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NATURAL PRODUCTS FOR BIOCIDES DISCOVERY: DISCOVERY OF ARUNDINE AND IT'S DERIVATIVES AS NOVEL ANTIVIRAL AND ANTI-PHYTOPATHOGENIC-FUNGUS AGENTS

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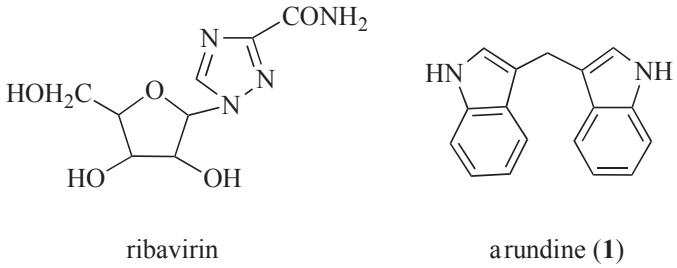
Abstract – Plant diseases are one of the natural disasters that seriously harm agricultural production, and it is very difficult to control. The discovery of new antiviral and antifungal lead compounds becomes more and more important. Natural product arundine was found to have anti-tobacco mosaic virus (TMV) activity and anti-phytopathogenic-fungus activity for the first time. A series of arundine analogues were designed, synthesized and evaluated for their antiviral and fungicidal activities. Compound **6** with excellent antiviral activity emerged as novel antiviral lead compound. Compound **7** with 12.6−38.3 μg/mL EC_{50} values against 14 plant pathogens emerged as novel antifungal lead compound. This work laid a foundation for promoting the application of arundine analogues in plant protection.

INTRODUCTION

Plant diseases, caused by plant virus and phytopathogenic-fungus, seriously affect grain yield and economic income, threatening social security.[1-3] Tobacco mosaic virus (TMV), the earliest and deepest studied virus, can infect more than 400 crops including tobacco, pepper, tomato and so on.[4] Ribavirin (Figure 1), the widely used antiviral agent, displays less than 50% TMV inhibitory effect at 500 μg/mL.

Control of TMV disease has long been a challenging task. The development of novel, efficient and environmentally friendly pesticides is still needed urgently.[5]



ribavirin arundine (**1**)

Figure 1. Structures of ribavirin and arundine (**1**)

Natural products (NPs) have a long history as the primary source of agrochemicals for the treatment of a wide range of plant disease. NPs have experienced biological selection in bio-synthesis, so they have good bio-compatibility.[6,7] On the other hand, due to increasingly stringent environmental, toxicological and regulatory requirements, the discovery of novel, environmentally friendly and efficient pesticides based on NPs is becoming more and more important.[8-10] A series of NPs, such as gramine,[11] pimprinine,[12] kealiinines A−C,[13] and topsentin alkaloids,[14] have been found to have good antiviral and fungicidal activities.

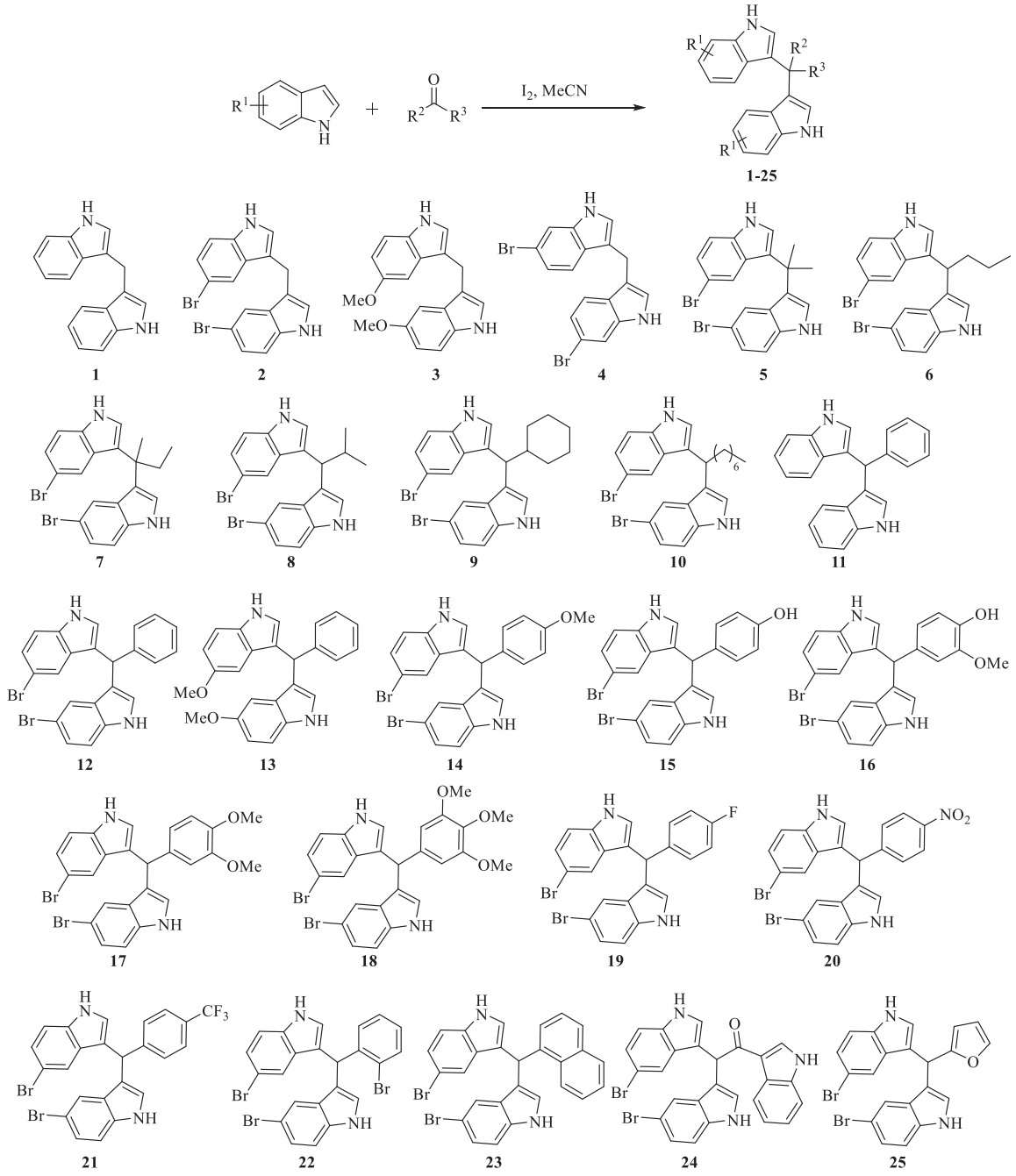
In a large number of indole derivatives, bis(indolyl)methanes (BIMs), a class of C3 substituted indole alkaloids, were found to have a great deal of interest for their numerous biological activities, such as anti-bacterial, anti-tumor and anti-leishmanial properties.[15,16] Arundine (**1**, Figure 1) was first isolated from the roots of *Arundo donax* in 1994 by Khuzhaev and co-workers.[17] Nine years later, Veluri and co-workers isolated this metabolite from the North sea bacterium *Vibrio parahaemolyticus* Bio249.[18] This was the first report on the occurrence of arundine from a microorganism. In 2017, arundine was also isolated from sea bacterium *Pseudovibrio denitrificans* BBCC725 by Rodrigues and co-workers.[19] Biological activity study showed that arundine exhibited good anti-tumor activity.[20,21] However, there is no report on the anti-TMV activity and anti-phytopathogenic-fungus activity of arundine till now.

Considering the above findings, natural product arundine was selected as parent compound for antiviral and fungicidal activities research. A series of aromatic or fat substituted arundine derivatives were designed, synthesized and evaluated the structure-activity relationship (SAR).

RESULTS AND DISCUSSION

Chemistry. BIMs have symmetric structure, and can be easily synthesized from two molecules of indole and an aldehyde/ketone using an acid or base catalyst.[22] As shown in Scheme 1, compounds **1**−**25** were prepared in 43−96% yields with I_2 as catalyst.

Antiviral Activity *in vivo*. As shown in Table 1, compounds **1**−**25** were first tested the inactive activities at 500 μg/mL. Compounds with good inactive activities (inactive effect > 40%) were further tested the curative activities and protective activities. The commercial plant virucide ribavirin was used as the control.



Scheme 1. Synthesis of compounds **1**−**25**

Most of the compounds displayed good antiviral activities *in vivo*. Compounds **5**, **6**, **9**−**11**, **13**, **16**, **17** and **22** exhibited higher inactive activities than ribavirin. Compound **6** with excellent antiviral activities

emerged as novel antiviral lead compound. The main difference between compounds **1**−**4** lies in the different substituents on the indole ring. Compound **2** with Br at 5-position of indole ring displayed higher inactive activity than the others. The introduction of methoxy at 5-position of indole ring or bromine at 6-position of indole ring displayed about similar activities with arundine. The above results showed that the electronic effect of indole ring has a significant effect on the activity. The introduction of alkyl group into the methylene of compound **2** is beneficial to the improvement of activity (inhibitory effect: **5**−**10** > **2**). Compound **5** with two methyl groups and compound **6** with propyl group exhibited significantly higher activities than ribavirin. Substitution of one methyl group of compound **5** with ethyl led to a significant decrease in activity, which indicated that the methylene region of arundine is the active sensitive region. Substituting isopropyl for propyl of **6** also resulted in decreased activity (inhibitory effect: **8** < **6**). Using cyclohexyl instead of isopropyl can obviously improve the biological activity (inhibitory effect: **9** > **8**). Compounds **10** and **6** displayed similar level of inactive activities, which indicated that the chain length of monoalkyl has little effect on biological activity, but steric hindrance seriously reduces biological activity. We further investigated the effect of the introduction aryl into the methylene on biological activity. The introduction of aryl is beneficial to the improvement of activity, except for compound **18**. Among the aryl substituted compounds, **11** with no substituents in indole ring showed the best activity. Substitution of benzene ring for cyclohexyl group resulted in obvious decrease of biological activity (inhibitory effect: **12** < **9**). Compound **13** containing methoxy at 5-position of indole ring showed about similar biological activity as compound **11**. The substituents on the aromatic ring also have obvious influence on the biological activity. Compounds **14**, **15** and **19**−**21** with electron withdrawing or electron donating groups at 4-position exhibited similar biological activity as compound **12**. Compounds **16** and **17** containing two electron donating groups at 3 and 4 positions and compound **22** substituted by *ortho*-bromine displayed higher bio-activities than compound **12**. Further increase of electron cloud density on benzene ring results in significant decrease of biological activity (inhibitory effect: **18** < **17**). The substitution of naphthalene ring or furan ring for benzene ring is beneficial to the improvement of biological activity. Indole formyl containing compound **24** exhibited similar biological activity as compound **12**. Compounds **5**, **6**, **9**−**11**, **13**, **16**, **17** and **22** also showed very good curative activities and protective activities.

Preliminary Mode of Action. Compound **6** was selected for further mode of action study via TEM using our previously reported method.[11] The test results showed that 20S CP Disk and TMV RNA can assemble into TMV rod effectively (Figure 2, B). Compound **6** can significantly inhibit the assembly of TMV rod (Figure 2, C). The above results indicated that compound **6** likely exerted it's virus inhibition by inhibiting virus assembly.

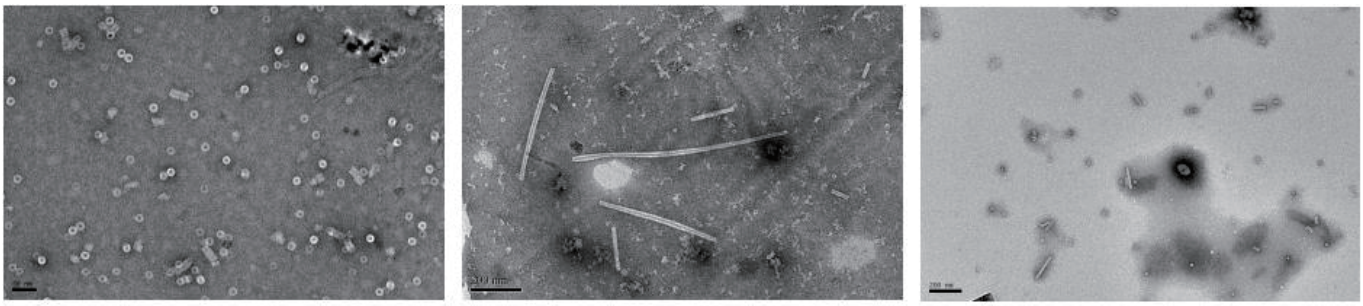


Figure 2. TMV rod assembly inhibition of compound **6**: (A) 20S CP Disk (50 nm scale bar); (B) 20S CP Disk + RNA (200 nm scale bar); (C) 20S CP Disk + RNA + **6** (200 nm scale bar).

Table 1. *In Vivo* Antiviral Activities of Compounds **1**−**25** 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]
1	22±1	—	—	**14**	35±2	—	—
2	30±2	—	—	**15**	32±1	—	—
3	23±2	—	—	**16**	**42±3**	35±2	47±4
4	20±2	—	—	**17**	**41±3**	30±2	31±1
5	**47±2**	**43±1**	**50±3**	**18**	24±4	—	—
6	**50±2**	**46±3**	**47±2**	**19**	31±1	—	—
7	34±2	—	—	**20**	37±4	—	—
8	35±4	—	—	**21**	32±3	—	—
9	**44±4**	46±3	39±2	**22**	**40±2**	41±3	37±3
10	**47±4**	37±2	38±3	**23**	39±3	—	—
11	**45±3**	**42±4**	**45±2**	**24**	33±2	—	—
12	34±1	—	—	**25**	36±3	—	—
13	**42±2**	40±1	37±2	**Ribavirin**	38±1	38±2	40±1

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

Fungicidal Activity. Compounds **1**−**25** were also evaluated for their fungicidal activities against 14 plant pathogens with commercial fungicides carbendazim and chlorothalonil as controls.

The results showed that these compounds also exhibited broad-spectrum fungicidal activities (Table 2). The fungicidal activities of **7** against *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Phytophthora capsici* and *Watermelon anthracnose* and the fungicidal activities of **2** and **6** against *Sclerotinia sclerotiorum* are higher than that of carbendazim and chlorothalonil. Compound **7** exhibited higher fungicidal activities against *Rhizoctonia solani* and *Fusarium moniliforme* than carbendazim. The fungicidal activities of **7** against *Alternaria solani* and *Fusarium graminearum* and the fungicidal activity of **15** against *Alternaria solani* are higher than that of chlorothalonil. The EC_{50} values of compound **7** against 14 plant pathogens

were further evaluated which range from 12.6 μg/mL to 38.3 μg/mL. Compound **7** with excellent fungicidal activity emerged as novel antifungal lead compound.

Table 2. *In Vitro* Fungicidal Activities of Compounds **1**−**25**, Carbendazim and Chlorothalonil against 14 Kinds of Fungi

Compd	Fungicidal activities (%)[a]/ 50 μg/mL													
	B.C[b]	*S.S*[b]	*R.S*[b]	*C.H*[b]	*F.M*[b]	*F.C*[b]	*P.C*[b]	*W.A*[b]	*B.M*[b]	*P.I*[b]	*A.S*[b]	*P.P*[b]	*F.G*[b]	*R.C*[b]
1	31±1	40±2	37±2	12±1	13±1	19±2	24±3	33±1	16±2	12±1	15±1	43±2	56±1	54±1
2	53±2	**66±2**	52±1	**62±2**	29±2	24±1	29±1	41±2	26±2	27±3	55±1	**73±2**	59±3	**93±1**
3	42±1	33±1	30±3	29±1	11±1	12±2	10±2	37±2	21±3	15±1	40±1	**64±1**	46±2	**68±2**
4	33±1	15±1	15±1	15±1	10	8±1	6±2	17±1	11±2	10±1	25±1	43±2	26±1	46±1
5	26±2	16±1	8±1	37±2	14±2	27±1	11±1	11±1	25±1	17±2	13±2	**68±3**	19±1	26±2
6	51±2	**72±2**	52±1	27±2	43±1	38±3	29±1	38±2	54±2	33±2	48±3	46±2	56±2	**95±1**
7	**100**	**86±1**	**97±1**	**69±3**	**81±2**	**91±1**	**94±1**	**78±2**	**71±1**	**70±3**	**93±1**	**93±1**	**90±1**	**97±1**
EC_{50} of **7** (μg/mL)	**13.3**	**16.0**	**12.6**	**13.7**	**18.3**	**26.3**	**25.1**	**21.3**	**22.6**	**38.5**	**25.0**	**14.5**	**28.9**	**15.0**
8	19±2	28±1	45±2	15±2	10±1	12±1	13±1	24±2	17±1	10±1	24±2	39±2	10±1	36±2
9	19±1	35±2	38±3	15±1	10±1	9±1	13±1	16±2	14±1	13±1	41±3	46±2	22±2	49±1
10	32±2	28±3	35±2	8±1	5±1	18±2	7±1	49±2	17±1	7±1	52±2	32±1	7±1	20±2
11	45±2	55±1	52±1	46±2	24±3	29±3	23±2	35±1	23±2	27±2	41±1	54±2	49±2	36±3
12	23±1	17±1	45±3	15±1	10±1	18±1	10±1	19±2	9±1	10±1	31±1	42±2	20±1	33±1
13	9±1	31±2	28±1	19±1	10±1	15±1	19±2	19±1	11±1	7±1	55±3	42±1	15±2	26±1
14	9±2	10±1	31±1	15±2	10±2	9±2	7±1	22±2	17±2	7±1	35±1	22±2	12±1	38±2
15	32±1	52±2	52±3	35±1	5±1	15±1	19±2	30±1	23±2	37±2	**62±2**	49±3	49±2	49±1
16	42±2	31±1	31±1	19±2	14±1	12±1	29±1	27±2	20±2	20±1	38±1	34±1	32±1	44±2
17	4±1	24±2	31±1	31±1	19±3	24±3	16±1	16±1	14±1	10±2	21±2	22±2	20±2	30±1
18	40±2	0	41±2	31±1	19±1	21±1	48±2	16±2	14±2	13±1	38±1	42±3	20±1	28±2
19	34±1	35±1	38±1	15±3	14±1	18±1	16±1	30±1	17±1	17±2	35±2	42±1	37±3	53±1
20	0	35±2	52±3	15±2	19±2	15±2	13±1	46±1	11±2	13±1	45±1	51±2	32±1	25±1
21	34±2	24±1	38±2	12±1	10±1	12±1	16±2	30±3	20±1	20±2	48±1	32±1	42±2	44±1
22	13±1	28±2	48±1	19±2	5±1	12±2	16±1	16±1	9±1	10±1	41±3	46±1	32±1	25±3
23	32±3	10±1	45±2	15±1	5±2	12±1	13±1	19±2	14±1	13±1	38±2	34±1	12±1	34±1
24	13±1	0	31±1	15±2	5±1	12±1	16±2	14±1	9±1	0	24±1	46±2	22±2	26±1
25	32±1	45±1	45±3	15±1	5±1	9±1	7±1	24±2	14±1	7±1	31±1	34±1	24±1	44±3
Carbendazim[c]	75±1	59±1	57±2	76±2	75±3	93±1	83±3	57±2	85±2	80±1	93±1	100	100	95±3
Chlorothalonil[c]	96±1	16±3	95±2	97±1	96±2	98±1	79±2	55±3	100	100	54±2	100	31±1	100

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

In summary, bisindole natural product arundine was found to have anti-TMV activity and anti-phytopathogenic-fungus activity for the first time. The molecular design and structure-activity relationship of arundine derivatives were curried out systematically. Compounds **5**, **6**, **9**−**11**, **13**, **16**, **17** and **22** exhibited higher inactive activities than ribavirin. Compound **6** with excellent antiviral activity emerged as novel antiviral lead compound. These compounds also displayed broad-spectrum fungicidal activities. The fungicidal activities of **7** against *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Phytophthora capsici* and *Watermelon anthracnose* and the fungicidal activities of **2** and **6** against *Sclerotinia sclerotiorum* are higher than that of carbendazim and chlorothalonil. Compound **7** with 12.6−38.3 μg/mL

EC_{50} values against 14 plant pathogens emerged as novel antifungal lead compound. Current research has laid a foundation for the application of arundine analogues in plant protection.

EXPERIMENTAL

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).

General Procedures for the Preparation of Compounds 1–25. To a solution of corresponding indole (5 mmol) and corresponding aldehyde (2.5 mmol) in MeCN (25 mL) was added I_2 (0.5 mmol) at 0 °C. The reaction mixture was stirred for 30 min, and quenched with 5% aqueous $Na_2S_2O_3$ solution (20 mL). The resulting solution was extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on a silica gel using petroleum ether and EtOAc (5:1, v/v) as the eluent to give corresponding compounds **1**−**25**.

Arundine (1). Pink solid; yield 61%; mp 161−163 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.72 (s, 2H), 7.52 (d, J = 7.9 Hz, 2H), 7.31 (d, J = 8.1 Hz, 2H), 7.13 (s, 2H), 7.05–6.99 (m, 2H), 6.94–6.88 (m, 2H), 4.12 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 136.4, 127.2, 122.7, 120.7, 118.6, 118.0, 114.2, 111.3, 20.9. $C_{17}H_{15}N_2$ $[M+H]^+$ 247.1230, found (ESI$^+$) 247.1235.

Bis(5-bromo-1*H*-indol-3-yl)methane (2). Pink solid; yield 53%; mp 172−173 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 2H), 7.73 (s, 2H), 7.31−7.24 (m, 4H), 6.96 (s, 2H), 4.16 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.1, 129.2, 124.9, 123.4, 121.8, 114.9, 112.6, 21.1. $C_{17}H_{13}Br_2N_2$ $[M+H]^+$ 402.9440, found (ESI$^+$) 402.9443.

Bis(5-methoxy-1*H*-indol-3-yl)methane (3). Pink solid; yield 49%; mp 169−170 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 2H), 7.21 (d, J = 8.7 Hz, 2H), 7.08 (s, 2H), 7.01 (s, 2H), 6.69 (dd, J = 8.7, 2.4 Hz, 2H), 4.05 (s, 2H), 3.70 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.7, 131.5, 127.5, 123.4, 113.9, 111.9, 110.7, 100.7, 55.3, 20.8. $C_{19}H_{19}N_2O_2$ $[M+H]^+$ 307.1441, found (ESI$^+$) 307.1448.

Bis(6-bromo-1*H*-indol-3-yl)methane (4). Pink solid; yield 61%; mp 206−207 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (s, 2H), 7.50 (d, J = 1.3 Hz, 2H), 7.43 (d, J = 8.4 Hz, 2H), 7.18 (s, 2H), 7.04 (dd, J = 8.4, 1.5 Hz, 2H), 4.10 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 137.2, 126.1, 123.9, 120.9, 120.3, 114.2, 113.8, 113.6, 20.6. $C_{17}H_{13}Br_2N_2$ $[M+H]^+$ 402.9440, found (ESI$^+$) 402.9444.

3,3'-(Propane-2,2-diyl)bis(5-bromo-1*H*-indole) (5). Brown oil; yield 68%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 2H, NH), 7.44 (s, 2H, Ar-H), 7.15 (d, 4H, Ar-H), 7.06 (d, J = 2.4 Hz, 2H, Ar-H), 1.83 (s, 6H,

CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.8, 127.9, 124.8, 124.4, 123.3, 121.7, 112.7, 112.1, 34.6, 29.8. $C_{19}H_{17}Br_2N_2$ $[M+H]^+$ 430.9753, found (ESI^+) 430.9757.

3,3'-(Butane-1,1-diyl)bis(5-bromo-1*H*-indole) (6). Brown oil; yield 74%; ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 2H), 7.66 (d, J = 1.7 Hz, 2H), 7.17−7.24 (m, 4H), 7.01 (d, J = 2.2 Hz, 2H), 4.34 (t, J = 7.5 Hz, 1H), 2.11−2.18(m, 2H), 1.36−1.42 (m, 2H), 0.96 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.3, 128.7, 124.7, 122.7, 122.1, 119.6, 112.7, 112.4, 37.5, 33.7, 21.4, 14.2. $C_{20}H_{19}Br_2N_2$ $[M+H]^+$ 444.9910, found (ESI^+) 444.9906.

3,3'-(Butane-2,2-diyl)bis(5-bromo-1*H*-indole) (7). Brown oil; yield 71%; ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 2H), 7.42 (s, 2H), 7.29−7.15 (m, 6H), 2.37 (q, J = 7.4 Hz, 2H), 1.77 (s, 3H), 0.76 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.7, 128.0, 124.4, 123.5, 123.4, 122.4, 112.6, 112.0, 38.3, 32.5, 26.0, 8.9. $C_{20}H_{19}Br_2N_2$ $[M+H]^+$ 444.9910, found (ESI^+) 444.9914.

3,3'-(2-Methylpropane-1,1-diyl)bis(5-bromo-1*H*-indole) (8). Yellow solid; yield 59%; mp 140−141 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 2H), 7.71 (s, 2H), 7.17−7.25 (m, 2H), 7.11 (d, J = 8.6 Hz, 2H), 7.04 (d, J = 1.55 Hz, 2H), 4.02 (d, J = 8.9 Hz, 1H), 2.54−2.59 (m, 1H), 0.95 (d, J = 6.5 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.9, 129.1, 124.6, 122.9, 122.1, 118.9, 112.6, 112.4, 41.4, 32.5, 21.9. $C_{20}H_{19}Br_2N_2$ $[M+H]^+$ 444.9910, found (ESI^+) 444.9907.

3,3'-(Cyclohexylmethylene)bis(5-bromo-1*H*-indole) (9). Brown oil; yield 43%; ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 2H), 7.73 (s, 2H), 7.12−7.21 (m, 4H), 7.07 (d, J = 1.41 Hz, 2H), 4.07 (d, J = 9.23 Hz, 1H), 2.13−2.22 (m, 1H), 1.65−1.77 (m, 6H), 0.96−1.19 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.9, 129.2, 124.6, 122.8, 122.0, 118.8, 112.6, 112.4, 42.4, 40.3, 32.4, 26.6. $C_{23}H_{23}Br_2N_2$ $[M+H]^+$ 485.0223, found (ESI^+) 485.0227.

3,3'-(Octane-1,1-diyl)bis(5-bromo-1*H*-indole) (10). Brown oil; yield 57%; ^1H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 2H), 7.65 (s, 2H), 7.14−7.24 (m, 4H), 6.97 (d, J = 2.0 Hz, 2H), 4.29 (t, J = 7.5 Hz, 1H), 2.10−2.16 (m, 2H), 1.22−1.33 (m, 10H), 0.85 (t, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.3, 128.7, 124.7, 122.7, 122.1, 119.7, 112.7, 112.4, 35.3, 34.0, 31.9, 29.6, 29.3, 28.2, 22.7, 14.2. $C_{24}H_{27}Br_2N_2$ $[M+H]^+$ 501.0536, found (ESI^+) 501.0541.

3,3'-(Phenylmethylene)bis(1*H*-indole) (11). Red solid; yield 86%; mp 146−148 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 2H, NH), 7.38 (d, J = 7.9 Hz, 2H, Ar-H), 7.31–7.34 (m, 4H, Ar-H), 7.20–7.28 (m, 3H, Ar-H), 7.17–7.14 (m, 2H, Ar-H), 6.97–7.01 (m, 2H, Ar-H), 6.60 (d, J = 1.5 Hz, 2H, Ar-H), 5.87 (s, 1H, Ar-CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.0, 136.7, 128.8, 128.3, 127.1, 126.2, 123.6, 121.9, 120.0, 119.7, 119.3, 111.1, 40.2. $C_{23}H_{19}N_2$ $[M+H]^+$ 323.1543, found (ESI^+) 323.1540.

3,3'-(Phenylmethylene)bis(5-bromo-1*H*-indole) (12). Red solid; yield 88%; mp 249−251 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 2H, NH), 7.50 (s, 2H, Ar-H), 7.32–7.33 (m, 3H, Ar-H), 7.29 (s, 2H, Ar-H), 7.26–7.28 (m, 3H, Ar-H), 6.67 (d, J = 1.9 Hz, 2H, Ar-H), 5.78 (s, 1H, Ar-CH); ^{13}C NMR (100 MHz,

DMSO-d_6) δ 144.3, 135.2, 128.4, 128.2, 126.0, 125.2, 123.4, 121.2, 117.6, 113.6, 110.9, 38.9. $C_{23}H_{17}Br_2N_2$ $[M+H]^+$ 478.9753, found (ESI^+) 478.9751.

3,3'-(Phenylmethylene)bis(5-methoxy-1*H*-indole) (13). Red solid; yield 81%; mp 224−225 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.79 (s, 2H, NH), 7.33 (d, J = 7.5 Hz, 2H, Ar-H), 7.28 (m, 1H, Ar-H), 7.23 (d, J = 4.7 Hz, 2H, Ar-H), 7.20 (d, J = 8.0 Hz, 2H, Ar-H), 6.81 (m, 2H, Ar-H), 6.79 (s, 2H, Ar-H), 6.64 (d, J = 1.3 Hz, 2H, Ar-H), 5.76 (s, 1H, CH), 3.67 (s, 6H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 144.3, 135.2, 128.4, 128.2, 126.0, 125.2, 123.4, 121.2, 117.6, 113.6, 110.9, 38.9. $C_{25}H_{23}N_2O_2$ $[M+H]^+$ 383.1754, found (ESI^+) 383.1758.

3,3'-((4-Methoxyphenyl)methylene)bis(5-bromo-1*H*-indole) (14). Red solid; yield 92%; mp 220−222 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 2H, NH), 7.47 (s, 2H, Ar-H), 7.26 – 7.23 (m, 4H, Ar-H), 7.19 (d, J = 8.6 Hz, 2H, Ar-H), 6.84 (d, J = 8.0 Hz, 2H, Ar-H), 6.64 (d, J = 1.6 Hz, 2H, Ar-H), 5.70 (s, 1H, CH), 3.80 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.4, 136.2, 135.2, 129.1, 128.3, 125.1, 123.4, 121.2, 118.0, 113.5, 110.8, 54.9, 38.0. $C_{24}H_{19}Br_2N_2O$ $[M+H]^+$ 508.9859, found (ESI^+) 508.9867.

4-(Bis(5-bromo-1*H*-indol-3-yl)methyl)phenol (15). Pink solid; yield 76%; mp 166−167 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 2H), 7.48 (s, 2H), 7.17−7.28 (m, 4H), 7.13 (d, J = 8.3 Hz, 2H), 6.74 (d, J = 8.3 Hz, 2H), 6.60 (s, 2H), 5.67 (s, 1H), 4.79 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.1, 135.45, 135.37, 129.7, 128.6, 125.0, 124.7, 122.3, 119.3, 115.3, 112.6, 39.1. $C_{23}H_{17}Br_2N_2O$ $[M+H]^+$ 494.9702, found (ESI^+) 494.9707.

4-(Bis(5-bromo-1*H*-indol-3-yl)methyl)-2-methoxyphenol (16). Purple solid; yield 85%; mp 233−234 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 2H), 7.49 (s, 2H), 7.23−7.26 (m, 4H), 6.73−6.85 (m, 3H), 6.65 (s, 2H), 5.68 (s, 1H), 5.53 (s, 1H), 3.79 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.9, 146.5, 135.4, 128.7, 125.0, 124.7, 122.3, 121.2, 119.4, 117.6, 114.2, 112.7, 112.6, 111.3, 55.9, 39.6. $C_{24}H_{19}Br_2N_2O_2$ $[M+H]^+$ 524.9808, found (ESI^+) 524.9811.

3,3'-((3,4-Dimethoxyphenyl)methylene)bis(5-bromo-1*H*-indole) (17). Red solid; yield 96%; mp 124−125 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 2H), 7.48 (s, 2H), 7.20−7.26 (m, 4H), 6.85 (s, 1H), 6.77 (s, 2H), 6.62 (s, 2H), 5.69 (s, 1H), 3.85 (s, 3H), 3.76 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.8, 147.5, 135.8, 135.4, 128.6, 124.9, 124.8, 122.3, 120.5, 119.2, 112.7, 112.0, 111.0, 55.9, 39.5. $C_{25}H_{21}Br_2N_2O_2$ $[M+H]^+$ 538.9964, found (ESI^+) 538.9967.

3,3'-((3,4,5-Trimethoxyphenyl)methylene)bis(5-bromo-1*H*-indole) (18). Pink solid; yield 78%; mp 258−259 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 2H), 7.50 (s, 2H), 7.34 (d, J = 8.6 Hz, 2H), 7.16 (dd, J = 1.5, 8.6 Hz, 2H), 6.97 (d, J = 1.9 Hz, 2H), 6.71 (s, 2H), 5.80 (s, 1H), 3.67 (s, 6H), 3.64 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.1, 140.5, 136.3, 135.7, 128.9, 125.7, 123.9, 121.7, 118.1, 114.0, 111.3, 106.3, 60.5, 56.3, 39.6. $C_{26}H_{23}Br_2N_2O_3$ $[M+H]^+$ 569.0070, found (ESI^+) 569.0065.

3,3'-((4-Fluorophenyl)methylene)bis(5-bromo-1*H*-indole) (19). Pink solid; yield 48%; mp 120−121 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 2H), 7.45 (s, 2H), 7.22−7.29 (m, 6H), 6.99 (d, J = 8.6 Hz, 2H), 6.63 (s, 2H), 5.74 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.4, 130.0, 129.9, 128.5, 125.1, 124.7, 122.2, 119.0, 115.4, 115.1, 112.8, 112.6, 39.2. $C_{23}H_{16}Br_2FN_2$ $[M+H]^+$ 496.9659, found (ESI^+) 496.9665.

3,3'-((4-Nitrophenyl)methylene)bis(5-bromo-1*H*-indole) (20). Yellow solid; yield 88%; mp 195−196 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13−8.16 (m, 4H), 7.44 (d, J = 8.65 Hz, 2H), 7.43 (s, 2H), 7.25−7.30 (m, 2H), 6.74 (d, J = 8.34 Hz, 2H), 6.65 (d, J = 2.09 Hz, 2H), 5.85 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.9, 146.8, 135.4, 129.4, 128.3, 125.4, 124.9, 123.9, 121.9, 117.4, 113.0, 112.9, 39.8. $C_{23}H_{16}Br_2N_3O_2$ $[M+H]^+$ 523.9604, found (ESI^+) 523.9607.

3,3'-((4-(Trifluoromethyl)phenyl)methylene)bis(5-bromo-1*H*-indole) (21). Pink solid; yield 49%; mp 129−131 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 2H), 7.55 (d, J = 8.1 Hz, 2H), 7.45 (s, 2H), 7.39 (d, J = 8.0 Hz, 2H), 7.23−7.28 (m, 4H), 6.61−6.62 (m, 2H), 5.81 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.2, 135.4, 128.9, 128.5, 125.5, 125.4, 125.3, 124.8, 122.1, 118.1, 112.9, 112.8, 60.5, 39.8. $C_{24}H_{16}Br_2F_3N_2$ $[M+H]^+$ 546.9627, found (ESI^+) 546.9631.

3,3'-((2-Bromophenyl)methylene)bis(5-bromo-1*H*-indole) (22). Pink solid; yield 65%; mp 227−228 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 2H, NH), 7.67 (d, J = 8.0 Hz, 1H, Ar-H), 7.36 (d, J = 8.6 Hz, 2H, Ar-H), 7.32 (d, J = 2.9 Hz, 2H, Ar-H), 7.29 (m, 1H, Ar-H), 7.21 (m, 1H, Ar-H), 7.19 (d, J = 2.0 Hz, 2H, Ar-H), 7.17 (d, J = 1.9 Hz, 1H, Ar-H), 6.79 (d, J = 1.9 Hz, 2H, Ar-H), 6.11 (s, 1H, CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.4, 133.1, 130.1, 128.6, 128.2, 127.4, 125.2, 125.0, 122.2, 117.8, 112.8, 112.6, 39.3. $C_{23}H_{16}Br_3N_2$ $[M+H]^+$ 556.8858, found (ESI^+) 556.8854.

3,3'-(Naphthalen-1-ylmethylene)bis(5-bromo-1*H*-indole) (23). Pink solid; yield 88%; mp 294−295 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 8.4 Hz, 1H), 7.96 (s, 2H), 7.90 (d, J = 8.2 Hz, 1H), 7.77 (d, J = 8.3 Hz, 1H), 7.45−7.49 (m, 3H), 7.40 (t, J = 8.0 Hz, 1H), 7.32 (t, J = 7.3 Hz, 1H), 7.24−7.29 (t, 4H), 7.16 (d, J = 6.9 Hz, 1H), 6.56 (s, 2H), 6.52 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.4, 134.1, 128.8, 128.7, 127.4, 126.1, 126.0, 125.5, 125.1, 124.1, 122.2, 118.7, 112.8, 112.7, 35.6. $C_{27}H_{19}Br_2N_2$ $[M+H]^+$ 528.9910, found (ESI^+) 528.9916.

2,2-Bis(5-bromo-1*H*-indol-3-yl)-1-(1*H*-indol-3-yl)ethan-1-one (24). Red solid; yield 65%; mp 194−195 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 1H), 8.45−8.47 (m, 1H), 8.15 (s, 2H), 7.85 (d, J = 3.1 Hz, 1H), 7.68 (d, J = 1.4 Hz, 2H), 7.38−7.41 (m, 1H), 7.29−7.31 (m, 1H), 7.20−7.23 (m, 3H), 7.12−7.14 (m, 2H), 6.81 (d, J = 2.0 Hz, 2H), 6.11 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.7, 135.1, 132.1, 128.4, 126.0, 125.1, 123.9, 122.9, 122.5, 121.4, 114.3, 113.0, 111.7, 43.4. $C_{26}H_{18}Br_2N_3O$ $[M+H]^+$ 545.9811, found (ESI^+) 545.9810.

3,3'-(Furan-2-ylmethylene)bis(5-bromo-1*H*-indole) (25). Grey solid; yield 93%; mp 179−180 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 2H, NH), 7.59 (s, 2H, Ar-H), 7.40 (d, J = 0.9 Hz, 1H, Ar-H), 7.28 (d, J = 1.7 Hz, 2H, Ar-H), 7.27 (d, J = 2.0 Hz, 2H, Ar-H), 6.91 (d, J = 1.8 Hz, 2H, Ar-H), 6.35 (m, 1H, Ar-H), 6.07 (d, J = 3.1 Hz, 1H, Ar-H), 5.83 (s, 1H, CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 141.6, 135.2, 128.4, 125.0, 124.2, 122.1, 116.5, 112.8, 112.7, 110.3, 107.0, 34.0. $C_{21}H_{15}Br_2N_2O$ $[M+H]^+$ 468.9546, found (ESI^+) 468.9553.

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

Specific steps for the anti-TMV[11] and fungicidal[13] activities were carried out in accordance with the literature method, also can be seen in Supporting Information.

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SUPPORTING INFORMATION

The detailed bio-assay procedures. The spectra data of arundine analogues **1**−**25**.

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