Synthesis and Antifungal Activity of the Derivatives of Novel Pyrazole Carboxamide and Isoxazolol Pyrazole Carboxylate

Jialong Sun and Yuanming Zhou *

College of Chemistry and Pharmaceutical Sciences, Qingdao Agricultural University, Qingdao 266109, China; E-Mail: sunjialong6289@163.com

* Author to whom correspondence should be addressed; E-Mail: zym7410@163.com; Tel.: +86-532-8608-0147.

Academic Editor: Derek J. McPhee

Received: 13 January 2015 / Accepted: 4 March 2015 / Published: 9 March 2015

Abstract: A series of pyrazole carboxamide and isoxazolol pyrazole carboxylate derivatives were designed and synthesized in this study. The structures of the compounds were elucidated based on spectral data (infrared, proton nuclear magnetic resonance and mass spectroscopy). Then, all of the compounds were bioassayed in vitro against four types of phytopathogenic fungi (Alternaria porri, Marssonina coronaria, Cercospora petroselini and Rhizoctonia solani) using the mycelium growth inhibition method. The results showed that some of the synthesized pyrazole carboxamides displayed notable antifungal activity. The isoxazole pyrazole carboxylate 7ai exhibited significant antifungal activity against R. solani, with an EC50 value of 0.37 μg/mL. Nonetheless, this value was lower than that of the commercial fungicide, carbendazol.

Keywords: pyrazole carboxamide; isoxazolol pyrazole carboxylate; antifungal activity; synthesis; fungi

1. Introduction

Phytopathogenic fungi, such as Rhizoctonia solani Kuhn, Alternaria porri (Ell) Ciferri, Marssonina coronaria (Ell.et Davis) Davis and Cercospora petroselini Saccardo, pose serious threats to agriculture. They are broad host-range pathogens and infect many crops worldwide, including rice, onions, apples and cucumbers. Many pesticides have been developed and applied to control these diseases with the
progression of the modern agrochemical industry. However, the increased microbial resistance of pathogens to known antibiotics facilitates the urgent need for new fungicides [1].

As with many other five-membered heterocyclic compounds, pyrazoles and their derivatives attract increasing attention in the fields of pharmacology and medicine because of their various bioactivities, including antifungal [2], anti-inflammatory [3], antiviral [4], antioxidant [5], cytotoxic [6], antihypertensive [7], A3 adenosine receptor antagonistic [8], antibacterial [9], tranquilizing, psychoanaleptic, muscle-relaxant, hypnotic, antidepressant, ulcerogenic and analgesic activities [10]. They are also highly significant in agrichemistry, and many of these compounds have been widely used, given their fungicidal [11], insecticidal [12] and herbicidal activities [13].

Pyrazole carboxamide derivatives are important heterocyclic compounds in the development of medicines and pesticides because of their broad spectrum of biological activities, including insecticidal [14], fungicidal [15], herbicidal [13] and acaricidal activity [16]. Many recent studies have been conducted on the synthesis and biological activity of these derivatives. Pyrazole carboxamide derivatives, such as penthiopyrad, furametpyr, penfluifen, isopyrazam and bixafen, which could inhibit the succinate dehydrogenase, have been developed and commercialized as fungicides in succession [17]. In addition, many isoxazole compounds, including oxacillin and sulfamethoxazole, have been developed as pesticides and drugs, because of their insecticidal, herbicidal, antiviral and antifungal activities. Isoxazole derivatives have received much attention, because of their wide application in medicine and pesticide chemistry [18].

In view of the facts and to explore the potential antifungal activity of pyrazole derivatives, a series of pyrazole carboxamide and isoxazolol pyrazole carboxylate derivatives are designed and synthesized in the current study. Pyrazole carbonyl chloride is synthesized from pyrazole carboxylic acid and thionyl chloride. Then, 18 novel pyrazole carboxamides and two isoxazolol pyrazole carboxylates are synthesized by reacting pyrazole carbonyl chloride with different substituted amines and with isoxazol-3-ol, respectively.

The synthesis of the derivatives of pyrazole carboxamide and isoxazolol pyrazole carboxylate is outlined in Scheme 1. Acetaoacetic ester (1a–b) and triethyl orthoformate were dissolved in acetic anhydride, refluxed and then converted into 2-ethoxymethylene acetooacetic ester derivatives (2a–b) [19]. Ethyl 1H-pyrazole-4-carboxylate (3a–b) was prepared by reacting Compounds 2a–b with hydrazine hydrate [20]. Intermediate 1H-pyrazole-4-carboxylic acids (5a–b) were obtained as light-yellow crystals from Compounds 3a–b by successively performing a substitution reaction using dimethyl sulfate, saponification with NaOH and acidification using HCl [21]. Subsequently, Compounds 5a–b were refluxed in SOCl2 to yield pyrazole acid chlorides (6a–b) [22]. Finally, the target compounds of pyrazole carboxamides and isoxazolol pyrazole carboxylates (7aa–bk) were obtained by reacting 6a–b with different substituted amines [23] and with isoxazol-3-ol, respectively.
IR, MS and $^1$H-NMR data were applied to confirm the structures of the new synthesized compounds. The synthesized compounds above were published in our Chinese Patents CN103554026A and CN103524417A [24,25].

Scheme 1. Synthesis route of the pyrazole derivatives.

2.2. Antifungal Activity

The initial concentration was set at 100 $\mu$g/mL for antifungal activity screening in vitro. If the percentage inhibition exceeded 50%, a series of concentrations of the compounds was tested to evaluate their EC$_{50}$ values. Carbendazol was selected as the positive control.

As suggested in Table 1, most of the synthesized pyrazole derivatives exhibited antifungal activity to some extent. Though the EC$_{50}$ value of these compounds was higher than that of the positive control, carbendazol, a few pyrazole carboxamides (7af, 7bc, 7bg, 7bh and 7bi) displayed remarkable antifungal activity. According to the literature [17] reported, a bigger group introduced into the ortho position of the aniline part of this type of compound would strengthen the antifungal activity. The simple anilines applied in this study only gave moderate antifungal activity, so the bioactivity and the structures of the pyrazole derivatives should be researched further.

| Compound | R$_1$ | R$_2$ | EC$_{50}$ ($\mu$g/mL) |
|----------|-------|-------|----------------------|
|          |       |       | A. porri | M. coronaria | C. petroselini | R. solani |
| 7aa      | CH$_3$ | -     | --       | --            | --            | 31.39     |
| 7ab      | CH$_3$ | -     | 80.76    | --            | 38.41         | --        |
| 7ac      | CH$_3$ | -     | 52.56    | 84.74         | --            | 40.00     |
| Compound | R₁ | R₂ | EC₅₀ (μg/mL) | A. porri | M. coronaria | C. petroselini | R. solani |
|----------|----|----|--------------|----------|--------------|---------------|-----------|
| 7ad      | CH₃ |   | --           | --       | --           | 6.32          | 18.15     |
| 7ae      | CH₃ |   | 65.12        | --       | --           | --            | 14.89     |
| 7af      | CH₃ |   | 63.04        | 7.87     | 35.90        | 5.23          |           |
| 7ag      | CH₃ |   | 54.86        | 76.12    | 51.00        | 16.91         |           |
| 7ah      | CH₃ |   | --           | --       | --           | --            | 69.45     |
| 7ai      | CH₃ |   | 2.24         | 3.21     | 10.19        | 0.37          |           |
| 7ba      | CF₃ |   | --           | 35.94    | 22.47        | 16.81         |           |
| 7bb      | CF₃ |   | --           | 74.54    | 27.82        | 19.47         |           |
| 7bc      | CF₃ |   | 10.10        | 14.92    | 5.43         | 3.40          |           |
| 7bd      | CF₃ |   | --           | --       | 74.38        | 27.37         |           |
| 7be      | CF₃ |   | 23.12        | 13.00    | 13.44        | 8.55          |           |
Table 1. Cont.

| Compound | R₁ | R₂ | EC₅₀ (µg/mL) | A. porri | M. coronaria | C. petroselini | R. solani |
|----------|----|----|-------------|---------|-------------|--------------|---------|
| 7bf      | CF₃⁻³ |    | 72.20       | 61.29   | --          | 81.72       |
| 7bg      | CF₃⁻³ |    | 11.22       | 7.93    | 27.43       | 4.99        |
| 7bh      | CF₃⁻³ |    | 24.76       | 25.48   | 6.99        | 5.93        |
| 7bi      | CF₃⁻³ |    | 21.01       | 9.08    | 32.40       | 7.69        |
| 7bj      | CF₃⁻³ |    | 11.46       | 15.86   | --          | 8.32        |
| 7bk      | CF₃⁻³ |    | 35.05       | --      | --          | 28.88       |
| carbendazol | | | 0.99 | 0.96 | 0.96 | 1.00 |

"--" The percentage of inhibition is lower than 50% at 100 µg/mL.

It was interesting that the isoxazolol pyrazole carboxylate 7ai showed significant antifungal activity against A. porri, M. coronaria, C. petroselini and R. solani, with EC₅₀ values of 2.24, 3.21, 10.29 and 0.37 µg/mL, respectively. The EC₅₀ value of this compound against R. solani was lower than that of the positive control, carbendazol (EC₅₀, 1.00 µg/mL).

The EC₅₀ values of the isoxazolol pyrazole carboxylate 7bk were 35.05, over 100, over 100 and 28.88 µg/mL against A. porri, M. coronaria, C. petroselini and R. solani, respectively (Table 1). The results of the preliminary structure-activity relationship (SAR) analysis suggested that the antifungal activity of the synthesized isoxazolol pyrazole carboxylate was significantly weakened when the methyl group at the C-3 of the pyrazole ring (7ai) was substituted with a trifluoromethyl group (7bk).

3. Experimental Section

3.1. Chemistry

All of the reagents and solvents were either chemically pure or purified in accordance with standard methods. Reactions were monitored through thin-layer chromatography (TLC) using precoated silica gel plates (silica gel GF 254, Qingdao Marine Chemistry Co. Ltd., Qingdao, China), and the spots
Molecules 2015, 20 4388

were visualized with UV (254 nm). All of the melting points were detected with a WRS-1A type melting point apparatus (ShangHai Suoguang Electric Tech Co., Ltd., Shanghai, China), and the thermometer was not corrected. IR spectra were recorded on a Nicolet IR-200 (Thermo Electron, Madison, WI, USA) spectrophotometer. 1H-NMR spectra were captured with Bruker AV-500 and AV-400 spectrometers, and tetramethylsilane was applied as an internal standard. High-resolution electrospray ionization mass spectroscopy (HR-ESI-MS) spectra were observed with a Micromass Q-TOF spectrometer (Waters Corp., Manchester, UK).

3.2. General Procedure for the Preparation of 2a–b

A mixture of acetoacetic ester (0.60 mol) (1a–b), triethyl orthoformate (0.72 mol) and acetic anhydride (1.08 mol) was stirred and heated under reflux until the 1a–b was no longer monitored by TLC. Then, the reaction mixture was evaporated in vacuo. The distillates of T = 140 °C–160 °C (3 KPa) were collected to produce 2-ethoxymethylene acetoacetic esters (2a–b) as a light-yellow liquid with yields ranging from 70% to 90%.

3.3. General Procedure for the Preparation of 3a–b

The acetoacetic ester of 2-ethoxymethylene (0.2 mol) (2a–b) was dissolved in ethanol (150 mL) in an ice-water bath, and 80% hydrazine hydrate (0.4 mol) was added dropwise. The mixture reacted at room temperature until the 2a–b was fully consumed, as detected by TLC. Subsequently, the reaction mixture was concentrated in vacuo. The residue was extracted with 1,2-dichloroethane, washed with water and brine, dried over anhydrous sodium sulfate and then concentrated in vacuo to obtain ethyl 1H-pyrazole-4-carboxylate (3a–b) as either light-yellow liquids or solids.

3.4. General Procedure for the Preparation of 5a–b

Ethyl 1H-pyrazole-4-carboxylate (3a–b, 0.5 mol) and NaHCO3 (0.6 mol) were dissolved in toluene (120 mL). (CH3)2SO4 (0.24 mol) was dropped gradually into the solution while the temperature was maintained at 20 °C–30 °C via an ice-water bath. Then, the solution reacted at a temperature of 50 °C with a water bath and was monitored by TLC. Once the reaction was complete, the reaction solution was filtered. A light-yellow solution was then obtained and washed with ice water. The upper toluene solution was concentrated in vacuo to produce ethyl 1-methyl-1H-pyrazole-4-carboxylate (4a–b). Subsequently, sodium hydroxide solution (0.12 mol NaOH dissolved in 45 mL water) was added to a solution of Compounds 4a–b (0.1 mol) in EtOH (95%, 50 mL) and then reacted at room temperature for approximately 2 h. The solution was concentrated in vacuo to remove most of the ethanol. The pH level was then adjusted to 3–4 with HCl. The reaction mixture was filtered, and the filtrate was recrystallized with ethyl acetate to produce pyrazole acids (5a–b) as light-yellow crystalline solids.

3.5. General Procedure for the Preparation of 7aa–bk

Pyrazole acid chlorides 6a–b were prepared by refluxing 4a–b in thionyl chloride for 8 h. Pyrazole acid chlorides 6a–b (12 mmol) in anhydrous tetrahydrofuran (THF; 30 mL) were slowly added to a solution of amine derivatives or 5-methylisoxazol-3-ol (10 mmol) and K2CO3 (1.38 g, 10 mmol) in
anhydrous THF (30 mL) at a controlled temperature of 5 °C. The reaction proceeded at room temperature until 6a–b was no longer tested by TLC. The reaction solution was then filtered and the solvent distilled. The residue was dissolved in ethyl acetate, washed with water and brine, dried over anhydrous sodium sulfate and recrystallized to generate the target pyrazole carboxamides and isoxazolol pyrazole carboxylates (7aa–bk). The product yields ranged from 40% to 80%. All 20 compounds were novel, and the physical and spectral data for these compounds are listed below.

1,3-Dimethyl-N-(2-hydroxyl)benzyl-1H-pyrazole-4-carboxamide (7aa): Bright brown crystal, yield of 77.9%, m.p. 193.0–193.1 °C. 1H-NMR (CDCl3, 500 MHz) δ: 8.82 (s, 1H, NH), 7.86 (s, 1H, pyrazole H), 7.61 (s, 1H, -OH), 7.14–7.16 (m, 1H, Ar-H), 7.13–7.09 (m, 1H, Ar-H), 7.08–7.05 (m, 1H, Ar-H), 6.89 (t, J = 1.5 Hz, 1H, Ar-H), 3.89 (s, 3H, N-CH3), 2.56 (s, 3H, pyrazole CH3); IR (KBr): ν 3431, 1643, 1593, 1544, 1452, 1382, 1278 cm⁻¹; HR-ESI-MS m/z: 232.1084 [M+H]+ (calcd. for C12H14N3O2, 232.1081).

1,3-Dimethyl-N-(4-hydroxyl)benzyl-1H-pyrazole-4-carboxamide (7ab): Gray needle crystal, yield of 80.5%, m.p. 208.0–208.7 °C. 1H-NMR (CDCl3, 500 MHz) δ: 7.76 (s, 1H, pyrazole H), 7.43 (m, 2H, Ar-H), 6.84 (d, J = 8.5 Hz, 2H, Ar-H), 4.78 (s, 1H, -OH), 3.89 (s, 3H, N-CH3), 2.54 (s, 3H, pyrazole CH3); IR (KBr): ν 3510, 3290, 1639, 1539, 1515, 1448, 1245, 1166 cm⁻¹; HR-ESI-MS m/z: 232.1081 [M+H]+ (calcd. for C12H14N3O2, 232.1081).

1,3-Dimethyl-N-2',4'-dichlorobenzyl-1H-pyrazole-4-carboxamide (7ac): White lamellar crystal, yield of 77.9%, m.p. 169.1–169.3 °C. 1H-NMR (CDCl3, 500 MHz) δ: 8.52 (d, J = 9.0 Hz, 1H, Ar-H), 7.86 (s, 1H, pyrazole H), 7.42 (d, J = 2.5 Hz, 1H, Ar-H), 7.27–7.30 (m, 1H, Ar-H), 3.91 (s, 3H, N-CH3), 2.60 (s, 3H, pyrazole CH3); IR (KBr): ν 3241, 1648, 1499, 1279, 1096 cm⁻¹; HR-ESI-MS m/z: 284.0347 [M+H]+ (calcd. for C12H12Cl2N3O, 284.0352).

1,3-Dimethyl-N-3',5'-dichlorobenzyl-1H-pyrazole-4-carboxamide (7ad): Gray crystal, yield of 66.9%, m.p. 181.6–182.2 °C. 1H-NMR (CDCl3, 500 MHz) δ: 7.77 (s, 1H, pyrazole H), 7.54 (d, J = 1.5 Hz, 2H, Ar-H), 7.11 (s, 1H, Ar-H), 3.88 (s, 3H, N-CH3), 2.53 (s, 3H, pyrazole CH3); IR (KBr): ν 3265, 3211, 1698, 1614, 1503, 1403, 1142, 1063 cm⁻¹; HR-ESI-MS m/z: 284.0357 [M+H]+ (calcd. for C12H12Cl2N3O, 284.0352).

1,3-Dimethyl-N-(1,3,4-thiadiazole-2-yl)-1H-pyrazole-4-carboxamide (7ae): White lamellar crystal, yield of 57.2%, m.p. 278.4–278.9 °C. 1H-NMR (DMSO-d6, 500 MHz) δ: 12.52 (s, 1H, NH), 9.15 (s, 1H, pyrazole H), 8.55 (s, 1H, thiadiazole H), 3.82 (s, 3H, N-CH3), 2.50 (s, 3H, pyrazole CH3); IR (KBr): ν 3381, 3124, 2929, 1680, 1548, 1415, 1311 cm⁻¹; HR-ESI-MS m/z: 224.0597 [M+H]+ (calcd. for C8H10N5OS, 224.0601).

1,3-Dimethyl-N-(5-ethyl-1,3,4-thiadiazole-2-yl)-1H-pyrazole-4-carboxamide (7af): White powder, yield of 68.3%, m.p. 266.2–266.5 °C. 1H-NMR (CDCl3, 400 MHz) δ: 12.58 (s, 1H, NH), 9.13 (s, 1H, pyrazole H), 3.94 (s, 3H, N-CH3), 3.07 (q, J = 1.0 Hz, 2H, CH2), 2.56 (s, 3H, pyrazole CH3), 1.45 (t, J = 1.0 Hz, 3H, CH3); IR (KBr): ν 3129, 2971, 2934, 1673, 1544, 1420, 1316, 1241, 1179 cm⁻¹; HR-ESI-MS m/z: 252.0912 [M+H]+ (calcd. for C10H14N5OS, 252.0914).
1,3-Dimethyl-N-(5-methyl-1,3,4-thiadiazole-2-yl)-1H-pyrazole-4-carboxamide (7ag): White powder, yield of 61.4%, m.p. >300 °C. 1H-NMR (CDCl3, 400 MHz) δ: 9.12 (s, 1H, pyrazole H), 3.96 (s, 3H, N-CH3), 2.72 (s, 3H, thiadiazole CH3), 2.56 (s, 3H, pyrazole CH3); IR (KBr): v 3149, 3012, 2921, 1677, 1544, 1494, 1416, 1320, 1250, 1188 cm⁻¹; HR-ESI-MS m/z: 238.0755 [M+H]+ (calcd. for C9H12N5OS, 238.0757).

1,3-Dimethyl-N-(5-trifluoromethyl-1,3,4-thiadiazole-2-yl)-1H-pyrazole-4-carboxamide (7ah): White lamellar crystal, yield of 44.3%, m.p. 272.1–272.8 °C. 1H-NMR (CDCl3, 500 MHz) δ: 12.61 (s, 1H, NH), 9.12 (s, 1H, pyrazole H), 3.98 (s, 3H, N-CH3), 2.58 (s, 3H, pyrazole CH3); IR (KBr): ν 1668, 1519, 1303, 1195, 1137, 1029 cm⁻¹; HR-ESI-MS m/z: 292.0471 [M+H]+ (calcd. for C9H9F3N5OS, 292.0474).

5-Methylisoxazol-3-yl 1,3-dimethyl-1H-pyrazole-4-carboxylate (7ai): White powder, yield of 54.7%, m.p. 124.2–124.4 °C. 1H-NMR (CDCl3, 500 MHz) δ: 7.98 (s, 1H, pyrazole H), 6.23 (s, 1H, isoxazole H), 3.88 (s, 3H, N-CH3), 2.49 (s, 3H, pyrazole CH3), 2.43 (s, 3H, isoxazole CH3); IR (KBr): ν 3120, 1743, 1622, 1548, 1232, 1050 cm⁻¹; HR-ESI-MS m/z: 222.0871 [M+H]+ (calcd. for C10H12N3O3, 222.0873).

1-Methyl-N-(2-hydroxyl)benzyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (7ba): Light yellow crystal, yield of 41.8%, m.p. 192.8–193.2 °C. 1H-NMR (DMSO-d6, 500 MHz) δ: 9.24 (s, 1H, -OH), 8.54 (s, 1H, pyrazole H), 7.65 (d, J = 7.5 Hz, 1H, Ar-H), 6.98 (d, J = 7.5 Hz, 1H, Ar-H), 6.88 (d, J = 7.5 Hz, 1H, Ar-H), 6.79 (d, J = 8.0 Hz, 1H, Ar-H), 3.94 (s, 3H, N-CH3); IR (KBr): ν 3485, 3116, 1656, 1598, 1494, 1441, 1329, 1250, 1175, 1146 cm⁻¹; HR-ESI-MS m/z: 286.0804 [M+H]+ (calcd. for C12H11F3N3O2, 286.0798).

1-Methyl-N-(4-hydroxyl)benzyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (7bb): Light brown powder, yield of 67.9%, m.p. 206.4–206.6 °C. 1H-NMR (DMSO-d6, 500 MHz) δ: 9.88 (s, 1H, NH), 9.25 (s, 1H, -OH), 8.44 (s, 1H, pyrazole H), 7.38–7.43 (m, 2H, Ar-H), 6.69–6.72 (m, 2H, Ar-H), 3.98 (s, 3H, N-CH3); IR (KBr): ν 1627, 1553, 1436, 1329, 1142, 839, 781, 711, 619, 516 cm⁻¹; HR-ESI-MS m/z: 286.0800 [M+H]+ (calcd. for C12H11F3N3O2, 286.0798).

1-Methyl-N-2',4'-dichlorobenzyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (7bc): White crystal, yield of 49.0%, m.p. 147.4–147.8 °C. 1H-NMR (CDCl3, 400 MHz) δ: 8.43 (d, J = 7.2 Hz, 1H, Ar-H), 8.04 (s, 1H, pyrazole H), 7.43 (d, J = 2.0 Hz, 1H, Ar-H), 7.26–7.30 (m, overlapped, Ar-H), 4.02 (s, 3H, N-CH3); IR (KBr): ν 2958, 1785, 1640, 1565, 1424, 1333, 1291, 1146 cm⁻¹; HR-ESI-MS m/z: 338.0066 [M+H]+ (calcd. for C12H9Cl2F3N3O, 338.0069).

1-Methyl-N-3',5'-dichlorobenzyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (7bd): White powder, yield of 53.2%, m.p. 149.1–150.3 °C. 1H-NMR (CDCl3, 500 MHz) δ: 8.03 (s, 1H, pyrazole H), 7.42 (d, J = 2.0 Hz, 1H, Ar-H), 7.26–7.30 (m, overlapped, Ar-H), 4.01 (s, 3H, N-CH3); IR (KBr): ν 2958, 1785, 1640, 1565, 1424, 1333, 1291, 1146, 1047, 847, 781, 706 cm⁻¹; HR-ESI-MS m/z: 338.0075 [M+H]+ (calcd. for C12H9Cl2F3N3O, 338.0069).

1-Methyl-N-(1,3,4-thiadiazole-2-yl)-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (7be): White floccus, yield of 49.6%, m.p. >300 °C. 1H-NMR (DMSO-d6, 500 MHz) δ: 8.43 (d, J = 2.0 Hz, 1H, Ar-H), 7.26–7.30 (m, overlapped, Ar-H), 4.02 (s, 3H, N-CH3); IR (KBr): ν 2958, 1785, 1640, 1565, 1424, 1333, 1291, 1146, 1047, 847, 781, 706 cm⁻¹; HR-ESI-MS m/z: 338.0066 [M+H]+ (calcd. for C12H9Cl2F3N3O, 338.0069).
1-Methyl-N-(5-ethyl-1,3,4-thiadiazole-2-yl)-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (7bf): White powder, yield of 56.9%, m.p. 288.6–288.8 °C. \(^1\)H-NMR (DMSO-\(d_6\), 500 MHz) \(\delta\): 12.82 (s, 1H, NH), 8.72 (s, 1H, pyrazole H), 3.96 (s, 3H, N-CH\(_3\)), 2.98 (q, \(J = 7.5\) Hz, 2H, CH\(_2\)), 1.29 (t, \(J = 7.5\) Hz, 3H, CH\(_3\)); IR (KBr): \(\nu\) 3456, 3054, 2963, 1694, 1627, 1557, 1424, 1341, 1308, 1171, 1129 cm\(^{-1}\); HR-ESI-MS \(m/z\): 278.0320 [M+H]+ (calcd. for C\(_8\)H\(_7\)F\(_3\)N\(_5\)OS, 278.0318).

1-Methyl-N-(5-trifluoromethyl-1,3,4-thiadiazole-2-yl)-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (7bg): White crystal, yield of 71.2%, m.p. 234.9–235.1 °C. \(^1\)H-NMR (DMSO-\(d_6\), 500 MHz) \(\delta\): 13.68 (s, 1H, NH), 8.81 (s, 1H, pyrazole H), 4.01 (s, 3H, N-CH\(_3\)); IR (KBr): \(\nu\) 3456, 3070, 2963, 1694, 1627, 1557, 1424, 1341, 1308, 1183, 885, 711 cm\(^{-1}\); HR-ESI-MS \(m/z\): 346.0191 [M+H]+ (calcd. for C\(_9\)H\(_6\)F\(_6\)N\(_5\)OS, 346.0192).

1-Methyl-N-2'-fluorobenzyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (7bh): Brown crystal, yield of 74.3%, m.p. 148.4–148.9 °C. \(^1\)H-NMR (CDCl\(_3\), 500 MHz) \(\delta\): 8.38 (m, 1H, Ar-H), 8.03 (s, 1H, pyrazole H), 8.00–8.03 (m, 1H, Ar-H), 7.08–7.18 (m, 2H, Ar-H), 4.00 (s, 3H, N-CH\(_3\)); IR (KBr): \(\nu\) 3245, 1652, 1565, 1507, 1441, 1316, 1217, 1183 cm\(^{-1}\); HR-ESI-MS \(m/z\): 288.0757 [M+H]+ (calcd. for C\(_{12}\)H\(_{10}\)F\(_4\)N\(_3\)O, 288.0755).

1-Methyl-N-3'-fluorobenzyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (7bi): Brown crystal, yield of 47.9%, m.p. 137.8–138.3 °C. \(^1\)H-NMR (CDCl\(_3\), 500 MHz) \(\delta\): 8.04 (s, 1H, pyrazole H), 7.78 (s, 1H, Ar-H), 7.69 (d, \(J = 7.5\) Hz, 1H, Ar-H), 7.15 (d, \(J = 7.0\) Hz, 1H, Ar-H), 4.00 (s, 3H, N-CH\(_3\)); IR (KBr): \(\nu\) 3241, 1652, 1557, 1432, 1329, 1300, 856, 823, 752 cm\(^{-1}\); HR-ESI-MS \(m/z\): 288.0757 [M+H]+ (calcd. for C\(_{12}\)H\(_{10}\)F\(_4\)N\(_3\)O, 288.0755).

1-Methyl-N-3',4'-difluorobenzyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (7bj): Light brown crystal, yield of 63.2%, m.p. 155.7–155.8 °C. \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \(\delta\): 8.04 (s, 1H, pyrazole H), 7.78 (s, 1H, Ar-H), 7.69 (d, \(J = 7.5\) Hz, 1H, Ar-H), 7.15 (d, \(J = 7.0\) Hz, 1H, Ar-H), 4.00 (s, 3H, N-CH\(_3\)); IR (KBr): \(\nu\) 3241, 1652, 1557, 1519, 1432, 1329, 1300, 1188 cm\(^{-1}\); HR-ESI-MS \(m/z\): 306.0663 [M+H]+ (calcd. for C\(_{12}\)H\(_{9}\)F\(_5\)N\(_3\)O, 306.0660).

5-Methylisoxazol-3-yl 1-methyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxylate (7bk): White powder, yield of 58.9%, m.p. 130.7–131.1 °C. \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \(\delta\): 8.19 (s, 1H, pyrazole H), 6.28 (s, 1H, isoxazole H), 4.03 (s, 3H, N-CH\(_3\)); IR (KBr): \(\nu\) 3290, 1660, 1551, 1523, 1436, 1300, 1134, 1059, 856, 823, 711, 648 cm\(^{-1}\); HR-ESI-MS \(m/z\): 276.0595 [M+H]+ (calcd. for C\(_{10}\)H\(_{9}\)F\(_3\)N\(_3\)O\(_3\), 276.0591).

### 3.6. Antifungal Bioassays

The fungicidal activity of the target Compounds 7aa–bk were tested in vitro against the phytopathogenic fungi *A. porri*, *M. coronaria*, *C. petroselini* and *R. solani* using the mycelium growth rate method [26,27]. The commercially available agricultural fungicide, carbendazol, was used as the
positive control, whereas acetone served as the negative control. The compounds were dissolved in acetone to prepare a 100-μg/mL stock solution for the following antifungal test.

After the mycelia were incubated at 25 °C over a certain period, the diameter of each strain was measured. The percentage inhibition was calculated as follows:

\[ I = \frac{(B - A)}{B} \times 100\% \]

where \( I \) is the percentage of inhibition, \( A \) is the average mycelia diameter (mm) with the compounds in Petri dishes and \( B \) is the average mycelia diameter with the compounds in the blank Petri dishes.

The percentage inhibition of the compounds was determined at a dosage of 100 μg/mL. The compounds that displayed high activity (\( I > 50\% \) at 100 μg/mL) were evaluated further at concentrations of 100, 50, 25, 12.5, 6.25 and 0 μg/mL. Three replicates were applied in each treatment. The EC50 (μg/mL) values were estimated statistically by Probit analysis using SPSS (version 11.5) on a personal computer.

4. Conclusions

In conclusion, a series of novel pyrazole carboxamides and isoxazolol pyrazole carboxylates was synthesized and characterized based on the spectral data of 1H-NMR, IR and MS in this study. The antifungal activity of the compounds was evaluated in vitro against the phytopathogenic fungi A. porri, M. coronaria, C. petroselini and R. solani. Among these compounds, the pyrazole carboxamides 7af, 7bc, 7bg, 7bh and 7bi exhibited moderate antifungal activity. The isoxazolol pyrazole carboxylate 7ai displayed strong antifungal activity against R. solani, with an EC50 value of 0.37 μg/mL. This value was better than that of the commercial fungicide, carbendazol. SAR analysis results suggested that the antifungal activity of the synthesized isoxazolol pyrazole carboxylate was significantly weakened when the methyl at the C-3 of the pyrazole ring was substituted with trifluoromethyl. Thus, these novel antifungal molecules can be considered promising lead compounds with which to explore biological activity in future research.

Supplementary Materials

Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/20/03/4383/s1.

Acknowledgments

This work was supported by High Level Talents Fund of Qingdao Agricultural University (No. 6631110) and Wheat Innovation Team of Modern Agricultural Industry Technology System of Shandong Province.

Author Contributions

J.S. designed the research. J.S. and Y.Z. performed the research and analyzed the data. Y.Z. wrote the paper. All authors read and approved the final manuscript.
Conflicts of Interest

The authors declare no conflict of interest.

References

1. Sumangala, V.; Poojary, B.; Chidananda, N.; Fernandes, J.; Kumari, N.S. Synthesis and antimicrobial activity of 1,2,3-triazoles containing Quinoline moiety. *Arch. Pharm. Res.* **2010**, *33*, 1911–1918.

2. Mert, S.; Kasımoğulları, R.; İca T.; Çolak, F.; Altun, A.; Ok, S. Synthesis, structure activity relationships, and *in vitro* antibacterial and antifungal activity evaluations of novel pyrazole arboxylic and dicarboxylic acid derivatives. *Eur. J. Med. Chem.* **2014**, *78*, 86–96.

3. Gawad, N.M.A.; Georgey, H.H.; Ibrahim, N.A.; Amin, N.H.; Abdelsalam, R.M. Synthesis of novel pyrazole and dihydropyrazoles derivatives as potential anti-inflammatory and analgesic agents. *Arch. Pharm. Res.* **2012**, *35*, 807–821.

4. Ouyang, G.P.; Cai, X.J.; Chen, Z.; Song, B.A.; Bhadury, P.S.; Yang, S.; Jin, L.H.; Xue, W.; Hu, D.Y.; Zeng, S. Synthesis and antiviral activities of pyrazole derivatives containing an oxime moiety. *J. Agric. Food Chem.* **2008**, *56*, 10160–10167.

5. Rangaswamy, J.; Kumar, H.V.; Harini, S.T.; Naik, N. Synthesis of benzo[4,1-d]pyrimidines and related pyrazole hydrazones toward breast adenocarcinoma MCF-7 cell line. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4773–4777.

6. Hassan, G.S.; Kadry, H.H.; Abou-Seri, S.M.; Ali, M.M.; Mahmoud, A.E.E. Synthesis and *in vitro* cytotoxic activity of novel pyrazolo[3,4-ç]pyrimidines and related pyrazole hydrazones toward breast adenocarcinoma MCF-7 cell line. *Bioorgan. Med. Chem.* **2011**, *19*, 6808–6817.

7. Turan-Zitouni, G.T.; Chevallet, P.; Kiliç, F.S.; Erol, K. Synthesis of some thiazolyl-pyrazoline derivatives and preliminary investigation of their hypotensive activity. *Eur. J. Med. Chem.* **2000**, *35*, 635–641.

8. Baraldi, P.G.; Cacciari, B.; Romagnoli, R.; Spalluto, G.; Moro, S.; Klotz, K.; Leung, E.; Varani, K.; Gessi, S.; Merighi, S.; *et al.* Pyrazolo[4,3-e]1,2,4-triazolo[1,5-c]pyrimidine derivatives as highly potent and selective human A3 adenosine receptor antagonists: Influence of the Chain at the N8 pyrazole nitrogen. *J. Med. Chem.* **2000**, *43*, 4768–4780.

9. Lupsor, S.; Aonofriesei, F.; Iovu, M. Antibacterial activity of aminals and hemiaminals of pyrazole and imidazole. *Med. Chem. Res.* **2012**, *21*, 3035–3042.

10. Palaska, E.; Aytemir, M.; Uzbay, I.T.; Erol, D. Synthesis and antidepressant activities of some 3,5-diphenyl-2-pyrazolines. *Eur. J. Med. Chem.* **2001**, *36*, 539–543.

11. Sauter, H.; Steglich, W.; Anke, T. Strobilurins: Evolution of a new class of active substances. *Angew. Chem. Int. Ed.* **1999**, *38*, 1328–1349.

12. Shiga, Y.; Okada, I.; Ikeda, Y.; Takizawa, E.; Fukuchi, T. Insecticidal activity of *N*-[(4-aryloxybenzyl)pyrazole-5-carboxamides. *J. Pestic. Sci.* **2003**, *28*, 313–314.

13. Ohno, R.; Watanabe, A.; Matsukawa, T.; Ueda, T.; Sakurai, H.; Hori, M.; Hirai, K. Synthesis and herbicidal activity of new pyrazole-4-carboxamide derivatives. *J. Pestic. Sci.* **2004**, *29*, 15–26.
14. Zhou, Y.Y.; Li, Y.X.; Li, Y.M.; Yang, X.P.; Mao, M.Z.; Li, Z.M. Design, synthesis and structure-activity of \(N\)-glycosyl-1-pyridyl-1\(H\)-pyrazole-5-carboxamide as Inhibitors of Calcium channels. *Chem. Res. Chin. Univ.* 2013, 29, 249–255.

15. Lv, H.S.; Wang, L.Y.; Ding, X.L.; Wang, X.H.; Zhao, B.X.; Zuo, H. Synthesis and antifungal activity of novel (1-arylmethyl-3-aryl-1\(H\)pyrazol-5-yl)(4-arylpiperazin-1-yl)methanone derivatives. *J. Chem. Res.* 2013, 37, 473–475.

16. Okada, I.; Okui, S.; Sekine, M.; Takahashi, Y.; Fukuchi, T. Synthesis and acaricidal activity of bicyclic pyrazole-3-carboxamide derivatives. *J. Pestic. Sci.* 1992, 17, 69–73.

17. Lambeth, C.; Dinges, J. Pyrazol carboxamide fungicides inhibiting succinate dehydrogenase. *Bioactivity Heterocyclic Compound Classes: Agrochemicals*, 1st ed.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2012; pp. 175–194.

18. Liu, F.; Wang, M.Y.; Teng, X.H.; Zhang, P.Z.; Jiang, L. Synthesis and biological evaluation of novel 2-(substituted isoxazol-4-yl)-5-arylamino-1,3,4-oxadiazoles. *Res. Chem. Intermed.* 2014, 40, 1575–1581.

19. Cernuchova, P.; Vo-Thang, G.; Milata, V.; Loupy, A. Solvent-free synthesis of quinolone derivatives. *Heterocycles* 2004, 64, 177–191.

20. Nishiwaki, N.; Matsushima, K.; Chatani, M.; Tamura, M.; Ariga, M. New reactivity of nitropyrimidinone: Ring transformation and N-C transfer reactions. *Synlett* 2004, 15, 703–707.

21. Toshio, K.; Kenji, O.; Takao, O. Preparation of 1,3-Dialkylpyrazole-4-Carboxylic Acid Esters. Jpn. Kokai Tokkyo Koho JP 2,000,044,541, 15 February 2000.

22. Kenji, H.; Bunta, W.; Osamu, K.; Seiichi, K.; Takashi, K.; Junko, S. Preparation of N-(1-Phenyl-1\(H\)-pyrazol-4-yl)-1\(H\)-pyrazole-4-carboxamide Derivatives as Agrochemical Fungicides. Jpn. Kokai Tokkyo Koho JP 2,010,202,649, 16 September 2010.

23. Palanki, M.S.S.; Erdman, P.E.; Gayo, F.; Leah, M.; Shevlin, G.I.; Sullivan, R.W.; Suto, M.J.; Goldman, M.E.; Ransone, L.J.; Bennett, B.L.; et al. Inhibitors of NF-\(\kappa\)B and AP-1 gene expression: SAR studies on the pyrimidine portion of 2-chloro-4-trifluoromethyl pyrimidine-5-[\(N\)-(3',5'-bis(trifluoromethyl)-phenyl) carboxamide]. *J. Med. Chem.* 2000, 43, 3995–4004.

24. Sun, J.L. 3-Trifluoromethyl-4-formyl Pyrazol Compounds. CN Patent 103,554,026A, 5 February 2014.

25. Sun, J.L. 3-Methyl-4-formyl Pyrazol Compounds. CN Patent 103,524,417A, 22 January 2014.

26. Xu, G.F.; Song, B.A.; Pinaki, S.B.; Yang, S.; Zhang, P.W.; Jin, L.H.; Xue, W.; Hu, D.Y.; Lu, P. Synthesis and antifungal activity of novel s-substituted-6-fluoro-4-alkyl(aryl) thioquinazoline derivatives. *Bioorg. Med. Chem.* 2007, 15, 3768–3774.

27. Tarun, K.C.; Prem, D.J. Antifungal activity of 4-methyl-6-alkyl-2\(H\)-pyran-2-ones. *J. Agric. Food Chem.* 2006, 54, 2129–2133.

**Sample Availability:** Samples of the compounds **7aa–bk** are available from the authors.

© 2015 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/4.0/).