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Synthesis, Biological Activity and 3D-QSAR Study of Novel Pyrrolidine-2,4-dione Derivatives Containing *N*-Substituted Phenylhydrazine Moiety

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Abstract Twenty-seven novel pyrrolidine-2,4-dione derivatives containing *N*-substituted phenylhydrazine moiety were synthesized. Their structures were confirmed by ^{1}H NMR, ^{13}C NMR and MS. The half effective concentration (EC_{50}) values of the title compounds against the phytopathogenic fungi *Rhizoctonia cerealis* were evaluated. Compounds **6**l and **6**q displayed good bioactivity with EC_{50} values of 1.626 and 2.043 μg/mL, respectively. The 3D quantitative structure activity relationship(3D-QSAR) model of CoMFA was established with reliable cross-validated correlation coefficient q^2 value of 0.585 and Noncross-validated correlation coefficient r^2 value of 0.971. This model provided a tool for guiding further design and synthesis of novel pyrrolidine-2,4-dione derivatives with high fungicidal activity.

Keywords Pyrrolidine-2,4-dione; *N*-Substituted phenylhydrazine; Antifungal activity; 3D quantitative structure activity relationship(3D-QSAR)

1 Introduction

Heterocyclic compounds have attracted great attention of chemists for their wide biological activities. Tenuazonic acid is a natural tetramic acid with a heterocyclic core of pyrrolidine-2,4-dione[1], and exhibited antitumor, antiviral, antibacterial and herbicidal activities[2—5]. Many tetramic acid derivatives have been designed and synthesized with tenuazonic acid as the lead compound. 3-Enaminetetramic acids were synthesized and evaluated as fungicide, herbicide and antibiotic agents, some of the compounds showed good activity[6,7]. A series of 3-(hydroxymethylene)tetramic acids was researched as herbicidal inhibitors of *p*-hydroxyphenylpyruvate dioxygenase(HPPD), most of them showed higher inhibiting rates than sulcotrione[8]. Other tetramic acid derivatives with the methoxyacrylate group showed visible fungicidal activity[9].

N-substituted hydrazines are typical groups in the structures of the compounds having biological activity. Trifluoromethanesulfonanilide(TFMS) derivatives with a structure of *N*-alkyl-hydrazinobenzene exhibited high insecticidal and acaricidal activity[10]. The derivatives of malonohydrazides containing alkylcarbonothioyl groups at 1-position of hydrazinobenzene showed remarkable anticancer activity[11]. The compounds with *N*-methyl hydrazone were evaluated as interleukin-1 blockers on corneal fibroblast proliferation and ocular inflammation *in vivo*[12]. In this work, *N*-substituted phenylhydrazine groups were introduced to 3-position of pyrrolidine-2,4-dione ring to synthesize a series of novel tetramic acid derivatives. The fungicidal activities of the title compounds were evaluated. And a good CoMFA model implemented in the SYBYL software packages was established to provide practical guidance for further structure modification[13—15].

2 Experimental

2.1 Materials and Instruments

All the reagents and solvents were obtained from commercial suppliers. The reagents were analytically or chemically pure and were not further purified. All the solvents were dried by standard methods in advance.

The melting points of all the title compounds were determined on an uncorrected WRS-1B digital melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker 400 spectrometer with DMSO-d_6 as solvent and tetramethylsilane(TMS) as internal standard. Mass spectra(EI) were recorded on a GC/MS-QP2010 spectrometer with a direct

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injection technique. Progress of the reactions was monitored by thin layer chromatography(TLC).

2.2 General Synthetic Procedure

2.2.1 Synthesis of Compounds 3a—3c

Intermediates **2** were prepared according to the reported method[16,17]. A mixture of compound **2**(0.2 mol) and sodium methoxide solution(0.2 mol of Na metal, 60 mL of methanol) in benzene(60 mL) was refluxed for 4 h. After evaporating under reduced pressure to give the crude products, 100 mL of water was added. The aqueous layer was acidified to pH=2—3 to get yellow solids **3**a—**3**c in yields of 48.3%—57.4%.

2.2.2 Synthesis of Compounds 5a—5n, 5γ and 5δ

In a nitrogen atmosphere, powdered sodium amide(42.0 mmol) was added into tetrahydrofuran(30 mL) at 0—5 °C. Then substituted phenylhydrazine hydrochloride **4**(20 mmol) was added slowly. A gentle stream of nitrogen was passed through the solution for 1 h. With cooling, the halohydrocarbon R^4X(21.3 mmol) was added dropwise. After 1.5 h, the mixture was poured into water(50 mL). The tetrahydrofuran was removed under reduced pressure. The residue was extracted with ethyl acetate, washed with water and dried. Under reduced pressure, the crude product was obtained and purified by column chromatography on silica gel to give orange oils **5**a—**5**l, **5***γ* and **5***δ* with yields of 56.3%—87.5%[18]. A mixture of compound **4**(50 mmol), 10%(mass fraction) sodium hydroxide aqueous solution(40 mL) and dichloromethane(50 mL) was stirred at room temperature for 30 min to yield substituted phenylhydrazine. Potassium *tert*-butoxide was added slowly (13.6 mmol) to a stirring solution of substituted phenylhydrazine(13.6 mmol) in dimethyl carbonate(0.54 mol). After 30 min, diethyl ether(60 mL) was added. Then the mixture was poured on silica gel in beaker and set aside for 12 h. After filtration, the silica gel was washed with diethyl ether, and the combined filtrate was evaporated *in vacuo* to give orange oils **5**m and **5**n[19]. The yields were 78.1% and 85.3%, respectively.

2.2.3 Synthesis of Compounds 6a—6y, 6γ and 6δ

A mixture of compounds **3**(2.5 mmol) and **5**(2.6 mmol) in ethanol(15 mL) was stirred at room temperature. The reaction was monitored by TLC[*V*(ethyl acetate):*V*(light petroleum): *V*(methanol):*V*(acetic acid)=10:2:2:0.2]. After 1.5 h, the precipitate was given. The mixture was filtrated, then the residue was washed with diethyl ether and dried to obtain the title compounds **6**a—**6**y, **6***γ* and **6***δ*.

1-Methyl-3-[1-(2-methyl-2-phenylhydrazinyl)ethylidene]-pyrrolidine-2,4-dione(**6**a): a yellow solid, yield 23.9%, m. p. 137.5—139.7 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), *δ*: *E*-isomer: 11.32(s, 1H), 7.35—7.24(m, 2H), 6.93(t, *J*=7.3 Hz, 1H), 6.83(t, *J*=8.0 Hz, 2H), 3.68(s, 2H), 3.14(s, 3H), 2.85(s, 3H), 2.47(s, 3H); *Z*-isomer: 11.50(s, 1H), 7.35—7.24(m, 2H), 6.93(t, *J*=7.3 Hz, 1H), 6.83(t, *J*=8.0 Hz, 2H), 3.72(s, 2H), 3.15(s, 3H), 2.85(s, 3H), 2.52(s, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz), *δ*: *E*-isomer: 192.6, 172.3, 167.9, 149.8, 129.7, 121.1, 114.2, 95.0, 57.5, 41.9, 28.3, 12.9; *Z*-isomer: 195.4, 169.7, 168.8, 149.8, 129.7, 121.1, 114.1, 96.5, 55.3, 41.8, 28.8, 11.9; EI-MS, *m*/*z*: 259$(M)^+$.

1-Methyl-3-{1-[2-methyl-2-(4-methylphenyl)hydrazinyl]-ethylidene}pyrrolidine-2,4-dione(**6**b): a white solid, yield 66.4%, m. p. 147.8—148.9 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), *δ*: *E*-isomer: 11.29(s, 1H), 7.16—7.05(m, 2H), 6.82—6.70(m, 2H), 3.67(s, 2H), 3.10(s, 3H), 2.84(s, 3H), 2.47(s, 3H), 2.23(s, 3H); *Z*-isomer: 11.47(s, 1H), 7.16—7.05(m, 2H), 6.82—6.70(m, 2H), 3.71(s, 2H), 3.11(s, 3H), 2.84(s, 3H), 2.52(s, 3H), 2.23(s, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz), *δ*: *E*-isomer: 192.5, 172.4, 167.7, 147.6, 130.2, 130.1, 114.5, 94.8, 57.4, 42.2, 28.3, 20.5, 12.9; *Z*-isomer: 195.4, 169.7, 168.7, 147.6, 130.2, 130.1, 114.4, 96.4, 55.3, 42.1, 28.7, 20.5, 11.9; EI-MS, *m*/*z*: 273$(M)^+$.

1-Methyl-3-{1-[2-(4-fluorophenyl)-2-methylhydrazinyl]-ethylidene}pyrrolidine-2,4-dione(**6**c): a white solid, yield 76.3%, m. p. 154.0—154.6 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), *δ*: *E*-isomer: 11.30(s, 1H), 7.18—7.09(m, 2H), 6.91—6.82(m, 2H), 3.68(s, 2H), 3.12(s, 3H), 2.84(s, 3H), 2.48(s, 3H); *Z*-isomer: 11.48(s, 1H), 7.18—7.09(m, 2H), 6.91—6.82(m, 2H), 3.71(s, 2H), 3.13(s, 3H), 2.84(s, 3H), 2.53(s, 3H); ^{13}C NMR (DMSO-d_6, 101 MHz), *δ*: *E*-isomer: 192.6, 172.2, 167.7, 156.4, 146.5, 116.0, 115.9, 95.0, 57.4, 42.4, 28.3, 12.9; *Z*-isomer: 195.3, 169.6, 168.6, 158.7, 146.5, 116.3, 115.8, 96.5, 55.3, 42.3, 28.7, 11.9; EI-MS, *m*/*z*: 277$(M)^+$.

1-Methyl-3-{1-[2-(3-chlorophenyl)-2-methylhydrazinyl]-ethylidene}pyrrolidine-2,4-dione(**6**d): a white solid, yield 79.2%, m. p. 115.2—116.3 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), *δ*: *E*-isomer: 11.31(s, 1H), 7.34—7.24(m, 1H), 6.98—6.90(m, 1H), 6.87—6.80(m, 1H), 6.80—6.71(m, 1H), 3.69(s, 2H), 3.16(s, 3H), 2.85(s, 3H), 2.45(s, 3H); *Z*-isomer: 11.47(s, 1H), 7.34—7.24(m, 1H), 6.98—6.90(m, 1H), 6.87—6.80(m, 1H), 6.80—6.71(m, 1H), 3.72(s, 2H), 3.17(s, 3H), 2.85(s, 3H), 2.49(s, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz), *δ*: *E*-isomer: 192.7, 172.0, 167.8, 151.2, 134.4, 131.3, 120.3, 113.4, 112.5, 95.4, 57.5, 41.6, 28.3, 12.9; *Z*-isomer: 195.3, 169.6, 168.5, 151.3, 134.4, 131.3, 120.2, 113.3, 112.4, 96.8, 55.3, 41.6, 28.8, 11.9; EI-MS, *m*/*z*: 294$(M)^+$.

1-Methyl-3-{1-[2-(4-chlorophenyl)-2-methylhydrazinyl]-ethylidene}pyrrolidine-2,4-dione(**6**e): a colorless solid, yield 77.3%, m. p. 143.9—144.5 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), *δ*: *E*-isomer: 11.31(s, 1H), 7.36—7.29(m, 2H), 6.87—6.79(m, 2H), 3.68(s, 2H), 3.14(s, 3H), 2.85(s, 3H), 2.45(s, 3H); *Z*-isomer: 11.47(s, 1H), 7.36—7.29(m, 2H), 6.87—6.79(m, 2H), 3.72(s, 2H), 3.15(s, 3H), 2.85(s, 3H), 2.49(s, 3H); ^{13}C NMR (DMSO-d_6, 101 MHz), *δ*: *E*-isomer: 192.7, 172.1, 167.8, 148.7, 129.4, 124.7, 115.7, 95.2, 57.4, 41.8, 28.3, 12.9; *Z*-isomer: 195.3, 169.6, 168.6, 148.7, 129.4, 124.6, 115.6, 96.7, 55.3, 41.7, 28.8, 11.9; EI-MS, *m*/*z*: 294$(M)^+$.

1-Methyl-3-{1-[2-(4-bromophenyl)-2-methylhydrazinyl]-ethylidene}pyrrolidine-2,4-dione(**6**f): a yellow solid, yield 44.5%, m. p. 142.0—142.9 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), *δ*: *E*-isomer: 11.30(s, 1H), 7.48—7.39(m, 2H), 6.82—6.73(m, 2H), 3.68(s, 2H), 3.14(s, 3H), 2.85(s, 3H), 2.45(s, 3H); *Z*-isomer: 11.46(s, 1H), 7.48—7.39(m, 2H), 6.82—6.73(m, 2H), 3.71(s, 2H), 3.14(s, 3H), 2.85(s, 3H), 2.49(s, 3H); ^{13}C NMR (DMSO-d_6, 101 MHz), *δ*: *E*-isomer: 192.7, 172.0, 167.8, 149.1, 132.3, 116.1, 112.4, 95.2, 57.4, 41.7, 28.3, 12.9; *Z*-isomer: 195.3, 169.6, 168.6, 149.1, 132.3, 116.0, 112.3, 96.7, 55.3, 41.6, 28.8, 11.9; EI-MS, *m*/*z*: 338$(M)^+$.

5-Methyl-3-[1-(2-methyl-2-phenylhydrazinyl)ethylidene]-

pyrrolidine-2,4-dione(**6**g): a white solid, yield 44.4%, m.p. 203.9—205.1 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.45(s, 1H), 7.88(s, 1H), 7.30(t, *J*=7.9 Hz, 2H), 6.93(t, *J*=7.3 Hz, 1H), 6.84(t, *J*=7.7 Hz, 2H), 3.77—3.63(m, 1H), 3.14(s, 3H), 2.48(s, 3H), 1.16(t, *J*=6.0 Hz, 3H); *Z*-isomer: 11.54(s, 1H), 7.61(s, 1H), 7.30(t, *J*=7.9 Hz, 2H), 6.93(t, *J*=7.3 Hz, 1H), 6.84(t, *J*=7.7 Hz, 2H), 3.77—3.63(m, 1H), 3.15(s, 3H), 2.52(s, 3H), 1.16(t, *J*=6.0 Hz, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz), δ: *E*-isomer: 197.5, 173.9, 169.1, 149.7, 129.7, 121.1, 114.2, 93.8, 56.9, 41.8, 18.3, 12.9; *Z*-isomer: 200.1, 171.0, 169.6, 149.7, 129.7, 121.1, 114.1, 95.4, 54.5, 41.8, 18.4, 12.2; EI-MS, *m*/*z*: 259(M)$^+$.

5-Methyl-3-{1-[2-methyl-(4-methylphenyl)hydrazinyl]-ethylidene}pyrrolidine-2,4-dione(**6**h): a yellow solid, yield 33.8%, m. p. 209.0—209.4 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.42(s, 1H), 7.87(s, 1H), 7.11(d, *J*=8.2 Hz, 2H), 6.76(t, *J*=7.4 Hz, 2H), 3.78—3.61(m, 1H), 3.10(s, 3H), 2.48(s, 3H), 2.23(s, 3H), 1.20—1.12(m, 3H); *Z*-isomer: 11.52(s, 1H), 7.59(s, 1H), 7.11(d, *J*=8.2 Hz, 2H), 6.76(t, *J*=7.4 Hz, 2H), 3.78—3.61(m, 1H), 3.11(s, 3H), 2.52(s, 3H), 2.23(s, 3H), 1.20—1.12(m, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz), δ: *E*-isomer: 197.5, 174.0, 169.0, 147.6, 130.3, 130.2, 114.6, 93.7, 56.8, 42.2, 20.5, 18.3, 12.9; *Z*-isomer: 200.2, 171.0, 169.5, 147.5, 130.3, 130.2, 114.5, 95.3, 54.5, 42.1, 20.5, 18.4, 12.2; EI-MS, *m*/*z*: 273(M)$^+$.

5-Methyl-3-{1-[2-(4-fluorophenyl)-2-methylhydrazinyl]-ethylidene}pyrrolidine-2,4-dione(**6i**): a white solid, yield 77.4%, m. p. 198.7—199.1 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.43(s, 1H), 7.88(s, 1H), 7.19—7.10(m, 2H), 6.92—6.82(m, 2H), 3.77—3.62(m, 1H), 3.12(s, 3H), 2.48(s, 3H), 1.20—1.11(m, 3H); *Z*-isomer: 11.52(s, 1H), 7.60(s, 1H), 7.19—7.10(m, 2H), 6.92—6.82(m, 2H), 3.77—3.62(m, 1H), 3.13(s, 3H), 2.53(s, 3H), 1.20—1.11(m, 3H); ^{13}C NMR (DMSO-d_6, 101 MHz), δ: *E*-isomer: 197.6, 173.8, 168.9, 156.4, 146.4, 116.1, 116.0, 93.8, 56.8, 42.3, 18.3, 12.9. *Z*-isomer: 200.1, 171.0, 169.5, 158.7, 146.4, 116.3, 115.9, 95.4, 54.5, 42.2, 18.4, 12.2; EI-MS, *m*/*z*: 277(M)$^+$.

5-Methyl-3-{1-[2-(3-chlorophenyl)-2-methylhydrazinyl]-ethylidene}pyrrolidine-2,4-dione(**6**j): a white solid, yield 85.9%, m. p. 210.7—211.6 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.44(s, 1H), 7.91(s, 1H), 7.34—7.26(m, 1H), 6.98—6.92(m, 1H), 6.87—6.81(m, 1H), 6.81—6.72(m, 1H), 3.79—3.63(m, 1H), 3.16(s, 3H), 2.45(s, 3H), 1.21—1.11(m, 3H); *Z*-isomer: 11.51(s, 1H), 7.64(s, 1H), 7.34—7.26(m, 1H), 6.98—6.92(m, 1H), 6.87—6.81(m, 1H), 6.81—6.72(m, 1H), 3.79—3.63(m, 1H), 3.17(s, 3H), 2.49(s, 3H), 1.21—1.11(m, 3H, CH_3CH); ^{13}C NMR(DMSO-d_6, 101 MHz), δ: *E*-isomer: 197.7, 173.6, 169.0, 151.2, 134.4, 131.3, 120.3, 113.4, 112.6, 94.2, 56.9, 41.6, 18.3, 12.8; *Z*-isomer: 200.1, 170.9, 169.5, 151.2, 134.4, 131.3, 120.2, 113.3, 112.5, 95.7, 54.5, 41.6, 18.3, 12.2; EI-MS, *m*/*z*: 294(M)$^+$.

5-Methyl-3-{1-[2-(4-chlorophenyl)-2-methylhydrazinyl]-ethylidene}pyrrolidine-2,4-dione(**6**k): a white solid, yield 68.7%, m. p. 182.9—183.7 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.43(s, 1H), 7.90(s, 1H), 7.38—7.27(m, 2H), 6.88—6.79(m, 2H), 3.77—3.64(m, 1H), 3.14(s, 3H), 2.45(s, 3H), 1.16(t, *J*=6.2 Hz, 3H); *Z*-isomer: 11.51(s, 1H), 7.63(s, 1H), 7.38—7.27(m, 2H), 6.88—6.79(m, 2H), 3.77—3.64(m, 1H), 3.15(s, 3H), 2.50(s, 3H), 1.16(t, *J*=6.2 Hz, 3H); ^{13}C NMR (DMSO-d_6, 101 MHz), δ: *E*-isomer: 197.6, 173.9, 169.0, 148.7, 129.4, 124.7, 115.7, 94.1, 56.9, 41.8, 18.3, 12.8; *Z*-isomer: 200.1, 170.9, 169.5, 148.7, 129.4, 124.7, 115.6, 95.6, 54.5, 41.7, 18.4, 12.2; EI-MS, *m*/*z*: 294(M)$^+$.

5-Methyl-3-{1-[2-(4-bromophenyl)-2-methylhydrazinyl]-ethylidene}pyrrolidine-2,4-dione(**6**l): a white solid, yield 45.5%, m. p. 192.8—193.8 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.43(s, 1H), 7.90(s, 1H), 7.50—7.40(m, 2H), 6.83—6.73(m, 2H), 3.77—3.62(m, 1H), 3.14(s, 3H), 2.45(s, 3H), 1.16(t, *J*=6.2 Hz, 3H); *Z*-isomer: 11.50(s, 1H), 7.63(s, 1H), 7.50—7.40(m, 2H), 6.83—6.73(m, 2H), 3.77—3.62(m, 1H), 3.14(s, 3H), 2.49(s, 3H), 1.16(t, *J*=6.2 Hz, 3H); ^{13}C NMR (DMSO-d_6, 101 MHz), δ: *E*-isomer: 197.6, 173.7, 169.0, 149.1, 132.3, 116.1, 112.4, 94.1, 56.9, 41.7, 18.3, 12.8; *Z*-isomer: 200.1, 170.9, 169.5, 149.1, 132.2, 116.0, 112.3, 95.6, 54.5, 41.6, 18.4, 12.2; EI-MS, *m*/*z*: 338(M)$^+$.

3-[1-(2-Methyl-2-phenylhydrazinyl)ethylidene]pyrrolidine-2,4-dione(**6**m): a white solid, yield 69.2%, m. p. 216.8—17.5 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.44(s, 1H), 7.69(s, 1H), 7.33—7.26(m, 2H), 6.93(t, *J*=7.3 Hz, 1H), 6.88—6.80(m, 2H), 3.60(s, 1H), 3.15(s, 3H), 2.48(s, 3H); *Z*-isomer: 11.59(s, 1H), 7.42(s, 1H), 7.33—7.26(m, 2H), 6.93(t, *J*=7.3 Hz, 1H), 6.88—6.80(m, 2H), 3.64(s, 2H), 3.16(s, 3H), 2.52(s, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz), δ: *E*-isomer: 194.7, 175.0, 168.9, 149.7, 129.7, 121.1, 114.2, 94.9, 51.5, 41.8, 12.9; *Z*-isomer: 197.5, 172.2, 169.5, 149.7, 129.7, 121.1, 114.1, 96.5, 49.3, 41.7, 12.1; EI-MS, *m*/*z*: 245(M)$^+$.

3-{1-[2-Methyl-2-(4-methylphenyl)hydrazinyl]ethylidene}pyrrolidine-2,4-dione(**6**n): a white solid, yield 60.8%, m. p. 229.4—232.6 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.41(s, 1H), 7.71(s, 1H), 7.10(d, *J*=7.9 Hz, 2H), 6.76(d, *J*=7.7 Hz, 2H), 3.60(s, 2H), 3.10(s, 3H), 2.48(s, 3H), 2.23(s, 3H); *Z*-isomer: 11.56(s, 1H), 7.43(s, 1H), 7.10(d, *J*=7.9 Hz, 2H), 6.76(d, *J*=7.7 Hz, 2H), 3.60(s, 2H), 3.10(s, 3H), 2.50(s, 3H), 2.23(s, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz), δ: *E*-isomer: 194.6, 175.2, 168.7, 147.6, 130.1, 114.5, 94.8, 51.5, 42.1, 20.5, 12.9; EI-MS, *m*/*z*: 259(M)$^+$.

3-{1-[2-(3-Chlorophenyl)-2-methylhydrazinyl]ethylidene}pyrrolidine-2,4-dione(**6**o): a white solid, yield 37.8%, m. p. 237.0—237.6 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.42(s, 1H), 7.71(s, 1H), 7.34—7.25(m, 1H), 6.94(d, *J*=7.8 Hz, 1H), 6.87—6.81(m, 1H), 6.80—6.73(m, 1H), 3.61(s, 2H), 3.17(s, 3H), 2.45(s, 3H); *Z*-isomer: 11.56(s, 1H), 7.45(s, 1H), 7.34—7.25(m, 1H), 6.94(d, *J*=7.8 Hz, 1H), 6.87—6.81(m, 1H), 6.80—6.73(m, 1H), 3.64(s, 2H), 3.17(s, 3H), 2.50(s, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz), δ: *E*-isomer: 194.8, 174.8, 168.8, 151.2, 134.4, 131.3, 120.3, 113.4, 112.6, 95.3, 51.5, 41.6, 12.9; *Z*-isomer: 197.4, 172.1, 169.3, 151.2, 134.4, 131.3, 120.2, 113.4, 112.5, 96.8, 49.3, 41.6, 12.1; EI-MS, *m*/*z*: 280(M)$^+$.

3-{1-[2-(4-Chlorophenyl)-2-methylhydrazinyl]ethylidene}pyrrolidine-2,4-dione(**6**p): a white solid, yield 44.9%, m. p. 194.1—195.0 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.42(s, 1H), 7.73(s, 1H), 7.37—7.29(m, 2H), 6.89—6.79(m, 2H), 3.61(s, 2H), 3.15(s, 3H), 2.46(s, 3H); *Z*-isomer: 11.56(s, 1H), 7.47(s, 1H), 7.37—7.29(m, 2H),

6.89—6.79(m, 2H), 3.63(s, 2H), 3.15(s, 3H), 2.50(s, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz), δ: *E*-isomer: 194.7, 174.9, 168.8, 148.7, 129.4, 124.7, 115.7, 95.2, 51.5, 41.8, 12.9; *Z*-isomer: 197.4, 172.1, 169.3, 148.7, 129.4, 124.7, 115.6, 96.7, 49.3, 41.8, 12.1; EI-MS, *m*/*z*: 280(M)$^+$.

3-{1-[2-(4-Bromophenyl)-2-methylhydrazinyl]ethylidene}-pyrrolidine-2,4-dione(**6**q): a white solid, yield 43.8%, m. p. 198.7—199.9 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.41(s, 1H), 7.73(s, 1H), 7.46—7.41(m, 2H), 6.83—6.74(m, 2H), 3.61(s, 2H), 3.14(s, 3H), 2.46(s, 3H); *Z*-isomer: 11.56(s, 1H), 7.46(s, 1H), 7.46—7.41(m, 2H), 6.83—6.74(m, 2H), 3.63(s, 2H), 3.15(s, 3H), 2.50(s, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz), δ: *E*-isomer: 194.8, 174.8, 168.8, 149.1, 132.3, 116.1, 112.4, 95.2, 51.5, 41.7, 12.9; *Z*-isomer: 197.4, 172.1, 169.3, 149.1, 132.2, 116.0, 112.3, 96.7, 49.3, 41.6, 12.1; EI-MS, *m*/*z*: 324(M)$^+$.

3-{1-[2-(4-Chlorophenyl)-2-ethylhydrazinyl]ethylidene}-pyrrolidine-2,4-dione(**6**r): a white solid, yield 42.8%, m. p. 186.6—187.9 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.50(s, 1H), 7.80(s, 1H), 7.35—7.32(m, 2H), 6.91—6.87(m, 2H), 3.62(s, 2H), 3.60—3.57(m, 2H), 2.43(s, 3H), 1.13—1.04(m, 3H); *Z*-isomer: 11.62(s, 1H), 7.50(s, 1H), 7.35—7.32(m, 2H), 6.91—6.87(m, 2H), 3.67(s, 2H), 3.60—3.57(m, 2H), 2.47(s, 3H), 1.13—1.04(m, 3H); ^{13}C NMR (DMSO-d_6, 101 MHz), δ: *E*-isomer: 194.6, 175.1, 169.5, 147.7, 129.6, 125.1, 116.4, 95.4, 51.6, 48.5, 13.2, 10.8. *Z*-isomer: 197.9, 171.9, 170.3, 147.7, 129.6, 125.2, 116.4, 97.0, 51.6, 49.3, 48.5, 12.4, 10.8; EI-MS, *m*/*z*: 294(M)$^+$.

3-{1-[2-Butyl-2-(4-chlorophenyl)hydrazinyl]ethylidene}-pyrrolidine-2,4-dione(**6**s): a yellow solid, yield 82.6%, m. p. 194.2—195.1 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.52(s, 1H), 7.81(s, 1H), 7.33(d, *J*=8.5 Hz, 2H), 6.94—6.81(m, 2H), 3.62(s, 2H), 3.52(br, 2H), 2.40(s, 3H), 1.57—1.42(m, 2H), 1.41—1.26(m, 2H), 0.90(t, *J*=7.2 Hz, 3H); *Z*-isomer: 11.65(s, 1H), 7.51(s, 1H), 7.33(d, *J*=8.5 Hz, 2H), 6.94—6.81(m, 2H), 3.67(s, 2H), 3.52(br, 2H), 2.45(s, 3H), 1.57—1.42(m, 2H), 1.41—1.26(m, 2H), 0.90(t, *J*=7.2 Hz, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz), δ: *E*-isomer: 194.6, 175.1, 169.4, 148.0, 129.6, 125.0, 116.2, 95.3, 54.0, 51.5, 27.8, 20.0, 14.3, 13.1; *Z*-isomer: 197.9, 171.9, 170.1, 148.0, 129.6, 125.0, 116.2, 97.0, 54.0, 49.3, 27.9, 20.0, 14.3, 12.4; EI-MS, *m*/*z*: 322(M)$^+$.

3-[1-(2-Allyl-2-phenylhydrazinyl)ethylidene]pyrrolidine-2,4-dione(**6**t): a pink solid, yield 60.4%, m. p. 130.1—132.4 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.52(s, 1H), 7.70(s, 1H), 7.30(t, *J*=7.9 Hz, 2H), 6.98—6.86(m, 3H), 5.95—5.81(m, 1H), 5.33—5.25(m, 2H), 4.18(br, 2H), 3.60(s, 2H), 2.44(s, 3H); *Z*-isomer: 11.66(s, 1H), 7.43(s, 1H), 7.30(t, *J*=7.9 Hz, 2H), 6.98—6.86(m, 3H), 5.95—5.81(m, 1H), 5.33—5.25(m, 2H), 4.18(br, 2H), 3.65(s, 2H), 2.48(s, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz), δ: *E*-isomer: 194.6, 175.1, 169.3, 148.7, 132.1, 129.9, 121.5, 120.8, 114.8, 95.1, 56.6, 51.5, 13.2; *Z*-isomer: 197.8, 172.0, 170.0, 148.7, 132.0, 129.9, 121.5, 120.8, 114.8, 96.8, 56.7, 49.3, 12.4; EI-MS, *m*/*z*: 271(M)$^+$.

3-{1-[2-Allyl-2-(4-chlorophenyl)hydrazinyl]ethylidene}-pyrrolidine-2,4-dione(**6**u): a white solid, yield 32.5%, m. p. 203.6—204.0 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.51(s, 1H), 7.77(s, 1H), 7.34(d, *J*=8.9 Hz, 2H), 6.96—6.86(m, 2H), 5.95—5.81(m, 1H), 5.28(d, *J*=14.4 Hz, 2H), 4.20(br, 2H), 3.61(s, 2H), 2.43(s, 3H); *Z*-isomer: 11.63(s, 1H), 7.50(s, 1H), 7.34(d, *J*=8.9 Hz, 2H), 6.96—6.86(m, 2H), 5.95—5.81(m, 1H), 5.28(d, *J*=14.4 Hz, 2H), 4.20(br, 2H), 3.65(s, 2H), 2.47(s, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz) δ: *E*-isomer: δ 194.7, 175.0, 169.3, 147.7, 131.9, 129.6, 125.1, 120.8, 116.3, 95.4, 56.6, 51.5, 13.2; *Z*-isomer: 197.8, 171.9, 169.9, 147.7, 131.9, 129.6, 125.2, 120.9, 116.3, 97.0, 56.7, 49.3, 12.4; EI-MS, *m*/*z*: 306(M)$^+$.

3-[1-(2-Benzyl-2-phenylhydrazinyl)ethylidene]pyrrolidine-2,4-dione(**6**v): a white solid, yield 77.6%, m. p. 170.0—170.9 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.52(s, 1H), 7.66(s, 1H), 7.36—7.26(m, 7H), 7.07—6.92(m, 3H), 4.73(s, 2H), 3.56(s, 2H), 2.23(s, 3H); *Z*-isomer: 11.64(s, 1H), 7.38(s, 1H), 7.36—7.26(m, 7H), 7.07—6.92(m, 3H), 4.75(d, *J*=7.5 Hz, 2H), 3.62(s, 2H), 2.26(s, 3H); ^{13}C NMR (DMSO-d_6, 101 MHz), δ: *E*-isomer: 194.5, 175.0, 169.2, 149.1, 136.2, 129.9, 129.6, 128.9, 128.2, 121.8, 115.3, 95.0, 58.1, 51.5, 13.1; *Z*-isomer: 197.7, 171.9, 169.9, 149.1, 136.1, 129.9, 129.6, 128.9, 128.3, 121.8, 115.3, 96.6, 58.1, 49.2, 12.4; EI-MS, *m*/*z*: 321(M)$^+$.

3-{1-[2-Benzyl-2-(4-chlorophenyl)hydrazinyl]ethylidene}pyrrolidine-2,4-dione(**6**w): a white solid, yield 80.0%, m. p. 198.2—199.3 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.51(s, 1H), 7.72(s, 1H), 7.38—7.28(m, 7H), 7.05—6.97(m, 2H), 4.75(s, 2H), 3.56(s, 2H), 2.21(s, 3H); *Z*-isomer: 11.61(s, 1H), 7.44(s, 1H), 7.38—7.28(m, 7H), 7.05—6.97(m, 2H), 4.76(s, 2H), 3.62(s, 2H), 2.24(s, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz), δ: *E*-isomer: 194.6, 174.9, 169.1, 148.1, 135.9, 129.7, 129.6, 129.0, 128.3, 125.4, 116.8, 95.2, 58.0, 51.5, 13.1; *Z*-isomer: 197.7, 171.8, 169.8, 148.1, 135.9, 129.7, 129.6, 129.0, 128.3, 125.5, 116.9, 96.7, 58.0, 49.2, 12.3; EI-MS, *m*/*z*: 356(M)$^+$.

Methyl 2-[1-(2,4-dioxopyrrolidin-3-ylidene)ethyl]-1-phenylhydrazine-1-carboxylate(**6**x): a white solid, yield 47.1%, m. p. 172.0—173.8 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.81(s, 1H), 7.82(s, 1H), 7.49—7.36(m, 4H), 7.29—7.21(m, 1H), 3.74(s, 3H), 3.61(s, 2H), 2.37(s, 3H); *Z*-isomer: 11.93(s, 1H), 7.57(s, 1H), 7.49—7.36(m, 4H), 7.29—7.21(m, 1H), 3.74(s, 3H), 3.64(s, 2H), 2.41(s, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz), δ: *E*-isomer: 195.1, 174.0, 167.6, 154.7, 141.4, 129.4, 126.7, 123.8, 96.5, 54.3, 51.6, 13.0; *Z*-isomer: 197.6, 171.5, 167.9, 154.7, 141.4, 129.4, 126.7, 123.8, 97.8, 54.3, 49.3, 12.2; EI-MS, *m*/*z*: 289(M)$^+$.

Methyl 1-(4-chlorophenyl)-2-[1-(2,4-dioxopyrrolidin-3-ylidene)ethyl]hydrazine-1-carboxylate(**6**y): a white solid, yield 65.3%, m. p. 208.0—209.7 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.77(s, 1H), 7.83(s, 1H), 7.47(s, 4H), 3.74(s, 3H), 3.62(s, 2H), 2.36(s, 3H); *Z*-isomer: 11.89(s, 1H), 7.59(s, 1H), 7.47(s, 4H), 3.74(s, 3H), 3.65(s, 2H), 2.40(s, 3H); ^{13}C NMR (DMSO-d_6, 101 MHz), δ: *E*-isomer: 195.2, 173.9, 167.5, 154.5, 140.4, 130.7, 129.3, 125.3, 96.7, 54.5, 51.6, 13.0; *Z*-isomer: 197.6, 171.4, 167.8, 154.5, 140.3, 130.7, 129.2, 125.3, 98.0, 54.5, 49.3, 12.2; EI-MS, *m*/*z*: 324(M)$^+$.

3-{1-[2-Allyl-2-(4-bromophenyl)hydrazinyl]ethylidene}-pyrrolidine-2,4-dione(**6**γ): a white solid, yield 79.7%, m. p. 219.2—219.4 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.51(s, 1H), 7.77(s, 1H), 7.45(d, *J*=8.8 Hz, 2H), 6.90—6.81(m,

2H), 5.95—5.79(m, 1H), 5.30(s, 2H), 4.20(br, 2H), 3.61(s, 2H), 2.42(s, 3H); *Z*-isomer: 11.62(s, 1H), 7.51(s, 1H), 7.45(d, *J*=8.8 Hz, 2H), 6.90—6.81(m, 2H), 5.95—5.79(m, 1H), 5.26(s, 2H), 4.20(br, 2H), 3.65(s, 2H), 2.47(s, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz) δ: *E*-isomer: 194.7, 175.0, 169.3, 148.1, 132.5, 131.8, 120.8, 116.7, 112.9, 95.4, 56.5, 51.5, 13.2; *Z*-isomer: 197.8, 171.9, 169.9, 148.1, 132.5, 131.9, 120.8, 116.7, 112.9, 97.0, 56.6, 49.3, 12.4; EI-MS, *m*/*z*: 349(M)$^+$.

3-{1-[2-Benzyl-2-(4-bromophenyl)hydrazinyl]ethylidene}pyrrolidine-2,4-dione(**6**δ): a white solid, yield 63.2%, m. p. 210.9—211.8 °C; ^{1}H NMR(DMSO-d_6, 400 MHz), δ: *E*-isomer: 11.51(s, 1H), 7.69(s, 1H), 7.46(d, *J*=8.9 Hz, 2H), 7.38—7.26(m, 5H), 7.00—6.91(m, 2H), 4.74(s, 2H), 3.56(s, 2H), 2.21(s, 3H); *Z*-isomer: 11.61(s, 1H), 7.41(s, 1H), 7.46(d, *J*=8.9 Hz, 2H), 7.38—7.26(m, 5H), 7.00—6.91(m, 2H), 4.76(s, 2H), 3.61(s, 2H), 2.24(s, 3H); ^{13}C NMR(DMSO-d_6, 101 MHz), δ: *E*-isomer: 194.6, 174.9, 169.1, 148.5, 135.9, 132.5, 129.5, 129.0, 128.3, 117.2, 113.1, 95.2, 57.9, 51.5, 13.1; *Z*-isomer: 197.7, 171.8, 169.8, 148.5, 135.8, 132.5, 129.5, 129.0, 128.3, 117.2, 113.2, 96.8, 57.9, 49.2, 12.3; EI-MS, *m*/*z*: 399(M)$^+$.

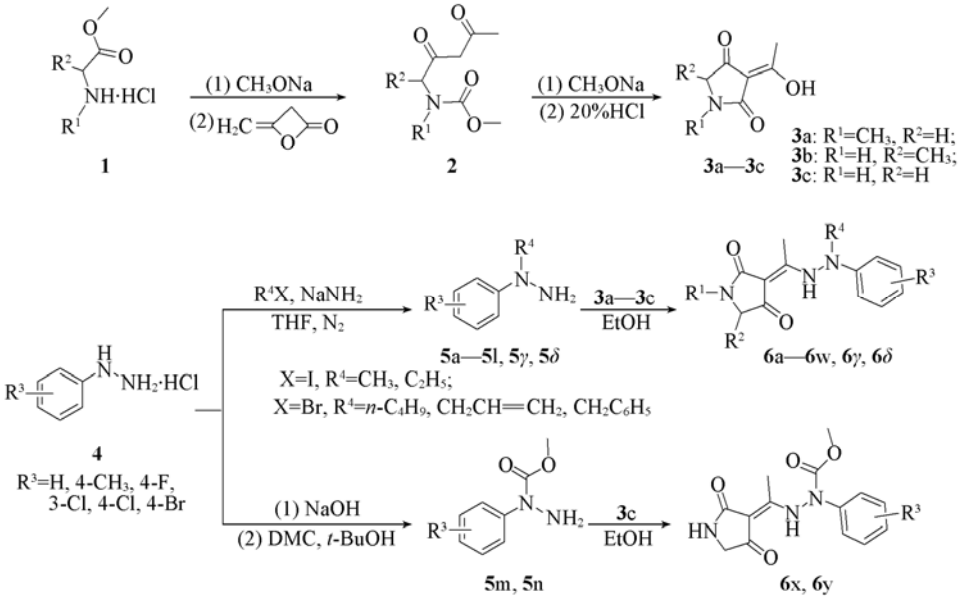
2.3 Antifungal Activity Bioassay

The inhibitory activities of the target compounds and the control fungicide drazoxolon against *R. Cerealis* were tested using a radial growth inhibition technique *in vitro*[6]. Every tested compound was dissolved in 0.5 mL of DMSO and mixed with potato sucrose agar(PSA) medium(45 mL). Meanwhile, 0.5 mL of DMSO in 45 mL of PSA medium was used as the control. The medium was poured into three 9 cm petri plates uniformly, cooled and solidified. The fungi was inoculated to the centre of the medium. Then the treatments were cultured at (25±1) °C for 3—5 d in the dark. The diameters of the fungal colonies were measured to calculate the growth inhibition rates when the petri dishes have been covered two-thirds by the fungal colonies in the control treatments. The EC_{50} values were determined using the double broth dilution method.

3 Results and Discussion

3.1 Chemistry

The synthetic routes of the title compounds are shown in Scheme 1. The intermediate tetramic acids **3**a—**3**c were prepared through the reactions of *N*-aceto-acetylation and cyclization with amino acid methyl ester hydrochlorides as the raw materials[16,17]. The alkyl groups were introduced to *N*-1-position of phenylhydrazines to prepare compounds **5**a—**5**l, **5**γ and **5**δ from phenylhydrazine hydrochlorides with different alkyl iodides or alkyl bromides in $NaNH_2$/THF at low temperature and a gentle stream of nitrogen was used to remove most of the dissolved ammonia[18]. Intermediates **5**m and **5**n were prepared by another method with dimethyl carbonate as the electrophilic reagent at room temperature[19]. The title compounds **6**a—**6**y, **6**γ and **6**δ were synthesized by reactions of compounds **5**a—**5**n, **5**γ and **5**δ with compounds **3**a—**3**c in ethanol, respectively. The reaction conditions were gentle and the yields of the title compounds ranged from 23.9% to 85.9%.



Scheme 1 General synthetic routes of the title compounds 6a—6y, 6γ and 6δ

The two tautomers, *E*-isomer and *Z*-isomer, of the title compounds could be observed by ^{1}H NMR and ^{13}C NMR[20]. The ^{1}H NMR spectra of the target compounds showed two pairs of signals at δ 12.0—11.0 for the protons of NH. These peaks appeared in the low field due to the influence of intramolecular hydrogen bond. In the ^{13}C NMR spectra, almost all the signals divided into two peaks due to the *E*-*Z*-isomerism. In order to distinguish between two existing forms of the tautomerism, the isomers of compound **6**q were calculated with the Gaussian 03W package[21—23]. HF/3-21G and B3LYP/6-31G* were successively performed to optimize the structures (Fig.1). In addition, density functional theory(DFT) method was used to calculate the single point energy at B3LYP/6-311++G** level including the solvent effect of DMSO. The relative energies of compounds **6**qα and **6**qβ were 0 and 1.21 kJ/mol, respectively. The result interpreted that *E*-isomer was more stable than *Z*-isomer. The ratios of *E*-isomers were estimated at 55% to 70% according to the intensities of the

two pairs.

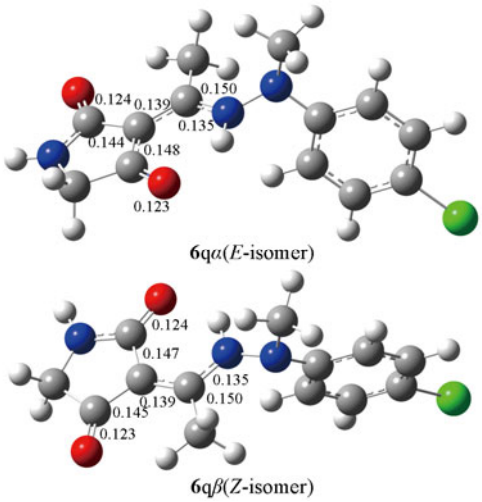


Fig.1 Optimized structures of *E*-isomer(*α*) and *Z*-isomer(*β*) of compound 6q

The key bond lengths(nm) were marked.

3.2 Antifungal Activity and 3D-QSAR Analysis

The EC_{50} values of the title compounds against *R. cerealis* were determined. As shown in Table 1, compounds **6**l and **6**q displayed remarkable activity, their EC_{50} values were 1.626 and 2.043 μg/mL, respectively, lower than 2.113 μg/mL of the control drug drazoxolon.

A 3D-QSAR CoMFA model was built to analyse the structure-activity relationship[13,14]. The experimental and predicted pEC_{50} values are reported in Table 1. Under Tripos force field, the charges of Gasteiger-Huckel were applied. The values of max. iterations and gradient were 1000 and 0.005, respectively. All the compounds used in the training set are aligned in Fig.2. Partial least-square(PLS) analysis was performed to establish a linear relationship between the molecular fields and the fungicidal activity against *R. cerealis*. Using leave-one-out method, the CoMFA model was obtained with optimal at 8 components, which showed good values of cross-validated correlation coefficient q^2(0.585), standard error of estimate(0.148), Noncross-validated correlation coefficient r^2 (0.971) and test value F(67.436). The contribution proportions made by steric and electrostatic fields were 60.8% and 39.2%, respectively. Furthermore, the contour maps of CoMFA steric and electrostatic fields are displayed in Fig.3 and Fig.4, which are helpful to identify important regions changing with antifungal activity against *R. cerealis*. The correlation plot of CoMFA model is shown in Fig.5.

In steric CoMFA map, a yellow region around *N*-1 of phenylhydrazine was depicted, which indicated that a bulky group in this region was unfavorable. For example, the activities of compounds **6**e, **6**k and **6**p were better than those of compounds **6**u and **6**w. A green contour around 4-position of phenyl ring indicated that a bulky group favored the activity. This explained that compounds **6**c and **6**i had not good fungicidal activity.

Table 1 Experimental and predicted pEC_{50} values of the title compounds against *R. cerealis*

Compd.	R^1	R^2	R^3	R^4	Actual EC_{50}/(μg·mL^{-1})	Actual pEC_{50}[a]	CoMFA	
							Predicted pEC_{50}	Residual
6a	CH_3	H	H	CH_3	64.685	4.189	4.412	0.2233
6b	CH_3	H	4-CH_3	CH_3	83.990	4.076	3.863	–0.2135
6c	CH_3	H	4-F	CH_3	142.873	3.845	3.852	0.0074
6d	CH_3	H	3-Cl	CH_3	32.359	4.490	4.400	–0.0898
6e	CH_3	H	4-Cl	CH_3	4.027	5.395	5.389	–0.0062
6f	CH_3	H	4-Br	CH_3	3.382	5.471	5.561	0.0900
6g	H	CH_3	H	CH_3	22.070	4.656	4.429	–0.2274
6h	H	CH_3	4-CH_3	CH_3	77.525	4.111	4.272	0.1608
6i	H	CH_3	4-F	CH_3	168.622	3.773	3.867	0.0945
6j	H	CH_3	3-Cl	CH_3	32.728	4.485	4.517	0.0316
6k	H	CH_3	4-Cl	CH_3	5.854	5.233	5.497	0.2643
6l	H	CH_3	4-Br	CH_3	1.626	5.789	5.612	–0.1767
6m	H	H	H	CH_3	54.835	4.261	4.348	0.0867
6n	H	H	4-CH_3	CH_3	99.034	4.004	4.085	0.0815
6o	H	H	3-Cl	CH_3	37.894	4.421	4.336	–0.0855
6p	H	H	4-Cl	CH_3	3.874	5.412	5.326	–0.0858
6q	H	H	4-Br	CH_3	2.043	5.690	5.560	–0.1305
6r	H	H	4-Cl	C_2H_5	34.532	4.462	4.437	–0.0252
6s	H	H	4-Cl	*n*-C_4H_9	2.657	5.576	5.586	0.0098
6t	H	H	H	$CH_2CH{=}CH_2$	189.355	3.723	3.695	–0.0275
6u	H	H	4-Cl	$CH_2CH{=}CH_2$	60.279	4.220	4.261	0.0413
6v	H	H	H	$CH_2C_6H_5$	610.739	3.214	3.212	–0.0015
6w	H	H	4-Cl	$CH_2C_6H_5$	58.265	4.235	4.239	0.0040
6x	H	H	H	$COOCH_3$	182.657	3.738	3.715	–0.0226
6y	H	H	4-Cl	$COOCH_3$	63.375	4.198	4.195	–0.0031
6γ[b]	H	H	4-Br	$CH_2CH{=}CH_2$	27.677	4.558	4.843	0.2849
6δ[b]	H	H	4-Br	$CH_2C_6H_5$	43.833	4.358	4.543	0.1852
Drazoxolon	—	—	—	—	2.113	—	—	—

a. pEC_{50}= –lgEC_{50}; *b*. compounds in the test set.

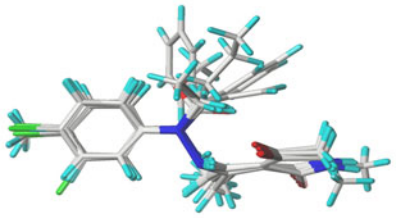


Fig.2 Alignment of all the compounds in training set

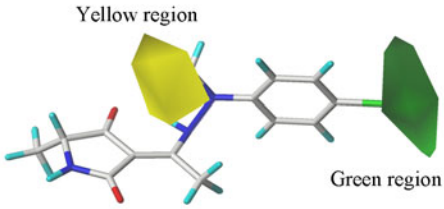


Fig.3 CoMFA model in steric fields

Sterically favored areas are in green, and sterically disfavored areas are in yellow.

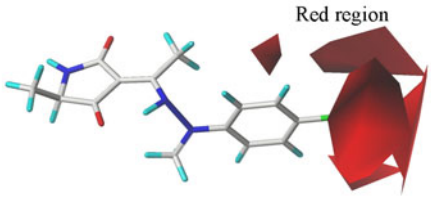


Fig.4 CoMFA model in electrostatic fields

Negative charge favored areas are in red.

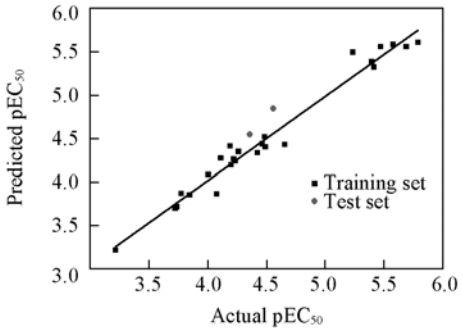


Fig.5 Correlation plot of actual and predicted activities for CoMFA model

In the electrostatic CoMFA contour map, a red region was shown around 4-position of phenyl ring, indicating that introducing the electronegative substitutions might improve the activity. It well explained that the compounds containing 4-Cl and 4-Br exhibited better antifungal activity than the compounds containing 4-CH_3. As a whole, a smaller group at *N*-1 of phenylhydrazine and a negative charge group at 4-position of phenyl ring could promote the antifungal activity, such as compounds **6**e, **6**f, **6**k, **6**l, **6**p and **6**q.

Compounds **6***γ* and **6***δ* were synthesized and their EC_{50} values against *R. cerealis* were predicted by the CoMFA model. Their actual EC_{50} values were 27.677 and 43.833 μg/mL, respectively, close to the predicted ones. Their EC_{50} values were higher than that of compound **6**q, but lower than those of compounds **6**t and **6**v, further confirming that a smaller group at *N*-1 of phenylhydrazine and a bulky negative charge group at 4-position of phenyl ring favored the activity.

In conclusion, *N*-Substituted phenylhydrazine groups were introduced to 3-position of pyrrolidine-2,4-dione ring to design and synthesize twenty-seven novel tetramic acid derivatives. Their structures were well confirmed by spectroscopic data. Compounds **6**l and **6**q displayed excellent activity against *R. cerealis*, their EC_{50} values were lower than that of the control drug drazoxolon. A 3D-QSAR CoMFA model was established with good q^2, r^2 and F values. It revealed that introducing a smaller group to *N*-1-position of phenylhydrazine and a negative charge group to 4-position of phenyl ring could increase the antifungal activity of the title compounds.

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