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Streptindole and Its Derivatives as Novel Antiviral and Anti-phytopathogenic-fungus Agents

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ABSTRACT: Plant diseases caused by plant viruses and pathogens seriously affect the production and storage of food crops. With the emergence of drug resistance, it is very difficult to control. Natural products are the source of new drug discovery. Here, natural product streptindole was found to have good antiviral activity against tobacco mosaic virus (TMV) and fungicidal activities against 14 kinds of phytopathogenic fungi. A series of derivatives of streptindole were designed, synthesized and evaluated for their antiviral and fungicidal activities. Compounds **4**, **5**, **11**, **12c**, **12d**, **13d** and **13i**–**13l** showed higher anti-TMV activities than ribavirin (inhibitory rate: 38%, 37%, 40% at 500 μg/mL for inactivation, curative, and protection activity *in vivo*, respectively), among which, compound **12d** (inhibitory rate: 57%, 55%, 53% at 500 μg/mL for inactivation, curative, and protection activity *in vivo*, respectively) with excellent antiviral activity was further evaluated mode of action. The mechanism research revealed that **12d** can break the three-dimensional structure of TMV coat protein (CP) through hydrogen bond, thus inhibiting the assembly of virus particles. Molecular docking result showed that compound **12d** did exhibit strong interaction with TMV CP. The derivatives of streptindole also displayed broad-spectrum fungicidal activities. Current study provided valuable insights into the antiviral and fungicidal activities of streptindole derivatives.

KEYWORDS: natural product, structure optimization, streptindole alkaloid, antiviral activity, fungicidal activity, mode of action

INTRODUCTION

Facing the continuous growth of global population, high efficiency, environmental protection and health will be the development trend of modern agriculture in the future.[1,2] Plant diseases caused by plant viruses and pathogens, known as plant cancer, are the main factors restricting the efficient production of agriculture.[3] Tobacco mosaic virus (TMV) is one of the earliest and most well-known viruses. It contains a single stranded RNA encapsulated in 2130 CP monomers. The particle of TMV is rod-shaped, with a total length of about 300 nm.[4] TMV is named for its first discovery on tobacco. Its host is very wide, and it can infect many plants including capsicum, cucumber, tomato, eggplant, solanum nigrum and so on, which brings great harm to agricultural production.[4] Although there are some commercial antiviral agents, efficient and practical varieties are few, the field control effect is mostly less than 60%. As a common antiviral agent, ribavirin can only give a inhibition rate of no more than 50% at 500 μg/mL. It is urgent to develop environment-friendly antiviral agents with simple structure and outstanding activity.[5,6]

Natural products play an important role in the process of new drug development because of their unique structures and wide range of biological activities, which alway offer a range of uncharted chemotypes for chemists to discover new drugs.[7-10] Through the unremitting efforts of scientists in recent years, some breakthroughs have been made in the development of new antiviral agents based on natural products. A series of natural products have been found to have antiviral activities, which may be developed into new

type of antiviral agents.[11-22] However, the new pesticide has the characteristics of long cycle, large investment and low success rate. It is necessary to continuously discover new drug candidates, so as to improve the success rate of creation. Streptindole, a genotoxic metabolite of human intestinal bacteria *Streptococcus faecium* IB 37, was isolated and determined structure by Osawa and Namiki in 1983.[23] Studies revealed that streptindole exhibited DNA-damaging and genotoxic properties.[24]

We have been committed to the development of new antiviral candidates based on indole natural products for a long time. Streptindole with simple structure has aroused our great interest. Considering the above findings and our work experiences, a series of streptindole derivatives were designed (Figure 1) and synthesized based on electronic effect, steric hindrance effect and pharmacophore splicing strategy. The activities, structure-activity relationship and mode of action 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 $CDCl_3$ or DMSO-d_6 as the solvent. High-resolution mass spectra were obtained with an FT-ICR mass spectrometer (Ionspec,

7.0 T). The *in vitro* TMV rod assembly inhibition and 20S CP disk assembly inhibition were tested via transmission electron microscopy (Tecnai G2 F20).

General Procedures for the Preparation of Compounds 3a–3c. To the stirred solution of corresponding indoles **1a**–**1c** (5 mmol) and ethyl glyoxylate **2** (0.26 g, 2.5 mmol) in acetonitrile (25 mL) was added I_2 (0.13 g, 0.5 mmol) at 0 °C. The reaction mixture was stirred for 0.5 h, quenched with 5 % $Na_2S_2O_3$ solution (20 mL) and extracted with ethyl acetate (50 mL × 3). The organic phase was washed with brine (100 mL), dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (5:1 PE/EA) to give **3a**–**3c**.

Ethyl 2,2-di(1*H*-indol-3-yl)acetate (3a). Brown oil, yield 91%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 2H, NH), 7.67 (d, J = 7.9 Hz, 2H, Ar-H), 7.30 (t, J = 8.1 Hz, 2H, Ar-H), 7.23−7.21 (m, 2H, Ar-H), 7.14−7.10 (m, 2H, Ar-H), 7.00 (d, J = 2.0 Hz, 2H, Ar-H), 5.53 (s, 1H, Ar_2-CH), 4.25 (q, J = 7.1 Hz, 2H, CH_2), 1.29 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 136.3, 126.7, 123.5, 122.1, 119.5, 119.3, 113.5, 111.3, 61.2, 40.7, 14.3.

Ethyl 2,2-bis(5-bromo-1*H*-indol-3-yl)acetate (3b). Brown oil, yield 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 2H, NH), 7.75 (d, J = 1.7 Hz, 2H, Ar-H), 7.27 (d, J = 1.8 Hz, 1H, Ar-H), 7.25 (d, J = 1.8 Hz, 1H, Ar-H), 7.17 (s, 1H, Ar-H), 7.15 (s, 1H, Ar-H), 7.00 (d, J = 2.3 Hz, 2H, Ar-H), 5.37 (s, 1H, Ar_2-CH), 4.26 (q, J = 7.1 Hz, 2H, CH_2), 1.31 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 135.1, 128.1, 125.1, 124.7, 121.8, 113.0, 112.9, 112.6, 61.7, 40.6, 14.2.

Ethyl 2,2-bis(6-bromo-1*H*-indol-3-yl)acetate (3c). Brown oil, yield 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 2H, NH), 7.46 (s, 2H, Ar-H), 7.44 (s, 1H, Ar-H), 7.20 (d, J = 1.4 Hz, 1H, Ar-H), 7.17 (d, J = 1.3 Hz, 1H, Ar-H), 6.98−7.07 (m, 3H, Ar-H), 5.42 (s, 1H, Ar_2-CH), 4.24 (q, J = 7.1 Hz, 2H, CH_2), 1.28 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 137.1, 125.4, 124.0, 122.9, 120.5, 115.7, 114.3, 113.4, 61.5, 40.5, 14.3.

Preparation of 2,2-di(1*H*-indol-3-yl)ethan-1-ol (4). At 0 °C, to the tetrahydrofuran (THF, 50 mL) was slowly added $LiAlH_4$ (0.57 g, 15 mmol) in the stirring state. After stirring for 10 min, ester **3a** (1.59 g, 5 mmol) was slowly added, then the reaction mixture was naturally rose to room temperature and stirred for further 3 h, quenched with water (10 mL). Diatomite was added into the reaction mixture to assist filtration, the filter cake was washed with ethyl acetate. The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (1:1 PE/EA) to give compound **4**: Yellow oil, yield 74%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 2H, NH), 7.60 (d, J = 7.9 Hz, 2H, Ar-H), 7.17−7.20 (m, 4H, Ar-H), 7.09 (t, J = 6.2 Hz, 2H, Ar-H), 6.72 (s, 2H, Ar-H), 4.72 (t, J = 5.9 Hz, 1H, Ar_2-CH), 4.20 (d, J = 5.8 Hz, 2H, CH_2), 2.32 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.5, 126.9, 122.9, 122.0, 119.4, 119.3, 115.9, 111.5, 85.7, 36.9. $C_{18}H_{17}N_2O$ $[M+H]^+$ 277.1335, found (ESI^+) 277.1331.

Preparation of streptindole (5). To a solution of **4** (1.38 g, 5 mmol) and Et_3N (0.76 g, 7.5 mmol) in CH_2Cl_2 (30 mL) was slowly added CH_3COCl (0.43 g, 5.5 mmol), then the

reaction mixture was naturally rose to room temperature and stirred for further 3 h, quenched with water (40 mL) and extracted with CH_2Cl_2 (30 mL × 2). The organic phase was washed with brine (100 mL), dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (2:1 PE/EA) to give streptindole (**5**): Brown oil, yield 58%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 2H, NH), 7.60 (d, J = 7.9 Hz, 2H, Ar-H), 7.28 (d, J = 8.1 Hz, 2H, Ar-H), 7.15 (t, J = 7.5 Hz, 2H, Ar-H), 7.05 (t, J = 7.6 Hz, 2H, Ar-H), 6.86 (d, J = 2.1 Hz, 2H, Ar-H), 4.92 (t, J = 7.1 Hz, 1H, Ar_2-CH), 4.71 (d, J = 7.1 Hz, 2H, CH_2), 1.95 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 136.5, 127.0, 122.2, 122.1, 119.5, 119.4, 116.2, 111.2, 67.5, 33.6, 21.2. $C_{20}H_{19}N_2O_2$ $[M+H]^+$ 319.1441, found (ESI^+) 319.1447.

Preparation of (*E*)-3-(2-nitrovinyl)-1*H*-indole (7). The mixture of indole formaldehyde (1.0 g, 6.9 mmol), ammonium acetate (0.53 g, 6.9 mmol), acetic acid (5.3 mL) and nitromethane (1.3 g, 21.3 mmol) was stirred at 120 °C for 4 h. After completion, most of the acetic acid was removed in vacuo, the residue was taken into H_2O (100 mL) and extracted with ethyl acetate (50 mL × 3). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (6:1 PE/EA) to give compound **7**: Red solid, yield 84%; mp 170−172 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H, NH), 8.29 (d, J = 13.5 Hz, 1H, NO_2CH), 7.80 (d, J = 13.5 Hz, 1H, Ar-CH), 7.76–7.80 (m, 1H, Ar-H), 7.67 (d, J = 2.9 Hz, 1H, Ar-H), 7.47–7.49 (m, 1H, Ar-H), 7.32–7.37 (m, 2H, Ar-H).

Preparation of 3,3'-(2-Nitroethane-1,1-diyl)bis(1*H*-indole) (8). To the stirred solution

of compound **7** (1.0 g, 5.32 mmol) in CH_2Cl_2 (100 mL) was slowly added indole (0.62 g, 5.32 mmol) and NBS (0.95 g, 5.32 mmol). The mixture was refluxed for 16 h, then cooled to room temperature and taken into H_2O (100 mL), extracted with CH_2Cl_2 (50 mL × 3). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (2:1 PE/EA) to give compound **8**: Red solid, yield 45%; mp 63−65 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 2H, NH), 7.62 (d, J = 7.9 Hz, 2H, Ar-H), 7.38 (d, J = 8.1 Hz, 2H, Ar-H), 7.23 (t, J = 7.8 Hz, 2H, Ar-H), 7.13 (t, J = 7.8 Hz, 2H, Ar-H), 7.05 (t, J = 2.3 Hz, 2H, Ar-H), 5.53 (t, J = 7.8 Hz, 1H, Ar-CH), 5.11 (d, J = 7.8 Hz, 2H, NO_2-CH_2).

Preparation of 2-(2,2-Di(1H-indol-3-yl)ethyl)isoindoline-1,3-dione (10). To the stirred solution of indole (0.59 g, 5 mmol) and aldehyde **9** (0.48 g, 2.5 mmol) in acetonitrile (25 mL) was added I_2 (0.13 g, 0.5 mmol) at 0 °C. The reaction mixture was stirred for 0.5 h, quenched with 5 % $Na_2S_2O_3$ solution (20 mL) and extracted with ethyl acetate (50 mL × 3). The organic phase was washed with brine (100 mL), dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (3:1 PE/EA) to give compound **10**: Pink solid, yield 96%; mp 210−211 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (s, 2H, NH), 7.75 (d, J = 8.0 Hz, 2H, Ar-H), 7.72 (dd, J = 3.2, 5.5 Hz, 2H, Ar-H), 7.61 (dd, J = 3.0, 5.4 Hz, 2H, Ar-H), 7.29 (d, J = 8.0 Hz, 2H, Ar-H), 7.13 (t, J = 7.2 Hz, 2H, Ar-H), 7.09 (d, J = 2.0 Hz, 2H, Ar-H), 7.03 (t, J = 7.5 Hz, 2H, Ar-H), 5.31 (t, J = 7.7 Hz, 1H, Ar_2-CH), 4.37 (d, J = 7.8 Hz, 2H, CH_2). $C_{26}H_{20}N_3O_2$ $[M+H]^+$ 406.1550, found (ESI^+) 406.1556.

Preparation of 2,2-Di(1*H*-indol-3-yl)ethan-1-amine (11). To the stirred solution of compound **10** (1 g, 2.5 mmol) in toluene (50 mL) was added 80% $NH_2NH_2 \cdot H_2O$ (0.78 g, 12.5 mmol). The mixture was refluxed for 20 h, then cooled to room temperature and evaporated under reduced pressure. The residue was taken into 2 mol/L NaOH solution (100 mL), extracted with CH_2Cl_2 (100 mL × 2). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give compound **11**: White solid, yield 91%; mp 164−165 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 2H, NH), 7.50 (d, J = 7.9 Hz, 2H, Ar-H), 7.30 (d, J = 8.1 Hz, 2H, Ar-H), 7.21 (s, 2H, Ar-H), 7.00 (t, J = 7.6 Hz, 2H, Ar-H), 6.87 (t, J = 7.6 Hz, 2H, Ar-H), 4.38 (t, J = 7.1 Hz, 1H, Ar_2-CH), 3.26 (d, J = 7.0 Hz, 2H, CH_2), 1.33 (s, 2H, NH_2). $C_{18}H_{18}N_3$ $[M+H]^+$ 276.1495, found (ESI^+) 276.1491.

General Procedures for the Preparation of Compounds 12a–12d. To a stirred solution of **11** (1 g, 3.64 mmol) and Et_3N (0.76 g, 7.5 mmol) in CH_2Cl_2 (80 mL) was added corresponding acyl chlorides (4 mmol) at 0 °C, then the reaction mixture was naturally rose to room temperature and stirred for further 3 h, quenched with water (40 mL) and extracted with CH_2Cl_2 (30 mL × 2). The organic phase was washed with brine (100 mL), dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (1:1 PE/EA) to give compounds **12a**–**12d**.

***N*-(2,2-Di(1*H*-indol-3-yl)ethyl)benzamide (12a).** Brown solid, yield 80%; mp 74−75 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H, NH), 8.30 (d, J = 7.8 Hz, 1H, NH), 7.84 (d, J

= 7.9 Hz, 1H, Ar-H), 7.78 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.69–7.63 (m, 2H, Ar-H), 7.57–7.51 (m, 4H, Ar-H), 7.47 (s, 1H, Ar-H), 7.39 (t, *J* = 7.3 Hz, 2H, Ar-H), 7.27–7.24 (m, 3H, Ar-H), 6.54 (t, *J* = 4.5 Hz, 1H, CH_2-*NH*), 5.07 (t, *J* = 6.9 Hz, 1H, Ar_2-CH), 4.45 (t, *J* = 6.3 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.8, 136.7, 133.7, 131.4, 130.2, 128.5, 126.9, 126.8, 122.3, 122.2, 119.6, 119.5, 116.7, 111.3, 44.2, 34.3. $C_{25}H_{22}N_3O$ $[M+H]^+$ 380.1757, found (ESI^+) 380.1751.

***N*-(2,2-Di(1*H*-indol-3-yl)ethyl)-4-methylbenzenesulfonamide (12b).** Brown solid, yield 90%; mp 108−109 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 2H, NH), 7.61 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.41–7.34 (m, 4H, Ar-H), 7.23–7.18 (m, 5H, Ar-H, CH_2-*NH*), 7.05–7.01 (m, 2H, Ar-H), 6.92 (d, *J* = 2.1 Hz, 2H, Ar-H), 4.66 (t, *J* = 6.7 Hz, 1H, Ar_2-CH), 3.69 (d, *J* = 6.7 Hz, 2H, CH_2), 2.43 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.4, 136.53, 136.45, 129.7, 127.1, 126.4, 122.8, 122.2, 119.5, 119.2, 115.6, 111.4, 46.6, 34.2, 21.6. $C_{25}H_{24}N_3O_2S$ $[M+H]^+$ 430.1584, found (ESI^+) 430.1588.

***N*-(2,2-Di(1*H*-indol-3-yl)ethyl)acetamide (12c).** Brown solid, yield 82%; mp 69−70 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21–8.15 (m, 2H, NH), 7.58 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.33 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.16 (t, *J* = 7.1 Hz, 2H, Ar-H), 7.04–6.95 (m, 4H, Ar-H), 5.59 (s, 1H, CH_2-*NH*), 4.71 (t, *J* = 6.8 Hz, 1H, Ar_2-CH), 4.01 (t, *J* = 6.4 Hz, 2H, CH_2), 1.85 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 136.6, 126.9, 122.1, 119.5, 119.4, 116.9, 111.3, 45.4, 34.3, 23.4. $C_{20}H_{20}N_3O$ $[M+H]^+$ 318.1601, found (ESI^+) 318.1607.

***N*-(2,2-Di(1*H*-indol-3-yl)ethyl)pivalamide (12d).** Brown solid, yield 87%; mp

125−126 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 2H, NH), 7.52 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.25 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.08 (t, *J* = 14.9 Hz, 2H, Ar-H), 6.95 (t, *J* = 14.8 Hz, 2H, Ar-H), 6.80 (s, 2H, Ar-H), 5.74 (t, *J* = 5.0 Hz, 1H, CH_2-*NH*), 4.65 (t, *J* = 7.1 Hz, 1H, Ar_2-CH), 3.92 (t, *J* = 6.5 Hz, 2H, CH_2), 0.97 (s, 9H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.8, 136.6, 126.9, 122.2, 122.0, 119.5, 119.3, 116.7, 111.4, 44.0, 34.1, 27.5. $C_{23}H_{26}N_3O$ $[M+H]^+$ 360.2070, found (ESI^+) 360.2075.

General Procedures for the Preparation of Compounds 13a–13l. To a stirred solution of **11** (1 g, 3.64 mmol) in CH_2Cl_2 (50 mL) was added corresponding isothiocyanates (3.64 mmol) at 0 ºC, then the reaction mixture was naturally rose to room temperature and stirred for further 3 h, quenched with water (40 mL) and extracted with CH_2Cl_2 (30 mL × 2). The organic phase was washed with brine (100 mL), dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (1:2 PE/EA) to give compounds **13a**–**13l**.

1-(2,2-Di(1*H*-indol-3-yl)ethyl)-3-phenylthiourea (13a). Brown solid, yield 70%; mp 214−215 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 2H, indole-NH), 7.64 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.36 (t, *J* = 8.4 Hz, 4H, Ar-H), 7.29 (d, *J* = 7.0 Hz, 1H, Ar-H), 7.18–7.24 (m, 3H, Ar-H), 6.99–7.08 (m, 5H, Ar-H), 6.63 (d, *J* = 7.8 Hz, 1H, CH_2-*NH*), 6.17 (s, 1H, CH_2NHCS-*NH*), 4.97 (t, *J* = 7.1 Hz, 1H, Ar_2-CH), 4.43 (t, *J* = 5.4 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.4, 139.6, 137.0, 130.4, 129.0, 127.2, 124.4, 123.2, 123.0, 121.4, 119.5, 118.7, 116.5, 111.9, 48.9, 33.5. $C_{25}H_{23}N_4S$ $[M+H]^+$ 411.1638, found (ESI^+) 411.1633.

1-(2,2-Di(1*H*-indol-3-yl)ethyl)-3-(*p*-tolyl)thiourea (13b). Yellow solid, yield 77%; mp 227−229 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 2H, indole-NH), 7.65 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.38 (d, *J* = 8.1 Hz, 4H, Ar-H), 7.16 (d, *J* = 3.4 Hz, 2H, Ar-H), 6.99 (d, *J* = 2.2 Hz, 2H, Ar-H), 6.84 (d, *J* = 8.2 Hz, 2H, Ar-H), 6.54 (d, *J* = 8.1 Hz, 2H, Ar-H), 6.20–6.25 (m, 1H, CH_2-*NH*), 6.12 (s, 1H, CH_2NHCS-*NH*), 4.97 (t, *J* = 7.2 Hz, 1H, Ar_2-CH), 4.41–4.44 (m, 2H, CH_2), 2.37 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.4, 137.0, 136.8, 133.7, 129.5, 127.2, 123.4, 123.1, 121.4, 119.5, 118.6, 116.6, 111.9, 48.9, 33.5, 20.9. $C_{26}H_{25}N_4S$ $[M+H]^+$ 425.1794, found (ESI^+) 425.1798.

1-(3-Chlorophenyl)-3-(2,2-di(1*H*-indol-3-yl)ethyl)thiourea (13c). Brown solid, yield 53%; mp 103−104 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 2H, indole-NH), 7.91 (s, 1H, Ar-H), 7.64 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.37 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.21 (t, *J* = 7.4 Hz, 2H, Ar-H), 7.07 (t, *J* = 7.3 Hz, 3H, Ar-H), 6.98 (d, *J* = 2.1 Hz, 2H, Ar-H), 6.94 (t, *J* = 8.0 Hz, 1H, Ar-H), 6.79 (s, 1H, Ar-H), 6.53 (d, *J* = 7.5 Hz, 1H, CH_2-*NH*), 6.20 (s, 1H, CH_2NHCS-*NH*), 4.96 (t, *J* = 7.0 Hz, 1H, Ar_2-CH), 4.43 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.9, 136.6, 130.7, 129.1, 128.3, 126.6, 124.4, 122.4, 122.33, 122.27, 119.65, 119.56, 116.3, 111.4, 49.5, 31.9. $C_{25}H_{22}ClN_4S$ $[M+H]^+$ 445.1248, found (ESI^+) 445.1257.

1-(2-Chlorophenyl)-3-(2,2-di(1*H*-indol-3-yl)ethyl)thiourea (13d). Brown solid, yield 25%; mp 62−63 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 2H, indole-NH), 7.62 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.34 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.28 (dd, *J* = 1.7, 7.9 Hz, 2H, Ar-H), 7.06–7.12 (m, 4H, Ar-H), 6.95 (d, *J* = 1.7 Hz, 2H, Ar-H), 6.71–6.80 (m, 4H, Ar-H,

232 CH_2-*NH*, CH_2NHCS-*NH*), 4.56 (t, *J* = 7.1 Hz, 1H, Ar_2-CH), 3.45 (d, *J* = 7.1 Hz, 2H,
233 CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.1, 142.9, 136.60, 136.55, 129.4, 127.8, 127.7,
234 122.2, 122.1, 119.6, 119.5, 119.2, 119.0, 115.9, 111.3, 37.1, 32.0. $C_{25}H_{22}ClN_4S$ $[M+H]^+$
235 445.1248, found (ESI^+) 445.1253.
236 **1-(2,2-Di(1*H*-indol-3-yl)ethyl)-3-(4-fluorophenyl)thiourea (13e).** Brown solid, yield
237 58%; mp 228−229 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 2H, indole-NH), 7.62 (d, *J*
238 = 7.9 Hz, 2H, Ar-H), 7.37 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.20 (t, *J* = 5.9 Hz, 2H, Ar-H), 7.06
239 (d, *J* = 7.7 Hz, 2H, Ar-H), 6.98 (s, 2H, Ar-H), 6.70 (t, *J* = 8.9 Hz, 2H, Ar-H), 6.58–6.61
240 (m, 2H, Ar-H), 6.28 (s, 1H, CH_2NHCS-*NH*), 5.93 (s, 1H, CH_2-*NH*), 4.95 (t, *J* = 7.5 Hz,
241 1H, Ar_2-CH), 4.39–4.42 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.5, 136.5,
242 127.3, 127.24, 127.16, 126.7, 124.5, 123.0, 122.4, 122.1, 119.7, 119.5, 116.5, 111.2, 37.1,
243 31.9. $C_{25}H_{22}FN_4S$ $[M+H]^+$ 429.1544, found (ESI^+) 429.1549.
244 **1-(3,5-Bis(trifluoromethyl)phenyl)-3-(2,2-di(1*H*-indol-3-yl)ethyl)thiourea (13f).**
245 Brown solid, yield 90%; mp 128−129 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 2H,
246 indole-NH), 7.54 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.52 (s, 1H, Ar-H), 7.39 (s, 1H, Ar-H), 7.26
247 (t, *J* = 8.3 Hz, 2H, Ar-H), 7.12–7.18 (m, 4H, Ar-H), 6.99 (t, *J* = 7.3 Hz, 2H, Ar-H), 6.85
248 (s, 2H, Ar-H, CH_2NHCS-*NH*), 6.18 (s, 1H, CH_2-*NH*), 4.86 (s, 1H, Ar_2-CH), 4.33 (s, 2H,
249 CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.4, 136.5, 129.1, 128.3, 126.5, 124.2, 123.6,
250 122.5, 122.4, 121.4, 119.7, 119.3, 111.5, 49.4, 29.7. $C_{27}H_{21}F_6N_4S$ $[M+H]^+$ 547.1386,
251 found (ESI^+) 547.1382.
252 **1-(2,2-Di(1H-indol-3-yl)ethyl)-3-(4-methoxyphenyl)thiourea (13g).** Brown solid, yield

66%; mp 265−266 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 2H, indole-NH), 7.65 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.39 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.20–7.23 (m, 3H, Ar-H), 7.06–7.10 (m, 2H, Ar-H), 7.01 (d, *J* = 2.1 Hz, 2H, Ar-H), 6.86 (t, *J* = 8.8 Hz, 1H, CH_2-*NH*), 6.58 (dd, *J* = 8.9 Hz, 4H, Ar-H), 5.95 (s, 1H, CH_2NHCS-NH), 4.97 (t, *J* = 7.3 Hz, 1H, Ar_2-CH), 4.42 (q, *J* = 5.8 Hz, 2H, CH_2), 3.75 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.5, 136.4, 126.7, 125.2, 122.6, 120.9, 118.9, 118.1, 116.01, 115.97, 113.8, 111.4, 55.1. $C_{26}H_{25}N_4OS$ $[M+H]^+$ 441.1744, found (ESI^+) 441.1741.

1-(2,2-Di(1*H*-indol-3-yl)ethyl)-3-(3,4-dimethoxyphenyl)thiourea (13h). Brown solid, yield 94%; mp 231−232 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 2H, indole-NH), 7.58 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.43 (s, 1H, Ar-H), 7.34 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.17 (t, *J* = 7.1 Hz, 2H, Ar-H), 7.03 (t, *J* = 7.4 Hz, 2H, Ar-H), 6.91 (d, *J* = 2.0 Hz, 2H, Ar-H), 6.75–6.82 (m, 1H, Ar-H), 6.48 (d, *J* = 8.2 Hz, 1H, Ar-H), 6.29 (s, 1H, CH_2NHCS-*NH*), 6.03–6.05 (m, 1H, CH_2-*NH*), 4.90 (t, *J* = 7.1 Hz, 1H, Ar_2-CH), 4.39 (t, *J* = 6.5 Hz, 2H, CH_2), 3.87 (s, 3H, CH_3), 3.81 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.6, 149.6, 148.2, 136.5, 126.7, 122.3, 122.1, 119.60, 119.56, 118.2, 116.6, 111.4, 111.1, 109.4, 56.0, 55.8, 49.3, 33.4. $C_{27}H_{27}N_4O_2S$ $[M+H]^+$ 471.1849, found (ESI^+) 471.1853.

1-Benzhydryl-3-(2,2-di(1*H*-indol-3-yl)ethyl)thiourea (13i). Brown solid, yield 39%; mp 89−90 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 2H, indole-NH), 7.54 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.30–7.39 (m, 7H, Ar-H), 7.10–7.20 (m, 4H, Ar-H), 7.04 (t, *J* = 7.6 Hz, 2H, Ar-H), 6.94–7.02 (m, 3H, Ar-H), 6.70 (s, 2H, Ar-H), 6.34 (s, 1H, CH_2NHCS-*NH*), 5.98 (s, 1H, CH_2-*NH*), 5.63 (s, 1H, Ph_2-CH), 4.73 (t, *J* = 6.9 Hz, 1H, Ar_2-CH), 4.28 (s,

2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.1, 139.2, 136.5, 129.0, 128.94, 128.86, 128.3, 128.0, 127.1, 126.6, 122.2, 119.5, 111.3, 64.6, 33.6, 32.0. $C_{32}H_{29}N_4S$ $[M+H]^+$ 501.2107, found (ESI^+) 501.2111.

1-Benzyl-3-(2,2-di(1*H*-indol-3-yl)ethyl)thiourea (13j). Brown solid, yield 69%; mp 93−94 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 2H, indole-NH), 7.56 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.29–7.43 (m, 5H, Ar-H), 7.16–7.21 (m, 4H, Ar-H), 7.02–7.06 (m, 2H, Ar-H), 6.75 (d, *J* = 2.0 Hz, 2H, Ar-H), 6.02 (s, 1H, CH_2NHCS-NH), 5.75 (s, 1H, CH_2NH), 4.76 (t, *J* = 6.7 Hz, 1H, Ar_2-CH), 4.73 (s, 2H, Ph-*CH_2*), 4.13–4.18 (m, 2H, Ar_2CH-*CH_2*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.6, 135.5, 133.2, 127.9, 127.7, 127.6, 127.4, 126.7, 126.4, 125.8, 121.3, 121.2, 118.5, 110.3, 47.6, 32.7, 28.7. $C_{26}H_{25}N_4S$ $[M+H]^+$ 425.1794, found (ESI^+) 425.1799.

1-Cyclohexyl-3-(2,2-di(1*H*-indol-3-yl)ethyl)thiourea (13k). Brown solid, yield 83%; mp 86−87 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 2H, indole-NH), 7.60 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.36 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.20 (t, *J* = 7.0 Hz, 4H, Ar-H), 7.08 (d, *J* = 7.7 Hz, 2H, Ar-H), 6.93 (s, 1H, CH_2NHCS-*NH*), 5.72 (s, 1H, CH_2-*NH*), 4.84 (s, 1H, Ar_2-CH), 4.14–4.23 (m, 2H, Ar_2CH-*CH_2*), 3.71 (t, *J* = 3.5 Hz, 1H, CSNH-*CH*), 1.40–1.92 (m, 10H, $(CH_2)_5$); ^{13}C NMR (100 MHz, DMSO-*d_6*) δ 181.8, 137.0, 127.3, 123.0, 121.3, 119.6, 118.6, 116.8, 111.8, 52.1, 48.7, 34.1, 32.8, 25.7, 24.9. $C_{25}H_{29}N_4S$ $[M+H]^+$ 417.2107, found (ESI^+) 417.2101.

1-Butyl-3-(2,2-di(1*H*-indol-3-yl)ethyl)thiourea (13l). Brown solid, yield 72%; mp 85−86 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 2H, indole-NH), 7.58 (d, *J* = 7.8 Hz,

2H, Ar-H), 7.33 (d, J = 8.1 Hz, 2H, Ar-H), 7.17 (t, J = 7.4 Hz, 2H, Ar-H), 7.04 (d, J = 7.3 Hz, 2H, Ar-H), 6.89 (s, 2H, Ar-H), 5.70 (s, 1H, CH_2NHCS-*NH*), 4.82 (s, 1H, CH_2-*NH*), 4.19 (s, 1H, Ar_2CH-*CH_2*), 3.51 (t, J = 6.6 Hz, 1H, Ar_2-CH), 3.00 (s, 2H, CSNH-*CH_2*), 0.78–1.71 (m, 8H, Ar_2CH-*CH_2*, $NHCH_2$-*$(CH_2)_2CH_3$*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.1, 136.5, 126.7, 122.4, 122.2, 119.5, 119.4, 116.3, 111.4, 33.7, 29.7, 22.7, 19.9, 14.2, 13.7. $C_{23}H_{27}N_4S$ $[M+H]^+$ 391.1951, found (ESI^+) 391.1956.

Preparation of 2,2-Di(1*H*-indol-3-yl)acetohydrazide (14). To the stirred solution of compound **3a** (1.59 g, 5 mmol) in ethanol (25 mL) was added 80% $NH_2NH_2 \cdot H_2O$ (25 mL). The mixture was refluxed for 3 h, then cooled to room temperature and evaporated under reduced pressure to remove ethanol. The resulting precipitate was collected by filtration in vacuo and was washed with H_2O to provide compound **14**: White solid, yield 83%; mp 234−235 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.87 (s, 2H, NH), 9.42 (s, 1H, NH_2-*NH*), 7.57 (d, J = 7.9 Hz, 2H, Ar-H), 7.34 (d, J = 8.1 Hz, 2H, Ar-H), 7.17 (d, J = 2.2 Hz, 2H, Ar-H), 7.07−7.03 (m, 2H, Ar-H), 6.96−6.92 (m, 2H, Ar-H), 5.26 (s, 1H, Ar_2-CH), 4.26 (s, 2H, NH-*NH_2*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 136.6, 127.1, 124.1, 121.3, 119.4, 118.6, 114.3, 111.8, 39.5.

General Procedures for the Preparation of Compounds 15a–15h. To a solution of **14** (1.52 g, 5 mmol) in ethanol (125 mL) was added corresponding aldehydes (5.5 mmol). The mixture was reluxed for 7 h, then cooled to room temperature and evaporated under reduced pressure to remove part of ethanol. The resulting precipitate was collected by filtration in vacuo and was washed with H_2O to provide compounds **15a**–**15h**.

316 ***N*'-Benzylidene-2,2-di(1*H*-indol-3-yl)acetohydrazide (15a).** Yellow solid, yield 87%;
317 mp 295−296 °C; *E*: *Z* = 5: 4, ^{1}H NMR (DMSO-d_6, 400 MHz): 11.86 (for *E* isomer, s,
318 0.53H, CO-*NH*), 11.40 (for *Z* isomer, s, 0.41H, CO-*NH*), 10.96 (s, 1.06H, NH), 10.92 (s,
319 0.84H, NH), 8.28 (for *E* isomer, s, 0.52H, CONHN-*CH*), 8.03 (for *Z* isomer, s, 0.41H,
320 CONHN-*CH*), 7.74 (d, *J* = 7.2 Hz, 0.90H, Ar-H), 7.63−7.69 (m, 3H, Ar-H), 7.36−7.45
321 (m, 5H, Ar-H), 7.26 (d, *J* = 1.8 Hz, 0.89H, Ar-H), 7.23 (d, *J* = 1.8 Hz, 1.11H, Ar-H),
322 7.05−7.10 (m, 2H, Ar-H), 6.94−7.00 (m, 2H, Ar-H), 6.57 (for *Z* isomer, s, 0.45H,
323 Ar_2-CH), 5.46 (for *E* isomer, s, 0.55H, Ar_2-CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.9,
324 168.8, 146.5, 142.5, 136.23, 136.17, 134.4, 134.3, 129.8, 129.6, 128.8, 128.6, 126.9,
325 126.7, 126.5, 124.0, 123.8, 120.9, 120.8, 118.85, 118.79, 118.3, 113.3, 113.2, 111.43,
326 111.40, 35.1. $C_{25}H_{21}N_4O$ $[M+H]^+$ 393.1710, found (ESI^+) 393.1706.
327 ***N*'-(4-Fluorobenzylidene)-2,2-di(1*H*-indol-3-yl)acetohydrazide (15b).** Brown solid,
328 yield 74%; mp 176−177 °C; *E*: *Z* = 3: 2, ^{1}H NMR (400 MHz, DMSO-d_6): 11.87 (for *E*
329 isomer, s, 0.61H, CO-*NH*), 11.38 (for Z isomer, s, 0.4H, CO-*NH*), 10.94 (s, 1H, NH),
330 10.91 (s, 1H, NH), 8.28 (s, 0.6H, CONHN-*CH*), 8.00 (s, 0.41H, CONHN-*CH*),
331 7.97−7.94 (m, 1H, Ar-H), 7.82−7.71 (m, 2H, Ar-H), 7.61 (s, 1H, Ar-H), 7.59 (s, 1H,
332 Ar-H), 7.38−7.20 (m, 5H, Ar-H), 7.08−6.92 (m, 4H, Ar-H), 6.52 (for *Z* isomer, s, 0.4H,
333 Ar_2-CH), 5.43 (for *E* isomer, s, 0.59H, Ar_2-CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.7,
334 160.3, 145.3, 141.3, 136.2, 136.1, 130.65, 130.56, 129.1, 129.0, 126.5, 124.0, 123.8,
335 120.9, 120.8, 118.82, 118.77, 118.3, 118.2, 115.84, 115.79, 113.2, 111.4, 35.1.
336 $C_{25}H_{20}FN_4O$ $[M+H]^+$ 411.1616, found (ESI^+) 411.1622.

2,2-Di(1*H*-indol-3-yl)-*N*'-(4-(trifluoromethyl)benzylidene)acetohydrazide (15c). White solid, yield 59%; mp 269−270 °C; *E*: *Z* = 5: 4, ^{1}H NMR (DMSO-d_6, 400 MHz): 12.02 (for *E* isomer, s, 0.53H, CO-*NH*), 11.57 (for *Z* isomer, s, 0.42H, CO-*NH*), 10.96 (s, 1.07H, NH), 10.92 (s, 0.85H, NH), 8.35 (s, 0.53H, CONHN-*CH*), 8.08 (s, 0.42H, CONHN-*CH*), 7.96 (d, *J* = 8.1 Hz, 0.87H, Ar-H), 7.90 (d, *J* = 8.2 Hz, 1.08H, Ar-H), 7.78−7.80 (m, 2H, Ar-H), 7.59−7.62 (m, 2H, Ar-H), 7.36 (t, *J* = 7.7 Hz, 2H, Ar-H), 7.26 (s, 0.85H, Ar-H), 7.22 (s, 1.06H, Ar-H), 7.04−7.09 (m, 2H, Ar-H), 6.93−6.99 (m, 2H, Ar-H), 6.54 (s, 0.42H, Ar_2-CH), 5.46 (s, 0.53H, Ar_2-CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.1, 169.0, 144.7, 140.7, 138.3, 136.22, 136.16, 127.5, 127.2, 126.6, 126.5, 125.6, 125.5, 124.0, 123.8, 120.9, 120.8, 118.8, 118.32, 118.27, 113.2, 113.0, 111.4, 35.2. $C_{26}H_{20}F_3N_4O$ $[M+H]^+$ 461.1584, found (ESI^+) 461.1588.

2,2-Di(1*H*-indol-3-yl)-*N*'-(3,4,5-trimethoxybenzylidene)acetohydrazide (15d). Pink solid, yield 88%; mp 265−266 °C; *E*: *Z* = 1: 1, ^{1}H NMR (DMSO-d_6, 400 MHz): 11.81 (for *E* isomer, s, 0.52H, CO-*NH*), 11.42 (for Z isomer, s, 0.48H, CO-*NH*), 10.92 (d, *J* = 12.7 Hz, 2H, NH), 8.20 (s, 0.50H, CONHN-*CH*), 7.91 (s, 0.47H, CONHN-*CH*), 7.37−7.34 (m, 2H, Ar-H), 7.23−7.20 (m, 2H, Ar-H), 7.08−7.03 (m, 2H, Ar-H), 6.99−6.91 (m, 4H, Ar-H), 6.51 (for *Z* isomer, s, 0.48H, Ar_2-CH), 5.42 (for *E* isomer, s, 0.51H, Ar_2-CH), 3.80 (s, 6H, OCH_3), 3.68 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.0, 168.7, 153.1, 146.6, 142.0, 136.2, 136.1, 129.9, 129.8, 126.7, 126.5, 123.9, 123.8, 120.94, 120.86, 118.9, 118.6, 118.3, 113.5, 113.2, 111.4, 105.6, 104.3, 103.8, 60.1, 55.9, 35.2. $C_{28}H_{27}N_4O_4$ $[M+H]^+$ 483.2027, found (ESI^+) 483.2021.

358 ***N*'-(Furan-2-ylmethylene)-2,2-di(1*H*-indol-3-yl)acetohydrazide (15e).** Yellow solid,
359 yield 49%; mp 285−286 °C; *E*: *Z* = 5: 3, ^{1}H NMR (DMSO-d_6, 400 MHz): 11.81 (for *E*
360 isomer, s, 0.57H, CO-*NH*), 11.37 (for *Z* isomer, s, 0.35H, CO-*NH*), 10.97 (d, *J* = 1.9 Hz,
361 1.24H, NH), 10.94 (d, *J* = 1.7 Hz, 0.68H, NH), 8.20 (s, 0.58H, CONHN-*CH*), 7.96 (s,
362 0.36H, CONHN-*CH*), 7.87 (d, *J* = 1.2 Hz, 0.34H, Ar-H), 7.81 (d, *J* = 1.2 Hz, 0.59H,
363 Ar-H), 7.61 (d, *J* = 7.9 Hz, 0.98H, Ar-H), 7.60 (d, *J* = 7.9 Hz, 0.93H, Ar-H), 7.34−7.44
364 (m, 3H, Ar-H), 7.26 (d, *J* = 2.1 Hz, 0.79H, Ar-H), 7.22 (d, *J* = 2.1 Hz, 1.22H, Ar-H),
365 7.05−7.11 (m, 2H, Ar-H), 6.96−7.01 (m, 2H, Ar-H), 6.92 (d, *J* = 3.3 Hz, 0.37H, Ar-H),
366 6.88(d, *J* = 3.3 Hz, 0.58H, Ar-H), 6.64 (q, *J* = 1.8 Hz, 0.36H, Ar-H), 6.61 (q, *J* = 1.8 Hz,
367 0.64H, Ar-H), 6.52 (s, 0.34H, Ar_2-CH), 5.43 (s, 0.54H, Ar_2-CH); ^{13}C NMR (100 MHz,
368 DMSO-d_6) δ 173.9, 168.8, 149.4, 149.3, 145.0, 144.8, 136.4, 136.2, 136.1, 132.7, 126.7,
369 126.5, 124.0, 123.8, 121.0, 120.9, 118.8, 118.4, 118.3, 113.3, 113.2, 113.1, 112.1, 111.5,
370 111.4, 34.8. $C_{23}H_{19}N_4O_2$ $[M+H]^+$ 383.1503, found (ESI^+) 383.1509.
371 **2,2-Di(1*H*-indol-3-yl)-*N*'-(thiophen-2-ylmethylene)acetohydrazide (15f).** Yellow solid,
372 yield 53%; mp 297−298 °C; *E*: *Z* = 1: 1, ^{1}H NMR (DMSO-d_6, 400 MHz): 11.80 (for *E*
373 isomer, s, 0.59H, CO-*NH*), 11.39 (for Z isomer, s, 0.41H, CO-*NH*), 10.96 (s, 2H, NH),
374 8.51 (s, 0.6H, CONHN-*CH*), 8.22 (s, 0.4H, CONHN-*CH*), 7.66−7.60 (m, 3H, Ar-H),
375 7.43−7.36 (m, 3H, Ar-H), 7.26−7.21 (m, 2H, Ar-H), 7.14−7.05 (m, 3H, Ar-H), 7.00−6.94
376 (m, 2H, Ar-H), 6.50 (for *Z* isomer, s, 0.41H, Ar_2-CH), 5.41 (for *E* isomer, s, 0.59H,
377 Ar_2-CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.7, 168.6, 141.7, 139.4, 139.1, 137.4,
378 136.2, 136.1, 130.4, 129.7, 128.5, 128.0, 127.8, 127.6, 126.7, 126.5, 124.0, 123.8, 120.9,

120.8, 118.8, 118.7, 118.3, 113.2, 113.1, 111.4, 34.6. $C_{23}H_{19}N_4OS$ $[M+H]^+$ 399.1274, found (ESI^+) 399.1277.

2,2-Di(1*H*-indol-3-yl)-*N*'-octylideneacetohydrazide (15g). White solid, yield 41%; mp 187−188 °C; *E*: *Z* = 3: 2, ^1H NMR (DMSO-d_6, 400 MHz): 11.36 (for *E* isomer, s, 0.58H, CO-*NH*), 10.88 (for Z isomer, s, 0.38H, CO-*NH*), 10.91 (d, *J* = 7.1 Hz, 2H, NH), 7.56−7.50 (m, 3H, Ar-H), 7.34 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.16−7.15 (m, 2H, Ar-H), 7.07−7.02 (m, 2H, Ar-H), 6.96−6.90 (m, 2H, Ar-H), 6.40 (for *Z* isomer, s, 0.38H, Ar_2-CH), 5.29 (for *E* isomer, s, 0.59H, Ar_2-CH), 2.27−2.15 (m, 2H, CONHNCH-CH_2), 1.52−1.41 (m, 2H, $CONHNCHCH_2$-CH_2), 1.35−1.20 (m, 8H, $CONHNCH(CH_2)_2$-$(CH_2)_4$), 0.84 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.4, 168.2, 150.7, 146.5, 136.2, 136.1, 126.7, 126.5, 123.8, 123.7, 120.8, 120.7, 118.8, 118.7, 118.2, 118.1, 113.5, 113.4, 111.4, 111.3, 34.5, 31.9, 31.2, 31.1, 28.6, 28.5, 28.4, 26.0, 25.7, 22.0, 13.9. $C_{26}H_{31}N_4O$ $[M+H]^+$ 415.2492, found (ESI^+) 415.2487.

***N*'-Butylidene-2,2-di(1*H*-indol-3-yl)acetohydrazide (15h).** Yellow solid, yield 33%; mp 198−199 °C; *E*: *Z* = 7: 4, ^1H NMR (DMSO-d_6, 400 MHz): 11.37 (for *E* isomer, s, 0.63H, CO-*NH*), 10.92 (for *Z* isomer, s, 0.39H, CO-*NH*), 10.90 (for *E* isomer, s, 1.21H, NH), 10.87 (for *Z* isomer, s, 0.80H, NH), 7.50−7.56 (m, 2.79H, Ar-H), 7.32−7.35 (m, 2.51H, Ar-H), 7.15−7.17 (m, 2H, Ar-H), 7.02−7.07 (m, 2H, Ar-H), 6.90−6.96 (m, 2H, Ar-H), 6.39 (for *Z* isomer, s, 0.38H, Ar_2-CH), 5.29 (for *E* isomer, s, 0.65H, Ar_2-CH), 2.14−2.24 (m, 2H, CONHNCH-CH_2), 1.43−1.55 (m, 2H, $CONHNCHCH_2$-CH_2), 0.86−0.93 (m, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.4, 168.2, 150.6, 146.5,

136.15, 136.09, 126.7, 126.5, 123.9, 123.7, 120.9, 120.8, 118.81, 118.76, 118.3, 118.2, 113.5, 113.4, 111.4, 111.3, 34.6, 33.8, 33.7, 19.3, 19.1, 13.6, 13.5. $C_{22}H_{23}N_4O$ $[M+H]^+$ 359.1866, found (ESI^+) 359.1860.

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

The tests of anti-TMV activity and mode of action[20] and fungicidal activity[25] were carried out using reported methods, which also can be seen in Supporting Information.

RESULTS AND DISCUSSION

Chemistry.

We first constructed the diindole framework by the reaction of indole with ethyl glyoxylate under the catalysis of I_2 (Figure 2). Compound **3a** was reduced to alcohol **4** by $LiAlH_4$, and then the natural product streptindole (**5**) was obtained by the reaction of alcohol **4** with acetyl chloride (Figure 3). In order to investigate the influence of the electronic effects on the biological activity, compound **8** with hydroxyl group of **4** changed into nitro group and compound **11** with hydroxyl group of **4** changed into amino group were designed and synthesized (Figures 4 and 5). Nitroalkene **7** was obtained by the reaction of indole formaldehyde with nitromethane, and compound **8** was obtained by the addition of indole with **7**. As the nitro group had a great influence on the reaction, the yield of **8** could not be improved. The plan of preparing compound **11** by reducing compound **8** was cancelled. We switched to Gabriel method to realize the efficient preparation of compound **11**. In order to investigate the effect of electronic effect,

421 aromaticity and steric hindrance of amino region on biological activity, we designed and
422 synthesized compounds **12a**−**12d** (Figure 5). Thiourea is a kind of pharmacophore
423 containing hydrogen bond donor and acceptor. Lu research group has done a very good
424 job in this respect. Based on the function of hydrogen bond, they designed and
425 synthesized a series of thiourea derivatives, and found that these compounds have very
426 good antiviral activities.[26,27] Inspired by the above work, we designed and synthesized
427 thiourea derivatives **13a**−**13l** by addition of compound **11** with isothiocyanate (Figure 6).
428 Acylhydrazone is a kind of good pharmacophore. In the previous work, we found that the
429 introduction of acylhydrazone can improve the antiviral activity of compounds. Under
430 the guidance of previous work experience, we designed and synthesized a series of
431 acylhydrazone derivatives **15a**−**15h** via the reaction of **14** with corresponding aldehydes
432 (Figure 7). The analysis of the ^{1}H NMR spectra revealed that compounds **15a**−**15h** exist
433 as Z/E isomers, and the proportion of isomers can be determined according to the
434 literature methods.[28,29]

435 **Phytotoxic Activity.**

436 The phytotoxic-activity tests (according to the criterion of safety evaluation of
437 pesticide to crops: NYT 1965.1-2010) revealed that compounds **3a**−**3c**, **4**, **5**, **8**, **10**, **11**,
438 **12a**−**12d**, **13a**−**13l**, **14** and **15a**−**15h** were safe for testing on plants at 500 μg/mL.[21]

439 **Antiviral Activity *in vivo*.** The activities of compounds **3a**−**3c**, **4**, **5**, **8**, **10**, **11**,
440 **12a**−**12d**, **13a**−**13l**, **14** and **15a**−**15h** against TMV are listed in Table 1 with commercial
441 viral inhibitor ribavirin as control. The inactive activity can best reflect the interaction

between compounds and TMV. We first tested the inactive activities of all compounds, and further tested the curative activities and protective activities of the compounds with good inactive activities (inactive effect > 40%).

As can be seen in table 1, most of the streptindole derivatives showed good anti-TMV activities, compounds **4**, **5**, **11**, **12c**, **12d**, **13d** and **13i–13l** displayed higher activities than ribavirin at 500 μg/mL, especially for compound **12d**. Compounds **3a**−**3c** displayed about similar antiviral activities, which indicated that the substitution effect on indole ring is not obvious, and the compound **3a** without substituents on indole ring has better activity. The antiviral activity is obviously improved after reduction of ester group to alcohol (inhibitory effect: **4** > **3a**). Natural product streptindole (**5**) showed 44% inactive effect, which is about similar to compound **4**. The antiviral activity decreased significantly when the electron deficient nitro group or *o*-phthalimide group replaced the acetoxy group of streptindole (inhibitory effect: **5** > **8** > **10**). Compound **11** showed slight higher activity than **4**, which revealed that it is feasible to replace hydroxyl with amino group. The main differences of compounds **12a**−**12d** are the substituents on the amino group. The introduction of benzoyl group or *p*-phenylsulfonyl group on amino group is not conducive to the antiviral activity (inhibitory effect: **11** > **12a** > **12b**). However, the introduction of fatty acyl group is beneficial to the improvement of activity and the larger steric hindrance is better (inhibitory effect: **12d** > **12c** > **11**). The introduction of thiourea functional groups did not significantly improve the activities of these compounds, compounds **13d** and **13j**−**13l** displayed similar activities as compound **11**, while the other

463 compounds showed lower activities. Conversion of ester group to hydrazide is beneficial
464 to the improvement of antiviral activity (inhibitory effect: **14** > **3a**). However, further
465 conversion of hydrazide to hydrazone did not improve the biological activity. Only
466 compound **15a** containing benzene ring and compound **15d** containing trimethoxy
467 substituted benzene ring maintain antiviral activity at the same level as compound **14**.
468 The above structure-activity relationship revealed that the ethyl in the structure of
469 streptindole (**5**) is very important to maintain the biological activity. The conversion of
470 ester group of streptindole (**5**) to fatty amide is beneficial to the improvement of activity.
471 Compound **12d** with excellent antiviral activity emerged as new antiviral candidate.

472 **Preliminary Mode of Action**.

473 Compound **12d** with excellent antiviral activity was further evaluated the mode of action
474 using our reported method.[20,21] As shown in Figure 8, 20S CP and TMV RNA can be
475 assembled into TMV particles with a total length of 300 nm, and DMSO had no effect on
476 the assembly. Compound **12d** can inhibit the assembly of the virus. No full-length virus
477 particles can be found in Figure 8C, most of them become fusion aggregates. Further
478 interaction test on the 20S CP disk and **12d** revealed that TMV CP can be incubated into
479 20S CP disk (Figure 9A), DMSO had no effect on the incubation of 20S CP disk (Figure
480 9B). Compound **12d** has strong interaction with 20S CP disk, which leads to the fusion
481 of 20S CP disk into aggregate (Figure 9C). These results suggest that compound **12d**
482 may act on the amino acid residues of TMV CP through hydrogen bonds, thus destroying
483 their three-dimensional structures.

Molecular Docking. To further confirm the interaction between these compounds and TMV CP (PDB code: 1EI7), we chose compounds **5** and **12d** for molecular docking via AutoDock-vina 1.1.2.[30] Compound **5** forms two conventional hydrogen bonds with the active site of ARG 261 at a distance of 2.4 Å and ASN 73 at a distance of 2.3 Å (Figure 10A). Compound **12d** forms three conventional hydrogen bonds with amino acids SER 138 (2.5 Å), GLY 135 (2.5 Å) and TYR 139 (2.7 Å). The above results further confirmed the mode of action of these compounds, and pointed out the direction for the later target determination.

Fungicidal Activity.

Plant fungal diseases are also persistent diseases that seriously affect plant growth.[3] We also evaluated the fungicidal activities of compounds **3a**−**3c**, **4**, **5**, **8**, **10**, **11**, **12a**−**12d**, **13a**−**13l**, **14** and **15a**−**15h** against 14 kinds of phytopathogenic fungi at 50 μg/mL with commercial fungicides carbendazim and chlorothalonil as controls.

As shown in Table 2, streptindole and its derivatives exhibited broad-spectrum fungicidal activities. Compounds **4** and **5** displayed higher fungicidal activities against *Alternaria solani* than carbendazim and chlorothalonil. The inhibitory effects of **3b**, **8**, **13a** and **15a** are better than that of chlorothalonil and carbendazim against *Phytophthora capsici*. The activities of compounds **3a**, **3b**, **4**, **5**, **12a** and **13i** are higher than that of chlorothalonil and carbendazim against *Botrytis cinereal*. These compounds have different structure-activity relationships on different plant fungi. Take *Botrytis cinereal* as an example: Compounds **3a**−**3c**, **4**, **5** and **8** shown good fungicidal activities; The fungicidal

activity decreased significantly when the *o*-phthalimide group or amino group replaced the acetoxy group of streptindole; Among amide substituted derivatives, benzamide compound **12a** showed higher fungicidal activity than the others; For thiourea derivatives, the diphenyl derivative **13i** displayed higher fungicidal activity; The fungicidal activities of hydrazone derivatives are not very prominent.

In summary, natural product streptindole and its derivatives were synthesized and evaluated for their antiviral activities and fungicidal activities. Compounds **4**, **5**, **11**, **12c**, **12d**, **13d** and **13i**–**13l** displayed higher antiviral activities than ribavirin. A systematic study on the structure-activity relationship of these compounds was carried out. Compound **12d** with significantly higher antiviral activities than ribavirin emerged as new antiviral candidate. The preliminary mode of action studies revealed that compound **12d** has strong interaction with 20S CP disk, which leads to the fusion of 20S CP disk into aggregate. The molecular docking results further confirmed the mode of action of these compounds. Streptindole and its derivatives also exhibited broad-spectrum fungicidal activities against 14 kinds of plant fungi at 50 μg/mL. Some compounds displayed higher fungicidal activities than chlorothalonil and carbendazim. Current research is expected to promote the application of these simple compounds in plant protection.

ASSOCIATED CONTENT

Supporting Information

The detailed bio-assay procedures. The spectra data of compounds **3a**−**3c**, **4**, **5**, **8**, **10**, **11**,

526 **12a**−**12d**, **13a**−**13l**, **14** and **15a**−**15h**. This material is available free of charge via the
527 Internet at http://pubs.acs.org.

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Figure Captions

Figure 1. Design of Streptindole Analogues.

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

Figure 3. Synthesis of Streptindole.

Figure 4. Synthesis of Compound **8**.

Figure 5. Synthesis of Compounds **12a**–**12d**.

Figure 6. Synthesis of Compounds **13a**–**13l**.

Figure 7. Synthesis of Compounds **15a**–**15h**.

Figure 8. TMV Rod Assembly Inhibition of Compound **12d** (200 nm scale bar): (A) 20S CP disk + RNA, (B) 20S CP disk + RNA + 2/100 DMSO, (C) 20S CP disk + RNA + 10 μM **12d**.

Figure 9. 20S CP Disk Assembly Inhibition of Compound **12d** (100 nm scale bar): (A) CP, (B) CP + 2/100 DMSO, (C) CP + 10 μM **12d**.

Figure 10. Molecule Docking Results of Compounds **5** and **12d** with TMV CP.

Table 1. *In Vivo* Antiviral Activities of Compounds **3a**–**3c**, **4**, **5**, **8**, **10**, **11**, **12a**–**12d**, **13a**–**13l**, **14**, **15a**–**15h**, and Ribavirin Against TMV at 500 μg/mL.

compd	inactive effect (%)[a]	curative effect (%)[a]	protective effect (%)[a]	compd	inactive effect (%)[a]	curative effect (%)[a]	protective effect (%)[a]
3a	29±4	—	—	**13f**	34±3	—	—
3b	27±1	—	—	**13g**	17±2	—	—
3c	25±3	—	—	**13h**	13±2	—	—
4	**40±4**	**39±3**	**43±2**	**13i**	11±2	—	—
5	**44±4**	**33±2**	**41±3**	**13j**	**48±2**	**45±1**	**49±3**
8	31±2	—	—	**13k**	**44±4**	**47±3**	**41±2**
10	11±2	—	—	**13l**	**40±1**	**39±2**	**42±1**
11	**42±1**	**40±1**	**46±4**	**14**	37±5	—	—
12a	33±5	—	—	**15a**	36±3	—	—
12b	17±3	—	—	**15b**	28±5	—	—
12c	**45±3**	**43±2**	**46±3**	**15c**	13±2	—	—
12d	**57±3**	**55±2**	**53±1**	**15d**	34±1	—	—
13a	27±1	—	—	**15e**	25±4	—	—
13b	30±3	—	—	**15f**	29±1	—	—
13c	32±3	—	—	**15g**	8±1	—	—
13d	**40±4**	**38±3**	**45±2**	**15h**	21±1	—	—
13e	11±2	—	—	ribavirin	38±1	37±3	40±4

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

Table 2. *In Vitro* Fungicidal Activities of Compounds **3a**−**3c**, **4**, **5**, **8**, **10**, **11**, **12a**−**12d**, **13a**−**13l**, **14**, **15a**−**15h**, Chlorothalonil and Carbendazim against 14 Kinds of Fungi.

Compd	Fungicidal activities (%)[a] at 50 μg/mL													
	FC[b]	CH[b]	PP[b]	RC[b]	BM[b]	WA[b]	FM[b]	AS[b]	FG[b]	PI[b]	PC[b]	SS[b]	BC[b]	RS[b]
3a	35±2	46±2	**51±1**	41±3	26±3	27±2	24±1	38±3	42±2	23±1	42±2	**59±1**	**57±1**	**55±2**
3b	24±1	42±2	**53±2**	43±1	26±2	21±2	29±2	35±1	49±3	27±3	**65±3**	**66±2**	**53±2**	**52±1**
3c	12±2	29±1	44±1	38±2	21±3	37±2	11±1	40±1	46±2	15±1	19±1	33±1	42±1	30±3
4	18 ±1	42±2	46±2	**67±3**	26±3	14±3	38±3	**52±3**	42±2	33±1	7±2	**52±1**	**53±2**	41±1
5	35±2	46±1	**61±2**	**62±1**	37 ±1	**51±2**	38±1	**55±1**	37±1	47±2	10±2	**59±2**	**57±1**	**59±1**
8	44±3	**50±2**	24±3	**89±2**	31±2	35±2	38±2	41±1	49±1	43±3	**55±1**	**66±2**	40±2	48±2
10	16±1	28±1	23±1	24±2	14±2	12±1	8±3	15±2	8±2	13±1	3±1	41±1	14±3	38±1
11	9±2	15±2	42±2	33±3	11±1	16±2	14±2	31±1	24±1	3±1	19±2	28±2	23±2	45±2
12a	29±1	31±3	**61±2**	**54±1**	20±2	32±1	19±2	35±1	37±2	27±2	10±1	**52±1**	**57±1**	**52±1**
12b	15±2	23±2	22±2	28±1	9±1	14±3	19±3	41±2	27±3	7±2	3±1	45±2	0	35±2
12c	12±3	12±1	29±1	**56±3**	17±3	27±3	14±1	45±1	37±1	10±1	23±2	24±1	32±2	45±2
12d	21±1	27±1	37±2	46±2	23±1	30±2	24±2	45±1	39±1	27±2	16±2	48±2	23±1	38±1
13a	13 ±1	20 ±1	19±1	32±1	19±2	24±1	17±1	22±3	16±2	16±1	**74±2**	46±1	29±1	44±3
13b	13±3	16±1	35±1	15±3	14±3	18±1	8±1	22±2	39±3	23±1	13±3	39±1	13±2	0
13c	16±2	16±2	33±2	21±1	14±3	15±3	13±2	19±1	10±1	13±2	10±1	39±1	19±1	31±1
13d	16±1	20±2	31±1	18±2	11±1	12±1	17±1	26±3	10±2	13±1	7±1	36±1	27±3	19±3
13e	19±2	16±2	21±3	42±1	14±1	21±2	17±1	33±1	14±1	19±2	29±2	46±2	0	31±1
13f	6±1	28±1	27±2	12±3	17±1	24±2	13±2	30±3	6±1	19±1	4±1	32±2	16±1	31±1
13g	13±1	16±3	23±1	18±3	11±3	15±1	13±3	19±3	6±1	16±1	29±2	34±3	19±1	16±1
13h	18±1	25±1	33±1	14±2	14±2	12±1	11±3	15±2	8±2	16±1	16±2	45±1	18±3	48±1
13i	16±1	16±2	46±2	15±2	17±3	24±2	8±1	33±2	8±1	16±1	10±1	14±1	**73±3**	47±2
13j	28±2	20±3	33±3	15±1	28±1	27±2	17±1	19±2	8±2	19±1	10±2	46±3	34±2	25±2
13k	6±1	20±2	21±1	21±3	14±1	12±2	8±1	19±1	10±1	16±2	16±2	30±2	14±1	16±1
13l	16±1	32±2	25±1	38±1	25±2	24±1	13±1	26±2	14±2	19±1	7±1	**50±2**	9±1	44±2
14	12±1	23±2	37±1	23±1	14±2	14±1	0	28±2	22±2	10±1	26±2	14±3	13±1	33±1
15a	12±2	19±1	**51±1**	25±2	9±1	24±2	9±1	41±2	32±1	7±1	**71±2**	17±1	45±2	48±2
15b	12±2	15±2	49±1	30±1	11±1	19±2	5±1	28±2	37±2	10±1	3±1	31±2	6±1	45±1
15c	12±1	35±1	37±1	31±2	17±2	27±1	10±2	41±2	22±2	20±1	16±2	45±3	6±1	48±2
15d	12±2	19±3	44±2	31±1	9±1	19±2	10±1	28±2	7±1	10±1	7±1	17±1	23±2	38±2

15e	12±1	19±2	42±1	31±2	9±1	14±2	0	28±2	37±1	7±1	13±2	17±2	26±1	41±1
15f	12±2	23±2	46±1	30±1	9±2	11±1	5±1	48±1	22±2	10±2	16±1	17±1	19±2	48±3
15g	9±1	15±2	39±2	23±3	11±1	16±3	10±3	45±2	32±1	10±1	13±2	17±2	47±1	**55±1**
15h	12±2	15±3	**56±1**	23±3	9±2	8±1	5±1	35±2	12±3	3±1	10±3	10±3	32±1	24±1
Chlorothalonil[c]	95±1	19±2	98±1	98±1	97±1	98±1	83±2	38±2	100	100	16±1	100	25±1	100
Carbendazim[c]	100	28±3	98±1	98±1	97±1	98±1	90±2	13±1	100	100	12±2	100	18±2	100

[a]Average of three replicates; all results are expressed as mean ± SD. [b]Abbreviations: FC, *Fusarium oxysporum f. sp. cucumeris*; CH, *Cercospora arachidicola Hori*; PP, *Physalospora piricola*; RC, *Rhizoctonia cerealis*; BM, *Bipolaris maydis*; WA, *watermelon anthracnose*; FM, *Fusarium moniliforme*; AS, *Alternaria solani*; FG, *Fusarium graminearum*; PI, *Phytophthora infestans*; PC, *Phytophthora capsici*; SS, *Sclerotinia sclerotiorum*; BC, *Botrytis cinereal*; RS, *Rhizoctonia solani*. [c]The commercial agricultural fungicides were used for comparison of antifungal activity.

Figure 1.

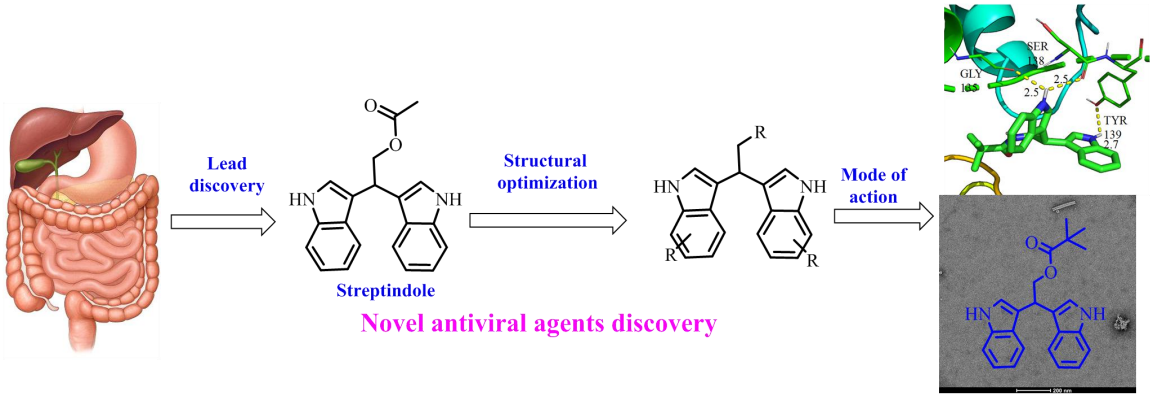
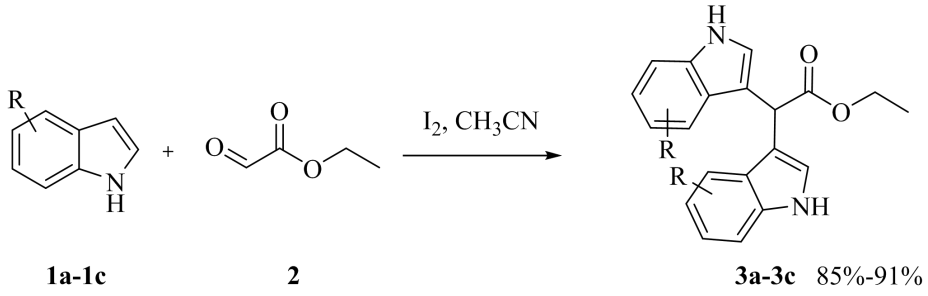


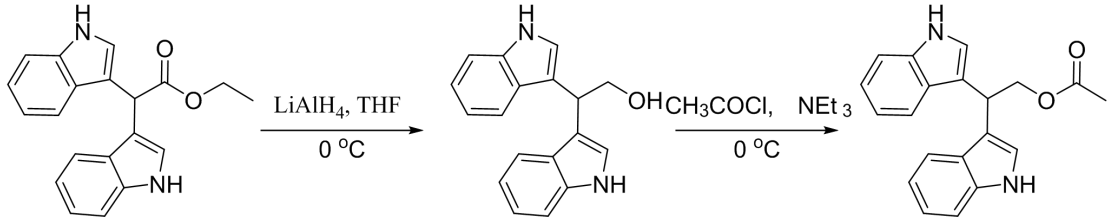
Figure 2.



1a-1c **2** **3a-3c** 85%-91%

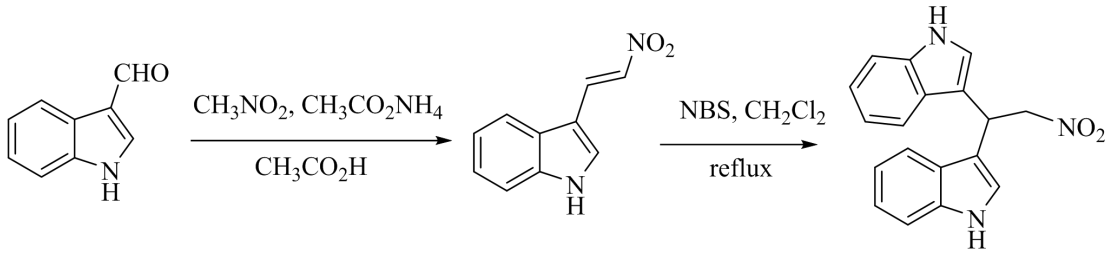
a: R = H; b: R = 5-Br; c: R = 6-Br

Figure 3.



3a **4** 74% **Streptindole (5)** 58%

Figure 4.



6 **7** 84% **8** 45%

Figure 5.

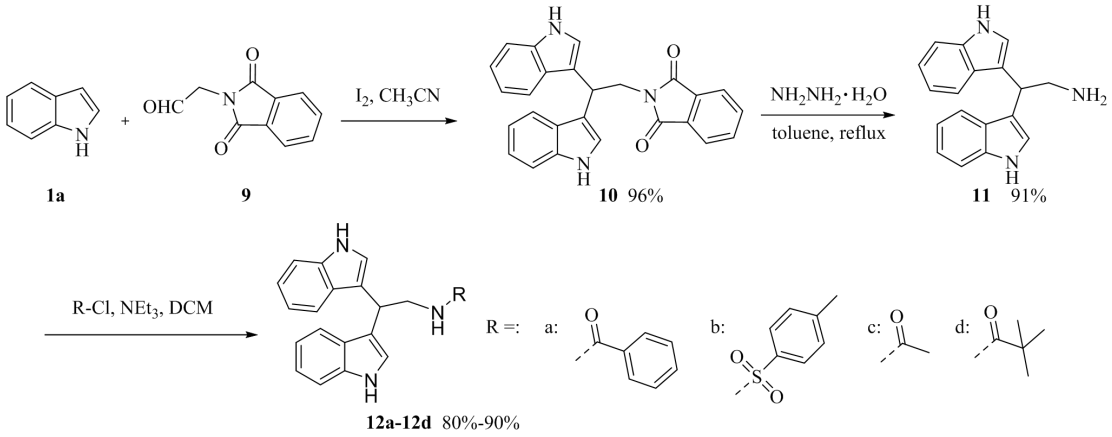


Figure 6.

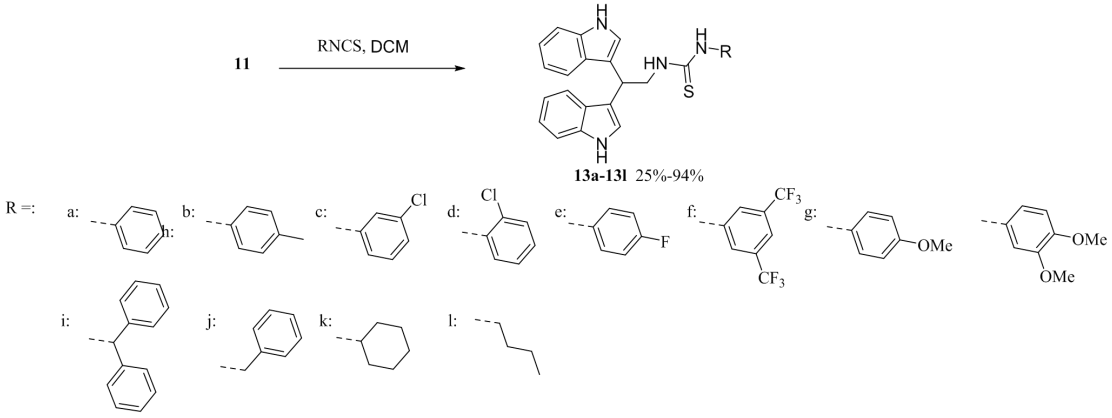


Figure 7.

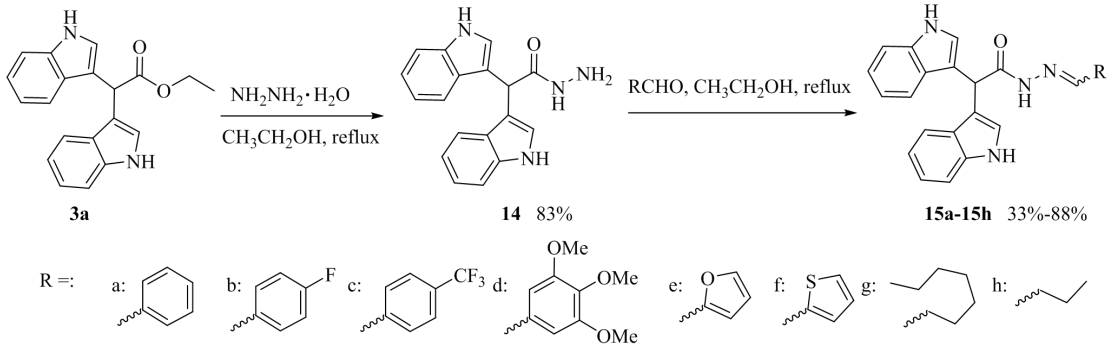


Figure 8.

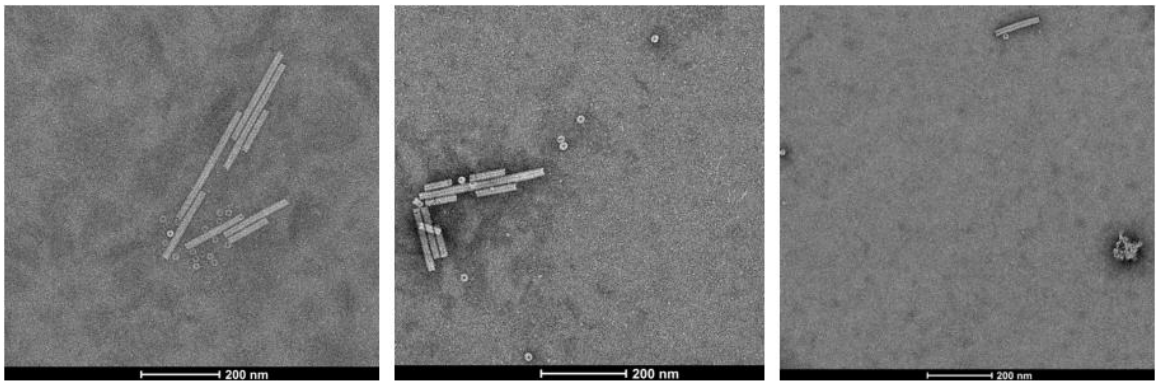


Figure 9.

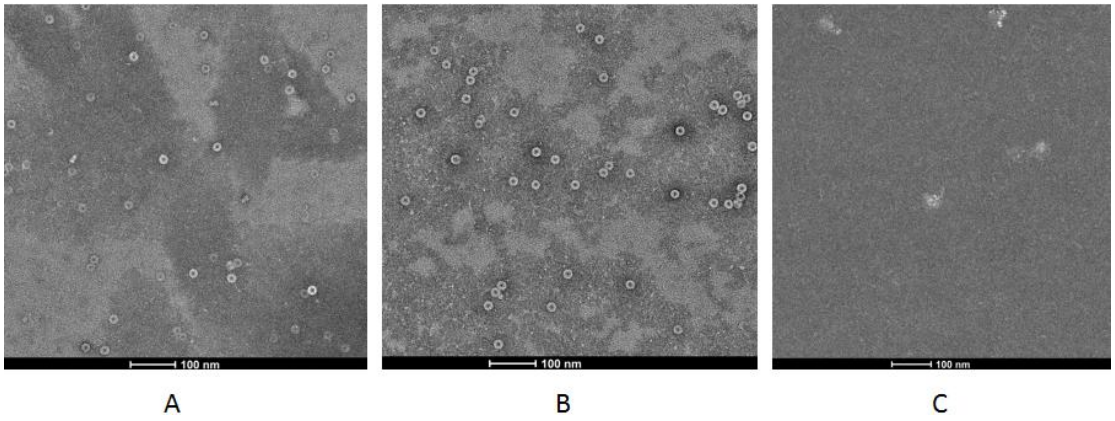
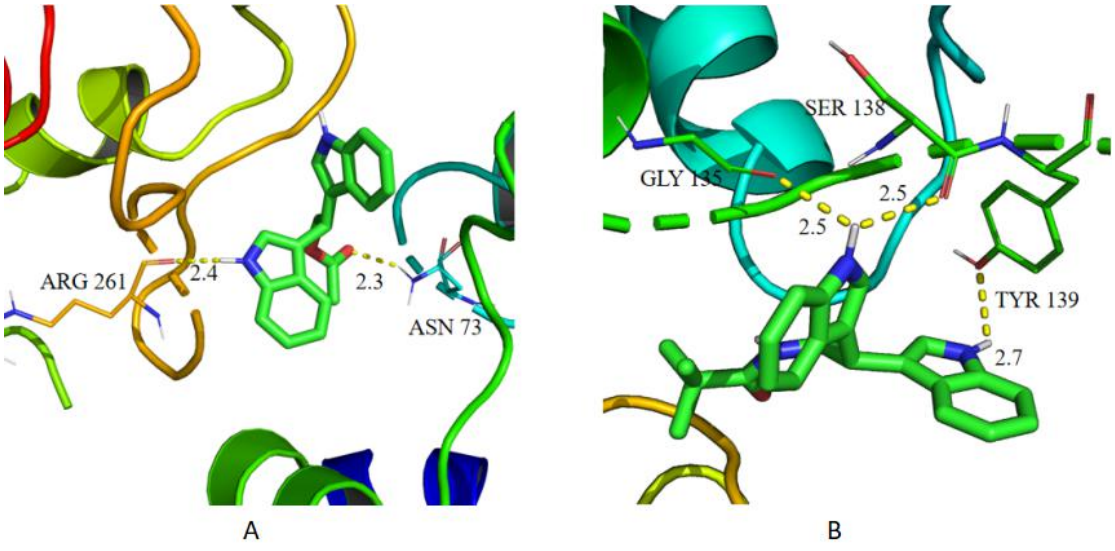


Figure 10.



TOC ***graphic***

Agrochemical Bioregulators