DOI: 10.1002/cjoc.201500436

Synthesis and Biological Activity of Novel Furan/Thiophene and Piperazine-Containing (Bis)1,2,4-triazole Mannich Bases

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A series of novel furan/thiophene and piperazine-containing 1,2,4-triazole Mannich bases and bis(1,2,4-triazole) Mannich bases have been conveniently synthesized *via* Mannich reaction with triazole Schiff bases, various piperazine derivatives, and formaldehyde as intermediates in good yields. Their structures were characterized by melting points, ^{1}H NMR, ^{13}C NMR, IR and elemental analysis. The preliminary bioassay showed that most compounds exhibited significant *in vitro* and *in vivo* fungicidal activity against several test plant fungi. Among 32 new compounds, the trifluoromethyl-containing compounds showed superior activity than the methyl-containing ones. Several compounds, such as **F8**, **F9**, **F10**, **G5**, **H7**, **H8**, **I3** and **I4**, were comparable with some commercial fungicides against different fungi during the present study and could be further structurally optimized. Meanwhile, several compounds showed good herbicidal activity against *Brassica campestris* at 100 μg/mL and KARI inhibitory activity at 200 μg/mL. However, compounds exhibited poor insecticidal activity against oriental armyworm at 200 μg/mL in the preliminary studies. The research results will provide useful information for the design and discovery of new agrochemicals with novel heterocyclic structures.

Keywords 1,2,4-triazole, Mannich base, piperazine derivative, biological activity

Introduction

It is well known that the application of agrochemicals has led to healthy crops, increased yields and economic benefits. In view of the resistance and eco-biological problems associated with conventional pesticides, the research and development of new leads with novel structures, potent activities and eco-friendly properties are urgently needed.[1,2]

Due to the interesting structural properties and versatile biological activities, heterocyclic compounds have attracted much attention in diverse areas. Particularly, they are very important part in almost all kinds of agrochemicals. For examples, xazole fungicides Triadimefon and Triflumizole are an important class of heterocyclic compounds that have long, protective, and curative activity against a broad spectrum of foliar, root, and seedling diseases caused by many ascomycetes, basidiomycetes, and imperfect fungi.[3-5] The carbamoyl triazole herbicides are some of the most important agrochemicals for controlling grass in rice fields which are widely used as pre-emergence herbicides to reduce grass and some dicotyledonous weeds in oilseed rape, soybean, maize, and other crop fields.[6,7] In spite of having been widely applied in pharmaceutical area, piperazine-containing compounds also play an important role in some agrochemicals, such as the systemic fungicide Triforine, which has been found effective for the control of a number of diseases in ornamentals, cereal grains, fruits, and vegetables.[8] Likewise, the furan and thiophene rings are also associated with various useful pesticidal activities, Pefurazoate and Trifensulfuron are heterocyclic fungicide and herbicide containing a furan ring and a thiophene ring, repectively.[9,10]

Furthermore, there are several reports about the synthesis and properties of heterocyclic ketone or thione with substituted amino methyl at α-carbon, however most of the researches focused on the pharmacological activities of these heterocyclic Mannich bases, such as antimicrobial,[11] antituberculous,[12] antitumor,[13] and anticancer activities.[14] Overall, the researches of this kind of compounds in agrochemical area are relatively few, so further exploration for this topic might provide novel agrochemical candidates. Therefore, it is necessary to carry out extensive study on the various agrochemical activities of heterocyclic Mannich bases.

In our previous work, we reported some interesting

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Received June 17, 2015; accepted July 30, 2015; published online September 29, 2015.
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cjoc.201500436 or from the author.

CHINESE JOURNAL OF CHEMISTRY

1,2,4-triazole Mannich base structures containing trifluoromethyl, piperazine and substituted phenyl groups derived from heterocyclic Schiff base. Some of those compounds displayed favorable herbicidal activity against dicotyledon plant, and inhibitory activity against ketol-acid reductoisomerase (KARI), one of the key enzyme involved in the biosynthesis route of chain amino acids leucine, isoleucine and valine.[15,16] Particularly, those compounds possessed significant fungicidal activities against *Pseudoperonospora cubensis*, *Corynespora cassiicola* and some other fungi as well, which provided valuable information for us to further structural modifications. The preliminary SAR studies encouraged us to optimize the benzene ring and substituent on the piperazine ring.[15] In view of the purpose to explore the biological activity related to agrochemical properties of heterocyclic Mannich bases, a series of novel methyl- and trifluoromethyl-containing triazole Mannich bases with furan/thiophene and various substituted piperzaine moieties, and some novel bis(triazole) Mannich bases were synthesized, the fungicidal, herbicidal, KARI inhibitory and insecticidal activities of these new compounds were investigated and the structure-activity relationships were discussed.

Experimental

Materials and methods

The melting points were determined on an X-4 binocular microscope melting point apparatus (Beijing Tech Instrument Co., Beijing, China) and are uncorrected. ^{1}H NMR spectra were measured on a Bruker AC-P500 instrument (400 MHz) using TMS as an internal standard and DMSO-d_6 or $CDCl_3$ as solvent. Infrared spectra were recorded on a Nicolet MAGNA-560 spectrophotometer as KBr tablets. Elemental analysis was performed on a Vario EL elemental analyzer. Partial 4-substituted piperazine intermediates were purchased from Aladin and Alfa Aesar reagent companies. Anhydrous piperazine and other materials were purchased from Nanjing Duodian Reagent Co. Ltd. All solvents were dried by standard methods in advance and distilled before use.

General synthetic procedure

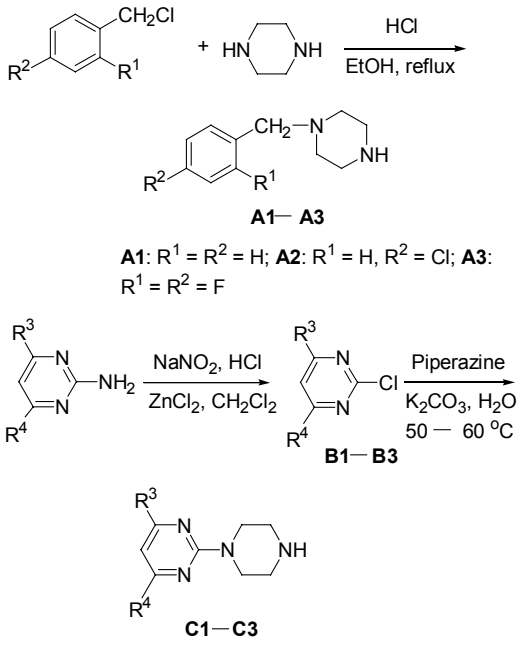
4-Amino-5-methyl/trifluoromethyl-4*H*-1,2,4-triazole-3-thiol **D** was prepared according to the literature.[17]

Preparation of 4-(2,4-disubstituted benzyl)piperazine A

Referring to the literature method,[18] to a solution of anhydrous piperazine (50 mmol) in 20 mL 96% of ethanol was added con. HCl (25 mmol) (Scheme 1). The mixture was stirred under reflux and substituted benzyl chlorine (25 mmol) added dropwise over 5 min. The mixture was refluxed for 4－8 h with TLC monitoring, then left overnight at room temperature. The solid precipitated was filtered and washed with ethanol, the filtrate was evaporated in vacuum and the residue was dissolved in 30 mL saturated K_2CO_3 aq., extracted with chloroform (8 mL×5). The chloroform solution was dried with anhydrous Na_2SO_4 and evaporated in vacuum. The residue was then distilled under reduced pressure to give compound **A** as a colorless liquid.

A1: yield 58%, b.p. 131－134 ℃/10 mmHg; **A2**: yield 41%, b.p. 138－141 ℃/10 mmHg; **A3**: yield 36%, b.p. 147－150 ℃/6 mmHg.

Scheme 1 The synthetic routes of the intermediates **A1**－**A3** and **C1**－**C3**



General synthetic procedures for 4-(4,6-disubstituted pyrimidin-2-yl)piperazine C

2-Chloro-4,6-disubstituted pyrimidines **B** were prepared by reaction of the diazonium salts of 4,6-disubstituted pyrimidin-2-amines with concentrated HCl and $ZnCl_2$.[19] Compounds **C** were prepared according to reference[18] and the method was improved. To a stirred solution of piperazine (45 mmol) and K_2CO_3 (16.5 mmol) in water (20 mL), was added chloropyrimidine **B** (18 mmol) in small portions at 50－65 ℃. The mixture was stirred for 1 h at 60－65 ℃ and cooled to 35 ℃. The yellow solid, 1,4-bis(4,6-disubstituted pyrimidin-2-yl)piperazine, was filtered off, and the filtrate was then extracted three times with chloroform, dried over Na_2SO_4, evaporated in vacuum to give **C**, which was used for the following reactions without further purification.

C1: yellow oil, yield 88%; **C2**: yellow solid, yield 81%, m.p. 45－48 ℃; **C3**: yellow solid, yield 79%, m.p. 82－84 ℃.

General synthetic procedures for 4-[(furan/thiophen-2-ylmethylene)amino]-5-methyl/trifluoromethyl-4*H*-1,2,4-triazole-3-thiol E

As shown in Scheme 2, Compound **D** (10 mmol) and furan-2-carbaldehyde or thiophene-2-carbaldehyde (10.5 mmol) were mixed in acetic acid (15 mL). After having been stirred and refluxed for 20 min, the reaction mixture was cooled to room temperature. The resulting crystals were filtered and washed with ethanol to give Schiff base **E**.

E1 (X=O, R^5=H): The compound was obtained in 84.6% yield as colorless crystals; m.p. 177—179 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.73 (s, 1H), 9.87 (s, 1H), 8.05 (s, 1H), 7.33 (d, J=3.4 Hz, 1H), 6.76 (dd, J=3.3, 1.7 Hz, 1H), 2.31 (s, 3H).

E2 (X=S, R^5=H): The compound was obtained in 89.1% yield as colorless crystals; m.p. 165—167 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.75 (s, 1H), 10.08 (s, 1H), 7.96 (d, J=4.9 Hz, 1H), 7.81 (d, J=3.5 Hz, 1H), 7.28 (t, J=4.3 Hz, 1H), 2.32 (s, 3H).

E3 (X=O, R^5=F): The compound was obtained in 80.5% yield as colorless crystals; m.p. 146—148 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.85 (s, 1H), 9.75 (s, 1H), 8.11 (s, 1H), 7.44 (d, J=3.6 Hz, 1H), 6.80 (dd, J=3.0, 1.6 Hz, 1H).

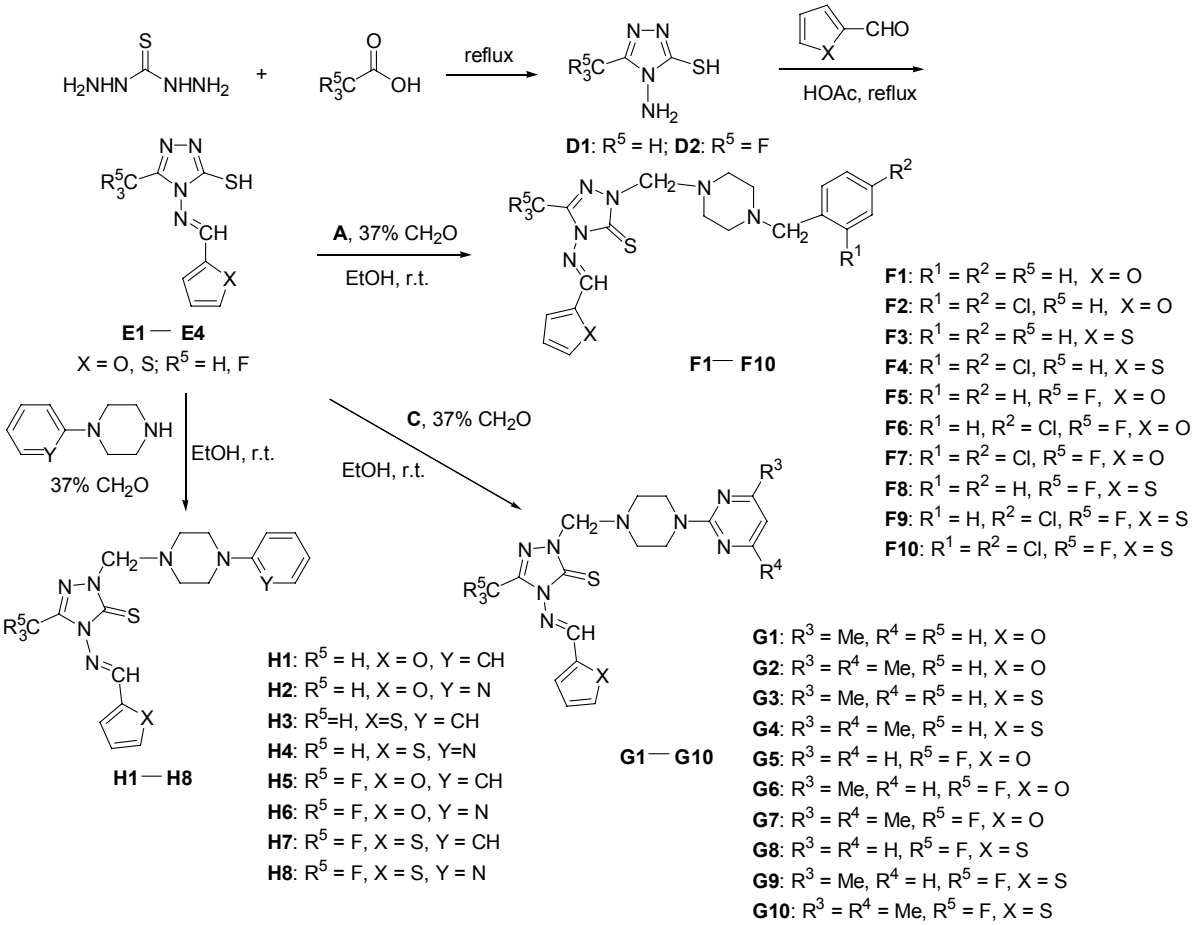
E4 (X=S, R^5=F): The compound was obtained in 88.6% yield as colorless crystals; m.p. 178—180 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.85 (s, 1H), 10.05 (s, 1H), 8.01 (d, J=5.2 Hz, 1H), 7.87 (dd, J=4.8, 3.6 Hz, 1H), 7.28 (dd, J=5.0, 4.0 Hz, 1H).

General synthetic procedures for 1-[(4-(2,4-disubstituted benzyl)piperazin-1-yl)methyl]-4-(furan/thiophen-2-ylmethylene)amino-3-methyl/trifluoromethyl-1*H*-1,2,4-triazole-5(4*H*)-thione F

As shown in Scheme 2, Schiff base **E** (1 mmmol), 37% formalin (1.5 mmol) were dissolved in ethanol (15 mL), and the mixture was stirred at room temperature for 5 min. A solution of 4-(2,4-disubstituted benzyl)piperazine **A** (1 mmol) in ethanol (2 mL) was slowly added dropwise. Then the reaction mixture was stirred for 2—3 h and placed at room temperature or in a refrigerator overnight. The resulting precipitate was filtered and recrystallized from ethanol to give novel 1,2,4-triazole Mannich base **F**.

F1: The compound was obtained in 60.1% yield as colorless crystals; m.p. 105—106 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.38 (s, 1H), 7.67 (d, J=1.5 Hz, 1H), 7.32—7.20 (m, 5H), 7.04 (d, J=3.4 Hz, 1H), 6.59 (dd, J=3.5, 1.8 Hz, 1H), 5.11 (s, 2H), 3.49 (s, 2H), 2.85 (br s, 4H), 2.47 (br s, 4H), 2.44 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 162.64, 148.87, 148.09, 147.77, 146.71, 138.00, 129.17, 128.20, 127.05, 118.58, 112.55, 68.67,

Scheme 2 The synthetic routes of compounds **F1—F10**, **G1—G10** and **H1—H8**



63.05, 52.97, 50.49, 11.08. Anal. calcd for $C_{20}H_{24}N_6OS$: C 60.58, H 6.10, N 21.20; found C 60.39, H 6.14, N 21.13.

F2: The compound was obtained in 67.9% yield as colorless crystals; m.p. 117—118 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.38 (s, 1H), 7.67 (s, 1H), 7.36 (dd, J=10.6, 5.2 Hz, 2H), 7.18 (dd, J=8.3, 2.1 Hz, 1H), 7.04 (d, J=3.4 Hz, 1H), 6.59 (dd, J=3.5, 1.7 Hz, 1H), 5.11 (s, 2H), 3.55 (s, 2H), 2.85 (br s, 4H), 2.52 (br s, 4H), 2.45 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 162.66, 148.84, 148.07, 147.83, 146.72, 134.76, 134.56, 133.00, 131.37, 129.12, 126.85, 118.56, 112.55, 68.66, 58.61, 52.99, 50.50, 11.11. IR (KBr) v: 2939, 2813, 1612, 1588, 1517, 1471, 1170 cm^{-1}. Anal. calcd for $C_{20}H_{22}Cl_2N_6OS$: C 51.62, H 4.76, N 18.06; found C 51.16, H 4.99, N 17.65.

F3: The compound was obtained in 61.8% yield as yellow crystals; m.p. 143—145 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.97 (s, 1H), 7.97 (d, J=5.0 Hz, 1H), 7.82 (d, J=3.3 Hz, 1H), 7.37—7.09 (m, 6H), 5.02 (s, 2H), 3.42 (s, 2H), 2.71 (br s, 4H), 2.34 (br s, 7H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 162.72, 154.30, 147.68, 138.02, 137.46, 134.21, 131.40, 129.17, 128.21, 128.03, 127.06, 68.68, 63.04, 52.98, 50.49, 11.02. IR (KBr) v: 2949, 2815, 1610, 1518, 1495, 1452, 1171 cm^{-1}. Anal. calcd for $C_{20}H_{24}N_6S_2$: C 58.22, H 5.86, N 20.37; found C 57.99, H 6.26, N 20.20.

F4: The compound was obtained in 71.5% yield as a light yellow solid; m.p. 142—144 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.67 (s, 1H), 7.60—7.51 (m, 2H), 7.38—7.34 (m, 2H), 7.19—7.14 (m, 2H), 5.11 (s, 2H), 3.55 (s, 2H), 2.86 (br s, 4H), 2.53 (br s, 4H), 2.44 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 162.75, 154.28, 147.73, 137.45, 134.76, 134.60, 134.20, 133.01, 131.41, 131.36, 129.13, 128.03, 126.86, 68.68, 58.62, 53.01, 50.52, 11.05. Anal. calcd for $C_{20}H_{22}Cl_2N_6S_2$: C 49.89, H 4.61, N 17.46; found C 49.54, H 4.68, N 17.30.

F5: The compound was obtained in 64.7% yield as white crystals; m.p. 134—136 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.20 (s, 1H), 7.71 (s, 1H), 7.30—7.24 (m, 5H), 7.12 (d, J=3.2 Hz, 1H), 6.61 (s, 1H), 5.21 (s, 2H), 3.49 (s, 2H), 2.86 (br s, 4H), 2.47 (br s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.12, 150.71, 147.58, 147.55, 147.42, 138.28 (m, Triazole-C_3), 129.29, 128.29, 127.23, 119.60, 119.54, 116.82 (q, J=271.69 Hz, CF_3), 112.78, 69.92, 62.99, 52.85, 50.26. Anal. calcd for $C_{20}H_{21}F_3N_6OS$: C 53.32, H 4.70, N 18.66; found C 53.01, H 4.86, N 18.32.

F6: The compound was obtained in 80.3% yield as white crystals; m.p. 99—100 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.21 (s, 1H), 7.71 (s, 1H), 7.27—7.21 (m, 4H), 7.12 (d, J=2.8 Hz, 1H), 6.61 (s, 1H), 5.20 (s, 2H), 3.45 (s, 2H), 2.84 (br s, 4H), 2.45 (br s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.14, 150.64, 147.54, 147.44, 138.78 (q, J=42.42 Hz, Triazole-C_3), 136.47, 132.84, 130.41, 128.39, 119.46, 116.83 (q, J=272.7 Hz, CF_3), 112.77, 69.93, 62.21, 52.86, 50.33. Anal. calcd for $C_{20}H_{20}ClF_3N_6OS$: C 49.54, H 4.16, N 17.33; found C 49.13, H 4.29, N 17.22.

F7: The compound was obtained in 72.1% yield as white crystals; m.p. 97—99 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.21 (s, 1H), 7.71 (s, 1H), 7.38—7.12 (m, 4H), 6.61 (s, 1H), 5.21 (s, 2H), 3.56 (s, 2H), 2.86 (br s, 4H), 2.53 (br s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.15, 150.65, 147.56, 147.41, 138.79 (q, J=42.42 Hz, Triazole-C_3), 134.82, 134.34, 133.16, 131.46, 129.18, 126.94, 119.51, 116.83 (q, J=272.7 Hz, CF_3), 112.78, 69.95, 58.58, 52.91, 50.36. Anal. calcd for $C_{20}H_{19}Cl_2F_3N_6OS$: C 46.25, H 3.69, N 16.18; found C 45.88, H 3.91, N 16.02.

F8: The compound was obtained in 66.8% yield as white crystals; m.p. 135—137 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.50 (s, 1H), 7.64 (d, J=4.8 Hz, 1H), 7.59 (d, J=3.6 Hz, 1H), 7.30—7.26 (m, 5H), 7.17 (t, J=4.8 Hz, 1H), 5.21 (s, 2H), 3.49 (s, 2H), 2.86 (br s, 4H), 2.48 (br s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.16, 156.10, 137.85 (m, Triazole-C_3), 136.45, 135.16, 132.80, 132.75, 129.23, 128.26, 128.13, 127.16, 116.79 (m, CF_3), 69.91, 63.02, 52.88, 50.31. Anal. calcd for $C_{20}H_{21}F_3N_6S_2$: C 51.49, H 4.54, N 18.01; found C 49.99, H 4.90, N 17.95.

F9: The compound was obtained in 78.9% yield as white crystals; m.p. 132—133 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.50 (s, 1H), 7.64 (d, J=4.8 Hz, 1H), 7.59 (d, J=3.6 Hz, 1H), 7.27—7.16 (m, 5H), 5.21 (s, 2H), 3.45 (s, 2H), 2.85 (br s, 4H), 2.45 (br s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.19, 156.08, 138.68 (q, J=42.42 Hz, Triazole-C_3), 136.50, 136.44, 135.16, 132.82, 132.78, 130.41, 128.39, 128.15, 116.82 (q, J=272.7 Hz, CF_3), 69.91, 62.21, 52.86, 50.33. Anal. calcd for $C_{20}H_{20}ClF_3N_6S_2$: C 47.95, H 4.02, N 16.77; found C 47.50, H 4.49, N 16.72.

F10: The compound was obtained in 77.6% yield as white crystals; m.p. 121—122 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.51 (s, 1H), 7.64 (br s, 1H), 7.60 (br s, 1H), 7.38—7.17 (m, 4H), 5.21 (s, 2H), 3.56 (s, 2H), 2.86 (br s, 4H), 2.53 (br s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.21, 156.07, 138.71 (q, J=42.42 Hz, Triazole-C_3), 136.45, 135.16, 134.81, 134.45, 133.13, 132.78, 131.42, 129.18, 128.16, 126.94, 116.83 (q, J=272.7 Hz, CF_3), 69.97, 58.61, 52.95, 50.40. Anal. calcd for $C_{20}H_{19}Cl_2F_3N_6S_2$: C 44.86, H 3.58, N 15.70; found C 44.53, H 4.11, N 16.06.

General synthetic procedures for 1-[(4-(4,6-disubstituted pyrimidin-2-yl)piperazin-1-yl)methyl]-4-(furan/thiophen-2-ylmethylene)amino-3-methyl/tri-fluoromethyl-1*H*-1,2,4-triazole-5(4*H*)-thione G

It was similar with that of **F** using pyrimidyl-piperazine **C** as amine material (Scheme 2).

G1: The compound was obtained in 64.9% yield as a white solid; m.p. 119—120 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.38 (s, 1H), 8.13 (d, J=5.0 Hz, 1H), 7.67 (d, J=1.6 Hz, 1H), 7.04 (d, J=3.4 Hz, 1H), 6.59 (dd, J=3.5, 1.8 Hz, 1H), 6.34 (d, J=5.0 Hz, 1H), 5.16 (s, 2H), 3.92—3.78 (m, 4H), 2.92—2.79 (m, 4H), 2.43 (s,

3H), 2.30 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.53, 162.65, 161.51, 157.16, 148.86, 148.04, 147.85, 146.71, 118.57, 112.53, 109.38, 68.84, 50.54, 43.52, 24.29, 11.07. Anal. calcd for $C_{18}H_{22}N_8OS$: C 54.25, H 5.57, N 28.12; found C 54.03, H 5.65, N 28.08.

G2: The compound was obtained in 65.2% yield as a white solid; m.p. 146－147 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.37 (s, 1H), 7.66 (d, J=1.5 Hz, 1H), 7.03 (d, J=3.4 Hz, 1H), 6.58 (dd, J=3.5, 1.7 Hz, 1H), 6.23 (s, 1H), 5.15 (s, 2H), 3.93－3.78 (m, 4H), 2.93－2.78 (m, 4H), 2.42 (s, 3H), 2.25 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.90, 162.58, 161.52, 148.75, 147.99, 147.74, 146.70, 118.54, 112.54, 108.79, 68.81, 50.57, 43.46, 24.05, 11.06. IR (KBr) v: 2939, 2877, 1569, 1502, 1448, 1167 cm^{-1}. Anal. calcd for $C_{19}H_{24}N_8OS$: C 55.32, H 5.86, N 27.16; found C 55.20, H 5.88, N 27.02.

G3: The compound was obtained in 68.5% yield as yellow crystals; m.p. 147－149 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.94 (s, 1H), 8.17 (d, J=4.8 Hz, 1H), 7.96 (d, J=4.8 Hz, 1H), 7.82 (d, J=2.8 Hz, 1H), 7.27 (dd, J=4.9, 3.8 Hz, 1H), 6.48 (d, J=4.8 Hz, 1H), 5.09 (s, 2H), 3.74－3.72 (m, 4H), 2.75－2.72 (m, 4H), 2.32 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.57, 162.72, 161.51, 157.17, 154.37, 147.78, 137.39, 134.25, 131.42, 128.02, 109.40, 68.85, 50.54, 43.53, 24.32, 11.02. Anal. calcd for $C_{18}H_{22}N_8S_2$: C 52.15, H 5.35, N 27.03; found C 52.11, H 5.20, N 26.98.

G4: The compound was obtained in 71.8% yield as light yellow crystals; m.p. 143－145 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.95 (s, 1H), 7.96 (d, J=4.0 Hz, 1H), 7.81 (s, 1H), 7.27 (d, J=3.3 Hz, 1H), 6.35 (s, 1H), 5.09 (s, 2H), 3.73 (br s, 4H), 2.72 (br s, 4H), 2.31 (s, 3H), 2.19 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.99, 162.76, 161.63, 154.31, 147.73, 137.44, 134.18, 131.38, 128.01, 108.83, 68.91, 50.65, 43.52, 24.06, 11.00. IR (KBr) v: 2913, 2843, 1600, 1575, 1496, 1157 cm^{-1}. Anal. calcd for $C_{19}H_{24}N_8S_2$: C 53.25, H 5.64, N 26.15; found C 53.17, H 5.48, N 25.94.

G5: The compound was obtained in 60.6% yield as white crystals; m.p. 141－143 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.21 (s, 1H), 8.28 (d, J=4.7 Hz, 2H), 7.71 (d, J=1.3 Hz, 1H), 7.12 (d, J=3.5 Hz, 1H), 6.60 (dd, J=3.5, 1.7 Hz, 1H), 6.47 (t, J=4.7 Hz, 1H), 5.26 (s, 2H), 3.87－3.85 (m, 4H), 2.90－2.87 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.15, 161.52, 157.73, 150.65, 147.54, 147.43, 139.52 (m, Triazole-C_3), 119.46, 116.79 (q, J=272.7 Hz, CF_3), 112.76, 109.96, 70.12, 50.37, 43.55. Anal. calcd for $C_{17}H_{17}F_3N_8OS$: C 46.57, H 3.91, N 25.56; found C 46.67, H 4.05, N 25.23.

G6: The compound was obtained in 69.2% yield as white crystals; m.p. 126－128 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.21 (s, 1H), 8.14 (d, J=4.8 Hz, 1H), 7.70 (s, 1H), 7.12 (d, J=3.2 Hz, 1H), 6.61 (bs, 1H), 6.36 (d, J=4.8 Hz, 1H), 5.27 (s, 2H), 3.87－3.86 (m, 4H), 2.88－2.87 (m, 4H), 2.31 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.66, 165.14, 161.49, 157.20, 150.65, 147.54, 147.42, 138.83 (q, J=42.42 Hz, Triazole-C_3), 119.47, 116.79 (q, J=272.7 Hz, CF_3), 112.76, 109.57, 70.15, 50.44, 43.51, 24.29. Anal. calcd for $C_{18}H_{19}F_3N_8OS$: C 47.78, H 4.23, N 24.77; found C 47.53, H 4.23, N 24.40.

G7: The compound was obtained in 60.5% yield as white crystals; m.p. 132－134 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.21 (s, 1H), 7.71 (br s, 1H), 7.12 (d, J=3.2 Hz, 1H), 6.60 (br s, 1H), 6.24 (s, 1H), 5.27 (s, 2H), 3.88－3.87 (m, 4H), 2.88－2.87 (m, 4H), 2.26 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.09, 165.11, 161.56, 150.65, 147.55, 147.39, 138.79 (q, J = 42.42 Hz, Triazole-C_3), 119.55, 116.78 (q, J=272.7 Hz, CF_3), 112.77, 109.04, 70.17, 50.50, 43.45, 24.06. Anal. calcd for $C_{19}H_{21}F_3N_8OS$: C 48.92, H 4.54, N 24.02; found C 48.80, H 5.01, N 23.85.

G8: The compound was obtained in 64.1% yield as white crystals; m.p. 140－142 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.50 (s, 1H), 8.28 (d, J=4.8 Hz, 2H), 7.63 (d, J=4.4 Hz, 1H), 7.59 (d, J=4.4 Hz, 1H), 7.17 (t, J=4.4 Hz, 1H), 6.47 (t, J=4.8 Hz, 1H), 5.26 (s, 2H), 3.87－3.85 (m, 4H), 2.90－2.87 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.20, 161.49, 157.74, 156.12, 138.77 (q, J=42.42 Hz, Triazole-C_3), 136.41, 135.18, 132.78, 128.15, 116.78 (q, J=273.7 Hz, CF_3), 109.96, 70.09, 50.36, 43.54. Anal. calcd for $C_{17}H_{17}F_3N_8S_2$: C 44.92, H 3.77, N 24.65; found C 44.60, H 4.03, N 24.23.

G9: The compound was obtained in 62.7% yield as white crystals; m.p. 129－131 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.50 (s, 1H), 8.14 (d, J=4.8 Hz, 1H), 7.63 (d, J=3.6 Hz, 1H), 7.59 (d, J=3.6 Hz, 1H), 7.16 (br s, 1H), 6.36 (d, J=4.8 Hz, 1H), 5.27 (s, 2H), 3.87－3.85 (m, 4H), 2.88－2.86 (m, 4H), 2.31 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.67, 165.19, 161.48, 157.21, 156.11, 138.75 (q, J=42.42 Hz, Triazole-C_3), 136.43, 135.15, 132.77, 128.14, 116.78 (q, J=272.7 Hz, CF_3), 109.57, 70.13, 50.44, 43.50, 24.30. Anal. calcd for $C_{18}H_{19}F_3N_8S_2$: C 46.14, H 4.09, N 23.92; found C 45.88, H 4.47, N 23.92.

G10: The compound was obtained in 66.1% yield as white crystals; m.p. 150－151 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.49 (s, 1H), 7.63 (d, J=3.6 Hz, 1H), 7.59 (d, J=3.6 Hz, 1H), 7.17 (t, J=3.6 Hz, 1H), 6.24 (s, 1H), 5.27 (s, 2H), 3.87 (br s, 4H), 2.88 (br s, 4H), 2.26 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.09, 165.19, 161.57, 156.10, 138.71 (q, J=42.42 Hz, Triazole-C_3), 136.44, 135.15, 132.76, 128.14, 116.78 (q, J=272.7 Hz, CF_3), 109.03, 70.17, 50.52, 43.47, 24.06. Anal. calcd for $C_{19}H_{21}F_3N_8S_2$: C 47.29, H 4.39, N 23.22; found C 47.65, H 4.05, N 22.95.

General synthetic procedures for 1-[(4-phenyl/(pyridin-2-yl)piperazin-1-yl)methyl]-4-(furan/thiophen-2-ylmethylene)amino-3-methyl/trifluoro-methyl-1*H*-1,2,4-triazole-5(4*H*)-thione H

It was similar with that of **F** using 4-phenyl-piperazine or 4-(pyridine-2-yl)piperazine as amine material (Scheme 2).

H1: The compound was obtained in 83.5% yield as colorless crystals; m.p. 110－111 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.40 (s, 1H), 7.69 (d, J=1.5 Hz, 1H),

7.32－7.20 (m, 2H), 7.07 (d, *J*＝3.4 Hz, 1H), 6.93 (d, *J*＝7.9 Hz, 2H), 6.88 (t, *J*＝7.3 Hz, 1H), 6.62 (dd, *J*＝3.5, 1.8 Hz, 1H), 5.19 (s, 2H), 3.26－3.17 (m, 4H), 3.05－2.95 (m, 4H), 2.48 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 162.73, 151.34, 149.01, 148.07, 147.95, 146.77, 129.11, 119.89, 118.68, 116.37, 112.57, 68.65, 50.53, 49.34, 11.11. Anal. calcd for $C_{19}H_{22}N_6OS$: C 59.66, H 5.80, N 21.97; found C 59.58, H 5.83, N 21.75.

H2: The compound was obtained in 85.6% yield as colorless crystals; m.p. 144－145 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 10.37 (s, 1H), 8.17 (dd, *J*＝4.9, 1.2 Hz, 1H), 7.67 (d, *J*＝1.6 Hz, 1H), 7.46 (ddd, *J*＝8.9, 7.1, 2.0 Hz, 1H), 7.05 (d, *J*＝3.4 Hz, 1H), 6.72－6.51 (m, 3H), 5.16 (s, 2H), 3.62－3.48 (m, 4H), 3.00－2.89 (m, 4H), 2.44 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 162.65, 159.29, 148.95, 148.01, 147.89, 146.74, 137.41, 118.61, 113.22, 112.56, 107.08, 68.71, 50.33, 45.12, 11.08. Anal. calcd for $C_{18}H_{21}N_7OS$: C 56.38, H 5.52, N 25.57; found C 56.20, H 5.54, N 25.33.

H3: The compound was obtained in 87.2% yield as white crystals; m.p. 109－111 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 10.66 (s, 1H), 7.57 (dd, *J*＝7.1, 4.4 Hz, 2H), 7.28－7.24 (m, 2H), 7.17－7.14 (m, 1H), 6.92－6.84 (m, 3H), 5.17 (s, 2H), 3.27－3.14 (m, 4H), 3.03－2.91 (m, 4H), 2.44 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 162.78, 154.52, 151.33, 147.89, 137.40, 134.34, 131.49, 129.13, 128.05, 119.92, 116.39, 68.64, 50.52, 49.34, 11.07. Anal. calcd for $C_{19}H_{22}N_6S_2$: C 57.26, H 5.56, N 21.09; found C 57.09, H 5.39, N 21.22.

H4: The compound was obtained in 88.2% yield as a white solid; m.p. 152－154 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 9.96 (s, 1H), 8.07 (d, *J*＝4.0 Hz, 1H), 7.97 (d, *J*＝4.8 Hz, 1H), 7.82 (d, *J*＝3.2 Hz, 1H), 7.49 (t, *J*＝7.2 Hz, 1H), 7.27 (t, *J*＝4.4 Hz, 1H), 6.80 (d, *J*＝8.8 Hz, 1H), 6.62－6.59 (m, 1H), 5.10 (s, 2H), 3.48 (br s, 4H), 2.78 (br s, 4H), 2.33 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 162.73, 159.31, 154.46, 147.93, 147.83, 137.44, 137.36, 134.29, 131.48, 128.04, 113.24, 107.11, 68.74, 50.35, 45.15, 11.04. Anal. calcd for $C_{18}H_{21}N_7S_2$: C 54.11, H 5.30, N 24.54; found C 53.89, H 5.23, N 24.69.

H5: The compound was obtained in 82.4% yield as colorless crystals; m.p. 121－122 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 10.21 (s, 1H), 7.71 (s, 1H), 7.28－7.24 (m, 2H), 7.14 (d, *J*＝3.5 Hz, 1H), 7.03－6.71 (m, 3H), 6.61 (dd, *J*＝3.4, 1.7 Hz, 1H), 5.27 (s, 2H), 3.25－3.11 (m, 4H), 3.09－2.90 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 165.18, 151.26, 150.76, 147.62, 147.39, 138.88 (q, *J*＝42.42 Hz, Triazole-C_3), 129.16, 120.14, 119.64, 116.83 (q, *J*＝272.7 Hz, CF_3), 116.51, 112.82, 69.89, 50.43, 49.41. IR (KBr) *ν*: 2945, 2829, 1602, 1484, 1455, 1317, 1213, 1172 cm^{-1}. Anal. calcd for $C_{19}H_{19}F_3N_6OS$: C 52.29, H 4.39, N 19.26; found C 52.07, H 4.55, N 19.16.

H6: The compound was obtained in 79.6% yield as colorless crystals; m.p. 86－87 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 10.20 (s, 1H), 8.17 (dd, *J*＝4.8, 1.2 Hz, 1H), 7.71 (d, *J*＝1.3 Hz, 1H), 7.46 (ddd, *J*＝8.8, 7.2, 1.9 Hz, 1H), 7.13 (d, *J*＝3.5 Hz, 1H), 6.71－6.53 (m, 3H), 5.27 (s, 2H), 3.61－3.51 (m, 4H), 2.97－2.87 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 165.15, 159.26, 150.79, 147.95, 147.61, 147.36, 138.85 (q, *J* ＝ 42.42 Hz, Triazole-C_3), 137.53, 119.63, 116.80 (q, *J*＝272.7 Hz, CF_3), 113.46, 112.80, 107.20, 70.01, 50.26, 45.17. Anal. calcd for $C_{18}H_{18}F_3N_7OS$: C 49.42, H 4.15, N 22.41; found C 49.15, H 4.48, N 22.22.

H7: The compound was obtained in 84.3% yield as white crystals; m.p. 131－133 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 9.99 (s, 1H), 8.03 (d, *J*＝4.2 Hz, 1H), 7.91 (s, 1H), 7.30 (s, 1H), 7.19 (t, *J*＝7.4 Hz, 2H), 6.92 (d, *J*＝7.8 Hz, 2H), 6.77 (t, *J*＝7.0 Hz, 1H), 5.24 (s, 2H), 3.13 (br s, 4H), 2.90 (br s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 165.24, 156.22, 151.26, 138.81 (q, *J*＝42.42 Hz, Triazole-C_3), 136.42, 135.21, 132.84, 129.16, 128.17, 120.13, 116.81 (q, *J*＝272.7 Hz, CF_3), 116.50, 69.88, 50.44, 49.41. IR (KBr) *ν*: 2944, 2829, 1597, 1522, 1500, 1461, 1314, 1214, 1172 cm^{-1}. Anal. calcd for $C_{19}H_{19}F_3N_6S_2$: C 50.43, H 4.23, N 18.57; found C 50.33, H 4.25, N 18.24.

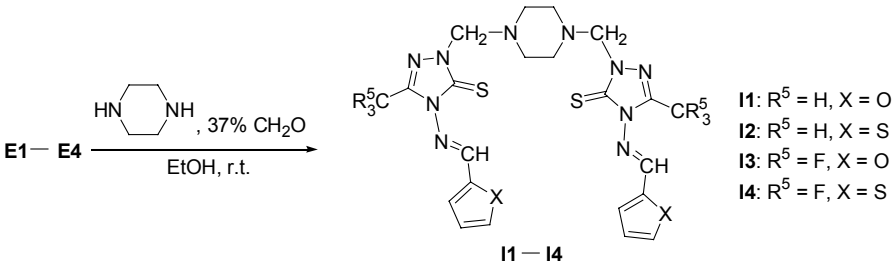
H8: The compound was obtained in 87.1% yield as a white solid; m.p. 124－126 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 9.97 (s, 1H), 8.08 (d, *J*＝4.0 Hz, 1H), 8.03 (d, *J*＝4.4 Hz, 1H), 7.91 (s, 1H), 7.51 (t, *J*＝7.2 Hz, 1H), 7.30 (s, 1H), 6.81 (d, *J*＝8.4 Hz, 1H), 6.61 (t, *J*＝6.0 Hz, 1H), 5.24 (s, 2H), 3.50 (br s, 4H), 2.84 (br s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 165.22, 159.27, 156.20, 147.98, 138.78 (q, *J*＝42.42 Hz, Triazole-C_3), 137.50, 136.41, 135.20, 132.82, 128.16, 116.79 (q, *J*＝272.7 Hz, CF_3), 113.44, 107.16, 70.01, 50.27, 45.16. Anal. calcd for $C_{18}H_{18}F_3N_7S_2$: C 47.67, H 4.00, N 21.62; found C 47.42, H 4.15, N 21.39.

General synthetic procedures for 1,1'-[piperazin-1,4-diylbis(methylene)]bis[4-(furan/thiophen-2-ylmethylene)amino-3-methyl/trifluoromethyl-1*H*-1,2,4-triazole-5(4*H*)-thione] I

As shown in Scheme 3, Schiff base **E** (1.8 mmmol), 37% formalin (2.5 mmol) were dissolved in ethanol (30 mL), and the mixture was stirred at room temperature for 5 min. A solution of piperazine (0.9 mmol) in ethanol (2 mL) was slowly added dropwise. Then the reaction mixture was stirred for 2－3 h at room temperature. The resulting precipitate was filtered and recrystallized from ethanol to give novel bis(1,2,4-triazole) Mannich base **I**.

I1: The compound was obtained in 80.3% yield as colorless crystals; m.p. 216－217 ℃ (Dec.). ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 9.74 (s, 2H), 8.08 (s, 2H), 7.38 (d, *J*＝3.6 Hz, 2H), 6.79 (t, *J*＝3.6 Hz, 2H), 4.99 (s, 4H), 2.70 (s, 8H), 2.35 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 162.57, 148.98, 148.04, 147.86, 146.75, 118.73, 112.56, 68.63, 50.39, 11.07. Anal. calcd for $C_{22}H_{26}N_{10}O_2S_2$: C 50.18, H 4.98, N 26.60; found C 50.03, H 5.17, N 26.28.

Scheme 3 The synthetic routes of compounds **I1**—**I4**



I2: The compound was obtained in 84.5% yield as a white solid; m.p. 198—200 ℃ (Dec.). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.63 (s, 2H), 7.53—7.57 (m, 4H), 7.14 (t, J=4.0 Hz, 2H), 5.06 (s, 4H), 2.84 (s, 8H), 2.41 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 162.65, 154.45, 147.79, 137.41, 134.30, 131.43, 128.03, 68.63, 50.39, 11.01. IR (KBr) v: 2939, 2823, 1600, 1526, 1451, 1171 cm^{-1}. Anal. calcd for $C_{22}H_{26}N_{10}S_4$: C 47.29, H 4.69, N 25.07; found C 46.94, H 4.73, N 24.84.

I3: The compound was obtained in 72.9% yield as white crystals; m.p. 199—200 ℃ (Dec.). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.21 (s, 2H), 7.71 (s, 2H), 7.13 (d, J=3.2 Hz, 2H), 6.61 (d, J=3.2 Hz, 2H), 5.17 (s, 4H), 2.86 (s, 8H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.08, 150.68, 147.58, 147.40, 138.86 (q, J=42.42 Hz, Triazole-C_3), 119.57, 116.80 (q, J=272.7 Hz, CF_3), 112.79, 69.85, 50.28. Anal. calcd for $C_{22}H_{20}F_6N_{10}O_2S_2$: C 41.64, H 3.18, N 22.07; found C 41.37, H 3.47, N 21.99.

I4: The compound was obtained in 78.4% yield as white crystals; m.p. 207—208 ℃ (Dec.). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.51 (s, 2H), 7.59—7.65 (m, 4H), 7.17 (t, J=4.0 Hz, 2H), 5.17 (s, 4H), 2.86 (s, 8H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.14, 156.14, 138.78 (m, Triazole-C_3), 136.43, 135.19, 132.80, 128.16, 116.79 (m, CF_3), 69.85, 50.28. Anal. calcd for $C_{22}H_{20}F_6N_{10}S_4$: C 39.63, H 3.02, N 21.01; found C 39.17, H 3.49, N 20.78.

The *in vitro* fungicidal activity assay

The *in vitro* fungicidal activity of compounds **F**, **G**, **H** and **I** against *Gibberella sanbinetti*, *Alternaria solani* Sorauer, *Cercospora arachidicola*, *Physalospora piricola*, *Fusarium omysporum* and *Rhizoctonia cerealis* were evaluated using the mycelium growth rate test.[20] The method for testing the primary biological activity was performed in an isolated culture.

The *in vivo* fungicidal activity assay

The *in vivo* fungicidal activity of compounds against *Corynespora cassiicola*, *Pseudomonas syringae* pv. *lachrymans*, *Ascochyta citrallina* Smith, *Pseudoperonospora cubensis*, and *Sclerotinia sclerotiorum* were evaluated according to the reference,[21] and a potted plants test method was adopted. Six commericial fungicides, Chlorothalonil, Dimethomorph, Thiophanate-methyl, Iprodione, Validamycin and Zhongshengmycin were evaluated as contrasts at the same condition. The *in vivo* fungicidal activities of compounds against *Puccinia sorghi* Schw. were tested according to the reference,[22] and the commercial fungicides Triadimefon and Azoxystrobin were used as contrasts.

The herbicidal activity assay

The *in vivo* herbicidal activity of compounds **F**, **G**, **H** and **I** was determined by the inhibition of the root-growth of rape (*Brassica campestris*) and inhibition of the seedling-growth of barnyardgrass (*Echinochloa crusgalli*) tests according to the reported method.[23]

KARI inhibitory activity assay

The cloning of rice KARI has been described previously,[24] and enzyme expression and purification followed that protocol. KARI activity was measured with a continuous assay method,[24] following the consumption of NADPH at 340 nm and 30 ℃. Assay solutions contained 0.2 mmol/L NADPH, 1 mmol/L $MgCl_2$, 0.1 mmol/L substrate (2-acetolactate), and inhibitor (synthesized compounds or CPD), in 0.1 mol/L phosphate buffer (pH 8.0). Inhibitors were preincubated with the enzyme, NADPH and $MgCl_2$ in phosphate buffer at 30 ℃ for 10 min. The reaction was then started by adding the substrate. The percentage of the inhibition was calculated.

Insecticidal activity assay

The larvicidal activities of the selected compounds **F5**, **F8**, **G5**, **G6**, **G7**, **G8**, **G9** and contrast Chlorantranili-prole against oriental armyworm (*Mythimna separate* Walker) were tested according to the leaf-dip method using the reported procedure.[25,26] Assessments were made on a dead/alive basis, and mortality rates were corrected using Abbott's formula.[27]

Results and Discussion

Synthesis and spectroscopy

The synthesis procedures for piperazine inter-mediates and target compounds **F**, **G**, **H** and **I** were shown in Scheme 1, Scheme 2 and Scheme 3. The Schiff base intermediates (**E1**—**E4**) were prepared by the condensation of 4-amino-5-methyl/trifluoromethyl-4*H*-1,2,4-trizole-3-thiol (**D1**—**D2**) and furfural or 2-thenaldehyde in acetic acid, according to the similar method reported in Lit.[15] These intermediates can be efficiently obtained

with yields 84%－89% and reaction time 20 min. The Mannich reaction of Schiff base **E** with formaldehyde and benzylpiperazine **A**, or pyrimidylpiperazine **C**, or 4-phenyl/pyridylpiperazine in ethanol at room temperature led to novel triazole heterocyclic Mannich bases **F**, **G** and **H**, respectively in satisfying yields. Under conditions of excess formaldehyde and 2∶1 molar ratio of Schiff base **E** and piperazine, bis heterocyclic Mannich base **I** was synthesized also with high yield (72%－85%) at room temperature. The attempts to obtain the possible mono heterocyclic Mannich base with different mole ratio of **E** and piperazine (1∶1－1∶3) indicate that the major product obtained still was bis-Mannich base. This may be due to that after the produce of mono-Mannich base (the tertiary amine part introduced) the activity of the secondary amine part on piperazine ring would be enhanced, which makes it easier to undergo further Mannich reaction than piperazine.

Compounds **F**, **G**, **H** and **I** were identified by melting point, ^{1}H NMR, ^{13}C NMR and IR spectra. The measured elemental analyses were also consistent with the corresponding calculated ones. In ^{1}H NMR, the －CH＝N－ proton appeared at δ 9.75－10.08 as a singlet in the Schiff base **E**, which shifted downfield to δ 9.94－10.67 in most of Mannich bases **F**－**I**. As having a －NH－C(＝S)－ function of thioamide, the Schiff bases **E** can exist either as a thione or the thiol tautomeric forms or as an equilibrium mixture of both forms. In view of the chemical shift at δ 13.73－14.85 as a singlet in **E** which is due to SH proton, it can be inferred that **E** did not exist as thione but as the thiol tautomeric forms in solution.[15,16] In the ^{1}H NMR spectra of the Mannich bases products, neither a NH signal nor a thiol SH signal is visible. The signal of CH_2 protons neighboring to the triazole ring was observed at δ 4.99－5.27 as a singlet. The piperazine ring proton (CH_2) in Mannich bases **F**, **G**, and **H** appeared at δ 2.71－3.93 and 2.34－2.93 as two broad singlets or multiplets, respectively. Whereas in the case of bis-Mannich base **I**, the piperazine ring proton appeared at δ 2.70－2.86 as a singlet due to the symmetric structure. In the ^{13}C NMR spectra of compounds **F**, **G**, **H** and **I**, the typical carbon resonance at δ 162.57－167.67 was indicative of a thiocarbonyl group (C＝S). The carbon signals of －CH＝N－ group in furan-containing compounds and thiophene-containing compounds were observed at δ 146.70－147.44 and 126.94－128.17, respectively. The piperazine carbons appeared at δ 50.28－50.39 as one singlet in compounds **I1**－**I4** owing to the symmetric structure of bis-Mannich base, while they appeared at δ 50.26－53.01 and δ 43.45－50.52 as two singlets in other compounds. The signals of C_3 in triazole ring and carbon adjacent to it were observed at δ 139.52－157.17 and 11.00－11.11 (CH_3) as singlet respectively in **F1**－**F4**, **G1**－**G4**, **H1**－**H4**, **I1** and **I2**, while at δ ~138.53 and ~116.80 (CF_3) as quartet or multiplet, respectively in **F5**－**F10**, **G5**－**G10**, **H5**－**H8**, **I3** and **I4**, which is due to the "F" splitting of the latter.

The IR spectra of representative compounds **F2**, **F3**, **G2**, **G4**, **H5**, **H7** and **I2** showed bands at 1569－1612 cm^{-1} for C＝N stretching. The characteristic stretching vibrations ν(C － F) (for trifluoromethyl-containing compounds) and ν(C ＝ S) appear at 1314 － 1317, 1213－1214 and 1157－1172 cm^{-1}, respectively.

Fungicidal activity

The *in vitro* fungicidal results of the Mannich bases **F**, **G**, **H** and bis-Mannich base **I** in inhibiting the mycelial growth of six test fungi were listed in Table 1. The commercial fungicides Triadimefon, Carbendazim and Chlorothalonil were used as contrasts. As indicated in Table 1, most of compounds showed obvious *in vitro* funicidal acitivity against several tested fungi at 50 μg/mL, especially for *Physalospora piricola*. In most cases, trifluoromethyl-containing compounds at 3-position of triazole ring exhibited higher activity than those of methyl-containing compounds, and thiophene-containing compounds exhibited higher activity than those of furan-containing compounds with same R^5 and piperazine substituents, such as **F1** vs. **F5**, **F4** vs. **F10**. Furthermore, trifluoromethyl-containing compounds **F5**－**F10** also showed good activity against *Cercospora arachidicola* with inhibitory rate 50%－71%, and **F8**, **F9**, **F10** whose data excessed 70% were more active than Triadimefon. In addition, several compounds such as **H7** and **H8** exhibited good fungicidal activity against *Rhizoctonia cerealis*, with inhibitory rate 86.3% and 92.2%, respectively. **F10** against *Fusarium omysporum* and **H1** against *Physalospora piricola* possessed 80.0% and 83.3% activity respectively, which were more active than Triadimefon. It was worthy of note that **F8**, **F10**, **H7** and **H8** exhibited higher and wider fungicidal activity than others, and were comparable with those of contrast fungicides Triadimefon and Chlorothalonil.

In view of the comparatively favorable fungicidal activity of trifluoromethyl-containing compounds, several of them were further investigated for the *in vivo* fungicidal effect. From Table 2, we can see that most of tested compounds exhibited significant control effect against *Corynespora cassiicola*, *Pseudomonas syringae* pv. *lachrymans*, *Ascochyta citrullina* Smith, *Pseudoperonospora cubensis* and *Sclerotinia sclerotiorum* at a test concentration of 500 μg/mL. Compounds **F8** and **I3** had 66.21% and 70.58% activity against *Corynespora cassiicola*, better than contrast Iprodione (53.52%). For *Pseudomonas syringae* pv. *lachrymans*, contrasts Chlorothalonil, Dimethomorph, Iprodione and Validamycin did not exhibit good control effect (＜39%), whereas most of tested compounds possessed 53%－77% activity. Especially **F10** and **G5** whose control effects were 76.07% and 71.27% were more effective than all the contrasts including Thiophanate-methyl (54.15%) and Zhongshengmycin (69.69%). For *Ascochyta citrullina* Smith, **F9**, **F10**, **I3** and **I4** had control effect of 76.93%, 73.73%, 77.62% and 85.34%, more effective than the contrast Validamycin. For *Pseudoperonospora*

cubensis, all the compounds showed 63.41%—84.52% activity, better than most of the contrasts. Among them, **I3** had 84.52% of control effect and was more effective than the best active contrast Dimethomorph (83.75%). For *Sclerotinia sclerotiorum*, **F5**, **F6**, **F7**, **F8**, **F9**, and **F10** possessed 74%—83% activity, similar to those of Chlorothalonil (74.89%), Thiophanate-methyl (83.39%) and Zhongshengmycin (84.43%).

Table 1 The biologicial activity data of compounds (% inhibiton)

Compd	*In vitro* fungicidal activity						Herbicidal activity		*In vitro* KARI inhibitory activity
	Gibberella sanbinetti	*Alternaria solani Sorauer*	*Cercospora arachidicola*	*Physalospora piricola*	*Fusarium omysporum*	*Rhizoctonia cerealis*	*Brassica campestris*	*Echinochloa crusgalli*	
	50 μg/mL	50 μg/mL	50 μg/mL	50 μg/mL	50 μg/mL	50 μg/mL	100 (10) μg/mL	100 (10) μg/mL	200 μg/mL
F1	24.4	15.8	25.0	30.2	19.4	37.5	0	10.0	nt[g]
F2	34.1	10.5	20.0	51.2	16.1	28.6	13.8	5.0	nt
F3	36.8	26.7	33.3	63.8	3.7	nt	0	10.0 (5.0)	nt
F4	31.7	15.8	15.0	48.8	16.1	33.9	0	0	nt
F5	30.5	31.2	51.4	10.1	30.0	nt	33.4	15.0	0
F6	21.0	30.0	50.2	40.4	50.4	nt	34.5	10.0	42.0
F7	20.2	40.4	60.0	11.3	31.3	nt	40.8	10.0	42.7
F8	70.3	61.2	70.2	50.2	60.4	nt	45.0 (4.5)	25.0 (5.0)	7.23
F9	21.3	31.3	70.4	21.4	20.0	nt	49.3 (13.0)	0	20.1
F10	50.4	50.0	70.3	50.5	80.0	nt	61.9 (2.2)	10.0	38.8
G1	24.4	10.5	25.0	37.2	16.1	33.9	14.5	25.0 (5.0)	nt
G2	24.4	10.5	30.0	25.6	16.1	37.5	5.5	25.0 (5.0)	nt
G3	21.1	40.0	23.8	44.7	3.7	nt	0	0	nt
G4	45.8	56.3	42.1	49.0	10.3	19.6	49.4	0	nt
G5	25.0	17.6	0.0	55.6	14.3	nt	61.3 (43.5)	14.5	32.7
G6	0.0	5.9	0	66.7	19.0	nt	0	18.9 (3.6)	0
G7	0.0	17.6	18.2	66.7	23.8	nt	30.1	2.3	47.4
G8	31.3	35.3	18.2	77.8	28.6	nt	68.1 (54.7)	10.9	71.9
G9	37.5	35.3	27.3	61.1	19.0	nt	0.4	26.5	0
G10	37.5	29.4	18.2	38.9	28.6	nt	0	11.2 (1.1)	58.1
H1	43.8	23.5	9.1	83.3	19.0	nt	42.0	5.0	nt
H2	50.0	17.6	27.3	66.7	14.3	nt	32.7	10.0	nt
H3	57.9	46.7	23.8	42.6	3.7	nt	37.4	15.0	nt
H4	26.3	46.7	23.8	57.4	18.5	nt	0	0	nt
H5	50.0	23.5	27.3	50.0	14.3	nt	37.2	10.0	nt
H6	50.0	17.6	9.1	66.7	14.3	nt	29.5	0	nt
H7	70.8	37.5	15.8	72.5	17.2	86.3	42.5	5.0	nt
H8	54.2	43.8	42.1	58.8	10.3	92.2	36.7	10.0	nt
I1	18.8	11.8	27.3	61.1	23.8	59.2	42.9 (3.3)	10.0	nt
I2	37.9	23.8	50.0	62.5	11.1	55.1	0	10.0	nt
I3	31.3	5.9	27.3	61.1	23.8	nt	20.0	20.0 (10.0)	0
I4	31.3	17.6	9.1	66.7	9.5	nt	23.8	5.0	0
C-1[a]	14.6	15.8	25.0	30.2	19.4	26.8	76.8 (40.2)	7.0 (2.0)	100
C-2[b]	nt	nt	nt	nt	nt	nt	80.4 (76.0)	29.9 (8.2)	nt
C-3[c]	31.3	52.9	63.6	66.7	71.4	98.0	nt	nt	nt
C-4[d]	100	nt	nt	100	nt	nt	nt	nt	nt
C-5[e]	nt	72.2	75.0	nt	100	nt	nt	nt	nt
C-6[f]	nt	94.4	87.5	nt	nt	nt	nt	nt	nt

[a] CPD; [b] Chlorsulfuron; [c] Triadimefon; [d] Carbendazim; [e] Chlorothalonil; [f] Thiram; [g] nt=not tested.

Table 2 *In vivo* fungicidal activity data of compounds at 500 μg/mL (% relative control efficacy)

Compd	*Corynespora cassiicola*	*Pseudomonas syringae* pv. *lachrymans*	*Ascochyta citrullina* Smith	*Pseudoperonospora cubensis*	*Sclerotinia sclerotiorum*
F5	27.50	62.62	43.76	64.69	77.16
F6	24.57	54.92	36.01	79.23	82.48
F7	–3.77	58.22	58.88	72.38	75.60
F8	66.21	53.57	63.00	63.41	74.17
F9	42.34	67.43	76.93	71.06	81.80
F10	37.83	76.07	73.73	71.16	78.56
G5	39.35	71.27	30.35	62.98	47.87
G6	–16.31	49.00	3.67	67.17	48.09
G7	9.27	56.37	34.70	68.28	30.74
G8	41.96	68.66	31.54	76.05	33.11
G9	31.50	40.40	57.27	71.16	69.30
G10	31.98	56.13	50.83	74.05	51.06
I3	70.58	53.95	77.62	84.52	36.06
I4	53.15	26.27	85.34	72.88	54.32
Chlorothalonil	95.16	26.88	nt	45.22	74.89
Dimethomorph	95.31	13.98	98.35	83.75	98.35
Thiophanate-methyl	78.75	54.15	100.00	68.71	83.39
Iprodione	53.52	38.49	98.35	55.92	97.84
Validamycin	78.20	–11.42	48.82	56.31	26.89
Zhongshengmycin	90.15	69.69	100.00	62.57	84.43

In Table 3 it showed that compounds **F10**, **H7** and **H8** against *Puccinia sorghi* Schw. at 200 μg/mL exhibited good fungicidal activity, with control efficacy of 90%, 98% and 60%, respectively. Especially **F10** has the same fungicidal level with Triadimefon at lower test concentrations (100 μg/mL and 50 μg/mL). The fungicidal effects of **F10**, **H7**, CK and contrasts were illustrated in Figure 1.

Table 3 *In vivo* fungicidal activity data of compounds against *Puccinia sorghi* Schw. (% control efficacy)

Compd	200 μg/mL	100 μg/mL	50 μg/mL
F10	90	50	20
H7	98	40	0
H8	60	nt	nt
Azoxystrobin	99 (20 μg/mL)	nt	nt
Triadimefon	95	70	30

Herbicidal and KARI inhibitory activity

To investigate the herbicidal activity and KARI inhibitory activity of the compounds referring to those of triazole Mannich bases we reported before during our search for novel KARI inhibitors,[16,28] herbicide Chlorsulfuron and 1,1-cyclopropanedicarboxylic acid (CPD),[24] potent inhibitor of KARI *in vitro*, were used as contrasts.

It was observed from Table 1 that most of compounds showed obvious herbicidal activity according to

Figure 1 Fungicidal effect of compounds against *Puccinia sorghi* Schw.: (a), (b) CK; (c) **F10**, 90% (200 μg/mL); (d) **H7**, 98% (200 μg/mL); (e) Triadimefon, 95% (200 μg/mL); (f) Azoxystrobin, 99% (20 μg/mL).

the rape (*Brassica campestris*) root and barnyardgrass (*Echinochloa crusgalli*) cup tests, especially in the former. Trifluoromethyl-containing compounds with substituted benzylpiperazine group **F5**—**F10** displayed significantly better herbicidal activity than methyl-containing compounds **F1**—**F4** against the dicotyledon rape. **G5** and **G8** containing both a trifluoromethyl group on triazole and a pyrimidine moiety on piperazine ring possessed 61.3% and 68.1% activity against rape, which are close to that of the contrast CPD at 100 μg/mL. While the activities of these two compounds are higher than that of CPD at 10 μg/mL. For phenylpiperazine- and pyridylpiperazine-containing compounds **H1**—**H8**, most of them showed moderate inhibitory activities for the growth of rape root, but there is no great activity difference. Several compounds were tested the *in vitro* inhibitory activity on rice KARI enzyme. From Table 1 we can see that some compounds exhibited significant KARI inhibitory activity at 200 μg/mL, such as **F6**, **F7**, **F9**, **F10**, **G5**, **G7**, **G8** and **G10** (20%—72%), and there are some relativity reflected between *in vivo* and *in vitro* herbicidal activity to a certain extent.

Insecticidal activity

It was found that compounds **F5**, **F8**, **G5**, **G6**, **G7**, **G8** and **G9** displayed death rate of 10%—20% at a test concentration of 200 μg/mL against the oriental armyworm larvae, which indicates such structure may lead to weak insecticidal activity.

Conclusions

In summary, a series of novel furan/thiophene and piperazine-containing 1,2,4-triazole Mannich bases and bis(1,2,4-triazole) Mannich bases have been conveniently synthesized *via* Mannich reaction in good yields and structurally confirmed. The preliminary bioassay showed that most compounds exhibited significant *in vitro* and *in vivo* fungicidal activity towards several test plant fungi. Especially, **F8**, **F9**, **F10**, **G5**, **H7**, **H8**, **I3** and **I4**, were comparable with the contrast commercial fungicides against different fungi during the present study and could be further structurally optimized. Meanwhile, several compounds showed good herbicidal and KARI inhibitory activity. However, compounds displayed weak insecticidal activity against oriental armyworm at 200 μg/mL. The research in this manuscript will provide useful information for the design and discovery of new agrochemicals with novel heterocyclic structures.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (No. 21372133) and "111" Project of Ministry of Education of China (No. B06005).

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(Cheng, F.)

