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Luotonin A and Its Derivatives as Novel Antiviral and Antiphytopathogenic Fungus Agents

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ABSTRACT: Plant diseases caused by viruses and fungi have posed a serious threat to global agricultural production. The discovery of new leads based on natural products is an important way to innovate pesticides. In this work, natural product luotonin A was found to have good antiviral activity against tobacco mosaic virus (TMV) for the first time. A series of luotonin A derivatives were designed, synthesized, and evaluated for their antiviral activities and fungicidal activities systematically. Most compounds displayed better antiviral activities against TMV than commercial ribavirin. Compounds **9k**, **12b**, and **12d** displayed about similar inhibitory effects as ningnanmycin (inhibitory rates of 55, 57, and 59% at 500 μg/mL for inactivation, curative, and protection activities *in vivo*, respectively), the best antiviral agent at present, and emerged as novel antiviral leads for further research. We selected **9k** for further antiviral mechanism research *via* transmission electron microscopy and molecular docking, which revealed that compound **9k** can interact with TMV coat protein through the hydrogen bond, leading to its polymerization, thus preventing virus assembly. Further fungicidal activity tests showed that these compounds also showed broad-spectrum fungicidal activities against 14 kinds of phytopathogenic fungi. Especially, compound **14** with a 100% antifungal effect against *Botrytis cinereal* emerged as a lead for further research. This work provides a reference for the development of agricultural active ingredients based on Chinese medicine plants.

KEYWORDS: *lead discovery, structure optimization, luotonin A, antiviral activity, fungicidal activity, mode of action*

INTRODUCTION

In the coming decades, the global demand for crops will continue to grow, which is affected by the growth of population and the improvement of per capita consumption.[1] Also every day, a large area of normal land is becoming marginal, which greatly reduces productivity and endangers food security.[2] We are facing one of the greatest challenges of the 21st century: reducing the harm of agriculture to the environment while meeting the growing food demand of society.[3] Plant diseases caused by viruses and fungi have posed a serious threat to global agricultural production. Tobacco mosaic virus (TMV), one of the most widely studied plant pathogens in the world, has the most extensive host range of more than 885 species in 65 families.[4] However, there are few commercially available antiviral agents. At present, the most effective antiviral agent ningnanmycin has an inhibitory effect of 50−60% at 500 μg/mL, and the most widely used antiviral agent ribavirin has a less than 50% inhibitory effect at 500 μg/mL.[5] Therefore, it is urgent to develop novel and more effective antiviral agents.

Natural products are the metabolites of animals and plants, which have the advantages of biocompatibility, structural diversity, and unique mechanism.[6,7] The discovery of new leads based on natural products is an important way to innovate pesticides. There have been many studies on the structural derivation of natural products in an attempt to find highly active compounds.[8−13] Quinazolinone and its derivatives are the basic skeletons of about 150 natural alkaloids isolated from plants, animals, and microorganisms.[14] As a representative molecule of quinazolone, luotonin A was isolated from *Peganum nigellastrum Bunge* (a Chinese medicinal plant) for the first time in 1997.[15] Studies have shown that luotonin A has certain inhibitory activity on topoisomerase I and topoisomerase II.[16−19] The unique molecular structure and spectral biological activity of luotonin A have attracted more and more attention of researchers. However, the researchers mainly focus on the antitumor research of luotonin A, rarely on the agricultural activity research at present. In particular, there is no report on the study of antiplant virus activity.

Our group has long been committed to the separation of active components of traditional Chinese medicine plants and the creation of antiviral agents based on the active ingredients. We have discovered that Chinese medicine plant *Cynanchum komarovii AL* has good antiviral activity against TMV, and the active components were tylophora alkaloids, such as tylophorine.[20] However, the tylophorine content of the plants is very low, and the molecule is light-sensitive and poorly soluble. In efforts to overcome these disadvantages, various tylophora alkaloids[21] and their simplified salt forming and sugar forming derivatives were designed and synthesized and evaluated for their anti-TMV activities.[22−24] A racemate of

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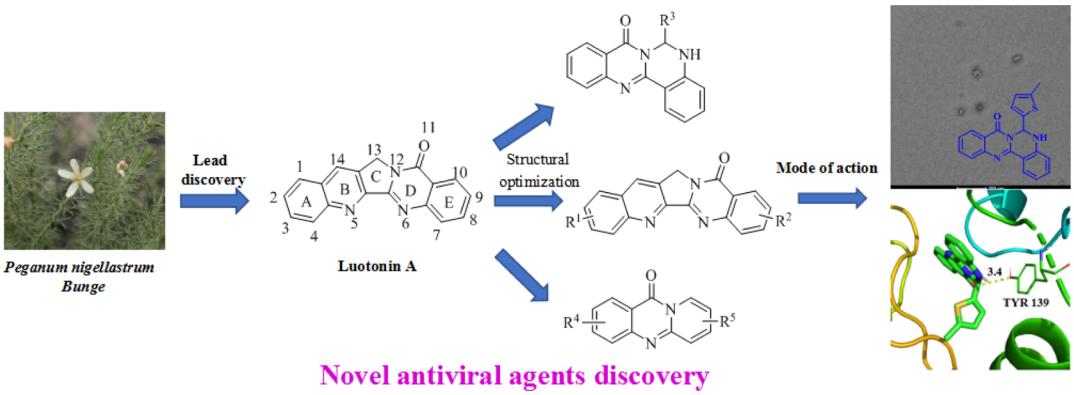

Figure 1. Design of luotonin A analogues.

tylophorine malate (designated NK007), an optimized compound, is being industrialized as an anti-TMV candidate.

Luotonin A also has a five-ring planar structure, which is low in content and poor in solubility. Based on our work experience and the above findings, a series of luotonin A derivatives were designed (Figure 1) and synthesized through ring expansion and ring contraction strategies. The anti-TMV and fungicidal activities and structure−activity relationships of these compounds were systematically evaluated.

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.

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 coat protein (CP) disk assembly inhibition were tested *via* transmission electron microscopy (Tecnai G2 F20).

*Preparation of Ethyl 4-Oxo-3,4-dihydroquinazoline-2-carboxylate (**2**).* A mixture of anthranilamide (**1**, 2 g, 14.7 mmol) and diethyl oxalate (50 mL) was refluxed at 185−186 °C for 5 h. Then, it was cooled to room temperature and filtered to give compound **2**: white solid, yield 87.5%; mp 190−192 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.65 (s, 1H), 8.19 (dd, J = 7.9, 1.1 Hz, 1H), 7.94−7.88 (m, 1H), 7.86−7.82 (m, 1H), 7.69−7.62 (m, 1H), 4.39 (q, J = 7.1 Hz, 2H), 1.36 (t, J = 7.1 Hz, 3H).

General Procedures for the Preparation of Compounds 3a−3d. To a solution of corresponding phenylamines (22 mmol) in anhydrous dichloroethane (60 mL) was added 2 M $AlMe_3$ (22 mL, 11 mmol) dropwise under an Ar atmosphere. The reaction mixture was stirred at room temperature for 0.5 h and then added compound **2** (3 g, 13.76 mmol). The mixture was heated at reflux for 5 h and then cooled to 0 °C and quenched by slow addition of 2 M HCl (20 mL) and H_2O (100 mL). Resulting precipitates were collected by filtration and washed with water to give **3a−3d**.

*4-Oxo-N-phenyl-3,4-dihydroquinazoline-2-carboxamide (**3a**).* It is obtained as a white solid, yield 97%; mp 247−249 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.52 (s, 1H), 10.79 (s, 1H), 8.21 (d, J = 7.6 Hz, 1H), 7.97−7.86 (m, 4H), 7.66 (t, J = 7.2 Hz, 1H), 7.41 (t, J = 7.9 Hz, 2H), 7.18 (t, J = 7.4 Hz, 1H).

*4-Oxo-N-(p-tolyl)-3,4-dihydroquinazoline-2-carboxamide (**3b**).* It is obtained as a light pink solid, yield 95%; mp 197−199 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.48 (s, 1H), 10.71 (s, 1H), 8.21 (d, J = 7.8 Hz, 1H), 7.95−7.87 (m, 2H), 7.76 (d, J = 8.4 Hz, 2H), 7.65 (t, J = 7.0 Hz, 1H), 7.21 (d, J = 8.4 Hz, 2H), 2.30 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.5, 158.3, 147.4, 146.4, 135.6, 135.3, 134.2, 129.7, 128.8, 128.4, 126.6, 123.2, 121.0, 21.0. $C_{16}H_{14}N_3O_2$ [M + H]$^+$, 280.1081; found, 280.1077.

*N-(3,4-Dimethoxyphenyl)-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**3c**).* It is obtained as a yellow green solid, yield 65%; mp 176−178 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.45 (s, 1H), 10.66 (s, 1H), 8.21 (d, J = 7.7 Hz, 1H), 7.96−7.87 (m, 2H), 7.68−7.62 (m, 1H), 7.58 (d, J = 2.3 Hz, 1H), 7.51 (dd, J = 8.7, 2.3 Hz, 1H), 6.98 (d, J = 8.8 Hz, 1H), 3.77 (d, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.5, 158.0, 149.0, 147.4, 146.4, 146.3, 135.3, 131.5, 128.7, 128.3, 126.7, 123.1, 112.9, 112.2, 106.0, 56.1, 55.9. $C_{17}H_{16}N_3O_4$ [M + H]$^+$, 326.1135; found, 326.1131.

*N-(4-Chlorophenyl)-4-oxo-3,4-dihydroquinazoline-2-carboxamide (**3d**).* It is obtained as a white solid, yield 54%; mp 294−296 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.55 (s, 1H), 10.96 (s, 1H), 8.21 (d, J = 7.8 Hz, 1H), 7.97−7.90 (m, 4H), 7.66 (t, J = 6.7 Hz, 1H), 7.50−7.44 (m, 2H).

General Procedures for the Preparation of Compounds 4a−4d. To a solution of compounds **3a−3d** (28.3 mmol) in dimethylformamide (DMF) (200 mL) was successively added K_2CO_3 (4.3 g, 31.1 mmol) and propargyl bromide (2.7 mL, 31.1 mmol). The reaction mixture was stirred at room temperature overnight and then poured into ice water. The resulting precipitates were collected by filtration and purified by flash chromatography on a silica gel using dichloromethane and petroleum ether (4:1, v/v) as the eluent to give **4a−4d**.

*4-Oxo-N-phenyl-3-(prop-2-yn-1-yl)-3,4-dihydroquinazoline-2-carboxamide (**4a**).* It is obtained as a white solid, yield 78%; mp 175−177 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.15 (s, 1H), 8.25 (d, J = 7.0 Hz, 1H), 7.99−7.92 (m, 1H), 7.85 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 7.7 Hz, 2H), 7.69 (t, J = 7.5 Hz, 1H), 7.41 (t, J = 7.9 Hz, 2H), 7.19 (t, J = 7.4 Hz, 1H), 5.05 (d, J = 2.4 Hz, 2H), 3.35 (t, J = 2.4 Hz, 1H).

*4-Oxo-3-(prop-2-yn-1-yl)-N-(p-tolyl)-3,4-dihydroquinazoline-2-carboxamide (**4b**).* It is obtained as a light pink solid, yield 70%; mp 178−180 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.07 (s, 1H), 8.24 (d, J = 7.8 Hz, 1H), 7.95 (t, J = 7.6 Hz, 1H), 7.85 (d, J = 7.9 Hz, 1H), 7.69 (t, J = 7.6 Hz, 1H), 7.64 (d, J = 7.1 Hz, 2H), 7.21 (d, J = 7.4 Hz, 2H), 5.04 (s, 2H), 3.34 (s, 1H), 2.30 (s, 3H). $C_{19}H_{16}N_3O_2$ [M + H]$^+$, 318.1237; found, 318.1233.

*N-(3,4-Dimethoxyphenyl)-4-oxo-3-(prop-2-yn-1-yl)-3,4-dihydroquinazoline-2-carboxamide (**4c**).* It is obtained as a yellow green solid, yield 50%; mp 160−162 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.98 (s, 1H), 8.24 (d, J = 7.0 Hz, 1H), 7.95 (dd, J = 11.9, 4.8 Hz, 1H), 7.85 (d, J = 8.0 Hz, 1H), 7.69 (t, J = 7.6 Hz, 1H), 7.42 (d, J = 2.3 Hz, 1H), 7.32 (dd, J = 8.7, 2.3 Hz, 1H), 6.98 (d, J = 8.7 Hz, 1H), 5.06 (d, J = 2.3 Hz, 2H), 3.77 (d, J = 2.1 Hz, 6H), 3.34 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.0, 159.1, 148.5, 148.3, 145.9, 145.7, 135.3, 131.0, 128.7, 127.7, 126.5, 120.9, 112.4, 111.9, 105.1, 78.5, 74.9, 55.7, 55.4, 33.1. $C_{20}H_{18}N_2O_4$ [M + H]$^+$, 364.1292; found, 364.1288.

*N-(4-Chlorophenyl)-4-oxo-3-(prop-2-yn-1-yl)-3,4-dihydroquinazoline-2-carboxamide (**4d**).* It is obtained as a white solid, yield 54%; mp 186−188 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.30 (s, 1H),

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8.25 (d, J = 6.8 Hz, 1H), 7.99−7.93 (m, 1H), 7.88−7.83 (m, 1H), 7.83−7.77 (m, 2H), 7.70 (t, J = 7.6 Hz, 1H), 7.51−7.46 (m, 2H), 5.05 (d, J = 2.4 Hz, 2H), 3.35 (t, J = 2.5 Hz, 1H).

General Procedures for the Preparation of Compounds 5a−5d.[25] To a solution of triphenylphosphine oxide (9.7 g, 34.8 mmol) in anhydrous CH_2Cl_2 (300 mL) was added trifluoromethanesulfonic anhydride (3 mL, 18 mmol) slowly at 0 °C under an Ar atmosphere. After the mixture was stirred at 0 °C for 10 min, one of compounds **4a−4d** (11.6 mmol) was added. The reaction mixture was stirred at room temperature and quenched by addition of 10% aqueous $NaHCO_3$ solution. The resulting precipitates were collected by filtration and washed with $CHCl_3$ to give **5a−5d**.

*Quinolino[2′,3′:3,4]pyrrolo[2,1-b]quinazolin-11(13H)-one (**5a**).* It is obtained as a white solid, yield 78%; mp 208−210 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.78 (s, 1H), 8.32−8.27 (m, 2H), 8.18 (d, J = 8.1 Hz, 1H), 7.98−7.89 (m, 3H), 7.78 (t, J = 7.5 Hz, 1H), 7.68−7.62 (m, 1H), 5.33 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.2, 153.6, 152.0, 149.5, 148.8, 135.0, 132.3, 131.5, 133.0, 130.2, 129.0, 128.8, 128.5, 127.7, 126.4, 48.0.

*2-Methylquinolino[2′,3′:3,4]pyrrolo[2,1-b]quinazolin-11(13H)-one (**5b**).* It is obtained as a light red solid, yield 70%; mp 272−274 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.66 (s, 1H), 8.30 (d, J = 7.8 Hz, 1H), 8.18 (d, J = 8.7 Hz, 1H), 7.96−7.93 (m, 3H), 7.78−7.74 (m, 1H), 7.67−7.61 (m, 1H), 5.31 (s, 2H), 2.58 (s, 3H).

*2,3-Dimethoxyquinolino[2′,3′:3,4]pyrrolo[2,1-b]quinazolin-11-(13H)-one (**5c**).* It is obtained as a yellow green solid, yield 76%; mp 280−282 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.55 (s, 1H), 8.29 (d, J = 7.6 Hz, 1H), 7.97−7.88 (m, 2H), 7.68−7.60 (m, 2H), 7.54 (s, 1H), 5.28 (s, 2H), 4.00 (d, J = 10.1 Hz, 6H).

*2-Chloroquinolino[2′,3′:3,4]pyrrolo[2,1-b]quinazolin-11(13H)-one (**5d**).* It is obtained as a white solid, yield 99%; mp >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.74 (s, 1H), 8.35 (d, J = 2.2 Hz, 1H), 8.31−8.28 (m, 2H), 7.97−7.90 (m, 3H), 7.68−7.62 (m, 1H), 5.33 (s, 2H).

*Preparation of N-(2-Carbamoylphenyl)-2-nitrobenzamide (**6**).* To a solution of compound 2-aminobenzamide (5 g, 37 mmol) in $CHCl_3$ was added triethylamine (14 mL), 2-nitrobenzoyl chloride (7 mL, 52 mmol). The reaction mixture was stirred at room temperature for 4 h, and the resulting precipitates were collected by filtration to give **6**: white solid, yield 80%; mp 195−197 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.52 (s, 1H), 8.48 (d, J = 8.2 Hz, 1H), 8.38 (brs, 1H), 8.11 (d, J = 8.1 Hz, 1H), 7.92−7.75 (m, 5H), 7.59 (t, J = 7.7 Hz, 1H), 7.23 (t, J = 7.6 Hz, 1H).

*Preparation of 2-(2-Nitrophenyl) Quinazolin-4(3H)-one (**7**).* Compound **6** (4 g, 14 mmol) was dissolved in a solution of 10% aqueous KOH solution (40 mL) and ethanol (20 mL). The mixture was stirred at reflux for 5 h. After completion, the ethanol was concentrated *in vacuo*, and the residue was extracted with ethyl acetate, dried over anhydrous Na_2SO_4, filtered, and concentrated to give **7**: light brown solid, yield 92%; mp 178−180 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.89 (s, 1H), 8.33−8.19 (m, 2H), 8.00−7.83 (m, 4H), 7.71 (d, J = 8.2 Hz, 1H), 7.64 (t, J = 7.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.6, 151.8, 148.4, 147.4, 134.7, 133.9, 131.5, 131.4, 129.2, 127.3, 127.1, 125.9, 124.5, 121.1.

*Preparation of 2-(2-Aminophenyl) Quinazolin-4(3H)-one (**8**).* To a solution of compound **7** (1.2 g, 4.5 mmol) in ethanol was added $SnCl_2{\cdot}2H_2O$ (5.1 g, 22.5 mmol). The mixture was stirred at reflux for 6 h. After completion, the solvent was concentrated *in vacuo*, and the residue was extracted with ethyl acetate, dried over anhydrous Na_2SO_4, filtered, and concentrated to give **8**: yellow solid, yield 80%; mp 155−157 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.18 (brs, 1H), 8.19 (d, J = 7.9 Hz, 1H), 7.88 (t, J = 7.6 Hz, 1H), 7.84−7.73 (m, 2H), 7.55 (t, J = 7.5 Hz, 1H), 7.26 (t, J = 7.6 Hz, 1H), 7.12 (brs, 1H), 6.89 (d, J = 8.3 Hz, 1H), 6.66 (t, J = 7.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.6, 154.1, 149.9, 148.5, 135.0, 132.3, 129.3, 127.4, 126.7, 126.2, 120.9, 117.1, 115.5, 112.8.

General Procedures for the Preparation of Compounds 9a−9m. To a solution of compound **8** (500 mg, 2.11 mmol) in [BmIm]Br (4 mL) was added I_2 (27 mg, 0.11 mmol) and corresponding aldehydes (2.11 mmol). After completion, the reaction was quenched by H_2O (50 mL). The resulting precipitates were collected by filtration to give **9a−9m**.

*5,6-Dihydro-8H-quinazolino[4,3-b]quinazolin-8-one (**9a**).* It is obtained as a yellow solid, yield 63%; mp 178−180 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 8.27−8.16 (m, 2H), 7.81−7.76 (m, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.48−7.42 (m, 1H), 7.40−7.34 (m, 1H), 6.99−6.92 (m, 1H), 6.88 (d, J = 8.1 Hz, 1H), 5.31 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.8, 148.6, 148.4, 148.2, 135.1, 133.7, 127.7, 127.5, 126.8, 126.4, 120.8, 119.5, 116.6, 116.1, 52.9. HRMS (ESI): calcd for $C_{15}H_{12}N_3O$ [M + H]$^+$, 250.0975; found, 250.0974.

*6-Methyl-5,6-dihydro-8H-quinazolino[4,3-b]quinazolin-8-one (**9b**).* It is obtained as a yellow solid, yield 65%; mp 116−118 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 8.27−8.16 (m, 2H), 7.83−7.76 (m, 1H), 7.72 (d, J = 8.1 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.40−7.33 (m, 1H), 6.90 (t, J = 7.6 Hz, 1H), 6.84 (d, J = 8.1 Hz, 1H), 6.32 (q, J = 6.1 Hz, 1H), 1.43 (d, J = 6.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.5, 148.1, 147.3, 145.7, 135.1, 134.1, 127.5, 127.4, 126.8, 126.4, 120.5, 118.9, 116.4, 115.1, 59.3, 19.9. HRMS (ESI): calcd for $C_{16}H_{14}N_3O$ [M + H]$^+$, 264.1131; found, 264.1131.

*6-Cyclohexyl-5,6-dihydro-8H-quinazolino[4,3-b]quinazolin-8-one (**9c**).* It is obtained as a yellow solid, yield 83%; mp 235−237 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.17 (d, J = 7.6 Hz, 2H), 7.83 (t, J = 7.6 Hz, 1H), 7.70 (d, J = 8.2 Hz, 1H), 7.57−7.44 (m, 2H), 7.37 (t, J = 7.6 Hz, 1H), 6.92 (d, J = 8.1 Hz, 1H), 6.84 (t, J = 7.5 Hz, 1H), 5.96−5.86 (m, 1H), 1.78 (d, J = 10.0 Hz, 2H), 1.69−1.44 (m, 3H), 1.22−1.11 (m, 1H), 1.11−0.88 (m, 5H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.2, 148.0, 147.6, 145.6, 135.2, 134.1, 127.5, 127.2, 126.3, 120.5, 118.6, 115.9, 115.8, 65.9, 28.7, 28.7, 26.0, 25.5, 25.5. HRMS (ESI): calcd for $C_{21}H_{22}N_3O$ [M + H]$^+$, 332.1757; found, 332.1757.

*6-Cyclopropyl-5,6-dihydro-8H-quinazolino[4,3-b]quinazolin-8-one (**9d**).* It is obtained as a yellow solid, yield 97%; mp 98−100 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32−8.26 (m, 1H), 8.19 (d, J = 8.2 Hz, 1H), 7.74−7.64 (m, 2H), 7.42−7.31 (m, 2H), 6.88 (t, J = 7.6 Hz, 1H), 6.64 (d, J = 8.2 Hz, 1H), 5.45−5.38 (m, 1H), 3.60−3.50 (m, 1H), 3.38−3.29 (m, 1H), 3.28−3.18 (m, 1H), 2.22−1.93 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.9, 146.9, 146.9, 144.5, 133.3, 132.5, 127.1, 126.1, 125.4, 125.0, 120.0, 118.0, 115.5, 111.8, 71.0, 44.6, 31.5, 19.8. HRMS (ESI): calcd for $C_{18}H_{16}N_3O$ [M + H]$^+$, 290.1288; found, 290.1287.

*6-Phenyl-5,6-dihydro-8H-quinazolino[4,3-b]quinazolin-8-one (**9e**).* It is obtained as a red brown solid, yield 71%; mp 188−190 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.23−8.18 (m, 1H), 8.15−8.10 (m, 1H), 8.02 (d, J = 3.3 Hz, 1H), 7.91−7.85 (m, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.53 (t, J = 7.1 Hz, 1H), 7.37−7.30 (m, 1H), 7.30−7.21 (m, 4H), 7.18 (d, J = 6.8 Hz, 2H), 6.93 (d, J = 8.0 Hz, 1H), 6.82 (t, J = 7.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.7, 147.7, 147.3, 145.1, 139.4, 135.0, 133.7, 128.6, 128.2, 127.2, 126.9, 126.7, 126.2, 125.7, 119.9, 118.8, 115.9, 115.5, 62.5. HRMS (ESI): calcd for $C_{21}H_{16}N_3O$ [M + H]$^+$, 326.1288; found, 326.1288.

*6-(2,4-Dimethoxyphenyl)-5,6-dihydro-8H-quinazolino[4,3-b]-quinazolin-8-one (**9f**).* It is obtained as a green solid, yield 65%; mp 193−195 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (d, J = 7.7 Hz, 1H), 8.10 (d, J = 7.7 Hz, 1H), 7.86 (t, J = 7.1 Hz, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.48 (t, J = 7.4 Hz, 1H), 7.33 (d, J = 2.6 Hz, 1H), 7.31−7.24 (m, 2H), 6.89−6.78 (m, 2H), 6.61 (d, J = 1.9 Hz, 1H), 6.47 (d, J = 8.5 Hz, 1H), 6.29−6.22 (m, 1H), 3.90 (s, 3H), 3.65 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.6, 159.1, 157.4, 148.0, 147.7, 145.0, 134.9, 133.4, 127.2, 126.7, 126.5, 126.1, 125.8, 119.9, 119.5, 118.4, 115.8, 115.0, 104.0, 98.9, 59.2, 55.8, 55.1. HRMS (ESI): calcd for $C_{23}H_{20}N_3O_3$ [M + H]$^+$, 386.1499; found, 386.1500.

*6-(3-Nitrophenyl)-5,6-dihydro-8H-quinazolino[4,3-b]quinazolin-8-one (**9g**).* It is obtained as a yellow solid, yield 85%; mp 183−185 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (t, J = 6.9 Hz, 2H), 7.81 (d, J = 8.6 Hz, 2H), 7.58 (s, 2H), 7.33−7.18 (m, 4H), 7.16 (t, J = 7.5 Hz, 1H), 6.80 (t, J = 7.6 Hz, 1H), 6.67 (d, J = 8.0 Hz, 1H), 5.13 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.8, 148.2, 147.9, 146.5, 146.2, 142.7, 135.0, 133.8, 127.9, 127.8, 127.3, 127.1, 126.6, 123.9, 121.6, 120.4, 118.2, 117.1, 62.8. HRMS (ESI): calcd for $C_{21}H_{15}N_4O_3$ [M + H]$^+$, 371.1139; found, 371.1140.

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*6-(2,4-Dichlorophenyl)-5,6-dihydro-8H-quinazolino[4,3-b]-quinazolin-8-one (**9h**).* It is obtained as a yellow solid, yield 82%; mp 237−239 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.25 (d, J = 7.0 Hz, 1H), 8.09 (dd, J = 7.9, 1.0 Hz, 1H), 7.91−7.85 (m, 1H), 7.79 (d, J = 8.0 Hz, 1H), 7.77−7.70 (m, 2H), 7.54−7.47 (m, 1H), 7.38 (d, J = 3.8 Hz, 1H), 7.36−7.29 (m, 1H), 7.22 (dd, J = 8.4, 2.1 Hz, 1H), 6.93−6.83 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.2, 147.7, 147.3, 143.5, 135.8, 135.1, 133.9, 133.8, 132.4, 129.6, 127.7, 127.3, 127.1, 126.9, 126.4, 119.8, 119.2, 116.2, 115.1, 60.7. HRMS (ESI): calcd for $C_{21}H_{14}Cl_2N_3O$ $[M + H]^+$, 394.0508; found, 394.0509.

*6-(Pyridin-4-yl)-5,6-dihydro-8H-quinazolino[4,3-b]quinazolin-8-one (**9i**).* It is obtained as a yellow solid, yield 84%; mp 114−116 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.54−8.43 (m, 2H), 8.23 (d, J = 7.2 Hz, 1H), 8.15−8.07 (m, 2H), 7.89 (t, J = 7.7 Hz, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.55 (t, J = 7.5 Hz, 1H), 7.36 (t, J = 7.1 Hz, 1H), 7.29 (d, J = 3.6 Hz, 1H), 7.17 (d, J = 5.3 Hz, 2H), 6.98 (d, J = 8.1 Hz, 1H), 6.92−6.81 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.2, 150.5, 148.6, 148.2, 147.4, 145.3, 135.6, 134.3, 127.8, 127.4, 127.2, 126.9, 121.4, 120.3, 119.7, 116.6, 116.2, 62.2. HRMS (ESI): calcd for $C_{20}H_{15}N_4O$ $[M + H]^+$, 327.1240; found, 327.1239.

*6-(1H-Imidazol-5-yl)-5,6-dihydro-8H-quinazolino[4,3-b]-quinazolin-8-one (**9j**).* It is obtained as a yellow solid, yield 72%; mp 211−243 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (d, J = 7.8 Hz, 2H), 7.88 (t, J = 7.1 Hz, 1H), 7.77 (d, J = 8.0 Hz, 1H), 7.59 (d, J = 3.6 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 7.37 (t, J = 7.6 Hz, 1H), 7.26 (d, J = 3.7 Hz, 1H), 6.98−6.83 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 159.6, 147.8, 147.5, 144.9, 144.4, 134.8, 133.2, 127.2, 126.8, 126.5, 126.0, 120.3, 119.3, 116.4, 116.3, 58.4. HRMS (ESI): calcd for $C_{18}H_{14}N_5O_3$ $[M + H]^+$, 316.1193; found, 316.1192.

*6-(5-Methylthiophen-2-yl)-5,6-dihydro-8H-quinazolino[4,3-b]-quinazolin-8-one (**9k**).* It is obtained as a brown solid, yield 88%; mp 153−155 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.23−8.14 (m, 2H), 7.93 (d, J = 3.0 Hz, 1H), 7.88−7.82 (m, 1H), 7.73 (d, J = 8.1 Hz, 1H), 7.54−7.48 (m, 1H), 7.45−7.34 (m, 2H), 6.98 (d, J = 8.0 Hz, 1H), 6.93−6.85 (m, 1H), 6.74 (d, J = 3.4 Hz, 1H), 6.57−6.53 (m, 1H), 2.25 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.1, 147.6, 146.6, 145.0, 140.2, 139.3, 135.0, 133.8, 127.2, 126.9, 126.6, 126.2, 126.0, 124.6, 119.9, 119.1, 116.1, 115.3, 59.9, 14.7. HRMS (ESI): calcd for $C_{20}H_{16}N_3OS$ $[M + H]^+$, 346.1009; found, 346.1009.

*6-(5-Bromofuran-2-yl)-5,6-dihydro-8H-quinazolino[4,3-b]-quinazolin-8-one (**9l**).* It is obtained as a brown solid, yield 78%; mp 168−170 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 8.24 (d, J = 8.0 Hz, 2H), 7.86−7.80 (m, 1H), 7.76 (d, J = 8.1 Hz, 1H), 7.50 (t, J = 7.5 Hz, 1H), 7.38 (t, J = 7.1 Hz, 1H), 7.26 (s, 1H), 6.96−6.87 (m, 2H), 6.21 (d, J = 3.4 Hz, 1H), 6.05 (d, J = 3.3 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.6, 154.1, 148.1, 147.2, 145.2, 135.5, 134.1, 127.8, 127.4, 127.1, 126.8, 121.9, 120.4, 119.8, 116.4, 116.0, 113.0, 111.5, 58.2. HRMS (ESI): calcd for $C_{19}H_{13}BrN_3O_2$ $[M + H]^+$, 394.0186; found, 394.0185.

*6-(4-Methylthiazol-5-yl)-5,6-dihydro-8H-quinazolino[4,3-b]-quinazolin-8-one (**9m**).* It is obtained as a brown solid, yield 78%; mp 168−170 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.62 (s, 1H), 8.26 (d, J = 7.0 Hz, 1H), 8.13 (dd, J = 8.0, 1.1 Hz, 1H), 7.88−7.82 (m, 1H), 7.78 (d, J = 3.0 Hz, 1H), 7.73 (d, J = 7.9 Hz, 1H), 7.54−7.45 (m, 2H), 7.45−7.39 (m, 1H), 6.97 (dd, J = 11.2, 4.0 Hz, 1H), 6.88 (d, J = 7.9 Hz, 1H), 2.63 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.0, 151.1, 150.5, 147.4, 144.2, 135.1, 134.0, 129.2, 127.2, 127.0, 126.5, 119.8, 119.5, 116.3, 115.2, 58.2, 15.3. HRMS (ESI): calcd for $C_{19}H_{15}N_4OS$ $[M + H]^+$, 347.0961; found, 347.0959.

*Preparation of 6-(2,4-Dichlorophenyl)-8H-quinazolino[4,3-b]-quinazolin-8-one (**9n**).* The compound **9h** (600 mg, 2 mmol) was dissolved in tetrahydrofuran (THF) solution (40 mL), and the mixture was stirred at 100 °C under an O_2 atmosphere. After completion, the mixture was diluted with water and extracted with methyl *tert*-butyl ether (3 × 50 mL). The combined organic phase was washed with brine (3 × 100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography on a silica gel using petroleum ether and ethyl acetate (10:1, v/v) as the eluent to give **9n**: light yellow solid, yield 61%; mp 131−133 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.82 (d, J = 7.9 Hz, 1H), 8.23 (d, J = 8.0 Hz, 1H), 7.87−7.76 (m, 4H), 7.65 (t, J = 6.8 Hz, 1H), 7.59 (d, J = 7.9 Hz, 1H), 7.50−7.38 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.8, 146.9, 146.6, 145.3, 142.0, 136.1, 135.7, 135.4, 133.7, 131.9, 129.8, 129.3, 129.0, 128.1, 127.6, 127.5, 127.3, 126.7, 126.2, 121.7, 119.8. HRMS (ESI): calcd for $C_{21}H_{12}Cl_2N_3O$ $[M + H]^+$, 392.0352; found, 392.0353.

*Preparation of 8H-Quinazolino[4,3-b]quinazolin-8-one (**9o**).* To a solution of compound **8** (500 mg, 2.1 mmol) in toluene (50 mL) was added DMF-dimethylacetal (500 mg, 4.2 mmol) and acetate (0.1 mL). The mixture was stirred at room temperature for 10 h. After completion, the mixture was diluted with water and extracted with methyl *tert*-butyl ether (3 × 50 mL). The combined organic phase was washed with brine (3 × 100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated to give **9o**: yellow solid, yield 89%; mp 200−202 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.29 (s, 1H), 8.72 (d, J = 7.9 Hz, 1H), 8.32 (d, J = 7.9 Hz, 1H), 7.97 (t, J = 7.6 Hz, 1H), 7.90 (t, J = 7.5 Hz, 1H), 7.84 (d, J = 7.9 Hz, 2H), 7.73 (t, J = 7.5 Hz, 1H), 7.60 (t, J = 7.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.7, 147.1, 144.5, 142.9, 138.1, 135.9, 133.8, 128.9, 127.7, 127.3, 127.0, 126.5, 125.4, 121.2, 118.7. HRMS (ESI): calcd for $C_{15}H_{10}N_3O$ $[M + H]^+$, 248.0818; found, 248.0820.

General Procedures for the Preparation of Compounds 12a−12h, 14, 16.[26] To a solution of corresponding isatins (3.4 mmol), corresponding 2-bromide pyridines or 2-bromthiazols or 2-brombenzothiazoles (2.1 mmol) in dry THF (50 mL) was added $NaHCO_3$ (0.57 g, 6.8 mmol) and $Cu(OAc)_2{\cdot}H_2O$ (0.14 g, 0.68 mmol). The mixture was heated at reflux for 10 h. After completion, the mixture was diluted with water and extracted with methyl *tert*-butyl ether (3 × 50 mL). The combined organic phase was washed with brine (3 × 100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. 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 **12a−12h**, **14**, **16**.

*11H-Pyrido[2,1-b]quinazolin-11-one (**12a**).* It is obtained as a yellow solid, yield 84%; mp 208−210 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.85 (d, J = 7.2 Hz, 1H), 8.42 (d, J = 8.0 Hz, 1H), 7.87−7.72 (m, 2H), 7.54−7.40 (m, 3H), 6.91−6.80 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.0, 148.6, 147.8, 135.1, 134.1, 127.4, 126.9, 126.7, 126.4, 125.3, 116.3, 112.5.

*2-Fluoro-11H-pyrido[2,1-b]quinazolin-11-one (**12b**).* It is obtained as a yellow solid, yield 81%; mp 164−166 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.89 (d, J = 7.5 Hz, 1H), 8.10 (d, J = 8.6 Hz, 1H), 7.90−7.80 (m, 1H), 7.69−7.61 (m, 1H), 7.54 (s, 2H), 6.97−6.86 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.9, 158.4, 158.4 (d, J = 3.8 Hz), 147.1 (d, J = 1.9 Hz), 145.4, 133.9, 129.5 (d, J = 8.1 Hz), 126.4 (d, J = 6.3 Hz), 124.5 (d, J = 25.1 Hz), 117.0 (d, J = 8.6 Hz), 112.9, 111.2 (d, J = 23.5 Hz).

*2-Chloro-11H-pyrido[2,1-b]quinazolin-11-one (**12c**).* It is obtained as a yellow solid, yield 79%; mp 122−124 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81 (d, J = 7.3 Hz, 1H), 8.35 (d, J = 2.1 Hz, 1H), 7.76−7.65 (m, 2H), 7.54−7.41 (m, 2H), 6.90−6.79 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.1, 146.8, 146.1, 134.6, 133.4, 127.7, 125.7, 125.4, 125.3, 116.0, 112.0.

*2-Methyl-11H-pyrido[2,1-b]quinazolin-11-one (**12d**).* It is obtained as a yellow solid, yield 80%; mp 135−137 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.88 (d, J = 7.4 Hz, 1H), 8.25 (s, 1H), 7.75−7.65 (m, 2H), 7.55−7.45 (m, 2H), 6.85 (s, 1H), 2.54 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.9, 147.1, 146.7, 136.9, 135.4, 133.6, 126.7, 126.7, 126.3, 126.2, 116.1, 112.3, 21.4.

*2-Methoxy-11H-pyrido[2,1-b]quinazolin-11-one (**12e**).* It is obtained as a yellow solid, yield 79%; mp 158−160 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.85 (d, J = 7.4 Hz, 1H), 7.77−7.69 (m, 2H), 7.51−7.40 (m, 3H), 6.87−6.80 (m, 1H), 3.94 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.7, 157.4, 146.1, 143.7, 132.8, 128.7, 126.8, 126.4, 116.9, 112.6, 105.1, 55.9.

*8-(Trifluoromethyl)-11H-pyrido[2,1-b]quinazolin-11-one (**12f**).* It is obtained as a yellow solid, yield 73%; mp 155−157 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.20 (s, 1H), 8.45 (d, J = 8.1 Hz, 1H), 7.89 (t, J = 7.7 Hz, 1H), 7.80 (d, J = 8.3 Hz, 1H), 7.58−7.46 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.5, 147.1, 145.6, 134.7, 127.9, 126.7,

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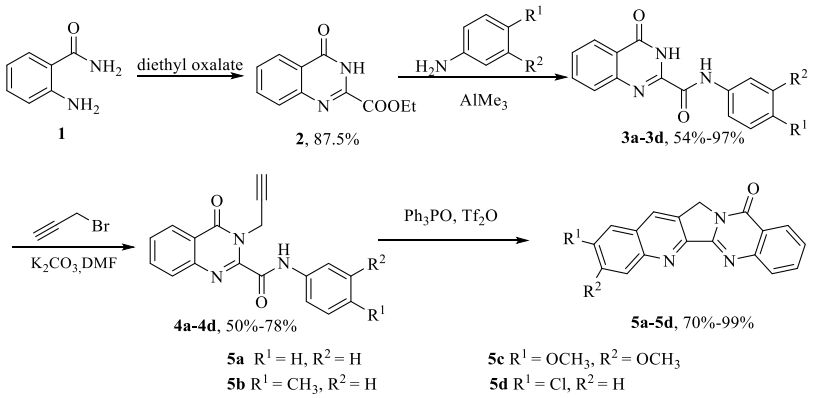

Figure 2. Synthesis of compounds **5a−5d**.

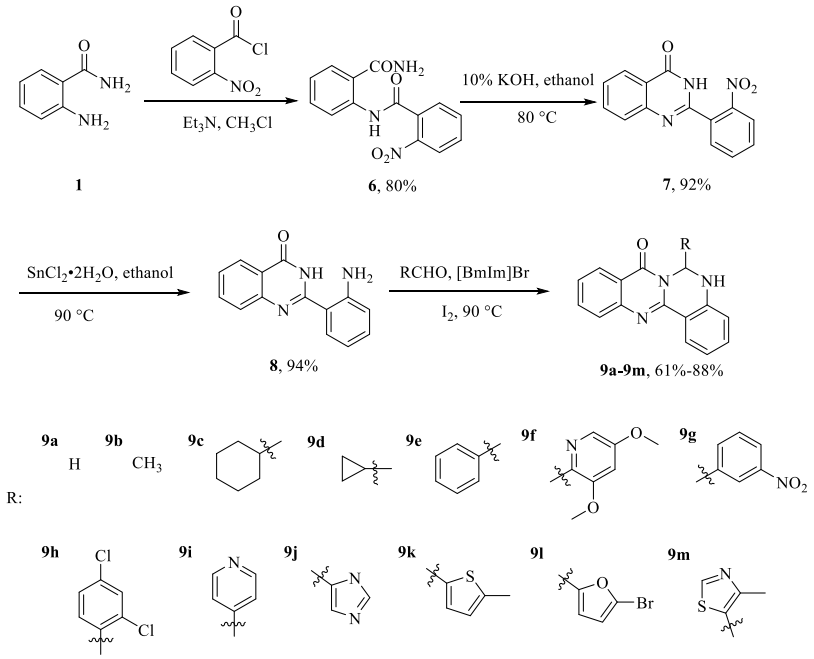

Figure 3. Synthesis of compounds **9a−9m**.

126.5, 126.3, 125.6 (q, J = 5.8 Hz), 125.3, 121.7 (q, J = 342.5 Hz), 115.6, 115.5.

*8-Bromo-11H-pyrido[2,1-b]quinazolin-11-one (**12g**).* It is obtained as a yellow solid, yield 76%; mp 162−164 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.99 (d, J = 1.8 Hz, 1H), 8.44 (dd, J = 8.2, 1.1 Hz, 1H), 7.88−7.82 (m, 1H), 7.77 (d, J = 8.3 Hz, 1H), 7.54−7.46 (m, 2H), 7.38 (d, J = 9.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.0, 148.2, 145.9, 137.5, 135.3, 127.5, 127.4, 127.2, 126.6, 126.0, 116.3, 107.7.

*8-Methyl-11H-pyrido[2,1-b]quinazolin-11-one (**12h**).* It is obtained as a yellow solid, yield 85%; mp 148−150 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.63 (s, 1H), 8.31 (d, J = 8.1 Hz, 1H), 7.89 (t, J = 7.7 Hz, 1H), 7.74 (d, J = 8.3 Hz, 1H), 7.62 (d, J = 9.3 Hz, 1H), 7.54−7.46 (m, 2H), 2.34 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.1, 153.3, 151.7, 143.3, 139.9, 131.8, 130.5, 130.0, 128.2, 127.8, 125.7, 120.8, 22.9.

*5H-Thiazolo[2,3-b]quinazolin-5-one (**14**).* It is obtained as a yellow solid, yield 89%; mp 142−144 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 (d, J = 8.2 Hz, 1H), 7.95 (d, J = 5.0 Hz, 1H), 7.81 (t, J = 7.1 Hz, 1H), 7.69 (d, J = 8.3 Hz, 1H), 7.47 (t, J = 7.6 Hz, 1H), 6.85 (d, J = 5.1 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.7, 158.1, 148.4, 135.0, 127.1, 126.2, 125.4, 121.2, 116.5, 109.5.

*12H-Benzo[4,5]thiazolo[2,3-b]quinazolin-12-one (**16**).* It is obtained as a white solid, yield 87%; mp 188−190 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.05 (d, J = 8.5 Hz, 1H), 8.45 (d, J = 7.5 Hz, 1H), 7.82 (t, J = 7.1 Hz, 1H), 7.75−7.60 (m, 2H), 7.55−7.41 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.7, 155.9, 146.2, 135.1, 133.9, 126.1, 125.8, 125.7, 124.9, 124.8, 122.7, 120.7, 118.2, 117.6.

Biological Assay. Each test was repeated three times at 25 ± 1 °C. Active effect expressed in the 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 the literature method,[9,27] which is also seen in Supporting Information. Ningnanmycin aqueous solution (2%) and 99% of ribavirin were purchased from Beijing Hwrkchemical Co., Ltd.

RESULTS AND DISCUSSION

Chemistry. In order to investigate the effect of substituents of quinoline ring on the biological activity, luotonin A (**5a**) and the designed compounds **5b−5d** were synthesized (Figure 2) *via* the reported method with small modification.[25] Quinazolinone **2** was synthesized by condensation of *o*-aminobenzoamide with diethyl oxalate. Ammonolysis of **2** with corresponding amines gave compounds **3a−3d**. The key intermediates **4a−4d** were obtained by the reaction of compounds **3a−3d** with propargyl bromide. The Ph_3PO-

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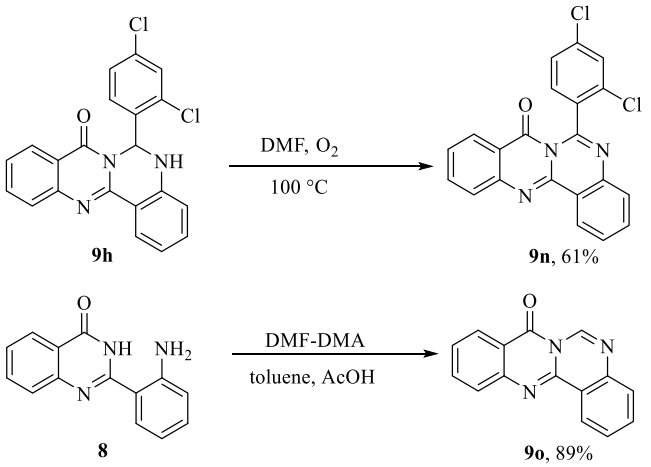

Figure 4. Synthesis of compounds **9n** and **9o**.

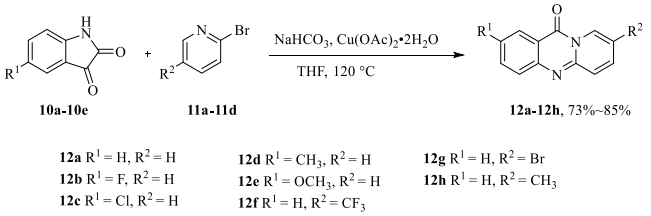

Figure 5. Synthesis of compounds **12a**−**12h**.

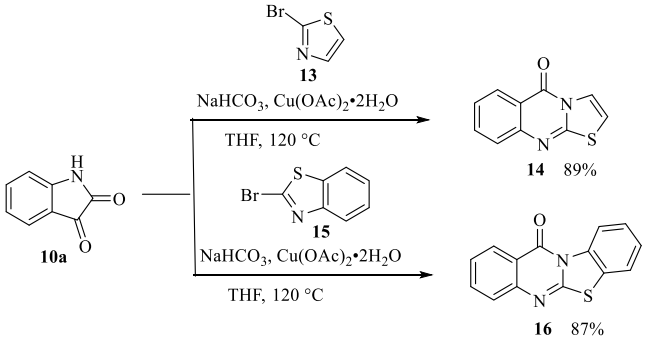

Figure 6. Synthesis of compounds **14** and **16**.

catalyzed tandem cyclization of **4a**−**4d** gave compounds **5a**−**5d**. Solubility has always been a barrier to the application of polycyclic aromatic hydrocarbons. To improve the solubility of luotonin A, the 6-aryl-5*H*-quinazolino[4,3-*b*]quinazolin-8(6*H*)-one derivatives **9a**−**9m** (Figure 3) were designed and synthesized using ionic liquid as the solvent.[28] Condensation of *o*-aminobenzoamide with *o*-nitrobenzoyl chloride gave **6**, which underwent cyclization in the presence of KOH and gave compound **7**. Compound **7** was reduced and then condensed with corresponding aldehydes to obtain compounds **9a**−**9m**. To explore the effect of N−H bond on biological activity, the oxidation products **9n** and **9o** were synthesized (Figure 4). Reducing the number of rings is the most effective way to simplify the molecular structure. In order to study the number of rings on the biological activity, we designed and synthesized **12a**−**12h**, **14**, and **16**, a series of 3/4-membered ring derivatives, by condensation of 2-bromo heterocycles with isatin (Figures 5 and 6).[26]

Phytotoxic Activity. The phytotoxic activity tests (according to the criterion of safety evaluation of pesticide to crops, NYT 1965.1-2010), showed that the luotonin A and its derivatives **5a**−**5d**, **9a**−**9o**, **12a**−**12h**, **14**, and **16** were safe for testing on plants at 500 μg/mL.

Table 1. *In Vivo* Antiviral Activities of Compounds 5a−5d, 9a−9o, 12a−12h, 14, 16, Ningnanmycin, and Ribavirin against TMV

compd	concn (μg/mL)	inactive effect (%)[a]	curative effect (%)[a]	protective effect (%)[a]
5a	500	41±1	45±3	36±4
	100	0	9±1	0
5b	500	35±4	—	—
5c	500	39±1	—	—
5d	500	43±3	38±1	37±3
	100	10±1	0	11±1
9a	500	44±5	35±1	52±4
	100	13±1	0	0
9b	500	49±4	40±5	47±3
	100	8±1	5±1	12±2
9c	500	45±1	39±3	35±5
	100	5±1	0	0
9d	500	48±4	45±3	37±3
	100	9±1	13±2	19±1
9e	500	36±1	—	—
9f	500	46±3	49±2	36±4
	100	0	17±2	0
9g	500	41±4	53±5	51±1
	100	14±2	0	51±1
9h	500	43±3	33±1	41±2
	100	4±1	0	8±1
9i	**500**	**50±2**	**43±4**	**42±2**
	100	**15±1**	**17±1**	**6±2**
9j	500	49±3	30±5	33±4
	100	4±2	0	10±1
9k	**500**	**54±4**	**51±1**	**43±2**
	100	**21±1**	**13±1**	**15±1**
9l	**500**	**53±1**	**47±4**	**56±5**
	100	**19±2**	**8±3**	**21±1**
9m	500	39±1	—	—
9n	500	26±2	—	—
9o	500	22±2	—	—
12a	500	46±2	42±1	48±4
	100	13±3	7±1	15±1
12b	**500**	**52±1**	**54±4**	**47±2**
	100	**20±4**	**16±1**	**16±1**
12c	500	25±2	—	—
12d	**500**	**53±2**	**51±4**	**54±1**
	100	**20±1**	**21±1**	**16±2**
12e	500	22±3	—	—
12f	500	34±1	—	—
12g	500	31±3	—	—
12h	500	42±4	38±1	32±2
	100	7±1	0	0
14	500	12±1	—	—
16	500	39±4	—	—
ningnanmycin	500	55±1	57±2	59±4
	100	26±1	26±1	28±1
ribavirin	500	40±1	37±3	40±2
	100	13±1	9±2	11±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.

Antiviral Activity *In Vivo*. The activities of luotonin A and its derivatives **5a**−**5d**, **9a**−**9o**, **12a**−**12h**, **14**, and **16** against TMV are listed in Table 1 with commercial viral inhibitors ningnanmycin and ribavirin as controls. The inactive activities

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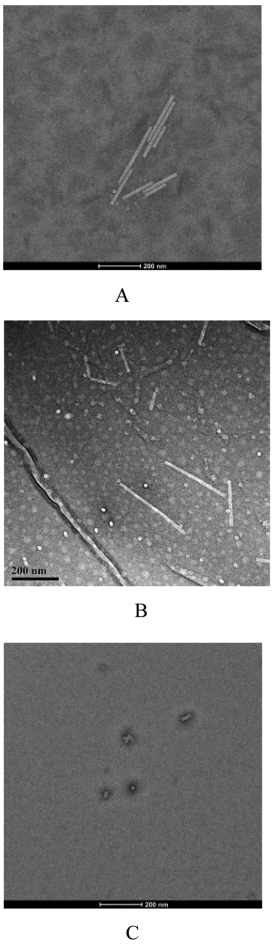

Figure 7. TMV rod assembly inhibition of compound **9k** (200 nm scale bar): (A) 20S CP disk + RNA, (B) 20S CP disk + RNA + 1/100 DMSO, (C) 20S CP disk + RNA + 10 μM **9k**.

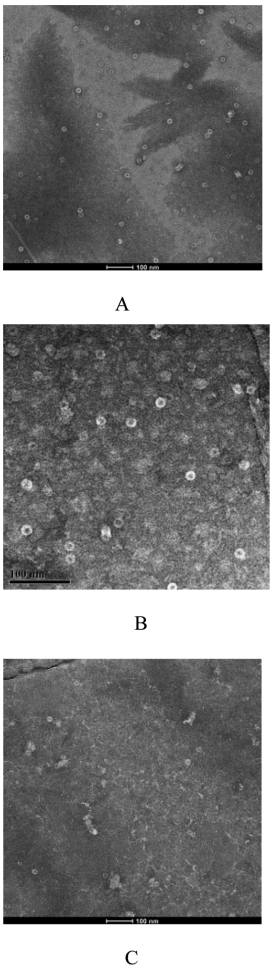

Figure 8. 20S CP disk assembly inhibition of compound **9k** (100 nm scale bar): (A) CP, (B) CP + 1/100 DMSO, (C) CP + 10 μM **9k**.

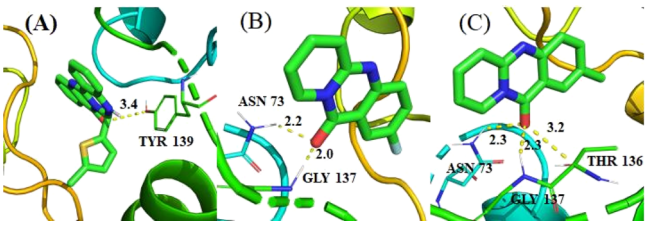

Figure 9. Molecule docking results of compounds **9k** (A), **12b** (B), and **12d** (C) with TMV CP.

of all the compounds were first tested at 500 μg/mL, and the curative activities and protective activities of compounds with good inactive activities (inactive effect > 40%) were further tested.

As shown in Table 1, most of these compounds exhibited better antiviral activities than ribavirin. What is more, compounds **9i**, **9k**, **9l**, **12b**, and **12d** showed about similar level of antiviral activities with ningnanmycin, which may be the most active antiviral agent at present. Natural product luotonin A (**5a**) displayed similar antiviral activity (inhibitory rates of 41, 45, and 36% at 500 μg/mL for inactivation, curative, and protection activities *in vivo*, respectively) with ribavirin (inhibitory rates of 40, 37, and 40% at 500 μg/mL for inactivation, curative, and protection activities *in vivo*, respectively). The effect of introducing push−pull electronic groups into the A-ring on the activity is not obvious, and compounds **5a**, **5c**, and **5d** exhibited about similar level of antiviral activities, and the activity of **5b** is slightly lower. The increase of solubility is beneficial to improve the biological activity of luotonin A, and most of the four-membered fused ring compounds **9a**−**9o** showed better biological activities than luotonin A. Among **9a**−**9o**, compounds **9a**−**9d** containing hydrogen or alkyl groups at 6-postion showed better inhibitory activities than luotonin A. The substitution of alkyl with the benzene ring resulted in a significant decrease in activity (inhibitory effect: **9a**−**9d** > **9e**). The introduction of electron-withdrawing group into the 6-position benzene ring is beneficial for the improvement of activity (inhibitory effect: **9g** and **9g** ≈ **5a** > **9e**). The addition of pyridine, imidazole, thiophene, or furan rings at 6-position is beneficial to the activity improvement (inhibitory effect: **9f**, **9i**, **9j**, **9k**, **9l** > **9a**). Compound **9m** with the thiazole ring at 6-position showed less activity than **9a**. By comparing **9h** and **9n** aand **9a** and **9o** (inhibitory effect: **9h** > **9n**, **9a** > **9o**), it can be found that the N−H bond has a great influence on biological activity. Linear quaternary-fused ring compound **16** showed the same level of activity as luotonin A. By further reducing the number of fused rings, the ternary-fused ring compounds **12a**−**12h** and **14** were obtained. Compounds **12a**, **12b**, and **12d** with more simple structures also exhibited significantly higher antiviral activities than luotonin A. The activity of compound **12h** is similar to

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Table 2. *In Vitro* Fungicidal Activities of Compounds 5a−5d, 9a−9o, 12a−12h, 14, 16, Chlorothalonil, Carbendazim, and Pyrimethanil against 14 Kinds of Fungi

	fungicidal activities (%)[a] at 50 μg/mL													
compd	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.G[b]	P.C[b]	S.S[b]	R.S[b]	B.C[b]
5a	23 ± 4	30 ± 4	74 ± 3	39 ± 2	26 ± 4	31 ± 1	30 ± 1	13 ± 3	4 ± 2	13 ± 3	16 ± 1	6 ± 4	6 ± 3	29 ± 3
5b	25 ± 1	26 ± 1	66 ± 1	39 ± 2	15 ± 2	29 ± 3	30 ± 1	31 ± 1	38 ± 2	38 ± 3	26 ± 2	62 ± 3	50 ± 1	45 ± 1
5c	20 ± 1	22 ± 2	51 ± 1	61 ± 2	28 ± 2	31 ± 1	33 ± 3	13 ± 3	4 ± 2	6 ± 3	10 ± 2	6 ± 3	19 ± 2	10 ± 2
5d	13 ± 3	22 ± 2	53 ± 2	51 ± 3	23 ± 1	26 ± 2	30 ± 1	6 ± 3	12 ± 2	6 ± 3	16 ± 1	38 ± 3	3 ± 1	4 ± 1
9a	36 ± 1	54 ± 2	62 ± 3	36 ± 1	39 ± 0	42 ± 2	34.6	50 ± 1	12 ± 2	8 ± 3	11.1	44 ± 1	14 ± 1	61 ± 1
9b	44 ± 3	29 ± 2	59 ± 3	61 ± 3	56 ± 1	53 ± 2	46 ± 2	39 ± 1	31 ± 2	33 ± 3	7 ± 2	44 ± 1	19 ± 3	50 ± 1
9c	18 ± 1	29 ± 2	57 ± 3	**87±1**	27 ± 2	19 ± 4	11 ± 3	28 ± 2	4 ± 2	8 ± 3	33 ± 3	44 ± 1	22 ± 2	11 ± 1
9d	36 ± 1	29 ± 2	66 ± 1	46 ± 1	42 ± 3	36 ± 1	39 ± 3	28 ± 2	12 ± 3	8 ± 3	7 ± 2	49 ± 2	6 ± 3	30 ± 3
9e	36 ± 1	50 ± 1	66 ± 1	53 ± 3	39 ± 1	39 ± 1	42 ± 3	44 ± 4	19 ± 2	33 ± 3	22 ± 2	44 ± 1	25 ± 1	57 ± 3
9f	41 ± 1	29 ± 2	64 ± 2	57 ± 3	39 ± 1	28 ± 2	31 ± 2	39 ± 1	12 ± 3	17 ± 2	11 ± 1	24 ± 3	14 ± 1	30 ± 3
9g	39 ± 3	54 ± 2	**81±1**	**81±1**	48.8	42 ± 3	46 ± 2	39 ± 1	19 ± 2	33 ± 3	19 ± 3	78 ± 1	56 ± 3	46 ± 3
9h	33 ± 3	33 ± 3	49 ± 1	70 ± 3	39 ± 1	31 ± 3	27 ± 1	33 ± 3	4 ± 2	33 ± 3	4 ± 3	61 ± 1	22 ± 2	33 ± 2
9i	46 ± 2	67 ± 3	70 ± 2	79 ± 3	61 ± 1	44 ± 3	54 ± 2	50 ± 1	19 ± 2	50 ± 1	56 ± 3	9 ± 2	44 ± 3	50 ± 1
9j	18 ± 1	21 ± 2	61 ± 3	51 ± 2	39 ± 1	31 ± 3	35 ± 3	22 ± 2	8 ± 3	17 ± 3	11 ± 1	49 ± 2	28 ± 2	22 ± 3
9k	25 ± 3	50 ± 1	66 ± 1	39 ± 3	39 ± 1	36 ± 1	31 ± 2	28 ± 2	8 ± 3	17 ± 3	**89 ± 1**	**85 ± 3**	42 ± 2	37 ± 1
9l	41 ± 1	33 ± 3	70 ± 2	56 ± 3	39 ± 1	36 ± 1	35 ± 3	50 ± 1	27 ± 1	50 ± 1	26 ± 1	63 ± 3	42 ± 3	52 ± 2
9m	54 ± 2	50 ± 1	**81±1**	57 ± 3	59 ± 3	53 ± 2	58 ± 2	61 ± 1	31 ± 2	50 ± 1	52 ± 1	**85 ± 3**	50.0	56.5
9n	23 ± 1	33 ± 3	49 ± 1	43 ± 2	44 ± 1	44 ± 3	23 ± 1	11 ± 1	4 ± 2	17 ± 3	4 ± 3	56 ± 1	28 ± 2	9 ± 3
9o	21 ± 3	29 ± 2	43 ± 3	20 ± 2	32 ± 3	25 ± 1	31 ± 2	22 ± 2	8 ± 2	8 ± 3	11 ± 1	7 ± 3	11 ± 1	22 ± 3
12a	25 ± 1	33 ± 3	68 ± 1	39 ± 2	31 ± 2	6 ± 2	40 ± 1	44 ± 2	46 ± 2	13 ± 3	29 ± 1	25 ± 1	44 ± 2	61 ± 2
12b	40 ± 1	44 ± 4	79 ± 2	57 ± 1	44 ± 3	31 ± 1	43 ± 3	81 ± 3	46 ± 2	75 ± 1	48 ± 3	75 ± 1	50 ± 1	**88 ± 2**
12c	33 ± 3	51 ± 2	72 ± 2	36 ± 1	44 ± 1	39 ± 1	42 ± 3	78 ± 2	12 ± 2	17 ± 3	26 ± 1	66 ± 1	28 ± 2	46 ± 3
12d	40 ± 1	41 ± 3	75 ± 1	39 ± 2	36 ± 1	55 ± 2	57 ± 2	69 ± 2	23 ± 1	38 ± 3	61 ± 3	63 ± 3	44 ± 2	78 ± 3
12e	80 ± 3	50 ± 1	**89±2**	44 ± 3	49 ± 2	50 ± 1	57 ± 3	28 ± 2	12 ± 3	17 ± 3	19 ± 3	24 ± 3	6 ± 3	4 ± 3
12f	23 ± 1	18 ± 3	51 ± 1	12 ± 1	27 ± 2	28 ± 2	27 ± 1	44 ± 3	8 ± 3	8 ± 3	4 ± 3	34 ± 1	19 ± 3	26 ± 1
12g	33 ± 3	38 ± 3	59 ± 3	39 ± 3	42 ± 3	36 ± 1	46 ± 2	56 ± 3	12 ± 3	17 ± 3	11 ± 1	39 ± 1	33 ± 3	33 ± 3
12h	51 ± 3	71 ± 2	**91±3**	36 ± 1	51 ± 2	53 ± 2	58 ± 3	61 ± 1	42 ± 3	67 ± 3	30 ± 3	2 ± 2	22 ± 2	65 ± 2
14	41 ± 1	50 ± 1	76 ± 3	36 ± 1	39 ± 1	39 ± 1	39 ± 3	67 ± 3	51 ± 1	50 ± 1	41 ± 3	61 ± 1	63 ± 1	**100**
16	15 ± 2	25 ± 1	59 ± 2	30 ± 3	34 ± 1	17 ± 3	19 ± 2	33 ± 3	16 ± 2	17 ± 3	11 ± 1	37 ± 3	14 ± 1	39 ± 1
chlorothalonil[c]	87 ± 2	37 ± 1	94 ± 2	99 ± 3	65 ± 1	98 ± 3	77 ± 1	22 ± 2	74 ± 2	100	88 ± 2	100	92 ± 3	94 ± 3
carbendazim[c]	100.0	19 ± 3	94 ± 2	99 ± 3	98 ± 2	98 ± 3	85 ± 3	11 ± 1	100	100	88 ± 2	91 ± 2	42 ± 3	15 ± 3
pyrimethanil[c]	66 ± 1	82 ± 3	96 ± 2	81 ± 3	63 ± 3	66 ± 1	58 ± 3	83 ± 3	48 ± 2	44 ± 2	85 ± 3	95 ± 2	83 ± 3	86 ± 3

[a]Average of three replicates; all results are expressed as mean ± SD. [b]Abbreviations: F.C, *Fusarium oxysporium 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. G, *Pyricularia grisea*; P.C, *Phytophthora capsici*; S.S, *Sclerotinia sclerotiorum*; R.S, *Rhizoctonia solani*. B.C, *Botrytis cinereal*. [c]The commercial agricultural fungicides were used for comparison of antifungal activity; activity data with prominent results are presented in bold.

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that of luotonin A. Compounds **12c**, **12e**, **12f**, **12g**, and **14** displayed significantly lower activity than luotonin A. From the above activity data, it can be seen that for ternary-fused ring compounds, hydrogen, fluorine, and methyl at the 2-position are beneficial to the activity while the introduction of substituents at 8-position is unfavorable to the activity. From the above structure−activity relationship, it can be found that small structural changes can lead to large changes in activity. The optimized compounds **9k**, **12b**, and **12d** may have stronger interaction with virus particles, showing excellent antiviral activity, which can be used as a new antiviral candidate for further research. In order to confirm the above conjecture, we further studied the mechanism of action.

Preliminary Mode of Action. We chose compound **9k** to study preliminary antiviral mechanism research using our reported method.[5] The test results show that the 20S CP disk can be assembled with RNA to form rod-shaped TMV virus particles with a length of about 300 nm (Figure 7A). A small amount of dimethyl sulfoxide (DMSO) does not inhibit the assembly of virus particles (Figure 7B). Compound **9k** can cause a reduction in the length and number of virus particles, indicating that they can inhibit the assembly of viruses (Figure 7C). At the same time, we can see from Figure 7C that the TMV particles are broken, fused, and aggregated, indicating that compound **9k** is likely to act on the TMV CP. We further designed 20S CP disk assembly experiments to test the interaction between **9k** and 20S CP disk. The TMV protein can form the disc structure (Figure 8A), and a small amount of DMSO does not affect the formation process (Figure 8B). Compound **9k** does interact with TMV CP, leading to its polymerization.

Molecular Docking. To further explore the interaction of **9k**, **12b**, **12d**, and TMV CP (PDB code: 1EI7), we performed molecular docking using AutoDock-vina 1.1.2.[29] Compound **9k** forms one conventional hydrogen bond with the active site of TYR 139 at a distance of 3.4 c5 (Figure 9A). Compound **12b** forms two conventional hydrogen bonds with amino acids ASN 73 (2.2 c5) and GLY 137 (2.0 c5). Compound **12d** forms three conventional hydrogen bonds with amino acids ASN 73 (2.3 c5), GLY 137 (2.3 c5), and THR 136 (3.2 c5). The molecular docking results indicate that these compounds interact with CP through hydrogen bonding.

Fungicidal Activity. Luotonin A and its derivatives **5a−5d**, **9a−9o**, **12a−12h**, **14**, and **16** were also evaluated for their fungicidal activities with commercial fungicides, carbendazim, chlorothalonil, and pyrimethanil, as controls (Table 2). At the concentration of 50 μg/mL, luotonin A and its derivatives showed broad spectrum inhibitory activity against all 14 plant pathogens. As a whole, most of the derivatives showed good inhibitory effect on *Physalospora piricola*, especially the inhibitory rates of compounds **9g**, **9m**, **12e**, and **12h** were over 80%. Compounds **9c** and **9g** exhibited higher fungicidal activities than pyrimethanil against *Rhizoctonia cerealis*. Compound **9k** showed better inhibition effect than commercial fungicides chlorothalonil, carbendazim, and pyrimethanil on *Phytophthora capsici* with an inhibition rate of 89%. The fungicidal activities of compounds **12b** and **14** are higher than that of carbendazim and pyrimethanil against *Botrytis cinereal*. The ternary-fused ring compound **14** did not give good antiviral activity, but its fungicidal activities are very prominent, which can be used as a lead for further research.

In summary, based on our work experience, a series of luotonin A derivatives were designed and synthesized through ring expansion and ring contraction strategies. The anti-TMV and fungicidal activities and structure−activity relationships of these compounds were systematically evaluated. The increase of solubility is beneficial to improve the biological activity of luotonin A, and most of the four-membered fused ring compounds **9a−9o** showed better biological activities than luotonin A. For ternary-fused ring compounds, hydrogen, fluorine, and methyl at the 2-position are beneficial to the activity while the introduction of substituents at the 8-position is unfavorable to the activity. The optimized compounds **9k**, **12b**, and **12d** may have stronger interaction with virus particles, showing excellent antiviral activity, which can be used as a new antiviral candidate for further research. Further antiviral mechanism research revealed that **9k** can inhibit the assembly of TMV particles by hydrogen bonding interaction with TMV CP. These compounds also showed broad spectrum inhibitory activities against all 14 plant pathogens. Structurally simplified compound **14** with prominent fungicidal activity emerged as a lead for further research. This work broadened the biological activity spectrum of alkaloid luotonin A and laid a foundation for the application of these compounds in plant virus protection.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c04278.

Detailed bio-assay procedures and spectra data of luotonin A and its derivatives **5a−5d**, **9a−9o**, **12a−12h**, **14**, and **16** (PDF)

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Godfray, H. C. J.; Beddington, J. R.; Crute, I. R.; Haddad, L.; Lawrence, D.; Muir, J. F.; Pretty, J.; Robinson, S.; Thomas, S. M.; Toulmin, C. Food security: the challenge of feeding 9 billion people. *Science* **2010**, *327*, 812−818.

(2) Rodríguez, J. P.; Rahman, H.; Thushar, S.; Singh, R. K. Healthy and resilient cereals and pseudo-cereals for marginal agriculture: Molecular advances for improving nutrient bioavailability. *Front. Genet.* **2020**, *11*, 49.

(3) Foley, J. A.; Ramankutty, N.; Brauman, K. A.; Cassidy, E. S.; Gerber, J. S.; Johnston, M.; Mueller, N. D.; O'Connell, C.; Ray, D. K.; West, P. C.; Balzer, C.; Bennett, E. M.; Carpenter, S. R.; Hill, J.; Monfreda, C.; Polasky, S.; Rockström, J.; Sheehan, J.; Siebert, S.; Tilman, D.; Zaks, D. P. M. Solutions for a cultivated planet. *Nature* **2011**, *478*, 337−342.

(4) Ge, Y.-h.; Liu, K.-x.; Zhang, J.-x.; Mu, S.-z.; Hao, X.-j. The limonoids and their antitobacco mosaic virus (TMV) activities from Munronia unifoliolate Oliv. *J. Agric. Food Chem.* **2012**, *60*, 4289−4295.

(5) Lu, A.; Wang, T.; Hui, H.; Wei, X.; Cui, W.; Zhou, C.; Li, H.; Wang, Z.; Guo, J.; Ma, D.; Wang, Q. Natural products for drug discovery: discovery of gramines as novel agents against a plant virus. *J. Agric. Food Chem.* **2019**, *67*, 2148−2156.

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

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

(8) Wang, T.; Yang, S.; Li, H.; Lu, A.; Wang, Z.; Yao, Y.; Wang, Q. Discovery, structural optimization and mode of action of essramycin alkaloid and its derivatives as anti-tobacco mosaic virus (TMV) and anti-phytopathogenic-fungus agents. *J. Agric. Food Chem.* **2020**, *68*, 471−484.

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

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

(11) Liu, B.; Li, R.; Li, Y.; Li, S.; Yu, J.; Zhao, B.; Liao, A.; Wang, Y.; Wang, Z.; Lu, A.; Liu, Y.; Wang, Q. Discovery of pimprinine alkaloids as novel agents against aplant virus. *J. Agric. Food Chem.* **2019**, *67*, 1795−1806.

(12) Wang, Y.; Zhang, J.; He, F.; Gan, X.; Song, B.; Hu, D. Design, synthesis, bioactivity and mechanism of dithioacetal derivatives containing dioxyether moiety. *Bioorg. Med. Chem. Lett.* **2019**, *29*, 2218−2223.

(13) Zhao, L.; Zhang, J.; Liu, T.; Mou, H.; Wei, C.; Hu, D.; Song, B. Design, synthesis and antiviral activity of coumarin derivatives containing dithioacetal structures. *J. Agric. Food Chem.* **2020**, *68*, 975−981.

(14) Shang, X.-F.; Morris-Natschke, S. L.; Liu, Y.-Q.; Guo, X.; Xu, X.-S.; Goto, M.; Li, J.-C.; Yang, G.-Z.; Lee, K.-H. Biologically active quinoline and quinazoline alkaloids part I. *Med. Res. Rev.* **2018**, *38*, 775−828.

(15) Nomura, T.; Ma, Z.-Z.; Hano, Y.; Chen, Y.-J. Two new pyrroloquinazolinoquinoline alkaloids from Peganum nigellastrum. *Heterocycles* **1997**, *46*, 541−546.

(16) Cagir, A.; Jones, S. H.; Gao, R.; Eisenhauer, B. M.; Hecht, S. M. Luotonin A. A naturally occurring human DNA topoisomerase I poison. *J. Am. Chem. Soc.* **2003**, *125*, 13628−13629.

(17) Cagir, A.; Eisenhauer, B. M.; Gao, R.; Thomas, S. J.; Hecht, S. M. Synthesis and topoisomerase I inhibitory properties of luotonin A analogues. *Bioorg. Med. Chem. Lett.* **2004**, *12*, 6287−6299.

(18) Ma, Z.; Hano, Y.; Nomura, T.; Chen, Y. Novel quinazoline−quinoline alkaloids with cytotoxic and DNA topoisomerase II inhibitory activities. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1193−1196.

(19) Almansour, A. I.; Arumugam, N.; Suresh Kumar, R.; Mahalingam, S. M.; Sau, S.; Bianchini, G.; Menéndez, J. C.; Altaf, M.; Ghabbour, H. A. Design, synthesis and antiproliferative activity of decarbonyl luotonin analogues. *Eur. J. Med. Chem.* **2017**, *138*, 932−941.

(20) An, T.-y.; Huang, R.-q.; Yang, Z.; Zhang, D.-k.; Li, G.-r.; Yao, Y.-c.; Gao, J. Alkaloids from cynanchum komarovii with inhibitory activity against the tobacco mosaic virus. *Phytochemistry* **2001**, *58*, 1267−1269.

(21) Wang, Z.; Li, Z.; Wang, K.; Wang, Q. Efficient and chirally specific synthesis of phenanthroindolizidine alkaloids by Parham-type cycloacylation. *Eur. J. Org. Chem.* **2010**, *2010*, 292−299.

(22) Wang, Z.; Wei, P.; Liu, Y.; Wang, Q. D and E rings may not be indispensable for antofine: discovery of phenanthrene and alkylamine chain containing antofine derivatives as novel antiviral agents against tobacco mosaic virus (TMV) based on interaction of antofine and TMV RNA. *J. Agric. Food Chem.* **2014**, *62*, 10393−10404.

(23) Wang, K.; Su, B.; Wang, Z.; Wu, M.; Li, Z.; Hu, Y.; Fan, Z.; Mi, N.; Wang, Q. Synthesis and antiviral activities of phenanthroindolizidine alkaloids and their derivatives. *J. Agric. Food Chem.* **2010**, *58*, 2703−2709.

(24) Wu, M.; Han, G.; Meng, C.; Wang, Z.; Liu, Y.; Wang, Q. Design, synthesis, and anti-tobacco mosaic virus (TMV) activity of glycoconjugates of phenanthroindolizidines alkaloids. *Mol. Divers.* **2014**, *18*, 25−37.

(25) Zhou, H.-B.; Liu, G.-S.; Yao, Z.-J. Short and efficient total synthesis of luotonin A and 22-hydroxyacuminatine using a common cascade strategy. *J. Org. Chem.* **2007**, *72*, 6270−6272.

(26) Liu, M.; Shu, M.; Yao, C.; Yin, G.; Wang, D.; Huang, J. Synthesis of pyrido-fused quinazolinone derivatives via copper-catalyzed domino reaction. *Org. Lett.* **2016**, *18*, 824−827.

(27) Lv, P.; Chen, Y.; Shi, T.; Wu, X.; Li, Q. X.; Hua, R. Synthesis and fungicidal activities of sanguinarine derivatives. *Pestic. Biochem. Phys.* **2018**, *147*, 3−10.

(28) Ma, Y.-G.; Zhang, Y.; Feng, B.-B.; Wang, X.-S. Synthesis of 6-aryl-5H-quinazolino [4, 3-b] quinazolin-8 (6H)-one derivatives in ionic liquids catalyzed by iodine. *Res. Chem. Intermed.* **2016**, *42*, 1045−1055.

(29) Trott, O.; Olson, A. J. Autodock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **2010**, *31*, 455−461.