

Accepted Manuscript

Title: Synthesis and antiviral bioactivity of novel chalcone derivatives containing purine moiety

Authors: Yan-Jiao Wang, Da-Gui Zhou, Fang-Cheng He, Ji-Xiang Chen, Yong-Zhong Chen, Xiu-Hai Gan, De-Yu Hu, Bao-An Song



PII: S1001-8417(17)30242-5
DOI: <http://dx.doi.org/doi:10.1016/j.cclet.2017.07.006>
Reference: CCLET 4124

To appear in: *Chinese Chemical Letters*

Received date: 9-4-2017
Revised date: 17-5-2017
Accepted date: 5-7-2017

Please cite this article as: Yan-Jiao Wang, Da-Gui Zhou, Fang-Cheng He, Ji-Xiang Chen, Yong-Zhong Chen, Xiu-Hai Gan, De-Yu Hu, Bao-An Song, Synthesis and antiviral bioactivity of novel chalcone derivatives containing purine moiety, *Chinese Chemical Letters* <http://dx.doi.org/10.1016/j.cclet.2017.07.006>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Communication

Synthesis and antiviral bioactivity of novel chalcone derivatives containing purine moiety

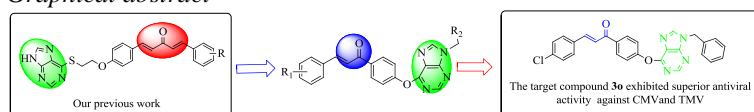
Yan-Jiao Wang, Da-Gui Zhou, Fang-Cheng He, Ji-Xiang Chen, Yong-Zhong Chen, Xiu-Hai Gan, De-Yu Hu*, Bao-An Song*

State Key Laboratory Breeding Base of Green Pesticide and Agricultural Bioengineering, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Research and Development Center for Fine Chemicals, Guizhou University, Guiyang 550025, China

* Corresponding authors.

E-mail addresses: *basong@gzu.edu.cn* (B.-A. Song), *dyhu@gzu.edu.cn* (D.-Y. Hu).

Graphical abstract



A series of novel chalcone derivatives containing purine group was synthesized and evaluated for their antiviral activities against cucumber mosaic virus and tobacco mosaic virus. Compound **3o** exhibited remarkable antiviral activities and strong combining capacity to tobacco mosaic virus coat protein.

ABSTRACT

A series of novel chalcone derivatives containing purine moiety was designed and synthesized, and their antiviral activities against cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV) were evaluated *in vivo*. Bioactivity assays indicated that some compounds showed good antiviral activities against CMV and TMV. It is worth mentioning that compounds **3o**, **3s**, **3w**, and **3x** exhibited excellent curative activity against CMV, with EC₅₀ values of 301.1 µg/mL, 315.7 µg/mL, 282.3 µg/mL, 230.5 µg/mL, respectively, which were better than that of Dufulin (373.7 µg/mL) and Ribavirin (726.3 µg/mL). In addition, the fluorescence spectroscopy experiment results showed that compound **3o** showed strong combining capacity to tobacco mosaic virus coat protein.

Keywords: Chalcone derivatives
6-Chloro-purine

Antiviral activity
Cucumber mosaic virus
Tobacco mosaic virus
Interaction mechanisms

ARTICLE INFO

Article history:

Received
Received in revised form
Accepted
Available online

Cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV) are two important plant viruses, which can cause serious plant disease in plants and can cause considerable damage to agriculture with those parasitic [1,2]. Up to now, only a few antiviral agents can be widely used to prevent and control CMV and TMV, such as Dufulin and Ribavirin. However, their inhibitory activities were from 40% to 50% at 500 µg/mL [3,4] and it is difficult to effectively control the spread of the virus in the field. Therefore, it is imperative to vigorously develop the new,

high-efficiency and low toxicity antiviral agents. As positive-sense single-stranded RNA virus, TMV can infect a wide range of plants, especially tobacco, vegetable and other members of the Solanaceae family. This virus is globally ubiquitous and unmanageable, which can cause great crop loss [1] and was named “plant cancer”. Ningnanmycin was found to be more effective in the treatment of TMV. However, it was not used in field trials because of unstable and expensive [2,3]. Therefore, the development of stable, natural product-based, and economical antiviral agents is a challenge in pesticide science [4].

Chalcone, as a natural flavonoids, widely exist in medicinal plants [5] with broad-spectrum bioactivity, such as antifungal [6], anticancer [7,8], antibacterial [9], insecticidal [10,11], and antiviral [12] activities. Some reports indicated that these bioactivity is due to their molecular flexibility and their ability to bind to different receptors [13,14]. In our previous study, a number of chalcone derivatives containing malonate (Fig. 1A and B) and quinazoline (Fig. 1C) groups have been designed and synthesized, and these compounds exhibited potent antiviral activities against CMV and TMV [15–17].

Meanwhile, purine is an important heterocyclic compounds, which has been widely used as antiviral agent in medicine [18], such as ganciclovir [19], abacavir [20], famciclovir [21] and so on. Additionally, purine and its derivatives possess well others pharmacological properties, including anticancer [22], anticonvulsant [23], antimicrobial [24], herbicidal activity [25], and growth regulating effects [26]. However, the application of purine and its derivatives in pesticide field was extremely rare, especially in controlling plant virus disease aspect. Our group found that a series of purine derivatives containing 1,4-pentadien-3-one moiety (Fig. 1D) exhibited potential antiviral activities against CMV [27].

To extend our study, 1,4-pentadien-3-one moiety was replaced with chalcone structure, and a series of novel chalcone derivatives containing purine group (Scheme 1) were designed and their antiviral activities against CMV and TMV were evaluated *in vivo*. Moreover, the interaction between the target compounds and the tobacco mosaic virus coat protein (TMV-CP) was tested by means of the fluorescence titration.

The synthetic route of the target compounds was depicted in Scheme 1. Based on reported method [28,29], intermediates **1** and **2** were prepared. Then, a mixture of intermediates **1** (1.1 mmol) and potassium carbonate (1.8 mmol) in acetonitrile was stirred for 1 h, and 9-substituted-6-chloro-9H-purine (1 mmol) was added and refluxed for 8 h. The solvent was evaporated *in vacuo*, and the residue was isolated by column chromatography on silica gel using petroleum ether/ethyl acetate (2:1, v/v) to obtain the title compounds (**3a–3z**). Their antiviral activities against CMV and TMV *in vivo* were evaluated by literature methods [27,30], with Dufulin and Ribavirin as controls. Meanwhile, the interaction between the target compound and TMV-CP was test by fluorescence spectroscopy

t i t r a t i o n [1 6]

During the synthesis of the target compounds, it is worth mentioning that the corresponding target compounds were not obtained in most reaction conditions when the 9-position of 6-chloropurine was hydrogen including different solvent, catalyst, and temperature (Table 1). However, it is exciting that the target product was obtained successfully when the 9-position was substituted with alkyl, in order to obtain the target compounds with high yields, the reaction conditions of compound **3a** were optimized. The results showed that the maximum yield of **3a** up to 67.3% was achieved when solvent, catalyst and temperature was CH₃CN, K₂CO₃ and 75 °C, respectively (Table 1). Under these conditions, other target compounds were synthesized with corresponding chalcone and 6-chloro-9-substituted-9H-purine. The structures of target compounds were identified by ¹H NMR, ¹³C NMR and HRMS. These data were collected in the Supporting information. Take compound **3e** as an example, the two single peaks at δ 8.53, 8.07 in the ¹H NMR spectra indicated the presence of Purine-H. The doublets at δ 7.82 (d, 1H, *J* = 15.7 Hz) and δ 7.51 (d, 1H, *J* = 15.7 Hz) indicated the presence of C=CH. The doublets at δ 8.13, 7.55, 7.42, and 7.22 were assigned to the Ar-H protons. Besides, the spectra shown the presence of -N-CH₂- in the form of a quartet at δ 4.36, the triplet at δ 1.59 and the single at δ 2.39 indicated the presence of -N-CH₂CH₃ and -CH₃, respectively. In addition, the ¹³C NMR spectra showed the presences of -C=O, -N-CH₂-, Ar-CH₃, and -NCH₂CH₃ at δ 189.4, 39.5, 21.7, and 16.5, respectively.

The antiviral activity of the title compounds *in vivo* was tested and shown in Table 2. The results revealed some compounds exhibited obvious inhibitory activities against CMV and TMV at 500 μ g/mL. Notably, compounds **3k**, **3o**, **3p**, **3s**, **3w**, and **3x** demonstrated good curative activities against CMV with the values of 52.3%, 58.3%, 51.3%, 52.5%, 53.3%, and 58.7%, respectively, which were better than that of Dufulin (50.6%) and Ribavirin (40.8%). Besides, the protective effects of compounds **3e**, **3k**, **3n**, **3o** and **3t** was 51.9%, 49.0%, 50.5%, 52.4%, and 48.5%, respectively, which were similar to that of Dufulin (51.5%) and Ribavirin (50.5%). Meanwhile, compounds **3d**, **3f**, **3p**, **3u**, **3x**, and **3y** exhibited good curative activities against TMV with the inhibition ratios of 50.8%, 50.3%, 52.0%, 48.5%, 49.3%, and 49.4%, respectively, which were equivalent to that of Dufulin (49.3%) and superior to Ribavirin (38.3%). The protective effects of compounds **3e**, **3t**, and **3w** against TMV was 49.2%, 53.7%, and 55.8%,

respectively, which were similar to that of Dufulin (48.1%) and Ribavirin (50.2%). Compounds **3i** (80.9%), **3o** (89.1%), **3p** (88.2%) and **3u** (82.0%) could effectively inactivate TMV, better than Dufulin and Ribavirin.

The EC_{50} values of curative activities against CMV of all the compounds indicated that compounds **3o**, **3s**, **3w**, and **3x** displayed excellent curative effects against CMV with EC_{50} values of 301.1, 315.7, 282.3, and 230.5 $\mu\text{g/mL}$, respectively, which were better than that of Dufulin ($EC_{50} = 373.7 \mu\text{g/mL}$) and Ribavirin ($EC_{50} = 726.3 \mu\text{g/mL}$). The preliminary structure-activity relationships indicated that different substituents had great influences on antiviral activity. Compounds **3w** and **3x** in which R_2 was 2-chloropyridin, R_1 was substituted with 2-fluoro and 4-chloro groups, showed better curative activity against CMV. In addition, the title compounds displayed notable inactivation activities against TMV when R_2 was phenyl and R_1 was 4-chloro or 4-nitro group, but the curative and protection activity did not showed the evident trend with electron-donating or electron-withdrawing substituents. When R_2 was 2-chloropyridin and R_1 was substituted with 2-methoxyl, 4-chloro or 4-nitro group, the target compounds showed good curative activities. As for the protection activity, when R_2 was 2-chloropyridin and R_1 was substituted with 4-methyl or 2-fluorine groups, the corresponding compounds displayed better antiviral activities than other compounds.

The results of fluorescence spectroscopy titration (Fig. S1 in Supporting information) revealed that compound **3o** exhibited strong combining capacity to TMV-CP, with the binding constant (K_a) value of $1.95 \times 10^5 \text{ L/mol}$, which was superior to that of Dufulin ($2.40 \times 10^4 \text{ L/mol}$) and Ribavirin ($3.31 \times 10^3 \text{ L/mol}$). However, compound **3s** ($6.46 \times 10^3 \text{ L/mol}$) and **3h** ($1.50 \times 10^2 \text{ L/mol}$) showed moderate and weak combining capacity to TMV-CP.

In conclusion, a series of novel chalcone derivatives with purine ring were designed, synthesized according to the substructure link principle. Compounds **3o**, **3s**, **3w** and **3x** exhibited satisfactory curative activities against CMV and TMV. In addition, compound **3o** exhibited strong combining capacity to TMV-CP. This finding indicated that chalcone derivatives containing purine moiety could be considered as novel lead structures to further research on new antiviral agents.

Acknowledgment

This research was supported by National Natural Science Foundation of China (Nos. 21562013 and 21362004), Subsidy Project for Outstanding Key Laboratory of Guizhou Province in China (No. 20154004), the Provincial University Cooperation Plan of Guizhou Province in China (No. 20147001) and Collaborative Innovation Center for Natural Products and Biological Drugs of Yunnan.

References

- [1] D.R. An, Chin. Bull. Life Sci. 2 (1994) 15–18.
- [2] L.H. Xie, Q.Y. Lin, Z.J. Wu, Plant virus, 2th. ed., Science Press, Beijing, 2009.
- [3] G.P. Zhang, G.F. Hao, J.K. Pan, et al., J. Agric. Food. Chem. 64 (2016) 4207–4213.
- [4] X.H. Gan, D.Y. Hu, P. Li, et al., Pest Manag. Sci. 72 (2015) 534–543.
- [5] S. Zhang, X.Y. Liu, Prog. Pharm. Sci. 36 (2012) 241–251.
- [6] H. Jin, Y.C. Geng, Z.Y. Yu, et al., Pestic. Biochem. Physiol. 93 (2009) 133–137.
- [7] V.R. Solomon, H. Lee, Biomed. Pharmacother. 66 (2012) 213–220.
- [8] H. Xu, X.M. Wang, X. Wei, et al., Chin. Chem. Lett. 20 (2009) 576–578.
- [9] B.T. Yin, C.Y. Yan, X.M. Peng, et al., Eur. J. Med. Chem. 71 (2014) 148–159.
- [10] R. Kumar, P. Sharma, A. Shard, et al., Med. Chem. Res. 21 (2012) 922–931.
- [11] G. Pasquale, G.P. Romanelli, J.C. Autino, et al., J. Agric. Food. Chem. 60 (2012) 692–697.
- [12] G. Du, J.M. Han, W.S. Kong, et al., Bull. Korean Chem. Soc. 34 (2013) 1263–1265.
- [13] Z.Y. Liang, X.S. Yang, Y. Wang, et al., Chin. Chem. Lett. 21 (2010) 818–820.
- [14] Y.W. Zhang, Foreign Medical Sciences Section on Pharmacy 23 (1996) 218–223.
- [15] Z.W. Chen, P. Li, B.A. Song, et al., Arab. J. Chem. (2015) doi:10.1016/j.arabjc.2015.05.003.
- [16] M.H. Chen, P. Li, D.Y. Hu, et al., Bioorg. Med. Chem. Lett. 26 (2016) 168–173.
- [17] Z.H. Wan, D.Y. Hu, P. Li, et al., Molecules 20 (2015) 11861–11874.
- [18] X.Z. Fu, F.J. Jiang, Y. Ou, et al., Chin. Chem. Lett. 25 (2014) 115–118.
- [19] J.P. Verheyden, J.C. Martin, Patent, US 4355032 A, 1982, CAN 98: 53544.
- [20] S.M. Daluge, Patent, EP 434450 A2, 1998, CAN 115: 208462.
- [21] S. Antebi, B.Z. Dolitzky, D. Ioffe, et al., Patent, WO 2005026167 A1, 2005, CAN 142: 316618.
- [22] A. Conejo-García, M.E. García-Rubiño, J.A. Marchal, et al., Eur. J. Med. Chem. 46 (2011) 3795–3801.
- [23] S.B. Wang, X.Q. Deng, D.C. Liu, et al., Med. Chem. Res. 23 (2014) 4619–4626.
- [24] S. Kinali-Demirci, Ö. İdil, A. Dişli, Med. Chem. Res. 24 (2015) 1218–1225.
- [25] H.P. Hu, Synthesis and bioactivity of APP-like derivatives containing purine group, Master dissertation, Central China Normal University, 2009.
- [26] D.Y. Zhao, M. Li, J.L. Yu, et al., Agrochemicals 39 (2000) 36–39.
- [27] F. Wu, P. Li, D.Y. Hu, et al., Res. Chem. Intermed. 42 (2016) 7153–7168.

- [28] B. Nijampatnam, L. Casals, R.W. Zheng, et al., *Bioorg. Med. Chem. Lett.* 26 (2016) 3508–3513.
- [29] G.D. Celik, A. Disli, Y. Oner, et al., *Med. Chem. Res.* 22 (2013) 1470–1479.
- [30] B.A. Song, H.P. Zhang, H. Wang, et al., *J. Agric. Food Chem.* 53 (2005) 7886–7891

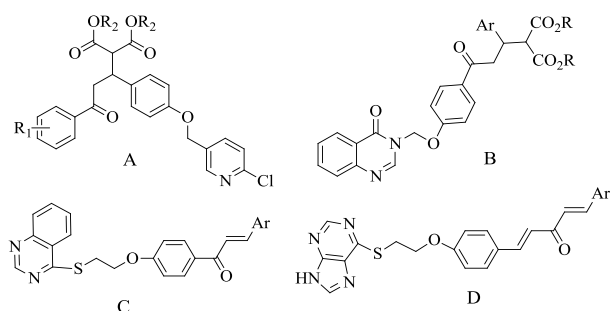
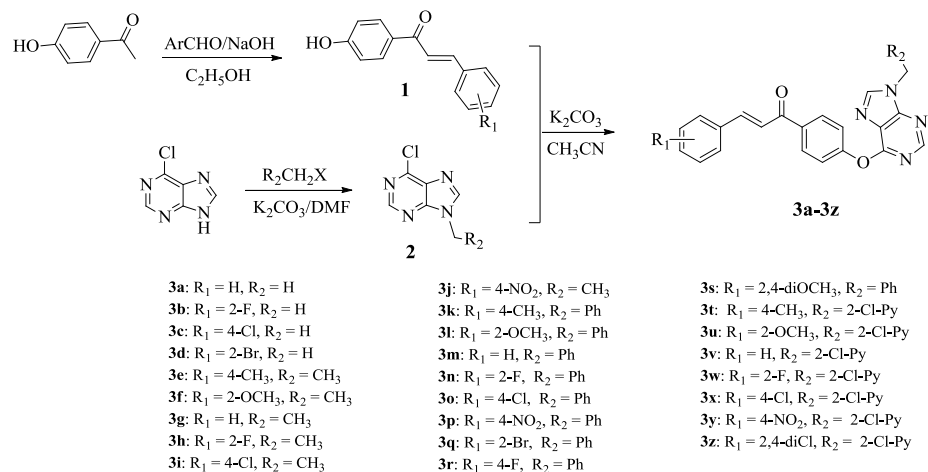
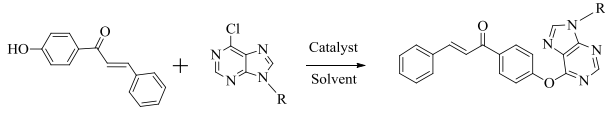


Fig. 1. Chemical structures of known antiviral molecules.



Scheme 1. Synthesis route of compounds **3a** to **3z**.

Table 1Effect of different conditions for synthesis of title compounds. ^a


Entry	R	Solvent	Catalyst	T (°C)	Yield ^b
1	H	DMF	K ₂ CO ₃	65	0
2	H	DMF	KOH	80	0
3	H	Acetone	KOH/KI	50	0
4	H	CH ₃ OH	KOH	65	0
5	H	CH ₃ CN	DBU ^c	80	0
6 ^d	H	DMSO	KOH	180	0
7	CH ₃	DMF	K ₂ CO ₃	25	20.8
8	CH ₃	DMSO	K ₂ CO ₃	25	45.7
9	CH ₃	CH ₃ CN	K ₂ CO ₃	25	48.3
10	CH ₃	CH ₃ CN	K ₂ CO ₃	75	67.3

^a Reactions were performed with the molar ratio of 6-chloro-9*H*-purine: chalcone: catalyst was 1:1.1:1.8.^b Yield of isolated product.^c 1, 8-Diazabicyclo[5.4.0]undec-7-ene.^d Microwave synthesis method.

Table 2Antiviral activity of the title compounds against CMV and TMV *in vivo*.^a

Compd.	CMV		TMV			
	Curative activity ^b (%)	Protection activity ^b (%)	Curative activity ^b (%)	Protection activity ^b (%)	Inactivation activity ^b (%)	EC ₅₀ values of curative activity (µg/mL)
3a	42.8 ± 2.3	44.9 ± 2.4	43.0 ± 2.8	42.3 ± 3.1	69.7 ± 2.7	1026.2 ± 4.9
3b	47.2 ± 1.9	44.5 ± 1.7	32.9 ± 2.0	33.8 ± 2.1	58.9 ± 3.3	752.3 ± 5.9
3c	43.9 ± 3.3	44.7 ± 2.0	44.5 ± 1.8	36.8 ± 3.1	56.6 ± 2.1	838.2 ± 3.9
3d	46.4 ± 2.1	42.1 ± 2.8	50.8 ± 1.4	44.8 ± 1.7	68.9 ± 3.2	823.9 ± 6.5
3e	49.4 ± 2.6	51.9 ± 3.5	41.2 ± 2.1	49.2 ± 2.3	60.2 ± 3.7	613.5 ± 9.2
3f	48.1 ± 3.1	40.2 ± 1.2	50.3 ± 3.2	47.4 ± 1.8	60.5 ± 3.1	518.2 ± 8.9
3g	46.8 ± 2.2	38.3 ± 1.1	47.6 ± 2.4	36.8 ± 2.9	51.3 ± 1.9	490.9 ± 10.3
3h	49.3 ± 1.6	39.3 ± 2.3	41.2 ± 2.3	44.8 ± 3.1	48.1 ± 2.0	407.9 ± 6.8
3i	43.3 ± 3.4	40.5 ± 1.9	39.4 ± 3.1	40.3 ± 2.1	80.9 ± 1.1	720.5 ± 11.9
3j	42.6 ± 3.7	43.8 ± 1.8	38.1 ± 2.7	43.5 ± 1.8	65.7 ± 1.4	712.9 ± 5.9
3k	52.3 ± 2.5	49.0 ± 2.0	44.2 ± 1.1	40.5 ± 1.1	56.7 ± 2.1	588.5 ± 7.2
3l	43.3 ± 1.4	47.3 ± 1.4	41.9 ± 1.0	39.6 ± 1.4	64.4 ± 1.6	1022.5 ± 11.1
3m	44.2 ± 1.1	45.9 ± 1.2	43.7 ± 1.3	37.7 ± 2.8	67.8 ± 2.5	802.9 ± 10.0
3n	45.2 ± 1.9	50.5 ± 1.0	46.2 ± 1.7	43.4 ± 1.0	56.0 ± 2.7	744.4 ± 5.7
3o	58.3 ± 1.3	52.4 ± 1.6	42.1 ± 3.2	46.3 ± 2.1	89.1 ± 2.1	301.1 ± 4.7
3p	51.3 ± 2.1	40.3 ± 2.9	52.0 ± 1.1	43.2 ± 2.8	88.2 ± 1.8	398.0 ± 3.8
3q	48.5 ± 2.0	43.9 ± 2.4	43.3 ± 2.1	40.2 ± 2.7	50.9 ± 2.5	395.0 ± 4.8
3r	47.4 ± 1.7	47.5 ± 1.1	44.7 ± 1.4	42.6 ± 1.3	54.4 ± 2.7	536.4 ± 7.0
3s	52.5 ± 2.7	45.8 ± 1.9	41.9 ± 2.1	39.7 ± 2.3	75.2 ± 1.7	315.7 ± 3.1
3t	49.4 ± 1.4	48.5 ± 1.7	43.0 ± 1.8	53.7 ± 1.0	68.3 ± 1.3	561.7 ± 7.7
3u	49.2 ± 1.9	44.0 ± 2.1	48.5 ± 2.5	31.3 ± 3.1	82.0 ± 1.9	725.2 ± 4.8
3v	41.3 ± 3.1	44.5 ± 2.4	36.4 ± 2.1	31.7 ± 2.2	70.0 ± 2.1	1111.1 ± 8.6
3w	53.3 ± 1.9	45.2 ± 3.1	46.4 ± 1.5	55.8 ± 1.2	64.4 ± 1.7	282.3 ± 4.6
3x	58.7 ± 1.3	41.5 ± 2.7	49.3 ± 1.3	42.1 ± 2.1	58.8 ± 2.5	230.5 ± 3.0
3y	50.5 ± 3.1	43.2 ± 2.9	49.4 ± 1.8	43.6 ± 2.3	76.9 ± 1.3	430.9 ± 6.6
3z	48.9 ± 3.9	38.4 ± 3.1	34.8 ± 3.8	37.3 ± 2.9	65.8 ± 2.7	372.5 ± 4.8
Dufulin	50.6 ± 2.4	51.5 ± 1.6	49.3 ± 2.1	48.1 ± 1.5	80.5 ± 2.1	373.7 ± 4.9
Ribavirin	40.8 ± 1.3	50.5 ± 3.0	38.3 ± 1.9	50.2 ± 2.1	71.9 ± 2.5	726.3 ± 3.5

^a Average of three replicates; ^b The concentration of compound is 500 µg/mL.