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Design, Synthesis, and Evaluation of 3-((4-(*t*-Butyl)-2-(2-benzylidenehydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-ones as Neuraminidase Inhibitors

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A series of novel 3-((4-(*t*-butyl)-2-(2-benzylidenehydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-ones (**7a**—**7z**) were designed, synthesized and evaluated for their ability of inhibiting neuraminidase (NA) of influenza H1N1 virus. Some compounds displayed moderate influenza NA inhibitory activity. Compound **7l** with the scaffold of 2-(2-(2-methoxybenzylidene)hydrazinyl)thiazole was the best one, exhibiting moderate NA inhibitory activity with IC_{50} of 44.66 μmol/L. Structure-activity relationship showed that compounds with methoxy or hydroxy groups at the *ortho* position, fluorine and nitro groups at the *meta* position and chlorine and bromine groups at the *para* position of phenyl ring were more active. Docking study indicated that compound **7l** has important interactions with some key residues (including Asp151, Glu119, Arg292, Tyr406, and Asn347) and binds to 430-cavity adjacent to NA active site.

Keywords neuraminidase inhibitor, 3-((4-(*t*-butyl)-2-(2-benzylidenehydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-ones, 2-(2-hydrazinyl)thiazole scaffold, synthesis, docking

Introduction

The influenza virus, a viral respiratory pathogen responsible for frequent seasonal epidemics, can cause grave threat to human health and economic problems.[1] Despite advances in the understanding of molecular and cellular aspects of influenza, the disease remains the major cause of mortality and morbidity among patients with respiratory diseases.[2] The viral functions of influenza contain two major glycoproteins, hemagglutinin and neuraminidase (NA),[3] and NA plays at least two critical roles in the virus life cycle, including the facilitation of virion progeny release and general mobility of the virus in the respiratory tract.[4] Hence, NA has been considered as an important target for designing agents against influenza viruses.

Currently, two major classes of anti-influenza medications have been approved by the Food and Drug Administration (FDA) for the treatment of influenza (Figure 1): M2 protein ion channel inhibitors (amantadine and rimantadine),[5] and NA inhibitors (Oseltamivir, Zanamivir and Peramivir).[6-8] Zanamivir and Peramivir are rarely used because of their low bioavailability and rapid elimination analysis. Oseltamivir is the only orally available drug currently in use.[9] However, the emergence of viruses resistance during the treatment of influenza infections has been widely reported in recent years.[10-13] With the emergence of drug-resistant influenza strains[14-16] and in view of a highly pathogenic flu pandemic, it is urgent to develop new antiviral strategies to fight against potential human influenza pandemic.

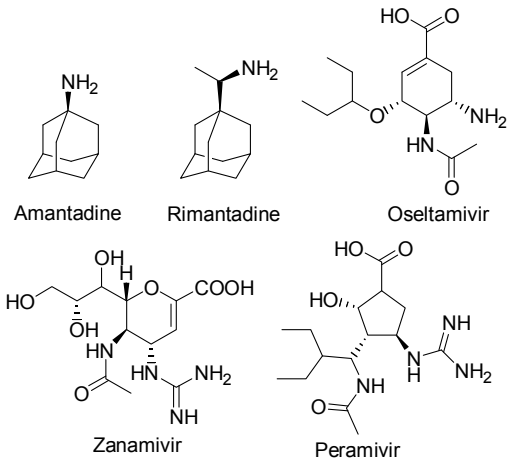

Figure 1 Chemical structures of clinically used anti-influenza drugs approved by the FDA.

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2-Oxoquinoline is an important heterocyclic compound, which shows a wide variety of pharmacological properties. Many 2-oxoquinoline derivatives have been reported to have antimicrobial,[17] anti-angiogenic,[18] anticancer,[19] antioxidant,[20] antimalarial,[21] and anti-inflammatory properties.[22,23] The thiazole nucleus is a multifunctional group and used to modify pharmacophores and improve their biological and pharmacological activities, such as anticancer,[24-26] antimalarial,[27] antimicrobial,[28] and anti-inflammatory activities.[29] Recently, thiazole derivatives were reported as NA inhibitors.[30] In our previous study,[31] 2-(2-hydrazinyl)-thiazole derivatives were found to exhibit inhibitory effects against the influenza A NA *in vitro*. Hence, it is considered that simultaneous administration of 2-oxoquinoline and 2-(2-hydrazinyl)thiazole structures in a single molecule, would lead to a rapid onset of NA inhibitory effect. So a series of novel 3-((4-(*t*-butyl)-2-(2-benzylidenehydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-ones **7a**—**7z** (Figure 2) were designed as NA inhibitors. The overall strategies for the synthesis of **7a**—**7z** were outlined in Scheme 1. The NA inhibitory activity was evaluated *in vitro*.

Experimental

All chemicals and solvents were analytical reagents and used directly without further depuration. Melting points were determined using an X-4 electrothermal digital melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) was recorded on a Varian INOVA-400 spectrometer in DMSO-d_6 or $CDCl_3$, whereas tetramethylsilane (TMS) (δ 0) was used as an internal standard for spectra recorded. Elemental analysis was carried out on an Elementar Vario EL CHNS Elemental Analyzer. Analyses for C, H and N were within 0.4% of the theoretical values.

Synthesis of 2-chloro-3-formylquinoline (1) and 2-oxo-3-formyquinoline (2)

Compounds **1** and **2** were synthesized according to the literature.[32] The Vilsmeier cyclisation of acetanilide was carried out by adding $POCl_3$ (17 mL) to the substrate in DMF (5 mL) at 0—5 ℃, followed by heating at 90 ℃ for 16 h. After the reaction, the mixture was poured into ice water and then filtered to offer pale powder of compound **1**. Yellow solid, yield 80.3%, m.p. 149—152 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.39 (s, 1H, O=CH), 8.99 (s, 1H, C=CH), 8.29 (d, J=8.1 Hz, 1H), 8.07—7.96 (m, 2H), 7.77 (t, J=7.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 189.64, 149.26, 148.82, 141.64, 134.15, 130.49, 128.53, 128.05, 126.61. Anal. calcd for $C_{10}H_6ClNO$: C 62.68, H 3.16, N 7.31; found C 62.66, H 3.18, N 7.29.

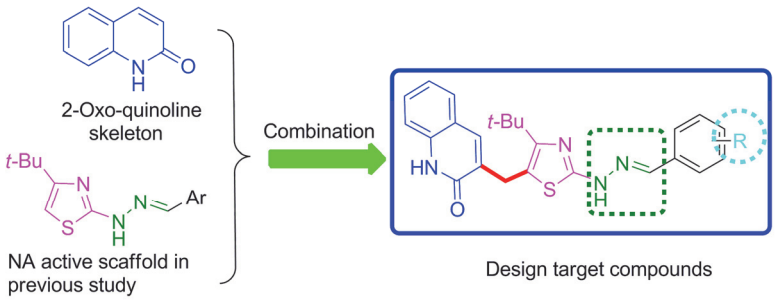
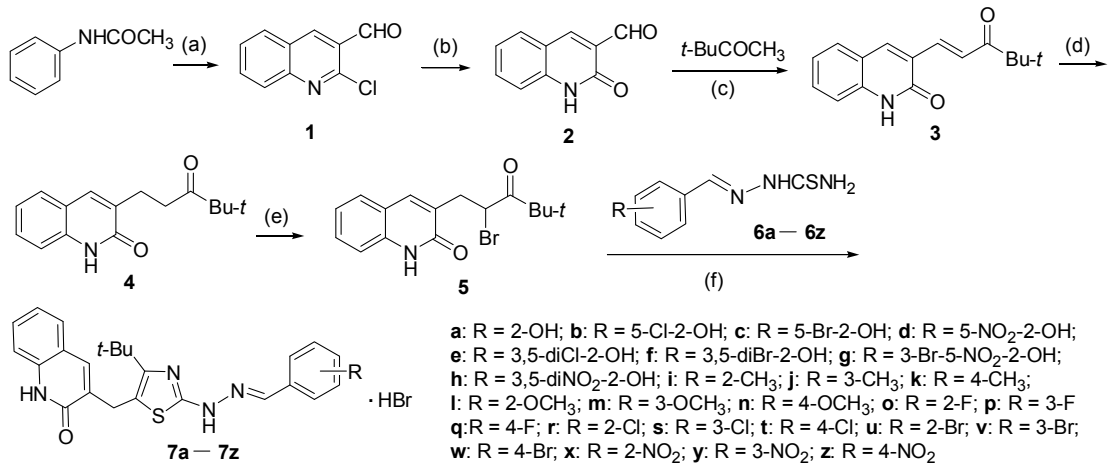


Figure 2 Design of novel 3-((4-(*t*-butyl)-2-(2-benzylidenehydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-ones as NA inhibitors.

Scheme 1 Synthesis of 3-((4-(*t*-butyl)-2-(2-benzyli-denehydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one



Reagents and conditions: (a) DMF, $POCl_3$, 90 ℃, 16 h; (b) 70% AcOH, 95 ℃, 6 h; (c) NaOH, EtOH, reflux 2 h; (d) Raney Ni, EtOH, 90 ℃, 8 h; (e) $CuBr_2$, $CHCl_3$-EtOAc, 80 ℃, 4 h; (f) EtOH, reflux

Then compound **1** (10 mmol) was treated with 70% acetic acid aqueous solution (200 mL) at 90 ℃ for 6 h and the solution was cooled down to room temperature to offer needle crystals of compound **2**. Yellow solid, yield 81.0%, m.p. 304－306 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.19 (s, 1H, NH), 10.25 (s, 1H, O＝CH), 8.49 (s, 1H, C＝CH), 7.91 (d, J＝7.8 Hz, 1H), 7.66 (t, J＝7.8 Hz, 1H), 7.37 (d, J＝8.3 Hz, 1H), 7.25 (t, J＝7.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 190.05, 161.76, 142.69, 141.43, 133.97, 131.21, 125.87, 122.94, 118.42, 115.69. Anal. calcd for $C_{10}H_7NO$: C 69.36, H 4.07, N 8.09; found C 69.34, H 4.11, N 8.07.

Synthesis of (*E*)-3-(4,4-dimethyl-3-oxopent-1-en-1-yl)quinolin-2(1*H*)-one (3)

The mixture of compound **2** (1 mmol), ethanol (20 mL), pinacolone (1.2 mmol) and 0.5 g NaOH was blended together and reacted at 80 ℃ for 2 h. After cooling down to room temperature, powders or crystals of compound **3** were obtained. Orange solid, yield 87.2%, m.p. 209－211 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.07－7.97 (m, 2H), 7.71 (d, J＝15.5 Hz, 1H), 7.61 (d, J＝7.7 Hz, 1H), 7.53 (t, J＝8.1 Hz, 1H), 7.37 (d, J＝8.1 Hz, 1H), 7.23 (d, J＝7.4 Hz, 1H), 1.27 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 200.36, 158.61, 136.74, 133.09, 126.45, 123.34, 121.27, 119.51, 117.78, 115.10, 111.37, 38.36, 21.40. Anal. calcd for $C_{16}H_{17}NO_2$: C 75.27, H 6.71, N 5.49; found C 75.23, H 6.72, N 5.47.

Synthesis of 3-(4,4-dimethyl-3-oxopentyl)quinolin-2 (1*H*)-one (4)

Compound **3** (0.1 mol), Raney Ni 1.28 g and EtOH (120 mL) were added into autoclave and the mixture was stirred at 80 ℃ under 2.5 MPa H_2 for 3 h. After the reaction was completed, the mixture was reduced to normal pressure, and cooled down to room temperature to offer needle crystals of compound **4**. Yellow solid, yield 90.0%, m.p. 183－185 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.53 (s, 1H, NH), 7.74 (s, 1H), 7.54 (d, J＝7.2 Hz, 1H), 7.46 (t, J＝7.6 Hz, 1H), 7.34 (d, J＝8.1 Hz, 1H), 7.20 (t, J＝7.5 Hz, 1H), 2.95 (s, 4H, 2×CH_2), 1.13 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 210.47, 159.31, 133.25, 132.65, 127.84, 124.66, 122.36, 117.64, 115.32, 110.64, 39.26, 30.33, 21.45, 20.86. Anal. calcd for $C_{16}H_{19}NO_2$: C 74.68, H 7.44, N 5.44; found C 74.65, H 7.42, N 5.46.

Synthesis of 3-(2-bromo-4,4-dimethyl-3-oxopentyl)-quinolin-2(1*H*)-one (5)

To a solution of compound **4** (0.02 mol) in a mixture of $CHCl_3$ (15 mL) and EtOAc (15 mL), $CuBr_2$ (0.04 mol) was added in portions during a period of 0.5 h at 80 ℃, the reaction mixture was allowed to reflux for 4 h. The solution was washed with HCl (aq) until the blue faded. The aqueous phase was then made neutral with water, and the product was extracted with EtOAc (10 mL×3). The combined organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to offer compound **5**. White solid, yield 69.1%, m.p. 190－193 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.89 (s, 1H), 7.86 (d, J＝8.5 Hz, 1H), 7.69 (d, J＝7.1 Hz, 1H), 7.62－7.57 (m, 1H), 7.40－7.36 (m, 1H), 5.57 (dd, J＝9.3, 6.8 Hz, 1H), 3.58－3.52 (m, 2H, CH_2), 1.31 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 205.85, 161.56, 141.98, 128.14, 124.41, 122.68, 122.43, 120.67, 119.45, 115.39, 39.05, 25.83, 21.73, 21.16. Anal. calcd for $C_{16}H_{18}BrNO_2$: C 57.16, H 5.40, N 4.17; found C 57.12, H 5.42, N 4.15.

General procedure for the synthesis of (*E*)-2-benzylidenehydrazinecarbothioamide (6a－6z)

Compounds **6a**－6**z** were synthesized according to the literature.[33] Aldehyde (0.01 mmol) was added to a stirring mixture of thiosemicarbazide (2 mmol) and EtOH (20 mL). The resulting mixture was heated at 80 ℃ and stirred for 2 h, and the reaction mixture was cooled down to room temperature to offer needle crystals of compound **6**.

General procedure for the synthesis of 3-((4-(*t*-butyl)-2-(2-benzylidenehydrazinyl)thiazol-5-yl)methyl)-quinolin-2(1*H*)-ones (7a－7z)

Compound **5** (1 mmol) was added into a refluxing solution of an appropriate compound **6** (1.2 mmol) in EtOH (15 mL). The mixture was kept at this temperature for more than 4 h. After completion of the reaction as indicated by TLC, the mixture was cooled to room temperature, then filtered to get the crude products. The crude products were recrystallized from hot DMSO and 95% alcohol, then the mixture was cooled to offer compounds **7a**－**7z**.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(2-hydroxybenzylidene)-hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7a) Yellow solid, yield 64.6%, m.p. 257－259 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.93 (s, 1H, NH), 10.25 (br, 1H, NH), 8.40 (s, 1H, N＝CH), 7.67 (d, J＝8.7 Hz, 2H), 7.55 (d, J＝7.7 Hz, 1H), 7.48 (t, J＝7.3 Hz, 1H), 7.33 (d, J＝8.2 Hz, 1H), 7.21 (t, J＝7.0 Hz, 1H), 7.16 (t, J＝7.6 Hz, 1H), 6.88 (d, J＝8.2 Hz, 1H), 6.84 (t, J＝7.5 Hz, 1H), 4.02 (s, 2H, CH_2), 1.35 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.97, 165.17, 163.63, 147.53, 143.76, 141.50, 140.29, 138.60, 137.06, 134.38, 132.27, 129.53, 127.73, 124.82, 124.58, 122.39, 121.70, 120.42, 66.13, 52.99, 35.47, 30.52, 21.52. Anal. calcd for $C_{24}H_{24}N_4O_2S$: C 66.64, H 5.59, N 12.95; found C 66.68, H 5.57, N 12.92.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(5-chloro-2-hydroxybenzylidene)hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7b) Beige solid, yield 50.0%, m.p. 275－278 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.93 (s, 1H, NH), 10.41 (br, 1H, NH), 8.21 (s, 1H, N＝CH), 7.67 (d, J＝7.2 Hz, 1H), 7.57 (s, 1H), 7.52 (s, 1H), 7.47 (t, J＝7.2 Hz, 1H), 7.31 (d, J＝8.4 Hz, 1H), 7.21 (dd, J＝2.8, 2.4 Hz, 1H), 7.15 (t, J＝7.2 Hz, 1H), 6.88 (d, J＝8.8 Hz, 1H), 3.99 (s, 2H, CH_2), 1.32 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.49, 165.87, 162.11, 143.86, 143.06, 141.63, 140.31, 137.98, 137.12, 134.96, 132.79, 132.21, 129.89, 128.36, 127.76, 127.03, 124.31,

123.67, 120.04, 40.64, 35.53, 32.27. Anal. calcd for $C_{24}H_{23}ClN_4O_2S$: C 61.73, H 4.96, N 12.00; found C 61.68, H 4.93, N 12.02.

(*E*)-3-((2-(2-(5-Bromo-2-hydroxybenzylidene)-hydrazinyl)-4-(*t*-butyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7c) Yellow solid, yield 67.7%, m.p. 259—261 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.91 (s, 1H, NH), 10.42 (br, 1H, NH), 8.19 (s, 1H, N=CH), 7.67 (d, J=10.8 Hz, 2H), 7.56 (s, 1H), 7.56 (t, J=7.6 Hz, 1H), 7.33—7.30 (m, 2H), 7.15 (t, J=7.2 Hz, 1H), 6.83 (d, J=8.4 Hz, 1H), 3.99 (s, 2H, CH_2), 1.31 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.44, 166.03, 164.59, 143.81, 141.49, 137.71, 137.10, 135.48, 134.58, 134.32, 132.41, 132.26, 129.42, 127.69, 126.90, 124.55, 124.25, 120.39, 119.94, 35.52, 31.05, 23.72. Anal. calcd for $C_{24}H_{23}BrN_4O_2S$: C 56.36, H 4.53, N 1095; found C 56.17, H 4.51, N 10.93.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(2-hydroxy-5-nitrobenzylidene)hydrazinyl)thiazol-5-yl)methyl) quinolin-2(1*H*)-one (7d) Orange solid, yield 72.2%, m.p. 261—263 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.92 (s, 1H, NH), 11.70 (s, 1H, OH), 8.42 (d, J=2.9 Hz, 1H), 8.29 (s, 1H, N=CH), 8.07 (dd, J=9.1, 2.9 Hz, 1H), 7.67 (d, J=7.5 Hz, 1H), 7.59 (s, 1H), 7.47 (t, J=7.7 Hz, 1H), 7.32 (d, J=8.2 Hz, 1H), 7.15 (t, J=8.0 Hz, 1H), 7.06 (d, J=9.1 Hz, 1H), 4.01 (s, 2H, CH_2), 1.33 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.68, 166.58, 145.19, 143.00, 141.36, 137.85, 134.88, 132.75, 130.88, 127.00, 126.57, 126.26, 124.33, 121.84, 120.01, 40.80, 35.45, 32.29. Anal. calcd for $C_{24}H_{23}N_5O_4S$: C 60.36, H 4.85, N 14.67; found C 60.19, H 4.83, N 14.65.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(3,5-dichloro-2-hydroxybenzylidene)hydrazinyl)thiazol-5-yl)methyl) quinolin-2(1*H*)-one (7e) Red solid, yield 62.0%, m.p. 245—247 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.07 (s, 1H, NH), 8.31 (s, 1H, N=CH), 7.66 (s, 1H), 7.60—7.55 (m, 2H), 7.41 (d, J=2.4 Hz, 1H), 7.34 (d, J=8.5 Hz, 1H), 7.29 (d, J=7.6 Hz, 1H), 7.20 (d, J=2.4 Hz, 1H), 4.19 (s, 2H, CH_2), 1.58 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 161.81, 151.19, 138.11, 136.34, 132.90, 130.00, 129.13, 127.90, 123.60, 122.12, 121.82, 119.46, 115.13, 35.86, 30.28, 27.58. Anal. calcd for $C_{24}H_{22}Cl_2N_4O_2S$: C 57.49, H 4.42, N 11.17; found C 57.30, H 4.39, N 11.15.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(3,5-dibromo-2-hydroxybenzylidene)hydrazinyl)thiazol-5-yl)methyl) quinolin-2(1*H*)-one (7f) Yellow solid, yield 46.2%, m.p. 258—260 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.92 (s, 1H, NH), 8.25 (s, 1H, N=CH), 7.71 (s, 1H), 7.70 (d, J=2.4 Hz, 1H), 7.65 (d, J=8.4 Hz, 1H), 7.60 (s, 1H), 7.46 (t, J=7.7 Hz, 1H), 7.32 (d, J=8.2 Hz, 1H), 7.15 (t, J=7.5 Hz, 1H), 4.05 (s, 2H, CH_2), 1.33 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 161.81, 152.72, 138.12, 136.34, 134.47, 132.84, 130.81, 130.01, 127.91, 122.77, 122.13, 119.46, 118.60, 115.14, 111.45, 111.13, 110.91, 35.82, 30.00, 27.46. Anal. calcd for $C_{24}H_{22}Br_2N_4O_2S$: C 48.83, H 3.76, N 9.49; found C 48.63, H 3.74, N 9.46.

(*E*)-3-((2-(2-(3-Bromo-2-hydroxy-5-nitrobenzylidene)hydrazinyl)-4-(*t*-butyl)thiazol-5-yl)methyl)-quinolin-2(1*H*)-one (7g) Red solid, yield 61.5%, m.p. 267—270 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.91 (s, 1H, NH), 8.48 (d, J=2.5 Hz, 1H), 8.34 (d, J=2.7 Hz, 1H), 7.69 (d, J=7.9 Hz, 1H), 7.62 (s, 1H), 7.47 (t, J=7.6 Hz, 1H), 7.32 (d, J=8.2 Hz, 1H), 7.15 (t, J=7.6 Hz, 1H), 3.98 (s, 2H, CH_2), 1.34 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 161.80, 140.56, 138.16, 136.39, 130.03, 127.92, 127.87, 122.13, 119.55, 115.14, 56.34, 30.30, 18.86. Anal. calcd for $C_{24}H_{22}BrN_5O_4S$: C 51.80, H 3.99, N 12.59; found C 51.61, H 3.96, N 12.57.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(2-hydroxy-3,5-dinitrobenzylidene)hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7h) Red solid, yield 43.3%, m.p. 259—262 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.91 (s, 1H, NH), 11.64 (s, 1H, OH), 9.05 (s, 1H), 8.68—8.62 (m, 1H), 8.46 (d, J=9.1 Hz, 2H), 7.67 (d, J=7.7 Hz, 1H), 7.62 (s, 1H, C=CH), 7.47 (t, J=7.7 Hz, 1H), 7.32 (d, J=8.2 Hz, 1H), 7.15 (t, J=7.5 Hz, 1H), 4.00 (s, 2H, CH_2), 1.34 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 178.43, 161.75, 157.58, 157.35, 138.09, 137.31, 137.24, 137.13, 136.40, 132.75, 129.99, 127.87, 126.09, 125.83, 122.28, 122.10, 121.35, 119.41, 115.10, 35.79, 30.34, 27.53. Anal. calcd for $C_{24}H_{22}N_6O_6S$: C 55.17, H 4.24, N 16.08; found C 55.21, H 4.26, N 16.04.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(2-methylbenzylidene)-hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7i) Beige solid, yield 48.0%, m.p. 225—228 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.02 (s, 1H, NH), 12.91 (s, 1H, OH), 11.60 (s, 1H, NH), 8.41 (s, 1H, N=CH), 7.68—7.63 (m, 2H), 7.60 (d, J=8.6 Hz, 1H), 7.56 (d, J=7.3 Hz, 1H), 7.42 (d, J=8.1 Hz, 1H), 7.30 (d, J=7.6 Hz, 2H), 7.16 (dd, J=15.1, 7.5 Hz, 2H), 4.19 (s, 2H, CH_2), 2.43 (s, 3H, CH_3), 1.56 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.54, 162.89, 149.55, 144.05, 138.33, 138.05, 137.69, 131.19, 131.01, 130.87, 130.38, 129.91, 127.82, 127.57, 126.24, 123.20, 119.56, 116.43, 115.55, 35.20, 30.28, 27.71, 19.91. Anal. calcd for $C_{25}H_{26}N_4OS$: C 69.74, H 6.09, N 13.01; found C 69.56, H 6.07, N 12.97.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(3-methylbenzylidene)-hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7j) Yellow solid, yield 42.2%, m.p. 221—224 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.03 (s, 1H, NH), 12.76 (s, 1H, OH), 10.98 (s, 1H, NH), 8.16 (s, 1H, N=CH), 7.63—7.53 (m, 3H), 7.50 (d, J=7.9 Hz, 2H), 7.35 (d, J=8.2 Hz, 1H), 7.29 (d, J=7.6 Hz, 1H), 7.16 (d, J=7.8 Hz, 2H), 4.17 (s, 2H, CH_2), 2.36 (s, 3H, CH_3), 1.55 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.25, 163.13, 150.67, 143.77, 141.96, 138.44, 137.83, 130.88, 129.84, 129.58, 129.52, 127.79, 127.71, 123.19, 119.56, 116.49, 115.70, 35.33, 30.24, 27.75, 21.58. Anal. calcd for $C_{25}H_{26}N_4OS$: C 69.74, H 6.09, N 13.01; found C 69.54, H 6.08, N 12.99.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(4-methylbenzylidene)-hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7k) Yellow solid, yield 38.3%, m.p. 176—178 ℃.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.04 (s, 1H, NH), 12.79 (s, 1H, OH), 11.58 (s, 1H, NH), 8.16 (s, 1H, N=CH), 7.65 (s, 1H), 7.63−7.56 (m, 2H), 7.40 (d, J=8.1 Hz, 1H), 7.33−7.27 (m, 2H), 7.19−7.14 (m, 2H), 6.96 (dd, J=8.6, 2.2 Hz, 1H), 4.18 (s, 2H, CH_2), 3.78 (s, 3H, CH_3), 1.56 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.54, 162.89, 149.55, 144.05, 138.33, 138.05, 137.69, 131.19, 131.01, 130.87, 130.46, 130.38, 127.82, 127.57, 126.24, 123.20, 119.56, 116.43, 115.55, 35.39, 30.28, 27.64, 19.97. Anal. calcd for $C_{25}H_{26}N_4OS$: C 69.74, H 6.09, N 13.01; found C 69.56, H 6.06, N 12.97.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(2-methoxybenzylidene)-hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7l) Orange solid, yield 49.9%, m.p. 239−241 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.86 (s, 1H, NH), 11.67 (s, 1H, NH), 8.57 (s, 1H, N=CH), 7.72 (dd, J=7.7, 1.4 Hz, 1H), 7.65 (s, 1H), 7.58 (dd, J=14.2, 7.5 Hz, 2H), 7.41 (d, J=8.2 Hz, 1H), 7.36 (t, J=8.7 Hz, 1H), 7.30 (d, J=7.5 Hz, 1H), 6.90−6.82 (m, 2H), 4.18 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 1.57 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.40, 158.25, 153.60, 141.77, 138.89, 133.50, 132.88, 127.81, 125.93, 124.95, 122.86, 121.46, 118.26, 115.81, 115.70, 114.64, 111.47, 110.74, 106.23, 50.56, 30.43, 25.36, 22.78. Anal. calcd for $C_{25}H_{26}N_4O_2S$: C 67.24, H 5.87, N 12.55; found C 67.05, H 5.85, N 12.53.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(3-methoxybenzylidene)-hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7m) Beige solid, yield 42.3%, m.p. 209−212 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.13 (s, 1H, NH), 12.81 (s, 1H, OH), 11.24 (s, 1H, NH), 8.16 (s, 1H, N=CH), 7.65 (s, 1H), 7.63−7.56 (m, 2H), 7.40 (d, J=8.1 Hz, 1H), 7.33−7.27 (m, 2H), 7.19−7.14 (m, 2H), 6.96 (dd, J=8.6, 2.2 Hz, 1H), 4.18 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 1.56 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 189.28, 174.53, 161.19, 161.10, 159.85, 138.90, 135.68, 135.31, 132.19, 130.41, 129.69, 127.36, 124.31, 122.86, 121.25, 119.40, 117.88, 115.50, 114.06, 55.60, 30.65. Anal. calcd for $C_{25}H_{26}N_4O_2S$: C 67.24, H 5.87, N 12.55; found C 67.36, H 5.89, N 12.52.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(4-methoxybenzylidene)-hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7n) Yellow solid, yield 52.0%, m.p. 178−181 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.98 (s, 1H, NH), 12.69 (s, 1H, OH), 11.60 (s, 1H, NH), 8.14 (s, 1H, N=CH), 7.65 (s, 1H), 7.63−7.56 (m, 2H), 7.54 (d, J=8.8 Hz, 2H), 7.42 (d, J=8.2 Hz, 1H), 7.31 (d, J=7.6 Hz, 1H), 6.86 (d, J=8.7 Hz, 2H), 4.18 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3), 1.55 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 173.35, 162.54, 161.22, 160.78, 138.84, 138.66, 135.84, 132.09, 130.95, 129.66, 127.50, 126.60, 123.65, 122.80, 119.42, 115.50, 114.85, 55.79, 38.51, 30.64, 27.48. Anal. calcd for $C_{25}H_{26}N_4O_2S$: C 67.24, H 5.87, N 12.55; found C 67.07, H 5.84, N 12.51.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(2-fluorobenzylidene)hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7o) White solid, yield 65.1%, m.p. 207−210.5 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.18 (s, 1H, NH), 13.05 (s, 1H, OH), 10.63 (s, 1H, NH), 8.43 (s, 1H, N=CH), 7.79 (t, J=7.6 Hz, 1H), 7.68−7.57 (m, 3H), 7.43−7.31 (m, 3H), 7.14−7.08 (m, 2H), 4.18 (s, 2H, CH_2), 1.55 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 189.20, 175.34, 162.87, 161.08, 153.49, 138.88, 135.53, 134.20, 132.17, 129.66, 128.25, 127.24, 125.34, 124.68, 122.75, 119.28, 116.62, 116.41, 115.44, 38.56, 30.59, 29.72. Anal. calcd for $C_{24}H_{23}FN_4O_2S$: C 66.34, H 5.34, N 12.89; found C 66.15, H 5.31, N 12.87.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(3-fluorobenzylidene)-hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7p) Yellow solid, yield 44.2%, m.p. 248−251 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.27 (s, 1H, NH), 12.91 (s, 1H, OH), 10.39 (s, 1H, NH), 8.17 (s, 1H, N=CH), 7.65−7.54 (m, 3H), 7.37−7.29 (m, 5H), 7.12 (d, J=8.5 Hz, 1H), 4.18 (s, 2H, CH_2), 1.56 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.78, 161.69, 161.37, 138.12, 136.71, 132.48, 131.19, 131.11, 130.02, 127.85, 123.11, 122.10, 119.37, 116.72, 116.63, 115.11, 112.88, 112.65, 35.76, 30.34, 27.25. Anal. calcd for $C_{24}H_{23}FN_4O_2S$: C 66.34, H 5.34, N 12.89; found C 66.15, H 5.31, N 12.86.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(4-fluorobenzylidene)-hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7q) Beige solid, yield 68.6%, m.p. 266−269 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.91 (s, 1H, NH), 7.97 (s, 1H, N=CH), 7.67−7.59 (m, 4H), 7.47 (t, J=7.6 Hz, 1H), 7.32 (d, J=8.0 Hz, 1H), 7.22 (t, J=8.8 Hz, 1H), 7.15 (t, J=7.2 Hz, 1H), 4.00 (s, 2H, CH_2), 1.32 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.14, 161.13, 159.98, 138.86, 135.64, 132.16, 131.43, 131.35, 130.57, 129.66, 127.34, 124.26, 122.77, 119.34, 116.54, 116.32, 115.47, 38.53, 30.46. Anal. calcd for $C_{24}H_{23}FN_4O_2S$: C 66.34, H 5.34, N 12.89; found C 66.14, H 5.32, N 12.87.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(2-chlorobenzylidene)-hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7r) Beige solid, yield 72.7%, m.p. 250−253 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.10 (s, 1H, NH), 11.37 (s, 1H, NH), 8.56 (s, 1H, N=CH), 7.81 (d, J=7.8 Hz, 1H), 7.66 (s, 1H), 7.58 (dd, J=16.4, 7.8 Hz, 2H), 7.42−7.36 (m, 2H), 7.34−7.28 (m, 2H), 7.18 (t, J=7.6 Hz, 1H), 4.18 (s, 2H, CH_2), 1.55 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 189.85, 175.78, 161.16, 156.87, 139.10, 135.58, 134.76, 133.51, 132.30, 130.97, 130.53, 129.79, 128.84, 128.13, 127.28, 124.98, 122.84, 119.37, 115.53, 38.66, 30.67. Anal. calcd for $C_{24}H_{23}ClN_4O_2S$: C 63.92, H 5.14, N 12.42; found C 63.73, H 5.12, N 12.40.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(3-chlorobenzylidene)-hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7s) Beige solid, yield 53.0%, m.p. 241−243 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.24 (s, 1H, NH), 12.85 (s, 1H, OH), 11.16 (s, 1H, NH), 8.14 (s, 1H, N=CH), 7.65 (s, 1H), 7.61 (d, J=4.8 Hz, 1H), 7.59−7.53 (m, 2H), 7.43 (d, J=7.7 Hz, 1H), 7.37 (d, J=7.8 Hz, 2H), 7.33−7.27 (m, 2H), 4.19 (s, 2H, CH_2), 1.57 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 189.58,

175.20, 161.16, 159.87, 139.02, 138.95, 136.06, 134.06, 132.23, 131.63, 131.18, 128.44, 127.35, 127.29, 124.71, 122.84, 119.40, 115.52, 38.61, 30.67. Anal. calcd for $C_{24}H_{23}ClN_4O_2S$: C 63.92, H 5.14, N 12.42; found C 63.75, H 5.11, N 12.41.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(4-chlorobenzylidene)-hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7t) Yellow solid, yield 62.6%, m.p. 254－257 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.91 (s, 1H, NH), 7.98 (s, 1H, N＝CH), 7.66 (d, J＝7.9 Hz, 1H), 7.61－7.58 (m, 3H), 7.49－7.43 (m, 3H), 7.32 (d, J＝8.3 Hz, 1H), 7.15 (t, J＝7.5 Hz, 1H), 4.01 (s, 2H, CH_2), 1.33 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.24, 165.85, 164.95, 143.81, 141.49, 137.71, 135.48, 134.82, 134.58, 134.32, 132.41, 129.42, 127.69, 126.90, 124.51, 124.25, 120.39, 119.94, 35.52, 31.15, 23.63. Anal. calcd for $C_{24}H_{23}ClN_4O_2S$: C 63.92, H 5.14, N 12.42; found C 63.72, H 5.11, N 12.39.

(*E*)-3-((2-(2-(2-Bromobenzylidene)hydrazinyl)-4-(*t*-butyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7u) Yellow solid, yield 48.1%, m.p. 168－171 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.16 (s, 1H, NH), 8.22 (s, 1H, N＝CH), 7.94 (dd, J＝7.9, 1.5 Hz, 1H), 7.55－7.50 (m, 3H), 7.47 (d, J＝7.3 Hz, 1H), 7.33 (d, J＝8.1 Hz, 1H), 7.25－7.13 (m, 3H), 4.19 (s, 2H, CH_2), 1.39 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 189.83, 175.78, 161.14, 159.16, 139.06, 138.97, 135.56, 133.75, 133.67, 132.41, 132.27, 129.75, 129.18, 128.60, 127.26, 125.13, 124.96, 122.81, 119.35, 115.52, 38.65, 30.67. Anal. calcd for $C_{24}H_{23}BrN_4OS$: C 58.18, H 4.68, N 11.31; found C 58.00, H 4.66, N 11.29.

(*E*)-3-((2-(2-(3-Bromobenzylidene)hydrazinyl)-4-(*t*-butyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7v) Yellow solid, yield 55.0%, m.p. 181－183 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.90 (s, 1H, NH), 7.95 (s, 1H, N＝CH), 7.75 (s, 1H), 7.66 (d, J＝7.4 Hz, 1H), 7.57 (d, J＝4.3 Hz, 2H), 7.51 (d, J＝7.1 Hz, 1H), 7.46 (t, J＝7.7 Hz, 1H), 7.36－7.31 (m, 2H), 7.14 (t, J＝7.5 Hz, 1H), 4.01 (s, 2H, CH_2), 1.32 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.58, 161.82, 138.13, 137.05, 136.53, 132.99, 132.09, 131.28, 130.03, 128.67, 127.90, 125.69, 122.46, 122.15, 119.47, 116.35, 115.15, 35.95, 30.62, 27.48. Anal. calcd for $C_{24}H_{23}BrN_4OS$: C 58.18, H 4.68, N 11.31; found C 57.98, H 4.67, N 11.27.

(*E*)-3-((2-(2-(4-Bromobenzylidene)hydrazinyl)-4-(*t*-butyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7w) Yellow solid, yield 45.1%, m.p. 264－267 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.21 (s, 1H, NH), 12.81 (s, 1H, OH), 11.09 (s, 1H, NH), 8.14 (s, 1H, N＝CH), 7.67 (s, 1H), 7.59 (dd, J＝18.4, 7.7 Hz, 2H), 7.50－7.44 (m, 4H), 7.39 (d, J＝8.3 Hz, 1H), 7.31 (t, J＝7.3 Hz, 1H), 4.19 (s, 2H, CH_2), 1.56 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.54, 162.89, 149.55, 144.05, 138.33, 138.05, 131.19, 131.01, 130.87, 130.38, 127.82, 127.57, 126.24, 123.20, 119.56, 116.43, 115.55, 35.39, 30.28, 27.64. Anal. calcd for $C_{24}H_{23}BrN_4OS$: C 58.18, H 4.68, N 11.31; found C 58.01, H 4.65, N 11.28.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(2-nitrobenzylidene)-hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7x) Yellow solid, yield 59.2%, m.p. 189－192 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.18 (s, 1H, NH), 13.05 (s, 1H, OH), 10.61 (s, 1H, NH), 8.43 (s, 1H, N＝CH), 7.79 (t, J＝7.6 Hz, 1H), 7.68－7.57 (m, 3H), 7.34 (dd, J＝15.8, 7.7 Hz, 2H), 7.10 (dd, J＝17.2, 9.3 Hz, 2H), 4.18 (s, 2H, CH_2), 1.55 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.86, 162.15, 147.87, 145.64, 143.88, 143.82, 139.63, 139.24, 138.95, 137.28, 135.82, 135.41, 134.53, 132.99, 130.10, 129.87, 127.75, 124.20, 120.43, 61.20, 35.54, 23.71. Anal. calcd for $C_{24}H_{23}N_5O_3S$: C 62.46, H 5.02, N 15.17; found C 62.37, H 4.98, N 15.19.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(3-nitrobenzylidene)-hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7y) Yellow solid, yield 62.9%, m.p. 242－245 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.93 (s, 1H, NH), 10.63 (s, 1H, NH), 8.38 (s, 1H), 8.15 (d, J＝5.7 Hz, 1H), 8.10 (s, 1H), 8.01 (d, J＝8.0 Hz, 1H), 7.70－7.64 (m, 1H), 7.58 (s, 1H), 7.47 (t, J＝7.7 Hz, 1H), 7.32 (d, J＝8.4 Hz, 1H), 7.15 (t, J＝7.5 Hz, 1H), 4.02 (s, 2H, CH_2), 1.33 (s, 1H, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 161.61, 148.56, 138.11, 137.11, 136.84, 136.35, 135.28, 134.77, 132.45, 132.40, 130.65, 129.96, 123.30, 121.25, 120.25, 117.24, 113.77, 36.05, 30.84, 27.43. Anal. calcd for $C_{24}H_{23}N_5O_3S$: C 62.46, H 5.02, N 15.17; found C 62.58, H 5.04, N 15.14.

(*E*)-3-((4-(*t*-Butyl)-2-(2-(4-nitrobenzylidene)-hydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-one (7z) Orange solid, yield 81.3%, m.p. 237－240 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.90 (s, 1H, NH), 8.22 (d, J＝8.6 Hz, 2H), 8.13－8.08 (m, 1H), 8.04 (s, 1H), 7.80 (d, J＝8.7 Hz, 2H), 7.60 (s, 1H), 7.47 (t, J＝7.7 Hz, 1H), 7.32 (d, J＝8.2 Hz, 1H), 7.15 (t, J＝7.5 Hz, 1H), 4.02 (s, 2H, CH_2), 1.33 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.36, 161.83, 147.13, 141.39, 138.14, 136.36, 133.29, 130.01, 127.89, 127.00, 124.42, 122.15, 119.47, 115.15, 35.89, 30.83, 27.20. Anal. calcd for $C_{24}H_{23}N_5O_3S$: C 62.46, H 5.02, N 15.17; found C 62.25, H 5.00, N 15.14.

NA inhibition assay

The NA inhibition assay was conducted using NA isolated from a subtype of the influenza A/PR/8/34 (H1N1) virus. The substrate used in the enzyme inhibition assay, 2'-(4-methylumbelliferyl)-*α*-*D*-acetylneuraminic acid sodium salt hydrate (MUNANA), was purchased from Sigma. The NA inhibition assay was performed according to the standard method.[34] The NA substrate (MUNANA) was cleaved by NA to generate a quantifiable fluorescent product.[35] The reaction mixture was dissolved in DMSO consisting of the test compound, a viral suspension (as the source of NAs), and MUNANA (32.5 mmol/L) in MES buffer containing 4 mmol/L $CaCl_2$ (pH 6.5). After incubation for 60 min at 37 ℃ in a black 96-well plate, then the reaction was terminated by adding 150 μL of 34 mmol/L NaOH. The resulting fluorescence was quantified at an excitation

wavelength of 360 nm and an emission wavelength of 450 nm.[36] The 50% inhibitory concentration (IC_{50}) was calculated by plotting the percent of inhibition of NA activity versus the inhibitor concentration.

Docking study

In order to determine the interaction between 3-((4-(*t*-butyl)-2-(2-benzylidenehydrazinyl)thiazol-5-yl)methyl)-quinolin-2(1*H*)-ones possessing 2-(2-hydrazinyl)thiazole scaffold and the NA active site, the most potent compound **7l** was docked into the active sites of NA (PDB entry: 3TI6) using LeDock (http://www.lephar.com/) with default parameters. The binding pocket was set as [X_{min}=–37.4, X_{max}=–19.8, Y_{min}=4.0, Y_{max}=21.5, Z_{min}=12.0, Z_{max}=32.2] around the active site. The results were analyzed and visualized using PyMOL (http://pymol.sourceforge.net/).

Results and Discussion

Compounds **7a**—**7z** were accomplished using the general method outlined in Scheme 1. Starting materials, 2-chloro-3-formylquinoline (**1**) and 2-oxo-3-formylquinoline (**2**) were prepared according to the reported procedures.[32] Compound **3** was synthesized by the aldol condensation reaction of pinacolone and 2-oxo-3-formylquinoline (**2**) under basic conditions, and then compound **4** was prepared by the reduction of compound **3** with H_2 in EtOH. Compound **5** was synthesized by the reaction of compound **4** with $CuBr_2$ in $CHCl_3$ and EtOAc. Compounds **6a**—**6z** were prepared by thiosemicarbazide and different substituted benzaldehyde or substituted salicylaldehyde in EtOH. Target compounds **7a**—**7z** were obtained in a simple one-pot cyclization reaction of compounds **6a**—**6z** and compound **5**.

In our study, compounds **7a**—**7z** were evaluated using an NA activity assay. The inhibition rates of the NA inhibitory activity of target compounds **7a**—**7z** and Oseltamivir (as a positive control) were shown in Table 1. As shown in Table 1, all target compounds showed inhibition effects on influenza H1N1 virus NAs (inhibition rates 20.03%—54.83%), while the inhibition rate of the positive control Oseltamivir was 72.6%, at the concentration of 40 μg/mL and 4 ng/mL, respectively.

Compounds **7a**, **7c**, **7e**, **7l**, **7p** and **7w** had moderate NA inhibitory activity, their IC_{50} values were calculated and shown in Table 2. As shown in Table 2, the preliminary influenza NA inhibition assay indicated that compound **7l** had mild inhibitory activity (with IC_{50} value of 44.66 μmol/L), other compounds **7a**, **7c**, **7e**, **7p** and **7w** had less inhibitory activity relative to compound **7l** (with IC_{50} values of 62.56, 55.90, 78.41, 69.32 and 49.08 μmol/L, respectively).

Structure-activity relationship (SAR)

From the above results, some interesting structure-activity relationships could be concluded. Table 2 also revealed that the substituents of R had great influence on the inhibition activity. For the scaffold of substituted salicylaldehyde, non-substituted group was better than substituted salicylaldehyde group, such as compounds **7a**, **7c** and **7e** with IC_{50} of 62.56, 55.90 and 78.41 μmol/L, respectively. For the scaffold of substituted benzaldehyde, electron donor substituents of R seemed to decrease the activity, except compound **7l** (with IC_{50} of 44.66 μmol/L), other compounds had low inhibitory activity. Moreover, electron withdrawing substituents of R had important effects on the inhibition activity and halogen groups were better than nitro groups, such as compounds **7p** and **7w** with IC_{50} of 69.32 and 49.08 μmol/L respectively. Also, as shown in Table 1, we found that most compounds with chlorine and bromine

Table 1 The inhibitory activities of the 3-((4-(*t*-butyl)-2-(2-benzylidenehydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-ones **7a**—**7z** and reference compound Oseltamivir against NA *in vitro*

Compd.	R	Inhibition rate/% (40 μg/mL)	Compd.	R	Inhibition rate/% (40 μg/mL)
7a	2-OH	48.57	**7n**	4-OCH_3	36.22
7b	5-Cl-2-OH	39.81	**7o**	2-F	45.24
7c	5-Br-2-OH	47.63	**7p**	3-F	47.56
7d	5-NO_2-2-OH	35.29	**7q**	4-F	41.41
7e	3,5-diCl-2-OH	47.12	**7r**	2-Cl	37.35
7f	3,5-diBr-2-OH	40.20	**7s**	3-Cl	31.53
7g	3-Br-5-NO_2-2-OH	33.09	**7t**	4-Cl	41.87
7h	3,5-diNO_2-2-OH	44.86	**7u**	2-Br	21.29
7i	2-CH_3	20.03	**7v**	3-Br	25.56
7j	3-CH_3	34.52	**7w**	4-Br	43.36
7k	4-CH_3	37.66	**7x**	2-NO_2	39.50
7l	2-OCH_3	54.83	**7y**	3-NO_2	45.16
7m	3-OCH_3	34.04	**7z**	4-NO_2	35.17
Oseltamivir		72.6[a]			

[a] Oseltamivir at the concentration of 4 ng/mL.

Table 2 IC_{50} values of the NA inhibitory activity of compounds **7a**, **7c**, **7e**, **7l**, **7p**, and **7w**

Compound	R	IC_{50}/(μmol•L^{-1})
7a	2-OH	62.56
7c	5-Br-2-OH	55.90
7e	3,5-diCl-2-OH	78.41
7l	2-OCH_3	44.66
7p	3-F	69.32
7w	4-Br	49.08
Oseltamivir		0.00159

groups at *para* positions of benzene ring had better inhibitory activity than those with chlorine and bromine groups at *ortho* and *meta* position. For the substituted fluorine and nitro, it could be easy found that fluorine groups had important effect on the inhibition activity than nitro groups. And fluorine or nitro substituent at the *meta* position of benzene ring would increase the activity. Therefore, in the present study, compound **7l** exhibited mild inhibitory activity, and it could be used as a good representative of compounds **7a**—**7z** for further studies.

Molecular docking

In this study, the most potent compound **7l** was docked into the active site of NA to understand their binding modes. Comparison of the interaction models of **7l** and Oseltamivir with A/H1N1-NA is shown in Figures 3A—3D. The predicted binding mode of **7l** indicates a binding interface largely non-overlapping with that of Oseltamivir (Figure 3D). What's more, differing from the binding pocket of Oseltamivir, sialic acid binding cavity (SA cavity), compound **7l** targets both SA cavity and adjacent hydrophobic cavity (430-cavity), which is a promising dual-site mode in NA inhibitor design (Figure 3C). As shown in Figure 3A, 3C, the amide group of the quinoline ring formed hydrogen bonds with Arg292 and Asn347. The thiazole ring formed a hydrogen bond with Asp151, and the *t*-butyl group may accommodate a small hydrophobic pocket formed by the hydrocarbon chains of Ile149 and Pro431.

In addition, a C═N···HO hydrogen bonding interaction between the —NH—N═C group and the phenolic hydroxyl group of Tyr406 was formed, the —NH— group could interact with Glu119 residue by hydrogen bond for stabilizing the interaction. And the methoxy oxygen of benzene ring formed hydrogen bonds with Arg152. Also, the 2-(1*H*)-quinoline ring could binds to the 430-cavity formed by Pro431, Lys432, Arg371 and Asn347. All in all, compound **7l** forms 7 hydrogen bonds with side chains of key residues of Asp151, Glu119, Arg292, Tyr406, and Asn347. Therefore, compound **7l** is most likely to be capable of inhibiting the catalytic action of NA by a tight binding in the

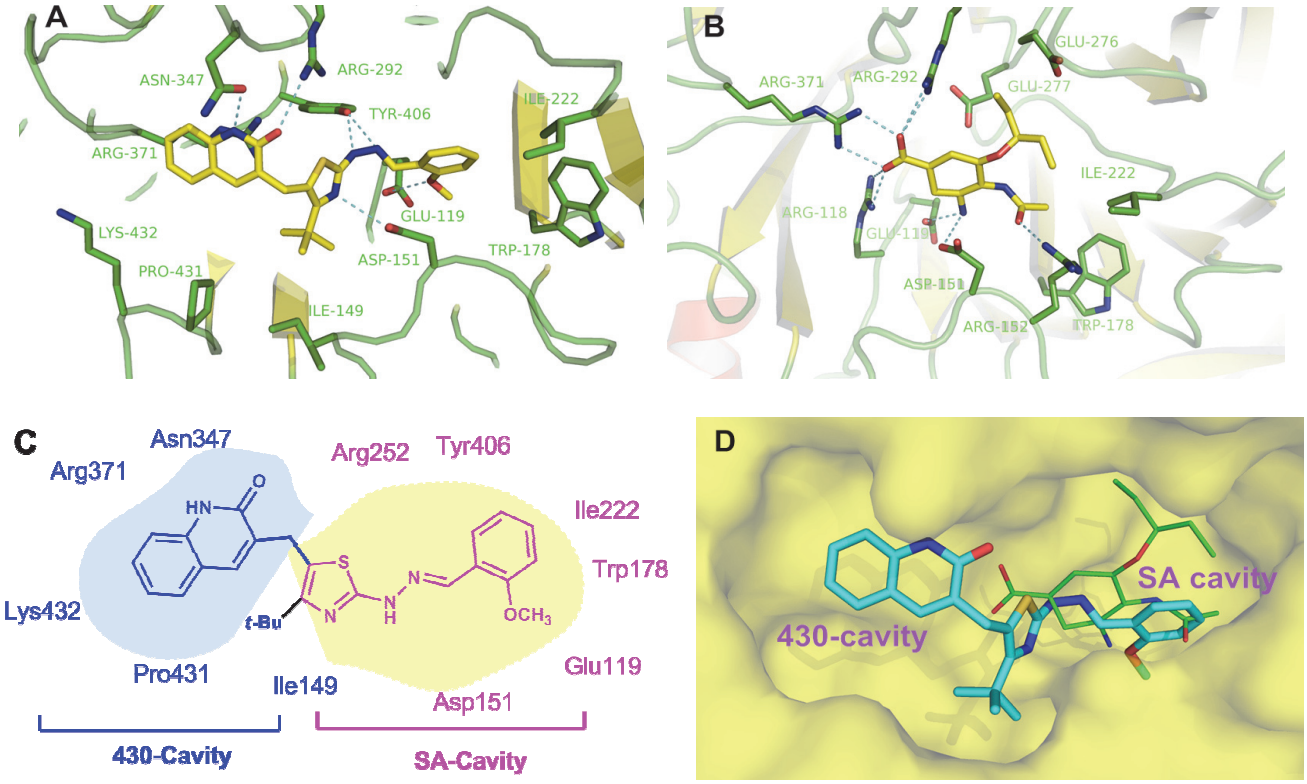


Figure 3 Comparison of the interaction models of **7l** and Oseltamivir with A/H1N1-NA (3TI6). (A-B) Binding models of **7l** (A) and Oseltamivir (B) with A/H1N1-NA. **7l** and Oseltamivir are shown in yellow sticks. The H-bond interactions are depicted as blue dashed lines. (C) Diagram of neuraminidase active site with **7l**. (D) Detailed comparison of the interactions of **7l** (in cyan stick) and Oseltamivir (in green thin stick) with A/H1N1-NA by structural superposition.

active site through the multiple hydrogen bond and hydrophobic interactions. As shown in Figure 3B, Oseltamivir forms 9 solid hydrogen bonds with side chains of key residues of Asp151, Arg152, Glu119, Arg292, Arg371 and Arg118. Thus, Comparing to Oseltamivir, compounds **7l** could not fit very well into the catalytic site of the NA, which may lead to mild NA inhibitory activity. Although compound **7l** exhibited mild inhibitory activity of NA, this compound differs from conventional active NA inhibitors in terms of shape, size and binding modes, making it fascinating to further explore this novel scaffold as NA inhibitors.

Conclusions

In this paper, a series of novel 3-((4-(*t*-butyl)-2-(2-benzylidenehydrazinyl)thiazol-5-yl)methyl)quinolin-2(1*H*)-ones were synthesized and evaluated for their ability to inhibit NA of influenza H1N1 virus. Among them, compound **7l** (IC_{50}=44.66 μmol/L) was the best compound with mild NA inhibitory activity. The docking analysis indicated that it has important interactions with some key residues (including Asp151, Glu119, Arg292, Tyr406, and Asn347) in the NA active site and 2-(1*H*)-quinoline ring could binds to the 430-cavity adjacent to the active site. Compound **7l** showed moderate potent NA inhibitory activity, which can provide attractive structural bases to explore novel NA inhibitors.

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