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Article

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Application of "hydrogen bonding interaction" in new drug development: Design, synthesis, antiviral activity and SARs of thiourea derivatives

Aidang Lu[†,*], Ziwen Wang[‡,§,*], Zhenghong Zhou[§], Jianxin Chen[†], Qingmin Wang[§,*]

[†]School of Marine Science and Engineering, Hebei University of Technology, Tianjin 300130, China

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

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

* To whom correspondence should be addressed. For Aidang Lu, E-mail: luaidang@hebut.edu.cn; Phone: 0086-22-60302241 Ext 205; Fax: 0086-22-60204274; For Ziwen Wang, E-mail: wangziwen2725@163.com; Phone: 0086-22-23503792; Fax: 0086-22-23503792; For Prof. Qingmin Wang, E-mail: wangqm@nankai.edu.cn; Phone: 0086-22-23503952; Fax: 0086-22-23503952.

ABSTRACT: A series of simple thiourea derivatives were designed based on the structure of nature product harmine and lead compound and synthesized from amines in one step. The antiviral activity of these thiourea derivatives was evaluated. Most of them exhibited significantly higher anti-TMV activity than commercial plant virucides Ribavirin, harmine and lead compound. Hydrogen bond was found to be important but not the more the better. The optimal compound (*R,R*)-**20** showed the best anti-TMV activity in vitro and in vivo (in vitro activity: 75%/500 μg/mL and 39%/100 μg/mL; inactivation activity: 71%/500 μg/mL and 35%/100 μg/mL; curative activity: 73%/500 μg/mL and 37%/100 μg/mL; protection activity: 69%/500 μg/mL and 33%/100 μg/mL) which is significantly higher than that of Ningnanmycin. The systematic study provides strong evidence that these simple thiourea derivatives could become potential TMV inhibitors.

KEYWORDS: tobacco mosaic virus, nature product, thiourea derivatives, anti-TMV activity, SARs

INTRODUCTION

Plant viruses have a great opportunity to infect their hosts due to their lower immunity. It is extremely difficult to controlling virus diseases effectively. *Tobacco mosaic virus* (TMV) may cause severe losses to agriculture, but its control is still a challenge. So far, many plant viral inhibitors (BTH, Ningnanmycin, Ribavirin, etc) have been used to combat TMV disease. However, all of chemical treatments can only reduce the degree of plants' infection.[1]

The agrochemicals derived from botanical have many advantages for its low toxicity to humans. Another characteristic is their degradable ability which can reduce their environmental risks. However, inevitably botanical agrochemicals also have some disadvantages such as too complex to synthesize; active ingredients easy to decompose; most botanical agrochemicals play a pharmacodynamics slowly, need to spray frequently due to very short residual efficient duration, and so on. These shortcomings limit the widespread use of botanical pesticides. Therefore, to discover new pesticide based on a precursor from active substances of plant origin is an important and a feasible means to solve the problem above.[2]

In our previous work, we found that nature product harmine (**Figure 1**) exhibited excellent anti-TMV activity (antiviral activity against TMV in vitro and inactivation, curative and protection activity in vivo were 45%, 41%, 39%, and 42%, respectively, at 500 μg/mL). Meanwhile, (1*S*,3*S*)-1-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carbohydrazide (**Figure 1**,

Lead compound) maintained high anti-TMV activity (the corresponding activity were 50%, 46%, 48%, and 50%, respectively, at 500 μg/mL), and it exhibited excellent stability and solubility; these advantages made it an ideal lead compound for further derivation.[3]

Chiral thioureas and their derivatives possess many different catalytic and biological activities. Part of them can not only support help to control configuration of compounds as catalysts but also have anti-HIV activity as medicines.[4–6] Some compounds bearing thiourea against TMV have been reported by Jin,[7] Li,[8] Song,[9] Yang,[10] and Yuan.[11]

From theoretical and experimental points of view, much attention was focused on intermolecular forces for their essential role in determining the three-dimensional structure. They are commonly seen in Proteins, DNA as well as enzyme-substrate complexes.[12] There are widespread reports about hydrogen bonding over the past ten years. However, the applications of hydrogen bonding in drug design are only now coming to light. Thiourea derivatives can bind to the nitrogen or oxygen atom serving as catalysts.[13] TMV, composing primarily with protein and RNA, contains a series of amino acid residues.[14] If they can bind to the residues of TMV, simple thiourea derivatives would exhibit antiviral activity.

A series of simple thiourea derivatives were designed based on the structure of nature product harmine and lead compound and synthesized as potential TMV inhibitors (**Figure 1**). The structures of these thiourea derivatives were confirmed and assessment for their anti-TMV was systematically evaluated.

MATERIALS AND METHODS

Instruments. The melting points of the products were determined on an X-4 binocular microscope (Gongyi Yuhua Instrument Co., China) and the thermometer was not corrected. NMR spectra were acquired with a Bruker 400 MHz (100 MHz for ^{13}C) instrument at room temperature. Chemical shifts were measured relative to residual solvent peaks of $CDCl_3$ (^1H: δ = 7.26 ppm; ^{13}C: δ = 77.0 ppm) or d_6-DMSO (^1H: δ = 2.50 ppm; ^{13}C: δ = 39.5 ppm) with tetramethylsilane as internal standards. The following abbreviations were used to designate chemical shift multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, and bs = broad singlet. All first-order splitting patterns were assigned on the basis of multiplet appearance. Splitting patterns that could not be easily interpreted were designated multiplet (m) or broad (br). HRMS data were obtained with an FT-ICR MS spectrometer (Ionspec, 7.0 T). Analytical TLC was performed on silica gel GF 254. Column chromatographic purification was performed using silica gel. All reagents were of analytical reagent grade or chemically pure and purified prior to use when necessary. Compounds **18**–**22** (**Figure 2**) were prepared according to our previously reported procedure.[15] The isothiocyanates were prepared according to the literature.[16]

General Experimental Procedures

General Procedures for the Preparation of Compounds 1–8, 13–17 and 20.[17]

To a mixture of CH_2Cl_2 (10 mL) and amines (5.0 mmol) was added the corresponding isothiocyanate (5.0 mmol or 10 mmol), and the resulting mixture was

stirred at room temperature until TLC indicated the reaction was complete. The reaction mixture was evaporated in vacuum. The pure products **1**–**8** and **13**–**17** were obtained by recrystallization from petroleum ether and ether. Compounds **20** were purified or chromatography on a column of silica gel with PE-EtOAc (v/v = 1: 1).

1,3-Diphenylthiourea (**1**): white solid; yield, 99%; mp 144−145 °C (lit.[18] 140−142 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 2H), 7.35−7.40 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.8, 137.2, 129.6, 127.1, 125.3; HRMS (ESI) calcd for $C_{13}H_{12}N_2S$ $(M+H)^+$ 229.0794, found 229.0791.

1,3-Dibenzylthiourea (**2**): white solid; yield, 98%; mp 142−144 °C (lit.[19] 145−146 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28−7.34 (m, 6H), 7.02 (d, J = 6.8 Hz, 4H), 6.13 (br s, 2H), 4.61 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.9, 136.6, 129.0, 128.0, 127.5, 48.6; HRMS (ESI) calcd for $C_{15}H_{16}N_2S$ $(M+H)^+$ 257.1107, found 257.1101.

1,3-Dicyclohexylthiourea (**3**): white solid; yield, 99%; mp 176−177 °C (lit.[18] 174−176 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 (br s, 2H), 3.84 (s, 2H), 1.99−2.02 (m, 4H), 1.60−1.73 (m, 6H), 1.32−1.41 (m, 4H), 1.13−1.24 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.0, 52.9, 32.9, 25.4, 24.7; HRMS (ESI) calcd for $C_{13}H_{24}N_2S$ $(M+H)^+$ 241.1733, found 241.1738.

1-(3,5-Bis(trifluoromethyl)phenyl)-3-phenylthiourea (**4**): white solid; yield, 99%; mp 135−136 °C (lit.[17] 141−143 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (s, 1H), 7.97 (s, 1H), 7.35 (s, 1H), 7.68 (s, 1H), 7.51 (t, J = 6.4 Hz, 2H), 7.40−7.42 (m, 1H), 7.32−7.34 (d, J = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.8, 139.5, 135.5, 132.1 ($J_{C\text{-}F}$ = 33.6 Hz),

130.6, 128.4, 125.6, 124.7 ($J_{C\text{-}F}$ = 2.5 Hz), 124.3 and 121.6 ($J_{C\text{-}F}$ = 271.1 Hz), 119.82 (m) ; HRMS (ESI) calcd for $C_{15}H_{10}F_6N_2S$ $(M+H)^+$ 365.0542, found 365.0546.

1-(3,5-Bis(trifluoromethyl)phenyl)-3-cyclohexylthiourea (**5**): white solid; yield, 100%; mp 159−160 °C (lit.[17] 164−165 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.76 (s, 2H), 6.13 (s, 1H), 4.20 (s, 1H), 2.04−2.08 (m, 2H), 1.61−1.72 (m, 3H), 1.36−1.45 (m, 2H), 1.17−1.22 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.9, 139.1, 132.7 ($J_{C\text{-}F}$ = 34.7 Hz), 126.8, 124.1 and 121.4 ($J_{C\text{-}F}$ = 271.4 Hz), 118.7, 53.9, 32.3, 25.2, 24.5; HRMS (ESI) calcd for $C_{15}H_{16}F_6N_2S$ $(M+H)^+$ 371.1011, found 371.1017.

1-(3,5-Bis(trifluoromethyl)phenyl)-3-butylthiourea (**6**): white solid; yield, 98%; mp 94−97 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 2H), 7.70 (s, 1H), 7.60 (s, 1H), 6.13 (br s, 1H), 3.62 (s, 2H), 1.58−1.65 (m, 2H), 1.34 (m, 2H), 0.95 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.3, 139.1, 132.8 ($J_{C\text{-}F}$ = 33.6 Hz), 126.8, 124.1 and 121.4 ($J_{C\text{-}F}$ = 271.3 Hz), 118.7, 45.1, 30.7, 20.1, 13.6; HRMS (ESI) calcd for $C_{13}H_{14}F_6N_2S$ $(M+H)^+$ 345.0855, found 345.0852.

1-(3,5-Bis(trifluoromethyl)phenyl)-3-(*tert*-butyl)thiourea (**7**): white solid; yield, 98%; mp 160 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (br s, 1H), 7.77 (s, 2H), 7.72 (s, 1H), 6.13 (s, 1H), 1.55 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.7, 142.4, 130.5 ($J_{C\text{-}F}$ = 32.4 Hz), 127.8, 125.1 and 122.4 ($J_{C\text{-}F}$ = 271.1 Hz), 119.7, 53.6, 28.7; HRMS (ESI) calcd for $C_{13}H_{14}F_6N_2S$ $(M+H)^+$ 345.0855, found 345.0853.

1,3-Bis(3,5-bis(trifluoromethyl)phenyl)thiourea (**8**): white solid; yield, 99%; mp 174−176 °C (lit.[20] 170−172 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 2H), 8.21 (s,

4H), 7.85 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.5, 141.2, 130.3 ($J_{C\text{-}F}$ = 32.9 Hz), 124.4, 124.4 and 121.713 ($J_{C\text{-}F}$ = 271.1 Hz), 117.5; HRMS (ESI) calcd for $C_{17}H_8F_{12}N_2S$ $(M+H)^+$ 501.0289, found 501.0293.

1,1'-((1*R*,2*R*)-1,2-Diphenylethane-1,2-diyl)bis(3-phenylthiourea) (**13**): white solid; yield, 100%; mp 179−181 °C (lit.[21] 198 °C); $[\alpha]_D^{20}$ = +63.2 (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (s, 2H), 8.43 (s, 2H), 7.10−7.34 (m, 20H), 5.98 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.9, 134.0, 139.6, 129.1, 128.6, 128.5, 127.7, 124.8, 123.5, 62.7; HRMS (ESI) calcd for $C_{28}H_{26}N_4S_2$ $(M+H)^+$ 483.1672, found 483.1669.

1,1'-((1*R*,2*R*)-1,2-Diphenylethane-1,2-diyl)bis(3-benzylthiourea) (**14**): white solid; yield, 100%; mp 195−196 °C; $[\alpha]_D^{20}$ = +75.4 (*c* = 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (s, 4H), 7.08−7.27 (m, 20H), 5.87 (s, 2H), 4.72 (s, 2H), 4.57 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.6, 139.9, 139.0, 128.2, 128.0, 127.7, 127.2, 127.1, 126.8, 62.0, 47.1; HRMS (ESI) calcd for $C_{28}H_{26}N_4S_2$ $(M+H)^+$ 483.1672, found 483.1669.

1,1'-((1*R*,2*R*)-1,2-Diphenylethane-1,2-diyl)bis(3-benzhydrylthiourea) (**15**): white solid; yield, 93%; mp 109−111 °C; $[\alpha]_D^{20}$ = +84.2 (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06−7.39 (m, 30H), 6.75 (br s, 6H), 5.86 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.5, 140.5, 139.5, 137.7, 129.1, 128.4, 128.2, 128.0, 127.8, 127.7, 127.6, 127.1, 65.1, 62.2; HRMS (ESI) calcd for $C_{42}H_{38}N_4S_2$ $(M+H)^+$ 663.2611, found 663.2609.

1,1'-((1*R*,2*R*)-1,2-Diphenylethane-1,2-diyl)bis(3-(3,5-bis(trifluoromethyl)phenyl)thiourea) (**(1*R*,2*R*)-16**): white solid; yield, 92%; mp 192−194 °C (lit.[22] 194−196 °C); $[\alpha]_D^{20}$ = −50.7 (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (s, 2H), 8.77 (s, 2H), 8.16

(s, 4H), 7.69 (s, 2H), 7.22−7.38 (m, 10H), 5.97 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.2, 141.3, 138.4, 129.8−130.5 (m), 128.3, 128.1, 127.8, 127.6, 126.5, 124.3, 122.0, 121.5, 118.8, 116.0, 62.4; HRMS (ESI) calcd for $C_{32}H_{22}F_{12}N_4S_2$ $(M+H)^+$ 755.1167, found 755.1163.

1,1'-((1*R*,2*R*)-cyclohexane-1,2-diyl)bis(3-(3,5-bis(trifluoromethyl)phenyl)thiourea) ((1*R*,2*R*)-17): white solid; yield, 95%; mp 132−134 °C (lit.[23] 132−133 °C); $[\alpha]_D^{20}$ = −61.2 (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (s, 2H), 8.18 (s, 6H), 7.71 (s, 2H), 4.35 (s, 2H), 2.20 (s, 2H) , 1.72 (s, 2H) , 1.31 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.0, 141.6, 130.1 ($J_{C\text{-}F}$ = 32.7 Hz), 127.2, 124.5 and 121.8 ($J_{C\text{-}F}$ = 271.0 Hz), 119.1, 56.8, 31.1, 24.2; HRMS (ESI) calcd for $C_{24}H_{20}F_{12}N_4S_2$ $(M+H)^+$ 657.1011, found 657.1016.

1-((1R,2R)-2-Amino-1,2-diphenylethyl)-3-benzylthiourea ((*R,R*)-20): white solid; yield, 63%; mp 53−55 °C; $[\alpha]_D^{20}$ = +69.4 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (br s, 1H), 7.12−7.30 (m, 15H), 6.52 (br s, 1H), 5.09 (br s, 1H), 4.59−4.62 (m, 1H), 4.51 (dd, *J* = 5.2 and 15.2 Hz, 1H), 4.29 (d, *J* = 4.0 Hz, 1H), 1.50 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ181.8, 141.9, 139.8, 137.2, 128.81, 128.76, 128.6, 127.67, 127.62, 127.4, 126.7, 126.4, 64.0, 60.2, 48.2; HRMS (ESI) calcd for $C_{22}H_{23}N_3S$ $(M+H)^+$ 362.1685, found 362.1684.

1-((1S,2S)-2-Amino-1,2-diphenylethyl)-3-benzylthiourea ((*S,S*)-20): white solid; yield, 65%; mp 52−54 °C; $[\alpha]_D^{20}$ = −69.7 (*c* = 1.0, $CHCl_3$) (lit.[24] $[\alpha]_D^{24}$ = −74.3 (*c* = 1.0, $CHCl_3$)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (br s, 1H), 7.11−7.27 (m, 15H), 6.58 (br s, 1H), 5.12 (br

s, 1H), 4.58−4.62 (m, 1H), 4.50 (dd, J = 4.8 and 14.8 Hz, 1H), 4.28 (d, J = 4.0 Hz, 1H), 1.66 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.8, 141.8, 139.7, 137.2, 128.82, 128.75, 128.6, 127.7, 127.6, 127.4, 126.7, 126.3, 63.9, 60.2, 48.3; HRMS (ESI) calcd for $C_{22}H_{23}N_3S$ $(M+H)^+$ 362.1685, found 362.1683.

General Procedures for the Preparation of Componds 9–12.[25]

In a 100 mL three-necks flask, the isothiocyanatobenzene (20 mmol), amino acids or their derivatives (20 mmol), NaOH (22 mmol), water (20 mL) and THF (40 mL) were added and stirred at room temperature for 24 h. After completion of the reaction, the pH of the mixture was adjusted to pH 2-3 with aqueous 25% HCl, and then solvents were removed under vacuum. The remaining residue was recrystallized from absolute methanol to obtain the desired thiourea derivatives **9**–**12**.

(*S*)-2-(3-Phenylthioureido)propanoic acid (**9**): white solid; yield, 75%; mp 173−175 °C; $[\alpha]_D^{20}$ = −44.2 (c = 0.5, CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (br s, 1H), 7.46−7.53 (m, 3H), 7.32 (d, J = 8.0 Hz, 2H), 4.35 (q, J = 6.8 Hz, 1H), 1.59 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.5, 174.1, 132.5, 129.3, 129.2, 128.2, 55.5, 17.0; HRMS (ESI) calcd for $C_{10}H_{12}N_2O_2S$ $(M+H)^+$ 225.0692, found 225.0695.

(*S*)-3-Phenyl-2-(3-phenylthioureido)propanoic acid (**10**): white solid; yield, 78%; mp 182−184 °C (lit.[25] 184−185 °C); $[\alpha]_D^{20}$ = −43.2 (c = 0.5, CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.42−7.47 (m, 3H), 7.33−7.39 (m, 3H), 7.26 (d, J = 6.8 Hz, 3H), 7.05 (d, J = 6.8 Hz, 2H), 4.53 (dd, J = 4.0 and 7.6 Hz, 1H), 3.34 (dd, J = 4.0 and 14.0 Hz, 1H), 3.11 (dd, J = 7.6 and 14.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.7, 172.9,

133.9, 132.4, 129.7, 129.4, 129.2, 128.2, 127.8, 60.9, 37.4; HRMS (ESI) calcd for $C_{16}H_{16}N_2O_2S$ $(M+H)^+$ 301.1005, found 301.1004.

(*S*)-1-(3-Oxo-1-phenylpentan-2-yl)-3-phenylthiourea (**11**): white solid; yield, 65%; mp 152−154 °C; $[\alpha]_D^{20}$ = −55.0 (*c* = 0.1, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.79 (s, 1H), 7.27−7.39 (m, 10H), 6.68 (s, 1H), 4.06 (t, *J* = 6.4 Hz, 1H), 3.04−3.07 (m, 1H), 2.89−2.94 (m, 1H), 1.03−1.10 (m, 1H), 0.57 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.8, 137.8, 137.4, 129.9, 129.4, 128.1, 128.0, 127.0, 126.2, 94.4, 61.2, 34.7, 29.6, 8.0; HRMS (ESI) calcd for $C_{18}H_{20}N_2OS$ $(M+H)^+$ 313.1369, found 313.1368.

(*S*)-1-(1-Oxo-1,3-Diphenylpropan-2-yl)-3-phenylthiourea (**12**): white solid; yield, 68%; mp 152−154 °C; $[\alpha]_D^{20}$ = −58.2 (*c* = 0.1, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H), 7.53 (s, 1H), 7.06−7.30 (m, 15H), 4.31 (t, *J* = 7.2 Hz, 1H), 2.95−3.10 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.1, 140.7, 137.9, 137.1, 129.5, 129.1, 128.7, 127.8, 127.6, 127.4, 126.7, 126.4, 125.9, 94.7, 67.9, 33.8; HRMS (ESI) calcd for $C_{22}H_{20}N_2OS$ $(M+H)^+$ 361.1369, found 361.1367.

Antiviral Biological Assay. The procedure of purifying TMV and the method to test the anti-TMV activity of the thiourea derivatives were the same with those reported previously in the literature.[26]

Purification of Tobacco Mosaic Virus. The upper leaves of *Nicotiana tabacum* L. inoculated with TMV were selected and ground in phosphate buffer and then filtered through double-layer pledget. The filtrate was centrifuged at 10000g, treated with PEG twice, and centrifuged again. The whole experiment was processed at 4 °C. Absorbance

211 value was estimated at 260 nm by ultraviolet spectrophotometer.

212 $$Virus\ concn = (A_{260} \times dilution\ ratio)E\ /\ {}^{0.1\%,\,260nm}_{1cm}$$

213 *Antiviral Activity of Compounds against TMV in Vitro*. Fresh leaf of the 5-6 growth
214 stage of tobacco inoculated by the juice-leaf rubbing method (concentration of TMV is
215 5.88×10^{-2} μg/mL) was cut into halves along the main vein. The halves were immersed
216 into the solution of different concentrations (see **Table 1**) of the compounds and double
217 distilled water for 20min, respectively, and then cultured at 25 °C for 72 h. Every
218 concentration for each compound was replicated at least three times.

219 *Protective Effect of Compounds against TMV in Vivo*. The compound solution was
220 smeared on the left side and the solvent serving as control on the right side of growing *N.*
221 *tabacum* L. leaves of the same ages. The leaves were then inoculated with the virus after
222 12 h. A brush was dipped in TMV of 6×10^{-3} mg/mL to inoculate the leaves, which were
223 previously scattered with silicon carbide. The leaves were then washed with water and
224 rubbed softly along the nervature once or twice. The local lesion numbers appearing 3-4
225 days after inoculation were counted. There are three replicates for each compound.

226 *Inactivation Effect of Compounds against TMV in Vivo*. The virus was inhibited by
227 mixing with the compound solution at the same volume for 30min. The mixture was then
228 inoculated on the left side of the leaves of *N. tabacum* L., whereas the right side of the
229 leaves was inoculated with the mixture of solvent and the virus for control. The local
230 lesion numbers were recorded 3-4 days after inoculation. There are three replicates for
231 each compound.

Curative Effect of Compounds against TMV in Vivo. Growing leaves of *N. tabacum* L. of the same ages were selected. TMV (concentration of 6.0×10^{-3} mg/mL) was dipped and inoculated on the whole leaves. Then the leaves were washed with water and dried. The compound solution was smeared on the left side, and the solvent was smeared on the right side for control. The local lesion numbers were then counted and recorded 3-4 days after inoculation. There are three replicates for each compound. The in vitro and in vivo inhibition rates of the compound were then calculated according to the following formula ("av" means average, and controls were not treated with compound).

Inhibition rate (%) = [(av local lesion no. of control—av local lesion no. of drug-treated)/av local lesion no. of control] × 100%

RESULTS AND DISCUSSION

Chemistry.

Synthesis of the Compounds 1−17. Compounds **1**−**17** were synthesized according to procedures in **Figure 3**. Amines were reacted with corresponding isothiocyanate to give thioureas **1**−**8**, **13**−**17** in almost quantitatively yields. Compounds **9**−**12** were obtained from isothiocyanatobenzene and amino acids or their derivatives in the presence of NaOH in good yields.

Phytotoxic Activity. Compounds **1**−**22** were first tested for their phytotoxic activity against the test plant.[27] The results indicated that compounds **1**−**22** showed no phytotoxic activity at 500 μg/mL.

Antiviral Activity. The anti-TMV activity of compounds **1**−**22** was tested. The

commercial plant virucide Ningnanmycin, perhaps the most successful registered anti-plant viral agent, Ribavirin, nature product harmine and lead compound were used as the controls.

In Vitro Anti-TMV Activity. The in vitro antiviral assay results of the synthesized compounds were listed in Table 1. As the control, Ribavirin exhibited a 41% inhibitory effect at 500 μg/mL, whereas most of the synthesized compounds **1**−**22** exhibited higher antiviral activity than Ribavirin even at the concentration of 100 μg/mL. The optimal compound **20** exhibited significantly higher inhibitory effect than Ningnanmycin, harmine and lead compound emerging as a new lead compound for anti-TMV research. To investigate the influence of the hydrogen bond strength on the anti-TMV activity, compounds **1**, **4**, and **8** were tested. Thiourea **8** with the stronger hydrogen bond did show the best inhibitory effect (inhibitory effect: **8** > **1** ≈ **4**). For studying the influence of the steric effect, thioureas **4**−**7** were synthesized. Compounds **4**−**7** exhibited 45%, 56%, 32%, and 45% inhibitory effect at 500 μg/mL, respectively. Comparing the results of **4**−**7**, the anti-TMV activity was improved by increasing steric hindrance of the thiourea.

Compounds **9**−**12** were prepared from isothiocyanatobenzene and natural amino acids or their derivatives. Compound **11** showed the best in vitro anti-TMV activity among them with 44% inhibitory effect.

Bis-thiourea-type derivatives **13**−**17** displayed in vitro activity ranging from 37%−56% against TMV at 500 μg/mL. Amongst them, (*R*,*R*)-**16** bearing 1,2-diphenylethylenediamine skeleton showed higher activity than (*R*,*R*)-**17** containing

1,2-cyclohexanediamine(46% and 42%, respectively). Compound **13** afforded good result with inhibitory rate of 56%. All of primary amine thioureas **18**−**22** exhibited much higher activity than Ribavirin, harmine and lead compound. Compounds **20** exhibited best result with inhibitory rate of >70%. The optical pure compounds **16**, **18** and **20** were prepared to investigate the effect of configuration on antiviral activity. (*R*,*R*)-Configuration is confirmed to be the optimal in vitro antiviral configuration (inhibitory effect: (*R*,*R*)-**16** > (±)-**16** > (*S*,*S*)-**16**; (*R*,*R*)-**18** > (±)-**18** > (*S*,*S*)-**18**; (*R*,*R*)-**20** > (±)-**20**> (*S*,*S*)-**20**).

As shown in Table 1, most of the compounds **1**−**22** displayed good in vitro activity against TMV. Therefore, these compounds were bioassayed further to investigate their antiviral activity in vivo.

In Vivo Anti-TMV Activity. As shown in Table 2, most of the compounds also displayed higher in vivo activity than that of the Ribavirin. (*R*,*R*)-**20** showed the best result in vivo anti-TMV activity (inactivation activity: 71%/500 μg/mL and 35%/100 μg/mL; curative activity: 73%/500 μg/mL and 37%/100 μg/mL; and protection activity: 69%/500 μg/mL and 33%/100 μg/mL), which is significantly higher than that of Ribavirin (inactivation activity: 31%/500 μg/mL and 12%/100 μg/mL; curative activity: 39%/500 μg/mL and 18%/100 μg/mL; and protection activity: 47%/500 μg/mL and 17%/100 μg/mL) and Ningnanmycin (inactivation activity: 63%/500 μg/mL and 28%/100 μg/mL; curative activity: 54%/500 μg/mL and 22%/100 μg/mL; and protection activity: 64%/500 μg/mL and 26%/100 μg/mL).

Just as the antiviral activity in vitro, Compounds **1**−**8** displayed moderate to good in vivo activity, and the results disclosed that the anti-TMV activity related to the hydrogen bond strength and steric effect in molecules. Thiourea **8** bearing bis-3,5-Bis(trifluoromethyl)phenyl gave relatively higher activity (60%, 56%, and 58% at 500 μg/mL) than that of **1**−**7**.

Chiral thiourea derivatives **9**−**12** showed moderate antiviral activity ranging from 34%−49% against TMV at 500 μg/mL, and different activity patterns displayed different activity rule (inactivation activity: **9** > **11** > **10** > **12**; curative activity: **11** > **10**> **9** > **12**; protection activity: **11** > **10** > **12** > **9**).

The bis-thiourea derivatives showed relatively lower activity than corresponding single thiourea derivatives (in *vivo* activity: **19** > **13**; **20** > **14**; **18** > **16**) which indicated that the hydrogen bond is important but not the more the better. All of primary amine thioureas **18**−**22** exhibited much higher in vivo activity than Ribavirin, harmine and lead compound, especially for compounds **20**.

In summary, based on the structure of nature product harmine and lead compound, a series of simple thiourea derivatives were designed and synthesized from amines in one step. The antiviral activity of these compounds were evaluated in vitro and in vivo. Most of them exhibited significantly higher antiviral activity against TMV than commercial plant virucide Ribavirin. The optimal compound (*R*,*R*)-**20** showed excellent anti-TMV activity in vitro and in vivo (in vitro activity: 75%/500 μg/mL and 39%/100 μg/mL; inactivation activity: 71%/500 μg/mL and 35%/100 μg/mL; curative activity: 73%/500

μg/mL and 37%/100 μg/mL; and protection activity: 69%/500 μg/mL and 33%/100 μg/mL) which is significantly higher than that of Ribavirin, Ningnanmycin, harmine and lead compound. The hydrogen bond is favorable for antiviral activity within a certain range but not the more the better. Present study provides fundamental support for development and optimization of these simple thiourea derivatives as potential inhibitors of plant virus. Further studies on structural optimization and mode of action are currently underway in our laboratories.

ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **1**−**17** and **20**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors

*(A.L.) E-mail: luaidang@hebut.edu.cn; Phone: 0086-22-60302241 Ext 205; Fax: 0086-22-60204274

*(Z.W.) E-mail: wangziwen2725@163.com; Phone: 0086-22-23503792; Fax: 0086-22-23503792.

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

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Notes

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Figure Captions

Figure 1. Design of Thiourea Derivatives

Figure 2. Chemical Structures of **18**–**22**

Figure 3. Synthesis of Thiourea Derivatives **1**–**17**

Table 1. In Vitro Antiviral Activity of Compounds Ribavirin, Harmine, Ningnanmycin, Lead Compound and **1**−**22** against TMV

Compd	Concn (μg/mL)	Inhibition rate (%)[a]	compd	Concn (μg/mL)	Inhibition rate (%)[a]
1	500	45±1	(*S*,*S*)-**16**	500	36±1
	100	10±1		100	8±2
2	500	32±2	(±)-**16**	500	41±2
	100	10±2		100	16±1
3	500	18±1	(*R*,*R*)-**17**	500	42±2
	100	0		100	16±1
4	500	45±2	(*R*,*R*)-**18**	500	57±2
	100	5±1		100	29±1
5	500	56±2	(*S*,*S*)-**18**	500	51±1
	100	17±1		100	22±2
6	500	32±2	(±)-**18**	500	53±1
	100	9±1		100	25±2
7	500	45±1	(±)-**19**	500	57±1
	100	10±2		100	23±2
8	500	51±2	(*R*,*R*)-**20**	500	75±2
	100	32±2		100	39±2
9	500	36±1	(*S*,*S*)-**20**	500	70±1
	100	0		100	33±2
10	500	40±2	**(±)-20**	**500**	**73±2**
	100	6±1		**100**	**37±1**
11	500	44±2	(*R*,*R*)-**21**	500	44±2
	100	8±2		100	0
12	500	33±1	(*R*,*R*)-**22**	500	48±1
	100	0		100	13±1
13	500	56±2	**Lead**	500	50±2
	100	23±1	**compound**	100	21±1
14	500	37±1	**Harmine**	500	45±2
	100	15±1		100	20±1
15	500	40±1	**Ribavirin**	**500**	**41±2**
	100	8±1		**100**	**10±1**
(*R*,*R*)-**16**	500	46±2	**Ningnanmycin**	**500**	**61±1**
	100	18±2		**100**	**26±1**

[a]*Average of three replicates; All results are expressed as mean ± SD.*

Table 2. In Vivo Antiviral Activity of Compounds Ribavirin, Harmine, Ningnanmycin, Lead Compound and **1**−**22** against TMV

Compd	Concn (μg/mL)	Inactivation effect (%)[a]	Curative effect (%)[a]	Protection effect (%)[a]	Compd	Concn (μg/mL)	Inactivation effect (%)[a]	Curative effect (%)[a]	Protection effect (%)[a]
1	500	50±1	42±2	40±1	(*S*,*S*)-**16**	500	40±1	46±3	43±1
	100	18±3	13±1	15±1		100	19±1	20±1	13±1
2	500	36±1	43±3	40±1	(±)-**16**	500	46±2	48±1	49±2
	100	9±1	18±2	18±1		100	20±1	21±1	19±1
3	500	17±2	24±1	27±2	(*R*,*R*)-**17**	500	49±2	47±2	50±1
	100	0	0	0		100	16±2	18±2	19±1
4	500	49±1	41±2	42±1	(*R*,*R*)-**18**	500	**57**±1	**55**±1	**60**±1
	100	18±1	12±1	15±1		100	24±2	29±2	27±2
5	500	59±1	53±3	50±1	(*S*,*S*)-**18**	500	55±1	50±1	53±2
	100	20±1	28±1	24±1		100	19±2	25±2	21±2
6	500	38±2	43±1	35±2	(±)-**18**	500	56±2	53±2	58±1
	100	15±1	10±1	9±1		100	22±2	26±2	26±2
7	500	43±2	41±2	39±1	(±)-**19**	500	**61±2**	**63±1**	**61±2**
	100	18±1	11±1	16±1		100	27±1	32±2	26±1
8	500	**60**±1	**56**±1	**58**±1	(*R*,*R*)-**20**	500	**71±2**	**73±1**	**69±2**
	100	25±2	22±2	28±1		100	35±1	37±2	33±1
9	500	49±1	41±1	34±2	(*S*,*S*)-**20**	500	66±2	70±1	64±1
	100	12±1	17±1	11±1		100	29±1	30±2	31±2
10	500	46±2	47±2	42±1	**(±)-20**	500	69±2	70±1	68±2
	100	24±1	23±1	21±1		100	31±1	35±1	33±1
11	500	48±2	49±1	46±2	(*R*,*R*)-**21**	500	44±1	48±1	48±1
	100	13±1	20±2	15±1		100	19±2	18±2	20±1
12	500	41±2	36±1	37±2	(*R*,*R*)-**22**	500	45±1	50±1	48±2
	100	0	9±1	14±1		100	14±2	19±2	22±1
13	500	**58**±1	**61**±2	**60**±1	**Lead**	500	46±2	48±2	50±1
	100	26±1	30±1	24±1	**compound**	100	17±1	20±2	23±1
14	500	33±1	30±3	40±1	**Harmine**	500	41±1	39±1	42±2
	100	0	0	10±2		100	11±1	16±2	16±1
15	500	41±2	47±1	50±2	**Ribavirin**	500	31±2	39±2	47±1
	100	13±2	21±1	20±1		100	12±1	18±2	17±1
(*R*,*R*)-**16**	500	53±1	49±2	54±1	**Ningnanmy**	500	63±2	54±1	64±2
	100	25±1	23±1	20±1	**cin**	100	28±1	22±1	26±1

[a] *Average of three replicates; All results are expressed as mean ± SD.*

Figure 1.

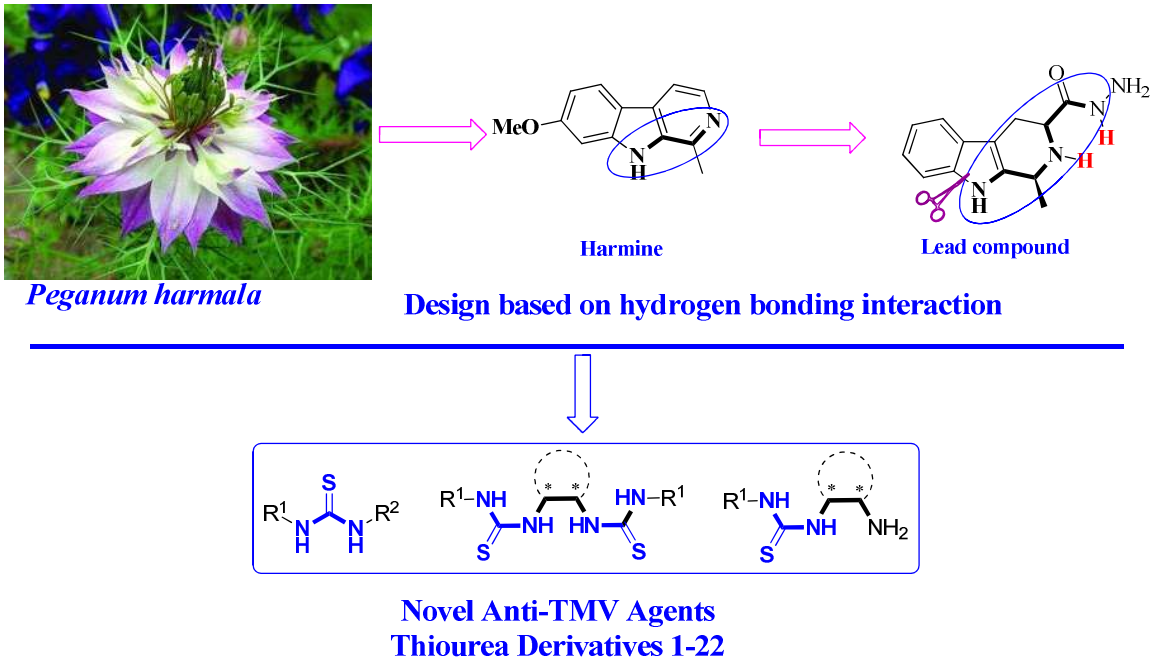

Peganum harmala
MeO
Harmine
Lead compound
Design based on hydrogen bonding interaction
Novel Anti-TMV Agents
Thiourea Derivatives 1-22

Figure 2.

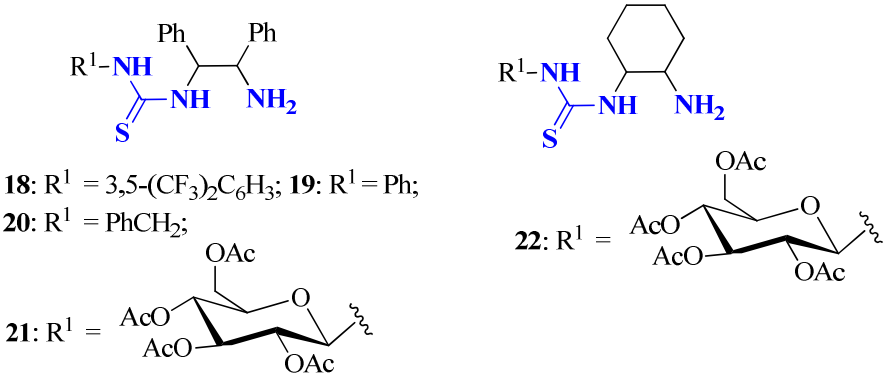
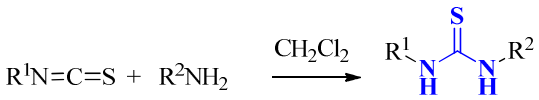
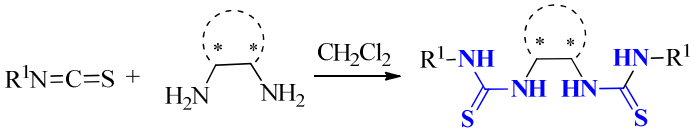

Ph
Ph
R1–NH
NH
NH2
S
18: R1 = 3,5-(CF3)2C6H3; 19: R1 = Ph;
20: R1 = PhCH2;
21: R1 =
OAc
AcO
AcO
O
OAc
22: R1 =
OAc
AcO
AcO
O
OAc

Figure 3.

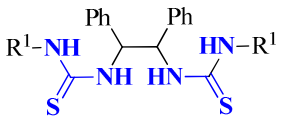


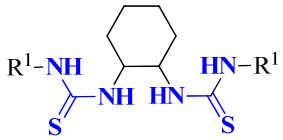
1-12

1: $R^1 = R^2 =$ Ph; **2**: $R^1 = R^2 = PhCH_2$; **3**: $R^1 = R^2 =$ cyclohexyl; **4**: $R^1 = 3,5\text{-}(CF_3)_2C_6H_3$, $R^2 =$ Ph;
5: $R^1 = 3,5\text{-}(CF_3)_2C_6H_3$, $R^2 =$ cyclohexyl; **6**: $R^1 = 3,5\text{-}(CF_3)_2C_6H_3$, $R^2 =$ *n*-butyl; **7**: $R^1 = 3,5\text{-}(CF_3)_2CH_3$, $R^2 =$ *t*-butyl;
8: $R^1 = R^2 = 3,5\text{-}(CF_3)_2C_6H_3$; **9**: $R^1 =$ Ph, $R^2 = (S)\text{-}PhCH_2CHCOOH$; **10**: $R^1 =$ Ph, $R^2 = (S)\text{-}CH_3CHCOOH$;
11: $R^1 =$ Ph, $R^2 = (S)\text{-}PhCH_2CHCOCH_2CH_3$; **12**: $R^1 =$ Ph, $R^2 = (S)\text{-}PhCH_2CHCOPh$;



13-17

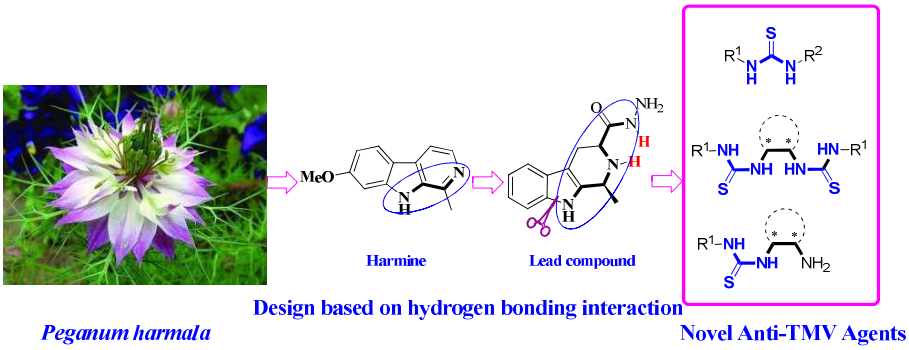




13: $R^1 =$ Ph; **14**: $R^1 = PhCH_2$;
15: $R^1 = Ph_2CH$; **16**: $R^1 = 3,5\text{-}(CF_3)_2C_6H_3$;

17: $R^1 = 3,5\text{-}(CF_3)_2C_6H_3$

TOC ***graphic***


MeO
Harmine
Lead compound
Design based on hydrogen bonding interaction
Peganum harmala
Novel Anti-TMV Agents