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Synthesis and Fungicidal Activity of Novel Substituted Spiro-Heterocycles Derivatives

FEIFEI LI*, DANPING CHEN and GUIPING OU YANG

State Key Laboratory Breeding Base of Green Pesticide and Agricultural Bioengineering, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Guiyang 550025, P.R. China

*Corresponding author: Fax: +86 851 3622211; Tel: +86 851 3620779; E-mail: xiaofei1986523@163.com

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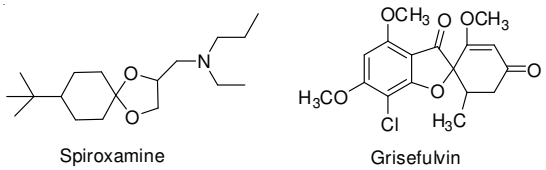
Some spiro-heterocycles compounds have been synthesized by the piperidone hydrochloride, glycerol, aromatic acid, nicotinic acid and pyrazole acid. The target compounds were characterized by spectral data, including ^{1}H and ^{13}C NMR, infrared, elemental analysis and mass spectra and were bioassayed *in vitro* against two kinds of phytopathogenic fungi *i.e.*, *B. cinerea* and *R. solani*. The results showed that some of the synthesized spiro-heterocycles derivatives exhibited moderate antifungal activities, among which compounds b, e, h, j, k and q displayed more than 80 % inhibition activities against *B. Cinerea.* at 50 µg/mL, which was better than that of the commercial fungicides spiroxamine and hymexazol.

Key Words: Synthesis, Spiro-heterocycles, Characterized, Antifungal activity.

INTRODUCTION

In the 21st century, a good pesticide is characterized by four important qualities which are high efficiency, low toxicity, economy and good environmental profile. In recent years, heterocyclic compounds have been the mainstream in pesticide discovery. More than 90 % of patented pesticides in the world are heterocyclic compounds, which are usually identified with their biological activities and features of broad spectrum and high efficiency. Spiro-heterocyclic[1-3] compounds are an important branch of heterocyclic compounds and play an important role in modern research and development of pesticides and medicine[4-6]. Recent years have witnessed significant development in the research of spiro-heterocyclic derivatives.

The results of structure-activity relationships (SAR) research revealed that highly active novel spiro-heterocyclic derivatives containing hetero atom (O) such as spiroxamine and grisefulvin (Fig. 1) show an expanded antifungal spectrum.



Spiroxamine Grisefulvin

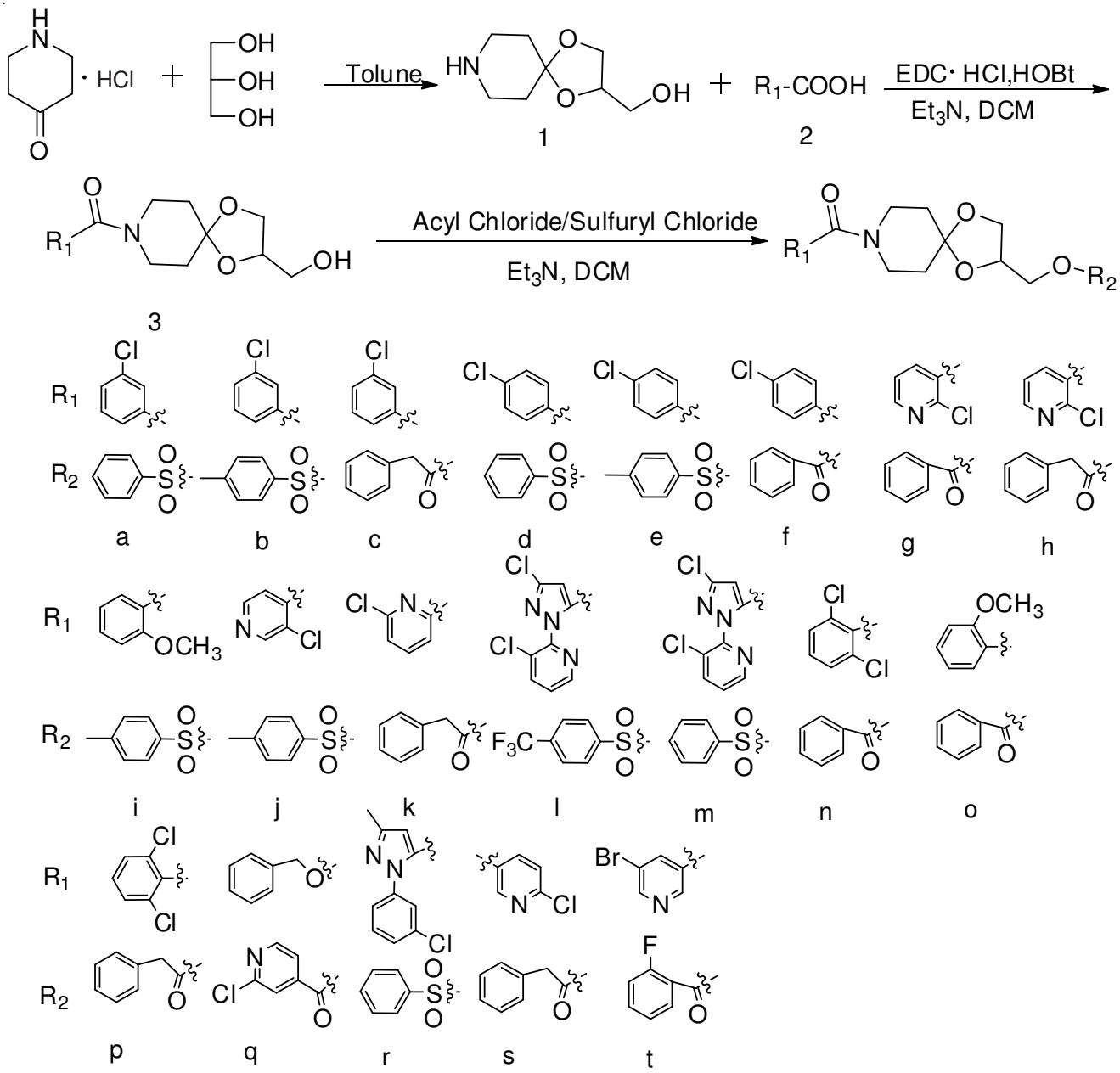
Fig. 1. Commercial spiro-heterocyclic fungicides

Here, a report is given on the preparation and antifungal activities of a series of spiro-heterocyclic compounds. Based on the molecular structure of spiroxamine, we introduce the N atom in the spiro ring in order to increase the antifungal activities of the compounds. The synthetic route to the title compounds was depicted in **Scheme-I**. The structures of all the compounds are characterized by the IR, NMR, MS and elemental analysis. Preliminary bioassay tests show that some of these compounds possess good antifungal activities against *B. cinerea* and *R. solani in vitro.*

EXPERIMENTAL

All the reagents and reactants were purchased from commercial suppliers. The ^{1}H and ^{13}C NMR spectra were recorded on a JEOL ECX 500 NMR spectrometer at room temperature operating at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR by using DMSO as the solvent and TMS as an internal standard; infrared spectra were recorded in KBr on a Bruker VECTOR 22 spectrometer; elemental analysis was performed on an Elemental Vario-III CHN analyzer; mass spectra were recorded by using Agilent LC/MSD(ion trap VL). The course of the reactions was monitored by TLC; analytical TLC was performed on silica gel GF254; thin layer chromatography purification was carried out using silica gel GF254 under normal pressure, the phytopathogenic fungi was obtained from Shenyang Research Institute of Chemical Industry.

General synthetic procedures for title compounds a-t: To a well stirred solution of different acid (1 mmol) and EDC·HCl/HOBt (1.2 mmol) in dichloromethane(25 mL) at



Scheme-I: Reagents and conditions: synthetic route to title compounds **a-t**

0 ºC, intermediate 1 (1 mmol) was added into flask at room temperature. Then we continued stirring for 4 h at room temperature and monitor it by TLC. Once the reaction is completed, the organic layer was extracted with distilled water (30 mL), saturation $NaHCO_3$ solution (30 mL) and brine (30 mL), respectively. The combined organic layer was removed under reduced pressure to get the intermediate **3**. Then the target compound was synthesized by reacting acyl chloride and sulfuryl chloride with intermediate **3** in dichloromethane in the presence of triethylamine at room temperature. We stirred it for 4 h and monitored by TLC.

After completion of the reaction, the organic layer was extracted with distilled water (30 mL), saturation $NaHCO_3$ solution (30 mL) and brine (30 mL), respectively. The residue was purified by thin layer chromatography(TLC) on silica gel (developing solvent: EtOAc/petroleum ether = 1/2) to afford product. The physical and spectral data for **a-t** are listed below.

[8-(3-Chlorobenzoyl)-1,4-dioxa-8-azaspiro[4,5]decan-2-yl]methylbenzenesulfonate (a): Light yellow oil substance; yield: 75 %; IR (KBr, ν_{max}, cm^{-1}): 3447, 2934, 2371, 1773, 1734, 1653, 1578, 1458, 1364, 1188, 1094, 980, 758, 669, 588; ^1H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.88 (s, 2H), 7.75 (s, 1H), 7.65 (s, 2H), 7.43-7.47 (br, m, 3H), 7.31 (d, 1H, J = 7.0 Hz, Ph-H), 4.30 (s, 1H), 4.14 (d, 1H, J = 8.0 Hz), 3.97-3.99 (br, m, 2H), 3.67 (s, 1H), 3.54 (s, 2H), 3.21 (s, 2H), 1.50-1.63 (br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 167.9, 138.6, 135.6, 134.9, 133.7, 130.9, 130.4, 129.8, 128.1, 127.1, 125.7, 108.3, 73.3, 71.2, 65.1, 45.6, 35.6; ESI-MS m/z: 452.4 $[M + 1]^+$. Anal. calcd. (%) for $C_{21}H_{22}NO_6SCl$: C 55.81, H 4.91, N 3.10; found (%): C 55.72, H 5.02, N 3.23.

[8-(3-Chlorobenzoyl)-1,4-dioxa-8-azaspiro[4,5]decan-2-yl]methyl4-methylbenzene-sulfonate (b): Light yellow oil substance; yield: 68 %; IR (KBr, ν_{max}, cm^{-1}): 3447, 2392, 2369, 1699, 1636, 1558, 1437, 1362, 1250, 1088, 978, 814, 741,

669, 554; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.74 (s, 2H), 7.30-7.37 (m, 5H), 7.22 (d, 1H, J = 7.5 Hz), 4.31 (s, 1H), 3.99-4.03 (m, 3H), 3.74-3.81 (m, 3H), 3.38 (s, 2H), 2.42 (s, 3H, CH_3-H), 1.72-1.85 (br, m, 4H, N-Spiro-H); ^{13}C NMR (DMSO, 125 MHz): 168.8, 145.2, 137.7, 134.5, 132.5, 129.9, 127.9, 126.9, 124.8, 108.2, 72.9, 69.0, 65.6, 45.1, 40.0, 21.5; ESI-MS m/z: 466.4 $[M + 1]^+$. Anal. calcd. (%) for $C_{22}H_{24}NO_6SCl$: C 56.71, H 5.19, N 3.01; found (%): C 56.60, H 5.27, N 3.15.

[8-(3-Chlorobenzoyl)-1,4-dioxa-8-azaspiro[4,5]decan-2-yl]methyl-2-phenylacetate (c): Light yellow oil substance; yield: 71 %; IR (KBr, ν_{max}, cm^{-1}): 3449, 2957, 2371, 1734, 1636, 1437, 1248, 1130, 1066, 930, 741, 696; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.92-7.94 (br, m, 2H), 7.57-7.63 (br, m, 1H), 7.51-7.53 (br, m, 2H), 7.47 (d, 2H, J = 8.2 Hz, Ph-H), 7.40 (m, 2H, Ph-H), 4.36 (s, 2H), 4.32(s, 1H), 4.16 (s, 1H), 4.04 (s, 1H), 3.64-3.68 (br, m, 5H), 3.38-3.40 (br, m, 2H), 1.54-1.74 (br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 171.2, 168.6, 137.7, 134.5, 133.7,129.8, 128.7, 127.3, 127.1, 126.7, 125.0, 108.0, 73.9, 71.3, 65.3, 45.5,33.8; ESI-MS m/z: 430.4 $[M + 1]^+$; Anal. calcd. (%) for $C_{23}H_{24}NO_5Cl$: C 64.26, H 5.63, N 3.26; found (%): C 64.40, H 5.42, N 3.20.

[8-(4-Chlorobenzoyl)-1,4-dioxa-8-azaspiro[4,5]decan-2-yl]methyl benzene-sulfonate (d): Light yellow oil substance; Yield: 72 %; IR (KBr, ν_{max}, cm^{-1}): 3445, 2932, 2369, 1734, 1636, 1558, 1506, 1437, 1362, 1269, 1188, 1070, 978, 833, 756, 689, 588; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.88 (d, 2H, J = 7.0 Hz), 7.76(s, 1H), 7.66(s, 2H), 7.45(d, 2H, J = 8.0 Hz, Ph-H), 7.38-7.40 (m, 2H, Ph-H), 4.29(s, 1H), 4.12-4.15 (dd, 1H, J_1=11.0 Hz, J_2=9.5 Hz), 3.94-3.97(br, m, 2H), 3.66 (s, 1H), 3.52-3.54 (br, m, 2H), 3.23 (s, 2H), 1.49-1.61 (br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 168.7, 139.1, 136.3, 134.2, 133.9, 130.9, 130.3, 129.9, 128.2, 127.1, 125.8, 108.4, 73.3, 71.2, 65.2, 45.6,34.8; ESI-MS m/z: 452.3 $[M + 1]^+$; Anal. calcd. (%) for $C_{21}H_{22}NO_6SCl$: C 55.81, H 4.91, N 3.10; found (%): C 55.60, H 4.62, N 3.25.

[8-(4-Chlorobenzoyl)-1,4-dioxa-8-azaspiro[4,5]decan-2-yl]methyl4-methylbenzene-sulfonate(e): Light yellow oil substance; yield: 68 %; IR (KBr, ν_{max}, cm^{-1}): 3445, 2959, 2367, 1636, 1437, 1362, 1250, 1177, 1092, 978, 837, 664, 556; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.76(s, 2H), 7.38-7.47 (m, 6H), 4.28 (s, 1H), 4.09-4.11(m, 1H), 3.92-3.97(m, 2H), 3.53-3.65 (br, m, 3H), 3.19-3.25 (m, 2H), 2.36 (br, s, 3H), 1.54-1.61 (br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm): 168.1, 145.9, 134.5, 132.1, 130.7, 129.3, 129.0, 128.3, 107.7, 73.1, 70.7, 65.3, 44.3, 35.6, 21.5; ESI-MS m/z: 466.7 $[M + 1]^+$; Anal. calcd. (%) for $C_{22}H_{24}NO_6SCl$: C 56.71, H 5.19, N, 3.01; found (%): C 56.53, H 5.33, N 3.14.

[8-(4-Chlorobenzoyl)-1,4-dioxa-8-azaspiro[4,5]decan-2-yl]methylbenzoate (f): Light yellow oil substance; Yield: 68 %; IR (KBr, ν_{max}, cm^{-1}): 3447, 2932, 2371, 1719, 1653, 1558, 1506, 1271, 1092, 920, 714, 669 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.90-7.94(br, m, 2H), 7.45 (d, 2H, J = 8.0 Hz, Ph-H), 7.57-7.63 (br, m, 1H), 7.50-7.52 (br, m, 2H), 7.39 (d, 2H, J = 8.5 Hz Ph-H), 4.45 (s, 1H), 4.36 (s, 1H), 4.26-4.29 (br, m, 1H), 4.11 (s, 1H), 3.84-3.87 (t, 1H), 3.52-3.69 (br, m, 4H), 1.63-1.69 (br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 169.8, 166.4, 139.1, 133.5, 132.2, 130.9, 129.7, 128.6, 127.2, 126.9, 125.0, 108.1, 73.9, 71.3, 65.3, 45.5,33.8; ESI-MS m/z: 416.4 $[M+1]^+$; Anal. calcd. (%) for $C_{22}H_{22}NO_5Cl$: C 63.54, H 5.33, N 3.37; found (%): C 63.40, H 5.02, N 3.40.

[8-(2-Chloronicotinoyl)-1,4-dioxa-8-aza spiro[4,5]decan-2-yl]methyl benzoate (g): Light yellow oil substance; Yield: 70 %; IR (KBr, ν_{max}, cm^{-1}): 3450, 2392, 2369, 1717, 1652, 1558, 1506, 1456, 1271, 1098, 1025, 922, 712, 669; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 8.42-8.44 (m, 1H, pyridine-H), 7.86-7.97(br, m, 3H), 7.44-7.65 (br, m, 4H), 4.29-4.49 (br, m, 3H), 4.09-4.15 (m, 1H), 3.83-3.88 (m, 3H), 3.15-3.19 (m, 2H), 1.61-1.78 (br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm): 166.1, 164.5, 150.7, 137.9, 134.0, 129.8, 129.7, 129.7, 129.4, 129.3, 129.1, 124.0, 107.9, 73.9, 65.9, 65.3, 65.1, 44.9, 36.1, 33.4; ESI-MS m/z: 417.5 $[M + 1]^+$; Anal. calcd. (%) for $C_{21}H_{21}N_2O_5Cl$: C 60.51, H 5.08, N 6.72; found (%): C 60.37, H 5.08, N 6.53.

[8-(2-Chloronicotinoyl)-1,4-dioxa-8-azaspiro[4,5]-decan-2-yl]methyl-2-phenyl-acetate (h): Light yellow oil substance; yield: 62 %; IR (KBr, ν_{max}, cm^{-1}): 3445, 2932, 2367, 1734, 1635, 1558, 1456, 1396, 1269, 1130, 1067, 922, 702; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 8.42-8.47 (m, 1H, pyridine-H), 7.87-7.91(m, 1H), 7.08-7.52 (br, m, 6H), 3.98-4.32 (br, m, 4H), 3.60-3.75 (br, m, 5H), 3.05-3.15 (m, 2H), 1.48-1.70 (br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm): 171.5, 164.9, 150.7, 137.9, 132.7, 129.9, 129.2, 129.1, 128.9, 128.8, 127.3, 124.1, 108.4, 73.8, 65.3, 64.1, 45.4, 34.4; ESI-MS m/z: 431.2 $[M + 1]^+$; anal. calcd. (%) for $C_{22}H_{23}N_2O_5Cl$: C 61.32, H 5.38, N 6.50; found (%): C 61.21, H 5.50, N 6.24.

[8-(2-Methoxybenzoyl)-1,4-dioxa-8-azaspiro[4.5]-decan-2-yl]methyl-4-methyl benzenesulfonate (i): Light yellow oil substance; yield: 70 %; IR (KBr, ν_{max}, cm^{-1}): 3445, 2932, 2367, 1628, 1558, 1437, 1362, 1244, 1177, 1066, 978, 831, 758, 669, 554; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.71-7.91(m, 2H), 7.32-7.49 (m, 3H), 6.91-7.16 (m, 3H), 4.23-4.32 (m, 1H), 3.88-4.12 (m, 3H), 3.49-3.79 (br, m, 6H), 2.94-3.11 (m, 2H), 2.28-2.34 (d, 3H, CH_3-H, J = 7.5 Hz), 1.36-1.60 (br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm): 166.8, 155.4, 145.5, 132.6, 130.8, 130.7, 130.6, 128.2, 126.2, 121.2, 111.8, 108.3, 73.3, 71.1, 65.2, 60.3, 55.9, 44.7, 36.2, 21.6, 21.5, 14.6; ESI-MS m/z: 462.4 $[M + 1]^+$; Anal. calcd. (%) for $C_{23}H_{27}NO_7S$: C 59.85, H 5.90, N 3.03; found (%): C 59.59, H 5.78, N 3.14.

[8-(3-Chloroisonicotinoyl)-1,4-dioxa-8-azaspiro[4,5]-decan-2-yl]methyl-4-methyl benzenesulfonate (j): Light yellow oil substance; yield: 71 %; IR (KBr, ν_{max}, cm^{-1}): 3445, 2967, 2363, 1635, 1558, 1396, 1362, 1271, 1177, 1096, 978, 814, 664, 554; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 8.43-8.44 (m, 1H, pyridine-H), 7.87-7.90 (m, 1H), 7.74-7.78 (m, 2H), 7.41-7.48 (m, 3H, Ph-H), 4.26-4.32 (m, 1H), 3.93-4.2 (m, 3H), 3.66-3.68 (m, 3H), 3.04-3.09 (m, 2H), 2.3 2 (s, 3H, CH_3-H), 1.48-1.56(br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm): 164.5, 150.7, 146.2, 146.0, 137.9, 132.6, 130.7, 128.3, 124.1, 108.1, 71.0, 65.3, 44.8, 31.2, 21.6; ESI-MS m/z: 467.9 $[M+1]^+$; Anal. calcd. (%) for $C_{21}H_{23}N_2O_6SCl$: C 54.02, H 4.96, N 6.00, found (%): C 54.14, H 4.87, N 5.82.

[8-(6-Chloropicolinoyl)-1,4-dioxa-8-azaspiro[4,5]-decan-2-yl]methyl-2-phenyl-acetate (k): Light yellow oil substance; Yield: 68 %; IR (KBr, ν_{max}, cm^{-1}): 3445, 2932, 2369,

1734, 1636, 1506, 1437, 1362, 1252, 1105, 1026, 920, 841, 756, 702; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 8.43 (s, 1H, pyridine-H), 7.88(d, 1H, *J* = 7.5 Hz), 7.55-7.61 (m, 1H), 7.24-7.32 (m, 5H, Ph-H), 4.29 (s, 1H), 4.13-4.15 (m, 2H), 4.03 (br, s, 1H), 3.52-3.67 (m, 5H), 3.19-3.23 (m, 2H), 1.55-1.67(br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm): 171.4, 166.5, 151.6, 148.5, 138.8, 134.8, 131.9, 129.8, 128.8, 124.8, 107.9, 73.8, 65.3, 64.4, 56.3, 45.6, 34.4; ESI-MS m/z: 431.4 $[M + 1]^+$; anal. calcd. (%) for $C_{22}H_{23}N_2O_5Cl$: C 61.32, H 5.38, N 6.50; found (%): C 61.12, H 5.50, N 6.73.

[8-(3-chloro-1-(3-chloropyridin-2-yl)-1*H*-pyrazole-5-carbonyl)-1,4-dioxa-8-azaspiro[4,5]decan-2-yl]methyl-4-(trifluoromethyl)benzenesulfonate (l): Light yellow oil substance; Yield: 60 %; IR (KBr, ν_{max}, cm^{-1}): 3445, 2932, 2365, 1734, 1653,1636, 1558, 1456, 1342, 1124, 1063, 889, 669; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 8.41(d, *J* = 4.0 Hz, 1H, pyridine-H), 8.20(d, *J* = 8.1 Hz, 2H, Ph-H), 8.05 (s, 2H, pyridine-H), 7.90(d, *J* = 8.0 Hz, 1H, Ph-H), 7.56(q, 1H, Ph-H), 6.92 (s, 1H, parazole-H), 4.33 (s, 1H), 4.24-4.26 (m,1H), 4.09 (q, 2H), 3.69(q, 1H), 3.41-3.50(br, m, 3H), 1.52-1.60(br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 158.8, 147.6, 147.4, 139.5, 138.9, 134.5, 129.2, 127.5, 126.9, 125.3, 124.4, 115.5, 108.2, 107.3, 73.4, 72.1, 65.2, 60.3, 45.5, 34.7; ESI-MS m/z:622.5 $[M + 1]^+$; Anal. calcd. (%) for $C_{24}H_{21}N_4O_6SCl_2F_3$: C 46.39, H 3.41, N 9.02; found (%): C 46.20, H 3.59, N 9.34.

[8-(3-Chloro-1-(3-chloropyridin-2-yl)-1*H*-pyrazole-5-carbonyl]-1,4-dioxa-8-azaspiro[4,5]decan-2-yl)methyl benzenesulfonate (m): Light yellow oil substance; Yield: 67 %; IR (KBr, ν_{max}, cm^{-1}): 3447, 2916, 2372, 1734, 1653, 1558, 1548, 1458, 1188, 1086, 889, 669; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 8.41 (d, *J* = 4.0 Hz, 1H, pyridine-H), 8.20 (s, 1H, Ph-H), 7.90 (d, *J* = 8.0 Hz, 2H, pyridine-H), 7.68-7.80 (m, 4H, Ph-H), 6.95 (d, 1H, *J* = 5.0 Hz parazole-H), 4.33(s, 1H), 4.14-4.20 (m, 1H), 3.97(q, 2H), 3.69(s, 1H), 3.41-3.50 (br, m, 4H), 1.45-1.62 (br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 158.7, 147.6, 147.4, 141.1, 140.1, 134.9, 130.3, 127.5, 126.9, 126.6, 125.3, 115.5, 108.1, 107.5, 73.4, 71.3, 65.3, 45.5, 34.7; ESI-MS m/z: 554.5 $[M + 1]^+$; Anal. calcd. (%) for $C_{23}H_{22}N_4O_6SCl_2$: C 49.92, H 4.01, N 10.12; found (%): C 49.70, H 4.15, N 10.01.

[8-(2,6-Dichlorobenzoyl)-1,4-dioxa-8-azaspiro[4.5]-decan-2-yl]methyl benzoate (n): Light yellow oil substance; yield: 66 %; IR (KBr, ν_{max}, cm^{-1}): 3447, 2937, 2367, 1765, 1635, 1420, 1362, 1234, 1070, 1015, 976, 787, 710; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.91-7.95(m, 2H), 7.60-7.64 (m, 1H), 7.39-7.50 (m, 5H), 4.31-4.48 (m, 2H), 4.26-4.29(m, 1H), 4.11-4.16(m, 1H), 3.85-3.88(m, 1H), 3.74-3.78 (m, 1H), 3.56-3.63(m, 1H), 3.15(s, 1H), 1.64-1.73 (br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm): 166.1, 162.8, 135.2, 134.0, 133.3, 131.8, 131.1, 130.0, 129.8, 129.1, 128.9, 107.4, 74.0, 66.0, 65.1, 44.4, 35.7; ESI-MS m/z: 450.2 $[M + 1]^+$; Anal. calcd. (%) for $C_{22}H_{21}NO_5Cl_2$: C 58.68, H 4.70, N 3.11; found (%): C 58.70, H 4.58, N 3.36.

[8-(2-Methoxybenzoyl)-1,4-dioxa-8-azaspiro[4.5]-decan-2-yl]methyl benzoate (o): Light yellow oil substance; Yield: 65 %; IR (KBr, ν_{max}, cm^{-1}): 3447, 2392, 2367, 1734, 1683, 1653, 1558, 1506, 1458, 1272, 1115, 922, 669 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.76 (d, 2H, *J* = 5.5 Hz), 7.65(s, 2H), 7.76(s, 1H), 7.43-7.46(br, m, 3H), 7.52-7.54 (m, 5H, Ph-H), 4.24-4.48(br, m, 3H), 4.08-4.14 (m, 1H), 3.96-4.01 (q, 1H), 3.82-3.87(m, 1H), 3.52(s, 1H), 3.35(s, 3H, OCH_3-H), 3.12(s, 2H,), 1.56-1.67(br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 167.8, 166.7, 166.1, 155.4, 134.1, 133.3, 130.7, 129.8, 129.4, 129.0, 128.0, 126.0,121.1, 111.6, 107.2, 74.1, 65.8, 64.9, 55.6, 45.2, 34.6; ESI-MS m/z: 412.6 $[M + 1]^+$; Anal. calcd. (%) for $C_{23}H_{25}NO_6$: C 67.14, H 6.12, N 3.40; found (%): C 67.01, H 6.35, N 3.58.

[8-(2,6-Dicholophenybenzoyl)-1,4-dioxa-8-azaspiro-[4.5]decan-2-yl]methyl phenylacetate (p): Light yellow oil substance; yield: 68 %; IR (KBr, ν_{max}, cm^{-1}): 3447, 2932, 2371, 1684, 1653, 1558, 1437, 1246, 1092, 922, 770; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.57-7.63(br, m, 1H), 7.51-7.52(m, 4H), 7.50(d, 2H, *J* = 8.5 Hz, Ph-H), 7.35(d, 2H, *J* = 8.5 Hz Ph-H), 4.15-4.19(br, m, 2H), 4.11 (s, 1H), 3.68-3.80 (m, 5H), 3.19-3.29(br, m, 2H), 1.73-1.74(br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 170.8, 166.9, 136.0, 133.7, 130.4, 130.2, 129.7, 129.3, 128.7, 127.6, 127.3, 108.0, 73.6, 66.3, 64.1, 44.7, 36.1; ESI-MS m/z: 430.4 $[M + 1]^+$; Anal. calcd. (%) for $C_{23}H_{23}NO_5Cl_2$: C 54.49, H 4.99, N 3.02; found (%): C 54.40, H 5.03, N 3.26.

Benzyl-2-[{(2-chloronicotinoyl)oxy}methyl]-1,4-dioxa-8-azaspiro[4.5]decane-8-carboxylate (q): Light yellow oil substance; yield: 68 %; IR (KBr, ν_{max}, cm^{-1}): 3447, 2934, 2371, 1717, 1653, 1558, 1508, 1458, 1364, 1233, 1111, 907, 758, 669; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 8.73(s, 1H), 7.92(m, 1H), 7.50-7.52(m, 4H), 7.13-7.14(m, 2H), 4.99(s, 2H, CH_2-H), 4.31-4.32(m, 2H), 4.21-4.23(m, 1H), 3.65-3.67(m, 3H), 3.340-3.42(m, 3H), 1.55-1.60 (br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 167.5, 159.9, 154.2, 150.1, 138.9, 136.6, 130.9, 130.4, 129.8, 128.1, 127.2, 125.8, 115.3, 76.5, 72.1, 65.7, 63.2, 43.9, 35.6; ESI-MS m/z: 430.2 $[M + 1]^+$; Anal. calcd. (%) for $C_{22}H_{23}N_2O_6Cl$: C 59.13, H 5.19, N 6.27; found (%): C 59.32, H 5.01, N 6.32.

[8-{1-(3-Chlorophenyl)-3-methyl-1*H*-pyrazole-5-carbonyl}-1,4-dioxa-8-azaspiro[4.5]decan-2-yl]methyl benzenesulfonate (r): Light yellow oil substance; yield: 60 %; IR (KBr, ν_{max}, cm^{-1}): 3438, 2932, 2369, 1734, 1635, 1506, 1464, 1188, 1094, 935, 740, 669; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.90 (d, *J* = 7.5 Hz, 2H, Ph-H), 7.70 (br, m, 4H, Ph-H), 7.56 (s, 3H, Ph-H), 4.33(br, m, 1H), 4.14-4.17(dd, 1H, J_1 = 10.0 Hz, J_2 = 10.1 Hz), 4.09(q, 2H), 3.55(s, 2H), 3.38(s, 2H), 2.18(s, 3H, parazole-CH_3-H), 1.53-1.60(br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 161.7, 148.9, 139.0, 135.0, 133.9, 131.5, 130.3, 129.2, 128.2, 125.6, 125.3, 124.4, 115.5, 108.2, 73.4, 71.3, 65.3, 45.2, 31.3, 13.1; ESI-MS m/z: 567.5 $[M + 1]^+$; Anal. calcd. (%) for $C_{25}H_{25}N_3O_6SCl_2$: C 53.01, H 4.45, N 7.42; found (%): C 53.15, H 4.32, N 7.20.

[8-(6-Chloronicotinoyl)-1,4-dioxa-8-azaspiro[4.5]-decan-2-yl]methyl-2-phenyl-acetate (s): Light yellow oil substance; yield: 60 %; IR (KBr, ν_{max}, cm^{-1}): 3445, 2932, 2369, 1734, 1636, 1506, 1437, 1362, 1252, 1105, 1026, 920, 841, 756, 702; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 8.43 (s, 1H), 7.88(d, 1H, *J*=7.5 Hz), 7.55-7.61(m, 1H), 7.24-7.32 (m, 5H, Ph-H), 4.29(s, 1H), 4.13-4.15(m, 2H), 4.03(br, s, 1H), 3.52-3.67(m, 5H), 3.19-3.23(m, 2H), 1.55-1.67(br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm): 171.4, 166.5, 151.6, 148.5, 138.8, 134.8, 131.9, 129.8, 128.8, 124.8,

107.9, 73.8, 65.3, 64.4, 56.3, 45.6, 34.4; ESI-MS m/z: 431.4 $[M + 1]^+$; Anal. calcd. (%) for $C_{22}H_{23}N_2O_5Cl$: C 61.32, H 5.38, N 6.50; found (%): C 61.12, H 5.50, N 6.73.

[8-(5-Bromonicotinoyl)-1,4-dioxa-8-azaspiro[4.5]-decan-2-yl]methyl-2-fluoro-benzoate (t): Light yellow oil substance; yield: 68 %; IR (KBr, ν_{max}, cm^{-1}): 3449, 2935, 2347, 1734, 1636, 1506, 1437, 1362, 1252, 1182, 1079, 921, 860; ^1H NMR (500 MHz, CH_3OH-d_4): δ (ppm) 8.76(s, 1H, pyridine-H), 8.58(m, 2H, pyridine-H), 7.83-7.88(t, 1H, Ph-H), 7.59-7.62(m, 2H, Ph-H), 7.50-7.52(m, 1H, Ph-H), 4.52 (s, 1H), 4.37-4.40(m, 2H), 4.19-4.24(m, 1H), 3.92(s, 1H), 3.84 (s, 2H), 3.49(s, 2H), 1.69-1.75(br, m, 4H, N-Spiro-H); ^{13}C NMR (125 MHz, CH_3OH-d_4): δ (ppm) 166.5, 163.4, 151.4, 145.9, 137.9, 137.3,134.4, 133.5, 132.8, 130.9, 128.4, 126.7, 120.6, 107.7, 74.2, 65.9, 64.5, 45.9, 40.7; ESI-MS m/z:478.3 $[M +1]^+$; Anal. calcd. (%) for $C_{21}H_{20}N_2O_5BrF$: C 52.62, H 4.21, N 5.84; found (%): C 52.48, H 4.25, N 5.57.

Antifungal bioassays: The fungicidal activity of the compounds **a-t** were tested *in vitro* against *B. cinerea* and *R. solani* and their relative inhibitory ratio (%) has been determined using the mycelium growth rate method *in vitro*[7]. Spiroxamine and Hymexazol was used as a control. After the mycelia grew completely, the diameters of the mycelia were measured and the inhibition rate is calculated according to the formula:

$$I = (M - N) = M \times 100\ \%$$

In the formula, I is the inhibition rate, M is the average diameter of mycelia in the blank test and N is the average diameter of mycelia in the presence of those compounds. The inhibition ratios of those compounds at the dose of 50 μg/mL have been determined and the experimental results are summarized in Table-2.

RESULTS AND DISCUSSION

Chemistry: As shown in **Scheme-I**, the starting material piperidone hydrochloride and glycerol can be easily converted into 1,4-dioxa-8-azaspiro[4.5]decan-2-ylmethanol (1) in 95 % yield by tolune in the presence of catalyst *para* toluene sulfonic acid[8,9]. The intemediate (1) was then treated with aromatic acid, pyrazol acid and nicotinic acid. Finally, substitution reaction of (3) with acyl chloride and sulfuryl chloride in dichloromethane produces the TM. To prepare the key intermediate (3), we optimized the reaction in different condensing agent, *e.g.*, HATU (*o*-(7-azabenzoriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate[10]; 2-(7-Aza-1*H*-benzotriaole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)/dichloromethane, DCC[11] (dicyclohexylcarbodiimide)/dichloromethane, EDC·HCl (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) and HOBt[12] (1-hydroxybenzotriazole)/dichloromethane. Compared to other reagents, EDC·HCl and HOBt/dichloromethane are readily available and cheap. They can also be more easily handled, stored and transported. The comparison results are provided in Table-1.

It can be easily observed that amongst all the condensing agents, EDC·HCl/HOBt gives the highest yield of the product (Table-2, Entry 3). In the reaction, we first activate carboxyl, HBOt and acid to form the relatively stable activeintermediate. The byproducts of the reaction have good water solubility. We can get pure product after washing without further purification. Since EDC·HCl has stronger polarity, it is easier to separate.

TABLE-1
EFFECT OF DIFFERENT CONDENSING AGENTS FOR THE SYNTHESIS OF INTERMEDIATE 3

Entry	Temp. (ºC)	Condensing agents	Time (h)	Solvent	Yield (%)
1	r.t	HATU	4	DCM	85.3
2	r.t	DCC	4	DCM	65.0
3	r.t	EDC·HCl/HOBt	4	DCM	91.2

Note: r.t : room temperature; DCM: dichloromethane.

TABLE-2
INHIBITION EFFECT OF TARGET COMPOUNDS TO *B. cinerea* AND *R. solani* AT 50 μg/mL *IN VITRO*

Compound	Concentration (μg/mL)	Inhibition rate[a] (%)	
		B. cinerea	*R. solani*
a	50	64.07	42.45
b	50	82.16	45.83
c	50	62.05	34.92
d	50	69.60	46.09
e	50	84.17	46.35
f	50	74.52	14.02
g	50	71.36	28.57
h	50	96.21	62.24
i	50	78.04	34.92
j	50	89.98	57.14
k	50	83.29	44.71
l	50	12.38	40.48
m	50	9.52	10.58
n	50	8.35	11.11
o	50	8.10	12.96
p	50	34.29	21.48
q	50	90.56	6.35
r	50	46.52	30.16
s	50	55.56	33.88
t	50	51.43	25.80
Spiroxamine	50	70.43	70.19
Hymexazol	50	65.51	65.02

[a]Every processing repeated three times.

Antifungal activity: The newly synthesized derivatives were evaluated for their antifungal activity against *B. cinerea* and *R. solani in vitro* by the mycelial growth rate method. The bioassay results (at 50 μg/mL) using spiroxamine and hymexazol as the control are shown in Table-2. It is clear that the title compounds **a-t** show a certain degree of antifungal activity against *B. cinerea.* The structural modification caused by changing the substituents (R_1 and R_2) in the 1,4-dioxa-8-azaspiro[4.5]decan moiety has a wide impact on antifungal activity of the title compounds. As compared to **l** and **m** which have only one substituted pyrazol ring, compound **j** and **k** which have chloride substituted pyridine and phenyl substituents show a higher inhibitory activity. Interestingly, the title compound **h** and **q** displayed reasonably good activity against *B. cinerea.*

Conclusion

In the previous paper, target compounds **a-t** were synthesized and we have described a mild, efficient and convenient method for the synthesis of 1,4-dioxa-8-azaspiro[4,5]decane derivatives from easily accessible piperidone hydrochloride and glycerol. In addition, in the process of the synthesis of intermediate **3**, the yield of production is satisfied without further purification. The incorporation of two different R_1 and

R_2 in a single structure leads to the development of novel derivatives with moderate activity. At the same time, we introduce the N atom in the spiro ring and this increases the fungicidal activities. Title compounds **b**, **e**, **h**, **j**, **k** and **q** exhibit favourable fungicidal activities against *B. cinerea,* which are better than that of the commercial fungicides spiroxamine and hymexazol. Therefore, the h could be developed as a leading compound for further structural optimization. The preliminary structure-activity relationship analysis indicates that the antifungal activity of the synthesized compounds shows significant increase when R_2 is phenylacetyl or *p*-methyl phenylsulfuryl. The structures of the target products need to be optimized to enhance their antifungal activity. Future structural modification and biological evaluation to explore the full potential of this novel class of antifungal molecules are currently ongoing.

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