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Synthesis of novel ribavirin hydrazone derivatives and anti-proliferative activity against A549 lung cancer cells

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ABSTRACT

A series of novel ribavirin hydrazone derivatives were synthesized by the reaction of ribavirin hydrazone with benzaldehyde or acetophenone derivatives. The structures of the compounds were determined by IR, ^{1}H NMR, and HRESIMS. Preliminary biological evaluation showed that one compound (**7h**) inhibits the growth of A549 cells at 20 μM.

1. Introduction

Lung cancer is one of the leading causes of death worldwide.[1] Our understanding of the biology of cancer has undoubtedly improved in the last decade. One characteristic of cancer cells is their highly proliferative nature. Consequently, inhibition of proliferative pathways is considered an effective strategy to fight cancer, and much attention has recently been paid to the discovery and development of new, more selective anticancer agents.[2–4]

In the search for more effective antiviral and antitumor agents, various modifications of nucleotides have been proposed. Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a nucleoside analog that has demonstrated efficacy in treating viral diseases both as monotherapy (respiratory syncytial virus) and in combination therapy with interferon alpha (hepatitis C virus) since it was initially synthesized in 1970.[5]

Structural modification of ribavirin represents a promising approach in the search for new antiviral agents. Much effort has been made to develop new ribavirin analogs with safer profiles. However, in the majority of the studies reported, the investigators focused on compounds having biologic nucleobase moieties with altered sugars, such as levovirin (1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide) and viramidine (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride). Another strategy is to replace the furanose oxygen in ribavirin with a methylene group, which gives the carbocyclic version of ribavirin.[6]

A number of hydrazide–hydrazone derivatives have been claimed to possess interesting bioactivity such as antibacterial–antifungal, anticonvulsant, antiinflammatory, antimalarial, analgesic, antiplatelets, antituberculosis, and anticancer activities.[7–12] Aroylhydrazide–hydrazones containing a heterocycle such as pyridine or an indole ring have attracted special attention.[13] A few pyrazole carbohydrazide hydrazone derivatives have also been reported.[14] In our effort to discover and develop apoptosis inducers as potential new anticancer agents, we recently synthesized a novel 3-aryl-1-arylmethyl-1*H*-pyrazole-5-carbohydrazide hydrazone and found that these compounds could inhibit proliferation of A549 cells.[15,16]

Based on a fragmented approach, we proposed that ribavirin hydrazone derivatives might have interesting bioactivities such as anticancer activity. Thus, we synthesized ribavirin hydrazone derivatives and carried out the preliminary biological evaluation.

2. Results and discussion

2.1. Synthesis of ribavirin hydrazone derivatives

Our synthetic approach for the preparation of ribavirin hydrazone derivatives is shown in Scheme 1. The key intermediate nucleoside, (2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(3-(ethoxycarbonyl)-1*H*-1,2,4-triazol-1-yl)tetrahydrofuran-3,4-diyl diacetate

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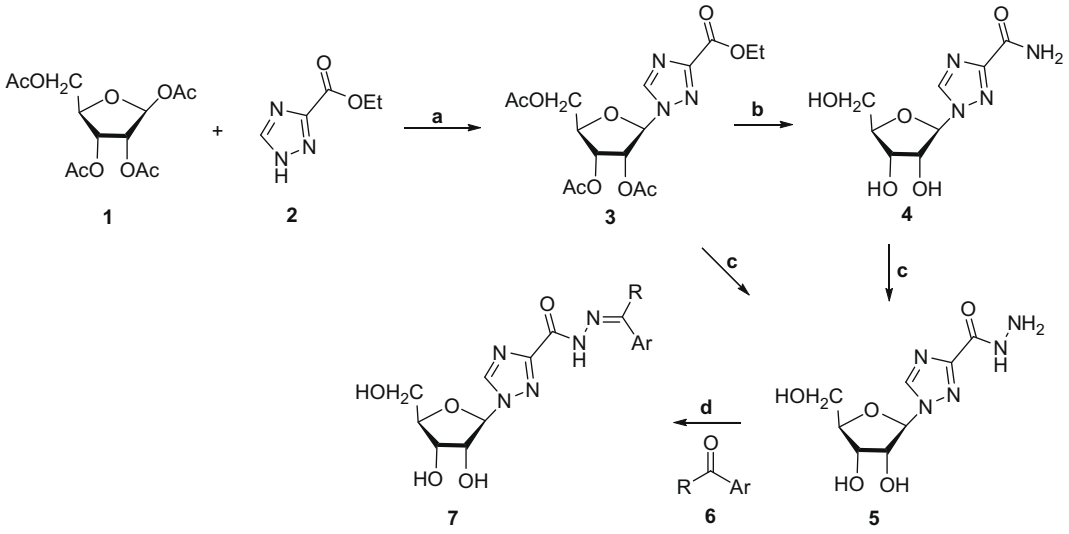
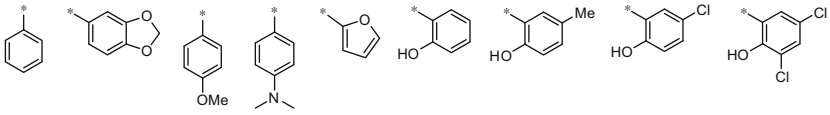


Compound	a	b	c	d	e	f	g	h	i
R	H	H	H	H	H	H	Me	Me	Me
Ar									

Scheme 1. Synthesis of ribavirin hydrazone derivatives. Reagents and conditions: (a) bis(4-nitrophenyl) hydrogen phosphate, 165–170 °C, vacuum (15–20 mmHg), 20 min, 60% yield; (b) MeOH, NH_3, 25 °C, 48 h, 90% yield; (c) 80% hydrazine hydrate, EtOH, reflux for 5 h; and (d) HOAc, EtOH, **6**, 0.5 h, 80% yield (for **c** and **d,** two steps).

(**3**), was synthesized from commercially available tetra-*O*-acetyl-β-D-ribofuranose (**1**) and ethyl 1,2,4-triazole-3-carboxylate (**2**) that was prepared according to the literature procedure.[17] Condensation of **1** with ethyl 1,2,4-triazole-3-carboxylate (**2**) in the presence of a catalytic amount of bis(*p*-nitrophenyl)phosphate at 165–170 °C for 20 min under vacuum, followed by workup provided the fully protected nucleoside (**3**) in 60% yield. Stirring a solution of **3** in methanolic ammonia in a flask at room temperature for 48 h, gave ribavirin, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (**4**) in 90% yield. Treatment of **3** or **4** with hydrazine hydrate in ethanol for 5 h at reflux under nitrogen afforded **5** (see Experimental Section for details of purification). The reaction of compound **5** with benzaldehyde or acetophenone derivatives **6** in ethanol furnished products **7** in 34–79% yields.

2.2. Structural characterization of the compounds

The structures of the products **7** were determined by the analyses of their spectral data including ^{1}H NMR, IR, and HRESIMS. For example, compound **7h**, obtained as yellow crystals, gave a [M+H]-ion peak at *m/z* 412.1016 in the HRESIMS, in accordance with the molecular formula $C_{16}H_{19}ClN_5O_6$. In the IR spectra, the carbonyl group absorptions in the hydrazide moiety and NH bands in CONH were observed in the 1694 cm^{-1} and 3096–2921 cm^{-1} region, respectively. The ^{1}H NMR chemical shift of the NH proton recorded as a singlet peak at δ 11.32 indicated a strong deshielding, which can be explained by hydrogen-bond formation with the phenol group of the aromatic moiety of **7h**. The signal of the hydroxyl group at δ 13.05 is also characteristic for the intramolecular bond formation. All other signals are consistent with the structure of **7h**.

2.3. Biological activities

The cytotoxicity of the compounds against A549 lung cancer cells was examined using the MTT assay and the results are shown in Figure 1. Compound **7h** showed cytotoxicity at 20 μM, although other compounds showed no cytotoxicity at 40 μM. Results from LDH assays showed that there was no significant difference in LDH release between the control group and the treatment groups with compounds **7** at 40 μM (except compound **7h** at 20 μM) for 48 h (Fig. 2), indicating that compounds **7** did not cause necrosis in A549 cells.

3. Conclusions

A series of novel ribavirin hydrazone derivatives have been synthesized by the reaction of ribavirin hydrazone with benzaldehyde or acetophenone derivatives. The anti-proliferative activity of these compounds against A549 lung cancer cells was examined by the MTT assay. Only compound **7h** showed inhibition of the growth of A549 lung cancer cells at a concentration of 20 μM, whereas other compounds were not active at a concentration of 40 μM.

4. Experimental

Thin-layer chromatography (TLC) was conducted on Silica Gel 60 F_{254} plates (E. Merck KgaA). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer, using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard. Melting points were determined on an XD-4 digital micro melting point apparatus. IR spectra were recorded with a VERTEX 70 FTIR spectrometer (Bruker Optics). MS spectra were recorded on a Trace DSQ mass spectrometer.

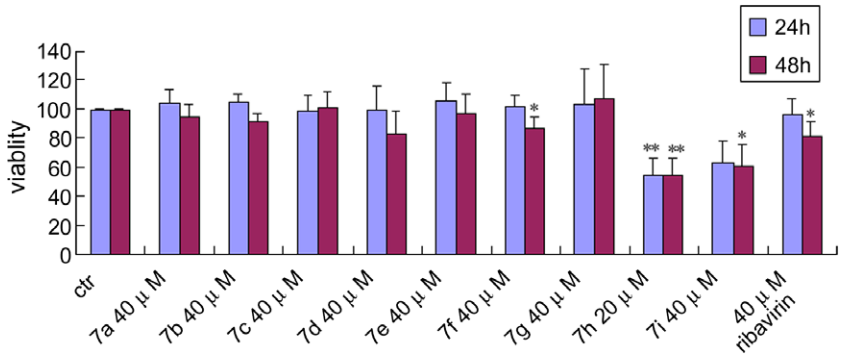


Figure 1. Viability of A549 cells treated with compounds **7a–i** and ribavirin. Cells were seeded in 96-well plates at the density of 6250/cm^2. Cells were treated with the compounds at concentrations of 40 μM (except compound **7h** at 20 μM) for 24 and 48 h. The cell viability was determined by MTT assay. ($^{*}p < 0.05$ and $^{**}p < 0.01$ vs control, $n = 3$).

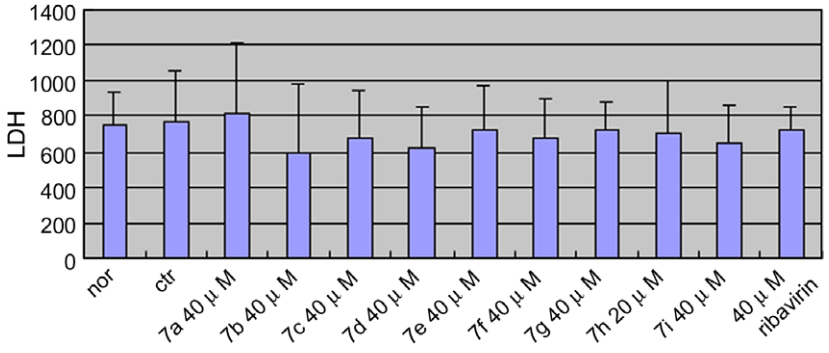


Figure 2. Effects of the compounds **7a–i** and ribavirin on the release of LDH from A549 cells. The culture media from the cells were treated with the compounds at 40 μM (except compound **7h** at 20 μM) for 48 h. Light absorption was analyzed at 340 nm using a model Cintra 5 UV–vis spectrometer. There was no significant difference in LDH release. ($p > 0.05$ vs control group, $n = 3$).

4.1. (2*R*,3*R*,4*R*,5*R*)-2-(Acetoxymethyl)-5-(3-(ethoxycarbonyl)-1*H*-1,2,4-triazol-1-yl)tetrahydrofuran-3,4-diyl diacetate (3)

(2*R*,3*R*,4*R*,5*R*)-2-(Acetoxymethyl)-5-(3-(ethoxycarbonyl)-1*H*-1,2,4-triazol-1-yl)tetrahydrofuran-3,4-diyl diacetate (**3**) was prepared according to the procedure for the preparation of (2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(3-(methoxycarbonyl)-1*H*-1,2,4-triazol-1-yl)tetrahydrofuran-3,4-diyl diacetate.[18] Briefly, tetra-*O*-acetyl-β-D-ribofuranose (**1**) (14.5 g, 45.5 mmol) and ethyl 1*H*-1,2,4-triazole-3-carboxylate (**2**) (6.7 g, 47.5 mmol) were added to a flask in an oil bath at 170 °C, and stirred until the sugar had melted. Then bis(4-nitrophenyl) hydrogen phosphate (0.12 g, 0.35 mmol) was added to the reaction mixture. The mixture was heated at 165–170 °C under vacuum at 15–20 mmHg for about 20 min. The crude product was dissolved in 100 mL of EtOH, and 125 mL of petroleum ether was added. The product was allowed to crystallize at 0 °C, and the first crop was filtered off. The mother liquor was then concentrated, and recrystallized again. The solid thus obtained was dried to give the target product: 11.3 g, 60%, mp 85–87 °C; IR (KBr): ν 3489, 3103, 2984, 1749 cm^{-1}; ^{1}HNMR (DMSO-d_6, 400 MHz): δ 8.92 (s, 1H, CH=N triazole), 6.35 (d, J 3.5 Hz, 1H), 5.69 (t, J 4.4 Hz, 1H), 5.53 (t, J 5.4 Hz, 1H), 4.43 (q, J 5.4 Hz, 1H), 4.39 (dd, J 3.4 Hz, J 12 Hz, 1H, CH_2), 4.35 (q, J 7.2 Hz, 2H, CH_2), 4.11 (dd, J 4.0 Hz, J 12 Hz, 1H, CH_2), 2.09, 2.08, 2.03 (s, 3 × 3H, CH_3C=O), 1.31 (t, J 7.2 Hz, 3H, CH_3); HRESIMS: calcd for $[M+Na]^+$ $C_{16}H_{21}N_3NaO_9$: 422.1175; found: 442.1180.

4.2. Preparation of 1-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-1,2,4-triazole-3-carboxamide (4)

A stirred solution of (2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(3-(ethoxycarbonyl)-1*H*-1,2,4-triazol-1-yl) tetrahydrofuran-3,4-diyl diacetate (9 g, 22.5 mmol) in 90 mL of MeOH was saturated with NH_3 for about 30 min in an ice bath, then stirred at rt for 48 h. The reaction mixture was condensed, and the residue was washed with 15 mL of MeOH, filtered, and dried to afford **4** as a white solid product: 4.9 g (90%); mp 177–179 °C (lit.[18] mp 174–176 °C); ^{1}HNMR (DMSO-d_6, 400 MHz): δ 8.86 (s, 1H, CH=N triazole), 7.80 (s, 1H, $CONH_2$), 7.59 (s, 1H, $CONH_2$), 5.81 (d, J 3.8 Hz, 1H), 5.55 (d, J 5.7 Hz, 1H, OH), 5.17 (d, J 5.6 Hz, 1H, OH), 4.89 (t, J 5.5 Hz, 1H, OH), 4.35 (q, J 4.8 Hz, 1H), 4.13 (q, J 5.2 Hz, 1H), 3.95 (q, J 4.6 Hz, 1H), 3.60–3.65 (m, 1H, CH_2), 3.48–3.53 (m, 1H, CH_2).

4.3. 1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-1,2,4-triazole-3-carbohydrazide (5)

Method A: 1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl)-1*H*-1,2,4-triazole-3-carboxamide (**4**) (0.5 g, 2.05 mmol), 80% hydrazine hydrate (1.3 g, 20.7 mmol), and 5 mL of EtOH were added to a flask. The mixture was stirred and heated at reflux for 5 h. The solvent was removed under reduced pressure, and the residue was then heated under reduced pressure at 100 °C to remove the remaining hydrazine. The product was used directly in the next step.

Method B: (2*R*,3*R*,4*R*,5*R*)-2-(Acetoxymethyl)-5-(3-(ethoxycarbonyl)-1*H*-1,2,4-triazol-1-yl) tetrahydrofuran-3,4-diyl diacetate (**3**) (150 mg, 0.375 mmol), 80% hydrazine hydrate (470 mg, 7.5 mmol), and 4 mL of EtOH were added to a flask, stirred, and heated to reflux for 5 h. The solvent was removed under reduced pressure, and then the residue was heated under reduced pressure at 140–150 °C to remove the remaining hydrazine and acetyl hydrazine. The product was used directly in the next step.

IR (KBr): ν 3416, 2930, 1668, 1494, 1265, 1103, 862, 659 cm^{-1}; ^{1}HNMR (DMSO-d_6, 400 MHz): δ 9.71 (s, 1H, NH), 8.85 (s, 1H, CH=N triazole), 5.81 (d, J 2.9 Hz, 1H), 5.56 (s, 1H, OH), 5.20 (s, 1H, OH),

4.89 (s, 1H, OH), 4.35 (s, 1H), 4.15 (q, *J* 4.3 Hz, 1H), 3.95 (q, *J* 4.1 Hz, 1H), 3.61–3.64 (m, 1H, CH_2), 3.49–3.52 (m, 1H, CH_2), 3.31 (s, 2H, NH_2); HRESIMS: calcd for $[M+Na]^+$ $C_8H_{13}N_5NaO_5$: 282.0814; found: 282.0804.

4.4. General procedure for the synthesis of ribavirin hydrazone derivatives

4.4.1. (*Z*)-*N*′-Benzylidene-1-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-1,2,4-triazole-3-carbohydrazide (7a)

1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-1,2,4-triazole-3-carbohydrazide (**5**) (530 mg, 2.04 mmol, theoretical quantity) was added to 7 mL of HOAc, with stirring and heating until **5** dissolved. Then PhCHO (350 mg, 3.3 mmol) and 12 mL of EtOH were added. The mixture was then heated to reflux for 0.5 h, at the end of which time it was filtered, and the solid was washed with 10 mL of EtOH and dried to afford **7a** as a white solid: 568 mg (79.8%); mp 245–249 °C; IR (KBr): *v* 3457, 3368, 3326, 3129, 2930, 1694, 1545, 1229, 1084 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 11.91 (s, 1H, CONH–N), 8.98 (s, 1H, CH=N triazole), 8.57 (s, 1H, ArCH=N), 7.70–7.72 (2 × 1H, ArH), 7.45–7.48 (3 × 1H, ArH), 5.87 (d, *J* 4.0 Hz, 1H), 5.55 (d, *J* 5.6 Hz, 1H, OH), 5.15 (d, *J* 5.5 Hz, 1H, OH), 4.89 (t, *J* 5.5 Hz, 1H, OH), 4.41 (q, *J* 4.8 Hz, 1H), 4.16 (q, *J* 5.1 Hz, 1H), 3.98 (q, *J* 4.6 Hz, 1H), 3.64–3.69 (m, 1H, CH_2), 3.51–3.56 (m, 1H, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 156.1, 155.0, 148.9, 144.9, 134.1, 130.1, 128.7 (2 × C), 127.1 (2 × C), 91.9, 85.4, 74.4, 69.7, 61.0. HRESIMS: calcd for $[M+H]^+$ $C_{15}H_{18}N_5O_5$: 348.1308; found: 348.1304.

4.4.2. (*Z*)-*N*′-(Benzo[*d*][1,3]dioxol-5-ylmethylene)-1-((2*R*,3*R*,4*S*,-5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-1,2,4-triazole-3-carbohydrazide (7b)

1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-1,2,4-triazole-3-carbohydrazide (**5**) (514 mg, 1.98 mmol, theoretical quantity) was added to 6 mL of HOAc, with stirring and heating until **5** dissolved. Then benzo[*d*][1,3]dioxole-5-carbaldehyde (358 mg, 2.38 mmol) and 25 mL of EtOH were added. The reaction mixture was heated to reflux for 2 h, and then the solvent was removed on a rotary evaporator. The crude residue was washed with 1:3 EtOAc–petroleum ether and subsequently, purified by column chromatography (silica gel; 1:2 EtOH–EtOAc) to afford **7b** as a white solid: 357 mg (61.7%); mp 190–199 °C; IR (KBr): *v* 3451, 3354, 3327, 3284, 3122, 2936, 2910, 1765, 1689 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 11.87 (s, 1H, CONH–N), 8.98 (s, 1H, CH=N triazole), 8.46 (s, 1H, ArCH=N), 7.28 (d, *J* 1.2 Hz, 1H, ArH), 7.14 (dd, *J* 8.1 Hz, 1.6 Hz, 1H, ArH), 6.99 (d, *J* 8 Hz, 1H, ArH), 6.09 (s, 2H, O–CH_2–O), 5.85 (d, *J* 3.9 Hz, 1H), 5.60 (d, *J* 5.5 Hz, 1H, OH), 5.21 (d, *J* 5.5 Hz, 1H, OH), 4.94 (t, *J* 5.5 Hz, 1H, OH), 4.40 (q, *J* 4.8 Hz, 1H), 4.15 (q, *J* 5.0 Hz, 1H), 3.97 (q, *J* 4.6 Hz, 1H), 3.63–3.68 (m, 1H, CH_2), 3.50–3.55 (m, 1H, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 156.8, 155.5, 149.7, 149.3, 148.5, 145.5, 129.0, 124.1, 108.9, 105.5, 102.1, 92.5, 86.0, 72.0, 70.3, 61.6; HRESIMS: calcd for $[M+H]^+$ $C_{16}H_{18}N_5O_7$: 392.1206; found: 392.1205.

4.4.3. (*Z*)-1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-*N*′-(4-methoxybenzylidene)-1*H*-1,2,4-triazole-3-carbohydrazide (7c)

Compound **7c** was prepared from 4-methoxybenzaldehyde (427 mg, 3.13 mmol) using the same procedure as described for **7a**. Purification of the solid resulted in **7c** as a white solid: 608 mg (78.7%); mp 241–244 °C; IR (KBr): *v* 3445, 3345, 3134, 2964, 2928, 1698, 1604, 1254, 1081, 850, 682 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 11.76 (s, 1H, CONH–N), 8.96 (s, 1H, CH=N triazole), 8.49 (s, 1H, ArCH=N), 7.65 (d, *J* 8.7 Hz, 2 × 1H, ArH), 7.02 (d, *J* 8.7 Hz, 2 × 1H, ArH), 5.86 (d, *J* 4.0 Hz, 1H), 5.54 (d, *J* 5.6 Hz, 1H, OH), 5.15 (d, *J* 5.6 Hz, 1H, OH), 4.89 (t, *J* 5.5 Hz, 1H, OH), 4.41 (q, *J* 4.8 Hz, 1H), 4.16 (q, *J* 5.1 Hz, 1H), 3.98 (q, *J* 4.6 Hz, 1H), 3.82 (s, 3H, OCH_3), 3.63–3.68 (m, 1H, CH_2), 3.50–3.56 (m, 1H, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 161.4, 156.8, 155.4, 149.3, 145.5, 129.3 (2 × C), 127.2, 114.8 (2 × C), 92.4, 86.0, 75.0, 70.3, 61.6, 55.8; HRESIMS: calcd for $[M+H]^+$ $C_{16}H_{20}N_5O_6$: 378.1414; found: 378.1404.

4.4.4. (*Z*)-1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-*N*′-(4-(dimethylamino)benzylidene)-1*H*-1,2,4-triazole-3-carbohydrazide (7d)

1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-1,2,4-triazole-3-carbohydrazide (**5**) (530 mg, 2.04 mmol, theoretical quantity) was added to 7 mL of HOAc, with stirring and heating until **5** dissolved. Then 4-(dimethylamino)benzaldehyde (458 mg, 3.07 mmol) and 12 mL of EtOH were added. The reaction mixture was heated to reflux for 1 h, and then the solvent was removed on a rotary evaporator. The crude residue was washed with 1:3 EtOAc–petroleum ether and subsequently purified by column chromatography (silica gel; 1:2 EtOH–EtOAc) to afford **7d** as a yellow solid: 342 mg (42.8%); mp 228–236 °C; IR (KBr): *v* 3463, 3402, 3351, 3322, 3288, 3107, 2934, 2903, 2854, 2820, 1681, 1667, 1599, 1365, 1215, 1181, 1093, 1032, 824, 607, 532 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 11.57 (s, 1H, CONH–N), 8.95 (s, 1H, CH=N triazole), 8.39 (s, 1H, ArCH=N), 7.52 (d, *J* 8.7 Hz, 2 × 1H, ArH), 6.76 (d, *J* 8.8 Hz, 2 × 1H, ArH), 5.85 (d, *J* 3.8 Hz, 1H), 5.53 (d, *J* 5.5 Hz, 1H, OH), 5.14 (d, *J* 5.6 Hz, 1H, OH), 4.89 (t, *J* 5.5 Hz, 1H, OH), 4.41 (q, *J* 4.8 Hz, 1H), 4.16 (q, *J* 5.1 Hz, 1H), 3.98 (q, *J* 4.5 Hz, 1H), 3.63–3.68 (m, 1H, CH_2), 3.50–3.56 (m, 1H, CH_2), 2.98 (s, 6H, $N(CH_3)_2$); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 157.0, 155.1, 152.1, 150.2, 145.4, 129.0 (2 × C), 121.8, 112.2 (2 × C), 92.4, 86.0, 75.0, 70.3, 61.6, 40.2 (2 × C); HRESIMS: calcd for $[M+H]^+$ $C_{17}H_{23}N_6O_5$: 391.1730; found: 391.1720.

4.4.5. (*Z*)-1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-*N*′-(furan-2-ylmethylene)-1*H*-1,2,4-triazole-3-carbohydrazide (7e)

Compound **7e** was prepared from furan-2-carbaldehyde (318 mg, 3.3 mmol) using the same procedure as described for **7d**. Purification by column chromatography resulted in **7e** as a white solid: 461 mg (66.8%); mp 243–246 °C; IR (KBr): *v* 3419, 3258, 3222, 3127, 2951, 2881, 1670, 1621, 1328, 1250, 1126, 1078, 1031, 766, 649, 596 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 11.93 (s, 1H, CONH–N), 8.97 (s, 1H, CH=N triazole), 8.46 (s, 1H, ArCH=N), 7.84 (s, 1H, ArH), 6.92 (d, *J* 3.0 Hz, 1H, ArH), 6.63 (s, 1H, ArH), 5.86 (d, *J* 3.7 Hz, 1H), 5.54 (d, *J* 5.6 Hz, 1H, OH), 5.15 (d, *J* 5.5 Hz, 1H, OH), 4.89 (t, *J* 5.2 Hz, 1H, OH), 4.40 (q, *J* 4.6 Hz, 1H), 4.16 (q, *J* 5.1 Hz, 1H), 3.98 (q, *J* 4.4 Hz, 1H), 3.64–3.69 (m, 1H, CH_2), 3.50–3.56 (m, 1H, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 156.6, 155.5, 149.8, 145.8, 145.5, 139.0, 114.2, 112.7, 92.4, 86.0, 75.0, 70.3, 61.6; HRESIMS: calcd for $[M+H]^+$ $C_{13}H_{16}N_5O_6$: 338.1101; found: 338.1098.

4.4.6. (*Z*)-1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-*N*′-(2-hydroxybenzylidene)-1*H*-1,2,4-triazole-3-carbohydrazide (7f)

1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-1,2,4-triazole-3-carbohydrazide (**5**) (530 mg, 2.04 mmol, theoretical quantity) was added to 7 mL of HOAc, with stirring and heating until **5** dissolved. Then 2-hydroxybenzaldehyde (542 mg, 4.43 mmol) and 12 mL of EtOH were added. The reaction mixture was heated to reflux for 4 h, and then the solvent was removed on a rotary evaporator. The crude residue was washed with 1:3 EtOAc–petroleum ether and subsequently purified by column chromatography (silica gel; 1:2 EtOH–EtOAc) to afford **7f** as a

yellow solid: 430 mg (57.8%); mp 216–222 °C; IR (KBr): ν 3443, 2925, 2874, 1685, 1613, 1549, 1490, 1453, 1344, 1272, 1211, 1083, 1033, 980, 915, 870, 755, 652, 521 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 12.32 (s, 1H, ArOH), 11.20 (s, 1H, CONH–N), 9.01 (s, 1H, CH=N triazole), 8.76 (s, 1H, ArCH=N), 7.50 (d, J 8.4 Hz, 1H, ArH), 7.31 (t, J 7.9 Hz, 1H, ArH), 6.92–6.95 (2 × 1H, ArH), 5.87 (d, J 3.8 Hz, 1H), 5.61 (d, J 5.4 Hz, 1H, OH), 5.21 (d, J 5.5 Hz, 1H, OH), 4.94 (t, J 5.5 Hz, 1H, OH), 4.40 (q, J 4.8 Hz, 1H), 4.16 (q, J 5.2 Hz, 1H), 3.98 (q, J 4.6 Hz, 1H), 3.63–3.69 (m, 1H, CH_2), 3.50–3.56 (m, 1H, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 157.1, 155.5, 154.6, 149.1, 144.7, 131.2, 129.2, 118.9, 118.2, 116.0, 91.6, 85.2, 74.2, 69.5, 60.7; HRESIMS: calcd for $[M+H]^+$ $C_{15}H_{18}N_5O_6$: 364.1257; found: 392.1255.

4.4.7. (*Z*)-1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-*N*′-(1-(2-hydroxy-5-methylphenyl)-ethylidene)-1*H*-1,2,4-triazole-3-carbohydrazide (7g)

1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-1,2,4-triazole-3-carbohydrazide (**5**) (424 mg, 1.63 mmol, theoretical quantity) was added to 6 mL of HOAc, with stirring and heating until **5** dissolved. Then 1-(2-hydroxy-5-methylphenyl)ethanone (369 mg, 2.45 mmol) and 10 mL of EtOH were added. The reaction mixture was heated to reflux for 1 h, and then the solvent was removed on a rotary evaporator. The crude residue was washed with 1:3 EtOAc–petroleum ether and subsequently purified by column chromatography (silica gel; 1:2 EtOH–EtOAc) to afford **7g** as a light yellow solid: 220 mg (34.3%); mp 228–247 °C; IR (KBr): ν 3406, 3359, 3089, 2914, 1713, 1692, 1542, 1494, 1212, 1106, 1052, 1020, 980, 514 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 12.73 (s, 1H, ArOH), 11.19 (s, 1H, CONH–N), 9.00 (s, 1H, CH=N triazole), 7.44 (s, 1H, ArH), 7.13 (d, J 8.1 Hz, 1H, ArH), 6.81 (d, J 8.2 Hz, 1H, ArH), 5.89 (d, J 3.7 Hz, 1H), 5.55 (d, J 5.5 Hz, 1H, OH), 5.16 (d, J 5.5 Hz, 1H, OH), 4.89 (t, J 5.4 Hz, 1H, OH), 4.41 (q, J 4.8 Hz, 1H), 4.17 (q, J 5.1 Hz, 1H), 3.99 (q, J 4.6 Hz, 1H), 3.65–3.69 (m, 1H, CH_2), 3.53–3.57 (m, 1H, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 160.5, 156.9, 156.3, 156.2, 145.6, 132.7, 129.2, 127.6, 119.3, 117.6, 92.5, 86.0, 75.1, 70.3, 61.5, 20.6, 14.5; HRESIMS: calcd for $[M+H]^+$ $C_{17}H_{22}N_5O_6$: 392.1570; found: 392.1573.

4.4.8. (*Z*)-*N*′-(1-(5-Chloro-2-hydroxyphenyl)ethylidene)-1-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-1,2,4-triazole-3-carbohydrazide (7h)

1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-1,2,4-triazole-3-carbohydrazide (**5**) (530 mg, 2.04 mmol, theoretical quantity) was added to 7 mL of HOAc, with stirring and heating until **5** dissolved. Then 1-(5-chloro-2-hydroxyphenyl)ethanone (422 mg, 2.47 mmol) and 12 mL of EtOH were added. The mixture was heated to reflux for 1 h and then stirred at rt for 10 h. The reaction mixture was then filtered, and the solid was washed with 20 mL of EtOH and dried to afford **7h** as a yellow solid: 549 mg (65.1%); mp 256–264 °C; IR (KBr): ν 3406, 3356, 3096, 2921, 1694, 1542, 1485, 1367, 1282, 1230, 1213, 1108, 1052, 1021, 904, 825, 750, 648, 590, 516 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 13.05 (s, 1H, ArOH), 11.32 (s, 1H, CONH–N), 9.01 (s, 1H, CH=N triazole), 7.65 (d, J 1.9 Hz, 1H, ArH), 7.35 (dd, J 8.7 Hz, 1.9 Hz, 1H, ArH), 6.95 (d, J 8.7 Hz, 1H, ArH), 5.89 (d, J 3.7 Hz, 1H), 5.55 (d, J 5.6 Hz, 1H, OH), 5.16 (d, J 5.5 Hz, 1H, OH), 4.89 (t, J 5.4 Hz, 1H, OH), 4.41 (q, J 4.8 Hz, 1H), 4.16 (q, J 5.2 Hz, 1H), 3.99 (q, J 4.6 Hz, 1H), 3.64–3.69 (m, 1H, CH_2), 3.51–3.56 (m, 1H, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 158.4, 157.2, 155.8, 155.6, 145.1, 131.0, 127.9, 122.1, 120.6, 119.0, 92.0, 85.5, 74.5, 69.7, 60.9, 14.1; HRESIMS: calcd for $[M+H]^+$ $C_{16}H_{19}ClN_5O_6$: 412.1024; found: 412.1016.

4.4.9. (*Z*)-*N*′-(1-(3,5-Dichloro-2-hydroxyphenyl)ethylidene)-1-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-1,2,4-triazole-3-carbohydrazide (7i)

1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-1,2,4-triazole-3-carbohydrazide (**5**) (424 mg, 1.63 mmol, theoretical quantity) was added to 6 mL of HOAc, with stirring and heating until **5** dissolved. Then 1-(5-chloro-2-hydroxyphenyl)ethanone (503 mg, 2.45 mmol) and 10 mL of EtOH were added. The reaction mixture was heated to reflux for 1 h, and then stirred at rt overnight. The solvent was removed on a rotary evaporator. The crude residue was washed with 1:3 EtOAc–petroleum ether and subsequently purified by column chromatography (silica gel; 1:2 EtOH–EtOAc) to afford **7i** as a yellow solid: 283 mg (38.7%); mp 192–199 °C; IR (KBr): ν 3426, 3090, 2926, 1700, 1535, 1441, 1366, 1245, 1189, 1094, 1038, 906, 859, 739, 662, 584, 552 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 14.21 (s, 1H, ArOH), 11.54 (s, 1H, CONH–N), 9.02 (s, 1H, CH=N triazole), 7.68 (d, J 1.6 Hz, 1H, ArH), 7.62 (d, J 1.7 Hz, 1H, ArH), 5.89 (d, J 3.9 Hz, 1H), 5.57 (d, J 5.3 Hz, 1H, OH), 5.18 (d, J 5.3 Hz, 1H, OH), 4.90 (t, J 5.3 Hz, 1H, OH), 4.41 (q, J 4.6 Hz, 1H), 4.17 (q, J 5.0 Hz, 1H), 4.00 (q, J 4.6 Hz, 1H), 3.65–3.70 (m, 1H, CH_2), 3.52–3.57 (m, 1H, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 157.8, 156.2, 155.6, 153.6, 145.1, 130.5, 126.9, 122.0, 121.7, 121.1, 92.1, 85.5, 74.5, 69.7, 61.0, 14.1; HRESIMS: calcd for $[M+H]^+$ $C_{16}H_{18}Cl_2N_5O_6$: 446.0634; found: 446.0633.

4.5. Biological activity assays

4.5.1. Cell culture

A549 lung cancer cells were cultured in RPMI 1640 medium at 37 °C with 5% CO_2 and 95% air, supplemented with 10% (v/v) bovine calf serum and 80 U/mL gentamicin. The cells were seeded onto 96-well plates or other appropriate dishes containing the medium at a density of 6250/cm^2.

4.5.2. Cell viability assay

As described in the previous report, cells were seeded onto 96-well plates and treated with compounds **7** and ribavirin at 40 μM (except compound **7h** at 20 μM) for 24 and 48 h, respectively. Cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) assay according to Price et al.[19] The light absorption was measured at 570 nm using a Spectra MAX 190 microplate spectrophotometer (GMI Co., USA).

4.5.3. LDH assay

Lactate dehydrogenase (LDH) assay was performed on cells treated with 40 μM compounds (except compound **7h** at 20 μM) for 48 h using a LDH kit (Nanjing Jiancheng, China) according to the manufacturer's protocol. Light absorption was measured at 340 nm using a model Cintra 5 UV–vis spectrometer (GBC, Australia).

4.5.4. Statistical analyses

Data were presented as means ± SE and analyzed by SPSS software. Pictures were processed with Photoshop software. Mean values were derived from at least three independent experiments. Differences at $p < 0.05$ were considered statistically significant.

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