Accepted Manuscript

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PII: S1001-8417(17)30091-8
DOI: http://dx.doi.org/doi:10.1016/j.cclet.2017.03.013
Reference: CCLET 4008

To appear in: *Chinese Chemical Letters*

Received date: 8-3-2017
Accepted date: 8-3-2017

Please cite this article as: Liang-Run Dong, De-Yu Hu, Zeng-Xue Wu, Ji-Xiang Chen, Bao-An Song, Study of the synthesis, antiviral bioactivity and interaction mechanisms of novel chalcone derivatives that contain the 1,1-dichloropropene moiety, Chinese Chemical Lettershttp://dx.doi.org/10.1016/j.cclet.2017.03.013

Original article

Study of the synthesis, antiviral bioactivity and interaction mechanisms of novel chalcone derivatives that contain the 1,1-dichloropropene moiety

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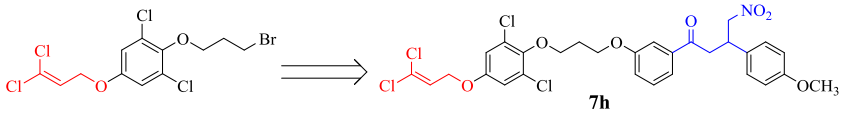
Graphical Abstract



7h

The compound **7h** showed the best inactivation activity against TMV.

A series of chalcone derivatives that contain the 1,1-dichloropropene moiety was synthesized and assayed for the antiviral activity against tobacco mosaic virus. Compound **7h** showed significant inactivation activity against TMV and strong interaction with the tobacco mosaic virus coat protein.

ABSTRACT

*A series of novel chalcone derivatives that contain the 1,1-dichloropropene moiety was designed and synthesized. Bioactivity assays showed that most of the target compounds exhibited moderate to good antiviral activity against tobacco mosaic virus (TMV) at 500 μg/mL. Among the target compounds, compound **7h** showed the highest* in vivo *inactivation activity against TMV with the EC_{50} and EC_{90} value of 45.6 and 327.5 μg/mL, respectively, which was similar to that of Ningnanmycin (46.9 and 329.4 μg/mL) and superior to that of Ribavirin (145.1 and 793.1 μg/mL). Meanwhile, the microscale thermophoresis and fluorescence spectroscopy experiments showed that the compound **7h** had a strong interaction with the tobacco mosaic virus coat protein.*

Keywords:
Chalcone derivatives
1,1-Dichloropropene moiety
Synthesis
Antiviral activity
Interaction mechanisms

ARTICLE INFO
Article history:
Received 15 November 2016
Received in revised form 15 December 2016
Accepted 16 January 2017
Available online

1. Introduction

Tobacco mosaic virus (TMV), a positive-sensesingle stranded RNA virus, infects a wide range of plants, especially tobacco, vegetable and other members of the Solanaceae family. The virus is globally ubiquitous and causes great crop loss [1]. Therefore, TMV is an uncontrollable form of "plant cancer". Ribavirin and Ningnanmycin are common treatments for TMV infections under field conditions. However, Ribavirin has poor inactivation activity, and Ningnanmycin unstable and expensive [2, 3]. Therefore, the development of a novel, simple, and high-efficiency antiviral agents is a significant challenge in pesticide science [4].

Chalcone is a natural product, that exists in licorice, saffron and other medicinal plants. Chalcones have a wide range of pharmacological activities [5], such as anti-Alzheimer's disease [6], antitumor [7], antibacterial [8], insecticidal [9], anti-inflammatory [10], anti-oxidation [11], antiplatelet [12], and antiviral activities [13–15]. In our previous work, we reported a variety of chalcone derivatives and evaluated for their antiviral activity [16–18].The results indicated that these compounds have moderate to good antiviral activity against TMV.

1,3-Dichloropropene is a water-soluble and volatile compound that is mainly used as a pre-planting fumigant and nematicide. It is widely used in the US and other countries [19]. Its analogue, 1,1-dichloropropene, also displays similar activities [20–23]. Pyridalyl, a commercially available 3,3-dichloro-2-propenyloxy-substituted compound, has excellent controlling effects on various lepidopterous and thysanopterous insects. It shows no cross-resistance with the existing insecticides, such as synthetic pyrethroids, organic phosphates, nicotinic insecticides and ryanodine insecticides [24]. 1,1-Dichloropropene compounds have drawn scientific attention as an interesting foundation for generating new lead compounds. Recently Liu *et al.* [25] synthesized several dichloro-allyloxy-phenol derivatives by introducing various substituted phenyl groups into the pyrazole ring. The resulting insecticidal compounds are active against *P. xylostella* at 6.25 μg/mL and are more potent than the reference pyridalyl. In 2015, Yang and co-workers synthesized a series of the novel 1,1-dichloropropene derivatives bearing diverse heterocycles with insecticidal activity against bollworm and *P. litura* [26]. Though, the 1,1-dichloropropene group is an important and applicable functional group for designing the insecticide, the corresponding derivatives that contain the 1, 1-dichloropropene moiety with antiviral activities have not been reported.

In order to evaluate antiviral activity of 1,1-dichloropropene compounds, the novel chalcone derivatives that contain the 1, 1-dichloropropene moiety were designed and synthesized. The physical characteristics were determined by ^{1}H NMR, ^{13}C NMR, IR and elemental analysis, and their antiviral activities were evaluated. The bioassay results indicated that the target compounds exhibited moderate to good inactivation activity against TMV. Compounds **7g**, **7h**, **7m**, **7n**, **7w**, and **7z** showed higher antiviral activity against TMV with EC_{50} values of 52.9, 45.6, 52.2, 53.8, 50.6, and 53.4 μg/mL, respectively, compared with Ribavirin (145.1 μg/mL). Furthermore, the interactions between target compounds and tobacco mosaic virus coat protein (TMV CP) were investigated by the fluorescence spectroscopy and microscale thermophoresis (MST).

2. Results and discussion

2.1. Chemistry

The synthetic route of chalcone derivatives that contain the 1, 1-dichloropropene moiety is summarized in Scheme 1. Intermediate **1** can be easily prepared with 1,1,1,3-tetrachloropropane [21]. Hydroquinone, 1,1,3-trichloroprop-1-ene **1** and K_2CO_3 were refluxed in CH_3CN for 5 h to obtain intermediate **2** after solvent removal and dichloromethane extraction. Intermediate **2** and diethylamine as a catalyst were stirred in toluene. The mixture was slowly added to a solution of SO_2Cl_2. Then, the reaction mixture was stirred at 56 °C for 5 h to produce intermediate **3** [27]. Intermediate **3**, K_2CO_3, KI, and 1,3-dibromopropane were stirred at room temperature for 12 h to yield intermediate **4**. Intermediates **5** and **6** were prepared in accordance with previously reported methods [25, 26]. Finally, intermediates **4** and **6** were reacted by etherification to yield the target compounds **7a**–**7z**. The target products were characterized by ^{1}H NMR, ^{13}C NMR, IR, and elemental analysis. The data of spectroscopic characterization of the target compounds can be found in the Supporting Information.

2.2. Antiviral activity

The antiviral activities against TMV of the title compounds **7a**–**7z** were determined *via* the half-leaf method [28,29]. As shown in Table 1, the bioassay results indicated that all of the target compounds exhibited moderate to good activity against TMV at the concentration of 500 μg/mL. Among the targets, compounds **7g**, **7h**, **7m**, **7n**, **7w**, and **7z** showed better inactivation activity against TMV, with the inhibition rates of 93.1%, 94.8%, 93.5%, 92.9%, 94.0%, and 93.1%, respectively. These inhibition rates were similar to that of Ningnanmycin (94.1%) and superior to that of Ribavirin (72.9%). The other compounds displayed moderate inactivation activity against TMV.

To establish the structure-activity relationships of the target compounds, the EC_{50} values of the target compounds' inactivation activities against TMV were evaluated. The evaluation results are presented in Table 2. All target compounds displayed excellent

inactivation activity. Compounds **7g**, **7h**, **7m**, **7n**, **7w**, and **7z** showed antiviral activity against TMV with EC_{50} and EC_{90} values of 52.9, 45.6, 52.2, 53.8, 50.6, 53.4 and 332.9, 327.5, 331.5, 333.7, 331.0, 333.2 μg/mL, respectively. The inactivation activity of these compounds was better than that of Ribavirin (145.1 and 793.1 μg/mL). Among these six compounds, compound **7h** exhibited the best inactivation activity against TMV and was similar to that of Ningnanmycin (46.9 and 329.4 μg/mL). Structure-activity relationship analysis revealed that differences in R groups have an important influence on inactivation activity. When the R groups were 4-OCH_3-Ph(**7h**), 3-OCH_3-Ph (**7m**), 4-OCH_2CH_3-Ph (**7q**), and 4-OCF_3-Ph (**7z**), the corresponding compounds displayed good activity compared with **7e** (2-Cl-Ph), **7f** (2-Br-Ph), **7g** (2-F-Ph) and **7k** (2-CF_3-Ph). Therefore, the introduction of electron-donating groups improves the anti-TMV activity of the chalcone compounds.

*2.3. Interaction between **7h** and TMV-CP*

Fluorescence spectroscopy and MST analysis were used to investigate the interactions between target compounds and TMV CP [30]. The test methods are shown in the supporting data. The fluorescence spectrum showed that the binding constant K_a between **7h** and TMV-CP was 2.63×10^5 L/mol, which was similar to that of Ningnanmycin (2.39×10^5 L/mol) and superior to that of Ribavirin (2.57×10^3 L/mol). Compound **7r** (3.98×10^4 L/mol) showed moderate affinity and **7j** (6.81×10^3 L/mol) showed weak affinity, as shown in Fig. 1 and Table 3. To further confirm the results of fluorescence spectroscopy, the K_d between compound **7h** and TMV CP was investigated with MST. The result of MST analysis was consistent with the data presented by the fluorescence spectrum, as shown in Fig. 2 and Table 3. The K_d between **7h** and TMV-CP was 9.51 μmol/L, which was higher than those of **7r** (34.8 μmol/L) and **7j** (162 μmol/L). Therefore, the result showed that compound **7h** strongly interacted with TMV CP.

3. Conclusion

In summary, we synthesized novel chalcone derivatives that contain a 1,1-dichloropropene moiety. The anti-TMV activity of the target compounds **7a**–**7z** was evaluated with the *in vivo* half-leaf method. Bioassay results showed that the target compounds were better than Ribavirin (145.1 μg/mL) against TMV. In particular, compound **7h** possessed excellent inactivation activity against TMV and had an EC_{50} value of 45.6 μg/mL, which was similar to that of Ningnanmycin (46.9 μg/mL). In addition, fluorescence spectroscopy and MST showed that compound **7h** strongly interacted with TMV CP. Further studies on the mechanism of inactivation are currently underway.

4. Experimental

4.1. Synthesis

Unless otherwise stated, allreactants andreagents were purchased from commercial suppliers. The melting points were determined on an XT-4 binocular microscope (Beijing Tech Instrument, China) and were not corrected. Mass Spectra were obtained with a LC-MS 1100/MSD spectrometer (Agilent company, America). ^{1}H NMR and ^{13}C NMR (solvent $CDCl_3$) spectral analyses were performed on a JEOL ECX 500 NMR spectrometer operated at 500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR at room temperature and TMS as the internal standard. Elements were performed with an Elemental Vario-III CHN analyzer, and IR spectra were acquired in KBr on a Bruker VECTOR 22 spectrometer. Analytical thin layer chromatography (TLC) was performed on silica gel GF_{254}.

General procedure for the preparation of the key intermediate **6a**–**6z**: Intermediates 6**a**–**6z** were prepared by performing previously reported synthetic procedures [24, 25]. A mixture of 4-hydroxychalcone (1.00 g, 4.2 mmol) and nitromethane (5.12 g, 83.9 mmol) in anhydrous ethanol was stirred. The mixture was refluxed for 2 h after the addition of KOH (0.28 g, 5.04 mmol). The reaction was monitored to completion *via* TLC. After the reaction system was cooled, its pH was adjusted with acid. Then, the mixture was filtered and the supernatant was discarded. The crude solid was purified via TLC on silica gel (petroleum ether/ethyl acetate = 3:1). The physical and chemical properties and spectroscopic characterization of the target compounds can be found in the Supporting information.

General synthetic procedures for the title compounds **7a**–**7z**: A mixture of intermediates **4** (1.2 mmol), **6** (1.0 mmol) and potassium carbonate (2.0 mmol), with KI in acetone as the catalyst, was stirred and refluxed for 8 h. The solvent was evaporated *in vacuo* and the residue was isolated *via* column chromatography on silica gel (petroleum ether/dichloromathane = 1:1) to obtain the title compounds. The physical and chemical properties and spectroscopic characterization of the target compounds can be found in the Supporting information.

4.2. Bioassay

The *in vivo* antiviral activities against TMV of the target compounds were examined. Ningnanmycin was used as the control. EC_{50} of the antiviral activity against TMV was determined. The assays were repeated thrice [28, 29].

*4.3. K_d of compound **7h** to TMV CP*

The *in vitro* binding affinity of the title compounds on TMV-CP was performed by MST and fluorescence spectroscopy measurement as previously described [30].

Acknowledgment

This research was supported by the National Natural Science Foundation of China (Nos. 21362004, 21562013) and Subsidy Project for Outstanding Key Laboratory of Guizhou Province in China (20154004).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://www.sciencedirect.com/

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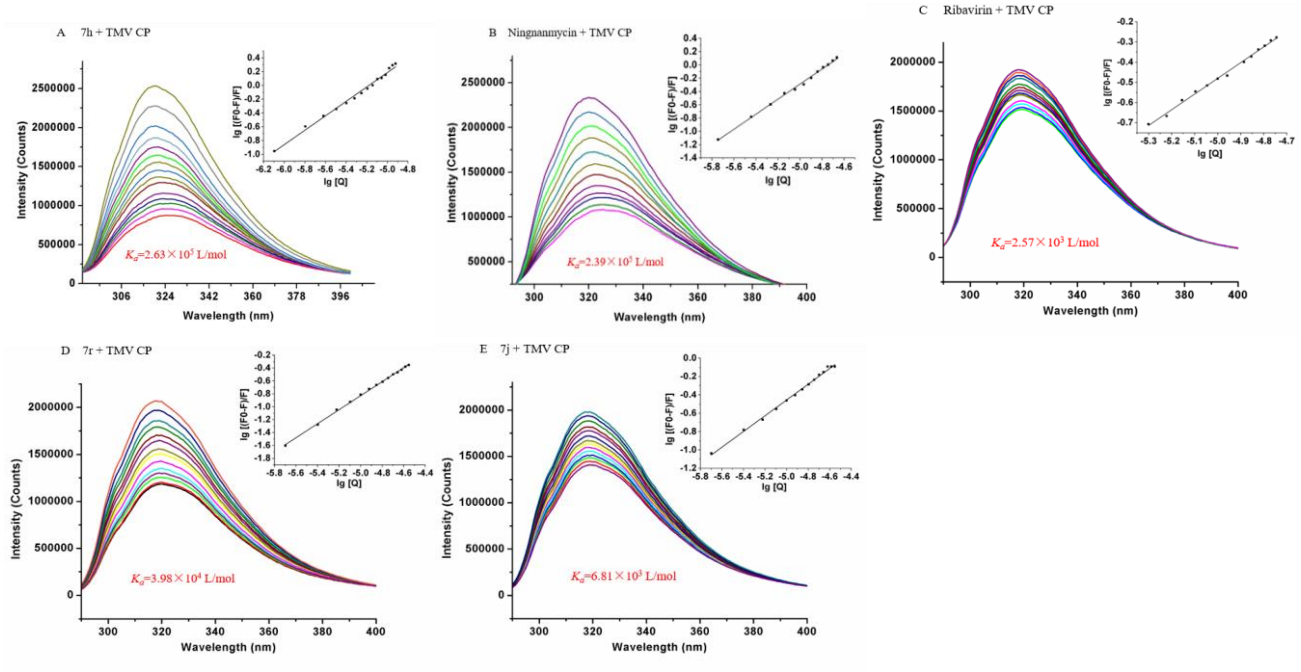


Fig. 1. Fluorescence emission spectra of TMV-CP in the presence of **7h** (A); Ningnanmycin (B); Ribavirin (C); **7r** (D); and **7j** (E)

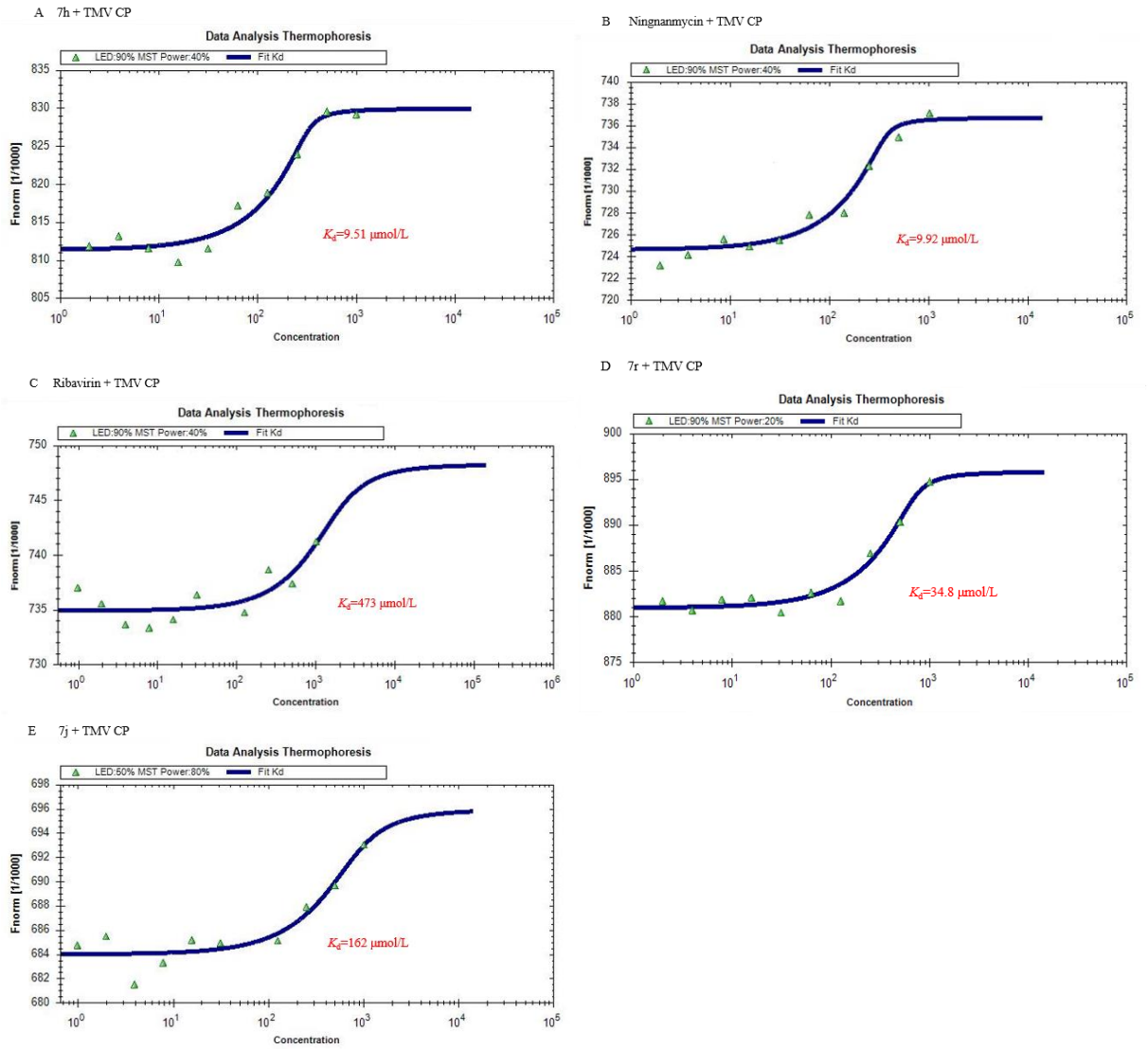
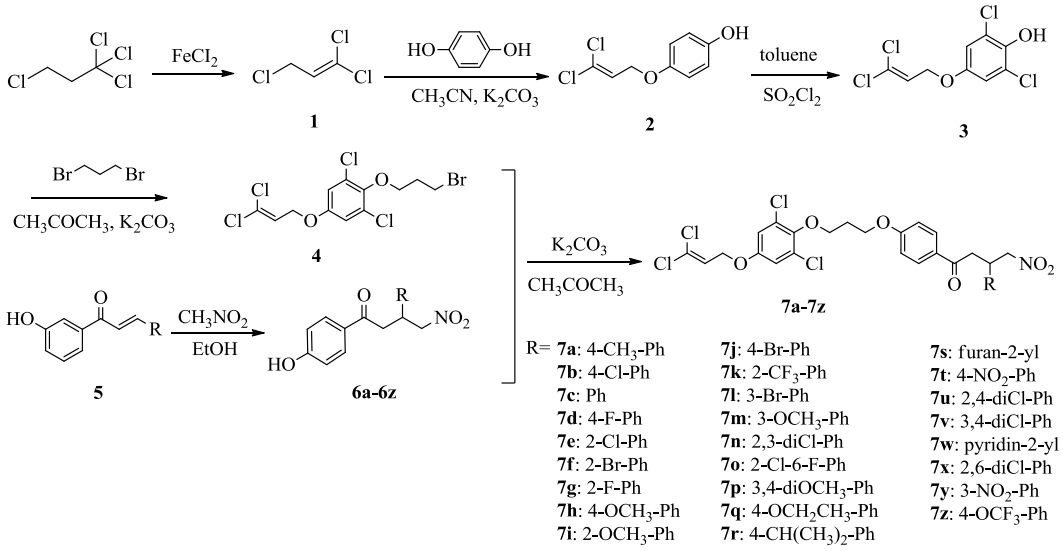


Fig. 2. Microscale thermophoresis (MST) of **7h** (A); Ningnanmycin (B); Ribavirin (C); **7r** (D); and **7j** (E)



Scheme 1. Synthesis of compounds **7a** to**7z**

Table 1. *In vivo* activity against TMV of the target compounds

Compd.	R	Concentration (μg/mL)	Inactivation effect (%)
7a	4-CH_3-Ph	500	90.9±2.4
7b	4-Cl-Ph	500	89.0±3.1
7c	Ph	500	89.9±1.9
7d	4-F-Ph	500	86.5±2.0
7e	2-Cl-Ph	500	90.8±3.2
7f	2-Br-Ph	500	91.5±5.4
7g	2-F-Ph	500	93.1±1.6
7h	4-OCH_3-Ph	500	94.8±2.8
7i	2-OCH_3-Ph	500	91.4±1.0
7j	4-Br-Ph	500	78.8±1.1
7k	2-CF_3-Ph	500	91.3±3.2
7l	3-Br-Ph	500	87.4±2.3
7m	3-OCH_3-Ph	500	93.5±4.1
7n	2,3-diCl-Ph	500	92.9±1.0
7o	2-Cl-6-F-Ph	500	90.8±1.7
7p	3, 4-diOCH_3-Ph	500	90.1±1.6
7q	4-OCH_2CH_3-Ph	500	91.6±1.3
7r	4-$CH(CH_3)_2$-Ph	500	87.7±3.8
7s	furan-2-yl	500	88.0±2.9
7t	4-NO_2-Ph	500	88.6±6.7
7u	2,4-diCl-Ph	500	83.2±5.3
7v	3,4-diCl-Ph	500	91.5±2.1
7w	pyridin-2-yl	500	94.0±1.5
7x	2,6-diCl-Ph	500	85.3±4.6
7y	3-NO_2-Ph	500	81.5±1.5
7z	4-OCF_3-Ph	500	93.1±2.0
Ningnanmycin	/	500	94.1±1.4
Ribavirin	/	500	72.9±2.4

Table 2. *In vivo* EC_{50} values of compounds **7a-7z** against TMV

Compd.	R	EC_{50}/EC_{90} (μg/mL)	Compd.	R	EC_{50}/EC_{90} (μg/mL)
7a	4-CH_3-Ph	63.2±2.6	**7o**	2-Cl-6-F-Ph	66.1±2.4
7b	4-Cl-Ph	77.7±3.4	**7p**	3,4-diOCH_3-Ph	71.7±3.1
7c	Ph	74.6±2.6	**7q**	4-OCH_2CH_3-Ph	56.3±2.8
7d	4-F-Ph	97.2±1.8	**7r**	4-$CH(CH_3)_2$-Ph	94.3±2.2
7e	2-Cl-Ph	65.5±3.3	**7s**	furan-2-yl	90.5±3.6
7f	2-Br-Ph	57.6±1.9	**7t**	4-NO_2-Ph	88.7±2.4
7g	2-F-Ph	52.9±3.7/332.9±3.8	**7u**	2,4-diCl-Ph	106.7±3.8
7h	4-OCH_3-Ph	45.6±2.8/327.5±2.7	**7v**	3,4-diCl-Ph	59.8±1.3
7i	2-OCH_3-Ph	60.8±1.3	**7w**	pyridin-2-yl	50.6±2.5/331.0±3.3
7j	4-Br-Ph	121.3±2.7	**7x**	2,6-diCl-Ph	102.5±2.1
7k	2-CF_3-Ph	61.5±3.1	**7y**	3-NO_2-Ph	110.6±3.7
7l	3-Br-Ph	95.1±1.6	**7z**	4-OCF_3-Ph	53.4±1.6/333.2±2.8
7m	3-OCH_3-Ph	52.2±3.2/331.5±3.1	Ningnanmycin	/	46.9±1.5/329.4±3.7
7n	2,3-diCl-Ph	53.8±1.7/333.7±3.4	Ribavirin	/	145.1±2.6/793.1±2.3

Table 3. Binding constant of **7h**, **7r**, **7j**, Ningnanmycin and Ribavirin

Compd.	Fluorescence spectroscopy (L/mol)	MST (μmol/L)
7h	2.63×10^5	9.51
7r	3.98×10^4	34.8
7j	6.81×10^3	162
Ningnanmycin	2.39×10^5	9.92
Ribavirin	2.57×10^3	473