Novel (Bis)1,2,4-Triazole Derivatives

Synthesis and Biological Activity of Novel Dimethylpyrazole and Piperazine -containing (Bis)1,2,4-Triazole Derivatives

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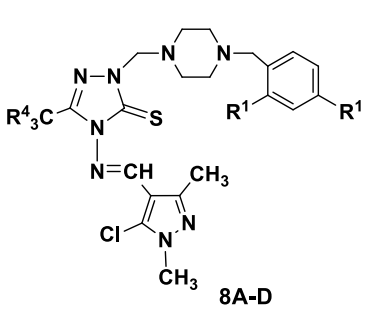
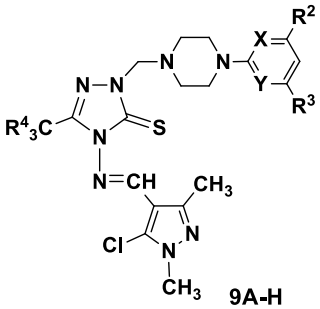
A series of novel dimethylpyrazole and piperazine -containing (bis)1,2,4-triazole derivatives have been conveniently synthesized via Mannich reaction in good yields. Their structures were identified by IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. The preliminary bioassays showed that among 14 new compounds, the trifluoromethyl-containing compounds exhibited superior activity than the methyl-containing ones; some of the compounds displayed significant in vitro and in vivo fungicidal activity against several plant fungi and were comparable with the control

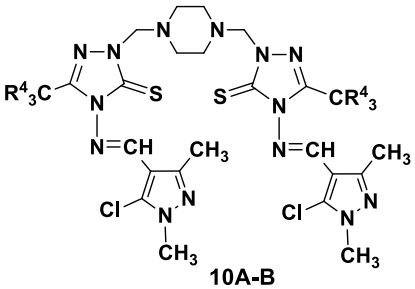
Triadimefon; several compounds exhibited certain herbicidal activity against Brassica campestris; several compounds possessed favorable KARI inhibitory activity, especially **8D** could be a promising KARI inhibitor for further study. The research results will provide useful information for the design and discovery of new agrochemicals with novel heterocyclic Mannich base structures containing piperazine moiety.



8A-D



9A-H



10A-B

Keywords

1,2,4-triazole, pyrazole, Mannich base, piperazine, biological activity

INTRODUCTION

As one of the nitrogen-containing heterocycles, pyrazole occupies an important position in the field of medicinal and pesticidal chemistry due to a variety of pharmacological and biological activity of its derivatives -- on one hand, pyrazole and its derivatives possess significant antibacterial, antiviral, anticoagulant, antimicrobial, anti-inflammatory and anticancer properties[1-3]; on the other hand, they also show versatile pesticidal activity and are used as insecticides, herbicides, fungicides and plant growth regulators. [4-9] For example, Fipronil, an insecticide that is used to control lepidopteran and coleopteran insects, is a type of pyrazole derivative[10]; Pyraclostrobin, a pyrazole-containing strobilurin fungicide, possesses a broad fungicidal spectrum with preventive, curative, translaminar and locosystemic properties[11]. Piperazine is another important nitrogen-containing heterocycle which has various drug-related properties and have attracted extensive investigation for small molecule drug discovery through decades. It has been found that *N*-substituted piperazine compounds have a wide range of biological activities, such as antimicrobial [12], anticancer [13], tuberculostatic [14], antifungal [15,16], herbicidal and insecticidal activities [17]. Comparatively, there are much less literatures about the the application of piperazine-containing compounds in agrochemical area than in medicinal area.

In addition, there are lots of heterocyclic Mannich bases been reported, however most of the researches focused on their pharmacological activities, such as antimicrobial [18], anticancer [19],

antitumor [20] and antituberculous [21] activities. Likewise, it is also necessary to carry out extensive study on the various agrochemical activities of heterocyclic Mannich bases, which might be a promising topic to produce more novel agrochemical candidates.

In our previous work, some interesting 1,2,4-triazole Mannich bases containing trifluoromethyl, piperazine and substituted phenyl groups were synthesized successfully [22,23,16] and found to exhibit 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 branched chain amino acids leucine, isoleucine and valine [24-26]. Particularly, those kinds of compounds possessed significant fungicidal activities against *Phytophthora infestans* (Mont.) de Bary, *G. zeae* Petch and other fungi, which provided an important reference for us to make some further structural modifications. The preliminary SAR studies illustrated the necessity for further optimization based on the general structure of this type of compounds, especially for the phenyl group and the substituent on the piperazine part [22,23]. Encouraged by this, some 1,2,4-triazole Mannich bases with a phenylpyrazolymethyleneamino group and a (substituted)piperazinomethyl group in the triazole 4-position and 1-position respectively were recently synthesized in our lab; they were also found to possess favorable fungicidal activities against some plant fungi [6]. In such type of compounds, there are phenyl, trifluoromethyl/methyl and chlorine moieties in the pyrazole ring, which mainly are electron-withdrawing groups. If more electron-donating groups could be

introduced into the pyrazole ring of this type of structure, e. g. two methyl groups, the corresponding intermediate probably would undergo the similar reaction as that of the phenylprazole-intermediate[6] in different reactivity, to generate some novel 1,2,4-triazole Mannich bases with good biological activities. By taking into account all the facts mentioned above, a series of novel methyl/trifluoromethyl-containing triazole Mannich bases with dimethylpyrazole and substituted piperzaine moieties were synthesized. Meanwhile, in piperazine ring there are two secondary amine structures (-NH-) that could both be used for undertaking Mannich reaction to generate some new structures, with this in mind, novel bis-1,2,4-triazole derivatives containing dimethylpyrazole and piperazine moieties were also synthesized. The fungicidal, herbicidal and KARI inhibitory activities of these new (bis)1,2,4-triazole derivatives were investigated in this paper.

RESULTS AND DISCUSSION

Synthesis and Spectra Characterization

The synthetic procedures for piperazine intermediates, triazole intermediates **7** and the title compounds **8**, **9** and **10** are shown in **SCHEME 1**, **SCHEME 2** and **SCHEME 3**. The novel triazole thione intermediates (**7A-B**) were prepared using an acetic acid catalysis method [6] *via* the condensation of thione intermediate **6** and 5-chloro-1,3-dimethyl-1*H*-pyrazole-4-carbaldehyde (**5**) in ethanol with ~ 80% yield. Foks *et al.* reported the similar Mannich reaction from

heterocycle-thiol intermediate using methanol or dioxane as solvent under reflux condition (1 h) [14], by contrast, our synthetic procedure for the title 1,2,4-triazole Mannich bases was to carry out the reaction in ethanol at room temperature. Using this method, **8** and **9** can be obtained in satisfying yields within 2-3 h, which results are similar with those of the Mannich reaction based on the phenylpyrazole-intermediats in our recent work [6]. Under conditions of excess formaldehyde and 2:1 mole ratio of **7** and piperazine, novel bis-1,2,4-triazole derivatives **10** can also be successfully synthesized at room temperature in high yield (82%-88%). It was found that this type of Mannich reaction based on the pyrazolymethyleneamino-triazole thione intermediates can undergo smoothly with high yield and under mild conditions in short time.

Intermediates **7** and the title compounds **8, 9** and **10** were identified by melting point, IR, ^{1}H NMR and ^{13}C NMR spectra. The measured elemental analyses were also consistent with the corresponding calculated values. In the IR spectra of the title compounds, the characteristic stretching vibration absorption peaks of C = S and C = N appeared at 1155-1176 cm^{-1} and 1592-1612 cm^{-1} respectively. The stretching vibrations *v* (C-F) of the trifluoromethyl-containing compounds were observed at 1186-1209 cm^{-1}. For intermediates **7**, absorption peaks of N-H and C = N appeared at 3452 cm^{-1} and 1638 cm^{-1}, respectively. In ^{1}H NMR, the --CH = N-- proton appeared at δ 9.77-10.29 as a singlet in **8**, **9** and **10**. The chemical shift at δ 13.71-14.83 (singlet) in **7** can be ascribed to the N-H active proton owing to the thione structure (--NH--C(= S)--). The piperazine ring proton (CH_2) in **8** and **9** appeared at δ 2.70-3.89 and δ 2.35-2.88 as two broad

peaks or multiplets, respectively; however, due to the symmetrical structure of bis-1,2,4-triazole derivatives **10**, they were observed as a singlet at δ ~ 2.85. In the ^{13}C NMR spectra of **8**, **9** and **10**, the typical carbon resonance at δ 160.3-167.7 was indicative of a thiocarbonyl group (C = S). The carbon signals of --CH = N-- group were observed at δ 143.2-150.0. The signals of C_3 in the triazole ring and carbon adjacent to it were observed at δ ~ 154.0 and δ ~ 14.9 (CH_3) as a singlet respectively, while at δ ~138.0 ($J \approx 40.4$ Hz) and δ ~116.0 (CF_3, $J \approx 273.0$ Hz) as quartet or multiplet, respectively in the trifluoromethyl-containing compounds, which is due to the "F" splitting of the latter.

In addition, the carbon signal at δ 162.1-169.6 (C = S) for the compounds **5**, **6** and **7** indicates these intermediates all existed in the thione tautomeric form [27]. It was worthy of note that the double bond in the imine part of these compounds (-CH = N-) should be all in *E*-configuration, which can be supported by the single crystal and calculation work (optimal conformation) of such a similar parent structure reported in our previous work [22].

Fungicidal activity

The *in vitro* fungicidal activity data of the title 1,2,4-triazole derivatives **8A-8D**, **9A-9H** and bis-1,2,4-triazole derivatives **10A-10B** for the inhibition of mycelial growth in six test fungi are listed in **Table S 1** (Supplemental Materials). The commercial fungicides Triadimefon, Carbendazim and Chlorothalonil were used as controls. It was found that at the test

concentration of 50 μg/mL, most of the compounds showed significant fungicidal activity against *Alternaria solani* Sorauer, *Gibberella sanbinetti*, *Physalospora piricola* and *Rhizoctonia cerealis* while some of these results were comparable with those of the control Triadimefon. For examples, **9B**, **9G**, **9H**, **10A** and **10B** possessed inhibition rates of 62% ~ 75% against *Physalospora piricola*; **9G** and **9H** held inhibition rates of 94% ~ 96% against *Rhizoctonia cerealis*. In addition, compound **10B** whose activity was 75.0% and 65.5% against *Cercospora arachidicola* and *Gibberella sanbinetti*, respectively, was more effective than Triadimefon.

According to the *in vitro* fungicidal activity data, several trifluoromethyl-containing compounds were chosen to be tested for *in vivo* fungicidal activity against *Puccinia sorghi* Schw.. From **Table S 2** (Supplemental Materials), it was shown that at 200 μg/mL concentration, compounds **8D**, **9E**, **9F**, **9G** and **9H** held good activity against *Puccinia sorghi* Schw. with control efficacy of 50% ~ 85%. At the test concentration of 100 μg/mL, **8D** and **9F** can still exhibit control efficacy of 20% and 30%, respectively.

As a whole, these dimethylpyrazole and piperazine -containing (bis)1,2,4-triazole derivatives displayed significant fungicidal activity against various plant fungi, while the CF_3 group at 3-position of 1,2,4-triazole ring (R^4 = F) made an apparent influence on their fungicidal activity, which could be well reflected particularly in the results of *Rhizoctonia cerealis* test (**Table S 1**). By analyzing the SAR based on the fungicidal data against *Rhizoctonia cerealis*, it was found that in most cases trifluoromethyl- containing compounds exhibit higher activity than methyl-

containing ones, such as **8D** vs. **8B**, **9H** vs. **9D**, **10B** vs. **10A**. The activity of compounds **8A-8D**, when R^4 is fixed as H or F atom, indicated the sequence of fungicidal activity is 2,4-dichlorobenzyl > benzyl in the substituted benzyl moiety in piperazine ring; in the case of compounds **9A-9H**, when R^4 is fixed as F atom, it showed the activity sequence of 4,6-dimethylpyrimidyl ≈ 4-methylpyrimidyl > pyridyl ≈ phenyl in the piperazine ring.

Herbicidal activity

As shown in **Table S 3** (Supplemental Materials), some of the title compounds exhibited certain herbicidal activity in rape (*Brassica campestris*) root and barnyardgrass (*Echinochloa crusgalli*) cup tests at 100 μg/mL concentration. For examples, **8C**, **8D** and **9G** against *Brassica campestris* possessed growth inhibition rates of 59.9%, 38.1% and 33.6%, respectively. Against *Echinochloa crusgalli*, however, all the compounds showed poor herbicidal activity. In general, trifluoromethyl-containing compounds displayed better herbicidal activity than methyl-containing ones.

KARI inhibitory activity

From **Table S 4** (Supplemental Materials), it was observed that most of the title compounds exhibited apparent KARI inhibitory activity at the concentration of 200 μg/mL, such as **8D**, **9A**, **9D**, **9F** and **10B** (activity 34-88%). It was worthy to note that compound **8D** whose *in vitro*

inhibitory rate towards rice KARI is 87.5%, could be a promising KARI inhibitor for further investigation in the future, although it is less active than the known potent inhibitor CPD [24].

EXPERIMENTAL

Melting points were determined using an X-4 binocular microscope apparatus and uncorrected. Infrared spectra were recorded on a Bruker Tensor 27 spectrophotometer as KBr tablets. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AV-400 instrument (400 MHz) using tetramethylsilane as an internal standard and DMSO-d_6 or $CDCl_3$ as solvent. Elemental analyses were performed on a Vario EL CUBE elemental analyzer. KARI inhibitory activity test was carried out on a VARIAN CARY 100 Bio Uv/Visibile spectrophotometer. 4-(Substituted)benzylpiperazine **1A** and **1B** were prepared according to the literature [28,23]. 4-(4,6-Disubstituted pyrimidin-2-yl)piperazine **3A** and **3B** were synthesized referring to the literature method [22]. 4-Phenylpiperazine (**3C**) and 4-(pyridine-2-yl)piperazine (**3D**) were purchased from Aladin and Alfa Aesar reagent companies. 4-Amino-3-methyl/trifluoromethyl-1*H*-1,2,4-triazole-5(4*H*)-thione were prepared according to the literature [29]. 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. The Supplemental Materials contains sample ^{1}H and ^{13}C NMR and IR spectra of products 7-10 (Figures S 1 -- S 54)

Preparation of 5-Chloro-1,3-dimethyl-1*H*-pyrazole-4-carbaldehyde 5

2,5-Dimethyl-2,4-dihydro-3*H*-pyrazol-3-one **4** was prepared by refluxing ethyl acetoacetate and methylhydrazine in ethanol [5]. Intermediate pyrazole carbaldehyde **5** was prepared according to reference [30] and the method was improved. To a violently stirred cold solution of DMF (23 mL) was added dropwise phosphorus oxychloride (64 mL) at 0-10 °C. The mixture was stirred at this temperature for 30 min then compound **4** (0.1 mol) was added in portions. The mixture was heated to 80-90 °C and stirred for 4 h. After being cooled to room temperature, the mixture was poured into ice-water (350 mL) and stirred for 10 min, then extracted with ethyl acetate (3 × 25 mL). The organic layer was washed with saturated $NaHCO_3$ solution (2 × 15 mL) and brine (15 mL) successively, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, the residue was recrystallized from ethyl acetate/petroleum ether (60-90°C) to give pyrazole carbaldehyde **5** as light yellow crystals, mp 77-78 °C, yield 81.5% (Lit. [30] mp 78-79 °C).

General Synthetic Procedure for Novel Triazole Thione Intermediates 7

Compound **6A** or **6B** (10 mmol), pyrazole carbaldehyde **5** (10.5 mmol) and acetic acid (10 drops) were mixed in absolute ethanol (25 mL). After having been stirred and refluxed for 4-5 h, the reaction mixture was cooled to room temperature. The resulting solid was filtered off and recrystallized from ethanol to give thione intermediate **7**.

(*E*)-4-((5-Chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methyleneamino)-3-methyl-1*H*-1,2,4-triazole-5(4*H*)-thione (7A)

Colorless crystals, yield 81.3%, mp 239-241 °C; IR (ν_{max}, cm^{-1}): 3452 (N-H), 1638(C = N), 1159(C = S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.71 (s, 1H, NH), 9.97 (s, 1H, CH = N), 3.81 (s, 3H, Pyrazole-CH_3), 2.39 (s, 3H, Triazole-CH_3), 2.32 (s, 3H, Pyrazole-CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ 162.1 (s, 1C, C = S), 155.9 (s, 1C, Triazole-C), 149.5 (s, 1C, Pyrazole-C), 149.2 (s, 1C, N = CH), 131.5 (s, 1C, Pyrazole-C), 111.0 (s, 1C, Pyrazole-C), 37.3 (s, 1C, Pyrazole-CH_3), 15.5 (s, 1C, Triazole-CH_3), 11.8 (s, 1C, Pyrazole-CH_3); Anal. Calcd. (%) for $C_9H_{11}ClN_6S$: C 39.93, H 4.10, N 31.04. Found: C 39.67, H 4.26, N 30.87.

(*E*)-4-((5-Chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methyleneamino)-3-(trifluoromethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (7B)

Colorless crystals, yield 78.8%, mp 214-216 °C; IR (ν_{max}, cm^{-1}): 3452 (N-H), 1639(C = N). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.84 (s, 1H, NH), 9.95 (s, 1H, CH = N), 3.82 (s, 3H, Pyrazole-CH_3), 2.36 (s, 3H, Pyrazole-CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ 163.7 (s, 1C, C = S), 156.9 (s, 1C, Pyrazole-C), 156.8 (s, 1C, Pyrazole-C), 148.3 (s, 1C, N = CH), 138.6 (d, J_{C-F} = 40.4 Hz, 1C, Triazole-C_3), 131.2 (s, 1C, Pyrazole-C), 114.1 (q, J_{C-F} = 273.7 Hz, 1C, CF_3), 36.2 (s, 1C, Pyrazole-CH_3), 14.0 (s, 1C, Pyrazole-CH_3); Anal. Calcd. (%) for $C_9H_8ClF_3N_6S$: C 33.29, H 2.48, N 25.88. Found: C 33.12, H 2.49, N 25.74.

General Synthetic Procedure for Novel 1,2,4-Triazole Derivatives 8

A solution of 4-(substituted)benzylpiperazine **1** (1 mmol) in ethanol (2 mL) was added dropwise to a mixture of thione intermediate **7** (1 mmol) and 37% formalin (1.5 mmol) in ethanol (15 mL). Then the reaction mixture was stirred for 2-3 h at room temperature. The resulting precipitate (in some cases, there is no solid precipitated out after reaction completed, the mixture can be placed in a fridge or concentrated to accelerate precipitating) was filtered off and recrystallized from ethanol to give novel 1,2,4-triazole derivative **8(A-D)**.

(*E*)-1-((4-Benzylpiperazin-1-yl)methyl)-4-((5-chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methylene amino)-3-methyl-1*H*-1,2,4-triazole-5(4*H*)-thione (8A)

White crystals, yield 62.4%, mp 152-154 °C; IR (ν_{max}, cm^{-1}): 2945, 2825(C-H), 1610(C = N), 1532, 1494, 1452(Ar), 1168(C = S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.83 (s, 1H, CH = N), 7.31-7.22 (m, 5H, Ph-H), 5.01 (s, 2H, NCH_2N), 3.81 (s, 3H, Pyrazole-CH_3), 3.42 (s, 2H, $PhCH_2$), 2.70 (bs, 4H, Piperazine-H), 2.40 (s, 3H, Pyrazole-CH_3), 2.35 (bs, 7H, Piperazine-H + Triazole-CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 162.5 (s, 1C, C = S), 153.9 (s, 1C, Triazole-C), 149.3 (s, 1C, Pyrazole-C), 147.5 (s, 1C, N = CH), 138.0 (1C, Ph-C), 131.3 (s, 1C, Pyrazole-C), 129.2 (s, 2C, Ph-C), 128.2 (s, 2C, Ph-C), 127.0 (s, 1C, Ph-C), 110.7 (s, 1C, Pyrazole-C), 68.6 (s, 1C, CH_2), 63.0 (s, 1C, CH_2), 53.0 (s, 2C, Piperazine-C), 50.5 (s, 2C, Piperazine-C), 36.2 (s, 1C, Pyrazole-CH_3), 14.8 (s, 1C, Triazole-CH_3), 11.1 (s, 1C, Pyrazole-CH_3); Anal. Calcd. (%) for $C_{21}H_{27}ClN_8S$: C 54.95, H 5.93, N 24.41. Found: C 54.78, H 5.99, N 24.32.

(*E*)-4-((5-Chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methyleneamino)-1-((4-(2,4-dichlorobenzyl)piperazin-1-yl)methyl)-3-methyl-1*H*-1,2,4-triazole-5(4*H*)-thione (8B)

White solid, yield 65.5%, mp 157-159 °C; IR (ν_{max}, cm^{-1}): 2929, 2840(C-H), 1606(C = N), 1528, 1474, 1453(Ar), 1166(C = S). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.29 (s, 1H, CH = N), 7.38-7.34 (m, 2H, Ph-H), 7.19-7.18 (m, 1H, Ph-H), 5.12 (s, 2H, NCH_2N), 3.84 (s, 3H, Pyrazole-CH_3), 3.55 (s, 2H, $ArCH_2$), 2.86 (bs, 4H, Piperazine-H), 2.53 (bs, 4H, Piperazine-H), 2.48(s, 3H, Pyrazole-CH_3), 2.41 (s, 3H, Triazole-CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 162.5 (s, 1C, C = S), 154.0 (s, 1C, Triazole-C), 149.3 (s, 1C, Pyrazole-C), 147.6 (s, 1C, N = CH), 134.8, 134.6, 133.0 (3C, Ph-C), 131.4 (s, 1C, Pyrazole-C), 131.3 (s, 1C, Ph-C), 129.1 (s, 1C, Ph-C), 126.8 (s, 1C, Ph-C), 110.7 (s, 1C, Pyrazole-C), 68.6 (s, 1C, CH_2), 58.6 (s, 1C, CH_2), 53.0 (s, 2C, Piperazine-C), 50.5 (s, 2C, Piperazine-C), 36.2 (s, 1C, Pyrazole-CH_3), 14.9 (s, 1C, Triazole-CH_3), 11.1 (s, 1C, Pyrazole-CH_3); Anal. Calcd. (%) for $C_{21}H_{25}Cl_3N_8S$: C 47.78, H 4.77, N 21.23. Found: C 47.87, H 4.57, N 21.14.

(*E*)-1-((4-Benzylpiperazin-1-yl)methyl)-4-((5-chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methyleneamino)-3-(trifluoromethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (8C)

White solid, yield 57.2%, mp 112-114 °C; IR (ν_{max}, cm^{-1}): 2935, 2813(C-H), 1606(C = N), 1533, 1492, 1455(Ar), 1313, 1190(C-F), 1157(C = S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.80 (s, 1H, CH = N), 7.32-7.22 (m, 5H, Ph-H), 5.15 (s, 2H, NCH_2N), 3.81 (s, 3H, Pyrazole-CH_3), 3.44 (s, 2H, $PhCH_2$), 2.75 (bs, 4H, Piperazine-H), 2.36 (bs, 7H, Piperazine-H + Pyrazole-CH_3). ^{13}C NMR (101 MHz,

$CDCl_3$): δ 165.0 (s, 1C, C = S), 155.4 (s, 1C, Pyrazole-C), 149.9 (s, 1C, N = CH), 138.5 (s, 1C, Triazole-C_3), 137.9 (s, 1C, Ph-C), 132.2 (s, 1C, Pyrazole-C), 129.2 (s, 2C, Ph-C), 128.3 (s, 2C, Ph-C), 127.1 (s, 1C, Ph-C), 118.8 (q, J_{C-F} = 273.7 Hz, 1C, CF_3), 110.2 (s, 1C, Pyrazole-C), 69.9 (s, 1C, CH_2), 63.1 (s, 1C, CH_2), 52.9 (s, 2C, Piperazine-C), 50.4 (s, 2C, Piperazine-C), 36.2 (s, 1C, Pyrazole-CH_3), 14.7 (s, 1C, Pyrazole-CH_3); Anal. Calcd. (%) for $C_{21}H_{24}ClF_3N_8S$: C 49.17, H 4.72, N 21.84. Found: C 49.34, H 4.90, N 22.01.

(*E*)-4-((5-Chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methyleneamino)-1-((4-(2,4-dichlorobenzyl)piperazin-1-yl)methyl)-3-(trifluoromethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (8D)

White solid, yield 66.1%, mp 139-141 °C; IR (ν_{max}, cm^{-1}): 2936, 2814(C-H), 1604(C = N), 1531, 1473, 1457(Ar), 1318, 1198(C-F), 1167(C = S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.80 (s, 1H, CH = N), 7.56 (s, 1H, Ph-H), 7.46-7.37 (m, 2H, Ph-H), 5.16 (s, 2H, NCH_2N), 3.81 (s, 3H, Pyrazole-CH_3), 3.51 (s, 2H, $ArCH_2$), 2.76 (bs, 4H, Piperazine-H), 2.43 (bs, 4H, Piperazine-H), 2.36 (s, 3H, Pyrazole-CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 165.0 (s, 1C, C = S), 155.4 (s, 1C, Pyrazole-C), 149.9 (s, 1C, N = CH), 138.8 (d, J = 40.4 Hz, 1C, Triazole-C_3), 134.8 (s, 1C, Ph-C), 134.4 (s, 1C, Ph-C), 133.1 (s, 1C, Ph-C), 132.2 (s, 1C, Pyrazole-C), 131.4 (s, 1C, Ph-C), 129.2 (s, 1C, Ph-C), 126.9 (s, 1C, Ph-C), 117.0 (q, J_{C-F} = 273.7 Hz, 1C, CF_3), 110.2 (s, 1C, Pyrazole-C), 69.9 (s, 1C, CH_2), 58.6 (s, 1C, CH_2), 53.0 (s, 2C, Piperazine-C), 50.4 (s, 2C, Piperazine-C), 36.2 (s, 1C, Pyrazole-CH_3), 14.7 (s, 1C, Pyrazole-CH_3); Anal. Calcd. (%) for $C_{21}H_{22}Cl_3F_3N_8S$: C 43.35, H 3.81, N 19.26. Found: C 43.53, H 3.62, N 19.09.

General Synthetic Procedure for Novel 1,2,4-Triazole Derivatives 9

It was similar with that of **8**, using 4-phenylpiperazine **3C**, or 4-(pyridin-2-yl)piperazine **3D**, or 4-(4,6-disubstituted-pyrimidin-2-yl)piperazine **3(A-B)** as amine material to give corresponding 1,2,4-triazole derivative **9(A-H)**.

(*E*)-4-((5-Chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methyleneamino)-3-methyl-1-((4-phenylpiperazin-1-yl)methyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (9A)

White solid, yield 71.6%, mp 190-192 °C; IR (ν_{max}, cm^{-1}): 2944, 2826(C-H), 1598(C = N), 1534, 1502, 1453(Ar), 1162(C = S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.83 (s, 1H, CH = N), 7.18 (bs, 2H, Ph-H), 6.91 (bs, 2H, Ph-H), 6.76 (bs, 1H, Ph-H), 5.10 (s, 2H, NCH_2N), 3.81 (s, 3H, Pyrazole-CH_3), 3.12 (bs, 4H, Piperazine-H), 2.85 (s, 3H, Pyrazole-CH_3), 2.40 (bs, 4H, Piperazine-H), 2.35 (s, 3H, Triazole-CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 162.6 (s, 1C, C = S), 154.1 (s, 1C, Triazole-C), 151.3 (s, 1C, Ph-C), 149.3 (s, 1C, Pyrazole-C), 147.7 (s, 1C, N = CH), 131.4 (s, 1C, Pyrazole-C), 129.1 (s, 2C, Ph-C), 119.9 (s, 1C, Ph-C), 116.4 (s, 2C, Ph-C), 110.7 (s, 1C, Pyrazole-C), 68.6 (s, 1C, CH_2), 50.5 (s, 2C, Piperazine-C), 49.3 (s, 2C, Piperazine-C), 36.2 (s, 1C, Pyrazole-CH_3), 14.9 (s, 1C, Triazole-CH_3), 11.1 (s, 1C, Pyrazole-CH_3); Anal. Calcd. (%) for $C_{20}H_{25}ClN_8S$: C 53.98, H 5.66, N 25.18. Found: C 53.68, H 5.62, N 24.92.

(*E*)-4-((5-Chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methyleneamino)-3-methyl-1-((4-(pyridin-2-yl)piperazin-1-yl)methyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (9B)

White solid, yield 76.2%, mp 160-162 °C; IR (ν_{max}, cm^{-1}): 2944, 2842(C-H), 1592(C = N), 1534, 1478, 1453(Ar), 1161(C = S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.81 (s, 1H, CH = N), 8.07 (d, J = 3.6 Hz, 1H, Pyridine-H), 7.49 (t, J = 7.2 Hz, 1H, Pyridine-H), 6.80 (d, J = 8.8 Hz, 1H, Pyridine-H), 6.61-6.58 (m, 1H, Pyridine-H), 5.09 (s, 2H, NCH_2N), 3.80 (s, 3H, Pyrazole-CH_3), 3.48 (bs, 4H, Piperazine-H), 2.78 (bs, 4H, Piperazine-H), 2.38 (s, 3H, Pyrazole-CH_3), 2.33 (s, 3H, Triazole-CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 162.6 (s, 1C, C = S), 159.3 (s, 1C, Pyridine-C) 154.1 (s, 1C, Triazole-C), 149.3 (s, 1C, Pyrazole-C), 148.0 (s, 1C, Pyridine-C), 147.7 (s, 1C, N = CH), 137.4 (s, 1C, Pyridine-C), 131.4 (s, 1C, Pyrazole-C), 113.2 (s, 1C, Pyridine-C), 110.7 (s, 1C, Pyrazole-C), 107.1 (s, 1C, Pyridine-C), 68.7 (s, 1C, CH_2), 50.4 (s, 2C, Piperazine-C), 45.2 (s, 2C, Piperazine-C), 36.2 (s, 1C, Pyrazole-CH_3), 14.8 (s, 1C, Triazole-CH_3), 11.1 (s, 1C, Pyrazole-CH_3); Anal. Calcd. (%) for $C_{19}H_{24}ClN_9S$: C 51.17, H 5.42, N 28.27. Found: C 50.98, H 5.39, N 27.99.

(*E*)-4-((5-Chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methyleneamino)-3-methyl-1-((4-(4-methylpyrimidin-2-yl)piperazin-1-yl)methyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (9C)

White solid, yield 74.3%, mp 151-153 °C; IR (ν_{max}, cm^{-1}): 2948, 2846(C-H), 1612(C = N), 1573, 1562, 1487, 1448(Ar), 1159(C = S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.81 (s, 1H, CH = N), 8.18 (d, J = 4.4 Hz, 1H, Pyrimidine-H), 6.48 (d, J = 4.8 Hz, 1H, Pyrimidine-H), 5.09 (s, 2H, NCH_2N), 3.81 (s, 3H, Pyrazole-CH_3), 3.74 (bs, 4H, Piperazine-H), 2.74 (bs, 4H, Piperazine-H), 2.39 (s, 3H, Pyrazole-CH_3), 2.32 (s, 3H, Triazole-CH_3), 2.25 (s, 3H, Pyrimidine-CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.6 (s, 1C, C = S), 162.6 (s, 1C, Pyrimidine-C_4), 161.5 (s, 1C, Pyrimidine-C_2), 157.9 (s,

1C, Pyrimidine-C_6), 154.1 (s, 1C, Triazole-C), 149.3 (s, 1C, Pyrazole-C), 147.7 (s, 1C, N = CH), 131.4 (s, 1C, Pyrazole-C), 110.7 (s, 1C, Pyrazole-C), 109.4 (s, 1C, Pyrimidine-C_5), 68.9 (s, 1C, CH_2), 50.6 (s, 2C, Piperazine-C), 43.5 (s, 2C, Piperazine-C), 36.2 (s, 1C, Pyrazole-CH_3), 24.3 (s, 1C, Pyrimidine-CH_3), 14.8 (s, 1C, Triazole-CH_3), 11.1 (s, 1C, Pyrazole-CH_3); Anal. Calcd. (%) for $C_{19}H_{25}ClN_{10}S$: C 49.50, H 5.47, N 30.38. Found: C 49.44, H 5.42, N 30.09.

(*E*)-4-((5-Chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methyleneamino)-1-((4-(4,6-dimethylpyrimidin-2-yl)piperazin-1-yl)methyl)-3-methyl-1*H*-1,2,4-triazole-5(4*H*)-thione (9D)

White solid, yield 80.4%, mp 189-191 °C; IR (ν_{max}, cm^{-1}): 2944, 2826(C-H), 1610(C = N), 1570, 1543, 1477, 1450(Ar), 1170(C = S). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.26 (s, 1H, CH = N), 6.23 (s, 1H, Pyrimidine-H), 5.17 (s, 2H, NCH_2N), 3.89-3.86 (m, 4H, Piperazine-H), 3.83 (s, 3H, Pyrazole-CH_3), 2.88-2.85 (m, 4H, Piperazine-H), 2.47 (s, 3H, Pyrazole-CH_3), 2.38 (s, 3H, Triazole-CH_3), 2.25 (s, 6H, Pyrimidine-CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.0 (s, 1C, C = S), 162.6 (s, 2C, Pyrimidine-$C_{4,6}$), 161.6 (s, 1C, Pyrimidine-C_2), 154.1 (s, 1C, Triazole-C), 149.3 (s, 1C, Pyrazole-C), 147.6 (s, 1C, N = CH), 131.4 (s, 1C, Pyrazole-C), 110.7 (s, 1C, Pyrazole-C), 108.8 (s, 2C, Pyrimidine-C_5), 68.9 (s, 1C, CH_2), 50.7 (s, 2C, Piperazine-C), 43.5 (s, 2C, Piperazine-C), 36.2 (s, 1C, Pyrazole-CH_3), 24.1 (s, 2C, Pyrimidine-CH_3), 14.8 (s, 1C, Triazole-CH_3), 11.1 (s, 1C, Pyrazole-CH_3); Anal. Cacld. (%) for $C_{20}H_{27}ClN_{10}S$: C 50.57, H 5.73, N 29.49. Found: C 50.63, H 5.65, N 29.25.

(*E*)-4-((5-Chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methyleneamino)-1-((4-phenylpiperazin-1-yl) methyl)-3-(trifluoromethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (9E)

White crystals, yield 69.4%, mp 140-142 °C; IR (ν_{max}, cm^{-1}): 2952, 2828(C-H), 1612(C = N), 1529, 1495, 1453(Ar), 1318, 1187 (C-F), 1164(C = S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.82 (s, 1H, CH = N), 7.19 (t, J = 7.1 Hz, 2H, Ph-H), 6.91 (d, J = 7.7 Hz, 2H, Ph-H), 6.76 (t, J = 7.0 Hz, 1H, Ph-H), 5.23 (s, 2H, NCH_2N), 3.81 (s, 3H, Pyrazole-CH_3), 3.13 (bs, 4H, Piperazine-H), 2.90 (bs, 4H, Piperazine-H), 2.36 (s, 3H, Pyrazole-CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 165.1 (s, 1C, C = S), 155.5 (s, 1C, Pyrazole-C), 151.3 (s, 1C, Ph-C), 150.0 (s, 1C, N = CH), 138.8 (d, J = 40.2, 1C, Triazole-C_3), 132.2 (s, 1C, Pyrazole-C), 129.1 (s, 2C, Ph-C), 120.1 (s, 1C, Ph-C), 116.5 (s, 2C, Ph-C), 114.1 (q, $J_{C\text{-}F}$ = 273.7 Hz, 1C, CF_3), 110.2 (s, 1C, Pyrazole-C), 69.8 (s, 1C, CH_2), 50.4 (s, 2C, Piperazine-C), 49.4 (s, 2C, Piperazine-C), 36.2 (s, 1C, Pyrazole-CH_3), 14.7 (s, 1C, Pyrazole-CH_3); Anal. Calcd. (%) for $C_{20}H_{22}ClF_3N_8S$: C 48.14, H 4.44, N 22.46. Found: C 48.11, H 4.46, N 22.21.

(*E*)-4-((5-Chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methyleneamino)-1-((4-(pyridin-2-yl)piperazin-1-yl)methyl)-3-(trifluoromethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (9F)

White crystals, yield 75.3%, mp 127-129 °C; IR (ν_{max}, cm^{-1}): 2953, 2829(C-H), 1612(C = N), 1592, 1530, 1478(Ar), 1317, 1209(C-F), 1163(C = S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.79 (s, 1H, CH = N), 8.08-8.06 (m, 1H, Pyridine-H), 7.50 (t, J = 8.0 Hz, 1H, Pyridine-H), 6.81 (d, J = 8.8 Hz, 1H, Pyridine-H), 6.60 (dd, J = 6.9, 5.0 Hz, 1H, Pyridine-H), 5.23 (s, 2H, NCH_2N), 3.81 (d, J = 8.4 Hz, 3H,

Pyrazole-CH_3), 3.51-3.48 (m, 4H, Piperazine-H), 2.85-2.82 (m, 4H, Piperazine-H), 2.35 (s, 3H, Pyrazole-CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 160.3 (s, 1C, C = S), 154.5 (s, 1C, Pyrazole-C), 150.7 (s, 1C, Pyridine-C), 145.2 (s, 1C, Pyridine-C), 143.2 (s, 1C, N = CH), 134.1 (d, J = 42.2 Hz, 1C, Triazole-C_3), 132.7 (s, 1C, Pyrazole-C), 127.4 (s, 1C, Pyridine-C), 111.1 (q, $J_{C\text{-}F}$ = 272.7 Hz, 1C, CF_3), 108.6 (s, 1C, Pyrazole-C), 105.4 (s, 1C, Pyridine-C), 102.4 (s, 1C, Pyridine-C), 65.2 (s, 1C, CH_2), 45.5 (s, 2C, Piperazine-C), 40.4 (s, 2C, Piperazine-C), 31.5 (s, 1C, Pyrazole-CH_3), 10.0 (s, 1C, Pyrazole-CH_3); Anal. Calcd. (%) for $C_{19}H_{21}ClF_3N_9S$: C 45.65, H 4.23, N 25.21. Found: C 45.66, H 4.35, N 24.99.

(*E*)-4-((5-Chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methyleneamino)-1-((4-(4-methylpyrimidin-2-yl)piperazin-1-yl)methyl)-3-(trifluoromethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (9G)

White solid, yield 77.3%, mp 114-116 °C; IR (ν_{max}, cm^{-1}): 2945, 2836(C-H), 1604(C = N), 1578, 1531, 1487, 1450(Ar), 1318, 1206(C-F), 1158(C = S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.79 (s, 1H, CH = N), 8.16 (d, J = 4.2 Hz, 1H, Pyrimidine-H), 6.46 (d, J = 4.2 Hz, 1H, Pyrimidine-H), 5.22 (s, 2H, NCH_2N), 3.80 (s, 3H, Pyrazole-CH_3), 3.75 (bs, 4H, Piperazine-H), 2.78 (bs, 4H, Piperazine-H), 2.35 (s, 3H, Pyrazole-CH_3), 2.24 (s, 3H, Pyrimidine-CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.7 (s, 1C, C = S), 165.0 (s, 1C, Pyrimidine-C_4), 161.5 (s, 1C, Pyrimidine-C_2), 157.2 (s, 1C, Pyrimidine-C_6), 155.4 (s, 1C, Pyrazole-C), 149.9 (s, 1C, N = CH), 138.6 (d, J = 40.4 Hz, 1C, Triazole-C_3), 132.2 (s, 1C, Pyrazole-C), 114.1 (q, $J_{C\text{-}F}$ = 273.7 Hz, 1C, CF_3), 110.2 (s, 1C, Pyrazole-C), 109.5 (s, 1C, Pyrimidine-C_5), 70.1 (s, 1C, CH_2), 50.5 (s, 2C, Piperazine-C), 43.5 (s, 2C, Piperazine-C), 36.2 (s, 1C,

Pyrazole-CH_3), 24.3 (s, 1C, Pyrimidine-CH_3), 14.7 (s, 1C, Pyrazole-CH_3); Anal. Calcd. (%) for $C_{19}H_{22}ClF_3N_{10}S$: C 44.31, H 4.31, N 27.20. Found: C 44.48, H 4.45, N 27.06.

(*E*)-4-((5-Chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methyleneamino)-1-((4-(4,6-dimethylpyrimidin-2-yl)piperazin-1-yl)methyl)-3-(trifluoromethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (9H)

White solid, yield 84.4%, mp 140-142 °C; IR (ν_{max}, cm^{-1}): 2948, 2847(C-H), 1605(C = N), 1579, 1530, 1488, 1449(Ar), 1318, 1195(C-F), 1155(C = S). ^{1}H NMR (400 MHz, DMSO-d_6): 9.77 (s, 1H, CH = N), 6.35 (s, 1H, Pyrimidine-H), 5.21 (s, 2H, NCH_2N), 3.80 (s, 3H, Pyrazole-CH_3), 3.75 (bs, 4H, Piperazine-H), 2.77 (bs, 4H, Piperazine-H), 2.35 (s, 3H, Pyrazole-CH_3), 2.19 (s, 6H, Pyrimidine-CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.1 (s, 1C, C = S), 165.0 (s, 2C, Pyrimidine-$C_{4,6}$), 161.5 (s, 1C, Pyrimidine-C_2), 155.4 (s, 1C, Pyrazole-C), 149.9 (s, 1C, N = CH), 139.5 (m, 1C, Triazole-C_3), 132.2 (s, 1C, Pyrazole-C), 116.9 (q, J_{C-F} = 272.7 Hz, 1C, CF_3), 110.2 (s, 1C, Pyrazole-C), 109.0 (s, 1C, Pyrimidine-C_5), 70.1 (s, 1C, CH_2), 50.5 (s, 2C, Piperazine-C), 43.4 (s, 2C, Piperazine-C), 36.2 (s, 1C, Pyrazole-CH_3), 24.1 (s, 2C, Pyrimidine-CH_3), 14.7 (s, 1C, Pyrazole-CH_3); Anal. Calcd. (%) for $C_{20}H_{24}ClF_3N_{10}S$: C 45.41, H 4.57, N 26.48. Found: C 45.52, H 4.60, N 26.22.

General Synthetic Procedure for Novel Bis-1,2,4-triazole Derivatives 10

A solution of anhydrous piperazine (0.9 mmol) in ethanol (2 mL) was added dropwise to a mixture of thione intermediate **7** (1.8 mmol) and 37% formalin (2.5 mmol) in ethanol (30 mL). After that, the reaction mixture was stirred at room temperature for 2-3 h. The resulting

precipitate was filtered off and recrystallized from ethanol to give novel bis-1,2,4-triazole derivative **10(A-B)**.

(*E*)-1,1'-(Piperazine-1,4-diylbis(methylene))bis(4-((*E*)-(5-chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methyleneamino)-3-methyl-1*H*-1,2,4-triazole-5(4*H*)-thione) (10A)

White solid, yield 87.6%, mp 226-228 °C (Dec.); IR (ν_{max}, cm^{-1}): 2937, 2826(C-H), 1610(C = N), 1541, 1475(Ar), 1176(C = S). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.23 (s, 2H, CH = N), 5.05 (s, 4H, NCH_2N), 3.82 (s, 6H, Pyrazole-CH_3), 2.83 (s, 8H, Piperazine-H), 2.46 (s, 6H, Triazole-CH_3), 2.37 (s, 6H, Pyrazole-CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 162.4 (s, 2C, C = S), 154.0 (s, 2C, Triazole-C), 149.3 (s, 2C, Pyrazole-C), 147.6 (s, 2C, N = CH), 131.4 (s, 2C, Pyrazole-C), 110.7 (s, 2C, Pyrazole-C), 68.6 (s, 2C, CH_2), 50.4 (s, 4C, Piperazine-C), 36.2 (s, 2C, Pyrazole-CH_3), 14.8 (s, 2C, Triazole-CH_3), 11.1 (s, 2C, Pyrazole-CH_3); Anal. Calcd. (%) for $C_{24}H_{32}Cl_2N_{14}S_2$: C 44.24, H 4.95, N 30.09. Found: C 44.31, H 5.04, N 30.02.

(*E*)-1,1'-(Piperazine-1,4-diylbis(methylene))bis(4-((*E*)-(5-chloro-1,3-dimethyl-1*H*-pyrazol-4-yl)methyleneamino)-3-(trifluoromethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione) (10B)

White solid, yield 81.7%, mp 193-195 °C (Dec.); IR (ν_{max}, cm^{-1}): 2938, 2854(C-H), 1605(C = N), 1528, 1489, 1459(Ar), 1317, 1186(C-F), 1161(C = S). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.18 (s, 2H, CH = N), 5.16 (s, 4H, NCH_2N), 3.83 (s, 6H, Pyrazole-CH_3), 2.86 (s, 8H, Piperazine-H), 2.45 (s, 6H, Pyrazole-CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 165.0 (s, 2C, C = S), 155.4 (s, 2C, Pyrazole-C), 149.9

(s, 2C, N = CH), 138.8 (q, J_{C-F} = 41.4 Hz, 2C, Triazole-C_3), 132.2 (s, 2C, Pyrazole-C), 116.9 (q, J_{C-F} = 272.7 Hz, 2C, CF_3), 110.2 (s, 2C, Pyrazole-C), 69.8 (s, 2C, CH_2), 50.3 (s, 4C, Piperazine-C), 36.2 (s, 2C, Pyrazole-CH_3), 14.7 (s, 2C, Pyrazole-CH_3); Anal. Calcd. (%) for $C_{24}H_{26}Cl_2F_6N_{14}S_2$: C 37.95, H 3.45, N 25.82. Found: C 38.11, H 3.59, N 25.60.

Fungicidal Activity Tests

The *in vitro* fungicidal activity of the compounds against *Fusarium omysporum*, *Cercospora arachidicola*, *Physalospora piricola*, *Alternaria solani Sorauer* and *Gibberella sanbinetti* were evaluated *via* the mycelium growth rate test according to the literature [31]. The method for testing the primary biological activity was performed in an isolated culture.

The *in vivo* fungicidal activity of the compounds against *Puccinia sorghi* Schw. were tested according to the reference [32], and the commercial fungicides Triadimefon and Azoxystrobin were used as controls.

Herbicidal Activity Tests

The *in vivo* herbicidal activity of compounds were 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 [33].

KARI Inhibitory Activity Test

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, following the consumption of NADPH at 340 nm and 30 °C [24]. 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 Tris-HCl buffer (pH 8.0). Inhibitor was preincubated with the enzyme, NADPH and $MgCl_2$ in Tris-HCl buffer at 30 °C for 10 min. The reaction was then started by adding the substrate. The percentage of the inhibition was calculated.

CONCLUSION

In summary, a series of novel dimethylpyrazole and piperazine -containing (bis)1,2,4-triazole derivatives have been conveniently synthesized *via* Mannich reaction in good yields. The preliminary bioassays showed that some of the compounds showed significant *in vitro* and *in vivo* fungicidal activity against several tested plant fungi and were comparable with the control Triadimefon. Several compounds also exhibited certain herbicidal activity against *Brassica campestris*. Furthermore, several compounds possessed favorable KARI inhibitory activity in the present study, especially **8D** showed superiority than others and could be a promising KARI inhibitor for further study. The research work in this manuscript will provide useful information for the design and discovery of new agrochemicals with novel heterocyclic Mannich base structures containing piperazine moiety.

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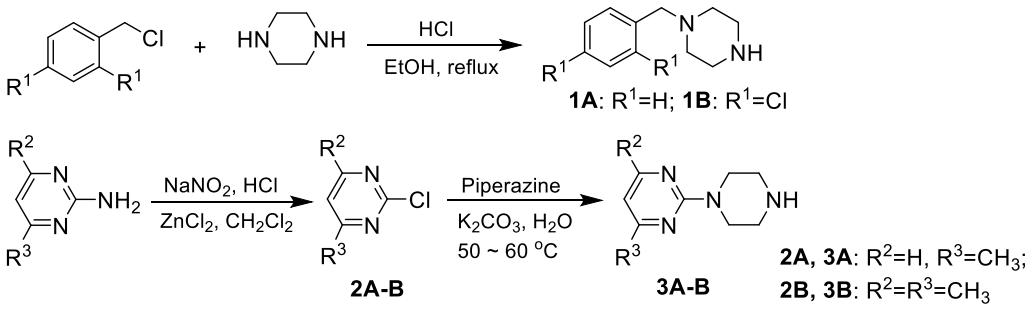
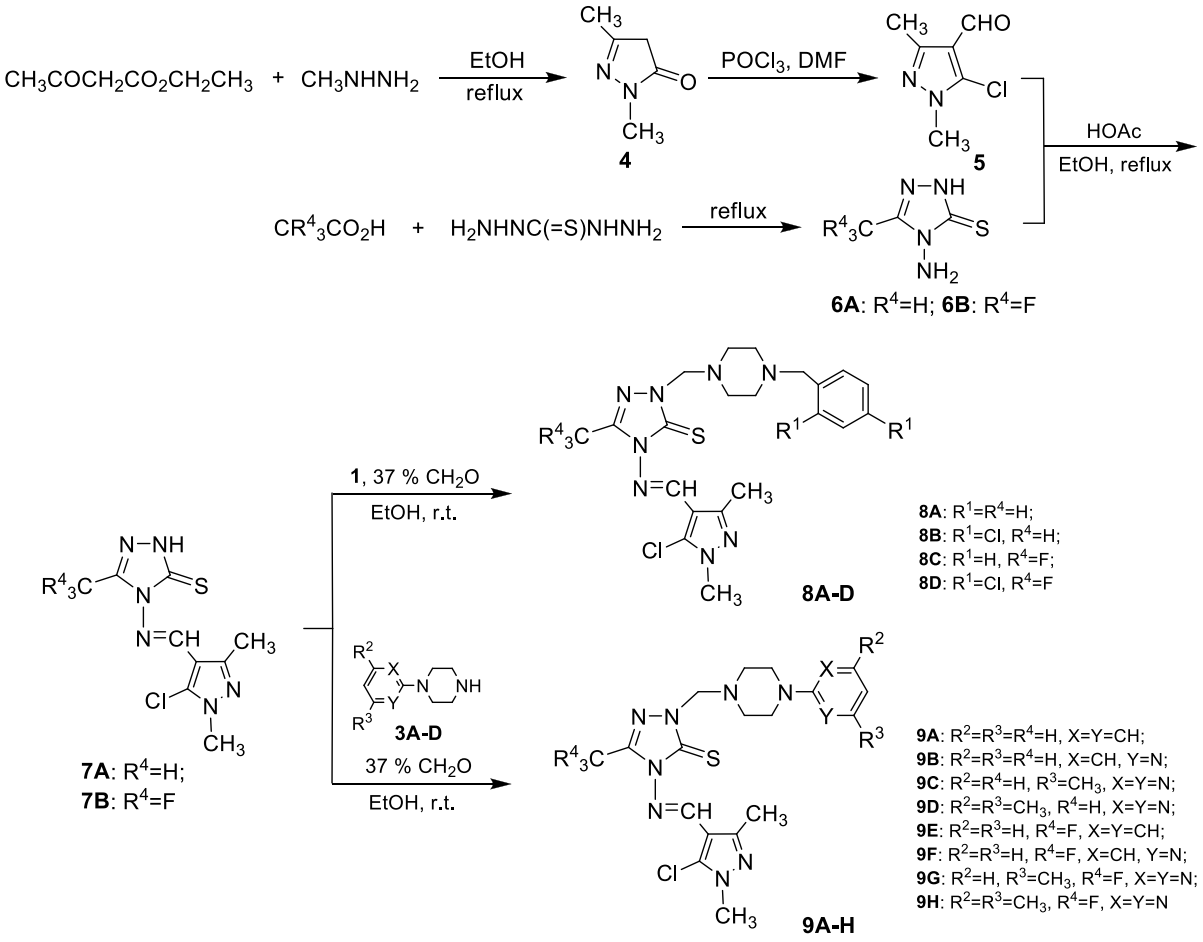
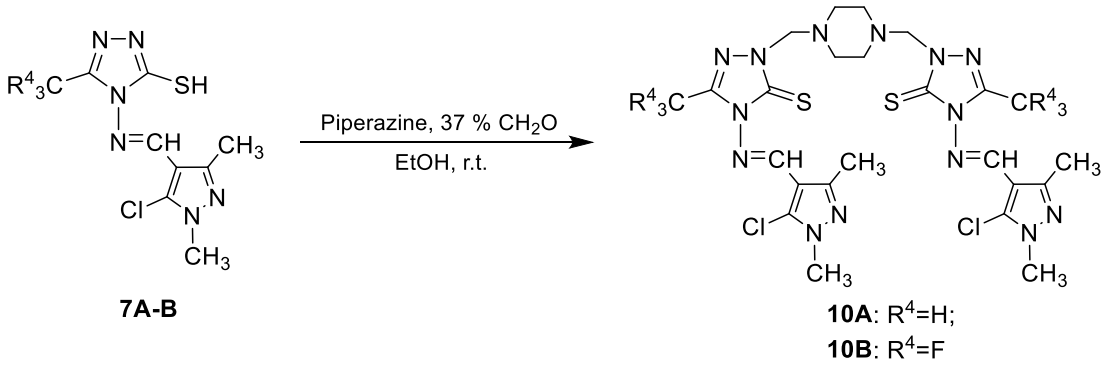


SCHEME 1: Synthesis of the intermediates **1A-B** and **3A-B**.



SCHEME 2: Synthesis of the intermediates **7A-B** and the title compounds **8A-D** and **9A-H**.



SCHEME 3: Synthesis of the title compounds **10A-B**.