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Synthesis and Fungicidal Activity of Pyrazolecarboxamide Containing α-Aminoacetanilide Moiety

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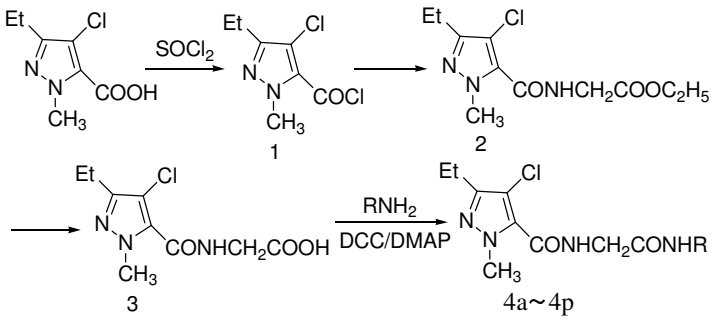
Abstract: A series of pyrazolecarboxamides containing α-aminoacetanilide moiety (**4**) were synthesized. These compounds were characterized by IR, MS and ^{1}H NMR. Their fugicidal activities against *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Monilinia fructicola* and *Alternaria brassicae* were evaluated. The results show that compound **4l** and **4p,** which bear only one electron-donating group on the *para*-position of phenyl or benzyl group, possessed excellent inhibitory activities against the above four fungi.

Keywords: Pyrazole, Glycine, Amide, Fugicidal

Introduction

Pyrazole derivatives have attracted considerable attention in chemical and medicinal research because of their diverse bioactivities. Particularly, they are widely used as herbicide, fungicide, insecticide, acaricide and antitumor agents[1-10]. Motivated by the aforementioned findings, we conceived that introduction of the α-aminoacetanilide moiety to pyrazole might result in novel compounds with good biological activities (Scheme 1). The present study reports the synthesis of a new series of pyrazole-carboxamides obtained by condensation of *N*-(4-chloro-3-ethyl-1-methyl-5-pyrazole carboxyl) glycine with substituted anilines or benzylamine, and the tests of their antifungal activities against hazardous fungi *i.e. Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Monilinia fructicola* and *Alternaria brassicae*, which cause great loss to crops like rice, *Brassica napus* L., peach, broccoli *etc*, were also carried out.



4a 2-ClC_6H_4, **4b** 2,6-$F_2C_6H_3$, **4c** 3-ClC_6H_4, **4d** 2,4-$Cl_2C_6H_3$, **4e** 2,4-$F_2C_6H_3$, **4f** 2-$CH_3OOCC_6H_4$, **4g** C_6H_5, **4h** 2-$CH_3C_6H_4$, **4i** 4-$CH_3C_6H_4$, **4j** 3-$NO_2C_6H_4$, **4k** 4-$NO_2C_6H_4$, **4l** 4-^{t}-butyl benzyl, **4m** 2-FC_6H_4, **4n** 3-FC_6H_4, **4o** 3-$CF_3C_6H_4$, **4p** 4-$CH_3OC_6H_4$

Scheme 1. Synthesis route for target compounds

Experimental

All the chemicals used were purchased from Shanghai Reagent Co. Melting points of all the compounds determined in WRS-1B digital melting apparatus were uncorrected. The ^{1}H NMR spectra were obtained with a DPX 600 MHz (or 400 MHz, 300 MHz) spectrometer with TMS as internal standard, $CDCl_3$ or DMSO-d6 was used as solvent. The IR spectra were recorded on an Avatar 330 instrument as potassium bromide pellets. The MS spectra were performed by using an Agilent 1100 LC/ MSD Trap. Yields refer to isolated products.

*Preparation of 4-chloro-3-ethyl-1-methyl-5-pyrazole carboxyl chloride (**1**)*

4-Chloro-3-ethyl-1-methyl-5-pyrazole carboxylic acid was prepared according to literature[11]. The pyrazole carboxylic acid (8.6 g, 0.0456 mol) and 2 drops of DMF was dissolved in toluene (60 mL), stirred at room temperature, thionyl chloride (8.0 mL, 0.1013 mol) was added dropwise in 1 h and the resultant reaction mixture was stirred for 6 h at 60 °C. After removal of the solvent and excess of thionyl chloride under reduced pressure, a viscous liquid residual was collected as the chloride compound (**1**) (8.85 g, 93.8%).

*Preparation of N-(4-chloro-3-ethyl-1-methyl-5-pyrazole carboxyl) glycine (**3**)*

100 mL two necked round bottom flask fitted with a reflux condenser, a dropping funnel was used, 0.68 g (0.0048 mol) ethyl glycinate hydrogen chloride and 10 mL dichloromethane was injected into it in ice cold water bath (-10~0 °C) and 2.5 mL triethylamine added drop wise with stirring. Then 1.0 g (0.0048 mol) 4-chloro-3-ethyl-1-methyl-5-pyrazole carboxyl chloride (**1**) in 10 mL dichloromethane was added through a funnel in 0.5 h. The mixture was stirred around 0 °C until the reaction was completed (TLC monitor, usually about 3 h). After filtration of the insoluble material from the mixture, the filtrate was washed with 15 mL 5% HCl, 15 mL 10% $NaHCO_3$, 15 mL water and finally 15 mL 20% brine. The solution was dried over magnesium sulphate and the solvent was evaporated under reduced pressure. Ethyl *N*-(4-chloro-3-ethyl-1-methyl-5-pyrazole carboxyl) glycinate (**2**) was obtained as white solid. Then 15 mL diethyl ether, 0.4 g NaOH and 5 drops of water were injected into the flask to make the ester hydrolyzed. After the completion of hydrolysis by TLC monitor, the corresponding sodium salt was collected by filtration. Finally the sodium salt was redissolved in 10 mL water and acidified to pH=2~3 by concentrated HCl. The precipitate was collected by filtration and dried at 80 °C. A white solid was collected as the *N*-(4-chloro-3-ethyl-1-methyl-5-pyrazole carboxyl) glycine (**3**) (0.53 g, 45.2%), m.p.175~176 °C. IR(KBr) *v*: 3321, 1736, 1643, 1554, 1513 and 1469 cm^{-1}.

*General procedure for the synthesis of **4a-p***

The synthesis method was exemplified by the preparation of compound **4a.** Compound **3** (1.0 g, 0.004 mol), *o*-chloroaniline (0.51 g, 0.004 mol), *N*, *N*'-dicyclo -hexyl carbodiimide (DCC, 0.99 g, 0.0048 mol) and 15 mL CH_2Cl_2 was added successively into 100 mL three necked round bottom flask. After stirring for 15 min in cold water bath (0~5 ^oC), 4-dimethyl-aminepyridine (DMAP, 0.05 g, 0.0004 mol) was added. Then the mixture was warmed to room temperature and stirred for 4 h. After filtration of insoluble material from the mixture, the solution was washed with 15 mL 5% NaOH, 15 mL 5% HCl, 15 mL 20% brine. The solution was dried over magnesium sulphate and the solvent was evaporated under reduced pressure. Compound **4a** was obtained as white solid. After recrystallization in ethanol, pure white **4a** (1.10 g, 77.6%) was provided. Others compounds were synthesized by the way similar to compound **4a**.

*N-[2-[(2-Chlorophenyl)amino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole-5-carboxamide (**4a**)*

Yield 77.6%, white solid, m.p.120.9~121.3 ^oC; ^1H NMR($CDCl_3$, 400 MHz) δ: 1.166~1.204 (t, J = 7.6 Hz, 3H, C-CH_3), 2.560~2.617 (q, J = 7.6 Hz, 2H, -CH_2CH_3), 4.081(s, 3H, N-CH_3), 4.261~4.275 (d, J = 5.6 Hz, 2H, -CH_2-N), 7.004~7.021 (d, J = 6.8 Hz, 1H, -Ph), 7.193~7.235 (t, J = 8.4 Hz, 1H, -Ph), 7.297~7.319 (d, J = 8.8 Hz, 1H, -Ph), 7.447(s, 1H, -Ph), 8.225 (br s, 1H, -NHPh), 8.264~8.285 (br s, 1H, -NHCH$_2$); IR (KBr) v: 3341, 3316, 1708, 1638, 1594, 1527, 1505, 1470, 758 cm^{-1}; MS *m/z* (%): 355 ($M^{+\cdot}$, 100), 228 (8.6).

*N-[2-[(2,6-Difluorophenyl)amino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole -5-carboxamide (**4b**)*

Yield 49.4%, white solid, m.p.161.7~162.3 ^oC; ^1H NMR($CDCl_3$, 600 MHz) δ: 1.231~1.256 (t, J = 7.2 Hz, 3H, C-CH_3), 2.628~2.665 (q, J = 7.2 Hz, 2H, -CH_2CH_3), 4.116(s, 3H, N-CH_3), 4.385~4.404 (d, J = 11.4 Hz, 2H, -CH_2-N), 6.957~6.984 (t, J = 7.8 Hz, 2H,-Ph), 7.236~7.240 (m, 1H, -Ph), 7.607~7.608 (d, J = 0.6 Hz, 1H, -NHCH$_2$), 7.850 (s, 1H, -NHPh); IR (KBr) v: 3417, 3255, 1682, 1669, 1625, 1603, 1536, 1505 cm^{-1}; MS *m/z* (%): 357 ($M^{+\cdot}$, 100), 228 (4.8).

*N-[2-[(3-Chlorophenyl)amino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole-5-carboxamide (**4c**)*

Yield 50.1%，white solid, m.p.146.5~147.3 ^oC; ^1H NMR($CDCl_3$, 400 MHz) δ: 1.157~1.194 (t, J = 7.4 Hz, 3H, C-CH_3), 2.547~2.604 (q, J = 7.4 Hz, 2H, -CH_2CH_3), 4.037(s, 3H, N-CH_3), 4.258~4.271 (d, J = 5.2 Hz, 2H, -CH_2-N), 7.004~7.026 (d, J = 8.8 Hz, 1H, -Ph), 7.130~7.193 (m, 1H, -Ph), 7.283~7.303 (d, J = 8 Hz, 1H, -Ph), 7.568 (s, 1H, -Ph), 7.631 (br s, 1H, -NH), 8.608 (br s, 1H, -NH); IR (KBr) v: 3366, 3328, 1704, 1644, 1596, 1530, 1501, 1479 cm^{-1}; MS *m/z* (%): 355 ($M^{+\cdot}$, 100).

*N-[2-[(2,4-Dichlorophenyl)amino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole-5-carboxamide (**4d**)*

Yield 63.3%, white solid, m.p.159.3~160.4 ^oC; ^1H NMR($CDCl_3$, 500 MHz) δ: 1.169~1.199 (t, J = 7.5 Hz, 3H, C-CH_3), 2.563~2.609 (q, J = 7.5 Hz, 2H, -CH_2CH_3), 4.079(s, 3H, N-CH_3), 4.247~4.258 (d, J = 5.5 Hz, 2H, -CH_2-N), 7.178~7.185 (d, J =3.5 Hz, 1H, -Ph), 7.193~7.200 (d, J = 3.5 Hz, 1H, -Ph), 7.323 (s, 1H, -Ph), 8.248(br s, 1H, -NH), 8.266 (br s, 1H, -NH); IR (KBr) v: 3407, 3319, 1672, 1650, 1584, 1527, 1505, 1470 cm^{-1}; MS *m/z* (%): 389 ($M^{+\cdot}$, 100).

*N-[2-[(2,4-Difluorophenyl)amino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole-5-carboxamide (**4e**)*

Yield 48.6%, white solid, m.p.147.9~148.0 °C; ^{1}H NMR($CDCl_3$, 500 MHz) δ: 1.166~1.196 (t, J = 7.5 Hz, 3H, C-CH_3), 2.561~2.606 (q, J = 7.5 Hz, 2H, -CH_2CH_3), 4.067(s, 3H, N-CH_3), 4.247~4.258 (d, J =5.5 Hz, 2H, -CH_2-N), 6.804~6.825 (m, 2H,-Ph), 7.448 (s, 1H, -Ph), 8.105~8.140 (m, 2H, -NH); IR (KBr) v: 3344, 3278, 1706, 1637, 1562, 1530, 1505 cm^{-1}; MS *m/z* (%): 357 ($M^{+\cdot}$, 100).

*N-[2-[(2-Methoxylcarbonylphenyl)amino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole-5-carboxamide (**4f**)*

Yield 49.6%, white solid, m.p.139.2~140.1 °C; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 1.246~1.271 (t, J = 7.5 Hz, 3H C-CH_3), 2.646~2.684 (q, J= 7.5 Hz, 2H, -CH_2CH_3), 3.877(s, 3H,-OCH_3), 4.151(s, 3H, N-CH_3), 4.354~4.362 (d, J = 4.8 Hz, 2H, -CH_2-N), 7.106~7.133 (m, 1H, -Ph), 7.513~7.574 (m, 2H, -Ph), 8.022~8.038 (m, 1H, -Ph), 8.694 (br s, 1H, -NH), 11.464 (br s, 1H, -NH);IR (KBr) v: 3290, 3265, 1691, 1660, 1590, 1524 cm^{-1}; MS *m/z* (%): 379 ($M^{+\cdot}$, 100),152(7.5).

*N-[2-[Phenylamino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole-5-carboxamide (**4g**)*

Yield 80.3%, white solid, m.p.136~137 °C; ^{1}H NMR($CDCl_3$, 400 MHz) δ: 1.232~1.270 (t, J = 7.6 Hz, 3H, C-CH_3), 2.623~2.680 (q, J = 7.6 Hz, 2H, -CH_2CH_3), 4.115(s, 3H, N-CH_3), 4.316~4.327 (d, J = 4.4 Hz, 2H, -CH_2-N), 7.114~7.150 (t, 1H), 7.306~7.344 (t, 2H, -Ph), 7.509~7.529 (d, J = 4.0 Hz, 2H, -Ph), 7.649~7.658 (br s, -NH), 8.258 (br s, 1H, -NH) ; IR (KBr) v: 3350, 3325, 1701, 1637, 1609, 1552, 1527, 1501 cm^{-1}; MS *m/z* (%): 321 ($M^{+\cdot}$, 100), 228(16.3).

*N-[2-[(2-Methylphenyl)amino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole-5-carboxamide (**4h**)*

Yield 61.8%, white solid, m.p.105~107 °C;^{1}H NMR($CDCl_3$, 400 MHz) δ: 1.228~1.266 (t, J = 7.6 Hz, 3H, C-CH_3), 2.261 (s, 3H, -CH_3Ph), 2.619~2.676 (q, J = 7.6 Hz, 2H, -CH_2CH_3), 4.128(s, 3H, N-CH_3), 4.304~4.318 (d, J = 5.6 Hz, 2H, -CH_2-N), 7.072~7.109 (t, 1H, -Ph), 7.179~7.228 (q, 2H, -Ph), 7.604-7.619 (d, J = 6 Hz, 1H, -Ph), 7.824 (br s, 1H, -NH), 7.943 (s, 1H, -NH); IR (KBr) v: 3417, 3259, 1663, 1590, 1536, 1508 cm^{-1}; MS (70eV) *m/z* (%): 335 ($M^{+\cdot}$, 100), 228(6.7).

*N-[2-[(4-Methylphenyl)amino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole-5-carboxamide (**4i**)*

Yield 62.4%, white solid, m.p.184~185 °C; ^{1}H NMR($CDCl_3$, 400 MHz) δ: 1.231~1.269 (t, J = 7.6 Hz, 3H, C-CH_3), 2.317 (s, 3H, -CH_3Ph), 2.624~2.681 (q, J = 7.6 Hz, 2H, -CH_2CH_3), 4.127 (s, 3H, N-CH_3), 4.280~4.293 (d, J = 5.2 Hz, 2H, -CH_2-N), 7.121~7.142 (d, J = 8.4 Hz, 2H, -Ph), 7.384~7.405 (d, J = 8.4 Hz, 2H, -Ph), 7.599 (br s, 1H, -NH), 7.982 (br s, 1H, -NH) ; IR (KBr) v: 3335, 3278, 1701, 1637, 1612, 1530, 1505, 814 cm^{-1}; MS *m/z* (%): 335 ($M^{+\cdot}$, 100), 228(6.5).

*N-[2-[(3-Nitrophenyl)amino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole-5-carboxamide (**4j**)*

Yield 33.0%, pale yellow solid, m.p.186~188 °C; ^{1}H NMR($CDCl_3$, 400 MHz) δ: 1.237~1.275 (t, J = 7.6 Hz, 3H, C-CH_3), 2.632~2.689 (q, J = 7.6 Hz, 2H, -CH_2CH_3), 4.157 (s, 3H, N-CH_3), 4.323~4.337 (d, J = 5.6 Hz, 2H, -CH_2-N), 7.485~7.526 (t, 1H, -Ph), 7.583 (s, 1H, -Ph), 7.919~7.939 (d, J = 8 Hz, 1H, -Ph), 7.975~7.996 (q, 1H, -Ph), 8.420 (s, 1H, -NH), 8.655 (s, 1H, -NH); IR (KBr) v: 3354, 3300, 1701, 1650, 1600, 1546, 1530, 1505 cm^{-1}; MS *m/z* (%): 366 ($M^{+\cdot}$, 100).

*N-[2-[(4-Nitrophenyl)amino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole-5-carboxamide (**4k**)*

Yield 32.3%, pale yellow solid, m.p.214~215 °C; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 1.167~1.193 (t, J = 7.8 Hz, 3H, C-CH_3), 2.565~2.603 (q, J = 7.6 Hz, 2H, -CH_2CH_3), 4.073(s, 3H, N-CH_3), 4.246~4.255 (d, J = 5.4 Hz, 2H, -CH_2-N), 7.513 (s, 1H, -NH), 7.649~7.664 (d, J = 9 Hz, 2H, -Ph), 8.133~8.148 (d, J = 9 Hz, 2H, -Ph), 8.851 (s, 1H, -NH);IR (KBr) v: 3379, 3347, 1713, 1656, 1615, 1562, 1530, 1505, 855 cm^{-1}; MS m/z (%): 366 ($M^{+\cdot}$, 100), 225(50).

*N-[2-[(4-tert-butylbenzylyl)amino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole-5-carboxamide (**4l**)*

Yield 96.0%, white solid, m.p.122~123 °C; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 1.232~1.257 (t, J = 7.5 Hz, 3H, C-CH_3), 1.304 (s, 9H, -$C(CH_3)_3$), 2.629~2.667 (q, J = 7.6 Hz, 2H, -CH_2CH_3), 4.078(s, 3H, N-CH_3), 4.142~4.150 (d, J = 4.8 Hz, 2H, -CH_2-N), 4.461~4.470 (d, J = 5.4 Hz, 2H，-CH_2Ph), 6.318(s, 1H, -NH), 7.211~7.225 (d, J = 8.4 Hz, 2H, -Ph), 7.351~7.365 (d, J = 8.4 Hz, 2H-Ph), 7.531 (s, 1H,-NH); IR (KBr) v: 3388, 3354, 2964, 1679, 1647, 1524, 1495 cm^{-1}; MS m/z (%): 391 ($M^{+\cdot}$, 100).

*N-[2-[(2-fluorophenyl)amino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole-5-carboxamide (**4m**)*

Yield 44.4%, white solid, m.p.163~164 °C; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 1.165~1.191 (t, J = 7.8 Hz, 3H, C-CH_3), 2.562~2.600 (q, J = 7.6 Hz, 2H, -CH_2CH_3), 4.069(s, 3H, N-CH_3), 4.252~4.261 (d, J = 5.4 Hz, 2H, -CH_2-N), 7.013~7.076 (m, 3H, -Ph), 7.536~7.545 (d, J = 5.4 Hz, 1H, -Ph), 8.256 (s, 1H, -NH), 8.283 (s, 1H, -NH);IR (KBr) v: 3341, 3290, 1707, 1637, 1619, 1596, 1533, 1508 cm^{-1}; MS m/z (%): 339 ($M^{+\cdot}$, 100), 225 (28.6).

*N-[2-[(3-fluorophenyl)amino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole-5-carboxamide (**4n**)*

42.9%, white solid, m.p.151~152 °C; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 1.165~1.191 (t, J = 7.8 Hz, 3H, C-CH_3), 2.562~2.600 (q, J =7.6 Hz, 2H, -CH_2CH_3), 4.061(s, 3H, N-CH_3), 4.214~4.222 (d, J = 4.8 Hz, 2H, -CH_2-N), 6.744~6.769 (t, 1H,-Ph), 7.169~7.183 (d, J = 8.4 Hz, 1H, -Ph), 7.269~7.275 (d, J = 3.6 Hz, 1H, -Ph),7.492~7.509 (d, J = 10.2 Hz, 1H, -Ph), 7.593 (s, 1H, -NH), 8.421 (s, 1H, -NH);IR (KBr) v: 3328, 2936, 1701, 1637, 1609, 1536, 1508, 1489 cm^{-1}; MS m/z (%): 339 ($M^{+\cdot}$, 100), 225 (51).

*N-[2-[(3-trifluoromethylphenyl)amino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole-5-carboxamide (**4o**)*

Yield 38.0%, brown solid, m.p.154~155 °C; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 1.244~1.269 (t, J = 7.5 Hz, 3H, C-CH_3), 2.647~2.685 (q, J = 7.6 Hz, 2H, -CH_2CH_3), 4.140(s, 3H, N-CH_3), 4.280~4.288 (d, J = 4.8 Hz, 2H, -CH_2-N), 6.846~6.861 (d, 2H, -Ph), 7.406~7.421 (m, 2H, -Ph), 7.637 (br s, 1H, -NH), 8.068 (br s, 1H, -NH);IR (KBr) v: 3357, 3281, 1694, 1641, 1609, 1555, 1527, 1505 cm^{-1}; MS m/z (%): 389 ($M^{+\cdot}$, 100).

*N-[2-[(4-methoxylphenyl)amino]-2-oxoethyl]-1-methyl-3-ethyl-4-chloro-1H-pyrazole-5-carboxamide (**4p**)*

Yield 75.2%, white solid, m.p.130~131 °C; ^{1}H NMR($CDCl_3$, 600 MHz) δ: 1.172~1.197 (t, J = 7.5 Hz, 3H, C-CH_3), 2.176 (s, 3H, O-CH_3), 2.571~2.609 (q, J = 7.6 Hz, 2H, -CH_2CH_3),

4.078 (s, 3H, N-CH_3), 4.233~4.242 (d, J = 5.4 Hz, 2H, -CH_2-N), 7.314~7.327 (d, J = 7.8 Hz, 1H, -Ph), 7.371~7.379 (t, J = 4.8 Hz, 1H, -Ph), 7.501~7.509 (d, J = 4.8 Hz, 1H, -Ph), 7.653~7.666 (d, J = 7.8 Hz, 1H, -Ph), 7.770 (s, 1H, -NH), 8.310 (s, 1H, -NH); IR (KBr) v: 3376, 3331, 1701, 1641, 1600, 1562, 1536, 1505 cm^{-1}; MS *m/z* (%): 379 ($M^{+\cdot}$, 100),152(15.5).

Antifungal activities

Radial fungal-growth assay

The antifungal activity was tested *in vitro* on *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Monilinia fructicola* and *Alternaria brassicae*. The activities of the compounds were assayed as growth inhibition. Each compound was dissolved in acetone plus Tween 80 (1.0%). Equal volumes of acetone containing tested compounds were added to sterile cool agar media (*Potato Dextrose Agar Difco*) to give final 50 mg/L concentration for each substance. The final acetone concentration did not exceed 1.0% of the final volume in both control and treated cultures. Compound-amended agar medium was dispersed aseptically onto 9 cm diameter plastic Petri dishes (10 mL/dish). Each dish was inoculated with a 0.5 cm diameter mycelial disc of actively growing colonies. Three replicates were used for each compound, together with controls containing toxicant-free medium. The growth inhibition was calculated from mean differences between treated and control cultures as a percentage of the latter. The results were compared with standard fungicide Carbendazim. The growth was determined after 2 days of incubation for *Rhizoctonia solani* and *Sclerotinia sclerotiorum*, 4 days for *Monilinia fructicola* and *Alternaria brassicae*.

Results and Discussion

Synthesis

For the synthesis of compound **2**, which was prepared by the amination of acyl chloride (**1**), the temperature should be controlled around 0 ^{0}C. When the temperature was higher, the side reaction of amination between glycinate would take place, leading to polymerization[12], that is, the amino group of one molecule ethyl glycinate could attack the ester group of another molecule of ethyl glycinate.

Two different strategies were applied to obtain the series of title compounds (**4a**-**4p**); the first one involved the acyl chloride of compound **3** with different anilines, however, there was side reaction between the acyl chloride group of compound **3** and the amine group on itself, moreover, the side product was hard to be removed; the second one, which was applied by this paper, exploited the DCC/DMAP system. DCC can transform the acid into corresponding anhydride and DMAP can efficiently promote acylation of aniline with acid anhydrides to give the corresponding amides in good yields[13].

The ^{1}H-NMR signals of the two NH units in title compounds were variable, which on the one hand, correlate with the chemical structure of the phenyl group connected to NH, on the other hand were strongly influenced by hydrogen bonding. It is well known that the more hydrogen bonding there is, the more the proton is deshielded and the higher its chemical shift will be. However, since the amount of hydrogen bonding is susceptible to factors such as solvation, concentration and temperature, so the chemical shift of NH units were not the same for all title compounds.

Fungicidal activities

The results of the *in vitro* fungistatic activity of compounds **4a–4p** and commercial fungicide Carbendazim at 50 mg/L against fungi *Rhizoctonia solani, Sclerotinia sclerotiorum, Monilinia*

fructicola and *Alternaria brassicae* are listed in Table 1, in which the fungistatic activity was expressed as inhibition percentage. The results in Table 2 showed that the synthesized target compounds were fairly active against nearly all four fungi to some extent. Compared to the control Carbendazim (58.7%), some of the compounds showed significant activities against *Sclerotinia sclerotiorum*, for example, the inhibition rates compounds **4c**, **4f**, **4l** and **4p** were all above 50% at 50 mg/L. Particularly, for compound **4l,** the inhibition rate on *Sclerotinia sclerotiorum* was above 80%. Furthermore, compounds **4l** and **4p**, exhibited very strong inhibition against the other three fungi. The common structural feature of both was that the benzene ring or benzyl group (**4l**) was substituted with only one strong electron-donating substitute at site-4. Furthermore, when R was benzyl group (*viz* **4l**) instead of a phenyl group, there was a dramatic increase in the activity, which suggests that the substitute on the amino moiety likely plays a positive role in the mechanism of their antifungal action.

Table 1. Antifungal activity of title compounds

Compound	Inhibitiory percentage, %			
	Rhizoctonia solani	*Sclerotinia sclerotiorum*	*Monilinia fructicola*	*Alternaria brassicae*
4a	0	21.0	26.5	17.8
4b	0	36.1	30.6	13.9
4c	57.8	50.6	31.6	48.9
4d	20.3	8.4	22.7	38.9
4e	24.3	16.0	39.4	32.2
4f	25.6	53.5	50.2	65.0
4g	0	22.7	32.8	15.0
4h	0	27.9	28.4	27.8
4i	41.9	23.8	43.8	36.1
4j	0	23.6	32.2	36.1
4k	0	17.1	18.9	9.4
4l	62.5	83.8	64.4	80.6
4m	19.9	29.0	34.4	17.8
4n	0	22.6	13.9	12.2
4o	57.4	18.2	32.7	52.2
4p	61.8	54.1	69.4	86.6
Carbendazim	100	58.7	100	92.8

Conclusion

On the whole, most of the title compounds exhibited good fungicidal activity. Hence, title compound could be useful for further optimization work in finding the potential antifungal compounds.

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