Article

JOURNAL OF THE CHINESE CHEMICAL SOCIETY

Synthesis and Antifungal Activity of Novel Furan-2,4-dione Derivatives Containing Substituted Phenylhydrazine Moiety

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(Received: Oct. 29, 2014; Accepted: Apr. 8, 2015; Published Online: May 19, 2015; DOI: 10.1002/jccs.201400463)

A series of novel 3-(2-(substituted phenyl)hydrazinylmethylidene)furan-2,4(3*H*,5*H*)-diones were designed and synthesized with ethyl 4-chloroacetoacetate as the starting material. Their structures were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, EI-MS and elemental analysis. Bioassay data demonstrated that these compounds exhibited remarkable antifungal activity against *Fusarium graminearum*, *Botrytis cinerea*, *Rhizoctonia cerealis* and *Colletotrichum capsici*. Compound 3-(2-(4-bromophenyl)hydrazinylmethylidene)furan-2,4(3*H*,5*H*)-dione (**5g**) had excellent bioactivity against *Botrytis cinerea* with an EC_{50} value of 0.18 μg/mL — markedly lower than the 0.24 μg/mL of the commercial fungicide procymidone. The result revealed that introducing the halogenated phenylhydrazine at the 3-position of furan-2,4(3*H*,5*H*)-dione was an effective way to design new tetronic acid derivatives as new fungicides.

Keywords: Tetronic acid; Furan-2,4-dione; Substituted phenylhydrazine; Synthesis; Antifungal activity.

INTRODUCTION

Natural tetronic acid derivatives with a heterocyclic core of furan-2,4(3*H*,5*H*)-dione have antioxidant,[1,2] antiviral,[3,4] antibacterial,[5] and antifungal effects,[6,7] which has attracted the interests of chemists. Many different tetronic acid derivatives have been designed and synthesized, and many of the compounds showed noticeable bioactivity especially when a proper substituent such as an aryl,[8] acyl,[9] and alkyl,[10] (compounds **a**, **b** and **c**, Figure 1) were introduced at the 3-position of furan-2,4-dione.

Phenylhydrazine is an important moiety often incorporated in bioactive molecules and has been described in reports about pharmaceutical and pesticidal chemicals (compounds **d** and **e**, Figure 1).[11-14] Recently, a series of phenol derivatives containing a moiety of substituted phenylhydrazine have been reported and some compounds showed remarkable antimicrobial activity across a broad spectrum (compound **f**, Figure 1).[15]

In this paper, some substituted phenylhydrazine groups were introduced to the 3-position of furan-2,4(3*H*,5*H*)-dione to synthesize a series of novel tetronic acid derivatives. The target compounds were screened for fungicidal activity against four selected phytopathogenic fungi. We close with a discussion of the preliminary structure-activity relationship.

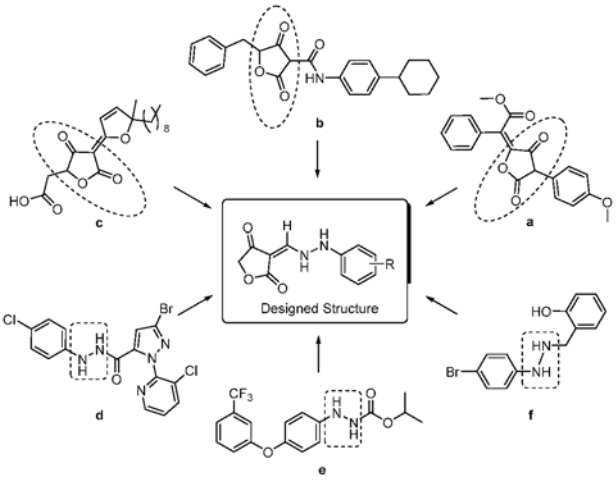

Fig. 1. Bioactive structures of tetronic acid and hydrazine as well as the design strategy of target compounds.

RESULTS AND DISCUSSION

Synthesis

Scheme 1 presents the synthetic route for the target compounds. The title compounds **5a**-**5k** were synthesized

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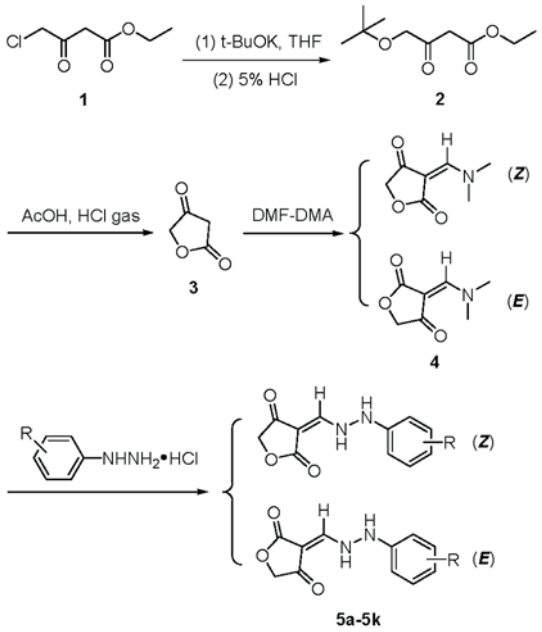
Supporting information for this article is available on the www under http://dx.doi.org/10.1002/jccs.201400463

with yields of 26.9% to 73.9%. The usual synthetic method for 3-substituted tetronic acids **4** included *O*-acylation, cyclization and acidification from α-hydroxy acids or their esters.[16-18] In this work, the furan ring was closed before the modification at the 3-position of the tetronic acid. According to the reported method,[19] ethyl 4-chloroacetoacetate was dissolved in 30% sodium hydroxide solution and refluxed for 1 h, acidified with 10% hydrochloride acid solution and then extracted with ethyl acetate. However, the crude product was too ropy for separation. Thus, we used ethyl 4-chloroacetoacetate that was oxyalkylated and then cyclized in acetic acid.[20] The entire synthetic route was efficient and simple. The cyclization of compound **2** was not a usual Lacey-Dieckmann reaction,[21] but rather an esterification. The ester group and the *tert*-butoxy group in compound **2** were converted to the carboxyl and hydroxyl group separately under acidic conditions. The esterification between the carboxyl and the hydroxyl group then gave ring-closure. Here, we studied the influence of feed ratio on yields. We used the preferred ratio 1:3 (n (**2**)/n (HCl)) and compared cost and the yield. Moreover, phenylhydrazine hydrochloride could not be replaced by phenylhydrazine in the final step, otherwise the product would become a critical complex that could not be separated and purified.

Scheme 1 Synthetic strategy for target compounds **5a-5k**.



5a: R = H; **5b**: R = 2-F; **5c**: R = 4-F; **5d**: R = 2-Cl; **5e**: R = 3-Cl; **5f**: R = 4-Cl; **5g**: R = 4-Br; **5h**: R = 2,4-Cl_2; **5i**: R = 4-CH_3; **5j**: R = 3,4-$(CH_3)_2$; **5k**: R = 4-OCH_3

Structure

The structures of the target compounds were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, EI-MS and elemental analysis. The IR spectrum showed peaks at 3208-3343 cm^{-1} due to N-H stretching vibration of the amino groups at phenylhydrazine. These peaks had low values because of the formation of intramolecular and intermolecular hydrogen bonds. The peaks at 3008-3098 cm^{-1} were attributed to C-H stretching vibration at the CH=C group. The ^{1}H NMR spectrum of the target compounds showed that the peaks at δ 7.76-7.85 ppm correspond to the protons of the CH=C. The peaks at δ 8.56-9.21 ppm were for the protons of NH close to the phenyl ring. The signals at δ 10.97-11.09 ppm were for the protons of NH close to the five-membered ring, which were shown in the low-field due to the intramolecular hydrogen bond. The ^{13}C NMR spectrum showed that the signals of the carbon atoms for methylidene at the 3-position were at δ 153.9-156.3 ppm. The peaks at δ 89.4-91.7 ppm correspond to the C-3 carbon atoms at the furan ring. Moreover, all the target compounds showed parent ion $[M]^+$ peaks in their MS spectra. All the elemental analysis data matched their molecular formulas.

In the ^{13}C NMR spectrum of the target compounds, almost all the C-3 carbon atoms at furan ring and the CH carbon atoms at benzene ring showed two pairs of signals — this indicated an isomeric mixture.[22] These compounds could possibly exist in four tautomeric forms (Figure 2). The internal equilibrium (**5**α ⇌ **5**β and **5**γ ⇌ **5**δ) was too fast on the NMR time scale to be seen and the two pairs of signals were caused by external equilibrium **5**α, **5**β ⇌ **5**γ, **5**δ.[23]

To distinguish between two existing forms of tautomerism, the isomers of compound **5f** were calculated with

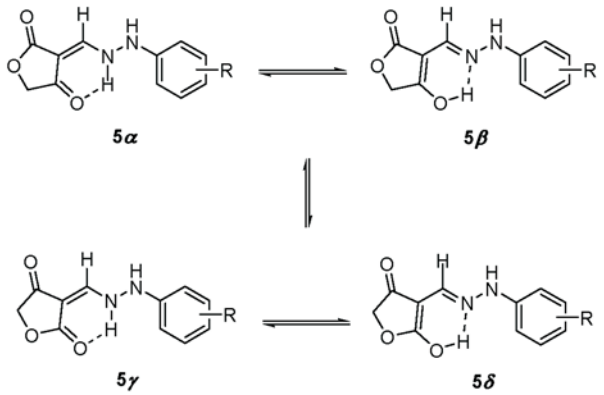


Fig. 2. Possible tautomers of target compounds **5a**-**5k**.

Gaussian 03W package using B3LYP/6-31G (d) for optimization. The solvent effect of DMSO was also considered. The calculated results showed that the relative energies of **5f**α, **5f**β, **5f**γ and **5f**δ were 0.00, 24.09, 0.86 and 71.32 kJ/mol, respectively. Therefore, **5f**α (i.e. ***E*-**isomer) and **5f**γ (i.e. ***Z*-**isomer) were comparably stable, and **5f**α predominated. The ratios of ***E***- and ***Z***-isomers were estimated to be 1:1 to 3:1 according to the intensities of the two pairs of peaks. We note that intermediate **4** should exist as ***E***/***Z***-isomers. However, the splitting peaks usually disappeared in the NMR spectrum because of the fast rotation around the C=C exocyclic bond from a dipolar resonance hybrid over the NMR time scale.[22]

Antifungal activity

Compounds **5a**-**5k** were evaluated for *in vitro* fungicidal activity against *F. graminearum*, *B. cinerea*, *R. cerealis* and *C. capsici* with EC_{50} values summarized in Table 1. Most compounds exhibited remarkable fungicidal activity. The EC_{50} values of compounds **5e** and **5f** were 0.51 μg/mL and 0.53 μg/mL, respectively. This became 0.38 μg/mL for carbendazim against *F. graminearum*. The EC_{50} values of compounds **5f**-**5h** were 0.29 μg/mL, 0.18 μg/mL and 0.31 μg/mL, respectively, which is approximately equal to that of 0.24 μg/mL for procymidone against *B. cinerea.*

The preliminary structure-activity relationship analysis revealed that both the substituent position and the electron effect at the phenyl ring were important influencing factors on fungicidal activity. These factors can be summarized relative to compound **5a**. Indeed, introducing halogen groups at the phenyl ring obviously increased fungicidal bioactivity, but introducing alkyls or alkoxy gave the opposite trend. Moreover, the introduction of halogen groups at *p*- and *m*-position of phenyl ring markedly increased the fungicidal activity against *F. graminearum* and *B. cinerea*. Compounds **5e** (*m*-Cl) and **5g** (*p*-Br) had the lowest EC_{50} values against the upper fungi. Halogen groups at the *o*-position and *m*-position improved the fungicidal activity against *R. cerealis* and *C. capsici*. Compounds **5d** (*o*-Cl) and **5b** (*o*-F) offered the best EC_{50} values against these two fungi, respectively. The results indicated that both the type and substituent of the halogens control bioactivity due to their electron-withdrawing properties.

EXPERIMENTAL

General: The melting points were measured on a WRS-1B digital melting point apparatus. The FT-IR spectra (4000-400 cm^{-1}) were recorded on a Thermo Nicolet 380 FT-IR spectrometer with the KBr disk method. The ^{1}H NMR and ^{13}C NMR spectra were collected on a Bruker AV 400 MHz spectrometer at room temperature with DMSO-d_6 as the solvent. Chemical shifts were given in δ units (ppm) relative to TMS as internal standard. Mass spectra were recorded on a GC/MS-Trace 2000 spectrometer using the direct injection technique. Elemental analyses were determined on an Elementar Vario EL cube analyzer. The solvents were dried prior to use as needed.

Synthesis of ethyl 4-*tert*-butoxyacetoacetate (2): The *t*-BuOK (59.0 mmol) was suspended in THF (42.9 g), to which a mixture of ethyl 4-chloroacetoacetate (24.3 mmol) and THF (7.1 g) was added dropwise such that the temperature fell 20-30 °C. After completion of the dropwise addition, the mixture was stirred at room temperature for 20 h. After condensing the THF, 5% hydrochloric acid (24 mL) was added dropwise under ice-cooling followed by extraction with ethyl acetate and separation. The organic layer was washed with water and concentrated to yield compound **2**.

Synthesis of furan-2,4(3*H*,5*H*)-dione (3): Compound **2** (18.8 mmol) was dissolved in acetic acid and treated with HCl gas (56.4 mmol) at 20-25 °C. After the air blowing, the mixture was stirred at room temperature for 3 h and concentrated *in vacuo* to one fifth of the original volume. The residue was stirred with a muddler at 0 °C to precipitate crystals. To this we added toluene (4.0 g) followed by stirring at room temperature for 2 h. The crystals were collected by filtration and yielded the compound **3**.

Synthesis of 3-(dimethylaminomethylidene)furan-2,4 (3*H*,5*H*)-dione (4): A solution of dimethylformamide dimethyl acetal (DMF-DMA) (3 mmol) and compound **3** (3 mmol) in abso-

Table 1. EC_{50} values of target compounds **5a-5k** against four plant pathogenic fungi (μg/mL)

Compd.	*F. graminearum*	*B. cinerea*	*R. cerealis*	*C. capsici*
5a	> 10	2.70	6.20	3.60
5b	> 10	0.69	1.03	0.63
5c	0.77	0.67	2.73	1.09
5d	> 10	1.15	0.93	0.97
5e	0.51	0.55	1.30	0.77
5f	0.53	0.29	2.24	0.94
5g	0.79	0.18	1.52	1.42
5h	2.55	0.31	0.93	0.99
5i	3.54	3.1124	> 10	> 10
5j	> 10	> 10	> 10	> 10
5k	> 10	> 10	> 10	> 10
Carbendazim	0.38	–	0.03	–
Procymidone	–	0.24	–	–
Azoxystrobin	–	–	–	0.29

lute ethanol was stirred for 30 min at room temperature. The crude product was washed with ether and recrystallized from ethanol to provide the solid **4**.

Synthesis of the title compounds 5a-5k: The intermediate **4** (2.5 mmol) was reacted with substituted phenylhydrazine hydrochloride (2.5 mmol) at room temperature for 3-6 h, respectively. The reaction was monitored by TLC (ethyl acetate:light petroleum:methanol:acetic acid = 10:2:2:0.2, *V*/*V*). The precipitate was filtered, washed with water, dried over $MgSO_4$ and concentrated *in vacuo* to give the desired products **5a**-**5k**.

Data for 4: Yellow powder, yield 52.6%, mp 147.7-148.6 °C; IR (KBr) *v*: 3039, 2978, 1729, 1604, 1420, 1393, 1256, 1038, 759 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 7.53 (s, 1H, CH=C), 4.36 (s, 2H, CH_2), 3.66 (s, 3H, NCH_3), 3.39 (s, 3H, NCH_3); ^{13}C (101 MHz, DMSO-d_6) δ: 191.1 (CH_2**C**O), 174.9 (COO), 156.6 (CH), 89.6 (CH=**C**), 71.0 (CH_2), 48.1 (CH_3); 44.1 (CH_3); MS (EI) *m/z*: 155.1 $(M)^+$. **Data for 5a:** Tan powder, yield 69.7%, mp 168.3-169.3 °C; IR (KBr) *v*: 3284, 3076, 2929, 1739, 1670, 1580, 1479, 1299, 1029, 752, 694 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 11.04 (s, 1H, ArNHN**H**), 8.93 (s, 1H, ArN**H**NH), 7.77 (s, 1H, CH=C), 7.24 (t, 2H, *J* = 7.9 Hz, ArH), 6.88 (t, 1H, *J* = 7.3 Hz, ArH), 6.76 (d, 2H, *J* = 7.9 Hz, ArH), 4.51 (s, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ: ***E***-isomer: 193.9 (CH_2**C**O), 172.2 (COO), 155.4 (CH), 147.9 (PhCNH), 129.5 (2C, 2 × PhCH), 121.1 (PhCH), 113.8 (2C, 2 × PhCH), 91.1 (CH=**C**), 71.1 (CH_2); ***Z***-isomer: 193.9 (CH_2**C**O), 172.2 (COO), 155.4 (CH), 147.9 (PhCNH), 129.8 (2C, 2 × PhCH), 121.5 (PhCH), 113.5 (2C, 2 × PhCH), 89.7 (CH=**C**), 71.1 (CH_2); MS *m/z* (EI): 218.2 $(M)^+$; Anal. Calcd. for $C_{11}H_{10}N_2O_3$ (218.21): C, 60.55; H, 4.62; N, 12.84. Found: C, 60.61; H, 4.65; N, 12.80. **Data for 5b:** Gray powder, yield 47.4%, mp 167.6-169.5 °C; IR (KBr) *v*: 3264, 3211, 3039, 2938, 1726, 1673, 1586, 1487, 1296, 1024, 735 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 11.08 (s, 1H, ArNHN**H**), 8.97 (s, 1H, ArN**H**NH), 7.84 (s, 1H, CH=C), 7.14-7.19 (m, 1H, ArH), 7.09 (t, 1H, *J* = 7.6 Hz, ArH), 6.84-6.92 (m, 2H, ArH), 4.52 (s, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ: ***E***-isomer: 193.7 (CH_2**C**O), 172.1 (COO), 155.3 (CH), 149.7 (PhCF), 135.6 (PhCNH), 125.2 (PhCH), 121.6 (PhCH), 115.9 (PhCH), 115.7 (PhCH), 91.2 (CH=**C**), 71.0 (CH_2); ***Z***-isomer: 193.7 (CH_2**C**O), 172.1 (COO), 155.3 (CH), 152.1 (PhCF), 135.5 (PhCNH), 125.2 (PhCH), 121.7 (PhCH), 115.8 (PhCH), 115.6 (PhCH), 90.0 (CH=**C**), 71.0 (CH_2); MS *m/z* (EI): 236.2 $(M)^+$; Anal. Calcd. for $C_{11}H_9FN_2O_3$ (236.20): C, 55.93; H, 3.84; N, 11.86. Found: C, 56.01; H, 3.85; N, 11.84. **Data for 5c:** Tan powder, yield 30.5%, mp 167.2-169.2 °C; IR (KBr) *v*: 3285, 3065, 2929, 1740, 1674, 1583, 1498, 1210, 1025, 826 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 11.05 (s, 1H, ArNHN**H**), 8.87 (s, 1H, ArN**H**NH), 7.78 (s, 1H, CH=C), 7.09 (t, 2H, *J* = 8.8 Hz, ArH), 6.77-6.80 (m, 2H, ArH), 4.51 (s, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ: ***E***-isomer: 193.9 (CH_2**C**O), 172.2 (COO), 156.3 (PhCF), 155.2 (CH), 144.4 (PhCNH), 115.9 (2C, 2 × PhCH), 115.4 (2C, 2 × PhCH), 91.0 (CH=**C**), 71.1 (CH_2); ***Z***-isomer: 193.9 (CH_2**C**O), 172.2 (COO), 158.7(PhCF), 155.2 (CH), 144.4 (PhCNH), 116.1 (2C, 2 × PhCH), 115.5 (2C, 2 × PhCH), 89.8 (CH=**C**), 71.1 (CH_2); MS *m/z* (EI): 236.2 $(M)^+$; Anal. Calcd. for $C_{11}H_9FN_2O_3$ (236.20): C, 55.93; H, 3.84; N, 11.86. Found: C, 55.80; H, 3.83; N, 11.89. **Data for 5d:** Light purple powder, yield 48.1%, mp 176.5-177.9 °C; IR (KBr) *v*: 3343, 3238, 3031, 2929, 1733, 1632, 1578, 1475, 1293, 1032, 743 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 11.06 (s, 1H, ArNHN**H**), 8.80 (s, 1H, ArN**H**NH), 7.84 (s, 1H, CH=C), 7.37 (d, 1H, *J* = 7.8 Hz, ArH), 7.25 (t, 1H, *J* = 7.7 Hz, ArH), 6.90 (t, 1H, *J* = 7.6 Hz, ArH), 6.83 (d, 1H, *J* = 7.2 Hz, ArH), 4.53 (s, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ: ***E***-isomer: 193.7 (CH_2**C**O), 172.0 (COO), 155.4 (CH), 143.7 (PhCNH), 129.9 (PhCH), 128.3 (PhCH), 121.9 (PhCH), 118.4 (PhCCl), 114.7 (PhCH), 91.4 (CH=**C**), 71.5 (CH_2); ***Z***-isomer: 192.8 (CH_2**C**O), 173.7 (COO), 155.4 (CH), 141.3 (PhCNH), 130.2 (PhCH), 128.4 (PhCH), 122.1 (PhCH), 119.2 (PhCCl), 114.4 (PhCH), 90.2 (CH=**C**), 71.0 (CH_2); MS *m/z* (EI): 252.1 $(M)^+$; Anal. Calcd. for $C_{11}H_9ClN_2O_3$ (252.65): C, 52.29; H, 3.59; N, 11.09. Found: C, 52.37; H, 3.56; N, 11.16. **Data for 5e:** Brilliant yellow powder, yield 26.9%, mp 199.0-121.0 °C; IR (KBr) *v*: 3265, 3184, 3019, 2934, 1728, 1664, 1599, 1494, 1296, 1033, 827, 781, 695 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 11.05 (s, 1H, ArNHN**H**), 9.21 (s, 1H, ArN**H**NH), 7.78 (s, 1H, CH=C), 7.25 (t, 1H, *J* = 8.0 Hz, ArH), 6.90 (d, 1H, *J* = 7.7 Hz, ArH), 6.77 (s, 1H, ArH), 6.71 (d, 1H, *J* = 8.2 Hz, ArH), 4.53 (s, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ: ***E***-isomer: 192.9 (CH_2**C**O), 172.1 (COO), 153.9 (CH), 149.4 (PhCNH), 134.0 (PhCCl), 131.1 (PhCH), 120.4 (PhCH), 113.1 (PhCH), 112.3 (PhCH), 91.6 (CH=**C**), 71.1 (CH_2); ***Z***-isomer: 192.9 (CH_2**C**O), 172.1 (COO), 153.9 (CH), 149.4 (PhCNH), 134.4 (PhCCl), 131.4 (PhCH), 120.7 (PhCH), 112.7 (PhCH), 112.1 (PhCH), 90.1 (CH=**C**), 71.1 (CH_2); MS *m/z* (EI): 252.2 $(M)^+$; Anal. Calcd. for $C_{11}H_9ClN_2O_3$ (252.65): C, 52.29; H, 3.59; N, 11.09. Found: C, 52.15; H, 3.59; N, 11.03. **Data for 5f:** Yellow powder, yield 49.6%, mp 195.3-196.3 °C; IR (KBr) *v*: 3243, 3184, 3008, 2935, 1751, 1643, 1572, 1480, 1240, 1031, 818 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 11.06 (s, 1H, ArNHN**H**), 9.09 (s, 1H, ArN**H**NH), 7.77 (s, 1H, CH=C), 7.28 (d, 2H, *J* = 8.7 Hz, ArH), 6.77 (d, 2H, *J* = 8.7 Hz, 2H, ArH), 4.52 (s, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ: ***E***-isomer: 193.4 (CH_2**C**O), 172.1 (COO), 154.4 (CH), 146.9 (PhCNH), 129.2 (2C, 2 × PhCH), 124.5 (PhCH), 115.1 (2C, 2 × PhCH), 91.3 (CH=**C**), 71.1 (CH_2); ***Z***-isomer: 193.1 (CH_2**C**O), 173.7 (COO), 154.4 (CH), 144.2

(PhCNH), 129.6 (2C, 2 × PhCH), 125.0 (PhCH), 115.3 (2C, 2 × PhCH), 90.0 (CH=**C**), 71.1 (CH_2); MS *m/z* (EI): 252.2 $(M)^+$; Anal. Calcd. for $C_{11}H_9ClN_2O_3$ (252.65): C, 52.29; H, 3.59; N, 11.09. Found: C, 52.28; H, 3.59; N, 11.03. **Data for 5g:** Yellow powder, yield 44.6%, mp 189.6-191.5 °C; IR (KBr) *v*: 3242, 3183, 3013, 2931, 1750, 1644, 1574, 1480, 1421, 1244, 1031, 817 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 11.06 (s, 1H, ArNHN**H**), 9.11 (s, 1H, ArN**H**NH), 7.78 (s, 1H, CH=C), 7.40 (d, 2H, *J* = 8.8 Hz, ArH), 6.72 (d, 2H, *J* = 8.7 Hz, ArH), 4.52 (s, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ: ***E***-isomer: 193.2 (CH_2**C**O), 172.1 (COO), 154.2 (CH), 147.3 (PhCNH), 133.1 (2C, 2 × PhCH), 115.7 (2C, 2 × PhCH), 112.1 (PhCBr), 91.4 (CH=**C**), 71.1 (CH_2); ***Z*-**isomer: 193.2 (CH_2**C**O), 172.1 (COO), 154.2 (CH), 144.7 (PhCNH), 132.4 (2C, 2 × PhCH), 115.4 (2C, 2 × PhCH), 112.1 (PhCBr), 90.0 (CH=**C**), 71.1 (CH_2); MS *m/z* (EI): 298.1 $(M + H)^+$; 296.1 $(M-H)^+$; Anal. Calcd. for $C_{11}H_9BrN_2O_3$ (297.10): C, 44.47; H, 3.05; N, 9.43. Found: C, 44.32; H, 3.07; N, 9.40. **Data for 5h:** Gray powder, yield 73.9%, mp 177.3-179.3 °C; IR (KBr) *v*: 3287, 3200, 3027, 2927, 1740, 1643, 1572, 1479, 1291, 1035, 822,762 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 11.09 (s, 1H, ArNHN**H**), 8.98 (s, 1H, ArN**H**NH), 7.85 (s, 1H, CH=C), 7.52 (d, 1H, *J* = 2.1 Hz, ArH), 7.31-7.33 (m, 1H, ArH), 6.85 (d, 1H, *J* = 8.1 Hz, ArH), 4.54 (s, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ: ***E***-isomer: 193.1 (CH_2**C**O), 172.0 (COO), 154.5 (CH), 142.9 (PhCNH), 129.1 (PhCH), 128.2 (PhCH), 124.4 (PhCCl), 118.9 (PhCCl), 115.9 (PhCH), 91.7 (CH=**C**), 71.3 (CH_2); ***Z*-**isomer: 192.2 (CH_2**C**O), 172.0 (COO), 154.5 (CH), 142.9 (PhCNH), 129.4 (PhCH), 128.3 (PhCH), 124.4 (PhCCl), 118.9 (PhCCl), 115.3 (PhCH), 90.6 (CH=**C**), 70.8 (CH_2); MS *m/z* (EI): 288.1 $(M + H)^+$; 286.1 $(M-H)^+$; Anal. Calcd. for $C_{11}H_8Cl_2N_2O_3$ (287.10): C, 46.02; H, 2.81; N, 9.76. Found: C, 45.96; H, 2.83; N, 9.71. **Data for 5i:** Brilliant yellow powder, yield 34.6%, mp 163.4-164.2 °C; IR (KBr) *v*: 3244, 3170, 3035, 2936, 1745, 1645, 1574, 1478, 1340, 1248, 1038, 808 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 10.98 (s, 1H, ArNHN**H**), 8.74 (s, 1H, ArN**H**NH), 7.76 (s, 1H, CH=C), 7.05 (d, 2H, *J* = 8.2 Hz, ArH), 6.67 (d, 2H, *J* = 8.3 Hz, ArH), 4.50 (s, 2H, CH_2), 2.21 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ: ***E***-isomer: 194.1 (CH_2**C**O), 172.2 (COO), 155.4 (CH), 145.5 (PhCNH), 130.1 (Ph**C**CH_3), 129.9 (2C, 2 × PhCH), 114.1 (2C, 2 × PhCH), 90.8 (CH=**C**), 72.3 (CH_2), 20.6 (CH_3); ***Z***-isomer: 194.1 (CH_2**C**O), 172.2 (COO), 155.4 (CH), 145.5 (PhCNH), 130.2 (Ph**C**CH_3), 129.9 (2C, 2 × PhCH), 114.1 (2C, 2 × PhCH), 89.5 (CH=**C**), 71.2 (CH_2), 20.6 (CH_3); MS *m/z* (EI): 232.2 $(M)^+$; Anal. Calcd. for $C_{12}H_{12}N_2O_3$ (232.24): C, 62.06; H, 5.21; N, 12.06. Found: C, 62.31; H, 5.20; N, 12.01. **Data for 5j:** Yellow powder, yield 36.6%, mp 171.1-172.9 °C; IR (KBr) *v*: 3208, 3182, 3008, 2966, 2929, 1738, 1614, 1582, 1479, 1356, 1293, 1038, 826, 768 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 10.97 (s, 1H, ArNHN**H**), 8.64 (s, 1H, ArN**H**NH), 7.76 (s, 1H, CH=C), 6.99 (d, 1H, *J* = 8.0 Hz, ArH), 6.56 (s, 1H, ArH), 6.49 (d, 1H, *J* = 8.0 Hz, ArH), 4.50 (s, 2H, CH_2), 2.16 (s, 3H, CH_3), 2.13 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ: ***E***-isomer: 194.2 (CH_2**C**O), 172.2 (COO), 155.6 (CH), 145.8 (PhCNH), 137.2 (Ph**C**CH_3), 130.4 (PhCH), 128.9 (Ph**C**CH_3), 115.4 (PhCH), 111.5 (PhCH), 90.8 (CH=**C**), 71.0 (CH_2), 20.0 (CH_3), 19.0 (CH_3); ***Z***-isomer: 194.2 (CH_2**C**O), 172.2 (COO), 155.6 (CH), 145.8 (PhCNH), 137.5 (Ph**C**CH_3), 130.6 (PhCH), 128.9 (Ph**C**CH_3), 115.4 (PhCH), 111.5 (PhCH), 89.5 (CH=**C**), 71.0 (CH_2), 20.0 (CH_3), 19.0 (CH_3); MS *m/z* (EI): 246.3 $(M)^+$; Anal. Calcd. for $C_{13}H_{14}N_2O_3$ (246.26): C, 63.40; H, 5.73; N, 11.38. Found: C, 63.06; H, 5.74; N, 11.28. **Data for 5k:** Yellow powder, yield 72.6%, mp 155.0-157.0 °C; IR (KBr) *v*: 3290, 3098, 2934, 1732, 1666, 1581, 1496, 1357, 1298, 1026, 824 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 11.00 (s, 1H, ArNHN**H**), 8.56 (s, 1H, ArN**H**NH), 7.81 (s, 1H, CH=C), 6.85 (d, 2H, *J* = 8.9 Hz, ArH), 6.74 (d, 2H, *J* = 8.9 Hz, ArH), 4.49 (s, 2H, CH_2), 3.69 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ: ***E***-isomer: 194.2 (CH_2**C**O), 172.3 (COO), 155.4 (CH), 154.7 (Ph**C**OCH_3), 141.4 (PhCNH), 115.9 (2C, 2 × PhCH), 114.9 (2C, 2 × PhCH), 90.6 (CH=**C**), 71.5 (CH_2), 55.7 (CH_3); ***Z***-isomer: 194.2 (CH_2CO), 172.3 (COO), 155.4 (CH), 154.7 ($PhCOCH_3$), 141.4 (PhCNH), 115.1 (2C, 2 × PhCH), 114.9 (2C, 2 × PhCH), 89.4 (CH=C), 70.9 (CH_2), 55.7 (CH_3); MS *m/z* (EI): 248.2 $(M)^+$; Anal. Calcd. for $C_{12}H_{12}N_2O_4$ (248.23): C, 58.06; H, 4.87; N, 11.29. Found: C, 58.25; H, 4.90; N, 11.22.

Antifungal Bioassay: The antifungal activities of the synthesized compounds against *F. graminearum*, *B. cinerea*, *R. cerealis* and *C. capsici* were determined *in vitro* using a radial growth inhibition technique according to the literature.[24,25] Each of the title compounds was first dissolved in 0.5 mL CH_3OH and mixed with 45 mL PSA (potato sucrose agar) medium. The medium was poured into three 9 cm petri dishes. After the mixture cooled, the fungi were inoculated in the center of the disks. The plates were cultured at 25 ± 1 °C for 3-5 days in the dark. The CH_3OH solution served as the blank control. The diameter of the fungal spread was measured to calculate growth inhibition. The commercial fungicides carbendazim, procymidone and azoxystrobin were used as control drugs. The EC_{50} values were tested using the double broth dilution method.

CONCLUSIONS

In summary, eleven novel tetronic acid derivatives bearing substituted phenylhydrazine moieties were designed and synthesized. Their structures are well supported by spectroscopic data and elemental analysis. The bioassay

indicated that the target compounds showed remarkable bioactivity against four plant pathogenic fungi. Compound **5g** had the best antifungal activity against *B. cinerea* with an EC_{50} value significantly higher than the fungicide procymidone. The results revealed that both the position and electron withdrawing effect of substituent groups at the phenyl ring influence fungicidal activity of the target compounds. These findings provide new information for further modification of the tetronic acid derivatives.

ACKNOWLEDGEMENTS

This work was funded by the National Key Technologies R&D Program of China (2011BAE06B04), 863 Program of China (2011AA10A206), Science & Technology Pillar Program of Jiangsu Province of China (BE2012371), the National Natural Science Foundation of China (31171889) and the Fundamental Research Funds for the Central Universities of China (KYZ201223).

REFERENCES

1. Schobert, R.; Schlenk, A. *Bioorg. Med. Chem.* **2008**, *16*, 4203.
2. EL-Gendy, K. S.; Aly, N. M.; Mahmoud, F. H.; Kenawy, A.; EI-Sebae, A. K. H. *Food Chem. Toxicol.* **2010**, *48*, 215.
3. Mittra, A.; Yamashita, M.; Kawasaki, I.; Murai, H.; Yoshioka, T.; Ohta, S. *Synlett* **1997**, *8*, 909.
4. Schobert, R.; Jagusch, C. *J. Org. Chem.* **2005**, *70*, 6129.
5. Gein, V. L.; Gein, L. F.; Sheptukha, M. A.; Voronina, E. V. *Pharm. Chem. J.* **2005**, *39*, 537.
6. Schobert, R.; Dietrich, M.; Mullen, G.; Urbina-Gonzalez, J. M. *Synthesis* **2006**, *22*, 3902.
7. Andrade, R.; Ayer, W. A.; Trifonov, L. S. *Aust. J. Chem.* **1997**, *50*, 255.
8. Dias, D.; White, J. M.; Urban, S. *Nat. Prod. Res.* **2007**, *21*, 366.
9. Peukert, S.; Sun, Y. C.; Zhang, R.; Hurley, B.; Sabio, M.; Shen, X. Y.; Gray, C.; Dzink-Fox, J.; Tao, J. S.; Cebula, R.; Wattanasin, S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1840.
10. Zhang, F.; Ding, G.; Li, L.; Cai, X. Y.; Si, Y. K.; Guo, L. D.; Che, Y. S. *Org. Biomol. Chem.* **2012**, *10*, 5307.
11. Yan, T.; Yu, S. J.; Liu, P. F.; Liu, Z.; Wang, B. L.; Xiong, L. X.; Li, Z. M. *Chin. J. Chem.* **2012**, *30*, 919.
12. Chee, G. L.; Dekeyser, M. A.; Seebold, K. W. J.; Osika, E. M.; Brouwer, W. G. WO 2005005376, **2005**. [CA. 142: P93517].
13. Hamid, A.; Sadiq, Z.; Yaqub, G.; Khan, N.; Iqbal, S.; Iqbal, K.; Ijaz, Z.; Bajwa, A. *Asian J. Chem.* **2013**, *25*, 5412.
14. Zareef, M.; Iqbal, R.; Mirza, B.; Khan, K. M.; Manan, A.; Asim, F.; Khan, S. W. *Arkivoc* **2008**, *ii*, 141.
15. Zou, M.; Lu, J. R.; Xin, C. W.; Bao, X. R.; Zhu, S. S.; Liu, Q.; Li, J. T.; Qiu, J. B. *J. Tianjin Univ. Technol.* **2010**, *26*, 1.
16. Mallinger, A.; Gall, T. L.; Mioskowski, C. *Synlett* **2008**, *3*, 386.
17. Tejedor, D.; Garcia-Tellado, F. *Org. Prep. Proced. Int.* **2004**, *36*, 35.
18. Royles, B. J. L. *Chem. Rev.* **1995**, *95*, 1981.
19. Brieden, W.; Schroer, J.; Lonza, A. CA 2211503, **1998**. [CA. 128: P230190].
20. Kouhei, F.; Mitsuru, O. EP 2163549, **2010**. [CA. 152: P357780].
21. Schobert, R. *Naturwissenschaften* **2007**, *94*, 1.
22. Gelin, S.; Chantegrel, B. *J. Heterocyclic Chem.* **1981**, *18*, 663.
23. Hofmann, J. P.; Duus, F.; Bond, A. D.; Hansen, P. E. *J. Mol. Struct.* **2006**, *790*, 80.
24. Lu, G. H.; Chu, H. B.; Chen, M.; Yang, C. L. *Chin. Chem. Lett.* **2014**, *25*, 61.
25. Liu, H. J.; Zhang, X.; Gao, Y. X.; Li, J. Z.; Wang, H. L. *J. Chin. Chem. Soc.* **2013**, *60*, 27.

