JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Agricultural and Environmental Chemistry

Discovery of Tryptanthrins as Novel Antiviral and Anti-phytopathogenic-fungus Agents

Yanan Hao, Jincheng Guo, Ziwen Wang, Yuxiu Liu, Yongqiang Li, Dejun Ma, and Qingmin Wang

J. Agric. Food Chem., **Just Accepted Manuscript** • Publication Date (Web): 01 May 2020

Just Accepted

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Discovery of Tryptanthrins as Novel Antiviral and Anti-phytopathogenic-fungus Agents

Yanan Hao[†], Jincheng Guo[†], Ziwen Wang[†,‡,*], Yuxiu Liu[†,*], Yongqiang Li[†], Dejun Ma[†], Qingmin Wang[†,*]

[†]State Key Laboratory of Elemento-Organic Chemistry, Research Institute of Elemento-Organic Chemistry, College of Chemistry, Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Nankai University, Tianjin 300071, China;

[‡]Tianjin Key Laboratory of Structure and Performance for Functional Molecules, College of Chemistry, Tianjin Normal University, Tianjin 300387, China;

* To whom correspondence should be addressed. For Ziwen Wang, E-mail: hxxywzw@tjnu.edu.cn; Phone: 0086-22-23766531; Fax: 0086-22-23766531; For Yuxiu Liu, E-mail: liuyuxiu@nankai.edu.cn; Phone: 0086-22-23503792; Fax: 0086-22-23503792; For Qingmin Wang, E-mail: wangqm@nankai.edu.cn; Phone: 0086-22-23503952; Fax: 0086-22-23503952.

ABSTRACT: Plant diseases seriously affect the yield and quality of crops and are difficult to control. Tryptanthrin and it's derivatives (tryptanthrins) were synthesized and evaluated for their antiviral activities and fungicidal activities. We found that tryptanthrins have good antiviral activities against tobacco mosaic virus (TMV) for the first time. Most of the tryptanthrins showed higher anti-TMV activities than ribavirin (inhibitory rate: 40%, 37%, 38% at 500 μg/mL for inactivation, curative, and protection activity *in vivo*, respectively). Compounds **3n** (inhibitory rate: 52%, 49%, 54% at 500 μg/mL for inactivation, curative, and protection activity *in vivo*, respectively) and **14** (inhibitory rate: 51%, 48%, 53% at 500 μg/mL for inactivation, curative, and protection activity *in vivo*, respectively) emerged as new antiviral lead compounds with excellent antiviral activities. Compound **16** was selected for further antiviral mechanism research, which revealed that **16** could inhibit virus assembly by decomposing 20S coat protein (CP) disk. Molecular docking results showed that compounds **3n** and **14**, which have higher antiviral activities in vivo than compound **16**, do show stronger interaction with TMV CP. Further fungicidal activity tests showed that tryptanthrins displayed broad-spectrum fungicidal activities, especially for **16**. These compounds showed good selectivity to a *Physalospora piricola*. In current study, a small molecular library of tryptanthrin was constructed and the bio-activity spectrum of these compounds was broadened, which lays a foundation for their application in plant protection.

KEYWORDS: lead discovery, structure optimization, tryptanthrin analogues, antiviral activity, fungicidal activity, mode of action

INTRODUCTION

Since the beginning of the 21st century, the total population of the world has exceeded 7 billion. Study suggests that the population is expected to reach 11.3 billion by the end of the century, and that the actual population will be much larger than currently projected.[1] Food is a basic human need, and meeting human demand for food while maintaining healthy ecosystems is a fundamental challenge of this century.[2] Plant diseases, caused by viruses and fungi, seriously affect the yield and quality of crops and are difficult to control. Tobacco mosaic virus (TMV) is one of the most widely studied plant viruses, which can cause deformation and stunting of the leaves, flowers and fruits of infected plants.[3] Diseases caused by TMV are difficult to control because TMV shows absolute parasitic and transmissibility to host cells and plants lack a complete immune system.[3] Although several commercial antiviral agents against TMV have been used, efficient and practical varieties are few. The widely used antiviral agent ribavirin only gave less than 50% anti-TMV effect at 500 μg/mL. The developing novel structure, remarkable effect and environmentally friendly anti-TMV agents are needed urgently.[3,4] On the other hand, the research on the mechanism of anti plant virus is not deep enough, and it is difficult to design a new type of anti-virus agent based on target.[5]

Natural products are important source for development of novel pesticides with unique mechanism due to their immense structural diversity and wide variety of biological activities.[6-8] Since Duggar and Armstrong found that pokeweed juice (*Phytolacca acinosa*) could inhibit TMV in 1925,[9] many other natural products and their

derivatives with anti-TMV activities have been reported,[10-15] however, there are only a few reported economically viable antiviral chemicals available for practical application in plant protection. Tryptanthrin, a kind of alkaloid with indolequinazolinone structure, mainly exists in blue plants such as marblea and indigo.[16,17] Studies revealed that tryptanthrin and its derivatives have a wide range of biological activities, such as anticancer activity,[18] anti-inflammatory activity,[19] insecticidal activity[20] and anti-allergy activity[21].

Our research group has long been committed to the discovery of new and efficient antiviral lead compounds based on natural products. Considering the above findings and our work experiences, we designed (Figure 1) and synthesized a series of tryptanthrin derivatives. The antiviral activities, structure-activity relationship and mechanism of these compounds were systematically evaluated. Moreover, the fungicidal activities of these compounds were also tested.

MATERIALS AND METHODS

Chemicals. The reagents were purchased from commercial sources and were used as received. All anhydrous solvents were dried and purified by standard techniques prior to use. Ribavirin was purchased from Beijing Hwrkchemical Company Limited with 99% purity.

Instruments. The melting points of the compounds were tested on an X-4 binocular microscope (Beijing Tech Instruments Company). NMR spectra were obtained with a Bruker AV 400 spectrometer with either $CDCl_3$, CD_3OD or DMSO-d_6 as the solvent.

High-resolution mass spectra were obtained with an FT-ICR mass spectrometer (Ionspec, 7.0 T). The *in vitro* TMV rod assembly inhibition and 20S CP disk assembly inhibition were tested via transmission electron microscopy (Tecnai G2 F20).

General Procedures for the Preparation of Compounds 3a–3i. A mixture of isatoic anhydride (0.5 g, 3.4 mmol), corresponding isatin derivatives **2a**–**2i** (3.4 mmol), and triethylamine (2.4 mL, 17 mmol) in toluene (40 mL) was refluxed for 6 h. After cooling, the reaction mixture was concentrated in vacuo. The resulting precipitates were filtrated. The filtrate was taken into ethyl acetate (250 mL) and washed with brine (2 ×100 mL). The organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (1:1 PE/EA) to give **3a**–**3i**.

Indolo[2,1-b]quinazoline-6,12-dione (3a). Yellow solid, yield 95%; mp 267−269 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (d, J = 7.9 Hz, 1H), 8.31 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 3.8 Hz, 2H), 7.91 – 7.84 (m, 2H), 7.78 – 7.69 (m, 1H), 7.48 (t, J = 7.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.4, 157.7, 146.4, 145.9, 145.0, 137.7, 135.1, 129.8, 126.9, 124.7, 123.2, 122.8, 117.0.

8-Fluoroindolo[2,1-b]quinazoline-6,12-dione (3b). Yellow solid, yield 95%; mp 282−284 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 – 8.45 (m, 1H), 8.32 (d, J = 7.8 Hz, 1H), 7.98 – 7.92 (m, 2H), 7.82 – 7.70 (m, 3H).

8-Chloroindolo[2,1-b]quinazoline-6,12-dione (3c). Yellow solid, yield 71%; mp 296−298 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (d, J = 8.4 Hz, 1H), 8.34 (d, J = 7.8

Hz, 1H), 8.02 –7.88 (m, 4H), 7.78 – 7.72 (m, 1H).

8-Bromoindolo[2,1-b]quinazoline-6,12-dione (3d). Yellow solid, yield 84%; mp 260−262 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, J = 8.4 Hz, 1H), 8.33 (d, J = 7.8 Hz, 1H), 8.09 – 8.04 (m, 2H), 7.97 (d, J = 3.7 Hz, 2H), 7.78 – 7.73 (m, 1H).

8-Methoxyindolo[2,1-b]quinazoline-6,12-dione (3e). Brown solid, yield 75%; mp 268−270 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.75 – 8.67 (m, 2H), 8.56 (d, J = 2.2 Hz, 1H), 8.37 (d, J = 7.8 Hz, 1H), 8.00 (d, J = 3.8 Hz, 2H), 7.81 – 7.77 (m, 1H).

8-Nitroindolo[2,1-b]quinazoline-6,12-dione (3f). Red solid, yield 43%; mp 270−272 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (d, J = 8.5 Hz, 1H), 8.30 (d, J = 7.9 Hz, 1H), 7.93 (d, J = 3.7 Hz, 2H), 7.73 (dt, J = 8.2, 4.2 Hz, 1H), 7.45 – 7.39 (m, 2H), 3.87 (s, 3H).

8-Methylindolo[2,1-b]quinazoline-6,12-dione (3g). Yellow solid, yield 67%; mp 256−258 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 – 8.29 (m, 2H), 7.95 (d, J = 3.8 Hz, 2H), 7.78 – 7.71 (m, 1H), 7.71 – 7.63 (m, 2H), 2.42 (s, 3H).

7-Chloroindolo[2,1-b]quinazoline-6,12-dione (3h). Yellow solid, yield 90%; mp 250−252 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (d, J = 7.8 Hz, 1H), 8.33 (d, J = 7.8 Hz, 1H), 7.97 (d, J = 3.7 Hz, 2H), 7.84 (t, J = 8.1 Hz, 1H), 7.79 – 7.72 (m, 1H), 7.52 (d, J = 7.9 Hz, 1H).

7-Bromoindolo[2,1-b]quinazoline-6,12-dione (3i). Yellow solid, yield 90%; mp > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (d, J = 7.9 Hz, 1H), 8.33 (d, J = 7.9 Hz, 1H), 8.00 – 7.94 (m, 2H), 7.79 – 7.72 (m, 2H), 7.70 – 7.66 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.5, 158.1, 148.1 146.9, 145.1, 138.8, 135.8, 131.6, 130.4, 127.5, 123.6,

121.1, 120.1, 116.6.

Preparation of 8-Hydroxyindolo[2,1-b]quinazoline-6,12-dione (3j). To a solution of compound **3e** (2 g, 7.2 mmol) in dry CH_2Cl_2 (100 mL) was added $AlCl_3$ (5 g, 37.6 mmol). The mixture was refluxed under Ar atmosphere. After the reaction was completed, the resulting precipitates were collected by filtration in vacuo and were washed with CH_2Cl_2 (3× 100 mL) to provide compound **3j**: orange solid, yield 70%; mp > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 – 10.21 (m, 1H), 8.28 (d, J = 6.6 Hz, 2H), 7.95 – 7.89 (m, 2H), 7.75 – 7.66 (m, 1H), 7.26 – 7.19 (m, 1H), 7.15 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.0, 157.6, 156.8, 146.9, 145.8, 139.1, 135.3, 130.3, 130.2, 127.2, 124.5, 123.9, 123.9, 118.8, 110.6. $C_{15}H_9N_2O_3$ $[M+H]^+$ 265.0608, found 265.0605.

Preparation of 8-Aminoindolo[2,1-b]quinazoline-6,12-dione (3k). To a solution of **3f** (1.0 g, 3.4 mmol) in ethanol was added $SnCl_2{\cdot}2H_2O$ (3.8 g, 17 mmol), and then the mixture was refluxed. After completion, the solvent was concentrated under reduced pressure. Then the mixture diluted with 200 mL of EtOAc, and the organic phases were washed with brine (3 × 100 mL). The organic layer was dried with $MgSO_4$, filtrated, and concentrated to provide compound **3k**: black green solid, yield 83%; mp > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (d, J = 7.6 Hz, 1H), 8.14 (dd, J = 7.8, 1.2 Hz, 1H), 7.91 – 7.89 (m, 2H), 7.72 – 7.68 (m, 1H), 7.03 – 6.95 (m, 2H), 5.67 (brs, 2H).

General Procedures for the Preparation of Compounds 3l–3n. To a solution of **3h** (200 mg, 1.8 mmol) in NMP (5 mL) was added **6a**–**6c**. and the mixture was stirred at 90 °C. After completion, the resulting precipitates were collected by filtration in vacuo

and was washed with MeOH (3× 50 mL) to provide compounds **3l**–**3n**.

7-Morpholinoindolo[2,1-b]quinazoline-6,12-dione (3l). Red solid, yield 90%; mp 265−267 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (d, J = 7.9 Hz, 1H), 7.98 (d, J = 7.6 Hz, 1H), 7.93 – 7.89 (m, 2H), 7.72 – 7.64 (m, 2H), 6.97 (d, J = 8.6 Hz, 1H), 3.86 – 3.78 (m, 4H), 3.42 – 3.35 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.64, 158.1, 151.6, 147.3, 147.1, 145.9, 139.1, 135.5, 130.0, 129.8, 127.4, 123.5, 116.0, 110.9, 108.1, 66.6, 51.1. $C_{19}H_{16}N_3O_3$ $[M+H]^+$ 334.1186, found 334.1182.

7-(4-Methylpiperazin-1-yl)indolo[2,1-b]quinazoline-6,12-dione (3m). Red solid, yield 85%; mp 272−274 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (d, J = 7.7 Hz, 1H), 7.96 – 7.91 (m, 3H), 7.72– 7.68 (m, 1H), 7.67 – 7.62 (m, 1H), 6.97 (d, J = 8.6 Hz, 1H), 3.40 – 3.37 (m, 4H), 2.55 – 2.53 (m, 4H), 2.26 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.4, 157.6, 151.1, 146.8, 146.6, 145.2, 138.5, 135.0, 129.5, 129.2, 126.8, 122.9, 115.8, 110.2, 107.2, 54.5, 50.1, 45.7. $C_{20}H_{19}N_4O_2$ $[M+H]^+$ 347.1503, found 347.1499.

7-(Pyrrolidin-1-yl)indolo[2,1-b]quinazoline-6,12-dione (3n). Red solid, yield 87%; mp 234−236 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (d, J = 7.6 Hz, 1H), 7.94 – 7.88 (m, 2H), 7.78 (d, J = 7.4 Hz, 1H), 7.72 – 7.65 (m, 1H), 7.55 – 7.48 (m, 1H), 6.79 (d, J = 8.8 Hz, 1H), 3.62 – 3.53 (m, 4H), 2.03 – 1.91 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.9, 157.7, 147.9, 146.7, 145.9, 145.6, 137.4, 134.9, 129.3, 128.9, 126.8, 122.8, 114.2, 106.5, 104.1, 51.6, 25.2. $C_{19}H_{16}N_3O_2$ $[M+H]^+$ 318.1237, found 318.1235.

General Procedures for the Preparation of Compounds 5a–5d. To a solution of **3k** (300 mg, 1.14 mmol) in CH_2Cl_2 was added triethylamine (0.1 mL). Then the solution

was cooled to 0 ºC, corresponding acyl chlorides **4a**–**4d** (2.4 mmol) was added dropwise. and the mixture was refluxed. After completion, the reaction was quenched with water, and extracted with CH_2Cl_2 (3×100 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to provide compounds **5a**–**5d**.

***N*-(6,12-Dioxo-6,12-dihydroindolo[2,1-b]quinazolin-8-yl) acetamide (5a).** Brown solid, yield 76%; mp 226−228 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (s, 1H), 8.41 – 8.34 (m, 1H), 8.30 (d, J = 7.5 Hz, 1H), 8.20 (s, 1H), 8.00 – 7.90 (m, 2H), 7.89 – 7.82 (m, 1H), 7.77 – 7.68 (m, 1H), 2.10 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.9, 169.3, 157.8, 146.9, 145.7, 141.6, 138.6, 135.5, 130.3, 127.7, 127.3, 123.8, 123.0, 118.0, 114.7, 24.5.

4-(*tert*-Butyl)-*N*-((4-(tert-butyl)phenyl)sulfonyl)-*N*-(6,12-dioxo-6,12-dihydroindolo[2, 1-b]quinazolin-8-yl)benzenesulfonamide (5b). Red brown solid, yield 65%; mp 155−157 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (t, J = 8.3 Hz, 1H), 8.33 (t, J = 6.8 Hz, 1H), 8.03 – 7.91 (m, 2H), 7.83 – 7.67 (m, 9H), 7.52 (t, J = 7.3 Hz, 1H), 7.30 (d, J = 6.2 Hz, 1H), 1.35 (d, J = 8.1 Hz, 18H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.7, 158.8, 158.2, 147.0, 146.8, 145.5, 140.8, 135.9, 135.7, 132.1, 130.6, 128.6, 127.6, 127.1, 127.1, 123.7, 123.5, 118.6, 35.7, 31.2. $C_{35}H_{34}N_3O_6S_2$ $[M+H]^+$ 656.1884, found 656.1881.

***N*-(6,12-Dioxo-6,12-dihydroindolo[2,1-b]quinazolin-8-yl)thiophene-2-carboxamide (5c).** Red brown solid, yield 60%; mp > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H), 8.44 (d, J = 8.7 Hz, 1H), 8.38 – 8.30 (m, 2H), 8.25 – 8.15 (m, 2H), 7.99 – 7.93 (m, 2H), 7.91 (d, J = 5.0 Hz, 1H), 7.76 – 7.72 (m, 1H), 7.25 (t, J = 4.2 Hz, 1H). ^{13}C NMR

(100 MHz, DMSO-d_6) δ 182.4, 160.3, 157.4, 146.4, 145.4, 141.5, 139.4, 137.7, 135.0, 132.4, 130.0, 129.9, 129.8, 128.7, 128.1, 126.9, 123.3, 122.5, 117.2, 115.7. $C_{20}H_{12}N_3O_3S$ $[M+H]^+$ 374.0594, found 374.0597.

***N*-(6,12-Dioxo-6,12-dihydroindolo[2,1-b]quinazolin-8-yl)-*N*-(furan-2-carbonyl)furan -2-carboxamide (5d).** Yellow solid, yield 60%; mp 235−237 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 (d, J = 8.5 Hz, 1H), 8.33 (d, J = 7.8 Hz, 1H), 8.01 – 7.90 (m, 5H), 7.81 (dd, J = 8.5, 2.0 Hz, 1H), 7.79 – 7.70 (m, 1H), 7.28 (d, J = 3.5 Hz, 2H), 6.68 (d, J = 1.9 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.6, 160.5, 157.7, 148.0, 146.6, 146.4, 145.2, 144.9, 137.2, 136.6, 135.3, 130.0, 129.9, 127.0, 124.0, 123.5, 123.1, 120.4, 118.0, 112.8. $C_{25}H_{14}N_3O_6[M+H]^+$ 452.0877, found 452.0877.

General Procedures for the Preparation of Compounds 8a–8b. A mixture of **3a** (2.5 g, 10 mmol), corresponding hydroxylamines **7a**–**7b** (50 mmol) in methanol (40 mL) was refluxed. After cooling, the resulting precipitates were collected by filtration in vacuo and washed with MeOH (3× 50 mL) to provide compounds **8a**–**8b**.

(*E*)-6-(Hydroxyimino)indolo[2,1-b]quinazolin-12(6*H*)-one (8a). White solid, yield 65%; mp 282−284 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.66 (s, 1H), 8.53 (d, J = 8.0 Hz, 1H), 8.37 (d, J = 7.6 Hz, 1H), 8.28 (d, J = 7.8 Hz, 1H), 7.89 (t, J = 7.6 Hz, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.63 (dt, J = 14.9, 7.6 Hz, 2H), 7.45 (t, J = 7.6 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.5, 148.3, 146.9, 144.2, 139.3, 134.7, 132.1, 128.1, 127.5, 127.4, 126.6, 126.5, 121.5, 118.8, 116.2.

(*E*)-6-(Methoxyimino)indolo[2,1-b]quinazolin-12(6*H*)-one (8b). Yellow solid, yield

62%; mp 207−209 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (s, 1H), 8.24 (d, J = 21.2 Hz, 2H), 7.86 (d, J = 24.0 Hz, 2H), 7.72 – 7.55 (m, 2H), 7.44 (s, 1H), 4.30 (s, 3H).

General Procedures for the Preparation of Compounds 10a–10b. To a solution of **3a** (500 mg, 2 mmol) in corresponding ketone (1.2 mmol) was added dimethylamine (0.33 g, 7.3 mmol). The mixture was stirred at room temperature for one day. Then the mixture diluted with 200 mL of CH_2Cl_2, and the organic phases were washed with brine (3× 100 mL). The organic layer was dried with $MgSO_4$, filtrated, and concentrated in vacuo. The residue was purified by flash chromatography on a silica gel using petroleum ether and ethyl acetate (4:1, v/v) as the eluent to give **10a**−**10b**.

6-Hydroxy-6-(2-oxo-2-phenylethyl)indolo[2,1-b]quinazolin-12(6*H*)-one (10a). Yellow solid, yield 48%; mp 184−186 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (d, J = 8.0 Hz, 1H), 8.35 (d, J = 7.8 Hz, 1H), 7.92 – 7.83 (m, 3H), 7.74 (d, J = 8.1 Hz, 1H), 7.70 (d, J = 7.4 Hz, 1H), 7.67 – 7.59 (m, 2H), 7.56 – 7.42 (m, 3H), 7.33 (t, J = 7.4 Hz, 1H), 6.62 (s, 1H), 4.56 (d, J = 18.3 Hz, 1H), 4.17 (d, J = 18.3 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.72, 161.5, 159.0, 147.1, 139.4, 135.5, 134.7, 134.1, 133.7, 129.7, 128.7, 128.0, 127.4, 127.3 126.5, 126.4, 123.7, 121.4, 116.0, 75.1, 47.7.

6-Hydroxy-6-(2-oxopropyl)indolo[2,1-b]quinazolin-12(6*H*)-one (10b). White solid, yield 46%; mp 170−172 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (d, J = 8.0 Hz, 1H), 8.34 – 8.29 (m, 1H), 7.92 – 7.85 (m, 1H), 7.78 – 7.74 (m, 1H), 7.66 – 7.59 (m, 2H), 7.54 – 7.47 (m, 1H), 7.40 – 7.32 (m, 1H), 6.46 (s, 1H), 3.82 (d, J = 17.9 Hz, 1H), 3.60 (d, J = 18.0 Hz, 1H), 1.98 (s, 3H).

Preparation of 6-Ethyl-6-hydroxyindolo[2,1-b]quinazolin-12(6*H*)-one (11). To a solution of **3a** (1.0 g, 4.0 mmol) in dry THF (25 mL) was added 1 M CH_3CH_2MgBr (9 mL, 9 mmol) dropwise at -78 ºC. The reaction mixture was stirred for three hours and NH_4Cl solution (1 mL) was added. After completion, the solvent were concentrated under reduced pressure. Then adding NH_4Cl solution (20 mL) gave a solid which was collected by filtration, and recrystallized from chloroform to give **11**: white solid, yield 33%; mp 188−190 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (d, J = 7.9 Hz, 1H), 8.30 (d, J = 7.8 Hz, 1H), 7.91 (t, J = 7.6 Hz, 1H), 7.86 – 7.80 (m, 1H), 7.66 – 7.60 (m, 2H), 7.54 (t, J = 7.8 Hz, 1H), 7.43 (t, J = 7.5 Hz, 1H), 6.36 (s, 1H), 2.21 – 2.11 (m, 2H), 0.57 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.7, 159.4, 147.5, 139.1, 135.3, 134.5, 130.1, 128.1, 127.9, 127.3, 126.9, 124.7, 121.8, 116.6, 78.6, 32.3, 8.2. $C_{17}H_{15}N_2O_2$ $[M+H]^+$ 279.1128, found 279.1128.

General Procedures for the Preparation of Compounds 13a–13b. To a solution of **3a** (1 g, 3 mmol) in MeOH (50 mL) was added corresponding hydrazines **12a**–**12b**, and the mixture was stirred at 70 °C. After completion, the resulting precipitates were collected by filtration in vacuo and was washed with MeOH (3 × 50 mL) to provide compounds **13a**–**13b**.

(*E*)-6-(2-Phenylhydrazono)indolo[2,1-b]quinazolin-12(6*H*)-one (13a). Red solid, yield 89%; mp 195−197 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.43 (s, 1H), 8.48 (d, J = 7.9 Hz, 1H), 8.35 (d, J = 7.8 Hz, 1H), 8.13 (d, J = 8.1 Hz, 1H), 7.99 – 7.90 (m, 2H), 7.69 – 7.60 (m, 3H), 7.54 (t, J = 7.9 Hz, 1H), 7.50 – 7.40 (m, 3H), 7.14 (t, J = 7.4 Hz, 1H). ^{13}C

NMR (100 MHz, $CDCl_3$) δ 158.7, 146.8, 145.9, 142.6, 137.0, 134.3, 129.8, 128.3, 127.3, 127.3, 127.2, 126.3, 126.2, 124.5, 123.5, 121.2, 119.0, 116.9, 114.5. $C_{21}H_{15}N_4O$ $[M+H]^+$ 339.1240, found 339.1246.

(*E*)-6-(2-(Pyridin-2-yl)hydrazono)indolo[2,1-b]quinazolin-12(6*H*)-one (13b). Red solid, yield 52%; mp 214−216 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 13.40 (s, 1H), 8.59 (d, J = 8.0 Hz, 1H), 8.46 (d, J = 7.7 Hz, 1H), 8.36 (d, J = 4.5 Hz, 1H), 7.99 (d, J = 8.1 Hz, 1H), 7.90 (d, J = 7.5 Hz, 1H), 7.82 (t, J = 7.6 Hz, 1H), 7.78 – 7.73 (m, 1H), 7.72 – 7.68 (m, 1H), 7.58 (t, J = 7.5 Hz, 1H), 7.48 (t, J = 7.7 Hz, 1H), 7.39 (t, J = 7.5 Hz, 1H), 7.01 (t, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.7, 155.5, 148.2, 146.8, 145.7, 138.3, 137.9, 134.4, 129.3, 128.4, 128.1, 127.8, 127.2, 126.4, 124.2, 121.5, 119.5, 118.6, 117.0, 108.2. $C_{20}H_{14}N_5O$ $[M+H]^+$ 340.1193, found 340.1195.

Preparation of 6-Hydroxy-5a,6-dihydroindolo[2,1-b]quinazolin-12(5*H*)-one (14). To a mixture of **3a** (0.5 g, 2 mmol) and acetic acid (50 mL) was added $NaBH_4$ (0.75 g, 20 mmol) in small portions in ice bath. The reaction mixture was allowed to warm to ambient temperature and stir for 4 h. After completion, the mixture was poured into 100 mL of ice-water and extracted with EtOAc (3 × 150 mL). The combined organic phase was washed with brine (3 × 50 mL), dried over anhydrous Na_2SO_4, filtrated, and concentrated in vacuo to give **14**: yellow solid, yield 85%; mp 202−204 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 8.09 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 7.8 Hz, 1H), 7.60 (s, 1H), 7.46 (d, J = 7.4 Hz, 1H), 7.40 – 7.34 (m, 2H), 7.16 (t, J = 7.4 Hz, 1H), 6.98 (d, J = 8.1 Hz, 1H), 6.87 (t, J = 7.5 Hz, 1H), 6.18 (d, J = 4.8 Hz, 1H), 5.30 – 5.22 (m, 1H) , 5.13 (d, J =

253 6.6 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.5, 148.8, 140.3, 134.1, 132.7, 129.6,
254 128.2, 125.3, 124.4, 119.2, 117.0,116.0, 115.3, 78.4, 77.1.
255 **Preparation of Indolo[2,1-b]quinazolin-12(5*H*)-one (15).** Compound **14** (1.0 g, 4
256 mmol) was taken into polyphosphoric acid (20 mL). The mixture was heated under N_2 for
257 1 h at 110 °C, then poured into ice-water and extracted with EtOAc (3 × 150 mL). The
258 combined organic phase was washed with brine (3 × 50 mL), dried over anhydrous
259 Na_2SO_4, filtrated, and concentrated in vacuo to give **15**: black solid, yield 82%; mp
260 265−267 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.77 (s, 1H), 8.61 (d, J = 8.1 Hz, 1H),
261 8.17 (d, J = 7.6 Hz, 1H), 7.76 – 7.66 (m, 1H), 7.55 (d, J = 7.7 Hz, 1H), 7.38 – 7.26 (m,
262 2H), 7.19 – 7.14 (m, 2H), 6.04 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.4, 140.9,
263 137.8, 135.3, 130.7, 129.6, 128.0, 124.6, 120.5, 119.9, 118.4, 115.8, 115.5, 111.6, 80.8.
264 $C_{15}H_{11}N_2O$ $[M+H]^+$ 235.0866, found 235.0865.
265 **Preparation of Indolo[2,1-b]quinazolin-6(12*H*)-one (16).** Step 1: To a solution of **3a** (2
266 g, 8 mmol) in ether (50 mL) was added $LiAlH_4$ (1 g, 24 mmol) at 0 °C. The reaction
267 mixture was refluxed overnight. The reaction was cooled to 0 ºC and quenched by slow
268 addition of H_2O (100 mL). The solution extracted with EtOAc (2×150). The organic
269 phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give
270 red mixture. Step 2: To a solution of the red mixture (1.4 g) in dry CH_2Cl_2 (50 mL) was
271 added MnO_2 (0.7 g) at room temperature. The reaction mixture was stirred at room
272 temperature for 48 h. Then the mixture was poured into ice-water and extracted with
273 CH_2Cl_2 (3 × 150 mL). The organic layer was dried with $MgSO_4$, filtrated, and

concentrated in vacuo. The residue was purified by flash chromatography on a silica gel using petroleum ether and ethyl acetate (3:1, v/v) as the eluent to give **16**: red solid, yield 73%; mp 226−228 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 7.5 Hz, 1H), 7.60 (t, J = 7.7 Hz, 1H), 7.49 (d, J = 7.5 Hz, 1H), 7.33 – 7.17 (m, 2H), 7.14 – 7.04 (m, 2H), 6.85 (d, J = 7.9 Hz, 1H), 4.94 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.7, 151.0, 146.2, 140.6, 137.7, 129.5, 129.1, 128.6, 126.7, 125.4, 122.5, 120.5, 119.6, 108.9, 42.9.

11*H*-Indeno[1,2-b]quinoxalin-11-one (19). A mixture of *o*-phenylenediamine (1.1 g, 10 mmol) and ninhydrin (1.8 g, 10 mmol) in ethanol/acetic acid (5:1, 36 mL) was refluxed for 5 h. The reaction contents were cooled and the resulting precipitates were collected by filtration in vacuo to give **19**: yellow solid, yield 95%; mp 217−219 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 – 8.11 (m, 2H), 8.08 (d, J = 7.8 Hz, 1H), 7.95 – 7.82 (m, 4H), 7.71 (t, J = 7.5 Hz, 1H).

Biological Assay. Each test was repeated three times at 25±1 °C. Active effect expressed in percentage scale of 0−100 (0: no activity; 100: total inhibited).

Specific steps for the anti-TMV and fungicidal activity tests and mode of action studies were carried out using literature method,[14] also can be seen in Supporting Information.

RESULTS AND DISCUSSION

Chemistry.

In order to investigate the influence of the electronic effects on the biological activities of these compounds, tryptanthrin (**3a**) and a series of it's designed derivatives (**3b**−**3i**) were synthesized via the reaction of isatoic anhydride with corresponding isatin

295 derivatives (Figure 2).[22] These compounds have rigid planar structure, and hydrogen
296 bond effect is also an important factor affecting biological activities for these compounds.
297 Compounds **3j**, **3k** and **5a**−**5d** containing hydrogen bond donor and acceptor were
298 designed and synthesized to investigate the effect of hydrogen bond on the biological
299 activities of these compounds (Figures 3 and 4). In order to investigate the effect of
300 heterocyclic pharmacophore on biological activity, compounds **3l**−**3n** were designed and
301 synthesized (Figure 5). Carbonyl group has a great influence on the electronic effect and
302 rigid planar structure of these compounds. Compounds **8a**−**8b**, **10a**−**10b**, **11**, **13a**−**13b**,
303 and **14**−**16** were designed and synthesized. The influence of 6 and 12 carbonyl groups on
304 the biological activities of these compounds was investigated. As shown in Figure 6, the
305 6-carbonyl group of tryptanthrin was reacted with hydroxylamines, ketones, Grignarg
306 reagent and hydrazines, respectively to give oximes **8a**−**8b**, alcohols **10a**−**10b**, **11** and
307 hydrazones **13a**−**13b**. The 6-carbonyl group was reduced to hydroxyl by sodium
308 borohydride to obtain **14**. Dehydroxylation of **14** gave **15**. The 12-carbonyl group was
309 reduced to methylene by lithium tetrahydroaluminum to obtain **16**. In order to further
310 investigate the influence of pyrimidinone ring on biological activity, compound **19**
311 containing pyrazine structure was designed and synthesized (Figure 7).

312 **Phytotoxic Activity.**

313 The phytotoxic-activity tests (according to the criterion of safety evaluation of
314 pesticide to crops: NYT 1965.1-2010) showed that the tryptanthrins **3a**−**3n**, **5a**−**5d**,
315 **8a**−**8b**, **10a**−**10b**, **11**, **13a**−**13b**, **14**−**16** and **19** were safe for testing on plants at 500

316 μg/mL.[14]

317 **Antiviral Activity *in vivo*.** The activities of tryptanthrins **3a**–**3n**, **5a**–**5d**, **8a**–**8b**,
318 **10a**–**10b**, **11**, **13a**–**13b**, **14**–**16** and **19** against TMV are listed in Table 1 with
319 commercial viral inhibitor ribavirin as control. The inactive activities of all the
320 compounds were first tested at 500 μg/mL, and the curative activities and protective
321 activities of the compounds with good inactive activities (inactive effect > 40%) were
322 further tested.

323 As can be seen from the table 1, most of the tryptanthrin derivatives showed good
324 anti-TMV activities, compounds **3e**, **3g**, **3i**, **3j**, **3k**, **3l**–**3n**, **5a**, **5d**, **8a**, **10a**, **13a**, **14**–**16**
325 and **19** displayed higher or similar inactive effects with ribavirin at 500 μg/mL,
326 especially for compounds **3n** and **14**. Natural product tryptanthrin (**3a**) showed 37%
327 inactive effect. The introduction of halogen into the 8-position of tryptanthrin has little
328 effect on its activity (inactive effect: **3a** ≈ **3b**–**3d**). Compound **3e** with strong electron
329 absorbing group showed higher activity than tryptanthrin, which indicated that the
330 introduction of strong electron withdrawing group at 8-position is beneficial to biological
331 activity. Compound **3f** with strong electron donating group exhibited similar level of
332 biological activity as tryptanthrin, however, compound **3g** with medium electron
333 donating group showed higher biological activity than tryptanthrin. The above results
334 revealed that the electronic effect of 8-position substituents has a great influence on
335 biological activity. The activity of tryptanthrin decreased with the introduction of Cl at
336 7-position, but increased with the introduction of Br (inactive effect: **3i** > **3a** > **3h**). The

337 introduction of hydroxyl and amino groups at the 8-position of tryptanthrin is beneficial
338 to the increase of activity (inactive effect: **3k** > **3j** > **3a**). Tryptanthrin derivatives with
339 heterocyclic rings at 7-position showed good bioactivity (inactive effect: **3l**, **3m** and **3n** >
340 **3a**), in particular, **3n** with tetrahydropyrrole ring exhibited significantly higher antiviral
341 activity than ribavirin. The 8-aminoacyl derivatives **5a**−**5d** of **3k** showed obvious
342 difference in activity (inactive effect: **5a** > **3k** > **5d** > **5b** ≈ **5c**), which revealed that this
343 region is highly sensitive to electronic effects and steric hindrance. By comparing
344 compounds **8a**, **8b**, **10a**, **10b**, **11**, **13a** and **13b**, it was found that the carbonyl group at
345 the 6-position of tryptanthrin was not irreplaceable (inactive effect: **13a** > **8a** ≈ **10a** > **3a**
346 ≈ **11** ≈ **13b** > **8b** > **10b**), among which compound **13a** containing phenylhydrazine
347 structure displayed the best biological activity. In addition, the antiviral activities of the
348 reduction products (**14**, **15** and **16**) of tryptanthrin were significantly higher than that of
349 tryptanthrin, especially for **14**. Compound **19** with pyrazine structure showed higher
350 antiviral activity than compound **16** with pyrimidine structure. Compounds **3n** and **14**
351 emerged as new antiviral leads due to their excellent antiviral activities.

352 **Preliminary Mode of Action**.

353 Considering the physical and chemical properties and biological activity, we chose
354 compound **16** which displayed the similar activity as ribavirin to study the mode of
355 action using our reported method.[5,14] And *N*′-(4-chlorobenzylidene)-(*3S*)-1-methyl-
356 2,3,4,9-tetrahydro-1*H*-pyrido[3,4-b]indole-3-carbohydrazide (NK0209), a highly
357 effective virus inhibitor independently developed by our group, was used as control.[23]

The test results showed that 20S CP could be assembled with TMV RNA into TMV particles of about 300 nm in length, and DMSO had no effect on the assembly (Figures 8A and 8B). NK0209 and compound **16** can inhibit the assembly of the virus because a decrease in the number of viruses and a short in their length can be observed (Figures 8C and 8D). In Figure 8D, we can clearly see the broken and fused virus particles. We further designed the interaction experiment between the 20S CP disk and **16** using our reported method.[5,14] The results showed that TMV CP could be incubated into 20S CP disk (Figure 9A), and the addition of DMSO had no effect on the results (Figure 9B). The control NK0209 can induce the aggregation and fusion of 20S CP disks (Figure 9C). As can be seen in Figure 9D, compound **16** can cause the 20s protein disk to fuse and then decompose.

Molecular Docking. In order to further study the mechanism of the interaction between tryptanthrins and TMV CP, we chose AutoDock-vina 1.1.2 for molecular docking.[24] Compounds **3n**, **14** and **16** were selected for molecular docking with TMV CP (PDB code: 1EI7). Compound **3n** forms one conventional hydrogen bond with the active site of ARG 341 at a distance of 2.5 Å (Figure 10A). Compound **14** forms one conventional hydrogen bond with the active site of ARG 341 at a distance of 2.2 Å (Figure 10B). Compound **16** forms two conventional hydrogen bonds with SER 146 at a distance of 3.1 Å and 3.3 Å. As the control, NK0209 forms two conventional hydrogen bonds with amino acids SER 147 (2.6 Å), GLN 257 (2.1 Å). Compounds **3n** and **14**, which have higher antiviral activities in vivo than compound **16**, do show stronger interaction with

379 TMV CP.

380 **Fungicidal Activity**.

381 It's also a very efficient method to find a new type of fungicidal lead compound based on
382 natural products.[25] Tryptanthrins **3a**–**3n**, **5a**–**5d**, **8a**–**8b**, **10a**–**10b**, **11**, **13a**–**13b**, **14**–**16**
383 and **19** were also evaluated for their fungicidal activities with commercial fungicides
384 carbendazim, chlorothalonil and pyrimethanil as controls.
385 Tryptanthrins exhibited broad-spectrum fungicidal activities against 14 kinds of
386 phytopathogenic fungi at 50 μg/mL (Table 2). On the whole, tryptanthrins showed
387 excellent fungicidal activities against *Physalospora piricola*. Most derivatives displayed
388 more than 60% inhibitory effects, especially, compounds **3c** and **3k** exhibited higher
389 fungicidal activities than pyrimethanil against *Physalospora piricola*. Compound **16**
390 displayed higher fungicidal activity against *Rhizoctonia cerealis* than pyrimethanil. The
391 inhibitory effects of **3g**, **5d**, **16** and **19** were better than that of chlorothalonil and
392 carbendazim against *Alternaria solani*. In addition, the inhibition rates of most
393 compounds on *Phytophthora capsici* and *Botrytis cinerea* were higher than that of
394 chlorothalonil and carbendazim.

395 In summary, tryptanthrins **3a**–**3n**, **5a**–**5d**, **8a**–**8b**, **10a**–**10b**, **11**, **13a**–**13b**, **14**–**16** and
396 **19** were synthesized and evaluated for their antiviral activities and fungicidal activities.
397 Most of these compounds displayed higher antiviral activities than ribavirin. Compounds
398 **3n** and **14** with significantly higher antiviral activities than ribavirin emerged as new
399 antiviral lead compounds for further research. A systematic study on the structure-activity

relationship of these compounds was carried out. The preliminary mode of action studies revealed that compound **16** can inhibit virus assembly by decomposing the 20s protein disk. We further study the mechanism of the interaction between tryptanthrins and TMV CP by molecular docking. Further fungicidal test revealed that these compounds exhibited broad-spectrum fungicidal activities against 14 kinds of plant fungi at 50 μg/mL. Tryptanthrins showed excellent fungicidal activities against *Physalospora piricola*. Compounds **3c** and **3k** exhibited higher fungicidal activities than pyrimethanil against *Physalospora piricola*. Current research has laid a foundation for the application of tryptanthrins in plant protection.

ASSOCIATED CONTENT

Supporting Information

The detailed bio-assay procedures. The spectra data of tryptanthrins **3a**−**3n**, **5a**−**5d**, **8a**−**8b**, **10a**−**10b**, **11**, **13a**−**13b**, **14**−**16** and **19**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors

*(Z.W.) E-mail: hxxywzw@tjnu.edu.cn; Phone: 0086-22-23766531; Fax: 0086-22-23766531.

*(Y.L.) E-mail: liuyuxiu@nankai.edu.cn; Phone: 0086-22-23503792; Fax: 0086-22-23503792.

*(Q.W.) E-mail: wangqm@nankai.edu.cn. Phone: 0086-22-23503952. Fax:

0086-22-23503952.

Funding

This study was supported by Natural Science Fund of China (21772145, 21732002, 21772104).

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Collins, J.; Page, L. The heritability of fertility makes world population stabilization unlikely in the foreseeable future. *Evol. Hum. Behav.* **2019**, *40*, 105−111.

(2) Poore, J.; Nemecek, T. Reducing food's environmental impacts through producers and consumers. *Science* **2018**, *360*, 987−992.

(3) Song, B. A.; Yang, S.; Jin, L. H.; Bhadury, P. S. Environment friendly anti-plant viral agents; *Chemical Industry Press & Springer Press*: Beijing, **2009**, 1−305.

(4) Liu, L. R. The control of disease and pests of tobacco. Beijing, China, Science Press Beijing, **1998**, 31.

(5) Guo, J. C.; Hao, Y. N.; Ji, X. F.; Wang, Z. W.; Liu, Y. X.; Ma, D. J.; Li, Y. Q.; Pang, H. L.; Ni, J. P.; Wang, Q. M. Optimization, structure-activity relationship and mode of action of nortopsentin analogues containing thiazole and oxazole moieties. *J. Agric. Food Chem.* **2019**, *67*, 10018−10031.

(6) Rodrigues, T.; Reker, D.; Schneider, P.; Schneider, G. Counting on natural products for drug design. *Nat. Chem*. **2016**, *8*, 531−541.

442 (7) Wang, S. Z.; Dong, G. Q.; Sheng, C. Q. Structural simplification of natural products.
443 *Chem. Rev.* **2019**, *119*, 4180−4220.
444 (8) Wu, W. B.; Tang, Y.; Yang, J. L.; Idehen, E.; Sang, S. M. Avenanthramide aglycones
445 and glucosides in oat bran: Chemical profile, levels in commercial oat products, and
446 cytotoxicity to human colon cancer cells. *J. Agric. Food Chem.* **2018**, *66*, 8005−8014.
447 (9) Duggar, B. M.; Armstrong, J. K. The effect of treating the virus of tobacco mosaic
448 with the juices of various plants. *Ann. Mo. Bot. Gard.* **1925**, *12*, 359−366.
449 (10) Ouyang, M. A.; Wein, Y. S.; Zhang, Z. K.; Kuo, Y. H. Inhibitory activity against
450 tobacco mosaic virus (TMV) replication of pinoresinol and syringaresinol lignans and
451 their glycosides from the root of *rhus javanica* var. *roxburghiana*. *J. Agric. Food Chem.*
452 **2007**, *55*, 6460–6465.
453 (11) Chen, J.; Yan, X. H.; Dong, J. H.; Sang, P.; Fang, X.; Di, Y. T.; Zhang, Z. K.; Hao, X.
454 J. Tobacco mosaic virus (TMV) inhibitors from *picrasma quassioides* benn. *J. Agric.*
455 *Food Chem.* **2009**, *57*, 6590–6595.
456 (12) Ji, X. F.; Wang, Z. W.; Dong, J.; Liu, Y. X.; Lu, A. D.; Wang, Q. M. Discovery of
457 topsentin alkaloids and their derivatives as novel antiviral and anti-phytopathogenic
458 fungus agents. *J. Agric. Food Chem*. **2016**, *64*, 9143–9151.
459 (13) Liu, B.; Li, R.; Li, Y. N.; Li, S. Y.; Yu, J.; Zhao, B. F.; Liao, A. C.; Wang, Y.; Wang,
460 Z. W.; Lu, A. D.; Liu, Y. X.; Wang, Q. M. Discovery of pimprinine alkaloids as novel
461 agents against aplant virus. *J. Agric. Food Chem.* **2019**, *67*, 1795–1806.
462 (14) Lu, A. D.; Wang, T. N.; Hui, H.; Wei, X. Y.; Cui, W. H.; Zhou, C. L.; Li, H. Y.; Wang,

463 Z. W.; Guo, J. C.; Ma, D. J.; Wang, Q. M. Natural products for drug discovery: discovery
464 of gramines as novel agents against a plant virus. *J. Agric. Food Chem*. **2019**, *67*,
465 2148–2156.
466 (15) Zhao, L.; Zhang, J.; Liu, T.; Mou, H. L.; Wei, C. L.; Hu, D. Y.; Song, B. A. Design,
467 synthesis, and antiviral activities of coumarin derivatives containing dithioacetal
468 structures. *J. Agric. Food Chem.* **2020**, *68*, 975−981.
469 (16) Honda, G.; Tabata, M. Isolation of antifungal principle tryptanthrin, from
470 *Strobilanthes cusia O. Kuntze*. *Planta medica* **1979**, *36*, 85−86.
471 (17) Honda, G.; Tosirisuk, V.; Tabata, M. Isolation of an antidermatophytic, tryptanthrin,
472 from indigo plants, Polygonum tinctorium and Isatis tinctoria. *Planta medica* **1980**, *38*,
473 275−276.
474 (18) Jun, K. Y.; Park, S. E.; Liang, J. L.; Jahng, Y.; Kwon, Y. Benzo [b] tryptanthrin
475 inhibits MDR1, topoisomerase activity, and reverses adriamycin resistance in breast
476 cancer cells. *ChemMedChem* **2015**, *10*, 827−835.
477 (19) Pergola, C.; Jazzar, B.; Rossi, A.; Northoff, H.; Hamburger, M.; Sautebin, L.; Werz,
478 O. On the inhibition of 5-lipoxygenase product formation by tryptanthrin: mechanistic
479 studies and efficacy in vivo. *Br. J. Pharmacol.* **2012**, *165*, 765−776.
480 (20) Krivogorsky, B.; Nelson, A. C.; Douglas, K. A., Grundt, P. Tryptanthrin derivatives
481 as Toxoplasma gondii inhibitors-structure–activity-relationship of the 6-position. *Bioorg.*
482 *Med. Chem. Lett*. **2013**, *23*, 1032−1035.
483 (21) Han, N. R.; Moon, P. D.; Kim, H. M.; Jeong, H. J. Tryptanthrin ameliorates atopic

484 dermatitis through down-regulation of TSLP. *Arch. Biochem. Biophys.* **2014**, *542*, 14−20.

485 (22) Sharma, V. M.; Prasanna, P.; Seshu, K. V. A.; Renuka, B.; Rao, C. V. L.; Kumar, G.

486 S.; Narasimhulu, C. P.; Babu, P. A.; Puranik, R. C.; Subramanyam, D.; Venkateswarlu,

487 A.; Rajagopal, S.; Kumar, K. B. S.; Rao, C. S.; Mamidi, N. V. S. R.; Deevi, D. S.;

488 Ajaykumar, R.; Rajagopalan, R. Novel indolo [2, 1-b] quinazoline analogues as

489 cytostatic agents: synthesis, biological evaluation and structure–activity relationship.

490 *Bioorg. Med. Chem. Let.* **2002**, *12*, 2303−2307.

491 (23) Liu, Y.; Song, H.; Huang, Y.; Li, J.; Zhao, S.; Song, Y.; Yang, P.; Xiao, Z.; Liu, Y.;

492 Li, Y.; Shang, H.; Wang, Q. Design, synthesis, and antiviral, fungicidal, and insecticidal

493 activities of tetrahydro-*β*-carboline-3-carbohydrazide derivatives. *J. Agric. Food Chem.*

494 **2014**, *62*, 9987−9999.

495 (24) Trott, O.; Olson, A. J. Autodock vina: Improving the speed and accuracy of docking

496 with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.*

497 **2010**, *31*, 455–461.

498 (25) Lv, P.; Chen, Y. L.; Shi, T. Z.; Wu, X. W.; Li, Q. X.; Hua, R. M. Synthesis and

499 fungicidal activities of sanguinarine derivatives. *Pestic. Biochem. Phys.* **2018**, *147*, 3–10.

500

501

Figure Captions

Figure 1. Design of Tryptanthrin Analogues.

Figure 2. Synthesis of Compounds **3a**–**3i**.

Figure 3. Synthesis of Compound **3j**.

Figure 4. Synthesis of Compounds **3k** and **5a**–**5d**.

Figure 5. Synthesis of Compounds **3l**–**3n**.

Figure 6. Synthesis of Compounds **8a**–**8b**, **10a**–**10b**, **11**, **13a**–**13b** and **14**–**16**.

Figure 7. Synthesis of Compound **19**.

Figure 8. TMV Rod Assembly Inhibition of Compound **16** and NK0209: (A) 20S CP disk + RNA (200 nm scale bar), (B) 20S CP disk + RNA + 1/100 DMSO(100 nm scale bar), (C) 20S CP disk + RNA + 10 μM NK0209 (100 nm scale bar), (D) 20S CP disk + RNA + 10 μM **16** (200 nm scale bar).

Figure 9. 20S CP Disk Assembly Inhibition of Compound **16** and NK0209 (100 nm scale bar): (A) CP, (B) CP + 1/100 DMSO, (C) CP +10 μM NK0209, (D) CP + 10 μM **16**.

Figure 10. Molecule Docking Results of Compounds **3n** (A), **14** (B), **16** (C) and Ribavirin (D) with TMV CP.

Table 1. *In Vivo* Antiviral Activities of Compounds **3a**–**3n**, **5a**–**5d**, **8a**–**8b**, **10a**–**10b**, **11**, **13a**–**13b**, **14**–**16**, **19** and Ribavirin Against TMV.

compd	concn (μg/mL)	inactive effect (%)[a]	curative effect (%)[a]	protective effect (%)[a]
3a	500	37±1	—	—
3b	500	39±3	—	—
3c	500	31±2	—	—
3d	500	31±5	—	—
3e	500	46±4	39±4	47±2
	100	8±2	13±1	18±1
3f	500	35±1	—	—
3g	500	44±2	42±2	35±4
	100	14±1	7±1	0
3h	500	32±1	—	—
3i	500	45±3	37±3	43±1
	100	16±1	11±2	7±1
3j	500	41±4	32±3	47±3
	100	8±2	0	11±1
3k	500	46±2	47±2	39±2
	100	15±3	11±1	0
3l	500	44±4	46±3	43±5
	100	0	5±1	9±1
3m	500	45±1	42±4	48±1
	100	14±1	11±1	19±2
3n	**500**	**52±2**	**49±5**	**54±4**
	100	**21±1**	**20±1**	**24±3**
5a	500	49±1	47±2	40±1
	100	21±2	17±3	13±1

5b	500	37±1	—	—
5c	500	36±3	—	—
5d	500	41±3	31±2	40±4
	100	10±1	—	8±1
8a	500	43±5	43±4	39±4
8b	500	33±4	—	—
10a	500	41±2	34±2	37±1
10b	500	23±1	—	—
11	500	35±5	—	—
13a	500	47±4	41±1	51±4
	100	10±4	14±3	17±1
13b	500	37±1	—	—
14	**500**	**51±3**	**48±1**	**53±4**
	100	**18±1**	**19±1**	**21±2**
15	500	44±3	36±2	42±3
	100	9±1	0	6±2
16	500	44±3	36±2	42±3
	100	9±1	0	6±2
19	500	48±2	41±4	43±4
	100	11±1	5±2	0
ribavirin	500	40±2	37±3	38±2
	100	12±1	10±1	9±1

[a] Average of three replicates; All results are expressed as mean ± SD; Activity Data with prominent were presented in pink bold with blue color.

Table 2. *In Vitro* Fungicidal Activities of Compounds **3a**–**3n**, **5a**–**5d**, **8a**–**8b**, **10a**–**10b**, **11**, **13a**–**13b**, **14**–**16**, **19**, Chlorothalonil, Carbendazim and Pyrimethanil against 14 Kinds of Fungi.

Compd	Fungicidal activities (%)[a] at 50 μg/mL													
	F.C[b]	C.H[b]	P.P[b]	R.C[b]	B.M[b]	W.A[b]	F.M[b]	A.S[b]	F.G[b]	P.I[b]	P.C[b]	S.S[b]	B.C[b]	R.S[b]
3a	20±1	30±2	**70±3**	**60±1**	28±2	29±2	20±1	31±2	8±2	13±1	42±2	13±1	16±1	4±1
3b	20±2	30±1	**79±1**	**61±2**	36±4	29±2	20±1	25±2	23±2	**63±3**	**65±3**	25±2	16±1	39±2
3c	20±1	19±2	**85±2**	46±1	23±2	26±1	17±2	31±1	12±1	25±2	19±1	38±2	13±2	35±1
3d	15±1	22±2	**76±2**	54±2	23±3	29±2	27±1	13±2	12±2	25±1	7±2	38±1	13±1	39±2
3e	15±1	56±2	**79±2**	48±2	23±2	21±3	27±2	13±2	4±1	38±1	10±2	13±2	6±2	4±1
3f	25±2	30±1	**79±1**	**67±1**	41±1	31±2	20±2	31±2	27±2	**63±2**	55±1	50±2	34±1	**82±2**
3g	18±1	22±2	**70±2**	44±2	31±1	36±2	27±1	50±2	31±1	50±1	3±1	50±2	19±1	59±2
3h	43±2	31±1	**71±1**	**60±2**	36±2	52±3	47±2	25±2	6±1	50±1	19±2	**85±3**	46±2	5±1
3i	15±1	30±2	**79±2**	37±1	23±2	26±3	27±2	13±1	19±2	31±2	10±1	13±2	9±1	4±1
3j	15±2	30±2	**70±1**	34±1	18±2	33±1	27±2	13±1	19±2	38±2	3±1	38±3	9±2	29±1
3k	23±1	52±3	**89±2**	**67±2**	28±1	29±2	50±1	25±2	12±1	38±2	23±2	13±1	25±2	20±2
3l	15±2	26±2	49±2	51±2	18±1	26±1	20±1	31±2	23±2	13±2	16±2	50±1	19±2	**61±2**
3m	33±1	26±1	**60±3**	34±2	44±2	41±2	37±2	25±1	19±1	**63±3**	**74±2**	38±2	19±2	**67±1**
3n	20±2	19±2	**60±2**	58±1	**81±2**	33±3	33±2	19±2	8±2	13±1	13±3	38±2	31±1	20±2
5a	15±1	22±1	**77±2**	42±2	31±1	29±1	17±1	13±2	8±2	25±2	10±1	13±1	13±2	14±1
5b	10±2	19±2	**60±1**	37±1	39±2	31±2	23±2	19±3	4±1	13±2	7±1	38±2	13±2	6±1
5c	8±1	19±1	45±2	38±2	10±2	21±2	33±3	25±1	8±1	25±1	29±2	13±1	19±1	20±2
5d	19±2	45±2	55±2	23±2	19±1	30±1	17±1	50±2	35±2	33±2	4±1	28±2	36±2	10±1
8a	23±1	22±2	**70±3**	57±2	36±2	36±2	37±1	31±1	23±1	25±2	29±2	13±1	16±2	29±2
8b	35±2	**67±1**	55±1	**66±1**	21±2	33±1	50±2	31±2	19±2	25±3	16±2	25±1	31±2	35±1
10a	13±1	19±2	**60±2**	39±2	23±1	19±2	17±2	13±2	19±2	38±1	10±1	38±2	19±3	58±2
10b	10±2	22±2	**66±2**	29±2	13±2	26±2	27±1	13±1	39±1	13±2	10±2	38±1	19±2	26±1
11	30±1	33±2	**79±2**	**66±1**	36±2	38±1	40±2	25±2	19±2	6±1	16±2	38±2	16±1	17±2
13a	13±2	19±1	59±1	32±2	18±1	24±2	33±3	6±2	4±1	6±2	7±1	6±1	6±2	6±1
13b	10±1	0	**70±2**	35±1	23±2	26±2	33±1	13±1	15±2	13±2	26±2	38±2	19±1	39±2
14	33±2	33±2	**62±1**	35±2	23±2	36±1	33±2	38±2	23±2	**75±1**	**71±2**	**63±2**	31±2	53±1
15	20±1	26±1	**76±2**	52±1	15±1	21±2	33±2	31±2	8±1	25±2	3±1	13±1	13±2	26±2

16	40±2	33±2	**74±3**	**86±2**	49±2	52±2	50±1	50±1	46±2	**63±1**	16±2	**63±2**	44±1	**67±1**
19	35±1	30±2	51±2	35±1	23±2	33±1	37±2	56±2	15±2	13±2	7±1	**63±2**	41±2	49±3
Chlorothalonil[c]	95±1	19±2	98±1	98±1	97±1	98±1	83±2	38±2	100	100	16±1	100	25±1	100
Carbendazim[c]	100	28±3	98±1	98±1	97±1	98±1	90±2	13±1	100	100	12±2	100	18±2	100
Pyrimethanil[c]	19±2	83±2	71±2	84±3	28±2	21±1	27±2	88±2	59±2	100	100	100	100	100

[a]Average of three replicates; all results are expressed as mean ± SD. [b]Abbreviations: F.O, *Fusarium oxysporum f. sp. cucumeris*; C.H, *Cercospora arachidicola Hori*; P.P, *Physalospora piricola*; R.C, *Rhizoctonia cerealis*; B.M, *Bipolaris maydis*; W.A, *watermelon anthracnose*; F.M, *Fusarium moniliforme*; A.S, *Alternaria solani*; F.G, *Fusarium graminearum*; P.I, *Phytophthora infestans*; P.C, *Phytophthora capsici*; S.S, *Sclerotinia sclerotiorum*; B.C, *Botrytis cinereal*; R.S, *Rhizoctonia solani*. [c]The commercial agricultural fungicides were used for comparison of antifungal activity; activity data with prominent results are presented in bold.

Figure 1.

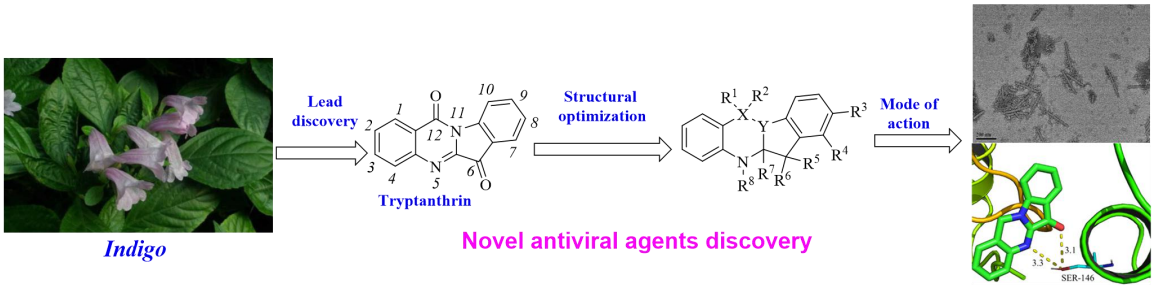
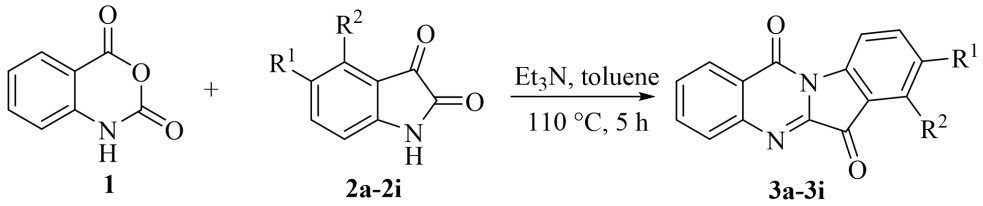


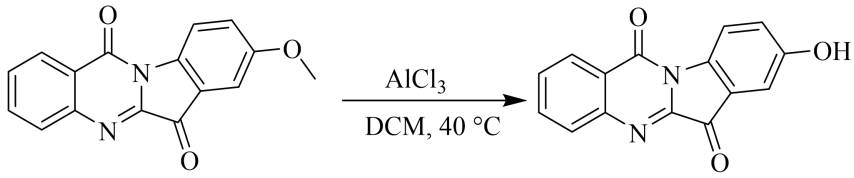
Figure 2.



1 **2a-2i** **3a-3i**

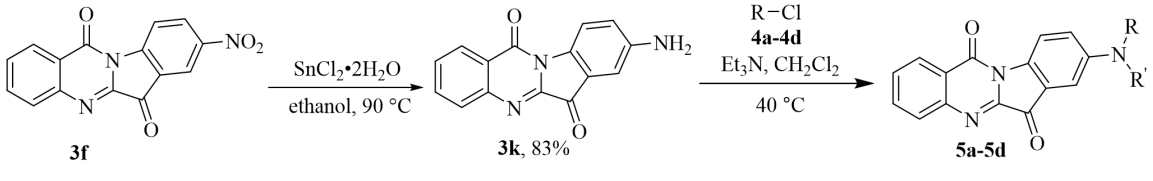
a R^1 = H, R^2 = H
b R^1 = F, R^2 = H
c R^1 = Cl, R^2 = H
d R^1 = Br, R^2 = H
e R^1 = NO_2, R^2 = H
f R^1 = OCH_3, R^2 = H
g R^1 = CH_3, R^2 = H
h R^1 = H, R^2 = Cl
i R^1 = H, R^2 = Br

Figure 3.



3e **3j**, 70%

Figure 4.



3f **3k**, 83% **5a-5d**

a R = , R' = H

b R , R' =

c R = , R' = H

d R , R' =

Figure 5.

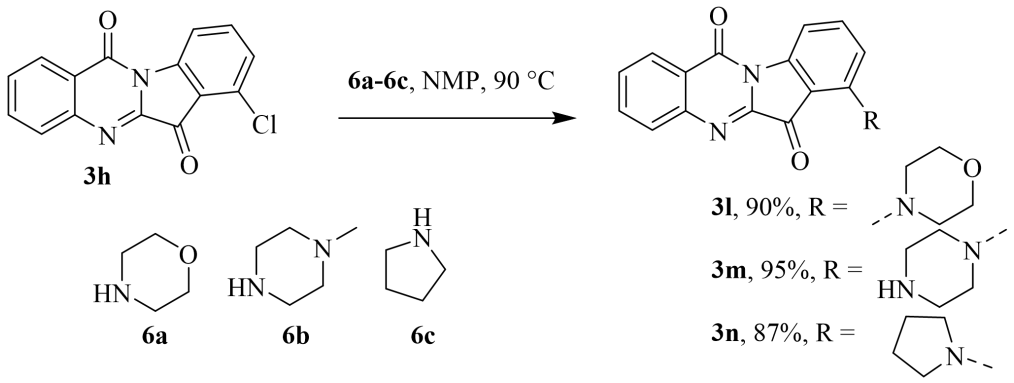


Figure 6.

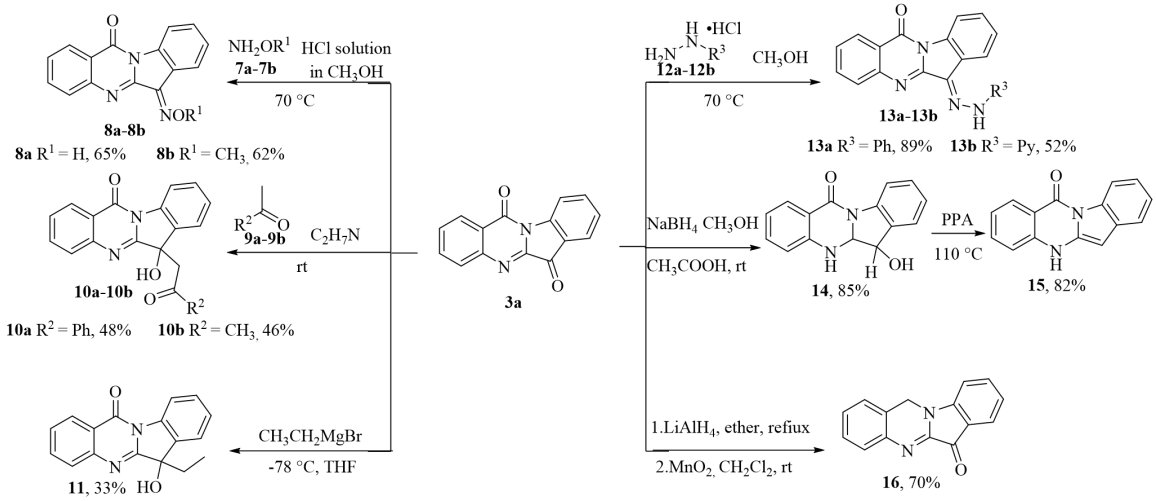


Figure 7.

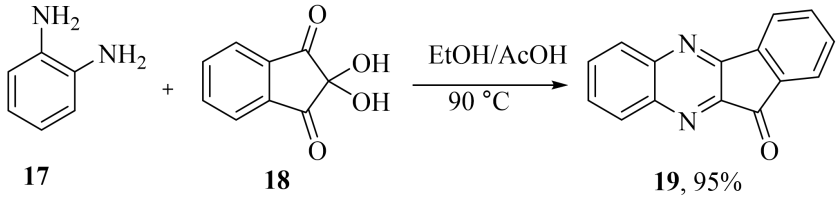


Figure 8.

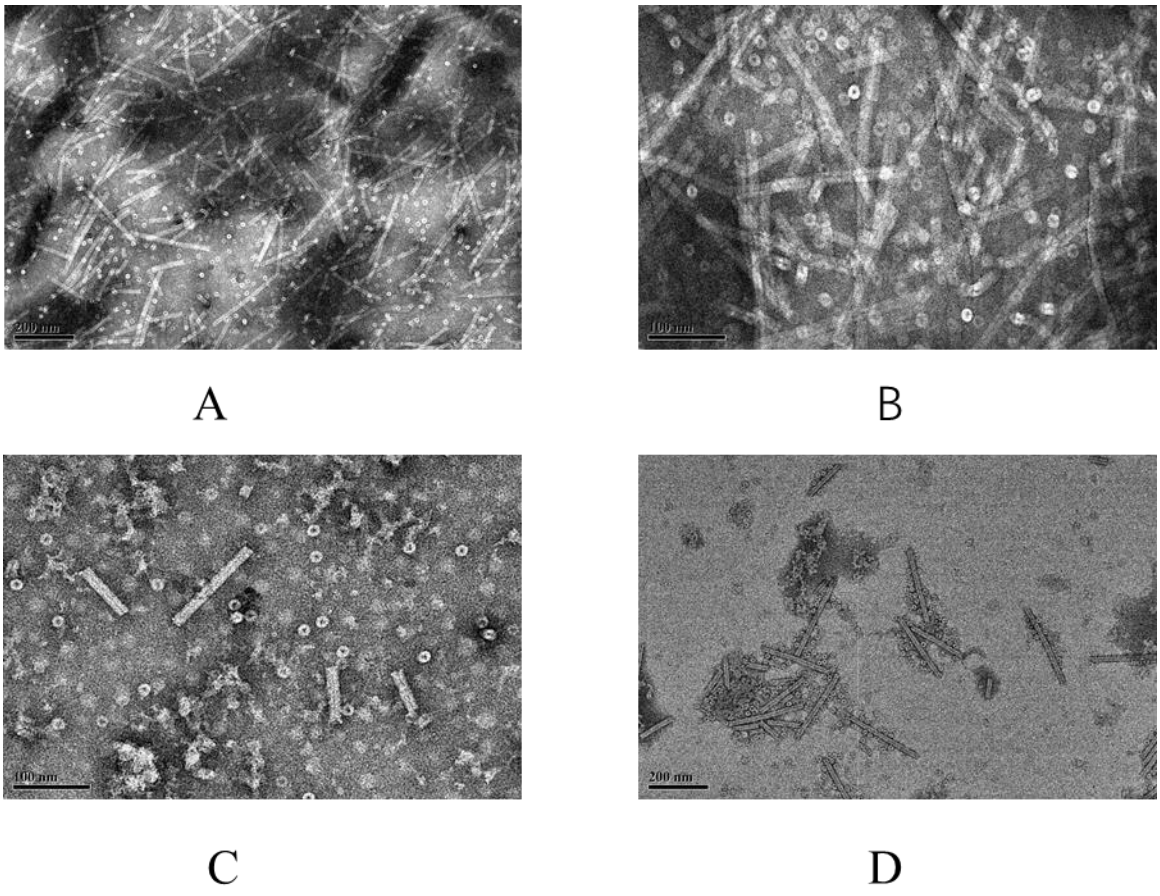


Figure 9.

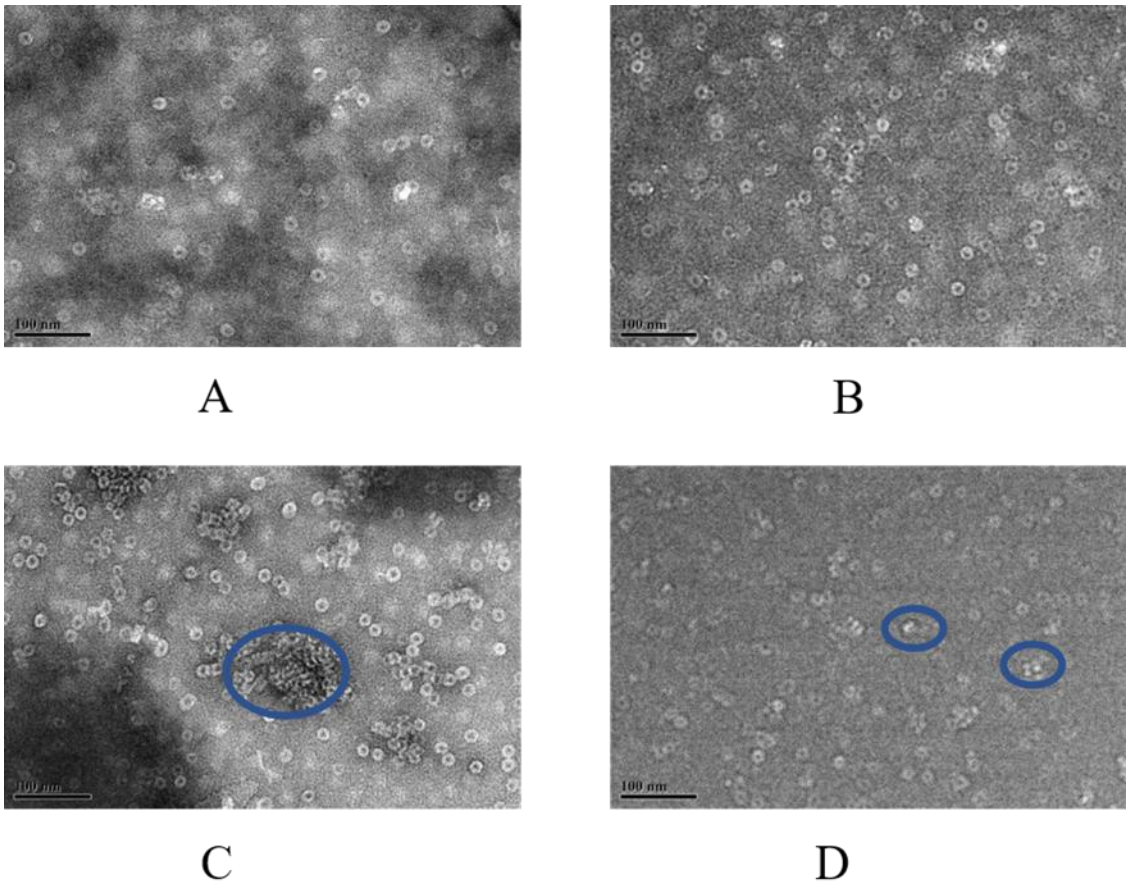
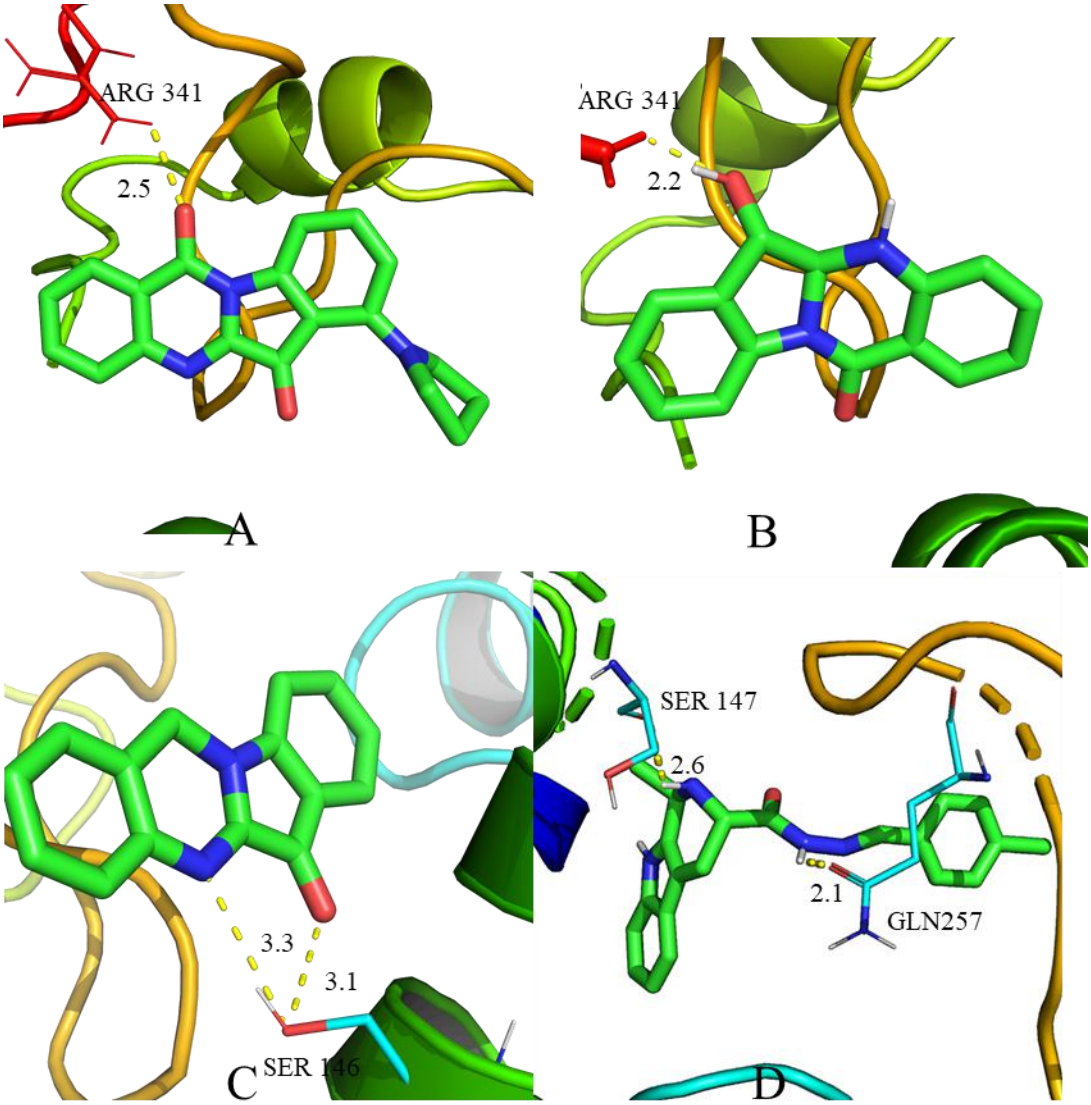


Figure 10.



TOC ***graphic***

Agrochemical Bioregulators