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Design, Synthesis, and Biological Activities of Aromatic Gossypol Schiff Base Derivatives

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Supporting Information

ABSTRACT: A series of aromatic gossypol Schiff bases have been successfully synthesized via a feasible chemical modification. The antiviral activity against tobacco mosaic virus (TMV) of these gossypol Schiff bases has been tested for the first time. The bioassay studies indicated most of these derivatives exhibited excellent anti-TMV activity, in which *o*-trifluoromethylaniline Schiff base (**19**) displayed the best antiviral activities. Furthermore, compound **19** exhibited an eminent anti-TMV effect in the field and low toxicity to mice. These results suggest it is a promising candidate for the inhibitor of plant virus.

KEYWORDS: *gossypol, Schiff base, synthesis, anti-TMV activity, structure−activity relationships*

INTRODUCTION

The discovery of gossypol (**1**, Figure 1) has attracted considerable attention due to its applications in antitumor activity.[1] Such a compound embedded in the cottonseed pigment glands exists in three forms,[2] which can be categorized as the aldehyde tautomer **1a**, the ketone (quinoid) tautomer **1b**, and the lactol (hemiacetal) tautomer **1c** (Figure 1). In most cases, as a part of the plant's defense system against pathogens (*Verticillium dahliae*, *Rhizoctonia solani*, and *Xanthomonas campestris*)[3] and herbivorous insects such as *Spodoptera* species and *Heliothis* species,[4] gossypol exhibits multiple biological properties,[1d] including spermicidal, antitumor, antiparasitic, and antiprotozoan activities. It also reported to have antiviral activity toward enveloped viruses such as HIV-1,[5] H_5N_1,[5] HSV-2,[6] and influenza virus[7] rather than nonenveloped viruses, such as poliovirus. However, a recent patent revealed that the gossypol fatty acid reagent had antiviral activity against tobacco mosaic virus (TMV),[8] which is classified as a nonenveloped virus.

The plant disease caused by TMV is found worldwide. It is well-known that TMV can infect members of 36 plant families and at least 400 individual species, including tobacco, tomato, pepper, cucumber, and a number of ornamental flowers.[9] TMV disease can cause vast losses in agriculture. It is found that in certain fields 90−100% of the plants showed mosaic or leaf necrosis by harvesting time. Ribavirin is one of the most widely used plant virus inhibitors, even though its inhibitory effects are <50% at 500 μg/mL. Ningnanmycin, perhaps the most successful registered antiplant viral agent, displayed a 56% in vivo curative effect at 500 μg/mL. Recently, a number of chemicals[10] were reported to exhibit antiviral activities; however, few of them have been utilized successfully in agriculture. As a result, the field of TMV inhibitors still deserves to be further exploited.

Knowledge of the molecular mechanism of the biological effects for gossypol is still limited. Some literature studies show that gossypol exerts its pharmacological actions by an interaction with biomolecular targets, such as enzymes, signal transduction mediators, or membranes.[11] Kovacic[12] in 2003 discussed the mechanism of biological properties of gossypol as well and pointed out that gossypol and its metabolites could produce reactive oxygen species (ROS) through redox cycling by electron transfer functions. Additionally, Bacsó in 2011 reported that external application of ROS in tobacco may control virus replication and symptom development.[13] On the basis of such interpretation, the antiviral activity of gossypol toward a nonenveloped virus, TMV, can be inferred as being relevant to ROS.

Numerous studies suggest gossypol is toxic to monogastric animals, but its toxicity can be reduced while its therapeutic effects are retained by modification of gossypol's reactive aldehyde groups.[14] A few gossypol Schiff bases were synthesized and tested for their biological activities including antitumor, antimalaria, and interferon-inducing activities.[15] However, reports of the anti-TMV activity of the gossypol Schiff bases are rather rare, and no examples are documented in the recent literature.[5,15,16] Gossypol analogues with quinones and conjugated imine groups have electron transfer functionalities to generate ROS, which may control virus replication and symptom development.[13] Therefore, condensation products of

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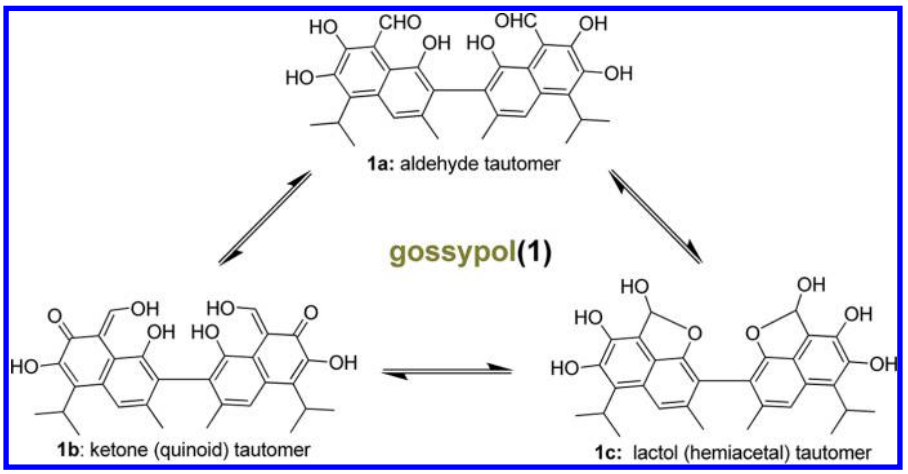

Figure 1. Structures of gossypol and its tautomeric forms.

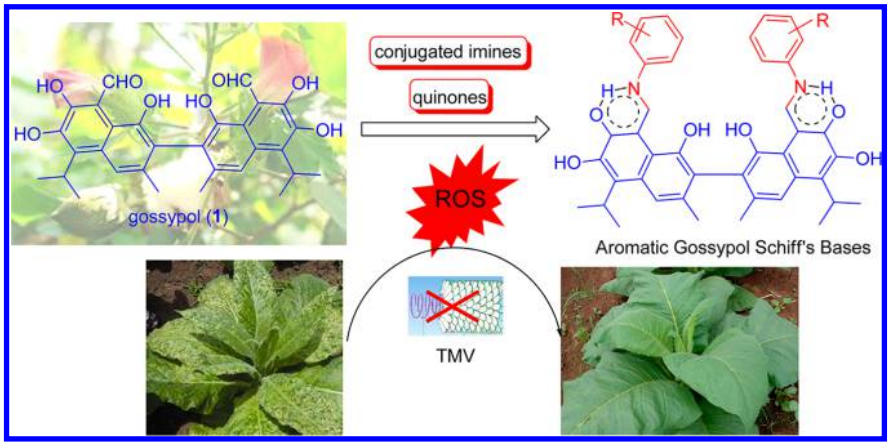

Figure 2. Design of compounds **3**–**25**.

gossypol and aromatic amines are expected to adjust gossypol to produce ROS and thus modulate the anti-TMV activity level. (Figure 2) In this contribution, a family of aromatic gossypol Schiff base derivatives has been synthesized and their anti-TMV activities have been determined using both in vitro and in vivo test methods. The structure–activity relationships of these derivatives were discussed as well. Field trial and toxicity evaluation were also conducted for the compound with high anti-TMV activity primarily to assess its potential as an antivirus candidate. Additionally, the synthesized derivatives were also investigated for their potential as fungicidal, insecticidal, or herbicidal reagents (see the Supporting Information).

MATERIALS AND METHODS

General. Reagents were purchased from commercial sources and were used as received. All anhydrous solvents were dried and purified by standard techniques just before use. Reaction progress was monitored by thin-layer chromatography on silica gel GF-254 with detection by UV. Melting points were determined on an X-4 binocular microscope melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Ascend 300/400 MHz spectrometer. Chemical shift values (δ) are given in parts per million and downfield from internal tetramethylsilane. High-resolution mass spectra (HRMS) were recorded on FT-ICR MS (Ionspec, 7.0 T).

General Procedure for the Synthesis of Aromatic Gossypol Schiff Bases 3–10, 12–14, 19, 21, 22, 24, and 25. A solution of gossypol acetic acid (**2**, 0.50 g, 0.86 mmol) and aromatic amine (1.73 mmol) in ethanol (50 mL) was stirred and refluxed for 5 h, and then the reaction mixture was cooled to room temperature. The resulting solid was filtered, washed with ethanol, and recrystallized to give the desired gossypol derivatives.

General Procedure for the Synthesis of Aromatic Gossypol Schiff Base Derivatives 11 and 23. A mixture of aromatic amine (1.73 mmol), NaOH (2.59 mmol), and ethanol (50 mL) was stirred and refluxed for 1 h. Then gossypol acetic acid (**2**, 0.50 g, 0.86 mmol) was added, and the reaction liquid was refluxes for a further 5 h. The reaction mixture was cooled to room temperature, and then the resulting solid was filtered, washed with ethanol, and then recrystallized to give the desired gossypol derivatives.

General Procedure for the Synthesis of Aromatic Gossypol Schiff Bases 15–18 and 20. A solution of gossypol acetic acid (**2**, 0.50 g, 0.86 mmol) and aromatic amine (3.46 mmol) in ethanol (50 mL) was stirred and refluxed for 5 h and then the reaction mixture was cooled to room temperature. The resulting solid was filtered, washed with ethanol, and then recrystallized to give the desired gossypol derivatives.

Data for ***3****:* chocolate crystal recrystallized from benzene; yield, 95%; mp, 252–253 °C (lit.[17] 258–260 °C); ^{1}H NMR (300 MHz, $CDCl_3$), δ 14.92 (d, J = 12.3 Hz, 2H), 10.18 (d, J = 12.3 Hz, 2H), 7.88 (s, 2H), 7.63 (s, 2H), 7.41–7.28 (m, 8H), 7.18 (t, J = 7.1 Hz, 2H), 5.76 (s, 2H), 3.79–3.67 (m, 2H), 2.16 (s, 6H), 1.58–1.53 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$), δ 174.34, 154.14, 149.39, 147.00, 139.47, 132.76, 129.87, 129.78, 128.76, 125.88, 118.68, 118.21, 116.31, 114.46, 105.09, 27.57, 20.32, 20.29, 20.10; HRMS (ESI) calcd for $C_{42}H_{39}N_2O_6$ $(M - H)^-$ 667.2814, found 667.2812.

Data for ***4****:* orange crystal recrystallized from chloroform; yield, 95%; mp, 246–248 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 14.97 (d, J = 12.4 Hz, 2H), 10.15 (d, J = 12.4 Hz, 2H), 7.91 (s, 2H), 7.63 (s, 2H), 7.23–7.13 (m, 8H), 5.74 (s, 2H), 3.80–3.69 (m, 2H), 2.33 (s, 6H), 2.15 (s, 6H), 1.58–1.53 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$), δ

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173.91, 154.21, 149.35, 147.03, 137.00, 135.95, 132.61, 130.42, 129.66, 128.49, 118.62, 118.14, 116.17, 114.49, 104.84, 27.55, 20.94, 20.35, 20.31, 20.13; HRMS (ESI) calcd for $C_{44}H_{43}N_2O_6$ $(M - H)^-$ 695.3127, found 695.3139.

Data for ***5:*** dark orange crystal recrystallized from tetrahydrofuran and ethanol; yield, 60%; mp, 272−274 °C (lit.[18] 287−289 °C); ^{1}H NMR (400 MHz, $CDCl_3$), δ 15.08 (d, J = 12.0 Hz, 2H), 10.09 (d, J = 12.0 Hz, 2H), 7.90 (s, 2H), 7.63 (s, 2H), 7.24 (d, J = 9.0 Hz, 4H), 6.89 (d, J = 9.0 Hz, 4H), 5.75 (s, 2H), 3.79 (s, 6H), 3.77−3.70 (m, 2H), 2.15 (s, 6H), 1.57−1.54 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 173.44, 157.94, 154.43, 149.30, 147.03, 132.82, 132.48, 129.54, 128.31, 119.65, 118.56, 116.09, 115.09, 114.50, 104.74, 55.59, 27.54, 20.37, 20.33, 20.13; HRMS (ESI) calcd for $C_{44}H_{45}N_2O_8$ $(M + H)^+$ 729.3170, found 729.3163.

Data for ***6:*** orange crystal recrystallized from pyridine; yield, 79%; mp, 241−243 °C (lit.[19] 291 °C); ^{1}H NMR (400 MHz, DMSO-d_6), δ 15.18 (d, J = 11.8 Hz, 2H), 10.30 (d, J = 11.8 Hz, 2H), 9.66 (s, 2H), 8.52 (s, 2H), 8.15 (s, 2H), 7.51 (s, 2H), 7.21 (d, J = 8.8 Hz, 4H), 6.83 (d, J = 8.8 Hz, 4H), 3.79−3.70 (m, 2H), 1.99 (s, 6H), 1.49−1.44 (m, 12H); ^{13}C NMR (100 MHz, DMSO-d_6), δ 172.52, 155.74, 153.64, 149.98, 146.16, 132.00, 131.27, 127.99, 127.47, 120.75, 119.36, 116.90, 116.46, 115.49, 104.98, 26.56, 20.27, 20.24, 20.21; HRMS (ESI) calcd for $C_{42}H_{39}N_2O_8$ $(M - H)^-$ 699.2712, found 699.2712.

Data for ***7:*** yellow crystal recrystallized from pyridine and ethanol; yield, 78%; mp, 266 °C (dec) (lit.[20] 203−204 °C); ^{1}H NMR (400 MHz, DMSO-d_6), δ 14.96 (d, J = 11.6 Hz, 2H), 10.31 (d, J = 11.6 Hz, 2H), 8.54 (s, 2H), 8.22 (s, 2H), 7.52 (s, 2H), 7.45−7.38 (m, 4H), 7.30 (t, J = 8.5 Hz, 4H), 3.79−3.71 (m, 2H), 2.00 (s, 6H), 1.49−1.43 (m, 12H); ^{13}C NMR (100 MHz, DMSO-d_6), δ 173.56, 160.93, 158.52, 153.87, 150.11, 146.10, 136.30, 132.46, 128.86, 127.77, 121.16, 119.83, 119.75, 117.10, 116.89, 116.66, 115.35, 105.61, 26.60, 20.22, 20.19; HRMS (ESI) calcd for $C_{42}H_{37}F_2N_2O_6$ (M -H)$^-$ 703.2625, found 703.2603.

Data for ***8:*** yellow crystal recrystallized from chloroform and ethanol; yield, 64%; mp, 286−287 °C (lit.[19] 302 °C); ^{1}H NMR (400 MHz, $CDCl_3$), δ 14.95 (d, J = 12.0 Hz, 2H), 10.09 (d, J = 12.0 Hz, 2H), 7.82 (s, 2H), 7.63 (s, 2H), 7.33 (d, J = 8.6 Hz, 4H), 7.23 (d, J = 8.6 Hz, 4H), 5.75 (s, 2H), 3.80−3.64 (m, 2H), 2.15 (s, 6H), 1.56−1.53 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 174.65, 153.69, 149.37, 146.90, 138.13, 133.01, 131.13, 129.97, 129.91, 129.13, 119.29, 118.82, 116.29, 114.21, 105.30, 27.58, 20.28, 20.24, 20.10; HRMS (ESI) calcd for $C_{42}H_{37}Cl_2N_2O_6$ $(M - H)^-$ 735.2034, found 735.2034.

Data for ***9:*** orange-red crystal recrystallized from pyridine and ethanol; yield, 81%; mp, 284−286 °C (lit.[19] 305−306 °C); ^{1}H NMR (400 MHz, $CDCl_3$), δ 14.84 (d, J = 11.7 Hz, 2H), 10.12 (d, J = 11.7 Hz, 2H), 8.24 (d, J = 9.0 Hz, 4H), 7.69 (s, 2H), 7.64 (s, 2H), 7.36 (d, J = 9.0 Hz, 4H), 5.82 (s, 2H), 3.75−3.66 (m, 2H), 2.17 (s, 6H), 1.58−1.52 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 176.46, 155.92, 151.73, 149.87, 149.67, 146.75, 137.66, 135.94, 134.01, 130.47, 125.94, 123.74, 117.51, 107.20, 106.02, 27.69, 20.19, 20.14, 20.12; HRMS (ESI) calcd for $C_{42}H_{38}N_4NaO_{10}$ $(M + Na)^+$ 781.2480, found 781.2486.

Data for ***10:*** gold crystal recrystallized from pyridine and ethanol; yield, 79%; mp, 261−263 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 14.89 (d, J = 11.2 Hz, 2H), 10.15 (d, J = 12.0 Hz, 2H), 7.77 (s, 2H), 7.65−7.55 (m, 6H), 7.37 (d, J = 8.4 Hz, 4H), 5.75 (s, 2H), 3.78−3.68 (m, 2H), 2.16 (s, 6H), 1.58−1.55 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 175.51, 153.08, 149.89, 149.48, 146.85, 142.44, 140.87, 133.41, 130.20, 129.67, 127.21, 127.19, 127.15, 119.01, 117.90, 116.45, 105.87, 105.85, 27.62, 20.25, 20.19, 20.10; HRMS (ESI) calcd for $C_{44}H_{38}F_6N_2NaO_6$ $(M + Na)^+$ 827.2526, found 827.2520.

Data for ***11:*** purple solid; yield, 36%; mp, >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6), δ 14.94 (d, J = 11.7 Hz, 2H), 10.41 (d, J = 11.7 Hz, 2H), 8.58 (s, 2H), 8.34 (s, 2H), 7.64 (d, J = 8.0 Hz, 4H), 7.52 (s, 2H), 7.29 (d, J = 8.0 Hz, 4H), 3.80−3.70 (m, 2H), 2.00 (s, 6H), 1.52−1.43 (m, 12H); ^{13}C NMR (100 MHz, DMSO-d_6), δ 174.71, 153.23, 146.68, 145.89, 139.81, 133.05, 129.55, 128.31, 127.85, 127.12, 121.85, 117.28, 115.75, 112.67, 106.31, 27.12, 20.72, 20.68; HRMS (ESI) calcd for $C_{42}H_{39}N_2O_{12}S_2$ $(M - 2Na + H)^-$ 827.1950, found 827.1928.

Data for ***12:*** yellow solid; yield, 72%; mp, 251−253 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 14.94 (d, J = 12.0 Hz, 2H), 10.10 (d, J = 12.0 Hz, 2H), 7.81 (s, 2H), 7.63 (s, 2H), 7.31 (d, J = 8.6 Hz, 4H), 7.22 (d, J = 8.6 Hz, 4H), 5.80 (s, 2H), 3.77−3.67 (m, 2H), 2.16 (s, 6H), 1.56−1.53 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 174.77, 153.99, 149.39, 146.89, 146.59, 138.27, 133.08, 129.97, 129.27, 122.66, 121.67, 119.30, 119.11, 118.84, 116.36, 114.22, 105.42, 27.59, 20.28, 20.24, 20.10; HRMS (ESI) calcd for $C_{44}H_{37}F_6N_2O_8$ $(M - H)^-$ 835.2460, found 835.2441.

Data for ***13:*** gold solid; yield, 91%; mp, 262−263 °C (lit.[19] 268−268 °C); ^{1}H NMR (400 MHz, DMSO-d_6), δ 14.67 (d, J = 12.7 Hz, 2H), 10.48 (s, 2H), 10.43 (d, J = 12.7 Hz, 2H), 8.68 (s, 2H), 8.21 (s, 2H), 7.51 (s, 2H), 7.33 (d, J = 8.1 Hz, 2H), 7.07−6.98 (m, 4H), 6.86 (t, J = 7.3 Hz, 2H), 3.78−3.70 (m, 2H), 2.00 (s, 6H), 1.49−1.44 (m, 12H); ^{13}C NMR (100 MHz, DMSO-d_6), δ 174.09, 150.90, 150.09, 147.37, 146.49, 132.15, 128.02, 127.73, 127.39, 125.84, 120.87, 119.94, 117.04, 115.99, 115.38, 114.71, 105.64, 26.56, 20.23, 20.21; HRMS (ESI) m/z calcd for $C_{42}H_{39}N_2O_8$ $(M - H)^-$ 699.2712, found 699.2704.

Data for ***14:*** orange crystal recrystallized from pyridine; yield, 56%; mp, 259−262 °C (lit.[19] 287 °C); ^{1}H NMR (400 MHz, DMSO-d_6), δ 14.86 (d, J = 12.0 Hz, 2H), 10.34 (d, J = 12.0 Hz, 2H), 9.82 (s, 2H), 8.57(s, 2H), 8.32 (s, 2H), 7.51 (s, 2H), 7.22 (t, J = 8.1 Hz, 2H), 6.78−6.74 (m, 2H), 6.73 (t, J = 1.9 Hz, 2H), 6.63 (dd, J = 8.1, 1.9 Hz, 2H), 3.80−3.68 (m, 2H), 1.99 (s, 6H), 1.49−1.44 (m, 12H); ^{13}C NMR (100 MHz, DMSO-d_6), δ 174.04, 158.87, 152.99, 150.23, 146.21, 140.50, 132.42, 130.91, 128.80, 127.75, 121.20, 117.08, 115.31, 112.80, 108.52, 105.50, 104.19, 26.61, 20.20, 20.18; HRMS (ESI) m/z calcd for $C_{42}H_{39}N_2O_8$ $(M - H)^-$ 699.2712, found 699.2704.

Data for ***15:*** yellow solid; yield, 76%; mp, 252−254 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 14.75 (d, J = 12.8 Hz, 2H), 10.20 (d, J = 12.8 Hz, 2H), 7.97 (s, 2H), 7.63 (s, 2H), 7.37 (d, J = 7.5 Hz, 2H), 7.14 (t, J = 7.3 Hz, 2H), 6.98 (d, J = 7.8 Hz, 2H), 6.93 (t, J = 7.7 Hz, 2H), 5.76 (s, 2H), 4.03 (s, 6H), 3.78−3.69 (m, 2H), 2.15 (s, 6H), 1.58−1.52 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 174.31, 152.40, 149.78, 149.39, 147.18, 132.64, 129.78, 128.83, 128.29, 126.13, 121.27, 118.61, 116.20, 115.50, 114.52, 111.49, 105.41, 56.19, 27.53, 20.33, 20.28, 20.12; HRMS (ESI) calcd for $C_{44}H_{43}N_2O_8$ $(M - H)^-$ 727.3025, found 727.3002.

Data for ***16:*** yellow solid; yield, 83%; mp, 266−268 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 14.82 (d, J = 12.2 Hz, 2H), 10.14 (d, J = 12.2 Hz, 2H), 7.87 (s, 2H), 7.63 (s, 2H), 7.26−7.23 (t, J = 8.0 Hz, 2H), 6.89 (dd, J = 8.0, 1.4 Hz, 2H), 6.81 (s, 2H), 6.71 (dd, J = 8.0, 1.4 Hz, 2H), 5.83 (s, 2H), 3.79 (s, 6H), 3.76−3.67 (m, 2H), 2.15 (s, 6H), 1.57−1.52 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 174.33, 160.83, 154.04, 149.42, 146.94, 140.61, 132.82, 130.69, 129.73, 128.86, 118.66, 116.38, 114.40, 111.27, 110.48, 105.07, 104.17, 55.45, 27.56, 20.29, 20.13; HRMS (ESI) calcd for $C_{44}H_{43}N_2O_8$ $(M - H)^-$ 727.3025, found 727.3009.

Data for ***17:*** orange solid; yield, 97%; mp, 255−257 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 14.89 (d, J = 12.8 Hz, 2H), 10.13 (d, J = 12.8 Hz, 2H), 7.99 (s, 2H), 7.63 (s, 2H), 7.27 (d, J = 8.8 Hz, 2H), 6.54 (d, J = 2.4 Hz, 2H), 6.44 (dd, J = 8.8, 2.4 Hz, 2H), 5.76 (s, 2H), 3.99 (s, 6H), 3.79 (s, 6H), 3.77−3.69 (m, 2H), 2.15 (s, 6H), 1.57−1.52 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 173.35, 158.65, 152.58, 151.01, 149.33, 147.22, 132.35, 129.51, 127.85, 122.56, 118.46, 116.56, 116.06, 114.60, 105.06, 104.97, 99.27, 56.22, 55.63, 27.51, 20.38, 20.33, 20.14; HRMS (ESI) calcd for $C_{46}H_{47}N_2O_{10}$ $(M - H)^-$ 787.3236, found 787.3236.

Data for ***18:*** gold solid; yield, 89%; mp, 234−235 °C; ^{1}H NMR (400 MHz, DMSO-d_6), δ 14.62 (d, J = 11.6 Hz, 2H), 10.39 (d, J = 11.6 Hz, 2H), 8.72 (s, 2H), 8.35 (s, 2H), 7.52 (s, 2H), 7.12 (d, J = 6.8 Hz, 2H), 6.92 (s, 2H), 6.77 (d, J = 6.8 Hz, 2H), 3.90 (s, 6H), 3.69 (br s, 8H), 1.98 (s, 6H), 1.46 (br s, 12H); ^{13}C NMR (100 MHz, DMSO-d_6), δ 174.76, 154.00, 150.79, 150.35, 146.52, 143.23, 132.48, 129.00, 128.66, 128.00, 120.80, 117.14, 115.03, 113.19, 109.66, 106.21, 101.40, 56.49, 55.59, 26.61, 20.18; HRMS (ESI) calcd for $C_{46}H_{47}N_2O_{10}$ $(M - H)^-$ 787.3236, found 787.3219.

Data for ***19:*** chocolate solid; yield, 43%; mp, 251−253 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 15.21 (d, J = 11.0 Hz, 2H), 10.10 (d, J = 11.0 Hz, 2H), 7.74 (s, 2H), 7.68 (d, J = 7.7 Hz, 2H), 7.62 (s, 2H), 7.53 (t, J = 7.7 Hz, 2H), 7.46 (d, J = 7.7 Hz, 2H), 7.26 (t, J = 7.7 Hz, 2H),

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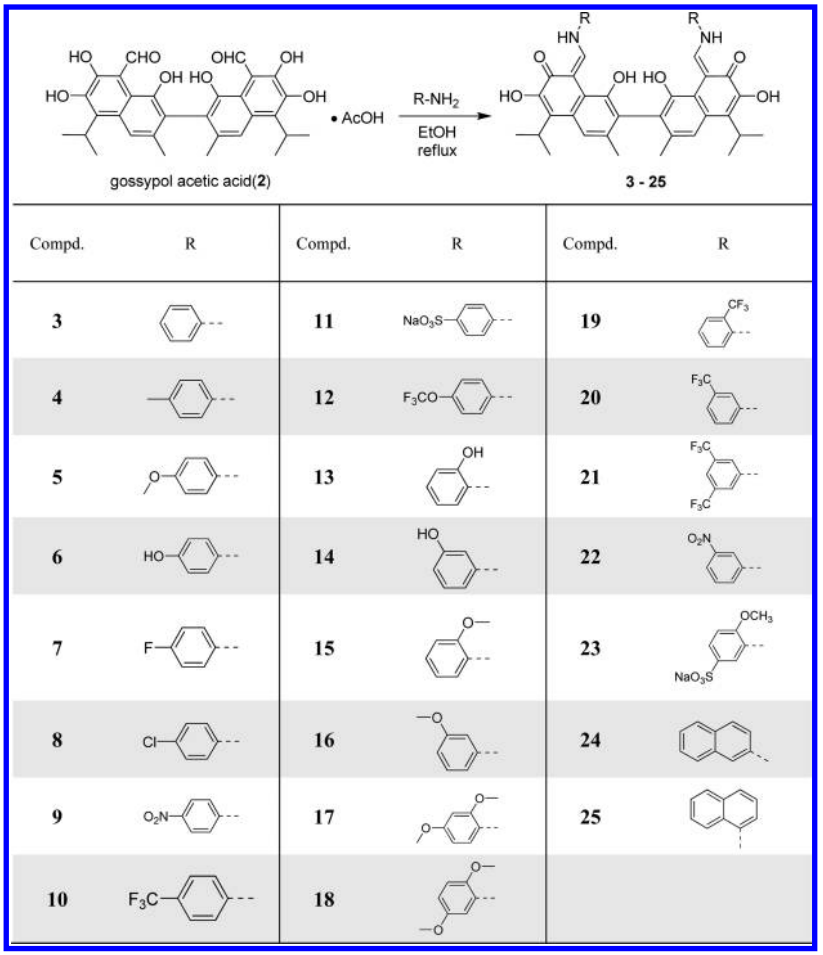
Scheme 1. General Synthetic Route for Target Compounds 3−25



Compd.	R	Compd.	R	Compd.	R
3		11	NaO_3S	19	CF_3
4		12	F_3CO	20	F_3C
5		13	OH	21	F_3C, F_3C
6	HO	14	HO	22	O_2N
7	F	15	O	23	OCH_3, NaO_3S
8	Cl	16	O	24	
9	O_2N	17	O, O	25	
10	F_3C	18	O, O		

5.77 (s, 2H), 3.76−3.66 (m, 2H), 2.15 (s, 6H), 1.57−1.51 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 174.94, 154.82, 149.50, 146.83, 138.56, 133.50, 133.34, 130.21, 129.54, 127.10, 127.05, 126.99, 126.94, 125.26, 125.00, 122.28, 121.15, 120.85, 119.03, 118.92, 116.41, 114.15, 106.43, 27.59, 20.23, 20.19, 20.12; HRMS (ESI) calcd for $C_{44}H_{37}F_6N_2O_6$ $(M - H)^-$ 803.2561, found 803.2533.

Data for ***20:*** yellow solid; yield, 81%; mp, 262−264 °C (lit.[21] 216−217.5 °C); ^{1}H NMR (400 MHz, $CDCl_3$), δ 15.02 (d, J = 11.7 Hz, 2H), 10.14 (d, J = 11.7 Hz, 2H), 7.79 (s, 2H), 7.65 (s, 2H), 7.54−7.41 (m, 8H), 5.79 (s, 2H), 3.77−3.69 (m, 2H), 2.16 (s, 6H), 1.58−1.53 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 175.19, 153.49, 149.49, 146.87, 140.23, 133.31, 133.11, 132.66, 132.32, 131.98, 130.54, 130.12, 129.55, 124.89, 122.18, 122.14, 122.10, 121.16, 118.97, 116.44, 114.91, 114.87, 114.11, 105.69, 27.63, 20.27, 20.24, 20.12; HRMS (ESI) calcd for $C_{44}H_{37}F_6N_2O_6$ $(M - H)^-$ 803.2561, found 903.2543.

Data for ***21:*** yellow crystal recrystallized from petroleum ether and ethyl acetate; yield, 49%; mp, 221−223 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 15.12 (d, J = 11.6 Hz, 2H), 10.10 (d, J = 11.6 Hz, 2H), 7.72−7.62 (m, 10H), 5.85 (s, 2H), 3.89−3.58 (m, 2H), 2.17 (s, 6H), 1.57−1.51 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 175.87, 152.50, 149.68, 146.72, 141.32, 133.99, 133.93, 133.66, 133.32, 130.49, 130.45, 124.15, 121.44, 119.22, 118.41, 117.71, 116.73, 113.77, 106.48, 27.71, 20.20, 20.17; HRMS (ESI) calcd for $C_{46}H_{35}F_{12}N_2O_6$ $(M - H)^-$ 939.2309, found 939.2283.

Data for ***22:*** orange-red crystal recrystallized from pyridine and ethanol; yield, 62%; mp, 269−270 °C (lit.[19] 280−282 °C); ^{1}H NMR (400 MHz, $CDCl_3$), δ 15.03 (d, J = 11.6 Hz, 2H), 10.16 (d, J = 11.6 Hz, 2H), 8.15 (s, 2H), 8.00 (d, J = 7.5 Hz, 2H), 7.73 (s, 2H), 7.66 (s, 2H), 7.62−7.50 (m, 4H), 5.79 (s, 2H), 3.76−3.70 (m, 2H), 2.17 (s, 6H), 1.56 (br s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 175.68, 152.92, 149.84, 149.57, 149.37, 146.75, 141.03, 133.63, 130.80, 130.32, 130.10, 124.12, 119.80, 119.13, 116.55, 112.09, 106.15, 27.65, 20.22, 20.20, 20.14.

Data for ***23:*** yellow solid; yield, 65%; mp, >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6), δ 14.93 (d, J = 12.2 Hz, 2H), 10.42 (d, J = 12.2 Hz, 2H), 8.66 (s, 2H), 8.62 (s, 2H), 7.69 (s, 2H), 7.48 (s, 2H), 7.43 (d, J = 8.5 Hz, 2H), 7.14 (d, J = 8.5 Hz, 2H), 3.97 (s, 6H), 3.80−3.68 (m, 2H), 1.97 (s, 6H), 1.51−1.41 (m, 12H); ^{13}C NMR (100 MHz, DMSO-d_6), δ 174.48, 150.97, 150.68, 149.01, 146.23, 141.88, 132.34, 128.66, 127.72, 127.38, 123.09, 121.61, 116.97, 115.30, 112.34, 111.12, 106.29, 56.38, 26.61, 20.32, 20.22, 20.19; HRMS (ESI) calcd for $C_{44}H_{43}N_2O_{14}S_2$ $(M - 2Na + H)^-$ 887.2161, found 887.2121.

Data for ***24:*** orange-red crystal recrystallized from pyridine and ethanol; yield, 82%; mp, 281 °C (lit.[19] 302−303 °C); ^{1}H NMR (400 MHz, $CDCl_3$), δ 15.17 (d, J = 12.2 Hz, 2H), 10.32 (d, J = 12.2 Hz, 2H), 7.92 (s, 2H), 7.84 (d, J = 8.8 Hz, 2H), 7.78 (d, J = 7.4 Hz, 4H), 7.69 (s, 2H), 7.67 (s, 2H), 7.51−7.38 (m, 6H), 5.85 (s, 2H), 3.82−3.70 (m, 2H), 2.19 (s, 6H), 1.59−1.55 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 174.36, 153.88, 149.81, 149.44, 147.01, 136.86, 133.82, 132.88, 131.46, 130.09, 129.82, 128.80, 127.85, 127.50, 127.25, 125.79, 118.74, 117.41, 116.31, 115.22, 105.33, 27.57, 20.33, 20.28, 20.14.

Data for ***25:*** orange-red solid; yield, 86%; mp, 280−281 °C (lit.[19] 283−285.5 °C); ^{1}H NMR (400 MHz, $CDCl_3$), δ 15.88 (d, J = 11.6 Hz, 2H), 10.36 (d, J = 11.6 Hz, 2H), 8.36 (d, J = 8.4 Hz, 2H), 7.99 (s, 2H), 7.91 (d, J = 8.4 Hz, 2H), 7.75−7.70 (m, 4H), 7.68 (s, 2H), 7.60 (t, J = 7.5 Hz, 2H), 7.52 (d, J = 7.4 Hz, 2H), 7.44 (t, J = 7.8 Hz, 2H), 5.86 (s, 2H), 3.85−3.73 (m, 2H), 2.20 (s, 6H), 1.60 (t, J = 6.1 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 174.30, 155.54, 149.84, 149.54, 147.03, 136.11, 135.94, 134.22, 132.86, 129.76, 128.93, 128.55, 127.34, 126.89, 126.38, 125.74, 125.59, 123.72, 121.22, 118.69, 116.31, 114.44, 113.69, 105.89, 27.59, 20.34, 20.29, 20.15.

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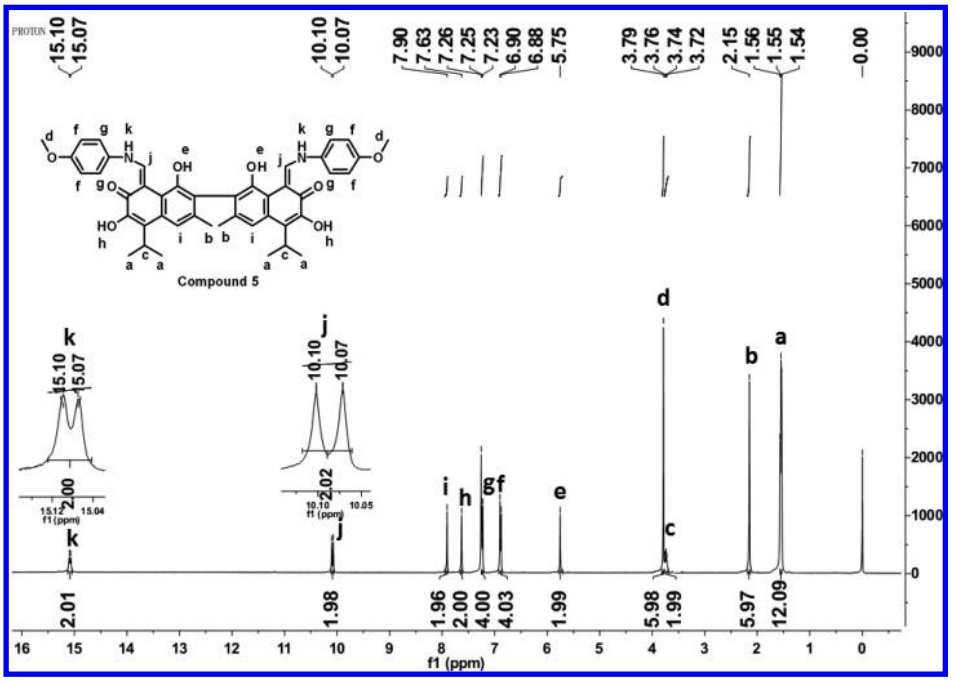

Figure 3. ^{1}H NMR spectra of compound **5**.

Biological Assay. The anti-TMV activity of all compounds was tested using our previously reported methods.[22]

The field trials were carried out in the trial area of the Institute of Agricultural Environment and Resource, Yunnan Academy of Agricultural Sciences, Kunming City, China, between June and July 2014. The formulation of compound **19** (1% emulsifiable concentrates (EC)) was prepared in our research group. It was diluted to 750 L/ha before use.

The rat oral gavage acute toxicity of pesticides was performed in Xu He (Tianjin) Pharmaceutical Technology Co., Ltd. in May 2014. Sixteen rats, half males and half females, were divided randomly into four groups. The samples were respectively configured to 5 and 50 mg/mL.

RESULTS AND DISCUSSION

Chemistry. Gossypol (**1**) was obtained using our previously reported method.[23] Gossypol acetic acid (**2**) was purchased from commercial sources. A series of Schiff bases of gossypol (**3**−**25**) were prepared by treating **2** with the corresponding amines in ethanol (Scheme 1). The Schiff bases had poor solubility in ethanol, so they were simply collected by filtration. Recrystallization of the products from suitable solvents could give purer samples. Unfortunately, recrystallization was not fit for compounds **15**−**18** and **20**. However, they were obtained via the employment of excess amine when the preparation was repeated. For compounds **11** and **23**, the sulfonic sodium salts of corresponding amines were prepared from homologous aminobenzenesulfonic acid and NaOH, for which were used 3 equiv of gossypol to ensure them a sodium sulfonate group. It is worth mentioning that if there are multiple electron withdrawing groups on the ortho and para positions of aromatic amine, such as 2,4-dinitroaniline and 2-nitro-4-(trifluoromethyl)aniline, the corresponding Schiff base of gossypol could not be achieved but the dehydration product of gossypol, anhydrogossypol,[24] was obtained. All of our synthetic gossypol Schiff bases exist in the enamine−enamine form[15,25] in solution as determined by ^{1}H NMR. For compound **5**, for example, the structure can be interpreted by the ^{1}H NMR spectrum (Figure 3). The signal peaks of symmetrical protons should appear at the same position and have identical coupling constants because of the axial symmetry of the structure. The presence of doublet peaks with the coupling constant of 12.0 Hz in the ^{1}H NMR spectrum can be attributed to the signals of **H**—C═C proton (j) and **H**—NAr proton (k). This behavior can be rationally explained by the fact that two protons are adjacent and coupled with each other.

Antiviral Biological Assay. Compounds **1**−**25** were initially tested for their phytotoxic activities against the test plant, *Nicotiana tabacum* L. The results indicated that these natural product-based compounds showed no phytotoxic activity at 500 μg/mL. In other words, the compounds caused no harm to the test plant; therefore, there would be no interference with the determination of anti-TMV data.

The antiviral bioassay of the compounds against TMV was carried out, and the antiviral results are listed in Tables 1 and 2. To make a judgment on the antiviral potency of the synthesized compounds, the commercially available plant virucide ribavirin[24] was used as the control.

The in vitro anti-TMV activities of compounds **1**−**25** and the contrast compound ribavirin are shown in Table 1. The results indicate that these target compounds exhibit moderate to excellent activities against TMV. Among these compounds, gossypol (**1**) and gossypol acetic acid (**2**) displayed similar in vitro anti-TMV activities (**1**, 32%; **2**, 28%); however, they were less effective than ribavirin (38%) at 500 μg/mL. In contrast, except for **6**, **22**, and **24**, other aromatic gossypol Schiff bases of **3**−**25** showed equivalent or even higher antiviral activities than ribavirin (38%); especially compounds **5**, **9**, **10**, **12**, **15**, **17**, **19**, and **23** displayed obviously higher anti-TMV activities at a concentration of 500 μg/mL, and the antiviral activities of compounds **9** and **19** at 100 μg/mL even reach the level of ribavirin at 500 μg/mL.

On comparison with the parent dianilinogossypol (**3**), except for *p*-hydroxy compound **6**, the activities of Schiff bases **4**−**12** with different substituent groups on the para position of aniline are higher. However, they displayed distinctly different activity levels against TMV in vitro. Compounds with methoxy (**5**),

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Table 1. In Vitro Antiviral Activity of Compounds 1−25 and Ribavirin against TMV

compd	concn (μg/mL)	in vitro curative effect (%)	compd	concn (μg/mL)	in vitro curative effect (%)
1	500	32	**14**	500	30
	100	0		100	0
2	500	28	**15**	500	58
	100	0		100	14
3	500	32	**16**	500	40
	100	0		100	0
4	500	40	**17**	500	53
	100	0		100	22
5	500	47	**18**	500	30
	100	10		100	0
6	500	24	**19**	500	62
	100	0		100	32
7	500	37	**20**	500	39
	100	6		100	0
8	500	44	**21**	500	32
	100	0		100	0
9	500	50	**22**	500	26
	100	39		100	0
10	500	57	**23**	500	47
	100	20		100	18
11	500	40	**24**	500	25
	100	0		100	0
12	500	52	**25**	500	44
	100	20		100	16
13	500	33	ribavirin	500	38
	100	0		100	14

nitro (**9**), trifluoromethyl (**10**), and trifluoromethoxy (**12**) showed excellent activities (48, 50, 57, and 52%, respectively). To investigate the influence of the position and the number of substituents on the activities, **13**−**23** were synthesized. Compared with their para-substitutional substrates, corresponding substituent groups on the ortho position were beneficial to the increase of their anti-TMV activities, whereas the substituent groups on meta positions were unfavorable to the anti-TMV activities (MeO, **15** > **5** > **16**; CF_3, **19** > **10** >**20**; NO_2, **9** > **22**). However, the substituent group of the hydroxyl was an exception, as the *o*-hydroxyl compound **13** and *m*-hydroxyl compound **14** had higher activities than *p*-hydroxyl compound **6**. Furthermore, the level of the anti-TMV activities could not be improved by increasing the number of substituent groups. Among methoxy-substituted compounds **5** and **15**−**18**, **15** with only one substituent group on the ortho position displayed the best inhibitory effect. However, **23** (18%) with *o*-methoxy and *m*-sodium sulfonate substituent groups exhibited better inhibitory effect than **11** (0%) and **15** (14%) at 100 μg/mL. The Schiff bases of naphthylamines were synthesized as well, and their anti-TMV activities were performed. Compound **25** (44% at 500 μg/mL), obtained by the condensation of gossypol and 1-naphthylamine, displayed a better inhibitory

Table 2. In Vivo Anti-TMV Activity of Compounds 1−25 and Ribavirin

		in vivo activity		
compd	concn (μg/mL)	inactivation effect (%)	curative effect (%)	protection effect (%)
1	500	35	29	29
	100	8	0	0
2	500	32	30	33
	100	0	0	0
3	500	52	34	44
	100	27	0	27
4	500	8	37	50
	100	0	0	17
5	500	46	42	44
	100	19	20	21
6	500	20	17	23
	100	0	0	0
7	500	33	36	35
	100	11	0	9
8	500	36	41	43
	100	9	19	20
9	500	36	47	42
	100	12	40	22
10	500	35	51	42
	100	25	22	19
11	500	38	35	36
	100	0	0	9
12	500	54	50	56
	100	26	21	23
13	500	35	36	34
	100	10	0	9
14	500	40	34	38
	100	10	8	15
15	500	51	55	56
	100	16	20	27
16	500	46	43	48
	100	18	9	23
17	500	56	48	50
	100	28	19	23
18	500	36	33	36
	100	8	0	0
19	500	60	57	62
	100	23	30	26
20	500	52	35	48
	100	17	13	22
21	500	41	35	37
	100	12	0	9
22	500	26	23	34
	100	0	0	0
23	500	46	43	38
	100	17	13	15
24	500	37	28	30
	100	8	0	0
25	500	39	37	43
	100	10	19	16
ribavirin	500	38	37	40
	100	12	17	18

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effect than dianilinogossypol (**3**, 32% at 500 μg/mL). At variance with **25**, the activity of 2-naphthylamine Schiff base **24** (25% at 500 μg/mL) was relatively lower than that of **3**.

Due to their well-performed behaviors of in vitro antiviral activities against TMV, the in vivo antiviral activities of these compounds deserve to be exploited further. As shown in Table 2, the majority of the compounds also displayed equivalent or higher in vivo activities than the control ribavirin at a concentration of 500 μg/mL. In particular, Schiff bases **12**, **15**, **17**, and **19** exhibited obviously higher anti-TMV activities than ribavirin at 500 μg/mL, of which compound **19** with an *o*-trifluoromethyl group showed the best activity (inactivation activity, 60%/500 μg/mL and 23%/100 μg/mL; curative activity, 57%/500 μg/mL and 30%/100 μg/mL; and protection activity, 62%/500 μg/mL and 26%/100 μg/mL), confirmed by the in vivo inactivation tests, curative tests, and protection tests. Additionally, the in vivo curative activities of these compounds displayed structure−activity relationships similar to the activities in vitro. More importantly, compound **9** with a *p*-nitro group showed a better effect at 100 μg/mL (40%), which is even higher than that of ribavirin at 500 μg/mL (37%).

Interestingly, the in vivo inactivation and protection activities of dianilinogossypol (**3**) displayed excellent inhibitory effects (inactivation activity, 52%/500 μg/mL and 27%/100 μg/mL; protection activity, 44%/ 500 μg/mL and 27%/100 μg/mL). However, only **12**, **15**, **17**, **19**, and **20** exhibited obviously better in vivo curative and protection activities at 500 μg/mL than parent dianilinogossypol (**3**). These results indicated the introduction of trifluoromethoxy, methoxy, and trifluoromethyl on the aromatic ring of aniline could increase the anti-TMV activities level of parent dianilinogossypol. Further analysis of the relationship between the positions of substituents and anti-TMV activities indicated that the order of methoxy and trifluoromethyl is ortho > meta > para position (**15** > **16** > **5**, **19** > **20** > **10**). Other groups and the number of them displayed similar SAR to anti-TMV activities in vitro. The derivatives of naphthylamines (**24** and **25**) had lower inhibitory effect than dianilinogossypol (**3**), but 1-naphthylamine (**25**) is relatively higher than that of 2-naphthylamine (**24**).

Evaluation of Anti-TMV Activity of 19 in the Field. Compound **19**, which had exhibited the best anti-TMV activity both in vitro and in vivo in the laboratory, was employed to evaluate the antiviral activity in the field. A 1% EC solution of **19** was prepared and evaluated for the control of TMV in Tengchong county of Yunnan province (southern China). The results of the field trials are shown in Table 3, which indicated that **19** at the amount of 10 g ai/ha had the same control effect (about 66%) as 5% amino glycan at 100 g ai/ha and 20% moroxydine hydrochloride−cupric acetate at 600 g ai/ha at 10 days after spraying. Therefore, it was suggested that **19** could be considered as an alternative to the employed plant virus inhibitor for further development on the basis of the field trials.

Table 3. Anti-TMV Effect in the Field in 2014

compd	amount (g ai/ha)	controlled effect (%)
19	10	66.61
	50	83.88
	100	94.56
5% amino glycan	100	65.70
20% moroxydine hydrochloride−cupric acetate	600	66.57

Rat Oral Gavage Acute Toxicity of Gossypol and 19. To balance the toxicity versus efficacy of compound **19**, we next explored the tolerated dose of **19** and gossypol using a group of rats. They were treated with a single dose of 500 or 50 mg/kg and observed for a period of 14 days, monitoring morbidity (body weight loss) and mortality. All rats were alive after 14 days. The weight of the rats that were treated with gossypol at 500 mg/kg slightly declined over the first 6 days. There is no obvious weight difference between any other two groups (Figure 4). Therefore, gossypol and **19** display low toxicity to rats, but the toxicity of **19** is lower.

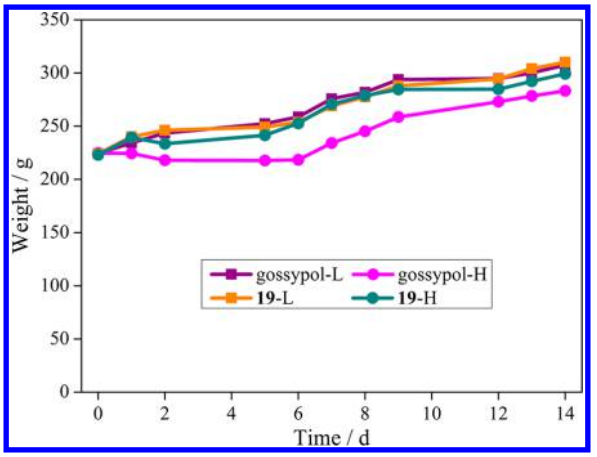

Figure 4. Average weight of rats. Gossypol-L, dosage of gossypol is 50 mg/kg; gossypol-H, dosage of gossypol is 500 mg/kg; **19**-L, dosage of **19** is 50 mg/kg; **19**-H, dosage of **19** is 500 mg/kg.

In summary, to eliminate the toxicity caused by the aldehyde group, retain the electron transfer functionalities to generate ROS, and adjust the anti-TMV activity level of gossypol, a series of aromatic gossypol Schiff bases **3**−**25** were prepared and investigated for their anti-TMV activity for the first time. The bioassay results indicated that most of these Schiff bases showed moderate to excellent activities against TMV and were more effective than gossypol (**1**) and gossypol acetic acid (**2**). The introduction of methoxy, trifluoromethyl, or trifluoromethoxy groups, positioned ortho to amine of enamine, could significantly increase the anti-TMV activity levels. Among all of the compounds, compound **19** with an *o*-trifluoromethyl group showed the best anti-TMV activities both in vitro and in vivo in the laboratory. Furthermore, the compound at 10 g ai/ha in the field trial had the same control effect as 5% amino glycan at 100 g ai/ha and 20% moroxydine hydrochloride−cupric acetate at 600 g ai/ha. More importantly, it showed lower toxicity to rats than gossypol. Because of the easy preparation, good anti-TMV activity, and low toxicity, the *o*-trifluoromethylaniline Schiff base (**19**) is expected to be developed as a promising candidate as an inhibitor of plant virus. Further studies on structural optimization and mechanism of action are currently underway in our laboratory.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Fungicidal, insecticidal, and herbicidal activities. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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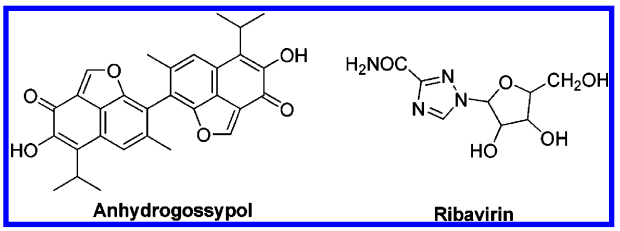

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