JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Agricultural and Environmental Chemistry

Discovery, Structural Optimization and Mode of Action of Essramycin Alkaloid and Its Derivatives as Anti-Tobacco Mosaic Virus (TMV) and Anti-phytopathogenic-Fungus Agents

tienan wang, shan yang, Hongyan Li, Aidang Lu, Ziwen Wang, Yingwu Yao, and Qingmin Wang

J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.9b06006 • Publication Date (Web): 16 Dec 2019

Just Accepted

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

Discovery, Structural Optimization and Mode of Action of Essramycin Alkaloid and Its Derivatives as Anti-Tobacco Mosaic Virus (TMV) and Anti-phytopathogenic-Fungus Agents

Tienan Wang[†], Shan Yang[†], Hongyan Li[†], Aidang Lu[†,*], Ziwen Wang[‡,*], Yingwu Yao[†,*], Qingmin Wang[§,*]

[†]National-Local Joint Engineering Laboratory for Energy Conservation in Chemical Process Integration and Resources Utilization, School of Chemical Engineering and Technology, Hebei University of Technology, Tianjin 300130, China;

[‡]Tianjin Key Laboratory of Structure and Performance for Functional Molecules, MOE Key Laboratory of Inorganic–Organic Hybrid Functional Material Chemistry, College of Chemistry, Tianjin Normal University, Tianjin 300387, China;

[§]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

* To whom correspondence should be addressed. For Aidang Lu, E-mail: luaidang@hebut.edu.cn;

Phone: 0086-22-60302812; Fax: 0086-22-60204274; For Ziwen Wang, E-mail: hxxywzw@tjnu.edu.cn; Phone: 0086-22-23766531; Fax: 0086-22-23766531; For Prof. Qingmin Wang, E-mail: wangqm@nankai.edu.cn; Phone: 0086-22-23503952; Fax: 0086-22-23503952.

ABSTRACT: Plant diseases seriously affect crop yield and quality and are difficult to control. Marine natural products (MNPs) have become an important source of drug candidates with new biological mechanisms. Marine natural product essramycin (**1**) was found to have good anti-tobacco mosaic virus (TMV) and anti-phytopathogenic-fungus activities for the first time. A series of essramycin derivatives were designed, synthesized and evaluated for their bio-activity. Most of these compounds exhibited higher antiviral effects than control ribavirin. Compounds **7e** and **8f** displayed higher antiviral activities than ningnanmycin (the most widely used antiviral agent at present), thus emerged as novel antiviral lead compounds. As the lead compound, **7e** was selected for further antiviral mechanism research. The results indicated that **7e** could inhibit virus assembly and promote 20S disk protein aggregation. Fungicidal activity tests against 14 kinds of phytopathogenic fungi revealed that essramycin analogues displayed broad-spectrum fungicidal activities. Compound **5b** displayed more than 50% inhibition rate against most of the 14 kinds of phytopathogenic fungi at 50 μg/mL. Current research lays a solid foundation for the application of essramycin alkaloids in crop protection.

KEYWORDS: marine natural product, essramycin, alkaloid, anti-TMV activity,

fungicidal activity, mode of action

INTRODUCTION

Plant diseases caused by plant viruses and pathogens have been recognized as a worldwide threat to the agricultural industry. Tobacco mosaic virus (TMV) is the first virus to be found, which is considered the most destructive disease of plant in the world. Because of its clear research, TMV is often used as a model virus in the development of antiviral agents. It has been found that TMV can infect more than 400 crops. As the widely used antiviral agents, ribavirin (less than 50% inhibitory effect at 500 μg/mL) and ningnanmycin (50-60% inhibitory effect at 500 μg/mL) (Figure 1) only gave moderate anti-TMV effects. How to control TMV is still a very challenging task.[1]

Natural products are still an important area to provide drug candidates with novel structure and unique mechanism.[2-4] However, the shortcomings of limited compound availability, poor solubility, and metabolic instability of natural products have always limited their direct application as drugs.[3] Till now, only a small amount of natural products are used directly as anti plant virus agents.[5-6] Structural optimization based on natural products is an important method to overcome these shortcomings.[7-13] Essramycin (Figure 1), obtained from the culture broth of the marine *Streptomyces sp*., is the first isolated triazolopyrimidine natural product. The bio-activity research revealed that essramycin

possesses antibacterial activity.[14-16] As the core framework, triazolopyrimidine is a well-known scaffold in agricultural and medicinal chemistry. These compounds have broad-spectrum biological activities, such as anticancer activity[17], phosphodiesterase inhibition activity[18] and anti-tubercular activity[19]. Triazolopyrimidine derivatives also have remarkable antibacterial activity[20], anti-epileptic activity[21], anti-mycobacterial activity[22], antimalarial activity[23-25]. As the widely used antiviral agent, ribavirin also contains triazole skeleton. Research revealed that ribavirin triphosphate (RTP) can interact with various viral RNA polymerases.[26,27] Essramycin and ribavirin contain the same core structural unit (Figure 1). Whether they have the same biological activity and mechanism of action is worth to further study.

Based on the above findings, natural product essramycin was selected as the parent structure. A series of essramycin analogues were designed (Figure 2), synthesized and evaluated for their anti-TMV and fungicidal activities. The preliminarily antiviral mechanism research was also carried out by transmission electron microscope (TEM).

MATERIALS AND METHODS

Instruments and Chemicals. The melting points of the products were determined on an X-4 binocular microscope (Gongyi Yuhua Instrument Co., China) and the thermometer was not corrected. NMR spectra were acquired with a Bruker 400 MHz (100 MHz for ^{13}C) instrument at room temperature. Chemical shifts were measured relative to residual solvent

peaks of $CDCl_3$ (^1H: δ = 7.26 ppm; ^{13}C: δ = 77.0 ppm) or d_6-DMSO (^1H: δ = 2.50 ppm; ^{13}C: δ = 39.5 ppm) with tetramethylsilane as internal standards. The following abbreviations were used to designate chemical shift multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, and bs = broad singlet. All first-order splitting patterns were assigned on the basis of multiplet appearance. Splitting patterns that could not be easily interpreted were designated multiplet (m) or broad (br). HRMS data were obtained with an FT-ICR MS spectrometer (Ionspec, 7.0 T). Procedures for the preparation of compounds **1**, **2**, 2-(5-amino-1*H*-1,2,4-triazol-3-yl)acetic acid (**3**), methyl 5-amino-1*H*-1,2,4-triazole-3-carboxylate (**4b**) and **5** (Figure 3) can been seen in Supporting Information. 5-Amino-4*H*-1,2,4-triazole-3-carboxylic acid (**4a**, Figure 3) was bought from *Bide Pharmatech Ltd*. All reagents were of analytical reagent grade or chemically pure. Among the synthesized compounds, **1**–**6**, **7a**–**7c**, **8a**, **8b**, **8d**, **8e**, **8g**, **9b**, **9c** and **9e** are reported compounds and their data were according with reference. The *in vitro* TMV rod assembly inhibition and 20S CP disk assembly inhibition were tested via transmission electron microscopy (Tecnai G2 F20).

Procedures for the Preparation of Compounds 6, 7a–7c.[15,16]

The solution of corresponding 3-substituted ethyl acetoacetate (2 mmol, 1.0 equiv.) and **3**, **4a** or **4b** (2 mmol, 1.0 equiv.) in CH_3COOH (20 mL) was refluxed for 10 h. The solvent was evaporated in vacuo and the residue was purified by recrystallization with ether to give compounds **6**, **7a**−**7c**.

2-(5-Methyl-7-oxo-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)acetic acid (**6**): white solid, 97% yield; mp 257−259 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 12.90 (br s, 2H, NH), 5.82 (s, 1H, C=CH), 3.73 (s, 2H, CH_2), 2.32 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 170.7, 159.2, 156.0, 151.8, 151.3, 98.7, 35.4, 19.1; HR-MS (ESI): Calcd for $C_8H_9N_4O_3$ $[M+H]^+$ 209.0669, found (ESI^+) 209.0672.

5-Methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(4*H*)-one (**7a**): white solid, 65% yield; mp 266−268 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 13.20 (br s, 1H, NH), 8.18 (s, 1H, N=CH), 5.82(s, 1H, C=CH), 2.31 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 155.8, 151.8, 151.6, 150.6, 98.1, 18.6; HR-MS (ESI): Calcd for $C_6H_7N_4O$ $[M+H]^+$ 151.0614, found (ESI^+) 151.0611.

5-Propyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(4*H*)-one (**7b**): white solid, 91% yield; mp 117−120 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 13.08 (br s, 1H, NH), 8.20 (s, 1H, N=CH), 5.84 (s, 1H, C=CH), 2.55 (t, J = 7.5 Hz, 2H, $CH_2CH_2CH_3$), 1.62−1.71 (m, 2H, $CH_2CH_2CH_3$), 0.90 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 156.0, 155.2, 151.9, 150.7, 97.6, 34.1, 21.2, 13.2; HR-MS (ESI): Calcd for $C_8H_{11}N_4O$ $[M+H]^+$ 179.0927, found (ESI^+) 179.0923.

Methyl 5-methyl-7-oxo-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidine-2-carboxylate (**7c**): white solid, 98% yield; mp 232−234 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 13.39 (br s, 1H, NH), 5.94 (s, 1H, C=CH), 3.91 (s, 3H, OCH_3), 2.35 (s, 3H, CCH_3); ^{13}C NMR (DMSO-d_6,

100 MHz): 160.0, 155.6, 153.0, 152.7, 151.1, 98.7, 52.6, 18.8; HR-MS (ESI): Calcd for $C_8H_9N_4O_3$ $[M+H]^+$ 209.0669, found (ESI^+) 209.0668.

Procedure for the Preparation of Compound 7d.

Compound **7c** (0.84 g, 4 mmol, 1.0 equiv.) and hydrazine hydrate (80% solution in water, 1.6 mL, 40 mmol, 10 equiv.) were dissolved in ethanol (20 mL). The mixture was refluxed for 16 h. The suspension was cooled to room temperature and filtered. Compound **7d** was obtained as a white solid, 98% yield; mp 213−214 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 9.79 (br s, 1H, CNHC=N), 7.27 (br s, 3H, $NHNH_2$), 5.54 (s, 1H, C=CH), 2.20 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 162.3, 159.7, 158.1, 157.9, 155.0, 95.4, 24.2; HR-MS (ESI): Calcd for $C_7H_9N_6O_2$ $[M+H]^+$ 209.0781, found (ESI^+) 209.0783.

Procedure for the Preparation of Compound 7e.

To a solution of **7c** (0.97 g, 5.0 mmol, 1.0 equiv.) in tetrahydrofuran (40 mL) and water (8 mL) was added lithium hydroxide hydrate (0.36 g, 15.0 mmol). The reaction mixture was stirred at room temperature for 12 h, then the mixture was evaporated in vacuo and the residue was diluted with ice water (20 mL) and adjusted to pH = 3 by 4 M HCl solution. The solution was refrigerated at 0 °C for 10 h. Compound **7e** was collected by filtration as a white solid, 91% yield; mp 187−190 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 13.40 (br s, 1H, NH), 5.90 (s, 1H, C=CH), 2.33 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 160.4, 155.2, 153.5, 152.0, 150.5, 98.0, 18.2; HR-MS (ESI): Calcd for $C_7H_7N_4O_3$ $[M+H]^+$

195.0514, found (ESI$^+$) 195.0518.

Procedures for the Preparation of Compounds 8a–8g.

The solution of corresponding **5** (2 mmol, 1.0 equiv.), ethyl acetoacetate (2.4 mmol, 1.2 equiv.) in CH_3COOH (2 mL) was refluxed for 6 h. After cooling, ethanol (15 mL) was added to the solution. The precipitate was filtered to obtain the target compounds **8a**–**8g**.

2-(Benzylthio)-5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(4*H*)-one: white solid (**8a**): 95% yield; mp 214−217 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 13.18 (s, 1H, NH), 7.44−7.46 (m, 2H, ArH), 7.26−7.34 (m, 3H, ArH), 5.81 (s, 1H, C=CH), 4.43 (s, 2H, CH_2), 2.29 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 162.0, 154.8, 151.1, 150.7, 137.4, 128.8, 128.5, 127.3, 98.5, 34.5, 18.5; HR-MS (ESI): Calcd for $C_{13}H_{13}N_4OS$ $[M+H]^+$ 273.0805, found (ESI$^+$) 273.0809.

5-Methyl-2-((4-methylbenzyl)thio)-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(4*H*)-one (**8b**): white solid, 91% yield; mp 248−250 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 13.18 (s, 1H, NH), 7.32 (d, J = 7.8 Hz, 2H, ArH), 7.12 (d, J = 7.7 Hz, 2H, ArH), 5.81 (s, 1H, C=CH), 4.38 (s, 2H, CH_2), 2.29 (s, 3H, CCH_3), 2.26 (s, 3H, $ArCH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz): 162.0, 154.8, 151.1, 150.7, 136.5, 134.2, 129.0, 128.8, 98.5, 34.3, 20.7, 18.5; HR-MS (ESI): Calcd for $C_{14}H_{15}N_4OS$ $[M+H]^+$ 287.0961, found (ESI$^+$) 287.0957.

2-((3-Methoxybenzyl)thio)-5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(4*H*)-one (**8c**): white solid, 91% yield; mp 153−155 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 13.19 (s, 1H,

NH), 7.23 (t, J = 7.8 Hz, 1H, ArH), 7.05 (s, 1H, , ArH), 7.01 (d, J = 7.5 Hz, 1H, ArH), 6.83 (d, J = 9.4 Hz, 1H, ArH), 5.81 (s, 1H, C=CH), 4.40 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), 2.29 (s, 3H, CCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 162.0, 159.2, 154.8, 151.1, 150.7, 138.9, 129.5, 121.0, 114.5, 12.8, 98.5, 55.0, 34.5, 18.5; HR-MS (ESI): Calcd for $C_{14}H_{15}N_4O_2S$ $[M+H]^+$ 303.0910, found (ESI^+) 303.0914.

2-((4-Fluorobenzyl)thio)-5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(4*H*)-one (**8d**): white solid, 93% yield; mp 214−217 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 13.19 (s, 1H, NH), 7.50 (t, J = 8.0 Hz, 2H, ArH), 7.16 (t, J = 8.8 Hz, 2H, ArH), 5.81 (s, 1H, C=CH), 4.43 (s, 2H, CH_2), 2.29 (s, 3H, CCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 161.9, 161.4 (d, J = 243.4 Hz), 154.8, 151.2, 150.7, 133.8 (d, J = 2.8 Hz), 130.8 (d, J = 8.2 Hz), 115.2 (d, J = 21.3 Hz), 98.5, 33.6, 18.5; HR-MS (ESI): Calcd for $C_{13}H_{12}FN_4OS$ $[M+H]^+$ 297.0710, found (ESI^+) 297.0708.

2-((4-Chlorobenzyl)thio)-5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(4*H*)-one (**8e**): white solid, 90% yield; mp 248−251 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 13.19 (s, 1H, NH), 7.36−7.48 (m, 4H, ArH), 5.81 (s, 1H, C=CH), 4.42 (s, 2H, CH_2), 2.28 (s, 3H, CCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 161.7, 154.8, 151.1, 150.7, 136.8, 131.9, 130.7, 128.4, 98.5, 33.7, 18.5; HR-MS (ESI): Calcd for $C_{13}H_{12}ClN_4OS$ $[M+H]^+$ 307.0415, found (ESI^+) 307.0413.

2-((2-Chloro-4-fluorobenzyl)thio)-5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(4*H*)-one

(**8f**): white solid, 94% yield; mp 216−219 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 13.21 (s, 1H, NH), 7.66 (t, J = 8.1 Hz, 1H, ArH), 7.50 (d, J = 8.5 Hz, 1H, ArH), 7.21 (t, J = 8.3 Hz, 1H, ArH), 5.83 (s, 1H, C=CH), 4.50 (s, 2H, CH_2), 2.29 (s, 3H, CCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 162.5, 161.4, 160.0, 154.8, 151.0 (d, J = 44.3 Hz), 134.0 (d, J = 10.7 Hz), 132.5 (d, J = 9.0 Hz), 131.1 (d, J = 3.3 Hz), 116.8 (d, J = 25.2 Hz), 114.5 (d, J = 21.1 Hz), 98.6, 32.0, 18.5; HR-MS (ESI): Calcd for $C_{13}H_{11}ClFN_4OS$ $[M+H]^+$ 325.0321, found (ESI^+) 325.0325.

5-Methyl-2-((4-nitrobenzyl)thio)-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(4*H*)-one (**8g**): white solid, 94% yield; mp 256−259 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 13.21 (s, 1H, NH), 8.18 (d, J = 8.5 Hz, 2H, ArH), 7.73 (d, J = 8.5 Hz, 2H, ArH), 5.82 (s, 1H, C=CH), 4.56 (s, 2H, CH_2), 2.28 (s, 3H, CCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 161.4, 154.8, 151.2, 150.8, 146.6, 146.0, 130.0, 123.5, 98.6, 33.7, 18.5; HR-MS (ESI): Calcd for $C_{13}H1_2N_5O_3S$ $[M+H]^+$ 318.0655, found (ESI^+) 318.0658.

Procedures for the Preparation of Compounds 9a–9o, 10.

The mixture of corresponding **5** (2 mmol, 1.0 equiv.), ethyl acetoacetate or acetylacetone (2.4 mmol, 1.2 equiv.), substituted aldehydes (1.0 mmol, 1.0 equiv.) in DMF (1.0 mL) was heated in oil bath at 140 °C for 2 h. After cooling, water (5 mL) was added to the solution. The precipitate was filtered and washed with ether to obtain the desired products **9a**–**9o**, **10**.

Ethyl 2-(benzylthio)-5-methyl-7-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxylate (**9a**): white solid, 90% yield; mp 171−173 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 10.85 (s, 1H, NH), 7.19−7.35 (m, 10H, ArH), 6.22 (s, 1H, CH), 4.23 (d, J = 13.2 Hz, 1H, SCH_2), 4.16 (d, J = 13.2 Hz, 1H, SCH_2), 3.92−3.97 (m, 2H, OCH_2CH_3), 2.40 (s, 3H, CCH_3), 1.04 (t, J = 7.1 Hz, 3H, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 165.0, 157.7, 147.7, 146.3, 141.8, 138.0, 128.7, 128.4, 128.2, 128.0, 127.0, 97.6, 59.5, 59.4, 34.7, 18.4, 13.8; HR-MS (ESI): Calcd for $C_{22}H_{23}N_4O_2S$ $[M+H]^+$ 407.1536, found (ESI^+) 407.1533.

Ethyl 2-(benzylthio)-5-methyl-7-(*p*-tolyl)-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxylate (**9b**): white solid, 90% yield; mp 157−159 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 10.84 (s, 1H, NH), 7.24−7.27 (m, 2H, ArH), 7.19−7.20 (m, 3H, ArH), 7.09−7.14 (m, 4H, ArH), 6.17 (s, 1H, CH), 4.23 (d, J = 13.2 Hz, 1H, SCH_2), 4.16 (d, J = 13.2 Hz, 1H, SCH_2), 3.92−3.97 (m, 2H, OCH_2CH_3), 2.39 (s, 3H, CCH_3), 2.26 (s, 3H, $ArCH_3$), 1.06 (t, J = 7.1 Hz, 3H, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 165.5, 158.1, 148.2, 146.6, 139.5, 138.4, 137.7, 129.4, 129.2, 128.7, 127.5, 127.4, 98.2, 59.9, 59.7, 35.3, 21.1, 18.8, 14.3; HR-MS (ESI): Calcd for $C_{23}H_{25}N_4O_2S$ $[M+H]^+$ 421.1693, found (ESI^+) 421.1688.

Ethyl 2-(benzylthio)-7-(4-methoxyphenyl)-5-methyl-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxylate (**9c**): white solid, 92% yield; mp 170−172 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 10.82 (s, 1H, NH), 7.25−7.27 (m, 2H, ArH), 7.19−7.21 (m, 3H, ArH), 7.14 (d, J = 8.7 Hz, 2H, ArH), 6.87 (d, J = 8.7 Hz, 2H, ArH), 6.17 (s, 1H, CH), 4.23 (d, J = 13.2

Hz, 1H, SCH_2), 4.16 (d, J = 13.2 Hz, 1H, SCH_2), 3.92−3.96 (m, 2H, OCH_2CH_3), 3.72 (s, 3H, OCH_3), 2.39 (s, 3H, CCH_3), 1.06 (t, J = 7.1 Hz, 3H, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 165.5, 159.4, 158.0, 148.1, 146.4, 138.5, 134.5, 129.2, 128.7, 127.5, 114.2, 98.3, 59.9, 59.4, 55.5, 35.2, 18.8, 14.4; HR-MS (ESI): Calcd for $C_{23}H_{25}N_4O_3S$ $[M+H]^+$ 437.1642, found (ESI^+) 437.1645.

Ethyl 2-(benzylthio)-7-(4-fluorophenyl)-5-methyl-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]

pyrimidine-6-carboxylate (**9d**): white solid, 89% yield; mp 153−156 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 10.90 (s, 1H, NH), 7.24−7.30 (m, 4H, ArH), 7.13−7.20 (m, 5H, ArH), 6.24 (s, 1H, CH), 4.23 (d, J = 13.2 Hz, 1H, SCH_2), 4.16 (d, J = 13.2 Hz, 1H, SCH_2), 3.90−4.00 (m, 2H, OCH_2CH_3), 2.40 (s, 3H, CCH_3), 1.03 (t, J = 7.1 Hz, 3H, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 165.4, 162.1 (d, J = 244.1 Hz), 158.3, 148.0, 147.0, 138.7, 138.5, 129.6 (d, J = 8.4 Hz), 129.2, 128.7, 127.5, 115.6 (d, J = 15.9 Hz), 97.8, 59.9, 59.3, 35.2, 18.9, 14.3; HR-MS (ESI): Calcd for $C_{22}H_{22}FN_4O_2S$ $[M+H]^+$ 425.1442, found (ESI^+) 425.1447.

Ethyl 2-(benzylthio)-7-(4-chlorophenyl)-5-methyl-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]

pyrimidine-6-carboxylate (**9e**): white solid, 90% yield; mp 150−152 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 10.92 (s, 1H, NH), 7.56 (d, J = 8.5 Hz, 2H, ArH), 7.19−7.25 (m, 7H, ArH), 6.22 (s, 1H, CH), 4.23 (d, J = 13.2 Hz, 1H, SCH_2), 4.14 (d, J = 13.3 Hz, 1H, SCH_2), 3.92−3.99 (m, 2H, OCH_2CH_3), 2.40 (s, 3H, CCH_3), 1.03 (t, J = 7.1 Hz, 3H, OCH_2CH_3);

210 ^{13}C NMR (DMSO-d_6, 100 MHz): 165.4, 158.3, 148.1, 147.2, 141.4, 138.5, 133.0, 131.6,
211 129.2, 128.9, 128.7, 127.5, 97.6, 60.0, 59.4, 35.2, 18.9, 14.4; HR-MS (ESI): Calcd for
212 $C_{22}H_{22}ClN_4O_2S$ $[M+H]^+$ 441.1147, found (ESI^+) 441.1153.

213 Ethyl 2-(benzylthio)-7-(4-bromophenyl)-5-methyl-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]
214 pyrimidine-6-carboxylate (**9f**): white solid, 93% yield; mp 143−147 °C; ^{1}H NMR (DMSO-
215 d_6, 400 MHz): 10.91 (s, 1H, NH), 7.53 (d, J = 8.4 Hz, 2H, ArH), 7.14−7.24 (m, 7H, ArH),
216 6.22 (s, 1H, CH), 4.23 (d, J = 13.3 Hz, 1H, SCH_2), 4.15 (d, J = 13.3 Hz, 1H, SCH_2),
217 3.91−3.98 (m, 2H, OCH_2CH_3), 2.40 (s, 3H, CCH_3), 1.04 (t, J = 7.1 Hz, 3H, OCH_2CH_3);
218 ^{13}C NMR (DMSO-d_6, 100 MHz): 165.4, 158.4, 148.1, 147.2, 141.7, 138.4, 131.8, 129.8,
219 129.2, 128.7, 127.5, 121.7, 97.5, 60.0, 59.5, 35.2, 18.9, 14.3; HR-MS (ESI): Calcd for
220 $C_{22}H_{22}BrN_4O_2S$ $[M+H]^+$ 485.0641 found (ESI^+) 485.0643.

221 Ethyl 2-(benzylthio)-7-(furan-2-yl)-5-methyl-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]
222 pyrimidine-6-carboxylate (**9g**): white solid, 82% yield; mp 195−197 °C; ^{1}H NMR (DMSO-
223 d_6, 400 MHz): 10.92 (s, 1H, NH), 7.56 (s, 1H, CH), 7.31−7.32 (m, 2H, ArH), 7.21−7.27
224 (m, 3H, ArH), 6.39−6.40 (m, 1H, ArH), 6.33 (m, 2H, ArH), 4.27 (d, J = 13.2 Hz, 1H, SCH_2),
225 4.20 (d, J = 13.2 Hz, 1H, SCH_2), 3.96−4.07 (m, 2H, OCH_2CH_3), 2.38 (s, 3H, CCH_3), 1.10
226 (t, J = 7.1 Hz, 3H, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 165.3, 158.3, 153.5, 148.5,
227 147.5, 143.2, 138.4, 129.3, 128.8, 127.6, 111.0, 108.0, 95.4, 59.9, 53.3, 35.2, 18.9, 14.4;
228 HR-MS (ESI): Calcd for $C_{20}H_{21}N_4O_3S$ $[M+H]^+$ 397.1329, found (ESI^+) 397.1327.

Ethyl 2-(benzylthio)-5-methyl-7-(thiophen-2-yl)-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]

pyrimidine-6-carboxylate (**9h**): white solid, 95% yield; mp 170−172 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 10.98 (s, 1H, NH), 7.43−7.45 (m, 1H, ArH), 7.31−7.33 (m, 2H, ArH), 7.21−7.27 (m, 3H, ArH), 6.94−6.96 (m, 2H), 6.53 (s, 1H, CH), 4.26 (d, J = 13.3 Hz, 1H, SCH_2), 4.21 (d, J = 13.3 Hz, 1H, SCH_2), 3.96−4.07 (m, 2H, OCH_2CH_3), 2.39 (s, 3H, CCH_3), 1.10 (t, J = 7.1 Hz, 3H, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 164.8, 157.9, 147.7, 145.0, 137.9, 128.8, 128.3, 127.1, 126.8, 125.9, 125.6, 97.6, 59.5, 54.3, 34.8, 18.3, 13.9; HR-MS (ESI): Calcd for $C_{20}H_{21}N_4O_2S_2$ $[M+H]^+$ 413.1100, found (ESI^+) 413.1101.

Ethyl 2-(benzylthio)-5-methyl-7-propyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine

-6-carboxylate (**9i**): white solid, 75% yield; mp 172−174 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 10.56 (s, 1H, NH), 7.36−7.38 (m, 2H, ArH), 7.23−7.30 (m, 3H, ArH), 5.27 (t, J = 3.92 Hz, 1H, CH), 4.30 (d, J = 13.4 Hz, 1H, SCH_2), 4.25 (d, J = 13.4 Hz, 1H, SCH_2), 4.05−4.17 (m, 2H, OCH_2CH_3), 2.30 (s, 3H, CCH_3), 1.71−1.80 (m, 1H,), 1.54−1.61 (m, 1H), 1.23 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 1.68−1.24 (m, 1H, $CH_2CH_2CH_3$), 0.75−0.82 (m, 4H, $CH_2CH_2CH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz): 165.7, 157.6, 149.0, 147.6, 138.5, 129.2, 128.8, 127.6, 96.8, 60.0, 56.0, 38.0, 35.5, 18.9, 17.2, 14.6, 14.0; HR-MS (ESI): Calcd for $C_{19}H_{25}N_4O_2S$ $[M+H]^+$ 373.1693, found (ESI^+) 373.1697.

Ethyl 5-methyl-2-((4-methylbenzyl)thio)-7-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]

pyrimidine-6-carboxylate (**9j**): white solid, 95% yield; mp 157−160 °C; ^{1}H NMR (DMSO-

248 d_6, 400 MHz): 10.87 (s, 1H, NH), 6.99−7.33 (m, 10H, ArH), 6.21 (s, 1H, CH), 4.18 (d, J =
249 12.8 Hz, 1H, SCH_2), 4.12 (d, J = 12.7 Hz, 1H, SCH_2), 3.93−3.96 (m, 2H, CH_2CH_3), 2.40
250 (s, 3H, CCH_3), 2.23 (s, 3H, $ArCH_3$), 1.04 (t, J = 5.6 Hz, 3H, CH_2CH_3); ^{13}C NMR (DMSO-d_6,
251 100 MHz): 165.0, 157.7, 147.6, 146.3, 141.8, 136.2, 134.8, 128.8, 128.6, 128.4, 127.9,
252 127.0, 97.6, 59.5, 59.4, 34.5, 20.6, 18.4, 13.8; HR-MS (ESI): Calcd for $C_{23}H_{25}N_4O_2S$
253 $[M+H]^+$ 421.1693, found (ESI^+) 421.1697.

254 Ethyl 5-methyl-2-((3-methoxybenzyl)thio)-7-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]

255 pyrimidine-6-carboxylate (**9k**): white solid, 93% yield; mp 151−153 °C; ^{1}H NMR (DMSO-
256 d_6, 400 MHz): 10.89 (s, 1H, NH), 7.21−7.32 (m, 5H, ArH), 7.11 (t, J = 7.9 Hz, 1H, ArH),
257 6.88 (s, 1H, ArH), 6.82 (d, J = 7.3 Hz, 1H, ArH), 6.77 (d, J = 7.9 Hz, 1H, ArH), 6.21 (s,
258 1H, CH), 4.20 (d, J = 13.4 Hz, 1H, SCH_2), 4.16 (d, J = 13.1 Hz, 1H, SCH_2), 3.93−3.97 (m,
259 2H, CH_2CH_3), 3.67 (s, 3H, OCH_3), 2.40 (s, 3H, CCH_3), 1.03 (t, J = 7.0 Hz, 3H, CH_2CH_3);
260 ^{13}C NMR (DMSO-d_6, 100 MHz): 164.9, 159.1, 157.8, 147.7, 146.3, 141.8, 139.3, 129.3,
261 128.4, 127.9, 127.0, 120.9, 114.2, 112.6, 97.7, 59.5, 59.4, 54.9, 34.8, 18.3, 13.8; HR-MS
262 (ESI): Calcd for $C_{23}H_{25}N_4O_3S$ $[M+H]^+$ 437.1642, found (ESI^+) 437.1639.

263 Ethyl 5-methyl-2-((4-fluorobenzyl)thio)-7-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]

264 pyrimidine-6-carboxylate (**9l**): white solid, 91% yield; mp 179−181 °C; ^{1}H NMR (DMSO-
265 d_6, 400 MHz): 10.87 (s, 1H, NH), 7.22−7.34 (m, 7H, ArH), 6.99 (t, J = 8.7 Hz, 2H, ArH),
266 6.21 (s, 1H, CH), 4.22 (d, J = 13.4 Hz, 1H, SCH_2), 4.14 (d, J = 13.4 Hz, 1H, SCH_2),

3.93−3.96 (m, 2H, CH_2CH$_3$), 2.40 (s, 3H, CCH$_3$), 1.02 (t, J = 7.0 Hz, 3H, CH$_2$CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 165.0, 161.4 (d, J = 243.1 Hz), 157.5, 147.7, 146.3, 141.8, 134.3 (d, J = 2.6 Hz), 130.7 (d, J = 8.1 Hz), 128.4, 128.0, 127.0, 114.9 (d, J = 21.3 Hz), 97.6, 59.5, 59.4, 33.8, 18.4, 13.8; HR-MS (ESI): Calcd for $C_{22}H_{22}FN_4O_2S$ $[M+H]^+$ 425.1442, found (ESI$^+$) 425.1447.

Ethyl 5-methyl-2-((4-chlorobenzyl)thio)-7-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]

pyrimidine-6-carboxylate (**9m**): white solid, 93% yield; mp 108−111 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): 10.58 (s, 1H, NH), 7.32 (s, 5H, ArH), 7.05−7.07 (m, 4H, ArH), 6.34 (s, 1H, CH), 4.23 (d, J = 13.4 Hz, 1H, SCH$_2$), 4.04−4.08 (m, 2H, CH_2CH$_3$), 4.02 (d, J = 13.4 Hz, 1H, SCH$_2$), 2.57 (s, 3H, CCH$_3$), 1.13 (t, J = 7.1 Hz, 3H, CH$_2$CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 164.6, 157.0, 147.3, 146.0, 141.5, 136.9, 131.2, 130.2, 128.1, 127.8, 127.6, 126.7, 97.2, 59.2, 59.0, 33.5, 18.0, 13.5; HR-MS (ESI): Calcd for $C_{22}H_{22}ClN_4O_2S$ $[M+H]^+$ 441.1447, found (ESI$^+$) 441.1145.

Ethyl 5-methyl-2-((2-chloro-4-fluorobenzyl)thio)-7-phenyl-4,7-dihydro-[1,2,4]triazolo

[1,5-*a*]pyrimidine-6-carboxylate (**9n**): white solid, 94% yield; mp 195−198 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): 10.78 (s, 1H, NH), 7.32 (s, 5H, ArH), 6.96−7.03 (m, 2H, ArH), 6.50 (t, J = 6.5 Hz, 1H, ArH), 6.34 (s, 1H, ArH), 4.31 (d, J = 13.7 Hz, 1H, SCH$_2$), 4.13 (d, J = 13.6 Hz, 1H, SCH$_2$), 4.04−4.07 (m, 2H, CH_2CH$_3$), 2.57 (s, 3H, CCH$_3$), 1.13 (t, J = 6.9 Hz, 3H, CH$_2$CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 164.9, 161.0 (d, J = 247.4 Hz), 157.0,

147.7, 146.3, 141.8, 133.8 (d, J = 10.6 Hz), 132.2 (d, J = 8.5 Hz), 131.7 (d, J = 3.1 Hz), 128.4, 128.0, 127.1, 116.6 (d, J = 25.1 Hz), 114.0 (d, J = 21.0 Hz), 97.6, 59.6, 59.4, 32.2, 18.3, 13.8; HR-MS (ESI): Calcd for $C_{22}H_{21}ClFN_4O_2S$ $[M+H]^+$ 459.1052, found (ESI^+) 459.1047.

Ethyl 5-methyl-2-((4-nitrobenzyl)thio)-7-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-*a*] pyrimidine-6-carboxylate (**9o**): white solid, 91% yield; mp 172−175 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 10.86 (s, 1H, NH), 8.00 (d, J = 8.5 Hz, 2H, ArH), 7.48 (d, J = 8.5 Hz, 2H, ArH), 7.27−7.36 (m, 3H, ArH), 7.20−7.22 (m, 2H, ArH), 6.20 (s, 1H, CH), 4.34 (d, J = 14.0 Hz, 1H, SCH_2), 4.26 (d, J = 13.9 Hz, 1H, SCH_2), 3.92−3.96 (m, 2H, CH_2CH_3), 2.39 (s, 3H, CCH_3), 1.02 (t, J = 7.1 Hz, 3H, CH_2CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 164.9, 162.3, 156.9, 147.8, 146.5, 146.3, 141.8, 129.9, 128.4, 127.9, 127.0, 123.2, 97.6, 59.5, 59.4, 33.8, 18.3, 13.8; HR-MS (ESI): Calcd for $C_{22}H_{22}N_5O_4S$ $[M+H]^+$ 452.1387, found (ESI^+) 452.1389.

(2-(Benzylthio)-5-methyl-7-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidin-6-yl)ethanone (**10**): white solid, 87% yield; mp 203−205 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): 10.85 (s, 1H, NH), 7.25−7.33 (m, 7H, ArH), 7.19−7.21 (m, 3H, ArH), 6.42 (s, 1H, CH), 4.25 (d, J = 13.3 Hz, 1H, SCH_2), 4.17 (d, J = 13.3 Hz, 1H, SCH_2), 2.42 (s, 3H, CCH_3), 2.13 (s, 3H, $C{=}OCH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz):195.2, 158.2, 147.9, 146.1, 141.8, 138.5, 129.1, 129.2, 128.9, 128.8, 127.8, 127.5, 108.2, 59.9, 35.2, 31.0, 19.9; HR-MS (ESI):

Calcd for $C_{21}H_{21}N_4OS$ $[M+H]^+$ 377.1431, found (ESI^+) 377.1437

Biological Assay. Each bioassay was repeated three times at 25±1 °C. Activity results were estimated according to a percentage scale of 0−100 (0: no activity; 100: total inhibited).

Detailed bioassay procedures for the anti-TMV[28] and and fungicidal[29-31] activities were described in literature and also can be found in the Supporting Information.

Mode of Action Studies. The detailed procedures of *in vitro* TMV rod and 20S CP disk assembly inhibition was described in literature[13] and also can been found in the Supporting Information.

RESULTS AND DISCUSSION

Chemistry.

*Synthesis of the Compounds **1**−**10***. Procedures for the preparation of compounds **1**, **2**, **3**, **4b** and **5a**−**5g** (Figure 3) can been seen in Supporting Information. Essramycin (**1**) and 2-(5-amino-4*H*-1,2,4-triazol-3-yl)-1-phenylethanone (**2**) were synthesized according to the method reported by Moody with modification.[15] Methyl ester **4b** was obtained in good yield by esterification of **4a** with methanol in the presence of $SOCl_2$.[32] Condensation of 3-amino-5-thiol-1,2,4-triazole and corresponding alkyl bromides in the presence of triethyl amine to give triazoles **5a**−**5g** in excellent yields.[20] Compounds **6**, **7a**−**7c** and **8a**−**8g** were prepared by condensation of corresponding 3-amino-1,2,4-triazoles and corresponding

323 ethyl acetoacetates in acetic acid (Figure 4). The tautmoeric structures (Figure 5) of a large
324 variety of 1,2,4-triazolo[1,5-*a*]pyrimidines have been determined in literature.[33,34] The
325 structures of **1**, **6**−**8** were validated as the lactam tautomeric forms via comparison of ^{1}H
326 NMR, ^{13}C NMR with literature. Compounds **7a** and **7b** were obtained as 2-decarboxylic
327 1,2,4-triazolo[1,5-*a*]pyrimidines rather than design products. In order to determine the
328 structures of **7a** and **7b**, the single crystal of **7a** was obtained (Figure 6, CCDC number:
329 1948036), which enabled the configuration of the product to be 2-decarboxylic 1,2,4-
330 triazolo[1,5-*a*]pyrimidine. The configuration of **7b** was assigned by analogy. Compound
331 **7d** was obtained by hydrazinolysis of **7c** with hydrazine hydrate in 98% yield (Figure 4).
332 Compound **7e** was gained by hydrolysis of **7c** with lithium hydroxide hydrate with 91%
333 yield (Figure 7).

334 Compounds **9a**−**9o** and **10** were obtained in excellent yields (Figure 8). The Biginelli-
335 like heterocyclization reaction of aldehyde, *β*-dicarbonyl compound and 3-alkylthio-5-
336 amino-1,2,4-triazole was used.

337 **Phytotoxic Activity.** Compounds **1**−**10** were first tested for their phytotoxic activities
338 against the test plant. The results indicated that compounds **1**−**10** showed no phytotoxic
339 activities at 500 μg/mL. There was no lesions on the tobacco leaves.

340 **Antiviral Activity.** The inhibition ratio of essramycin and its analogues against TMV
341 are shown in Tables 1 and 2 with the commercial plant virucides ribavirin and

ningnanmycin as the controls.

In Vitro Anti-TMV Activity. The natural alkaloid essramycin (**1**) and its analogues **2**−**10** were first investigated for their *in vitro* inhibition ratio against TMV using conventional half-leaf method.[28] Essramycin alkaloids were found to have good antiviral activities for the first time. Most of these compounds exhibited higher antiviral activities than ribavirin (Table 1). Compounds **7e** and **8f** exhibited significantly higher TMV inhibition effects than ningnanmycin (the most widely used antiviral agent at present), thus emerged as novel antiviral lead compounds. Essramycin (**1**) gave 39% inhibition ratio at 500 μg/mL, which about similar to that of ribavirin. Interestingly, pyrimidine ring opening compound **2** exhibited better activity than essramycin (**1**). The biological activity was further improved after the phenyl was converted into carboxyl group (inhibitory effect: **3** > **2**). Further reducing one methylene of compound **3** leaded to a decrease in activity (inhibitory effect: **3** > **4a**). However, **4b**, a carboxylic ester compound, exhibited the same level of biological activity as compound **3**. Above results indicate that minor changes of groups at 2-position can lead to significant changes in biological activity. A series of sulfur-containing functional groups were introduced into the 2-site to further investigate the structure-activity relationship in this region. Compounds **5a**−**5c** showed similar level of activities with **3** and higher than essramycin (**1**). Compounds **5d**, **5e** containing *p*-fluorophenyl and *p*-chlorophenyl exhibited the same biological activities as **1**. Among compounds **5a**−**5g**, compound **5f** containing 4-fluoro-2-chlorophenyl showed the best activity, while

compound **5g** containing *p*-nitrophenyl showed the lowest activity, indicated that the electron-withdrawing group was unfavorable to the activity. The 1,2,4-triazolo[1,5-*a*]pyrimidine compound **6** displayed the same activity as **3** but higher than compound **1**. Compounds **7a** and **7b** with a hydrogen atom, **7c** with methyl formyl, and **7d** with formylhydrazine showed a similar level of activities as ribavirin. Compared with **7a**, substitution of the *n*-propyl group (**7b**) in 5-position of 1,2,4-triazolo[1,5-*a*]pyrimidine resulted in sharp decline of anti-TMV activity, which indicated that addition of a lipophilic group on 5-position of 1,2,4-triazolo[1,5-*a*]pyrimidine is inadvisable. The COOH containing compound **7e** exhibited higher activity than compounds **6**, **4a** and ningnanmycin. Compound **8a** containing $-SCH_2Ph$ showed about similar level of TMV inhibition effect with compounds **6** and **4a**. Encouraged by these results, essramycin analogues **8b**−**8g** containing kinds of *S*-benzyl groups were further designed. Compounds with electron-withdrawing groups (**8d**, **8e** and **8g**) at the *S*-benzyl ring showed relatively lower activities than **8a**. However, **8f** with electron-withdrawing groups 4-F and 2-Cl performed excellent anti-TMV activity. Compounds **8b** and **8c** with electron-donating groups at *S*-benzyl ring displayed about similar level of activities as compound **8a**. From the above results, we can see that biological activity is very sensitive to structural changes. In order to further investigate the effect of substituent change at other sites on biological activity, compounds **9a**−**9o** and **10** were designed and synthesized. The results proved that the 5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(4*H*)-one is important core structure for activity

(inhibitory effect: **8a** > **9a**; **8b** > **9j**; **8c** > **9k**; **8d** > **9l**; **8f** > **9n**; **8g** > **9o**). Compounds **9b**−**9d** showed slightly higher activities than **9a**. The activities of **9e**, **9f** and **9i** are higher than that of ribavirin. Compounds **9g**, **9h**, **9j**−**9o** and **10** displayed relatively low activities.

In Vivo Anti-TMV Activity. All of target compounds and a part of intermediates were investigated *in vivo* antiviral activities against TMV using our reported method.[28] As shown in Table 2, most of the target compounds also exhibited higher *in vivo* TMV inhibitory effects than commercial ribavirin. Compounds **3**, **5f**, **6**, **8c** and **9e** showed about similar level of TMV inhibitory effects as ningnanmycin. Compounds **7e** and **8f** displayed significantly higher TMV inhibitory effects than ningnanmycin. Unlike the data of *in vitro* anti-TMV activity, the curative effects were slightly decreased. Other structure-activity relationships are consistent with *in vitro* activity. The EC_{50} value of compound **7e** for protection effect was further investigated to further confirm the activity data. As shown in Table 3, compound **7e** displayed 197 μg/mL EC_{50} value which is lower than that of ribavirin (711 μg/mL) and ningnanmycin (204 μg/mL).

Preliminary Mode of Action

With lead compounds in hand, we began to study their mechanism of action. Compound **7e** was selected to investigate preliminary mode of action with RNA inhibitor antofine[35] and CP disk assembly inhibitor NK0209[36] as controls. As shown in Figure 9, Figure 9A and Figure 9B revealed that 20S CP disk and TMV rod can be formed effectively. The use

of a small amount of DMSO did not affect virus assembly (Figure 9C). The bits and pieces of TMV rod in Figure 9D and Figure 9E revealed that antofine and NK0209 can obviously inhibit the assembly of TMV rods. From Figure 9E, we can also see a large number of 20S CP disk, which further proved that antofine had no effect on protein. By comparing the Figures 9D, 9E and 9F, we can see that compound **7e** has a better inhibitory effect on virus assembly than controls antofine and NK0209. Further 20S CP disk assembly inhibition tests were carried out to evaluate the interaction of compound **7e** with TMV CP. As depicted in Figure 10, the 20S CP disk can be gained by hatching TMV CP at 20 °C for 12 h (Figure 10A). The use of small amount of DMSO did not affect the formation of 20S CP disk (Figure 10B). Antofine has no impact on 20S CP disk assembly (Figure 10C). As CP disk assembly inhibitor, NK0209 exhibited obviously impact on the 20S CP disk, which can induce fusion and aggregation of 20S CP disks (Figure 10D). Comparing with Figure 10D, there are larger number of CP disks aggregated and fused in Figures 10E and 10F. The above results suggested that compound **7e** may inhibit viral assembly by fusing 20S CP disks, thus exerting its antiviral activity.

Fungicidal Activity. The fungicidal activities of compounds **1**−**10** on 14 kinds of plant fungi at 50 μg/mL were evaluated with commercial fungicides chlorothalonil and carbendazim as controls (Table 4). All of the compounds displayed broad spectrum fungicidal activities at 50 μg/mL. Compound **5b** displayed more than 50% inhibition rate against most of the 14 kinds of phytopathogenic fungi at 50 μg/mL. Compounds **5a**−**5c** and

421 **8e** displayed higher fungicidal activities than carbendazim against *Botrytis cinerea*. The
422 fungicidal activity of **5b** against *Cercospora arachidicola Hori* is higher than that of
423 commercial fungicides chlorothalonil and carbendazim. Compounds **5b** and **9n** displayed
424 higher fungicidal activities than chlorothalonil and carbendazim against *Rhizoctonia*
425 *cerealis*. Compound **5b** with broad spectrum and high effect fungicidal activity emerged
426 as new fungicidal lead compound.

427 In summary, marine natural product essramycin (**1**) was found to have good anti-TMV
428 activity for the first time. A series of [1,2,4]triazolo[1,5-*a*]pyrimidines were designed,
429 synthesized and evaluated for their anti-TMV activities systematically. Most of these
430 compounds exhibited higher antiviral activities than ribavirin. Compounds **3**, **5f**, **6**, **8c** and
431 **9e** showed about similar level of TMV inhibitory effects as ningnanmycin. Compounds **7e**
432 and **8f** exhibited significantly higher TMV inhibition effects than ningnanmycin, thus
433 emerged as novel antiviral lead compounds. The structure-activity relationship study
434 revealed that the biological activity is very sensitive to structural changes for these
435 compounds. Compound **7e** was selected to further investigate preliminary mode of action.
436 The results revealed that compound **7e** may inhibit viral assembly by fusing 20S CP disks,
437 thus exerting its antiviral activity. All of the compounds also displayed broad spectrum
438 fungicidal activities against 14 kinds of phytopathogenic fungi at 50 μg/mL. Compound **5b**
439 with broad spectrum and high effect fungicidal activity emerged as new fungicidal lead
440 compound. Current work demonstrated that these simple essramycin analogues could be

441 considered as potential candidates for the development of novel plant virus and
442 fungi inhibitors in the future.

443 **SUPPORTING INFORMATION**

444 Detailed preparation procedures of **1**−**5**, detailed bio-assay procedures and the spectra data
445 of compounds **1**−**10** are provided in Supporting Information. This material is available free
446 of charge via the Internet at http://pubs.acs.org.

447 **Funding**

448 This study was supported by Natural Science Fund of China (21772145, 21732002,
449 21672117) and the Research Innovation Program for Postgraduate of Hebei Province
450 (CXZZSS219014).

451 **Notes**

452 The authors declare no competing financial interest.

453 **REFERENCES**

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

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

458 (3) Wang, S. Z.; Dong, G. Q.; Sheng, C. Q. Structural simplification of natural products.
459 *Chem. Rev.* **2019**, *119*, 4180−4220.

460 (4) Wu, W. B.; Tang, Y.; Yang, J. L.; Idehen, E.; Sang, S. M. Avenanthramide aglycones
461 and glucosides in oat bran: Chemical profile, levels in commercial oat products, and
462 cytotoxicity to human colon cancer cells. *J. Agric. Food Chem.* **2018**, *66*, 8005−8014.

463 (5) Han, Y. G.; Luo, Y.; Qin, S. R.; Xi, L.; Wan, B.; Du, L. F. Induction of systemic
464 resistance against tobacco mosaic virus by Ningnanmycin in tobacco. *Pestic. Biochem.*
465 *Physiol*. **2014**, *111*,14−18.

466 (6) Martinez, M. J. A.; Del Olmo, L. M. B. D. Benito, P. B. Antiviral activities of
467 polysaccharides from natural sources. *Stud. Nat. Prod. Chem*. **2005**, *30*, 393−418.

468 (7) Guo, P. B.; Wang, Z. W.; Li, G.; Liu, Y. X.; Xie, Y. F.; Wang, Q. M. First discovery
469 of polycarpine, polycarpaurines A and C, and their derivatives as novel antiviral and
470 anti-phytopathogenic fungi agents. *J. Agric. Food Chem.* **2016**, *64*, 4264–4272.

471 (8) Li, G.; Guo, J. C.; Wang, Z. W.; Liu, Y. X.; Song, H. B.; Wang, Q. M. Marine natural
472 products for drug discovery: first discovery of kealiinines A−C and their derivatives
473 as novel antiviral and antiphytopathogenic fungus agents. *J. Agric. Food Chem.* **2018**,
474 *66*, 7310–7318.

475 (9) Guo, P. B.; Li, G.; Liu, Y. X.; Lu, A. D.; Wang, Z. W.; Wang, Q. M. Naamines and

476 naamidines as novel agents against a plant virus and phytopathogenic fungi. *Mar.*
477 *Drugs* **2018**, *16*, 311.

478 (10) Ji, X. F.; Wang, Z. W.; Dong, J.; Liu, Y. X.; Lu, A. D.; Wang, Q. M. Discovery of
479 topsentin alkaloids and their derivatives as novel antiviral and anti-phytopathogenic
480 fungus agents. *J. Agric. Food Chem.* **2016**, *64*, 9143–9151.

481 (11) Ji, X. F.; Guo, J. C.; Liu, Y. X.; Lu, A. D.; Wang, Z. W.; Li, Y. Q.; Yang, S. X.; Wang,
482 Q. M. Marine-natural-product development: First discovery of nortopsentin alkaloids
483 as novel antiviral, anti-phytopathogenic- fungus, and insecticidal agents. *J. Agric.*
484 *Food Chem.* **2018**, *66*, 4062–4072.

485 (12) Liu, B.; Li, R.; Li, Y. N.; Li, S. Y.; Yu, J.; Zhao, B. F.; Liao, A. C.; Wang. Y.; Wang,
486 Z. W.; Lu, A. D.; Liu, Y. X.; Wang, Q. M. Discovery of pimprinine alkaloids as novel
487 agents against aplant virus. *J. Agric. Food Chem.* **2019**, *67*, 1795–1806.

488 (13) Lu, A. D.; Wang, T. N.; Hui, H.; Wei, X. Y.; Cui, W. H.; Zhou, C. L.; Li, H. Y.; Wang,
489 Z. W.; Guo, J. C.; Ma, D. J.; Wang, Q. M. Natural products for drug discovery:
490 discovery of gramines as novel agents against a plant virus. *J. Agric. Food Chem.* **2019**,
491 *67*, 2148–2156.

492 (14) El-Gendy, M. M. A.; Shaaban, M.; Shaaban, K. A.; El-Bondkly, A. M.; Laatsch H.
493 Essramycin: A first triazolopyrimidine antibiotic isolatedfrom nature. *J. Antibiot.* **2008**,
494 *61*, 149–157.

(15) Battaglia, U.; Moody, C. J. A short synthesis of the triazolopyrimidine antibiotic essramycin, *J. Nat. Prod*. **2010**, *73*, 1938–1939

(16) Tee, E.; Karoli, T.; Ramu, S.; Huang, J.; Butler, M.; Cooper, M. Synthesis of essramycin and comparison of its antibacterial activity. *J. Nat. Prod.* **2010**, *73*, 1940–1942.

(17) Zhang, N.; Ayral-Kaloustian, S.; Nguyen, T.; Afragola, J.; Hernandez, R.; Lucas, J.; Gibbons, J.; Beyer, C. Synthesis and SAR of [1,2,4]triazolo[1,5-*a*]pyrimidines, a class of anticancer agents with a unique mechanism of tubulin inhibition. *J. Med. Chem.* **2007**, *50*, 319–327.

(18) DeNinno, M. P.; Wright, S. W.; Etienne, J. B.; Olson, T. V.; Rocke, B. N.; Corbett, J. W.; Kung, D. W.; DiRico, K. J.; Andrews, K. M.; Millham, M. L.; Parker, J. C.; Esler, W.; van Volkenburg, M.; Boyer, D. D.; Houseknecht, K. L.; Doran, S. D. Discovery of triazolopyrimidinebased PDE8B inhibitors: exceptionally ligand-efficient and lipophilic ligandefficient compounds for the treatment of diabetes. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5721–5726.

(19) Zuniga, E. S.; Korkegian, A.; Mullen, S.; Hembre, E. J.; Ornstein, P. L.; Cortez, G.; Biswas, K.; Kumar N.; Cramer, J.; Masquelin, T.; Hipskind, P. A.; Odingo, J.; Parish, T. The synthesis and evaluation of triazolopyrimidines as anti-tubercular agents. *Bioorg. Med. Chem*. **2017**, *25*, 3922–3946.

514 (20) Wang, H.; Lee, M.; Peng, Z. H.; Blzquez, Blas.; Wang, H.; Lee, M.; Peng, Z.; Blázquez,
515 B.; Lastochkin, E.; Kumarasiri, M.; Bouley, R.; Chang, M.; Mobashery, S. Synthesis
516 and evaluation of 1,2,4-triazolo[1,5-*a*]pyrimidines as antibacterial agents against
517 *Enterococcus faecium*. *J. Med. Chem.* **2015***, 58*, 4194–4203.

518 (21) Ding, J.; Cao, F. D.; Geng, Y. R.; Tian, Y.; Li, P.; Li, X. F.; Huang, L. J. Synthesis
519 and *in vitro* anti-epileptic activities of novel [1,2,4]-triazolo[1,5-*a*]pyrimidin-7(4*H*)-
520 one derivatives. *J. Asian Nat. Prod. Res*. **2018**, 1477–2213.

521 (22) Patil, V.; Kale, M.; Raichurkar, A.; Bhaskar, B.; Prahlad, D.; Balganesh, M.; Nandan,
522 S.; Hameed, P. S. Design and synthesis of triazolopyrimidine acylsulfonamides as
523 novel anti-mycobacterial leads acting through inhibition of acetohydroxyacid synthase.
524 *Bioorg. Med. Chem. Lett*. **2014**, *24*, 2222–2225.

525 (23) Gujjar, R.; Marwaha, A.; El Mazouni, F.; White, J.; White, K. L.; Creason, S.;
526 Shackleford, D. M.; Baldwin, J.; Charman, W. N.; Buckner, F. S.; Charman, S.; Rathod,
527 P. K.; Phillips, M. A. Identification of a metabolically stable triazolopyrimidine-based
528 dihydroorotate dehydrogenase inhibitor with antimalarial activity in mice. *J. Med.*
529 *Chem.* **2009**, *52*, 1864–1872.

530 (24) Gujjar. R.; El Mazouni, F.; White K. L.; White, J.; Creason, S.; Shackleford, D. M.;
531 Deng, X. Y.; Charman, W. N.; Bathurst, I.; Burrows, J.; Floyd, D. M.; Matthews, D.;
532 Buckner, F. S.; Charman, S. A.; Phillips, M. A.; Rathod, P. K. Lead optimization of

533 aryl and aralkyl amine-based triazolopyrimidine inhibitors of Plasmodium falciparum
534 dihydroorotate dehydrogenase with antimalarial activity in mice. *J. Med. Chem.* **2011**,
535 *54*, 3935–3949.

536 (25) Coteron, J. M.; Marco, M.; Esquivias, J.; Deng, X. Y.; White K. L.; White, J.; Koltun,
537 M.; El Mazouni, F.; Kokkonda, S.; Katneni, K.; Bhamidipati, R.; Shackleford, D. M.;
538 Angulo-Barturen, I.; Ferrer, S. B.; Jiménez-Díaz, M. B.; Gamo, F.; Goldsmith, E. J;
539 Charman, W. N.; Bathurst, I.; Floyd, D.; Matthews, D.; Burrows, J. N.; Rathod, P. K.;
540 Charman, S. A.; Phillips, M. A. Structure-guided lead optimization of
541 triazolopyrimidine-ring substituents identifies potent Plasmodium falciparum
542 dihydroorotate dehydrogenase inhibitors with clinical candidate potential. *J. Med.*
543 *Chem*. **2011**, *54*, 5540–5561.

544 (26) Parker, W. B. Metabolism and antiviral activity of ribavirin. *Virus Res.* **2005**, *107*,
545 165–171.

546 (27) Crotty, S.; Maag, D.; Arnold, J. J.; Zhong, W.; Lau, J. Y. N.; Hong, Z.; Andino, R.;
547 Cameron, C. E. The broad-spectrum antiviral ribonucleoside ribavirin is an RNA virus
548 mutagen. *Nat. Med.* **2000**, *6*, 1375–1379.

549 (28) Wang, Z. W.; Wei, P.; Wang, L. Z.; Wang, Q. M. Design, synthesis, and anti-tobacco
550 mosaic virus (TMV) activity of phenanthroindolizidines and their analogues. *J. Agric.*
551 *Food Chem*. **2012**, *60*, 10212−10219.

552 (29) Zhao, H. P; Liu, Y. X.; Cui, Z. P.; Beattie, D,; Gu, Y. C.; Wang, Q. M. Design,
553 synthesis, and biological activities of arylmethylamine substituted chlorotriazine and
554 methylthiotriazine compounds. *J. Agric. Food Chem.* **2011**, *59*, 11711−11717.

555 (30) Lv, P.; Chen, Y. L.; Shi, T. Z.; Wu, X. W.; Li, Q. X.; Hua, R. M. Synthesis and
556 fungicidal activities of sanguinarine derivatives. *Pestic. Biochem. Phys.* **2018**, *147*,
557 3−10.

558 (31) Lv, P.; Chen, Y. L.; Zhao, Z.; Shi, T. Z.; Wu, X. W.; Li, Q. X.; Hua, R. M. Design,
559 synthesis, and antifungal activities of 3-acyl thiotetronic acid derivatives: New fatty
560 acid synthase inhibitors. *J. Agric. Food Chem.* **2018**, *66*, 1023−1032.

561 (32) Chernyshev, V. M.; Chernysheva, A. V.; Taranushich, V. A. Synthesis of esters and
562 amides of 5-amino-1,2,4-triazole-3-carboxylic and 5-amino-1,2,4-triazol-3-ylacetic
563 acids. *Russ. J. Appl. Chem*. **2006**, *79*, 783−786.

564 (33) Kleinpeter, E.; Thomas, St.; Fischer, G. ^{13}C and ^{15}N NMR study of 1,2,4-triazolo[1,5-
565 *a*]pyrimidines with one tautomerism-introducing substituent. *J. Mol. Struct.* **1995**, *355*,
566 273−285.

567 (34) Kleinpeter, E.; Koch, A.; Fischer, G.; Askolin, C. P. ^{13}C NMR, ^{15}N NMR and
568 quantum-chemical study of the tautomerism of 2-substituted 5-Me-7-OH-1,2,4-
569 triazolo[1,5-*a*]pyrimidines. *J. Mol. Struct.* **1997**, *435*, 65−76.

570 (35) Xi, Z.; Zhang, R. Y.; Yu, Z. H.; Ouyang, D. The interaction between tylophorine B
571 and TMV RNA. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 4300–4304.

572 (36) Li, X. Y.; Hao, G. F.; Wang, Q. M.; Chen, Z.; Ding, Y.; Yu, L.; Hu, D. Y.; Song, B.
573 A. Ningnanmycin inhibits tobacco mosaic virus virulence by binding directly to its
574 coat protein discs. *Oncotarget* **2017**, *8*, 82446–82458.

575

Figure Captions

Figure 1. Structures of Essramycin, Ribavirin and Ningnanmycin.

Figure 2. Design of Essramycin Analogues.

Figure 3. Structures of Compounds **1**–**5**.

Figure 4 Synthesis of Compounds **6, 7a**–**7d** and **8a**−**8g**.

Figure 5. Different Tautomeric Forms of 1,2,4-Triazolo[1,5-*a*]pyrimidines.

Figure 6 X-ray Crystal Structure of **7a**.

Figure 7 Synthesis of Compound **7e**.

Figure 8 Synthesis of Compounds **9a**−**9o** and **10**.

Figure 9 TMV Rod Assembly Inhibition of Compound **7e**, NK0209 and Antofine.

Figure 10 20S CP Disk Assembly Inhibition of Compounds **7e**, NK0209, and Antofine.

Table 1. *In Vitro* Antiviral Activities of Compounds **1**−**10**, Ribavirin and Ningnanmycin Against TMV.

Compd	Concn	Inhibition	Compd	Concn	Inhibition

	(μg/mL)	rate (%)[a]		(μg/mL)	rate (%)[a]
	500	39±2		500	37±2
1			**8e**		
	100	12±2		100	14±3
	500	44±1		**500**	**63±3**
2			**8f**		
	100	13±2		**100**	**29±1**
	500	**56±1**		500	37±2
3			**8g**		
	100	**23±1**		100	14±1
	500	42±2		500	34±1
4a			**9a**		
	100	12±1		100	0
	500	53±1		500	41±1
4b			**9b**		
	100	19±1		100	15±2
	500	52±4		500	43±3
5a			**9c**		
	100	21±3		100	13±2
	500	51±1		500	46±1
5b			**9d**		
	100	21±2		100	16±2
	500	53±2		**500**	**56±2**
5c			**9e**		
	100	21±2		**100**	**25±2**
	500	42±2		500	48±1
5d			**9f**		
	100	15±1		100	18±1
	500	39±2		500	39±2
5e			**9g**		
	100	12±1		100	0
5f	**500**	**59±1**	**9h**	500	25±3

	100	**28±3**		100	0
	500	32±1		500	48±2
5g			**9i**		
	100	0		100	21±2
	500	**57±3**		500	39±1
6			**9j**		
	100	**27±1**		100	17±3
	500	40±3		500	41±1
7a			**9k**		
	100	13±1		100	19±2
	500	35±2		500	31±3
7b			**9l**		
	100	0		100	0
	500	32±1		500	38±1
7c			**9m**		
	100	0		100	15±1
	500	43±2		500	42±1
7d			**9n**		
	100	17±2		100	13±2
	500	**65±2**		500	23±2
7e			**9o**		
	100	**29±3**		100	0
	500	55±2		500	39±3
8a			**10**		
	100	23±1		100	12±1
	500	54±1		500	58±2
8b			Ningnanmycin		
	100	21±1		100	23±2
	500	**58±1**		500	41±1
8c			Ribavirin		
	100	**25±2**		100	15±2
8d	500	43±3			

[a]*Average of three replicates; All results are*

	100	19±2	*expressed as mean ± SD*; *Activity Data with prominent were presented in blue bold.*

Table 2. *In Vivo* Antiviral Activities of Compounds **1**−**10**, Ribavirin and Ningnanmycin Against TMV.

Compd	Concn (μg/mL)	Inactive effect (%)[a]	Curative effect (%)[a]	Protective effect (%)[a]	Compd	Concn (μg/mL)	Inactive effect (%)[a]	Curative effect (%)[a]	Protective effect (%)[a]
1	500	41±2	35±4	32±3	**8e**	500	41±3	33±3	47±4
	100	11±1	9±1	15±1		100	12±2	14±1	15±3
2	500	43±4	41±1	49±2	**8f**	**500**	**60±4**	**59±3**	**64±3**
	100	9±1	11±1	13±1		**100**	**28±1**	**25±1**	**26±1**
3	**500**	**55±3**	**50±4**	**60±2**	**8g**	500	35±2	33±1	40±1
	100	**23±1**	**16±1**	**27±1**		100	10±2	11±2	0
4a	500	43±2	39±1	44±2	**9a**	500	37±4	34±2	33±1
	100	10±1	6±1	13±1		100	0	0	0
4b	500	51±1	56±3	44±3	**9b**	500	43±1	39±2	46±3
	100	20±2	17±2	16±1		100	17±3	15±1	19±3
5a	500	50±4	45±4	55±3	**9c**	500	41±2	37±3	43±4
	100	19±1	16±1	23±1		100	12±2	0	19±3
5b	500	49±4	48±2	50±1	**9d**	500	49±2	43±3	48±2
	100	15±1	17±2	24±2		100	17±2	11±2	15±1
5c	500	55±1	46±3	53±3	**9e**	**500**	**53±5**	**56±3**	**58±4**
	100	20±2	12±1	19±1		**100**	**26±2**	**27±1**	**19±1**

5d	500	39±2	32±1	41±3	**9f**	500	45±1	40±2	50±1
	100	11±1	9±2	13±1		100	19±1	13±1	15±3
5e	500	40±1	30±1	42±2	**9g**	500	41±1	37±3	39±3
	100	11±3	9±1	8±1		100	13±1	0	12±4
5f	**500**	**55±2**	**56±1**	**58±2**	**9h**	500	29±4	23±3	23±2
	100	**26±1**	**19±1**	**24±1**		100	0	0	0
5g	500	35±1	30±2	34±2	**9i**	500	51±2	46±2	48±3
	100	0	0	0		100	24±1	19±1	23±1
6	**500**	**56±4**	**57±2**	**58±1**	**9j**	500	40±2	35±1	36±1
	100	**25±1**	**23±3**	**28±1**		100	18±1	13±3	12±2
7a	500	44±2	41±3	41±1	**9k**	500	39±2	40±1	41±3
	100	12±1	13±2	13±1		100	18±1	19±3	18±2
7b	500	37±1	39±1	35±1	**9l**	500	33±4	30±2	31±1
	100	11±1	12±3	0		100	0	0	0
7c	500	39±2	32±1	33±1	**9m**	500	39±2	33±1	36±1
	100	10±1	0	12±1		100	12±1	11±1	13±1
7d	500	46±1	40±2	43±2	**9n**	500	43±3	39±2	40±2
	100	13±1	15±2	17±1		100	15±1	13±1	15±1
7e	**500**	**62±3**	**64±2**	**68±4**	**9o**	500	27±3	21±3	23±2
	100	**31±2**	**28±1**	**29±1**		100	0	0	0
8a	500	59±3	52±4	50±3	**10**	500	40±2	38±1	42±2
	100	29±2	21±1	25±2		100	19±1	17±2	18±5

8b	500	55±3	53±1	53±1	Ningnanmycin	500	55±2	52±3	59±2
	100	23±1	19±2	23±2		100	28±2	22±2	26±3
8c	**500**	**60±1**	**53±3**	**55±1**	Ribavirin	500	39±2	38±3	40±1
	100	**27±4**	**19±2**	**23±2**		100	13±1	11±1	14±1
8d	500	45±5	36±2	47±3					
	100	13±1	17±2	12±1					

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

Table 3. The EC_{50} values of compound **7e**, ribavirin and ningnanmycin against TMV.

Compd.	regression equation	*r*	Protective effect EC_{50} (μg/mL)
7e	y =1.39+1.57x	0.9863	197
Ribavirin	y =1.64+1.18x	0.9717	711
Ningnanmycin	y =1.59+1.48x	0.9804	204

Table 4. Fungicidal Activities of the Compounds **1-10** against 14 Kinds of Fungi.

Compd	Fungicidal activity (%)[a] / 50 mg kg^{-1}													
	B.C[b]	*S.S*[b]	*R.S*[b]	*C.H*[b]	*F.M*[b]	*F.C*[b]	*P.C*[b]	*W.A*[b]	*B.M*[b]	*P.I*[b]	*A.S*[b]	*P.P*[b]	*F.G*[b]	*R.C*[b]
1	46±1	8±1	26±2	15±1	18±2	9±1	21±3	15±1	26±1	**58±2**	19±1	7±1	16±2	**57±1**
2	29±1	25±2	19±1	11±1	11±2	12±1	12±2	18±1	18±2	12±1	10±1	13±2	14±1	27±1
3	46±1	33±2	44±2	51±1	34±3	28±1	33±1	49±2	30±1	**55±2**	38±1	34±2	22±1	**56±1**
4a	27±1	26±2	15±1	7±1	19±2	11±1	0	6±1	17±1	16±1	35±2	45±3	16±2	27±1
4b	22±1	11±2	12±1	4±1	12±2	9±1	9±1	11±1	6±1	9±1	23±2	23±1	16±1	22±1
5a	**59±3**	30±1	30±2	26±3	12±1	3±1	3±1	17±1	11±1	9±1	27±1	29±1	15±2	**59±2**

5b	**57±2**	41±1	54±2	**75±1**	51±2	46±1	48±2	53±1	17±2	52±1	45±1	**58±2**	**59±1**	**81±3**
5c	**67±2**	25±2	41±1	37±3	32±1	18±4	12±1	28±1	10±1	15±3	29±3	19±2	23±3	54±1
5d	22±1	27±2	36±1	45±2	31±1	26±2	37±1	46±1	22±3	34±1	12±2	52±1	42±2	**56±3**
5e	12±2	8±1	12±3	25±1	17±1	11±2	17±1	13±2	17±1	18±2	22±1	6±1	16±2	**57±1**
5f	22±1	42±2	12±1	9±2	14±1	5±1	24±2	34±1	34±2	28±1	13±2	18±1	22±1	38±1
5g	21±2	36±2	22±1	33±2	29±1	36±2	34±1	30±3	46±2	39±1	37±1	21±1	35±2	50±1
6	12±1	11±2	18±1	4±1	12±1	14±1	9±1	6±1	3±1	3±1	46±3	10±1	15±1	12±1
7a	15±1	9±1	27±2	7±1	12±1	20±2	6±1	9±1	9±1	3±1	42±1	29±2	21±1	15±2
7b	32±2	18±1	12±1	0	23±2	17±1	6±1	3±1	0	9±1	23±2	48±1	15±1	27±1
7c	24±2	2±1	9±1	11±1	15±2	11±1	3±1	3±1	6±1	6±1	23±2	13±1	8±1	20±1
7d	37±2	40±1	27±2	11±1	19±2	17±1	6±1	11±1	3±1	16±2	54±1	45±2	7±1	37±2
7e	40±1	52±2	37±2	12±2	32±1	36±3	11±1	27±2	21±1	20±1	47±3	53±1	25±2	41±1
8a	37±1	21±1	30±2	15±1	27±3	9±1	6±1	6±1	9±1	6±1	23±1	32±2	12±1	37±2
8b	35±2	28±3	38±1	26±1	17±3	22±2	9±1	14±1	17±1	19±1	33±3	49±2	11±1	48±2
8c	44±3	23±1	26±1	11±1	29±1	17±2	7±1	13±1	15±1	25±1	17±1	39±3	22±1	**58±3**

8d	51±4	23±1	42±2	16±2	37±3	12±1	9±2	16±1	18±1	17±2	30±2	33±1	31±4	48±2
8e	**59±1**	39±1	39±2	27±1	43±3	21±1	16±1	21±1	0	5±1	39±1	28±2	22±1	51±1
8f	44±1	28±1	45±3	28±1	17±3	21±1	15±1	32±2	23±1	29±1	36±2	41±2	32±1	35±2
8g	27±2	9±1	36±2	28±1	37±3	27±2	28±1	30±1	18±1	16±1	43±1	30±2	23±1	46±2
9a	42±1	26±1	36±3	22±2	27±1	17±1	9±1	14±1	11±2	16±1	35±1	39±2	15±1	44±2
9b	24±1	0	27±1	7±1	8±1	6±1	9±1	6±1	11±1	13±2	27±1	16±1	18±1	32±1
9c	29±2	9±1	33±1	19±2	4±1	14±2	9±1	17±1	20±1	19±2	27±2	48±1	13±2	39±1
9d	24±1	26±2	27±3	4±1	23±2	11±1	6±1	9±1	9±1	16±1	35±3	48±1	12±1	39±2
9e	24±2	16±1	15±1	11±2	19±2	14±1	9±1	14±1	26±1	22±2	31±2	19±1	16±2	36±1
9f	27±1	11±1	36±2	7±1	19±2	17±1	6±1	11±1	14±2	16±1	27±1	29±1	20±1	54±2
9g	29±2	11±1	27±1	7±1	23±2	9±1	0	14±1	26±2	16±1	27±2	42±2	18±1	42±3
9h	22±2	19±2	0	15±1	19±2	11±2	6±1	9±1	11±1	6±1	0	19±2	16±1	42±1
9i	32±1	7±1	21±2	11±1	15±1	17±2	3±1	0	6±1	3±1	12±1	39±1	16±2	37±2
9j	29±1	10±1	46±2	17±2	21±2	17±1	9±1	13±1	18±2	15±1	33±1	31±2	24±2	**63±2**
9k	28±2	15±2	39±3	9±1	17±2	18±2	7±1	12±2	17±2	19±2	26±2	32±1	27±1	51±1

9l	22±1	17±2	32±2	8±1	22±3	25±1	5±1	16±2	16±1	19±2	32±3	27±2	22±1	**59±2**
9m	37±2	25±1	28±2	13±2	22±1	31±3	9±1	13±1	19±2	8±1	20±1	36±2	27±2	**68±1**
9n	33±1	26±2	56±1	11±1	25±2	25±1	6±1	18±2	22±1	9±1	17±1	20±1	42±1	**79±3**
9o	21±1	17±1	31±2	15±1	26±2	19±1	16±1	19±2	24±1	12±2	26±2	37±1	29±1	48±2
10	27±2	7±1	15±2	11±1	0	11±1	3±1	11±2	14±2	9±1	35±2	33±3	20±1	32±2
Chlorothalonil[c]	100	86±2	100	71±1	<50	100	92±2	100	<50	90±1	100	100	100	75±2
Carbendazim[c]	<50	100	100	<50	100	<50	100	<50	100	100	<50	<50	100	<50

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

Figure 1.

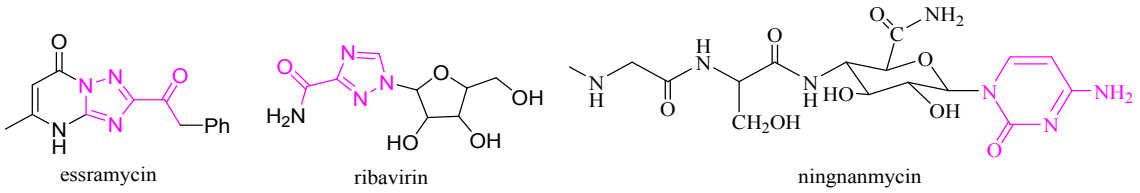


Figure 2.

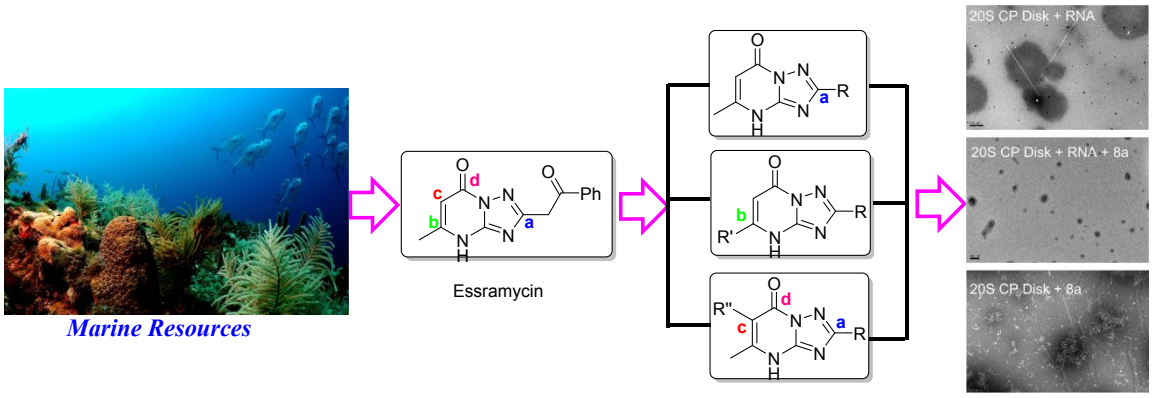


Figure 3.

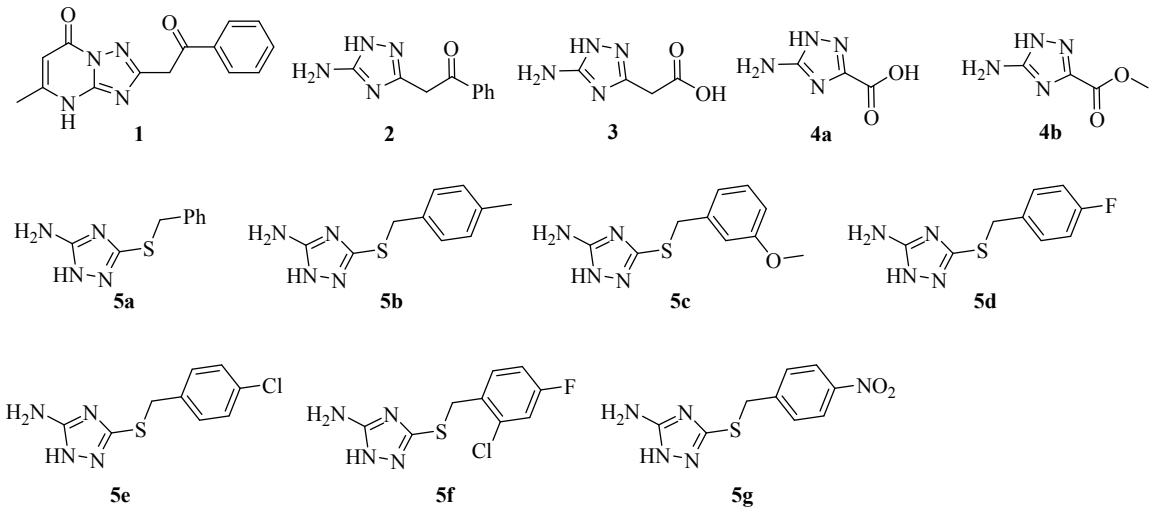

1
2
3
4a
4b
5a
5b
5c
5d
5e
5f
5g

Figure 4.

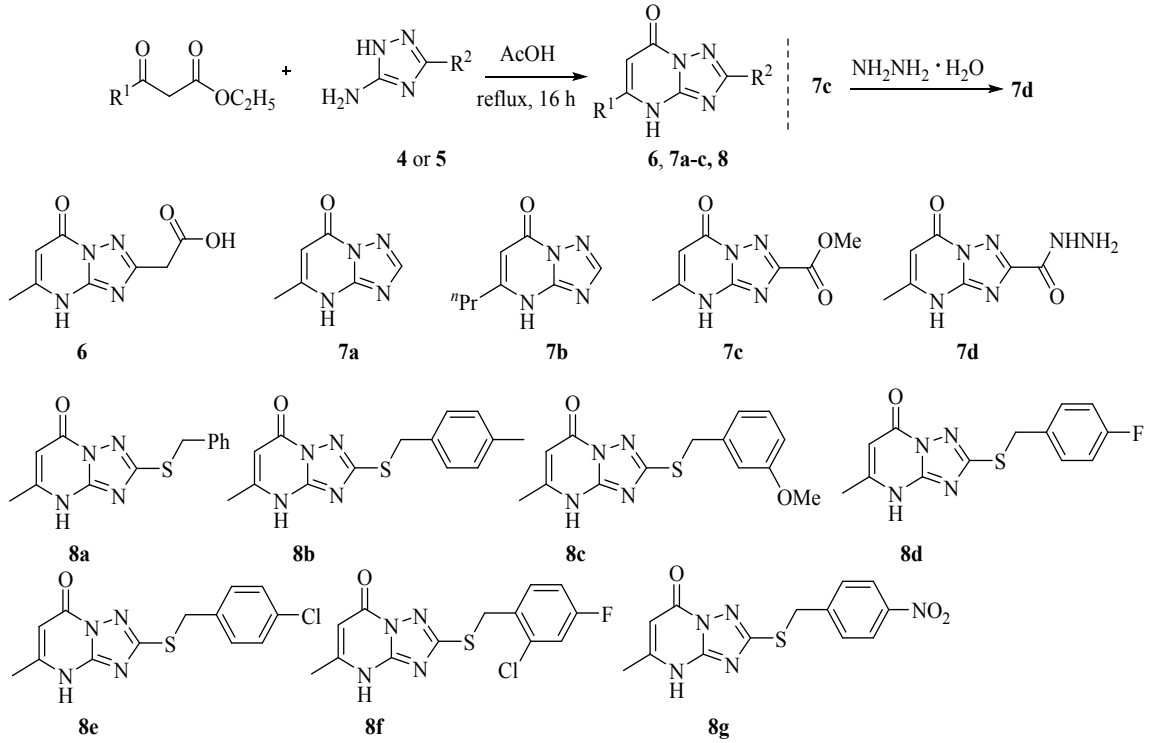

AcOH
reflux, 16 h
4 or 5
6, 7a-c, 8
7c
NH2NH2 · H2O
7d
6
7a
7b
7c
7d
8a
8b
8c
8d
8e
8f
8g

Figure 5.

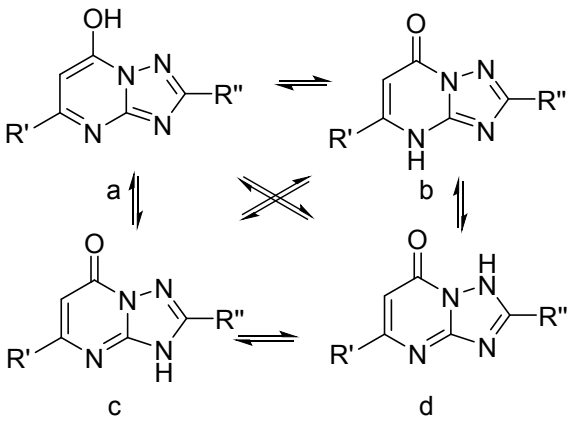


Figure 6.

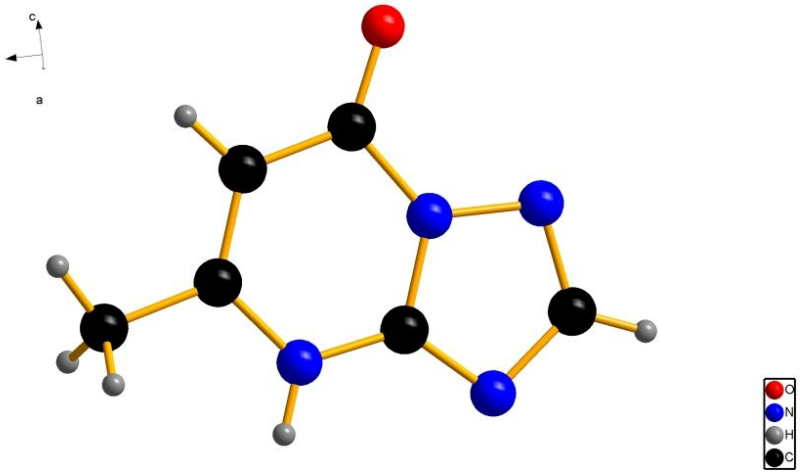


Figure7.

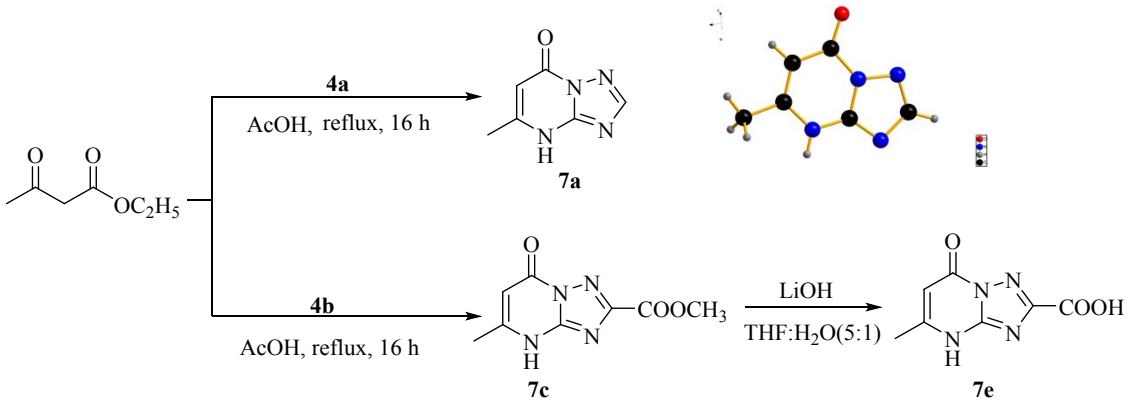
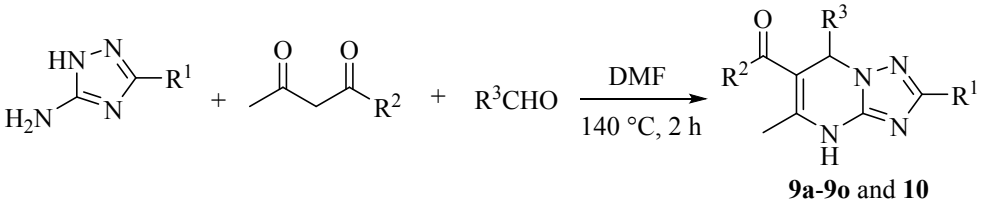
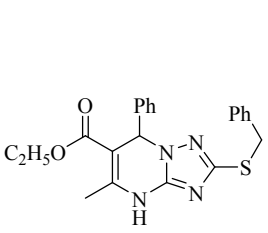
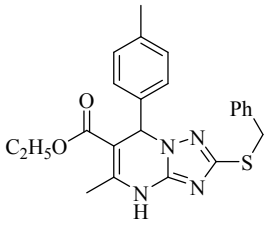


Figure 8.

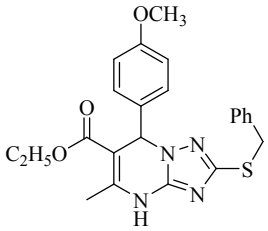




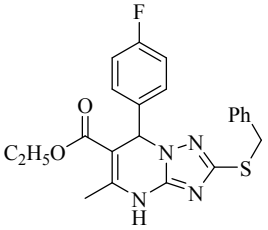
9a



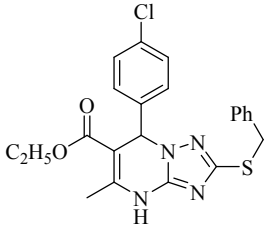
9b



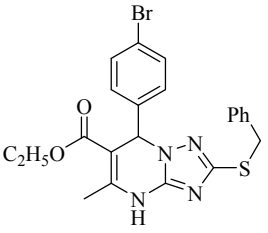
9c



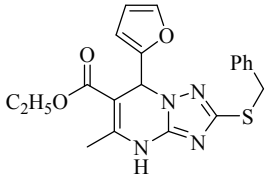
9d



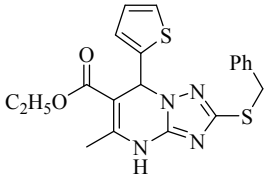
9e



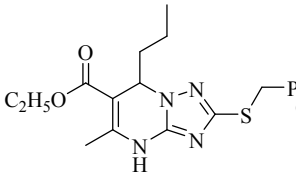
9f



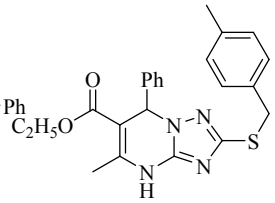
9g



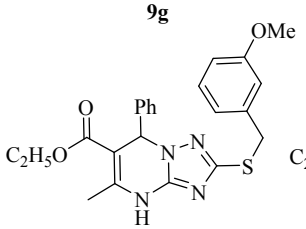
9h



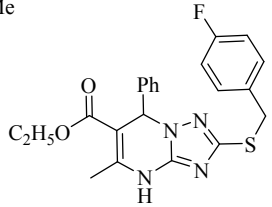
9i



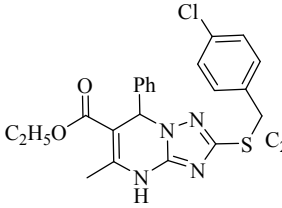
9j



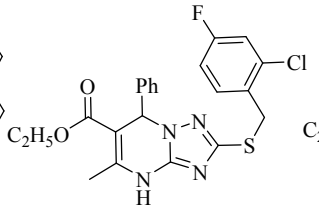
9k



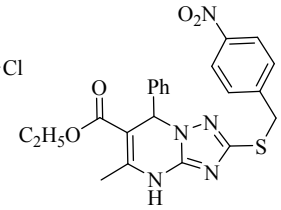
9l



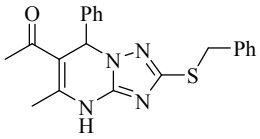
9m



9n

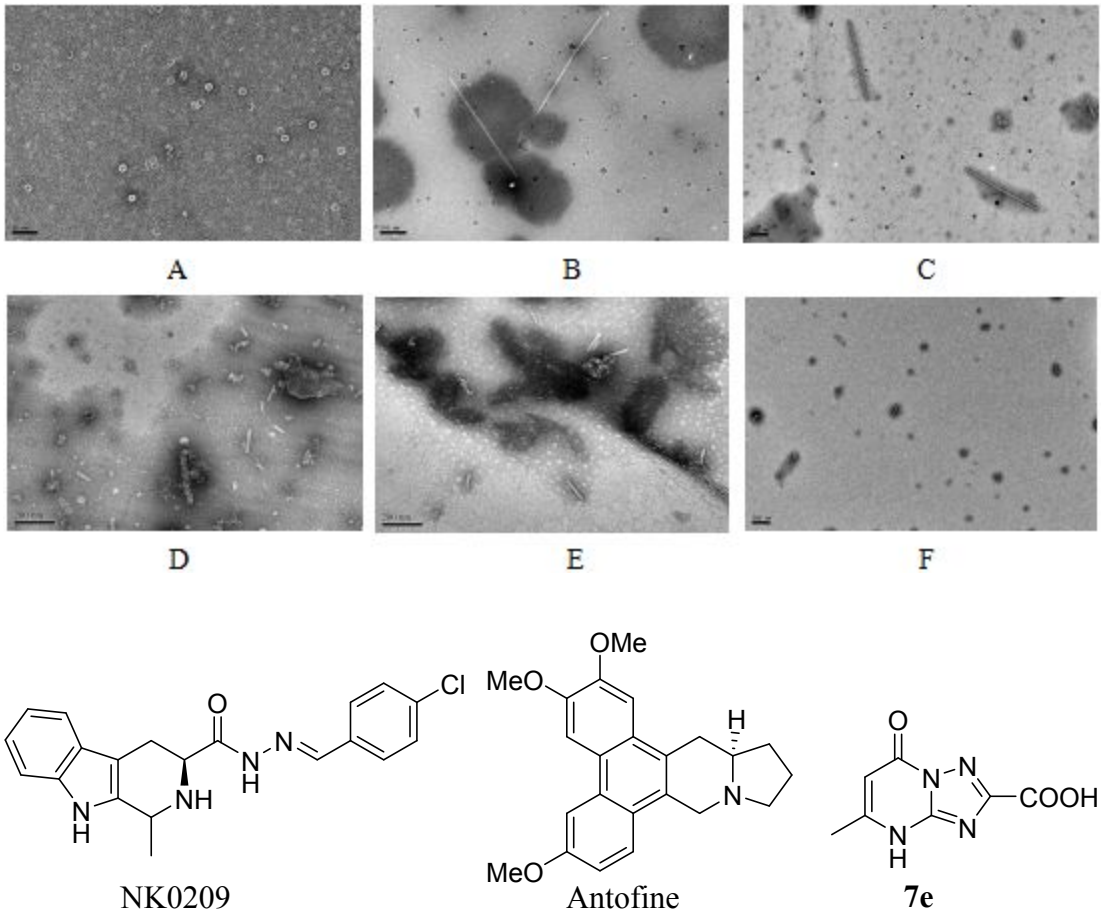


9o



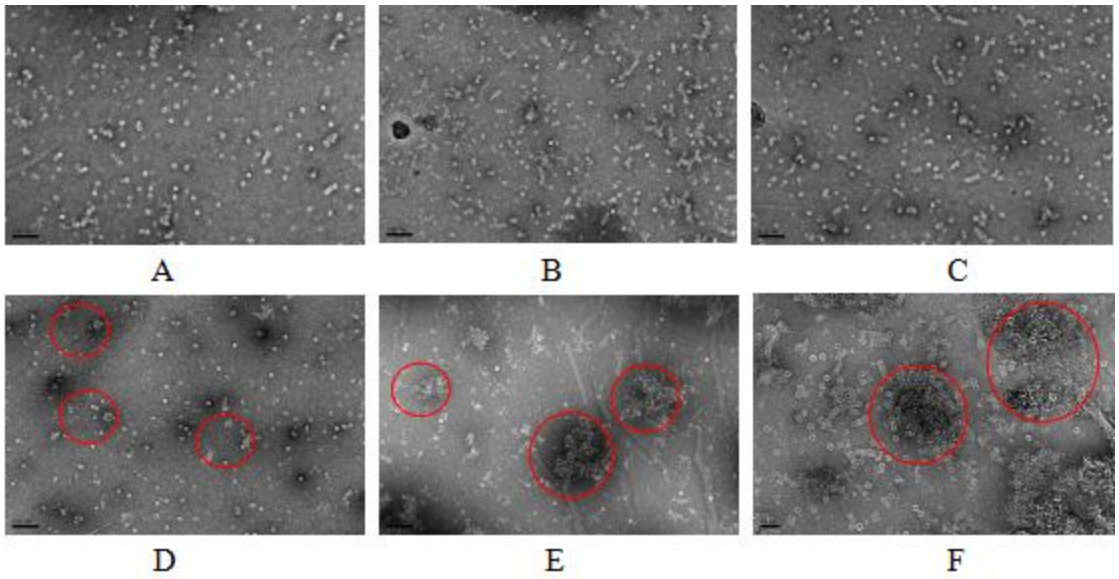
10

Figure 9.



(A) 20S CP disk (50 nm scale bar), (B) 20S CP disk + RNA (200 nm scale bar), (C) 20S CP disk + RNA + DMSO (200 nm scale bar), (D) 20S CP disk + RNA + antofine (200 nm scale bar), (E) 20S CP disk + RNA + NK0209 (200 nm scale bar), (F) 20S CP disk + RNA + **7e** (200 nm scale bar).

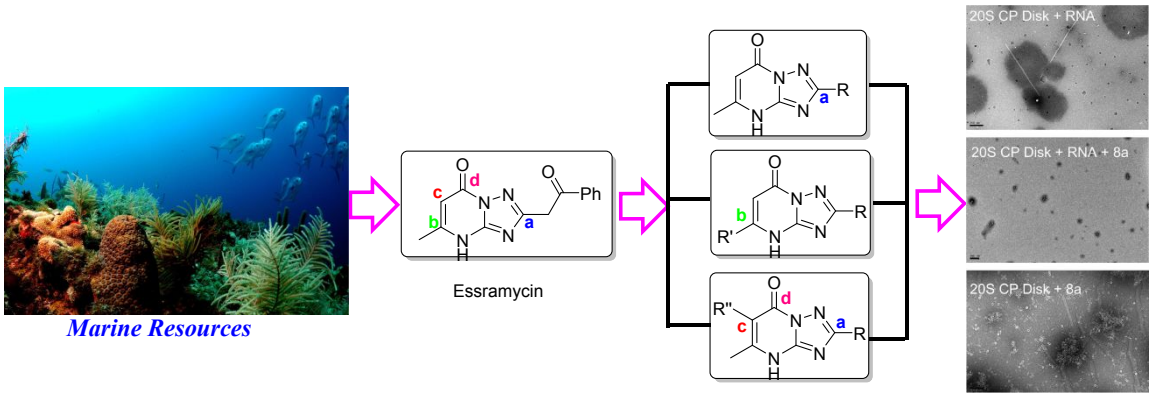
Figure 10.



(A) CP, (B) CP + DMSO (100 nm scale bar), (C) CP + antofine (100 nm scale bar), (D) CP + NK0209 (100 nm scale bar), (E) CP + **7e** (100 nm scale bar), (F) CP + **7e** (50 nm scale bar).

TOC ***graphic***

Agrochemical Bioregulators



Novel Antiviral and Anti-phytopathogenic-Fungus Agents Discovery