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Discovery of bitriazolyl compounds as novel antiviral candidates for combating the tobacco mosaic virus

Yi Xia,[a,†] Zhijin Fan,[b,†] Jianhua Yao,[c] Quan Liao,[c] Wei Li,[a] Fanqi Qu[a] and Ling Peng[a,d,*]

[a]*College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, PR China*
[b]*State Key Laboratory of Elemento-Organic Chemistry, Research Institute of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, PR China*
[c]*Department of Computer Chemistry and Chemoinformatics, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, PR China*
[d]*Département de Chimie, CNRS UMR 6114, 163, avenue de Luminy, 13288 Marseille, France*

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Abstract—Bitriazolyl compounds were synthesized and their activity against tobacco mosaic virus was assessed. Two of them showed promising antiviral activity and were more potent than the reference compounds. Moreover, these compounds are predicted not to be carcinogenic or mutagenic based on the prediction systems. Therefore, the bitriazolyl compounds may provide interesting new leads or scaffolds for use in further attempts to screen novel antiviral candidates.

Triazole units have been attracting considerable attention in fields such as medicinal and agrochemical research as well as in the material sciences due to their unique structure and properties. Ribavirin[1] was the first synthetic nucleoside found to show a broad spectrum of antiviral activity against many RNA and DNA viruses, and it is the only small-molecule drug available to date for treating patients infected with the hepatitis C virus.[2] Fluconazole, which bears two 1,2,4-triazole residues, is a powerful antifungal agent.[3] Recently, a 1,2,3-triazole ligand (*syn*-**1**) was found to be a potent acetylcholinesterase inhibitor.[4] In our ongoing project, which focuses on novel triazole compounds for use in the fields of medicine and agrochemicals, we are interested in developing bitriazolyl compounds (Scheme 1). Using Huisgen 1,3-dipolar cycloaddition,[5] we developed a simple and efficient procedure for synthesizing bitriazolyl compounds starting with azido-triazole and alkynes.[6] In a screening of antiviral candidates for combating the tobacco mosaic virus (TMV), we found that two of these compounds showed extremely promising antiviral activity. This suggests that bitriazolyl compounds may have considerable potential for use in the fields of agrochemical research. Here we report on the synthesis and characterization of these novel bitriazolyl compounds as well as on the assessment of their antiviral potential against the tobacco mosaic virus (TMV).

The synthesis of the bitriazolyl compounds is presented in Table 1. Under mild conditions, azido-triazole (**1**) readily engaged in a copper(I)-catalyzed Huisgen reaction with a variety of terminal acetylenes (Table 1), giving regioselectively 1,4-disubstituted 1,2,3-triazole products (**2**) with good yields.[7] The isomeric structure of the 1,4-disubstituted product was confirmed by the X-ray structure of **2f** (Fig. 1).[8] This regioselectivity is in agreement with the previously proposed reaction mechanism, where the copper (I) acetylide formed undergoes stepwise addition with the azide.[9] By treating with NH_3/MeOH, the bitriazolyl compounds (**2**) were further transformed into (**3**) with the exocyclic ester group being converted to an amide group (Table 1).[10]

Interestingly, the two triazole rings present in **2f** were found to be almost co-planar and at a dihedral angle of only 8.3 ° (Fig. 1B). This finding suggests that the bitriazolyl motif present in these compounds differs from the biphenyl motif. Biphenyl compounds are not co-planar because the four hydrogen atoms located in

Keywords: Triazoles; Antiviral candidates; Bitriazolyl compounds; Huisgen 1,3-cyclcoaddition; Tobacco mosaic virus.
* Corresponding author. Tel.: +33 4 91 82 91 54; fax: +33 4 91 82 93 01; e-mail: ling.peng@univmed.fr
† These authors contribute equally to this work.

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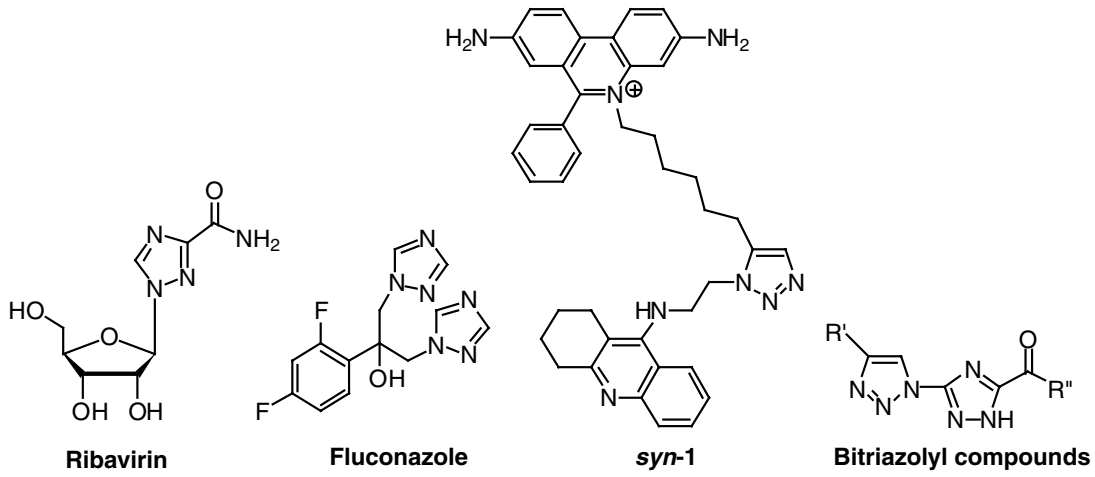
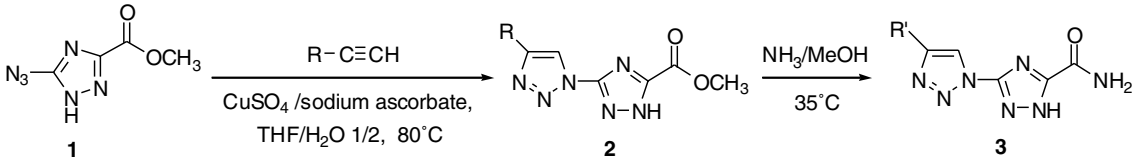
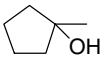
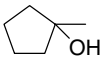
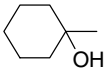
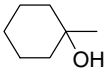


Scheme 1. Representative triazole compounds: ribavirin, fluconazole, the potent AChE inhibitor *syn*-**1** and bitriazolyl compounds.

Table 1. Bitriazolyl compounds **2** and **3** synthesized from **1**



Entry	R	Product	Yield (%)	R′	Product	Yield (%)
1	$CH_3CH_2OC(=O)-$	**2a**	85.3	$H_2N-C(=O)-$	**3a**	70.0
2	$CH_3C(=O)OCH_2-$	**2b**	92.4	$HO-CH_2-$	**3b**	71.5
3		**2c**	77.5		**3c**	81.0
4		**2d**	77.7		**3d**	77.4
5		**2e**	85.1		**3e**	89.8
6		**2f**	85.0		**3f**	95.0
7	$CH_3-C_6H_4-$	**2g**	84.5	$CH_3-C_6H_4-$	**3g**	77.0
8	$CH_3O-C_6H_4-$	**2h**	82.5	$CH_3O-C_6H_4-$	**3h**	92.4
9	$F-C_6H_4-$	**2i**	70.0	$F-C_6H_4-$	**3i**	83.0
10	$H_3C(H_2C)_4-C_6H_4-$	**2j**	81.5	$H_3C(H_2C)_4-C_6H_4-$	**3j**	90.0

the *ortho*-positions result in steric hindrance, making coplanarity impossible. In order to prevent the steric hindrance which occurs when the groups are in the *ortho*-position, the proton in the 1,2,4-triazole ring was located on N2 instead of N1, as confirmed by the high-resolution X-ray structure of **2f** (Fig. 1A).

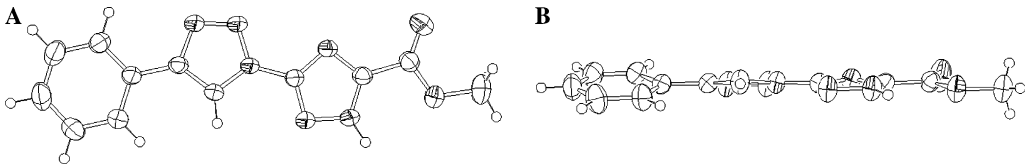


Figure 1. X-ray structure of **2f**.

Table 2. Antiviral activity of bitriazolyl compounds against TMV

Compound	Antiviral activity (%)
DHT	45 ± 5
2a	20 ± 7
2b	36 ± 8
2c	10 ± 5
2d	17 ± 8
2e	75 ± 11
2f	63 ± 5
2g	10 ± 7
2h	15 ± 11
2i	12 ± 6
2j	23 ± 12
Ribavirin	49 ± 8
3a	0
3b	8 ± 5
3c	13 ± 3
3d	0
3e	7 ± 6
3f	0
3g	6 ± 1
3h	0
3i	0
3j	28 ± 12

In our search for new antiviral candidates capable of combating the agricultural plant virus, we used the tobacco mosaic virus (TMV) as a model system for assessing the antiviral activity of the newly synthesized bitriazolyl compounds. The antiviral activity of the synthesized bitriazolyl compounds was assessed using the conventional half-leaf juice rubbing method[11,12] with the fresh leaves of tobacco plants and the commercial products, 2,4-dioxohexahydro-1,3,5-triazine (DHT) and ribavirin, as the control standards.[13,14] The antiviral activities of the bitriazolyl compounds are listed in Table 2. Compounds **2e** and **2f** showed excellent levels of activity against TMV, since the rates of antiviral efficiency of these compounds were found to be as high as 75 ± 11% and 63 ± 5%, respectively, whereas those of the reference standards, DHT and ribavirin, were only about 45 ± 5% and 49 ± 8%, respectively. The bitriazolyl compounds may therefore serve as interesting new leads for antiviral candidate capable of combating the plant virus. We are currently trying to obtain more bitriazolyl compounds with a view to establishing a reliable structure–activity study for lead development and lead optimization.

The antiviral effects of the bitriazolyl compounds **2e** and **2f** may result either directly from the inhibitory effects of the viral replication process or indirectly from the systemic acquired resistance (SAR) against TMV induced in the tobacco plant.[15] In order to elucidate the antiviral mechanism at work in **2e** and **2f**, studies were carried out

Table 3. Induction of systemic acquired resistance (SAR) of tobacco plant against TMV

Compound entry	Concentration (μg/mL)	Induction of SAR (%)	
		Leaf spray	Soil treatment
BTH	100	95 ± 4	96 ± 8
2e	100	0	0
2f	100	10 ± 5	10 ± 4

on how systemic acquired resistance against TMV was induced in tobacco, using acibenzolar-*S*-methyl (BTH, Syngenta product) as the control compound as previously described in the literature.[16,17] Our results (Table 3) indicated that, in both **2e** and **2f**, little if any induction of systemic resistance activity against TMV occurred, while the control compound, BTH, had extremely high induction effects, with the induction rate reaching more than 90%. These data indicate that the antiviral effects of compounds **2e** and **2f** are exerted via direct inhibition of the viral replication process, which requires to be studied in more detail.

With the view to undertaking further lead development and lead optimization using bitriazolyl compounds as antiviral agrochemicals capable of combating the plant virus, it is important to evaluate their toxicity due to the increasing concern about environment protection and human health. We therefore evaluated the mutagenicity/carcinogenicity of the newly synthesized bitriazolyl compounds using the programs of prediction system for carcinogenic toxicity (CISOC-PSCT) and prediction system for mutagenic toxicity (CISOC-PSMT), respectively.[18,19] Carcinogenic toxicity means the extent to which a compound is liable to cause cancer, while mutagenic toxicity indicates whether it has tendency to induce abnormal mutations. Both carcinogenicity and mutagenicity are important indicators to the toxic potential of a chemical compound. Based on the prediction systems, none of the newly synthesized bitriazolyl compounds are predicted to be carcinogenic or mutagenic (Table 4), suggesting that these compounds are promising candidates for further screening with a view to developing safe and efficacious antiviral candidates in the fields of agrochemical and possibly also pharmaceutical research.

In conclusion, we have discovered bitriazolyl compounds having antiviral activity against TMV. Two of them are of special interest, because they are more potent than the commercial products, DHT and ribavirin. These bitriazolyl compounds can be prepared conveniently and are predicted not to be carcinogenic or mutagenic based on the prediction systems. Therefore,

Table 4. Predicted results of carcinogenic and mutagenic toxicity of the bitriazolyl compounds using the CISOC-PSCT and CISOC-PSMT programs, respectively

Entry	Carcinogenicity			Mutagenicity		
	Toxicity	Non-toxicity	Predictability (%)	Toxicity	Non-toxicity	Predictability (%)
2a	0.001	0.951	78	0.01	0.50	82
2b	0.001	0.707	70	0.01	0.56	80
2c	0.001	0.952	82	0.01	0.71	84
2d	0.001	0.943	82	0.01	0.72	84
2e	0.001	0.654	81	0.01	0.57	85
2f	0.001	0.920	79	0.02	0.24	82
2g	0.001	0.837	81	0.01	0.24	84
2h	0.001	0.924	80	0.01	0.29	83
2i	0.001	0.904	80	0.02	0.23	83
2j	0.001	0.785	83	0.01	0.56	86
3a	0.001	0.979	76	0.06	0.10	80
3b	0.001	0.911	79	0.02	0.21	81
3c	0.001	0.981	82	0.01	0.47	84
3d	0.001	0.977	82	0.01	0.48	84
3e	0.001	0.791	82	0.01	0.33	86
3f	0.001	0.988	79	0.07	0.09	82
3g	0.001	0.935	81	0.07	0.09	84
3h	0.001	0.943	84	0.05	0.13	84
3i	0.001	0.982	80	0.08	0.09	83
3j	0.001	0.891	83	0.01	0.32	86

bitriazolyl compounds constitute interesting leads for the development of new antiviral candidates. Further studies on their structure–activity relationships, optimization of these compounds, and investigation of the mechanisms underlying the antiviral activity of these compounds, as well as screening bitriazolyl leads against other viruses, are actively underway in our laboratories.

Acknowledgments

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7. General procedure for preparing **2**: The azide (**1**, 0.4 mmol) and alkynes (0.48 mmol) were dissolved in a mixed solvent system (THF/H_2O, 1:2, 9 mL). Sodium ascorbate (0.2 mmol, freshly prepared solution in water) was added, followed by $CuSO_4{\cdot}5H_2O$ (0.064 mmol, freshly prepared solution in water). The yellowish mixture was stirred at 80 °C, at which point TLC analysis indicated complete consumption of **1**. The solvent in the reaction mixture was evaporated and the residue was purified by column chromatography with CH_2Cl_2/MeOH, 30:1. The product was dried in vacuo to afford **2**. Analytical data for **2a**, **2b**, **2d**, **2g**, **2h**, and **2i** can be found in Ref. 6. Compound **2c**: white solid. Mp: 143–145 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.41 (s, 1H), 5.18 (s, br, 1H), 3.94 (s, 3H), 1.98–2.03 (m, 2H), 1.78–1.93 (m, 4H), 1.71–1.76 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 157.8, 156.0, 121.0, 77.8, 53.7, 41.2, 23.9; MS (ESI): *m*/*z* 277.3 $(M-H)^-$. Compound **2e**: white solid. Mp: 161.5–162.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.69 (s, 1H), 6.58 (m, 1H), 3.91 (s, 3H), 2.35 (m, 2H), 2.14 (m, 2H), 1.67–1.68 (m, 2H), 1.58–1.60 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.1, 155.7, 151.2, 148.8, 127.6, 125.3, 119.3, 52.6, 26.4, 25.4, 22.7, 22.5; MS (ESI): *m*/*z* 273.1 $(M-H)^-$. Compound **2f**: white solid. Mp: 175.5–177 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.33 (s, 1H), 8.00 (d, 2H, J = 7.8 Hz), 7.47 (t, 2H, J_1 = 7.2 Hz, J_2 = 7.8 Hz), 7.37 (t, 1H, $J_1 = J_2$ = 7.5 Hz), 3.95 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 157.8, 154.9, 148.2, 147.7, 130.3, 129.7, 129.2, 126.3, 121.1, 53.7; MS (FAB): *m*/*z* 270 $(M)^+$. Compound **2j**: white solid. Mp: 184.5–186.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.24 (s, 1H), 7.88 (d, 2H, J = 7.8 Hz), 7.26 (d, 2H, J = 7.8 Hz), 3.93 (s, 3H), 2.57 (t, 2H, J = 7.8 Hz), 1.53–1.59 (m, 2H), 1.22–1.27 (m, 4H), 0.83 (t, 3H, J_1 = 6.6 Hz, J_2 = 6.3 Hz); ^{13}C NMR (150 MHz, DMSO-d_6): δ 159.3, 155.4, 150.2, 147.5, 143.3, 129.5, 128.0, 126.2, 120.6, 53.0, 35.6, 31.5, 31.2, 22.6, 14.6; MS (ESI): *m*/*z* 339.0 $(M-H)^-$.
8. X-ray structural analysis of **2f**: $C_{12}H_{10}N_6O_2$ (M_r 270.26), monoclinic space group $P\,c$, Z = 2, a = 8.1410 (6), b = 7.4730 (2), c = 10.6310 (3) Å, α = 90.00, β = 105.7371 (10), γ = 90.00, V = 622.52 (3) Å^3, MoK_α radition,

$\lambda = 0.71073$ Å, $3.77° < \theta < 25.60°$, 1032 reflections, $T = 293$ K on a Brucker-Nonius Kappa CCD. The structure was solved using direct methods (SHELXS97) and refined with SHELXL97 to final R ($F^2 > 2\sigma F^2$) = 0.0285 and $wR = 0.0777$ [$w = 1/[\sigma^2(F_o^2) + (0.0516P)^2 + 0.0546P]$, where $P = (F_o^2 + 2F_c^2)/3$]. The X-ray structure data have been deposited in the Cambridge Crystallographic Data Center with deposition No. CCDC 297233. Copy of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ UK (e-mail: deposit@ccdc.cam.ac.uk).

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10. General procedure for preparing **3**: a solution of **2** (0.3 mmol) in methanol saturated with ammonia (20 mL) was kept at 35 °C for 36–72 h. The solvent was evaporated under reduced pressure and the residue was chromatographed on a silica gel column with CH_2Cl_2/MeOH, 4:1, yielding the product **3**. Compound **3a**: white solid. Mp: >295 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.78 (s, 1H), 8.01 (s, br, 1H), 7.58 (s, br, 2H), 7.22 (s, br, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 163.7, 162.0, 157.5, 155.7, 143.1, 125.3; MS (ESI): *m*/*z* 221.1 $(M-H)^-$. Compound **3b**: white solid. Mp: 247–249 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.38 (s, 1H), 8.11 (s, br, 1H), 7.81 (s, br, 1H), 5.28 (s, br, 1H), 4.59 (s, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 159.6, 155.3, 152.6, 149.1, 122.6, 55.4; MS (ESI): *m*/*z* 208.0 $(M-H)^-$. Compound **3c**: white solid. Mp: 240–242 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.36 (s, br, 1H), 8.34 (s, 1H), 8.06 (s, br, 1H), 5.22 (s, br, 1H), 1.98–2.03 (m, 3H), 1.82–1.91 (m, 3H), 1.72–1.73 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 159.4, 155.4, 154.8, 151.9, 121.0, 78.1, 40.9, 23.7; MS (ESI): *m*/*z* 262.0 $(M-H)^-$. Compound **3d**: white solid. Mp: >216 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.32 (s, 1H), 8.26 (s, br, 1H), 7.96 (s, br, 1H), 5.04 (s, br, 1H), 1.91–2.01 (m, 3H), 1.65–1.78 (m, 4H), 1.33–1.47 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 159.2, 156.9, 155.3, 152.1, 120.9, 68.6, 38.3, 25.8, 22.3; MS (ESI): *m*/*z* 276.1 $(M-H)^-$. Compound **3e**: white solid. Mp: 233–235 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.58 (s, 1H), 8.35 (s, br, 1H), 8.04 (s, br, 1H), 6.58 (m, 1H), 2.36–2.37 (m, 2H), 2.16–2.17 (m, 2H), 1.69–1.72 (m, 2H), 1.59–1.63 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 157.8, 154.3, 150.8, 148.3, 126.5, 125.1, 118.7, 25.6, 24.6, 21.9, 21.7; MS (ESI): *m*/*z* 258.2 $(M-H)^-$. Compound **3f**: white solid. Mp: 246.5–248.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.16 (s, 1H), 8.25 (s, br, 1H), 7.98 (d, 3H, $J = 7.5$ Hz), 7.47 (t, 2H, $J_1 = 7.5$ Hz, $J_2 = 6.9$ Hz), 7.37 (t, 1H, $J_1 = J_2 = 6.6$ Hz); ^{13}C NMR (150 MHz, DMSO-d_6): δ 159.5, 155.2, 152.7, 147.3, 130.5, 129.7, 129.1, 126.2, 121.2; MS (ESI): *m*/*z* 254.1 $(M-H)^-$. Compound **3g**: white solid. Mp: 246–248 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.07 (s, 1H), 8.21 (s, br, 1H), 7.85 (d, 3H, $J = 8.1$ Hz), 7.27 (d, 2H, $J = 8.1$ Hz), 2.32 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.6, 155.5, 153.7, 147.2, 138.4, 130.2, 127.9, 126.1, 120.7, 21.6; MS (ESI): *m*/*z* 268.1 $(M-H)^-$. Compound **3h**: white solid. Mp: 241.5–243.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.99 (s, 1H), 8.11 (s, br, 1H), 7.89 (d, 2H, $J = 8.7$ Hz), 7.79 (s, br, 1H), 7.02 (d, 2H, $J = 8.7$ Hz), 3.78 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 163.7, 159.8, 157.3, 156.1, 146.5, 127.4, 123.8, 119.8, 115.0, 55.8; MS (ESI): *m*/*z* 284.2 $(M-H)^-$. Compound **3i**: white solid. Mp: >249 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.12 (s, 1H), 8.17 (s, br, 1H), 8.03 (m, 2H), 7.90 (s, br, 1H), 7.32 (dd, 2H, $^3J_{HF} = 8.7$ Hz, $^3J_{HH} = 8.7$ Hz); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.7 ($^1J_{CF} = 243.5$ Hz), 159.8, 155.3, 153.0, 146.4, 128.3 ($^3J_{CF} = 7.4$ Hz), 127.1, 121.1, 116.6 ($^2J_{CF} = 21.3$ Hz); MS (ESI): *m*/*z* 272.0 $(M-H)^-$. Compound **3j**: white solid. Mp: >235 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.09 (s, 1H), 8.26 (s, br, 1H), 7.97 (s, br, 1H), 7.88 (d, 2H, $J = 8.1$ Hz), 7.29 (d, 2H, $J = 7.8$ Hz), 2.60 (t, 2H, $J = 7.5$ Hz), 1.57–1.62 (m, 2H), 1.27–1.30 (m, 4H), 0.86 (t, 3H, $J_1 = 6.6$ Hz, $J_2 = 6.9$ Hz); ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.9, 155.1, 152.1, 147.5, 143.4, 129.6, 128.0, 126.2, 120.8, 35.5, 31.5, 31.2, 29.4, 22.6, 14.6; MS (ESI): *m*/*z* 324.1 $(M-H)^-$.
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12. Antiviral evaluation against TMV: antiviral activity of title compounds against TMV was performed by the conventional half-leaf method. Fresh leaves of tobacco plant with 4–6 leaves, which had been inoculated by the juice-leaf rubbing method with carborundum (Mesh 400) and mechanical inoculation according to Ref. 11, were cut into two halves along the main vein. Of the same leaf, one-half was immersed into the solution of 500 μg/mL title compound and the other half the double distilled water, respectively, for 20 min. The concentration of TMV inoculation was 5.88×10^{-2} μg/mL. Then, the two separated half leaves were cultured at 25 °C for 72 h. Standard of 2,4-dioxohexahydro-1,3,5-triazine (DHT) and ribavirin were used as control. All treatments were repeated for three times at least. The inhibition percentage was calculated by comparing the average numbers of the viral inflammations on the two halves of leaves according to Formula 1.

$$Y = \frac{CK - A}{CK} \times 100 \quad \text{(Formula 1)},$$

where Y is the antiviral inhibition percentage (%); CK is the average numbers of viral inflammations of the leaf treated with double distilled water; A is the average numbers of viral inflammations of the treated half leaf by title compound. The effects of direct antiviral activity were divided into four levels: excellent, $Y > 50\%$; good, Y: 50–30%; bad, Y: 20–30%; no activity, $Y < 20\%$.
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14. (a) Lerch, B. *Antiviral Res.* **1987**, *7*, 257; (b) Ribavirin is a reference standard used in our laboratories for more than 10 years and its average inhibition against TMV is around 50%.
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16. Siegrist, J.; Orober, M.; Buchenauer, H. *Physiol. Mol. Plant Pathol.* **2000**, *56*, 95.
17. Evaluation of induction of systemic acquired resistance against TMV: 20 mL of 100 μg/mL tested compound solution was sprayed to 4–5 leaves of tobacco plant of *Nicotiana tabacum* L. var. *Xanthi-NC* twice in the two consecutive days or by soil treatment twice. TMV of 5.88×10^{-2} μg/mL was inoculated 7 days later in the top leaf which has grown after the compound application. Each experiment was repeated for five times, while the double distilled water and acibenzolar-*S*-methyl (BTH) were used as reference control of standard plant activator. The treated tobacco plant was placed at 25 °C for 72 h of cultivation. The induction activity of antiviral efficacy was calculated by comparing the average numbers of the viral inflammations on the top leaf with corresponding control according to the following Formula 2:

$$I = \frac{CK - T}{CK} \times 100 \quad \text{(Formula 2)},$$

where I is the activities of systemic acquired resistance (%); CK is the average numbers of viral inflammations of control leaves; T is the average numbers of viral inflam-

mations of the inoculated leaves. The induction activities of systemic acquired resistance were divided into four levels: excellent, $I > 70\%$; good, I: 50–70%; bad, I: 30–50%; no induction activity, $I < 30\%$.

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19. The accuracy of the prediction model in carcinogenic toxicity is about 84% and that in mutagenic toxicity is about 85%. For the results of PSCT, if the predictability is greater than 70%, non-toxicity is greater than 0.65 and toxicity is less than non-toxicity, the compound is not carcinogenic and its reliability is about 85%. For PSMT, if the predictability is greater than 80% and toxicity is less than non-toxicity, the compound is not mutagenic and its reliability is about 84%.