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Note

Semisynthesis and acaricidal activities of isoxazole and pyrazole derivatives of a natural product bisdemethoxycurcumin

Jinxiang Luo, Wei Ding,* Yongqiang Zhang, Zhenguo Yang, Yang Li and Lijuan Ding

College of Plant Protection, Southwest University, Chongqing 400715, People's Republic of China

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Sixteen isoxazole and pyrazole derivatives of bisdemethoxycurcumin (BDMC) modified in β-diketone were synthesized, and their structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. Preliminary acaricidal activities against female adults of *Tetranychus cinnabarinus* (Boisduval) and *Panonychus citri* (McGregor) were evaluated using the slide-dip method. The results indicated that some of these compounds exhibit more pronounced acaricidal activity than BDMC, especially compound **4**, which displays acaricidal activity comparable to that of pyridaben.

Keywords: bisdemethoxycurcumin, semisynthesis, isoxazole, pyrazole, acaricidal activity.

Introduction

The carmine spider mite, *Tetranychus cinnabarinus* (Boisduval), and the citrus red mite, *Panonychus citri* (McGregor), are economically important mite pests of vegetable crops and fruit trees in China.[1–3] *T. cinnabarinus* has a worldwide distribution and infests over 100 crops or plants grown in the field or greenhouse.[4–8] This mite is a major pest, especially of cotton, beans, eggplants, tomatoes, peppers, and cucurbits.[9–11] *P. citri* is an important pest that attacks over 80 plant species worldwide.[12,13] This mite particularly devastates both deciduous and evergreen fruit trees, such as citrus, pear, peach, and holly.[14–16] Over the last several decades, the control of these two mite pests has depended mainly on sprays of synthetic chemical acaricides. However, the repeated application of those agrochemicals has led to the development of resistance in these two mite populations[17–24] as well as environmental problems. Thus, developing pest mite management methods from natural products is highly desirable.[25]

Curcuminoids, namely, curcumin, demethoxycurcumin, and bisdemethoxycurcumin (BDMC), are naturally occurring polyphenols that have a wide spectrum of biological activities,[26] such as acaricidal, anticarcinogenic, antioxidant, anticancer, and anti-inflammatory activities *in vitro* and *in vivo*.[27–34] Moreover, their activities differ according to the biological process and cell type involved.[34] Recent studies show that BDMC has more potent acaricidal effects than other curcuminoids.[27] However, the acaricidal activity of BDMC is still lower than that of registered miticides; thus, its use in agriculture is limited. In this study, BDMC was chosen as the lead compound and modified in β-diketone. A series of BDMC derivatives were designed by introducing isoxazole and pyrazole groups into the β-diketone moiety of BDMC. We also investigated whether the acaricidal activity of these derivatives could be improved compared with that of BDMC.

Materials and Methods

1. Instruments and reagents

Melting points were determined on a WRS-1A digital melting-point apparatus without calibration. IR spectra were recorded on a Bruker TENSOR 27 FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX400 instrument in DMSO-d_6 using tetramethylsilane as the internal standard. Mass spectra were recorded on a Shimadzu GCMS-QP2010 Ultra instrument. Elemental analyses were carried out using a Vario EL III Elemental Analyzer.

BDMC was purchased from Hebei Food Additive Co., Ltd. (China). Hydroxylamine hydrochloride and different hydrazine derivatives (hydrochloride or sulfate) were purchased from Taicang Hualian Chemical Industry Co., Ltd. (China). Pyridaben 95% TC was purchased from First Nanjing Pesticide Factory (China). BDMC was used after purification, and all other chemical materials were used as purchased and were of analytical grade. Analytical thin-layer chromatography (TLC) was performed on a glass plate coated with silica gel GF-254 and visualized in UV light (254 nm). Column chromatography was performed on silica gel (200 to 300 mesh).

2. Synthesis of compounds

2.1. Synthesis of 4-{(E)-2-[5-((E)-4-hydroxystyryl)isoxazol-3-yl]vinyl}phenol (**2**)

Sodium ethylare (0.21 g, 2.5 mmol) was added to a solution of hydroxylamine hydrochloride (0.18 g, 2.5 mmol) in EtOH (8 mL), and the reaction mixture was stirred at 65°C for 15 min. The resulting precipitate was removed by filtration to give a solution of hydroxylamine. This solution was directly added to a BDMC solution (0.62 g, 2 mmol) in AcOH (10 mL) and stirred at 85°C for 10 hr (the reaction progress was monitored by TLC

* To whom correspondence should be addressed.
E-mail: dwing818@163.com
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with UV detection). After completion of the reaction, the solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc, washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude products were purified by column chromatography using petroleum ether/ethyl acetate as the eluent to give compound (**2**) (0.36 g, 59% yield) as a white solid; m.p. 274–275°C; IR ν_{max} (KBr) cm^{-1}: 3323 (Ar-OH), 3111 (Ar-H), 1596, 1508, 1445 (Ar C-C); ^1H NMR (DMSO-d_6) δ: 6.81–6.83 (m, 4H, Ar-H), 6.88 (s, 1H, C_4-H), 7.03 (d, J=3.6 Hz, 2H, C_2-H and C_6-H), 7.30 (dd, J=4.9 Hz, 0.8 Hz, 2H, C_1-H and C_7-H), 7.49–7.54 (m, 4H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 97.80, 109.97, 112.20, 115.55, 126.56, 128.88, 134.34, 136.05, 158.26, 162.13, 168.22; ESI-MS m/z (%): 304 $[M–H]^+$ (100); Anal. Found: C, 74.62; H, 4.97; N, 4.62%; Calcd. for $C_{19}H_{15}NO_3$: C, 74.75; H, 4.92; N, 4.59%.

2.2. Synthesis of 4-{(E)-2-[5-((E)-4-hydroxystyryl)-1H-pyrazol-3-yl]vinyl}phenol (***3***)

Hydrazine hydrate (0.16 g, 2.5 mmol) was added to a BDMC solution (0.62 g, 2 mmol) in AcOH (10 mL) and the mixture was heated to reflux and kept for 10 hr. After completion of the reaction, the product was purified according to the aforementioned process to give (**3**) (0.37 g, 60% yield) as a white solid; m.p. 278–279°C; IR ν_{max} (KBr) cm^{-1}: 3588 (Ar-OH), 3284 (N-H), 3016 (Ar-H), 1594, 1497, 1461 (Ar C-C); ^1H NMR (DMSO-d_6) δ: 6.64 (s, 1H, C_4-H), 6.78–6.91 (m, 4H, Ar-H), 7.01 (d, J=4.2 Hz, 2H, C_2-H and C_6-H), 7.08 (d, J=4.1 Hz, 2H, C_1-H and C_7-H), 7.38 (d, J=1.3 Hz, 4H, Ar-H), 12.83 (s, 1H, N-H); ^{13}C NMR (DMSO-d_6) δ: 99.12, 112.30, 115.49, 118.07, 127.55, 128.78, 129.61, 141.69, 150.78, 156.92, 157.37; ESI-MS m/z (%): 303 $[M–H]^+$ (100); Anal. Found: C, 74.89; H, 5.31; N, 9.56%; Calcd. for $C_{19}H_{16}N_2O_2$: C, 75.00; H, 5.26; N, 9.21%.

2.3. General procedure for the synthesis of ***4–17***

NaOH (0.12 g, 3 mmol) was added to a solution of different hydrazine derivatives (hydrochloride or sulfate) (3 mmol) in water (6 mL) and stirred at 60°C for 15 min. The mixture was then extracted with EtOAc (2×5 mL) (the solution of containing methyl hydrazine was not extracted and was used directly for the next step), and the solvent of the combined organic layers was removed to give different hydrazine derivatives. These hydrazine derivatives were added to a BDMC solution (0.62 g, 2 mmol) in AcOH (10 mL), and the reaction mixture was heated to reflux and kept for 10–16 hr. After completion of the reaction, the product was purified according to the aforementioned process to yield the products **4**–**17**.

4-{(*E*)-2-[5-((*E*)-4-hydroxystyryl)-1-methyl-1*H*-pyrazol-3-yl]vinyl}phenol (**4**) Yield: 45%, white solid, m.p. 280.2–281.0°C; IR ν_{max} (KBr) cm^{-1}: 3340 (Ar-OH), 3015 (Ar-H), 2940 (–CH_3), 1594, 1515, 1446 (Ar C-C), 1371 (N-C); ^1H NMR (DMSO-d_6) δ: 3.86 (s, 3H, CH_3), 6.75–6.86 (m, 4H, Ar-H), 6.87 (s, 1H, C_4-H), 6.98 (d, J=1.2 Hz, 2H, C_2-H and C_6-H), 7.02 (d, J=1.2 Hz, 2H, C_1-H and C_7-H), 7.43 (dd, J=13 Hz, 2.1 Hz, 4H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 36.15, 98.38, 111.60, 115.39, 117.52, 127.51, 128.04, 128.60, 131.34, 142.09, 148.77, 156.91, 157.47; ESI-MS m/z (%): 318 $[M]^+$ (100); Anal. Found: C, 75.50; H, 5.71; N, 8.78%; Calcd. for $C_{20}H_{18}N_2O_2$: C, 75.47; H, 5.66; N, 8.81%.

4-{(*E*)-2-[5-((*E*)-4-hydroxystyryl)-1-*tert*-butyl-1*H*-pyrazol-3-yl]vinyl}phenol (**5**) Yield: 58%, white solid, m.p. 255–256°C; IR ν_{max} (KBr) cm^{-1}: 3525 (Ar-OH), 3015 (Ar-H), 2995 (–CH_3), 1594, 1512, 1446 (Ar C-C), 1357 (N-C); ^1H NMR (DMSO-d_6) δ: 1.63 (s, 9H, CH_3), 6.77–6.86 (m, 4H, Ar-H), 6.90 (s, 1H, C_4-H), 6.98 (dd, J=7.0 Hz, 2.8 Hz, 2H, C_2-H and C_6-H), 7.15 (d, J=3.9 Hz, 2H, C_1-H and C_7-H), 7.43 (dd, J=10.9 Hz, 2.2 Hz, 4H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 30.40, 59.55, 101.57, 114.06, 115.49, 117.96, 127.51, 128.51, 131.17, 141.67, 146.90, 156.86, 157.48; ESI-MS m/z (%): 360 $[M]^+$ (100); Anal. Found: C, 76.51; H, 6.58; N, 7.69%; Calcd. for $C_{23}H_{24}N_2O_2$: C, 76.67; H, 6.67; N, 7.78%.

4-{(*E*)-2-[5-((*E*)-4-hydroxystyryl)-1-phenyl-1*H*-pyrazol-3-yl]vinyl}phenol (**6**) Yield, 84%, white solid, m.p. 231–232°C; IR ν_{max} (KBr) cm^{-1}: 3237 (Ar-OH), 3013 (Ar-H), 1594, 1504, 1459 (Ar C-C), 1371 (N-C); ^1H NMR (DMSO-d_6) δ: 6.70–6.80 (m, 4H, Ar-H), 6.96 (d, J=4.1 Hz, 2H, C_2-H and C_6-H), 7.08 (s, 1H, C_4-H), 7.18 (dd, J=7.0 Hz, 3.0 Hz, 2H, C_1-H and C_7-H), 7.34 (d, J=2.2 Hz, 2H, Ar-H), 7.43–7.49 (m, 3H, Ar-H), 7.52–7.60 (m, 4H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 100.43, 111.70, 115.51, 116.98, 124.84, 127.15, 128.09, 129.27, 130.26, 132.29, 139.08, 142.19, 150.91, 157.18, 157.69; ESI-MS m/z (%): 380 $[M]^+$ (100); Anal. Found: C, 78.88; H, 5.22; N, 7.41%; Calcd. for $C_{25}H_{20}N_2O_2$: C, 78.95; H, 5.26; N, 7.37%.

4-{(*E*)-2-[5-((*E*)-4-hydroxystyryl)-1-*p*-tolyl-1*H*-pyrazol-3-yl]vinyl}phenol (**7**) Yield, 83%, light yellow solid, m.p. 252–253°C; IR ν_{max} (KBr) cm^{-1}: 3404 (Ar-OH), 3021 (Ar-H), 2949 (–CH_3), 1594, 1516, 1446 (Ar C-C), 1368 (N-C); ^1H NMR (DMSO-d_6) δ: 2.40 (s, 3H, Ar-CH_3), 6.67–6.79 (m, 4H, Ar-H), 6.95 (d, J=4.1 Hz, 2H, C_2-H and C_6-H), 7.05 (s, 1H, C_4-H), 7.16 (dd, J=7.4 Hz, 3.3 Hz, 2H, C_1-H and C_7-H), 7.31–7.44 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 20.55, 100.14, 111.75, 115.50, 117.07, 124.74, 127.17, 128.04, 129.67, 130.09, 132.08, 136.67, 137.13, 142.12, 150.68, 157.14, 157.65; ESI-MS m/z (%): 394 $[M]^+$ (100); Anal. Found: C, 79.22; H, 5.46; N, 7.20%; Calcd. for $C_{26}H_{22}N_2O_2$: C,79.19; H, 5.58; N, 7.11%.

4-{(*E*)-2-[5-((*E*)-4-hydroxystyryl)-1-(4-methoxyphenyl)-1*H*-pyrazol-3-yl]vinyl} phenol (**8**) Yield, 62%, light yellow solid, m.p. 245–246°C; IR ν_{max} (KBr) cm^{-1}: 3448 (Ar-OH), 3017 (Ar-H), 1593, 1516, 1455 (Ar C-C), 1369 (N-C); ^1H NMR (DMSO-d_6) δ: 3.84 (s, 3H, Ar-OCH_3), 6.63–6.80 (m, 4H, Ar-H), 6.95 (d, J=4.1 Hz, 2H, C_2-H and C_6-H), 7.03 (s, 1H, C_4-H), 7.09–7.19(m, 4H, Ar-H), 7.32 (d, J=2.1 Hz, 2H, C_1-H and C_7-H), 7.42–7.44 (m, 4H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 55.34, 99.81, 111.73, 114.31, 115.51, 117.13, 126.46, 127.20, 128.03, 129.92, 131.98, 132.12, 142.20, 150.45, 157.14, 157.64, 158.52; ESI-MS m/z (%): 410 $[M]^+$ (100); Anal. Found: C, 76.29; H, 5.33; N, 6.88%; Calcd. for $C_{26}H_{22}N_2O_3$: C,76.10; H, 5.37; N, 6.83%.

4-{(*E*)-2-[5-((*E*)-4-hydroxystyryl)-1-(3,4-dimethylphenyl)-1*H*-pyrazol-3-yl]vinyl} phenol (**9**) Yield, 76%, white solid, m.p. 249–250°C; IR ν_{max} (KBr) cm^{-1}: 3372 (Ar-OH), 3013 (Ar-H),

2950 (–CH_3), 1593, 1506, 1449 (Ar C-C), 1368 (N-C); ^{1}H NMR (DMSO-d_6) δ: 2.31 (s, 6H, Ar-CH_3), 6.66-6.80 (m, 4H, Ar-H), 6.94 (d, J=4.1 Hz, 2H, C_2-H and C_6-H), 7.04 (s, 1H, C_4-H), 7.16 (dd, J=7.2 Hz, 3.1 Hz, 2H, C_1-H and C_7-H), 7.31–7.44 (m, 7H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 18.94, 19.27, 100.02, 111.83, 115.45, 117.12, 122.19, 125.90, 127.22, 128.04, 129.93, 130.01, 131.96, 135.98, 136.88, 137.48, 142.10, 150.58, 157.17, 157.66; ESI-MS *m*/*z* (%): 408 $[M]^+$ 100; Anal. Found: C, 79.44; H, 5.85; N, 6.91%; Calcd. for $C_{27}H_{24}N_2O_2$: C, 79.41; H, 5.88; N, 6.86%.

4-{(*E*)-2-[5-((*E*)-4-hydroxystyryl)-1-(4-fluorophenyl)-1*H*-pyrazol-3-yl]vinyl} phenol (**10**) Yield, 56%, white solid, m.p. 231–232°C; IR ν_{max} (KBr) cm^{-1}: 3526 (Ar-OH), 3017 (Ar-H), 1597, 1511, 1446 (Ar C-C), 1366(N-C); ^{1}H NMR (DMSO-d_6, 400 Hz) δ: 6.66–6.80 (m, 4H, Ar-H), 6.95 (d, J=4.1 Hz, 2H, C_2-H and C_6-H), 7.07 (s, 1H, C_4-H), 7.17 (dd, J=6.9 Hz, 2.8 Hz, 2H, C_1-H and C_7-H), 7.35 (d, J=2.1 Hz, 2H, Ar-H), 7.39–7.44 (m, 4H, Ar-H), 7.55–7.59 (m, 2H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 100.34, 111.50, 115.51, 116.00, 116.93, 127.14, 127.78, 128.18, 130.34, 132.48, 135.55, 142.42, 150.93, 157.21, 157.73, 159.81, 162.24; ESI-MS *m*/*z* (%): 398 $[M]^+$ (100); Anal. Found: C, 75.24; H, 4.83; N, 7.10%; Calcd. for $C_{25}H_{19}N_2O_2F_1$: C, 75.38; H, 4.77; N, 7.04%.

4-{(*E*)-2-[5-((*E*)-4-hydroxystyryl)-1-(2-fluorophenyl)-1*H*-pyrazol-3-yl]vinyl} phenol (**11**) Yield, 67%, white solid, m.p. 229–230°C; IR ν_{max} (KBr) cm^{-1}: 3526 (Ar-OH), 3017 (Ar-H), 1597, 1511, 1446 (Ar C-C), 1366 (N-C); ^{1}H NMR (DMSO-d_6) δ: 6.74–6.80 (m, 4H, Ar-H), 6.94 (d, J=4.1 Hz, 2H, C_2-H and C_6-H), 7.09 (s, 1H, C_4-H), 7.17 (dd, J=5.2 Hz, 1.1 Hz, 2H, C_1-H and C_7-H), 7.29 (d, J=2.2 Hz, 2H, Ar-H), 7.41–7.54 (m, 4H, Ar-H), 7.58–7.60 (m, 2H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 99.50, 110.80, 113.52, 114.63, 115.55, 116.87, 118.56, 124.40, 125.23, 126.97, 127.81, 129.34, 130.49, 132.48, 136.69, 143.66, 148.81, 151.58, 154.93, 157.27, 157.41; ESI-MS *m*/*z* (%): 398 $[M]^+$ (100); Anal. Found: C, 75.31; H, 4.81; N, 7.08%; Calcd. for $C_{25}H_{19}N_2O_2F_1$: C,75.38; H, 4.77; N, 7.04%.

4-{(*E*)-2-[5-((*E*)-4-hydroxystyryl)-1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]vinyl}phenol (**12**) Yield, 89%, white solid, m.p. 261–262°C; IR ν_{max} (KBr) cm^{-1}: 3526 (Ar-OH), 3019 (Ar-H), 1597, 1510, 1445 (Ar C-C), 1373(N-C); ^{1}H NMR (DMSO-d_6) δ: 6.73–6.81 (m, 4H, Ar-H), 6.95 (d, J=4.2 Hz, 2H, C_2-H and C_6-H), 7.09 (s, 1H, C_4-H), 7.18 (dd, J=6.7 Hz, 3.1 Hz, 2H, C_1-H and C_7-H), 7.38–7.62 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 100.75, 111.47, 115.47, 116.79, 126.37, 127.10, 127.80, 128.24, 129.28, 130.57, 131.85, 132.68, 137.92, 142.41, 151.23, 157.22, 157.73; ESI-MS *m*/*z* (%): 414 $[M]^+$ (100); Anal. Found: C, 72.49; H, 4.76; N, 6.85%; Calcd. for $C_{25}H_{19}N_2O_2Cl_1$: C, 72.46; H, 4.59; N, 6.76%.

4-{(*E*)-2-[5-((*E*)-4-hydroxystyryl)-1-(3-chlorophenyl)-1*H*-pyrazol-3-yl]vinyl} phenol (**13**) Yield, 86%, white solid, m.p. 217.8–218.5°C; IR ν_{max} (KBr) cm^{-1}: 3524 (Ar-OH), 3025 (Ar-H), 1592, 1511, 1446 (Ar C-C), 1365 (N-C); ^{1}H NMR (DMSO-d_6) δ: 6.74–6.81 (m, 4H, Ar-H), 6.96 (d, J=4.2 Hz, 2H, C_2-H and C_6-H), 7.09 (s, 1H, C_4-H), 7.20 (dd, J=5.9 Hz, 1.8 Hz, 2H, C_1-H and C_7-H), 7.38 (d, J=2.0 Hz, 1H, Ar-H), 7.44 (d, J=2.0 Hz, 1H, Ar-H), 7.50–7.55 (m, 2H, Ar-H), 7.58–7.63 (m, 4H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 101.08, 111.49, 115.56, 116.76, 123.18, 124.47, 127.11, 127.85, 128.24, 130.80, 132.82, 133.57, 140.35, 142.52, 151.40, 157.31, 157.83; ESI-MS *m*/*z* (%): 414 $[M]^+$ (100); Anal. Found: C, 72.45; H, 4.61; N, 6.77%; Calcd. for $C_{25}H_{19}N_2O_2Cl_1$: C, 72.46; H, 4.59; N, 6.76%.

4-{(*E*)-2-[5-((*E*)-4-hydroxystyryl)-1-(4-bromophenyl)-1*H*-pyrazol-3-yl]vinyl} phenol (**14**) Yield, 90%, white solid, m.p. 272–273°C; IR ν_{max} (KBr) cm^{-1}: 3529 (Ar-OH), 3021 (Ar-H), 1596, 1504, 1446 (Ar C-C), 1371 (N-C); ^{1}H NMR (DMSO-d_6) δ: 6.72–6.80 (m, 4H, Ar-H), 6.95 (d, J=4.1 Hz, 2H, C_2-H and C_6-H), 7.09 (s, 1H, C_4-H), 7.19 (dd, J=7.5 Hz, 3.5 Hz, 2H, C_1-H and C_7-H), 7.41 (dd, J=8.0 Hz, 2.2 Hz, 4H, Ar-H), 7.50 (d, J=2.2 Hz, 2H, Ar-H), 7.76 (d, J=2.1 Hz, 2H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 100.87, 111.54, 115.55, 116.85, 120.30, 126.67, 127.16, 127.71, 128.31, 130.65, 132.26, 132.76, 138.39, 142.44, 151.34, 157.32, 157.83; ESI-MS *m*/*z* (%): 458 $[M-H]^+$ (100); Anal. Found: C, 65.11; H, 4.23; N, 6.12%; Calcd. for $C_{25}H_{19}N_2O_2Br_1$: C, 65.36; H, 4.14; N, 6.10%.

4-{(*E*)-2-[5-((*E*)-4-hydroxystyryl)-1-(4-nitrophenyl)-1*H*-pyrazol-3-yl]vinyl}phenol (**15**) Yield, 37%, light yellow solid, m.p. 229–230°C; IR ν_{max} (KBr) cm^{-1}: 3529 (Ar-OH), 3117 (Ar-H), 1595, 1506, 1446 (Ar C-C), 1323 (N-C); ^{1}H NMR (DMSO-d_6) δ: 6.78–6.84 (m, 4H, Ar-H), 6.99 (d, J=4.1 Hz, 2H, C_2-H and C_6-H), 7.12 (s, 1H, C_4-H), 7.23 (dd, J=9.7 Hz, 2.3 Hz, 2H, C_1-H and C_7-H), 7.42–7.58 (m, 4H, Ar-H), 7.84 (d, J=2.2 Hz, 2H, Ar-H), 8.42 (d, J=2.2 Hz, 2H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 102.28, 111.46, 115.49, 116.40, 122.87, 124.49, 125.65, 126.32, 127.05, 127.97, 128.56, 130.26, 131.58, 133.46, 137.71, 142.35, 144.13, 145.36, 150.55, 152.38, 155.44, 157.43, 157.91; ESI-MS *m*/*z* (%): 425 $[M]^+$ (100); Anal. Found: C, 70.43; H, 4.51; N, 9.86%; Calcd. for $C_{25}H_{19}N_2O_2$: C, 70.59; H, 4.47; N, 9.88%.

4-{(*E*)-2-[5-((*E*)-4-hydroxystyryl)-1-(2,4-dinitrophenyl)-1*H*-pyrazol-3-yl]vinyl} phenol (**16**) Yield, 31%, red solid, m.p. 247–248°C; IR ν_{max} (KBr) cm^{-1}: 3454 (Ar-OH), 3104 (Ar-H), 1603, 1505, 1446 (Ar C-C), 1330 (N-C); ^{1}H NMR (DMSO-d_6) δ: 6.81–6.89 (m, 4H, Ar-H), 6.94 (d, J=2.1 Hz, 2H, C_2-H and C_6-H), 7.09 (s, 1H, C_4-H), 7.21 (dd, J=6.7 Hz, 2.3 Hz, 2H, C_1-H and C_7-H), 7.71 (dd, J=14.1 Hz, 2.1 Hz, 4H, Ar-H), 8.06 (d, J=2.3 Hz, 1H, Ar-H), 8.35 (d, J=2.4 Hz, 1H, Ar-H), 8.86 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 101.98, 111.51, 115.71, 116.48, 123.01, 124.69, 128.45, 129.25, 132.02, 134.18, 136.44, 144.20, 149.84, 159.73; ESI-MS *m*/*z* (%): 470 $[M]^+$ (100); Anal. Found: C, 64.00; H, 3.88; N, 12.03%; Calcd. for $C_{25}H_{18}N_4O_6$: C, 63.83; H, 3.83; N, 11.91%.

4-[3,5-bis((*E*)-4-hydroxystyryl)-1*H*-pyrazol-1-yl]benzenesulfonamide (**17**) Yield, 60%, light yellow solid, m.p. 297–298°C; IR ν_{max} (KBr) cm^{-1}: 3345, 3310 (–NH_2), 3021 (Ar-H), 1596, 1506, 1447 (Ar C-C), 1326 (N-C); ^{1}H NMR (DMSO-d_6) δ: 2.51 (s, 2H, NH_2), 6.79–6.82 (m, 4H, Ar-H), 6.98 (d, J=4.1 Hz, 2H, C_2-H and C_6-H), 7.08 (s, 1H, C_4-H), 7.21 (dd, J=11.7 Hz, 3.6 Hz, 2H, C_1-H and C_7-H), 7.42 (dd, J=7.6 Hz, 2.1 Hz, 4H, Ar-H), 7.76 (d, J=2.1 Hz, 2H, Ar-H), 8.01 (d, J=2.2 Hz, 2H, Ar-H);

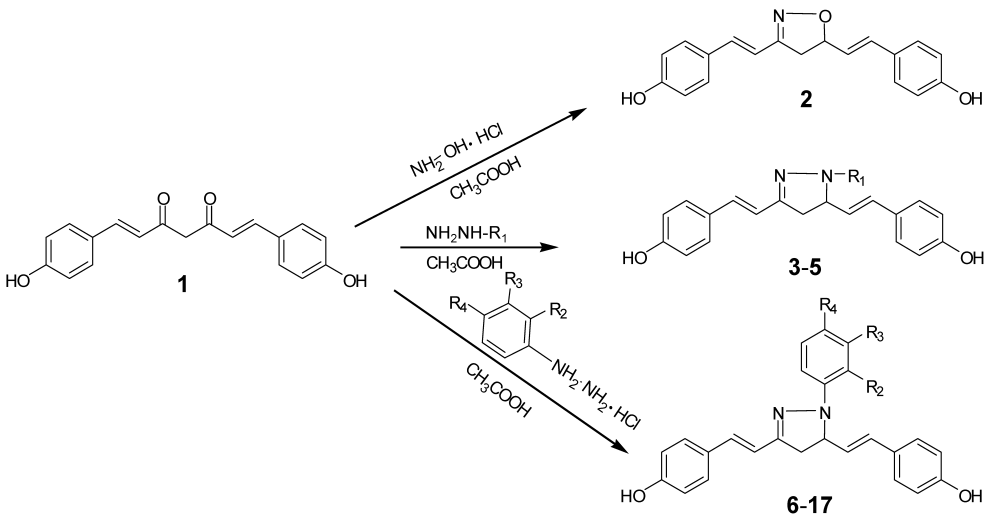

Fig. 1. Synthetic route for the preparation of compounds **2** to **17**.

^{13}C NMR (DMSO-d_6) δ: 101.39, 111.41, 115.53, 116.57, 124.57, 126.97, 127.54, 127.86, 128.29, 131.03, 133.07, 141.50, 142.31, 142.63, 151.73, 157.23, 157.84; ESI-MS *m/z* (%): 458 $[M-H]^+$ (100); Anal. Found: C, 65.26; H, 4.68; N, 9.26%; Calcd. for $C_{25}H_{21}N_3O_4S_1$: C, 65.36; H, 4.58; N, 9.15%.

3. Acaricidal activity assay

T. cinnabarinus was raised on potted young cowpea plants in the laboratory at (26±1)°C and 75–80% relative humidity (R. H.) under a 14 hr : 10 hr (light : dark) cycle with no acaricide exposure for at least 13 years. *P. citri* was collected from citrus plants with no pesticide exposure for several years at the Citrus Research Institute of Southwest University.

The slide-dip method[35] was adopted to evaluate the acaricidal activity of 2 to 17 against female adults of *T. cinnabarinus* and *P. citri*. The appropriate amounts of target compounds were dissolved in water containing 2% acetone, 0.8% Tween-80, and 1% laurocapram to obtain the desired final concentration of 1000 mg/L for the preliminary screening. Based on preliminary test results, a series of five to seven concentrations of the tested compounds were chosen to determine the median lethal concentration (LC_{50}) values of the compounds. Pyridaben 95% TC and BDMC were used as controls. Acaricidal activity assays were performed in triplicate and repeated thrice. The LC_{50} values of the tested compounds were calculated using the probit analysis procedure of SPSS 13.0 for Windows.

Results and Discussion

1. Synthesis

The target compounds were synthesized by the reaction of BDMC with hydroxylamine and different hydrazine derivatives (Fig. 1). To increase the electronegativity of the nitrogen atom, commercially available starting materials, namely, hydroxylamine hydrochloride and different hydrazine derivatives (hydrochloride or sulfate) were first neutralized by sodium ethylate or NaOH to afford the desired hydroxylamine and different hydrazine derivatives, thereby facilitating the nucleophilic cyclization reaction. In this reaction, AcOH was used as both solvent and catalyst to promote the transformation of dicarbonyl from BDMC to enol and increase the electropositivity of the carbon atoms that facilitate the nucleophilic cyclization reaction. Reaction mixtures were maintained at 85°C or reflux, leading to the desired compounds in 31–90% yields.

2. Acaricidal activity

As shown in Table 1, the acaricidal activities of BDMC and its derivatives increased with increasing processing time against female adults of *T. cinnabarinus* and *P. citri*. Nine target compounds exhibited acaricidal activities that were more pronouced than or comparable to BDMC against *T. cinnabarinus*. In particular, compounds **3**, **4**, and **7** exhibited about 3.3-, 4.1-, and 2.3-fold and 5.4-, 10.0-, and 15.4-fold higher acaricidal potency than BDMC at 48 hr and 72 hr, respectively. In addition, the target compounds **2**, **4**, **5**, **8** and **9** displayed excellent activities against *P. citri*. Compound **4** possessed the most promising acaricidal activity and exhibited about 31.0- and 59.1-fold higher acaricidal potency against *P. citri* than BDMC, as well as about 2.2-fold higher potency than pyridaben, at 72 hr.

The structure-activity relationships of the derivatives were also investigated. From the data presented in Table 1, we found that the substituted pyrazole was most prominent in increasing activity. Introduction of small groups [–H, $-CH_3$, and $-C(CH_3)_3$] to the N-atom of the pyrazole ring generally led to compounds with more potency than compounds with substituted phenyl groups. The acaricidal activity of the benzene rings with electron donating groups (4-methyl, 3, 4-dimethyl, and 4-methoxy) was higher than that of rings with electron-withdrawing groups.

The 48 hr LC_{50} value of BDMC in the current study was lower than that in our previous reports,[27] which may be attributed to the differences in BDMC purity, the pesticide adjuvants, and the solvents used to prepare tested compounds. The contact activity

Table 1. Acaricidal activities of BDMC isoxazole and pyrazole derivatives

Compd.	R_1	R_2	R_3	R_4	Time (hr)	LC_{50} (mg/L)[a]	
						T. cinnabarinus	*P. citri*
2	—	—	—	—	48	247.2±21.5	56.1±15.2
					72	112.8±19.2	17.1±6.7
3	H	—	—	—	48	99.6±18.8	106.2±16.5
					72	40.4±12.9	36.2±13.8
4	CH_3	—	—	—	48	80.3±13.2	4.7±1.9
					72	21.8±7.6	1.3±0.5
5	$C(CH_3)_3$	—	—	—	48	329.3±18.1	55.8±15.8
					72	162.0±18.2	28.6±10.2
6	—	H	H	H	48	406.1±25.2	122.1±17.6
					72	113.1±20.2	80.5±15.6
7	—	H	H	CH_3	48	144.7±20.4	129.5±19.0
					72	14.2±5.6	43.5±14.9
8	—	H	H	OCH_3	48	250.3±20.0	97.8±16.3
					72	105.5±18.7	42.1±13.9
9	—	H	CH_3	CH_3	48	235.1±17.6	51.6±15.9
					72	104.6±18.2	20.8±7.9
10	—	H	H	F	48	271.4±23.5	175.7±17.5
					72	107.4±19.6	119.2±17.5
11	—	F	H	H	48	563.1±28.9	217.5±21.3
					72	355.1±21.8	117.4±18.7
12	—	H	H	Cl	48	470.5±26.4	317.9±26.4
					72	279.4±21.4	173.7±16.1
13	—	H	Cl	H	48	608.0±28.9	156.5±19.4
					72	379.8±22.2	76.0±16.8
14	—	H	H	Br	48	646.4±39.1	453.8±43.8
					72	253.6±27.5	242.3±25.1
15	—	H	H	NO_2	48	827.0±50.9	109.1±20.8
					72	402.2±30.0	37.7±12.7
16	—	NO_2	H	NO_2	48	431.1±19.7	496.7±46.0
					72	171.5±17.5	254.1±25.7
17	—	H	H	SO_2NH_2	48	325.6±22.0	188.6±20.5
					72	202.0±20.8	93.2±18.6
BDMC	—	—	—	—	48	330.1±21.3	144.5±17.5
					72	218.3±19.8	76.8±16.6
Pyridaben	—	—	—	—	48	21.1±1.6	3.2±0.5
					72	20.1±1.6	2.9±0.4

[a] Values are means±S.D. (n=3).

of all compounds against female adults of *P. citri* was higher than that against the female adults of *T. cinnabarinus*, and the 48 hr LC_{50} values of the target compounds were significantly higher than their 72 hr LC_{50} values. Thus, the target compounds are similar to other plant-derived pesticides with respect to their selectivity and slow efficacy.[36]

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