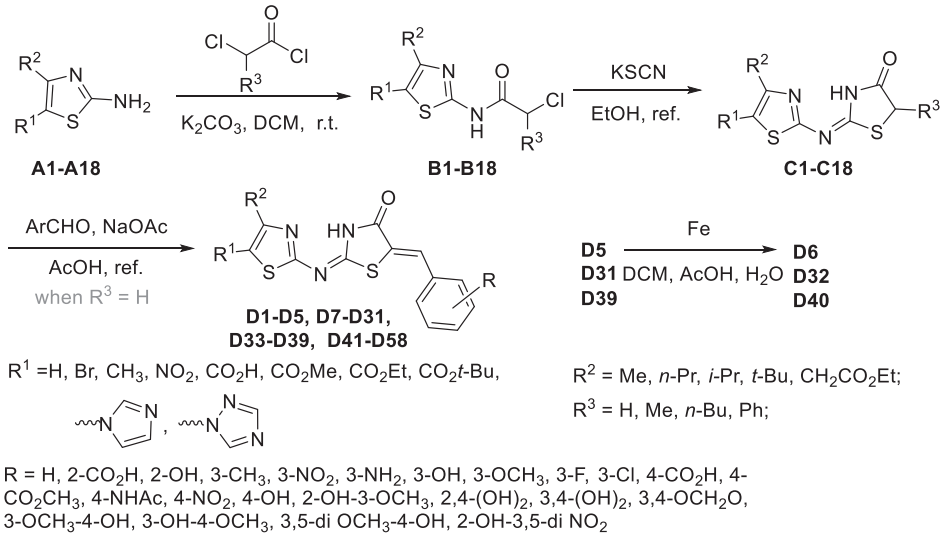


Fig. 2. Design of target compounds.



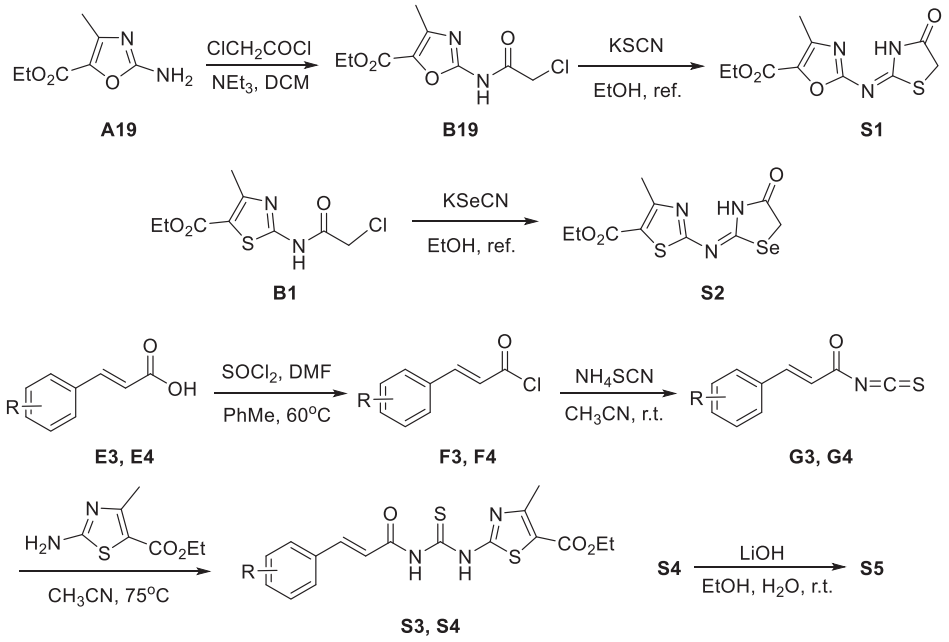
Scheme 1. Synthetic route of 5-benzylidene-4-thiazolinones.

2.2. Biological activity

All the target compounds (**C1**–**C18**, **D1**-**D58**, and **S1**–**S5**) were tested for NA inhibitory activity using human influenza virus A/PR/8/34 of subtype H1N1. Moreover, the most potent five compounds **D15**, **D18**, **D25**, **D41**, and **D44** were selected for further NA inhibition studies on multiple strains of influenza viruses. Finally, their toxicity and anti-influenza viral effect on drug-resistant influenza virus-infected Madin-Darby canine kidney (MDCK) cells were evaluated.

2.2.1. Structure-activity relationship of mother nucleus

Based on the literature study and the principle of bioisosterism, NA inhibitory fragments were introduced at the thiazole ring to systematically explore the structure-activity relationship of NA inhibitory activity of the mother nucleus. The results (Table 1) show that the intermediates **C1** to **C18** exhibited moderate NA inhibitory activity. Among them, **C4** displayed the best NA inhibitory activity with IC_{50} value of 33.60 μM, followed by **C1**, **C6**, **C10**, **C11** and **C16** with moderate activity (IC_{50} less than 60 μM). Comparing **S1** with **C1**, the activity was significantly reduced after the thiazole ring was replaced with an oxazole ring. Comparing **S2** with **C1**, the activity



Scheme 2. Synthesis route of 4-thiazolinone analogues.

Table 1
H1N1 NA inhibitory activities of **C1** − **C18** and **S1** − **S2**.

No.	X	Y	R^1	R^2	R^3	Inhibition Rates[a]/%	IC_{50}/μM
S1	O	S	CO_2Et	Me	H	34.77 ± 4.45	NT
S2	S	Se	CO_2Et	Me	H	17.92 ± 1.31	NT
C1	S	S	CO_2Et	Me	H	78.40 ± 1.01	58.84 ± 5.75
C2	S	S	CO_2H	Me	H	63.94 ± 3.53	97.01 ± 13.76
C3	S	S	CO_2Et	CF_3	H	37.05 ± 15.78	NT
C4	S	S	CO_2Me	*i*-Pr	H	68.02 ± 7.42	33.60 ± 8.89
C5	S	S	CO_2Me	*n*-Pr	H	36.99 ± 10.21	NT
C6	S	S	CO_2Me	Me	H	73.88 ± 5.97	53.41 ± 15.66
C7	S	S	CO_2t-Bu	Me	H	74.44 ± 1.43	66.05 ± 27.73
C8	S	S	CO_2Et	Me	Me	19.56 ± 5.76	NT
C9	S	S	CO_2Et	Me	*n*-Bu	52.12 ± 8.15	65.05 ± 15.43
C10	S	S	CO_2Et	Me	Ph	52.77 ± 6.71	55.36 ± 25.79
C11	S	S	$COCH_3$	Me	H	79.02 ± 3.04	48.68 ± 4.15
C12	S	S	H	CH_2CO_2Et	H	26.41 ± 7.62	NT
C13	S	S	Me	CH_2CO_2Et	H	43.82 ± 5.98	NT
C14	S	S	H	*t*-Bu	H	37.95 ± 9.22	NT
C15	S	S	Br	*t*-Bu	H	53.39 ± 2.74	104.38 ± 53.4
C16	S	S	NO_2	*t*-Bu	H	77.79 ± 1.38	41.62 ± 4.46
C17	S	S	imidazole-1-yl	*t*-Bu	H	36.81 ± 4.99	NT
C18	S	S	1,2,4-triazol-1-yl	*t*-Bu	H	18.75 ± 9.03	NT

* [a] NA inhibition rates at the concentration of 40 μg/mL.
[b] NT: Not tested.

was also significantly reduced after the thiazolidin-4-one ring was replaced with 1,3-selenazolidin-4-one ring. Comparing **C4** with **C5** and **C6**, The 4-position substituent of thiazole has the largest influence on the activity of the mother nucleus. The relative contribution of the substituents at 4-position to the activity in the mother nucleus was isopropyl (33.60 μM) > methyl (53.41 μM) > *n*-propyl. Comparing **C1** with **C3**, in terms of the 4-position substituents of thiazole, methyl (58.84 μM) is more active than trifluoromethyl. Comparing **C3** with **C4**, **C8**, **C9**, and **C13**, the activity contribution order of the 5-position group on thiazole is acetyl (48.68 μM) > methyl formate (53.41 μM) > ethyl formate (58.84 μM) and *tert*-butyl formate (66.05 μM) > carboxyl (97.01 μM). By comparing compounds **C14** − **C18**, it can be seen that when the 4-position of the thiazole ring is *tert*-butyl, the activity order of the 5-position substituted group is: nitro (41.62 μM) > bromine (104.38 μM) > hydrogen, imidazole-1-yl and 1,2,4-triazol-1-yl. Comparing **C1** with **C8**, **C9**, and **C10**, 5-position substitution has no obvious effect on activity. Comparing **C1** with **C6** and **C7**, the alkyl substitution of formate group also have little effect on the activity. In conclusion, the structure-activity relationship is summarized in Fig. 3.

2.2.2. Structure-activity relationship of side-chains

In order to further improve the activity of these compounds and explore the structure-activity relationship, through the aldol condensation reaction, different substituted benzylidene groups

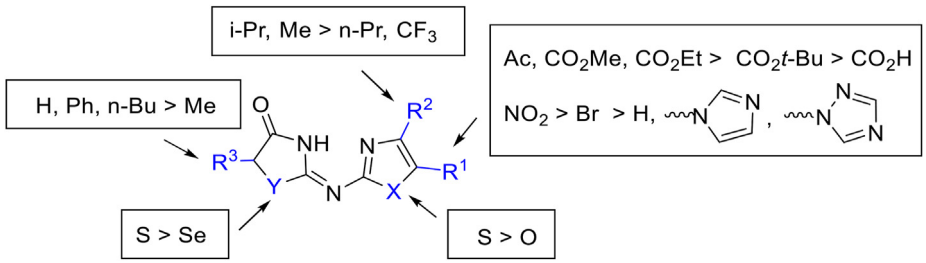


Fig. 3. Structure-activity relationship of the mother nucleus.

were introduced at the 5-position of 4-thiazolinone. This side-chain expansion may enhance the interaction with NA 430 cavity, a big hydrophobic cavity adjacent to NA catalytic cavity in both group-1 and group-2 NAs [36,37]. Herein, **C1**, **C4**, **C11**—**C13**, and **C16** were selected as the main intermediates for lower cost, better activity or easy synthesis. The activity results in Table 2 show that:

(1) The product after introduction of benzylmethylene is generally more active than its corresponding intermediate. The value of $\triangle IC_{50}$ (IC_{50} product-IC_{50} intermediates) was used to quantify the change in activity before and after modification. As shown in Table 2, 40/58 of ΔIC_{50} was below zero. For example, the activity of intermediate **C12** is low (IC_{50} > 100 μM), and all the products **D31**-**D37** are less than 50 μM;
(2) The most potent compounds was **D15** and **D18** with IC_{50} values of 16.33 and 13.06 μM, respectively. And compounds **D16**, **D25**, **D41** and **D44** also displayed good activity with IC_{50} values less than 20 μM;
(3) As shown in Table 3, by comparing the values of $\triangle IC_{50}$ before and after the modification of each series of compounds, we can find that the NA inhibitory activity of the substituted 4-thiazolinones, which introduced 4-CO_2H, 4-OH or 3-OCH_3-4-OH substituted benzyl methylene, was greatly improved.

2.2.3. Structure-activity relationship of the 4-thiazolinone ring

As shown in Table 4, the NA inhibitory activity values of the target compounds **D1** (64.21 μM) and **D18** (13.06 μM) are better than those of the corresponding 4-thiazolinone ring-opening analogues **S3** and **S5** (44.00 μM), therefore 4-thiazolinone is an essential NA inhibitory active fragment of such compounds. Moreover, by comparing the activities of **S5**, **S6** and ferulic acid, it found that the ferulic acid fragment is an important NA inhibitory activity fragment. Inspired by this finding, we discovered some novel ferulic acid derivatives as NA inhibitors recently [39].

2.2.4. Inhibitory activity against neuraminidase of influenza H3N2 and B virus strains

The five most potent compounds **D15**, **D18**, **D25**, **D41**, and **D44** were selected as representatives for the further NA inhibition studies on A/Minfang/151/2000 (H3N2) and B/Sichuan/01/96 (B) strains of influenza virus. As the result shown in Table 5, In general, the inhibitory activities of these compounds against influenza viruses H1N1 and H3N2 have little difference. For influenza B viruses, **D15**, **D18**, and **D25** (with carboxylic acid ester group substitution at the 5- position of the thiazole ring) show better activity than **D41** and **D44** (with acyl substitution at the 5-position of the thiazole ring). Among the selected compounds, **D18** exhibited the highest potency against NA from A/PR/8/34 (H1N1) (IC_{50} = 13.06 μM). **D18** and **D41** turned out to be the most potent inhibitors against H3N2 strains (IC_{50} = 15.00 μM and IC_{50} = 14.97 μM, respectively). **D18** and **D25** was the most potent compound against B strains (IC_{50} = 19.31 μM and IC_{50} = 16.09 μM, respectively). Therefore, **D18** possesses the best NA activity against the three influenza viruses overall. In order to better understand the relationship between the structure and the activity, then the logP values of Zanamivir and selected compounds were calculated by the XLOGP3 [40] (Table 5). The result demonstrated that **D41** (XLOGP3 = 3.46) showed a better lipid solubility than Zanamivir (XLOGP3 = −3.19).

2.2.5. Cytopathic effect reduction assay

Based on the NA inhibitory activity assay of the compounds, the active compounds were selected for cellular level activity against influenza virus. The anti-influenza activity was evaluated by the cytopathic effect of Madin-Darby canine kidney (MDCK) cells under viral infection. The selected drug-resistant virus, influenza virus A/Hebei Xinhua/SWL1106/2017H1N1pdm09, is NA H275Y variants [41] well known for its severe resistance to Oseltamivir and Peramivir. Replacement of the histidine residue at the 275-position of wild-type H1N1 virus by tyrosine will push the neighboring Glu-276 away, and cause a large dislocation of the side chain (i.e. pentoxy in Oseltamivir and pentyl in Peramivir), thus decreasing the hydrophobic interactions in the NA active site [42]. As shown in Table 6, this virus exhibited highly reduced inhibition by Oseltamivir and shows normal inhibition by Zanamivir, which is consistent with the literature report [41]. Among five selected potent compounds, we are lucky to find that the inhibition activity of **D41** (EC_{50} = 30.24 μM) is better than the existing anti-influenza drugs Oseltamivir (EC_{50} > 243.7 μM) and Amantadine (EC_{50} > 532.7 μM), and close to Zanamivir (EC_{50} = 5.09 μM). Besides, **D41** also has lower toxicity (TC_{50} > 278.2 μM, TC_0 = 41.73 μM) than other candidate compounds. These results indicate that **D41** could be a promising potential as candidates for further investigation, contributing to the development of more potent NA inhibitors to against mutant influenza viruses.

2.3. Molecular docking

For the purpose of better understanding on the potency of 4-thiazolinone derivatives and guiding further SAR studies, **D41,** the compound with low toxicity, good anti-influenza activity in both enzymatic assay and cellular assay, was docked into the active sites of NA (H1N1, PDB entry: 3TI57) using AutoDock Vina. Meanwhile, this compound differs from conventional active NA inhibitors in regard to shape and size, which makes the exploration of the binding mode of this novel class of inhibitors fascinating for further modeling studies. PyMOL and Ligplot+ [43] were used for docking and graphic display, respectively.

As shown in Fig. 4, the predicted binding mode of **D41** to the structure of H1N1 neuraminidase indicates a binding interface largely non-overlapping with that of Zanamivir. It is obvious that **D41** targets both the catalytic cavity (SA cavity) and the 430 Cavity (a large cavity adjacent to the SA cavity). This suggested new

Table 2
H1N1 NA inhibitory activity of target compounds **D1-D58**.

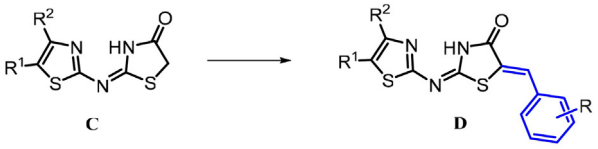
No.	R^1	R^2	R	Inhibition Rates [a]/%	IC_{50} /μM	$\triangle IC_{50}$ [b] /μM
D1	CO_2Et	Me	-H	72.4±6.10	64.21±6.64	5.37
D2	CO_2Et	Me	2-CO_2H	46.97±3.21	NT	>0
D3	CO_2Et	Me	2-OH	91.26±3.22	28.78±0.23	-30.06
D4	CO_2Et	Me	3-CH_3	35.42±6.67	NT	>0
D5	CO_2Et	Me	3-NO_2	43.48±1.08	NT	>0
D6	CO_2Et	Me	3-NH_2	77.43±1.73	31.25±4.38	-27.59
D7	CO_2Et	Me	3-OH	79.45±2.42	43.57±3.75	-15.27
D8	CO_2Et	Me	3-OCH_3	59.03±8.12	73.11±14.95	14.27
D9	CO_2Et	Me	3-F	54.48±3.37	64.38±6.26	5.54
D10	CO_2Et	Me	3-Cl	51.98±9.85	78.72±22.55	19.88
D11	CO_2Et	Me	4-CO_2H	79.11±3.95	36.46±2.85	-22.38
D12	CO_2Et	Me	4-CO_2Me	50.14±9.26	94.02±1.11	35.18
D13	CO_2Et	Me	4-NHAc	77.84±5.17	44.11±7.85	-14.73
D14	CO_2Et	Me	4-NO_2	40.38±2.24	NT	>0
D15	CO_2Et	Me	4-OH	94.12±3.27	**16.33±1.82**	-42.51
D16	CO_2Et	Me	2-OH-3-OCH_3	91.25±2.84	**19.21±5.91**	-39.63
D17	CO_2Et	Me	3,4-OCH_2O	44.98±1.24	NT	>0
D18	CO_2Et	Me	3-OCH_3-4-OH	83.12±5.08	**13.06±1.41**	-45.78
D19	CO_2Et	Me	2,4-$(OH)_2$	75.22±4.03	28.31±8.21	-30.53
D20	CO_2Et	Me	3,4-$(OH)_2$	91.34±2.87	27.11±4.37	-31.73
D21	CO_2Et	Me	2-OH-3,5-di NO_2	58.5±8.72	39.86±14.54	-18.98
D22	CO_2H	Me	3-OCH_3-4-OH	87.08±0.95	32.47±5.67	-64.54
D23	CO_2Me	Me	3-OCH_3-4-OH	88.21±0.44	30.39±1.87	-23.02
D24	CO_2*t*-Bu	Me	3-OCH_3-4-OH	89.03±0.92	27.53±1.70	-38.52
D25	CO_2Me	*i*-Pr	4-OH	98.92±1.74	**19.21±2.88**	-14.39
D26	CO_2Me	*i*-Pr	2-OH-3-OCH_3	98.53±3.35	28.90±2.68	-4.70
D27	CO_2Me	*i*-Pr	3,4-$(OH)_2$	89.57±5.86	21.81±3.03	-11.79
D28	CO_2Me	*i*-Pr	3-OCH_3-4-OH	97.94±6.75	22.98±3.09	-10.62
D29	CO_2Me	*i*-Pr	3,5-$(OCH_3)_2$-4-OH	70.37±12.43	28.67±9.71	-4.93
D30	CO_2Me	*n*-Pr	3-OCH_3-4-OH	87.57±0.69	30.43±1.94	< 0
D31	H	CH_2CO_2Et	3-NO_2	51.17±19.52	46.00±7.15	< 0
D32	H	CH_2CO_2Et	3-NH_2	86.44±3.52	35.76±3.96	< 0
D33	H	CH_2CO_2Et	4-CO_2H	89.62±3.82	34.71±1.37	< 0
D34	H	CH_2CO_2Et	4-OH	93.88±3.12	27.69±1.82	< 0
D35	H	CH_2CO_2Et	2-OH-3-OCH_3	93.82±3.44	33.90±3.74	< 0
D36	H	CH_2CO_2Et	3-OH-4-OCH_3	82.56±3.28	28.80±4.36	< 0
D37	H	CH_2CO_2Et	3-OCH_3-4-OH	86.23±5.99	28.04±2.05	< 0
D38	Me	CH_2CO_2Et	3-OCH_3-4-OH	95.94±1.54	20.30±2.86	< 0

D39	Ac	Me	3-NO_2	80.14±5.32	70.54±19.8	21.86
D40	Ac	Me	3-NH_2	24.33±7.34	NT	>0
D41	Ac	Me	3-OH	100.13±2.82	**19.09±3.70**	-29.59
D42	Ac	Me	4-CO_2H	89.12±6.18	31.75±6.04	-16.93
D43	Ac	Me	4-OH	105.78±3.67	20.53±2.62	-28.15
D44	Ac	Me	3-OH-4-OCH_3	101.01±4.34	**18.28±3.44**	-30.4
D45	Ac	Me	3-OCH_3-4-OH	100.29±3.22	26.11±4.7	-22.57
D46	Ac	Me	3,4-OCH_2O	88.76±3.29	42.28±9.34	-6.4
D47	Ac	Me	3,4-$(OH)_2$	95.83±2.04	25.30±5.17	-23.38
D48	NO_2	*t*-Bu	2-CO_2H	79.26±4.15	33.02±1.53	-8.60
D49	NO_2	*t*-Bu	2-OH	83.25±3.29	35.36±4.57	-6.26
D50	NO_2	*t*-Bu	3-NO_2	52.62±8.58	48.24±11.35	6.62
D51	NO_2	*t*-Bu	3-OCH_3	70.93±3.81	44.18±7.12	2.56
D52	NO_2	*t*-Bu	3-F	62.84±5.02	32.08±4.58	-9.54
D53	NO_2	*t*-Bu	3-Cl	45.86±7.19	NT	>0
D54	NO_2	*t*-Bu	4-NHAc	56.74±3.59	75.78±8.13	34.16
D55	NO_2	*t*-Bu	4-NO_2	46.12±1.34	NT	>0
D56	NO_2	*t*-Bu	4-OH	78.34±5.44	38.82±6.92	-2.80
D57	NO_2	*t*-Bu	2-OH-3-OCH_3	57.3±11.10	55.03±12.87	13.41
D58	NO_2	*t*-Bu	3-OCH_3-4-OH	77.1±6.20	35.21±4.17	-6.41

* [a] NA inhibition rates at the concentration of 40 μg/mL; [b] $\triangle IC_{50}$: The difference between the NA inhibitory activity of the product and the corresponding intermediates; NT: Not Tested; Compounds with $\triangle IC_{50} > 0.00\mu M$ are colored with Yellow; Compounds with $\triangle IC_{50} \leq 0.00\ \mu M$ are colored with Green;

opportunities to design highly effective NA inhibitors targeting both the 430 Cavity and the known active sites [36,37,44–47]. **D41** interacts with the NA active site by hydrogen bond interaction with Arg292 and Arg371, and hydrophobic interaction with Glu276, Glu277 and Tyr406. These are the key amino acid residues for Zanamivir to inhibit NA. It is obvious that in the SA cavity, the interaction between the thiazole moiety of **D41** and the key active site of NA is much weaker than that of Zanamivir. This is consistent

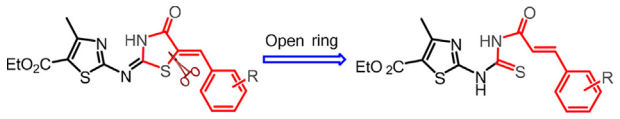
Table 3
Comparison of inhibitory activity between compound **D** and its corresponding intermediates **C**.

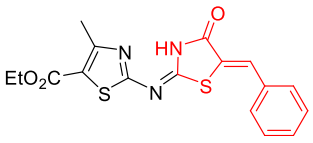
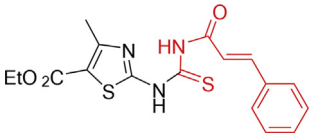
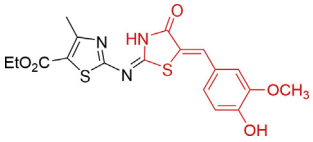
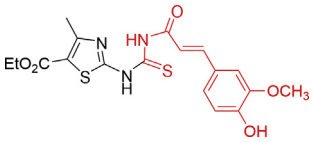
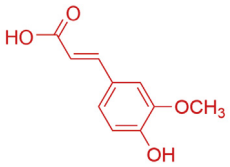
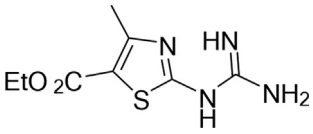


R	$\triangle IC_{50}/\mu M$ [a]								
R^1	Me	Me	Me	Me	Me	*i*-Pr	*n*-Pr	CH_2CO_2Et	*t*-Bu
R^2	CO_2H	CO_2Me	CO_2Et	CO_2t-Bu	Ac	CO_2Me	CO_2Me	H	NO_2
3-NH_2	—	—	-27.59	—	> 0	—	—	< 0	—
3-NO_2	—	—	> 0	—	21.86	—	—	< 0	6.62
4-CO_2H	—	—	-22.38	—	-16.93	—	—	< 0	—
4-OH	—	—	-42.51	—	-28.15	-14.39	—	< 0	-2.80
2-OH-3-OCH_3	—	—	-39.63	—	13.41	-4.70	—	< 0	13.41
3-OCH_3-4-OH	-64.54	-23.02	-45.78	-38.52	-22.57	-10.62	< 0	< 0	-6.41

* [a] $\triangle IC_{50}$: The difference between the NA inhibitory activity of the product and the corresponding reaction starting material; Compounds with $\triangle IC_{50} > 0.00\mu M$ are colored with Yellow; Compounds with $\triangle IC_{50} \leq 0.00\ \mu M$ are colored with Green; —: Not synthesized;

Table 4
NA inhibitory activity of **D1**, **D18**, **S3**, **S5**, and **S6**.



No	Structure	Inhibition Rates[a]/%	IC_{50}/μM
D1		72.4 ± 6.10	64.21 ± 6.64
S3		23.56 ± 2.10	NT
D18		83.12 ± 5.08	13.06 ± 1.41
S5		80.73 ± 1.07	44.00 ± 3.37
Ferulic acid		–	140 [38]
S6		−1.32 ± 5.51	NT

[a] NA inhibition rates at the concentration of 40 μg/mL; NT: Not tested.

with the conclusion drawn in Table 4 that the thiazole may not contribute much to the NA inhibitory activity. Therefore, we speculate that the activity of the target compound is related to the binding mode of the 5-benzylidenethiazolinone fragment on the cavity outside the active cavity. Notably, adjacent to the active site, the hydrophobic region formed by Ile149, Arg430, Pro431, and Thr439 accommodates the phenyl group with hydrophobic interaction. Besides, additional hydrogen bonds interaction with Arg371 significantly made benzene moiety more stable in 430 Cavity. Interestingly, the 4-thiazolinone group acting as a bridge connecting the active site and the 430 Cavity.

3. Conclusions

Based on our previous studies, 18 4-thiazolinone derivatives (**C1**−**C18**) and 58 5-benzyl-4-thiazolinone derivatives (**D1**-**D58**) were designed, synthesized, and evaluated for their influenza H1N1 NA inhibitory activity. A systematic structure-activity relationship analysis of the parent framework was conducted, and the following main conclusions were drawn: i. compound **C4** has the best NA inhibitory activity (IC_{50} = 33.60 μM) in C series; ii. When thiazole ring is replaced by oxazole ring, the activity is significantly reduced; iii. After S is replaced by Se on 4-thiazolinone, the activity is significantly reduced; iv. The 4-position substituent of thiazole has the largest influence on the activity of the mother nucleus. Generally, 5-benzyl-4-thiazolinone derivatives are more active than its corresponding thiazolinone fragment. Introducing 4-CO_2H, 4-OH, 3-OCH_3-4-OH substituted benzyl methylene to 5-position of the 4-thiazolinone ring brings significant activity improvement. The compound with the best H1N1 NA inhibitory activity in **D** series is **D18** (IC_{50} = 13.06 μM).

In the anti-NAs (H3N2 and B) assay, among the selected five most potent compounds, **D18** and **D41** turned out to be the most potent inhibitor against H3N2 strains (IC_{50} = 15.00 μM and IC_{50} = 14.97 μM, respectively). **D25** was the most potent compound against B strains (IC_{50} = 16.09 μM). The cell-based assays indicate

Table 5
The IC_{50} (μM) values of selected compounds against three NAs and their logP values.

compound	H1N1[a]	H3N2[b]	B[c]	XLOGP3[d]
D15	16.33 ± 1.82	15.45 ± 1.00	19.64 ± 0.75	4.00
D18	**13.06 ± 1.41**	**15.00 ± 0.88**	**19.31 ± 0.67**	3.97
D25	19.21 ± 2.88	15.49 ± 1.24	**16.09 ± 3.57**	4.39
D41	19.09 ± 3.70	**14.97 ± 0.22**	36.23 ± 2.78	3.46
D44	18.28 ± 3.44	24.44 ± 1.95	76.47 ± 0.82	3.43
Zanamivir	1.10×10^{-4}	1.31×10^{-3}	5.13×10^{-4}	−3.19

[a] A/PR/8/34 (H1N1).
[b] A/Minfang/151/2000 (H3N2).
[c] B/Sichuan/01/96 (B).
[d] The values were obtained using the XLOGP3 with the default settings.

Table 6
Inhibitory effects of compounds on the cytopathic effect induced by Oseltamivir and amantadine-resistant influenza virus A/Hebei Xinhua/SWL1106/2017H1N1pdm on MDCK cells

Compound	TC_{50} (μM) [a]	TC_0 (μM) [b]	EC_{50} (μM) [c]	SI [d]
D15	>256.8	20.54	>20.54	ND
D18	>238.4	19.07	>19.07	ND
D25	29.10±2.48	19.83	>19.83	<1.25
D41	>278.2	41.73	30.24±2.00	>9.85
D44	43.29±3.29	10.27	>10.27	<3.90
Oseltamivir	>243.7	>243.7	>243.7	ND
Amantadine	>532.7	>532.7	>532.7	ND
Zanamivir	>300.9	>300.9	5.09±0.69	>68.49

[a] TC_{50}: the average concentration of 50% cytotoxic compound;
[b] TC_0: Maximum non-toxic concentration;
[c] EC_{50}: the average concentration of 50% inhibition rate;
[d] SI: Selection index, TC_{50}/EC_{50}; ND: not determined.

that the activity of **D41** (EC_{50} = 30.24 μM) is better than the existing anti-influenza drugs Oseltamivir (EC_{50} > 243.7 μM) and Amantadine (EC_{50} > 532.7 μM), and close to Zanamivir (EC_{50} = 5.09 μM). Besides, **D41** also has lower toxicity (TC_{50} > 278.2 μM, TC_0 = 41.73 μM) than other candidate compounds. Further structure-activity relationship analysis and docking studies indicated that 4-thiazolinone and ferulic acid fragments are necessary NA inhibitory active fragments of such compounds. The 4-thiazolinone group can be used as a linker to design SA Cavity and 430 Cavity dual-site NA inhibitors. The results of this work may contribute to the development of more potent NA inhibitors to against mutant influenza virus.

4. Experiment section

4.1. Chemistry

Reagents were commercial grade and were used as supplied. Reactions were monitored by analytical thin-layer chromatography (TLC) on 0.25-mm F254 silica gel precoated sheets, and spots were visualized with ultraviolet (UV) light. Melting point (mp) was measured on an X-4 electrothermal digital melting point apparatus and uncorrected. In addition, The NMR spectra were recorded on a Bruker NMR AVANCE 400 (400 MHz) or a Bruker AVANCE III HD 600 NMR spectrometer (600 MHz equipped with an UltraCOOL probe) with TMS as internal standard with chemical shifts (δ) expressed in ppm. Signals are reported as m (multiplet), s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet), bm (broad

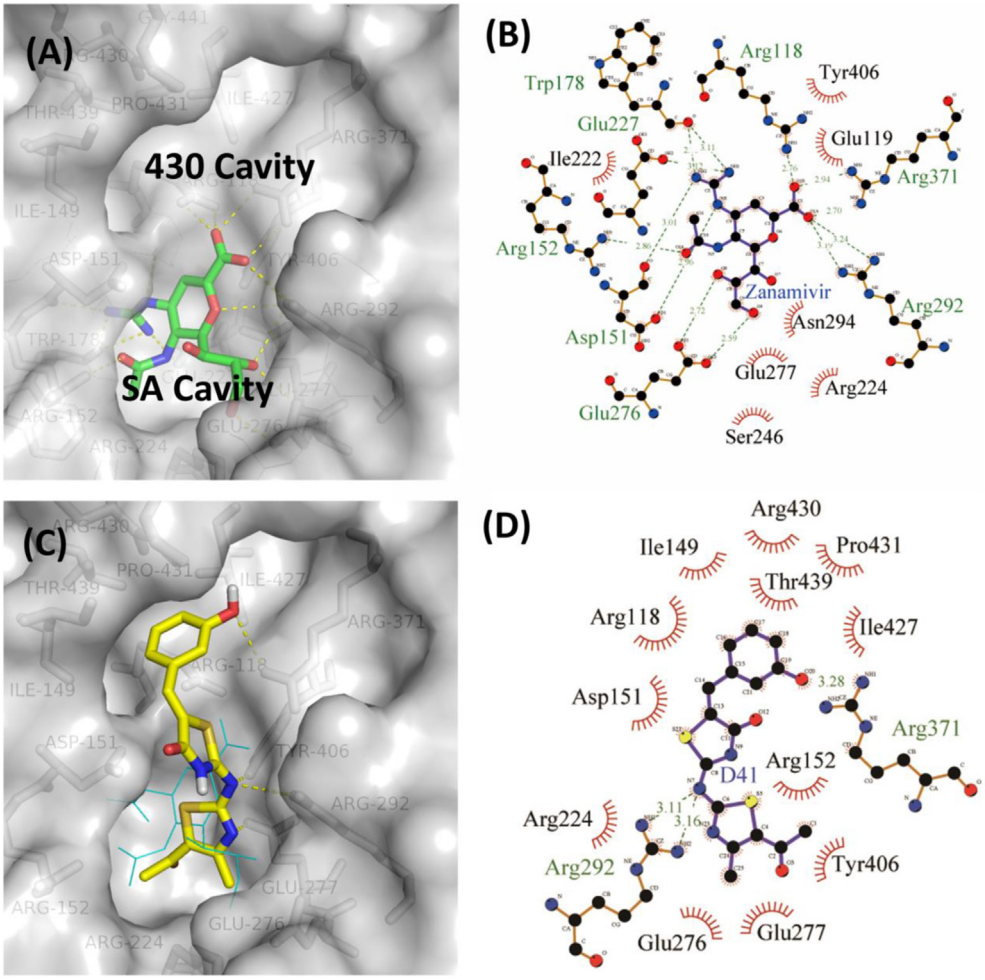


Fig. 4. Binding mode of Zanamivir (A, B) and **D41** (C, D).

multiplet), br (broad). Coupling constants (*J*) are given in hertz. Mass spectra were recorded on a Finnigan LCQ-advantage mass spectrometer. Elemental analyses were performed on a CHN–O-Rapid instrument.

*4.1.1. General procedure for synthesis of 4-thiazolinone intermediates (**C1** – **C18**)*

Aminothiazole (**A1-A18**, 50 mmol) and 100 mmol of potassium carbonate were dissolved in dichloromethane (100 mL), chloroacetyl chloride (8.0 mL) was added dropwise under ice bath, and reacted at room temperature monitored by TLC. When the reaction is completed, the reaction solution is poured into water, and washed with sodium bicarbonate solution, saturated saline and water successively, dry the organic phase with anhydrous sodium sulfate, remove the solvent under reduced pressure, and recrystallize with ethanol to obtain raw product **B1**–**B18**. Then intermediate (30 mmol) and KSCN (45 mmol) were dissolved in 100 mL of ethanol, and reflux for 6 h. The reaction mixture were cooled down, filtered, and washed with ethanol and water, the crude product was recrystallized from ethanol/water to afford the pure 4-thiazolinone intermediate **C1**–**C18**.

2-(4-Thiazolinone-2-imino)-4-methylthiazole-5-carboxylic acid ethyl ester (C1). Yellow solid. m.p. 203–204 °C, yield 81.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.27 (t, J = 7.2 Hz, 3H, CH_3), 2.58 (s, 3H, CH_3), 4.03 (s, 2H, SCH 2), 4.25 (q, J = 7.2 Hz, 2H, OCH_2), 12.28 (s, 1H, NH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.62, 17.84, 35.68, 61.29, 117.06, 158.03, 162.06, 166.69, 171.08, 174.58; Anal. Calcd. for $C_{10}H_{11}N_3O_3S_2$: C, 42.09; H, 3.89; N, 14.73; found C, 42.05; H, 3.40; N, 14.71.

2-(4-Thiazolinone-2-imino)-4-methylthiazole-5-carboxylic acid (C2). White solid, m.p. 250–251 °C, yield 47.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.56 (s, 3H, CH_3), 4.02 (s, 2H, SCH 2), 12.23 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) 1: 17.69, 35.62, 118.66, 157.29, 163.60, 166.17, 170.62, 174.63; Anal. Calcd. for $C_8H_7N_3O_3S_2$: C, 37.35; H, 2.74; N, 16.33; found C, 37.34; H, 2.77; N, 16.29.

2-(4-Thiazolinone-2-imino)-4-trifluoromethylthiazole-5-carboxylic acid ethyl ester (C3). Yellow solid. m.p. 228–229 °C, total yield of two steps is 26.4% (based on raw material **A3**). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.29 (t, J = 7.1 Hz, 3H, CH_3), 4.08 (s, 2H, CH_2), 4.32 (q, J = 7.1 Hz, 2H, OCH_2), 12.48 (s, 1H, NH); Anal. Calcd. for $C_{10}H_8F_3N_3O_3S_2$: C, 35.40; H, 2.38; N, 12.38; found C, 35.32; H, 2.47; N, 12.34.

2-(4-Thiazolinone-2-imino)-4-isopropyl Thiazole-5-carboxylic acid methyl ester (C4). Yellow solid, m.p. 205–207 °C, total yield of two steps is 64.3% (based on raw material **A4**). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.23 (d, J = 6.8 Hz, 6H, 2 × CH_3), 3.78 (s, 3H, OCH_3), 3.86 (hept, J = 6.8 Hz, 1H, CH), 4.02 (s, 2H, SCH 2), 12.26 (s, 1H, NH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 22.35, 28.90, 35.70, 52.52, 115.22, 162.25, 167.19, 167.66, 171.70, 174.76; Anal. Calcd. for $C_{11}H_{13}N_3O_3S_2$: C, 44.13; H, 4.38; N, 14.04; found C, 44.10; H, 4.41; N, 14.02.

2-(4-Thiazolinone-2-imino)-4-propyl Thiazole-5-carboxylic acid methyl ester (C5). Yellow solid, m.p. 211–212 °C, yield 82.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.92 (t, J = 7.4 Hz, 3H, CH_3), 1.66–1.78 (m, 2H, CH_2), 2.99 (t, J = 7.4 Hz, 2H, Thiazole-CH_2), 3.78 (s, 3H, OCH_3), 4.02 (s, 2H, SCH 2), 12.26 (s, 1H, NH) Anal. Calcd. for $C_{11}H_{13}N_3O_3S_2$: C, 44.13; H, 4.38; N, 14.04; found C, 44.09; H, 4.42; N, 14.01.

2-(4-Thiazolinone-2-imino)-4-methyl Thiazole-5-carboxylic acid methyl ester (C6). Yellow solid, m.p. 215–218 °C, yield 84.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.58 (s, 3H, CH_3), 3.78 (s, 3H, CH_3), 4.03 (s, 2H, CH_2), 12.28 (s, 1H, NH); Anal. Calcd. for $C_9H_9N_3O_3S_2$: C, 39.84; H, 3.34; N, 15.49; found C, 39.80; H, 3.36; N, 15.47.

2-(4-Thiazolinone-2-imino)-4-methyl Thiazole-5-carboxylic acid *tert*-butyl ester (C7). Yellow solid. m.p. 195–198 °C, yield 84.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.56 (s, 9H, 3 × CH_3), 2.63 (s, 3H, CH_3), 3.91 (s, 2H, SCH 2); Anal. Calcd. for $C_{12}H_{15}N_3O_3S_2$: C, 45.99; H, 4.82; N, 13.41; found C, 45.95; H, 4.84; N, 13.40.

2-(5-Methyl 4-thiazolinone-2-imino)-4-methyl Thiazole-5-carboxylic acid ethyl ester (C8). White solid **C8**, m.p. 183–185 °C, total yield of two steps is 74.3% (based on **A8**). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.36 (t, J = 7.1 Hz, 3H, CH_3), 1.73 (d, J = 7.3 Hz, 3H, $CH\underline{CH_3}$), 2.67 (s, 3H, CH_3), 4.16 (q, J = 7.3 Hz, 1H, CH), 4.32 (q, J = 7.1 Hz, 2H, OCH_2); Anal. Calcd. for $C_{11}H_{13}N_3O_3S_2$: C, 44.13; H, 4.38; N, 14.04; found C, 44.11; H, 4.40; N, 14.01.

2-(5-Butyl-4-thiazolinone-2-imino)-4-methyl Thiazole-5-carboxylic acid ethyl ester (C9). Pale yellow solid, m.p. 145–146 °C, total yield of two steps is 66.7% (based on raw material **A9**). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.94 (t, J = 7.1 Hz, 3H, CH_3), 1.36 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 1.38–1.43 (m, 2H, CH_2), 1.43–1.60 (m, 2H, CH_2), 2.18–2.22 (m, 2H, CH_2), 2.67 (s, 3H, CH_3), 4.14 (dd, J = 9.0, 4.1 Hz, 1H, CH), 4.32 (q, J = 7.1 Hz, 2H, OCH_2), 11.88 (s, 1H, NH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.17, 14.62, 17.85, 22.21, 28.55, 31.76, 50.22, 61.30, 117.21, 158.12, 162.04, 164.79, 170.97, 176.80; Anal. Calcd. for $C_{14}H_{19}N_3O_3S_2$: C, 49.25; H, 5.61; N, 12.31; found C, 49.21; H, 5.64; N, 12.29.

2-(5-Phenyl-4-thiazolinone-2-imino)-4-methyl Thiazole-5-carboxylic acid ethyl ester (C10). Yellow solid, m.p. 198–199 °C, total yield of two steps is 57.6% (based on raw material **A10**). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.41 (t, J = 7.0 Hz, 3H, CH_3), 2.56 (s, 3H, CH_3), 4.48 (q, J = 7.0 Hz, 2H, OCH_2), 5.62 (s, 1H, CH), 7.14–7.17 (m, 1H, C_6H_5 4-H), 7.34 (d, J = 7.6 Hz, 2H, C_6H_5 3,5-H), 7.58 (d, J = 7.6 Hz, 2H, C_6H_5 2,6-H), 12.53 (s, 1H, NH).

2-(4-Thiazolinone-2-imino)-4-methyl-5-acetylthiazole (C11). Yellow solid, m.p. 216–218 °C, yield 81.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.48 (s, 3H, CH_3), 2.60 (s, 3H, CH_3), 4.03 (s, 2H, CH_2), 12.29 (s, 1H, NH); Anal. Calcd. for $C_9H_9N_3O_2S_2$: C, 42.34; H, 3.55; N, 16.46; found C, 42.30; H, 3.58; N, 16.39.

2-(4-Thiazolinone-2-imino) Thiazole-4-ethyl acetate (C12). Yellow solid, m.p.162–165 °C, yield 81.4%; ^{1}H NMR (400 MHz, $CDCl_3$) δ:1.29 (t, J = 7.1 Hz, 3H, CH_3), 3.74 (s, 2H, $COCH_2$), 3.89 (s, 2H, SCH 2), 4.21 (q, J = 7.1 Hz, 2H, OCH_2), 6.91 (s, 1H, thiazole 5-H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.59, 35.34, 37.45, 60.85, 113.67, 146.24, 163.28, 169.18, 170.26, 174.56; Anal. Calcd. for $C_{10}H_{11}N_3O_3S_2$: C, 42.09; H, 3.89; N, 14.73; found C, 42.07; H, 3.89; N, 14.70.

2-(4-Thiazolinone-2-imino)-5-methyl Thiazole-4-ethyl acetate (C13). Yellow solid **C13**, m.p. 170–172 °C, yield 46.1%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.28 (t, J = 7.1 Hz, 3H, CH_3), 2.30 (s, 3H, CH_3), 3.68 (s, 2H, CH_2), 3.89 (s, 2H, SCH 2), 4.19 (q, J = 7.1 Hz, 2H, OCH_2); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.51, 15.44, 31.72, 35.32, 61.18, 119.82, 146.79, 162.19, 166.81, 170.51, 174.47; Anal. Calcd. for $C_{11}H_{13}N_3O_3S_2$: C, 44.13; H, 4.38; N, 14.04; found C, 44.10; H, 4.39; N, 14.02.

2-(4-Thiazolinone-2-imino)-4-*tert*-butylthiazole (C14). White solid, m.p. 200–202 °C, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.33 (s, 9H, 3 × CH_3), 3.88 (s, 2H, CH_2), 6.64 (s, 1H, thiazole-H), 12.24 (s, 1H, NH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 29.96, 34.94, 35.29, 107.81, 162.94, 168.68, 174.62; EI-MS (m/z): 255.3 $[M]^+$; Anal. Calcd. for $C_{10}H_{13}N_3OS_2$: C, 47.04; H, 5.13; N, 16.46; found C, 47.01; H, 5.16; N, 16.42.

2-(4-Thiazolinone-2-imino)-4-*tert*-butyl-5-bromothiazole (C15). Red solid, m.p. 202–203 °C, yield 83%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.47 (s, 9H, 3 × CH_3), 3.88 (s, 2H, CH_2), 11.92 (s, 1H, NH); Anal. Calcd. for $C_{10}H_{12}BrN_3OS_2$: C, 35.93; H, 3.62; N, 12.57; found C, 35.86; H, 3.69; N, 12.52.

2-(4-Thiazolinone-2-imino)-4-*tert*-butyl-5-nitrothiazole (C16). Yellow solid, m.p. 240–242 °C, total yield of two steps is 54.3% (based on raw material **A16**). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.46 (s, 9H, 3 × CH_3), 4.08 (s, 2H, CH_2), 12.54 (s, 1H, NH); ^{13}C NMR

(150 MHz, DMSO-d_6) δ: 28.62, 36.19, 37.30, 139.44, 164.19, 169.02, 170.34, 174.82; EI-MS (m/z): 300.0 $[M]^+$; Anal. Calcd. for $C_{10}H_{12}N_4O_3S_2$: C, 39.99; H, 4.03; N, 18.65; found C, 39.96; H, 4.05; N, 18.61.

2-((4-(*tert*-butyl)-5-(*1H*-imidazole-1-yl)thiazol-2-yl)imino) thiazolidin-4-one (C17). Yellow solid, m.p. 241−243 °C, yield 86%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.20 (s, 9H, 3 × CH_3), 3.90 (s, 2H, CH_2), 7.09 (s, 1H, imidazole 2-H), 7.19 (s, 1H, imidazole 4-H), 7.70 (s, 1H, imidazole 5-H), 12.08 (s, 1H, NH); Anal. Calcd. for $C_{13}H_{15}N_5OS_2$: C, 48.58; H, 4.70; N, 21.79; found C, 48.56; H, 4.72; N, 21.79.

2-((4-(*tert*-butyl)-5-(*1H*-1,2,4-triazol-1-yl)thiazol-2-yl) imino)thiazolidin-4-one (C18). Light yellow solid, m.p. 182−185 °C, yield 86%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.19 (s, 9H, 3 × CH_3), 3.90 (s, 2H, CH_2), 8.11 (s, 1H, triazole 3-H), 8.28 (s, 1H, triazole 5-H), 12.01 (s, 1H, NH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 29.64, 35.60, 36.23, 120.70, 149.18, 152.80, 157.77, 165.57, 166.16, 174.69; Anal. Calcd. for $C_{12}H_{14}N_6OS_2$: C, 44.71; H, 4.38; N, 26.07; found C, 44.67; H, 4.39; N, 26.06.

*4.1.2. General procedure for synthesis of 5-benzylidene-4-thiazolinone derivatives (**D**)*

To a solution of intermediate **C** (1 mmol) and benzaldehydes (2 mmol) in 15 mL of acetic acid, anhydrous sodium acetate (6.0 mmol) was added. The mixture was refluxed for 10 h and then cooled at room temperature. The precipitate was filtered, washed with ethanol, saturated brine and water in sequence to obtain pure product **D**. For the mixture without precipitate, it was poured into water, filtered, washed with water, and then the filter residue was recrystallized with ethanol to obtain the pure product **D**.

2-(5-Benzylidene-4-thiazolinone-2-imino)-4-methyl Thiazole-5-carboxylic acid ethyl ester (D1). Yellow solid, m.p. 215−216 °C, yield 75.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (t, J = 7.0 Hz, 3H, CH_3), 2.65 (s, 3H, CH_3), 4.25 (q, J = 7.0 Hz, 2H, CH_2), 7.47−7.54 (m, 1H, C_6H_5 4-H), 7.57 (d, J = 7.2 Hz, 2H, C_6H_5 3,5-H), 7.65 (d, J = 7.2 Hz, 2H, C_6H_5 2,6-H), 7.75 (s, 1H, =CH), 12.90 (s, 1H, NH); Anal. Calcd. for $C_{17}H_{15}N_3O_3S_2$: C, 54.68; H, 4.05; N, 11.25; found C, 54.63; H, 4.08; N, 11.22.

2-[5-(2-Carboxybenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D2). Yellow solid, m.p. 246−247 °C, yield 88.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.29 (t, J = 7.1 Hz, 3H, CH_3), 2.54 (s, 3H, CH_3), 4.23 (q, J = 7.1 Hz, 2H, OCH_2), 7.52−7.55 (m, 1H, C_6H_4 4-H), 7.69 (d, J = 7.6 Hz, 1H, C_6H_4 6-H), 7.71−7.75 (m, 1H, C_6H_4 5-H), 7.95 (d, J = 7.6 Hz, 1H, C_6H_4 3-H), 8.23 (s, 1H, =CH), 13.16 (s, 1H, NH); Anal. Calcd. for $C_{18}H_{15}N_3O_5S_2$: C, 51.79; H, 3.62; N, 10.07; found C, 51.74; H, 3.62; N, 10.04.

2-[5-(2-Hydroxybenzylidene)-4-thiazolinone-2-imino]-4-methyl thiazole-5-carboxylic acid ethyl ester (D3). Yellow solid, m.p. 252−254 °C, yield 69.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (t, J = 7.1 Hz, 3H, CH_3), 2.62 (s, 3H, CH_3), 4.25 (q, J = 7.1 Hz, 2H, CH_2), 7.02 (d, J = 7.6 Hz, 1H, C_6H_4 3-H), 7.34 (t, J = 7.6 Hz, 1H, C_6H_4 5-H), 7.59 (t, J = 7.6 Hz, 1H, C_6H_4 4-H), 7.75 (d, J = 7.6 Hz, 1H, C_6H_4 6-H), 8.08 (s, 1H, =CH), 10.63 (s, 1H, OH), 12.81 (s, 1H, NH); Anal. Calcd. For $C_{17}H_{15}N_3O_4S_2$: C, 52.43; H, 3.88; N, 10.79; found C, 52.40; H, 3.92; N, 10.75.

2-[5-(3-Methylbenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D4). Yellow solid, m.p. 246−247 °C, yield 88.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (t, J = 7.0 Hz, 3H, CH_3), 2.38 (s, 3H, $PhCH_3$), 2.65 (s, 3H, CH_3), 4.26 (q, J = 7.0 Hz, 2H, OCH_2), 7.32 (s, 1H, C_6H_4 2-H), 7.39−7.49 (m, 3H, C_6H_4 4,5,6-H), 7.70 (s, 1H, =CH), 12.88 (s, 1H, NH); Anal. Calcd. for $C_{18}H_{15}N_3O_5S_2$: C, 55.80; H, 4.42; N, 10.84; found C, 55.79; H, 4.45; N, 10.81.

2-[5-(3-Nitrobenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D5). Yellow solid, m.p. 279−280 °C, yield 89.0%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (t, J = 6.8 Hz, 3H, CH_3), 2.62 (s, 3H, CH_3), 4.24 (q, 2H, J = 6.8 Hz, OCH_2), 7.80−7.82 (m, 1H, C_6H_4 5-H), 7.82 (s, 1H, =CH), 8.04 (d, J = 8.0 Hz, 1H, C_6H_4 6-H), 8.27 (d, J = 8.0 Hz, 1H, C_6H_4 4-H), 8.46 (s, 1H, C_6H_4 2-H), 13.00 (s, 1H, NH); Anal. Calcd. for $C_{17}H_{14}N_4O_5S_2$: C, 48.80; H, 3.37; N, 13.39; found C, 48.80; H, 3.41; N, 13.36.

2-[5-(3-Hydroxybenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D7). Yellow solid, m.p. 266−268 °C, yield 70.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 4.25 (s, 2H, OCH_2), 6.91 (d, J = 7.8 Hz, 1H, C_6H_4 3-H), 7.04 (s, 1H, C_6H_4 2-H), 7.09 (d, J = 7.8 Hz, 1H, C_6H_4 6-H), 7.36 (t, J = 7.8 Hz, 1H, C_6H_4 5-H), 7.65 (s, 1H, =CH), 9.89 (s, 1H, OH), 12.85 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.57, 18.00, 61.33, 116.80, 117.78, 118.19, 121.86, 124.43, 130.76, 133.33, 134.83, 158.32, 159.09, 161.94, 167.29, 170.15; Anal. Calcd. for $C_{17}H_{15}N_3O_4S_2$: C, 52.43; H, 3.88; N, 10.79; found C, 52.41; H, 3.92; N, 10.76.

2-[5-(3-Methoxybenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D8). Yellow solid, m.p. 222−223 °C, yield 86.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (t, J = 7.0 Hz, 3H, CH_3), 2.62 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 4.25 (q, J = 7.0 Hz, 2H, OCH_2), 7.07 (d, J = 7.6 Hz, 1H, C_6H_4 4-H), 7.21−7.23 (m, 2H, C_6H_4 2,6-H), 7.48 (t, J = 7.6 Hz, 1H, C_6H_4 5-H), 7.71 (s, 1H, =CH), 12.88 (s, 1H, CONH); Anal. Calcd. for $C_{18}H_{17}N_3O_4S_2$: C, 53.58; H, 4.25; N, 10.41; found C, 53.55; H, 4.29; N, 10.38.

2-[5-(3-Fluorobenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D9). Yellow solid, m.p. 240−242 °C, yield 83.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.27 (t, J = 6.6 Hz, 3H, CH_3), 2.61 (s, 3H, CH_3), 4.23 (q, J = 6.6 Hz, 2H, OCH_2), 7.33 (t, J = 7.6 Hz, 1H, C_6H_4 4-H), 7.44 (s, 1H, C_6H_4 2-H), 7.46 (s, 1H, C_6H_4 6-H), 7.58−7.63 (m, 1H, C_6H_4 5-H), 7.70 (s, 1H, =CH), 12.92 (s, 1H, NH); Anal. Calcd. for $C_{17}H_{14}FN_3O_3S_2$: C, 52.16; H, 3.61; N, 10.74; found C, 52.10; H, 3.56; N, 10.70.

2-[5-(3-Chlorobenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D10). Yellow solid, m.p. 218−219 °C, yield 82.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (t, J = 6.8 Hz, 3H, CH_3), 2.62 (s, 3H, CH_3), 4.24 (q, J = 6.8 Hz, 2H, OCH_2), 7.46−7.64 (m, 4H, C_6H_4 2,4,5,6-H), 7.70 (s, 1H, =CH), 12.95 (s, 1H, NH); Anal. Calcd. for $C_{17}H_{14}ClN_3O_3S_2$: C, 50.06; H, 3.46; N, 10.30; found C, 50.01; H, 3.50; N, 10.28.

2-[5-(4-Carboxybenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylate (D11). Yellow solid, m.p. 320−321 °C, yield 87.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.29 (t, J = 7.1 Hz, 3H, CH_3), 2.62 (s, 3H, CH_3), 4.26 (d, J = 7.1 Hz, 2H, OCH_2), 7.73 (d, J = 8.0 Hz, 2H, C_6H_4 2,6-H), 7.75 (s, 1H, =CH), 8.07 (d, J = 8.0 Hz, 2H, C_6H_4 3,5-H); Anal. Calcd. for $C_{18}H_{15}N_3O_5S_2$: C, 51.79; H, 3.62; N, 10.07; found C, 51.73; H, 3.67; N, 10.04.

2-[5-(4-Methoxycarbonylbenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D12). Yellow solid, m.p. 222−224 °C, yield 78.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.29 (t, J = 6.8 Hz, 3H, CH_3), 2.66 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 4.26 (q, J = 6.8 Hz, 2H, OCH_2), 7.77 (d, J = 7.2 Hz, 2H, C_6H_4 2,6-H), 7.78 (s, 1H, =CH), 8.09 (d, J = 7.2 Hz, 2H, C_6H_4 3,5-H), 13.01 (s, 1H, NH); Anal. Calcd. for $C_{19}H_{17}N_3O_5S_2$: C, 52.89; H, 3.97; N, 9.74; found C, 52.84; H, 4.02; N, 9.71.

2-[5-(4-Acetylaminobenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D13). Yellow solid, yield 85.7%, m.p. 288−289 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.29 (t, 8 = 6.9 Hz, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.67 (s, 3H, CH_3), 4.26 (d, J = 6.9 Hz, 2H, OCH_2), 7.60 (d, 0 = 7.2 Hz, 2H, C_6H_4 3,5-H), 7.68 (s, 1H, =CH), 7.77 (d, J = 7.2 Hz, 2H, C_6H_4 2,6-H), 10.29 (s, 1H, NHAc), 12.82 (s, 1H, NH); Anal. Calcd. for $C_{19}H_{18}N_4O_4S_2$: C, 53.01; H, 4.21; N, 13.01; found C, 52.98; H, 4.29; N, 12.96.

2-[5-(4-Nitrobenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D14). Yellow solid, m.p. 279−280 °C, yield 87.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.29 (t, J = 6.8 Hz, 3H, CH_3), 2.67 (s, 3H, CH_3), 4.27 (q, 2H, J = 6.8 Hz,

OCH_2), 7.86 (s, 1H, =CH), 7.91 (d, J = 8.0 Hz, 2H, C_6H_4 2,6-H), 8.40 (d, J = 8.0 Hz, 2H, C_6H_4 3,5-H), 13.09 (s, 1H, NH); Anal. Calcd. for $C_{17}H_{14}N_4O_5S_2$: C, 48.80; H, 3.37; N, 13.39; found C, 48.83; H, 3.43; N, 13.35.

2-[5-(4-Hydroxybenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D15). Red solid, m.p. 291–293 °C, yield 67.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (t, J = 7.0 Hz, 3H, CH_3), 2.66 (s, 3H, CH_3), 4.25 (q, J = 7.0 Hz, 2H, OCH_2), 6.95 (d, J = 8.2 Hz, 2H, C_6H_4 3,5-H), 7.52 (d, J = 8.2 Hz, 2H, C_6H_4 2,6-H), 7.66 (s, 1H, =CH), 10.36 (s, 1H, OH), 12.75 (s, 1H, NH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.58, 18.02, 61.29, 116.79, 117.45, 120.11, 124.61, 133.07, 133.67, 158.30, 159.31, 160.42, 161.98, 167.48, 170.29; **Anal**. Calcd. for $C_{17}H_{15}N_3O_4S_2$: C, 52.43; H, 3.88; N, 10.79; found C, 52.40; H, 3.90; N, 10.78.

2-[5-(2-Hydroxy-3-methoxybenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D16). Yellow solid, m.p. 242–244 °C, yield 75.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (t, J = 6.6 Hz, 3H, CH_3), 2.64 (s, 2H, CH_3), 3.85 (s, 3H, OCH_3), 4.25 (q, J = 6.6 Hz, 2H, OCH_2), 6.97 (t, J = 7.6 Hz, 1H, C_6H_3 5-H), 7.06 (d, J = 7.6 Hz, 1H, C_6H_3 4-H), 7.10 (d, J = 7.6 Hz, 1H, C_6H_3 6-H), 8.02 (s, 1H, =CH), 9.75 (s, 1H, OH), 12.80 (s, 1H, NH); Anal. Calcd. for $C_{18}H_{17}N_3O_5S_2$: C, 51.54; H, 4.09; N, 10.02; found C, 51.45; H, 4.15; N, 9.98.

2-[5-(Benzo[d][1,3]dioxol-5-ylmethylene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D17). Yellow solid, m.p. 255–256 °C, yield 73.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.30 (t, J = 7.0 Hz, 3H, CH_3), 2.65 (s, 3H, CH_3), 4.27 (q, J = 7.0 Hz, 2H, OCH_2), 6.16 (s, 2H, OCH_2O), 7.14 (d, 8 = 8.0 Hz, 1H, 1,3-Benzodioxole 6-H), 7.17 (s, 1H, 1,3-Benzodioxole 2-H), 7.22 (d, J = 8.0 Hz, 1H, 1,3-Benzodioxole 7-H), 7.67 (s, 1H, =CH), 12.82 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.63, 18.03, 61.40, 102.54, 109.69, 117.67, 122.01, 126.76, 127.84, 133.36, 148.60, 149.81, 158.30, 159.10, 162.01, 167.39, 170.26; Anal. Calcd. for $C_{18}H_{15}N_3O_5S_2$: C, 51.79; H, 3.62; N, 10.07; found C, 51.73; H, 3.68; N, 10.05.

2-[5-(3-Methoxy-4-hydroxybenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D18). Yellow solid. m.p. 237–239 °C, yield 77.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (t, J = 6.9 Hz, 3H, CH_3), 2.64 (s, 2H, CH_3), 3.85 (s, 3H, OCH_3), 4.26 (q, J = 6.9 Hz, 2H, OCH_2), 6.98 (d, J = 7.8 Hz, 1H, C_6H_3 5-H), 7.05–7.11 (m, 2H, C_6H_3 2,6-H), 8.03 (s, 1H, =CH), 9.75 (s, 1H, OH), 12.81 (s, 1H, NH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.60, 17.86, 39.96, 55.77, 61.35, 114.09, 116.62, 117.46, 120.41, 125.10, 125.57, 133.99, 148.40, 149.97, 158.11, 159.66, 162.00, 167.48, 170.40; Anal. Calcd. for $C_{18}H_{17}N_3O_5S_2$: C, 51.54; H, 4.09; N, 10.02; found C, 51.44; H, 4.12; N, 4.02.

2-[5-(2,4-Dihydroxybenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D19). Red solid, m.p. > 300 °C, yield 51.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 4.24 (s, 2H, OCH_2), 6.43 (s, 1H, C_6H_3 3-H), 7.10 (s, 1H, C_6H_3 5-H), 7.63–7.68 (m, 1H, C_6H_3 6-H), 8.43 (s, 1H, =CH), 10.36 (s, 1H, OH), 11.06 (s, 1H, OH), 12.57 (s, 1H, NH); Anal. Calcd. for $C_{17}H_{15}N_3O_5S_2$: C, 50.36; H, 3.73; N, 10.36; found C, 50.27; H, 3.81; N, 10.31.

2-[5-(3,4-Dihydroxybenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D20). Red-brown solid, m.p. > 300 °C, yield 39.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.29 (t, J = 6.6 Hz, 3H, CH_3), 2.68 (s, 3H, CH_3), 4.26 (q, J = 6.6 Hz, 2H, OCH_2), 6.90 (d, J = 7.4 Hz, 1H, C_6H_3 5-H), 7.03 (d, J = 7.9 Hz, 1H, C_6H_3 6-H), 7.07 (s, 1H, C_6H_3 2-H), 7.58 (s, 1H, =CH), 9.57 (s, 1H, OH), 9.95 (s, 1H, OH), 12.77 (s, 1H, NH); Anal. Calcd. for $C_{17}H_{15}N_3O_5S_2$: C, 50.36; H, 3.73; N, 10.36; found C, 50.31; H, 3.76; N, 10.32.

2-[5-(2-Hydroxy-3,5-dinitrobenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D21). Red solid, m.p. 258–260 °C, yield 45.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.29 (t, J = 7.1 Hz, 3H, CH_3), 2.63 (s, 3H, CH_3), 4.27 (q, J = 7.1 Hz, 2H, OCH_2), 7.93 (s, 1H, =CH), 8.28 (s, 1H, C_6H_2 6-H), 8.60 (d, J = 2.9 Hz, 1H, C_6H_2 5-H), 12.74 (s, 1H, NH); Anal. Calcd. for $C_{17}H_{13}N_5O_8S_2$: C, 42.59; H, 2.73; N, 14.61; found C, 42.51; H, 2.83; N, 14.59.

2-[5-(3-Methoxy-4-hydroxybenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid (D22). Off-white solid, m.p. 245–247 °C, yield 35.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.16 (s, 1H, CO_2H), 12.72 (s, 1H, NH), 10.01 (s, 1H, OH), 7.68 (s, 1H, =CH), 7.26 (s, 1H, C_6H_3 2-H), 7.16 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 6.96 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 3.87 (s, 3H, OCH_3), 2.62 (s, 3H, CH_3); Anal. Calcd. for $C_{16}H_{13}N_3O_5S_2$: C, 49.10; H, 3.35; N, 10.74; found C, 49.04; H, 3.41; N, 10.70.

2-[5-(3-Methoxy-4-hydroxybenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid methyl ester (D23). Yellow solid, m.p. 262–263 °C, yield 26.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.73 (s, 3H, OCH_3), 3.84 (s, 3H, CO_2CH_3), 6.91 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.07 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 7.13 (s, 1H, C_6H_3 2-H), 7.55 (s, 1H, =CH), 10.06 (s, 1H, NH); Anal. Calcd. for $C_{17}H_{15}N_3O_5S_2$: C, 50.36; H, 3.73; N, 10.36; found C, 50.34; H, 3.76; N, 10.32.

2-[5-(3-Methoxy-4-hydroxybenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid *tert*-butyl ester (D24). Yellow solid. m.p. 263–265 °C, yield 35.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.51 (s, 9H, $C(CH_3)_3$), 2.60 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 6.96 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.15 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 7.25 (s, 1H, C_6H_3 2-H), 7.66 (s, 1H, =CH), 10.02 (s, 1H, NH); Anal. Calcd. for $C_{20}H_{21}N_3O_5S_2$: C, 53.68; H, 4.73; N, 9.39; found C, 53.63; H, 4.77; N, 9.37.

2-[5-(4-Hydroxybenzylidene)-4-thiazolinone-2-imino]-4-isopropyl Thiazole-5-carboxylate (D25). Yellow solid, m.p. 250–252 °C, yield 77.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.31 (d, J = 6.6 Hz, 6H, 2 × CH_3), 3.80 (s, 3H, OCH_3), 3.84–3.91 (m, 1H, CH), 6.92 (d, J = 8.4 Hz, 2H, C_6H_4 3,5-H), 7.53 (d, J = 8.4 Hz, 2H, C_6H_4 2,6-H), 7.67 (s, 1H, =CH), 10.37 (s, 1H, OH), 12.78 (s, 1H, NH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 22.29, 29.03, 52.45, 115.64, 116.58, 120.74, 124.85, 132.94, 133.59, 160.10, 160.33, 162.13, 167.60, 170.91, 172.49; Anal. Calcd. for $C_{18}H_{17}N_3O_4S_2$: C, 53.58; H, 4.25; N, 10.41; found C, 53.55; H, 4.29; N, 10.38.

2-[5-(2-Hydroxy-3-methoxybenzylidene)-4-thiazolinone-2-imino]-4-isopropyl Thiazole-5-carboxylic acid methyl ester (D26). Red solid, m.p. 247–249 °C, yield 66.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (d, J = 6.6 Hz, 6H, 2 × CH_3), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, CO_2CH_3), 3.87–3.93 (m, 1H, CH), 6.93 (t, 1H, C_6H_3 5-H), 7.03–7.14 (m, 2H, C_6H_3 4,6-H), 8.02 (s, 1H, =CH), 9.77 (s, 1H, OH), 12.83 (s, 1H, NH); Anal. Calcd. for $C_{19}H_{19}N_3O_5S_2$: C, 52.64; H, 4.42; N, 9.69; found C, 52.63; H, 4.45; N, 9.65.

2-[5-(3,4-Dihydroxybenzylidene)-4-thiazolinone-2-imino]-4-isopropyl Thiazole-5-carboxylic acid methyl ester (D27). Yellow solid, m.p. 254–257 °C, yield 19.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.31 (d, J = 6.5 Hz, 6H, 2 × CH_3), 3.80 (s, 3H, OCH_3), 3.82–3.94 (m, 1H, CH), 6.89 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 7.03 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.08 (s, 1H, C_6H_3 2-H), 7.59 (s, 1H, =CH), 9.31 (s, 1H, OH), 10.07 (s, 1H, OH), 12.76 (s, 1H, NH); Anal. Calcd. for $C_{18}H_{17}N_3O_5S_2$: C, 51.54; H, 4.09; N, 10.02; found C, 51.50; H, 4.10; N, 9.99.

2-[5-(3-Methoxy-4-hydroxybenzylidene)-4-thiazolinone-2-imino]-4-isopropyl Thiazole-5-carboxylic acid methyl ester (D28). Red solid, m.p. 242–245 °C, yield 32.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (d, J = 6.6 Hz, 6H, 2 × CH_3), 3.80 (s, 3H, OCH_3), 3.89–3.87 (s, 3H, CO_2CH_3), 3.89–3.93 (m, 1 H, CH), 6.92 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.18–7.21 (m, 2H, C_6H_3 2,6-H), 7.68 (s, 1H, =CH), 10.07 (s, 1H, OH), 12.80 (s, 1H, NH); Anal. Calcd. for $C_{18}H_{17}N_3O_5S_2$: C, 51.54; H, 4.09; N, 10.02; found C, 51.50; H, 4.10; N, 10.00.

2-[5-(3,5-Dimethoxy-4-hydroxybenzylidene)-4-thiazolinone-2-imino]-4-isopropyl Thiazole-5- carboxylic acid methyl ester (D29). Red solid. m.p. 273–275 °C, yield 71.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.26 (d, J = 6.7 Hz, 6H, 2 × CH_3), 3.79 (s, 3H, CO_2CH_3), 3.85 (s, 6H, 2 × OCH_3), 3.87–3.92 (m, 1H, CH), 7.00 (s, 2H, C_6H_2 2,6-H), 7.69 (s, 1H, =CH), 9.43 (s, 1H, OH), 12.79 (s, 1H, NH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 22.46, 28.85, 52.61, 56.36, 108.69, 115.75, 120.67, 123.92, 134.51, 139.17, 148.69, 159.83, 162.18, 167.55, 167.73, 171.00; Anal. Calcd. for $C_{20}H_{21}N_3O_6S_2$: C, 51.82; H, 4.57; N, 9.07; found C, 51.76; H, 4.65; N, 9.02.

2-[5-(3-Methoxy-4-hydroxybenzylidene)-4-thiazolinone-2-imino]-4-propyl Thiazole-5-carboxylic acid methyl ester (D30). Yellow solid, m.p. 274–276 °C, yield 69.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.96 (t, J = 7.2 Hz, 3H, CH_3), 1.74–1.79 (m, 2H, CH_2), 2.99 (t, J = 7.2 Hz, 2H, Thiazole-CH_2), 3.76 (s, 3H, OCH_3), 3.86 (s, 3H, CO_2CH_3), 6.91 (d, J = 8.1 Hz, 1H, C_6H_3 5-H), 7.13 (d, J = 8.1 Hz, 1H, C_6H_3 6-H), 7.16 (s, 1H, C_6H_3 2-H), 7.61 (s, 1H, =CH), 9.99 (s, 1H, OH), 12.68 (s, 1H, CONH); Anal. Calcd. for $C_{19}H_{19}N_3O_5S_2$: C, 52.64; H, 4.42; N, 9.69; found C, 52.63; H, 4.47; N, 9.65.

2-[2-(3-Nitrobenzylidene)-4-thiazolinone-2-imino] Thiazole-4-ethyl acetate (D31). Yellow solid, m.p.170–172 °C, yield 89.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ:1.31 (t, J = 7.1 Hz, 3H, CH_3), 3.79 (s, 2H, $COCH_2$), 4.23 (q, J = 7.1 Hz, 2H, OCH_2), 7.00 (s, 1H, thiazole 5-H), 7.70 (t, J = 8.0 Hz, 1H, C_6H_4 5-H), 7.83–7.87 (m, 2H, =CH, C_6H_4 6-H), 8.28 (dd, J = 8.0, 1.3 Hz, 1H, C_6H_4 4-H), 8.42 (s, 1H, C_6H_4 2-H); Anal. Calcd. for $C_{17}H_{14}N_4O_5S_2$: C, 48.80; H, 3.37; N, 13.39; found C, 48.78; H, 3.43; N, 13.38.

2-[2-(4-Carboxybenzylidene)-4-thiazolinone-2-imino] Thiazole-4-ethyl acetate (D33). Yellow solid. m.p. 289–290 °C, yield 82.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.21 (t, J = 7.1 Hz, 3H, CH_3), 3.82 (s, 2H, $COCH_2$), 4.15 (q, J = 7.1 Hz, 2H, OCH_2), 7.28 (s, 1H, thiazole 5-H), 7.72–7.79 (m, 3H, C_6H_4 2,6-H, =CH), 8.06 (d, J = 8.4 Hz, 2H, C_6H_4 3,5-H), 12.78 (s, 1H, CO_2H), 13.23 (s, 1H, CONH); Anal. Calcd. for $C_{18}H_{15}N_3O_5S_2$: C, 51.79; H, 3.62; N, 10.07; found C, 51.75; H, 3.71; N, 10.03.

2-[2-(4-Hydroxybenzylidene)-4-thiazolinone-2-imino] Thiazole-4-ethyl acetate (D34). Yellow solid. m.p. 215–217 °C, yield 81.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ:1.23 (t, J = 7.1 Hz, 3H, CH_3), 3.81 (s, 2H, $COCH_2$), 4.16 (q, J = 7.1 Hz, 2H, OCH_2), 6.96 (d, = 8.4 Hz, 2H, C_6H_4 3,5-H), 7.24 (s, 1H, thiazole 5-H), 7.51 (d, J = 8.4 Hz, 2H, C_6H_4 2,6-H), 7.62 (s, 1H, =CH), 10.51 (s, 1H, OH), 12.56 (s, 1H, CONH); Anal. Calcd. for $C_{17}H_{15}N_3O_4S_2$: C, 52.43; H, 3.88; N, 10.79; found C, 52.40; H, 3.93; N, 10.75.

2-[2-(2-Hydroxy-3-methoxybenzylidene)-4-thiazolinone-2-imino] Thiazole-4-ethyl acetate (D35). Tan solid, m.p. 180–182 °C, yield 24.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ:1.21 (t, J = 7.1 Hz, 3H, CH_3), 3.78 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 4.14 (q, J = 7.1 Hz, 2H, OCH_2), 6.92 (t, J = 8.0 Hz, 1H, C_6H_3 4-H), 7.05 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.10 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 7.26 (s, 1H, thiazole 5-H), 7.99 (s, 1H, =CH), 9.70 (s, 1H, OH), 12.60 (s, 1H, CONH); Anal. Calcd. for $C_{18}H_{17}N_3O_5S_2$: C, 51.54; H, 4.09; N, 10.02; found C, 51.51; H, 4.05; N, 10.03.

2-[2-(4-Methoxy-3-hydroxybenzylidene)-4-thiazolinone-2-imino] Thiazole-4-ethyl acetate (D36). Off-white solid, m.p. 168–170 °C, yield 42.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ:1.22 (t, J = 6.8 Hz, 3H, CH_3), 3.82 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3), 4.17 (q, J = 6.8 Hz, 2H, OCH_2), 7.05–7.15 (m, 3H, C_6H_3 2,5,6-H), 7.25 (s, 1H, thiazole 5-H), 7.58 (s, 1H, =CH), 9.45 (s, 1H, OH), 12.58 (s, 1H, NH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 14.66, 37.44, 56.18, 60.96, 112.71, 114.36, 117.13, 121.62, 123.57, 126.59, 132.75, 146.74, 147.34, 150.35, 156.37, 167.59, 168.55, 170.31; Anal. Calcd. for $C_{18}H_{17}N_3O_5S_2$: C, 51.54; H, 4.09; N, 10.02; found C, 51.51; H, 4.12; N, 10.01.

2-[2-(3-Methoxy-4-hydroxybenzylidene)-4-thiazolinone-2-imino] Thiazole-4-ethyl acetate (D37). Yellow solid, m.p.157–159 °C, yield 33.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.20 (t, J = 7.0 Hz, 3H, CH_3), 3.78 (s, 2H, CH_2), 3.87 (s, 3H, OCH_3), 4.13 (q, J = 7.0 Hz, 2H, OCH_2), 6.95 (d, J = 8.2 Hz, 1H, C_6H_3 5-H), 7.14 (d, J = 8.2 Hz, 1H, C_6H_3 6-H), 7.23 (s, 1H, C_6H_3 2-H), 7.25 (s, 1H, thiazole 5-H), 7.63 (s, 1H, =CH), 10.02 (s, 1H, OH), 12.54 (s, 1H, NH); Anal. Calcd. for $C_{18}H_{17}N_3O_5S_2$: C, 51.54; H, 4.09; N, 10.02; found C, 51.50; H, 4.15; N, 9.98.

2-[2-(3-Methoxy-4-hydroxybenzylidene)-4-thiazolinone-2-imino]-5-methyl Thiazole-4-ethyl acetate (D38). Yellow solid, m.p. 138–140 °C, yield 41.6%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (t, J = 6.9 Hz, 3H, CH_3), 2.32 (s, 3H, CH_3), 3.70 (s, 2H, CH_2), 3.97 (s, 3H, OCH_3), 4.20 (q, J = 6.9 Hz, 2H, OCH_2), 7.01–7.03 (m, 2H, C_6H_3 5,6-H), 7.11 (s, 1H, C_6H_3 2-H), 7.77 (s, 1H, =CH); Anal. Calcd. for $C_{19}H_{19}N_3O_5S_2$: C, 52.64; H, 4.42; N, 9.69; found C, 52.68; H, 4.48; N, 9.64.

2-((5-Acetyl-4-methylthiazol-2-yl) imino)-5-(3-nitrobenzylidene) thiazolidin-4-one (D39). Yellow solid, m.p. 303–306 °C, yield 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.51 (s, 3H, CH_3), 2.67 (s, 3H, CH_3), 7.88 (s, 1H, =CH), 8.08–8.50 (m, 4H, C_6H_4), 13.04 (s, 1H, CONH); Anal. Calcd. for $C_{16}H_{12}N_4O_4S_2$: C, 49.48; H, 3.11; N, 14.42; found C, 49.43; H, 3.14; N, 14.40.

2-((5-Acetyl-4-methylthiazol-2-yl)imino)-5-(3-hydroxybenzylidene)thiazolidin-4-one(D41). Yellow solid, m.p. 246–249 °C, yield 31%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.49 (s, 3H, CH_3), 2.67 (s, 3H, CH_3), 6.90–7.38 (m, 4H, C_6H_4), 7.64 (s, 1H, =CH), 9.91 (s, 1H, OH), 12.88 (s, 1H, CONH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 19.03, 30.59, 116.74, 118.24, 121.94, 124.40, 129.44, 130.83, 133.41, 134.82, 156.23, 158.32, 159.27, 167.33, 170.07, 190.81; EI-MS (m/z): 359.0 [M]+; Anal. Calcd. for $C_{16}H_{13}N_3O_3S_2$: C, 53.47; H, 3.65; N, 11.69; found C, 53.45; H, 3.68; N, 11.67.

4-((2-((5-Acetyl-4-methylthiazol-2-yl)imino)-4-oxothiazolidin-5-ylidene)methyl)benzoic acid(D42). Yellow solid, m.p. 313–315 °C, yield 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.50 (s, 3H, CH_3), 2.67 (s, 3H, CH_3), 7.71–7.77 (m, 3H, =CH, C_6H_4 2,6-H), 8.07 (d, J = 5.2 Hz, 2H, C_6H_4 3,5-H), 12.99 (s, 1H, CONH), 13.25 (s, 1H, CO_2H); Anal. Calcd. for $C_{17}H_{13}N_3O_4S_2$: C, 52.70; H, 3.38; N, 10.85; found C, 52.67; H, 3.43; N, 10.82.

2-((5-Acetyl-4-methylthiazol-2-yl)imino)-5-(4-hydroxybenzylidene)thiazolidin-4-one(D43). Yellow solid, m.p. 302–305 °C, yield 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.50 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 6.95 (d, J = 8.0 Hz, 2H, C_6H_4 3,5-H), 7.53 (d, J = 8.0 Hz, 2H, C_6H_4 2,6-H), 7.67 (s, 1H, =CH), 10.38 (s, 1H, OH), 12.77 (s, 1H, CONH); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 19.07, 30.59, 116.84, 120.06, 124.62, 129.18, 133.13, 133.80, 156.28, 159.47, 160.45, 167.52, 170.21, 190.79; Anal. Calcd. for $C_{16}H_{13}N_3O_3S_2$: C, 53.47; H, 3.65; N, 11.69; found C, 53.41; H, 3.70; N, 11.65.

2-((5-Acetyl-4-methylthiazol-2-yl)imino)-5-(3-hydroxy-4-methoxybenzylidene)thiazolidin-4-one (D44). Yellow solid, m.p. 259–263 °C, yield 51%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.51 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 7.10 (s, 1H, C_6H_3 2-H), 7.13–7.16 (m, 2H, C_6H_3 5,6-H), 7.62 (s, 1H, =CH), 9.58 (s, 1H, OH), 12.80 (s, 1H, CONH); Anal. Calcd. for $C_{17}H_{15}N_3O_4S_2$: C, 52.43; H, 3.88; N, 10.79; found C, 52.41; H, 3.41; N, 10.76.

2-((5-Acetyl-4-methylthiazol-2-yl)imino)-5-(4-hydroxy-3-methoxybenzylidene)thiazolidin-4-one (D45). Yellow solid, m.p. 255–260 °C, yield 31%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.47 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 7.01 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.12 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 7.21 (s, 1H, C_6H_3 2-H), 7.63 (s, 1H, =CH), 10.17 (s, 1H, OH), 12.80 (s, 1H, CONH); Anal. Calcd. for $C_{17}H_{15}N_3O_4S_2$: C, 52.43; H, 3.88; N, 10.79; found C, 52.39; H, 3.40; N, 10.77.

2-((5-Acetyl-4-methylthiazol-2-yl)imino)-5-(benzo[d][1,3]dioxol-5-ylmethylene)thiazolidin-4-one (D46). Tan solid, m.p.

283–285 °C, yield 51%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.50 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 6.14 (s, 2H, CH_2), 7.13 (d, J = 7.2 Hz, 1H, C_6H_3 5-H), 7.17 (s, 1H, C_6H_3 2-H), 7.22 (d, J = 7.2 Hz, 1H, C_6H_3 6-H), 7.68 (s, 1H, =CH), 12.83 (s, 1H, CONH); Anal. Calcd. for $C_{17}H_{13}N_3O_4S_2$: C, 52.70; H, 3.38; N, 10.85; found C, 52.63; H, 3.46; N, 10.82.

2-((5-Acetyl-4-methylthiazol-2-yl)imino)-5-(3,4-dihydroxybenzylidene)thiazolidin-4-one (D47). Dark brown solid, m.p. 275–278 °C, yield 56%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.50 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 6.89 (d, J = 7.6 Hz, 1H, C_6H_3 5-H), 7.02 (d, J = 7.6 Hz, 1H, C_6H_3 6-H), 7.07 (s, 1H, C_6H_3 2-H), 7.57 (s, 1H, =CH), 9.55 (s, 1H, 3-OH), 9.93 (s, 1H, 4-OH), 12.75 (s, 1H, CONH); Anal. Calcd. for $C_{16}H_{13}N_3O_4S_2$: C, 51.19; H, 3.49; N, 11.19; found C, 51.14; H, 3.46; N, 11.17.

2-[5-(2-Carboxybenzylidene)-4-thiazolinone-2-imino]-4-*tert*-butyl-5-nitrothiazole (D48). Yellow solid, m.p. 278–279 °C, yield 89.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.43 (s, 9H, $C(CH_3)_3$), 7.61 (t, J = 7.6 Hz, 1H, C_6H_4 4-H), 7.68–7.73 (m, 2H, C_6H_4 5,6-H), 8.03 (d, J = 7.6 Hz, 1H, C_6H_4 3-H), 8.40 (s, 1H, =CH), 13.17 (s, 1H, CONH); Anal. Calcd. for $C_{18}H_{16}N_4O_5S_2$: C, 49.99; H, 3.73; N, 12.96; found C, 49.92; H, 3.77; N, 12.91.

2-[5-(2-Hydroxybenzylidene)-4-thiazolinone-2-imino]-4-*tert*-butyl-5-nitrothiazole (D49). Yellow solid, m.p. 262–264 °C, yield 84.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.50 (s, 9H, $C(CH_3)_3$), 6.91 (t, J = 7.7 Hz, 1H, C_6H_4 5-H), 6.99 (d, J = 7.7 Hz, 1H, C_6H_4 3-H), 7.33 (t, J = 7.7 Hz, 1H, C_6H_4 4-H), 7.42 (d, J = 7.7 Hz, 1H, C_6H_4 6-H), 8.03 (s, 1H, =CH), 10.58 (s, 1H, CONH); Anal. Calcd. for $C_{17}H_{16}N_4O_4S_2$: C, 50.48; H, 3.99; N, 13.85; found C, 50.45; H, 4.03; N, 13.81.

2-[5-(3-Nitrobenzylidene)-4-thiazolinone-2-imino]-4-*tert*-butyl-5-nitrothiazole (D50). Yellow solid, m.p. 260–261 °C, yield 88.2%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.50 (s, 9H, $C(CH_3)_3$), 7.67–7.81 (m, 1H, C_6H_4 5-H), 7.86 (s, 1H, =CH), 8.08 (d, J = 8.0 Hz, 1H, C_6H_4 6-H), 8.29 (d, J = 8.0 Hz, 1H, C_6H_4 4-H), 8.38 (s, 1H, C_6H_4 2-H), 13.27 (s, 1H, CONH); Anal. Calcd. for $C_{17}H_{15}N_5O_5S_2$: C, 47.11; H, 3.49; N, 16.16; found C, 47.08; H, 3.50; N, 16.10.

2-[5-(3-Methoxybenzylidene)-4-thiazolinone-2-imino]-4-*tert*-butyl-5-nitrothiazole (D51). Yellow solid, m.p. 255–256 °C, yield 84.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.50 (s, 9H, $C(CH_3)_3$), 3.80 (s, 3H, OCH_3), 7.07 (d, J = 7.6 Hz, 1H, C_6H_4 4-H), 7.17 (s, 1H, C_6H_4 2-H), 7.21 (d, = 7.6 Hz, 1H, C_6H_4 6-H), 7.42 (t, J = 7.6 Hz, 1H, C_6H_4 5-H), 7.74 (s, 1H, =CH), 13.13 (s, 1H, CONH); Anal. Calcd. for $C_{18}H_{18}N_4O_4S_2$: C, 51.66; H, 4.34; N, 13.39; found C, 51.62; H, 4.36; N, 13.34.

2-[5-(3-Fluorobenzylidene)-4-thiazolinone-2-imino]-4-*tert*-butyl-5-nitrothiazole (D52). Yellow solid, m.p. 244–245 °C, yield 86.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.49 (s, 9H, $C(CH_3)_3$), 7.32 (td, J = 8.0, 2.2 Hz, 1H, C_6H_4 4-H), 7.41 (d, 4 = 8.0 Hz, 1H, C_6H_4 2-H), 7.45 (d, J = 8.0 Hz, 1H, C_6H_4 6-H), 7.51–7.57 (m, 1H, C_6H_4 5-H), 7.74 (s, 1H, =CH), 13.18 (s, 1H, CONH)); Anal. Calcd. for $C_{17}H_{15}FN_4O_3S_2$: C, 50.24; H, 3.72; N, 13.78; found C, 50.16; H, 3.83; N, 13.76.

2-[5-(3-Chlorobenzylidene)-4-thiazolinone-2-imino]-4-*tert*-butyl-5-nitrothiazole (D53). Yellow solid, m.p. 253–254 °C, yield 85.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.50 (s, 9H, $C(CH_3)_3$), 7.49–7.55 (m, 2H, C_6H_4 2,5-H), 7.59 (d, J = 8.4 Hz, 1H, C_6H_4 4-H), 7.63 (s, 1H, C_6H_4 6-H), 7.72 (s, 1H, =CH), 13.18 (s, 1H, CONH); Anal. Calcd. for $C_{17}H_{15}ClN_4O_3S_2$: C, 48.28; H, 3.58; N, 13.25; found C, 48.29; H, 3.64; N, 13.21.

2-[5-(4-Acetamidobenzylidene)-4-thiazolinone-2-imino]-4-*tert*-butyl-5-nitrothiazole (D54). Yellow solid. m.p. 287–288 °C, yield 82.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.53 (s, 9H, $C(CH_3)_3$), 2.08 (s, 3H, $COCH_3$), 7.58 (d, J = 8.0 Hz, 2H, C_6H_4 3,5-H), 7.71 (d, 0 = 8.0 Hz, 2H, C_6H_4 2,6-H), 7.74 (s, 1H, =CH), 10.24 (s, 1H, NH), 13.02 (s, 1H, CONH); Anal. Calcd. for $C_{19}H_{19}N_5O_4S_2$: C, 51.22; H, 4.30; N, 15.72; found C, 51.20; H, 4.35; N, 15.69.

2-[5-(4-Nitrobenzylidene)-4-thiazolinone-2-imino]-4-*tert*-butyl-5-nitrothiazole (D55). Yellow solid. m.p. 288–290 °C, yield 88.0%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.53 (s, 9H, $C(CH_3)_3$), 7.56 (d, J = 8.0 Hz, 2H, C_6H_4 2,6-H), 7.68 (s, 1H, =CH), 7.71 (d, J = 8.0 Hz, 2H, C_6H_4 3,5-H), 12.99 (s, 1H, CONH); Anal. Calcd. for $C_{17}H_{15}N_5O_5S_2$: C, 47.11; H, 3.49; N, 16.16; found C, 47.09; H, 3.55; N, 16.13.

2-[5-(4-Hydroxybenzylidene)-4-thiazolinone-2-imino]-4-*tert*-butyl-5-nitrothiazole (D56). Yellow solid. m.p. 275–277 °C, yield 72.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.53 (s, 9H, $C(CH_3)_3$), 6.91 (d, J = 8.5 Hz, 2H, C_6H_4 3,5-H), 7.53 (d, = 8.5 Hz, 2H, C_6H_4 2,6-H), 7.72 (s, 1H, =CH), 10.39 (s, 1H, OH), 13.02 (s, 1H, CONH); Anal. Calcd. for $C_{17}H_{16}N_4O_4S_2$: C, 50.48; H, 3.99; N, 13.85; found C, 50.46; H, 4.05; N, 13.82.

2-[5-(2-Hydroxy-3-methoxybenzylidene)-4-thiazolinone-2-imino]-4-*tert*-butyl-5-nitro-thiazole (D57). Yellow solid, m.p. 274–275 °C, yield 74.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.51 (s, 9H, $C(CH_3)_3$), 3.86 (s, 3H, OCH_3), 6.90 (t, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.05 (d, J = 8.0 Hz, 1H, C_6H_3 4-H), 7.10 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 8.07 (s, 1H, =CH), 9.80 (s, 1H, OH), 13.08 (s, 1H, CONH); Anal. Calcd. for $C_{18}H_{18}N_4O_5S_2$: C, 49.76; H, 4.18; N, 12.90; found C, 49.80; H, 4.22; N, 12.86.

2-[5-(3-Methoxy-4-hydroxybenzylidene)-4-thiazolinone-2-imino]-4-*tert*-butyl-5-nitro-thiazole (D58). Yellow solid, m.p. 285–286 °C, yield 78.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.50 (s, 9H, $C(CH_3)_3$), 3.83 (s, 3H, OCH_3), 6.90 (s, 1H, C_6H_3 5-H), 7.05–7.23 (m, 2H, C_6H_3 2,6-H), 7.67 (s, 1H, =CH), 9.98 (s, 1H, OH), 12.94 (s, 1H, CONH); Anal. Calcd. for $C_{18}H_{18}N_4O_5S_2$: C, 49.76; H, 4.18; N, 12.90; found C, 49.74; H, 4.22; N, 12.88.

*4.1.3. General procedure for synthesis of **D6**, **D32** and **D40***

Nitro-substituted raw material **D5**, **D31** and **D39** (1 mmol) was dissolved in a mixed solution of 10 mL of acetic acid, 10 mL of dichloromethane and 1 mL of water, and add an appropriate amount of reducing iron powder. The reaction proceeded at room temperature and monitored by TLC. The reaction solution was filtered, washed with dichloromethane, water. Then the mixture was washed with sodium bicarbonate solution. The organic phase was dried with anhydrous sodium sulfate, filtered and evaporated to afford pure target compounds **D6**, **D32** and **D40**, respectively.

2-[5-(3-Aminobenzylidene)-4-thiazolinone-2-imino]-4-methyl Thiazole-5-carboxylic acid ethyl ester (D6). Yellow solid. m.p. 202–203 °C, yield 69.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.29 (t, J = 6.8 Hz, 3H, CH_3), 2.67 (s, 3H, CH_3), 4.26 (q, 2H, J = 6.8 Hz, OCH_2), 6.70 (d, J = 7.6 Hz, 1H, C_6H_4 2-H), 6.79 (s, 1H, C_6H_4 4-H), 6.81 (s, 1H, C_6H_4 6-H), 7.18–7.22 (m, 1H, C_6H_4 5-H), 7.82 (s, 1H, =CH); Anal. Calcd. for $C_{17}H_{14}N_4O_5S_2$: C, 48.80; H, 3.37; N, 13.39; found C, 48.77; H, 3.39; N, 13.35.

2-[2-(3-Aminobenzylidene)-4-thiazolinone-2-imino] Thiazole-4-ethyl acetate (D32). Yellow solid, m.p. 144–146 °C, yield 51.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.21 (t, J = 7.1 Hz, 3H, CH_3), 3.82 (s, 2H, $COCH_2$), 4.15 (q, J = 7.1 Hz, 2H, OCH_2), 5.41 (s, 2H, NH_2), 6.69 (d, J = 7.5 Hz, 1H, C_6H_4 4-H), 6.78–6.81 (m, 2H, C_6H_4 2,6-H), 7.18 (t, J = 7.5 Hz, 1H, C_6H_4 5-H), 7.25 (s, 1H, thiazole 5-H), 7.51 (s, 1H, =CH), 12.69 (s, 1H, CONH); Anal. Calcd. for $C_{17}H_{16}N_4O_3S_2$: C, 52.56; H, 4.15; N, 14.42; found C, 52.56; H, 4.15; N, 14.42.

2-((5-Acetyl-4-methylthiazol-2-yl)imino)-5-(3-nitrobenzylidene) thiazolidin-4-one (D40). Yellow solid, m.p. 225–227 °C, yield 24%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.50 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 6.79–7.19 (m, 4H, C_6H_4), 7.55 (s, 1H, =CH), 10.18 (s, 1H, CONH); Anal. Calcd. for $C_{16}H_{14}N_4O_2S_2$: C, 53.62; H, 3.94; N, 15.63; found C, 53.64; H, 3.96; N, 15.58.

*4.1.4. Procedure for synthesis of 4-thiazolinone analogue **S1***

Ethyl 2-amino-4-methyloxazole-5-carboxylate (**A19**, 50 mmol)

and potassium carbonate (100 mmol) were dissolved in dichloromethane (100 mL), chloroacetyl chloride (8.0 mL) was added dropwise under ice bath, and reacted at room temperature monitored by TLC. When the reaction is completed, the reaction solution is poured into water, and washed with sodium bicarbonate solution, saturated sodium chloride solution and water successively, dry the organic phase with anhydrous sodium sulfate, remove the solvent under reduced pressure to get the crude product, and the residue was purified by silica-gel column chromatography to give the intermediate **B19**. Yield 30.5%, mp 173–174 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.39 (t, J = 7.0 Hz, 3H, CH_3), 2.49 (s, 3H, oxazole-CH_3), 4.34 (s, 2H, CH_2Cl), 4.38 (q, J = 7.0 Hz, 2H, OCH_2). Similar to the method of 4.1.1, the intermediate **B19** (10 mmol) and KSCN (15 mmol) were refluxed for 6 h to obtain yellow solid **S1**. Yield 62.1%, m.p. 213–214 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.29 (t, J = 7.1 Hz, 3H, CH_3), 2.38 (s, 3H, oxazole-CH_3), 4.06 (s, 2H, SCH 2), 4.27 (q, J = 7.1 Hz, 2H, OCH_2), 12.44 (s, 1H, NH); Anal. Calcd. for $C_{10}H_{11}N_3O_4S$: C, 44.60; H, 4.12; N, 15.61; found C, 44.58; H, 4.16; N, 15.57.

4.1.5. Procedure for synthesis of 4-thiazolinone analogue ***S2***

Similar to the method of 4.1.1, the intermediate ethyl 2-(2-chloroacetamido)-4-methylthiazole-5- carboxylate **B1** (5 mmol) and KSeCN (5 mmol) were refluxed for 6 h to obtain yellow solid **S2**. Yield 78.9%, m.p. 254–255 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (t, J = 6.9 Hz, 3H, CH_3), 2.52 (s, 3H, CH_3), 3.94 (s, 2H, CH_2), 4.23 (q, J = 6.9 Hz, 2H, OCH_2), 12.55 (s, 1H, NH); Anal. Calcd. for $C_{10}H_{11}N_3O_3SSe$: C, 36.15; H, 3.34; N, 12.65; found C, 36.18; H, 3.36; N, 12.61.

4.1.6. Synthesis of 4-thiazolinone ring-opening analogue ***S3***

Acetoferulic acid **E3** (2 mmol) was suspended in toluene (2 mL), thionyl chloride (0.5 mL), 1 drop of *N*, *N*-dimethylformamide were added dropwise in turn. The mixture reacted at 60 °C for 5 h. Then remove the solvent and remaining thionyl chloride to obtain crude acetylferulyl chloride **F3**. Potassium thiocyanate (2.5 mmol) was dissolved in 5 mL acetonitrile, and 5 mL of the acetonitrile solution of the **F3** was added dropwise to it. After the addition was completed, the reaction was carried out at room temperature for 5 h, and then filtered to obtain acetyl ferulyl isocyanate (**G3**) acetonitrile solution. Ethyl 2-amino-4-methyl- thiazole-5-carboxylate (1.5 mmol, **A1**) was added to this solution in two batches, heated to 75 °C and reacted for 4 h, cooled to room temperature, sealed and refrigerated, filtered, washed with ethanol, and dried to obtain target compounds **S3**. m.p. 237–239 °C, yield 44.2%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.29 (t, J = 6.8 Hz, 3H, CH_3), 2.56 (s, 3H, thiazole-CH_3), 4.24 (q, J = 6.8 Hz, 2H, OCH_2), 6.99 (d, J = 15.8 Hz, 1H, =CH), 7.46–7.49 (m, 3H, C_6H_5), 7.60–7.68 (m, 2H, C_6H_5), 7.79 (d, J = 15.8 Hz, 1H, =CH), 12.24 (s, 1H, NH), 14.17 (s, 1H, NH); Anal. Calcd. for $C_{17}H_{17}N_3O_3S_2$: C, 54.38; H, 4.56; N, 11.19; found C, 36.13; H, 3.38; N, 12.64.

4.1.7. Synthesis of 4-thiazolinone ring-opening analogue ***S5***

The raw material **S4** (ethyl 2-(3-(3-(4-acetoxy-3-methoxyphenyl)acryloyl) thioureido)-4-methyl- thiazole-5-carboxylate) was synthesized according to the 4.1.6 method. Intermediate **S4** was dissolved in 3 mL tetrahydrofuran, and 2 mL 0.6 mol/L lithium hydroxide solution was added dropwise. The mixture reacted at room temperature for 5 h, and the pH was adjusted to 2 to 3 with dilute hydrochloric acid. The precipitate was filtered and washed with water to obtain a light yellow powder **S5**. Yield 75.0%, m.p. 230–231 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.32 (t, J = 6.6 Hz, 3H, CH_3), 2.59 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 4.29 (q, J = 6.6 Hz, 2H, OCH_2), 6.86 (d, J = 15.2 Hz, 1H, CH), 6.89 (d, J = 7.2 Hz, 1H, C_6H_3), 7.17 (d, 2 = 7.2 Hz, 1H, C_6H_3), 7.24 (s, 1H, C_6H_3), 7.76 (d, J = 15.2 Hz, 1H, CH), 9.87 (s, 1H, OH), 12.13 (s, 1H, NH), 14.33 (s, 1H, NH); Anal. Calcd. for $C_{18}H_{19}N_3O_5S_2$: C, 51.29; H, 4.54; N, 9.97; found C, 51.25; H, 4.60; N, 9.95.

4.2. Determination of NA inhibitory activity

All the target compounds were tested for their NA inhibitory activity *in vitro*. NA inhibition assays were performed using NA from influenza A/PR/8/34 (H1N1), A/Minfang/151/2000 (H3N2) and B/Si chuan/01/96 virus. Under the hydrolysis of NA, the NA substrate 2′-(4-methylumbellifer-yl)-α-D-acetyl neuraminic acid (MUNANA) generates a fluorescent product that could be quantified. The intensity of fluorescence can reflect the activity of NA sensitively [48].

Inhibition of influenza virus NA activity was determined by a standard fluorimetric method [49,50] using MUNANA as substrate, in 96-well microplates. The reaction mixture containing the compounds and NA enzyme in MES buffer (32.5 mM MES, 4 mM $CaCl_2$, pH 6.5) was incubated at 37 °C for 60 min. After incubation, the reaction was terminated by adding NaOH (34 mM). Fluorescence intensity (M) was quantified with excitation wavelength at 360 nm and emission wavelength at 450 nm. Percentage inhibition was calculated relative to a blank reaction mixture (solvent control) containing virus NA and solvent (Inhibition rate = [1- ($M_{compound}$/$M_{control}$)] × 100%). The 50% inhibitory concentration (IC_{50}) was defined as the concentration of NA inhibitor necessary to reduce NA activity by 50% relative to a blank reaction mixture. IC_{50} values displayed represent the mean of three individual determinations each performed in triplicate assays. Zanamivir (Sigma) was used as the reference compound.

4.3. Cytotoxicity test

Madin-Darby canine kidney (MDCK) cells grown in 96-well plates until reaching 100% confluence. Cells were treated with the test samples at concentrations ranging from 0.1 to 100 μg/mL at 37 °C and 5% CO_2 for 48 h. Blank medium was added to the control wells. Cell viability was determined using the crystal violet staining method. The crystal violet staining solution was added to each well and the plates were incubated for 30 min. The excess staining solution was wash off and ethanol-acetic acid eluent was used to dissolve the product. After being homogenized, the absorbance of plates was read at a wavelength of 570 nm using spectrophotometer. The maximal non-toxic concentration (TC_0) and the median toxic concentration (TC_{50}) values were calculated for all compounds.

4.4. Cytopathic effect (CPE) reduction assay

The antiviral activities of the test samples were measured using the CPE reduction assay. MDCK cells were seeded into 96-well plates and incubated until reaching monolayer. The 6 serial dilutions of each test sample were added to the cells 1 h after the adsorption of 100$TCID_{50}$ A/HebeiXinhua/SWL1106/2017 (Oseltamivir & amantadine-resistant H1N1). The TC_0 was used as the highest concentration. The control drugs (Oseltamivir, Amantadine and Zanamivir), negative control wells and viral wells were set as comparison on each plate. The plates were incubated at 34 °C for 48 h. The CPE was then assessed using the crystal violet staining method (as above). Resulting data were used to calculate the half inhibitory concentration (IC_{50}). The experiments were repeated at least three times.

4.5. Docking procedure

The crystal structure data of H1N1 neuraminidase-Zanamivir

complex (PDB Code: 3TI5) is downloaded from RSCB Protein Data Bank. The protein was prepared in the standard manner using AutoDock Tools (version 1.5.6) [51]. And the conformation of the compounds was optimized using Corina Classic online demo (https://www.mn-am.com/online_demos/corina_demo_interactive). Then AutoDock Vina [52] was used for docking **D41** to the X-ray structures. A grid of 19.4 Å × 17.5 Å × 18.7 Å was constructed by GetBox Plugin (https://github.com/MengwuXiao/GetBox-PyMOL-Plugin), centered on the active site [29.7, 12.9, −21.5]. Default settings were applied for molecular docking. We verified that the docking procedures are reliable using re-docking method (more details in supporting information). The results were visualized using PyMOL (https://pymol.org). The molecular interactions (hydrogen bonds and hydrophobic interactions between the target proteins and compounds) were studied using LigPlot + version 2.2 [39].

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.ejmech.2021.113161. These data include MOL files and InChiKeys of the most important compounds described in this article.

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